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Supplemental Data

CDYL Bridges REST and Histone Methyltransferases for Gene Repression and Suppression of Cellular Transformation

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Supplemental Experimental Procedures

Recombinant Protein Purification

Human CDYL isoform 2 was expressed in E. coli as a GST-fusion using pGEX4T1 (Amersham Biosciences), and purified with GSH-agarose beads (Sigma). Cell lysis and washing buffer contained 500 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl pH 8, 1 mM DTT, 1 mM PMSF and Complete Protease Inhibitor Cocktail (Roche). N-terminal Flag-tagged human G9a and EuHMT1 cDNAs (a gift from Yoshihiro Nakatani), were expressed in baculovirus transduced SF9 cells using the Bac-to-Bac expression system (Invitrogen), purified using anti-Flag M2 Agarose beads and eluted with Flag peptide. Cell lysis, bead washing and peptide elution buffer contained 300 mM NaCl, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 0.2% Triton X-100, 1 mM DTT, 1 mM PMSF and Complete Protease Inhibitor Cocktail (Roche). HA-tagged human REST cDNA was and expressed in SF9 cells using the Bac-N-Blue expression system (Invitrogen). Protein was purified using anti-HA agarose (Santa Cruz Biotechnology) as per Flag-G9a/EMT protocol, and eluted with HA peptide. Purified recombinant His6-tagged human CoREST was a gift from Jian Oyang and Grace Gill.

ChIP PCR Primers

TrkC RE1 Forward (CTTACTCAACTCCCAGCCTCC)

TrkC RE1 Reverse (AGCTGTTCATAGTTCACTGCCC)

NPTXR RE1 Forward (CTGCTGCCTTGTGCCCTTCA)

NPTXR RE1 Reverse (CAGAACAGGGCTGCCCAATG)

GAPDH Promoter Forward (ACCACAGTCCATCCCATCAC)

GAPDH Promoter Reverse (TCCACCACCCTGTTGCTGTA).

Supplemental Figures and Table

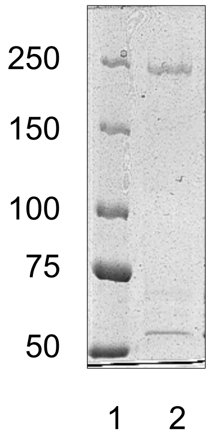

Figure S1. Purified Recombinant Flag-REST

Shown is a coomassie blue-stained SDS-PAGE gel of Flag-REST purified from Sf9 cells (lane 2). Molecular weight markers are in lane 1.

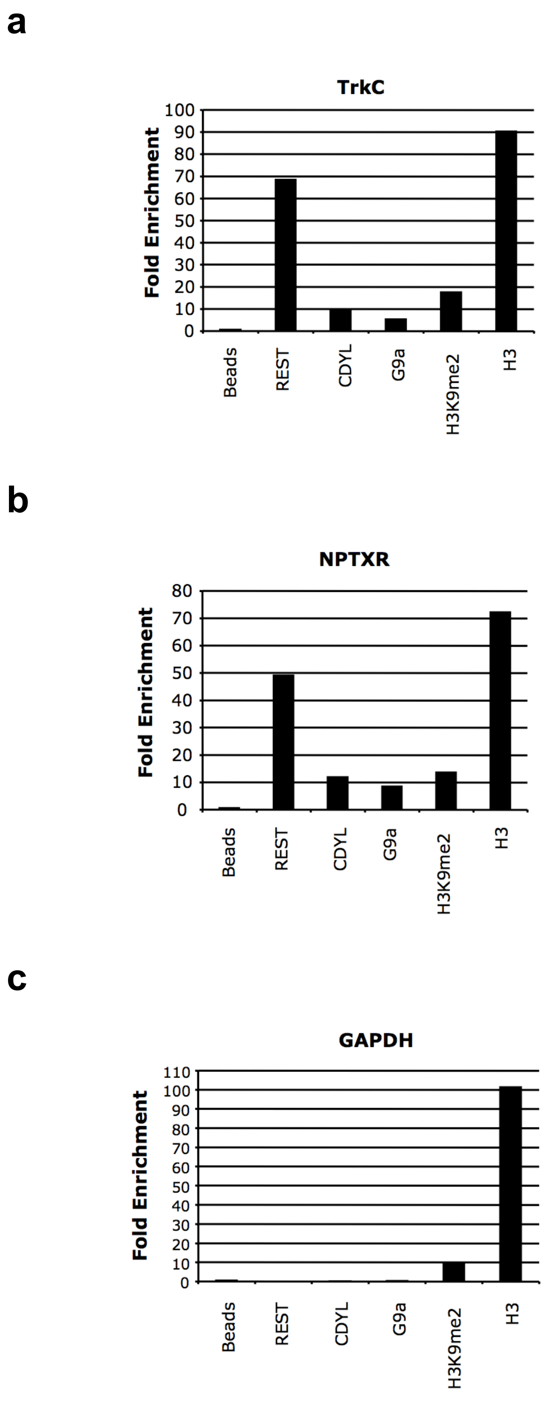

Figure S2. Enrichment of REST, CDYL, G9a and H3K9me2 at TrkC and NPTXR RE1 Site-Proximal Chromatin

Quantitative PCR was used to analyze the occupancy of TrkC and NPTXR RE1 site-proximal chromatin by REST, CDYL, G9a, H3K9me2 and H3. GAPDH promoter was used as a control. **(a, b)** TrkC RE1 site-proximal chromatin is enriched in REST, CDYL, G9a, H3K9me2 and H3, relative to beads alone. **(c)** GAPDH promoter showed no enrichment in REST, CDYL and G9a, while moderate enrichment of H3K9me2 and strong enrichment of H3 were observed.

Samples are expressed as fold-enrichment, relative to background PCR amplification from the beads alone control ChIP. Assay was repeated three times with similar results.

Table S1. Summary of Mass Spectrometry Analysis of Proteins Associated with Tandem-Affinity-Purified CDYL

[a] SDS-PAGE was used to resolve CDYL-associated proteins. Proteins eluted from gel slices were then trypsin-digested and fragments analyzed by MS-MS.

[b] Ions score is -10*Log(P), where P is the probability that the observed match is a random event as indicated by using MASCOT software (Matrixscience, London, UK). An individual Sample Ions Score greater than the Threshold Positive Ions Score for that sample indicates identity or extensive protein homology ($p<0.05$). In these cases, sample identity is confidently assigned.

Identified Protein	**Accession (gi)**	**Number of Peptides Identified [a]**	**Threshold Positive Ions Score [b]**	**Sample Ions Score [b]**	**Confident Assignment of Identity (p<0.05) [b]**
BAF200/ARID-2/Jumonji	42659931	1	40	42	Yes
hAM/MCAF/ATF-7 interacting protein	29611342	11	42	273	Yes
Polybromo-1	93102371	9	42	127	Yes
Brg1/SNF2-like 2	1711406	3	42	36	No
REST	15559211	22	42	475	Yes
SETDB1/ESET	25091210	5	42	69	Yes
KIAA1221	6330604	7	42	112	Yes
ZNF644	45946796	5	42	100	Yes
G9a iosform a	18373637	36	42	1015	Yes
EHMT1/EuHMTase1	40217808	20	42	539	Yes
BAF170	15341763	1	42	31	No
G9a isoform b	18426879	24	42	785	Yes
WIZ 135 Kda isoform	51474998	7	42	119	Yes
WIZ 125 Kda isoform	42661989	25	42	616	Yes
WIZ 115 kDa isoform	42661989	23	42	559	Yes
MI-ER2	45267839	8	44	193	Yes
lamin A/C	27436948	3	44	95	Yes
MI-ER1	21702659	22	41	531	Yes
CDYL	38494365	25	55	630	Yes
HDAC1	13128860	7	42	120	Yes
HDAC2	68068066	16	42	279	Yes
SNF5/INI1	3326993	1	44	65	Yes
CDYL, isoform c	25777621	5	44	126	Yes