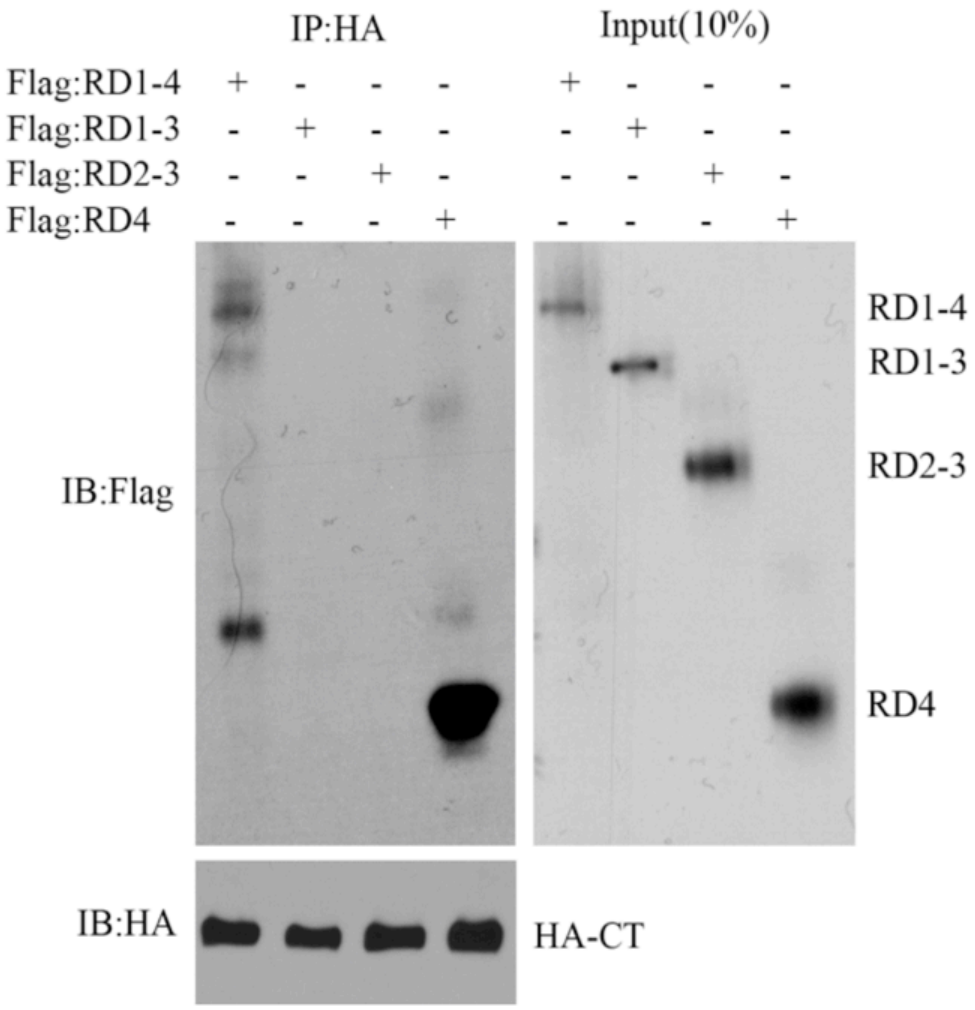
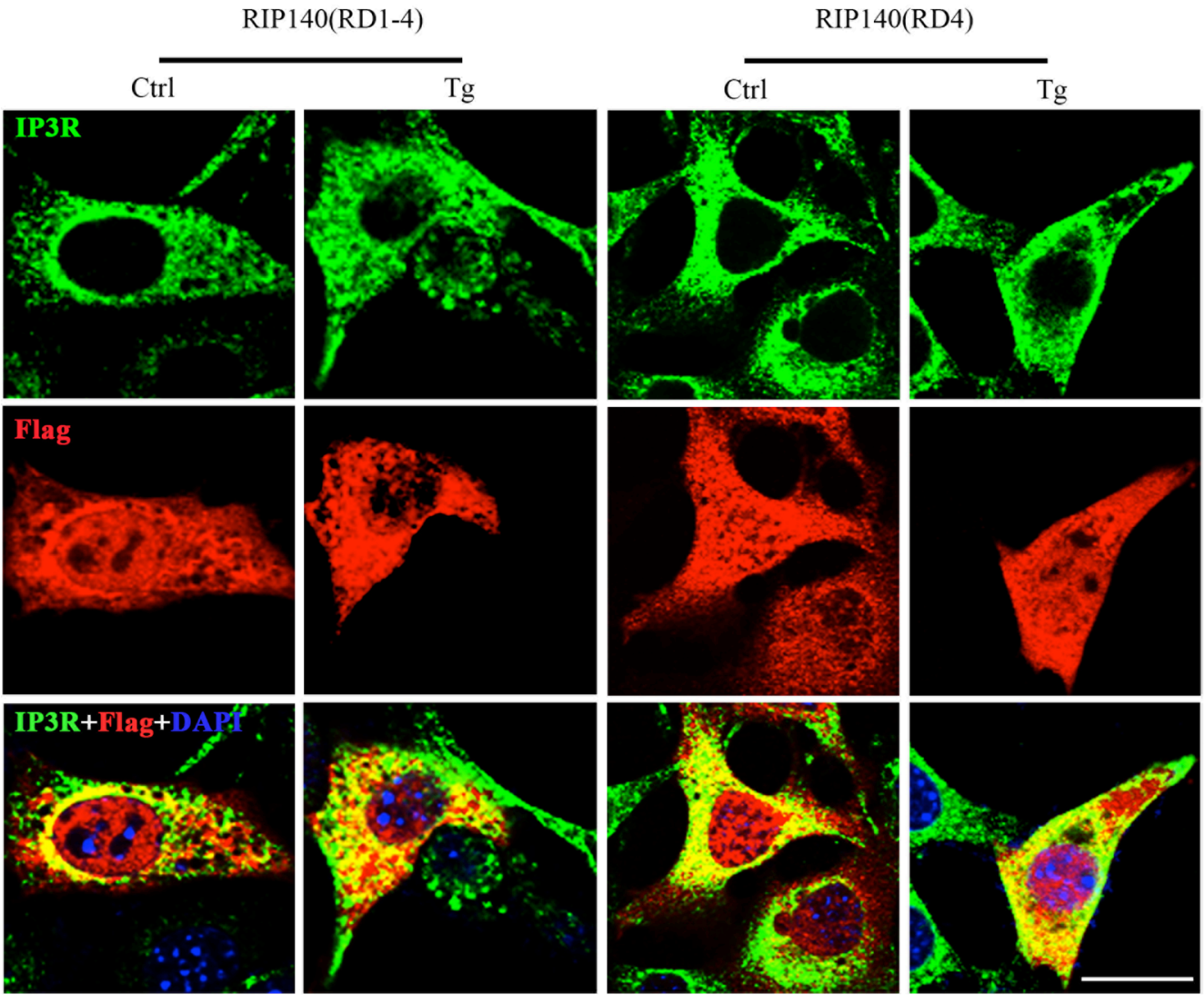
Supplemental Figures



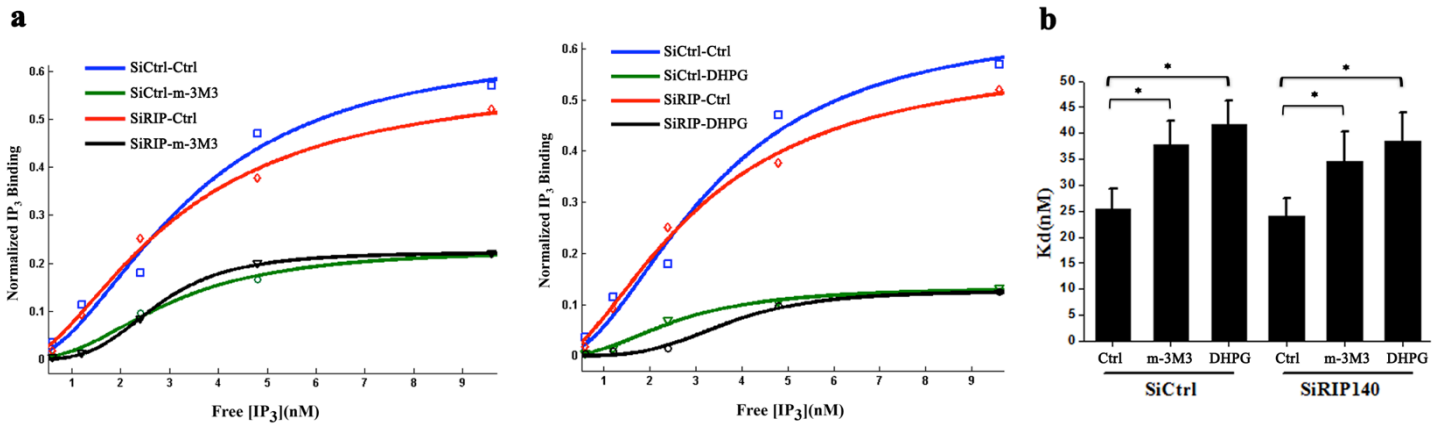
Supplementary Figure 1, RD4 of RIP140 mediates the binding of RIP140 to IP_3R.

Different flag-labeled RIP140 (as indicated) and HA-labeled C-terminal gate keeping domain of IP_3R (HA-CT) were *in vitro* translated (TNT, Promega). Flag-labeled RIP140 proteins were then used in pull-down assays with HA-CT. Inputs correspond to 10% of flag-labeled protein as indicated. As shown in the figure, both RD1-4 and RD4 of RIP140 interact with IP_3R-CT. RIP140 mutant lacking RD4 region failed to interact with IP_3R-CT, suggesting that RIP140's RD4 domain is essential for the interaction of RIP140 with IP_3R and sufficient for binding with IP_3R-CT.



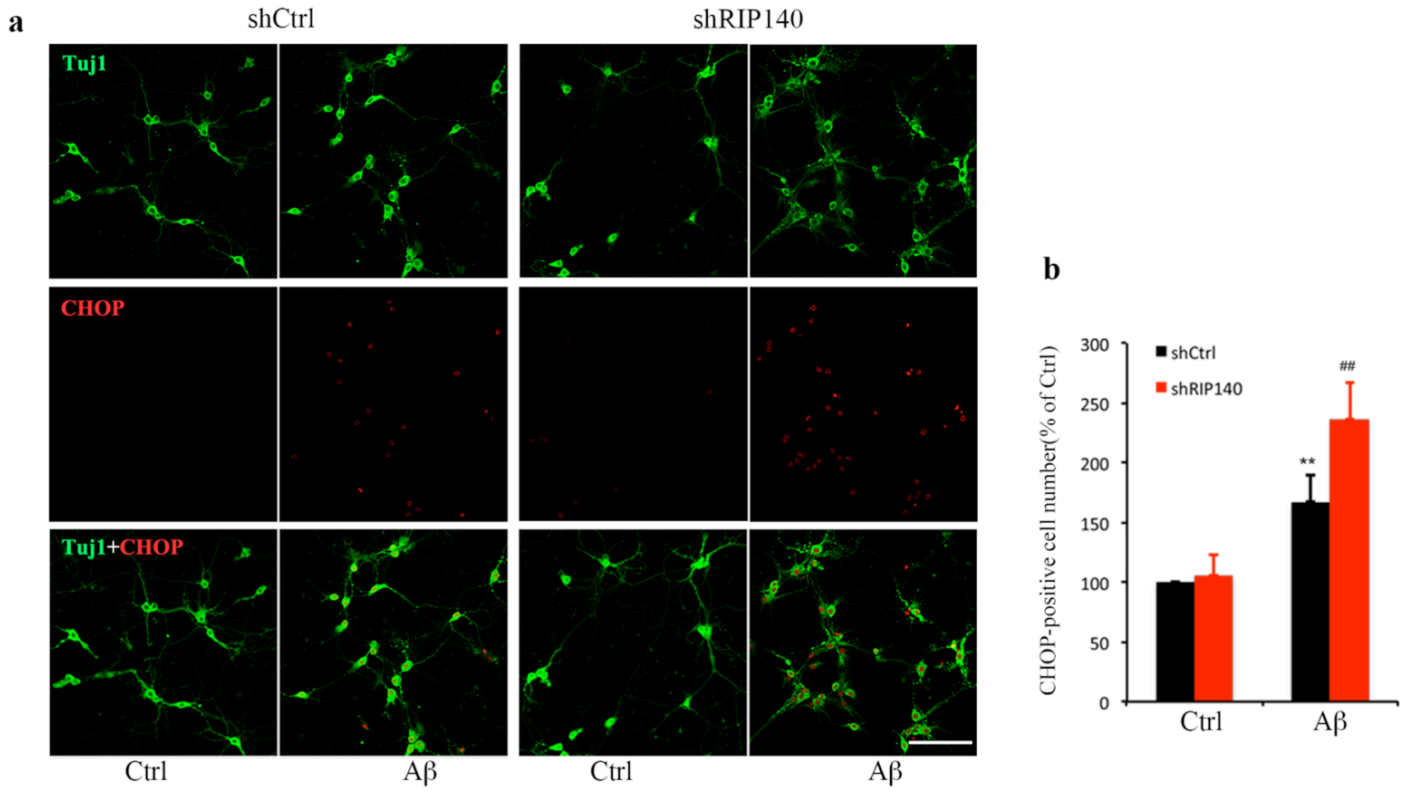
Supplementary Figure 2, ER stress induces full-length RIP140 cytoplasmic translocation and increases RIP140-IP_3R interaction.

HT22 cells were transfected with flag-tagged full-length RIP140 (RD1-4) or truncated RIP140 (RD4). Cells were then treated with Tg for 30 min and immunofluorescence was performed. The green fluorescence depicts IP_3R and red fluorescence shows RIP140 RD1-4 or RD4. Nuclei are labeled with DAPI (blue). Before stress, both RD1-4 and RD4 are distributed in the nucleus and cytoplasm and co-localized with IP_3R in the cytoplasm. After ER stress, interaction of RD1-4 with IP_3R was increased and nuclear RD1-4 was reduced as indicted by a decrease in red signals overlapping with the blue DAPI in the nuclei and an increase in red signals overlapping with green fluorescence in the cytoplasm. Interestingly, RD4 remains evenly distributed in the cytoplasm and nucleus after ER stress. Scale bar, 20 μm.

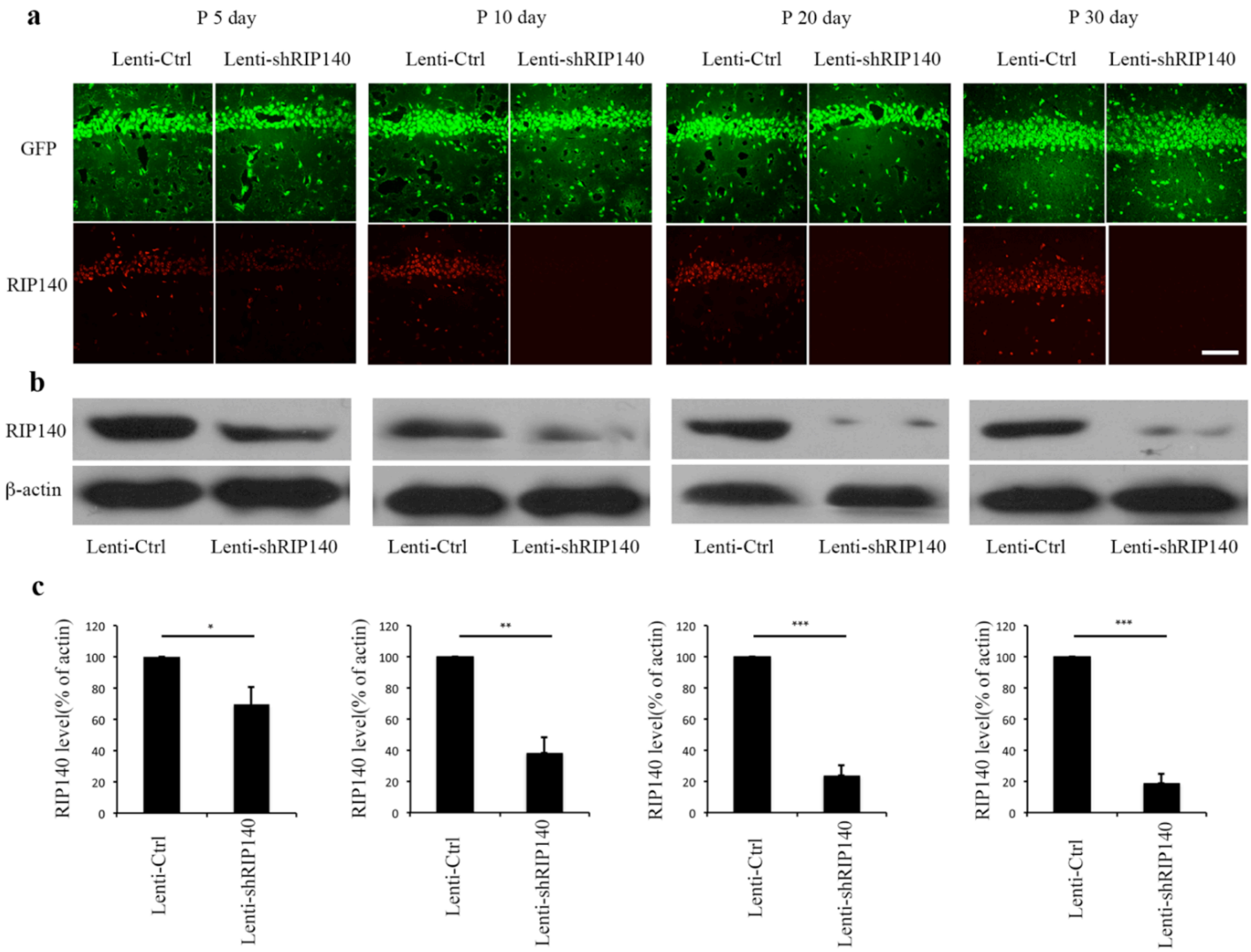


Supplementary Figure 3, IP_3-binding affinity of IP_3R1 from microsomal fraction of HT22 cells.

The IP_3 binding affinity assay of IP_3R1 was performed as described previously(Yamazaki et al, 2010). Microsomal fractions (50 µg) from HT22 cells suspended in the cytosol-like medium (pH 7.4) containing 0.5 mM EGTA were incubated with 1.2-9.6 nM [^{3}H]IP_3 (PerkinElmer Life Sciences) and various concentrations of unlabeled IP_3 on ice for 30 min. The mixture was centrifuged for 15 min at 20,000 g, 4 °C. Pellets were solubilized in 100 µl water and added to 2 ml ACS scintillation cocktail (Amersham Biosciences) and their activity was determined by liquid scintillation counting. The dissociation constant (Kd) was calculated by Scatchard analysis. **a,** Relationship of IP_3 binding and free IP_3 in HT22 cells with control or RIP140 knockdown after treatment with m-3M3 or DHPG. **b,** IP_3 dissociation constant of control or RIP140 knockdown cells after different treatment. The results presented by means ± SEM. from five repeated experiments, $^*p < 0.05$ relative control groups as determined by unpaired Student't *t*-test.

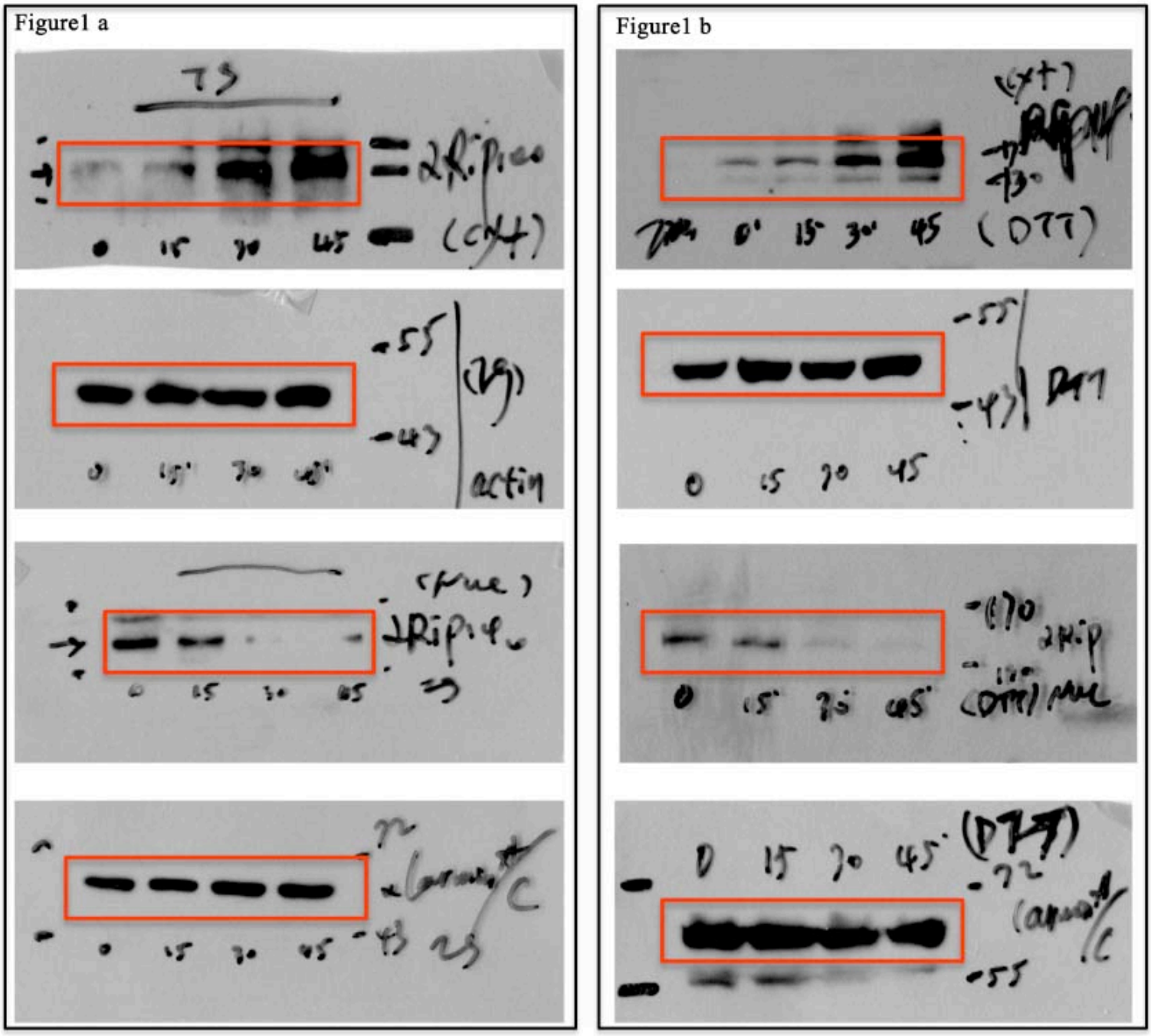


Supplementary Figure 4, RIP140-deficient hippocampal neurons are more susceptible to Aβ toxicity. Hippocampal neurons were transfected with control shRNA (shCtrl) or RIP140-specific shRNA (shRIP140) and then incubated with 10 μM Aβ (aggregates of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide) for 24 h. **a,** Confocal immunofluorescence microscopy shows neuron marker TUJ-1 (green) and ER stress marker CHOP (red). Treating cells with Aβ increased the expression of CHOP, indicating ER stress related cell apoptosis. Aβ–induced CHOP expression was further enhanced in RIP140 knockdown (shRIP140) cultures. Scale bar, 100 μm. **b**, Quantitative analysis of CHOP-positive cells and represent the mean ± SEM obtained from five independent experiments. The values obtained from untreated neurons were considered 100%. ***p*<0.01, different from untreated controls, ##p<0.01 relative to transfection control (shCtrl) treated with Aβ, results were analyzed by one-way ANOVA.

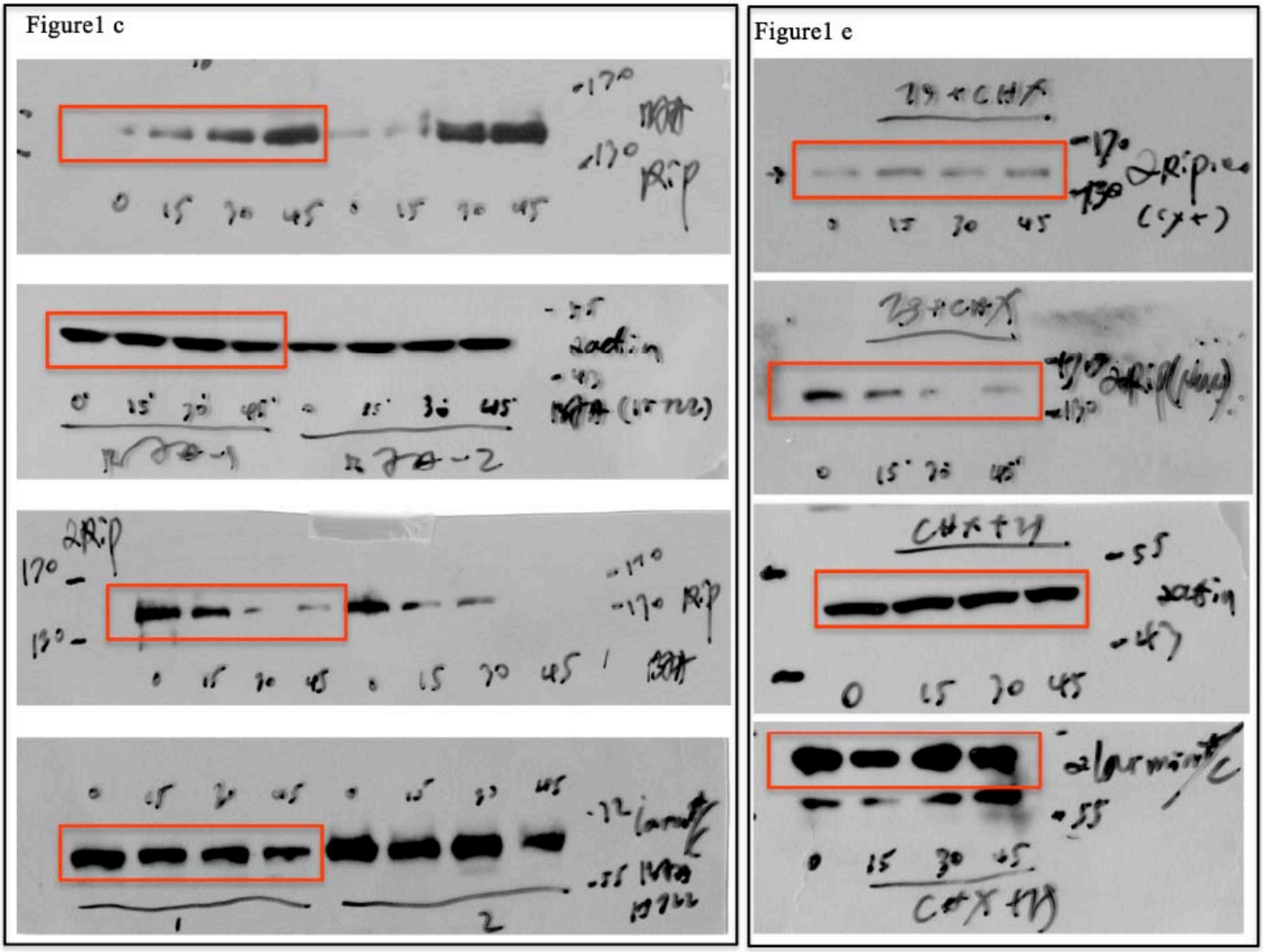


Supplementary Figure 5, Expression level of RIP140 protein from Lentivitus injected mouse hippocampus.

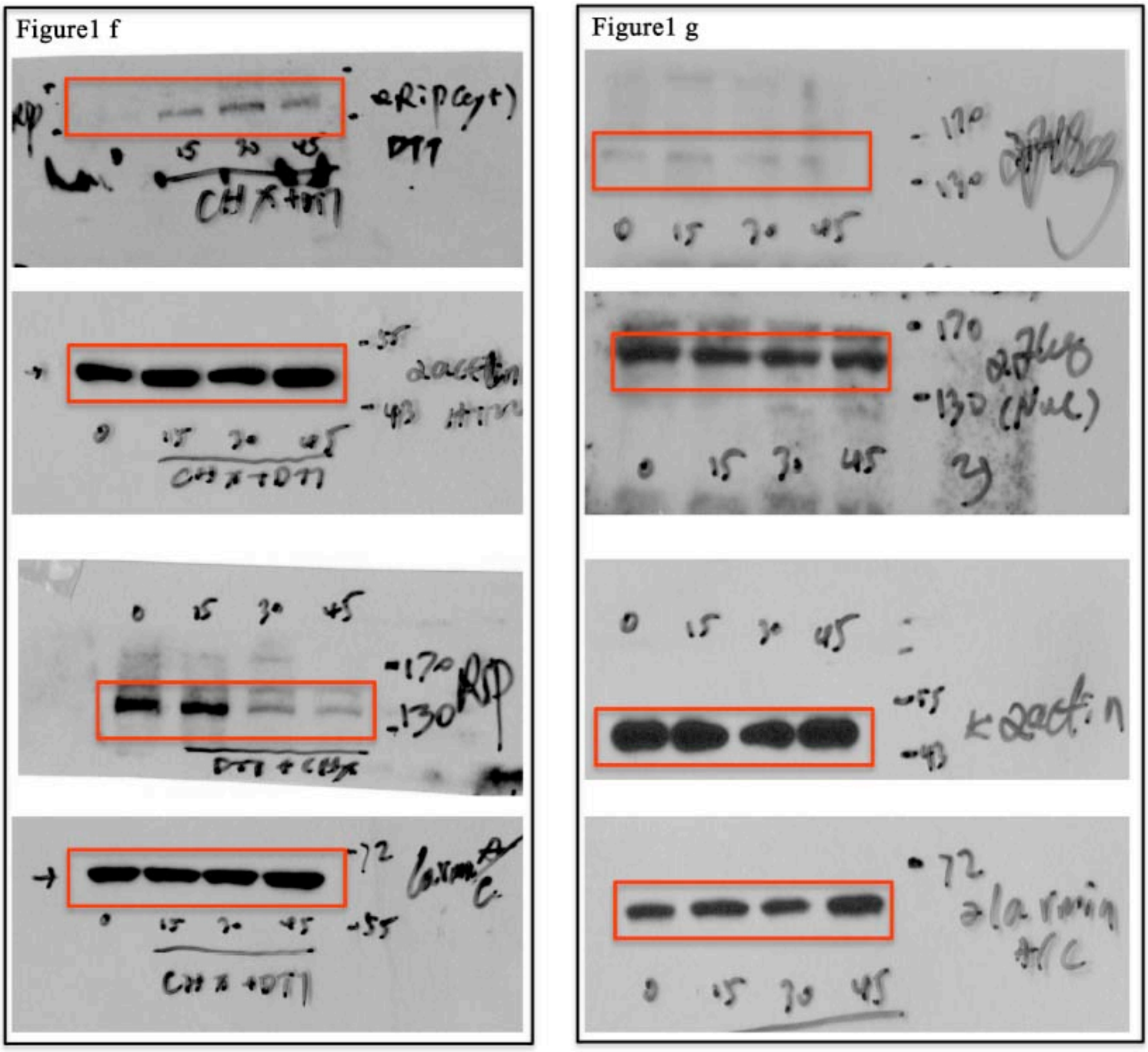
a, Confocal immunofluorescence microscopy shows GFP-Lentivirus (green) and RIP140 protein recognized by RIP140 antibody (red) in mouse hippocampus from the virus-injected hemisphere (Lenti-Ctrl and Lenti-shRIP140) at different time points following virus injection. Scale bar, 20 μm. **b,** Expression levels of RIP140 preotein detected by western blot in hippocampus extracted from control Lentivirus injected and shRIP140 lentivirus injected hippocampi. **c,** Quantitative analyses of RIP140 relative to β-actin. The results are presented as the means ± SEM obtained from three independent experiments. The values obtained from Lenti-Ctrl groups were considered 100%. **p*<0.05, ***p*<0.01, ****p*<0.001 relative to Lenti-Ctrl controls determined by Student's *t*-test.



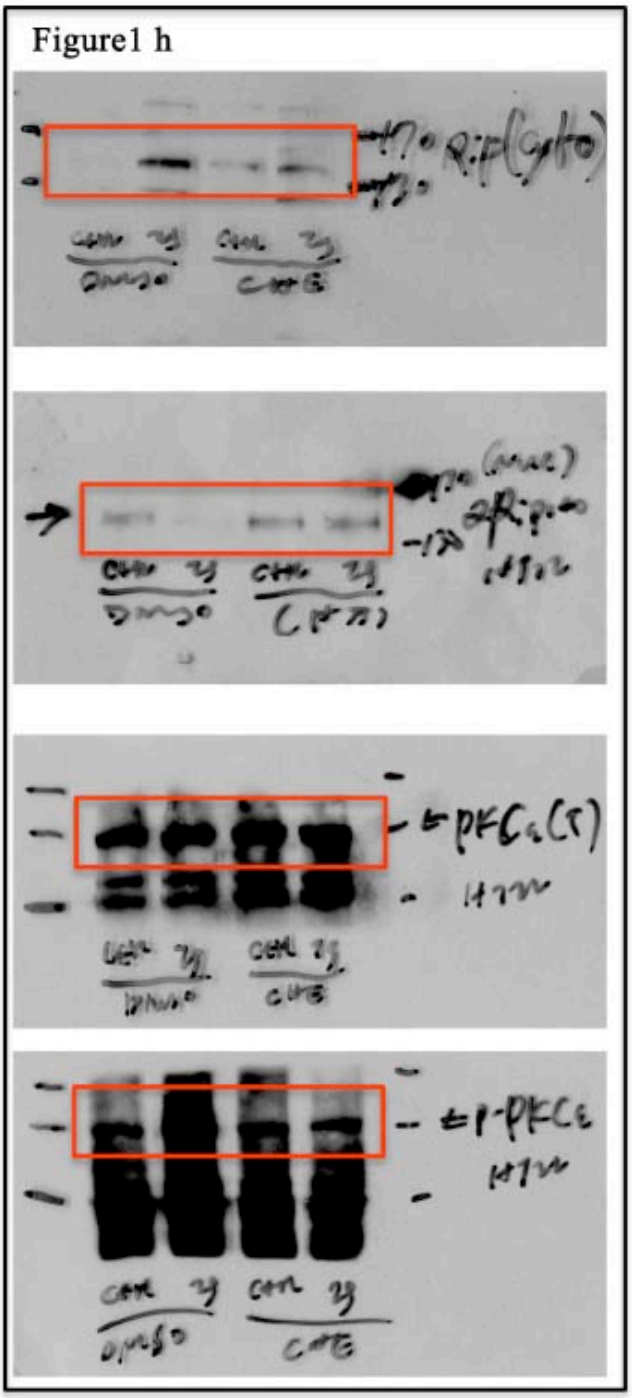
Supplementary Figure 6, Uncropped scans full scans of blots in figure 1a and b. To be continued....

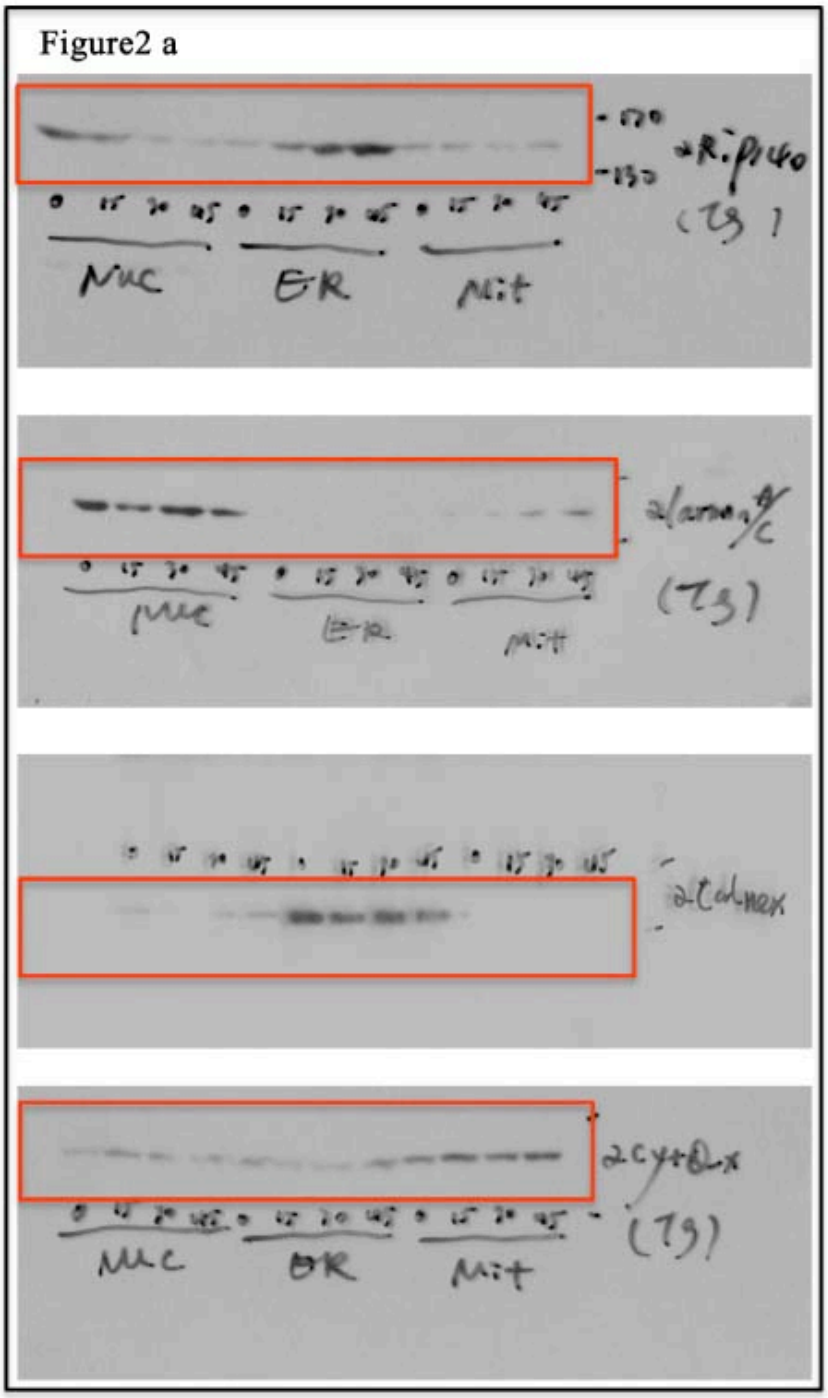


Supplementary Figure 7, Uncropped scans full scans of blots in figure 1c and e. To be continued....

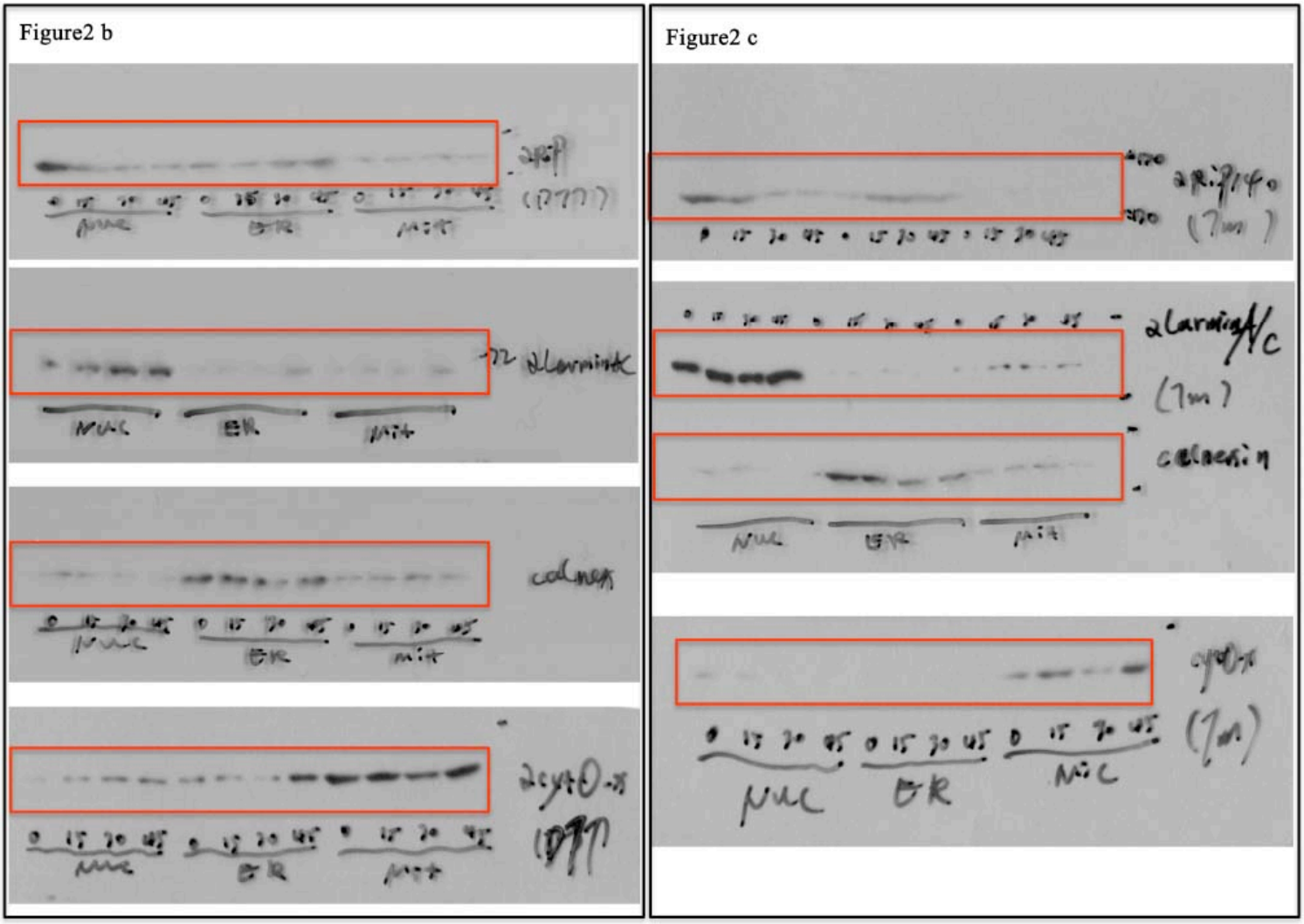


Supplementary Figure 8, Uncropped scans full scans of blots in figure 1f and g. To be continued....

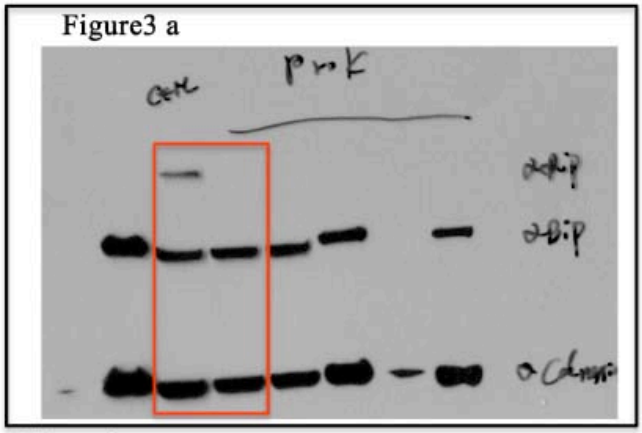


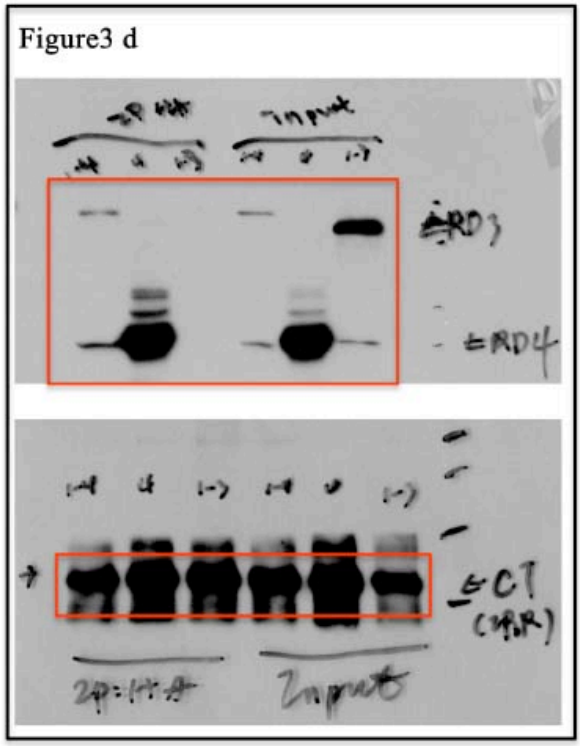


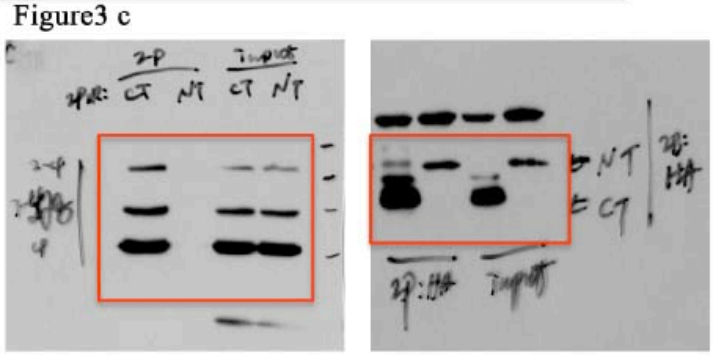
Supplementary Figure 9, Uncropped scans full scans of blots in figure 1h and figure2a. To be continued....

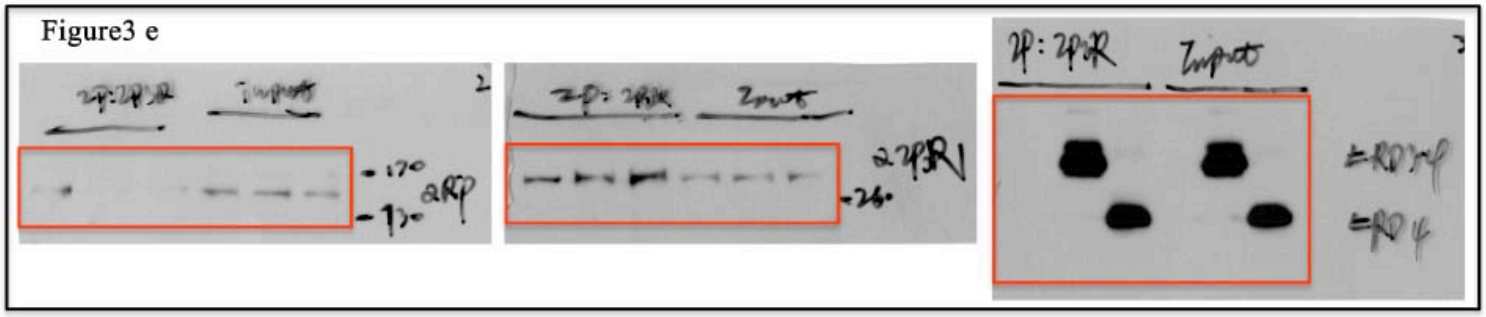


Supplementary Figure 10, Uncropped scans full scans of blots in figure 2b and c. To be continued....

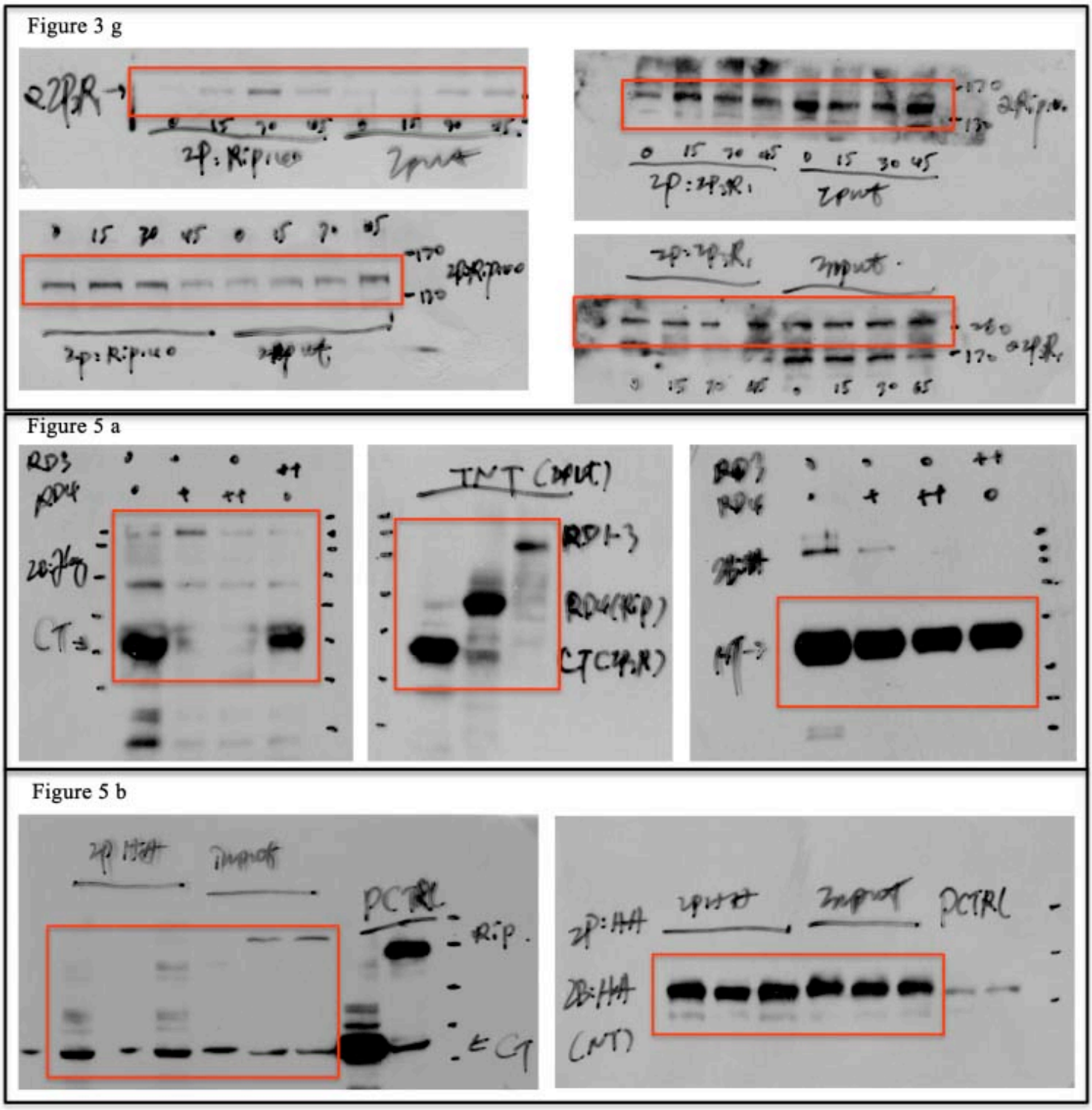




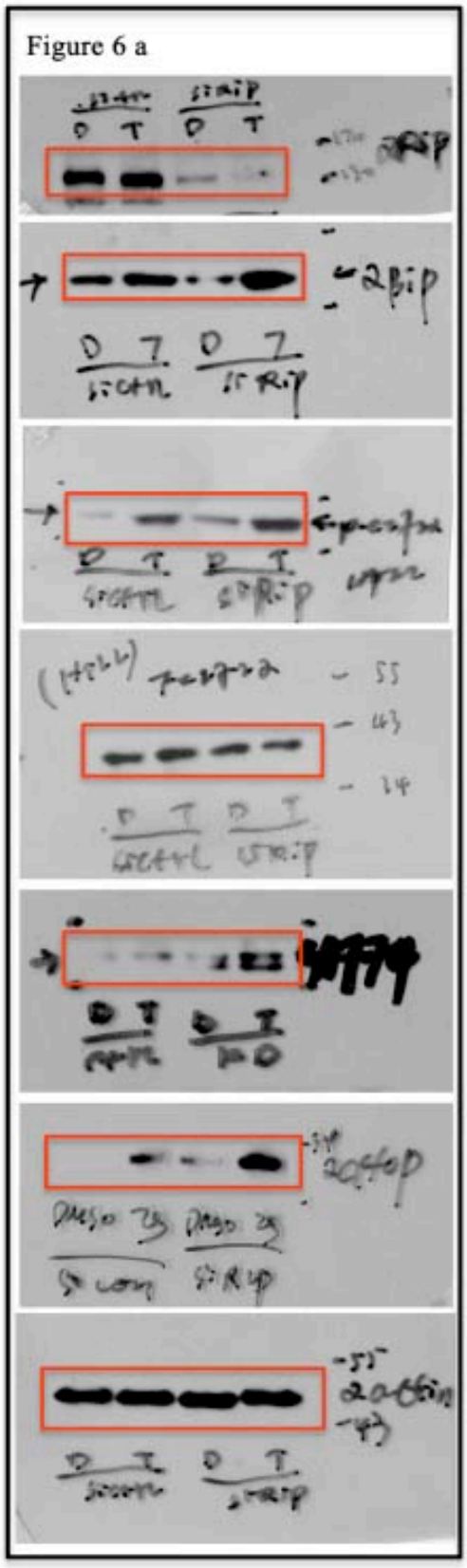


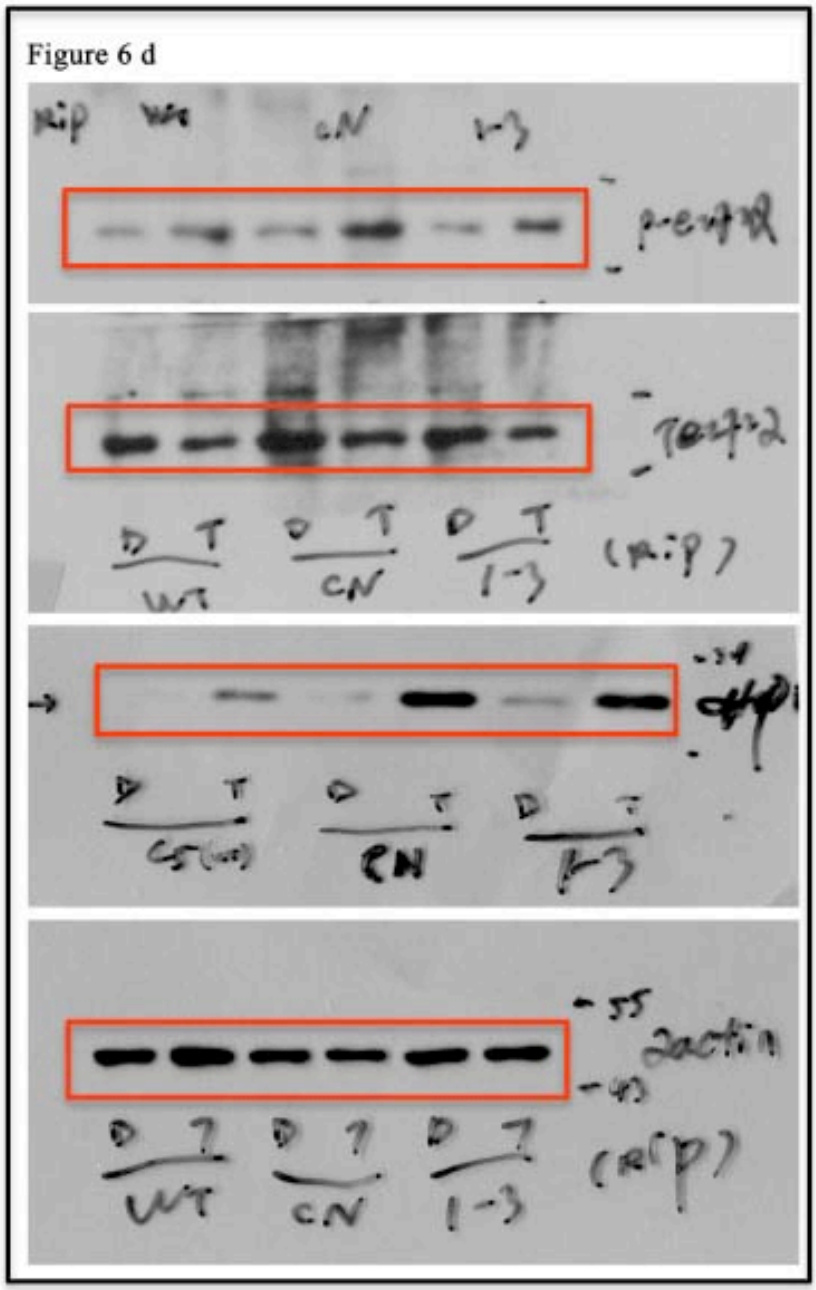


Supplementary Figure 11, Uncropped scans full scans of blots in figure 3a, c, d and e. To be continued....

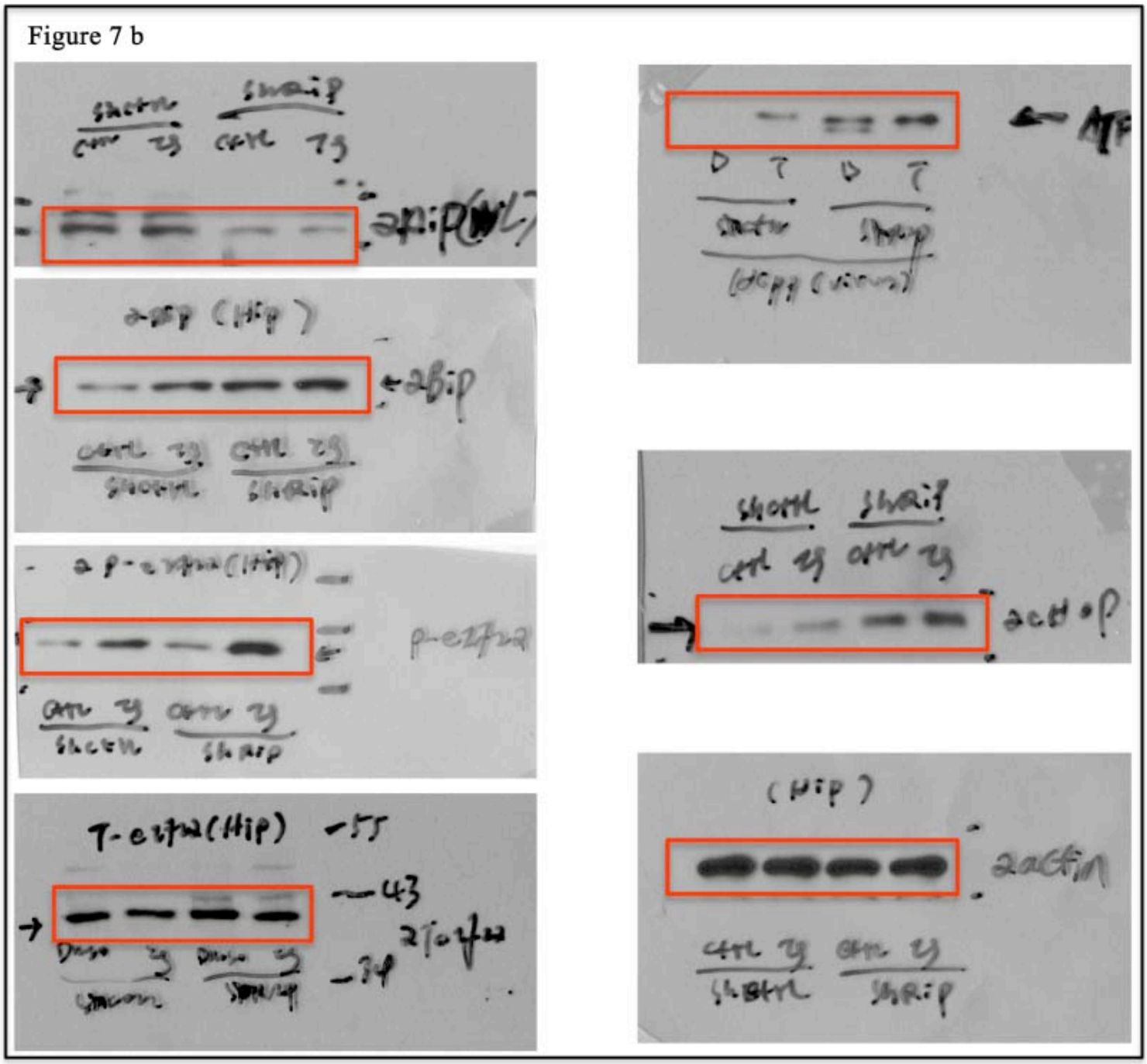


Supplementary Figure 12, Uncropped scans full scans of blots in figure 3g and figure5. To be continued....





Supplementary Figure 13, Uncropped scans full scans of blots in figure 6. To be continued....



Supplementary Figure 14, Uncropped scans full scans of blots in figure 7.

Antibody name	Supplier	Cat No	Concentration	Application
ATF-4	Abcam	ab-50546	1:2000	IB
Bip	Abcam	ab-21685	1:10000	IB
β-actin	Santa cruz	sc-47778	1:1000	IB
Calnexin	Sigma	C4731	1:5000	IB
CHOP	Cell signaling	2895	1:2000	IB
CytO-x(COX4)	Cell signaling	4844	1:2000	IB
eIF2a	Cell signaling	5324	1:1000	IB
phospho-eIF2a	Cell signaling	5119	1:1000	IB
Flag	Sigma	F3165	1:10000 1:400 1:1000	IB IF IP
HA	Santa cruz	sc-7392	1:8000 1:1000	IB IP
IP_3R1	Calbiochem	407140	1:400	IB
IP_3R1	Santa cruz	sc-271197	1:200	IF
IP_3R1	Lifespan	LS-C121863	1:1000 1:400	IB IF
LaminA/C	Santa cruz	sc-7293	1:1000	IB
RIP140	Abcam	ab-42126	1:1000 1:100	IB IP
RIP140	Abcam	ab-42125	1:1000	IB
RIP140	Santa cruz	sc-8997	1:200	IF

Supplementary Table 1, Primary antibodies and concentrations.

IB-immunoblot, IF-immunofluorescence, IP-immunoprecipitation

Supplementary references

1 Yamazaki H, Chan J, Ikura M, Michikawa T, Mikoshiba K (2010) Tyr-167/Trp-168 in type 1/3 inositol 1,4,5-trisphosphate receptor mediates functional coupling between ligand binding and channel opening. *The Journal of biological chemistry* **285:** 36081-36091