

CRISPR-Cas9 gene editing in primary and stem cells

Below is a list of publications which demonstrate the application of CRISPR-Cas9 genome engineering techniques for target gene knockout or precise knock-in in a variety of primary cell types, stem cells, and induced pluripotent stem cells.

Reference	Cells	Target gene(s)	Editing type	Cas9/guide RNA format	Delivery method	DOI
Primary cells						
Flamier <i>et al. npj Precision Oncology</i> 2020	Primary GBM (Glioblastoma multiforme)	<i>IBMI1</i>	Knockout	Plasmid and synthetic sgRNA	Lipofection	https://doi.org/10.1038/s41698-019-0106-1
Hendel <i>et al. Nature Biotechnology</i> 2015	Human primary T-cells, also CD34+ HSPCs	<i>IL2RG, HBB, CCR5</i>	Knockout	Cas9 protein, mRNA, plasmid/ synthetic sgRNA	Electroporation	http://dx.doi.org/10.1038/nbt.3290
Hou <i>et al. Scientific Reports</i> 2015	Human or Rhesus macaque CD4+ T cell	<i>CXCR4</i>	Knockout	Plasmid	Electroporation	http://dx.doi.org/10.1038/srep15577
Hultquist <i>et al. Nat Protoc.</i> 2019	Primary human CD4+ T cells	<i>CXCR4</i>	Knockout	Cas9 protein with synthetic crRNA:tracrRNA	Electroporation	https://doi.org/10.1038/s41596-018-0069-7
Liang <i>et al. J. Biotech.</i> 2015	Primary human keratinocytes, also mouse ESCs	<i>AAVS, HRPT, RELA</i>	Knockout	Cas9 protein complex with RNA	Electroporation	http://dx.doi.org/10.1016/j.jbiotec.2015.04.024
Shifrut <i>et al. Cell</i> 2018	Human T Cells	<i>CD8A</i>	Knockout	Protein and sgRNA lentivirus	Electroporation, transfection	https://doi.org/10.1016/j.cell.2018.10.024
Su <i>et al. Scientific Reports</i> 2016	Human T Cells	<i>PD-1 (PDCD1)</i>	Knockout	Cas9 plasmid/sgRNA plasmid	Electroporation	http://dx.doi.org/10.1038/srep20070
Van Overbeek <i>et al. Molecular Cell</i> 2016	HSCs	<i>BRCA2</i>	Knockin	Cas9 protein complex with RNA	Electroporation	http://dx.doi.org/10.1016/j.molcel.2016.06.037
Zhou <i>et al. Cell. Mol. Life Sci.</i> 2016	Porcine primary fibroblasts, human dermal fibroblasts	<i>MAPT, SORL1</i> (porcine) <i>MYH6, COL2A1</i> (human)	Knock-in/ knockout	Cas9 plasmid/ gRNA plasmid, donor plasmid	Electroporation, transfection	http://dx.doi.org/10.1007/s00018-015-2128-3

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Stem cells/Induced pluripotent stem cells						
Allende <i>et al. J Lipid Res.</i> 2018	Sandhoff iPSCs (to cerebral organoids)	<i>HEXB</i>	correction	plasmid	Electroporation	http://dx.doi.org/10.1194/jlr.M081323
Bressan <i>et al. Development</i> 2017	Neural stem cells	<i>SOX2, TP53, others</i>	several	Plasmid, Cas9 protein complex with RNA	Electroporation	http://dx.doi.org/10.1242/dev.140855
Bryne <i>et al. Curr. Prot. Stem Cell Biol.</i> 2015	Human induced pluripotent stem cells (iPSCs)	<i>THY1</i>	Knockout	Plasmid	Electroporation	http://dx.doi.org/10.1002/9780470151808.sc05a08s35
Chang <i>et al. Stem Cell Res.</i> 2018	Sandhoff iPSCs (to cerebral organoids)	<i>HEXB</i>	correction	plasmid	Electroporation	http://dx.doi.org/10.1016/j.scr.2017.12.015
Eggenschwiler <i>et al. Scientific Reports</i> 2016	Patient-derived iPSCs	<i>SERPINA1</i>	HDR	Plasmid	Electroporation, lipofection	http://dx.doi.org/10.1038/srep38198
Haupt <i>et al. J Vis Exp.</i> 2018	Human induced pluripotent stem cells (iPSCs)	<i>LMNB1</i>	HDR	Cas9 protein, synthetic sgRNA	Electroporation	https://doi.org/10.3791/58130
Kotini <i>et al. Cell Stem Cell</i> 2017	iPSCs staged from MDS and AML	<i>GATA2, ASXL1</i>	Knockout	Plasmid and lentivirus	Electroporation, transduction	http://dx.doi.org/10.1016/j.stem.2017.01.009
Liu <i>et al. Nature Protocols</i> 2015	Human embryonic stem cells (hESCs)	<i>EMX1, CCR5, AAVS-1, AAVS-2</i>	Knockout and HDR	Cas9 protein complex with RNA	Nucleofection	http://dx.doi.org/10.1038/nprot.2015.117
Marrone <i>et al. Stem Cell Rep.</i> 2018	iPSCs	<i>FUS</i>	GFP tagging	plasmid	Transfection	http://dx.doi.org/10.1016/j.stemcr.2017.12.018
Roberts <i>et al. Mol Biol Cell.</i> 2017	hiPSCs	<i>PXN, TUBA1B, others</i>	HDR	Cas9 protein complex with RNA	Transfection	https://doi.org/10.1091/mbc.E17-03-0209
Roberts <i>et al. Stem Cell Reports</i> 2019	hiPSCs	<i>TN, MYL7, MYL2, TNNI1, ACTN</i>	HDR	Cas9 protein, synthetic sgRNA	Transfection	http://doi.org/10.1016/j.stemcr.2019.03.001
Shimokawa <i>et al. Nature</i> 2017	cancer stem cell (CSC) organoids	<i>LGR5, KRT20</i>	knockout, knock-in	plasmid	Electroporation	http://dx.doi.org/10.1038/nature22081
Zhang <i>et al. Mol. Ther. Nucleic Acids</i> 2017 (review)	Multiple	Multiple	Multiple	Multiple	Multiple	http://dx.doi.org/10.1016/j.omtn.2017.09.009

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