SUPPLEMENTAL INFORMATION

$G_{q/11}\alpha$ and $G_s\alpha$ Mediate Distinct Physiological Responses to Central Melanocortins

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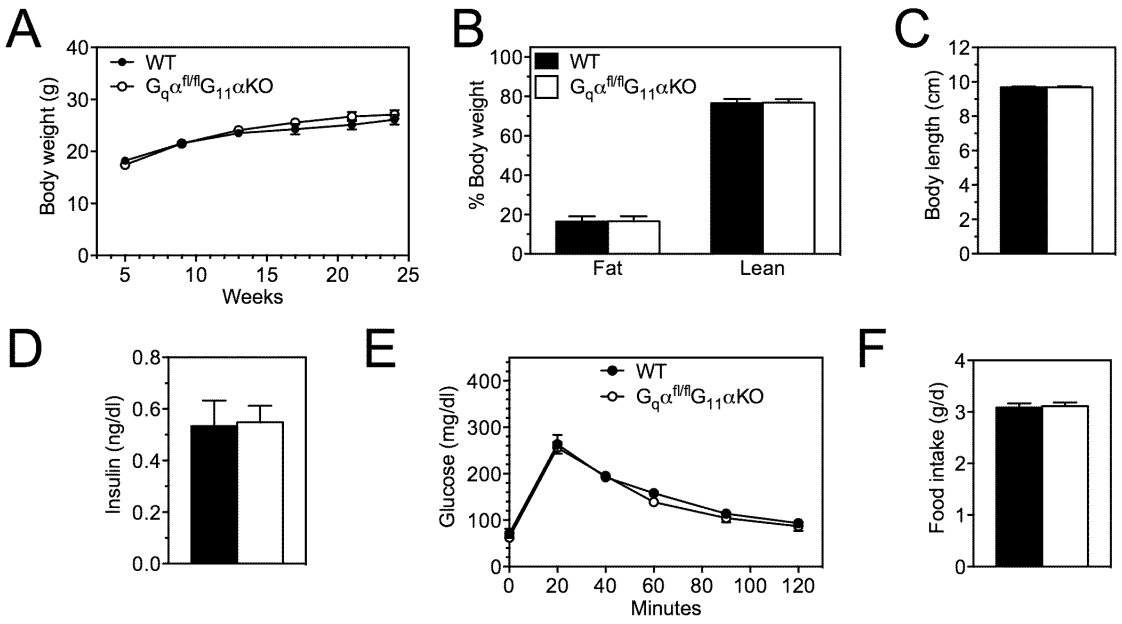
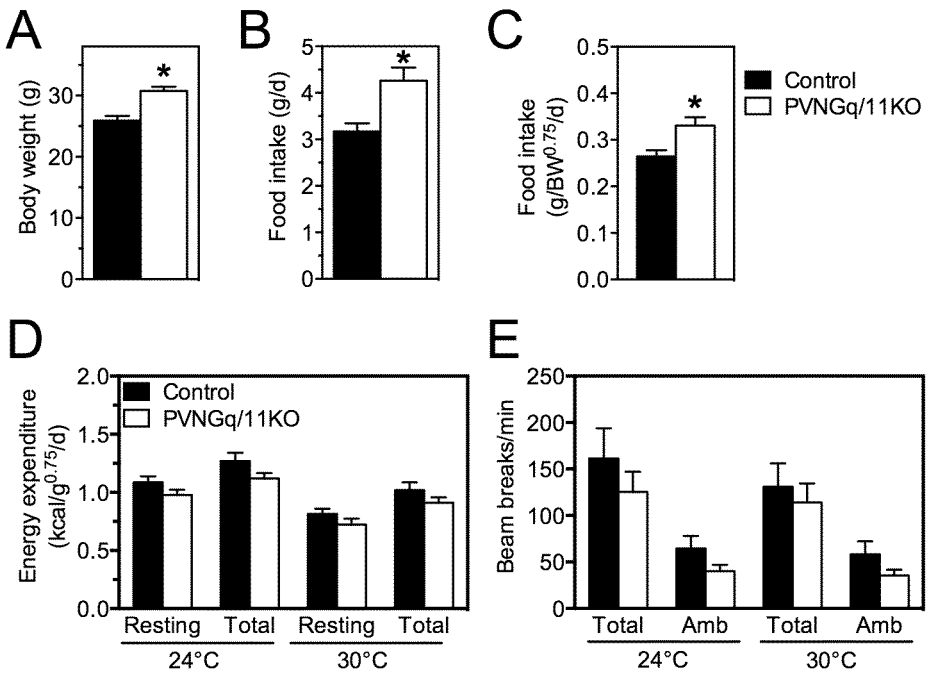
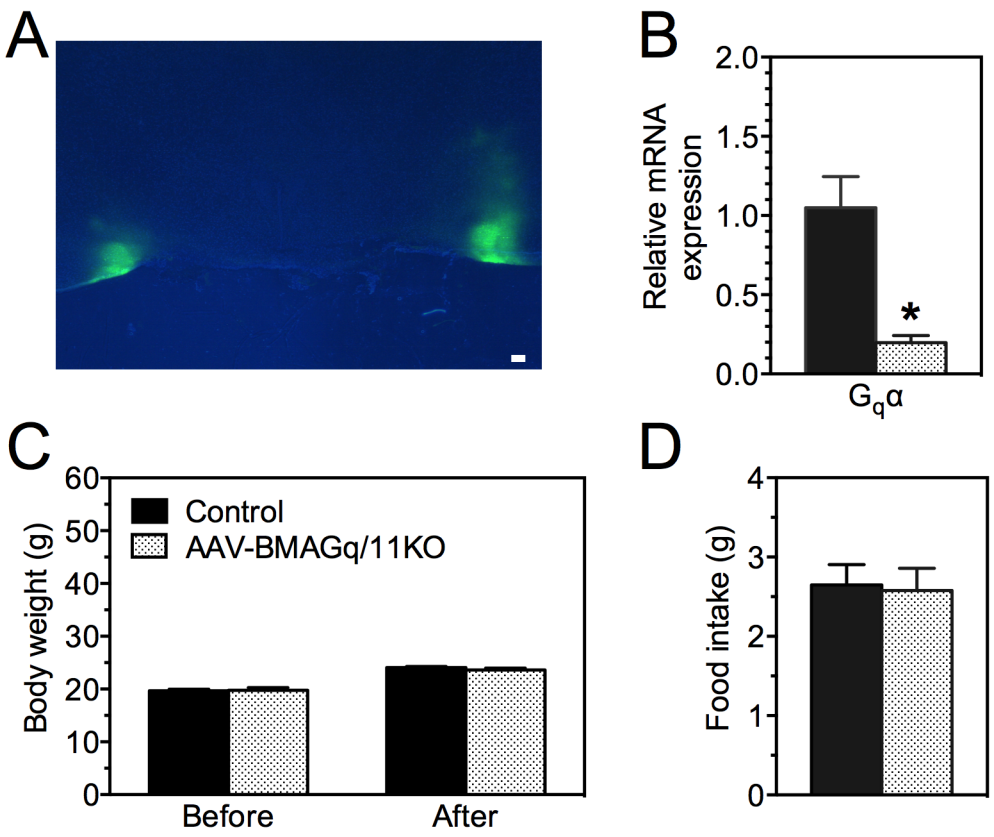


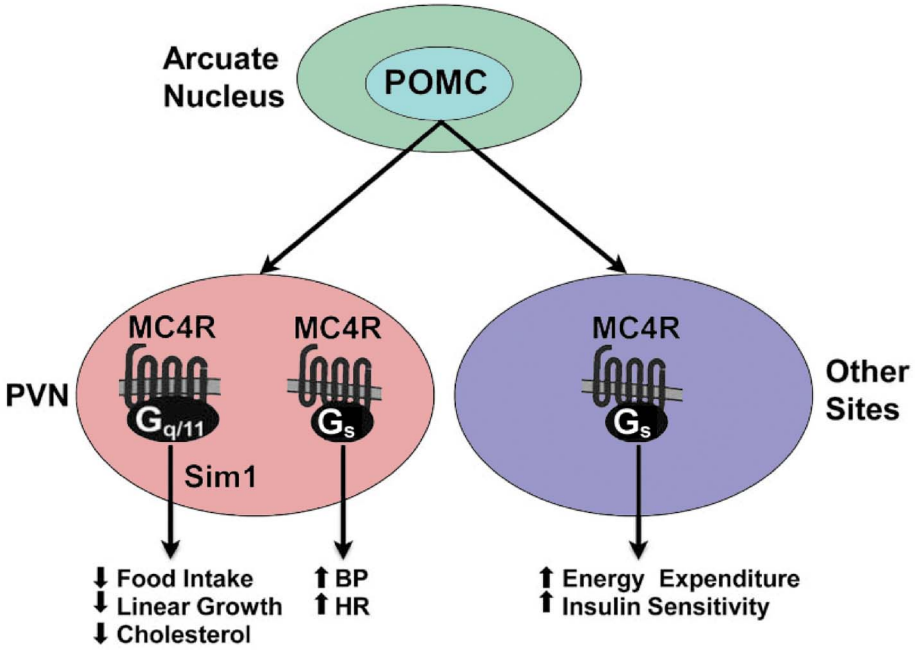
Supplemental Figure 1. $G_q\alpha^{flox/flox}G_{11}\alpha$KO mice have a normal metabolic phenotype. (**A**) Body weight curve of female $G_q\alpha^{flox/flox}$ $G_{11}\alpha$KO and wild type (WT) mice (n = 5/group). (**B-E**) (**B**) Body composition (n = 5-8/group), (**C**) body length (n = 5-9/group), (**D**) random serum insulin levels (n = 5-7/group), and (**E**) glucose tolerance test in 24-28 week old female $G_q\alpha^{flox/flox}$ $G_{11}\alpha$KO and WT mice (n=5/group). (**F**) Food intake in 6-8 week old female $G_q\alpha^{flox/flox}$ $G_{11}\alpha$KO and WT mice (n = 6-8/group). Data are expressed as mean ± SEM.



Supplemental Figure 2. Energy balance in older PVNGq/11KO mice. (**A**) Body weight (n = 8/group), (**B**) absolute food intake at 24°C (n = 6/group), (**C**) food intake normalized to body weight at 24°C (n = 6/group), (**D**) resting and total energy expenditure at 24°C and 30°C (n = 8/group) and (**E**) total and ambulatory activity levels at 24°C and 30°C (n = 8/group) in 12-16 wk-old female control and PVNGq/11KO mice. Data are expressed as mean ± SEM. **P*<0.05 vs. controls by Student's *t* test.



Supplemental Figure 3. Food intake and body weight are unaffected in AAV-BMAGq/11KO mice. (**A**) Representative image (2.5x, scale bar, 200 μM) showing fluorescence localized to basomedial amygdala, anterior (BMA) at 10 wks after bilateral injection of AAV-cre-GFP. (**B**) PVN mRNA levels of $G_q\alpha$ (*Gnaq*) measured 10 wks after viral injection in AAV-PVNGq/11KO and control mice (n = 4/group). (**C**) Body weight of AAV-BMAGq/11KO and control mice measured before and 10 wks after viral injection (n = 6/group). (**D**) Daily food intake of AAV-BMAGq/11KO and control mice measured 4 wks after viral injection (n = 6/group). Data are expressed as mean ± SEM. **P*<0.05 vs. controls by Student's *t* test.



Supplemental Figure 4. Proposed MC4R signaling pathways. Schematic diagram showing G protein pathways involved in physiological functions based upon our findings. In PVN neurons MC4R receptors bind melanocortins released from POMC neurons originating in the arcuate nucleus and activate $G_{q/11}\alpha$ to mediate effects on food intake, linear growth, and cholesterol metabolism and $G_s\alpha$ to mediate effects on BP and HR. Sim1 is likely involved in the $G_{q/11}\alpha$ pathway regulating food intake and linear growth. MC4R effects on energy expenditure and insulin sensitivity are mediated via $G_s\alpha$ at sites outside of the PVN. For these latter effects the activating POMC neurons likely originate from the arcuate nucleus as well as other sites.

Supplemental Table 1. Primer sequences used for real-time qRT-PCR.

Gene	Primer sequence	Product size (bp)
MC4R	Forward: CAGGCACAGGGACCATCCGC	94
	Reverse: AACGGGGCCCAGCAGACAAC	
Sim1	Forward: CCTGCGGTGGCTACAAGGTCA	131
	Reverse: CGGAGGCAGGGAGTGACCCA	
OXT	Forward: TCTCGCTTGCTGCCTGCTTGG	114
	Reverse: GGGAGACACTTGCGCATATCCAG	
AVP	Forward: GCAGCGACGAGAGCTGCGTG	86
	Reverse: TGTGGCGTTGCTTGGCTCCC	
CRH	Forward: AGGGAGGAGAAGAGAGCGCCCC	93
	Reverse: TGCAAGGCAGGCAGGACGAC	
TRH	Forward: ATCCTGCGCCTTGCTGGAAGC	134
	Reverse: CAAGGTCCCCTCGCACACGC	
BDNF	Forward: TTGGCAAGCTCCGGGTTGGT	108
	Reverse: ACCTGGTGGAACTTCTTTGCGGC	
SST	Forward: CTGGCTGCGCTCTGCATCGT	120
	Reverse: GGCCAGTTCCTGTTTCCCGGT	
β−actin	Forward: GACCTCTATGCCAACACAGT	94
	Reverse: TAGGAGCCAGAGCAGTAATC	
$G_q\alpha$	Forward: TGGACCGTGTAGCCGACCCT	135
	Reverse: GGCCCCCTACATCGACCATTCTGA	

Supplemental Table 2. Probe sequences for in situ hybridization

$G_s\alpha$ probe

GCTGCCTCGGCAACAGTAAGACCGAGGACCAGCGCAACGAGGAGAAGGCGCAGCG
CGAGGCCAACAAAAAGATCGAGAAGCAGCTGCAGAAGGACAAGCAGGTCTACCGG
GCCACGCA

$G_q\alpha$ probe

TCCTATCTGCCTACACAACAAGACGTGCTTAGAGTTCGAGTCCCCACTACAGGGATC
ATCGAATACCCCTTTGACTTACAAAGTGTCATTTTCAGAATGGTCGATGTAGGGGGC
CAAAGGTCAGAGAGAAGAAAATGGATACACTGCTTTGAAAATGTCACCTCCATCAT
GTTTCTAGTAGCGCTTAGCGAATATGATCAAGTTCTTGTGGAGTCAGACAATGAGAA
CCGCATGGAGGAGAGCAAAGCACTCTTTAGAACAATTATCACCTACCCCTGGTTCCA
GAACTCCTCTGTGATTCTGTTCTTAAACAAGAAAGATCTTCTAGAGGAGAAAATCAT
GTATTCCCACCTAGTCGACTACTTCCCAGAATATGATGGACCCCAGAGAGATGCCCA
GGCAGCTCGAGAATTCATCCTGAAAATGTTCGTGGACCTG

$G_{11}\alpha$ probe

TGCTGCTACTTGGCACTGGCGAGAGCGGGAAGAGTACCTTCATCAAGCAGATGCGC
ATCATCCACGGGGCCGGCTACTCGGAGGAGGACAAGCGCGGCTTCACCAAGTTGGT
GTACCAGAACATCTTTACCGCCATGCAGGCCATGGTGCGCGCCATGGAGACGCTCA
AGATCCTCTACAAGTATGAGCAGAACAAGGCCAATGCACTCCTGATCCGGGAGGTC
GATGTGGAGAAGGTCACAACTTTTGAGCACCAGTATGTGAATGCCATCAAGACGCT
GTGGAGTGACCCTGGTGTCCAGGAGTGTTACGATCGCAGGCGGGAGTTCCAGCTATC
TGACTCGGCTAAGTACTACTTGACGGACGTGGACCGCATCGCCACAGTA