

Supplementary Data

Activation of PPAR γ Induces Profound Multilocularization and Remodeling of Adipocytes in Adult Mouse White Adipose Tissues

(PPAR γ Induces Multilocularization in Adipocytes)

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Supplementary Table 1: Primers for sqRT-PCR and qrRT-PCR

| | | |
|---------------------------------|---------|--------------------------------|
| <i>ucp-1</i> | Forward | 5'-GTGAAGGTCAGAATGCAAGC-3' |
| | Reverse | 5'-AGGGCCCCCTTCATGAGGTC-3' |
| <i>pgc-1α</i> | Forward | 5'-CTCTCTGGAACTGCAGGCCT-3' |
| | Reverse | 5'-ATCCATGGCTAGTCCTGAAT-3' |
| <i>gapdh</i> | Forward | 5'-TTCCTACCCCAATGTGTCCGTC-3' |
| | Reverse | 5'-ACCCTGTTGCTGTAGCCGTATTCA-3' |

Supplementary Figure Legends

Supplementary Figure 1. ROSI induce profound multilocularization in the adipocytes of adult WAT of mice regardless of sex. The indicated adipose tissues were harvested from C57BL/6J male (M) and female (F) adult mice that had treated with nothing (Control) or ROSI (~15 mg/kg/day) for 3 weeks. **(A)** Tissues were whole-mounted, co-immunostained for perilipin (for lipid droplets) and collagen IV (the basement membrane that surrounds each adipocyte), and merged. Note that adipocytes with variable and smaller-sized perilipin⁺ lipid droplets are abundantly detected in ROSI-treated adipose tissues. Scale bars, 20 μ m. **(B)** Multilocular adipocytes were counted in 10 regions (~100 adipocytes/region) per indicated adipose tissue in each male and female mouse of Control (n=3) or ROSI (n=4), and presented as a percentage of the total adipocytes counted. Bars represent the mean \pm SD. *, $P < 0.01$ versus control.

Supplementary Figure 2. FENO does not induce multilocularization in the adipocytes of adult adipose tissues of mice. The indicated adipose tissues were harvested from C57BL/6J mice that had treated nothing (Control) or FENO (~150 mg/kg/day) for 3 weeks. **(A)** Tissues were whole-mounted, co-immunostained for perilipin and collagen IV, and merged. Note that few multilocular adipocytes are detected in FENO-treated adipose tissues. Scale bars, 20 μ m. **(B)** Multilocular adipocytes were counted in 10 regions (~100 adipocytes/region) per adipose tissue treated with nothing (n=3) or FENO (n=4), and presented as a percentage of the total counted adipocytes. Bars represent the mean \pm SD.

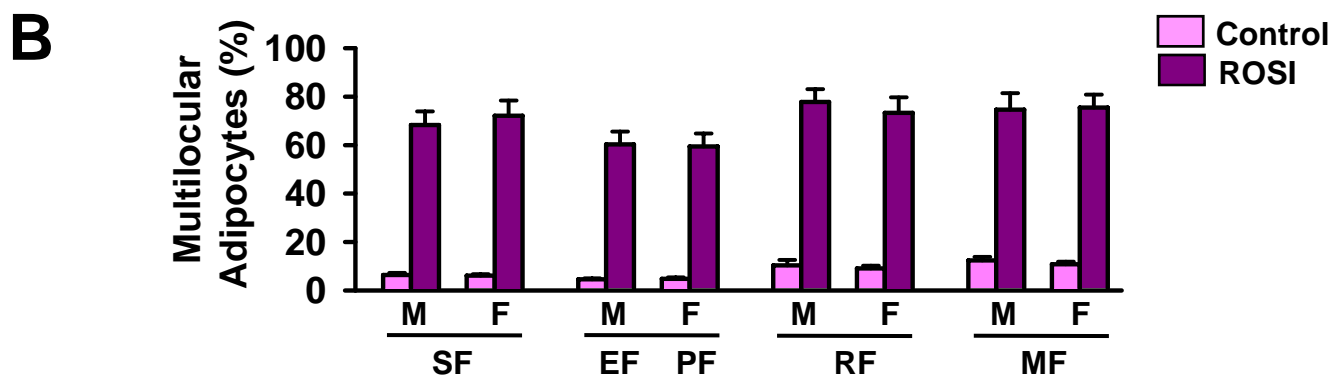
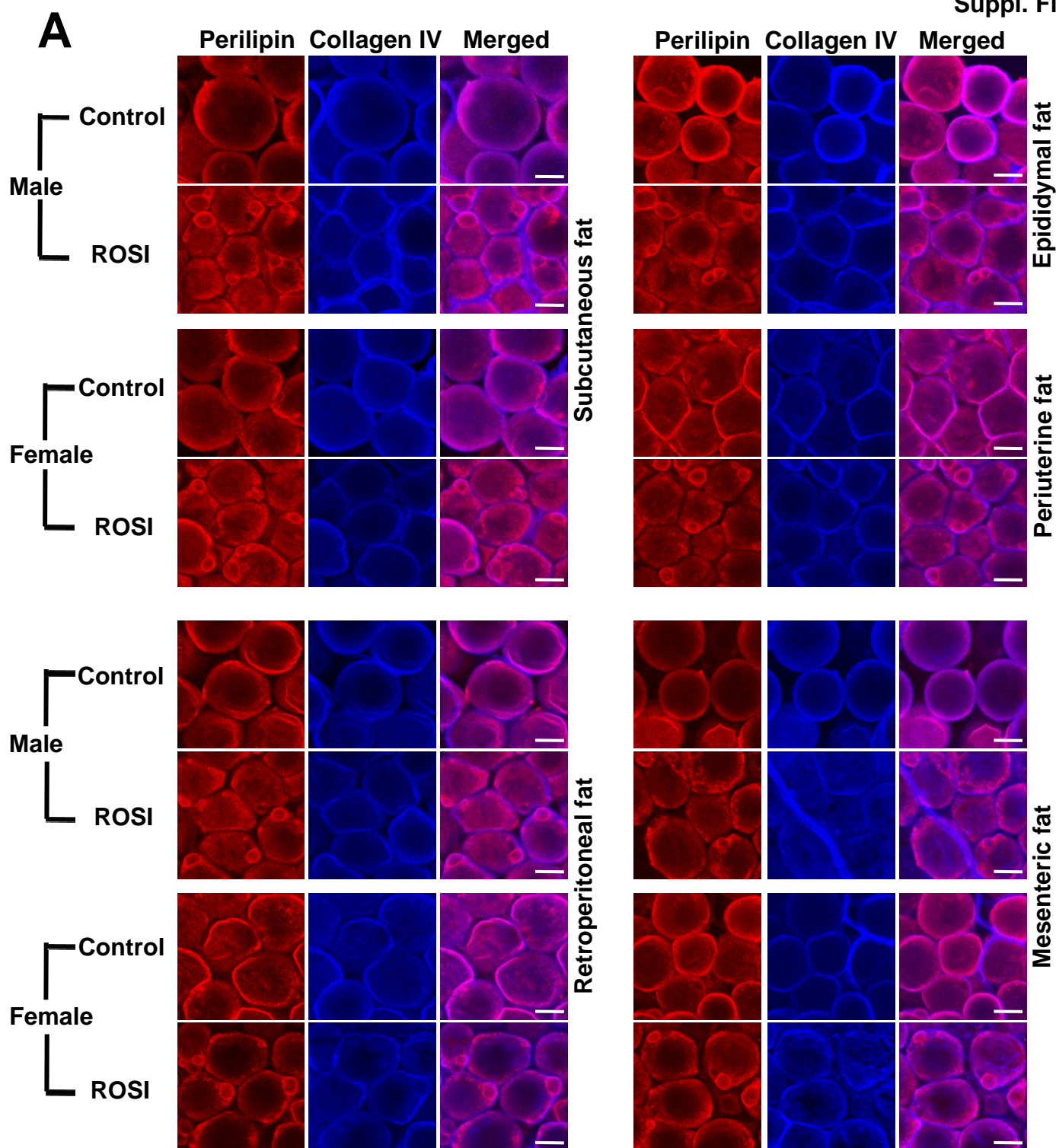
Supplementary Figure 3. ROSI induces remodeling in the form of reduced size of adipocytes in adult adipose tissues of mice. The indicated adipose tissues were harvested from C57BL/6J mice that had been treated with nothing (Control) or ROSI (~15 mg/kg/day) for 3 weeks. **(A)** Tissues were paraffin-embedded, sectioned, and HE stained. Note that smaller and more variably-sized adipocytes are detected in ROSI-treated adipose tissues. Scale bars, 20 μ m.

Supplementary Figure 4. β_3 -adrenoceptor CL316,243 increases mitochondrial content in multilocular adipocytes of adult adipose tissues. The indicated adipose tissues were harvested from C57BL/6J mice that had been treated with control buffer (Control) or CL316,243 (~0.1 mg/kg/day) for 3 weeks. Tissues were whole-mounted, co-immunostained for collagen IV and Mitotracker, and stained with BODIPY 493/503

(BD, for lipid droplet). Note that CL316,243-induced multilocular adipocytes contain substantially increased mitochondrial content. Scale bars, 20 μm .

Supplementary Figure 5. ROSI increases mitochondrial content in the multilocular adipocytes of the adult adipose tissues. The indicated adipose tissues were harvested from C57BL/6J mice that had treated with nothing (Control) or ROSI (~15 mg/kg/day) for 3 weeks. Tissues were whole-mounted, co-immunostained for collagen IV and Mitotracker, and stained with BODIPY 493/503 (BD, for lipid droplet). Note that ROSI-induced multilocular adipocytes contain substantially increased mitochondrial content. Scale bars, 20 μm .

Supplementary Figure 6. ROSI increases mRNA of *ucp-1* and *pgc-1 α* in the subcutaneous adipose tissues of mice. Subcutaneous adipose tissues were harvested from C57BL/6J mice that had treated with nothing (Con) or ROSI (~15 mg/kg/day) for 3 weeks. Total RNA were extracted from the isolated adipocytes and semi-quantitative RT-PCR performed with the appropriate primers. Each mRNA levels were normalized by *gapdh* and presented as relative density. The relative density measured for each control is arbitrarily presented as 1. Bars represent mean \pm SD (n=4). *, $P < 0.05$ versus Cont.



Supplementary Fig. 2

