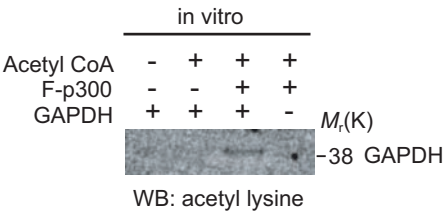
nature cell biology

a

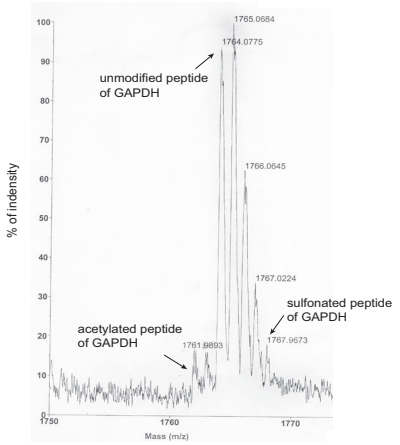


b

MALDI-TOF

	submitted (m/z) monoisotopic mass	matched (m/z) mass	sequence
acetylation of GAPDH	1,761.8846	1,761.8879	IISNASCTTNCLAPLAK$_{160}$

c



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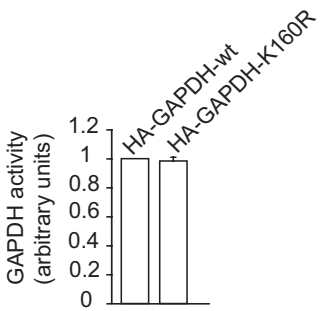

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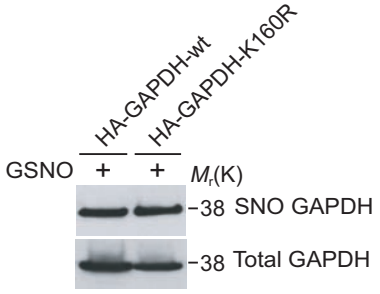

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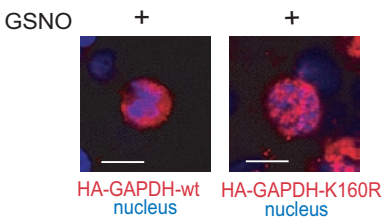

Figure S1 GAPDH is acetylated at its K160 residue, and effects of K160R on GAPDH's glycolytic activity, *S*-nitrosylation, and nuclear translocation. **a,** GAPDH is acetylated by p300 *in vitro*. **b,** Putative acetylation of GAPDH at K160 in HEK293 cells treated with an apoptotic inducer staurosporine, shown by mass spectrometry. Typically trypsin does not cleave after acetylated lysines but in some instances a minor proportion is cleaved, presumably accounting for our findings[1]. **c,** Original spectrum of mass spectrometry of acetylated GAPDH, unmodified GAPDH and sulfonated GAPDH. **d,** The K160R mutation that abolishes acetylation does not alter GAPDH catalytic activity in U2OS cells. Forty-eight h after transfection with HA-GAPDH-wt or HA-GAPDH-K160R, GAPDH was assayed in cell lysates. **e,** *S*-nitrosylation of GAPDH-K160R in HEK293 cells. Forty-eight h after transfection with GAPDH-wt or GAPDH-K160R, cells were treated with 200 μM GSNO for 1 h. Cell lysates were subjected to the biotin switch assay. **f,** Nuclear translocation of GAPDH-wt and GAPDH-K160R in HEK293 cells. Forty-eight h after transfection with HA-GAPDH-wt or HA-GAPDH-K160R, cells were treated with 200 μM GSNO for 24 h and stained with an anti-HA antibody. Red, GAPDH; blue, DAPI (nucleus). Scale bar, 10 μm.

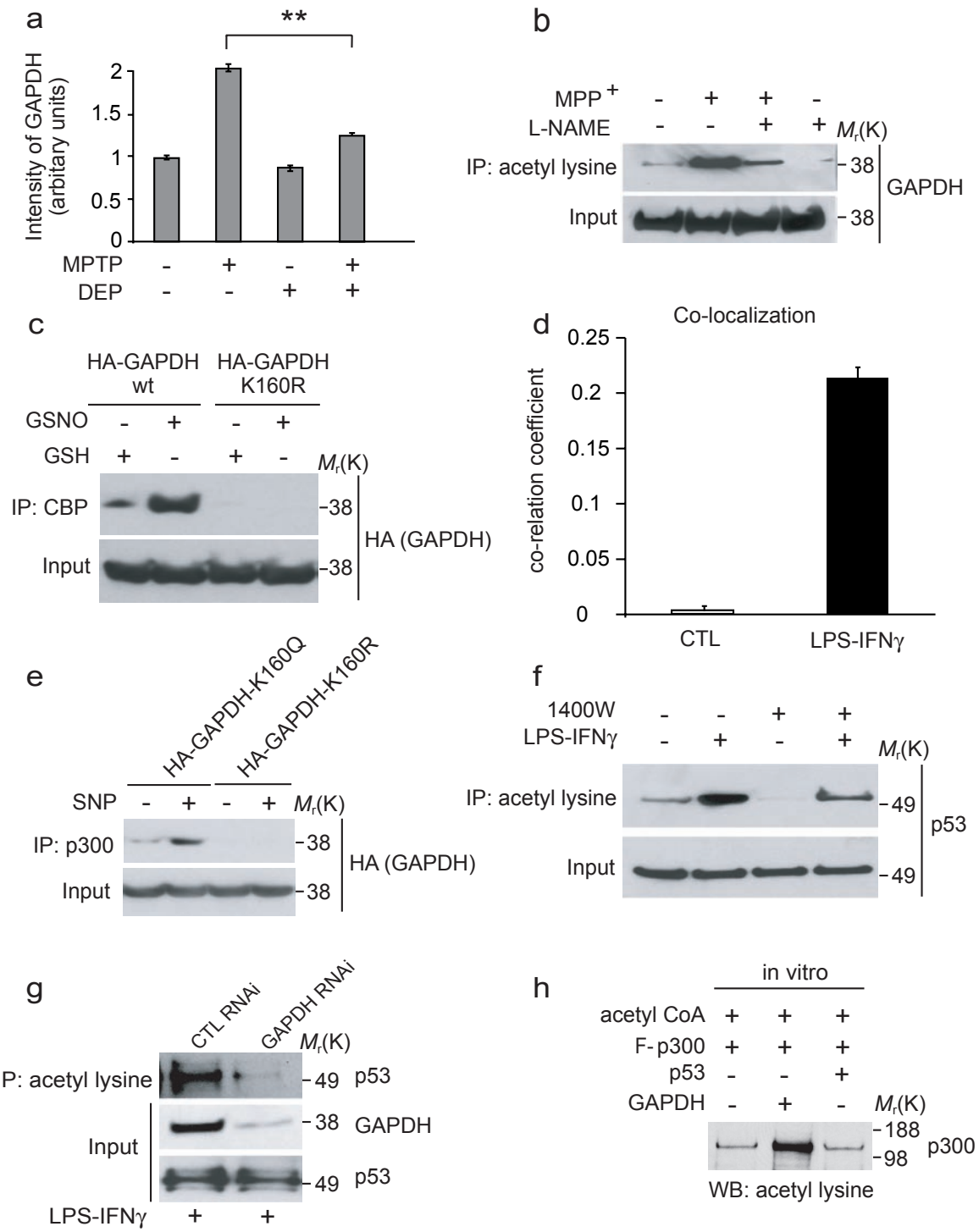

Figure S2 Effects of MPTP or MPP+ on acetylation of GAPDH, binding of p300/CBP to GAPDH, and effects of GAPDH on acetylation of p53 and p300. **a,** Quantitative assessment of GAPDH acetylation in brains from mice treated with MPTP in Fig. 1e. n=3, mean ± S.E.M., **p<0.001, one-way ANOVA. **b,** Acetylation of GAPDH is increased in SH-SY5Y cells treated with 5 μM MPP+ for 16 h, which is reversed by a NOS inhibitor L-NAME. Cell lysates were immunoprecipitated with an anti-acetyl lysine antibody, and the immunoprecipitates analyzed by Western blotting with an anti-GAPDH antibody. **c,** Wild-type GAPDH, but not GAPDH-K160R, binds to CBP in HEK293 cells treated with GSNO. **d,** Co-localization of p300 and GAPDH in the nucleus in control or LPS-IFNγ-treated RAW264.7 cells was assessed by correlation coefficient analysis. **e,** HA-GAPDH-K160Q (a pseudo-acetylation mutant), but not HA-GAPDH-K160R (acetylation-null mutant), binds to p300 in HEK293 cells exposed for 16 h to 200 μM sodium nitroprusside (SNP). **f,** Acetylation of p53 is increased in RAW264.7 cells treated with LPS-IFNγ for 16 h, which is blocked by 100 μM 1400W. **g,** Depletion of GAPDH by RNAi decreases acetylation of p53 in RAW 264.7 cells treated with LPS-IFNγ. **h,** GAPDH augments auto-acetylation of a fragment of p300 (F-p300) *in vitro*. Auto-acetylation of F-p300 (100 ng) with GAPDH (1 μg) or p53 (1 μg) was assessed by Western blotting with an anti-acetyl lysine antibody.

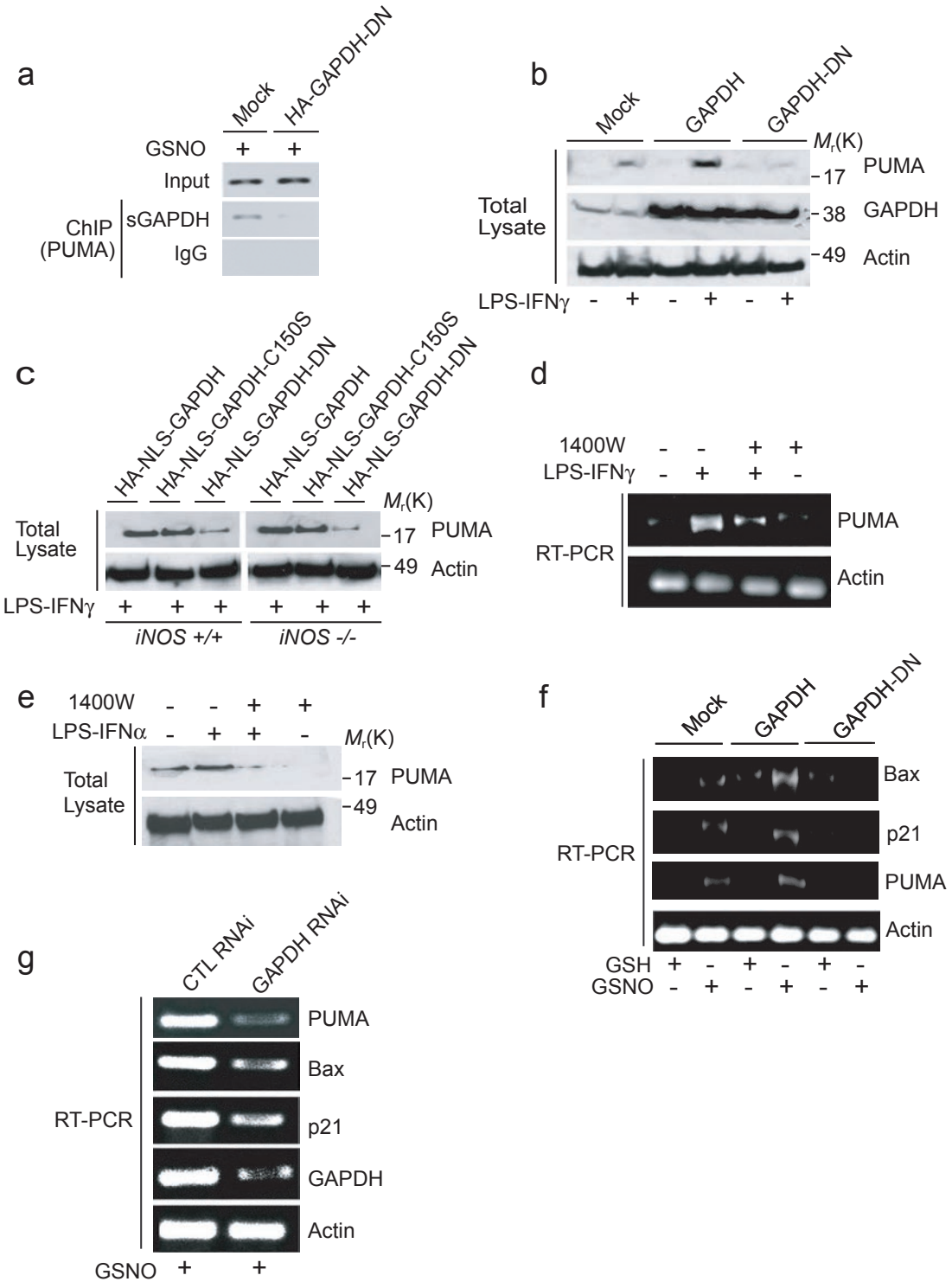

Figure S3 Influences of GAPDH on PUMA mRNA and protein levels. **a,** Interaction of sulfonated GAPDH (sGAPDH) at the *PUMA* promoter region is abolished by expression of GAPDH-DN in U2OS cells treated with GSNO. Cell lysates were used for ChIP assay with an sGAPDH antibody. **b,** Protein levels of PUMA are increased by expression of GAPDH, effects blocked by GAPDH-DN, in RAW264.7 cells treated with LPS-IFNγ for 16 h. **c,** Transfection of HA-NLS-GAPDH, HA-NLS-GAPDH-C150S, or HA-NLS-GAPDH-DN in peritoneal macrophages of wt or iNOS knockout mice. Both NLS-GAPDH and NLS-GAPDH-C150S elicit increased expression of PUMA, regardless of iNOS disposition, after treatment with LPS-IFNγ. **d, e,** Expression of PUMA mRNA and protein levels are increased in the presence of LPS-IFNγ, which is blocked by 1400W, in RAW 264.7 cells. **f,** Expression of PUMA, Bax, and p21 is increased in GSNO-treated U2OS cells expressing wild-type GAPDH, which is blocked by expression of GAPDH-DN. **g**, GAPDH augments induction of PUMA, Bax, and p21 in U2OS cells. Forty-eight h after transfection with shRNA to GAPDH, or control (CTL), transfected cells were treated with 200 μM GSNO for 24 h.

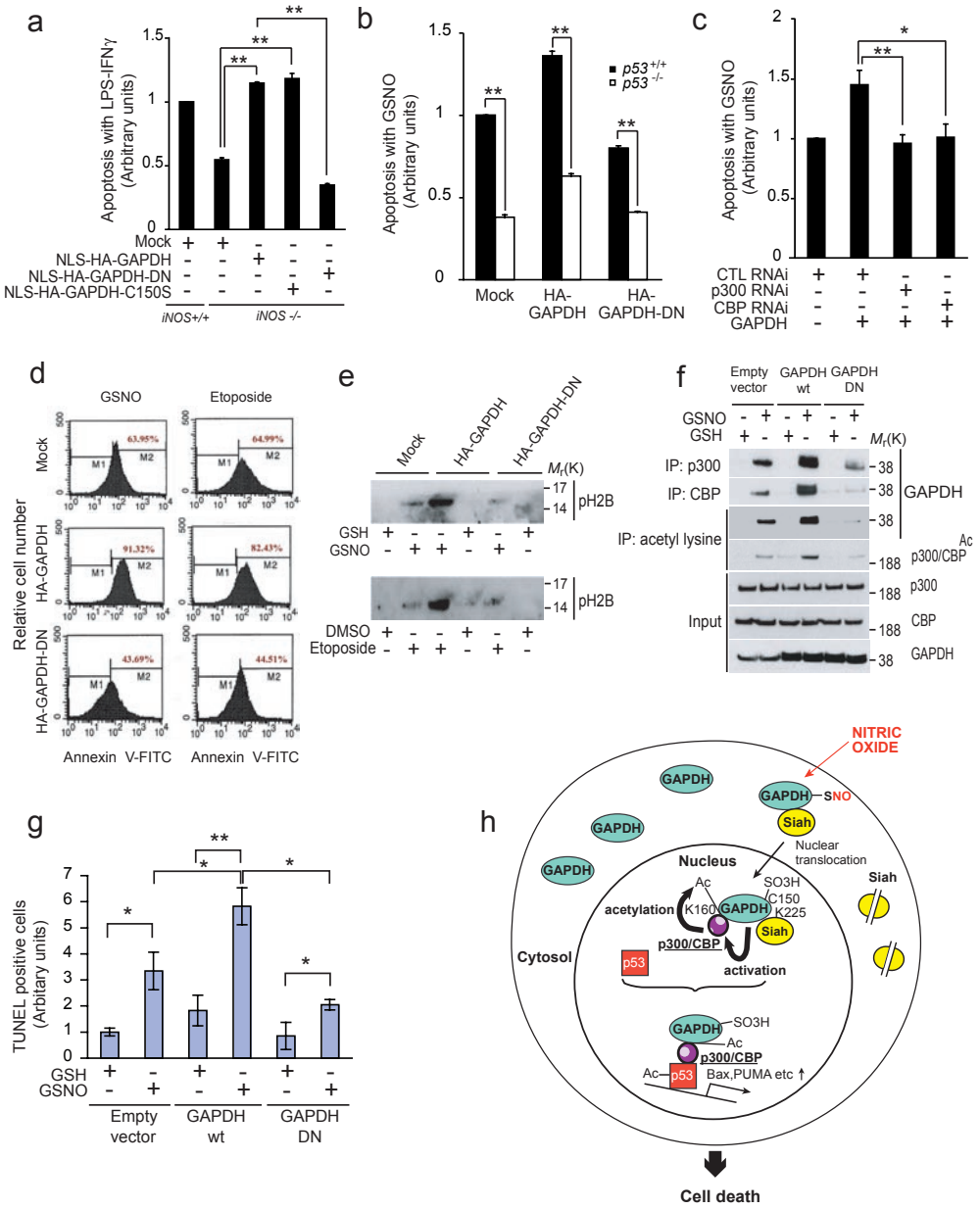

Figure S4 Acetylation and activation of CBP/p300 in cells stably overexpressing wild-type GAPDH, but not in cells with GAPDH-DN, and influences of GAPDH and GAPDH-DN on apoptosis. **a,** Nuclear localization of GAPDH augments apoptosis in peritoneal macrophages from iNOS knockout mice. Peritoneal macrophages from wt and iNOS knockout mice were transfected with HA-NLS-GAPDH, HA-NLS-GAPDH-C150S, or HA-NLS-GAPDH-DN. Forty-eight h after transfection, cells were treated with LPS-IFN-γ for 16 h. Apoptosis was assessed with cell death ELISA. n=3, mean ± S.E.M., $^{**}p<0.001$, one-way ANOVA. **b,** DNA fragmentation in HCT116 cells (wild-type or p53 null) was monitored by ELISA after transfection with GAPDH and GAPDH-DN in the presence of 200 μM GSNO for 24 h. n=3, mean ± S.E.M., $^{**}p<0.001$, one-way ANOVA. **c,** DNA fragmentation was measured by ELISA in U2OS cells after depletion of p300 or CBP during over-expression of GAPDH with 200 μM GSNO. n=3, mean ± S.E.M., $^{*}p<0.01$; $^{**}p<0.001$, one-way ANOVA. **d,** FACS analysis of apoptosis with annexin V indicates that the death triggered by GSNO or etoposide is blocked by GAPDH-DN. Forty-eight h after transfection with GAPDH-wt or GAPDH-DN, HEK293 cells were treated with either 200 μM GSNO or 100 μM etoposide. Cells were stained with annexin V-FITC to identify Annexin V-positive cells (M2 region, x axis). **e,** Forty-eight h after transfection with GAPDH-wt or GAPDH-DN, cells were treated with either 200 μM GSNO or 100 μM etoposide for 24 h. Apoptosis elicited by GSNO or etoposide is blocked by GAPDH-DN, which is assessed by another apoptotic marker, phosphorylation of histone H2B at serine-14 (pH2B)[2]. **f,** In SH-SY5Y cells stably overexpressing wild-type GAPDH exposed to 200 μM GSNO for 24 h, autoacetylation of CBP/p300 or GAPDH and GAPDH-CBP/p300 protein interactions are significantly increased, in comparison with cells stably overexpressing GAPDH-DN. **g,** Cell death in SH-SY5Y cells stably over-expressing GAPDH is increased compared to SH-SY5Y cells stably expressing GAPDH-DN. Cells were treated with 200 μM GSH or GSNO for 24 h. Cell death was measured by TUNEL assay. n = 4, mean ± S.E.M, $^{*}p<0.01$, $^{**}p<0.001$, one-way ANOVA. **h,** Schematic diagram of NO-GAPDH-p300/CBP cell death signaling. NO triggers *S*-nitrosylation/sulfonation of GAPDH, augments its binding to Siah, translocating the GAPDH-Siah protein complex to the nucleus. GAPDH is acetylated by p300/CBP at K160R. In turn, nuclear acetylated GAPDH augments acetyltransferase activity of p300/CBP. Acetylated p300 acetylates its substrates, such as p53, inducing downstream apoptotic genes and cell death.

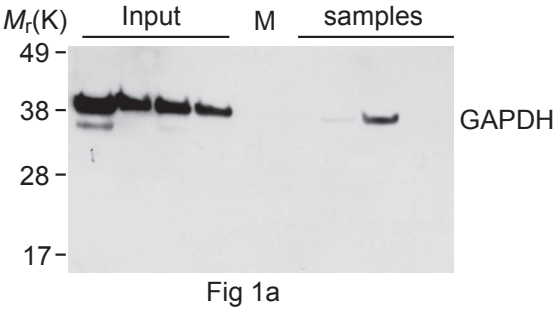

Fig 1a

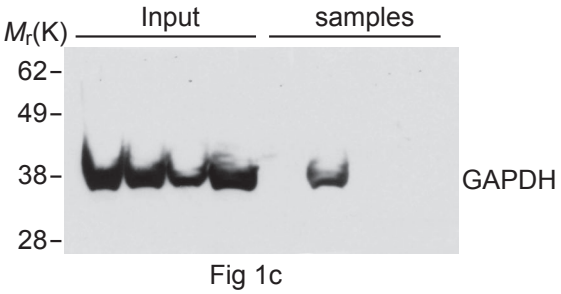

Fig 1c

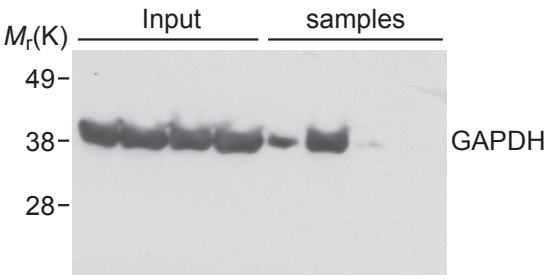

Fig 2e

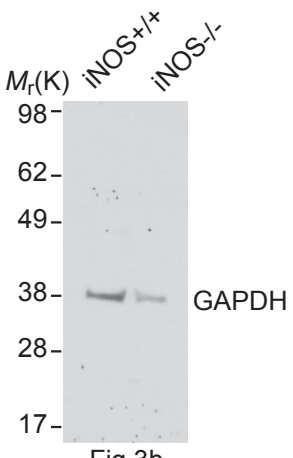

Fig 3b

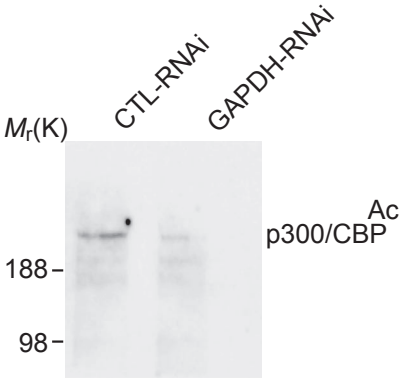

Fig 3c

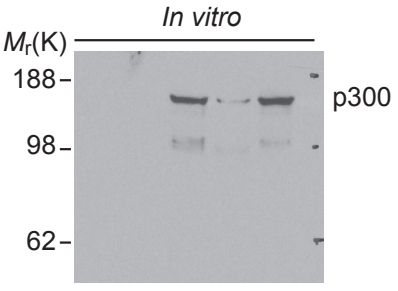

Fig 3f

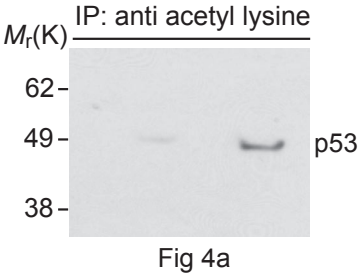

Fig 4a

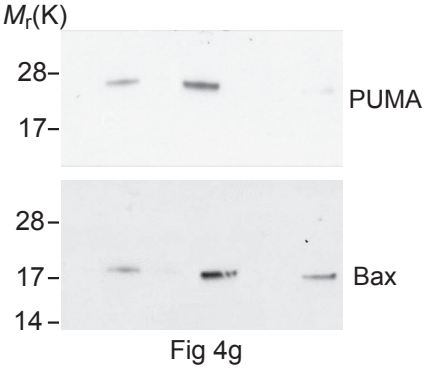

Fig 4g

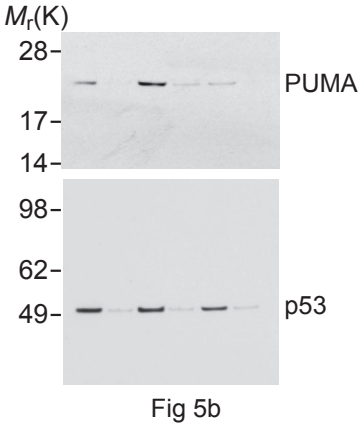

Fig 5b

Figure S5 Full scans of key Western blot data.

Supplementary References

1. Wang, D., Thompson, P., Cole, P.A. & Cotter, R.J. Structural analysis of a highly acetylated protein using a curved-field reflectron mass spectrometer. *Proteomics* **5**, 2288-2296 (2005).
2. Cheung, W.L. *et al.* Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* **113**, 507-517 (2003).