



Supplementary Figure 3 Several methods confirm protection of cells by H₂ against oxidative stress.

PC12 cells incubated in the presence of or absence of 0.6 mM H₂ were treated with the indicated concentration of antimycin A (**a**, **b**) or menadione (**c**), and maintained with each H₂ concentration for 24 h as described in **Methods**. (**a**) As another method, a modified MTT assay (WST-1 assay) was applied to the cell system according to a Cell Counting Kit (purchased from Wako) to ensure the protective effect by H₂ against oxidative stress (mean ± SD, $n = 4$). * $P < 0.05$, ** $P < 0.01$. (**b**) Lactate dehydrogenase (LDH) activities were measured to estimate cellular LDH leakage from damaged cells according to an LDH-Cytotoxic Test kit (Wako). LDH activity in medium of antimycin A- and H₂-untreated cells was taken as the background (mean ± SD, $n = 4$). * $P < 0.05$, ** $P < 0.01$. (**c**) Instead of antimycin A, menadione was used to induce oxidative stress for 24 h and living cells were enumerated as described in **Fig. 2f** (mean ± SD, $n = 4$). ** $P < 0.01$.