Online Resource 3

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Endothelial NOS (NOS 3) impairs myocardial function in developing sepsis

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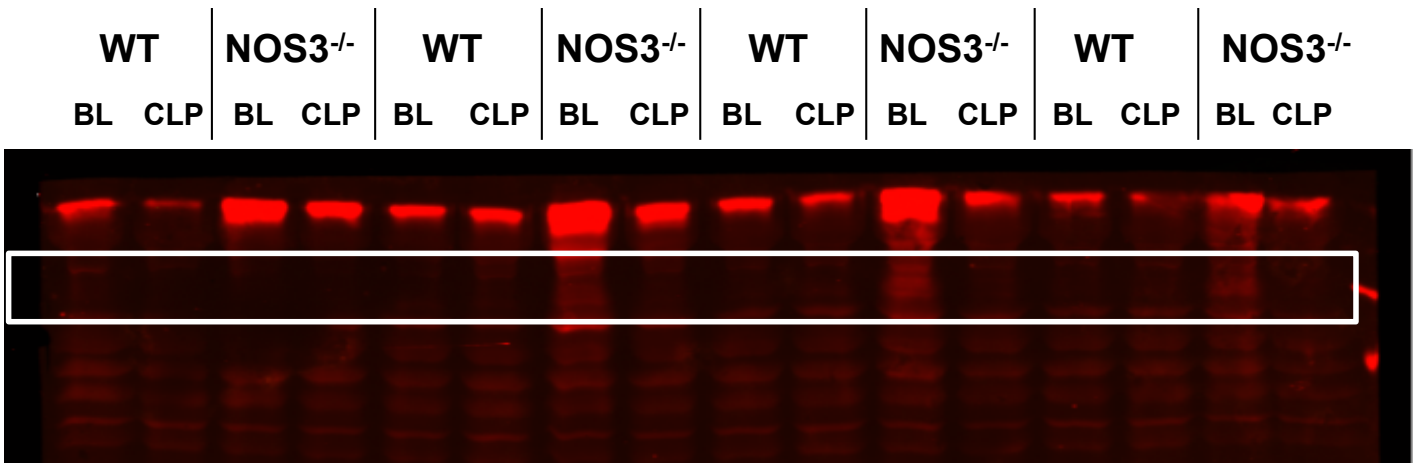
anti-Glutathione



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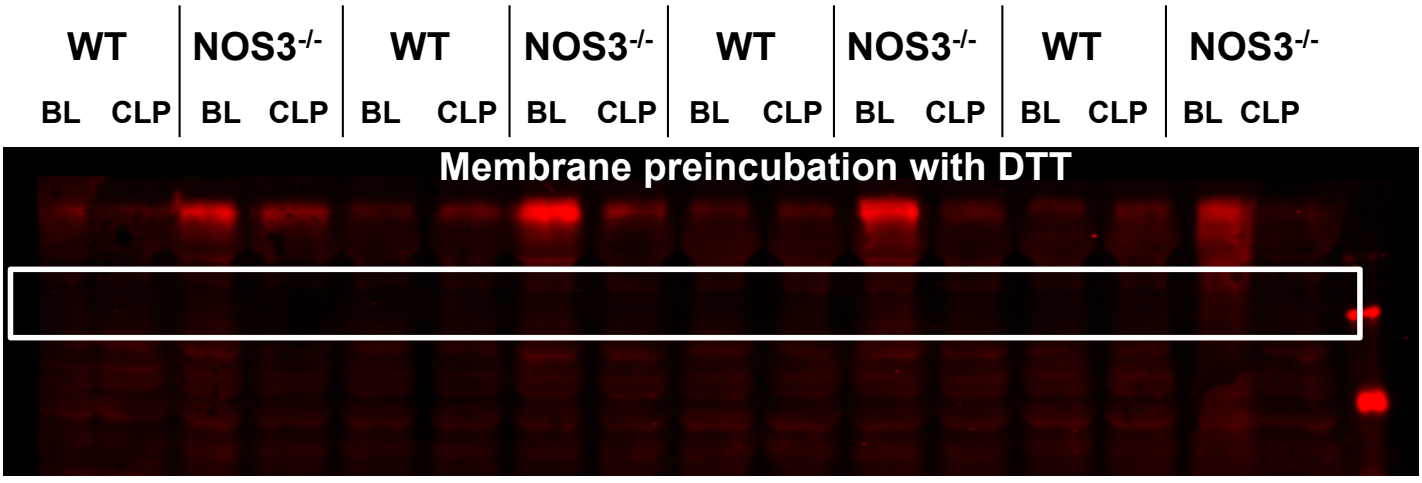

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Figure legend.

Online Resource 3. Global protein glutathionylation. Protein glutathionylation was not increased in sepsis developing mice. In WT mice no increase in glutathionylation could be observed at the level of eNOS/ NOS3.

Methods Online Resource 3.

eNOS/NOS3 glutathionylation. After in situ perfusion with NaCl supplemented with N-ethylmaleimide (NEM)/EDTA hearts were excised from the mice, snap frozen in liquid nitrogen and homogenized in N-ethylmaleimide (40 mM) and SDS (2%) containing HEPES (50 mM, pH7.0) buffer. NEM irreversibly alkylates thiol groups to prevent further S-glutathionylation during sample processing. Protein extracts were separated by SDS-PAGE under non reducing conditions and blotted onto nitrocellulose membranes. S-glutathionylated proteins were detected by Western Blot using an anti-glutathione monoclonal antibody (Thermo Scientific MA1-7620). As negative control the Western Blot membrane was pre-incubated with dithiothreitol (DTT).