SUPPLEMENTARY DATA

A protective effect of PPARα in endothelial progenitor cells through regulating metabolism

Running title: **PPARα in ECFC metabolism**

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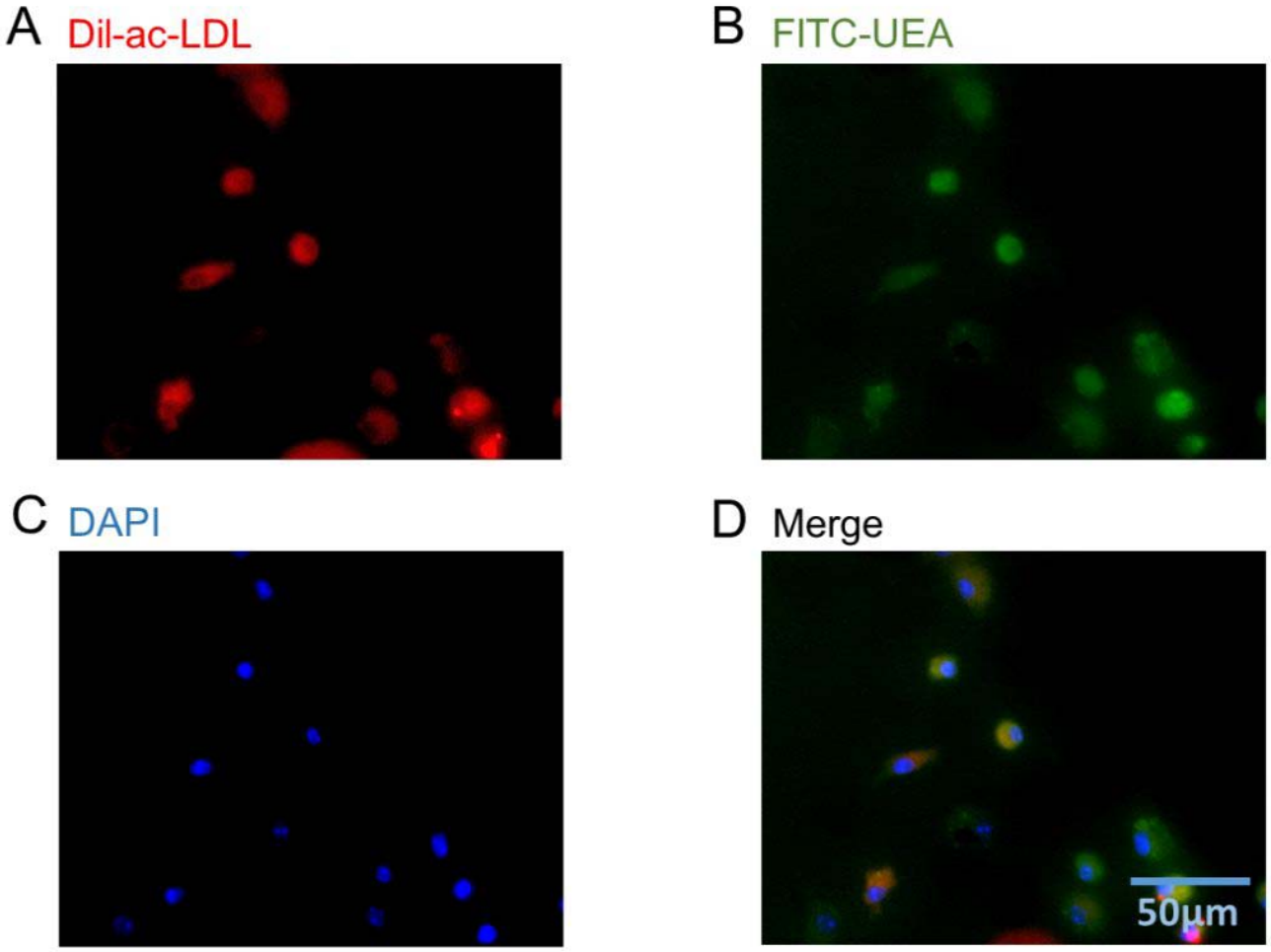
Supplementary Figure 1. ECFC identification. ECFC were washed twice (5 minu each) using PBS. The cells were incubated with Dil-ac-LDL (A: Red) at 37ºC for 2-hour and fixed with 4% paraformaldehyde for 20 min. The cells were then washed 3 times (5 min each), fixed with 2% paraformaldehyde (10 min). The cells were subsequently incubated with FITC-UEA (B: Green) and DAPI (C: Blue). The cells were visualized and photographed by fluorescence microscope (bar= 50 µm). D: Merged image.



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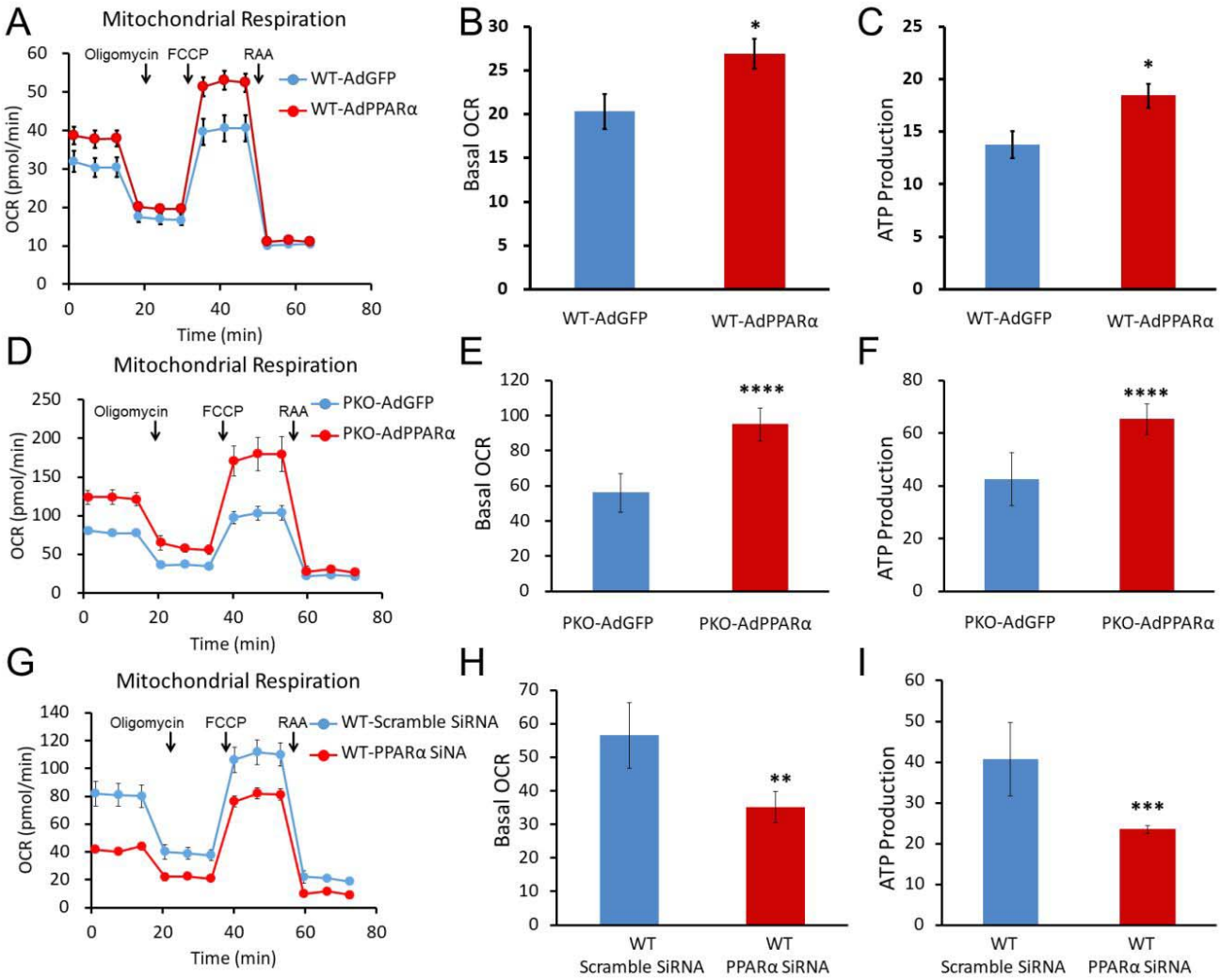
Supplementary Figure 2. PPARα regulated ECFC mitochondrial function. (A, D) Representative traces of OCR of WT (A) and *PPARα*$^{-/-}$ (PKO) (D) ECFC transfected with AdPPARα (MOI=50, 48-hour) or with AdGFP as control. (G) Representative traces of OCR of WT ECFC transfected with PPARα siRNA compared with scrambled siRNA control. The injections of reagents (oligomycin, FCCP, RAA) during the Seahorse analysis were indicated by arrows. Basal OCR (B, E and H) and ATP Production (C, F and I) (the basal respiration that potentially support ATP production) were calculated and compared. All values are mean±SEM. n≥3, *P<0.05. **P<0.01, ***P<0.001 and ****P<0.0001.



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Supplementary Figure 3. Effects of PPARα on mitochondrial mass. A&B: ECFC were immunostained for Tomm20 (Abcam, ab78547) following manufacturer's protocol. Images of ECFC were collected at the same setting in each experiment under an Olympus Fluoview (Version2.1a) (Confocal microscope, 100X objective). The fluorescence intensities of Tomm20 (green) were obtained using ImageJ software (NIH) and normalized by DAPI (blue) nuclei fluorescence intensity. A: Representative images showed the primary ECFC from WT and *PPARα*$^{-/-}$ (KO) mice stained with Tomm20 and DAPI. B: Area ratio of mitochondria and nuclear (mito/nucleus) were calculated using ImageJ (n=3). C: Total DNA was extracted from primary WT and *PPARα*$^{-/-}$ ECFC using ZR-*Duet*™ DNA/RNA MiniPrep Plus Kit (Zymo Research, Irvine, CA). The chip-based digital polymerase chain reaction (dPCR) was performed to quantify copies of mitochondrial DNA (mtDNA) as previously described (1). mtDNA copy numbers were calculated by qPCR and normalized by nuclear DNA concentration (n=5) (mean±SEM, NS, no significant difference).

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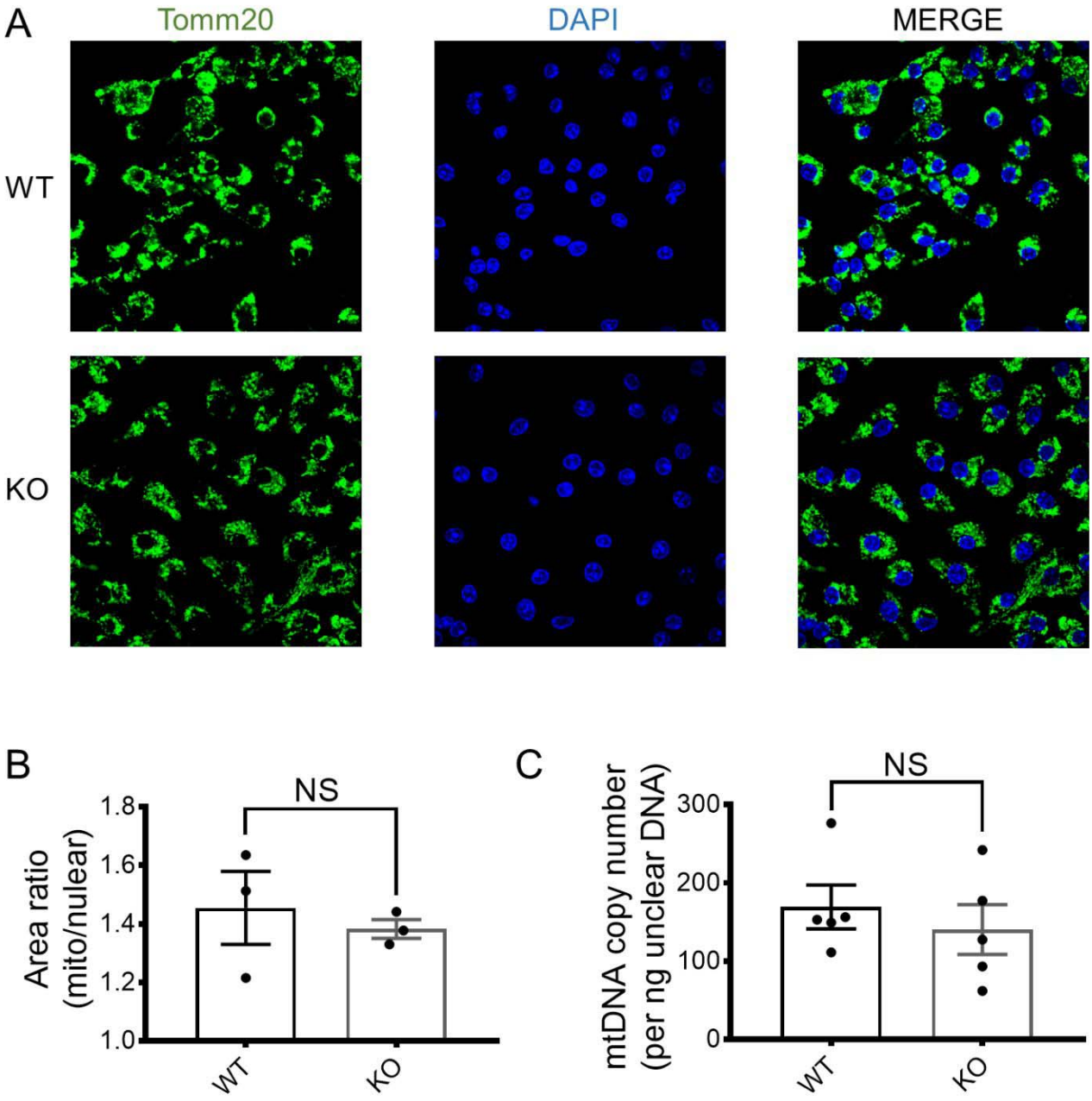

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Reference

1. Hong SN: [The usefulness of fecal calprotectin in differentiating between functional and organic bowel diseases: application in pediatric constipation patients]. Korean J Gastroenterol 2013;62:261-262