

Supplementary Figure Legends

Suppl. Fig. 1. LCN2 sensitizes astrocytes to both apoptosis and necrosis. Primary astrocyte cultures were exposed to SNP (0.5 mM), H₂O₂ (1 mM) or paraquat (PQ; 100 µM) with or without LCN2 proteins (10 µg/ml) for 24 hr, the nature of cell death was determined by PI and annexin V staining (**A**). Percentage of necrotic or apoptotic cell death was based on flow cytometric analysis (**B**).

Suppl. Fig. 2. The dose- and time-dependent effect of recombinant LCN2 protein on astrocyte morphology. Primary astrocytes were treated with different concentrations of recombinant LCN2 protein for 24 hr (**A**). Alternatively, primary astrocytes were treated with recombinant LCN2 protein (10 µg/ml) for different time periods (**B**). Cells were stained for GFAP (magnification, X 400) (**A, B**). Length of the longest astrocyte processes for each treatment was similarly measured (**C, D**). * $p < 0.05$; compared with untreated control.

Suppl. Fig. 3. Morphological changes of astrocytes following treatment with LCN2, LPS/IFN-γ, or gangliosides. Primary astrocyte cultures were exposed to LCN2 (10 µg/ml), a combination of LPS (100 ng/ml) and IFN-γ (50 units/ml), or ganglioside mixtures (Gmix; 50 µg/ml) for 24 hr, and cell morphology was examined by GFAP immunofluorescence analysis

(GFAP, green; Hoechst 33342, blue) (magnification, X 400).

Suppl. Fig. 4. The effect of recombinant LCN2 protein on GFAP expression. Primary astrocytes were treated with the indicated concentrations of LCN2 protein for the indicated time periods, and then GFAP mRNA levels were evaluated by RT-PCR (**A, B**). Results of densitometric analysis are also shown (**A, B; lower**). β -actin detection was done to confirm the equal loading of the samples.

Suppl. Fig. 5. Induction of reactive astrocyte morphology by NO donor SNP.

Treatment of primary astrocytes with 0.5 mM of SNP for 24 hr induced morphological changes similar to those of LCN2-treated cells. GFAP immunofluorescence analysis (magnification, X 400).

Suppl. Fig. 6. Inhibition of Rho-ROCK, but not Rac, abrogated LCN2-induced

morphological changes in astrocytes. A ROCK inhibitor Y27632 (100 μ M), but not Rac1 inhibitor NSC23766 (100 μ M), suppressed LCN2 (10 μ g/ml)-induced morphological changes as assessed by GFAP immunofluorescence analysis (GFAP, green; Hoechst 33342, blue) after 24 hr. Rac1 inhibition alone induced slight changes in astrocyte processes, suggesting that Rho and Rac play opposing roles in determining astrocyte morphology (magnification, X 400).

Suppl. Fig. 7. Stimulation of astrocyte migration by LCN2 protein. Primary astrocytes were treated with recombinant LCN2 protein (10 µg/ml), forskolin (100 nM), or dbcGMP (1 mM) for the indicated time periods, and wound healing assay (**A**) or Boyden chamber assay (**B**) was performed to evaluate cell migration (magnification, X 100).

Suppl. Fig. 8. No significant effect of LCN2 on phagocytic activity of astrocytes.

Primary astrocytes were treated with LCN2 (10 µg/ml) for 24 hr, and then incubated with fluorescence-labeled zymosan particles (20 µg/ml) for 1 hr in order to assess phagocytic functions. Uptake of zymosan particle was evaluated by fluorescence microscopy (**A**) or flow cytometric analysis (**B**). Alternatively, astrocytes were similarly incubated with zymosan particles for different time periods in order to assess phagocytic activities (**C**). Based on the results, 1 hr was selected to determine the effect of LCN2 on astrocyte phagocytic activities (magnification, X 200).