



**Supplementary Figure 1 Molecular hydrogen dissolved in culture medium does not reduce cellular hydrogen peroxide and nitric oxide.**

(a) PC12 cells were held in medium with or without 0.6 mM H<sub>2</sub>, and antimycin A (10 μg/ml) was added to the medium to induce O<sub>2</sub><sup>-•</sup>, which was rapidly converted into H<sub>2</sub>O<sub>2</sub>. Representative laser-scanning confocal images of the fluorescence of H<sub>2</sub>O<sub>2</sub> marker 2',7'-dichlorodihydrofluorescein (H<sub>2</sub>DCF) were taken 1 h after the addition of antimycin A. Scale bar: 100 μm. (b) DCF fluorescence in cells treated with antimycin A in the presence or absence of 0.6 mM H<sub>2</sub> was quantified from 100 cells from each independent experiment using NIH Image software (mean ± SD, *n* = 4). (c, d) Cellular NO• was detected with a cellular NO•-specific fluorescent probe, DAF-2 DA (diaminofluorescein-2 diacetate, purchased from Daiichi Pure Chemicals Co.) by laser-scanning confocal microscopy using excitation and emission filters of 488 and 510 nm, respectively. As a negative control, an inhibitor of NOS (L-NAME: N<sup>G</sup>-Nitro-L-arginine methyl ester, purchased from Sigma) was added so as not to generate NO•. Scale bar: 50 μm. (d) DAF-2 DA fluorescence was quantified as described in (b) (mean ± SD, *n* = 5). \*\*\**P* < 0.001.