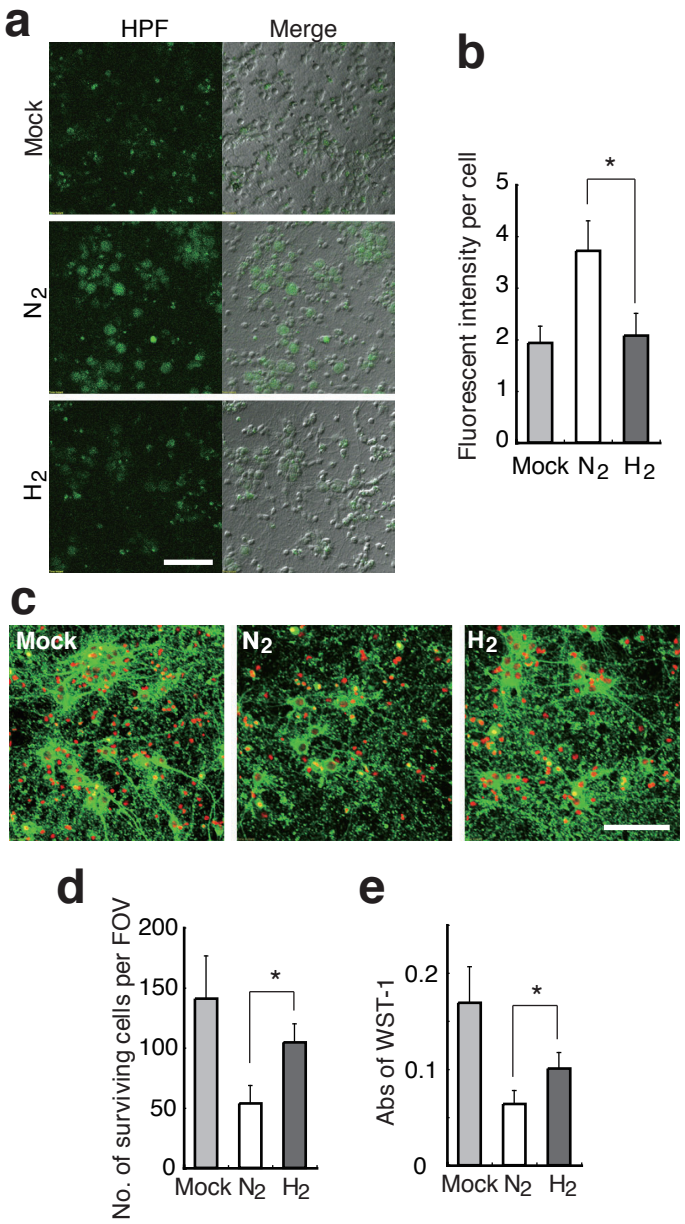

Supplementary Figure 4 Molecular hydrogen protects cultured neurons from ischemia and reperfusion *in vitro*.

A primary culture of rat neocortical cells was prepared and subjected to OGD (oxygen glucose deprivation) as described in **Supplementary methods**. (**a**) Ten min after reperfusion, cells were stained with HPF (left, fluorescent images; right, superimposition of the fluorescent HPF images with Nomarski differential interference contrast images). "Mock" indicates that cells were treated with DMEM medium containing glucose and oxygen instead of being subjected to OGD. Scale bar: 100 μm. (**b**) The average fluorescent intensity of HPF was measured in 100 cells (mean ± SD, $n = 4$). *$P < 0.05$. (**c**) Twenty hours after OGD, surviving neurons were fixed and immunostained with the neuron-specific antibody to TUJ-1 (green) and with PI (red). Scale bar: 100 μm. (**d**) Dead cells were washed out in the staining procedure and living cells were enumerated under a fluorescent microscope in four fields of view (FOV) per well (mean ± SD, $n = 4$). *$P < 0.05$. (**e**) Twenty hours after OGD, viability in a well was estimated by a modified MTT viability assay according to a Cell Counting Kit (WST-1 assay) (mean ± SD, $n = 4$). *$P < 0.05$.