**SUPPLEMENTARY FIGURE LEGENDS**

**Stomach-specific activation of oncogenic KRAS and STAT3-dependent inflammation cooperatively promote gastric tumorigenesis in a preclinical model**

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**Figure S1. Generation and validation of Tg(*Tff1-CreERT2*) mice.**

**(A)** Schematic illustration of the *Tff1-CreERT2* transgene. The BAC construct harbours substitutions of the indicated endogenous coding exons (*black boxes*) with cDNA encoding *CreERT2* alongside a polyadenylation (polyA) signal. The *CreERT2* cDNA was inserted at the transcriptional initiation sites of the *Tff1* loci.

**(B)** Schematic illustration of the construction of the *Tff1-CreERT2* BAC transgene using DNA recombineering technology to enable recombinase-mediated exchange between homologous regions (red lines) on cDNAs and endogenous loci on a BAC. The indicated *CreERT2* expression cassette (**i**) which included the ampicillin resistance marker (*Amp*, open box) flanked by *frt* recombination sites (*grey triangles*), were sequentially introduced into the *Tff1* locus of a BAC that carried the *Tff1* gene (**ii**, *black box*). Following recombination in *E.coli* and removal of the resistance markers, the *Tff1-CreERT2* BAC (**iii**) was liberated by *Not*I restriction digest from its vector backbone harbouring the chloramphenicol (*Cam*) resistance marker (*grey box*). Note, for simplification, recombination of the entire gene is depicted, rather than the exact parts of exons 1 and 2 that were replaced by each transgene (*refer to Fig. 1A*).

**(C)** Validation of the purified *Tff1-CreERT2* BAC (Clone E7) by PCR. The relative positions of the transgene-specific 5’ and 3’ primer pairs and the size of the corresponding PCR bands are indicated (M, DNA marker/standard).

**(D)** The *Not*I-liberated, linearized *Tff1-CreERT2* BAC fragment was injected into the male pronucleus of fertilized CBB6F1 mouse oocytes. Zygotes were transferred to the oviducts of pseudo-pregnant females and the pups were analysed by diagnostic PCR for the integration of the transgenic BAC into the genome. DNA from tail biopsies of four independently derived founder mice (#16, 47, 52 and 59) was analysed by PCR using the primers depicted in panel B. Purified BAC DNA was used as a positive control (PC) and DNA from wild-type mice (WT) or water (NC) as a negative control.

**(E)** Representative sections of stomachs from Tg(*Tff1-CreERT2*);*LacZ* mice stained for -galactosidase activity 6h and 120 days after a single injection of oil as vehicle controls (No TAM) for the stomach sections shown in **Fig1 A**. Scale bars represent 50µm.

**Figure S2. *Tff1-CreERT2* transgene, endogenous *Tff1* and *Lgr5* expression in the stomach.**

**(A)** Representative images of anti-Tff1 immunohistochemical stainings on stomachs from Tg(*Tff1-CreERT2*);*LacZ* mice. Scale bars represent 50µm.

**(B)** qRT-PCR expression analysis for endogenous *Tff1* and transgenic *Tff1-CreERT2* in three distinct regions of the stomach (fundus, corpus, antrum) and the proximal small intestine (prox. SI) of Tg(*Tff1-CreERT2*) mice (n≥6). Following normalization to 18S rRNA expression, results are shown as mean fold change ± SEM relative to the fundus samples (**\*\*** *P*<0.01, # *P*<0.02).

**(C)** Endogenous *Tff1* gene expression in Lgr5-positive cells was analysed via qPCR. Lgr5-CreGFP-positive and negative cells were sorted from colon or stomach tissue pooled from 6 Lgr5-CreGFP-positive mice. qPCR results are plotted as fold change of Lgr5-positive cell versus Lgr5-negative cells from colon and stomach.

**Figure S3. Tff1-CreERT2-mediated β-galactosidase reporter activity in various organs.**

**(A)** Representative whole-mount stomachs from vehicle-treated Tg(*Tff1-CreERT2*);*LacZ* mice (*Cre*+;*LacZ*) or tamoxifen-treated Tg(*Tff1-CreERT2*)-negative *LacZ* mice (*Cre*–;*LacZ*) stained for β-galactosidase activity.

**(B)** β-galactosidase activity was detected in the Brunner’s glands and lungs of Tg(*Tff1-CreERT2*);*LacZ* mice, collected three days after tamoxifen administration (5 consecutive days; 1 mg/20 g body weight; twice daily). Scale bars: 100 μm.

 **(C)** Summary of β-galactosidase activity staining observed in Tff1-CreERT2-negative (Cre-; n=3) and Tff1-CreERT2-positive (Cre+; n≥3) mice. Mice were ip injected twice daily for 5 consecutive days with 1mg/ 20g body weight of tamoxifen, and 3 days after the last tamoxifen injection organs were stained for β-galactosidase activity. Tissues showing blue stain are scored as “+” and as “-” when no blue staining was observed.

**(D)** No β-galactosidase activity was detected in the pancreas of representative low (left) and high (right) magnification images of X-gal stained pancreas from Tff1-CreERT2-negative and Tff1-CreERT2-positive mice (Scale bars 100µm).

**Figure S4. KrasG12D-induced gastric tumorigenesis is associated with aberrant mucous production, ectopic expression of intestinal genes and immune cell infiltration.**

**(A)** Tamoxifen-dependent activation of CreERT2 in Tg(*Tff1-CreERT2*);*Kras*LSL-G12D/+ mice and associated excision of the *loxP* flanked (*grey triangles*) transcriptional termination sequence (*red box*) enables expression of the conditional *Kras*G12D oncogene specifically in the glandular stomach. The presence of the recombined *Kras*G12D allele was confirmed by allele-specific PCR of DNA prepared from fundus (fun), antrum (ant) and tumor (tum) tissue using the indicated primers F1, F2 and R. The 651 bp band indicates recombination and activation of the *Kras*G12D allele and was only detected in the glandular stomach of Tg(*Tff1-CreERT2*);*Kras*LSL-G12D/+ mice following tamoxifen administration. The 623 bp band is specific for the *Kras* wild-type allele (*Kras*WT) and the 505 bp/751 bp bands indicate the non-recombined *Kras*LSL-G12D allele (M, DNA marker/standard).

**(B)** Representative Alcian Blue and Periodic acid-Schiff (PAS) stainings of gastric tumor and antrum sections from tamoxifen-treated *Kras*LSL-G12D/+ mice of the indicated *Tff1-CreERT2* genotypes demonstrating extensive production of acidic sulphated mucus (*blue*) and neutral glyco-/muco-proteins (*magenta*), respectively. Scale bars: 200 µm (*insets* 50 µm).

**(C)** Images of immunohistochemical stainings for immune cell infiltrates, macrophages (F4/80), T cells (CD3) and B cells (B220) are shown from tumors and tumor-adjacent submucosa of tamoxifen-treated Tg(*Tff1-CreERT2*);*Kras*LSL-G12D/+ mice. Scale bars: 100 μm (*insets* 20 μm).

**(D-F)** Representative immunohistochemical stainings for Tff2 (**D**), Cdx2 (**E**) and gpA33 (**F**) on stomach sections from tamoxifen-treated *Kras*LSL-G12D/+mice of the indicated *Tff1-CreERT2* genotypes. Ectopic expression of the intestinal markers gpA33 and Cdx2 is induced in gastric epithelial cells of tumor-bearing *Tff1-CreERT2*-positive mice (C-D). Arrows indicate regions of nuclear Cdx2 expression (D). Scale bars: 100 µm (*insets* 20 µm).

**Figure S5. Gastric-specific activation of oncogenic BrafV600E triggers tumorigenesis associated with increased Erk and Stat3 phosphorylation.**

**(A)** Representative whole-mount stomachs from *Braf* V600E/+ mice of the indicated Tff1-CreERT2 genotypes, collected 8 months after tamoxifen administration (arrowheads indicate tumors). H&E-stained cross-sections, cut along the dotted line, extend from the fore-stomach to the proximal end of the small intestine (SI). Boxed regions are shown at higher magnifications in panels B-C.

**(B-C)** The corpus and antrum of tamoxifen-treated Tff1-CreERT2-negative mice remain histologically normal (B), whereas the mucosa of tamoxifen-treated Tff1-CreERT2-positive mice shows severe pathological changes (C). The corpus exhibits inflammatory infiltrates as well as dilated and disorganized glands (i, ii); the distorted and hyperplastic glands in the antrum are characterized by chronic gastritis (iv) and extensive mucus metaplasia with goblet-like cells (iii). The tumors display glandular structures at the luminal edges (v) and mucus-containing, goblet-like cell clusters (arrows) in the core (vi). Scale bars: 50 μm.

**(D)** Representative pErk1/2 and pY-Stat3 immunostainings on tumor sections from Tff1-CreERT2-positive-*Braf* V600E/+ mice collected 9 months after tamoxifen administration. Scale bars: 200 μm (insets 50 μm).

**(E)** Immunoblot analysis of antral and pooled tumor tissue from individual *Braf* V600E/+ mice of the indicated Tff1-CreERT2 genotype collected nine months after tamoxifen administration.

**Figure S6. Tff1-CreERT2 activity in established gastric tumors of *gp130*F/F mice.**

**(A)** qRT-PCR expression analysis of endogenous *Tff1* in the antrum (ant) of wild-type (*gp130*+/+) mice and in the unaffected antrum and pooled tumors (tum) of 10-week old *gp130*F/F mice (n≥6). Following normalization to 18S rRNA expression, results are shown as mean fold change ± SEM relative to the *gp130*+/+ antrum samples (**\*** *P*<0.05).

**(B)** Representative whole-mount stomachs from *gp130*F/F;Tg(*Tff1-CreERT2*);*LacZ* mice stained for β-galactosidase activity following 5 consecutive days of tamoxifen administration (1 mg/20 g body weight; twice daily) and the indicated follow-up period (*top row*). Tumors are outlined with red circles. Corresponding sections were counterstained with nuclear fast red (*bottom rows*). Scale bars: 500 μm (*insets* 100 μm).

**(C)** Representative cross sections of tumors from gp130F/F;Tg(*Tff1-CreERT2*);*LacZ* mice 30 days after tamoxifen administration and stained for -galactosidase activity as well as for protein expression by immunohistochemical analysis with an anti--galactosidase antibody. Shown are images from Tff1-CreERT2 positive (left) and Tff1-CreERT2 negative (right) mice. Sections stained without the primary anti--galactosidase antibody are shown as controls. Slides were counter stained with Haematoxylin Scale bars: 100 μm.

**Figure S7. Co-activation of Kras and gp130 signalling induces intestinalization associated with severe atrophy.**

**(A)** H&E, Alcian Blue (AB) and Periodic-Schiff (PAS)-stained sections of the oxyntic gastric mucosa (corpus region) of *Tff1-CreERT2*-positive mice of the indicated genotypes, collected at the indicated time after tamoxifen administration (5 consecutive days; 1 mg/20 g body weight; twice daily). Boxed regions are shown at higher magnifications. Scale bars: 100 μm.

**(B)** H&E-stained sections of the oxyntic gastric mucosa (corpus region) of tamoxifen-treated *Tff1-CreERT2*-positive mice of the indicated genotypes. Boxed regions are shown at higher magnification and reveal a loss of parietal cells (*arrows*) in *gp130*F/F;*Kras*G12D/+ mice. Scale bars: 50 μm. Parietal cell (PC) atrophy was quantified by enumerating the average number of PCs per mm length of the mucosa. Results are mean ± SEM (n≥4 mice per cohort; \*\*\* *P*<0.001).