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A simple approach to generate transgenic cell lines with high efficiency using CRISPR and flippase-mediated recombination

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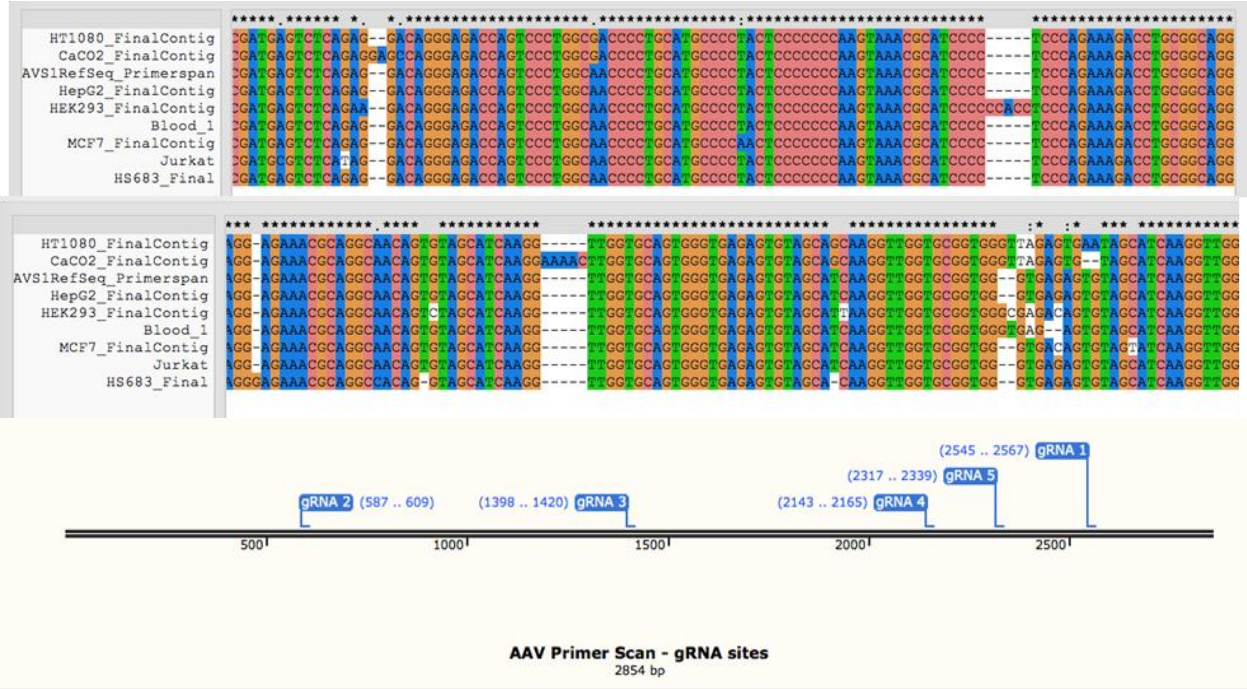
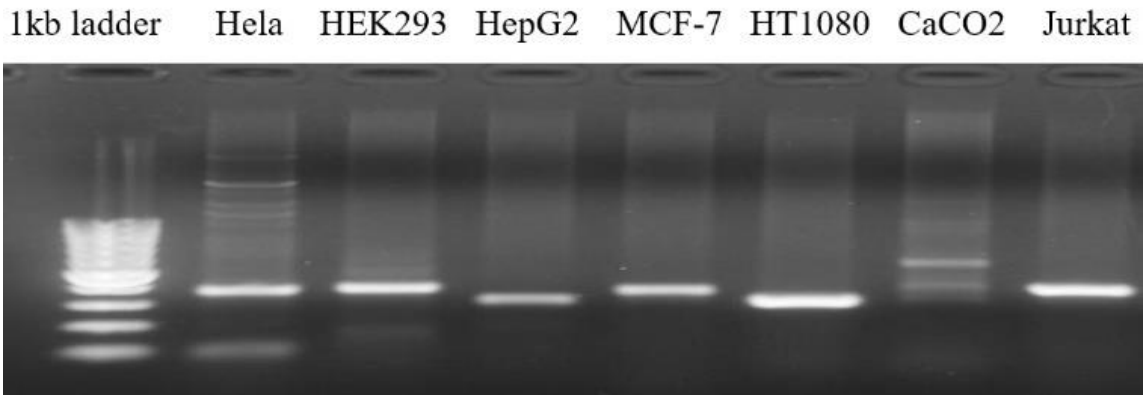
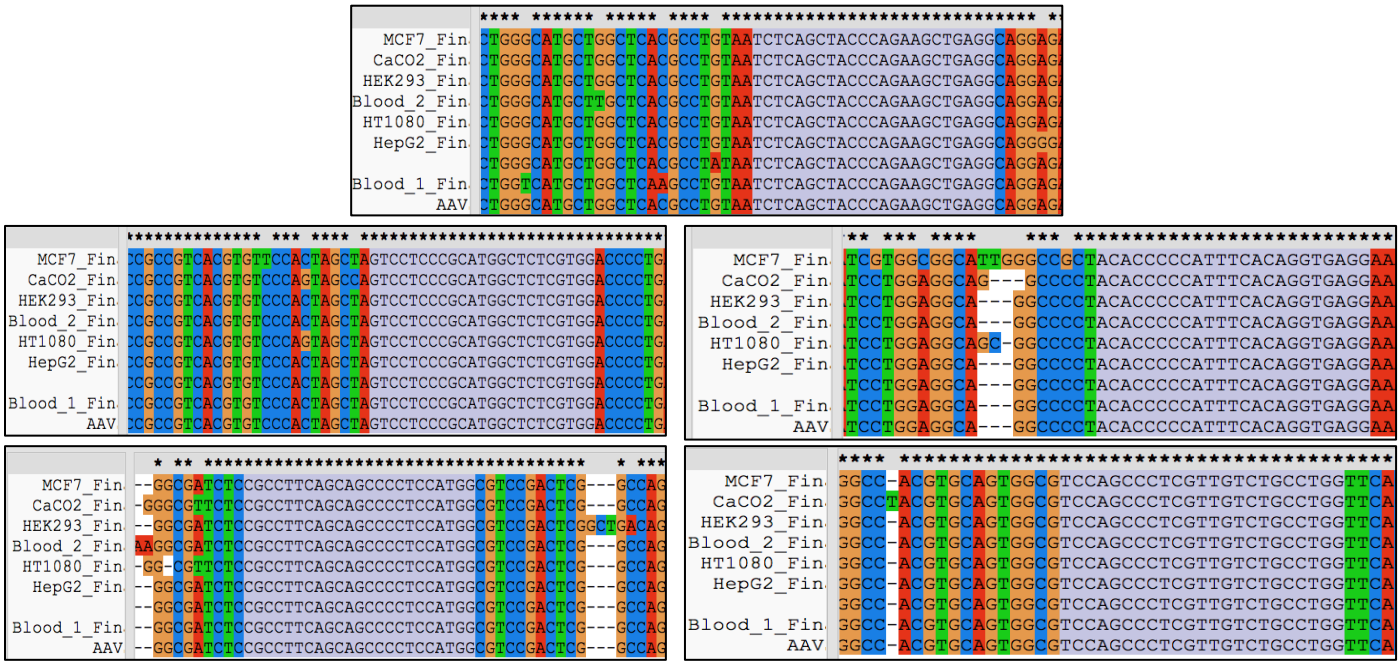
SUPPLEMENTARY MATERIAL



Supplementary Figure S1: Determination of homologous regions in chosen cell lines Hela, HEK293, HepG2, MCF-7, HT1080, CaCO2, Jurkat respectively.

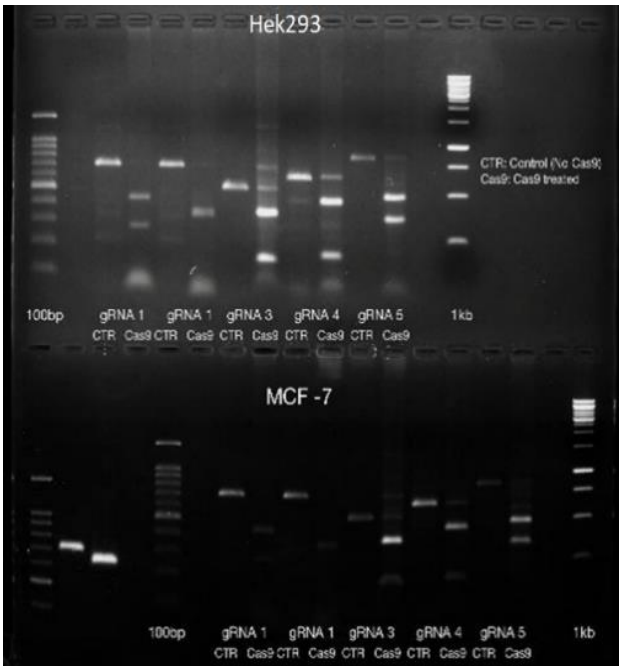


Supplementary Figure S2. Gel depicting the PCR amplicon of AAVS1 region across all the seven cell lines namely, Hela, HEK293, HepG2, MCF-7, HT1080, CaCO2, Jurkat respectively.

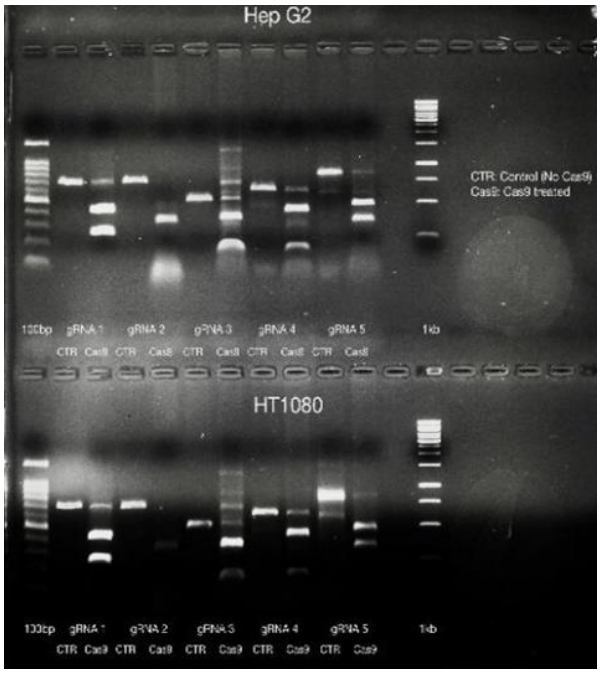


Supplementary Figure S3: The designing of 5 gRNAs from AAVS1 consensus of all cell lines determined from multiple sequence alignment.

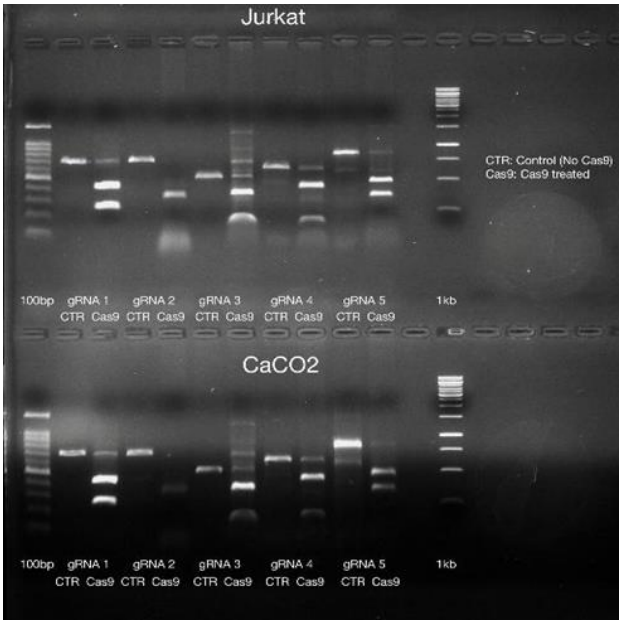
A.



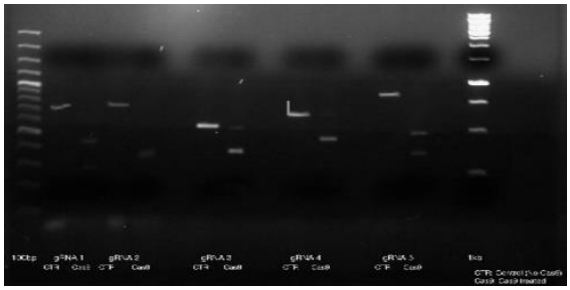
B.



C.

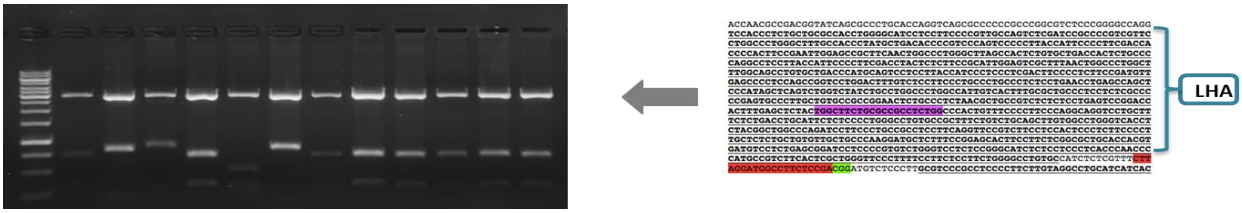


D.

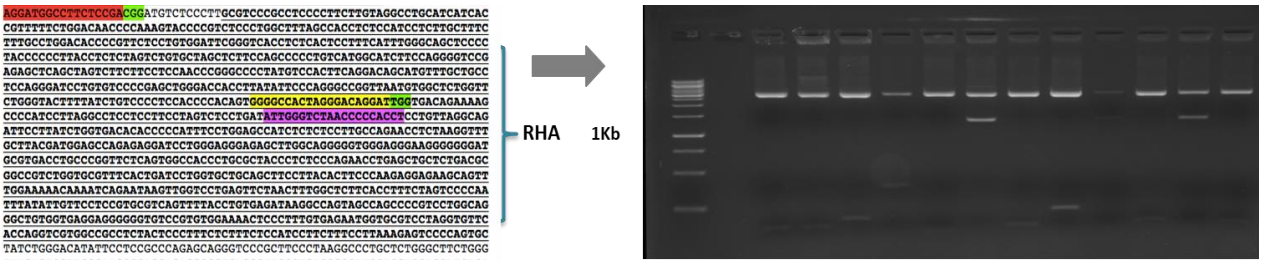


Supplementary Figure S4: Validation of the presence of all 5 gRNAs using Guide-It assay where, their presence is detected by the cleavage efficiency of host genome in A. HeK293 and MCF-7 B. HepG2 and HT1080 C. Jurkat and CaCO2 D. Hela cell lines. CTR: Untreated gRNA target; Cas9: Cas9 treated gRNA target showing cleavage

A.

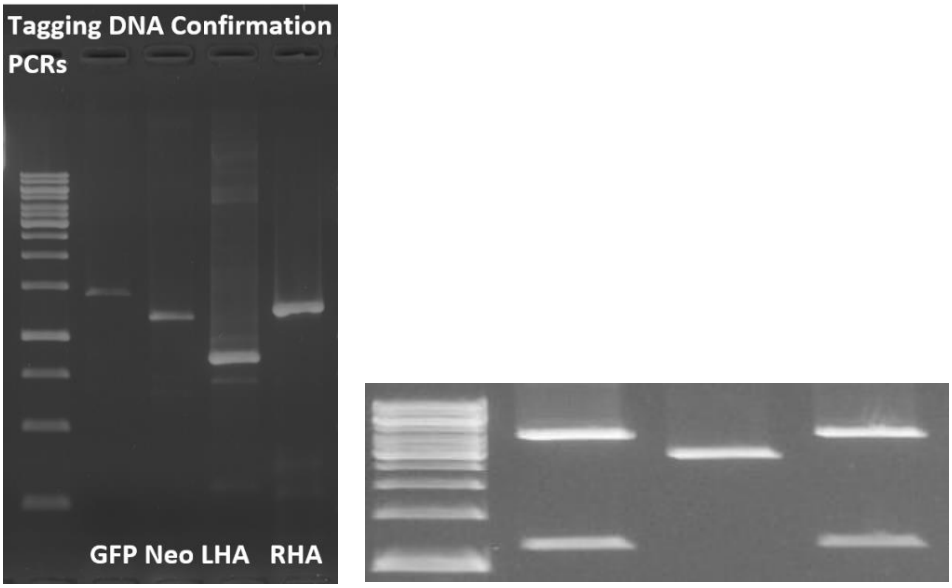


B.

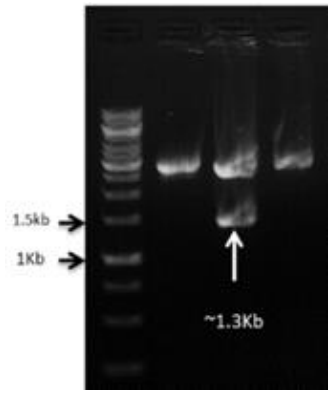


Supplementary Figure S5: A. Confirmation of the LHA (~1kb) cloned in tagging plasmid using restriction digestion using EcoRI and KpnI. B. Confirmation of the RHA (~1kb) cloned in tagging plasmid +LHA using restriction digestion using PacI and HindIII respectively.

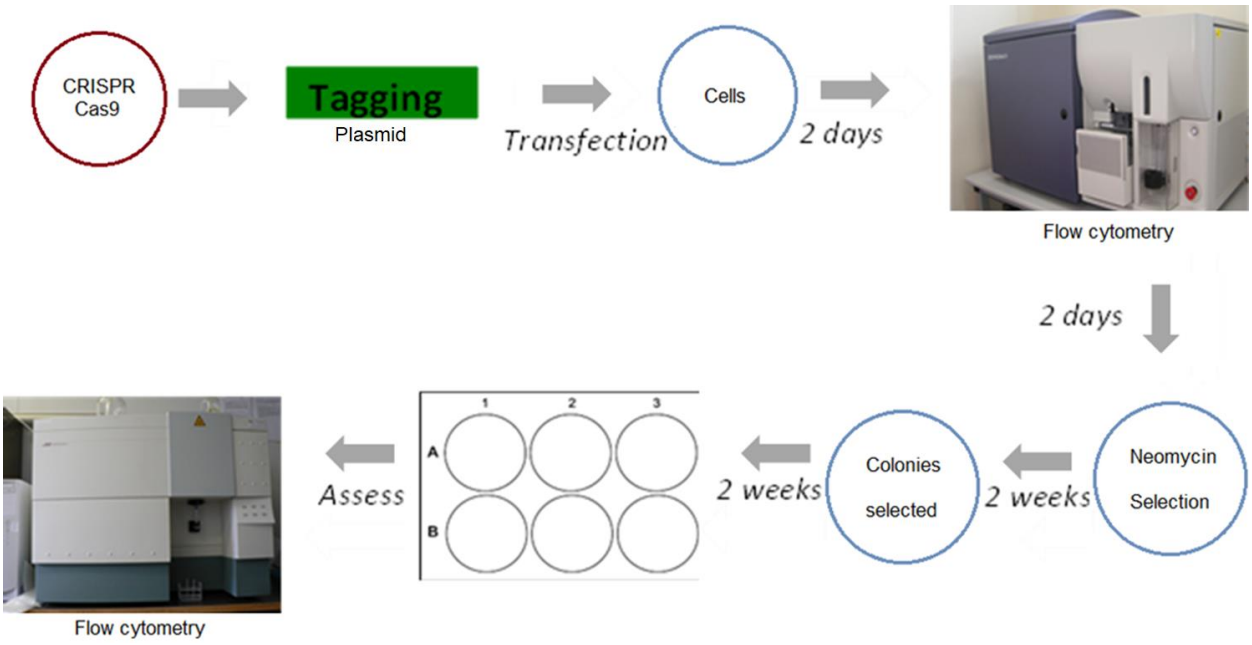
A. B.



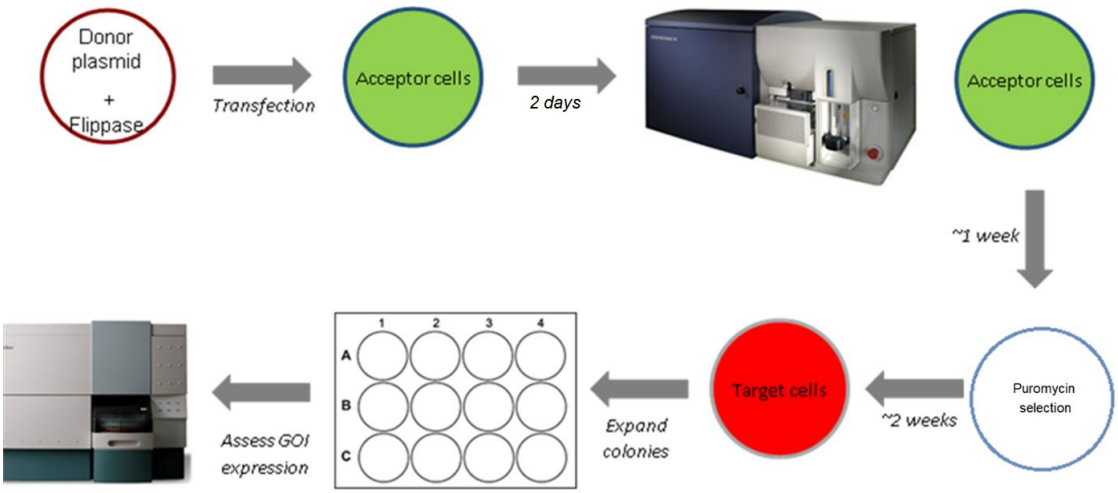
Supplementary Figure S6: A. PCR confirmation of the regions cloned inside tagging plasmid. B. Confirming the presence of Neomycin marker, SV40 polyA in the vector using restriction digestion.



Supplementary Figure S7: A. restriction digestion confirmation of mCherry and puromycin in the donor plasmid using NheI and BstBI.

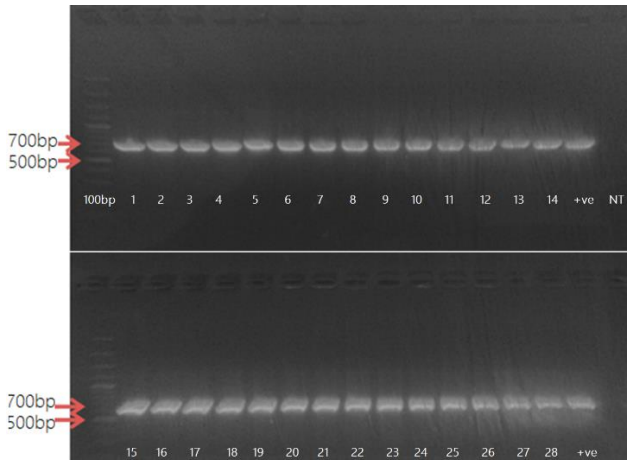


Supplementary Figure S8: Flowchart depicting the time and steps taken for generating acceptor cell lines.

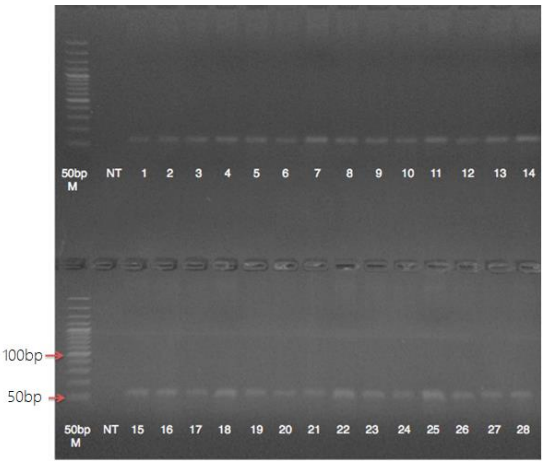


Supplementary Figure S9: Flowchart depicting the time and steps taken for generating target cell lines.

A



B



Supplementary Figure 10: A. PCR showing stable integration of mCherry in HEK293-mCherry (Target cell line) for 28 passages. B. GAPDH (Housekeeping gene) of HEK293-mCherry for 28 passages.

Primer name	Sequence (5'→3)
AAVS1.Reg.1.F	TTAGCCACTCTGTGCTGACC
AAVS1.Reg.1.R	AGGTGGGGGTTAGACCCAAT
gRNA1FP	CACCTCTCAGCTACCCAGAAGCTGAGG
gRNA1RP	AAACCCTCAGCTTCTGGGTAGCTGAGA
gRNA2FP	CACCGTCCTCCCGCATGGCTCTCGTGG
gRNA2RP	AAACCCACGAGAGCCATGCGGGAGGAC
gRNA3FP	CACCACACCCCCATTTCACAGGTGAGG
gRNA3RP	AAACCCTCACCTGTGAAATGGGGGTGT
gRNA4FP	CACCCGCCTTCAGCAGCCCCTCCATGG
gRNA4RP	AAACCCATGGAGGGGCTGCTGAAGGCG
gRNA5FP	CACCTCCAGCCCTCGTTGTCTGCCTGG
gRNA5RP	AAACCCAGGCAGACAACGAGGGCTGGA
Kpn1 FRT1 FP	GGGTACCGGCGCGCCGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGATAGTATAGG
Sal1RP	GCGTCGACAGGGCCGGGATTCTCTTCGACATCTCCACATGTCAGGAGG
Sal1FP	GCGTCGACATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCC
FRT2 1st RP	CCTTTTGAAGAATAGGAACTTCGGAATAGGAACTTCTAAGATACATTGATGAGTTTGG
FRT2 2nd RP	AAGCTTGGCCGGCCTTAATTAAGAAGTTCCTATACCTTTTGAAGAATAGGAACTTCG
AAV_LHA_FP	CGGAATTCTCCACCCTCTGCTGCGCCACC
AAV_LHA_RP	ATGGTACCGAGCTCGCACAGGCCCCAGAAGG
AAV_RHA_FP	GCTTAATTAAGCGTCCCGCCTCCCCTTCTTGTAGGCC
AAV_RHA_RP	AGGGCCGGCCGCACTGGGGACTCTTTAAGG
Puro_SalI_FP	ATGTCGACACCGAGTACAAGCCCACGGTGCGC
Puro_BstBI_RP	GCTTCGAATCAGGCACCGGGCTTGCGGGTC
NheI_mCherry_FP	ATGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGA
mcherry_RP1	GCAGGTGAGCAGGCTTCCCCTGCCCTCCTACTTGTACAGCTCGTCCATGC
mCherry_RP2_BamHI	TAGGATCCTGGGGCGGGGCCAGGGTTTTCCTCCACGTCGCCGCAGGTGAGCAGGCTTC

Supplementary Table 1: Sequences of the primers used for constructing transgenic cell lines