Figure S1 (Related to Figure 2)

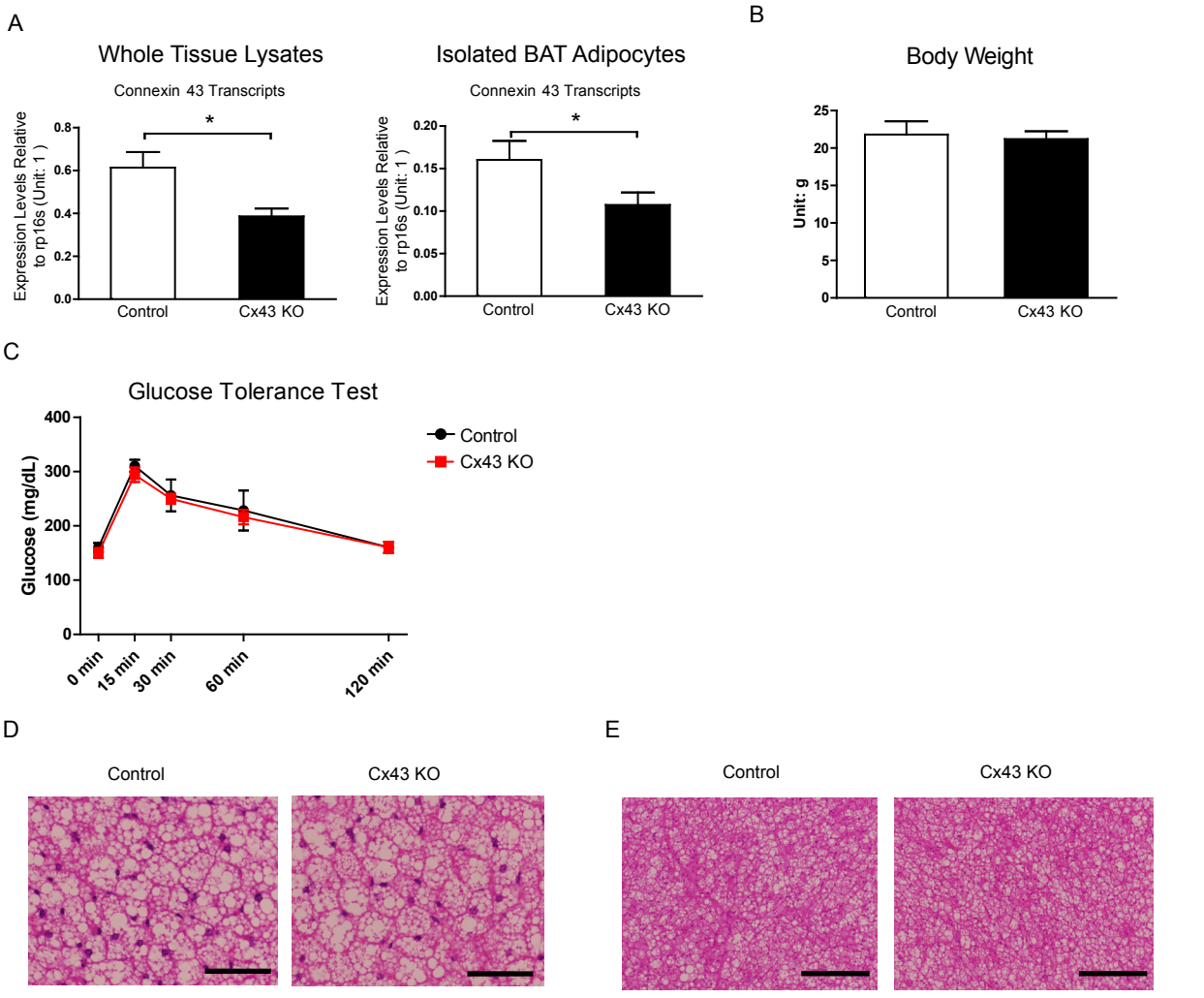
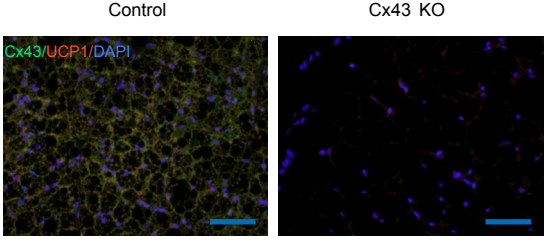


Figure S2 (Related to Figure 3)

A



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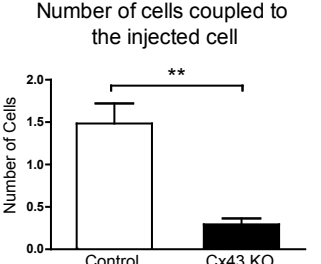

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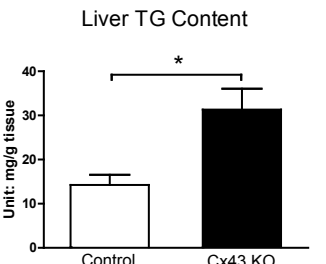


Figure S3 (Related to Figure 5)

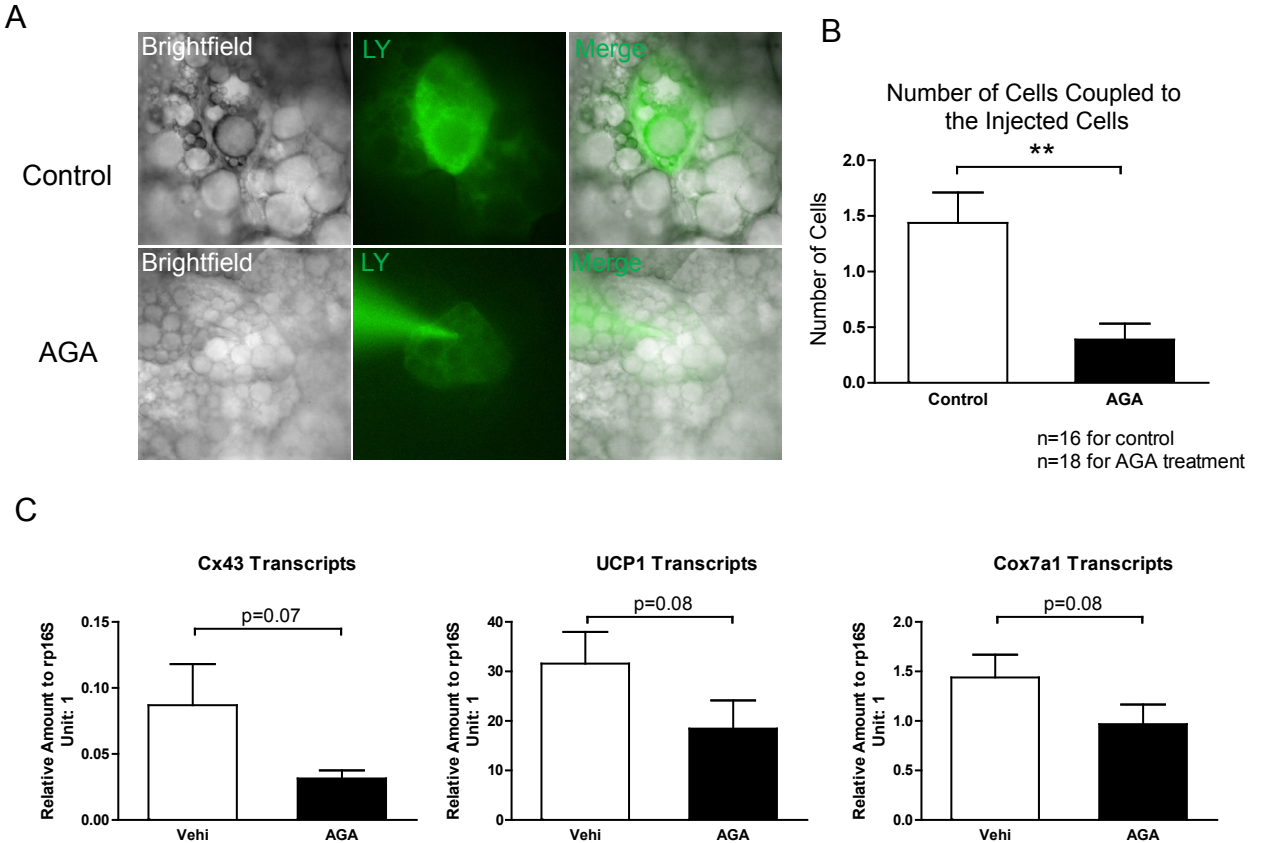
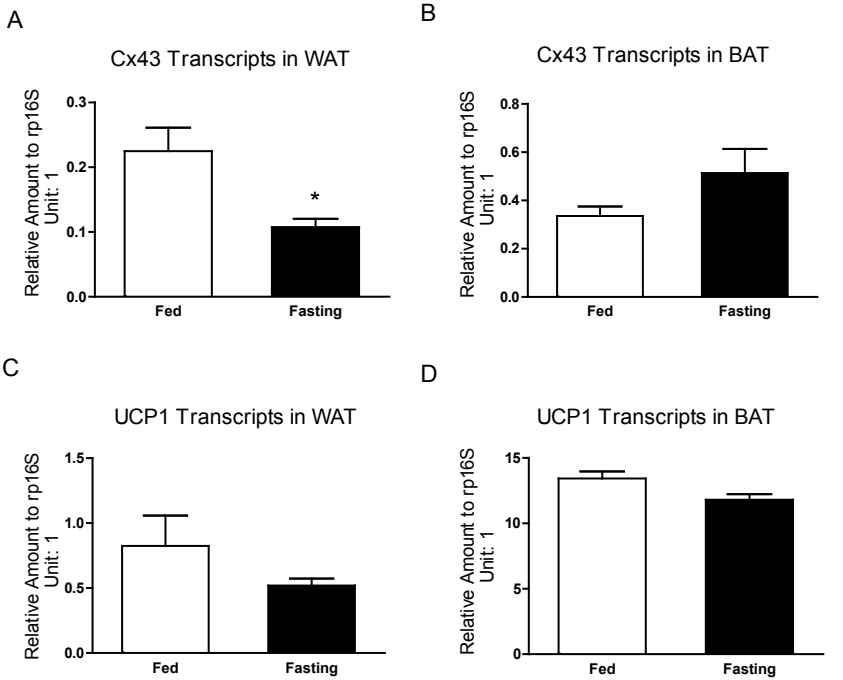


Figure S4 (Related to Figure 7)



SUPPLEMENTAL FIGURE LEGENDS

Suppl. Fig. 1 (Related to Fig. 2): A. Cx43 expression in BAT whole tissue lysates (Left panel, n = 4) or isolated brown adipocytes (Right panel, n = 4). **B.** Bodyweight of Cx43 mice after 3 weeks of DOX supplementation in the diet (n = 5-8). **C.** Glucose tolerance test of Cx43 KO mice after 3 weeks of Dox supplementation in the diet (n = 5-8). **D.** H&E staining of BAT from mice housed at room temperature. Scale bar = 50 µm. **E.** H&E staining of BAT from mice exposed to the cold for 3 weeks. Scale bar = 250 µm. Results are shown as mean ± SEM, *p < 0.05.

Suppl. Fig. 2 (Related to Fig. 3): A. Cx43 and UCP1 immunofluorescent co-staining. Scale bar = 50 µm. **B.** Quantification of the average number of cells coupled to the injected cell in control and Cx43 KO adipose depots. n = 30 for control, n = 51 for Cx43 KO. **C.** Liver triglyceride (TG) content after 3 weeks of cold exposure (n = 6-7). Results are shown as mean ± SEM, *p < 0.05, **p < 0.01.

Suppl. Fig. 3 (Related to Fig. 5): **A.** Lucifer Yellow coupling experiments of mice treated with AGA. **B.** Quantification of the average number of cells coupled to the injected cell in control and AGA treated adipose depots. **C.** Gene expression in wild-type mice treated with 2 days of AGA (n = 4-6). Results are shown as mean ± SEM, **p < 0.01.

Suppl. Fig. 4 (Related to Fig. 7): **A.** Cx43 expression in WAT after overnight fasting (n = 4). **B.** Cx43 expression in BAT after overnight fasting (n = 4). **C.** UCP1 expression in WAT after overnight fasting (n = 4). **D.** UCP1 expression in BAT after overnight fasting (n = 4). Results are shown as mean ± SEM, *p < 0.05.

SUPPLEMENTAL TABLE

<u>Suppl. Table 1 (Related to Fig. 1):</u> Genes differentially regulated in subcutaneous adipose depots derived from mice housed in the cold.

Whole genome profiling of genes differentially regulated in subcutaneous adipose depots. Data are presented as fold changes in gene expression levels in fat pads from mice housed in the cold (6°C) versus fat pads from mice housed at thermoneutrality (30°C). Original data have been deposited to GEO: GSE84860.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Immunofluorescence

Formalin-fixed, paraffin-embedded sections from either white adipose tissue or brown adipose tissue were blocked in PBST with 5% BSA. Primary antibodies used were perilipin (1:500 dilution, NB100-60554, NOVUS), UCP1 (1:250 dilution, ab10983, Abcam or 1:250 dilution, NB100-2828, Novus for UCP1 and Cx43 double staining) or Connexin43 (1:100 dilution; sc-6560-R, Santa Cruz); secondary antibodies (1:250 dilution) used were Alexa Fluor 488 or 594 donkey anti-rabbit IgG (HCL) or Alexa Fluor 488 or 594 donkey anti-goat IgG (HCL) (Invitrogen). Slides were counterstained with DAPI. Fluorescent Images were acquired using an AxioObserver Epifluorescence Microscope (Zeiss) or FSX100 microscope (Olympus). Confocal images were acquired using LSM510 (Zeiss), and were analyzed by ImageJ software.

rAAV Vector Construction and Packaging

The rAAV plasmid contains a vector expression cassette consisting of the CMV enhancer and chicken β-actin (CBA) promoter, woodchuck post-transcriptional regulatory element (WPRE) and bovine growth hormone (bGH) poly-A flanked by AAV2 inverted terminal repeats.

Transgenes encoding YFP or Cx43 were inserted into the multiple cloning sites between the CBA promoter and WPRE sequence. The engineered hybrid serotype Rec2 vectors were packaged and purified as previously described (Liu et al., 2014).

Gene Expression Analysis

Total RNA from mouse tissues was isolated using the TRIzol reagent (Thermo Fisher). First-strand cDNA was synthesized with reverse transcriptase and random hexamer primers (Invitrogen) from 1 μg of RNA. Real-time quantitative PCR was performed with the SYBR Green PCR system (Applied Biosystems), with ribosomal protein 16s (rp16s) as an internal control for

normalization. Data are presented as relative abundance to rp16s mRNA except that in Fig, 6E and Fig. 6J data are presented as fold changes to mRNA abundance in YFP AAV control samples. Primer sequences are available on request. Global gene expression profiling by Illumina MouseWG-6 V2 BeadChip microarray was conducted at the UTSW Genomics and Microarray Core using the manufacturer's protocols. Normalization and statistical analysis were performed using GenomeStudio (Illumina). Pathway analysis of genes changed in the microarray study was performed with Ingenuity IPA (Qiagen). All microarray data have been deposited to GEO: GSE84860.

SVF Culture and Adipocyte Differentiation

SVF culture and adipocyte differentiation were performed as described previously (Wang et al., 2015).

Brown Adipocyte Isolation

Brown adipose tissues were dissected from mice, minced into small pieces and digested with 10 mL liver digestion medium (Thermo Fisher) at 37°C for 1 – 1.5 hours with gentle shaking. Undigested BAT was removed by passing the samples through 250 μm mesh. About 10 mL liver digestion medium was added to each sample to make up to a total volume of 20 mL. The samples were then centrifuged at 800 g at room temperature for 1 minute. Floating brown adipocytes were collected from the top for following assays.

Body Temperature Measurements

IPTT-300 temperature transponders were implanted beneath the skin on the back (close to BAT); body temperature was assessed using a hand-held reader for the transponder (Bio Medic Data Systems).

Liver Lipid Assays

Frozen liver tissues were used for lipid extraction and measurement as previously described (Shao et al., 2014). In brief, 25-35 mg of liver tissue was homogenized in 0.5-0.7 ml PBS (20 X of liver weight in µl). 0.4 ml homogenates were then mixed with 1.6 ml of CHCl3-CH3OH (2:1, v/v), the suspension was centrifuged at 3,000 r.p.m. for 10 min at room temperature. The lower organic phase was transferred and air-dried overnight in a chemical hood. The residual liquid was re-suspended in 800 ml ethanol containing 1% Triton X-100, and the concentrations of triglyceride and cholesterol were determined using the serum triglyceride and cholesterol determination kit (Thermo Fisher Scientific, Waltham, MA).

SUPPLEMENTAL REFERENCES

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Shao, M., Shan, B., Liu, Y., Deng, Y., Yan, C., Wu, Y., Mao, T., Qiu, Y., Zhou, Y., Jiang, S.*, et al.* (2014). Hepatic IRE1alpha regulates fasting-induced metabolic adaptive programs through the XBP1s-PPARalpha axis signalling. Nat Commun *5*, 3528.

Wang, Q.A., Tao, C., Jiang, L., Shao, M., Ye, R., Zhu, Y., Gordillo, R., Ali, A., Lian, Y., Holland, W.L.*, et al.* (2015). Distinct regulatory mechanisms governing embryonic versus adult adipocyte maturation. Nat Cell Biol *17*, 1099-1111.