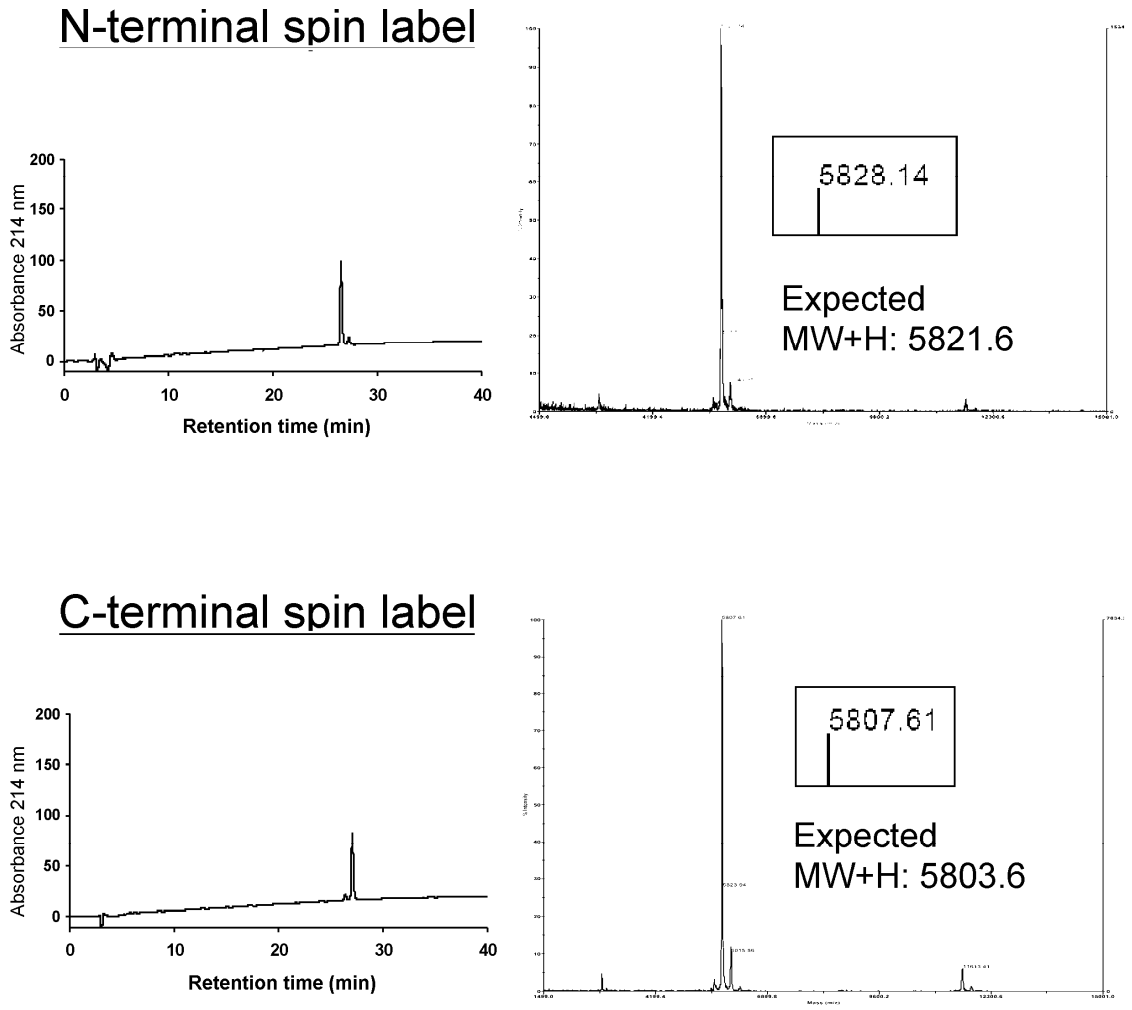
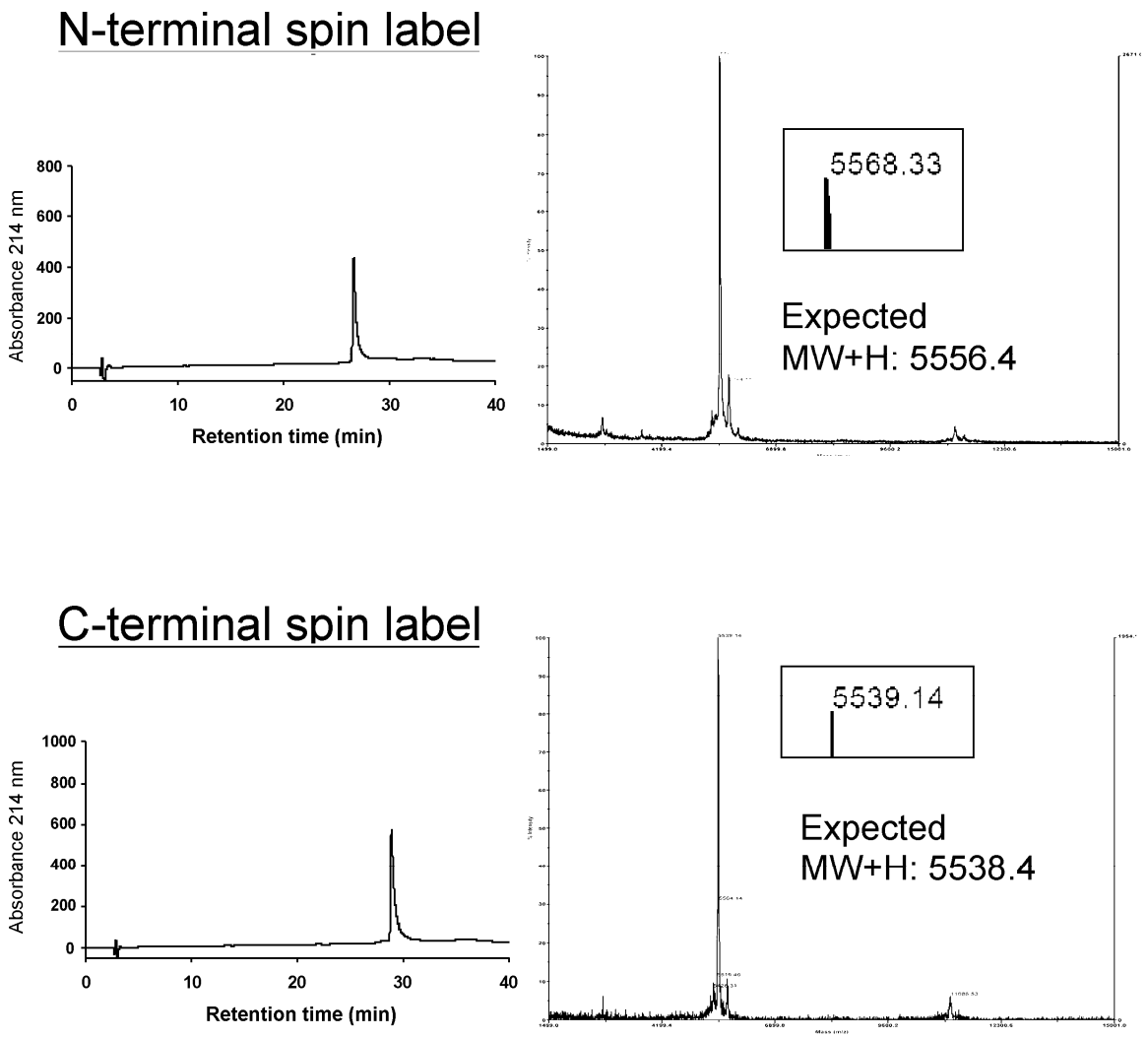
Supplementary Figure S1. Spin labeled p53(13-61)



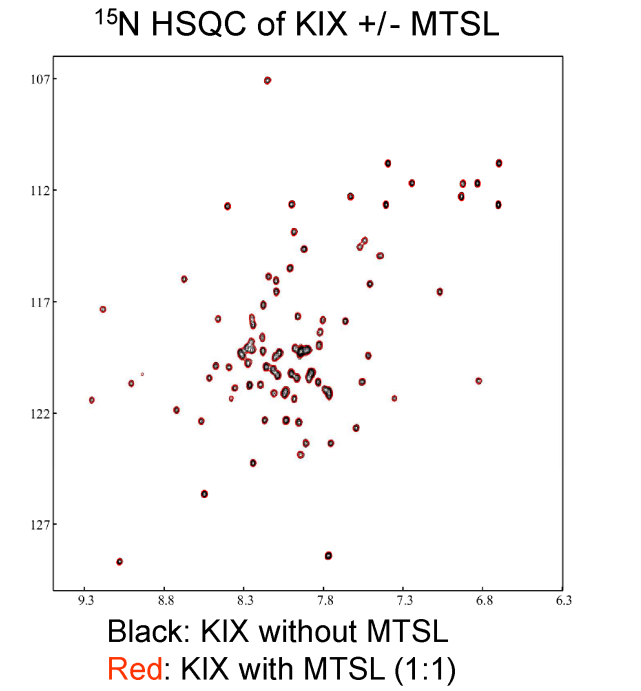
Supplementary Fig. S1. HPLC traces and mass spectra establishing the integrity of the spin labeled p53(13-61). (Top) Analytical reversed phase HPLC (left) and MALDI-TOF mass spectrum (right) of purified N-terminal spin labeled p53(13-61). **(Bottom)** Analytical reversed phase HPLC (left) and MALDI-TOF mass spectrum (right) of purified C-terminal spin labeled p53(13-61). The calculated mass (5821.6 for N-terminal spin labeled p53(13-61) and 5803.6 for C-terminal spin labeled p53(13-61)) matched the observed mass within error ranges.

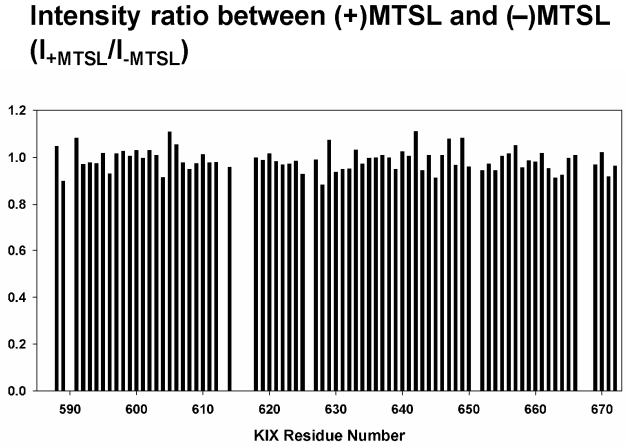
Supplementary Figure S2. Spin labeled p53(13-57)pT18pS20



Supplementary Fig. S2 HPLC traces and mass spectra establishing the integrity of the spin labeled p 53(13-57)pT18pS20. (Top) Analytical reversed phase HPLC (left) and MALDI-TOF mass spectrum (right) of purified N-terminal spin labeled p53(13-57)pT18pS20. **(Bottom)** Analytical reversed phase HPLC (left) and MALDI-TOF mass spectrum (right) of purified C-terminal spin labeled p53(13-57)pT18pS20. The calculated mass (5556.4 for N-terminal spin labeled p53(13-57)pT18pS20 and 5538.4 for C-terminal spin labeled p53(13-57)pT18pS20) matched the observed mass within error ranges.

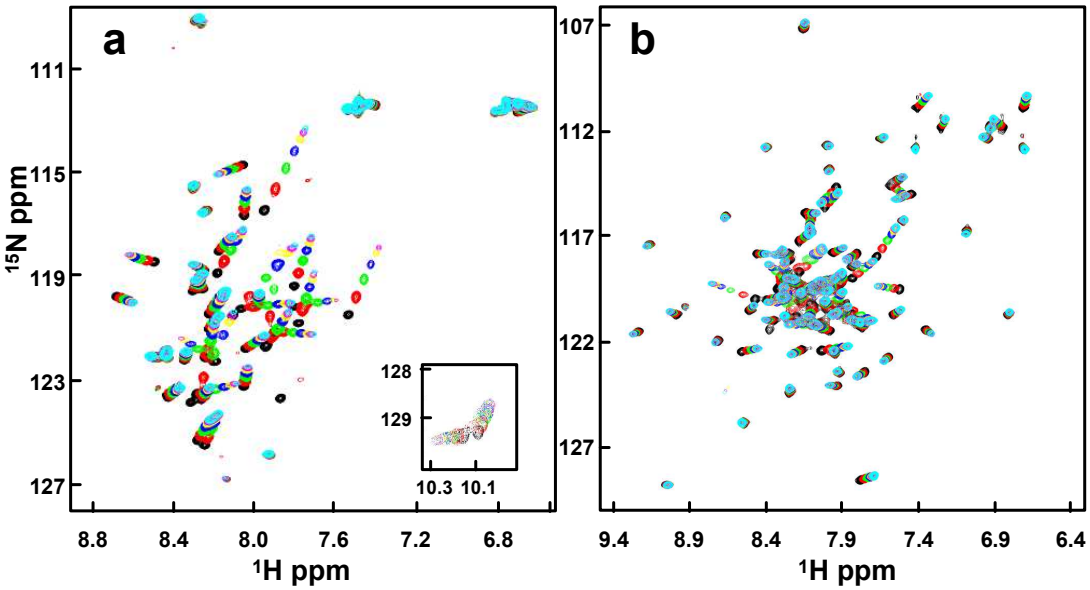
Supplementary Figure S3. Interaction of MTSL with KIX





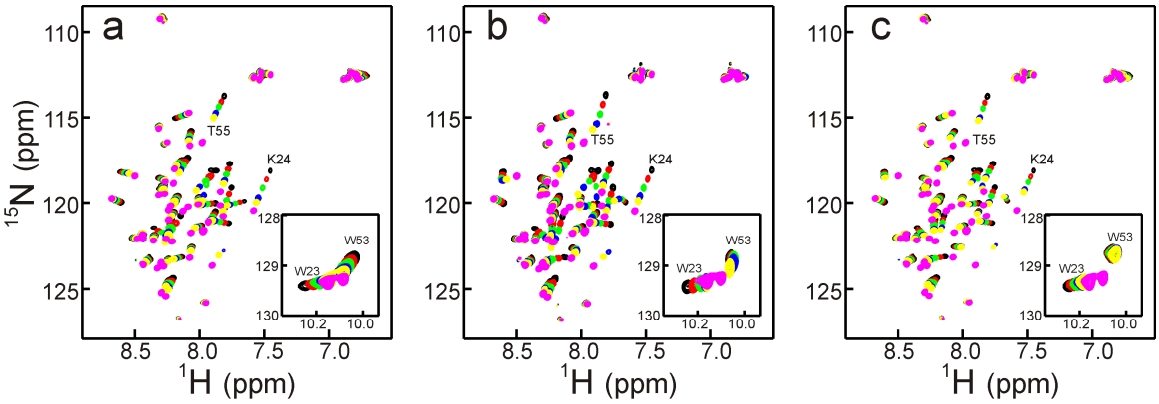
Supplementary Fig. S3 Control experiments showing that free MTSL spin label does not broaden KIX resonances. (Top) ^{1}H-^{15}N HSQC spectra of ^{15}N-KIX free (black) and in the presence of MTSL (1:1) (red). (Bottom) Histogram showing the experimental intensity ratios between with and without MTSL ($I = I_{+MTSL}/I_{-MTSL}$) for each residue with an adequately resolved cross peak in the ^{1}H-^{15}N HSQC spectrum of ^{15}N KIX.

Supplementary Figure S4. NMR titrations of KIX and p53



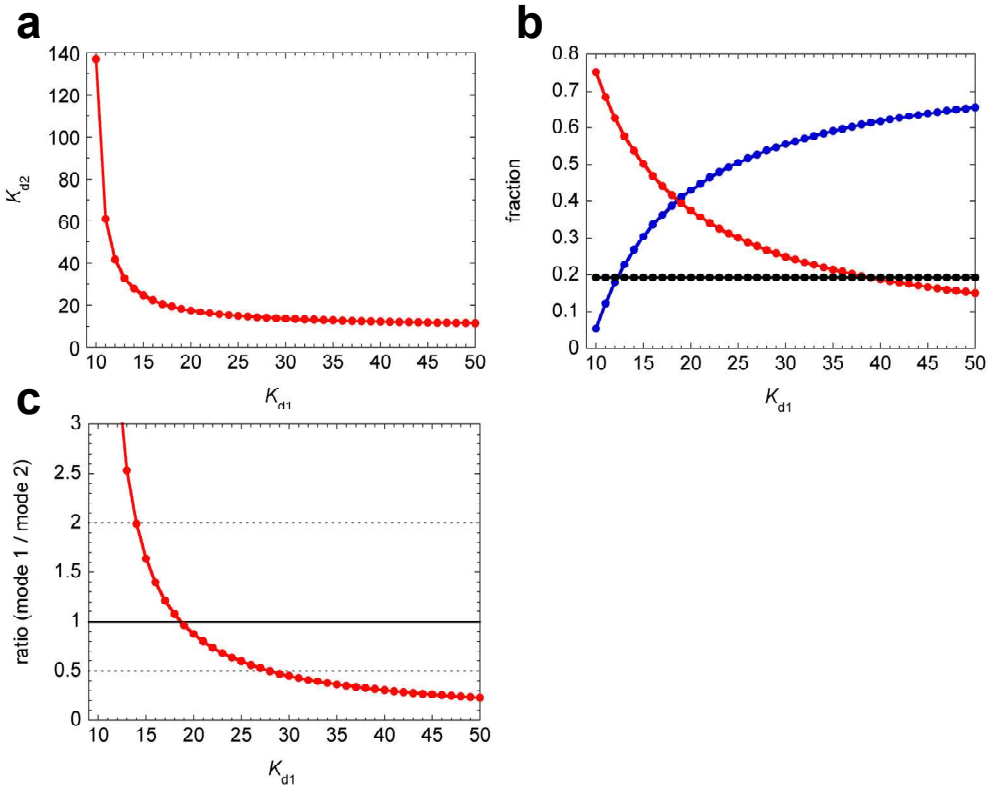
Supplementary Figure S4. ^{1}H-^{15}N HSQC spectra of p53(13-61) and KIX. a. Titration of ^{15}N-p53(13-61) with unlabeled KIX. Superposition of seven spectra at relative ratios of p53(13-61):KIX of 1:0 (*black*), 1:0.25 (*red*), 1:0.5 (*green*), 1:0.75 (*blue*), 1:1 (*yellow*), 1:1.25 (*magenta*) and 1:1.5 (*cyan*). Peaks from the two tryptophan side-chains are shown in the inset spectrum. b. Titration of ^{15}N-KIX with unlabeled p53(13-61). Superposition of seven spectra at relative ratios of KIX:p53(13-61) of 1:0 (*black*), 1:0.25 (*red*), 1:0.5 (*green*), 1:0.75 (*blue*), 1:1 (*yellow*), 1:1.25 (*magenta*) and 1:1.5 (*cyan*).

<u>Supplementary Figure S5</u>. Competition Experiments



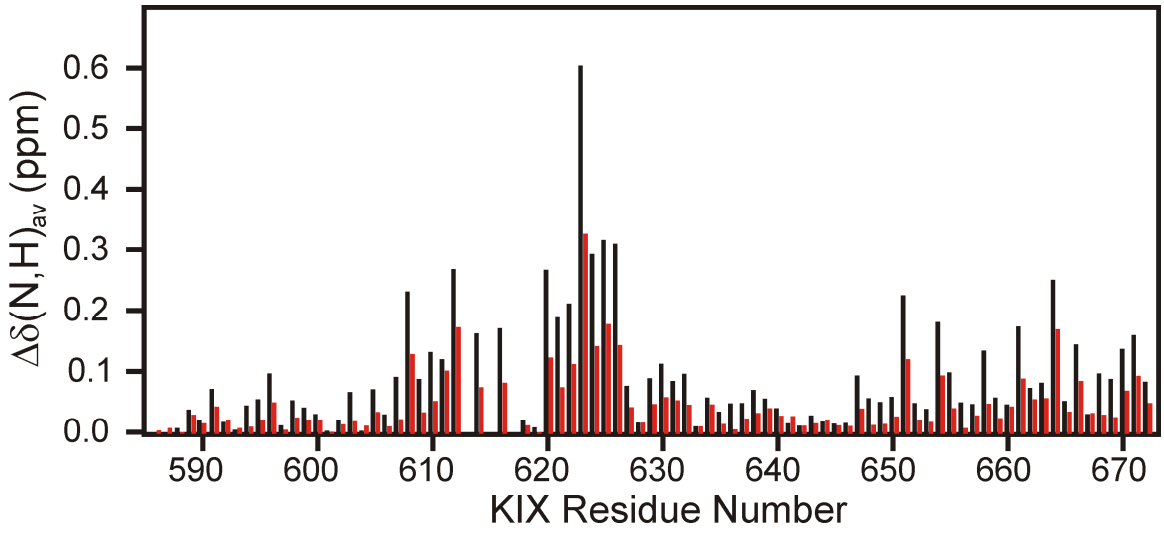
Supplementary Figure S5. Competition experiments between p53(13-61), MLL, and c-Myb or pKID. a. Titration of ^{15}N-p53(13-61) bound to KIX (1:1 ratio) with unlabeled MLL. Superposition of six spectra at relative ratios of p53(13-61):KIX:MLL of 1:1:0 (*black*), 1:1:0.25 (*red*), 1:1:0.5 (*green*), 1:1:1 (*blue*), 1:1:2 (*yellow*), and 1:0:0 (*magenta*). Peaks from two tryptophan side-chains are shown in the inset spectrum. b. Titration of ^{15}N-p53(13-61) bound to KIX (1:1 ratio) with unlabeled c-Myb. Superposition of six spectra at relative ratios of p53(13-61):KIX:c-Myb of 1:1:0 (*black*), 1:1:0.25 (*red*), 1:1:0.5 (*green*), 1:1:1 (*blue*), 1:1:2 (*yellow*), and 1:0:0 (*magenta*). Peaks from two tryptophan side-chains are shown in the inset spectrum. c. Titration of ^{15}N-p53(13-61) bound to KIX (1:1 ratio) with unlabeled pKID. Superposition of six spectra at relative ratios of p53(13-61):KIX: pKID of 1:1:0 (*black*), 1:1:0.25 (*red*), 1:1:0.5 (*green*), 1:1:1 (*blue*), 1:1:2 (*yellow*), and 1:0:0 (*magenta*). Peaks from two tryptophan side-chains are shown in the inset spectrum.

<u>Supplementary Figure S6</u>. Relationship between Kds



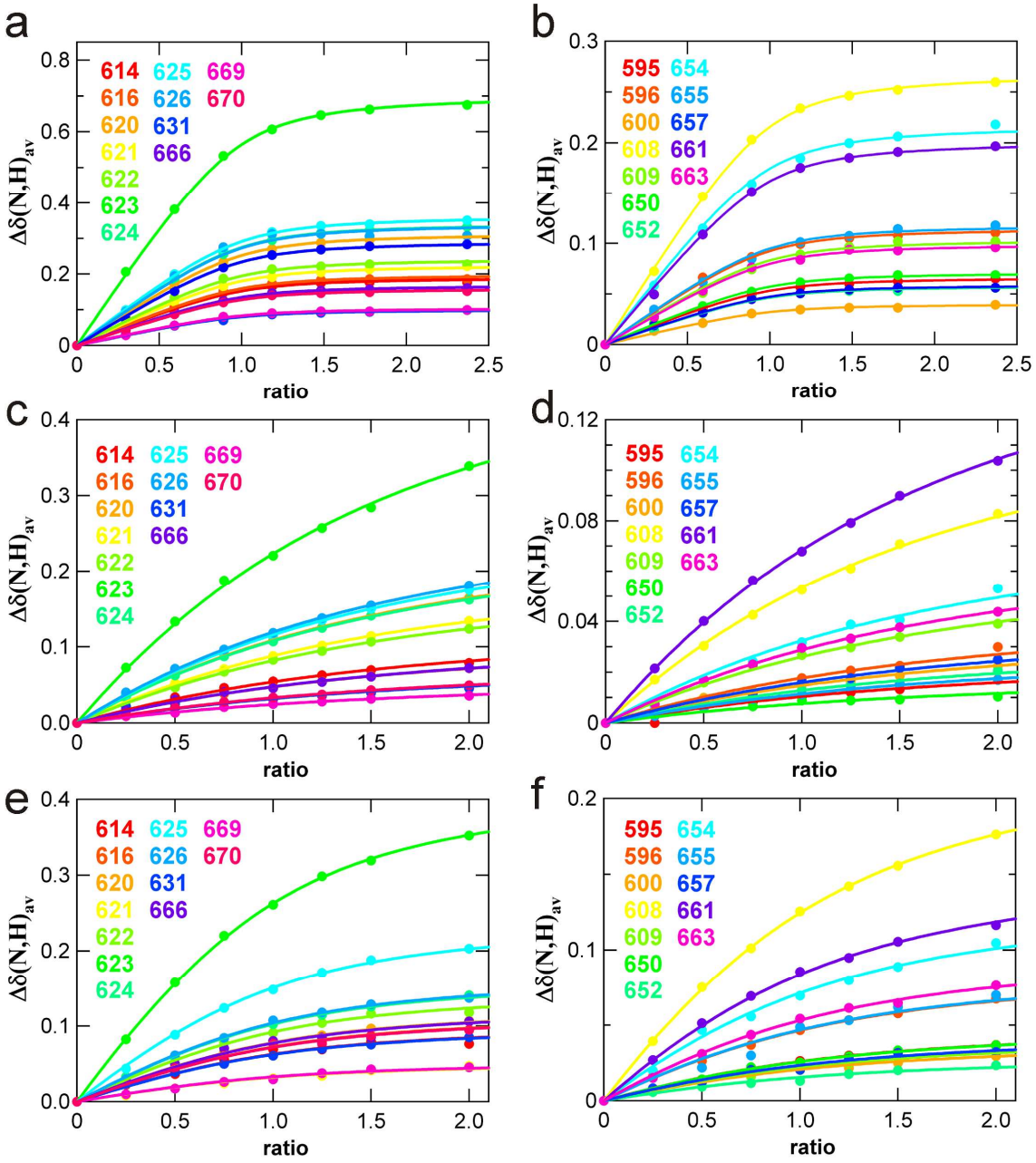
Supplementary Figure S6. a. Relationship between K_{d1} and K_{d2} under the restriction of $K_d = K_{d1}\ K_{d2}/(K_{d1}+K_{d2})$ = 9.32 μM. b. Changes in the population of the two binding modes depending on the K_{d1} value with the restriction of K_d = 9.32 μM. Red, blue, and black lines show the populations of binding mode 1, binding mode 2, and the free form of KIX. Concentrations of KIX and full-length p53 TAD were fixed at 200 μM. c. The ratio of the populations of the two binding modes.

Supplementary Figure S7. Chemical shift changes of KIX upon addition of p53



Supplementary Figure S7. Histogram showing weighted average chemical shift changes for KIX between free KIX and a 1:1 complex of p53(13-61) (black bar) and a 1:1 complex of a mixture of p53(14-28) and p53(38-61) (red).

Supplementary Figure S8. HSQC Titration Curves



Supplementary Figure S8. HSQC titration curves for titration of ^{15}N labeled KIX with (a, b) p53(13-61), (c,d) the AD1 peptide p53(14-28), and (e,f) the AD2 peptide p53(38-61). Values of $\Delta\delta$(N,H)$_{av}$ are plotted as filled circles and the continuous lines show the curves fitted globally or semi-globally to a one-site binding model. (a, b) ^{15}N KIX titrations with full-length p53 TAD fitted globally to the curves of all residues. The

curves corresponding to the MLL binding site (residues 614, 616, 620~626, 631, 666, 669, and 670) are shown in (a) and the curves corresponding to the pKID/c-Myb site (residues 595, 596, 600, 608, 609, 650, 652, 654, 655, 657, 661 and 663) in (b). (c) ^{15}N KIX titrations with AD1 fitted semi-globally for residues located in the MLL binding site. (d) ^{15}N KIX titrations with AD1 fitted semi-globally for residues located in the pKID/c-Myb binding site. (e) ^{15}N KIX titrations with AD2 fitted semi-globally for residues in the MLL binding site. (f) ^{15}N KIX titrations with AD2 fitted semi-globally for residues in the pKID/c-Myb binding site.