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Structural insight into p53 recognition by the 53BP1 tandem Tudor domain

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Summary

The tumor suppressor p53 and DNA repair factor 53BP1 regulate gene transcription and responses to genotoxic stresses. Upon DNA damage, p53 undergoes dimethylation at Lys382 (p53K382me2), and this posttranslational modification is recognized by 53BP1. The molecular mechanism of the nonhistone methyllysine mark recognition remains unknown. Here we report a 1.6 Å resolution crystal structure of the tandem Tudor domain of human 53BP1 bound to a p53K382me2 peptide. In the complex, dimethylated Lys382 is restrained by a set of hydrophobic and cation- π interactions in a cage formed by four aromatic residues and an aspartate of 53BP1. The signature HKKme2 motif of p53, which defines the specificity, is identified through a combination of NMR resonance perturbations, mutagenesis, measurements of binding affinities and docking simulations and analysis of the crystal structures of 53BP1 bound to p53 peptides containing other dimethyllysine marks, p53K370me2 and p53K372me2. Binding of the 53BP1 Tudor domain to p53K382me2 may facilitate p53 accumulation at DNA damage sites and promote DNA repair as suggested by chromatin immunoprecipitation and DNA repair assays. Together, our data detail the molecular mechanism of the p53-53BP1 association and provide the basis for deciphering the role of this interaction in regulation of p53 and 53BP1 functions.

Keywords

Tudor domain; methylated p53; 53BP1; structure; cancer

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Accession numbers Coordinates and structure factors have been deposited to the Protein Data Bank with accession numbers 3LGL, 3LGF and 3LH0.

Introduction

The tumor suppressor p53 is found mutated in about half of all human malignancies. It functions as a transcription factor that regulates expression of genes essential for the initiation of cell cycle arrest, DNA damage repair, cellular senescence and apoptosis^{1; 2}. The transcriptional activity of p53 is modulated by multiple post-translational modifications³. Over twenty Ser and Thr residues throughout the p53 sequence can be phosphorylated, which often leads to the elevated activity of p53. Ubiquitination of Lys residues in the carboxy-terminal region directs p53 for proteasomal degradation, whereas acetylation of these residues protects p53 from ubiquitination, promoting p53 stability and accumulation, and alters DNA binding and interactions with cofactors^{4; 5}.

Three lysine residues, Lys370, Lys372 and Lys382 of the p53 carboxy-terminal regulatory domain (CTD) undergo methylation^{6; 7; 8; 9}. Monomethylation of Lys372 by Set9 methyltransferase facilitates transcription of p53 target genes⁶, whereas monomethylation of either Lys370 by Smyd2 or Lys382 by Set8/PR-Set7 represses p53 function^{7; 8; 10}. Similarly to dimethylation of p53 at Lys370 (p53K370me2)⁷, dimethylation at Lys382 (p53K382me2)^{8; 11} enhances p53 stability and activation through association with the Tudor domain of p53 binding protein 1 (53BP1), a key DNA damage response mediator¹², however how the nonhistone methyllysine marks of p53 are recognized remains unknown.

Here, we elucidate the molecular mechanism of p53 recognition by 53BP1 by determining a 1.6 Å resolution crystal structure of the human 53BP1 tandem Tudor domain in complex with a p53K382me2 peptide, establish the determinants of specificity for the p53 sequence using a combination of NMR resonance perturbations, mutagenesis, measurements of binding affinities and docking simulations and analyzing the crystal structures of 53BP1 bound to p53K370me2 and p53K372me2 peptides, and propose the role of the p53K382me2-53BP1 interaction in p53 accumulation and DNA repair.

Results and Discussion

Overall structure and recognition of dimethylated Lys

The p53K382me2-bound 53BP1 tandem Tudor domain folds into two almost identical modules, each consisting of four twisted anti-parallel β -strands and a short α -helix (Fig. 1). A longer carboxy-terminal helix $\alpha 2$ is tightly packed against both modules. The overall fold of 53BP1 in complex with p53K382me2 is similar to the fold of the ligand-free or histone H4K20me2-bound protein (PDB 1xni, 2g3r and 2igo)^{13; 14}. The structures of the p53K382me2-bound and unbound states superimpose with a root mean square deviation (rmsd) of 1.1 Å and 0.2 Å over C α atoms, respectively, indicating that binding to the p53K382me2 peptide triggers only a minor conformational change in 53BP1.

The distinctive feature of p53 recognition by the 53BP1 tandem Tudor domain is an efficient coordination of dimethylated Lys382. The binding pocket for K382me2 is formed by four aromatic residues, W1495, Y1502, F1519 and Y1523, and an acidic residue D1521 of the amino-terminal Tudor1 module (Fig. 1c). The aromatic side chains of W1495, Y1502 and Y1523, positioned perpendicular to each other and orthogonally to the protein surface, create three walls of the aromatic cage, whereas F1519 lies at the bottom of the cage. The fourth wall is formed by D1521. The aromatic residues make favorable hydrophobic and cation- π contacts with the dimethylammonium group of Lys382, whereas the carboxylate of D1521 forms a hydrogen bond with the only amino proton and a salt bridge with the ion. This mode of dimethyllysine recognition is reminiscent of the mechanism for discrimination of the low lysinemethylation state, described previously for the interaction of 53BP1 tandem Tudor domain with H4K20me2 and binding of malignant brain tumor (MBT) repeats and engineered

plant homeodomain (PHD) to monomethylated and dimethylated histone tails¹⁴; 15. The presence of the dimethylated mark is required, as the 53BP1 tandem Tudor domain binds p53K382me2 with a K_d of 0.9 μ M but does not associate with the unmodified p53 peptide (Fig. 2a).

Determinants of specificity: the critical role of His380 and Lys381

The His380 and Lys381 residues preceding K382me2 in the p53 sequence are essential for strong interaction with 53BP1. Substitution of His380 and Lys381 with Ala decreased the binding affinity for p53K382me2 by 16- and 11-fold, respectively (Fig. 2a, b and Suppl. Fig. 1). On the contrary, replacement of Leu383 had virtually no effect on the K_d , implying that residues preceding but not following K382me2 significantly contribute to the binding energetics. The critical role of these residues in the formation of the complex was underscored by the fact that dimethylated lysine alone was found insufficient for the interaction (K_d of 2.9 ± 0.5 mM). The position of His380 and Lys381 in the complex was determined by NMR resonance perturbation analysis as the electron density of these residues was unclear. Large chemical shift changes were observed for several regions of ¹⁵N-labeled 53BP1 when the unlabeled p53K382me2 peptide was titrated in, suggesting that these regions are directly or indirectly involved in the interaction (Fig. 2c). Mapping the most perturbed residues onto the surface of 53BP1-p53K382me2 revealed an extended binding interface that spans both Tudor modules, outlining two apparent aromatic pockets (Fig. 2d). One pocket is formed by W1495, Y1502, D1521 and Y1523 and is occupied by K382me2 of the peptide in the crystal structure of the complex, another contains Y1500 and F1553.

The p53H380AK382me2 and p53K381AK382me2 peptides induced comparable resonance perturbations in the dimethyllysine-binding cage of the tandem Tudor domain, however significantly smaller chemical shift changes were detected for F1553 and the surrounding residues (Suppl. Fig. 2). Differences in the magnitude and pattern of perturbations, observed upon binding to p53H380AK382me2 and p53K381AK382me2 as compared to binding to p53K382me2, delineated the sites where His380 and Lys381 contact the tandem Tudor domain (Suppl. Fig. 3). NMR-data driven docking simulations of the 53BP1-p53K382me2 complex further suggested that Lys381 makes cation- π contacts with F1553 and Y1500 (Suppl. Fig. 4), likewise Arg19 of H4K20me2 is involved in a cation- π interaction with Y1500 in the 53BP1-H4K20me2 complex¹⁴. In contrast, Leu383 appeared to swing away from the protein surface (Suppl. Fig. 4). This is in agreement with the binding affinities and similar resonance perturbations caused by the p53K382me2L383A and p53K382me2 peptides (Fig. 2a, c and Suppl. Fig. 2), corroborating the notion that Leu383 is not required for the interaction.

Structural basis for association with p53K370me2 and p53K372me2

To further define the specificity, we examined interactions of the 53BP1 tandem Tudor domain with the other two methylated species of p53, known p53K370me2⁷, and yet to be identified p53K372me2, using NMR and fluorescence spectroscopy, and determined the 1.5 Å and 1.9 Å resolution crystal structures of the complexes (Fig. 3). The structures of the Tudor domain bound to p53K370me2 or p53K372me2 superimposed well with the structure of the p53K382me2-bound protein (rmsd = 0.1 Å over C α atoms). Notably, the side chains of the major aromatic cage residues occupied very similar positions in the complexes, demonstrating high conservation of the dimethyllysine readout (Fig. 3d). However, the p53K370me2 and p53K372me2 peptides were bound 22-fold weaker than p53K382me2 (Fig. 3e) and caused NMR resonance perturbations primarily in the dimethyllysine binding pocket (Suppl. Fig. 5). These results suggest that the HLKme2 and KSKme2 sequences of p53K370me2 and p53K372me2, respectively, cannot effectively substitute for the HKKme2 motif in p53K382me2. However, the HRKme2 sequence found in histone H4K20me2 can (Fig. 3b). Almost identical chemical shift changes, detected in the NMR spectra of the Tudor domain

upon binding to either p53K382me2 or H4K20me2, and comparable K_d values (0.9 μ M and 1.3 μ M) imply that these peptides interact with 53BP1 to the same extent (Fig. 3e). Furthermore, the crystal structures of the p53K382me2- and H4K20me2¹⁴-bound protein overlay with an rmsd of 0.2 Å, indicating that the mode of HK/RKme2 recognition is conserved. Taken together, these data demonstrate that both His and Lys/Arg residues preceding Kme2 define the specificity, and suggest that 53BP1 preferentially recognizes p53K382me2 and H4K20me2 as compared to other methylated p53 species or methylated histone H3, which lack the necessary HK/RKme2 motif (Fig. 3b).

p53 accumulation at DNA damage sites via p53K382me2-53BP1 interaction

Dimethylation at Lys382 is known to enhance the association of p53 with 53BP1 *in vivo*¹¹. 53BP1 co-immunoprecipitates with p53K382me2, generated by co-expression of p53 and K382me2-specific methyltransferase variant SET8(Y334F), but not with unmodified p53 (Suppl. Fig. 6 and ref.11). Two recent findings suggest a role for the p53K382me2-53BP1 interaction in the DNA damage signaling. Particularly, it has been found that the p53K382me2 levels increase upon DNA damage¹¹, and that the DNA damage triggers an acute mobilization of 53BP1 to the double-strand break (DSB) sites^{16, 17}, which in turn can promote accumulation of p53. To test whether p53 is concentrated at the DSB sites, we designed cell lines with an integrated I-SceI restriction site flanked by known ectopic sequences and performed chromatin immunoprecipitation (ChIP) experiments (Fig. 4a–c). As shown in Figure 4c, occupancy of both p53 and 53BP1 at the I-SceI-defined DSB sites increased substantially and concomitantly upon induction of DNA damage by I-SceI enzyme transfection as compared to mock transfection or the IgG control, demonstrating that p53 is targeted to the DSB sites. To explore the functional significance of p53K382me2 in the context of DSB, we measured DNA repair in HT1080 cells expressing I-SceI enzyme (Fig. 4d). We found that repair of the I-SceI-induced DSB in cells that express SET8(Y334F) was virtually 100% compared to 76% in cells lacking SET8(Y334F). This phenotype was not due to non-specific effects of SET8(Y334F), as it failed to promote repair in p53 knock-down cell lines (Fig. 4d). Together, these results suggest that generation of p53K382me2 facilitates DNA repair.

Conclusions

In spite of the fundamental role of p53 in gene repression, DNA damage repair and apoptosis, regulation of this tumor suppressor function by methylation remains unclear. In this study, we detail the molecular mechanism of recognition of dimethylated p53, a nonhistone methyllysine factor, by 53BP1. The crystal structure of the 53BP1 tandem Tudor domain in complex with the p53 peptide dimethylated at Lys382 demonstrates that K382me2 is caged by a set of cation- π hydrophobic, hydrogen-bonding and ionic interactions in an aromatic cage, whereas the HK sequence preceding K382me2 accounts for the specificity of 53BP1. Binding of 53BP1 to p53K382me2 facilitates p53 accumulation at DNA damage sites and promotes DNA repair. It has recently been shown in the T. Halazonetis laboratory that oligomerization of 53BP1 via the region N-terminal to the tandem Tudor domain is required for the efficient recognition of DSB¹⁸, whereas G. Mer and colleagues found that the 53BP1 tandem Tudor domain recognizes histone H4K20me2¹⁴. Accordingly, oligomerized 53BP1 may associate with p53K382me2 and H4K20me2 concurrently, recruiting p53 to the sites of DSBs and bridging other components of the p53 machinery to chromatin (Fig. 4e). Other methylated marks, such as Lys370me2, which plays an activating role in p53 regulation⁷ could contribute to the p53-53BP1-chromatin assembly by enhancing avidity of oligomeric 53BP1. Nearby post-translational modifications of p53, including acetylation of Lys381 and phosphorylation of Ser371, may further influence binding activity of 53BP1 and fine tune p53 function. The crosstalk between these modifications is currently under investigation in our laboratories. In conclusion, our results provide the first biochemical characterization of methylated-p53 interactions, suggest a novel mechanism for recruitment of p53 to the sites of DNA double

strand break, and offer new insights into the role of the p53-53BP1 complex formation in regulation of p53 activity. The mechanistic principles by which p53 interacts with 53BP1 and promotes accumulation of p53 at the sites of DNA damage may help in identification of new diagnostic markers and targets within cancer-critical p53-mediated signaling pathways.

Materials and Methods

Expression and purification of proteins

The human 53BP1 tandem Tudor domain (residues 1584–1603) was expressed in *E. coli* BL21 (DE3) pLysS (Stratagene) grown in LB media or $^{15}\text{NH}_4\text{Cl}$ -supplemented (Isotec) minimal media. Bacteria were harvested by centrifugation after IPTG induction (1 mM) and lysed by sonication. The unlabeled and uniformly ^{15}N -labeled GST-fusion proteins were purified on a glutathione Sepharose 4B column (Amersham), cleaved with precision protease and concentrated in Millipore concentrators (Millipore). The proteins were further purified by FPLC and concentrated into 50mM Tris buffer (pH 6.0, 7.0 and 8.0), containing 100 mM NaCl and 10 mM dithiothreitol in 7% $^2\text{H}_2\text{O}/\text{H}_2\text{O}$.

Peptide synthesis

Peptides [p53K382me2 (377–386; TSRHKKme2LMFK), p53H380AK382me2 (377–386), p53K381AK382me2 (377–386), p53K382me2L383 (377–386), p53K370me2 (366–375) and p53K382me0 (377–386)] were synthesized by 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis using an Applied Biosystem 431A peptide synthesizer. *N*- α -Fmoc protected amino acids and methylated *N*- α -Fmoc protected amino acids were purchased from Nobabiochem and Bachem. The peptides were purified by reverse phase (RP)-HPLC on preparative C4 and C18 columns using a water-acetonitrile mixture and trifluoroacetic acid and characterized by matrix-assisted laser desorption/ionization time of fly mass spectrometry and RP-HPLC on analytical C18 column. The purity of the peptides was > 95%. The p53K372me2 (367–377) and H4K20me2 (15–23) peptides were synthesized by the UCD Peptide Core Facility.

X-ray crystallography

The 53BP1 tandem Tudor domain (1.0 mM) was incubated with p53-dimethylated lysine peptides [p53K370me2 (residues 366–375), p53K372me2 (residues 367–377), p53K382me2 (residues 377–386)], in a 1:2 molar ratio prior to crystallization. Crystals of the complexes were grown using the microbatch method under oil at 25°C by mixing 2 μl of the protein-peptide solution with 2 μl of precipitant solution containing 0.1 M HEPES-Na pH 7.0, 2% PEG 400 and 2.4 M ammonium sulphate. Crystals grew in a monoclinic space group C2 with one molecule per asymmetric unit for all complexes. The complete data sets were collected at 100 K on a “NOIR-1” MBC system detector at beamline 4.2.2 at the Advanced Light Source in Berkeley, CA. The data were processed with D*TREK19. The molecular replacement solution was generated using the program Phaser20 and the crystal structure of 53BP1 (PDB 2G3R) as a search model. The initial models were build using COOT21 and refined with the program Phenix22. Statistics are shown in Supplementary Table 1.

NMR spectroscopy

NMR experiments were performed at 298 K on a Varian INOVA 600 spectrometer equipped with a cryogenic probe using uniformly ^{15}N -labeled 53BP1 tandem Tudor domain. The spectra were processed with NMRPipe and analyzed using nmrDraw and in-house software programs. The binding was characterized by monitoring chemical shift changes in ^1H , ^{15}N HSQC spectra of 0.1–0.2 mM 53BP1 Tudor domain as p53K382me2, p53H380AK382me2, p53K381AK382me2, p53K382me2L383, p53K370me2, p53K372me2 and H4K20me2

peptides, or Kme2 amino acid (Bachem) were added stepwise. The dissociation constant (K_D) for the association with Kme2 was determined by a nonlinear least-squares analysis using the program Kaleidagraph and the equation:

$$\Delta\delta = \Delta\delta_{\max} \left(([L] + [P] + K_d) - \sqrt{([L] + [P] + K_d)^2 - 4[P][L]} \right) / 2[P]$$

where [L] is concentration of Kme2, [P] is concentration of 53BP1, $\Delta\delta$ is observed chemical shift change, and $\Delta\delta_{\max}$ is the difference in chemical shifts of the free and the Kme2-bound protein. NMR assignments were taken from²³.

Fluorescence Spectroscopy

A Fluoromax-3 spectrofluorometer was used to carry out tryptophan fluorescence measurements. Spectra were recorded at 25°C on samples containing 0.5 μ M 53BP1 tandem Tudor domain and progressively increasing concentrations of p53K382me2, p53H380AK382me2, p53K381AK382me2, p53K382me2L383, p53K370me2, p53K372me2 and H4K20me2 peptides. Samples were excited at 295 nm and emission spectra were recorded between 305 and 405 nm with a 0.5 nm step size and a 1 s integration time, averaged over three scans. K_d values were determined by a nonlinear least-squares analysis in Kaleidagraph using the equation:

$$\Delta I = \Delta I_{\max} \left(([L] + [P] + K_d) - \sqrt{([L] + [P] + K_d)^2 - 4[P][L]} \right) / 2[P]$$

where [L] is the concentration of the peptide, [P] is the concentration of the protein, ΔI is the observed change of signal intensity, and $\Delta I_{\max}$ is the difference in signal intensity of the free and fully bound states of the tandem Tudor domain. The K_d values were averaged over three separate experiments, with error calculated as the standard deviation between the runs.

HADDOCK Docking calculations

Modeling of the 53BP1-p53K382me2 complex was performed using the flexible docking program HADDOCK (www.haddock.org). The protein coordinates were taken from the crystal structure of the 53BP1-p53K382me2 complex. Coordinates for the p53 peptide (residues 378–386) were generated using the Insight II molecular modeling system. NMR chemical shift perturbation data were used to restrain the calculation. Specifically, residues V1492, A1493, W1495, N1498, Y1500, F1501, Y1502, S1503, G1504, D1521, Y1523, E1524, C1525, F1553, S1554, A1546, L1547, S1548, and D1550 of 53BP1 and residues His380, Lys381, and Lys382me2 of p53 were identified as active residues. Additionally, the methyl-lysine head-group was restrained in the hydrophobic cage using unambiguous distance restraints in accordance with the crystal structure coordinates. The remainder of the 9-residue peptide was allowed full flexibility throughout the docking calculation.

Immunoprecipitation and Western Immunoblotting

Endogenous p53 or ectopically expressed FLAG-tagged p53 were immunoprecipitated with agarose conjugated p53 or FLAG antibodies from whole cell extracts in cell lysis buffer (50 mM Tris-HCl, pH 7.4, 250 mM NaCl, 0.5 % TritonX-100, 10 % glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride, and protease inhibitors). After an overnight incubation at 4°C, the beads were washed three times with the same buffer and boiled in 2X Laemmli buffer. The IP was resolved on sodium dodecylsulfate-polyacrylamide gel and detected by

α p53K382me2 and α 53BP1 antibodies, or horseradish peroxidase- α p53 to avoid cross-reactivity with IgG heavy chain.

RT-PCR, I-SceI DSB ChIP and Repair Assays

ChIP assays were performed as described²⁴. Quantitative RT-PCR was performed on the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Gene expression was calculated normalized to GAPDH levels by the comparative Cycle threshold method. The I-SceI recognition site was stably incorporated into the genome of HT1080 cells by retroviral transduction. Myc-NLS-I-SceI enzyme²⁵ was transfected for 24 hours to induce DSBs. Occupancy was determined as the % of ChIP signal to input. Repair assays were performed on purified genomic DNA. The relative extent of I-SceI induced break resolution was determined by a PCR strategy in which repair is measured as the ratio of PCR product generated with primers spanning the I-SceI DSB site to PCR product at an adjacent DSB-independent site, with the ratio obtained in control cells (i.e. not transfected with I-SceI enzyme) defined as 100% repair.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

53BP1	p53 binding protein 1
p53K382me2	p53 dimethylated at Lys382
p53K372me2	p53 dimethylated at Lys372
p53K370me2	p53 dimethylated at Lys370
MBT	malignant brain tumor
PHD	plant homeodomain
DSB	double-strand break
ChIP	chromatin immunoprecipitation
HSQC	heteronuclear single quantum coherence

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Figure 1. Structure of the 53BP1 tandem Tudor domain in complex with a p53 peptide dimethylated at Lys382. a, 53BP1 is shown as a solid surface with the residues comprising the K382me2-binding cage colored orange and labeled. Lys382me2 of the p53K382me2 peptide is shown as a stick model with C, O and N atoms colored green, red and blue, respectively. b, Ribbon diagram of the structure. c, Close view of the K382me2-binding cage.

Figure 2. His380 and Lys381 of p53K382me2 are necessary for the interaction with 53BP1. a, Binding affinities of the 53BP1 tandem Tudor domain for indicated wild type and mutant p53 peptides, measured by tryptophan fluorescence or Isothermal Titration Calorimetry (^a). b, Representative binding curves used to determine the K_d values of the interactions by tryptophan fluorescence. The K_d values were averaged over three separate experiments, with error calculated as the standard deviation between the runs. c, The histogram shows normalized ¹H, ¹⁵N chemical shift changes in backbone amides of the 53BP1 tandem Tudor domain observed upon addition of the p53K382me2 peptide. d, Residues that exhibit significant p53K382me2-induced resonance perturbations [more than average plus one (orange) and two (red) standard deviations] in (a) are mapped onto the surface of 53BP1-p53K382me2 and labeled.

Figure 3.

The Kme2-binding mode is conserved. **a**, Architecture of p53: the N-terminal transactivation (TA) domain, the DNA-binding domain (DBD), and the carboxyl-terminal domain (CTD). The methylation sites are indicated by red circles. **b**, Alignment of the p53 and histone H3 and H4 sequences with methylated lysine residues highlighted in orange. The His and Lys/Arg residues of the HK/RKme2 motif are colored in blue and yellow, respectively. **c**, Overlay of the structures of the 53BP1 tandem Tudor domain in complex with p53K382me2 (red), p53K370me2 (green), and p53K372me2 (yellow). **d**, Superimposed K382me2-binding cages of the three structures, colored as in (c). **e**, Binding affinities of the 53BP1 tandem Tudor domain for indicated p53 and H4 peptides, measured by tryptophan fluorescence.

Figure 4.

Functional significance of 53BP1-p53K382me2 interaction. a, Schematic of I-SceI restriction site and primer pairs used in the study. b, Expression level of I-SceI restriction enzyme in control or I-SceI-transfected HT1080 cells. Tubulin and p53 are shown as controls for loading. c, Detection of endogenous p53 at a defined DSB. Occupancy of p53 and 53BP1 adjacent to I-SceI induced DSB were determined by real-time (RT)-PCR analysis of ChIP in HT1080 cells stably expressing I-SceI restriction site, $\pm$ transfection of myc-tagged NLS I-SceI enzyme²⁵. ChIP antibodies: DO-1 for p53, monoclonal BP13 for 53BP1, IgG as the negative control. d, p53K382me2 generation promotes DNA repair. Cells as in (c) were transfected with I-SceI enzyme $\pm$ SET8(Y334F) and $\pm$ p53 shRNA. Error bars in (c) and (d) indicate the s.e.m. from at least three experiments. e, A model of p53 recruitment and bridging with H4K20me2 at the DSB sites via oligomerization of 53BP1¹¹.