SUPPLEMENTARY INFORMATION

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

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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

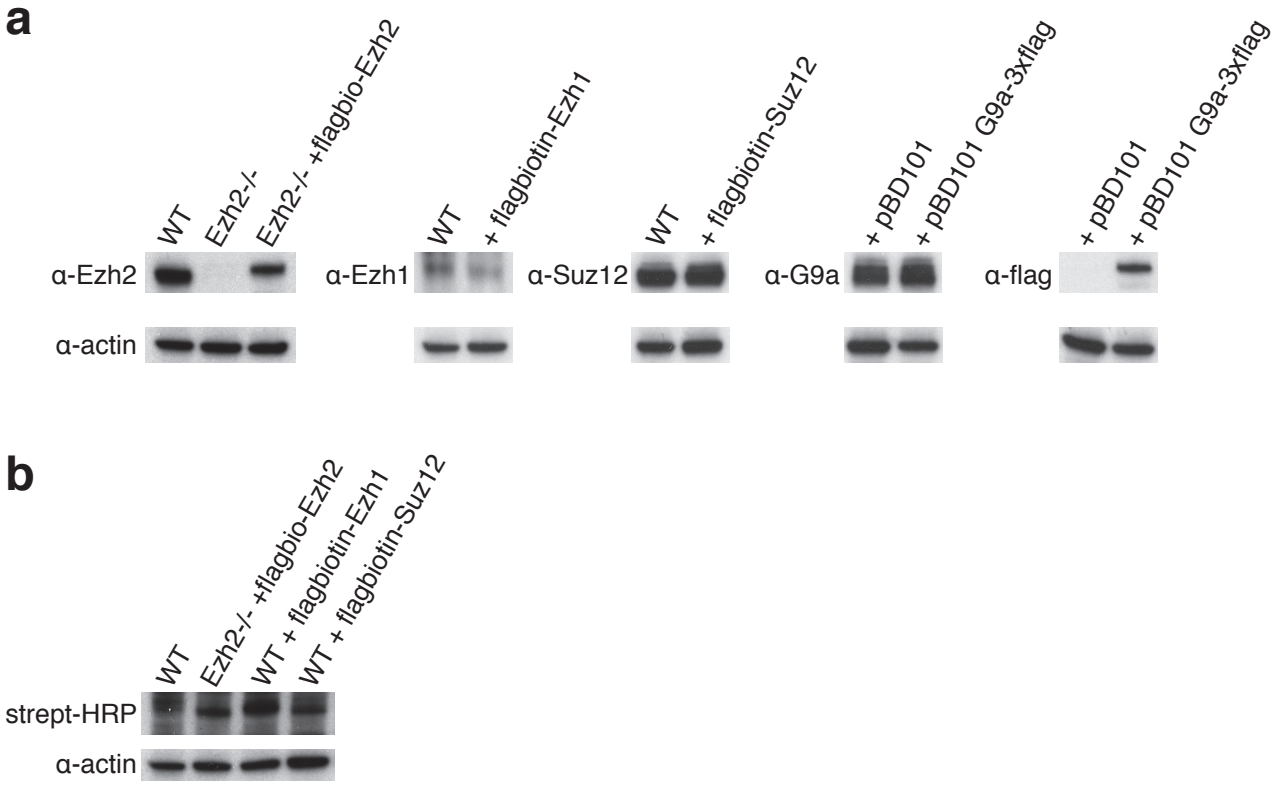
Supplementary Figure 1: Tagged transgenes were expressed at physiological or subphysiological levels in mESCs. (*a*) Western blots of nuclear extracts of *Ezh2*$^{-/-}$ mESCs or J1 mESCs expressing FLAGbio-tagged EZH2, EZH1 or SUZ12 probed with α-EZH2-, α-EZH1-, or α-SUZ12-antibody and of F1-2-1 mESCs 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_2 scale; below diagonal). Pearson correlation coefficients for pairwise comparisons are displayed above the diagonal. Frequency distribution histograms of the log_2 SILAC ratios are displayed on the diagonal boxes.

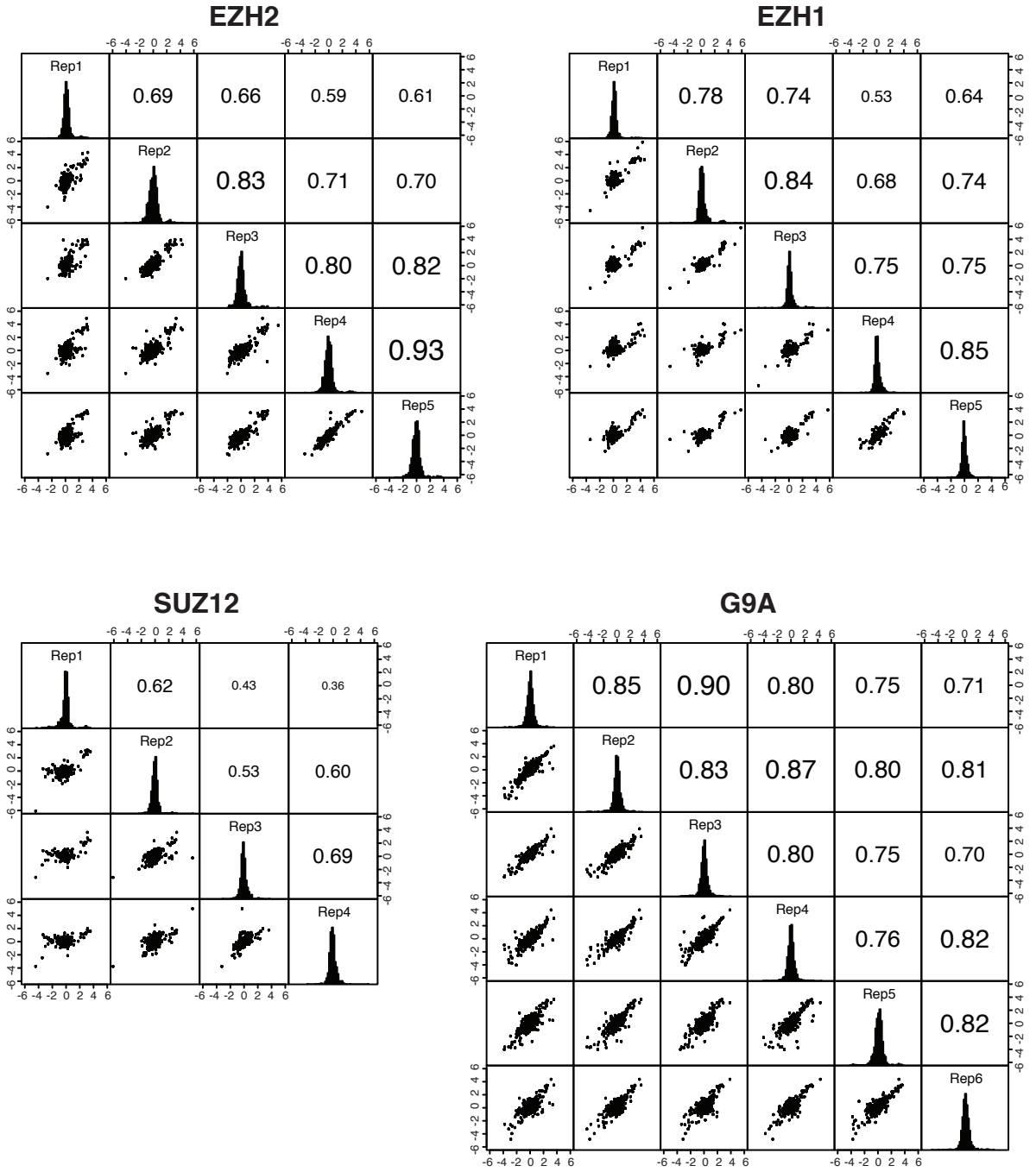
Supplementary Figure 3: Residual mRNA expression after shRNA knock-down in J1 mESCs measured by qPCR.

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.

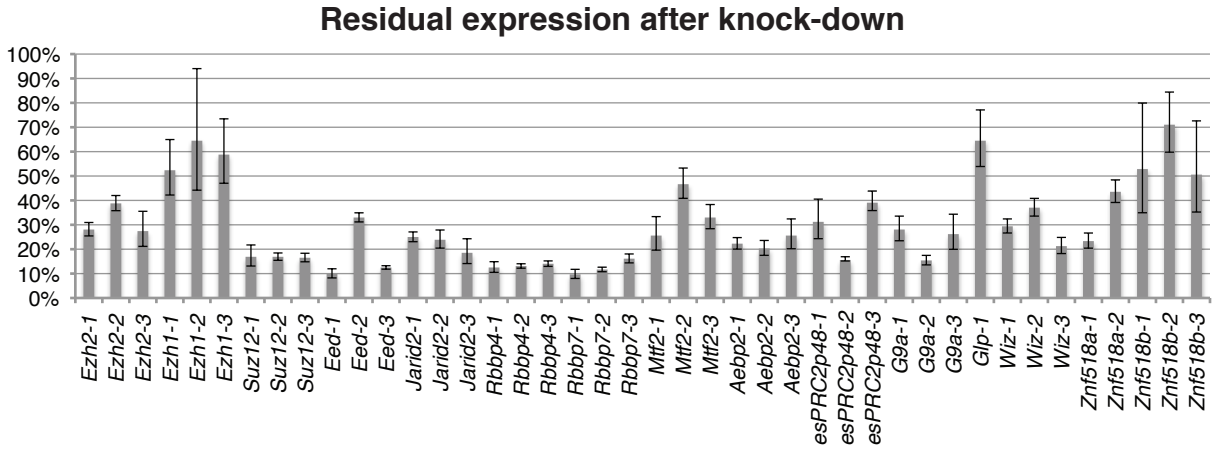
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4