Supporting Information

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SI Materials and Methods

Bacterial and Cell Culture. For bacterial culture, a single colony was inoculated into LB broth and grown for 8 h under aerobic conditions and then under oxygen-limiting conditions overnight. Under these conditions, bacteria correspond to 5 to 7×10^8 cfu/mL. Cells were infected at an MOI of 10 unless otherwise indicated. J774 cells and BAI1-overexpressing J774 cells were maintained in high-glucose DMEM containing 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, 4.5 g/L glucose, and antibiotics in a 5% CO_2 incubator at 37 °C. Cells were transfected using Lipofectamine 2000 (Invitrogen) as described previously (1).

PCR Primers. BAI1 and ELMO1 mRNA was amplified by using the primers BAI1-F 5′ aagttcttcggctacttctcg 3′ and BAI1-R 5′ cgggatccatccactgggcattgctgca 3′; ELMO1-F 5′ cgtgaaggtggccatagaat 3′ and ELMO1-R 5′ tcccctgaagatgaggaatg 3′.

Preparation of Recombinant RGD-TSR. The RGD-TSR region of BAI1 was subcloned into the vector pGEX-4T2, generating a fusion protein with an N-terminal GST tag. The plasmid was transformed into Arctic Express competent cells (DE3; Stratagene). Induction of protein was done with 1 mM IPTG for 24 h at 10 °C according to the manufacturer's instructions. Purified RGD-TSR was prepared in large scale by the Protein Biosynthesis and Biomarker Core Laboratory at the University of Texas Medical Branch (Galveston, TX).

Assessment of Rac1 Activation. Rac1 activity was assayed by pull-down assay. Infected cells were lysed in buffer containing 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 0.1 M NaCl, 1% Nonidet P-40, and 10% glycerol with protease inhibitors and incubated with GST coupled to the p21-binding domain of Pak to precipitate Rac-GTP. Blots were visualized using Pierce SuperSignal ECL reagents. The ratio of active Rac1 (GTP bound) to total Rac1 was quantified by using ImageQuant 5.2 (Molecular Dynamics).

BMDM Preparation. Marrow was flushed from mouse leg bones with medium (RPMI plus 10% FCS supplemented with 2 mM glutamine, 1 mM sodium pyruvate) and seeded onto Petri dishes. For growth of bone marrow macrophages, RPMI medium was supplemented with 20% of supernatant taken from L929 cells (containing murine granulocyte–macrophage colony stimulating factor, referred to as BMDM medium).

Gentamicin Protection Assay. Approximately 2×10^5 cells per well were seeded into 24-well culture dishes 18 h before infection at an MOI of 10 for 1 h in antibiotic-free DMEM in a 37 °C CO_2 incubator. Cells were then washed and incubated with gentamicin (500 μg/mL) for 90 min to kill extracellular bacteria. Subsequently, cells were lysed in 1% Triton-X 100, and lysates were serially diluted and plated directly onto LB agar plates. Total cfu values were enumerated the next day after overnight incubation at 37 °C. Values were standardized to levels of colonization in control cell preparations.

Preparation of Intestinal Macrophages. Gut antigen-presenting cells were isolated by using techniques described previously (2, 3). Briefly, small intestines were removed from C57BL/6 mice, opened longitudinally to flush out feces, cut into 15-mm pieces, and then incubated for 20 min at 37 °C on a shaker in HBSS supplemented with 5% heat-inactivated FBS and 2 mM EDTA. After passing the preparation through a metal filter, intestinal fragments were collected and the step was repeated. Subsequently, intestinal fragments were minced and incubated for 20 min at 37 °C on a shaker in HBSS supplemented with 5% heat-inactivated FBS and 1 mg/mL type VIII collagenase (Sigma-Aldrich). The cell suspension was passed through a cell strainer to remove debris and washed, and then $CD11b^+$ cells were enriched with a CD11b MACS kit (Miltenyi Biotec). From this enriched population, $CD11b^+$ intestinal macrophages were then sorted using a FACSVantage cell sorter (BD Biosciences).

1. Park D, et al. (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* 450:430–434.
2. Wilson JM, et al. (2009) The A_{2B} adenosine receptor impairs the maturation and immunogenicity of dendritic cells. *J Immunol* 182:4616–4623.
3. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 8:1086–1094.

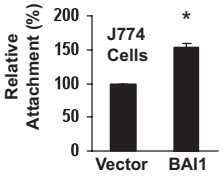

Fig. S1. Relative attachment of bacteria to control J774 cells (vector) or cells overexpressing BAI1. Bacterial counts were normalized to vector controls and represented as the mean (±SD) from three independent experiments (*$P \leq 0.05$).

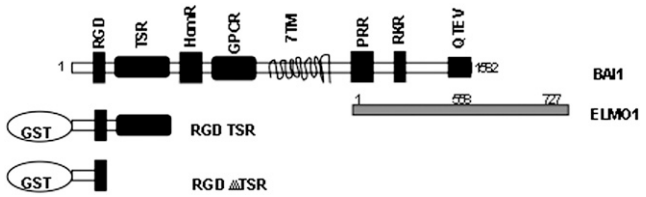

Fig. S2. Schematic representation of BAI1 domains. (RGD, putative integrin binding motif; TSR, five TSRs; HomR, hormone receptor homology; GPS, GPCR cleavage site; 7TM, seven transmembrane domains; PRR, proline rich region; RKR, ELMO binding site; QTEV, PDZ motif.) The RGD TSR construct contains an N-terminal GST tag, and includes the RGD motif and all five TSRs. Inclusion of the RGD is necessary to maintain solubility of the recombinant protein. The RGDΔTSR construct contains N-terminal GST and the RGD motif, but lacks the TSRs.

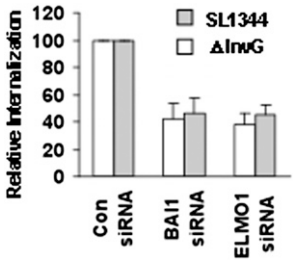

Fig. S3. Knockdown of BAI1 or ELMO1 inhibits internalization of both invasive (SL1344) and noninvasive (Δ*invG*) *Salmonella* Typhimurium. Bacterial internalization by BMDMs was assayed using the gentamicin protection assay. Data represent the mean ± SD of two replicates from three independent experiments.

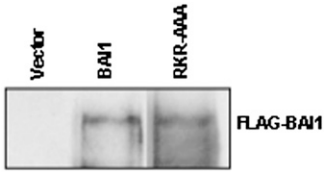

Fig. S4. Documentation of BAI1 expression levels in transfected CHO cells. Cells were transfected with vector control, full-length FLAG-BAI1, or FLAG-BAI1 with a mutation in the cytoplasmic ELMO-binding motif (RKR-AAA). Immunoblot with anti-FLAG antibody demonstrates equivalent levels of expression of two constructs.

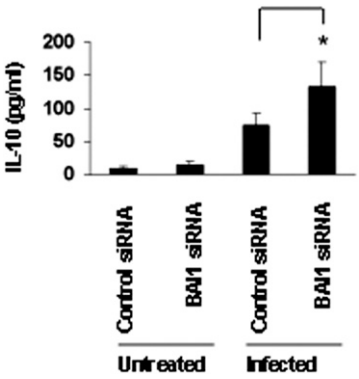

Fig. S5. Knockdown of BAI1 enhances IL-10 responses. IL-10 production was measured by ELISA in supernatants collected from control and BAI1-depleted BMDMs after 6 h of incubation with *Salmonella* Typhimurium (Δ*invG*). Data represent the mean ± SD of duplicate wells for each condition from three independent experiments (*$P \leq 0.05$).

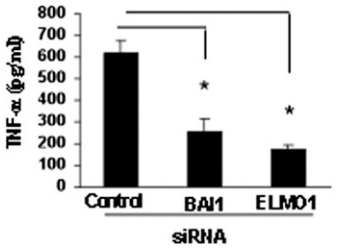

Fig. S6. Knockdown of BAI1 or ELMO1 inhibits TNF-α production induced by *S. typhimurium*. TNF-α secretion was measured by ELISA after infection of BMDM for 6 h.

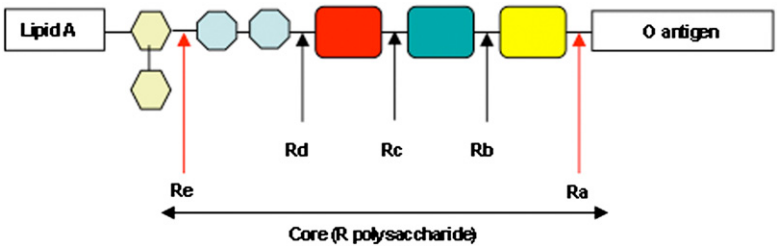

Fig. S7. LPS structure can be divided into three regions; the lipid A portion, the inner core (R polysaccharide), and the O-antigen. The core oligosaccharide can contain different lengths of oligosaccharide that attach to the lipid A as shown in the schematic. So-called rough strains (based on colony morphology) produce lipid A with variable amounts of core oligosaccharide, ranging from six (Ra) to none (Re), whereas "smooth" strains make LPS containing all the core oligosaccharides plus O-antigen.