SUPPLEMENTARY DATA

# The BBSome in POMC and AgRP Neurons is Necessary for Body Weight Regulation and Sorting of Metabolic Receptors

Deng-Fu Guo[1], Zhihong Lin[1], Yuanming Wu[2], Charles Searby[3], Daniel R. Thedens[4], George B. Richerson[2], Yuriy M. Usachev[1,7], Justin L. Grobe[1,6,7], Val C. Sheffield[3,6,7] and Kamal Rahmouni[1,5,6,7,*]

Department of Pharmacology[1], Department of Neurology[2], Department of Pediatrics[3], Department of Radiology[4], Department of Internal Medicine[5], Obesity Education and Research Initiative[6] and Fraternal Order of Eagles Diabetes Research Center[7], University of Iowa, Iowa City, Iowa

**Short title:** Neuronal BBSome and Energy Homeostasis.

**Contain:**

**Supplemental figures 1-8**

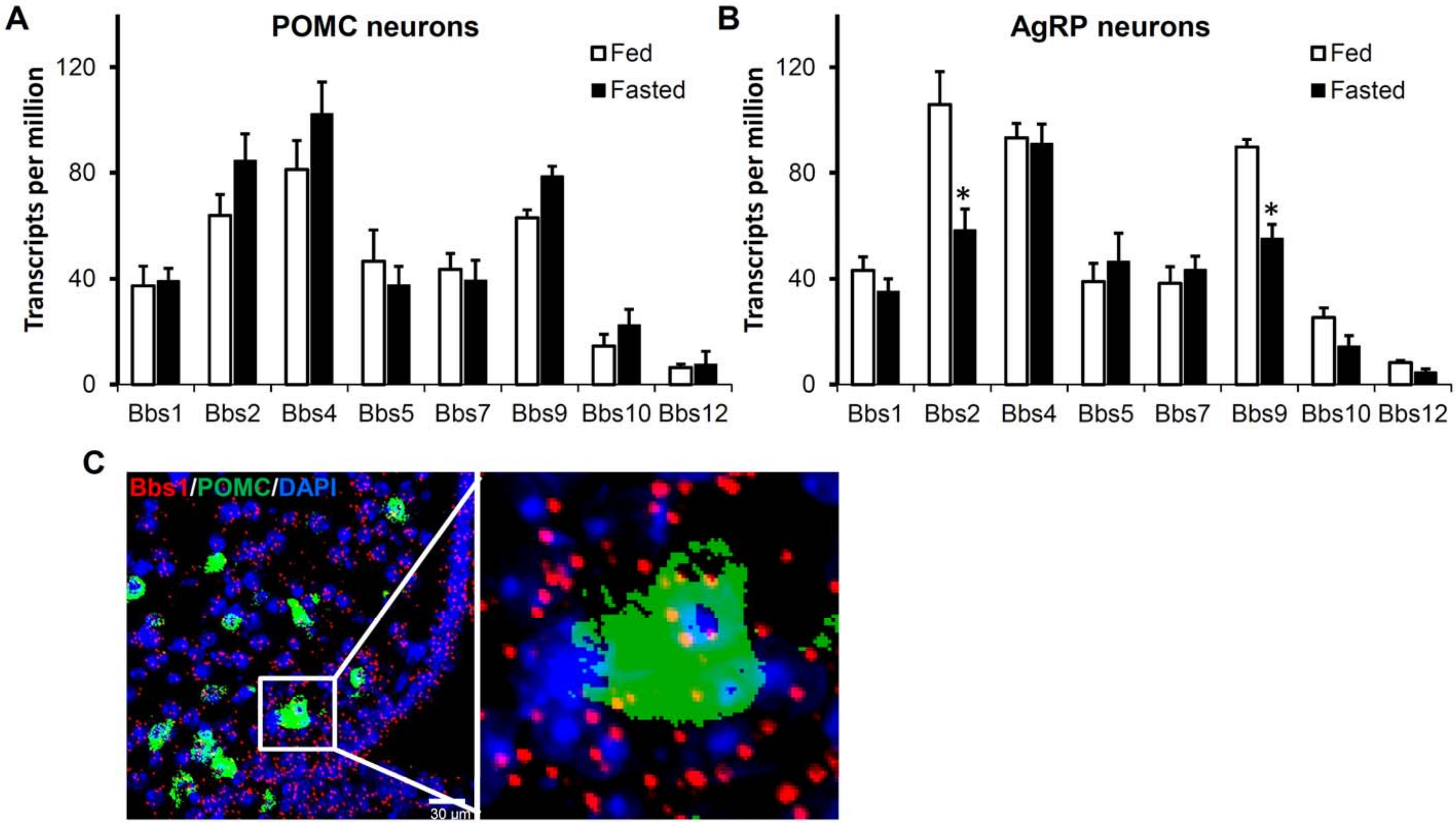
**Supplemental table 1**

***Corresponding author:**

Kamal Rahmouni, Ph.D.
Department of Pharmacology
University of Iowa Carver College of Medicine
Iowa City, IA, 52242, USA
e-mail: kamal-rahmouni@uiowa.edu
Tel: 319 353 5256
Fax: 319 353 5350

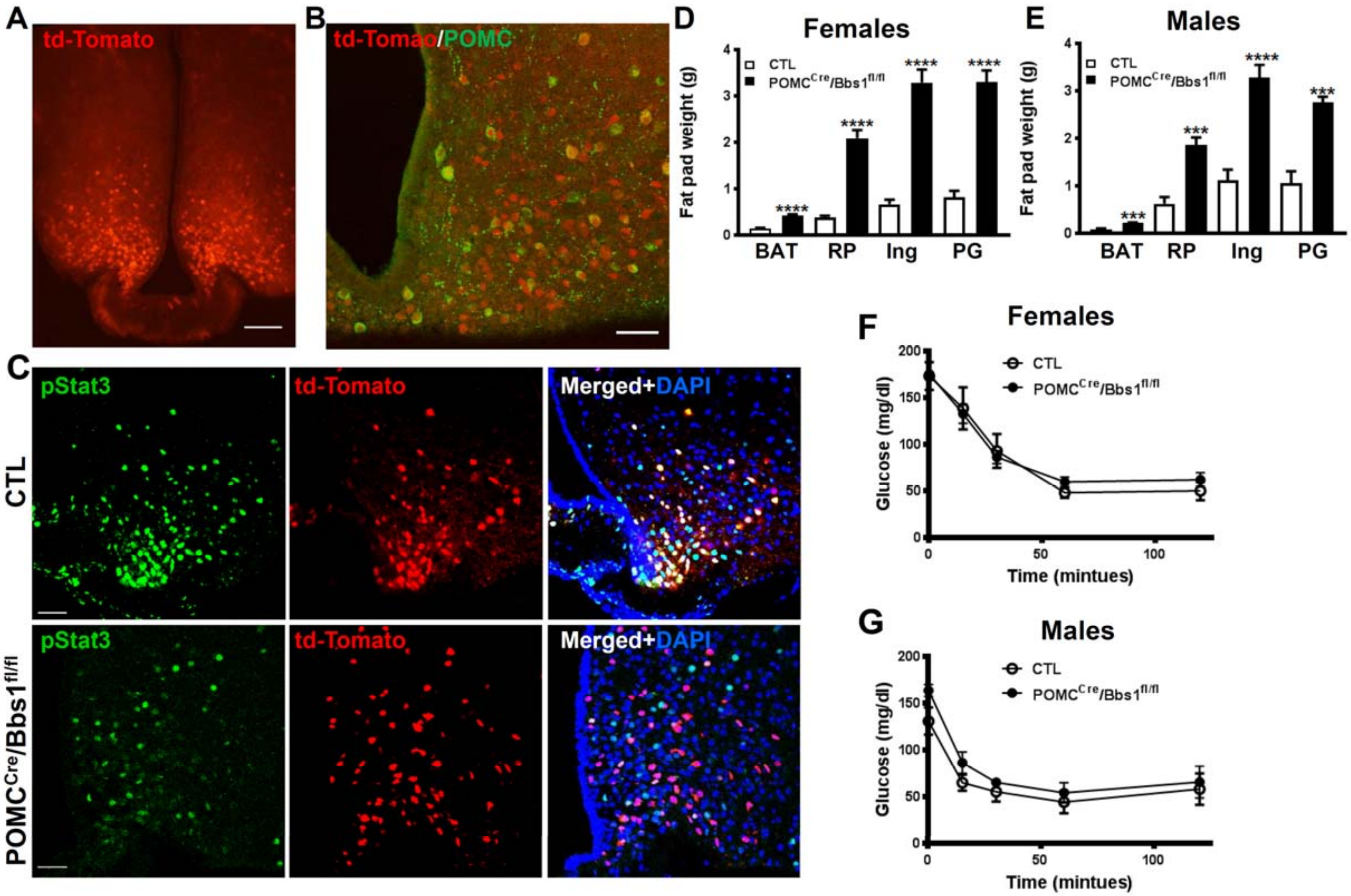
SUPPLEMENTARY DATA

**Supplementary Figure S1**. Expression of BBS genes in POMC and AgRP neurons is modulated by energy status. (A-B) In silico reanalysis of available data (ref 23) showing BBS gene expression in POMC and AgRP neurons in fed versus fasted mice (cells obtained from 6 fed mice and 5 fasted mice were used for single-cell RNA sequencing). (C) Co-localization of Bbs1 mRNA and POMC mRNA by RNAscope. *P< 0.05, vs. the fed group.



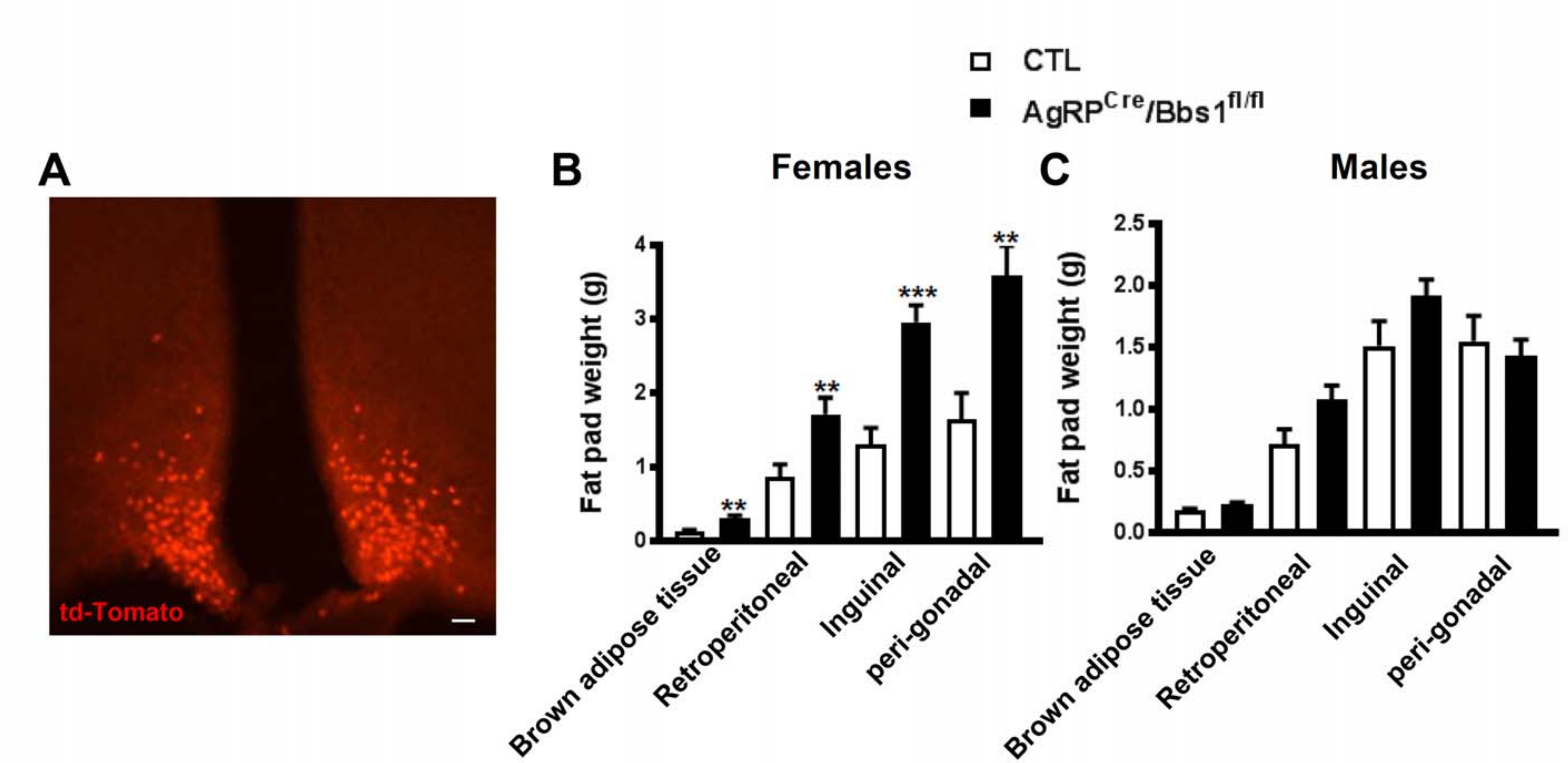
SUPPLEMENTARY DATA

**Supplementary Figure S2**. Evidence of Cre recombinase (presence of td-Tomado) (A) and its co-localization with POMC (B) in the arcuate nucleus of $POMC^{cre}$/Td-Tomato mice. (C) Intraperitoneal leptin increased p-Stat3 in POMC neurons of control mice, but not $POMC^{Cre}/Bbs1^{fl/fl}$ mice.(D-E) Weight of fat pads of $POMC^{Cre}/Bbs1^{fl/fl}$ mice compared to control littermates (n=6-8 per group: 7 males and 7 females for controls and 8 males and 6 females for $POMCcre/Bbs1^{fl/fl}$ mice). (F-G) Insulin tolerance test (n=10-12/group: 5 males and 5 females for controls and 6 males and 6 females for $POMC^{cre}/Bbs1^{fl/fl}$ mice). BAT: brown adipose tissue, RP: retroperitoneal fat, Ing: inguinal fat, PG: perigonaldal fat. ***P<0.001 and ****P<0.0001 vs. control group.



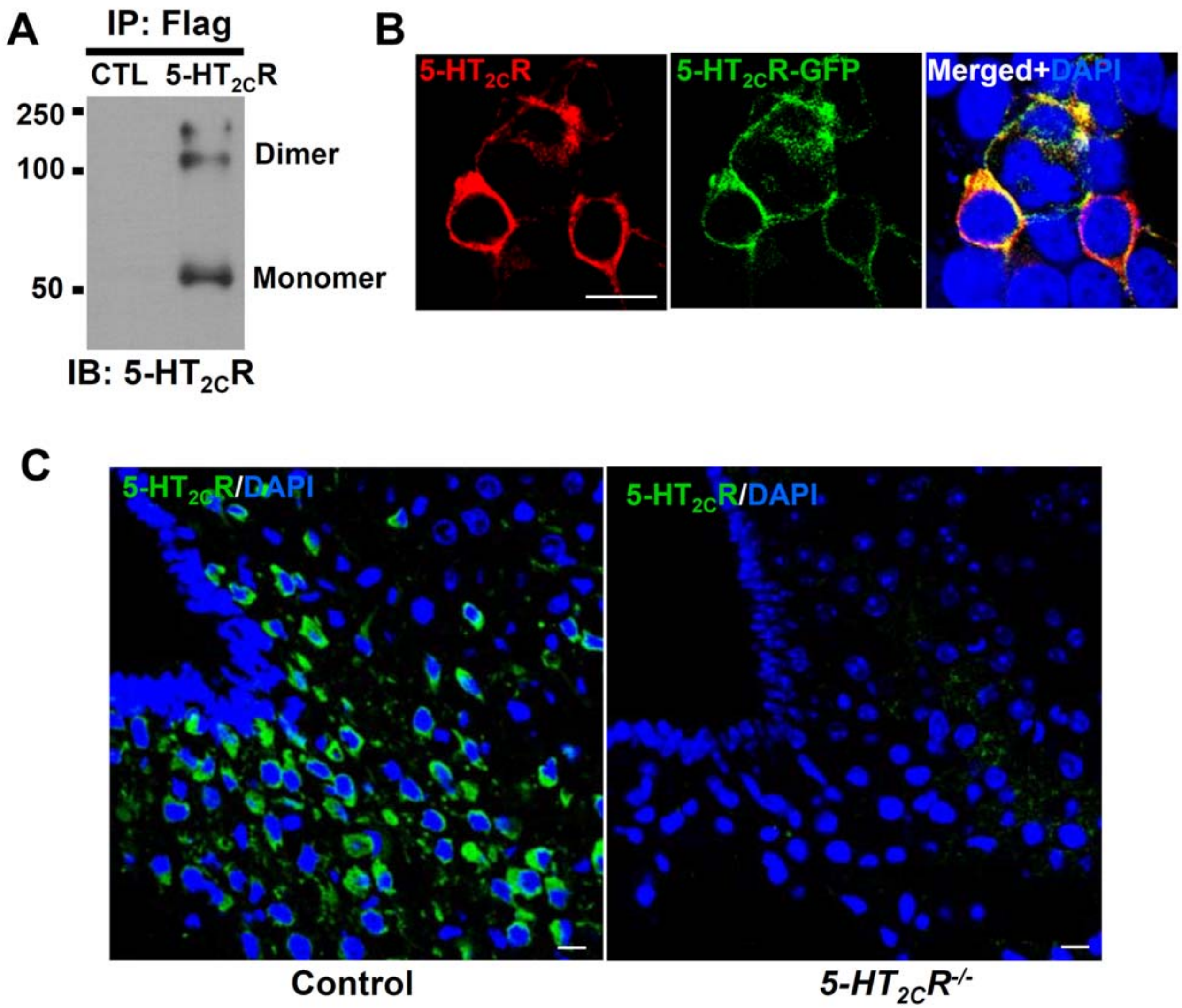
SUPPLEMENTARY DATA

**Supplementary Figure S3**. BBSome disruption in AgRP neurons causes obesity. (A) Evidence of Cre recombinase (presence of td-Tomato) in the arcuate nucleus of the hypothalamus (where AgRP neurons reside) in $AgRP^{Cre}$/ td-Tomato mice. (B-C) Weight of fat pads in female (B) and male (C) $AgRP^{cre}/Bbs1^{fl/fl}$ mice compared to control littermates (n=6-8 per group: 7 males and 9 females for control and 7 males and 8 females for $AgRP^{Cre}/Bbs1^{fl/fl}$ mice). **P< 0.01 and ***P<0.001 vs. control group. Scale bar: 100 mm.



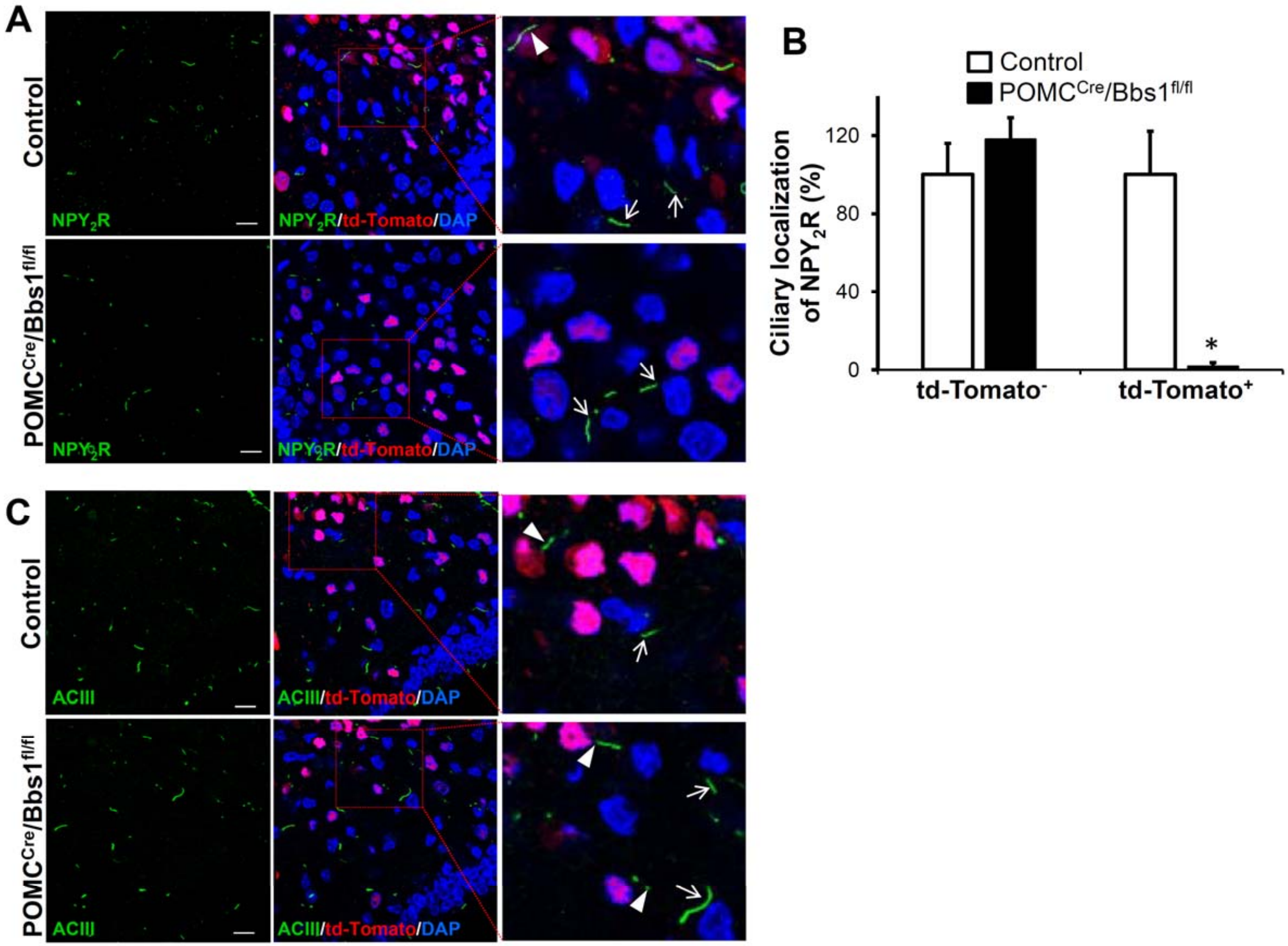
SUPPLEMENTARY DATA

**Supplementary Figure S4**. Validation of 5-$HT_{2C}$R antibody. (A) The anti-5-$HT_{2C}$R antibody detects monomer, dimer and oligomer forms of the Flag-tagged 5-$HT_{2C}$R in HEK 293 cells. (B) Staining with the anti-5-$HT_{2C}$R antibody co-localized with GFP in HEK 293 cells transfected with GFP tagged 5-$HT_{2C}$ R (C) The anti-5-$HT_{2C}$ R antibody detects the endogenous 5-$HT_{2C}$ R in the hypothalamic arcuate nucleus of control mice. This staining is absent in the arcuate nucleus of 5-$HT_{2C}$ R knockout mice. Scale bar: 10 mm

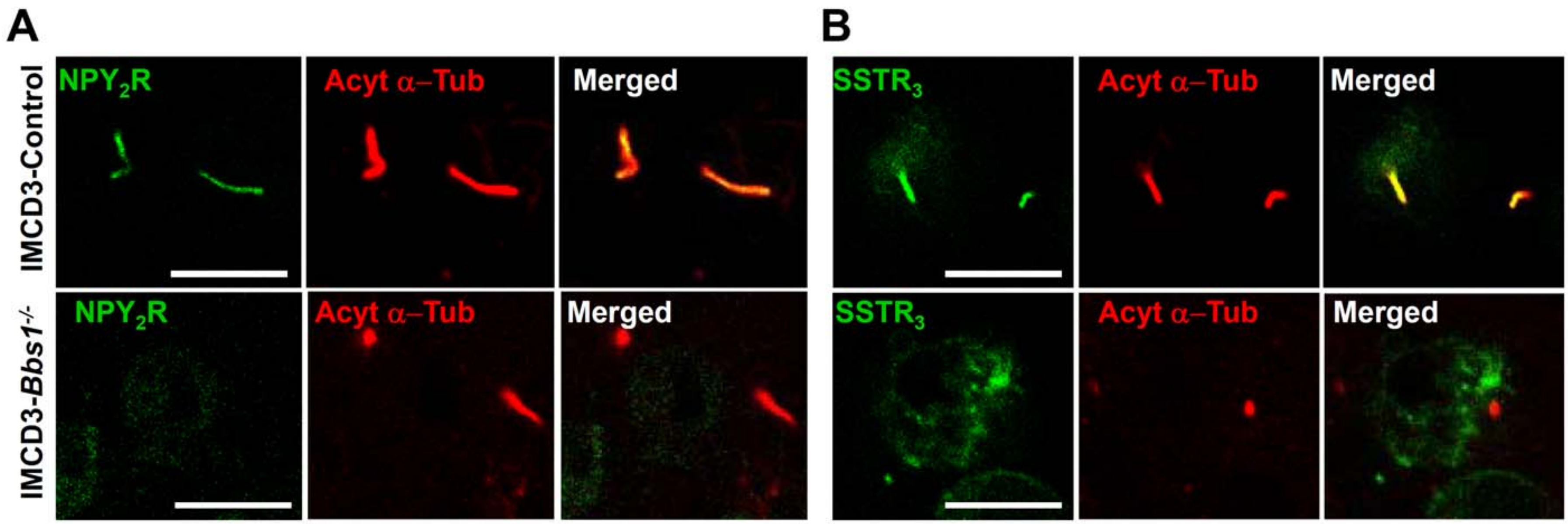


SUPPLEMENTARY DATA

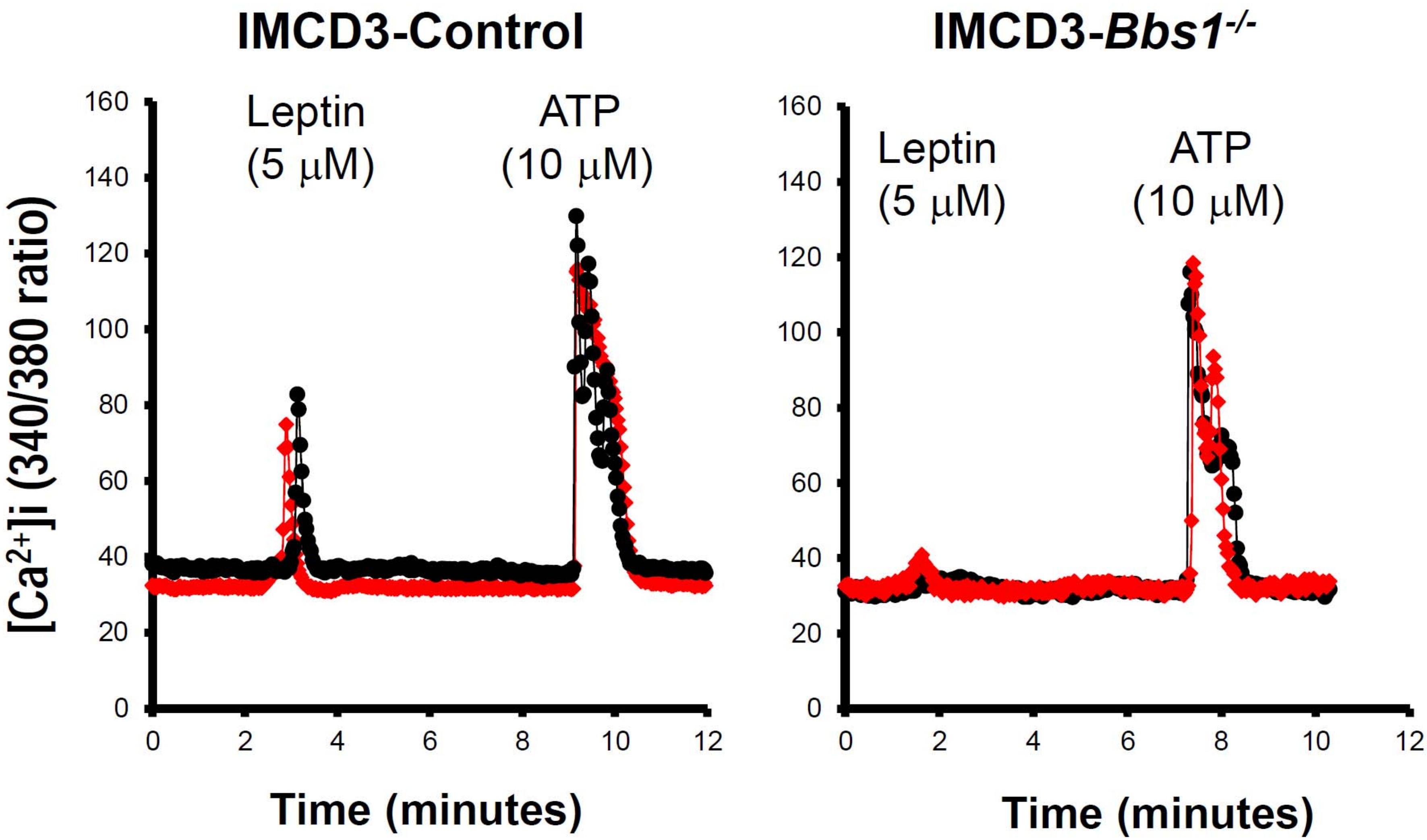
**Supplementary Figure S5**. The BBSome is required for the ciliary localization of $NPY_2R$ in POMC neurons. (A-B) Loss of $NPY_2R$ in cilia of td-Tomato$^+$ (red, arrow head), but not in td-Tomato$^-$ (non-red, arrow), cells in the arcuate nucleus of $POMC^{Cre}/Bbs1^{fl/fl}$ mice (n=3) vs controls (n=4). (C) Presence of cilia in td-Tomato$^+$ (arrow head) and td-Tomato$^-$ (arrow) cells of $POMC^{cre}/Bbs1^{fl/fl}$ mice. *$P<0.05$ vs Control. Scale bar: 10 μm.



**Supplementary Figure S6**. The BBSome is required for the ciliary localization of $NPY_2R$ and $SSTR_3$. (A-B) Loss of localization in cilia (stained with Acetylated α-Tub) of $NPY_2R$ (A) and $SSTR_3$ (B) in IMCD3-*Bbs1*$^{-/-}$ cells. Scale bar: 10 μm.

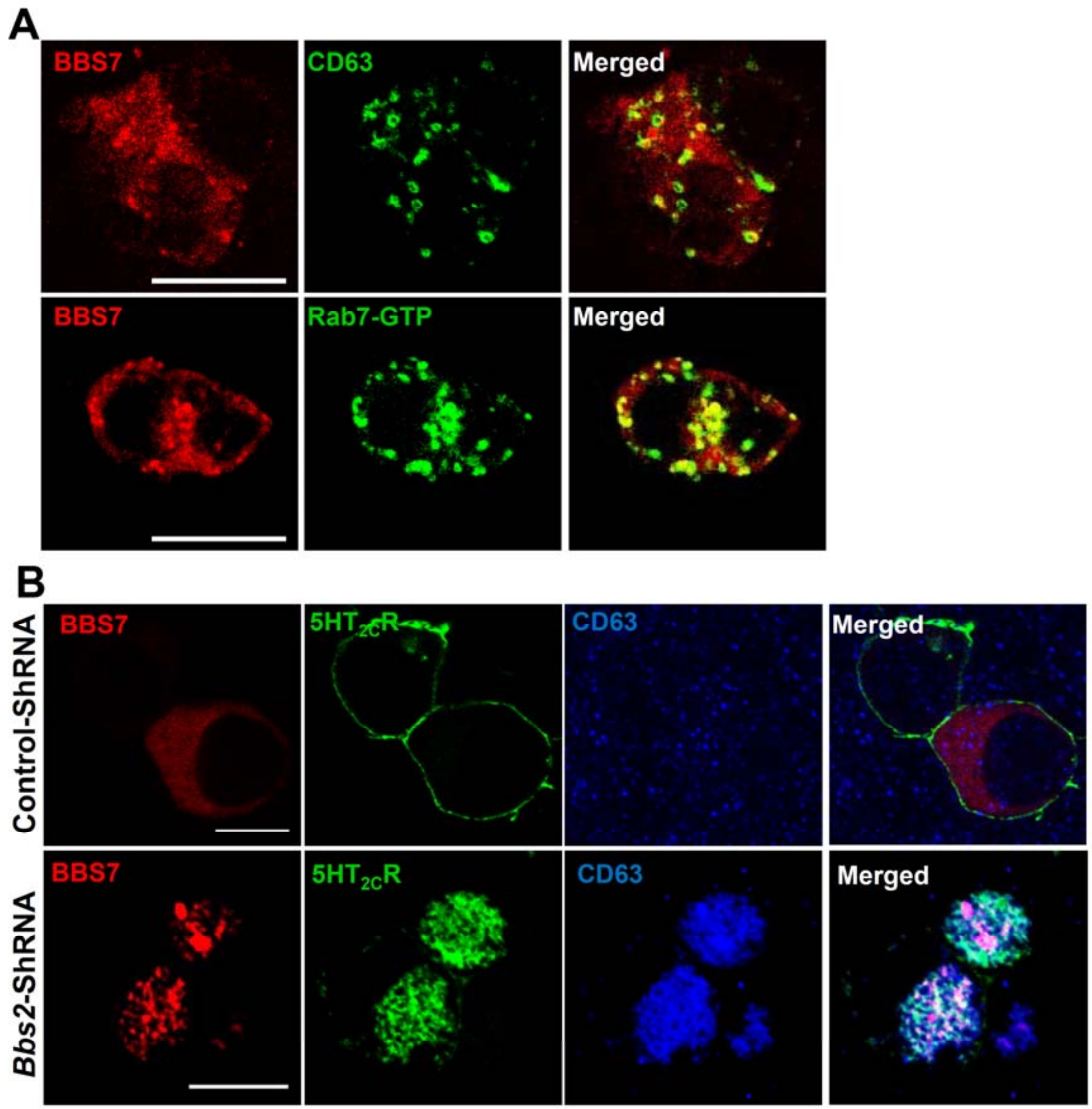


**Supplementary Figure S7**. Representative tracings showing reduced $[Ca^{2+}]i$ response to leptin in control and $Bbs1^{-/-}$ IMCD3 cells stably expressing the LepRb.



SUPPLEMENTARY DATA

**Supplementary Figure S8**. BBSome proteins interacts with 5-$HT_{2C}$R in the late endosome. (A) BBS7 (red) colocalizes with markers of the late endosome: CD63 (green) or Rab7-GTP (green). (B) Silencing *Bbs2* gene expression promotes BBS7 (red) and 5-$HT_{2C}$R (green) interaction in late endosome marked with CD63 (blue) in HEK 293 cells. Scale bar: 10 µm.



SUPPLEMENTARY DATA

**Supplementary Table S1.** Primer sequences used.

| Name | Sense (5'-3') | Anti-sense (5'-3') |
|---|---|---|
| Genotyping | | |
| *Cre* | ACCTGAAGATGTTCGCGATTATCT | ACCGTCAGTACGTGAGATATCTT |
| *Bbs1* | ACATCCACACCTTCTCCTCCT | GCCTACCTTGTACTCCCCATC |
| Hypothalamic gene expression | | |
| S18 | ACTGCCATTAAGGGCGTGG | CCATCCTTCACATCCTTCTG |
| AgRP | CAGAAGCTTTGGCGGAGGT | AGGACTCGTGCAGCCTTACAC |
| NPY | TCAGACCTCTTAATGAAGGAAAGCA | GAGAACAAGTTTCATTTCCCATCA |
| POMC | CTGCTTCAGACCTCCATAGATGTG | CAGCGAGAGGTCGAGTTTGC |
| Generation of Bbs1 knock-out cells | | |
| sgRNA | CCTTTGAGCACCTTCAGGCG | |
| *Bbs1* | ACTGCCATTAAGGGTGTGG | CCATCCTTTACATCCTTCTG |