

**Degradation of cofilin is regulated by Cbl, AIP4 and Syk resulting in increased
migration of LMP2A positive nasopharyngeal carcinoma cells**

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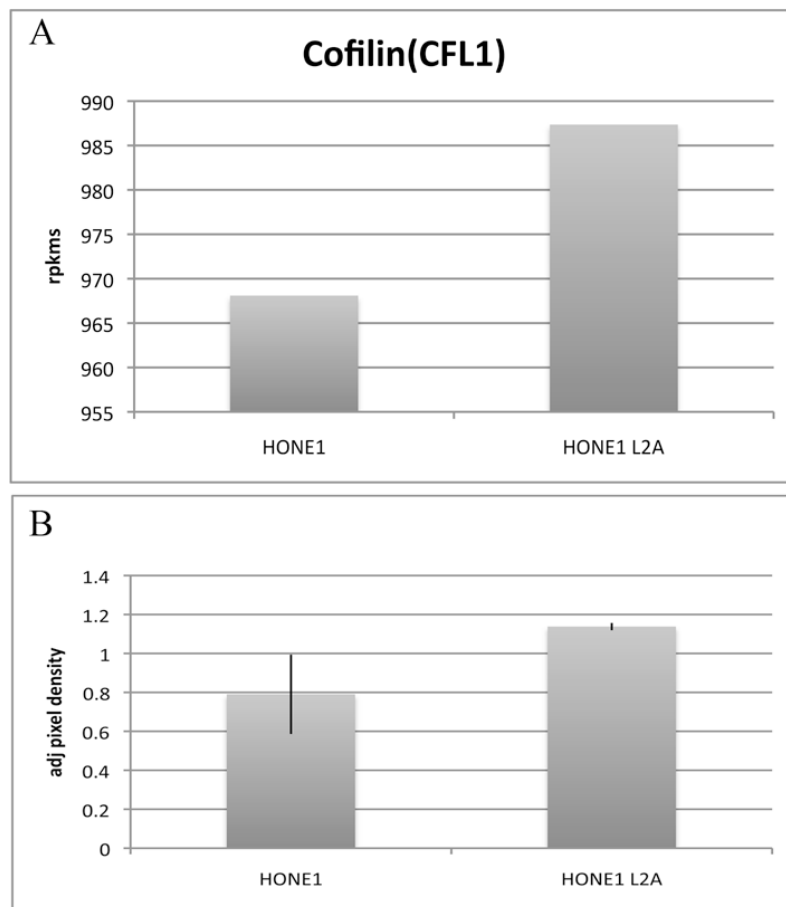


Figure S1. Cofilin gene expression is increased in the LMP2A positive HONE1 NPC cells.

A. RNAseq data (Astakhova et al, PLoS One 2016). B. RT-PCR results.

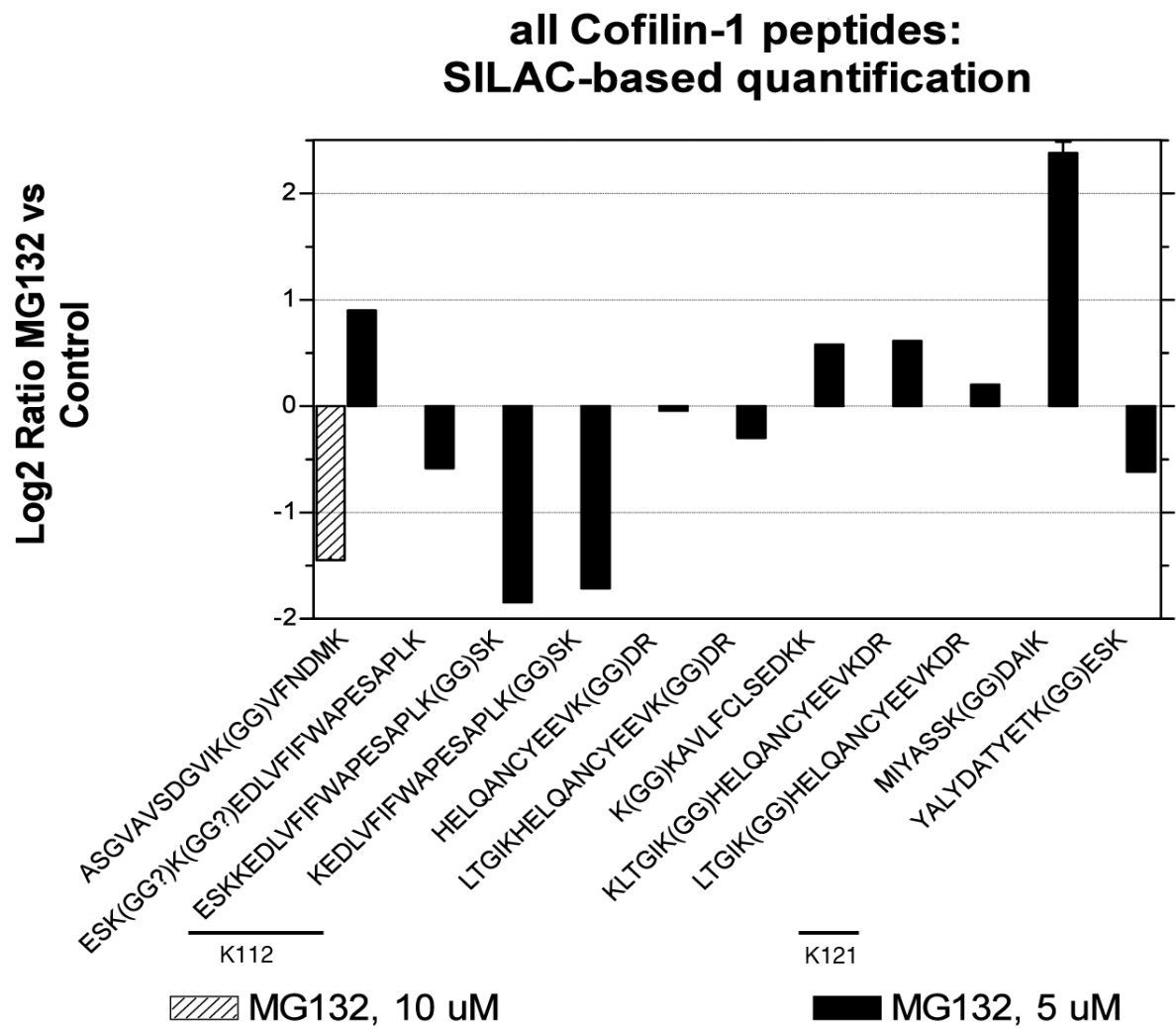


Figure S2. SILAC analysis (36) of cofilin peptide accumulation after tryptic digestion of Jurkat T cell lysates upon inhibition of proteasomal degradation with MG132 (24).

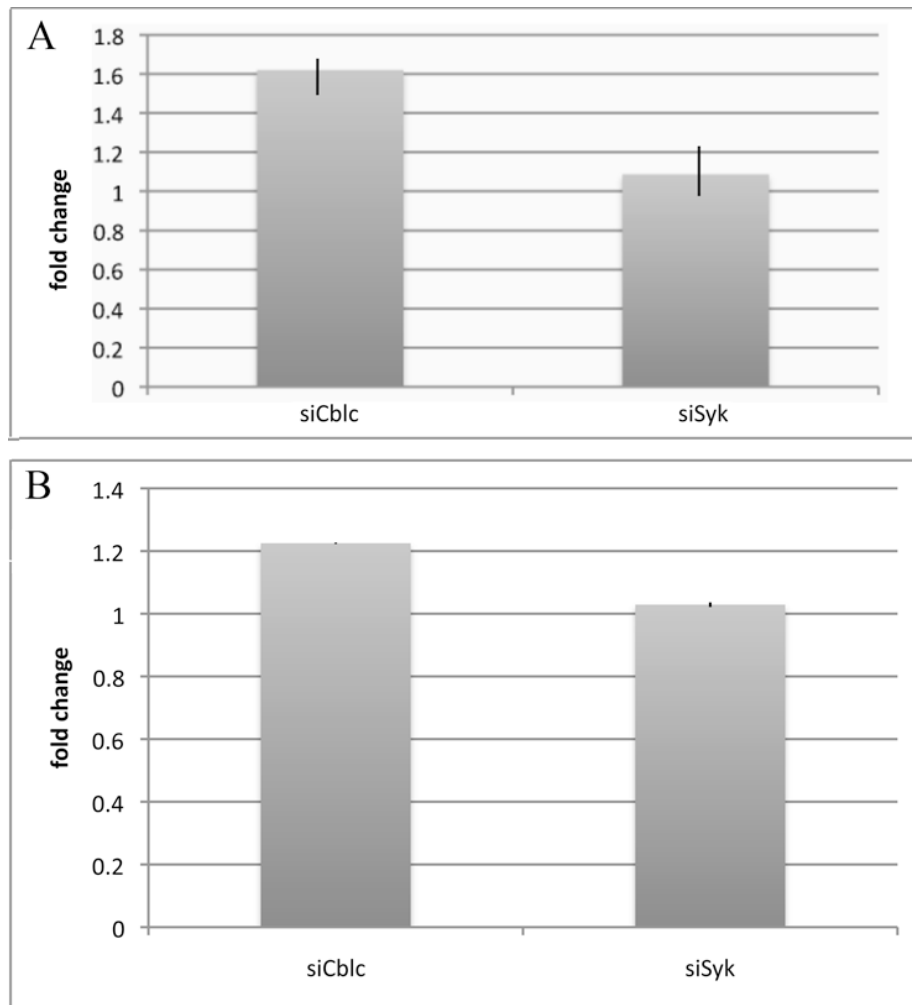


Figure S3. Down-regulation of Cbl and Syk expression results in increased cofilin expression. Western blot (A) and FACS (B) analysis of RFP-cofilin expression in CNE2 cells upon transient transfection with siRNA against either Cbl or Syk.

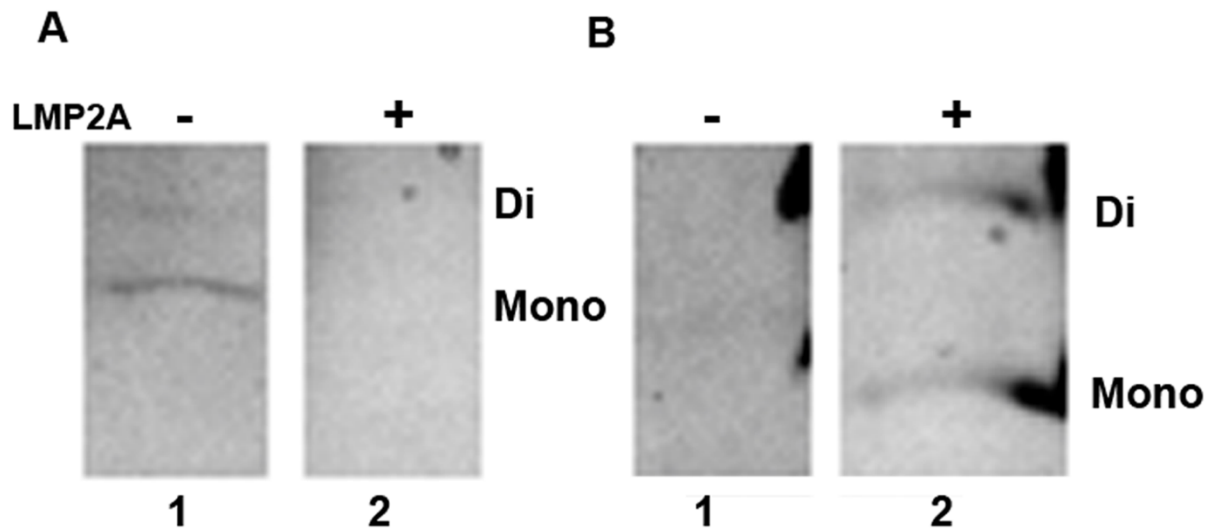


Figure S4. LMP2A expression influences sensitivity of Cofilin immunoprecipitates to different DUBs. Silver stained gel of supernatants of cofilin immunoprecipitates from LMP2A positive (lanes 2) and negative (lanes 1) CNE2 cells after treatment with the K11-ubiquitin linkage specific Cezanne DUB (panel A) and the K63-ubiquitin linkage specific AMSH DUB (panel B) from a panel of DUBs, which specifically recognize different ubiquitin linkages (UbiCREST DUB Enzyme Set, K-400, Bio-technique Ltd).

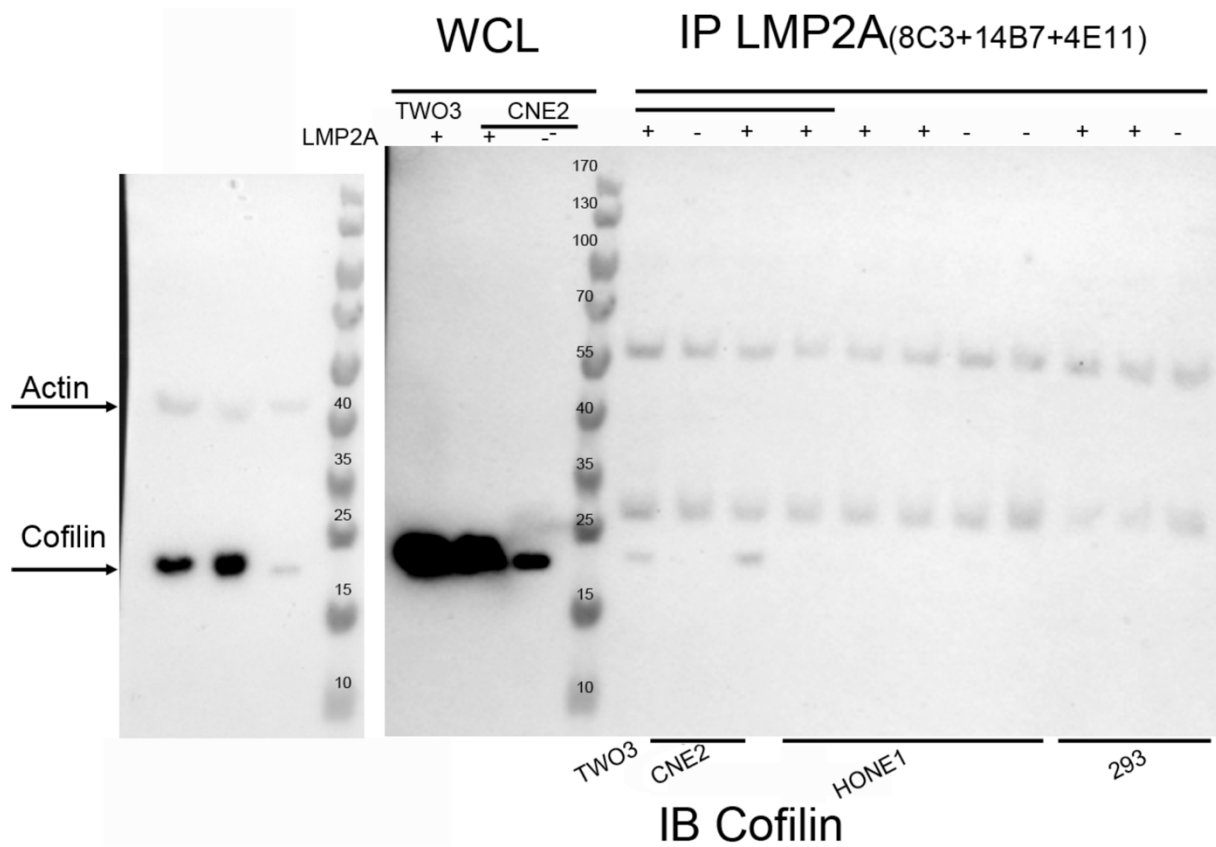
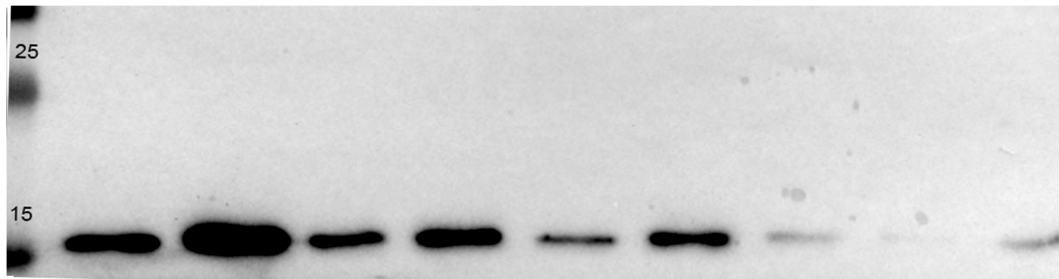


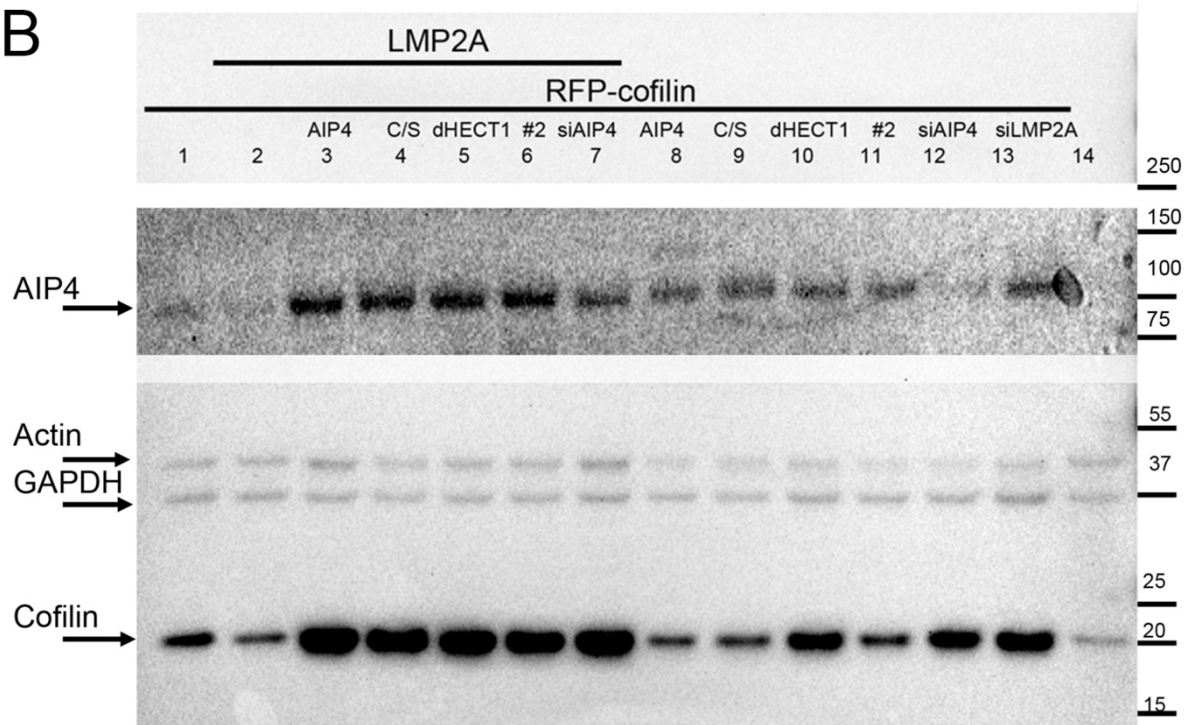
Figure S5. Full-length blot to Figure 1A. LMP2A binds cofilin and influences its stability. WB analysis of cofilin levels in wcl of the NPC cell line CNE2.

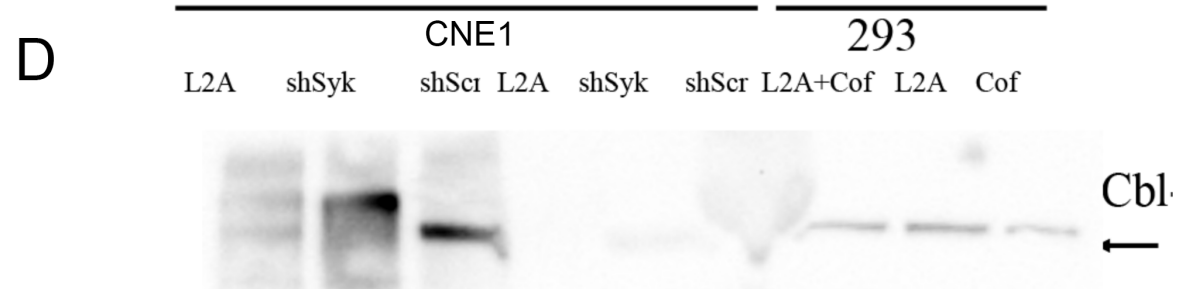
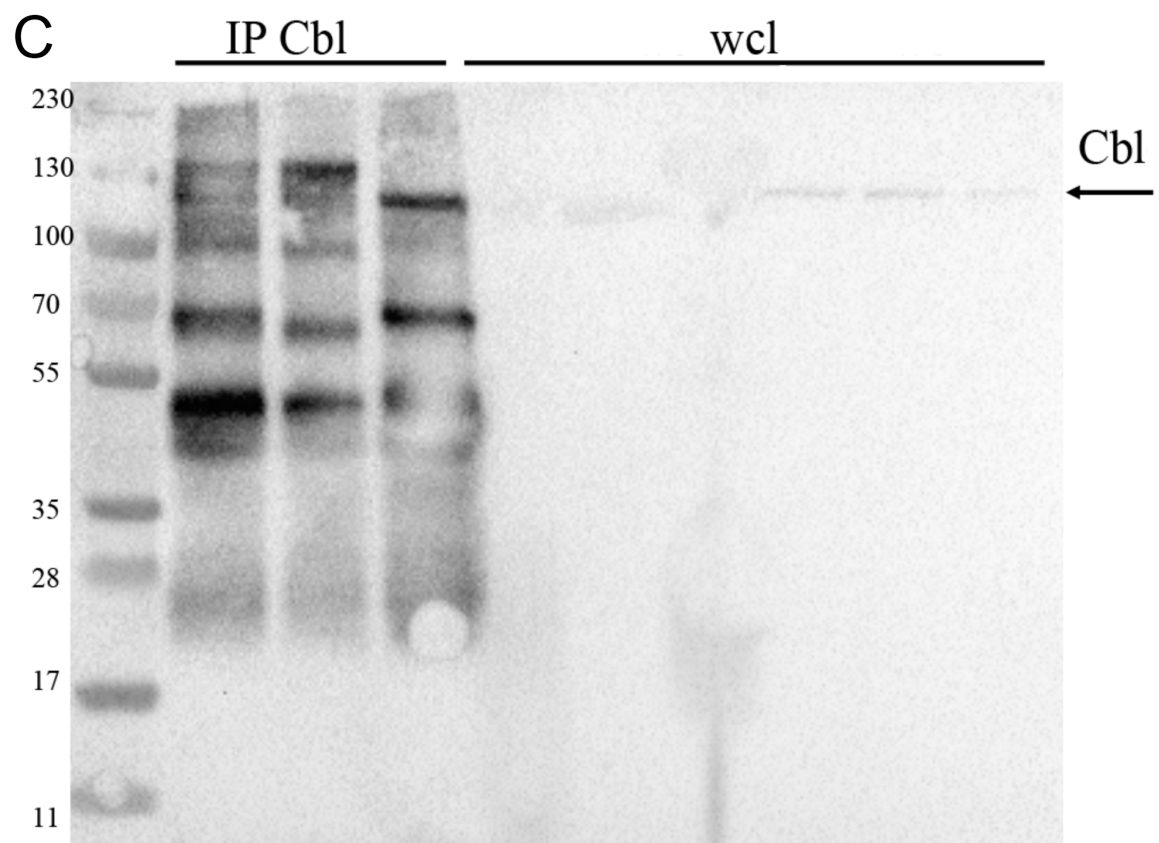
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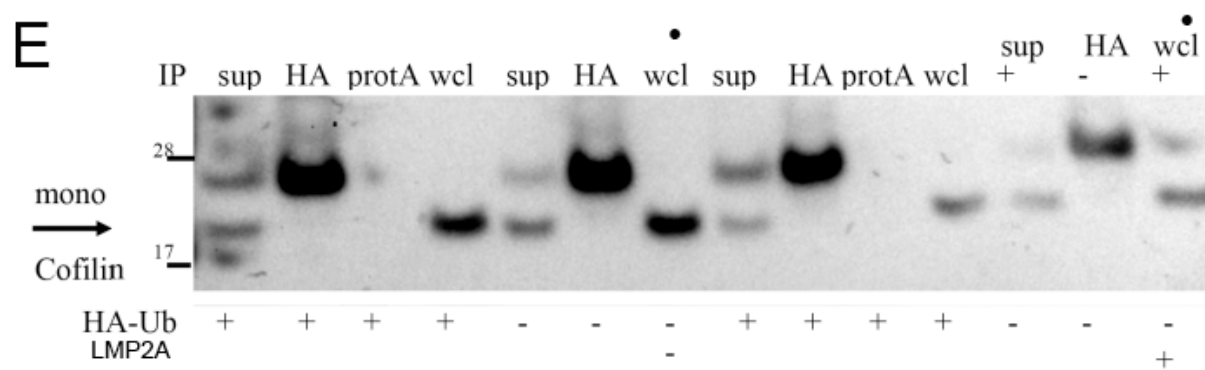
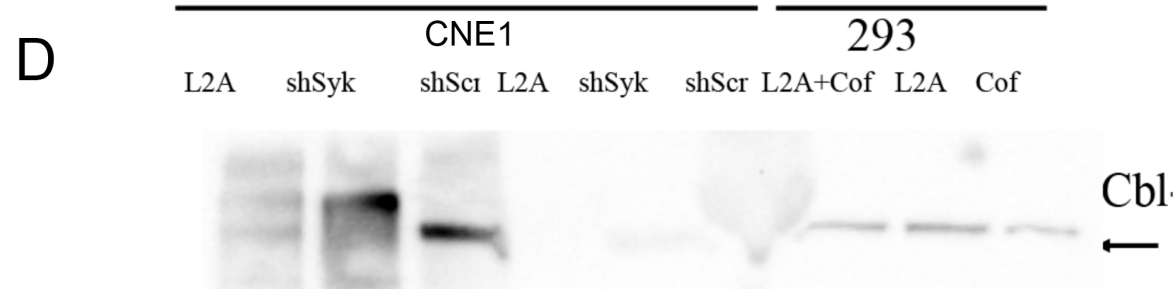
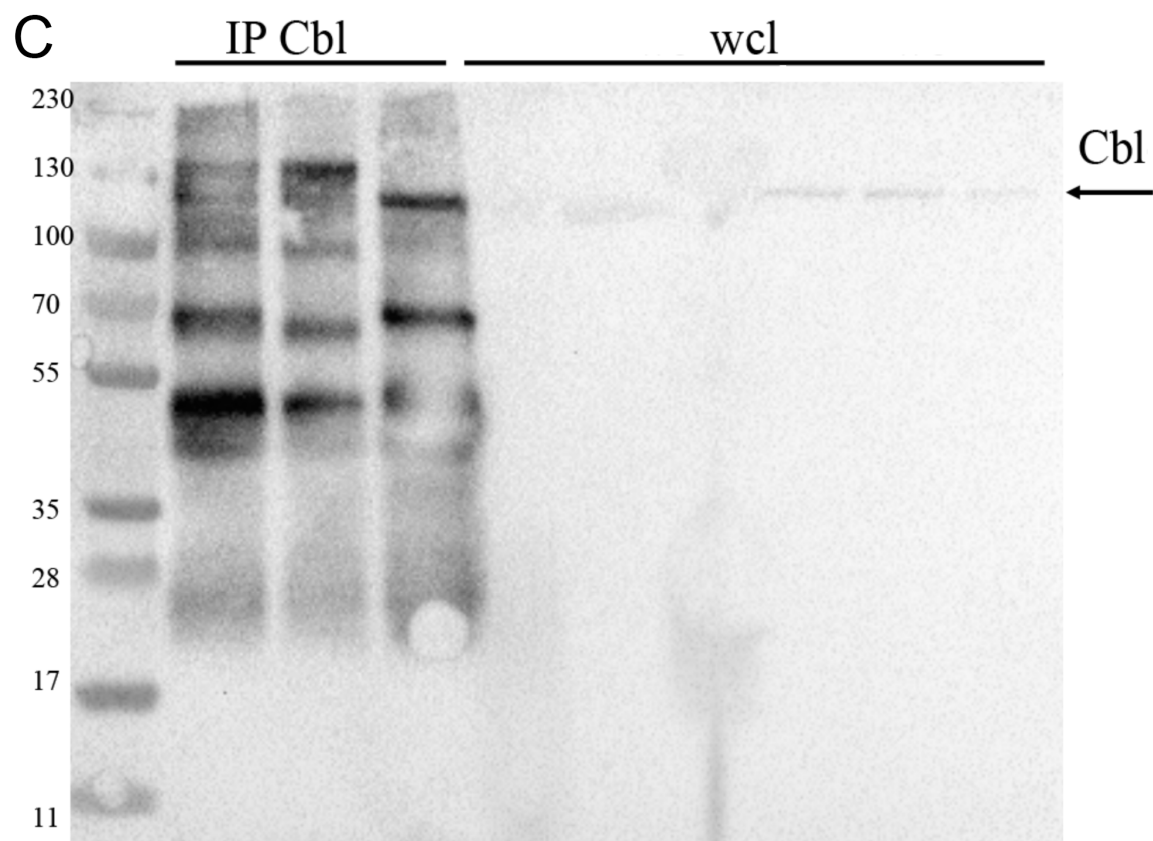


RFP-Cofilin	+	+	+	+	+	+	-	-	-
LMP2A	-	-	+	+	+	+	-	-	-
Cbl	-	wt	wt	G306E	70Z	LMP2A Δ noCT wt	wt	70Z	G306E

B







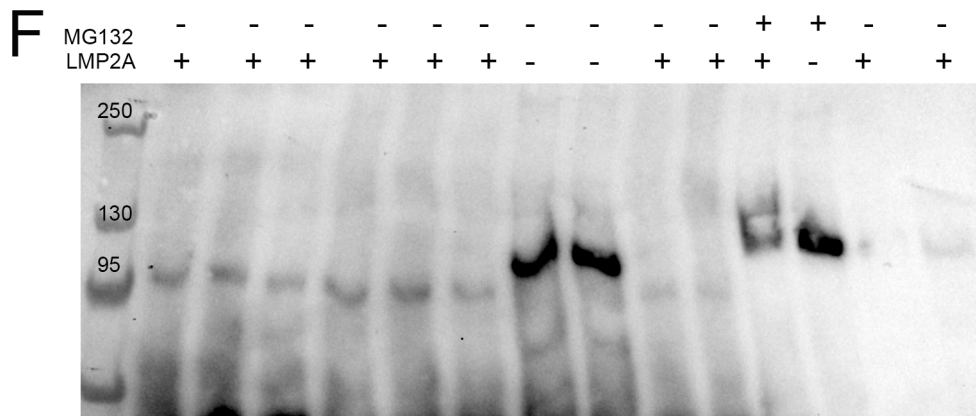


Figure S7. Full-length blots to Figure 3. Two ubiquitin E3 ligases are involved in the regulation of cofilin protein stability. WB analysis of cofilin expression in HEK293 cells upon transient expression of wt and mutant forms of Cbl (panel A) or AIP4 (panel B). The Cbl immunoprecipitations show the appearance of post-translationally modified (larger) forms of Cbl in LMP2A or shRNAsyk expressing cells. There two different exposures of the western blot are presented on figure S7C and D. Anti-cofilin immunoblot in Panel E show that additional larger form of cofilin (27 kDa) disappears in wcl of LMP2A positive TWO3 cells (compare lanes labeled with dots). Panel F presents anti-Cbl immunoblot of LMP2A positive and negative TWO3 cells.