

## **Supplementary Figures and Their Legends**

### **Little Evidence for Transdifferentiation of Bone-marrow-derived Circulating Progenitor Cells into Adipocytes in Adult Adipose Tissues**

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## Supplementary Figure Legends

**Supplementary Figure 1. Negative control displays no Cy3 signals.** Epididymal fats were harvested from C57BL/6J mice that had received BMT from GFP<sup>+</sup> mice 2 months previously and then were fed a normal diet (**A** and **B**) or a high-fat diet (**C**) for the 2-month period. Tissues were whole-mounted, immunostained without primary antibody against perilipin (**A** and **C**) or adipophilin (**B**), but with secondary antibodies, and merged. No Cy3 signals (red) were in the adipocytes of adipose tissues. Scale bars, 50  $\mu\text{m}$ .

**Supplementary Figure 2. No GFP<sup>+</sup>/adipophilin<sup>+</sup> adipocytes were detected in the adipose tissues of mice fed a normal or a high-fat diet.** Indicated adipose tissues (EF, epididymal fat; RF, retroperitoneal fat) were harvested from C57BL/6J mice that had received BMT from GFP<sup>+</sup> mice 2 months previously and then were fed a normal diet (**A**) or a high-fat diet (**B**) for the 2-month period. Tissues were whole-mounted, immunostained for adipophilin (for premature adipocytes, red), and merged. No GFP<sup>+</sup> cells (green) were adipophilin<sup>+</sup> adipocytes in the adipose tissues. A higher magnification of each area outlined by the dotted squares is shown in the lower panel. Scale bars, 50  $\mu\text{m}$ .

**Supplementary Figure 3. Most GFP<sup>+</sup> cells in the adipose tissues were macrophages.** Epididymal adipose tissues were harvested from C57BL/6J mice that had received BMT from GFP<sup>+</sup> mice 2 months previously and then were fed a normal diet. Tissues were whole-mounted, co-immunostained for CD11b, F4/80, LYVE-1, or CD45 and perilipin

and PECAM-1, and merged. Most GFP<sup>+</sup> cells were CD11b<sup>+</sup>, F4/80<sup>+</sup>, LYVE-1<sup>+</sup>, or CD45<sup>+</sup> macrophages (white arrowheads). Scale bars, 100  $\mu$ m.

**Supplementary Figure 4. Distributions of GFP<sup>+</sup> cells in several tissues.** Indicated tissues were harvested from C57BL/6J mice that had received BMT from GFP<sup>+</sup> mice 2 months previously and were then fed a normal diet. The tissues were whole-mounted, co-immunostained for perilipin and PECAM-1, and merged. The GFP<sup>+</sup> cells were distributed as residential macrophages, leukocytes, and perivascular cells. The GFP<sup>+</sup> cells were largely detected in spleen. Scale bars, 100  $\mu$ m.

**Supplementary Figure 5. Dissected visualization of clustered GFP<sup>+</sup> cells in the adipose tissues.** Epididymal adipose tissue was harvested from C57BL/6J mice that had received BMT from GFP<sup>+</sup> mice 2 months previously and were then fed a normal diet. Tissues were whole-mounted and immunostained for perilipin. (**A** and **B**). The clustered GFP<sup>+</sup> cells, visualized by sequential dissected images on this portion (**A**, white dotted rectangles), were revealed as clustered GFP<sup>+</sup>/perilipin<sup>-</sup> smaller cells (**B**). No GFP<sup>+</sup> cells were perilipin<sup>+</sup> adipocytes in the adipose tissues. White arrows indicate possible multilocular adipocytes. Scale bars, 100  $\mu$ m.

**Supplementary Figure 6. Comparison of body weight and epididymal fat weight.** (**A**) Body weights of C57BL/6J mice fed a normal diet (n= 6–8) or a high-fat (32% wt/wt) diet (n= 4–5) were monitored for 2 months from 8 weeks of age at the time of receiving BMT from the GFP<sup>+</sup> mice (blue arrow). At 16 weeks, the mice were photographed (**B**) and their epididymal fats harvested, photographed (**C**), and weighed

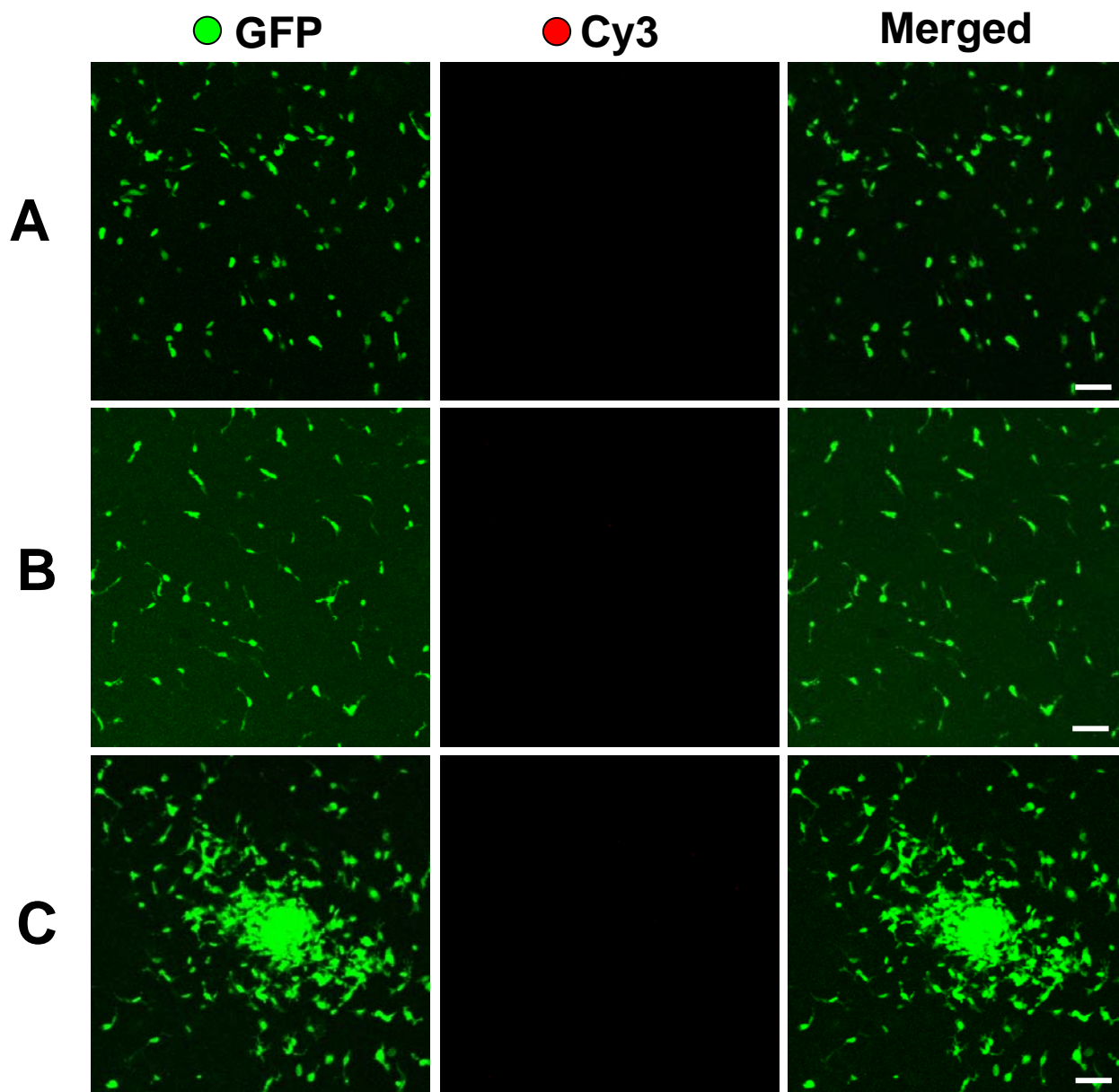
(D). Dots and bars represent means  $\pm$  SD from 4–8 mice. \*,  $P < 0.05$  versus normal diet.

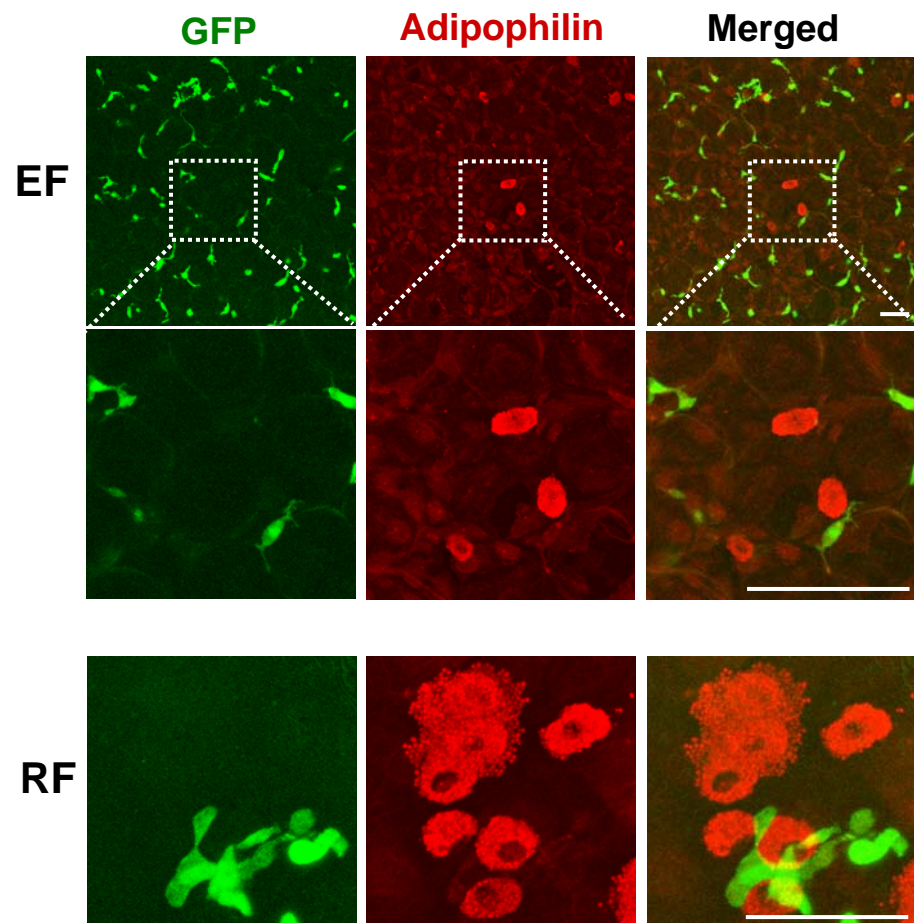
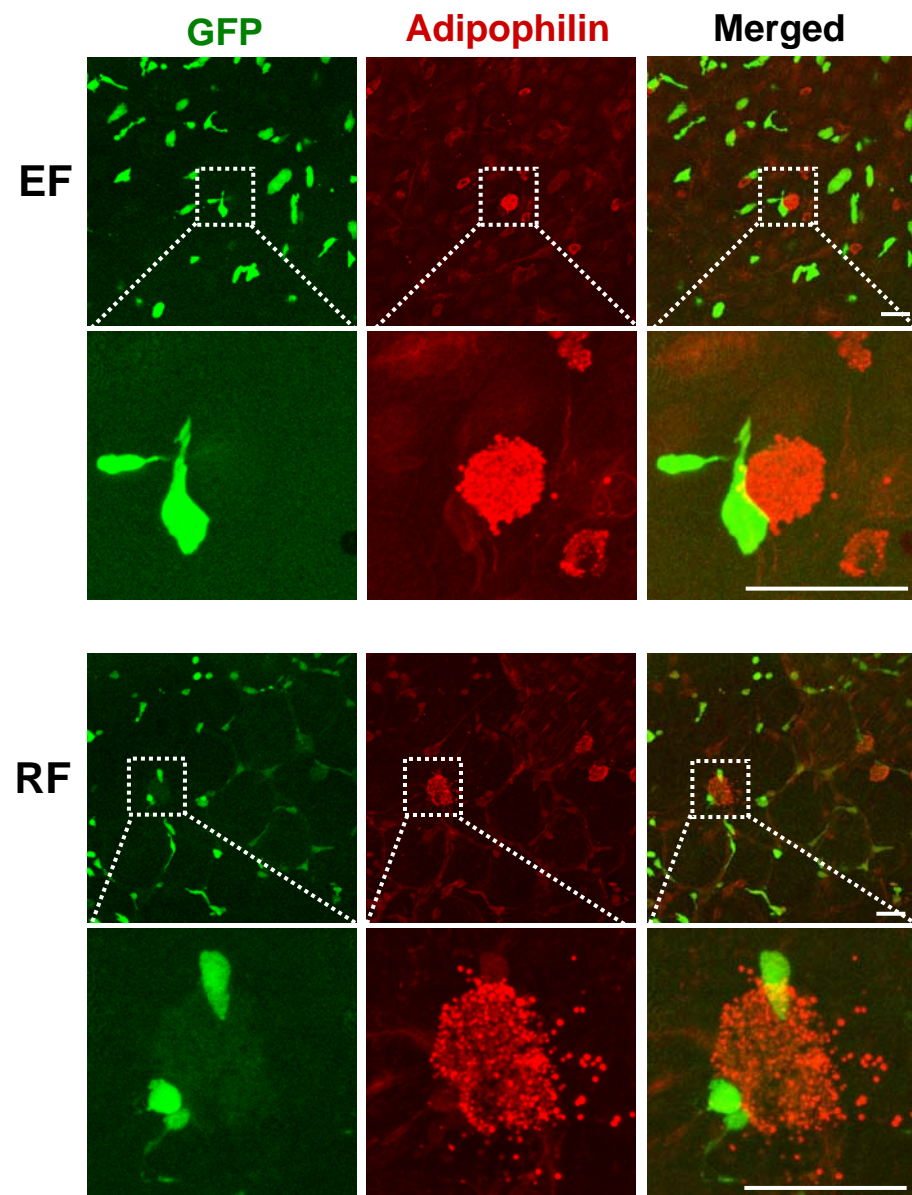
**Supplementary Figure 7. No GFP<sup>+</sup>/perilipin<sup>+</sup> unilocular and multilocular adipocytes were detected in the adipose tissues of mice treated with G-CSF.**

Indicated adipose tissues (EF, epididymal fat; MF, mesenteric fat; RF, retroperitoneal fat; SF, subcutaneous fat; BF, intersubscapular brown fat) were harvested from C57BL/6J mice that had received BMT from GFP<sup>+</sup> mice 2 months previously and then were treated with G-CSF (intraperitoneally 10  $\mu$ g/kg/day) for 2 weeks. Tissues were whole-mounted, immunostained for perilipin, and merged. No GFP<sup>+</sup>/perilipin<sup>+</sup> adipocytes were detected in the adipose tissues. Scale bars, 100  $\mu$ m. Right, higher magnifications of the merged images reveal clustered GFP<sup>+</sup> but perilipin<sup>-</sup> cells. Scale bars, 50  $\mu$ m.

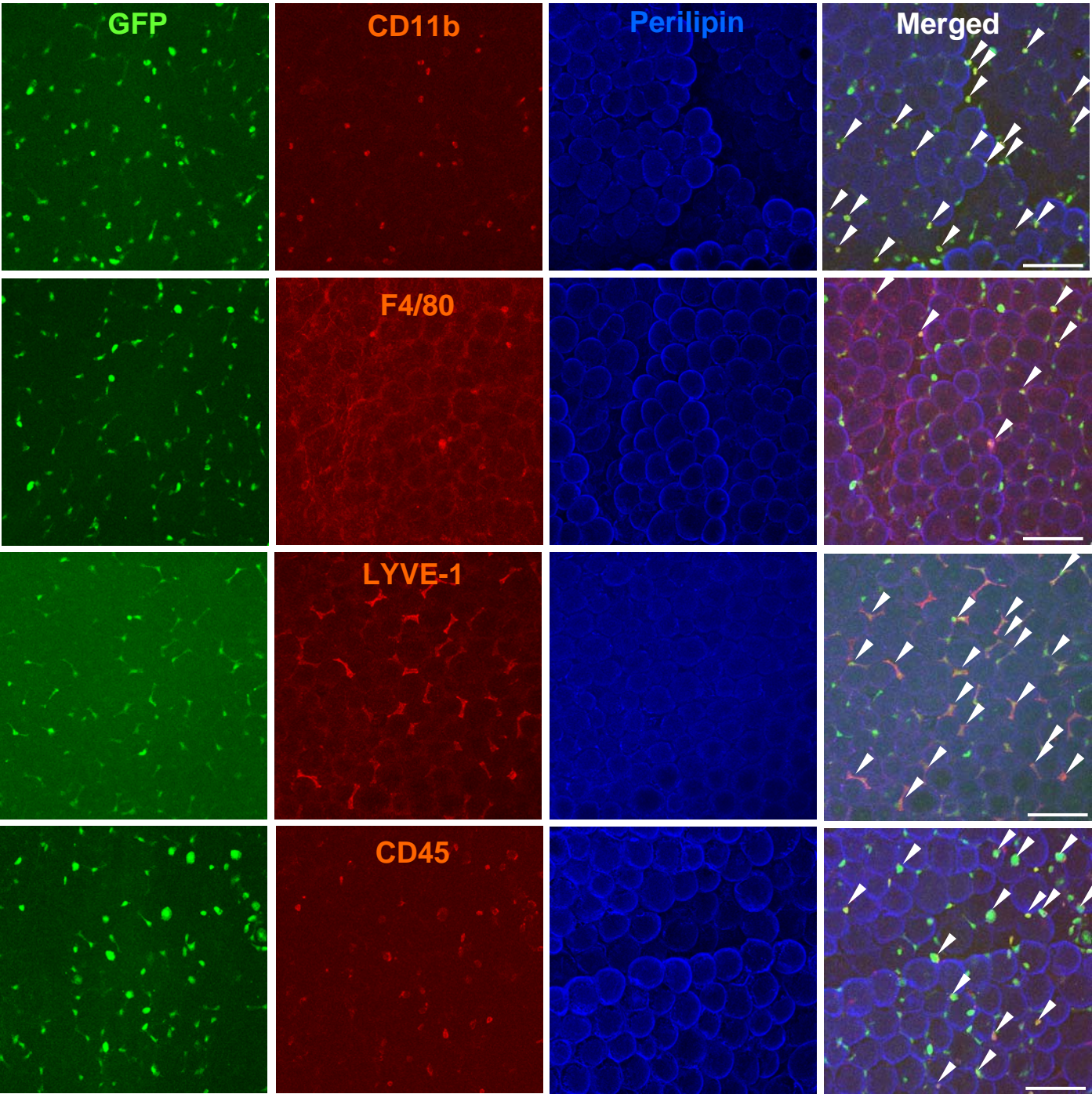
**Supplementary Figure 8. Cross-circulation was established by day 3 after the parabiosis surgical joining.**

The parabiosis between GFP<sup>+</sup> and GFP<sup>-</sup> C57BL/6J mice was conducted. At 3 days after the parabiosis surgical joining, indicated tissues were harvested, whole-mounted, co-immunostained for perilipin and CD45 or PECAM-1, and merged. The GFP<sup>+</sup> cells were detected, but no GFP<sup>+</sup> cells were perilipin<sup>+</sup> adipocytes. Scale bars, 50  $\mu$ m.

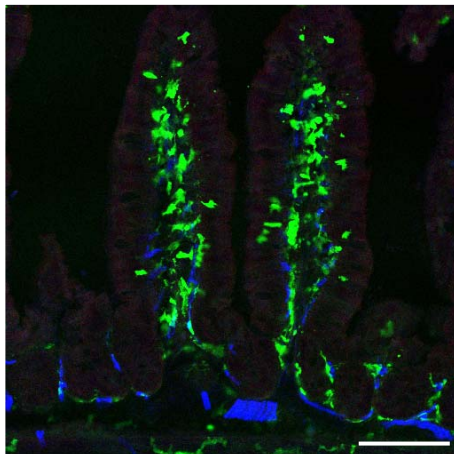


**A****B**

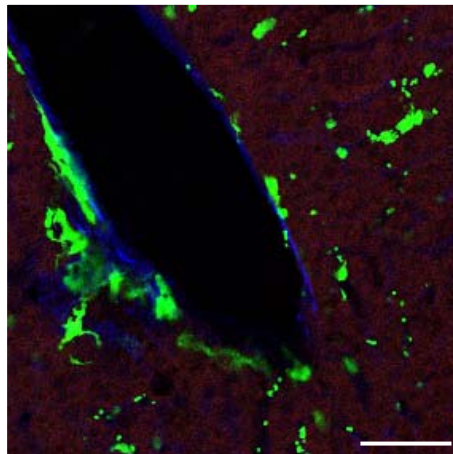
Supplemental  
Figure 3



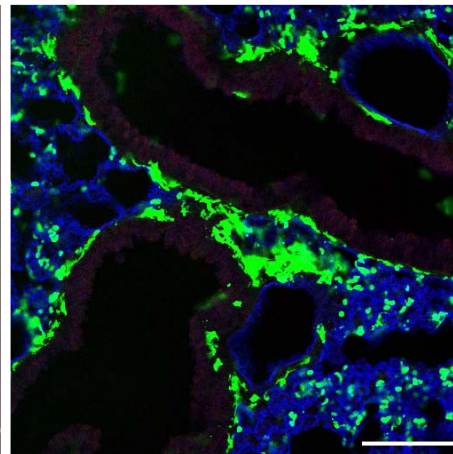
Intestine



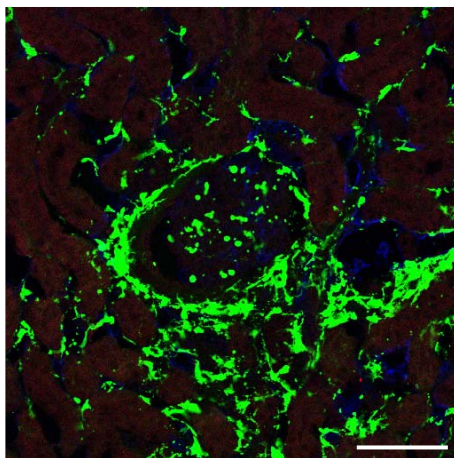
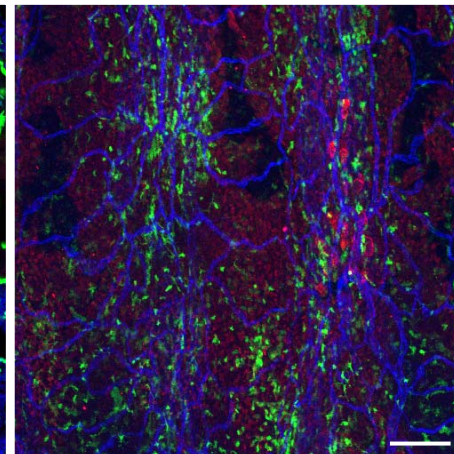
Liver



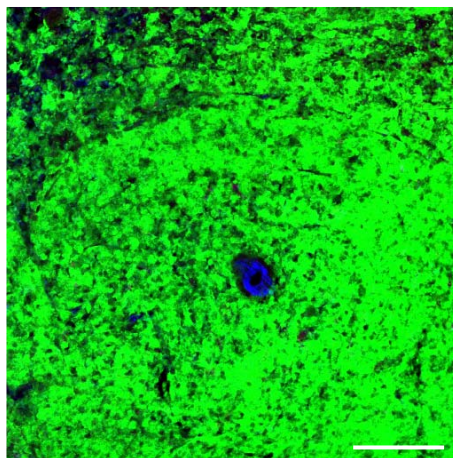
Lung



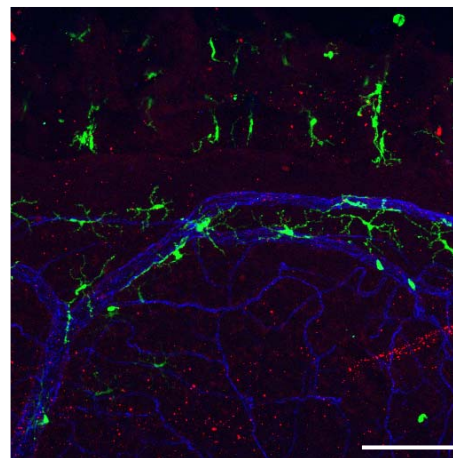
Trachea



Kidney



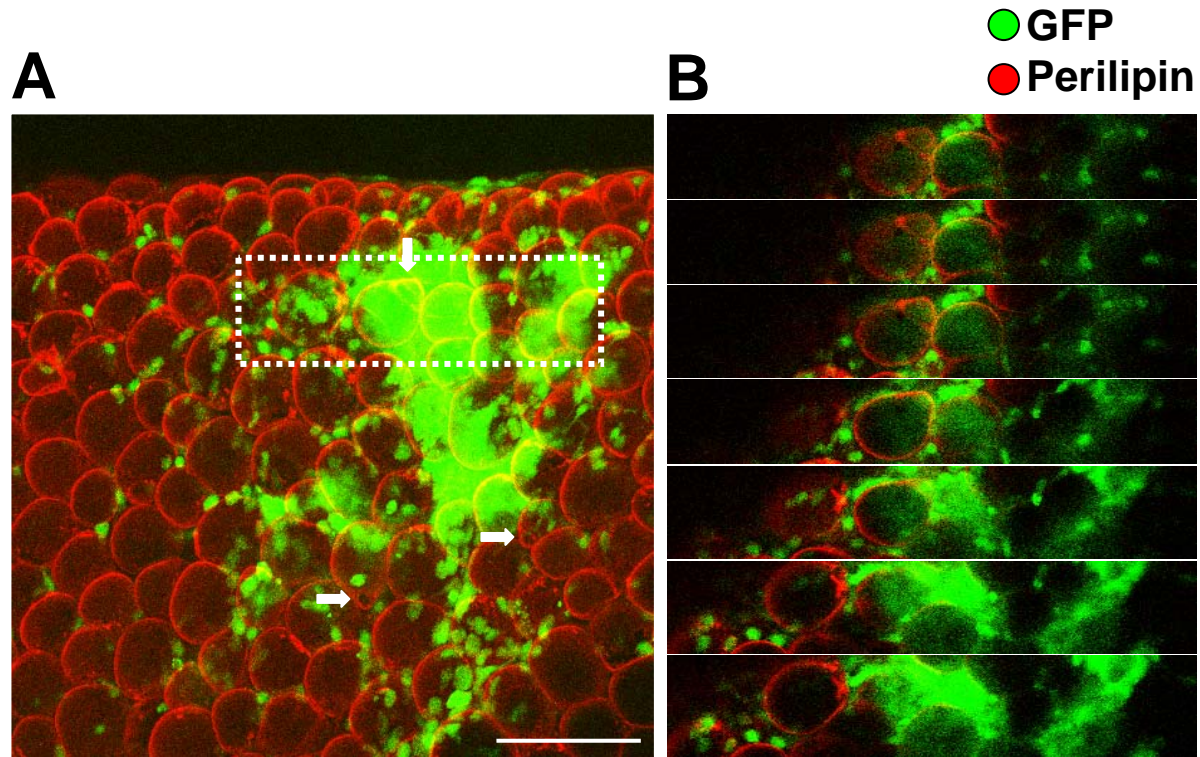
Spleen

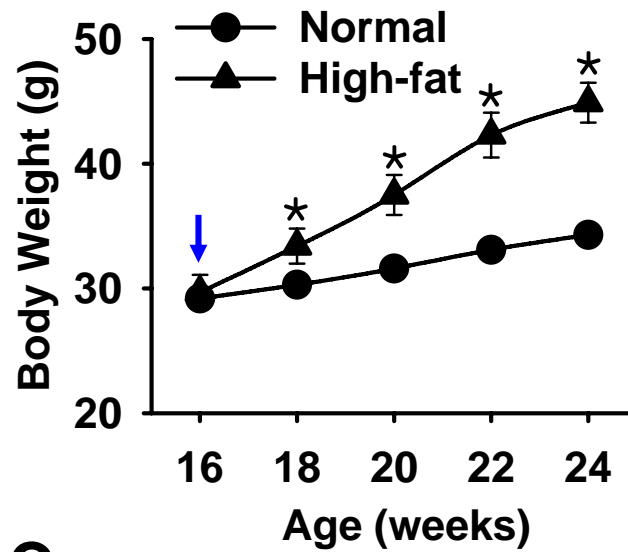
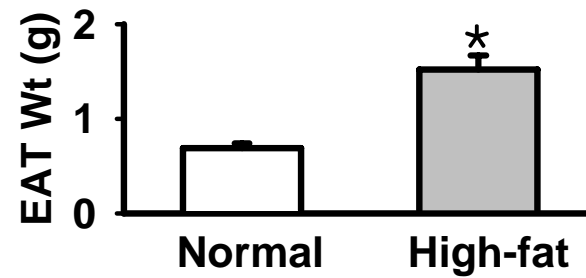
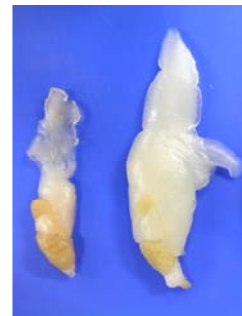


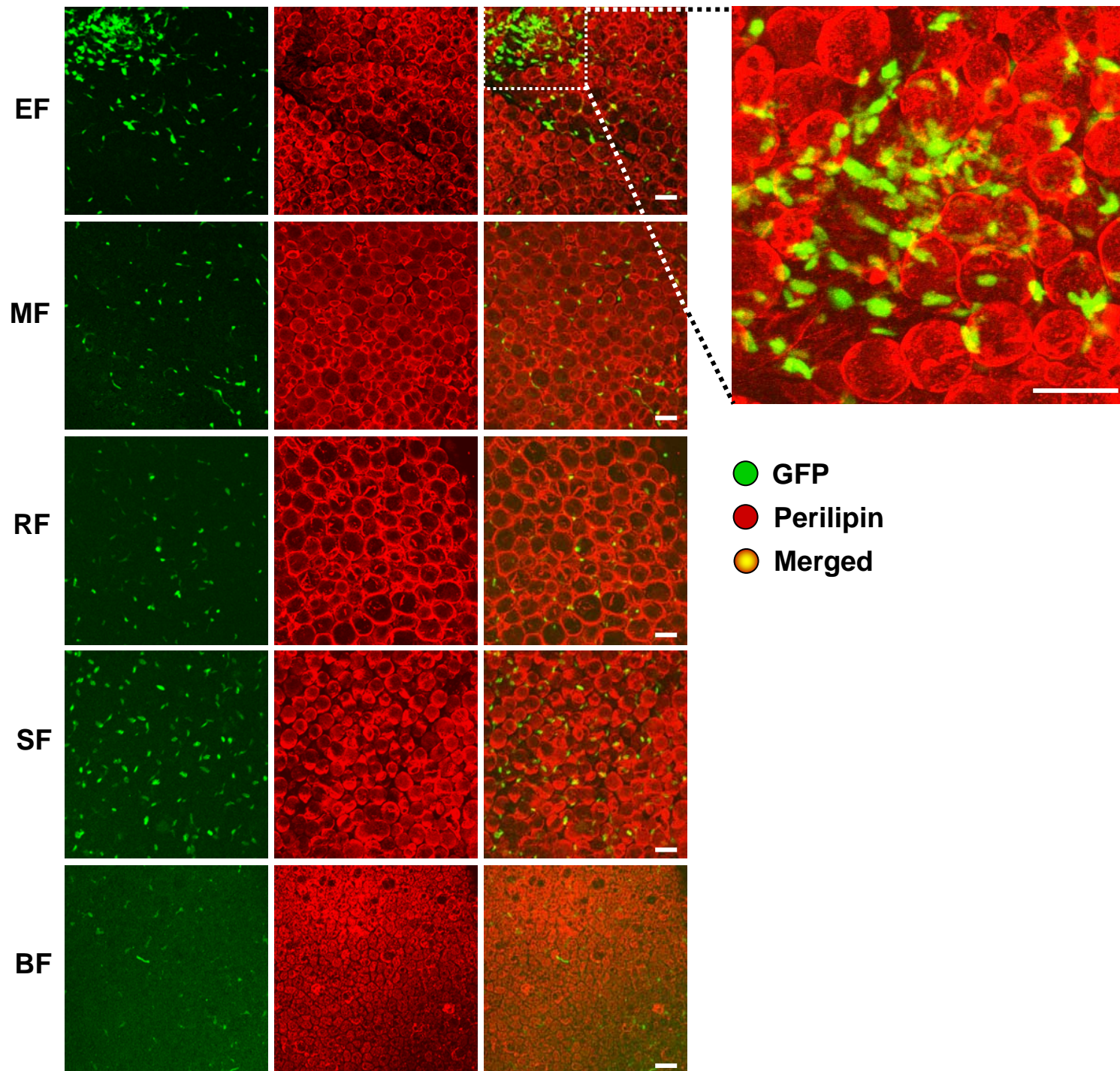
Retina

-  GFP
-  Perilipin
-  PECAM-1

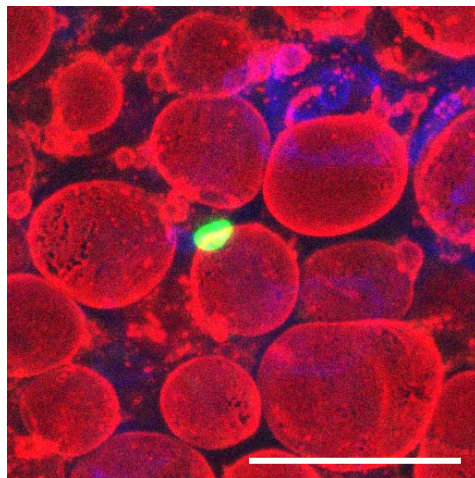




**A****B****C**

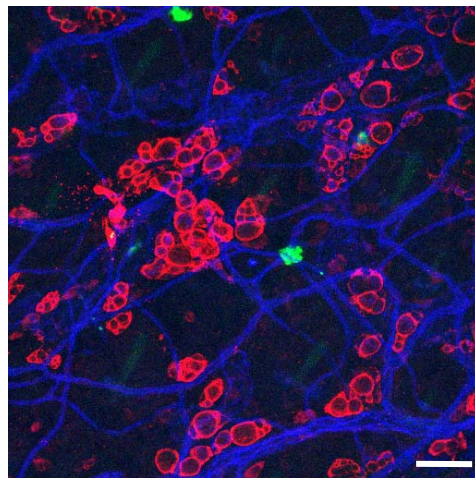


Adipose tissue



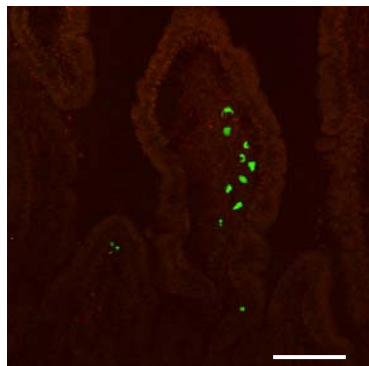
- GFP
- Perilipin
- CD45

Ear skin

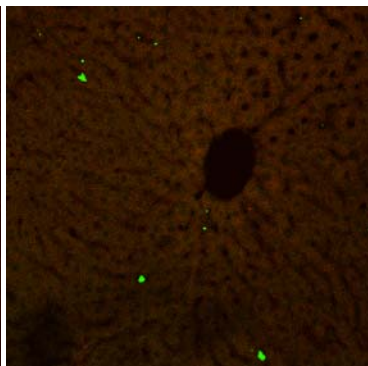


- GFP
- Perilipin
- PECAM-1

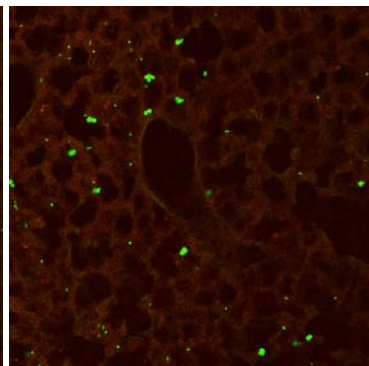
Intestine



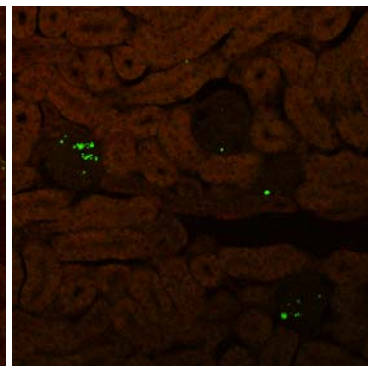
Liver



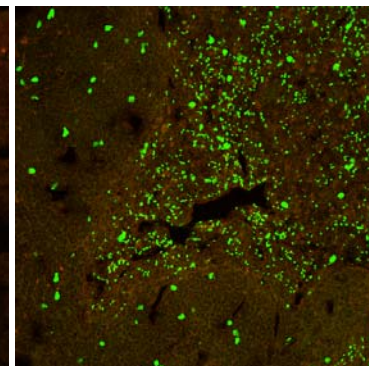
Lung



Kidney



Spleen



- GFP