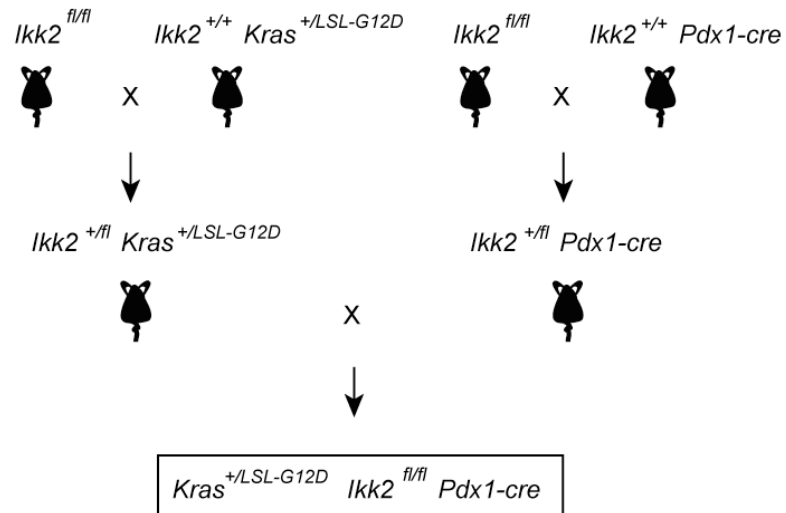
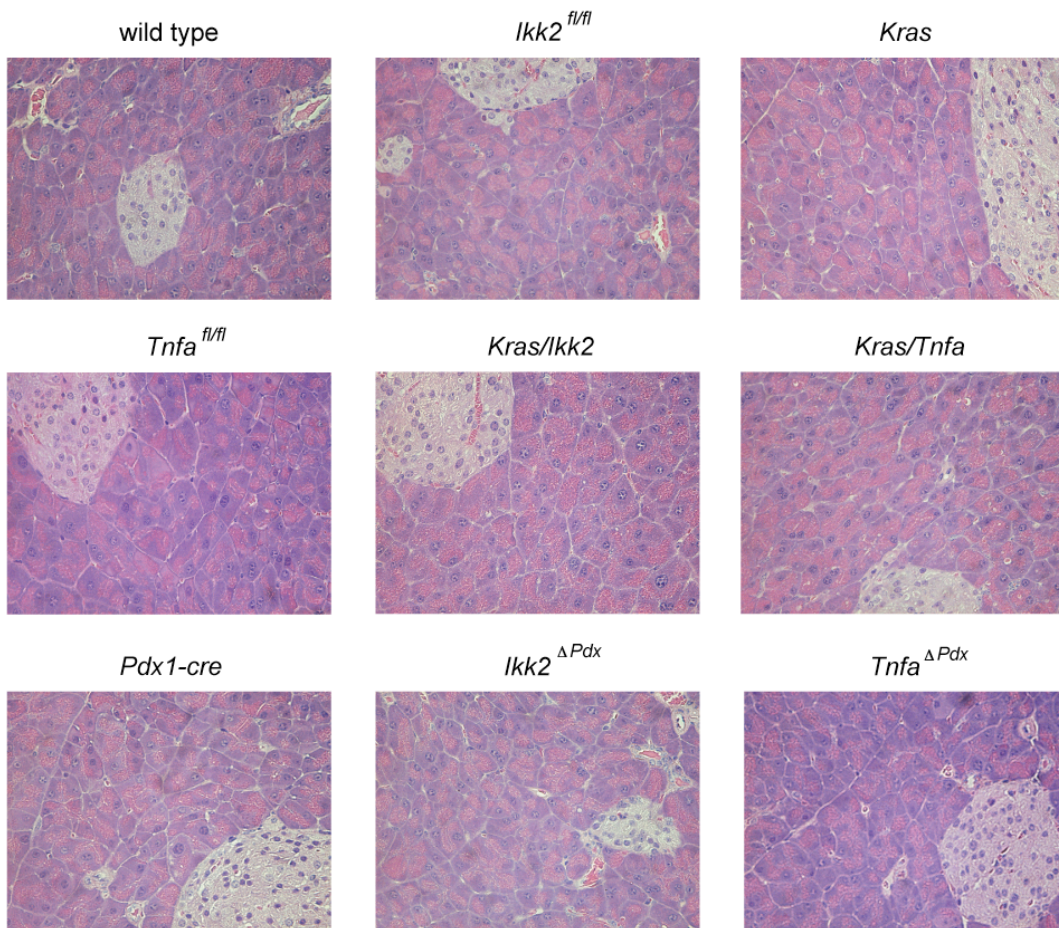


## Supplemental Figures

A

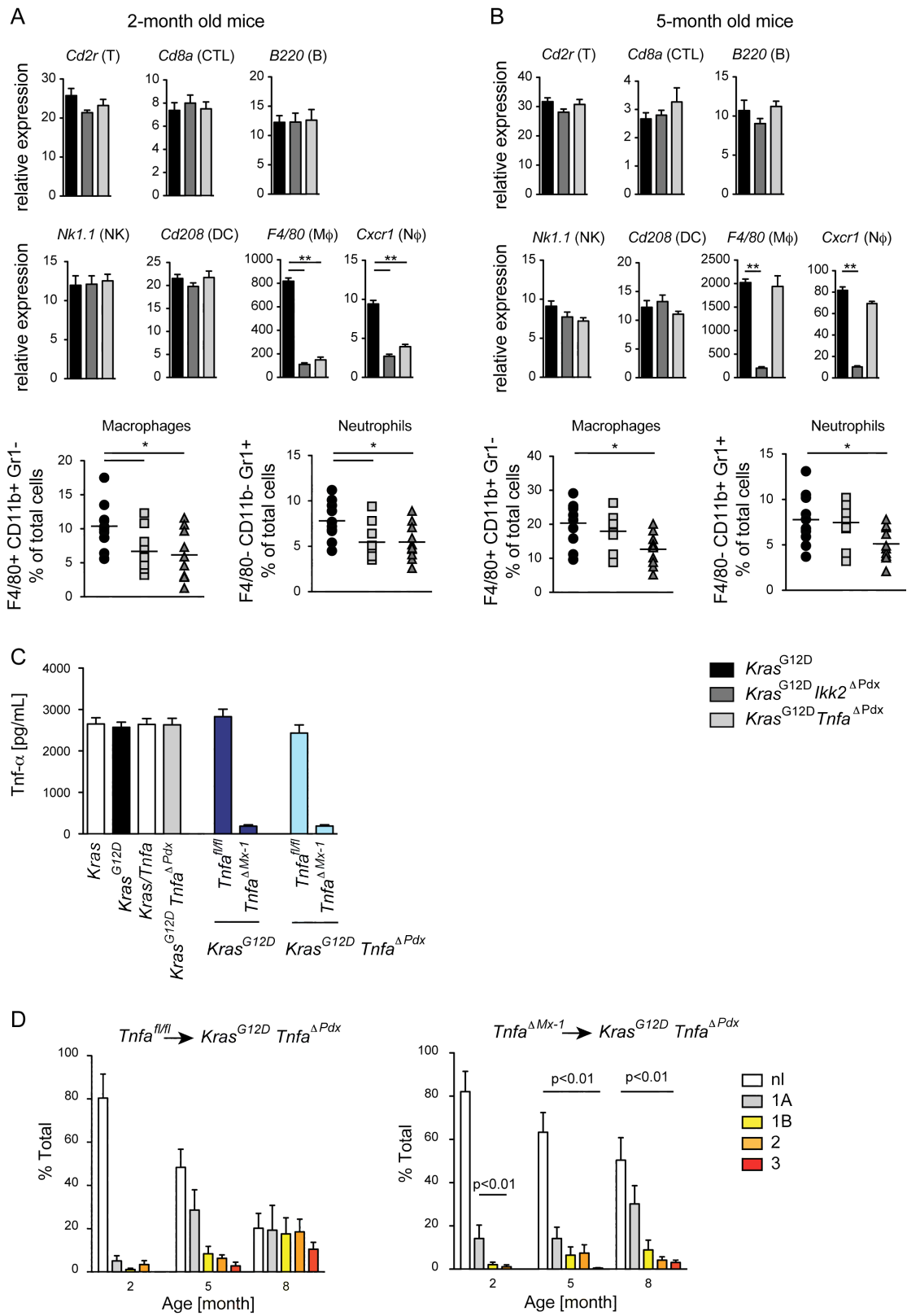


B

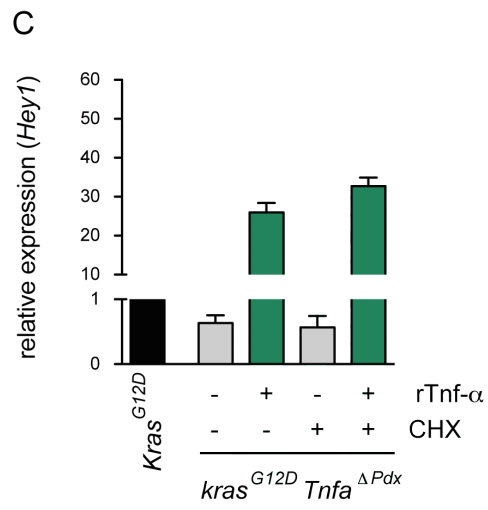
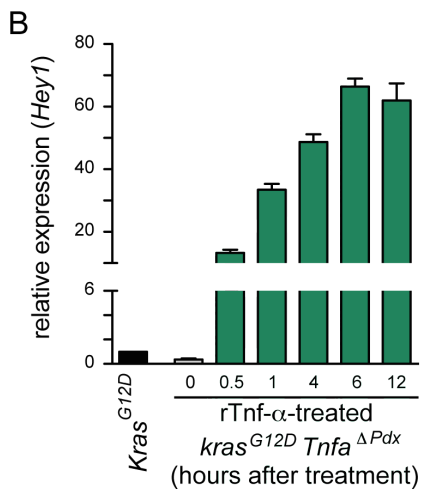
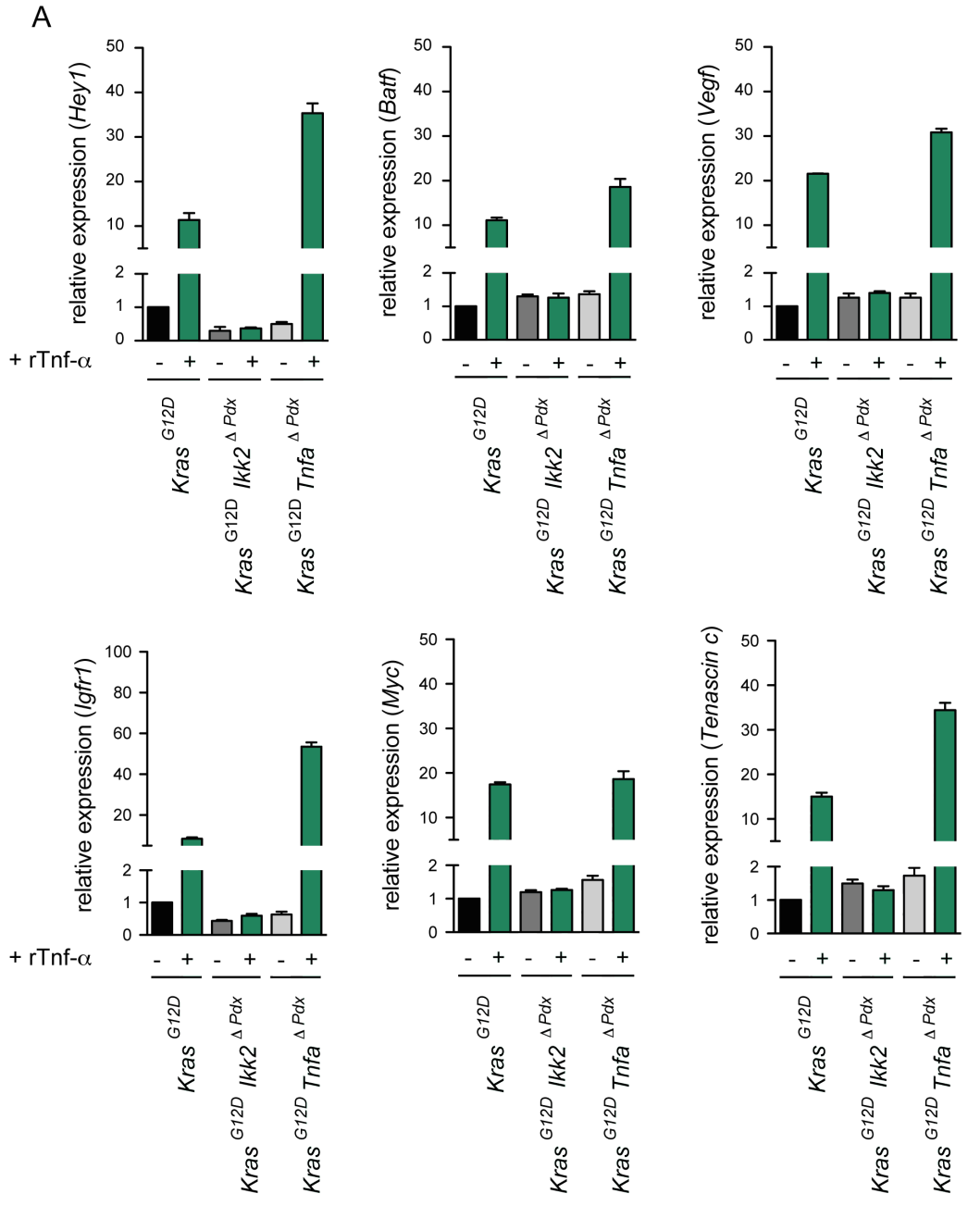


Supplemental Figure 1. Generation of  $Kras^{G12D}$  mice with targeted deletion of  $Ikk2$  or  $Tnfa$ . (A)  $Kras^{+LSL-G12D}$  and  $Pdx1-cre$  mice were crossed

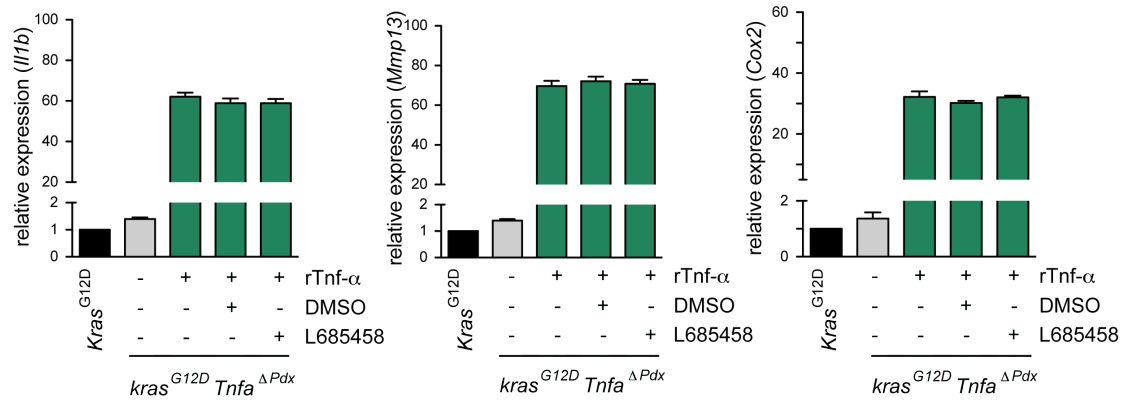
with the *Ikk2<sup>fl/fl</sup>* strain. The progeny was intercrossed to generate *Kras<sup>G12D</sup>Ikk2<sup>ΔPdx</sup>*. *Kras<sup>G12D</sup>Ikk2<sup>ΔPdx</sup>* were developed by a similar strategy. **(B)** H&E stained sections of pancreases from the indicated strains at 5 months of age. No signs of major abnormalities were detected in the pancreases of the indicated strains. Magnification x20.



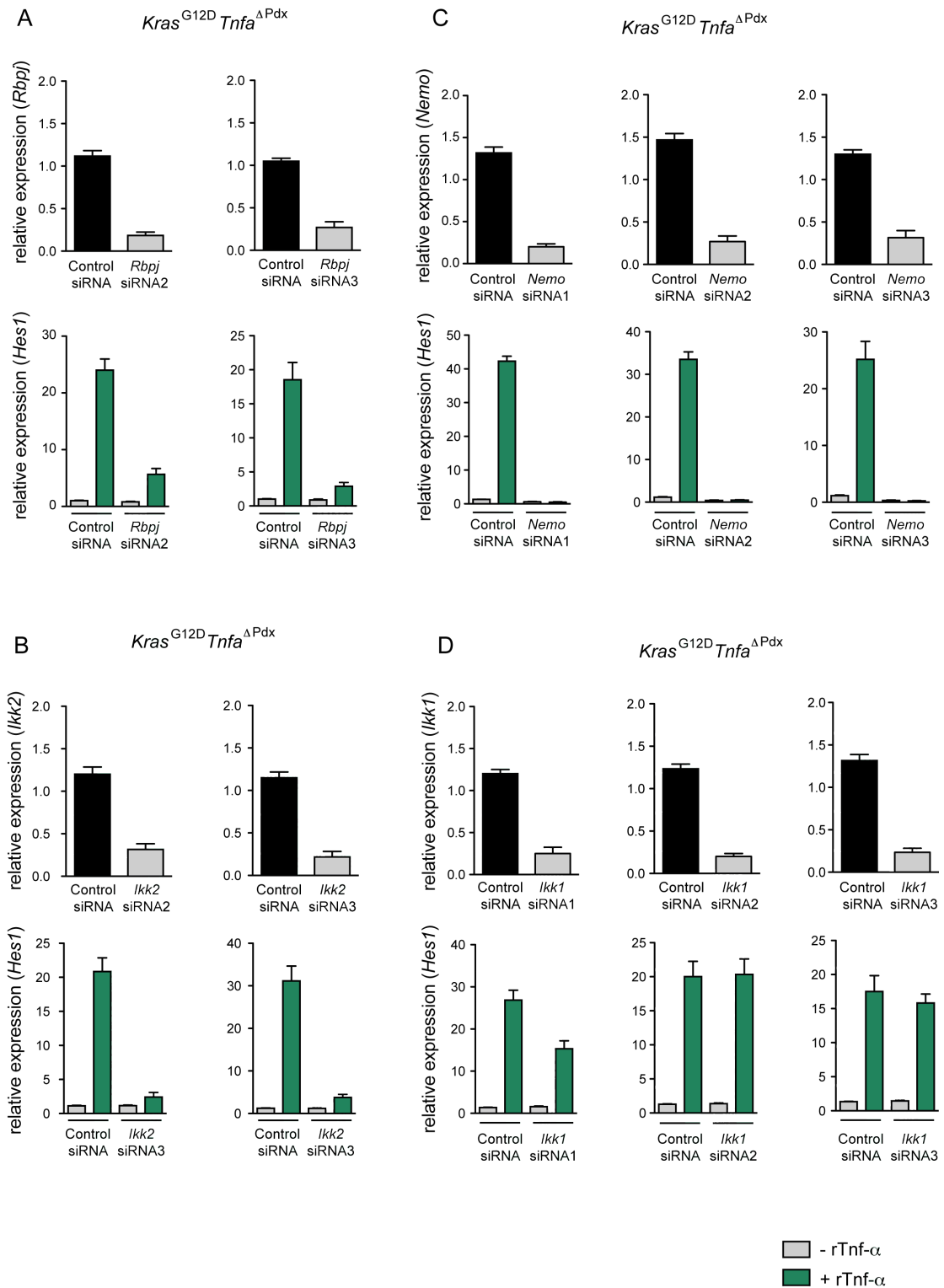
**Supplemental Figure 2. Genetic inactivation of the Tnf- $\alpha$ /Ikk2 axis in Kras induced pancreatic epithelial cells abrogates pancreas-specific infiltration of inflammatory cells.** Relative mRNA expression of *Cd2r*, *Cd8a*, *B220*, *Nk1.1*, *Cd208*, *F4/80* and *Cxcr1* in whole pancreases of (A) 2- and (B) 5-month old mice. Gene expression was quantified by real-time PCR normalized to mRNA levels of  $\beta$ -Actin. Percentage of F4/80<sup>+</sup>CD11b<sup>+</sup>Gr1<sup>-</sup> and F4/80<sup>-</sup>CD11b<sup>+</sup>Gr1<sup>+</sup> cells in the pancreas measured by flow cytometry. Each data point represents an individual mouse. Mean values are depicted by a horizontal line. Means + SD (n=8), \*p<0.05, \*\*p<0.01. (C) Six weeks old *Kras*<sup>G12D</sup> and *Kras*<sup>G12D</sup>*Tnfa* <sup>$\Delta$ Pdx</sup> female mice were lethally irradiated and transplanted with bone marrow of female *Tnfa*<sup>fl/fl</sup> or *Tnfa*<sup>fl/fl</sup>*Mx-1-Cre* mice (n=10 each group). Mice were thrice injected with 5  $\mu$ g/g body weight poly(I:C) to delete *Tnfa* in 2-month old mice. Deletion was examined by Tnf- $\alpha$  ELISA of blood leukocytes upon *ex vivo* LPS stimulation. (D) Quantification of the proportion of pancreas occupied by PanIN lesions. Frequency and grade of the lesions was quantified at 2, 5 and 8 months of age. Means + SD (n=10), p<0.01. nl, no lesion.



**Supplemental Figure 3. Tnf- $\alpha$ -induced Notch and NF- $\kappa$ B target gene expression in PanIN cell lines.** (A) Relative mRNA expression of *Hey1*, *Batf*, *Vegf*, *Igfr1*, *Myc* and *Tenascin c* in PanIN cell lines stimulated with 1 ng/ml rTnf- $\alpha$  for 6 h. Relative expression was calculated by setting expression of untreated *kras*<sup>G12D</sup> samples as 1. Data are shown as means + SD of triplicate determinants and one representative experiment out of three is shown. (B) Kinetic analysis of *Hey1* mRNA expression in *kras*<sup>G12D</sup>*Tnfa* <sup>$\Delta$ Pdx</sup> PanIN cell lines stimulated with 1 ng/ml rTnf- $\alpha$ . (C) Expression of *Hey1* in *Kras*<sup>G12D</sup>*Tnfa* <sup>$\Delta$ Pdx</sup> PanIN cells treated with 1 ng/ml rTnf- $\alpha$  in the presence or absence of 15  $\mu$ g/ml CHX. Relative expression was calculated by setting expression of untreated *Kras*<sup>G12D</sup> samples as 1. Data are shown as means + SD of triplicate determinants and one representative experiment out of three is shown.



**Supplemental Figure 4. Tnf- $\alpha$  induced NF- $\kappa$ B target genes expression is not affected by pharmacological inhibition of Notch signaling.** Expression of *Il1b*, *Mmp13* and *Cox2* in Tnf- $\alpha$ -induced *Kras*<sup>G12D</sup>*Tnfa*<sup>ΔPdx</sup> PanIN cells treated with the  $\gamma$ -secretase inhibitor L685458 (5 $\mu$ M). Cells were stimulated with 1 ng/ml rTnf- $\alpha$ . Relative expression was calculated by setting expression of untreated *Kras*<sup>G12D</sup> samples as 1. Data are shown as means + SD of triplicate determinants and one representative experiment out of three is shown.

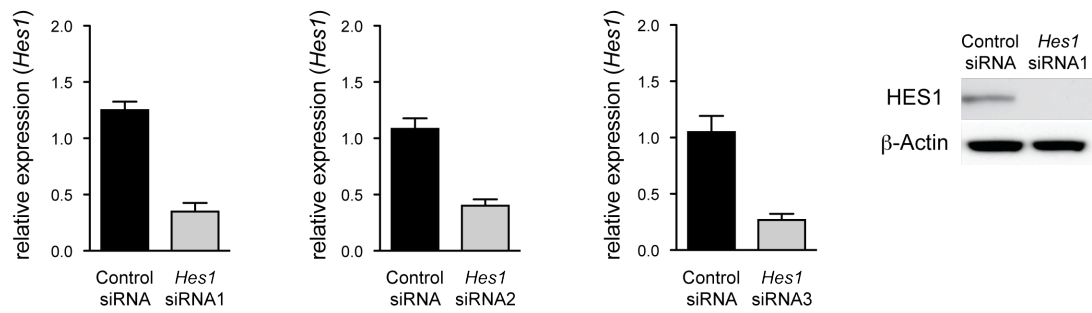


**Supplemental Figure 5. siRNA knockdown of *Rbpj*, *Ikk2*, *Nemo* and *Ikk1* in *Kras<sup>G12D</sup>Tnfa<sup>ΔPdx</sup>* cells. *Kras<sup>G12D</sup>Tnfa<sup>ΔPdx</sup>* PanIN cell lines transfected with (A) *Rbpj*, (B) *Ikk2*, (C) *Nemo* and (D) *Ikk1* specific siRNAs. Gene expression was evaluated 48 h post transfection and upon stimulation with 1 ng/ml rTnf-α**

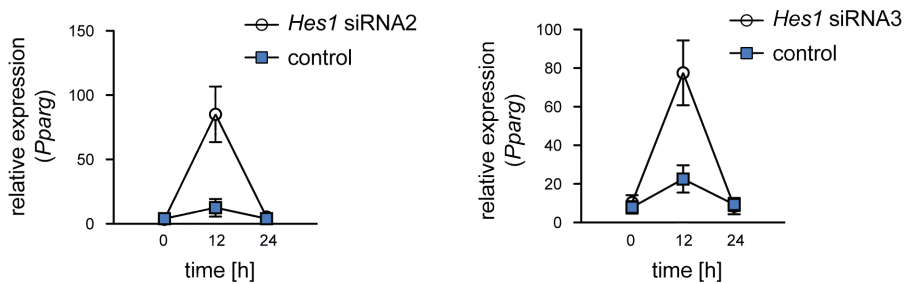


for 6 h. Non-targeting siRNA and/or unstimulated controls were included. Results were normalized to uninfected and unstimulated *Kras*<sup>G12D</sup>*Tnfa*<sup>ΔPdx</sup> cells. Data are shown as means + SD of triplicate determinants and are representative of three independent experiments.

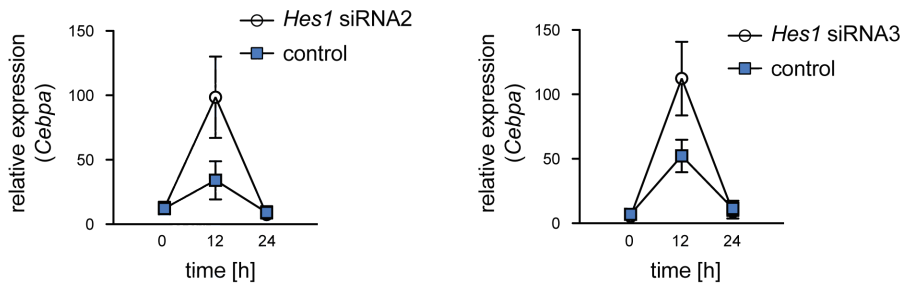
A



B



C



**Supplemental Figure 6. siRNA knockdown of *Hes1* results in upregulated *Pparg* and *Cebpa* expression.** (A) Downregulation of *Hes1* in *Kras*<sup>G12D</sup>*Tnfa*<sup>ΔPdx</sup> PanIN cell lines transfected with *Hes1* specific siRNAs. Results were normalized to uninfected *Kras*<sup>G12D</sup>*Tnfa*<sup>ΔPdx</sup> cells. (B) *Pparg* and (C) *Cebpa* downregulation in cells transfected with *Hes1* siRNA2 and siRNA3. Data are shown as means + SD of triplicate determinants and are representative of three independent experiments.

ID	Age (days)	PDAC	Histology	Liver	Lung	PD	Ascites	BO	Other
TH04-75	523	Y	undifferentiated	Y	N	N	N	Y	
TH04-81	514	Y	undifferentiated	Y	N	Y	N	N	LN
TH04-83	584	N		N	N	N	N	N	
TH04-111	408	N		N	N	N	N	N	
TH04-121	447	Y	undifferentiated	Y	N	Y	N	N	LN
TH04-122	432	N		N	N	N	N	N	
TH04-128	522	Y	undifferentiated	Y	Y	N	N	N	
TH04-175	167	N		N	N	N	N	N	
TH04-177	185	N		N	N	N	N	N	
TH04-190	224	N		N	N	N	N	N	
TH04-195	364	Y	glandular	N	N	N	Y	Y	
TH04-198	361	Y	glandular	N	N	N	Y	Y	
TH04-224	301	Y	undifferentiated	Y	Y	N	N	N	
TH04-230	254	N		N	N	N	N	N	
TH04-234	485	N		N	N	N	N	N	
TH04-236	203	N		N	N	N	N	N	
TH04-248	308	N		N	N	N	N	N	
TH04-249	388	Y	undifferentiated	Y	N	Y	N	Y	
TH04-255	365	Y	undifferentiated	Y	Y	N	N	N	
TH04-269	214	Y	undifferentiated	Y	N	N	Y	N	
TH04-271	394	Y	glandular	N	N	N	N	Y	
TH04-274	575	Y	undifferentiated	Y	N	N	N	N	
TH04-277	564	N		N	N	N	N	N	
TH04-294	467	N		N	N	N	N	N	
TH04-312	481	Y	undifferentiated	Y	N	N	N	Y	
TH04-318	348	N		N	N	N	N	N	
TH04-324	506	Y	undifferentiated	Y	Y	N	N	N	
TH04-328	407	N		N	N	N	N	N	
TH04-351	519	N		N	N	N	N	N	
TH04-361	522	N		N	N	N	N	N	
TH04-366	413	Y	glandular	N	N	N	Y	Y	
TH04-367	497	N		N	N	N	N	N	
TH04-372	463	Y	undifferentiated	Y	N	N	Y	N	
TH04-373	482	Y	undifferentiated	Y	Y	N	N	Y	
TH04-385	506	Y	undifferentiated	Y	N	N	N	N	
TH04-386	322	N		N	N	N	N	N	
TH04-387	471	Y	glandular	N	N	N	N	Y	
TH04-402	449	Y	undifferentiated	Y	Y	N	N	Y	
TH04-406	367	N		N	N	N	N	N	
TH04-425	308	N		N	N	N	N	N	
	Median		75% undifferentiated						
	422.5	50%	25% glandular	37.5%	15%	7.5%	12.5%	25%	

**Supplemental Table 1. Disease spectrum in *Kras*<sup>G12D</sup>**

PD, peritoneal disease; BO, biliary obstruction