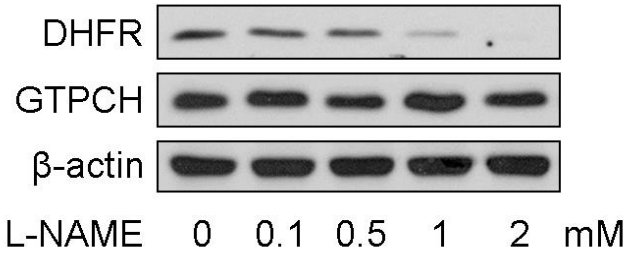
Supplement to

Endothelial Nitric Oxide Synthase-Derived Nitric Oxide Prevents Dihydrofolate Reductase Degradation by Promoting S-Nitrosylation

Zhejun Cai, Qiulun Lu, Ye Ding, Qilong Wang, Lei Xiao, Ping Song, Ming-Hui Zou

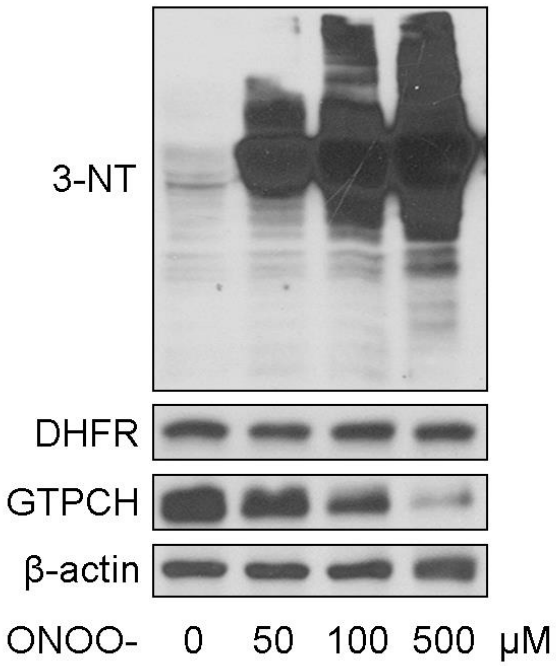
Supplemental Figures

Supplemental Figure I



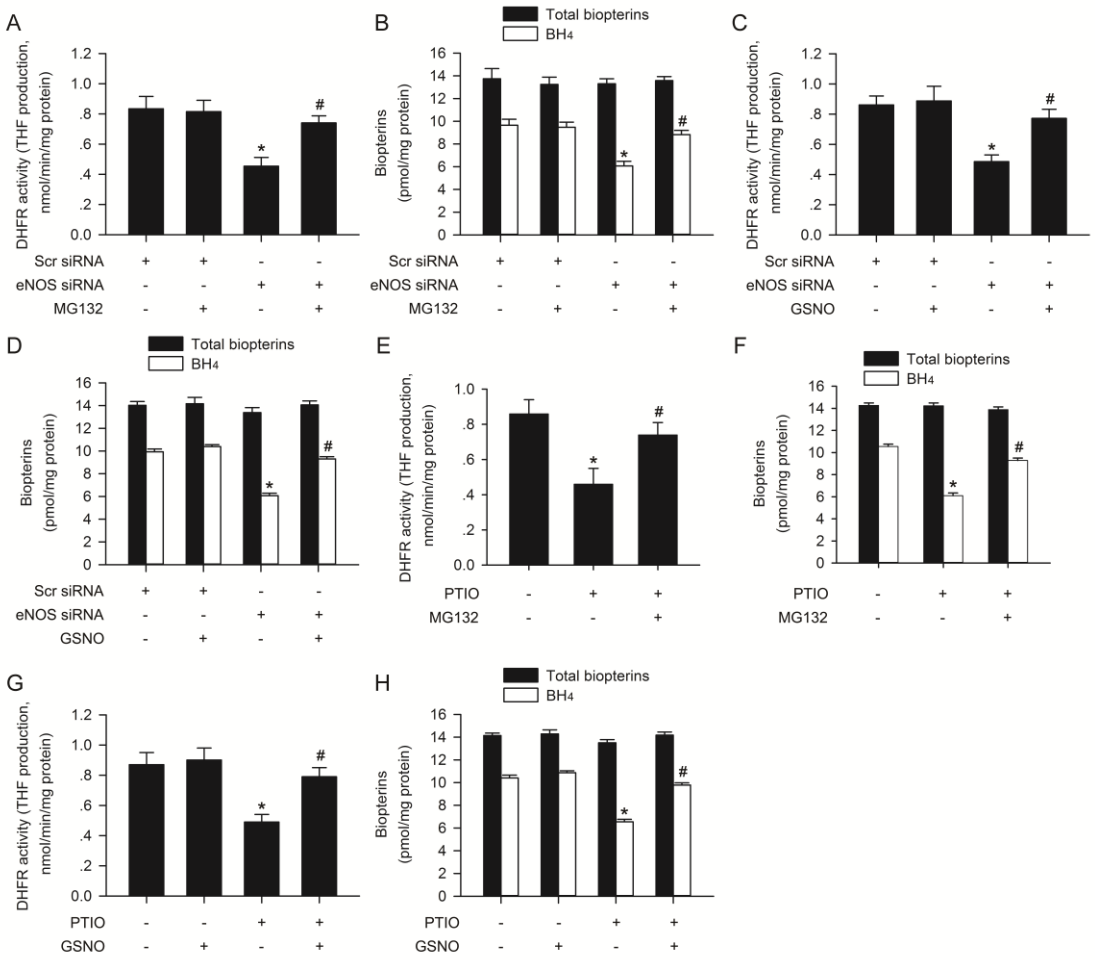
Supplemental Figure I. eNOS inhibitor L-NAME dose-dependently reduces DHFR expression in HUVECs. L-NAME reduced DHFR expression from 1mM to 2mM range, but had no effect on GTPCH expression.

Supplemental Figure II



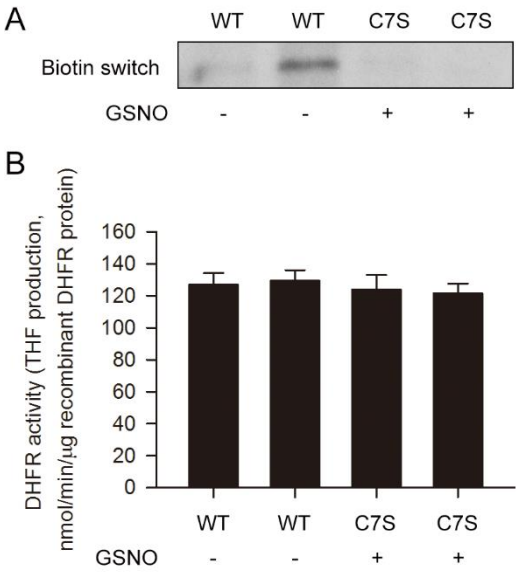
Supplemental Figure II. ONOO- does not alter DHFR expression in HUVECs. Tyrosine nitration of proteins as determined by 3-nitrotyrosine (3-NT) increased as the dose of ONOO- supplementation increased. ONOO- treatment reduced GTPCH expression in a dose-dependent manner, but had no effect on DHFR expression.

Supplemental Figure III



Supplemental Figure III. NO depletion reduces DHFR activity and BH_4 levels in HUVECs. MG132 (1μM, 6h) prevented eNOS silencing caused DHFR activity (**A**) and BH_4 contents (**B**) reduction. GSNO (100μM) supplementation reversed DHFR activity (**C**) and BH_4 contents (**D**) reduced by eNOS silencing. MG132 (1μM, 6h) and GSNO (100μM) also prevented PTIO (150μM) induced DHFR activity (**E and G**) and BH_4 contents (**F and H**) reduction in HUVEC. (n=3; *p<0.05 vs. Scr siRNA in **A**-**D**, or p<0.05 vs. control in **E**-**H**; #p<0.05 vs. eNOS siRNA in **A**-**D**, or p<0.05 vs. PTIO in **E**-**H**)

Supplemental Figure IV



Supplemental Figure IV. S-nitrosylation of DHFR does not affect its activity in vitro. (**A**) GSNO (100μM) incubation increased S-nitrosylation of WT DHFR but not C7S DHFR in vitro. (**B**) Recombinant WT and C7S DHFR exhibited no difference in activities. GSNO incubation did not alter their activities.