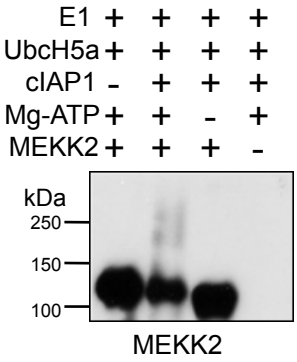
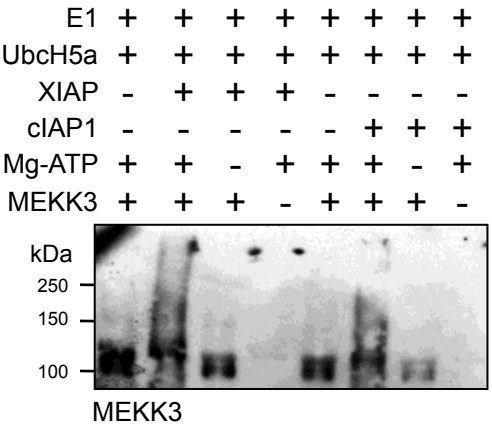
Supplementary Figure S3

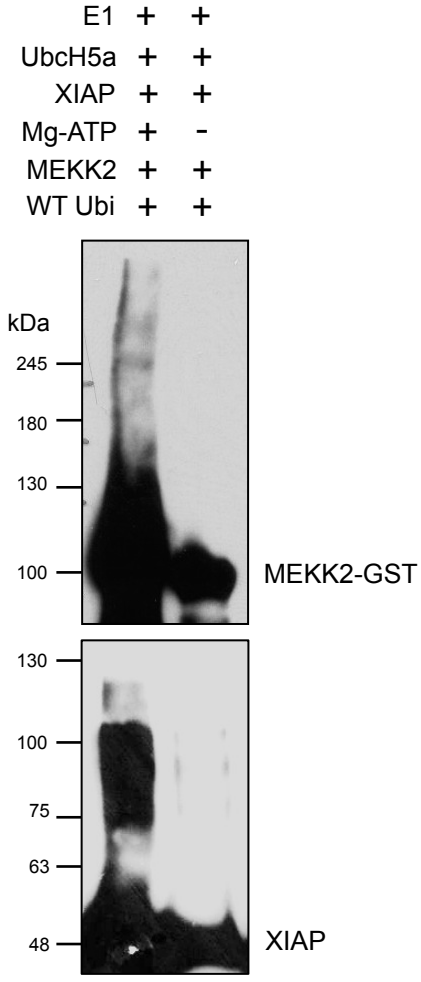
A

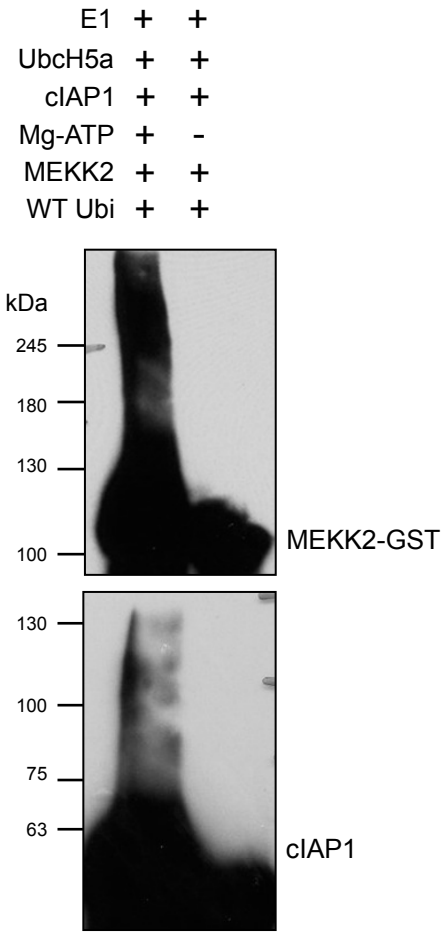


B

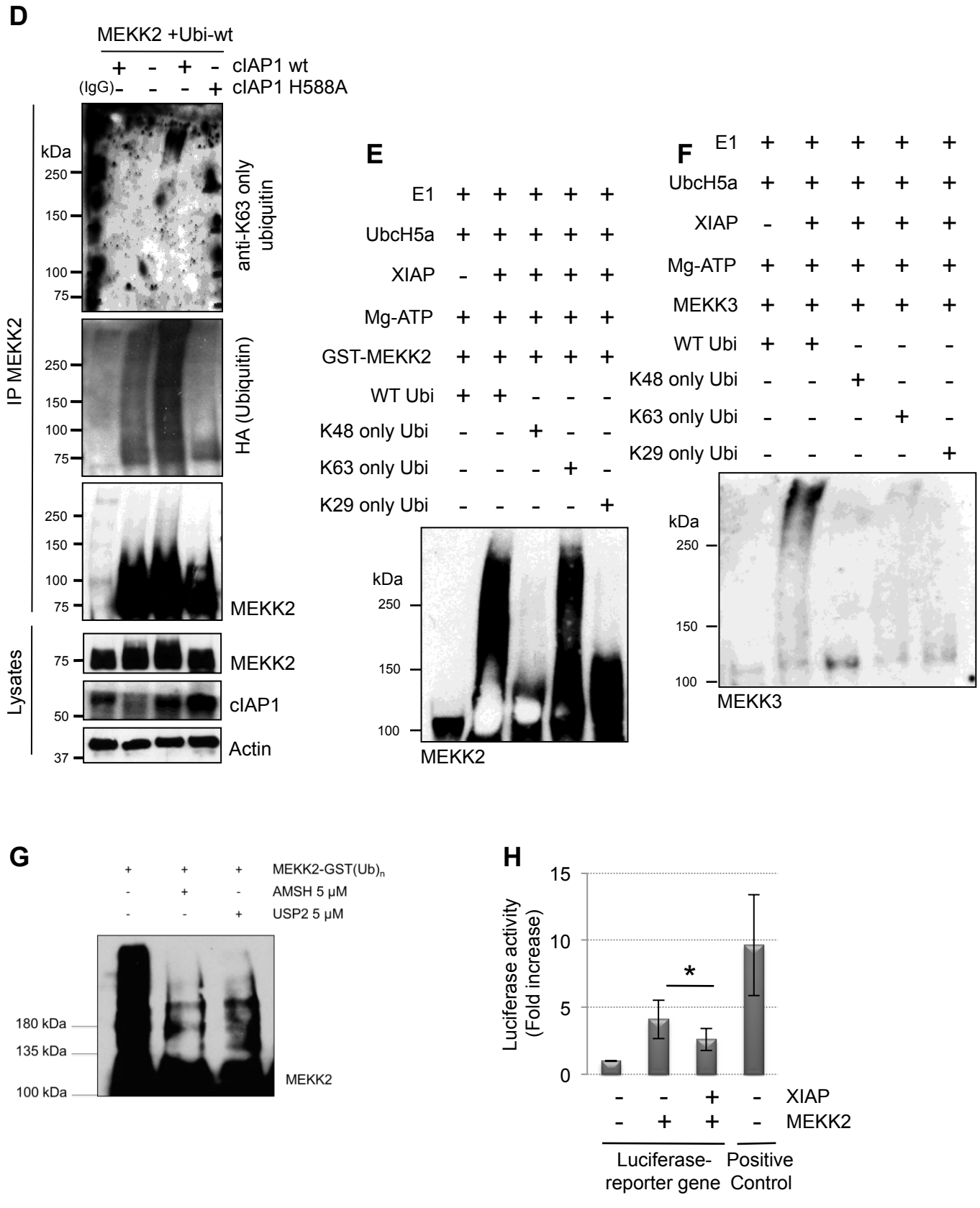


C





Supplementary Figure S3 (continued)



Supplementary Figure S3

(**A**) cIAP1 promotes ubiquitination of MEKK2 *in vitro*. *In vitro* ubiquitination of purified MEKK2 by cIAP1 was performed as stated in Materials and Methods section and analyzed by western blotting using MEKK2 antibody. (**B**) XIAP and cIAP1 promote ubiquitination of MEKK3 *in vitro*. Purified MEKK3 was subjected to *in vitro* ubiquitination by XIAP and cIAP1 and results were analyzed by western blotting using MEKK3 antibody. (**C**) MEKK2 was subjected to in vitro ubiquitination with XIAP and cIAP1 and the autoubiquitination of IAPs was monitored. (**D**) K63-ubiquitination of MEKK2 is dependent of cIAP1-E3 ligase activity. Flag-MEKK2 and HA-Ubiquitin were transfected in HEK293T cells with myc-EV, cIAP1 or cIAP1-H588A mutant. MEKK2 was immunoprecipitated using Flag antibody and ubiquitination was checked using western blot analysis with HA and K63-linkage specific antibodies. (**E and F**) XIAP promotes K63 specific-ubiquitination of MEKK2 and MEKK3 *in vitro*. In vitro ubiquitination of purified MEKK2 (E) and MEKK3 (F) by XIAP in the presence of various ubiquitin mutants was performed as stated in Materials and Methods section and analyzed by western blotting using MEKK2 (E) or MEKK3 (F) antibody. (**G**) MEKK2 was ubiquitinated with XIAP as mentioned earlier. The ubiquitinated MEKK2 was treated with AMSH and Usp2 as mentioned in the methods section. The ubiquitination of MEKK2 after treatment was monitored. (**H**) HEK293T cells were transfected with XIAP, MEKK2 or both, together with the Luciferase and the Renilla reporter genes. The luciferase activity was measured, normalized to Renilla activity and the fold increase of four independent experiments is shown (*=$p<0.05$).