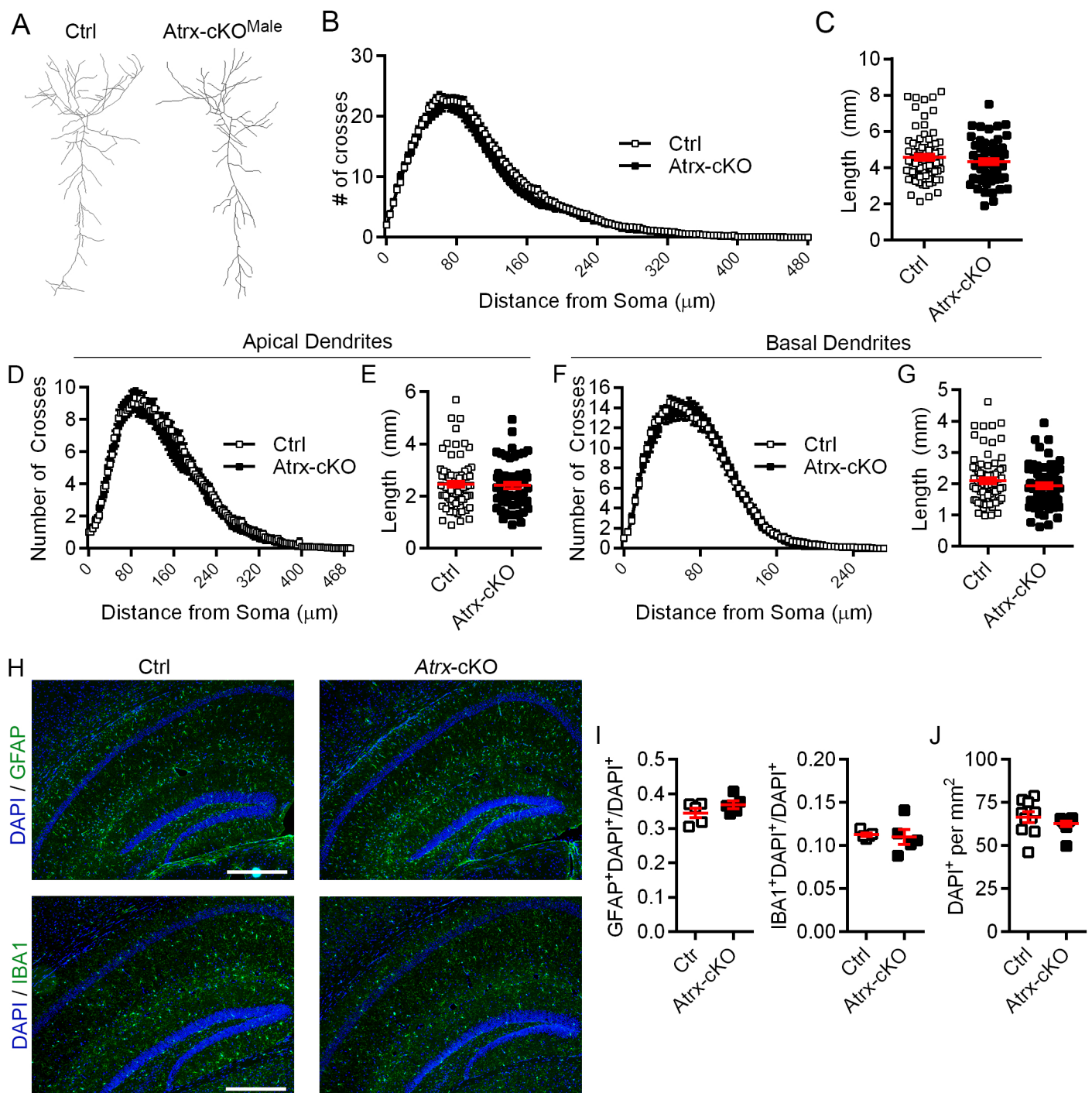# Supplemental Information

Atrx Deletion in Neurons Leads to Sexually

Dimorphic Dysregulation of miR-137

## and Spatial Learning and Memory Deficits

Renee J. Tamming, Vanessa Dumeaux, Yan Jiang, Sarfraz Shafiq, Luana Langlois, Jacob Ellegood, Lily R. Qiu, Jason P. Lerch, and Nathalie G. Bérubé

Figure S1. CA1 dendritic length and branching and the number of non-neuronal cells are not affected in Atrx-cKOMale mouse hippocampi. Related to Figure 1. (A) Representative Golgi traces from CtrlMale and Atrx-cKOMale hippocampal CA1 pyramidal neurons. (B) Sholl analysis of CtrlMale I (n=73) and Atrx-cKOMale (n=53) CA1 pyramidal neurons (p=0.08). (C) Total length of CtrlMale and Atrx-cKOMale CA1 dendrites (p=0.30). (D) Sholl analysis of CtrlMale and Atrx-cKOMale CA1 apical dendrites (p=0.39). (E) Total length of CtrlMale and Atrx-cKOMale CA1 apical dendrites (p=0.78). (F) Sholl analysis of CtrlMale and Atrx-cKOMale CA1 basal dendrites (p=0.73). (G) Total length of CtrlMale and Atrx-cKOMale CA1 basal dendrites (p=0.22). (H) Immunofluorescence staining of GFAP or IBA1 in CtrlMale and Atrx-cKOMale hippocampi. DAPI was used as a counterstain. Scale bar indicates 400 μm. (I) Quantification of the proportion of GFAP+ (p=0.22) and IBA1+ (p=0.79) cells in stratum radiatum/stratum lucidem moleculare of CtrlMale and Atrx-cKOMale hippocampi (n=5). (J) Quantification of DAPI+ cells per mm2 in stratum radiatum/stratum lucidem moleculare of CtrlMale and Atrx-cKOMale hippocampi (n=10, p=0.29). Data was analyzed by unpaired Student's T-Test (C,E,G,I-J) or two-way repeated measures ANOVA (B,D,F) with Sidak's multiple comparisons test, and asterisks indicate p<0.05. Data is displayed as mean +/-SEM.
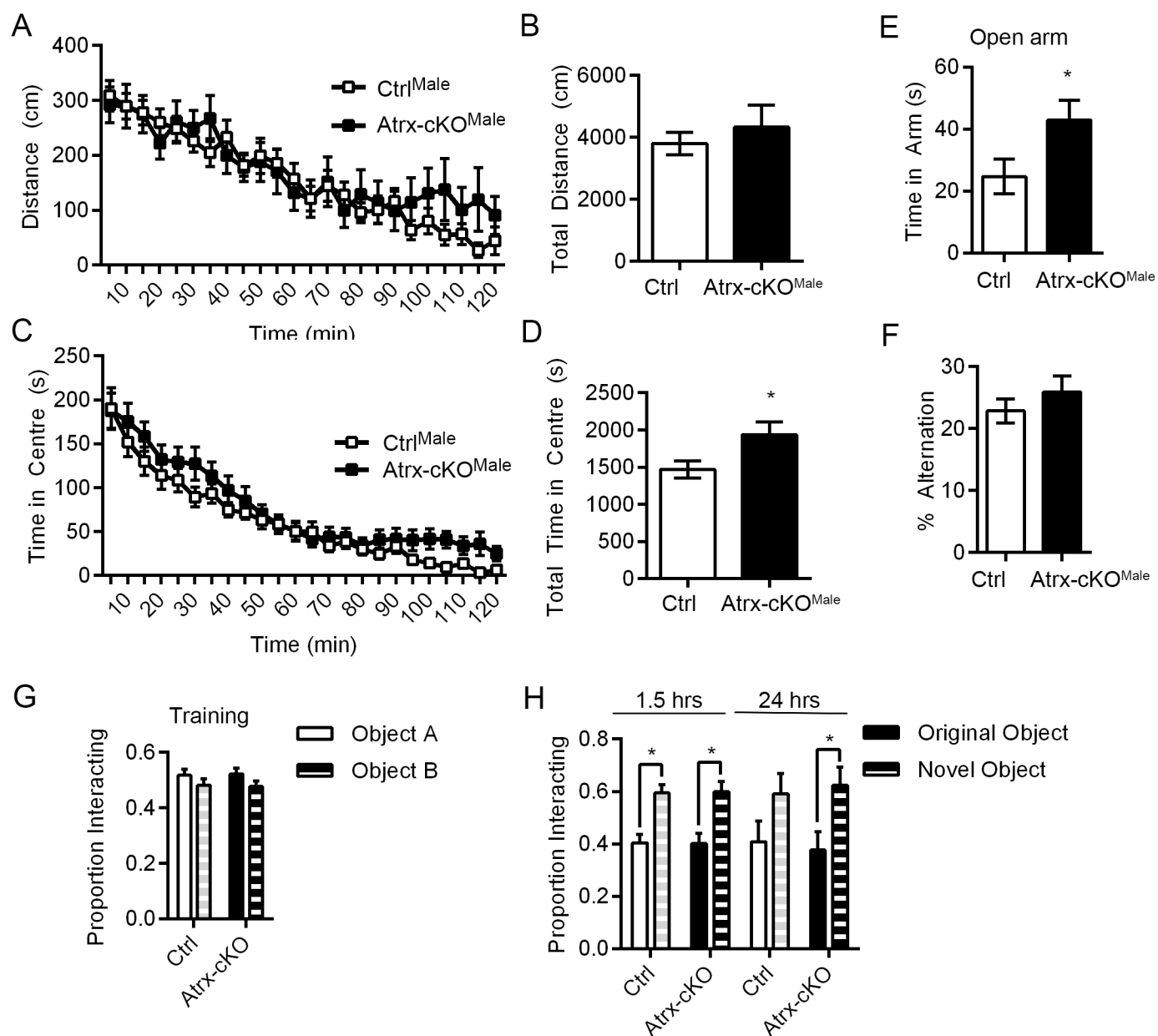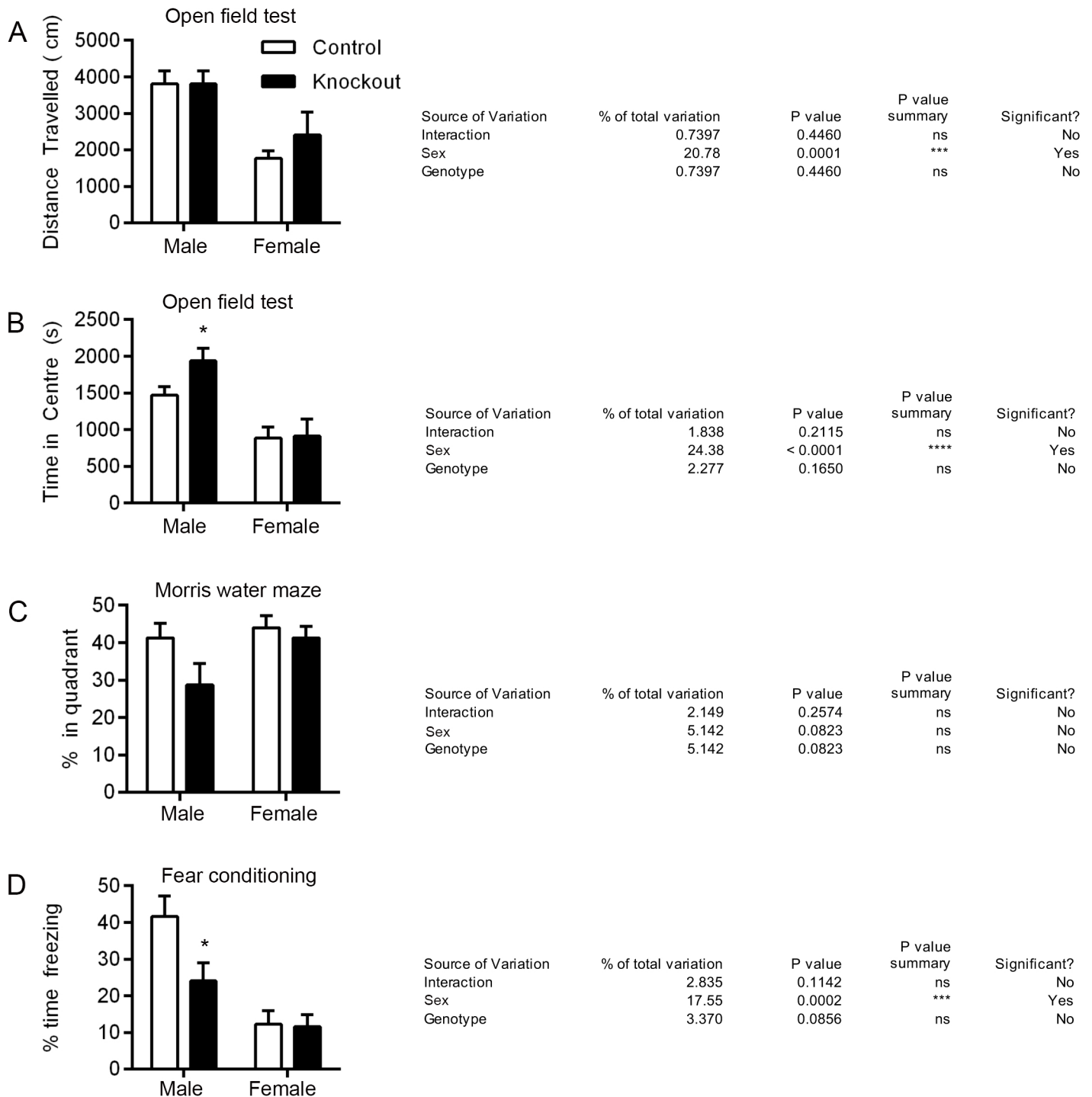
Figure S2. **Atrx-cKOMale mice show decreased levels of anxiety in the open field test and elevated plus maze, but no deficits in working or novel object recognition memory.** **Related to Figure 3.** (A) Distance travelled over 120 minutes in the open field test (p=0.68) (n=22 each genotype). (B) Total distance travelled (p=0.51). (C) Time spent in the center over 120 minutes in the open field test (p=0.09) (n=22 each genotype). (D) Total time spent in the center (p<0.05). (E) Time in the open arm of the elevated plus maze (p<0.05) (n=14 each genotype). (F) Percent alternation in the Y-maze (p=0.35) (n=14 each genotype). (G) Proportion of time interacting with object A or object B in the novel object recognition task with two identical objects (CtrlMale p=0.27, Atrx-cKOMale p=0.11) (n=14 each genotype). (H) Proportion of time interacting with the original object vs a novel object 1.5 hours (CtrlMale p<0.001, Atrx-cKOMale p<0.01) or 24 hours (CtrlMale p=0.11, Atrx-cKOMale p<0.05) after training (n=14 each genotype). Statistics by two-way repeated measures ANOVA with Sidak's multiple comparisons test were applied in A and C and Student's T-test in B,D,E-H. Asterisks indicate p<0.05.
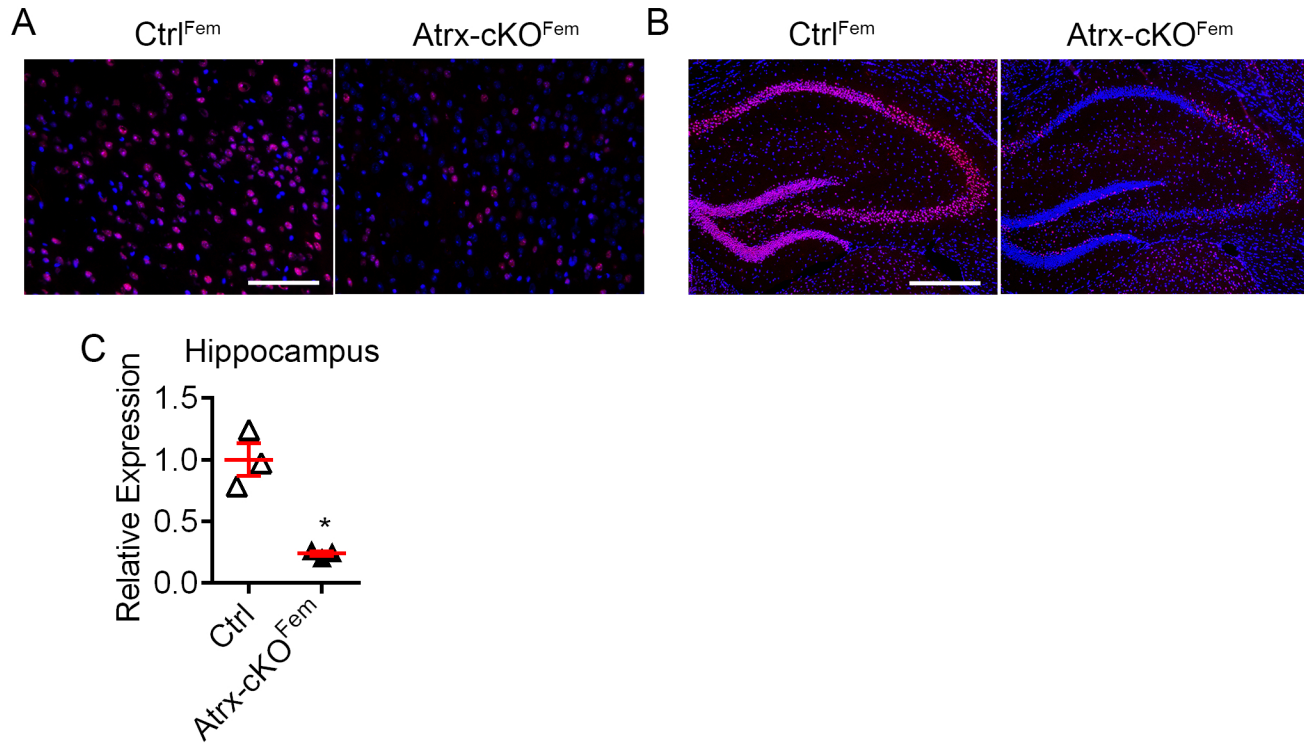
Figure S3. Atrx inactivation in Atrx-cKOFem mice. Related to Figure 3. (A) Immunofluorescence in cortex of control (CtrlFem) and knockout (Atrx-cKOFem) female mice. ATRX: red; DAPI: blue. Scale bar: 50 μm. (B) Immunofluorescence in hippocampus of CtrlFem and Atrx-cKOFem mice. ATRX: red; DAPI: blue. Scale bar: 200 μm. (C) Expression of hippocampal Atrx RNA transcripts as measured by qRT-PCR (n=4 each genotype) (p<0.005; unpaired Student's T-test).
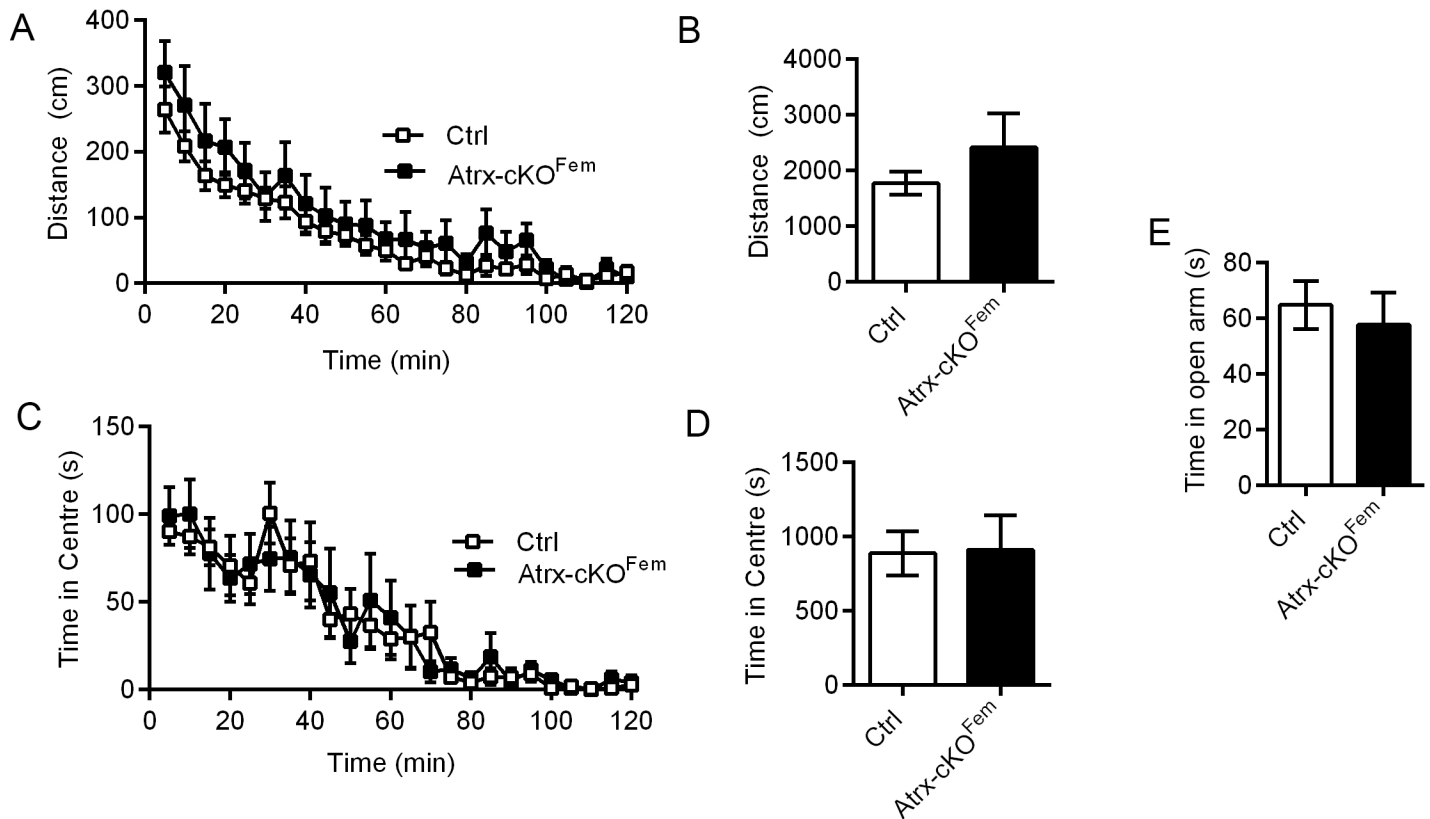
Figure S4: Atrx-cKOFem mice display normal anxiety levels in the open field test and the elevated plus maze. Related to Figure 3. (A) Distance travelled over 120 minutes in the open field test. p=0.28, two-way repeated measures ANOVA with Sidak's multiple comparisons test. (B) Total distance travelled (p=0.28, Student's T-test). (C) Time spent in the center over 120 minutes in the open field test. p=0.93, two-way repeated measures ANOVA with Sidak's multiple comparisons test. CtrlFem n=12, Atrx-cK-OFem n=9). (D) Total time spent in the center (p=0.93, Student's T-test). (E) Time spent in the open arm of the elevated plus maze. p=0.63, Student's T test. CtrlFem n=15, Atrx-cKOFem n=12.

A

Open field test

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	0.7397	0.4460	ns	No
Sex	20.78	0.0001	***	Yes
Genotype	0.7397	0.4460	ns	No

B

Open field test

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	1.838	0.2115	ns	No
Sex	24.38	< 0.0001	****	Yes
Genotype	2.277	0.1650	ns	No

C

Morris water maze

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	2.149	0.2574	ns	No
Sex	5.142	0.0823	ns	No
Genotype	5.142	0.0823	ns	No

D

Fear conditioning

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	2.835	0.1142	ns	No
Sex	17.55	0.0002	***	Yes
Genotype	3.370	0.0856	ns	No

Figure S5. Statistical analysis of behaviour data to identify sex or genotype differences. Related to Figure 3. Two-way ANOVA analyses of CtrlMale, Atrx-cKOMale, CtrlFem and Atrx-cKOFem mice in behavioural tests. (A) Distance travelled in the open field test (B) Time spent in center during the open field test (C) Percent time spent in the target quadrant on probe day 12 of the Morris water maze test and (D) Percent time spent freezing 24 hours after fear conditioning test. Asterisks indicate significance p<0.05 using Benjamini and Hochberg multiple comparison testing.
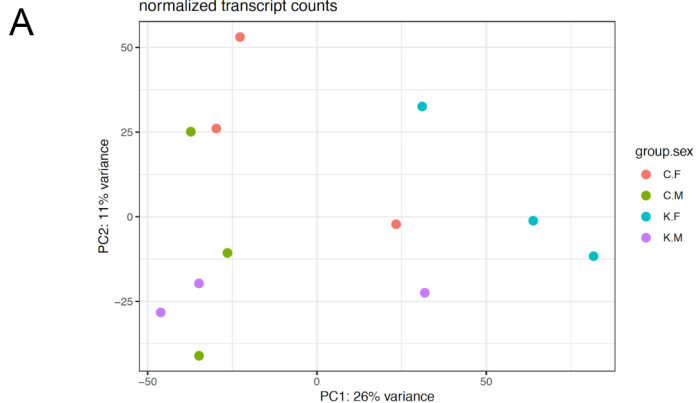
Figure S6. Details of RNA-sequencing analysis. Related fo Figure 5. (A) PCA plot of all RNAseq data. (B) Table summarizing the number of transcripts differentially expressed with condition (KO vs control or male vs female in each relevant group). In red, number of transcripts differentially expressed in both groups (with similar directionality; up=upregulated and dn=downregulated).
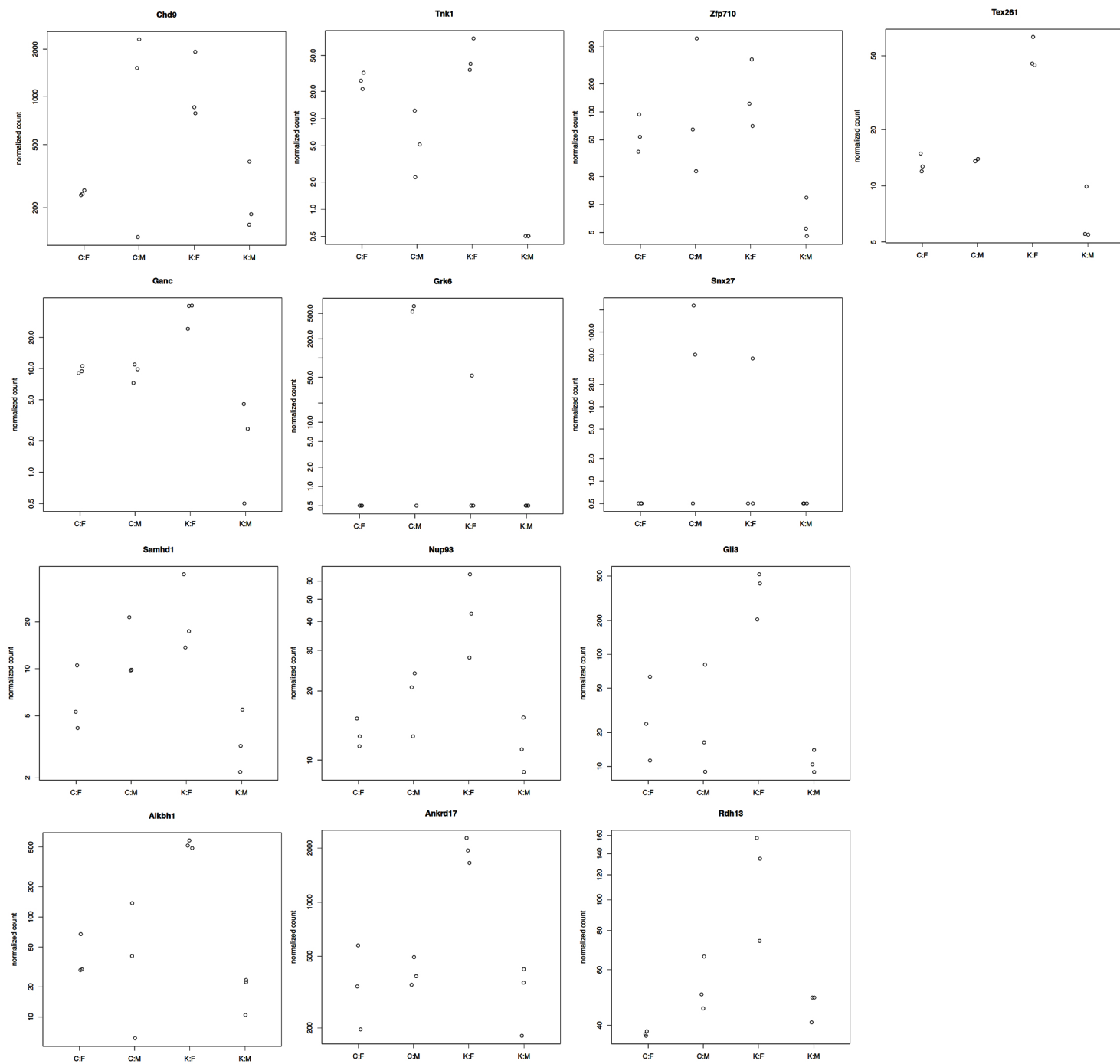
Figure S7. Additional examples of transcripts identified by RNA-sequencing that exhibit a sex and genotype interaction and that are predicted miR-137 targets. Related to Figure 6 and Table S3. CF:CtrlFem, CM:CtrlMale, KF: Atrx-cKOFem, KM: Atrx-cKOMale. Full gene list in Table S3.