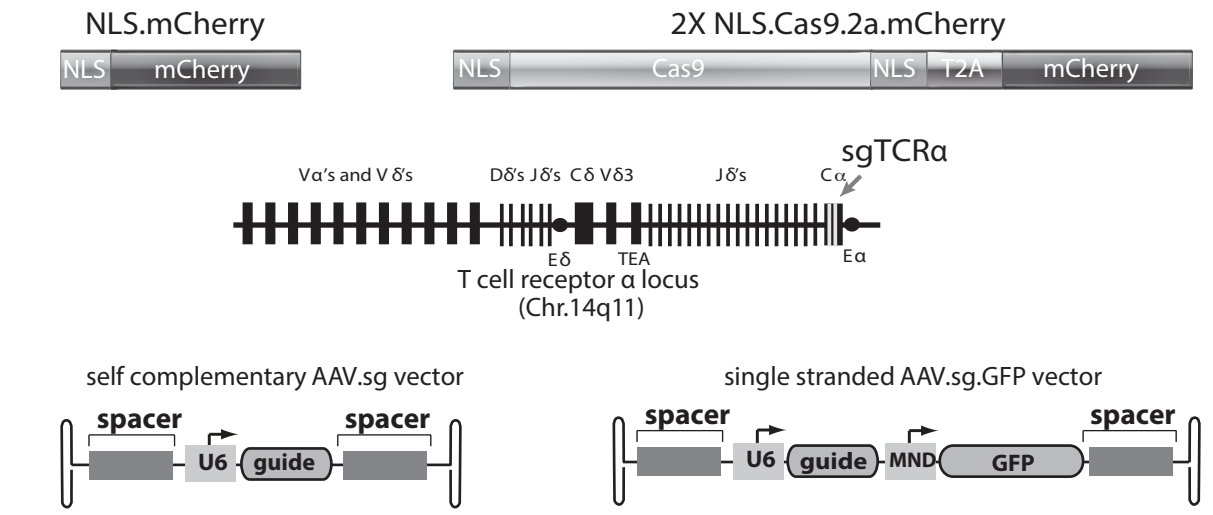
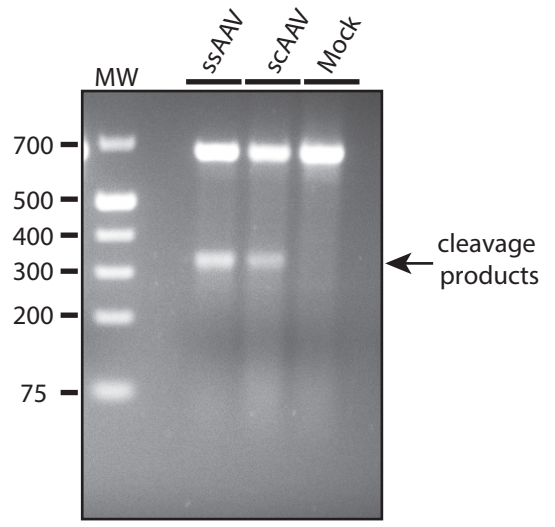
Supplementary Figure 1

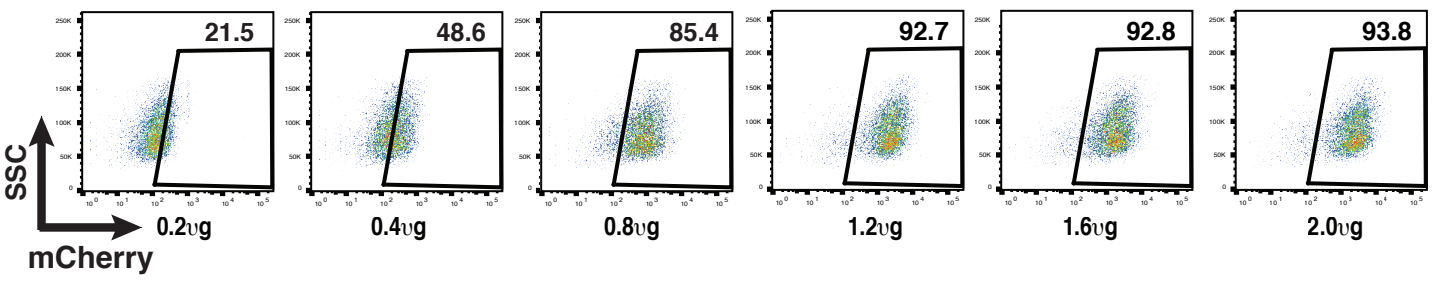
a **Schematic of mRNA and AAV vector constructs used for TCR locus targeting**



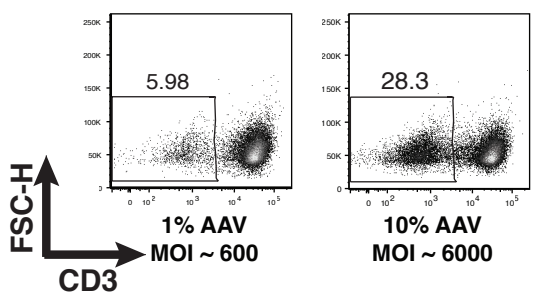
b **T7 assay of editing-induced indels at TCRa locus**



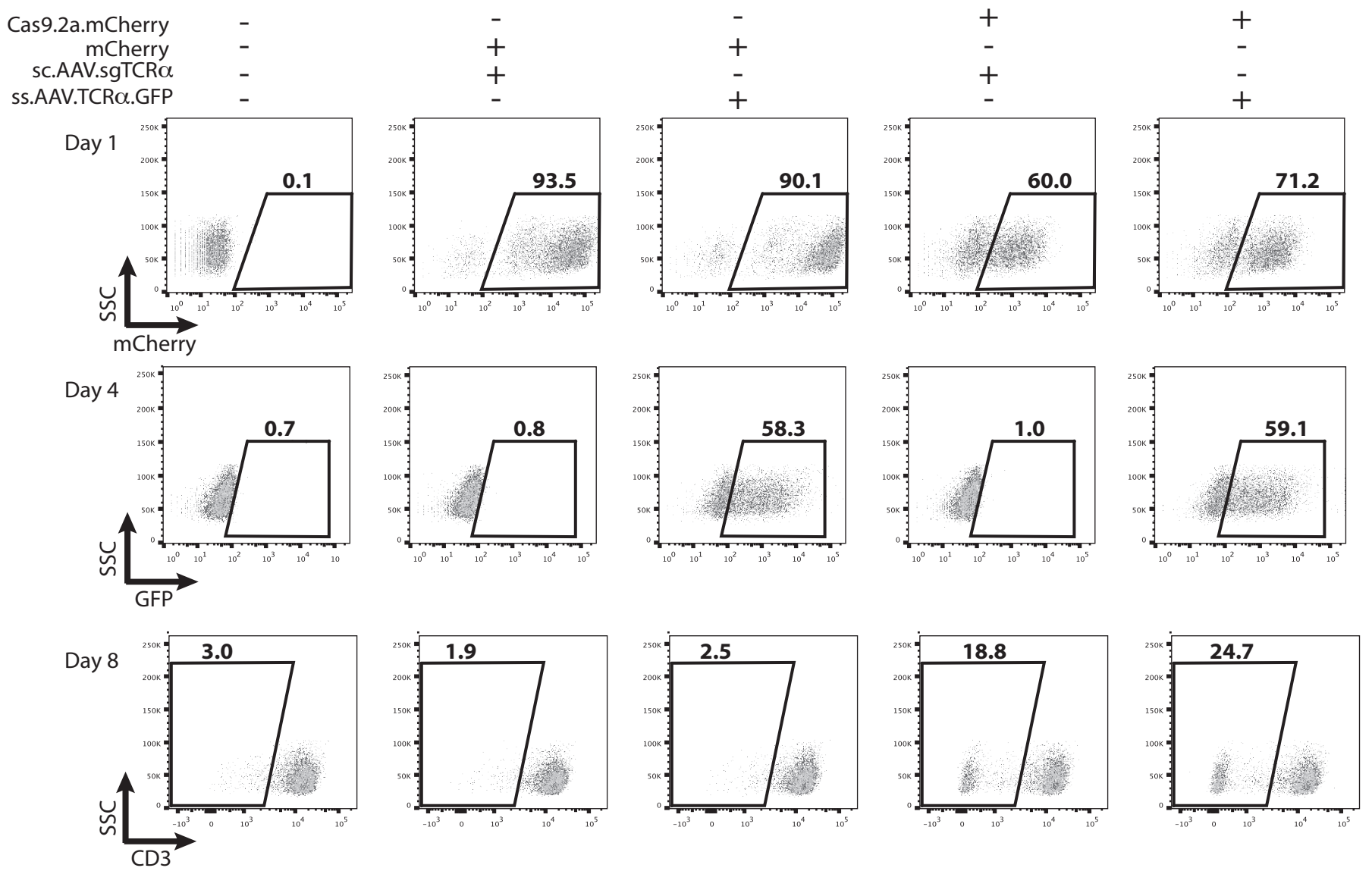
c **Effect of Cas9-T2A-mCherry mRNA dose on expression at 24h post transfection**



d **Increasing AAV dose increases TCRα knockout at day 7 post electroporation/transduc-**

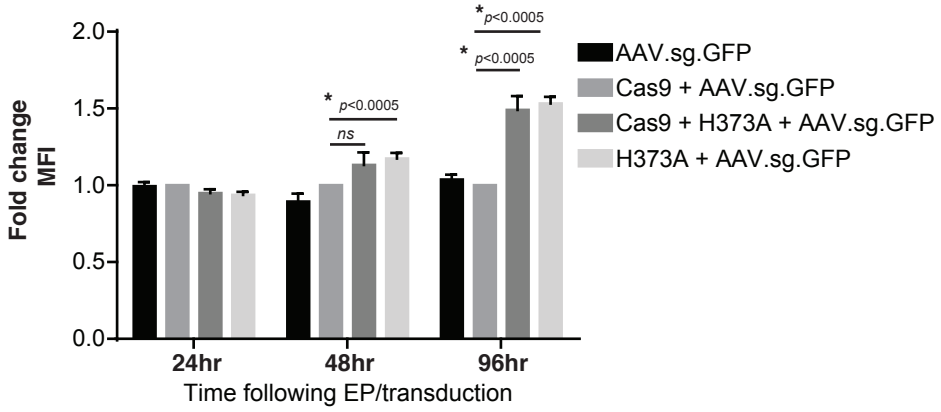


e **Flow cytometry comparison of TCRα KO with single stranded (ss) vs. self complementary (sc) AAV for guide expression**



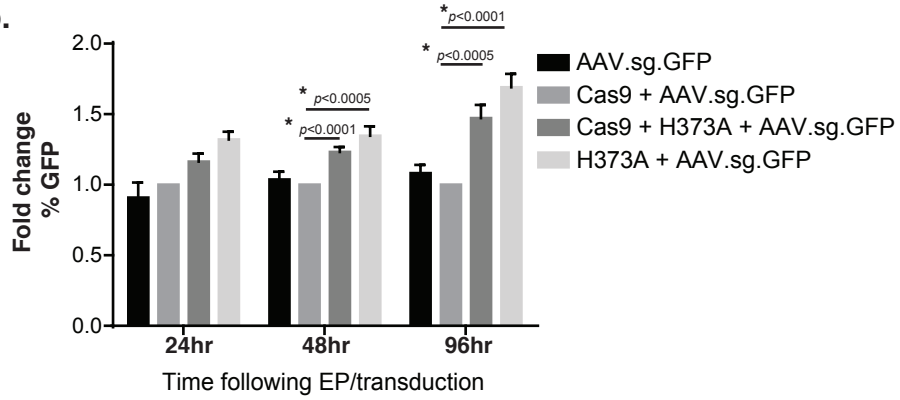
Supplementary Figure 2

a.



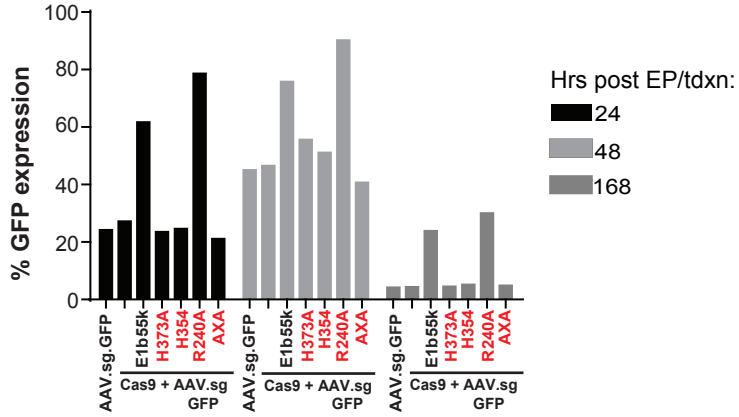
E4orf6/E1b55k-H373 expression significantly increases AAV-driven GFP expression at 48 and 96 hours

b.



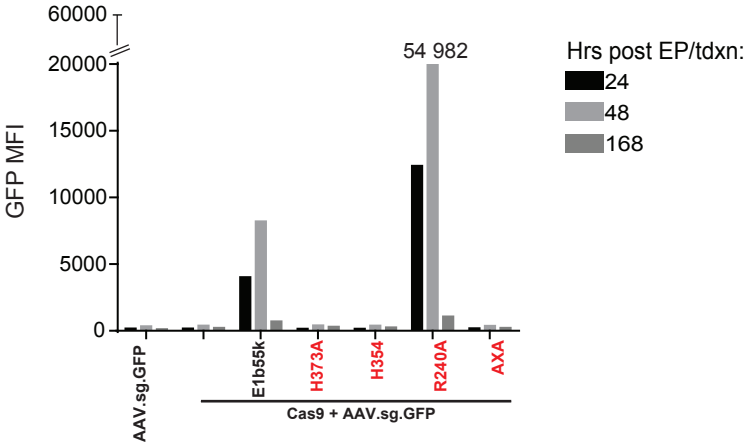
E4orf6/E1b55k-H373 expression significantly enhances AAV transduction at 48 and 96 hours

c.



Comparison of E1b55k, E4orf6 mutants effects on AAV transduction

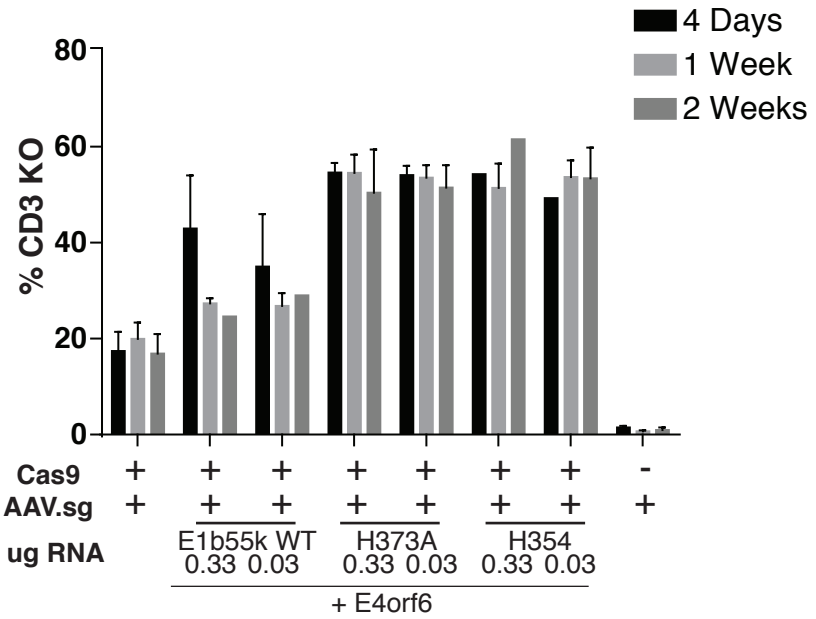
d.



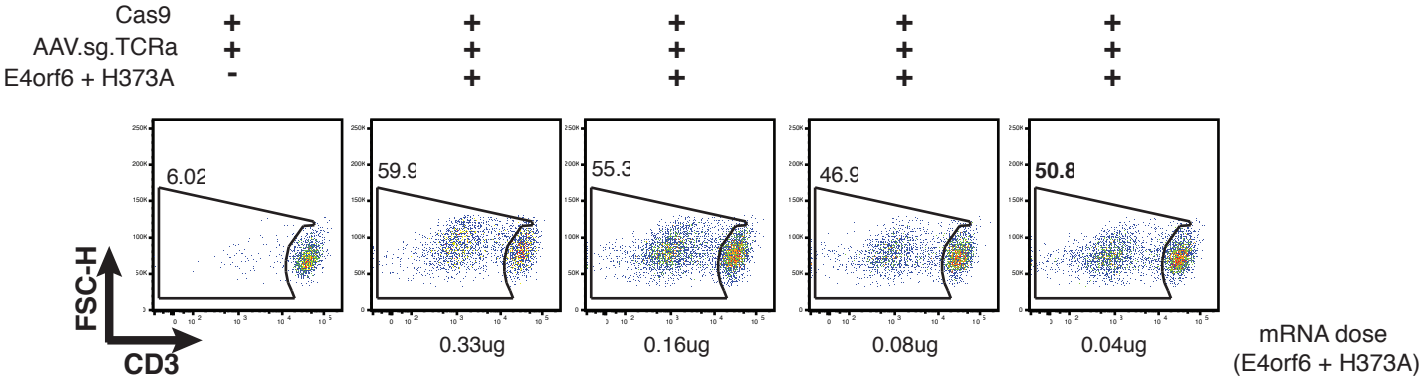
Comparison of E1b55k, E4orf6 mutants effects on AAV-driven GFP expression

Supplementary Figure 3

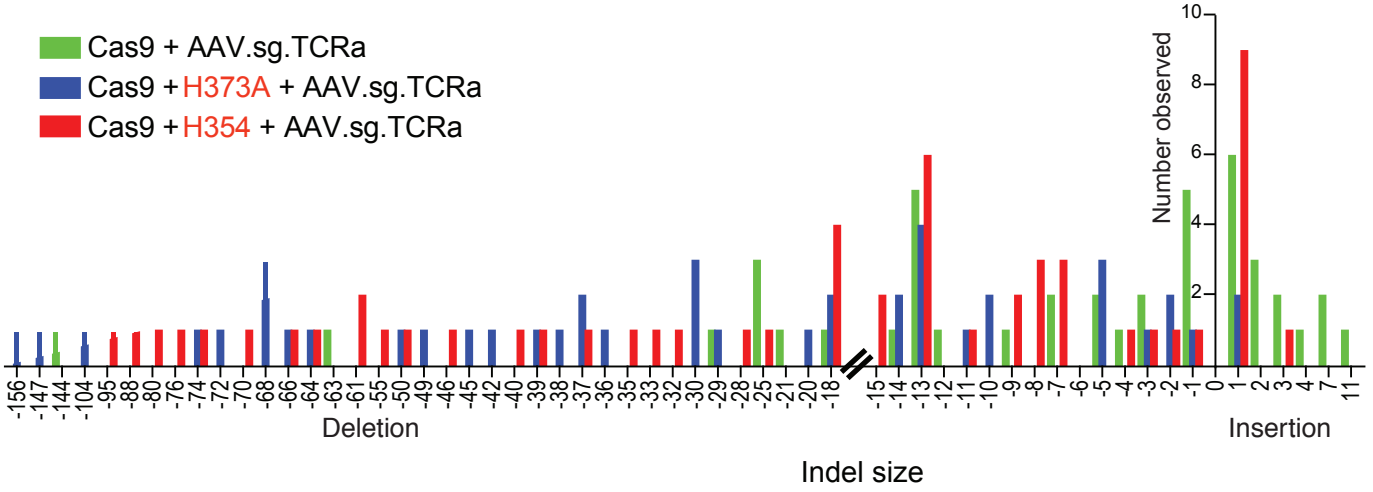
a **Stability of CD3 loss following editing with the indicated Cas9/E4orf6 + E1b55k protein combinations**



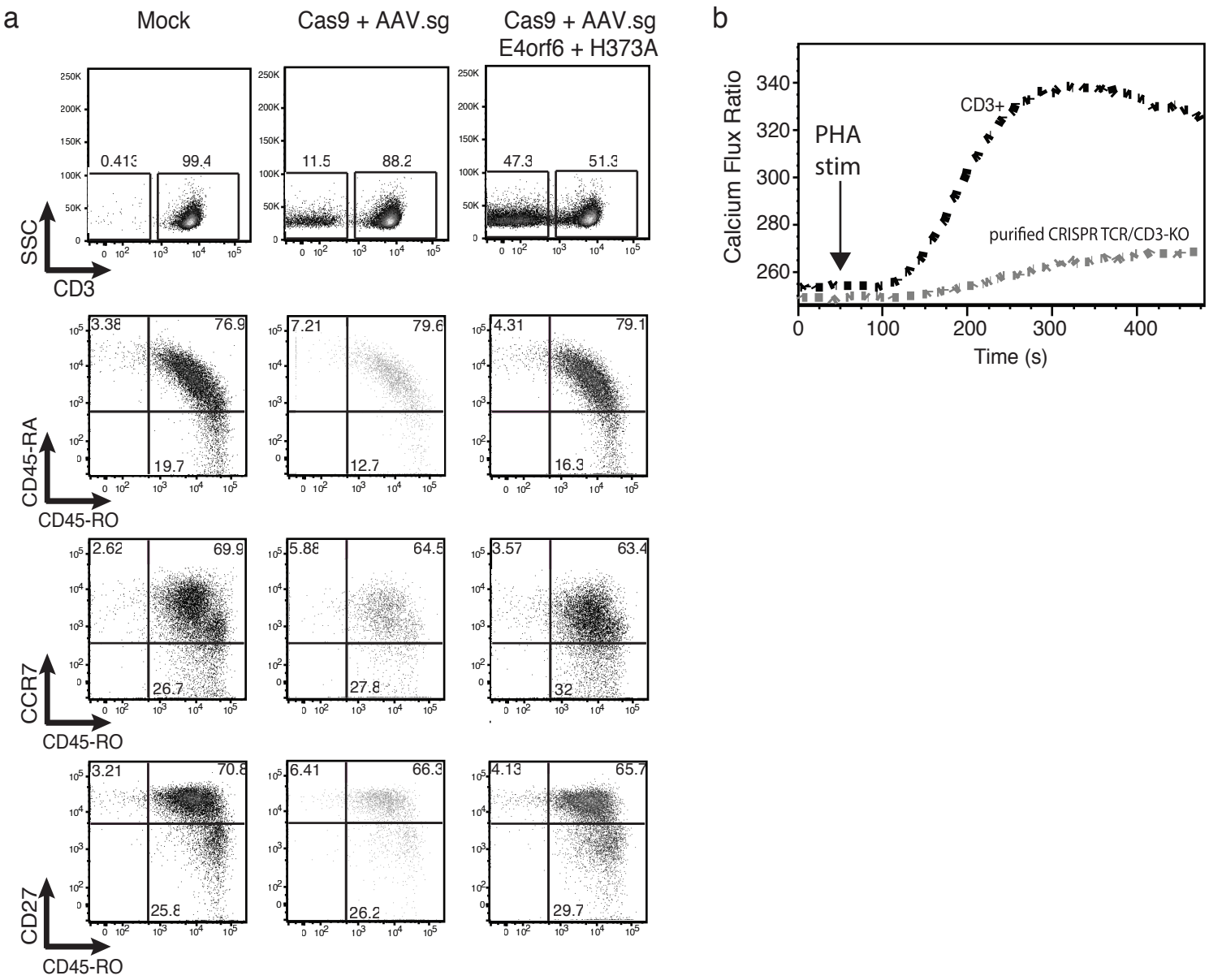
b **Dose response of E4orf6/E1b55k H373A mutant expression on rate of CD3 KO**



c **Indel sizes observed following editing with the indicated Cas9/E4orf6 +E1b55k mutant protein combinations**



Supplementary Figure 4

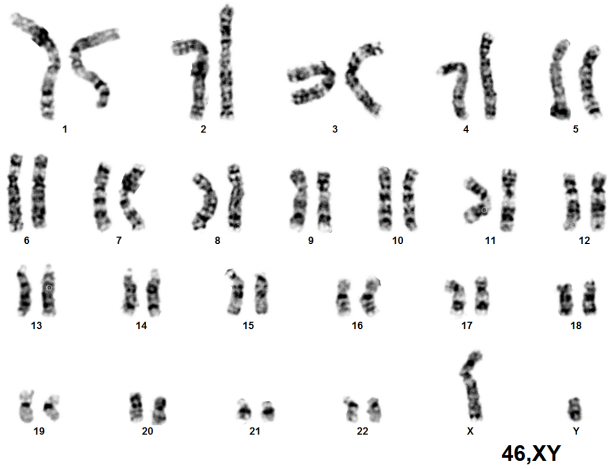


Supplementary Figure 5

T cells that have undergone CRISPR multiplex knockout with E4orf6/E1b55k-H373A display a normal karyotype

a

b

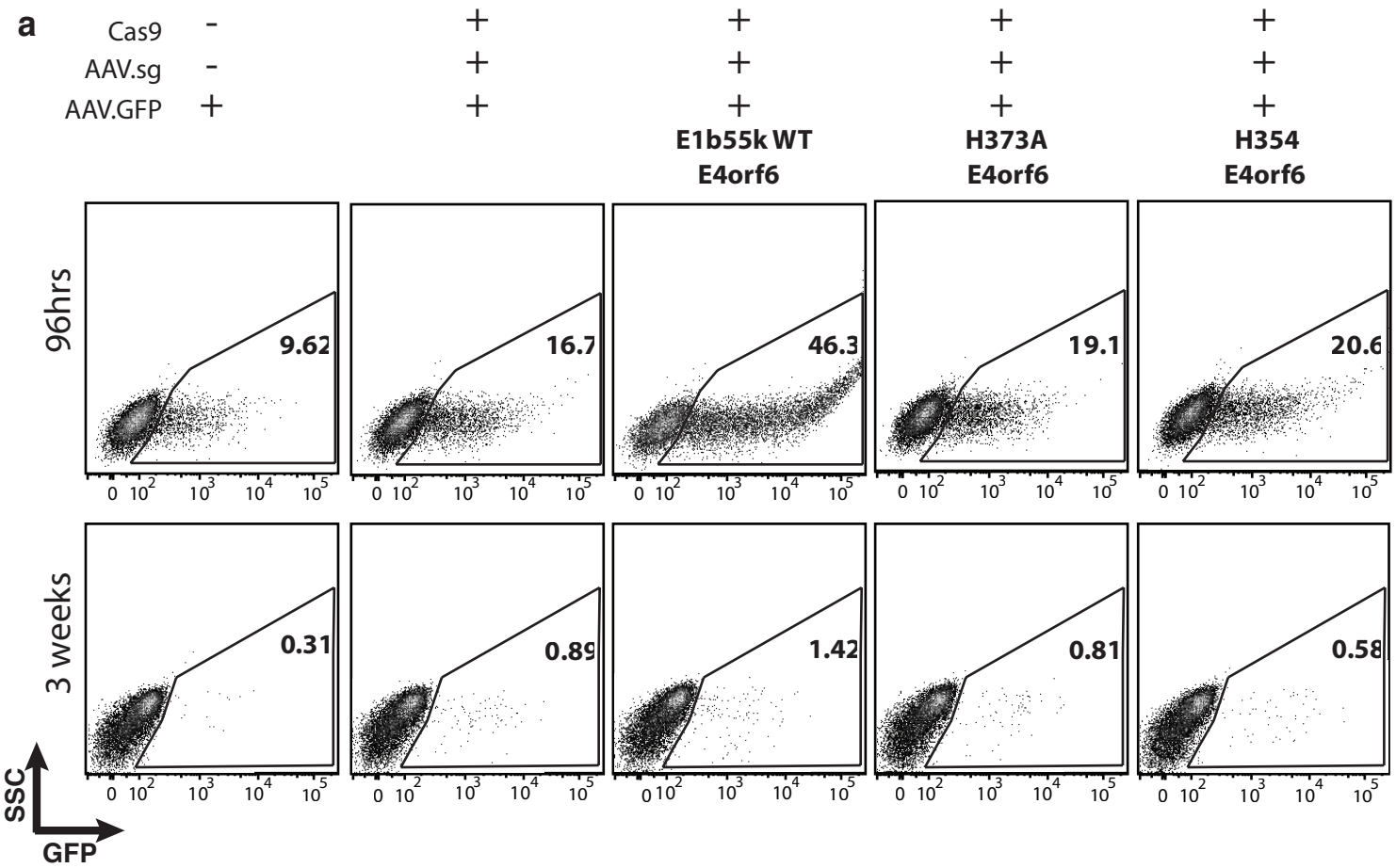


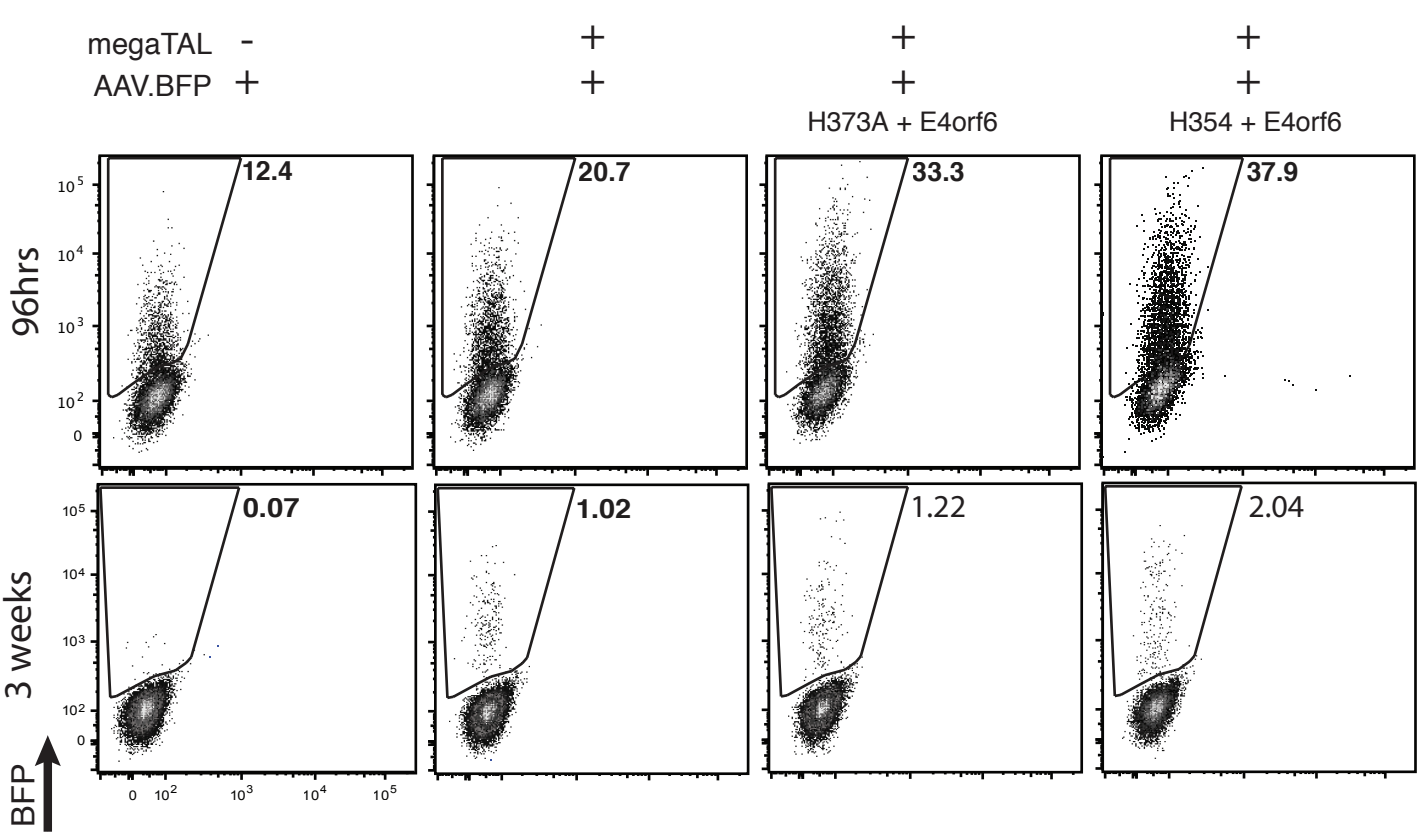
c Table 1. Summary data of karyotype results

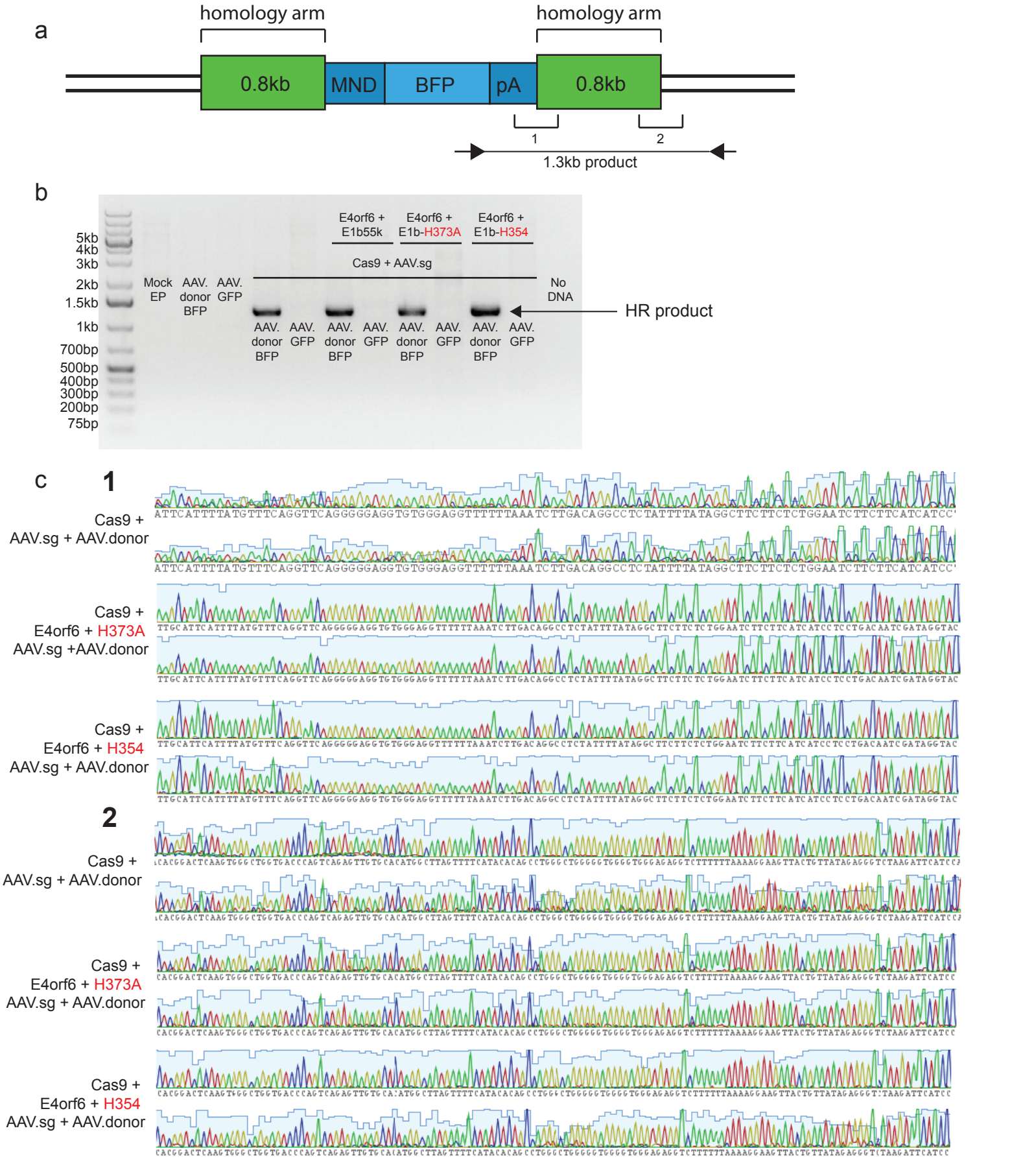
Sample	Band Level	Metaphase cells analyzed	Abnormalities detected (Y/N)
Untreated	400-450	20	N
AAV.sgTim3.GFP	300-450	20	N
AAV.sgTim3.sgTCRa.GFP	300-400	20	N
Cas9 alone	300-550	20	N
Cas9 + AAV.sgTim3.GFP	300-450	20	N
Cas9 + AAV.sgTim3.sgTCRa.GFP	450-650	20	N
Cas9 + E4orf6/E1b55k-H373A + AAV.sgTim3.GFP	300-400	20	N
Cas9 + E4orf6/E1b55k-H373A + AAV.sgTim3.sgTCRa.GFP	300-450	20	N

Supplementary Figure 6

Assessment of rate of stable integration of AAV-fluorophore lacking homology arms at CCR5 loci





Supplementary Figure 7

Molecular confirmation of HDR products