

Brain regions shown in Figure 3

MnPo: median preoptic nucleus, VOLT: vascular organ of the lamina terminalis. AVPV: anteroventral periventricular nucleus, VOLT: vascular organ of the lamina terminalis, H: hippocampus, DMH: dorsomedial nucleus of hypothalamus, ARC: arcuate nucleus, VMN: ventromedial nucleus, PLCo: posterolateral cortical amygdaloid nucleus, BMP: accessory basal amygdaloid nucleus, PH: posterior hypothalamic area, PMV: premammillary nucleus, ventral part, PMCo: posteromedial cortical amygdaloid nucleus, LV: lateral ventricle.

Supplementary Table 1: Primers used in this study

For qPCR

Species	Gene	Accession #	Forward Primer	Reverse Primer	Amplicon (bp)
Rhesus Monkey	<i>MKRN3</i>	XM_001106523.3	CAAGAGTAAATGTGGCAGAGT GAAAGGAGA	TGAAGGGGAAGGAGGGAAAGAA CAA	147
	<i>GAPDH</i>	NM_001195426.1	AAGGGCATCCTGGGCTACA	GAAGAGTGGGTGTCGCTGTTG	68
Rat	<i>Mkrn3</i>	XM_218735.9	AGAGGGAAGCGTGCTGTTA	AGCCTTAGCAGACCAGACCA	205
	<i>Ppia</i>	M19533.1	GGCAAATGCTGGACCAAACAC AA	GGTAAAATGCCCGCAAGTCAAAG A	222
Mouse	<i>Mkrn3</i>	NM_011746.3	AAGCGCATACTGGCATCAAG	AGCCAACGGTCATCAGAGAA	165
	<i>Rpl19</i>	NM_009078.2	CTGAAGGTCAAAGGGAATGTG	GGACAGAGTCTTGATGATCTC	195

For single cell RT-PCR

Species	Gene	Accession #	Forward Primer	Reverse Primer	Amplicon (bp)
Mouse	<i>Mkrn3</i>	NM_011746	GAAGTTGCTTTGCCTGAGAG	ATGCCACAGCGTGATACTAC	113
	<i>Kiss1</i>	NM_178260	TGCTGCTTCTCCTCTGT	ACCGCGATTCTTTTCC	120

For ChIP

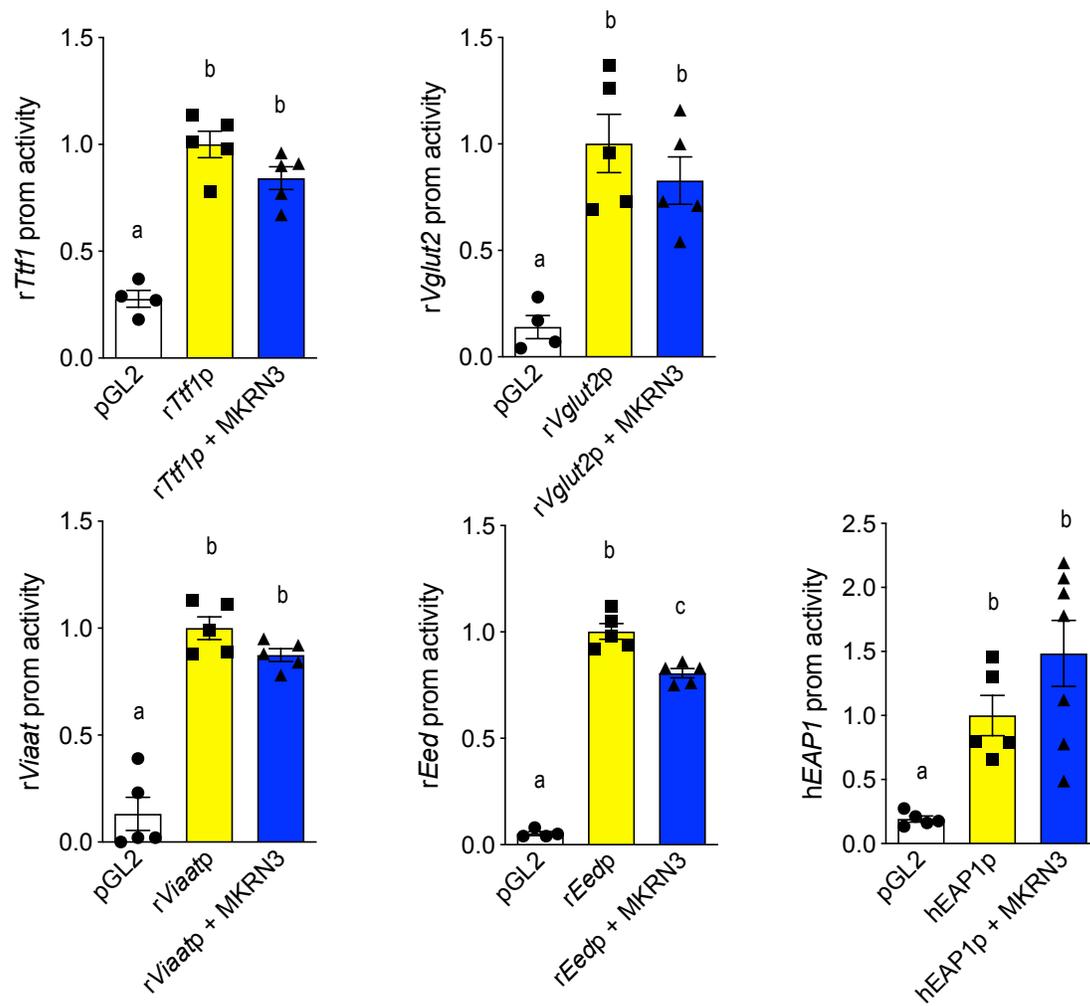
Species	Gene	Accession #	Forward Primer	Reverse Primer	Position from TSS	Amplicon (bp)
Human	<i>KISS1</i>	NM_002256	GTGCCCTTCTCCTCCCCTC TTCCT	GACCCATCCTGCCCCCA AACAA	-401/-244	158
	<i>TAC3</i>	NM_013251	GCTCGGGTGCTGTAGGAA ATGGT	GGGGGAGGAGGATGCA GACAGTAG	-178/-87	92

Supplementary Table 2: Promoters used in this study

The table below describes the genomic location of every sequence used in the promoter assays (* in reference to the transcriptional start site)

Gene Promoter	Species	Accession #	From*	To
<i>KISS1</i>	Human	NM_002256.3	-1317	+22
<i>TAC3</i>	Human	NM_001178054.1	-766	+69
<i>Tcf1</i>	Rat	XM_006233882.3	-5180	+1
<i>Vglut2</i>	Rat	NM_053427	-1113	-57
<i>Viaat1</i>	Rat	NM_031782	-1148	+1
<i>Eed</i>	Rat	NM_001106278.2	-1850	+256
<i>EAP1</i>	Human	NM_024496.4	-2345	+399
<i>PDYN</i>	Human	NM_001190892.1	-450	+340

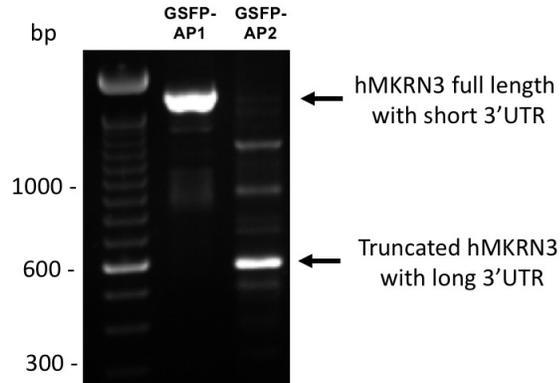
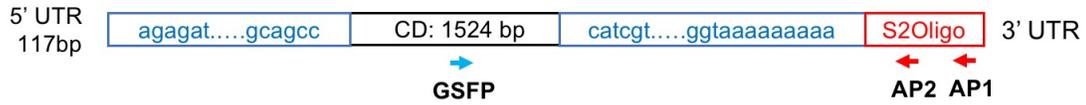
Supplementary Figure 1



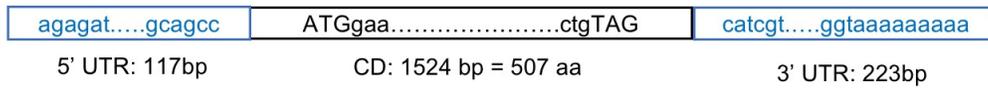
Supplementary Figure 1: MKRN3 does not inhibit the promoter activity of genes other than *KISS1* and *TAC3* implicated in stimulatory and inhibitory control of GnRH regulation. Lack of effect of MKRN3 on rat (*rTtf1*), *rVglut2*, *rViaat1*, *rEed*, and human (*hEAP1*) promoter (p) activity. A small but significant inhibitory effect was observed on *rEed* promoter activity. Bars represent mean \pm SEM ($n = 4-6$), white bars represent empty vector pGL1, yellow bars represent genes tested in luciferase and blue bars represent MKRN3 co-transfected with respective genes promoters. Groups with different letters are statistically different ($P < 0.05$), as determined by one-way ANOVA followed by Student–Newman–Keuls test.

Supplementary Figure 2

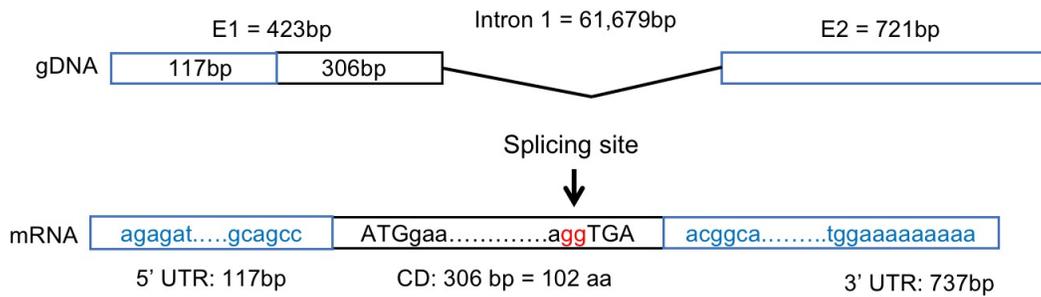
RACE PCR design



Full length hMKRN3



Truncated hMKRN3



Supplementary Figure 2: Identification of the *MKRN3* 3'-UTR in the human hypothalamus. (A) Structure of the *MKRN3* gene, 5'-UTR of 117 bp, coding domain (CD)

of 1524 bp and 3'-UTR. A nested PCR protocol with a gene-specific forward primer (GSFP) and two reverse AP1 and AP2 primers from the SMART™ RACE kit were used to amplify the 3'-UTR from human hypothalamic total RNA. **(B)** Gel image depicting the PCR products derived from RACE nested PCR. Two main transcriptional variants were detected, a full length *MKRN3* transcript in round one (primers GSFP and AP1) of the nested PCR and a shorter (truncated) version on the second round of PCR (primers GSFP and AP2). **(C)** Representation of the full length and truncated versions of *MKRN3* as determined by Sanger sequencing of at least 10 different clones for each. Black boxes represent coding domains, black lines are introns, and untranslated regions are represented by blue boxes. Numbers indicate the size of the exons and introns (in bp). *E1* = Exon 1, *E2* = exon 2, gDNA = genomic DNA, mRNA = messenger RNA.