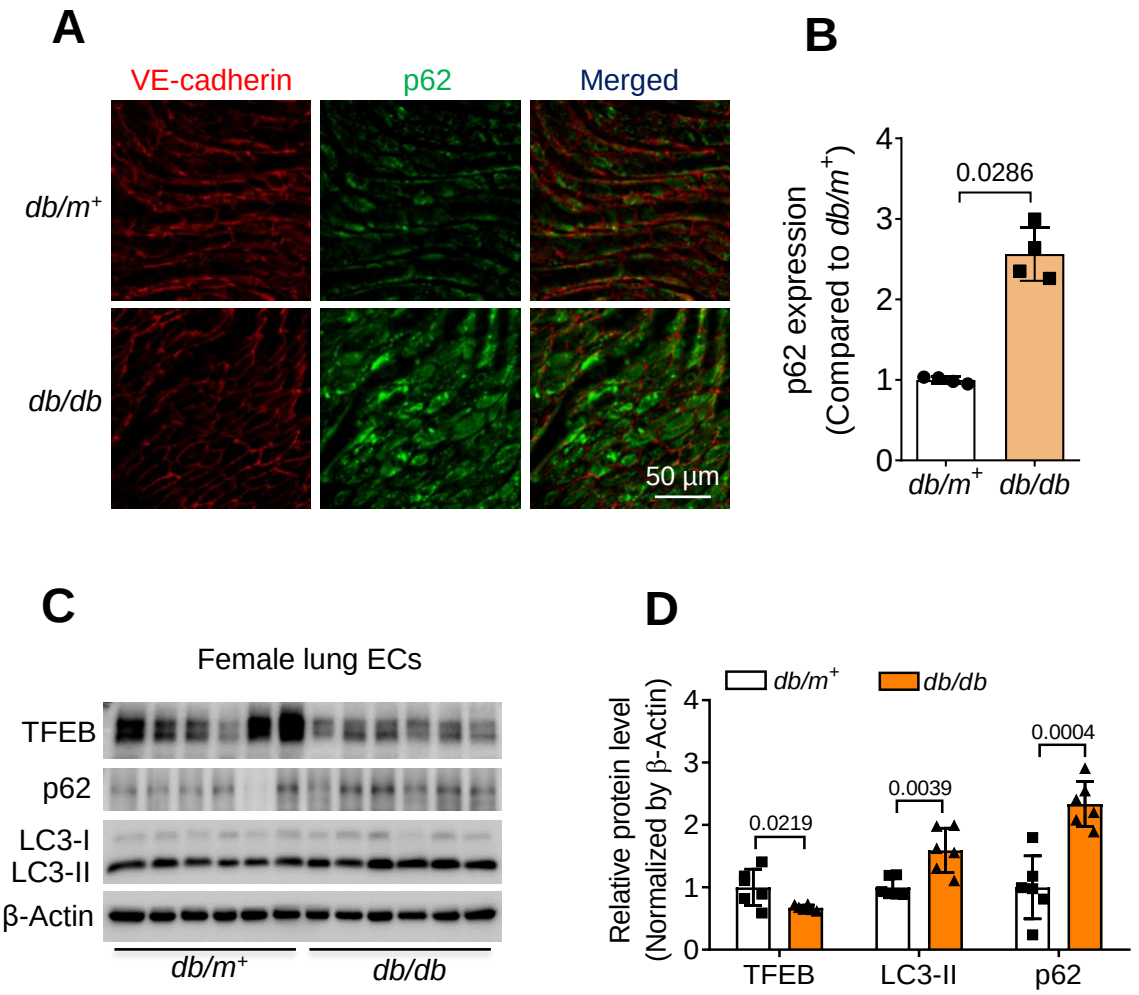
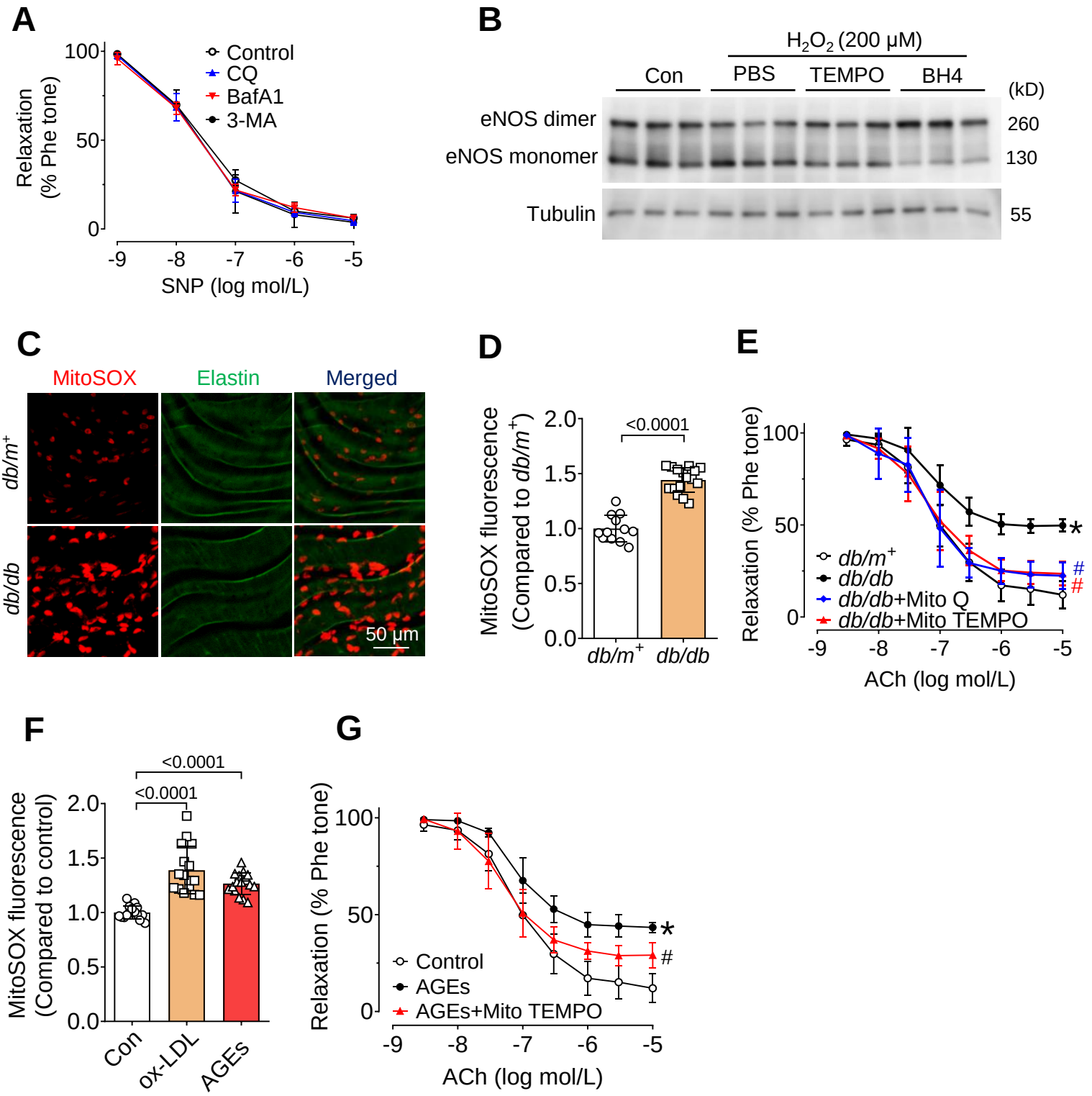
**Supplementary Figure 1**. Endothelial p62 level is increased in *db/db* mouse aortas.



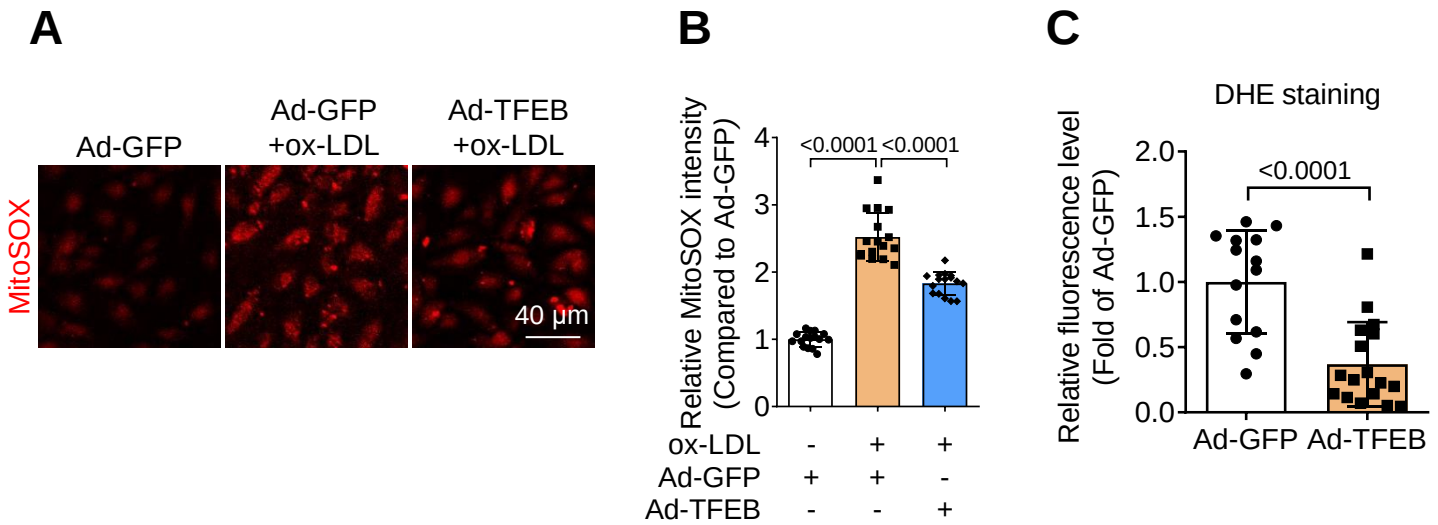

(A & B) Representative images and summarized *en face* immunofluorescence staining of p62 expression in the endothelium of intact male mouse aortas, n = 4. VE-cadherin: vascular endothelial-cadherin. (C & D) Lung ECs were isolated from female *db/m*$^+$ and *db/db* mice, Western blot analysis and summarized data of the protein levels of TFEB, p62, and LC3, n = 6. Results are means ± SD. Statistical analysis was performed using Mann-Whitney *U*-test for (B), unpaired two-tailed Student's *t*-test for (D).

**Supplementary Figure 2**. Suppression of ROS attenuates diabetic vascular dysfunction



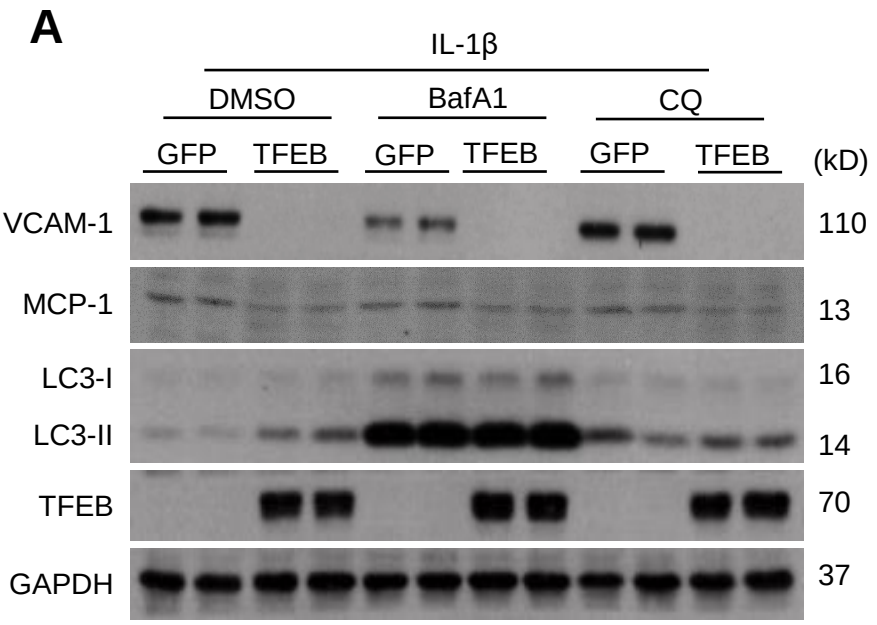

(A) Sodium nitroprusside (SNP)-induced endothelium-independent relaxations in male *db/m+* mouse aortas after treatment individually with 3-methyladenine (3-MA, 10 mmol/L, 24 h), CQ (10 μmol/L, 24 h) or BafA1 (10 nmol/L, 16 h), n = 3. (B) HUVECs were preincubated with Mito TEMPO (100 μmol/L) or tetrahydrobiopterin (BH4, 100 μmol/L), then exposed to $H_2O_2$ (200 μmol/L) for 3 h, low-temperature Western blot were used to measure the eNOS protein level. (C) Representative images of *en face* staining using MitoSOX red to determine mtROS production in sections of aortas from male *db/m*$^+$and *db/db* mice. (D) The statistical analysis results of (C), n = 12-16. (E) Acetylcholine-induced endothelium-dependent relaxations (EDR) in male *db/db* mouse aortas pretreated with mtROS scavengers, Mito-TEMPO (100 μmol/L) or MitoQ (10 nmol/L) for 24 h, n = 3-5, *$p$<0.05 vs *db/m*$^+$, #$p$<0.05 vs *db/db*. (F) mtROS production in HUVECs treated with ox-LDL (100 μg/ml) and AGEs (100 μg/ml) for 24 h, n = 16. (G) EDR in male *db/m+* mouse aortas pretreated with AGEs (100 μg/ml) for 24 h in control and the presence of Mito-TEMPO (100 μmol/L), n = 3-5, *$p$<0.05 vs control, #$p$<0.05 vs AGEs. Results are means ± SD. Statistical analysis was performed using two-way repeated measures ANOVA followed by Tukey's test for (A), (E), and (G), unpaired two-tailed Student's *t*-test for (D), and one-way ANOVA followed by Tukey's test for (F).

**Supplementary Figure 3**. TFEB lowers ROS level in HUVECs.



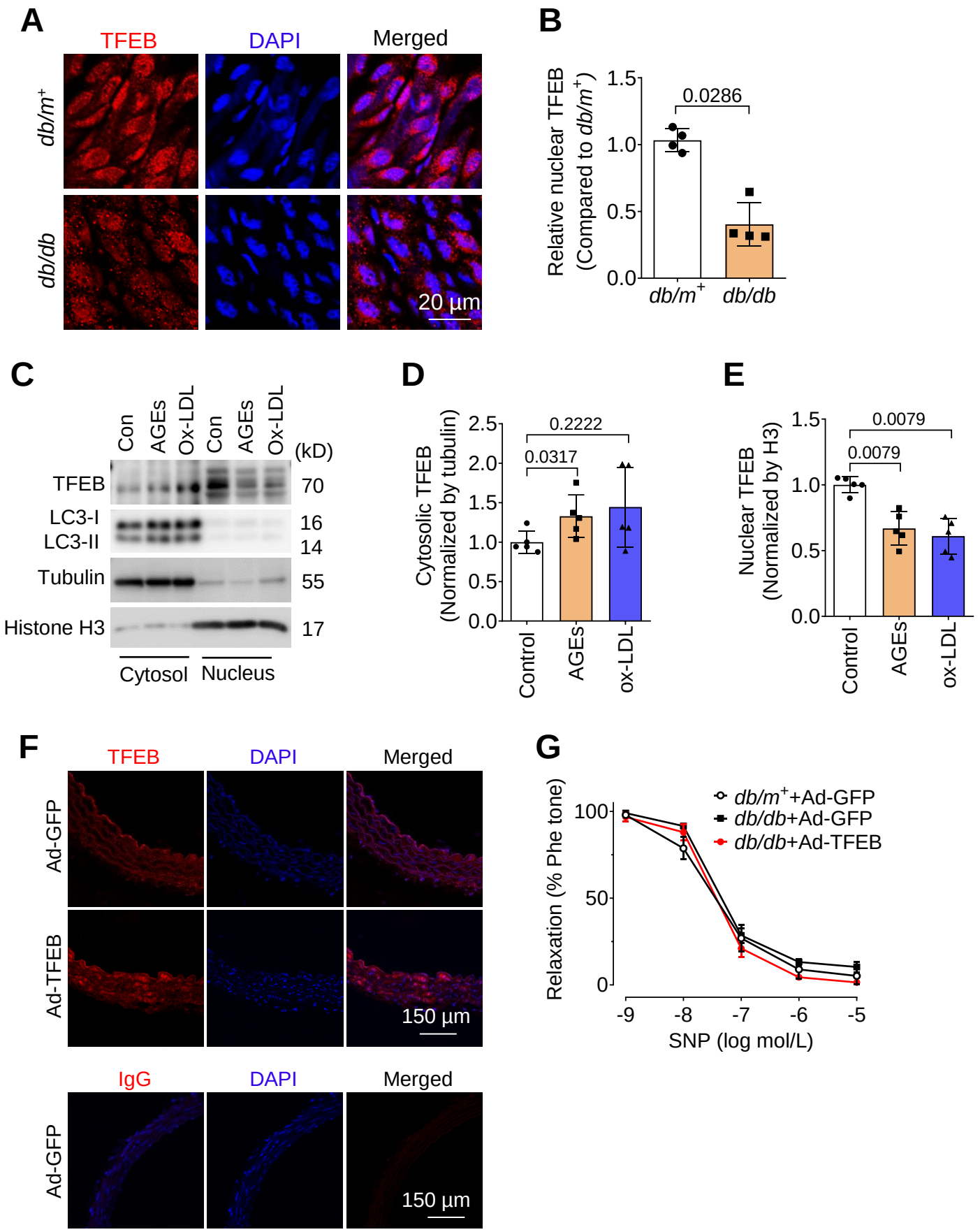

HUVECs were treated with Ad-TFEB for 24 h followed by 24 h-treatment of ox-LDL (100 μg/ml). (A) Representative images and (B) statistical analysis of mtROS production measured by MitoSOX red staining, n = 16. (C) HUVECs were treated with Ad-TFEB for 24 h, dihydroethidium (DHE) staining was used to measure the ROS level, n = 14-17. Results are means ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's test for (B), unpaired two-tailed Student's *t*-test for (C).

**Supplementary Figure 4**. The anti-inflammatory effect of TFEB is independent of its regulatory effect on autophagy.



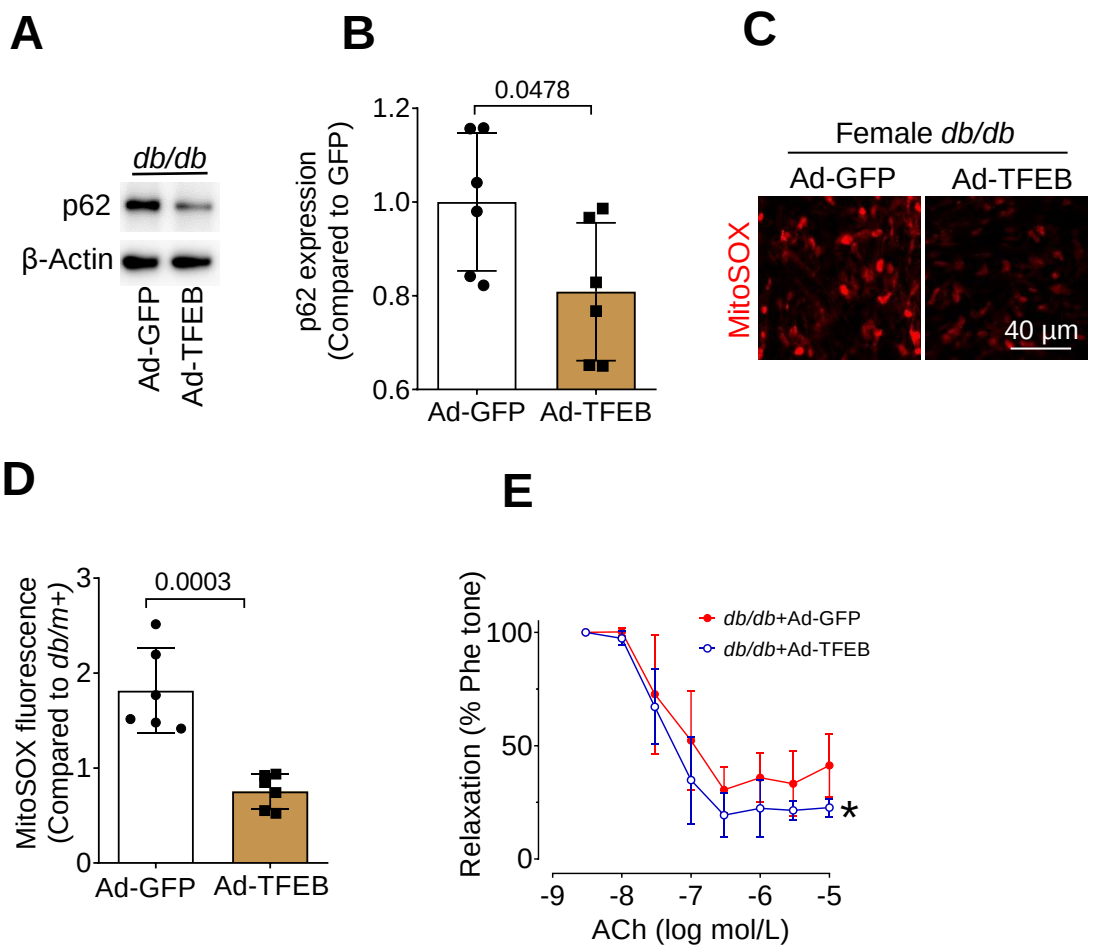

(A) HUVECs were treated first with Ad-TFEB for 24 h, then with IL-1β (2 ng/ml) plus CQ (10 μmol/L) or BafA1 (10 nmol/L) for another 12 h under FBS-free condition. The expressions of vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1), microtubule-associated protein 1 light chain 3 (LC3), and TFEB were measured using Western blot analysis.

**Supplementary Figure 5**. Nuclear TFEB is reduced in *db/db* mouse aortic endothelium



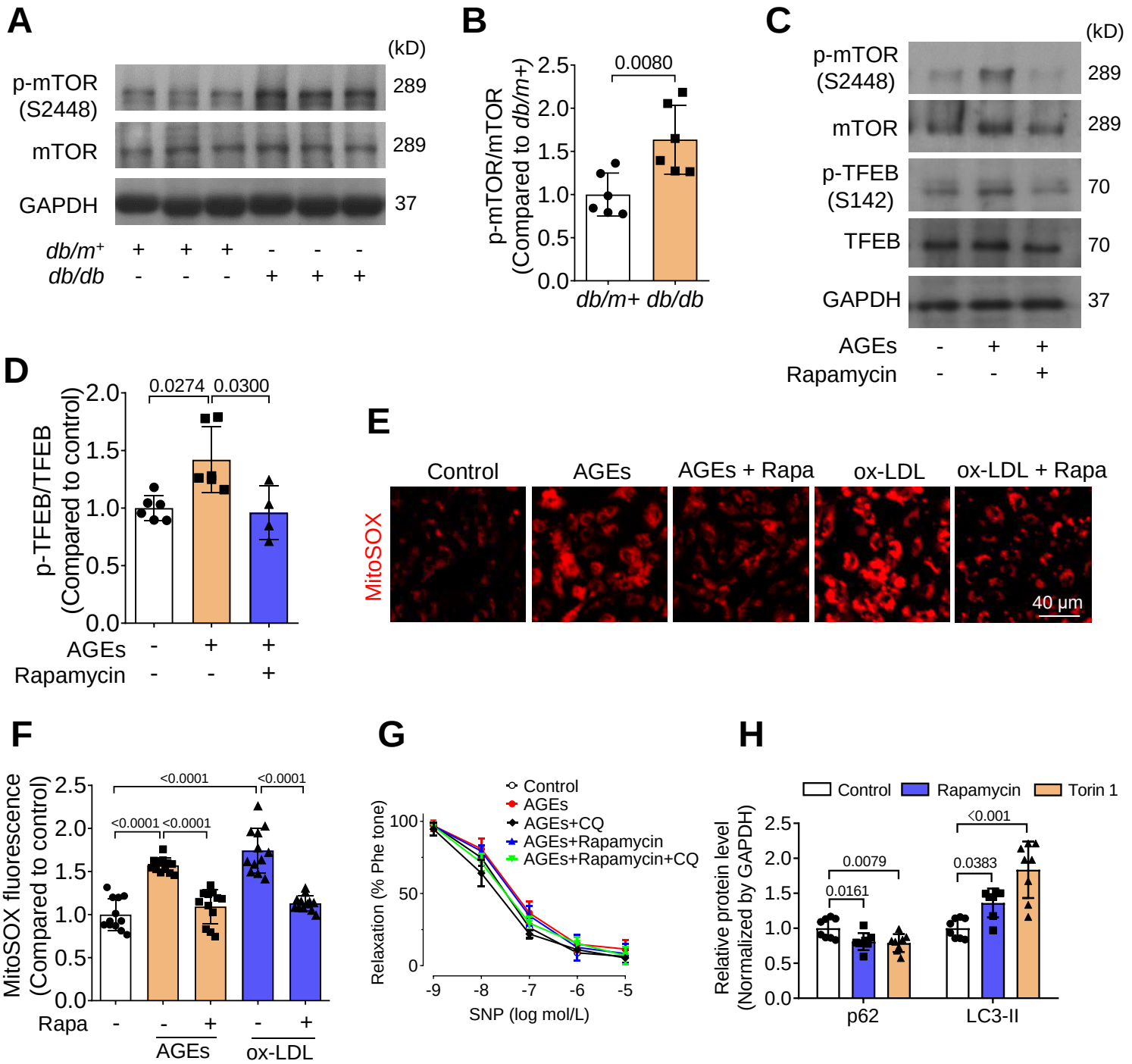

(A) *En face* staining of TFEB in male *db/m*$^+$ and *db/db* mouse aortic endothelium. (B) Statistical analysis results of (A), n = 4. (C-E) Western blot analysis of subcellular distribution of TFEB in HUVECs between nucleus and cytoplasm after 24 h exposure to AGEs (100 μg/ml) or ox-LDL (100 μg/ml), n = 5. (F) The overexpressing efficiency after 7 days Ad-TFEB injection as determined by immunofluorescence staining with anti-TFEB antibody. IgG was used as negative control. (G) Sodium nitroprusside (SNP)-induced endothelium-independent relaxations was unaltered by TFEB overexpression, n = 4. Results are means ± SD. Statistical analysis was performed using Mann-Whitney *U*-test for (B), one-way ANOVA followed by Tukey's test for (D) and (E), and two-way repeated measures ANOVA followed by Tukey's test for (G).

**Supplementary Figure 6**. TFEB overexpression lowers endothelium ROS level and attenuates endothelial dysfunction in female *db/db* aortas.



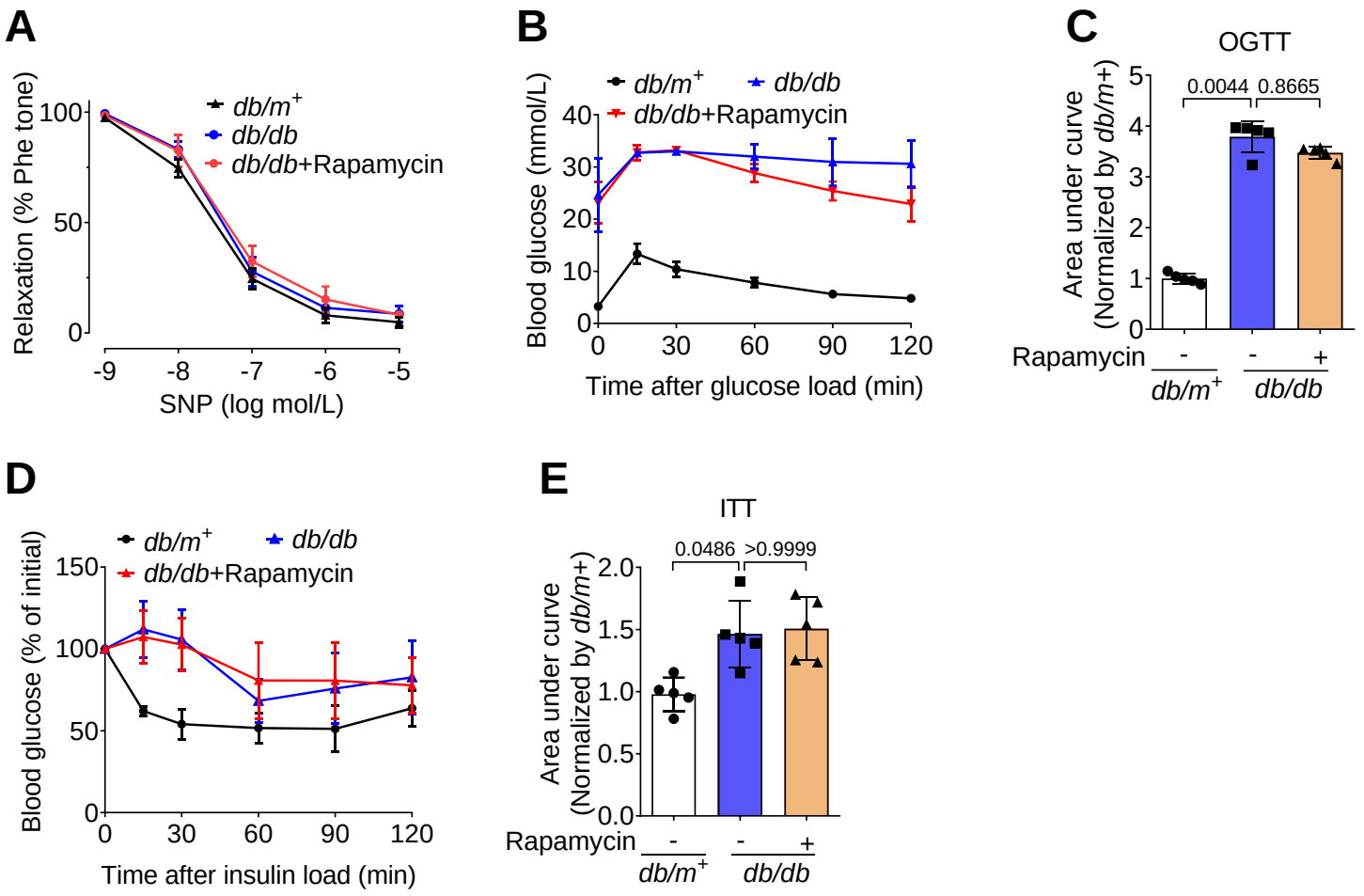

Female *db/db* mice were injected with Ad-GFP and Ad-TFEB for 1 week, (A & B) The protein level of p62 in aorta was measured using Western blot analysis. n = 6. (C & D) endothelial mtROS level was measured using *en face* staining of MitoSOX. (E) Endothelium-dependent relaxations by acetylcholine (ACh) were measured on wire myograph. Results are means ± SD, **p*<0.05 vs Ad-GFP. Statistical analysis was performed using unpaired two-tailed Student's *t*-test for (B) and (D), and two-way repeated measures ANOVA followed by Tukey's test for (E).

**Supplementary Figure 7.** Inhibition of mTOR reduces endothelial cell ROS production



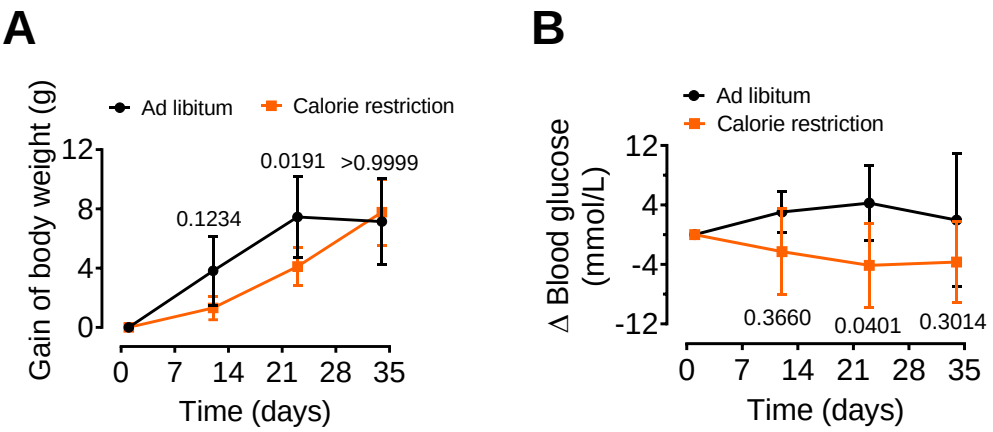

(A) Aortas of male *db/db* and *db/m*$^+$ mice were isolated for Western blot analysis, p-mTOR (S2448) and mTOR protein levels were measured. (B) The statistical analysis results of (A), n = 6. (C) The protein expression of p-mTOR (S2448), mTOR, p-TFEB (S142), and TFEB in HUVECs treated with AGEs (100 μg/ml) with and without rapamycin (300 nmol/L) for 24 h. (D) The statistical analysis results of (C), n = 4-6. (E) The mtROS production in HUVECs treated with ox-LDL (100 μg/ml) or AGEs (100 μg/ml) with or without rapamycin (300 nmol/L) for 24 h. (F) The statistical analysis results of (E), n = 12. (G) Sodium nitroprusside (SNP)-induced endothelium-independent relaxations were unaltered by AGEs, rapamycin or CQ, n = 3-4. (H) The statistical analysis data of main Figure 5C, n = 8. Results are means ± SD. Statistical analysis was performed using unpaired two-tailed Student's *t*-test for (B), Kruskal-Wallis test followed by Dunn comparison for (D), one-way ANOVA followed by Tukey's test for (F) and (H), and two-way repeated measures ANOVA followed by Tukey's test for (G).

**Supplementary Figure 8.** Inhibition of mTOR does not alter glucose metabolism in *db/db* mice




(A) Sodium nitroprusside (SNP)-induced endothelium-independent aortic relaxations were unaltered in male *db/m*$^{+}$ , *db/db* mice, or *db/db* mice after rapamycin treatment, n = 3. (B&C) Oral glucose tolerance test (OGTT) and (D&E) insulin tolerance test (ITT) of *db/db* mice after receiving rapamycin treatment (2 mg/kg every 2 days for 12 days), n = 5. Results are means ± SD. Statistical analysis was performed using two-way repeated measures ANOVA followed by Tukey's test for (A), (B), and (D), Kruskal-Wallis test followed by Dunn comparison for (C) and (E).

**Supplementary Figure 9**. Calorie restriction reduces body weight and blood glucose




Male *db/db* mice were exposed to 3 cycles calorie restriction. At the end of each cycle, (A) body weight and (B) blood glucose level were measured, n = 5-6. Results are means ± SD. Statistical analysis was performed using Mann-Whitney *U*-test for (A) and (B).