Supplemental Methods

Luminex assay

The Luminex assay was performed in the Human Immune Monitoring Center at Stanford University. Human 62-multiplex kit from Affymetrix was used to detect cytokines, chemokines and growth factors in a fluid based human sample. Patients' or donors' pancreas tissues were homogenized and lysed in RIPA buffer (Sigma, R0278), and the same amount of protein was used after estimating protein concentrations of tissue homogenates. The samples were mixed with antibody-linked polystyrene beads and incubated at room temperature for 2 hr. by shaking at 500rpm followed by overnight incubation at 4°C. Plates were then prewarmed for 30min without shaking and washed three times after the filtration. Next, biotinylated detection antibodies were added and incubated for 2 hr. at room temperature by shaking at 500rpm. Samples were then filtered and washed three times and resuspended with streptavidin-phycoerythrin (PE) for 40min at room temperature by shaking at 500rpm. Three additional vacuum washes were performed after the filtration, and the samples resuspended in Reading Buffer for 5min at room temperature by shaking at 500rpm. Each sample was run in duplicate. Plates were read using a Luminex 200 instrument.

Histology (H&E and Trichrome staining) and Immunohistochemistry (IHC)

Human pancreatic tissues were fixed with 10% formalin and transferred to 70% ethanol for paraffin-embedding and further processes. Paraffin-embedded tissue samples were sectioned and stained for hematoxylin and eosin, Trichrome staining and IHC were performed at Stanford human pathology/histology service center. IHC with anti-human CD45 antibody (Agilent, clone: 2B11+PD7/26, M070101-2) was performed at 1:100 dilution.

Hydroxyproline assay

Human pancreatic tissues were homogenized in 100μl of dH_2O for every 10mg of tissue, and 100 μl of tissue homogenate was transferred to a polypropylene vial and mixed with 100 μl of 10N NaOH. The mixture was heated at 120°C for 1 hour and placed on ice briefly before opened. Next, 100 μl of 10N HCl was added to neutralize NaOH, and the vial was vortexed and centrifuged at 10,000g for 5min. 10 μl of each neutralized sample hydrolysate was transferred to a clear, flat-bottom 96-well plate. By using Hydroxyproline Assay Kit (BioVision, #K226), transferred samples and standard dilutes underwent further reactions according to manufacturer's instructions. Hydrolyzed sample hydroxyproline concentration was calculated after measuring brightly colored chromophore with an absorbance peak at 560nm.

Immune cell isolation and flow cytometry analysis

Pancreas tissues were digested with 1.5 mg/ml of elastase (Worthington Biochemical, LS002294) in 10% BCS contained RPMI buffer for 45min at 37°C and filtered through100μm filter. Next, the undigested tissues were further digested with 2 mg/ml of DE collagenase 800 (VitaCyte, 011-1050) in 10% BCS contained RPMI buffer for 20min at 37°C and passed through 100μm filter. The filtered cells in buffer were centrifuged for 4 min at 50g to remove acini, and the supernatant was further centrifuged for 5min at 400g and the recovered pellet was added to an RBC lysis buffer (Sigma, R7757) to remove red blood cells. The recovered cells were then washed in RPMI buffer that contained 10% BCS and resuspended for flow cytometry antibody staining. Surface antibody staining was performed at room temperature (RT) for 30min and washed with 2% BCS containing HBSS buffer followed by fix/permeabilization at RT for 30min. Next, intracellular staining (e.g. for TNFα and CD68) was performed at RT for 30min and washed off with 1x wash solution in True-Nuclear Transcription Factor Buffer (BioLegend, #424401). Cells were fixed again with 4% paraformaldehyde at 4°C for 15min, and then the fixed cells were washed and resuspended in HBSS solution containing 2% BCS. Flow cytometry

was performed with BD LSRII flow cytometer at Stanford FACS facility, and data analysis performed using Flowjo (BD). Samples that had adequate live CD45 cells (minimum of 100 cells) were included in the analysis of immune cell subpopulation characterization of the study.

Antibodies used for flow cytometry

Flow cytometry antibodies against human surface and intracellular molecules are commercially available. Live/Dead (Zombie Aqua, #423102), anti-CD45 (HI30, PerCP-Cy5.5, #304028), anti-CD56 (5.1H11, APC-Cy5.5, #362522), anti-CD3 (UCHT1, Pacific Blue, #300431), anti-CD4 (RPA-T4, APC-Cy7, #300518), anti-CD4 (OKT4, BV605, #317438), anti-CD8 (SK1, PE-Cy7, #344750), anti-CD11c (3.9, APC, #301614), anti-CD117 (104D2, PE-Cy7, #313212), anti-CD68 (Y1/82A, FITC, #333806), anti-TNFα (Mab11, PE, #502909), anti-CD206 (15-2, APC-Cy7, #321120), anti-CD11b (M1/70, PE/Dazzle 594, #101256), anti-HLA-DR (L243, Pacific Blue, #307636), anti-T-bet (4B10, PE/Dazzle 594, #644828), anti-GATA3 (16E10A23, APC, #653806), anti-CD25 (BC96, BV605, #302632) and anti-FOXP3 (206D, AF488, #320112) were purchased from BioLegend, and anti-RORγt (Q21-559, PE, #563081) was from BD Biosciences.

TCRβ sequencing and data analyses

Genomic DNA (gDNA) was extracted from the human pancreas tissues using DNeasy Blood and Tissue Kit (Qiagen). Equal amount of gDNA from each sample was analyzed with high-throughput sequencing at the survey level of the TCRβ CDR3 regions using ImmunoSeq immune-profiling system (Adaptive Biotechnologies, Seattle, WA). Circos plots in Fig. 4d were made with a software downloaded from *circos.ca*. Total T cell numbers, productive rearrangements, and productive clonality were calculated using the Adaptive Biotechnologies Immunoseq Analyzer platform. Heatmap in Fig. 4g generated using Morpheus software (Broad Institute, Cambridge, MA) displays top non-ambiguous paired TRBV and TRBVJ gene usage

identified using Significance Analysis of Microarray T-test with 90th percentile of FDR=0 (samr R package). Full length CDR3 amino acid sequenced shared among patients, irrespective of gene usage, displayed in Fig. 4h.

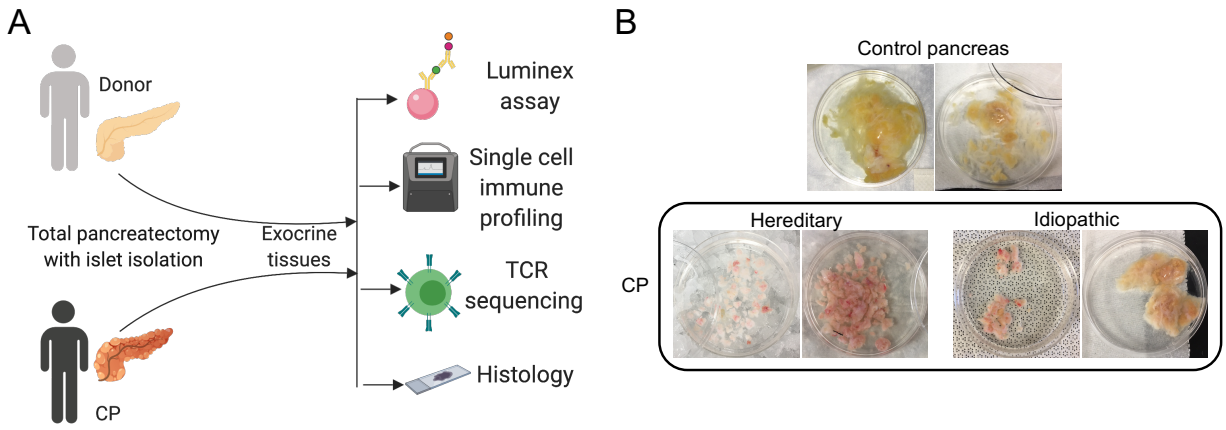
Statistics

Unless indicated, the mean ±SD was used, and comparisons were made by using the indicated statistical tests such as, two-tailed Student's t-test and one-way ANOVA with multiple comparison test using GraphPad software (version 8.1.0). Differences were considered to be statistically significant when $p<0.05$.

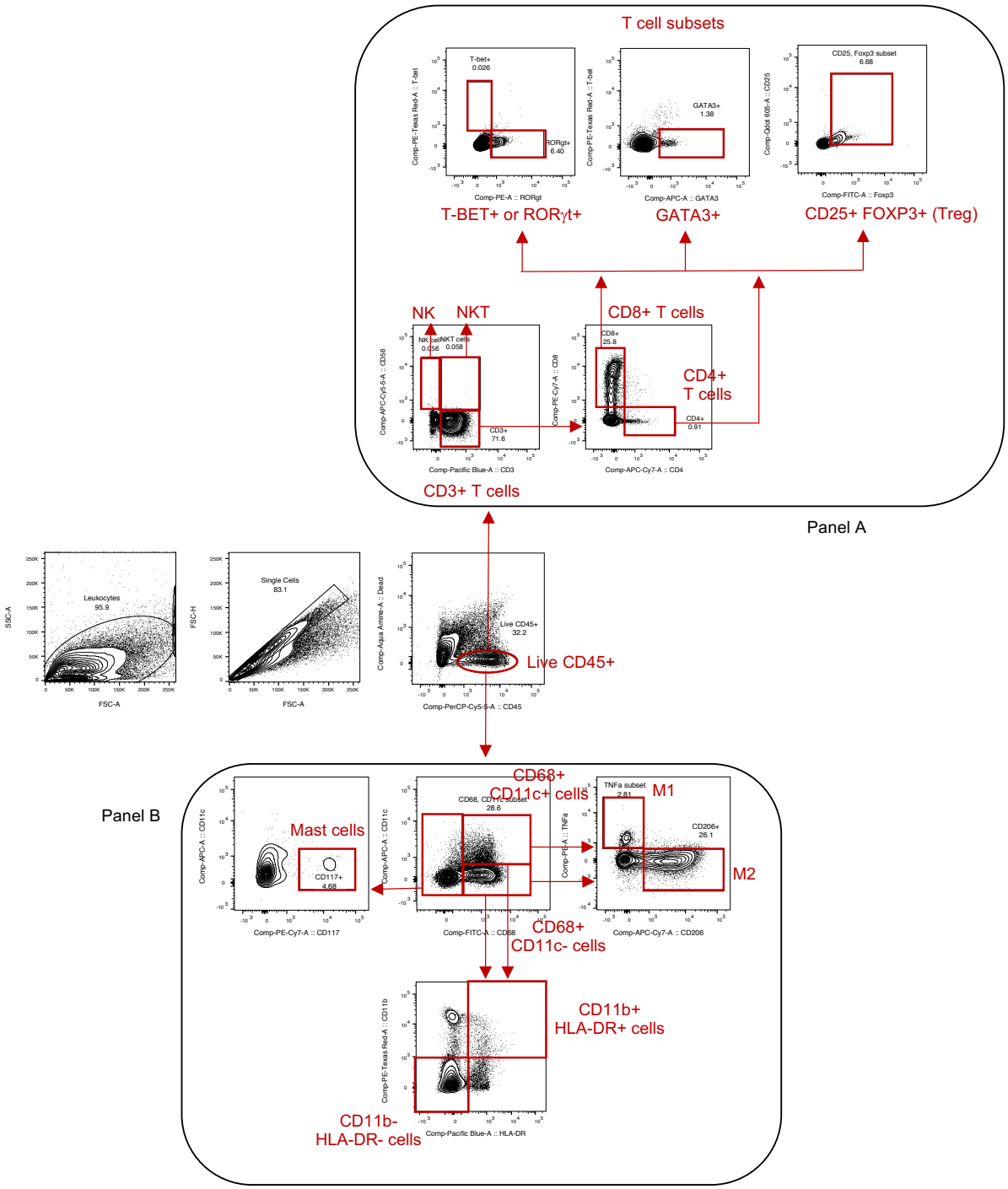
Study approval

Human pancreatic tissues from deceased donors (n=9) without any previous history of pancreatic disorders were obtained following total pancreatectomy with islet isolation procedure from the University of California, San Francisco and used as controls in this study. Human CP pancreatic tissues of hereditary and idiopathic patients (n=40) undergoing TPIAT (and with similar islet isolation procedure as in the controls) were received from the University of Minnesota, from exocrine tissue remaining after islet isolation. Prior to inclusion in the study, all patients with CP provided informed consent or parental consent and patient assent, as age appropriate. For tissue use in research, the protocol was reviewed and approved by the University of Minnesota (IRB #0609M91887) and Stanford University Institutional Review Board (IRB #43185).

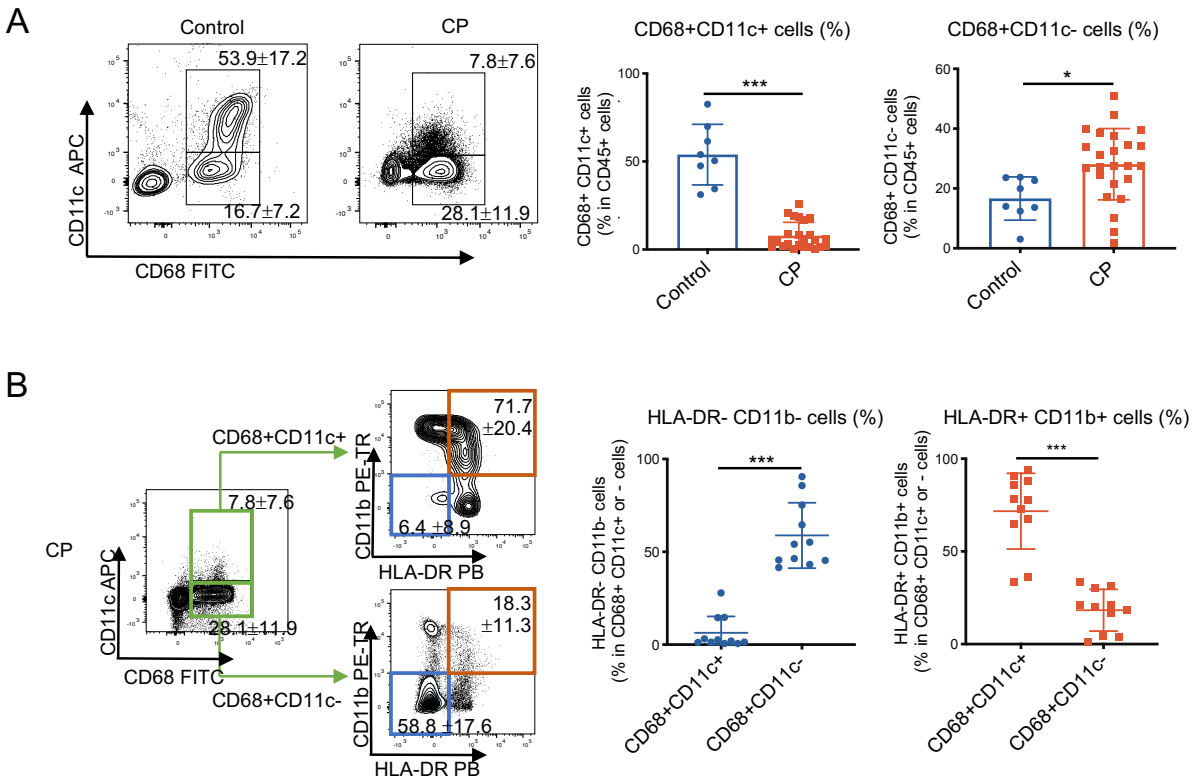
Supplemental Figures



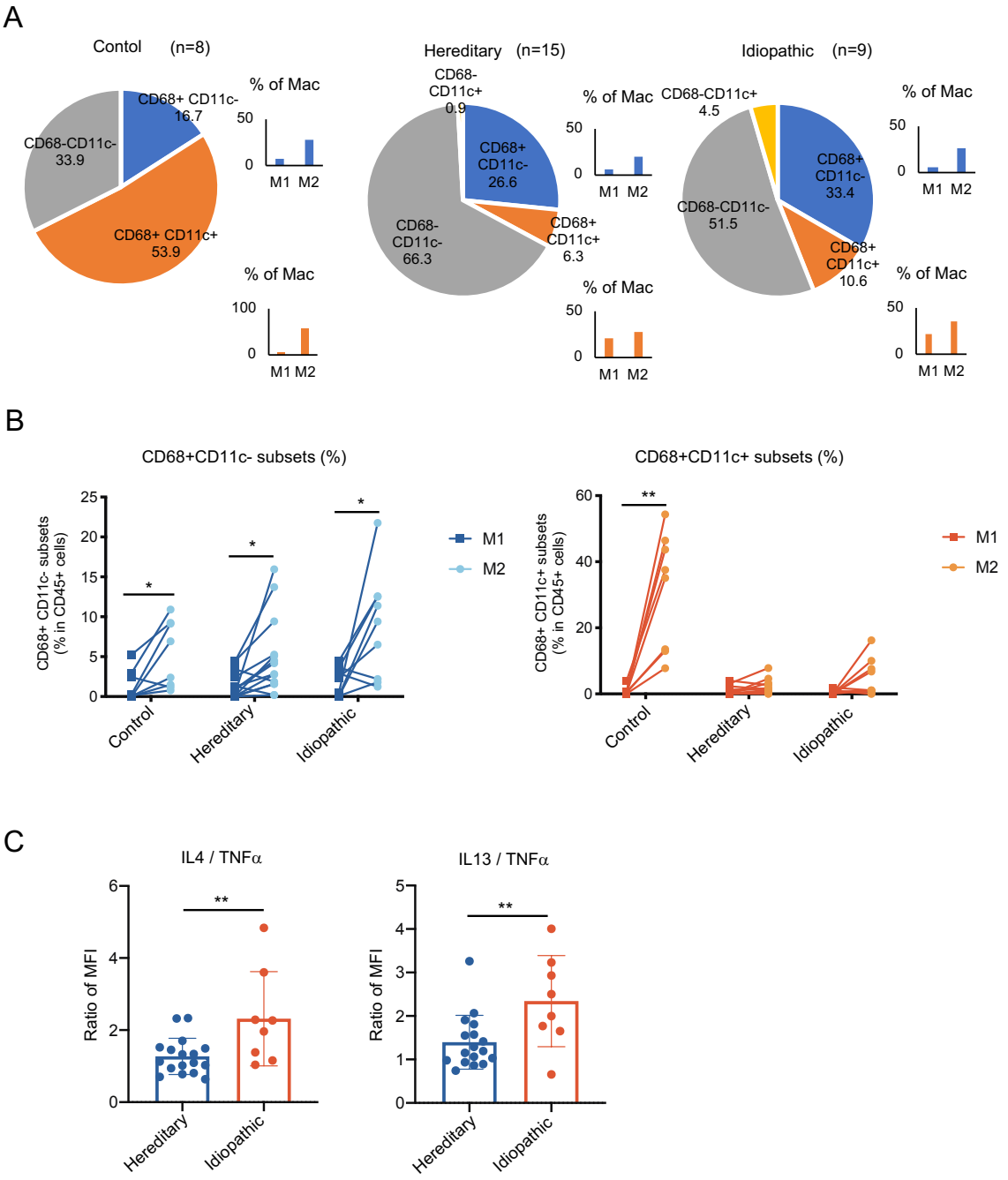
Supplemental Figure 1. Human CP and organ donor pancreas tissues recovered from total pancreatectomy and islet isolation (TPII) procedure. (**A**) Outline of experimental workflow (Created with BioRender). After TPII procedure, the leftover pancreas tissues were collected from control donors, hereditary or non-hereditary CP patients. Each donor or patient's tissue was divided for different experiments such as, Luminex assay, immune profiling by FACS, TCR sequencing, and histology. (**B**) Pictures of representative human pancreas tissues that were used for immune profiling.



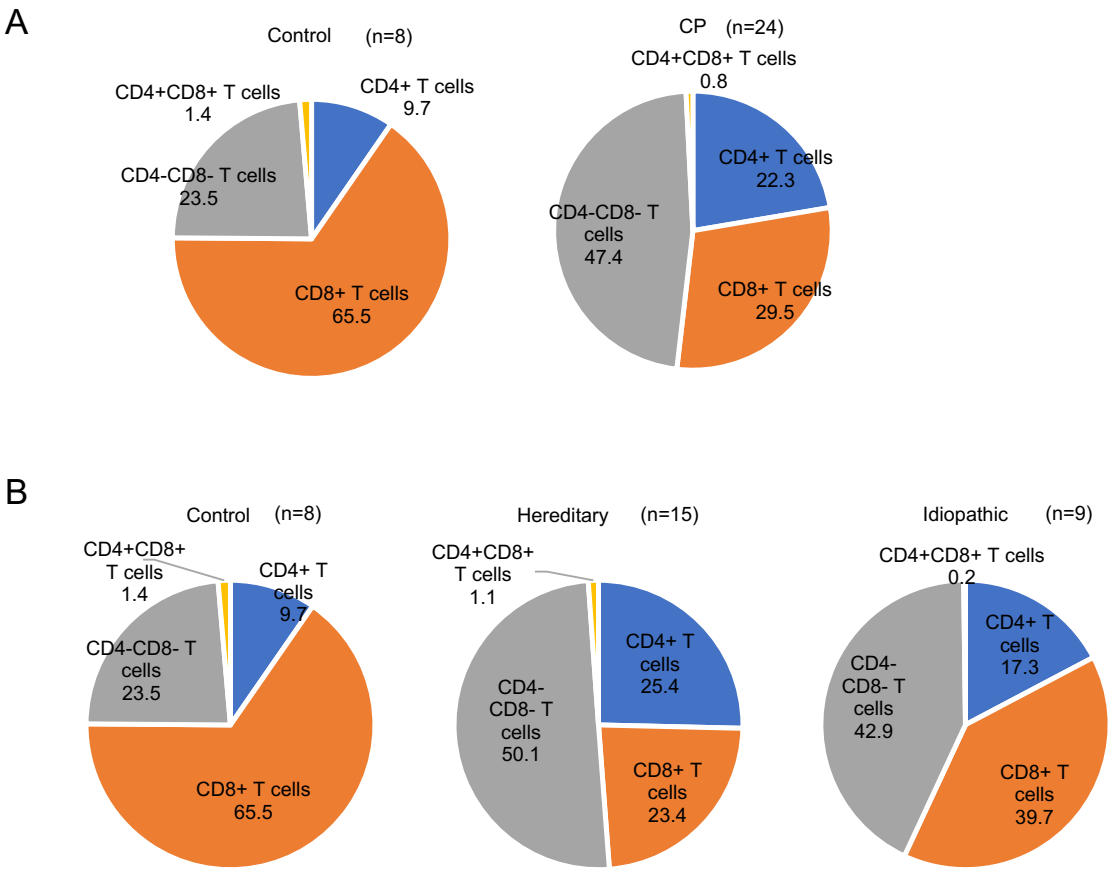
Supplemental Figure 2. Flow cytometry gating scheme. Flow cytometry gating strategies for Live CD45+ cells, NK cells, NKT cells, CD3+ T cells, CD4+ T cells, CD8+ T cells and T cell subsets (T-BET+, GATA3+, RORgt+, CD25/FOXP3+) in panel A and Mast cells, Macrophages (CD68+CD11c- and CD68+CD11c-), pro-inflammatory TNFα+ (M1) macrophages and anti-inflammatory CD206+ (M2) macrophages in panel B.



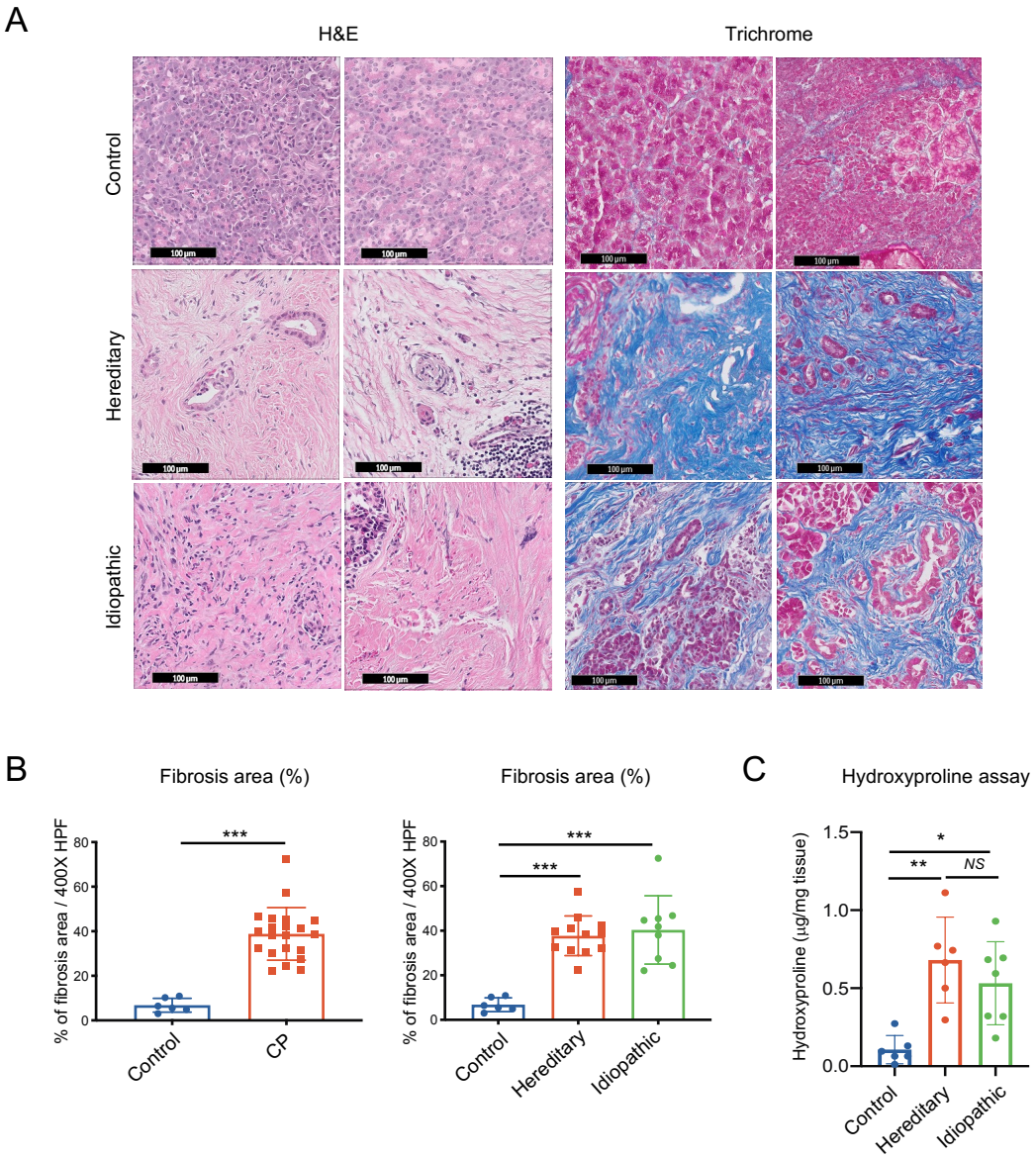
Supplemental Figure 3. Composition of CD68+ immune subsets in control versus CP pancreas tissues. (**A**) Representative plots of flow cytometry analyses of CD68+ subsets based on CD68 and CD11c expression in control and CP. Bar graphs show frequencies of CD68+CD11c- or CD68+CD11c+ cells from live CD45+ cells in control and CP pancreata (mean ±SD). (**B**) Representative plots of flow cytometry analyses of CD68+CD11c- and CD68+CD11c+ populations from CP pancreas tissues and their expression of HLA-DR and CD11b (mean ±SD). Dot plots show frequencies of HLA-DR and CD11b double negative or double positive cells in CD68+CD11c+ and CD68+CD11c- cells of CP pancreata. (The values were obtained by flow cytometry analyses. Unpaired two-tailed t-test was used, *p<0.05, **p<0.01 and ***p<0.001)



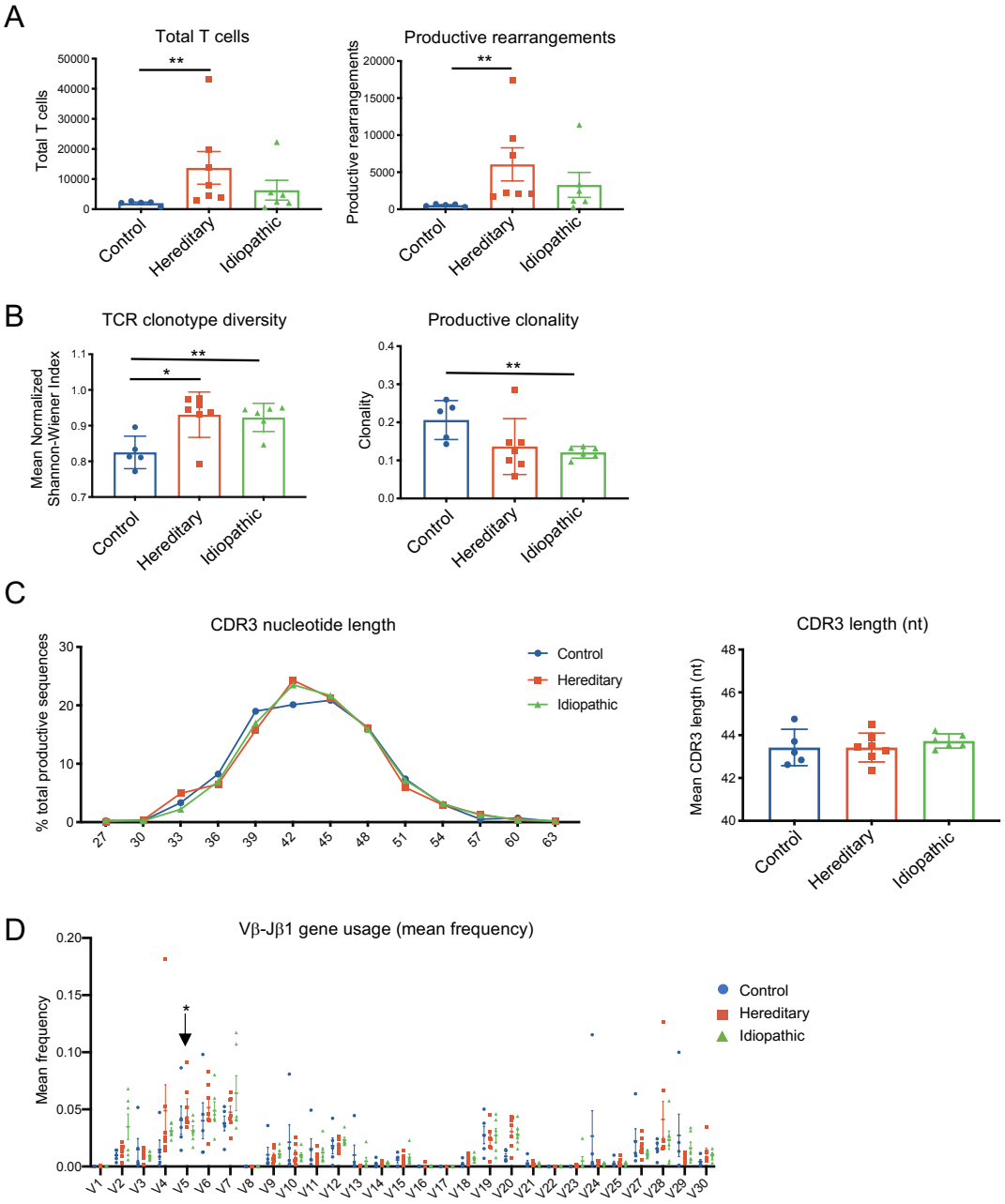
Supplemental Figure 4. Comparison of macrophage subsets infiltrating pancreas of control, hereditary and idiopathic CP subjects. (**A**) Pie charts show composition of macrophage subsets (CD68+CD11c- and CD68+CD11c+ cells) and their respective M1, M2 frequencies gated from live CD45+ cells in pancreas of control, hereditary and idiopathic CP. (**B**) Comparison of M1 and M2 frequencies among the CD68+ subsets (CD68+CD11c- and CD68+CD11c+) of each subject within the control, hereditary and idiopathic CP is shown (The values were obtained by flow cytometry analyses. mean ±SD, Paired two-tailed t-test was used, *p<0.05, **p<0.01 and ***p<0.001). (**C**) Comparison of the ratio of M2 to M1 cytokine expression (IL4/TNFA and IL13/TNFA) in hereditary and idiopathic CP by Luminex assay. (mean ±SD, Unpaired two-tailed t-test was used, **p<0.01)



Supplemental Figure 5. Comparison of T cell subsets infiltrating pancreas of control and CP subjects. (**A**) Pie graphs display mean frequencies of T cell subsets gated from live CD45+ cells in control (n=8) and CP (n=24) pancreata. (**B**) Pie graphs display mean frequencies of T cell subsets gated from live CD45+ cells in control (n=8), hereditary (n=15) and idiopathic (n=9) CP pancreata. The values were obtained by flow cytometry analyses.



Supplemental Figure 6. Histologic features of control, hereditary and idiopathic CP. (**A**) H&E and Trichrome staining of representative pancreas tissues from control (n=6) and CP (n=21) patients. Scale bars:100µm. (**B**) Degrees of pancreas fibrosis. Fibrosis extent was estimated by measuring collagen deposited area with a blue color per high power fields (400x). (Mean ±SD was used. Unpaired two-tailed t-test was used for the comparison of control and CP, One-way ANOVA with Tukey's multiple comparisons test was used for control, hereditary and idiopathic CP comparison, ***p<0.001) (**C**) Hydroxyproline quantification per pancreas tissue weight (µg/mg). (One-way ANOVA with Tukey's multiple comparisons test was used for control, hereditary and idiopathic CP comparison, *p<0.05,**p<0.01)



Supplemental Figure 7. Additional TCRβ sequencing data in control, hereditary and idiopathic CP. (**A**) Comparison of total T cell number and TCR productive rearrangements detected in equal amount (about 2.6 g) of gDNA extracted from pancreatic tissues of control (n=5), hereditary (n=7) and idiopathic (n=6) CP groups. **(**non-parametric Mann-Whitney U-test, **p<0.01) (**B**) TCRβ clonotype diversity and productive clonality of control (n=5), hereditary (n=7) and idiopathic (n=6) CP groups. (Mean normalized Shannon-Wiener diversity index with non-parametric Mann-Whitney U-test, *p<0.05 and **p<0.01) (**C**) CDR3 length (nucleotides) of productive templates. The percentage of each length of CDR3 starting from 27 nucleotides to 63 nucleotides in total productive sequences displayed with each dot, and the mean CDR3 length was compared among the groups on the right side (mean ±SD). (**D**) Comparison of Vβ-Jβ1 gene usage among the groups by displaying their mean frequencies. (Mean±SEM, One-way ANOVA with Kruskal-Wallis test, *p<0.05)

Supplemental Tables

Supplemental Table 1. Demographic and characteristics of control and CP subjects

Characteristics	Control (n=9)	CP (n=40)	P value
Age (years, mean ± SD)[A]	41.1 ±12.3	29.1 ±15.5	**0.035**
Sex, m/f [B]	5/4	20/20	0.763
Height (meters, mean ± SD) [A]	1.70 ±0.04	1.67 ±0.13	0.481
Weight (kg, mean ± SD) [A]	84.83 ±12.10	68.82 ±20.62	**0.030**
Body mass index [A] (kg/m^2, median, [IQR])	29.2 [25.85-32.48]	24.4 [19.18-28.70]	**0.004**
HbA1c (%, mean ±SD) [A]	5.45 ±0.43	5.32 ±0.47	0.470

SD, standard deviation; IQR, interquartile range.
[A]Unpaired two-tailed t-test; [B]Chi-square was used for p-values, Bold p value, *p<0.05*.

Supplemental Table 2. Demographic and CP patient characteristics

Characteristics	Hereditary (n=27)	Idiopathic (n=13)	P value
Age (years, mean ± SD) [A]	24.4 ±13.9	38.8 ±14.6	**0.005**
Sex, m/f [B]	17/10	3/10	**0.018**
Height (meters, mean ± SD) [A]	1.66 ±0.15	1.69 ±0.10	0.626
Weight (kg, mean ± SD) [A]	65.0 ±22.15	76.75 ±14.77	0.092
Body mass index [A] (kg/m^2, median, [IQR])	22.5 [19.1-24.65]	28.3 [25.27-29.56]	**0.009**
Severity gross fibrosis score [A] (median, IQR)	9 [8-9]	6 [2.5-9]	**0.007**
Pancreatitis Duration [A] (years, mean ± SD)	7.3 ±7.4	4.7 ±4.0	0.238
HbA1c (%, mean ±SD) [A]	5.3 ±0.40	5.4 ±0.59	0.554
Glucose (mg/dL, mean ±SD) [A]	90.6 ±9.6	95.8 ±11.9	0.145
MRCP use [B]	24 (88.9%)	11 (84.6%)	0.702
Atrophy by MRI [B]	10 (41.7%)	2 (18.2%)	0.174
Stone or Calcification by MRI [B]	5 (20.8%)	0 (0%)	0.102
Dilated Pancreatic Duct by MRI [B]	13 (54.2%)	6 (54.5%)	0.983
Side Branches by MRI [B]	11 (45.8%)	5 (45.5%)	0.983
Lifetime smoking more than 100 cigarettes (Yes/No) [B]	7/20	8/5	**0.029**

SD, standard deviation; IQR, interquartile range.
[A]Unpaired two-tailed t-test; [B]Chi-square was used for p-values, Bold p value, $p<0.05$.

Supplemental Table 3. Patient characteristics and general sample overview of TCRβ-sequencing analysis

Groups	#	Age (years)	Sex (M,F)	Mutations	Severity gross fibrosis score	Total templates	Productive templates	Unique Rearrange-ments
Control	1	43	M			3130	2325	451
	2	63	F			2881	2329	725
	3	35	M			3426	2922	681
	4	30	F			3407	2622	597
	5	48	F			1068	876	329
Hereditary	1	21	M	*PRSS1*	9.00	19831	15604	7287
	2	21	F	*PRSS1*	9.00	56605	45570	17456
	3	57	M	*PRSS1*	9.00	10069	8806	2202
	4	13	M	*PRSS1, CFTR*	8.00	4435	3475	2074
	5	18	F	*CFTR*	5.00	6281	4972	1749
	6	32	F	*CFTR*	3.00	5332	4149	2048
	7	41	M	*CFTR*	9.00	28533	21900	9579
Idiopathic	1	41	M		8.00	6713	5210	2477
	2	62	F		6.00	3329	2452	1136
	3	49	M		4.00	930	767	307
	4	17	M		7.50	7764	6135	3374
	5	58	F		3.00	3306	2653	1087
	6	42	F		9.00	29921	24252	11387