SUPPLEMENTARY INFORMATION

Supplementary Figure 1. Alignment of PHD domain sequences: absolutely, moderately and weakly conserved residues are colored brown, green and yellow, respectively. The residues involved in H3K4Me$_3$ binding are marked by pink and brown triangles. Brown triangles specify aromatic residues necessary for the recognition of trimethylammonium group of Lys 4. The residues of ING1, mutated in human malignancies, are indicated by yellow bars. Every tenth residue of the ING2 PHD is marked with a black dot. Secondary structure of the ING2 PHD finger is shown at the bottom.

Supplementary Figure 2. Determination of the specificity of the ING2 PHD finger. Histograms display normalized ^{1}H,^{15}N chemical shift changes observed in the ^{15}N-labeled PHD finger (0.2 mM) upon addition of 1 mM indicated histone peptides. Sequences of the methylated histone H3 and H4 peptides are shown above the histograms.

Supplementary Figure 3. Substitution of the active site residues of the ING2 PHD finger for Ala does not disrupt the structure. 1 Dimensional NMR spectra of wild type and mutant PHD fingers recorded at pH 6.5, 20ºC.

Supplementary Methods

Mutagenesis. Single, double and triple mutations of full length ING2 and PHD finger of ING2 (residues 205-265) were generated using QuikChange (Stratagene). The sequences were confirmed by DNA sequencing.

Expression and Purification of Proteins. The wild type and mutant ING2 proteins were expressed in *E. coli* BL21(DE3) pLysS (Stratagene) grown in zinc-enriched LB media or $^{15}NH_4Cl$-supplemented (Isotec) M9-minimal media. Bacteria were harvested by centrifugation after IPTG induction and lysed using a French press. The GST-fusion proteins were purified on a glutathione Sepharose 4B column (Amersham), cleaved with PreScission protease (Amersham) and concentrated in Millipore concentrators (Millipore). The proteins were further purified by FPLC and concentrated into Tris or d_{11}-Tris buffers containing 150 mM NaCl, 10 mM d_{10}-dithiothreitol and 1 mM NaN_3 in 7% $^2H_2O/H_2O$, pH 6.5. All tested mutant proteins appear to maintain the structure (Supplementary Fig. 3).

NMR spectroscopy. NMR spectra were collected at 25ºC on Varian INOVA 600 and 500 MHz spectrometers using unlabeled and uniformly ^{15}N-labeled ING2 PHD finger. The histone binding was characterized by monitoring chemical shift changes in $^1H,^{15}N$ HSQC spectra of 0.2 mM wild type or mutant proteins as histone peptides (up to 5 mM) were added stepwise. The dissociation constants (K_Ds) were determined by a nonlinear least-squares analysis using Xmgr program and the equation: $\Delta\delta=\Delta\delta_{max}((([L]+[P]+K_D)-$ sqrt(sqr([L]+[P]+K_D)+(4*[P]*[L])))/(2*[P])), where [L] is concentration of the peptide, [P] is concentration of PHD, $\Delta\delta$ is observed chemical shift change, and $\Delta\delta_{max}$ is the difference in chemical shifts of the free and the ligand-bound protein. The synthetic histone peptides: NH_2-ARTK(X)QTARKSTG-COOH, NH_2-ARTKQTARK(X)STG-COOH and Ac-AKRHRK(X)VLR-COOH, were X=Me, Me_2 and Me_3) were synthesized at the Biophysics Core Facility of the University of Colorado Health Sciences Center.

Cell-Culture and Western analyses were carried out as described[1].

Fluorescence spectroscopy. Tryptophan fluorescence spectra were recorded at 25˚C on Fluoromax3 spectrofluorometer. The samples of 10 μM wild type and mutant PHD fingers containing progressively increased concentration (up to 5 mM) of histone peptides were excited at 295 nm. Emission spectra were recorded between 305 and 405 nm with a 0.5 nm step size and a 1 s integration time and averaged over 3 scans. The K_Ds were determined by a nonlinear least-squares analysis using the equation: $\Delta I=(\Delta I_{max}*L)/(K_D+L)$, where L is concentration of the histone peptide, ΔI is observed change of signal intensity, and ΔI_{max} is the difference in signal intensity of the free and bound states of the protein. The K_D value was averaged over three separate experiments.

References for supplementary information

1. Gozani, O. et al. The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. *Cell* **114**, 99-111 (2003).