

SUPPLEMENTAL MATERIAL

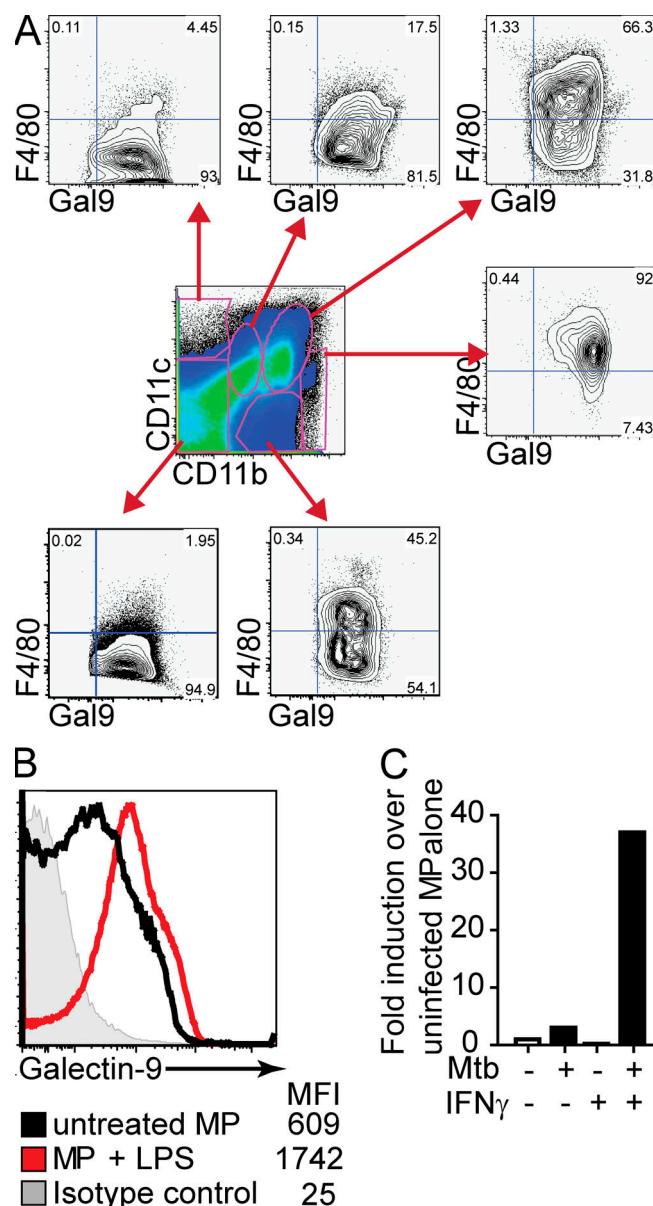
Jayaraman et al., <http://www.jem.org/cgi/content/full/jem.20100687/DC1>

Figure S1. Pulmonary myeloid cells express high levels of intracellular galectin-9. (A) Myeloid cell subsets in the lung were identified based on CD11c and CD11b staining. Further delineation of myeloid subsets was performed using F4/80 staining. The expression of intracellular Gal9 was determined. Representative FACS plots are shown for a cohort of mice ($n = 4\text{--}5$). Numbers indicate percentage of cells in each quadrant. (B) Uninfected thioglycolate-elicited peritoneal macrophages (MP) were treated with media alone or with 5 $\mu\text{g}/\text{ml}$ of LPS. 24 h after treatment, intracellular expression of Gal9 was determined. (C) Uninfected and *Mtb*-infected thioglycolate-elicited peritoneal macrophages were treated with media alone or with 10 U/ml of IFN- γ . 24 h after treatment, macrophages were lysed to extract RNA and expression of Gal9 mRNA was quantified using real-time quantitative RT-PCR. Data are representative of three independent experiments.

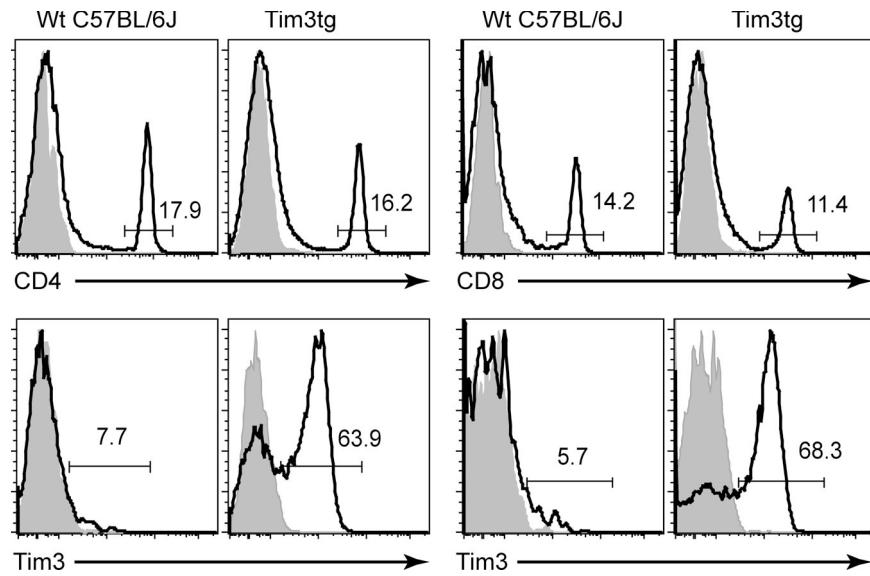


Figure S2. CD4⁺ and CD8⁺ T cells from Tim3tg mice overexpress Tim3. Total spleen cells were prepared from uninfected WT C57BL/6J and Tim3tg mice. Lymphocytes were gated based on size and by CD4⁺ and CD8⁺ staining (top). The percentage of CD4 and CD8 T cells that express Tim3 is expressed as histograms (bottom). Shaded histograms represent isotype control. Bars represent the positive gate based on isotype control. The numbers indicate the percentage of positively stained events. Representative FACS plots are shown for a cohort of mice ($n = 3-5$).

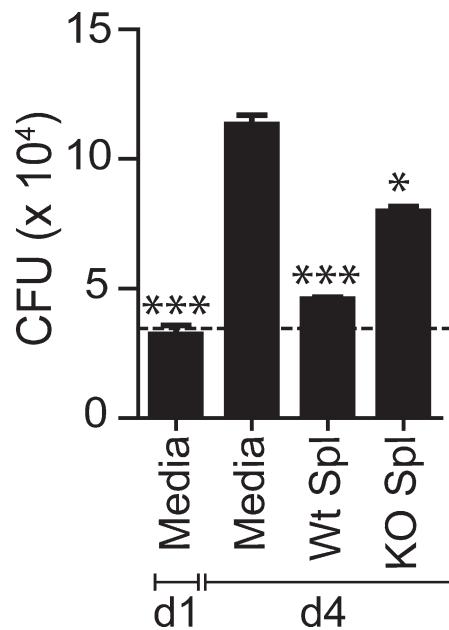


Figure S3. Tim3-mediated *Mtb* control is dependent on Tim3 expression by splenocytes. *Mtb*-infected macrophages (MP) cultured alone or with splenocytes from uninfected WT (WT Spl) or Tim3^{-/-} (KO Spl) mice. Day 1 (d1) is the CFU in infected macrophages alone 24 h after infection, before the addition of cells and represents initial inoculum, whereas day 4 (d4) is the CFU recovered from macrophages 4 d after infection in the absence of any treatment. Data are from a single experiment. Error bars indicate mean ± SEM from three replicate cultures. **, $P < 0.01$; ***, $P < 0.001$, one-way ANOVA compared with day 4 macrophages alone.

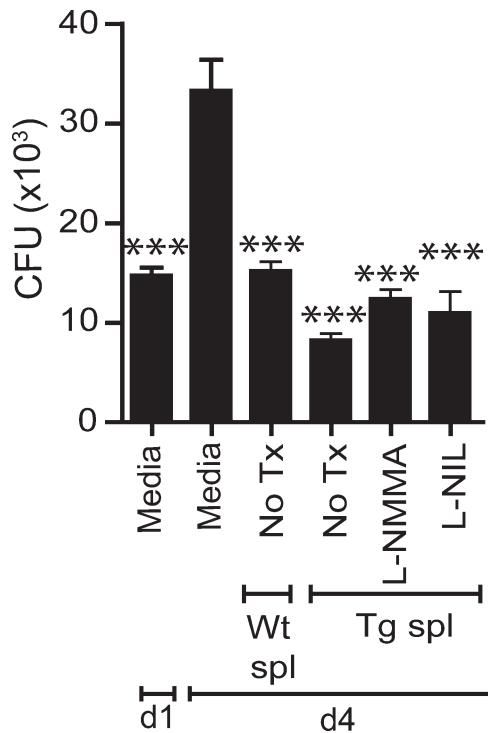


Figure S4. The action of Tim3-Ig is independent of iNOS. WT C57BL/6J macrophages were infected with H37Rv. 24 h after infection, the initial inoculum was determined (d1) and splenocytes from WT (WT Spl) and Tim3tg (Tg Spl) mice were added to infected macrophages with or without iNOS inhibitors, L-NMMA (2 mM), and L-NIL (0.5 mM). CFUs were determined 4 d after infection (d4). No Tx, No treatment. Data are representative of two independent experiments. Error bars represent mean \pm SEM. ***, P < 0.001, one-way ANOVA compared with day 4 macrophages alone.

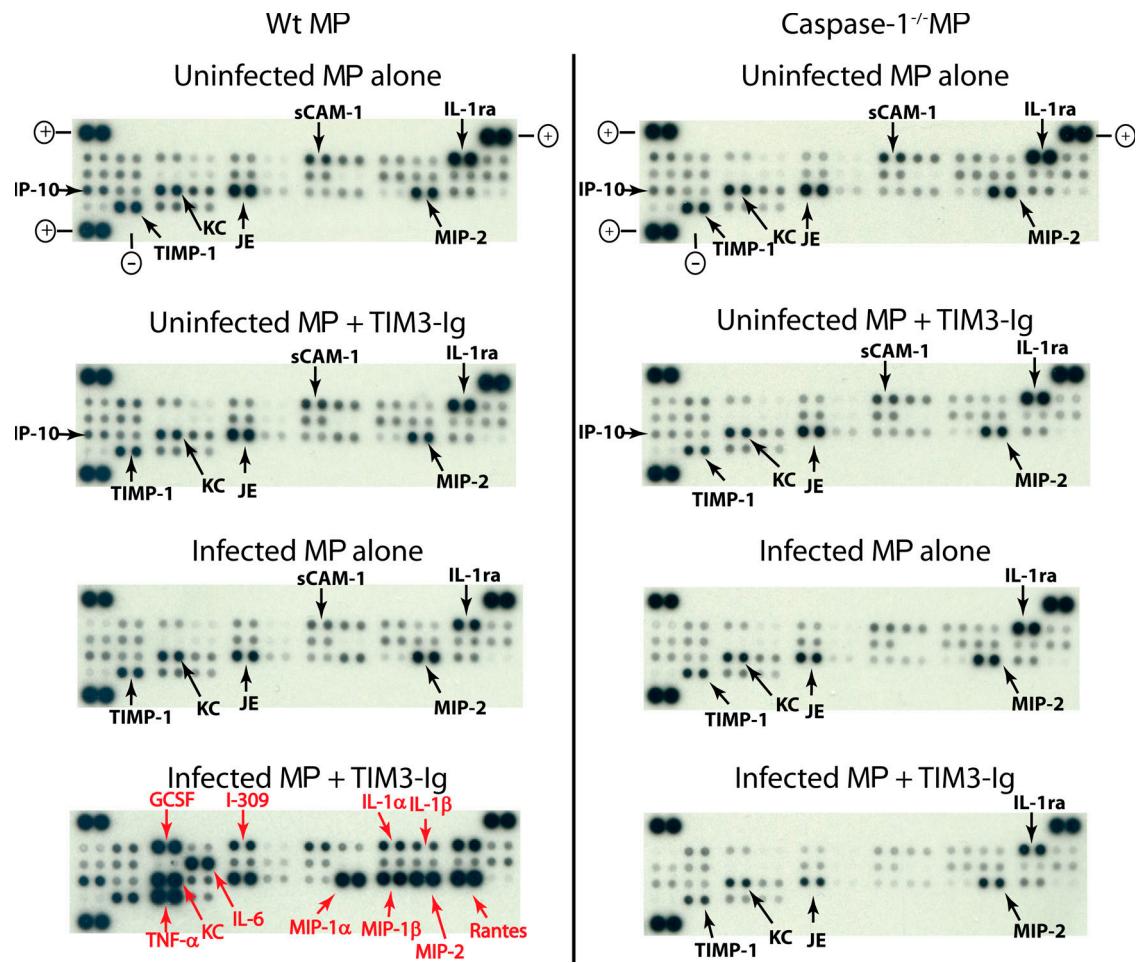


Figure S5. Tim3-Ig-induced macrophage activation state is dependent on caspase-1. WT C57BL/6J, caspase-1 $^{-/-}$ macrophages (MP) were infected with H37Rv in parallel and supernatants from uninfected and *Mtb*-infected macrophages treated with or without 10 μ g/ml Tim3-Ig were sampled 24 h after culture and analyzed using mouse cytokine blot. Black, present in uninfected macrophages; red, observed only in *Mtb*-infected macrophages treated with Tim3-Ig. Data are representative of two independent experiments.

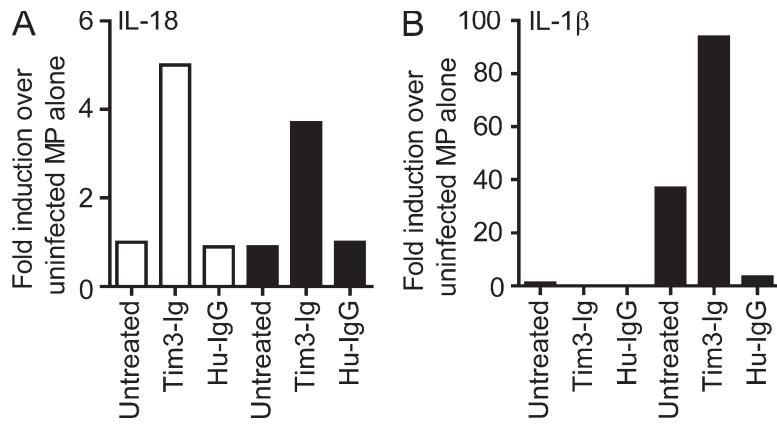


Figure S6. Treatment with Tim3-Ig induces IL-18 and IL-1 β transcription. Real-time PCR analysis of the expression of IL-18 (A) and IL-1 β (B) in WT C57BL/6J uninfected and *Mtb*-infected macrophages (MP) left untreated or treated with 10 μ g/ml Tim3-Ig fusion protein or human IgG (control). Expression of IL-18 and IL-1 β is presented relative to uninfected/untreated macrophages after accounting for expression of the house-keeping gene β -actin. Open bars indicate uninfected macrophages and closed bars indicate *Mtb*-infected macrophages. Data are representative of eight independent experiments.

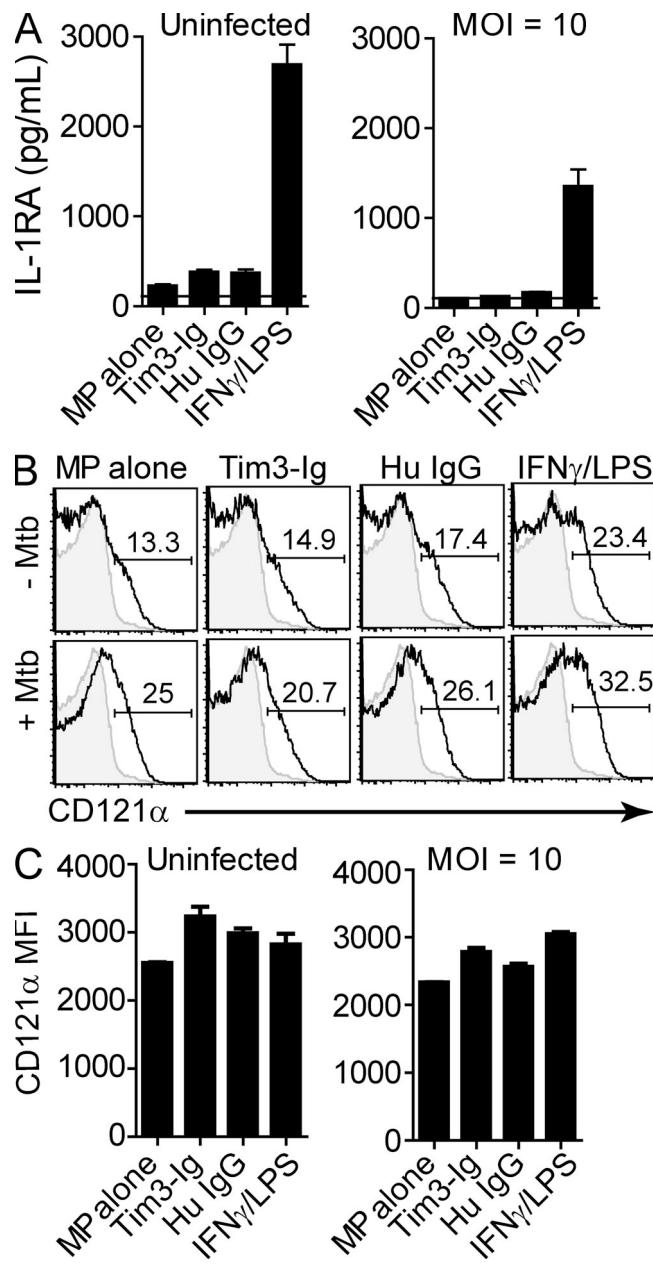


Figure S7. Tim3–Gal9 interaction does not regulate IL-1RA secretion and cell surface expression of IL-1R. (A) ELISA for IL-1RA in cell culture supernatants of uninfected or *Mtb*-infected macrophages (MP) treated for 48 h with media, Tim3-Ig, HulgG (control), or 10 U/ml IFN- γ /5 μ g/ml LPS. (B) Uninfected or *Mtb*-infected macrophages treated for 48 h with media, Tim3-Ig, HulgG (control), or 10 U/ml IFN- γ /5 μ g/ml LPS were stained for surface IL-1R expression. The expression of IL-1R is shown as a thick line in the histograms. Shaded histograms represent isotype control. Representative histograms from triplicate conditions are shown. (C) Mean fluorescence intensity of IL-1R $^+$ populations shown as histograms in C is graphically plotted for uninfected and *Mtb*-infected macrophages. Data in A–C are from one experiment with triplicate cultures.

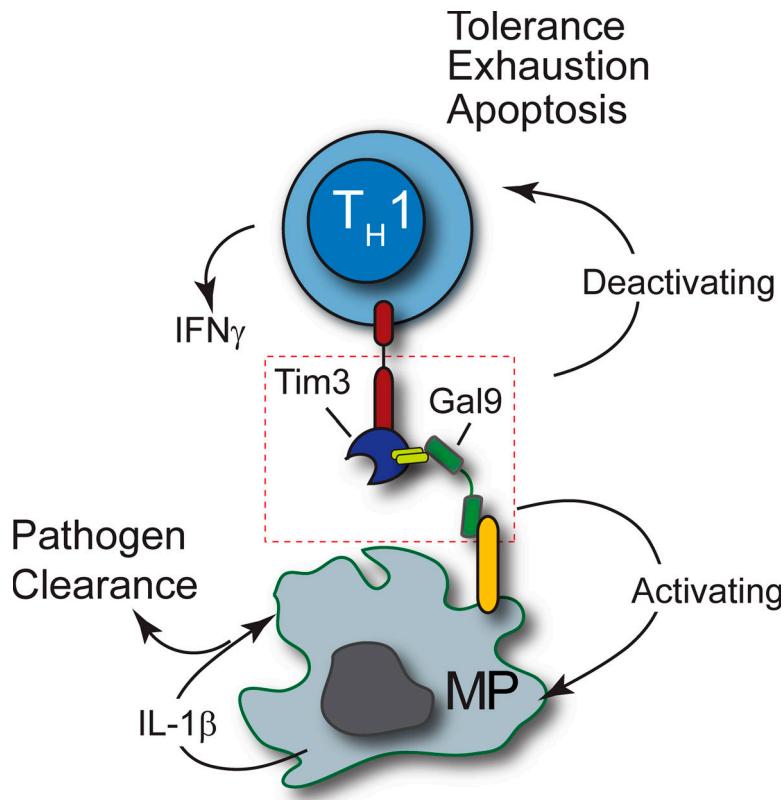


Figure S8. Proposed model of bidirectional regulatory circuit mediated by Tim3–Gal9 interaction. During infection with the intracellular pathogen *Mtb*, T cell-derived cytokine IFN- γ and innate cell-derived IL-1 β both induce the expression of Tim3 ligand Gal9. During the effector phase of infection, Tim3⁺ T cells that traffic to the site of infection (lungs) come in contact with Gal9 expressing infected alveolar macrophages. Data presented in this work suggest that the ensuing Tim3–Gal9 interaction delivers an unknown activating signal via Gal9 into infected macrophages, resulting in the activation of caspase-1 and IL-1 β secretion. Secreted IL-1 β acts in an autocrine fashion to further activate innate pathways of pathogen clearance. Data based on other previously published work indicate that Tim3–Gal9 interaction also delivers a negative deactivating signal into the effector T cell via Tim3 culminating in apoptosis, exhaustion or tolerance dependent on the disease model (Monney et al., 2002; Sabatos et al., 2003; Sanchez-Fueyo et al., 2003; Zhu et al., 2005; Koguchi et al., 2006; Jones et al., 2008; Golden-Mason et al., 2009). Therefore, the cell surface molecule Tim3 could potentially have evolved to inhibit growth of intracellular pathogens via its ligand Gal9, while at the same time inhibiting effector T_H1 cells to prevent further tissue inflammation and immunopathology.

REFERENCES

- Golden-Mason, L., B.E. Palmer, N. Kassam, L. Townshend-Bulson, S. Livingston, B.J. McMahon, N. Castelblanco, V. Kuchroo, D.R. Gretch, and H.R. Rosen. 2009. Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. *J. Virol.* 83:9122–9130. doi:10.1128/JVI.00639-09
- Jones, R.B., L.C. Ndhlovu, J.D. Barbour, P.M. Sheth, A.R. Jha, B.R. Long, J.C. Wong, M. Satkunarajah, M. Schweneker, J.M. Chapman, et al. 2008. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *J. Exp. Med.* 205:2763–2779. doi:10.1084/jem.20081398
- Koguchi, K., D.E. Anderson, L. Yang, K.C. O'Connor, V.K. Kuchroo, and D.A. Hafler. 2006. Dysregulated T cell expression of TIM3 in multiple sclerosis. *J. Exp. Med.* 203:1413–1418. doi:10.1084/jem.20060210
- Monney, L., C.A. Sabatos, J.L. Gaglia, A. Ryu, H. Waldner, T. Chernova, S. Manning, E.A. Greenfield, A.J. Coyle, R.A. Sobel, et al. 2002. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature*. 415:536–541. doi:10.1038/415536a
- Sabatos, C.A., S. Chakravarti, E. Cha, A. Schubart, A. Sánchez-Fueyo, X.X. Zheng, A.J. Coyle, T.B. Strom, G.J. Freeman, and V.K. Kuchroo. 2003. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat. Immunol.* 4:1102–1110. doi:10.1038/ni988
- Sánchez-Fueyo, A., J. Tian, D. Picarella, C. Domenig, X.X. Zheng, C.A. Sabatos, N. Manlongat, O. Bender, T. Kamradt, V.K. Kuchroo, et al. 2003. Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. *Nat. Immunol.* 4:1093–1101. doi:10.1038/ni987
- Zhu, C., A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, and V.K. Kuchroo. 2005. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat. Immunol.* 6:1245–1252. doi:10.1038/ni1271