Supplementary Figure S1. Protein expression data accompanying **(A)** Figure 1A, **(B)** Figure 1B, **(C)** Figure 1C, **(D)** Figure 1D, and **(E)** Figure 2C. IB, immunoblot.

Supplementary Figure S2. **HERC3 does not impair AP-1 transcriptional activity.** BAEC were transfected with AP-1-luciferase reporter plasmid (200ng) with or without MEKK inducer (20ng) and HERC3 (140ng). The constitutively expressed RSV-β-galactosidase plasmid (40ng) was used as normalization control. Luciferase activities were measured 24 hours after transfection and normalized to β-galactosidase activities. MEKK-induced reporter activity was set to 100 and activity of all other datasets was calculated relative to this value. Bars represent mean + SEM (n=9, derived from 3 independent experiments).

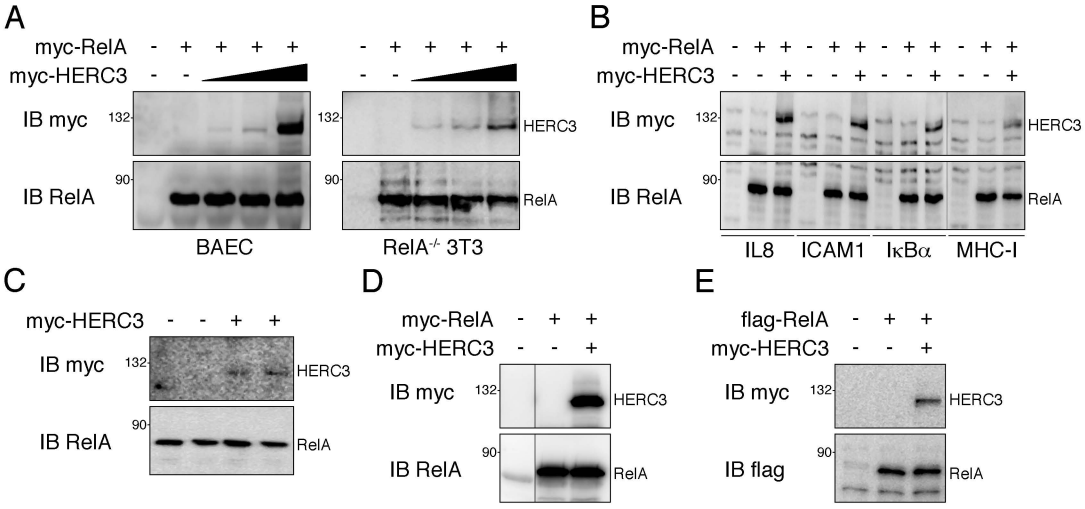
Supplementary Figure S3. **HERC3-RelA interaction and mediated ubiquitination are inhibited in presence of IκBα. (A)** HEK293T were transfected with myc-RelA/myc-HERC3, with and without HA-IκBα. Interaction of proteins was determined by co-immunoprecipitation of HERC3 and IκBα with RelA. **(B)** HEK293T were transiently transfected with his-ubiquitin, myc-RelA, myc-HERC3, and HA-IκBα. Ubiquitination of RelA was assessed by pull down under denaturing conditions as described in Fig. 3C. IB, immunoblot; IP, immunoprecipitation. All experiments were carried out at least 3 times.

Supplementary Figure S4. **HERC3-mediated RelA ubiquitination is partly dependent on RelA lysines 195/315. (A)** MS/MS spectra of 2 identified triply-charged peptides are shown. In panel 1 at m/z 462.91^{3+} covering residues 188 to 198, RelA lysine 195 is identified as ubiquitination site. In panel 2 at m/z 583.97^{3+} within residues 315 to 329, RelA ubiquitination on lysine 315 is uncovered. **(B)** HEK293T transiently transfected with his-ubiquitin, myc-RelA wild type and mutants as well as myc-HERC3 were lysed and ubiquitination of RelA was tested by denaturing pull down as described in Fig. 3C. **(C)** Transcriptional activities of RelA wild type and KR double mutant in presence of HERC3 were compared analogous to Fig. 1A. Bars represent mean +SEM (n=18, derived from 6 independent experiments). All shown experiments were at least performed in

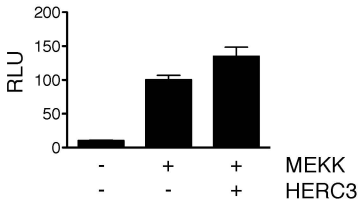
triplicates. KR, lysine to arginine; IB, immunoblot; IP, immunoprecipitation; RLU, relative luciferase units; wt, wild type.

Supplementary Figure S5. **Interactions between HERC3, RelA and UBQLN1 are not direct. (A) (B)** Myc-RelA, flag-HERC3, HA-IκBα and flag-UBQLN1 were *in vitro* translated using wheat germ extract. After combining the extracts containing the indicated proteins (input), IκBα, HERC3 and UBQLN1 were precipitated with HA- and flag-antibodies, respectively. Presence of RelA in pull downs was detected with RelA-specific antibody. IB, immunoblot; IP, immunoprecipitation. The experiments were carried out 4 times for (A) and 2 times for (B).

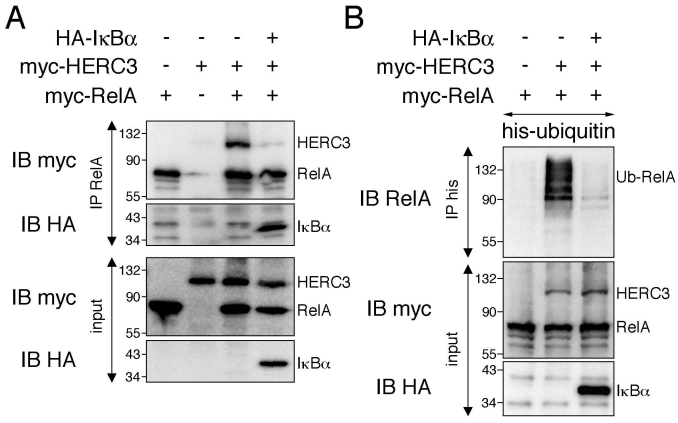
Supplementary Figure S1



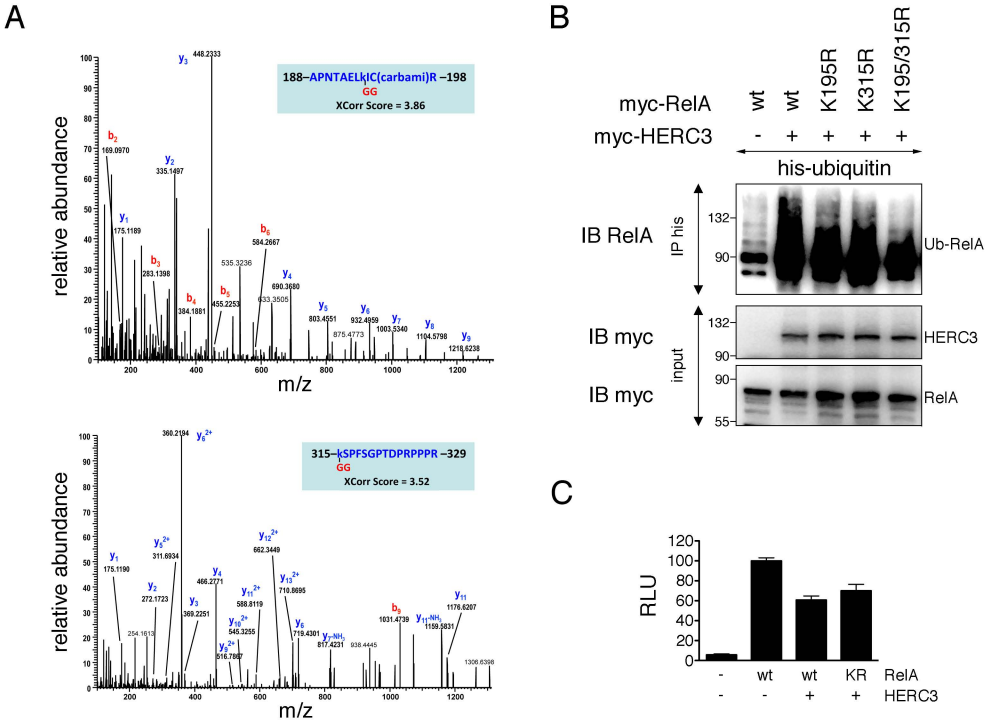
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5