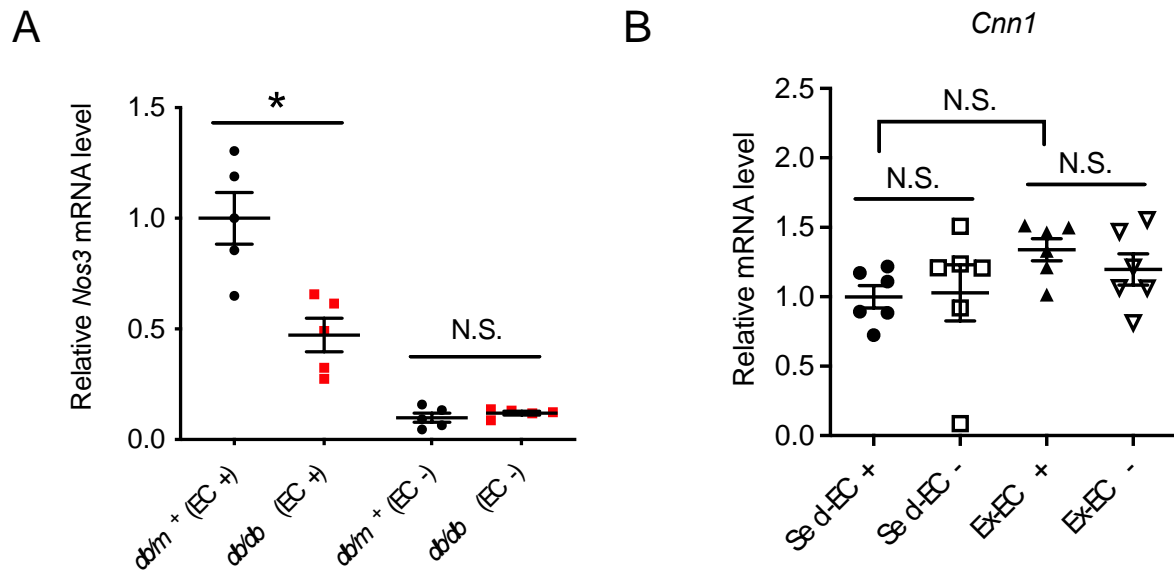


Supplementary Figures and Tables

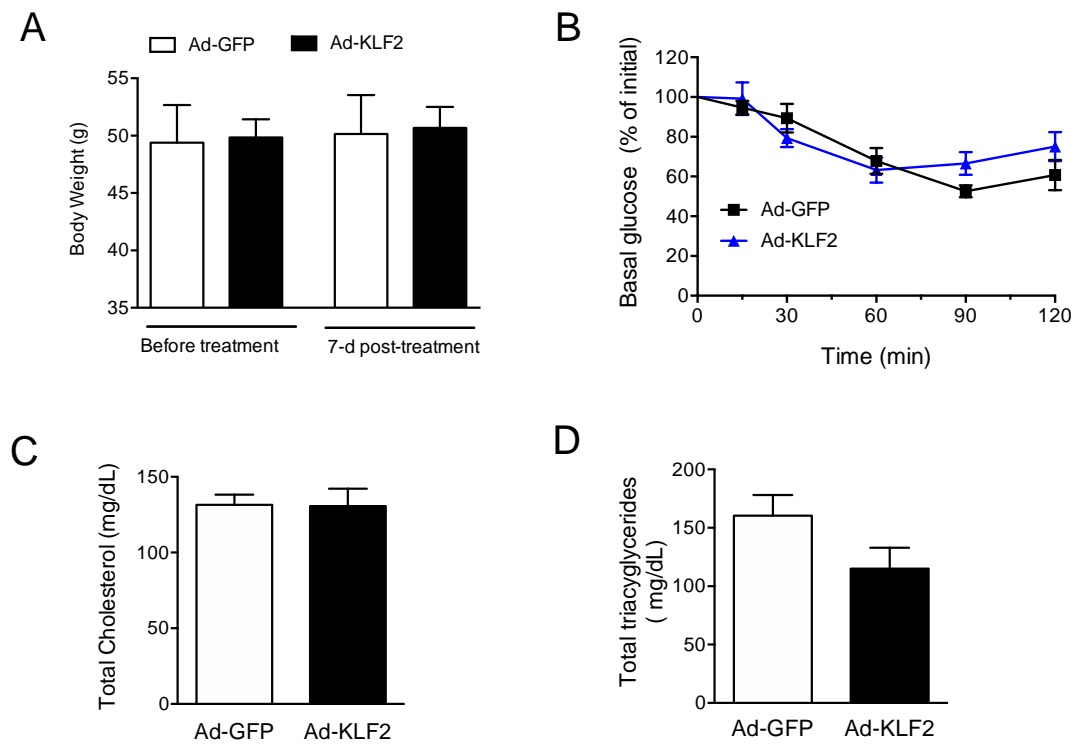
Supplementary Figure 1



Supplementary Figure 1. The mRNA expression level of *Nos3* and *Cnn1* in mouse aortas.

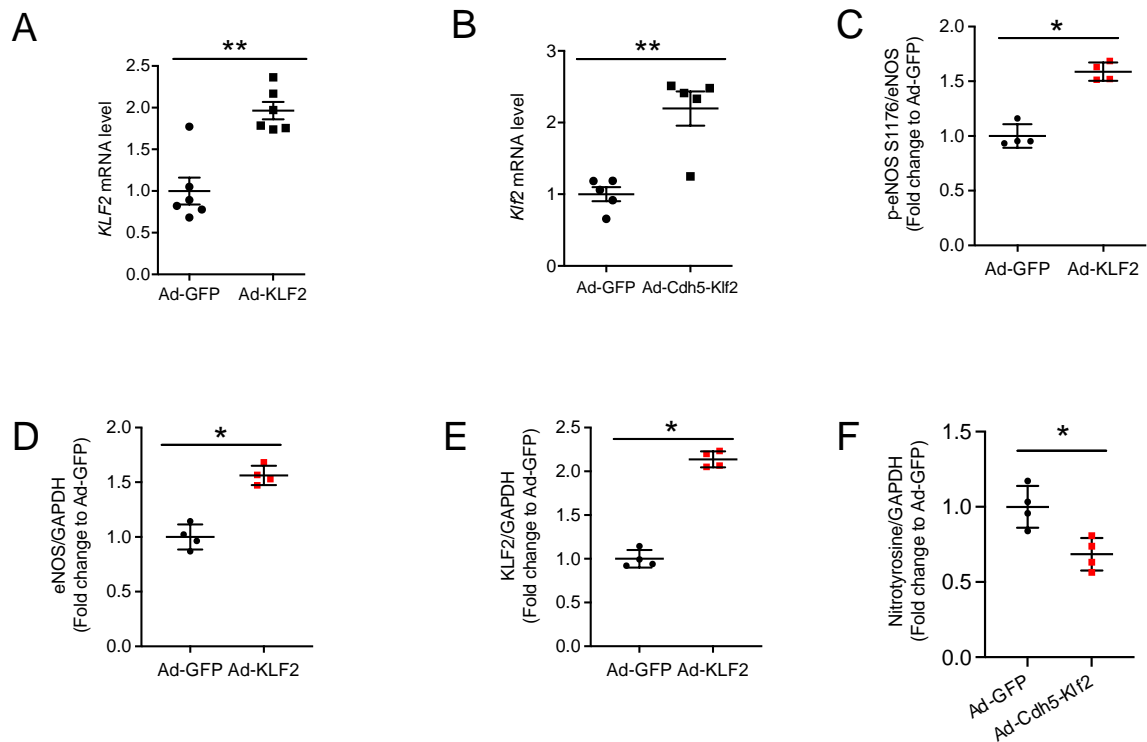
Real-time qPCR data showing *Nos3* (A) gene expression in aortas from *db/m*⁺ and *db/db* mice and *Cnn1* (B) gene expression in aortas from C57BL/6 mice subjected to 7-day treadmill exercise. EC⁺ indicates intact mouse aortas with endothelium, EC⁻ indicates mouse aortas with endothelium denuded. **p*<0.05 vs *db/m*⁺ (EC⁺), unpaired two-tailed *t* test.

Supplementary Figure 2



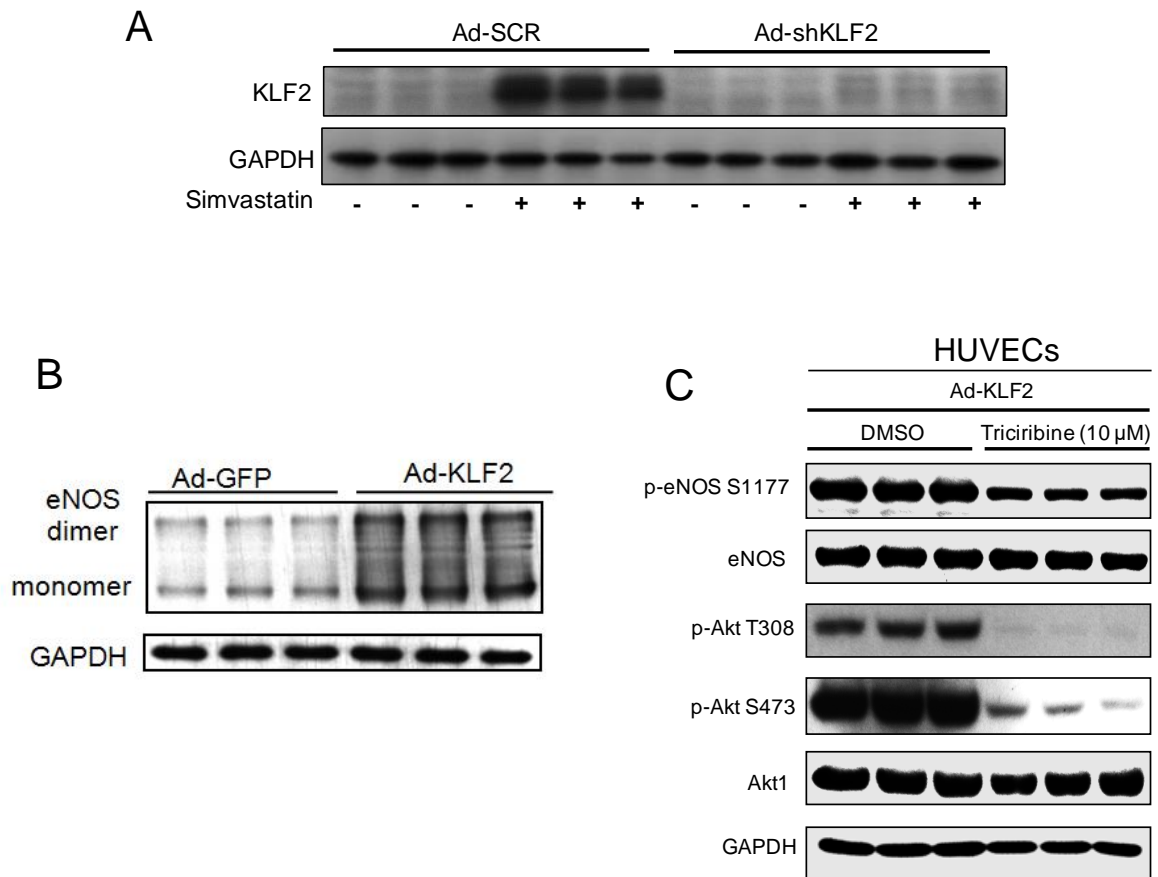
Supplementary Figure 2. The effect of Ad-KLF2 overexpression on the body weight, insulin sensitivity and lipid profile of *db/db* mice. Ad-KLF2 administration for 7 days to *db/db* mice does not affect body weight (A), insulin sensitivity indicated by Insulin Tolerance Test (B), total plasma cholesterol (C) and total plasma triacylglyceride levels (D).

Supplementary Figure 3



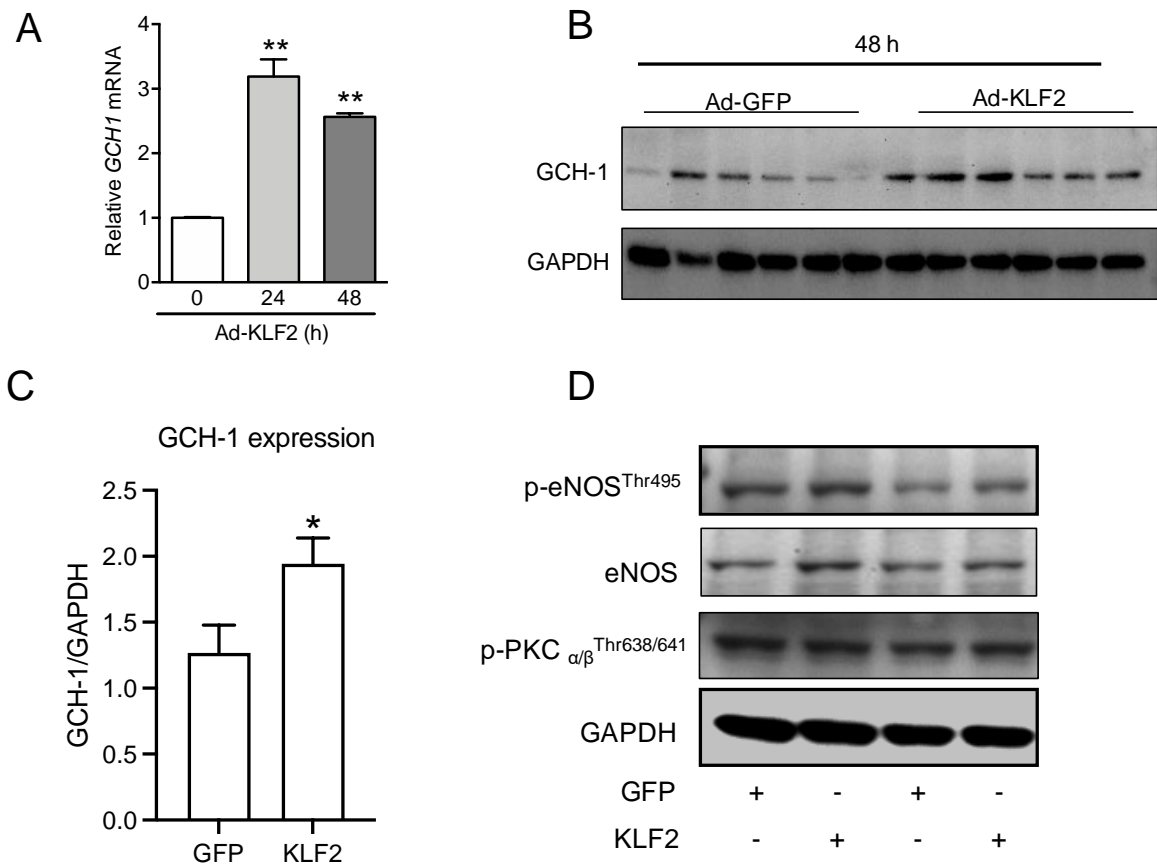
Supplementary Figure 3. KLF2 overexpression increases levels of KLF2, eNOS and p-eNOS S1176. QPCR results showing Ad-KLF2 (A) and Ad-Cdh5-Klf2 (B) increase KLF2 mRNA level in mouse aortas from *db/db* mice. Summarized western blotting data showing Ad-KLF2 upregulates levels of p-eNOS S1176 (C), total eNOS (D) and KLF2 (E) in mouse aortas from *db/db* mice. Summarized data showing Ad-Cdh5-Klf2 decreases the level of tyrosine nitrated proteins in aortas from *db/db* mice (F). ** $p < 0.001$ vs Ad-GFP, * $p < 0.05$ vs Ad-GFP, unpaired two-tailed *t* test.

Supplementary Figure 4



Supplementary Figure 4. KLF2 increases eNOS dimerization and Akt inhibitor Triciribine abolished KLF2-induced p-Akt and p-eNOS. (A) Ad-KLF2-shRNA (Ad-shKLF2) abolished simvastatin-induced expression of KLF2 in HAECs. (B) Ad-Klf2 increased level of eNOS monomer and dimer in HUVECs. (C) Triciribine (10 μM) treatment for 12 hours inhibited KLF2-induced p-Akt T308, p-Akt S473 and p-eNOS S1177 but not total Akt and eNOS.

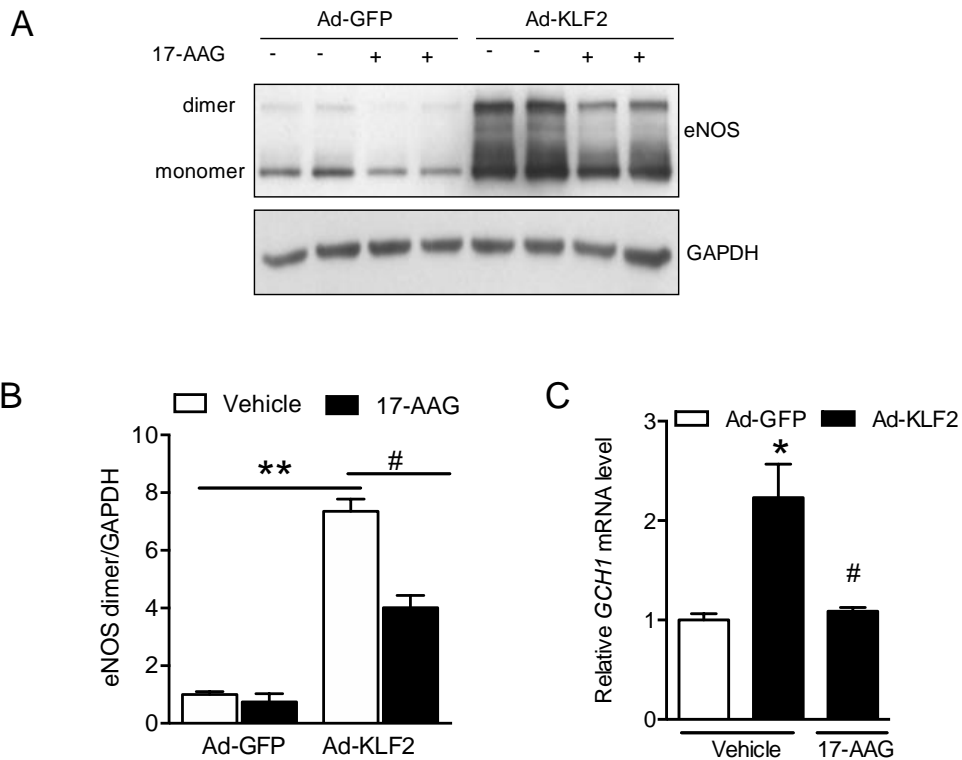
Supplementary Figure 5



Supplementary Figure 5. KLF2 upregulated mRNA and protein expression of GCH-1.

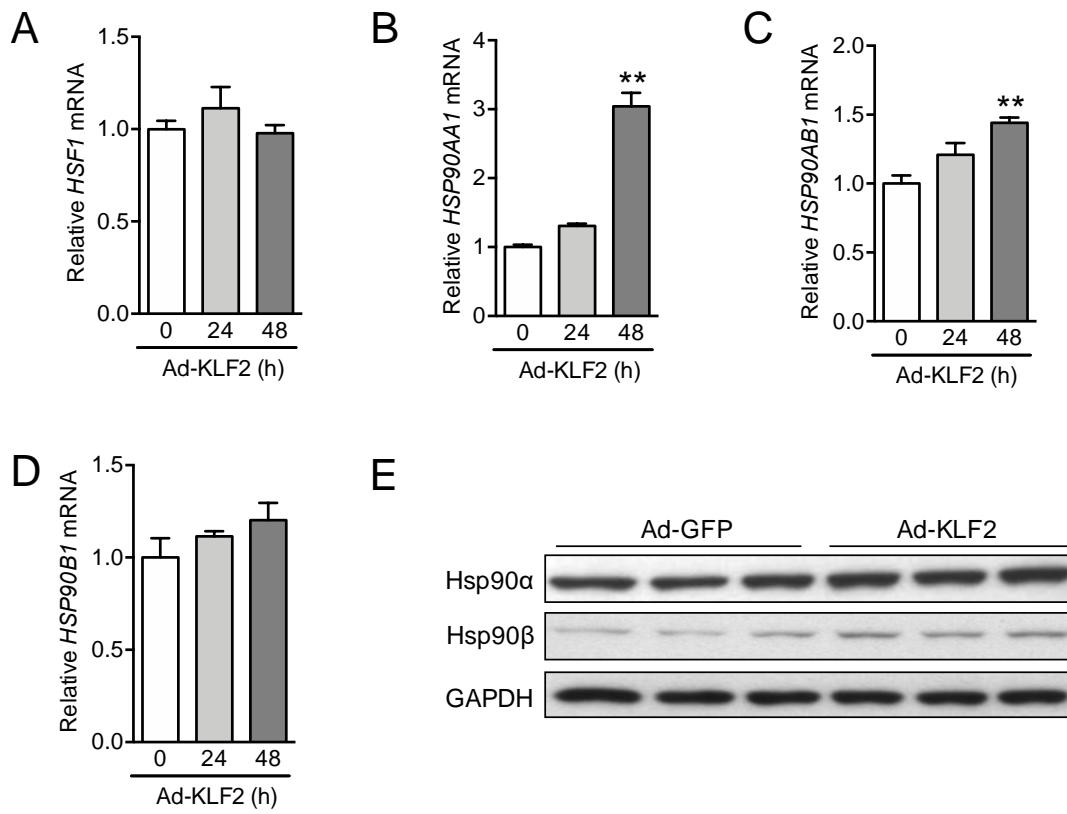
The qPCR results (**A**) and western blotting data (**B** and **C**) showing KLF2 overexpression increased GCH-1 mRNA and protein expression in HUVECs. ** $p < 0.01$ vs Ad-GFP, One-way ANOVA with Tukey's multiple comparison test. * $p < 0.05$ vs Ad-GFP, unpaired two-tailed t test. (**D**) The effect of KLF2 overexpression for 24 hours on levels of p-eNOS threonine 495 and p-PKC α/β threonine 638/641 in HUVECs.

Supplementary Figure 6



Supplementary Figure 6. KLF2-induced eNOS dimerization and GCH1 mRNA expression were suppressed by Hsp90 inhibitor 17-AAG. Western blotting images (A) and summarized data (B) showing 17-AAG (1 μ M) treatment for 12 hours attenuated KLF2-induced eNOS dimer formation. ** $p < 0.01$ vs Ad-GFP+ Vehicle, # $p < 0.05$ vs Ad-KLF2+Vehicle, Two-way ANOVA with Tukey's multiple comparison test. (C) *GCH1* mRNA expression induced by KLF2 was inhibited by 17-AAG (1 μ M) treatment in HUVECs. * $p < 0.01$ vs Ad-GFP+ Vehicle, # $p < 0.05$ vs Ad-KLF2+Vehicle, Two-way ANOVA with Tukey's multiple comparison test.

Supplementary Figure 7



Supplementary Figure 7. KLF2 increased the mRNA and protein level of Hsp90 α and Hsp90 β but did not affect HSF1 mRNA level. The qPCR results showing KLF2 overexpression for 24- and 48-hours upregulated mRNA expression of *HSP90AA1* (B) and *HSP90AB1*(C) but did not change the mRNA expression of *HSF1*(A) and *HSP90B1* (D). ** $p < 0.01$ vs Ad-GFP, One-way ANOVA with Tukey's multiple comparison test. (E) Western blotting images showing KLF2 upregulated Hsp90 α and Hsp90 β protein levels in HUVECs.

Supplementary Table 1

Gene name	Primer sequences	Species
<i>Klf2</i>	F: CTCAGCGAGCCTATCTTGCC R: CACGTTGTTTAGGTCCTCATCC	Mouse
<i>Nos3</i>	F: TGTGACCCTCACCGCTACAA R: GCACAATCCAGGCCCAATC	Mouse
<i>Gapdh</i>	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA	Mouse
<i>GAPDH</i>	F: CCACTCCTCCACCTTTGAC R: ACCCTGTTGCTGTAGCCA	Human
<i>KLF2</i>	F: CTACACCAAGAGTTCGCATCTG R: CCGTGTGCTTTCGGTAGTG	Human
<i>NOS3</i>	F: TGATGGCGAAGCGAGTGAAG R: ACTCATCCATACACAGGACCC	Human
<i>PLCB2</i>	F: ATCCGGGATACTCGCTTTGG R: CACCACCGTGAGTGTCTTCAG	Human
<i>PLCB3</i>	F: TTGAGCGGTTCTGAACAAG R: CACTTCGTTGAGTCTCGGGT	Human
<i>PLCB4</i>	F: TATTCGGTCCGGAGCCATAC R: GACACAAACTATCCGCCCTTC	Human
<i>PLCD3</i>	F: CCCAAGCTGAATGCCGAGAA R: TTGAGCACGTAGTCAGTCTCC	Human
<i>PLCG2</i>	F: CATCCTATATGGCACTCAGTTTCG R: TCCTGGTGTAAGATTTTCAAGCC	Human
<i>INSR</i>	F: CATCCGGGGATCACGACTG R: ATCAGGTTGTAGAGGCCGAGT	Human
<i>FLT1</i>	F: TTTGCCTGAAATGGTGAGTAAGG R: TGGTTTGCTTGAGCTGTGTTT	Human
<i>TEK</i>	F: CAGGATACGAACCATGAAGATGC R: GGGGCACTGAATGGATGAAG	Human
<i>ITGA3</i>	F: CTGCACCCCCAAAACATCACC R: CTGCCACCCATCATTGTTCA	Human
<i>ITGA6</i>	F: CAGTGGAGCCGTGGTTTTG R: CCACCGCCACATCATAGCC	Human
<i>ITGB4</i>	F: GCAGCTTCCAAATCACAGAGG R: CCAGATCATCGGACATGGAGTT	Human
<i>ADCY4</i>	F: ACCTGGCCCCGAGAGATGAA R: CAGCTCCTTAGGGGAACACTC	Human
<i>RAPGEF3</i>	F: CCTCTCCAACCTCGGTGAAGC R: CTGGCTGAACAACACGGTC	Human
<i>AKT1</i>	F: TCCTCCTCAAGAATGATGGCA R: GTGCGTTCGATGACAGTGGT	Human
<i>GCH1</i>	F: CAGCACAATGTTGGGTGTGTT R: AGTACGATCGGCAACCAACG	Human
<i>HSF1</i>	F: GCACATTCCATGCCCAAGTAT R: GGCCTCTCGTCTATGCTCC	Human
<i>HSP90AA1</i>	F: AGGAGGTTGAGACGTTTCGC R: AGAGTTCGATCTTGTGTTGTTCCG	Human
<i>HSP90AB1</i>	F: CATCTCCATGATTGGGCAGTT R: CTTGACCCGCCTCTCTTCTA	Human
<i>HSP90B1</i>	F: CCAGTTTGGTGTGCGTTTCTAT R: CTGGGTATCGTTGTTGTGTTTTG	Human

Supplementary Table 2

Target antigen	Vendor or Source	Catalog #	Working concentration
GAPDH	Cell Signaling Technology	2118	WB (1:2000)
KLF2	Millipore	09-820	WB (1:3000) IHC (1:500)
eNOS	Cell Signaling Technology	9572	WB (1:2000) IP (1:100)
Hsp90	Abcam	ab1429	WB (1:1000) IP (1:100)
p-eNOS S1177	BD transduction	612392	WB (1:1000)
p-eNOS S615	Millipore	07-561	WB (1:1000)
p-eNOS T495	Cell Signaling Technology	9574	WB (1:200)
p-Akt S473	Cell Signaling Technology	4060	WB (1:1000)
p-Akt T308	Cell Signaling Technology	9275	WB (1:1000)
Akt1	Cell Signaling Technology	2967	WB (1:1000)
GCH-1	Abcam	ab236387	WB (1:500)
p-PKC α/β T638/641	Cell Signaling Technology	9375	WB (1:1000)
Hsp90 α	Cell Signaling Technology	8165	WB (1:1000)
Hsp90 β	Cell Signaling Technology	5087	WB (1:1000)
Insulin receptor β	Cell Signaling Technology	23413	WB (1:1000)
PLCB3	GeneTex, Inc	GTX111100	WB (1:1000)
PLCD3	GeneTex, Inc	GTX123172	WB (1:1000)
Tyrosine nitrated proteins	Sigma-Aldrich	05-233	WB (1:1000)