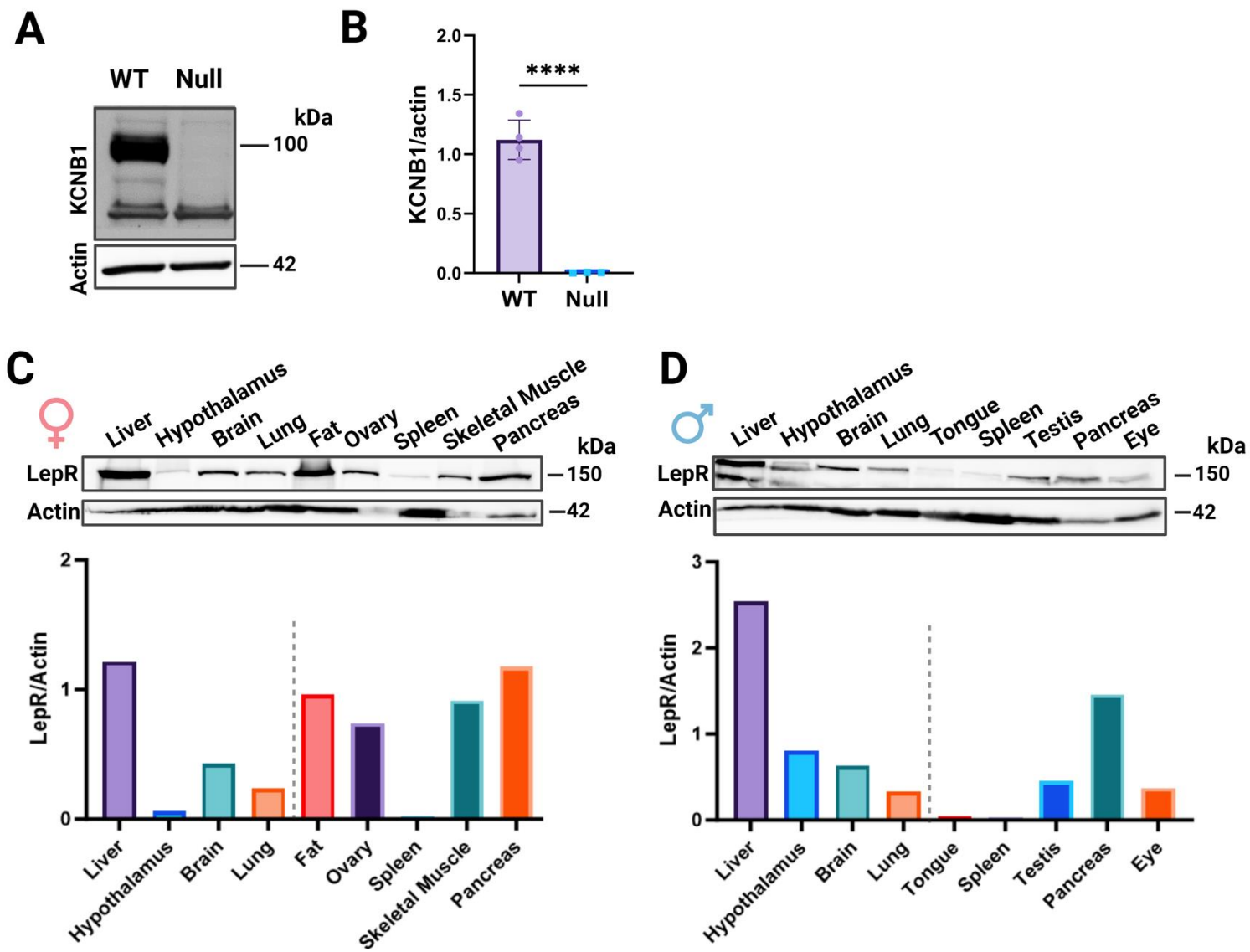

Figure S1 KCNB1 protein is detected in hypothalamic lysates

A-B) Representative Western blots of KCNB1 protein in WT and NULL hypothalamic lysates and densitometric quantification. N=3-4 mice/genotype. *P<0.001* (two-tailed Student's t-test).

C-D) Representative Western blots of LepR protein in lysates of the indicated tissues from female and male mice and quantifications. The levels of expression of the LepR match those reported in the Human Protein Atlas (https://www.proteinatlas.org/ENSG00000116678-LEPR/tissue). LepR expression is maximal in liver, white adipocytes and pancreas and minimal in spleen, tongue and eye.

Loading controls were actin and Bradford colorimetric assay.

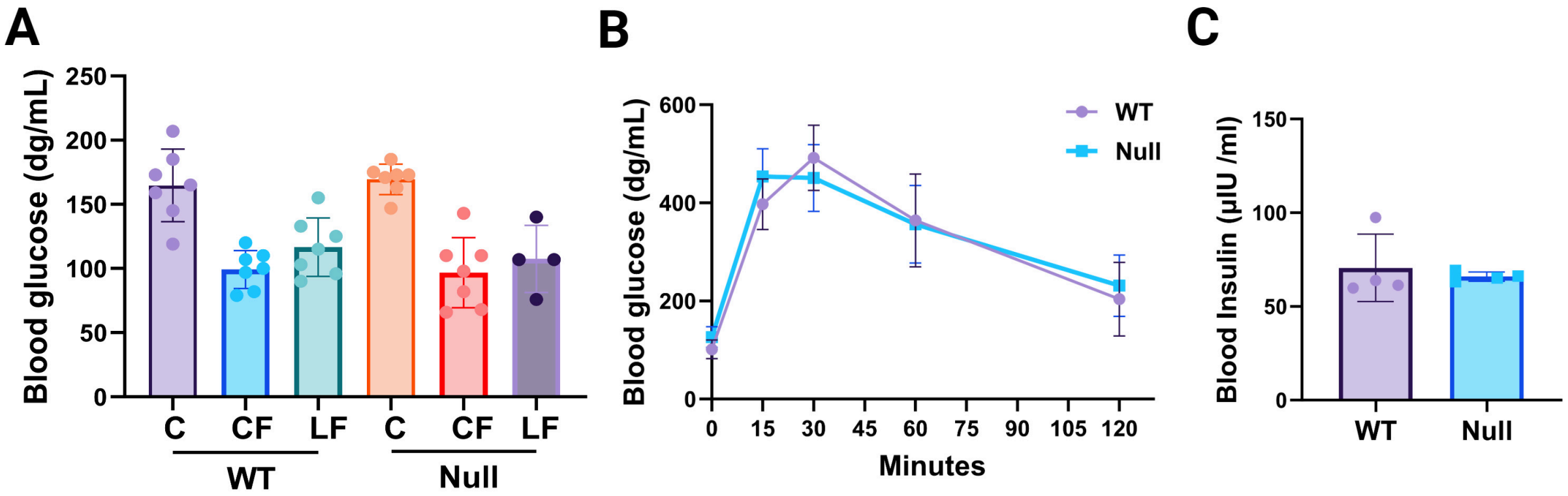

Figure S2 Glucose and insulin levels are normal in the NULL

A) Blood glucose in 12 weeks old WT and NULL mice fed *ad libitum* (C) or following 24 hours fasting (CF) or following 24 hours fasting in the presence of acute leptin administration (1.0 mg/kg body weight) at the beginning and the end of the fasting period (LF). N=4-7 mice/genotype.

B) Glucose load test on 12 weeks old WT and NULL mice. Single-caged mice was fasted for 16 hours. At t=0 min, mice was intraperitoneally injected 2.0 g/kg body weight of glucosium dissolved in drinking water. Blood glucose was measured with a glucose monitor/strips. N=7 mice/genotype.

C) Insulin levels in 12 weeks old WT and NULL mice under normal conditions (*ad libitum*).

Data are not statistically significant (two-tailed t-test , one-way ANOVA).

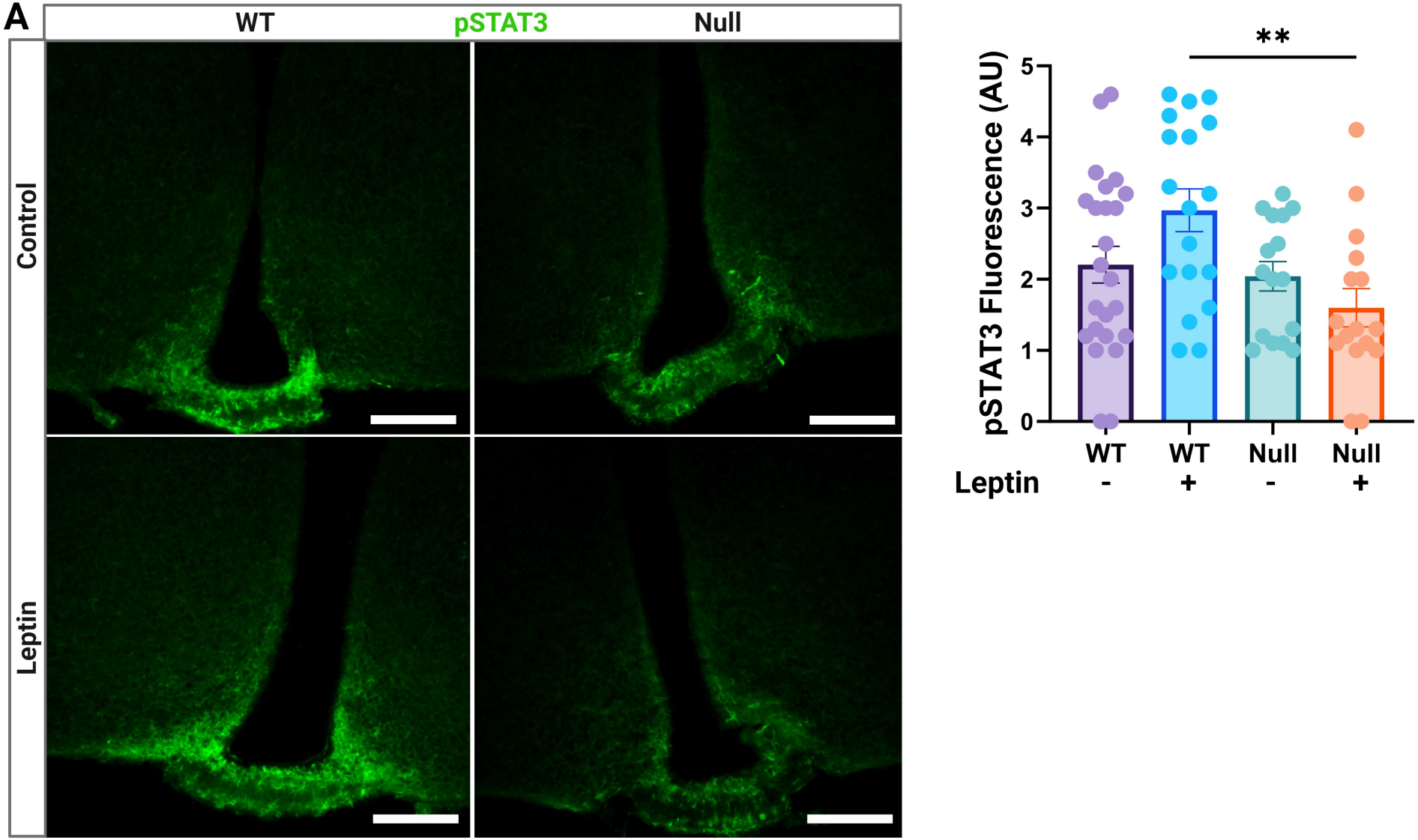

Figure S3 Leptin does not alter pSTAT3 immunoreactivity in NULL

A) Representative images of WT and NULL mediobasal hypothalamic slices stained with phospho Y705 STAT3 antibody (green) in the absence/presence of leptin and quantifications. Scale bars 500 μm. Animals were injected with either vehicle or 1.0 mg/kg/body weight leptin and sacrificed 90 min later. N=2 mice/genotype/experimental condition. **P<0.05* (ANOVA with Šídák's post hoc)

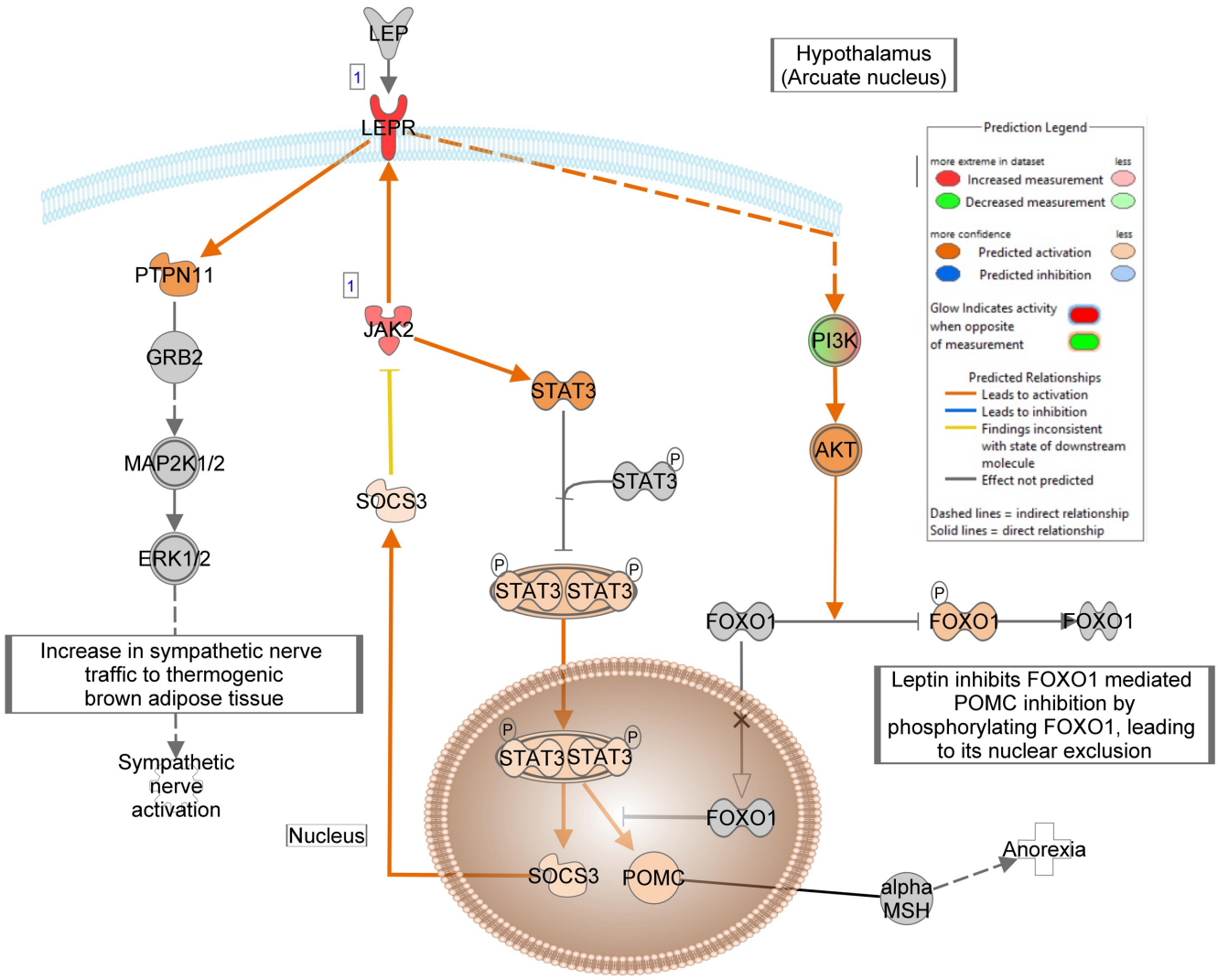

Figure S4 Ingenuity analysis predicts increased POMC production in the NULL. QIAGEN IPA was performed using NULL transcriptome data. In addition to the canonical STAT3 pathway the algorithm predicts increased POMC synthesis via Akt/FOXO1 and ERK1/2 signaling.

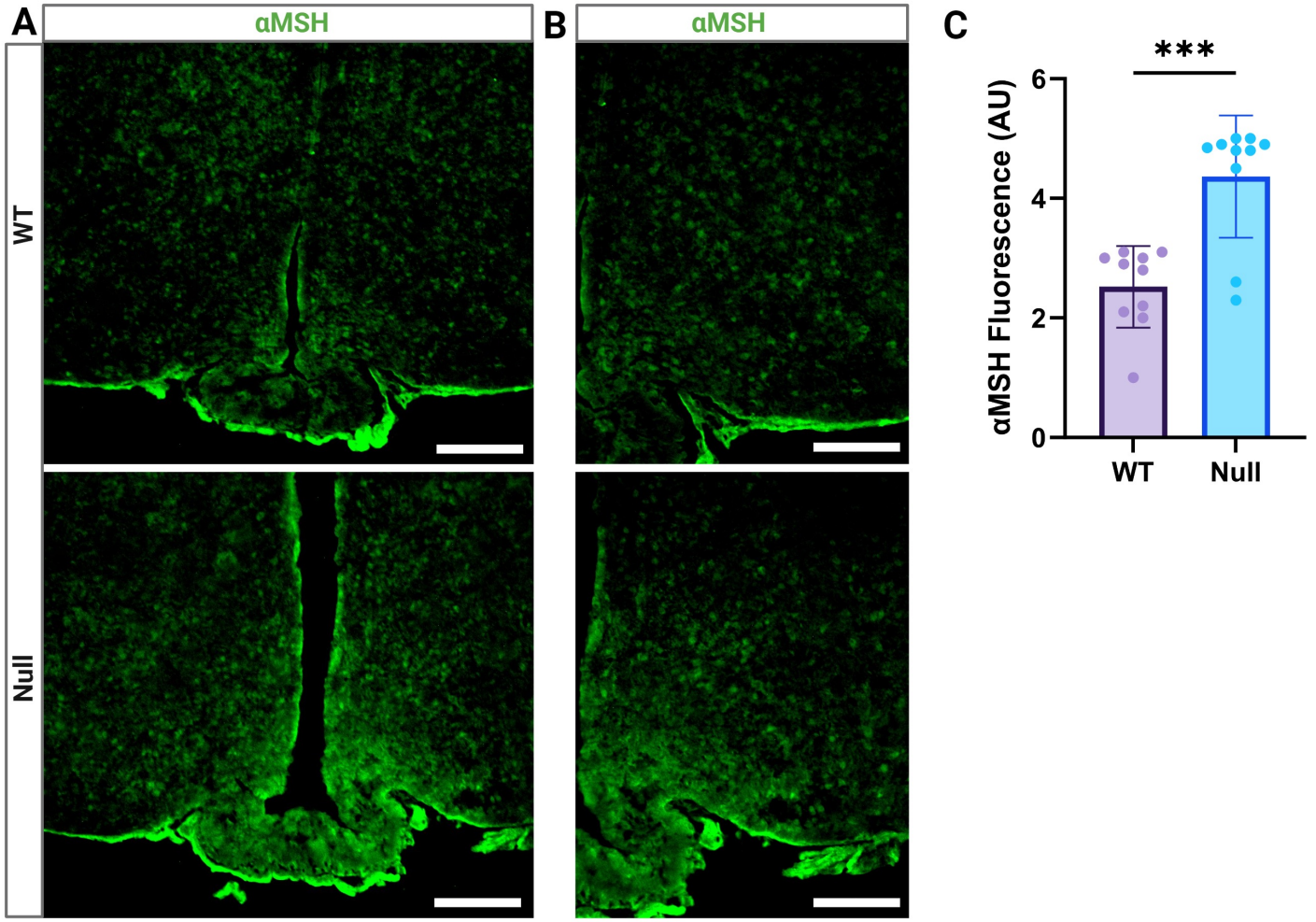

Figure S5 αMSH is increased in hypothalamic brain slices of the NULL.
A) Representative images of WT and NULL mediobasal hypothalamic slices stained with αMSH antibody (green). Scale bars 500 μm.
B) Magnifications of the ARH area. Scale bar 200 μm.
C) Quantifications of αMSH immunofluorescence in WT and NULL slices. Animals were injected with either vehicle or 1.0 mg/kg/body weight leptin and sacrificed 90 min later. N=2 mice/genotype. ****P<0.001,* two-tailed t-test.

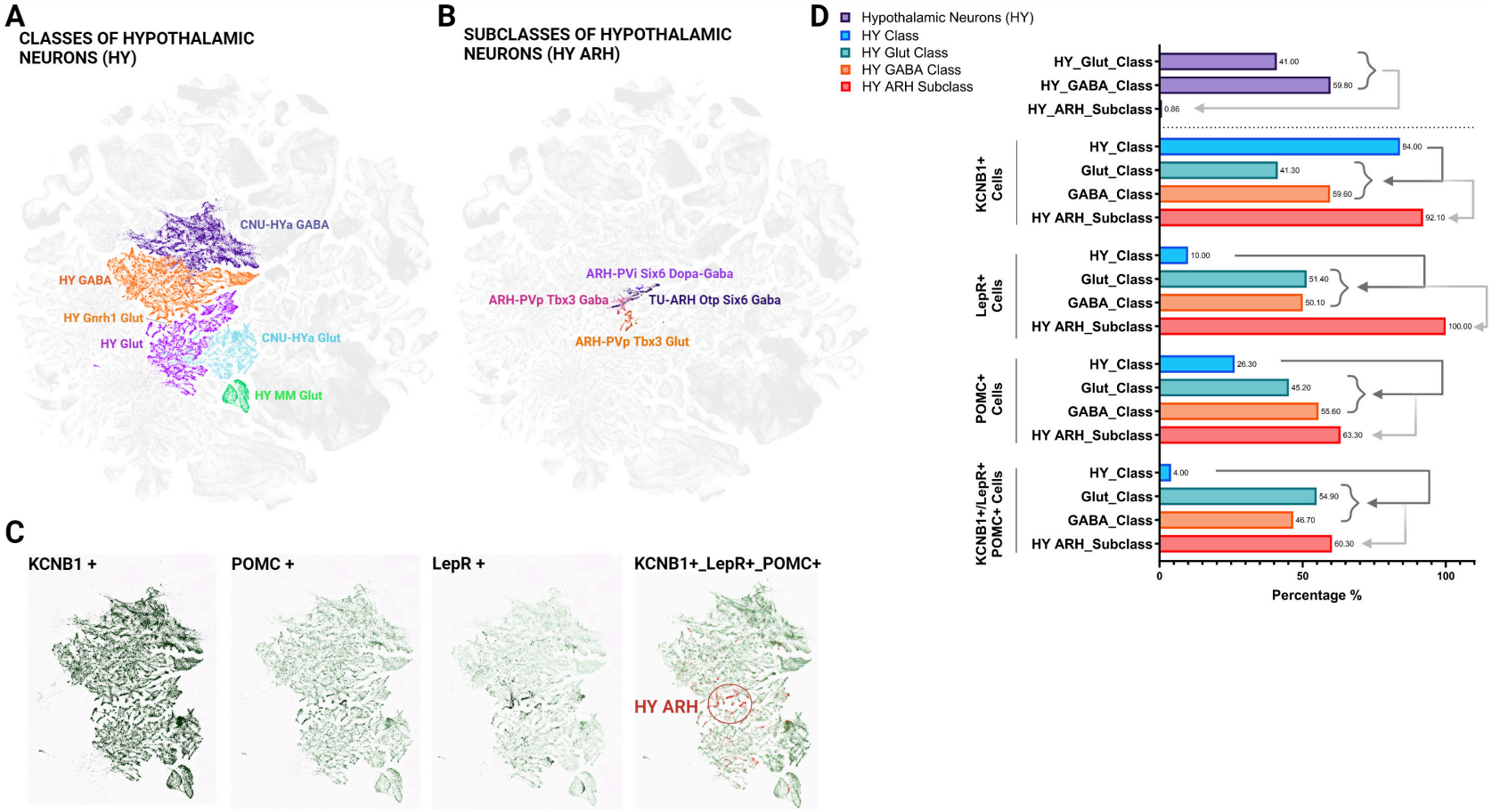

Figure S6 KCNB1, LepR and POMC transcripts expression in hypothalamic neurons from single-cell sequencing data of the Allen Institute Brain Atlas Open Source.
A) Visualization of the major classes of hypothalamic neurons.
B) Visualization of the major sub-classes of hypothalamic neurons in the ARH (HY ARH).
C) Visualization of KCNB1, POMC and LepR mRNA expression and coexpression in hypothalamic neurons. Overlap between cell expressing the three transcripts are shown in red. Maximal co-expression occurs in the ARH area (circled).
D) Fraction of hypothalamic neurons, segregated per classes and sub-classes expressing POMC, KCNB1, LepR transcripts or co-expressing all the three.
Purple represents Hypothalamic Neurons (HY) divided in Glut and GABA. 0.86% of HY neurons are HY ARH. Blue represents HY class neurons that express either KCNB1, LepR, POMC or all three together. Green represents the Glut portion of HY neurons that express either KCNB1, LepR, POMC or all three together. Orange represents the GABA portion of HY neurons that express either KCNB1, LepR, POMC or all three together. Red represents the HY ARH subclass of HY neurons that express either KCNB1, LepR, POMC or all three together. The figure was constructed by analysis of the mouse brain transcriptomic and cell type atlas, available at https://knowledge.brain-map.org/abcatlas?state=AQIBQVA4Sk5ONUxZQUJHVk1HS1kxQgACUTFOQ1dXUEc2RlowRE5JWEpCUQADAAQBAQKBLoApf%2FKQMAOE8nifhfo5DAAFAQFDMjMwMDk5RDA4UmlrAAAGAQECRlMwMERYVjBUOVIxWDlGSjRRRQADfgAAAAQAAAhHNEk0R0ZKWEpCOUFUWjNQVFgxAAlMVkRCSkFXOEJJNVlTUzFRVUJHAAoAAAFaSTNSUjBGWEwzSFlYR1ZFMlM1AAJBTkgzVVRTNkRTOTJYQlRPRTRLAAMABAEAAoJ%2BDz6D8ExsA4JqyMiDbuUmBDJOUVRJRTdUQU1QOFBRQUhPNFAABYHr20GBBacGgVgbSYC8Y%2BQGAAAFAQFDY25kMgAABgEBAkZTMDBEWFYwVDlSMVg5Rko0UUUAA34AAAEAAAIRFRWTEUxWUdOVEpRTVdWTUtFVQAJTFZEQkpBVzhCSTVZU1MxUVVCRwAKAAACAwA%3D

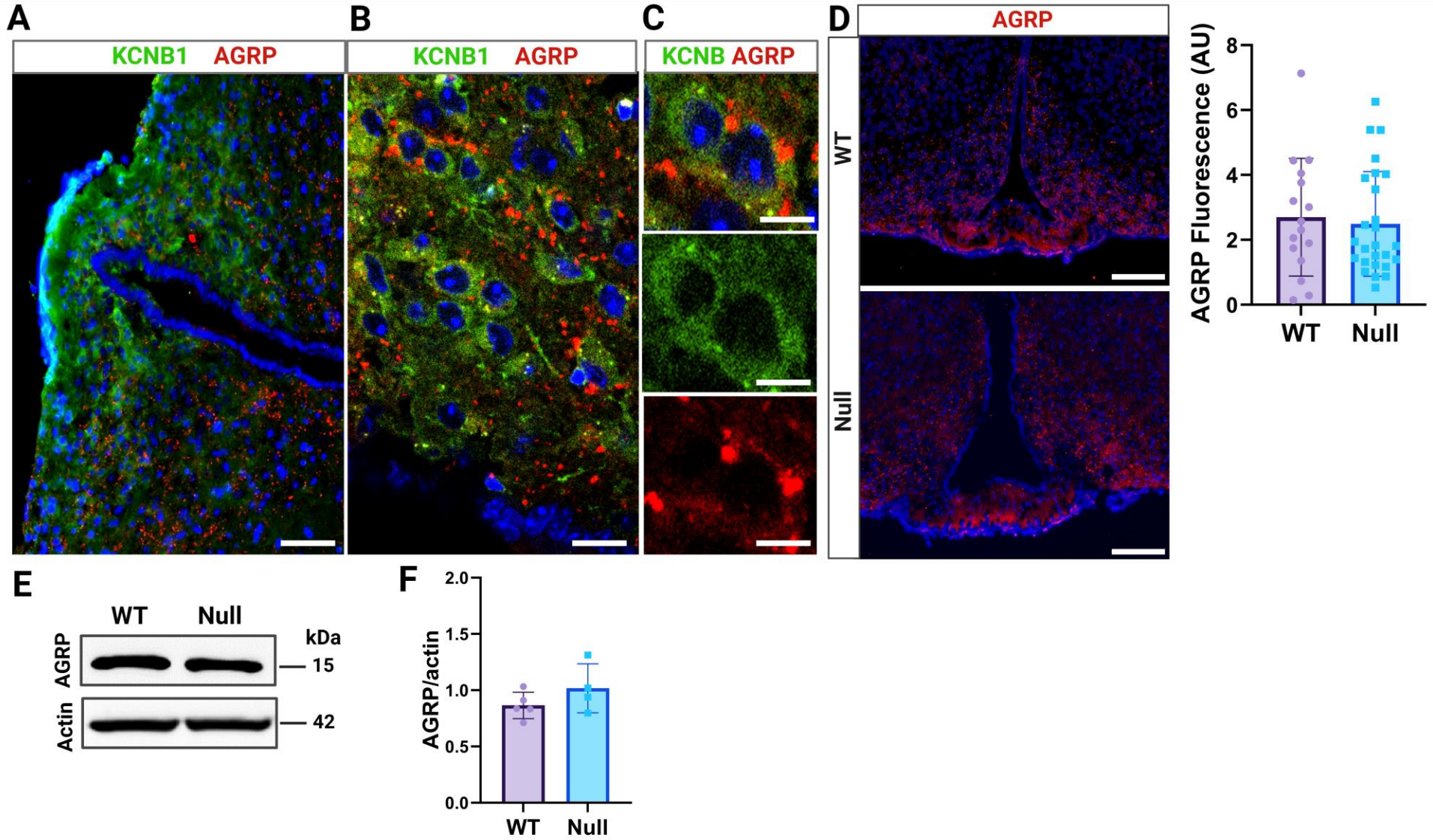

Figure S7 KCNB1 is detected in ARH^{AgRP} neurons

A) Representative confocal image of the mediobasal region of a WT slice stained with KCNB1 (green) and AgRP (red) antibody. DAPI blue color. Scale bar 300 μm.

B) Magnification of the ARH showing KCNB1-LepR signals overlap in individual neurons. Scale bar 25 μm.

C) Two neurons in (B) showing KCNB1-LepR overlap. Scale bar 10 μm.

D) Representative Nomarski images of a WT and a NULL mediobasal area stained with AgRP (red) antibody and quantifications. Scale bars 200 μm. N=3 mice/genotype.

E) Representative Western blot of AgRP protein in hypothalamic lysates of WT or NULL. Loading controls were actin and Bradford colorimetric assay. N=4-5 mice/genotype.

F) Densitometric quantification of Western blot experiments as shown in (E).

Data not statistically significant, two-tailed t-test.

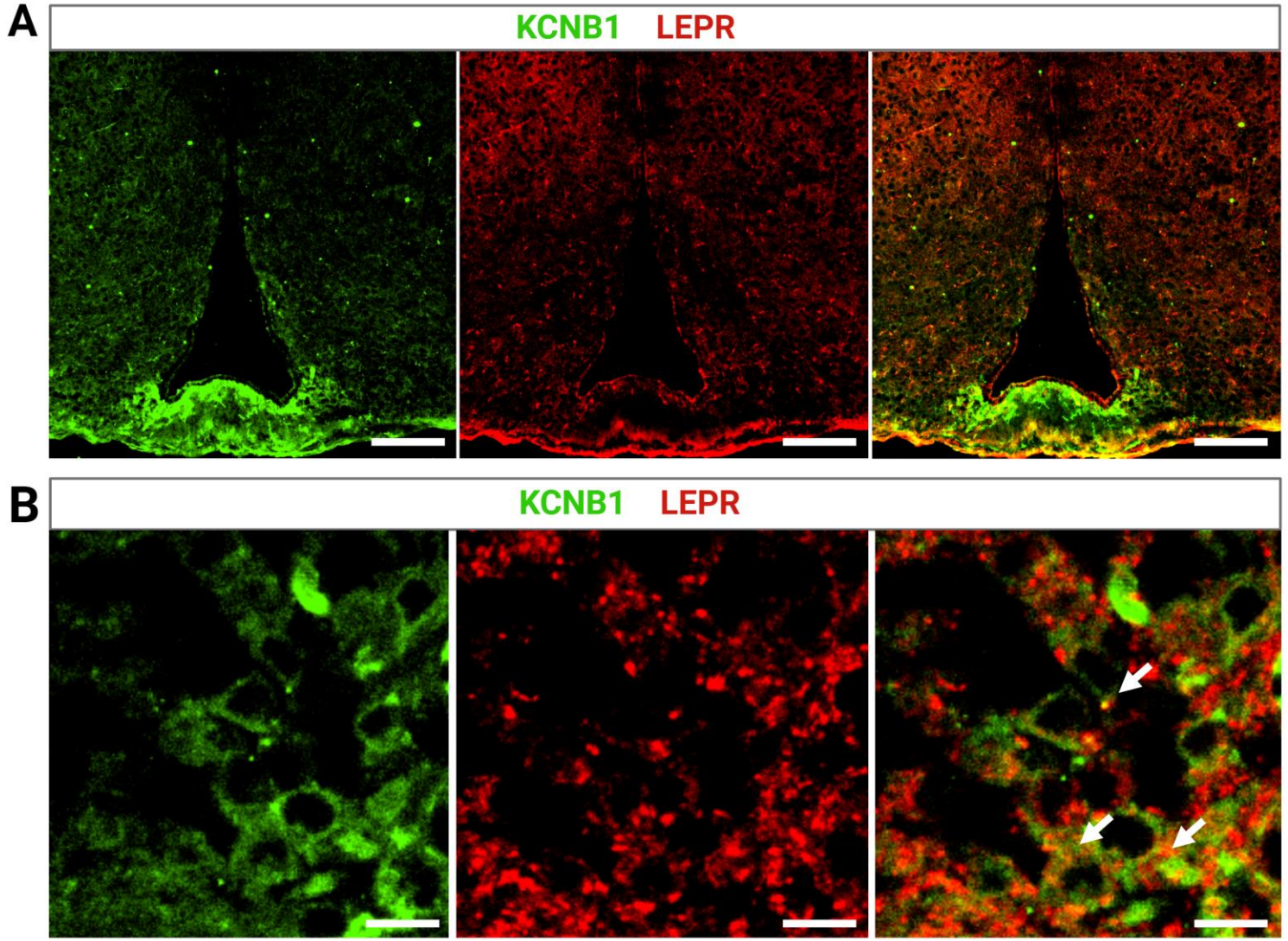

Figure S8 KCNB1 and LepR co-localize in the hypothalamus
A) Representative confocal images of a WT mediobasal hypothalamic slice showing immunofluorescence to KCNB1 (green), LepR (red) or both (composite). Scale bars 250 μm.
B) Representative confocal images showing overlap of KCNB1 and LepR signals in individual cells (arrows). Scale bars 25 μm.
N=2 mice.

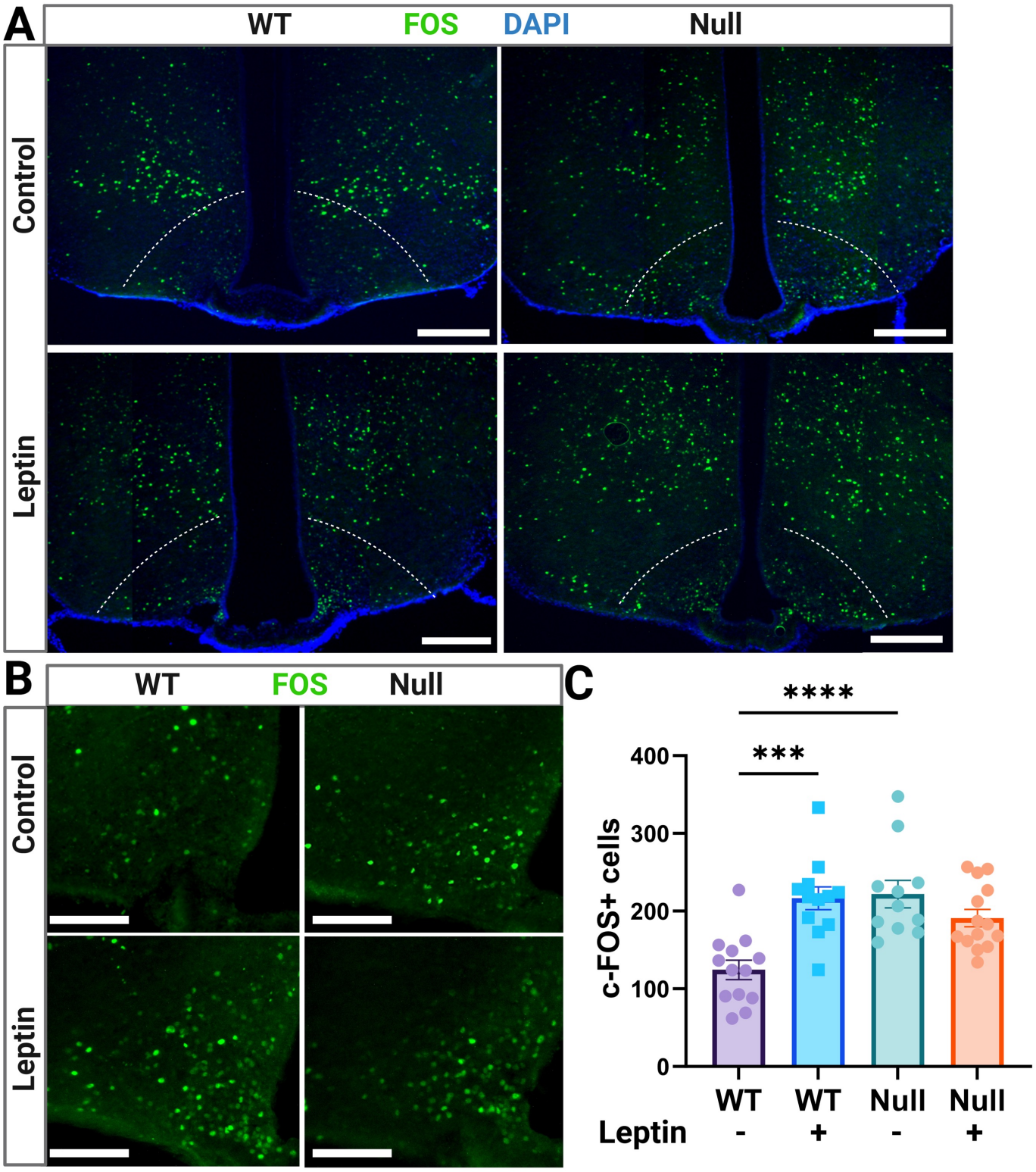

Figure S9 c-Fos expression is constitutively elevated in NULL ARH neurons

A) Representative images of c-Fos (green) immunofluorescence in the WT and NULL mediobasal area of the hypothalamus in the absence/presence of leptin. The ARH area is indicated. DAPI, blue color. Scale bars 300 μm.

B) Representative images of an ARH area stained for c-Fos (green) in the absence/presence of leptin. Scale bars 150 μm.

C) Quantifications of c-Fos immunofluorescence in WT or NULL in the absence/presence of leptin, calculated from images as those shown in (B).

Animals were injected with either vehicle or 1.0 mg/kg body weight leptin and sacrificed 90 min later. All the slices used for c-Fos analysis are from the same brains used for POMC analysis. N=3 mice/genotype/experimental condition. ****P<0.001, ****P<0.0001* (ANOVA with Tukey's post hoc).

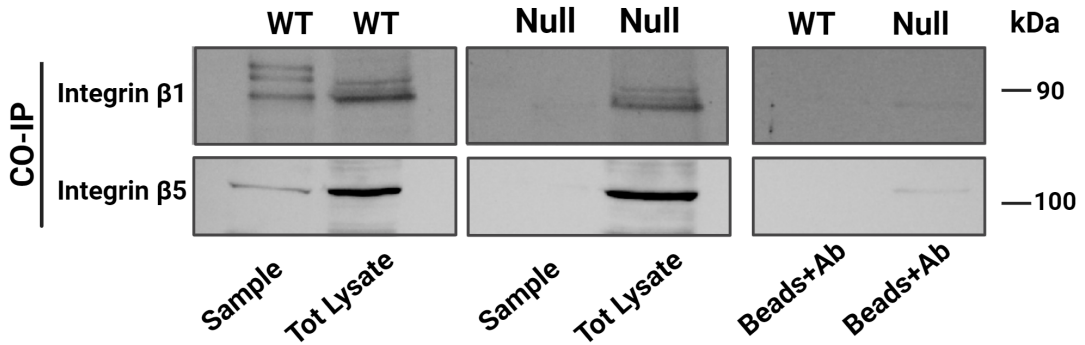

Figure S10 Integrins co-immunoprecipitate with the LepR
Representative co-IPs of integrin-β1 and integrin-β5 with the long isoform of the leptin receptor. Immunoprecipitations and co-immunoprecipitations were carried out on the same antibody-stripped membrane. The membrane used for the integrins co-IPs illustrated in this figure is the same membrane used in Figure 10, which also shows the LepR immunoprecipitate. Control: beads conjugated to mouse GFP antibody.
N=3-4 brains/genotype/experimental condition.