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Figure S1. Related to Figure 1

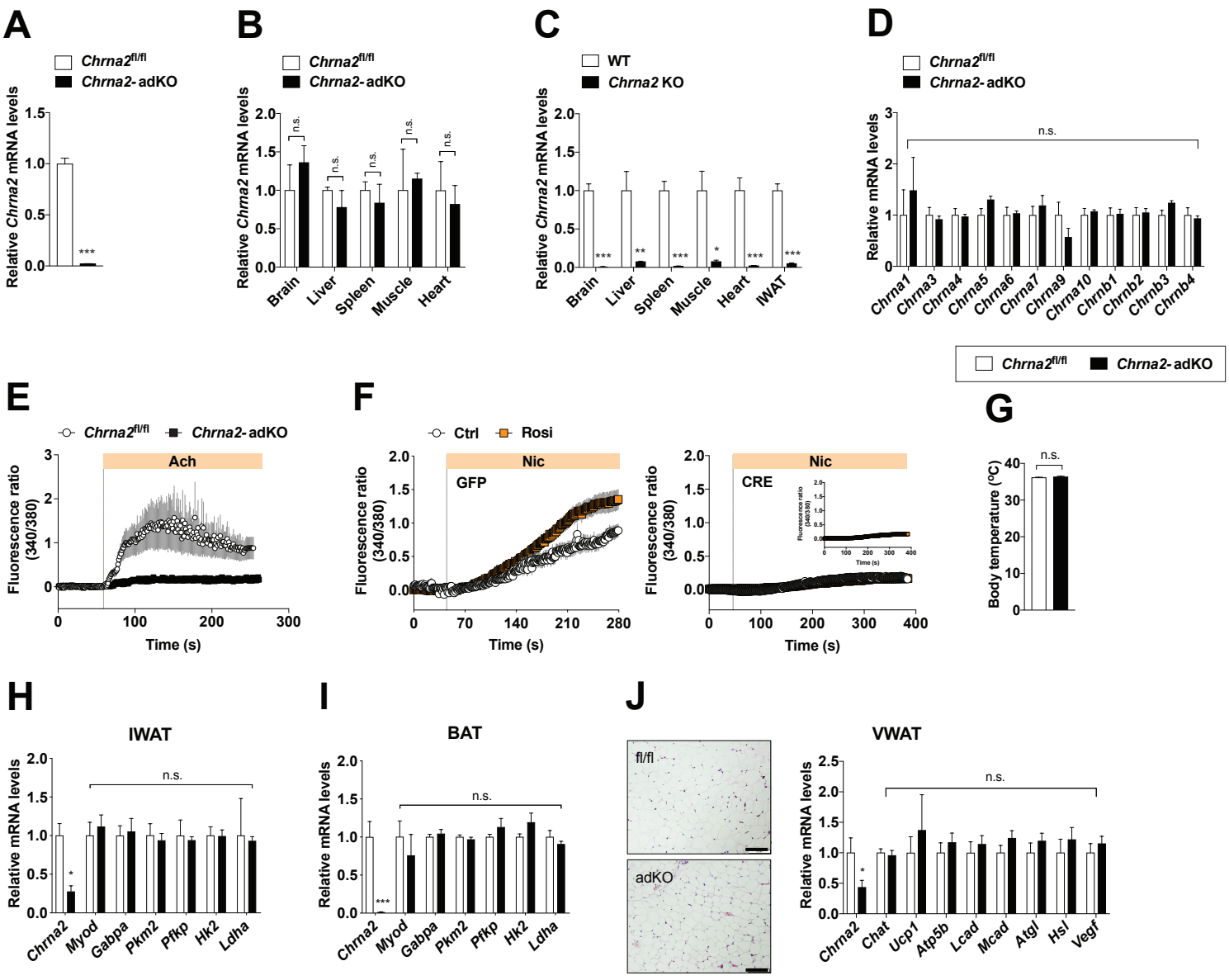

Figure S1. Related to Figure 1. Disruption of CHRNA2 signaling in adipose tissue.

(A) qPCR analyses of *Chrna2* mRNA levels in differentiated primary inguinal preadipocytes from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice (n = 6).

(B and C) qPCR analyses of *Chrna2* mRNA expression across tissues from *Chrna2*-adKO and littermate *Chrna2*$^{fl/fl}$ mice (n = 3) (B) and from *Chrna2* whole-body KO and WT counterparts (n = 4) (C).

(D) qPCR analyses of genes encoding nAChR subunits in IWAT of *Chrna2*$^{fl/fl}$ (n = 8) and *Chrna2*-adKO (n = 4) mice housed at room temperature on a chow diet.

(E) Intracellular calcium levels following stimulation with the CHRNA2 agonist acetylcholine (ACh, 100 μM) in differentiated primary inguinal preadipocytes from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice (n = 12).

(F) Intracellular calcium levels upon stimulation with the CHRNA2 agonist nicotine (Nic, 500 μM) in differentiated primary inguinal preadipocytes from *Chrna2*$^{fl/fl}$ mice after transduction with adenoviral GFP (n = 10 for Ctrl and 11 for Rosi) (left) or Cre recombinase (n = 14 for Ctrl and 15 for Rosi) (right) and treatment with vehicle (Ctrl) or rosiglitazone (Rosi, 1 μM) for 4 days. An insert graph in the right panel shows response with Rosi treatment in cells infected with adenoviral Cre separately, since it overlaps with the response with Ctrl treatment.

(G) Rectal core body temperature of *Chrna2*$^{fl/fl}$ (n = 14) and *Chrna2*-adKO (n = 6) mice at RT.

(H and I) qPCR analyses of glucose metabolism gene expression in IWAT (H) (n = 8 for fl/fl and 4 for adKO) and BAT (I) (n = 8 for fl/fl and 4 for adKO) from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice housed at room temperature.

(J) Representative H&E-stained images (left) and qPCR analyses (right) of VWAT (n = 9 for fl/fl and 13 for adKO) from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice under the basal condition. Scale bar, 100 μm.

(K) qPCR analyses of shivering-related gene expression in the skeletal muscle of *Chrna2*$^{fl/fl}$ (n = 4) and *Chrna2*-adKO (n = 6) mice under the basal condition.

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.005 by an unpaired two sample Student's *t*-test for two-group comparisons. n.s., not significant (p > 0.1).

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Figure S2. Related to Figure 2

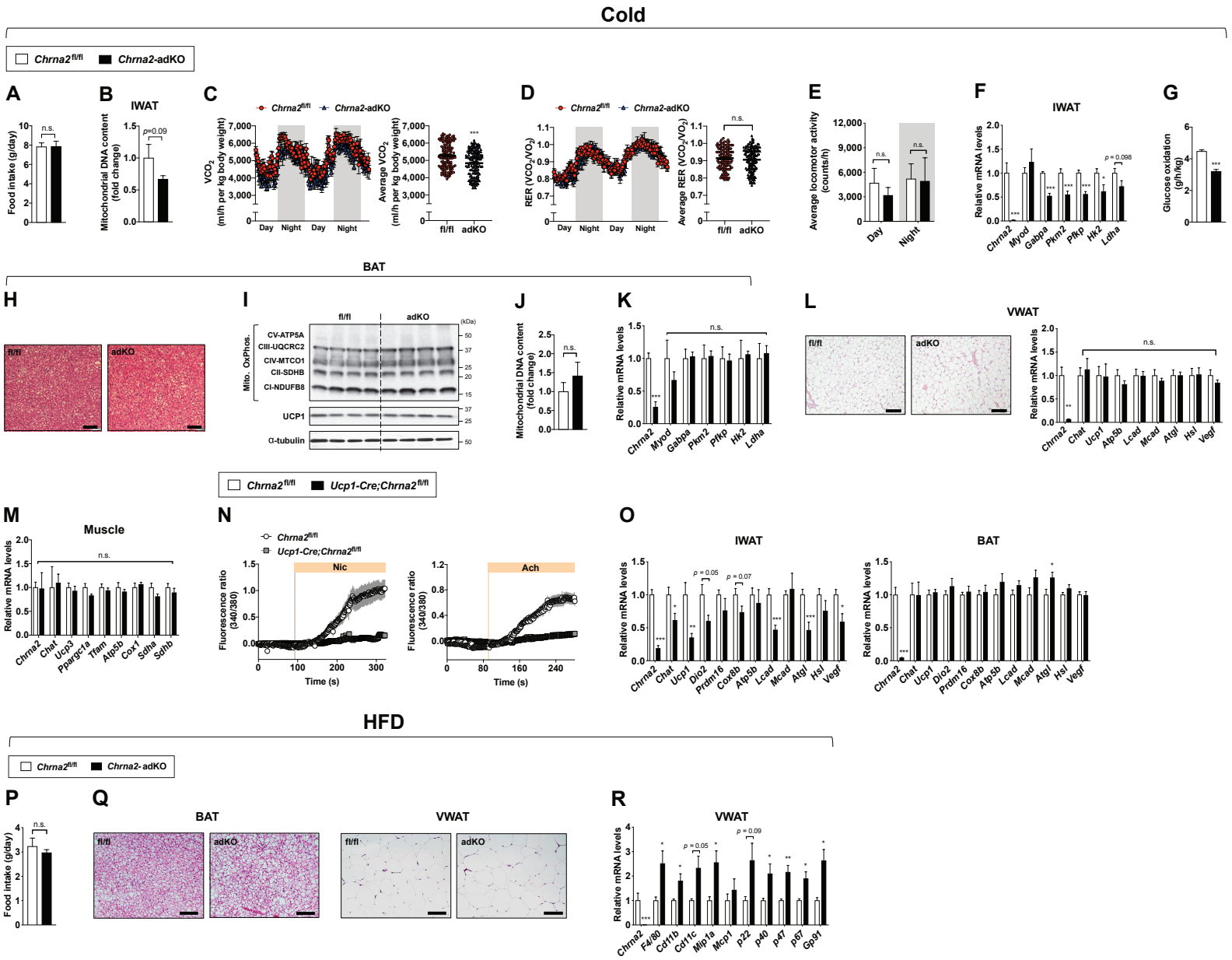

Figure S2. Related to Figure 2. Inguinal thermogenic defects by adipose-specific *Chrna2* deletion.

(A) Daily food intake on a chow diet in *Chrna2*$^{fl/fl}$ (n = 19) and *Chrna2*-adKO (n = 17) mice upon cold exposure (CE).

(B) Mitochondrial DNA content in IWAT of *Chrna2*$^{fl/fl}$ (n = 12) and *Chrna2*-adKO (n = 14) mice following CE.

(C) Whole-body CO_2 production (VCO_2) (left) and averaged VCO_2 (right) of *Chrna2*$^{fl/fl}$ (n = 7) and *Chrna2*-adKO (n = 5) mice after CE.

(D) Whole-body respiratory exchange ratio (RER) and averaged RER of *Chrna2*$^{fl/fl}$ (n = 7) and *Chrna2*-adKO (n = 5) mice after CE.

(E) Total locomotor activity (at x-axis and y-axis) of *Chrna2*$^{fl/fl}$ (n = 7) and *Chrna2*-adKO (n = 5) mice upon CE.

(F) qPCR analyses of glucose metabolism gene expression in IWAT of *Chrna2*$^{fl/fl}$ (n = 9) and *Chrna2*-adKO (n = 11) mice after CE.

(G) Whole-body glucose oxidation of *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice after CE (n = 6).

(H) Representative H&E-stained images of BAT from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice after CE. Scale bar, 100 μm.

(I) Immunoblot analyses of UCP1 and mitochondrial OxPhos components in BAT from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice after CE (n = 4). α-tubulin served as a loading control.

(J) Mitochondrial DNA content in BAT of *Chrna2*$^{fl/fl}$ (n = 12) and *Chrna2*-adKO (n = 14) mice following CE.

(K) qPCR analyses of glucose metabolism gene expression in BAT of *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice after CE (n = 12).

(L) Representative H&E-stained images (left) and qPCR analyses of thermogenic gene expression (n= 10) (right) of VWAT from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice after CE. Scale bar, 100 μm.

(M) qPCR analyses of shivering-related gene expression in the skeletal muscle of *Chrna2*$^{fl/fl}$ (n = 8) and *Chrna2*-adKO (n = 6) mice after CE.

(N) The absence of calcium uptake in response to the CHRNA2 agonist nicotine (Nic, 500 μM) (n = 12 for fl/fl and 20 for Cre) (left) or acetylcholine (Ach, 100 μM) (n = 15 for fl/fl and 22 for Cre) (right) in differentiated primary inguinal preadipocytes from *Ucp1-Cre; Chrna2*$^{fl/fl}$ mice compared to the control cells from *Chrna2*$^{fl/fl}$ mice.

(O) qPCR analyses of thermogenic markers of IWAT (left) and BAT (right) from *Chrna2*$^{fl/fl}$ (n = 10) and *Ucp1-Cre; Chrna2*$^{fl/fl}$ (n = 8) mice exposed to cold (10°C) for 2 weeks.

(P) Daily food intake on a HFD in *Chrna2*$^{fl/fl}$ (n = 10) and *Chrna2*-adKO (n = 16) mice.

(Q) Representative H&E-stained images of BAT (left) and VWAT (right) from *Chrna2*$^{fl/fl}$ and *Chrna2*-adKO mice after HFD feeding. Scale bar, 100 μm.

(R) qPCR analyses of macrophage-related inflammatory and NADPH oxidase-related oxidative stress gene expression in VWAT of *Chrna2*$^{fl/fl}$ (n = 5) and *Chrna2*-adKO (n = 7) mice following HFD challenge.

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.005 by an unpaired two sample Student's *t*-test for two-group comparisons. n.s., not significant (p > 0.1).

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Figure S3. Related to Figure 3

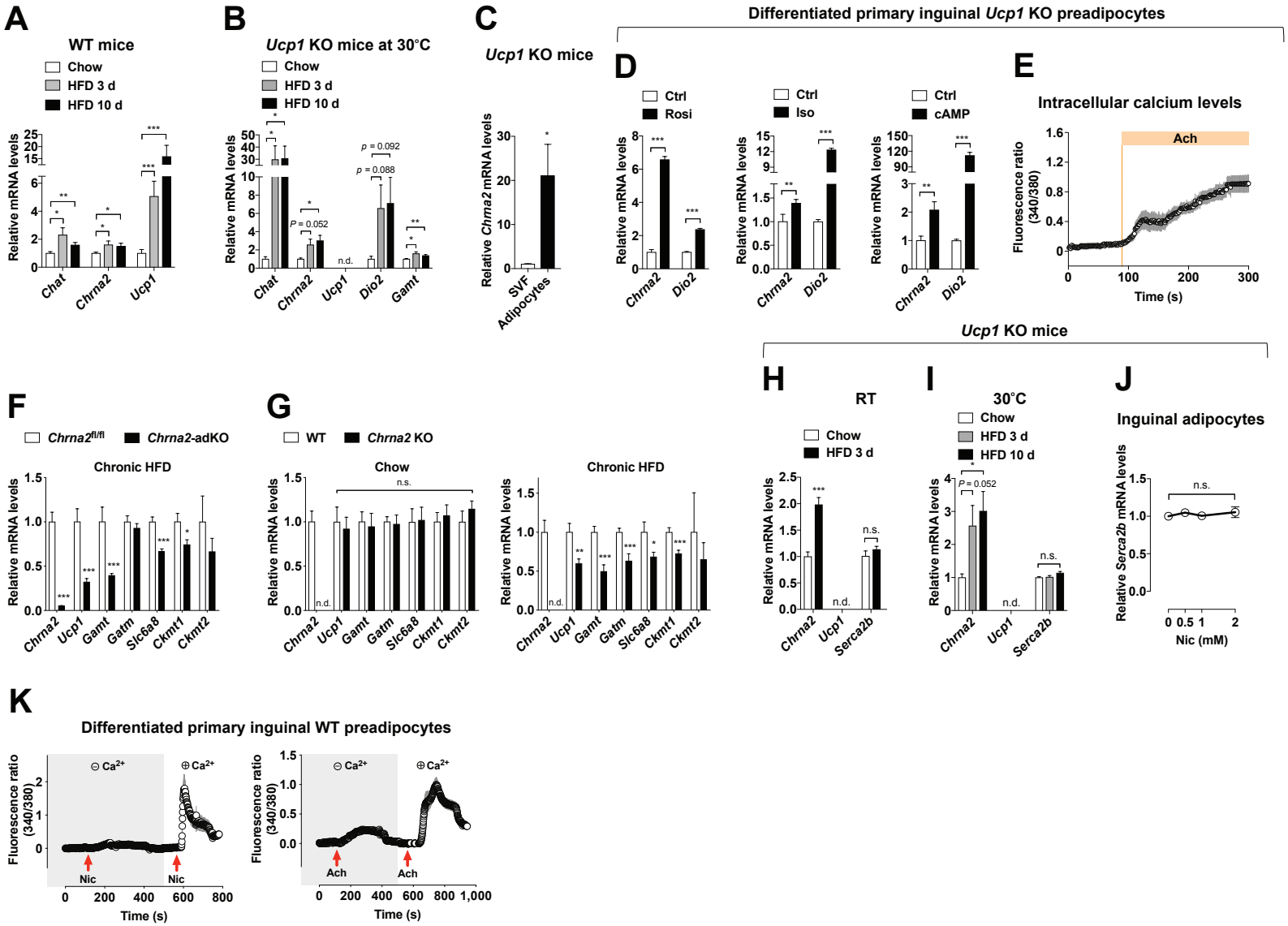

Figure S3. Related to Figure 3. Activation of CHRNA2 signaling in IWAT of *Ucp1* KO mice during calorie overload.

(A) qPCR analyses of *Chat*, *Chrna2* and *Ucp1* mRNA levels in IWAT of WT mice fed chow diet (n = 21) or HFD for 3 days (n = 20) or 10 days (n = 21) at room temperature (RT).

(B) qPCR analyses of *Chat*, *Chrna2* and representative thermogenic (*Dio2*) and creatine metabolism (*Gamt*) markers in the IWAT of *Ucp1* KO mice fed chow diet (n = 6) or HFD (n = 8) for 3 days or 10 days (n = 8) at thermoneutrality (30ºC).

(C) qPCR analyses of *Chrna2* mRNA levels in the SVF and mature adipocytes of IWAT from *Ucp1* KO mice housed at room temperature on a chow diet (n = 4).

(D) qPCR analyses of *Chrna2* and *Dio2* mRNA levels in differentiated primary inguinal preadipocytes from *Ucp1* KO mice after treatment with vehicle (Ctrl), 1 μM rosiglitazone (Rosi) for 2 days, 10 μM isoproterenol (Iso) for 4 hours or 500 μM dibutyryl-cAMP (cAMP) for 4 hours (n = 6).

(E) Increased intracellular calcium levels in the presence of the CHRNA2 agonist acetylcholine (Ach, 100 μM) in differentiated *Ucp1* KO inguinal preadipocytes (n = 11).

(F) qPCR analyses of *Chrna2*, *Ucp1* and creatine metabolism gene expression in IWAT of *Chrna2*$^{fl/fl}$ (n = 5) and *Chrna2*-adKO (n = 7) mice after 11 weeks on a HFD (45% of calories from fat) at RT.

(G) qPCR analyses of *Chrna2*, *Ucp1* and creatine metabolism gene expression in IWAT of WT control and *Chrna2* KO mice after 10 weeks on a chow diet (left) or HFD (right) at RT (n = 9).

(H) qPCR analyses of *Chrna2*, *Ucp1* and calcium cycling marker *Serca2b* mRNA levels in IWAT of *Ucp1* KO mice fed chow diet or HFD for 3 days at RT (n= 12).

(I) qPCR analyses of *Chrna2*, *Ucp1* and *Serca2b* in IWAT of *Ucp1* KO mice fed chow diet (n = 6) or HFD for 3 days (n = 8) or 10 days (n = 8) at thermoneutrality (30ºC).

(J) qPCR analyses of *Serca2b* mRNA levels in differentiated primary inguinal preadipocytes from *Ucp1* KO mice after treatment with vehicle (Ctrl) (n = 8) or the indicated concentration of nicotine (Nic) (n = 9) for 6 hours.

(K) The absence of calcium uptake under Ca^{2+}-free condition (medium containing EGTA) in differentiated WT inguinal adipocytes stimulated with the CHRNA2 agonist Nic (500 μM) (n= 15) or Ach (100 μM) (n = 16).

Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.005 by an unpaired two sample Student's *t*-test for two-group comparisons. n.s., not significant (p > 0.1). n.d., not detected.

Figure S4. Related to Figure 4

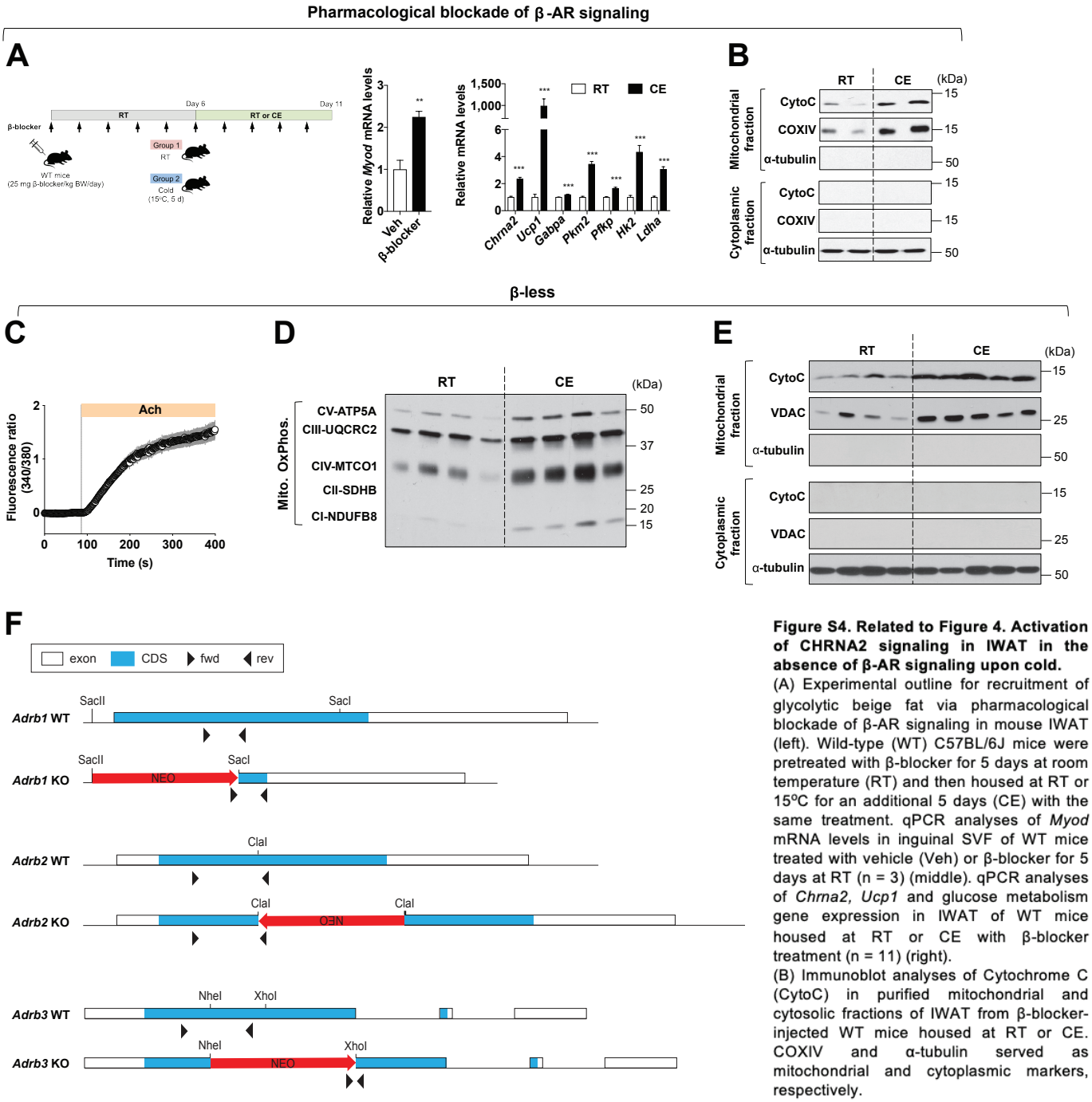

Figure S4. Related to Figure 4. Activation of CHRNA2 signaling in IWAT in the absence of β-AR signaling upon cold.

(A) Experimental outline for recruitment of glycolytic beige fat via pharmacological blockade of β-AR signaling in mouse IWAT (left). Wild-type (WT) C57BL/6J mice were pretreated with β-blocker for 5 days at room temperature (RT) and then housed at RT or 15ºC for an additional 5 days (CE) with the same treatment. qPCR analyses of *Myod* mRNA levels in inguinal SVF of WT mice treated with vehicle (Veh) or β-blocker for 5 days at RT (n = 3) (middle). qPCR analyses of *Chrna2*, *Ucp1* and glucose metabolism gene expression in IWAT of WT mice housed at RT or CE with β-blocker treatment (n = 11) (right).

(B) Immunoblot analyses of Cytochrome C (CytoC) in purified mitochondrial and cytosolic fractions of IWAT from β-blocker-injected WT mice housed at RT or CE. COXIV and α-tubulin served as mitochondrial and cytoplasmic markers, respectively.

(C) Upregulation of intracellular calcium levels in response to the CHRNA2 agonist acetylcholine (ACh, 100 μM) in differentiated primary inguinal preadipocytes from β-less (n = 24), indicating the presence and activation of CHRNA2 signaling in β-ARs-deleted adipocytes.

(D) Immunoblot analyses of mitochondrial OxPhos components of IWAT from β-less mice at RT or CE.

(E) Immunoblot analyses of CytoC in purified mitochondrial and cytosolic fractions of IWAT from β-less mice at RT or CE. VDAC and α-tubulin served as mitochondrial and cytoplasmic markers, respectively.

(F) Generation of β1WT and β3WT mice. β1WT and β3WT mice were generated by crossing β-less mice and 129SVE mice. β1WT mice expressed *Adrb1*, but not *Adrb2* and *Adrb3*. *Adrb1* and *Adrb2* were knocked-out in β3WT mice. Primers to detect WT or knockout (KO) alleles in *Adrb1*, *Adrb2* and *Adrb3* were developed based on the gene targeting strategy described previously (Chruscinski et al., 1999; Rohrer et al., 1996; Susulic et al., 1995). *Adrb1* WT primers were targeted to the region between SacII and SacI cut sites within the *Adrb1* transcript. *Adrb1* KO primers combined a forward primer targeted to the Neo cassette with a reverse primer at the end of the *Adrb1* coding sequence (Rohrer et al., 1996). Primers to detect the *Adrb2* WT and KO were developed based on the targeting construct described in Chruscinski *et al*. *Adrb2* WT primers were targeted to the *Adrb2* transcript, specifically surrounding the ClaI cut site. *Adrb2* KO primers combined a forward primer before the ClaI cut site with a forward primer within the Neo cassette (the Neo cassette is reversed in these animals). Based on the targeting strategy for deletion of *Adrb3* (Susulic et al., 1995), *Adrb3* WT primers were targeted to the region around the NheI cut site within the *Adrb3* transcript. *Adrb3* KO primers combined a forward primer targeted to the Neo cassette with a reverse primer after the XhoI cut site in the *Adrb3* sequence.

Data are presented as mean ± SEM. **p < 0.01, and ***p < 0.005 by an unpaired two sample Student's *t*-test for two-group comparisons.

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Figure S5. Related to Figure 5

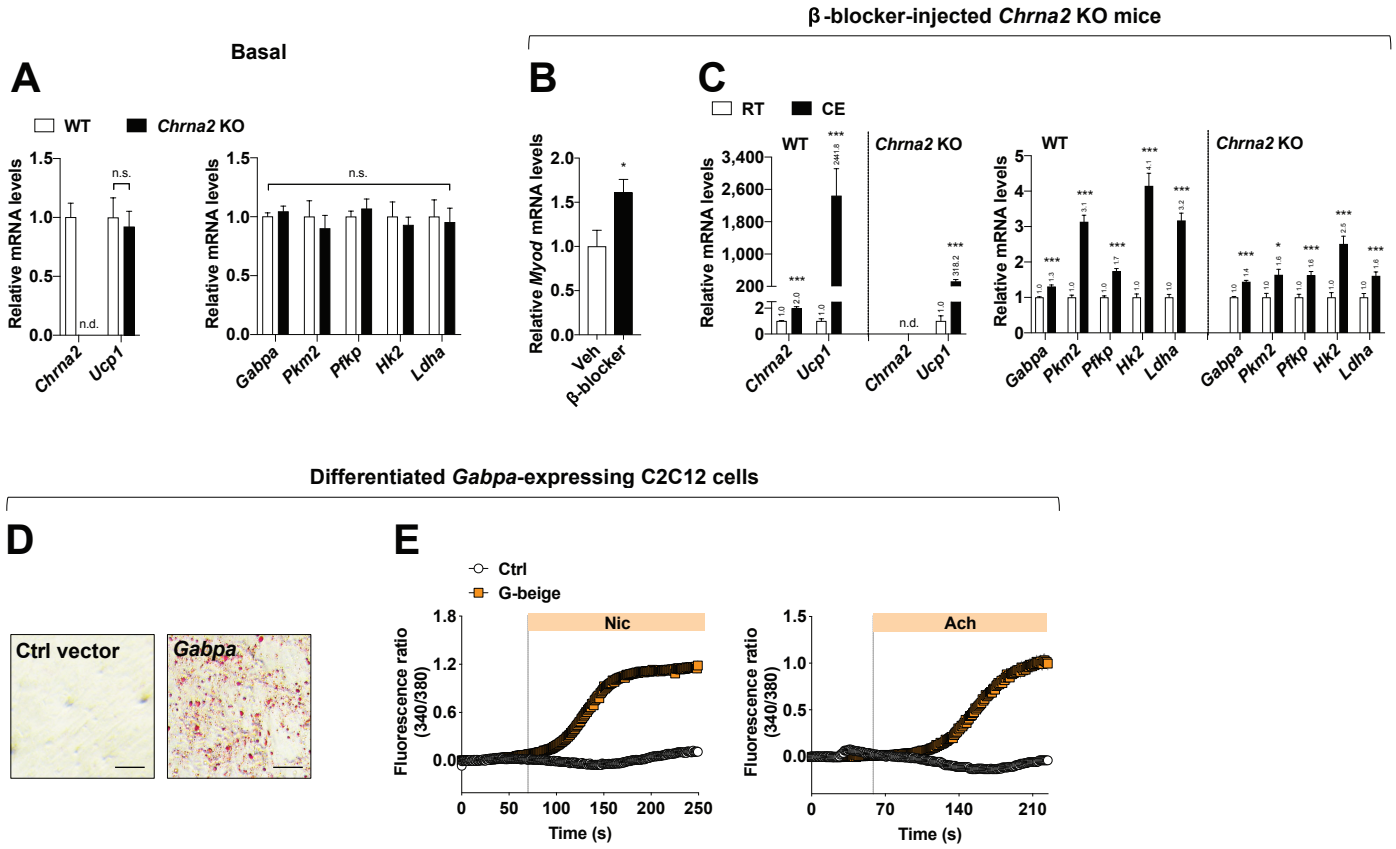

Figure S5. Related to Figure 5. The role of CHRNA2 in glycolytic beige fat recruitment.

(A) qPCR analyses of *Chrna2*, *Ucp1* (left) and glucose metabolism gene (right) expression in IWAT of WT control (n = 8) and *Chrna2* KO (n = 9) mice housed at room temperature (RT) on a chow diet.

(B) qPCR analyses of *Myod* mRNA levels in inguinal stromal vascular fraction (SVF) of *Chrna2* KO mice injected with vehicle or β-blocker for 5 days (n = 5).

(C) qPCR analyses of *Chrna2*, *Ucp1* (left) and glucose metabolism gene (right) expression in IWAT of WT (n = 19 for RT and 18 for CE) and *Chrna2* KO (n = 9) mice housed at RT or 15°C for 5 days (CE) with β-blocker treatment.

(D) Oil Red O staining in differentiated C2C12 cells expressing control vector or *Gabpa* under pro-adipogenic conditions. Scale bar, 50 μm.

(E) Increased intracellular calcium levels in the presence of the CHRNA2 agonist nicotine (Nic, 500 μM) (n = 15 for Ctrl and 22 for *Gabpa*) (left) or acetylcholine (ACh, 100 μM) (n = 11 for Ctrl and 30 for *Gabpa*) (right) in differentiated C2C12 cells expressing control vector or *Gabpa*, indicating the presence and activation of CHRNA2 signaling in g-beige adipocytes.

Data are presented as mean ± SEM. *p < 0.05, and ***p < 0.005 by an unpaired two sample Student's *t*-test for two-group comparisons. n.s., not significant (p > 0.1). n.d., not detected.