Supplementary Methods

Construction of recombinant *Salmonella* Typhimurium 14028s strains

Recombineering constructs were designed using Benchling's cloud-based software platform and were engineered with primers synthesized by Integrated DNA Technologies (**supplementary table 2).** Plasmids used in fluorescent reporter strain construction were obtained through Addgene.org and are listed in **supplementary table 1.**

Salmonella Typhimurium 14028s Δ*hilD* strain construction

An unmarked *hilD* deletion was engineered into the *Salmonella* Typhimurium 14028s (STm 14028s) genetic background **(PMID: 19897643)** using the FRUIT method of recombineering **(PMID: 23028641)**. Briefly, a recombination fragment was created by amplifying the *thyA* cassette from pAMD001 **(PMID: 23028641)** using Quick-load Taq 2x Master Mix (New England Biolabs) and tailed primers (hilD_KO_TU_F & hilD_KO_TD_R) designed to delete the entire *hilD* open reading frame (ORF). The resulting *hilD-thyA* recombination fragment was gel extracted via Thermo's GeneJET kit and subsequently electrotransformed into ST14028s *ΔthyA* harboring pKD46 **(PMID: 23028641)** that had been cultured at 30 °C in LB broth supplemented with carbenicillin and 0.2% arabinose to promote lambda Red-mediated recombination. Recombinants were then selected at 30°C on M9 agar supplemented with 1 % casamino acids and 100 µg/ml carbenicillin as previously described **(PMID: 23028641).** Colonies that emerged were replated on the same media to confirm the loss of thymine auxotrophy, and then screened via colony PCR using Quick-load Taq 2x Master Mix (New England Biolabs) and the hilD_KO_scrn_F1 & hilD_KO_Scrn_R1 primer set to confirm replacement of the *hilD* ORF with *thyA*. The resulting ST14028s *ΔthyA ΔhilD::thyA* intermediate strain was then subjected to a second round of lambda Red recombination to generate the scarless *hilD* deletion strain. To accomplish this, ~200 bp regions immediately upstream and downstream of the *hilD* ORF were first amplified using Quick-load Taq 2x Master Mix and the hilD_KO_scrn_F1/hilD_KO_MU_R and

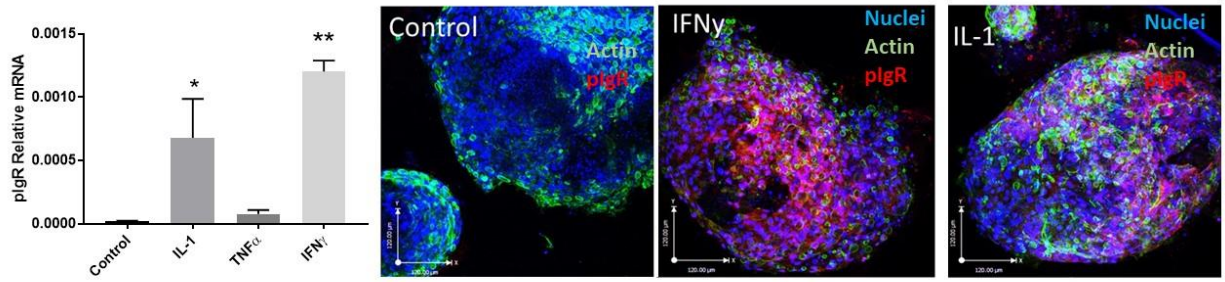
hilD_KO_MU_F/hilD_KO_Scrn_R1 primer sets. The amplified DNA fragments were then gel purified via Thermo's GeneJET kit and used as templates for overlap extension PCR with Q5 High-Fidelity 2x Master Mix (New England Biolabs) and the hilD_KO_scrn_F1/hilD_KO_Scrn_R1 primer set. The resulting PCR fusion product was then purified via Zymo Research's DNA Clean & Concentrator-5 kit and then electrotransformed into ST14028s *ΔthyA ΔhilD::thyA* + pKD46 cells that had been cultured at 30 °C in LB carbenicillin broth supplemented with 0.2 % arabinose to induce recombination. Recombinants were then selected at 30 °C on M9 agar supplemented with 1 % casamino acids,100 µg/ml carbenicillin, 100 µg/ml thymine, and 20 µg/ml trimethoprim as previously described **(PMID: 23028641).** The colonies that emerged following overnight incubation were passaged again on the same media to confirm trimethoprim resistance, and then screened by PCR to confirm removal of *thyA* from the *hilD locus* using Quick-load Taq 2x Master Mix (New England Biolabs) and the hilD_KO_scrn_F2 & hilD_KO_scrn_R2 primer set. The STm 14028s *ΔthyA ΔhilD* intermediate strain that resulted was then subjected to a third round of lambda Red recombination to restore the native *thyA* ORF as previously described **(PMID: 23028641).** Both the *thyA* and Δ*hilD* loci were then sequenced to confirm successful recombination using the hilD_KO_scrn_F2/hilD_KO_scrn_R2 primer sets. The resulting STm 14028s *ΔhilD* strain was then cured of pKD46 through passage on LB agar at 42 ˚C prior to experimentation.

Creation of *Salmonella* Typhimurium fluorescent reporter strains

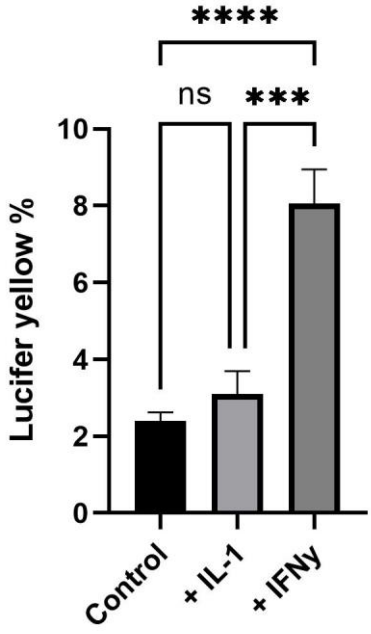
Constitutive *sGFP2 and mScarlet-I* reporter cassettes were stably integrated into the chromosome of *Salmonella* Typhimurium strains through Tn7 transposition **(PMID: 27260360) (PMID: 30631309)**. To accomplish this, STm 14028s WT, *ΔoafA* **(PMID: 23028641)**, and *ΔhilD* genetic backgrounds were electroporated with either pMRE-Tn7-152 (sGFP2) or pMRE-Tn7-145 (mScarlet-I) **(PMID: 30631309)** and transformants were selected for at 30 °C on LB agar containing carbenicillin and 0.2 % arabinose to induce TN7 transposition. Individual colonies that

emerged were picked and grown in antibiotic-free LB broth at 42 °C and 225 RPM to promote loss of the Tn7 plasmids. Four hours later the cultures were plated on LB agar supplemented with either kanamycin (pMRE-Tn7-152-sGFP2), or gentamycin (pMRE-Tn7-145 mScarlet-I) and then incubated overnight at 37 °C to select for recombinants harboring chromosomally integrated reporter cassettes. Transformants that emerged the following day were patch-plated on LB carbenicillin and either LB kanamycin or LB gentamycin and incubated overnight at 30 °C to confirm loss of the temperature-sensitive plasmids and chromosomal integration of the reporter cassettes. Recombinants were then assessed for fluorescence via Spectramax ID3 plate reader and customized excitation/emission spectra (sGFP2: 480nm/530nm, mScarlet-I: 570nm/620nm).

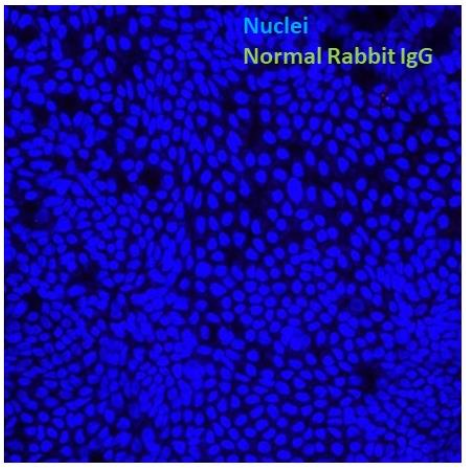
Supplementary Data



Supplementary Figure 1: preliminary experiment to determine best pIgR induction in HIOs. Results show both IL-1 and IFNγ significantly increase pIgR mRNA, but IFNγ also causes tight junction disruption (confocal image, second panel). IL-1 leaves tight junction intact.



Supplementary Figure 2: preliminary experiment to determine best pIgR induction in HIOs. Results show both IFNγ significantly increases Lucifer Yellow permeability in HIOs whereas IL-1 does not increase Lucifer Yellow permeability



Supplementary Figure 3: Normal IgG control staining to show GP2 specificity