Figure S1. Trasfection efficiency of pHA-uL3 evaluated by western blotting. HCT 116[p53-/-] cells were transiently transfected with 1 μg of pHA-uL3. 24 h later, cells were lysated and protein extracts from the samples were analyzed by western blotting with antibodies against uL3 and GAPDH as loading control. Full-length blots are presented in Figure S9.
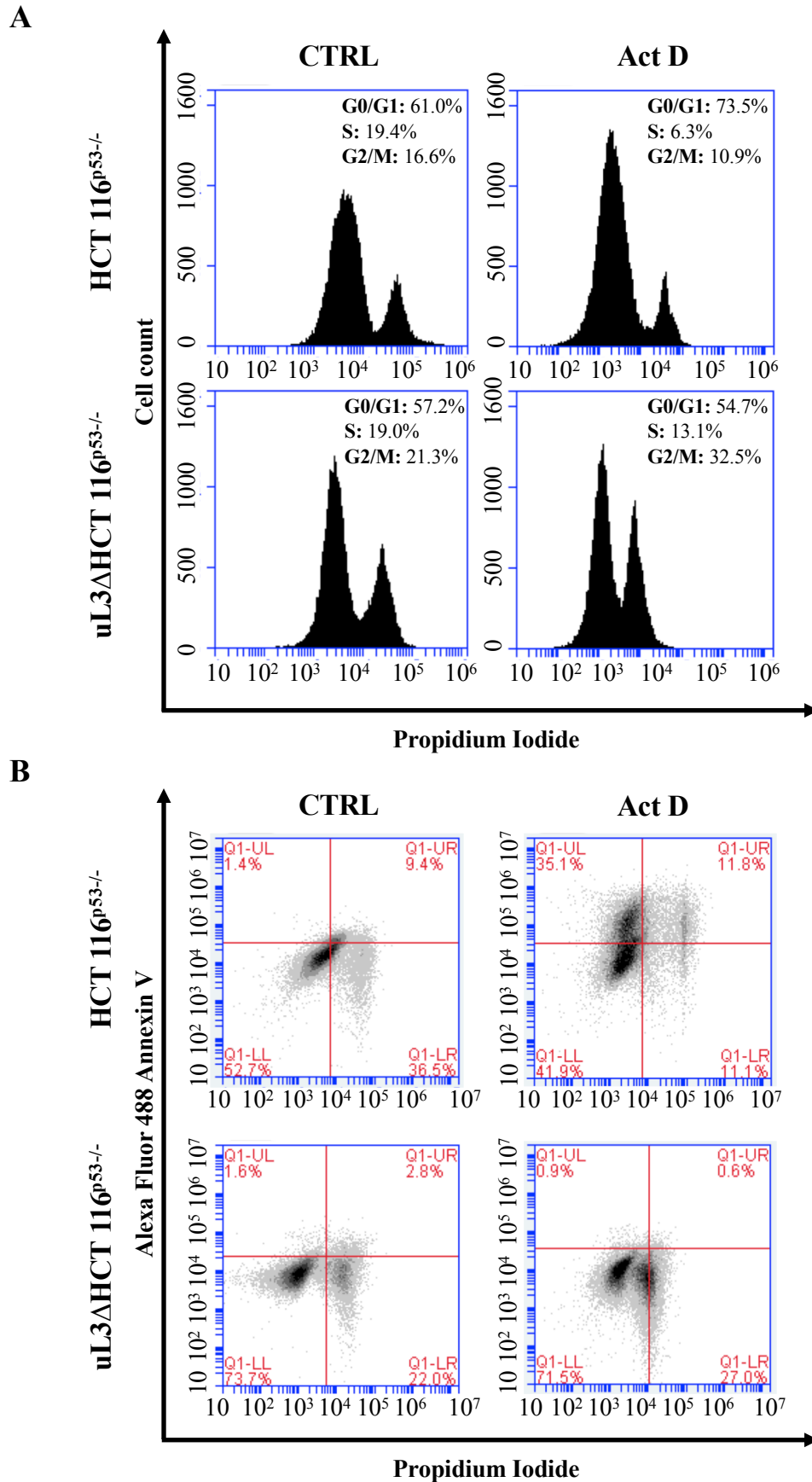
Figure S2. Effect of Act D treatment on cell cycle and apoptosis in uL3ΔHCT 116$^{p53-/-}$ cells. (**A**) HCT 116$^{p53-/-}$ and uL3ΔHCT 116$^{p53-/-}$ cells were treated with 5 nM Act D. 24 h later, cells were stained with PI and analysed using FACS. Peaks representing histograms of cell numbers and percentages in G1, S, and G2/M phases are shown. (**B**) Representative flow cytometry dot blots with double Annexin V-Alexa Fluor 488/PI staining for HCT 116$^{p53-/-}$ and uL3ΔHCT 116$^{p53-/-}$ treated with 5 nM Act D for 24 h.
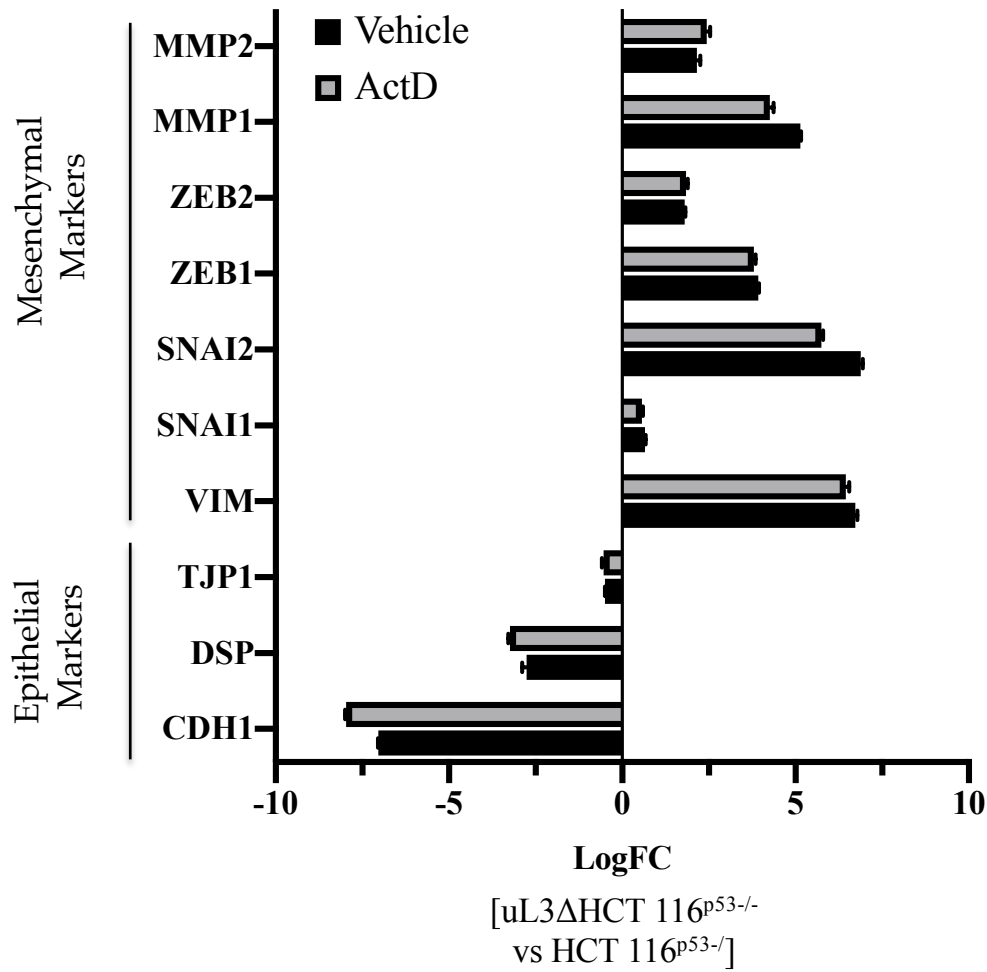
Figure S3. Epithelial-mesenchymal transition (EMT) related differential expressed genes between uL3ΔHCT 116$^{p53-/-}$ and HCT 116$^{p53-/-}$ cells treated or not with 5nM Act D for 24 h by RNA-seq analysis. Mesenchymal markers: MMP2, MMP1, ZEB2, ZEB1, SNAI2, SNAI1, VIM; epithelial markers: TJP1, DSP, CDH1; x-axis represents log 2 fold change of expressed genes vs average expression normalized to log 2 scale for each genes.
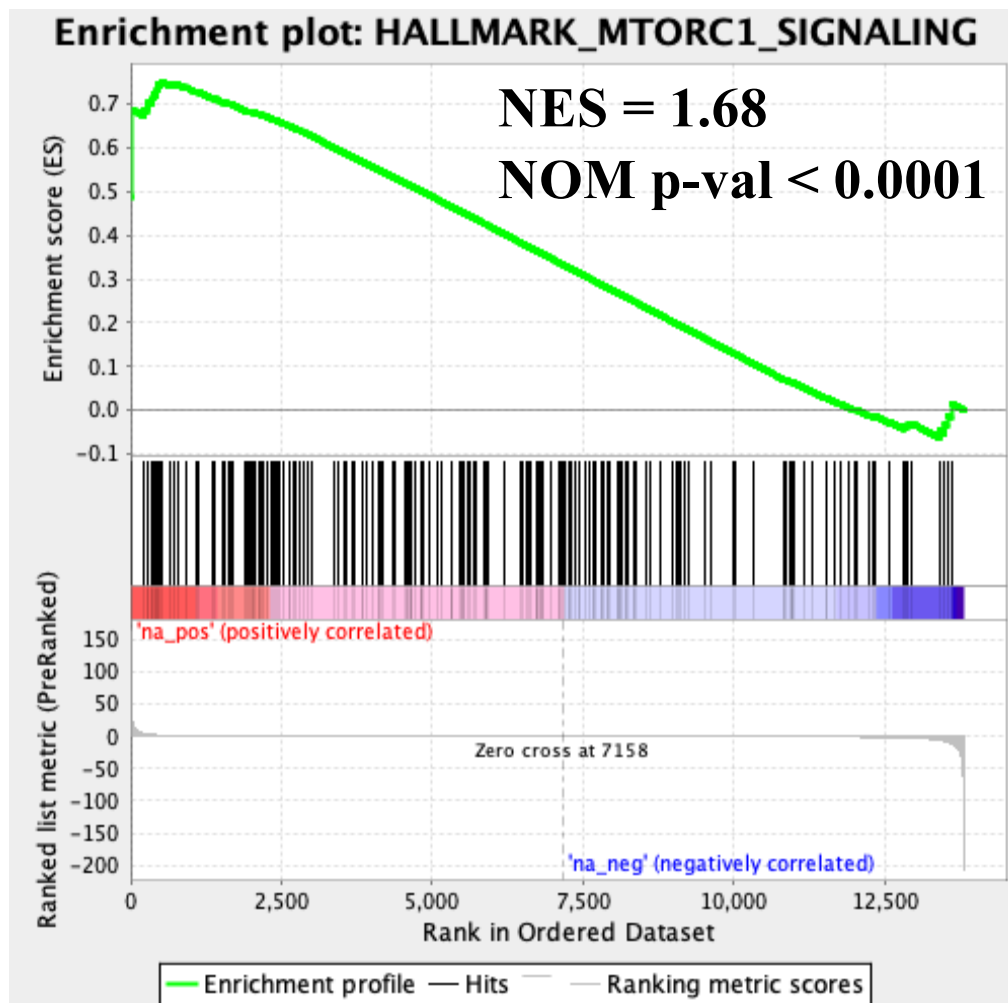
Figure S4. mTORC pathway regulated by Act D treatment in HCT 116[p53-/-] cells. The GSEA (Gene Set Enrichment Analysis) plots showing MTORC1_SIGNALING hallmark gene set in HCT 116[p53-/-] cells treated with Act D. The normalized enrichment scores (NES) and p value were indicated.
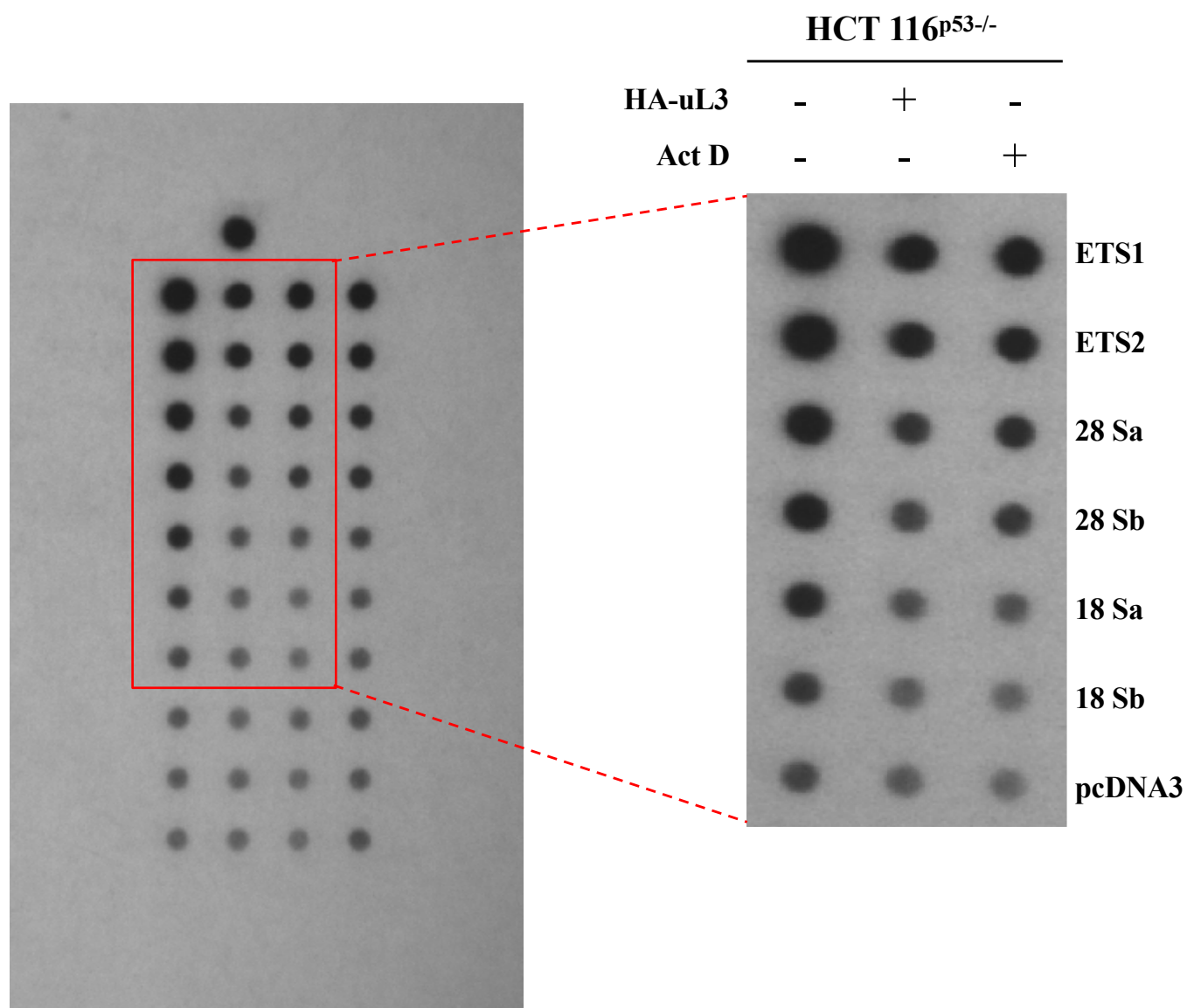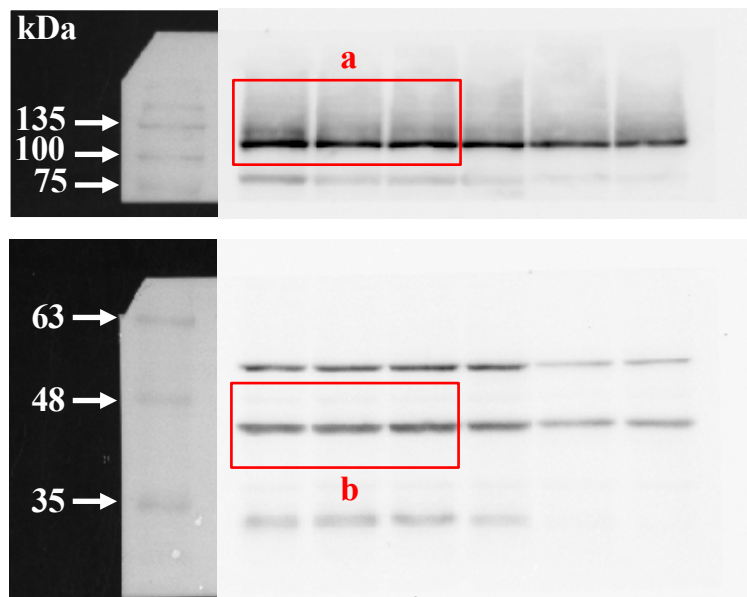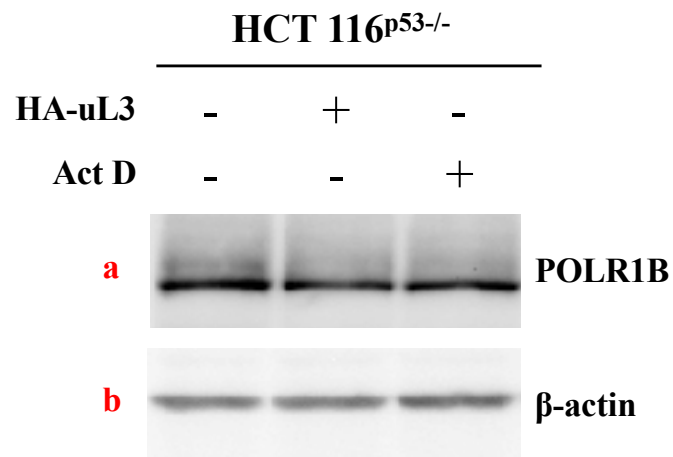
Figure S5. Full length blot of Figure 2B.

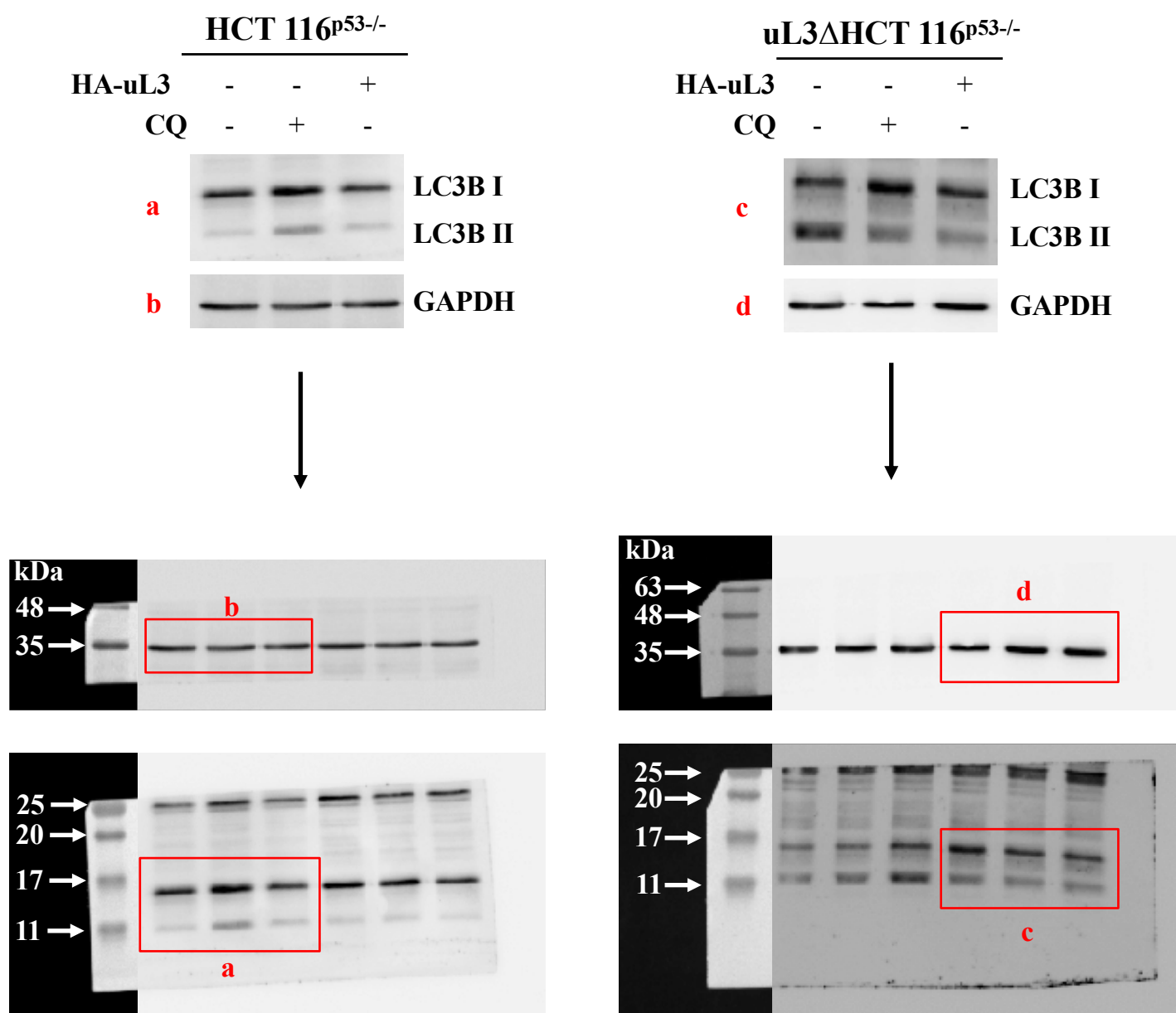**Figure S6.** Full length blots of Figure 2C.

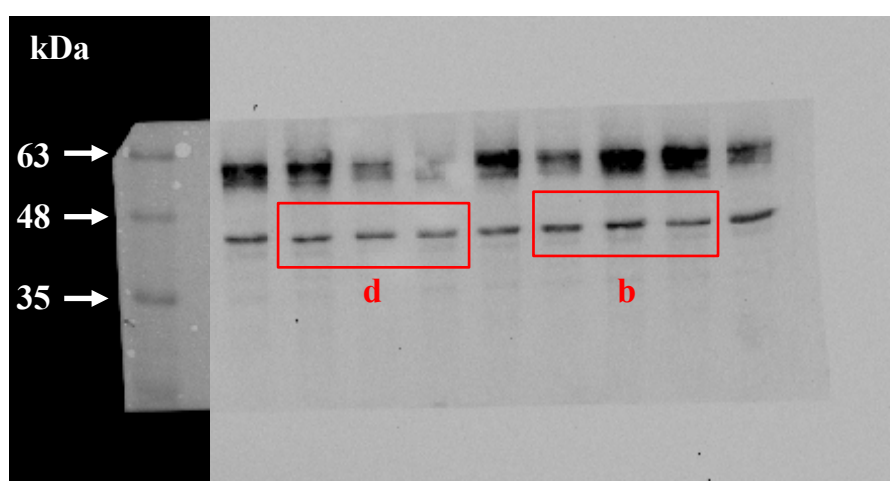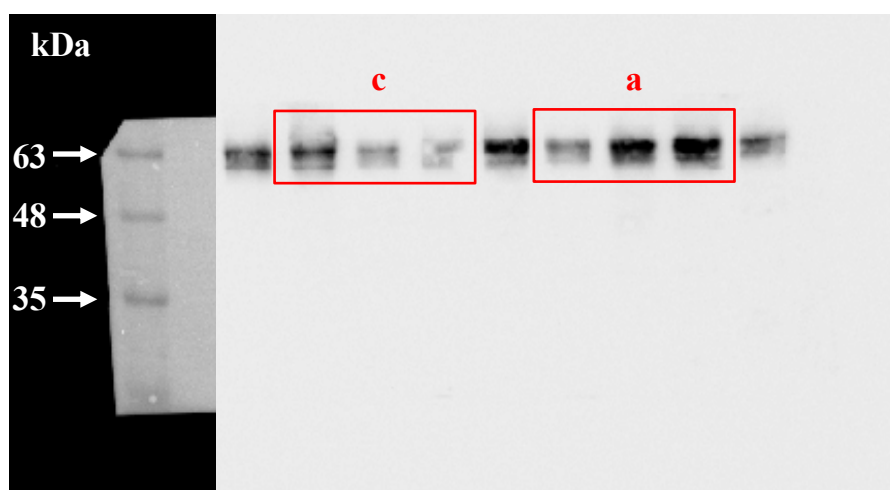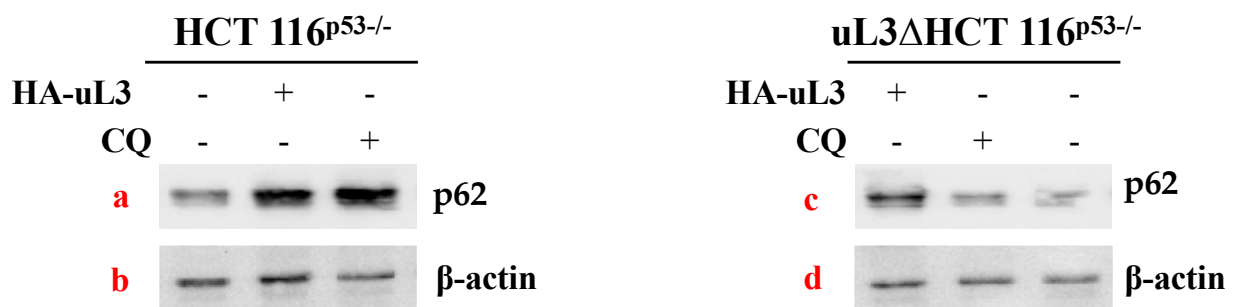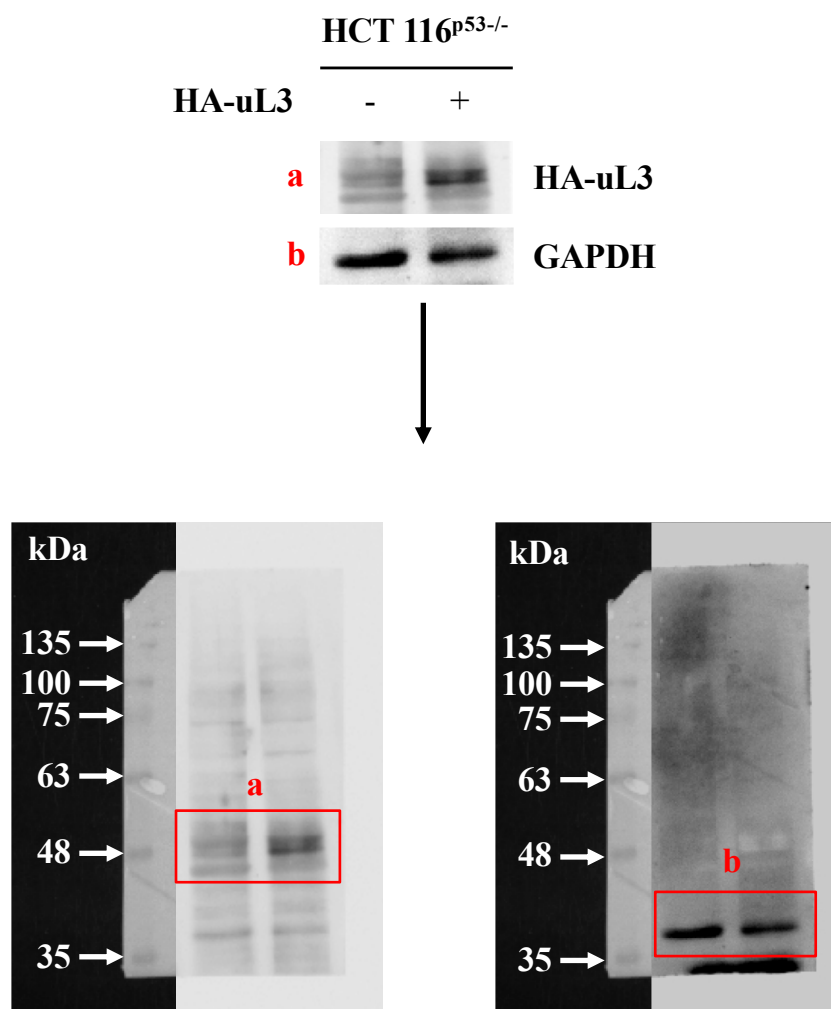**Figure S7.** Full length blots of Figures 6A and 6B.

Figure S8. Full length blots of Figures 6C and 6D .

Figure S9. Full length blots of Figure S1.