Molecular Cell, Volume *33*

Supplemental Data

ING4 Mediates Crosstalk between Histone H3 K4 Trimethylation and H3 Acetylation to Attenuate Cellular Transformation

Tiffany Hung, Olivier Binda, Karen S. Champagne, Alex J. Kuo, Kyle Johnson, Howard Y. Chang, Matthew D. Simon, Tatiana G. Kutateladze, and Or Gozani

Supplemental Experimental Procedures

Crystallization and Data Collection

The ING4 PHD finger (1.0 mM) was combined with H3K4me3 peptide (residues 1-12) in a 1:1.5 molar ratio prior to crystallization. Initial crystals of the complex were grown using the sitting drop vapor diffusion method at 18^{o}C by mixing 1 ul of the protein-peptide solution with 1 ul of a well solution containing 90% 1.6 M sodium citrate tribasic dihydrate pH 6.5, and 10% of condition 6 from Hampton crystal screen 1 (0.2 M magnesium chloride hexahydrate, 0.1 M Tris HCL pH 8.5, and 30% (w/v) polyethylene glycol 4000). All crystals grew to ~ 0.05x0.05x0.3 mm^3 in a tetragonal space group ($P4_3$) with unit cell parameters of a=68.16 Å, b=68.16 Å, c=27.96 Å. There are 2 similar molecules of the ING4:peptide complex per asymmetric unit (AB and CD; only the AB molecule complex is discussed in the text for clarity), with an estimated solvent content of 42%. Crystals were flash cooled in liquid nitrogen, and X-ray data were collected at 100K on a "NOIR-1" MBC system detector at beamline 4.2.2 at the Advanced Light Source (ALS) in Berkeley, CA. A complete Zn MAD dataset to 1.8Å was collected for

peak, inflection and remote wavelengths. Data were processed with the DTREK. The coordinates have been deposited in the Protein Data Bank under accession number 2pnx.

ChIP and mRNA expression RT-PCR

The following primers were used: EXT1(5'-CCAATCAATGGCAAGACGAA-3', 5'-CACGCTGCCGAGAGGAAT-3'), EGLN1(5'-GGGCCTGTCCAGCACAAA3', 5'-CAATGGCCGGACGAAAGT-3'), SMC4L1 (5' CGCGGAGTTGACATCTGAAG -3', 5' CATCGTTCCCTGGCTGCTTA-3') with Taqman Power Sybr Green PCR master mix (Applied Biosystems). For quantification of gene expression, cells were treated with 400ng/ml of Doxorubicin for 4 hours or mock treated, and total RNA was collected using Rneasy Mini Kit (Qiagen). mRNA expression levels were determined using the Taqman One-Step RT-PCR Assay (Applied Biosystems) using the comparative cycle threshold (Ct) method and normalized to GAPDH. Fold enrichment with DNA damage was calculated as ΔCt (Dox/control).

Peak Finding and promoter occupancy profiling

To identify ING4 binding sites from the Nimblegen Whole Genome array data, we used the Nimblescan program according to the standard manufacturer's protocol. Firstbinding sites must have a minimum of four consecutive probes where the log2 ratio of ChIP to input signal is between 20-90% of the hypothetical maximum log2 ratio, (the mean log2ratio per array + 6 standard deviations). Next, the false discovery rate (FDR) for each peak, calculated by permutating the data within each chromosome, must be less than 0.2. The FDR for each peak represents the probability of finding a similar peak by

chance, estimated by searching for peaks through randomly permuted log-ratio data. We defined this set of Nimblescan targets as “preliminary target genes”. To search for high confidence targets for further validation, we applied a third cutoff, which required the average log2ratio of all the probes within the peak be >1.5, corresponding to a 2.83 enrichment of ChIP/Input. . To obtain the occupancy profile of ING4, all ING4+doxorubicin high confidence promoters (as listed in Supplementary Table 2) were aligned at their Transcriptional Start Sites, and the log2ratio value every 100 bp along the promoters were averaged across the 292 genes. This process was repeated for all four ChIP-chip data sets. For single promoter occupancy profiling, log2ratio values along the promoters were plotted for individual genes.

Supplementary Figure 1

Figure S1. Sequence alignment of ING4$_{PHD}$ with ING2$_{PHD}$. Asterisks indicate residues that have been previously been shown to be critical for the interaction with H3K4me3 for ING2.

Supplementary Figure 2

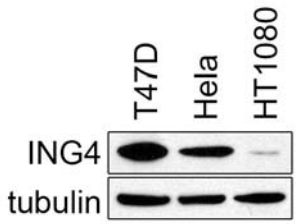


Figure S2. ING4 protein expression. Western blot analysis of ING4 protein levels in T47D, Hela, and HT1080 cell lysates.

Supplementary Figure 3

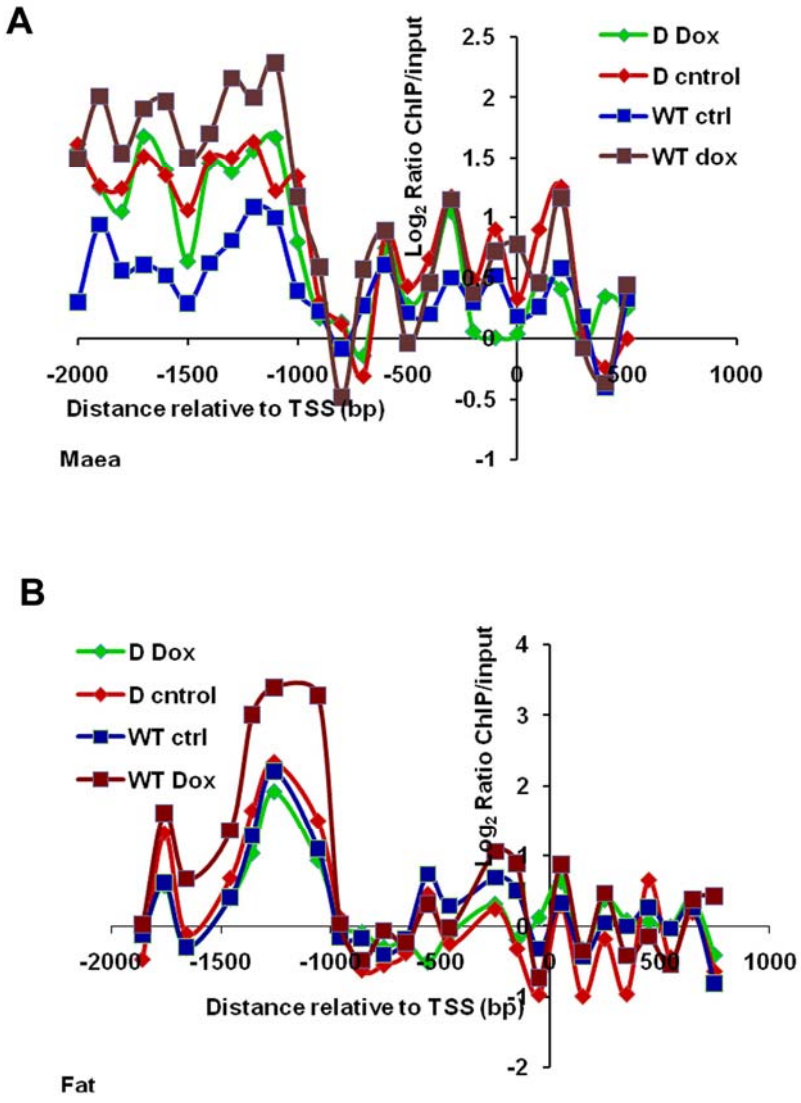
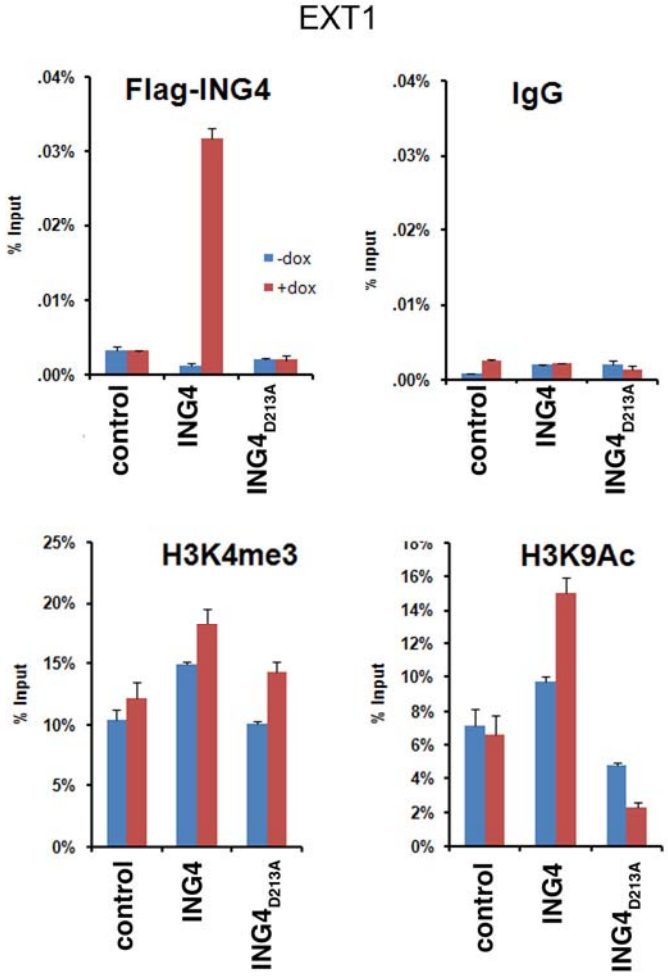


Figure S3. ING4 occupancy at two top promoters in the cell adhesion family.

Occupancy at the (A) *Maea* and (B) *Fat* promoters, as in figure 4b. Note the enrichment at 1000 bp upstream of the transcription start site.

Supplementary Figure 4

A



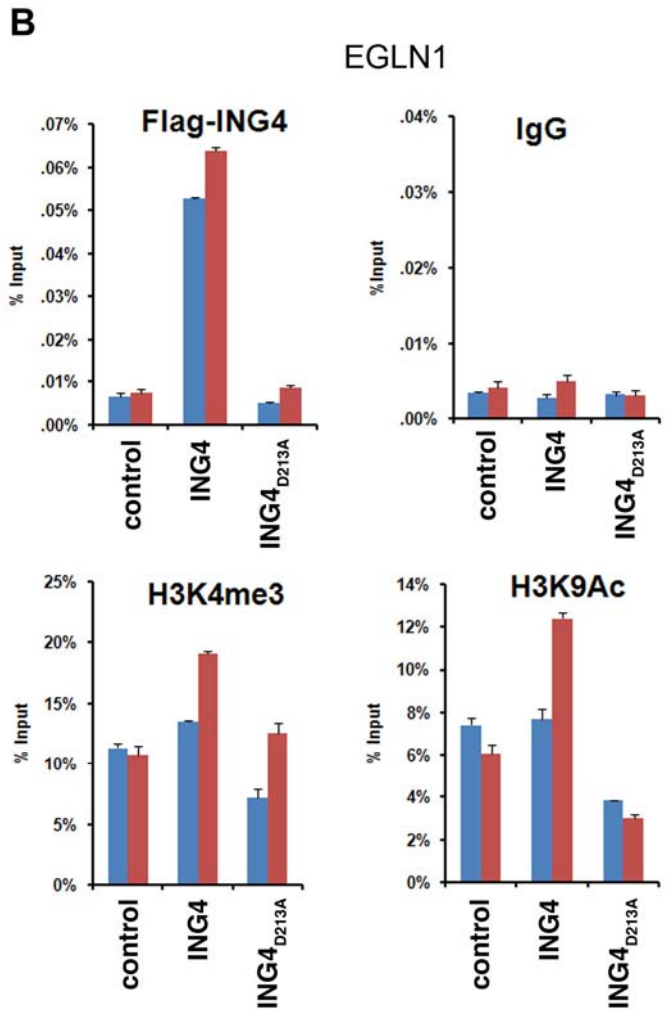


Figure S4. ING4 occupancy correlates with H3 acetylation at target promoters.

Realtime PCR of ChIP assays on the *Egln1* and *Ext1* promoters with the indicated antibodies and cell lines (as in Figure 4c). IgG is used as the negative control. Values = ChIP/input percent. Error bars indicate s.e.m.

Supplementary Figure 5

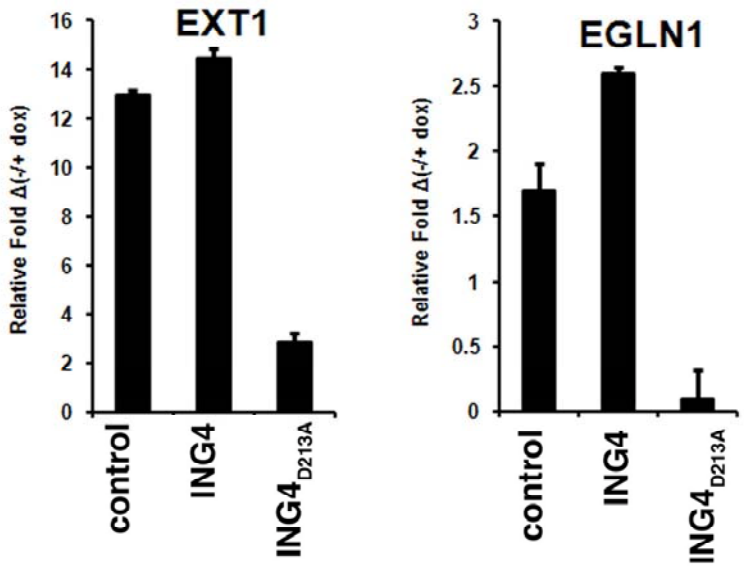


Figure S5. DNA damage increases transcription of ING4 target genes. Realtime PCR analysis of *Egln1* and *Ext1* transcript levels in control, ING4 and $ING4_{D213A}$ cells with DNA damage**.** Values shown represent the relative change of transcript with DNA damage as compared to untreated wild-type cells. Error bars indicate s.e.m.

Table S1. ING4 doxorubicin-sensitive target promoters in HT1080 cells.

High confidence target promoters as determined from ING4+doxorubicin ChIP-chip data from Nimblegen Whole Genome Promoter arrays. See methods for further information.

Table S2. ING4 target promoters in HT1080 cells.

High confidence target promoters as determined from ING4 ChIP-chip data.

Table S3. ING4$_{D213A}$ doxorubicin sensitive target promoters in HT1080 cells. High confidence target promoters as determined from ING4$_{D213A}$+ doxorubicin ChIP-chip data.

Table S4. ING4$_{D213A}$ target promoters in HT1080 cells.

High confidence target promoters as determined from ING4$_{D213A}$ ChIP-chip data.

Table S5. ING4 doxorubicin-sensitive targets in the cell adhesion family. All ING4+doxorubicin ChIP-chip-identified preliminary targets (all promoters and primary transcripts) were subjected to functional annotation clustering (http://david.abcc.ncifcrf.gov/) as in Krig et al., 2007. The most enriched gene set consisted of 150 genes associated with cell adhesion (11.8 fold enriched over the *homo sapiens* gene set, p=2.1E-2). Enrichment for cell-adhesion genes was not seen in the ING4D213A, ING4D213A+doxorubicin, or ING4 ChIP target genes.

1. Krig SR, Jin VX, Bieda MC, O'Geen H, Yaswen P, Green R, Farnham PJ, Identification of Genes Directly Regulated by the Oncogene ZNF217 Using Chromatin Immunoprecipitation (ChIP)-Chip Assays J. Biol. Chem. 282:9703-9712, 2007