

## SUPPLEMENTAL FIGURE AND VIDEO LEGENDS

**Supplemental Figure 1: Gp78 rescues mitochondrial phenotype of Gp78 KO HT-1080 cells in a RING domain-dependent manner.** (A) Representative maximum projections of mitochondria labelled with TOMM20 from wild type HT-1080 and Gp78 KO clones transiently transfected with wild type FLAG-Gp78, catalytically inactive RING finger (RF) mutant Gp78, or empty vector pcDNA3. (B) Bar graph shows quantification of total mitochondrial volume per cell (n=3 independent biological replicates; 10-14 cells/condition per experiment; \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001, \*\*\*\*, p<0.0001; Mean  $\pm$  SEM; Scale bar: 10  $\mu$ m).

**Supplemental Figure 2: The effect of variable Z-values on SPECHT spot detection.** Raw images of grey scale and merged GFP-mRFP tflLC3 of DMSO and CCCP/BafA1 treated HT-1080 cells. Corresponding Laplacian filter is presented with its resultant z-values of 1.50, 1.75, 2.0. Scale bar, 10  $\mu$ m.

**Supplemental Figure 3: Impact of CCCP on MitoView 633 mitochondrial labeling.** HT-1080 and Gp78 knockout clone g2-41 cells were treated with CCCP for 4 hours and labeled with the mitochondrial potential reporter MitoView 633 for 30 minutes. MitoView 633 integrated density per mitochondrial object was quantified. (n=3 independent biological replicates; >30 cells/condition per experiment; \*\*\*\*p<0.001).

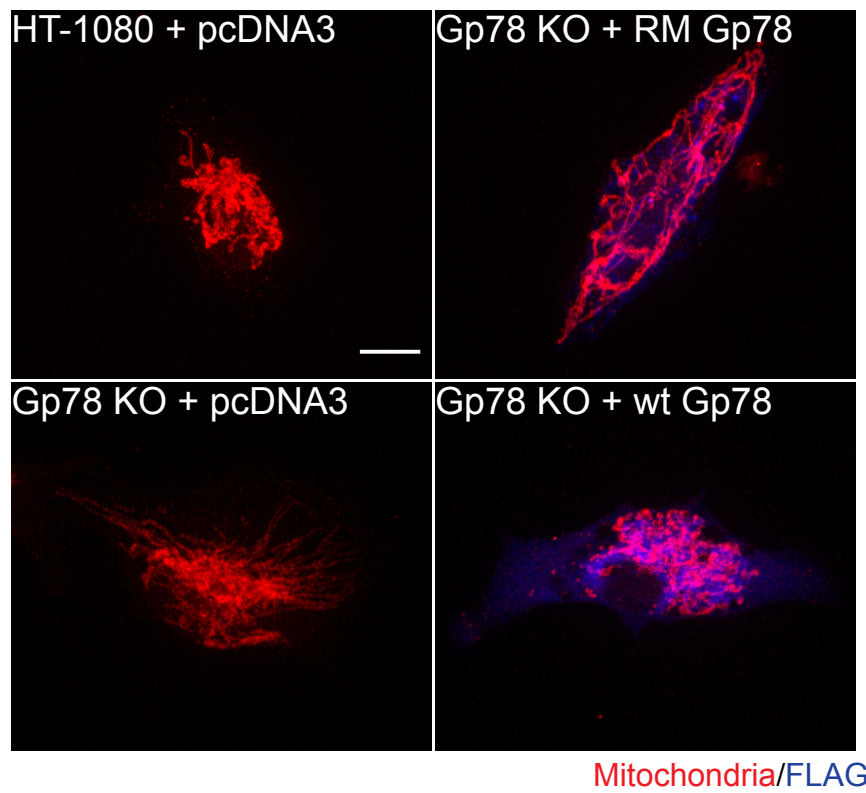
**Supplemental Figure 4:** Full Western blots for the Gp78 and  $\beta$ -actin blots included in Figure 1A.

**Supplemental Figure 5:** Full Western blots for the ATG5, ATPB and  $\beta$ -actin blots included in Figure 2A.

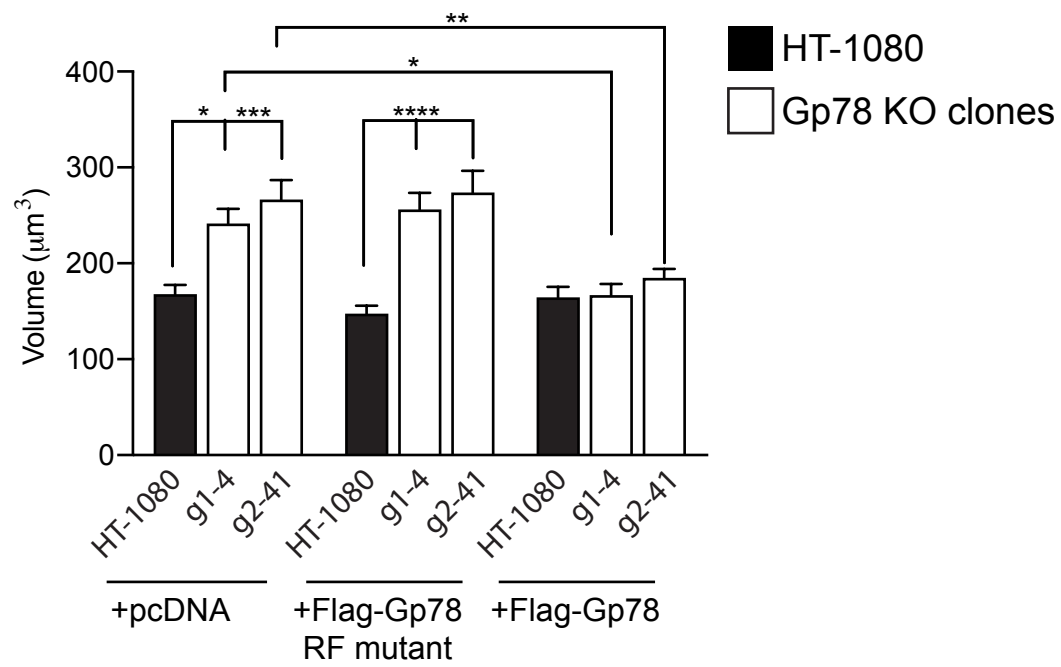
**Supplemental Figure 6:** Full Western blots for the LC3B-II and  $\beta$ -actin blots for DMSO, CCCP and starvation treated cells included in Figure 3.

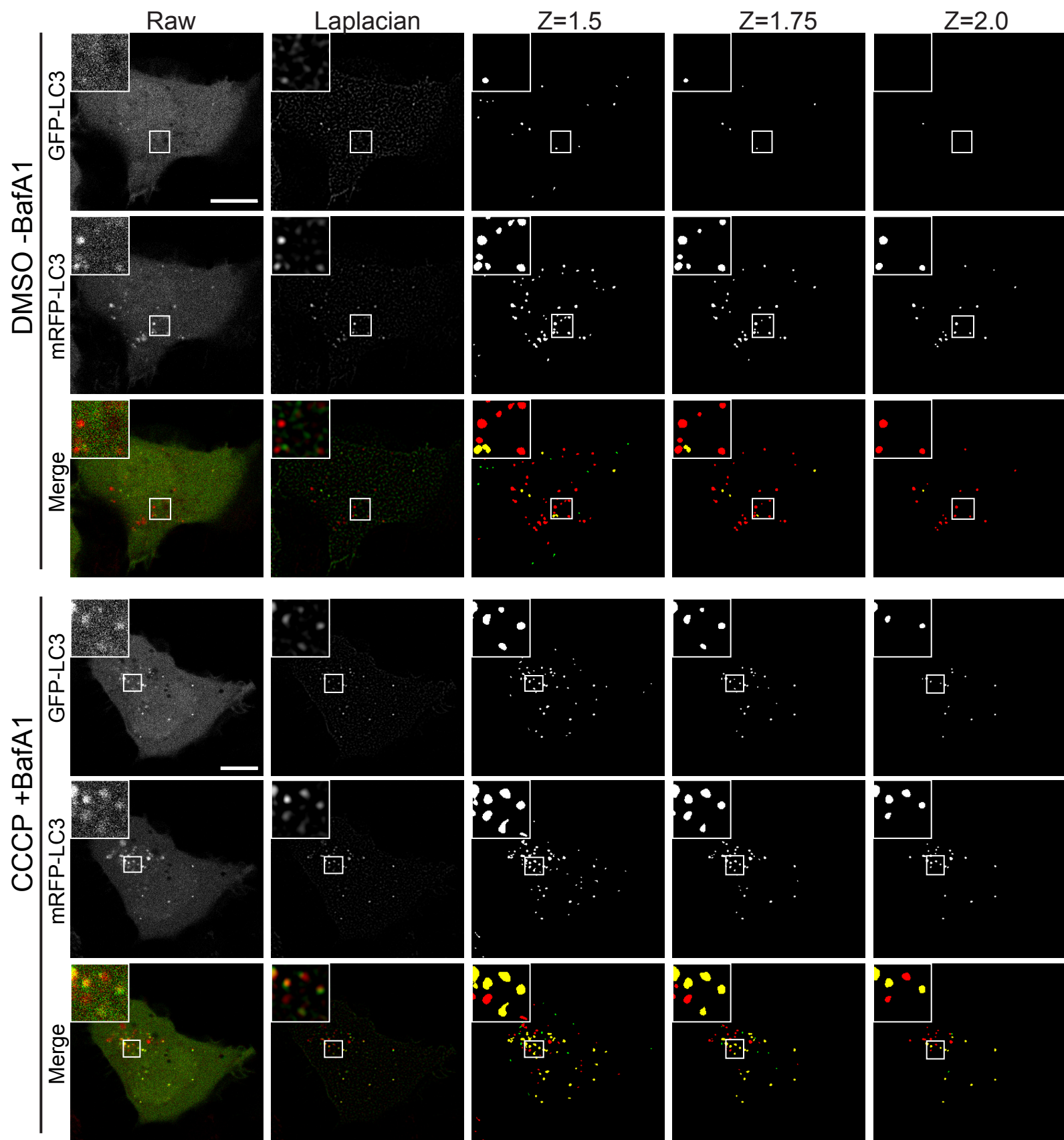
**Supplemental Video 1:** Time lapse movie of GFP-mRFP-positive tflLC3 puncta (yellow) and MitoView 633 labeled mitochondria in an HT-1080 cell (blue SPECHT outline overlaid on raw MitoView 633 image). Images acquired every 10 seconds.

A

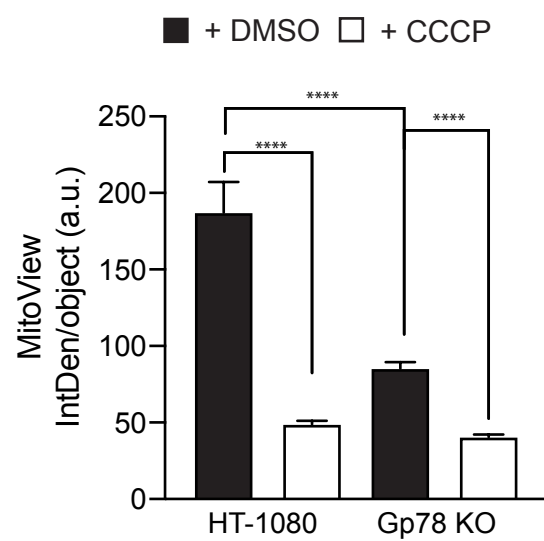


B

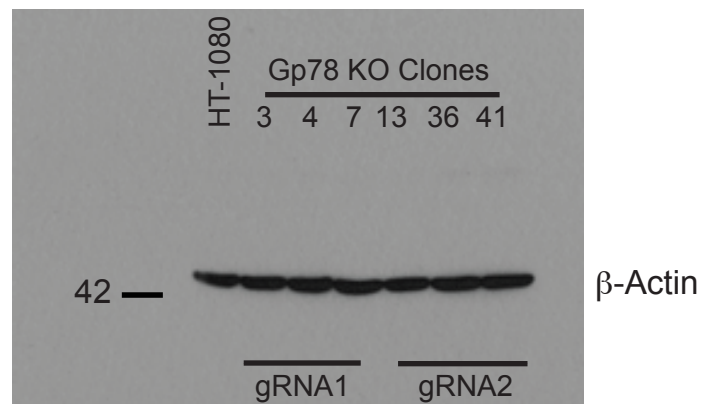
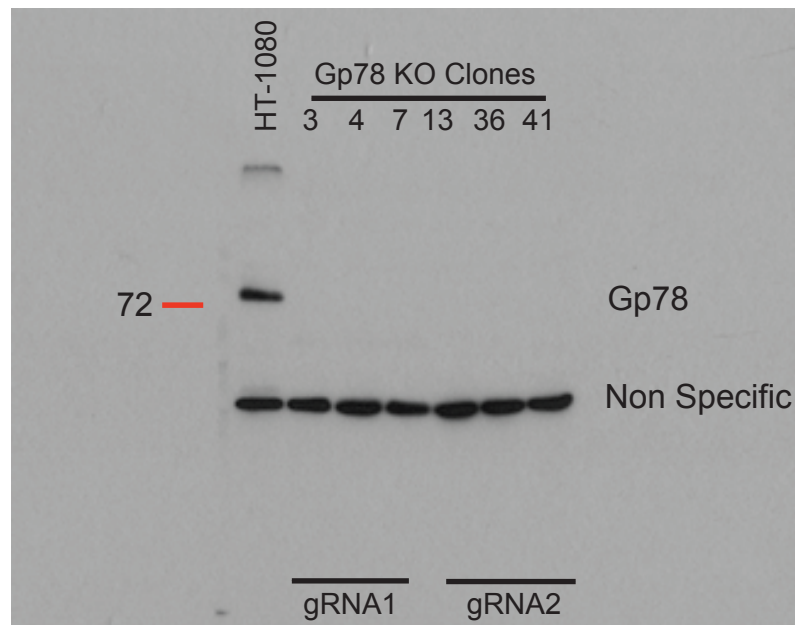




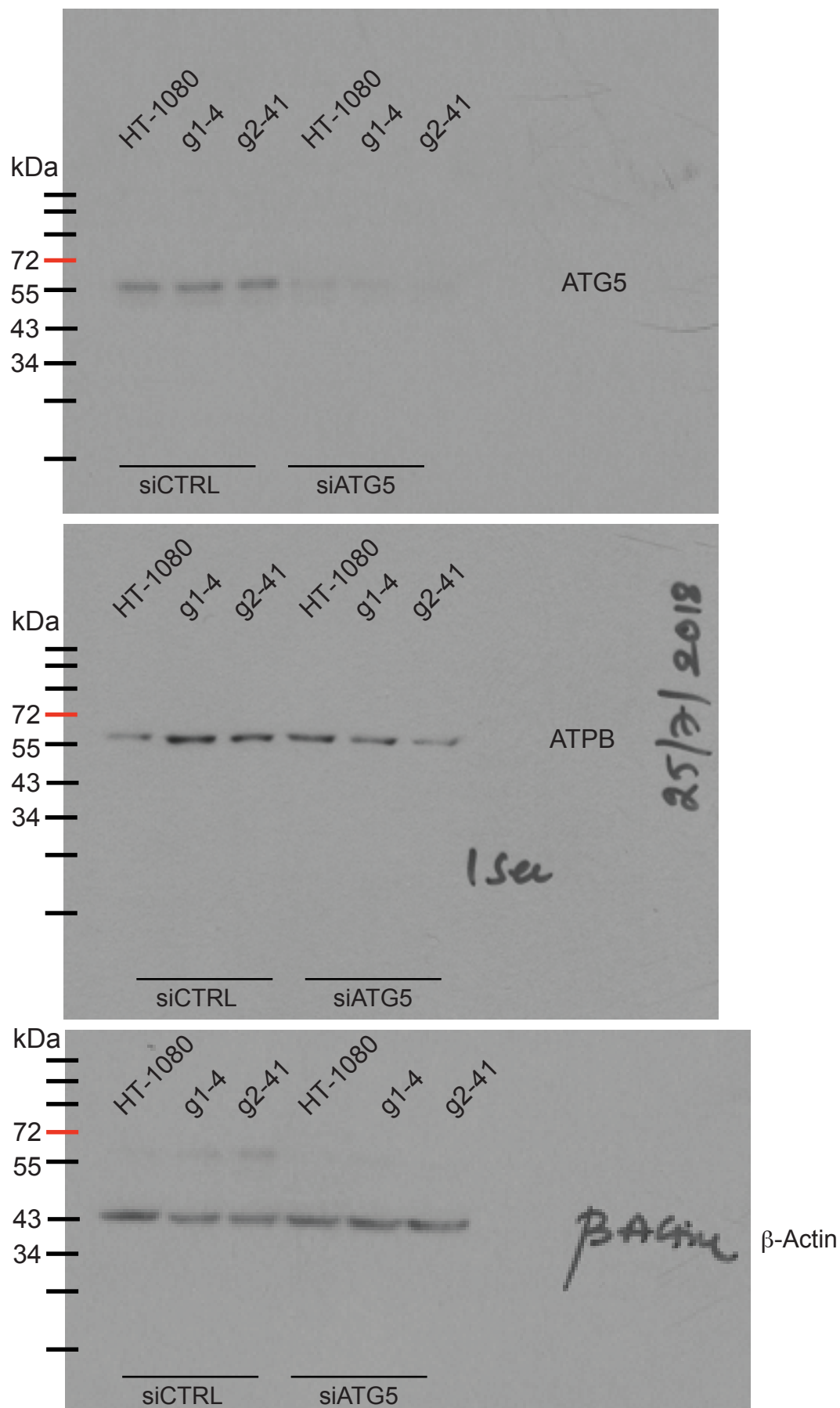
Supplementary Figure 2



**Supplemental Figure 3**

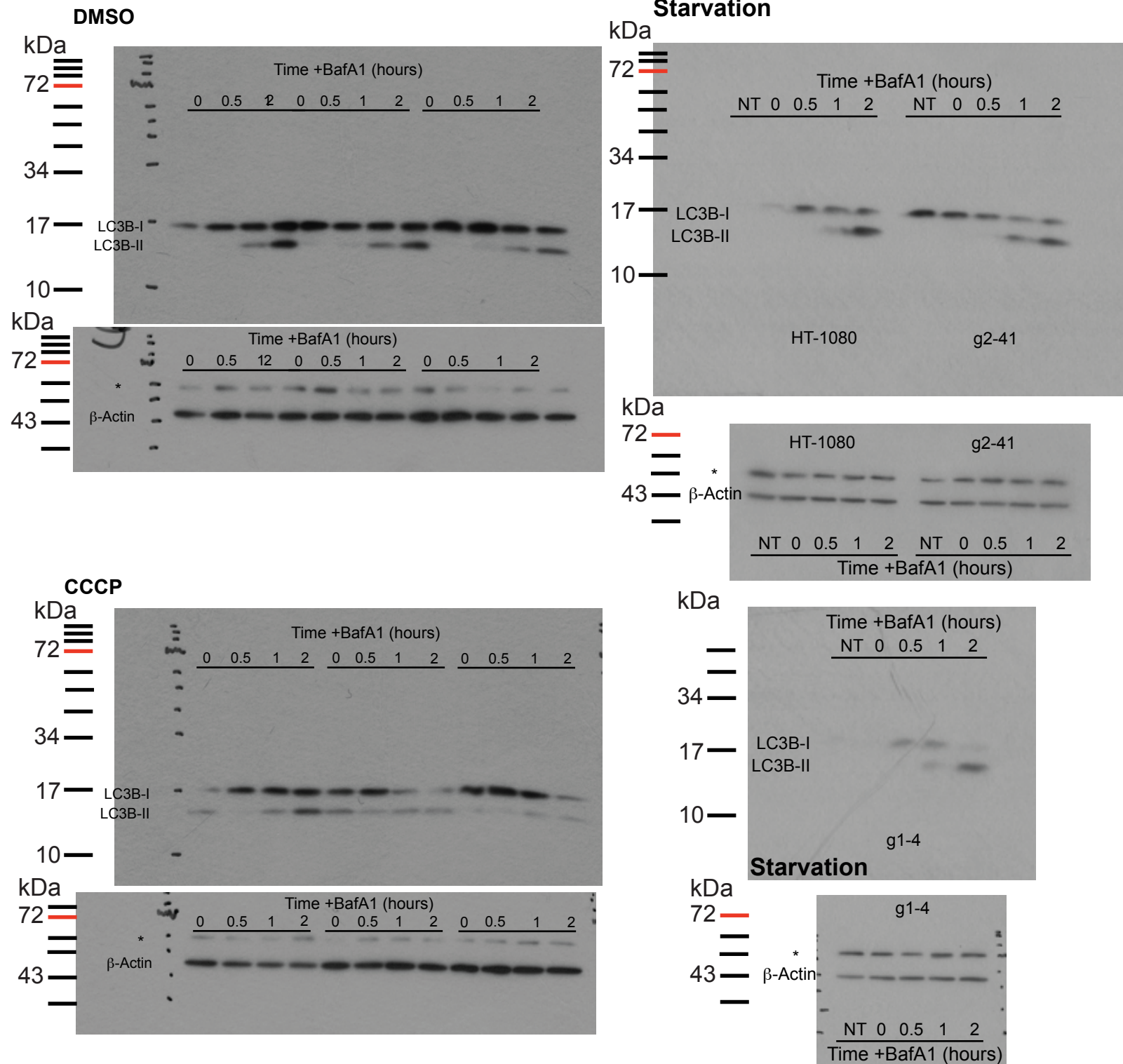


**Supplemental Figure 4 (Full blots for Figure 1A)**



Supplemental Figure 5 (Full blots for Figure 2A)

**FIGURE 3-DMSO-CCCP**



**Supplemental Figure 6 (Full blots for Figure 3)**