

Supplementary Materials for

NK Cell Adoptive Transfer Suppresses Metastasis and Prolongs Survival in Pancreatic Cancer

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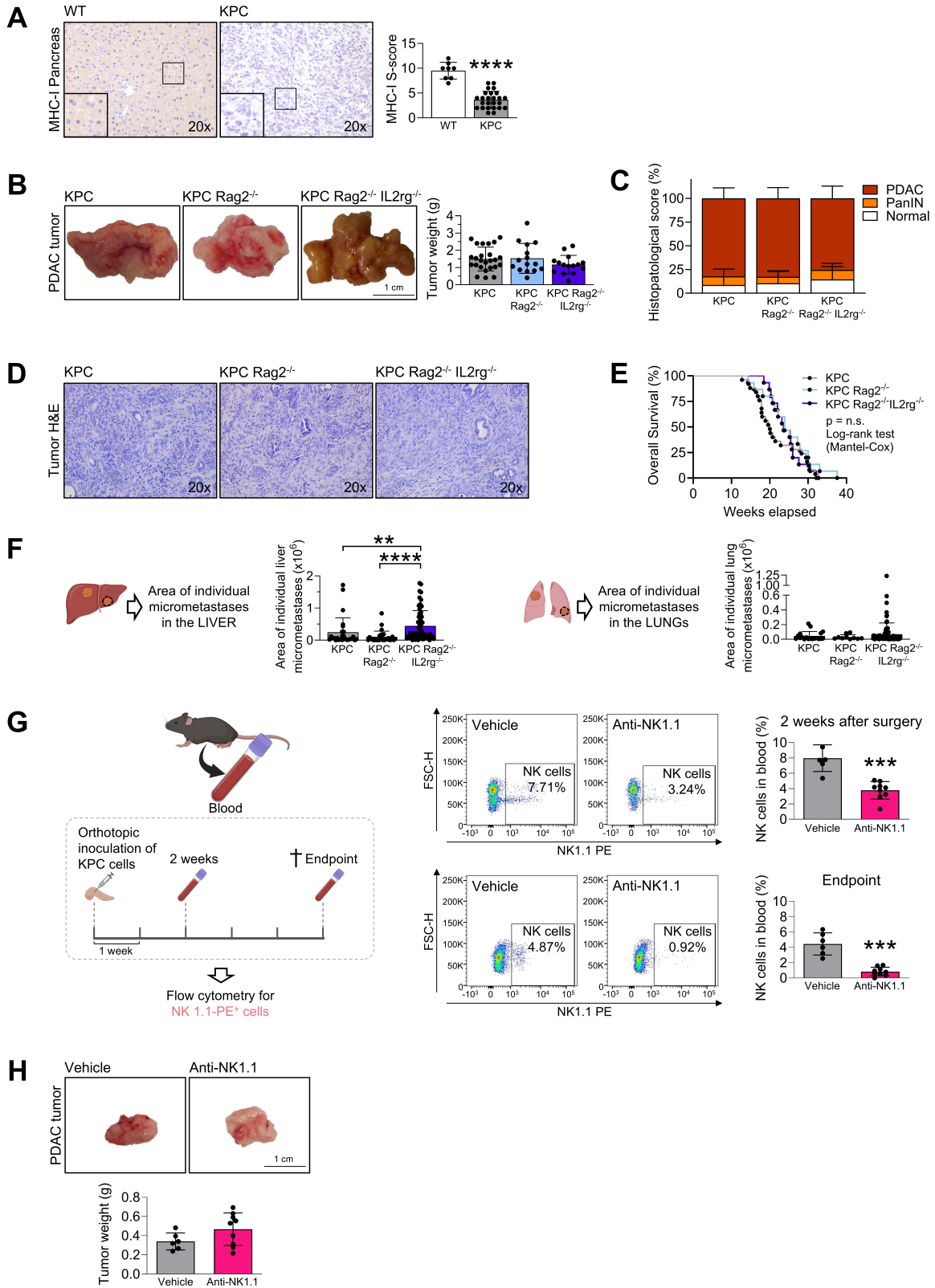
This PDF file includes:

Figures. S1 to S8

Other Supplementary Materials for this manuscript include the following:

Source Data File (excel format) containing the raw data of each figure panel
Scripts used for analysis using R and Python

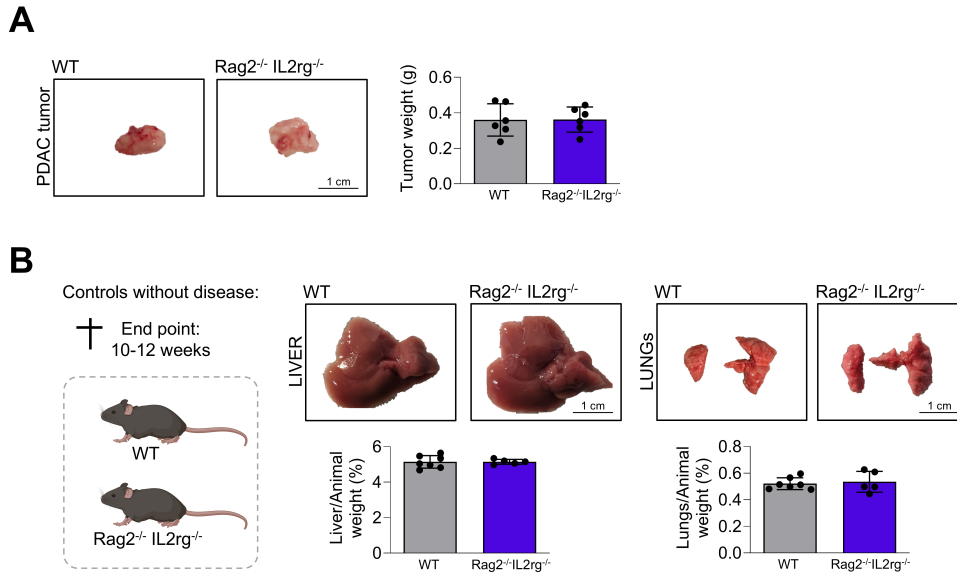
Figure. S1.



Supplementary Figure 1

(A) Representative IHC images (20x) of MHC-I in the pancreas of WT and KPC mice and respective S-score given to the staining throughout the whole tissue (Mann-Whitney test, **** $p < 0.0001$). (B) Representative photos of tumors from KPC, KPC Rag2^{-/-} and KPC Rag2^{-/-} IL2rg^{-/-} mice (left, scale bar: 1 cm) and respective graphical representation of the tumor weight (right, Mann-Whitney test, p -values not significant). (C) Histopathological evaluation of the percentage of the pancreas that showed no histological disease/Normal, PanINs and PDAC area in KPC, KPC Rag2^{-/-} and KPC Rag2^{-/-} IL2rg^{-/-} mice (Mann-Whitney test, p -values not significant). (D) Representative H&E images (20x) of the tumors from KPC, KPC Rag2^{-/-} and KPC Rag2^{-/-} IL2rg^{-/-} mice. (E) Kaplan-Meier curve of the overall survival of KPC (n=25), KPC Rag2^{-/-} (n=15) and KPC Rag2^{-/-} IL2rg^{-/-} (n=15) mice (log-rank Mantel-Cox test, p -values not significant). (F) Graphical representation of the area of individual micrometastases found in the liver (left) and lung (right) tissue of KPC, KPC Rag2^{-/-} and KPC Rag2^{-/-} IL2rg^{-/-} mice. (Mann-Whitney test, ** $p < 0.01$ and **** $p < 0.0001$). (G) Representative FACS plots and analysis of the percentage of NK1.1⁺ cells (i.e., NK cells) found in the circulation at 2 weeks after orthotopic inoculation of KPC cells (top) and at euthanasia in WT mice treated with vehicle and anti-NK1.1 (Mann-Whitney test, *** $p < 0.001$). (H) Representative photos of tumors from WT mice treated with vehicle and anti-NK1.1 (top, scale bar: 1 cm) and respective graphical representation of the tumor weight (bottom, Mann-Whitney test, p -value not significant). Schemes created with BioRender.com.

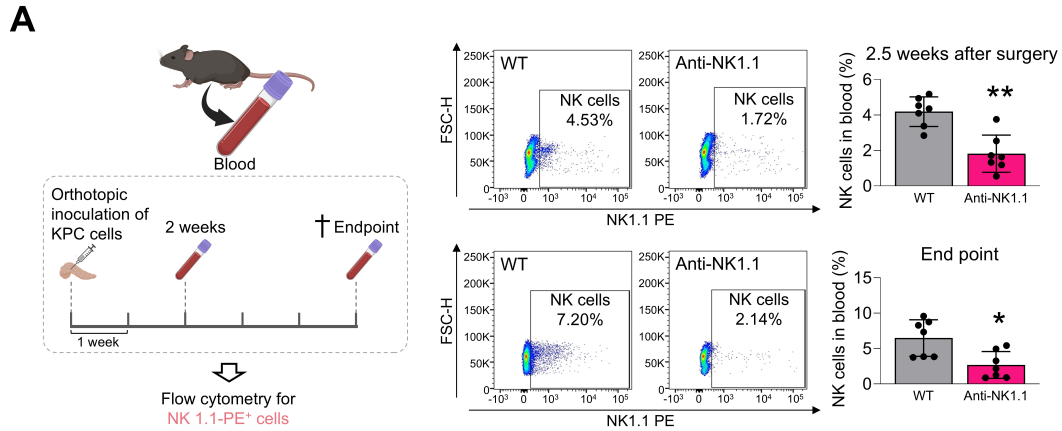
Figure. S2.



Supplementary Figure 2

(A) Representative photos of tumors from WT and Rag2^{-/-} IL2rg^{-/-} mice that were orthotopically inoculated with KPC cells in the pancreas (left, scale bar: 1 cm) and respective graphical representation of the tumor weight (right, Mann-Whitney test, *p*-value not significant). (B) Schematic representation of the experiment layout (left): in order to control for organ weight and morphology differences due to genotype, control WT (n=7) and Rag2^{-/-} IL2rg^{-/-} (n=5) mice without disease were euthanized at 10-12 weeks and their organs evaluated. Representative photos of liver and lungs (right) from control WT and Rag2^{-/-} IL2rg^{-/-} mice without disease (scale bar: 1 cm) and respective graphical representation of the liver/animal weight and lung/animal weight ratios (Mann-Whitney test, *p*-values not significant). Schemes created with BioRender.com.

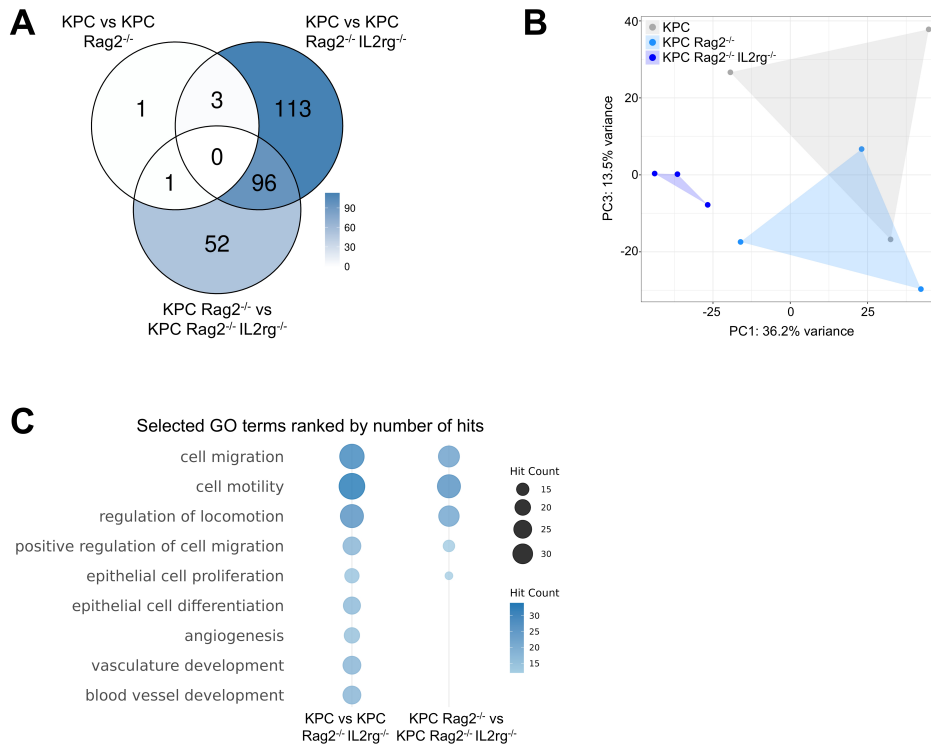
Figure. S3.



Supplementary Figure 3

(A) Representative FACS plots and analysis of the percentage of NK1.1⁺ cells (i.e., NK cells) found in circulation at 2.5 weeks after orthotopically inoculation of KPC GFP-LUC cells and at euthanasia in WT and anti-NK1.1 mice (Mann-Whitney test, * $p < 0.05$ and ** $p < 0.01$). Schemes created with BioRender.com.

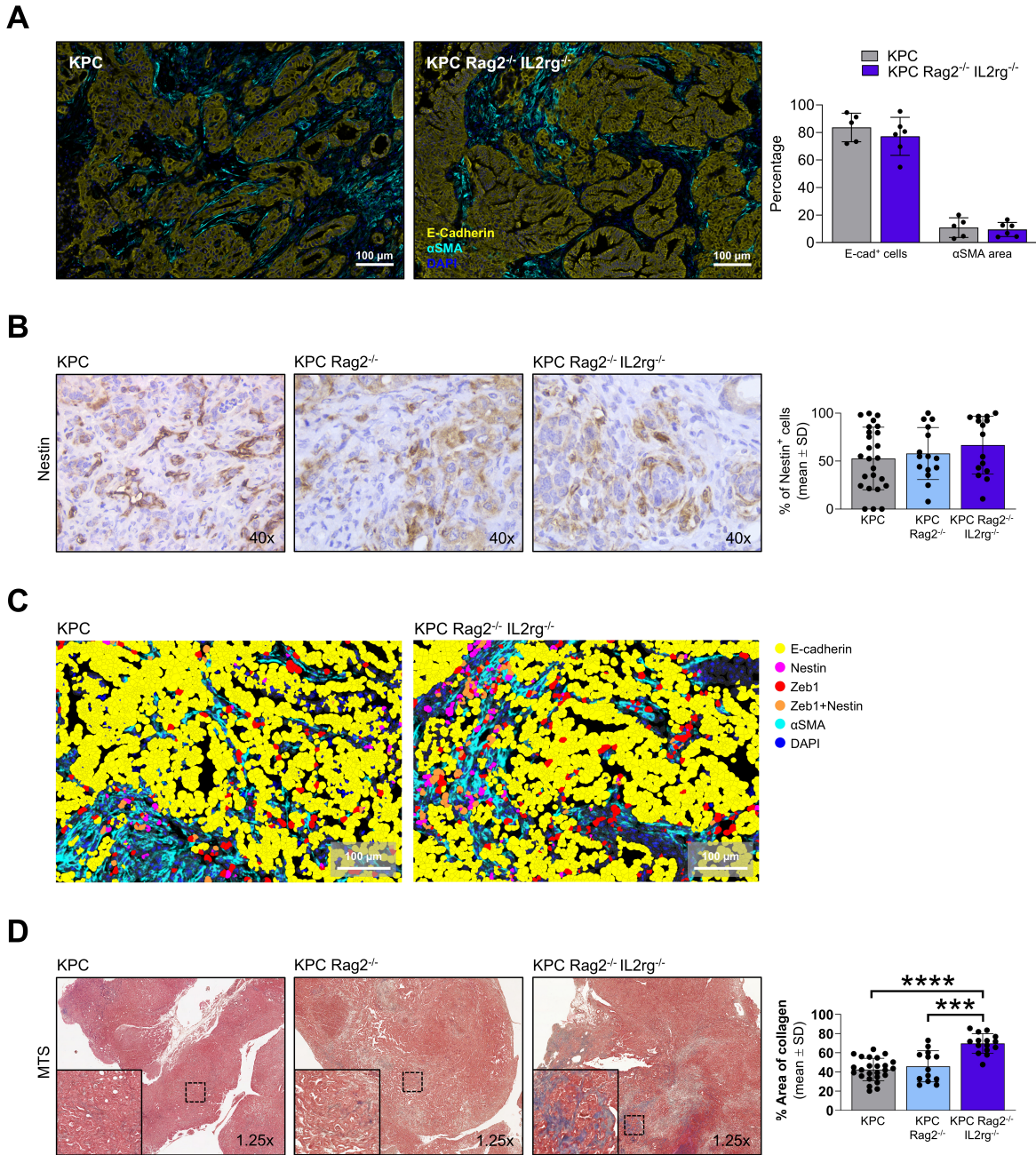
Figure. S4.



Supplementary Figure 4

(A) Venn diagram illustrating the number of DEGs found in the comparison of all the groups: KPC $Rag2^{-/-}$ IL2rg $^{-/-}$, KPC $Rag2^{-/-}$ and KPC. (B) Principal component analysis (PCA) of transcriptomic profiles showing separation between KPC $Rag2^{-/-}$ IL2rg $^{-/-}$, KPC $Rag2^{-/-}$ and KPC, with each point representing an independent sample. Clustering reflects distinct molecular signatures of tumor cells. (C) Selected pathways related to metastasis significantly enriched in KPC $Rag2^{-/-}$ IL2rg $^{-/-}$ cancer cells in comparison to KPC and KPC $Rag2^{-/-}$ ranked by number of hits.

Figure. S5.

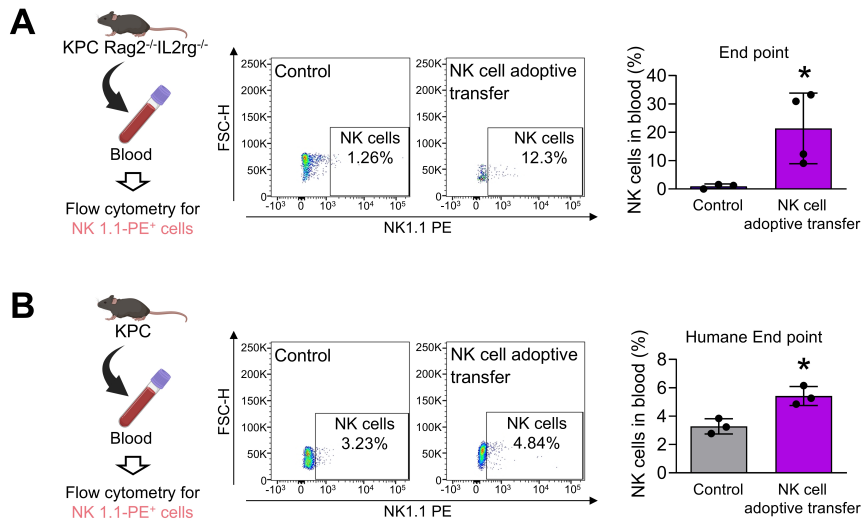


Supplementary Figure 5

(A) Representative images (left; Scale bar: 100 μ m) of multiplex immunofluorescence panel of E-cadherin (cancer cells) and α SMA (CAFs) in tumors from KPC and KPC Rag2^{-/-} IL2rg^{-/-} mice and respective quantification (right) of the percentage of cancer cells positive for the epithelial marker E-cadherin and quantification of the area of α SMA staining in tumors from KPC and KPC

Rag2^{-/-} IL2rg^{-/-} mice. **(B)** Representative IHC images (40x, left) of Nestin in the primary tumors of KPC (n=25), KPC Rag2^{-/-} (n=15) and KPC Rag2^{-/-} IL2rg^{-/-} (n=15) mice and respective quantification (right) of the percentage of positive cells per field. **(C)** Representation of the spatial distribution of α SMA⁺ CAFs (cyan) in relation to cancer cells (yellow – E-cadherin⁺ cancer cells, magenta – Nestin⁺ cancer cells, red – Zeb1⁺ cancer cells, orange – Zeb1⁺ Nestin⁺ cancer cells). Scale bar: 100 μ m. **(D)** Representative images (1.25x, left) of Masson's Trichrome (MTS) staining of primary tumors of KPC, KPC Rag2^{-/-} and KPC Rag2^{-/-} IL2rg^{-/-} mice and respective quantification (right) of the amount of collagen (blue) per field (Mann-Whitney test, * $p < 0.05$).

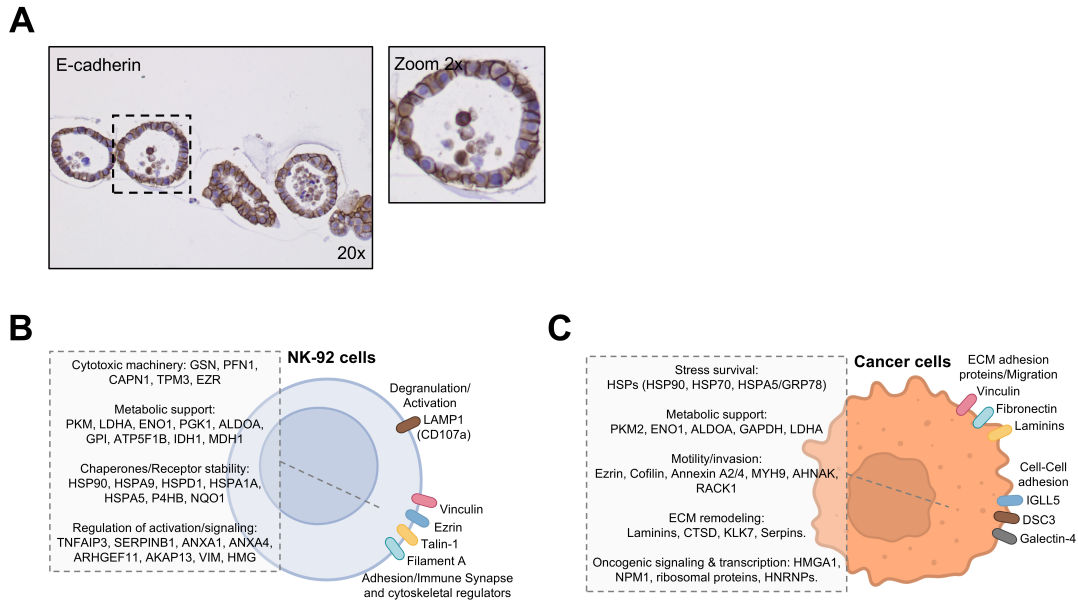
Figure. S6.



Supplementary Figure 6

(A-B) Representative FACS plots and analysis of the percentage of NK1.1⁺ cells (i.e., NK cells) found in the circulation of KPC Rag2^{-/-} IL2rg^{-/-} (A) and KPC (B) mice that received NK cell adoptive transfer versus control (Mann-Whitney test, * $p < 0.05$ and ** $p < 0.01$). Schemes created with BioRender.com.

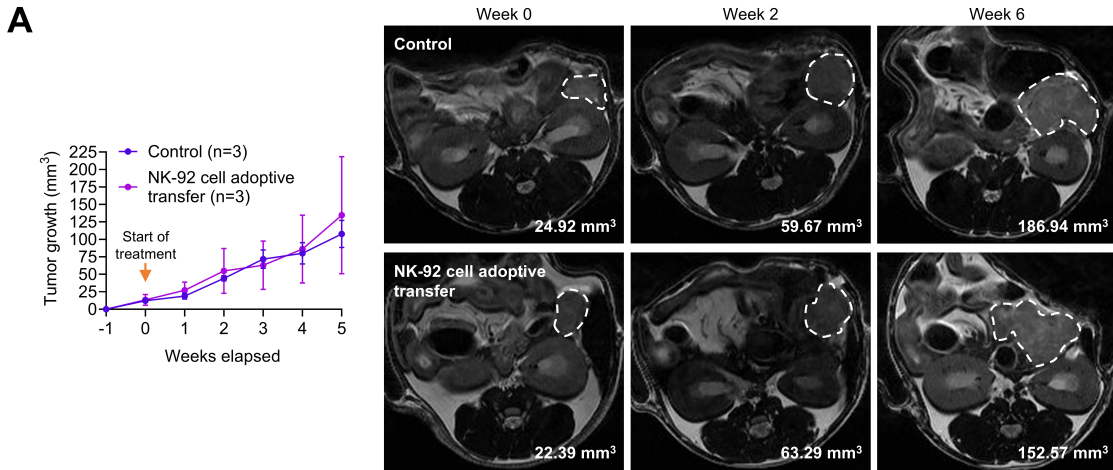
Figure. S7.



Supplementary Figure 7

(A) Representative IHC image (20x, with a 2x zoom) of E-cadherin staining in patient-derived organoids. (B-C) Schematic representation of baseline top 10% expressed membrane and intercellular proteins found through proteomic profiling of NK-92 cells (B) and organoids (C) cultured alone. Schemes created with BioRender.com.

Figure. S8.



Supplementary Figure 8

(A) Evaluation of tumor growth by MRI (fold change of tumor volume in relation to initial tumor volume, Mean \pm SEM), performed through the duration of the experiment, of mice orthotopically inoculated with PDX cells and treated with vehicle (control) or NK-92 cells upon tumor detection (Two-way ANOVA, *p*-values not significant). Representative MRI images (right; axial orientation) of the tumor of mice treated with vehicle (control) or NK-92 cell adoptive transfer,

with tumor borders outlined in white. **(B)** Table representing the top 25 expressed genes in each NK cell state obtained by cell state discovery using Statescope within TCGA (State 1: n=34; State 2: n=28; State 3: n=75).