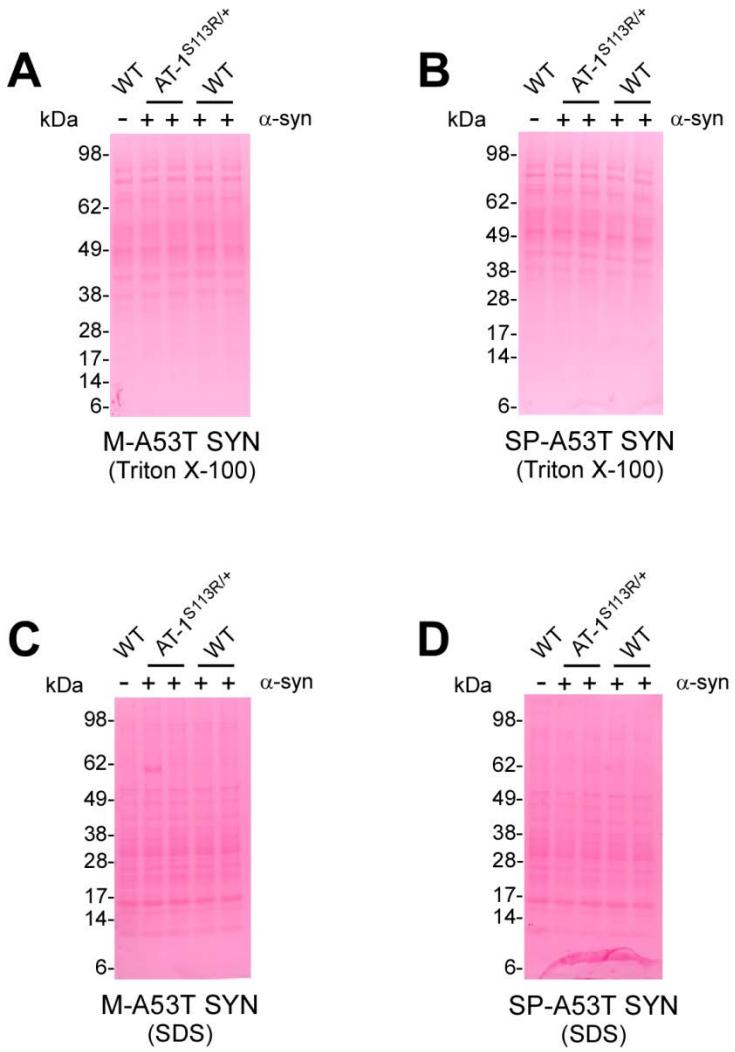
SUPPLEMENTARY MATERIAL

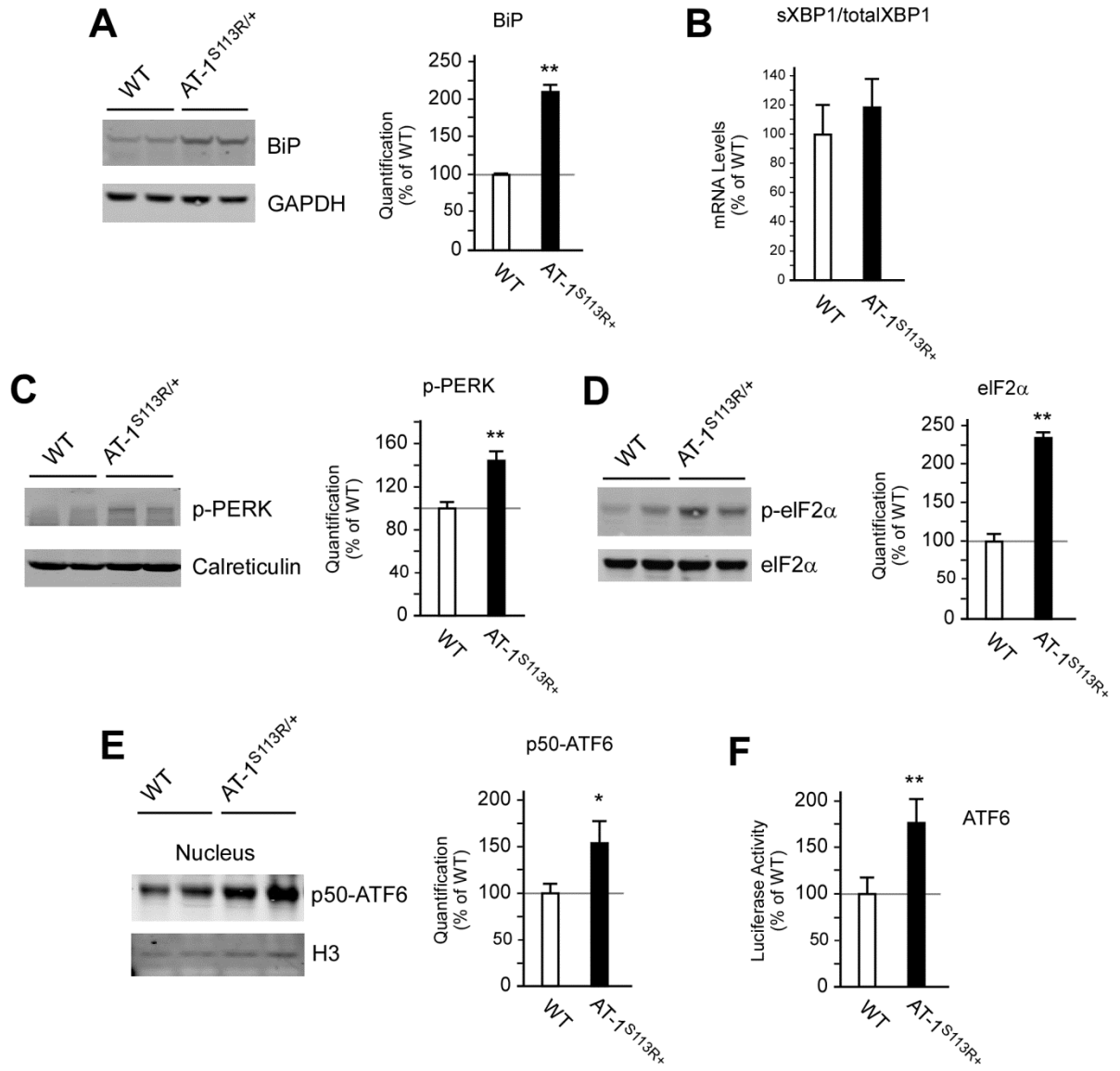
Improved Proteostasis in the Secretory Pathway Rescues Alzheimer's Disease in the Mouse

Yajing Peng, Mi Jin Kim, Rikki Hullinger, Kenneth J. O'Riordan, Corinna Burger, Mariana Pehar, Luigi Puglielli



Supplementary Figure 1. **Loading controls for Fig. 1, B-E.**

(A-D) Mouse embryo fibroblasts (MEFs) from wild-type (WT) and AT-$1^{S113R/+}$ mice were transfected with a mutant pro-aggregating version of α-synuclein (A53T syn) associated with Parkinson's disease. Loading controls (Ponceau Red staining) for soluble (Triton X-100) and insoluble/aggregated (SDS) extractions are shown.



Supplementary Figure 2. AT-1$^{S113R/+}$ mice display general activation of the UPR.

(A) Western blot determination of BiP.

(B) Real-time quantitation of sXBP1 and totalXBP1 mRNA levels.

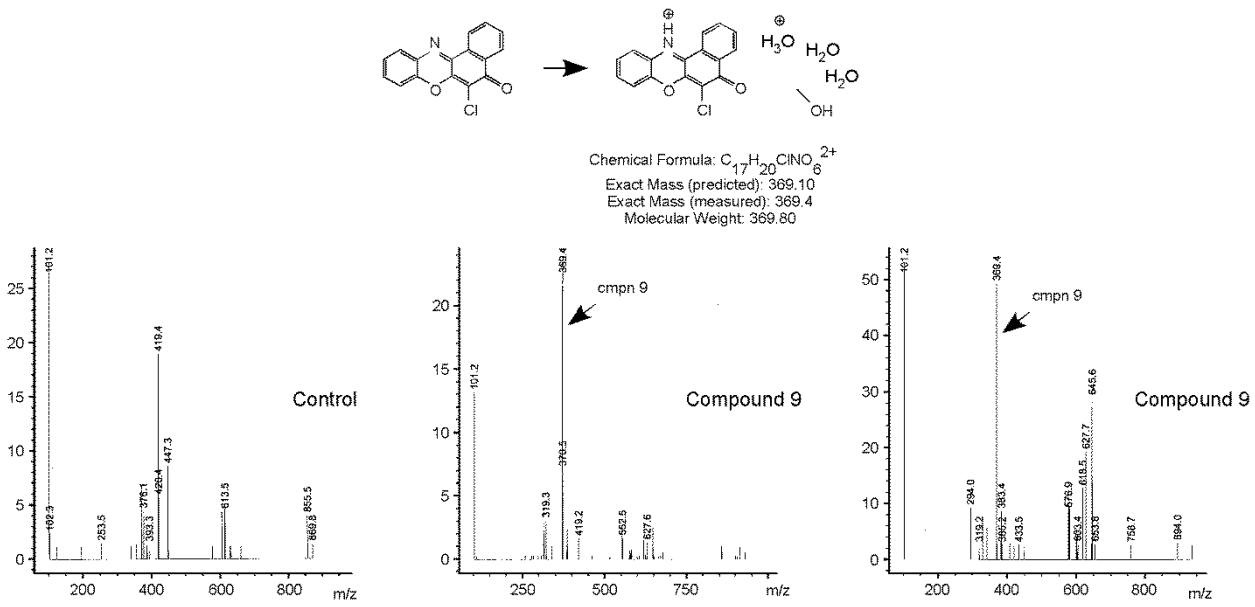
(C) Western blot determination of p-PERK.

(D) Western blot determination of eLF2α.

(E) Western blot determination of p50-ATF6.

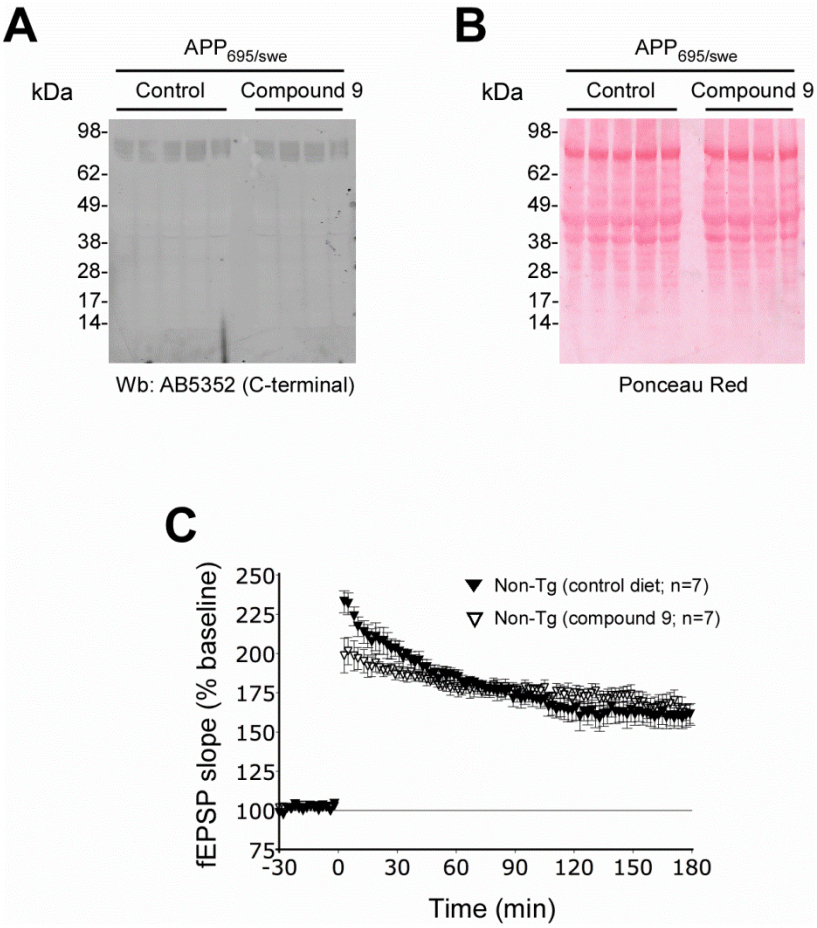
(F) Luciferase activity of ATF6 following transfection in MEF.

Due to the unique characteristics of the tissue, (A-E) were performed in liver. Values are mean ± SD. *, $p<0.05$; ** $p<0.005$.



Supplementary Figure 3. **Compound 9 is found in the CSF.**

Mass spectrometry determination of compound 9 in the cerebrospinal fluid of WT mice fed control or compound 9 diets. One control- and two compound 9-treated animals are shown.

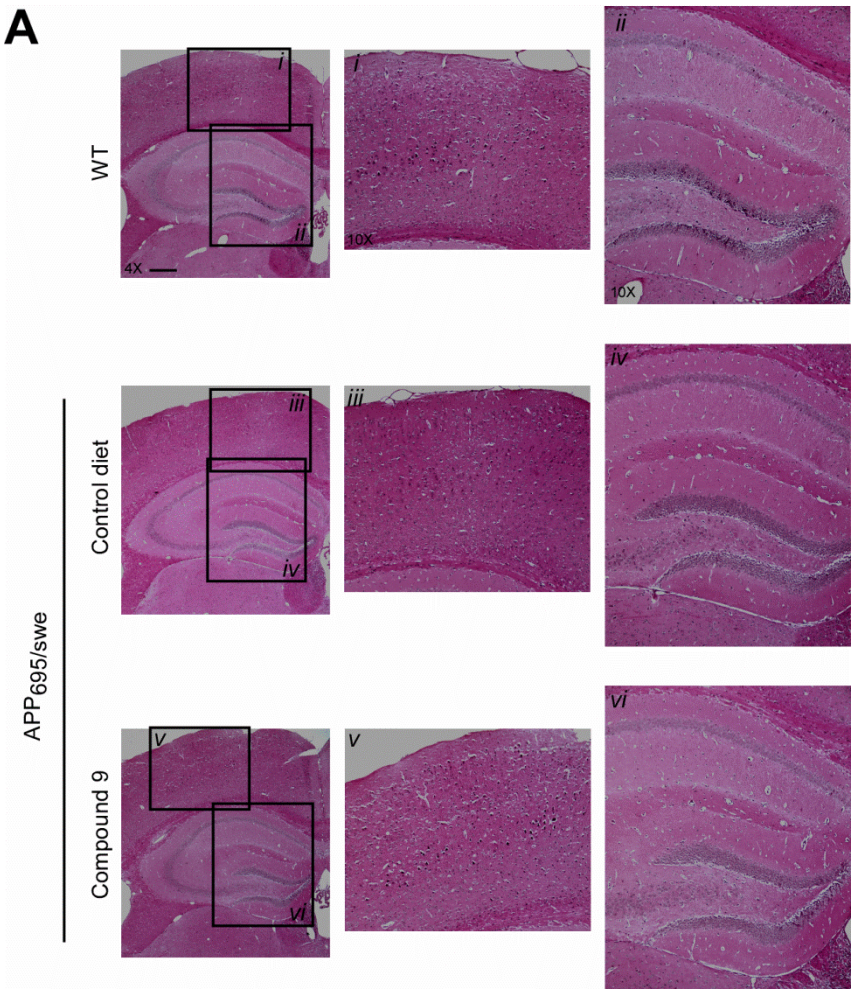


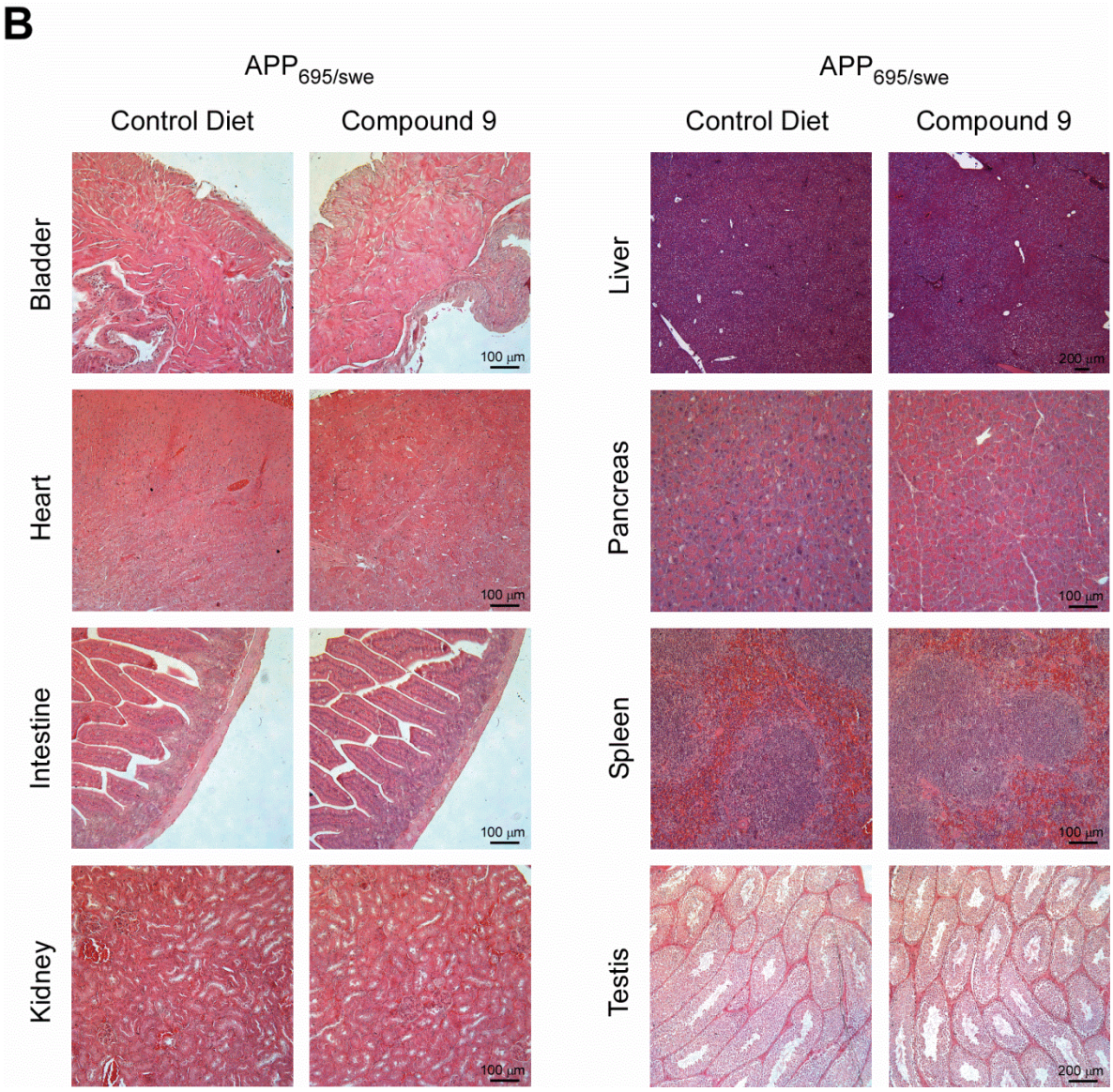
Supplementary Figure 4. **Loading controls for Fig. 6A and LTP of Non-Tg mice.**

(**A)** Blot shown in Fig. 6A was probed with an antibody against the C-terminus of APP to confirm that bands shown in Fig. 6A are not C-terminal membrane-bound fragments of APP.

(B) Loading control for the same blot.

(C) LTP induction in hippocampal slices of Non-Tg mice. Values are mean ± SD.


A
WT
Control diet
Compound 9
APP695/swe
i
ii
iii
iv
v
vi
4X
10X
10X

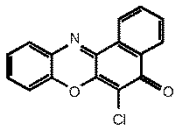
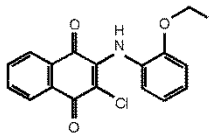


Supplementary Figure 5. Central and peripheral tissues in compound 9-treated mice appear normal.

(A) H&E staining of the brain of the indicated animals show no significant differences.

(B) H&E staining of peripheral tissues in compound 9-treated animals show no evident signs of toxicity.

Supplementary Table 1. Drug-likeness properties of compound 9 and 19.

	Compound 9	Compound 19	Lipinski's rule (modified *)
			
Hydrogen bond donors:	0	1	<5
Hydrogen bond acceptor:	3	4	<10
MW:	281.698	327.767	160 to 480 Da
cLog*P*:	+3.71	+2.53	-0.4 to +5.6

* McFarland, J.W., *et al.* J Med Chem 1997; 40:1340-1346.
Ghose, A.K., *et al.* J Comb Chem 1999; 1:55-68.
Lipinski, C.A., *et al.* Adv Drug Deliv Rev 2001; 46:3-26.

Supplementary Table 2. Solubility of compound 9 and 19.

	Solubility (mg/ml)	
	Compound 9	Compound 19
0.1 M HCl	Not Detected	0.0004
Citrate Buffer pH=4	Not Detected	0.0004
Phosphate Buffer pH=7	Not Detected	0.0003
PEG 400	0.58	4.7
Transcutol HP	2.4	7.9
Ethanol	0.32	2.2

Supplementary Table 3. Diet composition (BioServ #F0078).

Crude Protein	18.7%
Fat	5.6%
Fibers	4.7%
Ash	6.5%
Moisture	<10%
Carbohydrate	59.1%

Caloric Profile:	
Protein	0.75 kcal/gm
Fat	0.50 kcal/gm
Carbohydrate	2.36 kcal/gm
Total	3.60 kcal/gm

ORIGINAL GELS

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Figure 1A

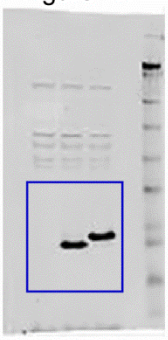


Figure 1A

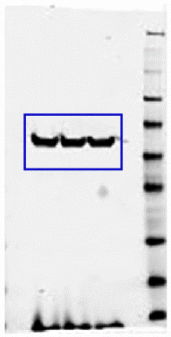


Figure 1B

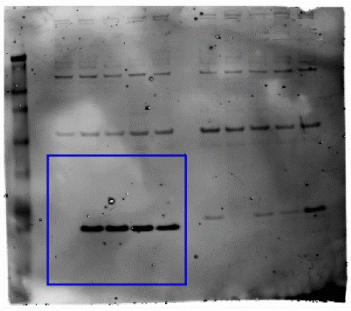


Figure 1C

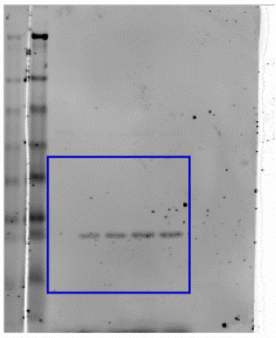


Figure 1D

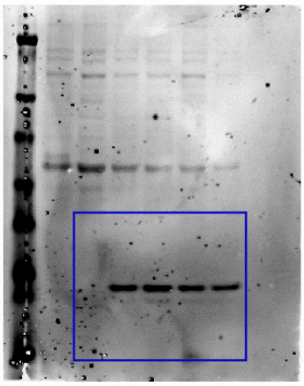


Figure 1E

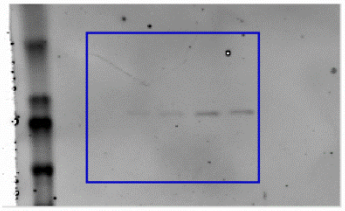


Figure 2D

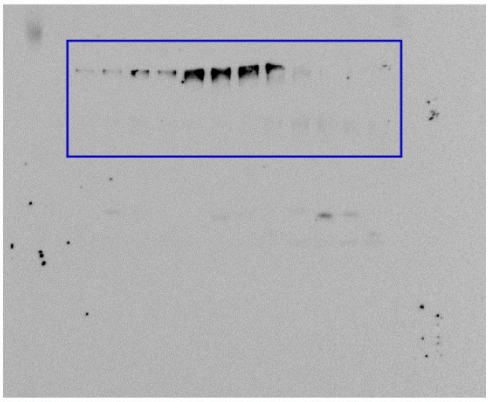
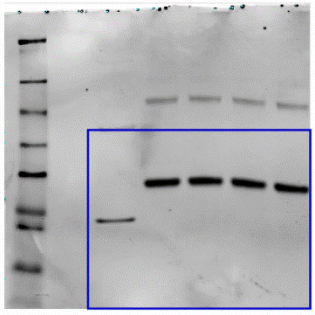


Figure 1G



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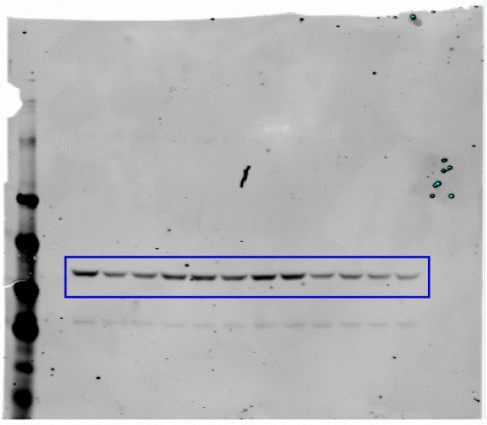

Figure 1J

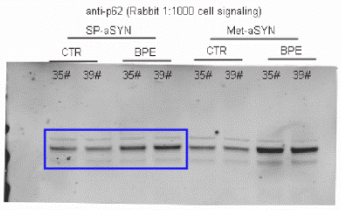

Figure 1J

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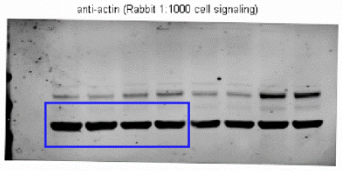


Figure 3D

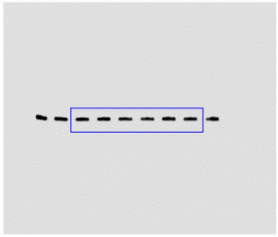


Figure 3D

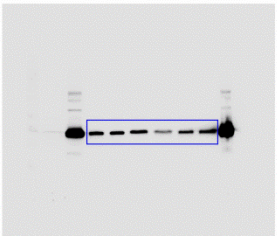


Figure 4C

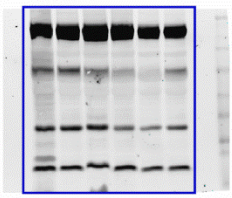
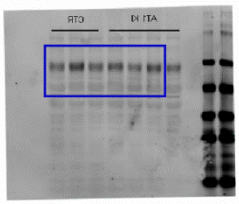


Figure 4F



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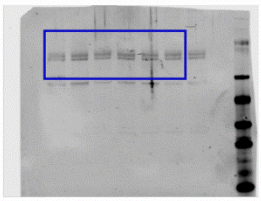

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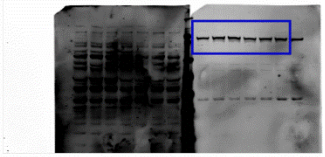

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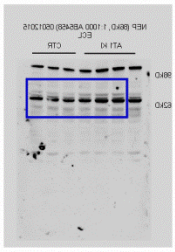

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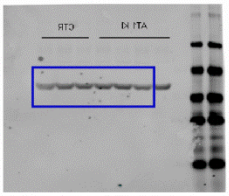


Figure 4F

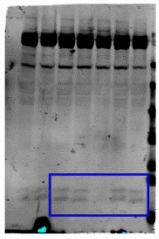


Figure 4F

actin cell signaling (1:1000)

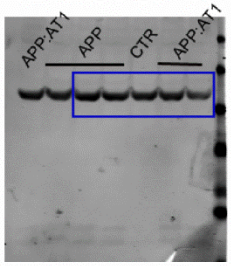
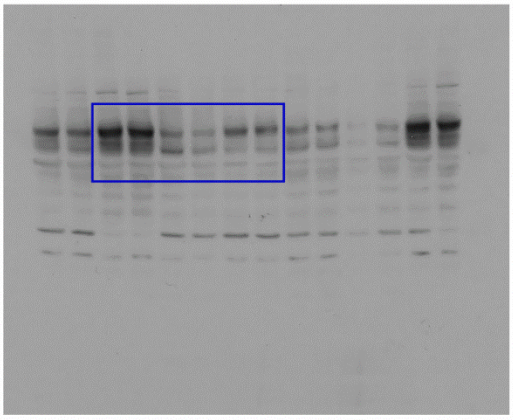


Figure 5B



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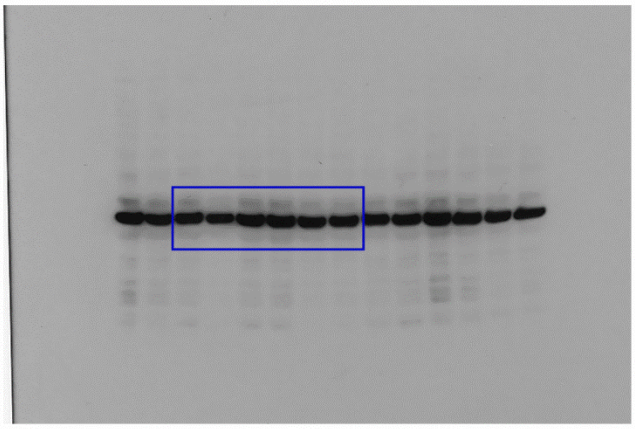

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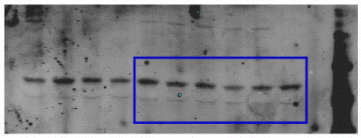

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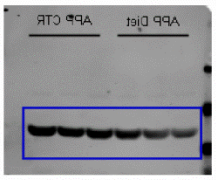

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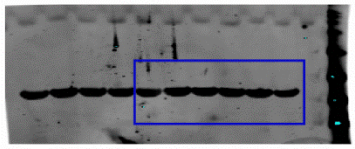

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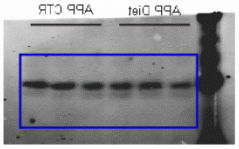

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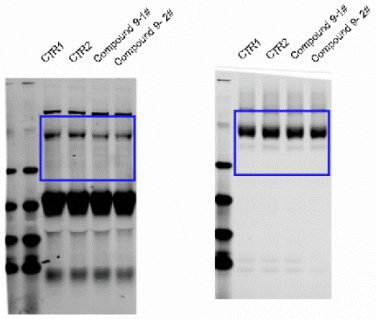


Figure 6A

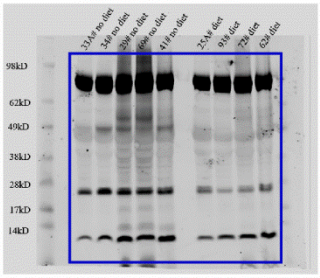
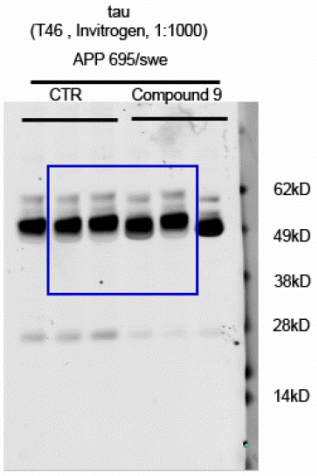


Figure 7C



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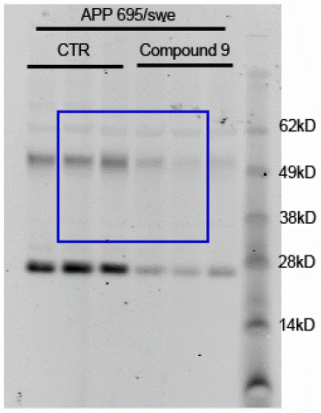

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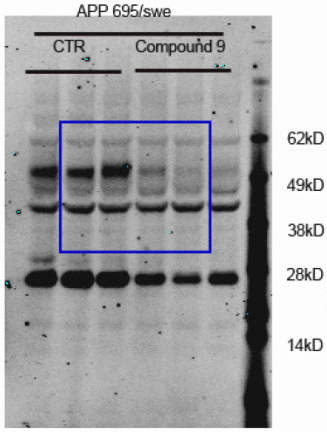

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