Editing of the TRIM5 gene decreases the permissiveness of human T lymphocytic cells to HIV-1



Figure S1. HDR editing-specific PCR screening of Jurkat cells transfected with CRISPR-Cas9 RNPs and donor ssDNA. 47 clonal cell populations were lysed and subjected to a PCR assay in which one of the primers is complementary to the correctly HDR-edited *TRIM5* region targeted for mutagenesis. A PCR product of the expected size was found in clones 6, 8, 12, 17, 30, 38. Ctl+ consisted of Jurkat cells following CRISPR components transfection but prior to the isolation of clones.

Editing of the TRIM5 gene decreases the permissiveness of human T lymphocytic cells to HIV-1



Figure S2. HaeIII screening of selected Jurkat clones. The 6 clones found to be positive in the HDR editingspecific PCR test, along with 8 randomly chosen negative clones, were subjected to a PCR assay using primers that bind outside of the genomic region complementary to the HDR donor DNA, followed by digestion with HaeIII. U and D indicates bands of the expected size for the undigested PCR product and the HaeIII digestion products, respectively.

Editing of the TRIM5 gene decreases the permissiveness of human T lymphocytic cells to HIV-1

	gRNA target Cut site PAM			
WT	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT			
Donor DNA	CCGAAACCACAAATAATCTACGGGGGCCGGCGGCACAGGATACCAGACATTT			
001101 0101				
	gRNA blocking HaeIIIR332G PAM blocking R335G			
	mutation mutation	N mutations	Indels	Subst.
Clone6	CCGAAACCACAGATAATATA C GGGGGC CGGC GG C ACAAGATACCAGACATTT	5/8	n	n
	CCGAAACCACAGATAATATATGGGGGCCGGCGGCACAGGATACCAGACATTT	5/8	n	n
Clone 7	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 8	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGGCCGGCGGCGGCGGCGGCGGCACACACA	4/8	n	у
Clone 12	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGGCCGGCGG <mark>T</mark> ACAGGATACCAGACATTT	4/8	n	у
Clone 17	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGGC CGGC GG <mark>T</mark> ACAGGATACCAGACATTT	4/8	n	У
Clone 30	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGG <mark>T</mark> ACAGGATACCAGACATTT	4/8	n	У
Clone 31	CCGAAACCACAGATAATATATGGG <mark>-</mark> CACGAGGGACAAGATACCAGACATTT	0/8	у	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 32	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 33	CCGAAACCACAGATAATATATGGG <mark>-</mark> CACGAGGGACAAGATACCAGACATTT	0/8	У	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 34	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 35	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 36	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 38	CCGAAACCACAGATAATATATGGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCC <mark>GGC</mark> GG <mark>T</mark> ACAGGATACCAGACATTT	4/8	n	У
		a (a		
	UUGAAAUUATA	0/8	у	У
Clone 43		0/8	n	n
	CCGAAACCACAGATAATATGGGGGCACGAGGGACAAGATACCAGACAT"T"	0/8	n	n

Figure S3. MiSeq sequencing results for all clones analyzed. This is an extended version of Figure 1, showing sequencing results for the 8 randomly chosen clones negative for HDR editing in the specific PCR test, in addition to the 6 clones found to be positive.

Editing of the TRIM5 gene decreases the permissiveness of human T lymphocytic cells to HIV-1



Figure S4. Permissiveness of HDR-edited clone 17 to HIV-1_{NL-GFP} infection. Jurkat clonal cell populations were analyzed for permissiveness to HIV-1 infection. Clones 17 and 30 are monoallelically edited to express R332G-R335G TRIM5 α whereas clones 32 and 36 are unedited. Cells were infected with increasing doses of HIV-1_{NL-GFP} in the presence or not of IFN- β . The percentage of cells expressing GFP was determined by FACS two days later.