Supplemental Data

TFF2 inhibits tumour development and is a target for epigenetic silencing in gastric cancer

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Figure S1. Comparison of EPITYPER and bisulphite sequencing analysis for the determination of *TFF2* promoter methylation. *TFF2* promoter methylation profiles in (A) normal gastric antrum; (B) gastric cancer. Bisulphite mutagenesis and sequencing strands (n=16) are shown; unmethylated CpGs are represented as open circles; methylated CpGs are represented as filled black circles (see bisulphite key). EPITYPER epigrams (n=2) are shown beneath bisulphite tracks; CpG methyaltion levels are indicated by the epigram colour key. Relative base pair position within the promoter is shown.

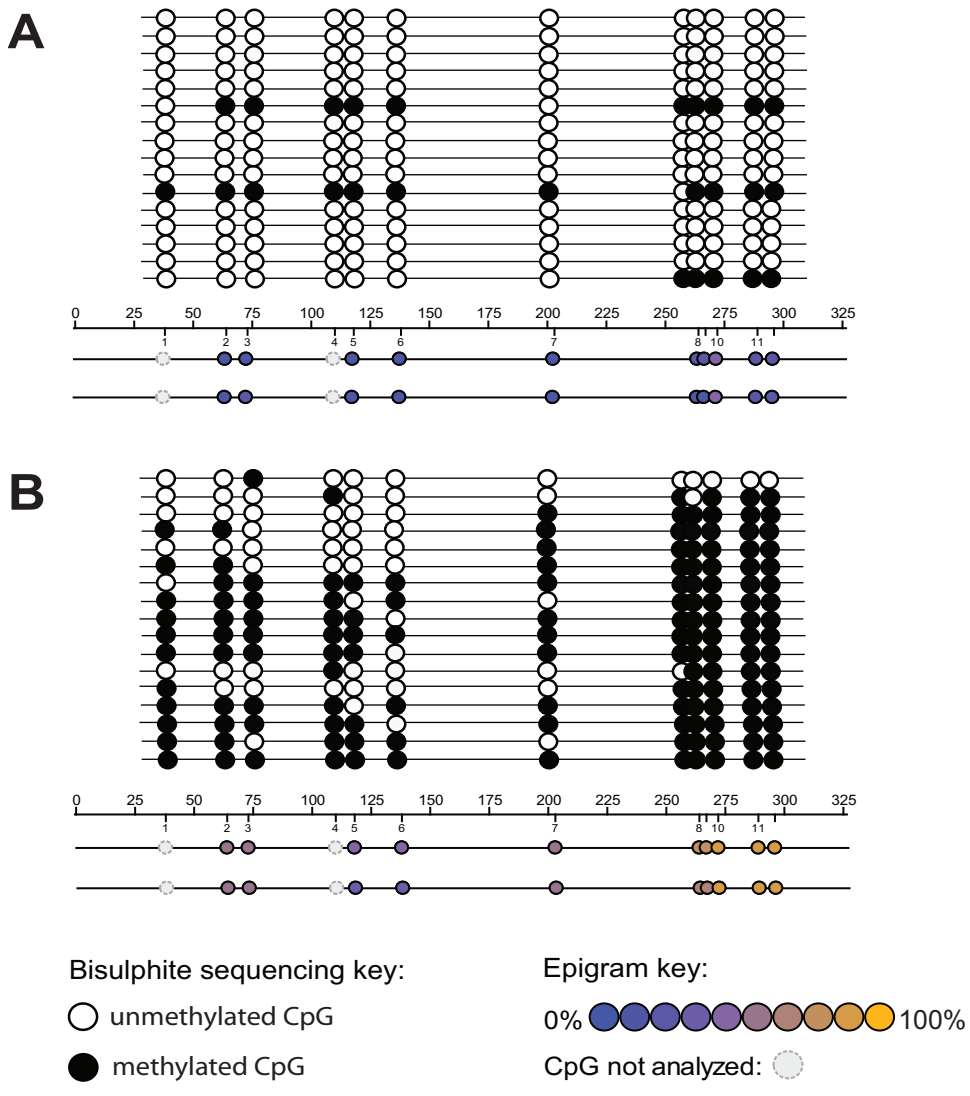

Figure S2. Increased antral tumour growth in *gp130*$^{F/F}$/*Tff2*$^{-/-}$ mice is independent of Stat3 and ERK signalling downstream of the gp130 co-receptor. Immunoblot analysis of (A) phosphorylated (activated) Stat3 and (B) phosphorylated ERK protein in antral stomach tissue. There is no significant difference in the abundance of activated Stat3 or ERK proteins between *gp130*$^{F/F}$/*Tff2*$^{-/-}$ compound mutants and *gp130*$^{F/F}$ single mutant antral tumours.

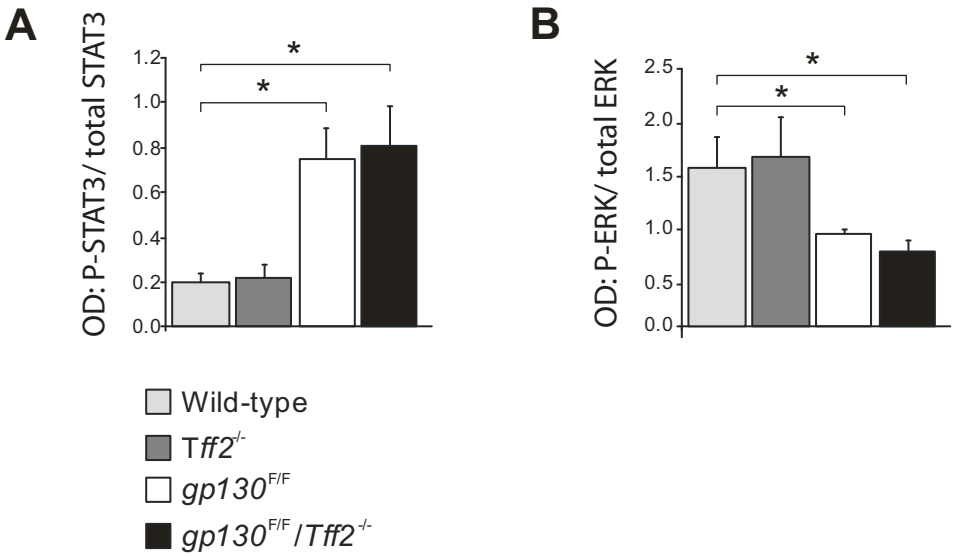

Figure S3. TFF2 dose effect on proliferation of AGS cells. AGS cells were treated with recombinant human (rh) TFF2 in 10-fold concentration increments from 0.05 to 50 μg/mL for 48 hours and the viable cell number determined by 0.4% trypan blue dye exclusion on a haemocytometer. Asterisk shows $P<0.05$.

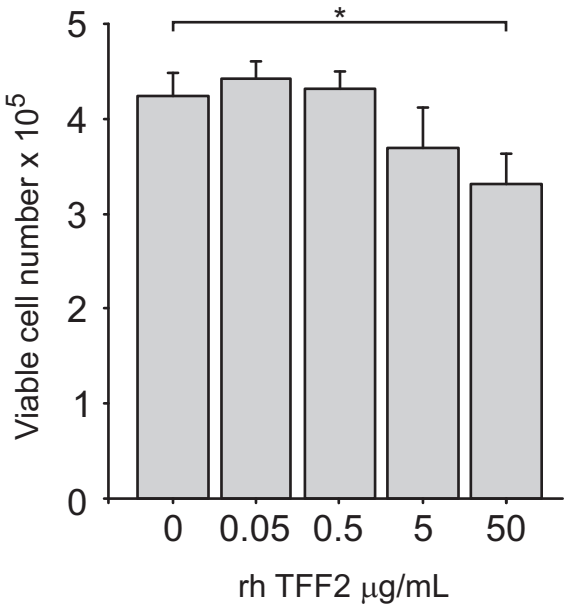

Figure S4. Tff2 expression and methylation in *gp130*$^{F/F}$ antrum at 3-months post infection with *H. pylori*. (A) Quantitative RT-PCR analysis of *Tff2* mRNA expression in antral stomach from wild-type and *gp130*$^{F/F}$ mice infected for 3-months with *H. pylori* SS1. Black horizontal bars show mean fold-change relative to the respective non-infected controls. (B) EPITYPER analysis of *Tff2* promoter methylation in samples shown in 'A'. Error bars show SEM. There is no significant effect of either the *gp130*$^{F/F}$mutation, *H. pylori* infection or both factors combined, on *Tff2* expression or methylation.

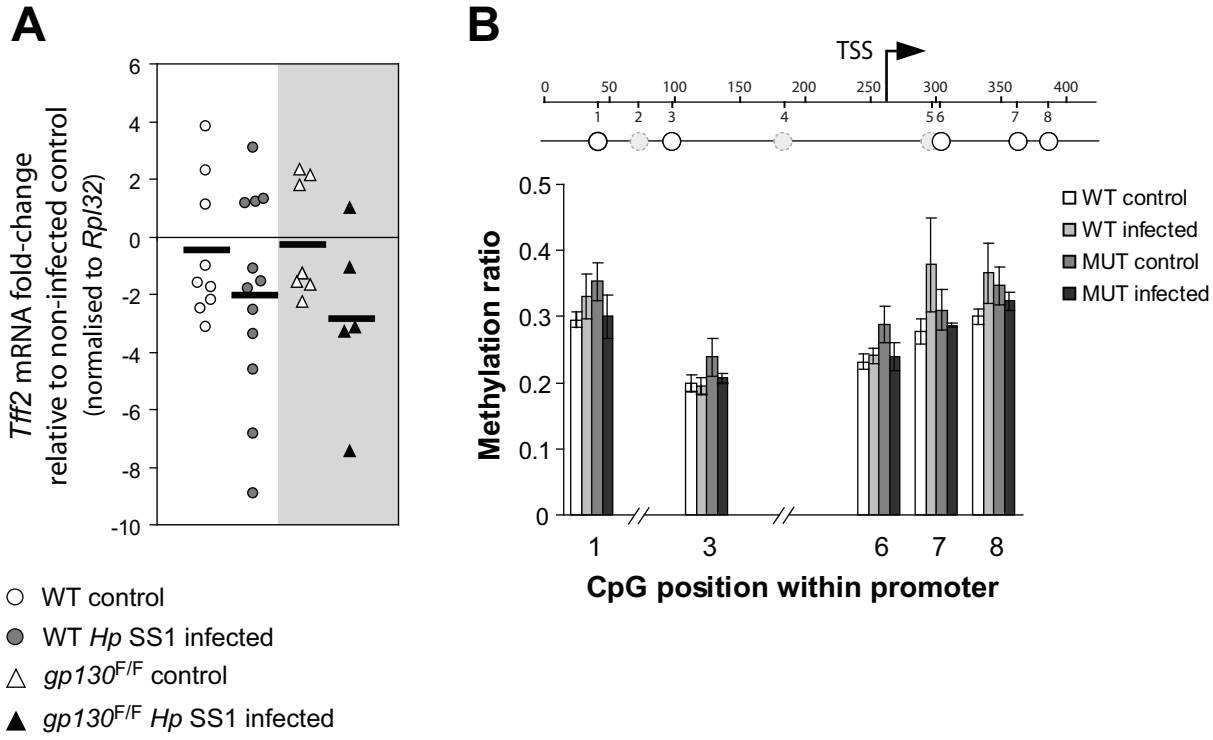

Figure S5. Subversion of *TFF2* activity in the stomach by epigenetic silencing during neoplastic progression. (A) *TFF2* assists in the maintenance of gastric homeostasis by repairing minor ulceration and preventing low grade inflammation becoming chronic, as well as promoting gastrin (G) and somatostatin (S) regulation of proximal stomach secretion, epithelial cell maturation and stomach tumour suppressor gene (*TFF1*, *GKN1*/*2*) expression. (B) *H. pylori* infection initiates *TFF2* epigenetic silencing by inducing promoter methylation, thereby allowing local inflammation and atrophy to proceed unchecked and thus compromising the gastric repair process. These factors, in conjunction with the loss of key stomach tumour suppressor genes, contribute to neoplastic progression.