

LysMD3 is a type II membrane protein without an *in vivo* role in the response to a range of pathogens

Christine C. Yokoyama¹, Megan T. Baldrige², Daisy W. Leung¹, Guoyan Zhao¹, Chandni Desai¹, Ta-Chiang Liu¹, Vladimir E. Diaz-Ochoa⁷, Jeremy P. Huynh³, Jacqueline M. Kimmey³, Erica L. Sennott⁸, Camaron R. Hole³, Rachel A. Idol⁴, Sunmin Park¹, Kelly M. Storek¹³, Caihong Wang⁵, Seungmin Hwang¹⁰, Ashley Viehmann Milam¹, Eric Chen¹¹, Tobias Kerrinnes⁷, Michael N. Starnbach⁸, Scott A. Handley¹, Indira U. Mysorekar^{1,5}, Paul M. Allen¹, Denise M. Monack⁹, Mary C. Dinauer⁴, Tamara L. Doering³, Renee M. Tsois⁷, Jonathan E. Dworkin¹², Christina L. Stallings³, Gaya K. Amarasinghe¹, Craig A. Micchelli⁶, Herbert W. Virgin¹

¹Department of Pathology and Immunology, ²Department of Medicine, Division of Infectious Diseases, ³Department of Molecular Microbiology, ⁴Department of Pediatrics, ⁵Department of Obstetrics and Gynecology, ⁶Department of Developmental Biology, Washington University School of Medicine, Saint Louis, MO; ⁷Department of Medical Microbiology and Immunology, University of California, Davis, CA; ⁸Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA; ⁹Department of Microbiology and Immunology, Stanford University, Stanford, CA; ¹⁰Department of Pathology, University of Chicago, Chicago IL; ¹¹Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; ¹²Department of Microbiology & Immunology, College of Physicians and Surgeons, Columbia University, New York, NY, ¹³Genentech, Inc, South San Francisco, CA.

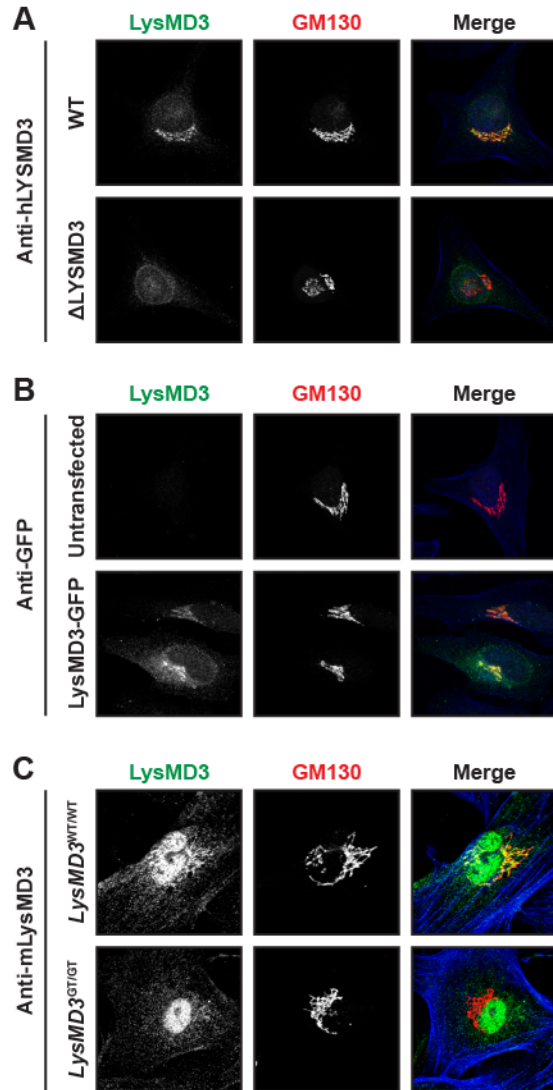


Figure S1. LysMD3 is a type II integral membrane protein that co-localizes with GM130+ structures. (A) WT and hLYSMD3-deficient Δ LYSMD3 cells were stained for LysMD3 using a polyclonal anti-hLYSMD3 antibody from Proteintech. (B) Δ LYSMD3 HeLa cells were transfected with a construct coding for GFP-tagged LysMD3 and stained for GFP. (C) LysMD3^{WT/WT} and LysMD3^{GT/GT} MEFs from a Het x Het mating were harvested and genotyped at approximately embryonic day 15. Cells were stained for LysMD3 using an affinity-purified rabbit polyclonal antibody raised to amino acids 1-210 of mLysMD3. For all images in A-C, cells were co-stained for GM130 and Phalloidin (blue) was used to visualize F-actin. Representative confocal images are shown.

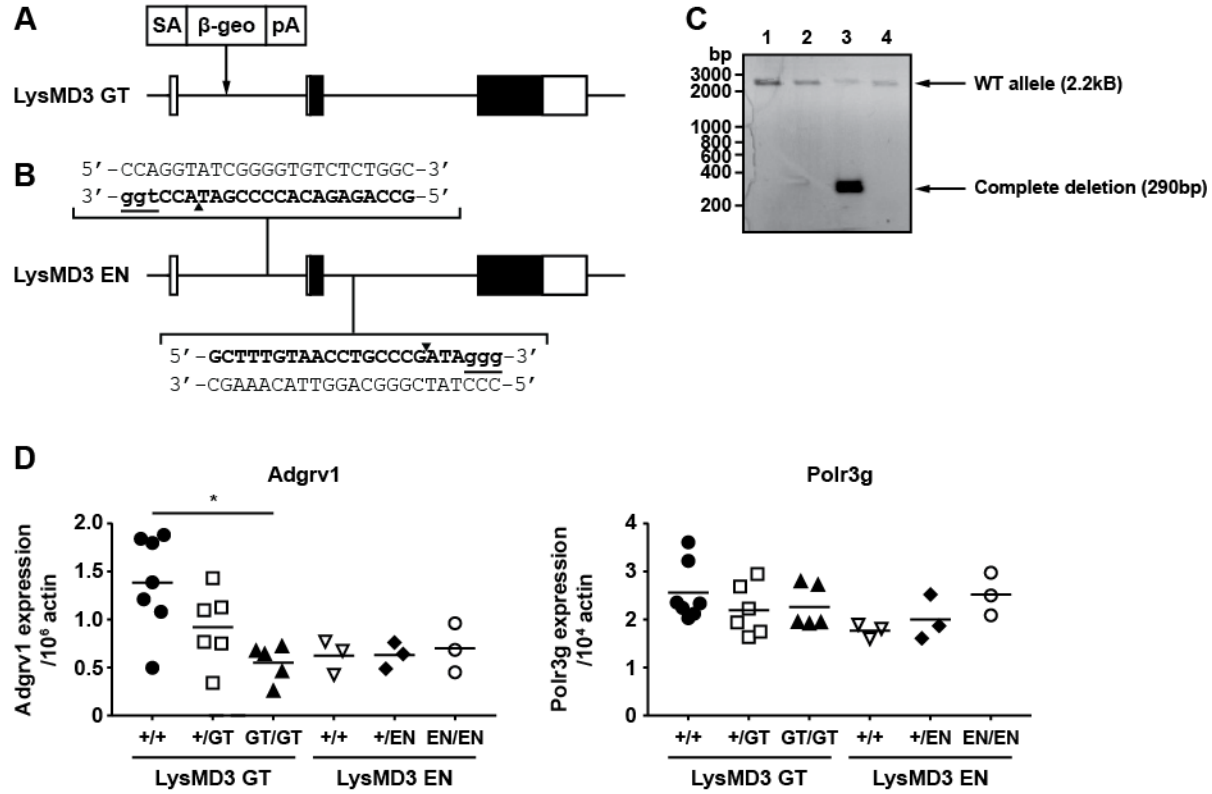


Figure S2. Generation of *LysMD3* deficient mice. (A) A gene trap vector encoding a β -galactosidase/neomycin resistance cassette inserted between exons 1 and 2 of the *LysMD3* gene, disrupting *LysMD3* mRNA and protein expression. (B) Exon 2 of *LysMD3* was targeted using gRNAs of the indicated sequences in B6 embryos. (C) Primers flanking the gRNA target sides were used to screen live pups for correct targeting. Samples from 4 representative mice are shown, with mouse 3 demonstrating a correctly targeted allele. (D) qRT-PCR analysis of *Adgrv1* and *Polr3g*, genes immediately flanking *LysMD3*, in ileum tissue from *LysMD3* GT and *LysMD3* EN mouse lines. All experimental mice were littermate-matched. Data were analyzed by ANOVA with Tukey post test. *, $p < 0.01$.

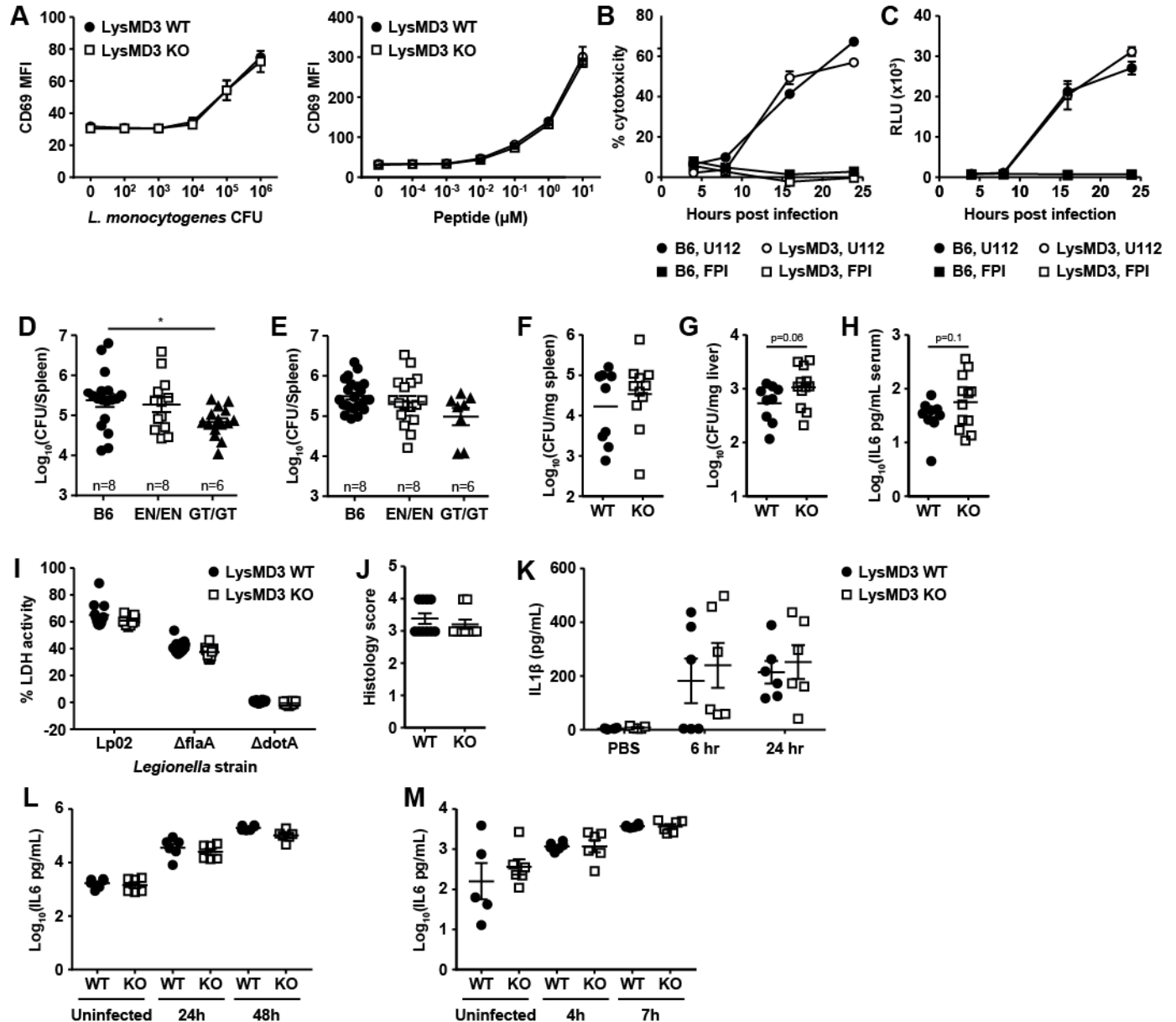


Figure S3. No role for mLysMD3 in the response to multiple models of bacterial infection. (A) BMMo from BMMo from the LysMD3 EN line were pulsed with *L. monocytogenes* or LLO peptide and LLO-specific T cell activation was evaluated by staining for CD69. (B-C) BMMo from the LysMD3 GT mouse line were infected with *F. novicida* strain U112 or isogenic mutant FPI at an MOI of 10. Cell death (B) and type I IFN production (C) were evaluated at the time points indicated. (D-E) Mice were aerosolically infected with *M. tuberculosis* strain Erdman and titers in the spleen were determined at 3 (D) and 13 (E) WPI. (F-H) Mice from the LysMD3 GT mouse line were infected with 1×10^6 CFU *B. abortus* IP and titers in the spleen (F) and liver (G) and serum IL6 levels (H) were determined at 3 DPI. Data are combined from 2 experimental replicates. (I) BMMo from the LysMD3 EN mouse line were infected with *L. pneumophila* strains Lp02, Δ dotA, or Δ flaA and LDH production was measured. (J) Female mice were inoculated transurethrally with 1×10^7 CFU of UPEC strain UTI89. Bladders were harvested at 24 HPI for histological analysis of inflammation severity. A semiquantitative scoring system, with a scale of 0 (normal) to 5 (necrosis with full-thickness inflammatory infiltration), was used to evaluate bladder inflammation (71). (K) BMMo were infected with UTI89 at an MOI of 0.1. Supernatant fluids were

evaluated for the presence of IL1 β at the indicated time points post infection. Mice and BMMo from the LysMD3 GT mouse line were used for UPEC experiments. (L-M) MEFs from the LysMD3 GT mouse line were infected with *C. trachomonatis* at an MOI of 10 (L) or *S. flexneri* at an MOI of 1 (M) and levels of secreted IL6 were evaluated by ELISA.