

SUPPORTING INFORMATION LEGENDS

Figure S1. Sperm motility in (a) 129 vinclozolin, (b) 129 flutamide, and (c) CD-1 vinclozolin. Sperm concentration in (d) 129 vinclozolin, (e) 129 flutamide, and (f) CD-1 vinclozolin. White bar represents control, black bar represents V1 or flutamide, gray bar represents V2. (*) Significantly different ($p \leq 0.05$) by Student's t-test compared to control.

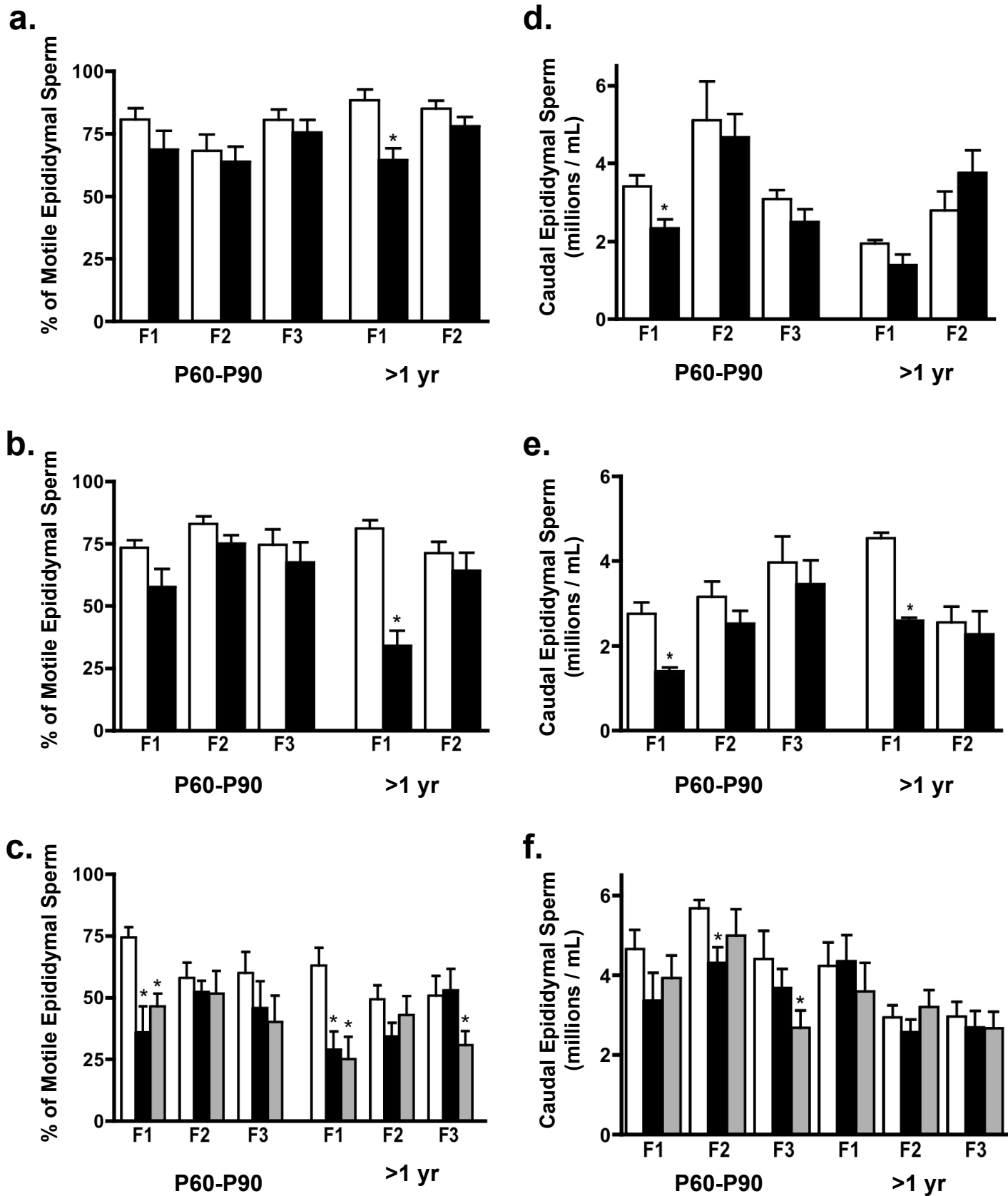
Figure S2. Tiling Array Profiles for all 66 promoter differential methylation regions. The log signal intensity for vinclozolin lineage blue line, or control lineage, red line, for F3 generation sperm MeDIP-Chip analysis versus chromosomal localization site is presented. The bar represents the site of the qPCR analysis. Comparative MeDIP-chip array signals of 68 regions in 65 genes showing significant vinclozolin-induced transgenerational change in DNA methylation. (a1-a35) depict DNA methylation changes confirmed with real-time qPCR. (b1-b15) depict DNA methylation changes not able to be tested with real-time qPCR. (c1-c15) depict DNA methylation changes not confirmed with real-time qPCR.

Table S1. (S1A) Adult male body weight, testes index, and kidney index for mouse 129 strain control, vinclozolin and flutamide F1, F2, and F3 generations. (S1B) Adult male body weight, testes index, and kidney index for mouse CD-1 strain control, vinclozolin V1 (100 mg/kg/day) and V2 (200 mg/kg/day) F1 and F2 generations.

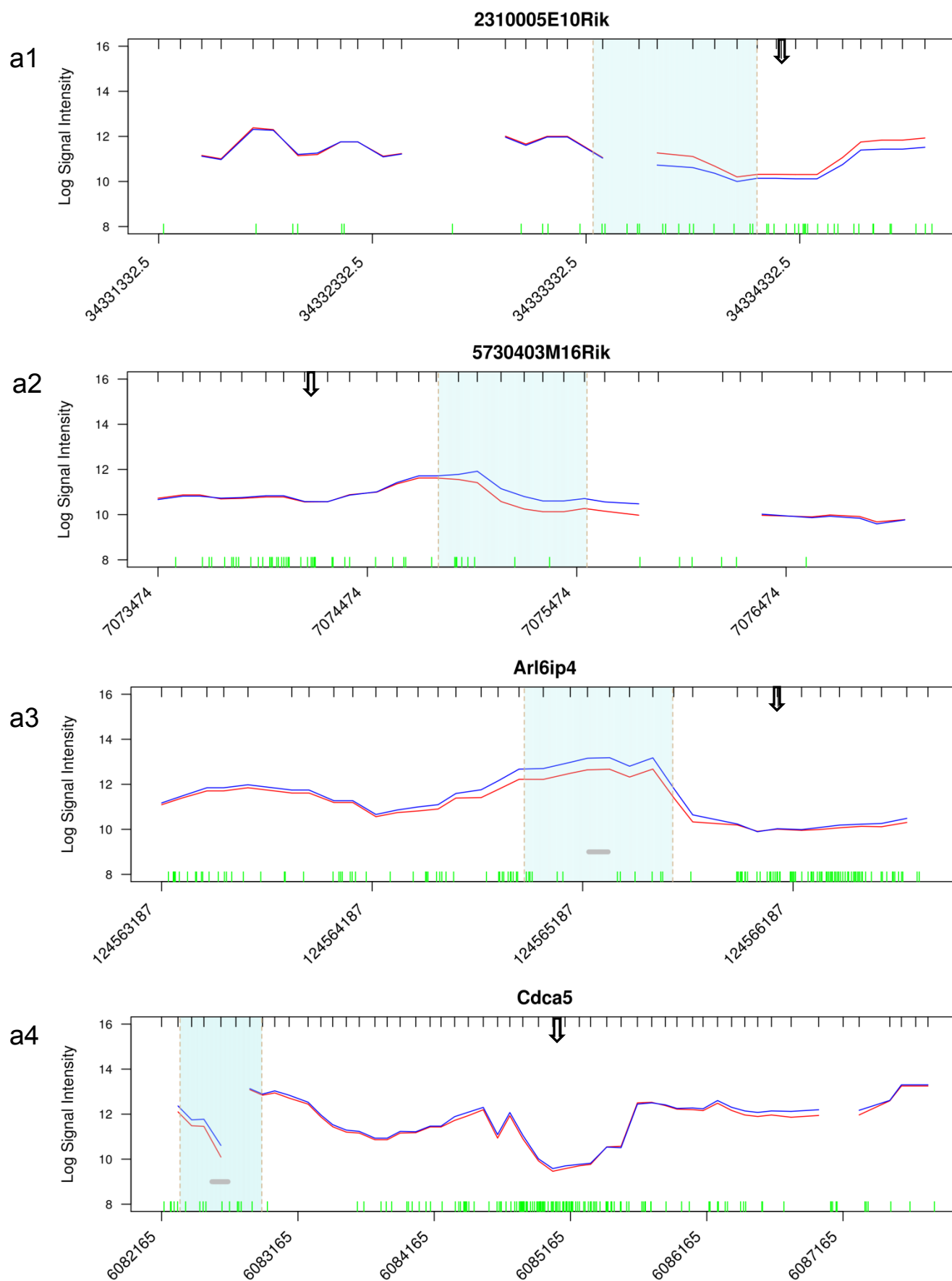
Table S2. The V1 (100/mg/kg/day) and V2 (200 mg/kg/day) doses of vinclozolin lineage to control lineage F3 generation animals. Testis, prostate and kidney disease assessment is described in the Materials and Methods section.

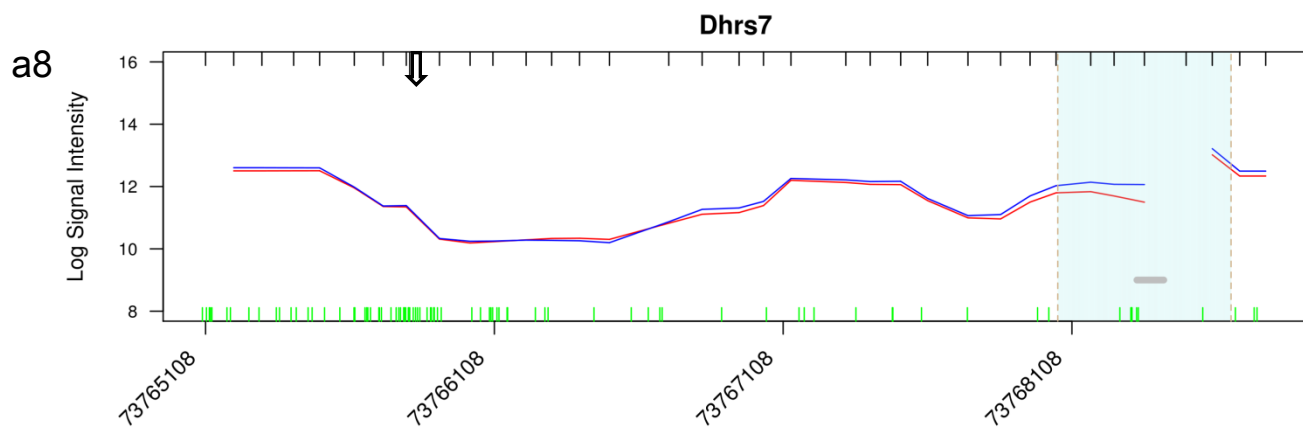
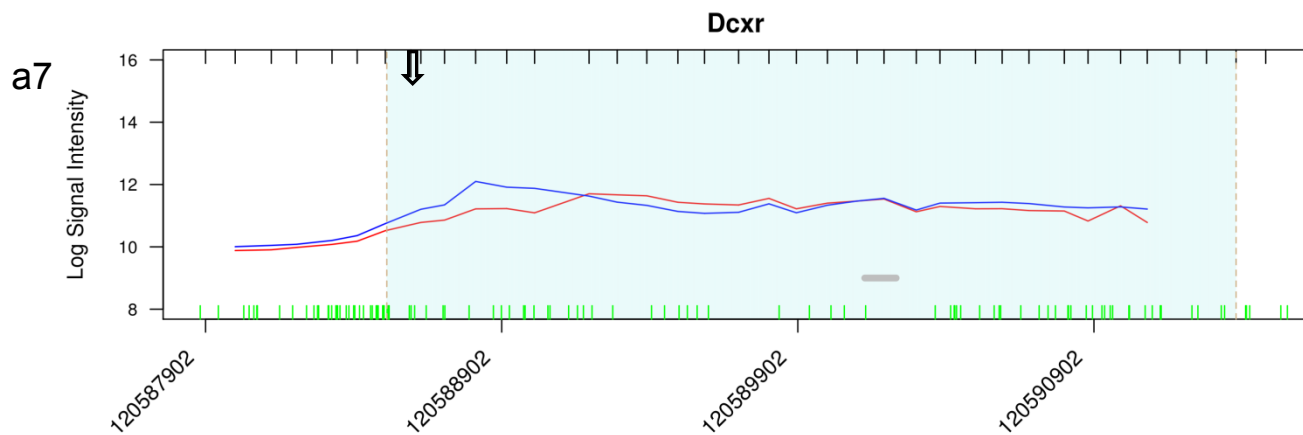
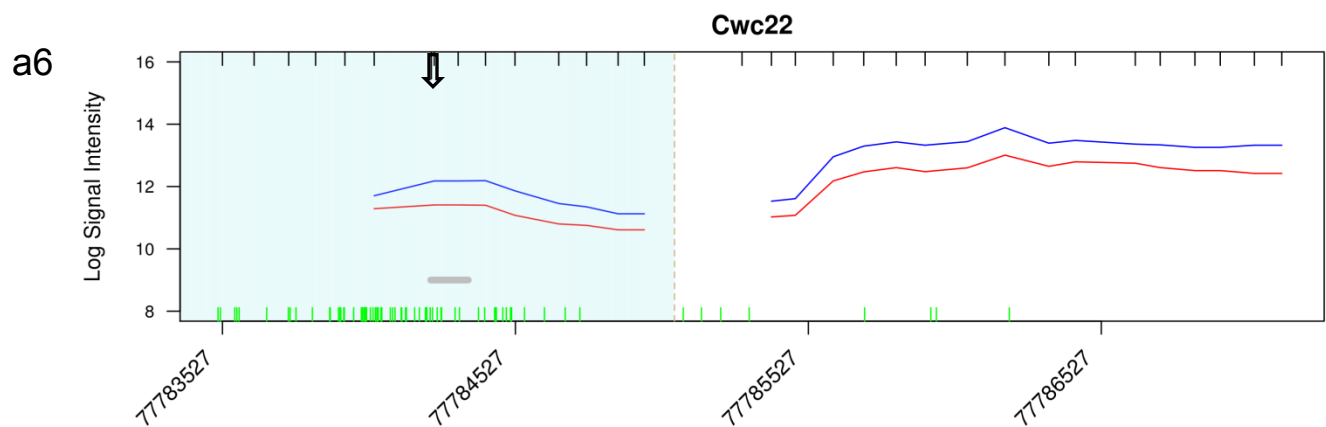
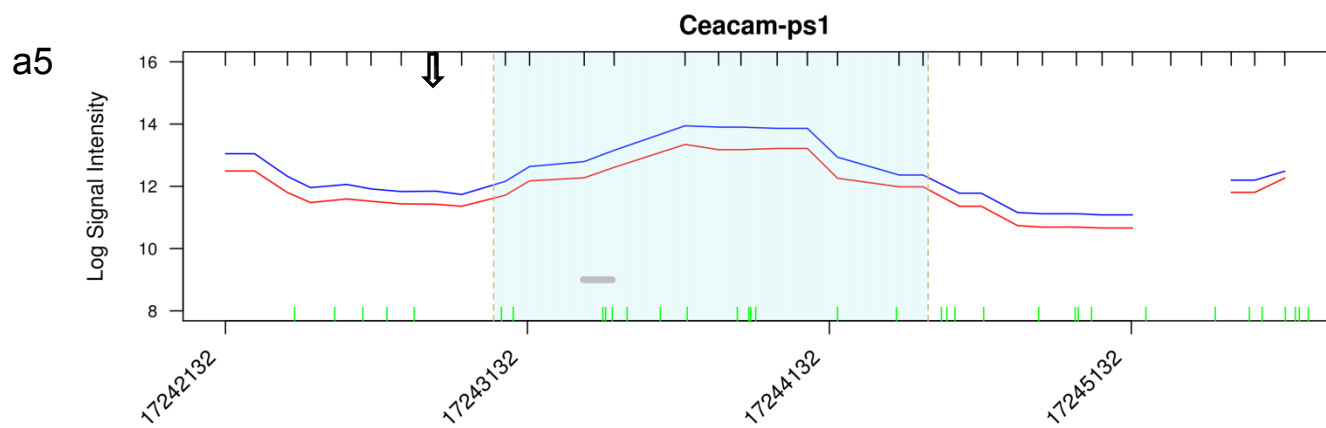
Table S3. Regions showing vinclozolin-induced transgenerational change with MeDip-chip and not confirmed Real Time qPCR.

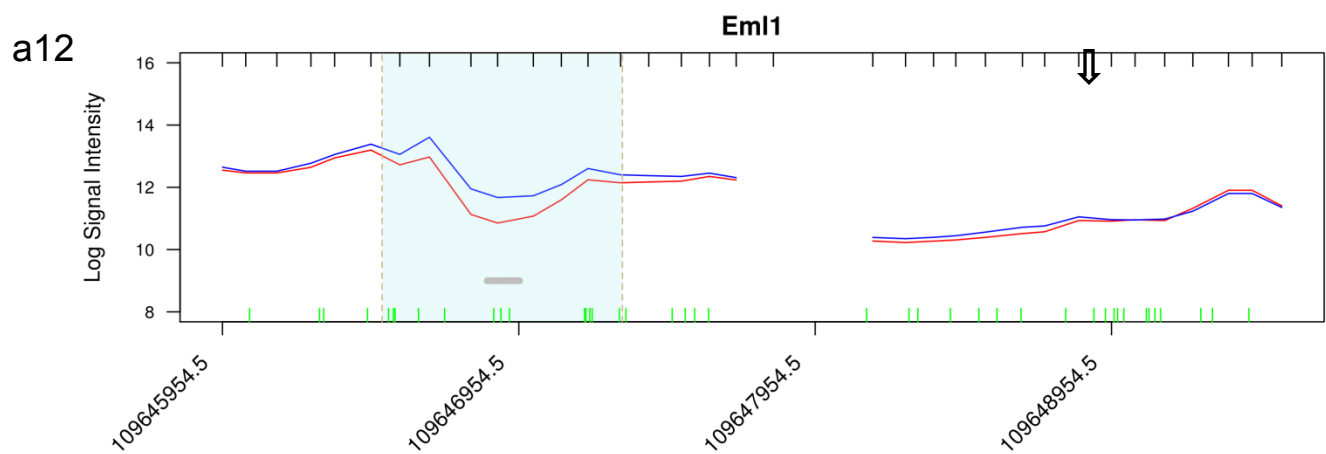
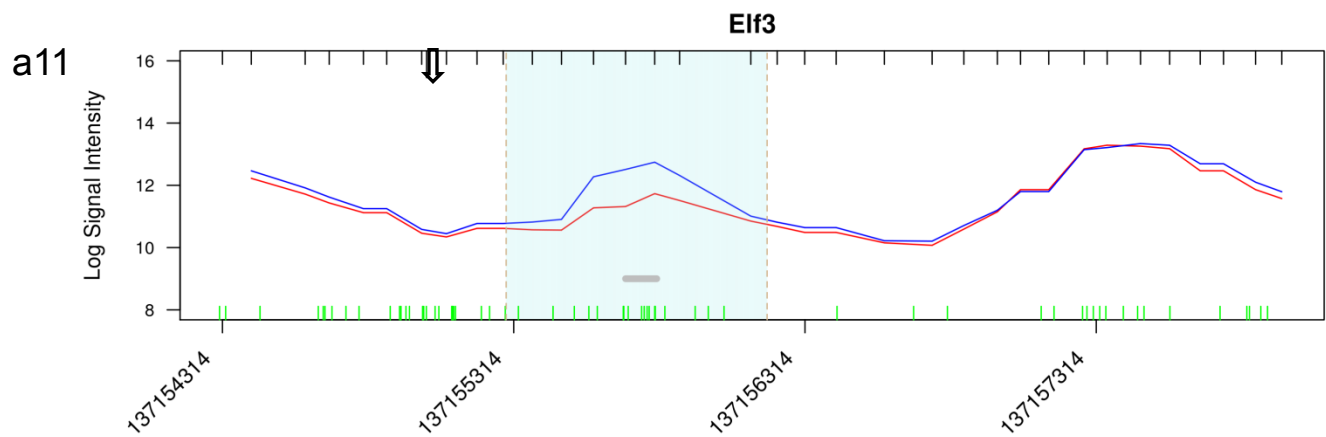
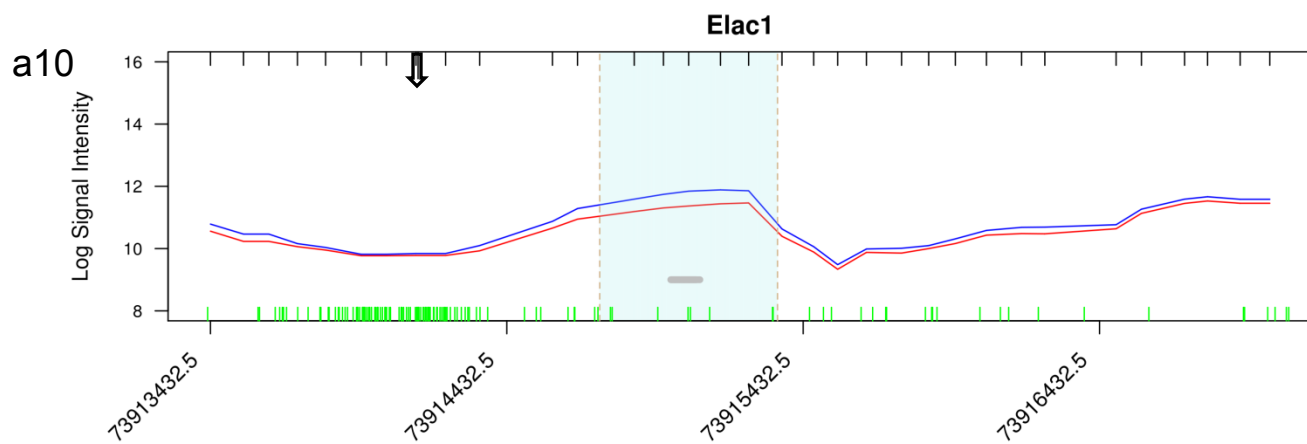
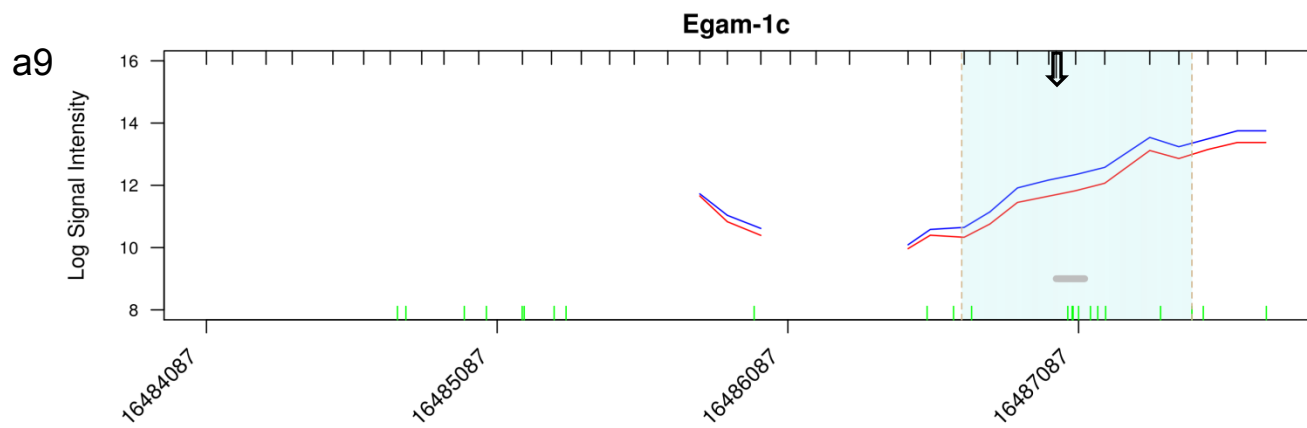
Supplemental Figure S1. Sperm motility in **(a)** 129 vinclozolin, **(b)** 129 flutamide, and **(c)** CD-1 vinclozolin. Sperm concentration in **(d)** 129 vinclozolin, **(e)** 129 flutamide, and **(f)** CD-1 vinclozolin. White bar represents control, black bar represents V1 or flutamide, gray bar represents V2. (*) Significantly different ($p \leq 0.05$) by Student's t-test compared to control.

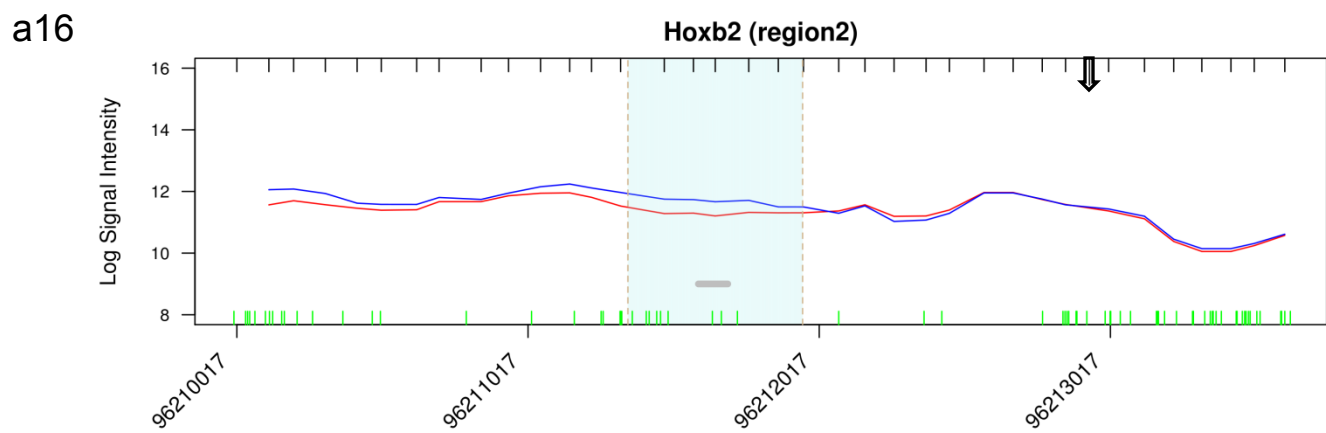
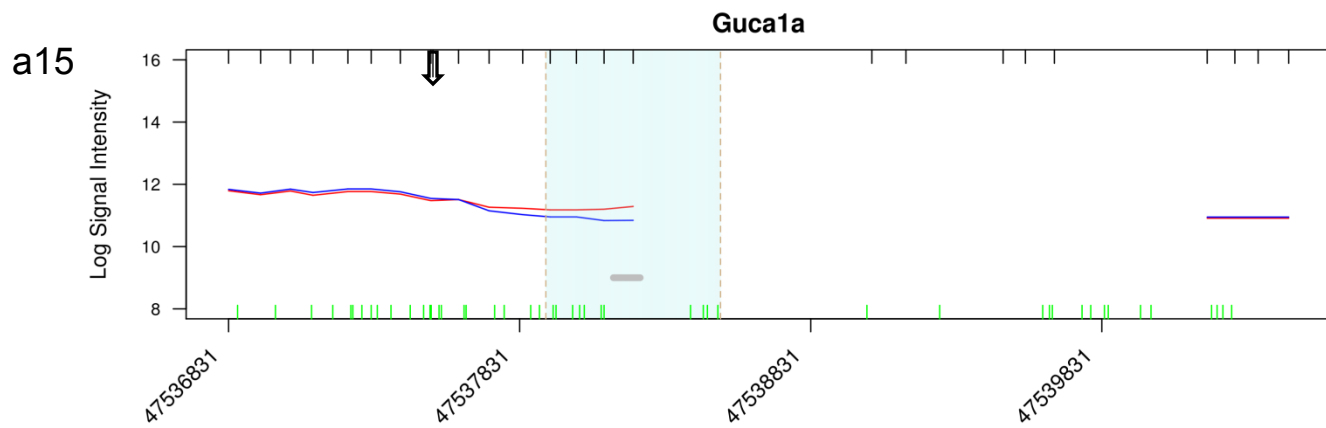
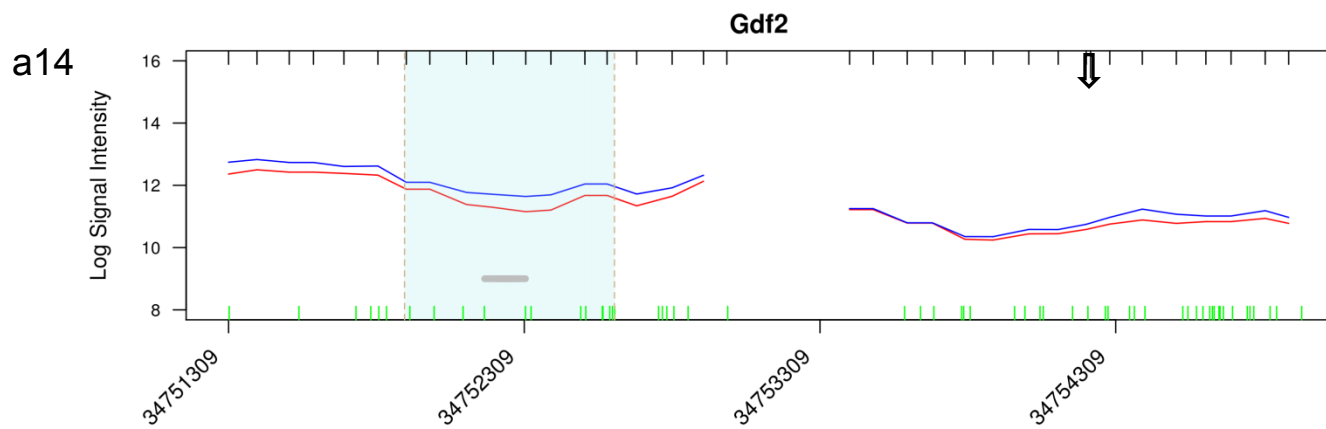
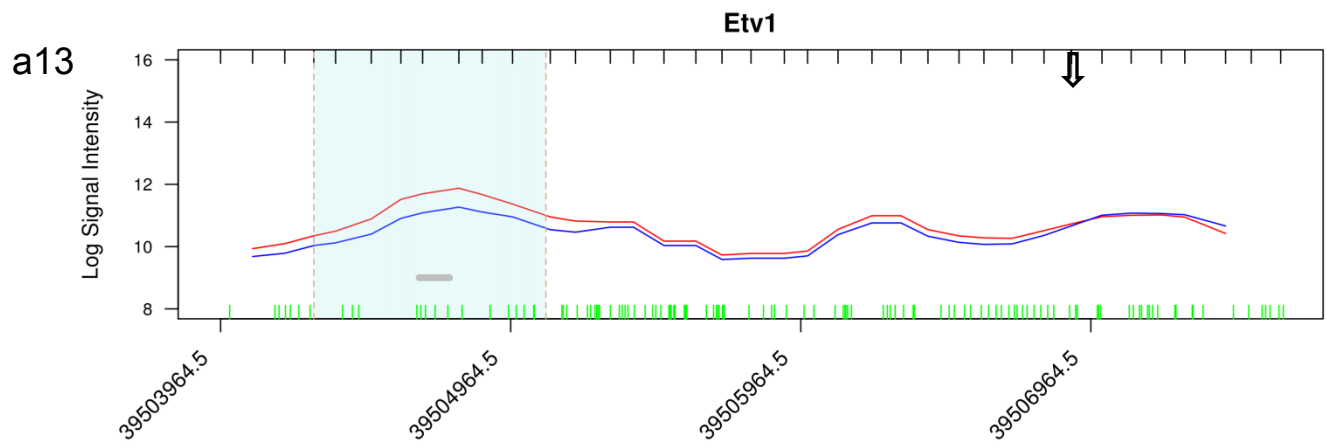


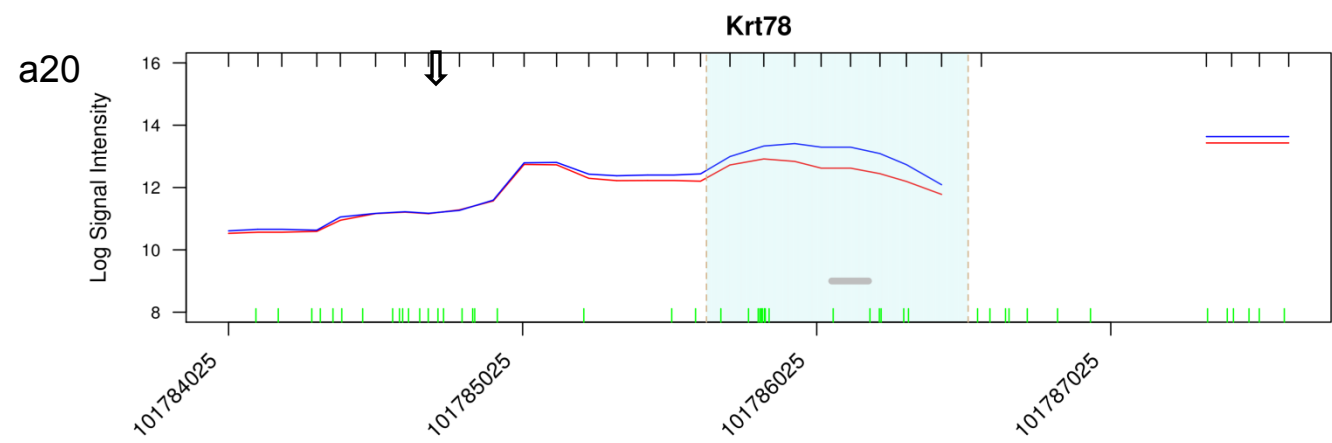
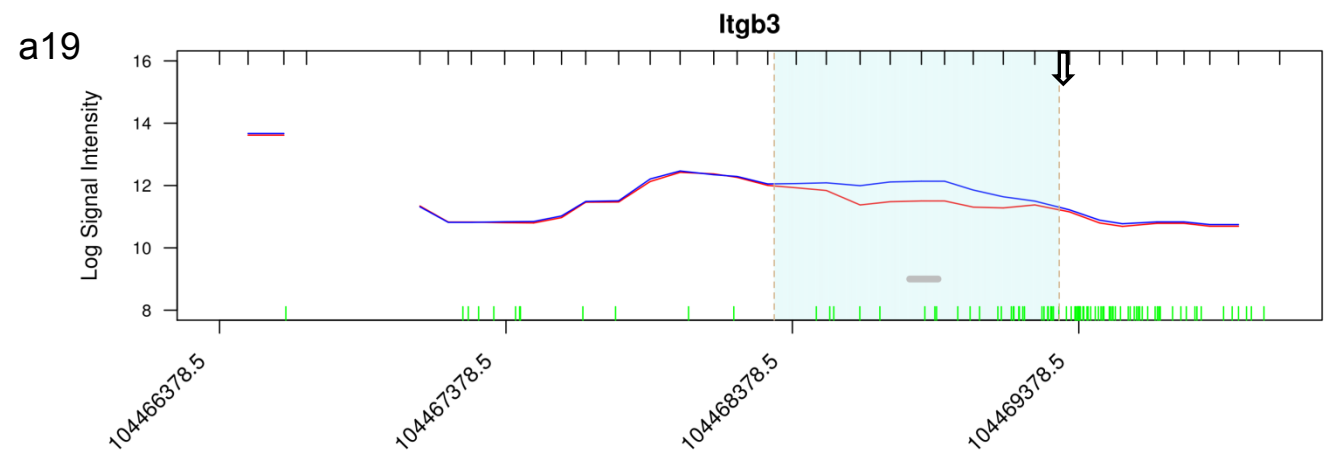
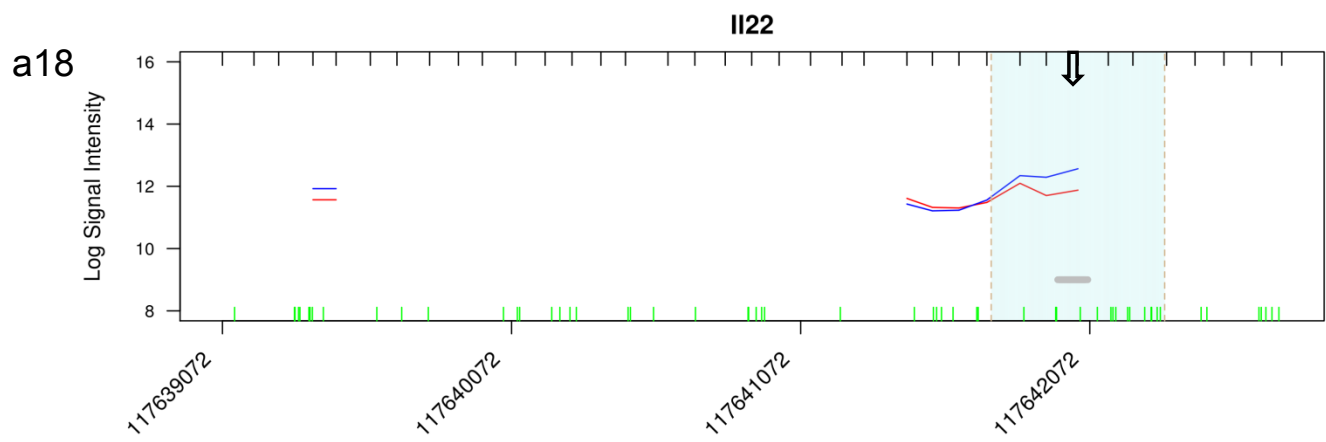
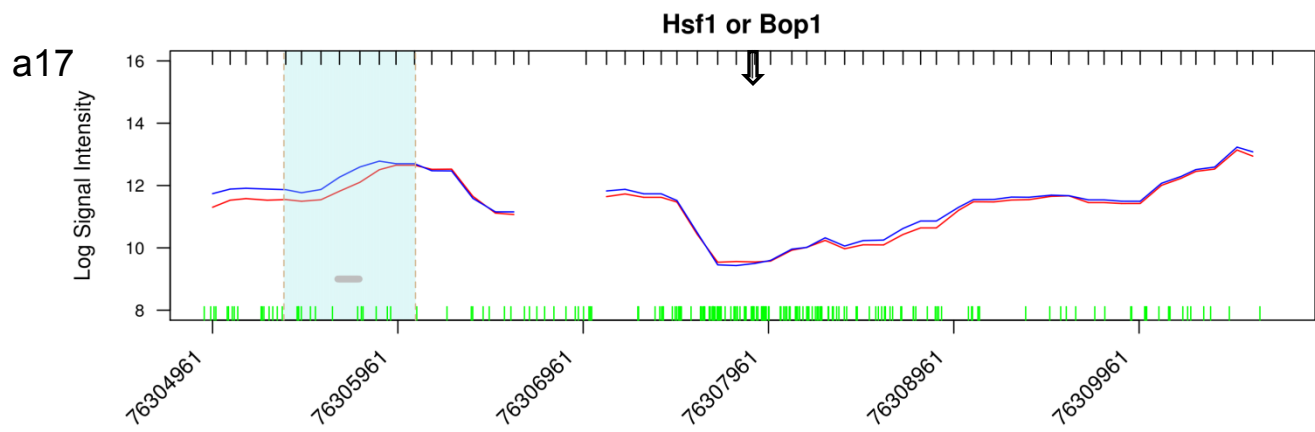
Supplementary Figure S2 – Tiling Array Profiles for all 66 promoter differential methylation regions. The log signal intensity for vinclozolin lineage blue line, or control lineage, red line, for F3 generation sperm MeDIP-Chip analysis versus chromosomal localization site is presented. The bar represents the site of the qPCR analysis. Comparative MeDIP-chip array signals of 68 regions in 65 genes showing significant vinclozolin-induced transgenerational change in DNA methylation. (a1-a40) depict DNA methylation changes confirmed with real-time qPCR. (b1-b10) depict DNA methylation changes not able to be tested with real-time qPCR. (c1-c17) depict DNA methylation changes not confirmed with real-time qPCR.

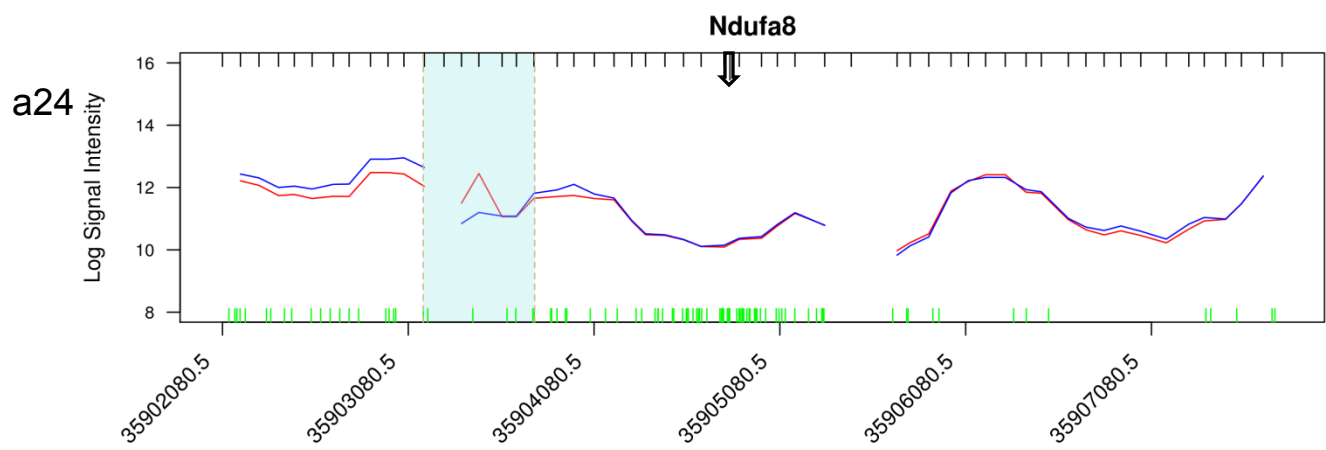
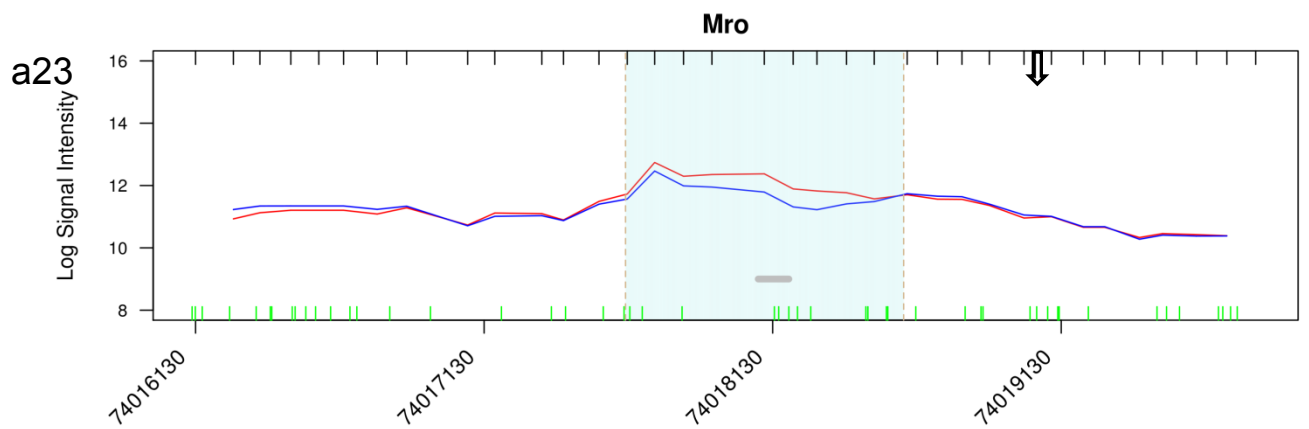
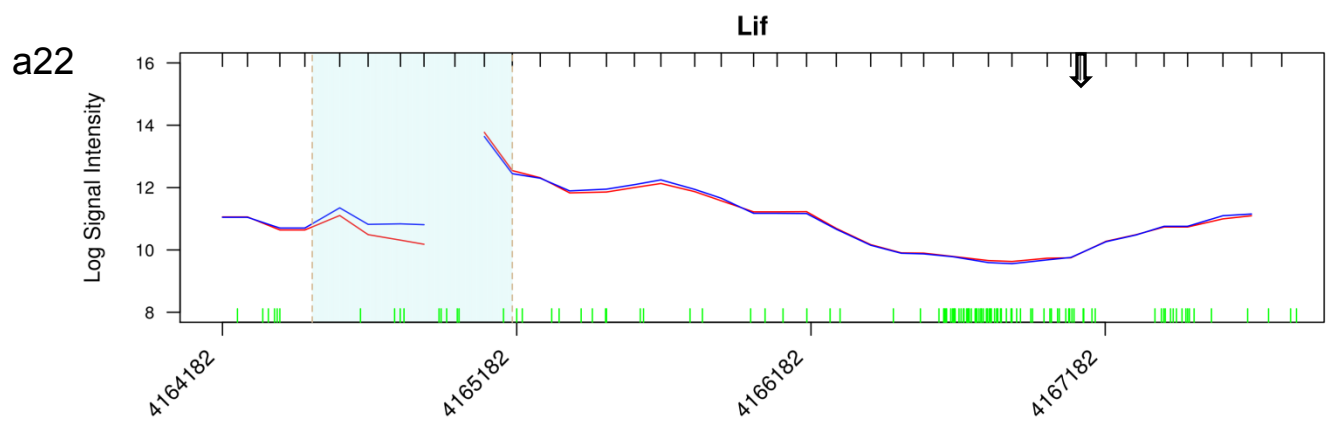
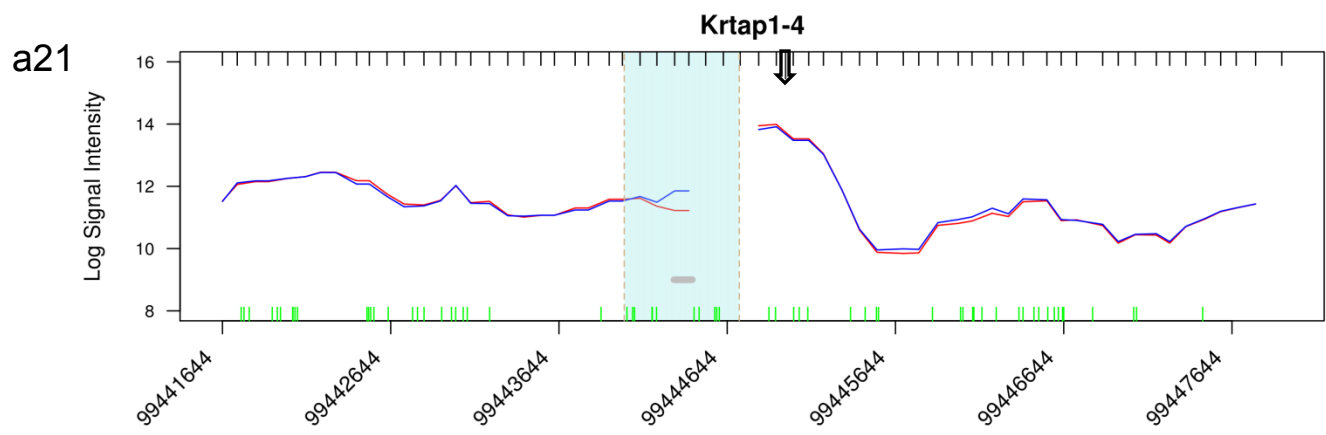


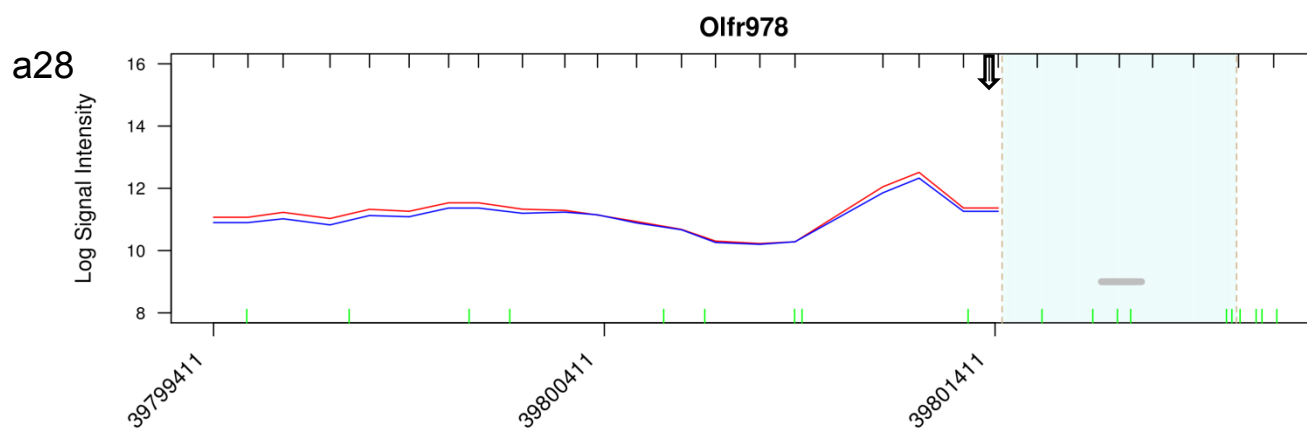
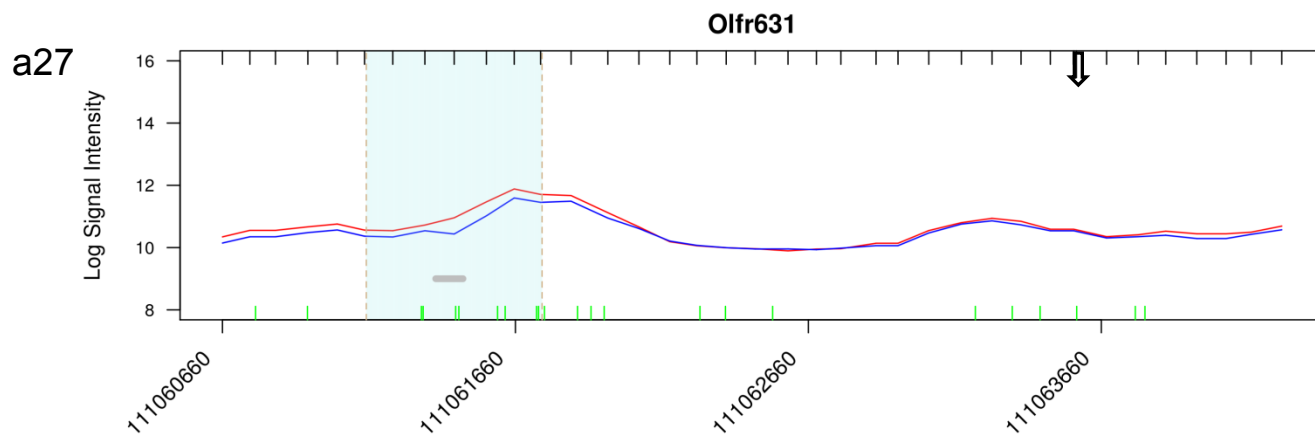
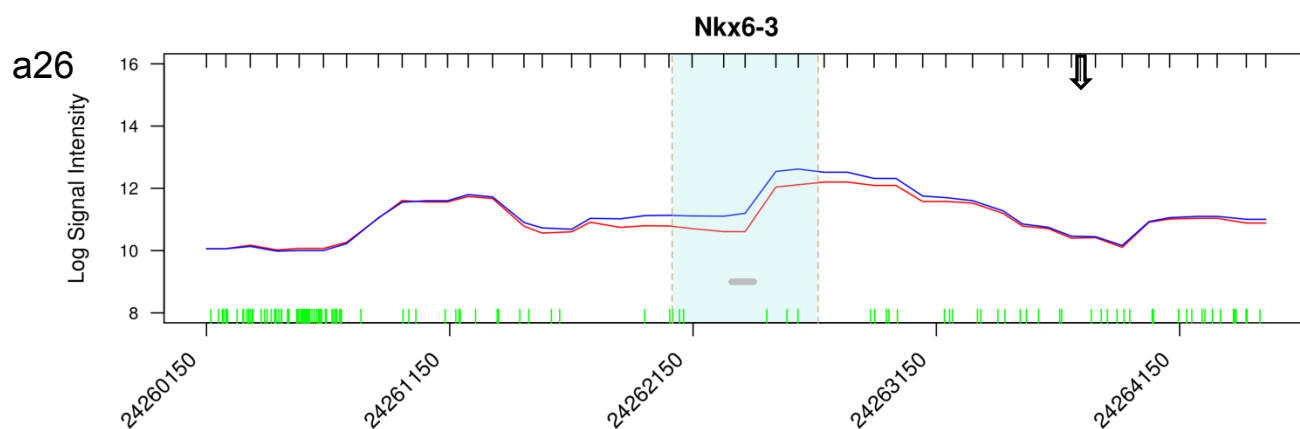
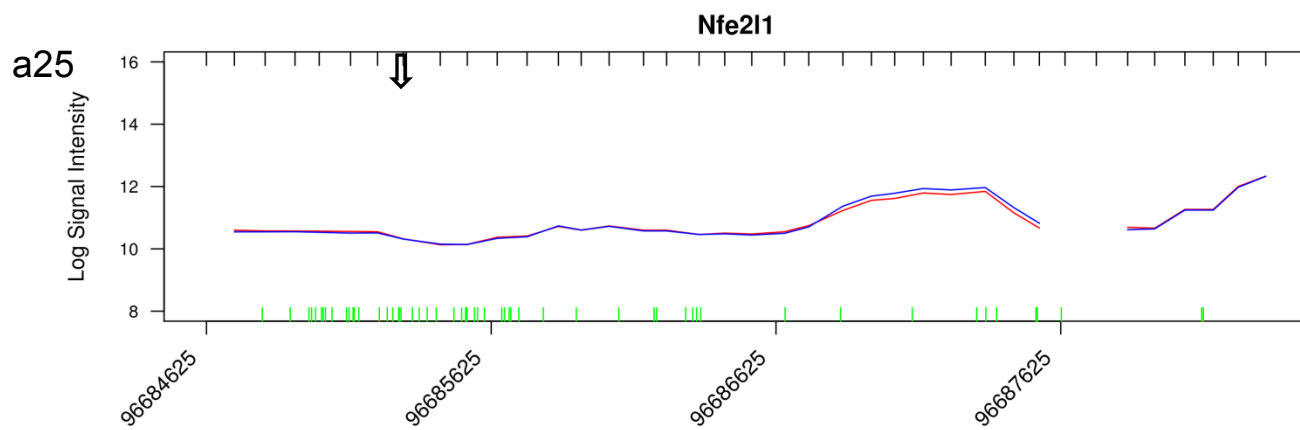


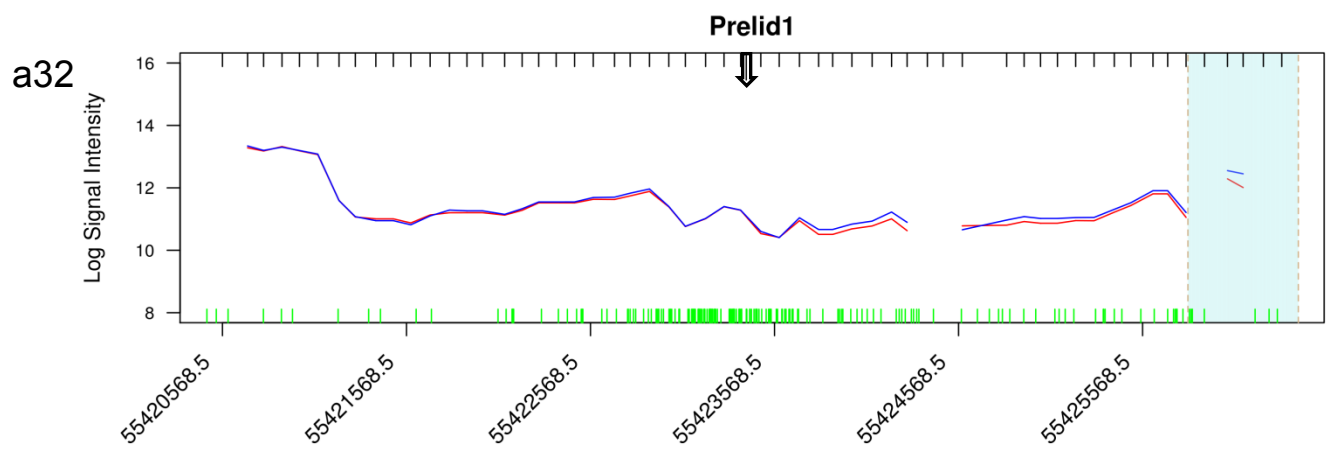
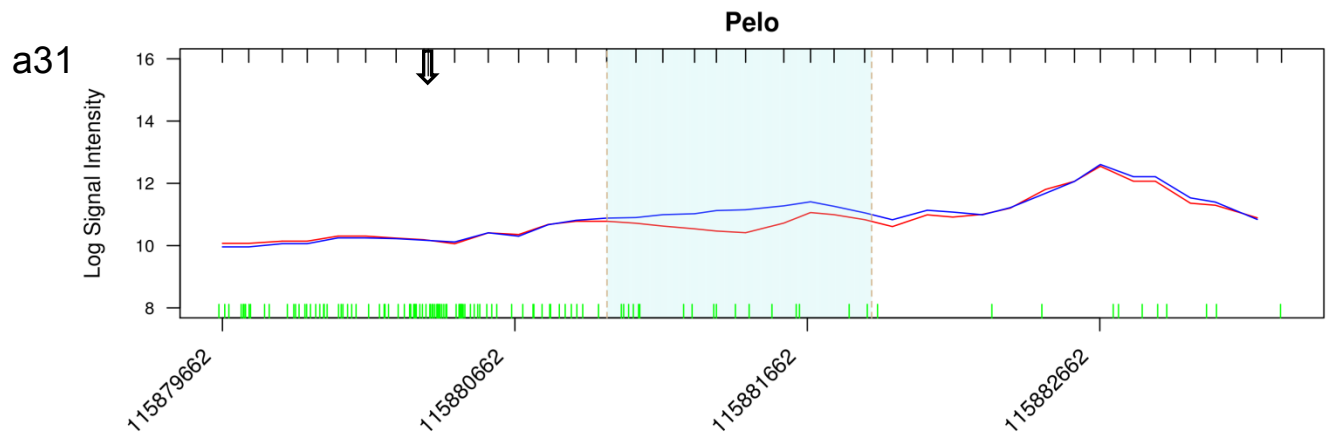
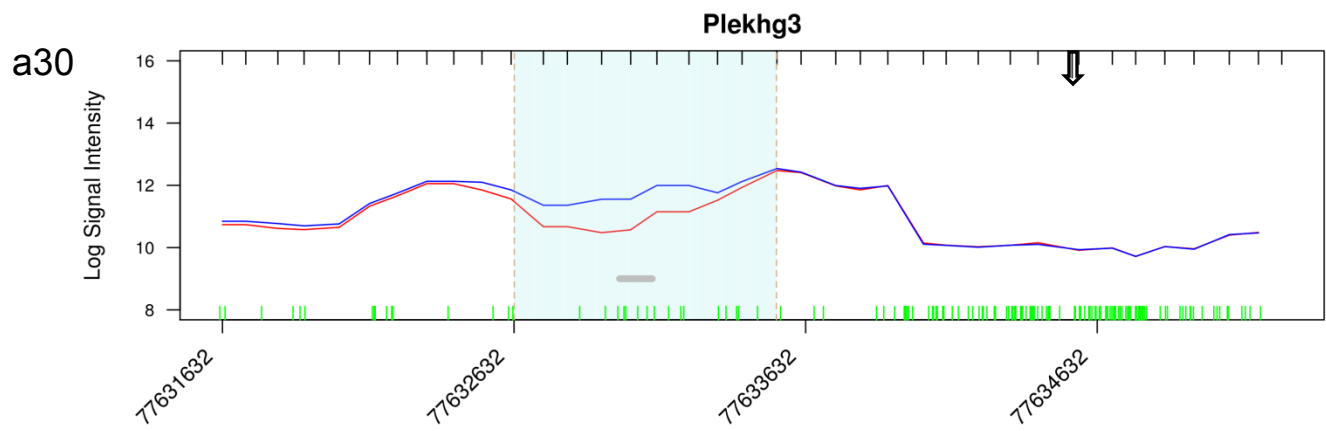
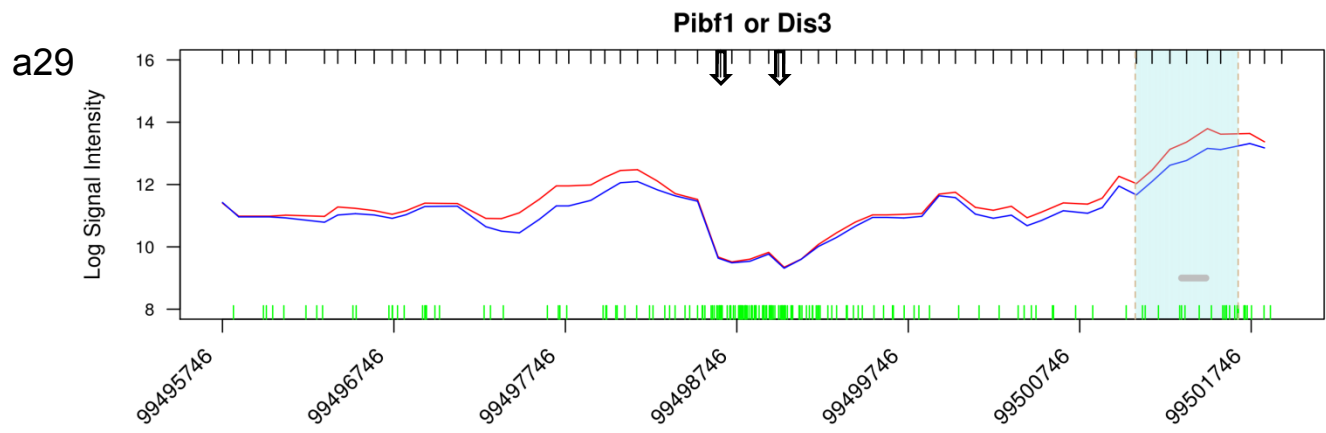


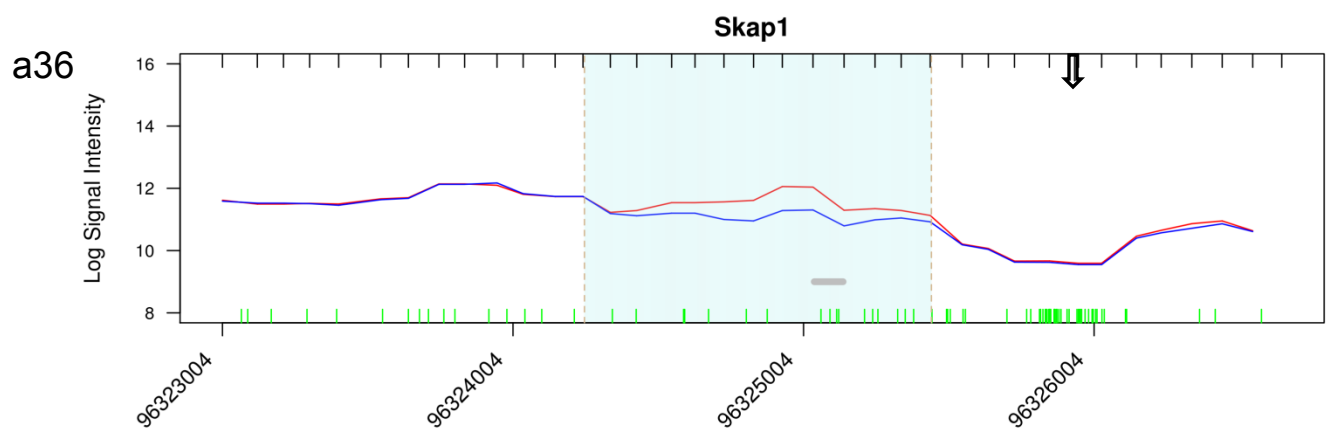
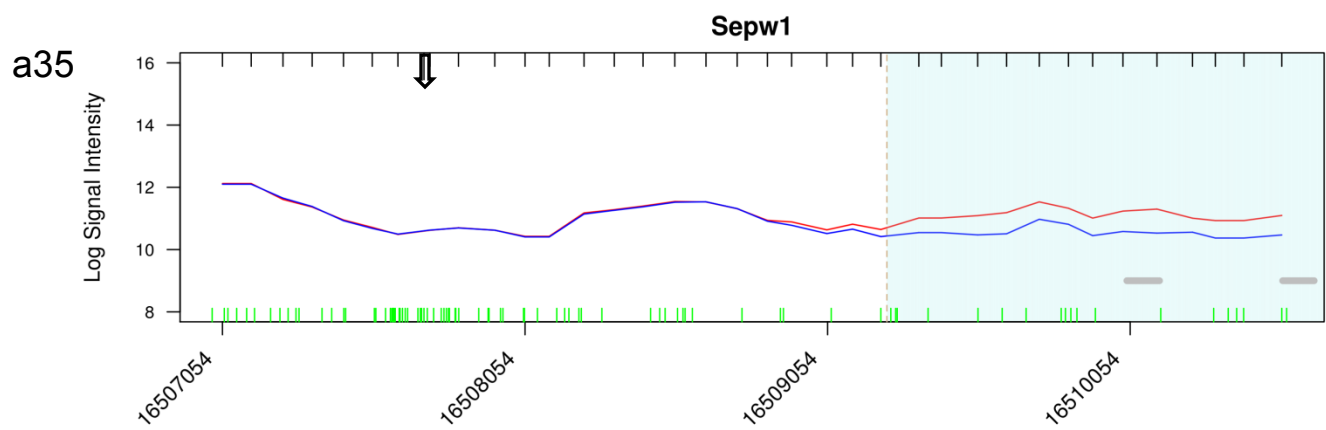
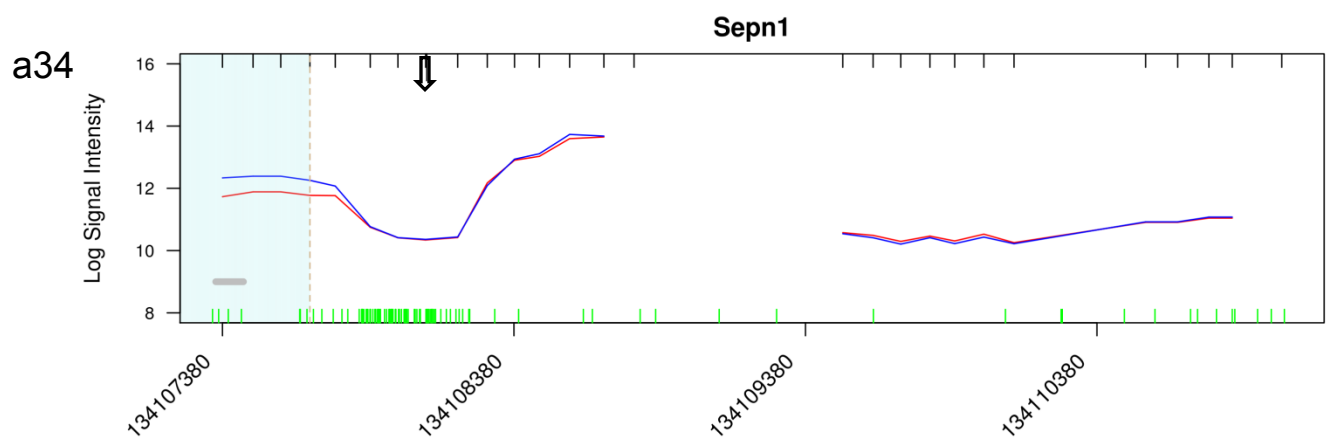
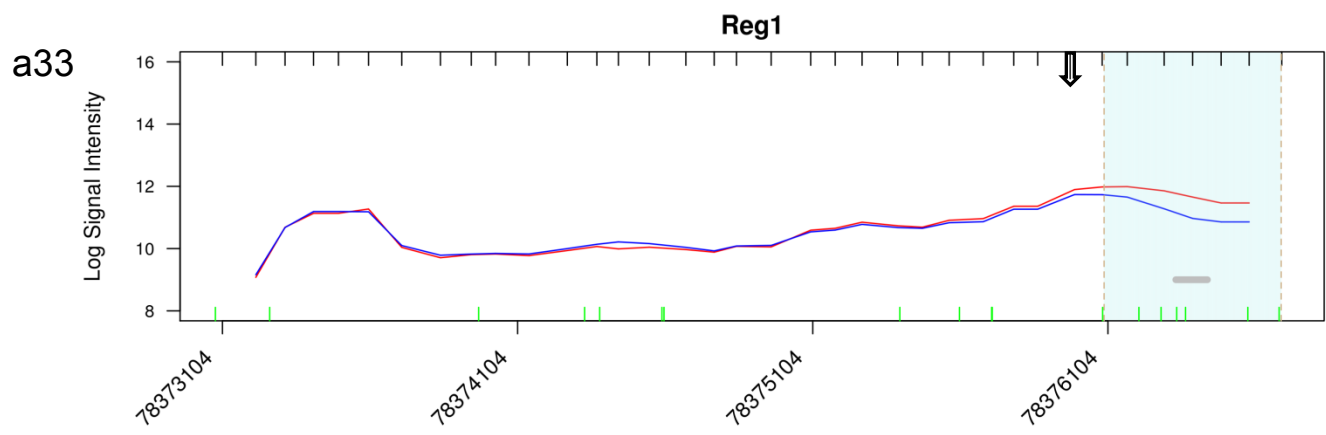




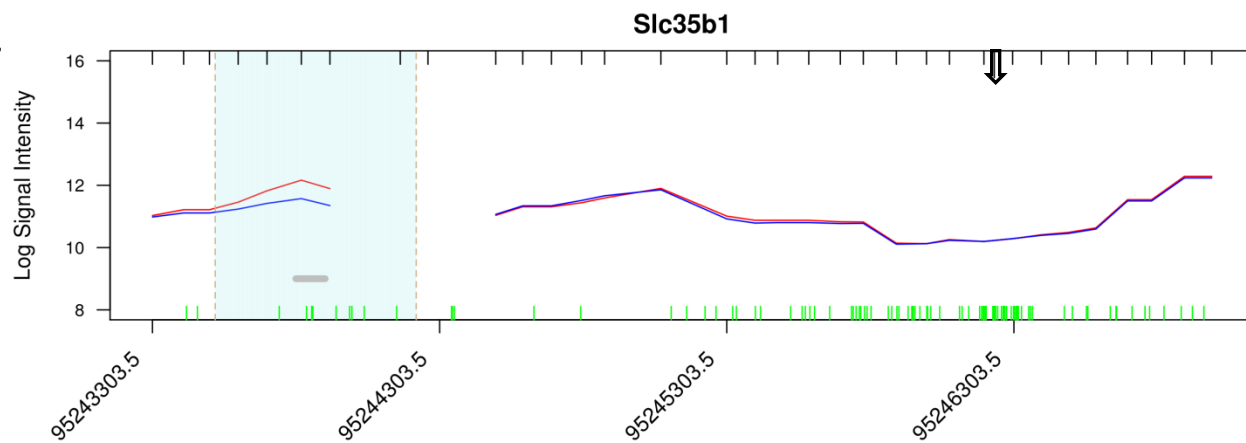




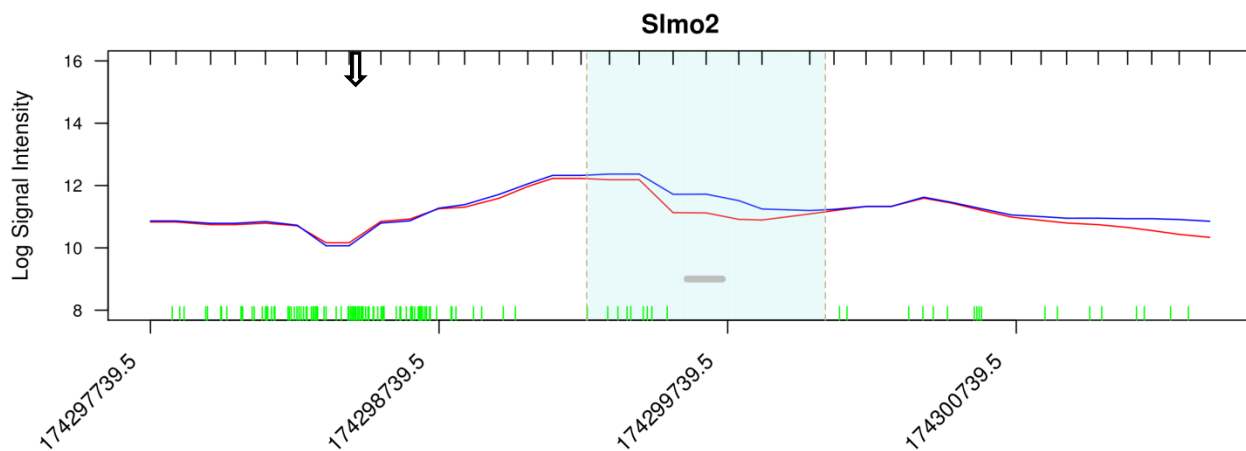




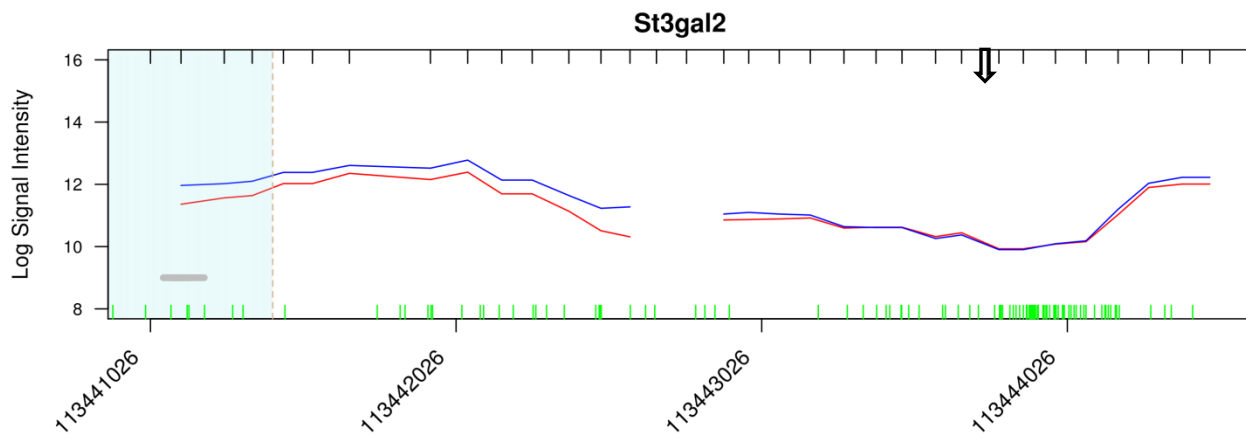
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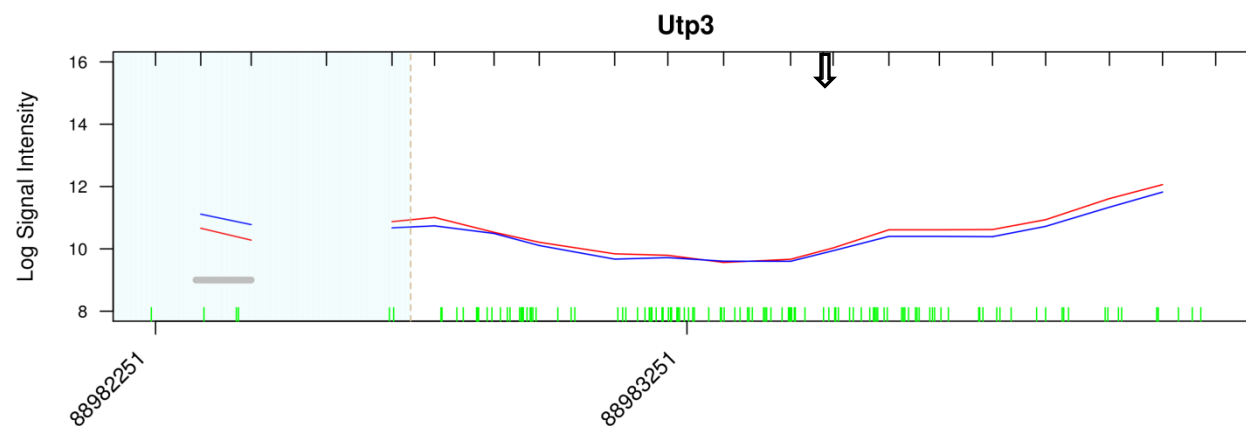
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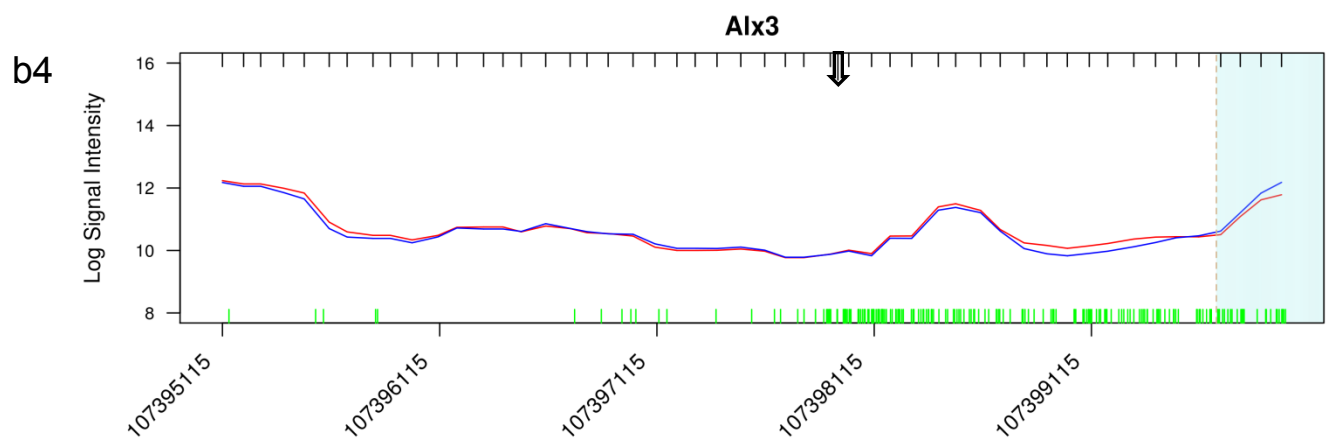
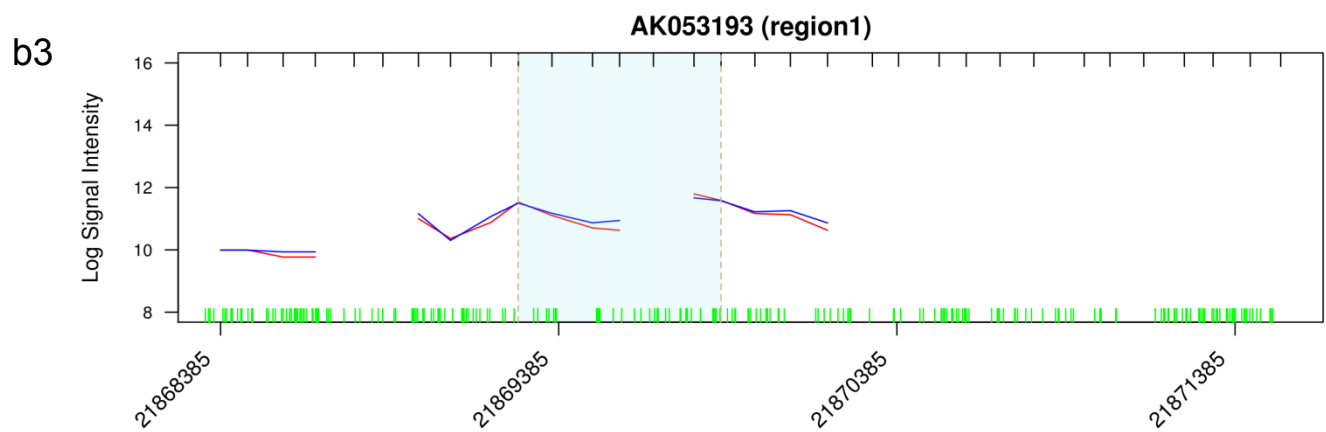
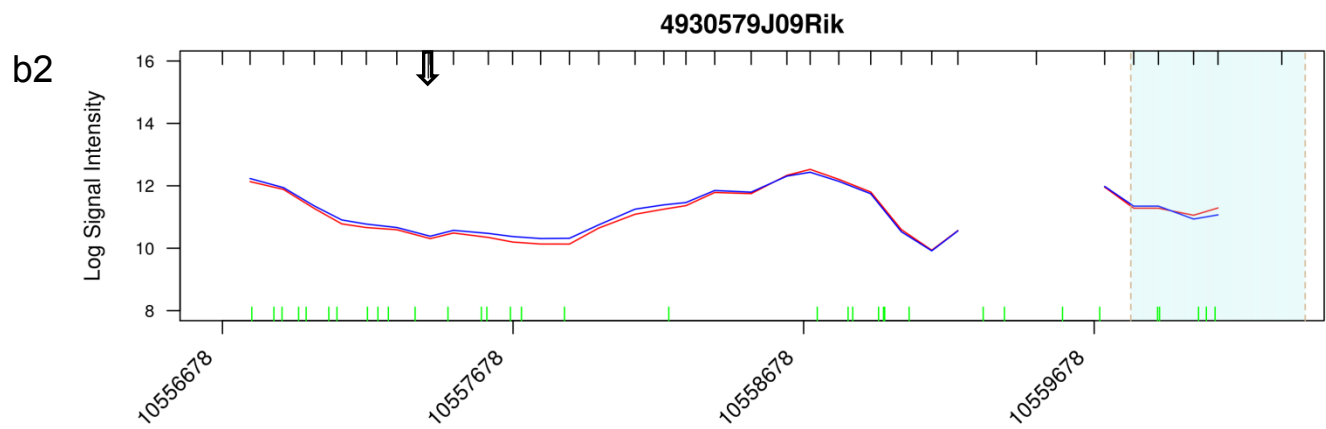
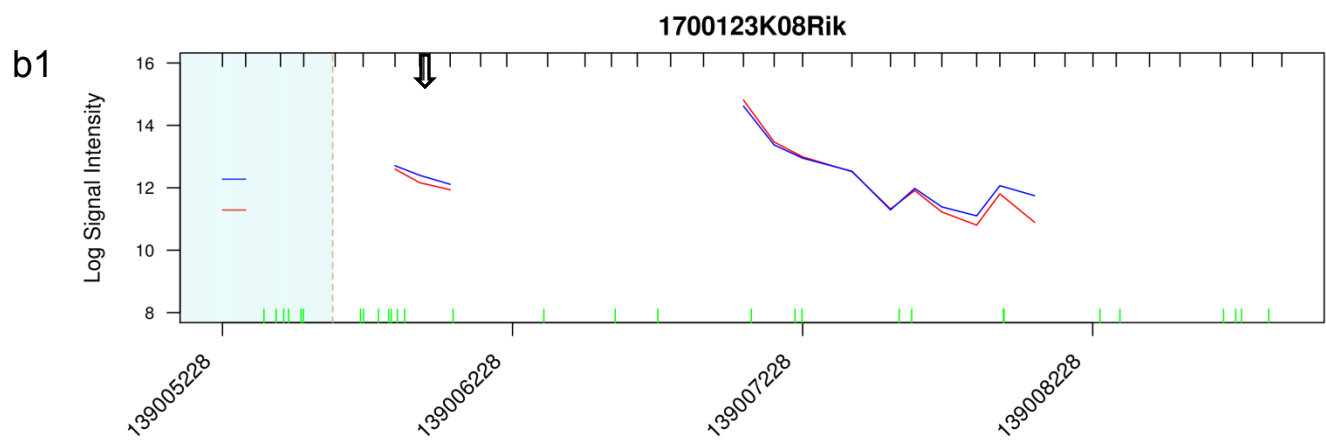


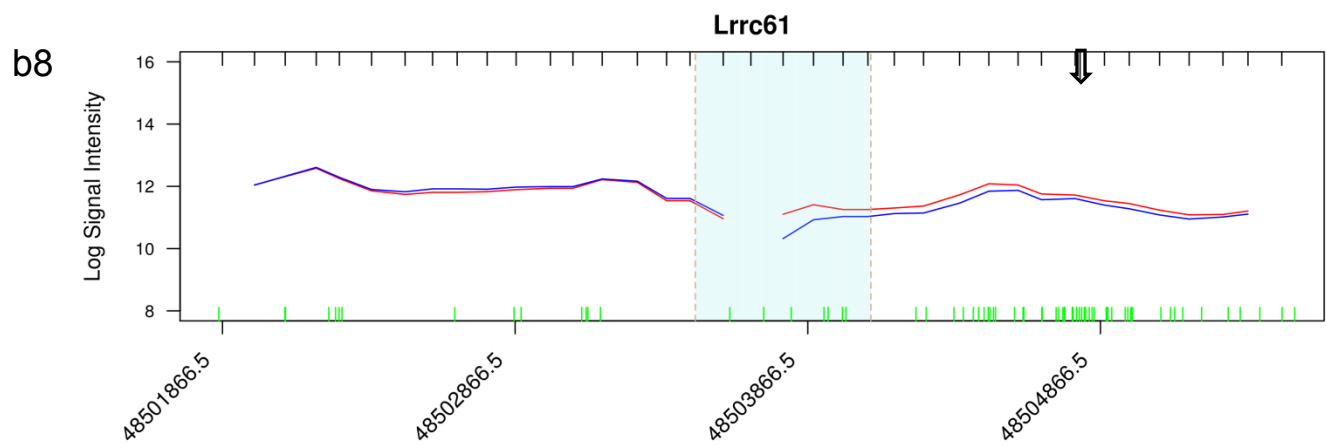
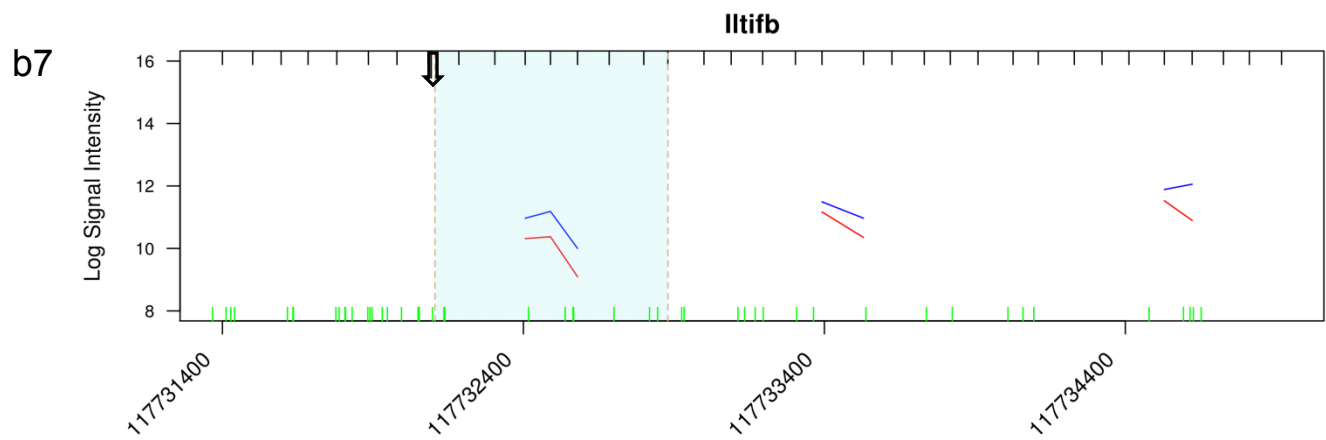
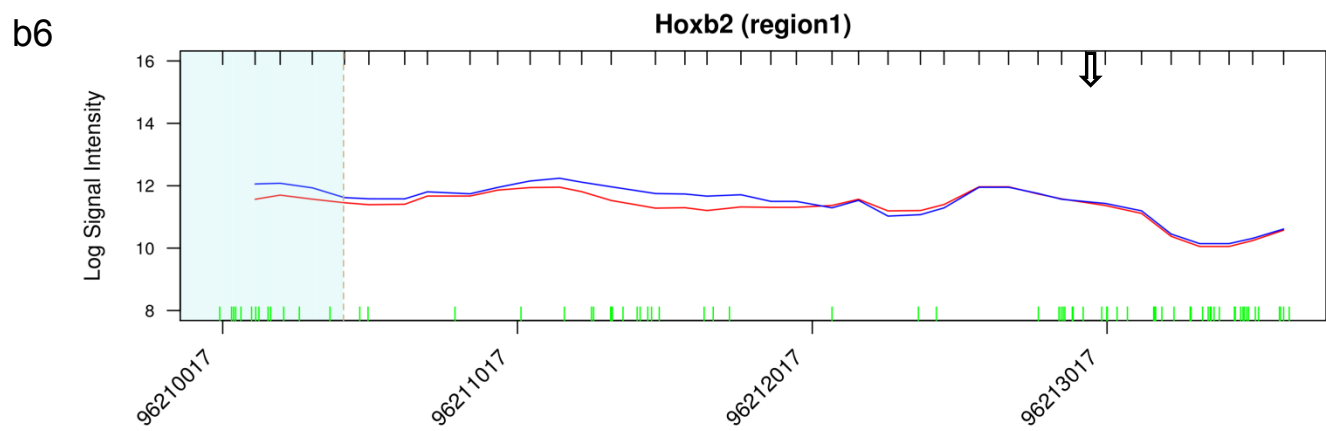
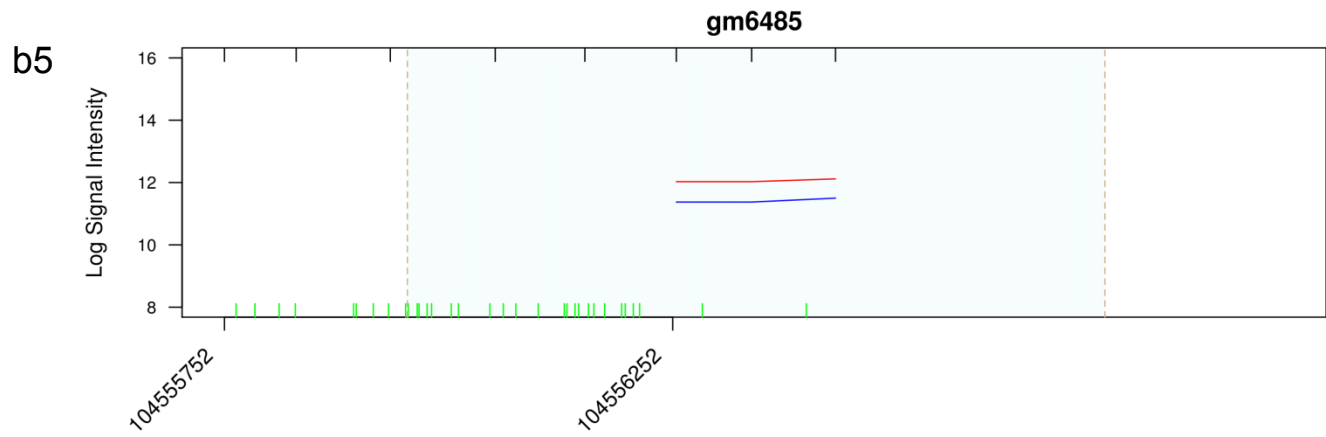
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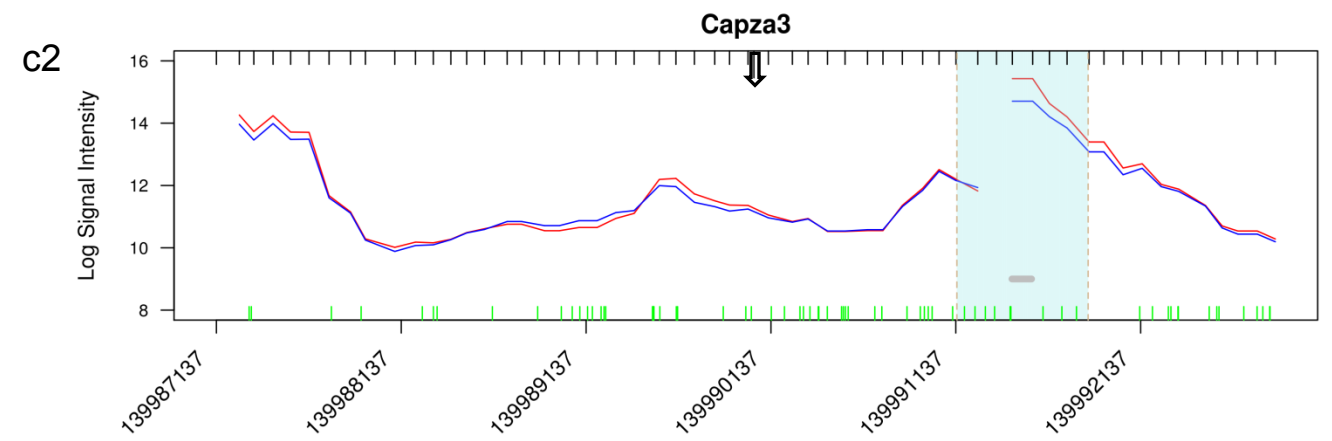
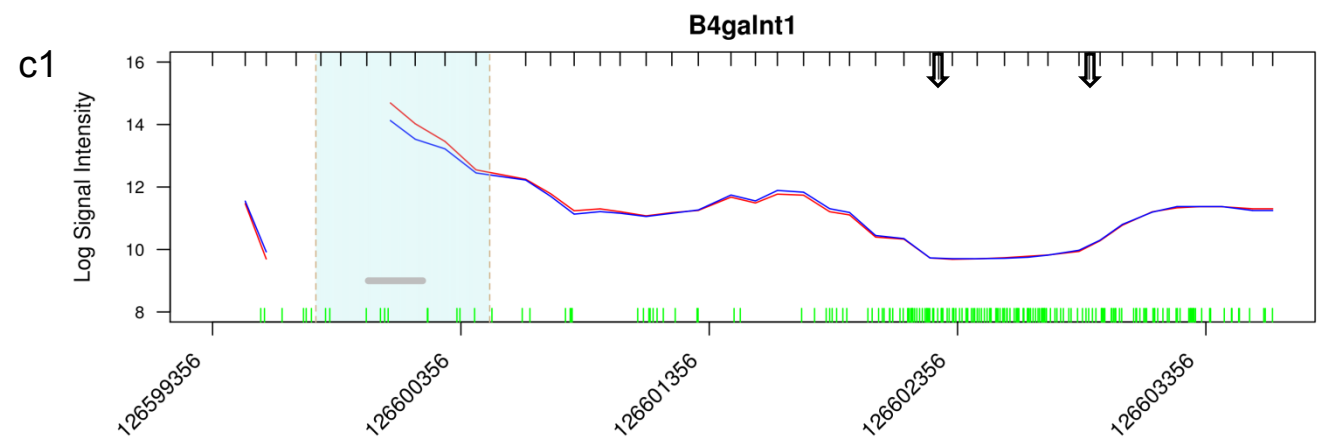
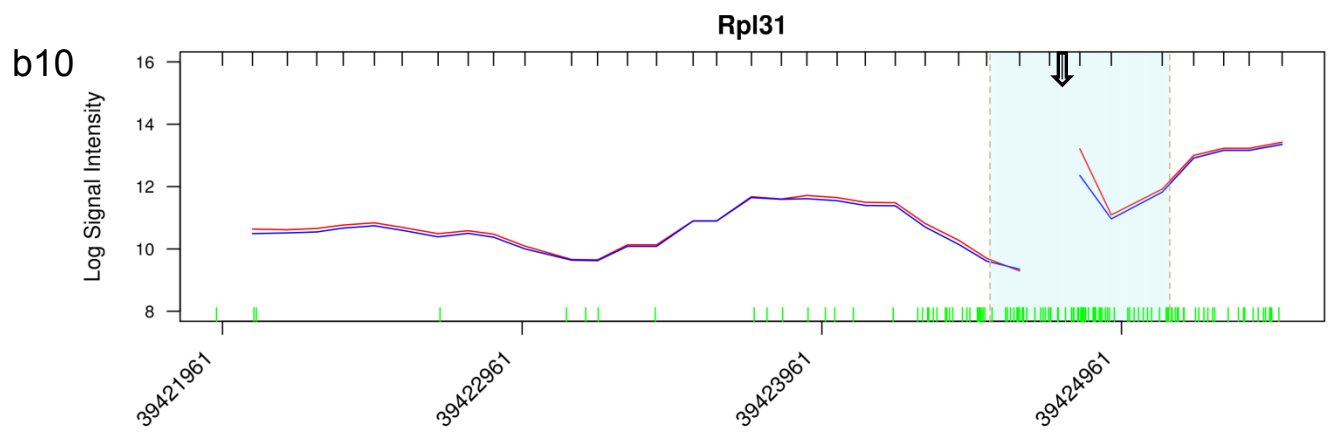
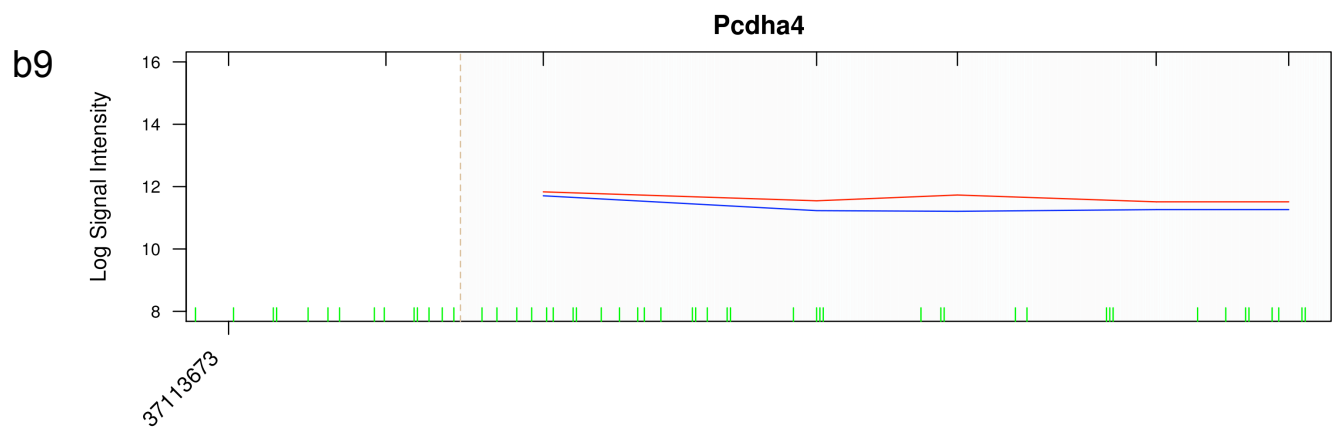


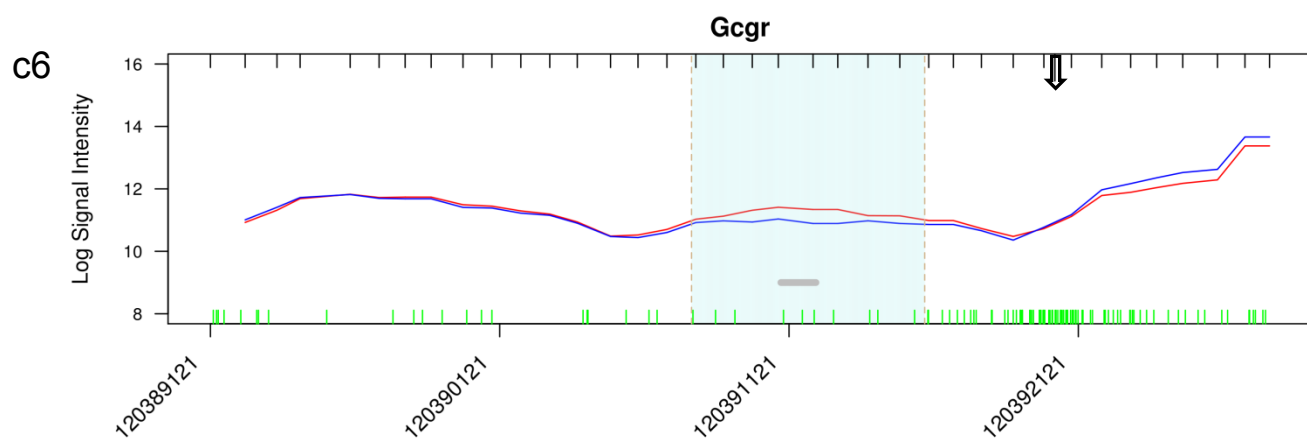
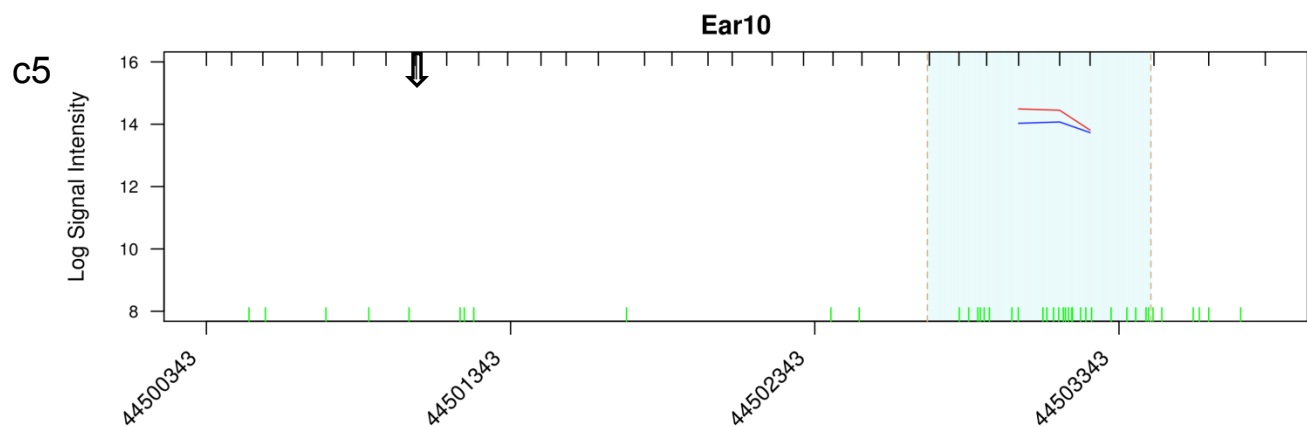
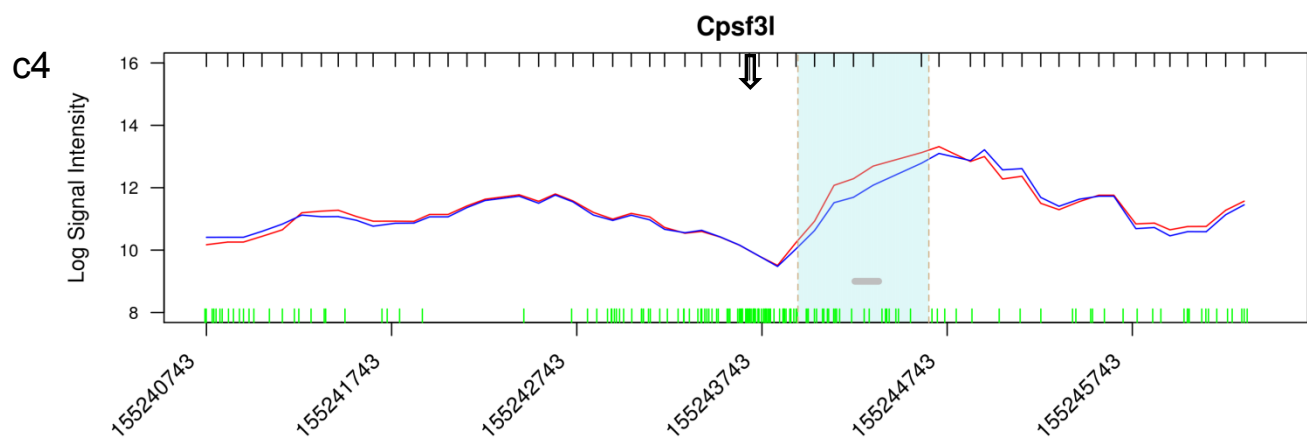
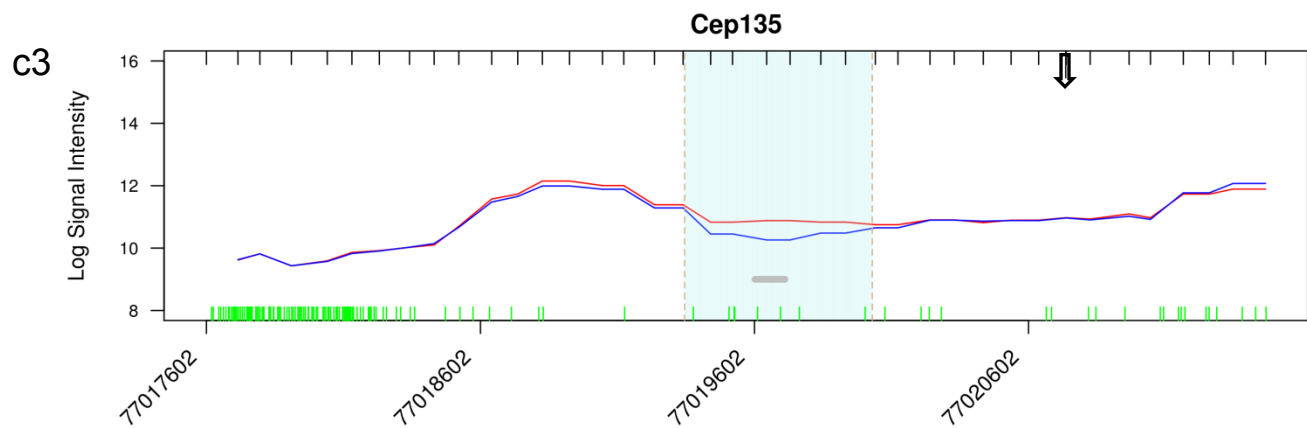
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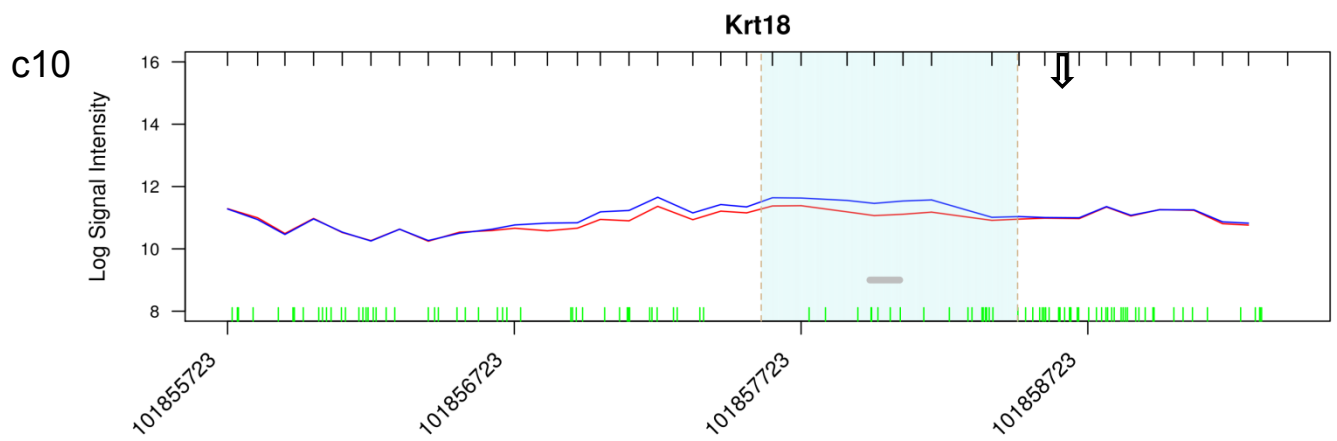
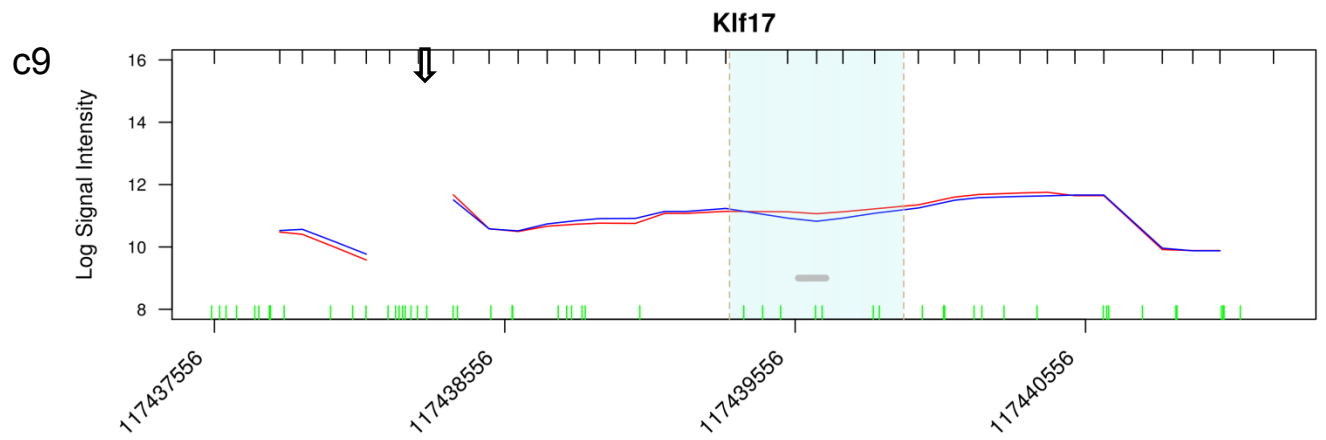
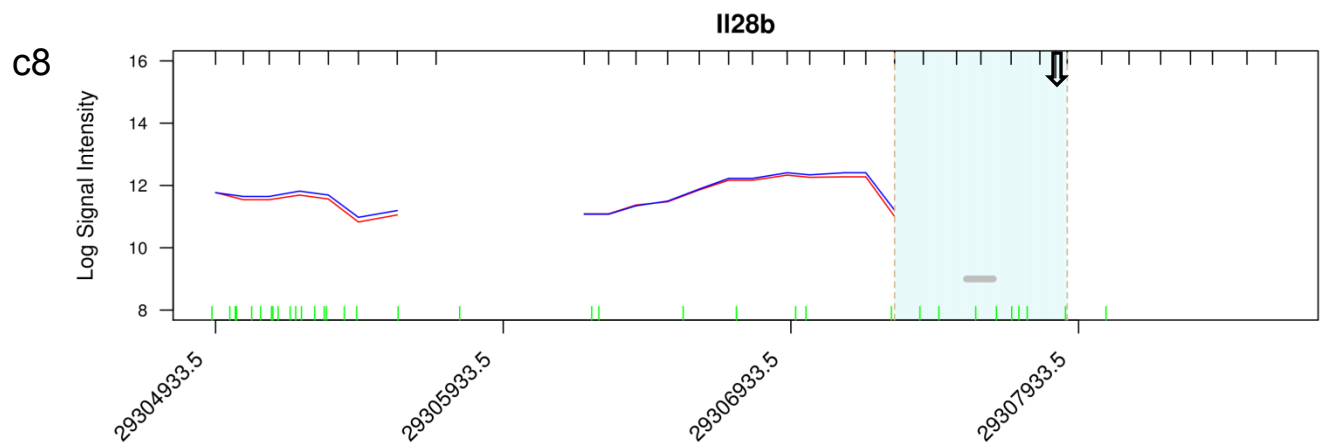
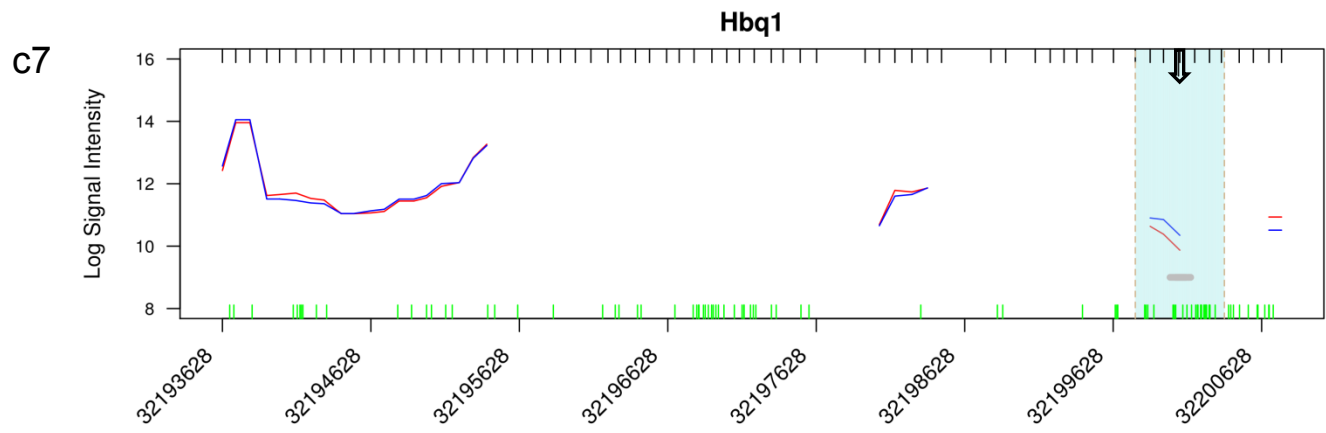


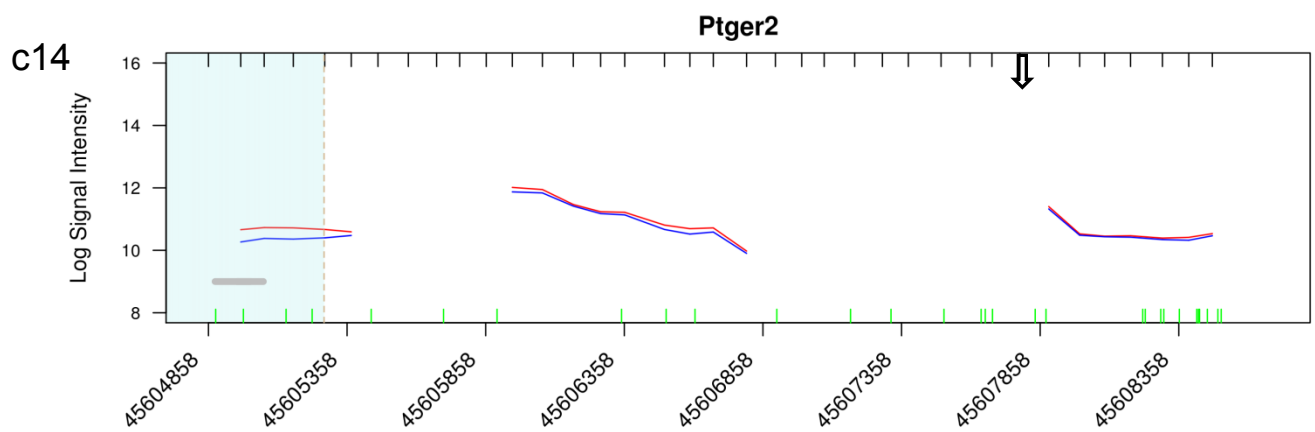
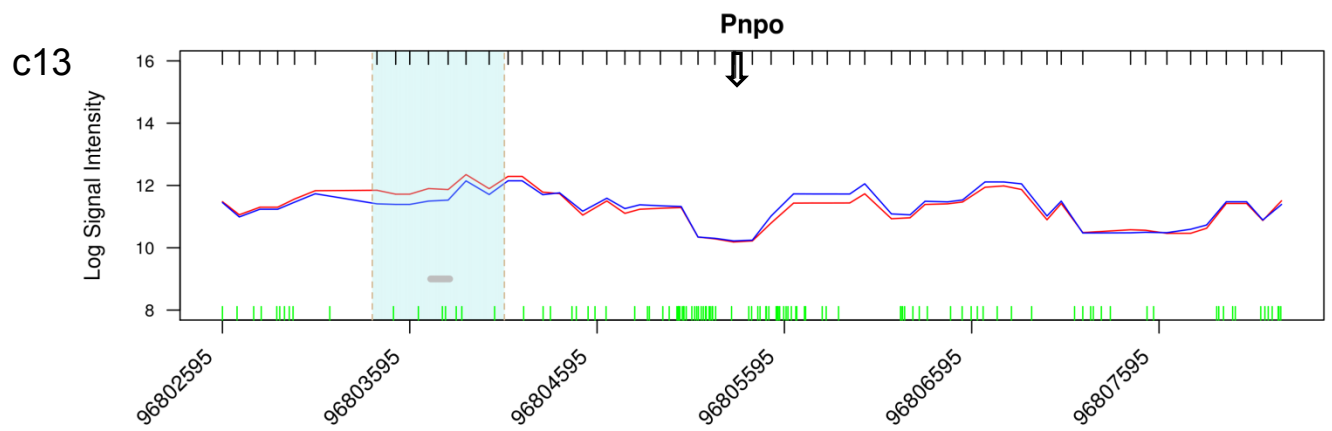
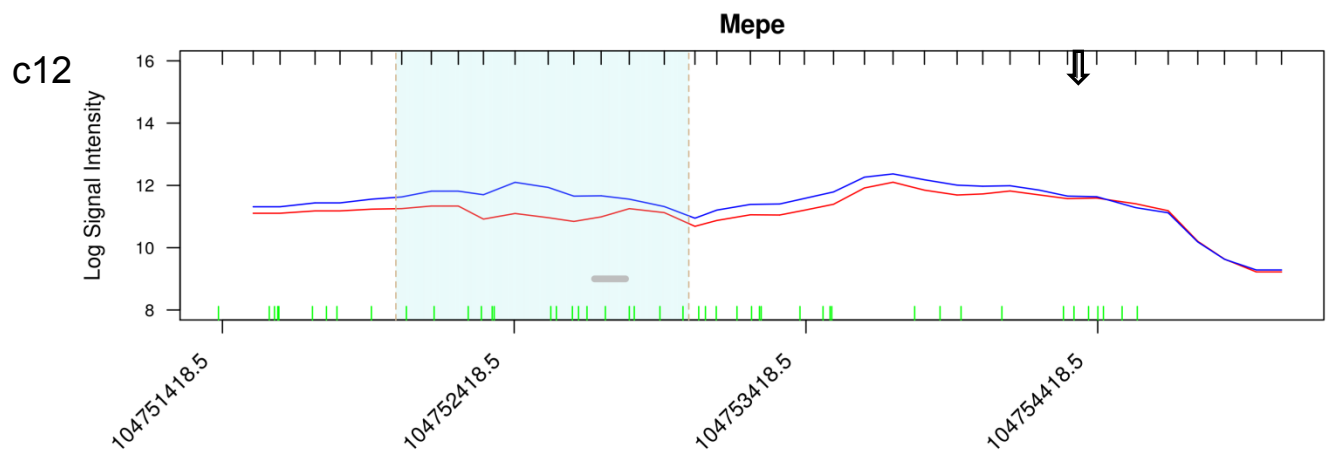
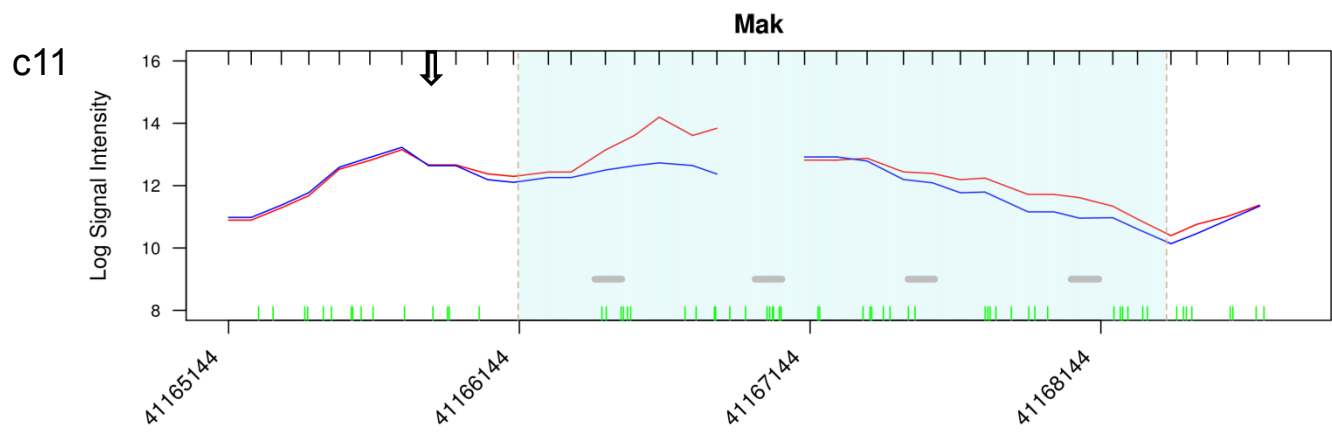


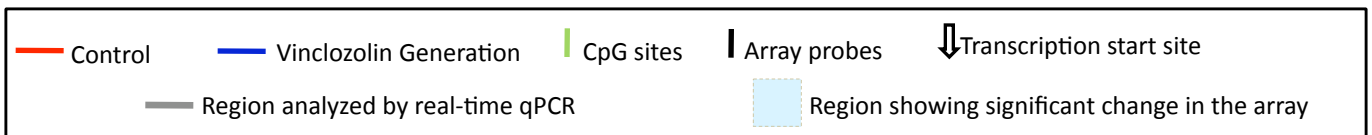
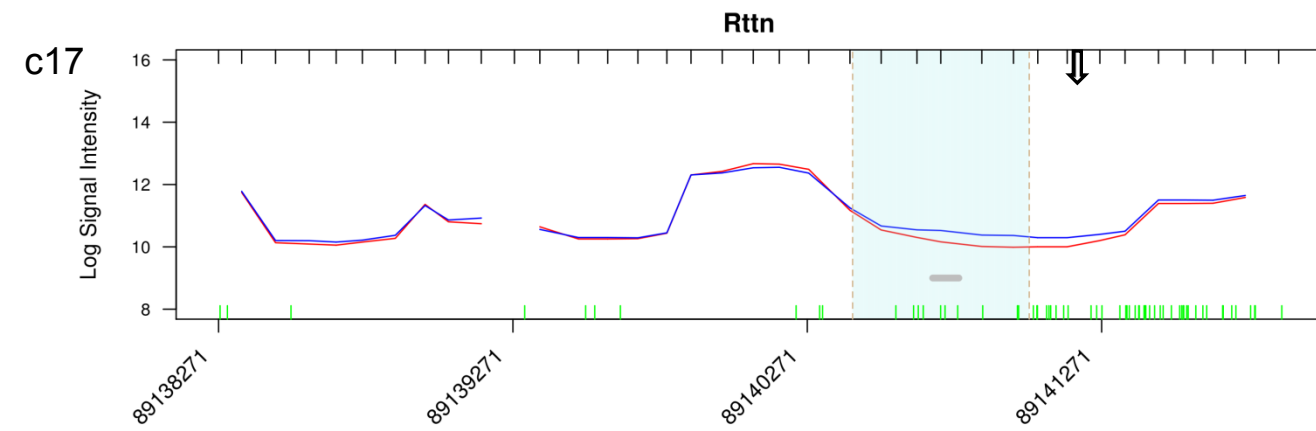
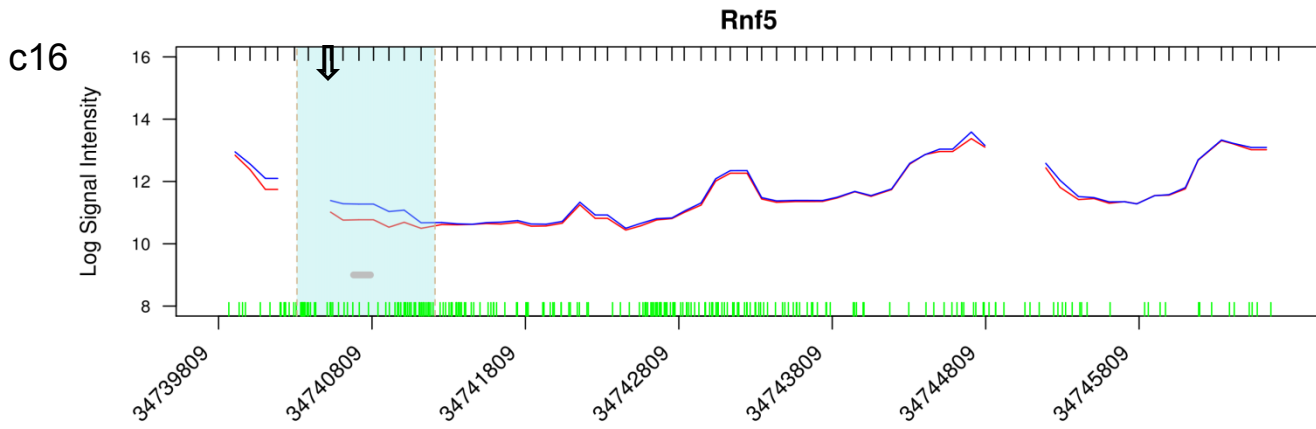
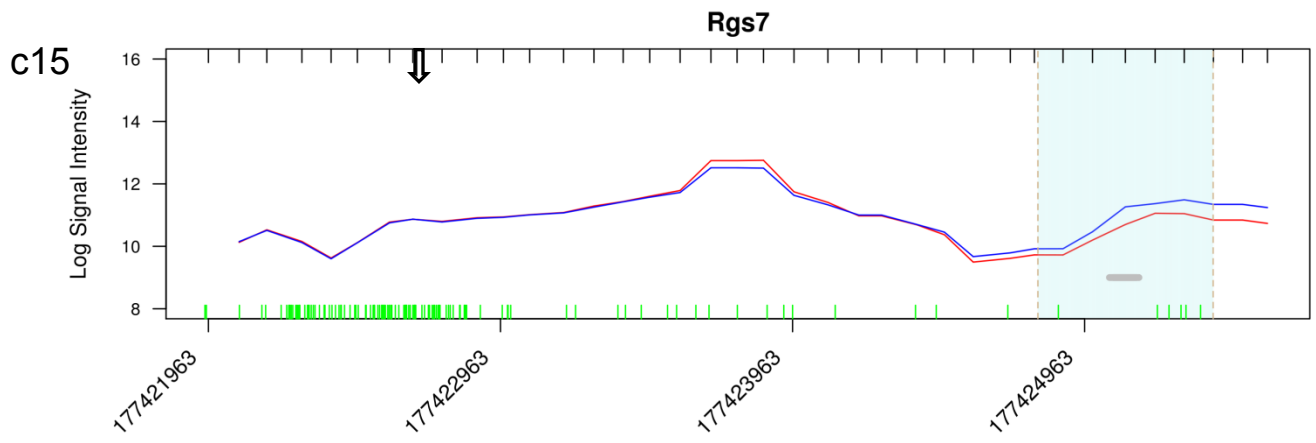












Supplemental Table S1A. Adult male body weight, testes index, and kidney index for P60-P90 mouse 129 strain control, vinclozolin and flutamide F1, F2, and F3 generations.

Generation	# of Animals	Body Weight (g)	Testes Index [†]	Kidney Index ^{††}
Vinclozolin				
Control F1	8	25.51 ± 0.89	8.27 ± 0.38	8.04 ± 0.34
Control F2	11	26.25 ± 0.72	8.52 ± 0.37	8.12 ± 0.29
Control F3	11	26.21 ± 0.98	9.10 ± 0.33	8.21 ± 0.16
Vinclozolin F1	11	25.85 ± 0.79	9.64 ± 0.40*	8.70 ± 0.30
Vinclozolin F2	22	28.91 ± 0.59*	8.61 ± 0.22	8.56 ± 0.19
Vinclozolin F3	11	24.64 ± 0.65	9.43 ± 0.37	8.59 ± 0.35
Flutamide				
Control F1	8	25.45 ± 0.39	8.88 ± 0.25	8.26 ± 0.51
Control F2	9	26.07 ± 0.53	8.67 ± 0.35	7.41 ± 0.31
Control F3	9	26.91 ± 1.31	10.27 ± 0.66	9.33 ± 0.50
Flutamide F1	8	23.73 ± 0.42*	8.71 ± 0.18	6.82 ± 0.22*
Flutamide F2	8	27.53 ± 1.31	8.08 ± 0.63	7.19 ± 0.14
Flutamide F3	10	26.75 ± 1.02	8.44 ± 0.22*	8.32 ± 0.39

Data presented as Mean ± SEM, asterisks (*) indicate statistically significant differences between control and vinclozolin or flutamide generation mice P<0.05. The # of animals listed represents the total number of animals analyzed (n value).

[†] Testis index is calculated as testis weight (g) / body weight (g) x 1000.

^{††} Kidney index is calculated as kidney weight (g) / body weight (g) x 1000.

Supplemental Table S1B. Adult male body weight, testes index, and kidney index for P60-P90 mouse CD-1 strain control, vinclozolin V1 (100 mg/kg/day) and V2 (200 mg/kg/day) F1, F2 and F3 generations.

Generation	# of Animals	Body Weight (g)	Testes Index [†]	Kidney Index ^{††}
Control F1	8	36.04 ± 0.97	7.43 ± 0.38	9.16 ± 0.44
Control F2	10	36.77 ± 1.16	6.95 ± 0.31	10.12 ± 0.41
Control F3	8	38.32 ± 1.60	7.68 ± 0.42	9.24 ± 0.45
Vinclozolin V1 F1	6	34.87 ± 1.08	7.52 ± 0.26	9.45 ± 0.44
Vinclozolin V1 F2	10	35.02 ± 0.92	6.70 ± 0.41	9.18 ± 0.24
Vinclozolin V1 F3	8	34.72 ± 1.63	7.43 ± 0.31	8.26 ± 0.36
Vinclozolin V2 F1	8	34.84 ± 0.87	7.31 ± 0.45	9.49 ± 0.28
Vinclozolin V2 F2	6	34.56 ± 0.99	8.14 ± 0.34*	9.25 ± 0.39
Vinclozolin V2 F3	8	38.55 ± 2.23	7.03 ± 0.20	10.35 ± 0.35

Data presented as Mean ± SEM, asterisks (*) indicate statistically significant differences between control and vinclozolin generation mice P<0.05. The # of animals listed represents the total number of animals analyzed (n value).

[†] Testis index is calculated as testis weight (g) / body weight (g) x 1000.

^{††} Kidney index is calculated as kidney weight (g) / body weight (g) x 1000.

Supplemental Table S2.

F3 Animal	Age (mos.)	Spermatogenic Apoptosis	Sperm Concentration	Sperm Motility	Testis Disease	Prostate Disease	Kidney Disease
Control 1	13	+	0.8	10			
Control 2	13	-	4.76	20			
Control 3	13	+	1.28	80			
Control 4	13	-	5.32	65			
Control 5	15	-	2.44	30	+		
Control 6	15	+	3.76	70	+		
Control 7	15	-	2.12	20			+
Control 8	15	-	2.72	17.5			+
Control 9	17	-	2.84	35		+	
Control 10	17	-	3.04	7.5			
Control 11	17	-	1.88	70			
Control 12	17	-	4.08	75		+	
V1 1	13	+	0.24	5	+		
V1 2	13	+	2.72	10			
V1 3	13	+	3.28	30			
V1 4	13	+	3.52	80			+
V1 5	14	+	2.76	65	+	+	
V1 6	15	-	3.64	25	+		+
V1 7	14	-	4	40	+		
V1 8	14	-	5.88	80	+	+	+
V1 9	14	+	2.6	65	+		
V1 10	15	+	2.08	90			+
V1 11	15	+	2.28	55	+		+
V1 12	15	+	1.32	90	+	+	+
V1 13	17	+	0.72	7.5		+	
V2 1	13	-	1.4	10			
V2 2	13	+	1.04	10		+	
V2 3	13	+	1.56	15			
V2 4	13	-	1.52	20			
V2 5	15	+	2.2	25	+		
V2 6	14	-	2.28	90	+		+
V2 7	14	-	1.32	25	+		+
V2 8	15	-	4.52	60	+	+	
V2 9	17	-	4.84	12.5		+	
V2 10	17	-	6.12	25		+	+
V2 11	17	-	2.64	30			
V2 12	17	-	3.64	30		+	
V2 13	17	-	3.44	15			
V2 14	17	-	1	0	+		+
V2 15	17	-	3.88	20			+
V2 16	17	-	2.64	45			+

The V1 (100/mg/kg/day) and V2 (200 mg/kg/day) doses of vinclozolin lineage to control lineage F3 generation animals. Testis, prostate and kidney disease assessment is described in the Materials and Methods section.

Supplementary Table S3:**Regions showing vinclozolin-induced transgenerational change with MeDIP-Chip not confirmed with Real Time qPCR**

Gene symbol	Description	MGI ID	Entrez gene ID	Significance (p ≤)	Changed region coordinates			Region size (bp)
					Chr	Start	End	
B4galnt1	Beta-1,4-N-acetyl-galactosaminyl transferase 1	1342057	14421	7.74E-11	10	126599772	126600472	700
Capza3	Capping protein (actin filament) muscle Z-line, alpha 3	106221	12344	2.22E-10	6	139991143	139991853	710
Cep135	Centrosomal protein 135	2681869	381644	1.17E-14	5	77019347	77020032	685
Cpsf3l	Cleavage and polyadenylation specific factor 3-like	1919207	71957	8.52E-08	4	155243937	155244644	707
Ear10	Eosinophil-associated, ribonuclease A family, member 10	1890464	93725	1.99E-08	14	44502713	44503448	735
Gcgr	Glucagon receptor	99572	14527	5.19E-08	11	120390784	120391590	806
Hbq1	Hemoglobin, theta 1	2685722	216635	2.87E-08	11	32199777	32200377	600
Il28b	Interleukin 28B	2450574	338374	5.70E-10	7	29307294	29307894	600
Klf17	Kruppel-like factor 17	2181068	75753	2.82E-10	4	117439330	117439930	600
Krt18	Keratin 18	96692	16668	1.70E-09	15	101857584	101858478	894
Mak	Male germ cell-associated kinase	96913	17152	1.95E-85	13	41166140	41168370	2230
Mepe	Matrix extracellular phosphoglycoprotein with ASARM motif (bone)	2137384	94111	3.30E-21	5	104752013	104753017	1004
Pnp0	Pyridoxine 5'-phosphate oxidase	2144151	103711	1.19E-10	11	96803395	96804100	705
Ptger2	Prostaglandin E receptor 2 (subtype EP2)	97794	19217	1.11E-08	14	45604675	45605275	600
Rgs7	Regulator of G protein signaling 7	1346089	24012	2.79E-08	1	177424803	177425403	600
Rnf5	Ring finger protein 5	1860076	54197	2.01E-09	17	34740320	34741220	900
Rtnn	Rotatin	2179288	246102	3.32E-09	18	89140425	89141025	600

Description and annotation of the regions showing vinclozolin-induced transgenerational change with MeDIP-chip but that were not confirmed with Real Time qPCR validation.

These regions did not meet the following criteria for a RT-qPCR value to be considered as a change:

i) passed t-test with $p < 0.05$,

ii) the trend of the change observed in the qPCR is the same as the observed in the MeDIP Chip array