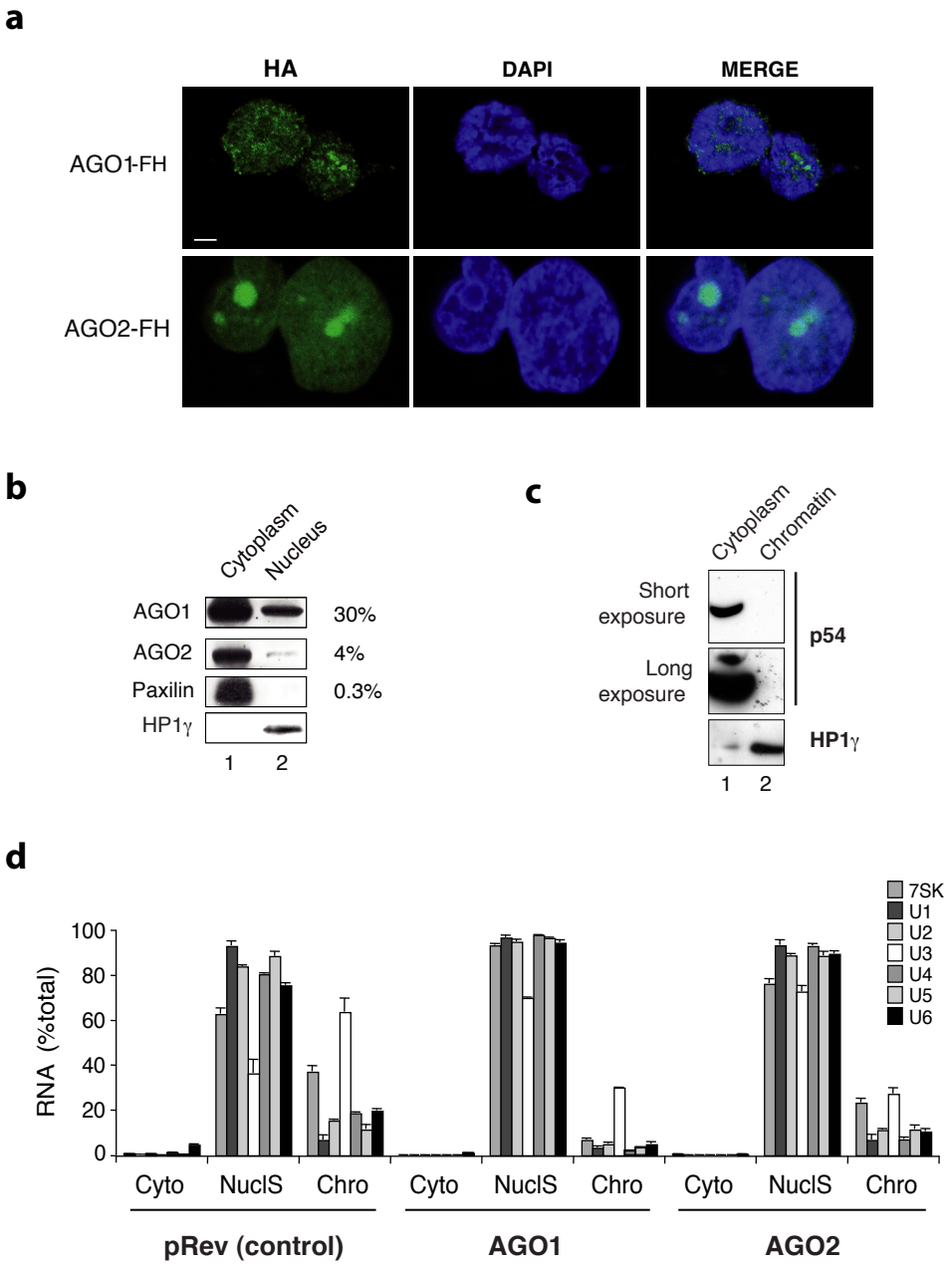

Supplementary Figure 1. Tagged AGO proteins are partially located in the nucleus. a) Nuclei of HeLa cells stably expressing AGO1-FH or AGO2-FH were purified on sucrose gradients and analyzed by indirect immunofluorescence using anti-HA antibodies; scale bar: 20 µm. b) Cytoplasmic or nuclear (following sucrose gradients purification) fractions were analyzed by western blot with the indicated antibodies. Paxillin and HP1γ are cytoplasmic and nuclear markers respectively. c-d) Characterization of subcellular fractions: c) RCK/P54, a protein of the P-bodies, is not detected in the chromatin fraction. Fractionated extracts of HeLa cells (nuclei were purified on sucrose gradients prior to chromatin extraction) were analyzed by western blot with anti-P54/RCK antibodies (two exposures are shown) and with an antibody against the nuclear protein HP1γ. d) U-snRNAs and 7SK detection. Subcellular fractions of HeLa cells (as in b), stably transduced with an empty vector, AGO1-FH or AGO2-FH, were analyzed by RT-qPCR; Cyto: cytoplasm; NuclS : Nuclear soluble ; Chro : chromatin.

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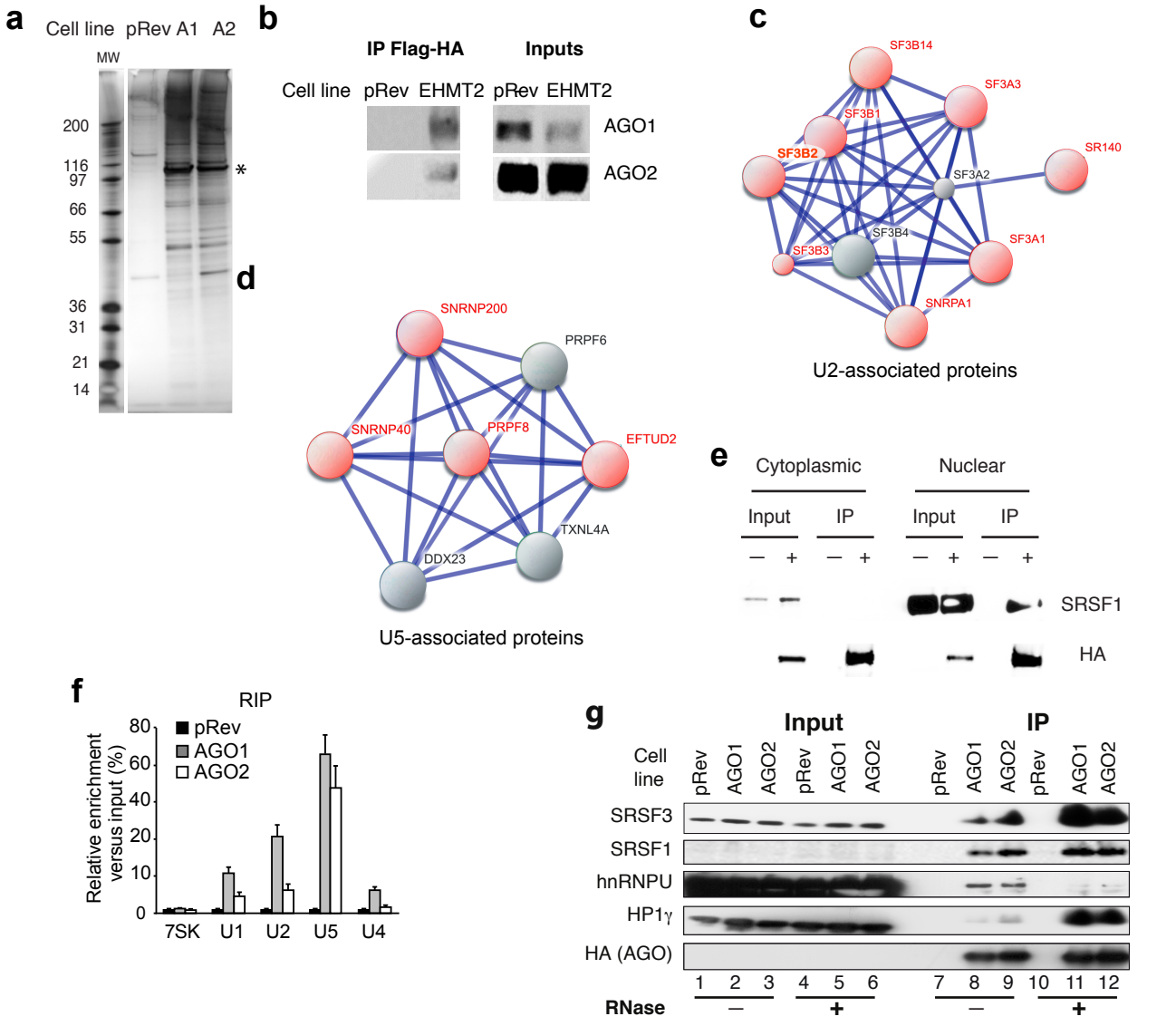
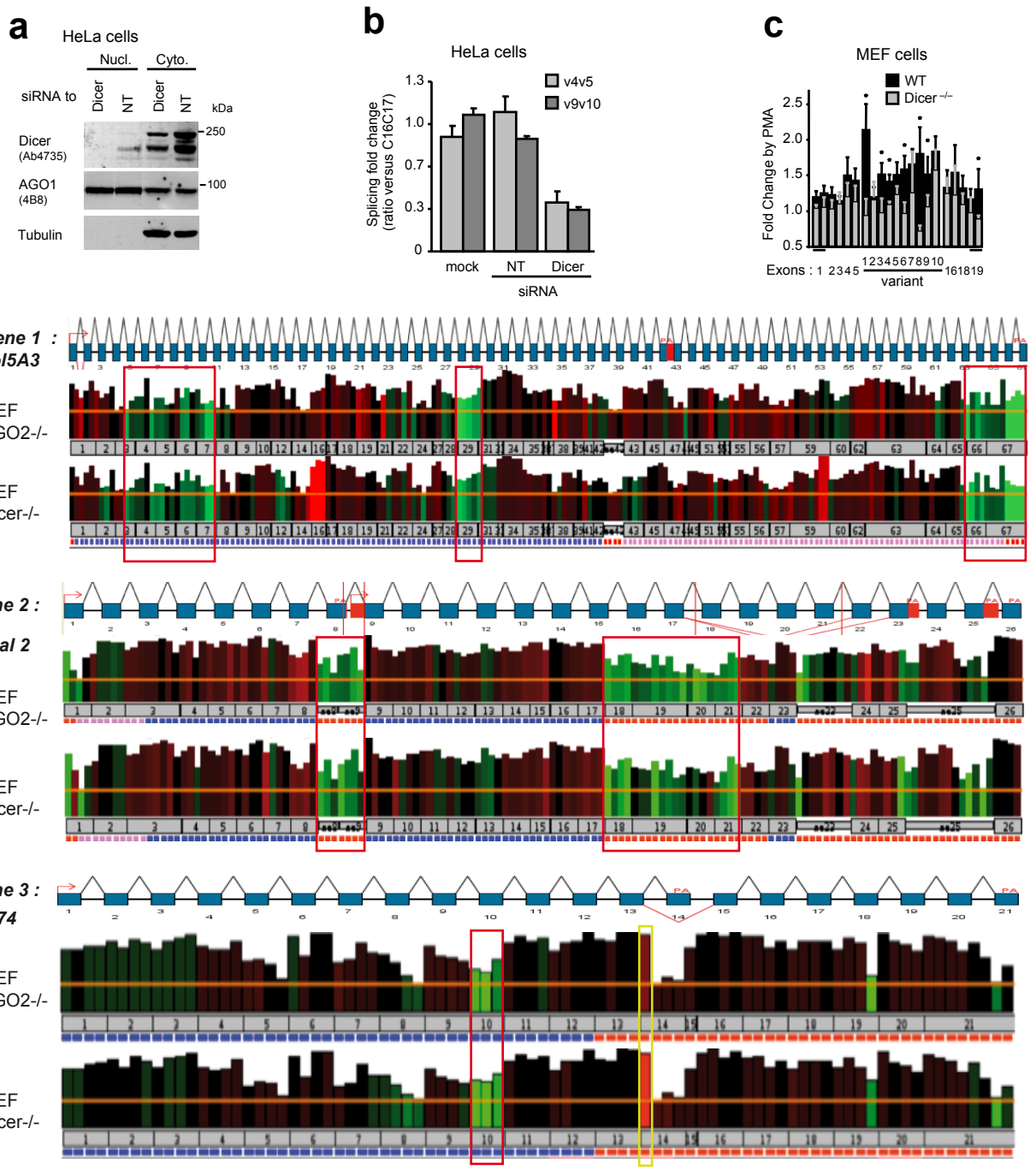


Supplementary Figure 2. Characterization of chromatin AGO1 and -2 complexes (a) Silver stain of FLAG-HA tandem affinity purified chromatin-AGO complexes from HeLa cells stably transduced with an empty vector (pRev), or expression vectors for AGO1-FH (A1) or AGO2-FH (A2). The major bands (*) correspond to AGO1-FH and AGO2-FH. (b) EHMT2 interacts with AGO1-2. Extracts from HeLa S3 cells stably transduced with an empty vector (pRev) or a Flag-HA-EHMT2 (G9a) expression vector were used for tandem immunoprecipitations. Proteins were detected using the indicated antibodies. (c-d) U2 and U5 snRNPs as defined by Will and Lührmann (Cur. Opin. Cell Biol. 2001), modified with information from the « String » database (string-db.org); proteins detected in the human nuclear AGO1 and -2 complexes are indicated in red. Blue lines indicate physical interactions as documented in the String database. (e) SRSF1 is detected in AGO2 complexes in mouse cells. Nuclear extracts from C2C12 mouse myoblasts stably transduced with an empty vector (pRev) or an expression vector for AGO2-FH were used for immunoprecipitation assays with anti-HA antibodies, and analyzed by western blotting. (f) snRNAs associated with AGO1and -2 complexes. Chromatin AGO1 and AGO2 complexes were isolated by tandem immunoprecipitation and snRNAs were quantified by RT-qPCR; Enrichments (relatively to the inputs) were normalized to control cells (empty vector, pRev) set to 1. (g) AGO association with SR protein resists RNase treatment. Extracts from Hela-S3 transduced with an empty vector (pRev) or expression vectors for either AGO1-Flag-HA (AGO1-FH) or AGO2-Flag-HA (AGO2-FH) were treated (+) or not (+) with a mixture of RNases (controlled by detecting various RNAs, not shown); immunoprecipitated proteins were analyzed by western blotting using the indicated antibodies.



Supplementary Figure 3. Dicer and AGO2 dependent alternative splicing (a)Dicer is present in HeLa cell nucleus; HeLa cells were transfected with siRNA to Dicer or with control siRNA (siNT- non-targeting) as a negative control. Cytosolic (Cyto.) or Nuclear (Nucl.) extracts were analyzed by Western blotting using the indicated antibodies. (b-c) Dicer knockdown affects CD44 variant exon splicing. b) HeLa cells were transfected as in a) with indicated siRNAs and treated with PMA overnight; total RNA was used for RT-qPCR reactions with the indicated primers; shown are the ratio between the variant forms v4v5 or v9v10 and the constant exons C16C17 normalized to control cells; (c) Dicer -/- MEFs were treated with PMA for 15 min and total RNA was analyzed on exon arrays (Genosplice technology); shown are the ratio between untreated and PMA-treated samples (mean +/- s. e. m. of three biological replicates). (d) Genes with impaired splicing in AGO2 -/- or Dicer -/- MEFs. Total RNA from MEF was analyzed as in b (except that cells were not treated with PMA). Top: Exon-intron structure of the gene (blue boxes: exons; horizontal lines: introns). Below: results of exon arrays; Each bar corresponds to one probe, and four probes were used for each exon; Bar height (log 2 scale) corresponds to signal intensity. Red bars correspond to up-regulated probes, green bars to down-regulated probes and black bars correspond to unmodified probes. Background signal is indicated by an orange line. Exon numbers are indicated below, in grey rectangles; Color underneath indicates the splicing status: blue: region shared by at least 75% of transcripts, purple: regions shared by at least 50% and less than 75% of the transcripts; red: region shared by less than 50% of transcripts (according to Easana Visualiation Module).

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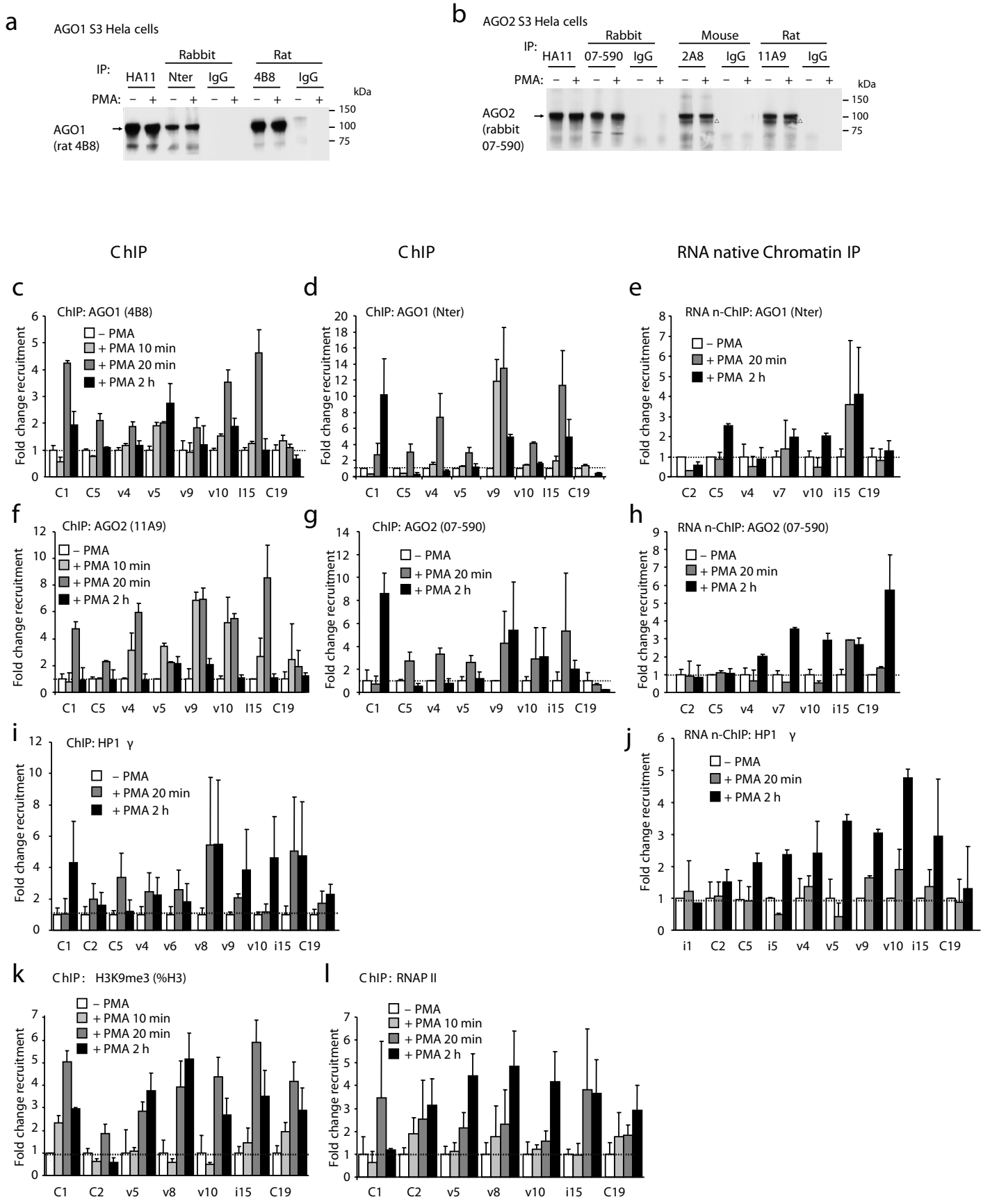
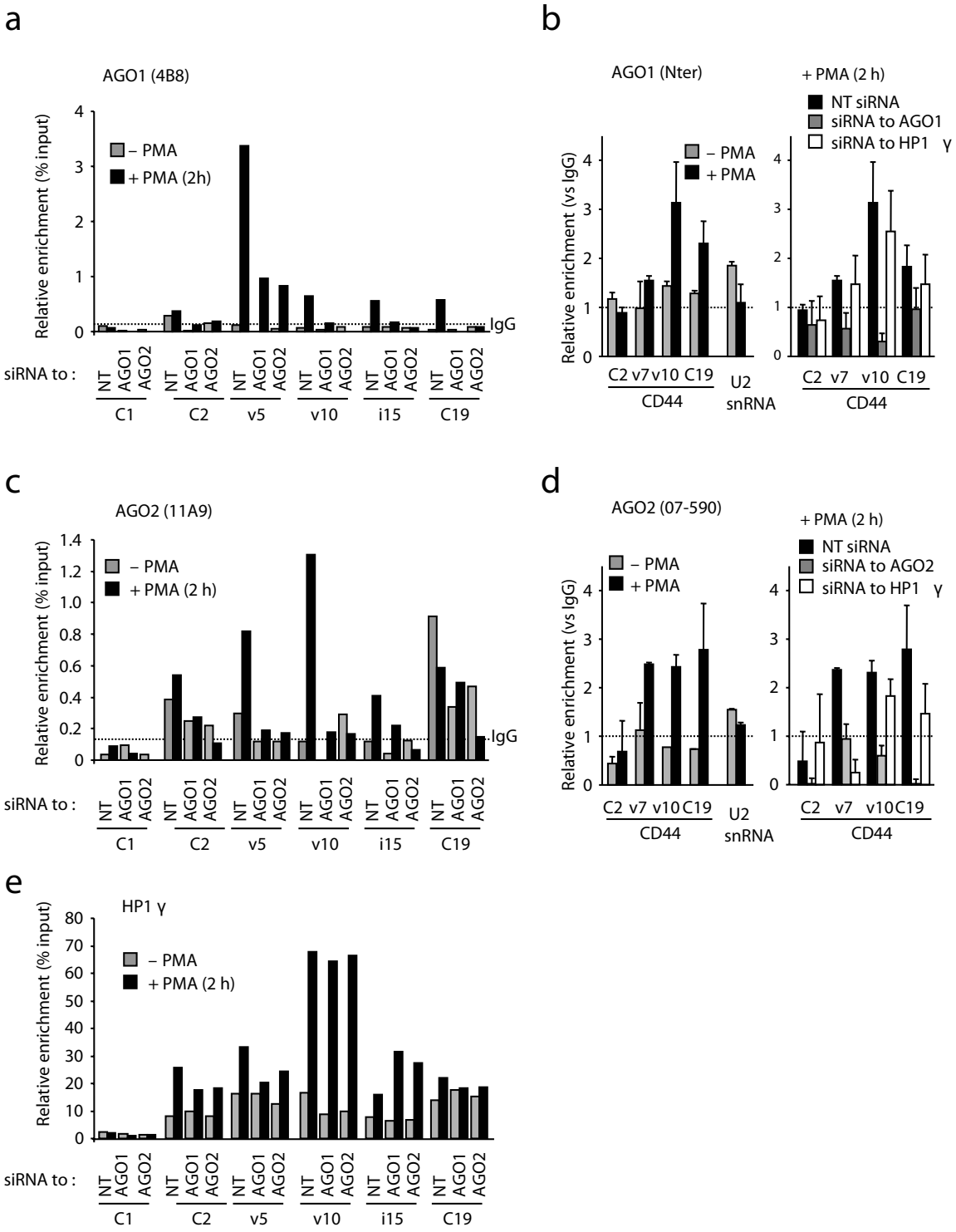
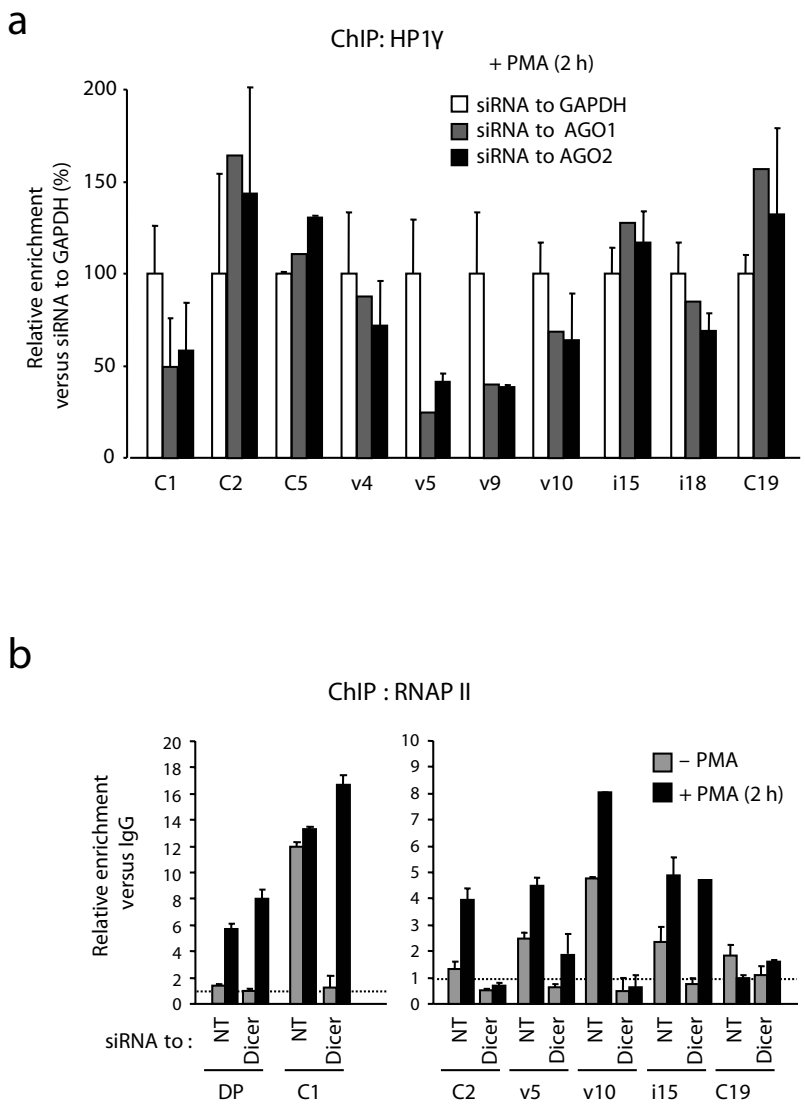


Supplementary Figure 4. Kinetics of AGO and HP1γ recruitment, of H3K9 methylation and of RNAPol II accumulation a-b) Immunoprecipitation efficiencies of anti-AGO1 or anti-AGO2 antibodies. HeLa S3 cells expressing AGO1-Flag-HA (a) or AGO2-Flag-HA (b) were treated (+), or not (–), with PMA for 2 h. Whole cell extracts were used for immunoprecipitation assays using specific antibodies or species-matched non immune IgGs as indicated. AGO1 and 2 proteins were detected by Western blotting using indicated antibodies. Arrows indicate tagged AGO proteins and triangles show the position of endogenous AGO2 protein. (c-l) : Kinetics of AGO1, AGO2, HP1γ, H3K9Me3 and RNAPII recruitment to *CD44* chromatin and nascent mRNA. HeLa cells were treated (+) or not (–) with PMA for the indicated periods of time and subjected to ChIp assays (c-i) or RNA native Chromatin IP (RNA n-ChIP) assays (panels j-l). ChIP assays (c-i): Chromatin from formaldehyde-fixed cells was prepared as described in the Methods section. Extracts were used for immunoprecipitation, using indicated antibodies or species-matched non-immune IgGs. Note that different antibodies were used for ChIP: rat and rabbit anti-AGO1 (c,d) ; rabbit anti-AGO2 (e,f). Immunoprecipitated DNA was isolated and detected by qPCR using primers in the indicated regions of *CD44*. RNA n-ChIP (j-l): DNase-solubilized chromatin from non-fixed cells was prepared as described in the Methods section. Immunoprecipitated RNAs were isolated, treated with DNase, and detected by RT-qPCR using primers in the indicated regions of *CD44*. Values are presented as fold-change normalized to species-matched non immune IgGs, and expressed relatively to control samples (without PMA, dotted line). Note that H3K9me3 is expressed as a percentage of H3, and normalized to the control samples (without PMA). Data are means ± s.e.m. from at least 4 independent experiments.

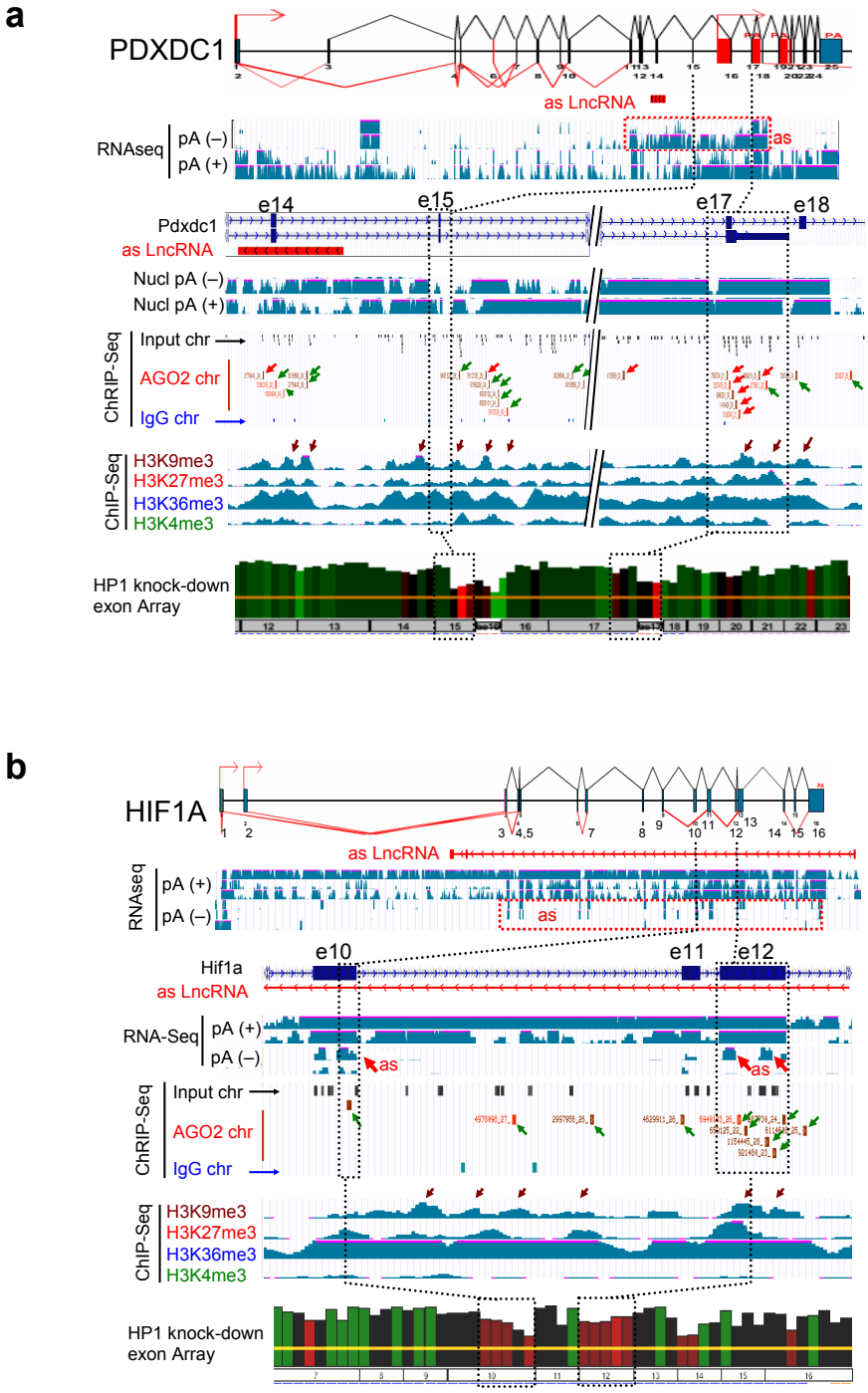
Nature Structural & Molecular Biology: doi:10.1038/nsmb.2373



Supplementary Figure 5. RNA native ChIP (RNA nCHIP) of AGO1-2 proteins and HP1γ HeLa cells transfected with siRNAs to AGO1, AGO2, HP1γ or control siRNAs (siNT, non-targetting), or non-transfected cells were treated (+) or not (-) with PMA for 2h. DNAse-solubilized chromatin was immunoprecipitated using the indicated antibodies or species matched non-immune IgGs. RNA was isolated, treated with DNase and detected by RT-qPCR unsing primers in the indicated regions of CD44. Values (a, c, and e: from one representative experiment; b, d: mean ± SEM of two independent experiments) are relative to the inputs of control cells (not treated with PMA); Backgrounds levels (IgGs) are indicated by dotted lines.



Supplementary Figure 6. HP1γ recruitment depends on AGO protein and RNAPII accumulation depends on Dicer a) ChIP assays were carried out with chromatin from HeLa cells transfected with siRNA targeting GAPDH, AGO1 or AGO2, and further treated by PMA during 2 h; Binding of HP1γ was calculated relative to non-immune IgG and to siRNA-to-GAPDH-transfected cells. Values are means ± s.e.m. of 2 experiments.
b) HeLa cells were transfected with the indicated siRNAs (siNT = control, non-targeting siRNA) and treated with PMA for 2 hrs. Chromatin was immunoprecipitated with either anti-RNAP II antibodies or non immune IgG, and used for qPCR amplification using indicated primers. Values were normalized to IgGs (dotted lines) and correspond to one representative experiment out of three.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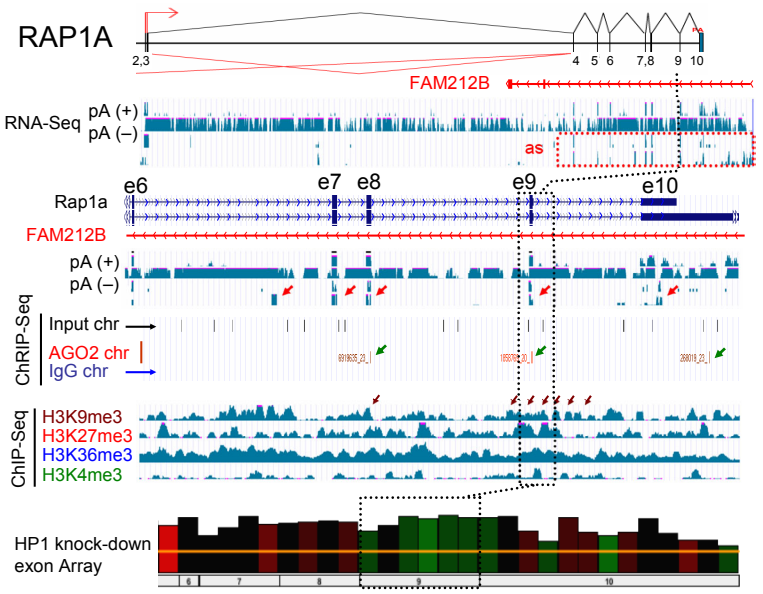
PDXDC1
as LncRNA
RNAseq
pA (–)
pA (+)
as
e14
e15
e17
e18
Pdxdc1
as LncRNA
Nucl pA (–)
Nucl pA (+)
ChRIP-Seq
Input chr
AGO2 chr
IgG chr
ChIP-Seq
H3K9me3
H3K27me3
H3K36me3
H3K4me3
HP1 knock-down
exon Array
b
HIF1A
as LncRNA
RNAseq
pA (+)
pA (–)
as
e10
e11
e12
Hif1a
as LncRNA
RNA-Seq
pA (+)
pA (–)
as
as
ChRIP-Seq
Input chr
AGO2 chr
IgG chr
ChIP-Seq
H3K9me3
H3K27me3
H3K36me3
H3K4me3
HP1 knock-down
exon Array

c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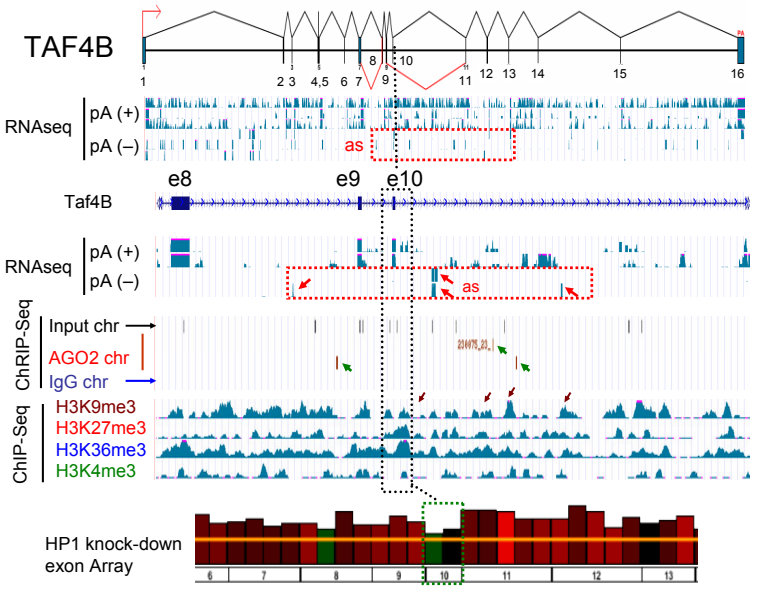
2,3
4 5 6 7,8 9 10
FAM212B
RNA-Seq
pA (+)
pA (–)
as
e6 e7 e8 e9 e10
Rap1a
FAM212B
pA (+)
pA (–)
ChRIP-Seq
Input chr
AGO2 chr
IgG chr
ChIP-Seq
H3K9me3
H3K27me3
H3K36me3
H3K4me3
HP1 knock-down
exon Array

d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pA (–)
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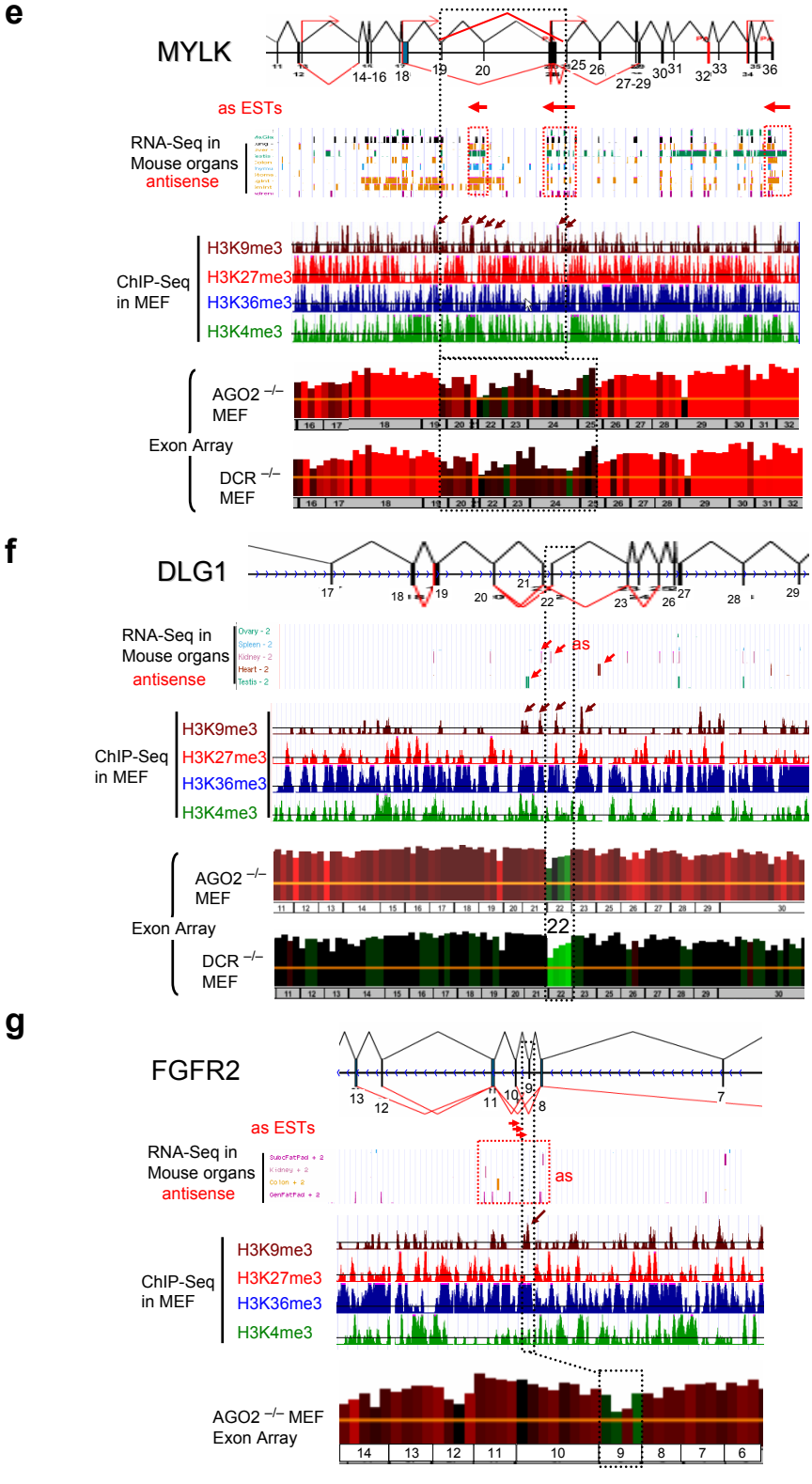
pA (–)
as
ChRIP-Seq
Input chr
AGO2 chr
IgG chr
ChIP-Seq
H3K9me3
H3K27me3
H3K36me3
H3K4me3
HP1 knock-down
exon Array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MYLK
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19
20
25
26
27-29
30
31
32
33
36
as ESTs
RNA-Seq in Mouse organs antisense
ChIP-Seq in MEF
H3K9me3
H3K27me3
H3K36me3
H3K4me3
AGO2 −/− MEF
Exon Array
DCR −/− MEF
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DLG1
17
18
19
20
21
22
23
26
27
28
29
RNA-Seq in Mouse organs antisense
Ovary - 2
Spleen - 2
Kidney - 2
Heart - 2
Testis - 2
as
ChIP-Seq in MEF
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H3K27me3
H3K36me3
H3K4me3
AGO2 −/− MEF
Exon Array
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DCR −/− MEF
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FGFR2
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9
8
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as ESTs
RNA-Seq in Mouse organs antisense
as
ChIP-Seq in MEF
H3K9me3
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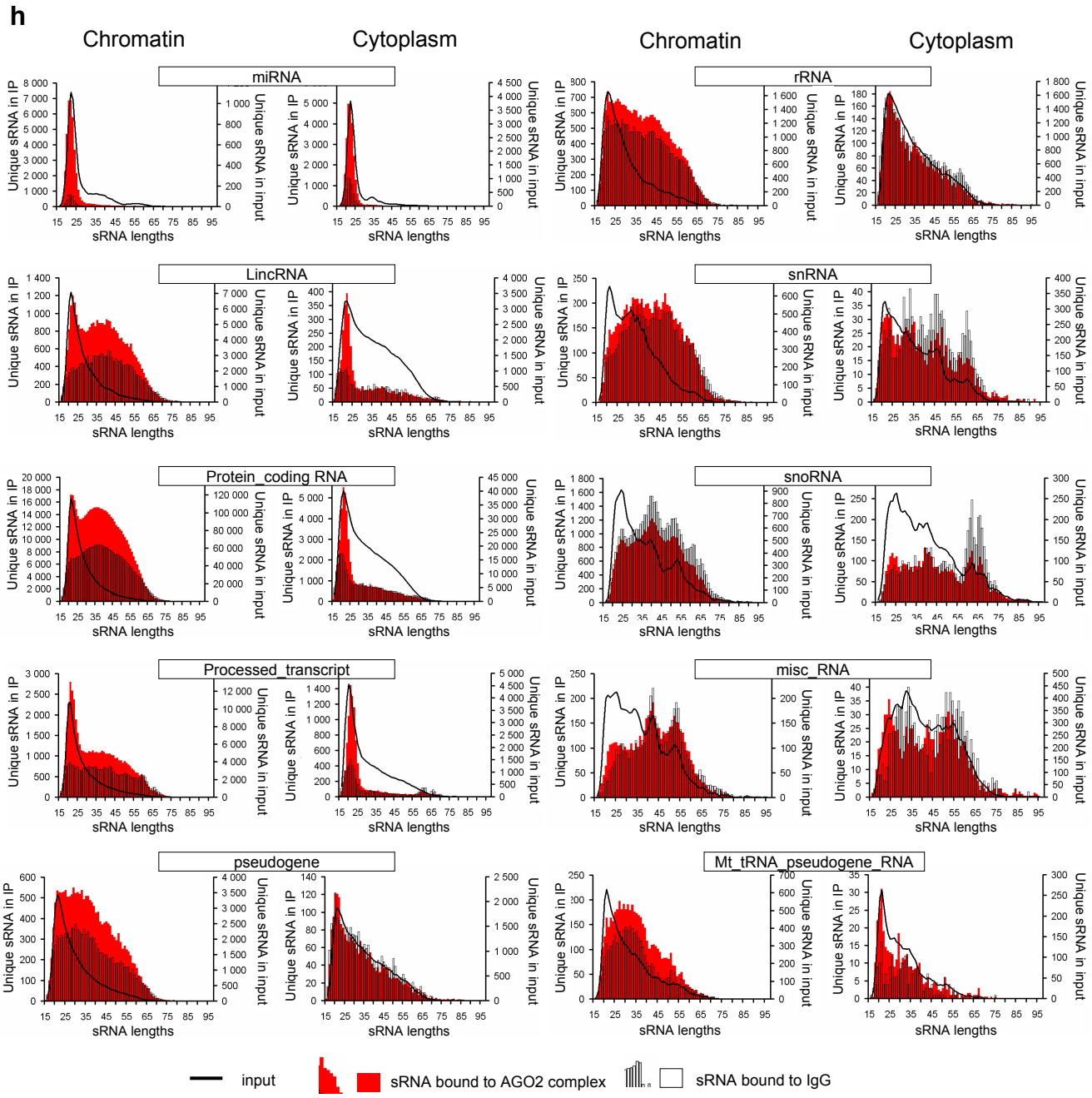
6


h
Chromatin
Cytoplasm
Chromatin
Cytoplasm
miRNA
rRNA
LincRNA
snRNA
Protein_coding RNA
snoRNA
Processed_transcript
misc_RNA
pseudogene
Mt_tRNA_pseudogene_RNA
Unique sRNA in IP
Unique sRNA in input
sRNA lengths
input
sRNA bound to AGO2 complex
sRNA bound to IgG

Supplementary Figure 7. Examples of AGO2- or HP1γ-dependent genes with peaks of H3K9me3 and intragenic antisense transcripts. Genes are PDXDC1 (pyridoxal-dependent decarboxylase) (**a**), HIF1A (hypoxia inducible factor 1, alpha) (**b**), RAP1A (RAS-related protein-1a) (**c**), and TAF4B (TATA box associated factor 4B) (**d**), MYLK (myosin, light polypeptide kinase) (**e**), DLG1 (Discs, large homolog 1 (Drosophila)) (**f**), and FGFR2 (**g**).

For each gene pictured, **a map** in the top section indicates the position of exons with alternative events in red. Antisense transcripts are indicated in red below the maps (Lnc RNA), annotated either as ESTs (human expressed sequence tag in GenBank) or in UCSC genecode genes V11 (Human GRCh37/hg19 assembly) (**a-d**) or in mouse (Mouse NCBI37/mm9 genome assembly) (**e-g**). The **RNA-Seq** tracks below the map show the sense (+) and antisense (–) transcripts from poly-adenylated (pA) transcripts in Hela cells in CSHL Long RNA-Seq (human GRCh37/hg19 genome assembly) (**a-d**). RNA sequencing data from different organs in CHSL Long RNA-Seq (Mouse NCBI37/mm9 genome assembly).. Only antisense transcript signals are shown (**e-g**). The red dotted boxes and red arrows delineate antisense transcription.

In human genes (**a-d**) an enlargement of the region affected by HP1 knock-down with exons and the position of the intragenic antisense transcript in red, followed by the localization of sRNA in our **ChRIP-Seq** experiment. Deep sequencing was performed in HeLa cells on RNA from input (black) or extracts immunoprecipitated with either IgGs (blue) or with anti-AGO2 antibodies (red), from chromatin (chr) fractions (as indicated in Fig 2). Bars indicate the positions of sRNA reads (19-30 nt). Green and red arrows point to the positions of sRNAs in the sense and antisense orientations, respectively.

Below, the **ChIP-Seq** tracks show histone H3 methylation marks in HeLa cells (from ENCODE/Broad Institute, on the Human hg19 assembly, **a-d**) or in MEFs (data from Broad Institute on the Mouse NCBI36/mm8 assembly, **e-g**). Peaks of H3K9me3 signal are highlighted by brown arrows.

In human genes (**a-d**), at the bottom are shown the results of **Affymetrix exon arrays** performed using RNA extracted from HeLa cells treated with siRNAs to HP1γ or GAPDH (Saint André et al., 2011). In mouse genes (**e-g**) at the bottom are shown the exon arrays performed using RNAs from $AGO2^{-/-}$ and $Dicer^{-/-}$ MEFs as described in Supplementary figure 3. Figures were obtained using the Easana visualization interface (GenoSplice technology). Each bar corresponds to one probe. Bar height corresponds to the intensity of the signal (indicated in a log 2 scale); black bars indicate unmodified signal, red bars indicate signal up-regulated and green bars indicate signal down-regulated in the indicated cells. The orange line indicates the background signal. Exons, delineated below the bars graph by white rectangles, were detected by at least four different probes (bars). **h)** Lengths of the sRNAs bound to AGO2.ca (red) or to control IgGs (grey) were aligned versus the indicated Ensembl biotypes; read numbers are represented by bar length. Black line: sRNA lengths in input

Supplementary Tables 1, 2 and 3. Mass spectrometry analysis of AGO1 and AGO2-chromatin associated complexes.

Supplementary Table 1 : RNA Processing and Splicing

	Entry name	Accession number	Accession number	MW	AGO1 IP[a]	AGO2 IP[a]	REF
Core of the RISC Complex	Argonaute-1	NP_036331	Q9UL18	97.0	23/27,3	5/4,6	**1**
	Argonaute-2	NP_036286	Q9UKV8	97.0	2/2,9	23/25,7	**1**
	Argonaute-4	NP_060099	Q9HCK5	96.9	1/1,4	-	-
	TNR6A	NP_055309	Q8NDV7	21.0	-	2/1,7	**1**
	TNC6B	NP_001155973	Q9UPQ9	18.2	-	7/1,7	**1,2,3**
hnRNP	hnRNPA3	NP_919223	P51991	39.4	4/7,7	3/6,9	**1**
	hnRNPC	NP_112604	P07910	33.5	5/14,7	4/14,1	**1.2**
	hnRNPCL1	NP_001139653	O60812	32.1	7/18,8	3/11,3	-
	hnRNPD	NP_112738	Q14103	38.3	2/6,2	2/6,2	**1**
	hnRNPF	NP_001091678	P52597	45.5	-	1/4,1	**1.2**
	hnRNPH1	NP_005511	P31943	49.0	3/9,6	5/16,5	**1**
	hnRNPH3	NP_036339	P31942	36.9	-	2/8,4	-
	hnRNPK	NP_112553	P61978	50.9	5/14,7	9/24,6	**1**
	hnRNPL	NP_001524.2	P14866	64	4/12,4	1/4,7	**1.2**
	hnRNPM	NP_005959	P52272	77.4	7/12,3	8/15,8	**1**
	hnRNPR	NP_001095868	O43390	70.8	4/7,4	2/4,1	**1**
	hnRNPU	NP_114032	Q00839	90.4	9/12,5	9/12,3	**1.2**
	hnRNPUL2	NP_001073027	Q1KMD3	88.1	4/3,6	1/1,9	-
	PCBP2	NP_005007	Q15366	35.3	-	1/3,6	**1.2**
	RALY	NP_057951	Q9UKM9	32.4	4/13,4	-	**1**
	RALYL	NP_001093861	Q86SE5	32.3	1/4,1	-	-
	hnRNPG	NP_002130	P38159	55.8	2/6,9	2/6,9	**1**
	hnRNPQ	NP_006363	O60506	69.5	5/9,3	-	**1**
U2 snRNP	SF3A1	NP_005868	Q15459	88.9	-	3/7,1	-
	SF3A3	NP_006793	Q12874	58.8	-	1/2,4	-
	SF3B1	NP_036565	O75533	145.8	-	3/3,1	-
	SF3B2	NP_006833	Q13435	100.2	3/6,1	1/3,3	-
	SF3B3	NP_036558	Q15393	135.6	-	5/5,3	-
	SF3B14	NP_057131.1	Q9Y3B4	14.6	-	1/9,6	-
	SNRPA1	NP_003081	P09661	28.4	-	3/16,5	-
U5 snRNP	U5-116 kDa	NP_004238	Q15029	109.4	-	2/3,1	-
	PRP8	NP_006436.3	Q6P2Q9	273.6	-	2/0,6	-
	U5-200KD	NP_054733	O75643	67.9	-	2/1,1	-
	SNRNP40	NP_004805	Q96DI7	39.3	-	1/2,8	-
SR/Sm proteins	RBM39	NP_909122	Q14498	59.3	-	2/4,9	-
	SNRPD1	NP_008869	P62314	13.3	1/10,9	-	-
	SNRPD2	NP_004588	P62316	13.5	01/16,2	-	-
	SNRPD3	NP_004166	P62318	13.9	1/7,9	1/7,9	-
	SNRPEL1	CAH73282	Q5VYJ4	10.7	2/12,1	2/25,01	-
	SRSF1	NP_008855	Q07955	27.7	1/5,7	-	-
	SRFS3	NP_003008	P84103	19.3	1/5,8	1/8,5	-
	SRSF7	NP_001026854	Q16629	27.3	4/21,4	-	-
	SRSF10	NP_004584	P62995	33.6	-	1/5,6	-
Other Splicing Factors	TARDBP	NP_031401	Q13148	44.7	1/2,9	2/5,1	-
	RBM8	NP_001244.1	Q99459	140.5	-	1/1,6	-
	PRP19	NP_055317.1	Q9UMS4	55.1	-	2/4,2	-
	PTBP1	NP_002810.1	P26599	57.2	1/3,8	5/11,7	-
	PTBP2	NP_067013.1	Q9UKA9	57.4	-	1/1,5	-
	RBM16	NP_055707.3	Q9UPN6	52.1	-	1/0,9	-
	SF4	NP_757386	Q8IWZ8	72.4	-	1/2,1	-
	SKIV2L2	NP_056175	P42285	117.8	-	1/1,7	-
	ACINUS	NP_055792	Q9UKV3	151.8	-	1/0,9	-
RNA metabolism and processing	ELAV1	NP_001410	Q15717	36.0	6/25,8	6/20,6	**1,2**
	ILF2	NP_004506	Q12905	43.0	6/20,8	6/19,7	**1,2**
	ILF3	NP_036350	Q12906	95.3	3/4,7	-	**1,2**
	ROD1	BAA75466	O95758	59.6	-	1/3,8	-
	SKIV2L2	NP_056175	P42285	117.8	-	1/1,1	-
	SON	NP_620305	P18583	263.8	-	1/0,6	-
RNA Helicases	DDX17	NP_006377	Q92841	72.3	1/1,8	2/3,5	**1**
	DDX21	NP_004719	Q9NR30	87.3	3/4,7	-	**2**
	DDX39	NP_005795	O00148	49.1	-	1/2,3	**2**
	DDX48	NP_055555	P38919	46.8	-	3/6,8	**2**
	DHX15	NP_001349	O43143	90.9	1/1,5	3/3,1	-
	DHX9	NP_001348	Q08211	140.9	9/8,4	4/4,7	**1,2**
Editing factor	DSRAD	NP_001102	P55265		-	1/1,2	**1**

Identified proteins were classified in specific tables according to their molecular functions.a number of peptides identified/ % protein coverage.
Previously characterized AGO proteins partners are referenced

Supplementary Table 2 : Chromatin organization

	Entry name	Accession number	Accession number	MW	AGO1 IP[a]	AGO2 IP[a]	REF
Histones	H2A1A	NP_734466	Q96QV6	14,2	-	2/12,3	-
	H1.1	Q02539	Q02539	21,8	-	1/5,1	-
	H2A1D	NP_066409	P20671	14,1	-	1/8,5	-
	macro-H2A.1	NP_001035248	O75367	39,6	-	1/5,4	-
	H2B.1	NP_066406	P33778	13.9	1/12,1	4/12,1	-
	H4	NP_778224	P62805	11,3	1/9,7	5/40,8	-
	H4-B	AAH69467	Q6DRA9	11,3	-	1/9,7	-
Transricptional regulation	HDAC1	NP_004955	Q13547	55.1	-	2/6,4	-
	RBM14	NP_006319.1	Q96PK6	69.4	-	1/2,2	-
	Sam68	NP_006550	Q07666	48.2	1/3,2	-	-
	SMRC2	NP_003066	Q8TAQ2	132.6	-	1/1,7	-
	RUVB1	NP_003698	Q9Y265	50.2	-	3/9,1	-
	RUVB2	NP_006657	Q9Y230	51.1	-	3/5,8	**2**
	MATR3	NP_001181884	P43243	94.6	6/10,9	8/15,8	**2**
	ERBB4	NP_005226	Q15303	146.8	-	01/16,01	-
	DBC-1	NP_066997	Q8N163	102.9	-	1/16,01	-
	RBBP7	CAG46502	Q16576	47.8	-	1/3,1	-
	ZN326	NP_892021	Q5BKZ1	65.6	1/2,7	-	**2**
HP1 and its partners	CBX3	NP_057671	Q13185	20.8	-	1/7,1	-
	TIF1−β	NP_005753	Q13263	88.5	-	1/1,1	-
	Suv39H1	NP_003164	O43463	47.9	-	5/14,8	-
DNA dammage	PRKDC	NP_008835	P78527	469.0	-	1/0,3	-
	TOP2A	NP_001058	P11388	174.3	-	1	-
Nuclear Organization	Lamin A/C	NP_733821	P02545	74.1	-	33/50,2	-
Chromosome segregation	SMC-3	NP_005436	Q9UQE7	141.5	-	1/1,6	-

Supplementary Table 3 :

	Entry name	Accession number	Accession number	MW	AGO1 IP[a]	AGO2 IP[a]	REF
Chaperons	HS90A	NP_005339	P07900	84.6	-	1/1,9	1
	DNAJB6	CAG38529	O75190	36	-	1/4,1	-
	HSP71	NP_005336	P08107	70.0	-	1/2,5	1
	HSP7C	NP_006588	P11142	70.0	-	1/1,9	-
	NPM	NP_002511	P06748	32.5	-	1/7,1	-
	TCPE	NP_036205	P48643	59.6	-	1/3,01	-
Cytoskeleton organization	Tubulin beta	CAA56071	Q5JP53	49.9	1/3,5	-	-
Apoptosis	DBC-1	NP_066997	Q8N163	102.9	-	1	-
Nuclear miRNA processing	SRRT	NP_001122325	Q9BXP5	100.6	-	1	-
Protein and mRNA nuclear	XPO1	NP_003391	O14980	123.3	-	1/1,1	-
Protein nuclear import	IMB1	NP_002256	Q14974	97.1	-	1/1,7	-
GTPase activity	SI1L2	NP_065859	Q9P2F8	190.4	-	-	-
Defense response	MLF2	NP_005430	Q15773	28.1	-	2/8,5	-
Unknown function	CU070	Q9NSI2	Q9NSI2	25.4	-	1/6,1	-

Identified proteins were classified in specific tables according to their molecular functions.a number of peptides identified/ % protein coverage.

Previously characterized AGO proteins partners are referenced

Supplementary Table 4. Peptides detected by Spectrometry in extracts from control cells.

Entry name	Accession number	peptide number
PGRC1	NG_016756.1	1
H2A1A	NP_734466.1	1
H4 (Fragment)	Q6fgb8	1
cDNA FLJ78387 : Ig-G like	A8K008	1
Mus musculus anti-human apolipoprotein A monoclonal antibody mAb	AF178454	1
anti-colorectal carcinoma heavy chain	AAB28159.1	5

HeLa S3 cells transduced with an empty vehicle vector were processed as in Supplementary Table 1-3 (all samples were run in parallel) and analyzed by mass spectrometry.

Supplementary Table 5. Effect of AGO2 or Dicer knockout on exon splicing.

	MEF AGO2 -/-	MEF DICER -/-	Common to both
Number of regulated exons	6768	7875	2340
down-regulated	1862	2587	479
up-regulated	1690	2258	259

Fold change mutant cells vs. knockout cells > 1.5; p-value <0.05

Supplementary Table 6 . Sequences of siRNAs used in this study.

siRNA	SEQUENCES (sense)
scramble	UAGCAAUGACGAAUGCGUA
EIF2C1	CCAAGAAUUGUGCAAGUAA
EIF2C1	GCUACAACUUAGAUCCCUA
EIF2C1pool of 4 different siRNA	GCACAGUAUUUCAAGCAGA,
	CAACGAACGGGUCGACUUU,
	UGACAAGAAUGAGCGAAUU,
	GGAAGUACCGCGUGUGUAA
EIF2C2	AGAUCCAUACGUCCGUGA
EIF2C2 pool of 2 different siRNA	GCACGACUGUGGACACGAA
	CAAGCAGGCCUUCGCACUA
HP1 γ pool of 2 different siRNA	AUCUGACAGUGAAUCUGAU
	AGUACUAGAUCGACGUGUA
Dicer	UGCUUGAAGCAGCUCUGGA
GAPDH	UGGUUUACAUGUUCCAAUA

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