Massachusetts Institute of Technology Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight

Biological Research Registration Form

Instructions:
This form is available at: https://cabescro.mit.edu/home
Please download and save this form to your computer. When completing this document please retain the format as nearly as possible and answer questions thoroughly. To modify the checkboxes, double-click on them and select "Not checked" or "Checked". Complete the appropriate sections as outlined below.
All information in this form is considered confidential.

Please indicate all relevant biosafety levels and research descriptors that describe your research.

Biosafety Level ☒BL1 ☒BL2 ☒BL2+ ☐BL3

Research Descriptors	X	rDNA/sNA	X	Biological Agents BL1	X	Other		Human embryonic stem cells		Induced pluripotent stem cells
Plants		Pathogens	X	Human Materials	X	Viral Vectors	X	Nanotechnology		Toxin Use

Required information: Every Principal Investigator must complete Sections 1, 2, 3, 11, 12 and 13. **Research Specific Sections.** Please complete the following sections if they are applicable to the research described here. **Please indicate either that the section has been completed or is not applicable (N/A).**		
Section 4	Teaching Laboratory/Biomaker space Information	☐ Completed ☒ N/A
Section 5	Use of Recombinant and Synthetic Nucleic Acid	☒ Completed ☐ N/A
Section 6	Biological Agent Use	☒ Completed ☐ N/A
Section 7	Use of Human Source Materials	☒ Completed ☐ N/A
Section 8	Use of Human Embryonic Stem (hES) Cells or Induced Pluripotent Stem (iPS) Cells	☐ Completed ☒ N/A
Section 9	Occupational Health Assessment, Medical Surveillance and Monitoring	☐ Completed ☒ N/A
Section 10	Use of Toxins	☐ Completed ☒ N/A

When you have completed the form, please email the completed form to your BSP contact or to BSP@mit.edu. Print and sign Section 13, the Certification and Signature page and mail this page to Biosafety Program, N52-496.

Section 1. General Information (required):

Current Registration number: **691**	Title of Registration: **Synthetic Biology for Clinical Applications - Mammalian**	
Principal Investigator **Timothy Lu**	Departmental Affiliation/DLC: **EECS/RLE/BE/SBC**	
PI email address: **timlu@mit.edu**	PI office phone: 715-4808	PI email address: **timlu@mit.edu**
Lab Research Contact: Ky Lowenhaupt	Lab Administrative Contact: Rashmi Karki	
Lab Research Contact email address: kytsing@mit.edu	Lab Administrative Contact email address: rkarki@mit.edu	
Lab Research Contact phone: 4-8149	Lab Administrative Contact phone: 4-6492	

Section 2. Laboratory Information (required):

a. List ALL Laboratories/Facilities where research is to be conducted and the corresponding biosafety level: include cold/warm rooms, equipment rooms as appropriate. Please indicate room(s) where biosafety cabinets (BSC) are located. Please include the location(s) of the autoclave used for laboratory waste sterilization prior to disposal. (The box is expandable.)

		Check box if applicable					
Room Number	Biosafety Level	BSCs in room	Warm/ Cold Room	Equipment Room	Human Materials	hES/iPS cells used	Autoclave location
NE47-017B	BL1	1 BSC					Biowaste boxes
NE47-019	BL1						Biowaste boxes
NE47-209 – shared lab	BL2	1 BSC					Biowaste boxes
NE47-217 – shared tissue culture room	BL2	5 BSCs			X		Biowaste boxes
NE47-216	BL2/BL2+ (under develop-ment)	2 BSCs			X		Biowaste boxes
NE47-235	BL2						Biowaste boxes
NE47-265	BL2						Biowaste boxes
NE47-290C – shared cold room	BL2		Cold Room				Biowaste boxes
NE47-290D – shared autoclave/dish washing room	BL2			X			Biowaste boxes
NE47-335B	BL2						Biowaste boxes
NE47-335C	BL2						Biowaste boxes
36-797	BL2						Biowaste boxes
36-799 – autoclave/dish washing room	BL2			X			Biowaste boxes
36-781	BL2						Biowaste boxes
36-789	BL2						Biowaste boxes

b. Please list or attach a list of all laboratory personnel working on this Registration at MIT, to include faculty, technical staff, graduate students, UROPS, etc. (The box is expandable.)

Laboratory Personnel			Research Materials Used (place an X in the appropriate box)			Training Completed (Enter most recent date of training)	
Name	Kerberos	UROP (Y/N)	Uses BL1, BL2 material	Uses BL2+, BL3 material	Uses human material	General Biosafety training (260c)	Bloodborne Pathogens training
Cao, JiCong	jicong	N	X	NA	X	03/09/16	03/07/17

Chang, Cheng	Cchang1	N	X	N/A	X	04/24/15	03/07/17
Chen, Ying-Chou	Yjoechen	N	X	N/A		03/09/16	03/07/17
Chen, Willam	wcwchen	N	X	N/A		03/09/16	03/07/17
Citorik, Robert	Rcitorik	N	X	N/A	X	03/09/16	03/07/17
Da Luz Areosa Cleto, Sara	scleto	N	X	N/A		03/09/16	03/07/17
Farzadfard, Fahim	Ffard	N	X	N/A		03/09/16	03/07/17
De la Fuentes Nunez, Cesar	cfuente	N	X	N/A		03/09/16	03/07/17
Higashikuni, Yasutomi	tommyh	N	X	N/A	X	03/09/16	03/07/17
Jerger, Logan	ljerger	N	X	N/A		07/14/16	03/07/17
Jung, Giyoung	giyoung	N	X	N/A		03/09/16	03/07/17
Jusiak, Barbara	jusiak	N	X	N/A	X	03/09/16	03/07/17
Lemire, Sebastien	Sele	N	X	N/A		03/09/16	03/07/17
Lowenhaupt, Ky	kytsing	N	X	N/A	X	03/09/16	03/07/17
Lu, Timothy	Timlu	N	X	N/A	X	03/09/16	03/07/17
Mimee, Mark	Mmimee	N	X	N/A	X	03/09/16	03/07/17
Mueller, Isaak	imueller	N	X	N/A		03/09/16	03/07/17
Nissim, Lior	Liorni	N	X	N/A	X	03/09/16	03/07/17
Nissim, Adina	anissim	N	X	N/A	X	03/09/16	03/07/17
Park, Heechul	heechul	N	X	N/A		03/09/16	03/07/17
Pery, Erez	erezpery	N	X	N/A	X	03/09/16	03/07/17
Sun, Qing	sunqing	N	X	N/A		03/09/16	03/07/17
Tang, Tzu-Chieh	tctang	N	X	N/A		03/09/16	03/07/17
Tham, Eleonore	etham	N	X	N/A		03/09/16	03/07/17
Weisinger, Karen	karen_w	N	X	N/A	X	03/09/2016	03/07/2017
Wu, Ming-Ru	mingru	N	X	N/A	X	03/09/16	03/07/17
Yehl, Kevin	kyehl	N	X	N/A		03/09/16	03/07/17

Section 3. Research Description (required):

a. In lay terms, briefly describe your research as you would to a friend who is not a scientist.

The Synthetic Biology Group is focused on advancing fundamental designs and applications for synthetic biology. Using principles inspired by electrical engineering and computer science, we are developing new techniques for constructing, probing, modulating, and modeling engineered biological circuits. Our current application areas include infectious diseases, amyloid-associated conditions, and nanotechnology.

Research in the Lu Lab that use mammalian cells can be divided into three classes. One class comprises the development and optimization of tools that will allow the rapid, economical production of therapeutic proteins in the context of biomanufacturing. Therapeutic proteins include hormones such as insulin, erythropoietin and follicle stimulation hormone, produced normally in the human body, but required in the disease states diabetes, renal failure, and infertility respectively. They also include the burgeoning class of therapeutic antibodies being developed for infectious disease and for targeted killing of cancer cells.
The second group of projects looks at the role of combinations of gene in the initiation and progress of disease. Current knowledge is based on careful studies of the effect of single genes in pathogenesis. The Lu lab has developed a strategy to observe the combined effect of two or more genes on the disease process in a high-throughput manner, allowing the screening of tens of thousands of combinations of genes and looking for synergies. This strategy, called CombiGEM, is being used to look at diabetes, heart disease, and ALS. It is also being applied to the investigation of therapies for cancer.
The third class of projects comprises experiments aimed at developing treatments for human disease using the tools of synthetic biology. Some of these experiments aim to develop robust, versatile tools that can be used to engineer mammalian cells and tissues. Others use the tools already in existence to develop gene therapies and cell therapies using genetically tailored cells. Projects involve the treatment of heart disease with gene therapy, immune cell therapy for the treatment of cancer, and gene therapy for kidney stones and obesity.

b. Outline the overall goal(s) of the research in the space below. Give enough information to assure that the purpose of the experiments and the techniques used are clear. Please use reasonably non-technical terms.

Section 4. Teaching Laboratory / Biomaker space Information: Check box if not applicable ☒

a. Please complete the bulleted points below for a Teaching Lab.

Teaching Lab: ☐

- Number of students in the class:
- Category of students/participants (e.g. high school, undergraduate, graduate student):
- Student-Instructor Ratio:
- Experience level of course Teaching Assistants:
- Describe how any biological materials will be handled and who will handle the biological materials:

b. Please complete the bulleted points below for a Biomaker Space.

Biomaker Space: ☐

- Number of user in the space:
- Minimum experience of the participants (undergraduate, graduate student):

- Average Participant/Supervisor Ratio:
- Experience level of Supervisors:
- Estimated Core hours of operation:
- Briefly describe the training process and how proficiency will be verified for users; include any equipment specific training that will be given and by whom:
- Describe how access to the Biomaker space is granted to new users:
- List the equipment available in the Biomaker Space for use in biological experiments:
- Describe how inventory of biological material will be kept and the process for new material to be added to the Biomaker Space:

Section 3. Research Description (required):

Outline the overall goal(s) of the research in the space below. Give enough information to assure that the purpose of the experiments and the techniques used are clear. Please use reasonably non-technical terms.

Project 1: The production and secretion of therapeutic proteins in CHO cells

1.1) Optimizing stable gene expression in CHO cells

In order to optimize production of recombinant therapeutic antibodies (such as the cancer treatment Nivolumab or the antibodies that make up the Ebolavirus vaccine Zmapp, c13C6, c2G4, and c4G7) in Chinese hamster ovary (CHO) cells, we are developing a recombinase-based gene integration strategy for simple and rapid CHO cell pool engineering for biomanufacturing applications. This strategy can reduce the timeline of cell line construction from 6-10 months to 2-4 weeks. Unlike random gene integration, recombinase-based site-specific gene integration allows one copy of foreign gene to be inserted at a pre-selected chromosomal site. The pre-selected site is a characterized genomic hot spot, which will allow reproducible and high protein expression; thus, we will produce stable, high-producing, and homogeneous CHO cell pools expressing high levels of proteins. One application will be to produce homogeneous pools of therapeutic antibodies, thereby greatly reducing the time of clonal expansion from single cell clones.

For this project, we have made CHO-K1 cells lines with transgenic “landing pad” (LP) cassettes inserted into the Rosa26 locus. An LP cassette consists of the constitutive Hef1a promoter followed by an attP site (a 60-80bp DNA sequence recognized by an integrase) and an EYFP-2A-Hygro coding region (enhanced yellow fluorescent peptide and hygromycin resistance gene, co-expressed from the same promoter by using a self-cleaving 2A peptide linker). The attP site is recognized by site-specific integrases that catalyze recombination between attP and attB sites. Co-transfection of an LP cell line with an attB site-carrying payload vector and an integrase expression vector leads to stable integration of the payload into the LP. We have built attB payload vectors that encode mKate-2A-Puro (mKate is a red fluorescent protein). Thus, following payload integration, cells go from yellow fluorescent and hygromycin-resistant (EYFP-2A-Hygro expression) to red fluorescent and puromycin-resistant (mKate-2A-Puro expression).

We obtained the LP-attP vector and attB-payload vector from Ron Weiss's lab, and modified the vectors with attP and attB sites for different integrases.

Personnel contributing to this project are Barbara Jusiak, William Chen, JiCong Cao, Giyoung Jung and Tim Lu.

1.2) Control of glycosylation of proteins in CHO cells

The production of antibodies in CHO cells is discussed **1.**1. In order for these antibodies to be active, precise glycosylation is required. The goal of this project is to use genetic engineering tools to controlling the glycosylation profile of therapeutic proteins in Chinese Hamster Ovary (CHO) cells. To accomplish this goal, we will construct circuits that are able to target glycosylation genes and knock down its expression using genome editing tools such as CRISPR/Cas9 and RNAi systems, which are delivered using FuGene (Promega) transfection reagent or by electroporation of mRNA into cells (Hashimoto and Takemoto, 2015 Nature Reports article 11315). CRISPR/Cas9 system will provide precise editing that is stably maintained, while the RNAi system will allow us to tune the expression level of genes with an inducible promoter or provide transient expression. The functional knockdown of glycosylation genes will be evaluated by measuring levels of both RNA and protein. Overall, this project will identify and validate an efficient method to engineer post-translational modification of a therapeutic protein. This will have applications not only for antibody production, but also for other proteins such as erythropoietin, used to treat patients on renal dialysis, and FSH use in fertility treatments and in vitro fertilization.

Personnel contributing to this project are Giyoung Jung , Barbara Jusiak, and Tim Lu.

1.3) Development of a tunable synthetic transcription system for expression in CHO cells

We will develop a tunable synthetic transcription system based on deactivated Cas9 (dCas9) activators to be used in the landing-pad CHO cells. The goal of this project is to develop a synthetic transcription system that can be precisely tuned with a number of parameters, such as guide RNA sequences, design of the operators, addition of enhancer sequences, etc, for biomanufacturing purposes. This synthetic transcription system can be expression transiently or integrated into the landing pad(s) for long-term expression. This system will enable a precise control of the protein expression level in CHO cells episomally and chromosomally.

Personnel contributing to this project are William Chen, and Tim Lu.

1.4) Optimizing stable expression in other mammalian cell lines

Similar to the project described in section 1.1 above for mammalian cells other than CHO cells, we will develop a recombinase-based gene integration strategy for simple and rapid mammalian cell pool engineering for biomanufacturing, tissue engineering, and therapeutic applications. This approach will reduce the timeline of cell line construction from 6-10 months to 2-4 weeks and enable stable integration of complex gene circuits. Unlike random gene integration, recombinase-based site-specific gene integration allows one copy of foreign gene to be inserted at the same pre-selected

chromosomal site. Thus, we will obtain stable, high-producing, and homogeneous mammalian cell pools expressing high levels of proteins and/or non-coding RNAs, as the pre-selected site will be a genomic hot spot, which allows reproducible and high protein/RNA expression. Moreover, we will use the homogeneous pools to produce therapeutic antibodies and proteins, which will further reduce the time of clonal expansion from single cell clones. The mammalian cell lines we plan to work with include mouse MS1 endothelial cells, C2C12 muscle cells, mesenchymal stromal cells, macrophage, and rat H9c2 heart muscle cells.

Personnel contributing to this project are JiCong Cao, William Chen, Yasutomi Higashikuni, and Tim Lu.

Section 5. Use of Recombinant or Synthetic Nucleic Acid: **Check box if not applicable** ☐ **(Please complete this section if you use or generate recombinant microorganisms, cells, animals, plants, etc.)**

Please indicate the Section of the NIH Recombinant DNA Guidelines that applies to this research. If you have questions Biosafety Staff will be glad to assist you. Note that the City of Cambridge requests that we register all recombinant work even if it is exempt under section III-F or Appendix C. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OBA website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

Section III-A ☐ **Section III-B** ☐ **Section III-C** ☐ **Section III-D** ☒ **Section III-E** ☐ **Section III-F** ☐

a. Source of Gene, Insert or Clone:

1. Specify DNA/RNA source (or probe), nature of insert, is a protein expressed, and percent of any viral genome in construct (for viral vectors see 5.b.2):

Insert	Cloned into	Under promoter control	Insert source	Nature of insert	Protein expressed?	% viral genome
Selection markers for E. coli including Bla, Kan, Zeocin resistance, etc	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	Native promoter	Plasmid collection	Antibiotic resistance	yes	<1%
Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	SV40	Plasmid collection	Antibiotic resistance	yes	<1%

Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Plasmid collection, Ron Weiss lab	Recombinases and integrases	yes	<15%
Integrase and recombinase recognition sites such as attP and attB	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	Hef1a	Ron Weiss lab	Integrase and recombinase recognition sites	no	<1%
gRNAs	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	pSNR52	Synthetic DNA	Guide RNAs	no	<1%
Cas9 and its derivatives	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	pEF1a	Plasmid collection (originally from S. aureus)	Enzymatic portion of CRISPR/Cas9 system	yes	<1%
Fluorescent proteins such as EGFP and EYFP	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Plasmid collection	Marker for FACS	yes	<1%
Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Synthetic DNA	Therapeutic proteins	yes	<1%

2. Are any sequences from select agents and toxins? Yes ☐ No ☒; If yes, please specify.

3. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

4. Is the DNA source from a USDA-regulated plant, animal or insect? Yes ☐ No ☒; If the regulated organism is grown or stored at MIT, please include a copy of the USDA permit. (Link to USDA site: http://www.aphis.usda.gov/brs/index.html)

b. Vectors and Host Cells:

1. Identify cloning/expression/transfection vectors used, recipient bacterial strains used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, plant, etc.). Provide a restriction map of vector if it is new or not published. If commercially available vector or plasmid, please indicate vendor. Describe the location and type of promoters and other control sequences and percent of any viral genome in construct.

Vector name	Recipient Bacterial strain	Reporter gene?	% viral genes	promoters	Host cell
pBluescript	DH5α	Fluorescent proteins	0	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	CHO, CHO-K1, mouse MS1 endothelial cells, C2C12 muscle cells, mesenchymal stromal cells, macrophage, and rat H9c2 heart muscle cells.

2. If using viral vectors, fill out the table below. If using multiple types of viral vectors, please add a new table per vector type; multiple variations of the same viral vector type can be in the same table. (If using retroviral or lentiviral vectors additional requirements apply. Please see MIT CAB/ESCRO policy at the following URL: https://cabescro.mit.edu/home

Viral Vector type	Adenovirus expression vector and its derivatives (E.g., pacAd5 and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Adenovirus serotype 5 based delivery system
Source (vendor / collaborator)	RaPAd Adenoviral system from Cell Biolabs and related products
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	Infectivity PCR Ishii-Watabe, A., E. Uchida, A. Iwata, R. Nagata, K. Satoh, K. Fan, M. Murata, H. Mizuguchi, N. Kawasaki, T. Kawanishi, T. Yamaguchi, and T. Hayakawa. 2003. Detection of replication-competent adenoviruses spiked

	into recombinant adenovirus vector products by infectivity PCR. *Mol Ther* 8:1009-1016.
Pseudotype	**No**
Host range	**Mammals including humans**
Safety feature (e.g. self-inactivating)	**Two plasmid system. Vector is replication deficient. There is no integration into the host genome.**
Integrate into genome (yes/no)	**No**
Exposure hazard (e.g. insertional mutagenesis)	Eye infection and common cold
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	AAV CMV expression vector and its derivatives (E.g.,pAAV-MCS expression vector and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Helper free adeno-associated virus system
Source (vendor / collaborator)	AAV helper free expression system from another MIT Lab or UPenn or VectorCore
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	AAV is naturally replication incompetent and no helper virus will be used
Pseudotype	**No**
Host range	Primate cells, including human
Safety feature (e.g. self-inactivating)	Does not cause disease in human. Three plasmid system.
Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Not associated with human disease
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	3rd generation lentiviral vector and its derivatives (E.g., pFUGW vector and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	HIV-based lentiviral system, pLV CAG CN-2A-CN (AAVS1 Zinc finger) This lentiviral backbone is derived from HIV-1 genome (contains 5', and 3'Long terminal repeats, Rev Response Elements (RRE), Psi packaging element, Woodchuck Hepatitis virus post-transcriptional response element). However, the elements in the lentiviral backbone are insufficient to cause viral replication and virulence. This lentiviral genome is devoid of virulence genes and essential genes involved in replication (such as gag, pol, env, tat, etc.).
Source (vendor / collaborator)	Addgene
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	To test for the presence of replication-competent or helper virus, marker rescue experiments will be performed. Culture supernatants of 293T cells which have been infected with a replication-defective virus expressing the puromycin resistance gene will be used to infect a second set of 293 cells. The second set of 293 cells will be selected in puromycin for several weeks to identify infected cells. This is a sensitive technique since a single colony, arising from a single replication-competent virus, can be detected readily or In order to test for the presence of replication competent or helper virus, we perform standard RT-PCR or real time PCR to determine the presence of lentiviral-related RNA transcripts. In addition, we test for horizontal transfer from supernatant of infected cells; supernatant of 293T cells, infected with replication-defective virus expressing GFP, is harvested and used to infect a second set of 293T cells. Test cells are prepared for FACS analysis at various timepoints for several weeks to determine the presence of GFP+ cells, an indication of infection by replication competent virus. This is a sensitive technique since a single GFP+ cell, arising from a single replication-competent virus, can be detected readily. These tests will be performed for each construct. The properties of particular plasmids should not change over time since they are stable at -20 Dull, T., R. Zufferey, M. Kelly, R. J. Mandel, M. Nguyen, D. Trono, and L. Naldini. 1998. A third-generation lentivirus vector with a conditional packaging system. *J Virol* 72:8463-8471.
Pseudotype	VSV-G
Host range	Mammals including humans
Safety feature (e.g. self-inactivating)	SIN, 3-plasmid system

Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Insertional mutagenesis, HIV virus may occur in HIV+ individuals or by in vitro recombination resulting in replication competent lentivirus.
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	HSV-1 based amplicon vector and its derivative (E.g., pA-EUA1 and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Herpes-simplex virus-1 based system
Source (vendor / collaborator)	Professor Alberto Epstein
Packaging cell line(s), if applicable	VERO2-2
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	Plaque assay (3) Lieber, D., and S. M. Bailer. 2013. Determination of HSV-1 infectivity by plaque assay and a luciferase reporter cell line. *Methods Mol Biol* 1064:171-181
Pseudotype	No
Host range	Mammals including humans and vertebrates
Safety feature (e.g. self-inactivating)	Most adults have pre-existing immunity. Replication incompetent virus.
Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Cold sores
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

3. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

c. Use of Animals (including invertebrates) or Insects: Check box if not applicable ☒
Most research using animals also needs the approval of the MIT Committee on Animal Care (IACUC). Contact them to register your animal research; If you have CAC protocol no(s), list them here: _____. The questions below are intended to deal with the use of potentially biohazardous agents in animals/insects or the creation of transgenic animals/insects.

1. Will rDNA technologies (such as producing transgenics), recombinant microbes/cells be used in animals or insects? Yes ☐ No ☒

   a. If yes, please describe objective of the research. Include recipient species, and list pathogen, rDNA technology, or recombinant microbe or human materials that will be used in animal/plant/ insect, and resulting genotype. What selection marker will be used?

2. If transgenic, including "knockout", animals/insects will be generated, please provide information on the transgene and vector as well as the expected phenotype of the recombinant animal/insect.

3. Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory or animal facility? Yes ☐ No ☒ If yes, explain:

d. Large-Scale Research: Check box if not applicable ☒

Do experiments involve growth of 10 liters or more of culture at a time? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

1. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

2. Describe the steps used to handle and contain spills:

e. Use of Plants: Check box if not applicable ☒
Describe experiments that involve recombinant work and plants, whether the plant itself is made recombinant or recombinant cell or microorganisms are used with plants.

Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory? Yes ☐ No ☐ If yes, explain:

Section 6. Biological Agent Use: Check box if not applicable ☐

(Please complete this section if you work with viable microorganisms or viruses.)

a1. Agent identification. List biological agent(s)/microorganism(s) genus/animals/plants/species/strain and genotypic markers, host range; any antibiotic resistance; recommended Biosafety Level (CDC); the source of the agent (e.g. new isolate from human tissue, blood, animal, tissue culture, another laboratory, ATCC, etc.).

Genus and species	Strain	Bio safety level (BSL) / Risk group (RG)	Source	Max volume used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
E. coli	DH5α	BL1	Personal collection	1L	1e12	80% ethanol, 10% bleach, Sklar, Preempt	N

For agents that are pathogens, please complete the following table:

Organism	Infectious Dose	Route of exposure	Host range	Clinically relevant Drug resistance	Availability of treatment	Special equipment /PPE used (above standard PPE)	Disease state and symptoms of exposure

a2. List and describe any other biological material not covered in the tables above:

Established cell lines from Mus musculus (MS1 endothelial cells, C2C12 muscle cells, mesenchymal stromal cells, macrophages), Chinese hamster (CHO), rat H9c2 heart muscle cells. All cell lines are unable to survive outside the experimental environment. Tissue culture cells are disinfected with 10%bleach, Sklar or Preempt.

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

1. Briefly describe experiment and procedures involving use of biological agents?

E. coli will be grown and transfected using standard methods, namely transfection into chemically competent cells (2002. *Escherichia coli*, Plasmids, and Bacteriophages. Current Protocols in Molecular Biology. 59:1.0:1.0.1–1.0.3.)

Mammalian cell lines will be grown as described in Current Protocols in Cell Biology; ISBN: 978047114303. Cell will be transfected according to manufacturer's protocols (FuGene (Promega), Viafect (Promega) or transduced as described in Harford, J. B. 2011. Viruses. Current Protocols in Cell Biology. 51:26.0:26.0.1–26.0.3. and as specified by the Addgene protocols.

2. Will experiments result in acquisition of new characteristics such as enhanced virulence, infectivity, drug or antibiotic resistance, or change in host range? Yes ☒ No ☐ If so, explain:

Experiments will result in the production of therapeutic proteins. Cells will gain resistance to drugs such as hygromycin, G418 or puromycin; however this has no health or environmental consequences because of the fragility of tissue culture cells. Cells will epress fluorescent proteins.

3. Will you introduce viable biological material (microorganisms or cells) into animals?

   Yes ☐ No ☒ If so:

   a) Please describe:

   b) List your MIT Committee on Animal Care (IACUC) protocol no(s), if your animal model also needs MIT Committee on Animal Care (IACUC) approval: _____

Section 3. Research Description (required):

Outline the overall goal(s) of the research in the space below. Give enough information to assure that the purpose of the experiments and the techniques used are clear. Please use reasonably non-technical terms.

2: Use of CombiGEM to characterize disease and identify druggable gene targets

The bacterial immune system, **CRISPR/Cas9**, and its derivatives has provided a powerful toolkit for Synthetic Biology. In the experiments described in this section and in section 3, this system is used in the following ways. In CombiGEM screens (as described in Wong, et al, 2016, Proc Natl Acad Sci USA 113:2544-2549) gRNAs targeting selected genes are expressed with barcodes, which allows high-throughput next-generation sequencing screens of selected cell populations. The gRNAs and the Cas9 are introduced using lentiviral libraries built on the pFUGW scaffold. This technique is important when stable integration is needed for experiments. In other experiments, either gRNA, Cas9 mRNA and derivatives, or both will be introduced by electroporation (Hashimoto and Takemoto, 2015 Nature Reports article 11315). A third delivery method will be to use FuGENE (Promega) or ViaFECT (Promega). In the experiments described below, in most cases genes in the target cells are knocked out; in some instances, Cas9 derivatives (dCas9) are used to regulate gene expression without making changes in the genome. gRNAs are designed to target only the relevant species in all cases.

In order to comply with regulations, we are in the process of setting up a BL2+ space in which tissue culture can be performed. This will be in NE47-216. The room has been assessed by EHS employees and deemed appropriate. This room will have two hoods, one for use at BL2+ and the other for BL2 experiments. It will contain a dedicated BL2+ incubator, and all work performed in the room will follow BL2+ guidelines and the SOP to be developed in collaboration with EHS.

The delivery of neither CRISPR nor Cas9 using the latter two protocols cannot have environmental or human disease consequences short of a major spill. In fact, it is almost impossible that RNA will survive even if grams were spilled. The pFUGW system has been selected as the safest viral vector for this work. It is a third generation, three vector system, which is self-inactivating; there are many commercial lentiviral vectors with a Cas9 payload based on this system in use in the scientific

community. Work with all lentiviral constructs in our lab is done only in a BSC and everything is decontaminated daily with Preempt, as well as immediately after all spills.

2.1) Use of CombiGEM to study of combinations of genes in disease development, including diabetes, and heart disease

The orchestrated action of genes controls complex biological phenotypes, yet the systematic discovery of gene and drug combinations that modulate these phenotypes in human cells is labor intensive and challenging to scale. We have created a platform for the massively parallel screening of barcoded combinatorial gene perturbations in human cells and translated these hits into effective drug combinations. This technology leverages the simplicity of the CRISPR-Cas9 system for multiplexed targeting of specific genomic loci and the versatility of combinatorial genetics en masse (CombiGEM) to rapidly assemble barcoded combinatorial genetic libraries that can be tracked with high-throughput sequencing. We applied CombiGEM-CRISPR to create a library of 23,409 barcoded dual guide-RNA (gRNA) combinations and then perform a high-throughput pooled screen to identify gene pairs that inhibited ovarian cancer cell growth when they were targeted. We validated the growth-inhibiting effects of specific gene sets, including epigenetic regulators KDM4C/BRD4 and KDM6B/BRD4, via individual assays with CRISPR-Cas-based knockouts and RNA-interference-based knockdowns. We also tested small-molecule drug pairs directed against our pairwise hits and showed that they exerted synergistic antiproliferative effects against ovarian cancer cells. We envision that the CombiGEM-CRISPR platform will be applicable to a broad range of biological settings and will accelerate the systematic identification of genetic combinations and their translation into novel drug combinations that modulate complex human disease phenotypes

We have plans to generate a lentiviral vector library in order to study the unfolded protein response (UPR) in immortalized mouse pancreatic beta-cell lines. I will use the FuGW vector backbone, which is currently used by other members of the Lu lab. The lentiviral vectors will express guide RNAs (gRNAs) for editing/mutating endogenous genes using the CRISPR-Cas9 system. I will use previously characterized strong constitutive promoters such as *CMV* and *Hef1a* in my lentiviral library. I will construct the lentiviral vector library using the same methods and following the same safety precautions as those used previously by Lu lab members who developed CombiGEM in mammalian cells. The miRNAs, ORFs, and gRNAs in the vector library will be either PCR-amplified from a mouse DNA library (genomic DNA for miRNAs and cDNA library for ORFs) or ordered from a DNA-synthesis company such as Integrated DNA Services (gRNAs).

In addition to the lentiviral vector library, we will also build recombinant DNA reporter constructs for stable integration into the mouse beta-cell lines. The purpose of the reporter constructs will be to differentially turn on fluorescent protein expression on/off in healthy vs. stressed cells. In addition to fluorescent proteins, the reporter constructs will also encode human codon-optimized *Streptococcus pyogenes* Cas9 protein, for CRISPR-mediated editing of the beta cell genome with gRNAs in my lentiviral library. The Cas9 coding region will be derived from a vector published by Feng Zhang's lab at the Broad Institute and available through Addgene.

Chronic hyperactivation of the UPR has been implicated in neurodegenerative diseases, including prion diseases. In pancreatic beta-cell lines, it is implicated in diabetes. The effect of modulating the expression of endogenous genes in beta-cells will be investigated. No experiments will be conducted in neural cells or with PrP (prion protein), and all gRNAs will target mouse genes specifically. These experiments are highly unlikely to result in potentially infectious prion disease or activation of the UPR in humans, even were no other precautions taken.

Mammalian cell lines to be used in this project:

- HEK293 (Human Embryonic Kidney) – immortalized, easy-to-grow cell line for use as a "test bed" for new reporter constructs. Obtained from Lior Nissim in the Lu lab (who got it from ATCC).
- MIN6-m9 – immortalized mouse insulinoma cell line, extensively used for in vitro studies of pancreatic beta-cells. Gift from Rohit Kulkarni, Senior Investigator at the Joslin Diabetes Center of Harvard Medical School.
- IRlox – immortalized pancreatic beta-cell line from mice with a floxed insulin receptor (IR) gene. The floxed allele is not excised; hence the cells are wild-type, like MIN6-m9 (insofar as an immortalized cell line can be considered "wild-type"). Gift from Rohit Kulkarni, Senior Investigator at the Joslin Diabetes Center of Harvard Medical School.
- 3T3 mouse embryonic fibroblasts (MEFs) – immortalized, easy-to-grow mouse cell line to be used as a "test bed" for the reporter constructs. Obtained from ATCC.

The same experimental design, using cardiomyocytes and fibroblast cell lines will be used to identify combinations of genes implicated in the progression of heart disease. Personnel contributing to this project are Barbara Jusiak, Yasutomi Higashikuni, William Chen, and Tim Lu.

2.2) Development of genetic circuits for treatment of heart disease

Our gene circuits will provide self-repairing capacity to mammalian cells under pathological conditions. We will transfect our gene circuits with viral vectors into mammalian cells, and assess their therapeutic efficacy by using in vitro disease models. Genes targeted by these circuits will include both single genes and combinations identified by CombiGEM, as described above.

Our gene circuits will consist of cell-type- and/or disease-specific promoters, effectors such as microRNAs and shRNAs as well as endogenous proteins, and transcriptional and/or translational regulators. Cell-type specific promoters allow us to design gene circuits that work only in our desired cells, which is important for clinical use. Disease-specific promoters work as sensors of pathological conditions and activate expression of effectors in mammalian cells only under diseased conditions such as heart failure and obesity, and can be natural and/or synthetic promoters. Effectors will be combinations of endogenous proteins, microRNAs, and shRNAs that counteract pathological changes in mammalian cells such as cell death and dysfunction. We will also use recombinases and the CRISPR/Cas9 system for genome editing and multiple gene expression system. Transcriptional and translational regulators such as ribozymes

and unique structural motifs in RNAs are used to build complex logic gate-based gene circuits.

Our projects consist of 4 parts: 1) finding good sensors, 2) finding good effectors, 3) building gene circuits, 4) assessment of therapeutic gene circuits in disease models. These experiments will be performed in the following mammalian cells: 1) cardiomyocyte cell line such as HL-1 (mouse) and H9c2 (rat), 2) murine fibroblast cell line such as 3T3-L1, 3) human vascular endothelial cell line such as HUVEC, 4) human embryonic cell line such as HEK293, 5) primary mammalian cells such as rat neonate cardiomyocytes, murine adult cardiomyocytes, human and murine preadipocytes and adipocytes. Primary human cells will be provided by collaborators at MGH. For transfection of gene circuits into mammalian cells, we will use viral vectors such as AAV and lentivirus. For transfection of microRNAs or shRNAs, a non-viral liposome-based transfection reagents will be used. To detect outputs of transfected gene circuits, reporter proteins will be integrated into gene circuits such as fluorescent proteins, luciferase and SEAP. For detection of effector proteins and non-coding RNAs, Western blot analysis and quantitative PCR will be performed, respectively.

To induce pathological stress in mammalian cells, we use drugs such as angiotensin 2, endothelin-1, norepinephrine, hydrogen peroxide, phenylephrine, isoproterenol and Cobalt chloride. Cellular function will be assessed by physiological assessment such as calcium handling and contractile strength in cardiomyocytes, migration capacity in endothelial cells, fibroblasts and immune cells and thermogenesis in preadipocytes and adipocytes. Gene expression and protein production in mammalian cells will be assessed by PCR and Western blot analysis, respectively. Cell viability will be assessed by ATP, MTT or MTS assay.

Personnel contributing to this project are Yasutomi Higashikuni, William Chen, Lior Nissim, Adina, Nissim, Karen Weisinger and Tim Lu.

2.3) Use of CombiGEM to study kinases that cause synthetic lethality in cancer cells

Our works will involve combinatorial genetic screening that screens for combinations of kinases whose inhibitions cause synthetic lethality in triple negative breast cancer. Since we are screening for kinases, we are not specifically studying certain signaling pathways. The cell lines used for this xenograft assay will be MDA-MB-231 (ATCC HTB-26), BT-549 (ATCC HTB-122) or HMLER (obtained from Dr. Robert Weinberg, Whitehead Institute, MIT).

The cell lines will contain dCas9 protein deficient in nuclease activity (D10A/H841A mutant) obtained from addgene, fused with KRAB (Kruppel associated box) also obtained from Addgene. dCas9 is derived from pathogen Streptococcus Pyogenes. However Cas9 does not contribute to pathogenesis of S. Pyogenes, and thus introduction of dCas9 into cells will not induce any pathogenicity. dCas9-KRAB gene is delivered to the cancer cells using replication incompetent lentivirus derived from FUGW vector (Addgene #14883, from Dr. David Baltimore). (See section 5 for further description of our lentiviral vectors.) Infection of lentivirus into cancer cells is not expected to cause any gain of pathogenicity to cancer cell lines. The same cells are

also expressing mixed library of sets of single guide RNAs (sgRNAs) against selected kinases driven by the U6 promoter derived from the human genome. The library vector involves the identical HIV-1 genome derived lentiviral backbone with U6 promoter-sgRNA and random barcode sequences. Since our screen searches for synthetic lethality in breast cancer, the sgRNAs will suppress expression of oncogenes, and thus will not cause tumor if not protect against tumor. Finally barcodes are extremely short (8~12nt) stretch of random sequence, which is extremely unlikely to have any meaningful function. The library will be delivered to cancer cells via lentivirus.
Another combinatorial genetic screening will involve screens for combinations of immunosuppressive receptors whose inhibitions cause active tumor immunity against cancer. BALB/c mice derived primary mouse T cells will be engineered to express dCas9 protein and sgRNAs against selected immunosuppressive receptors. The methods of gene delivery to primary mouse T cells are identical to those done for breast cancer cells for synthetic lethality screen, except the sgRNAs will target immunosuppressive receptors. These sgRNAs are expected to boost anti-tumor immunity, and thus is unlikely to cause cancer.
adverse effect to human when infected.
Personnel contributing to this project are Tackhoon Kim, Yasutomi Higashikuni, William Chen, and Tim Lu.

Section 5. Use of Recombinant or Synthetic Nucleic Acid: Check box if not applicable ☐ (Please complete this section if you use or generate recombinant microorganisms, cells, animals, plants, etc.)

Please indicate the Section of the NIH Recombinant DNA Guidelines that applies to this research. If you have questions Biosafety Staff will be glad to assist you. Note that the City of Cambridge requests that we register all recombinant work even if it is exempt under section III-F or Appendix C. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OBA website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

Section III-A ☐ Section III-B ☐ Section III-C ☐ Section III-D ☒ Section III-E ☐ Section III-F ☐

a. Source of Gene, Insert or Clone:

5. Specify DNA/RNA source (or probe), promoter used, nature of insert (genes families are acceptable, e.g. fluorescent proteins), if a protein expressed, and percent of any viral genome in construct (for viral vectors see 5.b.2):

Insert	Cloned into	Under promoter control	Insert source	Nature of insert	Protein expressed?	% viral genome
gRNAs	pAWp12 (Addgene)	pSNR52	Synthetic DNA	Guide RNAs	no	<1%
Cas9 and its derivatives	pFUGW	pEF1a	Plasmid collection (originally from S. aureus)	Enzymatic portion of CRISPR/Cas9 system	yes	<1%

Selection markers for E. coli including Bla, Kan, Zeocin resistance, etc	pFUGW, and derivatives	Native promoter	Plasmid collection	Antibiotic resistance	yes	<1%
Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, or puromycin	pFUGW, and derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Plasmid collection	Antibiotic resistance	yes	<1%
Fluorescent proteins such as EGFP and EYFP	pFUGW	Endogenous promoters activated/repressed in UPR, such as the BiP or GRP94 promoter	Plasmid collection, synthetic	Marker for FACS	yes	<1%
Validating gene sets	pFUGW, derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Synthetic DNA, mRNA libraries	Genes implicated as sensors of heart failure, genes that provide resistance to heart failure, therapeutic genes	yes	<1%

6. Are any sequences from select agents and toxins? Yes ☐ No ☒; If yes, please specify.

7. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

8. Is the DNA source from a USDA-regulated plant, animal or insect? Yes ☐ No ☒; If the regulated organism is grown or stored at MIT, please include a copy of the USDA permit. (Link to USDA site: http://www.aphis.usda.gov/brs/index.html)

b. Vectors and Host Cells:

3. Identify cloning/expression/transfection vectors used, recipient bacterial strains used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, plant, etc.). Provide a restriction map of vector if it is new or not published. If commercially available vector or plasmid, please indicate vendor. Describe the location and type of promoters and other control sequences and percent of any viral genome in construct.

Vector name	Recipient Bacterial strain	Reporter gene?	% viral genes	promoters	Host cell
pBluescript	DH5α	Fluorescent proteins	0	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	MIN6-m9, IRlox, 3T3 mouse embryonic fibroblasts (MEFs), HL-1, 3T3-L1, H9c2, HEK293, HUVEC, MDA-MB-231, BT-549, HMLER, Primary cells including rat neonate cardiomyocytes, murine adult cardiomyocytes, human and murine preadipocytes and adipocytes and BALB/c mice derived primary mouse T cells
Viral Vectors (see below)	NA	Fluorescent proteins	<50%	See below	MIN6-m9, IRlox, 3T3 mouse embryonic fibroblasts (MEFs), HL-1, 3T3-L1, H9c2, HEK293, HUVEC, MDA-MB-231, BT-549, HMLER, Primary cells including rat neonate cardiomyocytes, murine adult cardiomyocytes, human and murine preadipocytes and adipocytes and BALB/c mice derived primary mouse T cells

4. If using viral vectors, fill out the table below. If using multiple types of viral vectors, please add a new table per vector type; multiple variations of the same viral vector type can be in the same table. (If using retroviral or lentiviral vectors additional requirements apply. Please see MIT CAB/ESCRO policy at the following URL: https://cabescro.mit.edu/home

Viral Vector type	Adenovirus expression vector and its derivatives (E.g., pacAd5 and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Adenovirus serotype 5 based delivery system
Source (vendor / collaborator)	RaPAd Adenoviral system from Cell Biolabs and related products

Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	Infectivity PCR Ishii-Watabe, A., E. Uchida, A. Iwata, R. Nagata, K. Satoh, K. Fan, M. Murata, H. Mizuguchi, N. Kawasaki, T. Kawanishi, T. Yamaguchi, and T. Hayakawa. 2003. Detection of replication-competent adenoviruses spiked into recombinant adenovirus vector products by infectivity PCR. *Mol Ther* 8:1009-1016.
Pseudotype	**No**
Host range	**Mammals including humans**
Safety feature (e.g. self-inactivating)	**Two plasmid system. Vector is replication deficient. There is no integration into the host genome.**
Integrate into genome (yes/no)	**No**
Exposure hazard (e.g. insertional mutagenesis)	Eye infection and common cold
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	3rd generation lentiviral vector and its derivatives (E.g., pFUGW vector and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	HIV-based lentiviral system, pLV CAG CN-2A-CN (AAVS1 Zinc finger) This lentiviral backbone is derived from the HIV-1 genome (contains 5', and 3' Long terminal repeats, Rev Response Elements (RRE), Psi packaging element, Woodchuck Hepatitis virus post-transcriptional response element). However, the elements in the lentiviral backbone are insufficient to cause viral replication and virulence. This lentiviral genome is devoid of virulence genes and essential genes involved in replication (such as gag, pol, env, tat, etc.).
Source (vendor / collaborator)	Addgene
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	To test for the presence of replication-competent or helper virus, marker rescue experiments will be performed. Culture supernatants of 293T cells which have been infected with a replication-defective virus expressing the puromycin

	resistance gene will be used to infect a second set of 293 cells. The second set of 293 cells will be selected in puromycin for several weeks to identify infected cells. This is a sensitive technique since a single colony, arising from a single replication-competent virus, can be detected readily or In order to test for the presence of replication competent or helper virus, we perform standard RT-PCR or real time PCR to determine the presence of lentiviral-related RNA transcripts. In addition, we test for horizontal transfer from supernatant of infected cells; supernatant of 293T cells, infected with replication-defective virus expressing GFP, is harvested and used to infect a second set of 293T cells. Test cells are prepared for FACS analysis at various timepoints for several weeks to determine the presence of GFP+ cells, an indication of infection by replication competent virus. This is a sensitive technique since a single GFP+ cell, arising from a single replication-competent virus, can be detected readily. These tests will be performed for each construct. The properties of particular plasmids should not change over time since they are stable at -20 Dull, T., R. Zufferey, M. Kelly, R. J. Mandel, M. Nguyen, D. Trono, and L. Naldini. 1998. A third-generation lentivirus vector with a conditional packaging system. *J Virol* 72:8463-8471.
Pseudotype	VSV-G
Host range	Mammals including humans
Safety feature (e.g. self-inactivating)	SIN, 3-plasmid system
Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Insertional mutagenesis, HIV virus may occur in HIV+ individuals or by in vitro recombination resulting in replication competent lentivirus.
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

4. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	

Exposure hazard	

c. Use of Animals (including invertebrates) or Insects: Check box if not applicable ☒
Most research using animals also needs the approval of the MIT Committee on Animal Care (IACUC). Contact them to register your animal research; If you have CAC protocol no(s), list them here:_____. The questions below are intended to deal with the use of potentially biohazardous agents in animals/insects or the creation of transgenic animals/insects.

2. Will rDNA technologies (such as producing transgenics), recombinant microbes/cells be used in animals or insects? Yes ☐ No ☒

   a. If yes, please describe objective of the research. Include recipient species, and list pathogen, rDNA technology, or recombinant microbe or human materials that will be used in animal/plant/ insect, and resulting genotype. What selection marker will be used?

4. If transgenic, including "knockout", animals/insects will be generated, please provide information on the transgene and vector as well as the expected phenotype of the recombinant animal/insect.

5. Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory or animal facility? Yes ☐ No ☒ If yes, explain:

d. Large-Scale Research: Check box if not applicable ☒

Do experiments involve growth of 10 liters or more of culture at a time? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

3. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

4. Describe the steps used to handle and contain spills:

e. Use of Plants: Check box if not applicable ☒
Describe experiments that involve recombinant work and plants, whether the plant itself is made recombinant or recombinant cell or microorganisms are used with plants.

Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory? Yes ☐ No ☐ If yes, explain:

Section 6. Biological Agent Use: Check box if not applicable ☐

(Please complete this section if you work with viable microorganisms or viruses.)

a1. Agent identification. List biological agent(s)/microorganism(s) genus/animals/plants/species/strain and genotypic markers, host range; any antibiotic resistance; recommended Biosafety Level (CDC); the source of the agent (e.g. new isolate from human tissue, blood, animal, tissue culture, another laboratory, ATCC, etc.).

Genus and species	Strain	Bio safety level (BSL) / Risk group (RG)	Source	Max volume used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
E. coli	DH5α	BL1	Personal collection	1L	1e12	80% ethanol, 10% bleach, Sklar, Preempt	N

For agents that are pathogens, please complete the following table:

Organism	Infectious Dose	Route of exposure	Host range	Clinically relevant Drug resistance	Availability of treatment	Special equipment /PPE used (above standard PPE)	Disease state and symptoms of exposure

a2. List and describe any other biological material not covered in the tables above:

Mouse cell lines including MIN6-m9, IRlox, 3T3 mouse embryonic fibroblasts (MEFs), HL-1, 3T3-L1
Rat cell lines including H9c2
Human cell lines including HEK293, HUVEC, MDA-MB-231, BT-549, HMLER
Primary cells including rat neonate cardiomyocytes, murine adult cardiomyocytes, human and murine preadipocytes and adipocytes and BALB/c mice derived primary mouse T cells

All cell lines are unable to survive outside the experimental environment. Tissue culture cells are disinfected with 10%bleach, Sklar or Preempt.

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

4. Briefly describe experiment and procedures involving use of biological agents?

E. coli will be grown and transfected using standard methods, namely transfection into chemically competent cells (2002. *Escherichia coli*, Plasmids, and Bacteriophages. Current Protocols in Molecular Biology. 59:1.0:1.0.1–1.0.3.)

Mammalian cell lines will be grown as described in Current Protocols in Cell Biology; ISBN: 978047114303. Cell will be transfected according to manufacturer's protocols (FuGene (Promega), Viafect (Promega) or transduced as described in Harford, J. B. 2011. Viruses. Current Protocols in Cell Biology. 51:26.0:26.0.1–26.0.3. and as specified by the Addgene protocols.

5. Will experiments result in acquisition of new characteristics such as enhanced virulence, infectivity, drug or antibiotic resistance, or change in host range? Yes ☒ No ☐ If so, explain:

Experiments will result in the production of therapeutic proteins. Cells will gain resistance to drugs such as hygromycin, G418 or puromycin; however this has no health or environmental consequences because of the fragility of tissue culture cells. Cells will epress fluorescent proteins.

6. Will you introduce viable biological material (microorganisms or cells) into animals?

Yes ☐ No ☒ If so:

a) Please describe:

b) List your MIT Committee on Animal Care (IACUC) protocol no(s), if your animal model also needs MIT Committee on Animal Care (IACUC) approval: _____

Section 3. Research Description (required):

Outline the overall goal(s) of the research in the space below. Give enough information to assure that the purpose of the experiments and the techniques used are clear. Please use reasonably non-technical terms.

3: Treatment of human diseases using synthetic biology tool and circuits ##

3.1) Development of genetic circuits for advanced immunotherapy for cancer

Our goal is to engineer genetic circuits that can secrete immunostimulatory proteins to recruit immune cells to kill tumors. We will be testing the therapeutic efficacy of genetic circuits on murine tumor models. We will use commercially available DNA delivery materials and lentivirus, adenovirus, AAV, or HSV vehicles to deliver the genetic circuits locally within tumors or systemically to identify the optimal delivery strategies for different disease status.

Immunostimulatory proteins of human and murine origins will be expressed by commercially available DNA delivery material, lentivirus, adenovirus, AAV expression vectors from various vendors to identify the optimal system of delivery (E.g., DNA delivery material from Life Technologies, Lentivirus from addgene, Adeno-X system from Clontech and Cell Biolabs, AAV system from Cell Biolabs and etc.) HSV vector from collaborators may also be used for gene delivery. The viral genome in genetic constructs will be maintained minimally for virus to maintain a basic replicative-deficient life cycle. The manipulating of viral vector will be done as commercially protocol suggested. The promoters used to express immunostimulatory proteins will be promoters that have strong activity in tumor cells but very low or no activity in normal cells. The safety concern of exposure of immunostimulatory proteins should be minimal, since immunostimulatory protein are produced by mice at a very low level and cannot mediate effect through aerosols or direct animal contact. In some experiments, non-viral delivery vehicles may be used to compare the delivery efficiency to viral vehicles (E.g., direct recombinant DNA injection, bio-materials carrying DNA, DNA coated bio-materials, and etc.)

We will establish murine tumor models in vivo and then use commercially available lentivirus, adenovirus, AAV, or HSV vehicles to deliver genetic circuits to test the optimal circuit design for treating cancers. We will deliver the genetic circuits locally and systemically to identify the optimal therapeutic strategies for different stage of cancer in vivo.

Lentivirus, adenovirus, AAV, or HSV will be used. All four types of vectors are commonly used for in vivo gene delivery and are considered very safe. All viral production systems will be purchased from commercial sources or from collaborators as described above. All the viral vectors used are replication-incompetent. The packaging

of virus and the viral packaging cells to be used will follow the commercial protocols and will be operated in BSL2 facility to maintain high safety.

For establishing various tumor models in mice and treating mice with immunotherapy, we will use commercially available tumor cells lines and primary cells listed in the table below (all the cells in the table and the procedure to establish these tumor models in their respective murine hosts are already approved by DCM in the animal protocol).

Tumor model	Cell lines
Ovarian cancer	OVCAR8 and its variant ID8 and its variant
Breast cancer	MDA-MB453 and its variant MDA-MB231 and its variant SKBR3 and its variant MCF7 and its variant 4T1 and its variant
Brain tumor	MGG4 and its variant MGG8 and its variant
Prostate cancer	TRAMP series and their variant PC3 and its variant DU145 and its variant LNCaP and its variant
Pancreatic cancer	NB508 and its variant

Personnel contributing to this project are Lior Nissim, Adina, Nissim, Erez Pery, Ming-Ru Wu, Karen Weisinger and Tim Lu.

3.2) Development of regulatory genetic circuits in mammalian cells

In lay terms, our goal is design genetic circuits in HEK293T human cell line that serve to record physiologically relevant information on to the DNA.

Cas9 and small molecule responsive proteins including TetR and LacI of bacterial origins will be expressed by commercially available lentivirus vectors from Addgene. The viral genome in genetic constructs will be maintained minimally for virus to maintain a basic replicative-deficient life cycle. The manipulating of viral vector will be done as commercially protocol suggested. The promoters used to express the bacterial proteins will be promoters that have medium activity in HEK 293T cells. The safety concern of exposure of the bacterial proteins should be minimal, since the proteins are produced by cells at a very moderate level and cannot mediate effect through aerosols or direct animal contact.

Lentiviral vectors are commonly used for in vivo gene delivery and are considered very safe. All viral production systems will be purchased from commercial sources as described above. All the viral vectors used are replication-incompetent. The

packaging of virus and the viral packaging cells to be used will follow the commercial protocols and will be operated in BSL2 facility to maintain high safety.

Personnel contributing to this project are Lior Nissim, Adina, Nissim, Erez Pery, Ming-Ru Wu, Karen Weisinger and Tim Lu.

3.3) State machines in mammalian cells

Our works will involve novel synthetic tools called recombinase based state machine to investigate tumor heterogeneity. The cell lines used for this xenograft assay will be MDA-MB-231 (ATCC HTB-26), BT-549 (ATCC HTB-122) or HMLER (obtained from Dr. Robert Weinberg, Whitehead Institute, MIT).

The cancer cell lines will express Cre and Flp recombinases derived from P1 bacteriophage and Yeast (Saccharomyces Cerevisiae), respectively, driven by cell type specific promoters derived from human genome. They will also express fluorescent proteins (EBFP2, EGFP (from jellyfish Aequorea Victoria), mCherry (from mushroom anemone Discosoma Sp.), NirFP (from sea anemone Entacmaea Quadricolor)), driven by promoters derived from human genome. The Cre and Flp recombinases will excise fluorescent genes to turn on or turn off fluorescent protein expression, but will not cause any genetic alterations to the genomes of cancer cell lines. Fluorescent proteins are highly orthogonal to endogenous mammalian cell signaling, and is unlikely to cause adverse effect to human when infected.

Personnel contributing to this project are Tackhoon Kim, Fahim Farzadfard and Tim Lu.

3.4) Complex, sequential gene expression circuit in mammalian cells

Our work will involve a series of recombinases (Cre, Flp, BxbI (from Mycobacteriophage), phiC31 (from bacteriophage phiC31), TP901 (from bacteriophage TP901-1)) fused with ligand binding domains of gibberellin or abscisic acid (GID/GAI and ABA/ABI, respectively). Those ligand binding domains for plant hormones are derived from Arabidopsis Thaliana, and enable instantaneous activation of recombinases upon treatment of these plant hormones. We will utilize this system to design genetic circuit for mammalian cells to enable sequential, multiplexed gene expression inducible by sequential treatment of plant hormones. The multiplexed gene expression control is enabled by nuclease deficient Cas9 (dCas9) fused with VP64 for gene activation and KRAB for gene repression. Although VP16 is part of gene in Herpes Simplex virus, this gene itself is not virulent. sgRNAs will be expressed to specify the genes to be activated/repressed to enable directed differentiation of cells into certain cell types, such as cardiomyocyte, or to establish colon cancer models with sequential inactivation of tumor suppressor genes. In the latter genetic circuit, the sgRNAs may inactivate tumor suppressors (such as P53, SMAD4, APC). These genetic circuits will NOT be introduced through any infectious particles (e.g., lentivirus). Rather, we will integrate plasmids containing these constructs into defined genomic landing pads within cell lines using recombinase-mediated integration. These plasmids will be introduced through electroporation, chemical transformation, and/or nucleofection, and will not be capable of self-propagation or infecting other cells through viral particles.

Personnel contributing to this project are Tackhoon Kim, Fahim Farzadfard, Eleonore Tham, Tzu-Chieh Tang, Giyoung Jung and Tim Lu.

3.5) Creation of synthetic receptors for detection of extracellular environment

Our goal is to create synthetic receptors that can detect extracellular disease markers including soluble molecules and/or cell surface antigens. Our synthetic receptors can convert extracellular stimuli into intracellular signals in an orthogonal manner, which enables us to integrate extracellular signals into gene circuit therapy or engineered cell therapy for various diseases. We will utilize natural tyrosine kinase receptors as a template, and modify extracellular ligand recognition domains and intracellular transcriptional regulation domains to create novel synthetic receptors. For extracellular ligand recognition domains, we will utilize amino acid sequences of ligand recognition domains from natural receptors or scFvs. For intracellular domains, we will use gene activator elements including tetR-VP64, Gal4-VP64, ZFHD-VP64 and Gal4-KRAB so that these can activate synthetic promoters. These receptors will be tested in HEK 293T cells, and will be applied to various cell lines including cardiomyocytes, adipocytes and immune cells.
Personnel contributing to this project are Yasutomi Higashikuri, William Chen, Tackhoon Kim, Mark Mimee, Eleonore Tham, Tzu-Chieh Tang, Giyoung Jung and Tim Lu.

3.6) Development of gene circuit therapy for obesity

Obesity is one of big issues world-wide because it will cause various life style-related diseases including cardiovascular diseases and cancers. To develop novel therapies for obesity, we will engineer adipocytes. Adipocytes consist of 2 cellular types, white adipocytes and brown adipocytes. White adipocytes are responsible for energy storage, sequestering lipids, but in the presence of excess food, are the hallmark and pathogenic cells of obesity. Brown adipocytes promote energy expenditure, the utilization of lipids and weight loss. It has been shown that the transcription factors, IRX3 and IRX5, play critical roles in differentiation into white adipocytes from preadipocytes and/or transdifferentiation from brown adipocytes to white adipocytes. By using shRNAs against IRX3 and IRX5 and our synthetic receptors detecting obesity markers such as leptin, adiponectin and FABP4, we will create gene circuits in adipocytes that can detect obesity markers and induce differentiation and/or transdifferentiation of preadipocytes and white adipocytes into brown adipocytes. These gene circuits will allow cells to respond to their environment and encourage the accumulation of brown adipocytes and the decrease in white adipocutes. We will test gene circuits in adipocytes and/or preadipocytes in culture, and apply this technology for animal models of obesity.

Personnel contributing to this project are Yasutomi Higashikuri, William Chen, and Tim Lu.

3.7) Development of engineered cell therapy for chronic inflammatory disease

Chronic inflammation has been shown to contribute to various diseases including cardiovascular diseases and gastrointestinal diseases. We will create anti-inflammatory engineered cell therapies that enable fine-tuning of inflammatory responses specifically in the diseased tissues. By using atherosclerosis and inflammatory bowel diseases as disease models, we will engineer immune cells such as T cells and regulatory T cells so

that these cells can localize into the diseased tissues and suppress inflammatory responses. For specific localization of these cells, we will use synthetic receptors that can detect disease markers or diseased cells. For anti-inflammatory effectors, we will utilize anti-inflammatory cytokines, scFvs against proinflammatory cytokines and natural anti-inflammatory activity of regulatory T cells. For detection of inflammation, we will use synthetic promoters that can detect proinflammatory transcriptional factors such as NF-kB. Synthetic receptor genes will be transfected into cells using pFUGW. Once we create engineered immune cells, we will assess the effects of these cells in animal models of atherosclerosis and inflammatory bowel diseases.

Personnel contributing to this project are Yasutomi Higashikuri, William Chen, Mark Mimee, Logan Jerger, Chang Cheng, and Tim Lu.

3.8) Engineering Synthetic Promoters and Viruses for Pediatric Glioma-Specific Immunotherapy

We aim to develop effective immunotherapies for pediatric high-grade glioma (pHGG) through the use of highly specific and active promoters and viruses that drive the expression of immunomodulators within the cancer cells themselves. We shall generate and test large libraries of synthetic promoters (up to 70,000 different designs) and identify those that are selectively active in pHGG cancer stem-like cells (pGSCs), including from diffuse intrinsic pontine gliomas (DIPG). This screen will be carried out using transient transfection of barcoded promoters linked to fluorescent reporters. Sorted cells will be analyzed to identify the barcodes, and therefore the promoters that drive strong expression in the pHGG cells. These promoters will be used in future studies to develop two types of complementary immunotherapies: 1) novel oncolytic HSVs that selectively replicate in and kill pHGG; 2) genetic circuits that achieve pHGG-specific expression of combinatorial immunotherapies. We shall confirm the selectivity of these therapeutic constructs and test their ability to trigger effective anti- cancer responses using *in vitro* models. Ultimately, this innovative approach will identify highly active and highly pHGG-specific promoters. These promoters should have broad utility for the research community, and enable specific and effective triggering of the immune system to target brain cancers, including pHGG. These results will provide the necessary preliminary data to apply for further funding to take this new technology into the clinic. This interdisciplinary proposal brings together an Engineering PI (Lu) with expertise in synthetic biology and a Cancer Research PI (Rabkin) with expertise in brain tumor stem cells and oncolytic virus therapy.

Personnel contributing to this project are Lior Nissim, Adina, Nissim, Erez Pery, Ming-Ru Wu, Karen Weisinger and Tim Lu.

3.9) Development of 2D self-organizing surface of mammalian epithelial cell monolayer

The aim of this project is to generate a gene circuit that is capable of generating consistent self-organizing pattern on 2D surface. This self-organizing pattern involves short-range diffusing activator and long-range diffusing repressor. The gene circuits will be implemented in Madin Darby Canine Kidney (MDCK) cells derived from dog and obtained from ATCC. The gene circuit will consist of synthetic secreted peptide ligands

involving Suntag (tandem GCN4 peptides, originated from yeast transcription factor GCN4, from addgene), GFP, myc tag (synthesized from Integrated DNA Technologies (IDT) Inc.), and CD19 (from human, obtained from addgene). These ligands will be secreted in the form of exosomes by tagging them with exosome targeting sequence isolated from MFG-E8 gene from mouse. The synthetic receptors for those ligands will be chimeric proteins consisting of ScFvs that bind to aforementioned ligands (obtained from addgene), and TEV proteases (from Tobacco Etch virus), tetR-VP64, LacI-VP64 as cytoplasmic effector proteins. TEV protease does not cause any virulence, and has no putative cleavage sites in human proteome. Finally, gene circuit will be expressing synthetic miRNA (from Dr. Ron Weiss) for gene repression in response to repressing ligands.

Personnel contributing to this project are Yasutomi Higashikuri, William Chen, Tackhoon Kim, Heechul Park, Eleonore Tham, Mark Mimee, Lior Nissim, Adina, Nissim, Erez Pery, Ming-Ru Wu, Karen Weisinger and Tim Lu.

3.10) Developing genetic and epigenetic molecular recorders for lineage tracing and recording temporal dynamics of gene networks

We aim to develop advanced molecular recorders for recording temporal dynamics of gene networks and recording molecular events during development. We use CRISPR-Cas9 gene editing (either Cas9 nuclease, Cas9 nickase or recently described variants of Cas9 fused to cytidine deaminases) to introduce mutations into synthetic or natural memory registers in mammalian cell genome. The mutations can be made conditional by driving the expression of guide RNA (or Cas9) by inducible promoters. We trace the accumulation of these genetic changes in the target locus over time by high-throughput sequencing. We initially plan to apply the system to mammalian cell lines (HEK) cells, and after optimizing the system, we will apply it to trace cellular lineages during development. Initially the gRNA is introduced by lentivirus and Cas9 by transient transfection with FuGene transfection reagent.

Personnel contributing to this project are Fahim Farzadfard, Mark Mimee, and Tim Lu.

3.11) Genetic elements screening to enhance protein expression in human muscle cells

In this work, we aim to develop a comprehensive strategy to identify novel 5’UTR sequences that can enhance protein expression from a human CMV promoter. We first design a library of 12,000 5’UTR sequences based on genomic searches and in silicon computations, then develop screening strategies to screen the library in human rhabdomyosarcoma cells (RD cells), as well as develop next-generation sequencing (NGS) analysis methods that allows us to investigate the correlation between 5’UTR regions and protein expression. The top 5’UTR candidates are then experimentally validated in RD cells.

Personnel contributing to this project JiCong Cao and Tim Lu

3.12) Development of gene circuit therapy for kidney stones

The objective of this research is to develop gene circuit therapy to prevent kidney stone from recurring. Kidney stone is one of the most common urologic diseases in a developed country. Current treatments of urolithiasis are limited to mainly the removal of renal stone which cause severe pain. Because pathological mechanism has not yet been elucidated, progress towards preventing for kidney stone remains slow. We will construct a mammalian expression vector which has oxalate-degrading enzymes under constitutive promoter. Then, we shall evaluate the activity by measuring RNA, protein expression and oxalate concentration after transfection into TERT-NHUC (telomerase-immortalized normal human urothelial cells). These cells were obtained from a collaborator. And then, we will investigate whether oxalate-degrading enzyme expression will reduce or prevent the kidney stone formation using in vitro microfluidic device.
Personnel contributing to this project are Giyoung Jung and Tim Lu

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Section 5. Use of Recombinant or Synthetic Nucleic Acid: Check box if not applicable ☐ (Please complete this section if you use or generate recombinant microorganisms, cells, animals, plants, etc.)

Please indicate the Section of the NIH Recombinant DNA Guidelines that applies to this research. If you have questions Biosafety Staff will be glad to assist you. Note that the City of Cambridge requests that we register all recombinant work even if it is exempt under section III-F or Appendix C. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OBA website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

Section III-A ☐ **Section III-B** ☐ **Section III-C** ☐ **Section III-D** ☒ **Section III-E** ☐ **Section III-F** ☐

a. Source of Gene, Insert or Clone:

1. Specify DNA/RNA source (or probe), nature of insert, is a protein expressed, and percent of any viral genome in construct (for viral vectors see 5.b.2):

Insert	Cloned into	Under promoter control	Insert source	Nature of insert	Protein expressed?	% viral genome
Selection markers for E. coli including Bla, Kan, Zeocin resistance, etc	pFUGW, and derivatives	Native promoter	Plasmid collection	Antibiotic resistance	yes	<1%
Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin	pFUGW, and derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Plasmid collection	Antibiotic resistance	yes	<1%
Synthetic receptors, incorporating transcriptional activation domains such as tetR-VP64, Gal4-VP64,	pFUGW, and derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Plasmid collection, synthetic DNA	Gene activators and repressors	yes	<1%

ZFHD-VP64 and Gal4-KRAB, and engineered tyrosine kinase receptors						
Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901	pFUGW, and derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Plasmid collection, Ron Weiss lab	Recombinases and integrases	yes	<15%
HSVIntegrase and recombinase recognition sites such as attP and attB	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Ron Weiss lab	Integrase and recombinase recognition sites	no	<1%
gRNAs	pAWp12 (Addgene)	pSNR52	Synthetic DNA	Guide RNAs	no	<1%
shRNAs	pFUGW and derivatives	pSNR52	Synthetic DNA	RNAi effectors	no	<1%
Immunostimulatory proteins	pBluescript, pacAd5, pAAV-MCS, pA-EUA1, pFUGW, and their derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	cDNA libraries	Immunostimulatory proteins	yes	<1%
Cas9 and its derivatives	pFUGW and derivatives	pEF1a	Plasmid collection (originally from S. aureus or S. pyogenes)	Enzymatic portion of CRISPR/Cas9 system	yes	<1%
Fluorescent proteins such as EGFP and EYFP	pFUGW and derivatives	Endogenous promoters activated/repressed in UPR, such as the BiP or GRP94 promoter	Plasmid collection, synthetic	Marker for FACS	yes	<1%
Barcoded synthetic promoters	pBluescript	no	Synthetic DNA	promoters	no	<1%
Enhancer sequences	pFUGW and derivatives	no	Synthetic DNA, cDNA library	5' UTR sequences	no	<1%

Oxalate degrading enzymes	pFUGW and derivatives	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Synthetic DNA	Bacteria from microbiome	yes	<1%

b. Vectors and Host Cells:

2. Are any sequences from select agents and toxins? Yes ☐ No ☒; If yes, please specify.

3. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

4. Is the DNA source from a USDA-regulated plant, animal or insect? Yes ☐ No ☒; If the regulated organism is grown or stored at MIT, please include a copy of the USDA permit. (Link to USDA site: http://www.aphis.usda.gov/brs/index.html)

b. Vectors and Host Cells:

5. Identify cloning/expression/transfection vectors used, recipient bacterial strains used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, plant, etc.). Provide a restriction map of vector if it is new or not published. If commercially available vector or plasmid, please indicate vendor. Describe the location and type of promoters and other control sequences and percent of any viral genome in construct.

Vector name	Recipient Bacterial strain	Reporter gene?	% viral genes	promoters	Host cell
pBluescript	DH5α	Fluorescent proteins	0	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic	Mouse tumor cells including ovarian, breast, prostate, and pancreatic cancer cell lines, and brain tumor lines (see table 3,1) Human cell lines including HEK293T, MDA-MB-231, BT-549, and HMLER, pHGG cells, rhabdomyosarcoma cells, telomerase-immortalized normal human urothelial cells Primary cells including rat neonate cardiomyocytes, murine adult cardiomyocytes, human and murine preadipocytes and adipocytes, T-cells,

Viral vectors see below					

6. If using viral vectors, fill out the table below. If using multiple types of viral vectors, please add a new table per vector type; multiple variations of the same viral vector type can be in the same table. (If using retroviral or lentiviral vectors additional requirements apply. Please see MIT CAB/ESCRO policy at the following URL: https://cabescro.mit.edu/home

Viral Vector type	Adenovirus expression vector and its derivatives (E.g., pacAd5 and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Adenovirus serotype 5 based delivery system
Source (vendor / collaborator)	RaPAd Adenoviral system from Cell Biolabs and related products
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	Infectivity PCR Ishii-Watabe, A., E. Uchida, A. Iwata, R. Nagata, K. Satoh, K. Fan, M. Murata, H. Mizuguchi, N. Kawasaki, T. Kawanishi, T. Yamaguchi, and T. Hayakawa. 2003. Detection of replication-competent adenoviruses spiked into recombinant adenovirus vector products by infectivity PCR. *Mol Ther* 8:1009-1016.
Pseudotype	**No**
Host range	**Mammals including humans**
Safety feature (e.g. self-inactivating)	**Two plasmid system. Vector is replication deficient. There is no integration into the host genome.**
Integrate into genome (yes/no)	**No**
Exposure hazard (e.g. insertional mutagenesis)	Eye infection and common cold
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	AAV CMV expression vector and its derivatives (E.g.,pAAV-MCS expression vector and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Helper free adeno-associated virus system

Source (vendor / collaborator)	AAV helper free expression system from another MIT Lab or UPenn or VectorCore
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	AAV is naturally replication incompetent and no helper virus will be used
Pseudotype	**No**
Host range	Primate cells, including human
Safety feature (e.g. self-inactivating)	Does not cause disease in human. Three plasmid system.
Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Not associated with human disease
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	3rd generation lentiviral vector and its derivatives (E.g., pFUGW vector and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	HIV-based lentiviral system, pLV CAG CN-2A-CN (AAVS1 Zinc finger) This lentiviral backbone is derived from HIV-1 genome (contains 5', and 3'Long terminal repeats, Rev Response Elements (RRE), Psi packaging element, Woodchuck Hepatitis virus post-transcriptional response element). However, the elements in the lentiviral backbone are insufficient to cause viral replication and virulence. This lentiviral genome is devoid of virulence genes and essential genes involved in replication (such as gag, pol, env, tat, etc.).
Source (vendor / collaborator)	Addgene
Packaging cell line(s), if applicable	HEK-293T (ATCC® CRL-3216™)
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	To test for the presence of replication-competent or helper virus, marker rescue experiments will be performed. Culture supernatants of 293T cells which have been infected with a replication-defective virus expressing the puromycin resistance gene will be used to infect a second set of 293 cells. The second set of 293 cells will be selected in puromycin for several weeks to identify infected cells. This is a sensitive technique since a single colony, arising from a single replication-competent virus, can be detected readily or In order to test for the presence of replication competent or

	helper virus, we perform standard RT-PCR or real time PCR to determine the presence of lentiviral-related RNA transcripts. In addition, we test for horizontal transfer from supernatant of infected cells; supernatant of 293T cells, infected with replication-defective virus expressing GFP, is harvested and used to infect a second set of 293T cells. Test cells are prepared for FACS analysis at various timepoints for several weeks to determine the presence of GFP+ cells, an indication of infection by replication competent virus. This is a sensitive technique since a single GFP+ cell, arising from a single replication-competent virus, can be detected readily. These tests will be performed for each construct. The properties of particular plasmids should not change over time since they are stable at -20 Dull, T., R. Zufferey, M. Kelly, R. J. Mandel, M. Nguyen, D. Trono, and L. Naldini. 1998. A third-generation lentivirus vector with a conditional packaging system. *J Virol* 72:8463-8471.
Pseudotype	VSV-G
Host range	Mammals including humans
Safety feature (e.g. self-inactivating)	SIN, 3-plasmid system
Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Insertional mutagenesis, HIV virus may occur in HIV+ individuals or by in vitro recombination resulting in replication competent lentivirus.
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	HSV-1 based amplicon vector and its derivative (E.g., pA-EUA1 and etc.)
Description (Plasmids used, viral vector generation for lentiviral vector)	Herpes-simplex virus-1 based system
Source (vendor / collaborator)	Professor Alberto Epstein
Packaging cell line(s), if applicable	VERO2-2
Replication competent or incompetent	**Replication incompetent**
Assays for detecting replication competent virus, if applicable	Plaque assay (3)

	Lieber, D., and S. M. Bailer. 2013. Determination of HSV-1 infectivity by plaque assay and a luciferase reporter cell line. *Methods Mol Biol* 1064:171-181
Pseudotype	No
Host range	Mammals including humans and vertebrates
Safety feature (e.g. self-inactivating)	Most adults have pre-existing immunity. Replication incompetent virus.
Integrate into genome (yes/no)	**Yes**
Exposure hazard (e.g. insertional mutagenesis)	Cold sores
Promoters to be used with viral vector	CMV, RSV, EFS, Tet-on, ERT, SV40, Hef1a, pSNR52, pEF1a, synthetic
Inserts to be used with viral vector[1,2]	Recombinases and integrases, such as BxB1, ϕC31, ϕBT1, Benedict, Theia, Sleeping Beauty, Veracruz, Wβ, Flp-e, Cre, and TP901, Antibiotic resistance genes for eukaryotes, such as resistance to G418, Zeocin, hygromycin, puromycin, Fluorescent proteins such as EGFP and EYFP, Therapeutic proteins, including antibodies such as Nivolumab, c13C6, c2G4, and c4G7, and other proteins such as erythropoetin and insulin-like growth factors, Cas9 and its derivatives (constitutive promoter)

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

Viral Vector type	**Oncolytic Herpes simplex (oHSV)**
Description (Plasmids used, viral vector generation for lentiviral vector)	**Armed HSV (G47Δ) vectors will constructed using the flip-flop HSV BAC system as follows:** **G47Δ, derived from G207, contains deletions of both copies of γ34.5 and α47, and a LacZ insertion inactivating ICP6. G47ΔBAC was derived by homologous recombination between G47Δ DNA and pBAC-ICP6EF and contains a cytomegalovirus promoter driving expression of the enhanced green fluorescent protein (EGFP) in place of LacZ/** **G47ΔUs11fluc was derived from G47Δ with the addition of the firefly luciferase gene driven by the true late Us11 gene promoter of herpes simplex virus 1 (HSV-1). The Us11 promoter was polymerase chain reaction (PCR)-cloned from the genome of wild-type HSV-1 strain F using the following primers: forward primer, CCGGATCCTGAGATCAATAAAAGGGGGCGTGAG, and reverse primer, CCGCCATGGTCCGCCCAGAGACTCGGGTGATG. This promoter sequence contains the following cis-acting regulatory elements of HSV-1 late promoters: the TATA box and the cap/leader sequences (initiator element), which together confer true late regulation, as well as the downstream activation sequence that allows transcriptional activation. The Us11 promoter and the luciferase gene**

	codon-optimized for mammalian expression (from pGL4.10[luc2]; Promega, Madison, WI, http://www.promega.com) were subcloned into shuttle plasmid pFLS-IE4-Express, removing the HSV IE4 promoter. This pFLS-Us11luc shuttle plasmid was recombined with pG47Δ-BAC using Cre recombinase. The resulting bacterial artificial chromosome (BAC) plasmid and an FLPe-expressing plasmid were cotransfected to Vero cells to remove BAC-derived sequences and the EGFP gene, which enables efficient HSV packaging. The resulting virus progeny was plaque-purified, and clones selected based on luciferase expression were expanded.
Source (vendor / collaborator)	**Samuel Rabkin - Collaborator**
Packaging cell line(s), if applicable	**Vero cells**
Replication competent or incompetent	**Competent**
Assays for detecting replication competent virus, if applicable	**Plaque assay**
Pseudotype	
Host range	**Humans, but non-human primates in captivity can be accidentally infected. Rabbits and rodents can be infected experimentally.**
Safety feature (e.g. self-inactivating)	**1. Conditional replication in tumor cells through deletion of viral genes that are essential for viral replication in non-dividing cells** **2. Targeting by transcription specificity has to limit virus infection to tumor cells only.** **3. antiviral drugs are available that enable optional termination of the therapy**
Integrate into genome (yes/no)	**No**
Exposure hazard (e.g. insertional mutagenesis)	**Primary hazards: Direct contact with clinical material or viral isolates, inhalation of concentrated aerosolized materials, droplet exposure of mucous membranes of the eyes, nose, or mouth, ingestion, accidental parenteral inoculation are the primary hazards associated with herpes viruses including HSV 1 and 2.**
Promoters to be used with viral vector	**To be determined experimentally (see section 3.**
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

5. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

c. Use of Animals (including invertebrates) or Insects: Check box if not applicable ☐
Most research using animals also needs the approval of the MIT Committee on Animal Care (IACUC). Contact them to register your animal research; If you have CAC protocol no(s), list them here:__ **1215-114-18; 0817-080-20; 0915-092-18** _____. The questions below are intended to deal with the use of potentially biohazardous agents in animals/insects or the creation of transgenic animals/insects.

3. Will rDNA technologies (such as producing transgenics), recombinant microbes/cells be used in animals or insects? Yes ☒ No ☐

   a. If yes, please describe objective of the research. Include recipient species, and list pathogen, rDNA technology, or recombinant microbe or human materials that will be used in animal/plant/ insect, and resulting genotype. What selection marker will be used?

Research described in sections 3.1, 3.6 and 3.7 should yield results that will be tested in mice. At this time, only 3.1 is ready to test; this is described in protocol **0817-08-20**. Briefly, the object of the research is to treat cancer using engineered T-cells or 3T3 cells containing immunostimulatory gene circuits. No pathogens will be used. Engineered cells will be produced and clonally expanded and selected to eliminate residual vector. No transgenic mice will be produced. Selection will consist of examination of effects on tumors induced in the mice.

6. If transgenic, including “knockout”, animals/insects will be generated, please provide information on the transgene and vector as well as the expected phenotype of the recombinant animal/insect.

7. Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory or animal facility? Yes ☐ No ☒ If yes, explain:

d. Large-Scale Research: Check box if not applicable ☒

Do experiments involve growth of 10 liters or more of culture at a time? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

5. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

6. Describe the steps used to handle and contain spills:

e. Use of Plants: Check box if not applicable ☒
Describe experiments that involve recombinant work and plants, whether the plant itself is made recombinant or recombinant cell or microorganisms are used with plants.

Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory? Yes ☐ No ☐ If yes, explain:

Section 6. Biological Agent Use: Check box if not applicable ☐

(Please complete this section if you work with viable microorganisms or viruses.)

a1. Agent identification. List biological agent(s)/microorganism(s) genus/animals/plants/species/strain and genotypic markers, host range; any antibiotic resistance; recommended Biosafety Level (CDC); the source of the agent (e.g. new isolate from human tissue, blood, animal, tissue culture, another laboratory, ATCC, etc.).

Genus and species	Strain	Bio safety level (BSL) / Risk group (RG)	Source	Max volume used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
E. coli	DH5α	BL1	Personal collection	1L	1e12	80% ethanol, 10% bleach, Sklar, Preempt	N

For agents that are pathogens, please complete the following table:

Organism	Infectious Dose	Route of exposure	Host range	Clinically relevant Drug resistance	Availability of treatment	Special equipment /PPE used (above standard PPE)	Disease state and symptoms of exposure

a2. List and describe any other biological material not covered in the tables above:

Mouse tumor cells including ovarian, breast, prostate, and pancreatic cancer cell lines, and brain tumor lines (see table 3,1)

Human cell lines including HEK293T, MDA-MB-231, BT-549, and HMLER, pHGG cells, rhabdomyosarcoma cells, telomerase-immortalized normal human urothelial cells

Primary cells including rat neonate cardiomyocytes, murine adult cardiomyocytes, human and murine preadipocytes and adipocytes, T-cells.

All cell line and primary cells are unable to survive outside the experimental environment. Tissue culture cells are disinfected with 10%bleach, Sklar or Preempt.

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

7. Briefly describe experiment and procedures involving use of biological agents?

E. coli will be grown and transfected using standard methods, namely transfection into chemically competent cells (2002. *Escherichia coli*, Plasmids, and Bacteriophages. Current Protocols in Molecular Biology. 59:1.0:1.0.1–1.0.3.)

Mammalian cell lines will be grown as described in Current Protocols in Cell Biology; ISBN: 978047114303, and in instructions from ATCC. Cell will be transfected according to manufacturer's protocols (FuGene (Promega), Vivafect (Clontech) or transduced as described in Harford, J. B. 2011. Viruses. Current Protocols in Cell Biology. 51:26.0:26.0.1–26.0.3. and as specified by the Addgene protocols.

8. Will experiments result in acquisition of new characteristics such as enhanced virulence, infectivity, drug or antibiotic resistance, or change in host range? Yes ☒ No ☐ If so, explain:

Experiments will result in the production of therapeutic proteins. Cells will gain resistance to drugs such as hygromycin, G418 or puromycin; however this has no health or environmental consequences because of the fragility of tissue culture cells. Some cells will express fluorescent proteins.

9. Will you introduce viable biological material (microorganisms or cells) into animals?

   Yes ☒ No ☐ If so:

   a. Please describe:

Research described in sections 3.1, 3.6 and 3.7 should yield results that will be tested in mice. At this time, only 3.1 is ready to test; this is described in protocol 0817-080-20-. Briefly, the object of the research is to treat cancer using engineered T-cells or 3T3 cells containing immunostimulatory gene circuits. No pathogens will be used. Engineered cells will be produced and clonally expanded and selected to eliminate residual vector. No transgenic mice will be produced. Selection will consist of examination of effects on tumors induced in the mice.

b) List your MIT Committee on Animal Care (IACUC) protocol no(s), if your animal model also needs MIT Committee on Animal Care (IACUC) approval: **1215-114-18; 0817-080-20; 0915-092-18**

Section 7. Use of Human Source Material: Check box if not applicable ☐

a. Do you have an Exposure Control Plan (ECP) on file with the MIT EHS office? Yes ☒ No ☐

b. If no, then how has the material been treated prior to use in the lab (such as formalin fixing or heat treatment)? Please describe how material will be rendered noninfectious prior to use.

c. Human material used (check all that apply):

X	Established human cell lines		Human blood, serum, plasma, blood products, or components
X	Primary human cell lines		Human bodily fluids
	Unfixed human tissues or organs		Cells, tissues, or organs containing HIV or HBV

Section 8. Human Embryonic Stem Cells or Induced Pluripotent Stem Cells (hES or iPS Cells respectively): Check box if not applicable ☒

Investigators should be aware of the NIH Guidelines on Human Stem Cell Research if they plan to work with either cell type (http://stemcells.nih.gov/policy/2009-guidelines.htm).

1. **Do you plan to derive human embryonic stem (hES) cells?**
   Yes ☐ No ☐ If yes, please describe the technology e.g. single cell nuclear transplantation, derivation from a donated embryo, etc. Please contact the Biosafety Office at 2-3477 as we will need further information. Please note that NIH will not fund derivation of hES cell lines.

2. **Are the human embryonic stem cells (hES) with which you plan to work on the NIH Registry of federally approved lines?** Yes ☐ No ☐ If yes, please list cell line(s) and indicate where you will get the hES cell line.

hES cell line name	NIH registry #	Source (vendor / collaborator / Institution)

3. **If you plan to use an already existing human stem cell line that is not on the NIH Registry please provide the following information as an attachment to this Biological Research Registration:**
   a. Please list hES Cell Lines and source: another laboratory or investigator
   b. Documentation required as part of registration (from the source investigator and institution)
      i. Please submit a Letter of Assurance from the investigator supplying the cells. This letter should document that the hES cell lines were generated with Institutional Review Board (IRB) oversight and approval. Please be sure that the source investigator includes the name of the approving IRB and the IRB OHRP assurance number.
      ii. Please submit the approval letter from the Institutional ESCRO Committee.
   c. MIT investigators must document that a source of non-federal funding for research involving these particular hES cell lines is in place.
   d. A plan must be developed to ensure separation of supporting materials and equipment for work with all non-federally approved hES cell lines.
   e. Contact the Office of Sponsored Programs for information on funding issues surrounding research involving non-federally approved hES cell lines.

4. **Funding Information: Please list all grants and contracts, including pending grants or contracts, that describe the use of any hES cell based research. Please include funding source e.g., NIH, HHMI, JDRF, etc., and start and end dates. If departmental or MIT funds are used please indicate that as well (enter "internal MIT funds" into grant/contract space).** (The box is expandable.)

Funding Agency	Grant/ Contract Number	Internal Cost Object	Date Grant Submitted	Date Grant begins	Date Grant ends

Does the information in this biological research registration form include all of the laboratory research section(s) described in the grants or contacts listed above?

☐ Yes ☐ NA (internal MIT funds to support this research only) ☐ No (Explain)

5. **Do you plan to inject or transplant hES cells in any animal, animal embryo or at any stage or prenatal development where the hES cells may contribute to the animal germline?** Yes ☐ No ☐ Please outline the animal research in the space below.

6. **Do you have human embryonic stem cells stored that are no longer used in your laboratory?**
   Yes ☐ No ☐ If yes, please explain.

7. **Will you receive iPS cells from a vendor or collaborator in an induced form?** Yes ☐ No ☐ If yes, please state the method of induction.

   If viral transduction is used, please state whether any residual virus is expected to be present in the induced cells.

8. **Do you plan on isolating and developing human induced pluripotent stem (iPS) cells or cell lines?** Yes ☐ No ☐ If yes, please describe the source of the somatic cells to be used and complete Section 7 of this form. In addition you will need to develop an Exposure Control Plan. Is the donor traceable? Yes ☐ No ☐

9. **What methods will be used to generate the induced pluripotent stem cells?** If you use viral vectors to generate the iPS cells please complete the detailed questions in Section 5 of this form.

10. **Do you plan to inject or transplant iPS cells in any animal, animal embryo or at any stage or prenatal development where the iPS cells may contribute to the animal germline?** Yes ☐ No ☐ Please outline the animal research in the space below.

Section 9. OCCUPATIONAL HEALTH ASSESSMENT, MEDICAL SURVEILLANCE & MONITORING: **Check box if not applicable** ☒

Some research may involve the need for a health assessment or vaccination prior to the initiation of the project. If there is a health risk associated with this research, please check the appropriate box below and contact Occupational Medicine. You can contact Occupational Medicine at 617-253-8552 to arrange an appointment. Occupational Medicine assessment is available to all MIT employees/investigators regardless of the biosafety level of the research

- ❑ **Pre-project serum samples.** These samples of blood serum are collected prior to beginning work with some types of infectious materials to serve as a reference should an infection occur during the course of work with an agent.

- ❑ **Pre-project vaccinations other than the Hepatitis B vaccine.** A vaccination may be warranted based on the nature of the work being done and the availability of an appropriate vaccine. Check the box if you need a vaccine other than Hepatitis B. Type of Vaccine:_____

- ❑ **Medical Surveillance monitoring**. This may include a baseline assessment, periodic evaluations during the experiment time period, and a final evaluation at the end of the experiment. **Note:** This type of surveillance is not usually indicated for research below Biosafety Level 3.

Section 10. Use and/or Storage of Toxins: Check box if not applicable ☒

This section reflects requirements at the federal and Institute level regarding risk assessment, training, procurement, use, storage and disposal of biological originated toxins used in research.

a. Please describe the specific use of toxin(s) in your research. Provide enough information to understand how the toxin will be handled in terms of safety and security.

b. Please complete the following table for each Select Toxin used.

Select Toxin name	Serotype	Proposed max. qty. at all times (mg)	Research Use (concentration in dilutions)		
			In-vivo	In-vitro	Other (please write in)
Abrin					
Botulinum neurotoxins					
Conotoxin					
Diacetoxyscirpenol (DAS)					
Ricin					
Saxitoxin					
Staphylococcal enterotoxins					
T-2 toxin					
Tetrodotoxin (TTX)					

1. For current approved holders of select toxins, are you storing select toxins in your laboratory space, but not actively using them? Yes ☐ No ☐ If yes, you are still required to keep these materials accounted for and registered in this BRR.

2. Please complete a Standard Operation Procedure (SOP) template and read the MIT EHS Select Agent Toxin Control and Containment Program: Purchasing, Inventory, Shipping and Receiving Procedures SOP. Both can be found here (https://ehs.mit.edu/site/content/select-agent-toxins). Please complete, print and share this SOP with the users to provide steps to the safe handling of these products.

c. Please complete the following table for each biological toxin or component (e.g. toxoid, subunit) of a biological toxin. This table covers toxins that are not regulated but that may represent a risk to personnel and/or the laboratory environment.

Toxins name	Subunit/ Toxoid name	Functional toxin?	Source (vendor)	LD_{50} (mg/kg)	Toxicity mechanism (e.g. B subunit mediates receptor binding and cell entry, A subunit carries toxic activity) and symptoms of exposure

Note: No SOP is required for biological toxins that are not select toxins. A written procedure is recommended for the lab to maintain locally reflecting consistent levels of safety practices and methods to properly handle, store and dispose of these products.

d. What protocol will be used to inactivate the toxins? List the toxins* under the select method(s):

	Autoclave:		Other (please describe):

(*) please see https://ehs.mit.edu/site/content/disposal for specific information about inactivation methods.

Section 11. Dual-Use Assessment (required): ☐ Yes ☒ No. Please complete all sub-sections below.

"Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. The United States Government's oversight of DURC is aimed at preserving the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research." (NIH Office of Science Policy)

a. Do you work with or have any of the following 15 agents or toxins in storage? Yes ☐ No ☒

	Avian influenza virus (highly pathogenic)		Marburg virus
	Bacillus anthracis		Reconstructed 1918 Influenza virus
	Botulinum neurotoxin		Rinderpest virus
	Burkholderia mallei		Toxin-producing strains of *Clostridium botulinum*
	Burkholderia pseudomallei		
	Ebola virus		Variola major virus
	Foot-and-mouth disease virus		Variola minor virus
	Francisella tularensis		*Yersinia pestis*

b. If you answered yes for any of the boxes in (a) above, are the strains used attenuated?
Yes ☐ No ☐ **If so, explain:**

c. If you answered yes for any of the boxes in (a) above, are your experiment(s) expected to:

1. Enhance the harmful consequences of the agent or toxin, such as result in acquisition of new characteristics such enhanced virulence, infectivity, stability, transmissibility, or the ability to be disseminated? Yes ☐ No ☐ If so, explain:
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification? Yes ☐ No ☐ If so, explain:
3. Confer to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies? Yes ☐ No ☐ If so, explain:
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin? Yes ☐ No ☐ If so, explain.
5. Alter the host range or tropism of the agent or toxin? Yes ☐ No ☐ If so, explain:
6. Enhance the susceptibility of a host population to the agent or toxin? Yes ☐ No ☐ If so, explain:
7. Generate or reconstitute an eradicated or extinct agent or toxin listed in (a), above? Yes ☐ No ☐ If so, explain.

d. If you answered no to all of the boxes in (a) above, are your experiment(s) expected to result in the development of materials or technologies with "dual use" potential listed in (c)?
Yes ☐ No ☒ **If so explain:**

e. If your research does not use any of the materials listed in (a) above and you answered "No" to (d) above, then check the "No" box at the top of Section 11.

Section 12. Safe Practices and Procedures (required): Please complete this section for all viable biological research agents or materials including human-derived materials.

1. Please identify and discuss the health and safety risks associated with the proposed research use of this biological agent or recombinant materials. (Please refer to Sections above if relevant):

2. What procedures create the greatest risk of exposure or infection e.g. aerosolization of materials, and how will this risk be minimized during the course of the research:

3. Outline protective equipment required to minimize exposure of laboratory personnel during all procedures requiring handling or manipulation of biological agent e.g. use of gloves, lab coats, safety glasses, etc.

X	Lab Coat	X	Gloves
	Safety glasses		Safety Goggles
	Face shield (please specify procedure):		Other (Please describe PPE and procedure):

4. Outline decontamination procedures and disinfectant(s) to be used for work surfaces, instruments, equipment, liquid containing biological materials and glassware:

Liquid waste decontamination	
X	10% final concentration of household bleach (0.5% NaOCl), 20 minute contact time
	Wescodyne (1%), 20 minute contact time
	Autoclave
	Other (Please describe rationale):
Work surfaces and equipment decontamination	
X	10% final concentration of household bleach (0.5% NaOCl)
X	70% ethanol (Note: ethanol is not an appropriate disinfectant for work involving human materials)
X	EPA approved product such as Sklar, Lysol spray, PREempt, etc. (Please list product(s)):
	Other (Please describe):

5. Outline disposal/decontamination procedures for contaminated sharps, contaminated solid waste, tissues, pipette tips, etc.

X	Solid waste	Placed in EHS provided biowaste boxes for disposal
	Solid Waste	Autoclave (please describe rationale):
X	Animal or human tissue	Placed in EHS provided biowaste boxes dedicated for incineration
X	Sharps	Placed in puncture resistant biosharps container and dispose of full containers in EHS provided biowaste box
	Other (Please specify):	Describe process:

6. Will mixed waste be generated (radioactive/biological or chemical/biological)? YES ☐ NO ☒
If yes, please indicate how you will inactivate the biological component of the mixed waste in the box below. (For information on waste management, please see: http://ehs.mit.edu/site/waste. If you have questions, contact EHS at 2-3477).

Section 13. Certification and Signatures

The information contained in this application is accurate and complete. I am familiar with and agree to abide by all guidelines and regulations pertaining to this research. These guidelines and regulations include the current NIH Guidelines for Research Involving Recombinant DNA Molecules; CDC and NIH guidance documents such as “Biosafety in Microbiological and Biomedical Laboratories”; the DHHS and USDA Select Agents and Toxin regulations; OSHA Bloodborne Pathogen Standard; the provisions of the City of Cambridge Ordinance on Recombinant DNA Research; the Massachusetts State Sanitary Code Chapter VIII, 105 CMR 480, “Minimum Requirements for the Management of Medical or Biological Waste; Massachusetts law, M.G.L. c.111L, “Biotechnology” for human embryonic stem cell research; as well as any MIT Policies and Procedures and other local, state and federal regulations that may be applicable.

Specifically I agree to abide by the following requirements:

a. I will not initiate any biological research subject to the regulations and guidelines mentioned above until that research has been registered, reviewed and approved by the Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight (CAB/ESCRO). The purview of the MIT CAB/ESCRO includes biological research involving recombinant DNA; biological agents and pathogens; human cells, tissues, materials and embryonic stem cells; select agents and toxins, and the use of any of the above in animal research.
b. I will assure that personnel have received appropriate information about the biological hazards of the research outlined in this registration by making available copies of approved protocols, Biosafety Manuals, and Biological Research Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.
c. I am familiar with and will ensure use of appropriate biosafety level laboratory practices and procedures in the conduct of this research.
d. I certify that laboratory personnel have appropriate technical expertise.
e. I will ensure that laboratory personnel know the procedures for dealing with incidents and spills of biological materials, and know the appropriate waste management procedures.
f. I will comply with all shipping requirements for biohazardous materials.
g. I will ensure that all laboratory personnel working with biological materials are listed on this registration.
h. I will assure that all laboratory personnel have completed all necessary training and that their training records are up to date.
i. I certify that all laboratory spaces associated with the research described in this registration are listed.
j. If this research involves recombinant or synthetic nucleic acid technologies, I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the “NIH Guidelines for Research Involving Recombinant DNA Molecules” (a copy of this section is available from the MIT Biosafety Program)
k. I will assure adequate supervision of personnel, and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.
l. I will inform the MIT Biosafety Program of any serious spills, potential exposures or breaches of the guidelines and regulations listed above.

_____ _____

Principal Investigator Date

_____ _____

MIT Biosafety Officer Date