Supplemental Table and figure legends

Table S1 – Patient clinical information, Related to Figures 2, 4, 5, 6

Table S2 – Single-cell RNA sequencing cluster assignment, Related to Figure 2 (Excel file)

Table S3 – HLA class I genotype of bladder cancer patients, Related to Figure 2

Table S4 – Mass Cytometry panels, Related to Figures 3, 4, 6

Table S5 – RNA velocity analysis, Related to Figure 5 (Excel file)

Figure S1. Improved overall survival in NKG2A$^+$ (*KLRC1* ^{high}) CD8 T cell-infiltrated bladder tumors, Related to Figure 1
Immunofluorescence staining of *KLRC1*^{high} and *KLRC1*^{low} bladder tumors.
(A) Representative staining of *KLRC1*^{high} bladder tumors (n=1). Green: NKG2A, Red: NKp46, Blue: DAPI
(B) Representative staining of *KLRC1*^{low} bladder tumors (n=1). Green: NKG2A, Red: NKp46, Blue: DAPI, White: CD3
(C) Summary graph of NKG2A expression in CD8 T cells from *KLRC1*^{high} (n=6) and *KLRC1*^{low} (n=4) bladder tumors. Error bars represent the SEM.

Figure S2. Bladder tumor cell-surface expression of HLA class I and ligands for PD-1 and activating NK cell receptors, Related to Figure 2.
(A) Representative staining for HLA-ABC, HLA-E and the tumor marker EpCAM by immunohistochemistry, alongside Halo AI™-derived classification and segmentation of bladder tumor cells (n=1).
(B) Differentially expressed genes in CD8 T cells from *HLA-E*^{high} (n=1973 cells from 4 donors) versus *HLA-E*^{low} (n=869 cells from 3 donors) bladder tumors.
(C) The expression of HLA class I and ligands for PD-1 (PD-L1/L2), DNAM-1 (CD112, CD115), NKG2D (ULBPs, MICA/B), NKp30 ("NKp30-L"), NKp44 ("NKp44-L"), NKp46 ("NKp46-L") was assessed by flow cytometry on n=11 bladder cancer lines from histological grade 1 to 3 as well as on the control cell line K562 (n=1 replicate). Grey: unstained control. Red: stained cells.

Figure S3. NKG2A-expressing CD8 T cells are differentiated and possess TCR-independent NK-like functions in healthy individuals, Related to Figure 3
(A-D) Mass cytometry was performed *ex vivo* on HD PBMCs (n=20).
(A) CMV and gender effect on NKG2A expression on CD8 T cells from HD PBMCs depending on differentiation stage.
(B) Correlation between age and NKG2A expression on CD8 T cells depending on differentiation stage.
(C) Correlation between HLA-E expression on lymphocytes and NKG2A expression on CD8 T cells depending on their differentiation stage.
(D) NKp30, NKp46, NKG2D and DNAM-1 expression on NKG2A$^{+/-}$ CD8 T cells depending on their differentiation stage.
(E) Representative NKG2A and CD28 staining on CD8 T cells (n=1).
(F-H) CD8 T cells were isolated from HD PBMCs and recovered overnight with IL-12, IL-15, IL-18 10ng/mL prior to a 5h co-culture with K562 in the presence of brefeldin and monensin (n=20)
(F) Degranulation, production of IFN-γ, TNF-α, XCL1, IL-2 and cytolytic content of CD8 T cells upon K562 stimulation.
(G) Amount of functional markers acquired or released upon K562 stimulation in CD8 T cells and NK cells.
(H) Effect of CD8 T cell NKG2A expression, lymphocyte HLA-E expression, age, gender and combined effect of NKG2A with HLA-E on the upregulation of functional markers upon K562 stimulation.
(I-J) CD8 T cells were isolated from HD PBMCs and recovered overnight with IL-12, IL-15, IL-18 10ng/mL prior to a 5h co-culture with K562 in the presence of brefeldin and monensin in the presence or absence of anti-DNAM-1 blocking antibody (n=10).
(I) Correlation between NKG2A or CD28 expression within each cluster and Log2(Fold change) of the expression of XCL1 upon K562 stimulation.
(J) Log2(Fold change) of IFN-γ and TNF-α expression upon anti-DNAM-1 blocking antibody addition, versus isotype control, in all clusters.
Pearson correlation was used in (B), (C), (I), paired t-tests in (F), linear models in (H) and unpaired t-tests in (I), (J). All statistical tests were adjusted for multiple comparison. Only the significant comparisons are displayed in (H). (A), (D), (F), (G): Error bars represent the SEM. "ns" $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Figure S4. NKG2A defines a subset of PD-1$^+$ TRM CD8$^+$ T cells that retain TCR-independent functions in bladder tumors, Related to Figure 4
(A-D) Mass cytometry was performed on *ex vivo* bladder tumor-draining lymph nodes, tumor and adjacent tissue from bladder cancer patients.
(A) Summary *ex vivo* expression of cytotoxic mediators on CD8 T cells from bladder tumor-draining lymph

nodes (n=5), tumor (n=7) and adjacent tissue (n=6). Lines show matching samples from a unique donor.
(B) CD8 T cell phenotype in bladder tumor-draining lymph nodes that are tumor-infiltrated ("TILN", n=2) or not tumor-infiltrated ("non-TILN", n=3).
(C) Association between normalized mean signal intensity of key markers on CD8 T cells with pseudotime (n=7).
(D) Phenotype of $PD1^+$ $NKG2A^-$ and $PD1^+$ $NKG2A^+$ CD8 T cells in the tumors (n=6). Only the nonsignificant markers are displayed.
Paired t-tests were used in (A), (D).

Figure S5. *KLRC1* (NKG2A) expression in CD8 T cells does not associate with a skewed TCR clonal repertoire, Related to Figure 4
(A-D) *KLRC1* $^{low/high}$ CD8 T cell clonality was determined by analyzing publicly available scRNAseq and TCRseq data from bladder tumors (n=7) or non-involved adjacent tissue (n=2) (Oh *et al.*, 2020).
(A) Frequency of TCRs present in 1, 2 or at least 3 cells per sample, depending on *KLRC1* expression.
(B) Summary data in tumor and non-involved adjacent tissue. Error bars represent the SEM.
(C) Frequency of TCRs present in 1 cell depending on *KLRC1* expression.
(D) Number of TCRs present in at least 2 cells that are unique or common to *KLRC1* ^{low} and *KLRC1* ^{high} cells per sample. Only samples with at least 2 TCRs present in at least 2 *KLRC1* ^{high} cells were selected.

Figure S6. TCR-independent function is acquired by CD8 T cells upon NKG2A acquisition and is enhanced with NKG2A blockade in bladder cancer, Related to Figure 6
(A-F) CD8 T cells were isolated from bladder tumor-draining lymph nodes and expanded for 8-13 days with IL-2, IL-7, IL-15 and CD3/CD28 tetramer. $CD49a^-$ $NKG2A^-$ $PD1^-$ (n=5), $CD49a^+$ $NKG2A^-$ $PD1^-$ (n=6), $CD49a^{+/-}$ $NKG2A^-$ $PD1^+$ (n=6) and $CD49a^{+/-}$ $NKG2A^+$ $PD1^-$ (n=5) cells were then FACS-sorted and expanded during 3 days with IL-2, IL-7, IL-15 and CD3/CD28 tetramer with or without TGF-β prior to coculture with K562. Mass cytometry was performed at all timepoints.
(A) Representative staining of NKG2A/PD1 expression on FACS-sorted cells upon 3-day expansion (n=1 patient).
(B) Summary data of NKG2A/PD1 expression on FACS-sorted cells upon 3-day expansion.
(C) Upregulation of functional markers upon K562 co-culture of CD8 T cells that have been FACS-sorted as $CD49a^-$ or $CD49a^+$ $NKG2A^-$ $PD1^-$ CD8 T cells and expanded during 3 days with TGF-β.
(D) Expression of markers associated with tissue-residency, exhaustion and NK/CD8T cell function on $CD49a^{+/-}$ $NKG2A^-$ $PD1^-$ CD8 T cells before and after 3-day expansion with or without TGF-β.
(E) Expression of markers associated with tissue-residency, exhaustion and NK/CD8T cell function alongside NKG2A and PD-1 acquisition upon 3-day expansion of $NKG2A^-$ $PD1^-$ CD8 T cells with TGF-β.
(F) Expression of functional markers alongside NKG2A and PD-1 acquisition upon 3-day expansion of $NKG2A^-$ $PD1^-$ CD8 T cells with TGF-β and 5h co-culture with K562.
(G-H) CD8 T cells were isolated from bladder tumors (n=6) and expanded for 11-17 days with IL-2, IL-7, IL-15 and CD3/CD28 tetramer, prior to 5h co-culture with Wild-type (WT) or $HLA\text{-}E^+$ K562.
(G) Degranulation of $NKG2A^+$ CD8 T cells upon co-culture with WT K562 in the presence or absence of monalizumab.
(H) Production of IFN-γ, TNF-α, XCL1 and IL-2 by $NKG2A_+$ CD8 T cells upon co-culture with $HLA\text{-}E^+$ K562 in the presence or absence of monalizumab.
Paired t-tests were used in (B)-(H). P-values were corrected for multiple comparisons in (B), (D), (E), (F).
(B), (G), (H): Error bars represent the SEM. "ns" $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Figure S1 Improved overall survival in NKG2A^{+} (*KLRC1*^{high}) CD8 T cell-infiltrated bladder tumors, Related to Figure 1

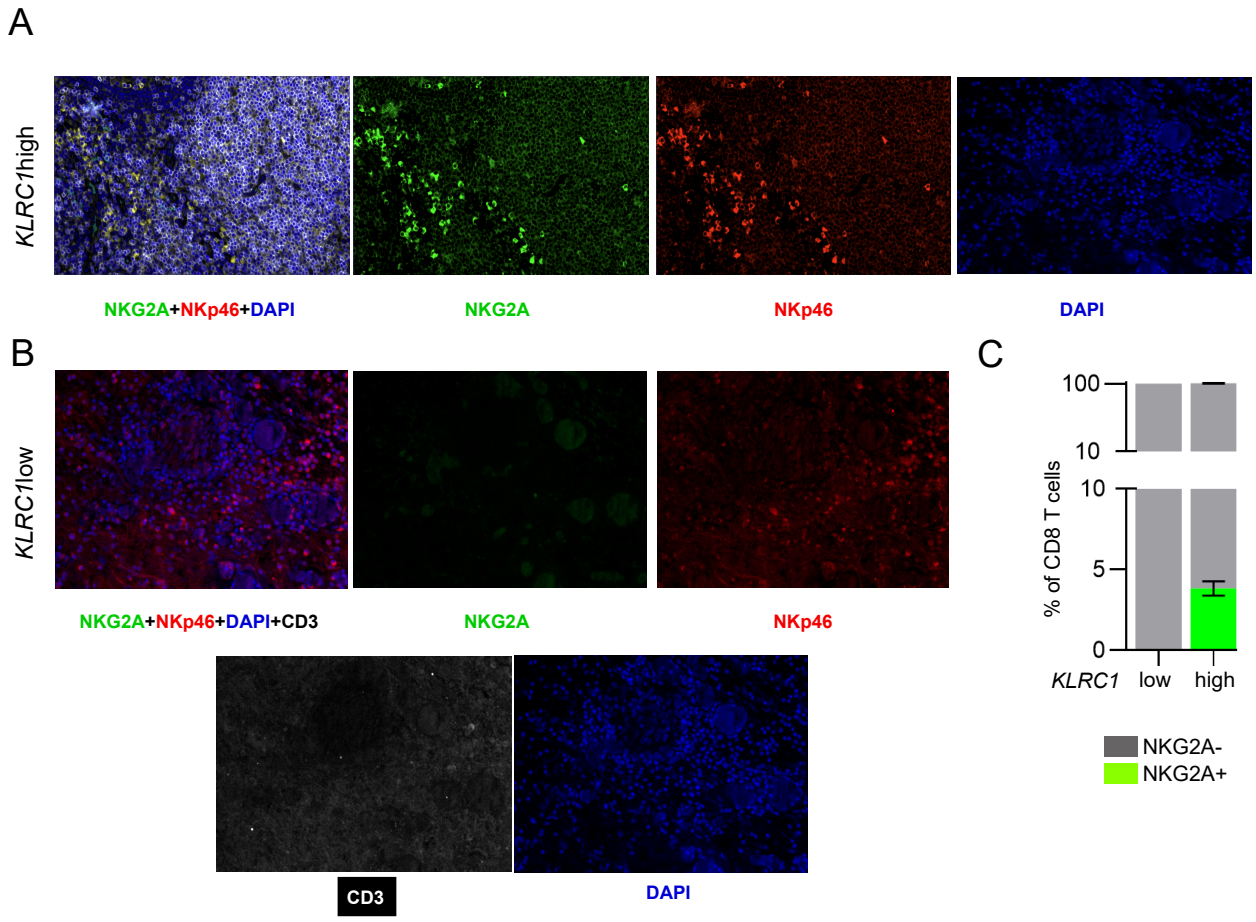

Table S1 Patient Clinical Information, Related to Figures 2, 4, 5, 6

ID	scRNAseq - bulk	Genotyped	Flow tumor	*ex vivo* CyTOF	scRNAseq - CD8T	TGF	Monalizumab	HTG / IF	IHC	NMI / MI	Pathology T stage	Pathology N stage	Surgery	Treatment history	Age	Sex	Note	Isolation (scRNAseq)
1										NMI	pT1	Nx	TURBT	BCG	85	M	Prostate cancer history	CD45+/- FACS
2										MI	pT3	N3	cystectomy	BCG, Gemcitabin/ Cisplatin/ Nivolumab	67	M		CD45+/- FACS
3										MI	pT4	N0	cystectomy	Gemcitabin/ Cisplatin	68	M		none
4										NMI	pT1	N0	cystectomy	Gemcitabin/ Cisplatin/ Nivolumab	73	M		none
5										MI	pT3b	N2	cystectomy	Gemcitabin/ Cisplatin/ Nivolumab	46	F		none
6										MI	pT3b	N2	cystectomy	none	71	M		none
7										NMI	pT0	N0	cystectomy	Gemcitabin/ Cisplatin/ Nivolumab	52	F		none
8										MI	pT2	N0	TURBT	none	60	M		none
9										NMI	pT1	N0	cystectomy	none	65	M		N/A
10										NMI	pT1	N0	cystectomy	BCG	85	M		N/A
11										MI	pT4a	N2	cystectomy	none	76	F		N/A
12										MI	pT2+	Nx	TURBT	none	56	M		N/A
13										NMI	pTa	Nx	TURBT	intravesical chemotherapy	62	M		N/A
14										NMI	pT1	Nx	TURBT	BCG	61	M		N/A
15										NMI	pT1	N0	cystectomy	BCG, radiation, chemotherapy	82	M		N/A
16										NMI	pT1	Nx	cystectomy	none	71	M		N/A
17										NMI	pTis	Nx	TURBT	none	74	M		N/A
18										MI	pT4a	N2	cystectomy	BCG, Gemcitabin/Cisplatin	61	M		N/A
19										MI	pT2a	N0	cystectomy	Gemcitabin/Cisplatin	69	M		N/A
20										MI	pT3a	N0	cystectomy	none	73	M	HIV+	N/A
21										NMI	pTis	N2	cystectomy	Methotrexate/Vinblastine/ Doxorubicin/Cisplatin	75	F		N/A
22										NMI	pTa	Nx	TURBT	none	84	F		N/A
23										NMI	pTa	Nx	TURBT	Gemcitabin	80	M		N/A
24										MI	pT3a	N1	cystectomy	none	67	F		N/A
25										NMI	pTa	Nx	TURBT	none	67	M		N/A
26										MI	pT2a	N0	cystectomy	none	74	M		N/A
27										NMI	pT1	Nx	TURBT	none	81	M		N/A
28										MI	pT3a	N0	cystectomy	none	73	M		N/A
29										MI	pTis	N2	cystectomy	chemotherapy	75	F		N/A
30										MI	pT0	N0	cystectomy	Gemcitabin/ Cisplatin/ Nivolumab	57	M		N/A
31										NMI	pTa	Nx	TURBT	none	84	F		N/A
32										NMI	pTa	Nx	TURBT	Gemcitabin	81	M		N/A
33										MI	pT3b	N1	cystectomy	none	73	M		N/A
34										NMI	pTa	Nx	TURBT	none	76	M		N/A
35										NMI	pT0	Nx	cystectomy	none	50	F		N/A
36										MI	pT3b	N2	cystectomy	none	71	M		N/A
37										MI	pT3a	N0	cystectomy	none	87	M		N/A
38										MI	pT3a	N0	cystectomy	none	76	M		N/A
39										NMI	pT1	Nx	TURBT	none	48	M		N/A
40										MI	pT3	N0	cystectomy	none	54	M		N/A
41										MI	pTis	N2	cystectomy	none	64	M		N/A
42										MI	pT2a	N0	cystectomy	none	84	M	Prostate cancer history	N/A
43										MI	pT4a	N0	cystectomy	none	65	M		N/A
44										NMI	pTa	N0	cystectomy	BCG	65	M		N/A
45										MI	pT3+	Nx	TURBT	BCG	78	M	Prostate cancer history	N/A
46										MI	pT3a	N2	cystectomy	none	75	F		N/A
47										MI	pT2a	N0	cystectomy	none	51	M		N/A
48										MI	pT3b	Nx	cystectomy	none	66	F		N/A

49										MI	pT2	Nx	TURBT	none	59	M	HIV+, not detectable; Renal cancer history	CD45+ FACS
50										NMI	pT0	Nx	TURBT	unknown	73	M		CD45+ FACS
51										MI	pT2	Nx	TURBT	none	66	M		CD45+ FACS
52										NMI	pTa	Nx	TURBT	none	82	F		CD45+ FACS
53										MI	pT1	Nx	TURBT	ipilimumab/nivolumab	67	M		CD45+ FACS
54										MI	pT3b	N0	cystectomy	BCG	82	M		CD45+ FACS
55										MI	pT3	N0	TURBT	none	85	F	Uterine cancer history	none
56										NMI	pTis	N0	cystectomy	BCG, intravesical chemotherapy	88	M		N/A
57										NMI	pT0	N0	cystectomy	BCG	58	M	Concomittant prostate cancer	N/A
58										MI	pT3a	N1	cystectomy	BCG	71	M		N/A
59										NMI	pT1	N0	TURBT	none	60	M		N/A
60										NMI	pTis	N0	TURBT	none	89	M		N/A
61										NMI	pT1	N0	TURBT	none	86	M		N/A
62										NMI	pTa	N0	TURBT	BCG	61	M		N/A
63										NMI	pT1	N0	TURBT	BCG/interferon	64	M		N/A
64										NMI	pT1	N0	TURBT	BCG	54	M		N/A
65										NMI	pT1	N0	TURBT	BCG	84	M		N/A
66										NMI	pTa	N0	TURBT	none	60	M		N/A
67										NMI	pT1	N0	TURBT	BCG	71	M		N/A
68										NMI	pTis/Ta	N0	TURBT	BCG, Mitomycin	81	F		N/A
69										NMI	pTis/Ta	N0	TURBT	none	66	M		N/A
70										NMI	pT1	N0	TURBT	none	75	M		N/A
71										NMI	pT1	N0	TURBT	none	50	M		N/A
72										NMI	pTis/Ta	N0	TURBT	none	74	M		N/A
73										NMI	pT1	N0	TURBT	BCG	84	M		N/A
74										NMI	pT2	N0	cystectomy	BCG	61	M		N/A
75										NMI	pT2	N0	TURBT	BCG, anti-PD-1	66	M		N/A
76										NMI	pTis/T1	N0	cystectomy	BCG	76	M		N/A
77										NMI	pT1	N0	TURBT	BCG	50	M		N/A
78										NMI	pTis/Ta	N0	TURBT	BCG	75	M		N/A
79										MI	pT2a	N3	cystectomy	platinum-based chemotherapy, pembrolizumab	75	M		N/A
80										MI	pT3a	N0	cystectomy		61	M		N/A
81										MI	pTa	N0	TURBT		75	M		N/A
82										MI	pT3	N2	Resection		64	F		N/A
83										MI	?	?	Resection		42	F		N/A
84										MI	?	?	TURBT		?	M		N/A
85										MI	pT3b	N3	cystectomy		86	M		N/A
86										MI	pT4b	N0	Anterior exenteration		?	F		N/A
87										MI	?	?	TURBT		47	F		N/A
88										MI	?	?	Ureteral biopsy	platinum-based chemotherapy	?	M		N/A
89										MI	pT2	N0	cystectomy	none	53	M		N/A

Figure S2 Bladder tumor cell-surface expression of HLA class I and ligands for PD-1 and activating NK cell receptors, Related to Figure 2

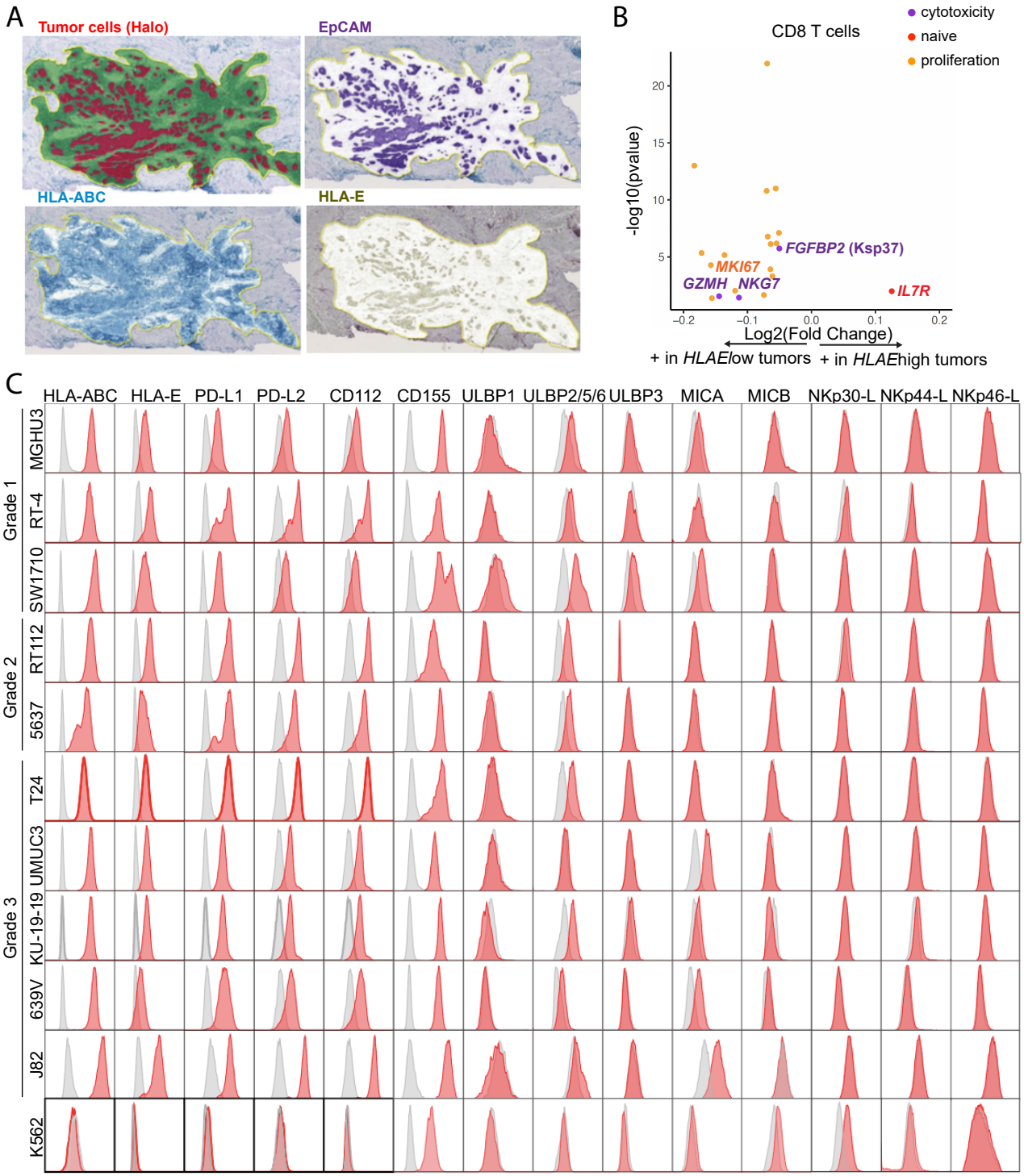

Table S3 HLA class I genotype of bladder cancer patients, Related to Figure 2

ID	HLA-A_1	HLA-A_2	HLA-B_1	HLA-B_2	HLA-C_1	HLA-C_2
#9	24:02	25:01	41:01	51:01	15:02	17:01
#10	02:01	02:01	38:01	48:01	08:03	12:03
#11	02:01	31:01	35:02	52:01	04:01	12:02
#12	23:01	26:01	35:01	53:01	04:01	06:02
#13	11:01	24:20	35:03	48:01	04:01	08:03
#14	01:01	23:01	53:01	81:01	04:01	18:01
#15	02:01	32:01	15:71	18:01	03:03	07:01
#16	02:01	02:05	35:01	58:01	04:01	07:01
#17	01:01	26:01	35:02	38:01	06:02	12:03
#18	11:01	24:02	13:02	52:01	06:02	12:02
#19	02:01	02:11	35:01	49:01	04:01	07:01
#20	02:01	23:01	42:01	44:03	04:01	17:01
#21	23:01	26:01	35:01	53:01	04:01	06:02
#22	01:01	02:05	35:02	41:01	04:01	07:01
#23	02:10	11:02	40:06	55:02	01:02	08:01
#24	02:05	23:01	44:03	49:01	04:01	07:01

Table S4 Mass Cytometry panels, Related to Figures 3, 4, 6

	Panel 1	Panel 2	Panel 3	Panel 4
89 Y	CD45			
111Cd	HLA-DR			
112Cd	Granzyme A			
113Cd	CD38			
114Cd	γδTCR			
115 In	KLRG1, CRTH2	IFN-γ		
116Cd	CD57			
141 Pr		Ksp37		SLAMF6
142Nd	CD4, CD14, CD19, Vα24 TCR			
143 Nd	CD45RA			
144 Nd	CD122	CD69	CD122	CD69
145 Nd	NKG2D			
146 Nd	CD8			
147 Sm	NKp80	CD107a		
148 Nd	CCR7			
149 Sm	CTLA-4			
150 Nd	ROR-γt	IL-2		
151 Eu	TCF-1			
152 Sm	CD25	TNF-α		
153 Eu	PD1			
154 Sm	CXCR5			
155 Gd	NKp46			
156 Gd	Tim-3			
158 Gd	KIR2DL1			CD49a
159 Tb	CD56			
160 Gd	CD39			
161 Dy	NKG2A			
162 Dy	NKG2C			
163 Dy	EOMES			
164 Dy	CD28			
165 Ho	NKp30			
166 Er	Fas-L	IgG4	Fas-L	IgG4
167 Er	KIR2DL2/L3			CD103
168 Er	TRAIL			KI67
169 Tm	Tbet			
170 Er	CD3			
171 Yb		XCL1		
172 Yb	Perforin			
173 Yb	Granzyme B			
174 Yb	TOX			
175 Lu	TIGIT			
176 Yb	DNAM1			
209 Bi	CD16			

Figure S3. NKG2A-expressing CD8 T cells are differentiated and possess TCR-independent NK-like functions in healthy individuals, Related to Figure 3.

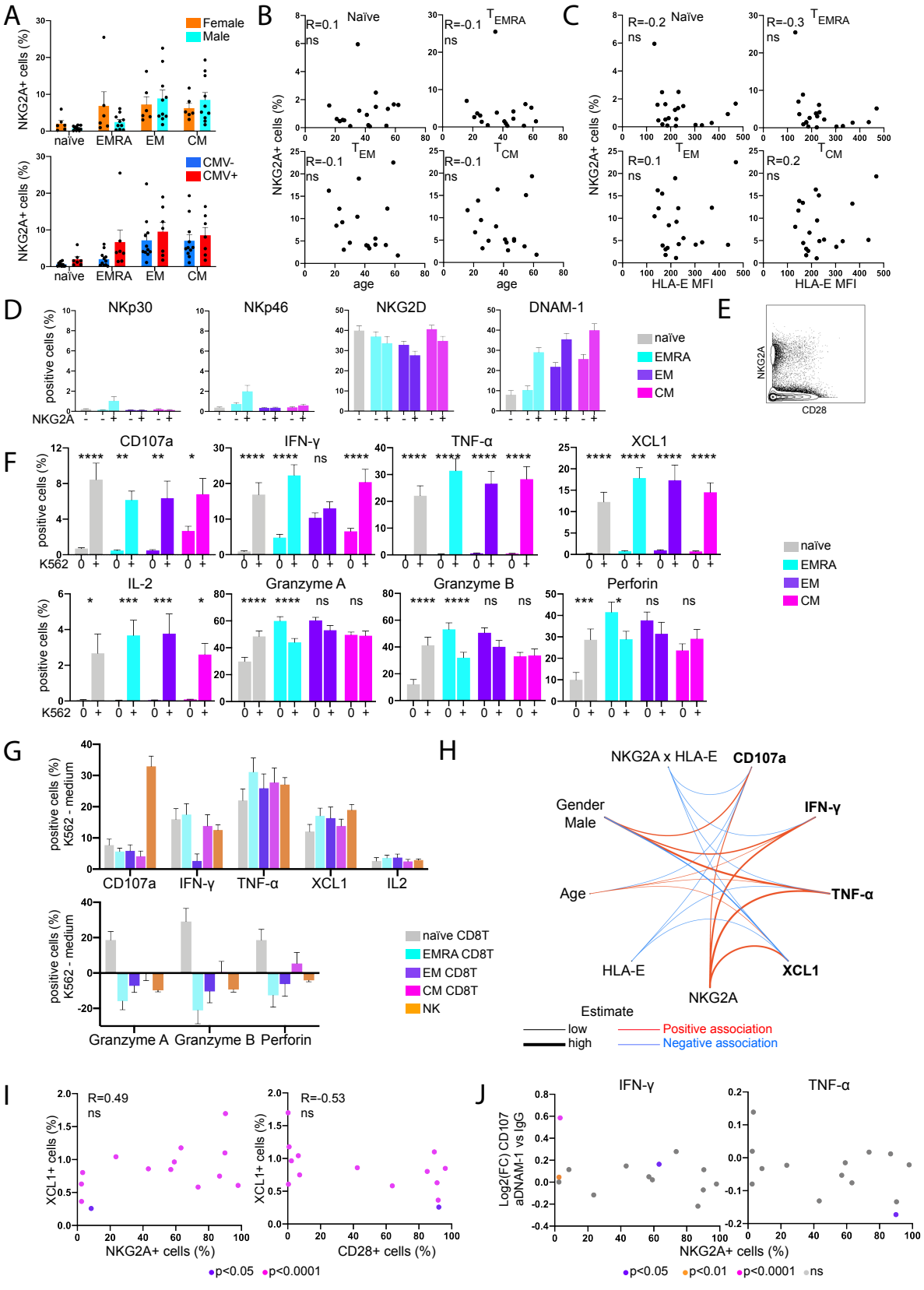

Figure S4. NKG2A defines a subset of PD-1+ TRM CD8+ T cells that retain TCR-independent functions in bladder tumors, Related to Figure 4

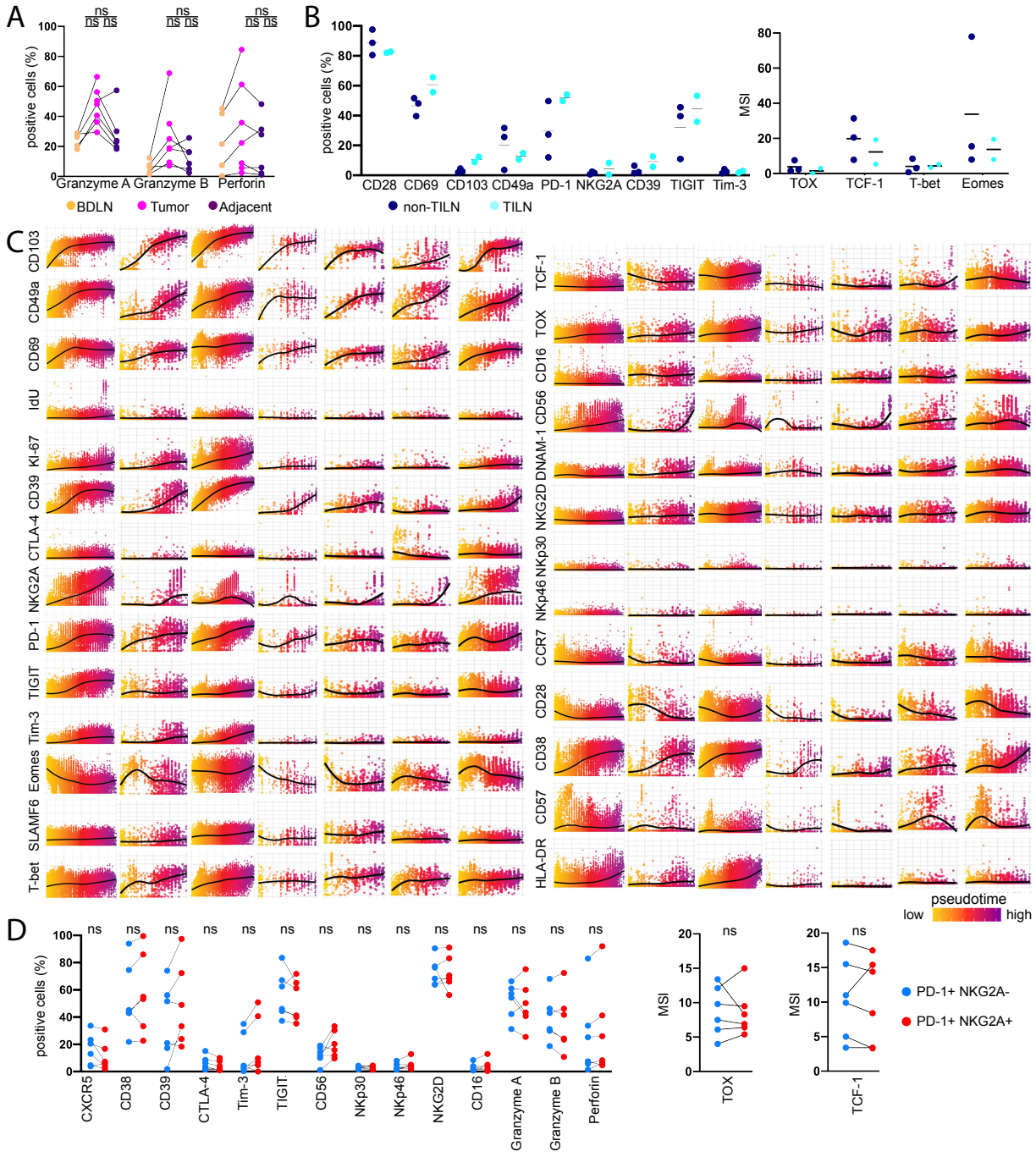

Figure S5. *KLRC1* (NKG2A) expression in CD8 T cells does not associate with a skewed clonal repertoire, Related to Figure 4.

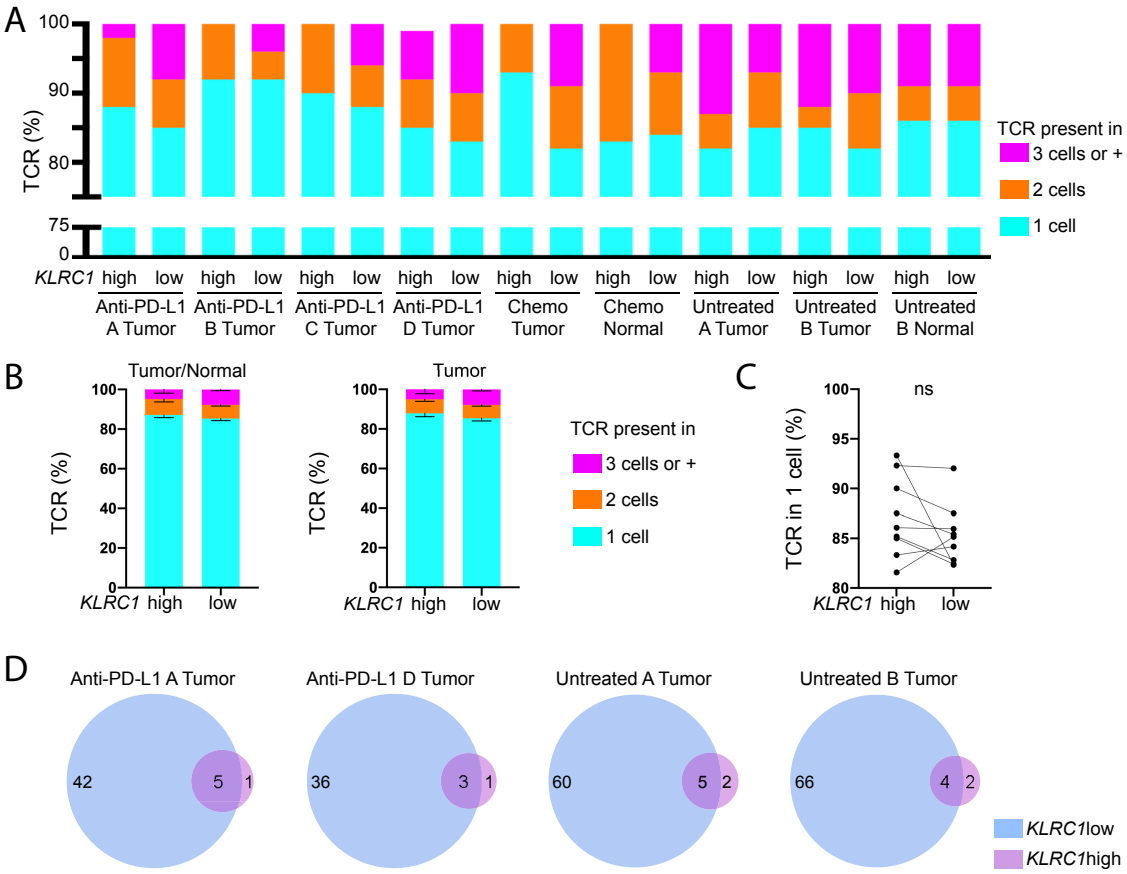

Figure S6. TCR-independent function is acquired by CD8 T cells upon NKG2A acquisition and is enhanced with NKG2A blockade in bladder cancer, Related to Figure 6