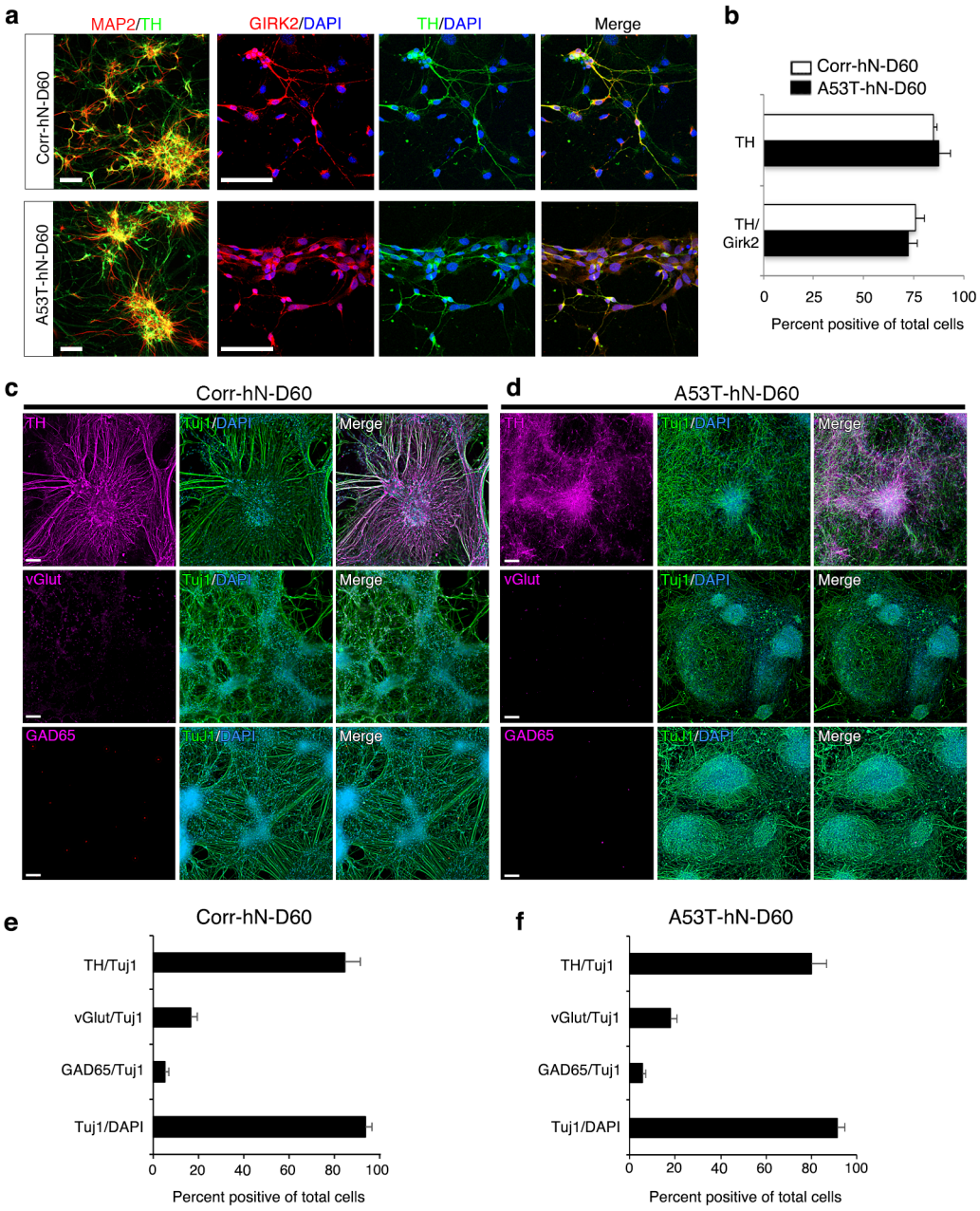
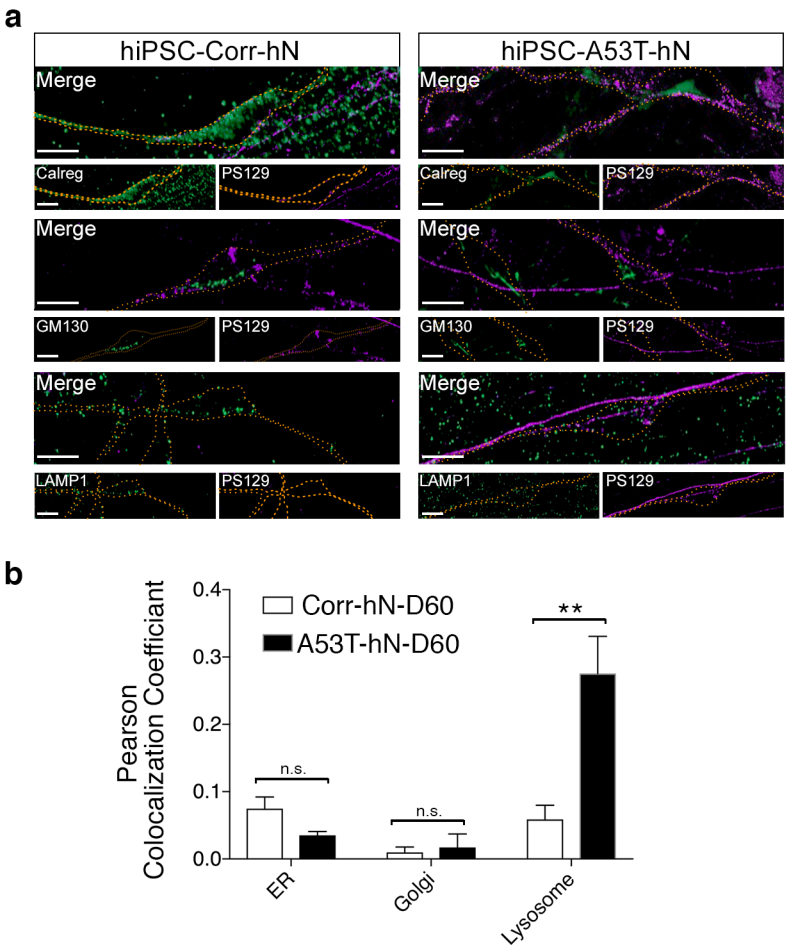
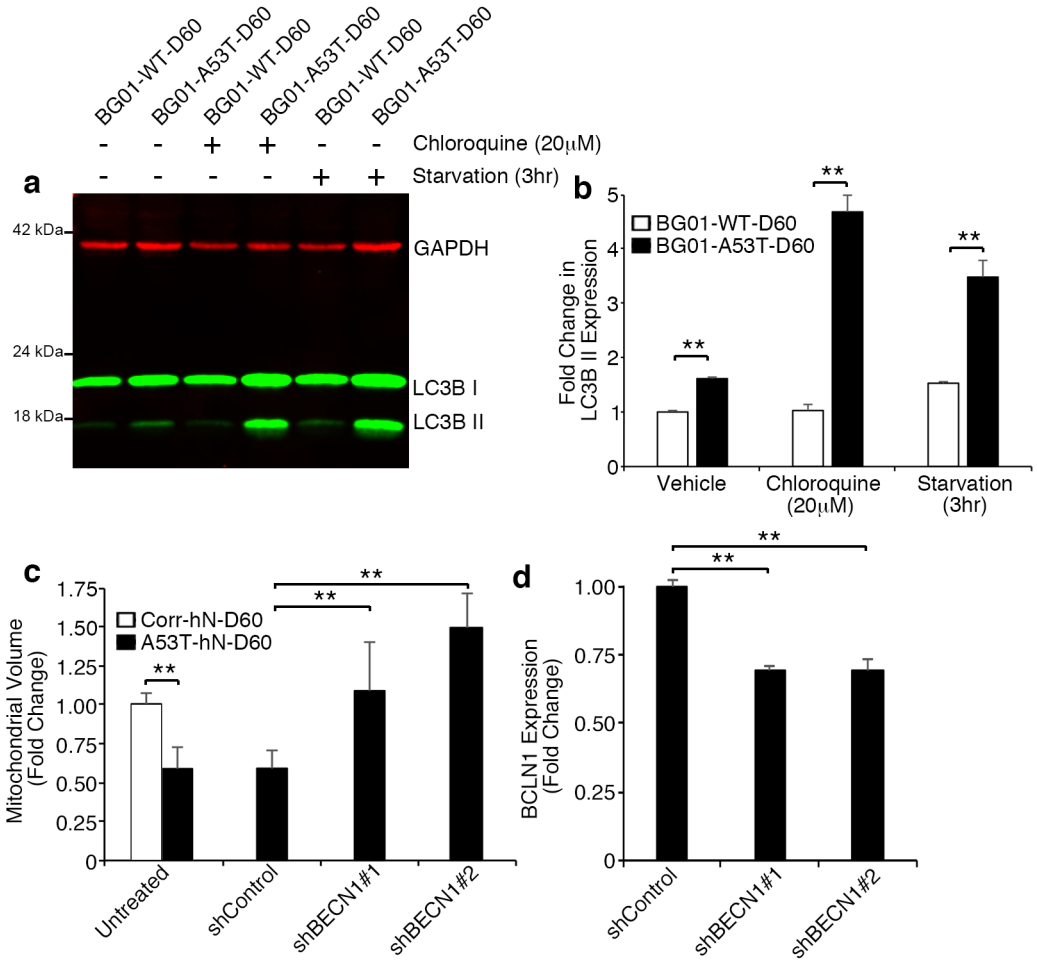
## Supplementary Information



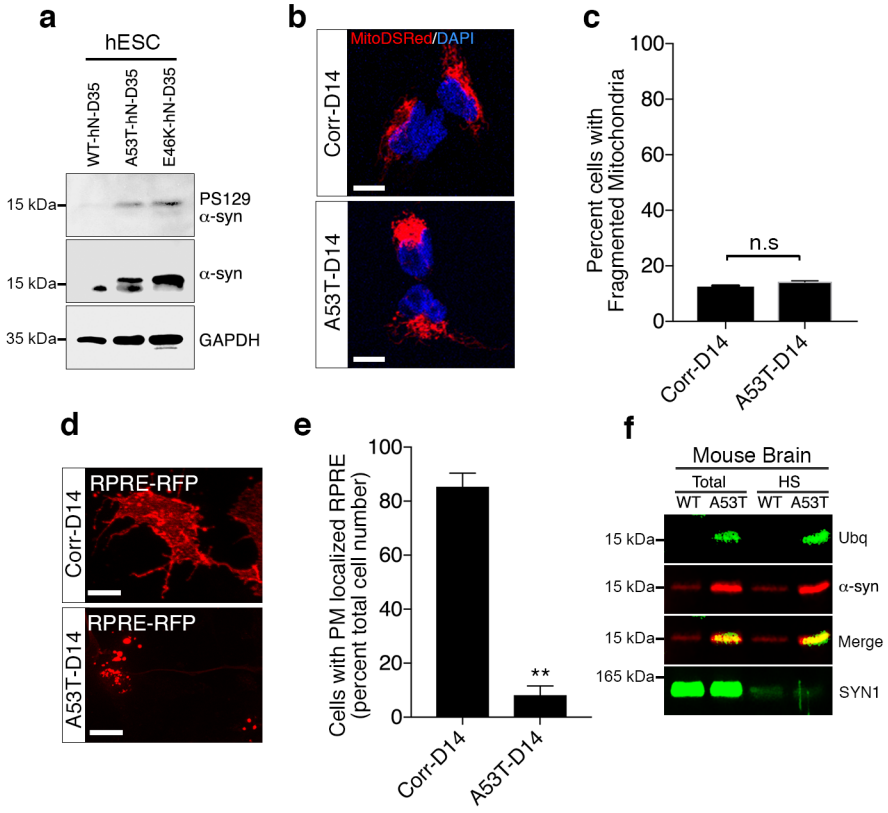

**Supplementary Figure 1 | Characterization of floor plate differentiation. a-f,** Quantification of neuronal subtypes present in differentiated hNs. hiPSC-derived corrected hNs (top panels) and A53T hNs (bottom panels) are both MAP2/TH double-positive and Girk2/TH double-positive at 60 days *in vitro* (DIV). Blue: DAPI. Scale bar: 50 µm (**a**). Quantification of TH-positive and TH-GIRK2 double positive populations. Data represent mean ± s.e.m., *n* = 6 coverslips over three independent differentiations, DIV: 60 (**b**). A53T and corrected hNs were antigenically labeled for Tuj1 and either vGlut, Gad65 or TH (**c,d**) and the percentage of cells expressing each marker was quantified (**e,f**). *n* = 6 coverslips from 2 independent differentiations, Blue=DAPI.



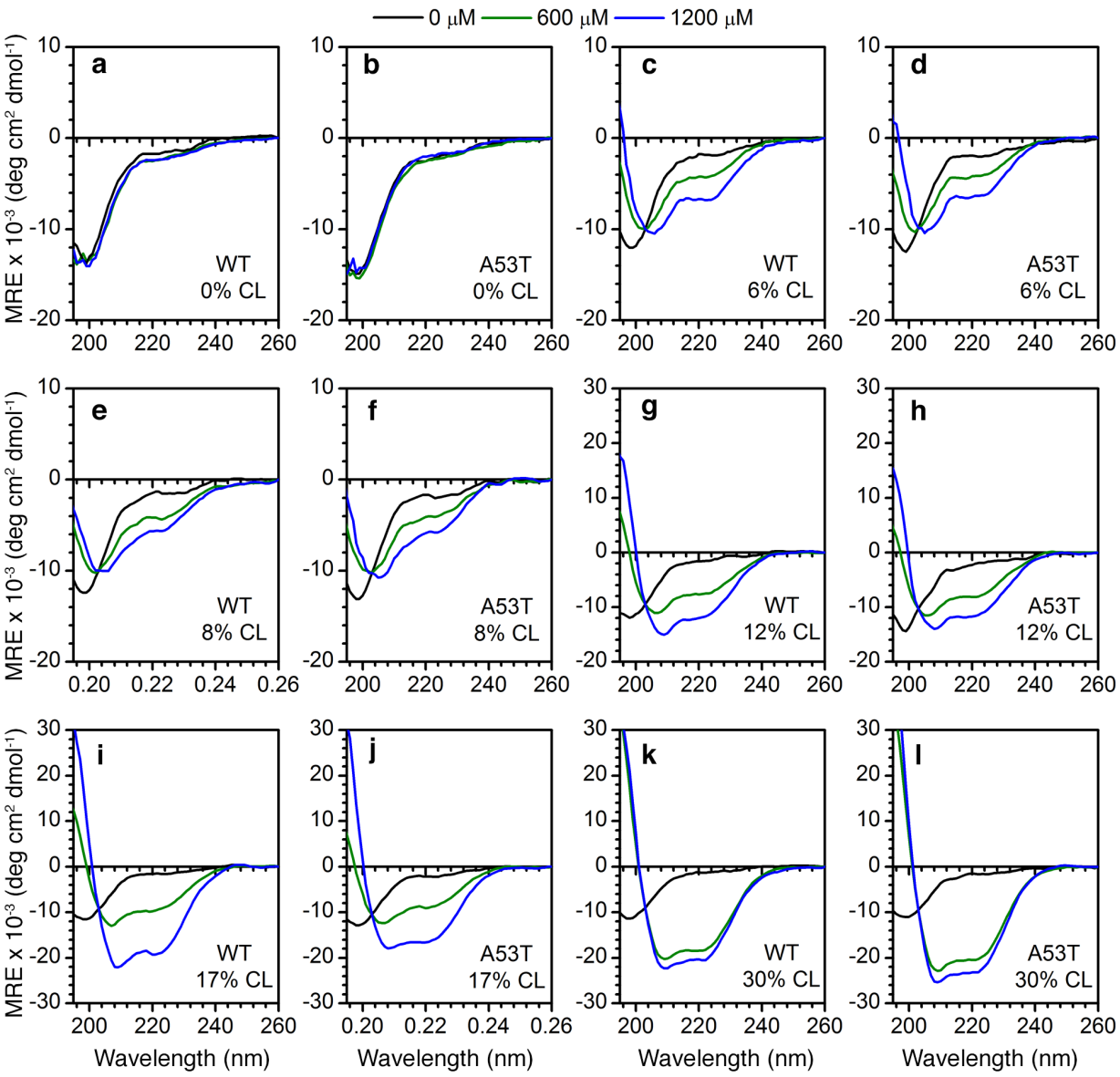

**Supplementary Figure 2 | Subcellular localization of α-syn-PS129 in α-syn mutant hNs. a,b,** Micrographs depicting α-syn-PS129 co-localization with the endoplasmic reticulum (Calregulin), the Golgi Apparatus (GM130) and the lysosomal compartment (LAMP1) (**a**). Quantification of Pearson co-localization coefficients from samples in panel a (**b**). Data represent mean + s.e.m. $^{**}P < 0.01$ by ANOVA, posthoc Tukey. $n = 4$ coverslips from 2 independent differentiations, DIV: 60.



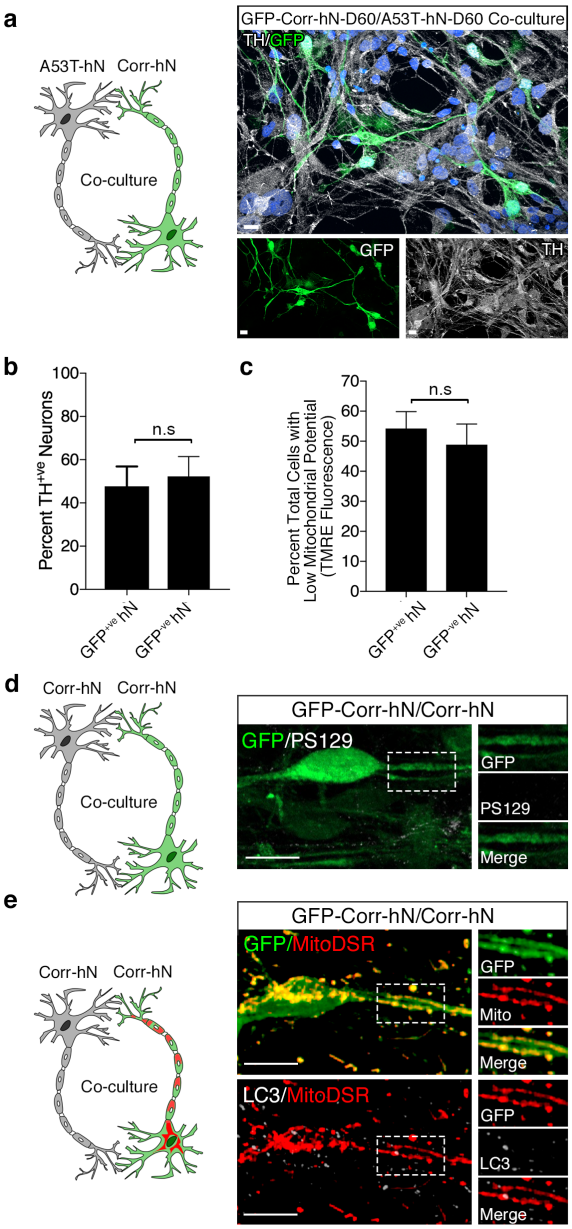

**Supplementary Figure 3 | Characterization of mitochondrial pathology in BG01-WT and BG01-A53T hNs. a-b** Steady-state levels of LC3I and LC3II protein expression were monitored by lysosomal inhibition with 20μM chloroquine (**a**). Turnover was induced with 3hrs of starvation. Triplicate experiments were quantified (**b**) relative to GAPDH expression. Data represent mean + s.e.m. $^{**}P < 0.01$ by Student t-test, $n = 3$. **c,** Quantification of the average mitochondrial volume per neurons for A53T and WT hNs following knock down of the mitophagy gene, Beclin-1 (*BECN1*) or scrambled shRNA control. Data represent mean + s.e.m. $^{**}P < 0.01$ by ANOVA followed by Dunnett, $n = 3$ neurons from 2 independent differentiations, DIV: 60. **d**, Knockdown efficiency of Beclin1 as measured by fold change in mRNA expression. Data represent mean + s.e.m. $^{**}P < 0.01$ by ANOVA followed by Dunnett, $n = 3$ independent experiments.



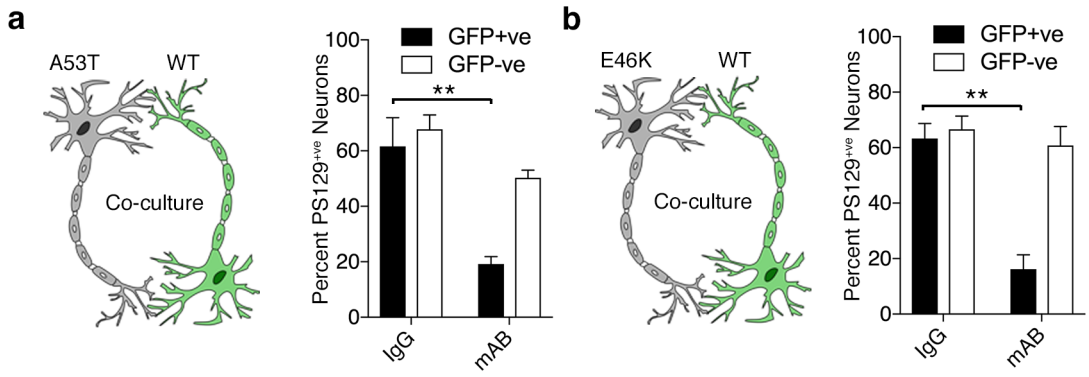

**Supplementary Figure 4 | Characterization of α-syn accumulation and mitochondrial fragmentation. a**, Western blot analysis of lysates from hESC-derived WT, A53T or E46K hNs at DIV 35 labeled for total α-syn, PS129, or GAPDH shows that levels of PS129 modified α-syn (14 kDa) are elevated in A53T and E46K hNs. **b,c,** Mitochondrial structure is normal in in hiPSC-derived A53T cells at the NPC stage (DIV14) as shown by labelling with MitoDSRed (**b**). Quantification of data (**c**) represent mean ± s.e.m. $P = 0.1647$ by Student $t$-test, $n = 8$ coverslips over 2 independent differentiations, DIV: 14; scale bar: 10 μm. **d,e,** Micrographs of hiPSC-derived corrected (left panel) and A53T mutant NPCs at DIV 14, expressing the negative charge probe RPRE-RFP (**d**). RPRE shifts from a primarily plasma membrane localization in isogenic-corrected hNs to a punctate intracellular localization in A53T hNs at DIV 14. Quantification of percentage of hNs with plasma membrane localized RPRE-RFP at DIV14 (**e**). Data represent mean ± s.e.m. $^{**}P < 0.0001$ by Student $t$-test, $n = 6$ coverslips over 2 independent differentiations, DIV: 14; scale bar: 10 μm. **f,** Western blot analysis of total and heat stable (HS) lysate from WT and A53T transgenic brain probed for ubiquitylation (Ubq), α-syn or synaptophysin 1. Merge represents overlay of α-syn and Ubq.



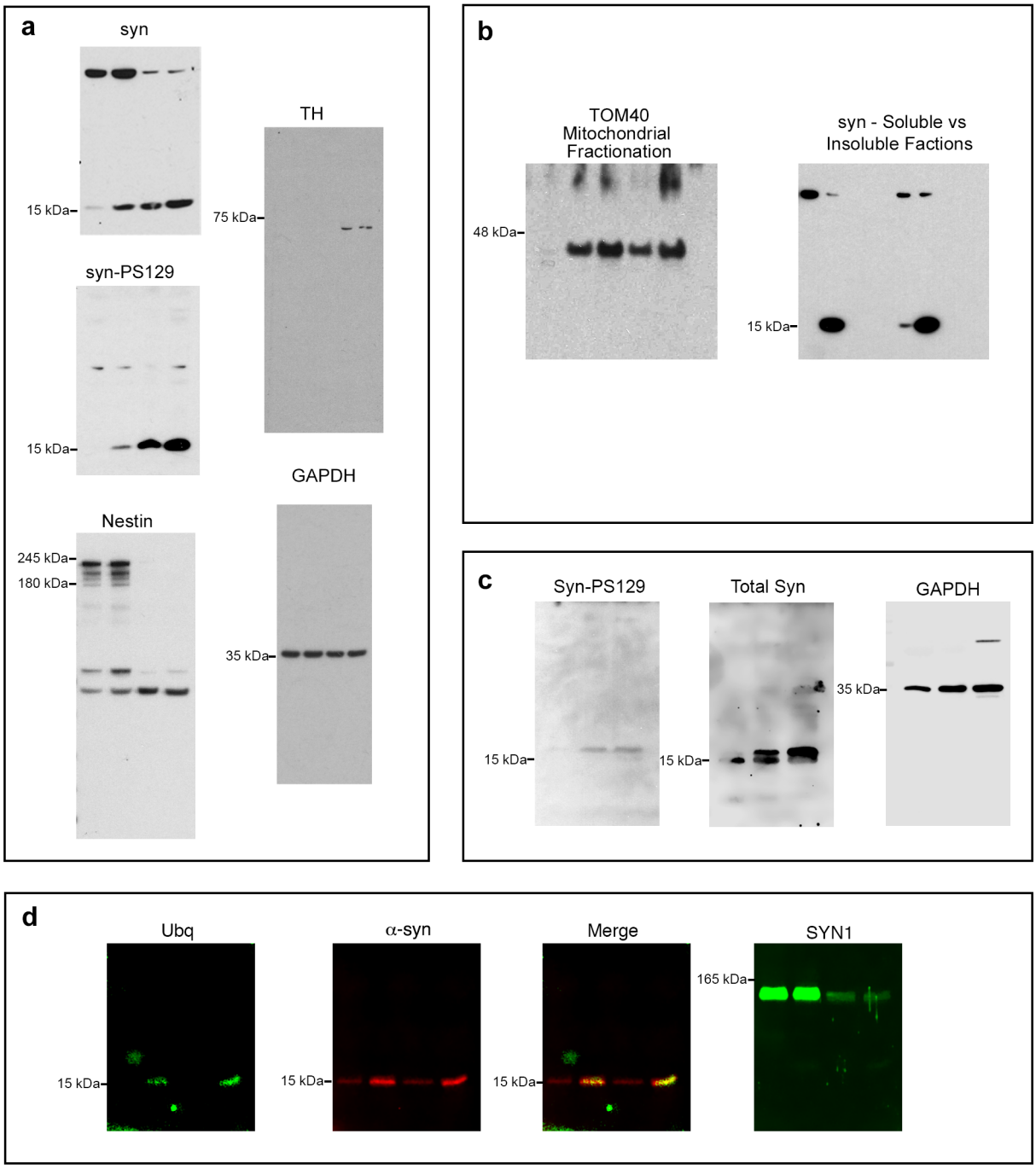

**Supplementary Figure 5 | Effect of large unilamellar lipid vesicles (LUVs) with different cardiolipin (CL) content on folding of WT and A53T α-syn monomers. a-l,** Six lipid compositions of PI:PC:PS:PE:CL with increasing molar ratio of cardiolipin were used, namely, 9.4:55.2:7.3:28.1:0 (**a,b**); 8.8:51.9:6.9:26.4:6 (**c,d**); 8.6:50.8:6.7:25.9:8 (**e,f**); 8.3:48.6:6.4:24.7:12 (**g,h**); 7.8:45.8:6.1:23.3:17 (**i,j**); and 6.6:38.6:5.1:19.7:30 (**k,l**). Monomeric WT (**a,c,e,g,i,k**) or A53T (**b,d,f,h,j,l**) α-syn (4 μM) was incubated in the presence of an increasing concentration of LUVs at 37°C, and the CD spectrum was collected to follow the relative redistribution of the secondary structure components (from mostly random coil in the absence of LUVs to α-helical structures in their presence).




**Supplementary Figure 6 | Characterization of differentiation of A53T and isogenic-corrected hNs in co-culture. a,b,** Micrographs of GFP$^{+ve}$ isogenic-corrected hNs and GFP$^{-ve}$ A53T mutant hNs in co-culture, antigenically-labeled for TH (**a**). Percentage of total TH positive neurons that are GFP$^{+ve}$ and GFP$^{-ve}$ (**b**). Data represent mean + s.e.m. *P* = 0.3022 by Student *t*-test, *n* = 10 coverslips over 2 independent differentiations, DIV: 60. **c**, Percentage of total neurons with depolarized mitochondria. Data represent mean + s.e.m. *P* = 0.5764 by Student *t*-test, *n* = 3 cultures from independent differentiations, DIV: 60.**d,** GFP$^{+ve}$ isogenic-corrected hNs and GFP$^{-ve}$ isogenic-corrected hNs in co-culture, antigenically-labeled for PS129 modified $\alpha$-syn. **e,** GFP$^{+ve}$, MitoDSRed$^{+ve}$ isogenic-corrected hNs and GFP$^{-ve}$ isogenic-corrected hNs in co-culture, antigenically-labeled for LC3. Scale Bar: 10 $\mu$m.




**Supplementary Figure 7** | **Blocking α-syn transmission blocks α-syn PS129 accumulation. a-b**, hESC-derived WT cells expressing GFP were co-cultured with either A53T cells (**a**) or E46K cells (**b**) at DIV 14 and differentiated to hNs (DIV 60) together in the presence of either monoclonal anti-α-syn or IgG control to block α-syn seeding and percentage PS129 positive neurons was quantified. Data represent mean + s.e.m. ***P* < 0.0001 by ANOVA, posthoc Tukey, *n* = 5 coverslips from 3 independent differentiations, DIV: 60.




**Supplementary Figure 8** | **Uncropped blots. a-d**, Uncropped blots from figure 4a (**a**), figure 4b (**b**), and supplementary figures S4a (**c**) and S4f (**d**) are depicted.

**Supplementary Table 1: Binding parameters of WT, A53T and E46K α-syn to cardiolipin-containing LUVs**

| Type of LUV | WT | | | | A53T | | | | E46K | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $K_D$, μM | n | L | $R^2$ | $K_D$, μM | n | L | $R^2$ | $K_D$, μM | n | L | $R^2$ |
| 8% CL | ~ 3.7 mM | N.D. | N.D. | 0.946 | ~ 4.2 mM | N.D. | N.D. | 0.961 | 140.74 ±27.14 | 166.27 ±19.77 | 13.30 ±1.58 | 0.994 |
| 12% CL | 199.69 ±22.46 | 296.00 ±16.22 | 35.52 ±1.95 | 0.998 | 438.11 ±54.61 | 202.00 ±32.24 | 24.24 ±3.87 | 0.996 | 56.59 ±12.74 | 146.77 ±11.44 | 17.61 ±1.37 | 0.996 |
| 17% CL | 52.02 ±11.62 | 220.98 ±11.04 | 29.57 ±2.31 | 0.997 | 114.44 ±24.68 | 163.91 ±18.11 | 27.87 ±3.08 | 0.995 | 11.29 ±6.18 | 130.85 ±3.72 | 22.25 ±1.32 | 0.995 |
| 30% CL | 1.11 ±2.33 | 98.51 ±7.71 | 29.57 ±2.31 | 0.993 | 2.36 ±4.13 | 95.34 ±10.31 | 28.6 ±3.09 | 0.987 | 8.37 ±4.69 | 86.34 ±6.21 | 25.90 ±1.86 | 0.994 |

Binding parameters ($K_D$, n, and L) were obtained from fitting of the data presented in Figure 5 (a-c) to equation 1 (see Methods). s.e.m was estimated by the fitting program (OriginPro 8) at the confidence level of 95%. n – the total number of lipid molecules in the bilayer that are involved in binding one molecule of protein (stoichiometry of binding). L – the number of cardiolipin (CL) molecules in the bilayer that are involved in binding one molecule of protein. Date represent mean ± s.e.m. of 3 replicates.

**Supplementary Table 2: Efficacy of mAB-α-syn mediated capture of α-syn from conditioned media**

| | Corr-hN-D60 | A53T-hN-D60 | Co-Culture |
|---|---|---|---|
| **α-synuclein captured (ng ml$^{-1}$)** | 10.85±4.75 | 6.05±2.80 | 6.98±1.43 |

One mL of conditioned media from hiPSC-derived A53T, Corrected or Co-Cultured hNs was incubated for 24 hours at 37°C with 1ug of α-syn-mAB conjugated to protein G dynabeads. The quantity of captured α-syn was normalized against a standard curve of recombinant human α-syn. Date represent mean ± s.d. of 4 replicates. Mean values do not differ significantly.