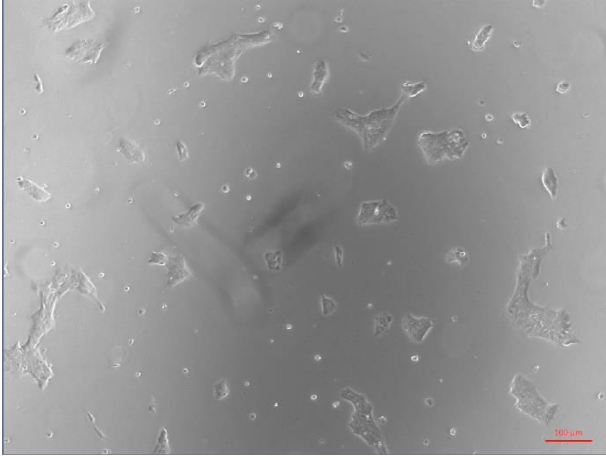


Supplemental information

**Excess of guide RNA reduces
knockin efficiency and drastically
increases on-target large deletions**

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2 μ M ssODN



3 μ M ssODN



2 μ M ssODN



4 μ M ssODN



Figure S1: Microscopic observation with Zeiss Axio vert.A1 microscope (5X) of hiPSC 2 days post-electroporation with Cas9 0.4 μ M, RNP ratio Cas9/dgRNA 1/1 and 2, 3 or 4 μ M of ssODN, related to Figure 2. Electroporation of 3 μ M and 4 μ M of ssODN with Cas9 0.4 μ M and RNP ratio 1/1 shows low confluences (10% and 5% respectively compared to their 2 μ M control (25% and 20%, respectively). Moreover, with 2 μ M of ssODN, hiPSC form numerous colonies compared to higher concentrations. A 100 μ m scale bar is displayed in red on each picture. dgRNA, dual guide RNA; ssODN, single-stranded oligonucleotide, hiPSC, human induced pluripotent stem cells.

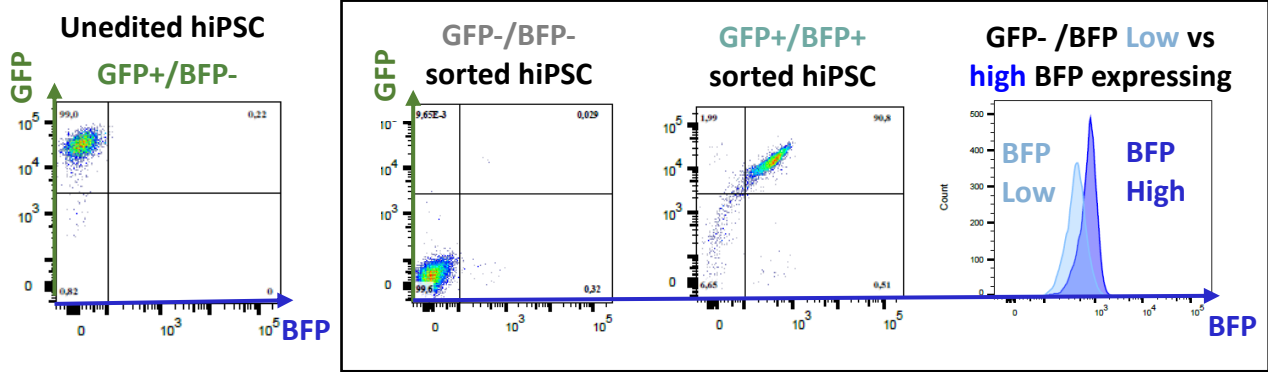
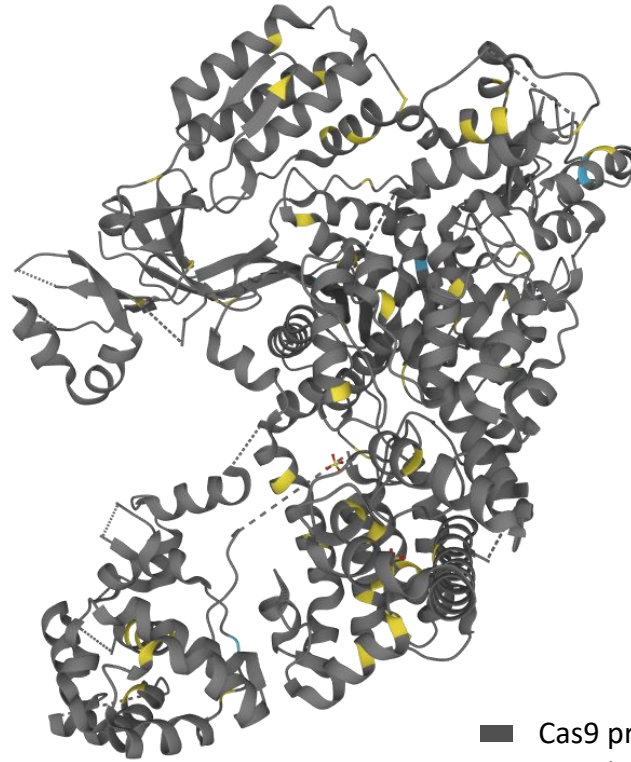


Figure S2: Cell-sorted subsets phenotyping by flow cytometry to verify purity of each subset, related to Figure 3. Cell sorting results of GFP- /BFP-, GFP+/BFP+, GFP-/BFP low and GFP-/BFP high subsets. hiPSC, human induced pluripotent stem cells; GFP, Green Fluorescent Protein; BFP, Blue Fluorescent Protein.

A.



■ Cas9 protein
■ Tyrosine residues
■ Tryptophan residues
■ guide RNA

B.

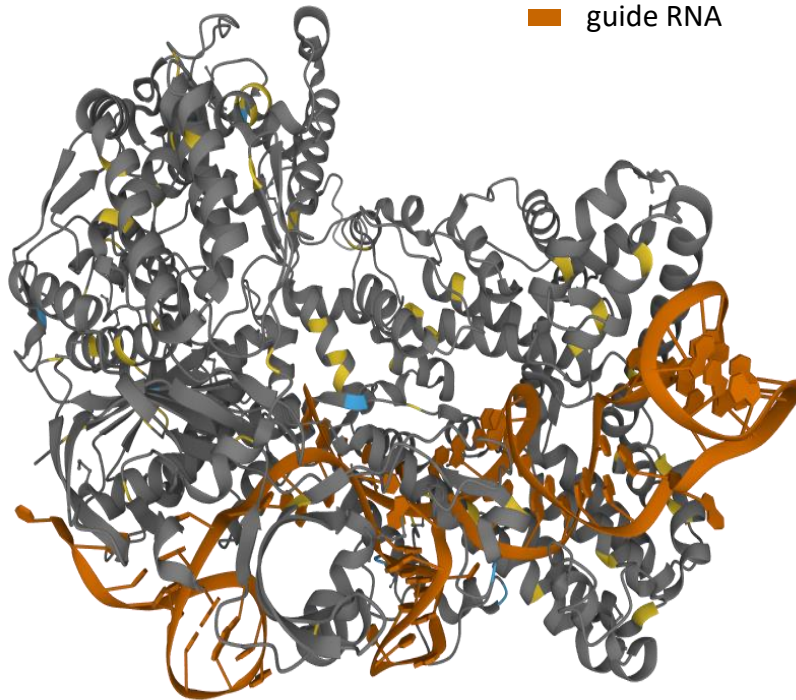


Figure S3: Tyrosine and tryptophan residues visualization on Cas9 from *Streptococcus Pyogenes* and RNP complex, related to Figure 5. A. ApoCas9 conformation (PDB: 4CMP) or **B.** in complex with a gRNA (orange) forming the RNP complex conformation (PDB: 4ZT0). Tyrosine (yellow) and tryptophan (blue) residues visualization. Figure made with PDB visualization online software. PDB, protein data base; dgRNA, dual guide RNA.

Table S2. CRISPR/Cas9 mediated edition and KI generation on rat embryos with dgRNA rEphx2 and ssODN XbaI, related to Figure 6.

No., number; ssODN, single-stranded oligonucleotide; KI, knockin.

Cas9 (μM)	Cas9/dgRNA molar ratio	ssODN (μM)	No. of electroporation experiments	No. of foster females used	No. of analyzed /No. of transferred embryos (%)	No. edited embryos, including KI /No. of analyzed embryos (%)	No. KI embryos /No. edited embryos, including KI (%)	No. of embryos with large deletion /No. KI embryos/ (%)
0.2	1/1	2	3	4	30 / 108 (27.8%)	29 (96.7%)	15 (50.0%)	5 (16.7%)
0.2	1/2	2	3	3	24 / 88 (27.3%)	24 (100%)	9 (37.5%)	18 (75.0%)