SUPPLEMENTAL MATERIAL

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

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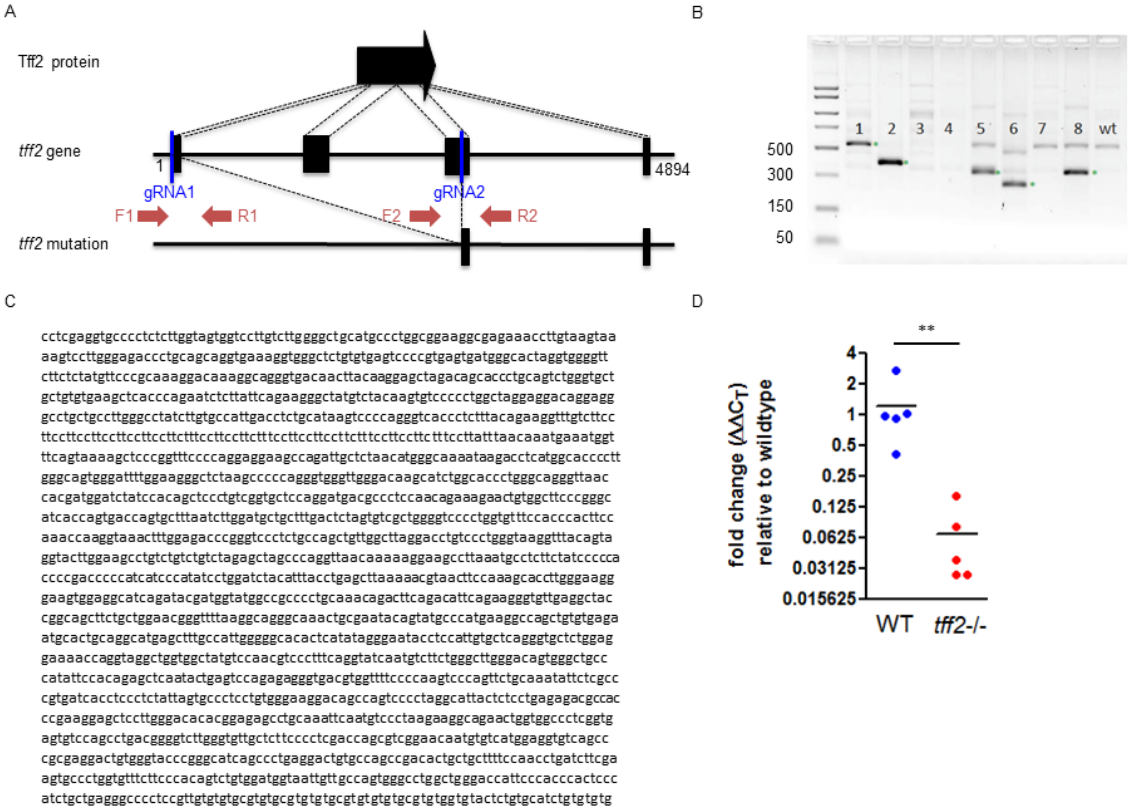

Fig. S1. Validation of tff2-/- rats. A, Design strategy for mutating the *tff2* gene. Two pairs of sgRNAs were designed to cleave together to generate a 1.8 kb deletion between the target sites. gRNA1 and gRNA2 were targeted to exon 1 and 3, respectively, of the rat *tff2* gene sequence (GenBank accession 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 Δ_{CT} method for relative quantification of qPCR data. Mean ± SD; $n = 5$. Δ_{CT} values were compared by *t* test. $^{**}P<0.01$

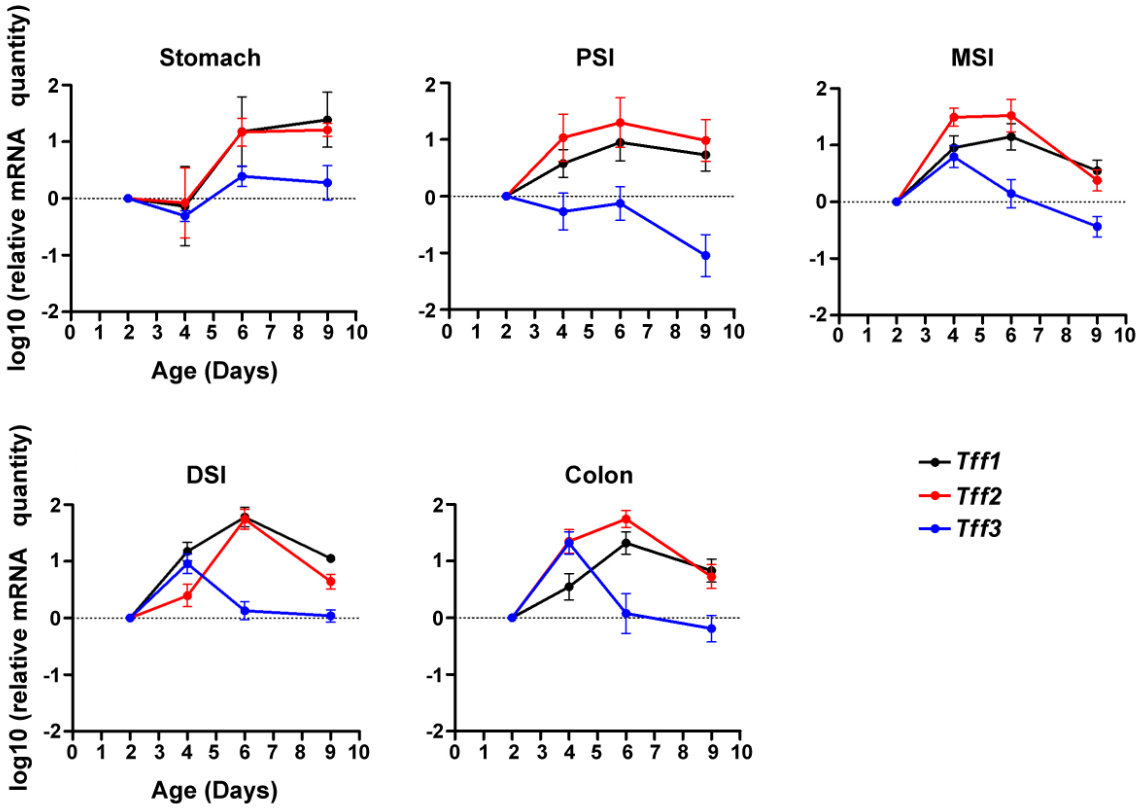

Fig. S2. Temporal changes in trefoil factor expression in the neonatal rat gastrointestinal tract. The normal developmental expression of *tff1*, *tff2* and *tff3* over the period P2 to P9. qRT-PCR data for Tff1, Tff2 and Tff3 was normalized to data obtained for the *rspS23* gene, and standardised to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

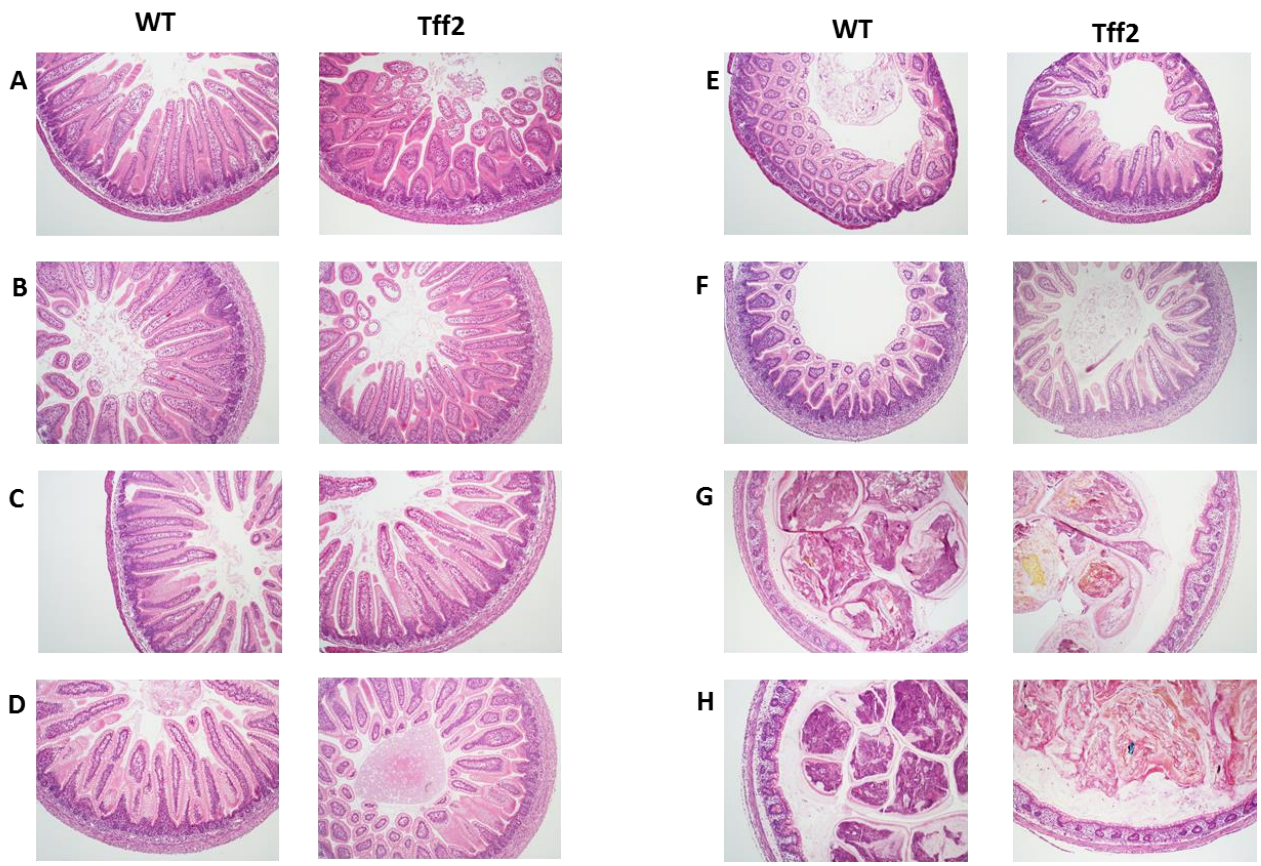

Fig. S3. Colonization of the P9 gastrointestinal tract of neonatal rats with *E. coli* A192PP does not alter the integrity of the intestinal epithelium in either wildtype (WT) or *tff2-/-* homozygotes (Tff2). Micrographs of H&E-stained sections from tissues removed 48 h after colonization or sham-colonization (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 primer	Concentration	Annealing temperature	Efficiency
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