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Supplemental Information

Reprogramming of Polycomb-Mediated Gene Silencing in Embryonic Stem Cells by the miR-290 Family and the Methyltransferase Ash1l

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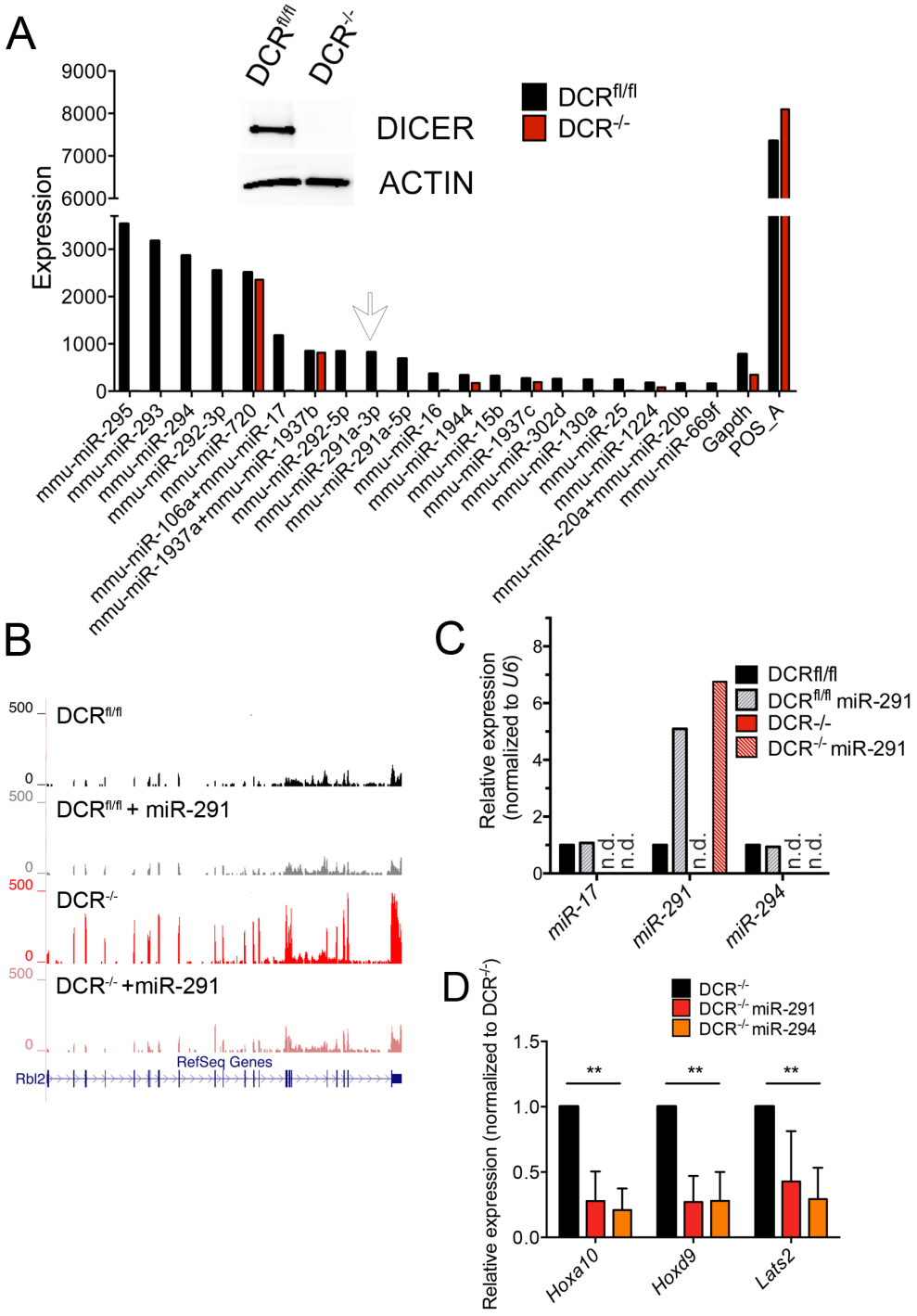

Figure S1. Global miRNA levels in DCR$^{fl/fl}$ and DCR$^{-/-}$ ESCs and effect of miR-291 transfection. A) Expression levels of the 20 most highly expressed miRNAs in ESCs as assessed by Nanostring. Arrow indicates the levels of mature *miR-291-3p*. miR-720 and 1937 are tRNA fragments. Inset: western blot with an anti-Dicer antibody (right panel). Actin is shown as a loading control. B) Genome browser screenshot of the reads aligning to the *Rbl2* locus in DCR$^{fl/fl}$ and DCR$^{-/-}$ ESCs before and 48 h after transfection with a synthetic miR-291 mimic. (n.d. not detected) C) qRT-PCR analysis of mature *miR-17*, *miR-291* and *miR-294* in DCR$^{fl/fl}$ and DCR$^{-/-}$ ESCs mock transfected or transfected with a miR-291 mimic. D) qRT-PCR analysis of *Hoxa10*, *Hoxd9* and *Lats2* transcript levels in DCR$^{-/-}$ ESCs ± miR-291 or miR-294 mimic. Data are represented as fold change compared to DCR$^{-/-}$ ESCs. Bars represent mean ± SD of three independent experiments. **p<0.01

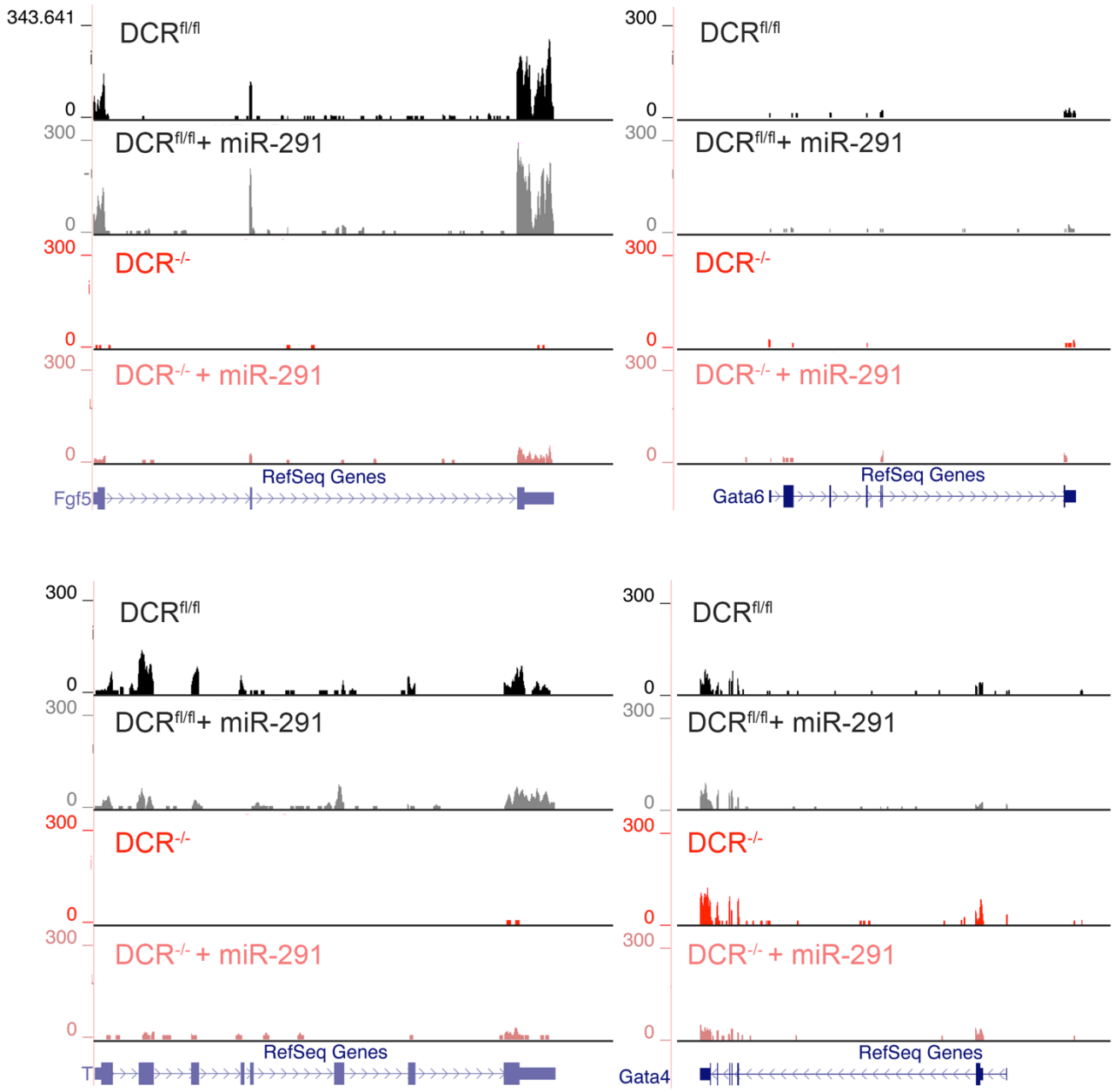

Figure S2. Differentiation genes are not universally regulated by miR-291. Genome browser screenshot of the reads aligning to the *Fgf5*, *Gata6*, *T (Brachyury) and Gata4* locus in DCR$^{fl/fl}$ and DCR$^{-/-}$ ESCs before and 48 h after transfection with a synthetic miR-291 mimic.

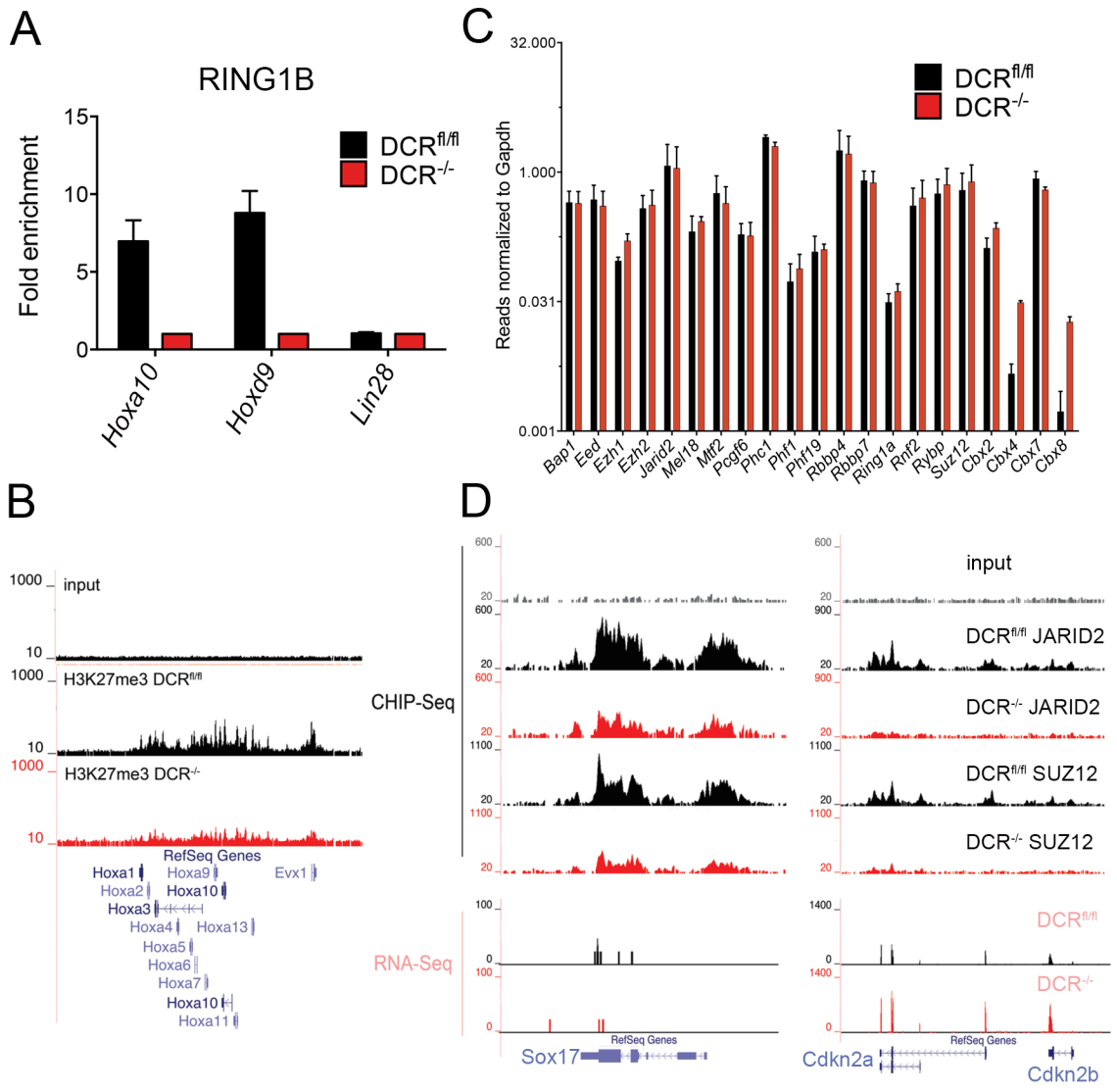

Figure S3. Loss of PRC1 components and H3K27me3 at Hox loci. A) ChIP-qPCR for Ring1b at *Hox* loci and *Lin28a*. Bars represent mean ± SD of two independent experiments. Data are represented as fold change of $DCR^{fl/fl}$ over $DCR^{-/-}$ signal. B) Genome browser screenshot of the reads aligning to the *Hoxa* locus after ChIP with anti-H3K27me3 antibody from $DCR^{fl/fl}$ (black) and $DCR^{-/-}$ (red) ESCs. C) Normalized read count (to Gapdh) of different PRC1 and PRC2 subunits in $DCR^{fl/fl}$ and $DCR^{-/-}$ ESCs. Bars (black, $DCR^{fl/fl}$ and red, $DCR^{-/-}$) represent mean ± SD of three independent RNA-Seq experiments. D) Jarid2 and Suz12 ChIP-seq (top) and RNA-seq (bottom) tracks of the reads aligning to the *Sox17* and *Cdkn2a,b* locus in $DCR^{fl/fl}$ and $DCR^{-/-}$ ESCs.

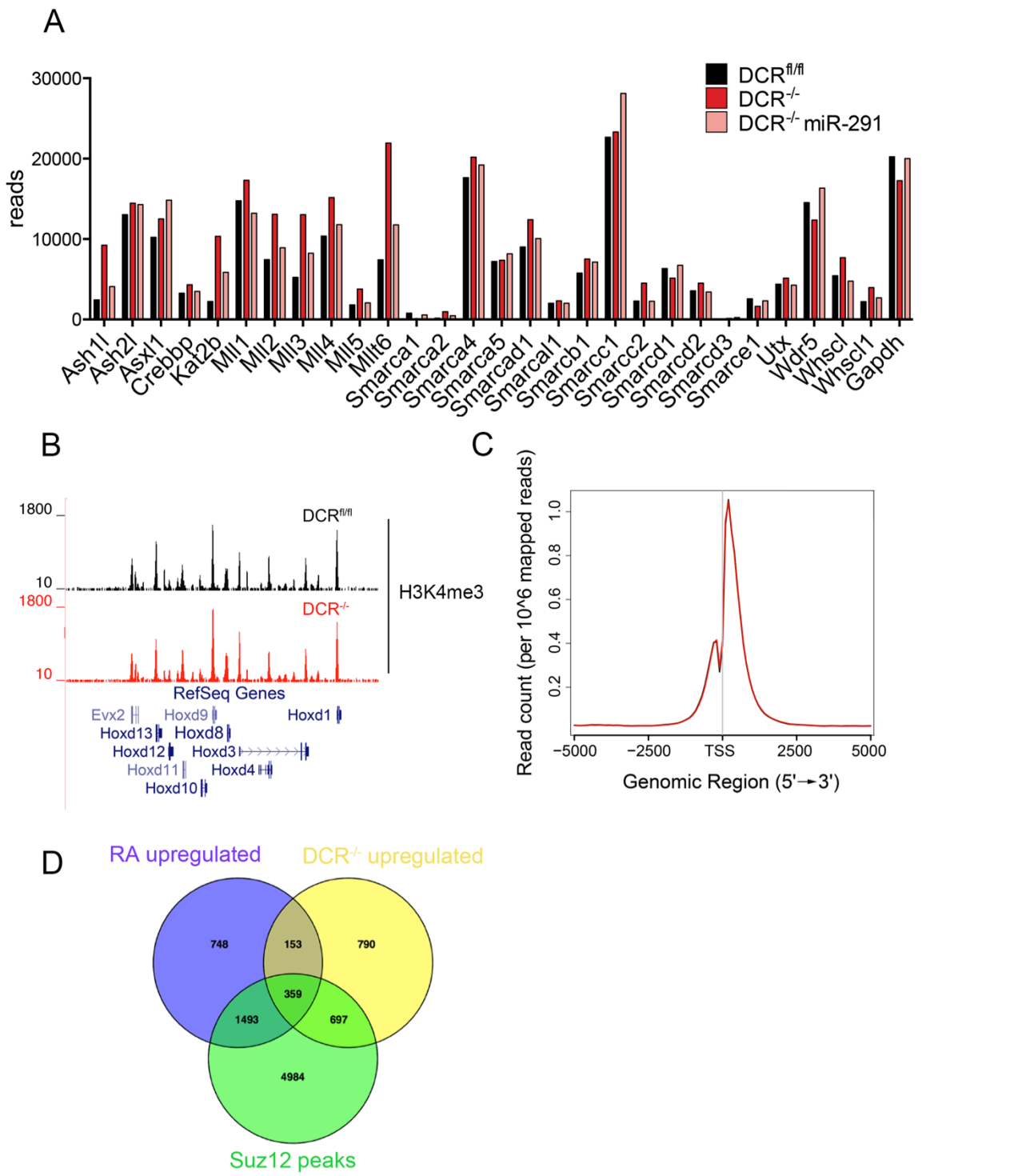

Figure S4. A) Analysis of RNA-seq data for activating histone modifiers in DCR$^{fl/fl}$, DCR$^{-/-}$ and DCR$^{-/-}$ ESCs 48h after transfection with miR-291. B) Genome browser screenshot of the reads aligning to the *Hoxd* locus after ChIP with anti-H3K4me3 antibody from DCR$^{fl/fl}$ (black) and DCR$^{-/-}$ (red) ESCs C) Line plot depicts distribution of H3K4me3 (localization at TSS± 5 kb of all RefSeq genes from DCR$^{fl/fl}$ (black lines) and DCR$^{-/-}$ (red lines) ESCs. D) The majority of differentiation genes are not significantly upregulated in DCR-deficient ESCs. Venn diagram depicting the overlap of genes upregulated (>2-fold change) 48h after retinoic acid (RA) differentiation with the genes upregulated in DCR$^{-/-}$ vs. DCR$^{fl/fl}$ ESCs and the genes that have Suz12 peaks in DCR$^{fl/fl}$ ESCs.

Extended experimental procedures

Luciferase assays

3' UTR containing consructs were generated by Infusion cloning (Clontech) of relevant UTRs in the psiCheck2 reporter vector (Promega). Reporter constructs were transfected ± indicated miRNAs into $DCR^{-/-}$ ESCs (to avoid effects of endogenous miRNAs) using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Cells were harvested 24h after transfection and Renilla and Firefly luciferase values were measured using the Dual Luciferase Reporter Assay kit (Promega). Renilla values were normalized to Firefly levels to correct for differences in transfection efficiency. Bars are plotted as fold change of the normalized luciferase ratio to the no miRNA control.

Chromatin immunoprecipitation

Briefly, cells were fixed with 1% paraformaldehyde for 10 min, washed with ice cold PBS and harvested by scraping. Cell pellets were resuspended in lysis buffer 1 (50mM Hepes, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100), pelleted and then resuspended in lysis buffer 2 (200 mM NaCl, 1mM EDTA, 10 mM Tris pH 8). Nuclei were pelleted and resuspended in lysis buffer 3 (100 mM NaCl, 1mM EDTA, 10 mM Tris pH 8, 0.1% Na-deoxycholate and 0.5% N-lauroyl sarcosine), sonicated for 5x5min in a Bioruptor (Diagenode, Seraing, Belgium). After sonication Triton X-100 was added to a final concentration of 1% and insoluble material was pelleted by centrifugation. Chromatin was incubated overnight with 2-5 mg of antibodies pre-bound to protein G Dynalbeads (Invitrogen). The following antibodies were used: a-Jarid2 (Abcam, ab48137), a-Suz12 (Cell Signaling, #3737), a-Ring1b (Abcam, ab101273), a-RNA PolII (Covance, MMS-126R), a-H3K27me3 (Millipore) and a-Ash1l (Bethyl labs or Santa Cruz). Specifically for the Ash1l ChIP cells were additionally crosslinked with 2 mM ethylene glycol bis(succinimidyl succinate) (EGS, Pierce) for 45 min at RT, prior to paraformaldehyde fixation. Sonication for these samples was increased to 40 min.

For ChIP-Seq libraries were constructed using either the ChIP-Seq DNA Sample Prep Kit (Illumina, San Diego, CA) or Next Ultra DNA Library Kit for Illumina (NEB, Ipswich, MA). ChIP-Seq was preformed on a GAIIx genome analyzer (Illumina). Reads were mapped to the mouse genome (NCBI 37, mm9) using the short read Bowtie aligner (version-0.12.7) with option -p 8 --chunkmbs 256 -a --best --strata -m 20. Unique reads are considered any reads that do not align to more than 20 locations in the genome. Aligned reads and

coverage tracks were then visualized on a local mirror of the UCSC Genome Browser. Read densities and regions of statistically significant enrichment ("peaks") were determined using MACS (Model-Based Analysis of ChIP-Seq, version 1.4.2) using default settings (Zhang et al., 2008). Peaks of ChIP-seq enrichment with p < 1e-9, relative to input, were considered significant and included in subsequent analyses. For the read density plots around TSSs ngs.plot software (Shen et al., 2014) was used with the options "-G mm9 -R tss -L 5000 -MW 3 -D refseq"

For western blotting the same antibodies as for ChIP were used; in addition we performed WB for a-Eed (R&D, Minneapolis, MN), Dicer (Kanellopoulou et al., 2005) and Ago2 (rabbit sera generated from rabbits immunized with an Ago2 specific peptide).

RNA analyses

All qRT-PCR values were normalized to the signal from *Hprt1* and fold change was calculated using the ΔΔCT method.

For single molecule RNA-FISH, cells were grown overnight on gelatinized glass coverslips, fixed, permeabilized and digested for 30 min with protease QS at room temperature (1:8000). Probes were then added and hybridized for 3h at 40 °C, followed by washes, incubation with preamplification, amplification and detection reagents as described in the Quantigene RNA VIEW ISH assay kit protocol (Panomics). Hoxa9 and Hprt probes were mixed and acquired at 488 and 568 nm laser respectively; the Hprt signal was used as a positive control while DAPI costaining was used to visualize the nucleus.

Table S1. List of top one hundred differentially expressed genes between $DCR^{fl/fl}$ and $DCR^{-/-}$ ESCs from two independent RNA-seq experiments. Related to Figure 2.

PRIMER	Sequence 5'-3'	
Hoxa10-UTR-f	TAGGCGATCGCTCGAGTGAAACTTCCAGACAACGTC	cloning
Hoxa10-UTR-r	TTGCGGCCAGCGGCCGCTTCATTCCACAGCTTTTATTC	cloning
Hoxd9-UTR-F	TAGGCGATCGCTCGAGCTGGCCTGCAGCACTCAAAAG	cloning
Hoxd9-UTR-r	TTGCGGCCAGCGGCCGCCTCCATTTGTTCAGATCAGC	cloning
Lats2-UTRf	ATACTCGAGGCCTCAGTTAACCACAACTCGAG	cloning

Lats2-UTRr	TATCTCGAGATTGTGCCAGTAGAAGCTTTC	cloning
Cdkn1a-UTR	ATACTCGAGAAGTGCCCACGGGAGCCCCG	cloning
Cdkn1a-UTR	TATCTCGAGAGAAGCGGCCGCAATCATCGAGAAGTATTTATTGAG	cloning
Hoxa10-CHIPf	CCTCCAGAAACTTTGAAAAACG	qPCR
Hoxa10-CHIPr	GCAGATAGCACGGATGTTTGTA	qPCR
Hoxd9-CHIPf	CTGATTTACTCCGGGTATTGGT	qPCR
Hoxd9-CHIPr	CGAGTCCACGTAGTAGTTGCTG	qPCR
Lin28CHIP-f	AAGATGTAGCAGCCTCTTCTCC	qPCR
Lin28CHIP-r	AAGCTCGAACCTGCAAACTG	qPCR
Hoxa10-intronic-f	GCTAAAACAGGTGCCTGGAA	qPCR
Hoxa10-intronic-r	GGATGCCCGAAGTCATAGAG	qPCR
Hoxd9-intronic-f	AGAAATTGCCCCCTGATTTATT	qPCR
Hoxd9-intronic-r	CAAGGTAGGTGGTTATGGGAAG	qPCR
qHoxa10-f	CAGCCCCTTCAGAAAACAGTAA	qPCR
qHoxa10-r	AGAAACTCCTTCTCCAGCTCCA	qPCR
qHoxa7-f	GAAGCCAGTTTCCGCATCTAC	qPCR
qHoxa7-r	ATGGAATTCCTTCTCCAGTTCC	qPCR
qHoxc6-f	CCAGAAAGCCAGTATCCAGATT	qPCR
qHoxc6-r	CGAGTTAGGTAGCGGTTGAAGT	qPCR
qHoxd9-f	AGC AGC AAC TTG ACC CAA AC	qPCR
qHoxd9-r	CGG GTG AGG TAC ATG TTG AA	qPCR
qHoxb9-f	CTG GCT ACG GGG ACA ATA AA	qPCR
qHoxb9-r	TCC AGC GTC TGG TAT TTG GT	qPCR
qHoxd3-f	GATGAAAGAATCCCGACAGAAC	qPCR
qHoxd3-r	GATAGCGGTTGAAGTGGAACTC	qPCR
qAsh1l-f	AGTGAAAGGGCAATACAGTCGT	qPCR
qAsh1l-r	TCTGGAAGGAACTCCATTCACT	qPCR

Ash1l-UTR-f	AAACCTAGAGCGGCCGCCTCTCAAAAGGATGAGAACCGCG	cloning
Ash1l-UTR-r	TTGCGGCCAGCGGCCGCGCAGGAAAAGTTGTTTCCATTTAAT	cloning

Table S2. Primer sets used in this study, related to Figures 1, 2, 3 and 4.