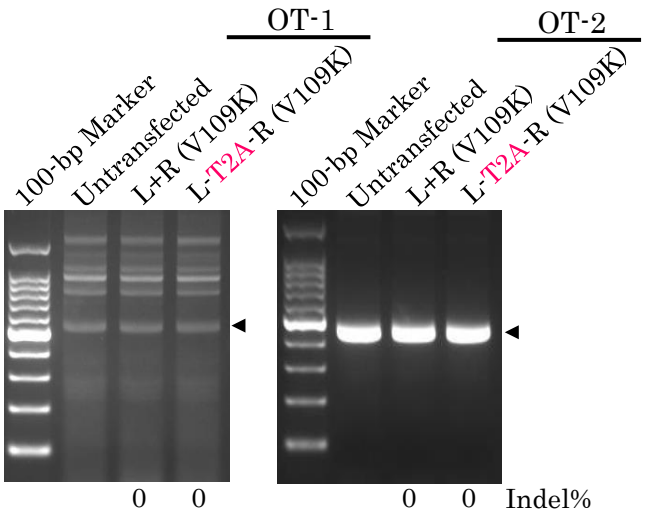
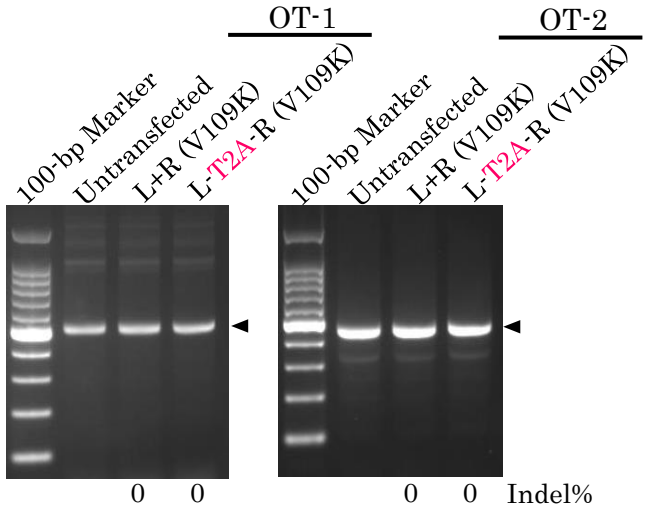
Figure S1 (Katayama et al.)

(a)



(b)



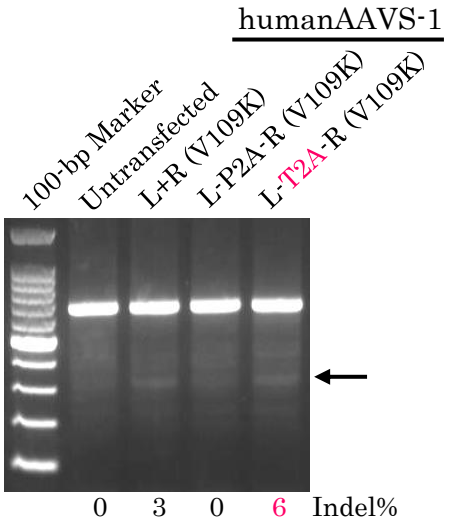
(c)

Target gene	Prototype	Name	Sequence (Top strand: 5' to 3', Bottom strand: 3' to 5')	Coordinate	Genomic region	Mutation	Identities (%)
Human AAVS-1	2027L+R	On-target	CAG CGC CCC CCG CCC GGC GTCTCC CGG GGC CAG GTC CAC CCT GTC GCG GGG GGC GGG CCG CAGAGG GCC CCG GTC CAG GTG GGA	Chr19: 55117179-55117220	Intron	-	-
		OT1	CAG CGC CCC CCG CCC GGC CGCCGC TCG GAG CCA CCG CCC GCC GTC GCG GGG GGC GGG CCG GCGGCG AGC CTC GGT GGC GGG CGG	Chr3: 46882282-46882323	Intergenic	Not detected	66.7
		OT2	TCT GGA GGA CGG GGA GGC AGCCCC AGG GGC CAG GTC CAC CCT AGA CCT CCT GCC CCT CCG TCGGGG TCC CCG GTC CAG GTG GGA	Chr8: 143876342-143876383	Intron	Not detected	63.9
	9299L(v2)+R	On-target	CCC GTT GCC GTC TCG ATG CGCCCC GTC GTT CCT GGC CCT GGG GGG CAA CGG CAG AGC TAG GCGGGG CAG CAA GGA CCG GGA CCC	Chr19: 55117107-55117149	Intron	-	-
		OT1	GCC GTT GCC GTC TAG ATC CAGCCG ATA AAG GGC TGG GCT GGC CGG CAA CGG CAG ATC TAG GTCGGC TAT TTC CCG ACC CGA CCG	Chr7: 28956967-28957008	Exon	Not detected	61.1
		OT2	GCA TAG GAT TGC CAA GTA CCAGGA CTC GTT CCT GGC CCT GGA CGT ATC CTA ACG GTT CAT GGTCCT GAG CAA GGA CCG GGA CCT	Chr12: 78280388-782803429	Intergenic	Not detected	55.6

Figure S1. Off-target analysis of ZF-ND1s. (**a, b**) T7E1 assay. A gel image of T7E1-treated PCR products amplified from the target sites. The arrowheads indicate the corresponding band for the target site. OT1 and 2 indicate the off-target candidate sites 1 and 2, respectively. The cells untransfected and transfected with L+R (V109K) or L-T2A-R (V109K) (**a**) were subjected to a T7E1 assay. The cells untransfected and transfected with L(v2)+R or L(v2)-T2A-R (**b**) were subjected to a T7E1 assay. (**c**) Summary of an off-target analysis of ZF-ND1s. The red and blue letters indicate left and right ZF-ND1 target sequences, respectively. Mismatches are underlined. Identities were calculated as follows: ((the number of match sequences) / 36) × 100.

Figure S2 (Katayama et al.)

(a)



(b)

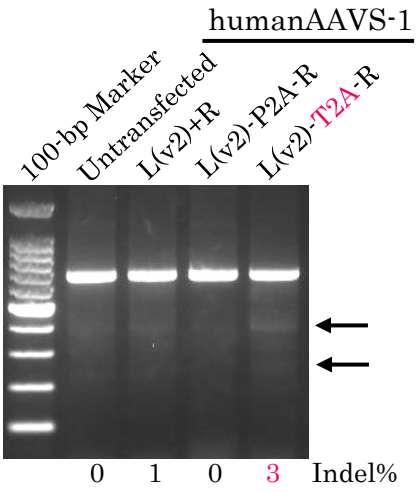


Figure S2. 2A-coupled co-expression of two ZF-ND1 monomers at a lower dose. (**a, b**) The T7E1 assay. Gel images of T7E1-treated PCR products amplified from the target sites. Arrowheads indicate the cleaved DNA band. (**a**) L+R (V109K) indicates the separately expressed ZF-ND1 monomers. L-P2A-R (V109K) and L-T2A-R (V109K) indicate the P2A- and T2A-coupled ZF-ND1 monomers, respectively. The total amount of transfected plasmid DNA: L+R (V109K), 50 ng; L-P2A-R (V109K), 50 ng; L-T2A-R (V109K), 50 ng. (**b**) L(v2)+R indicates the separately expressed ZF-ND1 monomers. L(v2)-P2A-R and L(v2)-T2A-R indicate the P2A- and T2A-coupled ZF-ND1 monomers, respectively. The total amount of transfected plasmid DNA: L(v2)+R, 50 ng; L(v2)-P2A-R, 50 ng; L(v2)-T2A-R, 50 ng.

Figure S3 (Katayama et al.)

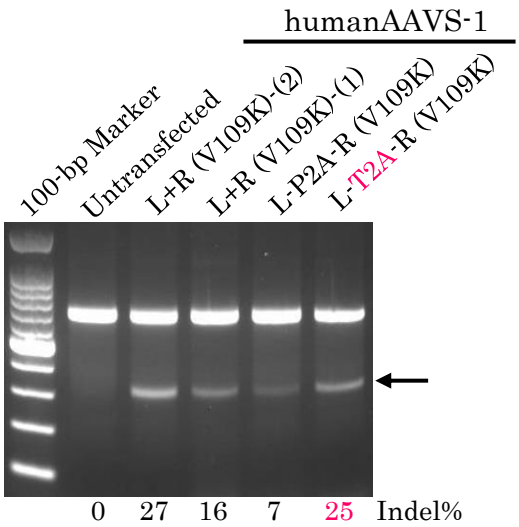


Figure S3. 2A-coupled co-expression of two ZF-ND1 monomers in Jurkat cells.
The T7E1 assay. Gel images of T7E1-treated PCR products amplified from the target sites. Arrowheads indicate the cleaved DNA band. L+R (V109K) indicates the separately expressed ZF-ND1 monomers. L-P2A-R (V109K) and L-T2A-R (V109K) indicate the P2A- and T2A-coupled ZF-ND1 monomers, respectively. The total amount of transfected plasmid DNA: L+R (V109K), 2 μg (2); L+R (V109K), 1 μg (1); L-P2A-R (V109K), 1 μg; L-T2A-R (V109K), 1 μg.