Leukotriene B_4-induced reduction of SOCS1 is required for murine macrophage MyD88 expression and NFκB activation

Carlos H. Serezani[1,3], Casey Lewis[1], Sonia Jancar[2] and Marc Peters-Golden[1,3]

[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Health System, Ann Arbor, MI 48109 USA. [2]Department of Immunology, Institute of Biomedical Science IV, University of São Paulo, São Paulo, Brazil

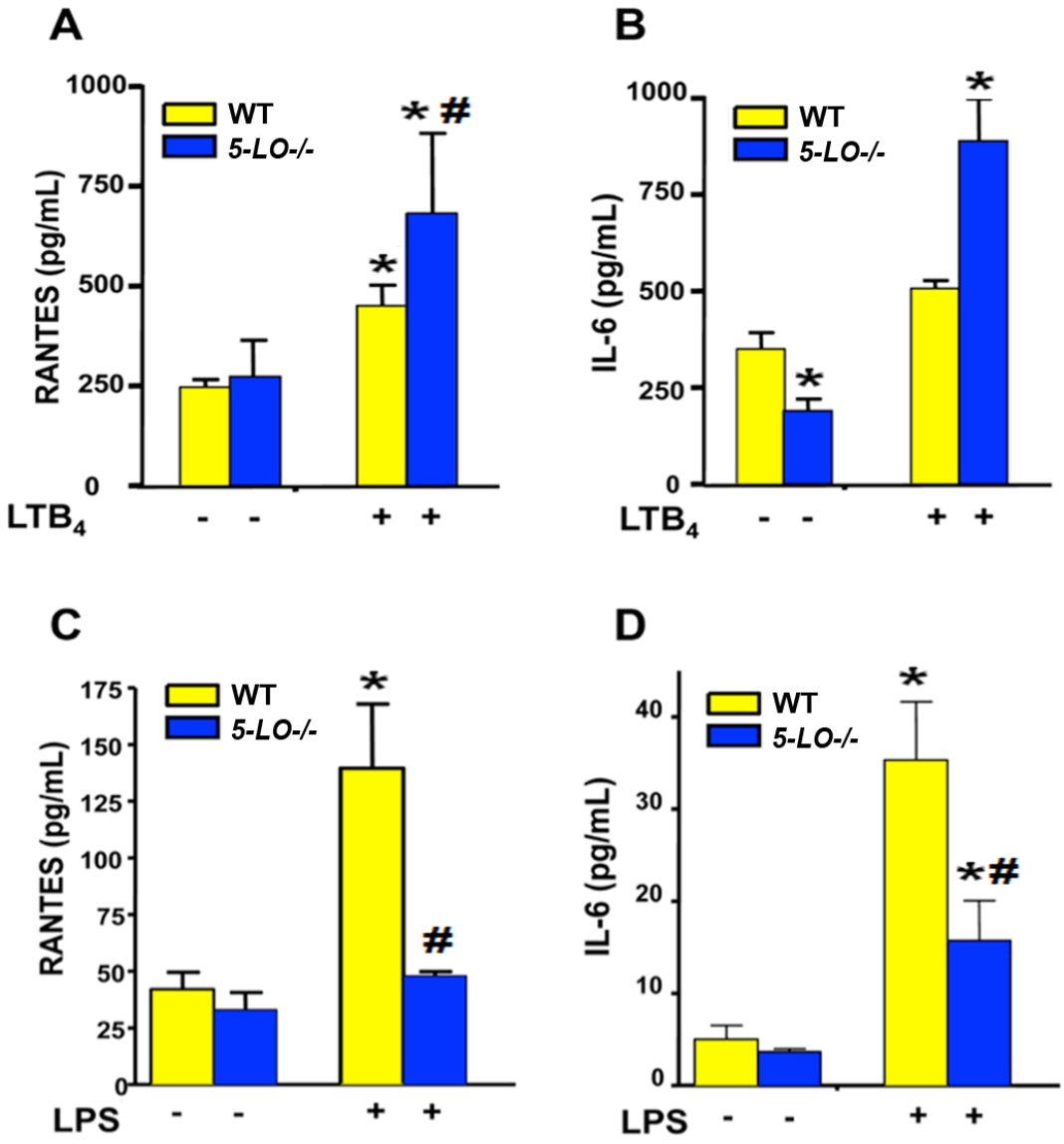
[3]Address correspondence to: Marc Peters-Golden, M.D., 6301 MSRB III, 1150 W. Medical Center Drive, University of Michigan Health System, Ann Arbor, MI 48109-5642. E-mail: petersm@umich.edu; Phone: 734-763-9077; Fax: 734-764-4556. Or to,

Carlos H. Serezani, Ph. D., 5501B MSRB I 1150 W. Medical Center Drive, University of Michigan Health System, Ann Arbor, MI 48109-5642. E-mail: cserezan@med.umich.edu; Phone: 734-615-5523; Fax: 734-764-4556.

Conflict of interest: Research support exceeding $50,000 was provided by Nycomed to MP-G in the year prior to submission of this manuscript.

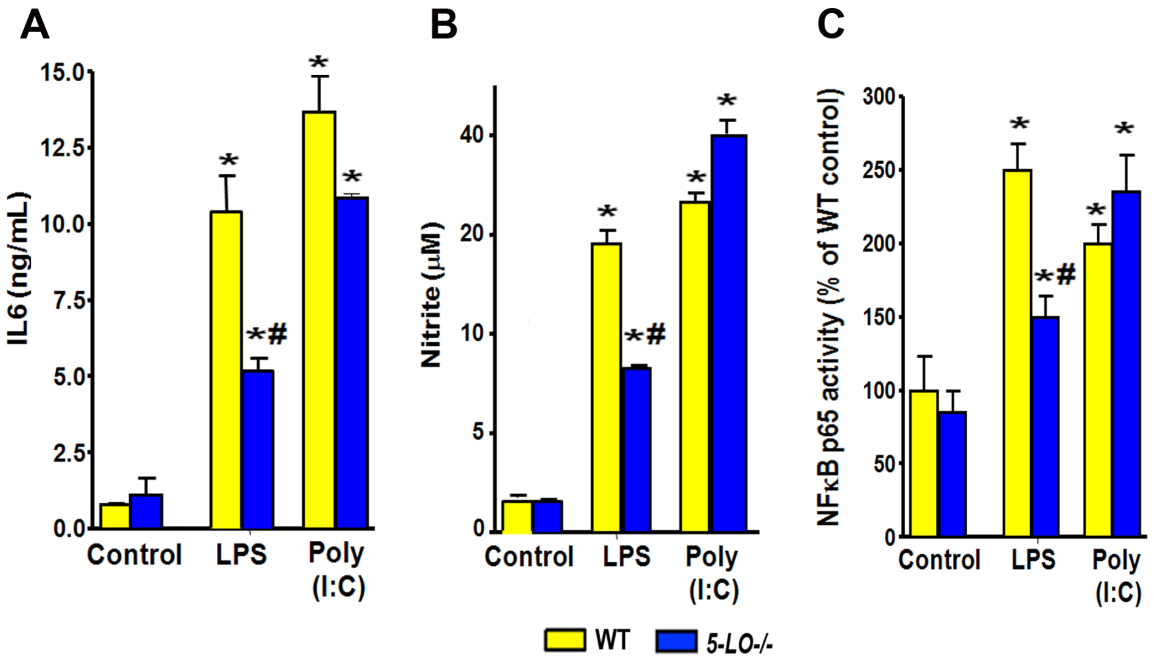
Running title: LTB_4 increases MyD88 expression and NFκB activation

Supplemental Figure 1



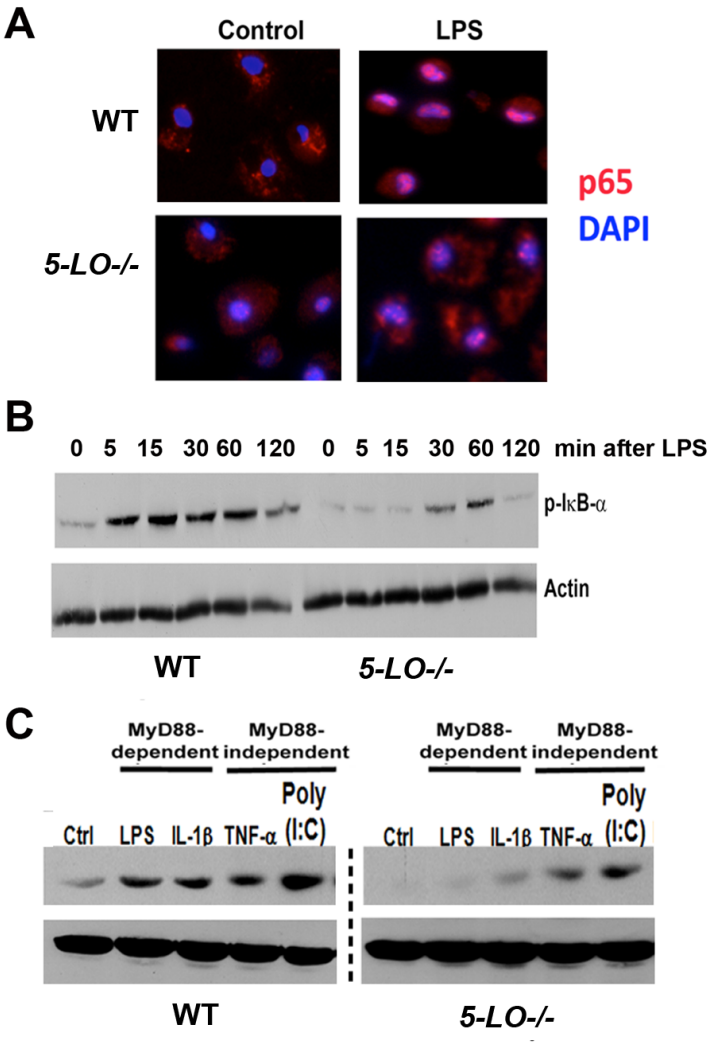
Supplemental Figure 1. LTB_4 amplifies production of NFκB-regulated gene products in vitro and in vivo. (**A, B**) WT and *5-LO-/-* MΦs were treated for 24 h with or without LTB_4 and RANTES (**A**) and IL-6 (**B**) levels were determined by ELISA. (**C, D**) WT and *5-LO-/-* mice were challenged i.p. with LPS and peritoneal lavage fluid was harvested 6 h thereafter for determination of RANTES (**C**) and IL-6 (**D**) levels by ELISA. Values represent the mean ± SEM from 3 individual experiments, each performed in triplicate.*$p < 0.05$ versus control WT; #p<0.05 versus LPS- or LTB_4-challenged cells.

Supplemental Figure 2



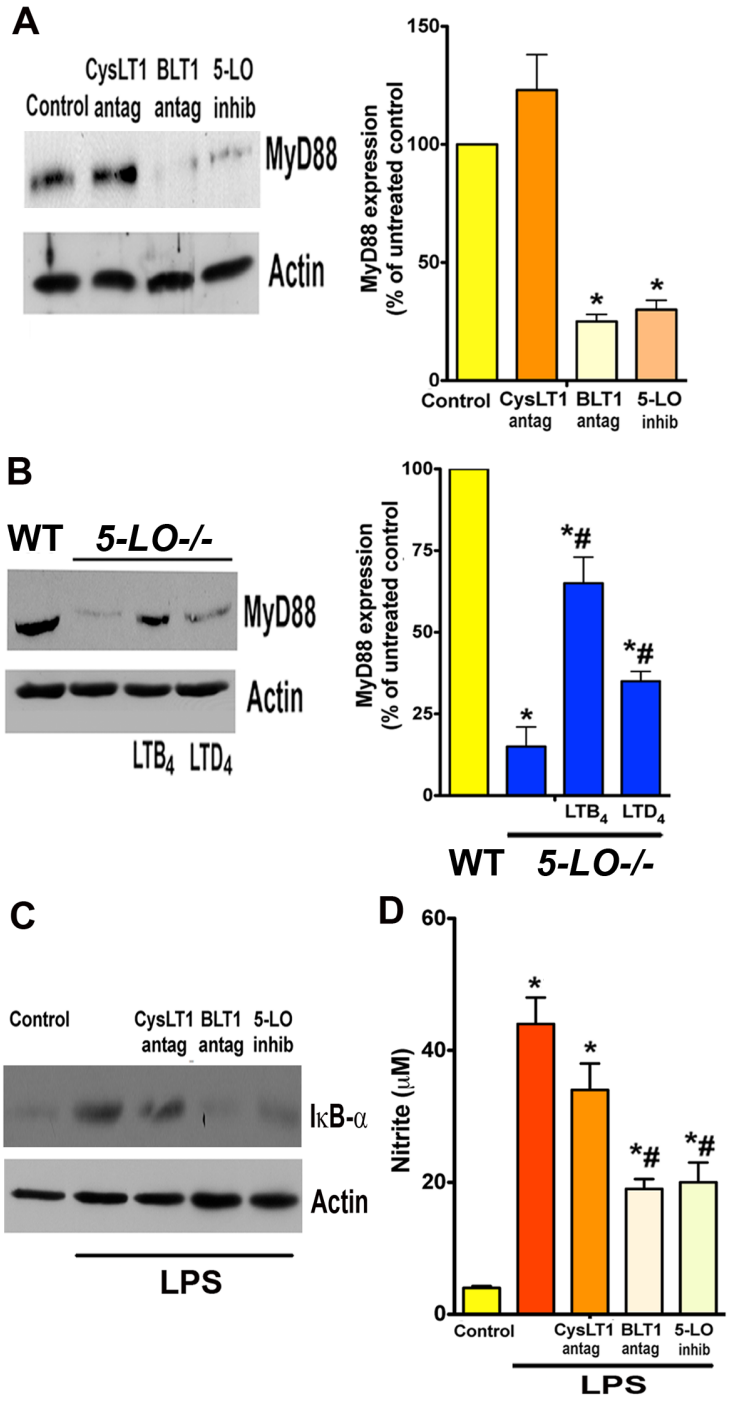
Supplemental Figure 2. 5-LO products are required for MyD88-dependent inflammatory responses and NFκB activation in BMDMs. (**A, B**) WT and *5-LO-/-* cells were treated with or without LPS or Poly (I:C) for 24 h and RANTES (**A**) and IL-6 (**B**) levels were determined by ELISA. (**C**) WT and *5-LO-/-* BMDMs were stimulated with or without LPS and Poly (I:C) for 24 h, and nuclear p65 DNA-binding activity was determined as described in Methods. Values represent the mean ± SEM from 3 individual experiments, each performed in triplicate.*$p < 0.05$ versus control WT; #p<0.05 versus LPS-treated WT.

Supplemental Figure 3



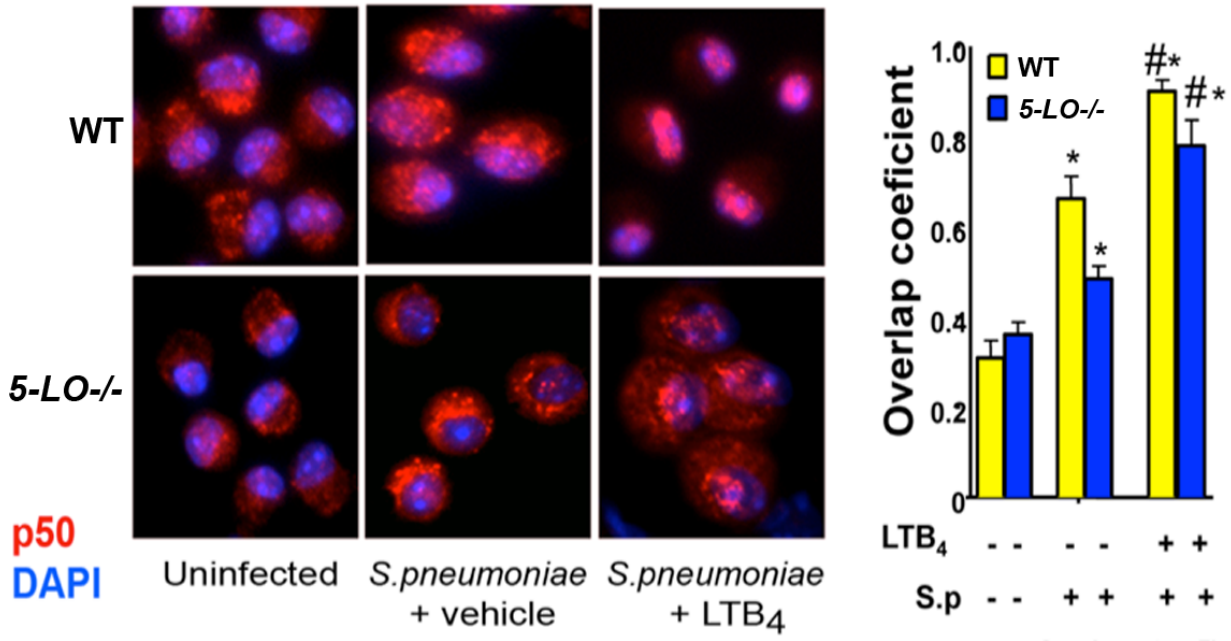
Supplemental Figure 3. 5-LO metabolism is required for MyD88-dependent NFκB p65 nuclear translocation and IκB phosphorylation. (**A**) NFκB p65 localization by immunofluorescence microscopy in WT (top) and *5-LO-/-* (bottom) MΦs incubated for 24 h with (right) or without (left) LPS. p65 is stained red and DAPI stains nuclei blue; each field depicted is representative of 100 examined, from images captured at 400 x magnification and are representative of those obtained from 3-5 animals per experimental group. (**B**) Detection of phosphorylated IκB-α by immunoblot in WT and *5-LO-/-* MΦs incubated for the indicated time points with LPS or (**C**) for 24 h with MyD88-dependent and -independent agonists. Data are representative of 2-3 independent experiments. In (**B**), WT and *5-LO-/-* blots were from the same gel but were noncontiguous; the dashed line indicates missing lanes between the two halves.

Supplemental Figure 4



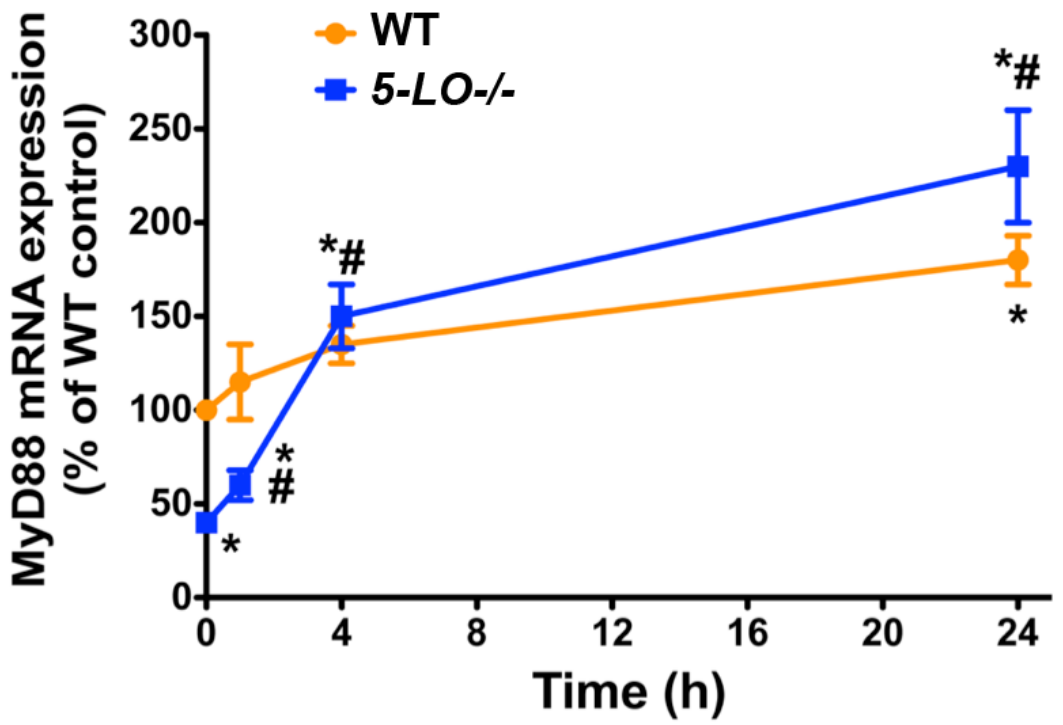
Supplemental Figure 4. CysLTs are not required for MyD88 expression and LPS-induced IκB-α phosphorylation. (**A** and **B**) MyD88 expression by immunoblot analysis in WT MΦs incubated for 48 h with (**A**) the 5-LO inhibitor AA-861 (1 μM), the BLT1 antagonist CP105,696 (1 μM) and the CysLT1 antagonist MK571 (1 μM), or (**B**) LTB_4 and LTD_4. Data are from one experiment representative of three independent experiments. (**A** and **B,** right) Mean ± SEM densitometric values for MyD88 protein in WT MΦs from 3 independent experiments, normalized for actin and expressed relative to the untreated control.*p < 0.05 versus WT control; #p < 0.05 versus 5-LO-/- control. (**C**) LPS-induced IκB-α phosphorylation assessed at 1 h in WT MΦs pretreated with the 5-LO inhibitor, the BLT1 antagonist, or the CysLT1 antagonist as described in **A** for 48 h. (**D**) Nitrite generation from WT MΦs pretreated as in **A** and then stimulated with LPS for 24 h. *p < 0.05 versus control; #p < 0.05 versus LPS-treated cells

Supplemental Figure 5



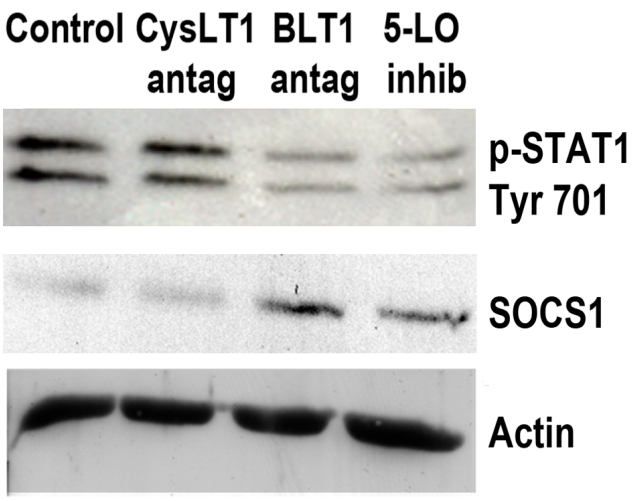
Supplemental Figure 5. Endogenous and exogenous LTB_4 regulates in vivo NFκB nuclear translocation during *S. pneumoniae* infection. (left) Ex-vivo NFκB p50 localization in MΦs from lungs of WT and *5-LO-/-* mice uninfected or infected intranasally with *S. pneumoniae*, treated 24 h thereafter with aerosolized vehicle or LTB_4, and harvested by lavage 24 later. p50 is stained red and DAPI stains nuclei blue; each field depicted is representative of 100 examined, from images captured at 400x magnification and are representative of those obtained from 3-5 animals per experimental group. (right) Quantification of p50/DAPI colocalization was determined as described in the Methods. Data represent the mean ± SEM from 3 individual experiments, with ~100 cells analyzed in each experimental group.*$p < 0.05$ versus control WT; #p<0.05 versus *S. pneumoniae* + vehicle-challenged WT or *5-LO-/-* mice.

Supplemental Figure 6



Supplemental Fig. 6. LTB_4 enhances MyD88 mRNA expression in a time-dependent manner. WT and *5-LO-/-* MΦs were incubated for various times with or without LTB_4 and MyD88 mRNA was measured by real time RT-PCR; data represent the mean ± SEM from 3 individual experiments, each performed in triplicate, and values are expressed relative to those in untreated WT MΦs. *p < 0.05 versus WT control; #p < 0.001 versus *5-LO-/-* control.

Supplemental Figure 7



Supplemental Figure 7. Basal CysLT production is not required for STAT1 phosphorylation and SOCS1 expression. WT MΦs were treated for 48 h with or without the 5-LO inhibitor AA-861 (1 μM), the BLT1 antagonist CP105,696 (1 μM), or the CysLT1 antagonist MK571 (1 μM) and p-STAT1 (Tyr 701), SOCS1 and actin expression were determined by immunoblotting. Data are representative of two independent experiments.