Supplemental Movie Legends

Movie S1. Isolectin-labeled vasculatures in mouse inguinal WAT are visualized via 3D volume fluorescence-imaging.

The wild-type mice were intravenously injected with Isolectin GS-IB4 Alexa647. Inguinal WAT were processed for tissue optical clearing and were imaged at 1.26x magnification on the lightsheet microscope.

Movie S2. $CD31^+$ vasculatures in mouse inguinal WAT are visualized via 3D volume fluorescence-imaging.

Inguinal WAT of the wild-type mice were processed for the whole-mount immunolabeling of CD31/PECAM-1 and were imaged at 12.6x magnification on the lightsheet microscope. Representative 500 μm x 500 μm x 500 μm cubic tissue volume is shown.

Movie S3. Isolectin-labeled vasculatures in mouse interscapular BAT are visualized via 3D volume fluorescence-imaging.

The wild-type mice were intravenously injected with Isolectin GS-IB4 Alexa647. Interscapular BAT were processed for tissue optical clearing and were imaged at 12.6x magnification on the lightsheet microscope. Representative lightsheet scanning of 500 μm-depth tissue is shown.

Movie S4. Spatial engagement of the sympathetic fibers with the vasculatures in mouse inguinal WAT is visualized via 3D volume fluorescence-imaging.

The wild-type mice were intravenously injected with Isolectin GS-IB4 Alexa647 (green). Inguinal WAT were processed for the whole-mount immunolabeling of tyrosine hydroxylase (magenta) and were imaged at 12.6x magnification on the lightsheet microscope. Representative 500 μm x 500 μm x 500 μm cubic tissue volume is shown.

Movie S5. Association of the increased vasculatures with the cold-induced beige cells in mouse inguinal WAT is visualized via 3D volume fluorescence-imaging.

$Ucp1^{CreERT2/+}$; Rosa26-LSL-EGFP mice daily treated with 4-hydroxytamoxifen were cold-challenged for 4 days. The mice were then intravenously injected with Isolectin GS-IB4

Alexa647 (green). Inguinal WAT were processed for the whole-mount immunolabeling of EGFP (magenta) and were imaged at 12.6x magnification on the lightsheet microscope. Representative 3D image of 500 μm-depth tissue is shown.

Supplemental Figures

Figure S1

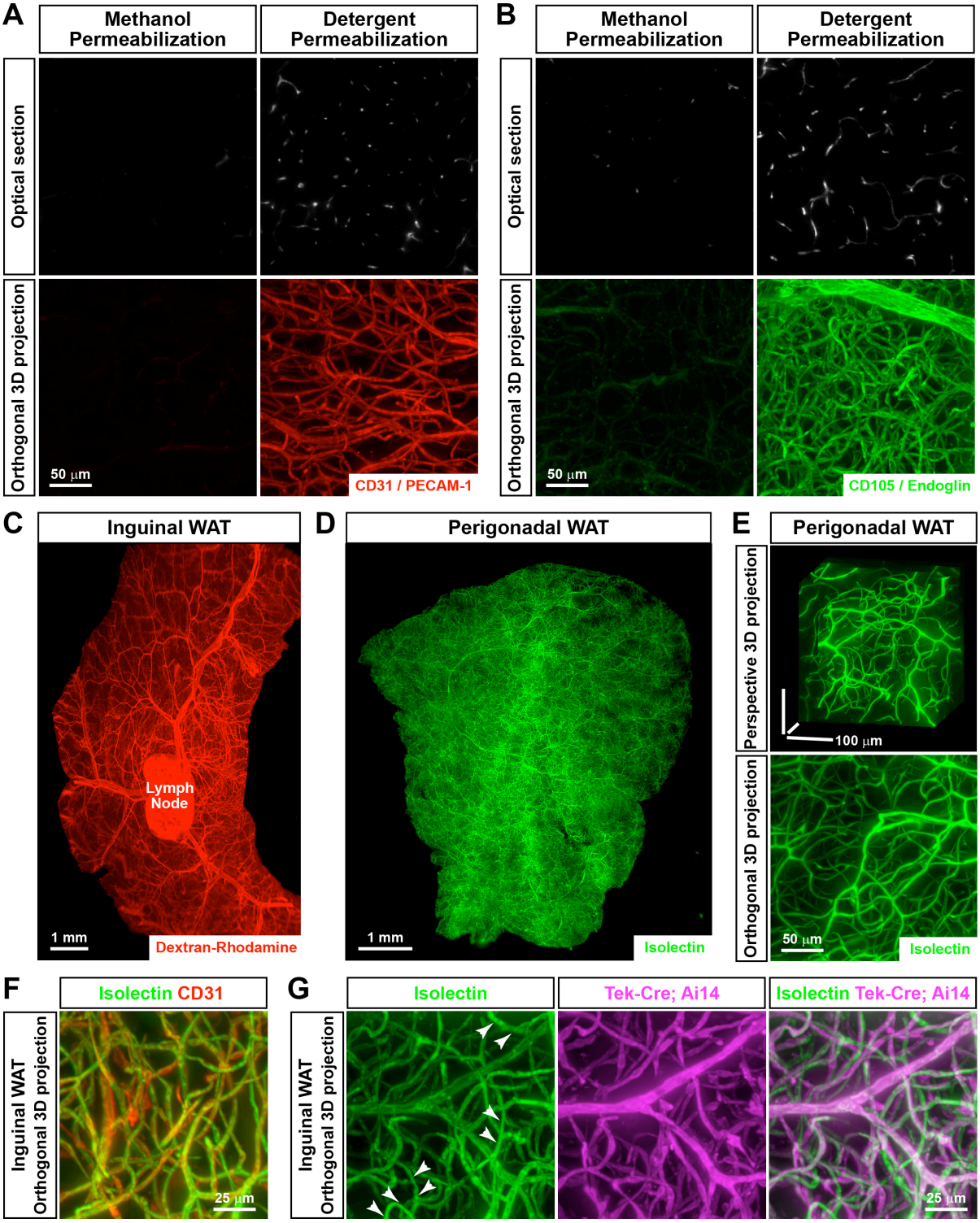

Figure S1. Three-dimensional volume fluorescence-imaging of the vasculatures in mouse adipose tissues.

(A and B) Comparison of the methanol- or detergent-based permeabilization steps for the whole-mount immunolabeling of vasculatures in adipose tissues. Inguinal WAT of the wild-type mice maintained at room temperature **(A)** or cold-challenged for 4 days **(B)** were processed for the whole-mount immunolabeling of CD31/PECAM-1 **(A)** or CD105/Endoglin **(B)** with the methanol- or detergent-based permeabilization steps, respectively. The immunolabeled and optically cleared tissues were imaged at 12.6x magnification on the lightsheet microscope. Representative optical sections of the lightsheet imaging (upper panels) or representative orthogonal 3D-projection images of 500 μm-depth tissues (lower panels) are shown. **(C)** The wild-type mice were intravenously injected with 2,000,000MW-Dextran Tetramethylrhodamine. Inguinal WAT were processed for tissue optical clearing and were imaged at 1.26x magnification on the lightsheet microscope. Representative orthogonal 3D-projection image is shown. **(D and E)** The wild-type mice were intravenously injected with Isolectin GS-IB4 Alexa647. Perigonadal WAT were processed for tissue optical clearing and were imaged at 1.26x **(D)** or 12.6x **(E)** magnification on the lightsheet microscope. **(D)** Representative orthogonal 3D-projection image is shown. **(E)** Representative perspective 3D-projection image of 300 μm x 300 μm x 300 μm cubic tissue volume (upper panel) or representative orthogonal 3D-projection image of 500 μm-depth tissue (lower panel) is shown. **(F)** The wild-type mice were intravenously injected with Isolectin GS-IB4 Alexa647 (green). Inguinal WAT were processed for the whole-mount immunolabeling of CD31/PECAM-1 (red) and were imaged at 12.6x magnification on the lightsheet microscope. Representative orthogonal 3D-projection image of 500 μm-depth tissue is shown. **(G)** *Tek-Cre; Rosa26-LSL-tdTomato (Ai14)* mice were intravenously injected with Isolectin GS-IB4 Alexa647 (green). Inguinal WAT were processed for the whole-mount immunolabeling of tdTomato (magenta) and were imaged at 12.6x magnification on the lightsheet microscope. Representative orthogonal 3D-projection images of 500 μm-depth tissue are shown. Arrowheads denote the examples of Isolectin^{+} tdTomato^{-} vasculatures.

Figure S2

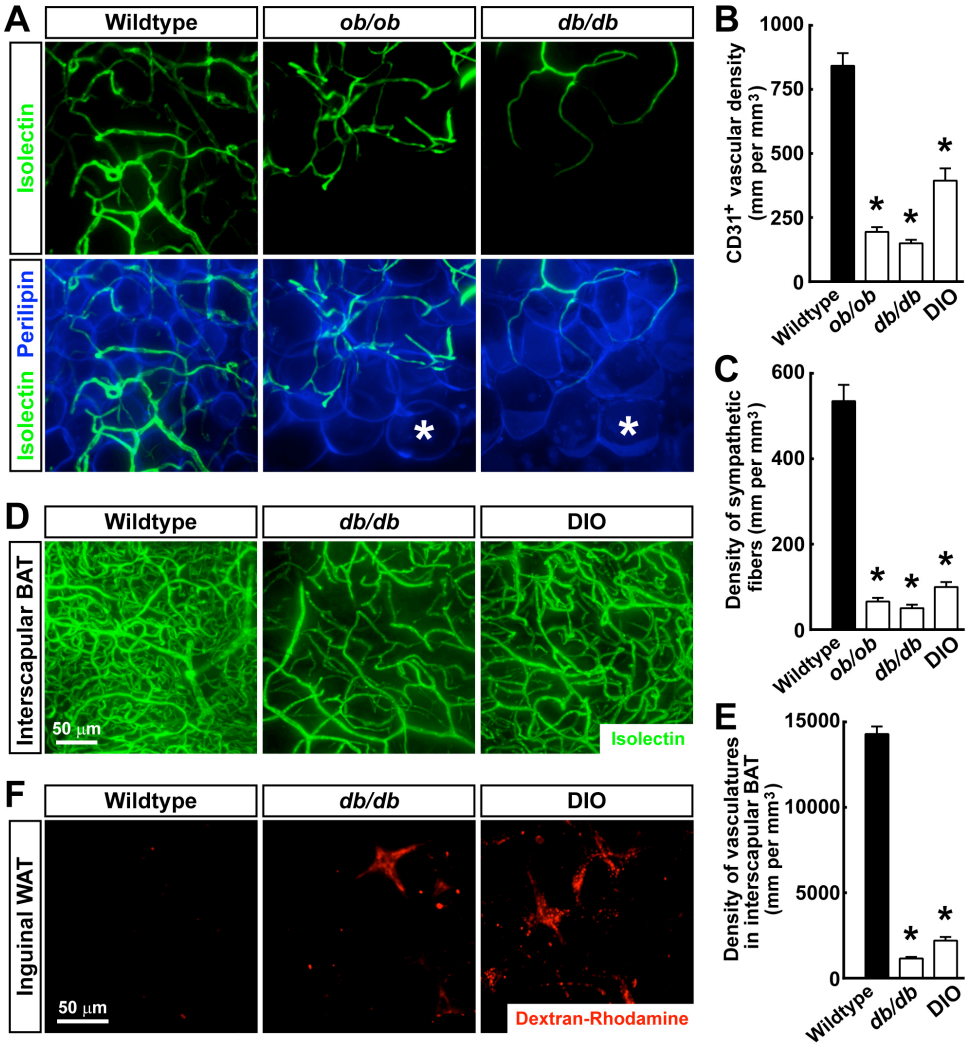
A
Wildtype
ob/ob
db/db
Isolectin
Isolectin Perilipin
B
CD31+ vascular density (mm per mm3)
1000
750
500
250
0
Wildtype
ob/ob
db/db
DIO
C
Density of sympathetic fibers (mm per mm3)
600
400
200
0
D
Wildtype
db/db
DIO
Interscapular BAT
50 μm
Isolectin
E
Density of vasculatures in interscapular BAT (mm per mm3)
15000
10000
5000
0
F
Inguinal WAT
50 μm
Dextran-Rhodamine

Figure S2. Pathological remodeling of the vasculatures in adipose tissues under the obese conditions.

(A) The wild-type, *ob*/*ob*, and *db*/*db* mice were intravenously injected with Isolectin GS-IB4 Alexa647 (green). Inguinal WAT were processed for the whole-mount immunolabeling of perilipin (blue) and were imaged at 12.6x magnification on the lightsheet microscope. Representative orthogonal 3D-projection images of 500 μm-depth tissues are shown. Asterisks denote the adipocytes without surrounding vasculatures (i.e., "vasculature-less" adipocytes). **(B and C)** Inguinal WAT of the wild-type, *ob*/*ob*, *db*/*db*, and diet-induced obesity (DIO) mice were processed for the whole-mount immunolabeling of CD31/PECAM-1 **(B)** or tyrosine hydroxylase **(C)** and were imaged at 12.6x magnification on the lightsheet microscope. Density of $CD31^+$ vasculatures **(B)** or the sympathetic fibers **(C)** was quantified. n = 3, mean ± SEM, * $p < 0.01$. **(D and E)** The wild-type, *db*/*db*, and diet-induced obesity (DIO) mice were intravenously injected with Isolectin GS-IB4 Alexa647. Interscapular BAT were processed for tissue optical clearing and were imaged at 12.6x magnification on the lightsheet microscope. **(D)** Representative orthogonal 3D-projection images of 100 μm-depth tissues are shown. **(E)** Density of the vasculatures in interscapular BAT was quantified. n = 3, mean ± SEM, * $p < 0.01$. **(F)** To assess the leakage of vasculatures, the wild-type, *db*/*db*, and diet-induced obesity (DIO) mice were intravenously injected with 2,000,000MW-Dextran Tetramethylrhodamine. The mice were thoroughly perfused with PBS at 10 min after the dextran-dye injection. Inguinal WAT were processed for tissue optical clearing and were imaged at 12.6x magnification on the lightsheet microscope. Representative orthogonal 3D-projection images of 500 μm-depth tissues are shown.

Figure S3

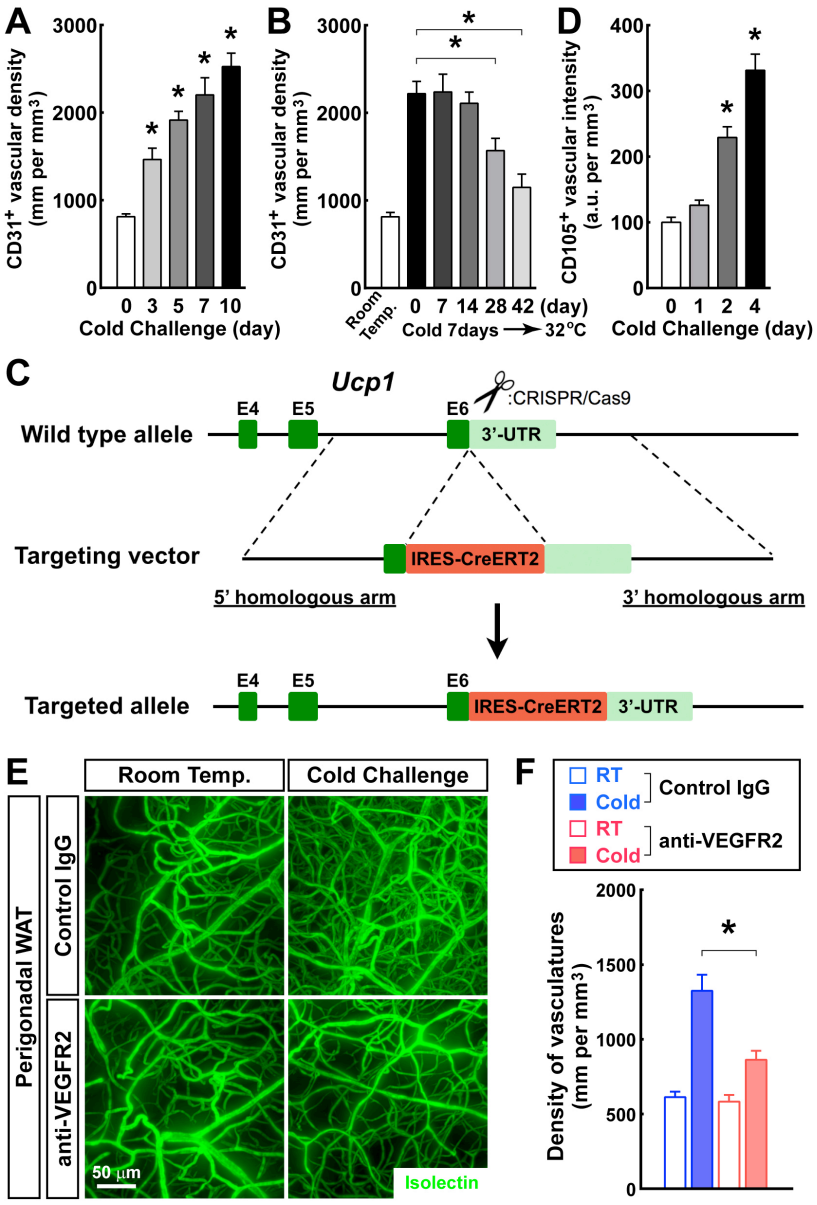
A
CD31+ vascular density (mm per mm3)
3000
2000
1000
0
0 3 5 7 10
Cold Challenge (day)
B
CD31+ vascular density (mm per mm3)
3000
2000
1000
0
Room Temp.
0 7 14 28 42 (day)
Cold 7days → 32°C
D
CD105+ vascular intensity (a.u. per mm3)
400
300
200
100
0
0 1 2 4
Cold Challenge (day)
C
Ucp1
:CRISPR/Cas9
E4
E5
E6
Wild type allele
3'-UTR
Targeting vector
IRES-CreERT2
5' homologous arm
3' homologous arm
Targeted allele
E4
E5
E6
IRES-CreERT2
3'-UTR
E
Room Temp.
Cold Challenge
Perigonadal WAT
Control IgG
anti-VEGFR2
50 μm
Isolectin
F
RT
Cold
Control IgG
RT
Cold
anti-VEGFR2
Density of vasculatures (mm per mm3)
2000
1500
1000
500
0

Figure S3. Vascular plasticity in WAT in response to cold challenge.

(A) The wild-type mice were subject to cold challenge (4°C). Inguinal WAT were harvested at the indicated time points, processed for the whole-mount immunolabeling of CD31/PECAM-1, and imaged at 12.6x magnification on the lightsheet microscope. Density of $CD31^+$ vasculatures was quantified. n = 4, mean ± SEM, * $p < 0.01$. **(B)** The wild-type mice were subjected to cold challenge for 7 days and then reacclimated at the thermal-neutral condition (32°C). Inguinal WAT were harvested at the indicated time points, processed for the whole-mount immunolabeling of CD31/PECAM-1, and imaged at 12.6x magnification on the lightsheet microscope. Density of $CD31^+$ vasculatures was quantified. n = 4, mean ± SEM, * $p < 0.01$. **(C)** Diagram of the generation of $Ucp1^{CreERT2/+}$ knock-in mouse line. **(D)** The wild-type mice were subjected to cold challenge. Inguinal WAT were harvested at the indicated time points, processed for the whole-mount immunolabeling of CD105/Endoglin, and imaged at 12.6x magnification on the lightsheet microscope. Intensity of CD105-immunolabeling was quantified. n = 4, mean ± SEM, * $p < 0.01$. **(E and F)** The wild-type mice administered VEGFR2-neutralizing antibody or control IgG were maintained at room temperature or subjected to cold challenge. The mice were then intravenously injected with Isolectin GS-IB4 Alexa647. Perigonadal WAT were processed for tissue optical clearing and were imaged at 12.6x magnification on the lightsheet microscope. **(E)** Representative orthogonal 3D-projection images of 500 μm-depth tissues are shown. **(F)** Density of the vasculatures in perigonadal WAT was quantified. n = 3, mean ± SEM, * $p < 0.01$.

Figure S4

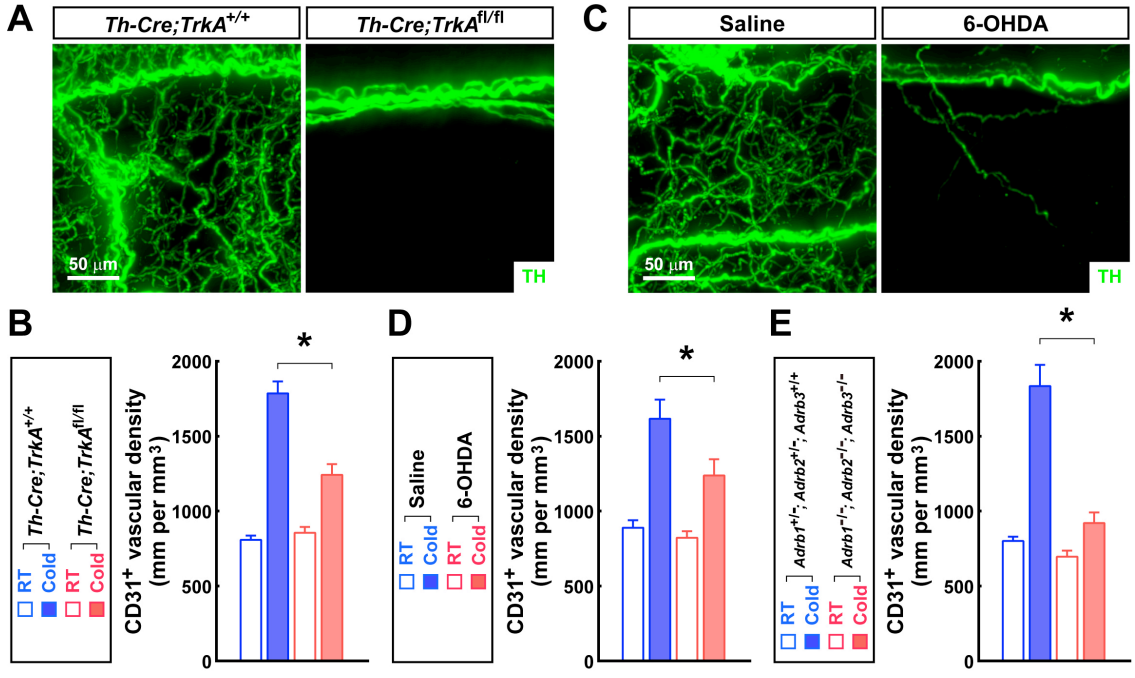
A
Th-Cre;TrkA+/+
Th-Cre;TrkAfl/fl
50 μm
TH
C
Saline
6-OHDA
50 μm
TH
B
RT
Cold
Th-Cre;TrkA+/+
RT
Cold
Th-Cre;TrkAfl/fl
CD31+ vascular density (mm per mm3)
2000
1500
1000
500
0
*
D
RT
Cold
Saline
RT
Cold
6-OHDA
CD31+ vascular density (mm per mm3)
2000
1500
1000
500
0
*
E
RT
Cold
Adrb1+/-; Adrb2+/-; Adrb3+/+
RT
Cold
Adrb1-/-; Adrb2-/-; Adrb3-/-
CD31+ vascular density (mm per mm3)
2000
1500
1000
500
0
*

Figure S4. Cold-induced vascular plasticity in WAT depends on the sympathetic-derived catecholamine signal.

(A) Inguinal WAT of *Th-Cre;TrkA*$^{+/+}$ or *Th-Cre;TrkA*$^{fl/fl}$ mice were processed for the whole-mount immunolabeling of tyrosine hydroxylase (TH) and were imaged at 12.6x magnification on the lightsheet microscope. Representative orthogonal 3D-projection images of 500 μm-depth tissues are shown. **(B)** *Th-Cre;TrkA*$^{+/+}$ or *Th-Cre;TrkA*$^{fl/fl}$ mice were maintained at room temperature or subjected to cold challenge. Inguinal WAT were processed for the whole-mount immunolabeling of CD31/PECAM-1 and were imaged at 12.6x magnification on the lightsheet microscope. Density of CD31^{+} vasculatures was quantified. n = 4, mean ± SEM, * $p < 0.01$. **(C and D)** Inguinal WAT of the wild-type mice were locally treated with 6-hydroxydopamine (6-OHDA) or saline control. **(C)** Inguinal WAT were harvested at 6 days after the treatment, processed for the whole-mount immunolabeling of tyrosine hydroxylase (TH), and imaged at 12.6x magnification on the lightsheet microscope. Representative orthogonal 3D-projection images of 500 μm-depth tissues are shown. **(D)** The mice were subjected to cold challenge at 6 days after the treatment. Inguinal WAT were processed for the whole-mount immunolabeling of CD31/PECAM-1 and were imaged at 12.6x magnification on the lightsheet microscope. Density of CD31^{+} vasculatures was quantified. n = 5, mean ± SEM, * $p < 0.01$. **(E)** *Adrb1*$^{+/-}$*; Adrb2*$^{+/-}$*; Adrb3*$^{+/+}$ or *Adrb1*$^{-/-}$*; Adrb2*$^{-/-}$*; Adrb3*$^{-/-}$ mice were maintained at room temperature or subjected to cold challenge. Inguinal WAT were processed for the whole-mount immunolabeling of CD31/PECAM-1 and were imaged at 12.6x magnification on the lightsheet microscope. Density of CD31^{+} vasculatures was quantified. n = 5, mean ± SEM, * $p < 0.01$.