

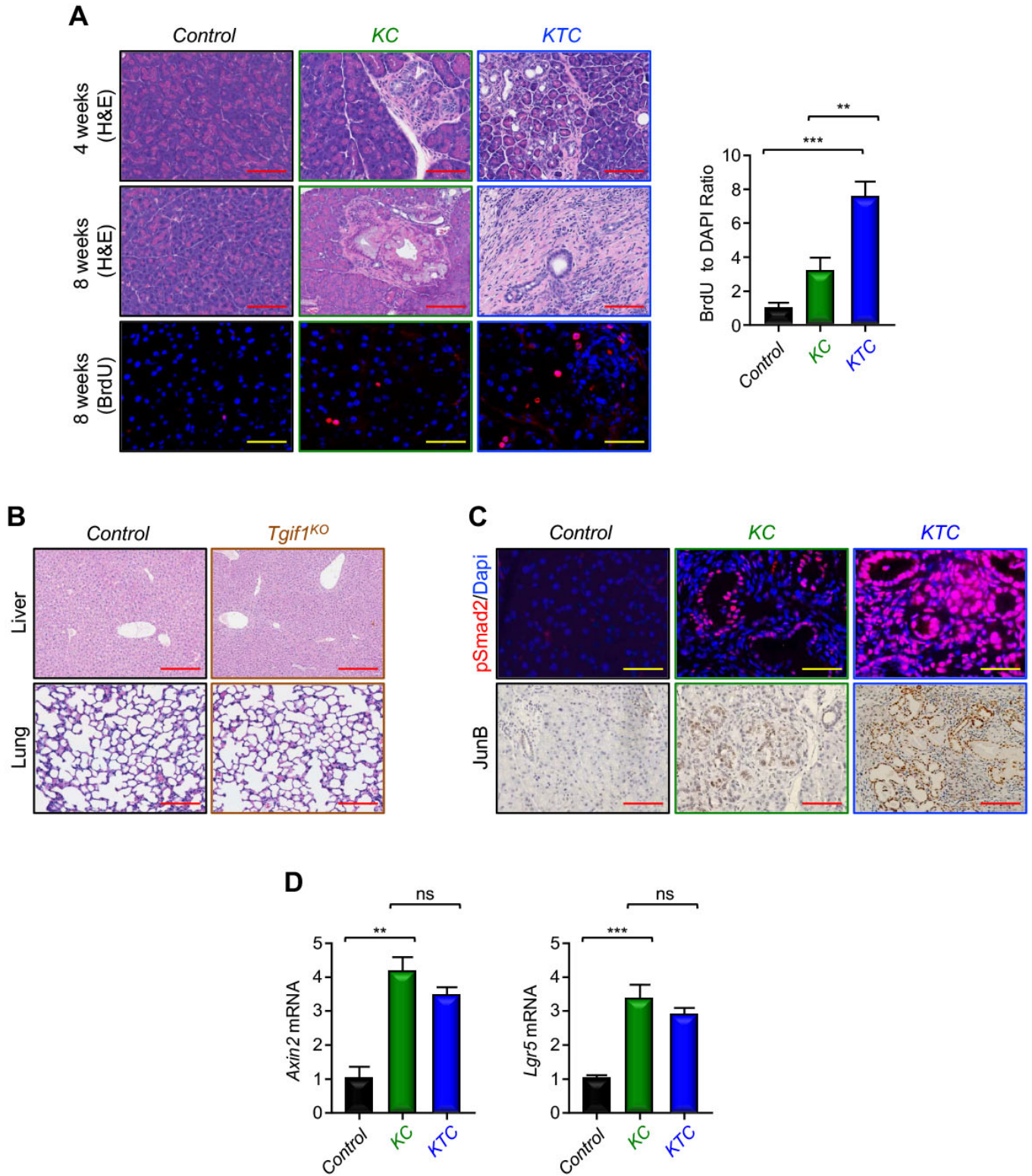
Appendix

Table of Contents

1. Appendix Figures S1-S6

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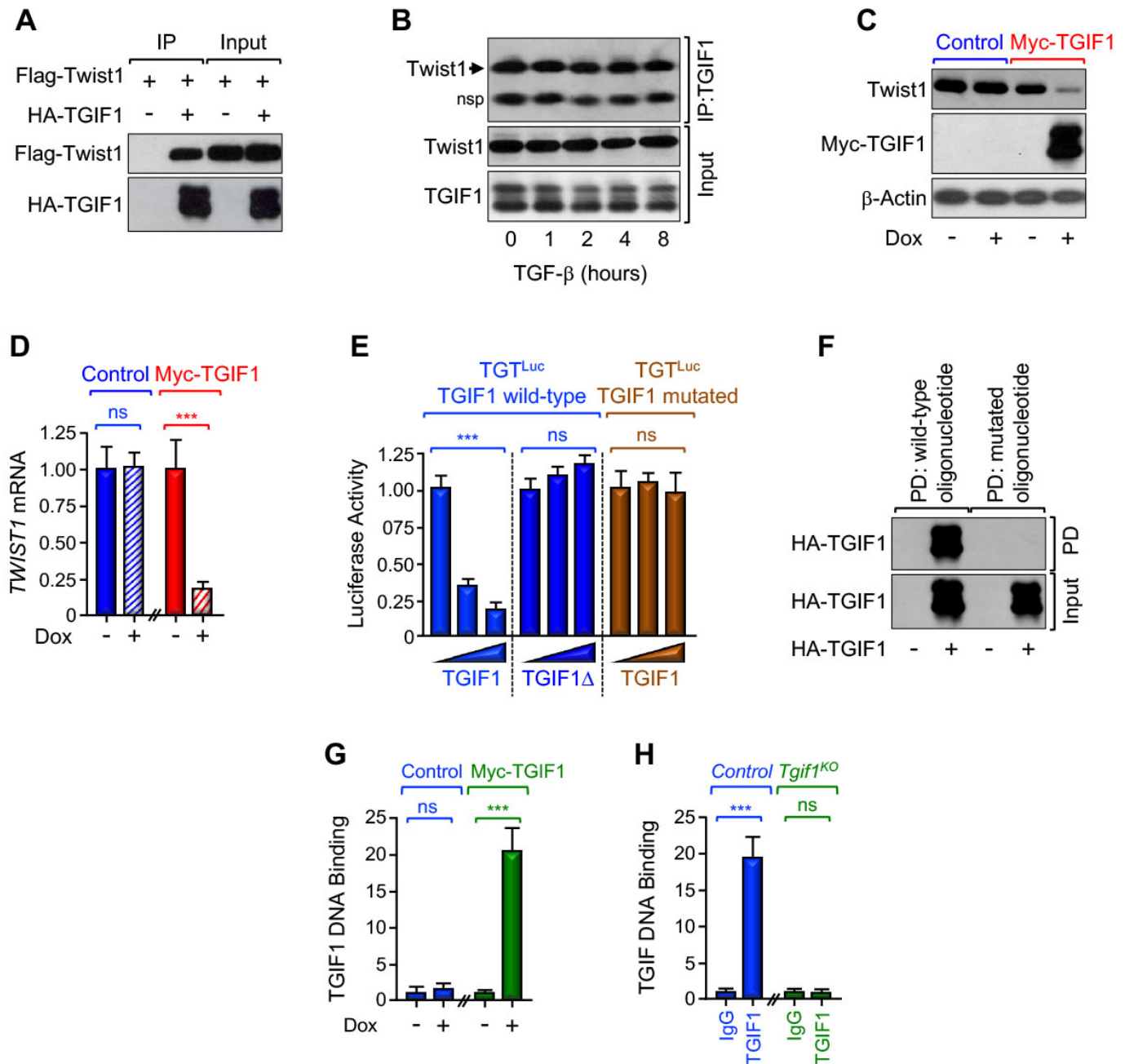
Appendix Fig S1



Appendix Figure S1. *Tgif1* deletion accelerates PDAC formation and progression.

- A** Control, *KC*, or *KTC* mice at 4 or 8 weeks of age were injected with BrdU, and FFPE pancreatic sections were stained with H&E or immunostained with anti-BrdU and revealed by IF and DAPI. Representative pictures at 20x (H&E) or 40x (IF-BrdU) are shown (n=30). Scale bars, 200 μ M (H&E) or 100 μ M (IF-BrdU).
- B** FFPE liver or lung sections from control or *Tgif1*^{KO} mice were stained with H&E. Representative pictures at 10x (Liver, H&E) or 20x (Lung, H&E) are shown (n=30). Scale bars, 400 μ M (Liver, H&E) or 200 μ M (Lung, H&E).
- C** FFPE pancreatic sections from control, *KC* or *KTC* mice were immunostained with antibodies to pSmad2 or JunB and revealed by IF and DAPI or IHC. Representative pictures at 20x (IHC-JunB) or 40x (IF-pSmad2) are shown (n=30). Scale bars, 200 μ M (IHC-JunB) or 100 μ M (IF-pSmad2).
- E** Expression of *Axin2* and *Lgr5* in pancreas from control, *KC* or *KTC* mice (same as in Fig 2D) was analyzed by qRT-PCR (n=6). Data are expressed as mean \pm SEM. **p< 0.01; ***p< 0.001; ns: not significant; based on a two-tailed Student's test.

Appendix Fig S2



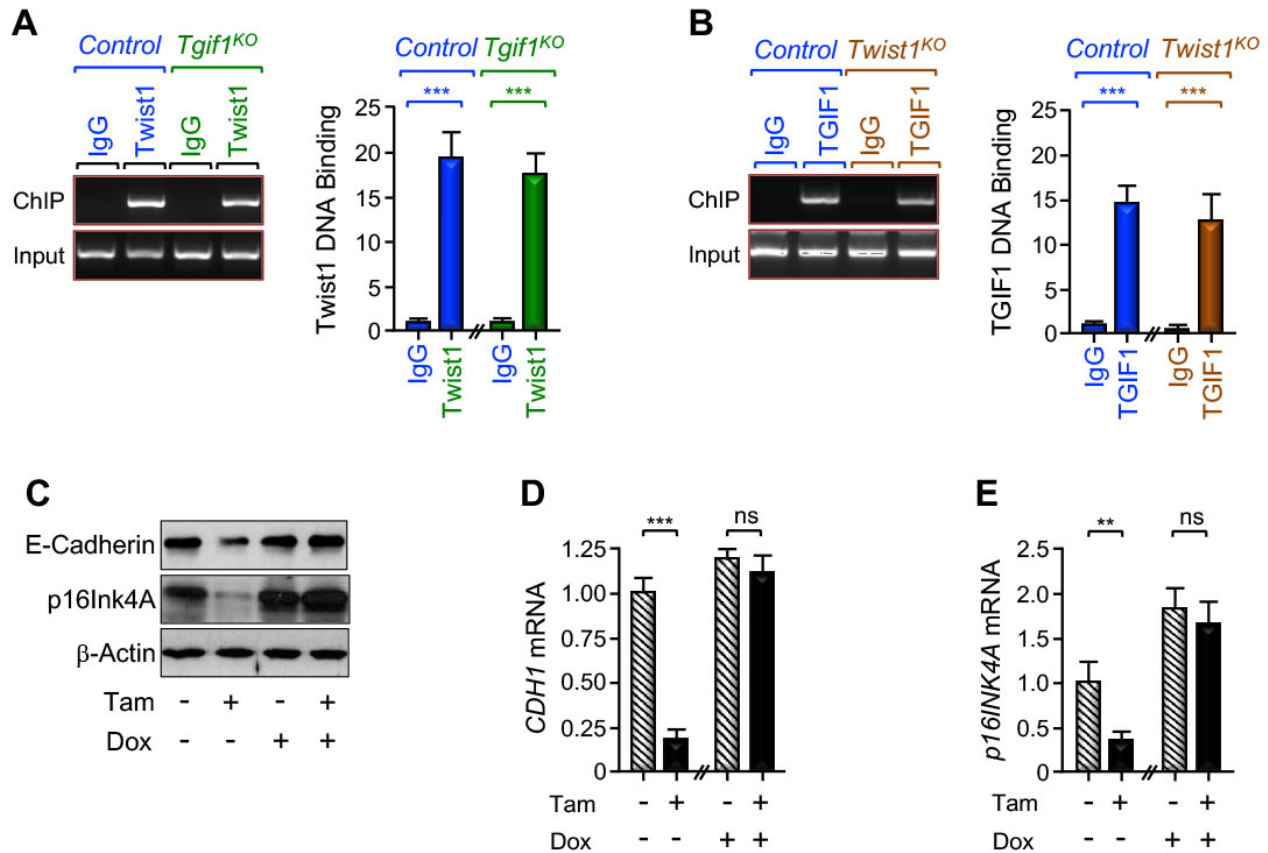
Appendix Figure S2. TGIF1 associates with and inhibits Twist1 expression.

- A** HEK293T cells were transfected with expression vectors encoding Flag-Twist1 and HA-TGIF1 and analyzed for the interaction of TGIF1 with Twist1 by co-immunoprecipitation followed by immunoblotting (IP: immunoprecipitation).
- B** Panc-1 cells were treated with TGF- β for increasing times, and protein extracts were immunoprecipitated with anti-TGIF1 antibody and analyzed by immunoblotting using anti-Twist1 antibody.
- C,D** PL45 stably expressing Dox-inducible TGIF1 were treated with vehicle or Dox for 48 hr and analyzed for Twist1 expression by immunoblotting (C) or qRT-PCR (D) (n=6).

- E** MIAPaCa-2 cells were transfected with wild-type or mutated Twist1 (TGIF1 binding site) luciferase reporters (TGT^{Luc}) together with increasing amounts of wild-type TGIF1 or a mutant defective in its ability to bind the general HDAC transcriptional corepressor complex. Luciferase activity was measured 48 hr following transfection and normalized on the basis of co-transfected Renilla luciferase (n=6).
- F** HA-TGIF1 was purified from transfected HEK293T cells, incubated with biotinylated oligonucleotides encompassing the TGIF1 binding site (wild-type or mutated), pulled down with streptavidin (PD), and analyzed by immunoblotting using anti-TGIF1 antibody.
- G** PL45 stably expressing Dox-inducible TGIF1 were treated with vehicle or Dox for 48 hr and analyzed for binding of TGIF1 to the *Twist1* promoter by CHIP and qPCR (n=6).
- H** Pancreatic chromatin from control or *Tgif1*^{KO} mice was analyzed for binding of TGIF1 to the *Twist1* promoter by CHIP and qPCR (n=6).

Data information: For (D, E, G and H), data are expressed as mean \pm SEM. *** $p < 0.001$; ns: not significant; based on a two-tailed Student's test.

Appendix Fig S3



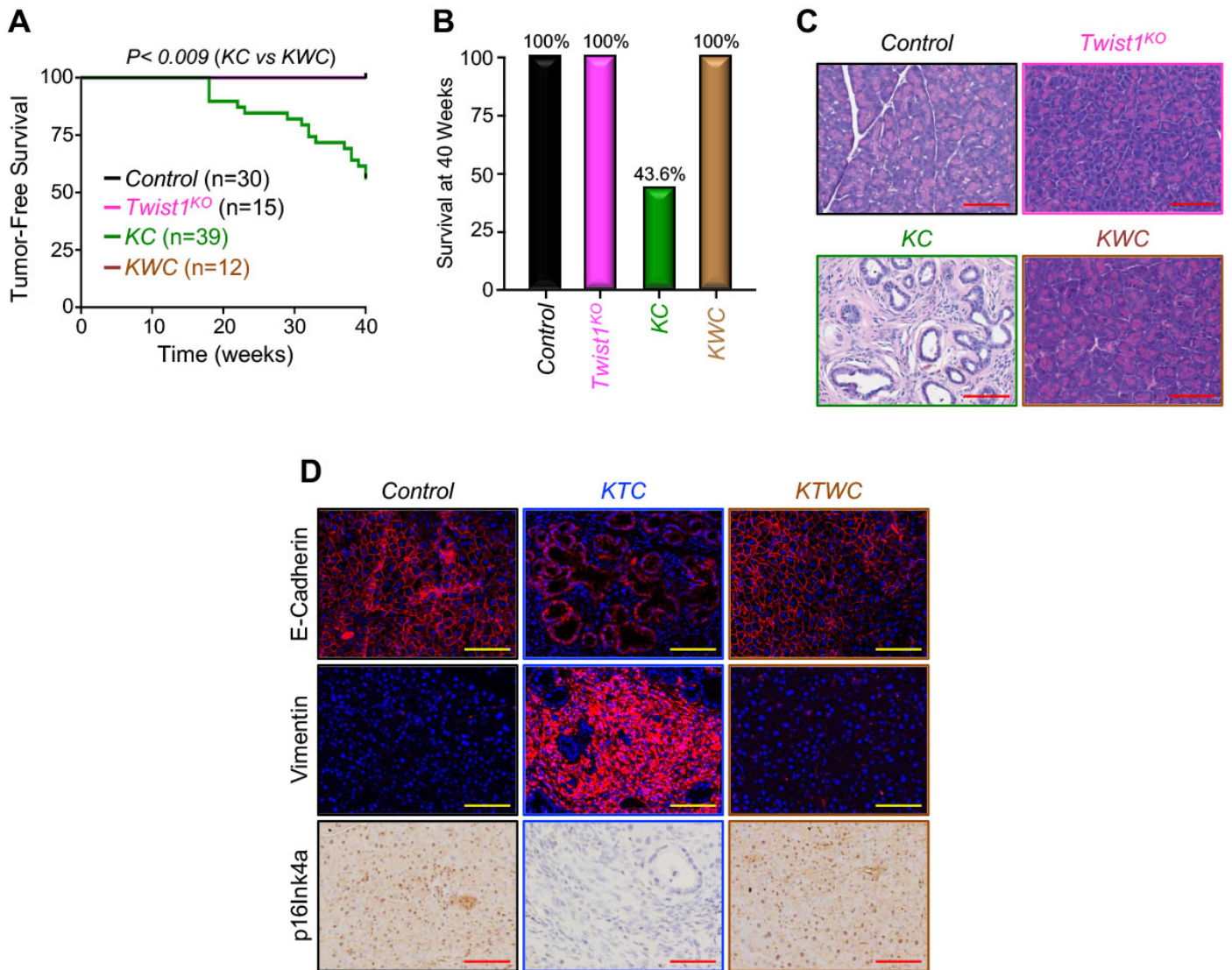
Appendix Figure S3. TGIF1 inhibits Twist1 transcriptional activity (related to Fig 4).

A,B Chromatin were prepared from pancreatic tissues of wild-type, *Tgif1*^{KO} or *Twist1*^{KO} mice and analyzed for binding of Twist1 (A) or TGIF1 (B) to the Twist1 promoter by ChIP and agarose gel or qPCR (n=6).

C-E PL45 stably expressing both Dox-inducible TGIF1 and Tam-inducible Twist1 (*Twist1*^{ER}) were treated with Tamoxifen (Tam) and/or Dox for 48 hr and analyzed for E-Cadherin or p16Ink4A expression by immunoblotting or qRT-PCR (n=6).

Data information: For (A, B, D and E), data are expressed as mean ± SEM. **p < 0.01; ***p < 0.001; ns: not significant; based on a two-tailed Student's test.

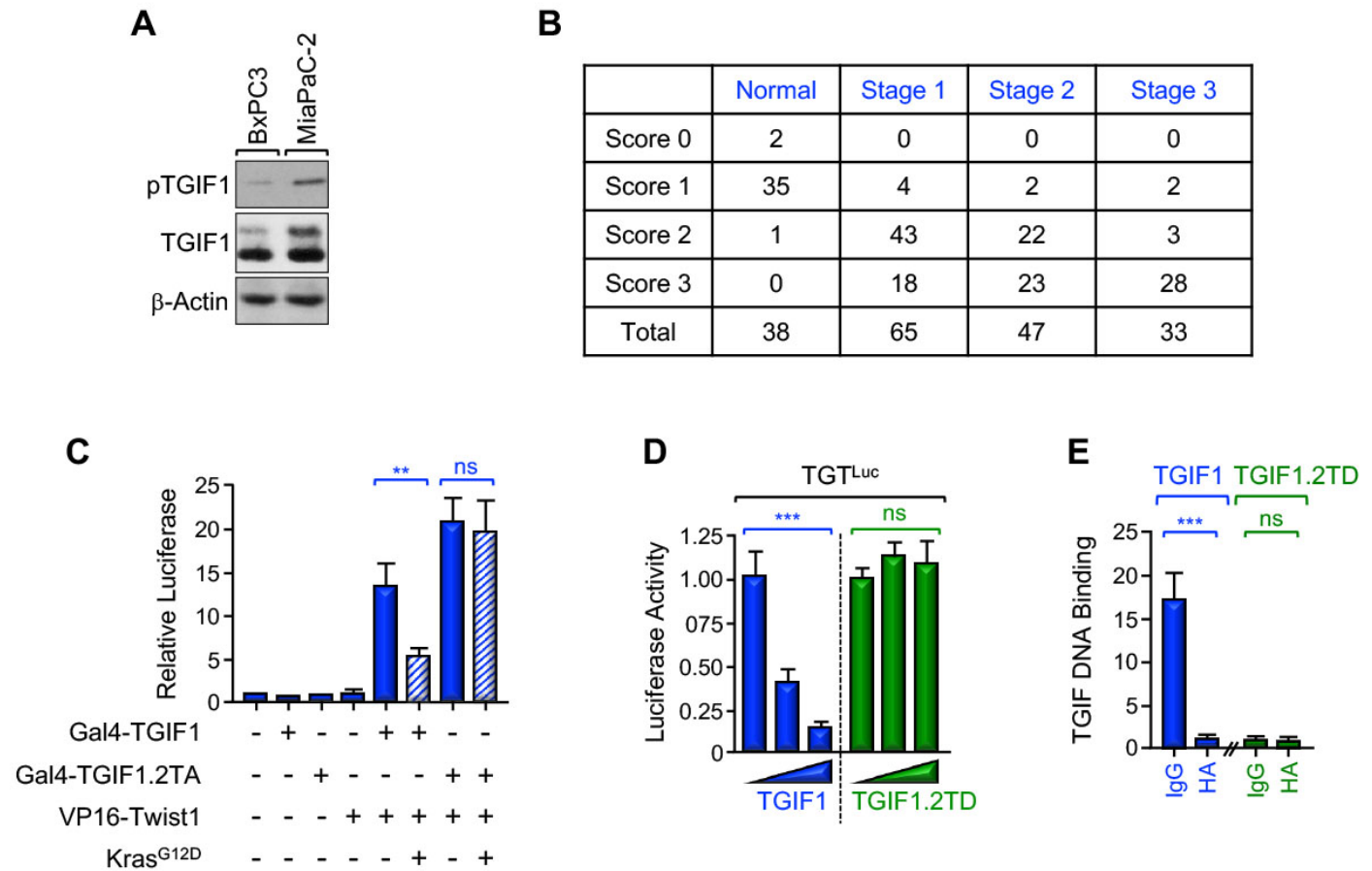
Appendix Fig S4



Appendix Figure S4. *Twist1* inactivation blocks PDAC formation in KC mice.

- A** Kaplan-Meier survival analysis of control, *Twist1*^{KO}, KC and KWC mice. $P < 0.009$ by log-rank test for significance
- B** Survival of control, *Twist1*^{KO}, KC and KWC mice at 40 weeks of age, time at which the experiment was terminated.
- C** FFPE sections of pancreas from control, *Twist1*^{KO}, KC and KWC mice were stained with H&E. Representative pictures at 20x are shown (n=12 to 30). Scale bars, 200 μ M. Some pictures were re-used from Fig 5B.
- D** FFPE pancreatic sections from control, KTC or KTWC were stained with antibodies to E-Cadherin, Vimentin or p16Ink4a, and revealed by IF or IHC. Representative pictures are at 20x shown (n=12 to 30). Scale bars, 200 μ M.

Appendix Fig S5

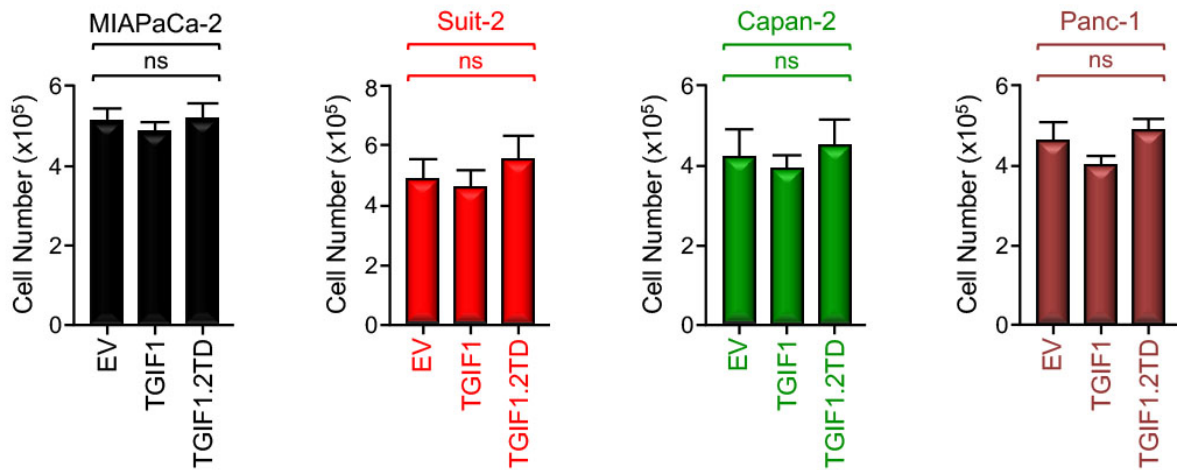


Appendix Figure S5. Phosphorylation-mediated inactivation of TGIF1 in PDAC.

- A** Expression of TGIF1 and pTGIF1 in MIAPaCa-2 and BxPC3 cells was examined by immunoblotting.
- B** Expression of pTGIF1 in human tissue microarrays of human PDAC samples was analyzed by immunohistochemistry. Representative pictures at different stages are shown. Samples were scored 0 (negative), 1 (low), 2 (moderate) and 3 (high) under a microscope in a blinded manner and results presented as dot plot.
- C** BxPC3 cells were transfected with pG5E1b-Luc together with VP16-Twist1 and Gal4-TGIF1 or Gal4-TGIF1.2TA in the presence or absence of Kras^{G12D}. Luciferase activity was measured 48 hr following transfection and normalized on the basis of co-transfected Renilla luciferase (n=6).
- D** MIAPaCa-2 cells were transfected with the Twist1 luciferase reporter TGT^{Luc} together with increasing amounts of TGIF1 or TGIF1.2TD. Luciferase activity was measured 48 hr following transfection as described in B (n=6).
- E** MIAPaCa-2 cells were transfected with TGIF1 or TGIF1.2TD expression vectors, selected with G418 and resistance colonies were pooled (n=6). Binding of TGIF1 to the Twist1 promoter was examined by ChIP and qPCR.

Data information: For (B-D), Data are expressed as mean \pm SEM. **p < 0.01; ***p < 0.001; ns: not significant; based on a two-tailed Student's test.

Appendix Fig S6



Appendix Figure S5. Analysis of the effects of short-term expression of TGIF1 or TGIF1.2TD on the proliferation of human PDAC cell lines.

MIAPaCa-2, Suit-2, Capan-2, and Panc-1 cells were transfected with empty vector (EV), wild-type (Flag-TGIF1) or phosphorylation mimic mutant (Flag-TGIF1.2TD) of TGIF1 and counted 48 hr later before starting selection with G418 (n=6). Data are expressed as mean \pm SEM. ns: not significant; based on a two-tailed Student's test.