

## Supplementary Information

### Supplementary Figure Legends

**Supplementary Figure 1. The kinetics of TRIM32 induction by p53 activation in HCT116 p53<sup>+/+</sup> and RKO p53<sup>+/+</sup> cells treated with Etoposide.** (a) The p53-dependent induction of TRIM32 mRNA in HCT116 and RKO cells at different time points after Etoposide treatment. (b) The p53-dependent induction of TRIM32 protein in HCT116 and RKO cells at different time points after Etoposide treatment. HCT116 p53<sup>+/+</sup>, HCT116 p53<sup>-/-</sup>, RKO p53<sup>+/+</sup>, RKO p53<sup>-/-</sup> cells were treated with Etoposide (20  $\mu$ M), and cells were collected at different time points after treatment. The TRIM32 mRNA levels were measured by Taqman real-time PCR assays and normalized with Actin (a). Data are presented as mean  $\pm$  SD (n = 3). The TRIM32 protein levels were measured by Western-blot assays (b).

**Supplementary Figure 2. Etoposide and 5-FU induce TRIM32 expression in a p53-dependent but p63- and p73-independent manner.** (a) TA-p63 $\alpha$  and TA-p73 $\alpha$  are the main p63 and p73 isoforms expressed in HCT116 p53<sup>+/+</sup> and RKO p53<sup>+/+</sup> cells. Cells were transfected with vectors expressing TA-p63 $\alpha$ ,  $\Delta$ N-p63 $\alpha$ , TA-p73 $\alpha$ , or  $\Delta$ N-p73 $\alpha$  as markers. (b,c) Etoposide and 5-FU induced TRIM32 expression in a p53-dependent but p63- and p73-independent manner in HCT116 p53<sup>+/+</sup> cells. HCT116 p53<sup>+/+</sup> cells were transfected with 2 different siRNA oligos to knock down the endogenous p53, p63 and p73, respectively (b). Cells were then treated with Etoposide (20  $\mu$ M) or 5-FU (100  $\mu$ M) for 24 h before TRIM32 mRNA levels were measured by Taqman real-time PCR assays,

and normalized with Actin (c). Data are presented as mean  $\pm$  SD (n = 3). **(d,e)** Etoposide and 5-FU induced TRIM32 expression in a p53-dependent but p63- and p73-independent manner in RKO p53+/+ cells. The endogenous p53, p63 and p73 were knocked down by siRNA oligos, respectively, in RKO p53+/+ cells (d). Cells were then treated with Etoposide (20  $\mu$ M) or 5-FU (100  $\mu$ M) for 24 h before TRIM32 mRNA levels were measured by Taqman real-time PCR assays and normalized with Actin (e). Data are presented as mean  $\pm$  SD (n = 3).

**Supplementary Figure 3. p53 induces TRIM32 expression in normal human neuronal CRL-10742 cells.** **(a)** The basal TRIM32 mRNA levels in CRL-10742, LN-2024 and LN-Z308 cells measured by real-time PCR assays. **(b)** The basal protein levels of TRIM32 and TA-p73 in CRL-10742, LN-2024 and LN-Z308 cells measured by Western-blot assays. **(c)** Etoposide induced TRIM32 expression in CRL-10742 cells in a p53-dependent manner. CRL-10742 cells transduced with control shRNA vectors or shRNA vectors against p53 were treated with Etoposide (20  $\mu$ M) for different hours before assays. Left panel: Knockdown of WT p53 by 2 different p53 shRNA vectors in cells detected by Western-blot assays. Left panel: Etoposide induced TRIM32 mRNA levels in a p53-dependent manner in cells. In a & c, the mRNA levels of TRIM32 were measured by Taqman real-time PCR assays and normalized with Actin. Data are presented as mean  $\pm$  SD (n = 3).

**Supplementary Figure 4. p53 induces TRIM32 expression in response to stress in normal human colorectal CRL-1831 cells.** **(a, b)** The basal mRNA (a) and protein

levels (b) of TRIM32 in CRL-1831, HCT116 p53<sup>+/+</sup> and RKO p53<sup>+/+</sup> cells. The mRNA levels were measured by Taqman real-time PCR assays and normalized with actin. **(c,d)** Etoposide induced TRIM32 mRNA (c) and protein (d) in CRL-1831 cells in a p53-dependent manner. CRL-1831 cells stably transduced with control shRNA vectors or shRNA vectors against p53 were treated with Etoposide (20  $\mu$ M) for different hours before assays. The mRNA levels of TRIM32 were measured by Taqman real-time PCR assays and normalized with Actin. Data are presented as mean  $\pm$  SD (n = 3). In c and d, two different p53 shRNA vectors were used and very similar results were observed. For the sake of clarity, only results from one p53 shRNA are presented.

**Supplementary Figure 5. p53 activation by stress induced TRIM32 whereas knockdown of endogenous TRIM32 induced p53 in human lung H460 and breast MCF7 cells.** **(a, b)** Etoposide induced TRIM32 expression in human lung H460 (a) and human breast MCF7 (b) cells in a p53-dependent manner. Left panels: Stable knockdown of WT p53 by two different shRNA vectors in H460 and MCF7 cells detected by Western-blot assays. Middle panels: Etoposide induced TRIM32 mRNA levels in a p53-dependent manner in cells treated with Etoposide. The mRNA levels of TRIM32 were measured by Taqman real-time PCR assays and normalized with Actin. Right panels: The induction of p21 mRNA levels by Etoposide in a p53-dependent manner in cells, acting as a positive control for p53 activation. **(c,d)** TRIM32 knockdown by two different siRNA oligos induced p53 protein levels in H460 (a) and MCF7 (b) cells. The TRIM32 knockdown was confirmed by Taqman real-time PCR and normalized with Actin (right panels). Data are presented as mean  $\pm$  SD (n = 3).

**Supplementary Figure 6. MDM2 displays a stronger effect on p53 ubiquitination and degradation than TRIM32.** (a) Ectopic expression of MDM2 and TRIM32 negatively regulated the exogenous p53 protein in p53-null H1299. Cells were transfected with pRC-WTp53 vectors (0.3  $\mu$ g) together with pCMV-TRIM32-Flag vectors (3  $\mu$ g) or pCMV-MDM2-Myc (3  $\mu$ g), and p53 levels were determined at 24 h after transfection by Western-blot assays. (b) Ectopic expression of TRIM32-Flag and MDM2-Myc promoted the ubiquitination of p53 in H1299 cells. Cells were transfected with HA-Ub vectors (1  $\mu$ g) and pRC-WTp53 vectors (0.3  $\mu$ g) together with pCMV-MDM2-Myc or pCMV-TRIM32-Flag (3  $\mu$ g) vectors. Cell lysates were immunoprecipitated with the p53 antibody (DO-1) and then immunoblotted with the HA antibody.

**Supplementary Figure 7. TRIM32 negatively regulates mutant p53.** The p53-null H1299 cells were transfected with pCMV-TRIM32-Flag vectors (3 and 6  $\mu$ g) together with pRC-WTp53 vectors, pRC-R175H, or pRC-R273H vectors (0.3  $\mu$ g). P53 protein levels were measured at 24 h after transfection by Western-blot assays. The pRC-WTp53 vectors express WT p53, whereas the pRC-R175H and pRC-R273H vectors express two tumor “hotspot” R175H and R273H mutant p53, respectively.

**Supplementary Figure 8. TRIM32 negatively regulates apoptosis, cell cycle arrest and senescence in response to stress in a p53-dependent and p63-, p73-independent manner.** (a) TRIM32 negatively regulated 5-FU-induced apoptosis in HCT116 p53<sup>+/+</sup> cells in a p53-dependent but p63- and p73-independent manner. HCT116 p53<sup>+/+</sup> cells with or without stable ectopic TRIM32 expression by pLPCX-TRIM32-Flag vectors were

transfected with siRNA oligos to knock down the endogenous p53, p63 and p73, respectively. Cells were then treated with 5-FU (300  $\mu$ M) for 24 h before cellular apoptotic analysis. **(b)** TRIM32 knockdown by siRNA induced cell cycle arrest in a p53-dependent but p63- and p73-independent manner in HCT116 p53<sup>+/+</sup> cells. HCT116 p53<sup>+/+</sup> cells were transfected with siRNA oligos against TRIM32 together with siRNA against p53, p63 and p73, respectively, for 48 h before cell cycle distribution was analyzed. Two different TRIM32 siRNA oligos were used and very similar results were observed. For the sake of clarity, only results from one siRNA were presented. **(c)** TRIM32 negatively regulated Doxorubicin-induced senescence in a p53-dependent but p63- and p73-independent manner in HCT116 p53<sup>+/+</sup> cells. HCT116 p53<sup>+/+</sup> cells with or without stable ectopic expression of TRIM32 were transfected with siRNA oligos against p53, p63 and p73, respectively. After transfection, cells were treated with Doxorubicin (100 nM) for 3 days before senescent cells were detected by SA- $\beta$ -gal staining. Left panels: represented images of SA- $\beta$ -gal staining of senescent cells. Right panel: the percentage of SA- $\beta$ -gal positive cells. Two different siRNA against p53, p63 and p73, respectively, were used and very similar results were observed. For the sake of clarity, only results from one siRNA were presented. In a-c, data are presented as mean  $\pm$  SD (n = 4). #:  $p < 0.05$ . \*:  $p < 0.01$ .

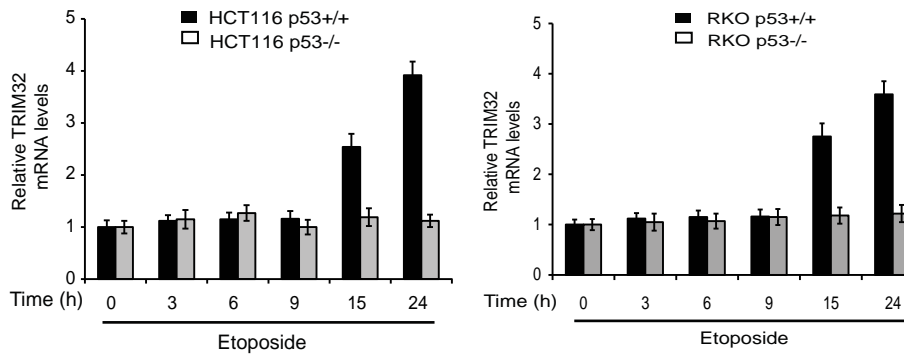
**Supplementary Figure 9. TRIM32 displays a stronger effect on p53 down-regulation than p63 and p73 down-regulation in cells.** **(a)** HCT116 p53<sup>+/+</sup> cells were transfected with pCMV-TRIM32-Flag vectors (3  $\mu$ g), and p53, p63 and p73 protein levels were measured at 24 h after transfection by Western-blot assays. **(b)** HCT116 p53<sup>+/+</sup> cells

were transfected with 2 different TRIM32 siRNA to knock down the endogenous TRIM32, and p53, p63 and p73 protein levels were measured at 48 h after transfection by Western-blot assays. (c) H1299 cells were transfected with TRIM32-HA vectors (3 and 6  $\mu$ g) together with vectors expressing p53, TA-p63 $\alpha$ -Flag, or TA-p73 $\alpha$ -Flag (0.3  $\mu$ g). p53, TA-p63 $\alpha$ -Flag, and TA-p73 $\alpha$ -Flag protein levels were measured at 24 h after transfection by Western-blot assays.

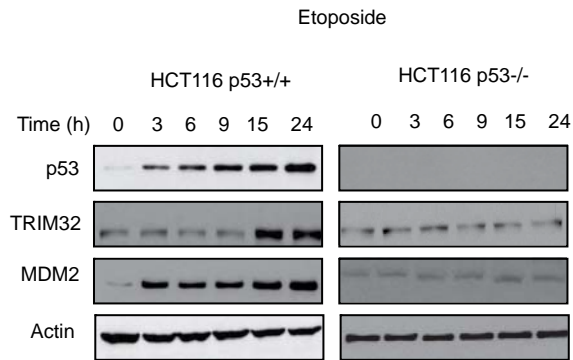
**Supplementary Figure 10. TRIM32 promotes cell oncogenic transformation in a p63- and p73-independent manner.** (a) Overexpression of TRIM32 promoted the cell oncogenic transformation in E1A/Ras-expressed p63 $^{-/-}$  or p73 $^{-/-}$  MEFs. (b) Knockdown of endogenous TRIM32 by stable transduction of 2 different shRNA vectors against TRIM32 reduced cell oncogenic transformation in E1A/Ras-expressed p63 $^{-/-}$  or p73 $^{-/-}$  MEFs. In a & b, data are presented as mean  $\pm$  SD (n = 6). \* $P$ <0.01.

## Supplementary Figure 1

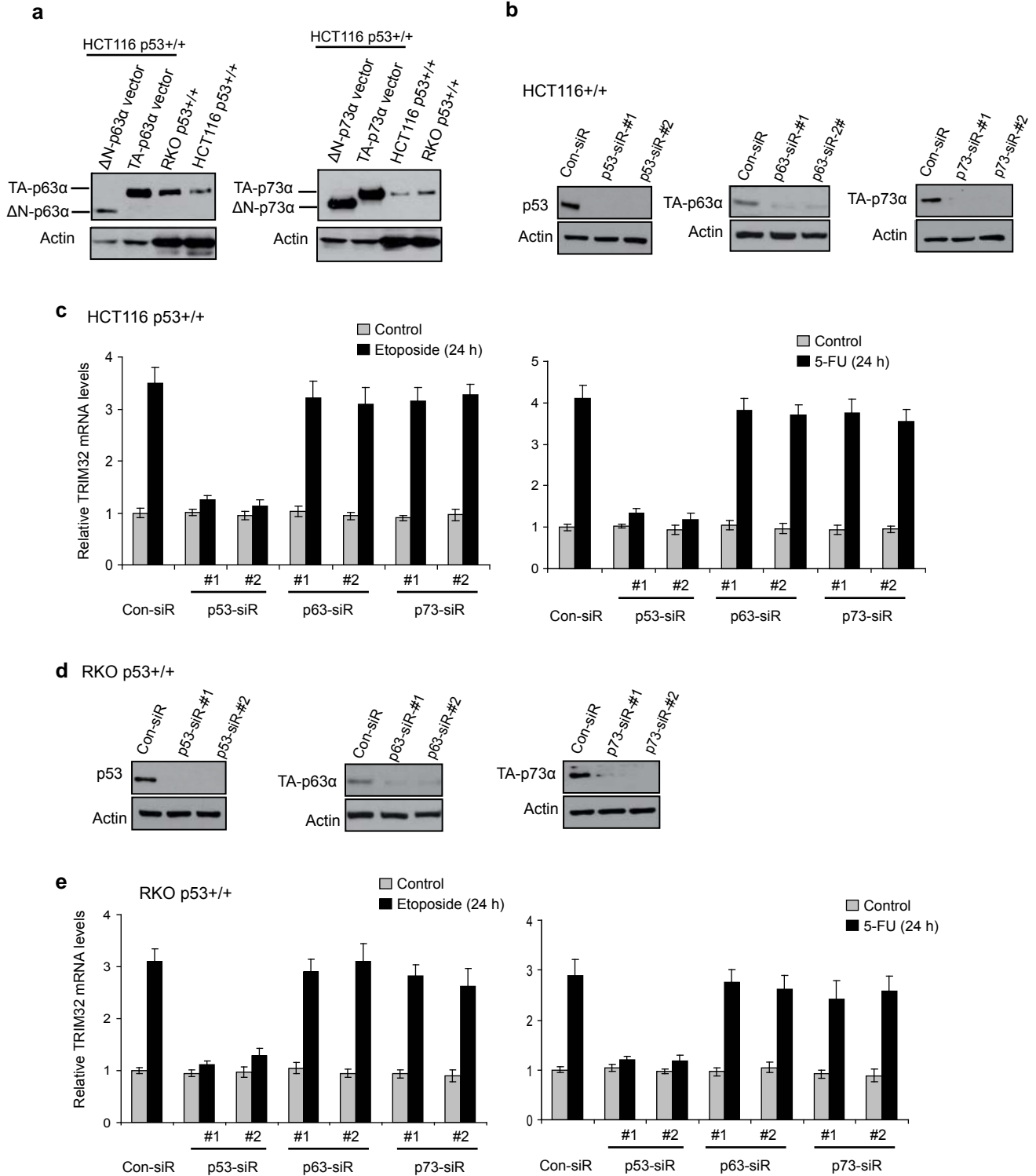
**a**



**b**

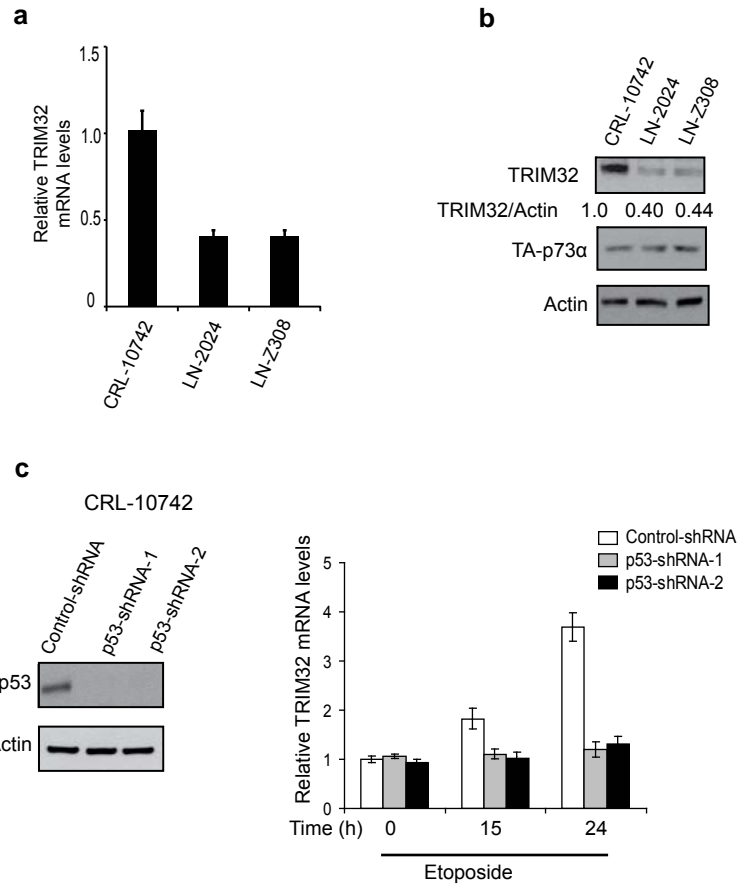


## Supplementary Figure 2



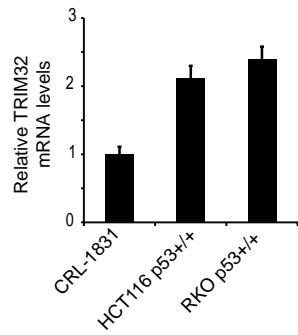


### Supplementary Figure 3

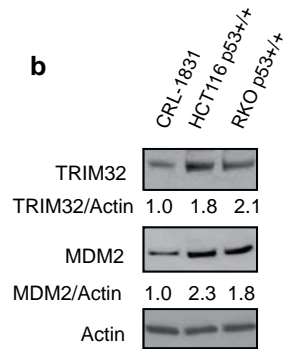


## Supplementary Figure 4

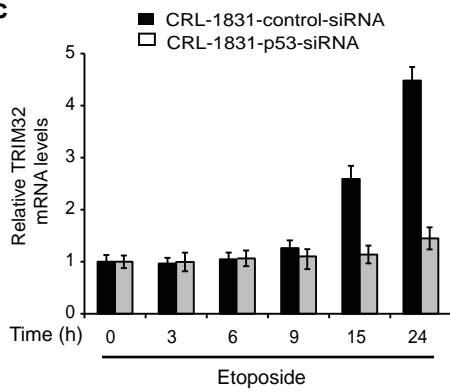
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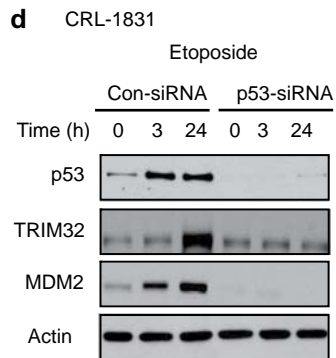
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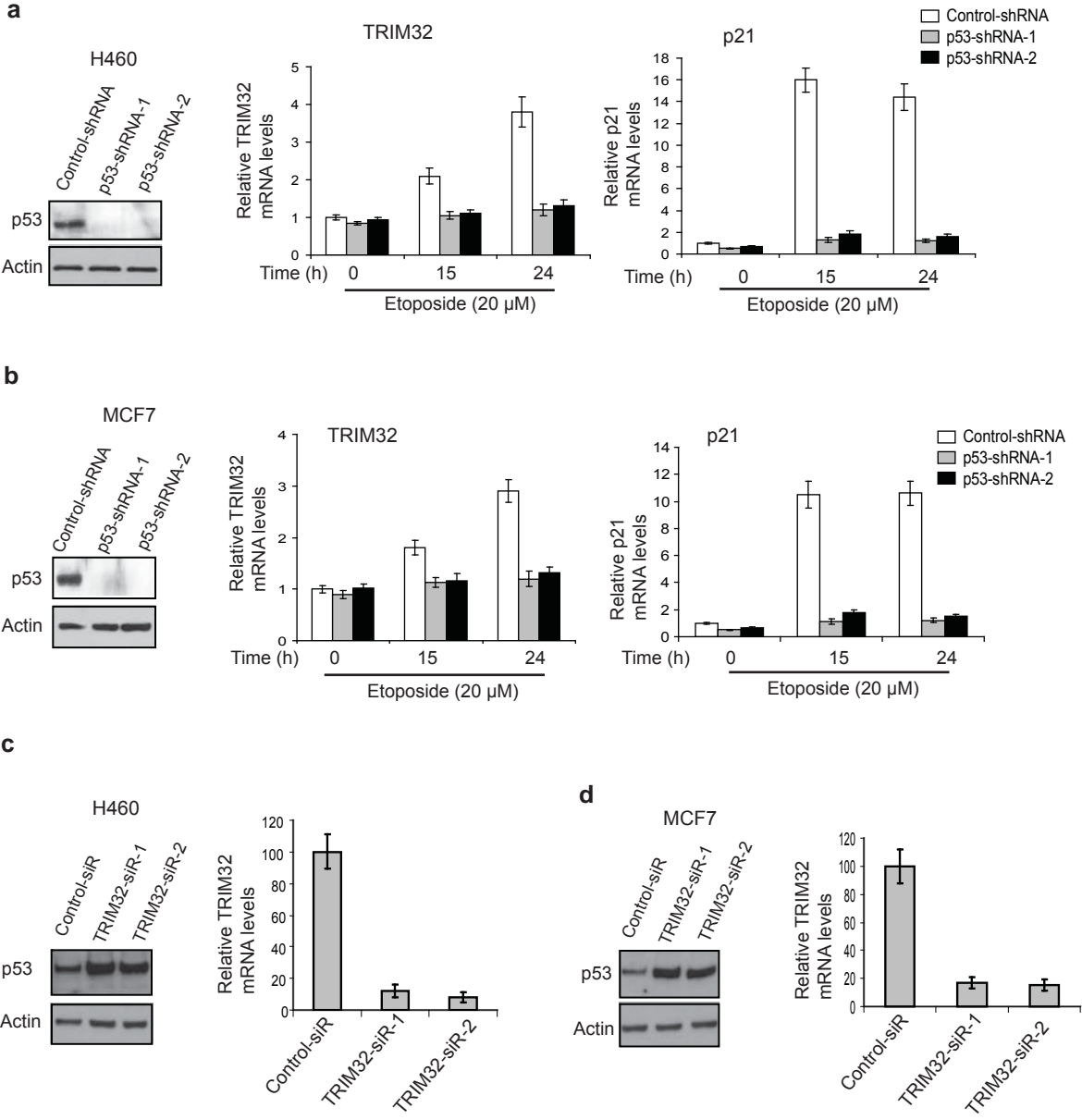
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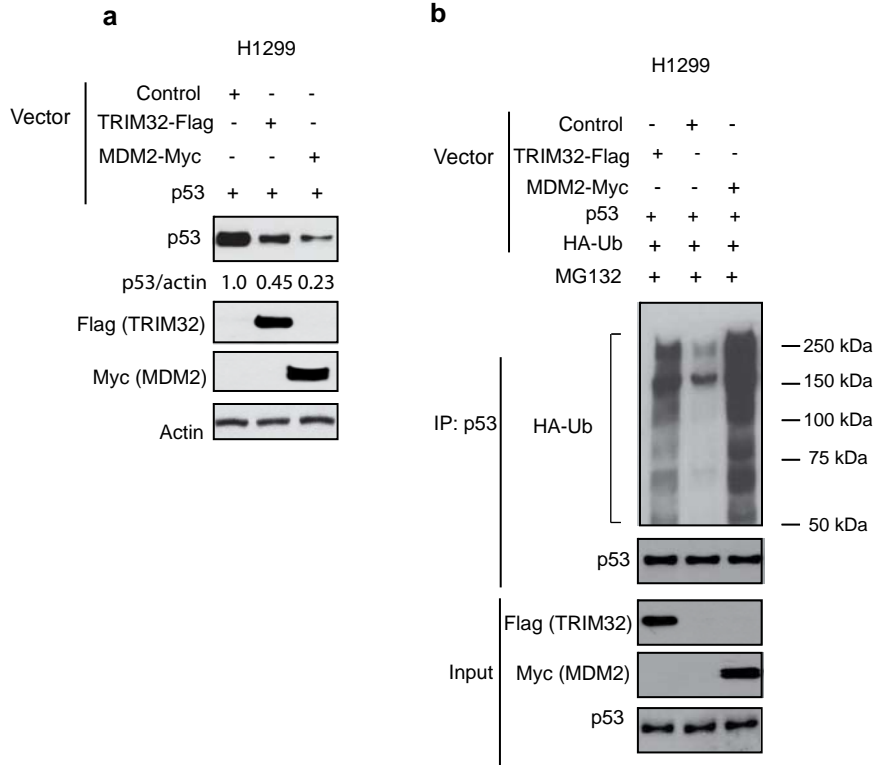
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# Supplementary Figure 5

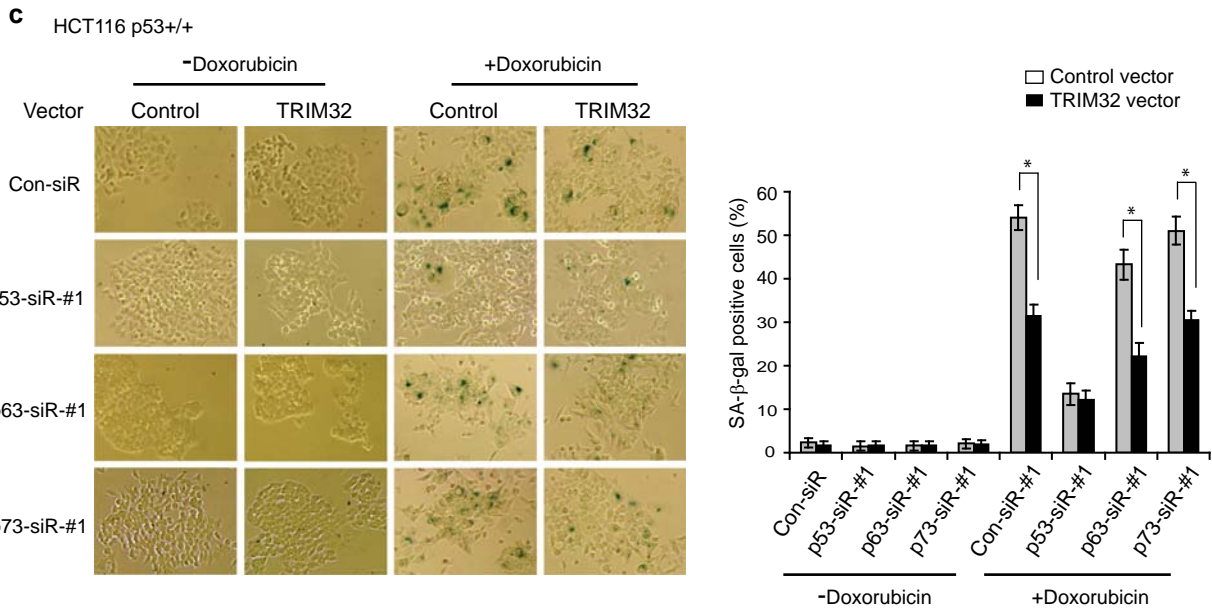
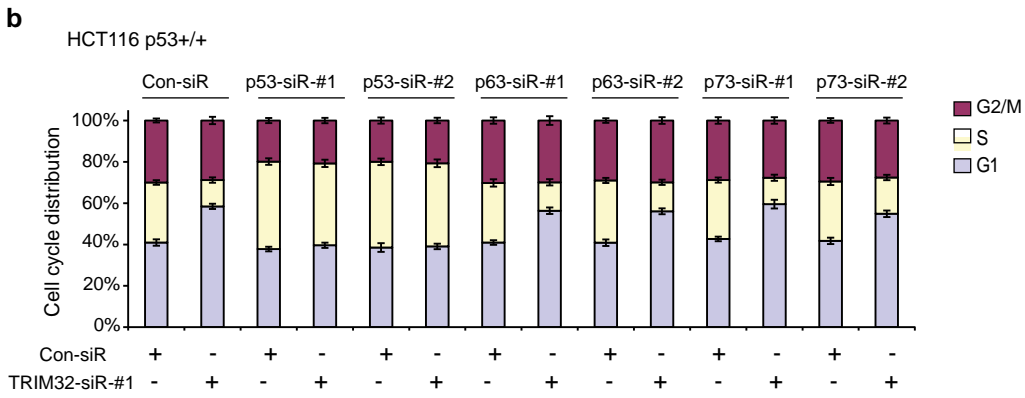
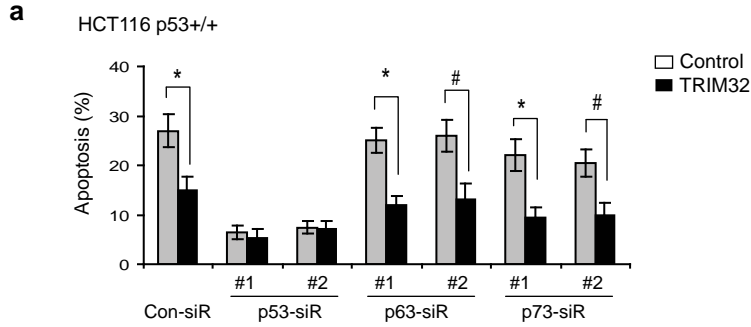


## Supplementary Figure 6

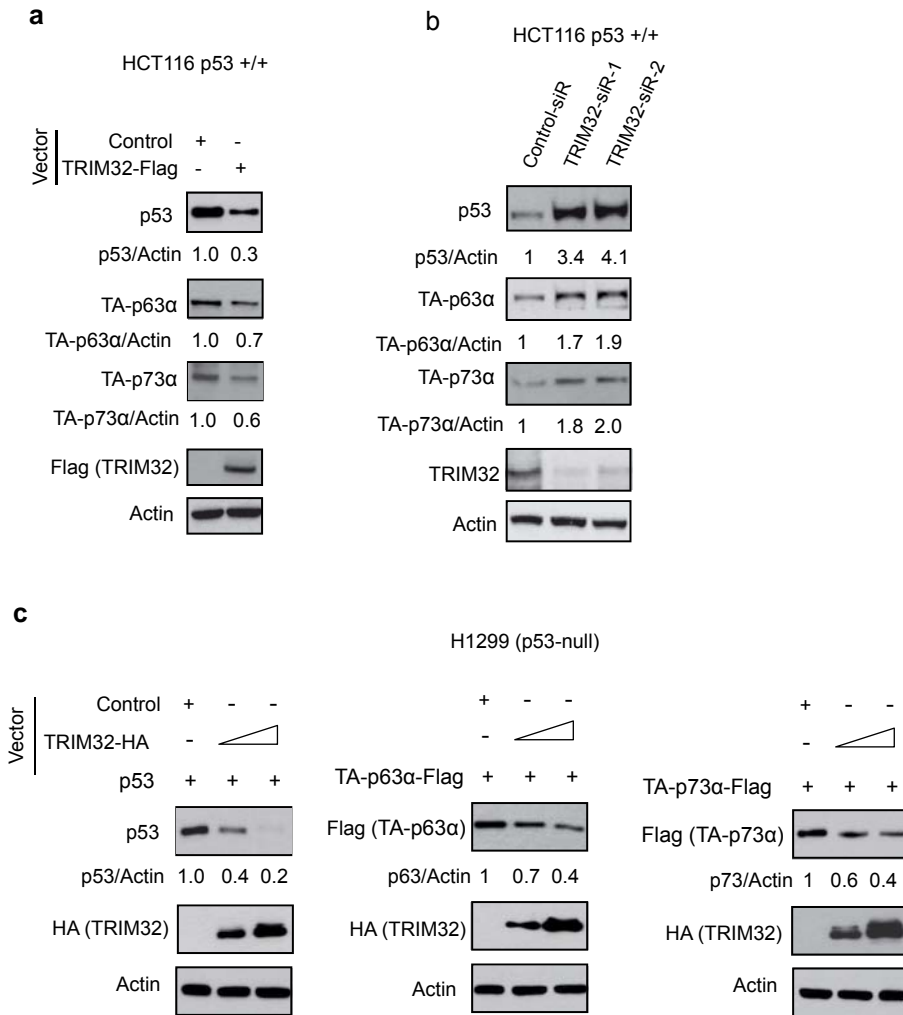




# Supplementary Figure 8

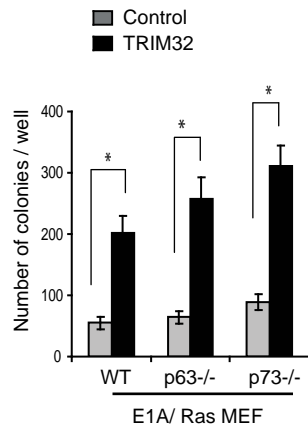


## Supplementary Figure 9



## Supplementary Figure 10

**a**



**b**

