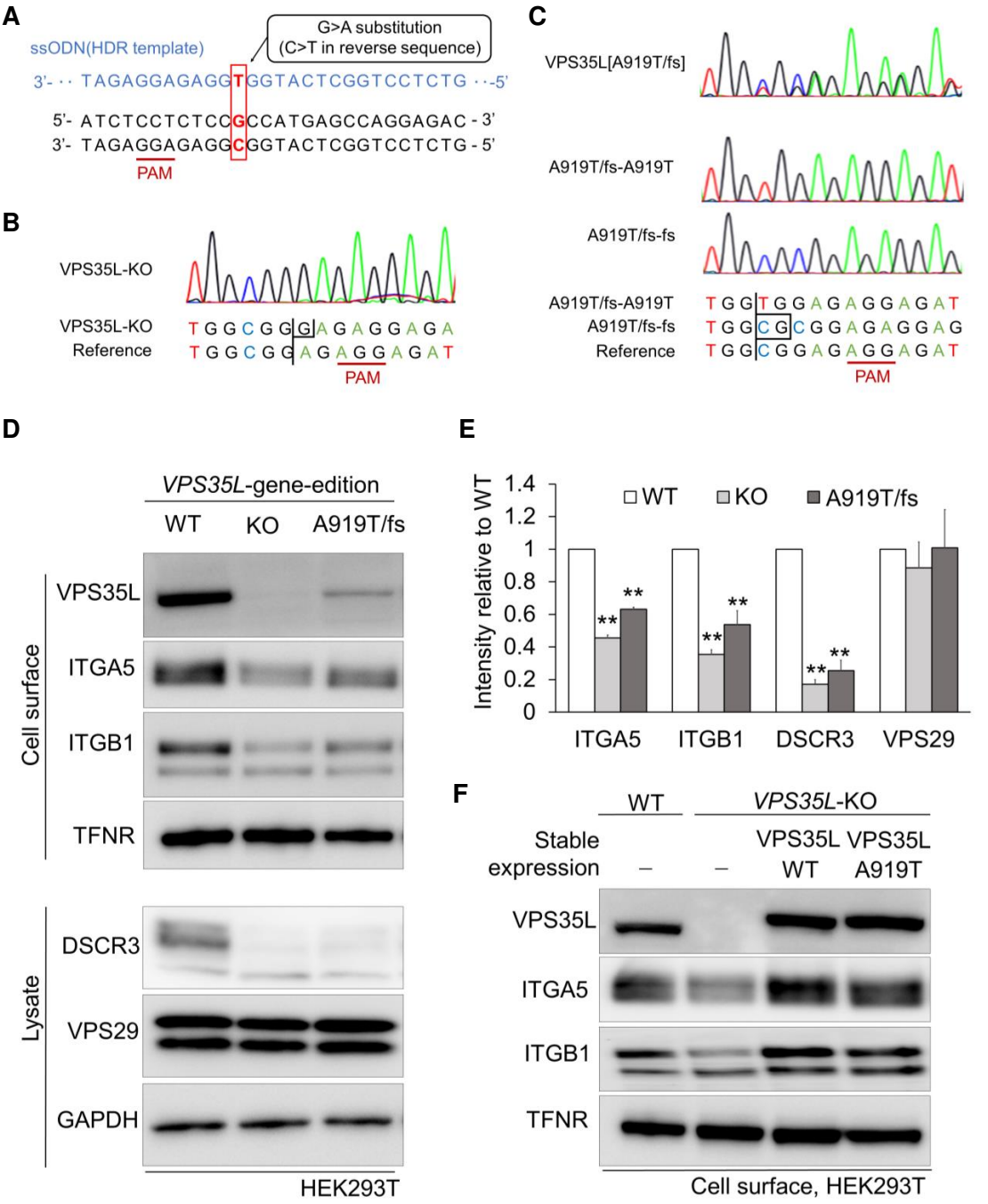
Supplementary Figure 1



Suppl. Figure 1. Western blot analysis of *VPS35L*-gene edited cells.
(A) gRNA target and single-stranded oligo DNA nucleotide (ssODN) template sequence for generating the VPS35L-A919T point mutation by CRISPR/Cas9-mediated homology-directed repair (HDR) system. Red underline indicates PAM sequence. (B) Genomic DNA sequence chromatograms (reverse-complementary sequence) indicate the homozygous frame shift variant with one base insertion (c.2752dup; p.Ala919Argfs*36) in VPS35L-KO cells. (C) Genomic DNA sequence chromatograms (reverse-complementary sequence) indicate compound heterozygous variants in VPS35L[A919T/fs] cells (upper, A919T/fs), which include the patient-derived missense variant (c.2755G>A; p.Ala919Thr; middle, A919T/fs-A919T) and the frameshift variant with two base insertion (c.2755_2756insCG; p.Met920Profs*2; lower, A919T/fs-fs). The frameshift variant is considered to induce mRNA degradation by nonsense mediated decay, therefore, the genotype of VPS35L[A919T/fs] mimics that of present patients. Clones were separated by TA-cloning. (D) Representative blots of VPS35L, ITGA5, and ITGB1, which proteins were extracted from membrane fraction, and DSCR3 and VPS29 from whole cell lysate. TFNR and GAPDH immunoreactivity indicates equivalent loading. (E) Protein levels as a proportion of TFNR or GAPDH levels averaged over three independent experiments. Significantly decreased expression of ITGA5, ITGB1, and DSCR3 was observed in VPS35L-KO and VPS35L[A919T/fs] cells. Bar graphs, means and s.e.m are shown, ** $P < 0.01$. (F) Representative blots of VPS35L, ITGA5, and ITGB1 from three experiments in HEK293T cells of wildtype (WT, ctrl), VPS35L-KO, VPS35L-KO with stable expression of VPS35L-WT, and VPS35L-KO with stable expression of VPS35L-A919T. Proteins were extracted from membrane fraction, and TFNR immunoreactivity indicates equivalent loading. Stable expression of both VPS35L-WT and VPS35L-A919T were able to restore expression levels of ITGB1 and ITGA5.