

SUPPLEMENTARY MATERIALS

Transcription factor trapping by RNA in gene regulatory elements

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MATERIALS and METHODS

Murine embryonic stem cells

Bio-YY1 murine embryonic stem cells (mESCs) (25), control mESCs expressing only biotin ligase *BirA* (25), and bio-OCT4 ESCs (26) were grown on irradiated murine embryonic fibroblasts (MEFs) unless otherwise stated. Cells were grown under standard mESC conditions as described previously (27). Briefly, cells were grown on 0.2% gelatinized (Sigma, G1890) tissue culture plates in ESC media; DMEMKO (Invitrogen, 10829-018) supplemented with 15% fetal bovine serum (Sigma, F4135-500), 1000 U/mL LIF (ESGRO, ESG1106), 100 μ M nonessential amino acids (Invitrogen, 11140-050), 2 mM L-glutamine (Invitrogen, 25030-081), 100 U/mL penicillin, 100 μ g/mL streptomycin (Invitrogen, 15140-122), and 8 nL/mL of 2-mercaptoethanol (Sigma, M7522).

Antibodies

Anti-RNA polymerase II (phospho CTD Ser-2) (Millipore, 04-1571), anti-YY1 (Santa Cruz Biotechnology, SC-1703), anti- β -Tubulin (Millipore, 05-661), anti-Histone H3 (Abcam, ab1791), anti-OCT4 (Santa Cruz, 8628X), and anti-EXOSC3 (Abcam, ab156683) antibodies were used for Western blot analyses.

Actinomycin D, THZ1, Triptolide, and DRB treatment

Bio-YY1 mESCs were treated with 1 μ M of Actinomycin D, 10 μ M THZ1, or 1 μ M triptolide prepared in 100% DMSO or with equal amount of DMSO by adding the drug or DMSO directly to the culture media and incubating cells with the drug for 6hrs at 37°C. Cells were washed with PBS and used for GRO-seq or ChIP-seq analysis. In the DRB experiment, cells were treated with 100 μ M DRB for 3hrs and YY1 ChIP-qPCR was performed after the first 10, 20, and 30 minutes of incubation with the drug, whereas

ChIP-seq was performed after 30-min of incubation with the drug. At the end of the 3hr-treatment, cells were washed two times with PBS, and YY1 ChIP-qPCR was performed after the first 10, 20, and 30 minutes after fresh media without the drug was added to the cells, whereas ChIP-seq was performed 30 minutes after fresh media was added as previously described (28).

Affinity purification of YY1-associated DNA (YY1 ChIP-seq)

Purification of YY1-associated chromatin was performed using bio-YY1 mESCs (25). Detailed protocol was described previously (29). Bio-YY1 is expressed at 10-20% of the endogenous YY1 protein level as has been estimated using quantitative Western blot analysis. mESCs were depleted of MEFs by splitting mESCs twice (1:5) when cells reached confluence onto newly gelatinized plates without MEFs and growing them until achieving confluence again. Approximately 5×10^7 mESCs were chemically crosslinked by the addition of one-tenth volume of fresh 11% formaldehyde solution for 10 minutes at room temperature. Cells were rinsed twice with 1X PBS and harvested using a silicon scraper and flash frozen in liquid nitrogen. Cells were stored at -80°C prior to use. Cells were sonicated (Misonix Sonicator 3000) in sonication buffer (20mM Tris-HCl pH 8.0, 150mM NaCl, 2mM EDTA, 1% Triton X-100, 0.1% SDS) for 10 cycles at 30 seconds each on ice at 21 watts (60 second pause between pulses). The resulting whole cell extract was cleared by centrifugation for 10 min at 12,000 g and then incubated overnight at 4°C with 50 μl streptavidin sepharose high performance (GE Healthcare, 17-5113-01). Beads were washed 2X with 2% (vol/vol) SDS, 1X with 50mM HEPES pH 7.5, 500mM NaCl, 1mM EDTA, 1% Triton X-100, 1X with 10mM Tris-HCl pH 8.0, 250 mM LiCl, 1mM EDTA, 0.5% NP40, 0.5% sodium deoxycholate, and 2X with TE buffer. The affinity-purified DNA was eluted and formaldehyde crosslinks were reversed overnight at 65°C . The DNA were purified by phenol/chloroform extraction and precipitated with ethanol. The ChIP-seq libraries were prepared with Illumina TruSeq DNA Sample Preparation kit, and sequenced on Illumina HiSeq 2000.

Analysis of the affinity purified YY1-associated DNA (YY1 ChIP-seq)

Acquired images were processed through the bundled Illumina image extraction pipeline, which identified polony positions, performed base calling and generated QC statistics. Sequences were aligned using Bowtie 1.0.1 (30) to NCBI Build 37 (UCSC 37, mm9) of the mouse genome. Alignments were performed with the following parameters: `-k 1 -m 1 -n 2 -p 4 -l 40 -best -sam`. WIG files for visualization were made using MACS (31) with parameters `-w -S -space=50 -nomodel -shiftsize=200 -keep-dup=1`. WIG files were uploaded and visualized on the UCSC Genome Browser (32). Peaks were identified using MACS with corresponding control and parameters `-p 1e-9` and `-keep-dup=1`.

Genome-wide YY1 binding motif identification

To identify DNA sequences in the mouse mm9 genome version predicted to bind YY1, we used FIMO (33). The genome was scanned with PWMs MA0142.1 from Jaspar (34) for OCTSOX, and YY1_full from (35) for YY1.

Targeting of ESCs with shRNA against an exosome component

The pLKO.1 shRNA constructs against firefly luciferase (as a negative control) and *Exosc3* were a gift from Dr. Phillip Sharp. The targeted sequence in the *Exosc3* mRNA is 5'-GGUGAAUUUCUCCUGGCAGAUC-3'. The targeted sequence in the firefly luciferase mRNA is 5'-GGACAUCACUUACGCUGAGU-3'. The puromycin resistance gene in pLKO.1 construct was replaced with the hygromycin B resistance gene using *BamHI* and *KpnI* sites. About 3 μ g pLKO.1 vector, 2.5 μ g pMD2, and 7.5 μ g pPAX plasmids were transfected into 6 million HEK293FT cells in T75 tissue culture flask using Lipofectamine 2000 (Life technologies) according to the manufacturer's instructions. Growth media was replaced with 10 ml fresh media 16hrs post-transfection. Lentivirus-containing supernatants were harvested 48hrs post-transfection and

centrifuged at 1200 rpm for 20 min at room temperature to pellet cell debris. About 3 million mESCs in 4 ml complete growth media were incubated with 3 ml lentivirus-containing supernatant with polybrene (final concentration 6 $\mu\text{g}/\text{ml}$). The next day, the media was replaced with complete mESC culture media containing 500 $\mu\text{g}/\text{ml}$ hygromycin B (Life Technologies, 10687-010). After 7 days of selection, mESCs were maintained in complete growth media containing 200 $\mu\text{g}/\text{ml}$ hygromycin B.

Total RNA was isolated from 10 million mESCs (luciferase control or *Exosc3* knockdown cells) using mirVana miRNA Isolation Kit (Ambion, AM1560) according to the manufacturer's protocol. About 7.6 μl of 1:100 fold dilution of ERCC RNA Spike-In Mix (Ambion, 4456740) was added to 1 μg total RNA. TruSeq Stranded RiboZero libraries were then prepared and sequenced on Illumina HiSeq2000.

For YY1 ChIP-Seq experiments, mESCs targeted with control and *Exosc3* shRNAs were counted and equal number of cells was plated 12hrs before crosslinking.

RNA-Seq analysis of changes in steady-state RNA levels in ESCs targeted with shRNA against an exosome component

RNA-seq reads were aligned to the non-random mm9 version of the mouse reference genome with added ERCC spike-in chromosomes using tophat (36) with a GTF of mouse RefSeq genes provided as a parameter. FPKM values were calculated twice using RPKM_count from the RSeQC package (37). The initial run used all mouse RefSeq genes and ERCC spike-ins. This allowed us to determine that exosome knockdown did not cause a loss of mRNA expression, because the ERCC spike-ins added in ratio to cell number provide a normalization method capable of detecting global effects on expression. We floored the expression of all RefSeq genes and ERCC probes at 0.01 and added a pseudocount of 0.1 to all entries. All values were normalized by equilibrating the expression of the ERCC probes between experiments as described previously (38). Briefly, normalize.loess from the affy R package was used to equilibrate

the expression of ERCC probes between experiments and used to normalize the expression of RefSeq genes. A one-tailed Wilcoxon rank sum test of whether RefSeq gene levels decreased upon exosome knockdown showed they did not decrease.

Since expression of RefSeq genes—largely mRNAs—is not lost upon exosome knockdown, we used the RefSeq genes as a normalization method to determine if enhancer RNAs and promoter RNAs tended to increase upon exosome knockdown. FPKM values were calculated for RefSeq genes, ERCC probes, promoters of 16,202 non-overlapping RefSeq genes, and all constituent enhancers. These values were floored at 0.01 and a pseudocount of 0.1 was added. After normalizing using expressed RefSeq genes (RPKM > 1 in both cases) as the constant, we noted that levels of these non-coding RNAs increased. The amount of non-coding RNA level increase is conservative and low because our evidence suggests that mRNA levels also increase upon exosome knockdown. Thus, our normalization shows that enhancer and promoter RNA levels become elevated even relative to the mRNA levels.

Metagene analysis

Enhancer metagenes were constructed using +/- 2000 bases from the centers of regions co-bound by OCT4, SOX2, and NANOG in mouse ESCs as defined in (23). Super-enhancer constituent metagenes were constructed using +/- 2000 bases from the centers of super-enhancer constituents, which were defined as enhancers contacting super-enhancers as calculated in (23). Promoter metagenes were constructed using +/- 2000 bases from RefSeq transcription start sites that had no other transcription start sites in that window.

Densities were calculated in bins using bamToGFF (39). Resulting values were plotted in R using matplotlib. Figure panel-specific adjustments are noted in the table below. Where noted, we disregarded the top 20 most signal-rich regions, because CLIP-seq signal was unusually concentrated therein, and all of these regions had at least one

RepeatMasker-annotated repeat, often somewhat divergent RNA repeats. Where noted, we used “transcribed” promoters, which had GRO-seq RPM-normalized density >1.

Where noted we used “not transcribed” promoters, which had zero GRO-seq reads and zero H3K4me3 reads. Where noted, multiple reads with the same position were collapsed to one using samtools rmdup except in CLIP-seq analysis where reads were manually collapsed if they had the same barcode and position, and in motif analyses.

Where noted, background signal was subtracted from ChIP or CLIP signal by either per-bin per-region normalization of counts in the exact same genomic location, or by subtracting the mean bin signals of input from the precipitated sample.

Here is the detailed description of the parameters used.

Figure	Factor	Treatment	Region Type	# Regions Used	Region Criteria	RPM	Positional Duplicates	Background Normalization
1B	GRO-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	None
1B	GRO-Seq	None	Enhancers	10627	All	Y	Removed	None
1D, SF1B	YY1 ChIP-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	None
1D, SF1B	YY1 ChIP-Seq	None	Enhancers	10627	All	Y	Removed	None
1D, SF1B	YY1 CLIP-Seq	None	Promoters	16182	Non-Overlapping, Discard top 20	Y	Removed if same barcode	None
1D, SF1B	YY1 CLIP-Seq	None	Enhancers	10607	Discard top 20	Y	Removed if same barcode	None
3B	GRO-Seq	DRB -> Release	Promoters	16202	Non-Overlapping	N	Removed	None
3B	GRO-Seq	DRB -> Release	Enhancers	10627	All	N	Removed	None
3B	GRO-Seq	DRB -> Release	SE Constituents	646	All	N	Removed	None
3B	GRO-Seq	DRB	Promoters	16202	Non-Overlapping	N	Removed	None
3B	GRO-Seq	DRB	Enhancers	10627	All	N	Removed	None
3B	GRO-Seq	DRB -> Release	SE Constituents	646	All	N	Removed	None
3B	YY1 ChIP-Seq	DRB -> Release	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
3B	YY1 ChIP-Seq	DRB -> Release	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
3B	YY1 ChIP-Seq	DRB -> Release	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
3B	YY1 ChIP-Seq	DRB	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
3B	YY1 ChIP-Seq	DRB	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
3B	YY1 ChIP-Seq	DRB	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
3C	YY1 ChIP-Seq	shLuc	Promoters	16202	Non-Overlapping	Y	Removed	per-bin per-region input subtraction
3C	YY1 ChIP-Seq	shLuc	Enhancers	10627	All	Y	Removed	per-bin per-region input subtraction
3C	YY1 ChIP-Seq	shLuc	SE Constituents	646	All	Y	Removed	per-bin per-region input subtraction
3C	YY1 ChIP-Seq	shEXO3	Promoters	16202	Non-Overlapping	Y	Removed	per-bin per-region input subtraction
3C	YY1 ChIP-Seq	shEXO3	Enhancers	10627	All	Y	Removed	per-bin per-region input subtraction
3C	YY1 ChIP-Seq	shEXO3	SE Constituents	646	All	Y	Removed	per-bin per-region input subtraction
SF1B	Oct4 ChIP-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	None
SF1B	Oct4 ChIP-Seq	None	Enhancers	10627	All	Y	Removed	None
SF1B	Oct4 Motifs	None	Promoters	16202	Non-Overlapping	N, no density	Kept	None
SF1B	Oct4 Motifs	None	Enhancers	10627	All	N, no density	Kept	None
SF1B	YY1 Motifs	None	Promoters	16202	Non-Overlapping	N, no density	Kept	None
SF1B	YY1 Motifs	None	Enhancers	10627	All	N, no density	Kept	None

SF2	GRO-Seq	None	Promoters	9709	Transcribed	Y	Removed	None
SF2	GRO-Seq	None	Promoters	1118	Not transcribed	Y	Removed	None
SF2	OCT4 ChIP-Seq	None	Promoters	9709	Transcribed	Y	Removed	None
SF2	OCT4 ChIP-Seq	None	Promoters	1118	Not transcribed	Y	Removed	None
SF2	YY1 ChIP-Seq	None	Promoters	9709	Transcribed	Y	Removed	None
SF2	YY1 ChIP-Seq	None	Promoters	1118	Not transcribed	Y	Removed	None
SF2	YY1 CLIP-Seq	None	Promoters	9709	Transcribed	Y	Removed	None
SF2	YY1 CLIP-Seq	None	Promoters	1118	Not transcribed	Y	Removed	None
SF5	YY1 CLIP	None	Promoters	16182	Discard top 20	Y	Removed if same barcode	Ctrl CLIP
SF5	YY1 CLIP	None	Enhancers	10607	Discard top 20	Y	Removed if same barcode	Ctrl CLIP
SF5	Oct4 CLIP	None	Promoters	16182	Discard top 20	Y	Removed if same barcode	Ctrl CLIP
SF5	Oct4 CLIP	None	Enhancers	10607	Discard top 20	Y	Removed if same barcode	Ctrl CLIP
SF10	GRO-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	None
SF10	GRO-Seq	None	Enhancers	10627	All	Y	Removed	None
SF10	GRO-Seq	None	SE Constituents	646	All	Y	Removed	None
SF10	GRO-Seq	DRB	Promoters	16202	Non-Overlapping	Y	Removed	None
SF10	GRO-Seq	DRB	Enhancers	10627	All	Y	Removed	None
SF10	GRO-Seq	DRB	SE Constituents	646	All	Y	Removed	None
SF10	YY1 ChIP-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10	YY1 ChIP-Seq	None	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10	YY1 ChIP-Seq	None	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
SF10	YY1 ChIP-Seq	DRB	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10	YY1 ChIP-Seq	DRB	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10	YY1 ChIP-Seq	DRB	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
SF10C	GRO-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	None
SF10C	GRO-Seq	None	Enhancers	10627	All	Y	Removed	None
SF10C	GRO-Seq	None	SE Constituents	646	All	Y	Removed	None
SF10C	GRO-Seq	ActD	Promoters	16202	Non-Overlapping	Y	Removed	None
SF10C	GRO-Seq	ActD	Enhancers	10627	All	Y	Removed	None
SF10C	GRO-Seq	ActD	SE Constituents	646	All	Y	Removed	None
SF10C	YY1 ChIP-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10C	YY1 ChIP-Seq	None	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10C	YY1 ChIP-Seq	None	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
SF10C	YY1 ChIP-Seq	ActD	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10C	YY1 ChIP-Seq	ActD	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10C	YY1 ChIP-Seq	ActD	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	None	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	None	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	None	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	THZ1	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	THZ1	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	THZ1	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	TPI	Promoters	16202	Non-Overlapping	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	TPI	Enhancers	10627	All	Y	Removed	Mean input signal in each bin subtracted
SF10D	YY1 ChIP-Seq	TPI	SE Constituents	646	All	Y	Removed	Mean input signal in each bin subtracted

Statistical tests for change in binding

p -values were calculated by comparing the distributions of values of drug-treated and control sequencing samples using one-tailed Student's t -test unless otherwise noted. Values were calculated using one value per sample for the whole displayed region.

Heatmap analysis

16,202 promoters (described above) and 10,627 enhancers (described above) were used for heatmap analysis in fig. S1C. RPM-normalized read densities per region were calculated using bamToGFF (-d -r). Regions are ordered by ChIP-seq values and visualized using heatmap.2 in R.

Read distribution analysis

For fig. S4, reads were categorized by the type of region they fell into: enhancer (+/- 2kb around the center of 10,627 OSN sites, described above), promoter (+/- 2kb around 16,202 filtered RefSeq transcription start site, described above), RefSeq intron, RefSeq exon, other. Reads that fell in multiple categories of DNA regions were sorted into the most former category on this list. Reads were categorized using bedtools intersect (40) and the amount of genome in each category was calculated using bedtools subtract. To normalize read counts, we divided the number of reads falling in a category of region by the total number of DNA bases in all regions of that type.

Global run-on sequencing (GRO-seq) sample preparation

GRO-seq analysis for Fig. 1, B and C, fig. S1A, fig. S2, and fig. S4 was performed as described in (3).

GRO-seq for fig. 3B, S10B, and S10C was performed as described previously (5) by using 1×10^8 bio-YY1 mESCs. Briefly, the nuclei were isolated first, and then *in vitro* nuclear run-on assay was performed with Br-UTP. The nascent RNA was labeled with Br-UTP, hydrolyzed with NaOH, and purified with anti-BrdU agarose beads (Santa Cruz

Biotech). The purified nascent RNA was then phosphorylated and polyA tail was added at the 3' end. Subsequently, the RNA was reverse transcribed, and the cDNA was purified through gel extraction and circularized by CirLigase. The circular single-stranded DNA was linearized by APE 1 endonuclease, and PCR amplified. The final PCR product was purified and sequenced on the Illumina HiSeq 2000.

Global run-on sequencing (GRO-seq) analysis

Once sequenced, the 40bp GRO-seq reads used in Fig. 1C and fig. S1A were first filtered off ribosomal RNAs using SortmeRNA (41) with the eukaryote rRNA databases to account for rRNA genes in the proximity of *Arid1a*. Reads used in metagenes in Fig. 1B, S2, S4 were not filtered in this manner. Both filtered and unfiltered reads were then trimmed at their 3' ends to remove residual sequencing adaptor sequences, and trimmed reads over 24bp were aligned to the mm9 version of the mouse genome using Bowtie 0.12.9 (30) as described in (3). Reads having a Phred score quality under 33 or aligning to more than 10 locations in the genome were discarded, and only the best mapping location was retained for each read. A high number of reads mapping to chr17:39979942-39985774 + strand region encoding a 45S rRNA precursor not entirely contained in the eukaryote rRNA database were additionally removed. Reads aligning to the + or - strand of the genome were respectively separated into Watson and Crick strand read containing files and wiggle tracks were generated for each file using bedtools (40). Wig format wiggle files were converted into BigWig format with wigToBigWig (42) and uploaded for visualization in the UCSC genome browser (32).

Untreated and ActD-treated GRO-seq reads in fig. S10C were trimmed at the 3 end at the sequence TGG AATTCTCGGGTGCCAAGGAACTCCAGTCAC using cutadapt -m 18. Trimmed reads were aligned with bowtie with parameters -p 20 -n 2 -S -k 1 -m 1 -best and these reads were discarded in favor of those that had polyA tails. PolyA tails for unmapped reads were trimmed using prinseq -trim_tail_right 1 (28). PolyA-trimmed

reads were aligned using bowtie -n 2 -S -k 1 -m 1 -best to retain reads that could be uniquely mapped. GRO-seq reads used in Fig. 3B and fig. S10B are from (24).

Affinity purification and sequencing of YY1-associated RNA (YY1 CLIP-seq)

CLIP-seq was performed as previously described (43) with the following modifications. Bio-YY1 ESCs and control ESCs expressing only biotin ligase *BirA* were grown in 15-cm plates. Each plate was washed twice with 30 ml of ice-cold PBS, and UV-crosslinking was conducted with 254 nm UV light when plates were placed on ice with lids off at 400 mJ/cm². Cells were scraped off each plate and collected in 15-ml Falcon tubes. Nuclei were purified using Nuclei EZ Prep nuclei isolation kit (Sigma) and flash frozen until ready to use. Three 15-cm plates (~1.5x10⁸ cells) of ESCs were used for each experiment. 50 μ l of Dynabeads® MyOne Streptavidin T1 was used for each 0.5x10⁸ cells. Beads were washed thrice with PBS, twice with DEPC-treated 0.1M NaOH, 0.05M NaCl, and once with DEPC-treated 0.1M NaCl. After blocking the beads with sterile-filtered blocking buffer (0.5% BSA in PBS) for 30 min at 4°C, beads were kept on ice in 100 μ l of the lysis buffer (1xPBS, 0.1% SDS, 0.5% sodium deoxycholate, 0.5% NP-40, 1 mM DTT, cOmplete EDTA-free protease inhibitor cocktail (Roche)) until ready to use.

Each aliquot of 0.5x10⁸ cells was thawed on ice and resuspended in 1 ml of the lysis buffer in 1.5 ml tubes. Cells were passed three times through the 26" needle to reduce viscosity, and tubes were rotated for 10 min at 4°C to complete the lysis. 30 μ l of RQ1 RNase-free DNase (Promega) and 1 μ l of RNase I (Life Technologies, 100U/ μ l) were added to each 1 ml of lysate and tubes were incubated at 37°C for 15 min in the ThermoMixer C (Eppendorf) (rock for 15 sec at 1,200 rpm, 75 sec off). RNase digestion was stopped by transferring the tubes to ice and adding 25 μ l Supersaseln RNase Inhibitor (Life Technologies, 20U/ μ l) to each aliquot of 0.5x10⁸ cells. Lysates were cleared up by centrifugation at 20,000g for 10min at 4°C, supernatants were added to the beads, and rotated overnight at 4°C. 2% of the supernatant was saved as input for Western blot analysis.

At the end of incubation, beads were washed twice by rotating them for 5 min each time with 1 ml of ice-cold wash buffer 1 containing 50mM Tris-HCl, pH 7.4, 1mM EDTA, 1M NaCl, 0.1% SDS, 1% NP-40, 1mM DTT, 10u/ml SupraseIN, and cOmplete protease inhibitor followed by two washes (each for 5 min at 4°C) with 1 ml of wash buffer 2 containing 50mM Tris-HCl, pH 7.4, 1mM EDTA, 300mM NaCl, 0.1% SDS, 1% NP-40, 1mM DTT, 10u/ml SupraseIN, and cOmplete protease inhibitor. The second DNase-treatment was conducted by incubating beads in 50- μ l reaction in presence of 5 μ l of 10X TurboDNase buffer and 2 μ l of TurboDNase (2u/ μ l) (Life Technologies) for 25 min at 37°C in the ThermoMixer C (rock for 15 sec at 1,000 rpm, 90 sec off). Beads were washed twice by rotating them for 5 min each time with 1 ml of ice-cold wash buffer 1 containing 50mM Tris-HCl, pH 7.4, 1mM EDTA, 500mM NaCl, 0.1% SDS, 1% NP-40, 1mM DTT, 10u/ml SupraseIN, and cOmplete protease inhibitor followed by two washes with 1 ml of wash buffer 2, two washes with 1 ml of RIPA-S buffer containing 50mM Tris-HCl pH 7.4, 1M NaCl, 2M Urea, 0.5% NP-40, 1% sodium deoxycholate, 5 mM EDTA, 0.1% SDS, 1mM DTT, and two washes with 1 ml of PNK buffer containing 50mM Tris-HCl pH 7.4, 10 mM MgCl₂, and 0.5% NP-40. Beads were resuspended in 1000 μ l of PNK buffer and 100 μ l of the beads was transferred to a clean tube. After removal of the PNK buffer, these beads were resuspended in 30 μ l of PNK labeling mix containing 3 μ l of 10X PNK buffer (NEB), 1 μ l of T4 PNK (NEB) and 1 μ l of ATP, g-³²P (Perkin Elmer, NEG035C001MC) and incubated for 20 min at 37°C in the ThermoMixer C (rock for 15 sec at 1,000 rpm, 90 sec off). Labeling mix was removed and beads were washed three times with 50mM Tris-HCl, pH 7.4, 1mM EDTA, 150mM NaCl, 0.1% SDS, 1% NP-40, 1mM DTT. Unlabeled 900- μ l worth of beads was washed in the same manner. Labeled RNA species cross-linked to YY1 were eluted off beads by incubating beads for 10 min at 95°C in 20 μ l of 1X NuPage LDS sample buffer (Life technologies) in presence of 10mM DTT. Of the remaining 900 μ l of unlabeled beads, 100 μ l was used for Western blot analysis, and protein-RNA complexes were eluted off the remaining beads in 40 μ l of 1X NuPage LDS sample buffer, 10mM DTT. Protein-RNA complexes were separated on a 10% Criterion XT Bis-Tris gel (Bio-Rad) and transferred to nitrocellulose membrane (Bio-Rad) for 2hrs (250mA) at 4°C. Samples for Western

blot analysis were resolved on SDS-PAGE and transferred to another membrane in parallel with the CLIP samples. After transfer, membrane was rinsed with TBS buffer, wrapped in Saran Wrap and exposed to film. Regions slightly above the radioactive band corresponding to YY1 protein based on results of the Western blot analysis, were excised from the membrane, sliced into thin strips, and transferred to 2ml tubes. RNA was isolated in the following way: 400 μ l of proteinase K buffer containing 100mM Tris-HCl pH 7.4, 50mM NaCl, 10mM EDTA and 4 mg/ml proteinase K (20mg/ml) (Life Technologies) was added to the tubes, which were rocked for 40 min at 37°C in the ThermoMixer C at 1,200 rpm. Once 400 μ l of PKU buffer containing 100mM Tris-HCl pH 7.4, 50mM NaCl, 10mM EDTA, 7M Urea was added to the tubes, they were incubated for another 40 min at 37°C in the ThermoMixer C at 1,200 rpm. Liquid fraction was transferred to a fresh 2ml tube, 800 μ l of phenol:chlorophorm:isoamyl alcohol (25:24:1 v/v) pH 8.0 (Sigma) was mixed with it and loaded to MaxTract High Density tubes (Quagen). After centrifugation at 16,000g for 5 min, aqueous phase was transferred to two clean tubes and RNA was precipitated in presence of 0.3 M sodium acetate, 1 μ l of GlycoBlue (Life Technologies) and 3X volume of 100% ethanol at -20°C overnight. Precipitated RNA pellets were washed with 70% ethanol and dried. At this point, UV-crosslinked RNA purified from all 1.5×10^8 cells was pulled from all six tubes and resuspended in 10 μ l of RNase-free H₂O. RNA was dephosphorylated in a 20- μ l reaction containing 70mM Tris-HCl pH 6.5, 10mM MgCl₂, 5mM DTT, 0.5 μ l SuperaseIN, and 2 μ l PNK (NEB) at 37°C for 1 hr. After phenol/chloroform extraction, RNA was ethanol precipitated and resuspended in 13 μ l H₂O. RNA was denatured by incubation at 70°C for 2 min and kept on ice until ready to use. Subsequently, adapter with sequence /5rApp/TG GAA TTC TCG GGT GCC AAG G/3ddC/ was ligated to the 3' end of the RNA in a 20 μ l reaction containing 20 μ M adapter, 1xRNA ligase buffer, 2 μ l PEG 8000, and 2 μ l truncated RNA ligase (NEB, M0373) at 16°C overnight. Reactions were ethanol precipitated and reverse transcription of the RNA was conducted using SuperScript III reverse transcriptase (Life Technologies) at 55°C for 1hr using RT primer [Phos]ANNNNagatcGGAAGAGCGTCGTGTAGGGAAAGAGTGT[Sp-C18]CACTCA[Sp-C18]CCTTGGCACCCGAGAATTCCA. Subsequently, RNA was removed by treating the

sample with 1 μ l RNase H and 1 μ l (0.3 mg/ml) RNase A at 37°C for 20 min. After phenol/chloroform extraction, cDNA was ethanol precipitated and resuspended in 10 μ l H₂O.

cDNA denatured in presence of gel loading dye II (Life Technologies) at 70°C for 3 min was separated from the adapter and the RT primer on a 10% TBE-Urea polyacrylamide gel (10 Watts, 1.5-2hrs). Gel was stained with SYBR Gold Nucleic Acid Stain (Life Technologies) and area corresponding to 100-200nt was excised from the gel. Excised gel fragments were further fragmented and cDNA was eluted in buffer containing 0.5M NaCl and 1mM EDTA by rotating tubes at room temperature overnight. Gel slurry was transferred to a Costar Spin-X 0.22 μ m column (Corning, 8161) and spun at 8,000g for 3min. cDNA was consequently ethanol precipitated, resuspended in 8 μ l H₂O, and circularized in presence of 1 μ l CircLigase II buffer, 0.5 μ l MnCl₂ and 0.5 μ l CircLigaseII (Epicentre, CL9021K) by incubating the cDNA at 60°C for 1hr. After CircLigaseII heat inactivation at 80°C for 10 min, DNA was ethanol precipitated and resuspended in 21 μ l H₂O. 5 μ l of the DNA was used as a template in each of two 50- μ l PCR reactions using Phusion High Fidelity DNA polymerase (NEB, M0530) (7 cycles, empirically determined) and the following primers:

DSFP5 (Aatgatacggcgaccaccgagatctacactctttccctacacgacgctcttcc) and the following bar-coded primers:

DSFP3_1 for control *BirA* sample

CAAGCAGAAGACGGCATACGAGATCGTGATCGGTCCTTGGCACCCGAGAATTCCA

DSFP3_2 for bio-YY1 sample

CAAGCAGAAGACGGCATACGAGATACATCGCGGTCCTTGGCACCCGAGAATTCCA

PCR products were ethanol precipitated and separated on a 10% TBE-Urea polyacrylamide gel. After gel staining with SYBR Gold, 150-250nt gel fragments were excised from a gel and DNA was eluted by incubating gel pieces in 0.5M NaCl and 1mM

EDTA by rotating tubes at room temperature overnight. DNA was ethanol precipitated and resuspended in 10 μ l H₂O. After diluting the DNA ten-fold, 1 μ l of the diluted DNA was used as a template in the next round of 15 cycles of PCR for bio-YY1 sample and 25 cycles of PCR for control *BirA* sample using either Phusion High-Fidelity DNA polymerase or GoTaq DNA polymerase (Promega) and the following primers: AATGATACGGCGACCACC and CAAGCAGAAGACGGCATAC. PCR products were resolved on 2% Agarose Resolute GPG gel (American Bioanalytical), isolated from a gel, and PCR products synthesized using GoTaq DNA polymerase was cloned into TOPO-TA vector (Life Technologies) and resulting plasmid was subjected to Sanger sequencing to confirm complexity of the insert. PCR product obtained using Phusion High Fidelity DNA polymerase was subjected to high-throughput sequencing on Illumina High-Seq genome analyzer. Insert sequencing primer was CTCTTTCCCTACACGACGCTCTTCCGATCT and barcode sequencing primer was TGGAATTCTCGGGTGCCAAGGACCG.

OCT4 CLIP-seq was conducted similarly to YY1 CLIP-seq using bio-OCT4 mESCs described in (26).

YY1 CLIP-seq analysis

CLIP-Seq reads were processed in a manner adapted from (43). Cutadapt (44) was used to trim the sequence TGGAATTCTCGGGTGCCAAGG off of the 3' end of CLIP-Seq reads with parameter $-m$ 18. First, bowtie was used to map trimmed reads to the mm9 mouse genome with parameters $-best -strata -m1 -n 2 -5 5 -p 50 -S$. Bowtie2 (45) was used to map those reads bowtie failed to map (accounting for deletions caused by UV crosslinking) with parameters $-5 5 -sensitive -p 50$. The resulting bowtie and bowtie2 mappings were combined and used for downstream analysis.

Multiple reads were collapsed to one if they had the same chromosome, start, end, and first four nucleotides (barcode) to account for PCR duplicates. RepeatMasker regions

for mm9 were downloaded from the UCSC Genome Browser and subsetted for rRNA repeats. Reads contacting these regions were discarded. Remaining reads were separated into + and – strand reads using samtools view (46). Enriched regions and WIG files were created on strand-separated mapping files using MACS with parameters –w –S –space=50 –nomodel –shiftsize=40 –keep-dup=1. Peaks identified separately on reads mapping to each strand were concatenated and used for Figure S3. Peaks were required to have at least one read mapped with bowtie2.

Purification and determination of concentration of the recombinant YY1

YY1 protein was purified using a method modified from (15). Briefly, a plasmid containing the N-terminal His₆-tagged YY1 coding sequence (a gift from Dr. Yang Shi) was transformed into BL21-CodonPlus (DE3)-RIL cells (Stratagene, 230245). A fresh bacterial colony was inoculated into LB media containing ampicillin and chloramphenicol and grown overnight at 37°C. Bacteria were pelleted, resuspended in 500ml of fresh pre-warmed LB media, and grown for 1.5 hours at 37°C. After induction of YY1 expression with 1mM IPTG, cells were grown for another 5 hours, collected, and stored frozen at -80°C until ready to use.

Pellets were resuspended in 5ml of Buffer A (6M GuHCL, 25mM Tris, 100mM NaCl, pH8.0) containing 10mM imidazole, 5mM 2-mercaptoethanol, and cOmplete protease inhibitors (Roche) and sonicated (ten cycles of 15 seconds on, 60 sec off). The lysate was cleared by centrifugation at 13,000g for 20 minutes at 4°C, 1ml of Ni-NTA agarose (Invitrogen, R901-15) pre-equilibrated with 10X volumes of buffer A was added to the cleared lysate, and tubes containing agarose lysate slurry were rotated at room temperature for 1 hour. The agarose slurry was poured into a column, and packaged agarose was washed with 15x volumes of Buffer A containing 10mM imidazole and 5mM DTT. Protein was eluted with 3ml Buffer A containing 500mM imidazole and 5mM DTT.

After adjusting the concentration of ZnCl₂ and DTT in the elution buffer to 0.1mM and 100mM, respectively, the purified protein was denatured at 60°C for 30 minutes and refolded by dialyzing it against 600 ml of the dialysis buffer containing 25mM Tris-HCl pH 8.5, 100mM NaCl, 10mM MgCl₂, 0.1mM ZnCl₂, and 5mM DTT at 4°C changing the buffer three times. At the end of the dialysis, precipitated material was removed by centrifugation at 1,800g for 15 minutes at 4°C. Soluble fraction was dialyzed against 600ml of the dialysis buffer containing 1mM DTT and 10% glycerol at 4°C changing the buffer three times. At the end of the dialysis, protein was stored in aliquots at -80°C.

Based on A260 to A280 ratio, less than 4% of protein is associated with nucleic acid. HPLC chromatograph does not show presence of soluble YY1 aggregate. Recombinant YY1 behaves similarly to endogenous YY1 in nuclear extract EMSA assays as shown in fig. S6 and S7.

Concentration of the full-length YY1 in the final protein preparation was determined by first resolving serial dilutions of BCA standard alongside YY1 on a 10% SDS-PAGE gel. Resolved proteins were then stained with the Bio-Safe Coomassie Stain (Bio-Rad), and YY1 amount was estimated by densitometry of the corresponding Coomassie-stained YY1 band relative to the BCA standards using ChemiDoc XRS+ system with Image Lab software (Bio-Rad).

The N-terminal (amino acids 1-277) and C-terminal (amino acids 271-420) portions of YY1 were purified in a fashion similar to the full-length YY1.

RNase treatment and YY1 chromatin binding assay

Chromatin binding assay was modified from a published protocol (47). Nuclei were prepared using hypotonic buffer and then half of the purified nuclei was left untreated (control), whereas another half was treated with 1: 100 dilution of RNase A (Sigma, R4642) for 10 min at 37 °C. After washing, both untreated and RNase A-treated nuclei were digested with 1:10 dilution of DNase I (Promega, M6101) in presence of (NH₄)₂SO₄

at 37 °C for 30 min to isolate soluble chromatin fraction. The supernatants (soluble chromatin fraction) were then analyzed by Western blotting.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed essentially as described in (48). To prepare nuclear extracts (NE), bio-YY1 mESCs were depleted of MEFs. Cells were then washed twice in cold PBS, collected, and resuspended in 5 ml ice-cold hypotonic lysis buffer (20 mM HEPES, pH 7.4, 20% glycerol, 10 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) in presence of cOmplete proteinase inhibitor. After 10 min incubation on ice, nuclei were spun down, resuspended in 0.5 ml of nuclear extraction buffer (hypotonic lysis buffer plus 420 mM NaCl) and rotated for 1 hr at 4°C. Supernatants were then clarified by centrifugation, aliquoted, and stored at –80°C. Protein concentrations were determined using the BCA protein assay (Life Technologies).

Oligonucleotide DNA probes containing YY1 binding sites were generated by first annealing 30-nt single-stranded oligonucleotides to obtain 100 μM stock of double-stranded oligonucleotides, and by then labeling 10 pmol of this stock with T4 polynucleotide kinase (New England Biolabs) and [γ -³²P]-ATP (Perkin Elmer). DNA-RNA chimeric oligonucleotides used in the tethering experiments were annealed and labeled in a similar way. The 30-nt single-stranded RNA probes were labeled in the same way as the DNA probes. Unincorporated [γ -³²P]-ATP was removed using G-25 spin columns (Roche). Labeled stocks were further diluted to obtain 0.1 μM stocks of labeled nucleic acids, 1 μl of which was used in the binding reactions.

EMSA with NE were performed as follows: DNA-binding reactions (20 μl) containing 20 mM HEPES-KOH, pH 7.5, 105 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.02% Triton X-100, 5% glycerol, 0.5 mM dithiothreitol, 500 ng poly(dI-dC), and 10 μg nuclear extract were pre-incubated with or without specific competitor (100-fold excess) at room temperature for 20 min. Following pre-incubation, 0.1 pmol of radiolabeled DNA probe was added to the reaction mixtures and incubation continued for another 80 min. Each

reaction mixture was then mixed with Triple Dye Loading Buffer (National Diagnostics), loaded onto a native 5% polyacrylamide gel (acrylamide:bis, 39:1, National Diagnostics) containing 0.5× TBE and 1% glycerol, and electrophoresed in 0.5× TBE at 300 V for 10 min at room temperature, followed by at 250 V for 75 min at 4°C for. After electrophoresis, the gels were dried at 80°C for 50 min and exposed to a phosphorimager screen (Fuji). For antibody super-shift assays, NEs were first incubated with radiolabeled DNA probe for 20 min before 0.5 µl of the YY1 antibody was added to the reaction mixtures and incubation continued for another 80 min.

EMSAs with the recombinant YY1 were performed in a similar way as those with the NE. DNA-binding reactions (20 µl) contained 10 mM HEPES-KOH pH 7.5, 12 mM Tris-HCl pH 7.4, 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM ZnCl₂, 0.01% NP-40, 5% glycerol, 500 ng poly(dI-dC), 0.5 mM DTT, and recombinant YY1. RNA-binding reactions (20 µl) contained 10 mM HEPES-KOH pH 7.5, 12 mM Tris-HCl pH 7.4, 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM ZnCl₂, 0.01% NP-40, 5% glycerol, 10 U SUPERase In RNase inhibitor (Life Technologies), 0.5 mM DTT, and recombinant YY1.

DNA oligonucleotides used as probes and competitors in EMSA

Rpl30 promoter with YY1 motif (labeled DNA probe in fig. S6, A and B, S7C, S14, A to C, and S15, A and B; cold specific competitor in fig. S6B, cold DNA competitor 3 in fig. S8C, and a part of cold competitor 2 in fig. S15, A and B):

Forward 5´-TCGCTCCCCGGCCATCTTGGCGGCTGGTGT-3´

Reverse 5´- ACACCAGCCGCCAAGATGGCCGGGGAGCGA-3

Rpl30 promoter with no YY1 motif (labeled probe in fig. S6A and cold non-specific competitor in fig. S6B):

Forward 5´- AGGCAGTGGcTAgcTcaCGTCCCTGGATCG-3´

Reverse 5' - CGATCCAGGGACGtgAgCTaGCCACTGCCT-3'

Arid1a promoter with YY1 motif (labeled DNA probe in Fig. 2, A and B, fig. S6A, S7B, and S9B; cold competitor in fig. S7B and DNA competitor 2 in fig. S7C):

Forward 5' -CTCTTCTCTCTTAAAATGGCTGCCTGTCTG- 3'

Reverse 5' -CAGACAGGCAGCCATTTTAAAGAGAGAAGAG- 3'

Arid1a promoter with no YY1 motif (labeled DNA probe in fig. S6A; cold non-specific competitor in fig. S7B):

Forward 5' -CCCGCCTCCCCAGGCCTACGCGCTGAGCTC -3'

Reverse 5' -GAGCTCAGCGCGTAGGCCTGGGGAGGCGGG -3'

Lefty1 enhancer (labeled DNA probe in fig. S5B and cold DNA competitor in fig. S5B):

Forward 5' - GGTGGGAGGGAGACTGCCCTTTGTCATGTAGAAGGAGCTT-3'

Reverse 5' - AAGCTCCTTCTACATGACAAAGGGCAGTCTCCCTCCCACC -3'

mut *Lefty1* enhancer (cold DNA competitor in fig. S5B):

Forward 5' -ACGTGCAGGGAGACTGCCCTTTGTCATGTAGAAGCAGTTG -3'

Reverse 5' -CAACTGCTTCTACATGACAAAGGGCAGTCTCCCTGCACGT -3'

RNA oligonucleotides used as probes and competitors in EMSA

Arid1a promoter RNA **A** (labeled probe in Fig. 2, A and B, in fig. S5B, S7, A to C, S9C, and as a part of probe 2 in fig. S14, A to C; cold competitor in fig. S5B, S7C, S8, B and C, a part of probe 3 in fig. S14, A to C, and a part of cold competitor 1 and 2 in fig. S15, A and B):

5' -rCrUrCrUrUrCrUrCrUrCrUrUrArArArArUrGrGrCrUrGrCrCrUrGrUrCrUrG-3'

Arid1a promoter RNA **1** (labeled probe in fig. S8A; cold competitor in fig. S8B):

5´ - rCrArGrArCrArGrGrCrArGrCrCrArUrUrUrUrArArGrArGrArGrArArGrArG -3´

Arid1a promoter RNA **B** (cold competitor in fig. S8, B and C):

5´ - rCrCrCrGrCrCrUrCrCrCrCrArGrGrCrCrUrArCrGrCrGrCrUrGrArGrCrUrC -3´

DNA-RNA chimeric oligonucleotides used in in vitro tethering experiments

Rpl30 promoter with YY1 motif – *Arid1a* promoter RNA (labeled probe 2 in fig. S14, A to C; cold competitor 1 in fig. S15, A and B):

Forward 5´ -

TCGCTCCCCG**GCCATCTTG****GCGGCTGGTGT**rCrUrCrUrUrCrUrCrUrCrUrUrArArArArU
rGrGrCrUrGrCrCrUrGrUrCrUrG -3´

Reverse 5´ -

ACACCAGCCG**CAAGATGG****CCGGGGAGCGA**rCrUrCrUrUrCrUrCrUrCrUrUrArArArA
rUrGrGrCrUrGrCrCrUrGrUrCrUrG-3´

Competition EMSA: in vitro RNA tethering experiments

For competition EMSA in fig. S15, single-stranded DNA-RNA chimeric oligonucleotides were annealed to obtain 50µM stock of the 30-bp *Rpl30* DNA containing 30-nt *Arid1a* RNA overhang on each 3´ end (competitor 1) and its serial dilutions immediately before the experiment. Thus, each molecule of competitor 1 contained three potential binding sites for YY1: one in DNA, and two in RNA. Competitor 2 was obtained by first annealing complementary single-stranded *Rpl30* DNA oligonucleotides to obtain 100µM stock of 30-bp *Rpl30* DNA, and by then mixing this stock with *Arid1a* promoter RNA to

obtain a final stock, which consisted of 50 μ M 30-bp *Rpl30* DNA and 100 μ M 30-nt *Arid1a* RNA. Thus, for each molecule of DNA, there were two molecules of RNA, which added up to three potential YY1 binding sites, as was the case in the competitor 1. The stock of the competitor 2 was further serially diluted before each experiment.

Binding reactions (20 μ l) containing 10 mM HEPES-KOH pH 7.5, 12 mM Tris-HCl pH 7.4, 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM ZnCl₂, 0.01% NP-40, 5% glycerol, 10 U SUPERase In RNase inhibitor (Life Technologies), 0.5 mM DTT, 500 ng poly(dI-dC), and recombinant YY1 were pre-incubated with or without different concentrations of either competitor 1 or competitor 2 at room temperature for 20 min. Following pre-incubation, 0.1 pmol of radiolabeled DNA probe was added to the reaction mixtures and binding reactions were incubated for another 80 min. Each reaction mixture was then mixed with Triple Dye Loading Buffer (National Diagnostics) and loaded onto a native 5% polyacrylamide gel (acrylamide:bis, 39:1, National Diagnostics) containing 0.5 \times TBE and 1% glycerol and electrophoresed in 0.5 \times TBE at 300 V for 10 min at room temperature, followed by electrophoresis at 250 V for 75 min at 4°C for. After electrophoresis, the gels were dried at 80°C for 50 min and exposed to a phosphorimager screen (Fuji). Screen were scanned using Typhoon FLA 9500 (GE Healthcare Life Sciences) and quantified using ImageQuant TL software (GE Healthcare Life Sciences). Graph in fig. 15B was created and IC₅₀ values were estimated using Prism 6 software. Error bars represent standard deviation from the mean value for each data point obtained in at least three replicates. Values were fit using non-linear regression function in the Prism program. The *p*-value for differences between log₁₀IC₅₀ values was calculated using the two-tailed *t*-test taking into account number of biological replicates for each data point.

Generation of stable mESC lines for in vivo tethering experiments

Genome editing was performed using CRISPR/Cas9 essentially as described (49, 50).

Lentiviral plasmid pHAGE-TRE-dCas9 containing tetracycline-inducible dCas9 and lentiviral stuffer plasmid pLKO.1-puro U6 sgRNA *BfuAI* were obtained from Addgene (50915 and 50920, respectively).

Target-specific DNA oligonucleotides were annealed and cloned into the pLKO.1-puro U6 sgRNA *BfuAI* plasmid digested with *BfuAI* to obtain pLKO.1-puro U6 sg*Suz12*, pLKO.1-puro U6 sg*Klf5*, pLKO.1-puro U6 sg*E2f3*, pLKO.1-puro U6 sg*Nufip2*, pLKO.1-puro U6 sg*Cnot6*, and pLKO.1-puro U6 sg*Pias1* plasmids. The genomic sequences complementary to guide RNAs are GTACTGGCTGCTCAAATGTC for *Suz12* enhancer, GTGATGTAGGTATAATTAGCC for *Klf5* enhancer, TTGCATGTTGTTCTCGGAGC for *E2f3* enhancer, CCATGTATGGTTACGGGGATC for *Nufip2* enhancer, GCTGAAAGACCACAGCTCCC for *Cnot6* enhancer, and TTGGGTGTTGAGAATAGGTCC for *Pias1* enhancer. Constructs for targeting the RNA tethering constructs to the six enhancers were ordered from Integrated DNA Technologies as gBlocks and cloned into the pLKO.1-puro U6 sgRNA *BfuAI* plasmid digested with *NdeI* and *EcoRI* to obtain pLKO.1-puro U6 sg*Suz12*-tracrRNA-*Arid1a* RNA **A** and pLKO.1-puro U6 sg*Suz12*-tracrRNA-*Arid1a* RNA **B** constructs, pLKO.1-puro U6 sg*Cnot6*-tracrRNA-*Arid1a* RNA **A** and pLKO.1-puro U6 sg*Cnot6*-tracrRNA-*Arid1a* RNA **B** constructs, pLKO.1-puro U6 sg*E2f3*-tracrRNA-*Arid1a* RNA **A** and pLKO.1-puro U6 sg*E2f3*-tracrRNA-*Arid1a* RNA **B** constructs, pLKO.1-puro U6 sg*Klf5*-tracrRNA-*Arid1a* RNA **A** construct, pLKO.1-puro U6 sg*Nufip2*-tracrRNA-*Arid1a* RNA **A**, and pLKO.1-puro U6 sg*Pias1*-tracrRNA-*Arid1a* RNA **A**, in which *Arid1a* RNA **A** is the RNA compatible with YY1 binding *in vitro*, and *Arid1a* RNA **B** is the RNA incompatible with YY1 binding.

Puromycin-resistance (*pac*) gene in all targeting plasmids was then replaced with hygromycin B-resistance gene (HygR) by first amplifying HygR gene from pLVX-Tet-On Advanced vector (Clontech) using Phusion High-Fidelity DNA polymerase (NEB), digesting it with *BamHI* and *KpnI* restriction enzymes, and then cloning the digested PCR product into the targeting plasmids digested with the same restriction enzymes to obtain pLKO.1-hygro U6 sg*Suz12*-tracrRNA-*Arid1a* RNA **A** and pLKO.1-hygro U6

sg*Suz12*-tracrRNA-*Arid1a* RNA **B** constructs, pLKO.1-hygro U6 sg*Cnot6*-tracrRNA-*Arid1a* RNA **A** and pLKO.1-hygro U6 sg*Cnot6*-tracrRNA-*Arid1a* RNA **B** constructs, pLKO.1-hygro U6 sg*E2f3*-tracrRNA-*Arid1a* RNA **A** and pLKO.1-hygro U6 sg*E2f3*-tracrRNA-*Arid1a* RNA **B** constructs, pLKO.1-hygro U6 sg*Klf5*-tracrRNA-*Arid1a* RNA **A** construct, pLKO.1-hygro U6 sg*Nufip2*-tracrRNA-*Arid1a* RNA **A**, and pLKO.1-hygro U6 sg*Pias1*-tracrRNA-*Arid1a* RNA **A** plasmids.

pHAGE-TRE-dCas9 DNA and each of the targeting plasmids were packaged into lentivirus by transfecting the corresponding plasmids into the HEK 293FT cells (Life Technologies) using Lipofectamine 2000 transfection reagent (Life Technologies) in the presence of packaging plasmids.

mESCs expressing bio-YY1 (25) were depleted of MEFs and transduced with the lentivirus for the expression of dCas9 and each of the targeting RNA constructs. Selection for cells expressing both dCas9 and each of the targeting constructs was conducted in the presence of 500 $\mu\text{g/ml}$ of geneticin (Life Technologies, 10131035) and 500 $\mu\text{g/ml}$ of hygromycin B (Sigma, H0654). Selection began 24hrs from the start of transduction and continued for another 7 days. Once selection was finished, cells were maintained on DR4 MEFs in media containing 100 $\mu\text{g/ml}$ of geneticin and 100 $\mu\text{g/ml}$ of hygromycin B. Expression of dCas9 was induced with 1 $\mu\text{g/ml}$ of doxycycline and cells were collected 72hrs later. Cells were crosslinked using formaldehyde in three biological replicates and the extent of YY1 binding at targeted and control untargeted enhancers in the same cells was evaluated after affinity purification of YY1-associated DNA followed by qPCR.

Affinity purification and qPCR analysis (ChIP-qPCR) of YY1-associated DNA in the in vivo tethering experiments

Approximately 2×10^7 mESCs were chemically crosslinked by the addition of one-tenth volume of fresh 11% formaldehyde solution for 10 minutes at room temperature. Formaldehyde was quenched by addition of one-twentieth volume of 2.5M Glycine and incubation for another 5 min at room temperature. Cells were rinsed twice with 25 ml of ice-cold 1X PBS and harvested

using a silicon scraper, pelleted down by centrifugation at 1,500 g for 5 min at 4°C, and pellets were flash-frozen in liquid nitrogen. Cells were stored at –80°C prior to use.

Cells were lysed in 4 ml of LB1 (50mM HEPES-KOH pH 7.5, 140mM NaCl, 1mM EDTA, 0.25% Triton X-100, 0.5% NP-40, 10% glycerol) in presence of cOmplete proteinase inhibitors (Roche) and incubated on a rotator at 4°C for 10 min. After centrifugation at 1350 g for 5 min at 4°C, nuclei were washed with 4 ml of LB2 (10mM Tris-HCl pH 8.0, 200 mM NaCl, 1mM EDTA pH 8.0, 1mM EGTA pH 8.0 in presence of cOmplete proteinase inhibitors (Roche)) by incubating them on a rotator at 4°C for 10 min. After centrifugation at 1350 g for 5 min at 4°C, nuclei were resuspended in 2 ml of the final sonication buffer (50mM HEPES-KOH pH 7.5, 140mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate in presence of cOmplete proteinase inhibitors (Roche)) and sonicated on ice (7 cycles at 30 seconds each at 18 watts 60 second pause between pulses, output level 4.5) using Misonix Sonicator 3000. The resulting whole cell extract was cleared by centrifugation for 10 min at 20,000 g and then incubated overnight at 4°C with 50 μ l Dynabeads® MyOne™ Streptavidin T1 (Life Technologies, 65601) magnetic beads pre-blocked with 0.5% BSA in PBS for 2hrs at 4°C. Beads were washed at room temperature for 8 min with each of the following buffers: 2X with 2% (vol/vol) SDS in PBS; 1X with 50mM HEPES pH 7.5, 350mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS; 1X with 20mM Tris-HCl pH 8.0, 250 mM LiCl, 1mM EDTA, 0.5% NP40, 0.5% sodium deoxycholate; 2X with TE.

DNA was eluted off beads in 300 μ l of elution buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1% SDS) by rotating the tubes containing the beads overnight at 65°C. SDS in the eluted sample was diluted two-fold with TE buffer, and RNA was digested by incubating the tubes at 37°C for 30 min in presence of 3 μ l of RNase A (30mg/ml). Following incubation, proteins were digested with 7 μ l of 20 mg/ml Proteinase K in presence of 3 μ l of 1M CaCl₂ at 55°C for 1.5hrs. After phenol/chloroform extraction, DNA was ethanol precipitated and resuspended in 50 μ l. DNA isolated from 5% of the initial whole-cell extract (input DNA) was similarly resuspended in 50 μ l of H₂O.

1 μ l of the affinity-purified YY1-associated DNA or input DNA was used in qPCR reactions containing 1x *Power* SYBR Green PCR master mix (Life Technologies). *Arid1a* enhancer, bound by YY1 in mESCs, was chosen as a positive control region, whereas olfactory receptor gene, which has no YY1 binding in mESCs, was chosen as a negative control region. First, fold enrichment in YY1 binding at *Arid1a* enhancer in each ChIP sample over the YY1 binding at the same enhancer in the corresponding input sample was estimated relative to the fold enrichment at the negative control region in the same sample as a measure of the affinity purification efficiency in different samples. As a result, to compensate for differences in affinity purification efficiency among different samples, normalization coefficients were derived for each ChIP sample. Then, fold enrichment in YY1 binding at each of the six targeted enhancers and at three not targeted enhancers in each ChIP sample over the corresponding input sample was estimated relative to the fold enrichment at the negative control region in the same sample. These fold enrichment values were then multiplied by the normalization coefficients to allow comparison between different samples. Finally, fold enrichment at the targeted enhancers in cells containing tethered RNA was compared to fold enrichment at these enhancers in cells containing only the corresponding guide RNA (sgRNA). The differences in the fold enrichments were expressed as fold change in YY1 binding in cells containing tethered RNA relative to cells containing only the corresponding guide RNA.

Primer sequences were the following:

***Arid1a* enhancer**

Forward GGCAAACCTTTCGGTTCAGTGG
Reverse TTTTCCTCCCCCAAGACAGG

Negative control region

Forward CCCACCTTGTGTTCAAATGCTGA
Reverse ACGCTTTTCTTCTGCCTTCTGC

***Cnot6* enhancer**

Forward AACCATCAGGAAGGCATCAGG
Reverse AGCTGATAGGCACTCTGGGTA

***Suz12* enhancer**

Forward GATCTCCGTACAAGCAGGAGG
Reverse ACTCACCTAAATCTGCCTGCC

***E2f3* enhancer**

Forward AGATGCCAGAGGTCACCTTTG
Reverse CAAGAGGATTTGTGGGGCTCT

***Nufip2* enhancer**

Forward TACACGTGTGCGCCAGAAGAG
Reverse TCTAGGAGCCCAGCCCCTTTT

***Klf5* enhancer**

Forward CTCCATTGTCCTAGGGATGCC
Reverse ACTTTATTCACGGGGCTCCAG

***Pias1* enhancer**

Forward ACTGACTTCCTCCAAGGCCAC
Reverse TGAATGCTTGGTCCCCAGTGT

p-values for the *in vivo* tethering experiments are shown below:

	Fold change in YY1 binding relative to control			<i>p</i> -value for changes relative to control
	exp1	exp2	exp3	
Targeted enhancer <i>Suz12</i>	1.64	1.36	1.54	0.01
not targeted enhancer <i>Cnot6</i>	0.75	0.85	0.91	0.96
not targeted enhancer <i>E2f3</i>	1.19	1.03	1.16	0.06
not targeted enhancer <i>Klf5</i>	1.22	0.80	1.13	0.37
Targeted enhancer <i>Cnot6</i>	1.14	1.34	1.32	0.03
not targeted enhancer <i>Suz12</i>	0.87	0.82	1.18	0.63
not targeted enhancer <i>E2f3</i>	0.81	0.72	0.91	0.96
not targeted enhancer <i>Klf5</i>	1.06	1.12	0.89	0.38

Targeted enhancer <i>E2f3</i>	1.5	1.58	1.43	0.00
not targeted enhancer <i>Cnot6</i>	1.30	1.22	1.31	0.01
not targeted enhancer <i>Suz12</i>	1.08	1.15	1.29	0.05
not targeted enhancer <i>Klf5</i>	0.74	1.01	0.75	0.90
Targeted enhancer <i>Klf5</i>	1.64	1.81	2.41	0.03
not targeted enhancer <i>Suz12</i>	0.96	0.69	0.87	0.91
not targeted enhancer <i>E2f3</i>	1.02	0.68	1.10	0.67
not targeted enhancer <i>Cnot6</i>	0.89	0.86	0.83	0.99
Targeted enhancer <i>Pias1</i>	1.51	1.39	1.31	0.01
not targeted enhancer <i>Suz12</i>	0.79	1.09	1.05	0.59
not targeted enhancer <i>E2f3</i>	1.10	1.12	0.82	0.45
not targeted enhancer <i>Cnot6</i>	1.19	0.98	1.15	0.12
Targeted enhancer <i>Nufip2</i>	1.87	2.18	1.42	0.03
not targeted enhancer <i>Suz12</i>	0.85	0.97	1.19	0.49
not targeted enhancer <i>E2f3</i>	1.05	1.07	1.02	0.04
not targeted enhancer <i>Cnot6</i>	0.82	0.92	0.76	0.96

SUPPLEMENTARY FIGURES

Fig. S1: YY1 binds to DNA and RNA at promoter-proximal and distal elements in murine embryonic stem cells (ESCs).

Fig. S2: OCT4 and YY1 are associated with promoters of active genes.

Fig. S3: YY1 CLIP-seq sample preparation and analysis.

Fig. S4: YY1 ChIP-seq and YY1 CLIP-seq reads are distributed similarly among various genomic regions.

Fig. S5: OCT4 does not bind to RNA *in vivo* or *in vitro*.

Fig. S6: YY1 binds to DNA probes containing consensus YY1 binding motif *in vitro* in murine ESC nuclear extracts.

Fig. S7: DNA-binding properties of recombinant YY1 are similar to properties of endogenous YY1 present in murine ESC nuclear extracts.

Fig. S8: YY1 binds to some RNA species *in vitro*.

Fig. S9: Different regions in YY1 are responsible for binding to DNA and RNA.

Fig. S10: Perturbation of RNA levels using transcription inhibitors affects YY1 binding to DNA.

Fig. S11: Treatment of ESCs with DRB does not change steady-state levels of YY1.

Fig. S12: RNA-seq and Western blot analyses of ESCs, in which an exosome component was targeted with shRNA.

Fig. S13. RNA sequences compatible with YY1 binding *in vitro* enhance YY1 binding *in vivo* when tethered near an YY1 binding site in DNA.

Fig. S14. Analysis of YY1 binding to probes utilized in the competition EMSA.

Fig. S15. DNA containing tethered RNA outcompetes DNA with untethered RNA in competition EMSA.

Fig. S16. EMSA of various transcription factors in complexes with RNA.

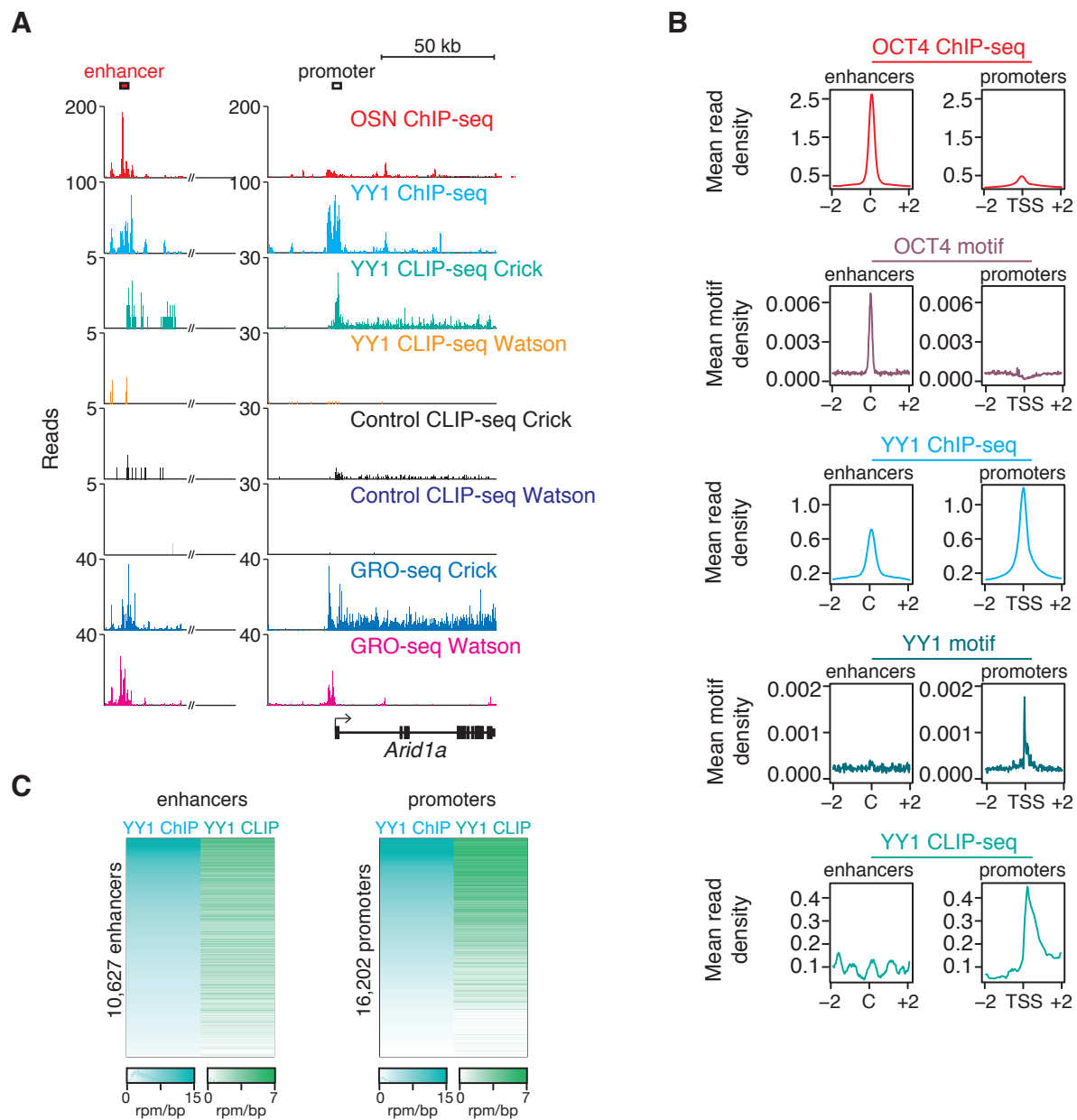


Fig. S1. YY1 binds to DNA and RNA at promoter-proximal and distal elements in murine embryonic stem cells (ESCs).

(A). Gene tracks for the *Arid1a* gene showing ChIP-seq reads for OCT4, SOX2, and NANOG (OSN) and bio-YY1, together with CLIP-seq data for bio-YY1 cells and control ESCs expressing only biotin ligase *BirA*. Bio-YY1 and control ESCs were previously described (25). Also shown are GRO-seq reads at the *Arid1a* gene and its enhancer. CLIP-seq and GRO-seq reads that map to Watson and Crick strands of DNA are shown separately. Portions of this panel are duplicated from Fig.1C for context.

(B). Mean read density of OCT4 ChIP-seq, YY1 ChIP-seq, and YY1 CLIP-seq reads as well as alignment of Oct4 and YY1 motif occurrences at enhancers and promoters of all RefSeq genes in ESCs. The x-axis indicates distance from either the enhancer center (C) or the transcription start site (TSS) in kilobases. The y-axis indicates mean density of uniquely mapped reads or average count of motifs per genomic bin. Portions of this panel are duplicated from Fig.1D for context.

(C). Heatmap comparing YY1 binding to DNA and RNA at enhancers and promoters of all RefSeq genes in ESCs using ChIP-seq and CLIP-seq data. Enhancers and promoters were ranked based on number of YY1 ChIP-seq reads.

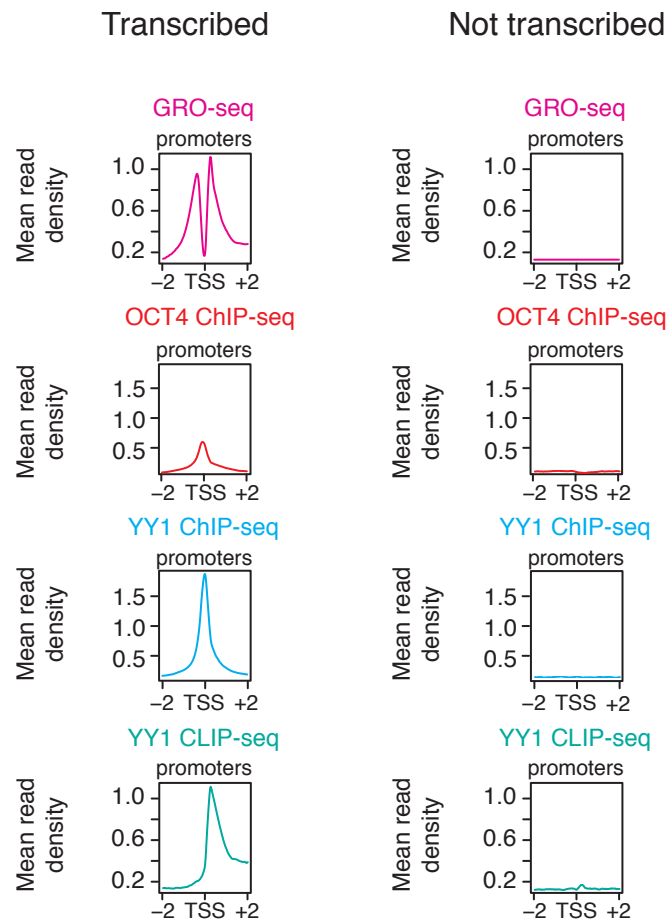


Fig. S2. OCT4 and YY1 are associated with promoters of active genes.

Alignment of GRO-seq, OCT4 ChIP-seq, YY1 ChIP-seq, and YY1 CLIP-seq at promoters of transcribed RefSeq genes and at promoters of genes that are not transcribed in murine ESCs. The x-axis indicates distance from the TSS in kilobases. The y-axis indicates average density of uniquely mapped reads per genomic bin. Transcribed promoters have more than one GRO-seq read assigned per kilobase of target per million mapped reads (RPKM), whereas not transcribed ones have no GRO-seq and no histone 3 trimethylated at lysine 4 (H3K4me3) ChIP-seq reads.

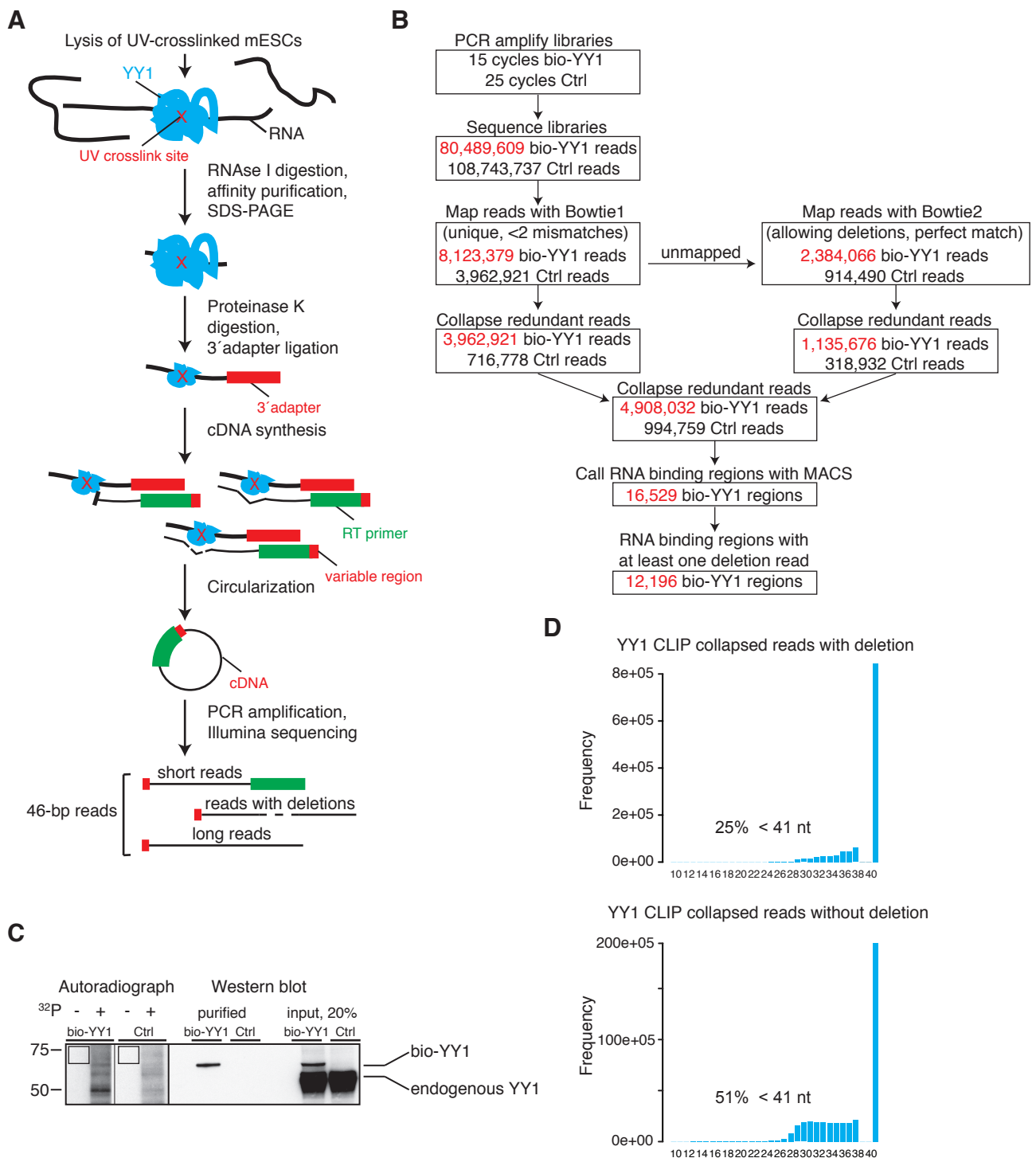


Fig. S3. YY1 CLIP-seq sample preparation and analysis.

(A). Schematic of CLIP-seq protocol used for identification of RNA species bound by YY1 *in vivo*.

(B). Flowchart of pipeline for identification of RNA regions associated with biotinylated-YY1 (bio-YY1). Each step of the analysis pipeline is indicated above rectangular boxes. Number of reads or regions at each step of the analysis pipeline is shown in red for bio-YY1 and in black for control (Ctrl) *BirA* mESCs.

(C). Western blot analysis of bio-YY1 purified during the CLIP procedure is shown alongside the autoradiograph of the nitrocellulose membrane obtained after transfer of RNA species UV-crosslinked to the affinity purified bio-YY1 protein from SDS-PAGE to the membrane. Control (Ctrl) CLIP procedure was conducted using cells containing only *BirA* biotin ligase and no bio-YY1. Rectangular boxes indicate regions of the membrane, which were excised to isolate the RNA.

(D). Distribution of lengths of YY1 CLIP-seq reads either containing or lacking deletions demonstrates that deletion reads are longer than reads without deletions consistent with both types of reads being derived from UV-crosslinked RNA species.

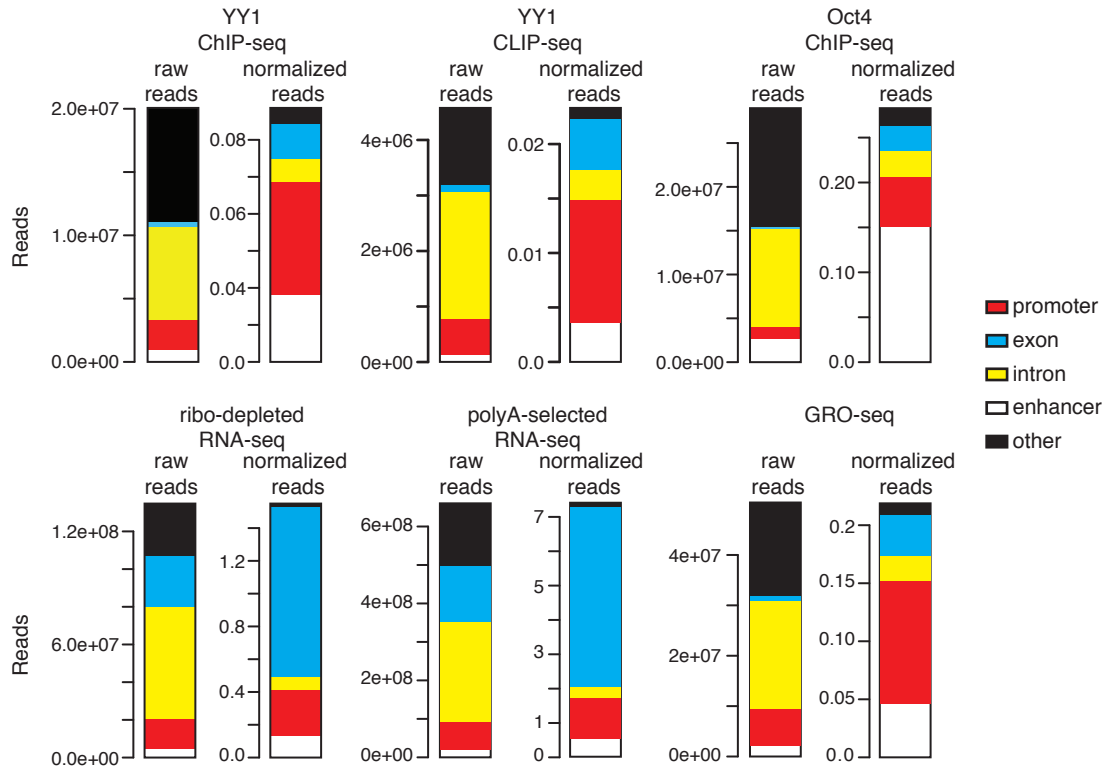


Fig. S4. YY1 ChIP-seq and YY1 CLIP-seq reads are distributed similarly among various genomic regions.

Stacked bar-plots comparing distributions of YY1 ChIP-seq and YY1 CLIP-seq reads among promoters, enhancers, exons, introns, and all other genomic locations. Distributions of Oct4 ChIP-seq reads, ribo-depleted RNA-seq reads, polyA-selected RNA-seq reads, and GRO-seq reads are shown as controls. Data are presented as either raw reads or reads normalized to the size of these regions in genomic DNA.

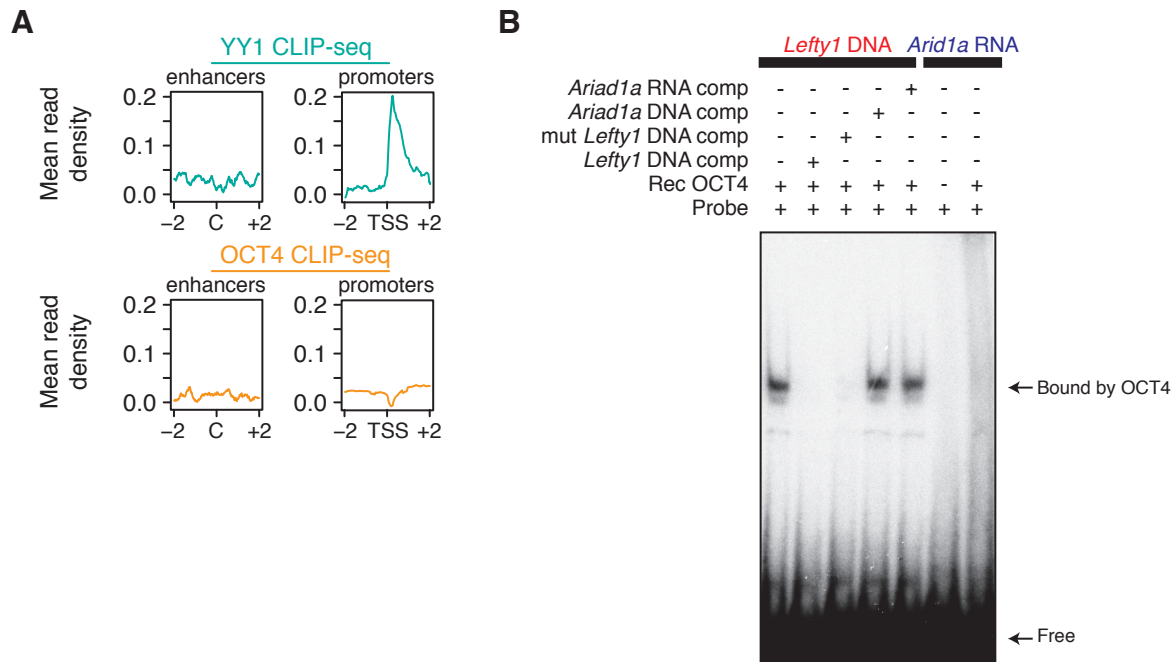


Fig. S5. OCT4 does not bind to RNA *in vivo* or *in vitro*.

(A). Comparison of YY1 CLIP-seq and OCT4 CLIP-seq reads at enhancers and promoters of all RefSeq genes. Background CLIP read densities obtained by sequencing of RNA isolated from control ESCs expressing only *BirA* biotin ligase were subtracted from CLIP read densities obtained by sequencing of RNA isolated from ESCs expressing biotinylated versions of YY1 (bio-YY1) or OCT4 (bio-OCT4). The x-axis indicates distance from the TSS in kilobases. The y-axis indicates average density of uniquely mapped reads per genomic bin.

(B). EMSA analysis of DNA in complex with recombinant human OCT4 protein (Rec OCT4) in the presence and absence of competitor DNA. A radioactively labeled 40-bp DNA probe containing OCT4 consensus binding motif derived from the enhancer of *Lefty1* gene was incubated with 250 nM of the recombinant human OCT4 (Abcam, #ab134876). In competition EMSA, 100-fold molar excess of cold competitor DNA containing the OCT4 motif (*Lefty1* DNA comp), cold DNA competitor, in which bases adjacent the OCT4 motif were randomly substituted (mut *Lefty1* DNA comp), cold DNA competitor lacking the OCT4 motif (*Arid1a* DNA comp), or cold RNA competitor (*Arid1a* RNA comp) was pre-incubated with the recombinant OCT4 before the radioactively labeled DNA probe was added to the binding reaction. The arrows indicate free probe and probe bound by OCT4. Incubation of a 30-nt RNA probe derived from the promoter region of *Arid1a* gene with 250 nM of the recombinant human OCT4 did not retard the probe.

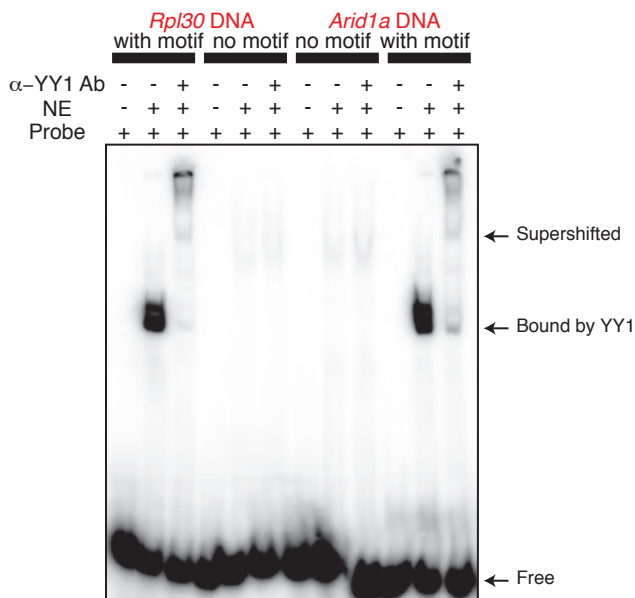
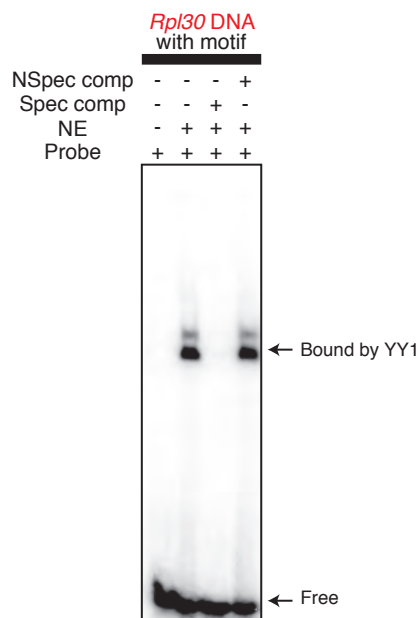
A**B**

Fig. S6. YY1 binds to DNA probes containing consensus YY1 binding motif *in vitro* in murine ESC nuclear extracts.

(A). EMSA of YY1-DNA complexes. 10 μ l of ESC nuclear extract (NE) was incubated with 30-bp radioactively labeled DNA probes. For supershift assay, 0.5 μ l of YY1 antibodies (#sc-7341) was added to the DNA probe pre-incubated with NE. Identity of the DNA probes is shown above the image. The arrows indicate free probe, probe bound by YY1, and supershifted probe.

(B). EMSA analysis of YY1-DNA complexes in the presence and absence of competitor DNA. Radioactively labeled 30-bp DNA probe containing YY1 consensus binding motif derived from the promoter region of *Rpl30* gene was incubated with 10 μ l of ESC nuclear extract (NE). In competition EMSA, 100-fold molar excess of cold competitor DNA containing YY1 motif (Spec comp) or lacking YY1 motif (Nspec comp) was pre-incubated with NE before the radioactively labeled DNA probe was added to the binding reaction. The arrows indicate free probe and probe bound by YY1.

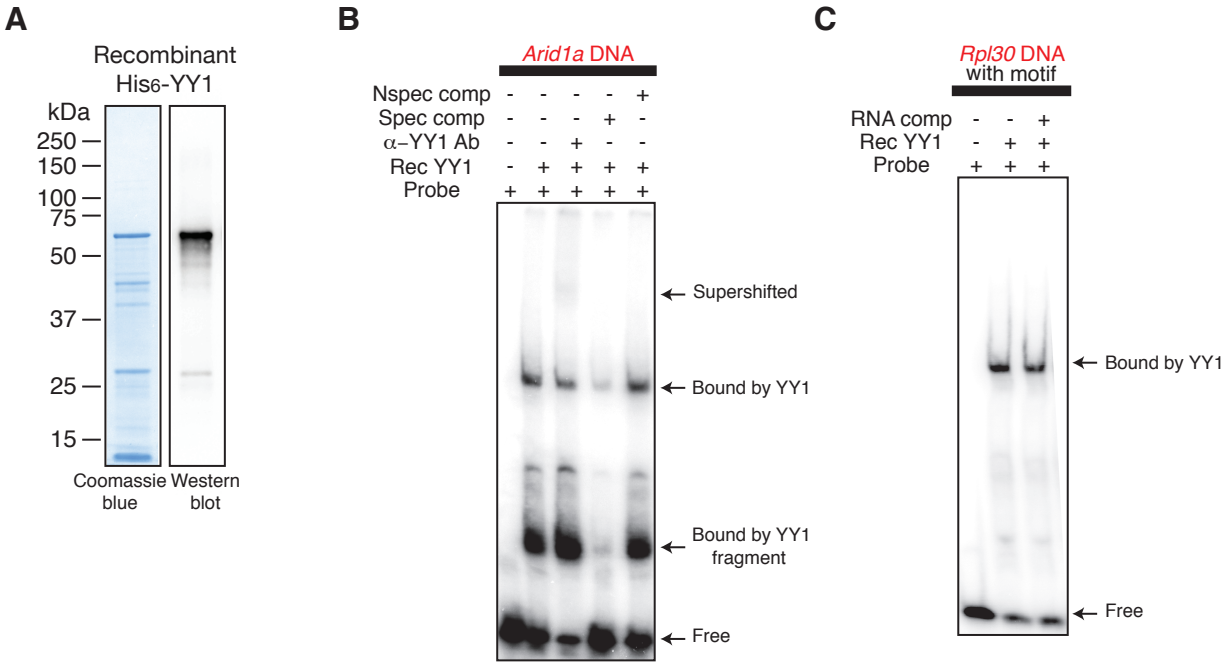


Fig. S7. DNA-binding properties of recombinant YY1 are similar to properties of endogenous YY1 present in murine ESC nuclear extracts.

(A). Coomassie Blue staining and Western blot analysis of recombinant YY1 used for *in vitro* studies.

(B). EMSA analysis of DNA in complex with recombinant YY1 protein (Rec YY1) in presence and absence of competitor DNA. A radioactively labeled 30-bp DNA probe containing YY1 consensus binding motif derived from the promoter region of *Arid1a* gene was incubated with 80 nM of the recombinant YY1. For supershift assay, 0.5 μ l of YY1 antibodies (#sc-7341) was added to the DNA probe pre-incubated with the recombinant protein. In competition EMSA, 100-fold molar excess of cold competitor DNA containing YY1 motif (Spec comp) or lacking YY1 motif (Nspec comp) was pre-incubated with the recombinant YY1 before the radioactively labeled DNA probe was added to the binding reaction. The arrows indicate free probe, probe bound by YY1, probe bound by YY1 fragment, and supershifted probe.

(C). EMSA analysis of YY1-DNA complexes in the presence and absence of competitor RNA. Radioactively labeled 30-bp DNA probe containing YY1 consensus binding motif derived from the promoter region of *Rpl30* gene was incubated with 240 nM of the recombinant YY1. In competition EMSA, 100-fold molar excess of cold competitor RNA derived from the promoter region of *Arid1a* gene was pre-incubated with the recombinant YY1 before the radioactively labeled DNA probe was added to the binding reaction. The arrows indicate free probe and probe bound by YY1.

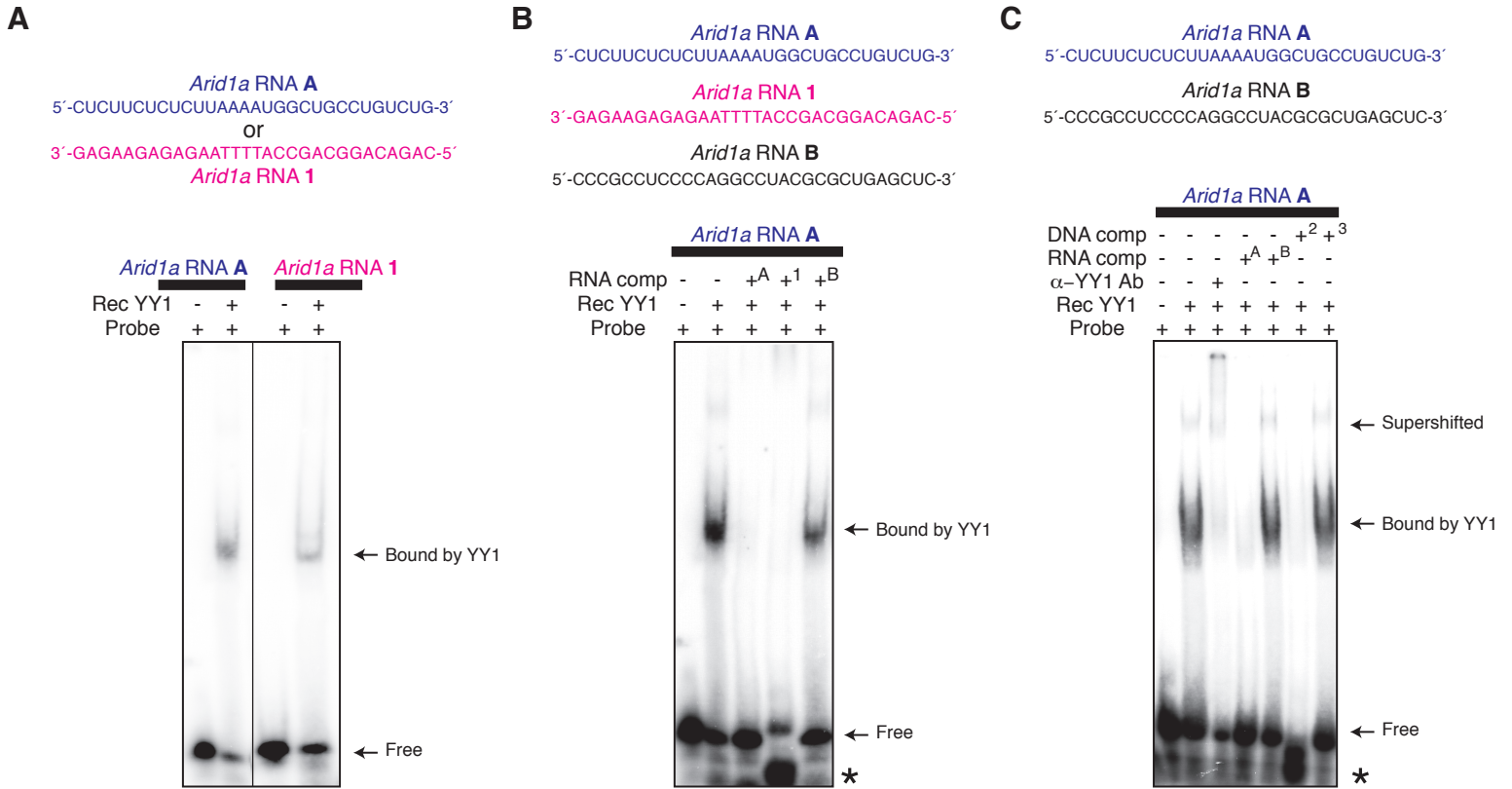


Fig. S8. YY1 binds to some RNA species *in vitro*.

(A). EMSA analysis of RNA in complex with recombinant YY1 protein (Rec YY1). A radioactively labeled 30-nt RNA probe containing sequence derived from the promoter region of *Arid1a* gene (shown in blue above the image) or the complementary sequence (shown in pink) was incubated with 400 nM of the recombinant YY1. The arrows indicate free probe and probe bound by YY1.

(B). EMSA analysis of YY1-RNA complexes in presence and absence of competitor RNA. Radioactively labeled 30-nt RNA probe derived from the promoter region of *Arid1a* gene was incubated with 400 nM of recombinant YY1. Sequence of the probe is shown above the image in blue. In competition EMSA, 100-fold molar excess of cold competitor RNA with the same sequence as the radiolabeled RNA (*Arid1a* RNA A), complementary sequence (*Arid1a* RNA 1), or a different sequence (*Arid1a* RNA B) was pre-incubated with the recombinant YY1 before the probe was added to the binding reaction. The arrows indicate free probe and probe bound by YY1. Asterisk indicates the position of double-stranded RNA formed when single-stranded cold RNA was incubated with the complementary labelled RNA probe.

(C). EMSA analysis of YY1-RNA complexes in presence and absence of competitor RNA. Radioactively labeled 30-nt RNA probe derived from the promoter region of *Arid1a* gene was incubated with 400 nM of recombinant YY1. Sequence of the probe and competitor RNA are shown above the image. For supershift assay, 0.5 μ l of YY1 antibodies (#sc-7341) was added to the RNA probe. In competition EMSA, 100-fold molar excess of cold competitor RNA with the same sequence as the radiolabeled RNA probe (*Arid1a* RNA A) or a different sequence (*Arid1a* RNA B) was pre-incubated with the recombinant YY1 before the probe was added to the binding reaction. Other cold competitors were double-stranded DNA derived from the promoter region of *Arid1a* gene containing the YY1 motif and the same sequence as the radiolabeled RNA probe (competitor 2) and double-stranded DNA derived from the promoter region of *Rpl30* gene containing the YY1 motif (competitor 3). The arrows indicate free probe, probe bound by YY1, and supershifted probe. Asterisk indicates the position of DNA-RNA hybrid probe formed when remnants of unannealed single-stranded DNA present in the cold double-stranded *Arid1a* DNA competitor interacted with the complementary labelled RNA probe.

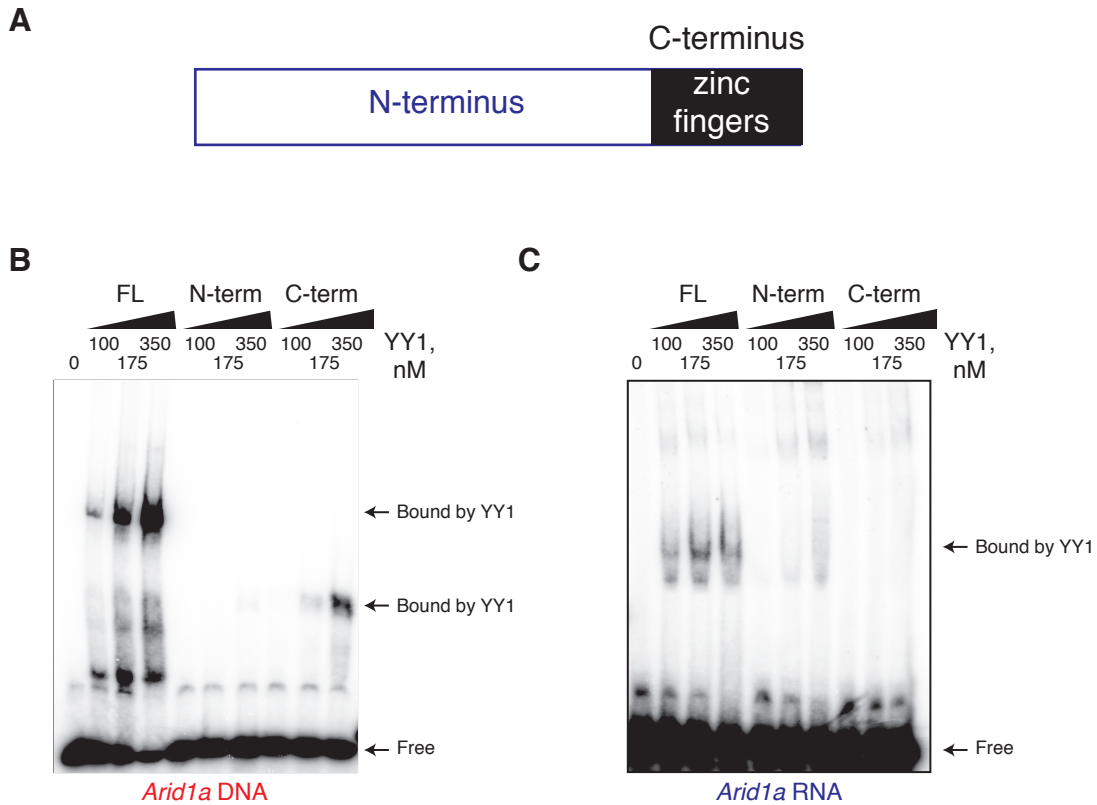


Fig. S9. Different regions in YY1 are responsible for binding to DNA and RNA.

(A). Cartoon depicting regions of YY1 used in EMSA. Sizes of N-terminal and C-terminal regions of YY1 are drawn to scale.

(B). EMSA of YY1-DNA complexes at different concentrations of recombinant YY1. 5 nM of radioactively labeled 30-bp DNA probe derived from the promoter region of *Arid1a* gene containing a consensus YY1 binding motif was incubated with increasing concentrations of full-length recombinant YY1 protein (FL), the N-terminal portion of YY1 (N-term) lacking the zinc-fingers or the C-terminal portion (C-term) containing the zinc fingers. Concentration of the YY1 protein in binding reactions is displayed above the image. The arrows indicate free probe and probe bound by YY1.

(C). EMSA of YY1-RNA complexes at different concentrations of recombinant YY1. 5 nM of radioactively labeled 30-nt RNA probe derived from the promoter region of *Arid1a* gene was incubated with increasing concentrations of full-length recombinant YY1 protein (FL), the N-terminal portion of YY1 (N-term) lacking the zinc-fingers or the C-terminal portion (C-term) containing the zinc fingers. Concentration of the YY1 protein in binding reactions are displayed above the image. The arrows indicate free probe and probe bound by YY1.

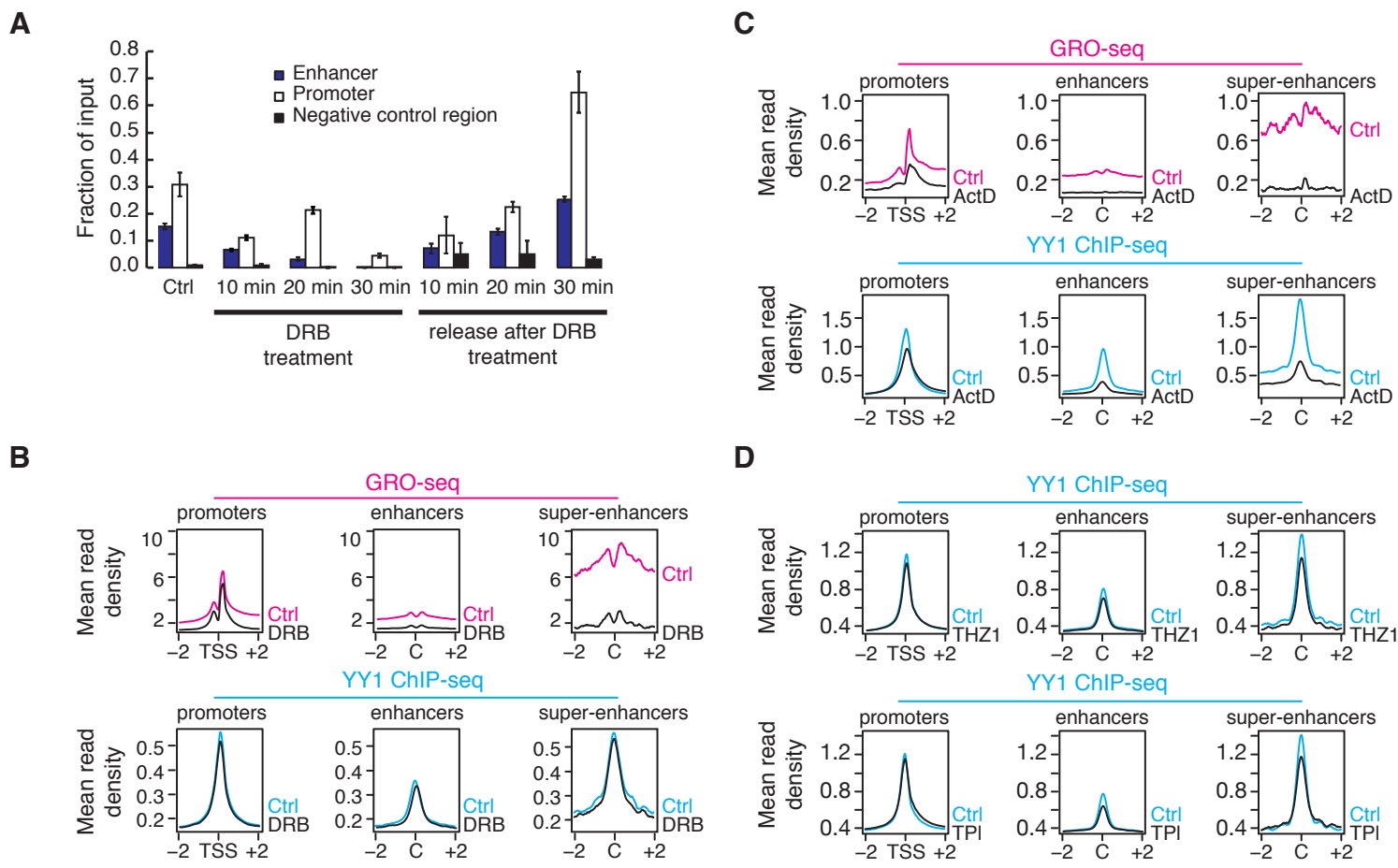


Fig. S10. Perturbation of RNA levels using transcription inhibitors affects YY1 binding to DNA.

(A). Results of YY1 ChIP followed by quantitative PCR (ChIP-qPCR) analysis at the *Arid1a* enhancer, promoter, and a negative control region in mESCs treated with D-rybofuranosylbenzimidazole (DRB) for various time intervals, and in ESCs at several time points after DRB was removed from the culture media (release after DRB treatment). The y-axis indicates YY1 binding at the specified genomic regions as a fraction of input.

(B). (Top) Alignment of GRO-seq reads at promoters of all RefSeq genes and at enhancers and super-enhancer constituents in control untreated ESCs and cells treated with DRB (24). (Bottom) Alignment of YY1 ChIP-seq reads at promoters of control untreated ESCs and in cells treated with DRB for 30 min. The x-axis indicates distance from either the TSS or from the enhancer or super-enhancer constituent center (C) in kilobases. The y-axis indicates average density of uniquely mapped GRO-seq or ChIP-seq reads per genomic bin. Decrease in YY1 binding in presence of DRB is significant: p -value $< 3.8 \times 10^{-15}$ for promoters, p -value $< 2.4 \times 10^{-22}$ for enhancers, p -value $< 2.7 \times 10^{-3}$ for super-enhancer constituents. p -values were calculated using the one-tailed t -test.

(C). Alignment of GRO-seq and YY1 ChIP-seq reads at promoters of all RefSeq genes as well as at enhancers and super-enhancer constituents in control DMSO-treated ESCs and in cells treated with actinomycin D (ActD) for 6 hrs. The decrease in YY1 binding in the presence of ActD is significant: p -value $< 4.5 \times 10^{-7}$ for promoters, p -value $< 1.0 \times 10^{-1023}$ for enhancers, p -value $< 3.8 \times 10^{-82}$ for super-enhancer constituents.

(D). Alignment of YY1 ChIP-seq reads at promoters of all RefSeq genes as well as at enhancers and super-enhancer constituents in control DMSO-treated ESCs and in cells treated with THZ1 or triptolide (TPI) for 6 hrs. Decrease in YY1 binding in presence of THZ1 is significant: p -value $< 2.1 \times 10^{-6}$ for promoters, p -value $< 2.5 \times 10^{-27}$ for enhancers, p -value $< 1.5 \times 10^{-8}$ for super-enhancer constituents. Decrease in YY1 binding in presence of TPI is significant: p -value $< 4.4 \times 10^{-18}$ for enhancers, p -value $< 2.8 \times 10^{-1}$ for super-enhancer constituents.

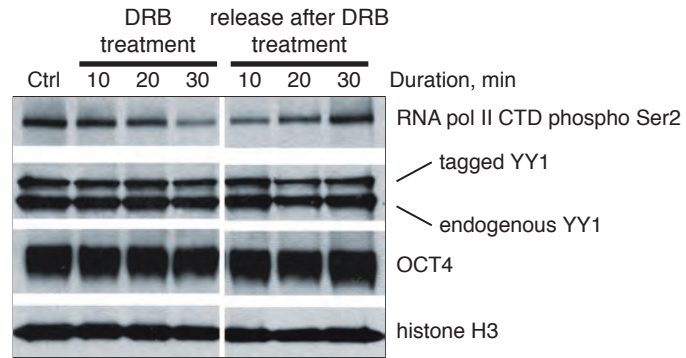


Fig. S11. Treatment of ESCs with DRB does not change steady-state levels of YY1.

Western blot analysis of protein levels of RNA polymerase II phosphorylated at serine 2 (RNA pol II CTD phospho Ser2), YY1, and OCT4 in control ESCs, in cells treated with DRB within intervals of time shown above the graph, and in ESCs at several time points once DRB was removed from the culture media (release of inhibition). Histone H3 and OCT4 serve as controls.

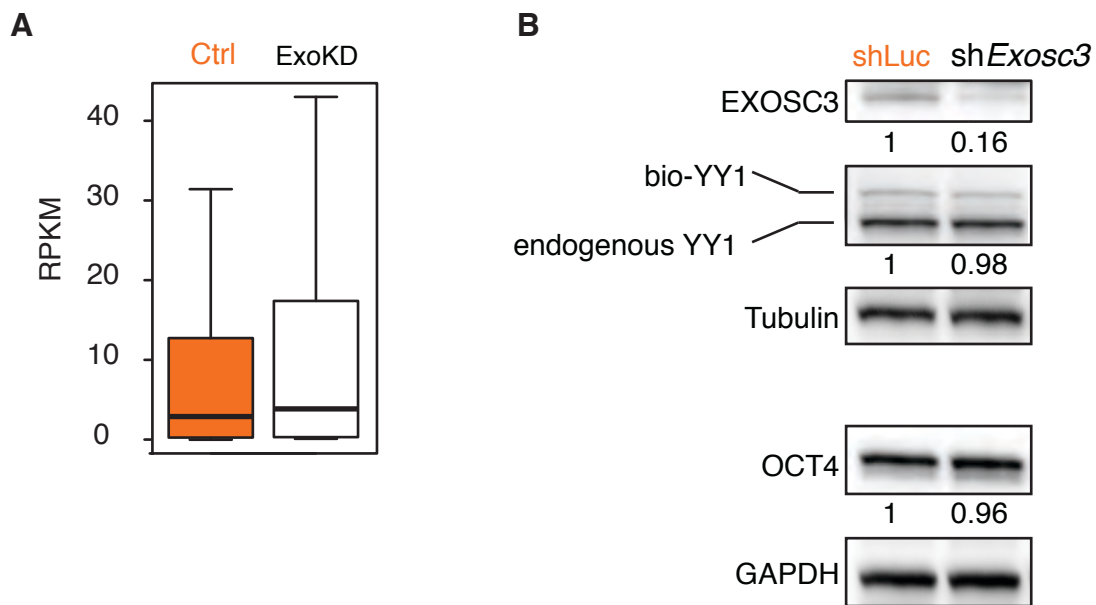


Fig. S12. RNA-seq and Western blot analyses of ESCs, in which an exosome component was targeted with shRNA.

(A). Box plot of changes in expression of all RefSeq transcripts in ESCs targeted with control shRNA against luciferase gene (Ctrl) or with shRNA against *Exosc3*. RPKM values for RefSeq transcripts and ERCC spike-in probes were calculated using RPKM_count.py (RSeQC). These values were then floored at 0.01 and a pseudocount of 0.1 was added to all entries. Using normalize.loess from the affy R package, RPKM values for the RefSeq transcripts were subsequently normalized using all ERCC probes as the normalization subset, and the distributions were further log-normalized using log.it. RefSeq transcripts were significantly up-regulated in cells targeted with shRNA against *Exosc3* relative to control cells (p -value $< 5.07 \times 10^{-25}$). The p -value was calculated using a one-tailed Wilcoxon rank sum test.

(B). Western blot analyses of protein levels of exosome component EXOSC3 as well as levels of YY1 and OCT4 in ESCs targeted with control shRNA against luciferase gene (Ctrl) or with shRNA against *Exosc3*. Levels of GAPDH and β -Tubulin serve as loading controls. Normalized values are shown below corresponding blots.

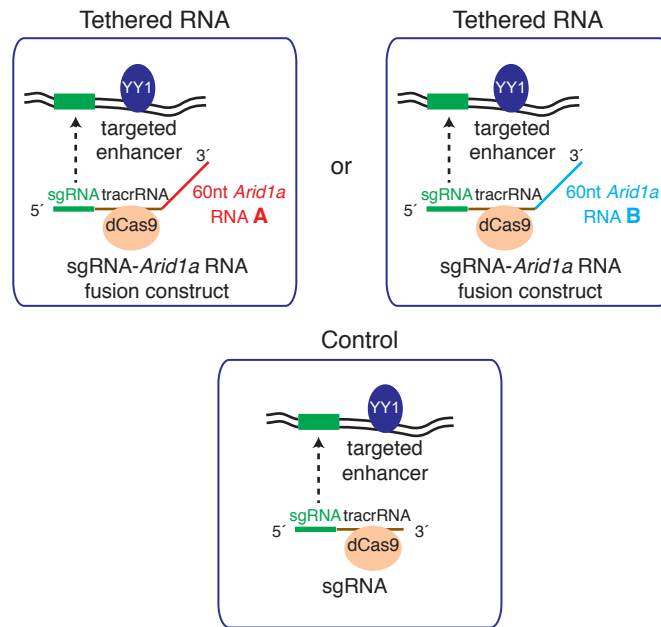
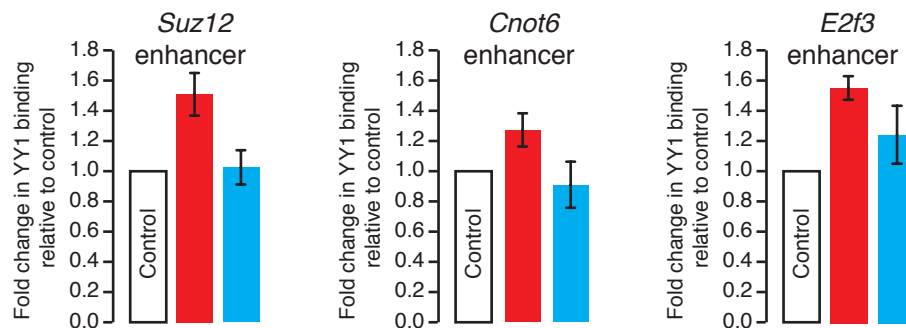
A**B**

Fig. S13. RNA sequences compatible with YY1 binding *in vitro* enhance YY1 binding *in vivo* when tethered near an YY1 binding site in DNA .

(A). Cartoon depicting strategy for tethering of RNA in the vicinity of YY1 binding site at three different enhancers *in vivo*. Control ESCs were engineered to express catalytically inactive endonuclease Cas9 (dCas9) and guide RNAs (sgRNA) fused to tracrRNA, permitting targeting of dCas9 near an YY1 binding site at the three enhancers. Experimental ESC lines were engineered to express dCas9 with the same sgRNAs and tracrRNA fused to either a 60-nt RNA from the promoter region of *Arid1a* gene containing RNA sequence compatible with YY1 binding *in vitro* (RNA A shown in red) or RNA sequence not compatible with YY1 binding *in vitro* (RNA B shown in blue), permitting the *Arid1a* RNAs to be targeted near the three enhancers (tethered RNA). (B). ChIP-qPCR analysis of YY1 binding at three enhancers targeted with a 60-nt RNA from the promoter region of *Arid1a* gene containing RNA sequence compatible with YY1 binding *in vitro* (shown in red) and RNA sequence not compatible with YY1 binding *in vitro* (shown in blue) in control ESCs and in ESCs containing tethered RNAs in three independent experiments. The y-axis indicates fold change in YY1 binding in ESCs expressing the sgRNA-*Arid1a* RNA fusion construct relative to cells expressing the sgRNA targeted to the same locus. The difference in YY1 binding was significant for the enhancers targeted with RNA A: *Suz12* enhancer (p -value=0.01), *Cnot6* enhancer (p -value=0.03), and *E2f3* enhancer (p -value=0.01), but not with RNA B: *Suz12* enhancer (p -value=0.4), *Cnot6* enhancer (p -value=0.8), and *E2f3* enhancer (p -value=0.1).

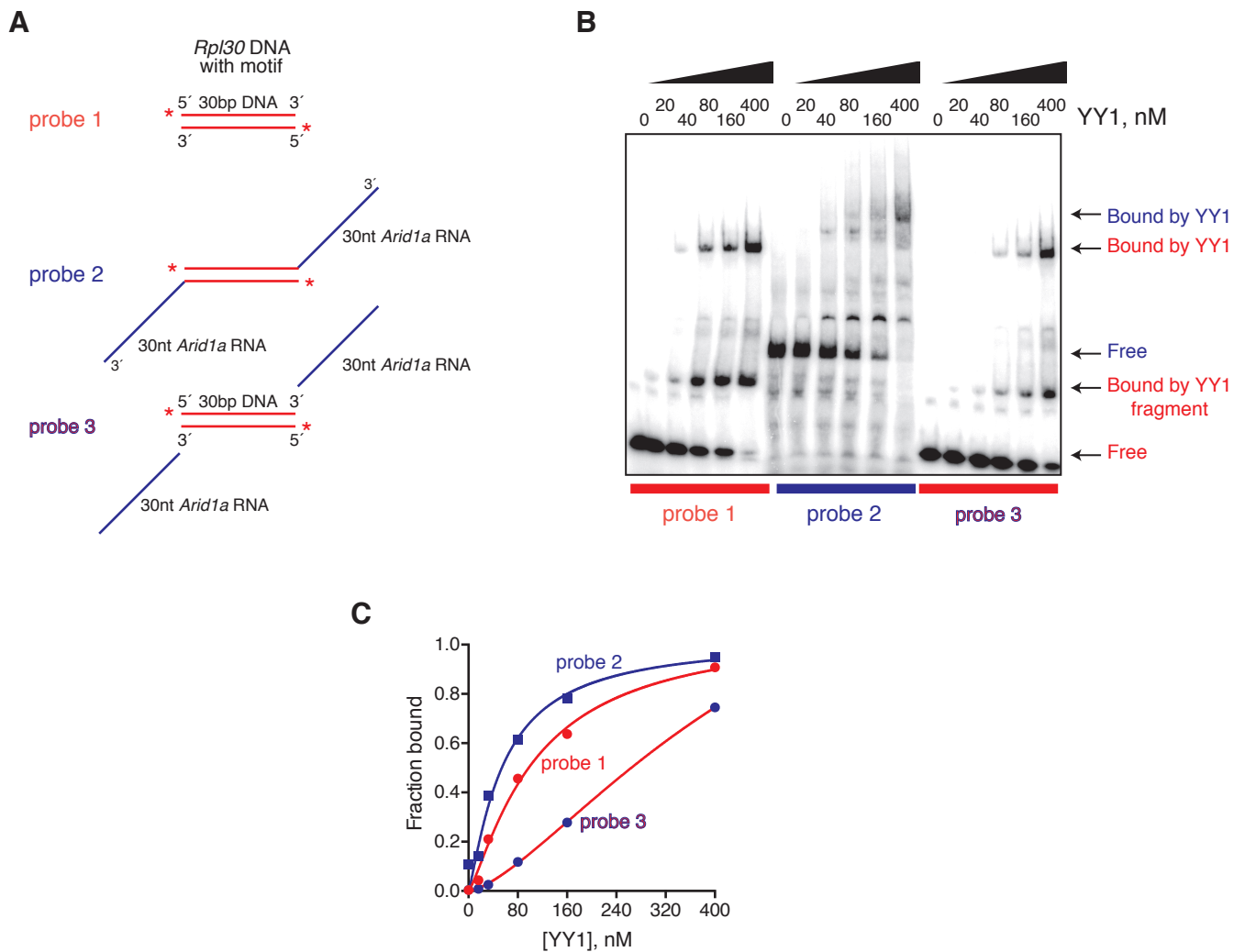


Fig. S14. Analysis of YY1 binding to probes utilized in the competition EMSA.

(A). Schematic of probes used in competition EMSA of DNA in complex with recombinant YY1. Radioactively labeled 30-bp DNA probe containing YY1 consensus binding motif derived from the promoter region of *Rpl30* gene (probe1), DNA probe with the same sequence containing 30-nt RNA derived from the promoter region of *Arid1a* gene at each 3' end of the DNA (probe 2), or 1:2 mixture of radioactively labeled 30-bp DNA containing YY1 consensus binding motif derived from the promoter region of *Rpl30* gene and 30-nt cold RNA derived from the promoter region of *Arid1a* gene (probe 3) was incubated with various concentrations of the recombinant YY1.

(B). EMSA of YY1-DNA complexes at different concentrations of recombinant YY1. 0.1 pmole of radioactively labeled probes described in **A** was incubated with increasing concentrations of recombinant YY1. Concentrations of the YY1 protein in binding reactions are displayed above the image. Arrows indicate positions of the corresponding free and bound probes.

(C). Graph depicting relationship between fraction of the radioactively labeled probes described in **A** bound by YY1 and concentration of YY1 in binding reactions.

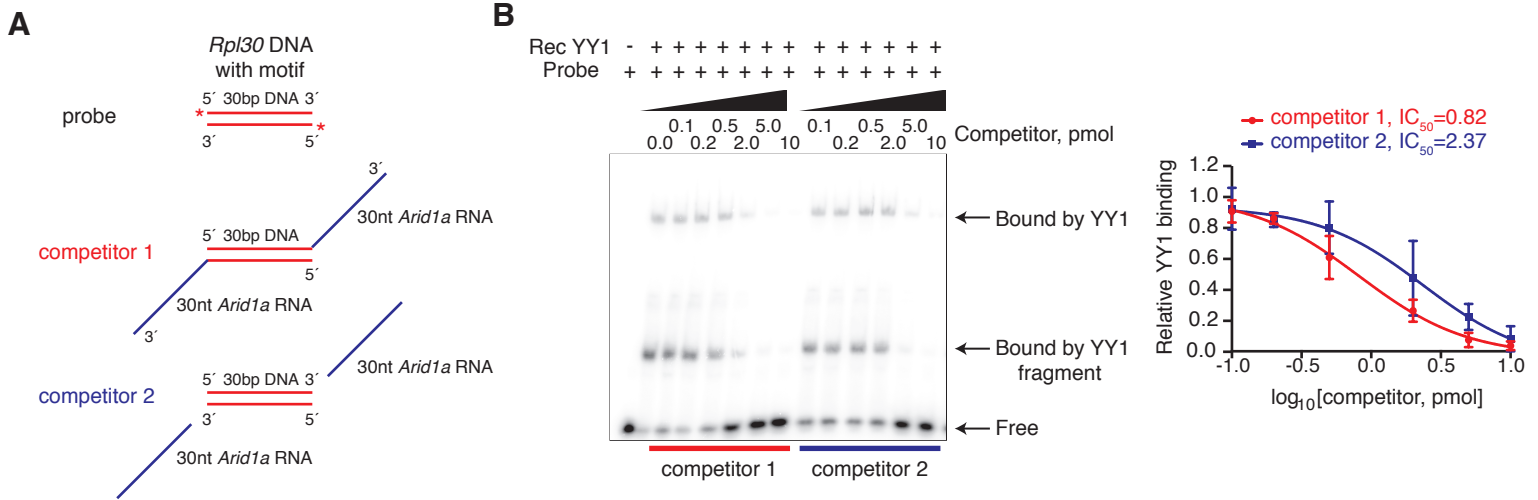


Fig. S15. DNA containing tethered RNA outcompetes DNA with untethered RNA in competition EMSA.

(A). Schematic of probes and competitors used in competition EMSA of DNA in complex with recombinant YY1. A radioactively labeled 30-bp DNA probe containing YY1 consensus binding motif derived from the promoter region of *Rpl30* gene was incubated with 200 nM of recombinant YY1 in the presence and absence of cold DNA competitor with the same sequence containing 30-nt RNA derived from the promoter region of *Arid1a* gene at each 3' end of the duplex DNA (competitor 1) or a 1:2 mixture of cold 30-bp DNA containing YY1 consensus binding motif derived from the promoter region of *Rpl30* gene and 30-nt RNA derived from the promoter region of *Arid1a* gene (competitor 2).

(B). (left) EMSA analysis of DNA in complex with recombinant YY1 (Rec YY1) in the presence and absence of competitor DNA. Competition assays were conducted with increasing amounts of the two competitors described in A. (right) Graph depicting the relationship between fraction of the radioactively labeled DNA probe bound by YY1 and the level of cold competitor in the binding reaction. IC_{50} values for the two competitors are shown above the graph. The difference between $\log(IC_{50})$ values for the two competitors is significant: p -value < 0.05. p -value is estimated using the two-tailed t -test.

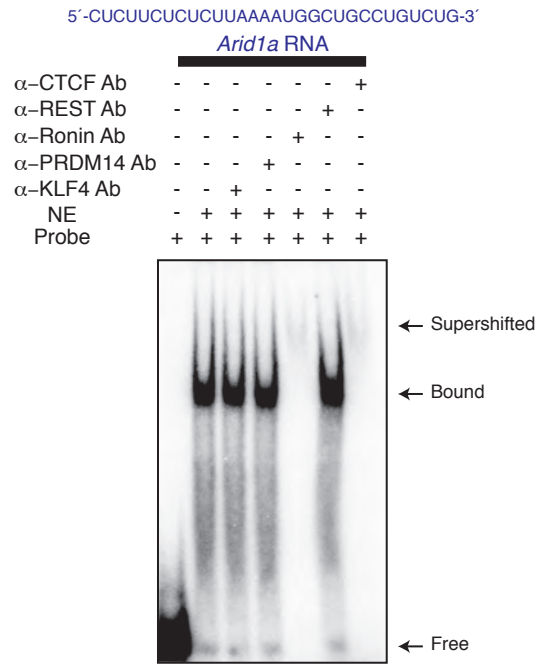


Fig. S16. EMSA of various transcription factors in complexes with RNA.

10 μ l of ESC nuclear extract (NE) was incubated with 30-nt radioactively labeled RNA probe derived from the promoter region of *Arid1a* gene, and specific antibodies recognizing various TFs were added to the EMSA reaction to identify TFs bound to the probe. Ronin and CTCF antibodies were able to retard the RNA-protein complexes formed in the NE, suggesting that both Ronin and CTCF were bound to the RNA. For supershift assay, 0.5 μ l of KLF4 antibodies (R&D, #AF3158), PRDM14 antibodies (Millipore, #4350), Ronin antibodies (Millipore, #ABE567), REST antibodies (Abcam, #26635), or CTCF antibodies (Millipore, #07-729) was added to the RNA probe pre-incubated with NE. Sequence of the RNA probe is shown above the image. The arrows indicate free probe, bound probe, and supershifted probe.

SUPPLEMENTARY TABLES

Table S1. GEO numbers for the datasets used in this study.

Table S2. YY1 ChIP-seq enriched regions.

Table S3. YY1 CLIP-seq enriched regions.

Table S1. GEO numbers for the datasets used in this study.

Experiment	IP Sample ID	Control Sample ID
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Fig. 1B metagenes

GRO-seq	GSM1665566	N/A
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Fig. 1C *Arid1a* track

YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A
GRO-seq	GSM1665566	N/A

Fig. 1D metagenes

YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A

Fig. 3B metagenes

DRB -> Release GRO-seq	GSM1579227	N/A
DRB GRO-seq	GSM1579224	N/A
DRB -> Release YY1 ChIP-seq	GSM1904538	GSM1665554, GSM1665557, GSM1665560
DRB YY1 ChIP-seq	GSM1665556	GSM1665554, GSM1665557, GSM1665560

Fig. 3C boxplots/metagenes

Control (shLuciferase) RNA-seq	GSM1904550	N/A
sh <i>Exosc3</i> RNA-seq	GSM1904549	N/A
Control (shLuciferase) YY1 ChIP-seq	GSM1904540, GSM1904541	GSM1904542, GSM1904539
sh <i>Exosc3</i> YY1 ChIP-seq	GSM1904546, GSM1904545	GSM1904544, GSM1904543

fig. S1A

OSN merged ChIP-seq	GSM1082340, GSM1082341, GSM1082342	N/A
YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A
Control CLIP-seq	GSM1665565	N/A
GRO-seq	GSM1665566	N/A

fig. S1B

Oct4 ChIP-seq	GSM1082340	N/A
YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A

fig. 1C heatmaps

YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A

Table S1. GEO numbers for the datasets used in this study.

fig. S2 metagenes		
GRO-seq	GSM1665566	N/A
OCT4 ChIP-seq	GSM1082340	N/A
YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A
H3K4me3 ChIP-seq	GSM1082344, GSM307149	N/A

fig. S3B flowchart		
YY1 CLIP-seq	GSM1665564	N/A
Control CLIP-seq	GSM1665565	N/A

fig. S4 bar-plots		
YY1 ChIP-seq	GSM1665561	N/A
YY1 CLIP-seq	GSM1665564	N/A
OCT4 ChIP-seq	GSM1082340	N/A
PolyA RNA-seq	GSM1246869	N/A
Ribo-depleted RNA-seq	GSM903663	N/A
GRO-seq	GSM1665566	N/A

fig. S5		
YY1 CLIP-seq	GSM1665564	GSM1665565
Oct4 CLIP-seq	GSE68196	GSE68196

fig. S10B		
Untreated GRO-seq	GSM1579223	N/A
Untreated YY1 ChIP-seq	GSM1665555	GSM1665554, GSM1665557, GSM1665560
DRB GRO-seq	GSM1579224	N/A
DRB YY1 ChIP-seq	GSM1665556	GSM1665554, GSM1665557, GSM1665560

fig. S10C		
Untreated GRO-seq	GSM1665567	
ActD GRO-seq	GSM1665568	
Untreated YY1 ChIP-seq	GSM1665558	GSM1665554, GSM1665557, GSM1665560
ActD YY1 ChIP-seq	GSM1665559	GSM1665554, GSM1665557, GSM1665560

fig. S10D		
YY1 ChIP-seq	GSM1665561	GSM1665554, GSM1665557, GSM1665560
THZ1 YY1 ChIP-seq	GSM1665562	GSM1665554, GSM1665557, GSM1665560

Table S1. GEO numbers for the datasets used in this study.

TPI YY1 ChIP-seq	GSM1665563	GSM1665554, GSM1665557, GSM1665560
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fig. S12A boxplot

Control (shLuciferase) RNA-seq	GSM1904550	N/A
sh <i>Exosc3</i> RNA-seq	GSM1904549	N/A

Table S2. YY1 ChIP-seq enriched regions.

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Table S3. YY1 CLIP-seq enriched regions.

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