SUPPLEMENTARY INFORMATION

Functional proteomic analysis of repressive histone methyltransferase complexes PRC2 and G9A reveals ZNF518B as a G9A regulator

Verena K. Maier¹, Caitlin M. Feeney², Jordan E. Taylor², Amanda L. Creech², Jana W. Qiao², Attila Szanto¹, Partha P. Das³, Nicholas Chevrier⁴, Catherine Cifuentes-Rojas¹, Stuart H. Orkin³, Steven A. Carr², Jacob D. Jaffe², Philipp Mertins²* and Jeannie T. Lee¹*

SUPPLEMENTARY METHODS

Western blotting

8 µg of nuclear extracts used as input for SILAC affinity proteomics experiments were separated by 4-12% SDS-PAGE and transferred to nitrocellulose membranes. Proteins were detected with α-Ezh2 antibody (BD Transduction Laboratories, 612667), α-Ezh1 antibody (abcam, ab13633), α-Suz12 antibody (Cell Signaling Technologies, D39F6), α-G9A antibody (Perseus Proteomics, PP-A8620A-00), M2 α-FLAG antibody (Sigma-Aldrich, F7425) or α-actin antibody (Sigma-Aldrich, 2066) and HRP conjugated secondary antibodies (Promega), and were visualized by Western Lightning Plus ECL (PerkinElmer). Biotin-tagged proteins were detected using HRP-coupled streptavidin.

Affinity purifications in the presence of RNase

Purifications with RNase were conducted in parallel with no RNase samples, but 200 µg RNase A (Invitrogen) and 200 units RNase T1 (Ambion) were added to the samples along with TURBO DNAse before the 25°C incubation step. Since no RNA-dependent interactors were identified, data is not shown in the main text, but can be found in the raw data table.
SUPPLEMENTARY TABLE LEGENDS

Table S1: Lists of all identified EZH2, EZH1, SUZ12 and G9A interaction partners in mouse ESCs and their stoichiometries with respect to the bait protein

Table S2: Data table of affinity proteomics mass spectrometry experiments

Table S3: List of lentiviral shRNA constructs, corresponding knock-down efficiencies in mouse ESCs and primer sequences used for measuring expression levels of target proteins

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Tagged transgenes were expressed at physiological or subphysiological levels in mESCs. (a) Western blots of nuclear extracts of Ezh2<sup>-/-</sup> mESCs or J1 mESCs expressing FLAGbio-tagged EZH2, EZH1 or SUZ12 probed with α-EZH2-, α-EZH1-, or α-SUZ12-antibody and of F1-2-1 mESCs expressing G9A-3xFLAG probed with α-G9A- or α-FLAG-antibody as indicated. ACTIN served as a loading control. (b) Western blot membrane with nuclear extracts from mESCs expressing FLAGbio-EZH2, -EZH1 or -SUZ12 probed with streptavidin-coupled HRP.

Supplementary Figure 2: SILAC affinity proteomics experiments are very reproducible. Bait pull-down to negative control protein ratios measured in individual replicates of affinity purifications with EZH2, EZH1, SUZ12 and G9A are plotted against each other (log<sub>2</sub> scale; below diagonal). Pearson correlation coefficients for pairwise comparisons are displayed above the diagonal. Frequency distribution histograms of the log<sub>2</sub> SILAC ratios are displayed on the diagonal boxes.
Supplementary Figure 3: Residual mRNA expression after shRNA knock-down in J1 mESCs measured by qPCR.

Supplementary Figure 4: Effects of individual knock-downs of members of PRC2, the G9A-complex and selected interaction partners on 42 histone H3 posttranslational modification signatures were measured by global chromatin profiling. Heatmap displaying relative changes of tail modifications caused by individual knock-down with up to three different shRNAs directed against genes of interest as indicated. Empty control lentivirus and shRNA against RFP served as negative controls.
Supplementary Figure 1

(a) 


α-Ezh2  α-Ezh1  α-Suz12  α-G9a  α-flag

α-actin

(b) 

WT  Ezh2-/-+flagbio-Ezh2  WT  flagbio-Ezh1  WT  flagbio-Suz12

strept-HRP  α-actin
Supplementary Figure 2
Supplementary Figure 3

Residual expression after knock-down