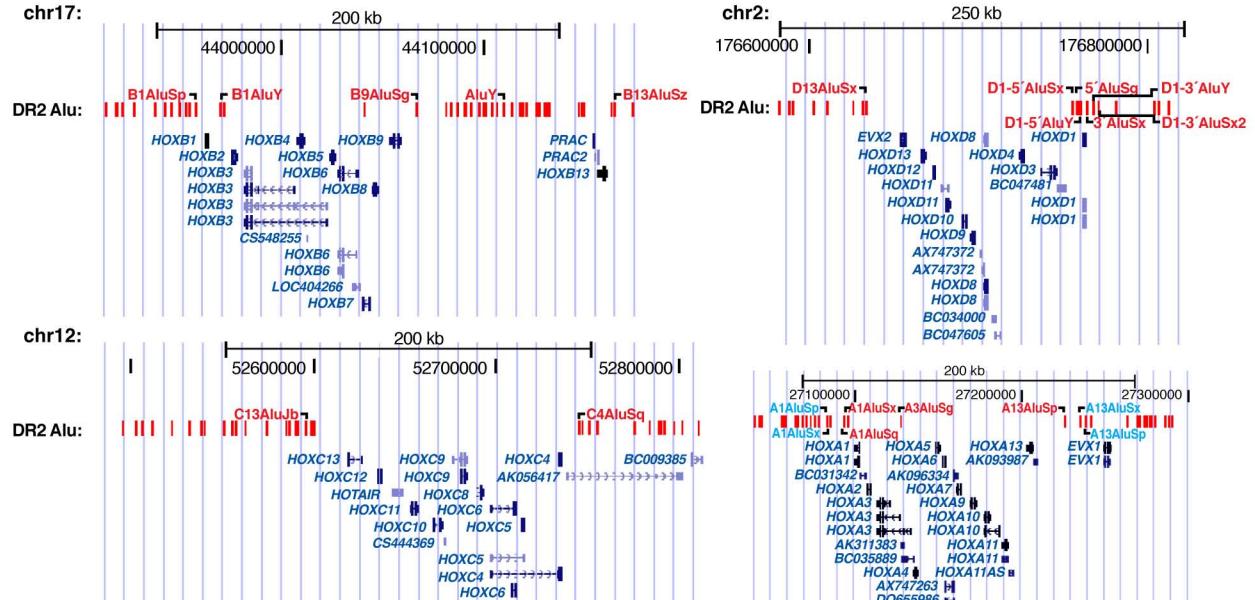
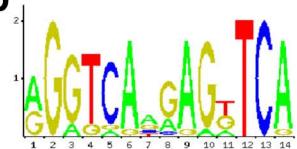
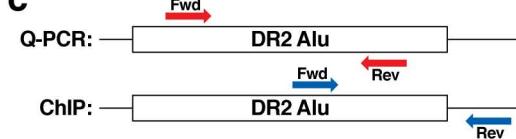
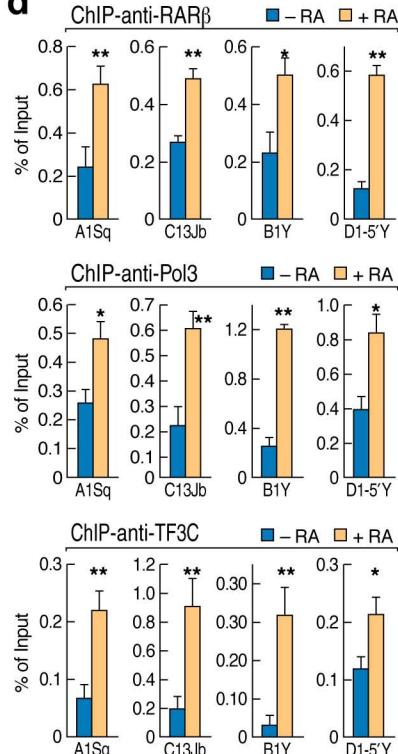
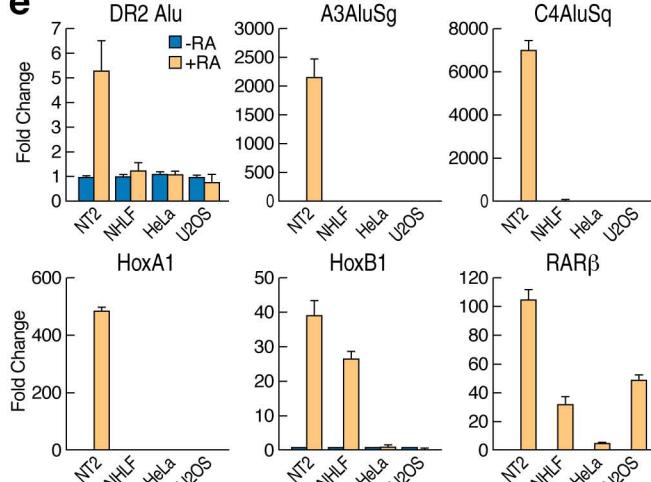
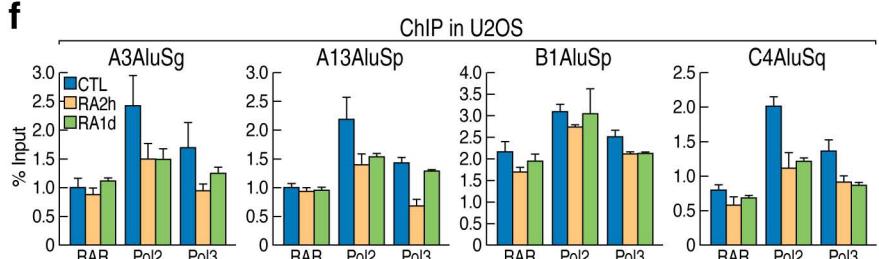
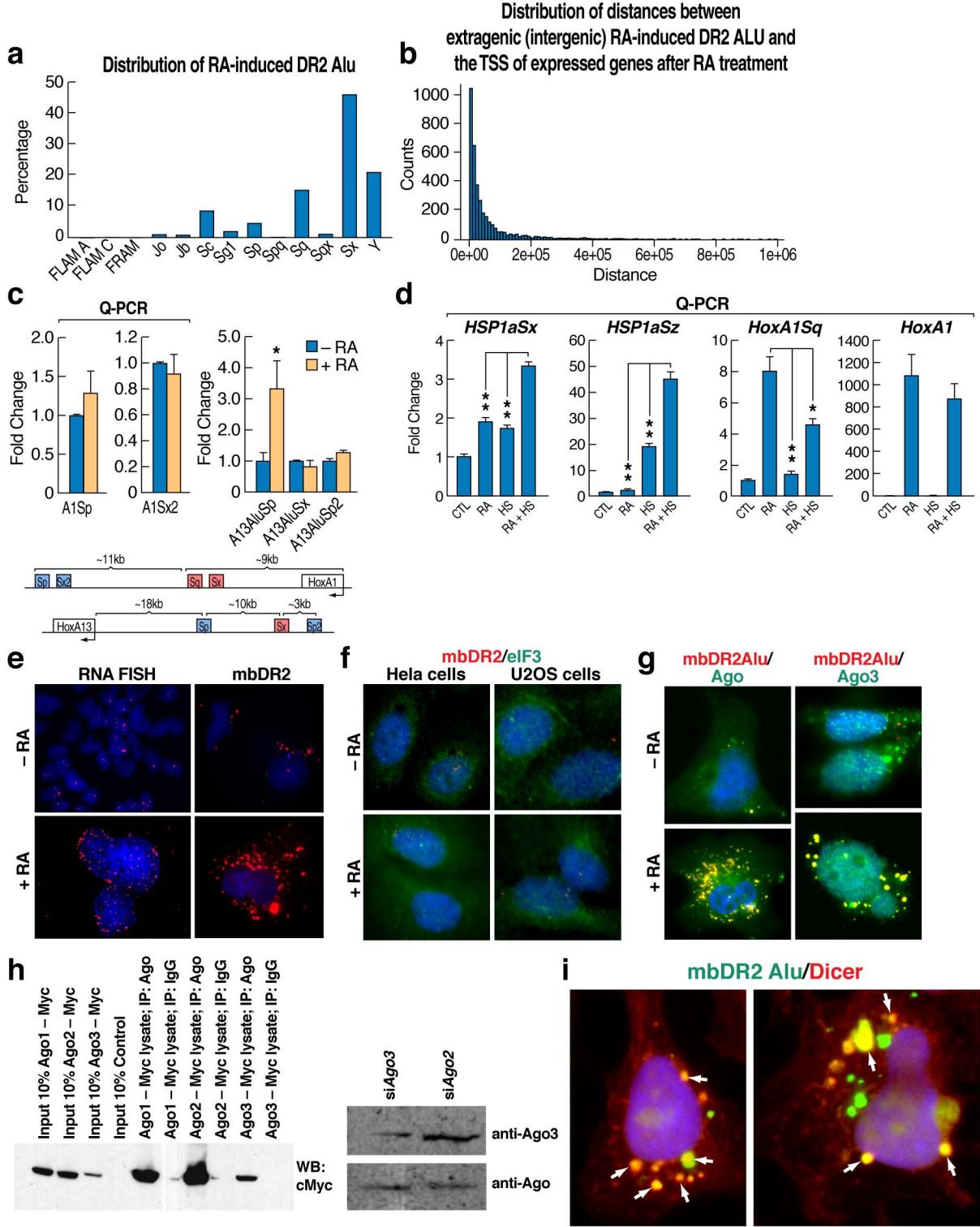
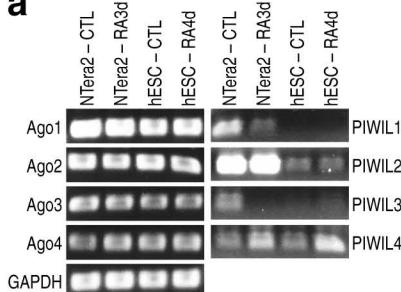
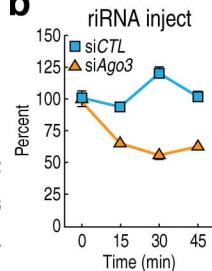
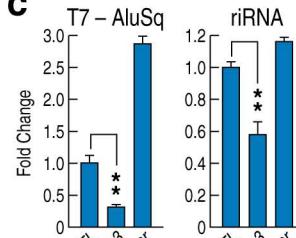


**a****b****c****d****e****f**

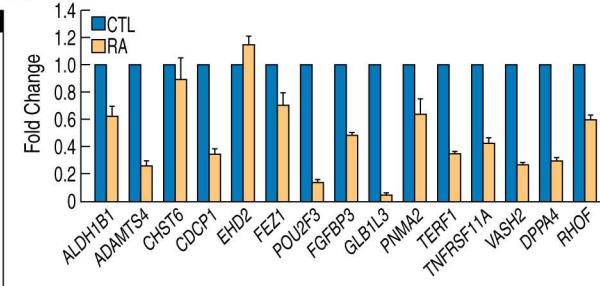
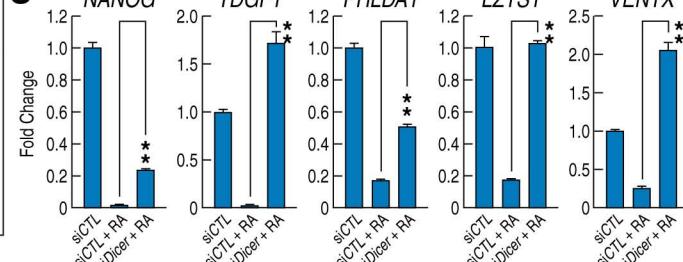
Supplementary Figure 1. DR2 Alu is activated by RA. (a) Distribution of all potential DR2 Alu elements flanking HoxA-D. Repeats highlighted in red are activated potentially by RA while those in aqua not. (b) The sequence logo of the DR2 position frequency matrix from PAZAR database. (c) The design of DR2 Alu primers used in this study. (d) RA-induced recruitment of RAR $\beta$ , Pol III and TF3C onto the DR2 Alu repeats flanking Hox. (e) RT-qPCR showing the RA-induced DR2 Alu and coding gene transcription in indicated cells. (f) Recruitment of RAR $\beta$ , Pol II and Pol III on DR2 Alu repeats was not enhanced in RA-treated U2OS cells. (mean+SEM; \*p<0.05, \*\*p<0.01)



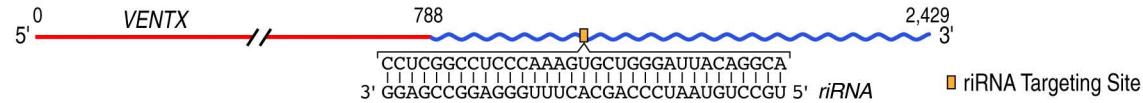
Supplementary Figure 2. DR2 Alu transcripts are localized in the cytoplasm. (a) The distribution of DR2 Alu transcripts among the Alu subfamilies. (b) The distribution of the distance between induced intergenic DR2 Alu and gene TSS. (c) DR2 Alu elements located further from *HoxA1* or *HoxA13* (>20kb) were not activated by RA. (d) Two DR2 Alu repeats near *Hsp70* showed a combinatorial response to heat shock plus RA treatment, while the repeat near *HoxA1* and *HoxA13* itself did not. (mean+SEM; \*p<0.05; \*\*p<0.01) (e) RNA FISH and molecular beacon (mbDR2) showed an increase of DR2 Alu transcripts in RA-treated cells. (f) In HeLa and U2OS cells, RA did not increase DR2 Alu RNAs determined by molecular beacons. (g) Immunostaining plus mbDR2 showed that DR2 Alu transcripts were colocalized with AGO proteins, including AGO3. (h) Validation of the AGO antibodies. Left: Myc-tagged AGO1-3 overexpressed in 293T were immunoprecipitated with the Ago antibody and immunoblotted with anti-Myc; right: Ntera2 transfected with AGO2 or 3 siRNAs were immunoblotted with the AGO3 or the generic AGO antibody. (i) Molecular beacon and immunostaining showed the partial colocalization of DR2 Alu transcripts with DICER (arrows) in the cytoplasm.

**a****b****c****d**

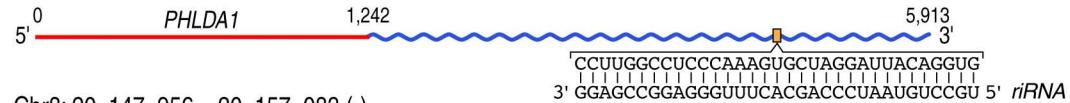
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AFMID	BC142973	KIAA1161	NM_020702	SAMHD1	NM_015474
AIM1	NM_001624	LIX1	NM_153234	SH2B3	AJ012793
AKNA	AK074040	LUZP2	AK309077	SLC12A8	BX538023
ALDH1B1	NM_000692	LZTS1	NM_021020	SLC15A1	NM_005073
ANKDD1A	AK075298	MALL	NM_005434	SLC47A1	AK225360
ARHGPAP26	BC068555	MARVELD2	DG682658	SYT13	NM_020826
C9orf91	AL834412	METTL7A	AY358797	TAL1	X51990
CD3EAP	AF017633	MYO5C	NM_018728	TDGF1	NM_003212
CDCP1	NM_022842	NANOG	NM_024865	TERF1	NM_003218
CGNL1	AY610514	NHEJ1	NM_024782	TMEM119	NM_181724
CHST6	NM_021615	NMUR1	NM_006056	TMEM170A	AK094930
CNTNAP2	CR933671	NR5A2	AK308446	TMEM53	AL832539
CR1	NM_000573	NXNL2	NM_145283	TNFRSF11A	NM_003839
CYorf15A	AK127387	OLFM2A	BC054001	TPCN2	AL137479
CYP51A1	AK091323	PAPLN	AY358330	UAPI1	CCDS7028
DPPA4	NM_018189	PDE6B	NM_000283	ZFAT	NM_032873
EHD2	NM_014601	PHLDA1	NM_007350	VASH2	BC053836
EMP2	NM_013447	PHYH1	AK054994	VENTX	NM_014668
FEZ1	NM_022549	PLEKHG5	AK091201	VGLL3	NM_016206
FGFBP3	NM_152429	PNNM2	NM_007257	WVA5B1	AK125833
GLB1L3	BC131702	POU2F3	AK097382	ZBTB3	NM_024784
GNPTAB	NM_024312	RASGRP4	BC150202	ZFP64	NM_018197
GPR26	NM_153442	RHD	AY751496	ZNF454	AX721148
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**e****f****f**

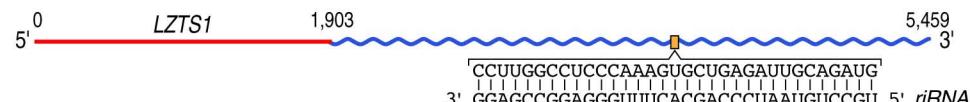
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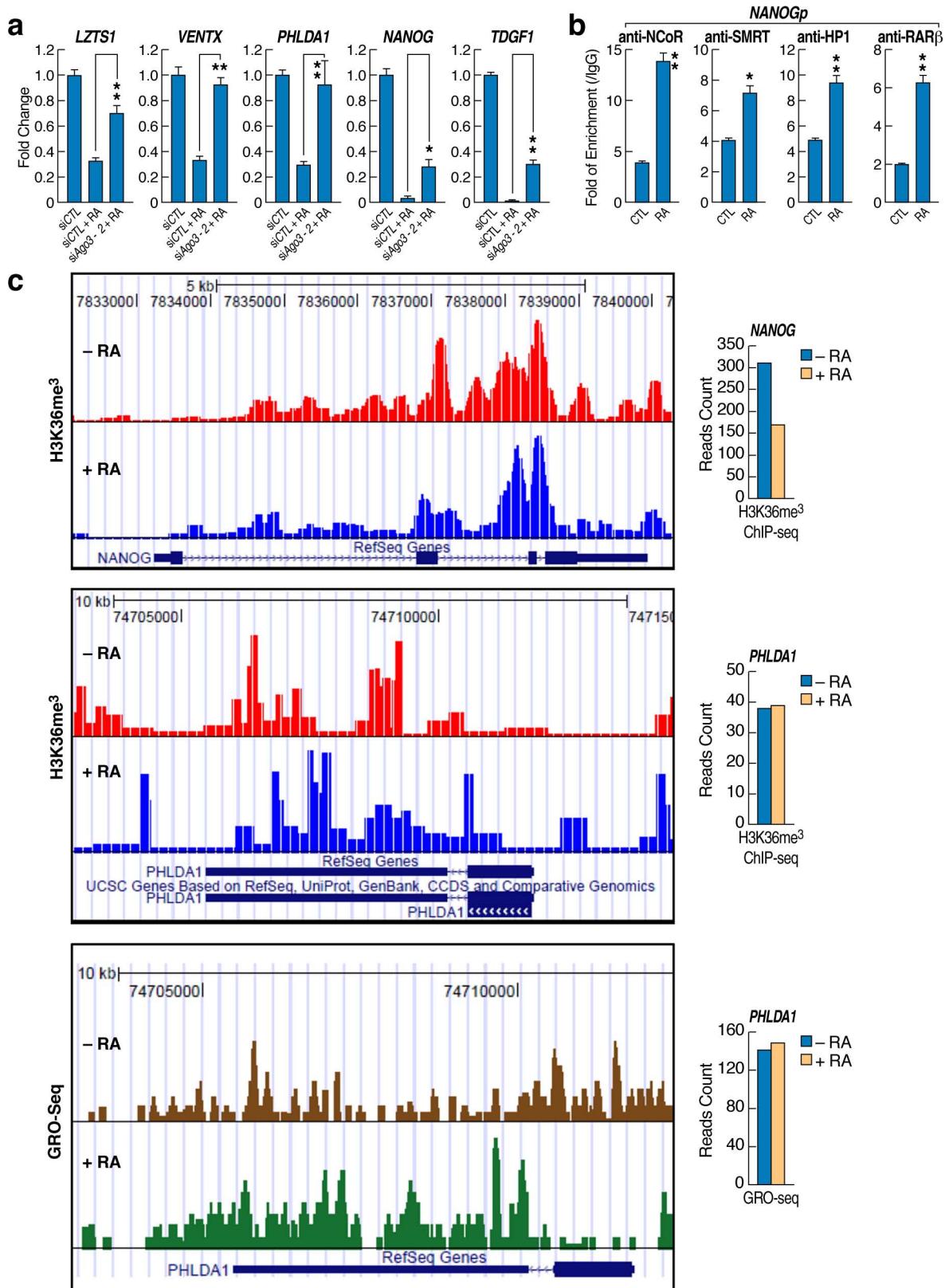
Chr12: 74, 705, 494 – 74, 711, 823 (-)



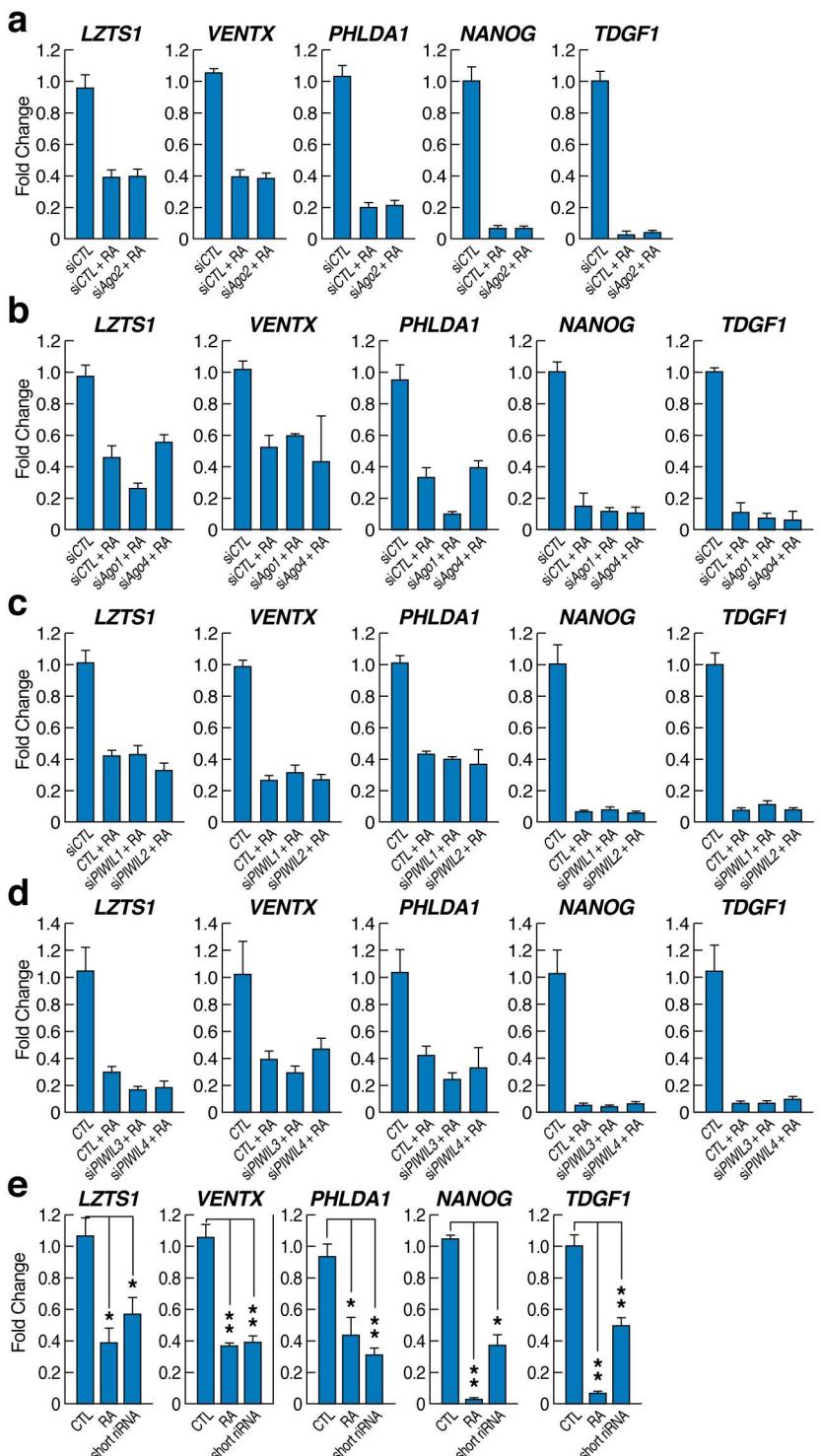
Chr8: 20, 147, 956 – 20, 157, 083 (-)



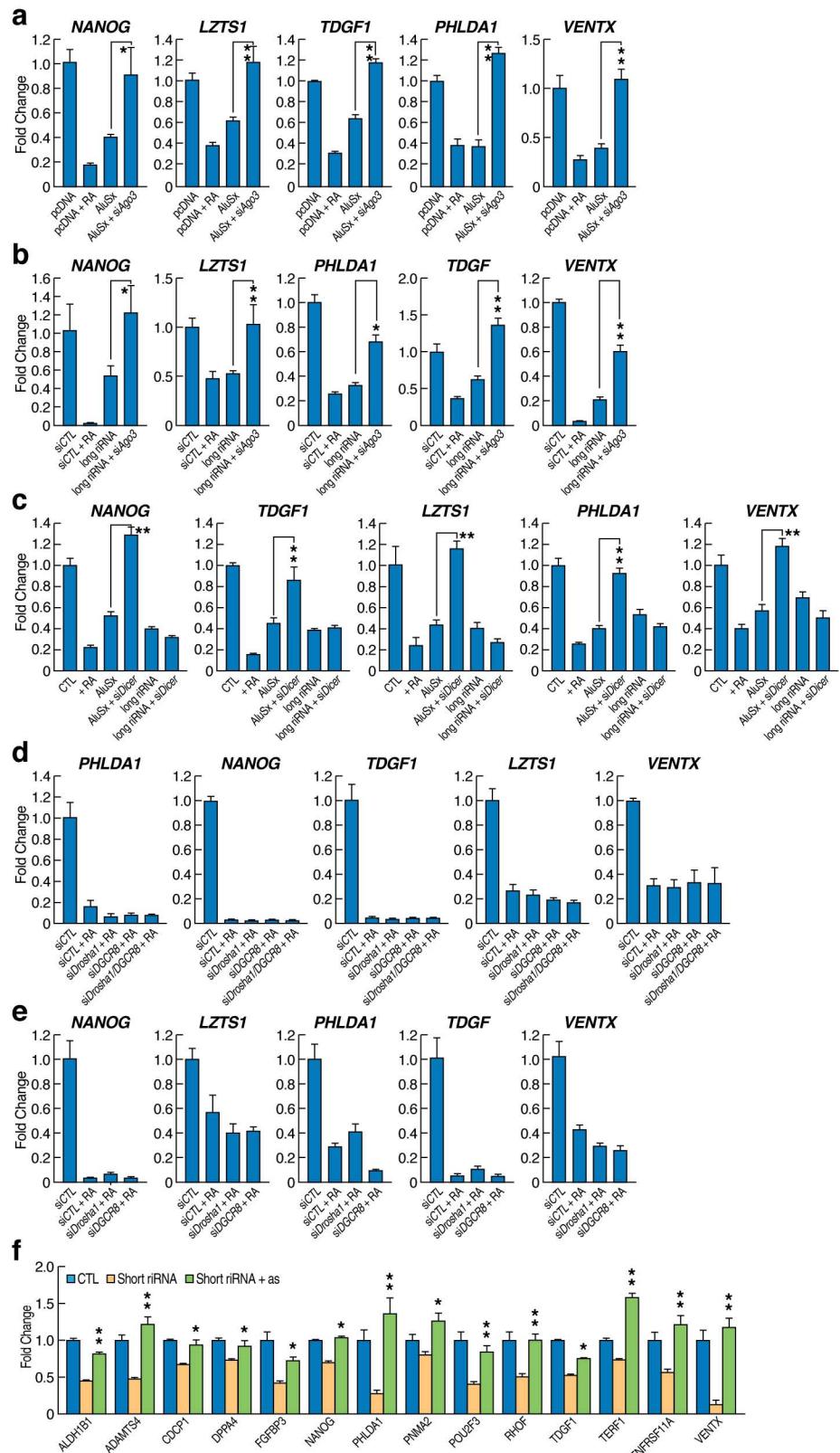
Supplementary Figure 3. AGO3 stabilizes riRNAs targeting 3'UTRs of mRNAs. (a) RT-PCR showed the expression of AGO and PIWIL homologs in Ntera2 and hESCs. (b) Ntera2 with AGO3 knockdown were micro-injected with a synthesized 69nt. riRNA oligonucleotide. The cells were harvested at indicated time points and analyzed by RT-qPCR. (c) Ntera2 with AGO3 or DICER knockdown were transfected with DR2 Alu transcripts or riRNA for four hours. The introduced RNAs were analyzed by RT-qPCR analysis. (d) List of 79 RA-downregulated transcripts (FC down-reg>1.5, and q-val<0.05) containing complementary sequences to riRNA in the 3'UTRs. (e) RT-qPCR confirmed that 13 out of 15 genes from the list (d) were downregulated by RA. (f) Structures of representative transcripts from the list (d). (g) RT-qPCR showed that knockdown of DICER reversed RA-induced expression downregulation. (mean+SEM; \*p<0.05, \*\*p<0.01)



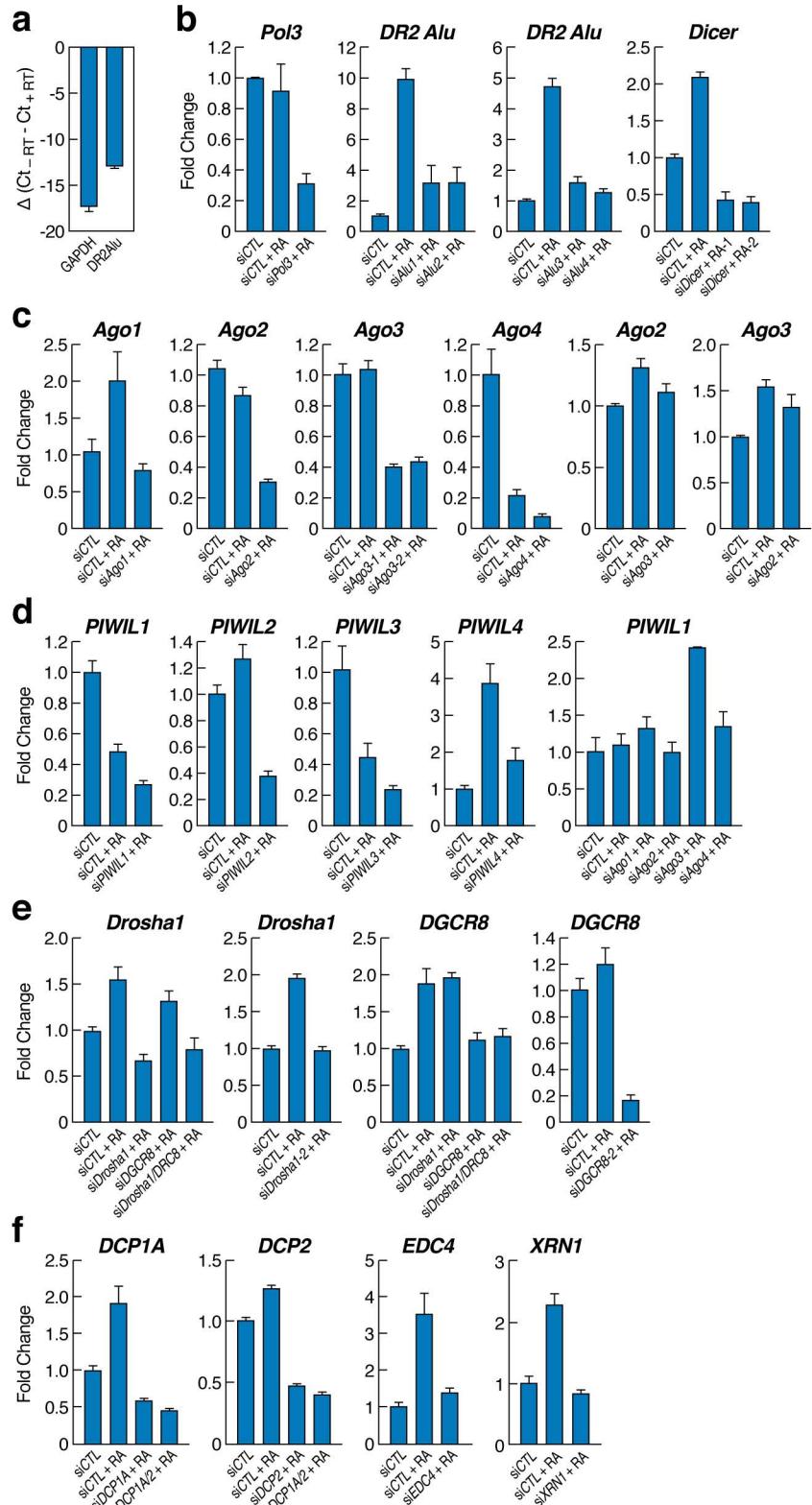
Supplementary Figure 4. Nanog is also regulated at the transcriptional level. (a) AGO3 knockdown by another siRNA (Dharmacon) abolished RA-induced expression downregulation. (b) ChIP assays showed RA-induced recruitment of NCoR-SMRT, HP1 and RAR $\beta$  at the *Nanog* promoter. (mean+SEM; \*p<0.05, \*\*p<0.01) (c) UCSC genome browser displays of the H3K36me3 ChIP-seq and GRO-seq reads over *Nanog* and *Phlda1* (H3K36me3 ChIP-seq: (-)RA in red, (+)RA in blue; GRO-seq: (-)RA in brown, and (+)RA in green).



Supplementary Figure 5. Other argonaute and piwi-like proteins did not restore transcript levels. (a-d) Knock-down of AGO1,2,4 and PIWI1-4 did not abolish RA-mediated downregulation of the indicated genes. (e) Transfection of a synthesized 26nt. riRNA oligonucleotide decreased the transcript levels of the indicated genes in Ntera2. (mean+SEM; \* $p<0.05$ , \*\* $p<0.01$ )



Supplementary Figure 6. AGO3 and DICER are involved in riRNA-mediated downregulation of transcripts. (a,b) AGO3 knockdown neutralized the repressive effects of overexpressed AluSx or riRNAs on genes. (c) DICER knockdown did not affect riRNA-mediated transcript down-regulation. (d,e) DROSHA1 or DGCR8 knockdown by two sets of different siRNAs did not release RA-mediated repression of these genes. (f) Nteria2 were co-transfected with the same molar amount of short riRNA and the antisense oligonucleotide. RT-qPCR showed the neutralizing effects of the antisense oligonucleotide. (mean+SEM; \* $p<0.05$ , \*\* $p<0.01$ )



Supplementary Figure 7. Validation of the siRNAs. (a) In RA-treated cells, minus RT control gave insignificant amplification signals for Gapdh and DR2 Alu, as exemplified by the huge negative  $\Delta(Ct_{\text{minusRT}} - Ct_{\text{plusRT}})$  in RT-qPCR. (b-f) RT-qPCR assays verified the efficiency of the siRNAs used.

**Table 1. Customized molecular beacons, oligonucleotides and siRNAs**

“m” indicates 2’O-methyl modification.

**Table 2. Primers used in RT-qPCR**

**Table 3. Primers used in ChIP and RIP assays**

Name	Sequences
HoxA1-AluSq	gcataagtgaacaaggacttggg+agagacaaggattcaccatgttg
HoxA3-AluSg	cactgagctctccagatacccta+ctcccaaagtgcgtggattac
HoxA13-AluSp	cactgtccctacccagcatattaaca+gttagagacagggtttctccatgttg
HoxB1-AluSp	cccttgtctgtcatctggagag+ctcccaaagtgcgtggattaca
HoxB1-AluY	actttctctcccccaacaac+aggaaaggttagcgtgacatga
HoxC4-AluSq	caaggaggactggtagagatgt+aagtgtaaagggtctgagacc
HoxC13-AluJb	caacagagcgataccctgtctc+ accccttaactgtcacagcttc
HoxD1-3'AluSx2	ctagaaaccctctgttagacacctca+ggattacaggcatgagccacta
HoxD1-5'AluY	ctaactgggtgttagggagaac+tttagtagagacggggttcacc
NANOGp	gacctgggaagaagctaaagagc+ggcattatagcatgaagccaag
NANOG-3'utr	agtctggacactggctgaatc+aaagaaaacctcgctgattagc
TDGF1-3'utr	aacatcttaaggggaggaacc+gaaacttgcctccatttagc

**Table 4. Probes for Northern blot**

Name	Sequences
radio-labeled DR2 Alu	5'- gagacggagttcactct-3'
biotin-labeled DR2 Alu	5'-cggcctccaaagtgcgtggattaca-3'
miR106a	5'-gtaagaagtgcatactgcag-3'
U6	5'-cgttccaaatttagtatatgtgctgccgaagcgagcac-3'