Ghrelin causes a decline in GABA release by reducing fatty acid oxidation in ortex

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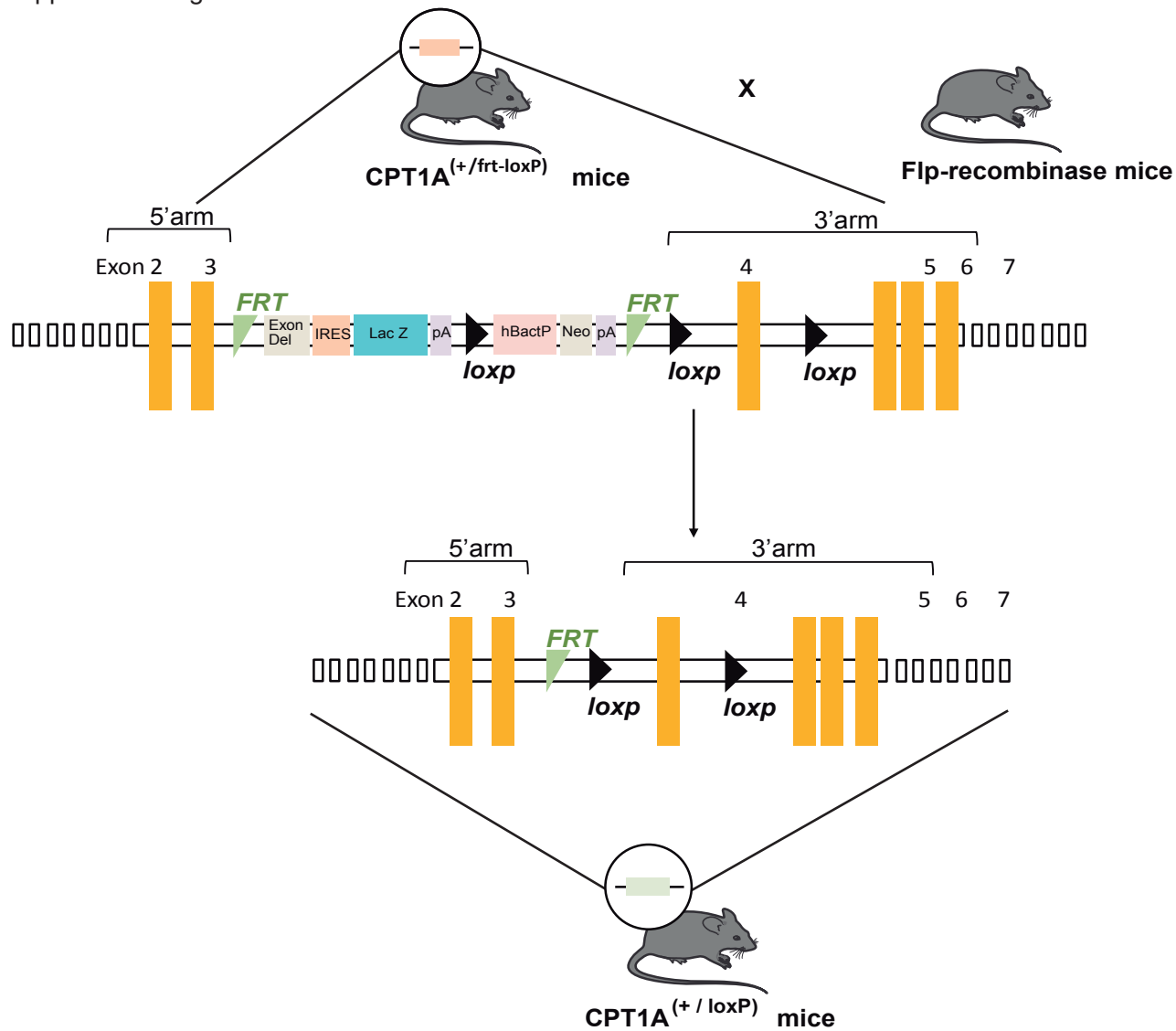
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Supplemental Figure 1. Scheme of the obtention of CPT1A$^{(loxP/loxP)}$ mice. CPT1A$^{(+/frt-loxP)}$ mice containing the CPT1A conditional expression cassette were crossbred with C57BL6/J Flp-frt recombinase-expressing mice to eliminate the frt-*lacz* cassette and obtain CPT1A$^{(+/loxP)}$ mice. FRT: target sequences for FLP recombinase are in green; Exon Del: internal exon deletion; IRES: internal ribosome entry site; LacZ: β-galactosidase gene; pA: polyadenylation site; loxp: target sequence for CRE recombinase; hBactP: human β-actin promoter; NEO: neomycine resistance gene.
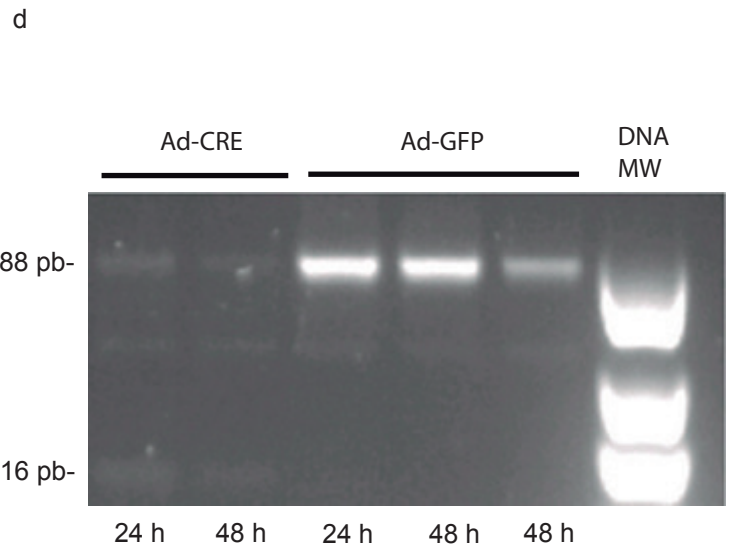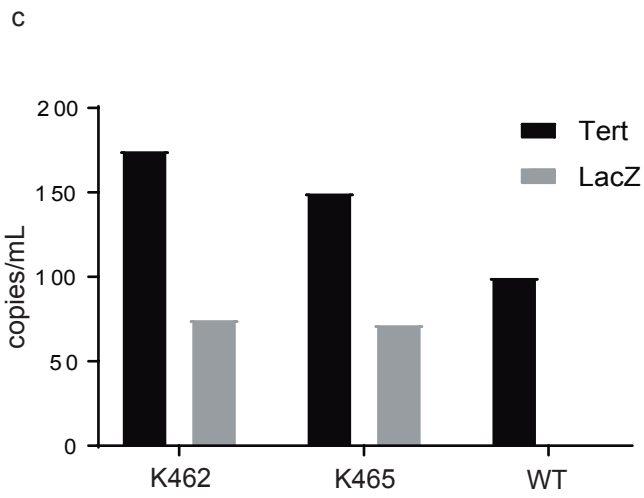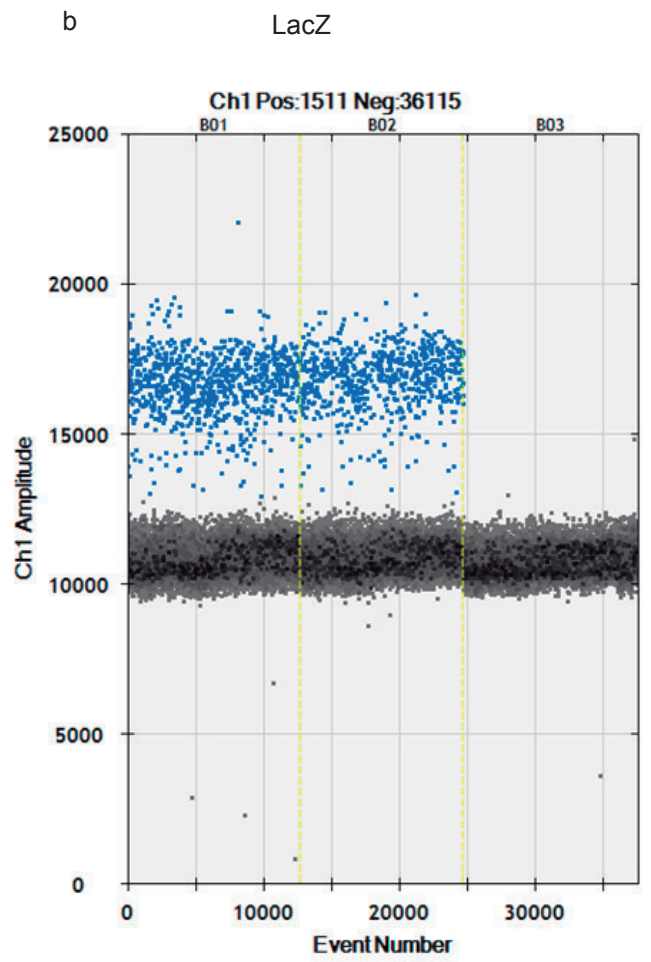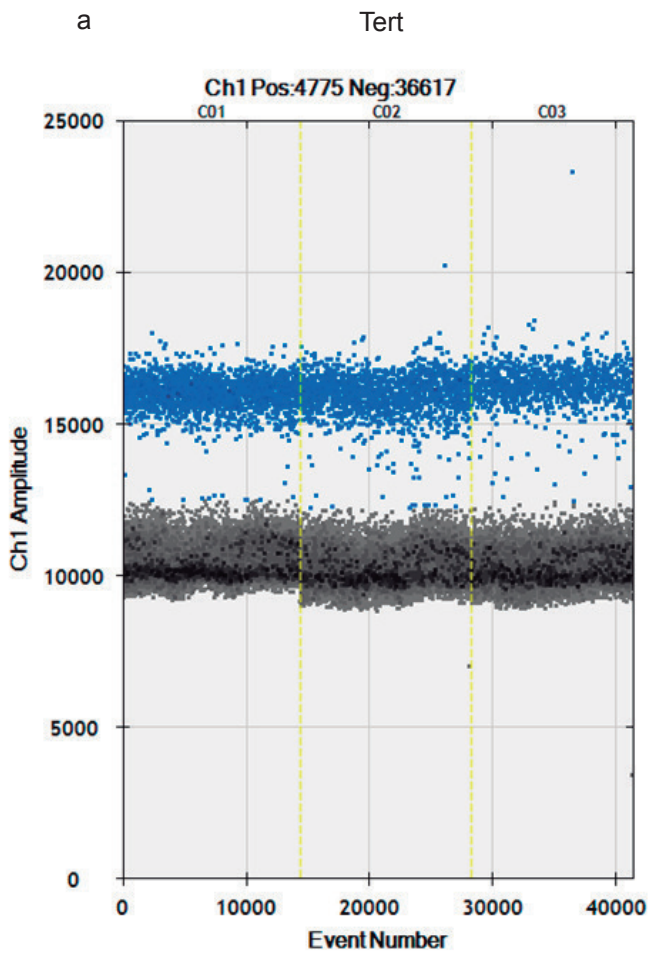
Supplemental Figure 2. ddPCR analysis of CPT1A conditional expression cassette copy number inserted into the genome of CPT1A$^{(+/frt-loxP)}$ mice. (a) Two CPT1A$^{(+/frt-loxP)}$ mice K462 and K465 and one wt mice were screened by ddPCR for the control gene *tert* and the *lacz*. (b) ddPCR quantification. (c) Electrophoretic analysis of the cDNA amplifications of the CPT1A mRNA levels in Cre-infected or GFP-infected primary cortical neurons for 24 and 48 h.

Supplemental Figure 3. Analysis of the mRNA levels of GABA metabolism genes in the primary cortical neurons with deleted CPT1A. Primary cortical neurons infected with Ad-CRE recombinase (CRE) or ad-GFP were treated with ghrelin (black bars) or without ghrelin (white bars). (a-c) Relative mRNA levels of GABA metabolism genes were analysed by qPCR. Results are represented as mean + SEM. n=4-6; * $p < 0.05$, ** $p < 0.01$ respect to the Ad-GFP control.

Supplemental Fig1

CPT1A$^{(+/frt-loxP)}$ mice

X

Flp-recombinase mice

5'arm

Exon 2   3

FRT

Exon Del   IRES   Lac Z   pA   hBactP   Neo   pA   FRT

loxp   loxp   loxp

3'arm

4   5   6   7

5'arm

Exon 2   3

FRT

loxp   loxp

3'arm

4   5   6   7
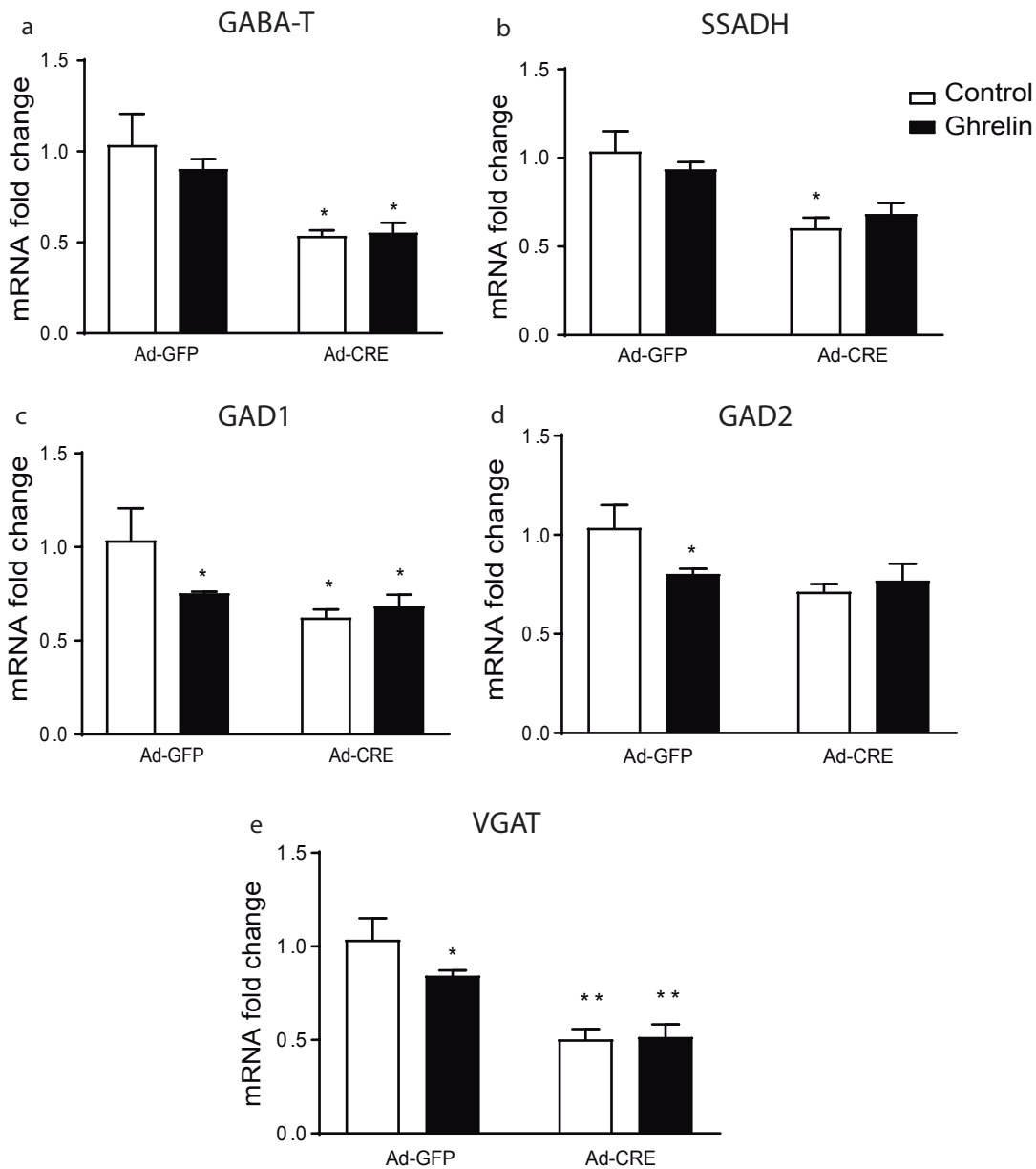
CPT1A$^{(+/loxP)}$ mice

Supplemental Fig2

a Tert

b LacZ

c

d

Supplemental Fig3

Supplemental table1:

Primers for qRT-PCR dectection and relative quantification of genes in mouse

GENE	SEQUENCE
CPT1A for	5'- GACTCCGCTCGCTCATTC
CPT1A rev	5'- AAGGCCACAGCTTGGTGA
AgRP for	5'- TGCAGACCGAGCAGAAGAAG
AgRP rev	5'- GACTCGTGCAGCCTTACACA
VGLUT1 for	5'- AAAAGCAGCAGCCAAGGTT
VGLUT1 rev	5'- CGCAGTTTAGCATTTCAGGAC
VGLUT2 for	5'- CTGGGGTCCTTGTGCAGTAT
VGLUT2 rev	5'- CCGAAGCTGCCATAGACATAG
VGLUT3 for	5'- TAAGGAGTAGCATGTTGCTCAAA
VGLUT3 rev	5'- GGCAACCACCATGTACTCTTCT
VGAT for	5'- GGCAACCACCATGTACTCTTCT
VGAT rev	5'- TGAGGAACAACCCCAGGTAG
GAD1 for	5'-ATACAACCTTTGGCTGCATGT
GAD1 rev	5'-TTCCGGGACATGAGCAGT
GAD2 for	5'-ACTAAAGAAAATGAGAGAAATCATTGG
GAD2 rev	5'-AGCATGGCATACATGTTGGA
GABAT for	5'-TGCCTCCAGAGAACTTTGTG
GABAT rev	5'-TGATGAGCTGGGACATGC
SSADH for	5'-AACAGCTGGAAAGGGGTCTC
SSADH rev	5'-CATTAAGTCGTACCATTTACGGAGT