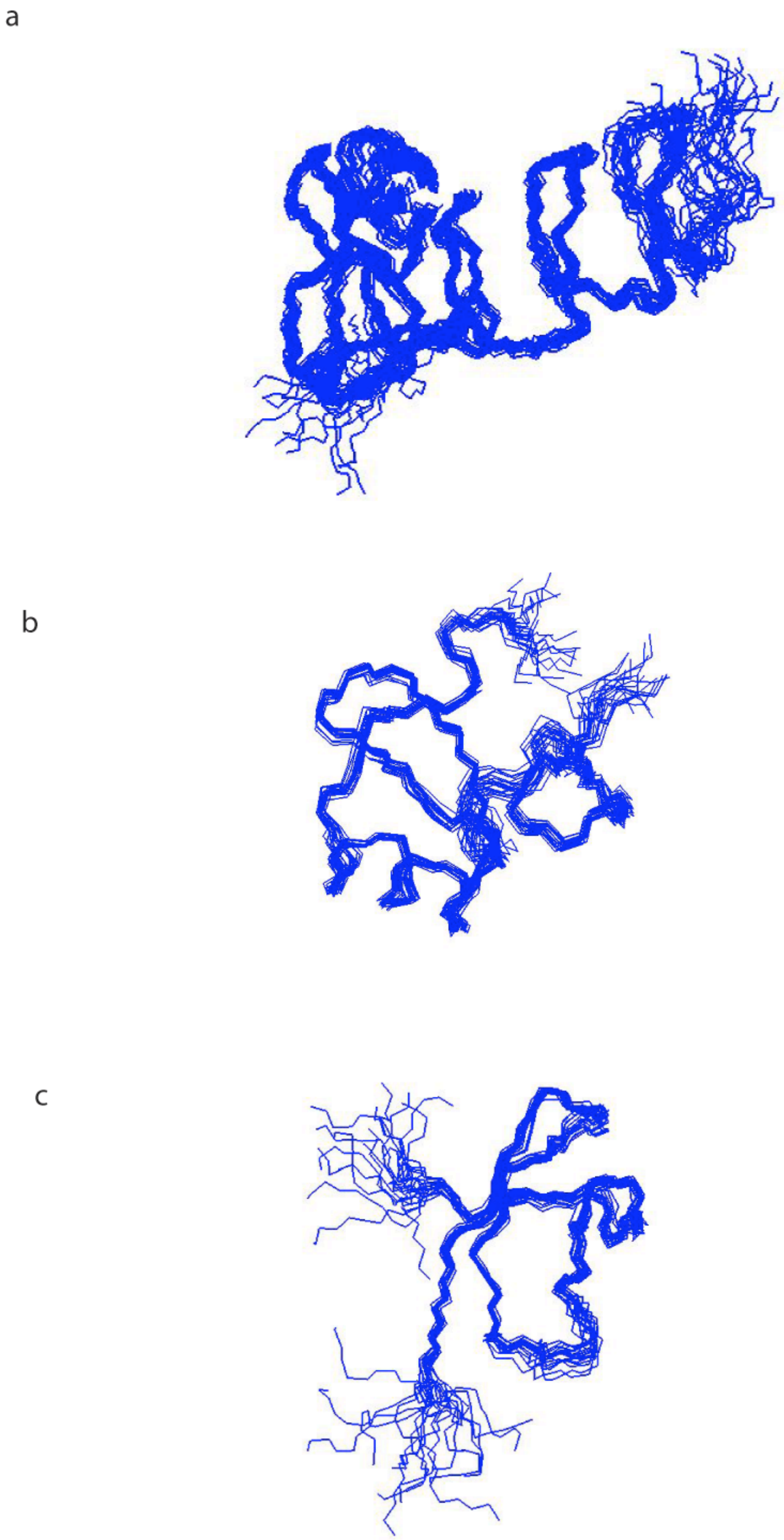
Molecular Basis of Pirh2-mediated p53 Ubiquitylation

Yi Sheng[1,3], Rob Laister[1,4], Alexander Lemak[1,4], Bin Wu[1], Elizabeth Tai[1], Shili Duan[1], Jonathan Lukin[1], Maria Sunnerhagen[2], Sampath Srisailam[1], Murthy Karra[1], Sam Benchimol[3] and Cheryl H. Arrowsmith[1]

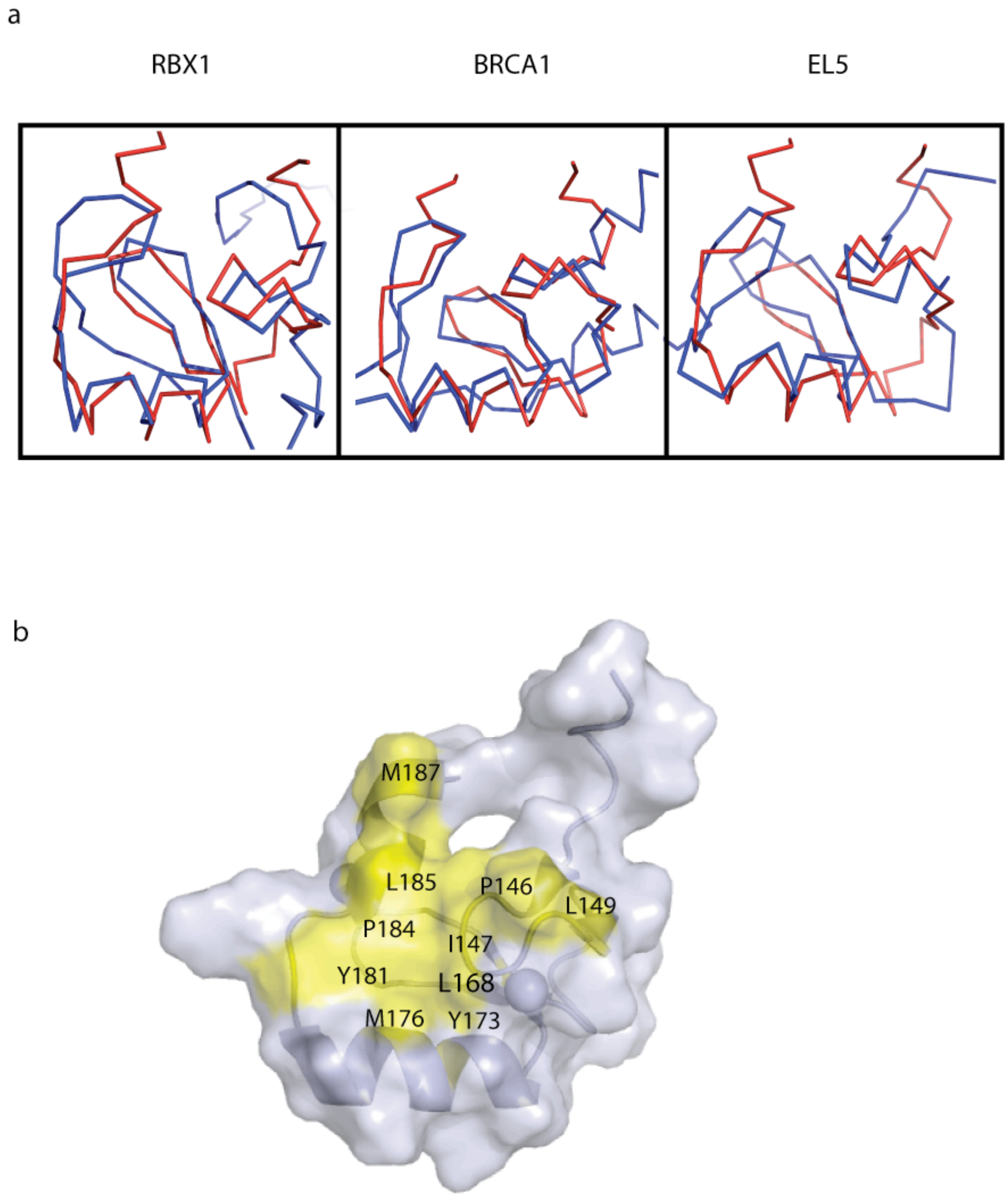
Supplementary Information

Supplementary figure 1



Supplementary figure 1. NMR-generated structure models of the three Pirh2 domains. The ensembles of top 20 NMR models from the final calculations are illustrated as wires for (a) the Pirh2 NTD (residues 15-137), (b) the Pirh2 RING (residues 142-187) and (c) the Pirh2 CTD (residues 215-255). For clarity, the disordered regions are not shown.

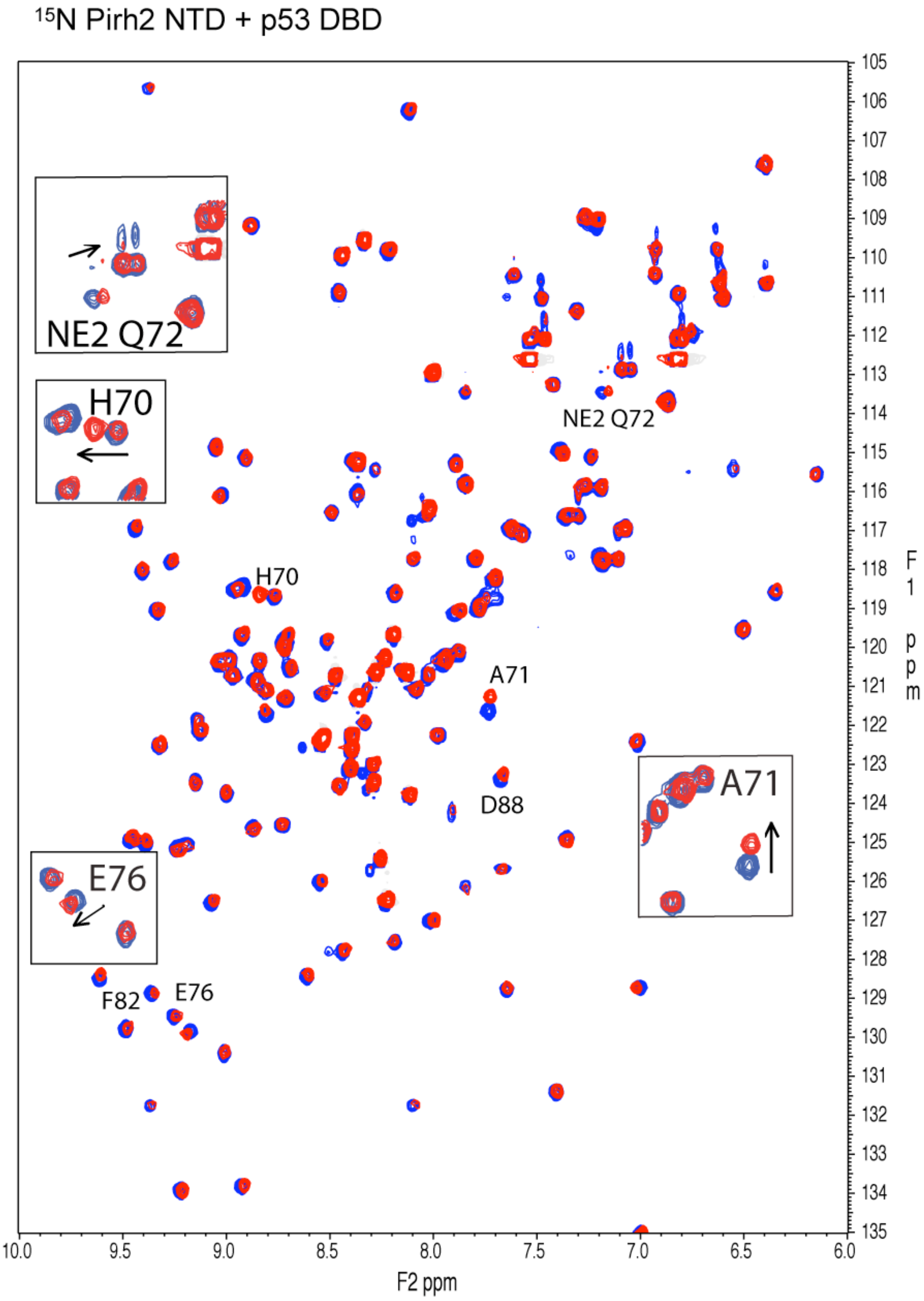
Supplementary figure 2



Supplementary figure 2. The solution structure of the Pirh2 RING domain. (a) Superposition of the Pirh2 RING domain (red) with the RING domains from RBX1 (PDB: 1LDJ-B), BRCA1 (PDB: 1JM7-A) and EL5 (PDB: 1IYM-A), colored in blue. (b)

Transparent surface representation of the Pirh2 RING domain with the surface hydrophobic residues colored in yellow.

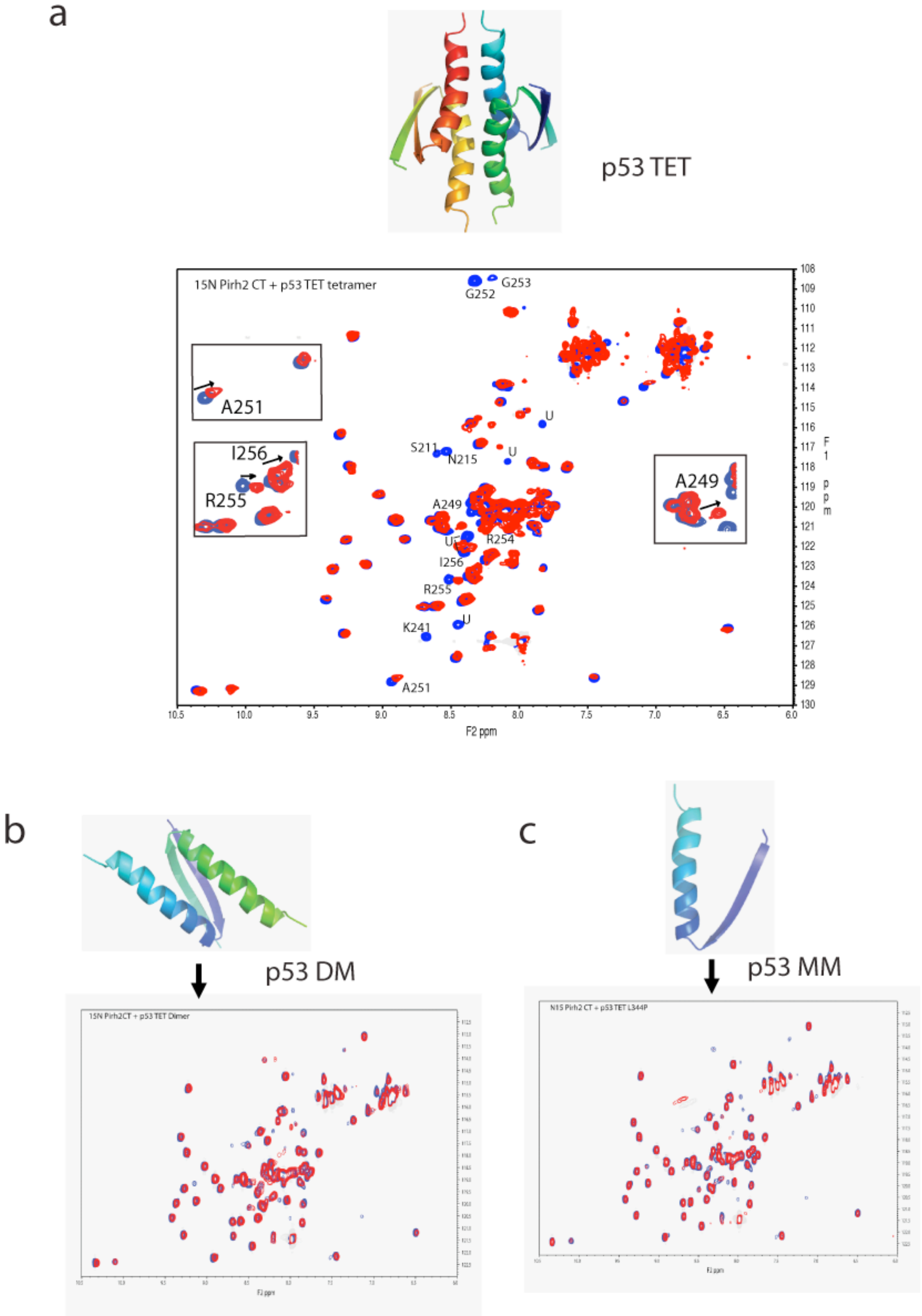
Supplementary figure 3



Supplementary figure 3. NMR titrations of the Pirh2 NTD with the p53 DBD. Overlay of the HSQC spectra for the ^{15}N -labeled Pirh2 NTD (blue) titrated with the p53 DBD (red).

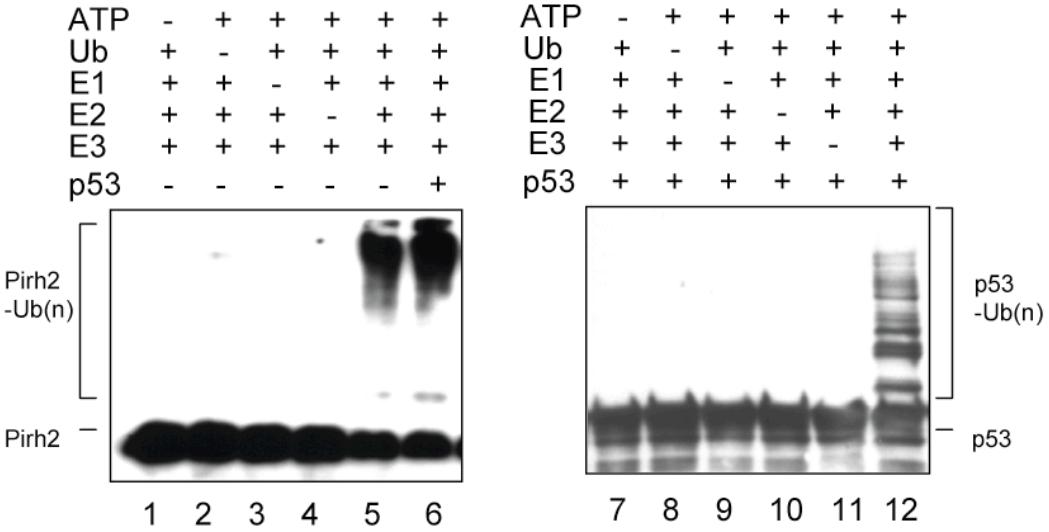
Residues H70, A71, Q72 and E76 are highlighted to demonstrate the peak shifts observed in the HSQC spectra of the Pirh2 NTD when it is bound by the p53 DBD.

Supplementary figure 4



Supplementary figure 4. NMR titrations of the Pirh2 CTD with the p53 TET domain proteins. (a) Overlay of the HSQC spectra for the ^{15}N -labeled Pirh2 CTD (blue) titrated with the p53 TET (red). Backbone resonances correspond to the residues A249, A251, R255 are shifted upon binding to the p53 TET (red). (b) and (c) Overlay of the HSQC spectra of the ^{15}N-labeled Pirh2 CTD (blue) titrated with p53 dimeric mutant (p53 DM) and p53 monomeric mutant (p53 MM) shown in red.

Supplementary figure 5

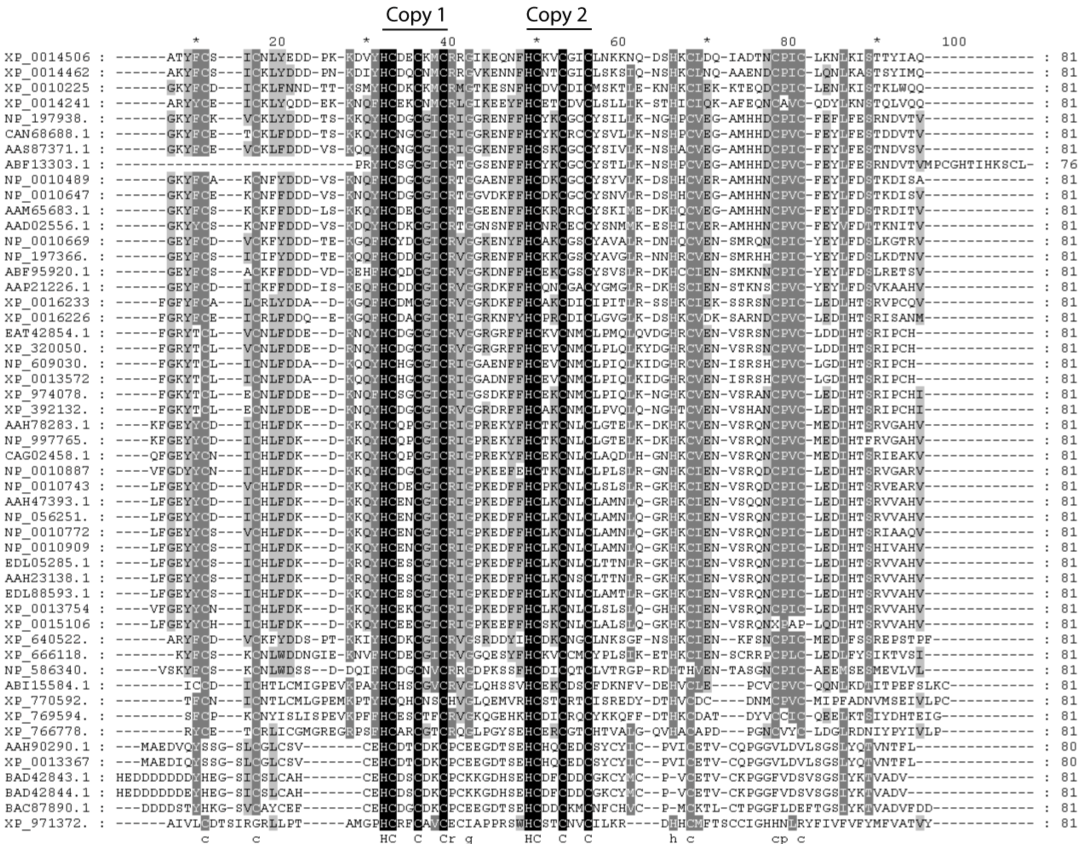


Supplementary figure 5. Autoubiquitylation and ubiquitylation of p53 by Pirh2 are dependent on the presence of ATP, Ub, E1, E2 and Pirh2. Full length Pirh2 protein was employed in an *in vitro* ubiquitylation assay in the presence and absence of ATP, E1, E2 (UBE2D2/UbcH5b), 6XHis-tagged ubiquitin and p53 as indicated. The reaction products were resolved on a 7.5% SDS-PAGE gel and detected by Western blot. Pirh2 autoubiquitylation (lanes 1-6, left panel) was detected by blotting with a monoclonal antibody against GST. Ubiquitylation of p53 was detected by blotting with a monoclonal antibody against p53 (PAB 1801) (lanes 7-12, right panel).

Supplementary figure 6

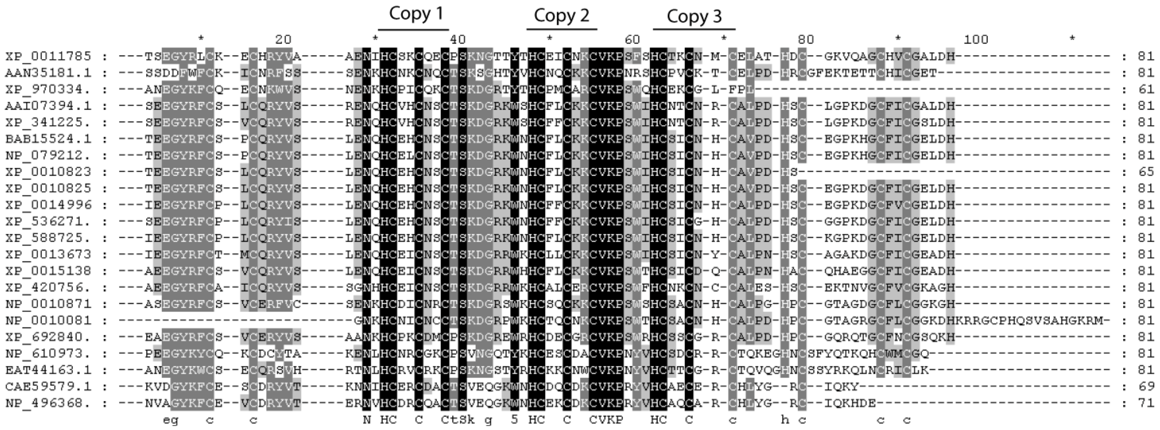
a

CXXCX(7-12)HCXXCXXCX(9)HCXXCXXCX(8)HXC



b

CXXCX(9)HCXXCXXCX(9)HCXXCXXCX(6)HCXXCXXCX(4-5)HXC



Supplementary figure 6. Sequence alignment of the zinc β-spiral motif in the Pirh2 NTD. (a) The protein sequences contain two copies of HCxxCxxC sequence element. (b) The sequences contain three copies of HCxxCxxC sequence element. The consensus sequence patterns for the zinc β-spiral motif are indicated.