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Supplemental Data

MicroRNA-15a/16 Regulates Apoptosis of Lung Epithelial Cells After Oxidative Stress

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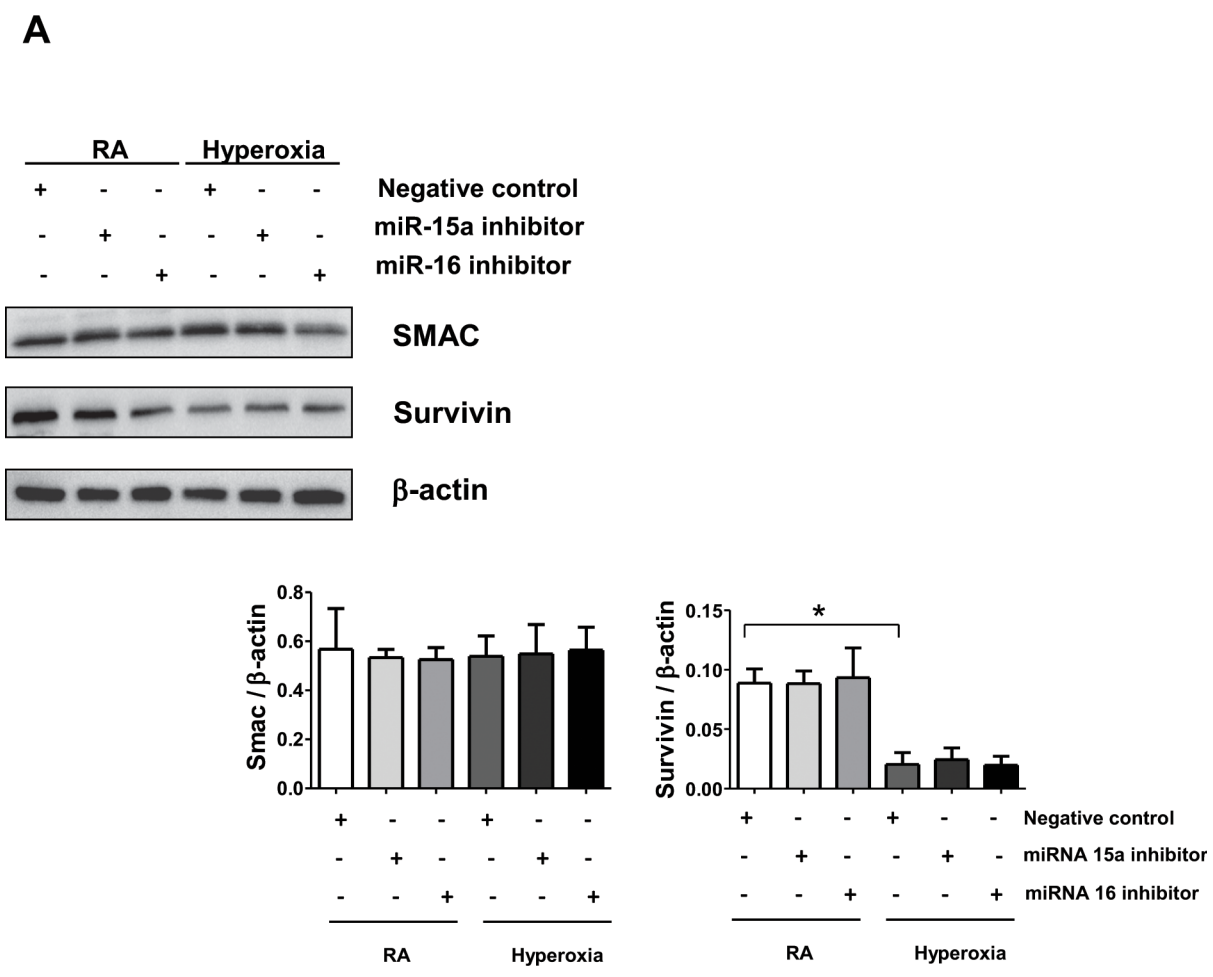

Supplementary Figure S1. Effects of miR-15a/16 inhibitors on the level of apoptosis- and necroptosis-associated proteins. Beas2B cells were transfected with miR-15a/16 inhibitors or negative controls. After hyperoxia (1 d), apoptosis or necroptosis-associated proteins were detected using Western Blot analysis. Statistical analysis was shown on the right. Figures represent three independent experiments with the similar results. *$p < 0.05$.

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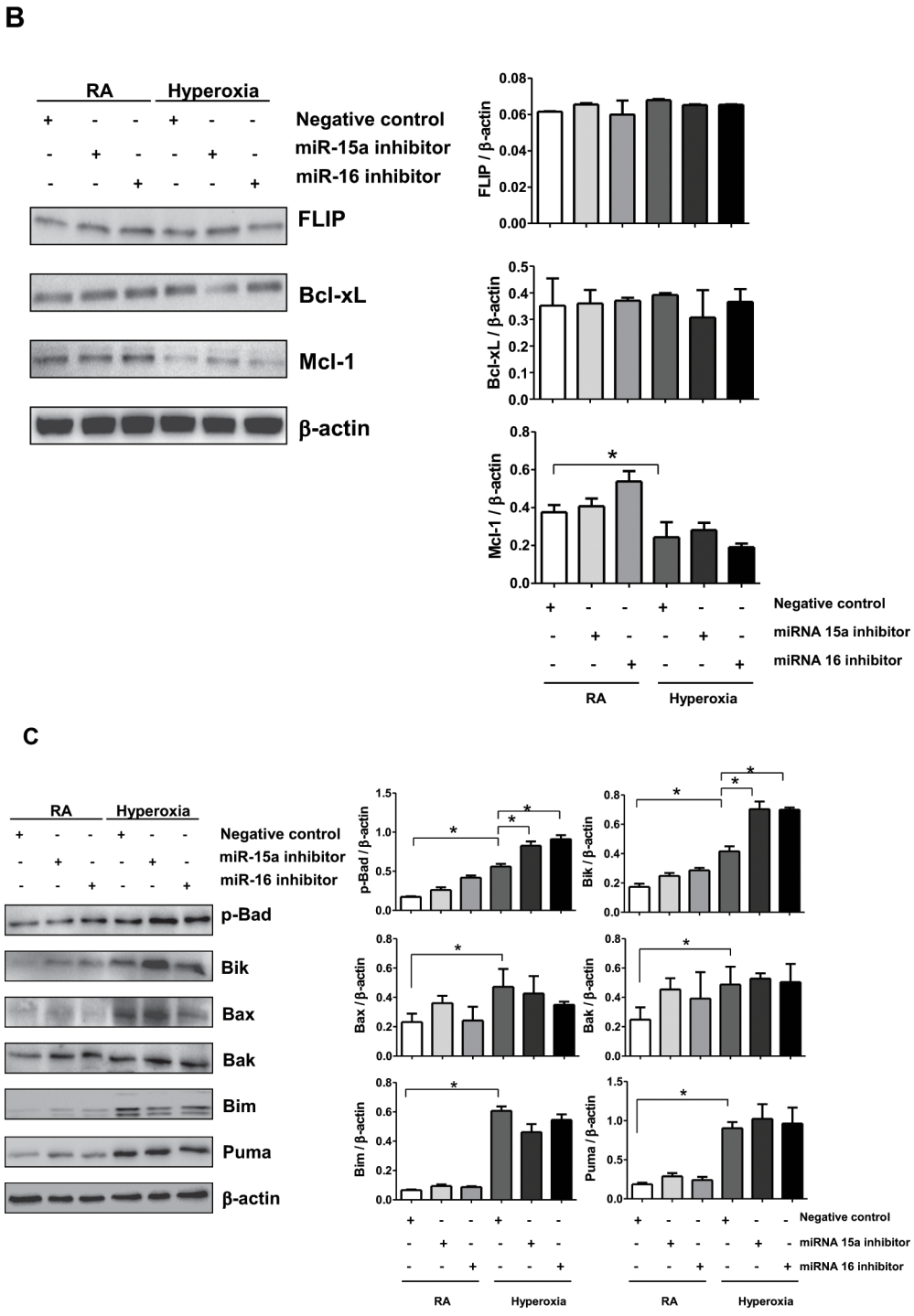
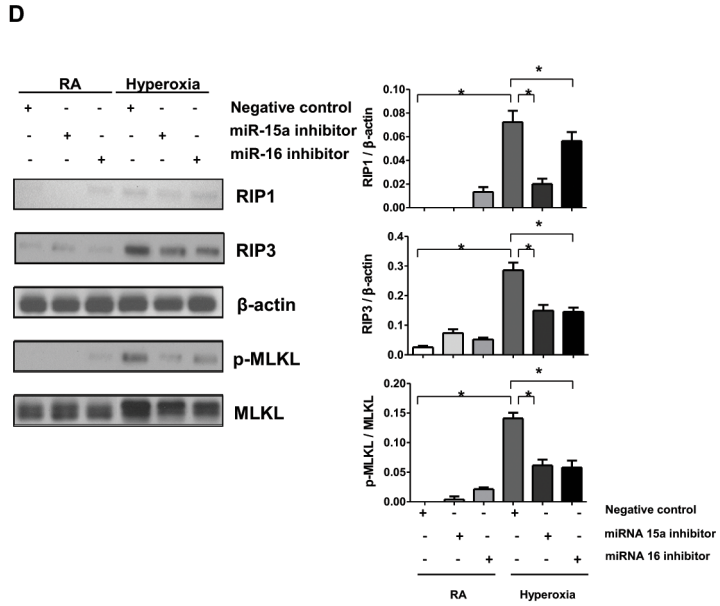
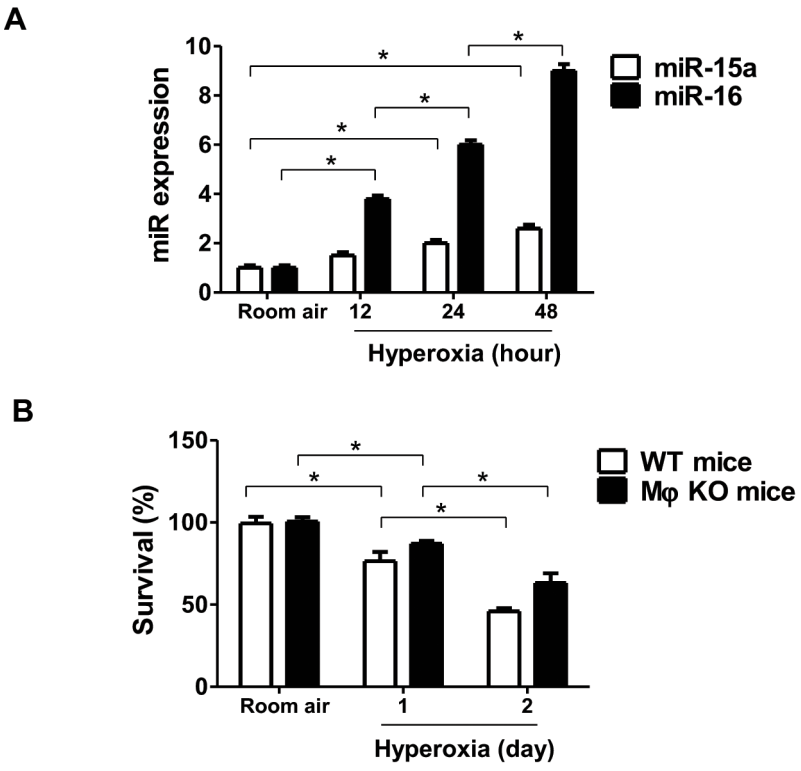


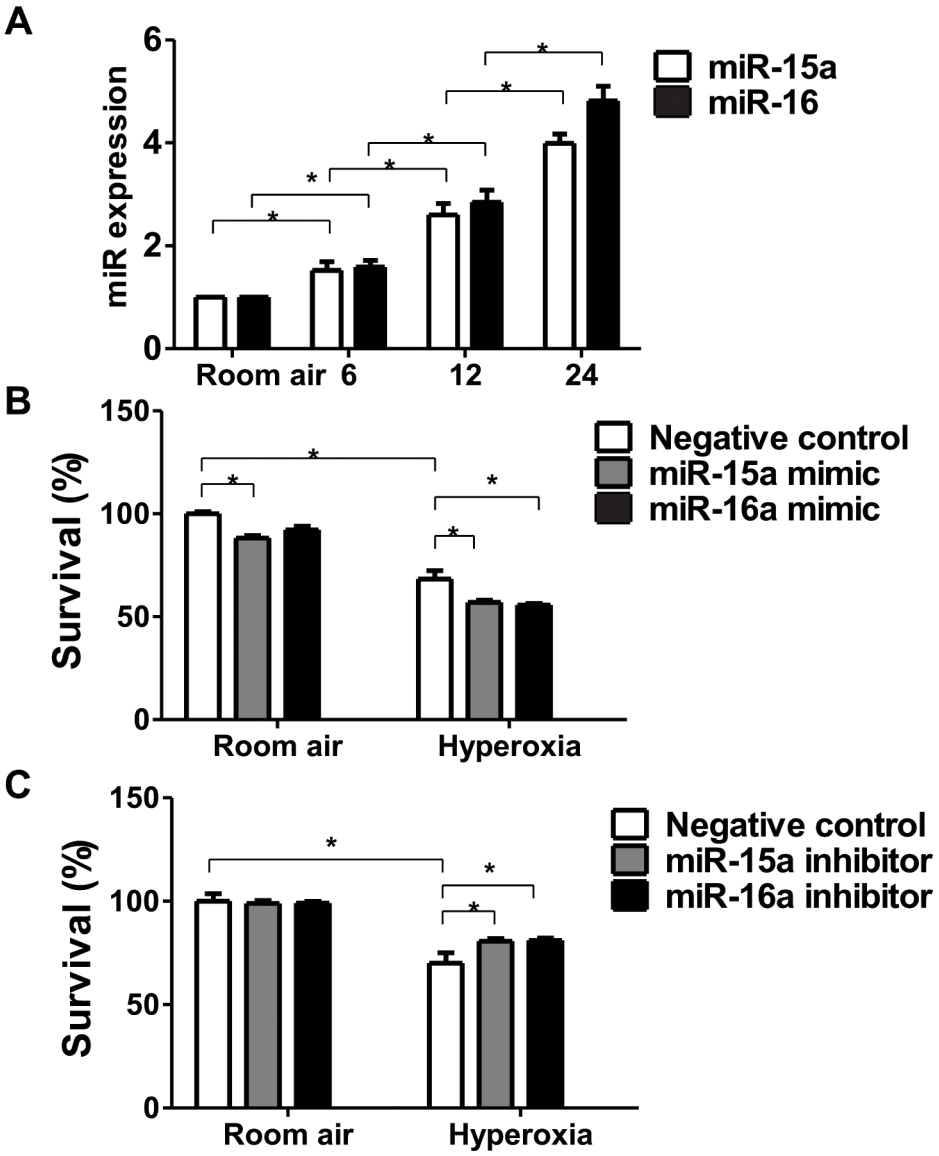
Supplementary Figure S1. *Continued.*



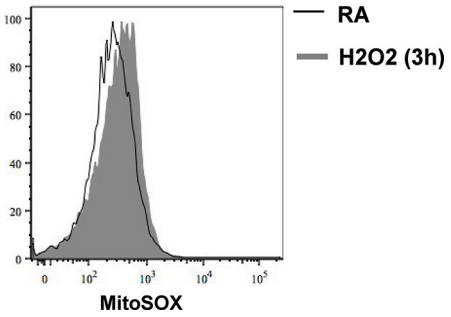
Supplementary Figure S1. *Continued.*



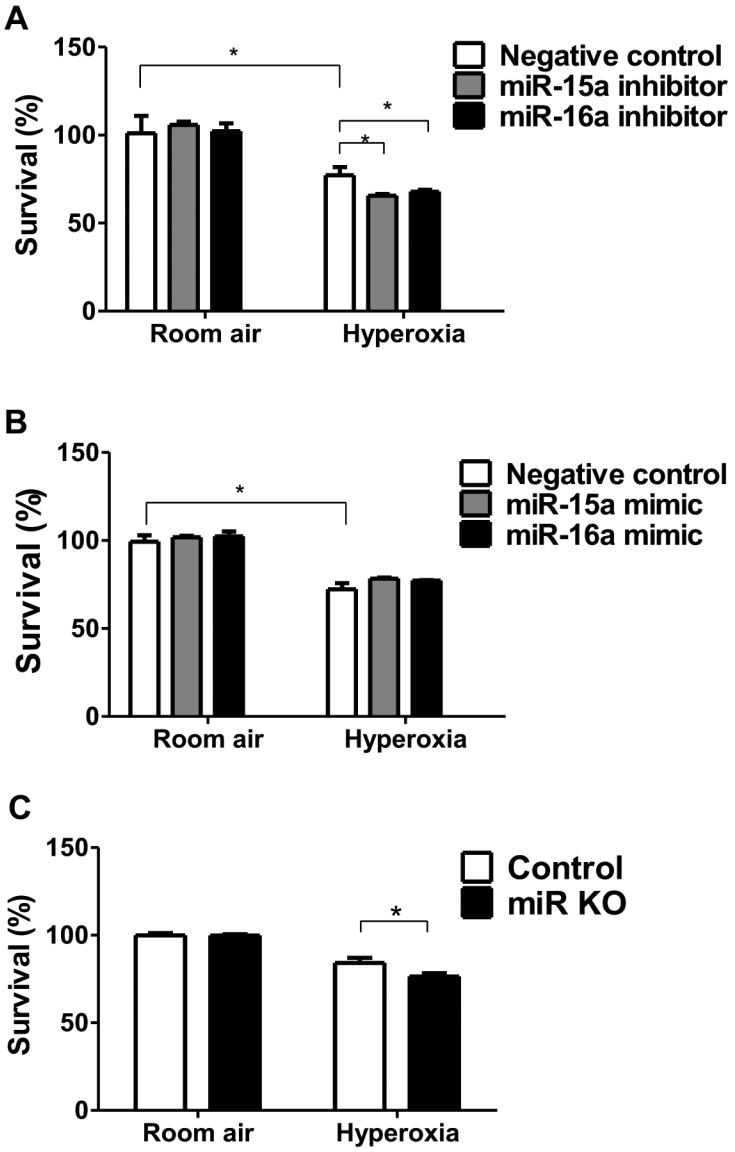
Supplementary Figure S2. Effects of miR-15a/16 on macrophages in hyperoxia. (A) Hyperoxia promoted miR-15a/16 level in macrophages. THP1 macrophages were exposed to hyperoxia in a time course. MiR-15a/16 level was analyzed using realtime PCR. Figures represent three independent experiments with the similar results. *$p < 0.05$. (B) Deletion of miR-15a/16 improved macrophage survival in the presence of hyperoxia. Bone marrow-derived macrophages were isolated from control or mononuclear cell-specific Cre-miR-15a/16$^{-/-}$ mice. After exposed to hyperoxia, cell survival was determined. Figures represent three independent experiments with the similar results. *$p < 0.05$.



Supplementary Figure S3. Effects of miR-15a/16 on A549 lung tumor cells in hyperoxia. (A) Hyperoxia promoted miR-15a/16 level in A549 cells. A549 cells were exposed to hyperoxia in a time course. MiR-15a/16 level was analyzed using realtime PCR. Figures represent two independent experiments with the similar results. *$p < 0.05$. (B, C) Effects of miR-15a/16 on A549 cell survival in the presence of hyperoxia. A549 cells were transfected with miR-15a/16 mimics (B) or miR-15a/16 inhibitors (C), along with their matching controls. Cells were then exposed to hyperoxia, cell survival was determined. Figures represent two independent experiments with the similar results. *$p < 0.05$.



Supplementary Figure S4. H_2O_2 induced Beas2B cells to produce ROS. Beas2B cells were exposed to control or H_2O_2 (100 nmol/L). After 3 h incubation, ROS was detected using MitoSOX and FACS.



Supplementary Figure S5. Effects of miR-15a/16 on the survival of lung epithelial cells in the presence of hyperoxia. (A, B) Beas2B cells were transfected with miR-15a/16 inhibitors or mimics, and exposed to RA or hyperoxia (95% oxygen). After 24 h, cell viability was determined. Figures represent three independent experiments with the similar results. *$p < 0.05$. (C) Alveolar epithelial cells (AEC) were isolated from hybrid mice (mice after cross-breeding C57BL/6J WT mice with CC10 Cre mice serve as control; mice after cross-breeding miR15a/16$^{-/-}$ loxP mice with CC10 Cre mice serve as lung epithelial cell-specific miR-15a/16$^{-/-}$ mice). AEC were cultured for 6 d and then were incubated in room air (21% oxygen) or hyperoxia (95% oxygen) for 24 h. Next, cell viability was analyzed. Figures represent three independent experiments with the similar results. *$p < 0.05$.