Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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* * * Amendment * * *

Amendment

List of Sections (and questions) that have been changed/modified

If you would like to make changes to the information in the protocol, add/remove/update personnel, add/modify a procedure, etc., click on the appropriate section/link on the left side menu.

1. Please provide a brief summary of the proposed amendment:

I am updating the AUP to reflect our new, approved funding source: NIH UG3AI150552-01.

2. Please provide a rationale for the proposed amendment:

Our funding was pending before, so we are updating the funding status to reflect the confirmed funding source.

3. Check all that apply. The proposed amendment involves:

- A change from non-survival to survival surgery
- A change that results in greater pain, distress, or degree of invasiveness
- A change in housing and/or use of animals in a location that is not part of the animal program overseen by the ACUC
- The addition of a new species
- A change in the objectives of a study
- A change in the Principal Investigator (PI)
- A change that impacts personnel safety
- A change in compounds or dosage of experimental substances which are fundamentally similar to compounds already approved in the protocol, and are documented in the literature regarding safety and toxicity in the same species
- A change in compounds or dosage of anesthesia, analgesia, or sedation that are consistent with UCB ACUC guidelines
- A change in euthanasia method to any method approved in the AVMA guidelines (including UCB ACUC euthanasia guidelines)
- A greater than () 10% increase in the originally approved number of animals of any one species
- Changes in personnel (other than PI)
- X Changes in funding sources

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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* * * Personnel Information * * *

Principal Investigator

(Must have PI status or Exceptional PI status at UC Berkeley)

Name
[redacted]

Title
[redacted]

Email
[redacted]

Office Phone
[redacted]

Lab Phone
[redacted]

Emergency Phone
[redacted]

Department
[redacted]

Mail Code
[redacted]

Campus Mailing Address
[redacted]

Will this individual be working directly with animals on this protocol? Yes X No

If "Yes" complete the following:

What species will this person use?:

Briefly list what procedures this person will perform (a full description of procedures is asked for later).:

Describe the experience/training this person has had with this/these species and procedures.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, "Investigators, Staff and Students - Basic Course" and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Laboratory Contact

Name
[redacted]

Title

Email

Office Phone

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

[redacted]

Lab Phone
[redacted]

[redacted]

Emergency Phone
[redacted]

Department
[redacted]

Mail Code
[redacted]

Campus Mailing Address
[redacted]

Will this individual be working directly with animals on this protocol? X **Yes** **No**

If "Yes" complete the following:

What species will this person use?: Mus musculus

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Mice: intravenous injection, euthanasia, blood removal, necropsy.

Describe the experience/training this person has had with this/these species and procedures.

[redacted] has completed training in animal care, use, and experimentation (through levels "B" & "C", see PDF attachments), consisting of 60 hours of lecture and 60 hours of practical experience. This training includes but is not limited to: Handling and basic principles of animal maintenance, Biology and maintenance of experimental animals, Anesthesia, analgesia and experimental procedures, Recognition of pain, suffering and anguish.

Before performing any work with animals at UC Berkeley, [redacted] will perform additional training as appropriate and [redacted] will be approved by OLAC in all procedures to be performed, including euthanasia and necropsy (with oversight from [redacted] for example).

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, "Investigators, Staff and Students - Basic Course" and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Other Personnel

Protocol Title: [redacted]

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* * * Species * * *

Species to be Used

Common Name	Genus & Species	Source
Mouse, Laboratory	Mus musculus	OLAC Approved Vendors, PI Breeding Colony
Humanized NSG mice	Mus musculus	OLAC Approved Vendors

Species to be Used

Common Name **Mouse, Laboratory**

Genus & Species Mus musculus

Strain(s) or Breed(s) C57BL/6N, Ai9

Animal Sex **Male**

Source OLAC Approved Vendors, PI Breeding Colony

Proposed Housing Location [redacted]

Building Name [redacted]

Room Number TBD

Maximum number of animals for three year project period [redacted]

Note: If breeding animals, the maximum number should include breeders plus all offspring produced.

Common Name **Other(Mouse)**
Humanized NSG mice

Genus & Species Mus musculus

Strain(s) or Breed(s) Hu-PBMC-NSG, Hu-CD34-NSG

Animal Sex **Female**

Source OLAC Approved Vendors

Proposed Housing Location [redacted]

Building Name [redacted]

Room Number [redacted]

Maximum number of animals for three year project period [redacted]

Note: If breeding animals, the maximum number should include breeders plus all offspring produced.

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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Protocol Title: [redacted]

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* * * Are You Using? * * *

NOTE: Select either "yes" or "no" for each question. If you select "yes", click on the "Add" button to provide required information.

Are You Using?

NOTE: The questions below are used to identify special circumstances where:

1) Animals are used in teaching

2) Additional oversight by regulatory agencies may be required

3) Coordination with campus compliance committees may be required

4) Personnel health and safety issues need to be addressed

1. Are you using live vertebrate animals for teaching?* N

2. Collaboration with Other Institution(s)* N

Animal transfers or changes in animal ownership between UC Berkeley PIs and collaborators at other institutions must comply with the ACUC policy on Changes in Animal Ownership and ACUC Guidelines on Animal Transportation.

3. Hazardous Agent(s) in Laboratory Animals

a) Infectious Agent(s) * N

Use of BSL-2 or 3 infectious agents in animals (including viral vectors; human cells, tissues or bodily fluids; and infectious select agents) requires approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site .

b) Recombinant DNA * Y

The introduction of recombinant DNA/RNA into animals and the generation of transgenic animals require approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site .

Recombinant Material

Recombinant Material	Source	BUA Number
Liver-targeted ctelCas9 RNP (RNA-protein complex)	[redacted] laboratory	[redacted]
T cell-targeted ctelCas9 RNP (RNA-protein complex)	[redacted] laboratory	[redacted]

NOTE: If breeding animals, create a "Breeding/Genotyping" Procedure and provide additional information and justification (including specific strains and phenotypes).

Transgenic Animals

Species	BUA Number
Mouse, Laboratory (OLAC Vivarium)	[redacted]
Humanized NSG mice (OLAC Vivarium)	[redacted]

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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c) Human Embryonic Stem Cells * N

NOTE: Use of Human Embryonic Stem Cells in animals requires approval by the UC Berkeley Stem Cell Research Oversight Committee (SCRO) and Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the SCRO web site and the EH&S Biosafety Program web site.

1. Do you have SCRO approval? *
2. BUA # *
3. Used In Which Species?

d) Biological Material/Animal Product(s) Not Described Above* N

NOTE: The use of biological materials in rodents must comply with the ACUC Policy on Testing Biologicals used in Laboratory Rodents. The use of human cells, tissues or bodily fluids requires approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site.

e) Toxic Agent(s) * N

This includes the use of carcinogens, reproductive hazards, and other biological toxins (including select agents) in laboratory animals. Standard Operating Procedures (SOPs) must be in place. For guidance, please refer to the EH&S SOP web site.

f) Controlled Substance(s) * N

NOTE: The Principal Investigator and any individuals using controlled substances in animals must be registered with EH&S prior using these agents. For guidance, please refer to the EH&S Controlled Substance Program web site.

g) Radiological Agent(s) * N

NOTE: Use of radiological agents in animals, radiation producing devices or lasers requires an approved Radiation Use Authorization(RUA) or Laser Use Registration (LUR) be in place prior to ACUC approval. For further guidance, please refer to the EH&S Radiation Safety Programs web site or Laser Safety Program web site.

4. Non-pharmaceutical Grade Compounds * Y

NOTE: Federal regulations require the use of pharmaceutical grade compounds in animals used for research and teaching unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. Please refer to the ACUC Policy on Use of Non-Pharmaceutical Grade Compounds

Non-pharmaceutical Grade Compounds

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Species	Specify Material	Please provide justification for use of non-pharmaceutical compounds
Mouse, Laboratory (OLAC Vivarium)	Liver-targeted ctelCas9 RNP (RNA-protein complex)	This enzyme complex is not available for purchase as a pharmaceutical-grade compound.
Mouse, Laboratory (OLAC Vivarium)	Buffered saline solution (vehicle)	For negative control experiments, we will inject a buffer (vehicle) that is matched to our experimental therapeutic enzyme. This will also be used to dilute our experimental compound for administration.
Humanized NSG mice (OLAC Vivarium)	T cell-targeted ctelCas9 RNP (RNA-protein complex)	This enzyme complex is not available for purchase as a pharmaceutical-grade compound.
Humanized NSG mice (OLAC Vivarium)	Buffered saline solution (vehicle)	For negative control experiments, we will inject a buffer (vehicle) that is matched to our experimental therapeutic enzyme. This will also be used to dilute our experimental compound for administration.

5. Field Study or Wildlife Study*

N

NOTE: Additional procedure-based information for field studies is requested under the Protocol Information section of the Protocol.

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* * * Funding Sources * * *

Funding Checklist

If the research is not funded, check the "Not Funded" box below.
If the research is funded, add the funding source to the appropriate table below.

NOTE: Only the Principal Investigator (PI) of the grant or subcontract can add his or her own SPO Funding information in this section. The PI of the grant or subcontract must also be listed in the Personnel Information section of the protocol in one of the following roles: Principal Investigator or Faculty Sponsor, Student or Postdoctoral Investigator, Co-Principal Investigator, Administrative Contact, or Other Contact. Training Grants can be added by anyone in one of the aforementioned roles. For step-by-step instructions, see eProtocol IACUC Quick Guides

Not Funded

SPO - Funding

SPO ID	Sponsor	Sponsor Award ID	Project Title
047842-001	NIH National Institute of Allergy & Infectious Diseases	UG3AI150552	Cas9 RNP Delivery to Immune Cells in Vivo via Molecular Targeting

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Protocol Title: [redacted]

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* * * Rationale * * *

Rationale

As you answer the questions in this section, please use language that can be understood by a layperson. r Avoid overly technical terms and define abbreviations.

1. STUDY OBJECTIVES

a) What is the overall aim and purpose of this research or teaching demonstration/exercise?*

Genetic diseases have been extremely difficult to treat. With the advent of genome editing, it is now possible to correct disease-causing genes with high efficiency. However, this is only possible if the genome editing enzyme, such as Cas9, is able to reach the cells in need of correction. This presents a massive technical challenge, and a barrier that currently prevents genome editing from realizing its full potential. Several existing technologies (e.g. adeno-associated virus (AAV) vectors or lipid nanoparticles (LNPs)) have shown promise for enabling in vivo delivery of Cas9, but each is hindered by substantial limitations (Wilson & Gilbert 2017 ACS Chem Bio PMID 29019396). AAV manufacture remains extremely expensive and its use can lead to increased unwanted mutations. LNPs can correct mutations with high efficiency, but their use is practically limited to the liver.

We aim to develop an emerging technology in the use of Cas9 delivery for therapeutic genome editing: cell-type targeted delivery of a pre-assembled Cas9 enzyme. In contrast to the AAV or lipid nanoparticle approaches, we will deliver an intact Cas9 enzyme consisting of RNA and protein (RNP) components. The Cas9 RNP has no inherent ability to enter cells, which means it can be engineered for targeted uptake via endocytosis. We have already established a robust method for selective uptake of Cas9 RNP into liver cells in a tissue culture setting (Rouet et al. JACS 2018 PMID 29668265), and we now intend to perform in vivo studies to validate this approach for potential therapeutic use.

b) How will the information gained be important to human or animal health, the advancement of knowledge, or the good of society?*

Our development of a novel platform for in vivo delivery of therapeutic genome editing enzymes holds immense potential for the advancement of medical science. There are thousands of genetic diseases that are currently untreatable or can only be treated, not cured, and genome editing can theoretically cure these diseases. Genome editing also holds the potential to reverse HIV infection, which currently impacts nearly 40 million people. Furthermore, safe and affordable genome editing could be used as preventative medicine to reduce risks of heart disease, cancer, and degenerative diseases of the CNS.

Our work strives to enable curative and preventative therapeutic genome editing with a new approach for delivery. If successful, this will facilitate efficient genetic correction that can conceivably be targeted to a diverse array of cell/tissue/organ targets as appropriate for the disease in question. Our platform will be easier to manufacture than AAV-based approaches, and more versatile than the intrinsically-limited LNPs. Finally, because the Cas9 RNP will have only a transient presence in the cell, it will be less prone to unwanted edits than AAV vectored editing is.

Because Cas9 can be readily reprogrammed to target any genetic locus needed, our focus is on enabling genome editing in vivo. Demonstrating that our platform is safe and efficient in vivo is a crucial and necessary developmental step following our promising tissue culture results.

2. RATIONALE FOR USE OF ANIMALS

a) Why do you need to use animals? Discuss why non-vertebrate alternatives (e.g., tissue culture, invertebrate animal models, computer simulations) are inappropriate or implausible to answer your scientific questions or meet your educational goals.*

We have chosen to use rodents (mice, specifically) for our work because there is no in vitro cell line (or other model) that accurately predicts the Cas9 RNP pharmacokinetics/pharmacodynamics (PK/PD) or the phenotypic effects of genome editing. In the case of our cell-type targeted platform, an in vivo model is especially important because we hope to maximize the targeted efficacy (in our case, editing in liver cells) while minimizing the editing taking place in the cell types we are not targeting. This interplay is impossible to ascertain in any system less complex - or less similar to humans - than a mammalian model.

In our recent collaboration with [redacted], we have used tissue culture to its fullest extent to characterize our platform before initiating mouse studies. Likewise, in the proposed independent research at UC Berkeley, we will continue this approach, by performing mouse studies only once the limits of tissue culture have been reached.

The use of primary hepatocytes has been considered and attempted, but it has been observed in our lab and by [redacted] that primary hepatocytes (both human and murine) are particularly intractable when it comes to genome editing assays (Rouet et al. JACS 2018 PMID 29668265). This is reflected by a striking paucity of primary hepatocyte editing reports in the literature, which is in stark contrast to the plethora of successful studies performed in cultured cells and in mammalian models. We suspect that cell health is a key determinant of the success of our receptor-mediated genome editing, and that primary hepatocytes (which do not thrive in culture) are thus an inappropriate model for our approach.

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b) **Why have you selected these particular species (and not others)?***

We have selected mice because they are the simplest, most well-established, and most appropriate in vivo model system in which to assess tissue-targeted delivery. Furthermore, this project has already established safety and nominal efficacy of the novel targeted genome-editing platform in mice (via collaboration with [redacted]), so we are extremely confident that this is an appropriate model system to continue similar experiments. In addition to standard breeds (e.g. C57BL/6N), we will use available fluorescent reporter Ai9 mice to inform on biodistribution of genome editing activity.

For recently-funded studies aiming to perform cell-targeted editing of immune cells in vivo, we require a distinct model, namely humanized immunodeficient (NSG) mice. NSG mice are immunodeficient and thus receptive to transplant of human immune cells. Thus we intend to use the Hu-PBMC-NSG and/or Hu-CD34-NSG mice available from The Jackson Laboratory.

3. JUSTIFICATION OF ANIMAL NUMBERS

For complete instructions and guidance on how to complete the section on justification of animal numbers, please refer to the ACUC guideline on Justification for Animal Numbers found on the ACUC website.

a) **How did you determine that the numbers provided in the Species section of this protocol are the smallest number of animals needed to fulfill the study goals over a three-year period? Please use the table below to graphically describe for reviewers how you arrived at your animal numbers. Regardless of species, please briefly describe the Experiments included in your protocol and complete the table below, FOR THE THREE-YEAR PERIOD OF THE PROTOCOL. Note: Experiments may consist of multiple procedures. For breeding colonies, enter these as a line item, with the total consisting of breeding stock plus offspring NOT used in any studies.**

Animal Groups for Procedures

Experiment	Maximum number of groups (Control)	Maximum number of groups (Experimental)	Maximum number of animals per group	Maximum number of replications needed	Total number of animals needed
In vivo targeted genome editing	1	4	5	5	125

b) **Please justify the proposed number of animals being used:**

For targeted genome editing studies in mice using our novel Cas9 RNP platform, we designed the studies with n = 5 mice per condition (control or experimental) based on the experience of the similar work performed by the Rader & Musunuru labs (Ding et al. Circ Res 2014, PMID: 24916110). Regarding the number of experimental groups, we will use 4 experimental conditions per experiment (with 1 "vehicle" negative control). For each experiment, we will use 2 iterations of the Cas9 RNP (different novel protein engineering to facilitate cell entry after molecular targeting to the liver), and for each iteration we will also include a corresponding "non-targeted" condition (2 total) to assess the impact of the tethered targeting molecule). Thus each experiment will use 25 mice, and we will perform a maximum of 5 experiments over the three-year period (125 mice total). 3 of these 5 experiments will test the efficacy of liver-targeted ctelCas9 RNP; 2 of these 5 experiments will test the efficacy of T cell-targeted ctelCas9 RNP.

c) **Method Used to Determine Group Size (check all that apply):**

X **Statistical estimates; please describe the power analysis and all other statical analyses used:**

Because we are developing tissue-targeted genome editing, we expect dramatic differences between the experimental samples intended to edit a targeted tissue and those that are not intended to edit the targeted tissue. Our establishing work in tissue culture (Rouet et al. JACS 2018 PMID 29668265, Figure 4B & Table S1) suggests that we might anticipate 0.5% (+/- 0.5%) "background" editing in the liver and 5% (+/- 1%) targeted editing. Similar rates in vivo would allow a study with alpha = 0.05 and 90% power to be conducted with just one mouse per group (CliniCalc.com). That said, we want to guarantee that we have at least three data-points per condition, and we want to safeguard the data against sample loss (e.g. one animal lost during experimentation, one sample lost during data collection). Therefore we propose 5 animals per condition. This will also allow us to detect smaller differences between conditions, which may result from unintended "background" editing. For example, with n = 5, we will be able to distinguish between 2% "background" editing in the liver and 4% (+/- 2%) targeted editing (alpha = 0.05 and 90% power, via CliniCalc.com).

This is a pilot study, as similarly established studies do not exist. The proposed study will use a small number of animals to determine the feasibility of a larger study.

X **Studies cited in the literature; please provide the literature citations here or as an attachment:**

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As noted above, similar work has been done by the Rader & Musunuru labs:
Ding et al. Circ Res 2014, PMID: 24916110

X **Previous experience by this PI. Please describe and cite references here or as an attachment.**

As noted above, we have performed preliminary studies of a similar nature in collaboration with [redacted] wherein we used 5 mice per experimental (or control) group.

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* * * Procedures * * *

Tissue Collection in Mice (Pre-filled)

Procedure Category	Rodent Blood and Tissue Collection Procedures		
Procedure Type:	Tissue Collection in Mice (Pre-filled)	**Procedure Title:**	Collection of Donor Cells/Tissue in Euthanized Mice
Species:	Mouse, Laboratory (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	75	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

* * * Procedure Description * * *

Procedure Description

Instructional Text (do not edit or delete):

These procedures can be used as general tissue collection procedures. They can also be used as a procedure for the collection of lymphoid cells from euthanized donor mice so that the adoptive cells can then be injected into a recipient mouse. This procedure can be used in conjunction with the Pre-filled Procedure, "Production of Hematopoietic Chimeric Mice."

Under "Procedural Steps," check the box(es) listed below for those collection methods that you will NOT be using and click on Delete. Please refer to the following ACUC eProtocol Quick Guide for additional information: http://acuc.berkeley.edu/eprotocol_guides/procedures.pdf

Make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

Procedural Steps

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Step Name	Step Description
General Tissue Collection From Donor Mice	**Note: Since this is a terminal procedure used to collect cells/tissues, ensure that the method of euthanasia specified here is also described in the Animal Disposition section of this protocol.** **Procedural Steps:** 1. Euthanize mouse outlined in the Animal Disposition section and confirm death. 2. Dip the euthanized mouse in 70% alcohol. 3. Cells/Tissues are collected from the donor mouse for analysis.

How does this procedure fit into or address your overall research goals?

Because we are developing a tissue-targeted genome editing platform, it will be crucial to assess the specificity of our liver-targeted ctelCas9 RNP. Thus comparison of editing rates in liver as compared to other tissues (e.g. heart, kidney, lung, spleen, stomach, testes) will be of great importance.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Tissue collection will be performed after euthanasia.

Describe post procedure monitoring that will be performed.

Tissue collection will be performed after euthanasia.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Tissue collection will be performed after euthanasia.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Compound/Drug Administration

Procedure Type:	Compound/Drug Administration	**Procedure Title:**	Intravenous injection of liver-targeted ctelCas9 RNP
Species:	Mouse, Laboratory (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	75	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

* * * Procedure Description * * *

Procedure Description

The experimental compound, liver-targeted ctelCas9 RNP (RNA-protein complex) (see "Non-pharmaceutical Grade Compounds"), will be prepared in a solution of Buffered saline solution (vehicle) (see "Non-pharmaceutical Grade Compounds"). The compound will be prepared at 4°C and allowed to reach room temperature before injection. The compound will be administered at 2.5 mg/kg, and the precise dose size will be determined based on body weight determination of each animal. The ~50 ug of material will be prepared in a volume no greater than 200 uL (and no more than 10% of the mouse's estimated total blood volume) and sterile filtered before administration. For control experiments, an equal volume of Buffered saline solution (vehicle) will be administered.

The mouse will be placed in a restrainer and the tail will be warmed with a lamp or warm towel, or immersed in warm water (40–45°C), order to dilate the vessels. The tail will be swabbed with 70% alcohol applied to a gauze sponge or swab. The compound (or buffer control) solution will be administered to each animal via tail vein injection using a 30G1/2 needle. This will be performed by keeping the needle parallel to the tail vein and

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penetrating 2–4 mm into the lumen while keeping the bevel of the needle facing upwards. The injection will be performed slowly, and performed such that no resistance (back-pressure) can be detected. After the intravenous administration is finished, the injection site will be pressed firmly with a swab or fingers to prevent backflow of the administered solution and/or blood.

How does this procedure fit into or address your overall research goals?

It is necessary to administer our experimental therapeutic material to test its PK/PD properties. An intravenous tail vein injection is an ideal route of administration in our model system because the anticipated final application of our experimental therapeutic material (in human patients) would be via intravenous injection.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Based on our prior experience, we do not anticipate any observable changes to the health, behavior, or clinical properties of the animal based on the administration of the experimental material. Our guide RNAs (which determine the genetic locus to be edited) will target either the developmental gene EMX1, which has no function in adult cells, or the TdTomato site of Ai9 mice. Therefore we don't anticipate any clinical, health, or behavioral changes based on the genome editing itself. These guide sequences are well validated and known to have only infrequent off-target effects, so this will not be a concern.

Regarding host response (i.e. immunological reaction), during our prior collaboration, we have empirically observed that mice fully tolerate the Cas9 RNP. No apparent immune responses have been noted.

As with any injection, there is risk of excessive bleeding, hematoma formation, tissue trauma, or infection.

Describe post procedure monitoring that will be performed.

On the day of the procedure, each animal will be checked immediately after administration and once more during the two-hour period following administration. Hemostasis will be verified before returning any animal to its home cage.We anticipate that any adverse effects of administration will manifest quickly, if at all. Following this two-hour window, the animals will be checked daily for clinical signs of pain, distress, and/or morbidity. This will include daily observations of behavior, appearance, and posture to check for signs of pain or distress, to assess clinical condition and homeostasis as well as other clinical parameters (e.g., mobility, locomotion, body temperature & hydration). Same-day and subsequent monitoring will check for all signs of pain, distress, or morbidity listed in the following section.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Any animal displaying clinical signs of pain, distress, illness, or evidence of a moribund state will be euthanized. These clinical signs include (but are not limited to): decreased food or water consumption; inability to ambulate to reach food or water; abnormal urine; chronic and/or severe diarrhea or constipation; weight loss; dehydration; hypothermia; marked change in behavior; progressive respiratory distress abnormal mucous membrane color; abnormal discharge; distinct icterus; severe and/or uncontrollable bleeding; central nervous system signs such as head tilt, tremors, spasticity, seizures, circling or paralysis or paresis; cardiovascular disease with related clinical signs (e.g. coughing, respiratory distress, cyanosis, limb edema); persistent self-induced trauma and/or excessive licking/scratching; abnormalities of the skin and/or musculoskeletal system (e.g. swelling, redness, discoloration, evidence of pain on palpation, ulceration, abnormally warm or cold to the touch); rapid growth of a mass or clinical signs of neoplasia; lesions interfering with eating or drinking; hematologic or biochemical parameters that indicate organ failure; evidence of infection that is not readily treatable; unconsciousness with no response to external stimuli such as handling or the toe-pinch withdrawal test; fluid accumulation in body cavities/subcutaneous tissue; untreatable rectal prolapse. The following signs will be cause for euthanasia if they are observed in conjunction with other clinical signs: porphyrin (red-tinged) staining around the eyes or nose in rodents; rough hair coat or hunched posture; distended abdomen.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Peri procedure Care/Analgesics * * *

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Post-procedure Monitoring

Recovery Location Building Name

Room Number

Responsible Personnel

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

Monitoring Duration

Monitoring Frequency

Describe what actions will be taken if parameters monitored fall outside normal ranges:

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Describe record keeping/documentation methods for post-procedure monitoring:

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Cas9 RNA-protein (RNP) complex	2.5 mg/kg in 200 uL or less	Intravenous (IV)	The dose will be administered one time via slow injection, following 2-3 days of acclimation.
Buffered saline solution (vehicle)	Dosage (N/A) in <200 uL	Intravenous (IV)	The dose will be administered one time via slow injection, following 2-3 days of acclimation.

Blood Collection in Conscious Mice (Pre-filled)

Procedure Category	Rodent Blood and Tissue Collection Procedures		
Procedure Type:	Blood Collection in Conscious Mice (Pre-filled)	**Procedure Title:**	Blood Collection in Conscious Mice
Species:	Mouse, Laboratory (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	75	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Procedure Description * * *

Procedure Description

Instructional Text (do not edit or delete):

Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

General blood withdrawal guidelines: Mice have an average circulating blood volume of 72 ml/kg (0.072 ml/g x 25 g mouse = 1.8 ml circulating blood volume for a 25 g adult mouse). 7.5% of the circulating blood volume can be safely removed with a recovery period of 7 days. If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week. All personnel performing blood withdrawal in conscious mice should be trained in proper restraint techniques.

Please check the box and click on Delete for those blood collection methods listed below that you will NOT be using.

Procedural Steps

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Step Name	Step Description
Lateral tail vein or tail artery	1. Restrain mouse in rodent restraint apparatus. 2. Warm tail to dilate vessels (heat lamp, warm water, or warm compress). 3. Moisten venipuncture site with alcohol. 4. Using a 25-27g needle on a 0.5 -1cc syringe, insert the needle, bevel facing up into vessel. Gently pull back on the plunger to avoid collapsing the blood vessel. 5. Alternatively, puncture the blood vessel with the needle and allow the blood to drip into a microcentrifuge tube or be collected by capillary action into a blood collection tube. 6. Remove needle if utilized and apply pressure to puncture site with a gauze pad until bleeding stops. **Potential Adverse Events:** Excessive bleeding, hematoma formation, tissue trauma, or infection.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Facial vein (submandibular)	1. Scruff mouse by grasping loose skin over the shoulders between thumb and index finger of non-dominant hand. 2. Puncture facial vein, located slightly behind the mandible, but in front of the ear canal near the bald spot or "dimple", in a swift, lancing motion with a 4.0-5.5mm lancet or tip of a 19-25g needle; blood will flow immediately if in the correct location. 3. Collect sample into a pipette via capillary action or allow blood to drip into a microcentrifuge or blood collection tube. 4. Apply pressure with a gauze pad until bleeding stops. **Potential Adverse Events:** Depth of the puncture must be controlled or excessive bleeding, entry into the ear canal, entry into the oral cavity, hematoma formation, trauma to the underlying muscles or infection can occur. **Note: Hemostasis may take longer than other methods of blood collection.**

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Lateral saphenous	1. Place mouse head first in restraint tube (a 50 ml plastic conical test tube or syringe casing works well). 2. Extend hind limb over top edge of the tube, applying gentle pressure above the knee joint or use a small tourniquet to hold off the vessel. 3. Apply sterile ophthalmic ointment to allow the blood to pool at the site, and part hair to visualize vessel. 4. Puncture vessel with 25g needle in a swift, lancing motion; blood will flow from site and pool on the ointment. 5. Collect sample into a pipette via capillary action or allow blood to drop into a microcentrifuge or blood collection tube. 6. Release downward pressure on leg and apply gentle pressure to venipuncture site with a gauze pad until bleeding stops. 7. Removal of the scab will enable serial sampling. **Potential Adverse Events:** Excessive bleeding, hematoma formation, tissue trauma, or infection.

How does this procedure fit into or address your overall research goals?

Blood draws will allow us to monitor PK of our experimental therapeutic material, and to determine the physiological impact of the material.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.

Hemostasis will be verified before returning any animal to their home cage. Mice will be examined immediately following blood collection and, as well as the following day, for general appearance and activity level, as well as potential adverse events based on blood collection method (see above).

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Blood collection amounts and frequency will not exceed stated guidelines. If moribund, or if any other abnormal signs are noted, an OLAC veterinarian will be contacted or the mouse will be euthanized immediately.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Identification of Conscious Mice (Pre-filled)

Procedure Category	Rodent Breeding Procedures		
Procedure Type:	Identification of Conscious Mice (Pre-filled)	**Procedure Title:**	Identification of Conscious Mice via Ear Notch
Species:	Mouse, Laboratory (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	75	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

Instructional Text (do not edit or delete):

Individual identification of animals plays a critical role in accurate record keeping. There are several optional methods for identification of rodents. Proper restraint plays an important role in most of these techniques.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

Please check the box and click on Delete for those identification methods listed below that you will NOT be using.

Procedural Steps

Step Name	Step Description
Ear notch punch	**Note: Tissue from punch can often be used for genetic analysis, so it is typically done on pups <3 weeks of age. With this method, mice can be identified with the numbers 1 through 299.** **Procedural Steps:** 1. Manually restrain the mouse. 2. Using a special ear punch tool, place the ear punch in the preselected locations on the ear, and engage the punch quickly and firmly to ensure a clean cut; sanitize the tool between mice. 3. Return mouse to its cage. When identifying neonates, it is very important to remove any blood from ears before returning to dam to discourage cannibalism. **Potential Adverse Events:** unreadable punches/notches, infection, cannibalism of neonates. **Note: Holes or notches can grow closed over a period of time. Please see Animal Identification for reference figure: http://www.olac.berkeley.edu/sites/default/files/doc/Animal_Identification.pdf.**

How does this procedure fit into or address your overall research goals?

This will allow identification of the animals, which is necessary to perform the experiments.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.

Protocol Title: [redacted]
Approval Period: 11/05/2019-11/30/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Mice will be examined immediately following the identification procedure, and weekly thereafter for general appearance and activity level, as well as potential adverse events based on identification method (see above).

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If any abnormal signs are noted, an OLAC veterinarian will be contacted or the animal will be euthanized immediately.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Tissue Collection in Mice (Pre-filled)

Procedure Category	Rodent Blood and Tissue Collection Procedures		
Procedure Type:	Tissue Collection in Mice (Pre-filled)	**Procedure Title:**	Collection of Donor Cells/Tissue in Mice
Species:	Humanized NSG mice (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	50	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

Instructional Text (do not edit or delete):

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

These procedures can be used as general tissue collection procedures. They can also be used as a procedure for the collection of lymphoid cells from euthanized donor mice so that the adoptive cells can then be injected into a recipient mouse. This procedure can be used in conjunction with the Pre-filled Procedure, "Production of Hematopoietic Chimeric Mice."

Under "Procedural Steps," check the box(es) listed below for those collection methods that you will NOT be using and click on Delete. Please refer to the following ACUC eProtocol Quick Guide for additional information: http://acuc.berkeley.edu/eprotocol_guides/procedures.pdf

Make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

Procedural Steps

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Step Name	Step Description
General Tissue Collection From Donor Mice	**Note: Since this is a terminal procedure used to collect cells/tissues, ensure that the method of euthanasia specified here is also described in the Animal Disposition section of this protocol.** **Procedural Steps:** 1. Euthanize mouse outlined in the Animal Disposition section and confirm death. 2. Dip the euthanized mouse in 70% alcohol. 3. Cells/Tissues are collected from the donor mouse for analysis.
General Collection of Tissues/Cells from Fetuses	**Collection of Fetal Tissue/Cells:** This procedure involves the collection of hematopoietic cells (i.e., fetal liver cells) from euthanized donor mice so that the hematopoietic cells can be injected into lethally irradiated recipient mice. **Procedural Steps:** 1. Euthanize timed pregnant mice on embryonic day 12-17 as outlined in the Animal Disposition section and confirm death. 2. Perform a laparotomy to harvest the embryos from the uterus. 3. Euthanize the embryos by decapitation using scissors or a sharp blade. 4. Cells/Tissues are collected from the donor embryos for analysis. **Note: Since this is a terminal procedure used to harvest donor fetuses for harvest of fetal liver cells, specify CO2 as the primary method of euthanasia for the pregnant dam, and decapitation as the method of euthanasia for embryonic day 12-17 fetuses, in the Animal Disposition section of this protocol.**

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Collection of Fetal Liver Cells from Donor Mice	**Collection of Fetal Liver Cells:** This procedure involves the collection of hematopoietic cells (i.e., fetal liver cells) from euthanized donor mice so that the hematopoietic cells can be injected into lethally irradiated recipient mice. **Procedural Steps:** 1. Euthanize timed pregnant mice on embryonic day 12-17 as outlined in the Animal Disposition section and confirm death. 2. Perform a laparotomy to harvest the embryos from the uterus. 3. Euthanize the embryos by decapitation using scissors or a sharp blade. 4. Using sterile instruments, harvest the fetal livers from the embryos. 5. Process livers by aseptically dissociating into a single cell suspension (e.g., 0.5-5 X 106 cells in 200-400 µl of sterile phosphate buffered saline) for transplantation per *in vitro* protocol. **Note: Since this is a terminal procedure used to harvest donor fetuses for harvest of fetal liver cells, specify CO2 as the primary method of euthanasia for the pregnant dam, and decapitation as the method of euthanasia for embryonic day 12-17 fetuses, in the Animal Disposition section of this protocol.**

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Collection of Lymphoid Cells from Donor Mice	**Note: The tracking of antigen-specific T cells in vivo is a useful approach for the study of the adaptive immune response. The methodology involves the adoptive transfer of T-cell clones or lines with defined epitope specificity into histocompatible, age-matched mice. These T-cell grafts can subsequently be distinguished from the host's T-cells through the use of congenic or clonotypic markers.** **Procedural Steps:** 1. Euthanize mouse as outlined in the Animal Disposition section and confirm death. 2. Soak the mouse with 70% ethanol; designate one pair of sterile dissection scissors and forceps as an outside pair, and a second set as an inside pair. 3. Use the outside forceps to lift the skin away from the abdomen and use outside scissors to make an incision, beginning at the urethral opening and extending to the chin of the mouse. 4. Next, make an incision down to the knee on either side of the first incision, resulting in an incision that looks like an upside-down Y. Turn the outside hair and skin under and away from the underlying structures to reduce the chance of bacterial contamination. This will expose the underlying structures, while leaving the peritoneal wall intact. Using the inside forceps and scissors, harvest the lymph nodes of interest that are present subcutaneously (e.g., cervical, brachial, inguinal, axillary, popliteal). Note: If the donor mouse has been immunized prior to harvest, only the draining lymph nodes near the injection site are usually removed. 5. Using the sterile inside forceps lift the peritoneal layer and incise with the inside scissors to expose the lymphoid organs. Gently dissect out and harvest the lymph nodes of interest (e.g., mesenteric, renal, etc). Note: If the donor mouse has been immunized prior to harvest, only the draining lymph nodes near to the injection sites need to be removed. 6. Gently grasp the spleen with the inside forceps and detach it from the pancreas and intestines with the inside scissors. 7. Aseptically process the spleen and lymph nodes by dissociating into a single cell suspension in sterile

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

	medium (e.g., phosphate buffered saline) for expansion and transplantation of T-cell clones per *in vitro* protocol.
Collection of Bone Marrow from Donor Mice	**Collection of Bone Marrow:** This procedure involves the collection of hematopoietic cells (i.e., bone marrow) from euthanized donor mice so that the hematopoietic cells can be injected into lethally irradiated recipient mice. **Procedural Steps:** 1. Euthanize mouse as outlined in the Animal Disposition section and confirm death. 2. Dip the euthanized mouse in alcohol prior to removing the femur and tibia from both hind legs, and clean off any soft tissue attachments. 3. Using sterile bone-cutting forceps (e.g., Rongeurs), cut off the extreme distal tip of each bone and force sterile media (e.g., Hank's balanced salt solution-EDTA) through the bone with a sterile syringe. 4. Disperse cell clumps into sterile media and aseptically prepare a single cell suspension (e.g., 1-5 X 106 cells in 200-400 µl of sterile phosphate buffered saline) for transplantation per *in vitro* protocol. **Note: Since this is a terminal procedure used to collect bone marrow from the donor mouse, specify CO2 as the primary method of euthanasia in the Animal Disposition section of this protocol.**

How does this procedure fit into or address your overall research goals?

Because we are developing a tissue-targeted genome editing platform, it will be crucial to assess the specificity of our T cell-targeted ctelCas9 RNP. Thus comparison of editing rates in T cells as compared to other tissues (e.g. liver, heart, kidney, lung, spleen, stomach, testes) will be of great importance.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Tissue collection will be performed after euthanasia.

Describe post procedure monitoring that will be performed.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Tissue collection will be performed after euthanasia.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Tissue collection will be performed after euthanasia.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Identification of Conscious Mice (Pre-filled)

Procedure Category	Rodent Breeding Procedures		
Procedure Type:	Identification of Conscious Mice (Pre-filled)	**Procedure Title:**	Identification of Conscious Mice
Species:	Mouse, Laboratory (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	50	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

Instructional Text (do not edit or delete):

Individual identification of animals plays a critical role in accurate record keeping. There are several optional methods for identification of rodents. Proper restraint plays an important role in most of these techniques.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

methods for identification of rodents. Proper restraint plays an important role in most of these techniques.

Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

Please check the box and click on Delete for those identification methods listed below that you will NOT be using.

Procedural Steps

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Step Name	Step Description
Metal ear tags	**Procedural Steps:** 1. Select the appropriate size tag and use a recommended applicator. 2. Manually restrain the mouse, allowing access to the ears. 3. Apply the tag to the distal 1/3 of the pinnae of the ear using gentle pressure. 4. Return mouse to its cage. **Potential Adverse Events:** missing tags, infection, chronic inflammation at the site of attachment. **Note: Placement of the ear tag must not impinge on the external ear canal. Correctly placed tags will last for at least 6 months.**
Ear notch punch	**Note: Tissue from punch can often be used for genetic analysis, so it is typically done on pups <3 weeks of age. With this method, mice can be identified with the numbers 1 through 299.** **Procedural Steps:** 1. Manually restrain the mouse. 2. Using a special ear punch tool, place the ear punch in the preselected locations on the ear, and engage the punch quickly and firmly to ensure a clean cut; sanitize the tool between mice. 3. Return mouse to its cage. When identifying neonates, it is very important to remove any blood from ears before returning to dam to discourage cannibalism. **Potential Adverse Events:** unreadable punches/notches, infection, cannibalism of neonates. **Note: Holes or notches can grow closed over a period of time. Please see Animal Identification for reference figure: http://www.olac.berkeley.edu/sites/default/files/doc/Animal_Identification.pdf.**

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Implantable transponders	**Note: Generally performed on an awake mouse, however anesthesia may facilitate handling (see Identification of Anesthetized Mice procedure).** **Procedural Steps:** 1. Load the sterilized transponder (e.g., BioMedic Data Systems or AVID Identification Systems) into the 12 gauge implantation device. 2. Manually restrain the mouse for subcutaneous injection. 3. Swab the mid-dorsal region between the shoulder blades with 70% ethanol on a gauze sponge or swab. 4. Inject the transponder implant using subcutaneous injection technique. 5. Scan the mouse using the receiving unit to confirm transponder function and identification information. 6. Return the mouse to the cage. **Potential Adverse Events:** tissue irritation, infection. **Note: Although more costly than other methods of identification, microchip transponders are biocompatible, can be used in long-term studies, and are especially useful in pigmented animals. They allow animal identification data to be added automatically to a computer database file.**

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Toe clipping	**Note: This procedure requires a written justification for why it is necessary, and why alternative methods of identification are unsatisfactory. It is typically done to identify neonates while collecting tissue for genotyping, should only be performed on mice up to 7 days of age, and is limited to one digit per extremity. For additional information, please refer to ACUC Guidelines on Antemortem Tissue Collection for Genotyping/Identification (http://www.acuc.berkeley.edu/guidelines/tissue_collection.pdf).** **Procedural Steps:** 1. Cleanse the foot with a dilute betadine solution or betadine swab. 2. Using sharp, sterile scissors or a sterile blade, amputate the digit. Amputating different digits will provide a unique identification method for each mouse, as well as ample tissue for genotyping. 3. Apply pressure to the paw with a sterile gauze pad until bleeding has stopped. Styptic powder or tissue adhesive can also be used to aid in achieving hemostasis. 4. Return pup to its cage once the bleeding has stopped. It is very important to remove any blood from the paws before returning to the dam to discourage cannibalism. **Potential Adverse Events:** tissue irritation, infection, cannibalism of neonates.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Microtattooing	**Note: Can be performed on adult or neonatal mice without anesthesia if only tattooing with a colored dot. Tattooing requires special training - please contact OLAC.** **Procedural Steps:** 1. Prepare the micro-tattooing forceps with colored tattooing paste (e.g., Ketchum Mfg.) and needles (e.g., 25 to 30 gauge, 0.5 inch) according to the manufacturer's instructions. 2. Manually restrain the mouse allowing access to the paws, ear, or tail. 3. Swab the area with 70% ethanol on a gauze sponge or swab. 4. Squeeze the forceps together, penetrating the toe/foot pad, ear, or tail to deposit a dot of colored tattoo ink; tattooing different toes and/or using different patterns will provide unique identifiers for each animal. 5. Gently wipe off excess ink and return mouse to its cage. **Potential Adverse Events:** tissue irritation, infection, cannibalism of neonates. **Note: Needles must be sterile and sharp, and should be changed between each group of animals or when the needle becomes blunted; neonates may need to be re-tattooed when they get older.**

How does this procedure fit into or address your overall research goals?

This will allow identification of the animals, which is necessary to perform the experiments.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.

Mice will be examined immediately following the identification procedure, and weekly thereafter for general appearance and activity level, as well as potential adverse events based on identification method (see above).

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If any abnormal signs are noted, an OLAC veterinarian will be contacted or the animal will be euthanized immediately.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Blood Collection in Conscious Mice (Pre-filled)

Procedure Category	Rodent Blood and Tissue Collection Procedures		
Procedure Type:	Blood Collection in Conscious Mice (Pre-filled)	**Procedure Title:**	Blood Collection in Conscious Mice
Species:	Humanized NSG mice (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	50	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

Instructional Text (do not edit or delete):

Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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General blood withdrawal guidelines: Mice have an average circulating blood volume of 72 ml/kg (0.072 ml/g x 25 g mouse = 1.8 ml circulating blood volume for a 25 g adult mouse). 7.5% of the circulating blood volume can be safely removed with a recovery period of 7 days. If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week. All personnel performing blood withdrawal in conscious mice should be trained in proper restraint techniques.

Please check the box and click on Delete for those blood collection methods listed below that you will NOT be using.

Procedural Steps

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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Step Name	Step Description
Lateral tail vein or tail artery	1. Restrain mouse in rodent restraint apparatus. 2. Warm tail to dilate vessels (heat lamp, warm water, or warm compress). 3. Moisten venipuncture site with alcohol. 4. Using a 25-27g needle on a 0.5 -1cc syringe, insert the needle, bevel facing up into vessel. Gently pull back on the plunger to avoid collapsing the blood vessel. 5. Alternatively, puncture the blood vessel with the needle and allow the blood to drip into a microcentrifuge tube or be collected by capillary action into a blood collection tube. 6. Remove needle if utilized and apply pressure to puncture site with a gauze pad until bleeding stops. **Potential Adverse Events:** Excessive bleeding, hematoma formation, tissue trauma, or infection.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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Facial vein (submandibular)	1. Scruff mouse by grasping loose skin over the shoulders between thumb and index finger of non-dominant hand. 2. Puncture facial vein, located slightly behind the mandible, but in front of the ear canal near the bald spot or "dimple", in a swift, lancing motion with a 4.0-5.5mm lancet or tip of a 19-25g needle; blood will flow immediately if in the correct location. 3. Collect sample into a pipette via capillary action or allow blood to drip into a microcentrifuge or blood collection tube. 4. Apply pressure with a gauze pad until bleeding stops. **Potential Adverse Events:** Depth of the puncture must be controlled or excessive bleeding, entry into the ear canal, entry into the oral cavity, hematoma formation, trauma to the underlying muscles or infection can occur. **Note: Hemostasis may take longer than other methods of blood collection.**

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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Lateral saphenous	1. Place mouse head first in restraint tube (a 50 ml plastic conical test tube or syringe casing works well). 2. Extend hind limb over top edge of the tube, applying gentle pressure above the knee joint or use a small tourniquet to hold off the vessel. 3. Apply sterile ophthalmic ointment to allow the blood to pool at the site, and part hair to visualize vessel. 4. Puncture vessel with 25g needle in a swift, lancing motion; blood will flow from site and pool on the ointment. 5. Collect sample into a pipette via capillary action or allow blood to drop into a microcentrifuge or blood collection tube. 6. Release downward pressure on leg and apply gentle pressure to venipuncture site with a gauze pad until bleeding stops. 7. Removal of the scab will enable serial sampling. **Potential Adverse Events:** Excessive bleeding, hematoma formation, tissue trauma, or infection.

How does this procedure fit into or address your overall research goals?

Blood draws will allow us to monitor PK of our experimental therapeutic material, to determine the genome editing efficiency in the targeted T cells, and to determine the physiological impact of the material.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.

Hemostasis will be verified before returning any animal to their home cage. Mice will be examined immediately following blood collection and, as well as the following day, for general appearance and activity level, as well as potential adverse events based on blood collection method (see above).

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Blood collection amounts and frequency will not exceed stated guidelines. If moribund, or if any other abnormal signs are noted, an OLAC veterinarian will be contacted or the mouse will be euthanized immediately.

* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Compound/Drug Administration

Procedure Type:	Compound/Drug Administration	**Procedure Title:**	Intravenous injection of T cell-targeted ctelCas9 RNP
Species:	Humanized NSG mice (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	50	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

* * * Procedure Description * * *

Procedure Description

The experimental compound, liver-targeted ctelCas9 RNP (RNA-protein complex) (see "Non-pharmaceutical Grade Compounds"), will be prepared in a solution of Buffered saline solution (vehicle) (see "Non-pharmaceutical Grade Compounds"). The compound will be prepared at 4°C and allowed to reach room temperature before injection. The compound will be administered at 2.5 mg/kg, and the precise dose size will be determined based on body weight determination of each animal. The ~50 ug of material will be prepared in a

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Approval Period: 11/05/2019-11/30/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

determined based on body weight determination of each animal. The ~50 ug of material will be prepared in a volume no greater than 200 uL (and no more than 10% of the mouse's estimated total blood volume) and sterile filtered before administration. For control experiments, an equal volume of Buffered saline solution (vehicle) will be administered.

The mouse will be placed in a restrainer and the tail will be warmed with a lamp or warm towel, or immersed in warm water (40–45°C), order to dilate the vessels. The tail will be swabbed with 70% alcohol applied to a gauze sponge or swab. The compound (or buffer control) solution will be administered to each animal via tail vein injection using a 30G1/2 needle. This will be performed by keeping the needle parallel to the tail vein and penetrating 2–4 mm into the lumen while keeping the bevel of the needle facing upwards. The injection will be performed slowly, and performed such that no resistance (back-pressure) can be detected. After the intravenous administration is finished, the injection site will be pressed firmly with a swab or fingers to prevent backflow of the administered solution and/or blood.

How does this procedure fit into or address your overall research goals?

It is necessary to administer our experimental therapeutic material to test its PK/PD properties. An intravenous tail vein injection is an ideal route of administration in our model system because the anticipated final application of our experimental therapeutic material (in human patients) would be via intravenous injection.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Based on our prior experience, we do not anticipate any observable changes to the health, behavior, or clinical properties of the animal based on the administration of the experimental material. Our guide RNAs (which determine the genetic locus to be edited) will target either the developmental gene EMX1, which has no function in adult mammals, or the TdTomato site of Ai9 mice. Therefore we don't anticipate any clinical, health, or behavioral changes based on the genome editing itself. These guide sequences are well validated and known to have only infrequent off-target effects, so this will not be a concern.

Regarding host response (i.e. immunological reaction), during our prior work with animals (in collaboration), we have empirically observed that mice fully tolerate the Cas9 RNP. No apparent immune responses have been noted.

As with any injection, there is risk of excessive bleeding, hematoma formation, tissue trauma, or infection.

Describe post procedure monitoring that will be performed.

On the day of the procedure, each animal will be checked immediately after administration and once more during the two-hour period following administration. Hemostasis will be verified before returning any animal to its home cage.We anticipate that any adverse effects of administration will manifest quickly, if at all. Following this two-hour window, the animals will be checked daily for clinical signs of pain, distress, and/or morbidity. This will include daily observations of behavior, appearance, and posture to check for signs of pain or distress, to assess clinical condition and homeostasis as well as other clinical parameters (e.g., mobility, locomotion, body temperature & hydration). Same-day and subsequent monitoring will check for all signs of pain, distress, or morbidity listed in the following section.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Any animal displaying clinical signs of pain, distress, illness, or evidence of a moribund state will be euthanized. These clinical signs include (but are not limited to): decreased food or water consumption; inability to ambulate to reach food or water; abnormal urine; chronic and/or severe diarrhea or constipation; weight loss; dehydration; hypothermia; marked change in behavior; progressive respiratory distress abnormal mucous membrane color; abnormal discharge; distinct icterus; severe and/or uncontrollable bleeding; central nervous system signs such

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as head tilt, tremors, spasticity, seizures, circling or paralysis or paresis; cardiovascular disease with related clinical signs (e.g. coughing, respiratory distress, cyanosis, limb edema); persistent self-induced trauma and/or excessive licking/scratching; abnormalities of the skin and/or musculoskeletal system (e.g. swelling, redness, discoloration, evidence of pain on palpation, ulceration, abnormally warm or cold to the touch); rapid growth of a mass or clinical signs of neoplasia; lesions interfering with eating or drinking; hematologic or biochemical parameters that indicate organ failure; evidence of infection that is not readily treatable; unconsciousness with no response to external stimuli such as handling or the toe-pinch withdrawal test; fluid accumulation in body cavities/subcutaneous tissue; untreatable rectal prolapse. The following signs will be cause for euthanasia if they are observed in conjunction with other clinical signs: porphyrin (red-tinged) staining around the eyes or nose in rodents; rough hair coat or hunched posture; distended abdomen.

* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Peri procedure Care/Analgesics * * *

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Post-procedure Monitoring

Recovery Location Building Name

Room Number

Responsible Personnel

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

Monitoring Duration

Monitoring Frequency

Describe what actions will be taken if parameters monitored fall outside normal ranges:

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Describe record keeping/documentation methods for post-procedure monitoring:

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Procedure Relationships * * *

Procedure Relationships

Please describe the sequence and timing of the manipulations:

If more than one surgery or procedure will be performed on some or all animals used under this protocol, describe the sequence and timing of these manipulations. Flow charts may be helpful and can be attached to the protocol.

Day 1:
Following animal identification notch (Identification of Conscious Mice via Ear Notch), and after 2–3 days of acclimating to cages, each mouse will have 30 µL blood drawn (Blood Collection in Conscious Mice). After hemostasis has been confirmed, each mouse will be administered either our experimental material or buffer (Intravenous injection of [cell type]-targeted ctelCas9RNP) via intravenous tail vein injection. At two hours post-injection, each animal will be examined for clinical signs of pain, distress, illness, or morbidity.

Four hours after administration of experimental material (or buffer as negative control), each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 2:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

24 hours after the Day 1 administration, each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 3:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

Day 4:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

96 hours after the Day 1 administration, each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 5:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events. Animals will be euthanized using CO_2 and necropsy will be performed (Collection of Donor Cells/Tissue in Euthanized Mice).

Procedures done on a single animal:

Please indicate how many and which procedures a single animal will go through. If applicable, please identify the strain/genotype/breed of animals that will be used in each procedure. Charts are highly recommended for clarity.

For each mouse (C57BL/6N or Ai9 for liver-targeted genome editing; Hu-PBMC-NSG or Hu-CD34-NSG for T cell-targeted genome editing), the following procedures will be performed:
"Ear notch" will be performed 1x
"Intravenous injection of liver-targeted ctelCas9RNP" OR "Intravenous injection of T cell-targeted ctelCas9RNP" will be performed 1x
"Blood Collection in Conscious Mice" will be performed 4x (30 µL each; 120 µL over 4 days)
"Euthanasia by CO_2" will be performed 1x
"Collection of Donor Cells/Tissue in Euthanized Mice" will be performed 1x

Multiple Major Survival Surgery Description:

Describe why it is necessary to perform multiple major surgical procedures on the same animal. Indicate the length of time between surgeries.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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* * * Husbandry * * *

Animal Transportation X None

If animals will be transported between facilities, laboratories or institutions (e.g., hand carried, vehicular, etc.), describe the methods and containment measures to be utilized. Transportation of animals must conform to the ACUC Animal Transportation Guidelines.

FIELD STUDIES: If animals (live or dead) will be transported to or from the field, describe how they will be transported and measures to be taken to avoid potential disease transmission to researchers and other animals. Transportation of animals must conform to the ACUC Animal Transportation Guidelines.

Non-Standard Housing Requirements X None

Please check and describe all non-standard housing requirements that apply. Provide justification for each. For guidance, please refer to ACUC's Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals, Aquatic Frog Housing Density, Guidelines for Investigators Who Manage Mouse Breeding Programs, and Rat Housing Guidelines.

Non-Standard Housing Requirements

Species	Cage/Pen Size	Cage sanitation interval	Wire-bottom rodent cages or grids	Animals outside dedicated animal housing for greater than 12 hours	Exemption from exercise (dogs only)
Mouse, Laboratory (OLAC Vivarium)					
Other(Mouse) (OLAC Vivarium)					

Description of Non-Standard Housing Requirements

Non-Standard Husbandry or Care X None

Please check and describe any non-standard environmental requirements, diets, husbandry equipment or animal care. Include which species are affected. For guidance, please refer to ACUC's Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals and Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Investigator Care of Animals (describe below and provide justification). For guidance, please refer to the ACUC Guidelines on Investigator Care of Vertebrate Animals.

Non-Standard Lighting Cycles, e.g., greater than twelve-hour light or dark cycles (describe below and provide justification).

Non-Standard Housing Temperature Ranges (describe below and provide justification).

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Non-Standard Diets (describe below and provide justification). For guidance, please refer to the ACUC Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Telemetry or Tether Devices (describe below and provide justification).

Running Wheels (describe below and provide justification).

Individually Housed (describe below and provide justification).

Exemption from Standard Enrichment (describe below and provide justification). For guidance, please refer to the ACUC Environmental Enrichment Guidelines.

Other - Please describe and provide justification.

Non-standard Experimental Requirements

Food or Fluid restriction X None

Complete all section below that apply. For guidance, please refer to the ACUC Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Food or Fluid restriction

Species	Food Restriction	Length of Restriction	Fluid Restriction	Length of Restriction	Reason for Restriction
Mouse, Laboratory (OLAC Vivarium)					
Other(Mouse) (OLAC Vivarium)					

Restraint of Conscious Animals None

Complete all section below that apply. For guidance, please refer to the ACUC guidelines on Physical Restraint of Unanesthetized Animals.

Restraint of Conscious Animals

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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Species	Type restraint (manual, commercial, manual and commercial)	Describe acclimation to restraint	Length of restraint
Mouse, Laboratory (OLAC Vivarium)	Manual and Commercial	None necessary, due to limited duration.	<15 min.
Other(Mouse) (OLAC Vivarium)	Manual and Commercial	None necessary, due to limited duration.	<15 min.

Description of Restraint

In addition to manual restraint, Broome-style restrainers may be used for administration of experimental material and/or during blood draws. Restraint will be limited to under 15 minutes in duration and therefore is not expected to have a substantial impact on animal well-being.

Protocol Title: [redacted]

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* * * Animal Disposition * * *

Please consult the ACUC Euthanasia Guidelines. Physical methods of euthanasia must be performed under anesthesia. Following euthanasia and prior to carcass disposal, an additional physical means of ensuring euthanasia must be performed. These physical methods vary by species but may include cervical dislocation for small rodents, bilateral thoracotomy, decapitation, exsanguination, double pithing for amphibians and reptiles, freezing for small ectotherms, or another AVMA-approved method. These must occur after the animal has been rendered non-responsive to noxious stimuli by the primary euthanasia agent.

Euthanasia

Protocol Title: [redacted]

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Species	Method of Euthanasia: Primary	Route of Administration	Dosage (in mg/kg if possible) and volume	Site	Building Name	Room Number	Method of Euthanasia: Secondary	Briefly describe the euthanasia procedure
Mouse, Laboratory (OLAC Vivarium)	Carbon Dioxide	Inhalation (IN)		[redacted]	[redacted]	[redacted]	Cervical Dislocation	Each animal will be placed in a chamber that has not been pre-charged. As a preferred alternative, the animal will be left in its cage for euthanasia, as facilitated by an apparatus that covers the animal's cage. 100% carbon dioxide will be introduced at a flow rate of 10-30% [chamber volume] per minute, with flow controlled to prevent

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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								loud noise. After respiration ceases, the carbon dioxide flow will continue for at least one minute. At that point, death will be confirmed by noting the animal's fixed and dilated pupils. Cervical dislocation will be used as the secondary method of euthanasia.

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Humanized NSG mice (OLAC Vivarium)	Carbon Dioxide	Inhalation (IN)		[redacted]	[redacted]	[redacted]	Cervical Dislocation	Each animal will be placed in a chamber that has not been pre-charged. As a preferred alternative, the animal will be left in its cage for euthanasia, as facilitated by an apparatus that covers the animal's cage. 100% carbon dioxide will be introduced at a flow rate of 10-30% [chamber volume] per minute, with flow controlled to prevent loud noise. After respiration ceases, the carbon dioxide

Protocol Title: [redacted]

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								flow will continue for at least one minute. At that point, death will be confirmed by noting the animal's fixed and dilated pupils. Cervical dislocation will be used as the secondary method of euthanasia.

Provide specific details for carcass disposal.

Carcasses will be securely placed in two leak proof bags. All bags with animal carcasses will be labeled with the following information: IACUC #, Method used to ensure death, Date, and Initials of person disposing of the carcass. The bags will be placed into a properly labeled dedicated refrigerator, freezer, or cold room that is easily sanitized. Carcasses will never be placed into the regular trash.

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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* * * Attachments * * *

NOTE: The following types of files can be attached here: pdf, gif, jpeg,jpg, docx, xlsx.

CITI Certificates

Document Type	Document Name	Attached Date	Submitted Date
CITI Certificates	[redacted]_CITI-cert	08/20/2018	08/21/2018
CITI Certificates	[redacted]_CITI-cert	08/20/2018	08/21/2018

Other Documents

Document Type	Document Name	Attached Date	Submitted Date
Other Documents	Procedure_Relationships	08/20/2018	08/21/2018
Other Documents	[redacted]_training-B	11/19/2018	11/26/2018
Other Documents	[redacted]_training-C	11/19/2018	11/26/2018
Other Documents	Procedure Relationships (amended)	06/24/2019	06/24/2019

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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* * * Certifications * * *

Certification

As Principal Investigator, I have ultimate responsibility for this study, the protection of animal subjects, and strict adherence by all co-investigators and research personnel to federal regulations, state statutes, and University of California (UC) Office of the President (UCOP) and UC Berkeley (UCB) policies pertaining to animal use in research and teaching.

I hereby assure the following:

1) As per the ACUC's Policy and Procedures on Protocol Review, any changes in the care and use of animals involved in this protocol will be promptly forwarded to the ACUC for review. Such changes will not be implemented until approval is obtained from the ACUC. I understand that the ACUC and Institutional Official (IO) have the authority to suspend a previously approved protocol if an activity is performed differently from that outlined in the protocol.

2) All procedures involving animal subjects will be performed under my supervision or that of another qualified professional listed on this protocol. Individuals listed on this protocol are qualified or will be trained to conduct procedures involving animals outlined under this proposal as per the ACUC's Training and Education Policy.

3) As per the ACUC's Training and Education Policy, all individuals listed on an this protocol have completed the required Collaborative Institutional Training Initiative (CITI) course, "Investigators, Staff, and Students - Basic Course".

4) As per the ACUC's Animal Occupational Health and Safety Program (AOHSP), all individuals working on this protocol have enrolled in the AOHSP by submitting an Occupational Health Surveillance System (OHSS). I understand that further participation in the AOHSP is voluntary unless required by the Occupational Health Physician or if the individual is working with specific species or research material.

5) The research proposed herein is not unnecessarily duplicative of previous reported research.

6) I ascribe to all of the responsibilities outlined in the ACUC's Principal Investigator Responsibilities policy.

X As Principal Investigator, I have read and agree to abide by the above obligations.

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Attached Document * * *

Document Name	Created Date
Procedure_Relationships.pdf	10/12/2019

Procedure Relationships

Please describe the sequence and timing of the manipulations:

Day 1:
Following ear notch, and after 2–3 days of acclimating to cages, each mouse will be administered either our experimental material or buffer (Intravenous Cas9 RNP injection) via intravenous tail vein injection. Over the subsequent two hours, each animal will be examined twice (once immediately) for clinical signs of pain, distress, illness, or morbidity.

Four hours after administration of experimental material (or buffer as negative control), each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 2:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

24 hours after the Day 1 administration, each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 3:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

Day 4:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

96 hours after the Day 1 administration, each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 5:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

Animals will be euthanized using CO_2 and necropsy will be performed.

Procedures done on a single animal:
Please indicate how many and which procedures a single animal will go through. If applicable, please identify the strain/genotype/breed of animals that will be used in each procedure. Charts are highly recommended for clarity.

Procedure	Strain	Number of times performed
Ear notch	C57BL/6N or Ai9	1
Intravenous Cas9 RNP injection	C57BL/6N or Ai9	1
Blood Collection in Conscious Mice	C57BL/6N or Ai9	3
Euthanasia by CO_2	C57BL/6N or Ai9	1
Collection of Donor Cells/Tissue in Euthanized Mice	C57BL/6N or Ai9	1

Multiple Major Survival Surgery Description:
Describe why it is necessary to perform multiple major surgical procedures on the same animal. Indicate the length of time between surgeries.

N/A

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NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

*** * * Attached Document * * ***

Document Name	Created Date
[redacted]_CITI-cert.pdf	10/12/2019

CITI PROGRAM

Completion Date 16-Aug-2018
Expiration Date 15-Aug-2023
Record ID [redacted]

This is to certify that:

[redacted]

Has completed the following CITI Program course:

Working with the IACUC (Curriculum Group)
Working with the IACUC (All Animal Users) (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

University of California, Berkeley

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/[redacted]

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

*** * * Attached Document * * ***

Document Name	Created Date
[redacted]_CITI-cert.pdf	10/12/2019

CITI PROGRAM

Completion Date 17-Aug-2018
Expiration Date 16-Aug-2023
Record ID [redacted]

This is to certify that:

[redacted]

Has completed the following CITI Program course:

Working with the IACUC (Curriculum Group)
Working with the IACUC (All Animal Users) (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

University of California, Berkeley

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/[redacted]

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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

*** * * Attached Document * * ***

Document Name	Created Date
[redacted]_training-B.pdf	10/12/2019

Animalaria
Formación y Gestión S.L.

Diploma del Curso

Personal que lleva a cabo los procedimientos con animales:

Categoría B

Animalaria Formación y Gestión, S.L. informa que

[redacted]

(NIF [redacted])

Ha aprobado el curso de Categoría B de experimentación animal, validado por la Consejería de Medio Ambiente y de Ordenación del Territorio de la Comunidad de Madrid con fecha 9 de Mayo de 2013, para la obtención de dicha categoría profesional.

(Referencia [redacted])

Y para que conste se expide en Madrid a 15/05/2015

Los Directores del Curso

[redacted] [redacted]

De acuerdo con el Real Decreto 1201/2005 de 10 de Octubre de 2005

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 11/05/2019-11/30/2021

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*** * * Attached Document * * ***

Document Name	Created Date
[redacted]_training-C.pdf	10/12/2019

Animalaria
Formación y Gestión S.L.

Diploma del curso

Personal que diseña y dirige los procedimientos con animales

Categoría C

Animalaria Formación y Gestión, S.L. informa que

[redacted]

(NIF [redacted]

Ha aprobado el curso de Categoría C de experimentación animal, validado por la Consejería de Medio Ambiente y de Ordenación del Territorio de la Comunidad de Madrid con fecha 9 de Mayo de 2013, para la obtención de dicha categoría profesional.

(Referencia [redacted] *)*

Y para que conste se expide en Madrid a 15/05/2015

Los Directores del Curso

[redacted]

De acuerdo con el R.D. 53/2013 de 1 de Febrero de 2013

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

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* * * Attached Document * * *

Document Name	Created Date
Procedure_Relationships_amend.pdf	10/12/2019

Procedure Relationships

Please describe the sequence and timing of the manipulations:

Day 1:
Following ear notch, and after 2–3 days of acclimating to cages, each mouse will have 30 µL blood drawn (Blood Collection in Conscious Mice). After hemostasis has been confirmed, each mouse will be administered either our experimental material or buffer (Intravenous injection of [cell-targeted] ctelCas9 RNP) via intravenous tail vein injection. At two hours post-injection, each animal will be examined for clinical signs of pain, distress, illness, or morbidity.

Four hours after administration of experimental material (or buffer as negative control), each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 2:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

24 hours after the Day 1 administration, each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 3:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

Day 4:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

96 hours after the Day 1 administration, each animal will have 30 µL blood drawn (Blood Collection in Conscious Mice).

Day 5:
Animals will be monitored for general appearance and activity level, as well as for potential adverse events.

Animals will be euthanized using CO_2 and necropsy will be performed.

Procedures done on a single animal:
Please indicate how many and which procedures a single animal will go through. If applicable, please identify the strain/genotype/breed of animals that will be used in each procedure. Charts are highly recommended for clarity.

Procedure	Strain	Number of times performed
Ear notch	C57BL/6N or Ai9 for liver-targeted genome editing; Hu-PBMC-NSG or Hu-CD34-NSG for T cell-targeted genome editing	1
Intravenous injection of [cell-targeted] ctelCas9 RNP	(same as above)	1
Blood Collection in Conscious Mice	(same as above)	4 (30 µL each; 120 µL over 4 days)
Euthanasia by CO_2	(same as above)	1
Collection of Donor Cells/Tissue in Euthanized Mice	(same as above)	1

Multiple Major Survival Surgery Description:
Describe why it is necessary to perform multiple major surgical procedures on the same animal. Indicate the length of time between surgeries.

N/A