Supplementary Materials for

Long-term monitoring of inflammation in the mammalian gut using programmable commensal bacteria

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Supplementary Figures:

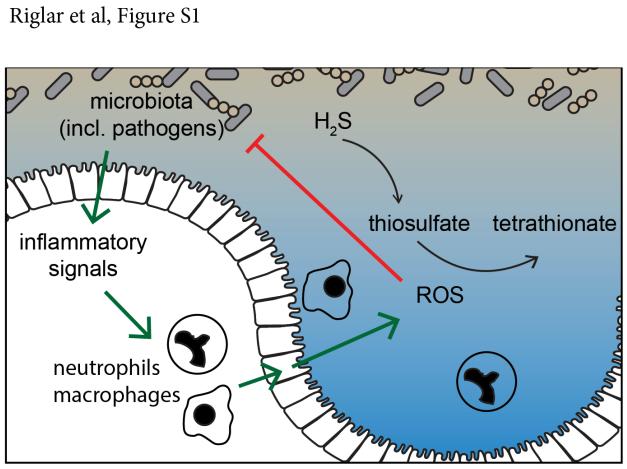

Figure S1: The inflammatory response in the mammalian gut leads to tetrathionate generation

Cytokine signaling following an inflammatory insult leads to, among other responses, release of reactive oxygen species (ROS) into the gut lumen that inhibit microbial growth, and can oxidize thiosulfate, generated from hydrogen sulfide, to tetrathionate.

Riglar et al, Figure S2

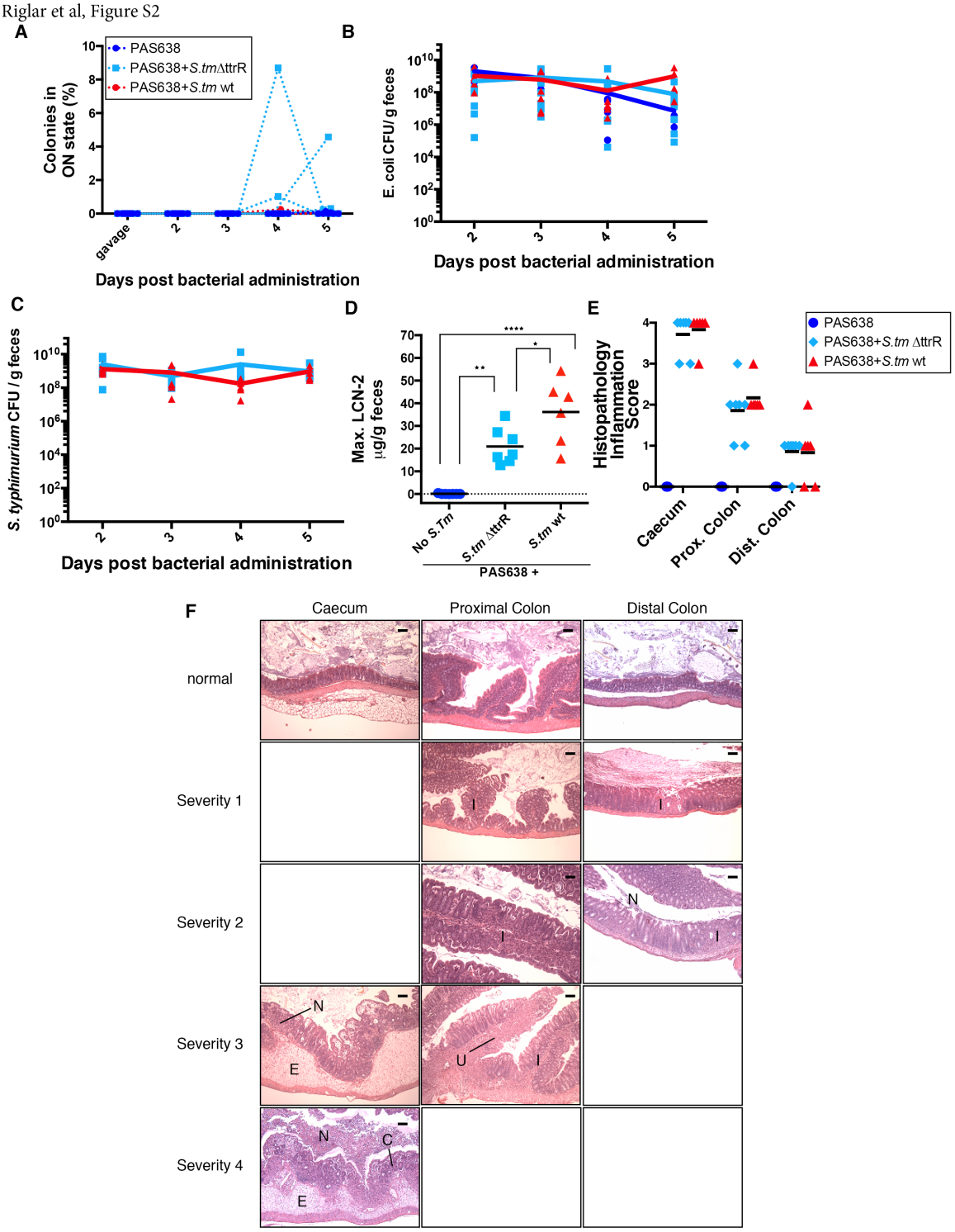

Figure S2:

A) Timecourse of PAS638 switching during infection of C57Bl/6 mice with wt and ΔttrR *S. typhimurium (S.tm)* (n= 6-7 per group). Switching was only apparent in

colonies from mice coinfected with *S. typhimurium* ΔttrR and on days 4 and/or 5 post administration. B) Enumeration of PAS638 *E. coli* and C) *S. typhimurium* variant levels by selective plating showed relatively consistent levels across experimental groups. D) LCN-2 quantification demonstrated inflammation in both *S. typhimurium* ΔTtrR and wt administered mice. Graphs show individual mouse values and mean. * $p<0.05$, ** $p<0.005$ *** $p<0.0005$ using one way ANOVA with Tukey's multiple comparisons test. E) Histology scoring of colon, proximal and distal colon, showed inflammation in the presence of both *S. typhimurium* variants decreasingly apparent from the caecum to the distal colon. F) Scoring used a 0-4 point scale with example images of each score by histology provided. For cecal samples, only severity 3 and 4 were observed during *S. typhimurium* infection, characterized by accumulation of neutrophils (N) in epithelial tissues, edema (E), mucosal thickening and at times low-level presence of neutrophils in exudate at severity 3 and edema (E), extensive neutrophils present in exudate and/or epithelia (N), mucosal thickening and signs of crypt damage or regeneration (C) at severity 4. Proximal and distal colon showed signs of low-level inflammation (I) at severity 1, more extensive inflammation (I) and neutrophils in exudate (N) at severity 2. Severity 3 was noted in the proximal colon only and showed signs of ulceration (U), inflammatory cell migration (I), and neutrophils commonly present in exudate. Scale bars = 10μm.

Table S1:

Table of strains constructed for use in this study.

Strain name	Background	Elements (template)
PAS637	*E. coli* NGF1	LAM14 memory element (*E. coli* PAS132/133) [5]
PAS638	*E. coli* NGF1	LAM14 memory element (*E. coli* PAS132/133)[5]; *ttrR/S-*P_{ttrBCA} (*S. typhimurium* LT2)
S. typhimurium wt	*S. typhimurium* 14028s	zhj-1401::Tn10 (*S. typhimurium* LT2 SA2700) (Salmonella Genetic Stock Database)
S. typhimurium ΔttrR	*S. typhimurium* 14028s	zhj-1401::Tn10 (*S. typhimurium* LT2 SA2700) (Salmonella Genetic Stock Database); ΔttrR (*S. typhimurium* LT2 TT22470) [16]

Table S2: PAS638 sensing of tetrathionate correlates broadly with LCN-2 levels in 129X1/SvJ mice. Following administration of PAS638 to five 129X1/SvJ mice, fecal samples were tested daily for the first week and at specific time-points over the following 200 days for PAS638 memory state LCN-2 levels. Time-points in which bacteria were more highly switched into the ON state (memory values >1% highlighted green) tended to correspond with higher LCN-2 levels (values >50ng/g highlighted red).

	Mouse 1		Mouse 2		Mouse 3		Mouse 4		Mouse 5	
Day	**Switch (%)**	**LCN-2 (ng/g)**	**Switch (%)**	**LCN-2 (ng/g)**	**Switch (%)**	**LCN-2 (ng/g)**	**Switch (%)**	**LCN-2 (ng/g)**	**Switch (%)**	**LCN-2 (ng/g)**
Pre	N/A	11.8	N/A	3.1	N/A	3.1	N/A	8.7	N/A	5.8
1	0.0	6.1	0.0	1.3	0.0	0.6	0.1	0.8	0.0	2.2
2	0.0	0.8	0.0	1.5	0.0	0.8	0.9	3.4	0.0	5.0
3	0.0	1.9	0.0	6.4	0.0	2.0	4.0	287.6	0.0	3.9
4	0.0	4.2	0.2	3.3	4.8	24.1	3.6	5.6	0.0	5.7
5	0.3	4.0	0.1	6.0	16.8	>500	2.1	288.5	N/A	8.0
6	0.0	1.5	0.9	10.1	N/A	11.6	4.4	6.3	0.0	5.6
7	0.0	5.1	0.7	5.5	0.0	4.3	0.1	3.5	0.0	2.9
159	1.7	130.6	1.0	335.4	0.0	80.6	18.5	31.6	0.0	26.4
200	2.7	>50	0.0	4.4	N/A	25.0	0.0	25.5	0.0	4.5

Table S3: Correct *in vitro* function of PAS638 remained after colonization of the murine gut for 200 days. Colonies were tested by replica plating on plates containing tetrathionate, which were grown anaerobically for ~12 hours to trigger P_{ttrBCA} expression, followed by aerobic growth to shut of P_{ttrBCA} expression. All colonies tested showed correct function.

Mouse	Colonies counted	ON colonies	Percent functional colonies (%)
M1	68	68	100
M2	250	250	100
M3	243	243	100
M4	136	136	100
M5	109	109	100

Table S4: GnotoComplex 2.0 strains. The strains chosen are human commensals selected to recapitulate key physiologic functions and phylogenetic diversity in the host.

Strain	**ID**
Akkermansia muciniphila	DSM 22959
Anaerostipes hadrus	DSM 3319
Bacteroides cellulosilyticus	DSM 14838
Bacteroides fragilis	ATCC 25285
Bacteroides ovatus	ATCC 8483
Bacteroides vulgatus	ATCC 8483
Bifidobacterium longum subsp. infantis	ATCC 15697
Bilophila wadsworthia	ATCC 51581
Blautia hansenii	DSM 20583
Clostridium hiranonis	DSM 13275
Clostridium ramosum	DSM 1402
Clostridium scindens	ATCC 35704
Coprococcus comes	ATCC 27758
Dorea formicigenerans	ATCC 27755
Eggerthella lenta	DSM 2243
Enterococcus faecalis	ATCC 29200
Escherichia coli	MG1655
Klebsiella oxytoca	ATCC 700324
Lactobacillus reuteri	DSM 20016
Parabacteroides distasonis	ATCC 8503
Prevotella melaninogenica	ATCC 25845
Proteus mirabilis	ATCC 29906
Roseburia hominis	DSM 16839
Ruminococcus obeum	ATCC 29174
Veillonella parvula	ATCC 10790