

Figure S1. Fast Red signals with RNAscope using a positive control probe (mouse *Polr2a* probe), a negative control probe (*Bacillus subtilis* *dapB*) or a mouse *Plag1* probe on light fields. LHA, lateral hypothalamic area; *Plag1*, pleomorphic adenoma gene-like 1; *Polr2a*, RNA polymerase II subunit A.

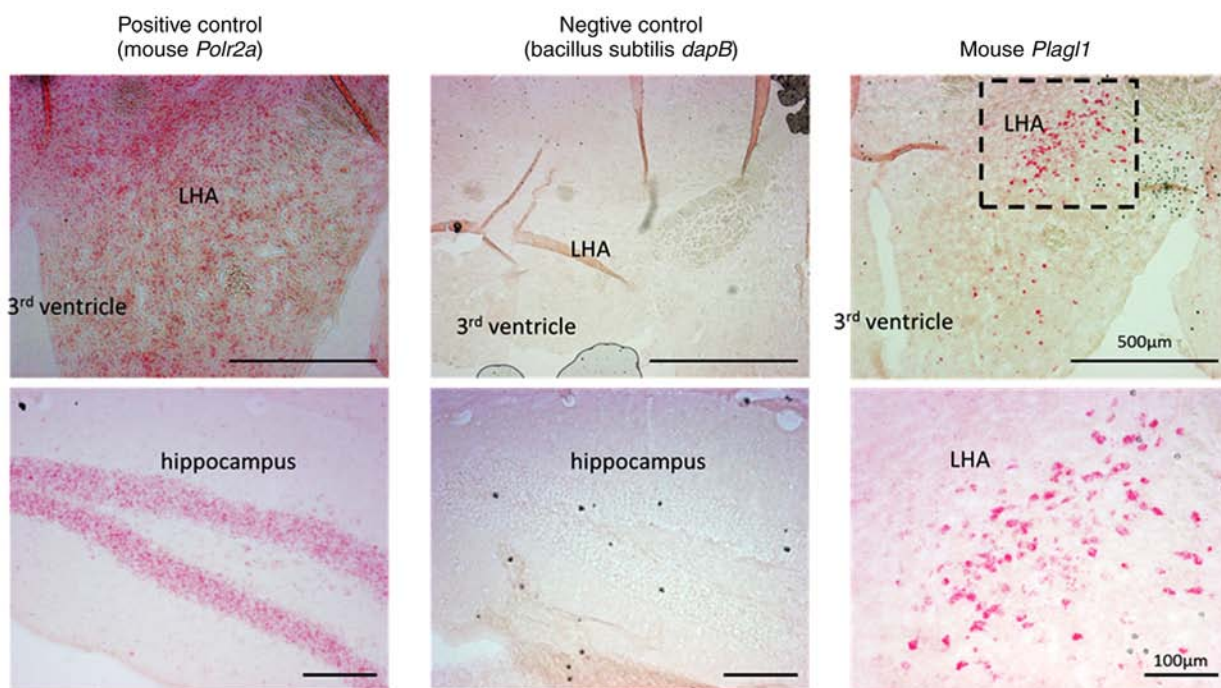


Figure S2. PLAGL1 staining and Plagl1 staining with an RNAscope probe in the murine lateral hypothalamic area at Zeitgeber time 6. (A) PLAGL1 was labeled with Cy5 (green). (B) Plagl1 was labeled with a Fast Red-conjugated RNAscope probe (red). (C) Merged image of A and B. The images are of the same area that is presented in Fig. 2. Scale bar, 50 μ m. Plagl1, pleomorphic adenoma gene-like 1.

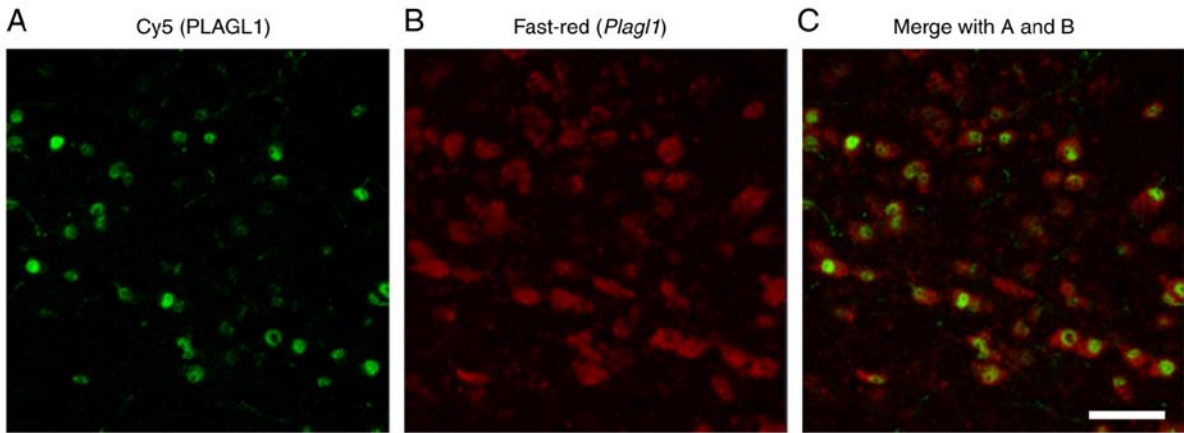


Figure S3. Hypocretin staining and *Plagl1* staining with an RNAscope probe in the murine lateral hypothalamic area at Zeitgeber time 6. (A) *Plagl1* was labeled with a Fast Red-conjugated RNAscope probe (red). (B) Hypocretin was labeled with Alexa Fluor[®]488 (green). (C) DAPI-labeled nuclei (blue). (D) Merged image of A-C. The images are of the same area that is presented in Fig. 2. Scale bar, 50 μ m. *Plagl1*, pleomorphic adenoma gene-like 1.

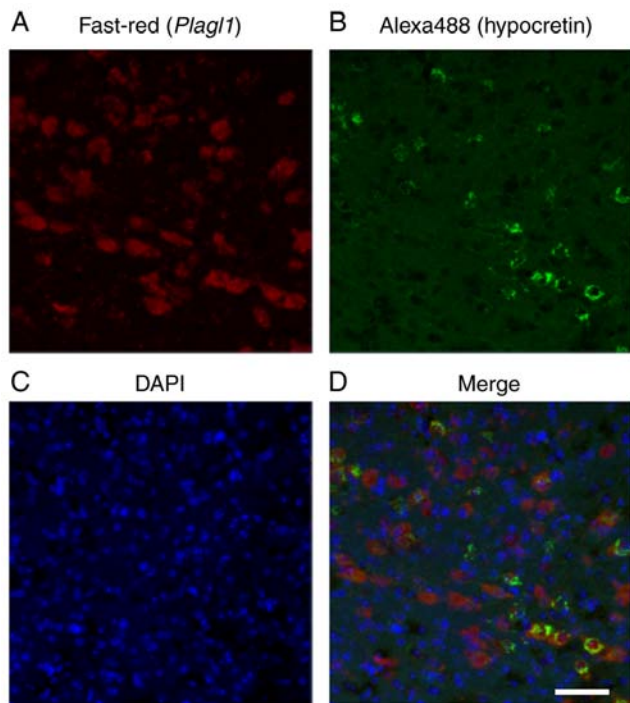


Figure S4. EGFP electroporation into one side of the hypothalamus. 3vt, third ventricle; EGFP, enhanced green fluorescent protein; Lv, lateral ventricle; PLAGL1, pleomorphic adenoma gene-like 1.

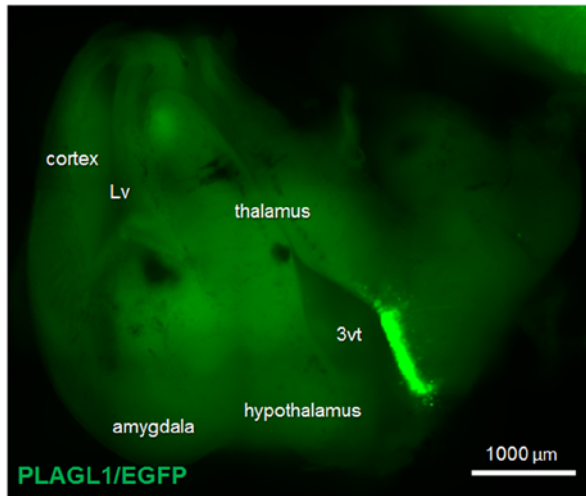


Figure S5. EGFP and Plagl1 electroporation into the cortex. *In utero* electroporation was performed with pCAGGS-EGFP and pCAGGS-mPlagl1 into the cortex at E12 to evaluate the effect of PLAGL1 using EGFP (green), Alexa Fluor[®]594 visualized murine HCRT or PLAGL1 (red), and DAPI (blue). Embryos were harvested at E15. There was no HCRT signal after pCAGGS-mPlagl1 overexpression in the cortex. Co-expression of PLAGL1 and EGFP following electroporation was detected in the cortex. In addition, some cells stained by Fast Red that depend on PLAGL1 antibody were not merged with EGFP signals. E, embryonic day; EGFP, enhanced green fluorescent protein; HCRT, hypocretin; PLAGL1, pleomorphic adenoma gene-like 1.

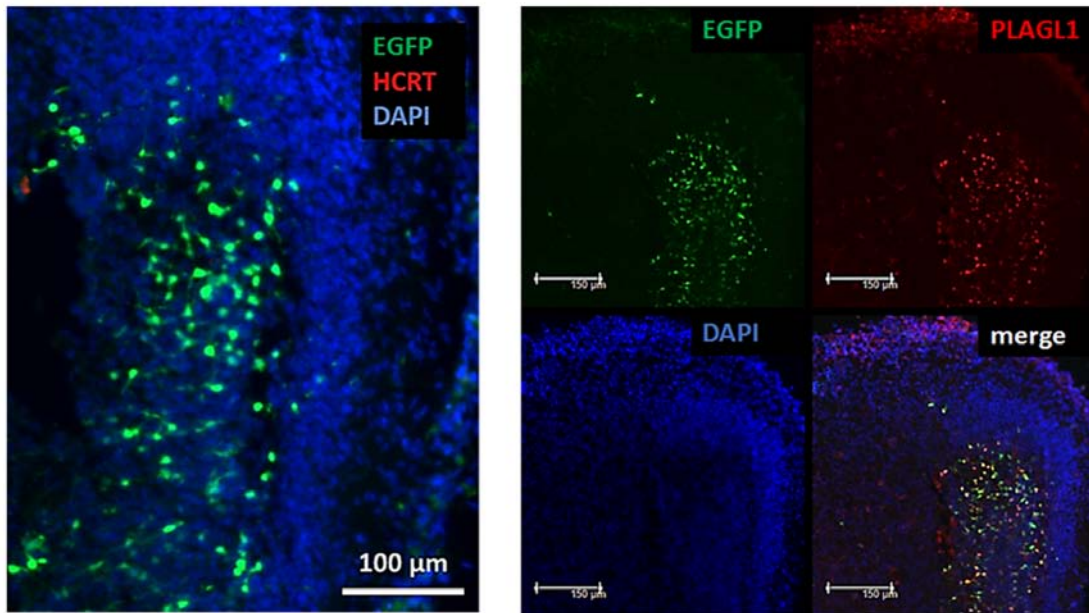


Figure S6. Embryonic hypothalamic hypocretin expression following PLAGL1 overexpression, using *in utero* electroporation. *In utero* electroporation was performed to evaluate the effect of PLAGL1 on HCRT transcription using EGFP (green), Alexa Fluor[®]594 visualized murine hypocretin (red) and DAPI (blue). A low magnification image of the hypothalamus at E15 after electroporation is shown (left panel). The two panels on the right are expanded views of the dotted-square fields in the left panel. Some electroporated-EGFP colocalized with HCRT expression (right panels, arrows). Arrowheads indicate single fluorescence of HCRT. Scale bar, 50 μ m. 3vt, third ventricle; E, embryonic day; EGFP, enhanced green fluorescent protein; HCRT, hypocretin; PLAGL1, pleomorphic adenoma gene-like 1.

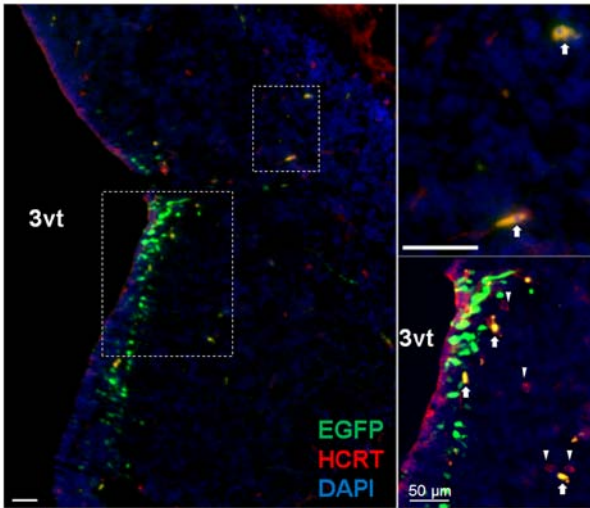


Figure S7. EGFP and Plag11 electroporation into the hypothalamus. pCAGGS-EGFP and pCAGGS-mPlag11 were injected into the 3vt and electroporated into one side of the hypothalamus at E12. Embryos were harvested at E18. HCRT was detected by immunofluorescence methods using rabbit anti-HCRT-1 and anti-rabbit-Alexa Fluor[®]488 antibodies. Left panel shows the low-power fields. Middle three panels are the expanded views of the square field in the left panel. Three panels on the right are the expanded views of the dotted-square field in the left panel. Arrows indicate the colocalization of EGFP within HCRT neurons. 3vt, third ventricle; cp, cerebral peduncle; EGFP, enhanced green fluorescent protein; HCRT, hypocretin; LH, lateral hypothalamus; Lv, lateral ventricle; STh, subthalamic nucleus.

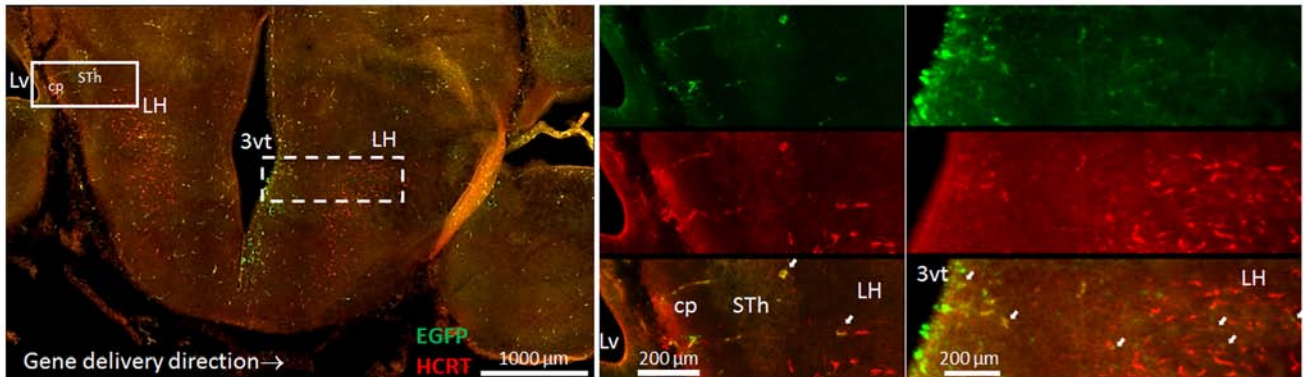


Table SI. Primer sequences.

A, Primers for ChIP-PCR analysis

Primer	Sequence
mHert_-1617F	5'-GGTAGTTCTTGACCTGCTTTGAAC-3'
mHert_-1433R	5'-GTTATAGAGAGTTGTGAGCCACCA-3'
mHert_-751F	5'-CTGGAGATGGCAGAGTAAGACTAC-3'
mHert_-649F	5'-AGAGTAAGACTACCTCTTCTCTACTGG-3'
mHert_-611F	5'-AGATGTCTACCCTTCATCAGCTTCT-3'
mHert_-440R	5'-GGAAACAGCTTTTGACACCCTACT-3'
mHert_-507R	5'-TTCTTCTGTCCCTTTACCAAACACT-3'
mHert_-355F	5'-AGACAGTAATTCTCAGACTGCCAT-3'
mHert_-205R	5'-TAGGGCAGTTTCCGAGTACTAATG-3'

B, Primers for murine mRNA quantification

Primer	Sequence
EGFP_418F	5'- CACAAGCTGGAGTACAACACTACAACAG-3'
EGFP_495R	5'-GTTACCTTGATGCCGTTCTTC-3'
mHprt_1009F	5'-CTCTGGTAGATTGTGCTTATCTTGTAAG-3'
mHprt_1237R	5'-CCTCTTAGATGCTGTTACTGATAGGAAATC-3'
mHert_10F	5'-GCCTCAGACTTCTTGGGTATTTGG-3'
mHert_79R	5'-CTCAGGGGAAAGTAATCTGGAGAG-3'
mPlagl1_1127F	5'-AGAGATTTGGGCGCAAAGAC-3'
mPlagl1_1255R	5'-ACGAAGTTGAAGGCGCAATG-3'
mPmch_383F	5'-GCCAACATGGTCGGTAGACT-3'
mPmch_555R	5'-CGCTCTGAAAGGATCCGTAG-3'
mNptx2_1102F	5'-GGCGATGTTGATGATCTCCT-3'
mNptx2_1347R	5'-CACATCTGCATCACCTGGAC-3'
mPdyn_49F	5'-TGTAGCCTTCTTCCAAAGCAA-3'
mPdyn_280R	5'-TCTAATGTTATGGCGGACTGC-3'
mNpy_254F	5'-CTACATCAATCTCATCACCAGACAGAG-3'
mNpy_323R	5'-GAGGTCTGAAATCAGTGTCTCAGG-3'
mTrh_869F	5'-CTCTGGAGGAGTAAGGTTAGAGTCAG-3'
mTrh_948R	5'-AGTGAAGGGACTGGGATAGGATAC-3'
mOxt_17F	5'-GGATCTCAGACTGAGCACCATC-3'
mOxt_164R	5'-ACACTTGCGCATATCCAGGTC-3'
mAgrp_642F	5'-TACTGCCGCTTCTTCAATGC-3'
mAgrp_742R	5'-GCCTTTGCCCAAACAACATC-3'
mB2m_157F	5'- TGGTGCTTGCTCACTGACC -3'
mB2m_308R	5'-CCGTTCTTCAGCATTGGAT-3'

C, Sequence primers for pCAG-mPlagl1

Primer	Sequence
mPlagl1_606F	5'-GAAGTTCACCATTACAATTATTCC-3'
mPlagl1_1992R	5'-AATGGCTATATTCACAGCATCTACC-3'
mPlagl1_1968F	5'-GGTAGATGCTGTGAATATAGCCATT-3'
mPlagl1_+576F	5'-AAAATGTGGCAAGTCCTTCG-3'
mPlagl1_+740R	5'-TGAATCTTCTGTGGCGAGTG-3'
mPlagl1_546F_ <i>Kpn</i> I	5'-CGGGGTACCAAAGGCCATGGCTCCATTCCGCTGTCA-3'
mPlagl1_2671R_ <i>Bam</i> HI	5'-TAGATCCGGTGGATCCTTATCTAAATGCGTGATGGA-3'
mPlagL1_+493F	5'-TTCTACACCCGGAAAGATGTG-3'
mPlagL1_+653R	5'-GCCTGCATATTCTTGCATC-3'
mPlagL1_+1142F	5'-CAATGCCAGAGCCGCTT-3'
mPlagL1_+1389R	5'-TTTCCCAAACCCCTCCAT-3'
mPlagL1_+1493F	5'-CTCCCCAGAATGGCTTTGT-3'

Table SI. Continued.

D, Sequence primers for murine reporter plasmid

Primer	Sequence
mPlagL1_+1691R	5'-GGCTGGATCTGCAACTGAA-3'
pGL3_MCS_AS	5'-CTCGAGGCTAGCGAGCTCAGGTACCGGCCA-3'
pGL3_MCS_S	5'-AGATCTGGCCTCGGCGGCCAAGCTTGGCAA-3'
mHert_+92_Bg/II	5'-GGAAGATCTGGAACCTTTGTAGAAGGAAAG-3'
mHert_-385_XhoI	5'-CCGCTCGAGAGGTACCCTCCCTACCTTCAA-3'

AS, antisense; F, forward; R, reverse; S, sense.

Table SII. Relative expression in the hypothalamus following *in utero* electroporation.

Gene	Mock group		PLAGL1 group	
	Contralateral side	EP side	Contralateral side	EP side
EGFP	1,383.9±1,978.6	42,006.1±75,966.2	723.4±1,057.6	38,586.3±53,484.7 ^a
Plagl1	2.8±2.4	2.6±1.8	3.7±1.4	7.9±1.5 ^a
Hcr1	27.6±20.1	22.7±18.6	21.9±15.3	83.5±25.9 ^a
Pmch	7.7±8.0	10.2±9.3	7.3±5.0	26.9±7.9 ^a
Nptx2	6.0±4.8	3.4±2.0	10.1±6.1	18.7±13.0
Pdyn	2.6±1.2	2.0±0.9	2.4±0.9	5.4±4.2 ^a
Trh	7.2±8.8	4.4±0.7	10.4±6.5	35.3±25.1 ^a
Oxt	9.6±10.0	8.3±4.3	17.9±15.0	64.5±57.4 ^a
Npy	7.2±11.2	4.0±2.3	11.3±15.5	18.0±11.1
Agrp	6.8±2.9	4.3±3.9	14.8±5.3	28.7±38.8
B2m	2.6±1.5	2.0±0.8	2.8±2.6	4.2±2.3

Data are presented as the means ± standard deviation. ^aP<0.05 vs. contralateral side. EP, electroporated side.