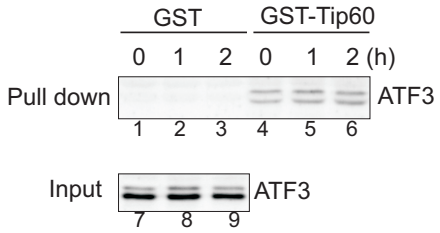
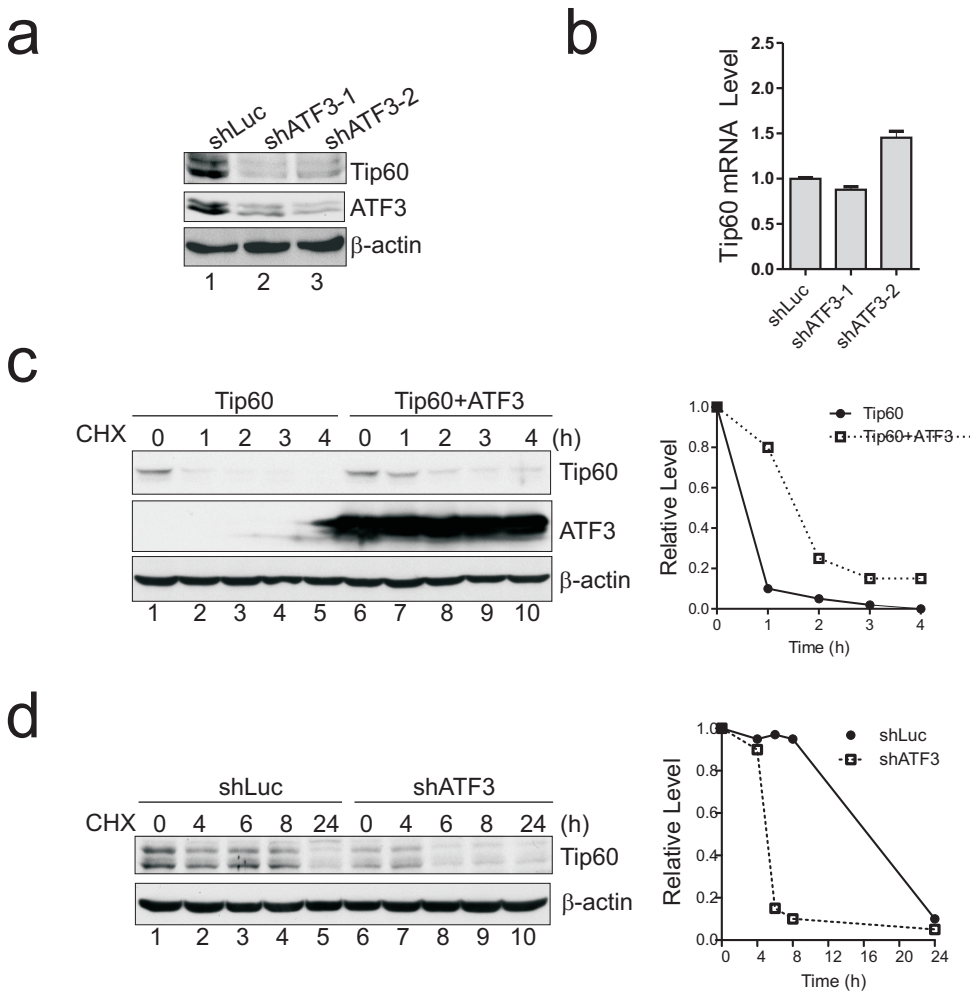
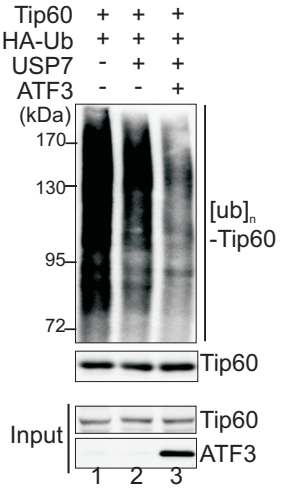
Supporting Information



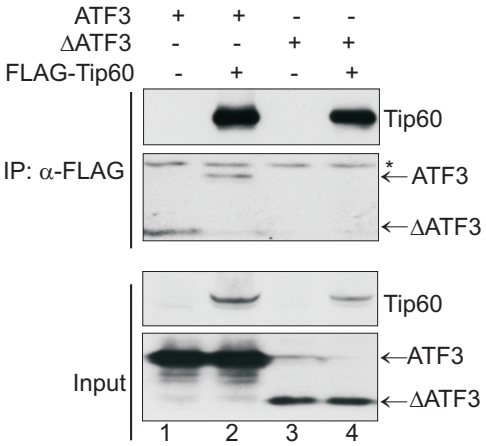
Supplementary Figure 1. **Irradiation does not alter the binding of ATF3 to immobilized GST-Tip60.** After 10 Gy of IR, HCT116 cells were lysed, and cell lysates containing an equal amount of ATF3 were incubated with GST-Tip60 immobilized onto glutathione agarose for 5 h. Beads were washes, and bound proteins were eluted for Western blotting.



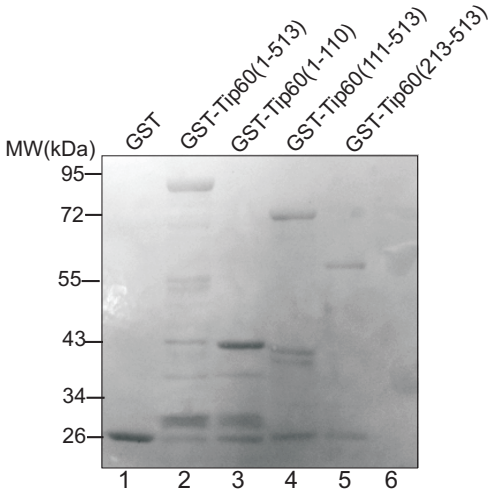
Supplementary Figure 2. **ATF3 stabilizes both endogenous and exogenously-expressed Tip60.** (**a**,**b**) Knockdown of ATF3 in HCT116 cells led to downregulation of the protein level but not the mRNA level of Tip60. HCT116 cells in which the endogenous Tip60 protein was tagged with a 3×FLAG peptide were infected with Lentiviruses expressing shLuc or shATF3. Stable clones were subjected to Western blotting for Tip60 expression using the FLAG antibody (A). The Tip60 mRNA level was determined by quantitative RT-PCR (B). (**c**) ATF3 increased the stability of exogenously-expressed Tip60. H1299 cells transfected with FLAG-Tip60 and/or ATF3 were treated with 100 μg/ml of cycloheximide, and subjected to Western blotting. (**d**) Knockdown of ATF3 decreased the half-life of the endogenous Tip60 protein in HCT116 cells. HCT116 cells expressing 3×FLAG-Tip60 was subjected cycloheximide-chase assays as described above. The half-life of exogenously-expressed Tip60 appeared to be shorter than that of the endogenous protein.



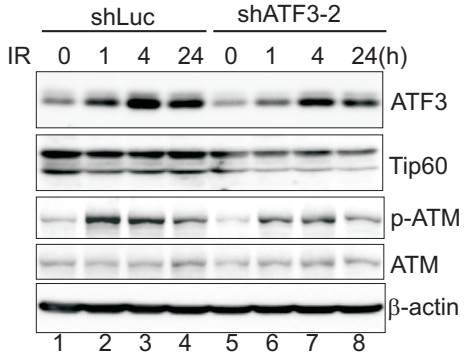
Supplementary Figure 3. **ATF3 promotes USP7-mediated decrease of the Tip60 ubiquitination level.** Lysates from transfected H1299 cells were immunoprecipitated with the FLAG antibody followed by Western blotting using the HA antibody.



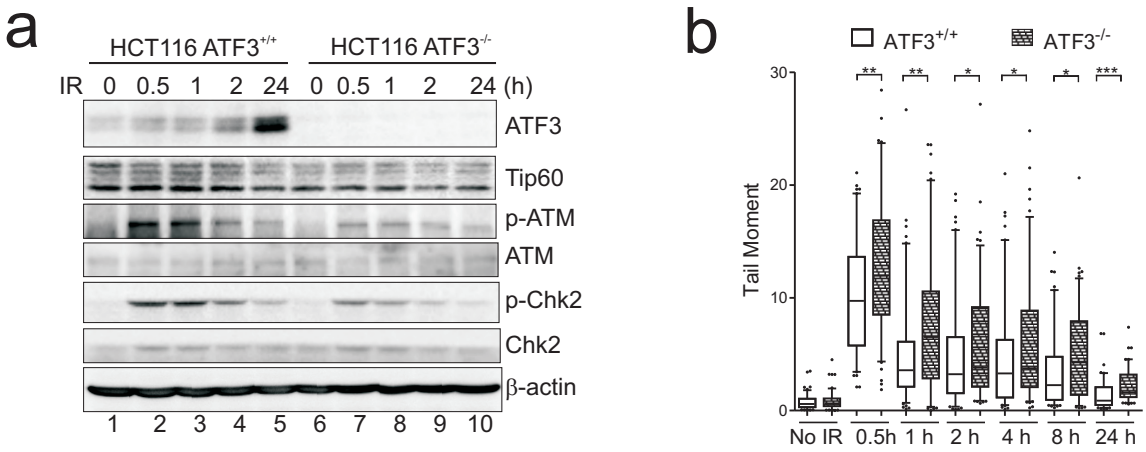
Supplementary Figure 4. **The ATF3 mutant with a deletion of the Zip domain (Δ102-139, ΔATF3) is deficient in binding to Tip60.** H1299 cells were transfected with FLAG-Tip60, ATF3, and ΔATF3 as indicated, and subjected to co-IP using the FLAG antibody. A non-specific band or the IgG light chain was indicated by a "*".



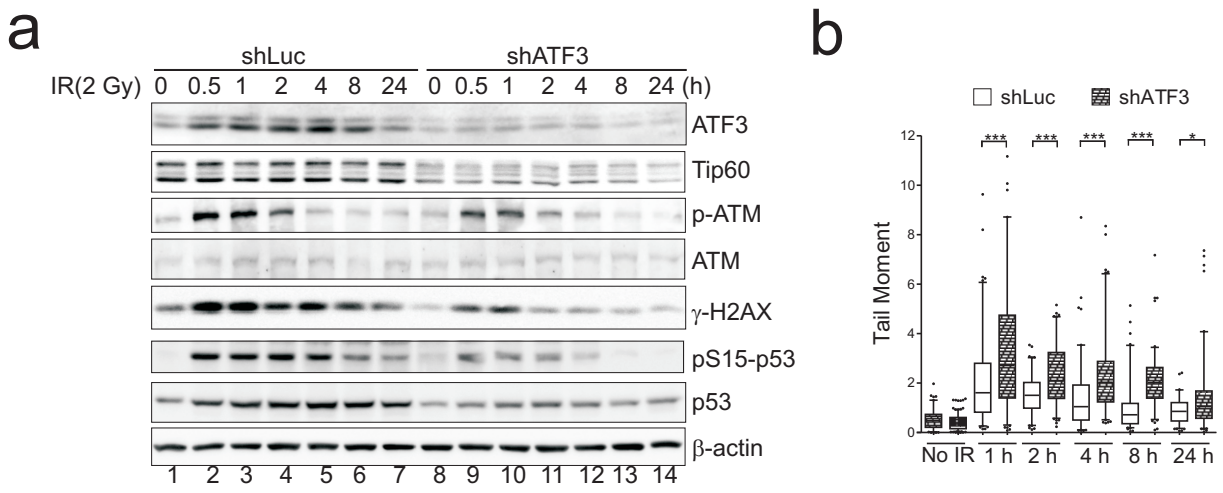
Supplementary Figure 5. **Ponceau S staining of GST-Tip60 fusion proteins.** After immunoblotted for ATF3 binding, the blot in Figure 5A was stained with Ponceau S as control.



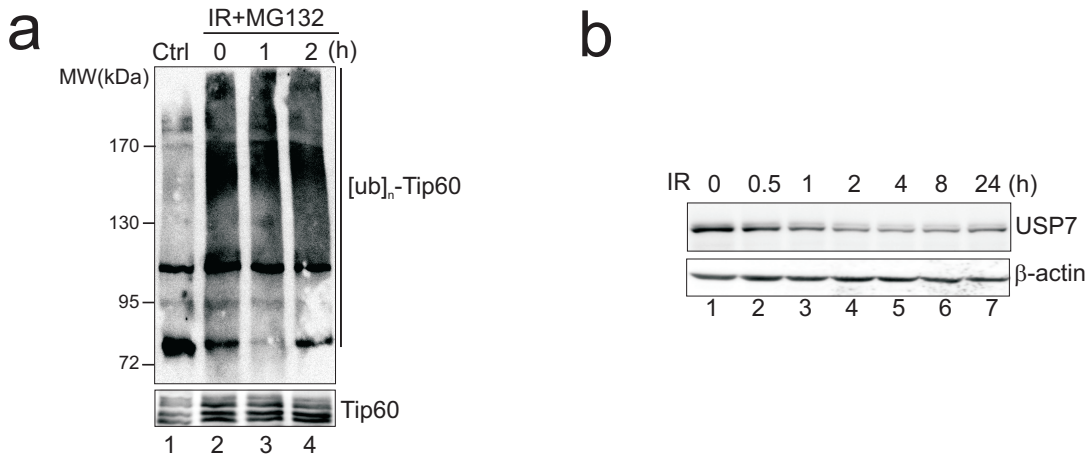
Supplementary Figure 6. **ATF3 knockdown impairs IR-mediated ATM activation in another U2OS clone stably expressing shATF3 (shATF3-2).** The cells were irradiated by 10 Gy of IR for Western blotting.



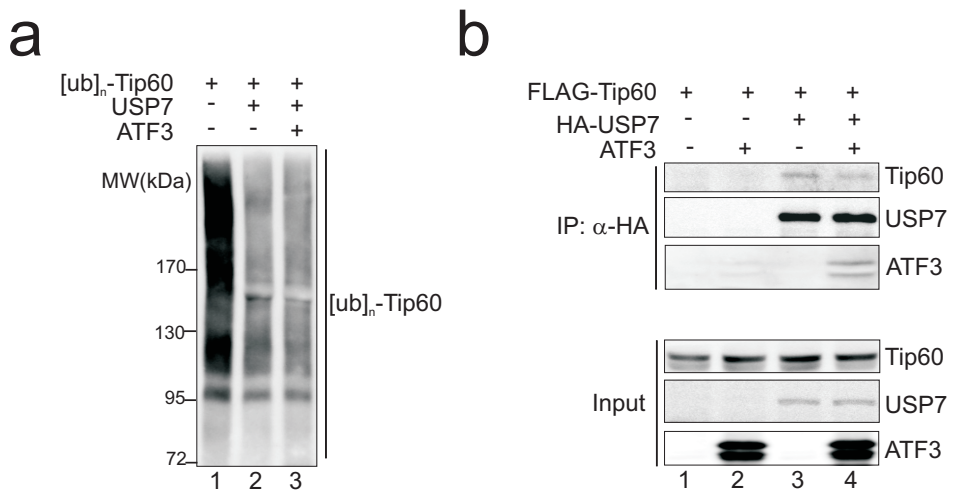
Supplementary Figure 7. **Knockout of ATF3 expression impairs IR-mediated ATM activation and subsequent DNA repair in HCT116 cells.** (**a**) ATM activation was impaired in HCT116 cells engineered to lose ATF3 expression. ATF3-deficient ($ATF3^{-/-}$) HCT116 were developed using homologous recombination mediated by adenoassociated viruses. These cells and wild-type cells were subjected to 10 Gy of IR for Western blotting. (**b**) ATF3 wildtype (ATF3+/+) and knockout cells (ATF3-/-) were irradiated by 10 Gy γ-rays, and subjected to comet assays after indicated periods of time. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Mann Whitney test.



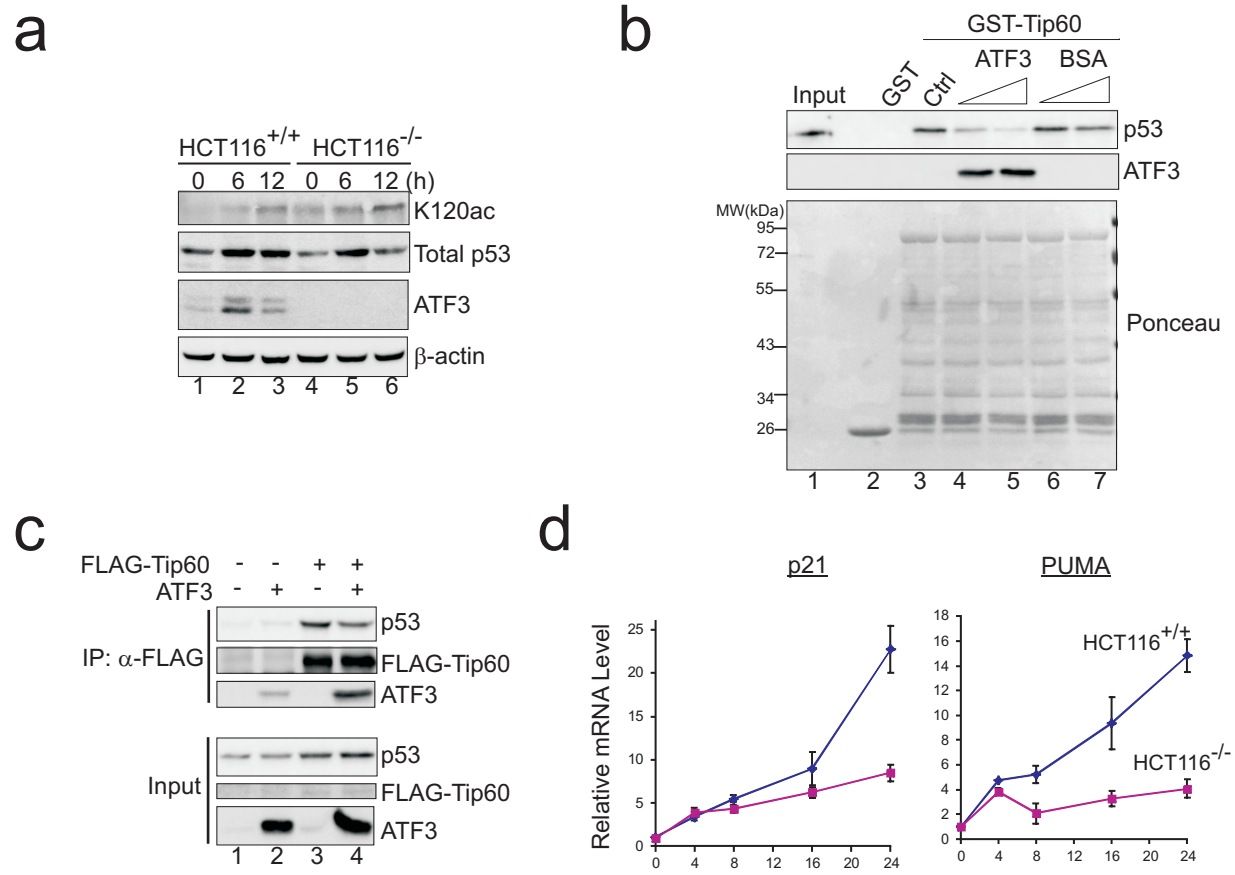
Supplementary Figure 8. **Knockdown of ATF3 expression in U2OS cells impairs ATM activation and DNA repair in response to a sublethal dose of IR (2 Gy).** U2OS cells expressing shATF3 or control shRNA were irradiated by 2 Gy of γ-rays, and then lysed for Western blotting (**a**), or subjected to comet assays (**b**) as indicated. *, $p < 0.05$; ***, $p < 0.001$; Mann Whitney test.



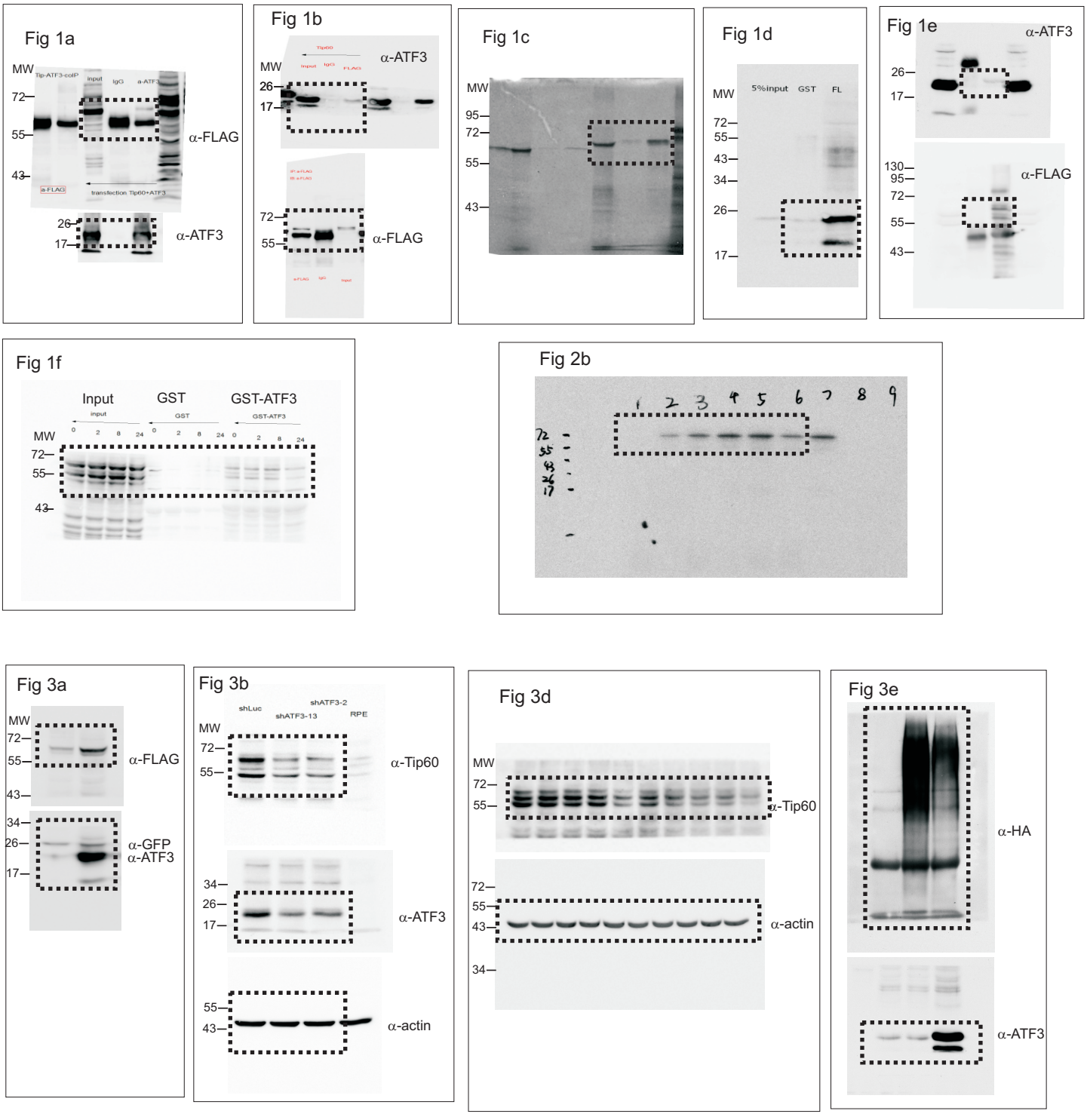
Supplementary Figure 9. **Irradiation does not affect Tip60 ubiquitination but downregulates USP7 expression.** (**a**) HCT116 3×FLAG cells transfected with HA-ubiquitin were treated with 10 μM of MG132 for 5 h, and then irradiated by 10 Gy of IR. Cell lysates were immunoprecipitated using the FLAG antibody, and subjected to Western blotting to detect ubiquitinated Tip60 using the HA-antibody. The "Ctrl" sample was not treated with MG132 or IR. (**b**) U2OS cells were irradiated by 10 Gy of IR, and subjected to Western blotting for USP7 expression.



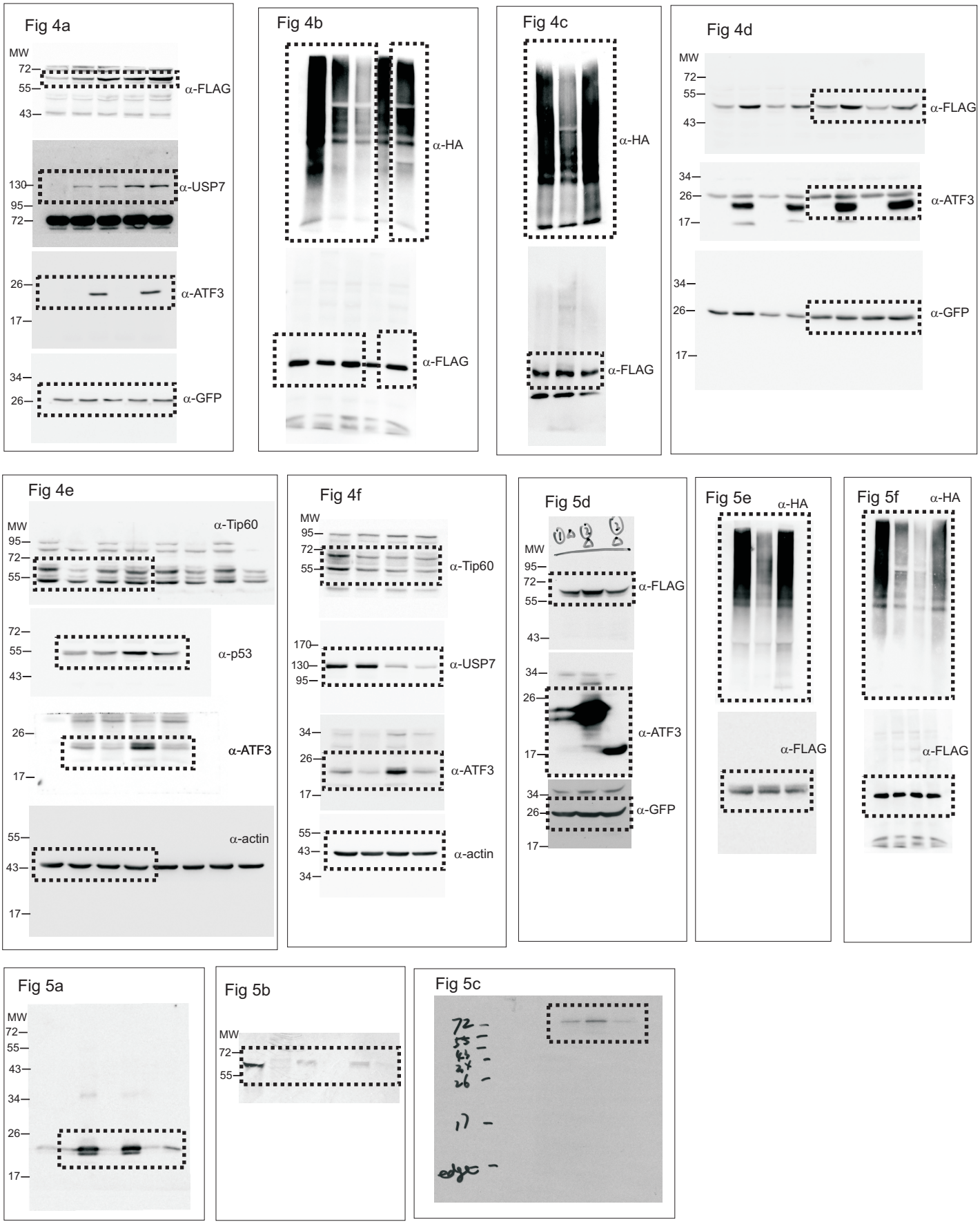
Supplementary Figure 10. **ATF3 does not directly affect USP7 activity, nor enhances the USP7-Tip60 interaction.** (**a**) Pre-incubation of USP7 with ATF3 did not further promote the USP7 deubiquitinase activity as determined by *in vitro* deubiquitination assays. (**b**) ATF3 did not enhance the USP7-Tip60 interaction. H1299 cells transfected with FLAG-Tip60, HA-USP7 and ATF3 as indicated, and subjected to co-IP using the HA antibody.



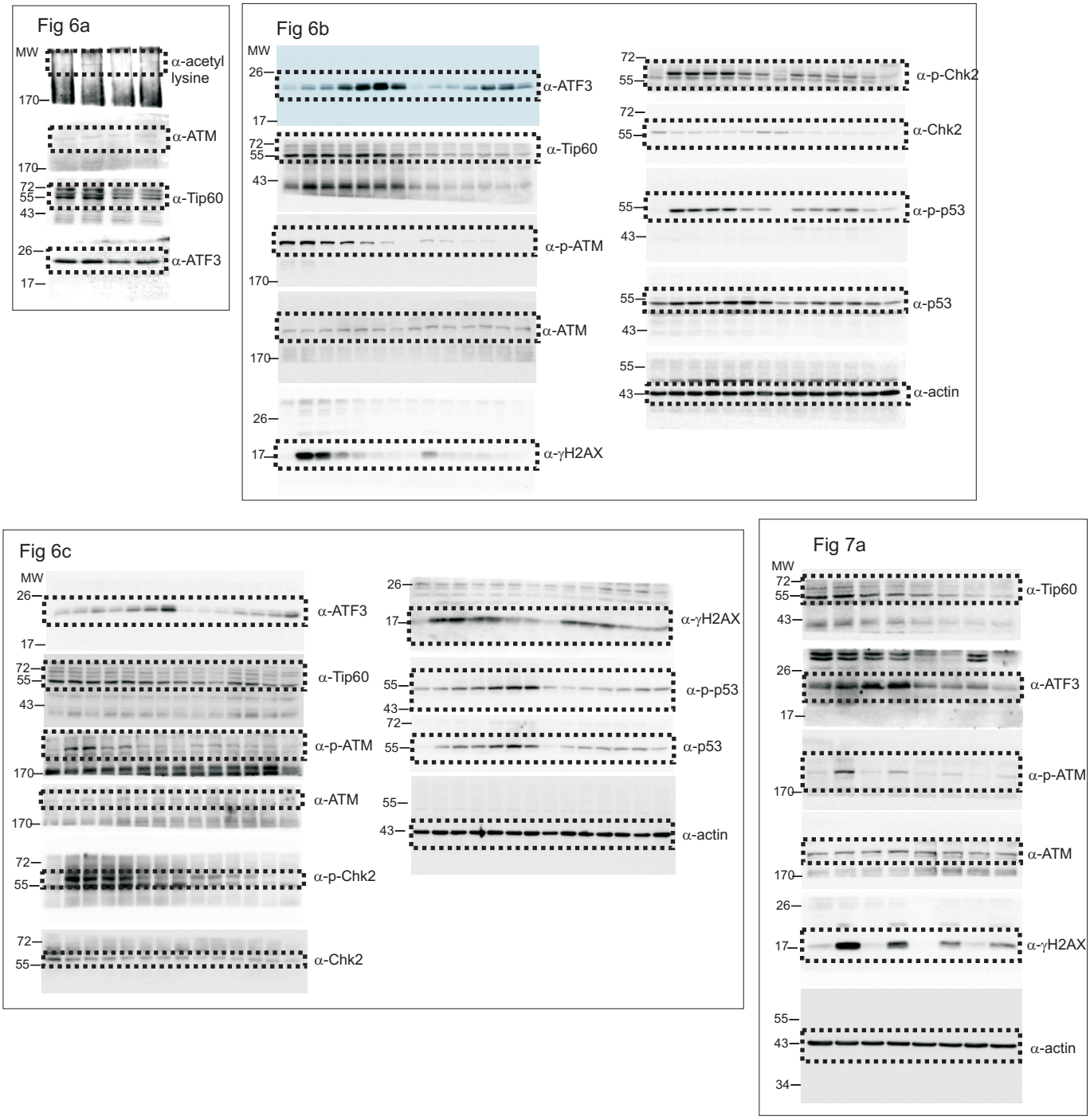
Supplementary Figure 11. **Effects of ATF3 on p53 K120 acetylation.** (**a**) The p53 K120 acetylation level appeared to be increased in ATF3-deficient cells. ATF3-deficient or wild-type HCT116 cells were treated with 5 μM of etoposide, and subjected to imunoprecipitation using the K120ac antibody. K120-acetylated p53 was detected using a p53 antibody (DO-1). (**b**) ATF3 blocked the p53-Tip60 interaction. GST-Tip60 immobilized onto glutathione agarose was incubated with *in vitro*-translated p53 in the presence of ATF3 (0.5, 1 μg) or equal amounts of BSA. Bound proteins were detected by Western blotting. (**c**) ATF3 interfered with the p53-Tip60 interaction as determined by co-IP assays. H1299 cells transfected with p53, FLAG-Tip60, and/or ATF3 were subjected to co-IP using the FLAG M2 affinity gel. Bound proteins were eluted with the FLAG peptide and subjected to Western blotting. (**d**) ATF3 promoted p21 and PUMA expression induced by DNA damage. ATF3-deficient or wildtype HCT116 cells were treated with 5 μM of etoposide, and lysed for quantitative RT-PCR to determine p21 and PUMA mRNA levels.



Supplementary Figure 12. **Original images of Western blots** – for Figure 1, 2, and 3



Supplementary Figure 12(cont.). **Original images of Western blots** – for Figure 4 and 5



Supplementary Figure 12(cont.). **Original images of Western blots** – for Figure 6 and 7