

Supplementary Tables, Figures, and Data Sources

Small RNA Pathways in the Nematode *Ascaris* in the Absence of piRNAs

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Data Source File

Source_Data.xlsx

Supplementary Table 1. *Ascaris* small RNA pathway proteins and RNA expression

Small RNA Pathways	C. elegans gene	Ascaris chromosome	Start	End	Ascaris gene	Strand	GenBank Accession	Description	M1	M2	M3	M4	M5	M6	M7	M8	F1	F2	F3	F4	F5	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	L1	L2	Muscle	Intestine	Carcass	Highly expressed	Enriched tissue	
Ascaris Argonautes	Catalytic Tetrad																																						
AsALG-1	Yes (D/E/DH)	chr19	4458043	4471466	ascaris_gene_12048	-	HQ611964	Argonaute ALG-1	22	23	31	28	12	1	1	5	93	54	56	12	3	10	13	18	28	26	39	42	42	58	79	40	8	0	3	2	late embryo	not enriched	
AsALG-4	Yes (D/E/DH)	chr7	12118054	12141251	ascaris_gene_05665	-	HQ611965	Argonaute ALG-4	3	2	1	3	34	63	41	4	1	1	1	1	2	2	4	3	3	3	0	0	1	0	0	0	1	0	0	0	1	testis	testis
AsALG-5	Yes (D/E/DH)	chr18	4714106	4733778	ascaris_gene_11603	-	HQ611966	Argonaute ALG-5	8	5	6	4	2	0	0	1	10	4	5	2	2	3	3	6	8	13	7	13	13	8	4	1	1	0	1	late embryo	not enriched		
AsALG-6	NO (D/E/DH)	chr5	5452999	5472595	ascaris_gene_03788	+	HQ611967	Argonaute ALG-6	24	28	29	21	12	2	1	5	30	16	17	5	5	23	22	26	34	21	25	18	5	2	1	2	0	1	0	1	zygote	not enriched	
AsWAGO-1	Yes (D/E/DH)	chr11	1414478	1436678	ascaris_gene_07699	+	HQ611968	Argonaute WAGO-1	40	44	25	17	20	6	3	2	10	8	4	3	9	8	9	15	14	14	10	17	15	10	9	2	2	2	2	3	testis	testis	
AsWAGO-2	NO (D/E/DH)	chr1	15908083	15930379	ascaris_gene_00855	-	HQ611970	Argonaute WAGO-1	81	81	48	92	65	8	4	11	17	12	13	6	10	88	89	80	125	106	75	51	23	10	6	5	1	8	28	65	zygote	not enriched	
AsWAGO-3	NO (S/T/G/S)	chr6	12439274	12465887	ascaris_gene_04899	+	HQ611971	Argonaute WAGO-2	25	26	24	24	10	1	1	5	33	31	33	11	10	49	47	42	37	26	31	17	8	6	5	4	1	1	10	4	zygote	not enriched	
AsCSR-1	Yes (D/E/DH)	chr17	2927127	2946141	ascaris_gene_11056	-	HQ611969	Argonaute CSR-1	109	132	105	122	141	70	35	18	180	96	104	12	29	127	206	241	336	193	131	52	8	3	4	5	1	2	6	3	zygote	zygote	
AsNRDE-3	NO (D/A/G/R)	chr5	4557517	4582095	ascaris_gene_03731	-	HQ611973	Argonaute NRDE-3 (Nuclear)	19	22	15	13	38	19	6	3	33	19	20	6	25	28	34	33	52	50	31	26	24	12	9	2	3	3	3	early embryo	not enriched		
Ascaris RdRPs																																							
RdRP 1	ego-1 or rrf-1	chr8	1412361	1451674	ascaris_gene_05790	-	HQ611977	RNA-dependent RNA polymerase RdRP-1	13	14	12	20	37	7	3	2	9	6	6	2	1	4	3	2	4	3	2	1	2	2	3	3	1	0	2	1	testis	testis	
RdRP 2	rrf-1 or ego-1	chr1	3602126	3640615	ascaris_gene_00191	+	HQ611978	RNA-dependent RNA polymerase RdRP-2	6	6	4	4	3	0	0	1	3	3	2	2	0	1	1	1	3	2	2	2	4	4	4	3	0	0	1	0	low expression	low expression	
RdRP 3	rrf-3	chr2	751145	785712	ascaris_gene_01050	+	HQ611979	RNA-dependent RNA polymerase RdRP-3	11	10	7	16	58	4	2	1	14	7	8	6	2	12	12	15	20	11	11	7	9	6	8	3	1	2	1	1	testis	not enriched	
miRNA																																							
alg-1	chr19	4458043	4471466	ascaris_gene_12048	-	HQ611964	Argonaute ALG-1	22	23	31	28	12	1	1	5	93	54	56	12	3	10	13	18	28	26	39	42	42	58	79	40	8	0	3	2	late embryo	not enriched		
dcr-1	chr12	1368927	1402556	ascaris_gene_08345	+	HQ611974	miRNA processing factor	26	26	22	17	15	3	2	2	3	1	1	3	3	2	3	2	5	8	13	11	23	21	22	15	2	7	5	5	late embryo	not enriched		
pasha	chr1	1826955	1897512	ascaris_gene_00093	-	HQ611976	miRNA processing factor	43	37	35	26	22	14	7	4	6	4	6	4	3	1	5	5	6	9	12	10	4	6	5	5	3	0	1	2	1	testis	testis	
drosna	chr15	5528651	5564322	ascaris_gene_10272	+	HQ611975	miRNA processing factor	24	29	26	39	32	4	1	5	13	8	4	6	20	16	16	21	41	26	28	41	44	40	28	8	6	3	9	late embryo	not enriched			
ain-1	chrX1	1594921	1610151	ascaris_gene_12286	-	J1160532	miRNA RISC complex factor	10	7	8	7	4	1	1	4	67	65	64	10	12	13	15	21	15	22	18	25	29	43	56	50	30	3	6	2	ovary	not enriched		
nhl-2	chr12	6339984	6370362	ascaris_gene_08672	-	J1165920	Nhl-2	21	22	17	13	12	3	1	3	25	15	16	3	3	13	11	12	11	19	12	11	9	11	11	7	2	1	1	2	early embryo	not enriched		
vig-1	chr18	5039655	5051965	ascaris_gene_11629	+		miRNA RISC complex factor, RNA-binding	45	58	53	64	71	23	9	8	32	18	7	36	144	199	293	182	236	267	318	191	129	88	51	29	45	41	51	late embryo	not enriched			
tsn-1	chrX5	2244380	2260469	ascaris_gene_15329	+		Nuclease domain-containing protein 1	24	28	20	40	35	4	4	21	70	42	43	27	28	36	36	35	36	32	29	21	28	28	42	30	7	114	39	66	muscle	muscle		
cgh-1	chr3	13262450	13996516	ascaris_gene_02947	-		ATP-dependent RNA helicase cgh-1	50	51	46	42	21	4	1	5	42	46	46	12	5	35	27	26	30	52	32	22	17	14	11	1	1	5	3	6	early embryo	not enriched		
cgh-1	chr1	36822	134440	ascaris_gene_00004	-		ATP-dependent RNA helicase cgh-1	41	47	40	50	22	3	2	11	62	65	63	20	10	62	54	55	64	76	77	50	33	24	20	13	3	8	6	10	early embryo	not enriched		
dcs-1	chr19	1845323	1849979	ascaris_gene_11857	+		Decapping scavenger enzyme, DcpS	4	3	5	9	4	0	0	1	9	4	4	1	1	4	7	6	16	31	20	21	26	20	14	10	1	1	1	1	early embryo	not enriched		
xm-2	chr13	11458424	11507380	ascaris_gene_02441	+		3'-5' exonuclease	26	34	24	26	13	1	1	28	35	29	27	14	8	17	15	14	20	17	24	21	21	19	12	8	4	7	13	10	ovary	not enriched		
ALG-3/4 26G	missing eri-3 and eri-5																																						
alg-3/4	chr7	12118054	12141251	ascaris_gene_05665	-	HQ611965	Argonaute ALG-4	3	2	1	3	34	63	41	4	1	1	1	1	1	2	2	4	3	3	3	3	0	0	1	0	0	0	0	1	testis	testis		
eri-3	chr5	751145	785712	ascaris_gene_01050	-	HQ611979	RNA-dependent RNA polymerase RdRP3	11	10	7	16	58	4	2	1	14	7	8	6	2	12	12	15	20	11	11	7	9	6	8	3	1	2	1	1	testis	not enriched		
dcr-1	chr12	1368927	1402556	ascaris_gene_08345	+	HQ611974	DICER	26	26	22	17	15	3	2	2	3	1	1	3	3	2	3	2	5	8	13	11	23	21	22	15	2	7	5	5	late embryo	not enriched		
eri-1	chr5	1827586	18320784	ascaris_gene_03925	+	J1173279	ERI1 exonuclease 3	15	13	7	11	16	8	6	13	29	20	23	6	6	26	27	21	24	23	25	17	15	24	27	11	6	26	11	4	intestine	not enriched		
rde-4	chr3	5134164	5146005	ascaris_gene_02096	-	J1170472	dsRNA binding domain-containing protein rde-4	14	17	19	20	11	2	2	2	11	5	6	2	2	7	9	11	11	15	15	16	19	16	13	9	5	3	11	4	late embryo	not enriched		
dth-3	chr9	10645994	10673120	ascaris_gene_06989	-	J1165061	ATP-dependent RNA helicase	41	47	41	71	70	10	5	10	15	13	14	11	28	23	16	20	17	14	19	21	24	16	14	8	6	11	13	13	testis	testis		
gstf-1	chr13	4404093	4411376	ascaris_gene_09121	-		Gametocyte-specific factor 1-like (GSTF-1)	13	15	10	12	7	2	1	1	4	3	2	2	4	14	10	8	12	13	10	8	17	19	14	12	1	2	1	3	late embryo	late embryo		
pr-1	chr17	1204210	1221260	ascaris_gene_10954	-	J1172438	RNAP complex-1-interacting phosphatase	75	74	70	77	89	12	24	9	22	19	21	28	71	150	83	59	67	75	76	64	78	66	62	34	16	22	34	40	zygote	not enriched		
CSR-1 22G																																							
csr-1	chr17	2927127	2946141	ascaris_gene_11056	-	HQ611969	Argonaute CSR-1	109	132	105	122	141	70	35	18	180	96	104	12	29	127	206	241	336	193	131	52	8	3	4	5	1	2	6	3	zygote	zygote		
ego-1	chr8	1412361	1451674	ascaris_gene_05790	-	HQ611977	RNA-dependent RNA polymerase	13	14	12	20	37	7	3	2	9	6	6	2	1	4	3	2	4	3	2	1	2	2	3	3	1	0	2	1	testis	testis		
wago-2	chr6	12439274	12465887	ascaris_gene_04899	+	HQ611971	Argonaute WAGO-2	25	26	24	24	10	1	1	5	33	31	33	11	10	49	47	42	37	26	31	17	8	6	5	4	1	1	10	4	zygote	zygote		
ekl-1	chr19	1737257	1746257	ascaris_gene_11849	+		Tudor domain containing protein	11	11	14	15	13	3	1	2	10	6	7	3	15	10	9	15	13	10	5	16	18	12	7	1	1	1	1	1	late embryo	not enriched		
dth-3	chr9	10645994	10673120	ascaris_gene_06989	-	J1165061	ATP-dependent RNA helicase	41	47	41	71	70	10	5	10	15	13	14	11	28	23	16	20	17	14	19	21	24	16	14	8	6	11	13	13	testis	testis		
cde-1	chr14	2083211	2109048	ascaris_gene_09522	-	J1167978	Terminal uridylyltransferase 7 (cde-1, cid-1, pup-1)	13	14	18	17	17	24	20	2	5	3	3	2	4	5	6	7	10	11	10	19	22	22	15	2	0	2	1	late embryo	not enriched			
pr-1	chr17	1204210	1221260	ascaris_gene_10954	-	J1172438	R/RNP complex-1-interacting phosphatase	75	74	70	77	89	12	24	9	22	19																						

Supplementary Table 2. *Ascaris* small RNA IP and sequencing libraries

Library	INPUT/AGO	Developmental stage	Reads # (18-30nt)	Norm (30 M)
ALG1_testis	ALG1	testis	20,258,575	1.48
ALG1_ovary	ALG1	ovary	25,894,531	1.16
ALG1_4cell	ALG1	4cell	6,041,327	4.97
ALG4_M1	ALG4	M1	21,920,380	1.37
ALG4_M2	ALG4	M2	17,303,681	1.73
ALG4_M3	ALG4	M3	20,147,308	1.49
ALG4_M4	ALG4	M4	17,019,710	1.76
ALG4_M5	ALG4	M5	14,594,971	2.06
ALG4_M6	ALG4	M6	33,061,039	0.91
ALG4_M7	ALG4	M7	20,076,957	1.49
ALG4_testis	ALG4	testis	24,413,409	1.23
ALG4_ovary	ALG4	ovary	13,781,368	2.18
ALG4_4cell	ALG4	4cell	11,785,658	2.55
WAGO1_M1	WAGO1	M1	64,894,596	0.46
WAGO1_M2	WAGO1	M2	27,650,140	1.08
WAGO1_M3	WAGO1	M3	78,808,041	0.38
WAGO1_M4	WAGO1	M4	51,542,968	0.58
WAGO1_M5	WAGO1	M5	46,436,116	0.65
WAGO1_M6	WAGO1	M6	34,158,119	0.88
WAGO1_M7	WAGO1	M7	35,436,541	0.85
WAGO1_testis	WAGO1	testis	116,225,461	0.26
WAGO1_ovary	WAGO1	ovary	81,649,947	0.37
WAGO1_4cell	WAGO1	4cell	45,550,387	0.66
WAGO2_M1	WAGO2	M1	19,368,275	1.55
WAGO2_M2	WAGO2	M2	12,643,822	2.37
WAGO2_M3	WAGO2	M3	17,922,560	1.67
WAGO2_M4	WAGO2	M4	12,396,772	2.42
WAGO2_M5	WAGO2	M5	21,504,190	1.40
WAGO2_M6	WAGO2	M6	41,636,168	0.72
WAGO2_M7	WAGO2	M7	29,775,560	1.01
WAGO2_testis	WAGO2	testis	41,814,032	0.72
WAGO2_ovary	WAGO2	ovary	32,028,287	0.94
WAGO2_4cell	WAGO2	4cell	27,291,379	1.10
CSR1_M1	CSR1	M1	49,341,217	0.61
CSR1_M2	CSR1	M2	10,181,731	2.95
CSR1_M3	CSR1	M3	56,729,787	0.53
CSR1_M4	CSR1	M4	45,346,435	0.66
CSR1_M5	CSR1	M5	63,015,414	0.48
CSR1_M6	CSR1	M6	6,587,238	4.55
CSR1_M7	CSR1	M7	20,430,927	1.47
CSR1_testis	CSR1	testis	84,083,235	0.36
CSR1_ovary	CSR1	ovary	36,661,269	0.82
CSR1_4cell	CSR1	4cell	86,131,360	0.35
WAGO3_M1	WAGO3	M1	41,338,185	0.73
WAGO3_M2	WAGO3	M2	13,452,144	2.23
WAGO3_M3	WAGO3	M3	23,253,295	1.29
WAGO3_M4	WAGO3	M4	16,275,541	1.84
WAGO3_M5	WAGO3	M5	44,131,606	0.68
WAGO3_M6	WAGO3	M6	20,300,969	1.48
WAGO3_M7	WAGO3	M7	20,213,402	1.48
WAGO3_testis	WAGO3	testis	29,132,141	1.03
WAGO3_ovary	WAGO3	ovary	22,265,707	1.35
WAGO3_4cell	WAGO3	4cell	40,916,382	0.73
NRDE3_M1	NRDE3	M1	24,032,409	1.25
NRDE3_M2	NRDE3	M2	28,945,864	1.04
NRDE3_M3	NRDE3	M3	15,718,655	1.91
NRDE3_M4	NRDE3	M4	6,300,983	4.76
NRDE3_M5	NRDE3	M5	21,045,060	1.43
NRDE3_M6	NRDE3	M6	30,055,113	1.00
NRDE3_M7	NRDE3	M7	17,823,901	1.68
NRDE3_testis	NRDE3	testis	70,567,291	0.43
NRDE3_ovary	NRDE3	ovary	62,473,304	0.48
NRDE3_4cell	NRDE3	4cell	17,267,975	1.74

Supplementary Figure legends:

Supplementary Figure 1. AsCSR-1 RNA expression and H3K9me3 marks. A genome browser view on Chr17 shows the AsCSR-1 gene (in between 2.9 - 2.95 Mb, marked by an arrow) and other gene models in this region of the genome, RNA-seq and H3K9me3 ChIP-seq from selected developmental stages. The gene models are largely based on RNA-seq (including ISO-seq) data. Red and blue indicate forward and reverse transcription, respectively. Note the high expression of AsCSR-1 in early embryos (1-4 cells) and the loss of its expression in 32-64 cells associated with heavy H3K9me3 marks.

Supplementary Figure 2. *Ascaris* Argonaute antibodies. A. Western blots illustrating the specificity of antibodies and the relative amount of Argonaute proteins in the total cell lysate (T), cytoplasmic (C), or nuclear (N) fraction. The size of the Argonaute proteins is at ~ 100 kDa. An H3 histone variant (CENP-A) is used as a control for the cell fractionation. Data are derived from two or more biological replicates. **B.** Argonautes IP small RNAs and Northern blots. Illustrated are small RNAs that co-IP with selected Argonautes antibodies on gels or from Northern blots. Note the high specificity of AsALG-1 IP for miRNAs and AsWAGO-1 for 22G-RNAs. CBP80 is a negative control that does not bind to any small RNAs. Data are derived from two biological replicates. miR-100 sequence: AACCCGTAGATCCGAACCTTGTTT; Northern probe: CACAAGTTCGGATCTACGG. 22G-49 sequence: GTTAACGTAGAGCTCGCCAGAG; Northern probe: TGGCGAGCTCTACGTTAAC.

Supplementary Figure 3. *Ascaris* male germline regions. Male germline removed and untangled in an 11"x10" tray in 1x PBS. The germinal tip is less than 0.15 mm in diameter and typically entangled around a downstream region of the germline; thus it requires careful dissection to obtain the intact region.

Supplementary Figure 4. *Ascaris* testes 5' all-phosphate vs. 5'-monophosphate small RNA libraries. Comparison of small RNA library preparation methods (see methods) that capture 5' all-phosphate vs. 5'-monophosphate in broad regions of the testis. Region 1 corresponds to the mitotic region, transition zone, and early pachytene, Region 2 to late pachytene and meiosis, and Region 3 to late meiosis and mature spermatids. We observed an increased amount of small RNAs matching rRNAs in region 3 that have a random size distribution, consistent with what is observed in spermatids (Fig. 4C). This may be associated with the turnover of rRNAs in *Ascaris* late meiosis. Note the enrichment of 5'-monophosphate small RNAs on right, i.e. miRNAs in Region 1 and 26G-RNAs in Region 2.

Supplementary Figure 5. Chromosomal location of small RNAs. Distribution of small RNAs associated with A) *Ascaris* CSR-1 (green) and B) WAGO-1 (Red) Argonautes on *Ascaris* chromosomes. The small RNAs are normalized to the total number of sequencing reads. The scale on Y-axis are (provide scale of 0- X reads) capped so plots at highly enriched regions will not go beyond the top lines. Note the relative even distribution of AsCSR-1 associated small RNAs (targeting mRNAs) and the more biased distribution of AsWAGO-1 associated small RNAs (targeting repeats) in the genomic regions that will be eliminated during programmed DNA elimination. These eliminated regions have higher levels of repetitive sequences. Also note that the sex chromosomes are largely silenced in the male germline, thus there is a lack of siRNAs associated with AsCSR-1 on these chromosomes in the testis.

Supplementary Figure 6. Distribution and strandedness of small RNAs to mRNAs. Meta-analysis of siRNA distribution across mRNAs (in 100 bins on x-axis). The relative levels of the siRNAs are shown on y-axis. Note the biased antisense targeting at the 5'-end of mRNAs at late pachytene and meiosis for many of the Argonaute associated small RNAs, suggesting a possible concerted effort to repress the mRNAs for their clearance. Blue represents antisense to mRNAs and red indicates sense mRNAs.

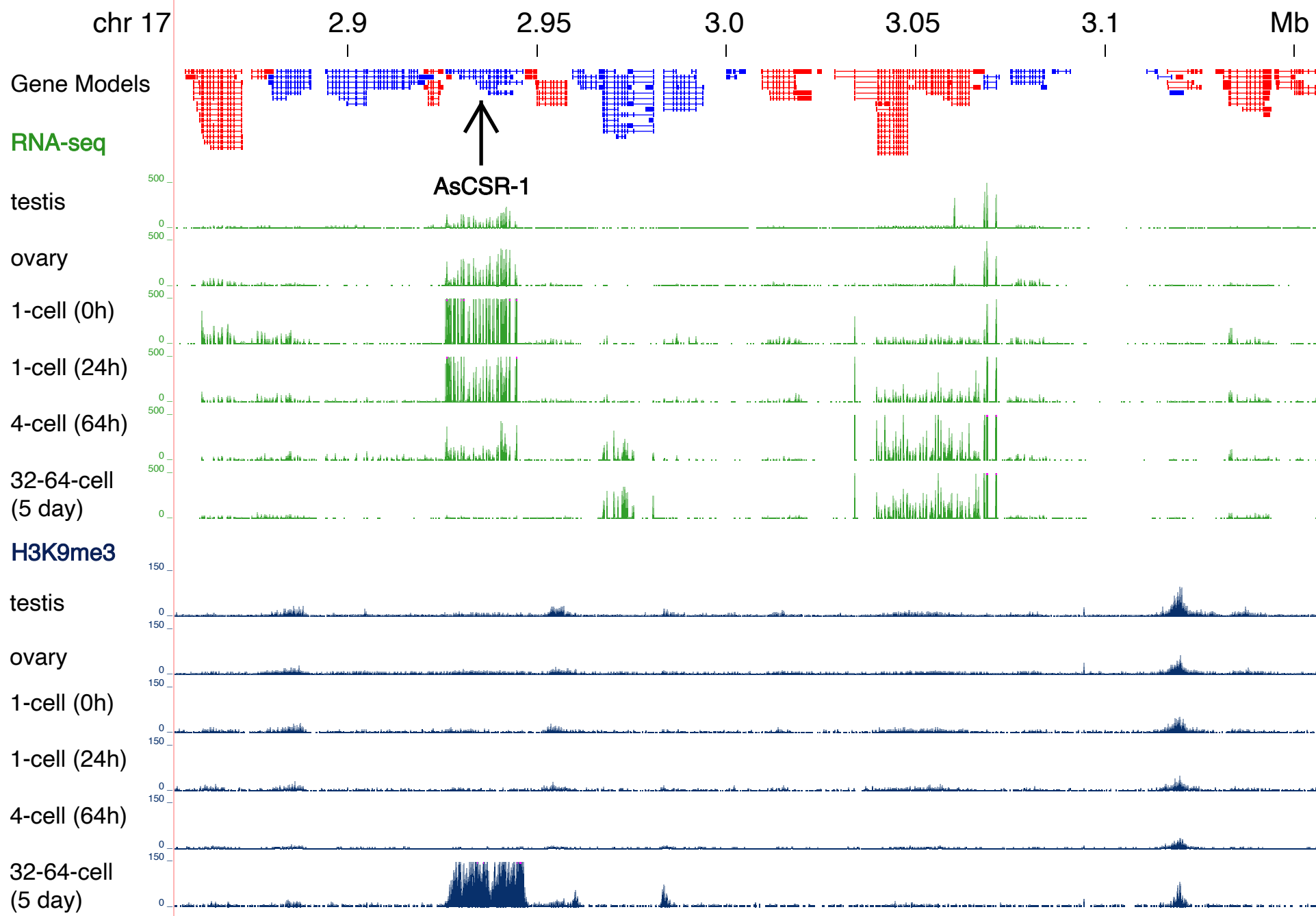
Supplementary Figure 7. Small RNA size distributions in INPUT and AGO IP libraries. The size distribution and frequency of small RNAs associated with the INPUT or a specific Argonaute IP. Small RNAs (18-30 nt) from input and Argonaute IP were plotted for the whole sequencing library (All-siRNAs) and different types of small RNA targets. Small RNAs starting with A, C, G, and U of different sizes (x-axis)

were plotted against their read frequencies (y-axis; raw reads in millions). For each library, the y-axis scale is set the same as All-siRNAs to provide a direct comparison between different types of siRNAs within the same library.

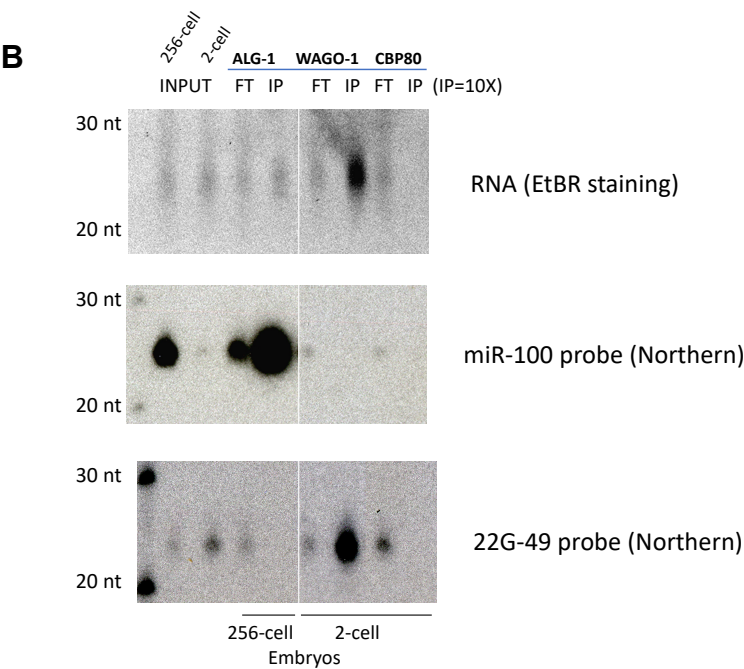
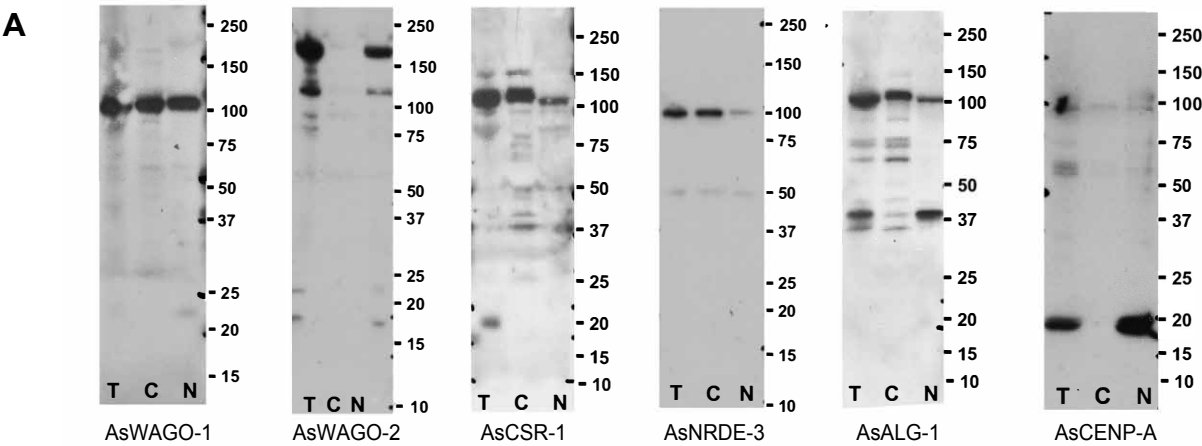
Supplementary Figure 8. Comparisons of siRNA levels and their target RNA expression. Pairwise comparisons of siRNAs associated with different argonautes and their targets. **A.** Repetitive sequences targeted by small RNAs in the testis (data from Figure 2A). The small RNAs are normalized to the total number of sequencing reads (see methods). The RNA level (in rpkm) for these repeat targets on the y-axis is derived from testis RNA-seq data. Overall, the RNA expression level from these repeats is low, suggesting a potential repression role of the siRNAs. Note the similarity between AsWAGO-1, AsWAGO-2 and AsNRDE-3. **B.** mRNA small RNA targets from the testis in late pachytene and meiosis (M6). The red indicates mRNAs that are specifically expressed during male meiosis. Note the differential targeting between AsCSR-1 and AsALG-4 and the overlap between AsNRDE-3 and AsCSR-1. The targeted mRNAs show complex RNA expression levels, suggesting a potential mix of licensing, tuning and repressive functions for the small RNAs.

Supplementary Figure 9. Argonaute associated small RNAs targeting Argonaute mRNAs. Genome browser tracks showing four Argonautes mRNAs targeted by their siRNAs in different developmental stages. The mRNA levels are normalized RNA-seq data from the M1-M8, ovary, and 4-cell embryos. PRO-seq data shows the nascent RNAs for these four Argonaute genes. The data below the PRO-seq are small RNA sequencing data from the corresponding Argonaute IPs. This illustrates potential auto-regulation of the expression of Argonautes by their associated small RNAs. For example, AsALG-4 is meiosis-specific and its expression in M5-M7 leads to the generation of 26G-RNAs that also target and repress AsALG-4 mRNA in M6-M7.

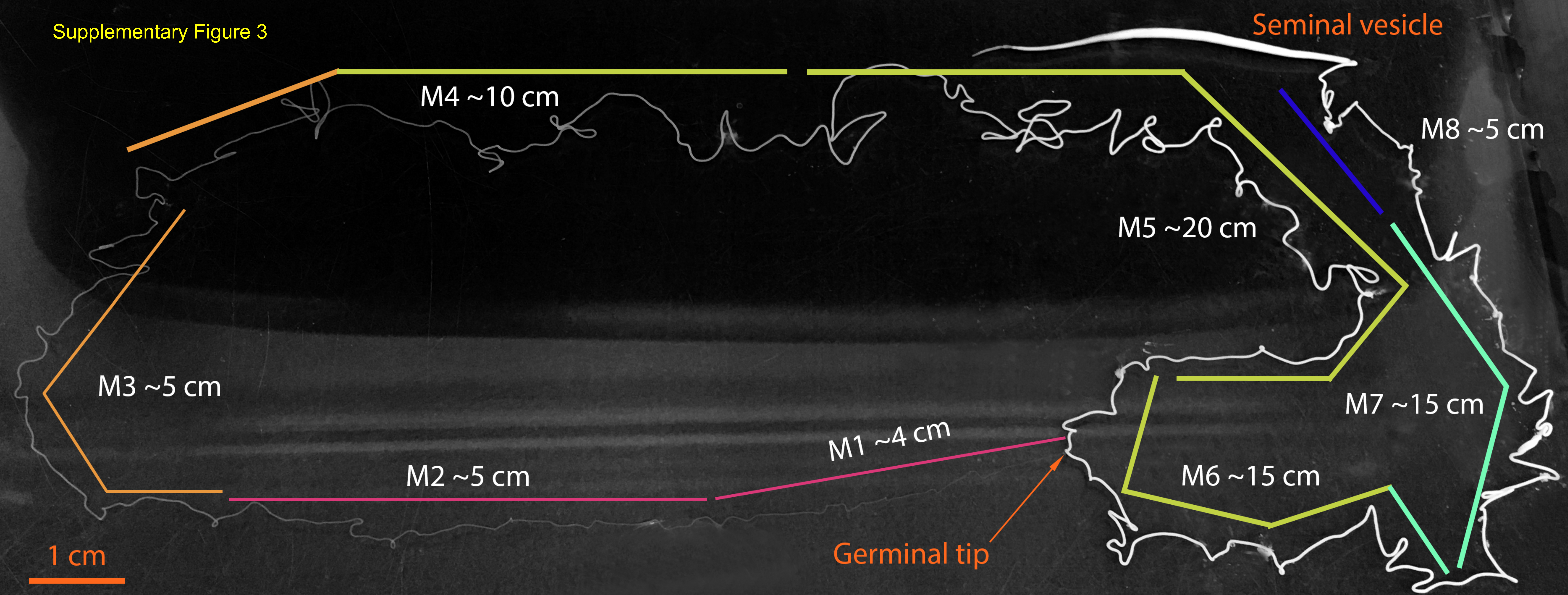
Supplementary Figure 1



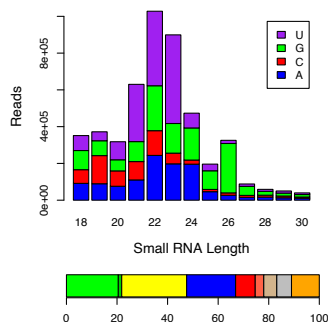
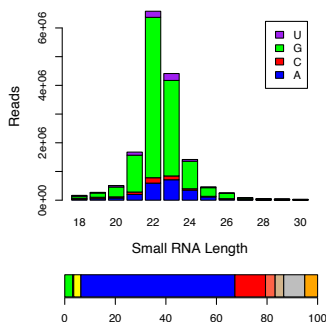
Supplementary Figure 2



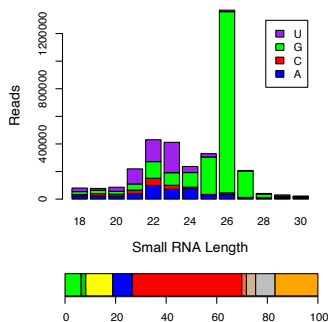
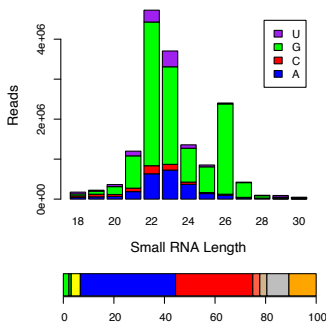
Supplementary Figure 3



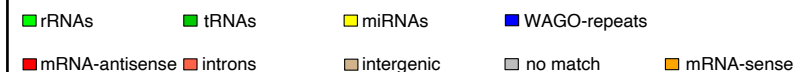
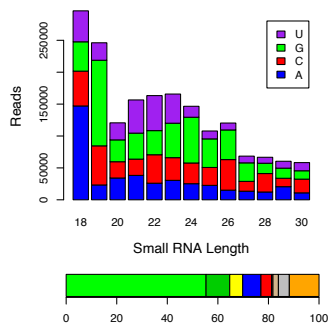
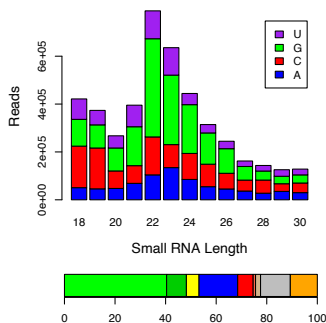
Testis Region 1



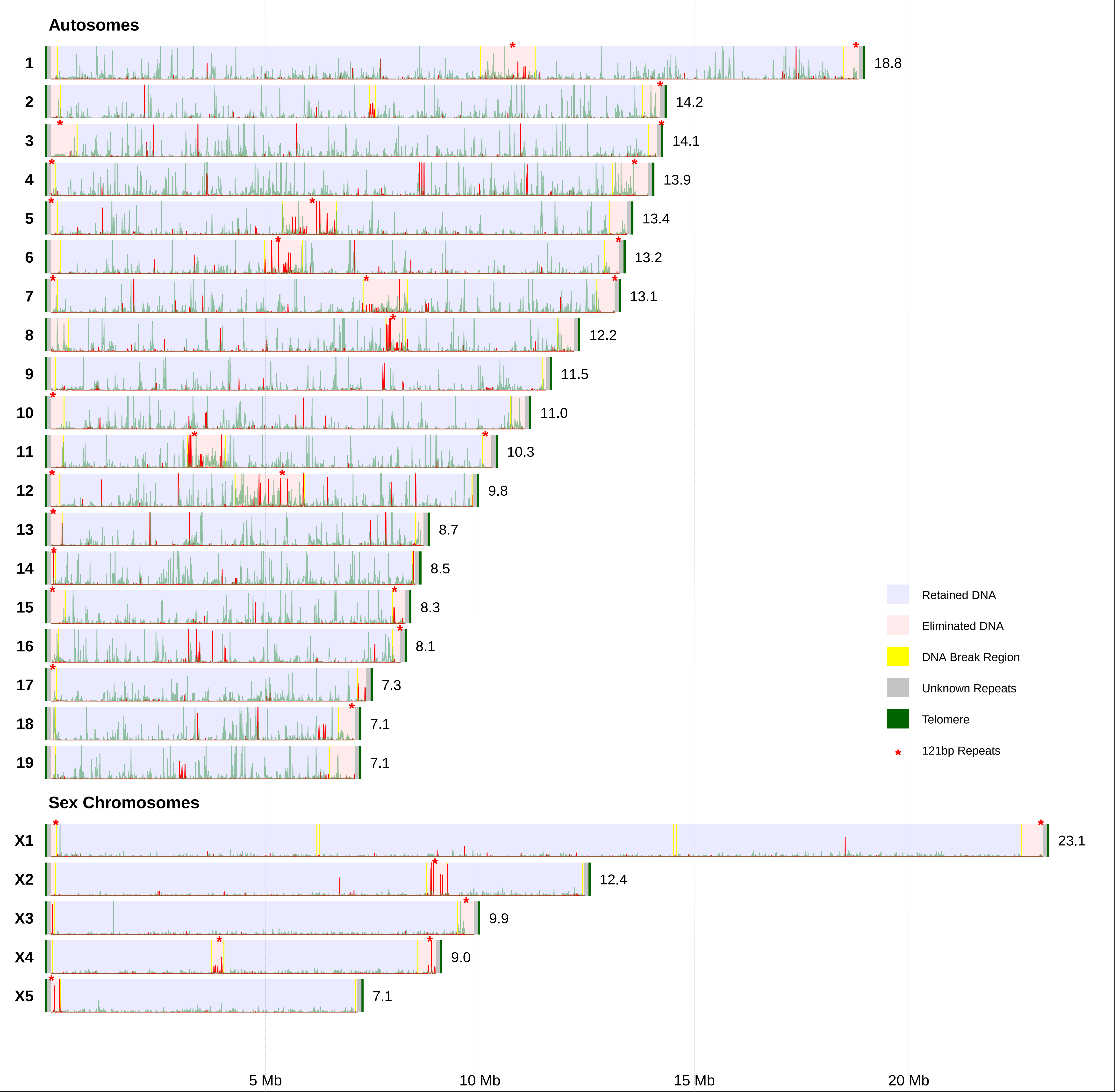
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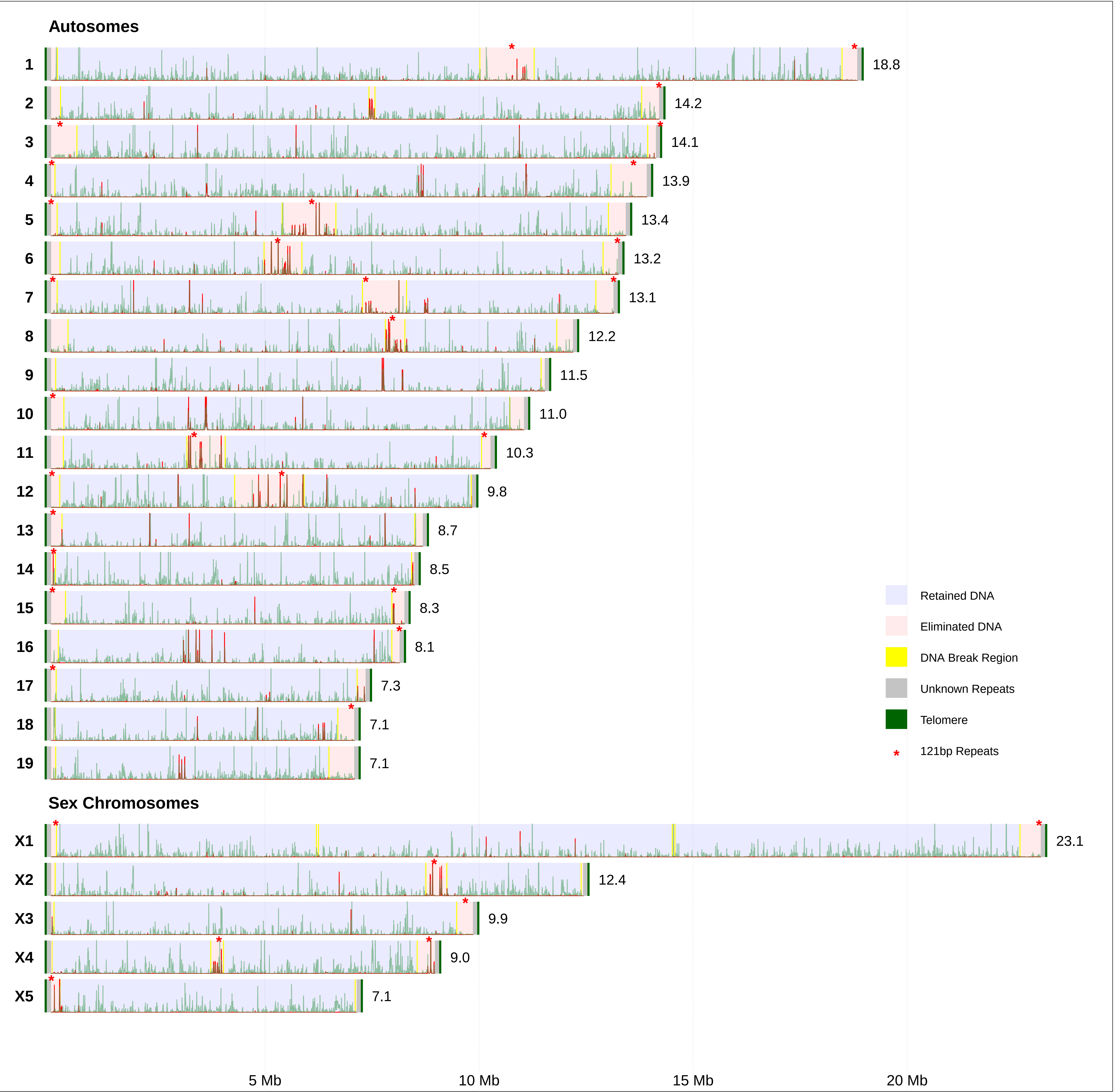
Testis Region 3



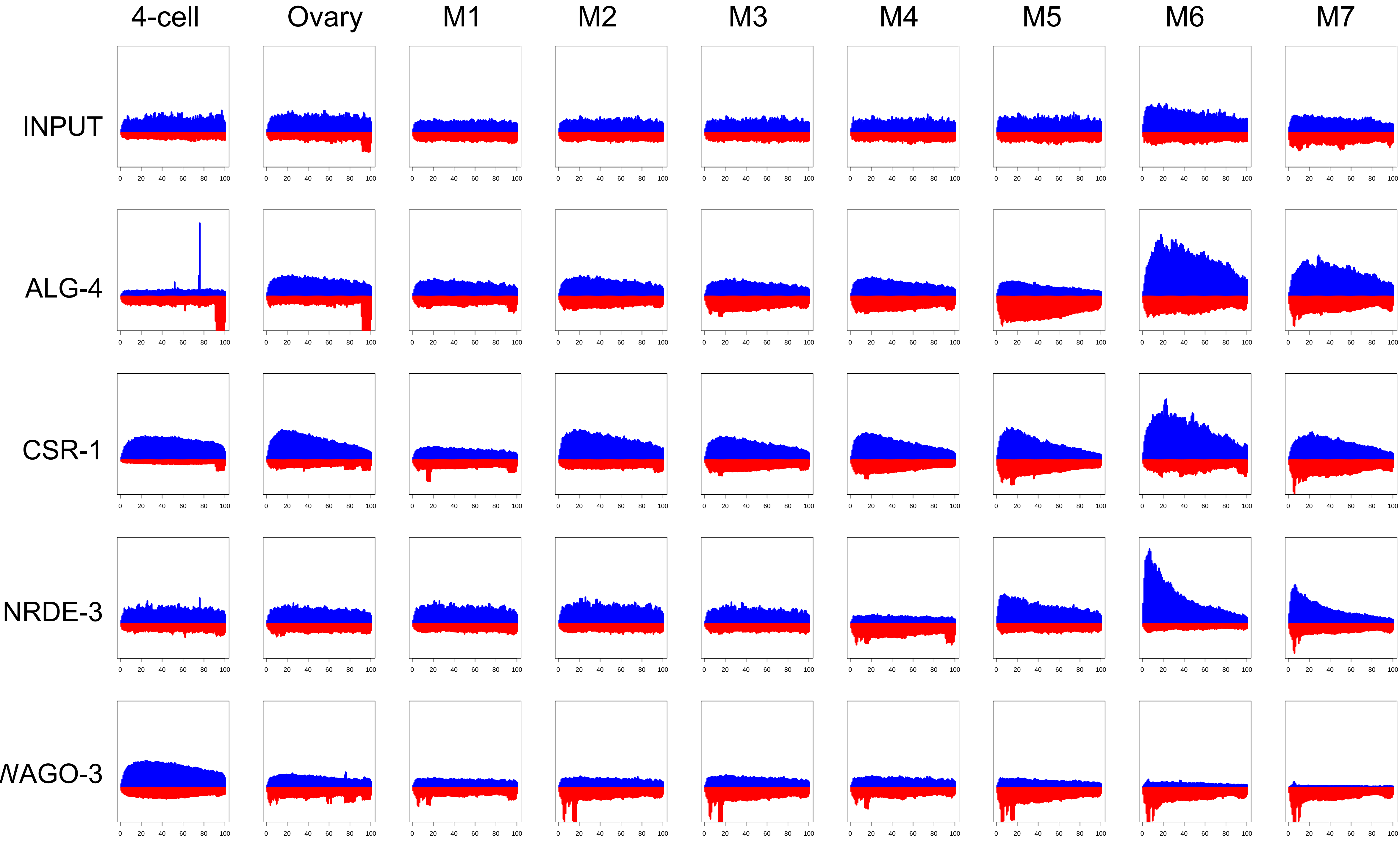
siRNAs associated with CSR-1 (green) and WAGO-1 (red) on *Ascaris* Chromosomes in Testis



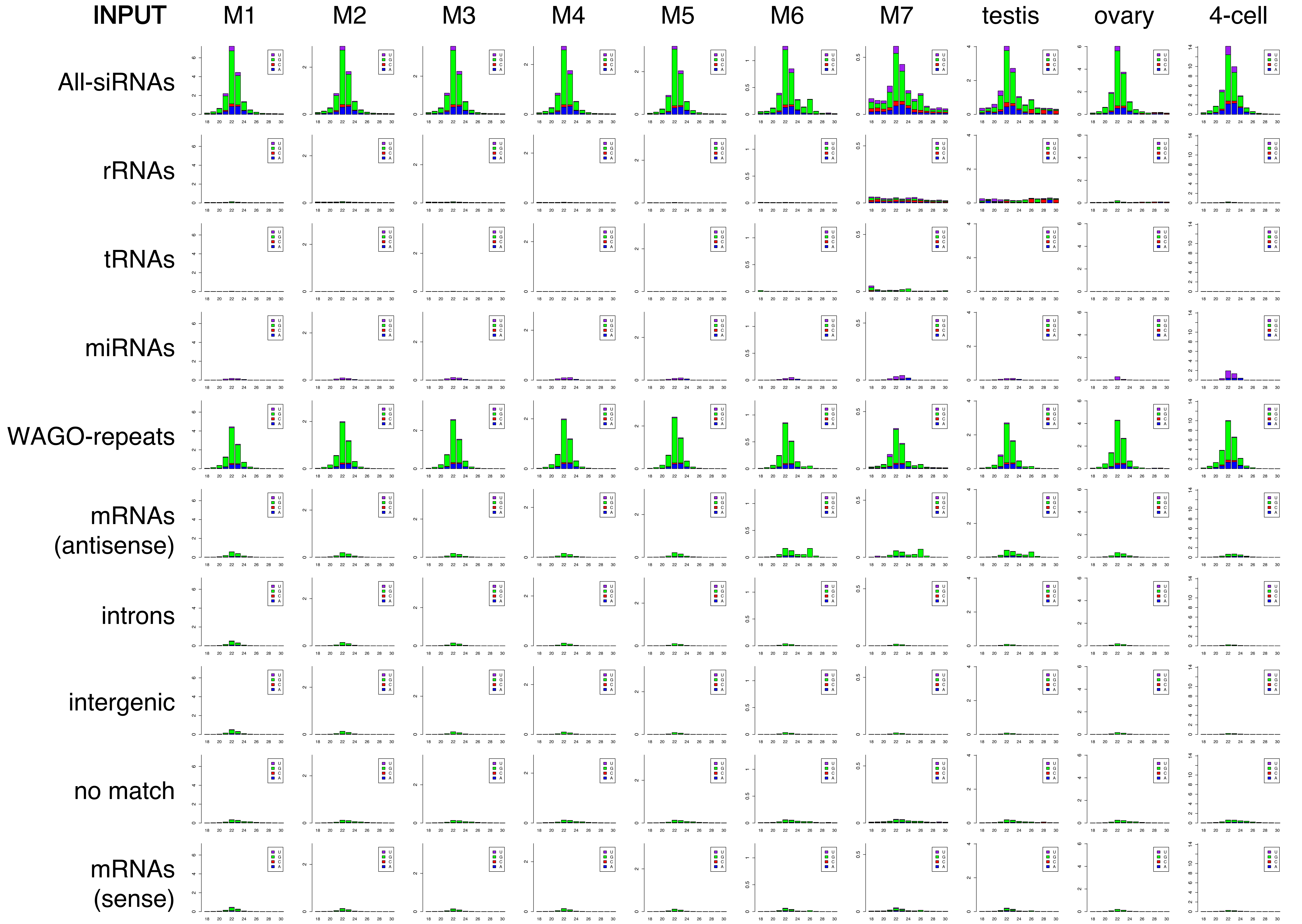
siRNAs associated with CSR-1 (green) and WAGO-1 (red) on *Ascaris* Chromosomes in Ovary



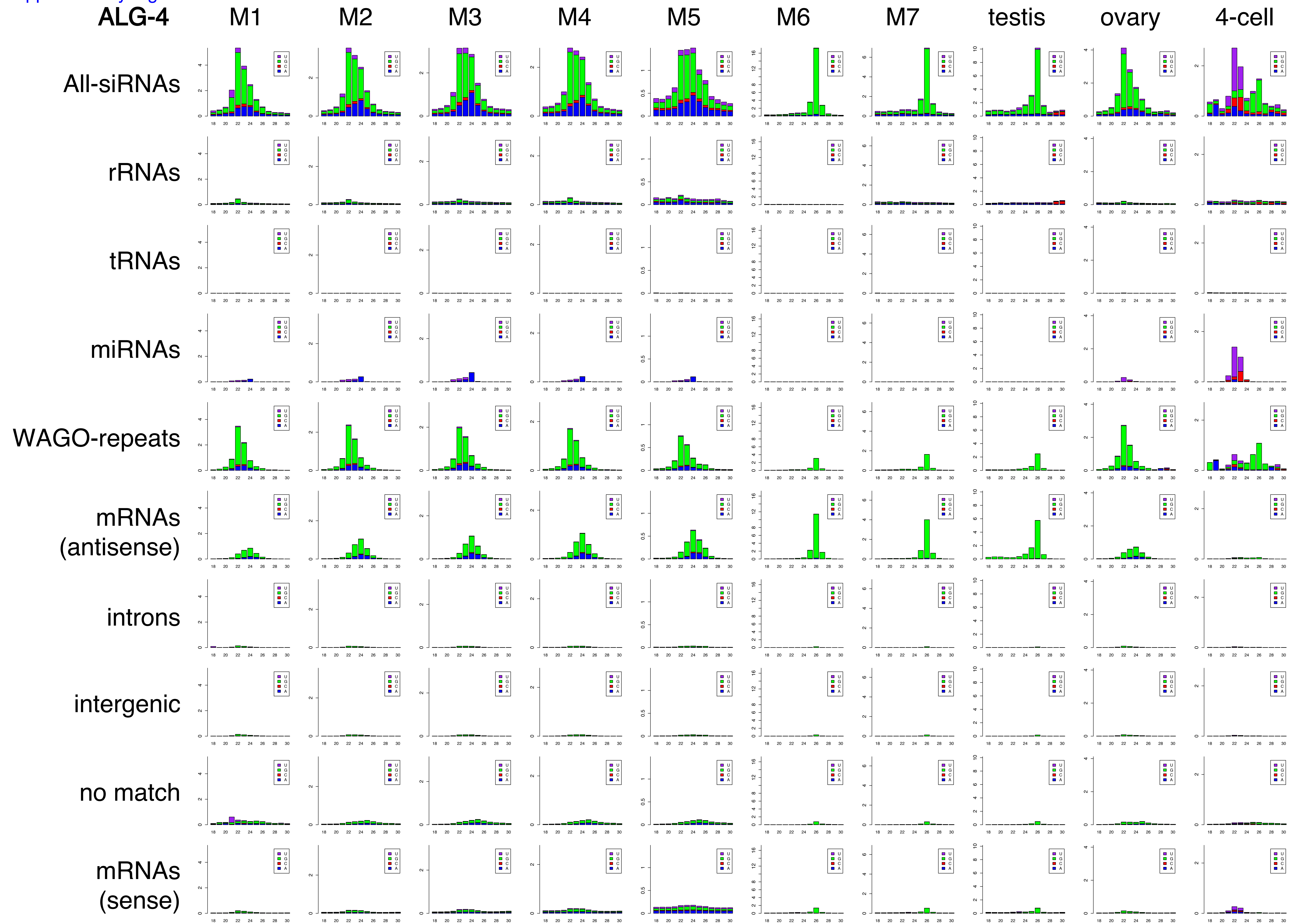
Supplementary Figure 6



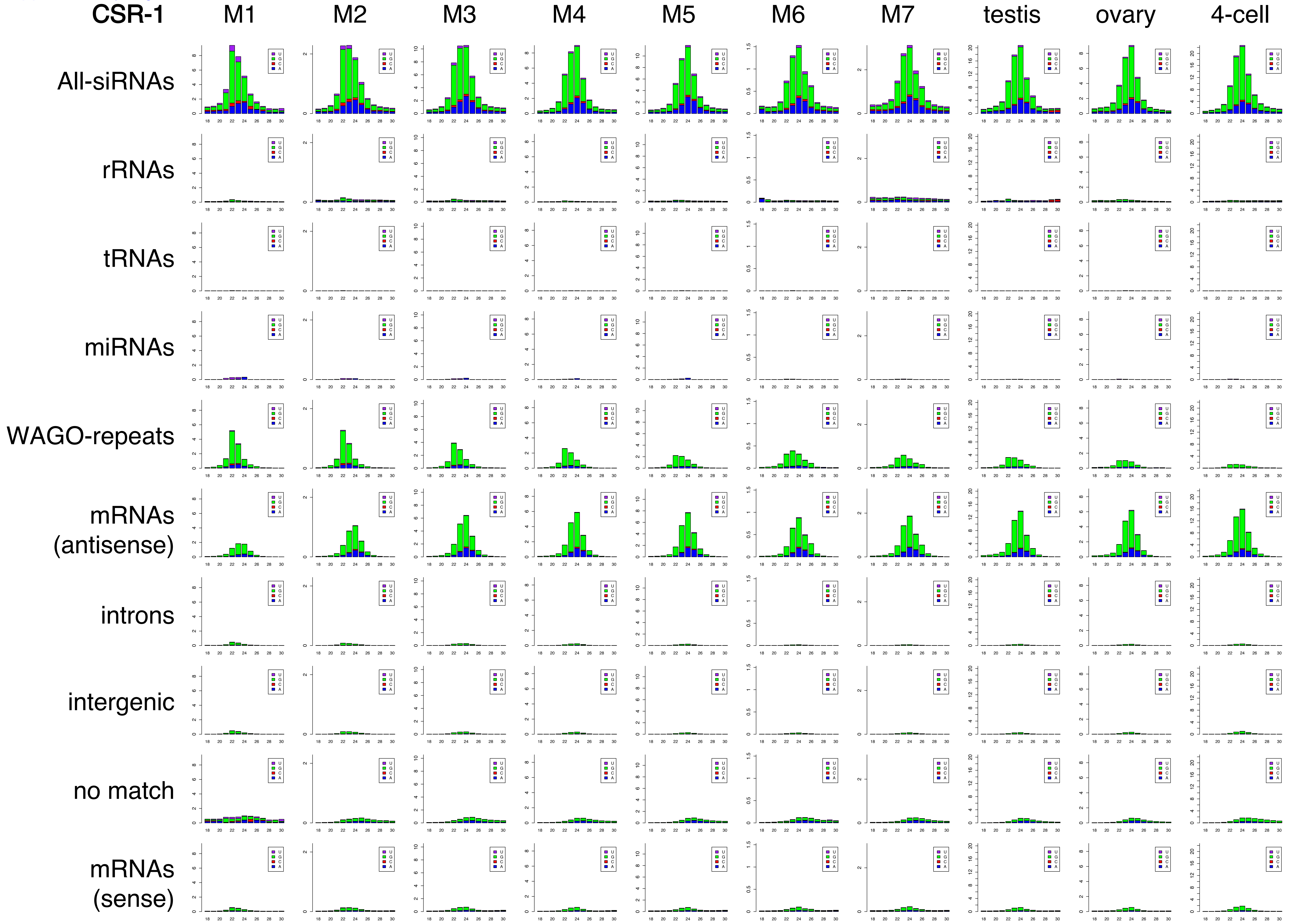
Supplementary Figure 7a



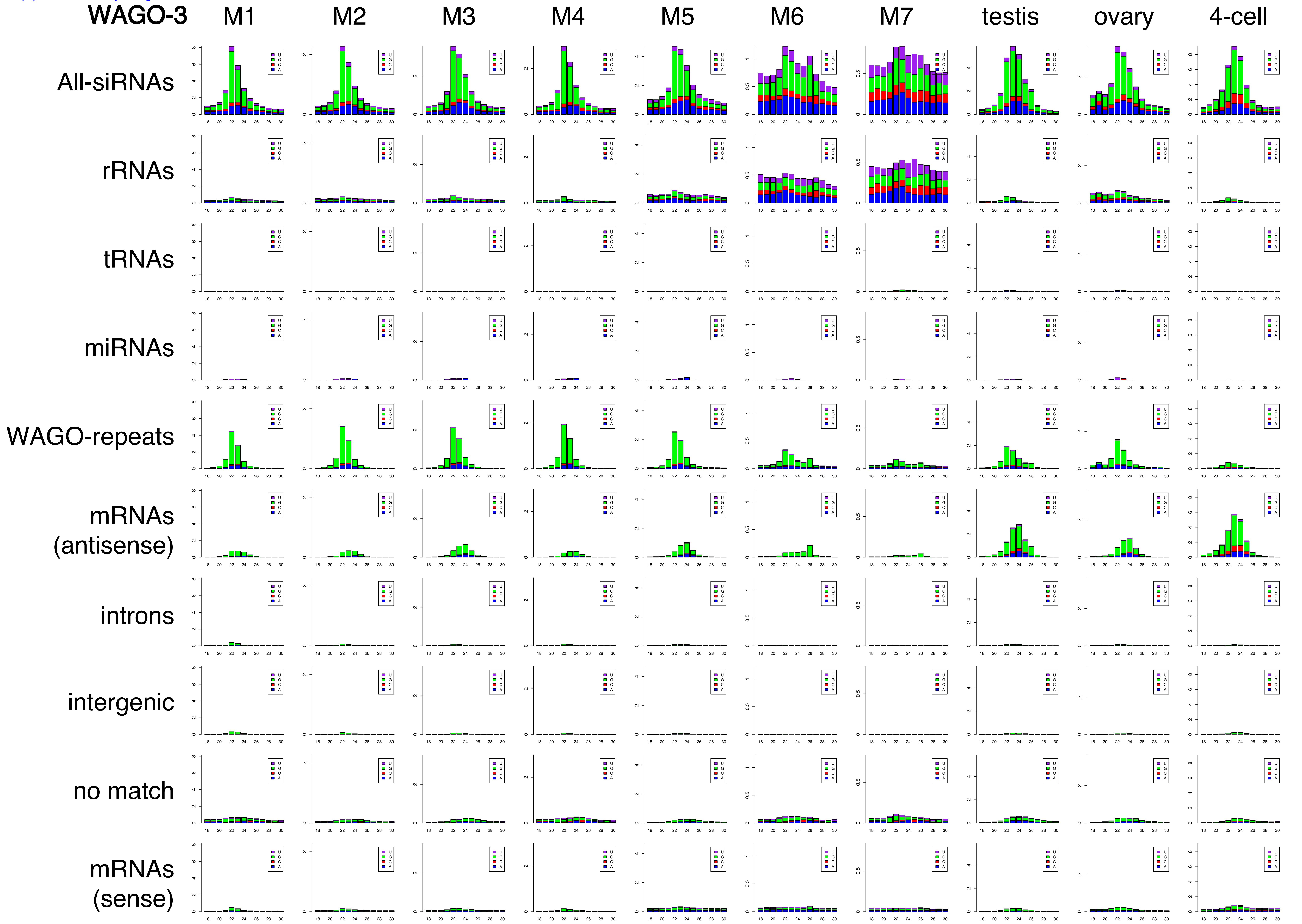
Supplementary Figure 7b



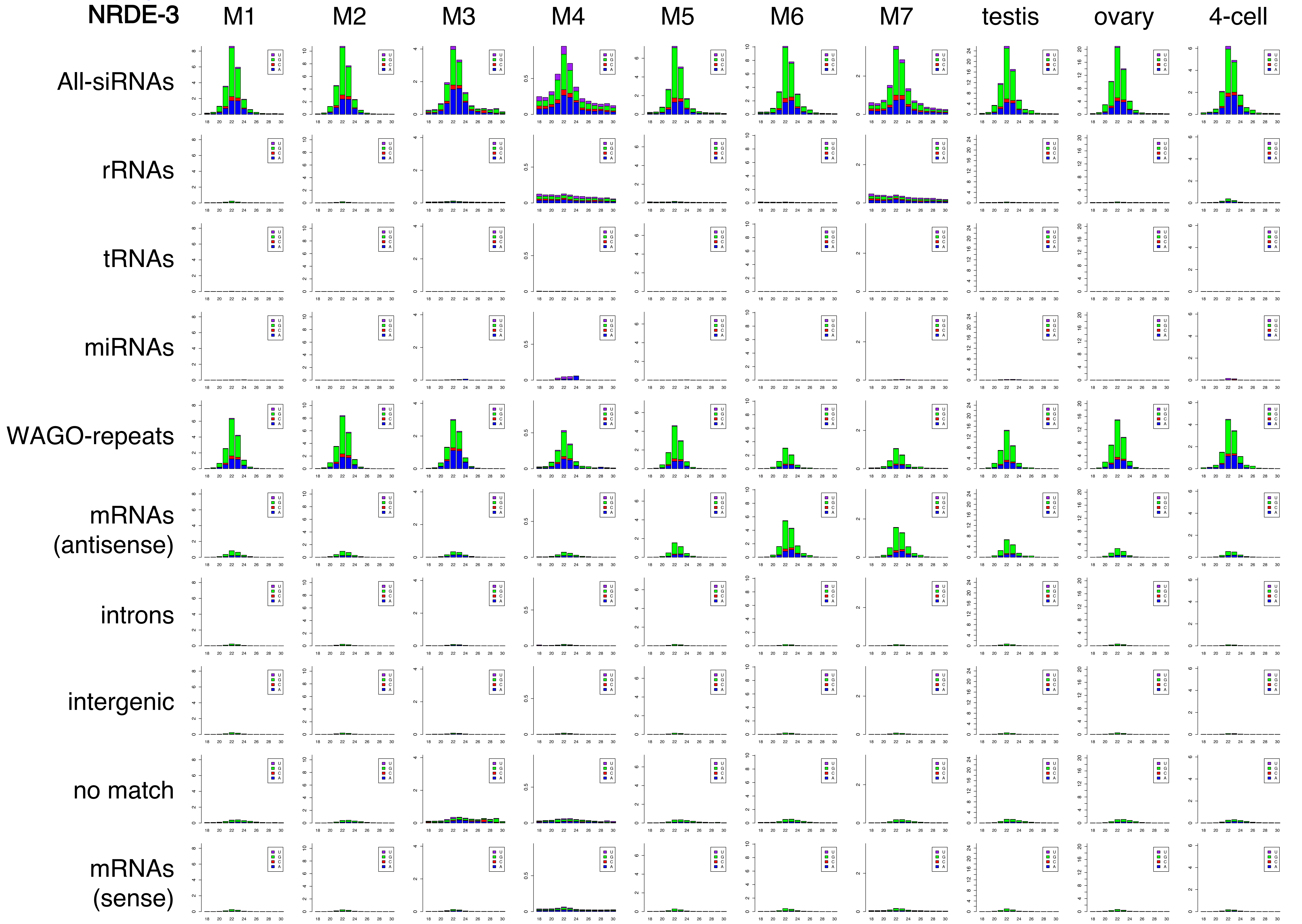
Supplementary Figure 7c

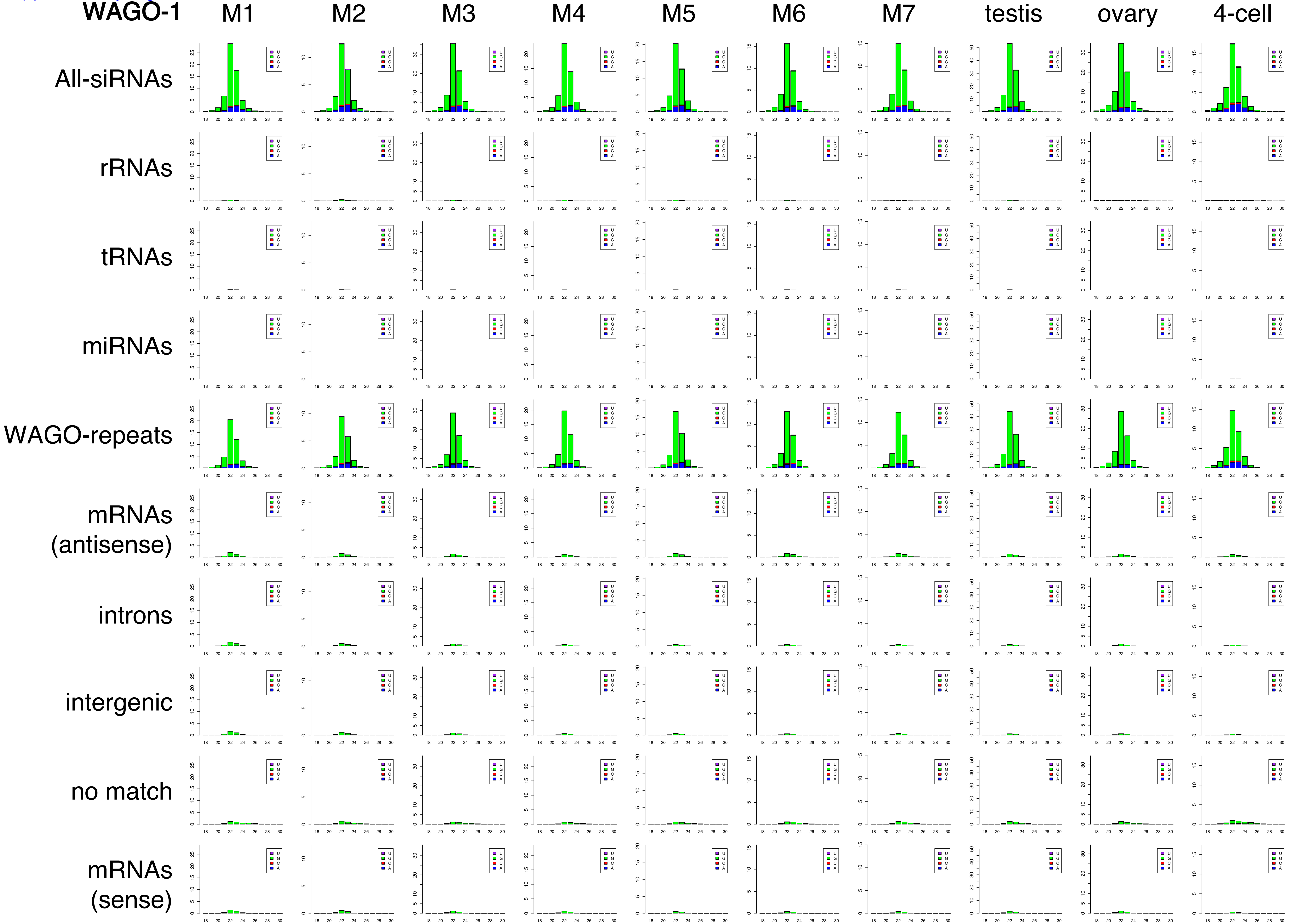


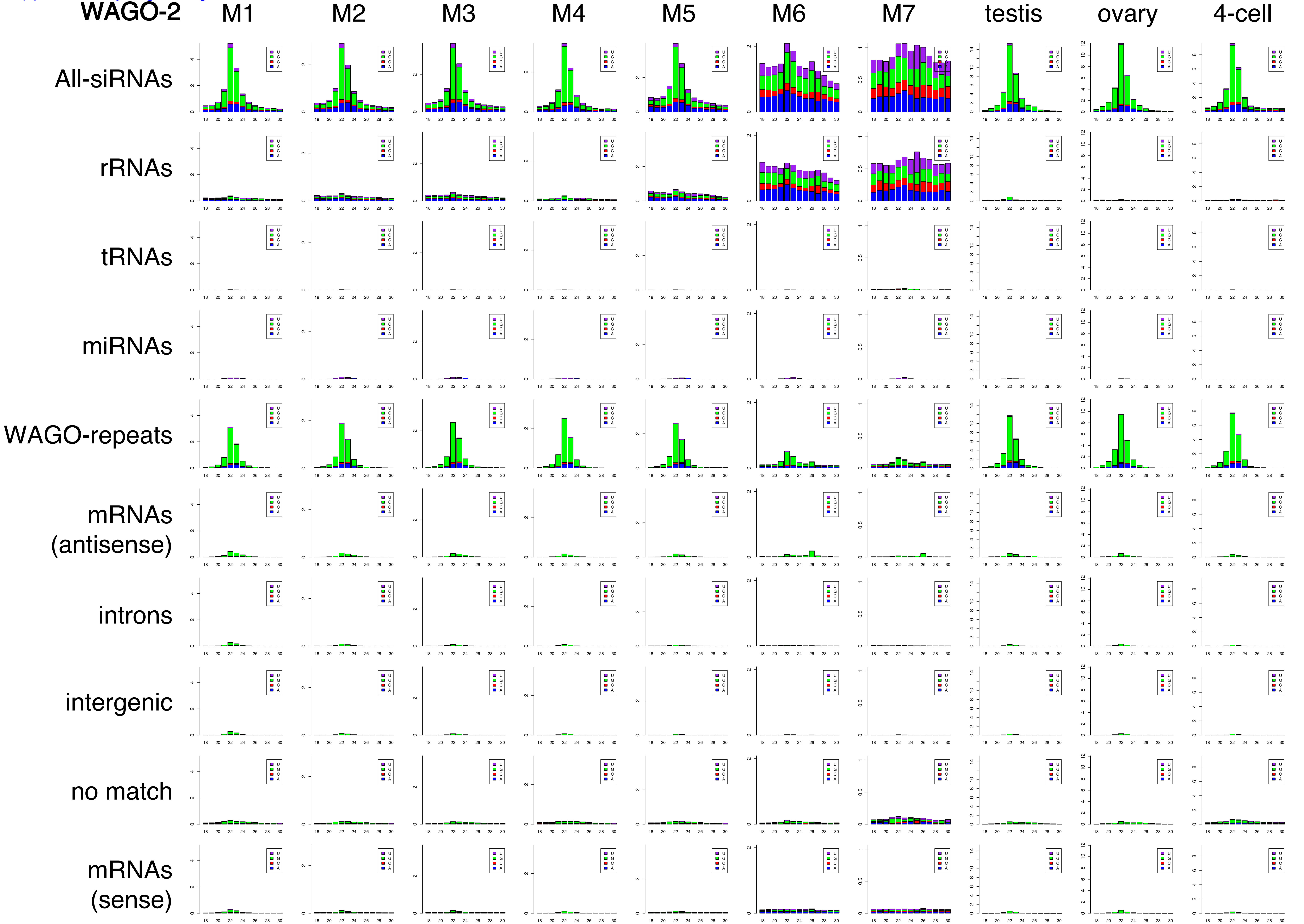
Supplementary Figure 7d



Supplementary Figure 7e

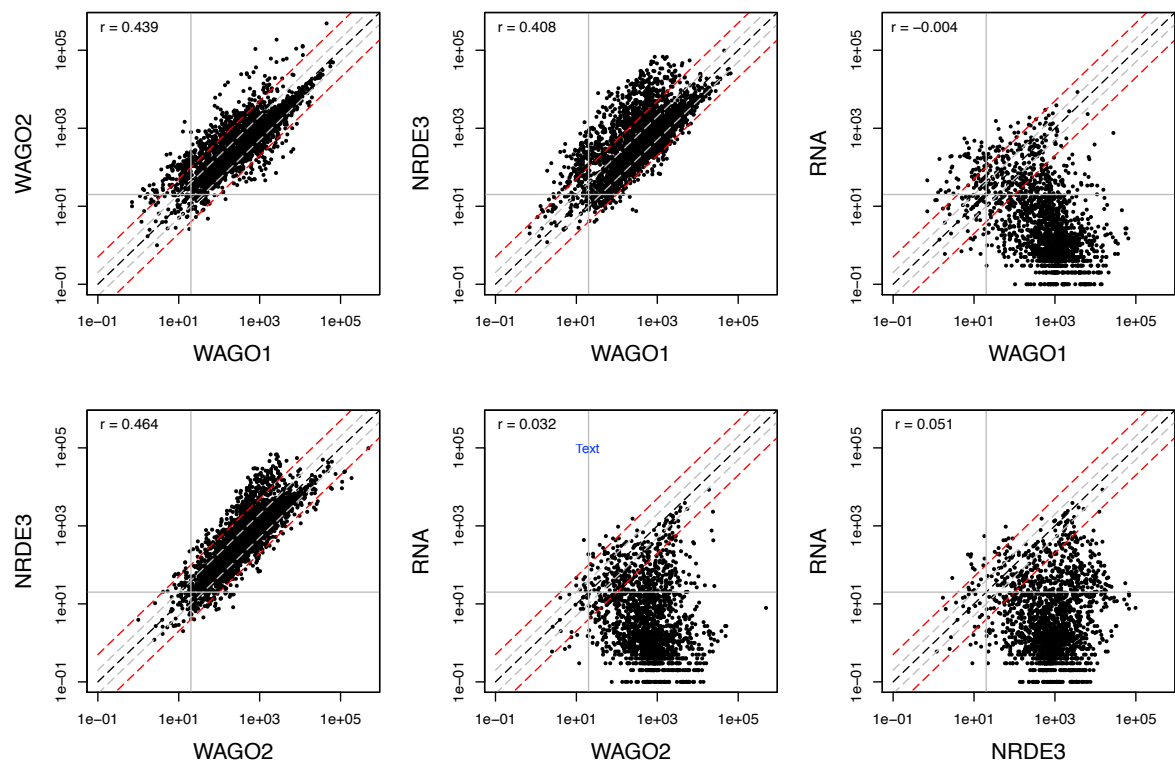




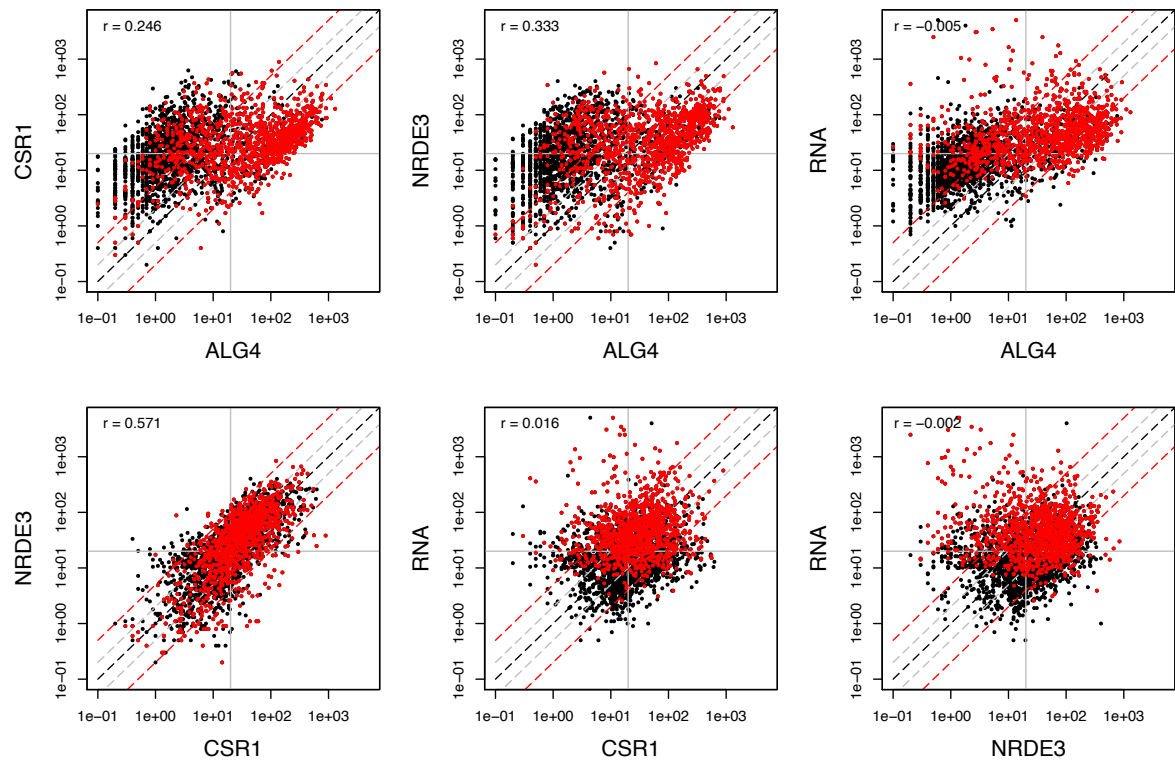


Supplementary Figure 8

A



B



Supplementary Figure 9

