

Tissue-specific anti-tumor NK cell subsets identified in colorectal cancer liver metastases express candidate therapeutic targets

Joanna Mikulak^{1*}, Domenico Supino^{2*}, Paolo Marzano^{1,3}, Sara Terzoli^{1,4}, Roberta Carriero⁵,
Valentina Cazzetta¹, Rocco Piazza⁶, Elena Bruni¹, Paolo Kunderfranco⁴, Alessia Donato², Sarah
Natalia Mapelli², Roberto Garuti⁴, Silvia Carnevale⁷, Francesco Scavello², Elena Magrini², Jelena
Zeleznjak^{2,8}, Clelia Peano^{9,10}, Matteo Donadon¹¹, Guido Costa¹², Guido Torzilli^{4,12}, Alberto
Mantovani^{2,4,13}, Cecilia Garlanda^{2,4†} and Domenico Mavilio^{1,3†}

¹Unit of Clinical and Experimental Immunology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

²Unit of Experimental Immunopathology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

³Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.

⁴Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy.

⁵Bionformatic Unit, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

⁶Department of Medicine and Surgery, University of Milan-Bicocca, Monza, Italy.

⁷Unit of Innate immunity in inflammation and cancer, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

⁸Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.

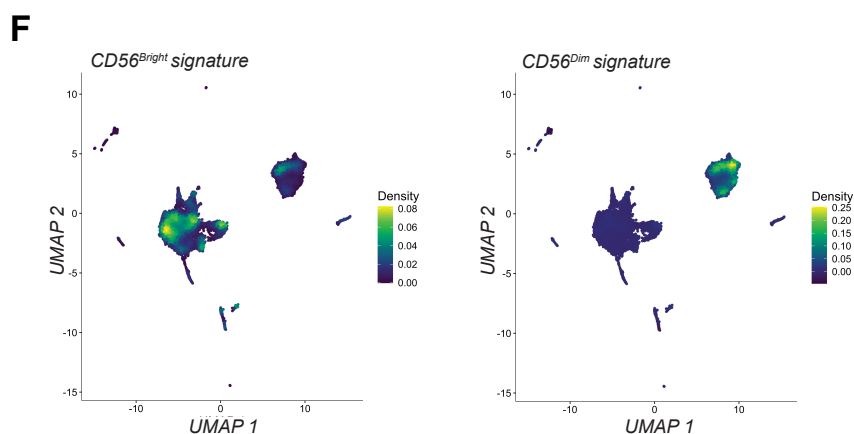
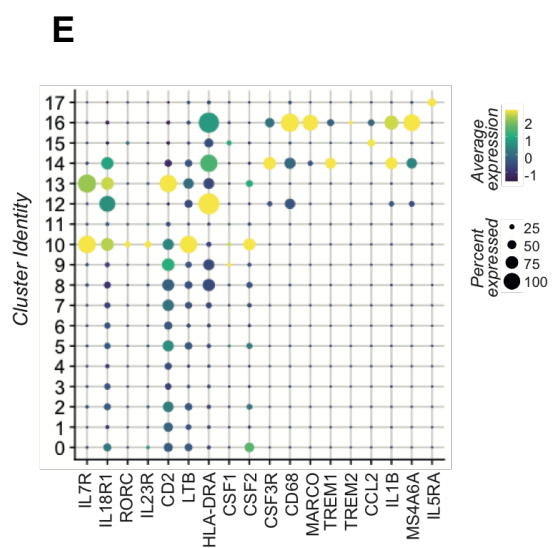
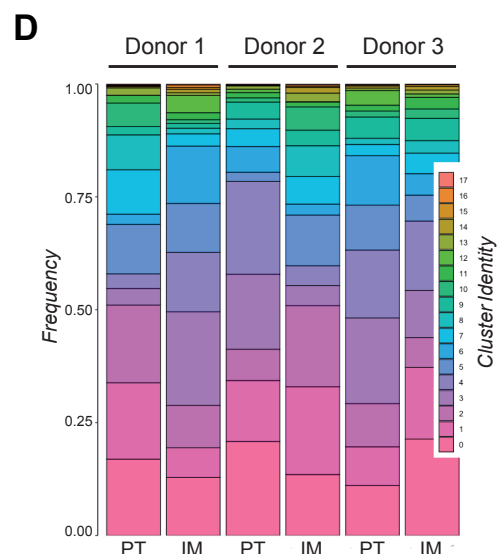
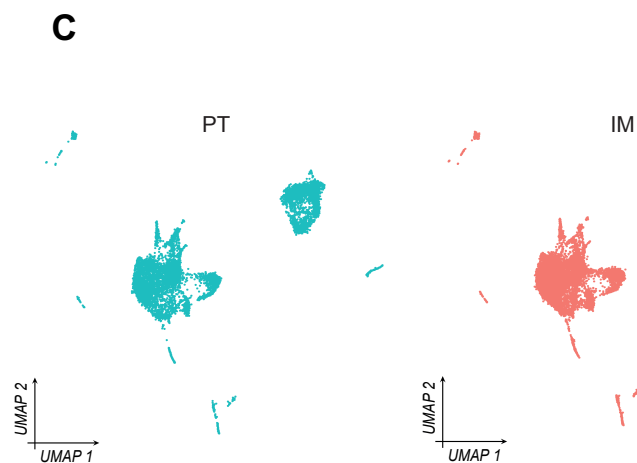
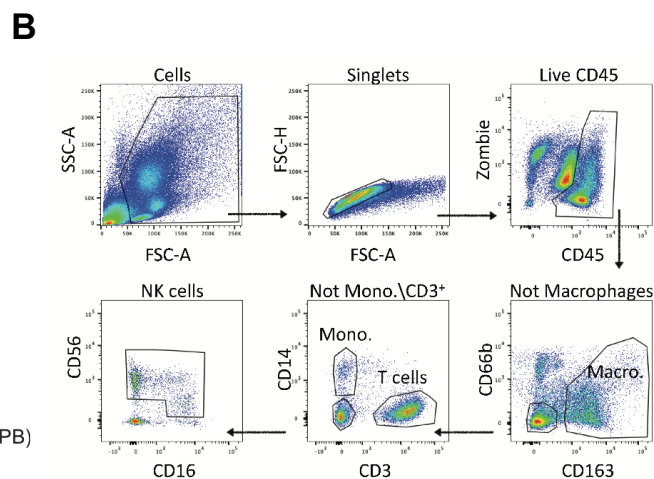
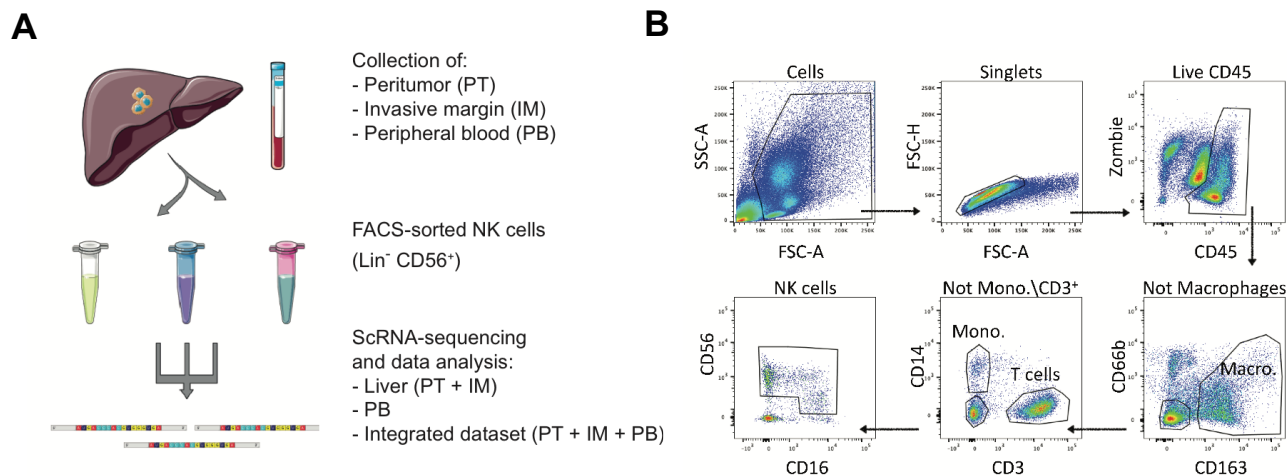
⁹Institute of Genetics and Biomedical Research, UoS of Milan, National Research Council, Rozzano, Milan, Italy.

¹⁰Human Technopole, Milan, Italy.

¹¹Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy; Department of General Surgery, University Maggiore Hospital Della Carità, Novara, Italy.

¹²Unit of Hepatobiliary and General Surgery, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

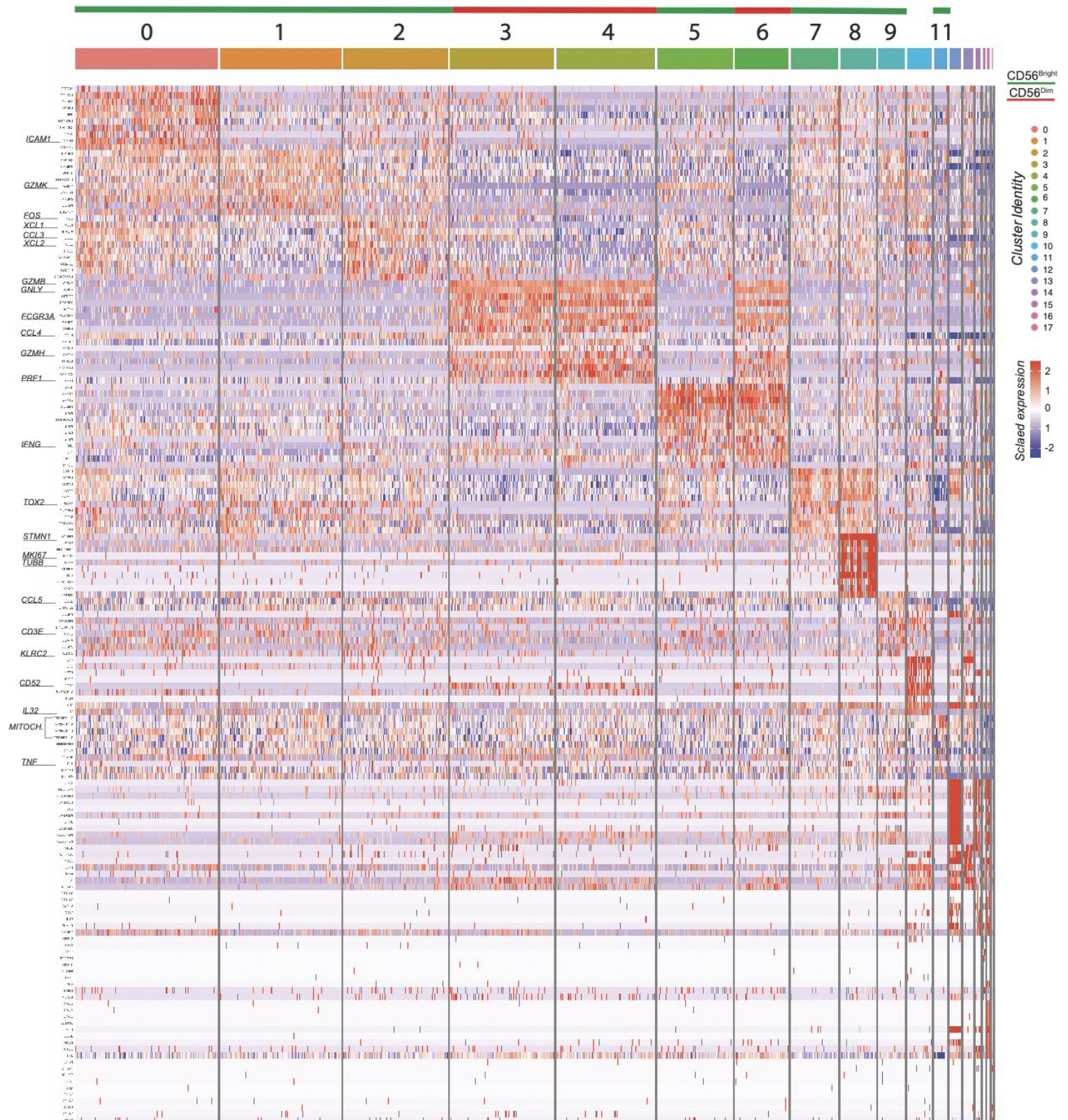
¹³The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom.



Supplementary Figure 1. Single cell RNA-seq analysis of NK cells in CRLM samples

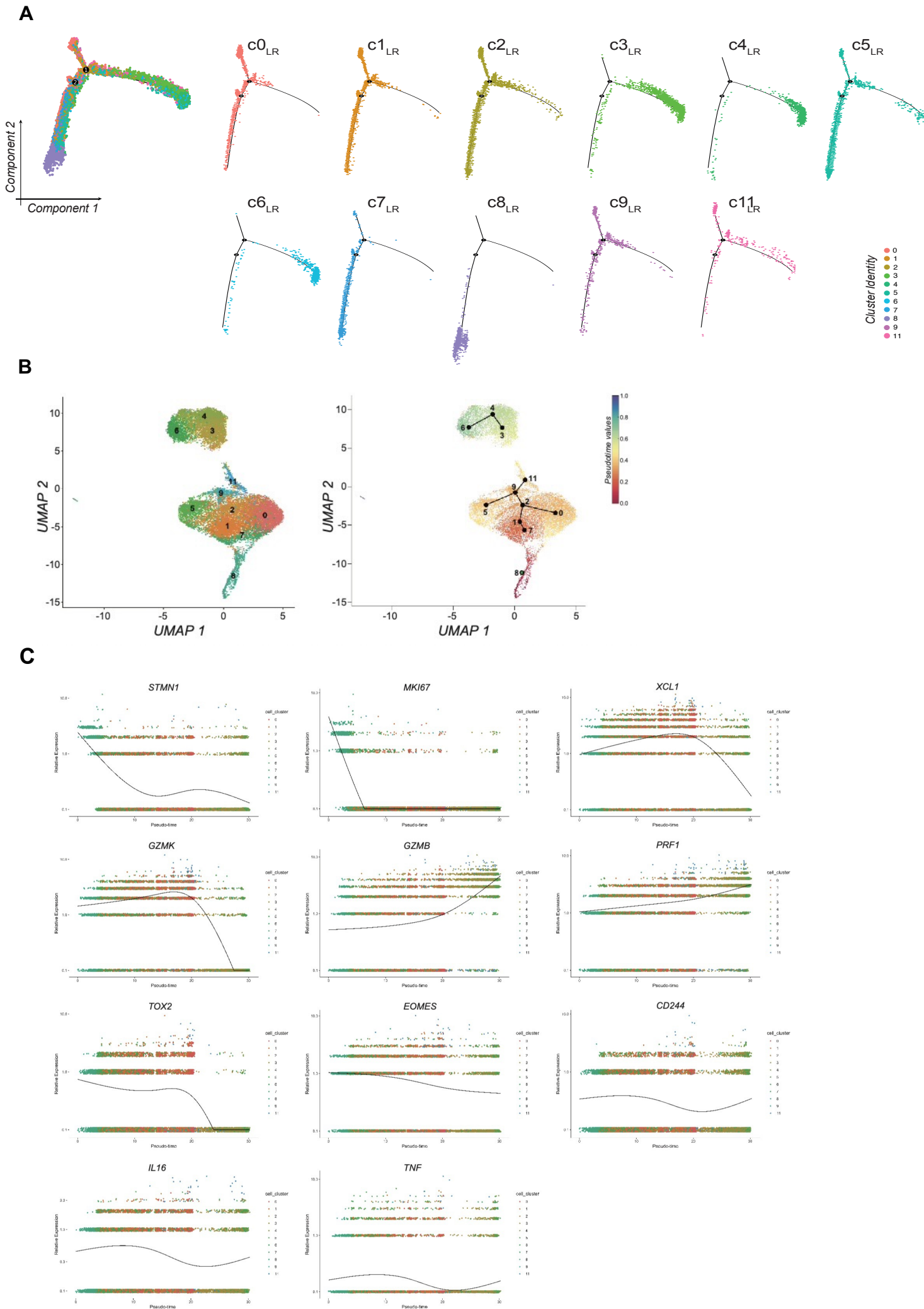
A. Schematic overview of the experimental design. **B.** One representative gating strategy (out of 9 samples) of flow cytometry-sorted peripheral blood NK cells to perform scRNA-seq analysis. NK cell purity after FACS-sorting was $\geq 97\%$. **C.** *UMAP* showing the distribution of NK_{LR} from peritumor (PT) and invasive margin (IM) tissues. **D.** Bar graph showing the frequency (%) of NK cells from the invasive margin (IM) and peritumor (PT) CRLM tissue clusters in each patient (Donor 1-3). Cell numbers were normalized to the total number of cells per tissue and patient. **E.** Dot plot showing the expression of selected myeloid and MAIT cell genes in all NK_{LR} cells. **F.** Kernel density of the *CD56^{Bright}* and *CD56^{Dim}* signature scores embedded on UMAP plot.

Supplementary Figure 2



40 **Supplementary Figure 2. Heatmap of the top 10 DEGs identified in each tissue NK_{LR} cell cluster**
41 NK_{LR} cell cluster characterization. The heatmap shows the scaled average expression of the top
42 10 DEGs (rows) for each identified NK_{LR} cell cluster shown in Figure 1A. CD56^{Bright} and CD56^{Dim}
43 NK_{LR} cell subsets are highlighted in green and red, respectively.

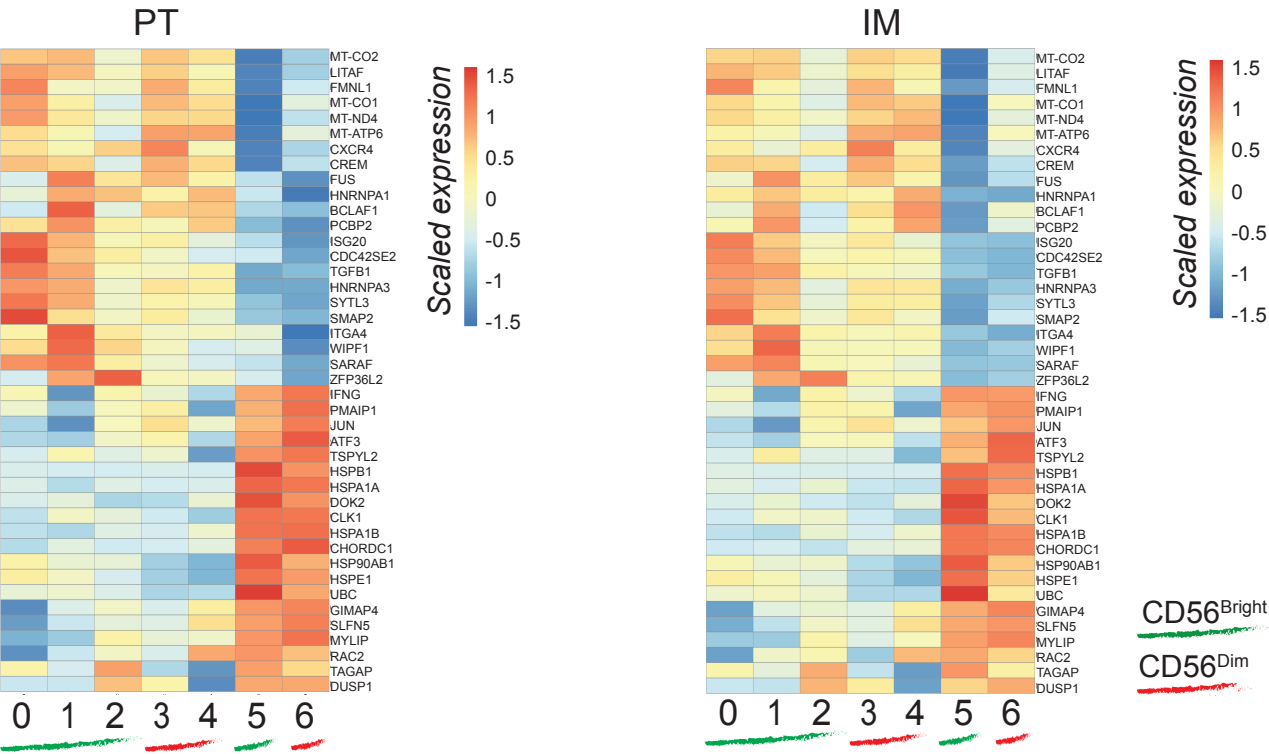
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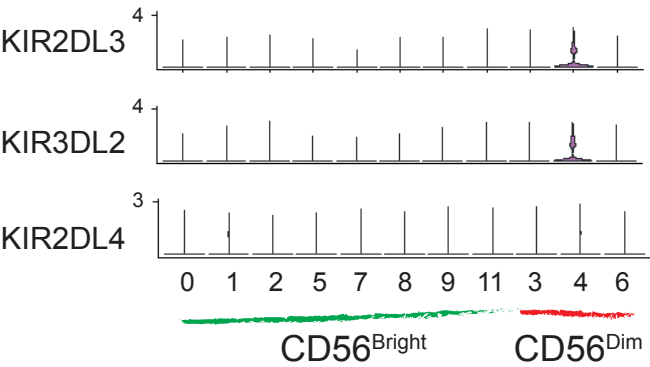
Supplementary Figure 3. Pseudotime trajectory of NK_{LR}

A. Pseudotime trajectory of NK_{LR}, where each cell is colored by its cluster identity (left panel). Pseudotime trajectory of each NK_{LR} cell cluster (right panel). **B.** Inferred developmental trajectory with Slingshot of the intrahepatic NK_{LR} cells embedded on the *UMAP*. Left panel, *UMAP* clustering projection; right panel, inferred trajectory displayed on the *UMAP*. **C.** DEGs were selected in cycling (*STMN1*, *MKI67*), CD56^{Bright} (*XCL1*, *GZMK*), CD56^{Dim} (*GZMB*, *PRF1*) NK_{LR} cells or associated with functional features of NK_{LR} from CRLM.

A



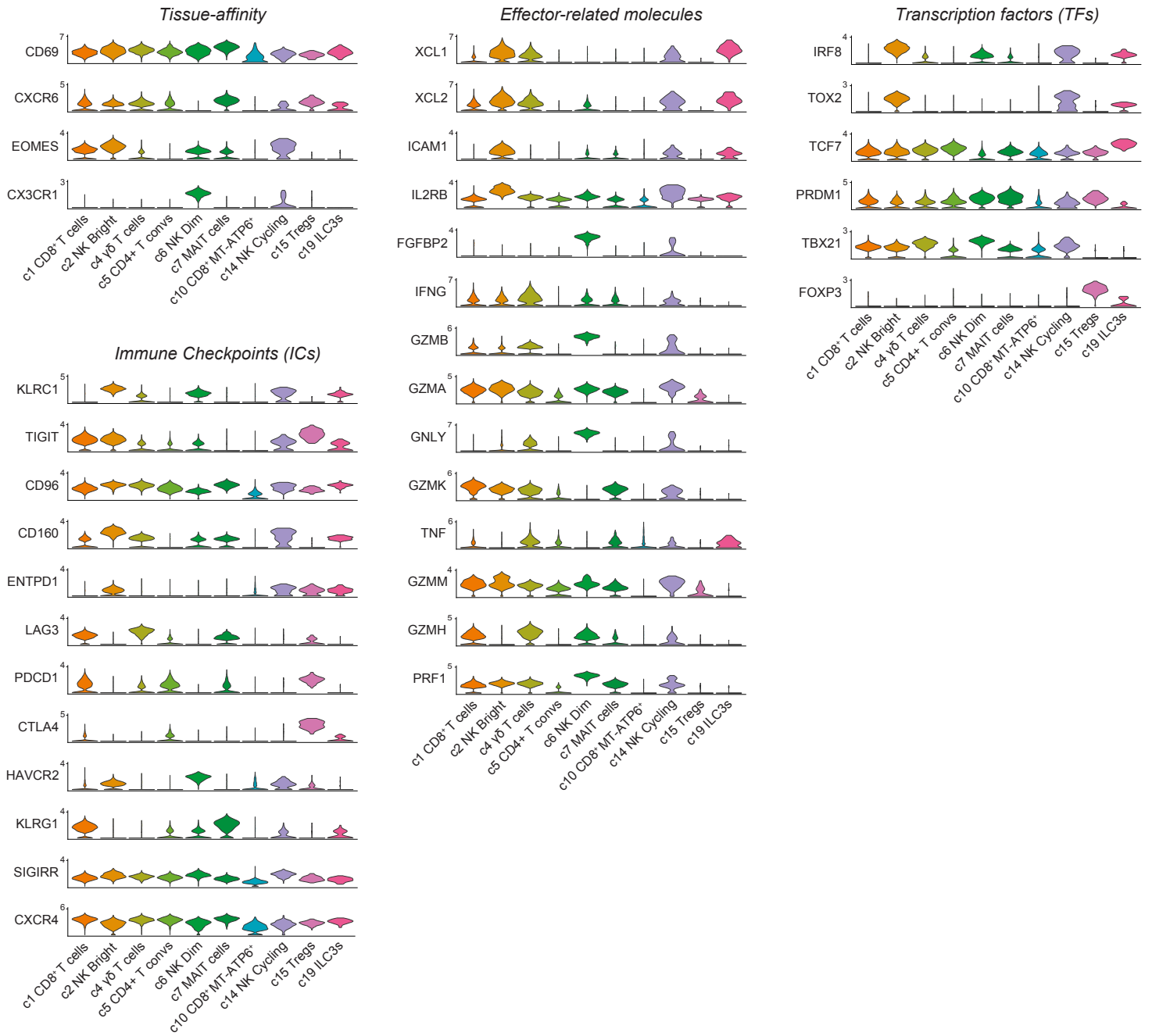
B



Supplementary Figure 4. Shared *IFNG*^{high} signature gene profile among NK_{LR} cell subsets

A. Heatmap showing *IFNG*^{high} signature gene expression in tissue identified NK_{LR} cell clusters for peritumor (PT) (left panel) and invasive margin (IM) (right panel). **B.** Violin plot displaying the expression of selected KIRs. CD56^{Bright} and CD56^{Dim} NK_{LR} cell subsets are highlighted in green and red, respectively.

Supplementary Figure 5



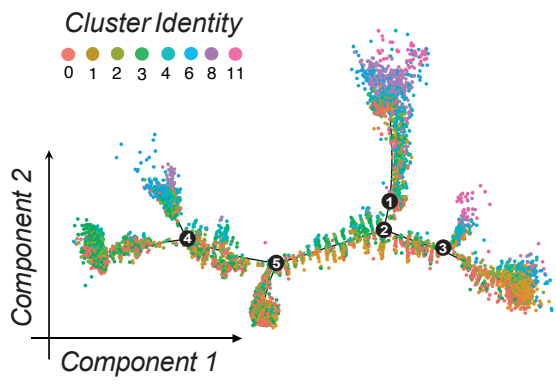
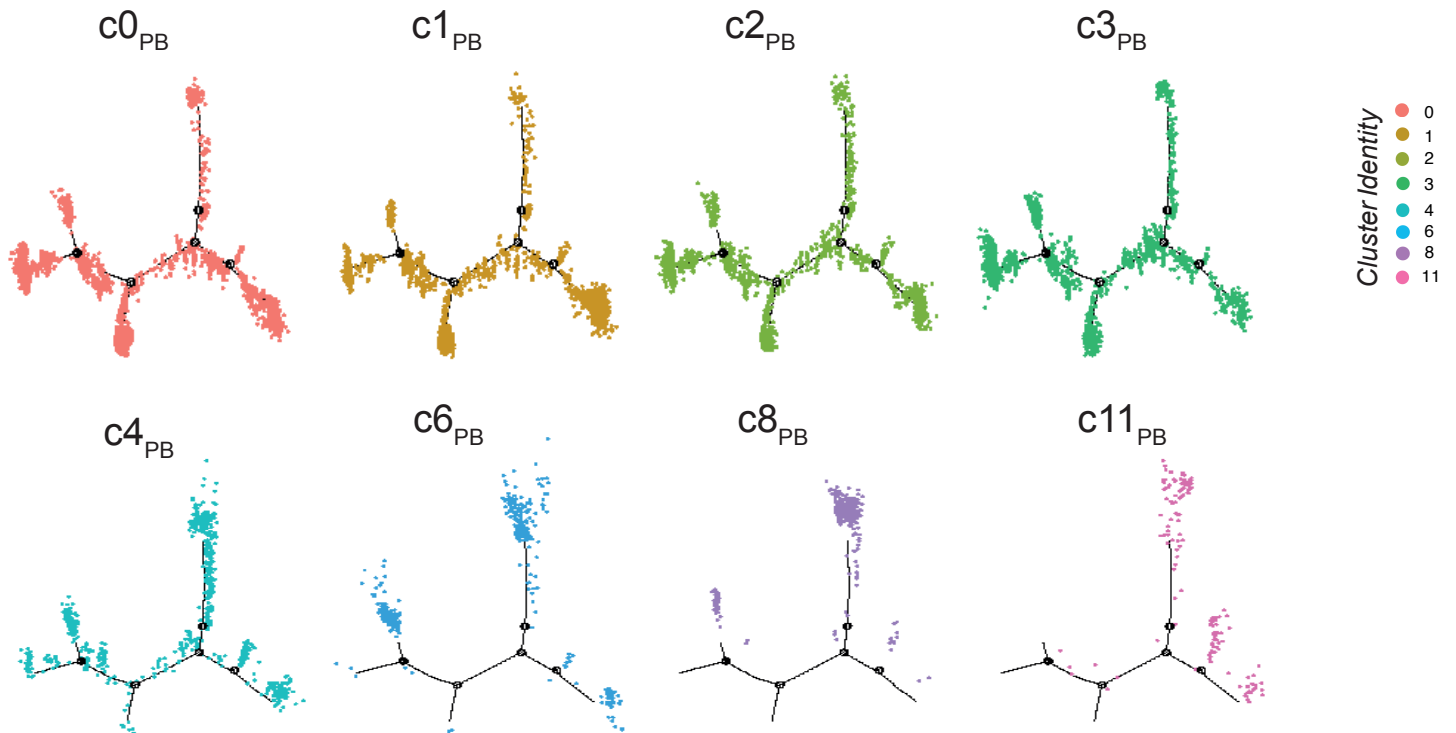
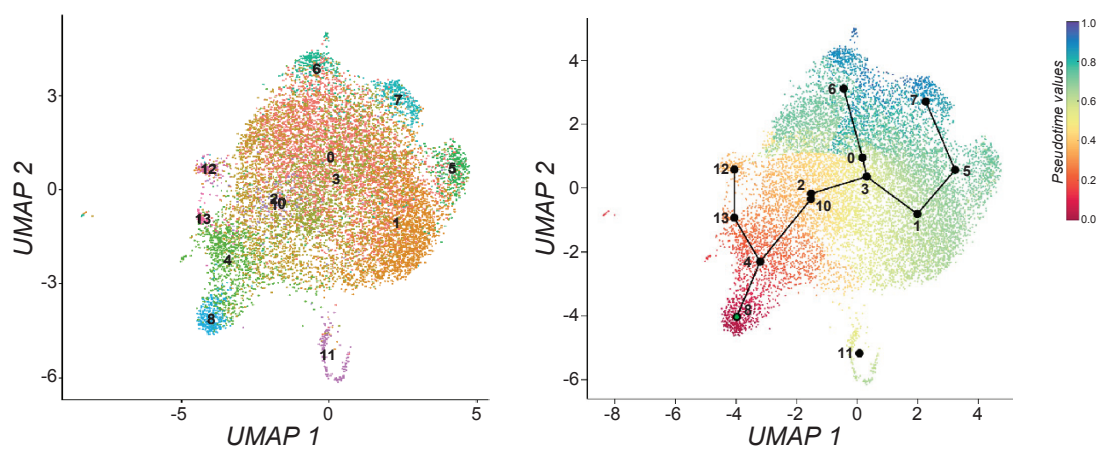
59 **Supplementary Figure 5. Comparison of hepatic NK cells, T cells and ILC3 in CRLM tissue.**

60 Violin plots of selected genes across the intrahepatic lymphocyte clusters

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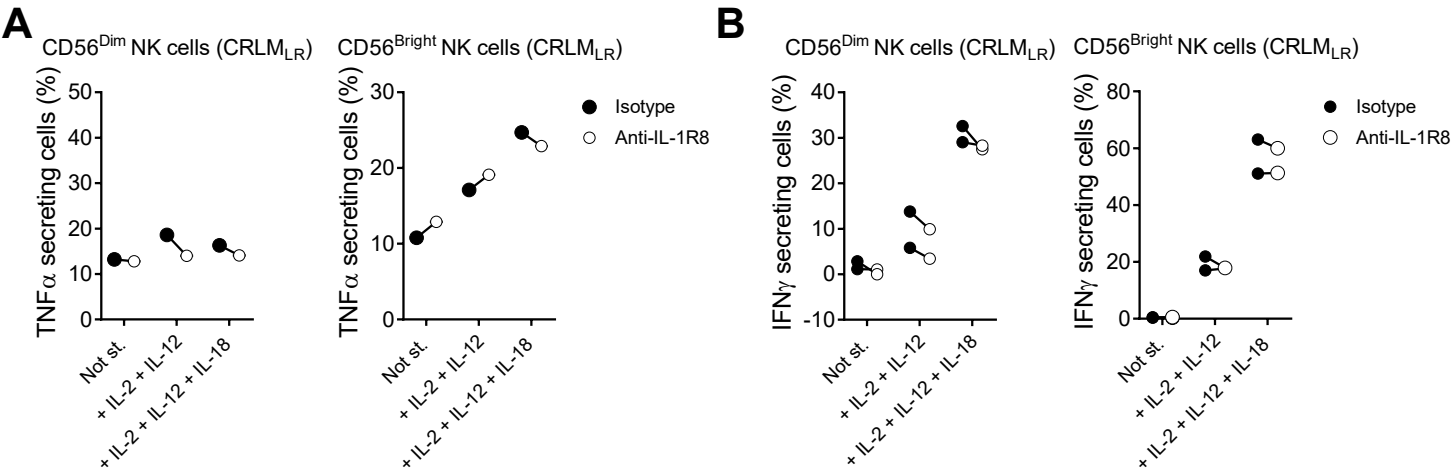
Supplementary Figure 6. Single cell RNA-seq analysis of blood NK_{PB} cells in CRLM patients

A. Blood NK_{PB} cell cluster characterization. Bar graph representing the frequency (%) of each NK_{PB} cell cluster for each CRLM patient. Cell numbers were normalized to the total number of cells per patient. **B.** Dotplot showing the expression of selected myeloid and MAIT cell genes. **C.** Kernel density of the *CD56^{Bright}* and *CD56^{Dim}* signature scores embedded on UMAP plot. **D.** Heatmap showing the scaled average expression of the top 10 DEGs (rows) for each identified NK_{PB} cell cluster shown in Figure 5A. *CD56^{Bright}* and *CD56^{Dim}* NK_{PB} cell subsets are highlighted in green and red, respectively.

A**B****C**

Supplementary Figure 7. Pseudotime trajectory analysis of blood NK_{PB} cells.

A. Pseudotime trajectory of merged NK_{PB} cell clusters. **B.** Pseudotime trajectory of each NK_{PB} cell cluster. Each cell is colored by its cluster identity. **C.** Inferred developmental trajectory of the circulating NK cells computed with Slingshot algorithm. Left panel, *UMAP* clustering projection; right panel, inferred trajectory displayed on the *UMAP*.



77 **Supplementary Figure 8. Not-responder patients stimulated with anti-hIL-1R8 mAb**

78 **A-B.** Evaluation of TNF α (A) and IFN γ (B) in CD56^{Dim} and CD56^{Bright} NK cells from CRLM patients
79 stimulated with an anti-hIL-1R8 mAb or its isotype control.

80

Supplementary Table 1
Monoclonal antibodies (mAbs) used for flow cytometry analysis.

Marker	Fluorochrome	Clone	Company	Catalogue number
CD3	BUV661	UCHT1	BD	565065
CD16	BUV737	3G8	BD	564434
CD56	BUV563	NCAM16.2	BD	565704
CXCR4	BUV395	12G5	BD	563924
CXCR6	BV650	13B	BD	743600
CTLA-4	APC	BNI3	BD	560938
ILT-2	PE-Cy5	GHI/75	BD	551054
Ki67	PE	B56	BD	51-36525X
LAG-3	APC-R700	T47-530	BD	565774
LAIR-1	PE	DX26	BD	550811
TIGIT	BV421	741182	BD	747844
TIM-3	BV711	7D3	BD	565566
CD14	BV570	M5E2	BioLegend	301832
CD19	BV570	HIB19	BioLegend	302236
CD39	PE/Dazzle594	A1	BioLegend	328224
CD96	PE-Cy7	NK92.39	BioLegend	338415
KLRG1	BV785	2F1/KLRG1	BioLegend	138429
IFN γ	PE-Cy7	B27	BioLegend	506518
TNF α	BV785	Mab11	BioLegend	502948
NKG2A	VioBright-FITC	REA110	Miltenyi	130105646
IL-1R8	Biotin		R&D	BAF990

Supplementary Table 2

NK Dim score	NK Bright score	Cell cycle score	
CD160	LTB	MCM5	NUSAP1
CTSD	FOS	PCNA	UBE2C
CCL4	IL2RB	TYMS	BIRC5
ADGRG1	IFITM3	FEN1	TPX2
CD38	COTL1	MCM2	TOP2A
CD247	IL7R	MCM4	NDC80
CHST2	PIK3R1	RRM1	CKS2
CX3CR1	AREG	UNG	NUF2
KLRB1	ZFP36L2	GIN52	CKS1B
LAIR2	DUSP2	MCM6	MKI67
IGFBP7	CD44	CDCA7	TMPO
AKR1C3	SELL	DTL	CENPF
FGFBP2	GPR183	PRIM1	TACC3
MYOM2	CMC1	UHRF1	FAM64A
CLIC3	KLRC1	HELLS	SMC4
GZMB	TCF7	RFC2	CCNB2
PRF1	TPT1	RPA2	CKAP2L
FCER1G	XCL2	NASP	CKAP2
NKG7	XCL1	RAD51AP1	AURKB
SPON2	GZMK	GMNN	BUB1
LGALS1	CCL3	WDR76	KIF11
GZMH	ZNF331	SLBP	ANP32E
S100A4	TOX2	CCNE2	TUBB4B
PRSS23	NR4A2	UBR7	GTSE1
GNLY	CD160	POLD3	KIF20B
S100A6	SERPINE2	MSH2	HJURP
SYNE2	FAM177A1	ATAD2	CDCA3
EMP3	ABHD15	RAD51	HN1
CD99	LEPROTL1	RRM2	CDC20
KIR2DL3	CD27	CDC45	TTK
S1PR5	CXCR6	CDC6	CDC25C
KIR2DL1	CD69	EXO1	KIF2C
FCRL6	ADGRG3	TIPIN	RANGAP1
HOPX	SPRY1	DSCC1	NCAPD2
GTF3C1	ALOX5AP	BLM	DLGAP5
	TIGIT	CASP8AP2	CDCA2
	IFRD1	USP1	CDCA8
	IRF8	CLSPN	ECT2
	TSC22D3	POLA1	KIF23
	KLF6	CHAF1B	HMMR
	ELF1	BRIP1	AURKA
	JUND	E2F8	PSRC1
	STAT4	HMGB2	ANLN
		CDK1	LBR
			CKAP5
			CENPE
			CTCF
			NEK2
			G2E3
			GAS2L3
			CBX5
			CENPA