

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Effect of 2-deoxy-D-glucose on cell viability. Cells were treated with PDGF in the absence or presence of 2-deoxy-D-glucose (2-DG, 20 mM) for 48 h. Cell viability was assessed by measuring LDH activity in the cell lysates and media. Data are presented as means \pm SEM; n=3–5 per group. N.S. is used to express non-significant differences between groups.

Supplementary Figure 2. Changes in cell cycle proteins in VSMC treated with PDGF: Effects of PI3K inhibitor and 2-deoxy-D-glucose. VSMC were treated with PDGF (10 ng/ml) for 24 h in the absence or presence of LY-294002 (LY, 10 μ M) or 2-DG (20 mM). Proteins in cell lysates were used to examine phospho-Rb (Ser^{807/811}), cyclin D₁ and β -actin (loading control) by Western blotting. **(B and C)** Bar graphs for grouped densitometric values of phospho-Rb (Ser^{807/811}) and cyclin D Western blots, respectively. Data are presented as means \pm SEM; n=3 per group, *p<0.05 vs. CTRL, #p<0.05 vs. PDGF, \$p<0.05 vs. LY-294002.

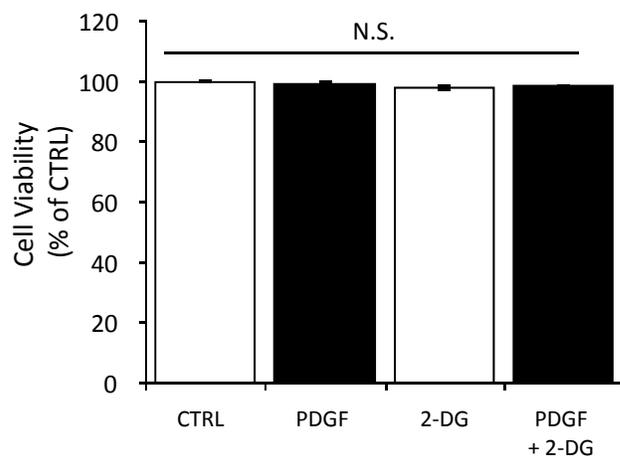
Supplementary Figure 3. Effect of PDGF on ATP and ATP/ADP ratios in VSMC. VSMC pre-treated with LY-294002 (LY, 10 μ M) or 2-DG (20 mM) for 1 h were exposed to PDGF (10 ng/ml, 24 h). Cellular nucleotides were isolated and analyzed by HPLC. **(A)** Total levels of intracellular ATP. **(B)** ATP/ADP ratios. Data are presented as means \pm SEM; n=3 per group, *p<0.05 vs. CTRL, #p<0.05 vs. PDGF.

Supplementary Figure 4. Mitochondrial function assay. Schematic showing parameters assessed by XF analysis. To probe individual components of respiration that contributed to the consumption of oxygen, oligomycin (1 μ g/ml), FCCP (1 μ M), and antimycin A (10 μ M) were injected sequentially. This allowed for an estimation of the contribution of non-ATP-linked oxygen consumption (proton leak; Leak) and ATP-linked mitochondrial oxygen consumption (ATP). The maximal respiratory capacity was determined using the FCCP-stimulated rate. The reserve capacity is represented by the maximal respiratory capacity subtracted from the baseline OCR. The residual oxygen consumption that occurred after addition of antimycin A was ascribed to non-mitochondrial sources and was subtracted from all values in the analysis.

Supplementary Figure 5. Effect of PDGF on mitochondrial membrane potential, protein expression, and enzymatic activity. Mitochondrial membrane potential, citrate synthase activity, complex IV activity (cytochrome c oxidase), mitochondrial protein expression, pyruvate dehydrogenase (PDH) E-1 α subunit expression and phosphorylation as well as pyruvate dehydrogenase kinase -1 (PDK-1) expression were determined in VSMC treated with PDGF (10 ng/ml) for 24 h. **(A)** Mitochondrial membrane potential was assessed using the ratio of red to green JC-1 fluorescence. **(B)** Citrate synthase activity was examined after incubation of VSMC with vehicle (CTRL) or PDGF (10 ng/ml). **(C)**

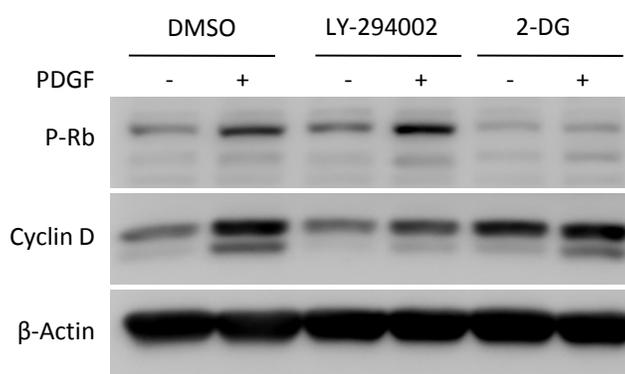
Cytochrome *c* oxidase activity was similarly determined after vehicle (CTRL) or PDGF treatment. Data for A, B and C are presented as means \pm SEM; n=3–6 per group, *p<0.05 vs. CTRL. **(D)** Representative Western blots are shown for Cytochrome *c* (Cyt-*c*), complex III core 2 protein (C III-core 2), complex II 70kD protein (C II-70kD), voltage-dependent anion channel (VDAC), complex IV subunit 1 (C IV-1), complex I 39kD protein (C I-39kD), and β -actin (loading control) after PDGF treatment for 24 h. Grouped densitometric values shown to the right of each representative Western blot are presented as mean fold change vs. control \pm SEM; n=3 per group. **(E)** Representative Western blots for PDH E-1 α subunit phosphorylation at Ser²⁹³ and total protein expression, as well as PDK-1 after pre-treatment of VSMC with LY-294002 (LY, 10 μ M) or 2-DG (20 mM) for 1 h followed by exposure to PDGF (10 ng/ml) for 24 h. Data values are presented as mean fold change vs. control \pm SEM; n=3 per group.

Supplementary Figure 1

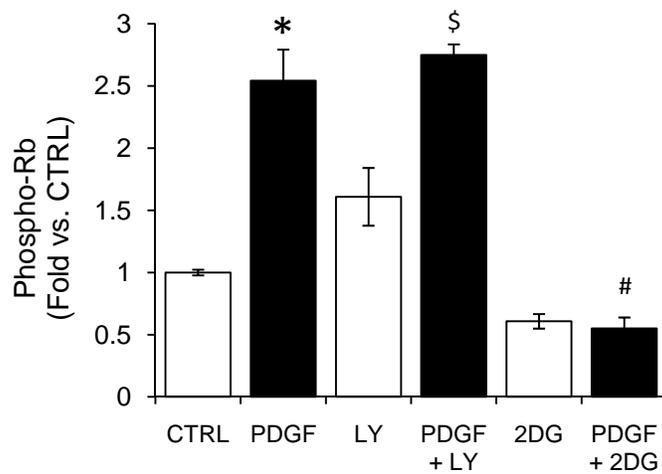


Supplementary Figure 2

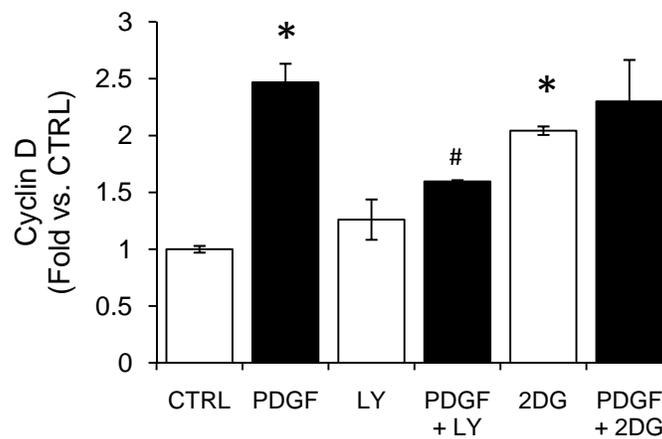
A.



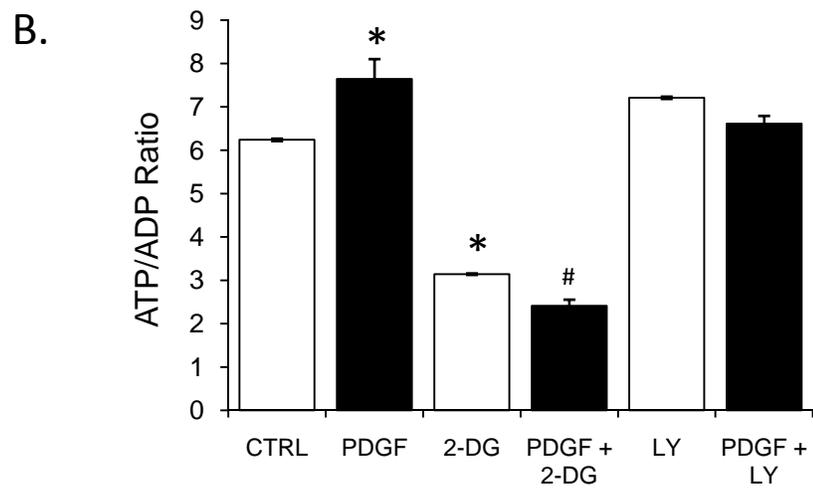
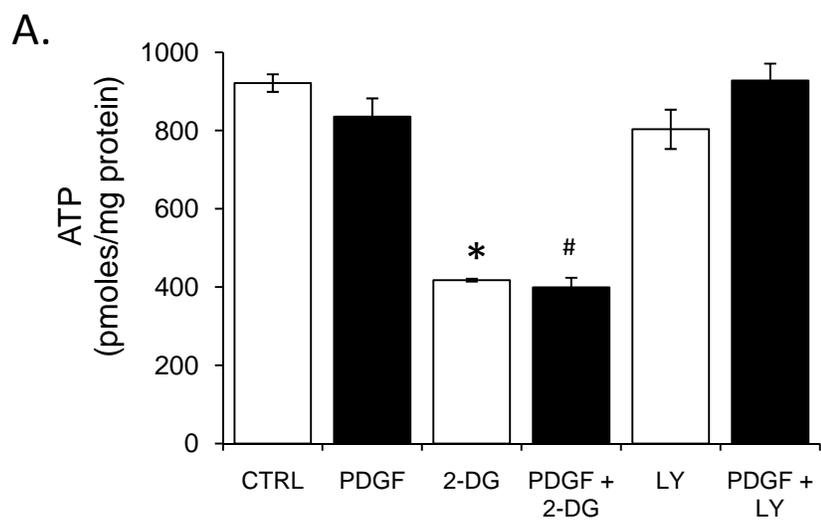
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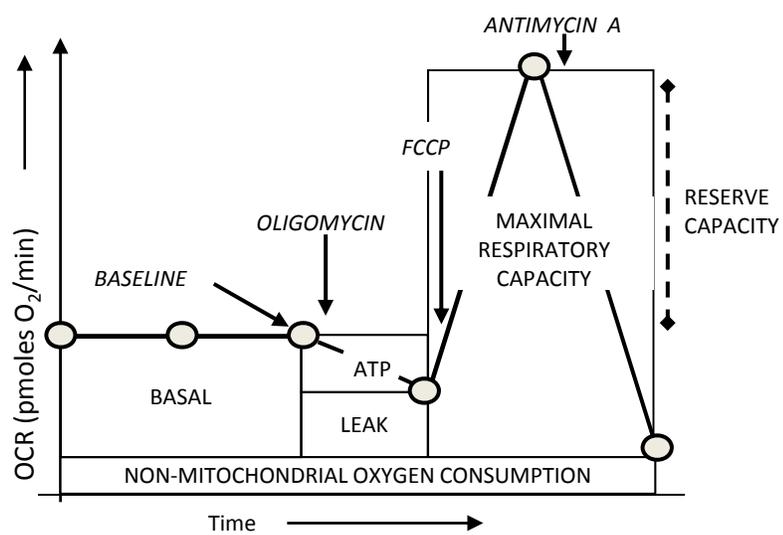
C.



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

