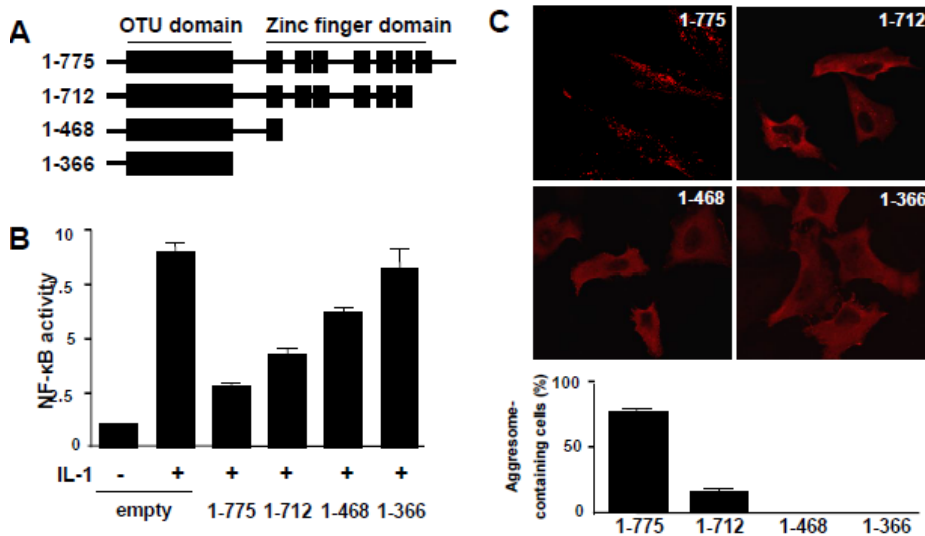


**Supplementary Figure 1 A20 ubiquitination is not required for NF- $\kappa$ B regulation in HeLa or A20<sup>-/-</sup> MEFs.**

(A) HeLa cells were co-transfected with varying amounts of pHM6-A20 wild-type (wt) or C624,627A A20 mutant together with fixed amounts of NF- $\kappa$ B reporter gene (pGL2) and Renilla luciferase control (pRL-TK). Transfected cells were then stimulated with TNF $\alpha$  or IL-1 for 16 hours. Cell lysates were analysed to determine the ratio of firefly/renilla luciferase, a measure of NF- $\kappa$ B activity normalised for transfection efficiency. Mean values calculated from triplicate wells were pooled from 4 experiments and are shown as mean  $\pm$  standard deviations. (B) A20<sup>-/-</sup> MEFs were transfected with pEGFP-A20 wild-type (WT) or with C103S or C624,627A A20 mutants. Transfected cultures (or untransfected controls) were stimulated with IL-1 (20ng/ml) or TNF $\alpha$  (10ng/ml) for 15 minutes. The intracellular localisation of RelA was assessed by immunofluorescence staining and confocal microscopy. Representative images are shown.



**Supplementary Figure 2**

**A20 activity correlated with punctate intracellular localisation.**

(A-C) HeLa cells were transfected with Flag-tagged total and A20 deletion mutants corresponding to residues 1-775 (wild-type), 1-712, 1-468 and 1-366 (or with empty vector as a control). (A) Schematic of the A20 deletion mutants in relation to A20 domain structure. (B) Cells were co-transfected with NF- $\kappa$ B reporter gene (pGL2) and Renilla luciferase control (pRL-TK) before stimulation with IL-1 for 16h. Cell lysates were analysed to determine firefly/renilla luciferase ratio which is a measure of NF- $\kappa$ B activity normalised for transfection efficiency. Mean values calculated from triplicate wells were pooled from 2 experiments and are shown with standard deviations. (C) The localization of A20 1-775, 1-712, 1-468 and 1-366 was determined by immunofluorescence using anti-FLAG antibodies. Representative images are shown. The proportion of cells containing punctate A20 were calculated and pooled from 3 experiments and reported as mean values  $\pm$  standard deviations.