SUPPLEMENTARY INFORMATION

PHF20 is an effector protein of p53 double lysine methylation that stabilizes and activates p53

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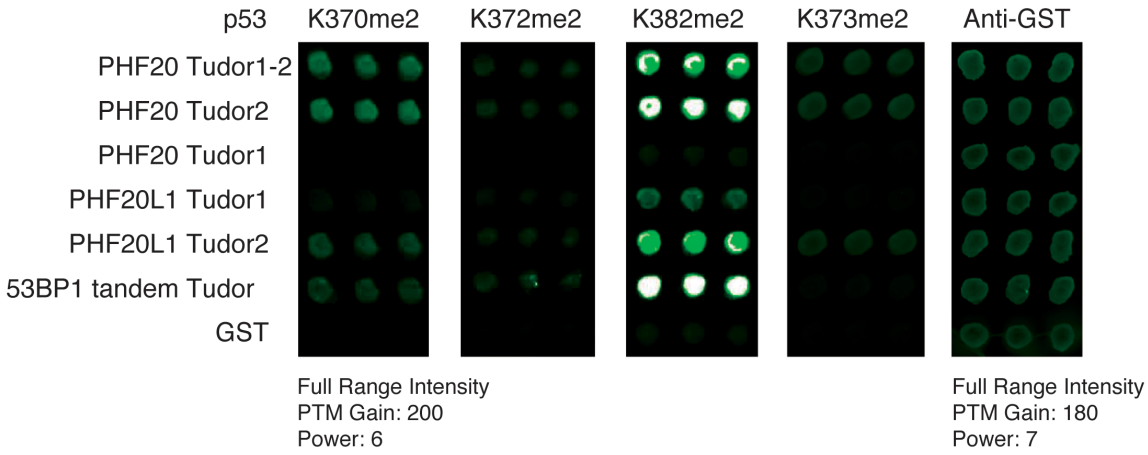
SUPPLEMENTARY FIGURES



Supplementary Figure 1 Interactions of selected Tudor domains with dimethylated peptides representing the known methylation sites of p53. All protein domains are fused to the C-terminus of GST and spotted on nitrocellulose-coated glass slides. These slides were probed with Cy3 dye-labeled peptides representing an 18-amino acid stretch of p53 centered on the denoted methylated lysine residue. An anti-GST antibody shows equal spotting of all domains.

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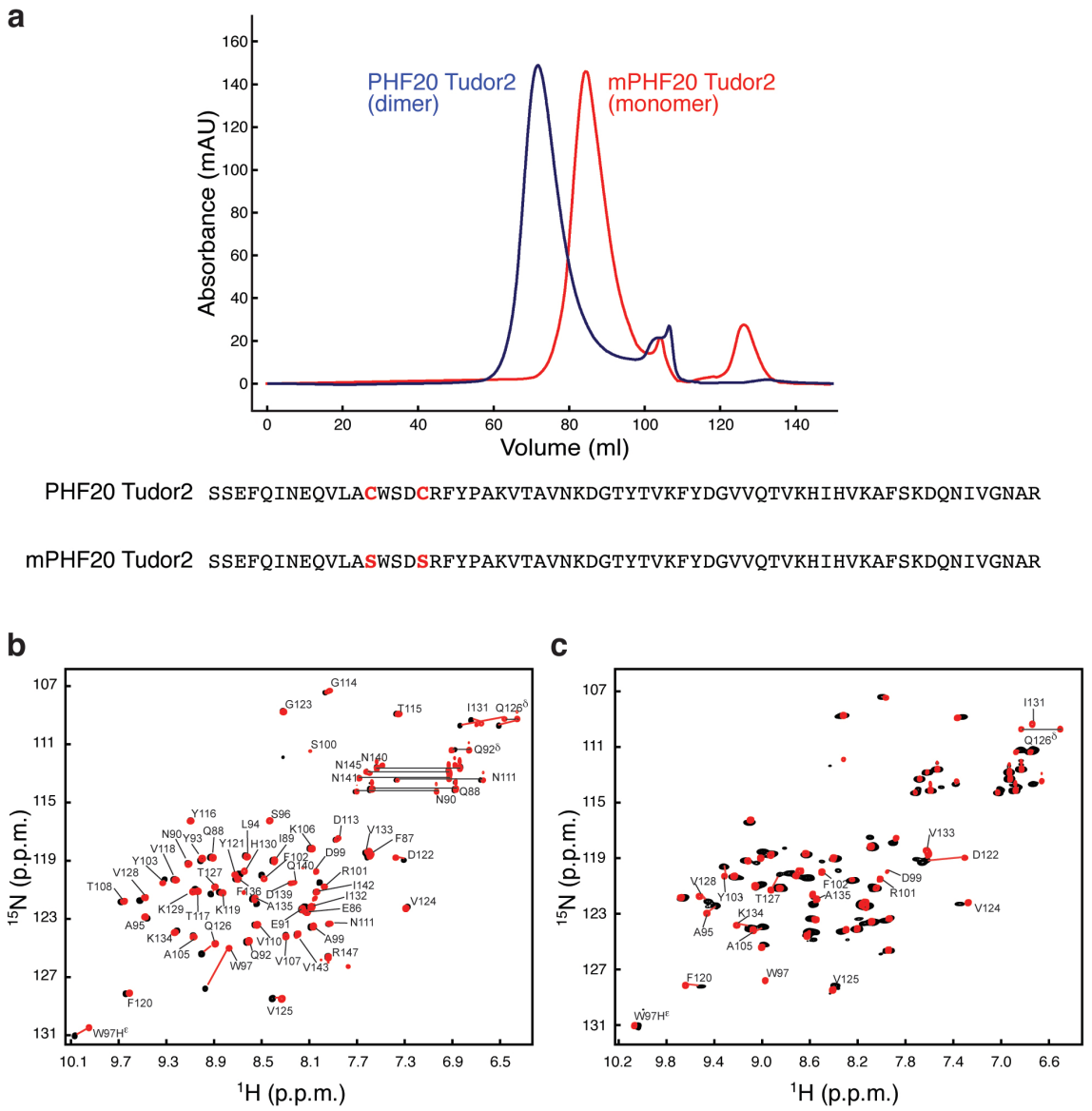
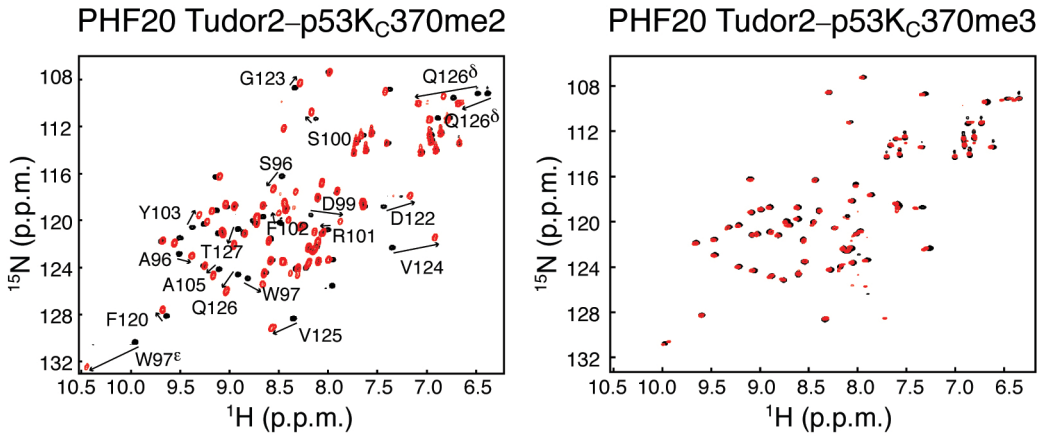


Supplementary Figure 2 Conversion of PHF20 Tudor2 dimer into a monomer. (**a**) FPLC chromatograms of wild-type PHF20 Tudor2 (dimeric) and mPHF20 Tudor2 C96S/C100S mutant (monomeric). Amino acid sequence of PHF20 Tudor2 and mutation sites are shown. (**b**) Overlay of ^{1}H-^{15}N HSQC spectra of PHF20 Tudor2 in the presence of 20 mM DTT (black) and mPHF20 Tudor2 (red). (**c**) Overlay of ^{1}H-^{15}N HSQC spectra of PHF20 Tudor2 in the absence (black) and presence of DTT (red). Residues displaying the largest changes in chemical shifts are labeled.



Supplementary Figure 3 PHF20 Tudor2 does not interact with trimethylated p53. Overlay of the ^{1}H-^{15}N HSQC spectra of PHF20 Tudor2–p53C370 linked construct (black) with, from left to right, the same construct after dimethylation (PHF20 Tudor2–p53K$_C$370me2) and trimethylation (PHF20 Tudor2–p53K$_C$370me3) (red). Shifts are only observed for the dimethylated protein.

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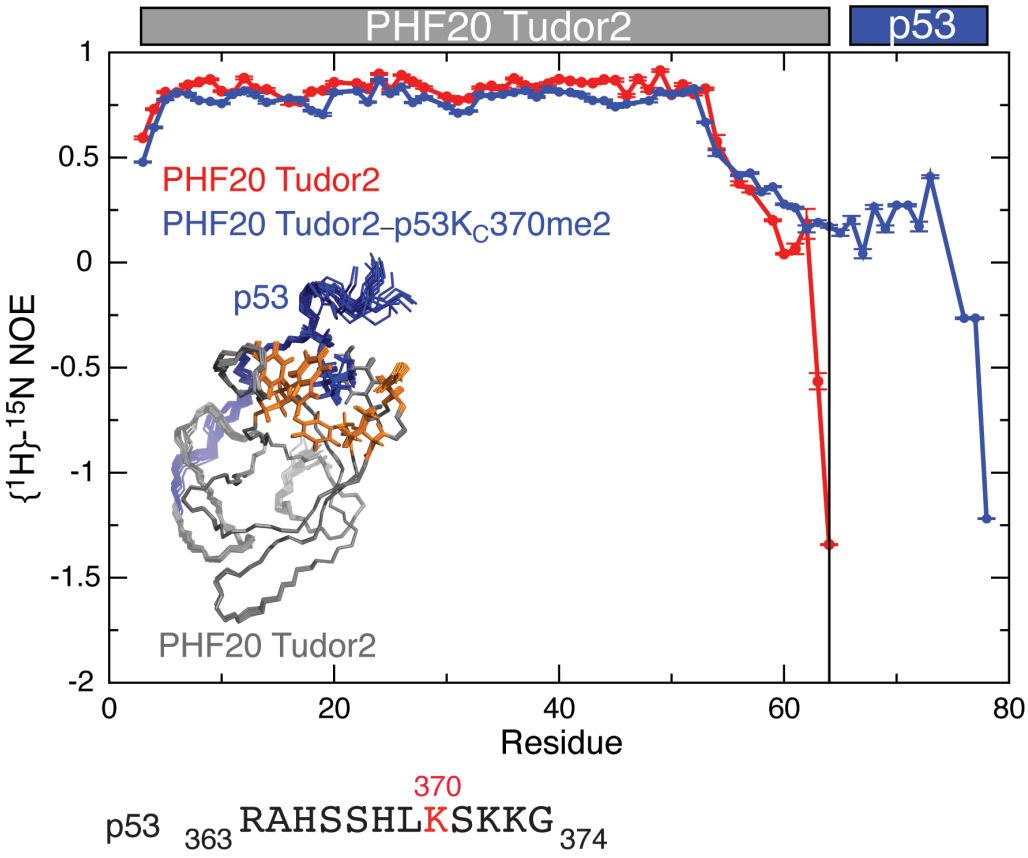

Supplementary Figure 4 Dynamics of free and p53K$_C$370-linked monomeric PHF20 Tudor2 (PHF20 Tudor2–p53K$_C$370). Shown are {^{1}H}-^{15}N heteronuclear NOE experiments performed at 700 MHz for free PHF20 Tudor2 (red graph) and PHF20 Tudor2–p53K$_C$370 (blue graph) with regions of Tudor2 and p53 indicated on top of the graph. Also shown are superimposed NMR structures of PHF20 Tudor2–p53K$_C$370me2 with PHF20 in gray and side chains of aromatic cage residues highlighted in orange. The p53K$_C$370me2 segment is in blue. The amino acid sequence of the p53 segment linked to PHF20 Tudor2 is shown (residues 363–374).

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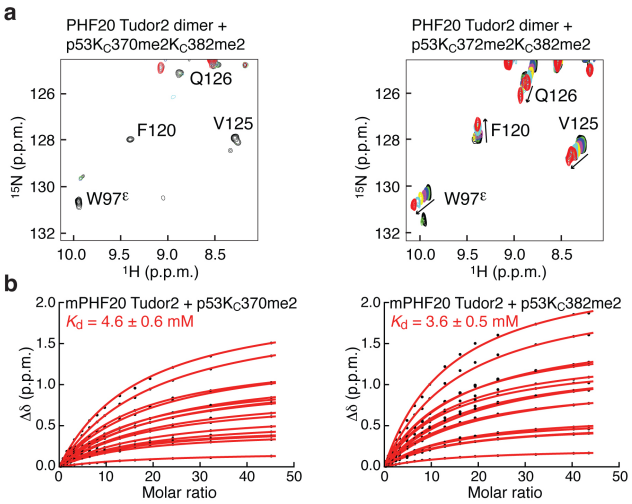
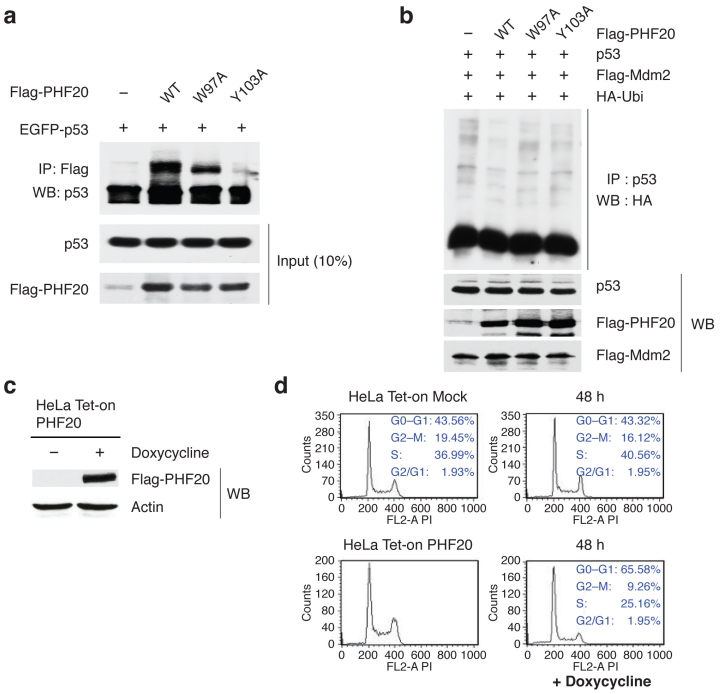


Supplementary Figure 5 Binding of PHF20 Tudor2 to methylated p53. (**a**) Titration of PHF20 Tudor2 dimer with p53K$_C$370me2K$_C$382me2 and p53K$_C$372me2K$_C$382me2 peptides. Shown are chemical shift changes from a selected region of the ^{1}H-^{15}N HSQC spectrum of PHF20 Tudor2 dimer upon titration with the p53K$_C$370me2K$_C$382me2 peptide (left) and p53K$_C$372me2K$_C$382me2 peptide (right). Black and red signals correspond to the spectra in the absence of peptide and at the end of the titration, respectively. The disappearance of signals for the titration of PHF20 Tudor2 with p53K$_C$370me2K$_C$382me2 due to exchange broadening indicates higher affinity of p53K$_C$370me2K$_C$382me2 compared to p53K$_C$372me2K$_C$382me2. (**b**) Determination of the dissociation constants for the interactions of mPHF20 Tudor2 with p53K$_C$370me2 and p53K$_C$382me2 peptides (amino acids 363–389). The K_ds were determined by non-linear least squares fitting of the equation:

$$\frac{\Delta\delta}{\Delta\delta_{max}} = 0.5\left[\left(1+\frac{K_d}{C_p}+M\right) - \sqrt{\left(\left(1+\frac{K_d}{C_p}+M\right)^2 - 4M\right)}\right]$$

where M is the molar ratio of p53 peptide to mPHF20 Tudor2, C_p, the concentration of PHF20 Tudor2 and $\Delta\delta$, the normalized chemical shift change calculated as: $\Delta\delta = \sqrt{(\delta_{HN})^2 + (\delta_N)^2}$ where δ_{HN} and δ_N denote the amide hydrogen and nitrogen atoms chemical shift differences between the free and peptide-bound states for PHF20 Tudor2. $\Delta\delta_{max}$ is the normalized difference in chemical shifts of the free and peptide-saturated PHF20 Tudor2. Errors associated with K_d values are the standard deviations from performing the fit for 16 non-overlapping ^{1}H-^{15}N resonance signals.



Supplementary Figure 6 Effects of PHF20 on p53 ubiquitylation and cell cycle regulation. (**a**) The W97A and Y103A Tudor2 mutations in full-length PHF20 reduce PHF20 binding to p53 in comparison to wild-type (WT) PHF20. MCF7 cells were transfected with Flag-PHF20 and EGFP-p53 plasmids, immunoprecipitated with an anti-Flag antibody and then immunoblotted with an anti-p53 antibody. The W97A and Y103A mutations when introduced in the isolated Tudor2 domain prevent interactions with p53K370me2 and p53K382me2 peptides but do not disrupt the Tudor domain fold. (**b**) WT full-length PHF20 protects p53 from ubiquitylation by Mdm2. The W97A and Y103A mutations in PHF20 decrease this protection effect. Following transfection of MCF7 cells with Flag-PHF20, Flag-Mdm2 and HA-Ubiquitin plasmids and treatment with proteasome inhibitor MG132, the cells were immunoprecipitated with an anti-p53 antibody and immunoblotted with an anti-HA antibody (top). Indicated proteins, including endogenous p53, were immunoblotted (bottom). (**c**) Doxycycline-induced expression of Flag-PHF20 in HeLa Tet-on cells (HeLa Tet-on PHF20). Proteins in the cell lysates were identified by WB using anti-Flag and anti-actin antibodies. (**d**) HeLa Tet-on PHF20 cells and control HeLa Tet-on cells (Mock) were treated with 1 μg ml^{-1} doxycycline for 12 h to induce expression of Flag-PHF20 and then with nocodazole for 12 h to synchronize cells to M phase. Cells were subsequently washed, cultured in fresh nocodazole-free medium, fixed and analyzed by flow cytometry.

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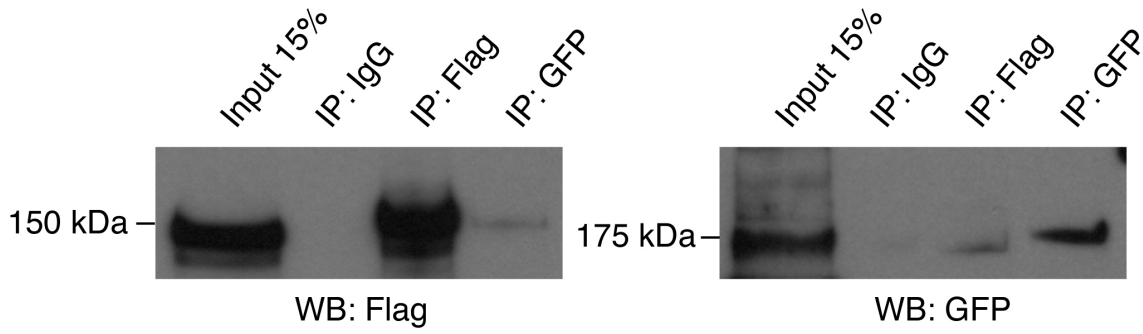

Supplementary Figure 7 Co-immunoprecipitations of full-length PHF20 constructs expressed with different tags in cells. HEK 293 cells were co-transfected with Flag-PHF20 and EGFP-PHF20 expression constructs. After a 24-h period to allow for protein expression, the cells were harvested and the lysate was used to perform immunoprecipitations using anti-Flag and anti-GFP antibodies. GFP-PHF20 pulls down Flag-PHF20 and vice versa, demonstrating an interaction between two molecules of PHF20.

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