Increased Expression of TGF-β1 by 4-hexylresorcinol Is Mediated by Endoplasmic Reticulum and Mitochondrial Stress in Human Umbilical Endothelial Vein Cells

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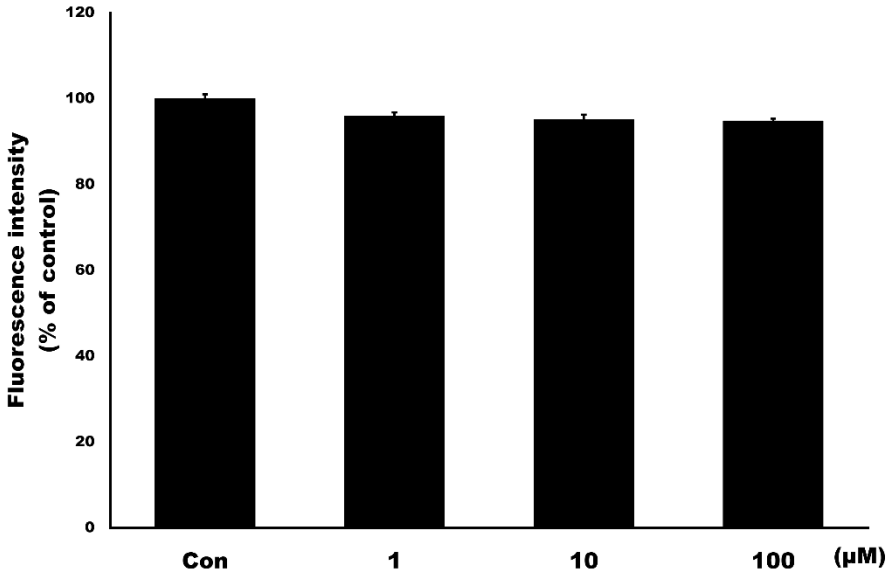
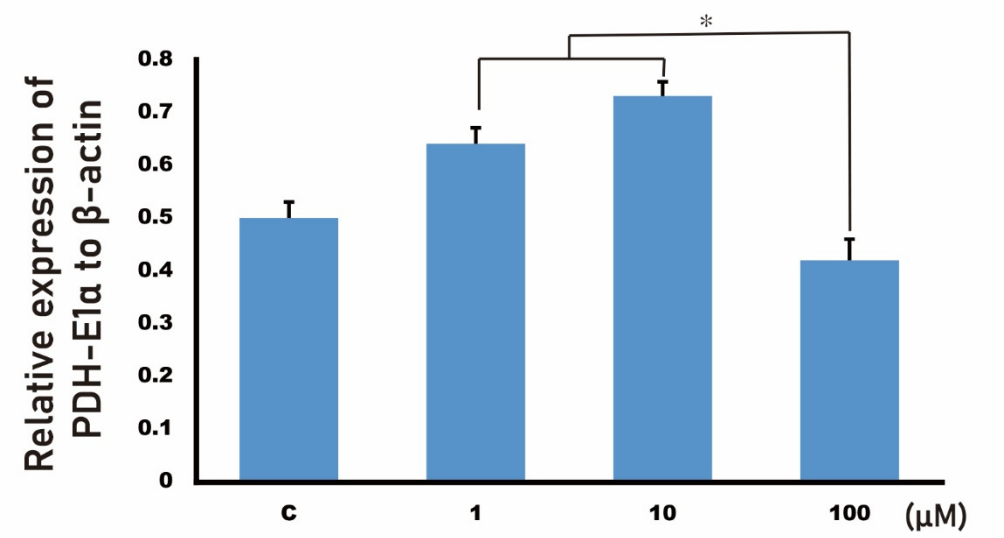
Supplementary Figure S1. Oxygen consumption assay.

The administration of 4-hexylresorcinol (4HR) decreased extracellular oxygen consumption in HUVECs. The relative oxygen consumption level compared to untreated control was 96.2 ± 2.1, 95.7 ± 2.5, and 94.7 ± 1.4 % for 1, 10, and 100 μM, respectively. Accordingly, 100 μM 4HR administration decreased 5.3% of oxygen consumption in HUVECs ($P<0.001$).



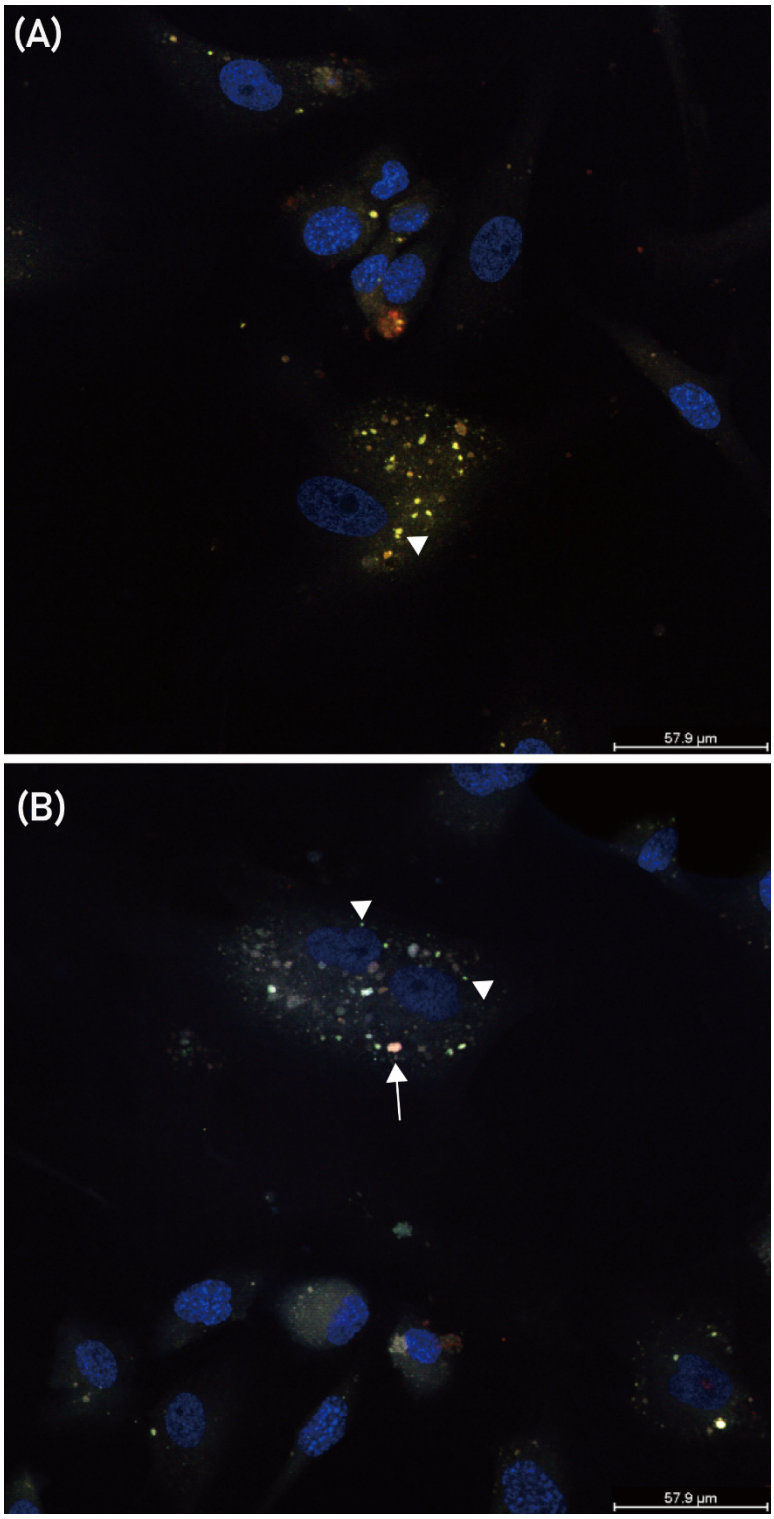
Supplementary Figure S2. Quantification of PDH-E1α in Figure 1C.

The level of PDH-E1α was significantly changed after 4HR administration (P<0.001). In post hoc test, 100 μM 4HR administration group showed significantly lower level of PDH-E1α when compared to 1 and 10 μM 4HR administration groups (*P<0.001). Decreased level of PDH-E1α in 100 μM 4HR administration might be causing the decreased ATP and oxygen consumption.



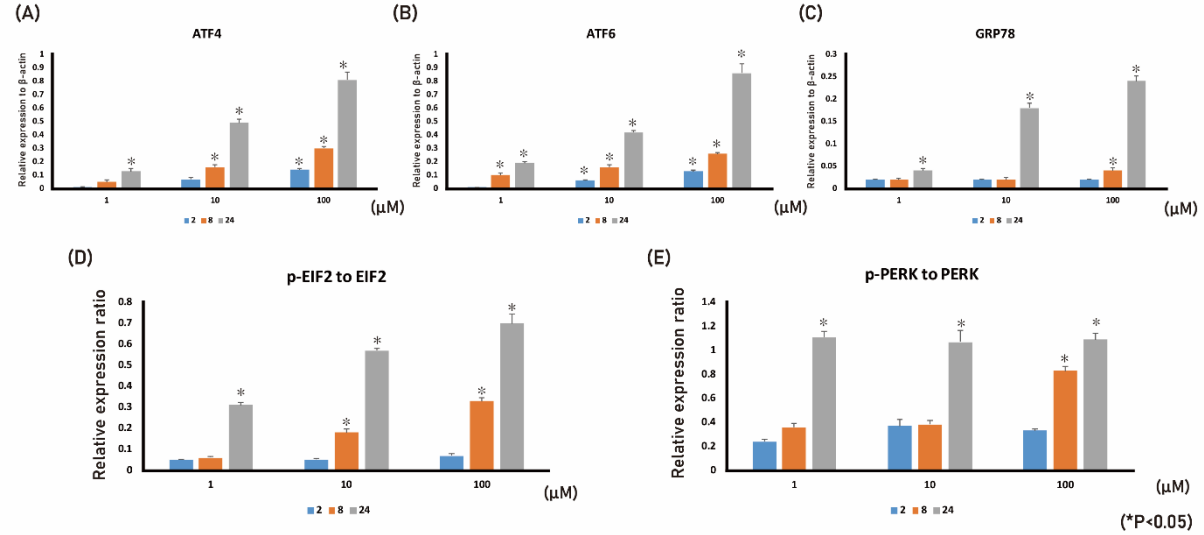
Supplementary Figure S3. Confocal microscopic findings after 4HR administration.

(A) Most mitochondria in the untreated control showed bright orange to yellow color (arrow head). (B) Administration of 4HR (100 μM) did not show prominent cytochrome c leakage from mitochondria. Cytochrome c leakage from mitochondria was shown as sporadic green dots (arrow heads).



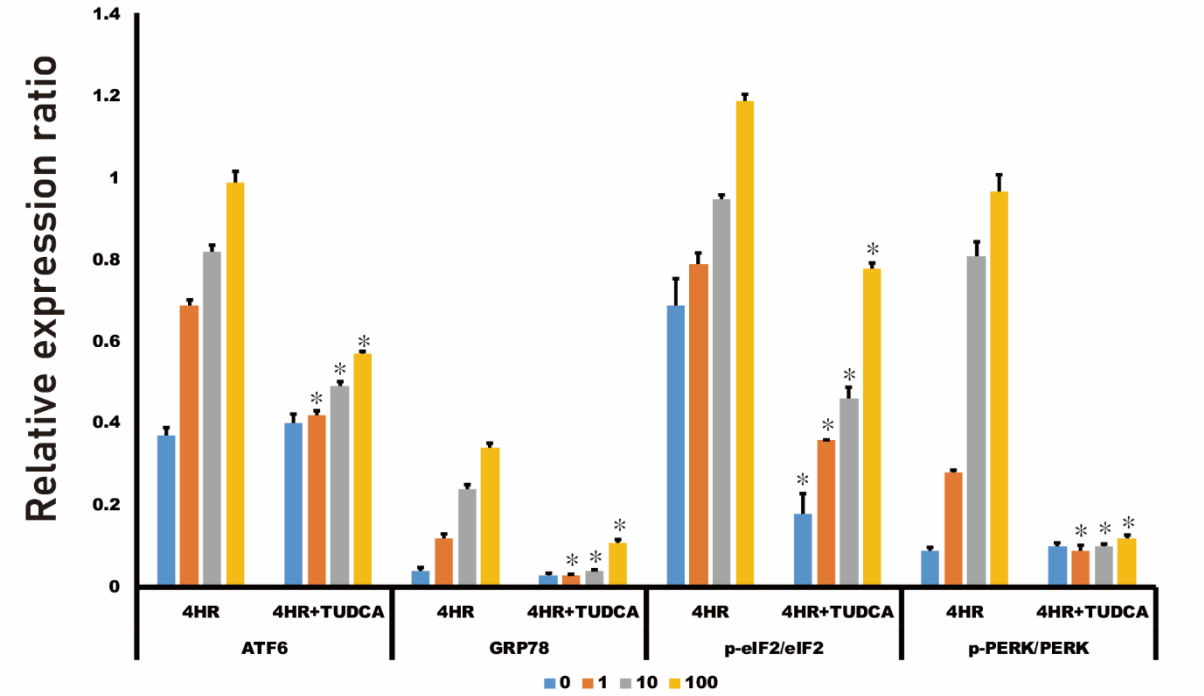
Supplementary Figure S4. Quantification of Western blot of Figure 3A.

As demonstrated by Western blot analysis, ATF4 (A), ATF6α (B), GRP78 (C) protein level in cultured HUVECs were increased by 4HR at concentrations from 1 to 100 μM application. The difference in ATF4, ATF6, and GRP78 content among the compared groups was statistically significant (P<0.001); the post hoc test showed differences between the groups treated with 1, 10, and 100 μM 4HR treatments resulted in significantly higher values compared to the untreated control (*P <0.05). The difference in the ratio of p-eIF2 to eIF2 (D) among the compared groups was statistically significant (P<0.001); the post hoc test showed differences between the groups treated with 1, 10, and 100 μM 4HR treatments resulted in significantly higher values compared to the untreated control (*P <0.05). The difference in the ratio of p-PERK to PERK (E) among the compared groups was statistically significant (p<0.001); the post hoc test showed differences between the groups treated with 1, 10, and 100 μM 4HR treatments resulted in significantly higher values compared to the untreated control (*P <0.05).



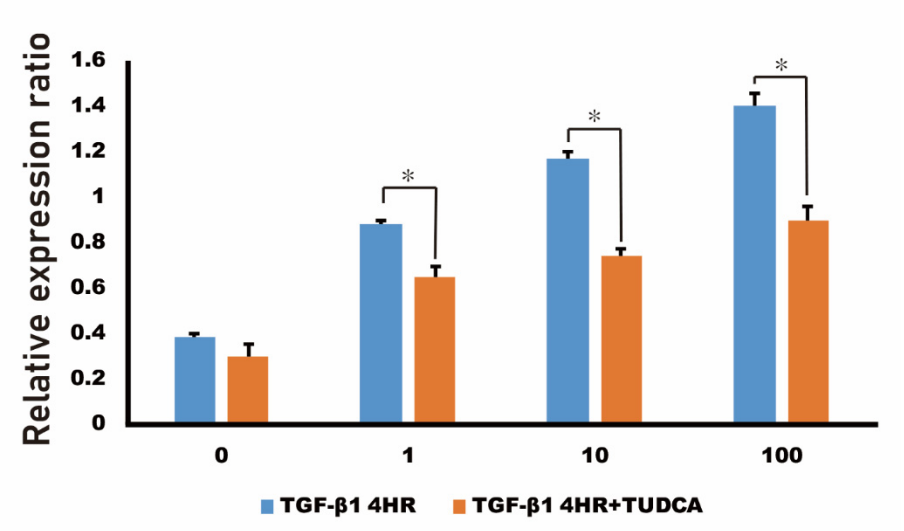
Supplementary Figure S5. Quantification of Western blot of Figure 3B.

As demonstrated by Western blot analysis, the application of ER stress reliever (TUDCA) decreased the expression level of ER stress markers such as ATF6α and GRP78 protein level in cultured HUVECs which were increased by 4HR at concentrations from 1 to 100 μM application. The differences in ATF6α and GRP78 content between the group treated with 4HR and the group treated with 4HR + TUDCA were statistically significant (*P <0.05). The ratios were also statistically significantly different between groups (*P<0.05). Interestingly, the application of TUDCA could significantly reduce the ratio of p-eIF2 to eIF2 in the 4HR untreated groups.



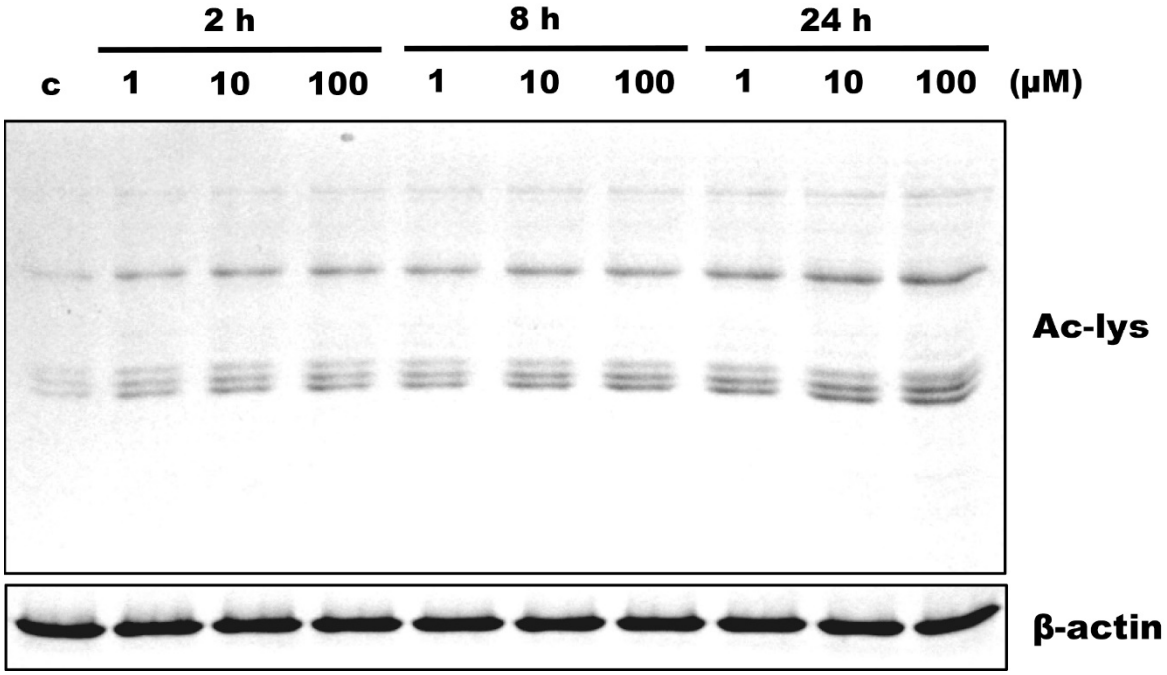
Supplementary Figure S6. Quantification of Western blot of Figure 3C.

As demonstrated by Western blot analysis, the application of ER stress reliever (TUDCA) decreased the expression level of TGF-β1 in cultured HUVECs which were increased by 4HR at concentrations from 1 to 100 μM application. The differences in TGF-β1 content between the group treated with 4HR and the group treated with 4HR + TUDCA were statistically significant (*P <0.05).



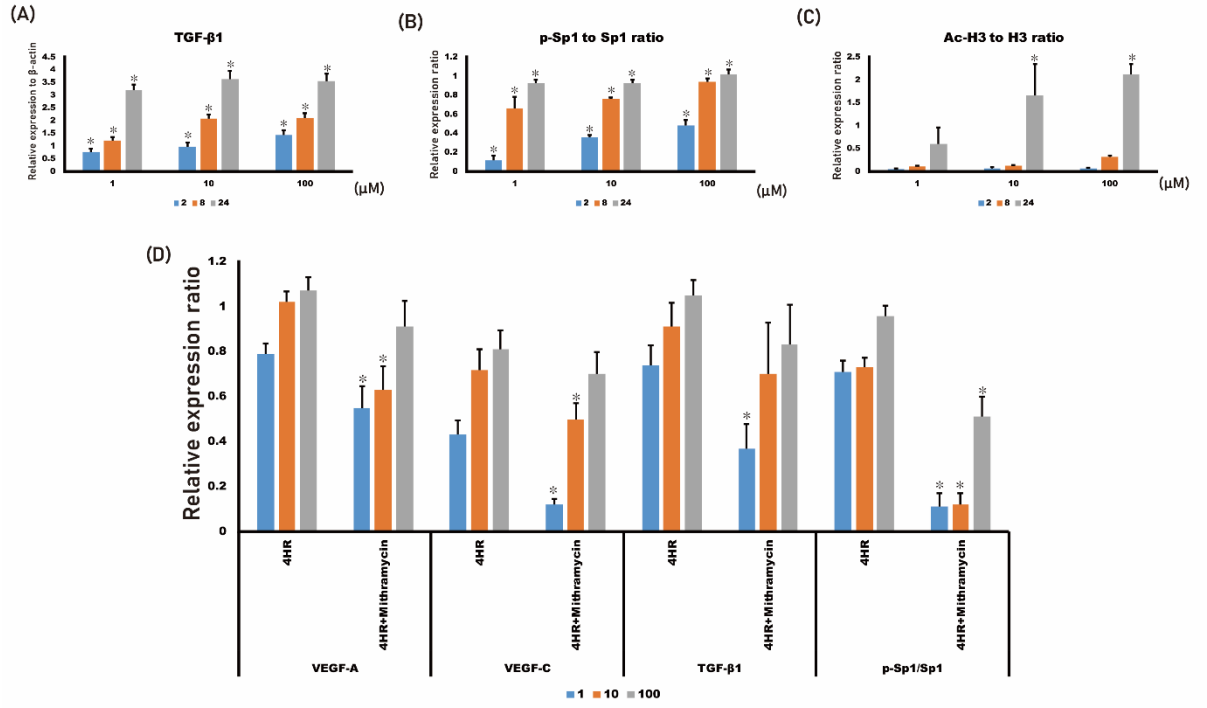
Supplementary Figure S7. Protein acetylation was increased by 4HR administration.

The administration of 4-hexylresorcinol (4HR) increased the acetylation level in the intra-cellular proteins. Whole cellular lysates were processed by anti-Ac-lys antibody application.



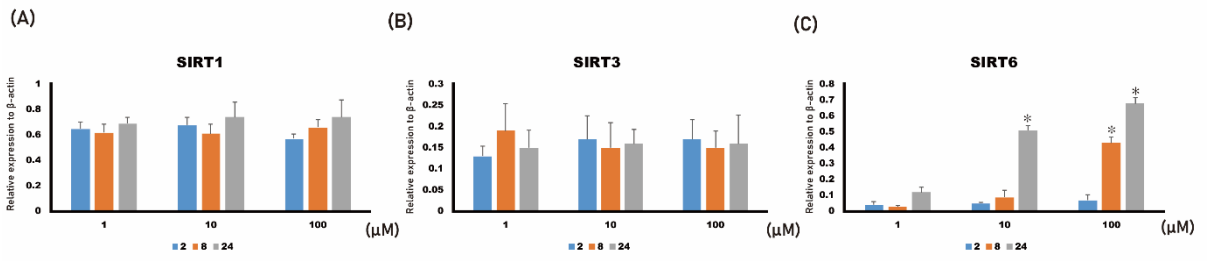
Supplementary Figure S8. Quantification of Western blot of Figure 4.

(A) As demonstrated by Western blot analysis, TGF-β1 protein level in cultured HUVECs were increased by 4HR at concentrations from 1 to 100 μM application. The difference in TGF-β1 content among the compared groups was statistically significant (P<0.001); the post hoc test showed differences between the groups treated with 1, 10, and 100 μM 4HR treatments resulted in significantly higher values compared to the untreated control (*P =0.018 for 1 μM 4HR at 2 hrs, P<0.001 for the others). (B) The difference in the ratio of p-Sp1 to Sp1 among the compared groups was statistically significant (P<0.001); the post hoc test showed differences between the groups treated with 1, 10, and 100 μM 4HR treatments resulted in significantly higher values compared to the untreated control (*P <0.05). (C) The difference in the ratio of Ac-H3 to H3 among the compared groups was statistically significant (P<0.001); the post hoc test showed differences between the groups treated with 10 and 100 μM 4HR treatments at 24 hrs resulted in significantly higher values compared to the untreated control (*P<0.001). (D) As demonstrated by Western blot analysis, the application of Sp1 inhibitor (mithramycin) decreased the expression level of TGF-β1, VEGF-A, and VEGF-C in cultured HUVECs which were increased by 4HR at concentrations from 1 to 100 μM application. The differences in TGF-β1, VEGF-A, and VEGF-C content between the group treated with 4HR and the group treated with 4HR + mithramycin were statistically significant (*P <0.05). Interestingly, the differences in TGF-β1, VEGF-A, and VEGF-C content were enhanced in lower dosage of 4HR (1 and 10 μM application). As mithramycin is Sp1 inhibitor, the differences in the ratio of p-Sp1 to Sp1 were significant in all tested dosage of 4HR (1 to 100 μM application).



Supplementary Figure S9. Quantification of Western blot of Figure 5.

The administration of 4-hexylresorcinol (4HR) did not change the expression level in SIRT1 (A) and SIRT3 (B) proteins, significantly (P>0.05). As demonstrated by Western blot analysis, SIRT6 (C) protein level in cultured HUVECs were increased by 4HR at concentrations from 10 to 100 µM application. The difference in SIRT6 content among the compared groups was statistically significant (P<0.001); the post hoc test showed differences between the groups treated with 10 and 100 µM 4HR treatments resulted in significantly higher values compared to the untreated control (*P<0.001).



Supplementary Figure S10. NADH level after 4HR administration.

NADH levels were increased significantly upon the administration of 100 µM 4HR after 8 h and 24 h.