Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/8/391/ra86/DC1

Supplementary Materials for

Extracellular signal–regulated kinase 5 promotes acute cellular and systemic inflammation

Kevin Wilhelmsen,* Fengyun Xu, Katherine Farrar, Alphonso Tran, Samira Khakpour, Shirin Sundar, Arun Prakash, Jinhua Wang, Nathanael S. Gray, Judith Hellman

*Corresponding author. E-mail: wilhelmsenk@anesthesia.ucsf.edu

Published 25 August 2015, *Sci. Signal.* **8**, ra86 (2015)
DOI: 10.1126/scisignal.aaa3206

The PDF file includes:

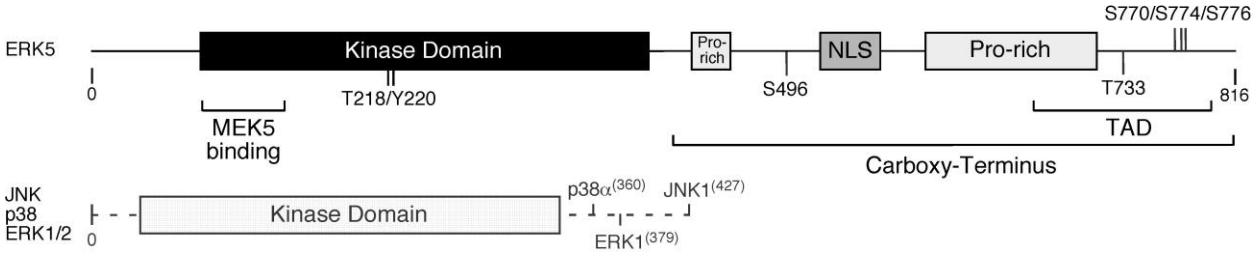

Fig S1. Domain organization of ERK5. ERK5 differs from the other MAPKs (including JNK1, p38α, and ERK1) because of its larger size and its distinct C-terminal domain, which contains proline-rich regions, a nuclear localization signal (NLS), and a transcriptional activation domain (TAD) that is regulated by autophosphorylation (*38-40*, *54*). The locations of the identified serine (S), threonine (T), and tyrosine (Y) phosphorylation sites are shown. All of the MAPK proteins depicted are human in origin.

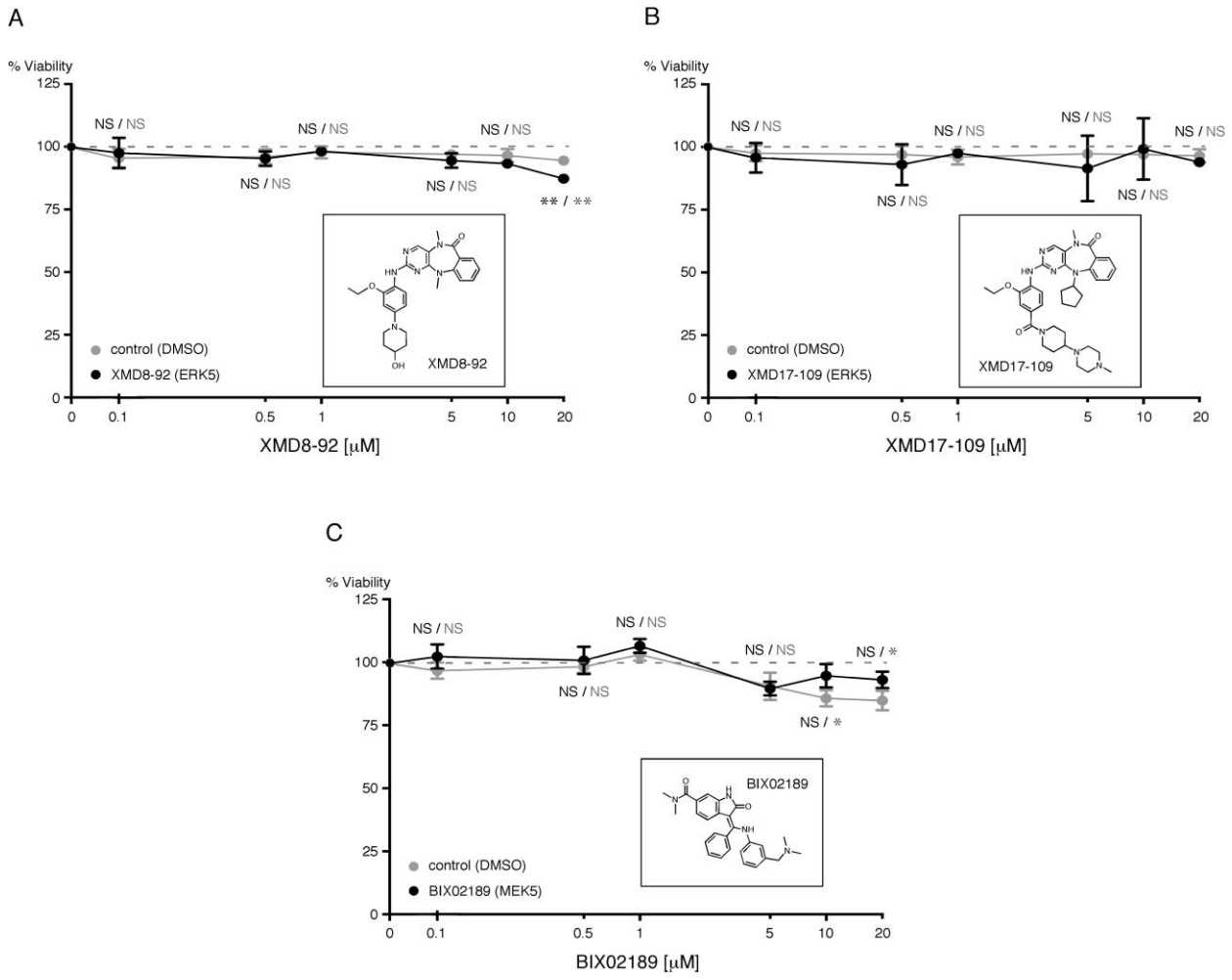

Fig S2. XMD8-92, XMD17-109, and BIX02189 have no substantial effects on cell viability. (**A** to **C**) Confluent monolayers of HMVEC-lung cells grown in 96-well plates were incubated with DMSO (vehicle control) or the indicated concentrations of (A) XMD8-92, (B) XMD17-109, or (C) BIX02189 for 7 hours. MTT assays were then performed to determine cell viability. NS, not significant; $*P < 0.05$ and $**P < 0.01$ when comparing untreated HMVEC-lung cells with inhibitor- or DMSO-treated cells. Data are means ± SD of four sample wells per group and are representative of two independent experiments. Insets: chemical formulas of the inhibitors.

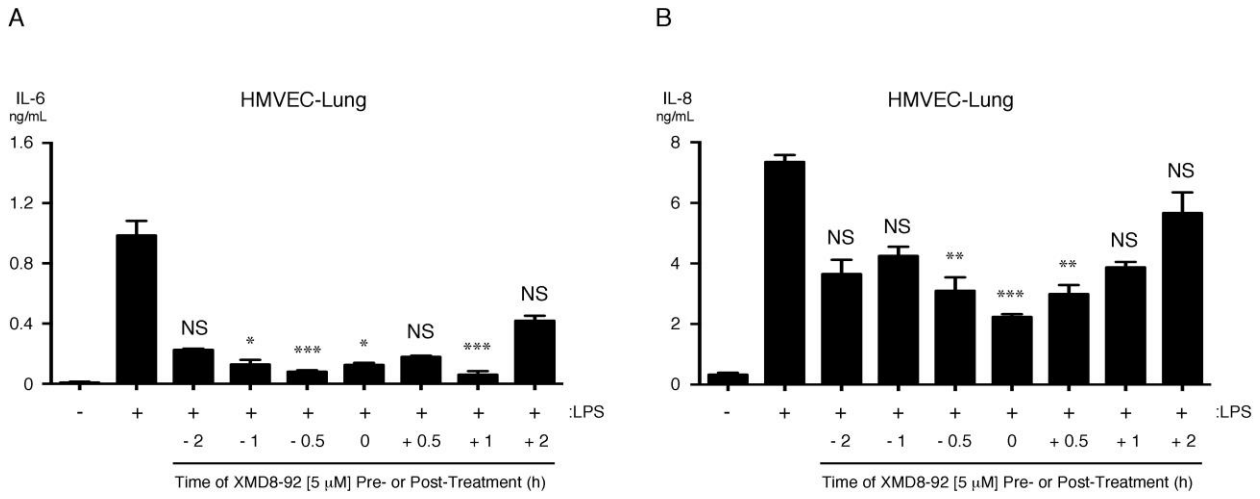

Fig S3. The ERK5 inhibitor XMD8-92 reduces the amounts of proinflammatory cytokines secreted by HMVEC-lung cells when it is added before or after they are treated with LPS. (**A** and **B**) HMVEC-lung cells were treated with 5 μM XMD8-92 for up to 2 hours before or 2 hours after the addition of LPS (10 μg/ml), which was added for exactly 6 hours. Once XMD8-92 was added, it was continuously present until the cell culture medium was collected. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 when comparing between cells treated with inflammatory stimulus in the absence or presence of inhibitor. Data are means ± SD of four sample wells per group and are representative of two independent experiments.

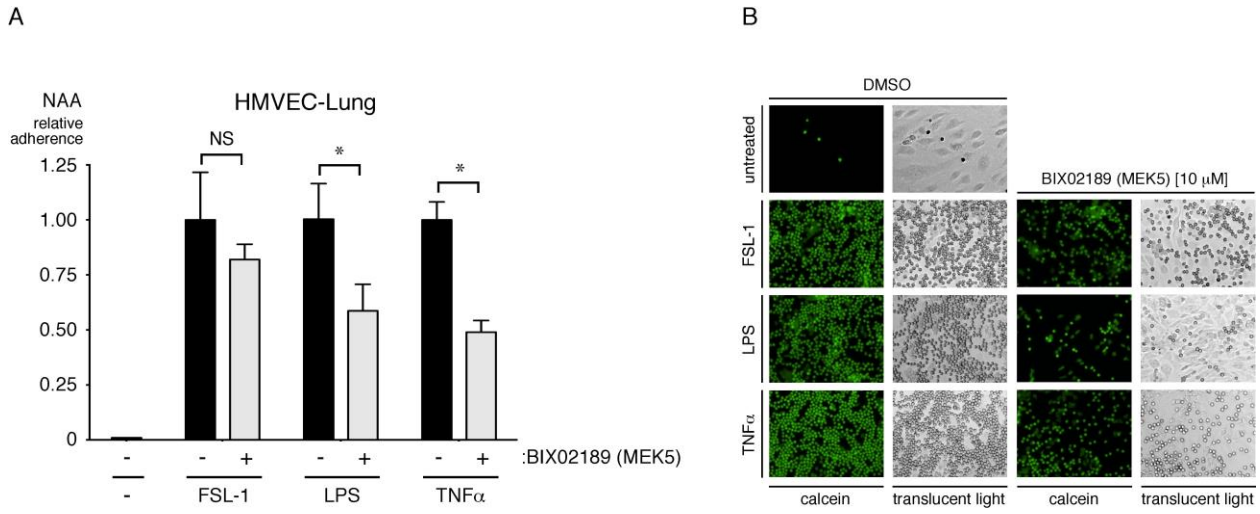

Fig S4. MEK5 promotes the binding of neutrophils to activated ECs. (**A** and **B**) HMVEC-lung cells were pretreated for 1 hour with DMSO or 10 μM BIX02189 before being treated with FSL-1 (10 μg/ml), LPS (10 μg/ml), or TNF-α (100 ng/ml) for an additional 3 hours while in the continuous presence of DMSO or BIX02189. The cells were then washed, and calcein AM–labeled primary human neutrophils were allowed to adhere to the HMVEC-lung cells for 20 min before any non-adherent neutrophils were removed. (A) The numbers of remaining neutrophils were counted and their relative adherence was then calculated. NS, not significant, $*P < 0.05$ when comparing between cells treated with inflammatory stimulus in the presence or absence of BIX02189. Data are means ± SD of four sample wells per group and are representative of two independent experiments. (B) Representative images of calcein AM–labeled neutrophils bound to HMVEC-lung cells. Fluorescence images taken with a fluorescein filter set are shown in the left columns of each group, whereas translucent light microscopy images of the same field of reference are shown in the right hand column. Magnification: 10×.

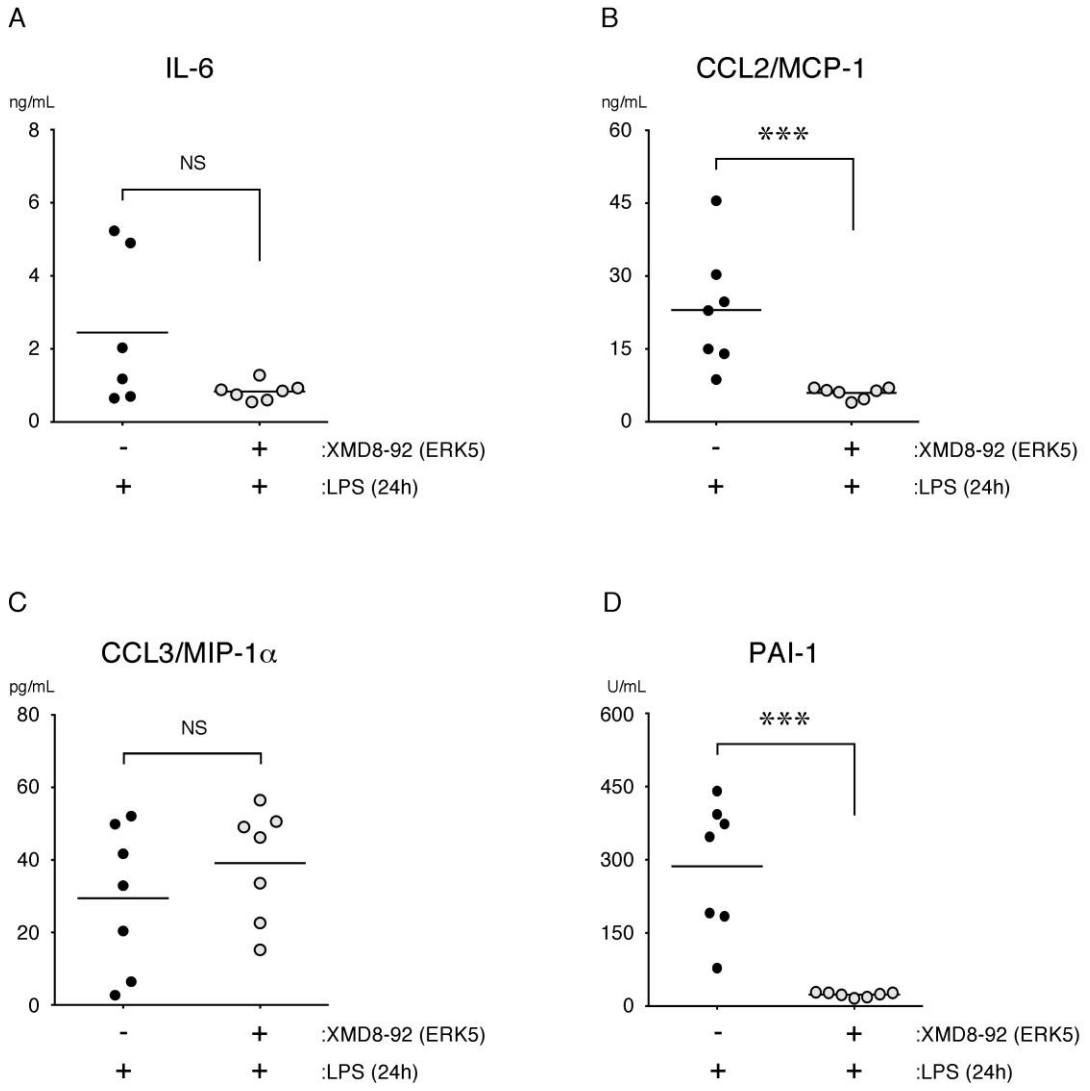

Fig S5. ERK5 promotes the secretion of CCL2 and enhances PAI-1 activity in mice 24 hours after they are challenged with LPS. (**A** to **D**) WT mice were treated IP with XMD8-92 (50 mg/kg) or vehicle 30 min before they were injected IV with LPS (10 mg/kg) or vehicle. The plasma concentrations of IL-6, CCL2, and CCL3 and the activity of PAI-1 were quantified 24 hours after challenge. NS, not significant, ***$P < 0.001$ when comparing between mice treated with LPS in the presence or absence of XMD8-92. Data are means ± SD of four mice per group and are representative of two independent experiments.