IL-35 producing B cells promote the development of pancreatic neoplasia.

Yuliya Pylayeva-Gupta[1], Shipra Das[1], Jesse S. Handler[1], Cristina H. Hajdu[2], Maryaline Coffre[2], Sergei B. Koralov[2], Dafna Bar-Sagi[1]

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Treatment with anti-CXCL13 antibody reduces B cell accumulation and growth of pancreatic neoplasia.

(A) Sections of human PanIN lesions were stained by immunofluorescence (CXCL13, red; vimentin, green; and DAPI, blue). Arrows indicate cells that are double-positive for CXCL13 and vimentin. Scale bar, 20μm.

(B) Sections of mouse PanIN lesions were stained by immunofluorescence (CXCL13, red; vimentin, green; and DAPI, blue). Arrows indicate cells that are double-positive for CXCL13 and vimentin. Scale bar, 50μm.

(C) Representative flow cytometry plots of B cells ($CD45^+CD19^+$) isolated from spleens of mice treated with control IgG and pancreata of *KC* mice treated with either anti-CXCL13 or control IgG. Cells were gated on CD45 and subsequently were analyzed for the presence of CD19.

(D) Graph depicts quantification of the data in (C) (n = 4-5 mice per group).

(E) Representative flow cytometry plots of B cells ($CD45^+CD19^+$) isolated from spleens and pancreata with orthotopic pancreatic grafts 2 weeks after *GFP-KRas^{G12D}*-PDEC implantation into WT mice treated with either anti-CXCL13 or control IgG. Cells were gated on CD45 and subsequently were analyzed for the presence of CD19.

(F) Graph depicts quantification of the data in (E) (n = 4-5 mice per group).

(G) Pancreas tissue sections from orthotopic pancreatic grafts 2 weeks after *KRas^{G12D}*-PDEC implantation into WT or *μMT* mice were stained with H&E. Scale bars, 100μm.

(H) Graph indicates the fraction of GFP+ signal per field of view (FOV); (10 FOV per animal; n=5 WT+IgG, n=5 WT+anti-CXCL13).

Error bars indicate SEM; p value: *<0.05; ***<0.001

Supplementary Figure S2. B cells promote growth of *Kras^{G12D}*-PDEC *in vivo*.

(A) Sections from orthotopic pancreatic grafts 4 weeks after GFP-*KRas*^{G12D}-PDEC implantation into WT or *μMT* mice were stained with anti-GFP antibody. Scale bars, 100μm.

(B) Graph depicts quantification of the data in (A) and indicates the fraction of GFP^{+} signal per field of view (FOV), (n=9-12 mice per group).

(C) Schematic of the protocol used for isolation and adoptive transfer of B cells into *μMT* mice followed by orthotopic implantation of GFP- *KRas*^{G12D}-PDEC.

(D) Pancreas tissue sections from orthotopic pancreatic grafts 2 weeks after GFP-*KRas*^{G12D}-PDEC implantation into *μMT* or *μMT* mice supplemented with *WT* B cells were stained with anti-B220 antibody. Arrows point to B220 positive cells. Scale bar, 50μm.

(E) Immunohistochemical detection of B cells (B220 staining) in pancreata from a *KPC* mouse. Scale bar, 50μm.

(F) Gross anatomical images of pancreata of WT or *μMT* mice implanted with KPC cells at 4 weeks post-implantation (tumors are outlined with dotted lines). Graph (right) depicts quantification of the data indicating lesion size (n=10 WT, n=10 μMT).

Error bars indicate SD; P values were determined by Student's t-test (unpaired, two-tailed); p value: *<0.05.

Supplementary Figure S3. Engraftment of GFP- *KRas*^{G12D}-PDEC or GFP-*KPC* cells in μMT mice is accompanied by a switch in the polarization of the macrophage population.

(A) Representative flow cytometry plots of pancreatic immune cells for the surface expression of CD11b, CD206 and CD86 at 2 weeks after implantation of GFP-*Kras*^{G12D}-PDEC into WT or *μMT* mice. After gating on the CD45^{+} F4/80^{+} population, CD11b^{+} macrophages were analyzed for the presence of CD206 or CD86. Uninjected WT mice were used as controls.

(B) The graph depicts quantification of the data shown in **(A)** and shows the percent of CD11b^{+}CD206^{+} or CD11b^{+}CD86^{+} cells out of all F4/80^{+} cells sorted from the pancreas.

(C) Representative flow cytometry plots of pancreatic immune cells for the surface expression of CD11b, CD206 and CD86 at 4 weeks after implantation of GFP-KPC into WT or *μMT* mice. After gating on the CD45^{+} F4/80^{+} population, CD11b^{+} cells were analyzed for the presence of CD206 or CD86.

(D) The graph depicts quantification of the data shown in **(C)** and shows the percent $CD11b^{+}CD206^{+}$ or $CD11b^{+}CD86^{+}$ cells out of all $F4/80^{+}$ cells sorted from the pancreas. Error bars indicate SD; p value *<0.05, **<0.01

Supplementary Figure S4. Systemic perturbation of B lymphocyte maturation and differentiation in mice with pancreatic neoplasia.

(A) Representative flow cytometric analysis of plasma cells from spleens, mesenteric lymph nodes (MLN), and pancreata of *p48^{Cre}* (control) or *KC* mice. Cells were gated on $CD19^{low/-}$and analyzed for expression of markers B220 and CD138. Frequency of plasma cells is indicated by percentage of $CD138^{+}B220^{low/-}$ cells out of all $CD19^{low/-}$cells (n=5 *p48^{Cre}*, n=5 *KC*).

(B) Representative flow cytometric analysis of B lymphocytes from spleens of *p48^{Cre}* (control) and *KC* mice. Cells were gated on CD19 and B220, and then analyzed for the presence of marker AA4.1. Percentages of $AA4.1^{+}B220^{+}$ immature and $AA4.1^{-}B220^{+}$ mature cells are indicated.

(C) Flow cytometric analysis of B lymphocytes from spleens of *p48^{Cre}* (control) and *KC* mice. Cells were gated on CD19 and B220, followed by gating on mature $AA4.1^{-}B220^{+}$ population, and then analyzed for the presence of markers CD1d and CD21. Percentages of $CD1d^{high}CD21^{high}$ marginal zone (MZ) cells are indicated.

(D) Representative flow cytometric analysis of immune cells from pancreata of control *p48^{Cre}* mice, *KC* mice (2.5mo), or *KRas^{G12D}*-PDEC orthotopic lesions (2 weeks), as indicated. After gating on CD19 and CD1d populations, cells were analyzed for the presence of CD5 marker. The percent of $CD1d^{high}$cells out of all $CD19^{+}$ cells is indicated in the upper plots, and the percent of $CD1d^{high}CD5^{+}$ cells out of all $CD19^{+}$ cells is indicated in the lower plots (n=8 *p48^{Cre}*, n=8 *Kras^{G12D}*-PDEC, n=8 *KC*). Fluorescence-minus-one (FMO) control was generated by omission of anti-CD1d antibody staining.

Supplementary Figure S5. Adoptive transfer of $CD1d^{high}CD5^{+}$ and $CD1d^{low}CD5^{-}$ B cell subsets into µMT mice.

(A) Schematic of the protocol used for isolation and adoptive transfer of $CD1d^{high}CD5^{+}$ and $CD1d^{low}CD5^{-}$ B cell subsets into *µMT* mice followed by orthotopic implantation of GFP- *KRas^{G12D}*-PDEC.

(B) Splenic B cells from WT C57BL/6 mice were isolated by MACS, and stained for cell surface markers CD1d, CD5, and CD19 before flow cytometry analysis. A

representative flow cytometry plot of the $CD19^+CD1d^{high}CD5^+$ and $CD19^+CD1d^{low}CD5^-$ B cell subpopulations that were purified by cell sorting is shown.

(C) Pancreata of *μMT* mice reconstituted with either no B cells, $CD1d^{high}CD5^+$ or $CD1d^{low}CD5^-$ cells were analyzed by flow cytometry for B cell specific CD19 marker. Graph enumerates $CD19^+$ cells as a percent of total gated live leukocytes within the pancreas of recipient mice.

Supplementary Figure S6. Expression of *IL-10* in B cells infiltrating pancreatic cancer.

(A) $CD19^+$ cells were isolated by FACS from pancreata of $p48^{Cre}$ (control), *KC* and GFP-$KRas^{G12D}$-PDEC orthotopically implanted mice, and analyzed for expression of *IL10* mRNA by PCR.

(B) Levels of *IL10* mRNA in immune cells from spleen and pancreata of $p48^{Cre}$ (control) and *KC* mice were assessed by quantitative RT-PCR. Error bars indicate SEM. p values: * < 0.05; ** < 0.01; NS - not significant.

(C) Levels of *IL10* mRNA in $CD19^+CD1d^{high}CD5^+$ and $CD19^+CD1d^{low}CD5^-$ sub-populations of B cells from pancreata of *KC* mice were assessed by quantitative RT-PCR. Error bars indicate SD. p values: * < 0.05.

(D) Immunofluorescence staining for IL-10 and B220 in pancreata from orthotopic pancreatic implants 4 weeks after GFP- $KRas^{G12D}$-PDEC implantation into WT mice, or IL-10 and CD20 in a sample of human pancreatic cancer containing PanIN lesions. Scale bar, 30 μm (mouse) and 100μm (human).

(E) Representative flow cytometry plots of B cells isolated from spleens and pancreata of mice of the indicated genotypes. Cells were gated on $CD45^+$ and subsequently were analyzed for the presence of CD19.

Supplementary Figure S7. Expression of p35, *IL12b* and *IL27* in B cells infiltrating pancreatic neoplasia.

(A) Immunofluorescence staining for p35 and CD20 in samples of human pancreatic cancer containing PanIN lesions. White boxes indicate the location of magnified images shown in **Figure 3E**. Two independent fields of view are shown.

(B) Immunofluorescence staining for p35 and B220 in samples of *KC* pancreata. White boxes indicate the location of magnified images shown in **Figure 3F**. Two independent fields of view are shown.

(C) Levels of *IL12b and IL27* mRNA in B cells and non-B cells from pancreata of *KC* mice were assessed by quantitative RT-PCR. Data are presented as fold change over non-B cells. Error bars indicate SD. p values: ***<0.001; NS - not significant.

(D) Levels of *IL12b and IL27* mRNA in $CD19^{+}CD1d^{high}CD5^{+}$ and $CD19^{+}CD1d^{low}CD5^{-}$ sub-populations of B cells from pancreata of *KC* mice were assessed by quantitative RT-PCR. Data are presented as fold change over $CD19^{+}CD1d^{low}CD5^{-}$ B cells. Error bars indicate SD. p values: NS - not significant.

Supplementary Figure S8. Adoptive transfer of wild-type and IL12a-/- B cells into *μMT* mice.

Representative flow cytometry plots of B cells isolated from spleens and pancreata of mice of the indicated genotypes. Cells were gated on $CD45^{+}$ and subsequently were analyzed for the presence of CD19.