Supporting Information

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SI Text

RT-PCR. Total cellular RNA was purified from HeLa cells. The following primer sets were used for PCR (25 cycles); *OPN*, forward 5′-CGGGGTACCCCGATGGGCCGAGGTGATAGT-3′ and reverse 5′-CCCAAGCTTGGGATTGACCTCAGAAGA-3′; *GAPDH*, forward 5′-GGTGAAGGTCGGAGTCAACG-3′ and reverse 5′-TCACACCCATGACGAACATG-3′.

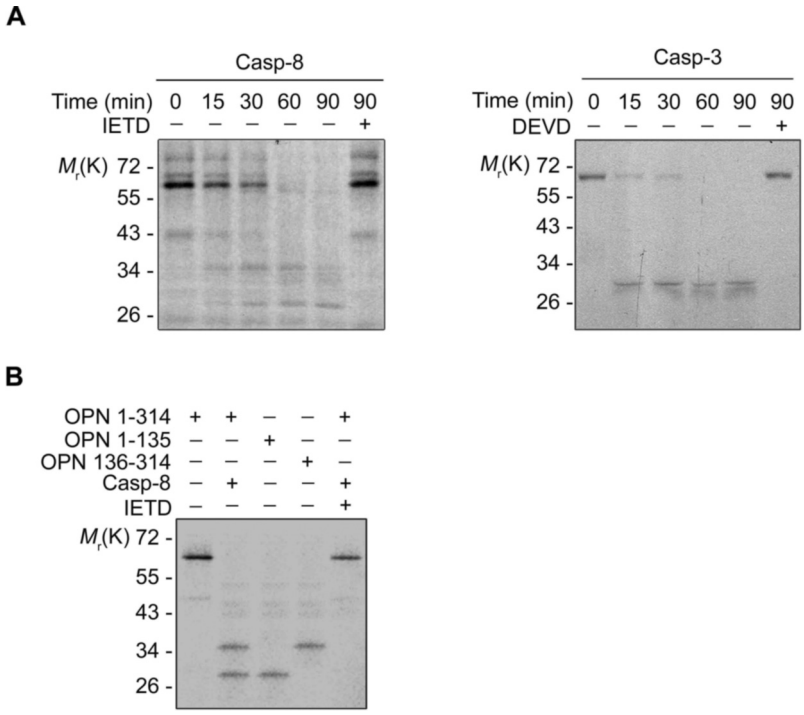

Fig. S1. Cleavage kinetics of OPN and mapping of cleavage site by caspases-8. (*A*) In vitro translated OPN was incubated with 10 ng of purified caspase-8 or -3 in the presence or absence of 25 μM IETD-fmk (caspase-8) and DEVD-fmk (caspase-3) for the indicated times. The reaction mixtures were then visualized by autoradiography. (*B*) OPN is cleaved at Asp-135 by caspase-8. OPN 1–314, OPN 1–135, and OPN 136–314 were left untreated or incubated with 10 ng of purified caspase-8 for 60 min in the presence or absence of 25 μM caspase inhibitor (IETD). Then, the reaction products were analyzed by autoradiography.

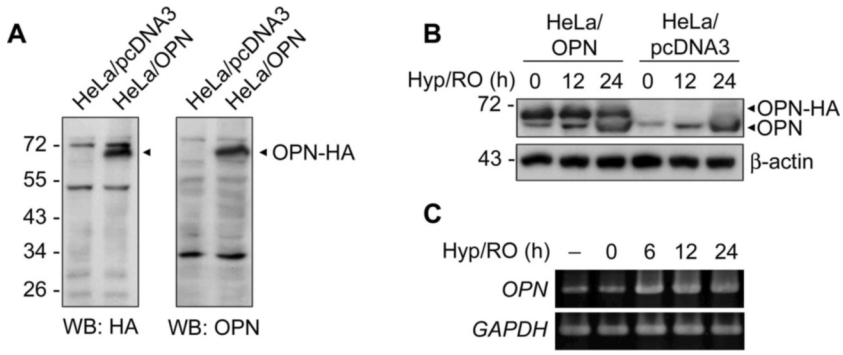

Fig. S2. Comparison of OPN induction during Hyp/RO. (*A*) HeLa cells stably expressing OPN-HA (HeLa/OPN) were generated and confirmed by Western blot analysis using both anti-HA and anti-OPN (LF123) antibodies. (*B*) Endogenous OPN is increased in a time-dependent manner during Hyp/RO. HeLa/pcDNA3 and HeLa/OPN cells were exposed to Hyp/RO for the indicated times. OPN level was detected using anti-OPN antibody. β-actin was used as an internal control. (*C*) OPN mRNA is increased during Hyp/RO. HeLa cells were exposed to Hyp/RO for the indicated times. Then, total RNAs were extracted and analyzed by RT-PCR.

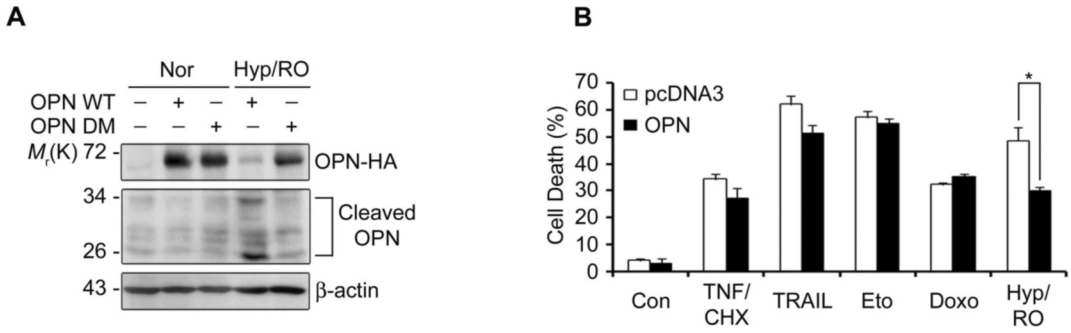

Fig. S3. Effects of OPN expression on various types of cell death. (*A*) Cleavage-defective OPN DM is resistant to degradation during Hyp/RO. SK Hep-1 cells were transfected with OPN WT or OPN DM expression vector for 24 h and then exposed to Hyp (12 h)/RO (30 h). Cell extracts were then examined with Western blot analysis using anti-HA or anti-OPN antibody. Cleaved forms of OPN are indicated and β-actin was used as an internal control. (*B*) Inhibitory effects of OPN expression on cell death induced by Hyp/RO. HeLa cells were transfected with GFP and either pcDNA3 or OPN expression vector for 24 h and then left untreated (Con) or exposed to various cell death stimuli, including 20 ng/mL TNF-α and 0.5 μg/mL cycloheximide (TNF/CHX) for 24 h, 100 ng/mL TRAIL for 6 h, 100 μM etoposide (Eto) for 24 h, 2 μg/mL doxorubicin (Doxo) for 24 h, or hypoxia for 12 h/reoxygenation for additional 18 h (Hyp/RO). Then, percentage of cell death was determined using EthD staining of GFP-positive cells. Bars, mean values $\pm$ SD; ($n = 3$). *, $P < 0.005$.

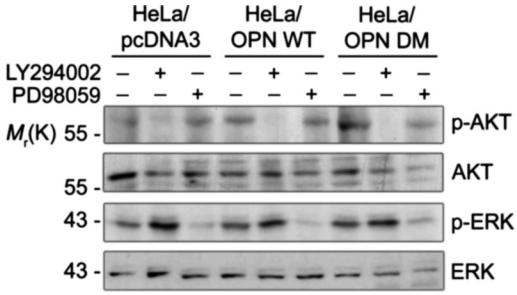

Fig. S4. Effects of LY294002 and PD98059 on the activation of AKT and ERK, respectively. HeLa/pcDNA3, HeLa/OPN WT, and HeLa/OPN DM cells were left untreated or exposed to Hyp (12 h)/RO (20 h) in the presence or absence of 40 μM LY294002 or 40 μM PD98059. Cell extracts were then prepared and examined with Western blot analysis using the indicated antibodies.

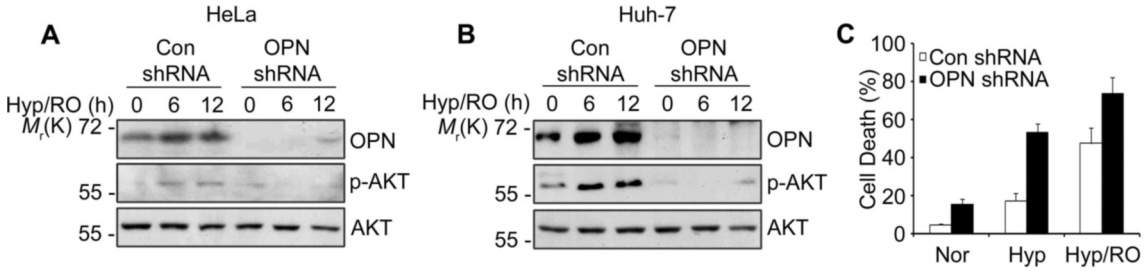

Fig. S5. Knockdown of OPN expression suppresses AKT activation but increases cell death in HeLa and Huh-7 cells. (*A* and *B*) AKT activation is inhibited by OPN shRNA during Hyp/RO. HeLa (*A*) and Huh-7 (*B*) cells were transfected with pSuper (Con shRNA) or pOPN shRNA for 48 h and then exposed to Hyp/RO for the indicated times. Cell extracts were analyzed with Western blotting using anti-OPN, anti-pAKT, and anti-AKT antibodies. (*C*) Huh-7 cells transfected with pSuper or pOPN shRNA were left untreated (Nor) or exposed to hypoxia for 24 h (Hyp) or additional reoxygenation for 18 h (Hyp/RO). Cell death assay was performed using EthD staining. Bars, mean values ± SD; ($n = 3$).

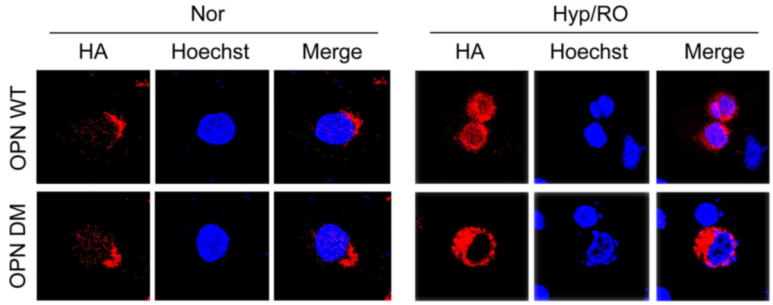

Fig. S6. Detection of OPN immunoreactivity in the nucleus during Hyp/RO. HeLa cells were transfected with OPN WT or OPN DM expression vector for 24 h and then left untreated (Nor) or exposed to Hyp (12 h)/RO (24 h). Cells were then immunostained using anti-HA antibody or Hoechst 33258 for the nucleus and then examined under a confocal microscope.

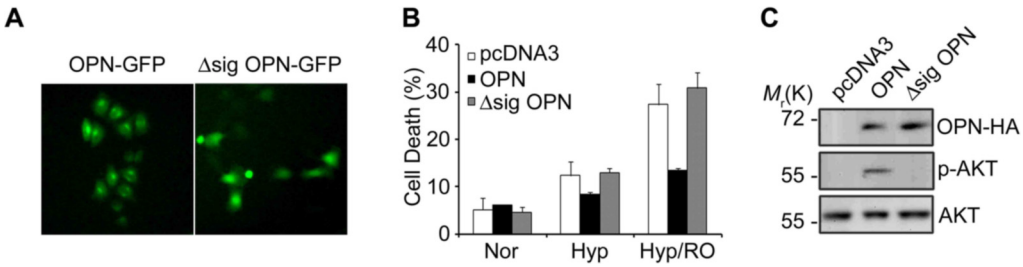

Fig. S7. OPN mutant lacking signal sequence loses anti-apoptotic function during Hyp/RO. (*A*) HeLa cells were transfected with pOPN-GFP or pΔsig OPN-GFP for 24 h and then observed under fluorescence microscope. (*B*) HeLa cells were transfected with pcDNA3, pOPN-HA, or pΔsig OPN-HA for 24 h, then left untreated (Nor) or exposed to hypoxia for 12 h (Hyp) or additional reoxygenation for 12 h (Hyp/RO), and cell death assay was performed as in *Materials and Methods*. (*C*) HeLa cells were transfected with pcDNA3-HA, pOPN-HA, or pΔsig OPN-HA for 36 h and cell extracts were analyzed by Western blot using anti-pAKT, anti-AKT, or anti-HA antibody.