

Sex-specific role for the long non-coding RNA LINC00473 in depression

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Supplemental items inventory

Supplementary Tables

Supplementary Table S1. Demographics of the human postmortem RNA-seq study cohort.

Supplementary Table S2. Demographics of the human postmortem subjects used for FISH study.

Supplementary Table S3. List of custom oligonucleotides used in the study

Supplementary Figures

Figure S1. Replication of the sex-specific pattern of lncRNA regulation in depression.

Figure S2. LINC00473 is enriched within glutamatergic neuronal nuclei in PFC.

Figure S3. LINC00473 expression in mPFC: validation and additional phenotyping.

Figure S4. No effect of LINC00473 expression on membrane excitability of mPFC pyramidal neurons in both sexes.

Figure S5. LINC00473 regulation by forskolin and its predicted binding motifs.

Supplementary Tables

Supplementary Table S1. Demographics of the human postmortem RNA-seq study cohort. Related to Figure 1.

RIN, RNA integrity number; PMI, postmortem interval; CA, history of child abuse or other early life adversity; AD, antidepressants.

Subject	Sex	Age	Race	RIN	PMI	pH	CA	AD	Alcohol	Drugs
CTRL1	F	44	C	6.8	29.5	6.7	N/A	no	yes**	no
CTRL2	F	79	C	6.3	7.5	6.4	no	yes*	no	no
CTRL3	F	72	C	5.9	17	6.1	no	no	no	no
CTRL4	F	45	C	7.2	44	6.5	yes	yes*	no	no
CTRL5	F	82	C	7.0	106	7.0	N/A	no	no	no
CTRL6	F	60	C	7.8	38	6.7	yes	no	yes**	no
CTRL7	F	22	C	7.0	2	7	no	yes*	no	no
CTRL8	F	68	C	7.7	8.5	6.2	N/A	no	no	no
CTRL9	F	51	C	7.7	16	6.7	yes	no	no	no
CTRL1	M	47	C	7.6	12	6.5	no	no	no	no
CTRL2	M	41	C	7.1	24	6	no	no	no	no
CTRL3	M	31	C	7.2	29.5	6.7	no	no	no	no
CTRL4	M	19	C	6.6	27.7	6.7	N/A	no	no	no
CTRL5	M	46	C	7.3	19.5	6.4	no	no	no	no
CTRL6	M	40	C	5.9	29.7	6.3	N/A	no	no	no
CTRL7	M	33	C	7.5	18	6.7	yes	no	no	no
CTRL8	M	38	C	7.0	44.5	6.8	N/A	no	no	no
CTRL9	M	55	C	6.5	24	6.7	yes	no	no	no
CTRL10	M	46	C	6.9	59	6.8	N/A	no	no	no
CTRL11	M	52	C	6.0	10	6.1	N/A	no	no	no
CTRL12	M	29	C	6.9	4	6.4	N/A	no	no	no
CTRL13	M	59	C	7.9	23.5	6.8	N/A	no	no	no
MDD1	F	25	C	6.6	20	6.7	N/A	no	no	no
MDD2	F	46	C	7.1	15	6.5	N/A	yes	yes**	no
MDD3	F	55	C	7.0	36	6.8	no	no	no	no
MDD4	F	40	DNK	7.0	49.5	6.8	N/A	yes	no	no
MDD5	F	25	C	6.6	56	6.5	N/A	no	no	no
MDD6	F	54	C	7.4	28.5	6.8	yes	yes	no	no
MDD7	F	32	C	7.2	41	6.9	N/A	no	no	no
MDD8	F	55	C	7.3	2.5	6.5	yes	no	no	no
MDD9	F	41	C	6.8	12	6.7	N/A	no	no	no
MDD10	F	48	C	7.4	17.5	6.5	N/A	yes	no	no
MDD11	F	36	C	7.5	7.5	6.9	no	yes	no	no
MDD12	F	52	C	7.5	4.5	6.5	N/A	yes	no	no
MDD13	F	59	C	8.1	3	6.3	no	yes	no	no
MDD1	M	49	C	7.4	32	6.6	no	no	no	no
MDD2	M	53	C	7.2	33.5	6.9	yes	no	no	no
MDD3	M	39	C	5.4	19	6	yes	yes	no	no
MDD4	M	38	C	7.3	30	6.9	N/A	no	no	no
MDD5	M	22	C	7.2	24	6.7	N/A	yes	no	no
MDD6	M	28	C	5.9	36	6.9	N/A	no	no	no

MDD7	M	29	C	6.8	27	7.0	no	no	no	no
MDD8	M	68	C	8.0	32	6.9	N/A	no	no	no
MDD9	M	39	C	7.5	18.5	6.4	yes	yes	no	no
MDD10	M	63	C	7.2	50	6.9	N/A	no	no	no
MDD11	M	48	C	7.1	49	6.6	no	no	no	no
MDD12	M	67	C	7.1	56	6.8	N/A	no	no	no
MDD13	M	64	C	7.5	6.5	6.2	no	yes	no	no

*Antidepressants were used by these individuals to help with sleep.

**Positive for alcohol in a postmortem toxicology screen, but did not have a substance use disorder.

RIN, age, AD and alcohol were used as covariates in the statistical models for the original analysis of the RNA-seq data (Labonté et al., 2017) on which the present study was based initially.

Supplementary Table S2. Demographics of the human postmortem subjects used for FISH study. Related to Figure 2.

RIN, RNA integrity number; PMI, postmortem interval; AD, antidepressants.

Subject	Sex	Age	Race	RIN	PMI	pH	AD
CTRL1	F	15	C	8.9	25.0	6.3	no
CTRL2	F	23	C	8.8	17.0	6.2	no
CTRL3	F	45	C	8.4	17.3	5.9	no
CTRL4	F	45	C	8.4	22.5	6.9	no
CTRL5	F	50	C	6.2	23.3	6.0	no
CTRL6	F	53	L	7.6	23.0	6.3	no
CTRL7	F	60	C	7.9	20.3	6.4	no
CTRL8	F	62	C	5.9	19.4	5.9	no
CTRL9	F	68	C	8.8	16.6	6.4	no
CTRL10	F	61	C	8.3	20.6	6.0	no
CTRL1	M	13	C	8.4	25.0	6.7	no
CTRL2	M	19	C	9.0	20.0	6.6	no
CTRL3	M	20	C	8.2	21.2	6.8	no
CTRL4	M	46	C	10.0	22.0	6.4	no
CTRL5	M	48	C	10.0	14.7	6.8	no
CTRL6	M	51	C	7.8	19.0	6.6	no
CTRL7	M	54	C	10.0	19.3	6.7	no
CTRL8	M	55	C	8.6	24.0	6.6	no
CTRL9	M	59	C	8.7	22.2	6.4	no
CTRL10	M	69	C	8.2	24.3	6.5	no
MDD1	F	14	C	9.3	17.0	6.8	no
MDD2	F	18	C	8.9	23.2	6.4	no
MDD3	F	38	C	9.0	18.0	6.8	no
MDD4	F	41	C	6.1	21.6	5.7	no
MDD5	F	53	C	8.0	13.4	6.0	yes
MDD6	F	58	C	6.3	14.0	6.4	no
MDD7	F	61	C	8.9	22.0	6.7	yes
MDD8	F	66	C	8.1	19.2	6.8	yes
MDD9	F	66	C	4.0	17.0	5.4	yes
MDD10	F	65	L	7.4	13.4	6.0	no
MDD1	M	18	C	8.5	21.1	6.7	no
MDD2	M	17	C	9.1	21.5	7.0	yes
MDD3	M	20	C	7.6	17.3	6.7	no
MDD4	M	44	C	9.1	19.5	6.1	yes
MDD5	M	53	C	9.3	21.3	6.8	yes
MDD6	M	53	C	8.1	24.5	6.4	yes
MDD7	M	53	C	7.9	24.0	6.1	no
MDD8	M	56	C	8.3	23.0	7.0	yes
MDD9	M	56	C	7.4	18.0	6.3	yes
MDD10	M	69	C	8.4	17.6	6.2	no

**Supplementary Table S3. List of custom oligonucleotides used in the study.
Related to Key Resource Table.**

Name	Sequence	Use
LINC00473 Forward primer	GCATACTTTGGCGGACCTTTT	qPCR
LINC00473 Forward primer	GCATACTTTGGCGGACCTTTT	qPCR
LINC00473 Revers primer	TGTGCCTCCCTGTGAATTCTC	qPCR
Human HPRT1 Forward primer	GACTAATTATGGACAGGACTGAACGTC	qPCR
Human HPRT1 Revers primer	TCTCCTTCATCACATCTCGAGC	qPCR
Mouse HPRT1 Forward primer	GCAGTACAGCCCCAAAATGG	qPCR
Mouse HPRT1 Revers primer	GGTCCTTTTCACCAGCAAGCT	qPCR
GFP Forward primer	CATGCCCGAAGGCTACGT	qPCR
GFP Revers primer	CGATGCCCTTCAGCTCGAT	qPCR
1_LINC00473_ChIRP	GTGCTTGTGCTCTCAGGAAC	ChIRP
2_LINC00473_ChIRP	CAGCAACTTCGGACTCAGAC	ChIRP
3_LINC00473_ChIRP	ATCTTCTCGAAAAGGCGAG	ChIRP
4_LINC00473_ChIRP	AACTGCGCAAAGCAAGTTGC	ChIRP
5_LINC00473_ChIRP	AAGTATGCTGACGCGCATAT	ChIRP
6_LINC00473_ChIRP	CGCAGTTTTTCATCGTGATG	ChIRP
7_LINC00473_ChIRP	AGGCCGAGCATAAAGTAGTA	ChIRP
8_LINC00473_ChIRP	CAGGGTTGGCCCAAATAAAC	ChIRP
9_LINC00473_ChIRP	TCCGCTTTGCATTCAGAATA	ChIRP
10_LINC00473_ChIRP	GTAAACCTTACACCGTGACA	ChIRP
11_LINC00473_ChIRP	GAGAATCCCGCACAAACCAAG	ChIRP
12_LINC00473_ChIRP	GAAAACCCGTCAGAAGGAGG	ChIRP
13_LINC00473_ChIRP	TATGACTTGGGTTCTTCTGG	ChIRP
1_LINC00473_ASO	TTTACTAGGTGTCCGC	KD
2_LINC00473_ASO	CCGAGCATAAAGTAGT	KD
3_LINC00473_ASO	TGTAGTGTCTTTAGTA	KD
4_LINC00473_ASO	GAGAACTGCGCAAAGC	KD
5_LINC00473_ASO	ACATGCAGTGGAAACT	KD

Supplementary Figures

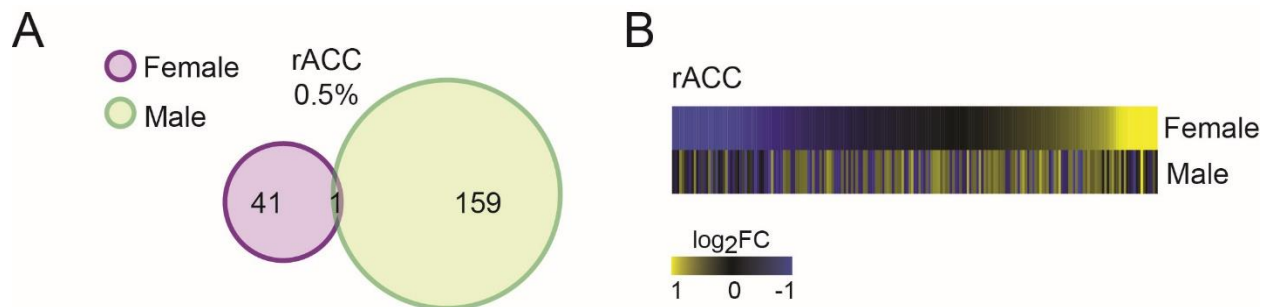
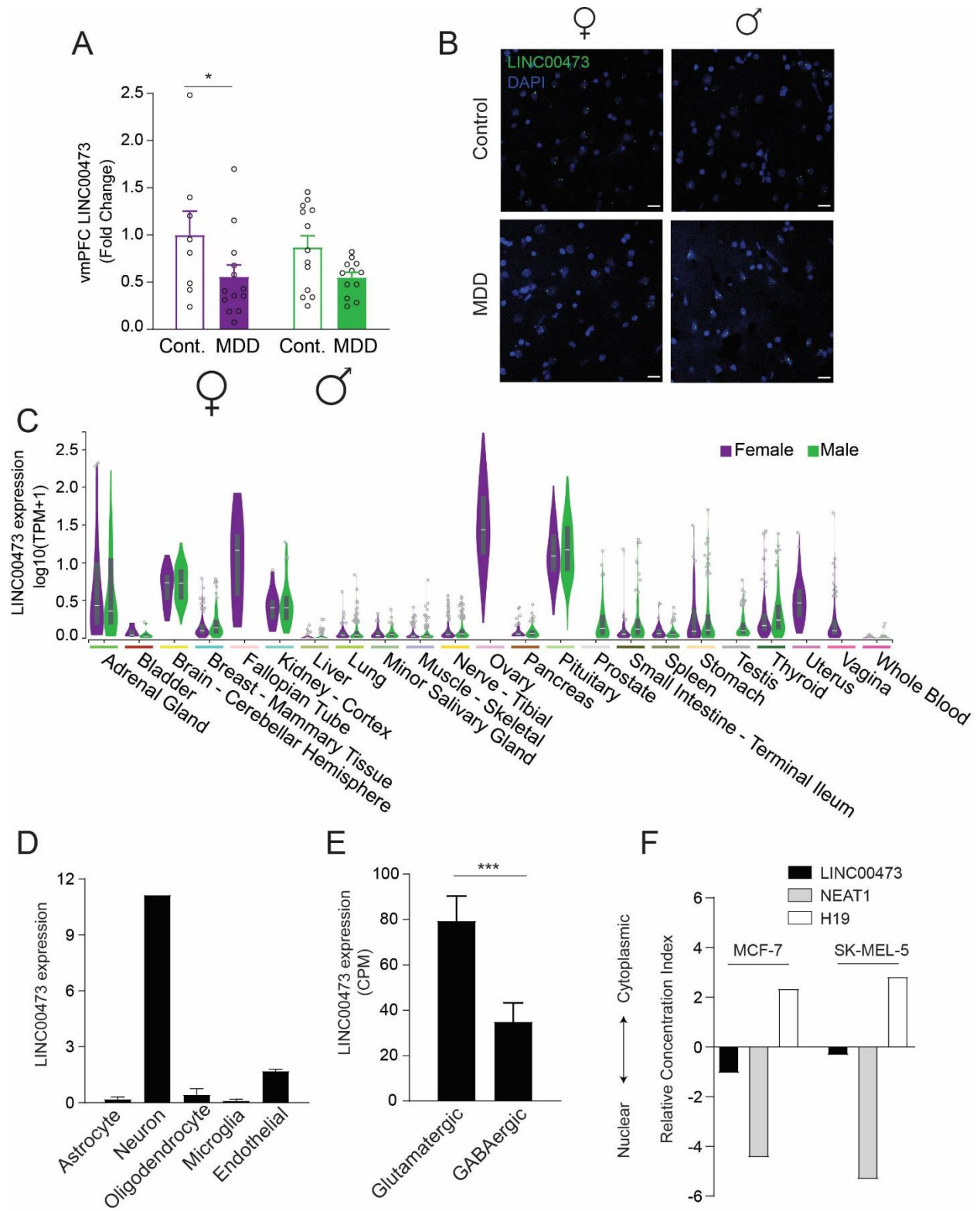


Figure S1. Replication of the sex-specific pattern of lncRNA regulation in depression. Related to Figure 1.

Venn diagram (A) and union heatmap (B) representing the low overlap between lncRNAs regulated in anterior cingulate cortex (rACC) of depressed female subjects compared to depressed males. Data analyzed from (Zhou et al., 2018). N= 5-7 in female, n=19 per group for male. Fold change defined by $P < 0.05$ $FC > 30\%$.



**Figure S2. LINC00473 is enriched within glutamatergic neuronal nuclei in PFC.
Related to Figure 2.**

(A) qPCR validation of LINC00473 downregulation in samples used for RNA-seq with stronger effect in females. N=9-13 per group. (B) Representative LINC00473 FISH images from rACC of depressed and control subjects from both sexes. Green, LINC00473; blue, Dapi. Scale bar=20 μ M. (C) LINC00473 expression profile across human tissue is similar between the sexes and is detected in brain. Data modified from (GTEx, 2013). (D) LINC00473 is expressed in human brain predominantly within neurons. Data modified from (Zhang et al., 2016). (E) LINC00473 is enriched in glutamatergic neurons compared to GABAergic neurons in human PFC. Data modified from (Kozlenkov et al., 2016). (F) LINC00473 is enriched in the nucleus of MCF-7 and SK-ML-5 cell lines. H19, cytoplasmic marker; NEAT1, nuclear marker. Data modified from LncATLAS (Mas-Ponte et al., 2017). **P<0.01, ***P<0.001.

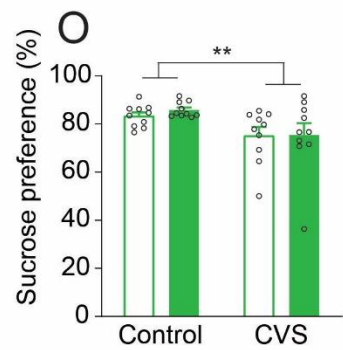
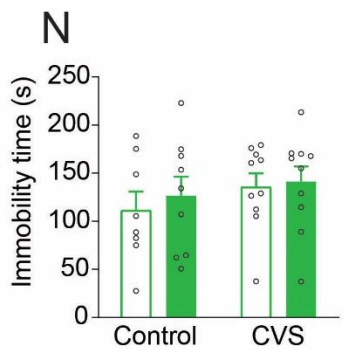
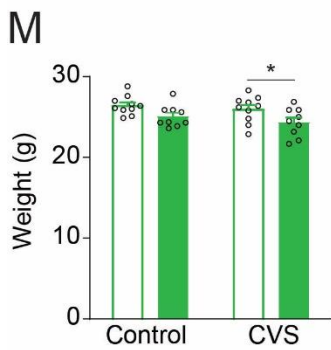
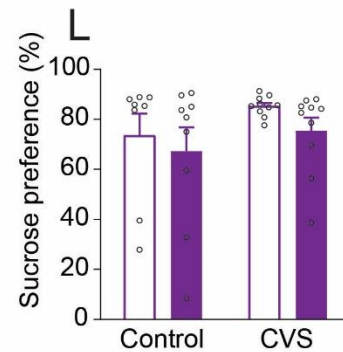
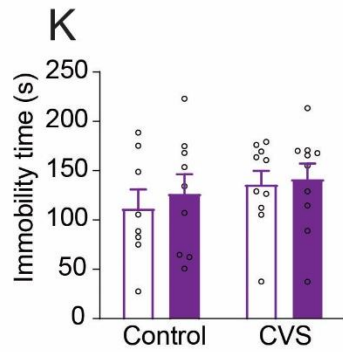
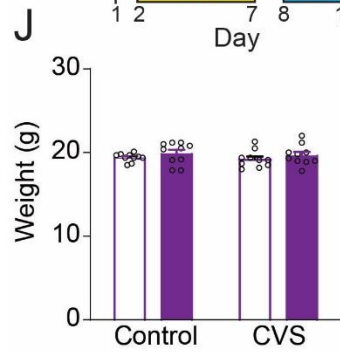
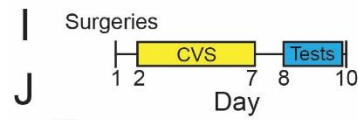
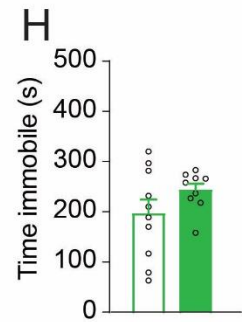
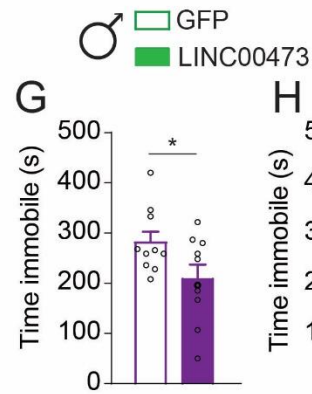
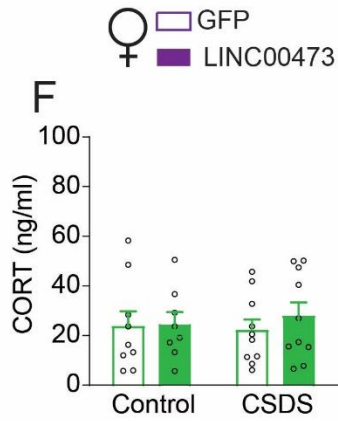
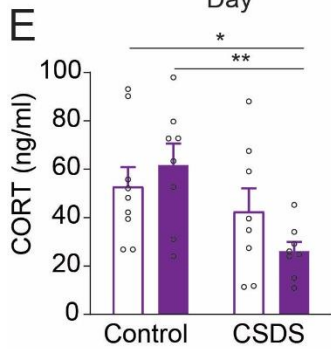
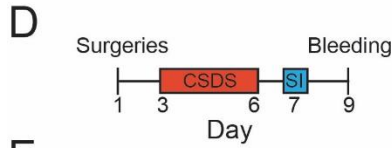
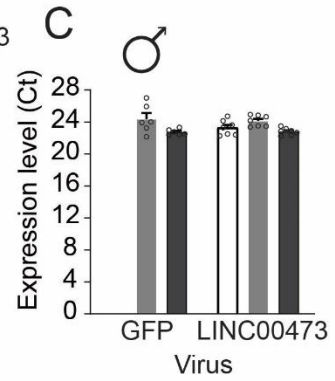
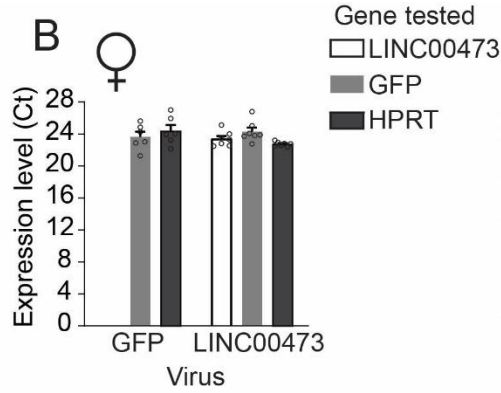
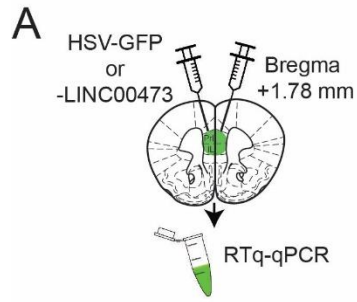


Figure S3. LINC00473 expression in mPFC: validation and additional phenotyping. Related to Figure 3.

(A) Schematic representation of LINC00473 validation experiment. (B-C) Validation of LINC00473 expression in mPFC of female (B) and male (C) mice. Only mice receiving the LINC00473 virus express it and to similar levels (based on C_t values) as GFP and the control gene *Hprt1*. N=6-7 per group. This was confirmed in our RNA-seq dataset (not shown). (D,I) Schematic representations of experimental designs. (E-F) Female (E), but not male (F) mice exposed to CSDS and expressing LINC00473 had lower levels of serum CORT compared to control mice. N=8-10 per group. (G,H) LINC00473 expression decreased immobility in the tail suspension test in female (G) but not male (H) mice. N=9-10 per group. (J-K) No effects of LINC00473 expression in females in weight (J), forced swim test (K), and the sucrose preference test (L). N=8-10 per group. (M) CVS led to decrease in body weight in male mice expressing LINC00473. (N) No effects of LINC00473 expression in males in the forced swim test. (M) Male mice exposed to CVS had lower levels of sucrose preference compared to control mice, regardless of viral treatment. N= 8-10 per group. ** $P < 0.01$. Bar graphs show mean \pm SEM.

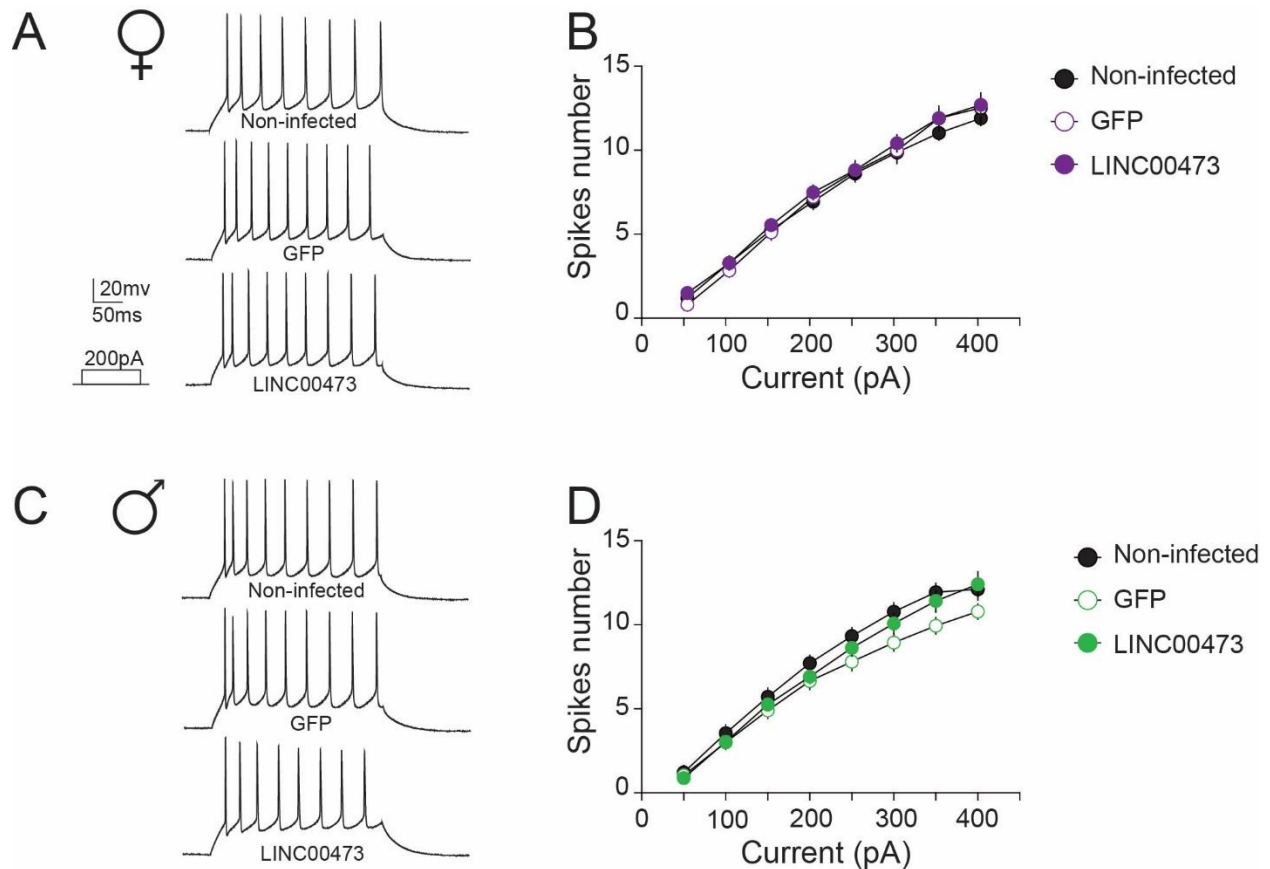
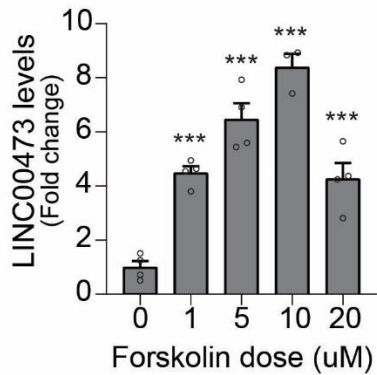


Figure S4. No effect of LINC00473 expression on membrane excitability of mPFC pyramidal neurons in both sexes. Related to Figure 4.

Representative recordings from current injection into non-infected (top), GFP-expressing (middle), or LINC00473/GFP-expressing (bottom) mPFC pyramidal neurons from female (A) and male (C) mice. No differences in membrane excitability between the groups in females (B; N=5-10 per group) and males (D; N=3-8 per group). Circles show mean \pm SEM.

A



B

LINC00473 DMSO	Rank	Motif	P-value	% of targets	% of background
	1		1e-8	15.22%	0.49%
	2		1e-7	10.87%	0.22%
	3		1e-7	13.04%	0.46%

C

LINC00473 forskolin	Rank	Motif	P-value	% of targets	% of background
	1		1e-8	15.00%	0.28%
	2		1e-5	7.50%	0.06%
	3		1e-5	7.50%	0.09%

Figure S5. LINC00473 regulation by forskolin and its predicted binding motifs. Related to Figure 7.

(A) qPCR data from a dose response test of LINC00473 upregulation by forskolin in SH-SY5Y cells. *** $P < 0.001$. Bar graphs show means \pm SEM. (B-C) Top de novo predicted DNA motifs for binding of LINC00473 in the DMSO (B) or forskolin (C) conditions. The table includes indication of statistical significance and the percentage of targets from LINC00473 ChIRP or genomic background, which include the motif.