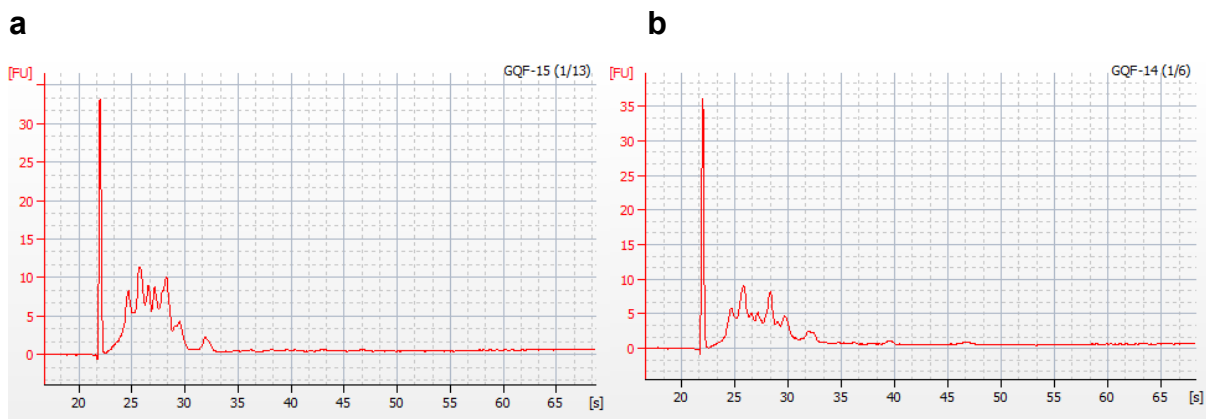


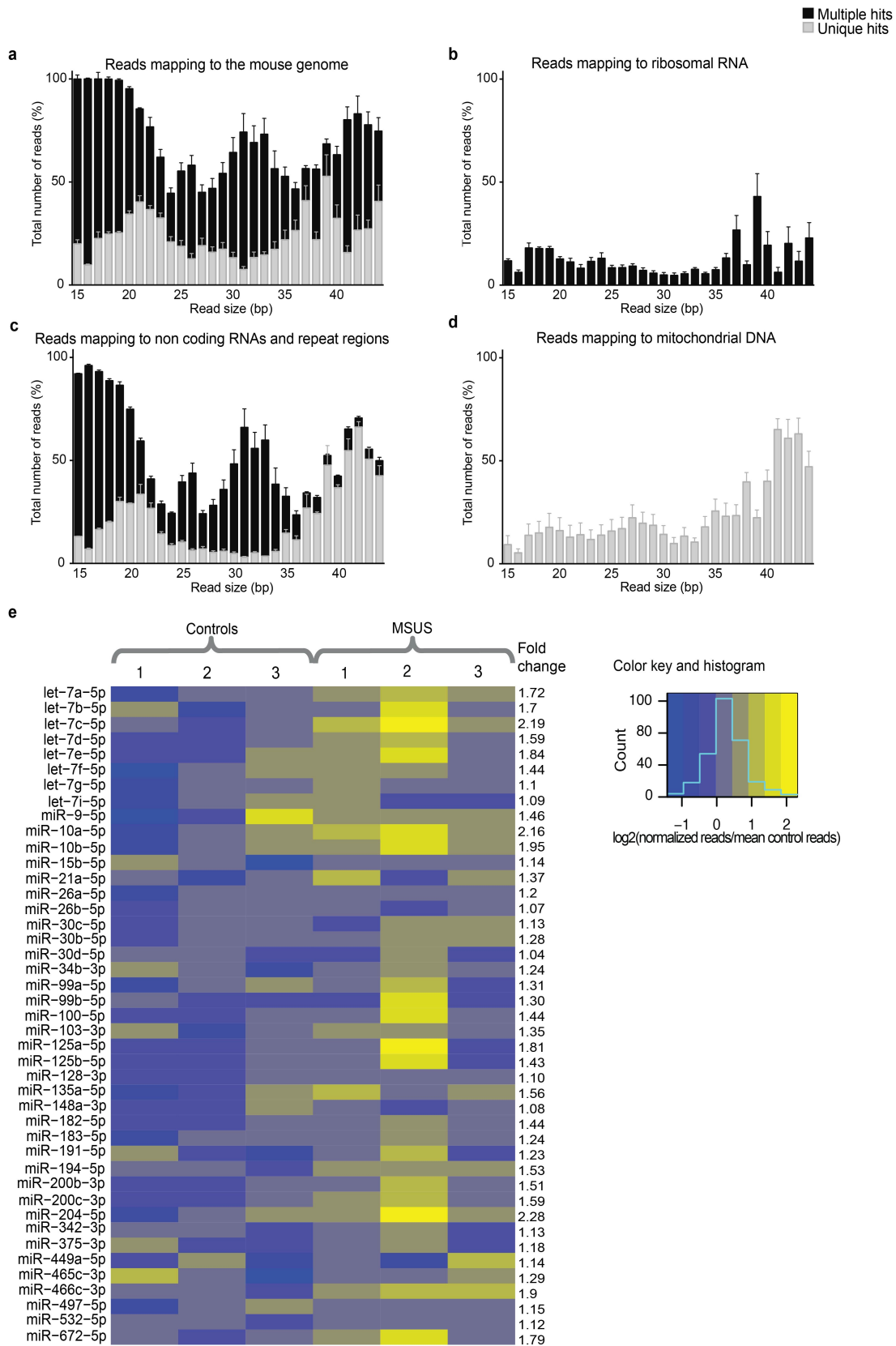
Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice

Katharina Gapp, Ali Jawaid, Peter Sarkies, Johannes Bohacek, Pawel Pelczar, Julien Prados, Laurent Farinelli, Eric Miska and Isabelle M. Mansuy

Supplementary Material

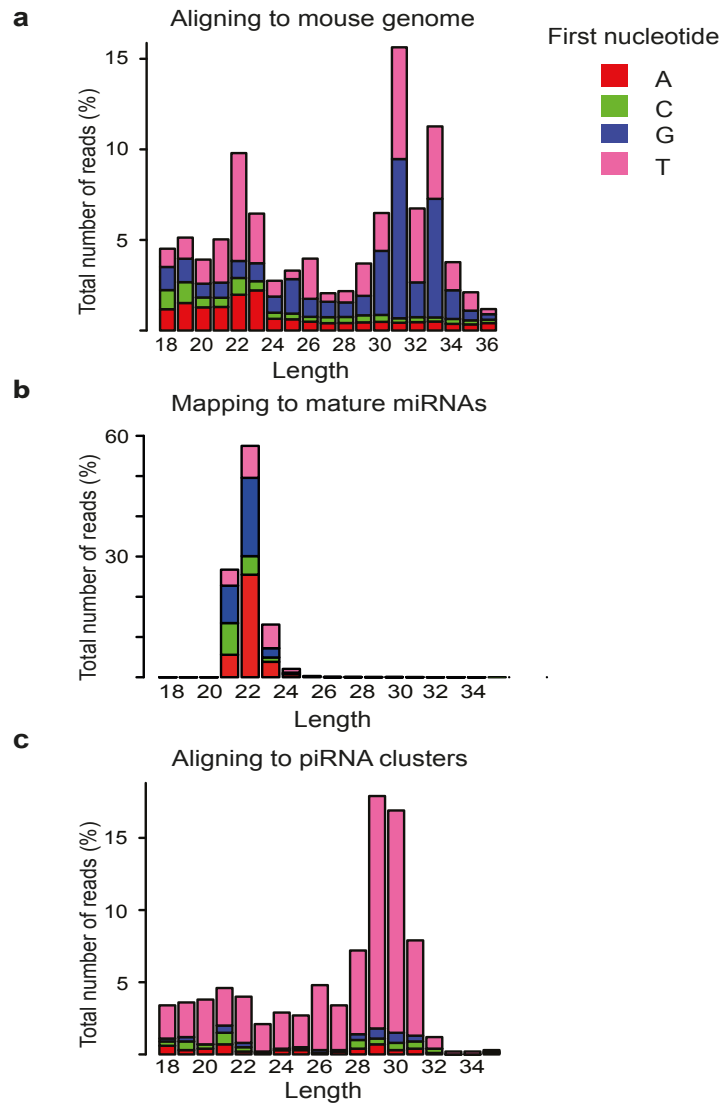


Supplementary Figure 1. Size and integrity profile of sperm RNAs used for deep sequencing and injected into fertilized oocytes. Representative bioanalyser electropherograms show fluorescence intensity (fluorescence unit, FU) over time (seconds) during the pulsing of an RNA sample through a separation microchannel. Small RNAs go through the microchannel faster than long RNAs and appear on the left of the x-axis (for instance, 25bp RNAs appear after about 23 seconds, 200bp RNAs after 28 seconds and 2kb RNAs after 44 seconds). GQF-15 in a) corresponds to a control sample and GQF-14 in b) to a MSUS sample (pooled RNA from 5 mice). The profiles indicate that both samples contain short RNAs but no apparent RNAs above 0,5-1kb. They also show no DNA contamination. This was confirmed by Q-bit analyses using a dsDNA HS assay (Life technologies 1, [DNA]<0,1ng/ul). No protein contamination was detectable by Q-bit assay (Life technologies Q33212, [protein]<1pg/ul) and was confirmed by mass spectrometry (MS).

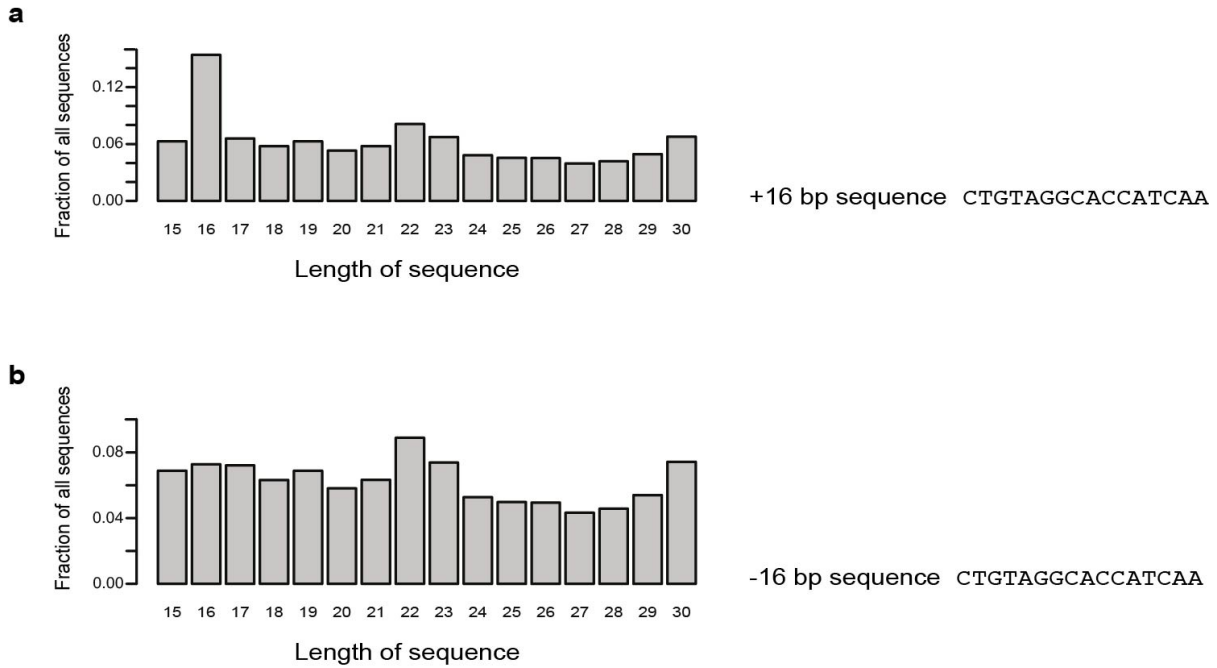


Supplementary Figure 2. SncRNAs in adult sperm. Mapping of 15–44bp

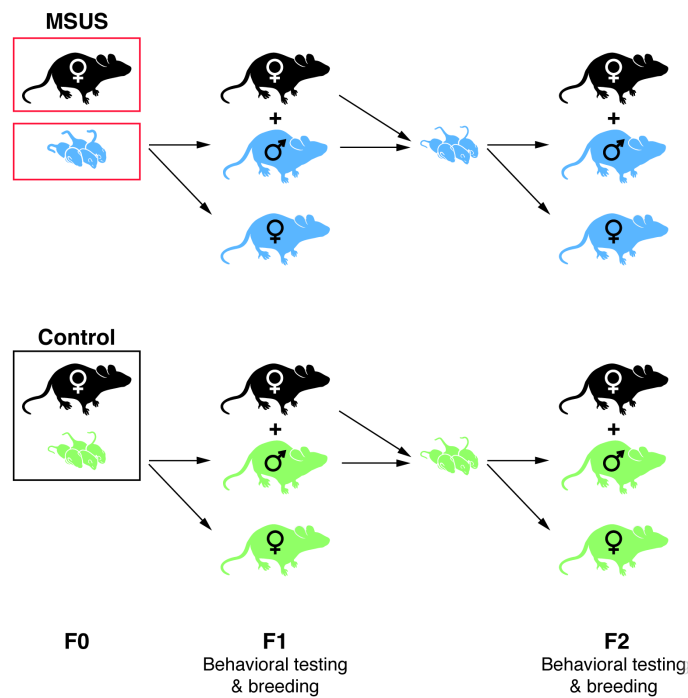
sequencing reads to a) the mouse reference genome, b) ribosomal RNAs, c) other non-coding RNAs and repeat regions and d) mitochondrial DNA, with multiple (black) or unique (grey) hits (n=16 mice, pooled in 4 samples). % total reads represents the proportion of reads with a given size mapping to the mouse genome or selected sequences over the total number of same-size reads. (e) Heatmap showing miRNAs (>100 reads) in control libraries which are altered by MSUS in adult sperm (n=3 each pooled from 5 mice). The blue-to-yellow scale is the number of normalized reads of a given sample over the mean normalized reads of all control samples for each miRNA. Bioinformatic analyses were performed twice using two independent methods. Data are mean \pm s.e.m.



Supplementary Figure 3. Illustration of short RNA reads in adult mouse sperm (a) aligning to the mouse genome, (b) mapping to mature miRNA sequences (allowing for overhanging 5' and 3' nucleotides) and (c) aligning to piRNA clusters. In (a), reads alignment shows peaks at the typical size of miRNAs (21-23bp) and piRNAs (26-31bp). In (b), mapping of 18-35bp reads (not mapping to the transcriptome) to annotated miRNAs shows a sharp peak at 22bp, the typical size of mature miRNAs. In (c), alignment of 18-35bp reads (not mapping to the transcriptome) to genomic regions annotated as piRNAs shows a peak at the typical size of piRNAs, starting with the nucleotide T indicative for piRNA identity. A concatenation of all reads detected in control libraries is shown. The size and first nucleotide are shown by position on the x-axis and color, respectively. The y-axis shows the percentage of reads relative to total reads for the combined libraries.



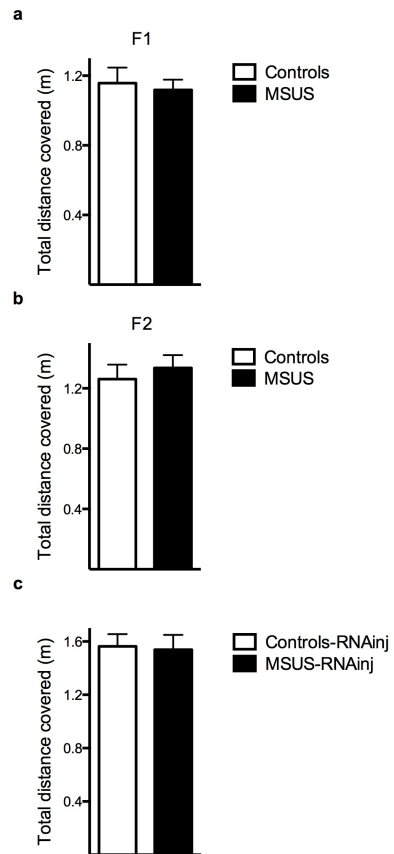
Supplementary Figure 4. Proportion of 15-30bp reads including or excluding a 16bp specific sequence. The y-axis represents the percentage of reads relative to total reads of combined control libraries either a) including an abundant 16bp sequence corresponding to an annotated piRNA sequence or b) excluding this 16bp sequence. Exclusion of this sequence results in a loss of the apparent enrichment of the 16bp peak, suggesting that the peak is not an artefact. A concatenation of all reads detected in control libraries is shown.



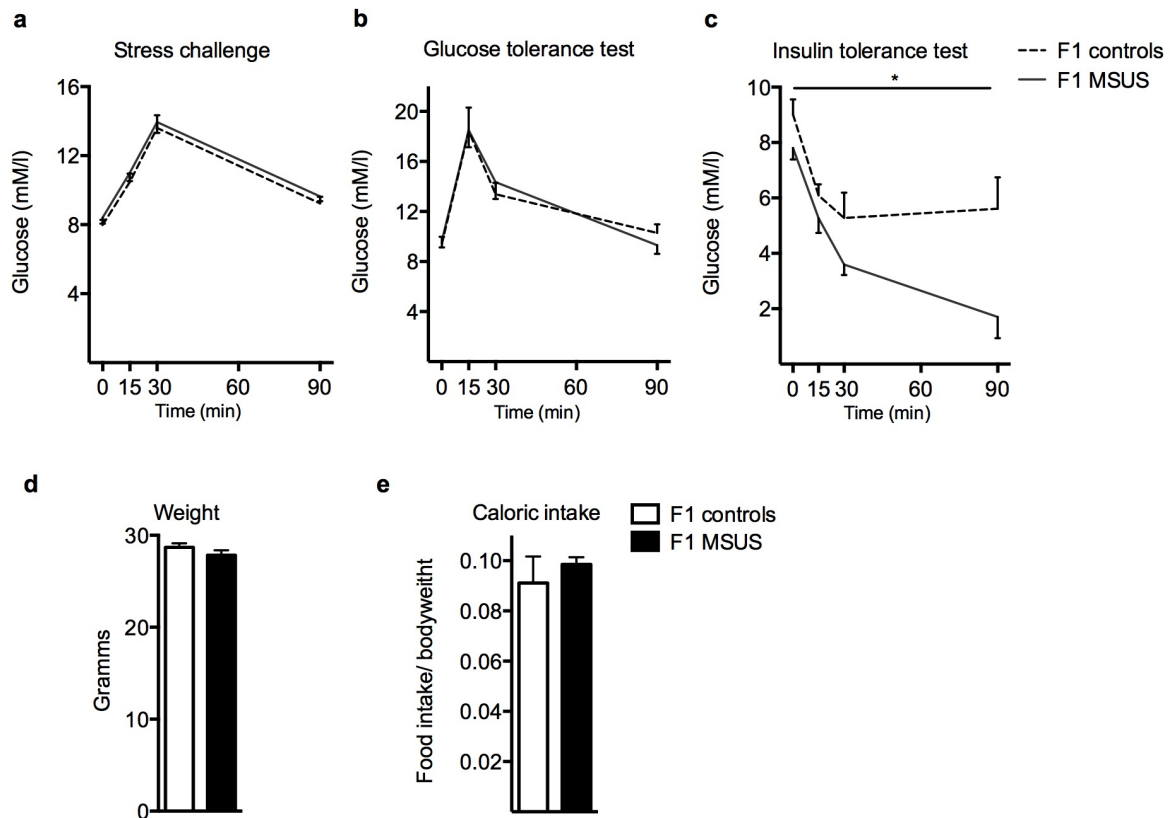
Supplementary Figure 5. Experimental design for MSUS treatment and breeding.

C57Bl/6J females (F0, left) were bred to C57Bl/6J males and their pups were subjected to MSUS from postnatal day (PND) 1 to 14 or raised in normal conditions (Control). Males from the F1 offspring were then bred to naïve C57Bl/6J females to obtain second-generation animals (F2) that were raised in normal conditions (no maternal separation or maternal stress). Animals from F1 and F2 generations were tested behaviorally then bred.

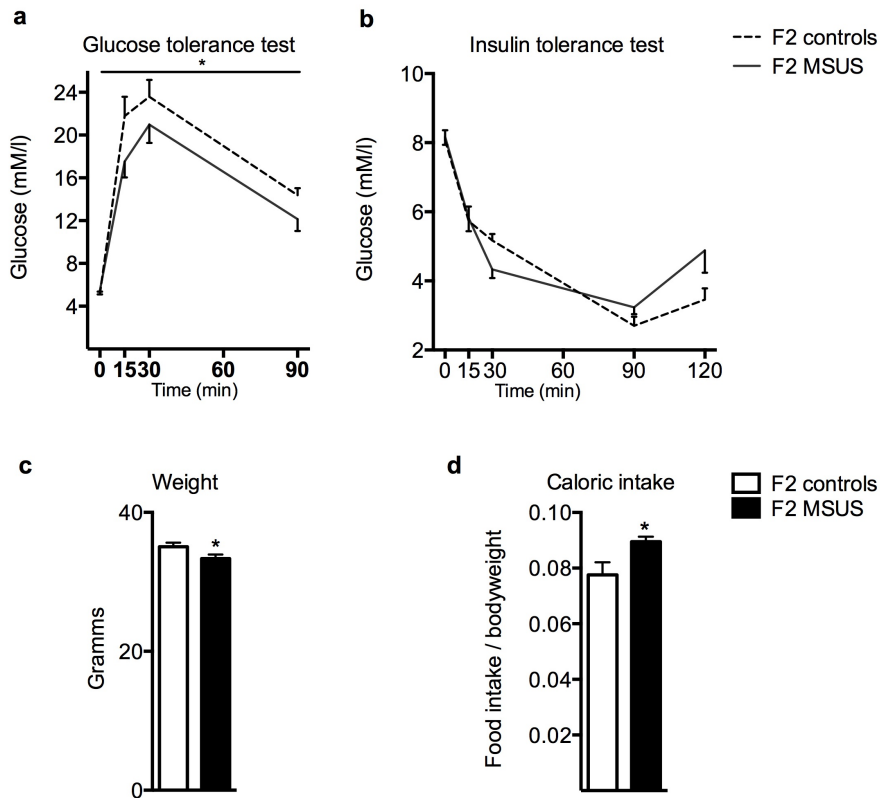
Illustration: University of Zürich informatics services, MELLS, Natasa Milosevic.



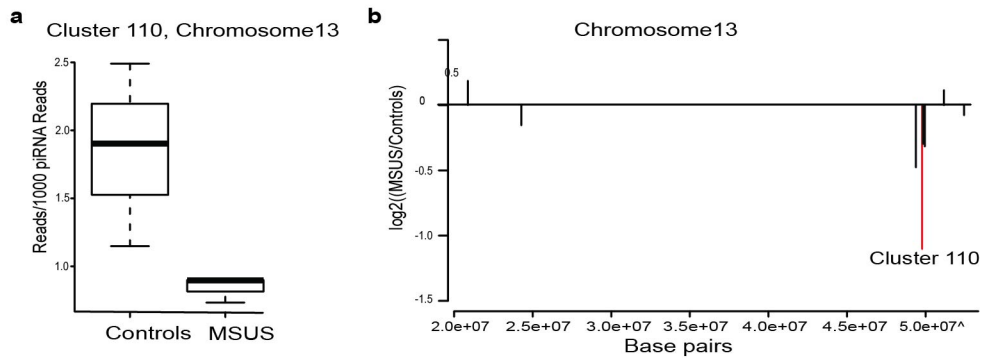
Supplementary Figure 6. Activity on an elevated plus maze. Total distance covered by adult (a) F1 (controls $n=8$, MSUS $n=17$; $t(23)=0.55$), (b) F2 (controls $n=30$, MSUS $n=30$; $t(53)=-1.06$) and (c) RNA-injected (controls-RNAinj $n=18$, MSUS-RNAinj $n=19$, $t(35)=0.18$) animals. Data are mean \pm s.e.m.



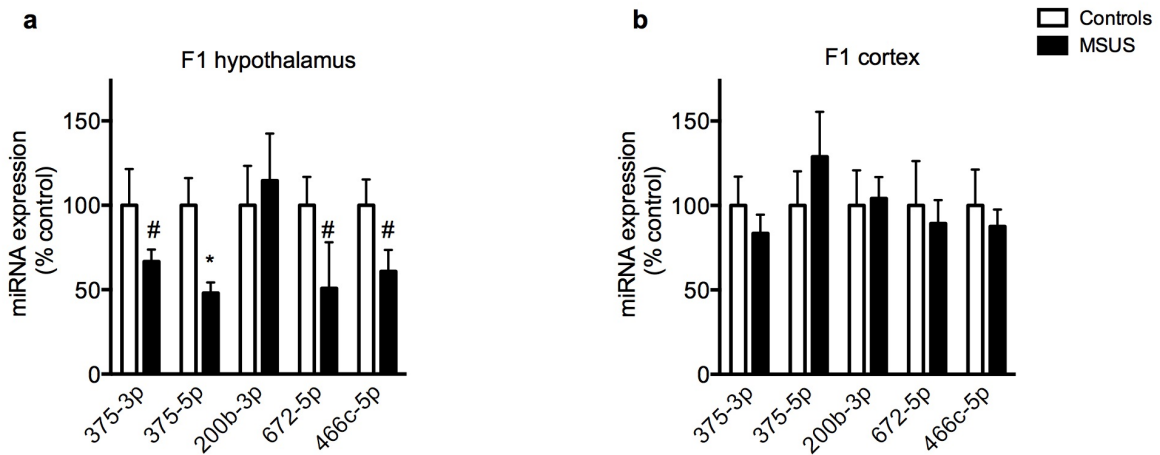
Supplementary Figure 7. Metabolic profile in F1 MSUS animals. (a-c) Glucose level in blood a) at baseline and during a glucose tolerance test (GTT) after an acute restraint stress in non-fasted F1 mice (control, n=8; MSUS, n=8; $F(1,22)=4.26$) b) at baseline and during GTT in fasted F1 mice (control, n=8; MSUS, n=8; $F(1,14)=0.01$) c) at baseline and during an insulin tolerance test (ITT) in fasted F1 mice (control, n=8; MSUS, n=6; $F(1,12)=5.38$). (d) Body weight (control, n=10; MSUS, n=13; $t(21)=1.82$) and (e) caloric intake (control, n=4; MSUS, n=6; $t(8)=-0.81$) in F1 adult animals. Data are mean \pm s.e.m. * $p < 0.05$ group effect repeated measures ANOVA and t-test.



Supplementary Figure 8. Metabolic profile in F2 MSUS animals. (a-b) Glucose level in blood (a) at baseline and during GTT in fasted F2 mice (control, n=8; MSUS, n=8; $F(1,14)=4.71$) and b) at baseline and during ITT in fasted F2 mice (control, n=7; MSUS, n=6; $F(4,44)=3.38$; 0 min: $t(11)=-2.5$, 15 min: $t(11)=-0.15$, 30 min: $t(11)=2.76$, 90 min: $t(11)=-1.58$). (c) Body weight (control, n=13; MSUS, n=11; $t(21)=2.09$) and (d) caloric intake (control, n=6; MSUS, n=6; $t(6.52)=-2.44$) in F2 adult animals. Data are mean \pm s.e.m, * $p<0,05$ group effect repeated measures ANOVA and t-test.

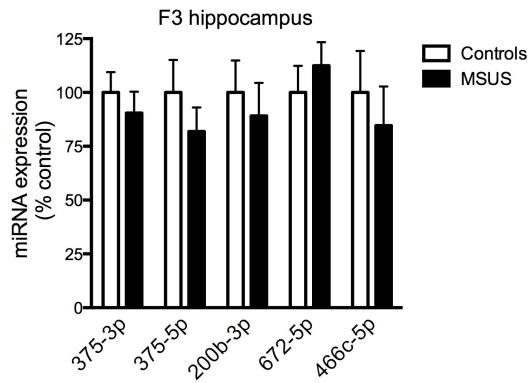


Supplementary Figure 9. Effect of MSUS on piRNAs in adult sperm. (a) Boxplot showing reads aligning to piRNA cluster 110 (on chromosome 13) per 1000 piRNAs reads in control and MSUS samples (negative binomial test $p < 0.1$ after Bonferonni multiple test correction). (b) Log₂ of the ratio of MSUS to control reads aligned to piRNA clusters on chromosome 13 showing that cluster 110 (in red) and two neighboring clusters (in black) are down-regulated in MSUS samples.



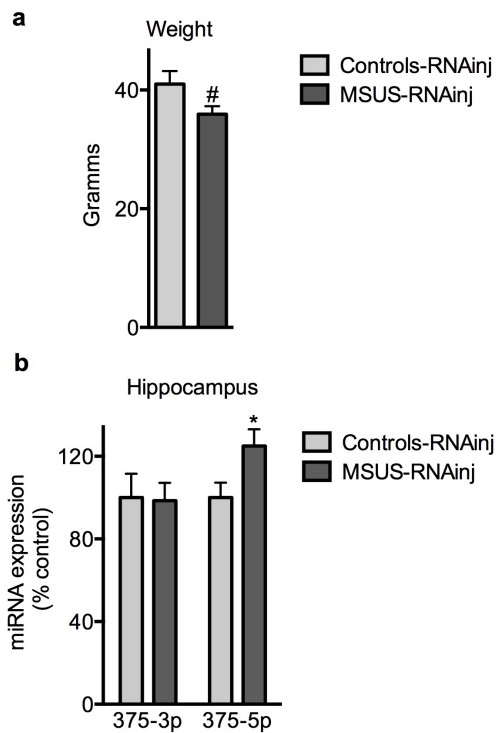
Supplementary Figure 10. MiRNA expression in hypothalamus and cortex in F1 mice.

Level of miRNAs expression in (a) hypothalamus (miR-375-3p: control, n=3; MSUS, n=4; $t(5)=1.68$; miR-375-5p: control, n=3; MSUS, n=4; $t(5)=3.38$; miR-200b-3p: control, n=3; MSUS, n=4; $t(5)=-0.38$; miR-672-5p: control, n=3; MSUS, n=4; $t(6)=2.02$; miR-466c-5p: control, n=3; MSUS, n=4; $t(5)=1.98$) and (b) cortex (miR-375-3p: control, n=4; MSUS, n=4; $t(6)=0.81$; miR-375-5p: control, n=4; MSUS, n=4; $t(6)=-0.86$; miR-200b-3p: control, n=4; MSUS, n=4; $t(6)=-1.17$; miR-672-5p: control, n=4; MSUS, n=4; $t(6)=0.36$; miR-466c-5p: control, n=4; MSUS, n=4; $t(6)=0.53$) in adult F1 control and MSUS males. Data are mean \pm s.e.m, * $p < 0,05$, # $p < 0,1$.

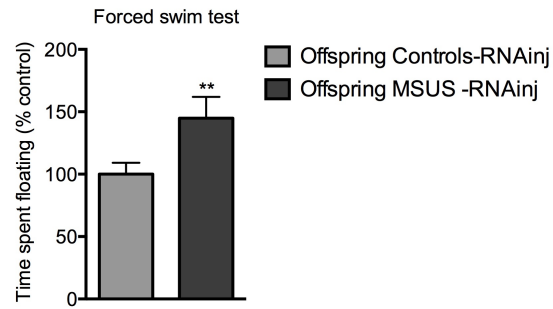


Supplementary Figure 11. MiRNAs expression in the hippocampus of F3 animals.

Similar miRNA expression in F3 control and MSUS adult males (miR-375-3p: control, n=9; MSUS, n=9; $t(16)=0.7$; miR-375-5p: control, n=10; MSUS, n=9; $t(17)=0.95$; miR-200b-3p: control, n=9; MSUS, n=9; $t(16)=0.51$; miR-672-5p: control, n=10; MSUS, n=9; $t(17)=-0.45$; miR-466c-5p: control, n=10; MSUS, n=9; $t(17)=0.58$). Data are mean \pm s.e.m.



Supplementary Figure 12. Analyses in MSUS-RNAinj males. (a) Body weight of adult controls-RNAinj (n=8) and MSUS-RNAinj (n=9) animals ($t(15)=1.9$). (b) Level of miR-375-3p (Controls-RNAinj n=7, MSUS-RNAinj n=8; $t(13)=.10$) and miR-375-5p (controls-RNAinj n=7, MSUS-RNAinj n=7; $t(12)=-2.3$) in the adult hippocampus. Data are mean \pm s.e.m, * $p<0,05$, # $p<0,1$.



Supplementary Figure 13. Performance of the offspring of RNAinj animals on a forced swim test. Time spent floating on the forced swim test in the offspring of Controls-RNAinj (n=19) and MSUS-RNAinj (n=12) animals ($t(29)=-3.369$). Data are mean \pm s.e.m.

Gene name	miRNA																												
	let-7-5p	9-p5	10a-5p	15b-5p	21a-5p	26a-5p	30b-5p	34b-5p	99a-5p	100-5p	103-3p	125a-5p	128-3p	135a-5p	148a-3p	182-5p	183-5p	194-5p	200b-3p	204-5p	342-3p	375-3p	449a-5p	465c-3p	466c-3p	497-5p	532-5p	672-5p	
ACSL4																													
ACVR2A																													
AI314180																													
AK220484																													
AQP11																													
ASH1L*																													
BC030336																													
BCL2																													
CADM2																													
CPEB2*																													
DCX																													
DYRK1B																													
E2F3*																													
EIF2C4*																													
EIF4E2*																													
ELAVL4																													
ELL2*																													
ESRRG																													
FMR1																													
FOXN2*																													
FRS2																													
GMFB																													
HS3ST3B1																													
IL1RAPL1																													
INHBB																													
INO80D*																													
JARID2*																													
LIN28*																													
LIN28B*																													
MACF1																													
MAP3K1																													
MMD																													
MSL2*																													
N4BP1*																													
NARG1																													
NFIA*																													
NR4A2*																													
NTNG1																													
ODZ2																													
OTUD4																													
PCMT1																													
PNPLA6																													
PPARGC1B																													
PRTG																													
PTPRD																													
PWWP2B*																													

Gene name	miRNA																												
	let-7-5p	9-p5	10a-5p	15b-5p	21a-5p	26a-5p	30b-5p	34b-5p	99a-5p	100-5p	103-3p	125a-5p	128-3p	135a-5p	148a-3p	182-5p	183-5p	194-5p	200b-3p	204-5p	342-3p	375-3p	449a-5p	465c-3p	466c-3p	497-5p	532-5p	672-5p	
RASGEF1B																													
RC3H2																													
RFX5 *																													
RNF138																													
RNF38																													
RORA *																													
RSPO3																													
SALL1 *																													
SEMA3A																													
SIRT1 *																													
SLC8A1																													
SOCS6																													
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TAOK1																													
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TNRC6B *																													
UBR3 *																													
WEE1																													
WIPI2																													
ZBTB34 *																													
ZBTB39 *																													
ZFHX4 *																													
ZFP462 *																													
ZFP711*																													
ZFPM2 *																													
ZFYVE26 *																													

Supplementary Table 1. Predicted targets of miRNAs with altered expression in MSUS sperm. Common predicted target genes shared by 3 or 4 miRNAs are indicated by grey boxes. Genes targeted by different miRNAs from the same family are counted as one. * highlights genes with a functional implication in epigenetic regulation, i.e. the histone deacetylase SIRT1, transcriptional control (transcription factors), DNA/RNA binding (zinc-finger proteins), and DNA synthesis and repair (DNA polymerases).