Supporting Information

Heat shock protein 90 increases superoxide generation from neuronal nitric oxide synthases

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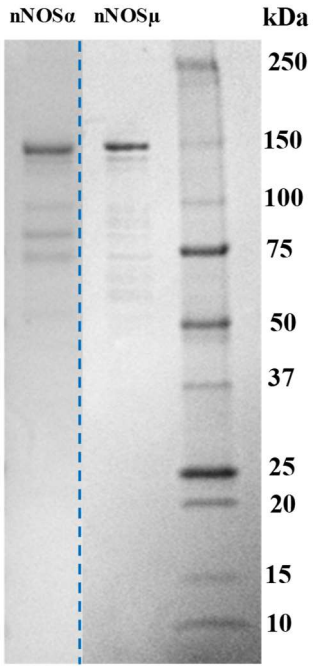

Figure S1. SDS-PAGE analysis of purified nNOSα and nNOSμ proteins used in this study. This confirms the size and the purity of the recombinant nNOS variants. Dotted lines are marks to show that the gel images have been modified to splice out empty or irrelevant lanes. Molecular weight standards are marked at the right side of the panel.

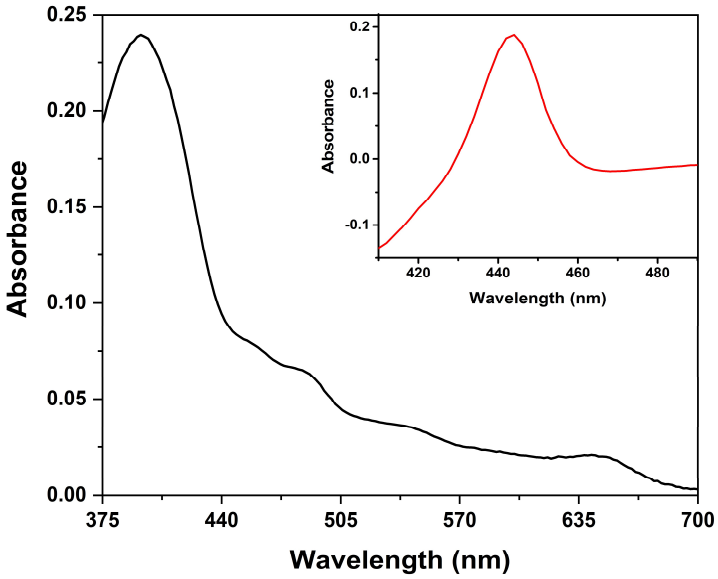

Figure S2. The absorption spectrum of purified nNOSμ protein showing a heme Soret peak at 396 nm and flavins' peaks in the 450 and 480 nm region. The inset is difference spectrum of reduced protein in the presence of CO.

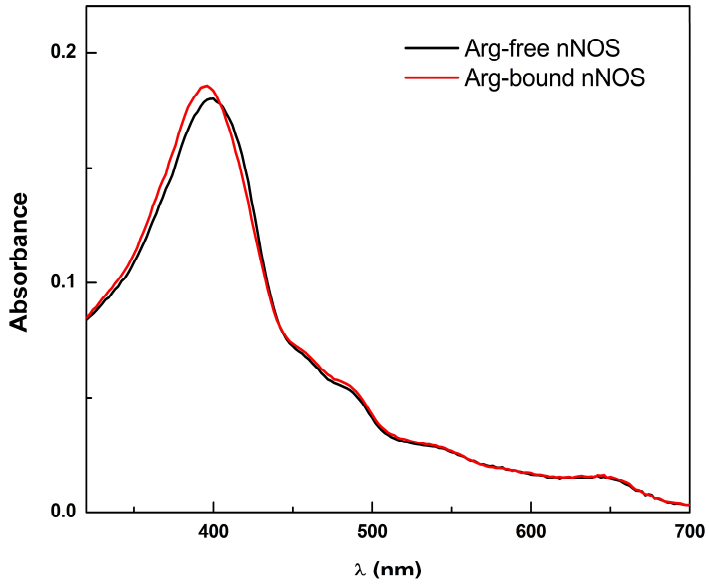

Figure S3. Absorption spectra of L-Arg free nNOS (black) and L-Arg bound nNOS (red) proteins. Note that upon L-Arg binding the Soret peak is blue-shifted to 396 nm and becomes sharper and more symmetric. L-Arg was removed from the purified protein by 3-cycle of buffer exchanging into L-Arg-free buffer.

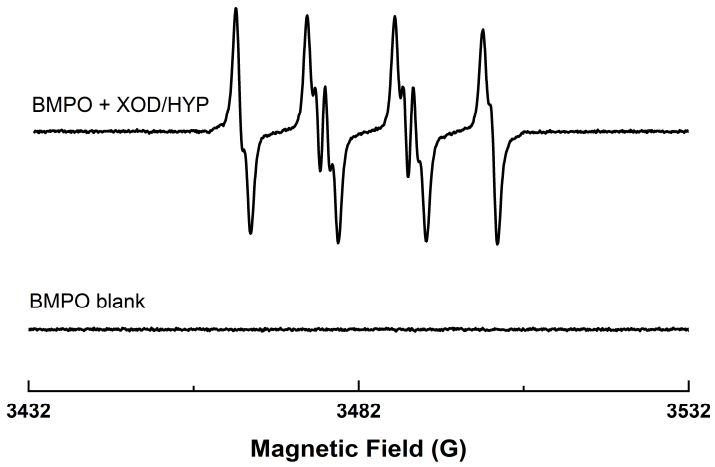

Figure S4. EPR detection of the BMPO-•OOH adduct formed from the reaction of BMPO with superoxide ($O_2^{\bullet-}$) in a XOD/HYP $O_2^{\bullet-}$ generating system. The system contained xanthine oxidase (1 mU/ml), hypoxanthine (50 μM), BMPO (20 mM) and DTPA (0.1 mM) in 50 mM sodium phosphate buffer (pH=7.4). EPR spectra of BMPO-•OOH adduct were recorded continuously, and the spectra presented here were collected 20 min after adding XOD into the system.

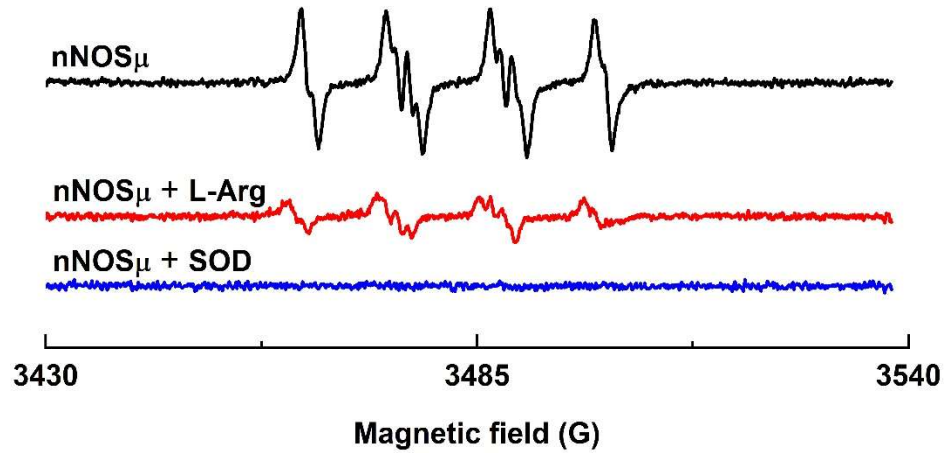

Figure S5. EPR signals of superoxide ($O_2^{\bullet-}$) adduct, BMPO-$^{\bullet}$OOH, observed in the reaction system containing 20 nM nNOSμ alone (i.e., without Hsp90). Strong EPR signals of superoxide adduct BMPO-$^{\bullet}$OOH were observed in the system containing 20 nM nNOSμ in the absence of L-Arg. The signal was significantly inhibited in the presence of 100 μM L-Arg. The EPR signal was predominantly quenched upon adding 200 units/mL SOD into the L-Arg-depleted sample prior to initiation of the reaction by adding NADPH, showing that the primary adduct is from superoxide. EPR spectra were recorded continuously, and the spectra presented here were collected 15 min after adding NADPH into the reaction system. The experimental conditions were the same as described in Figure 2 in main text.

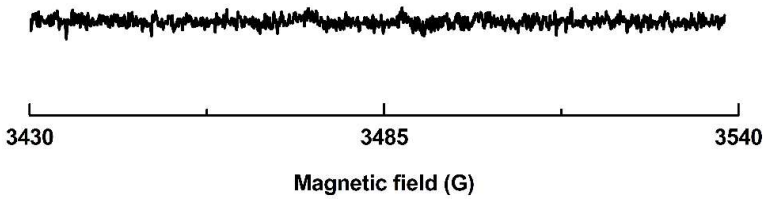

Figure S6. EPR signal for the system containing only the human Hsp90α protein. EPR spectra were recorded continuously, and the spectra presented here were collected 15 min after adding NADPH into the reaction system. The experimental conditions were the same as described in Figure 2 in main text.

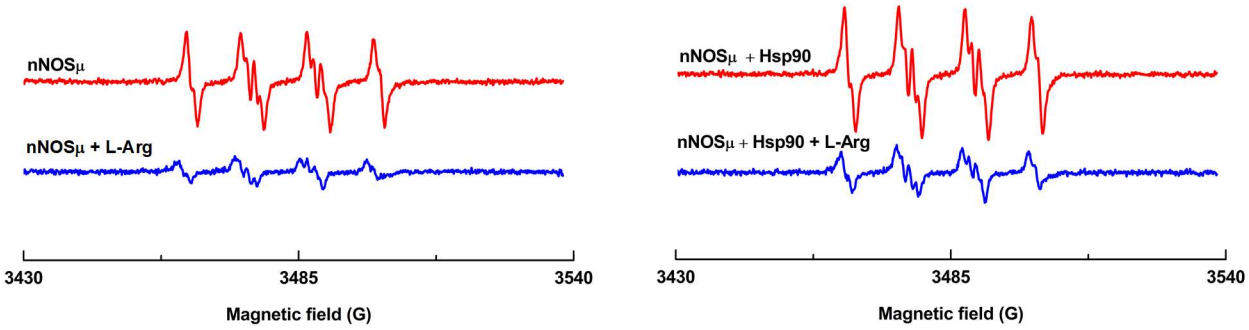

Figure S7. Comparing the superoxide ($O_2^{\bullet-}$) adduct BMPO-$^{\bullet}$OOH signal by the nNOSμ protein without (left panels) and with Hsp90α (right panels) under varying L-Arg concentrations (zero or 100 μM). EPR spectra were recorded continuously, and the spectra presented here were collected 15 min after adding NADPH into the reaction system. The experimental conditions were the same as described in Figure 2 in main text.