Supplemental Materials

Molecular Biology of the Cell

Langness *et al*.

Cholesterol Lowering Drugs Reduce APP Processing to Aβ

by Inducing APP Dimerization

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Supporting Information

Figure S1: Absolute (non-normalized) levels of Aβ42 for each patient cell line depicted in Figure 1A`

Figure S2: Absolute (non-normalized) levels of Aβ40 for each patient cell line depicted in Figure 1A

Figure S3: Effect of statin treatment on CTF levels

Figure S4: Representative flow cytometry gating scheme

Figure S5: Flow cytometry compensation controls

Figure S6: Atorvastatin does not affect transfection efficiency

Figure S7: Validation of flAPP-BACE1 split Venus interaction assay

Figure S8: Validation of APP dimerization split Venus assay

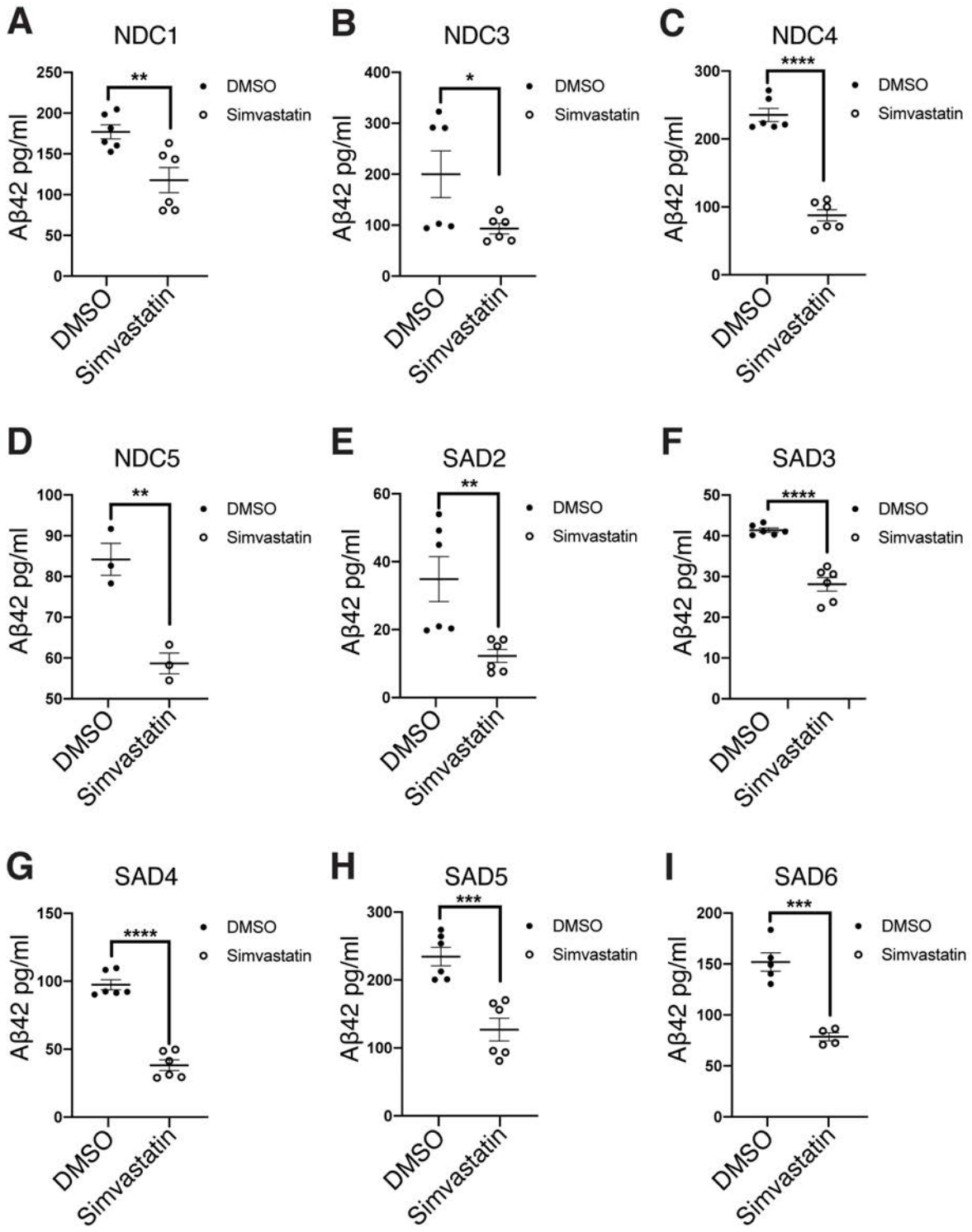

Figure S1: Absolute (non-normalized) levels of Aβ42 for each patient cell line depicted in Figure 1A. Effect of 5 day treatment with simvastatin (10 μM) on Aβ42 levels in (A-D) neurons derived from four different non-demented controls (NDC) (E-I) neurons derived from five different individuals with sporadic AD (SAD).

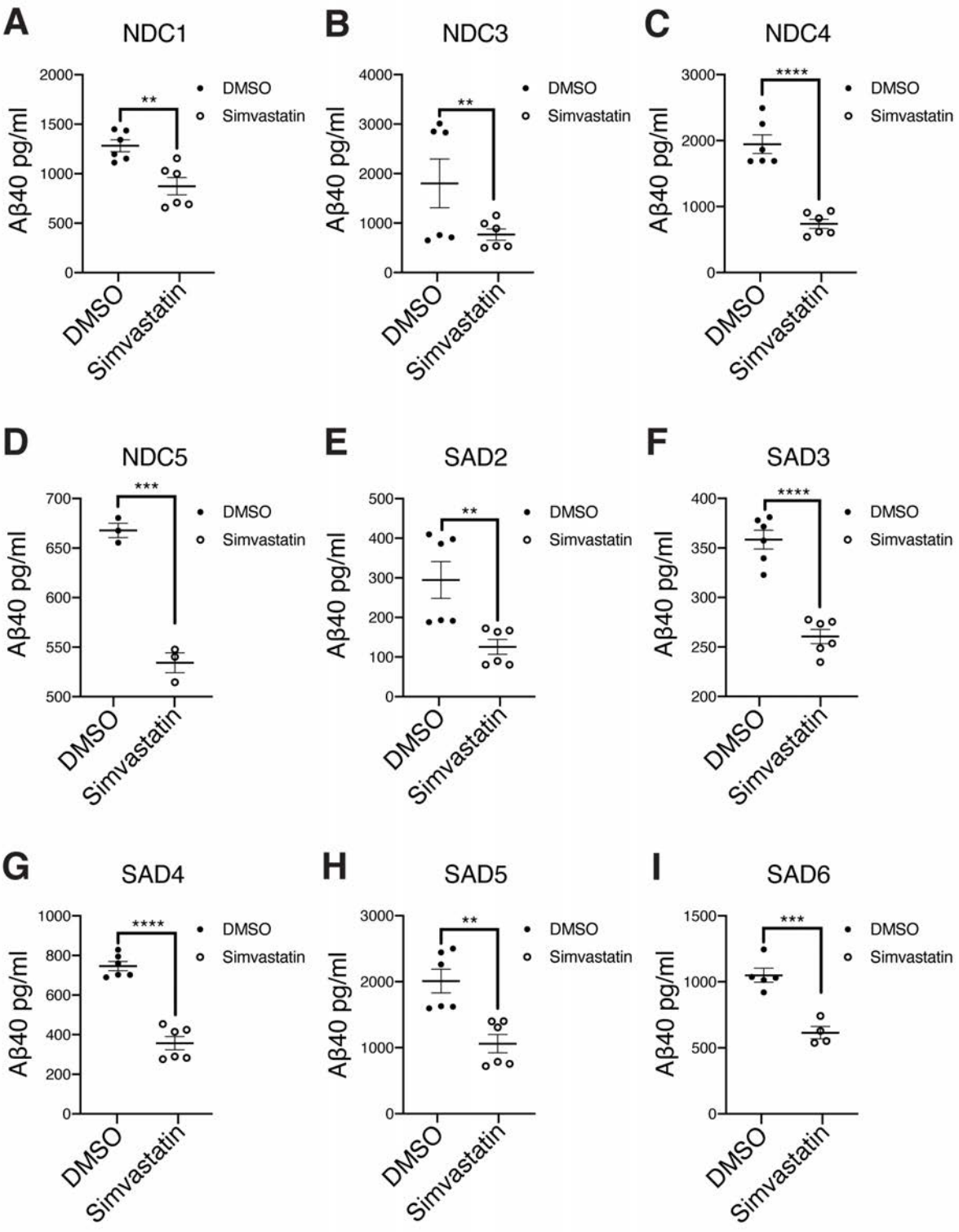

Figure S2: Absolute (non-normalized) levels of Aβ40 for each patient cell line depicted in Figure 1A. Effect of 5 day treatment with simvastatin (10 μM) on Aβ40 levels in (A-D) neurons derived from four different non-demented controls (NDC) (E-I) neurons derived from five different individuals with sporadic AD (SAD).

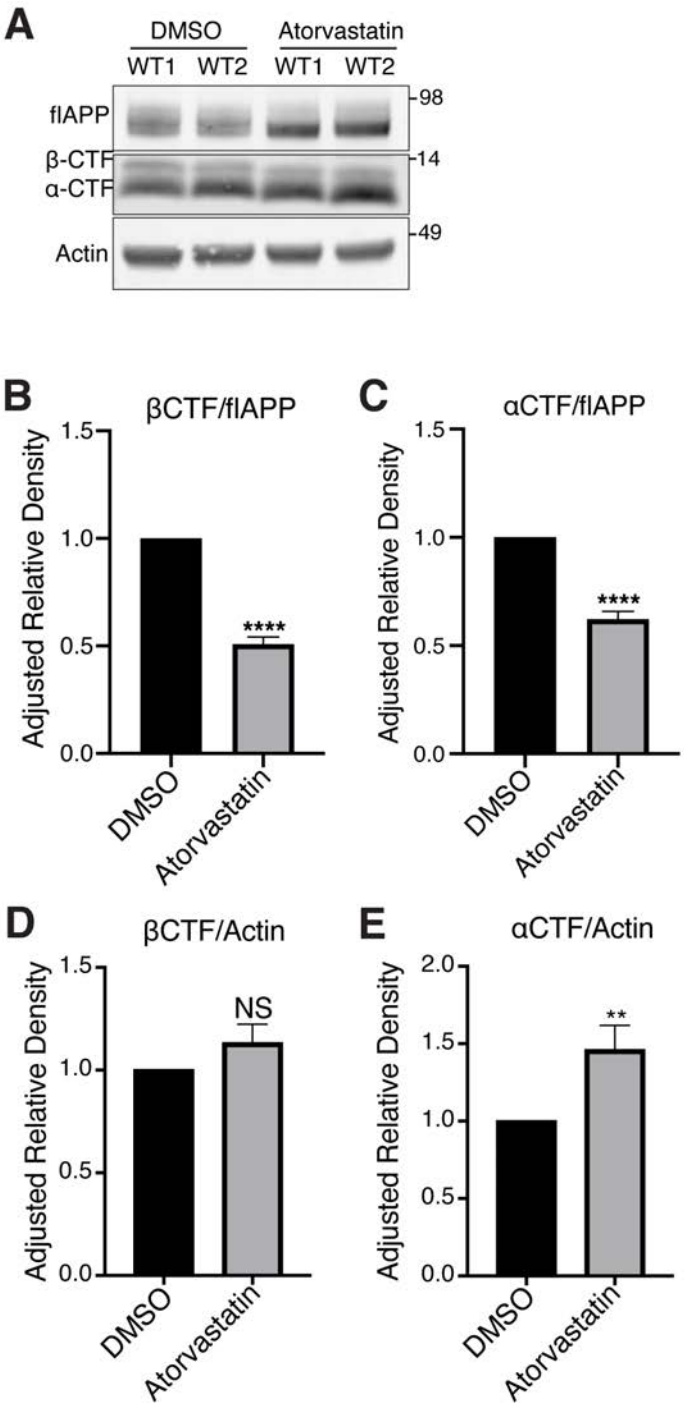

Figure S3: Effect of statin treatment on CTF levels. (A-H) WT neurons were treated with DMSO or 10 μM Atorvastatin for 5 days. (A) Levels of αCTF and βCTF were determined by western blot (B) quantification of βCTF normalized to flAPP (C) quantification of αCTF normalized to flAPP (D) quantification of βCTF normalized to actin (E) quantification of αCTF normalized to actin (mean ± SEM, n ≥ 3).

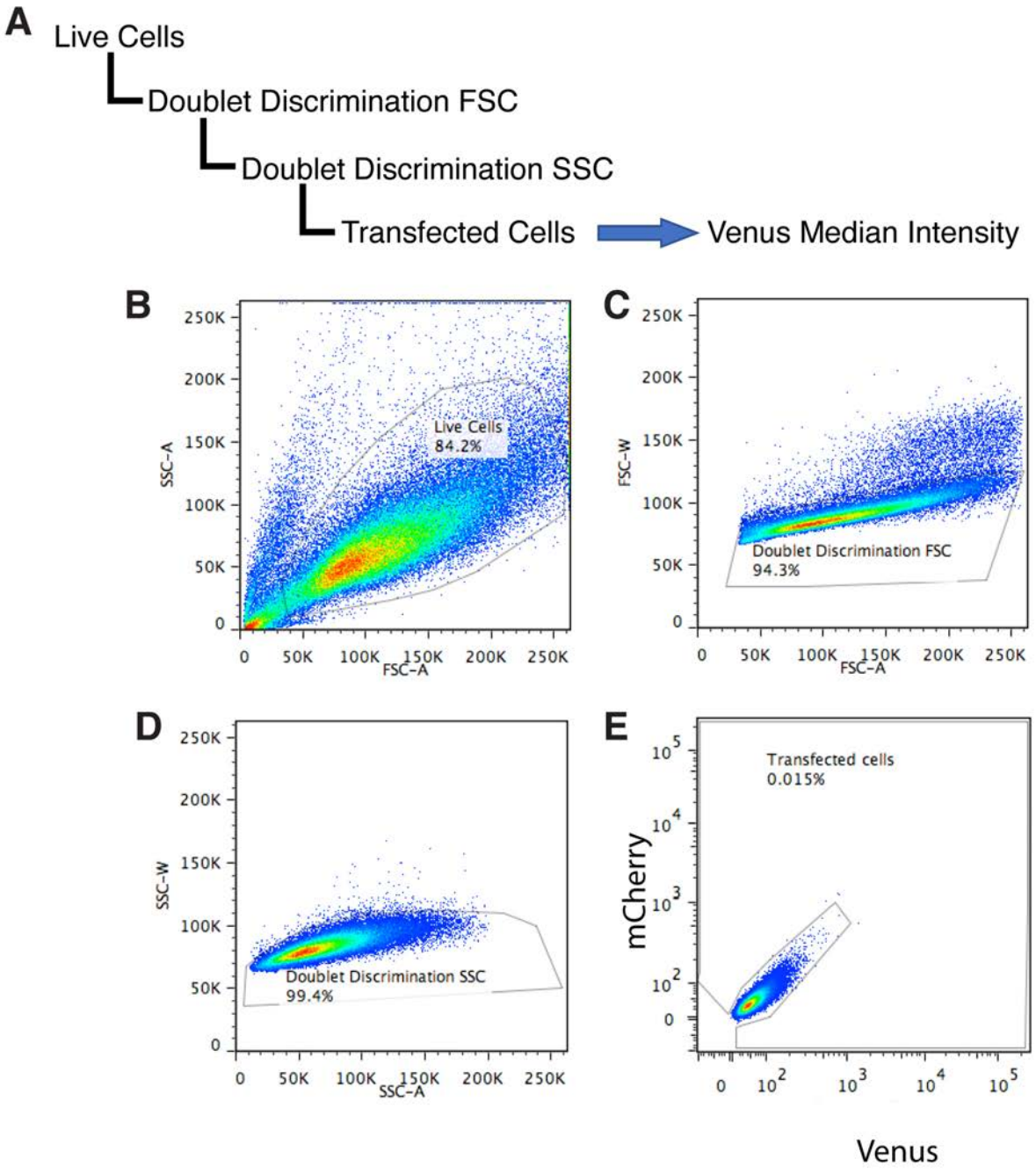

Figure S4: Representative flow cytometry gating scheme. Example of the gating scheme used for the split Venus flow cytometry assays. (A) Hierarchy showing the gating scheme used for analysis of all flow cytometry data in this manuscript. After gating out (B) cell debris, and (C&D) doublets, (E) an untransfected control was used to set a gate to discriminate transfected cells from untransfected cells. Venus median fluorescence intensity was measured in the transfected cell population. Untransfected cells were excluded from this measurement. Transfected cells were defined as those with mCherry and/or Venus signal above that of the untransfected control.

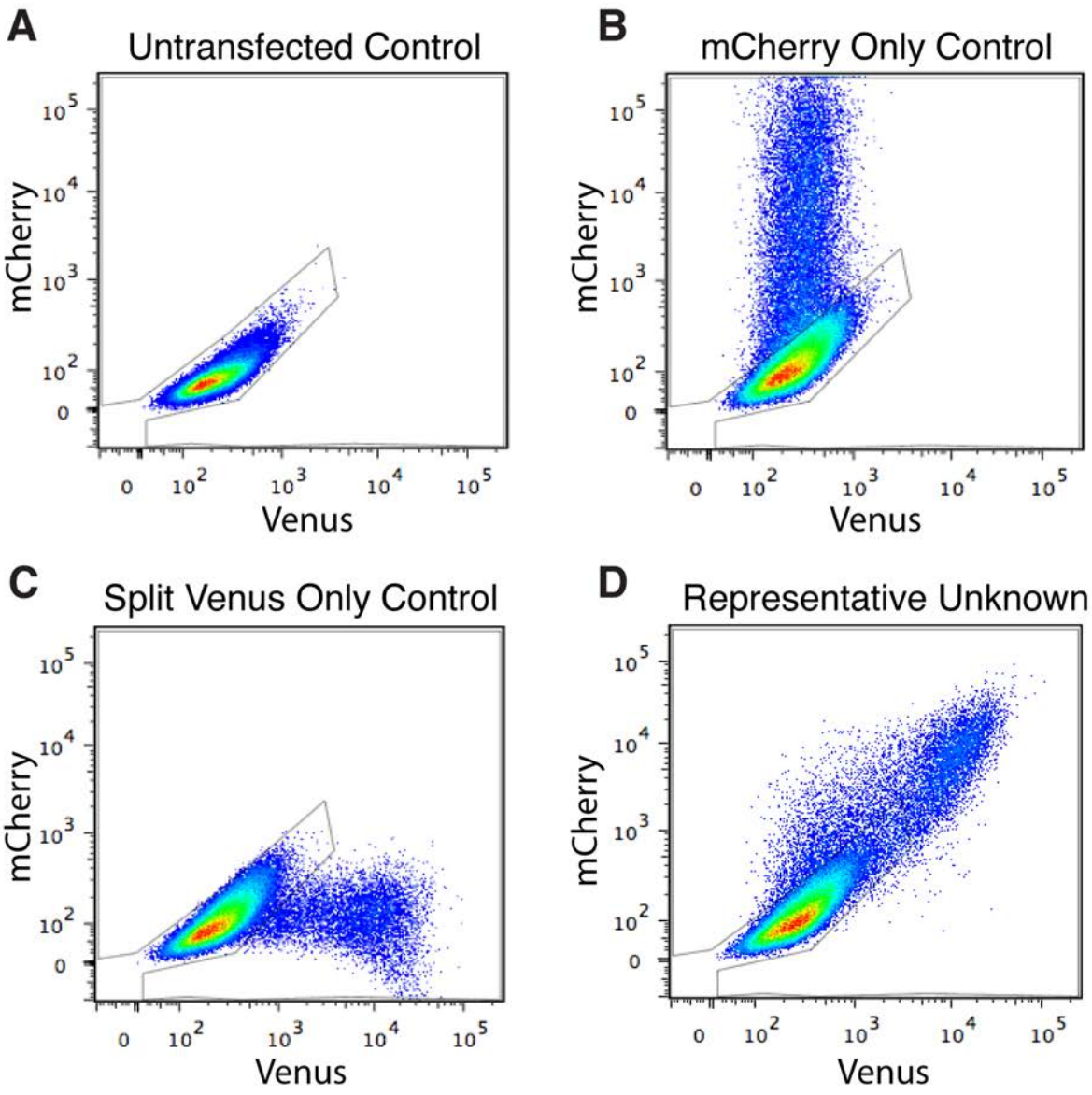

Figure S5: Flow cytometry compensation controls. (A-C) All flow cytometry experiments were run with compensation controls. The compensation controls were used to set up compensation in such a way that cells expressing only Venus showed signal only on the Venus axis and cells expressing only mCherry showed signal only on the mCherry axis. The untransfected control was used to gate out untransfected cells. Compensation controls are all shown as dot plots along with the transfected cell gate. (A) Representative untransfected control (B) Representative mCherry only control (C) Representative Venus only control (transfected with a split Venus pair) (D) Representative unknown sample transfected with both mCherry and a split Venus pair.

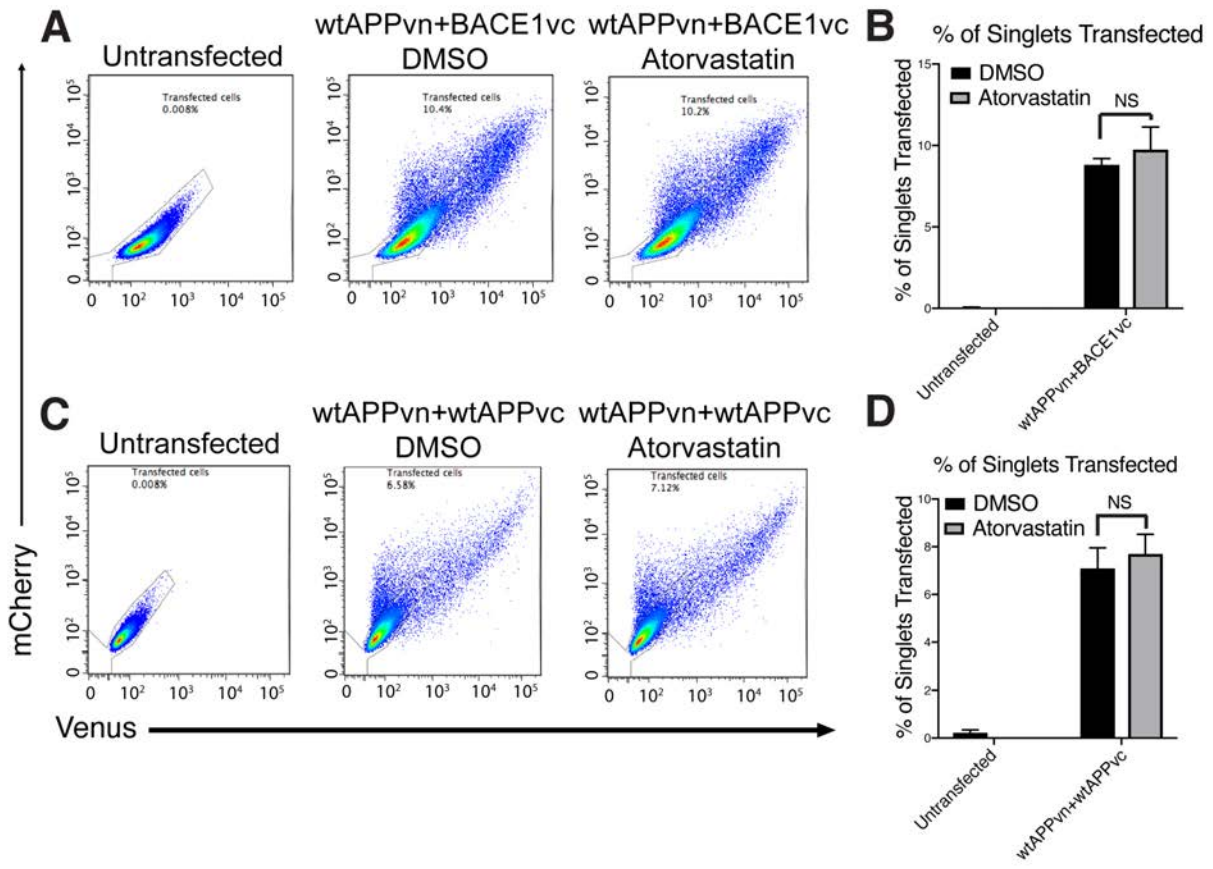

Figure S6: Atorvastatin does not affect transfection efficiency. HEK cells were plated in DMSO or 10 μM atorvastatin and then transiently transfected 24 hours later. Cells were analyzed 16 hours after transfection. (A) Dot plots showing untransfected controls, and BACE1vc+wtAPPvn+mCherry transfected cells treated with DMSO or Atorvastatin (as indicated in the figure). Each plot shows the transfected cell gate. (B) Quantification of the percentage of transfected singlets as a measure of transfection efficiency for each condition. (C) Dot plots showing untransfected controls, and wtAPPvc+wtAPPvn+mCherry transfected cells treated with DMSO or atorvastatin (as indicated in the figure). Each plot shows the transfected cell gate.(D) Quantification of the percentage of transfected singlets as a measure of transfection efficiency for each condition. (mean ± SEM, n ≥ 3).

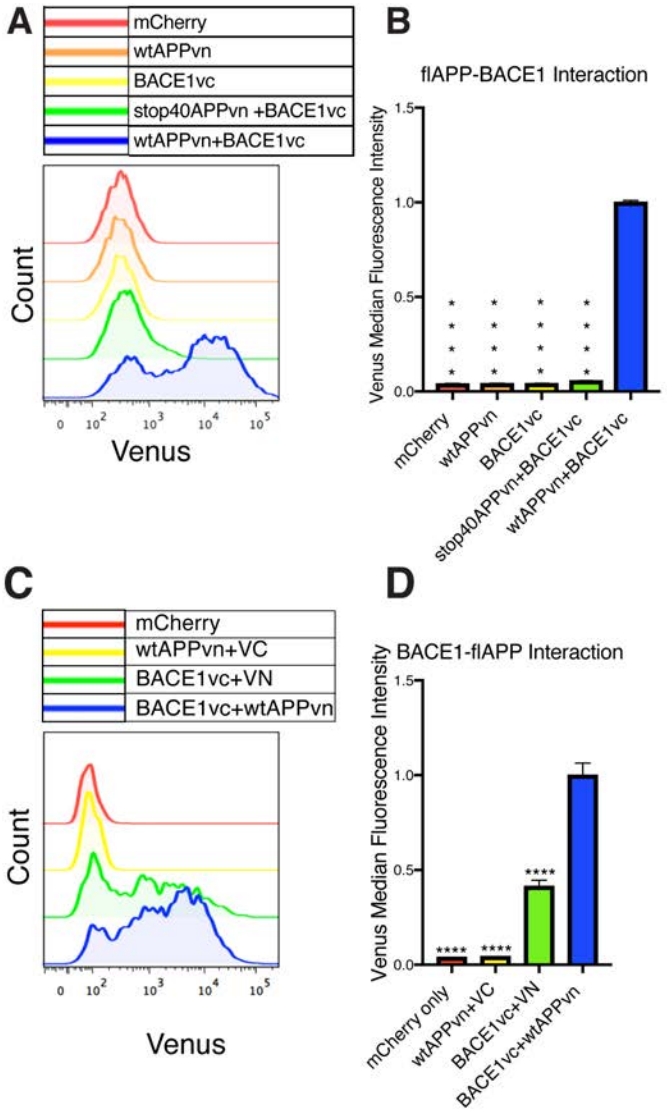

Figure S7: Validation of flAPP-BACE1 split Venus interaction assay (A-D) HEK cells were transiently transfected 24 hours after plating. Cells were analyzed 16 hours after transfection. HEK cells were transfected with mCherry in addition to the indicated construct(s). (A&B) None of the individually expressed split Venus tagged constructs generated Venus signal. When BACE1 is co-transfected with a truncated APP which lacks most of the C-terminal region (stop40APPvn) which is suspected to mediate APP-BACE1 interactions (Das et al., 2016), we see essentially no Venus signal. (C&D) When wtAPPvn is co-transfected with a plasmid expressing just the VC fragment and when BACE1vc is co-transfected with a plasmid expressing just the VN fragment, we see very little signal compared to when wtAPPvn and BACE1vc are co-transfected together. (A&C) Histograms showing flow cytometry analysis of APP-BACE1 interaction (Venus median fluorescence intensity). (B&D) Quantification of flow cytometry analysis of APP-BACE1 interaction (Venus median fluorescence intensity) (mean ± SEM, n ≥ 3).

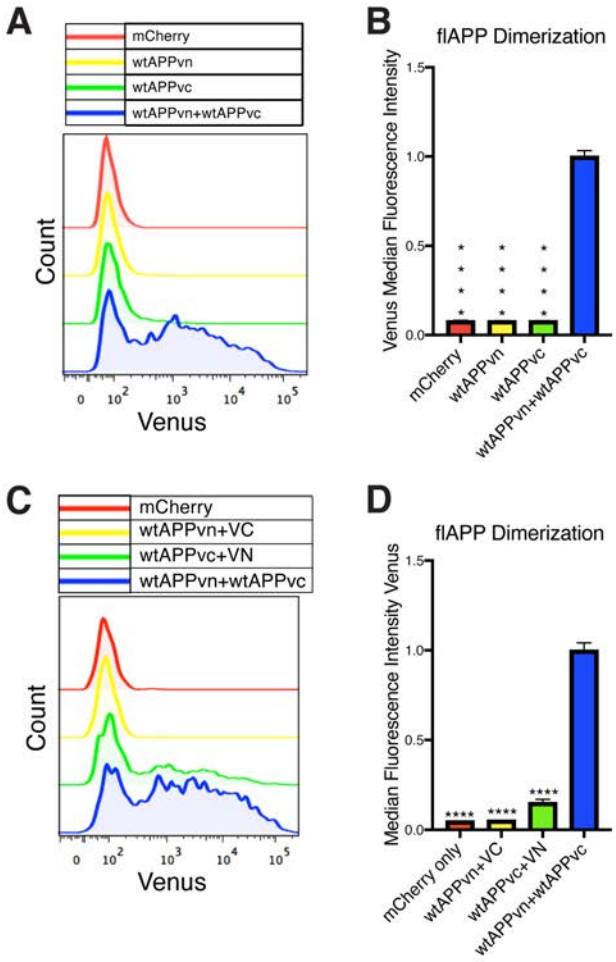

Figure S8: Validation of APP dimerization split Venus assay (A-D) HEK cells were transiently transfected 24 hours after plating. Cells were analyzed 16 hours after transfection. HEK cells were transfected with mCherry in addition to the indicated construct(s) (A&B) Neither of the split Venus tagged constructs expressed on their own generated Venus signal (C&D) When wtAPPvn is co-transfected with a plasmid expressing just the VC fragment and when wtAPPvc is co-transfected with a plasmid expressing just the VN fragment, we see very little signal compared to when wtAPPvn and wtAPPvc are co-transfected together indicating that neither split Venus dimer construct non-specifically interacts with co-transfected VN or VC protein. (A&C) Histograms showing flow cytometry analysis of dimerization (Venus median fluorescence intensity). (B&D) Quantification of flow cytometry analysis of APP dimerization (Venus median fluorescence intensity).