Cas9-knock-in mouse models, or other engineered murine genetic models (*e.g*. knockout (KO), inducible cell deletion, reporter).

SUMMARY

[0005] The instant disclosure generally relates to strategies for gene perturbation in primary myeloid cells, *e.g*. of human and murine origin. Using transfection techniques (e.g., electroporation/nucleofection) to deliver ribonuclear protein complexes (RNPs) including guide-RNAs non-covalently associated with recombinant Cas (e.g. Cas9), near population-level genetic knockout of single and multiple targets can be achieved in a range of cell types without the need for selection or enrichment of genetically modified cells. Cellular fitness and response to immunological stimuli of cells modified according to the methods described herein are not significantly affected by the gene editing process. Such advances enable pathway discovery and drug target validation across species in the field of innate immunity.

[0006] Delivery via transfection, e.g., nucleofection of Cas-RNP complexes to non-granulocytic cells (*i.e*. monocytes, macrophages and dendritic cells) in accordance with the methods described herein can generate up to at least about 90% knockout of single or multiple target genes without the need further selection of genetically modified cells. This can be achieved in both differentiated primary myeloid cell populations and freshly isolated cells. Provided herein are methods that enable rapid loss-of-function gene assessment within donor cell populations without substantially affecting normal cell function, including, for example, responsiveness to stimulation.

[0007] In an ~~aspect~~example, provided herein are methods for genetic modification of a myeloid cell including transfecting the myeloid cell with a gene editing reagent targeting a genetic site of interest, where the myeloid cell is not transduced with a viral vector. In one aspect, the present disclosure refers to a method for genetic modification of a myeloid cell, the method comprising transfecting the myeloid cell with a gene editing reagent targeting a genetic site of interest, wherein the myeloid cell is not transduced with a viral vector. In ~~embodiment~~examples, a gene editing reagent suitable for use with the methods described herein can include an RNP including a guide RNA non-covalently contacted with a Cas protein, such as a Cas9 protein.

[0008] In an ~~aspect~~example, provided herein are methods for genetic modification of a plurality of myeloid cells, including transfecting the plurality of myeloid cells with a gene editing reagent targeting a site of interest, wherein the myeloid cells are not transduced with a viral vector or viral delivery system. In one aspect, the present disclosure refers to a method for genetic modification of a plurality of myeloid cells, the method comprising transfecting the plurality of myeloid cells in the presence of a gene editing reagent targeting a site of interest, wherein the myeloid cells are not transduced with a viral vector.

[0009] In an ~~aspect~~example, provided herein are methods of genetically modifying a plurality of myeloid cells, including transfecting the myeloid cells with a gene editing reagent, wherein the myeloid cells are not transduced with a viral vector, and wherein the method does not include a selection step or enrichment step following myeloid cell transfection. In one aspect, the present disclosure refers to a method of genetically modifying a plurality of myeloid cells, comprising transfecting the myeloid cells with a gene editing reagent, wherein the myeloid cells are not transduced with a viral vector, and wherein the method does not comprise a selection step or enrichment step following myeloid cell transfection.

[0010] In an ~~aspect~~example, provided herein are systems for genetically modifying a myeloid cell in the absence of a viral vector. In ~~embodiment~~examples, the systems include electroporation systems. In ~~embodiment~~examples, the systems include nucleofection systems. In ~~embodiment~~examples, the system includes a chamber compatible with electroporation or nucleofection system, multiple myeloid cells within the chamber in a media compatible with electroporation/nucleofection, and at least one gene editing system designed to target at least one site of interest in the genome of myeloid cells. In one aspect, the present disclosure refers to a system for genetically modifying a myeloid cell in the absence of a viral vector, the system comprising a chamber compatible with a transfection system, multiple myeloid cells within the chamber in a media compatible with electroporation, and at least one gene editing system designed to target at least one site of interest in the genome of myeloid cells.

[0011] In an ~~aspect~~example, provided herein are methods of treating a disease treatable with a myeloid cell provided herein including ~~embodiment~~examples thereof. The methods include providing a genetically modified myeloid cell that has not been transformed with a virus, where the myeloid cell has been transfected with a gene editing reagent; and administering the myeloid cell to a patient in need thereof. In one aspect, the present disclosure refers to use of a composition comprising a genetically modified myeloid cell made by any one of the methods disclosed herein, and a pharmaceutically acceptable excipient, in the manufacture of a medicament for treating a disease treatable with a myeloid cell.

[0012] In an ~~aspect~~example, provided herein is a genetically modified myeloid cell made by a method provided herein including ~~embodiment~~examples thereof. In one aspect, the present disclosure refers to a genetically modified myeloid cell made by any one of the methods disclosed herein.

[0013] In an ~~aspect~~example, provided herein is an assay for drug discovery comprising screening the effect of one or more compounds on a genetically modified myeloid cell provided herein including ~~embodiment~~examples thereof. In one aspect, the present disclosure refers to an assay for drug discovery comprising screening the effect of one or more compounds on the myeloid cell disclosed herein.

[0014] In an ~~aspect~~example, provided herein is a method for target validation of a compound, including contacting a genetically modified myeloid cell provided herein including ~~embodiment~~examples thereof with the compound and monitoring an effect on the cell. In one aspect, the present disclosure refers to a method for target validation of a compound, comprising contacting a myeloid cell disclosed herein with the compound and monitoring an effect on the cell.

[0015] In an ~~aspect~~example, provided herein are compositions including a plurality of myeloid cells in contact with a gene editing reagent, a transfection buffer, and an electroporation enhancer, where the composition does not comprise a viral vector. In one aspect, the present disclosure refers to a composition comprising a plurality of myeloid cells, a gene editing reagent, a transfection buffer, and an electroporation enhancer, wherein the composition does not comprise a viral vector.