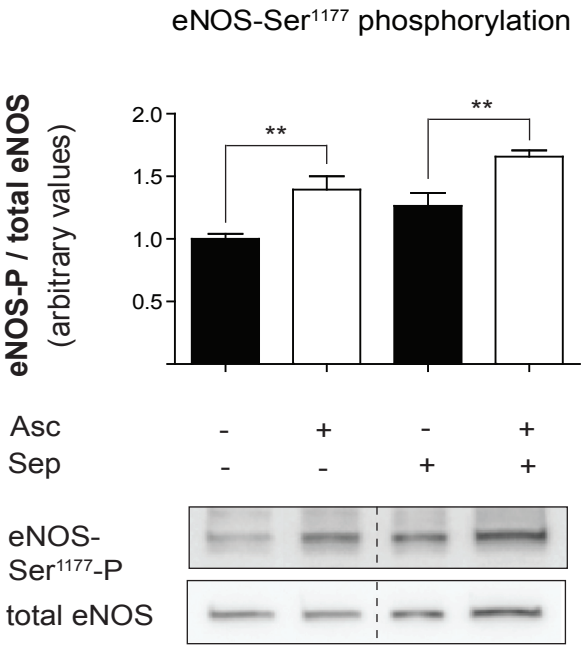
Figure Legends

Supplementary Figure 1: Short term modulation of eNOS phosphorylation by ascorbate is independent from BH4 levels.
Endothelial cells were pretreated with 10µM sepiapterin (Sep) for 30 min and then incubated with 100 µM ascorbate for 60 min as indicated before total cell lysates were determined and subjected to western blot analysis for (phospho)eNOS. Representative blots out of three independent experiments with consistent results are depicted. The dashed line indicates that interjacent lanes on the (same) respective membrane were cut out.

Supplementary Figure 2: H_2O_2 is not responsible for the effect of ascorbate on eNOS activity.
(A) EA.hy926 cells were pretreated with 300 U catalase per well for 35 min and then incubated for 4 h with 100 µM ascorbate as indicated before a [^{14}C]L-arginine/[^{14}C]L-citrulline conversion assay was performed. [^{14}C]L-citrulline production was normalized to the untreated control (***, $p < 0.001$; ns, not significant) (mean ± SEM, n =4). (B) EA.hy926 cells were treated with 100 µM ascorbate, 0.25 µM H_2O_2 or 1 µM H_2O_2 for 60 min as indicated before an Amplex Red assay was performed to determine extracellular levels of H_2O_2. As control supernatants of untreated cells were used. (C) EA.hy926 cells were pretreated with 0.1 µM wortmannin for 30 min and then incubated with 100 µM ascorbate for 30 min and subjected to Western blot analysis for the detection of (phospho-)eNOS levels. One representative blot is shown (n = 3).

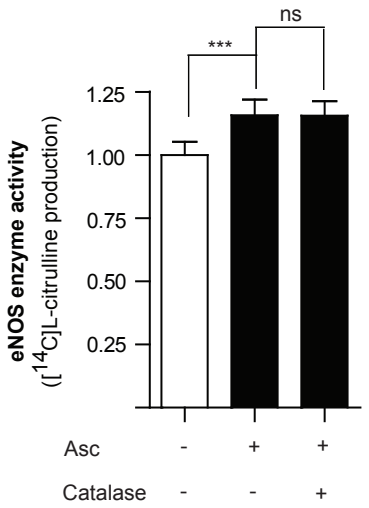
Supplementary Figure 3: Extent of eNOS activation by common known activators.
(A) EA.hy926 cells were treated with 50 ng/mL VEGF for 5 minutes before cell lysates were subjected to western blot analysis for (phospho-) eNOS and (phospho-) AMPK levels, respectively. EA.hy926 cells were treated with (B) 30 µM resveratrol (Res) or (C) 3 µM mevastatin (Mev) before eNOS enzyme activity was assessed in the arginine citrullin conversion assay. Respresentative blots are shown out of three independent experiments with consistent results.



--> Supplementation with sepiapterin cannot abrogate the rapid ascorbate-mediated increase in eNOS -Ser1177 phosphorylation, underlining the BH4 independency of early effects of ascorbate on eNOS function.

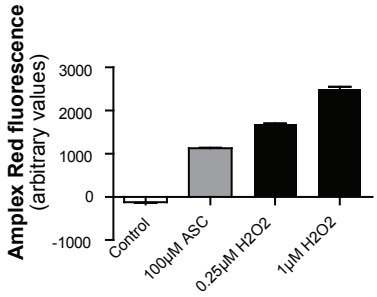
Supplementary Figure 1

A



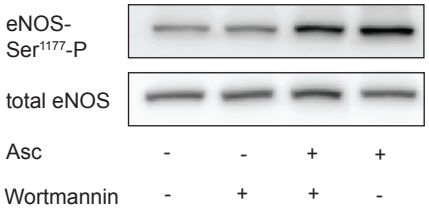
eNOS activation by ascorbate cannot be blocked by incubation with catalase.
--> exogenous H2O2 is not responsible for eNOS activation by vitamin C

B



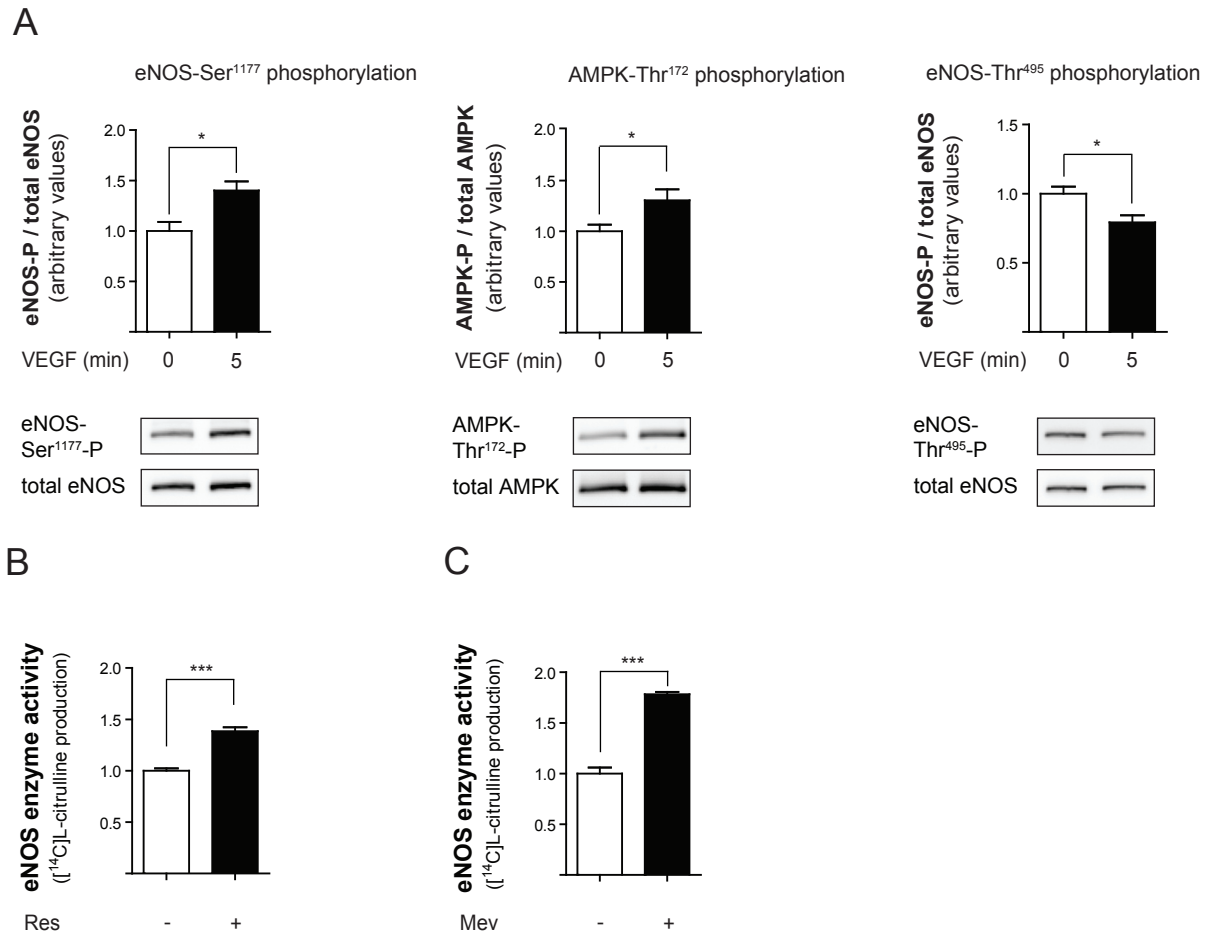
Incubation with ascorbate elicits only minute changes in the amounts of extra-cellular H2O2.

C



Wortmannin does not overcome the stimulating effect of ascorbate on eNOS Ser1177 phosphorylation
--> AKT is not involved in the ascorbate-triggered change in eNOS phosphorylation

Supplementary Figure 2



--> The extent of ascorbate-triggered eNOS activation (about 1.5-3 fold compared to the untreated control cells) is comparable to that elicited by VEGF, resveratrol and mevastatin, known activators of eNOS.

Supplementary Figure 3