Figure S1. Pathology development is dose, time, and conformation dependent. a, WT primary hippocampal neurons treated with Hu^{wt} or Hu^{S87N} PFFs immunostained for NFL, NeuN, pSyn, and DAPI. **b**, **c**, pSyn and neuronal viability (NeuN) of primary cultures fixed at 15 DPT after Ms^{wt} or Hu^{S87N} PFF treatment. N = 18 wells from 3 biological replicates per group. Data are mean ± SEM with Kruskall-Wallis Test with Dunn's Multiple Comparison Test. *p<0.05, **p<0.01, ***p<0.001. Scale bar, 100µm.

Figure S2. Endogenous aSyn and PFF fibrillar conformations are required to induce pathology and toxicity with mutant Hu^{S87N} PFFs. a, *$SNCA^{-/-}$* primary hippocampal neurons treated with 350nM Hu^{S87N} PFFs and immunostained for NeuN, pSyn, and DAPI at DPT 16. N = 10-11 wells from 3 biological replicates per group. Unpaired student t-test. **b**, WT primary hippocampal neurons treated with 350nM Hu^{S87N} PFFs or Hu^{S87N} monomer. Coverslips immunostained for NeuN, pSyn, and MAP2. Viability determined by number of $NeuN^+$ cells relative to PBS after treatment. Two-way ANOVA with Bonferroni post-tests. N= 6-7 wells from 2 biological replicates per group. Data are mean ± SEM. *p<0.05, **p<0.01. Scale bar, 100µm.

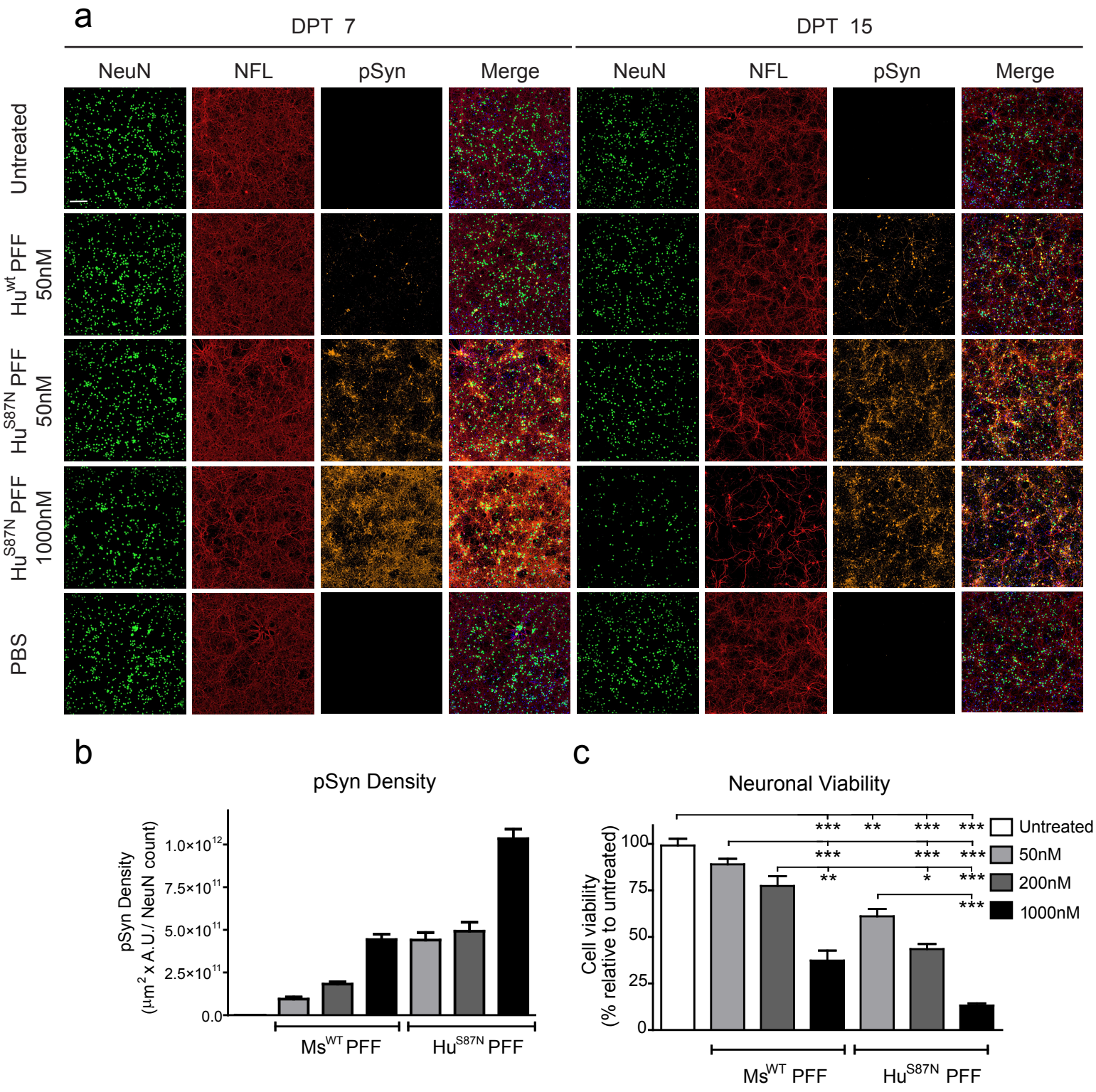
Figure S3. Mixed neuronal-glia cultures are predominantly comprised of glutamatergic neurons and GABAergic neurons are resistant to PFF induced toxicity. a, 14 DIV WT primary hippocampal neurons immunostained for NeuN, DAPI, and either Math2, Prox1 or CTIP2 transcription factors (TFs). Ratio as a percentage of each TF to NeuN is shown. Note that $CTIP2^+$ neurons also co-express either Math2 or Prox1. N = 11-12 wells from 3 biological replicates per group. Data are mean ± SEM. **b**, Immunostaining for GAD 67 and pSyn at 14 DPT. Arrows indicate GABAergic neurons without cell body aggregates. Arrowheads point to neurons that contain cell body aggregates. **c**, Immunostaining for GAD 67 and aSyn at 8 DIV. **d**, Immunostaining of WT primary hippocampal neurons for GAD 65/67, NeuN, pSyn, and DAPI treated with 350 nM Hu^{S87N} PFFs and incubated until 15 DPT. **e**, GAD $65/67^+$ area normalized to

PBS wells. N= 8-9 wells from 3 biological replicates per group. **f**, Neuronal viability determined by total NeuN counts of PFF relative to PBS. N= 4-6 wells from 2 biological replicates per group. **g**, Lysates of wt primary hippocampal neurons treated with 350 nM Hu^{S87N} PFFs and incubated until 14 DPT immunoblotted for GAD 65/67. **h**, Expression levels of GAD 65/67 to PBS treated group. N= 9 wells from 3 biological replicates per group. All data are mean ± SEM. Unpaired student t-test. *p<0.05, ***p<0.001. Scale bar, 100 µm.

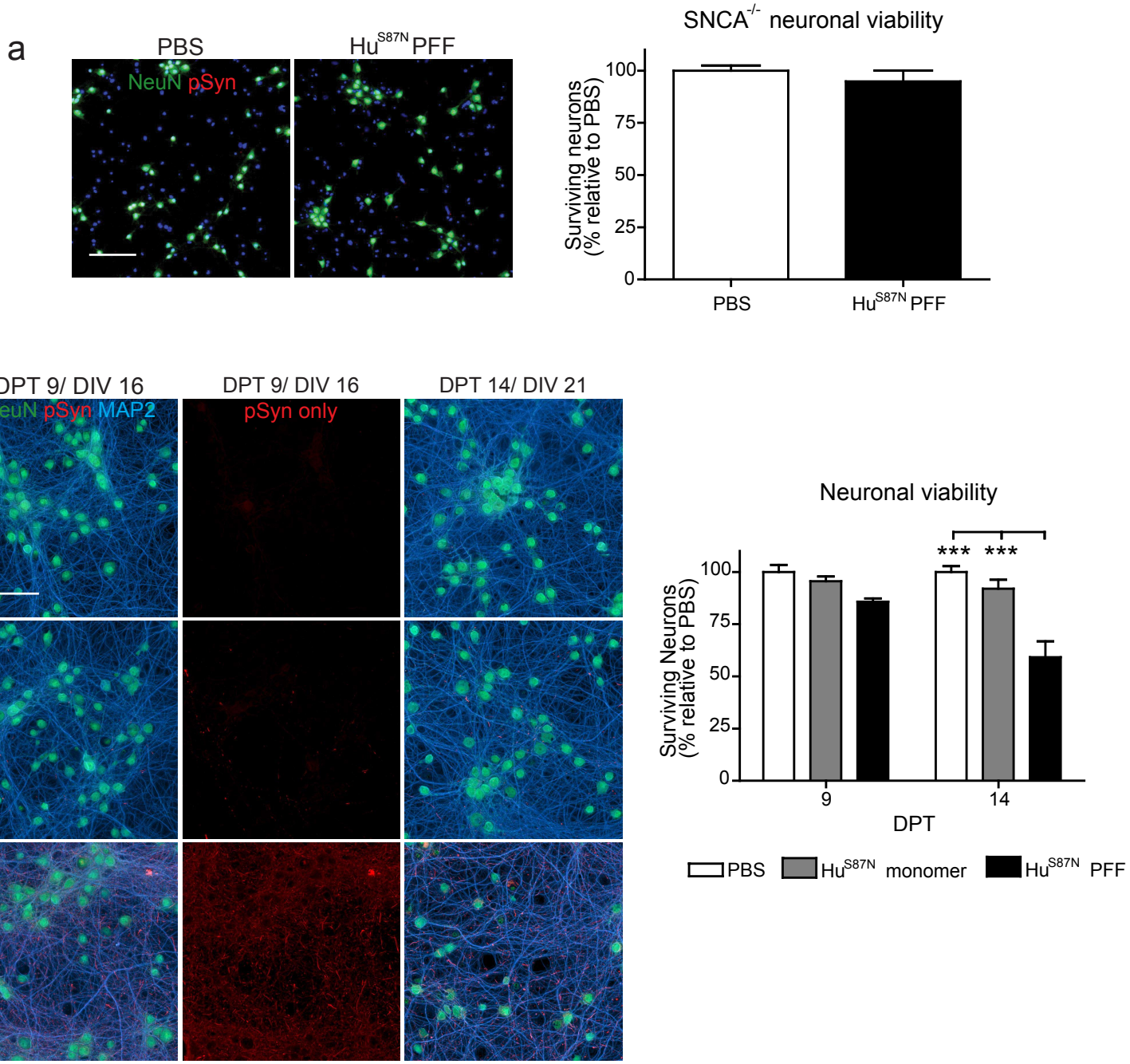
Figure S4. PFF induced toxicity is partially dependent on oxidative stress. a, Neurons treated with either PBS or 200 nM Hu^{S87N} PFFs and subsequently treated with vehicle or indicated treatment daily from 9-10 DPT to 13-14 DPT and fixed at 14-15DPT. Neuronal viability determined by NeuN counts of PFF group relative to PBS of treatment group and presented as percentage relative to PFF treatment + vehicle. Data are mean ± SEM. N= 8-15 wells from 3 biological replicates. One-way ANOVA with Dunnett's post-test comparing all groups to NAC only treatment. **p<0.01. **b**, Immunostaining of NeuN, pSyn, and DAPI of PBS and PFF treated neurons at 15 DPT after daily treatment from 9 -14 DPT with either vehicle, 100 µM NAC or 100 µM NAC + 5 µM BSO. Scale bar, 100 µm.

Figure S5. Differential expression of aSyn mRNA in mouse hippocampal neurons.

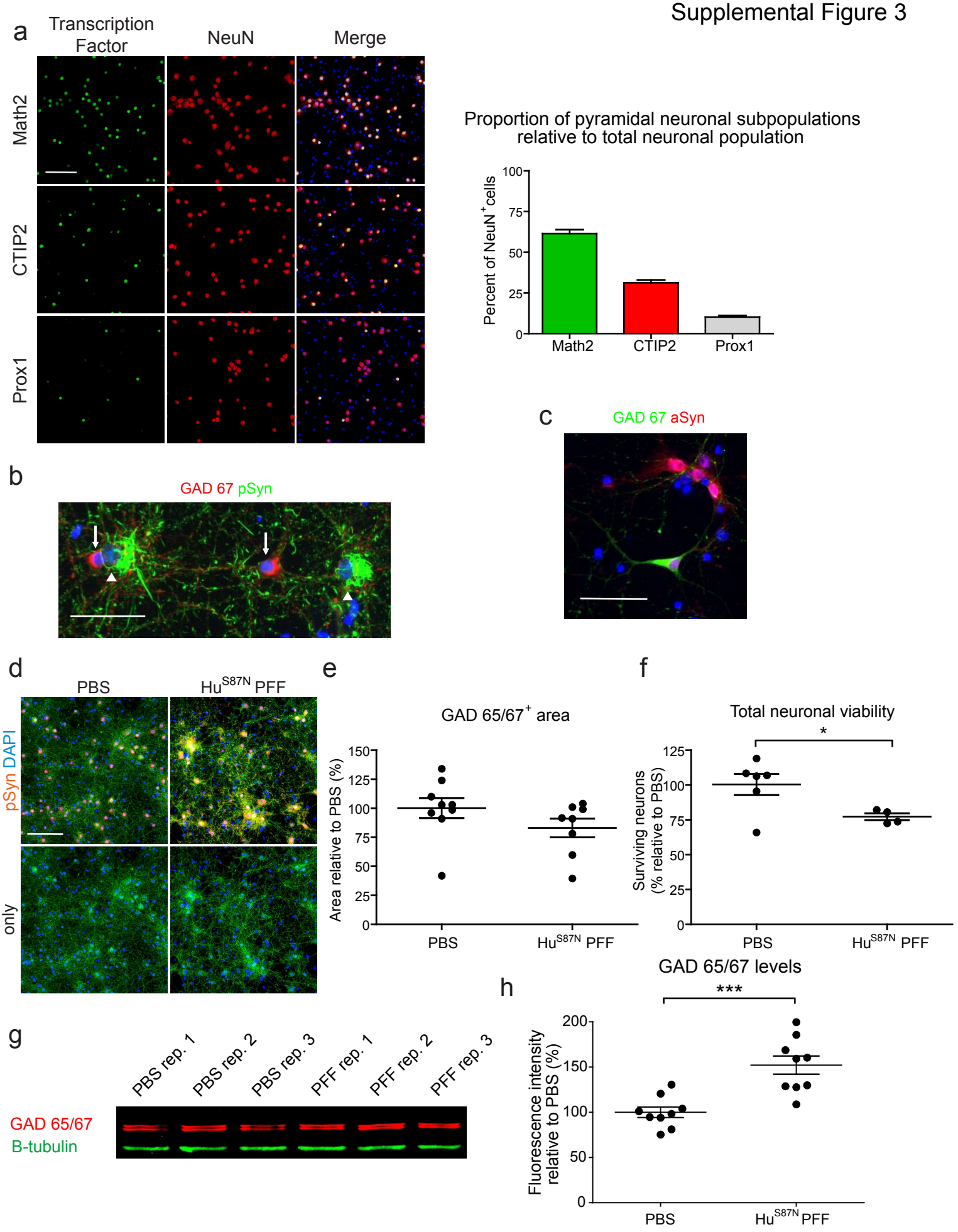
a, *In situ* hybridization of aSyn, Math2, CTIP2, and Prox1 mRNA showing relative expression levels in adult hippocampus (http://brain-map.org) [44]. **b**, Mean aSyn mRNA levels from RNA-seq of hippocampal neurons showing differential expression between CA and DG subpopulations (http://hipposeq.janelia.org) [14].

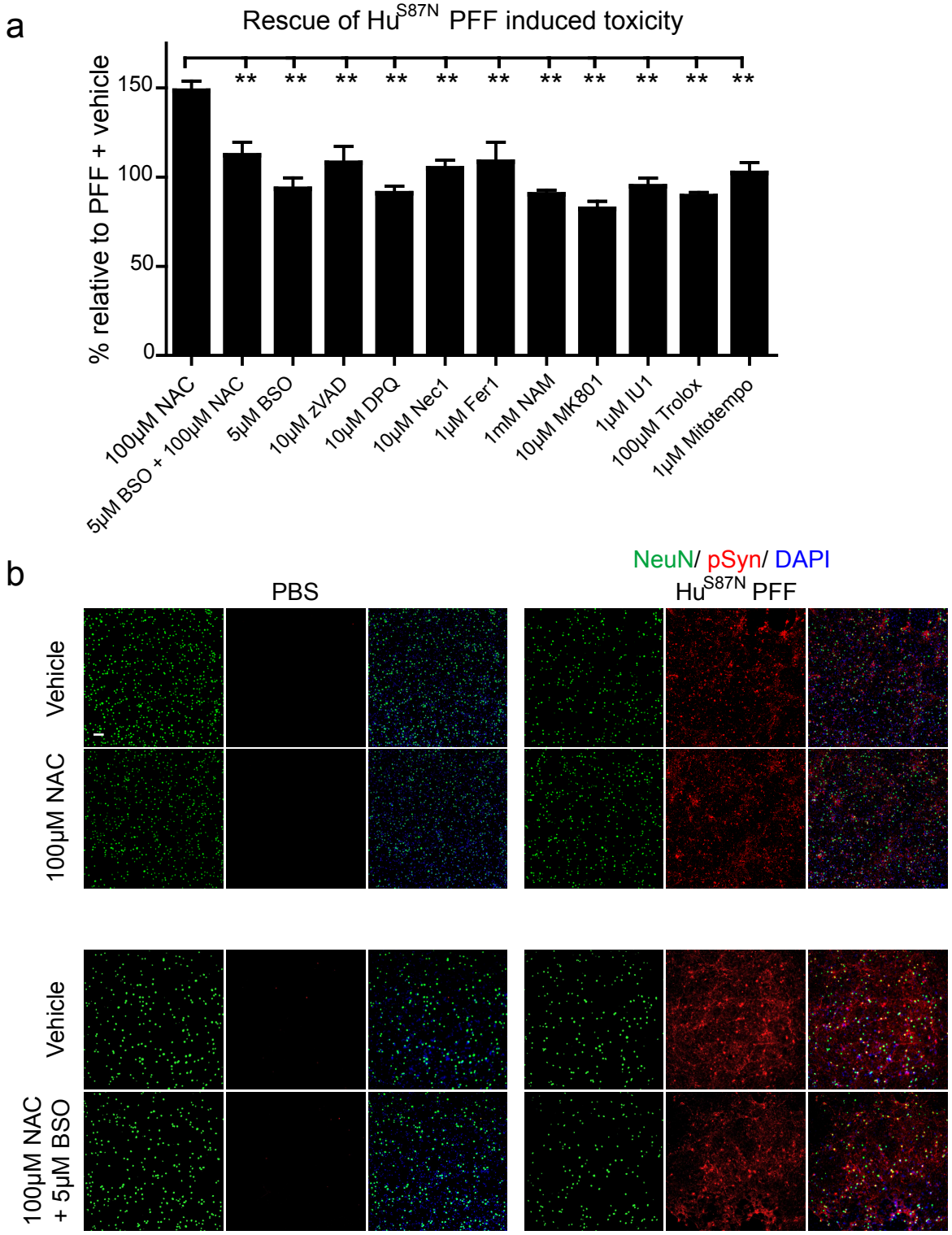

a
DPT 7
DPT 15
NeuN
NFL
pSyn
Merge
Untreated
Hu^wt PFF 50nM
Hu^S87N PFF 50nM
Hu^S87N PFF 1000nM
PBS
b
pSyn Density
pSyn Density (μm² x A.U./ NeuN count)
1.0×10^12
7.5×10^11
5.0×10^11
2.5×10^11
0.0
Ms^WT PFF
Hu^S87N PFF
c
Neuronal Viability
Cell viability (% relative to untreated)
100
75
50
25
0
Untreated
50nM
200nM
1000nM
Ms^WT PFF
Hu^S87N PFF

Supplemental Figure 2



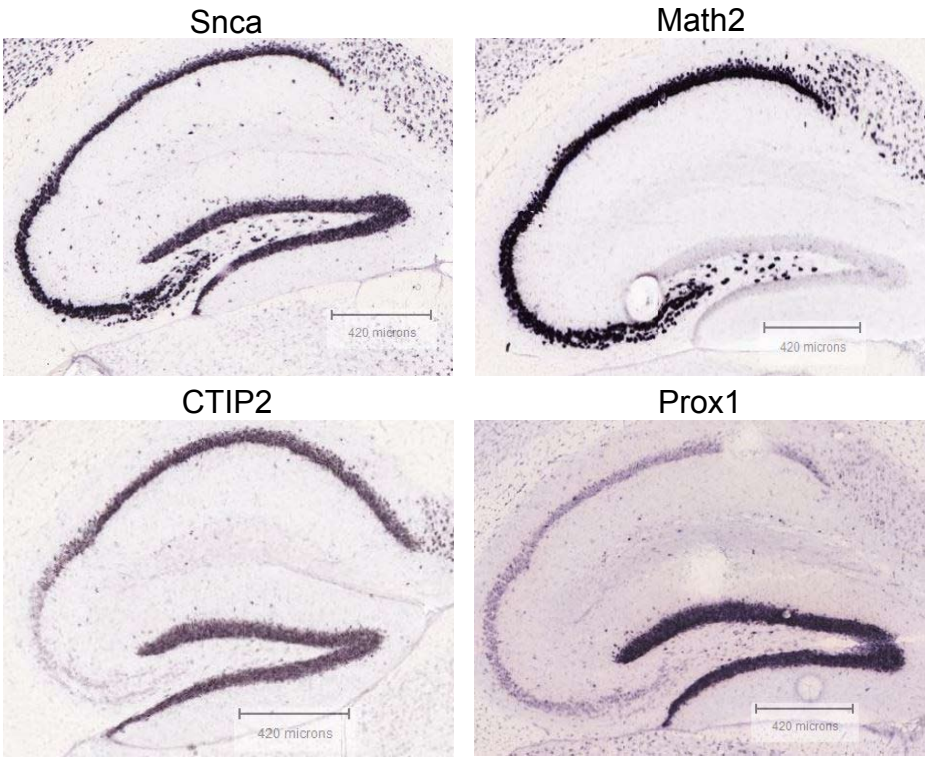
Supplemental Figure 3




a
Rescue of Hu^{S87N} PFF induced toxicity
% relative to PFF + vehicle
150
100
50
0
**
100μM NAC
5μM BSO + 100μM NAC
5μM BSO
10μM zVAD
10μM DPQ
10μM Nec1
1μM Fer1
1mM NAM
10μM MK801
1μM IU1
100μM Trolox
1μM Mitotempo
b
NeuN/ pSyn/ DAPI
PBS
Hu^{S87N} PFF
Vehicle
100μM NAC
Vehicle
100μM NAC
+ 5μM BSO

Supplemental Figure 5

a



b