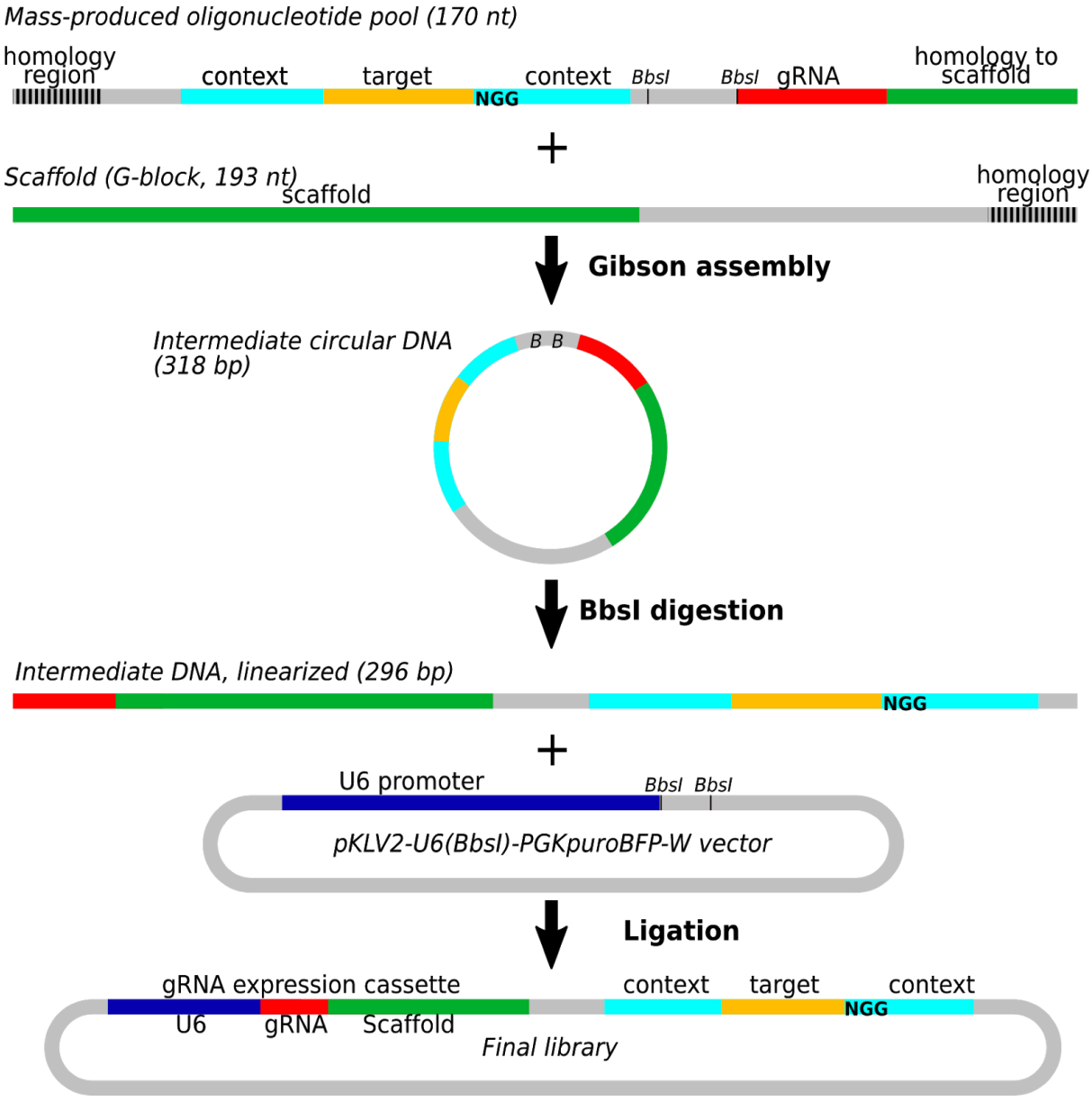
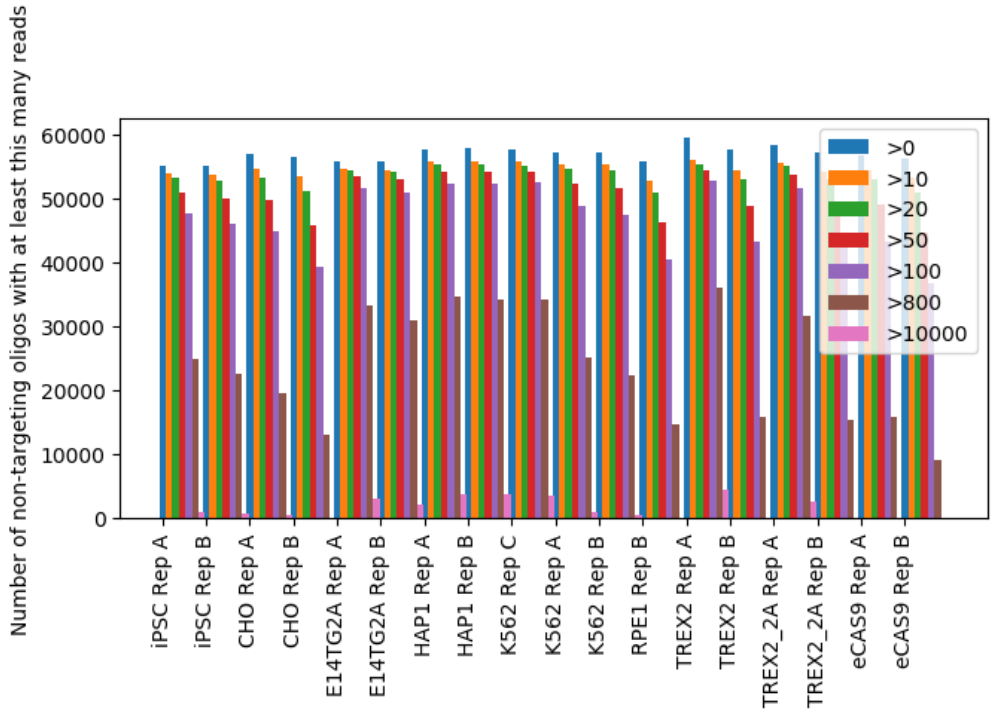
Supporting Information

Figure S1. Construct design and cloning approach



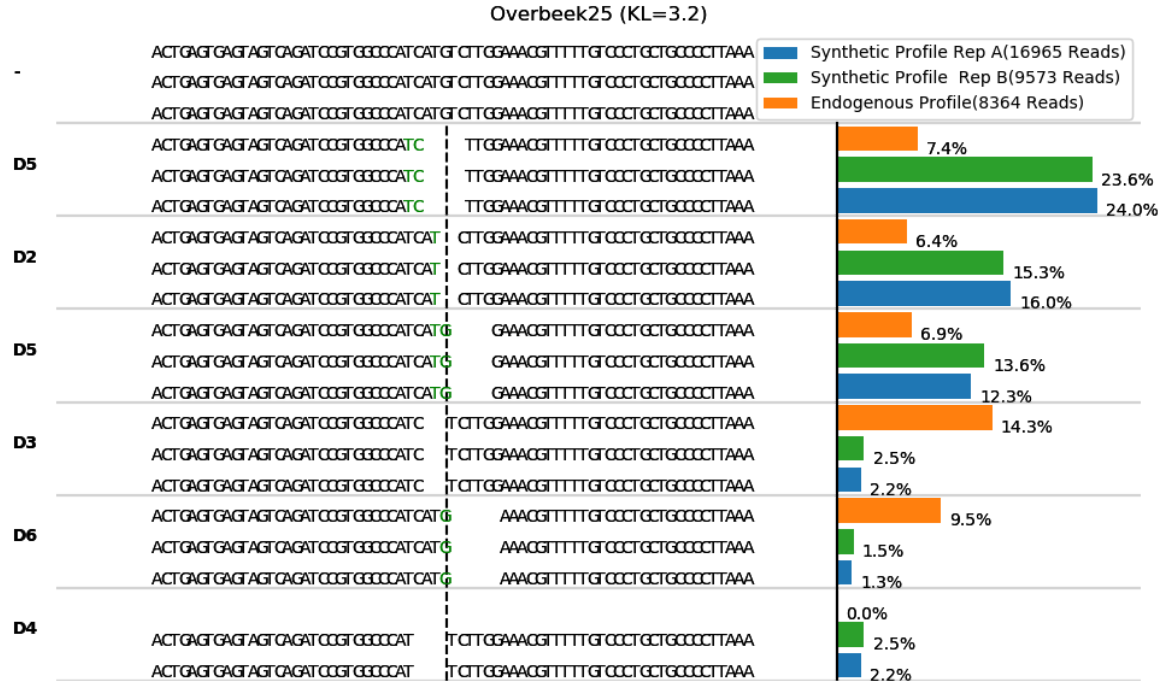
Library cloning started by PCR amplification of the 170 nt oligonucleotide pool of designed sequences encoding gRNA and target sequence, separated by a spacer harbouring two BbsI restriction sites. Gibson assembly was employed to fuse the amplified pool to a 193 nt G- block fragment encoding either a conventional or improved version of the gRNA scaffold and a spacer. The resulting 318 bp circular DNA was linearised with BbsI and the 296 bp linear product was ligated into scaffold-less pKLV2-U6(BbsI)-PKGpuro2ABFP-W, to produce the complete library constructs encoding a functional gRNA expression cassette and its target sequence.

Figure S2. Coverage across experiments



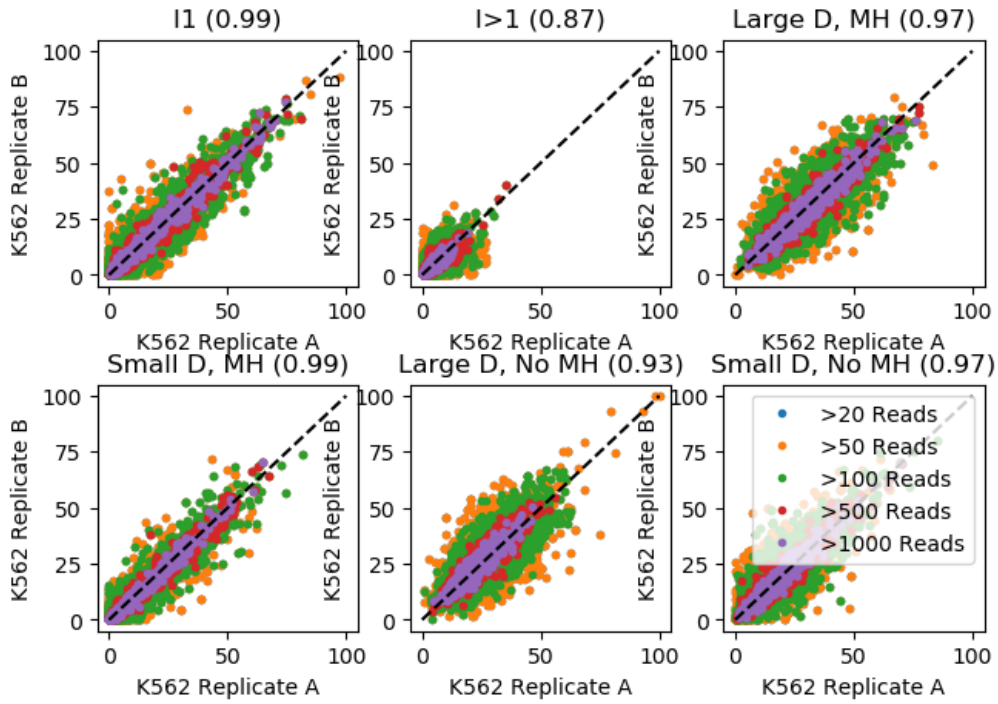
Number of constructs (y-axis) with a minimum number of mapped reads (color) in each experiment (x-axis).

Figure S3. Comparison of endogenous and synthetic repair profiles for Overbeek 25 gRNA



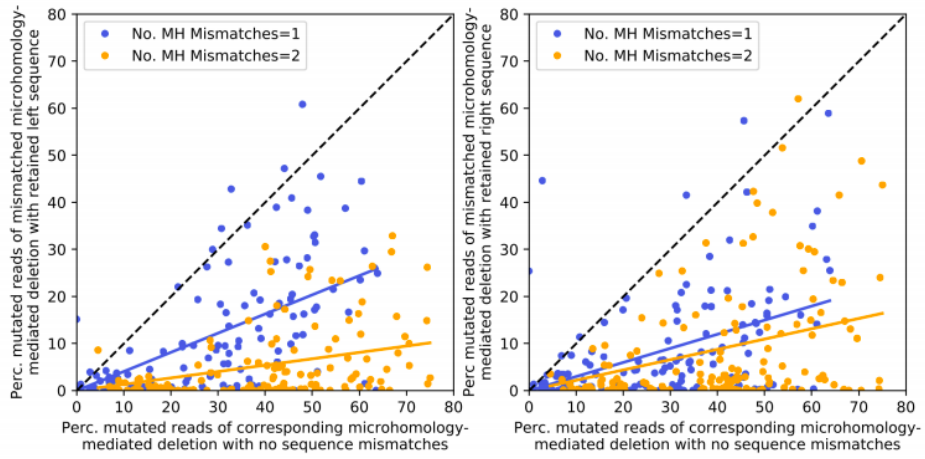
Measured repair profile reproducibility for the outlier, Overbeek 25, gRNA-target pair. DNA sequence of the target (top) is edited to produce a range of outcomes in two synthetic replicates (green, blue bars) and one endogenous measurement (orange bars). The proportions (x-axis) of the four largest mutational outcomes (e.g. "D3" - deletion of three base pairs, "I1" - insertion of one base pair, etc.; y-axis) is consistent between the experiments. Stretches of microhomology (green) and inserted sequences (red) are highlighted at the cut site (dashed vertical line).

mFigure S4. Reproducibility of indel class frequencies per gRNA



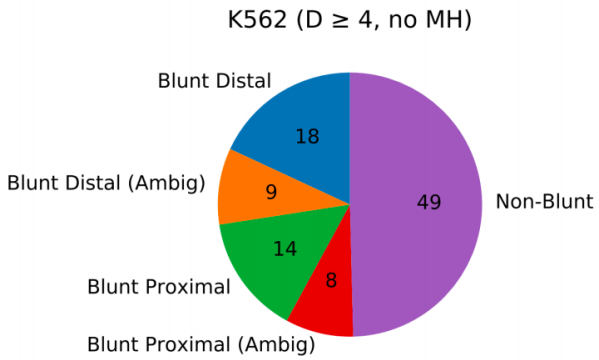
Frequency of different indel classes (panels) in replicate A (x-axis) or B (y-axis) of K562 cell line measurements (markers), annotated by sequencing coverage (color).

Figure S5. No bias in the side of sequence selected for MMEJ outcomes



Mutations in microhomology sequence reduce repair outcome frequency, but corresponding deletions are still present. For matched pairs of guides, with and without mutations in the microhomologous sequence, the fraction of mutated reads associated with the particular microhomology (y-axis) is smaller than without mismatches (x-axis) for most gRNAs (markers; blue: one mismatch, yellow: two mismatches). The rates of repair are not different depending on whether sequence was retained from PAM-distal (left panel) or PAM-proximal (right panel) side of the cut.

Figure S6. No bias in the side of deletions for NHEJ outcomes



Percent of alternative outcomes for large deletions without microhomology.

Figure S7. Example of profile measured across different cell lines (most frequent 4 indels only)

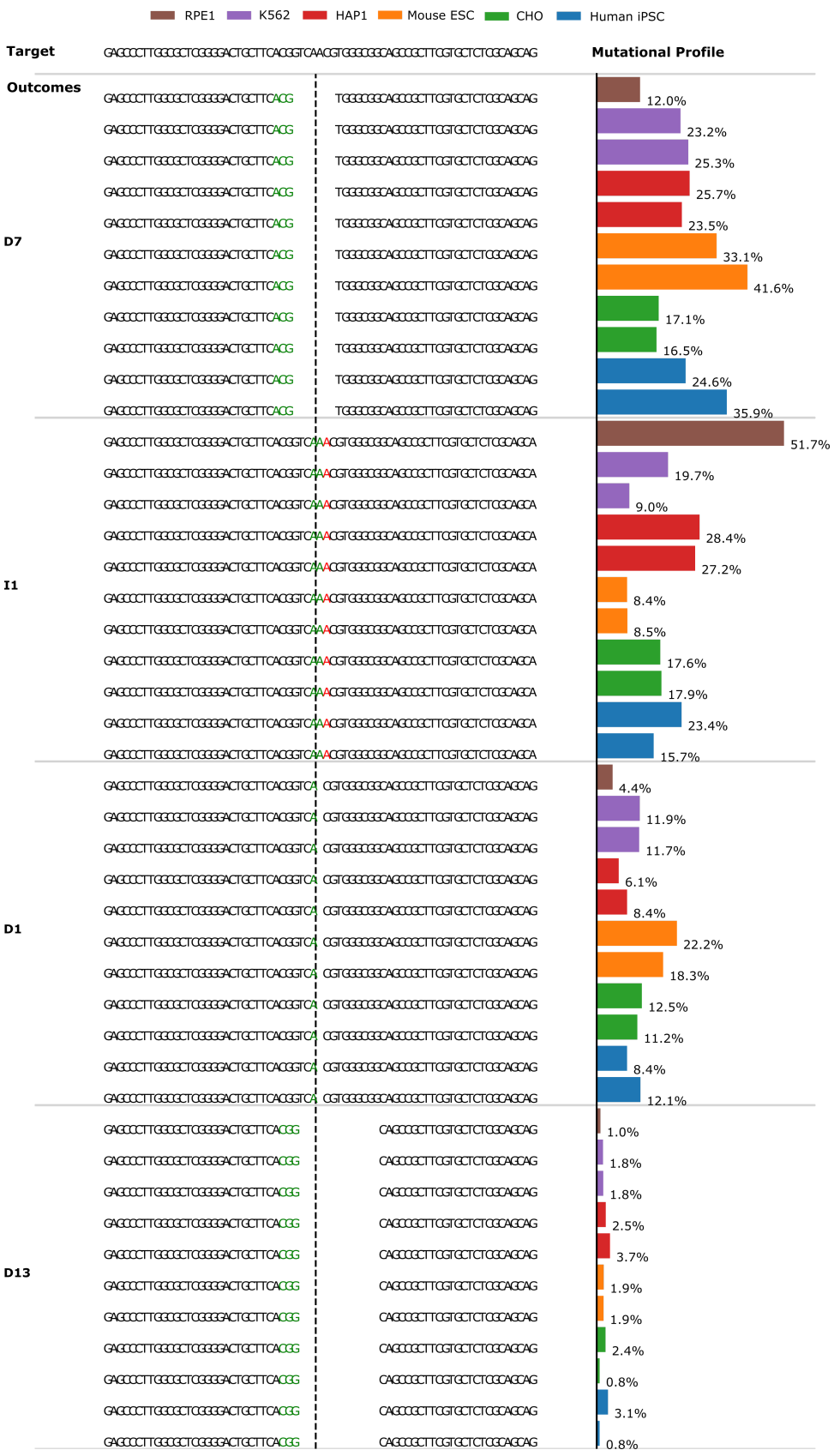
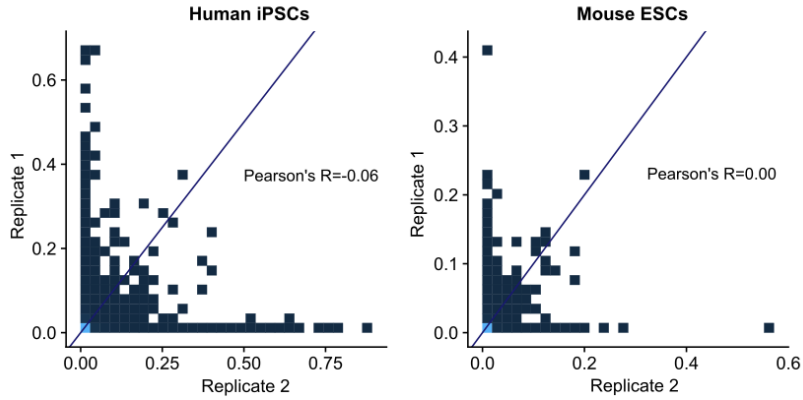
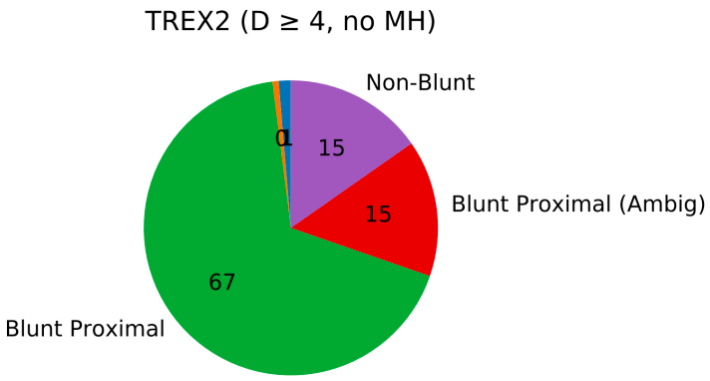


Figure S8. Lack of reproducibility in large insertions in human and mouse stem cells



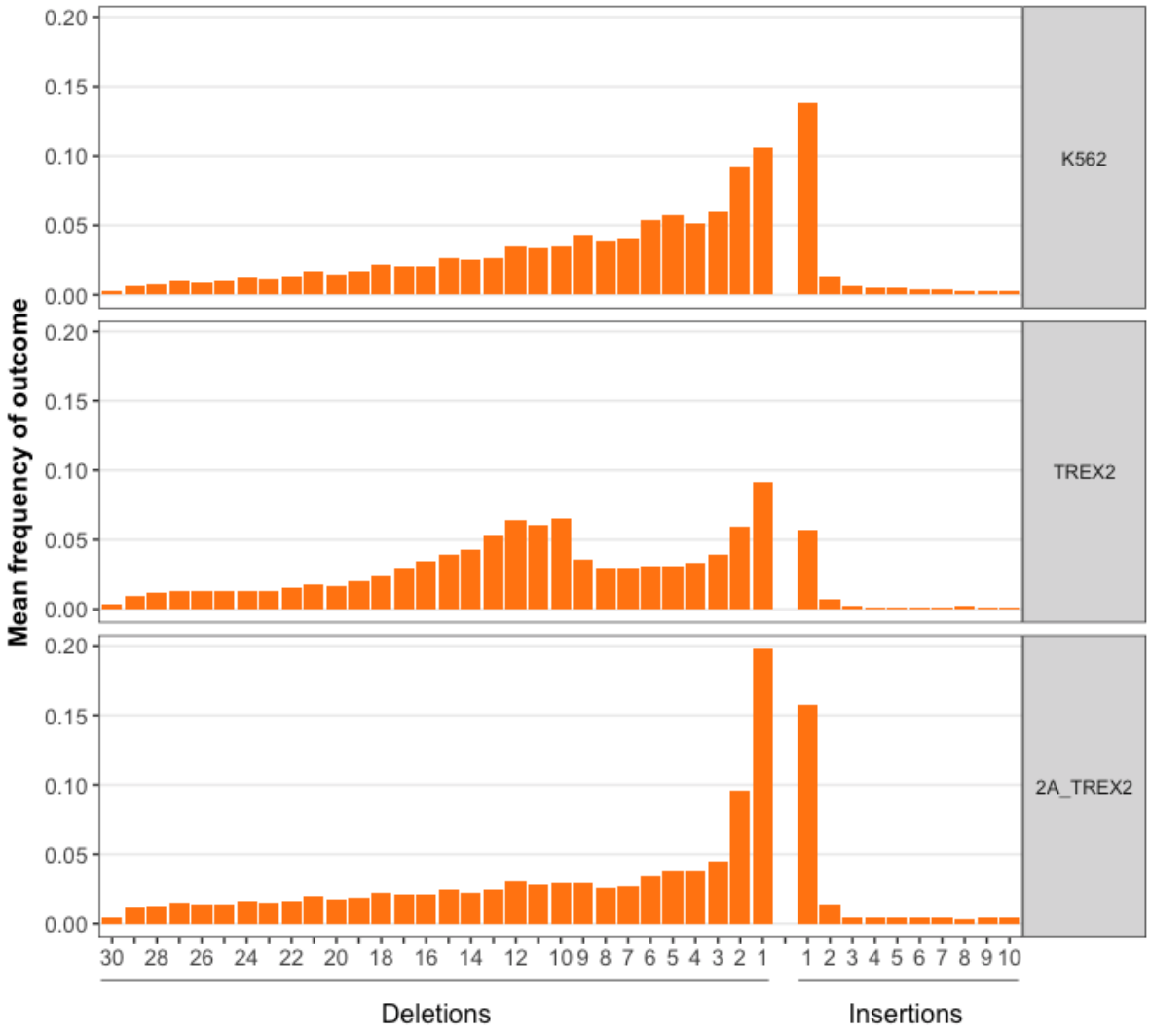
Proportion of individual indels in two replicates of human iPSCs (left) and mouse ESCs (right).

Figure S9. Repair outcomes from Cas9-TREX2 fusion favour blunt end joins



Percent of alternative outcomes for large deletions without microhomology.

Figure S10. Cas9-2A-TREX2 has smaller influence on repair outcomes than Cas9-TREX2



The mean frequency (y-axis) of deletion or insertion size (x-axis) across genomic sequence targets for three alternative Cas9 effector constructs (panels).

Figure S11. Training success depends on hyperparameters and dataset size

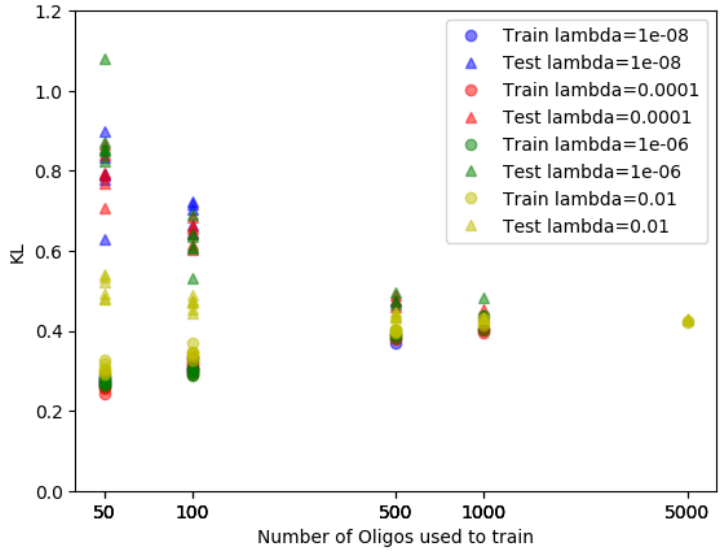


Table S1. Primer sequences (5’ > 3’)

Cloning of pKLV2-U6(BbsI)-PKGpuro2ABFP-W	
P1	GGCAGCACTGCATAATTCTCTTAC
P2	CCTACCCGGTAGAATTGGATCCAAACGTGTCTTCTCGAAGACCC
P3	GTAAGAGAATTATGCAGTGCTGCC
P4	GGGTCTTCGAGAAGACACGTTTGGATCCAATTCTACCGGGTAGG
Amplification of oligo pools for library cloning	
P5	GGAAACTACACTTGCCTGGC
P6	AACTTGCTATTTCTAGCTCTAAAAC
P7	GACGTCCAGAGCACAGATGG
P8	GCTGTTTCCAGCATAGCTCTTAAAC
Preparation of sequencing libraries	
P9	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGTGGAAAGGACGAAACA
P10	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAAACTACACTTGCCTGGC
P11	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACGTCCAGAGCACAGATGG
P12	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACCCGGTAGAATTGGATCCAAAC
P13	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
P14*	CAAGCAGAAGACGGCATACGAGATN_{10}GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT
Sequencing primers	
P15	TCTTCCGATCTCTTGTGGAAAGGACGAAACACCG
P16	CTCTTCCGATCTGACGTCCAGAGCACAGATGG
P17	GCTCTTCCGATCTGGAAACTACACTTGCCTGGC
P18	CGCTCTTCCGATCTACCCGGTAGAATTGGATCCAAAC

*: N_{10}, index for multiplexed sequencing.

Table S2. Screen conditions

Cell line	gRNA Scaffold	Cells ($x10^6$)	Multiplicity of infection	Coverage (cells per construct)	Replicates
K562-Cas9	Improved	70	0.6	800	2
K562-Cas9	Improved	140	0.6	1600	2
K562-Cas9	Conventional	32	0.5	1600	1
K562	Improved	70	0.6	800	1
K562	Conventional	16	0.5	800	1
K562-eCas9	Improved	70	0.6	800	2
K562-eCas9	Conventional	16	0.5	800	2
K562-Cas9-TREX2	Improved	70	0.6	800	2
K562-Cas9-TREX2	Conventional	16	0.5	800	2
K562-Cas9-2A-TREX2	Improved	70	0.6	800	2
K562-Cas9-2A-TREX2	Conventional	16	0.5	800	2
RPE-1-Cas9	Improved	52	0.5	500	2
HAP1-Cas9	Improved	83	0.5	800	2
CHO-Cas9	Improved	83	0.5	800	2
iPSC-Cas9	Improved	83	0.5	800	2
E14TG2a-Cas9	Improved	83	0.5	800	2

Table S3. Most important features for prediction

Feature Symbol	θ value/s	Description
I1Rpt	1.461	Single nucleotide insertion repeating the PAM distal nucleotide adjacent to the cut site
IL-1--1, IL-2--2	1.432, 0.838	Insertion at the cut site
PW_I1_T_vs_I1Rpt	0.692	Single nucleotide insertion repeating the PAM distal nucleotide adjacent to the cut site in which a T is inserted
PW_I1_A_vs_I1Rpt	0.544	Single nucleotide insertion repeating the PAM distal nucleotide adjacent to the cut site in which an A is inserted
PW_No MH_vs_DL-1--1	0.355	No microhomology, blunt-end deletion on PAM-proximal side
I1	0.354	Any single nucleotide insertion
PW_I1_C_vs_I1Rpt		Single nucleotide insertion repeating the PAM distal nucleotide adjacent to the cut site in which a C is inserted
PW_L0_NT=C_vs_DL-1--1	0.311	Blunt-end deletion on PAM-proximal side with a C adjacent to the cut site.
PW_CS0_NT=G_vs_I1	0.303	Any single nucleotide insertion when there is a G adjacent to the cut site on the PAM-proximal side
	...	
	...	
I2NonRpt	-0.195	Double nucleotide insertion that is not just an additional repeat of the repeat single nucleotide insertion
IL>=0, IL<-3	-0.249,-0.244	Any insertion away from the cut site
I2	-0.250	Any double nucleotide insertion
PW_D>12_vs_DR0-0	-0.364	Blunt-end deletion on PAM-distal side of cut of size greater than 12
I1_G	-0.473	Single nucleotide insertion of a G