

ONLINE SUPPLEMENT

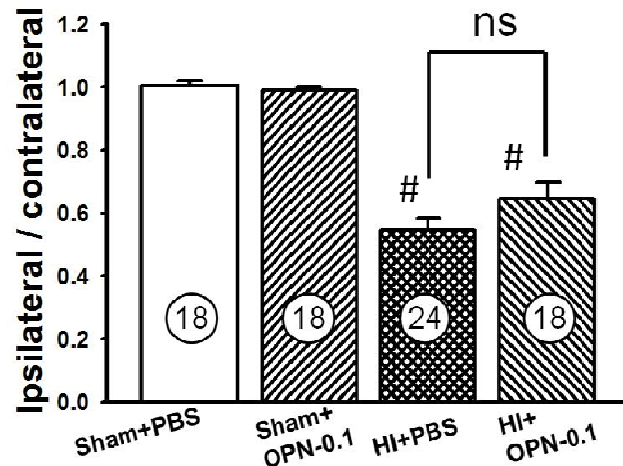
Title: “Osteopontin reduced hypoxia-ischemia neonatal brain injury by suppression of apoptosis in a rat pup model”

Supplemental Methods

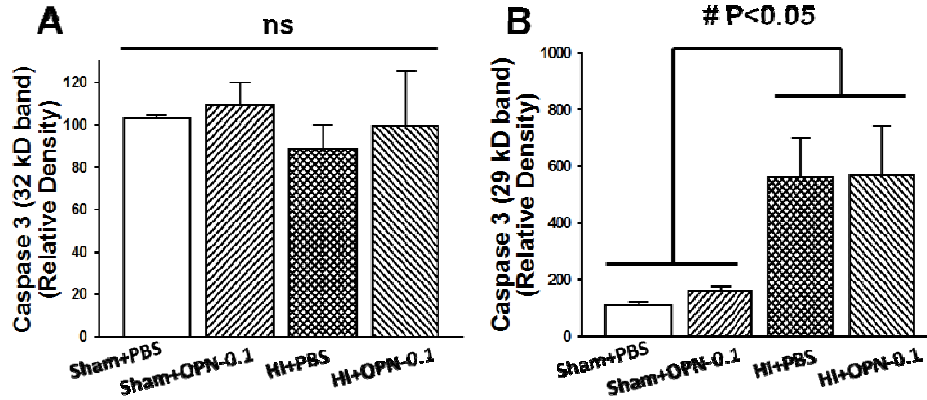
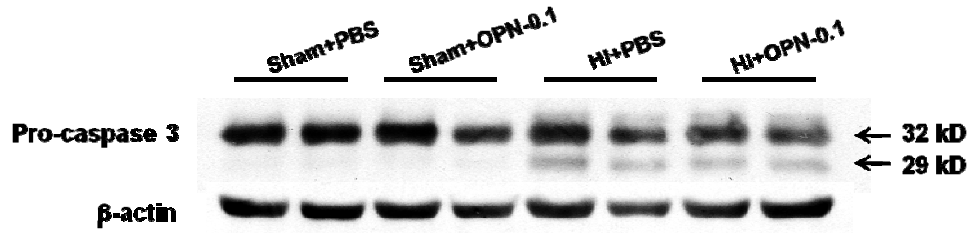
Brain Atrophy

Pups were euthanized and the brains were removed at 7 weeks post HI, after the Morris water maze test was done. The ipsilateral and contralateral hemisphere were separated by a midline incision and then weighed on a high precision balance (sensitivity $\pm 0.001\text{g}$). Brain atrophy was expressed as the mass ratio of the ipsilateral hemisphere compared to the contralateral hemisphere.

Supplemental Figures



Supplemental Figure S1. Brain atrophy at 7 weeks post HI. There was a significant tissue loss in the ipsilateral hemisphere in HI+PBS and HI+OPN-0.1 group ($\#P < 0.05$ vs. sham and sham+OPN-0.1). Brain atrophy in ipsilateral hemisphere was not attenuated after OPN treatment (ns = no significance). The data was expressed as the ratio of ipsilateral and contralateral hemisphere tissue mass. N = 18 animals for sham+PBS group. N = 18 animals for sham+OPN-0.1 group. N = 24 animals for HI+PBS group. N = 18 animals for HI+OPN-0.1 group. Vertical bars indicate SEM.



Supplemental Figure S2. OPN treatment didn't change the level of 32 kD pro-caspase-3 and 29 kD caspase-3 at 24 h post HI. (A) The level of pro-caspase-3 (32 kD) was not significantly different between all the groups (ns = no significance). (B) The level of 29 kD caspase-3 was significantly increased after HI injury ($\#P < 0.05$ vs. sham and sham+OPN-0.1). OPN treatment didn't significantly change the level of 29 kD caspase-3 expression compared with the HI+PBS group.