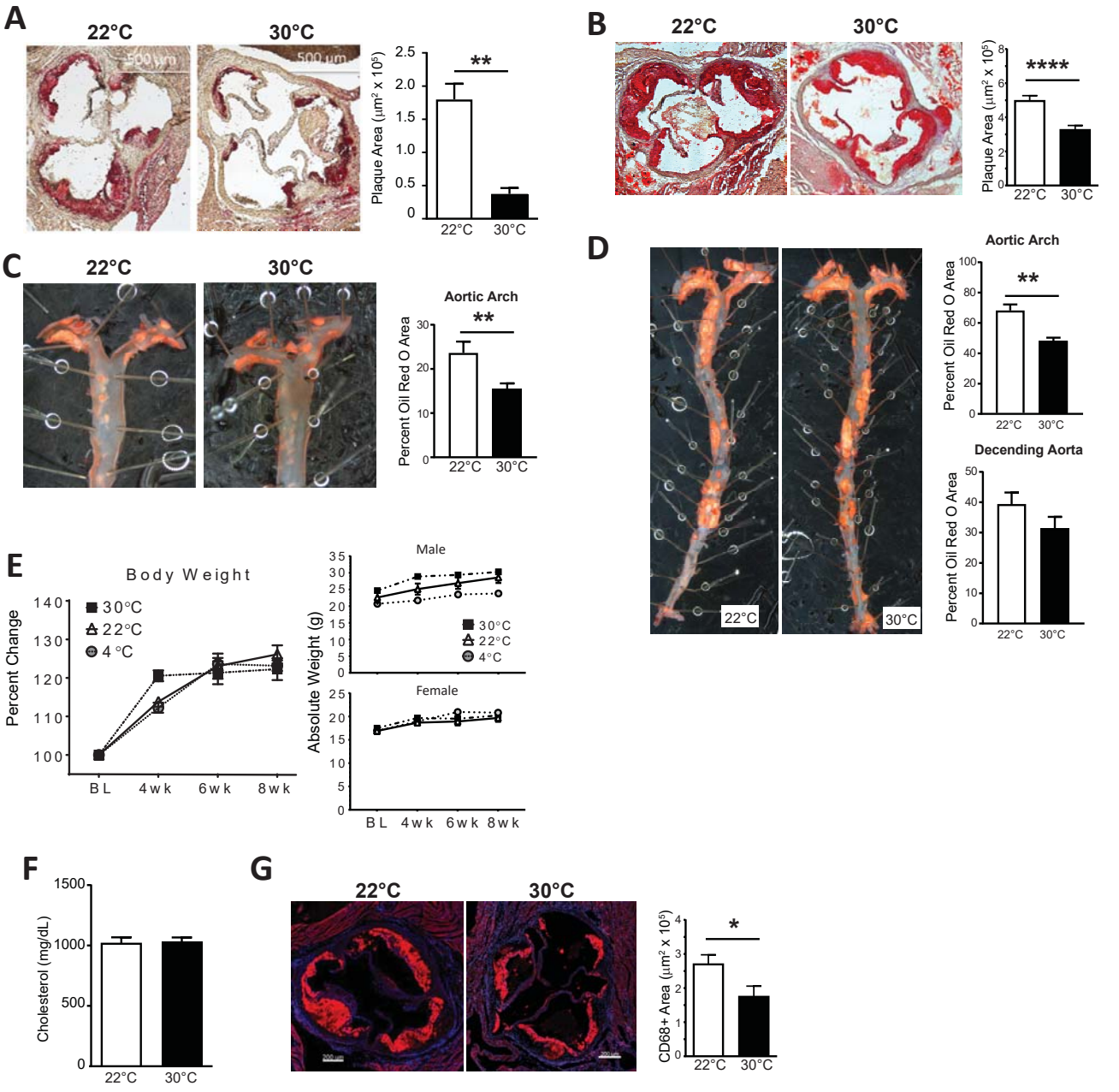
**Williams, et. al.**

# Thermoneutrality but not UCP1 deficiency suppresses monocyte mobilization into the blood

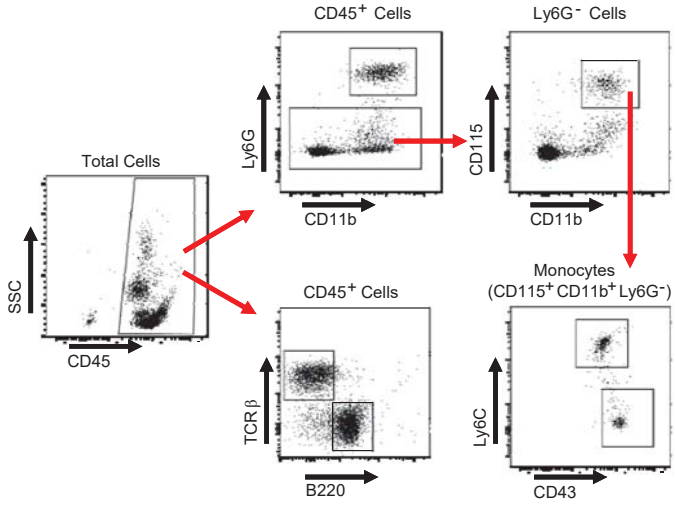
# Supplemental Material

# Online Figure I



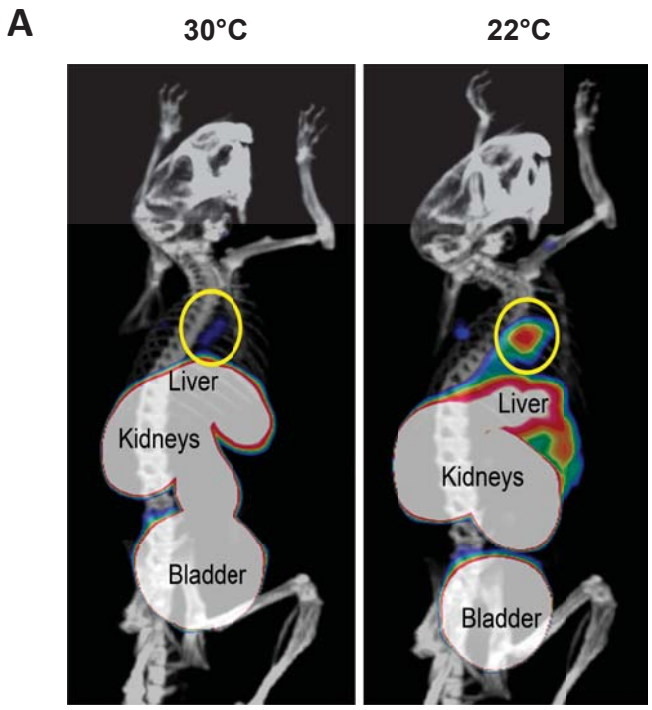

**Online Figure I. Reduced atherosclerosis at thermoneutral housing environment.** A) *Apoe*$^{-/-}$ mice were housed at the indicated temperatures and assayed for atherosclerosis progression following 8 weeks of HFD. *Ldlr*$^{-/-}$ mice were housed at indicated temperatures and fed HFD for 12 weeks, then assayed for plaque development at aortic sinus (B) and aortic arch (C). D) *Ldlr*$^{-/-}$ mice fed HFD for 28 weeks were assayed by en face oil red o staining for plaque area on aortic arch and descending aorta. E) Percentage change relative to starting body weight of *Ldlr*$^{-/-}$ mice housed at different ambient temperatures while on HFD, (right panel) absolute weight numbers for males (upper) and female (lower). F) Plasma cholesterol levels were assayed in *Apoe*$^{-/-}$ mice after 8 weeks HFD. G) *Ldlr*$^{-/-}$ mice fed HFD for 12 weeks were assayed for CD68+ area in aortic sinus. All data include n≥5 animals per group and are representative of two or three independent experiments each. Statistical analysis was performed by unpaired student T-test, * p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

# Online Figure II



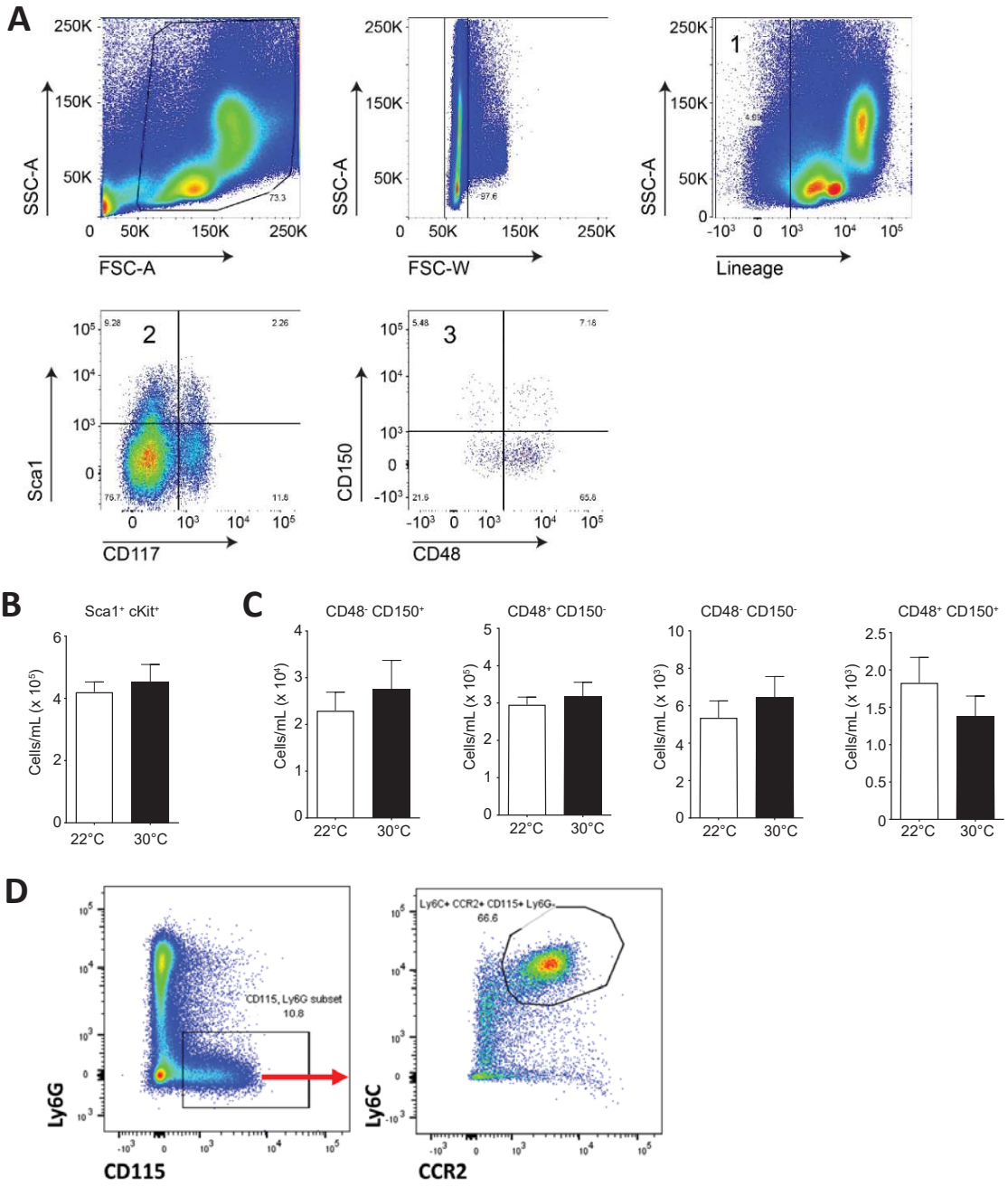

**Online Figure II. Gating scheme for blood monocytes.** Monocytes were identified as $CD45^+$, $Ly6G^-$, $CD115^+$, and $CD11b^+$. Monocytes were then separated into subsets by expression of Ly6C and CD43.

## Online Figure III



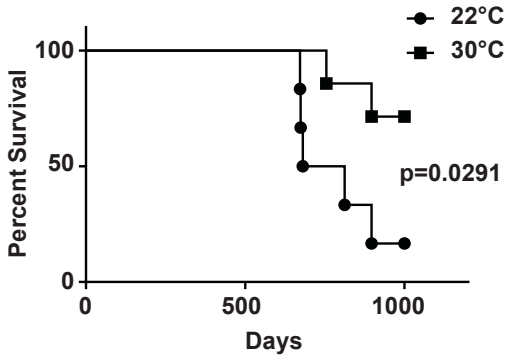

**Online Figure III. PET/CT Images of $^{64}$Cu-DOTA-ECL1i accumulation.** *Ldlr*$^{-/-}$ mice housed at the indicated temperature and fed a HFD for 8 weeks were imaged by PET/CT for $^{64}$Cu-DOTA-ECL1i tracer.

## Online Figure IV



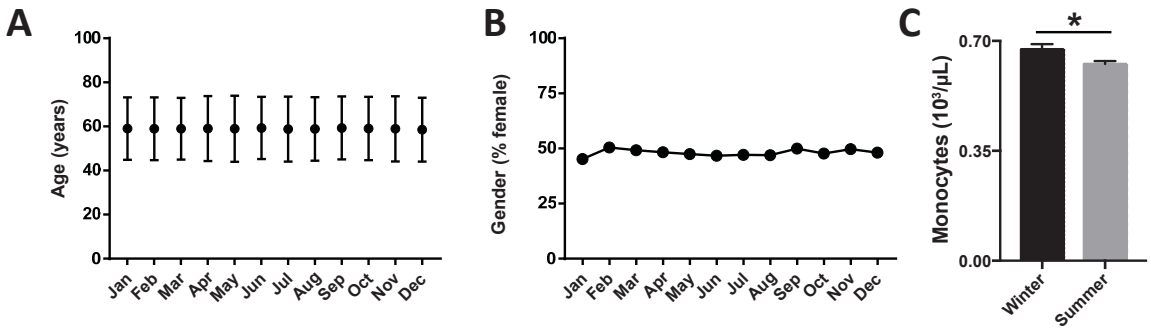

**Online Figure IV. Gating strategy for bone marrow precursor cells.** A) Bone marrow was isolated and stained for stem cell markers Sca1, CD117(cKit), CD150, and CD48. Gating was first performed on total cells by fsc and ssc, then singlet gate and selected on lineage negative cells ($CD3^-$ $B220^-$ $Gr1^-$ $Ter119^-$), as displayed in flow plot 1. B). $Sca1^+$ $CD1117^+$ bone marrow stem cell progenitors were identified in flow plot 2 and C) subpopulations in flor plot 3 to identify stem cells were then separated into $CD48^-$ $CD150^+$, $CD48^+$ $CD150^-$, $CD48^-$ $CD150^-$, and $CD48^+$ $CD150^+$, with gate 3 ($CD150^+$ $CD48^-$) cells being the least differentiated cell in gating panel. D) Bone marrow monocytes were identified by $CD115^+$, $Ly6G^-$. $CCR2^+$, and $Ly6C^+$ expression. A and D are representative gating panels, B and C are n= 5 mice per group and experiment was repeated two independent times.

## Online Figure V



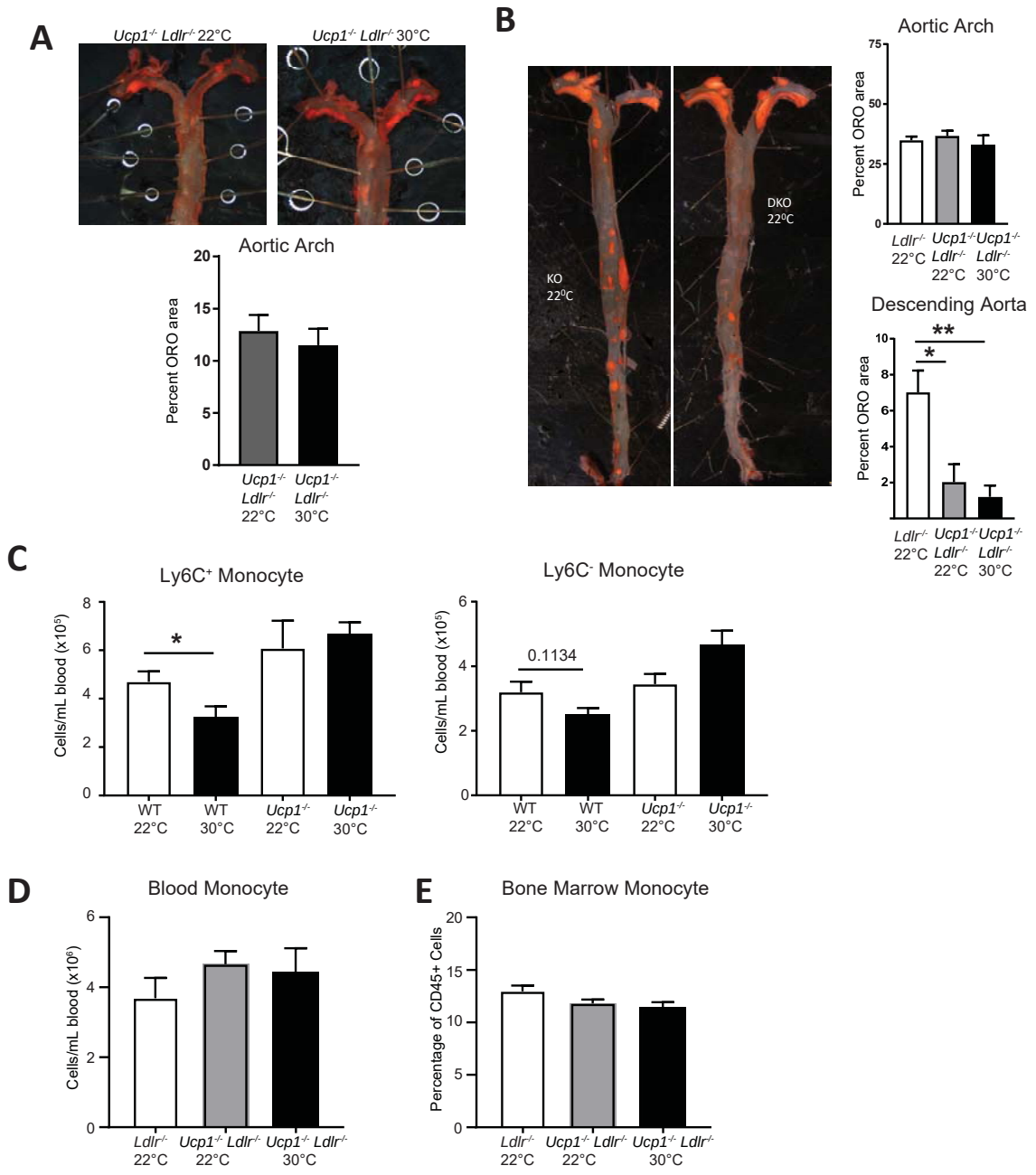

**Online Figure V. Thermoneutral housing increased lifespan of C57bl/6 mice.** Animals were placed in the indicated environmental temperatures at 6 weeks of age, under normal chow diet and SPF conditions. Animals were tracked for survival, plotted on Kaplan-Meir Curve. Statistical analysis performed by Gehan-Breslow Wilcoxon Test, p-value reported. n=6-7 animals per group.

## Online Figure VI



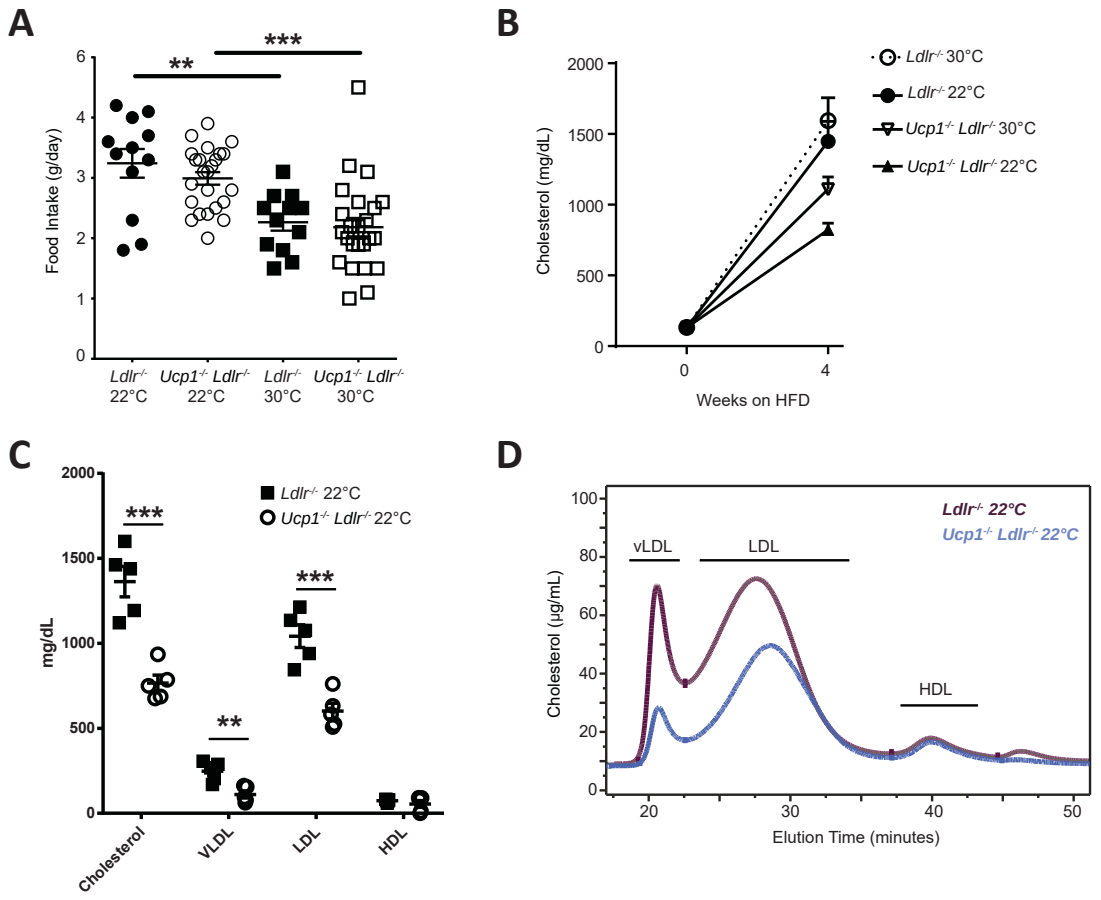

**Online Figure VI. Human peripheral blood analysis.** A) Average age of patients in relation to month in which blood was drawn (±SD). B) Gender (presented as % female) information for patients sampled across different months. C) Blood monocyte levels were separated by average cells in winter months vs. summer months. Data are averages of approximately 1200 samples per month.

# Online Figure VII



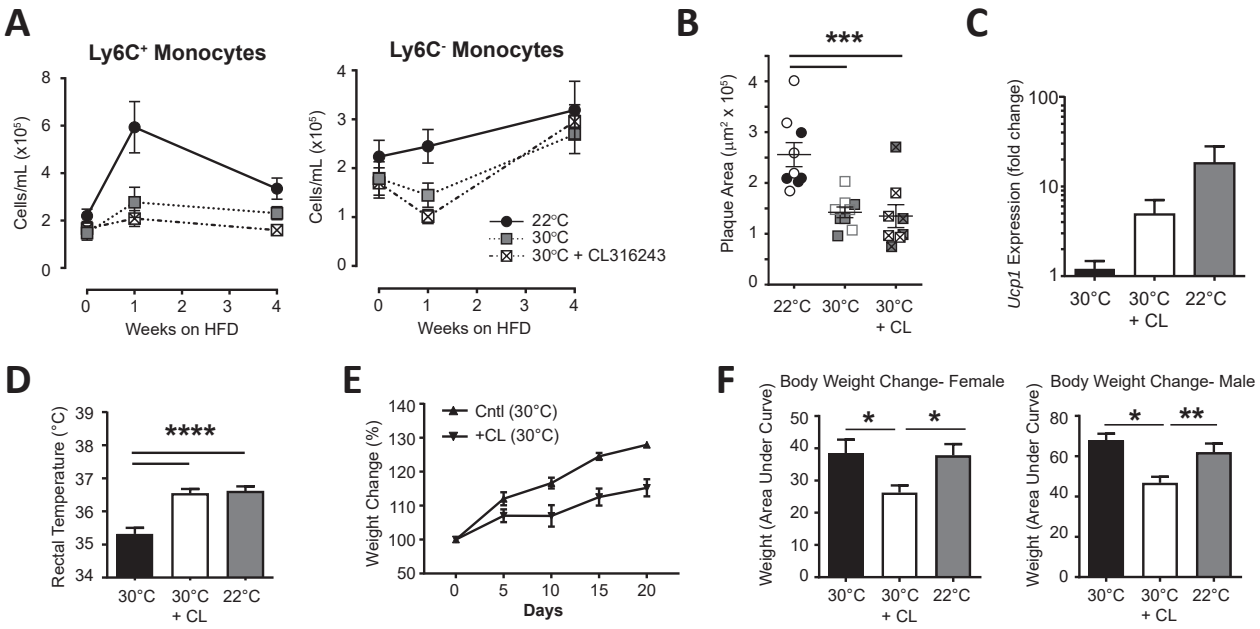

**Online Figure VII. UCP1-deficiency regulates atherosclerosis progression but not monocyte levels.** A) *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ mice were housed at 22°C or 30°C and fed HFD for 12 weeks, Aortic arches were assayed for plaque deposition by oil red o staining. B) *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice were housed at indicated temperature and fed HFD for 20 weeks and assayed by en face aorta analysis for plaque area in aortic arch and descending aorta. C) *Ucp1*$^{-/-}$ or C57bl/6 mice were housed at indicated temperatures for 7 days and assayed for changes in Ly6C$^{+}$ and Ly6C$^{-}$ blood monocyte levels. D) Blood and E) bone marrow monocyte analysis of *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ or *Ldlr*$^{-/-}$ mice housed at the indicated temperature and fed a HFD for 8 weeks, then assayed for monocyte levels. Data are representative experiments, n≥5 mice per group, and repeated in one or two independent experiments. Statistical analysis was performed by unpaired student T-test, * p≤0.05, **p≤0.01

# Online Figure VIII




**Online Figure VIII. *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ mice have reduced LDL levels following HFD compared to controls.** A) *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice were housed at indicated temperatures and fed a HFD for 8 weeks, then assayed for food consumption. B) Total cholesterol levels were measured at 0 and 4 weeks HFD. C) After 8 weeks HFD, plasma from *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice was analyzed for lipoprotein profiles of total cholesterol, vLDL, LDL, and HDL levels. D) Representative graph of cholesterol elution from *Ucp1*$^{-/-}$ *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ plasma samples. All data include n≥5 animals per group. Statistical analysis was performed by unpaired student T-test, * p≤0.05, **p≤0.01, ***p≤0.001

# Online Figure IX




**Online Figure IX. β3-adrenoreceptor signaling during thermoneutrality modulates food intake and body temperature but not monocyte counts or atherosclerosis progression.** A) *Ldlr*$^{-/-}$ mice were housed at the indicated ambient temperature and given twice-daily doses of β3-agonist CL or PBS for 4 weeks while on HFD. Animals were monitored for blood monocyte levels. B) Following 4 weeks of continuous treatment and HFD exposure, animals were assayed for plaque development in the aortic sinus, (C) *Ucp1* expression D) core body temperature measured by rectal probe, and (E) body weight measurements (F, separating female and male weight data). Data are combined to include n≥8 animals per group and is representative of two independent experiments. Statistical analysis was performed by unpaired student T-test, * p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

# Online Movie I

**Online Movie I. Bone marrow imaging of CXCR1$^{gfp/+}$ CXCL12$^{dsred}$ animals housed at different temperatures.** Animals were housed at 22°C or 30°C for 4 weeks, then assayed by intravital bone marrow imaging for monocyte (gfp$^{+}$) cell accumulation. Representative video shows dynamic behavior and organization within the bone marrow milieu.