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Supplemental Information

Stabilizing the Retromer Complex in a Human Stem Cell Model of Alzheimer's Disease Reduces TAU Phosphorylation Independently of Amyloid Precursor Protein

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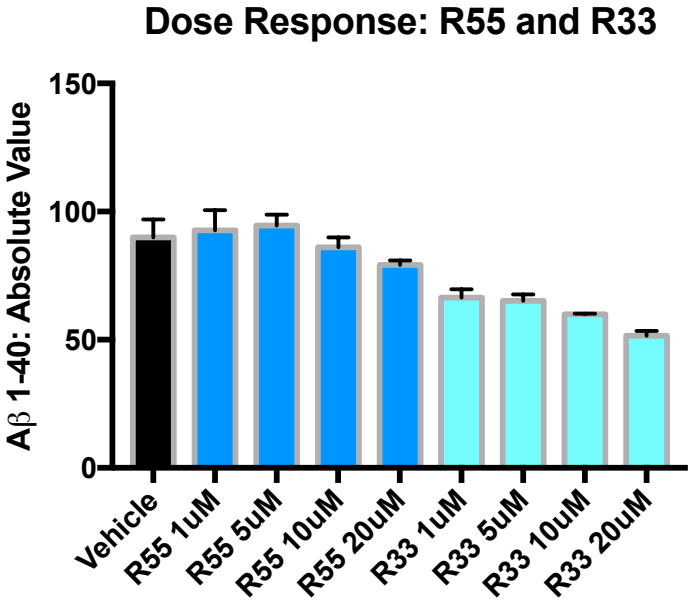
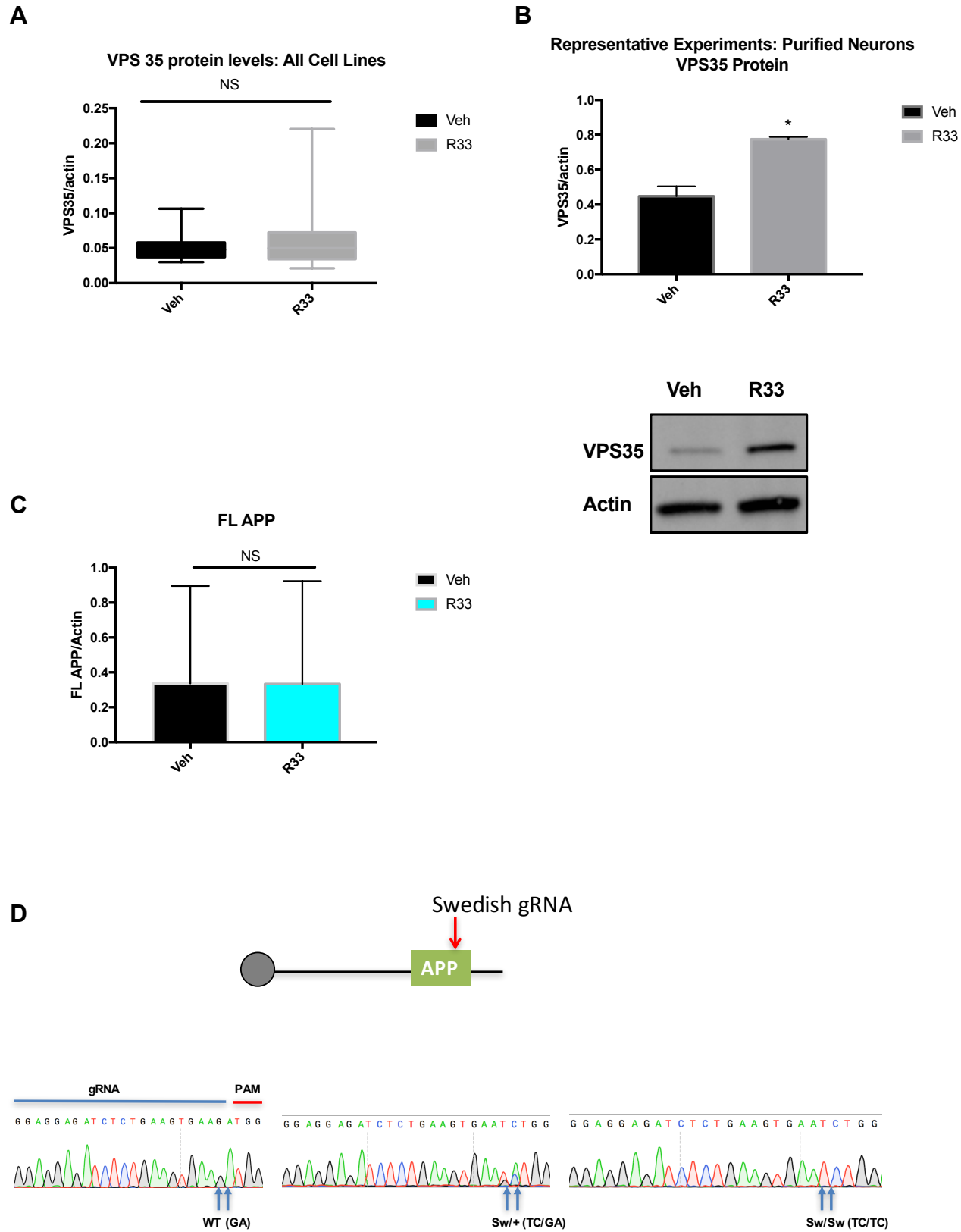
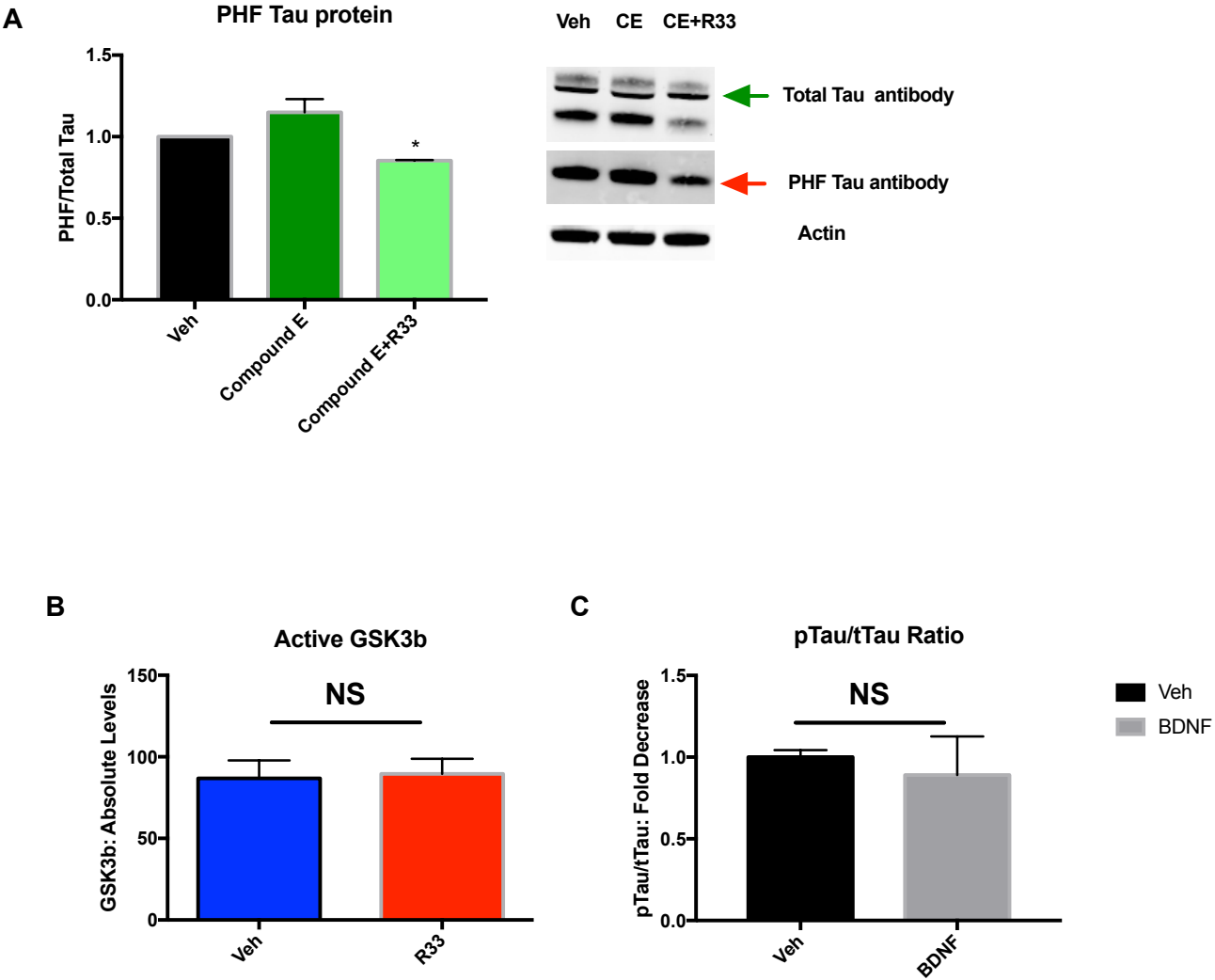
Supplemental Figure 1



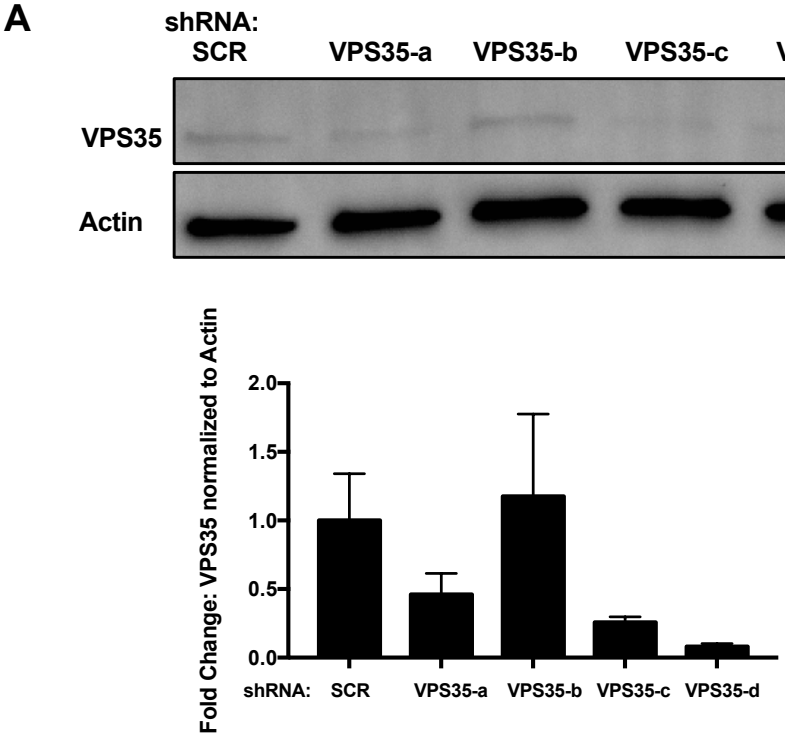
Supplemental Figure 2



Supplemental Figure 3

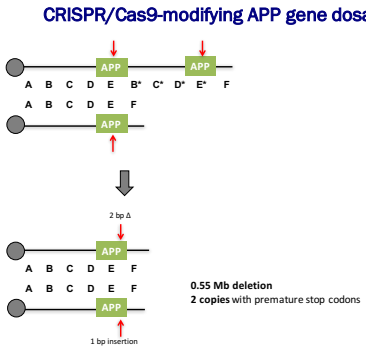


Supplemental Figure 4



Supplemental Figure 5

A



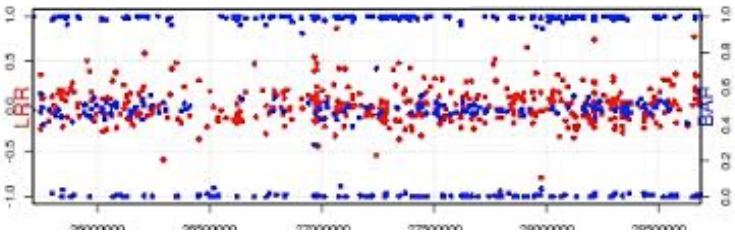
WT
APP exon 16: S G L T N I K T E E I S E V K **Met** D A E F R H D S G Y E V H H Q K L
APP exon 17: V F F A E D V G S N K G A I I G L **Met** V G G V V I A T V I V I T L V **Met** L K K K Q Y T S I H H G V V E
APP exon 18: V D A A V T P E E R H L S K **Met** Q Q N G Y E N P T Y K F F E Q **Met** Q N **Stop**

2 bp deletion
APP exon 16: S G L T N I K T E E I S E V *** D G C R I P T **Stop** L R I **Stop** S S S S K I
APP exon 17: G V L C R R C G F K Q R C N H W T H G G R C C H S D S D R H H L G D A E E E T V H I H S S W C G G G
APP exon 18: **Stop** R R C H P R G A P P V Q D A A E R L R K S N L Q V L **Stop** A D A E L

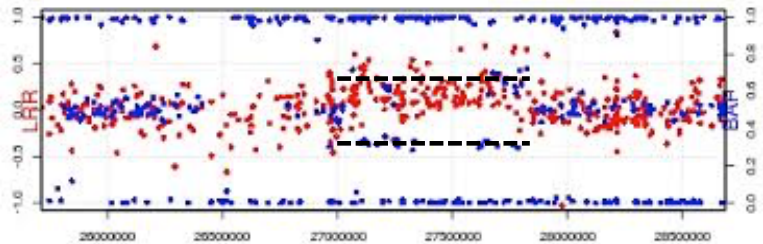
1 bp insertion
APP exon 16: S G L T N I K T E E I S E V K *** D G C R I P T **Stop** L R I **Stop** S S S S K I
APP exon 17: G V L C R R C G F K Q R C N H W T H G G R C C H S D S D R H H L G D A E E E T V H I H S S W C G G G
APP exon 18: **Stop** R R C H P R G A P P V Q D A A E R L R K S N L Q V L **Stop** A D A E L

B

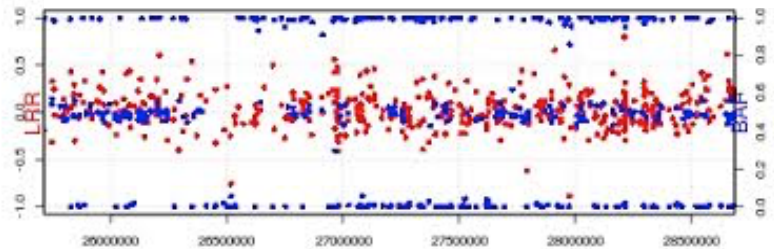
NDC (2 copies of APP)



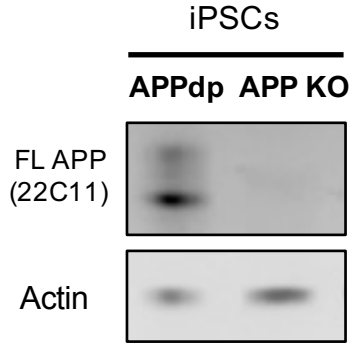
APPdp (3 copies of APP)



APPKO: 2 genomic copies of APP (contain indels)

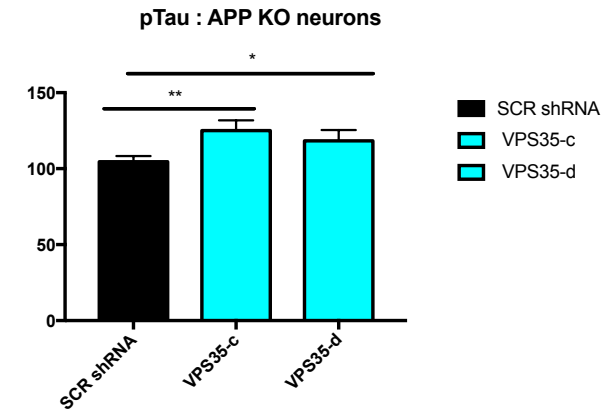


C



Supplemental Figure 6

A



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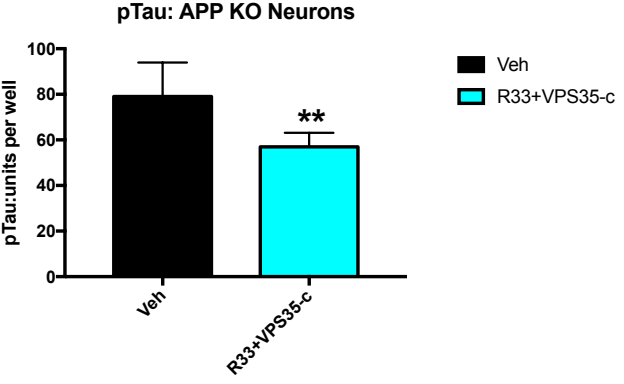

Supplemental Figure Legends

Supplemental Figure 1: Dose response of R55 and R33. Dose response in WT control neurons with different concentration of retromer stabilizing chaperone molecules R55 or R33. In these samples, R33 was more effective at lowering Aβ peptides than R55.

Error bars represent SD.

Relates to Figure 1.

Supplemental Figure 2

A-B: VPS35 protein stabilization. Quantification of Western blots for VPS35 levels across all cell lines. Although when analyzed together, the effect of R33 is non-significant (**A**), in representative cell lines, an increase in VPS35 protein levels can be detected (**B**).

C: FL APP expression. Across all patient cell lines treatment with R33 did not change levels of FL APP

D: CRISPR/Cas9 engineering of APP^{Swe}. Schematic of design for CRISPR/Cas9 mediated gene-editing for the APP Swedish (APP^{Swe}) in WT hiPSCs. Sanger sequencing confirms heterozygous and homozygous incorporation of the mutation.

For each comparison, a two-tailed T-test was performed or a Wilcoxon test was performed.

*p<0.05

NS: Non-significant

Error bars represent SD.

Relates to Figure 1 and 2.

Supplemental Figure 3

A-B: Analysis of PHF TAU and GSK3β. A. Quantification of Western blots for PHF and Total TAU of WT control cell lines treated with Compound E and R33. R33 reduces the PHF phospho epitope of TAU in hiPSC-derived purified neurons. Two-tailed t-test: *p<0.05 B. Analysis of TAU kinase GSK3β in hiPSC-derived purified neurons from all 13 individuals show no difference in activated GSKβ in response to R33 treatment. Wilcoxon test: NS: non-significant

Error bars represent SD

C. Previous work demonstrated that hiPSC-derived neurons with protective haplotypes in the *SORL1* gene respond to BDNF treatment with reduced Aβ peptides levels (Young et al., 2015). Treatment of hiPSC-derived neurons harboring *SORL1* protective haplotypes with the neurotrophin BDNF does not reduce the pTAU/tTAU ratio. N=3 independent experiments per condition Two-tailed t-test: NS: non-significant

Relates to Figure 3.

Supplemental Figure 4

Lentiviral knock-down of VPS35.

A. Four shRNA constructs against *VPS35* (sh*VPS35* a, b, c, d) were packaged into lentiviral vectors. hiPSC-derived purified neurons were transduced for 72 hours and VPS35 protein levels were analyzed by Western Blot. Three of four (sh*VPS35* a, c, d) constructs reduced VPS35 protein levels.

Error bars represent SD.

Relates to Figure 4 and Figure 6.

Supplemental Figure 5

CRISPR/Cas9 engineering of APP null cells.

A. Schematic of design for CRISPR/Cas9 mediated gene-editing for the APP KO cells from the APP duplication ($APP^{Dp}1$) parental cell line. ABCDE represent one region of APP locus, B*C*D*E* represent duplicated region of APP locus that was excised during the gene-editing process. The remaining two copies of APP sustained indels that lead to premature stop codons.

B. Infinium HumanExome Bead Chip (Illumina) array data showing the loss of 0.55Mb in APP^{Dp} hiPSCs. Top panel: Non-demented control cell line with two copies of APP. Middle panel: APP^{Dp} hiPSCs showing duplicated region (dashed black bars). Bottom panel: APPKO hiPSCs showing two copies of APP. LRR: Log R Ratio; BAF: B Allele Frequency.

C. Western blot analysis showing that APP null hiPSCs do not express APP due to excision of one copy of APP and NHEJ-mediated indels in the remaining two copies leading to premature stop codons.

Relates to Figure 6.

Supplemental Figure 6

A. Two shRNA constructs (sh*VPS35*-c and sh*VPS35*-d) increased phospho-TAU levels in

APP KO neurons. N=3 independent experiments per treatment/condition.

B. APP KO neurons expressing a *VPS35* shRNA (*VPS35*-c) still exhibited a significant decrease in pTAU after treatment with R33. N=3 independent experiments per treatment/condition.

A: For each comparison, a one-way ANOVA with a Tukey's multiple comparisons post-test was performed.

B: For each comparison, a two-tailed T-test was performed.

*p<0.05

**p<0.01

Error bars represent SD

Relates to Figure 6