

17 β -Estradiol phosphorylates cystathionine γ -lyase and induces vascular endothelial H₂S release via nongenomic modulation of pGC-A/cGMP/PKG signaling

Xingyan Xu^{1#}, Qing Yan^{1#}, Xiaoyun Liu¹, Ping Li¹, Xiaosa Li¹, Yiwen Chen¹, Tommaso Simoncini², Junxiu Liu^{3*}, Dongxing Zhu^{1*}, Xiaodong fu^{1*}

From the ¹Department of Gynecology and Obstetrics, The Sixth Affiliated Hospital, Key Laboratory of Cardiovascular Diseases, School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou 510260, China; ²Molecular and Cellular Gynecological Endocrinology Laboratory (MCGEL), Department of Reproductive Medicine and Child Development, University of Pisa, Pisa 56100, Italy; ³ Department of Gynecology and Obstetrics, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China.

Supporting Figure S1

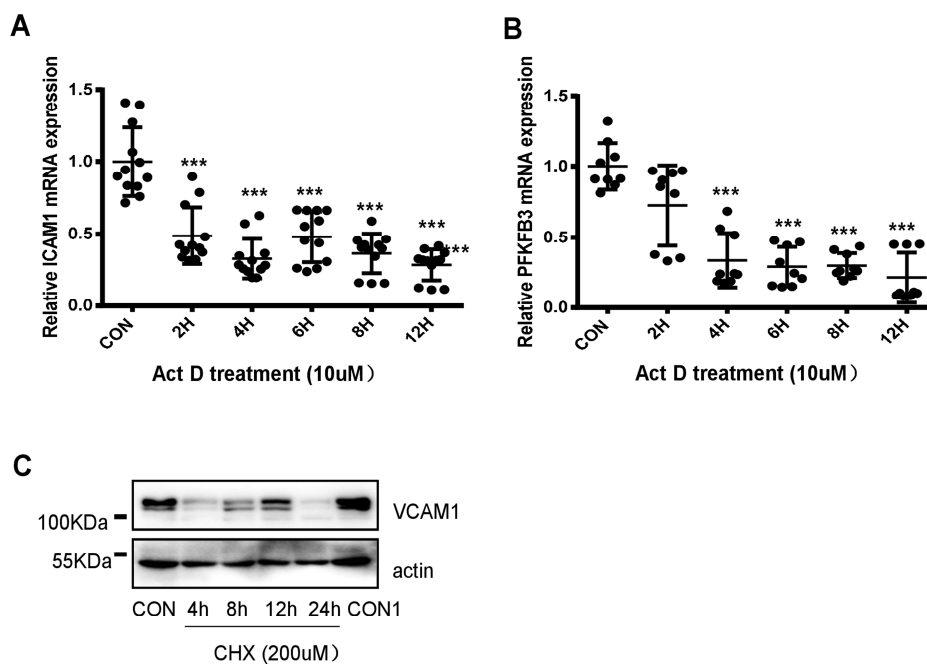


Figure S1. ActD and CHX used in the present study successfully prevented transcription of ICAM1 and PFKFB3 and translation of VCAM1. A-B. HUVECs were treated with 10 μ M Act D as the indicated time points, qPCR was performed to examine the expression of ICAM1 and PFKFB3 mRNA levels (mean \pm SD, n=12 technical replicates, ***P<0.001, vs CON, one way-ANOVA followed by the post-hoc LSD test). C, HUVECs were treated with 10 μ M Act D as the indicated time points, western blot was performed to examine the protein expression of VCAMC1.

Supporting Figure S2

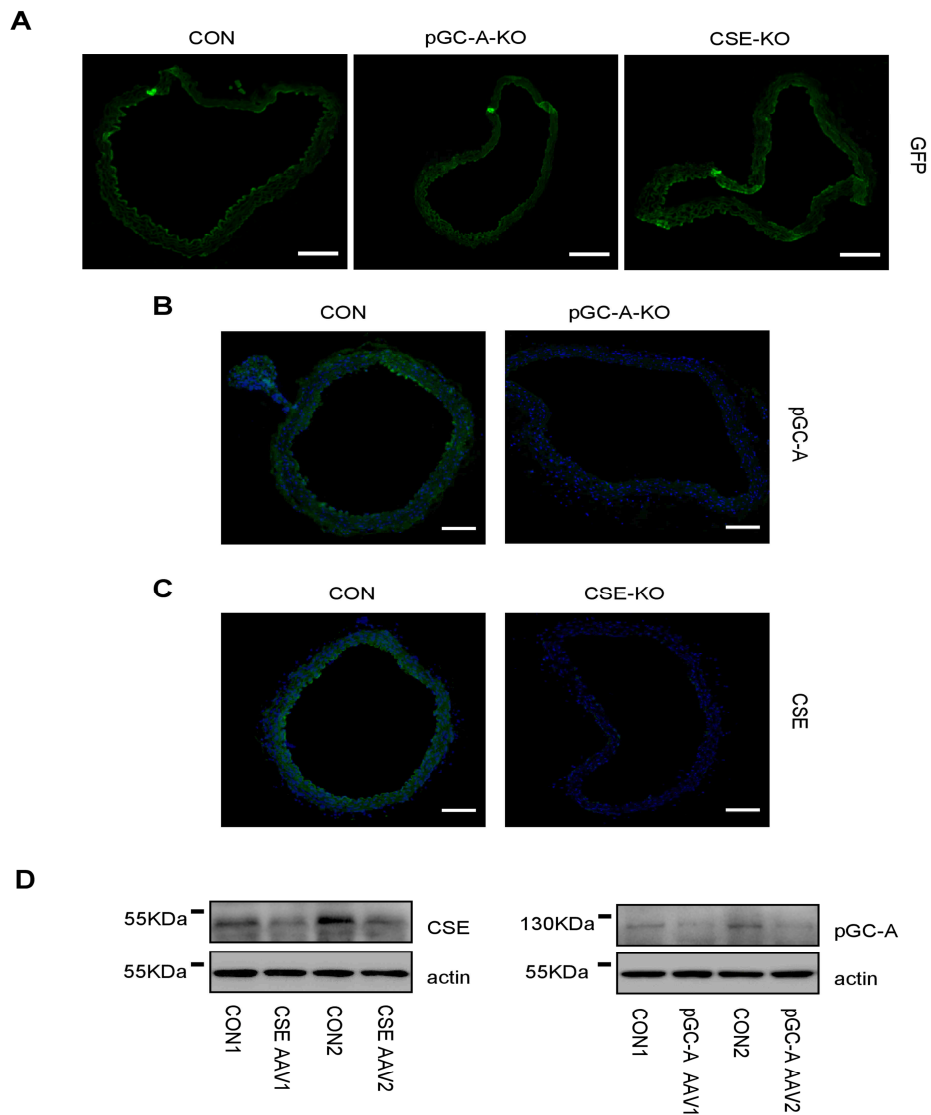


Figure S2. Validation of AAV mediated knockdown efficiency of pGC-A and CSE. Mice at 8 weeks of age were injected from the tail vein with AAV-control-shRNA, AAV-pGC-A-shRNA, AAV-CSE-shRNA. After 4 weeks, the aortae were dissected out for immunofluorescence staining and western blot. A. GFP positive cells showed that AAV-mediated shRNA was efficiently delivered to the aorta. B-C. immunofluorescence staining of pGC-A and CSE. D. Western blot of pGC-A and CSE.

Supporting Figure S3

Figure 2A

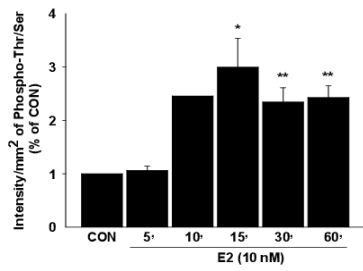


Figure 3A

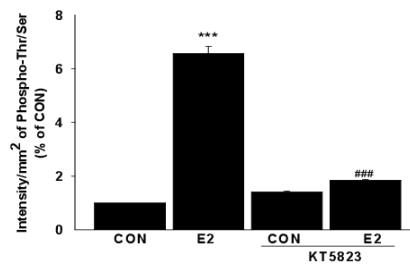


Figure 3C

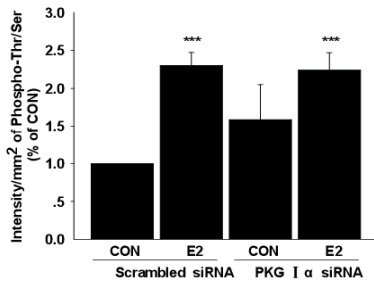


Figure 3D

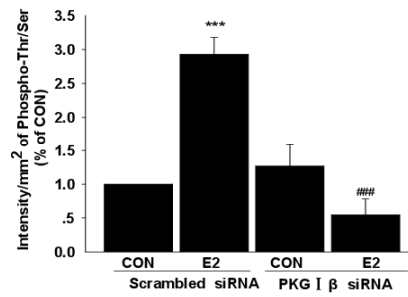


Figure 4C

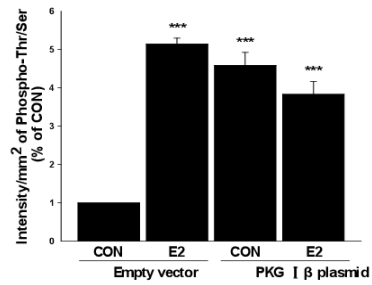


Figure 4D

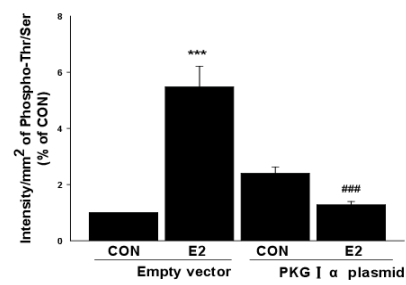


Figure S3. Semi-quantitative analysis of western blot for Figure 2 to Figure 4.

Supporting Figure S4

Figure 6E

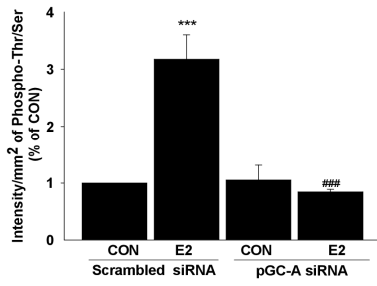


Figure 6G

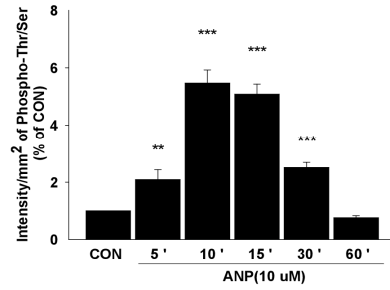


Figure 7D

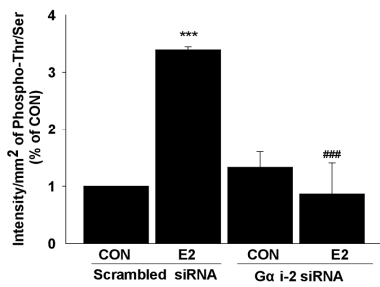


Figure 7E

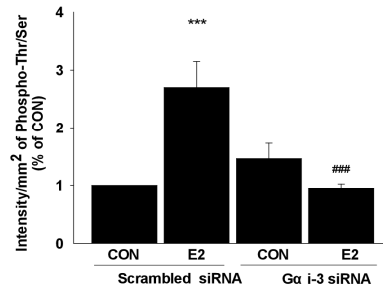


Figure 8C

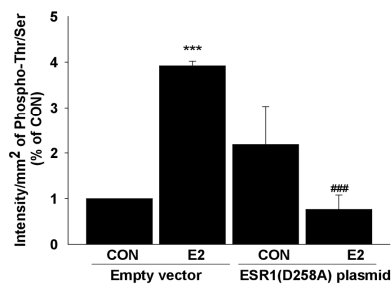


Figure S4. Semi-quantitative analysis of western blot for Figure 6 to Figure 8.