**Plasmids:** (also see Table of materials) Lentiviral packaging vectors: gag-pol packaging plasmid psPAX2, VSV-G envelop plasmid pMD2.G; pRSV-Rev plasmid. pBK43 (integrase-deficient) packaging cassette is used for generating IDLVs (see below). psPAX2 (second-generation LV packaging system) expresses the following genes: *gag* (encodes the capsid, nucleocapsid and matrix structural proteins), *pol* (encodes reverse transcriptase, protease and integrase proteins), *tat* (encodes protein for transactivation of transcription from viral LTR), and *rev* (encodes protein that mediates export of viral RNAs from nucleus).

 **Note**: (A) Tat is dispensable for production of the **s**elf-inactivated (SIN) vectors used in this study. (B) A third-generation packaging system can also be used to package the CRISPR/Cas9- genome, but results in lower viral titers compared to those obtained with second generation vectors. (C) VSV-G exhibits a broad host cell range; however, additional pseudotypes are available for efficient transduction in different cell types. (D) The pRSV-Rev plasmid from the second-generation packaging system has been successfully used to enhance production titers.

**Plasmid construction**: The catalytic domain of the integrase protein contains the D,D-35-E catalytic amino-acid triad motif, comprised of the highly conserved amino-acids D64, D116 and E152 29(Fig 1a). The IDLV packaging cassette was derived from psPAX2 and was created as follows. The *int* region was amplified with primers: F- 5’-GAAATTTGTACAGAAATGG-3’, and R- 5’-CTTCTAAATGTGTACAC-3’. The R-primer harbored a T🡪G mutation in the GAT codon, which created an Asp🡪Glu (D64E) substitution. The PCR product harboring the mutation was digested with BsrGI and cloned into psPAX2, replacing the corresponding region. The resulting i*ntD64E*-containing packaging plasmid was named pBK43. The plasmid sequence was confirmed by restriction enzyme digestion (Fig. 1b) and Sanger sequencing. To generate a shorter version of the pLenti-CRISPR/Cas9-expressing vector, the plasmid was digested with BsmBI and cloned with a pair of annealed and phosphorylated oligonucleotides, upper- 5’- CACCGGAGACGTGTACACGTCTCT-3’ and lower- 5’-AAACAGAGACGTGTACACGTCTCC-3’. The resulting plasmid, pBK109 harbored two BsmBI restriction sites separated by a short linker sequence. The BsrGI site created between BsmBI sites in the new vector allowed for easy screening of small guide RNA (sgRNA)-positive clones. The plasmid was further modified to include a pair of Sp1-binding sites. To this end, the plasmid was digested with KpnI and PacI and cloned with a pair of the annealed and the phosphorylated oligonucleotides: upper: 5’- TAATGGGCGGGACGTTAACGGGGCGGAACGGTAC-3’, lower: 5’- CGTTCCGCCCCGTTAACGTCCCGCCCATTAAT-3’. The resulting plasmid was named pBK301. The following oligos were used to introduce sgRNAs targeting GFP into pBK109 and pBK301 (sgRNA1): Upper: 5’-CACCGGGGCGAGGAGCTGTTCACCG-3’; lower: 5’-AAACCGGTGAACAGCTCCTCGCCCC-3’. The resulting plasmids were named pBK189 and pBK198, respectively.