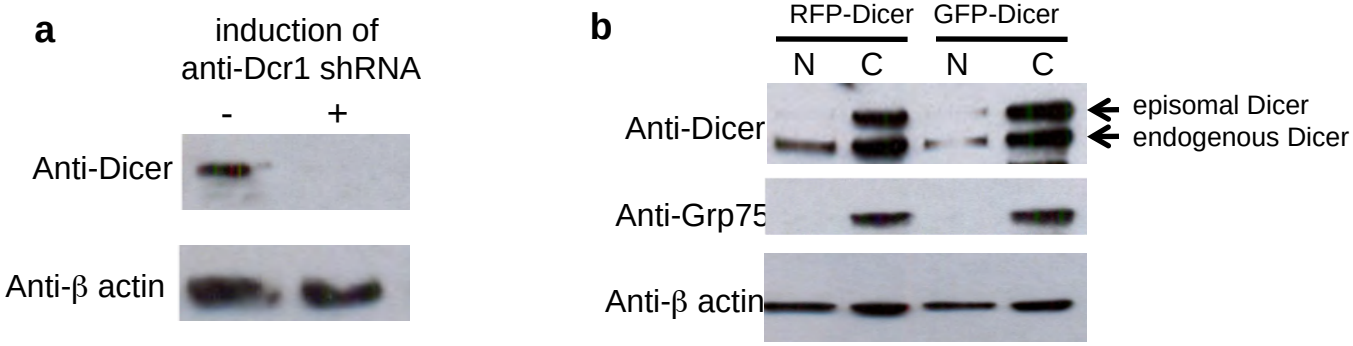
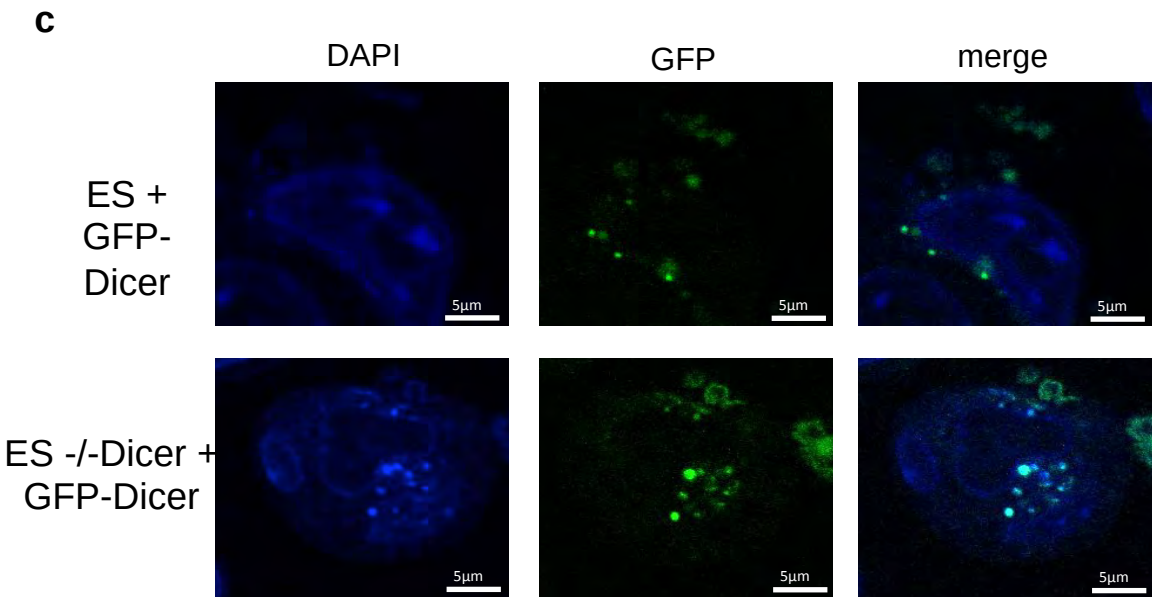
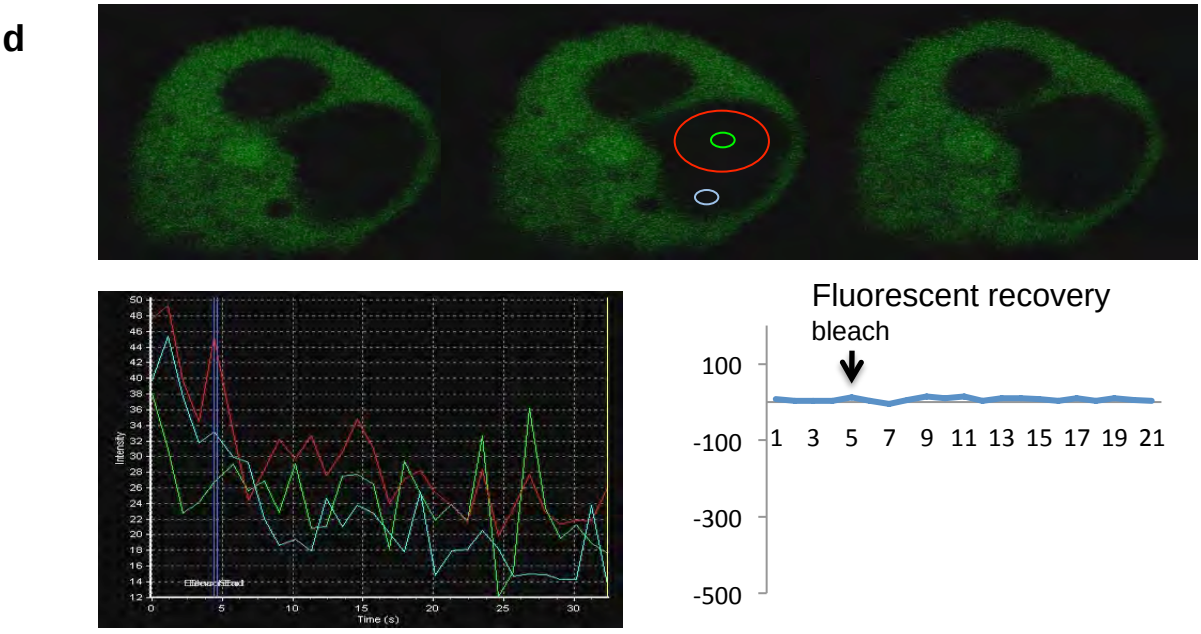
White_Supplementary Figure 1







Supplementary Figure 1. Dicer localizes in both nucleus and cytoplasm of HEK293 cells.

a) Western blot analysis of protein extracts from HEK293 cells with inducible integrated shRNA transgene. Induction by doxycycline leads to Dicer-specific shRNA production and consequent Dicer knockdown. Antibodies specific to Dicer and γ-actin were employed.

b) Western blot analysis of Dicer in nuclear and cytoplasmic extracts from HEK293 cells transfected with episomal GFP-Dicer or RFP-Dicer expressing plasmids. Grp75 and γ-actin were used as controls.

c) Expression of GFP-Dicer transgene (green) in normal and Dicer knockout murine embryonic stem cells as in Figure 1b.

d) Control FRAP experiment shows Dicer-GFP (green) recovery in nucleus of HEK293 cells (lacking GFP signal) after bleaching. This provides a background control to FRAP analysis of GFP-Dicer expression in the nucleus of Dicer knockdown cells. Here, only background signals can be detected. The cell is shown in the left hand panel before bleaching. In the middle panel, the red circle corresponds to bleached area in nucleus. Fluorescent recovery was measured in the green circle. Fluorescent background values were measured in the blue circle. Fluorescent recovery was measured in 20 frames, bleaching was initiated at frame 5. Right-hand panel shows the cell after recovery. Absolute levels of fluorescence and relative levels of recovery (background fluorescence subtracted from fluorescence levels in measured region) are shown in the left and right panels, respectively. All experiments described in Supplementary Fig. 1a-c, were replicated three times.

White_Supplementary Figure 2

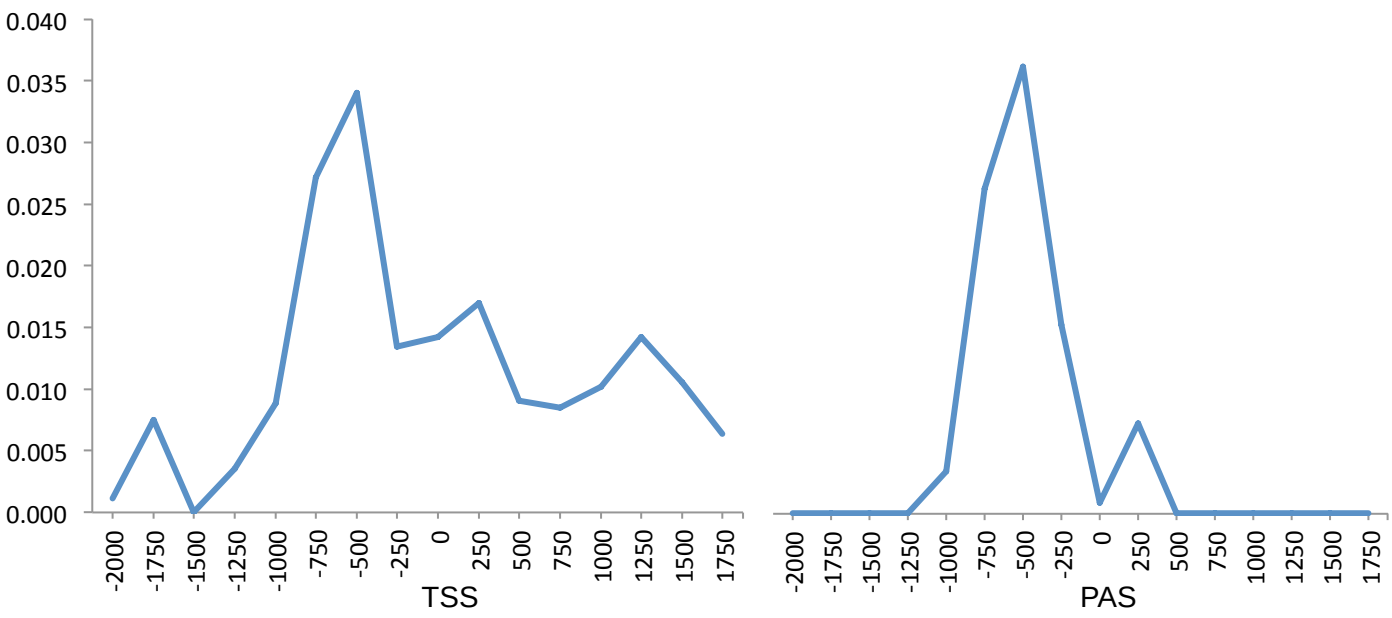
a

Genomic coordinates (hg19)			MACS score	genomic annotation	nearest gene	
chrom.	start	end			symbol	full name
chr7	5566155	5566639	922.62	terminator	ACTB	actin, beta
chr17	66015846	66016214	176.77	intergenic	KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
chr1	94312891	94313381	153.32	promoter	BCAR3	breast cancer anti-estrogen resistance 3
chr7	139025267	139025685	150.09	promoter	LUC7L2	LUC7-like 2 (S. cerevisiae)
chr12	120729400	120729672	137.94	intergenic	SIRT4	sirtuin 4
chr13	45491894	45492223	136.96	intergenic	NUFIP1	nuclear fragile X mental retardation protein interacting protein 1
chr11	66115407	66115892	122.53	promoter	B3GNT1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1
chr1	153643602	153643972	119.26	promoter	ILF2	interleukin enhancer binding factor 2, 45kDa
chr9	35657672	35658032	116.98	promoter	RMRP	RNA component of mitochondrial RNA processing endoribonuclease
chr2	43037481	43037893	115.62	intergenic	HAAO	3-hydroxyanthranilate 3,4-dioxygenase
chr6	126101203	126101805	114.93	promoter	NCOA7	nuclear receptor coactivator 7
chr2	70475997	70476371	106.97	promoter	TIA1	TIA1 cytotoxic granule-associated RNA binding protein

b

Genomic coordinates (hg19)			MACS score	genomic annotation	nearest gene
chr12	20704354	20704656	393.00	intron	PDE3A
chr11	10822850	10823053	187.94	terminator	SNORD97
chr7	142375331	142375541	213.69	terminator	MTRNR2L6
chr3	155378874	155379079	236.54	promoter	PLCH1

c

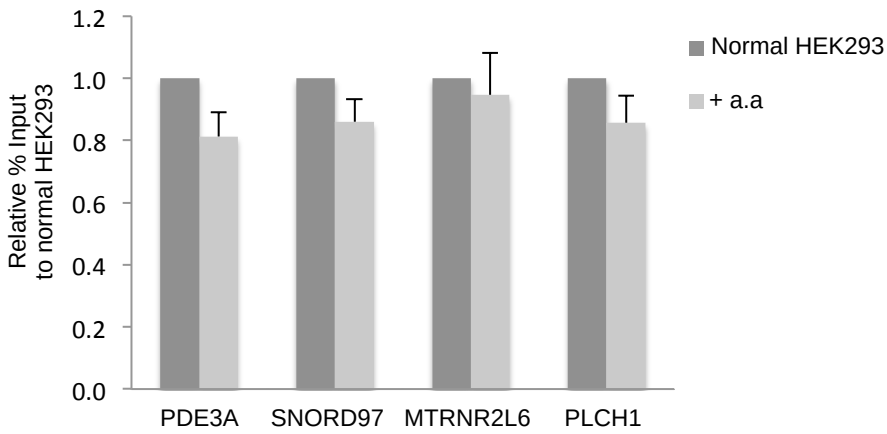


White_Supplementary Figure 2

d

type	number of dicer peaks overlapping the elements	expected	p-value (right handed)	significance
LINE	20	31	0.996	Significantly underrepresented
SINE	9	27	1	Significantly underrepresented
LTR	8	13	0.957	Significantly underrepresented
Satellite	16	1	0	Significantly overrepresented
rRNA	15	0	0	Significantly overrepresented
tRNA	38	0	0	Significantly overrepresented

e



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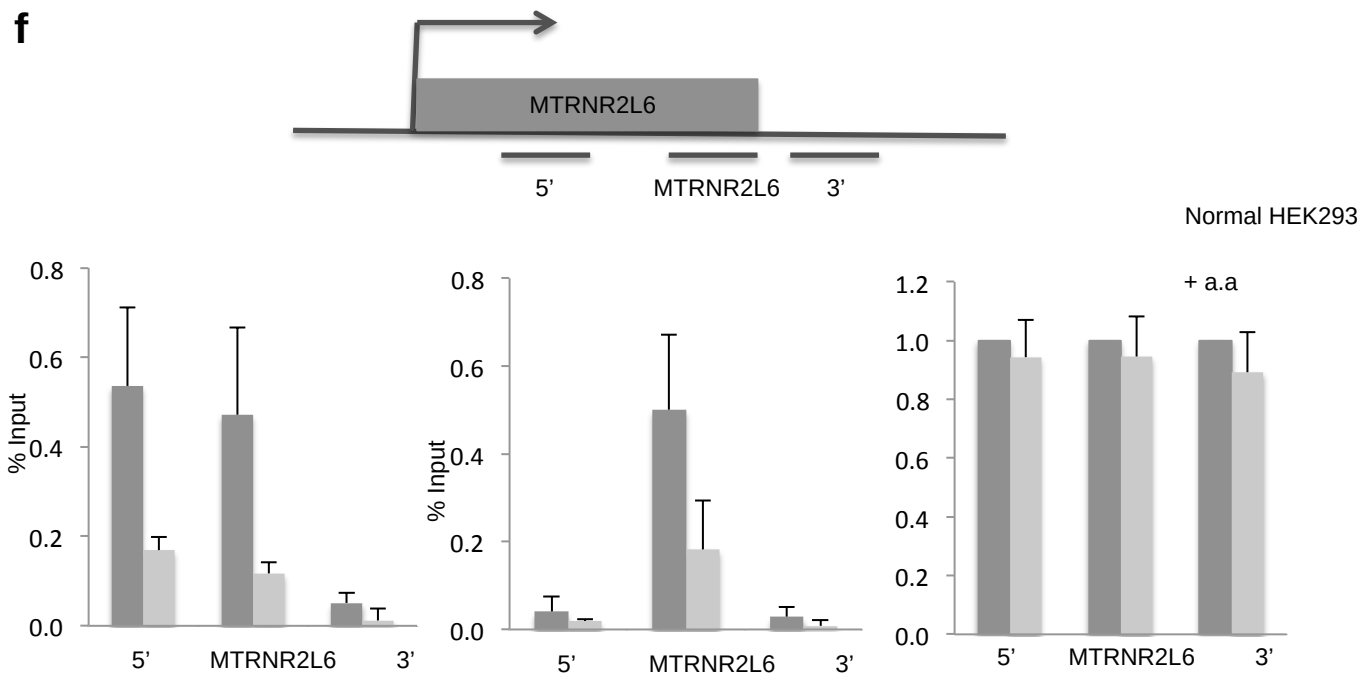


Supplementary Figure 2. Genomic annotation of Dicer ChIP-seq peaks.

a) Table showing the top 12 peaks of Dicer binding loci according to the ChIP sequencing data. Nearest gene identity is shown.

b) The 4 additional loci chosen for further analysis, showing a range of Dicer binding

c) Metagene analysis of top 118 Dicer binding sites (ChIP-seq peaks) showing their location distribution around TSS and PAS of coding genes in the human genome. Data were grouped into 250 nt bins. Each bin-midpoint was plotted on the graph.

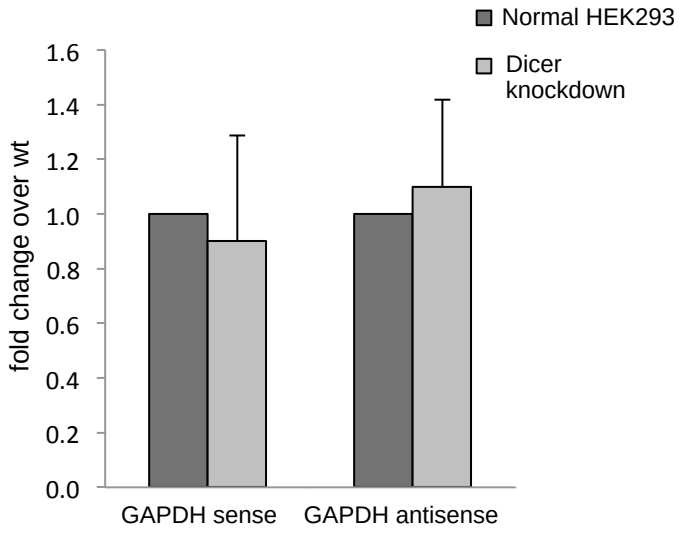
d) Table summarising the enrichment of repetitive DNA sequence elements in the Dicer ChIP-seq data. The RepeatMasker hg19 table was used to extract genomic repetitive elements. Repetitive element overlapping a Dicer peak with 5 nt or more was included in the analysis. Each category of repetitive elements as presented, was analysed separately. p-values are based on a background distribution of sampling 118 random genomic sequences of the same length as Dicer peaks. Sampling was performed more than 1000 times.

e) H3 control ChIP experiment as in Figure 2e.

f) Top panel: Diagram of the MTRNR2L6. The start site of transcription (arrow) and the position of the PCR amplicons (bold) are indicated. Bottom panels: Pol II, Dicer and H3 ChIP experiments as in Figure 2e.

White_Supplementary Figure 3

a



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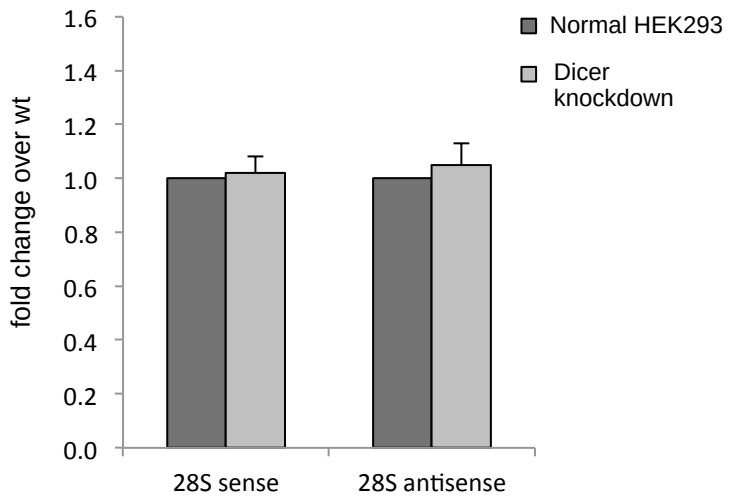
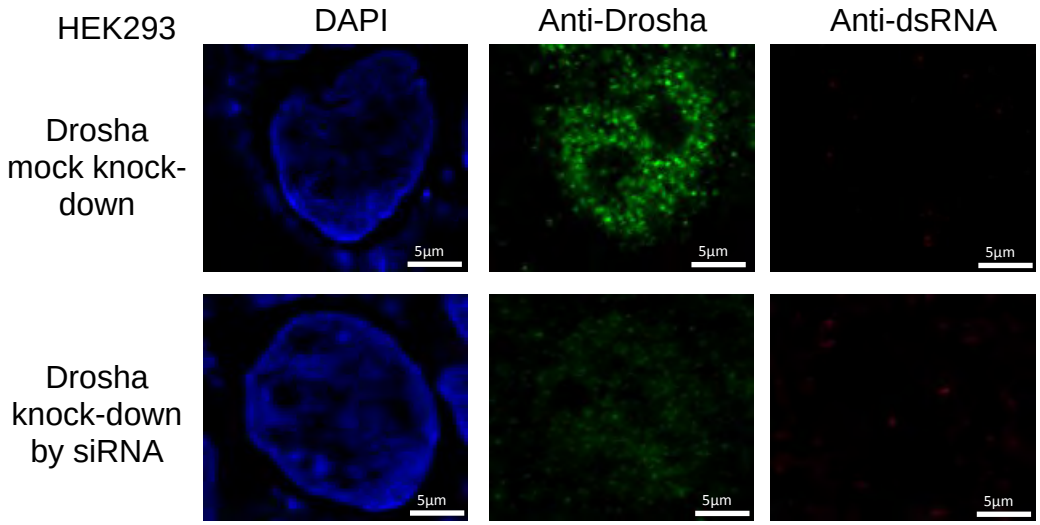


Supplementary Figure 3. Dicer and Pol II binding affect each other at selected loci.

a) qRT-PCR as in Fig 3d and 3e. Probes were specific to GAPDH and b) 28S rDNA.

White_Supplementary Figure 4

a



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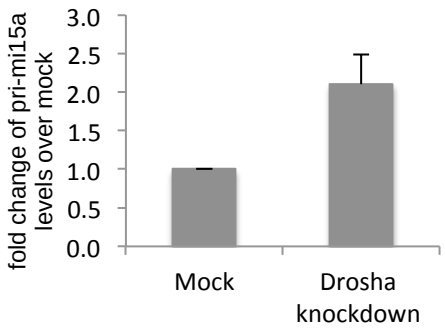
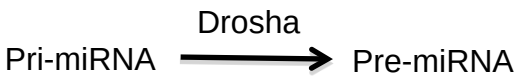


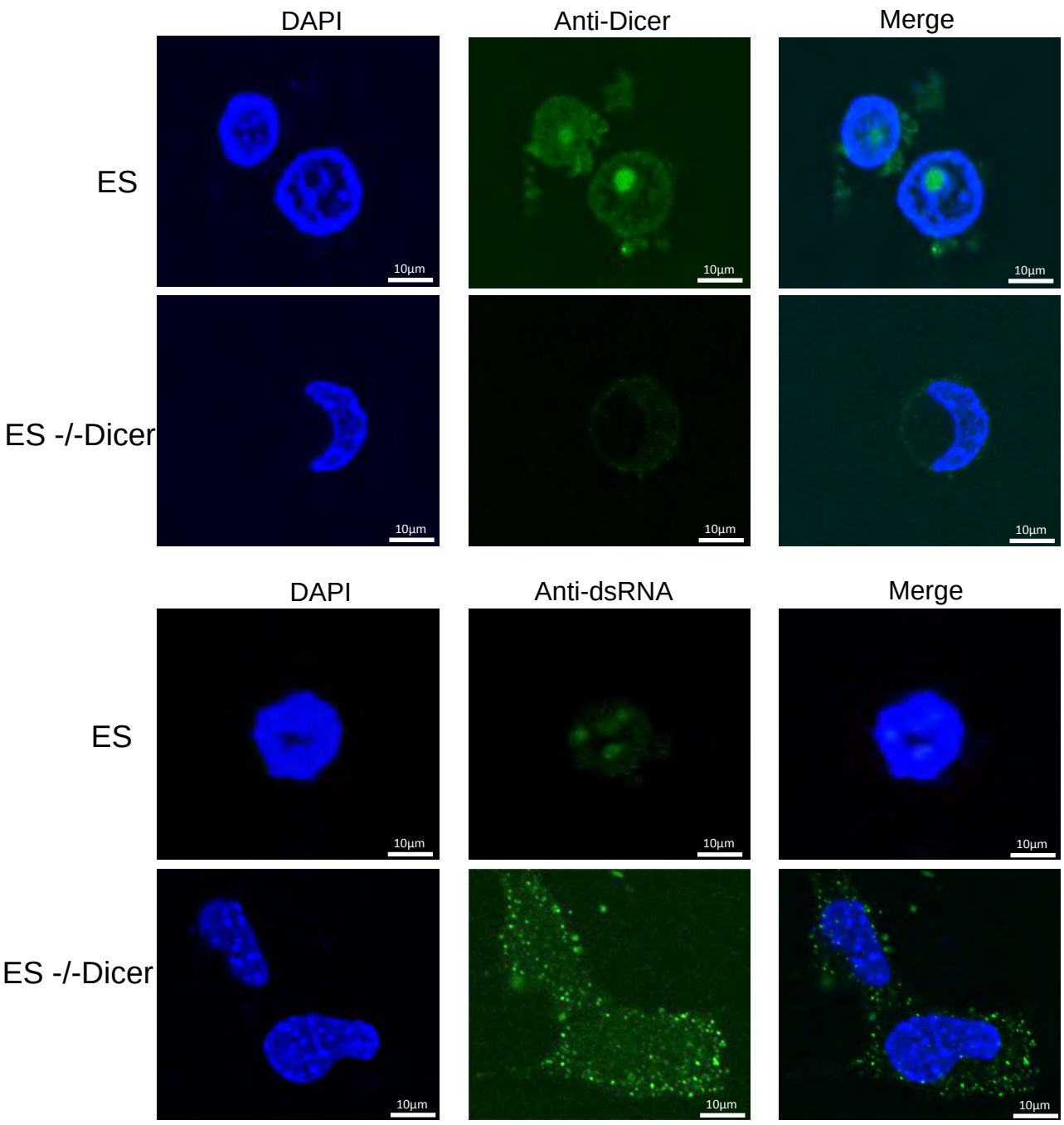


Supplementary Figure 4. Loss of Drosha has no effect on the accumulation of long dsRNA.

a) IF as in Fig. 1a using anti-Drosha and J2 antibodies on normal and Drosha knockdown HEK293 cells.

b) Levels of pri-mRNA15a transcripts as determined by qRT-PCR analysis using a specific primer for reverse transcription. RNA was isolated from normal HEK293 cells and cells treated with an shRNA against Drosha for 10 days. Signals are based on average values ± s.d. from three independent biological experiments and are normalised to the levels of transcripts in normal cells, set as 1.

White_Supplementary Figure 5

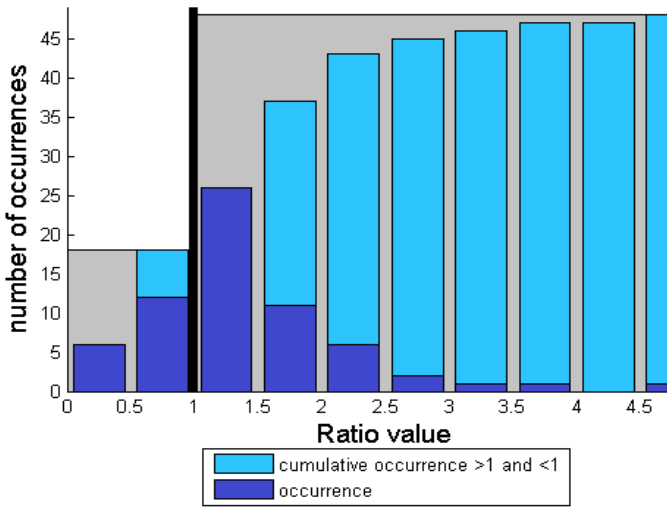


Supplementary Figure 5. Loss of Dicer leads to accumulation of long dsRNA.

IF as in Fig. 1a using anti-Dicer and J2 antibodies on Dicer knockout murine embryonic stem cells.

White_Supplementary Figure 6

a



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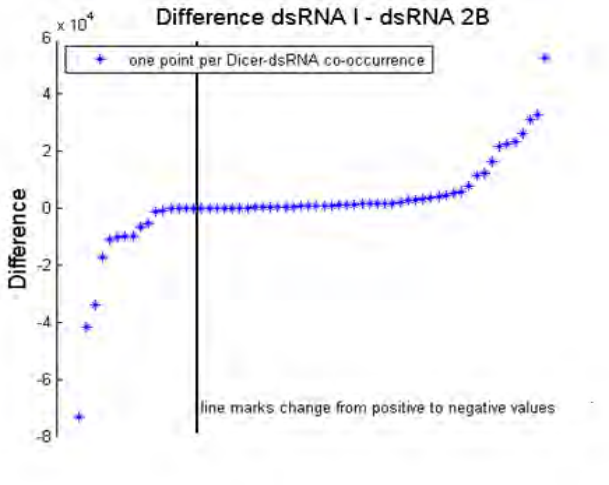

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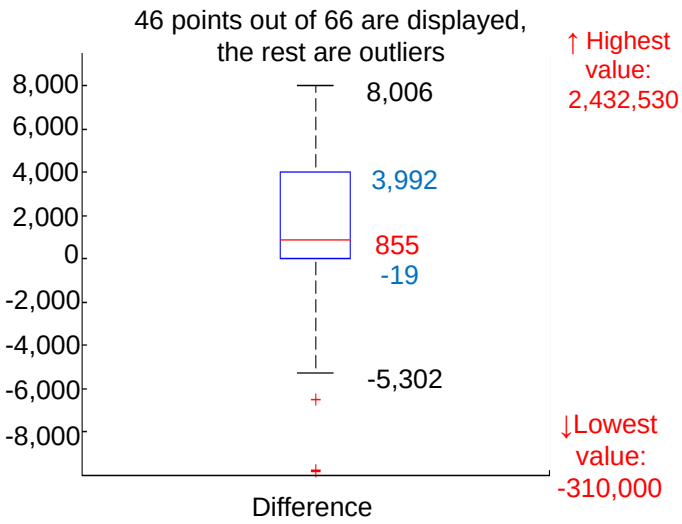
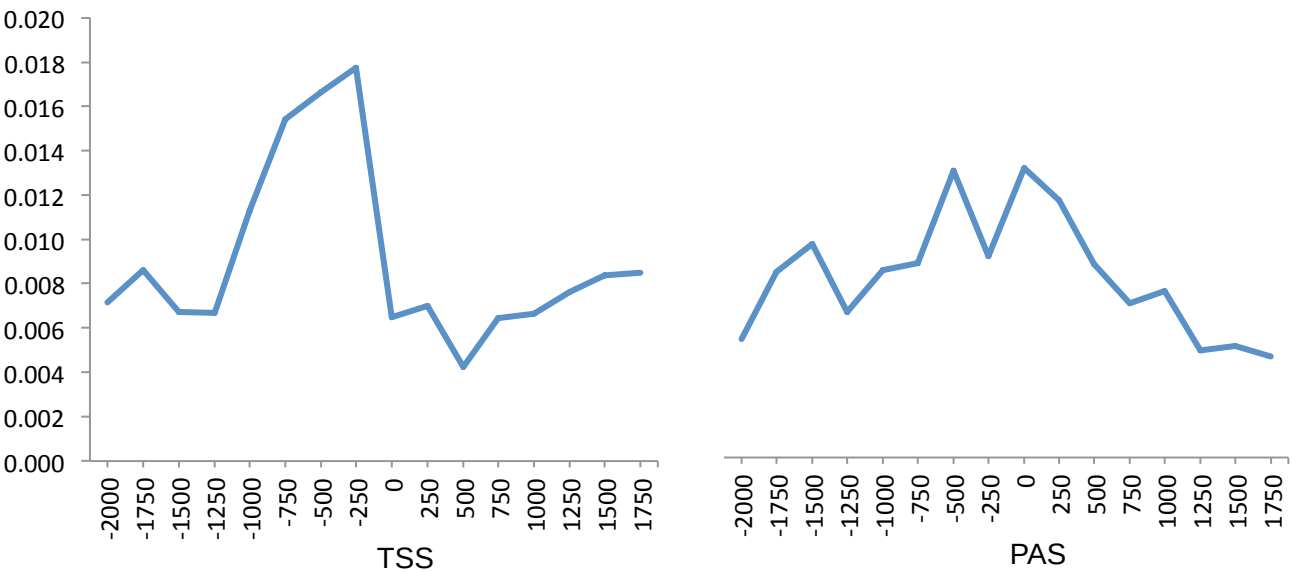


d

type	number of dicer peaks overlapping the elements	expected	p-value (right handed)	significance
LINE	71	211	1	Significantly underrepresented
SINE	70	153	1	Significantly underrepresented
LTR	17	90	1	Significantly underrepresented
Satellite	0	5	1	Significantly underrepresented
rRNA	200	0	0	Significantly overrepresented
tRNA	188	0	0	Significantly overrepresented

e



Supplementary Figure 6. Loss of Dicer leads to accumulation of dsRNA.

a) Histogram of peak summit ratio of dsRNA in Dicer knockdown cells to dsRNA in normal cells. Any dsRNA signal >10 in both uninduced and induced cells was considered. Value higher than 1 indicates accumulation of dsRNA and value lower than 1 indicates a decrease in dsRNA levels in Dicer knockdown cells. The black line marks the value of 1. Dark blue bins indicate the number of times a ratio falls in value span marked on the horizontal axis. Light blue bins represent a cumulative number for the values under 1 or over 1 separately. Centromeric repeat regions were excluded from analysis.

b) Diagram of the change level in dsRNA (according to peak summit value) accumulation in normal and Dicer knockdown cells. Any dsRNA signal >10 in both uninduced and induced cells was considered. Plotted levels from normal cells are subtracted Dicer knockdown cell levels. Value less than 0 (marked by a black vertical line) indicates the absolute value of the decrease in dsRNA, while a value greater than 0 indicates an increase. Each blue point depicts one locus of Dicer-dsRNA co-localization. The loci were ordered according to the difference value in ascending order. One point is too large to be depicted in the plot, it has value 2432530. Note the scale of the graph is ($x10^4$). Centromeric repeat regions were excluded from analysis. c) Box Plot of change in dsRNA levels (according to peak summit value) between Dicer knockdown and normal cells. Any dsRNA signal >10 in both uninduced and induced cells was considered. The red mark indicated the median, at a value of 855. The lower quartile q1 (25^{th} percentile) is at -19 and the upper quartile q3 (75^{th} percentile) at 3992 (blue box). The shift in the positive direction of the box shows a general increase in dsRNA at Dicer loci. 20 outlier

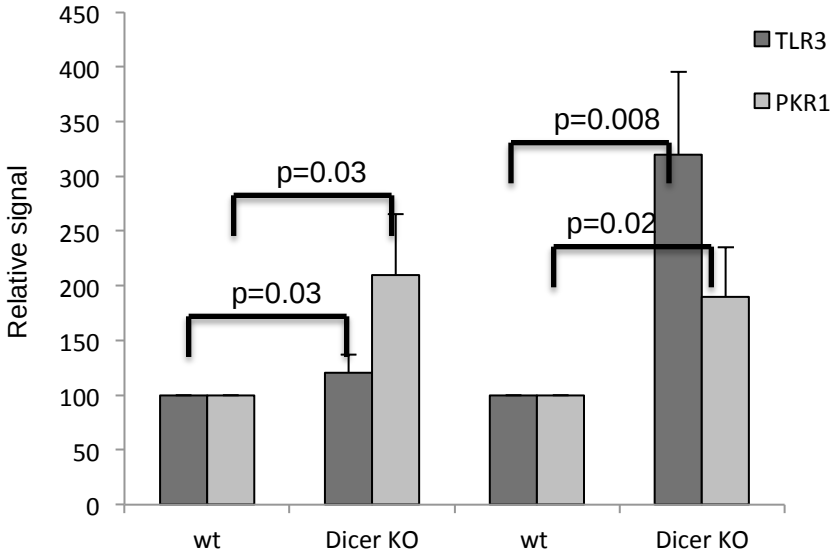
values were excluded from the plot. Centromeric repeat regions were excluded from analysis.

d) Tabular summary of enrichment of repetitive elements in dsRNA-seq data. The RepeatMasker hg19 table was used to extract genomic repetitive elements. Repetitive element overlapping a dsRNA peak with 5 nt or more was included in the analysis. Each category of repetitive elements as presented, was analysed separately. p-values are based on a background distribution of sampling 118 random genomic sequences of the same length as Dicer peaks. Sampling was performed more than 1000 times.

e) Metagene analysis of top dsRNA peaks (see Online Methods) showing location distribution around TSS and PAS of coding genes in the human genome. Data were grouped into 250 nt bins. Each bin-midpoint was plotted on graph.

White_Supplementary Figure 7

a



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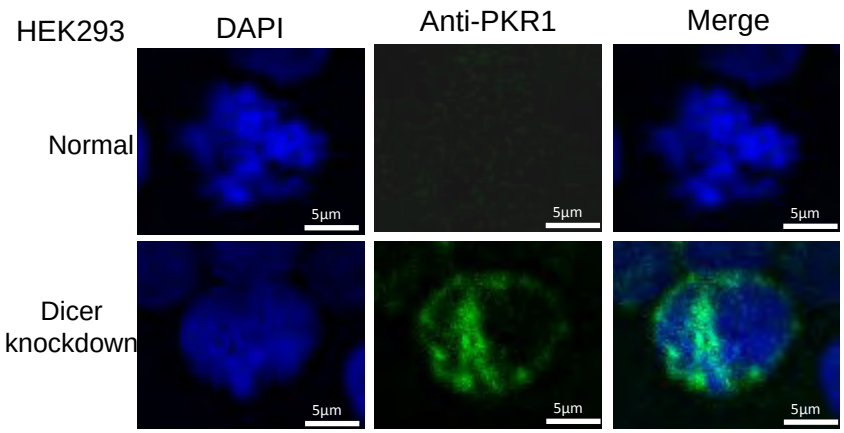


Supplementary Figure 7. Loss of Dicer triggers the interferon response.

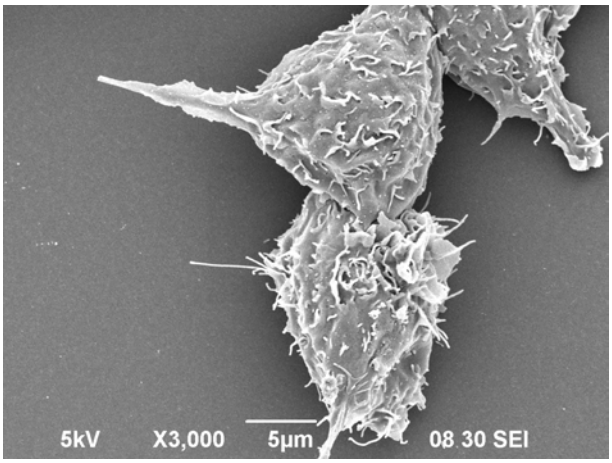
a) Quantitation of Western blot analysis of cell extracts isolated from normal and Dicer knockdown HEK293 cells, growing in tissue culture for 1 or 2 weeks using antibodies specific to Dicer, TLR3, PKR1 and γ-actin (see Figure 7a). Signals were measured by image-Quant software, are expressed as a % of input and are expressed as an average of three independent experiments, normalised to normal HEK293 cell levels, set as 1. * indicates statistical significance ($p < 0.05$), based on unpaired, two-tailed distribution Student's t-test.

b) IF as in Figure 4c using anti-PKR antibody (green).

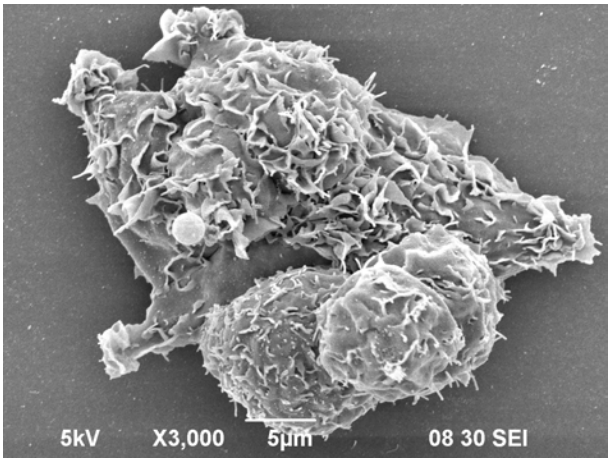
White_Supplementary Figure 8

a

Normal



Dicer knockdown



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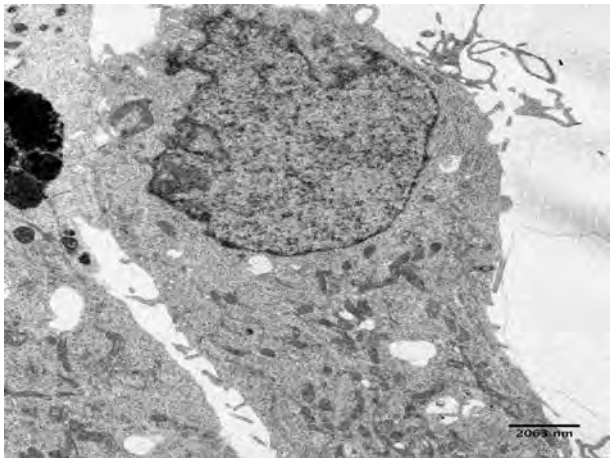

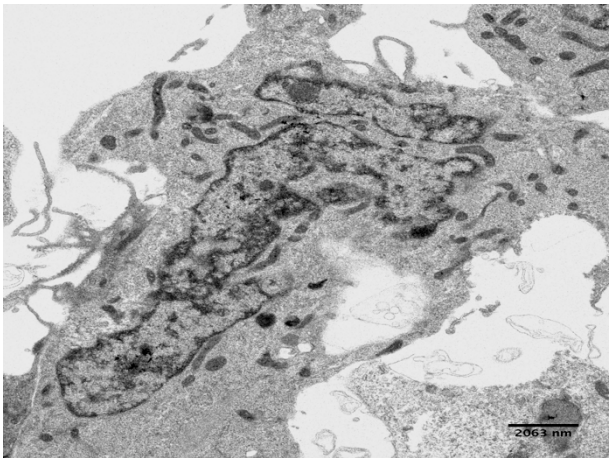

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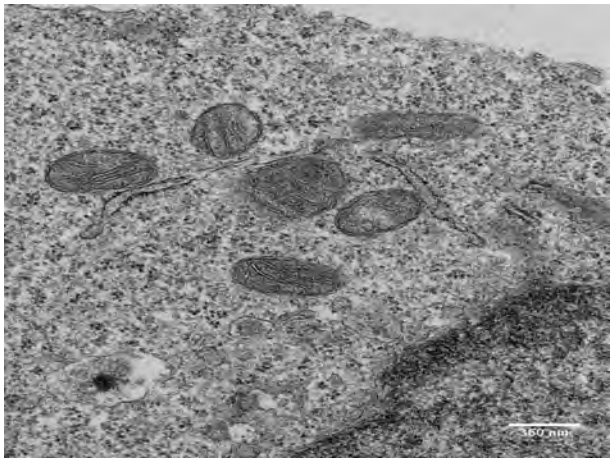
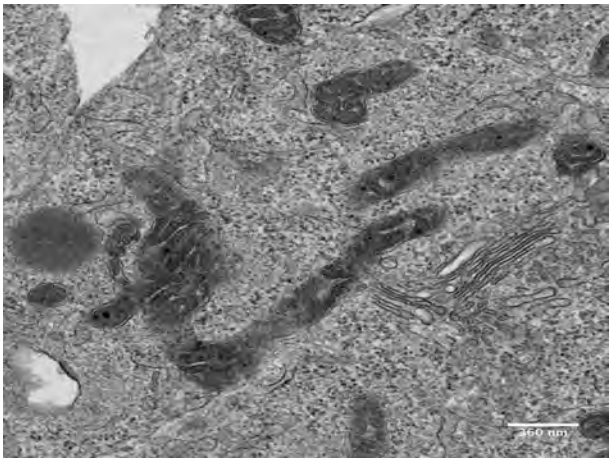




Supplementary Figure 8. Loss of Dicer triggers the interferon response.

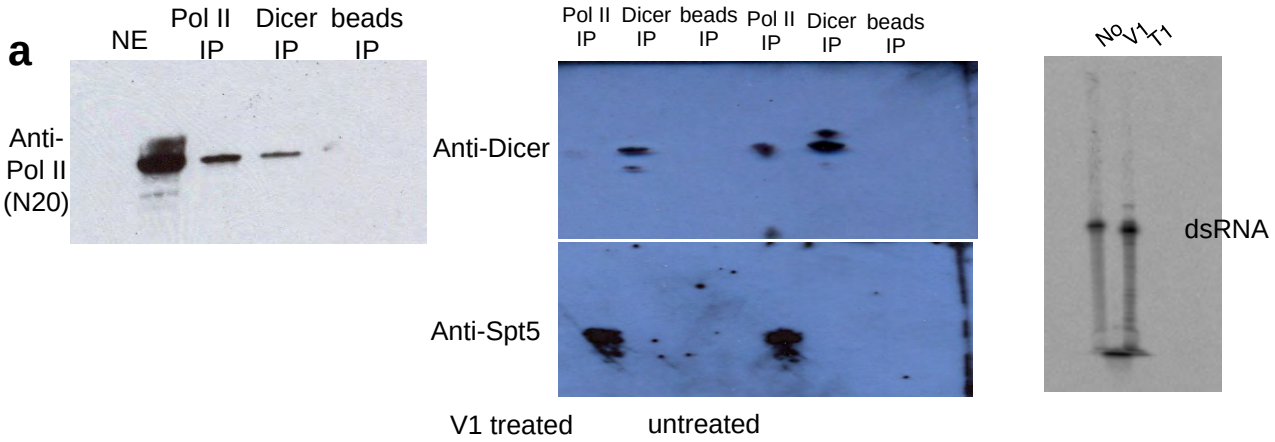
a) Morphological changes of normal and Dicer knockdown HEK293 cells using Electron Microscopy (SEM) at 3000-fold magnification.

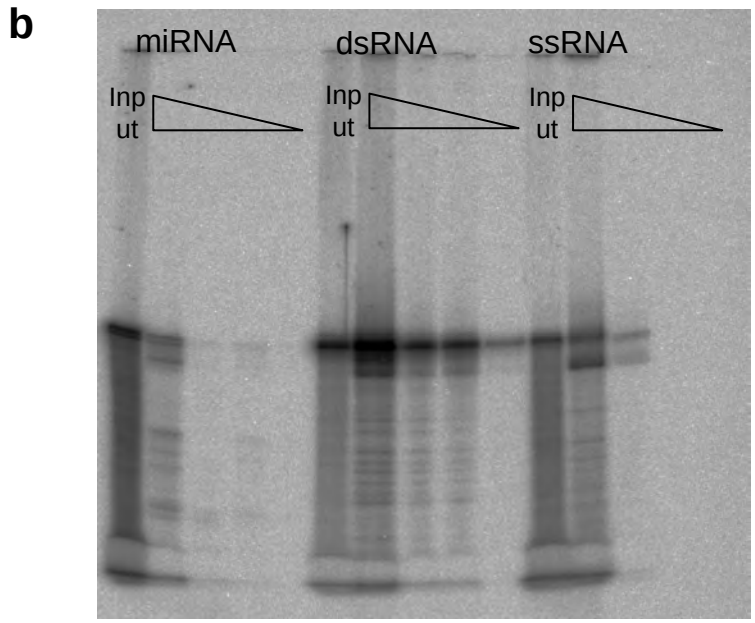
b) TEM analysis of nuclear structure in normal and Dicer knockdown HEK293.

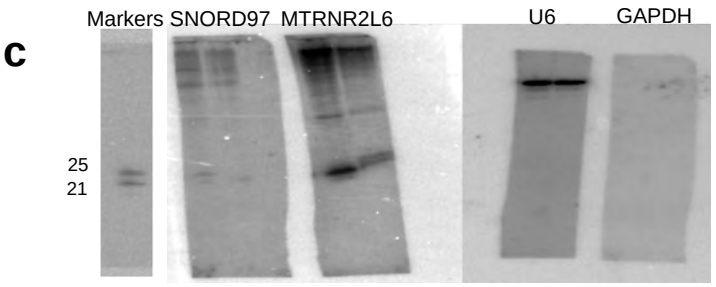
c) TEM as in middle panel focusing on mitochondria.

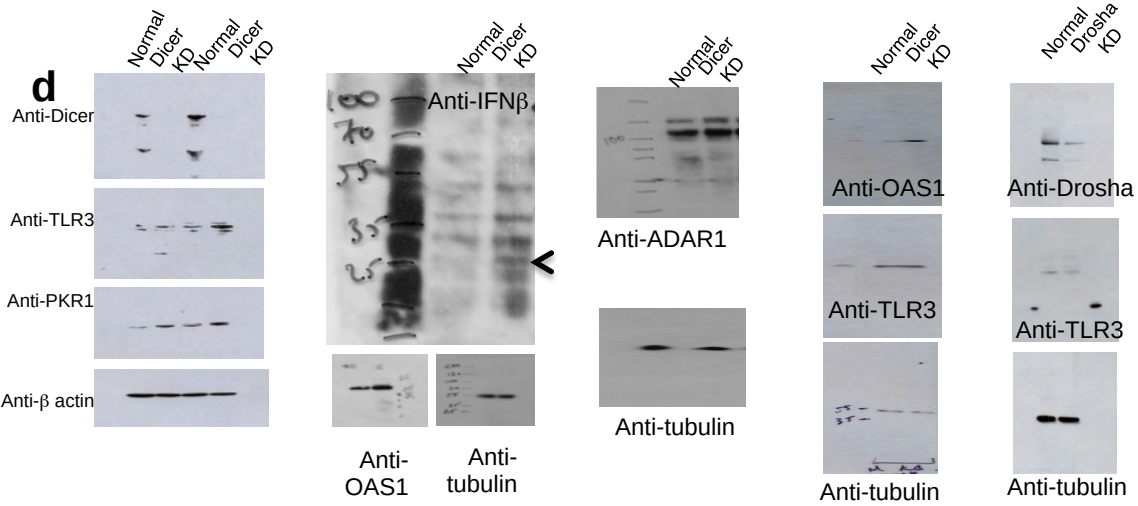
Representative images from 20 cells are presented.

White_Supplementary Figure 9









Supplementary Figure 9. Uncropped blot images.

a) Relating to Figure 2.

b) Relating to Figure 4.

c) Relating to Figure 6.

d) Relating to Figure 7.

Supplementary Table 1 – Primer sequences

Name	Sequence
PDE3A fw	5'gacatggccttccacatcta
PDE3A rev	5'ccatcttcgccttcttcttg
SNORD97 fw	5'gaaaatgccctgttttatcctt
SNORD97 rev	5'ctttgaatgtccagcgtcct
MTRNR2L6 fw	5'gggataacagcgcaatccta
MTRNR2L6 rev	5'tagatgggaggtgtggagga
5' MTRNR2L6 fw	5'tccaacacaggcatgctcta
5' MTRNR2L6 rev	5'aagagacagctgaaccctcg
5' MTRNR2L6 fw	5'tgcctggagtcctagttttagt
3' MTRNR2L6 rev	5'tctgcaaatgaggaccccat
PLCH1 fw	5'ttggctaaggcaatgaaag
PLCH1 rev	5'cctcctgggttcagtgtgtc
28S fw	5'gcaggaggtgtcagaaaagttaccacag
28S rev	5'ctaacctgtctcacgacggtctaaaccc
GAPDH fw	5'gatgacatcaagaaggtggt
GAPDH rev	5'ttgacaaagtggtcgttgag
pri-miRNA 15a fw	5'accttggagtaaagtagcagcac
pri-miRNA 15a rev	5'tattttcttcagaagatcag