SUPPLEMENTARY INFORMATION

Supplementary Figure Legends

Supplementary Figure 1. Identification of methylated p53 species in DO1 IPs from HeLa cells. **A.** Schematic of scheme to purify endogenous p53 from HeLa nuclear extract (NE). 10 mg of NE were incubated in batch with DO1 antibodies covalently coupled to agarose beads under stringent conditions to selectively purify endogenous p53 (see methods). **B.** Silver stain and Western analysis (with horse-radish peroxidase coupled DO1 antibody) of the purified p53 used for mass spectrometry analysis. **C.** Tryptic peptides containing a single lysine (and no arginines) were analyzed for a mass shift corresponding to one or two methyl groups. MS analysis identified potentially three additional methylated p53 species: p53K370me2, p53K386me1 and p53K386me2.

Supplementary Figure 2. The anti-p53K382me2 antibody does not recognize H4K20me2 peptides. Peptide dot blots were probed with the indicated antibodies, or a streptavidin-HRP control for peptide loading.

Supplementary Figure 3. 53BP1 knock-down does not impact expression of *PUMA*. Real-time PCR analysis of relative mRNA levels of *PUMA* in U2OS cells treated with control or 53BP1 siRNAs, ± 45 nM NCS.

Supplementary Figure 4. 53BP1 knock-down does not cause a decrease in *p53* expression. Real-time PCR analysis of relative mRNA levels of *p53* in U2OS cells treated with control or 53BP1 siRNAs, ± 45 nM NCS.

Supplementary Figure 5. Model of 53BP1-p53K382me2 interaction in transduction of DSB signals to p53 stabilization. In the absence of DNA damage, p53 levels are rendered low by a number of E3-ubiquitin ligases and factors that promote degradation (reviewed in [1]). In response to DSBs, multiple molecular mechanisms are activated to prevent p53 degradation. Two DNA damage-dependent events depicted here include the coordinated increase in dimethylation of p53 at K382 and the mobilization of 53BP1 and other DNA damage sensors and transducers at DSB foci. We propose that the DNA damage-triggered rapid accumulation of 53BP1 at DSB sites (in conjunction with the assembly of additional p53-regulatory factors such as ATM and Chk2) may cooperate to create a high-affinity site for p53 that facilitates accumulation of p53 by sequestering it away from proteins that target it for degradation. In this model, 53BP1 serves as an adaptor at DSB sites, with one 53BP1 molecule bound to H4K20me2 and a paired second 53BP1 molecule free to dock p53K382me2 [2].

Supplementary Methods

Fluorescence polarization measurements and protein purification

GST-tagged human 53BP1 (residues 1480-1526) was expressed in BL21 (DE3) *E. coli* cells at 37°C in LB medium. The protein was loaded to a glutathione sepharose 4B column equilibrated in 20 mM Tris-HCl pH 8.0, 0.15 M NaCl, 10 % glycerol, 1 mM

DTT, 1 mM EDTA and eluted by addition of 10 mM reduced glutathione. The GST tag was removed by overnight digestion with PreScission protease. The protein was further purified over a MonoQ column equilibrated in 20 mM Tris pH 8, 5% glycerol, 75 mM NaCl, 1 mM DTT and 0.1 mM EDTA and eluted in a gradient of salt.

Peptide synthesis and purification

Peptides were synthesized by the solid phase method with 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. Methylated amino acids were coupled as Fmoc-Lys(Me, Boc)-OH, Fmoc-Lys(Me)$_2$-OH, and Fmoc-Lys(Me)$_3$-OH (AnaSpec Inc., San Jose, CA). Fluorescence labeling of the peptide was achieved with 3 equivalent of 5-(6)-carboxyfluorescein succiniimidyl ester (Molecular Probes, Eugene, OR) in dimethylsulfoxide, and allowed to stir overnight (room temperature, in the dark). The peptides were cleaved with a 82.5% trifluoroacetic acid (TFA), 5% phenol, 5% thioanisole, 5% water, and 2.5% ethandithiol, and then purified by reversed-phase high-performance liquid chromatography (RP-HPLC) on a C-4 column with 0.05% TFA/water/acetonitrile. The purity of the peptides was determined to be >95% by analytical RP-HPLC. The mass of peptides was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Micromass, Beverly, MA).

The affinities (IC_{50} values) of the non-labeled methylated peptides (0.01-600 μM) to 53BP1 tudor domain (14 μM) were determined by competition assay using fluorescence anisotropy change of N-terminally fluorescein-labeled H4-K20me2 peptide (5 nM) as a tracer. Fluorescence anisotropy was measured at 25 °C on a Beacon 2000 (PanVera, Madison, WI). All samples were incubated for overnight at 4 °C in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl and 1mM DTT. The IC_{50} value was estimated by sigmoidal dose-response equation as a 1:1 stoichiometry.

Mass spectrometry

α-cyano-4-hydroxycinnamic acid (Sigma) matrix was prepared in a 60:40 (v/v) mixture of acetonitrile and 0.1% aqueous trifluoroacetic acid solution. Equal volumes of sample and matrix totaling 6 ml were deposited on the stainless steel target to form a sample spot 4 mm in diameter. Samples were analyzed on a reflectron time-of-flight mass spectrometer, MALDI-TOF instrument (Ultraflex, Bruker Daltonics, Billerica, MA), equipped with a 337 nm nitrogen laser and delayed ion extraction capability (delay times: 30-50 ns). Ion structure information was obtained by PSD using the mass gate feature to select specific *m/z* window for fragmentation. The mass gate resolution was 1% of the precursor mass. Data was recorded in both positive and negative ion modes at 20kV acceleration, and mass analysis of ions determined using a dual micro-channel plate detector. Detector output was collected with a 1 GHz digitizer and displayed directly on a Windows NT based computer. Ten positive ion reflectron TOF mass spectra of 1000 laser shots were accumulated and externally calibrated with commercial peptide mix (Bruker Daltonics, Billerica, MA).

References:

1 C. L. Brooks and W. Gu, *Mol Cell* **21** (3), 307 (2006).

2 I. Ward, J. E. Kim, K. Minn et al., *J Biol Chem* **281** (50), 38472 (2006).

3 X. Shi, T. Hong, K. L. Walter et al., *Nature* **442** (7098), 96 (2006).