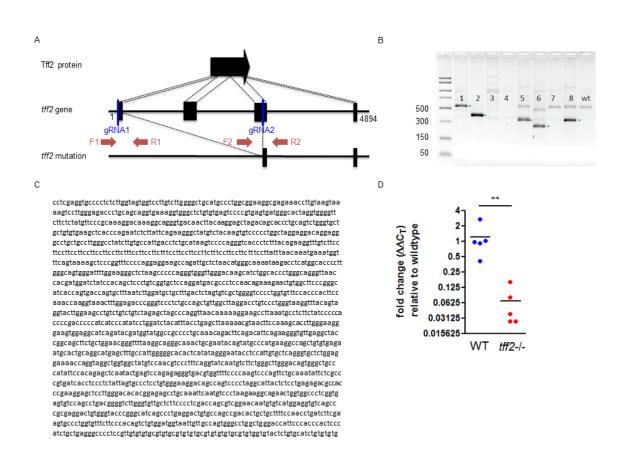
## **SUPPLEMENTAL MATERIAL**

## Loss of trefoil factor 2 sensitizes rat pups to systemic infection with the neonatal pathogen Escherichia coli K1

Alex J. McCarthy, George M. H. Birchenough & Peter W. Taylor



**Fig. S1. Validation of tff2-/- rats.** A, Design strategy for mutating the *tff2* gene. Two pairs of sgRNAs were designed to cleave together to generate a 1.8 kb deletion between the target sites. gRNA1 and gRNA2 were targeted to exon 1 and 3, respectively, of the rat *tff2* gene sequence (GenBank accession number NC\_005119). Primers flanking each gRNA1 (F1 and R1) and gRNA2 (F2 and R2) sites were designed to screen for deletion mutations between the two target sites. B, PCR amplification of the *tff2* gene identified 4 founders (founders 2, 5, 6 and 8) with desired *tff2* deletion mutations. Bands were excised and sequenced. C, Sequence of the 1933 bp deletion in founder 2. D, qRT-PCR analysis of *tff2* gene expression in stomach tissues of wildtype (WT) and *tff2-/-* Sprague-Dawley rats at P9. Data for *tff2* gene was normalized to *rspS*23 gene, and data from P9 *tff2-/-* pups standardized to expression levels in P9 wildtype animals using the  $\Delta_{CT}$  method for relative quantification of qPCR data. Mean  $\pm$  SD; n = 5.  $\Delta_{CT}$  values were compared by t test. \*\*P<0.01

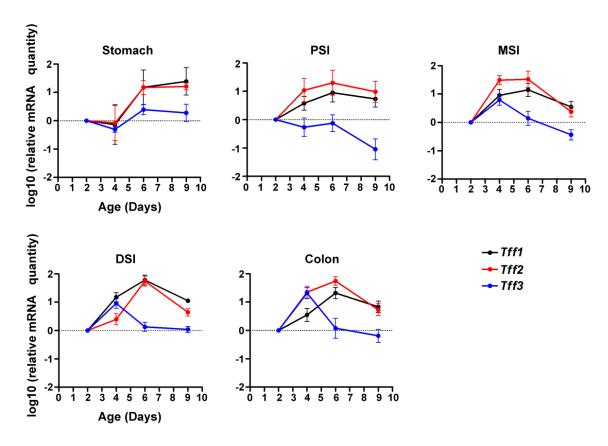


Fig. S2. Temporal changes in trefoil factor expression in the neonatal rat gastrointestinal tract. The normal developmental expression of tff1, tff2 and tff3 over the period P2 to P9. qRT-PCR data for Tff1, Tff2 and Tff3 was normalized to data obtained for the rspS23 gene, and standardised to expression levels at P2 using the  $\Delta C_T$  method. Error bars represent SEM of results from n=6 animals.

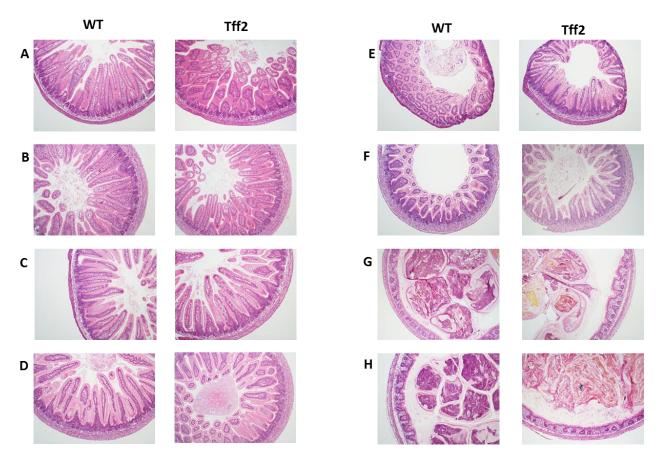


Fig. S3. Colonization of the P9 gastrointestinal tract of neonatal rats with *E. coli* A192PP does not alter the integrity of the intestinal epithelium in either wildtype (WT) or *tff2-/-* homozygotes (Tff2). Micrographs of H&E-stained sections from tissues removed 48 h after colonization or sham-colonization (broth vehicle alone) of P9 pups: A, sham-colonized PSI; B, A192PP-colonized PSI; C, sham-colonized MSI; D, A192PP-colonized MSI; E, sham-colonized DSI; F, A192PP-colonized DSI; G, sham-colonized distal colon; H, A192PP-colonized distal colon.

Amplicon	Forward primer	Concentration	Reverse	Concentration	Annealing	Efficiency
			primer		temperature	
rsp23	TGTGTCAGGGTGCAGCTCATTAAGAACG	200 μm	CTTTGCGACCAAATCCAGCAACCAGAAC	200 μm	60°C	97.20%
tff1	CAAGGTGACCTGTGTCCTC	200 μm	CTTGCTGGTTCTCAATGACC	200 μm	60°C	99.50%
tff2	GGCATCACCAGTGACCAGTGCTTTAATC	200 μm	GCAGTGCCCTTCAGTAGTGACAATCATC	200 μm	60°C	99.75%
tff3	ATGGAGACCAGAGCCTTCT	200 μm	GGATGCTGGAGTCAAAACAG	200 μm	60°C	100.00%
defaRS1	GACCAGGATGTGTTCTGTCTCCTTTG	100 μm	TGTGGACCTTGATAGCCGAATGC	100 μm	60°C	94.71%
defa24	TGATGAGCAGCCAGGGAAAGAG	400 μm	TCAGCGGCAACAGAGTATGAGC	400 μm	60°C	111.00%
TNF-α	ACTCCCAGAAAAGCAAGC AA	100 μm	CGAGCAGGAATGAGAAGAGG	100 μm	59°C	104.40%
IFN-γ	ATGAGTGCTACACGCCGCGTCTTGG	900 μm	GAGTTCATTGACAGCTTTGTGCTGG	900 μm	59°C	113.00%

TABLE S1. Primers used for RT-PCR