SUPPLEMENTARY INFORMATION FOR

BACTERIAL FLAVOHEMOGLOBIN: A MOLECULAR TOOL TO PROBE MAMMALIAN NITRIC OXIDE BIOLOGY

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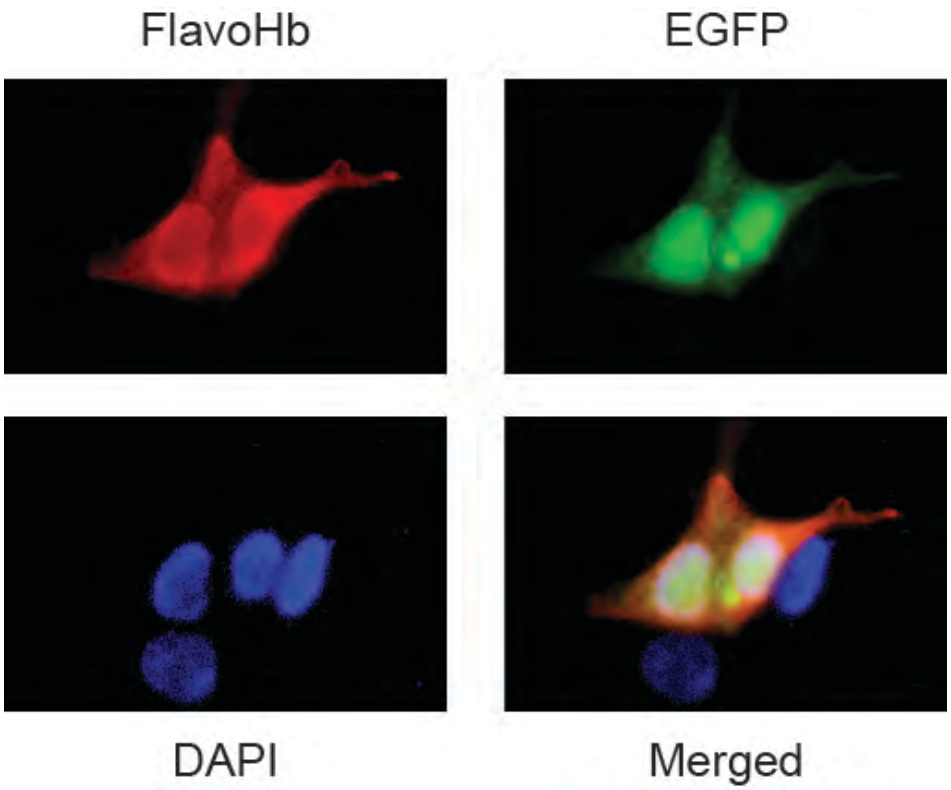
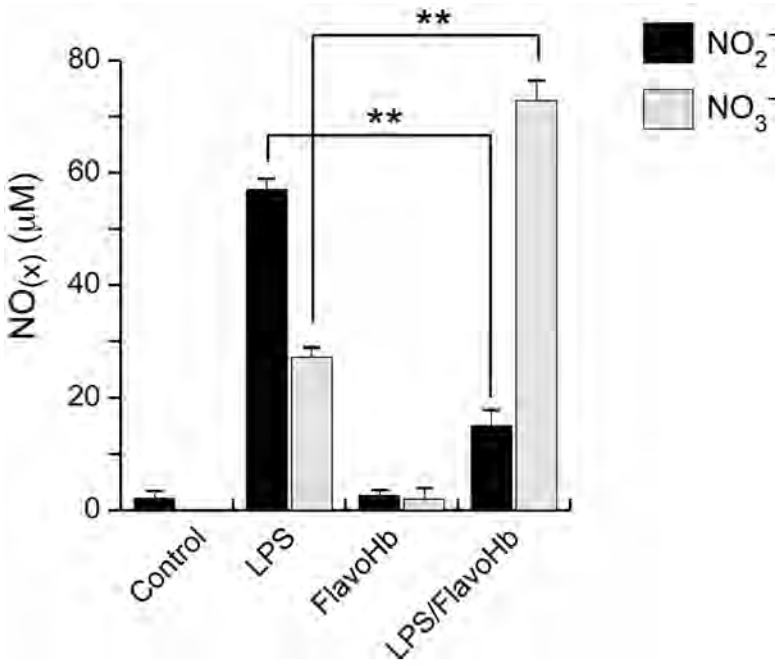
List of Supplementary Figures:



Supplementary Figure 1. Localization of FlavoHb by fluorescence microscopy. HEK293 cells were transiently transfected with vector bearing Flag-tagged FlavoHb for 24 h, then fixed and imaged as described in the Methods section.



Supplementary Figure 2. Assessment of FlavoHb activity in stably infected RAW264.7 murine macrophages. Cells expressing either empty vector or FlavoHb were grown to 80% confluence, treated -/+ 500 ng/ml LPS for 24 h, and media was collected. Nitrite and nitrate, products of NO auto-oxidation and flavoHb-based di-oxygenation, respectively, were measured as described in the Methods section (**, $p < 0.01$ by Student's t-test).

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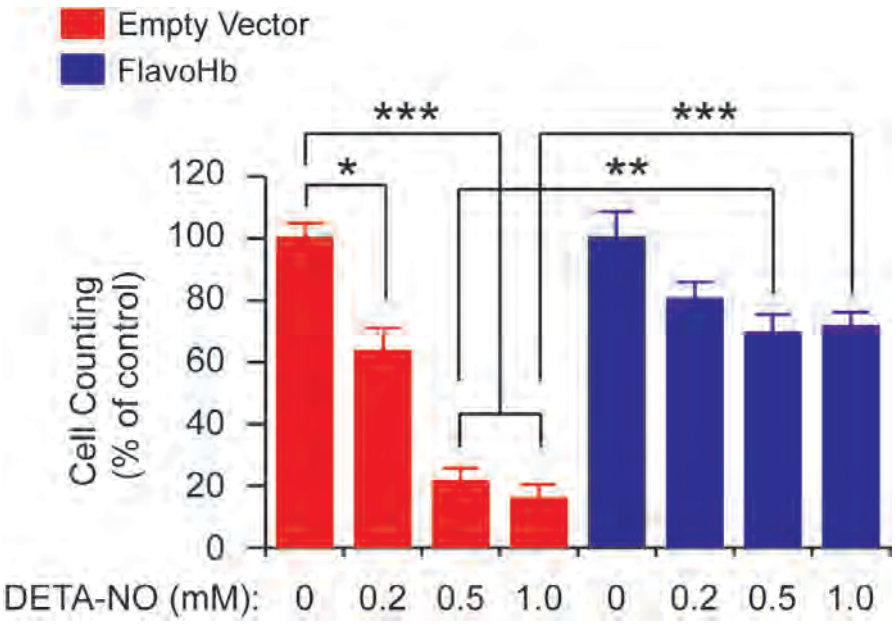
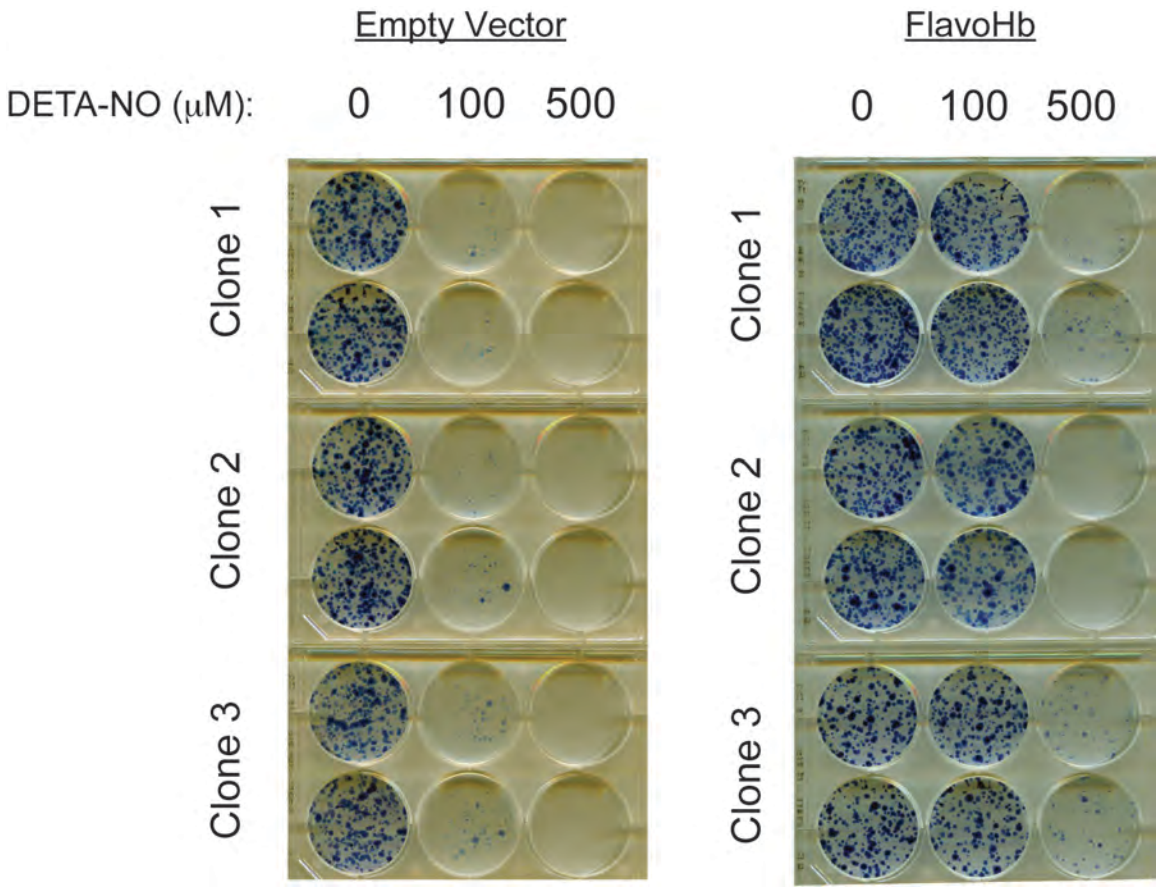


Supplementary Figure 3. Role of FlavoHb in protection from nitrosative stress: assessment by cell counting. HEK293 cells expressing either empty vector or FlavoHb were grown to 90% confluence, exposed to the indicated concentrations of DETA-NO for 48 h, and viable cells (i.e., trypan blue-excluding) were counted on a hemacytometer as described in the Methods section (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ by ANOVA).



Supplementary Figure 4. Colony formation assays of HEK293 cells exposed to nitrosative stress. Three different clones of HEK293 cells stably expressing either empty vector or FlavoHb were seeded into 6 well plates (500 cells per well), and treated with the indicated concentration of DETA-NO. After 1 week, wells were fixed and colonies visualized by methylene blue staining.