Supplemental Information

Shear stress regulates cystathionine γ lyase expression to preserve endothelial redox balance and reduce membrane lipid peroxidation

Supplementary Figure Legends

Supplementary Figure 1. Relationship between shear stress, KLF2 and miR-27b in human endothelial cells. Human endothelial cells (passage 1) were left untreated or incubated with a control siRNA (siCTL) or a siRNA directed against KLF2 (siKLF2) for 36 hours. (**A**) Effect of shear stress (12 dynes cm^{-2}) on miR-27b levels. (**B**) Consequence of siRNA-mediated KLF2 downregulation (siKLF2) on miR-27b mRNA levels under static conditions and following exposure to shear stress for 24 hours. (**C**) Sequence of the human wild-type (CSE-WT) versus the mutated CSE (CSE-mut) 3′UTR reporter and the seeding sequence of miR-27b. Graphs summarize data from n=6 different cell batches of endothelial cells. ***P*<0.01, ****P*<0.001 (ANOVA, Newman-Keuls).

Supplementary Figure 2. Shear stress-induced changes in CSE, eNOS and miR-27b in murine endothelial cells. (**A**) Effect of shear stress (12 dynes cm^{-2}) on the expression of CSE and eNOS protein. Endothelial cells from CSE^{iEC} mice (iEC) were included as a negative control. (**B**) Effect of shear stress on the expression of CSE and eNOS protein and the generation of H_2S_n; n=6 independent cell batches (ANOVA, Newman-Keuls). (**C**) Consequence of KLF2 downregulation (siKLF2) and a control siRNA (siCTL) on KLF2, eNOS and CSE protein levels in static conditions and after exposure to shear stress (12 dynes cm^{-2}, 24 hours); n=6 independent cell batches (ANOVA, Newman-Keuls). (**D**) Effect of shear stress on CSE, eNOS, miR-27b and KLF2 mRNA levels; n=6 Independent cell batches (ANOVA, Newman-Keuls). (**E**) Effect of pre-miR-27b (48 hours) on CSE protein levels; n=6 independent cell batches (Student's t-test). ***P*<0.01, ****P*<0.001.

Supplementary Figure 3. CSE activity and inflammation in human endothelial cells from healthy and atherosclerotic arteries. (**A**) Cystathionine levels in endothelial cells isolated from plaque-free (PF) and atherosclerotic plaque-containing arteries with (P+Sim) or without statin (P) treatment. (**B**) Circulating levels of IL-1β in the plaque-free (PF) individuals and patients with atherosclerotic plaques, with or without statin treatment from which endothelial cells (see Figure 4) were isolated; n=4-12 (Student's t-test). *P<0.05, ***P*<0.01.

Supplementary Figure 4. CSE deletion and Prx6 sulfhydration in murine arteries. (**A**) Prx6 sulfhydration (S-SH Prx6) in endothelial cells from wild-type (WT) and CSE^{iEC} mice. DTT was included to demonstrate the specificity of the signal. (**B**) Dihydroethidium derivatives generated by reactive oxygen species (O_2^- as the superoxide specific products and ethidium as a general ROS unspecific product) in the lesser curvature of aortae from wild-type and CSE^{iEC} mice. n=6 independent cell batches or animals (Student's t-test). ***P*<0.01, ****P*<0.001.

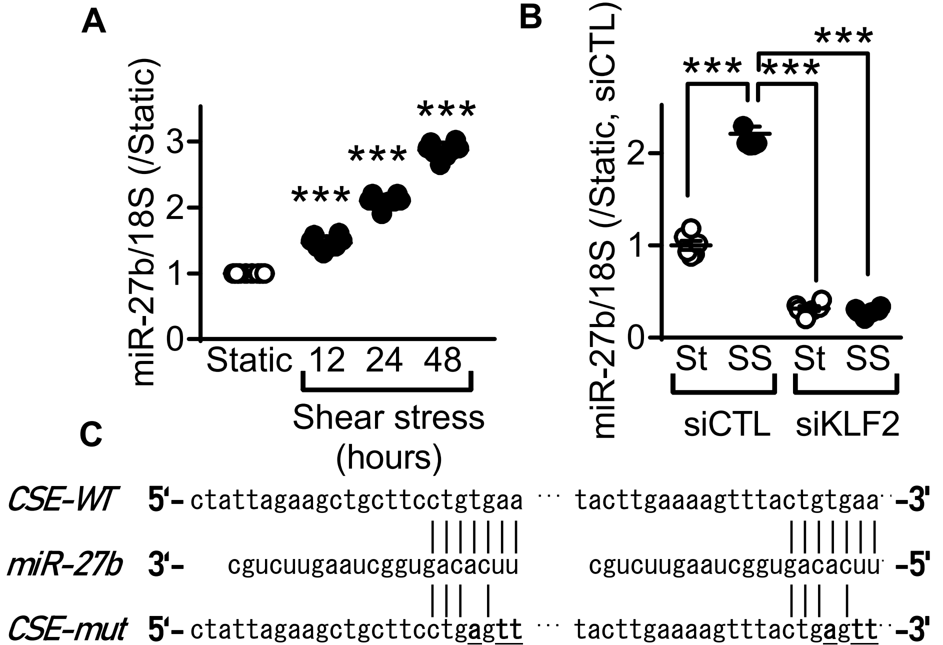
Supplementary Tables

Supplementary Table 1. Clinical and demographic data from the human subjects

	Healthy	Patients
Demographic data		
No	8	45
Mean age (range)	68 (55–76)	66 (56–78)
Male /female	8/0	35/10
Smokers	0	20
Clinical data		
Hypertension	0	35
Diabetes	0	5
Hyperlipidemia	0	45
Coronary disease	0	12
Myocardial Infraction	0	0
Valve insufficiency	0	0
Renal disease	0	0
Heart failure	0	0
Angiographic carotid stenosis		
<90%	0	45
Plaque histopathology		
Unstable	0	25
Stable	0	20
Medication		
ACE inhibitors	0	28
b-blockers	0	14
Simvastatin	0	15

Supplementary Table 3. Sulfhydrated proteins enriched in the healthy arterial endothelial cells involved in redox cellular homeostasis

Cysteine	Leading protein name	Gene names	T test	log2ratio
93	P27695	APEX1	0,000171	-9,65186
131	Q96HE7	ERO1L	0,002581	-10,6767
128	A0A087X247	GPX4	0,004496	-10,9816
85	H7BZJ3;P30101	PDIA3	0,007077	-12,0818
244	P30101	PDIA3	0,012486	-12,6647
92	H7BZJ3;P30101	PDIA3	0,000864	-11,5754
85	H7BZJ3;P30101	PDIA3	0,007077	-12,0818
231	Q14554	PDIA5	0,000888	-11,6465
465	Q14554	PDIA5	0,004625	-7,3713
47	P30041	PRDX6	0,00317	-3,09152
91	P30041	PRDX6	0,000648	-5,79393
128	Q8NBS9	TXNDC5	0,004663	-8,72552
121	Q8NBS9	TXNDC5	0,006241	-8,68166
254	Q8NBS9	TXNDC5	0,064368	-11,0099
247	Q8NBS9	TXNDC5	0,064368	-11,0099
388	Q8NBS9	TXNDC5	0,088504	-9,6878
381	Q8NBS9	TXNDC5	0,002877	-5,55595
470	A0A182DWF2	TXNRD2	0,058085	-10,4591



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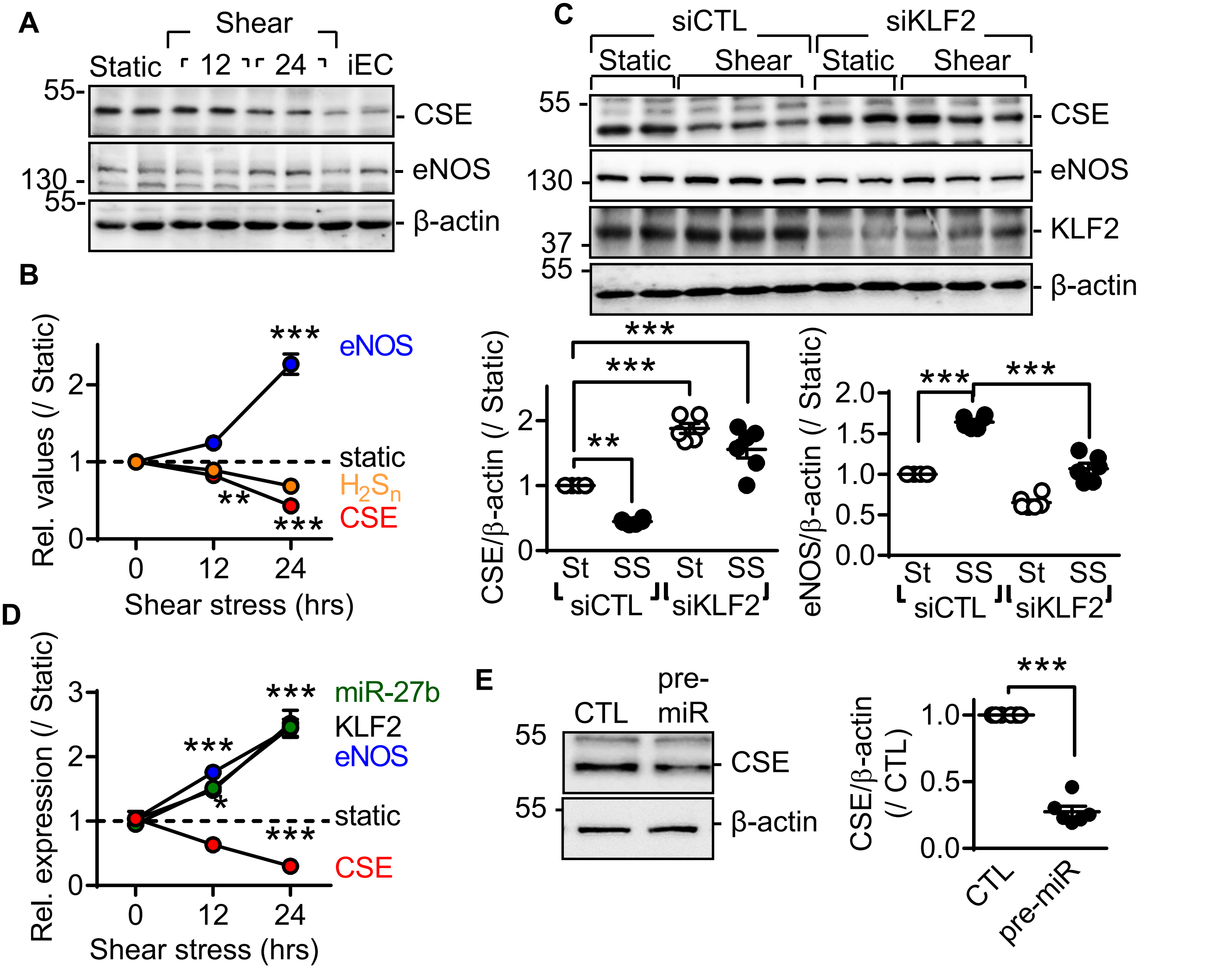

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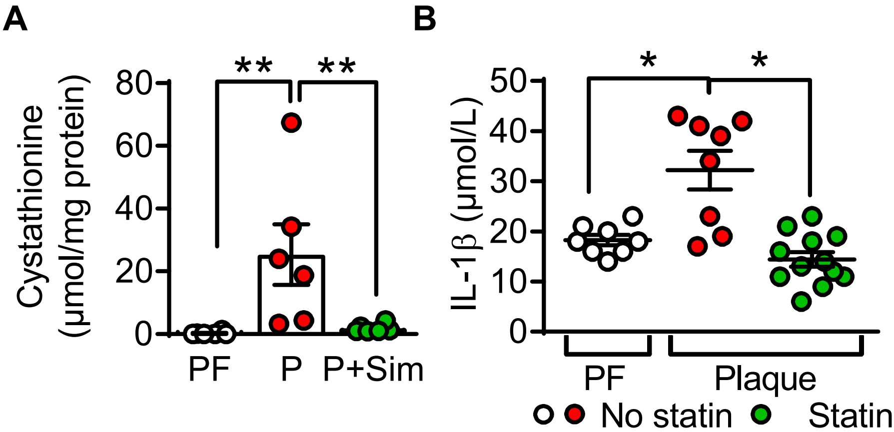

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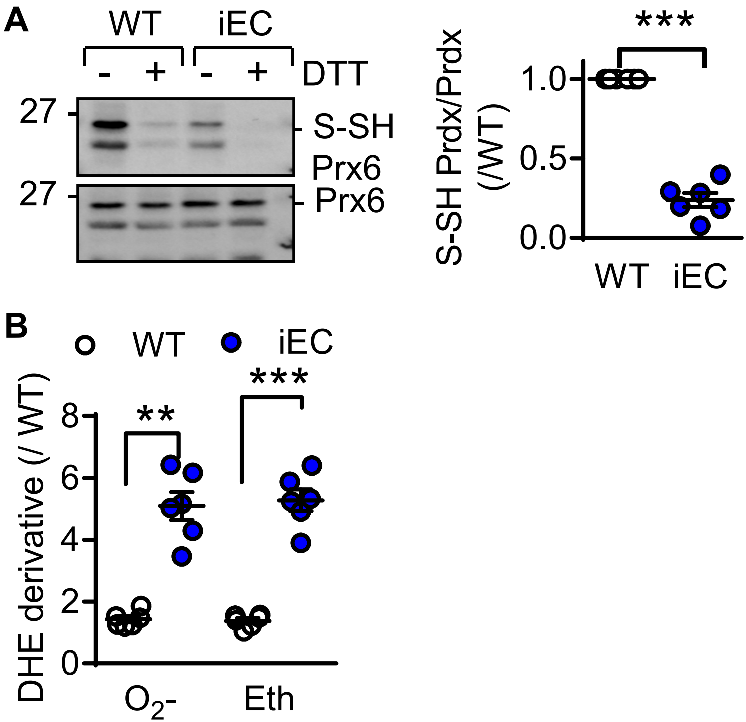

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