

## Supplemental Materials

### Estrogen Receptor- $\alpha$ in Medial Amygdala Neurons Regulates Body Weight

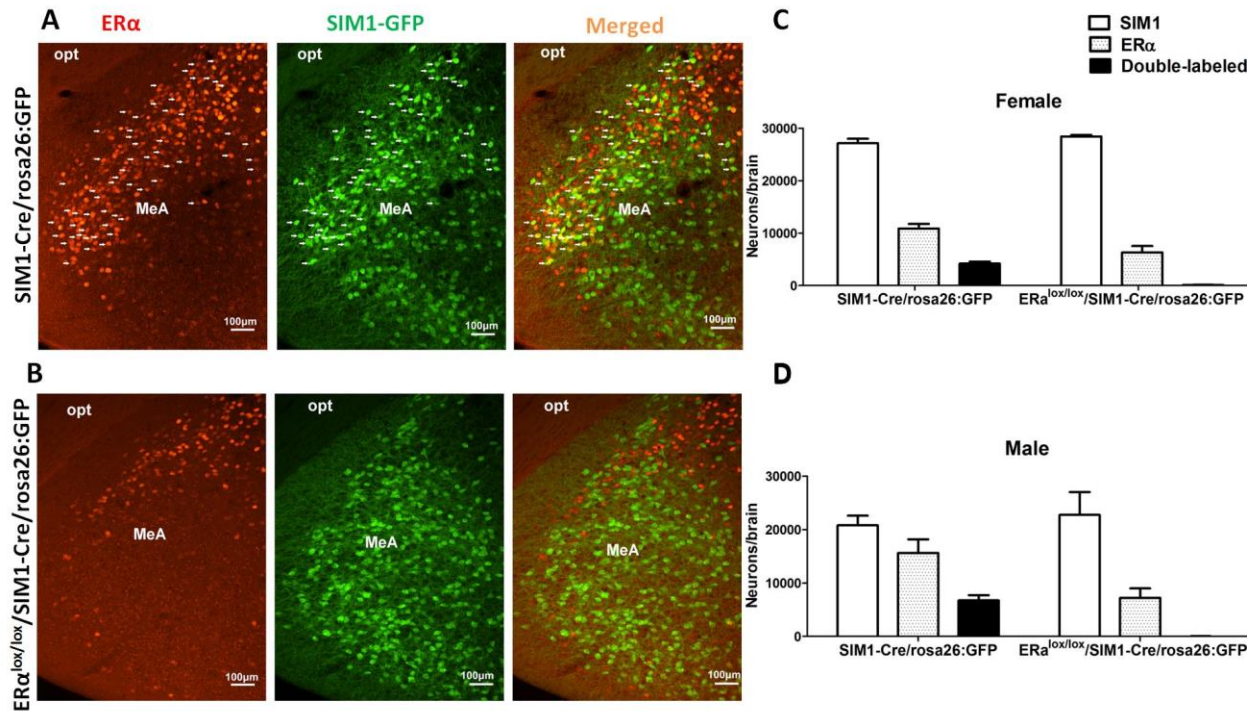
Pingwen Xu<sup>1,9</sup>, Xuehong Cao<sup>1,9</sup>, Yanlin He<sup>1</sup>, Liangru Zhu<sup>1,2</sup>, Yongjie Yang<sup>1</sup>, Kenji Saito<sup>1</sup>, Chunmei Wang<sup>1</sup>, Xiaofeng Yan<sup>1</sup>, Antentor Othrell Hinton Jr.<sup>1</sup>, Fang Zou<sup>1</sup>, Hongfang Ding<sup>1</sup>, Yan Xia<sup>1</sup>, Chunling Yan<sup>1</sup>, Gang Shu<sup>1</sup>, San-Pin Wu<sup>3</sup>, Bin Yang<sup>4</sup>, Yuxin Feng<sup>5</sup>, Deborah J. Clegg<sup>6</sup>, Richard DeMarchi<sup>4</sup>, Sohaib A. Khan<sup>7</sup>, Sophia Y. Tsai<sup>3</sup>, Francesco J. DeMayo<sup>3</sup>, Qi Wu<sup>1</sup>, Qingchun Tong<sup>8</sup>, Yong Xu<sup>1,3\*</sup>

<sup>1</sup>Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030; <sup>2</sup>Department of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, People's Republic of China; <sup>3</sup>Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; <sup>4</sup>Department of Chemistry, Indiana University, Bloomington, IN 47405; <sup>5</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, OH; <sup>6</sup>Department of Biomedical Research, Diabetes and Obesity Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA; <sup>7</sup>Department of Cell and Cancer Biology, Vontz Center for Molecular Studies, University of Cincinnati, College of Medicine, Cincinnati, OH 45267; <sup>8</sup>Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030.

<sup>9</sup>These authors equally contributed to this work.

\*Correspondence should be addressed to: 1100 Bates Street, Rm 8070, Houston, Texas 77030. E-mail: yongx@bcm.edu, Telephone: (713)-798-7199, Fax: (713)-798-7187.

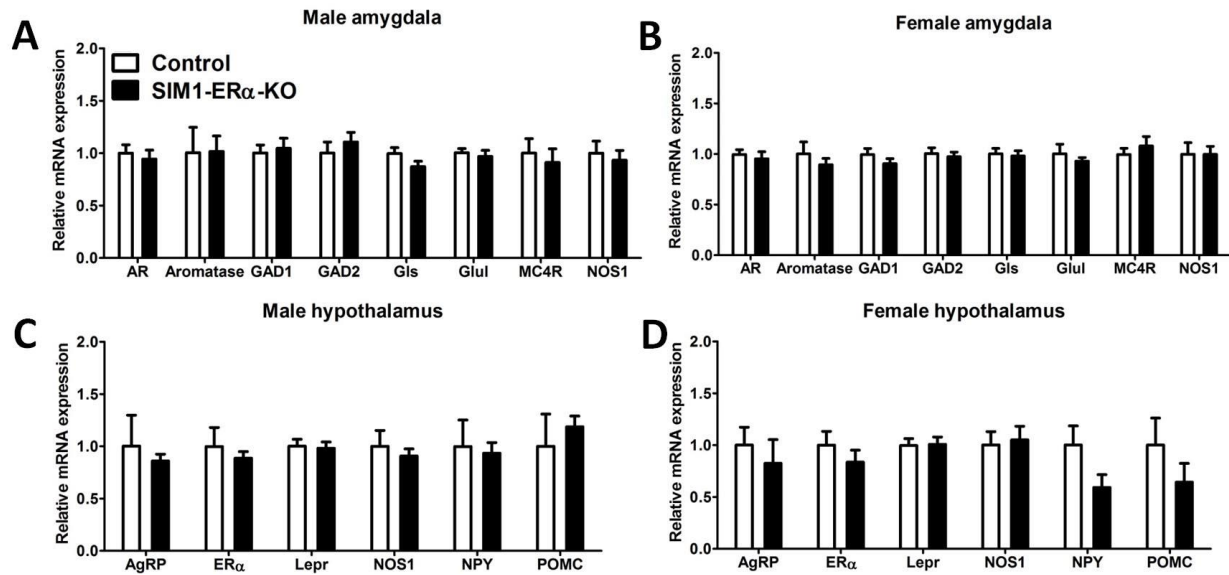
**Running Title:** MeA ER $\alpha$  regulates physical activity & body weight



**Supplemental Figure 1. Validation of selective deletion of ERα in SIM1-ERα-KO mice.**

(A-B) Dual immunofluorescence for GFP (green) and ERα (red) in the MeA in SIM1-Cre/Rosa26-GFP mice (control, A) and in ERα<sup>lox/lox</sup>/SIM1-Cre/Rosa26-GFP mice (SIM1-ERα-KO, B). Left panel: ERα immunoreactivity; middle panel: GFP immunoreactivity; right panel: merged images. Arrows point to double-labeled neurons. MeA, medial amygdala; opt, optic tract. Scale bars = 100 μm.

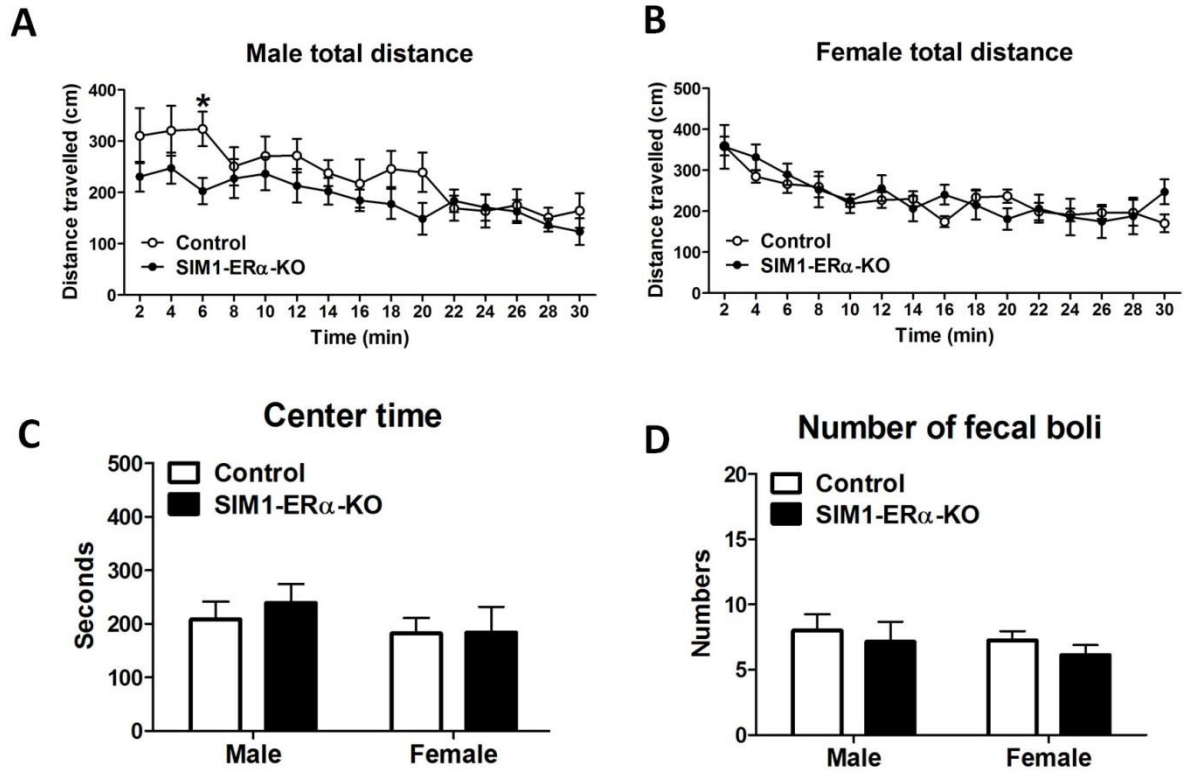
(C-D) Numbers of SIM1 (GFP) neurons, ERα neurons, and double-labeled neurons in the MeA in female (A) and male (B) SIM1-Cre/Rosa26-GFP or ERα<sup>lox/lox</sup>/SIM1-Cre/Rosa26-GFP mice. Data are presented as mean±SEM. N=3 in each group.



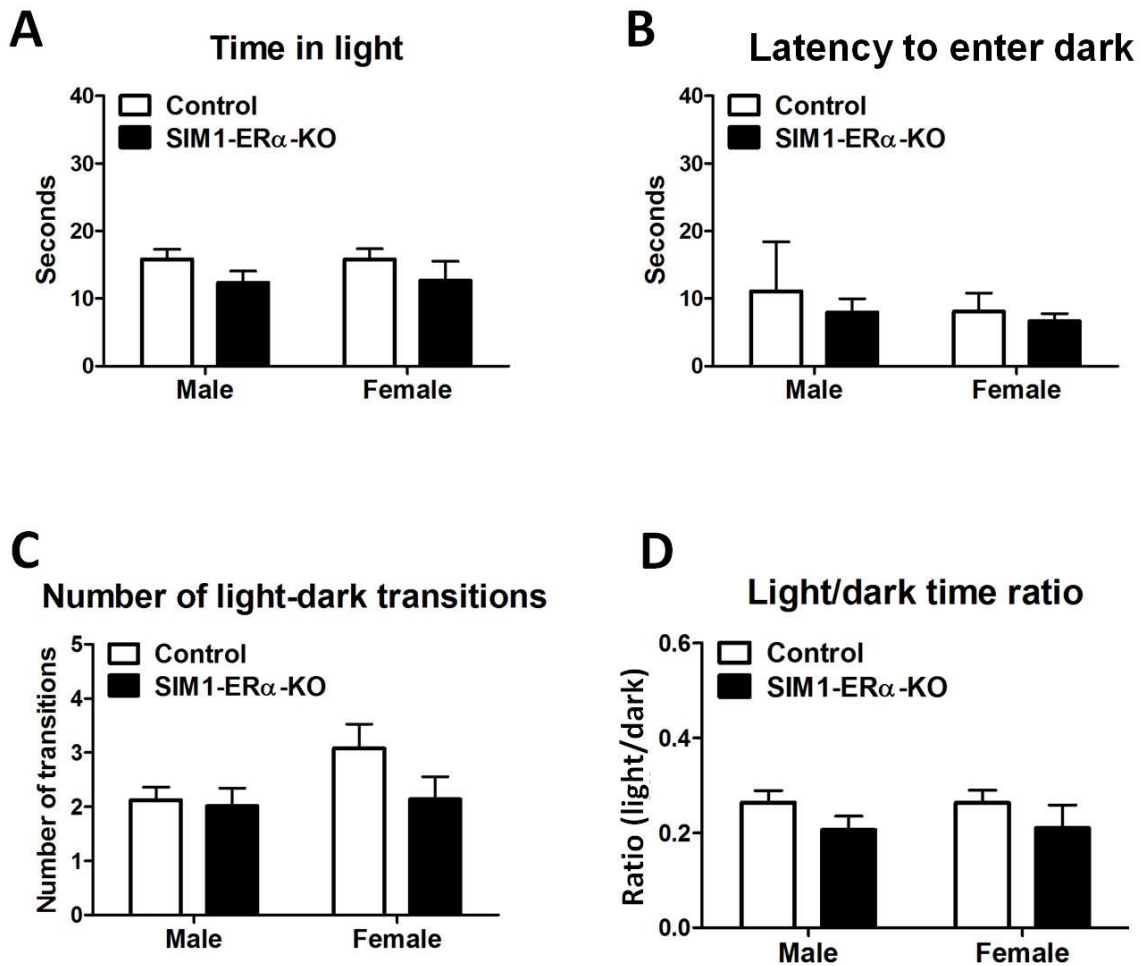
**Supplemental Figure 2. Gene expression in the amygdala and hypothalamus.**

(A-B) Relative mRNA levels of indicated genes in the MeA from chow-fed male (A) or female (B) control and SIM1-ER $\alpha$ -KO mice. Data are presented as mean $\pm$ SEM. N=6 in each group.

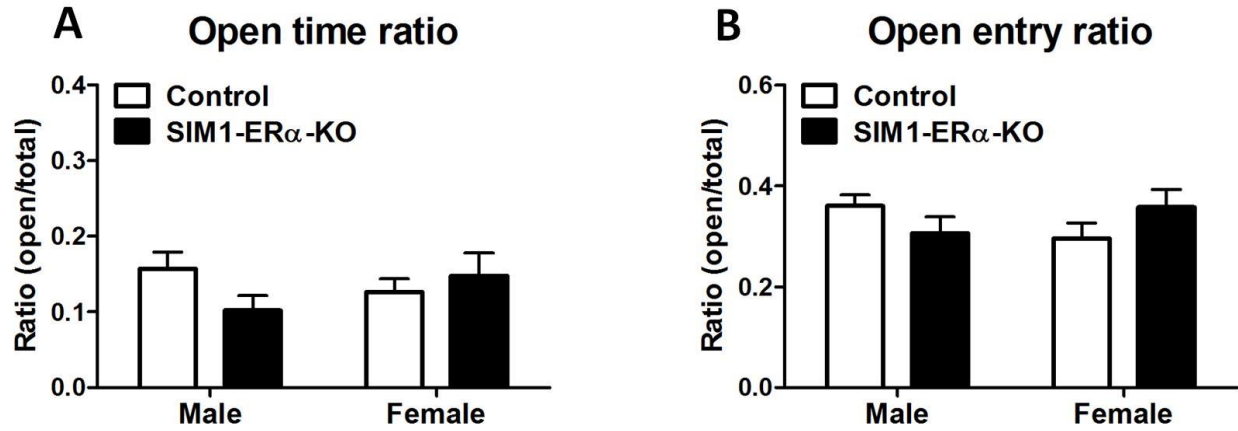
(C-D) Relative mRNA levels of indicated genes in the hypothalamus from chow-fed male (C) or female (E) control and SIM1-ER $\alpha$ -KO mice. Data are presented as mean $\pm$ SEM. N=6 in each group.



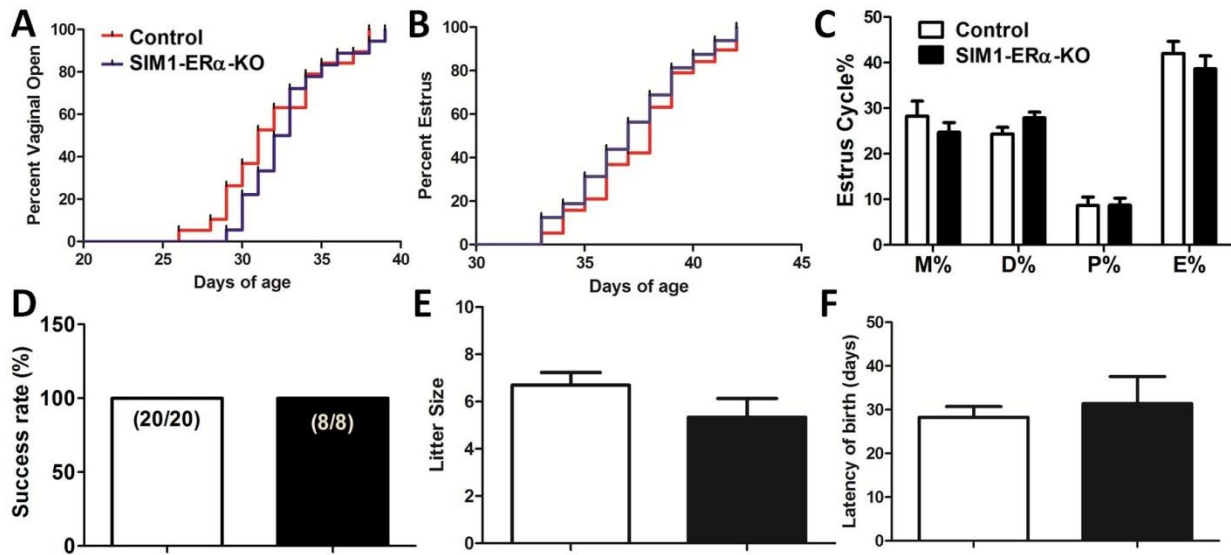
**Supplemental Figure 3. Open field tests.** Anxiety-related responses in open field tests in male and female control and SIM1-ER $\alpha$ -KO mice (8 weeks of age). (A-B) Distance travelled in male (A) and (B) mice. (C) Time spent in the center region of the open field. (D) Numbers of fecal boli. N=7-12/group. Results are shown as MEAN  $\pm$  SEM.



**Supplemental Figure 4. Light-dark tests.** Anxiety-related responses in light-dark tests in male and female control and SIM1-ER $\alpha$ -KO mice (8 weeks of age). (A) Time spent in the light chamber. (B) Latency to enter the dark chamber. (C) The total number of transitions between the light and dark chambers. (D) Ratio of time spent in the light chamber vs the dark chamber. N=7-12/group. Results are shown as MEAN  $\pm$  SEM.

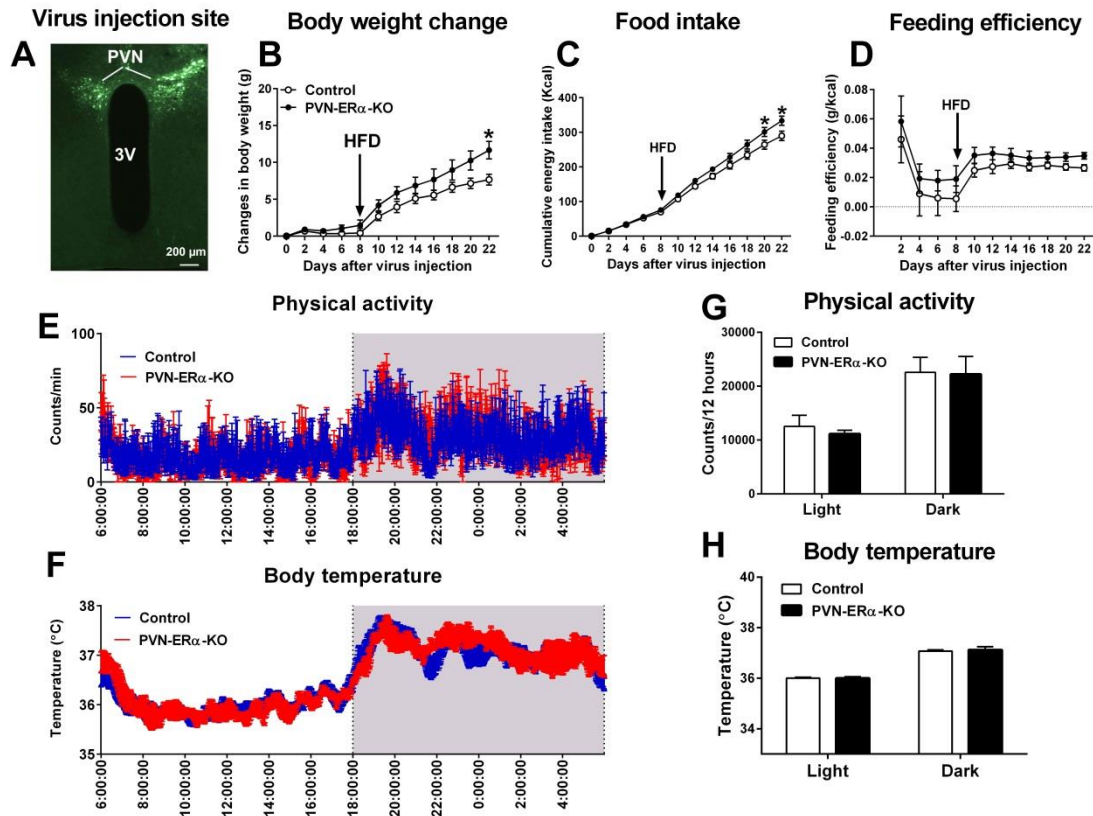


**Supplemental Figure 5. Elevated plus maze tests.** Anxiety-like behavior measured in male and female control and SIM1-ER $\alpha$ -KO mice (8 weeks of age). (A) Open time ratio. (B) Open entry ratio. N=7-12/group. Results are shown as MEAN  $\pm$  SEM.



**Supplemental Figure 6. Reproductive functions in female SIM1-ER $\alpha$ -KO mice.**

- (A) Appearance of vaginal opening. Data are presented as mean $\pm$ SEM. N=18 in each group.
- (B) Appearance of the first estrus. Data are presented as mean $\pm$ SEM. N=16 or 18 in each group.
- (C) Length of diestrus, proestrus and estrus relative to the entire estrous cycles. Data are presented as mean $\pm$ SEM. N=14 or 20 in each group.
- (D) Percentage of mice that successfully delivered pups. Data are presented as mean $\pm$ SEM. N=8 or 20 in each group.
- (E) Averaged litter size. Data are presented as mean $\pm$ SEM. N=20 or 6 in each group.
- (F) Averaged time period between mating day and birth day of pups. Data are presented as mean $\pm$ SEM. N=18 or 6 in each group.



### Supplemental Figure 7. Metabolic profiles of PVN-ER $\alpha$ -KO mice.

(A) Post hoc visualization of GFP in the PVN in an ER $\alpha^{lox/lox}$  mouse receiving AAV-Cre-GFP stereotaxic injections. 3V, 3rd ventricle; PVN, paraventricular nucleus of the hypothalamus. The scale bar = 200  $\mu$ m.

(B) Gains in body weight after virus injections. Note mice were fed with regular chow for the first 7 days and then with HFD for the rest period. Data are presented as mean $\pm$ SEM. N=4 in each group. \* P<0.05 in two-way ANOVA analysis followed by post hoc Bonferroni tests.

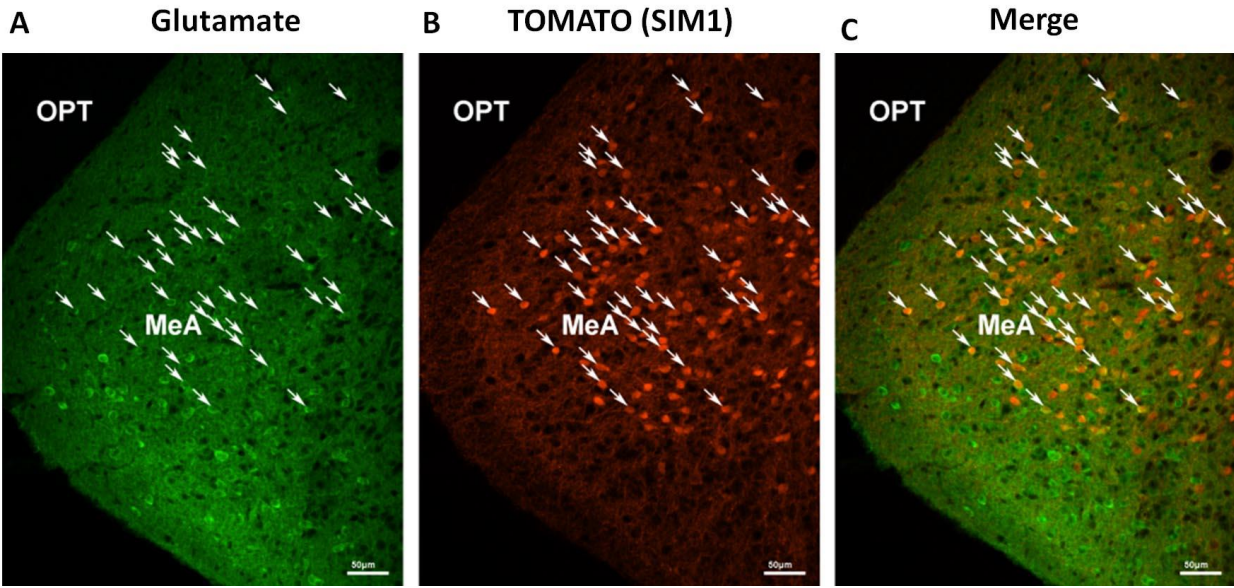
(C) Cumulative food intake. Data are presented as mean $\pm$ SEM. N=4 in each group. \* P<0.05 in two-way ANOVA analysis followed by post hoc Bonferroni tests.

(D) Feeding efficiency calculated as the ratio between changes in body weight (B) and cumulative food intake (C).

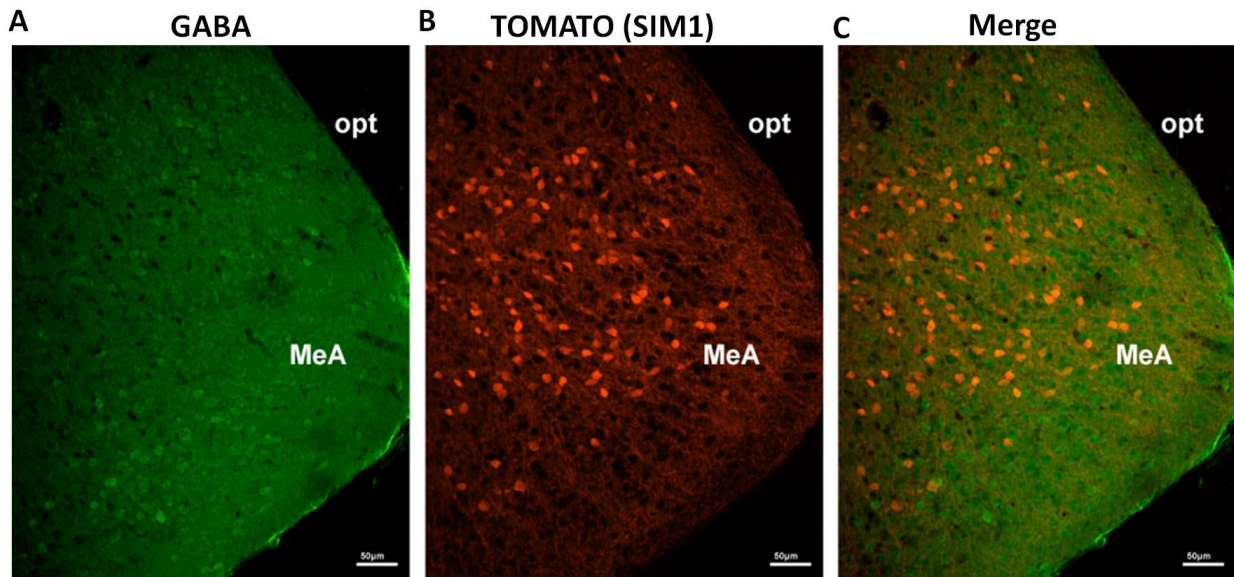
(E) 24-hour physical activity. (F) Sum physical activity during the light or dark cycles. Data are presented as mean $\pm$ SEM. N=4 in each group.

(G) 24-hour body temperature. (H) Averaged body temperature during the light or dark cycles. Data are presented as mean $\pm$ SEM. N=4 in each group.

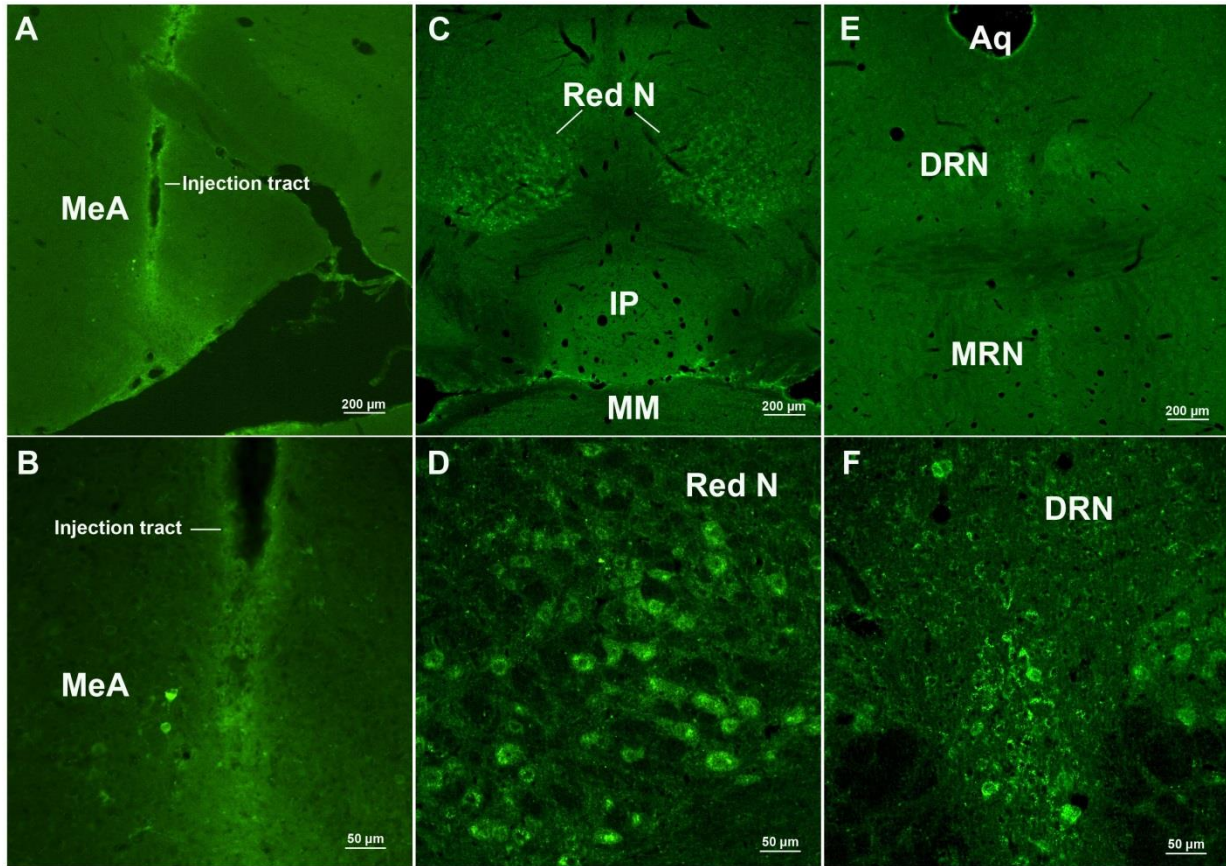




**Supplemental Figure 8. Co-localization of glutamate and SIM1 in the MeA.** Immunofluorescence for glutamate (green in A), TOMATO red fluorescence (B), and the merge (C) in the MeA in SIM1-Cre/Rosa26:tdTOMATO mice. Arrows point to double-labeled neurons. MeA, medial amygdala; opt, optic tract. Scale bars = 50  $\mu$ m.



**Supplemental Figure 9. Co-staining of GABA and SIM1 in the MeA.** Immunofluorescence for GABA (green in A), TOMATO red fluorescence (B), and the merge (C) in the MeA in SIM1-Cre/Rosa26:tdTOMATO mice. Note that there is no double-labelled neuron. MeA, medial amygdala; opt, optic tract. Scale bars = 50 µm.



**Supplemental Figure 10. Anterograde tracing of MeA SIM1 neurons.** (A-B) Injection tract showing accurate stereotaxic delivery of Ad-IN/W virus into the MeA of SIM-Cre mice. (C-F) Immunofluorescence for WGA in the Red N (C-D), DRN (E-F) and MRN (E). Aq, aqueduct; DRN, dorsal Raphe nuclei; MeA, medial amygdala; MRN, median Raphe nuclei; Red N, red nucleus. Values of scale bars are indicated for each panel.

**Supplemental Table 1: Serum hormones in male and female mice**

Parameter	Male		Female	
	Control	SIM1-ER $\alpha$ -KO	Control	SIM1-ER $\alpha$ -KO
T3 (ng/mL)	0.51 $\pm$ 0.05	0.4 $\pm$ 0.05	0.31 $\pm$ 0.05	0.3 $\pm$ 0.04
T4 (ng/mL)	34.28 $\pm$ 1.65	32.01 $\pm$ 2.29	25.46 $\pm$ 1.99	26.31 $\pm$ 2.15
Norepinephrine (pg/mL)	16002.5 $\pm$ 758.55	15298.75 $\pm$ 741.29	23611.88 $\pm$ 1352.62	23361 $\pm$ 2834.8
Epinephrine (pg/mL)	3315.25 $\pm$ 695.79	5195.25 $\pm$ 1377.85	13324.13 $\pm$ 3952.6	16838.25 $\pm$ 5029.73
Testosterone (ng/mL)	9.94 $\pm$ 5.42	6.32 $\pm$ 5.19	_____	_____
17 $\beta$ -estradiol (pg/ml)	_____	_____	50.86 $\pm$ 3.95	62.82 $\pm$ 7.07
Leptin	_____	_____	2.15 $\pm$ 0.40	5.87 $\pm$ 1.60*

All data are presented as mean  $\pm$  SEM. \*, P<0.05 between control and SIM1-ER $\alpha$ -KO in t-test.

**Supplemental Table 2: Primer sequence (add BAT gene primers)**

Target gene	Primer sequences		PCR products	GenBank accession
Androgen receptor (Ar)	Ar F	TCTTCAAGGGAGGTTACGC	101bp	<a href="#">NM_013476.3</a>
	Ar R	GAGGACGGGATCTCAAGTGTC		
Aromatase	Cyp19a1 F	ACGTGGATGTGTTGACCTC	93bp	<a href="#">NM_007810.3</a>
	Cyp19a1 R	TCACAATAGCACTTTCGTCCAG		
Glutamate decarboxylase 1 (Gad1)	Gad1 F	AGCCAGACAAGCAGTATGACG	126bp	<a href="#">NM_008077.4</a>
	Gad1 R	GGTTTTCAAATCCCACGGT		
Glutamic acid decarboxylase 2 (Gad2)	Gad2 F	ACAGGGCATCCCCGATAT	75bp	<a href="#">NM_008078.2</a>
	Gad2 R	TGTCAACCAGTCTGCTGCTAAT		<a href="#">NM_001081081.2</a>
Glutaminase (Gls)	Gls F	GCACAGACATGGTTGGGATAC	126bp	<a href="#">NM_001081081.2</a>
	Gls R	TTTACCAGTAATTGGGCAGA		
Glutamate-ammonia ligase (Glul)	Glul F	TCATCTTTGGATAGCCCGTTTT	132bp	<a href="#">NM_008131.3</a>
	Glul R	GCCTTGGTGCTGAAGTTGGTA		
Melanocortin 4 receptor (MC4R)	MC4R F	GGTCGGAACCATCGTCA	132bp	<a href="#">NM_016977</a>
	MC4R R	AAAGCAGGCTGCAAATGG		
Nitric oxide synthase 1 (Nos1)	NOS1 F	GATTGGCGTTCGTGATTACTG	69bp	<a href="#">NM_008712.2</a>
	NOS1 R	TCCATTTTCTGGCTACTTCCT		<a href="#">XM_006512632.1</a>
Single-minded 1 (SIM1)	SIM1 F	TGTCTCCCTTTGATGGAT	219bp	<a href="#">NM_001271806.1</a>
	SIM1 R	CATGCACGTGTAGGTACA		
Agouti related peptide (AgRP)	AGRP F	CGGCCACGAACCTCTGTAG	65bp	<a href="#">NM_001271806.1</a>
	AGRP R	CTCATCCCCTGCCTTTGC		
Estrogen receptor alpha (ERa)	ERa F	AAGAGAGTGCCAGGCTTT	223bp	<a href="#">NM_007956.4</a>
	ERa R	GGAACCGACTTGACGTAG		<a href="#">NM_001122899.1</a>
Leptin receptor (Lepr)	Lepr F	GGAGCCATTACCTAAGAACCC	156bp	<a href="#">NM_001122899.1</a>
	Lepr R	TGACATTCACATCCCCGAAG		
Neuropeptide (NPY)	NPY F	TCCGCTCTGCGACACTAC	230bp	<a href="#">NM_023456.2</a>
	NPY R	GGGACAGGCAGACTGGTT		
Cyclophilin (CYCLO)	CYCLO F	TGGAGAGCACCAAGACAGACA	66bp	<a href="#">NM_011149.2</a>
	CYCLO R	TGCCGGAGTGCACAATGAT		
Tripartite motif-containing 25	Trim25 F	TGATGTGGCTGTGCATGATA	91bp	<a href="#">NM_009546.2</a>

(Trim25)	Trim25 R	AAGACCTGCTCCCCTACGAC		
Pro-opiomelanocortin (POMC)	POMC F	GAGGCCACTGAACATCTTTGTC	252bp	<a href="#">NM_001278581.1</a>
Uncoupling protein 1 (UCP1)	POMC R	GCAGAGGCAAACAAGATTGG		
	UCP1F	GAGGTGTGGCAGTGTTCATTG	59bp	NM_009463
	UCP1R	GGCTTGCATTCTGACCTTCA		
CCAAT/enhancer binding protein, alpha (CEBP $\alpha$ )				
	CEBP $\alpha$ F	GCGCAAGAGCCGAGATAAAG	81bp	<a href="#">NM_001287514.1</a>
	CEBP $\alpha$ R	CGGTCATTGTCACTGGTCAACT		
Estrogen related receptor, alpha (ERR $\alpha$ )	ERR $\alpha$ F	AGCAAGCCCCGATGGA	105bp	NM_007953
	ERR $\alpha$ R	GAGATGCCTGGGATGCTCTT		
Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PGC-1 $\alpha$ )	PGC-1 $\alpha$ F	AACCACACCCACAGGATCAGA	73bp	NM_008904.2
	PGC-1 $\alpha$ R	TCTTCGCTTTATTGCTCCATGA		
Peroxisome proliferator activated receptor alpha (PPAR $\gamma$ )	PPAR $\gamma$ F	CGTACGGCAATGGCTTTATC	55bp	NM_011144.6
	PPAR $\gamma$ R	AACGGCTTCCTCAGGTTCTT		
Nuclear receptor interacting protein 1 (RIP140)		TTCTCAGGACATAATCCTTTAACAT		
	RIP140 F	TC	72bp	NM_173440.2
	RIP140 R	CGGGCCTCCTCAGTCAAC		
Uncoupling protein 3 (UCP3)	UCP3 F	TTTCTGCCACTGGGAGCTT	63bp	NM_009464.3
	UCP3 R	GGCCCTCTCAGTTGCTCAT		
Adrenergic receptor, beta 3 (ADRB3)	ADRB3 F	GACTACAGACCATAACCAACGTG	82bp	NM_013462
	ADRB3 R	CCTGGTGGCATTACGAGGA		
PR domain containing 16 (PRDM16)	PRDM16 F	CCACCAGCGAGGACTTCAC	107bp	NM_027504
	PRDM16 R	GGAGGACTCTCGTAGCTCGAA		

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