cancers

MDPI

Article

Supplementary Materials: USP47 Promotes Tumorigenesis by Negative Regulation of p53 through Deubiquitinating Ribosomal Protein S2

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Supplementary Materials

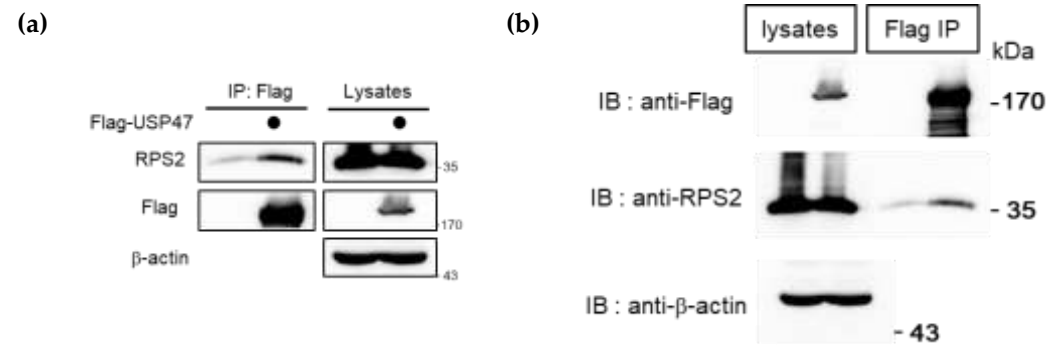


Figure S1. Overexpressed USP47 interacts with endogenous RPS2 in cells. (**a**) HEK293T cells were overexpressed with Flag vector or Flag-USP47, and the cell lysates were then immunoprecipitated with anti-Flag M2 agarose and detected with the indicated antibodies, and β-actin was used as a loading control. (**b**) Uncropped Western Blot figure.

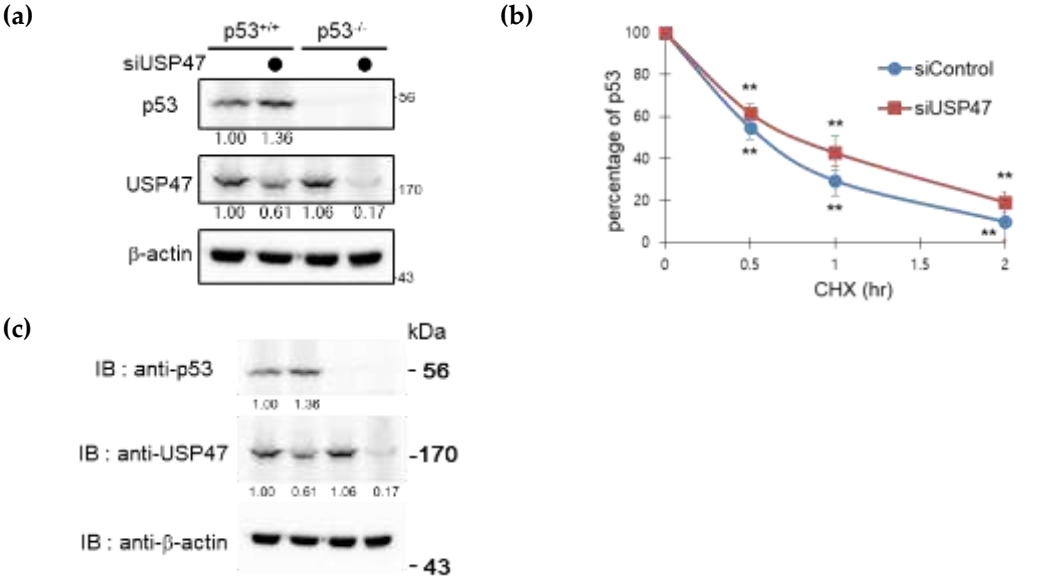


Figure S2. USP47 depletion induces p53 protein level. (**a**) HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells were transfected with siRNA against control or USP47 for 72 h. Cell lysates were immunoblotted with the indicated antibodies. (**b**) U2OS cells transfected with siRNA against control or USP47 were treated with 100 μg/ml cycloheximide and harvested at the indicated times. Cell lysates were immunoblotted with the indicated antibodies. The band intensity was measured by Image J and then p53 levels are normalized by HSP90. The data from three independent experiments represent the mean ± SD (* $p < 0.05$, ** $p < 0.01$, *t*-test). (**c**) Uncropped Western Blot figure.

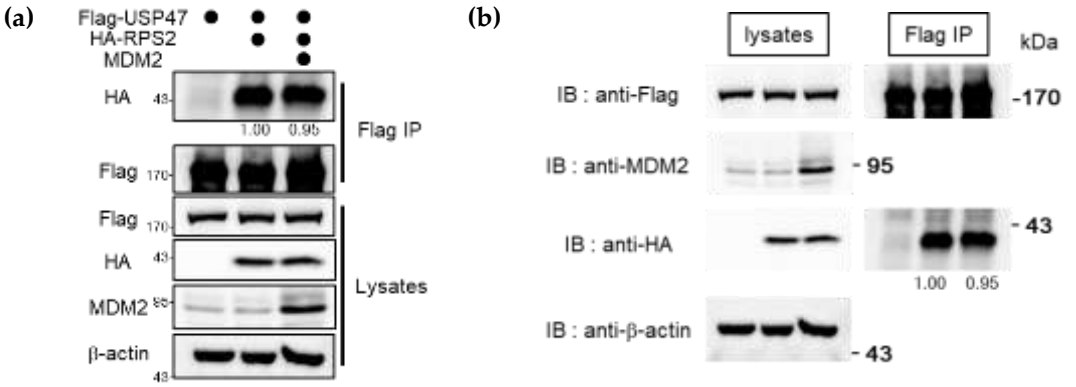


Figure S3. (**a**)MDM2 does not have any effect on the interaction between USP47 and RPS2. HEK293T cells were co-overexpressed with Flag-USP47, HA-RPS2, MDM2 and then, immunoprecipitation was performed with anti-Flag M2 agarose. Bound proteins were immunoblotted with the indicated antibodies. (**b**) Uncropped Western Blot figure.

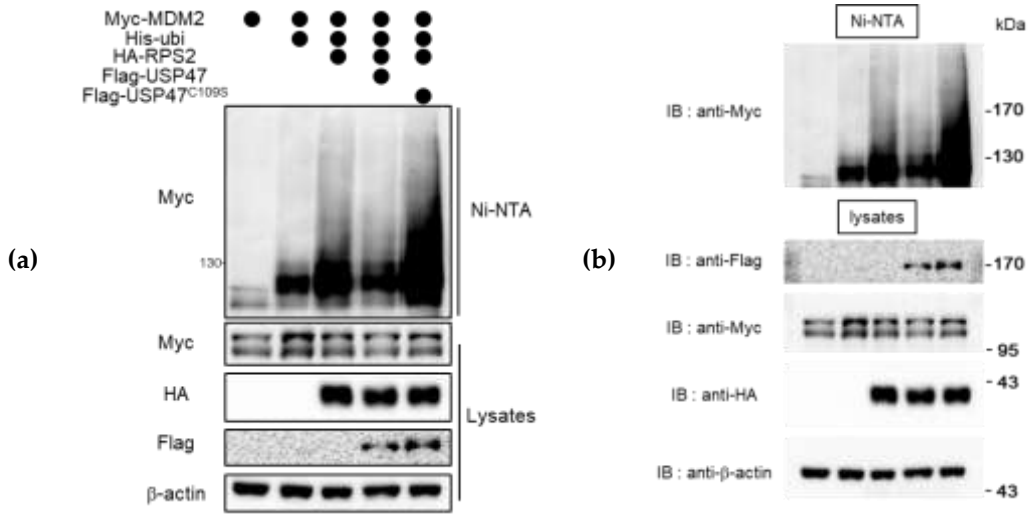


Figure S4. (**a**) MDM2 ubiquitination is increased by RPS2 and restored by USP47. HEK293T cells were co-overexpressed with Myc-MDM2, His-ubiquitin, HA-RPS2, Flag-USP47, or Flag-USP47C109S for 24 h as indicated and then treated with 10 μM MG132 for 4 h. Ubiquitin conjugates were purified on Ni-NTA-agarose under denaturing conditions, and ubiquitinated MDM2 was detected by anti-Myc antibodies. (**b**) Uncropped Western Blot figure.

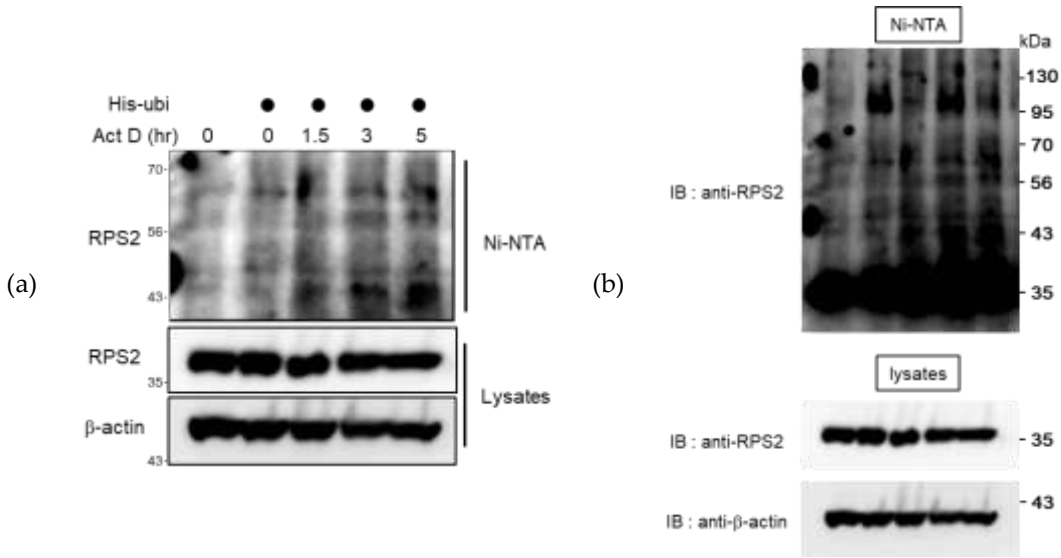


Figure S5. (a) The ubiquitination of endogenous RPS2 is accumulated with Actinomycin D treatment in a time-dependent manner. HEK293T cells were overexpressed with His-ubiquitin for 24 h and then treated with 10 μM MG132 for 4 h and 5 nM actinomycin D for indicated times. Ubiquitin conjugates were purified on Ni-NTA-agarose under denaturing conditions, and ubiquitinated endogenous RPS2 was detected by anti-RPS2 antibodies. (**b**) Uncropped Western Blot figure.

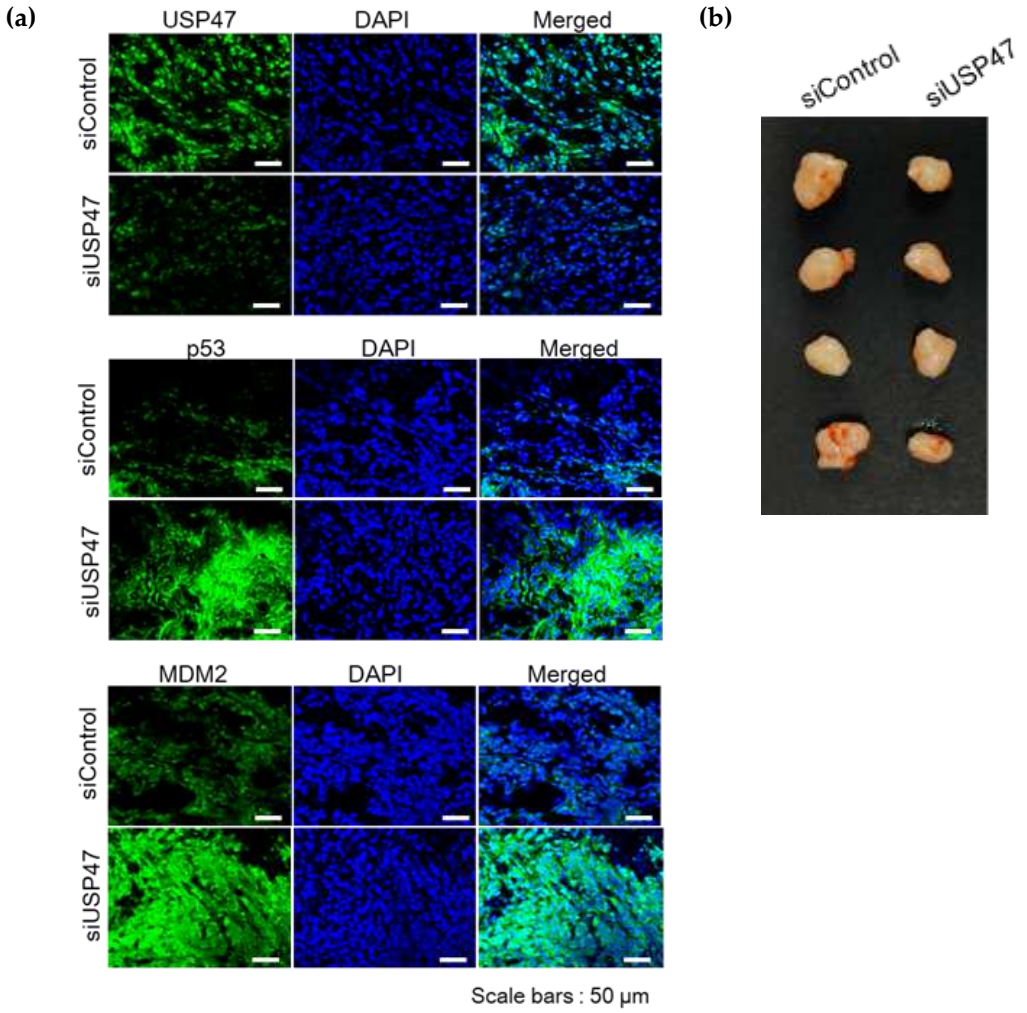
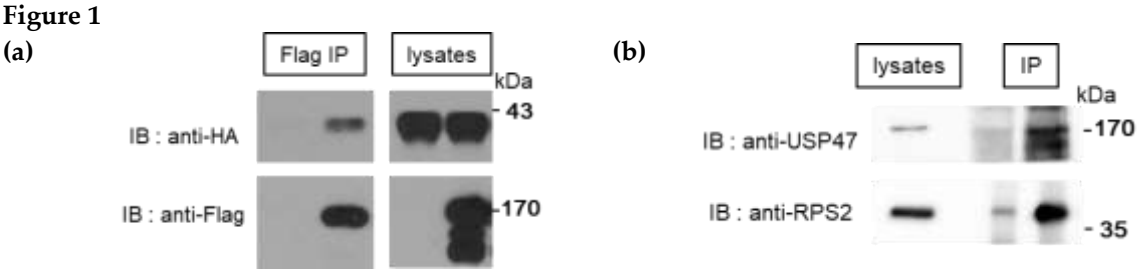


Figure S6. Inhibition of xenograft tumor by knockdown of USP47. (**a**,**b**) 2 mg/kg of siControl and siUSP47 were intratumorally administered into A549 xenografts every 3 days for 2 weeks. (**a**) Immunofluorescence staining was conducted in dissected tumors and detected by indicated antibodies. Immunofluorescence images were captured by confocal microscopy. Blue colors represent DAPI. Scale bars indicate 50 μm. (**b**) Xenograft tumor images.

Figure S7. The uncropped **Western Blots** and molecular weight markers of the Figures 1–7 in main text.

Figure 1



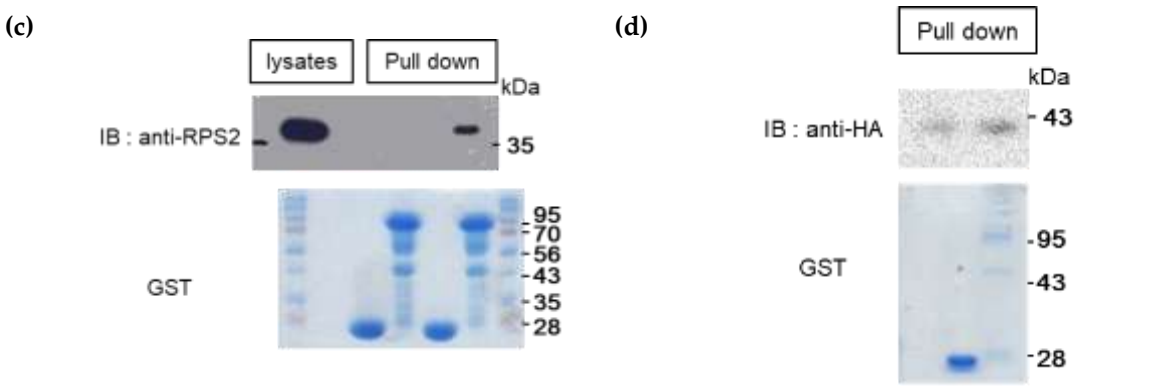


Figure 2

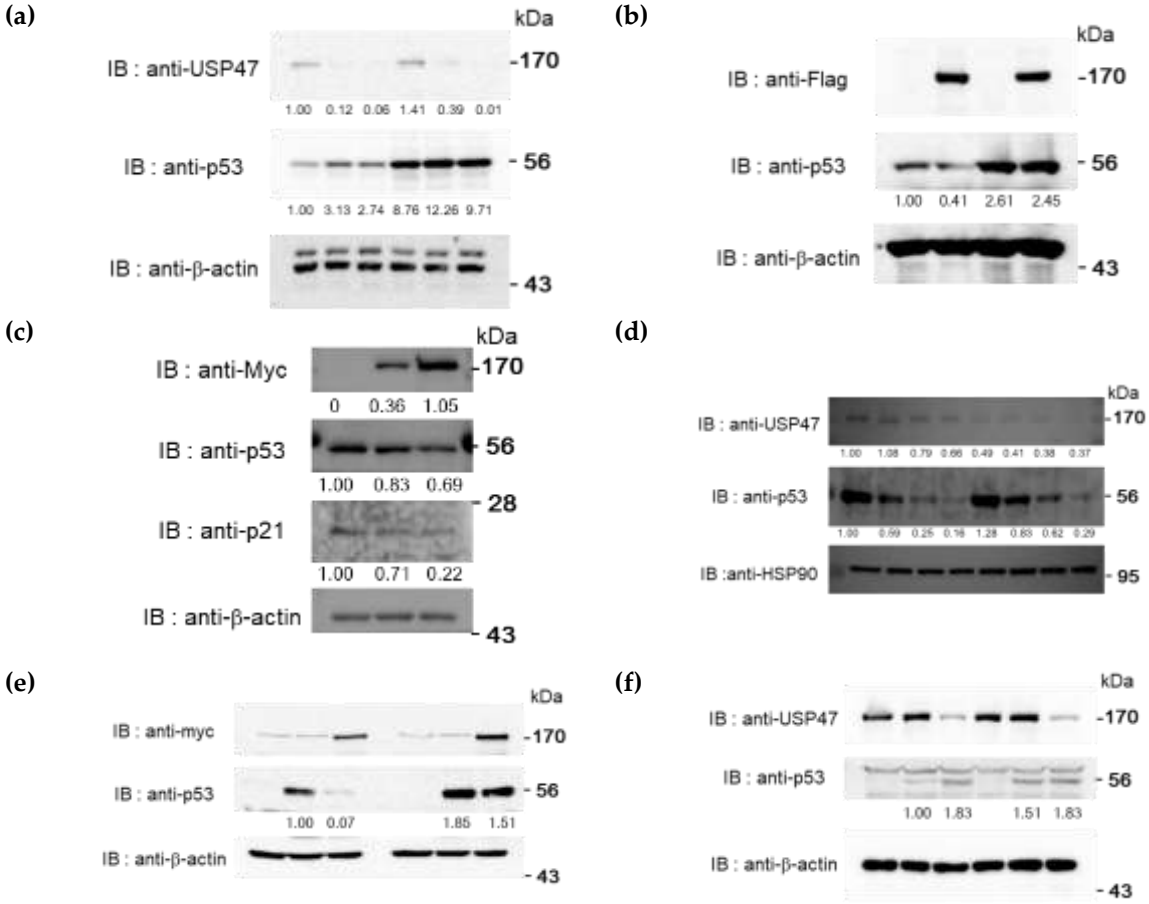


Figure 3

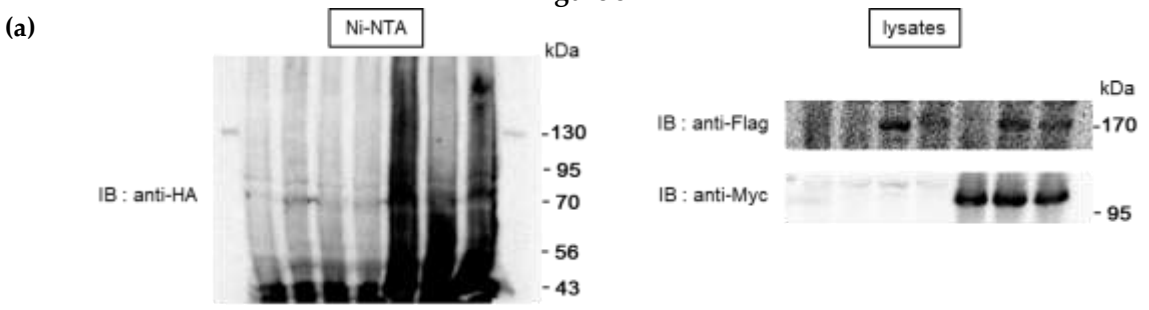


Figure 3

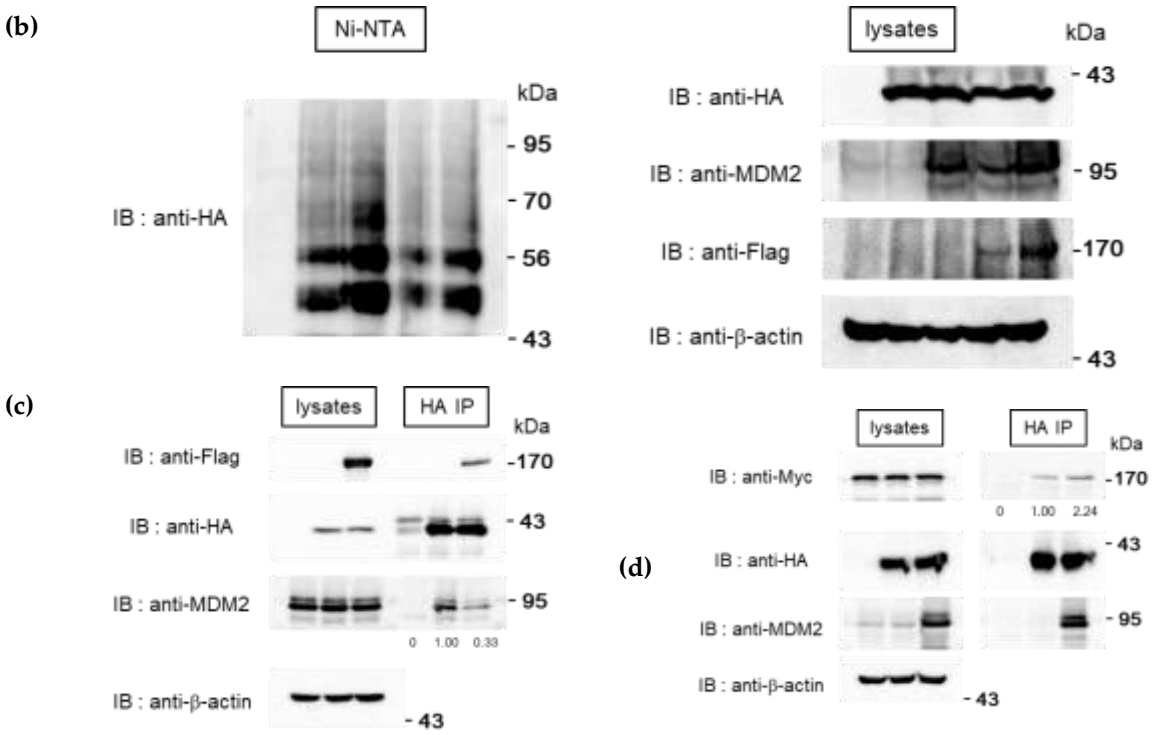
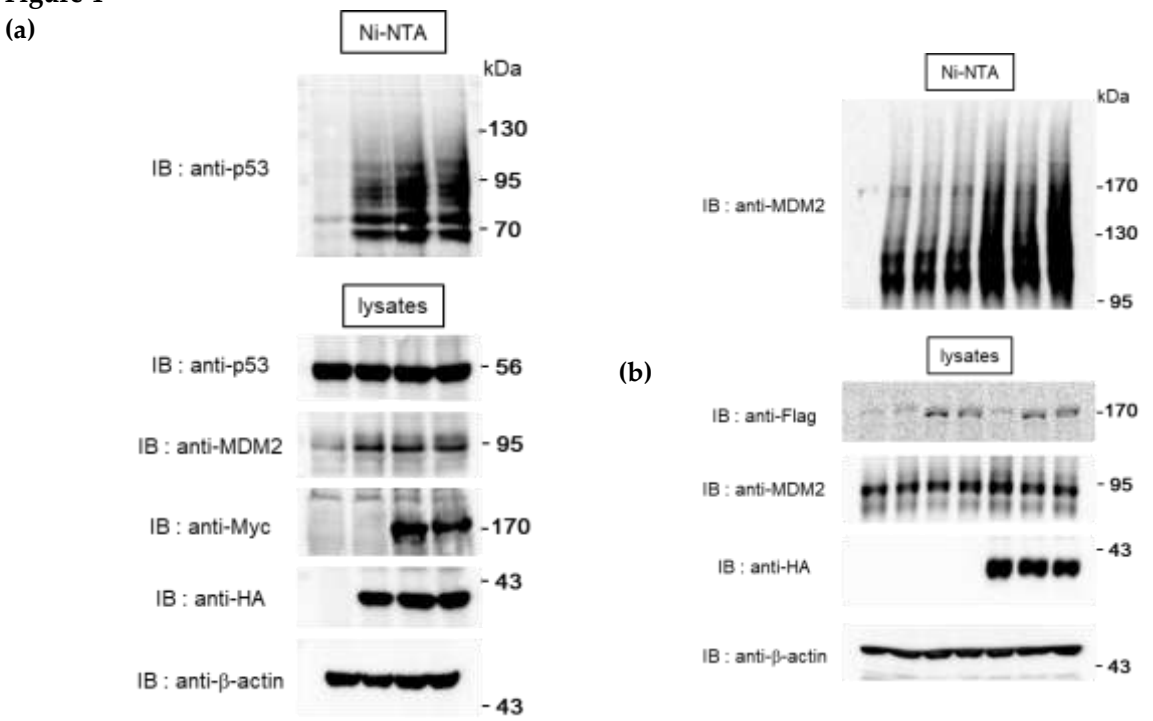

(b)
Ni-NTA
kDa
IB : anti-HA
95
70
56
43
lysates
kDa
IB : anti-HA
IB : anti-MDM2
IB : anti-Flag
IB : anti-β-actin
43
95
170
43
(c)
lysates
HA IP
kDa
IB : anti-Flag
IB : anti-HA
IB : anti-MDM2
IB : anti-β-actin
170
43
95
43
0 1.00 0.33
(d)
lysates
HA IP
kDa
IB : anti-Myc
IB : anti-HA
IB : anti-MDM2
IB : anti-β-actin
170
43
95
43
0 1.00 2.24

Figure 4


(a)
Ni-NTA
kDa
IB : anti-p53
130
95
70
lysates
IB : anti-p53
IB : anti-MDM2
IB : anti-Myc
IB : anti-HA
IB : anti-β-actin
56
95
170
43
43
(b)
Ni-NTA
kDa
IB : anti-MDM2
170
130
95
lysates
IB : anti-Flag
IB : anti-MDM2
IB : anti-HA
IB : anti-β-actin
170
95
43
43

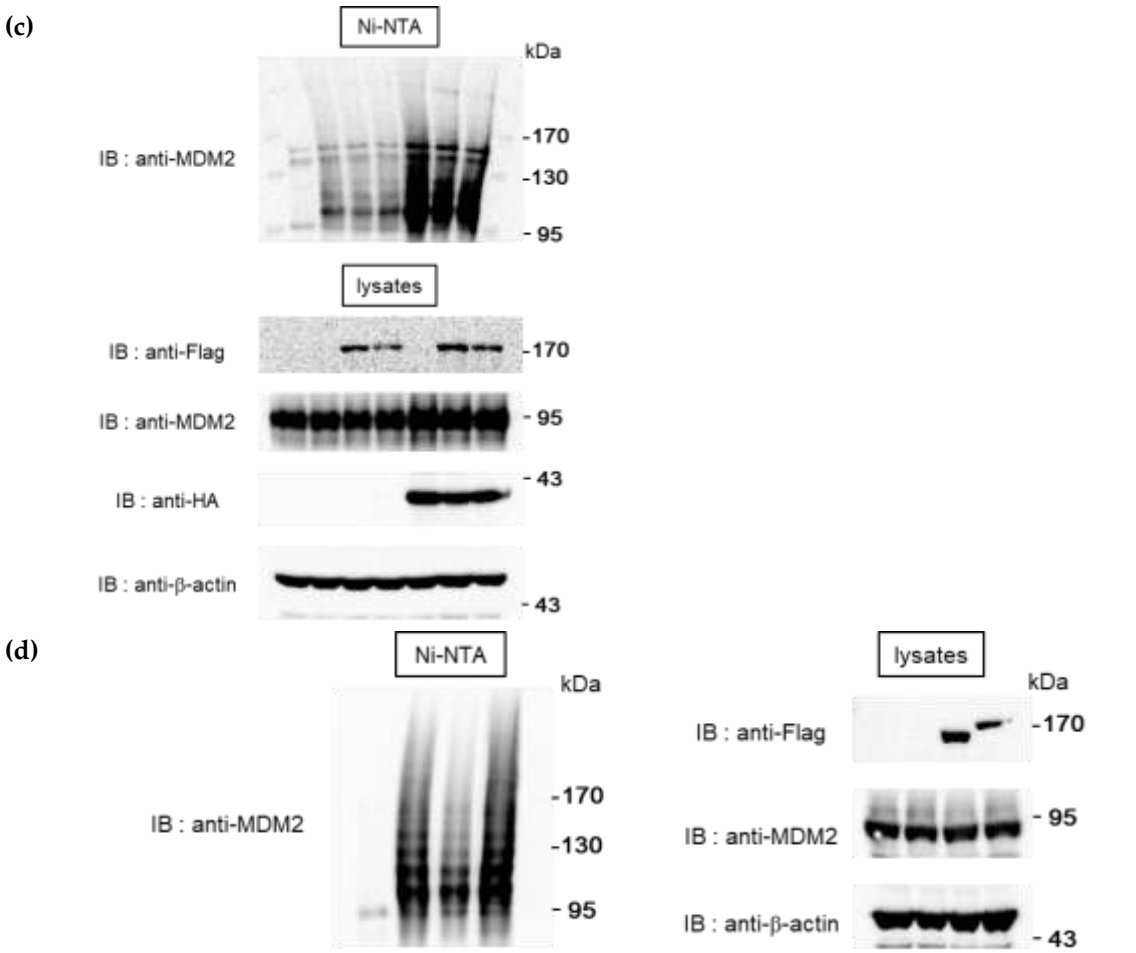
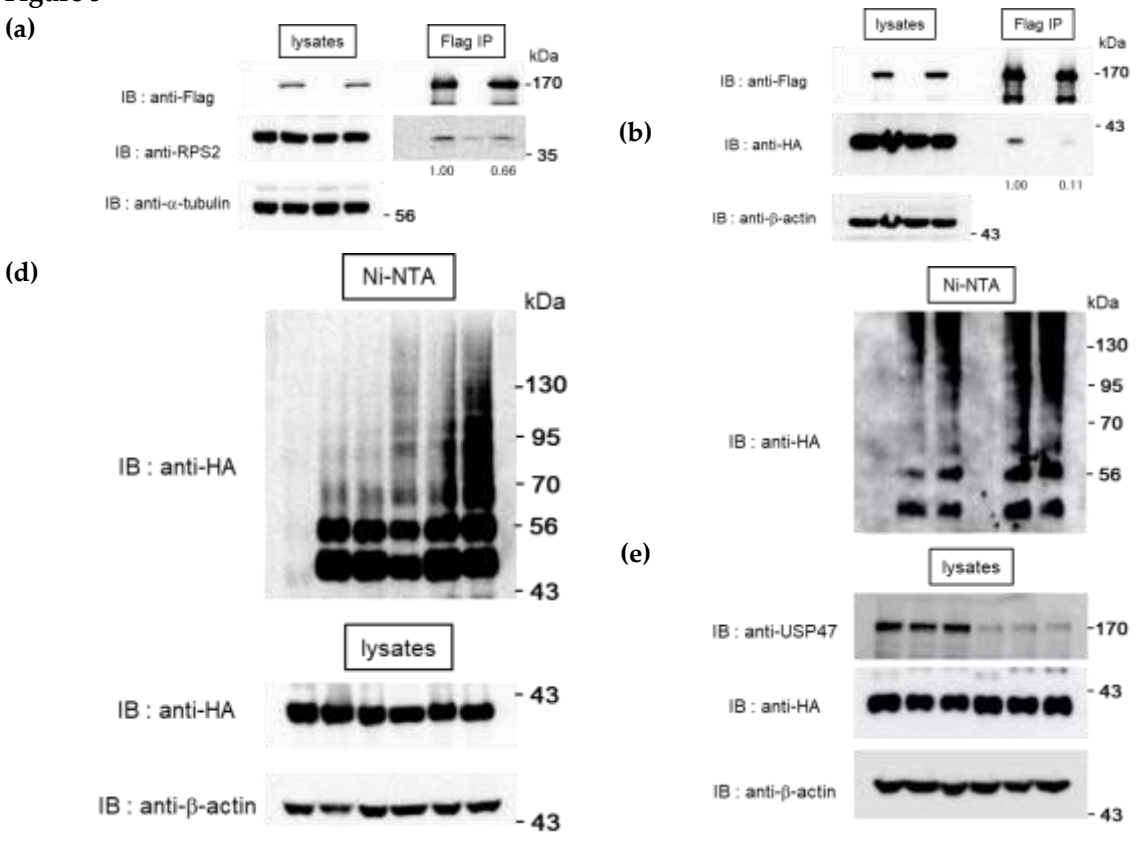

(c)
Ni-NTA
kDa
IB : anti-MDM2
-170
-130
-95
lysates
IB : anti-Flag
-170
IB : anti-MDM2
-95
IB : anti-HA
-43
IB : anti-β-actin
-43
(d)
Ni-NTA
kDa
IB : anti-MDM2
-170
-130
-95
lysates
kDa
IB : anti-Flag
-170
IB : anti-MDM2
-95
IB : anti-β-actin
-43

Figure 5


(a)
lysates
Flag IP
kDa
IB : anti-Flag
-170
IB : anti-RPS2
-35
1.00
0.66
IB : anti-α-tubulin
-56
(b)
lysates
Flag IP
kDa
IB : anti-Flag
-170
IB : anti-HA
-43
1.00
0.11
IB : anti-β-actin
-43
(d)
Ni-NTA
kDa
IB : anti-HA
-130
-95
-70
-56
-43
lysates
IB : anti-HA
-43
IB : anti-β-actin
-43
(e)
Ni-NTA
kDa
IB : anti-HA
-130
-95
-70
-56
lysates
IB : anti-USP47
-170
IB : anti-HA
-43
IB : anti-β-actin
-43

(f)

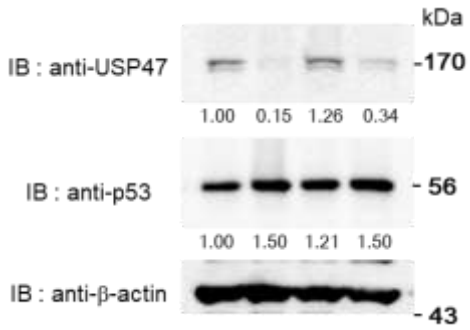


Figure 6

(a)

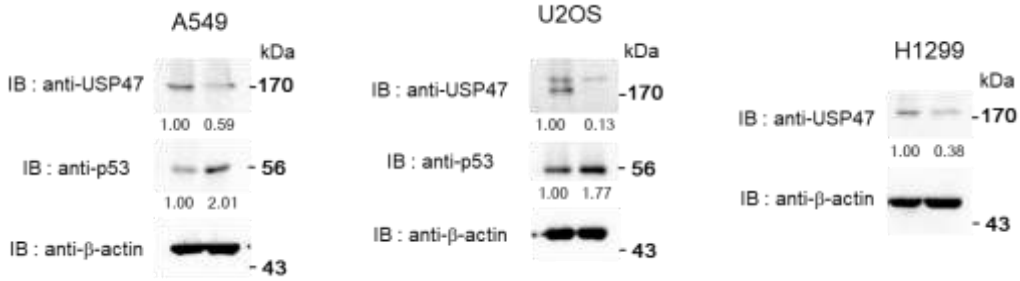
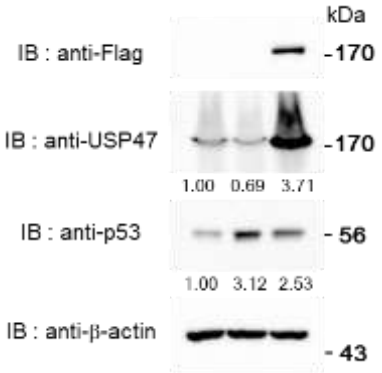


Figure 7

(e)



(a)