

## **Supplementary Materials**

### **Artifacts introduced by sample handling in chemiluminescence assays of nitric oxide metabolites**

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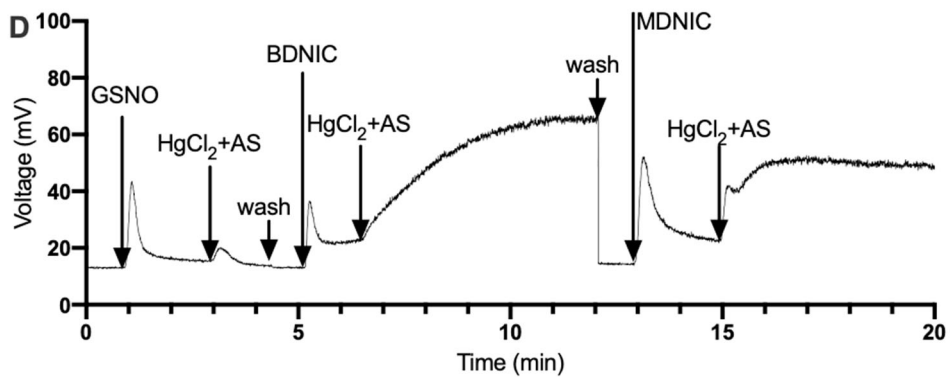
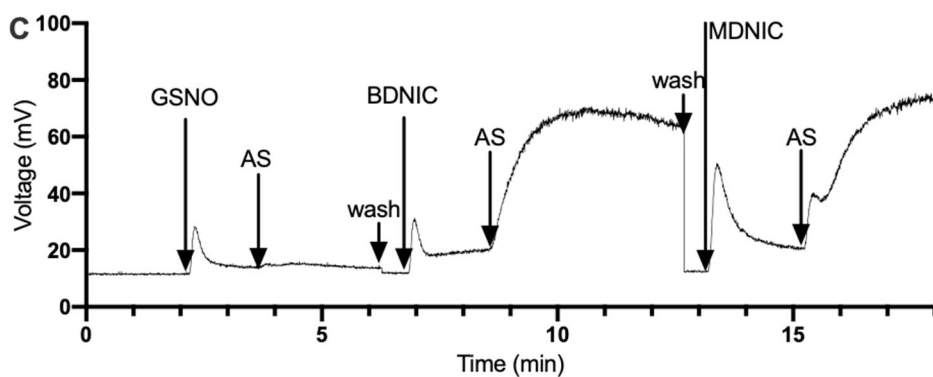
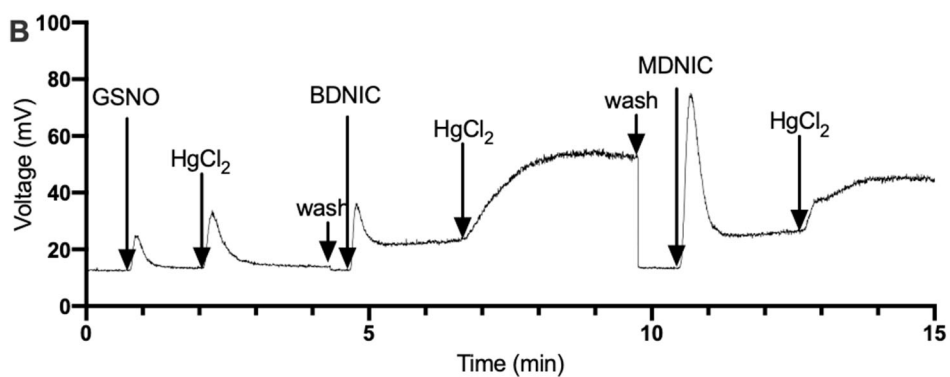
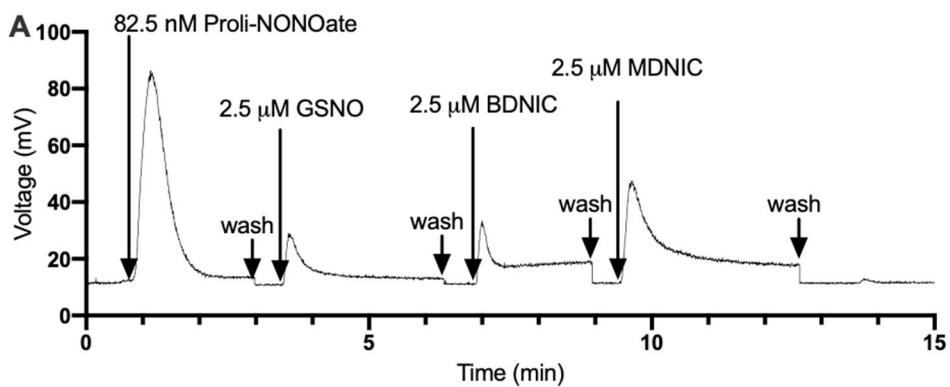
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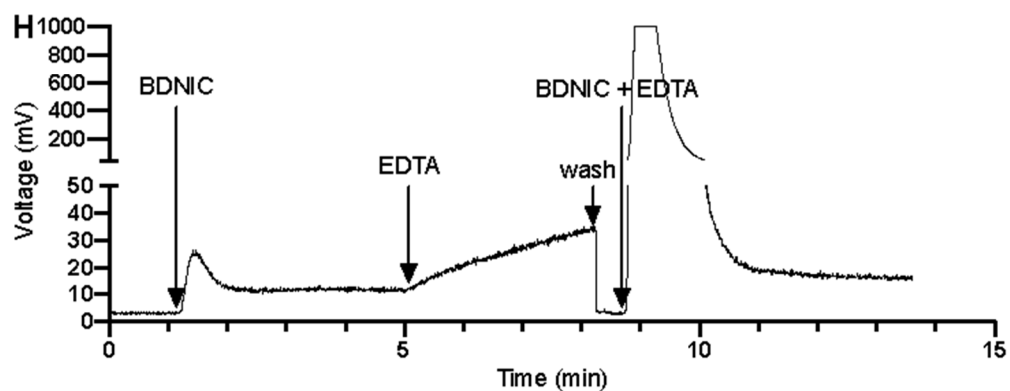
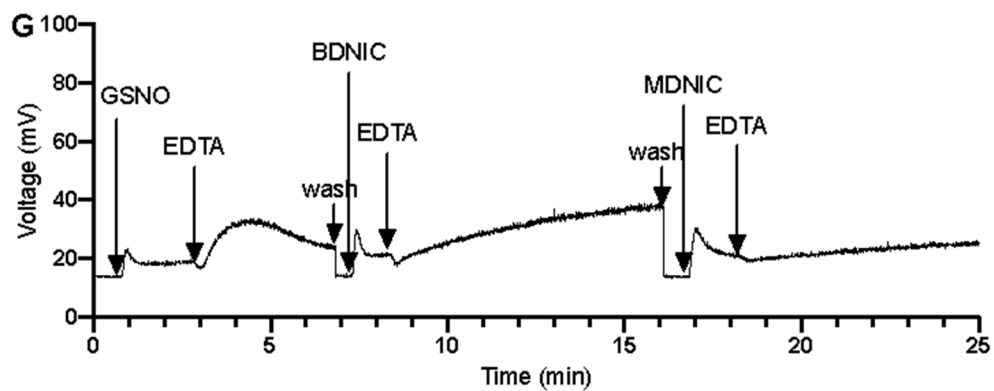
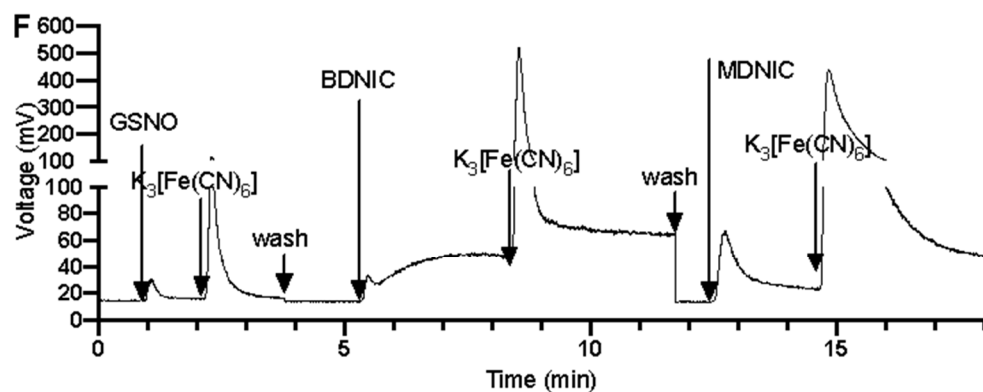
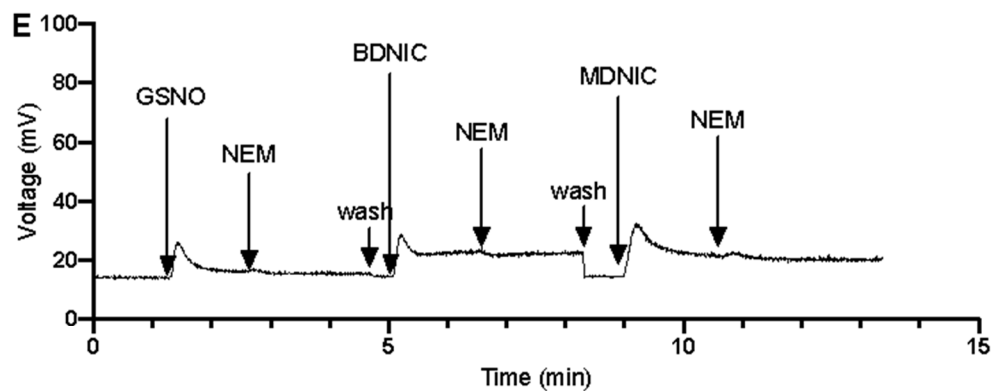
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<sup>†</sup>Passed away.

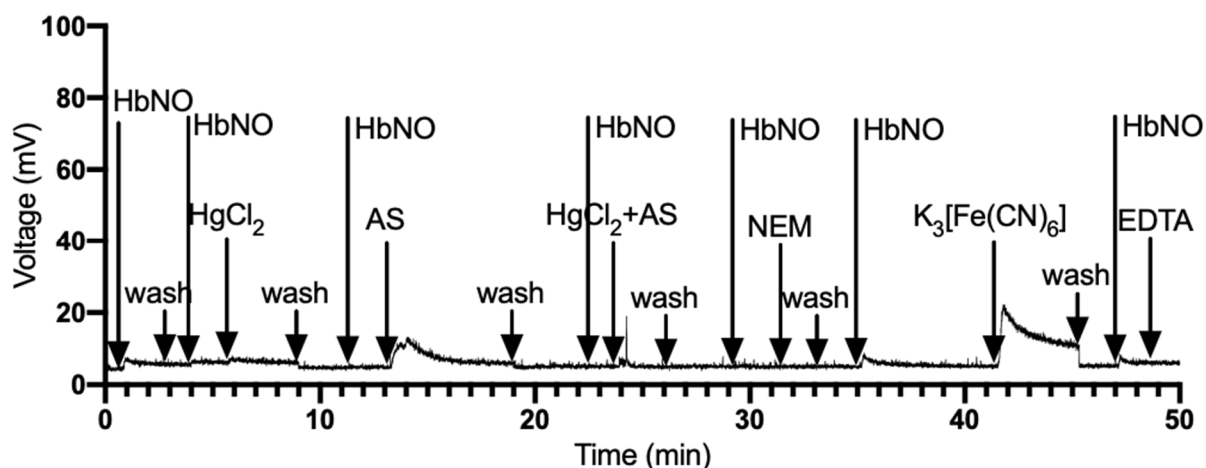
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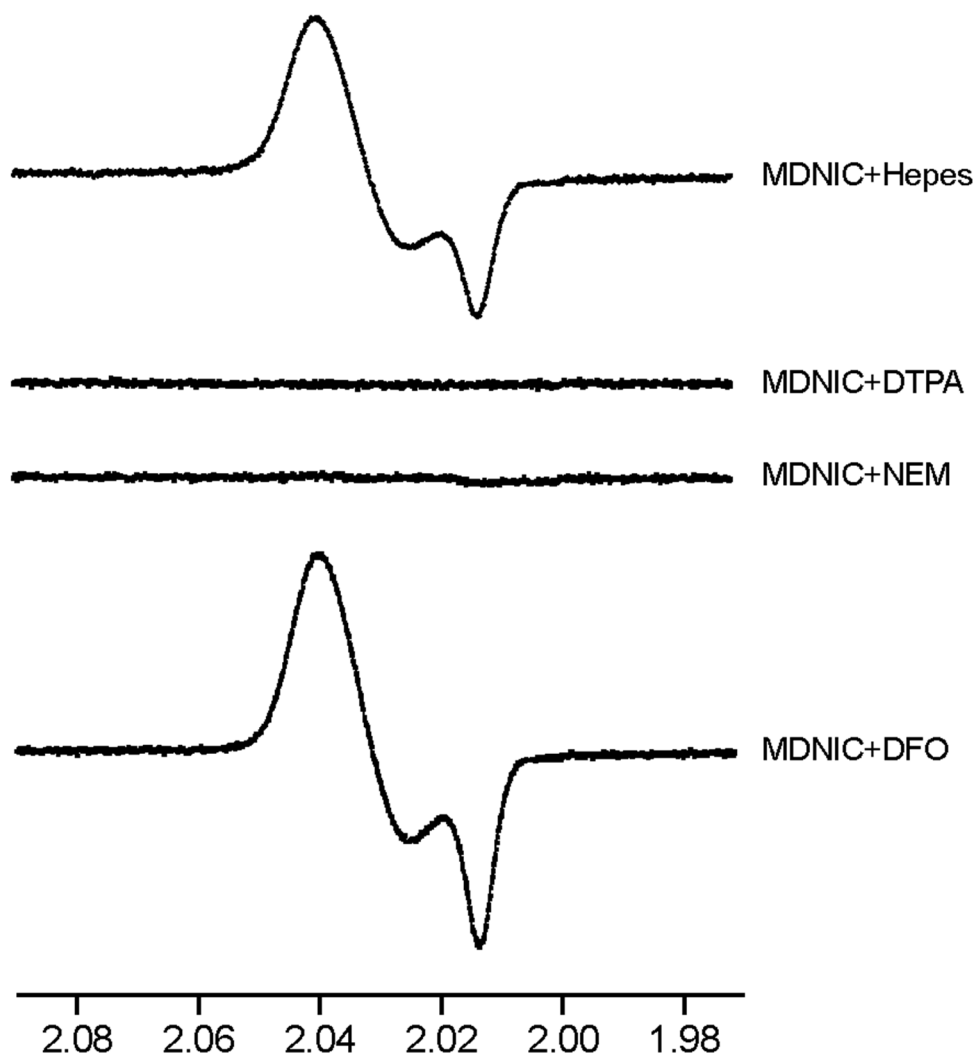




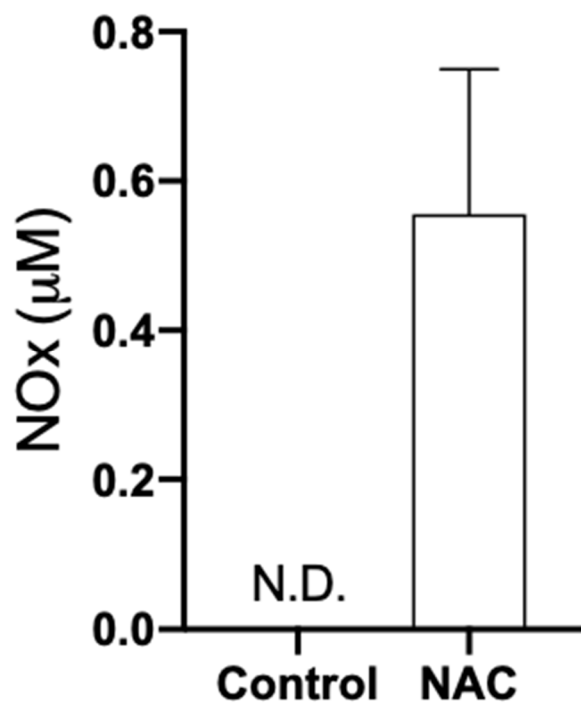
**Figure S1.** Effects of several chemicals, commonly used in processing of NO<sub>x</sub> samples, on the stability of GSNO, BDNIC, and MDNIC. n=3. All samples and reagents were injected into a purge vessel (never previously used for I<sub>3</sub><sup>-</sup> assay) containing HEPES buffer (no DTPA). Arrows indicate injections or washes. **A)** Representative traces of NO (Proli-NONOate), GSNO, BDNIC, and MDNIC. **B)** Effects of 2.5 mM HgCl<sub>2</sub>, **C)** Effects of AS (0.125% w/v sulfanilamide in 0.05 N HCl), **D)** Effects of HgCl<sub>2</sub>+AS, **E)** Effects of 100 μM NEM, **F)** Effects of 0.5 mM ferricyanide, **G-H)** Effects of 25 mM EDTA. All concentrations given above are the final concentrations in the purge vessel. Different from other injections that were directly injected into the purge vessel, the second injection of BDNIC in **(H)** was after its (50 μM) incubation (10 min; room temp in dark) with 500 mM EDTA at a volume ratio of 1:1 in an Eppendorf tube.



**Figure S2.** Effects of several chemicals commonly used in processing of NO<sub>x</sub> samples on the stability of heme-NO (HbNO). n=3. All samples and reagents were injected into a purge vessel (never previously used for I<sub>3</sub><sup>-</sup> assay) containing HEPES buffer (no DTPA). Arrows indicate injections or washes. Representative traces of heme-NO (2.5 μM), and the effects of 2.5 mM HgCl<sub>2</sub>, AS (0.125% w/v sulfanilamide in 0.05 N HCl), HgCl<sub>2</sub>+AS, 100 μM NEM, 0.5 mM ferricyanide, and 25 mM EDTA are shown.



**Figure S3.** Representative EPR traces showing effects of DTPA, NEM, and DFO on MDNIC signals.  $n=3$ . HEPES buffer, 100  $\mu\text{M}$  DTPA, 2 mM NEM, or 1 mM DFO was mixed with 50  $\mu\text{M}$  MDNIC at a volume ratio of 1:1 and measured with EPR at 150 K. All traces were shown in the same scale.



**Figure S4.** Ferricyanide releases NO from N-acetylcysteine (NAC). n=3. 10 mM of NAC and HEPES buffer (Control) were analyzed with  $K_3[Fe(CN)_6]$ /PBS assay. Parallel injection of NAC into  $I_3^-$  resulted in no detectable NO (not shown).