

Supplemental Text and Figures

ARF-BP1 enhances p42 ubiquitination

To explore whether ARF-BP1 mediates p42 ubiquitination, we cotransfected GST-p42 and HA-ubiquitin into HEK293 cells in the presence of ARF-BP1 or MDM2, followed by MG132 treatment. ARF-BP1 strongly stimulated p42 polyubiquitination, whereas MDM2 barely influenced its ubiquitination. MG132 treatment further elevated the ubiquitination effect (Supplemental Figure 2A, top left panel). Cotransfection of ARF-BP1 but not MDM2 also upregulated GST-p42 polyubiquitination by the endogenous ubiquitin (data not shown). To investigate whether ARF-BP1 interacts with p42, we conducted a GST-pull down assay. Compared with GST control, GST-p42 robustly associated with ARF-BP1, but it was unable to bind MDM2 (Supplemental Figure 2B, top left and middle panels). Interestingly, S360D mutant exhibited even stronger affinity than wild-type p42, whereas S360A failed to bind Arf-BP1, suggesting that Ebp1 phosphorylation promotes its association with ARF-BP1 (Supplemental Figure 2B, right panels). However, ablation of ARF-BP1 did not induce p42 expression in human cancer cells (Supplemental Figure 2C). These data suggest that ARF-BP1 might not be the physiological E3 ubiquitin ligase for p42.

Supplemental Figure 1. ARF-BP1 enhances p42 ubiquitination

A, ARF-BP1 promotes p42 ubiquitination. GST-p42 and HA-ubiquitin were cotransfected into HEK293 cells in the presence of ARF-BP1 or MDM2, followed by MG132 treatment.

Transfection of ARF-BP1 enhanced p42 ubiquitination, which was further augmented by MG132 treatment. In contrast, MDM2 failed (top panel). Verification of transfected constructs (2nd to bottom panels). **B**, ARF-BP1 binds p42. Glutathione beads pull-down reveals that GST-p42

bound to cotransfected ARF-BP1 but not MDM2. S360D exhibited the strongest binding affinity to ARF-BP1; in contrast, S360A did not bind to ARF-BP1. C, Depletion of ARF-BP1 does not affect p42 expression in human cancer cells. Human cancer cell lines were transfected with si-RNA of ARF-BP1, and ARF-BP1 was decreased in a dose-dependent manner (top panel). P53 was induced upon ARF-BP1 depletion, however, p42 expression was not detected (2nd to bottom panels).

Supplemental Figure 1

