

Lipopolysaccharide-induced Murine Lung Injury Results in Long-term Pulmonary Changes and Downregulation of Angiogenic Pathways

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Supplementary Figure Legends and Files

Supplementary Figure Legends

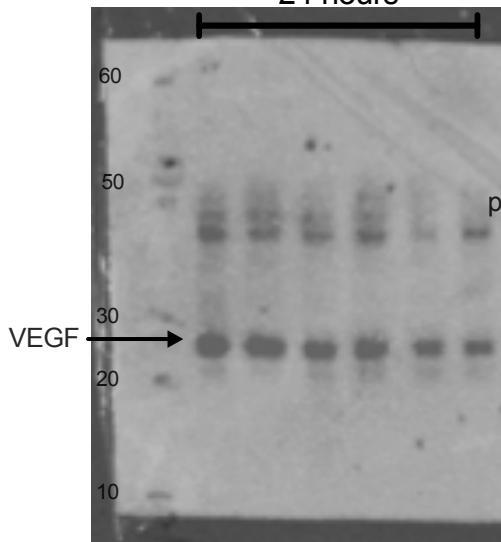
Supplemental Figure 1. Percent change in weight from baseline at the various time points. Lipopolysaccharide (LPS)-treated mice had significantly greater weight loss compared to control mice at 24 hours (**A**), 4 days (**B**), and 7 days (**C**) after instillation. Both groups of mice had similar changes in weight at the 4 week (**D**) time point. Statistical analysis of the experimental groups at each time-point was performed with Student's t-test. Results are expressed as mean $\pm$ SE. *** $P < 0.001$; **** $P < 0.0001$.

Supplemental Figure 2. Bronchoalveolar fluid (BALF) differential cell counts. Lipopolysaccharide (LPS)-treated mice had significantly increased amounts of neutrophils compared to controls at 4 days after instillation (**A**). Both groups had a similar number of neutrophils, monocytes, and lymphocytes at the 4 week time point (**B**). Neutrophils were the predominant cell type in LPS-treated mice at 4 days while monocytes became predominant at 4 weeks (**C, D**). Monocytes were the predominant cell type in control mice at both time points (**C, D**). Total and differential cell counts of BALF were determined using the May-Grunwald-Giemsa stain (300 cells per animal). Each count was performed in biological duplicate. Statistical analysis was done using a two-way analysis of variance (ANOVA) model with interaction for group and cell type with Sidak adjustment for multiple comparisons (**C, D**). Results are expressed as mean $\pm$ SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

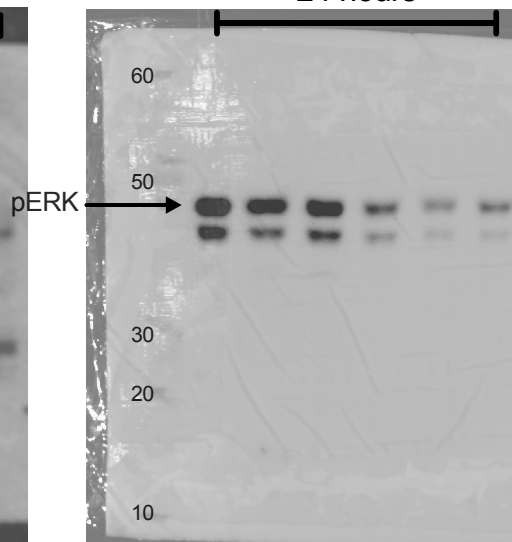
Supplemental Figure 3. Immunohistochemistry of lung tissue. Representative micrographs at 200x magnification (**A**) of co-stained lung tissue for type I collagen (COL1A1; green) and nuclear marker DAPI (blue) demonstrate similar amounts of COL1A1 in control (CTL) and lipopolysaccharide (LPS)-treated mice at the various time points (**B**). However, COL1A1 appears to redistribute from the alveoli to the nonparenchymal tissue in LPS-treated mice at 4 weeks. Slides co-stained for type 4 collagen (COL4A1; red) and nuclear marker DAPI (blue) demonstrate lower amount of COL4A1 in LPS-treated mice compared to controls at 4 weeks based on representative slides (**A**) and quantification (**C**). Statistical analysis of the experimental groups at each time point was performed with Student's t-test. Results are expressed as mean $\pm$ SE. * $P < 0.05$.

Supplemental File 1. Full length blots correspond to part of Figures 5A, 5C, 5E:

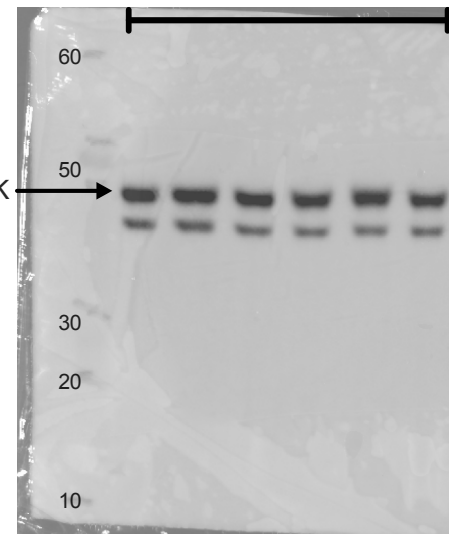
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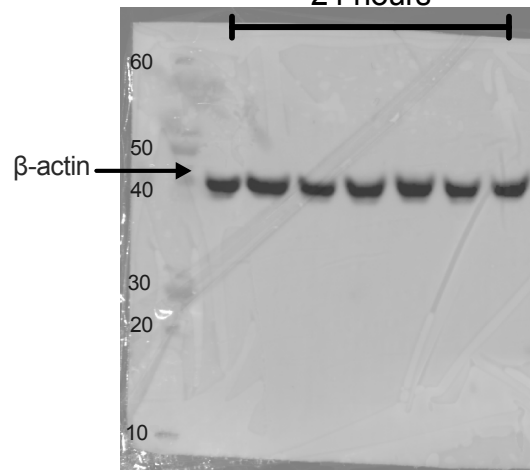
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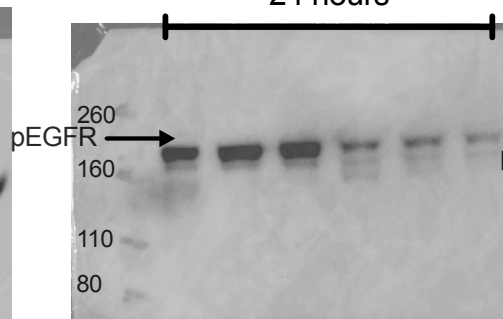
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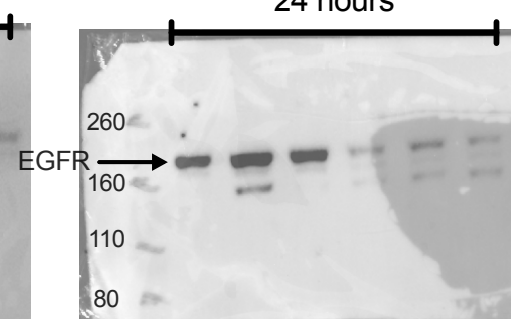
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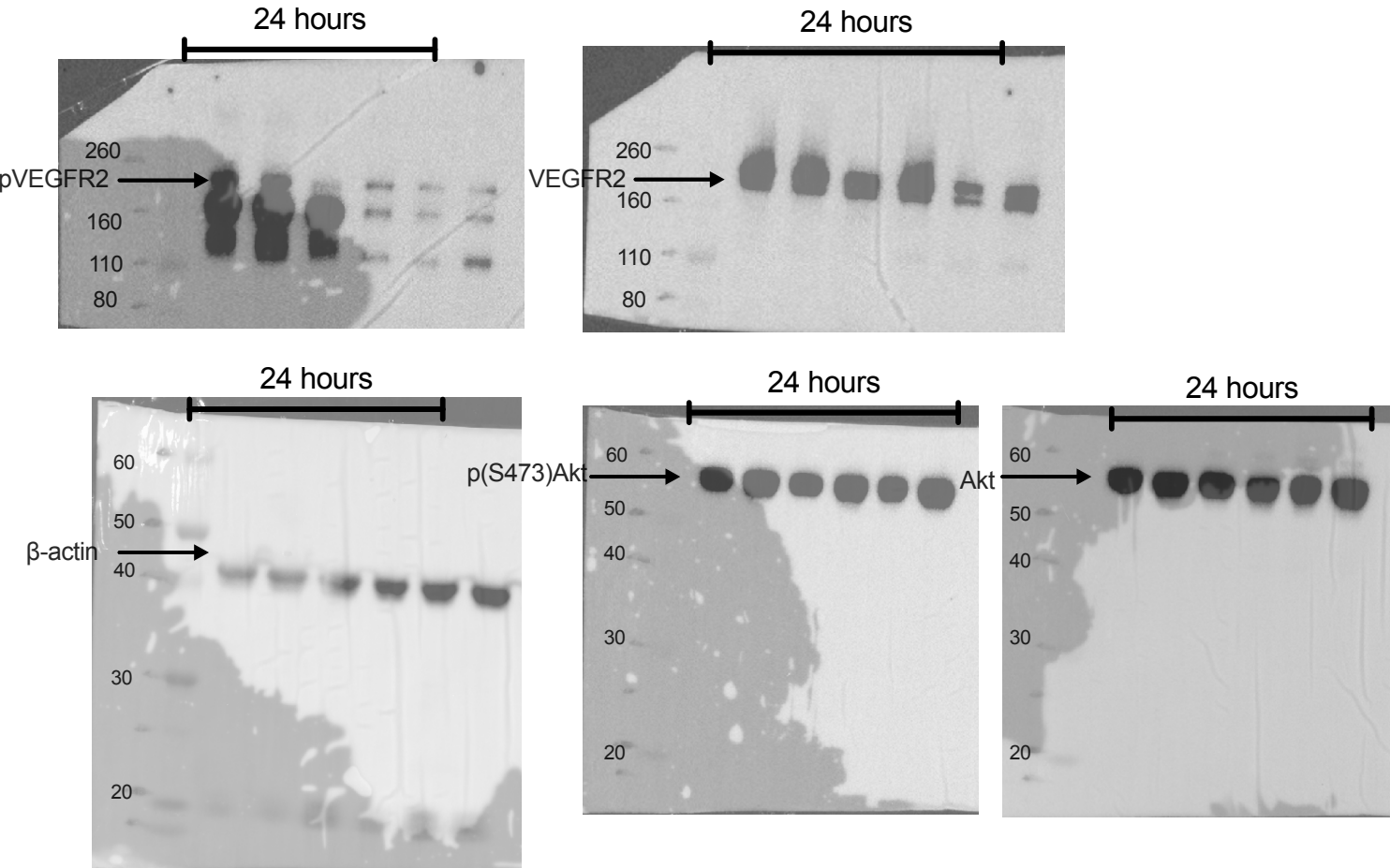
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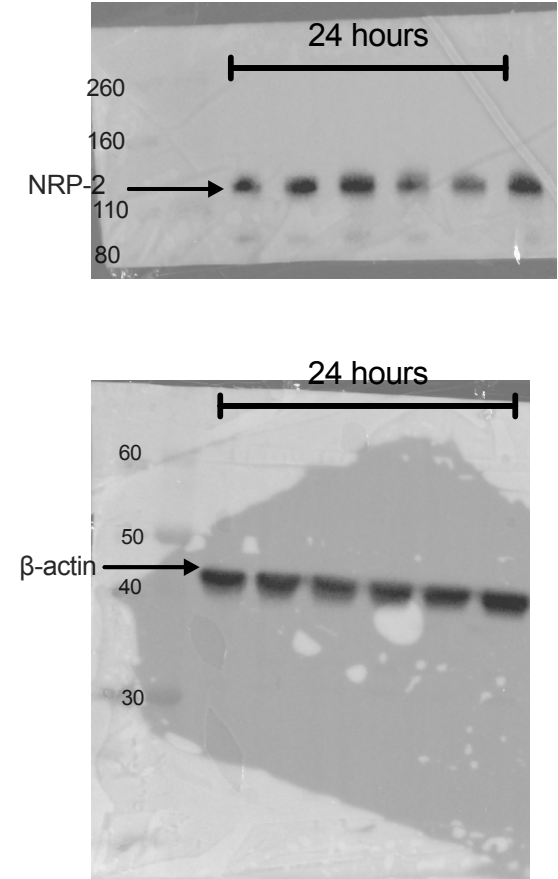
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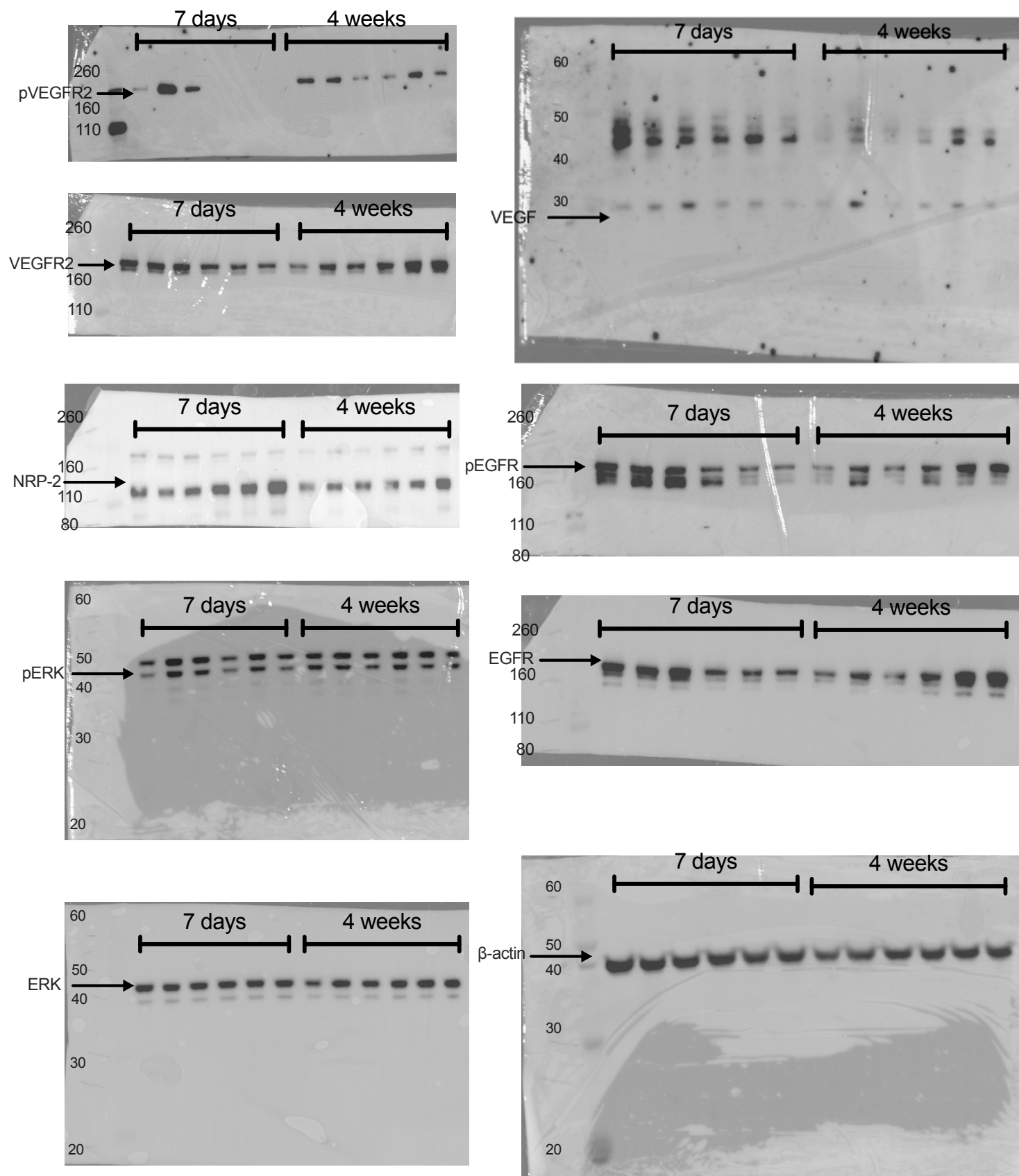
Supplemental File 2A. Full length blots correspond to part of Figure 5A:



Supplemental File 2B. Full length blots correspond to part of Figure 5B:



Supplemental File 3. Full length blots correspond to parts of Figure 5A, 5B, 5C, 5E:



Supplemental File 4. Full length blots correspond to parts of Figure 5D:

