

## Legend of Supplemental Figures

### **Supplemental Fig. S1. Elevated c-MYC expression in human pancreatic cancer cell lines**

Western blot analysis to determine the expression of c-MYC and Kras in pancreatic cancer cell lines compared to immortalized normal human pancreatic ductal cells expressing hTERT (HPNE). HPNE cells were kindly provided by Dr. Ouellette (University of Nebraska Medical Center).

### **Supplemental Fig. S2. c-Myc is highly upregulated in a mouse model for pancreatic cancer**

**A.** Western blot analysis to determine the expression of c-Myc in pancreatic tissues of 4- and 10-month-old animals and a pancreatic tumor of mice that express mutant Kras (Pdx1-Cre/*LSL-Kras*<sup>G12D</sup>); **C.** 4-month-old wildtype control mouse. **B.** Immunofluorescence staining of c-Myc (red, nuclear) and CK19 (green) in a mouse pancreatic precursor lesion, high-grade PanIN (**b**) and in late-stage ductal adenocarcinomas (**d**) of genetically engineered mice that express mutant *Kras* in the pancreatic ductal epithelium and that are haploinsufficient in *p53* (PTF1A/p48-Cre/*LSL-Kras*<sup>G12D</sup>/*p53*<sup>fl/+</sup>). Slides were counterstained with DAPI. Panels **a.** and **c.** are serial H&E-stained section of panels **b.** and **d.** The bar represents 50  $\mu$ m.

### **Supplemental Fig. S3. Levels of c-Myc (exogenous and endogenous) in ductal and poorly differentiated pancreatic adenocarcinomas that were induced through upregulation of this oncogene in comparison to pancreatic tumors from Pdx1-Cre *LSL-Kras*<sup>G12D</sup> animals that carry two wildtype or one mutant allele of *p53*.**

Note that, besides showing lower expression of CK19, poorly differentiated pancreatic carcinomas contain less infiltrating stroma (i.e., vimentin-expressing cells). Expression of E-Cadherin versus vimentin is indicative of the relative contribution of adenocarcinoma cells and tumor-associated stromal cells within individual pancreatic cancers. Since elevated expression of c-Myc (exogenous and/or endogenous) is mostly confined to carcinoma cells as apposed to cancer-associated fibroblasts (see Fig 1A and Supplemental Fig 2B), it is evident that the

levels of total c-Myc in cancers initiated through exogenous expression of this oncogene does not greatly exceed the levels of endogenous c-Myc in tumors associated with activation of Kras.

**Supplemental Fig. S4. Expression of Muc1, E-Cadherin, Pdx1, and Sox9 in the normal pancreas as well as ductal carcinomas and poorly differentiated carcinomas of transgenic mice expressing exogenous c-Myc.**

Arrows in the left panels point to the location of ducts in the normal pancreas; IC, islet cells; bars represent 50  $\mu$ m.

**Supplemental Fig. S5. Elevated levels of exogenous c-Myc induces expression of Bax and active Caspase-3.**

**A.** H&E and immunohistochemical staining of c-Myc and cleaved Caspase-3 in serial sections of an untransformed pancreatic duct (left panels) as well as ductal and poorly differentiated adenocarcinomas (middle and right panels) in mice that express exogenous c-Myc in the pancreas. **B.** Western blot analysis of Bax and active Caspases-3/7 in pancreatic cancers before (-Dox) and 3 days after ablation of c-Myc expression (+Dox). The levels of CK19 and E-Cadherin in comparison to vimentin were used to control equal contribution of cancer cells between the tumors prior to tumor regression in response to treatment with Dox.

**Supplemental Fig. S6. Ablation of c-Myc expression leads to reduced proliferation, increased expression of p53 as well as autophagy-related protein LC3 but a reduction in the levels of active Caspase-3.**

**A.** Immunofluorescence staining of Ki67 and E-Cadherin in PDAC induced by expression of exogenous c-Myc (-Dox) and 3 days after c-Myc ablation (+Dox). **B.** Western blot analysis of c-Myc, p53 as well as the Microtubule-associated protein light chain 3 (LC3B) to monitor autophagy in pancreatic cancers before (-Dox) and 3 days after ablation of c-Myc expression (+Dox). **C.** Immunofluorescence staining of Beclin (also known as autophagy-related gene 6, Atg6) in PDAC before and after ablation of c-Myc. **D.** Western blot analysis to monitor the expression of autophagy-

related proteins in pancreatic cancer cells cultured *ex vivo* prior to (-Dox) and after downregulation of c-Myc. **E.** Relative survival of cultured pancreatic cancer cells with or without c-Myc ( $\pm$ Dox) in the presence or absence of cloroquine.

**Supplemental Fig. S7. Downregulation of active Caspase-3 and upregulation of LC3 in pancreatic cancers that lack *Cdkn2a*.**

Western blot analysis to monitor changes in the expression of p53, Caspase-3, and LC3 in response to the downregulation of c-Myc in tumors that are wildtype or deficient in *Cdkn2a*.

**Supplemental Fig. S8. Residual cancer cells do not express c-Myc, lack nuclear staining of Ki67 and are TUNEL negative**

**Upper panels:** Immunofluorescence (IF) staining of c-Myc (red, nuclear) and GFP (green, cytoplasmic) in pancreatic cancers (-Dox, left) and residual cancer cells following tumor regression in response to downregulation of c-Myc (+Dox, right); arrows in the right panel indicate c-Myc-negative nuclei within residual cancer cells; T, tumor; N, adjacent normal tissue (left panels). **Middle panels:** IF staining of Ki67 (red, nuclear) and GFP (green, cytoplasmic), arrows indicate Ki67-negative nuclei within residual cancer cells. **Lower panels:** TUNEL labeling (green) of nuclei of apoptotic cells and IF staining against GFP (red, cytoplasmic), arrows indicate TUNEL-negative nuclei within residual cancer cells; bars in all panels represents 25  $\mu$ m.