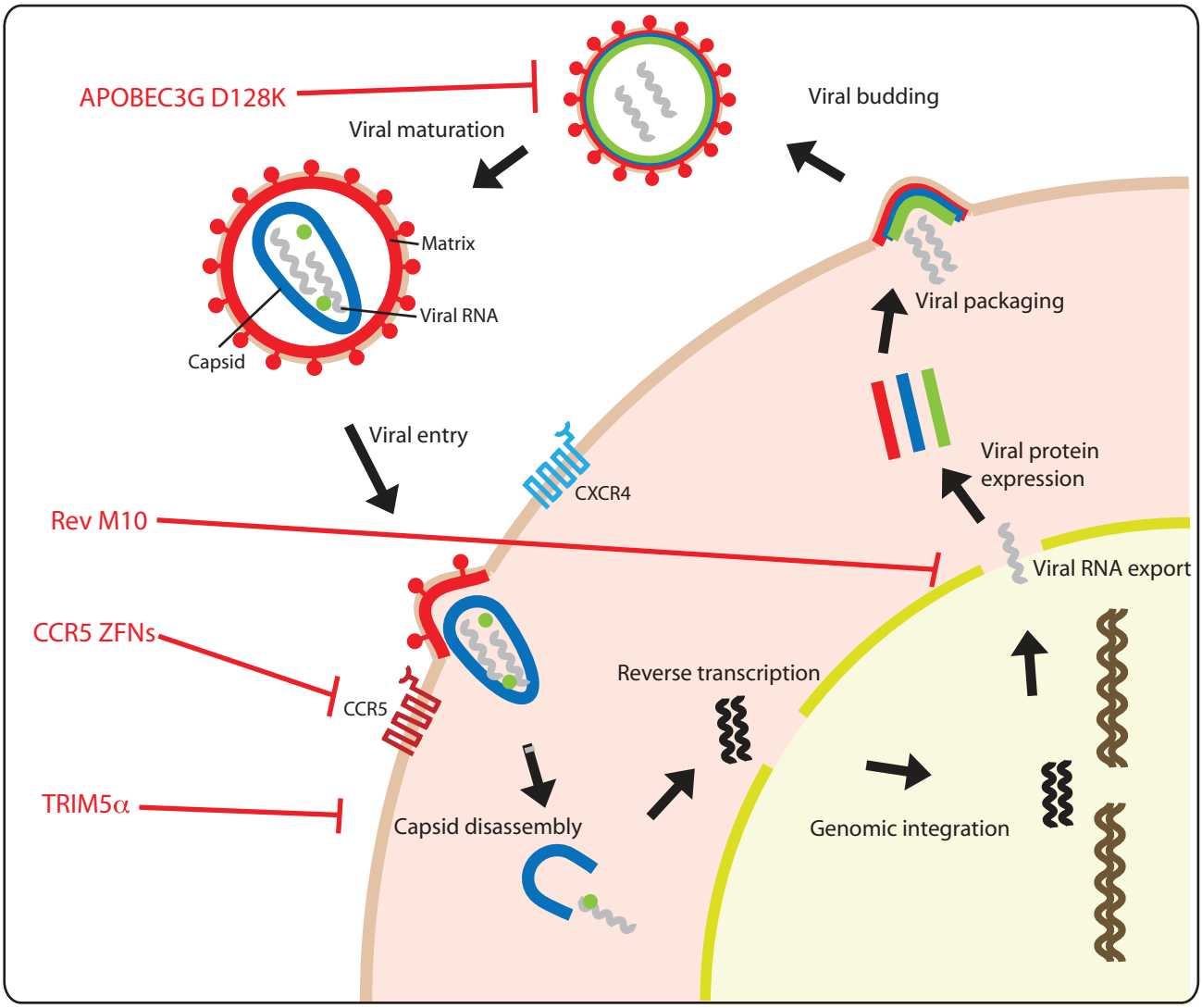
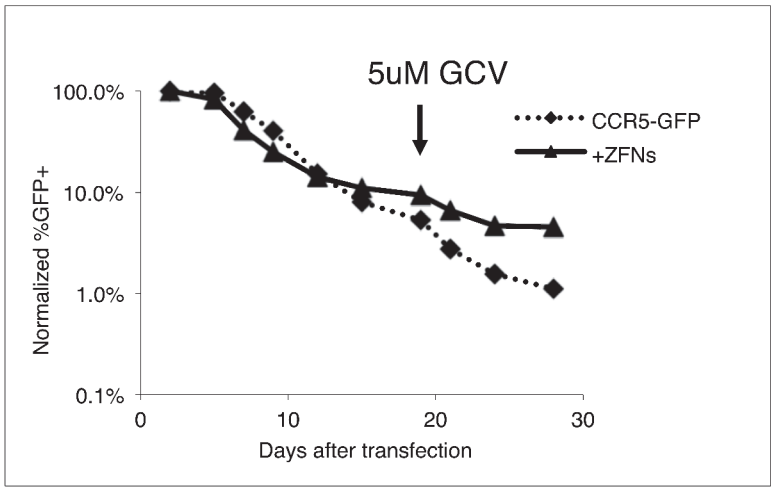
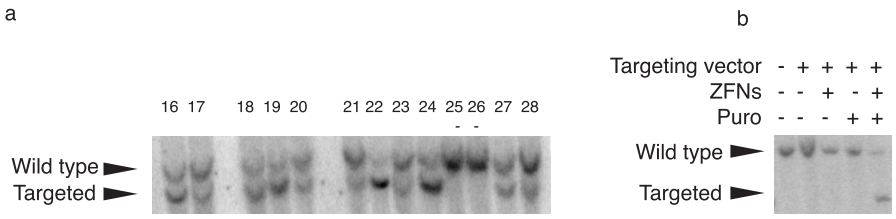


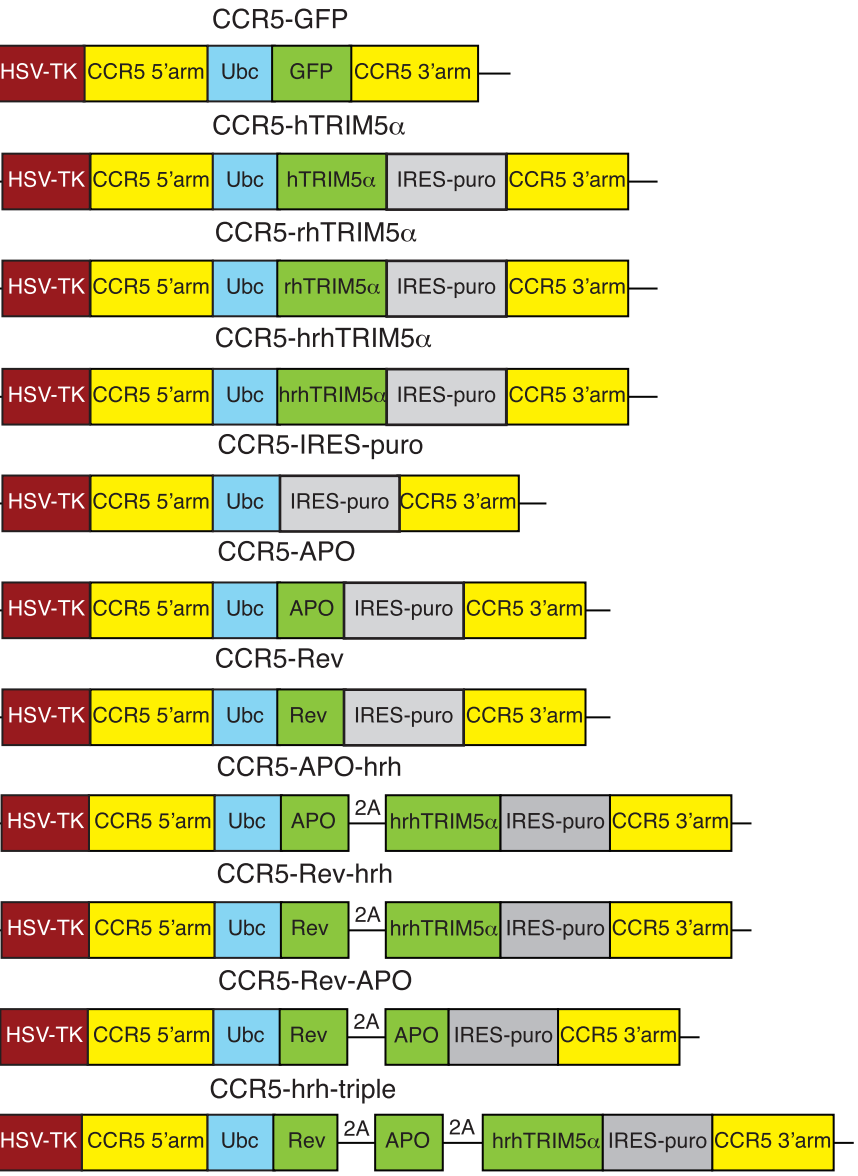
Supplementary Figure S1: Disruption of the HIV lifecycle by targeted gene therapy. Current pharmacological therapies simultaneously attack multiple stages of the HIV lifecycle. Similarly, we have designed a ZFN-based targeting strategy to insert into the CCR5 locus the genes APOBEC3G D128K, Rev M10, and human-rhesus TRIM5α. In doing so, we will stack genetic resistance to both R5-tropic and X4-tropic HIV.



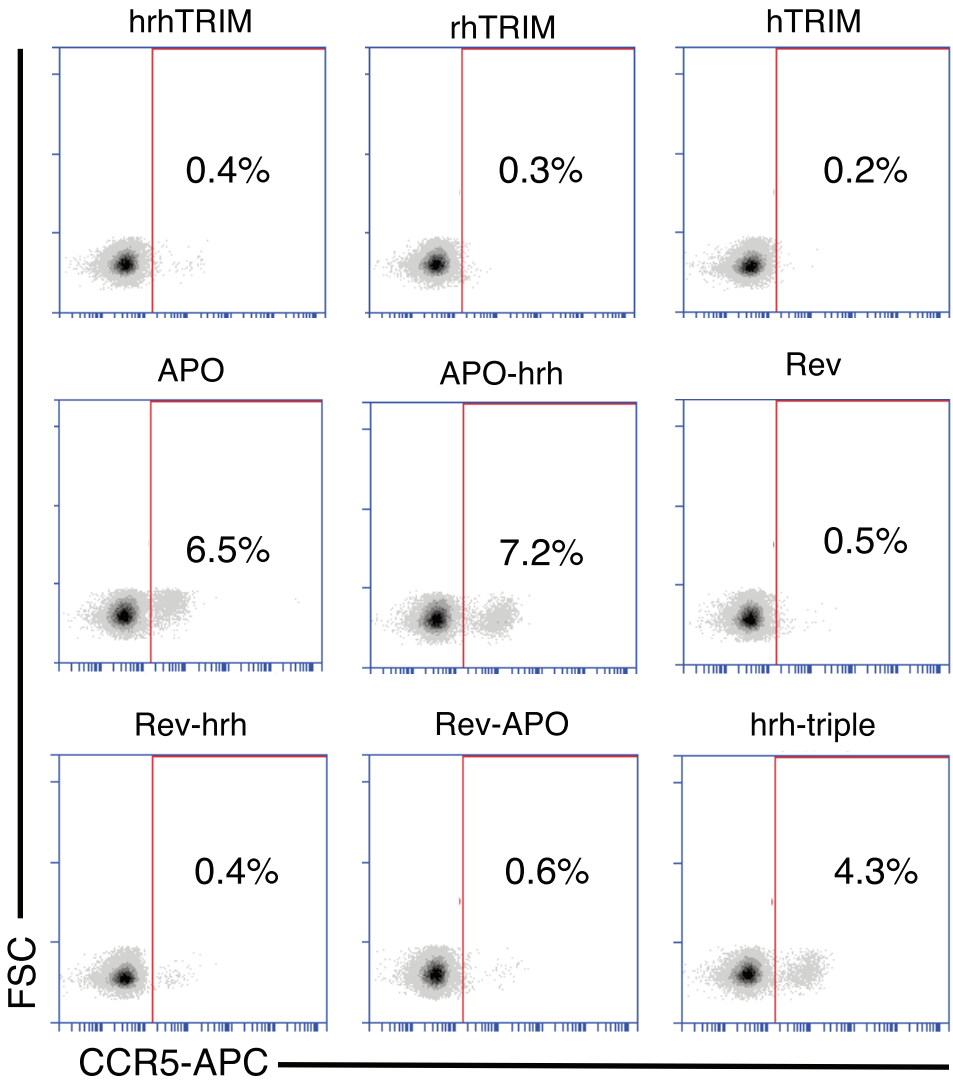
Supplementary Figure S2: Negative selection with ganciclovir enriches for targeted cells. On day 19 after transfection with or without ZFNs and with CCR5-GFP, random integrants that preserved the HSV-TK domain were killed by the addition of 5μM ganciclovir. Ganciclovir killed 2.5 fold more cells in the –ZFN sample, indicating a 2.5 fold enrichment of targeted to untargeted cells in the +ZFN sample.



Supplementary Figure S3: Clonal analysis of K562 cells targeted at CCR5. (**a**) Analysis of 13 additional clones targeted at CCR5 with the CCR5-GFP targeting vector using CCR5 probe, as in **Figure 1g.** (**b**) Southern blot analysis of populations of K562s treated with and without the CCR5-GFP targeting vector, ZFNs and puromycin (puro), as indicated.



Supplementary Figure S4: CCR5 targeting vectors. Schematic representations of CCR5 targeting vectors used in this study. (HSV-TK – herpes simplex virus thymidine kinase, Ubc – ubiquitin C promoter, hTRIM5α – human TRIM5α, rhTRIM5α – rhesus TRIM5α, hrhTRIM5α – human-rhesus hybrid TRIM5α, Rev – Rev M10, APO – APOBEC3G D128K, IRES-puro – internal ribosome entry site – puromycin N-acetyltransferase, 2A – 2A translation skipping peptide).



Supplementary Figure S5: CCR5 expression in targeted cell lines. ZFN-mediated disruption of CCR5 measured by antibody staining and FACS. Numbers indicate percent of cells with functional CCR5 expression. See also Figure 2d.