

Isoform-specific hyperactivation of calpain-2 occurs presymptotically at the synapse in Alzheimer's disease mice and correlates with memory deficits in human subjects

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SUPPLEMENTARY DATA

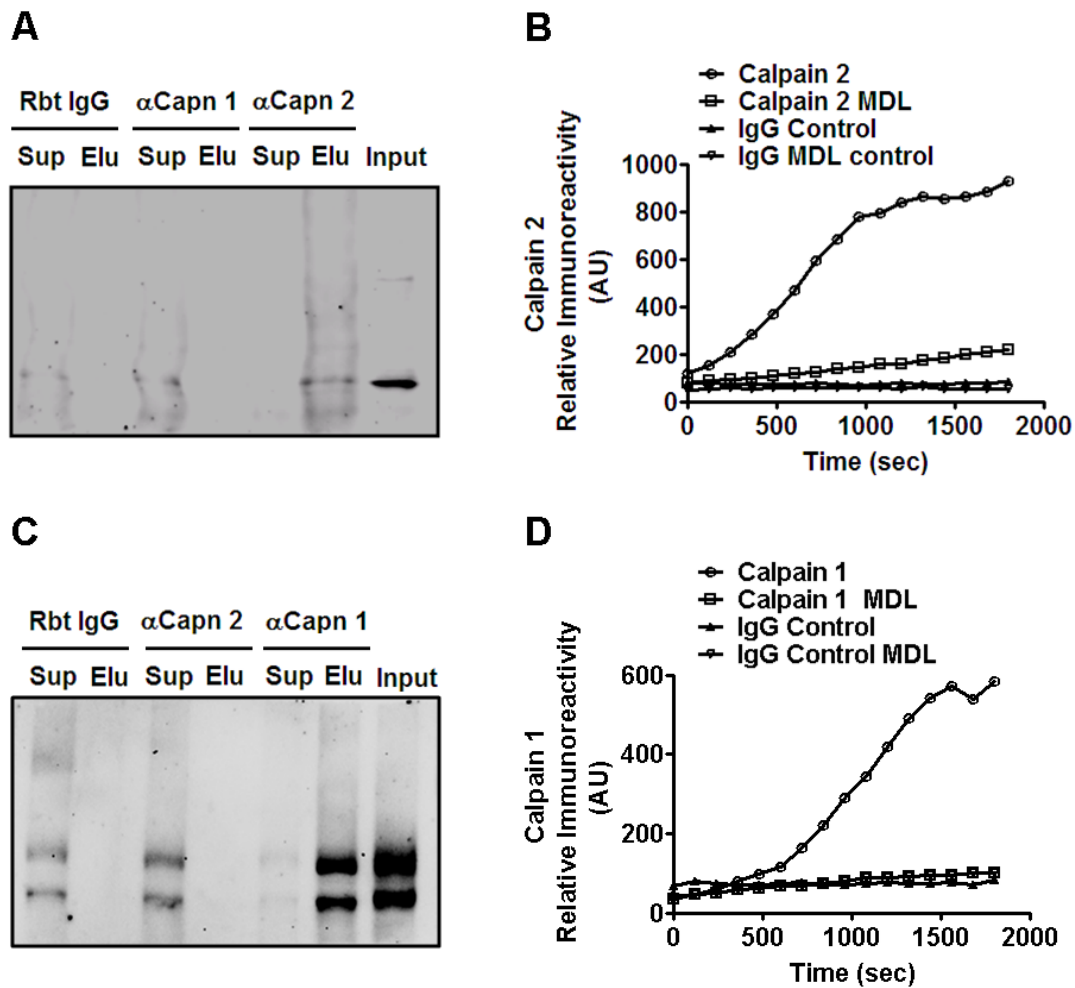
Supplementary Table 1

Case	Age at death (yr.)	Gender	PMI (h)	Braak stage
NCI 1	77.31	female	5.83	I
NCI 2	93.64	female	12.42	I
NCI 3	91.14	female	16.33	0
NCI 4	78.67	male	7.00	I
NCI 5	87.87	male	6.17	I
NCI 6	85.83	male	7.67	I
NCI 7	71.66	male	5.43	0
NCI 8	81.16	male	12.43	I
NCI 9	96.02	female	4.17	I
NCI 10	83.37	female	1.00	I
NCI 11	80.85	female	4.83	I
NCI 12	75.85	female	3.00	I
MEAN ± SD	83.61 ± 7.48		7.19 ± 4.42	
MCI 1	78.73	female	13.58	0
MCI 2	91.28	female	6.38	IV
MCI 3	93.63	female	12.33	II
MCI 4	74.96	male	16.00	III
MCI 5	85.85	male	10.63	IV
MCI 6	89.78	male	20.88	IV
MCI 7	85.32	male	5.00	III
MCI 8	96.32	male	5.92	III
MCI 9	90.70	female	4.33	III
MCI 10	99.36	female	4.33	III
MCI 11	90.67	female	4.55	III
MCI 12	80.09	female	4.78	III
MEAN ± SD	88.06 ± 7.32		9.06 ± 5.55	
AD 1	80.64	female	6.00	V
AD 2	90.10	female	4.97	V
AD 3	93.24	female	18.17	IV
AD 4	92.40	male	12.9	III
AD 5	90.31	male	2.75	V
AD 6	85.11	male	5.00	V
AD 7	92.33	female	7.42	V
AD 8	92.51	female	17.58	V
AD 9	94.98	female	5.25	V
AD 10	89.66	female	11.25	V
AD 11	88.18	female	5.08	V
AD 12	94.75	female	3.12	V
MEAN ± SD	90.35 ± 4.14		8.29 ± 5.38	

Supplementary Table 1. Details of patients with no cognitive impairment (NCI), mild cognitive impairment (MCI) and Alzheimer's disease dementia (AD).

PMI: post-mortem interval

Supplementary Figure 1

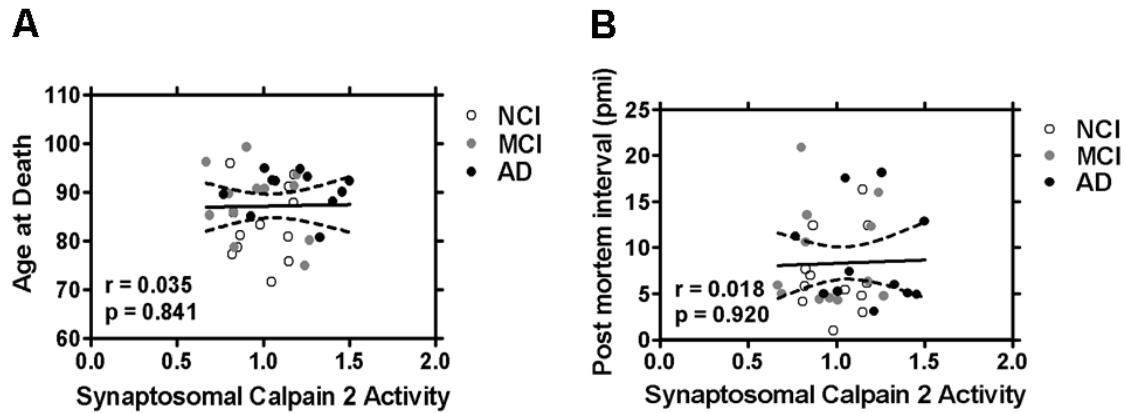


Supplementary Fig. 1. Immunoprecipitation was employed to assay isoform specific calpain-2 and calpain-1 activities in synaptosomes.

(A) Use of calpain-2 specific antibody allowed immunoprecipitation of calpain-2 (as seen as calpain-2 immunoreactive band in the eluent fraction) from synaptosomal samples prepared from mouse brain cortex. Normal rabbit IgG used as a negative control did not pull down calpain-2. Calpain-1 antibody used for immunoprecipitation was also unable to pull down calpain-2. 'Elu' represents eluent or immunoprecipitated fraction and 'Sup' represents supernatant or unbound fraction. 'Input' was used as a

marker for calpain-2. **(B)** Use of fluoregenic Suc-LLVY-AMC peptide substrate confirmed synaptosomal calpain-2 activation in samples immunoprecipitated with anti-calpain-2 antibody but not with normal rabbit IgG. Calpain inhibitor MDL 28170 blocked calpain-2 activation. **(C)** Anti-calpain-1 but not anti-calpain 2 antibody could immunoprecipitate calpain-1 from synaptosomal samples prepared from mouse brain cortex. Normal rabbit IgG was used as a negative control and did not pull down calpain-1. 'Elu' represents eluent or immunoprecipitated fraction and 'Sup' represents supernatant or unbound fraction. 'Input' was used a marker for calpain-1. **(D)** Fluoregenic Suc-LLVY-AMC peptide substrate was used to confirm activation of synaptosomal calpain-1 in samples immunoprecipitated with anti-calpain-1 antibody but not with normal rabbit IgG. Calpain inhibitor MDL 28170 blocked calpain-1 activation.

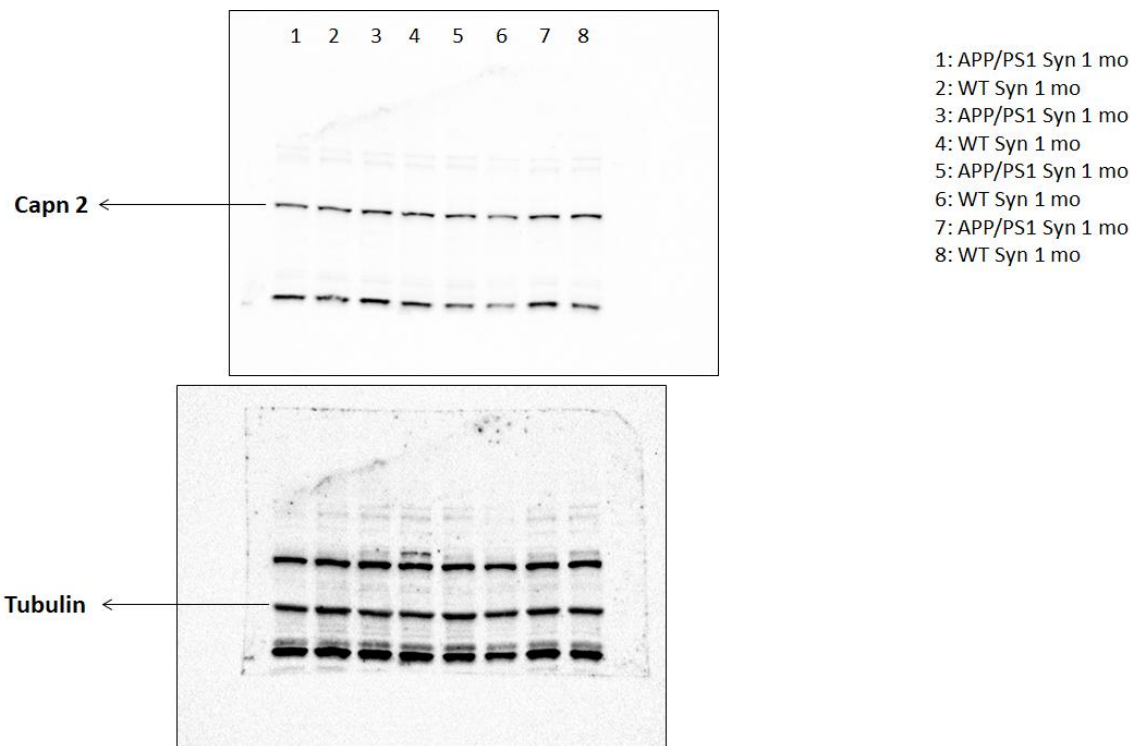
Supplementary Figure 2



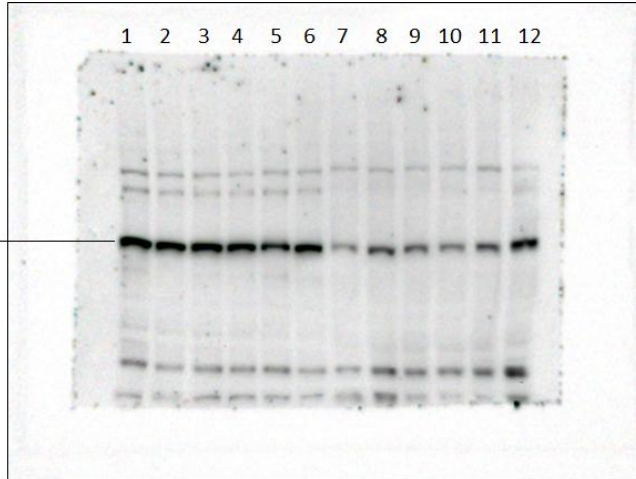
Supplementary Fig. 2. Activity levels of synaptosomal calpain-2 of frontal neocortical tissue samples of post-mortem brains from human subjects are not affected by post mortem indices or age at death.

No correlation was observed between synaptosomal calpain-2 activity and age of death **(A)** and post mortem index (pmi; **(B)**) of the subjects.

Supplementary Figure 3. Representative full blots for data in Figure 3A.

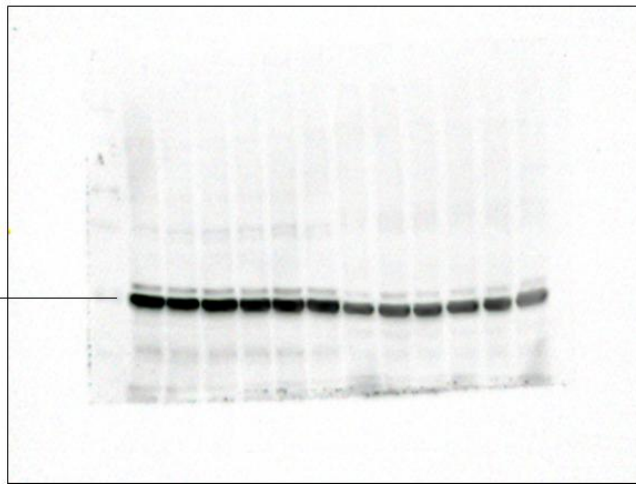


Capn 2 ←

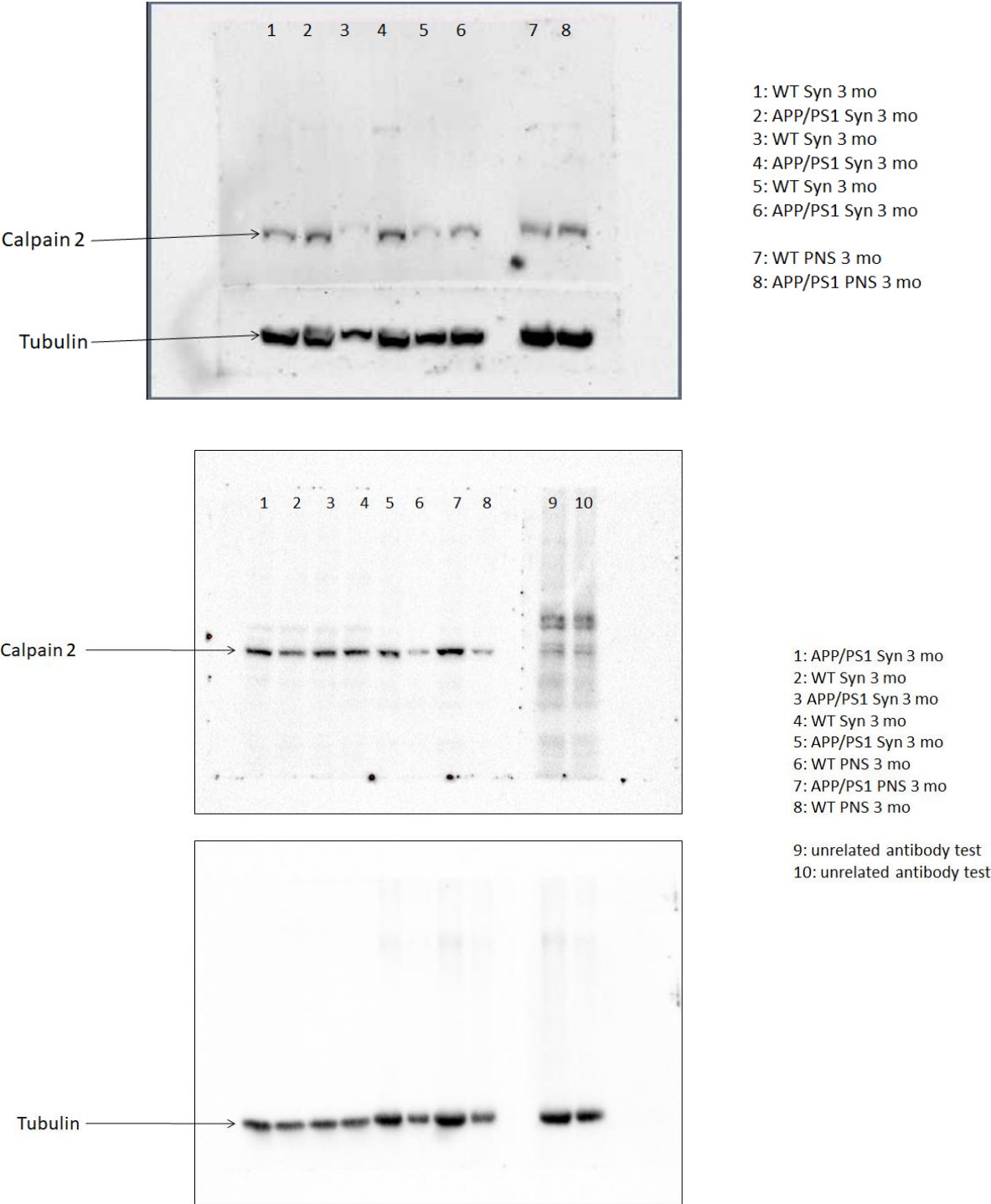


- 1: WT PNS 3 mo
- 2: APP/PS1 PNS 3 mo
- 3: WT PNS 3 mo
- 4: APP/PS1 PNS 3 mo
- 5: APP/PS1 PNS 3 mo
- 6: WT PNS 3 mo
- 7: WT Syn 1 mo
- 8: APP/PS1 Syn 1 mo
- 9: WT Syn 1 mo
- 10: APP/PS1 Syn 1 mo
- 11: WT Syn 1 mo
- 12: APP/PS1 Syn 1 mo

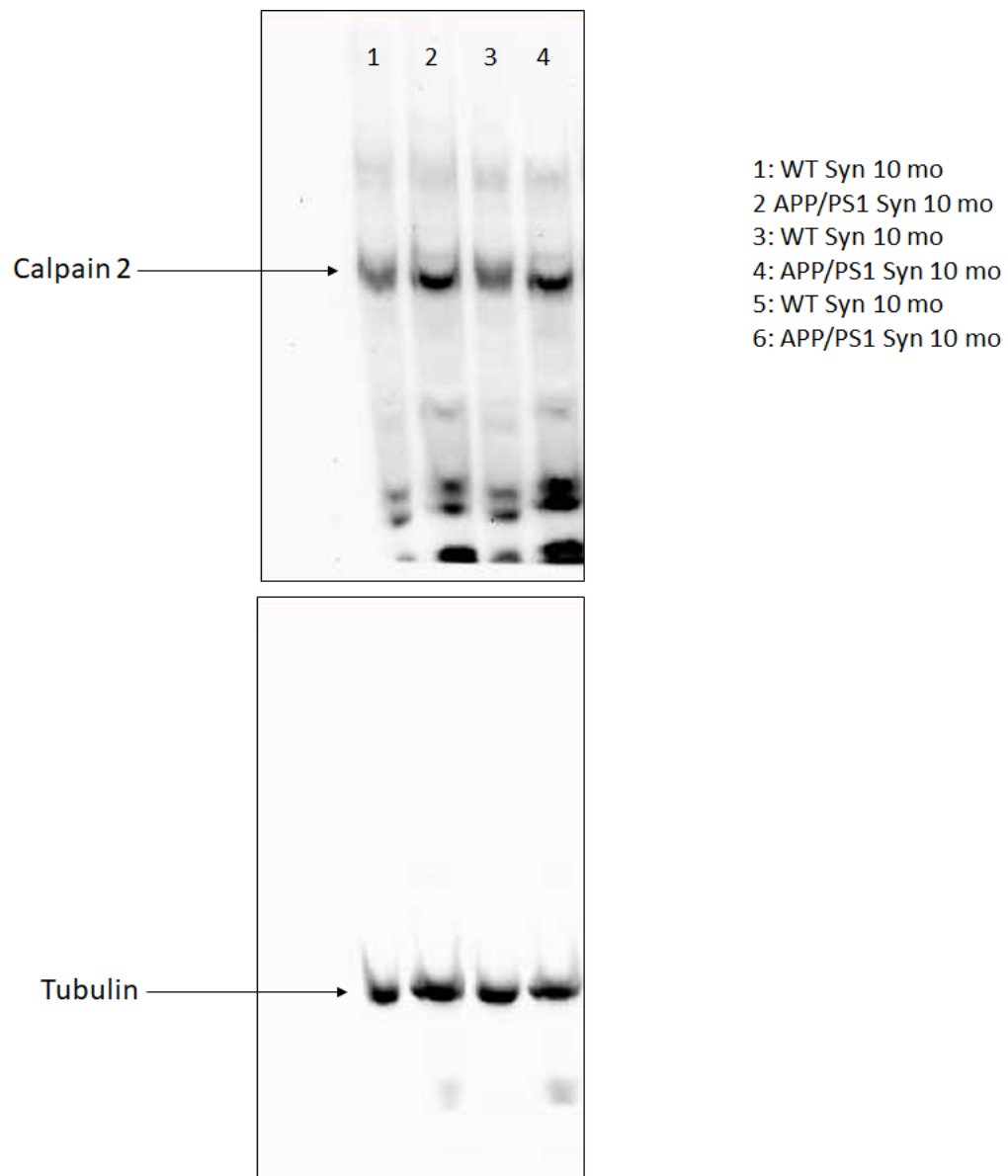
Tubulin ←

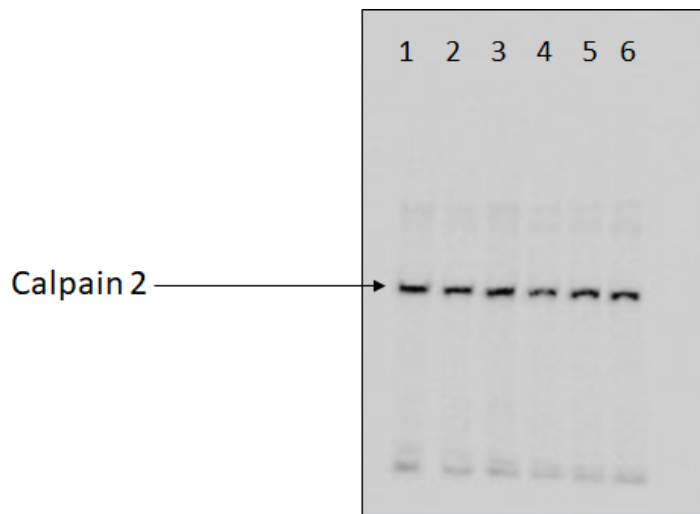


Supplementary Figure 4. Representative full blots for data in Figure 3B.

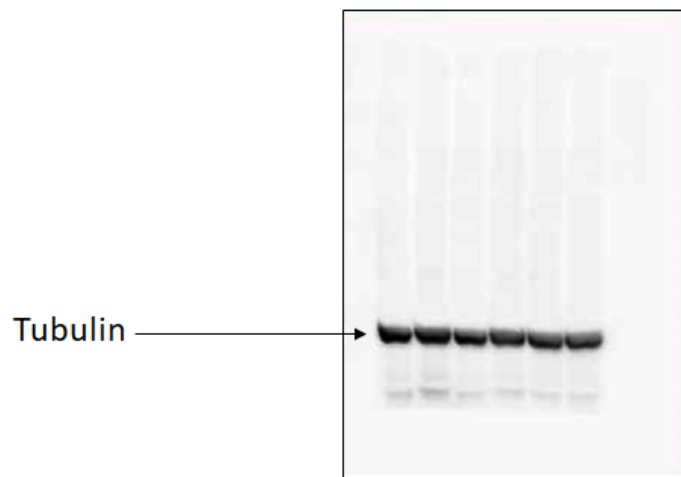


Supplementary Figure 5. Representative full blots for data in Figure 3C.

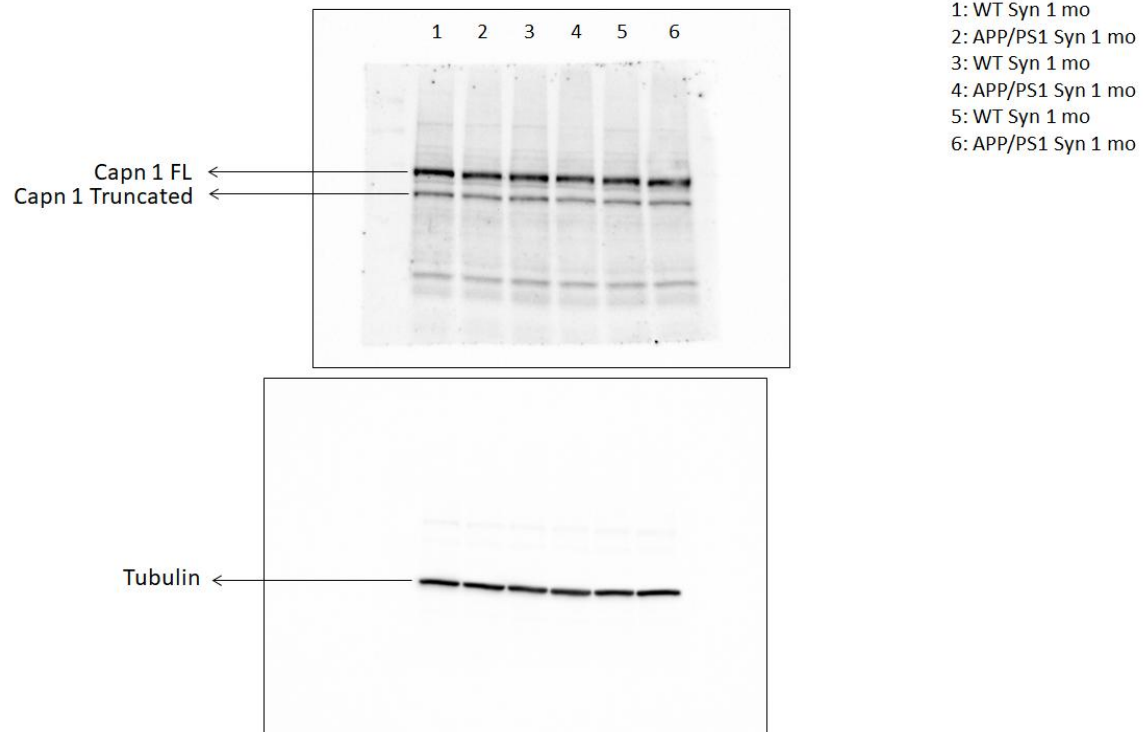


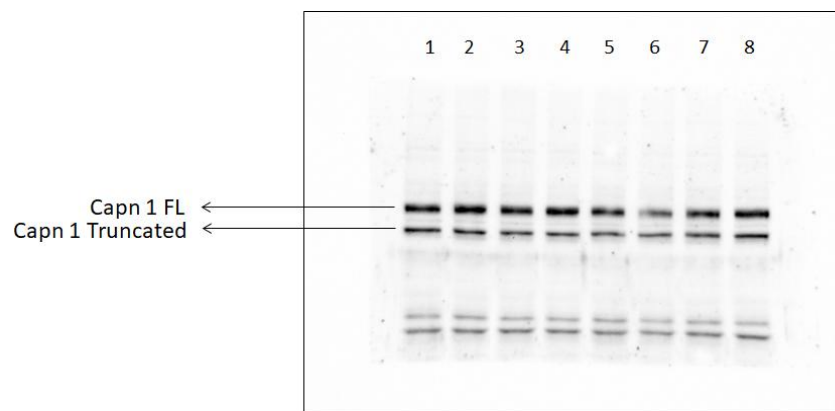


1: APP/PS1 Syn 10 mo
2: WT Syn 10 mo
3: APP/PS1 Syn 10 mo
4: WT Syn 10 mo
5: APP/PS1 Syn 10 mo
6: WT Syn 10 mo

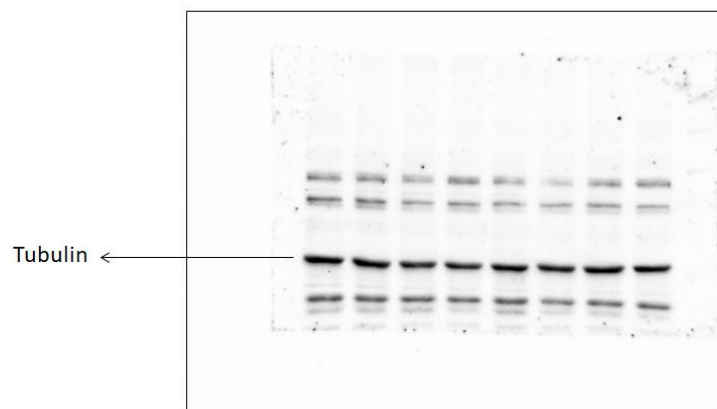


Supplementary Figure 6. Representative full blots for data in Figure 4A.

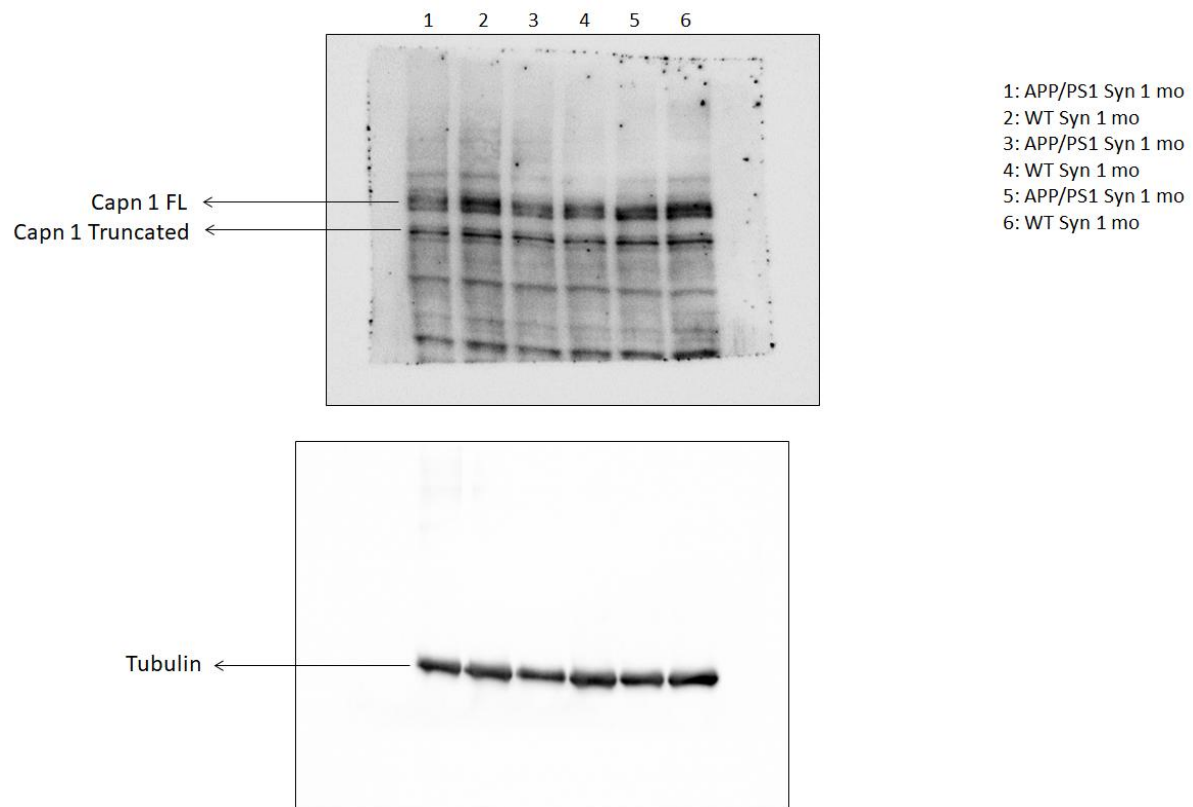




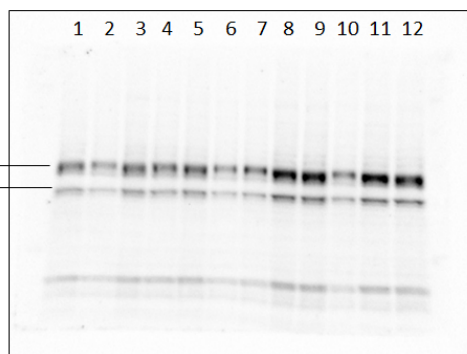
- 1: APP/PS1 Syn 1 mo
- 2: WT Syn 1 mo
- 3: APP/PS1 Syn 1 mo
- 4: WT Syn 1 mo
- 5: APP/PS1 Syn 1 mo
- 6: WT Syn 1 mo
- 7: APP/PS1 Syn 1 mo
- 8: WT Syn 1 mo



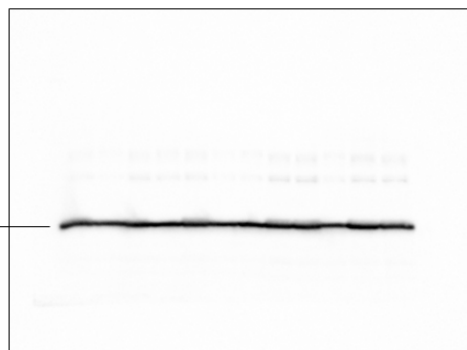
Supplementary Figure 7. Representative full blots for data in Figure 4B.



Capn 1 FL ←
Capn 1 Truncated ←

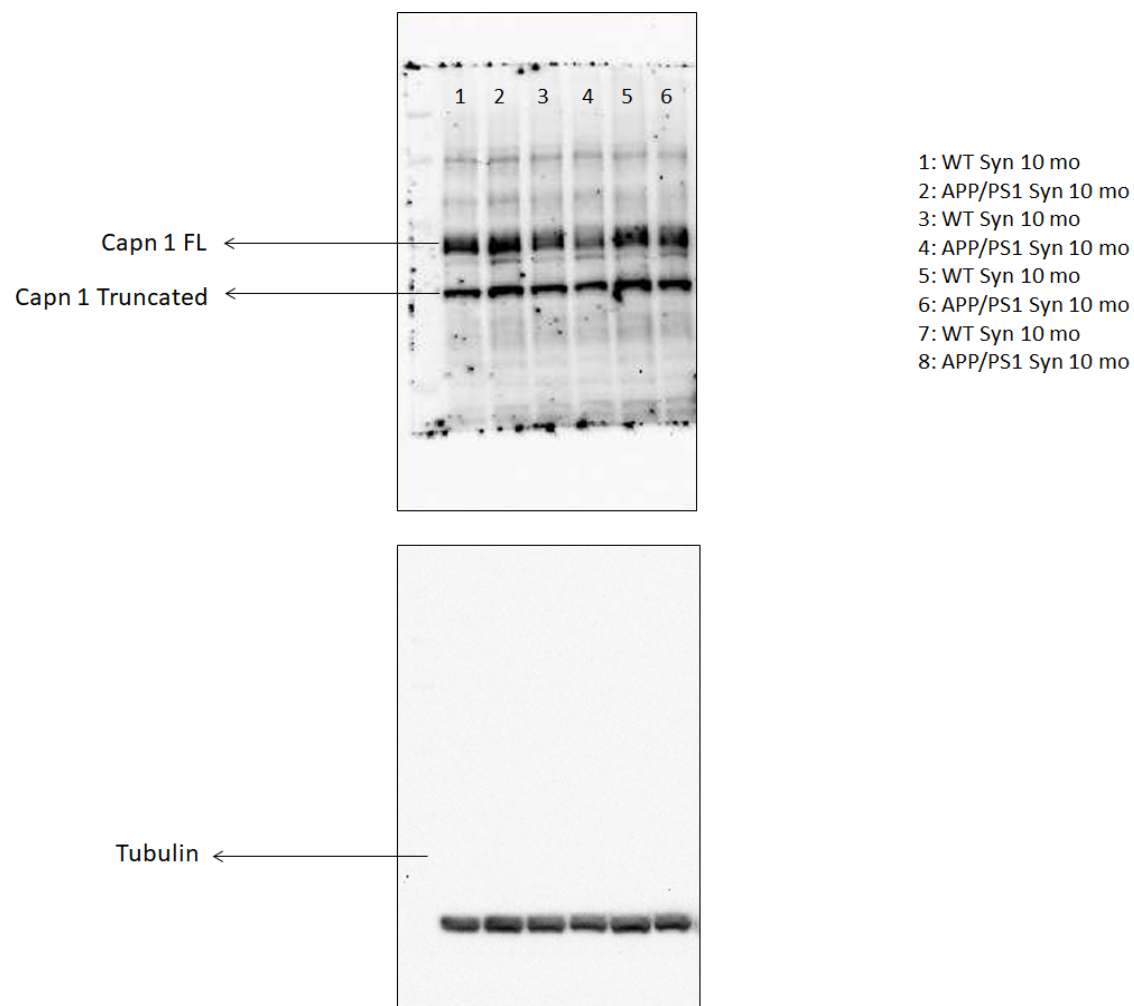


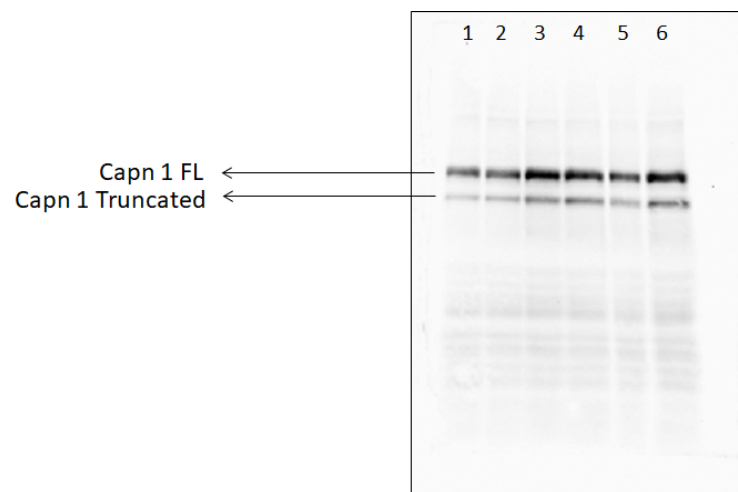
Tubulin ←



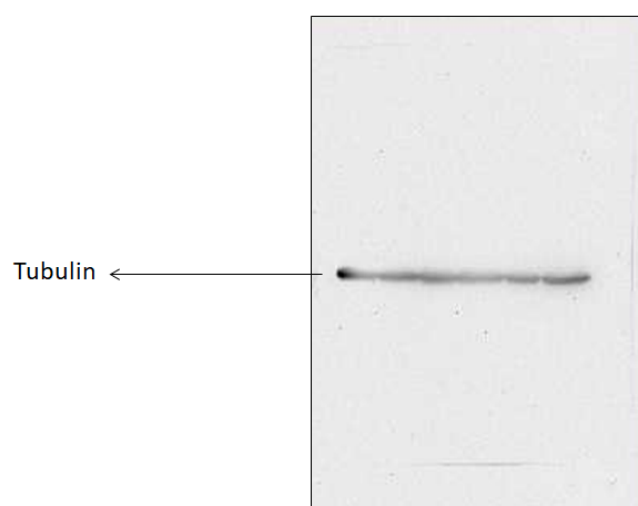
1: APP/PS1 Syn 3 mo
2: WT Syn 3 mo
3: APP/PS1 Syn 3 mo
4: WT Syn 3 mo
5: APP/PS1 Syn 3 mo
6: WT Syn 3 mo
7: APP/PS1 Syn 3 mo
8: WT Syn 3 mo
9: APP/PS1 Syn 3 mo
10: WT Syn 3 mo
11: APP/PS1 Syn 3 mo
12: WT Syn 3 mo

Supplementary Figure 8. Representative full blots for data in Figure 4C.

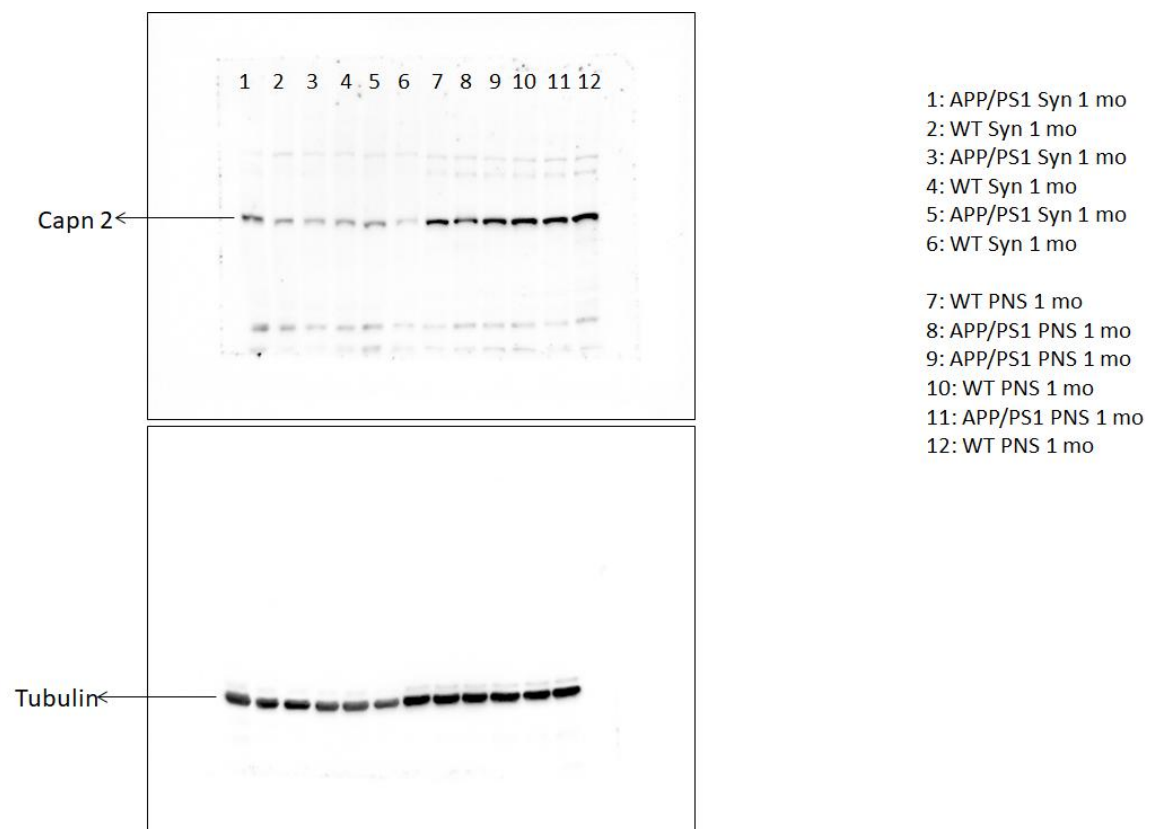


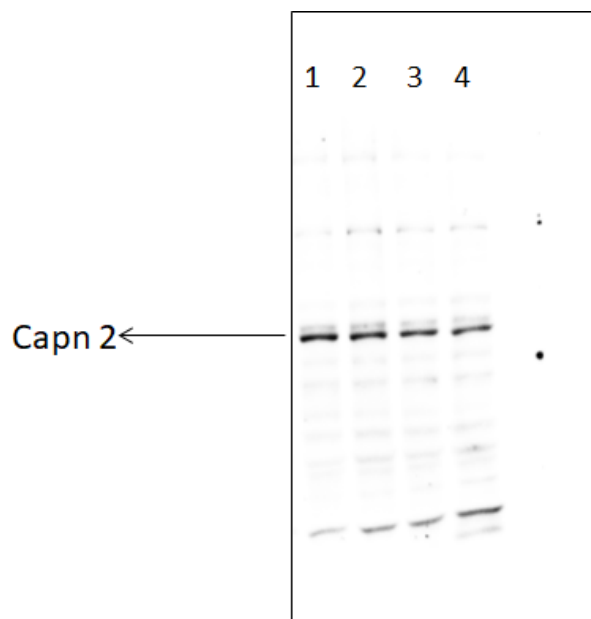


- 1: APP/PS1 Syn 10 mo
- 2: WT Syn 10 mo
- 3: APP/PS1 Syn 10 mo
- 4: WT Syn 10 mo
- 5: APP/PS1 Syn 10 mo
- 6: WT Syn 10 mo
- 7: APP/PS1 Syn 10 mo
- 8: WT Syn 10 mo

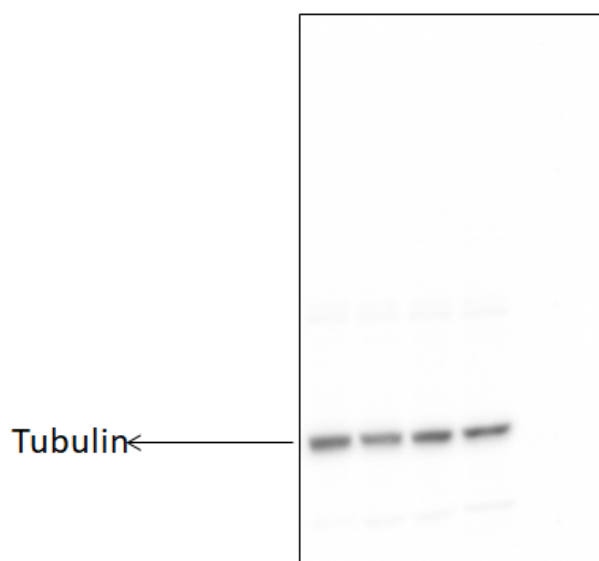


Supplementary Figure 9. Representative full blots for data in Figure 5A.

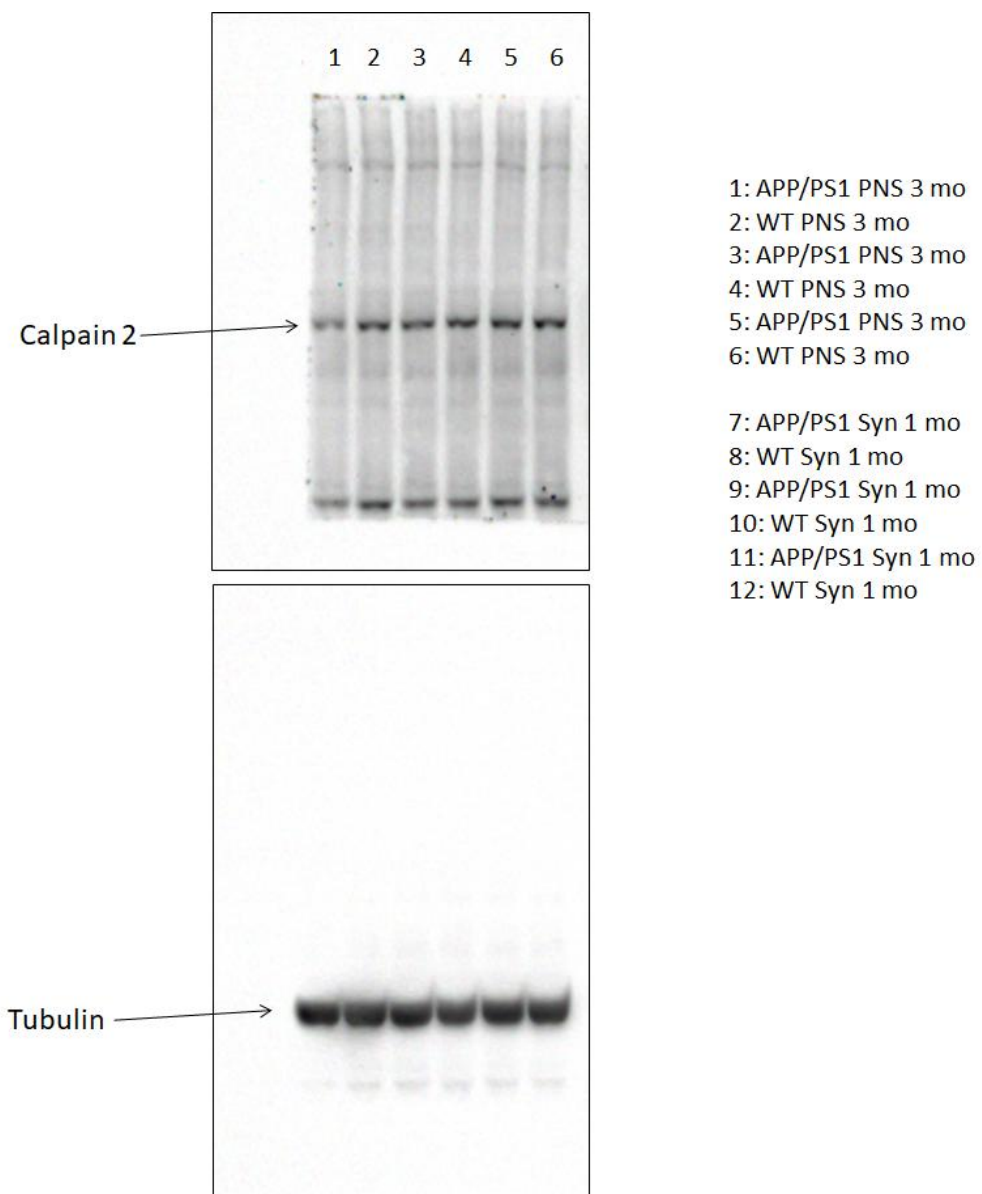


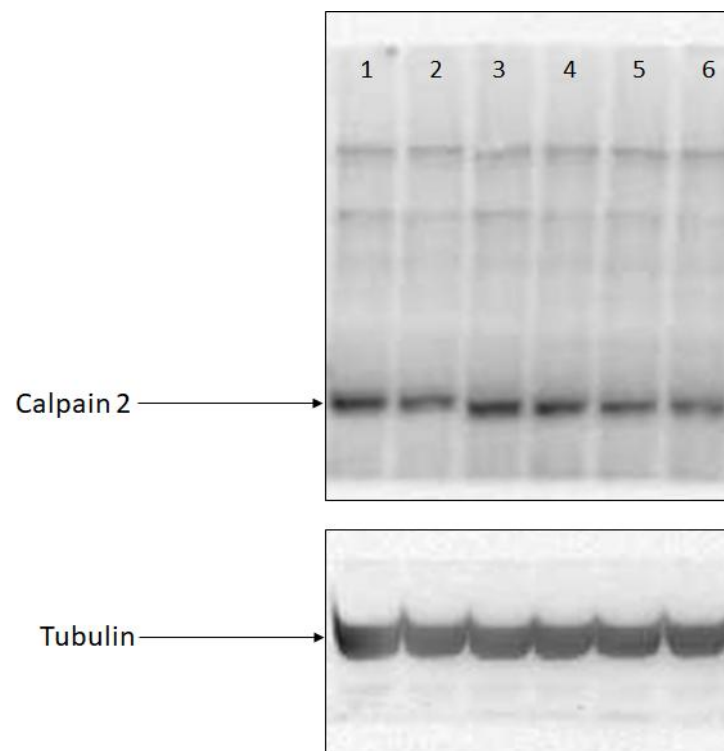


1: APP/PS1 PNS 1 mo
2: WT PNS 1 mo
3: APP/PS1 PNS 1 mo
4: WT PNS 1 mo



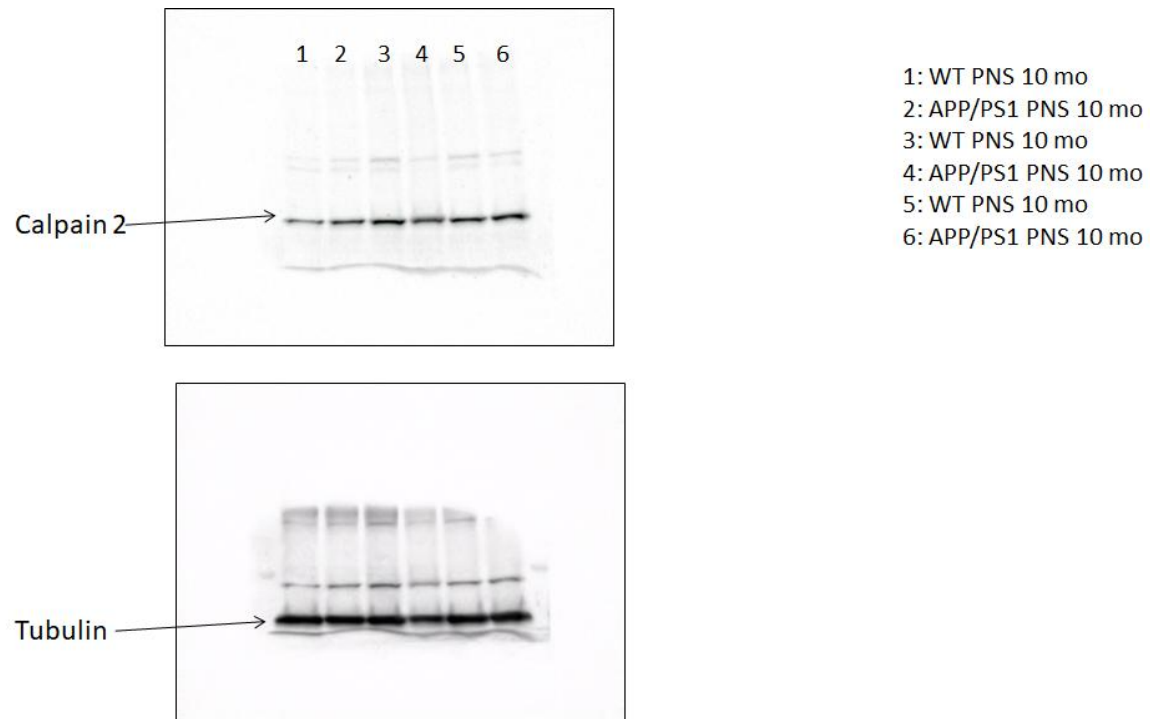
Supplementary Figure 10. Representative full blots for data in Figure 5B.

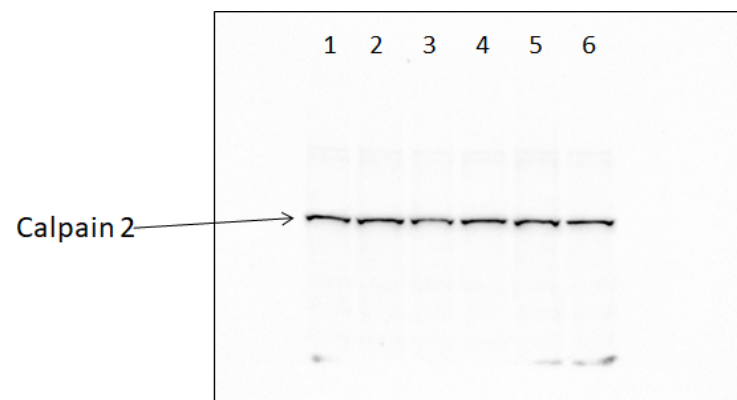




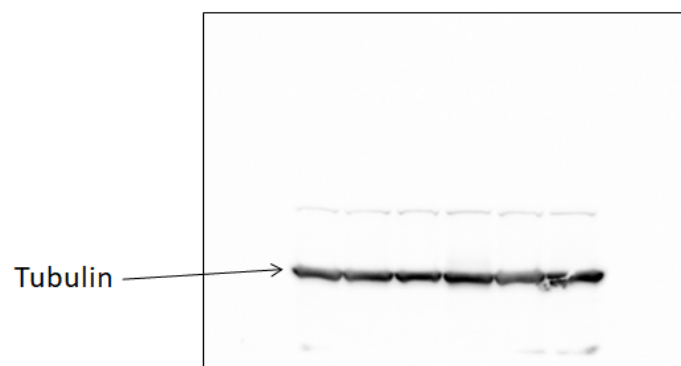
- 1: WT PNS 3 mo
- 2: APP/PS1 PNS 3 mo
- 3: WT PNS 3 mo
- 4: APP/PS1 PNS 3 mo
- 5: WT PNS 3 mo
- 6: APP/PS1 PNS 3 mo

Supplementary Figure 11. Representative full blots for data in Figure 5C.

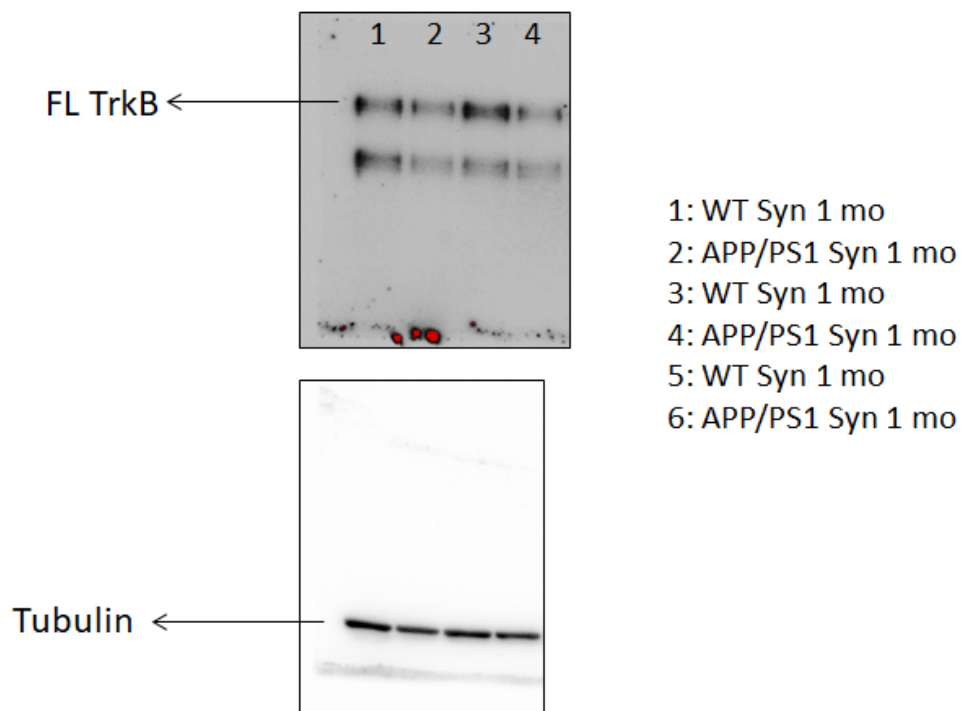


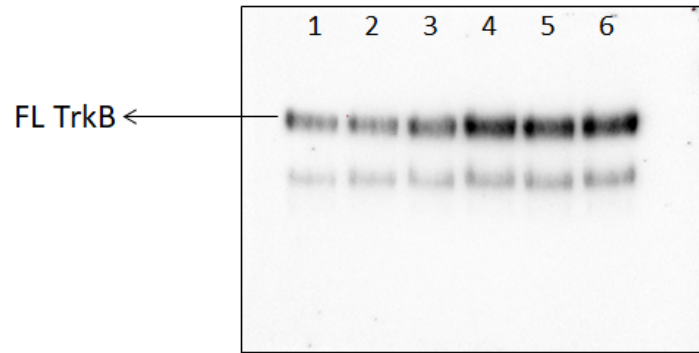


1: WT PNS 10 mo
2: APP/PS1 PNS 10 mo
3: WT PNS 10 mo
4: APP/PS1 PNS 10 mo
5: WT PNS 10 mo
6: WT PNS 10 mo

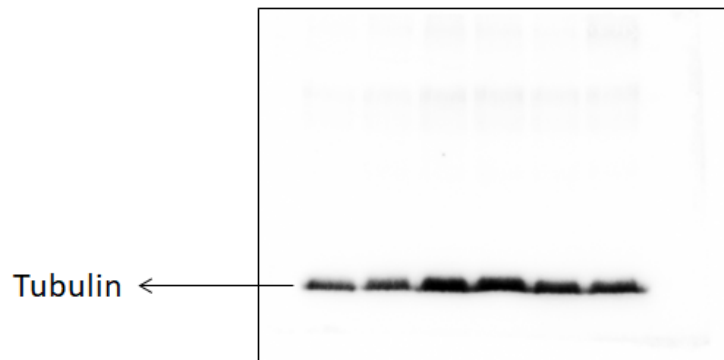


Supplementary Figure 12. Representative full blots for data in Figure 7A.

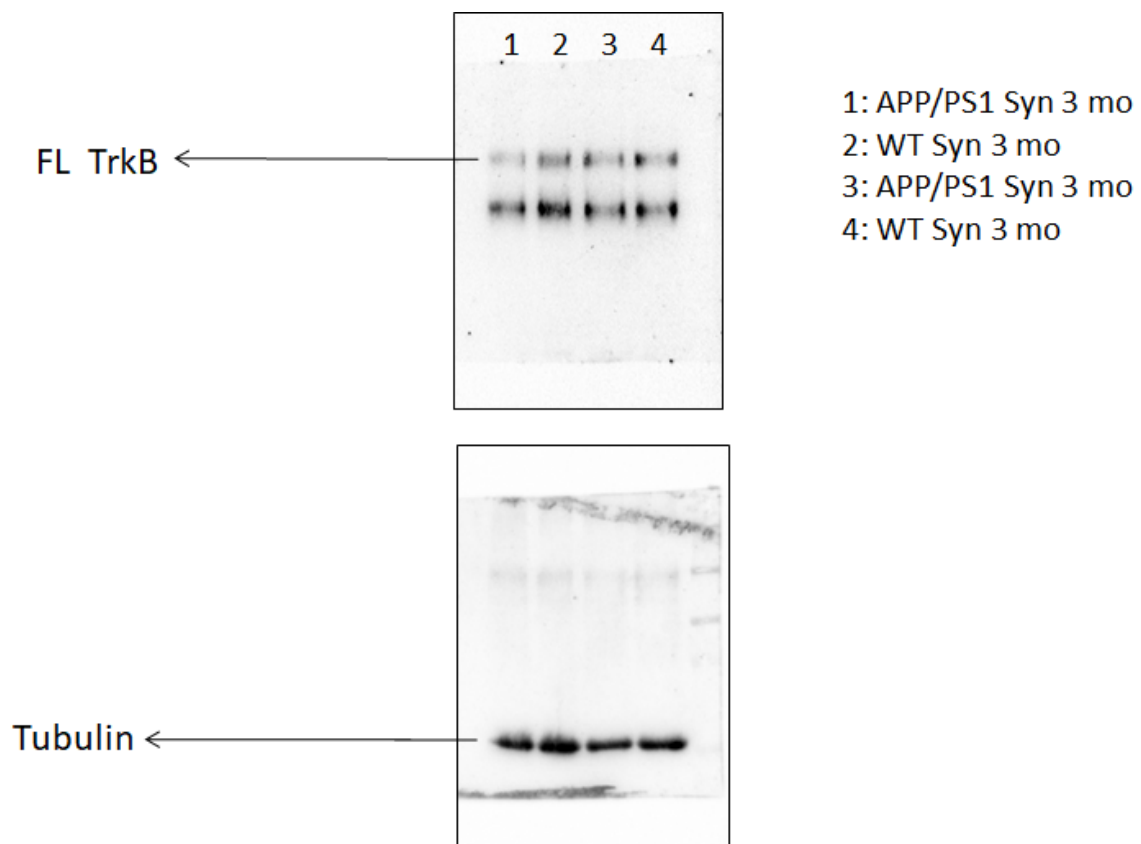


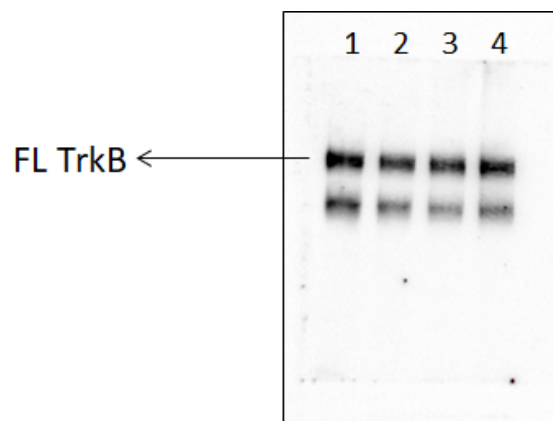


1: APP/PS1 Syn 1 mo
2: APP/PS1 Syn 1 mo
3: APP/PS1 Syn 1 mo
4: WT Syn 1 mo
5: WT Syn 1 mo
6: WT Syn 1 mo

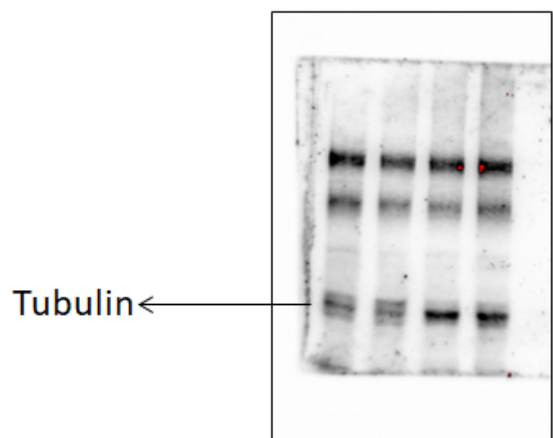


Supplementary Figure 13. Representative full blots for data in Figure 7B.

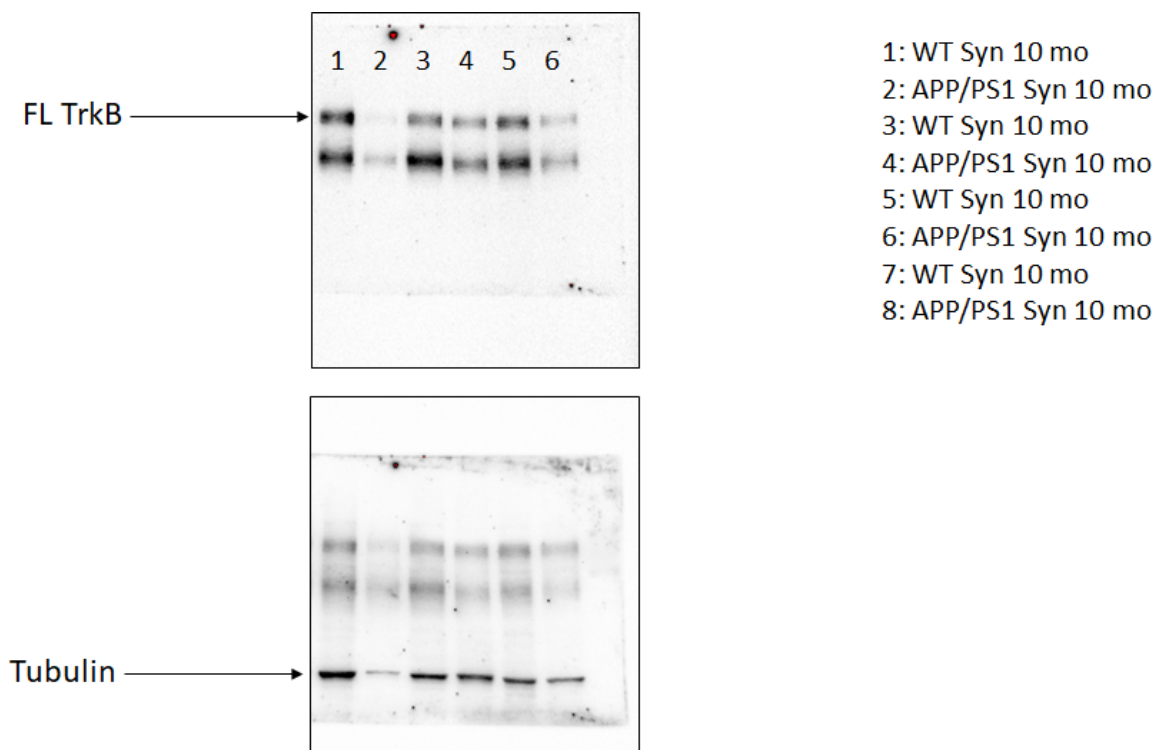


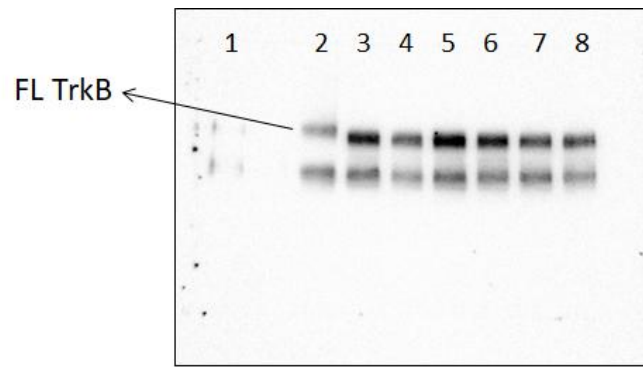


1: WT Syn 3 mo
2: APP/PS1 Syn 3 mo
3: WT Syn 3 mo
4: APP/PS1 Syn 3 mo



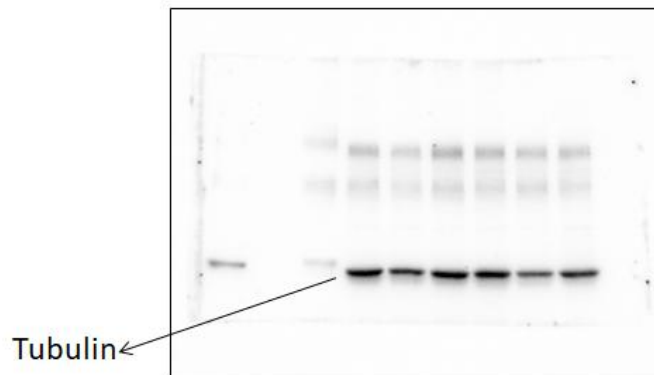
Supplementary Figure 14. Representative full blots for data in Figure 7C.



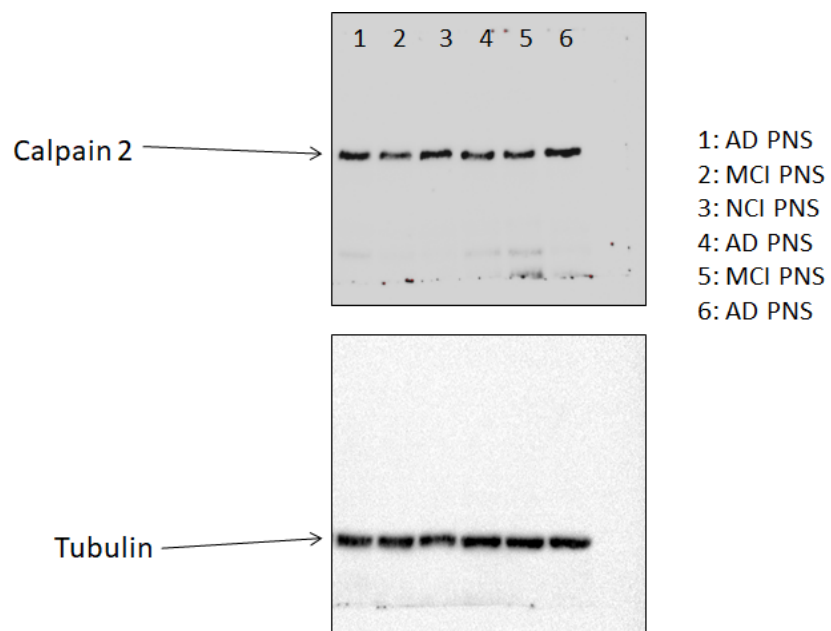


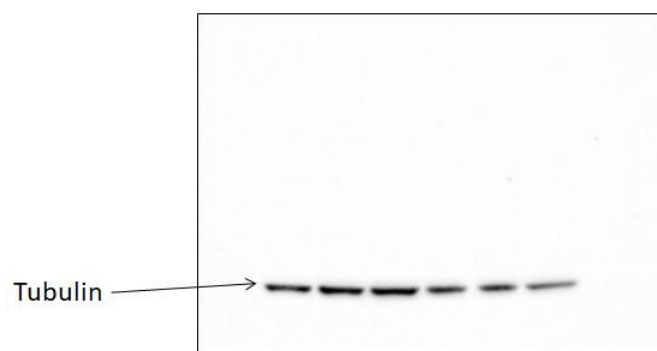
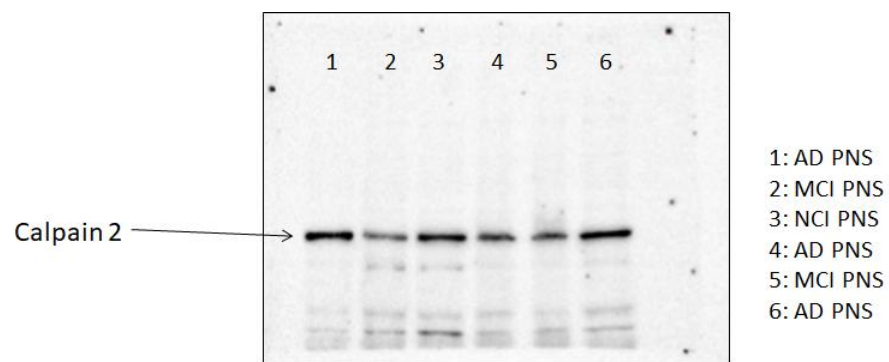
1: Human Syn test
2: Human PNS test

3: WT Syn 10 mo
4: APP/PS1 Syn 10 mo
5: WT Syn 10 mo
6: WT Syn 10 mo
7: APP/PS1 Syn 10 mo
8: APP/PS1 Syn 10 mo



Supplementary Figure 15. Representative full blots for data in Figure 9A.





Supplementary Figure 16. Representative full blots for data in Figure 9B.

