Supplemental Figure 1

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Supplemental Figure 1: Pyridine nucleotide loss after combined treatment with NO donor and redox cycler.

BAEC were exposed to Deta/NO (500 μ M) for 1 h prior to treatment with menadione (20 μ M, white bars) or DMNQ (20 μ M, grey bars) for an additional 4 h. Black bars represent control treatment. NADP+ (A), and NADPH (B) levels were measured by HPLC and normalized to total protein. Values represent means ± SE; n = 3. * p < 0.05 and ** p < 0.005 compared to samples without Deta/NO.



Supplemental Figure 2: The effects of NO donor and redox cyclers on plasma membrane integrity.

BAEC were exposed to Deta/NO (500 μ M) for 1 h prior to treatment with menadione (20 μ M, white bars) or DMNQ (20 μ M, grey bars) for an additional 4 h. Cells were trypsinized after the treatment and the integrity of plasma membrane was measured by trypan blue exclusion from the life cells and expressed as percentage of total cells. Values represent means ± SE; n = 3. * p < 0.05 compared to untreated control.

Supplemental Figure 3

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Supplemental Figure 3. The effect of PARP inhibitor on nucleotide depletion

BAEC were exposed to Deta/NO (500 μ M) for 1 h prior to incubation without (black bars) and with menadione (20 μ M, white bars) for an additional 4 h in the presence and absence of PARP-1 inhibitor, PJ-34 (10 μ M). ATP (A), ADP (B), and AMP (C) levels were measured by HPLC and normalized to total protein. Values represent means ± SE; n = 3. * p < 0.05 compared to samples without Deta/NO, **p < 0.05 compared to samples without PJ-34.



Supplemental Figure 4. Measurement of bioenergetics in BAEC using XF technology.

(A) A schematic representation of mitochondrial function assay obtained after sequential addition of oligomycin (1 μ g/ml), FCCP (1 μ M), and antimycin A (10 μ M). These OCR measurements allow for determination of basal OCR, ATP-linked OCR (ATP), proton leak (Leak), reserve capacity (Reserve) and non-mitochondrial OCR. (B) BAEC were exposed to menadione (20 μ M) or DMNQ (20 μ M) for 4 h and mitochondrial function assay was performed. (C) BAEC were expose Deta/NO (500 μ M) for 1 h prior to treatment with menadione (20 μ M) or DMNQ (20 μ M) for an additional 4 h and mitochondrial function assay was performed. Proteon leak (D) and non-mitochondrial OCR (E) were calculated. OCRs were normalized to total protein per well after completion of assay. Values represent means ± SE; n = 3 to 4.