Supplemental Data

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Mediobasal Hypothalamic p70 S6 Kinase 1 Modulates the Control of Energy Homeostasis

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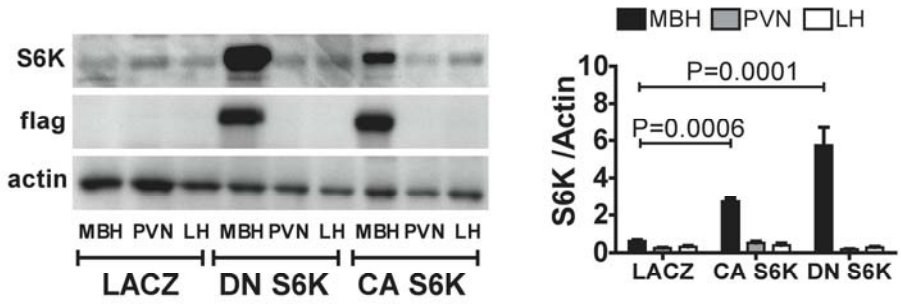
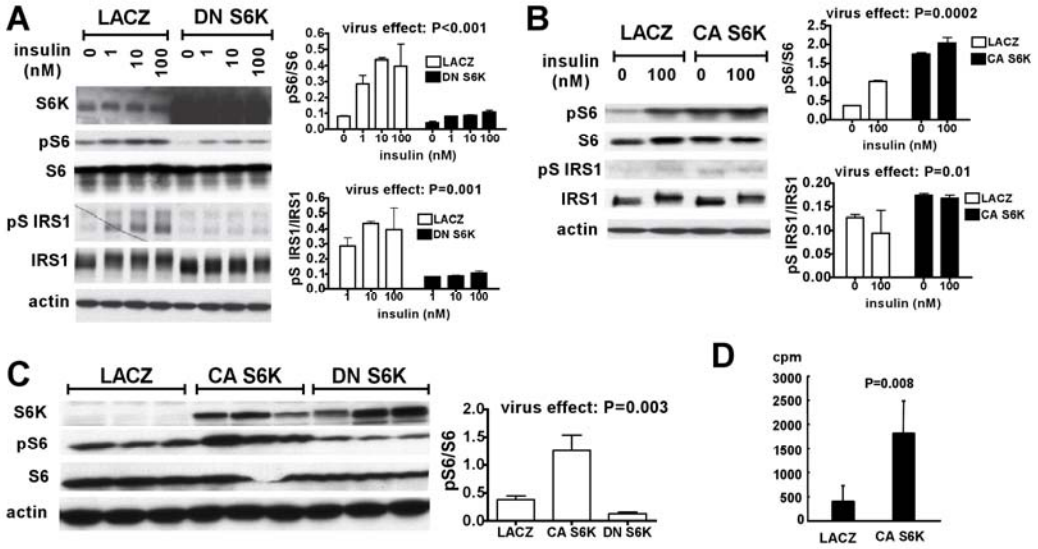
Supplementary Figure 1



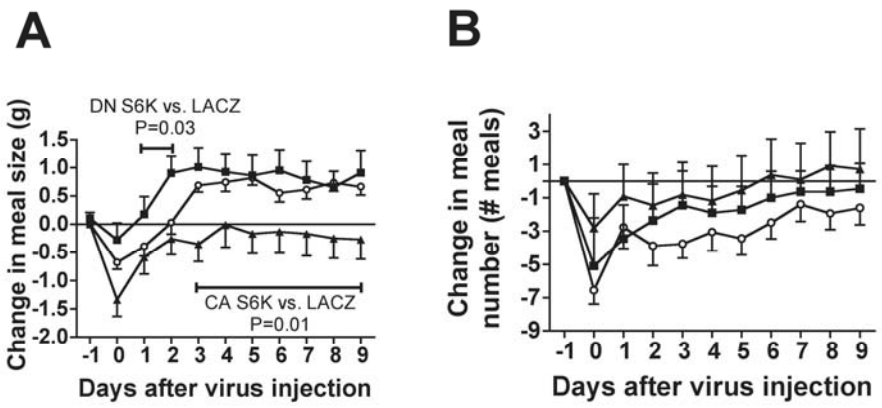
Supplementary Figure 1: Adenovirus spread control. S6K protein level in MBH, PVN (paraventricular nuclei of the hypothalamus) and LH (lateral hypothalamus) of rats expressing LACZ (n=4), CA S6K (n=4) or DN S6K (n=4). All data are means ± SEM.

Supplementary Figure 2



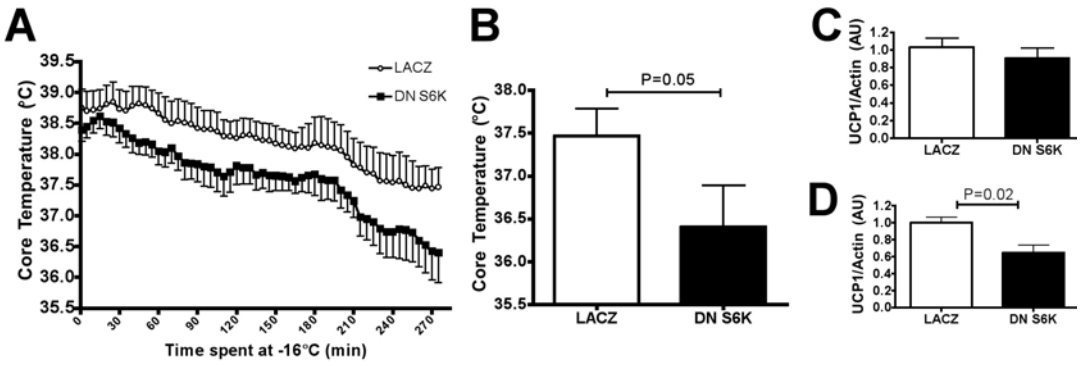
Supplementary Figure 2: Adenovirus functional validation. (A) Insulin-induced phosphorylation of S6 ribosomal protein (S6) and serine-phosphorylation of IRS1 in hypothalamic GT1-7 cells infected with LACZ or DN S6K. (B) Insulin-induced phosphorylation of S6 and serine-phosphorylation of IRS1 in hypothalamic GT1-7 cells infected with LACZ or CA S6K. (C) S6 phosphorylation in MBH of rats expressing LACZ (n=6), CA S6K (n=6) or DN S6K (n=6) rats. (D) S6K enzymatic activity in MBH of rats expressing LACZ (n=4) or CA S6K (n=4). All data are means ± SEM.

Supplementary Figure 3



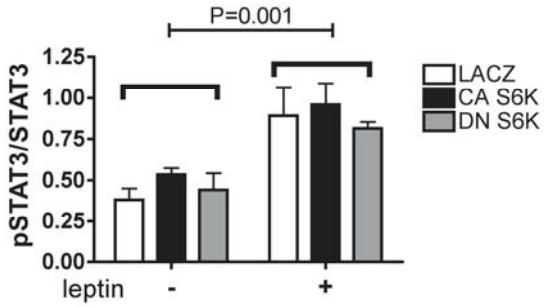
Supplementary Figure 3: (A) changes in mean meal size and (B) changes in daily mean number after MBH injection of CA S6K (n=12, filled triangle), DN S6K (n=13, filled square) or LACZ (n=18, open circle). All data are means ± SEM.

Supplementary Figure 4



Supplementary Figure 4: Core temperature (A) during a 5 hr cold challenge at -16°C 4 days after adenoviral injection in DN S6K (filled square, n=5) and LACZ (open circle, n=5) rats and (B) after the 5 hr cold challenge. Intrascapular brown adipose tissue UCP1 (C) mRNA level normalized to actin and (D) protein level normalized to actin in LACZ (n=5), and DN S6K rats (n=5). All data are means ± SEM.

Supplementary Figure 5



Supplementary Fig. 5: MBH leptin-induced STAT3 activation in rats expressing LACZ (n=4), CA S6K (n=4) and DN S6K (n=4) 30 min after MBH aCSF or leptin injection (150 ng in 150 nl per side). All data are means ± SEM.

Supplementary table 1

Supplementary Table 1	PCR primer sequences
AGRP	Forward TGGCAGAGGTGCTAGATCCA
	Reverse GCACAGGTCGCAGCAAGGTA
NPY	Forward CCGCCATGATGCTAGGTAAC
	Reverse TGTCGCAGA GCGGAGTAGTA
POMC	Forward: ACCTCCGAGAAGAGCCAGAC
	Reverse: GGCCTTGGAGTGAGAAGACC
UCP1	Forward: GCCTTCAGATCCAAGGTGAA
	Reverse: CGAGATCTTGCTTCCCAAAG
Actin	Forward: CTGGAGAAGAGCTATGAGCTGCCT
	Reverse: CTCCTGCTTGCTGATCCACATCTG