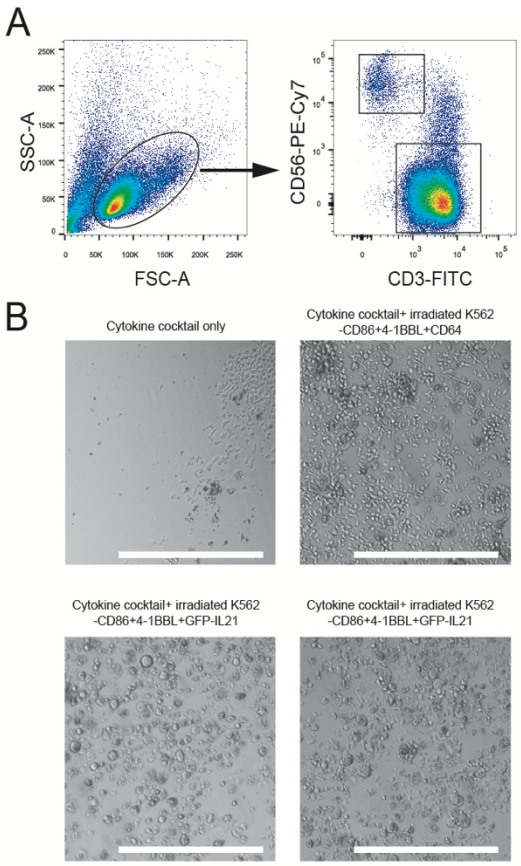
Figure S1

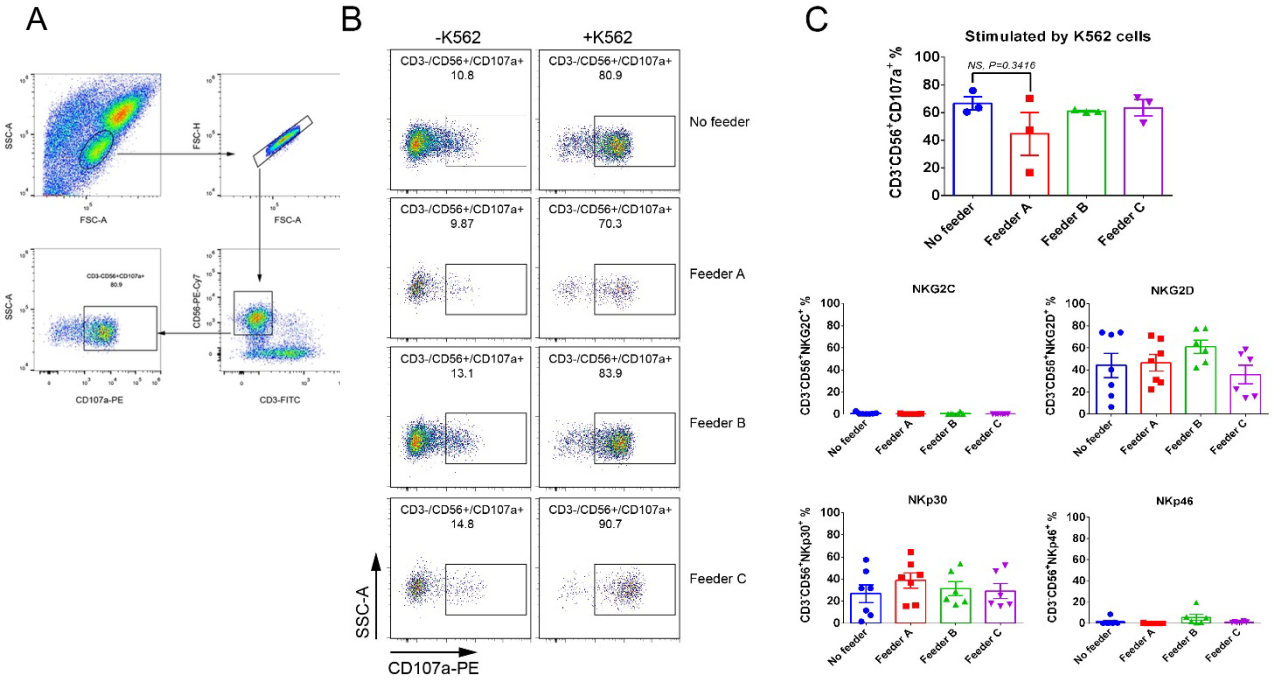


K562 based feeder cells greatly enhanced the proliferation of TIL fragment cultures.

A. Gating strategy for phenotype analysis of TIL cultures.

B. B. Representative microscopic photogram of individual well of TIL cultures showing the effect of different K562 based feeder cells on growth of activated lymphocytes. The shinning “thumderball” like cells are lymphocytes expanded from tumor fragments, whereas the large granular “fat” cells are K562 feeder cells.

Figure S2



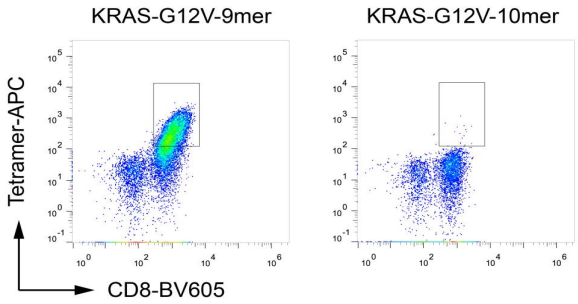
NK cells expanded from tumor fragments are functional and express multiple NK receptors. TIL cultures were subjected to flow cytometry analysis to measure the upregulation of CD107a. The NK cell population were gated based on size and expression of CD56 and negative expression of CD3.

A． NK functional activity was measured by flow cytometry analysis. One representative result was shown. CD107a functional assay showed NK cells generated from fragment cultures using different feeder cells were capable to degranulate when they were re-stimulated by K562 cells.

B． Summary of data from multiple samples from different patients. The quantified cell surface levels of CD107a were analyzed by FlowJo. Data represent mean ±SD, n = 3. P values were determined by ordinary (no pairing) one-way ANOVA test.

C． NKG2C, NKG2D, NKp30 and NKp46 expression on the NK cells generated from different feeder cells. Bar graphs represents the percentage of NK cells expressing corresponding NK receptors.

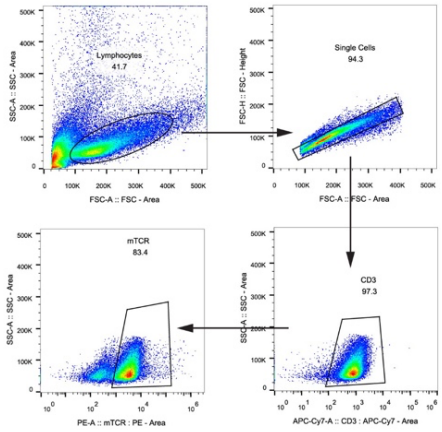
Figure S3



Indentify the specificity of KRAS-G12V-9mer and KRAS-G12V-10mer tetramer.

PBMCs from a human donor were transduced with the PCV TCR and stained with KRAS-G12V-9mer or KRAS-G12V-10mer tetramer, shown by flow cytometric plot.

Figure S4



Gating strategy for identification of T cells that express mouse TCR (mTCR).

After *in vitro* sanitization with TMG-T cells and expansion in defined medium, we analyze the percentage of T cells that express mouse TCR by flow cytometry.

Table S1

Patient clinical information: Cancer Clinical stage, CA 19-9, KRAS mutation type.

Tumor tissues of 70 patients were collected. 64/70 patients with PDAC, 6/70 patients with other diseases determined by pathological section after the surgery. 7/64 patients received neoadjuvant therapy before tumor resection.

Patient ID	Cancer Type	Clinical Stage	CA 19-9	KRAS Mutation
NJ001	PDAC, moderate differentiation	III	192	G12D
NJ002	PDAC, moderate to poorly differentiation	II	278	G12V
NJ003	PDAC, moderate to poorly differentiation	N/A	>1000	G12D
NJ004	PDAC, moderate differentiation	III	459	WT
NJ005**	PDAC, moderate differentiation	N/A	633	WT
NJ006	PDAC, moderate to poorly differentiation	I	>1000	G12D
NJ007	PDAC, moderate to poorly differentiation	III	22	G12V
NJ008	PDAC, moderate differentiation	N/A	137	G12D
NJ009	PDAC, moderate differentiation	I	3.7	WT
NJ010	PDAC, moderate differentiation	IV	>1000	G12D
NJ011	PDAC, moderate to poorly differentiation	N/A	127	G12D
NJ012	PDAC	N/A	371	G12D
NJ013*	spindle cell carcinoma of the pancreas	N/A	13	WT
NJ014	PDAC, well differentiation	IV	>1000	G12R
NJ015	PDAC, poor differentiation	III	864	G12R
NJ016	PDAC, moderate to poorly differentiation	III	313	G12V
NJ017	PDAC, moderate differentiation	N/A	>1000	G12V
NJ018	PDAC, moderate differentiation	II	>1000	G12D
NJ019	PDAC, liver metastases	IV	113	WT
NJ020*	chronic pancreatitis	N/A	54	N/A
NJ021	PDAC, moderate differentiation	I	39	G12D
NJ022	PDAC, squamous cell carcinoma 30%	III	184	G12D
NJ023*	cystadenoma of carcinogenesis	I	23	WT
NJ024	PDAC, moderate to poorly differentiation	III	15	G12D
NJ025	PDAC, moderate differentiation	II	83	G12D
NJ026	PDAC	III	458	G12D
NJ027	PDAC, moderate differentiation	III	195	G12D
NJ028	PDAC, moderate to poorly differentiation	II	228	WT
NJ029	PDAC	III	19.8	WT
NJ030	PDAC, moderate differentiation	IV	198	G12V
NJ031	PDAC, moderate differentiation	I	431	WT
NJ032**	PDAC, with metastases	N/A	>1000	G12D
NJ033	PDAC, poor differentiation	II	95	WT
NJ034	PDAC, moderate differentiation	II	217	G12V
NJ035	PDAC, moderate differentiation	II	>1000	G12R
NJ036**	PDAC, moderate differentiation	III	43	G12D
NJ037	PDAC, moderate differentiation	III	287	WT
NJ038	PDAC, moderate to poorly differentiation	II	8.59	WT
NJ039	PDAC, moderate differentiation	I	432	G12D
NJ040	PDAC, moderate differentiation	II	18	G12D
NJ041	PDAC, moderate differentiation	III	214	G12D
NJ042	PDAC, moderate to poorly differentiation	III	170	G12D
NJ043	PDAC, moderate differentiation	I	58.25	G12D
NJ044	PDAC, poor differentiation	III	88.92	G12V
NJ045	PDAC, moderate differentiation	III	457	Q61H
NJ046	PDAC, moderate to poorly differentiation	III	131	WT
NJ047	PDAC, poor differentiation	III	>1000	G12V
NJ048*	chronic pancreatitis	N/A	59	WT
NJ049	PDAC, with metastases	Ⅳ	>1000	G12V
NJ050**	PDAC, moderate differentiation	Ⅱ	74	G12R
NJ051**	PDAC, moderate differentiation	Ⅲ	43	G12V
NJ052*	Duodenal carcinoma	N/A	150	G12D
NJ053	PDAC, poor differentiation	Ⅱ	289	G12D
NJ054	PDAC, moderate to well differentiation	Ⅲ	277	G12V
NJ055	PDAC, moderate differentiation	Ⅲ	>1000	WT
NJ056	PDAC, moderate differentiation	Ⅱ	3.5	G12V
NJ057	PDAC, moderate to poorly differentiation	Ⅰ	33	G12V
NJ058**	PDAC, poor differentiation	Ⅳ	>1000	WT
NJ059	PDAC, moderate differentiation	Ⅳ	>1000	WT
NJ060*	Breast cancer, pancreatic metastases	N/A	340	WT
NJ061	PDAC, moderate to poorly differentiation	Ⅱ	16	G12D
NJ062	PDAC, moderate differentiation	Ⅱ	20	G12D
NJ063	PDAC, moderate to poorly differentiation	Ⅱ	129	G12D
NJ064	PDAC, moderate to poorly differentiation	Ⅱ	208	G12V
NJ065	PDAC, moderate to poorly differentiation	Ⅱ	130	G12D
NJ066**	PDAC, moderate differentiation	N/A	2.97	WT
NJ067	PDAC, moderate differentiation	Ⅰ	74	Q61H
NJ068	PDAC, moderate differentiation	Ⅱ	69	G12V
NJ069	PDAC, moderate differentiation	Ⅲ	20	G12D
NJ070	PDAC, moderate differentiation	Ⅱ	753	G12V

*Note: * patients with other diseases determined by pathological section after the surgery, ** patients received neoadjuvant therapy before tumor resection.*

Note

TILs expanded with feeder cells are highly cytolytic

To determine the ability of TILs to degranulate upon incubation with an NK cell target, we mixed 4×105 TILs with 4×105 K562. We incubated them for four hours in the presence of anti-CD107a antibody (1:100) and protein transport inhibitor (1:1000) at a 96-well plate. A culture containing 4×105 TILs only was included as the negative control. After incubation, cells were washed with PBS containing 0.1% BSA, then 100 ul of antibody diluent (1:200 anti-CD3, 1:200 anti-CD4, 1:200 anti-CD8, and 1:200 anti-CD56) was added to the cells and incubated for 30 min at 40°C. Cells were then subjected to flow cytometry analysis to determine the ability of NK cell degranulation, as shown in supplementary data Figure S2

Macron et al. found the relative abundance of NK cells in pancreatic tumors to be lower than in peripheral blood, and these NK cells in PDAC are typically dysfunctional due to the downregulation of activation receptors and CD16 [(1)]. The phenotype of ex-vivo expanded TIL-NK was assessed by flow cytometry using antibodies against NK receptors or activation markers – NKG2C, NKG2D, NKp30, and NKp46. We found no difference in expression of these four markers between NK expanded with different feeder cells (Figure S2C). Surprisingly, we found no NKG2c and NKp46 expression on these ex-vivo expanded TILs. On average, around 50% of ex-vivo expanded TILs expressed NKG2D and NKp30 on their surface. To determine the functional activity of ex-vivo expanded TIL-NK cells, we measured the degranulation marker CD107a expression on NK cells after challenge with K562 target cells (Figures S2A and 3). Around 60% of NK cells rapidly degranulated. However, the NK cells

expanded most vigorously by ECCE feeder cells were less functional at degranulation (30%) (Figure S2B). The reduced NK degranulation reactivity could be explained by NK desensitizing effect by feeder cells which are based on K562 cells [2 3]. However, this desensitizing effect might be negated by the expression of mbIL-21 on feeder cells.

References:

bm1. Marcon F, Zuo J, Pearce H *et al.* NK cells in pancreatic cancer demonstrate impaired cytotoxicity and a regulatory IL-10 phenotype. *Oncoimmunology*. 2020;**9**:1845424.doi:10.1080/2162402X.2020.1845424

bm2. Thompson TW, Kim AB, Li PJ *et al.* Endothelial cells express NKG2D ligands and desensitize antitumor NK responses. *Elife*. 2017;**6**:e30881.doi:10.7554/eLife.30881

bm3. Ardolino M, Azimi CS, Iannello A *et al.* Cytokine therapy reverses NK cell anergy in MHC-deficient tumors. *J Clin Invest*. 2014;**124**:4781-4794.doi:10.1172/JCI74337