

Table S1. Characteristics of HNSCC patients assessed for phenotypic and functional properties.

		Number (%)
Gender	Male	14 (63.6%)
	Female	8 (36.4%)
Average age (years)		64.5
Ethnicity	Caucasian	22 (100%)
Primary Site	Larynx	0 (0%)
	Oral cavity	13 (59.1%)
	Oropharynx	9 (40.9%)
HPV/p16 status	Negative	15 (68.2%)
	Positive	7 (31.8%)
Disease stage	I	2 (9.1%)
	II	3 (13.6%)
	III	5 (22.7%)
	IVA	11 (50%)
	IVC	1 (4.6%)

Table S2. Antibody panels used to assess NK cell phenotype.

PANEL I: CELL SURFACE STAINING

Antibody	Clone	Isotype	Flouochrome	Company	Dilution
Zombie NIR	NA	NA	NA	Thermofisher	1:1000
LAG-3	T47-530	IgG1k	PE-CF594	BD	1:20
Lineage 3	CD3 SK7	IgG1k	FITC	BD	1:5
	CD19 SJ25C1	IgG1k			
	CD20 L27	IgG1k			
	CD14 MφP9	IgG2b			
CD16	3G8	IgG1k	Percp-cy5.5	Biolegend	1:20
CTLA-4	BNi3	IgG2a	BV421	Biolegend	1:20
CD56	5.1H11	IgG1k	BV510	Biolegend	1:20
TIM-3	7D3	IgG1k	BV711	BD	1:20
LAG-3	11C3C65	IgG1k	BV785	BD	1:20
CD45	HI30	IgG1k	BUV395	BD	1:20

PANEL II: CELL SURFACE STAINING

Marker	Clone	Isotype	Flouochrome	Company	Dilution
CD45	HI30	Mouse IgG1	BUV395	BD Biosciences	1:20
KLRC1	131411	Mouse IgG2a, k	BUV737	BD Biosciences	1:40
PD-1	MIH4	Mouse (BALB/c) IgG1	BV421	BD Biosciences	1:20
CD56	NCAM16.2	mlgG2b	BV510	BD Biosciences	1:20
TIM-3	F38-2E2	mlgG1	BV711	BioLegend	1:20
CD44	BJ18	mlgG1	BV785	BioLegend	1:20
CD16	3G8	mlgG1	FITC	BioLegend	1:20
KIR2DL3	CH-L	mlgG2b	PE	BD Biosciences	1:5
CD3	SK7	mlgG1	PE-Fire 700	BioLegend	1:20
CD314	1D11	IgG1	PE-Cy7	BioLegend	1:20
TIGIT	A15153G	mlgG2	APC	BioLegend	1:20
Zombie NIR	N/A	N/A	Zombie NIR	BioLegend	1:1000
CD57	REA769	rhlgG1	APC-Vio770	Miltenyi	1:50

PANEL III: INTRACELLULAR STAINING

Marker	Clone	Isotype	Flouochrome	Company	Dilution
CD45	HI30	Mouse IgG1	BUV395	BD Biosciences	1:20
IFN-gamma	4S.B3	mlgG1	BUV737	BD Biosciences	1:13
Lag-3	11C3C65	mlgG1	BV421	BioLegend	1:20
CD56	NCAM16.2	mlgG2b	BV510	BD Biosciences	1:20
TIM-3	F38-2E2	mlgG1	BV711	BioLegend	1:20
CD16	3G8	mlgG1	FITC	BioLegend	1:20
Granzyme B	QA16A02	mlgG1	PE	BioLegend	1:20
TNF	MAb11	mlgG1	PE-CF594	BD Biosciences	1:20
CD3	SK7	mlgG1	PE-Fire 700	BioLegend	1:20
Perforin	B-D48	mlgG1	APC	BioLegend	1:40
Zombie NIR	N/A	N/A	Zombie NIR	BioLegend	1:1000
HLA DR	REA805	rhlgG1	APC-Vio770	Miltenyi	1:50

PANEL IV: Galectin-9 ligation experiments

Antibody	Clone	Isotype	Flouochrome	Company	Dilution
Perforin	dG9	mlgG2b	APC	BioLegend	1:20
Zombie NIR	N/A	N/A	Zombie NIR	Thermo fisher	1:1000
Granzyme B	QA16A02	mlgG1	PE-Cy7	BioLegend	1:20
CD16	3G8	mlgG1	PerCP-Cy5.5	BioLegend	1:20
Ki-67	B56	mlgG1	BV421	BioLegend	1:20
CD56	5.1H11	mlgG1	BV510	BioLegend	1:20
TIM-3	7D3	mlgG1	BV711	BD Biosciences	1:20
CD3	UCHT1	mlgG1	BUV395	BD Biosciences	1:20

Table S3. Characteristics of HNSCC patients assessed by scRNAseq.

		Number (%)
Gender	Male	23 (79.3%)
	Female	6 (20.7%)
Average age (years)		59.5
Ethnicity	Caucasian	29 (100%)
Primary Site	Larynx	2 (6.9%)
	Oral cavity	17 (58.6%)
	Oropharynx	10 (34.5%)
HPV/p16 status	Negative	18 (62.1%)
	Positive	11 (37.9%)
Disease stage	I	6 (20.7%)
	II	6 (20.7%)
	III	5 (17.2%)
	IVA	10 (34.5%)

Table S4. Antibodies and dilutions used for DSP.

Name	Channel	Host	Company	Clone #	Cat. #	Conc. used
Syto13	488	-	Thermofisher	-	S7575	1:25
PanCK	532	Mouse	Novus	AE1 + AE3	NBP2-33200AF532	1:40
CD20	594	mouse	Novus	IGEL/773	NBP2-47840DL594	1:200
CD3E	647	mouse	Origene	UMAB54	UM00048	1:200

Table S5. Top 100 DEG for 12 NK cell clusters (part 1)

DEG rank	Cluster 0 (cNK)				Cluster 1 (cNK)				Cluster 2 (cNK)				Cluster 3 (tNK)			
	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value
0	NKG7	0.436	18.854	9.96E-72	MYOM2	5.468	103.637	0	SH2D1B	3.566	105.731	0	XCL1	5.066	44.339	8.64E-247
1	FGFBP2	0.791	12.845	2.13E-33	FGFBP2	1.489	27.989	4.08E-134	NKG7	0.460	18.673	3.42E-69	CD44	2.876	39.277	3.58E-219
2	KLRF1	0.629	11.326	4.82E-26	NKG7	0.540	23.204	2.51E-102	FGFBP2	0.764	12.206	5.84E-30	XCL2	3.430	38.788	3.20E-211
3	CMC1	0.790	11.166	2.74E-25	CST7	0.780	19.910	2.63E-74	MT-CO2	0.288	11.760	6.17E-28	FOS	2.891	33.633	3.43E-174
4	B2M	0.135	10.664	3.98E-23	SPON2	1.168	17.830	3.30E-60	MT-CO3	0.283	11.673	1.13E-27	DUSP1	1.756	23.433	6.01E-99
5	GZMH	0.729	10.226	3.16E-21	KLRB1	0.919	16.624	1.88E-53	CST7	0.448	11.481	1.16E-26	RGS1	2.581	22.867	1.28E-93
6	CST7	0.411	9.646	5.70E-19	FCGR3A	0.877	14.230	7.76E-40	ITGB2	0.565	11.326	7.00E-26	COTL1	2.115	21.682	4.14E-86
7	ITGB2	0.488	9.557	1.25E-18	B2M	0.163	12.572	1.38E-31	SPON2	0.736	11.232	2.01E-25	LINC00996	3.561	21.472	1.72E-83
8	MT-CO2	0.231	8.829	6.11E-16	ITGB2	0.631	12.267	3.69E-30	MT-CO1	0.255	10.949	2.69E-24	AREG	2.107	21.376	7.15E-84
9	GNLY	0.343	8.658	2.40E-15	TMSB10	0.281	11.581	4.48E-27	EEF1A1	0.232	10.756	1.69E-23	CD2	1.730	21.333	4.13E-84
10	MT-CO1	0.211	8.562	5.74E-15	PLAC8	0.712	11.181	3.64E-25	CMC1	0.760	10.488	3.13E-22	NFKBIA	1.653	21.297	3.78E-84
11	HLA-C	0.212	8.186	1.08E-13	MALAT1	0.225	10.981	2.28E-24	KLRF1	0.564	10.385	7.46E-22	JUN	2.030	21.203	5.52E-83
12	MT-CYB	0.307	8.112	1.80E-13	TXNIP	0.697	10.911	5.20E-24	HLA-C	0.233	10.222	3.15E-21	IL7R	4.197	20.942	7.47E-80
13	CYBA	0.309	8.036	3.24E-13	GZMM	0.697	10.410	6.20E-22	CYBA	0.336	9.975	2.91E-20	PPP1R15A	2.003	20.775	6.66E-80
14	MT-ND3	0.291	8.031	3.24E-13	MT-CO1	0.271	10.313	1.27E-21	LITAF	0.576	9.849	1.09E-19	SELL	2.132	20.227	3.79E-76
15	SPON2	0.524	7.834	1.40E-12	S100A4	0.532	10.237	2.85E-21	MT-ND3	0.309	9.322	9.25E-18	KLRC1	1.592	20.210	4.95E-77
16	MT-CO3	0.216	7.543	1.07E-11	AES	0.640	9.925	5.30E-20	FCGR3A	0.584	9.278	1.60E-17	FOSB	2.765	19.746	1.32E-72
17	FCGR3A	0.464	7.280	6.75E-11	HLA-B	0.278	9.655	5.87E-19	TXNIP	0.483	8.626	3.55E-15	GZMK	2.335	18.962	5.02E-68
18	MT-ND4	0.224	7.269	7.15E-11	MT-CO2	0.259	9.523	1.47E-18	HLA-E	0.256	8.075	2.53E-13	BTG1	0.858	18.642	6.39E-67
19	LYAR	0.647	7.144	1.66E-10	MT-ND2	0.338	9.510	1.74E-18	RPL3	0.182	8.025	3.63E-13	KLF6	1.731	18.361	2.08E-64
20	MALAT1	0.126	6.821	1.35E-09	IGFBP7	0.796	9.219	2.47E-17	MT-ND4	0.234	8.010	4.13E-13	TNFAIP3	1.864	18.163	4.56E-63
21	MT-ATP6	0.242	6.340	2.54E-08	CYBA	0.328	8.994	1.32E-16	MT-ATP6	0.294	7.691	4.46E-12	GPR183	3.955	18.157	2.23E-62
22	PATL2	0.835	6.140	8.41E-08	CLIC3	0.665	8.787	8.41E-16	MT-CYB	0.263	7.500	1.71E-11	TNFRSF18	1.676	17.821	3.16E-61
23	MYO1F	0.377	5.997	1.86E-07	FTL	0.344	8.664	2.16E-15	PLAC8	0.466	7.472	2.17E-11	CAPG	2.263	17.674	4.98E-60
24	ARL4C	0.411	5.883	3.55E-07	MT-CO3	0.256	8.476	8.65E-15	RPS17	0.275	6.945	7.76E-10	TSC22D3	1.225	16.946	4.35E-56
25	KLF2	0.442	5.874	3.72E-07	NBEAL1	0.856	8.039	3.06E-13	KLF2	0.519	6.921	9.36E-10	VIM	1.308	16.825	2.36E-55
26	MT-ND2	0.203	5.790	5.79E-07	CND3	0.552	7.953	5.46E-13	GZMM	0.427	6.850	1.44E-09	PLP2	1.837	16.646	5.57E-54
27	S1PR5	0.497	5.775	6.34E-07	NACA2	1.122	7.909	8.12E-13	PFN1	0.219	6.800	1.92E-09	SPTSSB	5.010	16.152	1.29E-50
28	C12orf75	0.402	5.640	1.30E-06	AKR1C3	0.839	7.826	1.41E-12	EFHD2	0.449	6.612	6.40E-09	IFH4L	2.666	15.763	1.26E-48
29	PRSS23	0.513	5.639	1.31E-06	MT-CYB	0.296	7.628	5.26E-12	RPS3A	0.171	6.575	7.76E-09	LTB	1.965	15.538	1.62E-47
30	HOPX	0.314	5.605	1.55E-06	MT-ND4	0.249	7.568	8.13E-12	UCP2	0.447	6.516	1.16E-08	IFITM3	1.483	15.480	2.19E-47
31	RPL3	0.146	5.525	2.36E-06	BIN2	0.497	7.452	1.93E-11	ARL4C	0.454	6.484	1.41E-08	TMEM123	1.636	15.451	3.61E-47
32	TXNIP	0.305	5.170	1.33E-05	MT-ATP6	0.315	7.419	2.32E-11	MT-ND2	0.206	6.363	2.81E-08	CD69	1.239	15.311	1.43E-46
33	LITAF	0.311	5.122	1.67E-05	KLRF1	0.464	7.419	2.34E-11	MYO1F	0.390	6.295	4.37E-08	FXYD5	0.957	15.224	3.01E-46
34	GZMM	0.321	5.027	2.60E-05	CHST2	0.746	7.402	2.75E-11	ABHD17A	0.448	6.248	5.82E-08	DUSP2	1.257	14.952	1.35E-44
35	EFHD2	0.314	4.677	0.0001	PRF1	0.376	7.384	2.90E-11	ARHGDIIB	0.261	6.219	6.76E-08	TCF7	2.078	14.752	3.27E-43
36	CEP78	0.503	4.629	0.0001	PFN1	0.250	7.199	1.00E-10	HOPX	0.350	6.140	1.09E-07	ITM2C	2.241	14.515	6.15E-42
37	KLRC1	0.382	4.601	0.0002	CALM1	0.397	7.160	1.33E-10	AES	0.367	5.965	2.91E-07	RPS18	0.509	14.319	4.77E-41
38	CCL5	0.287	4.593	0.0002	RPS10	0.311	7.123	1.67E-10	CLIC3	0.417	5.898	4.20E-07	RPLP1	0.432	14.087	6.80E-40
39	ANKRD20A4	0.855	4.360	0.0004	CD160	0.778	7.042	3.03E-10	S1PR5	0.501	5.844	5.58E-07	LMNA	3.250	13.758	7.84E-38
40	TRBC1	0.316	4.348	0.0005	UBB	0.302	7.015	3.47E-10	B2M	0.072	5.744	9.43E-07	TNFSF10	2.087	13.586	4.48E-37
41	PRF1	0.208	4.244	0.0007	ZNF90	0.898	6.921	6.70E-10	RPL7	0.168	5.698	1.20E-06	SPINK2	6.852	13.356	9.57E-36
42	LINC00623	0.400	4.192	0.0008	PTGDS	1.049	6.921	6.70E-10	PRMT2	0.438	5.685	1.31E-06	DUSP4	2.830	13.350	7.95E-36
43	RPS27	0.082	4.110	0.0011	GZMH	0.533	6.904	7.10E-10	RPL4	0.236	5.670	1.39E-06	HIF1A	2.053	13.347	6.96E-36
44	RAP1B	0.260	4.109	0.0011	S100A9	0.810	6.861	9.68E-10	FTL	0.206	5.541	2.69E-06	RGCC	2.702	13.196	4.68E-35
45	PRKCB	0.416	4.080	0.0012	ABHD17A	0.529	6.843	1.06E-09	PRF1	0.271	5.513	3.11E-06	RPL13A	0.365	13.064	1.11E-34
46	OR2A25	0.814	4.070	0.0013	PRSS23	0.665	6.684	2.95E-09	TPT1	0.171	5.511	3.11E-06	RPS25	0.417	12.966	2.59E-34
47	RPL7	0.131	4.053	0.0014	DBI	0.471	6.648	3.67E-09	CND3	0.345	5.482	3.65E-06	MCL1	1.143	12.684	8.58E-33
48	GZMB	0.188	4.004	0.0016	EFHD2	0.473	6.546	6.79E-09	APMAP	0.387	5.355	7.06E-06	CXCR4	0.965	12.680	7.67E-33
49	ASCL2	0.440	3.899	0.0024	MYO1F	0.438	6.445	1.26E-08	ASCL2	0.628	5.354	7.14E-06	MAFF	1.464	12.572	3.29E-32
50	PCSK7	0.279	3.753	0.0039	RAP1B	0.438	6.438	1.31E-08	RPS3	0.126	5.325	8.10E-06	RPL36	0.430	12.563	2.51E-32
51	TPST2	0.296	3.705	0.0045	FCRL6	0.653	6.355	2.13E-08	BIN2	0.321	5.280	1.02E-05	IGKC	1.331	12.528	4.65E-32
52	SORL1	0.356	3.669	0.0051	XIST	1.021	6.334	2.44E-08	MT-ND1	0.227	5.262	1.10E-05	BHLHE40	1.401	12.239	1.24E-30
53	SELL	0.373	3.655	0.0053	ADGRG1	0.603	6.302	2.90E-08	PRSS23	0.482	5.258	1.13E-05	STK17A	1.009	12.227	1.17E-30
54	AES	0.226	3.629	0.0057	MAPK1	0.650	6.278	3.36E-08	MAF1	0.457	5.245	1.20E-05	RPS27	0.285	12.108	4.20E-30
55	CX3CR1	0.453	3.623	0.0058	MT-ND3	0.243	6.144	7.07E-08	EIF3G	0.346	5.218	1.38E-05	RPLP0	0.590	12.103	3.76E-30
56	DTHD1	0.675	3.612	0.0060	SERF2	0.233	6.080	1.04E-07	RPL10	0.095	5.170	1.71E-05	RPL13	0.339	11.868	5.80E-29
57	TTG38	0.320	3.611	0.0060	PPIA	0.336	6.079	1.05E-07	DHRS7	0.392	5.149	1.93E-05	IRF7	1.534	11.828	1.09E-28
58	VAMP2	0.234	3.572	0.0067	ARHGDIIB	0.276	6.033	1.35E-07	RPS10	0.213	5.138	2.00E-05	CXCR3			

Table S5. Top 100 DEG for 12 NK cell clusters (part 2)

DEG rank	Cluster 4 (tiNK)				Cluster 5 (cNK)				Cluster 6 (cNK)				Cluster 7 (cNK)			
	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value
0	TMSB4X	1.031	55.835	0	KIR2DL1	10.413	143.288	0	KIR2DL3	5.522	126.775	0	B3GNT7	5.115	103.066	0
1	HLA-DRB1	3.758	48.365	3.40E-289	NKG7	0.538	22.407	2.13E-96	NKG7	0.567	24.126	8.52E-110	MT-CO3	0.394	13.749	1.18E-36
2	CCL5	2.044	46.790	0	FGFBP2	1.119	19.045	6.43E-69	FGFBP2	1.325	24.030	4.31E-104	MT-ND3	0.479	13.330	1.50E-34
3	KRT81	6.201	44.635	7.55E-252	CST7	0.646	17.264	7.42E-58	CST7	0.608	15.599	1.36E-47	MT-CO2	0.336	12.663	2.12E-31
4	CD74	2.371	44.331	3.11E-273	KIR2DL3	2.102	16.776	4.30E-53	SPON2	0.975	14.351	2.79E-40	EEF1A1	0.293	12.235	2.23E-29
5	CKLF	2.815	44.177	1.01E-263	HLA-C	0.353	15.914	1.69E-49	ITGB2	0.703	14.189	1.22E-39	MT-CO1	0.285	12.062	1.04E-28
6	IGKC	3.706	43.455	3.20E-252	FCGR3A	0.895	14.623	8.52E-42	FCGR3A	0.825	12.756	3.07E-32	MT-ND4	0.359	12.062	1.29E-28
7	HLA-DRA	3.675	43.268	1.16E-249	KIR3DL1	2.425	13.824	5.90E-37	HLA-C	0.270	11.428	3.68E-26	MT-ATP6	0.443	11.912	6.22E-28
8	KRT86	5.514	42.917	2.59E-240	ITGB2	0.662	13.005	1.96E-33	B2M	0.146	11.243	2.65E-25	NKG7	0.337	11.736	1.95E-27
9	COTL1	3.547	42.298	5.66E-243	CYBA	0.394	11.226	3.70E-25	S100A4	0.528	10.658	9.19E-23	MT-ND2	0.373	11.376	1.22E-25
10	CAPG	4.632	38.846	1.45E-211	IGFBP7	0.834	10.622	2.20E-22	GZMM	0.660	10.190	8.37E-21	RPL3	0.282	11.357	1.72E-25
11	GZMA	1.927	36.430	8.17E-203	PRF1	0.501	10.425	1.22E-21	AES	0.629	10.099	1.88E-20	RPS3A	0.301	10.902	1.38E-23
12	CD52	1.883	35.063	4.39E-200	SPON2	0.704	10.017	5.80E-20	GZMH	0.738	9.628	1.33E-18	CMC1	0.878	10.291	6.36E-21
13	ALOX5AP	2.033	34.656	2.20E-191	GZMH	0.712	9.814	3.55E-19	CYBA	0.343	9.547	2.22E-18	MT-ND1	0.464	10.011	5.83E-20
14	GAPDH	1.392	33.643	6.94E-183	S100A4	0.481	9.778	4.42E-19	IGFBP7	0.798	9.313	2.08E-17	RPS10	0.424	9.566	2.88E-18
15	ACP5	4.326	33.193	5.16E-169	HLA-E	0.291	9.411	9.81E-18	PFN1	0.312	8.828	1.04E-15	FGFBP2	0.670	8.984	3.84E-16
16	VIM	1.991	33.163	2.56E-179	GZMB	0.405	9.288	2.82E-17	EFHD2	0.592	8.625	5.44E-15	C1orf56	0.956	8.959	5.41E-16
17	ZNF683	4.358	31.632	2.25E-157	B2M	0.118	9.136	1.07E-16	APRC2	0.366	8.525	1.11E-14	MT-CYB	0.359	8.943	4.97E-16
18	GZMK	3.852	31.064	5.72E-154	MT-ND3	0.303	9.111	1.15E-16	PLAC8	0.536	8.351	4.24E-14	RPL17	0.511	8.659	5.27E-15
19	TNFRSF18	2.720	31.052	1.85E-156	PLAC8	0.557	9.012	2.89E-16	MALAT1	0.169	8.279	7.02E-14	RPL7	0.268	8.546	1.15E-14
20	LGALS1	2.253	30.435	8.04E-154	ALDOA	0.761	8.445	3.13E-14	PRF1	0.424	8.268	7.85E-14	RPS17	0.372	8.265	9.23E-14
21	KLRC1	2.118	29.910	2.76E-151	MALAT1	0.166	8.297	1.40E-13	MT-CO2	0.220	8.193	1.28E-13	SPON2	0.642	8.056	4.59E-13
22	HLA-DQB1	3.261	29.276	4.95E-141	PRSS23	0.755	8.130	2.13E-13	AKR1C3	0.843	8.146	2.12E-13	PPP2R5C	0.668	7.863	1.89E-12
23	KIR2DL4	3.568	29.075	3.32E-139	GZMM	0.523	8.185	2.13E-13	MT-CYB	0.307	8.081	3.05E-13	ITGB2	0.459	7.817	2.36E-12
24	TYMP	2.842	29.014	8.67E-140	S100A6	0.430	8.104	3.65E-13	LAIR2	0.929	7.681	6.30E-12	RPL9	0.258	7.815	2.36E-12
25	RPLP1	0.550	28.624	8.93E-147	KLRF1	0.448	8.048	5.42E-13	ADGRG1	0.689	7.459	2.84E-11	PLAC8	0.542	7.636	8.40E-12
26	CD44	2.090	27.976	7.57E-135	LAIR2	0.888	7.604	1.53E-11	HLA-E	0.250	7.391	4.24E-11	LITAF	0.542	7.594	1.12E-11
27	LGALS3	3.392	27.932	1.07E-130	MT-CO3	0.223	7.492	3.06E-11	TMSB10	0.206	7.346	5.84E-11	HNRNP1	0.791	7.567	1.39E-11
28	HLA-DPA1	2.057	27.057	8.08E-127	UCP2	0.486	7.190	2.50E-10	S100A6	0.413	7.244	1.20E-10	DUSP2	0.721	7.538	1.67E-11
29	CSF1	7.200	26.782	6.30E-121	BIN2	0.435	7.143	3.41E-10	ABHD17A	0.549	7.241	1.23E-10	CDC42	0.510	7.422	3.63E-11
30	ITM2C	4.024	26.557	3.66E-120	HLA-B	0.194	7.056	6.14E-10	GZMB	0.337	7.235	1.21E-10	ARL4C	0.630	7.401	4.18E-11
31	JUN	2.130	26.499	1.10E-123	NBEAL1	0.662	6.957	1.14E-09	CLIC3	0.529	7.091	3.24E-10	EIF1	0.239	7.361	5.23E-11
32	KLRC2	3.363	25.895	8.00E-116	LITAF	0.408	6.858	2.11E-09	MYO1F	0.464	7.071	3.64E-10	S1PR5	0.707	7.011	5.73E-10
33	HLA-DRB5	3.326	25.444	1.41E-112	PTGER2	0.649	6.851	2.24E-09	MT-CO3	0.203	7.064	3.60E-10	MAP3K8	0.744	6.869	1.40E-09
34	PLP2	2.532	25.433	1.42E-113	ANXA1	0.463	6.799	3.02E-09	CHST2	0.695	7.008	5.67E-10	ZFP36	0.505	6.766	2.15E-09
35	ITGA1	4.720	25.316	6.13E-111	LAPTM5	0.391	6.742	4.28E-09	FCRL6	0.705	6.954	8.11E-10	RPL21	0.171	6.637	5.45E-09
36	SAMS1	2.789	25.286	5.29E-112	CD99	0.377	6.670	6.71E-09	CD247	0.391	6.930	9.15E-10	B4GALT1	0.945	6.634	5.85E-09
37	CD63	1.294	25.253	1.05E-115	EIF1	0.177	6.645	7.54E-09	MYO1G	0.593	6.908	1.08E-09	CD160	0.813	6.603	6.95E-09
38	CD7	0.968	25.206	4.73E-116	S1PR5	0.572	6.579	1.18E-08	FTL	0.264	6.853	1.45E-09	IFTIM1	0.473	6.579	7.81E-09
39	LAG3	4.638	24.959	2.16E-108	EFHD2	0.438	6.551	1.37E-08	PRSS23	0.654	6.850	1.54E-09	KLRB1	0.498	6.564	8.54E-09
40	PGK1	1.691	24.939	1.36E-111	UBB	0.256	6.506	1.77E-08	PTGDS	1.002	6.788	2.32E-09	CLIC3	0.560	6.530	1.05E-08
41	TRAC	2.670	24.463	3.44E-106	PSMB10	0.471	6.400	3.37E-08	ARHGDI	0.304	6.779	2.32E-09	RPS13	0.233	6.399	2.19E-08
42	IFI6	2.558	24.250	8.33E-105	ARHGDI	0.274	6.349	4.50E-08	IFTIM1	0.425	6.570	8.61E-09	H3F3B	0.250	6.276	4.66E-08
43	MT2A	1.609	24.088	6.75E-106	ARPC2	0.261	6.250	8.06E-08	CALM1	0.341	6.410	2.31E-08	MYO1F	0.459	6.269	4.93E-08
44	LDHA	1.489	23.810	1.12E-103	MT-CO2	0.165	6.220	9.47E-08	TTC38	0.606	6.358	3.16E-08	AES	0.443	6.266	4.95E-08
45	ISG15	2.135	23.787	4.04E-102	ADGRG1	0.546	6.211	1.04E-07	TGFBI	0.459	6.282	4.80E-08	RPL10	0.131	6.200	7.13E-08
46	SLC16A3	2.787	23.659	2.70E-100	POLR2J3	0.900	6.195	1.15E-07	MT-ND2	0.199	6.267	4.95E-08	ZFP36L2	0.418	6.121	1.14E-07
47	CTSA	2.503	23.522	1.50E-99	LYAR	0.563	6.159	1.37E-07	CLF1	0.224	6.197	7.61E-08	EFHD2	0.471	6.104	1.26E-07
48	PKM	1.869	23.162	6.49E-98	RPS17	0.253	6.099	1.86E-07	DSTN	0.511	6.177	8.78E-08	RBM38	0.733	5.920	3.65E-07
49	APOBEC3G	1.509	22.798	3.28E-96	CD247	0.318	5.956	4.14E-07	FLNA	0.505	6.141	1.08E-07	GZMM	0.438	5.895	4.00E-07
50	NDIFP2	3.930	22.721	5.12E-93	MT-CO1	0.143	5.944	4.31E-07	EIF1	0.175	6.136	1.08E-07	RBM39	0.429	5.848	5.20E-07
51	RPS26	0.964	22.719	4.33E-98	EMP3	0.362	5.896	5.70E-07	MT-CO1	0.145	6.088	1.38E-07	RPS27A	0.145	5.775	7.44E-07
52	S100A11	1.556	22.324	1.35E-92	MT-ATP6	0.216	5.892	5.70E-07	BIN2	0.391	5.931	3.45E-07	CDC42SE1	0.564	5.658	1.41E-06
53	HLA-DMA	3.161	22.117	3.60E-89	CALM1	0.302	5.869	6.46E-07	ZEB2	0.563	5.871	4.91E-07	RPL30	0.189	5.607	1.78E-06
54	HLA-DQA1	3.621	22.015	2.84E-88	DSTN	0.458	5.819	8.62E-07	RPS17	0.241	5.838	5.65E-07	TXNIP	0.387	5.530	2.64E-06
55	CLEC2B	1.493	21.555	3.34E-87	TMSB10	0.148	5.752	1.20E-06	S1PR5	0.534	5.836	5.83E-07	RPL6	0.195	5.503	2.99E-06
56	ACTB	0.728	21.398	3.05E-87	MYL12A	0.236	5.708	1.54E-06	LITAF	0.377	5.816	6.37E-07	BIN2	0.396	5.462	3.76E-06
57	CCND2	2.267	21.191	1.88E-83	PLEK	0.523	5.653	2.07E-06	MT-ATP6	0.240	5.806	6.64E-07	C12orf75	0.448	5.410	4.88E-06
58	PTMA	0.615	21.083	4.77E-84	MT-CYB	0.206	5.538	3.70E-06	MYOM2	0.767	5.723	1.07E-06	PRKCB	0.648	5	

Table S5. Top 100 DEG for 12 NK cell clusters (part 3)

DEG rank	Cluster 8 (cNK)				Cluster 9 (cNK)				Cluster 10 (cNK)				Cluster 11 (cNK)			
	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value	Gene name	Log FC	Score	Adj p-value
0	CD160	3.865	81.242		KIR3DL2	6.080	97.150	0	PTGDR	3.426	69.761	1.14E-266	PTGDS	5.672	67.428	6.12E-234
1	NKG7	0.405	14.666	1.35E-41	FGFBP2	1.284	18.082	2.01E-56	NKG7	0.477	13.976	2.74E-35	FGFBP2	1.513	20.630	3.95E-65
2	KLRB1	0.889	13.096	9.47E-33	NKG7	0.485	16.329	9.18E-49	FGFBP2	0.813	7.938	8.27E-15	NKG7	0.483	14.923	1.05E-39
3	MT-ND2	0.416	11.205	1.76E-24	MT-ND3	0.435	10.969	3.29E-23	FCGR3A	0.756	8.041	1.12E-12	SPON2	1.280	13.447	3.45E-32
4	MT-CO3	0.361	11.135	3.28E-24	SPON2	0.955	10.937	7.38E-23	HLA-C	0.285	7.984	1.57E-12	B2M	0.226	11.544	1.44E-24
5	MT-ND3	0.422	10.430	2.22E-21	MT-CYB	0.421	10.315	1.07E-20	B2M	0.141	7.938	2.06E-12	S100A6	0.787	10.359	2.80E-20
6	CMC1	0.829	9.407	2.00E-17	AES	0.775	9.974	3.01E-19	ITGB2	0.584	7.638	1.57E-11	CST7	0.606	10.243	5.99E-20
7	MT-CO2	0.307	9.302	4.02E-17	ITGB2	0.625	9.924	3.84E-19	PRF1	0.457	6.445	2.00E-08	ITGB2	0.682	9.135	3.87E-16
8	KLRF1	0.607	9.253	5.81E-17	MT-CO2	0.283	9.668	2.53E-18	PFN1	0.298	6.218	6.96E-08	S100A4	0.669	8.902	2.34E-15
9	MT-CO1	0.259	8.839	1.52E-15	CST7	0.482	9.086	3.60E-16	MT-CO2	0.244	6.148	1.00E-07	FCGR3A	0.820	8.589	2.15E-14
10	PFN1	0.321	8.387	5.13E-14	B2M	0.145	8.916	1.39E-15	MT-CO1	0.213	6.005	2.11E-07	MT-CO2	0.324	7.832	3.52E-12
11	GZMM	0.643	8.233	1.70E-13	FCGR3A	0.679	8.351	8.95E-14	CYBA	0.314	5.364	5.19E-06	FTL	0.419	7.640	1.28E-11
12	CLIC3	0.704	8.096	4.66E-13	S100A4	0.509	8.232	2.08E-13	CST7	0.352	5.346	5.53E-06	MALAT1	0.242	6.627	6.36E-09
13	PLAC8	0.589	7.941	1.39E-12	PTGDS	1.462	7.475	4.47E-11	EMP3	0.500	5.332	5.97E-06	CYBA	0.394	6.615	6.62E-09
14	MT-ND4	0.278	7.836	2.73E-12	PFN1	0.290	7.439	4.82E-11	MYL12A	0.331	5.294	7.07E-06	TMSB10	0.264	6.518	1.13E-08
15	MT-ATP6	0.351	7.437	4.46E-11	MT-ATP6	0.324	7.339	9.17E-11	SPON2	0.559	5.254	8.57E-06	PRSS23	0.919	6.450	1.70E-08
16	SPON2	0.613	7.381	6.57E-11	TMSB10	0.209	7.192	2.40E-10	MT-ND4	0.221	5.132	1.47E-05	ARPC2	0.397	6.217	5.95E-08
17	EEF1A1	0.231	7.369	7.02E-11	GZMH	0.687	7.140	3.59E-10	CCND3	0.517	5.130	1.51E-05	TXNIP	0.583	6.151	8.45E-08
18	CST7	0.354	7.232	1.69E-10	GZMM	0.596	7.097	4.68E-10	MALAT1	0.145	5.126	1.51E-05	KLRB1	0.551	6.115	9.99E-08
19	ABHD17A	0.623	7.160	2.85E-10	HLA-E	0.271	6.959	1.03E-09	KLRF1	0.448	5.036	2.26E-05	S100B	1.728	5.996	1.99E-07
20	MT-ND1	0.387	7.057	5.41E-10	MT-ND2	0.273	6.725	4.21E-09	MT-ND3	0.268	4.847	4.99E-05	EFHD2	0.654	5.911	2.99E-07
21	ITGB2	0.444	6.827	2.27E-09	MT-ND4	0.240	6.701	4.83E-09	S1PR5	0.674	4.806	6.10E-05	AES	0.562	5.810	5.07E-07
22	RPL3	0.192	6.567	1.08E-08	EFHD2	0.583	6.594	9.81E-09	ANXA1	0.492	4.758	7.44E-05	PLAC8	0.566	5.803	5.25E-07
23	RPL17	0.392	6.156	1.15E-07	KLRB1	0.519	6.428	2.50E-08	HLA-E	0.226	4.694	9.55E-05	IGFBP7	0.728	5.739	7.34E-07
24	KLRG1	1.046	6.144	1.27E-07	LITAF	0.516	6.364	3.62E-08	MT-CO3	0.192	4.557	0.0002	MT-CO1	0.220	5.719	7.87E-07
25	HOPX	0.435	6.065	1.89E-07	RPS17	0.337	6.239	7.23E-08	TXNIP	0.420	4.374	0.0004	KLRF1	0.514	5.695	8.99E-07
26	LITAF	0.442	5.942	3.67E-07	BIN2	0.507	6.217	8.21E-08	IFITM1	0.420	4.204	0.0007	FCRL6	0.857	5.661	1.09E-06
27	AES	0.443	5.788	8.11E-07	MT-CO3	0.205	5.993	2.60E-07	MT-CYB	0.227	3.999	0.0014	MT-ATP6	0.332	5.627	1.26E-06
28	MYL12A	0.281	5.658	1.54E-06	PLAC8	0.494	5.981	2.89E-07	EFHD2	0.416	3.851	0.0024	MT-CYB	0.355	5.541	1.94E-06
29	FKBP11	0.591	5.578	2.35E-06	CYBA	0.293	5.966	3.10E-07	CLIC3	0.450	3.847	0.0024	PRF1	0.405	5.411	3.52E-06
30	TXNIP	0.398	5.466	4.02E-06	HLA-C	0.205	5.880	4.92E-07	ASCL2	0.713	3.842	0.0024	HLA-C	0.188	5.238	7.97E-06
31	EFHD2	0.452	5.399	5.61E-06	RPL17	0.389	5.846	5.81E-07	GZMH	0.440	3.787	0.0029	CALM1	0.430	5.224	8.65E-06
32	MT-CYB	0.247	5.377	6.17E-06	CD99	0.443	5.745	9.79E-07	PLAC8	0.371	3.778	0.0030	GZMH	0.601	5.201	9.63E-06
33	RPS3A	0.184	5.210	1.38E-05	EEF1A1	0.163	5.709	1.13E-06	IFITM2	0.249	3.697	0.0039	GZMM	0.512	5.134	1.28E-05
34	TMSB10	0.176	5.140	1.91E-05	S1PR5	0.654	5.678	1.38E-06	PDLIM1	0.987	3.570	0.0060	MT-ND4	0.241	5.098	1.49E-05
35	RPL5	0.252	5.123	2.06E-05	RPS10	0.302	5.635	1.67E-06	STK10	0.528	3.451	0.0087	PFN1	0.275	5.090	1.55E-05
36	ARL4C	0.449	4.999	3.65E-05	CLIC3	0.539	5.624	1.79E-06	TRBC1	0.401	3.382	0.0106	CD6	1.181	5.076	1.68E-05
37	IGF2R	0.877	4.982	3.95E-05	MYO1F	0.463	5.577	2.23E-06	HLA-A	0.133	3.355	0.0115	FEZ1	1.201	5.008	2.24E-05
38	DOK2	0.430	4.743	0.0001	TXNIP	0.444	5.574	2.24E-06	S100A4	0.265	3.253	0.0155	MYO1F	0.501	4.957	2.77E-05
39	MAP3K8	0.536	4.661	0.0002	ABHD17A	0.533	5.527	2.85E-06	CORO1A	0.250	3.242	0.0160	AK5	1.468	4.886	3.81E-05
40	TYROBP	0.212	4.641	0.0002	MT-CO1	0.158	5.521	2.81E-06	HOPX	0.289	3.208	0.0177	CCND3	0.488	4.878	3.87E-05
41	C1orf162	0.482	4.633	0.0002	ADGRG1	0.659	5.460	3.97E-06	GZMM	0.333	3.190	0.0187	AKR1C3	0.771	4.875	3.95E-05
42	ZFPF36	0.376	4.610	0.0002	AKR1C3	0.738	5.412	4.92E-06	DSTN	0.402	3.182	0.0192	PPP2R5C	0.564	4.794	5.61E-05
43	KLF2	0.438	4.589	0.0002	MALAT1	0.141	5.359	6.12E-06	UCP2	0.352	3.158	0.0205	BIN2	0.445	4.459	0.0002
44	HLA-E	0.190	4.580	0.0002	FCRL6	0.696	5.352	6.46E-06	LINC00861	0.550	3.156	0.0206	APMAP	0.516	4.423	0.0003
45	JUNB	0.362	4.492	0.0003	PPP2R5C	0.525	5.262	9.80E-06	MYO1F	0.322	3.102	0.0238	SYNGR1	0.867	4.357	0.0003
46	HCST	0.243	4.487	0.0003	UBB	0.261	5.088	2.19E-05	MYO1G	0.403	3.045	0.0279	MARCKSL1	1.028	4.243	0.0005
47	MYO1F	0.346	4.404	0.0004	PRSS23	0.628	5.015	3.09E-05	CX3CR1	0.618	3.040	0.0283	KLF2	0.500	4.123	0.0008
48	H3F3B	0.185	4.351	0.0005	RPS4Y1	0.486	4.992	3.37E-05	ARL4C	0.348	2.983	0.0332	RASSF4	1.065	4.078	0.0010
49	RPL7	0.156	4.317	0.0006	ITGAL	0.668	4.956	3.91E-05	RAB9A	0.648	2.969	0.0343	FLNA	0.506	4.045	0.0011
50	HNRNPDL	0.318	4.312	0.0006	GPSM3	0.429	4.909	4.80E-05	RORA	0.484	2.938	0.0370	ABHD17A	0.485	4.015	0.0012
51	RPS10	0.232	4.299	0.0007	TRBC2	0.477	4.872	5.61E-05	CMC1	0.360	2.930	0.0376	CFL1	1.234	3.997	0.0013
52	RNPEL1	0.555	4.220	0.0009	SYTL1	0.582	4.817	7.12E-05	MBNL1	0.420	2.890	0.0420	NCR3	0.563	3.948	0.0015
53	RPS6	0.126	4.206	0.0009	DDX5	0.272	4.793	7.80E-05	ARHGAP25	0.602	2.861	0.0455	HBA1	1.400	3.924	0.0017
54	PRKCH	0.413	4.150	0.0011	S100A6	0.351	4.743	9.63E-05	DRG2	0.763	2.861	0.0456	ACTB	0.243	3.918	0.0017
55	TTC38	0.454	4.131	0.0012	UCP2	0.446	4.694	0.0001	SMIM10L1	0.603	2.848	0.0472	CD247	0.332	3.895	0.0018
56	CALM1	0.275	4.131	0.0012	IGFBP7	0.504	4.630	0.0002	PPP2R5C	0.324	2.842	0.0479	CAPZB	0.411	3.802	0.0025
57	ARHGDB1	0.227	4.127	0.0012	EIF1	0.176	4.599	0.0002	EPB41	0.565	2.773	0.0558	CTBP2	0.729	3.701	0.0035
58	RPS17	0.223	4.120	0.0013	ARPC2	0.263	4.594	0.0002	DTHD1	0.876	2.770	0.0561	MT-CO3	0.158	3.639	0.0042
59	TPT1	0.186	4.118	0.0013	PRF1	0.299	4.454	0.0002	NMUR1	0.753	2.748	0.0592	LAIR1	0.727	3.625	0.0044
60	RPL9	0.154	4.074	0.0015	FTL	0.242	4.499	0.0003	S100A6	0.242	2.707	0.0654	R			

Table S6. HAVCR2+ tiNK cell top 100 DEG

Gene	LogFC	Score	Adj p-val	Gene	LogFC	Score	Adj p-val
HAVCR2	32.194756	45.710583	0	MRPL52	0.6706374	5.074549	0.000125407
GZMB	0.9150915	11.895086	1.24E-28	H2AFZ	0.49579915	5.0682507	0.000127537
GZMA	0.74313956	8.794015	9.47E-15	BST2	0.4744695	5.0186005	0.000162716
GAPDH	0.47882807	7.8991413	1.38E-11	KRT81	0.67595625	4.9910727	0.000184779
PRF1	0.61246496	7.6077704	1.10E-10	LCP2	0.65554357	4.9007144	0.00027695
MT2A	0.69921005	7.2965126	8.31E-10	HMGB2	0.54502213	4.8793693	0.000303776
ENO1	0.64844966	7.2282968	1.20E-09	PPDPF	0.43442893	4.8666553	0.000319348
PKM	0.7213339	6.830763	1.85E-08	ZNF683	0.67295045	4.84183	0.000356865
HLA-DPA1	0.6507694	6.57559	8.68E-08	CFL1	0.3100587	4.8298936	0.000372063
TMSB4X	0.16584985	6.4637113	1.58E-07	APOBEC3G	0.43617132	4.8254027	0.000372063
CCL5	0.5348597	6.4606786	1.58E-07	MT1X	0.6947274	4.8241587	0.000372063
ARPC1B	0.5773926	6.4488006	1.59E-07	TUBB	0.51530147	4.821359	0.000372063
ACTB	0.3012748	6.28315	4.36E-07	SLC16A3	0.6142851	4.819979	0.000372063
GNLY	0.44241986	6.21371	6.28E-07	SAMSN1	0.5529425	4.81578	0.000375039
CCL3	0.7480147	6.2064586	6.28E-07	PGK1	0.44420397	4.793832	0.000413154
TNFRSF18	0.6084601	6.0810685	1.31E-06	RAN	0.46902293	4.782226	0.000432204
HLA-DRB5	0.92368203	5.9224753	2.97E-06	CTSD	0.41237557	4.757401	0.000478288
HLA-DQB1	0.7120477	5.8814564	3.64E-06	HNRNPF	0.46544448	4.7567787	0.000478288
CAPG	0.6920501	5.8724747	3.68E-06	RANBP1	0.53602535	4.7492557	0.000490374
KLRC2	0.81117415	5.86501	3.69E-06	PTPN6	0.58653694	4.743929	0.000497388
TYMP	0.66775995	5.829842	4.37E-06	KIR2DL4	0.5860569	4.7380967	0.000505821
RBX1	0.5729994	5.7631426	6.09E-06	MYL6	0.26660421	4.730457	0.000519057
GBP2	0.66808236	5.756397	6.09E-06	CTSC	0.47971207	4.727113	0.000521539
CD74	0.49604735	5.7490096	6.10E-06	RGS10	0.6772499	4.6889324	0.000607744
HLA-DRB1	0.57917213	5.717322	7.10E-06	PSMB8	0.4583393	4.647194	0.000736332
ACP5	0.7662849	5.699709	7.62E-06	CCL4L2	0.7116423	4.644045	0.000739436
ACTG1	0.34933412	5.569926	1.57E-05	IFI6	0.5662297	4.6271706	0.000788358
LGALS1	0.55327296	5.5431566	1.77E-05	KRT86	0.5858917	4.6262956	0.000788358
COTL1	0.5765388	5.5379663	1.77E-05	PSMB9	0.4142323	4.621183	0.000799445
TPI1	0.46375707	5.5193424	1.91E-05	GABARAPL2	0.5218126	4.6036477	0.00086068
ID2	0.41496018	5.4932923	2.16E-05	IFNG	0.7861706	4.598224	0.000874186
CLEC2B	0.53723115	5.475446	2.32E-05	LDHB	0.4741107	4.5882125	0.000902557
TIGIT	0.83007675	5.4585724	2.48E-05	RAC2	0.44522634	4.58726	0.000902557
KLRC1	0.51576835	5.4543343	2.48E-05	CORO1A	0.29975098	4.5725045	0.000958746
CKLF	0.51071626	5.417631	2.97E-05	PTMS	0.8511223	4.543208	0.001091192
LGALS3	0.69297546	5.355461	4.00E-05	TRAC	0.54969734	4.53794	0.001107726
DEK	0.5922593	5.3137617	4.92E-05	HLA-DPB1	0.46540582	4.516886	0.001211738
COPE	0.5198143	5.3051496	4.99E-05	HLA-DRA	0.45735323	4.460645	0.001520452
IDH2	0.6095712	5.3030114	4.99E-05	SH3BGRL3	0.3084114	4.4603343	0.001520452
PGAM1	0.5594271	5.296324	5.06E-05	ARPC2	0.32532158	4.448709	0.001590103
STARD3NL	0.65810674	5.270429	5.70E-05	PPP1CA	0.4389116	4.4466095	0.001590849
LAG3	1.0257359	5.259893	5.91E-05	ID3	1.0262344	4.440078	0.001624864
ACTN4	0.61191064	5.2456236	6.26E-05	DNPH1	0.713087	4.434984	0.001648633
CD52	0.4387858	5.238975	6.36E-05	SERBP1	0.43418556	4.4119477	0.001817706
COX6B1	0.43989512	5.1867585	7.82E-05	HNRNPM	0.45797524	4.4056487	0.001854631
LDHA	0.44788957	5.1862726	7.82E-05	NPM1	0.37267724	4.3981256	0.001886398
NDFIP2	0.88944024	5.1757746	8.13E-05	MX1	0.60775787	4.395695	0.00189104
FKBP1A	0.5229083	5.1656466	8.43E-05	ARPC3	0.40174562	4.3895717	0.001928312
YWHAH	0.513552	5.1407433	9.46E-05	NOP10	0.44626138	4.381232	0.001986505
ALOX5AP	0.4622448	5.0772905	0.000125407	PSMB3	0.4389584	4.3675847	0.002096817

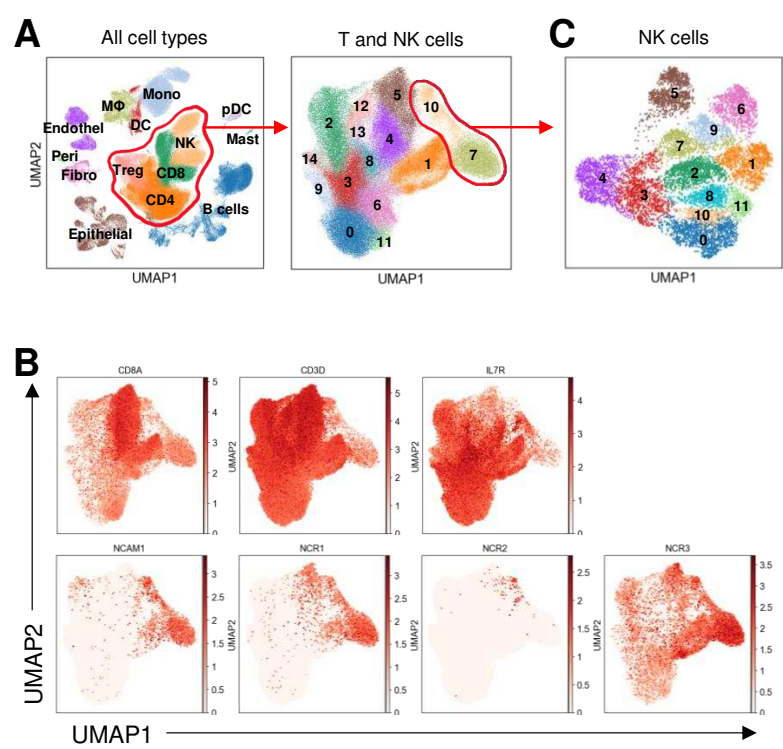


Figure S1. Identification and visualization of circulating and tumor-infiltrating NK cells by scRNAseq and flow cytometry. (A-C) Our published scRNAseq datasets generated from a cohort of 29 treatment-naïve HNSCC patients were used to evaluate circulating and tumor infiltrating NK cell profiles. (A) CD45⁻ non-immune and CD45⁺ immune cell subsets were identified using our previously published strategy (3). To extract NK cells, first we had to subcluster all of cells labeled as T and NK cells. (B-C) From the T/NK cell UMAP, NK cell clusters were identified using canonical NK cell lineage markers NCAM1 (CD56), NCR1 (NKp46), NCR2 (NKp44) and NCR3 (NKp30), and by excluding clusters expressing high levels of the canonical T cell marker CD3D.



Figure S2. Identification and visualization of circulating and tumor-infiltrating NK cells by flow cytometry. (A) Flow cytometry gating strategy used to identify circulating and tumor-infiltrating NK cell subsets throughout the paper is shown. Panels II and III (**Table S2**) data for patient HN# 3 are shown. Viable CD45⁺ singlets were evaluated for CD56 and CD3 expression. CD56⁺ CD3⁻ NK cell subsets were identified based on CD56 and CD16 expression levels. Subsequently, expression of different markers expressed by (B) the total population or (C-D) specific subsets of NK cell was evaluated.

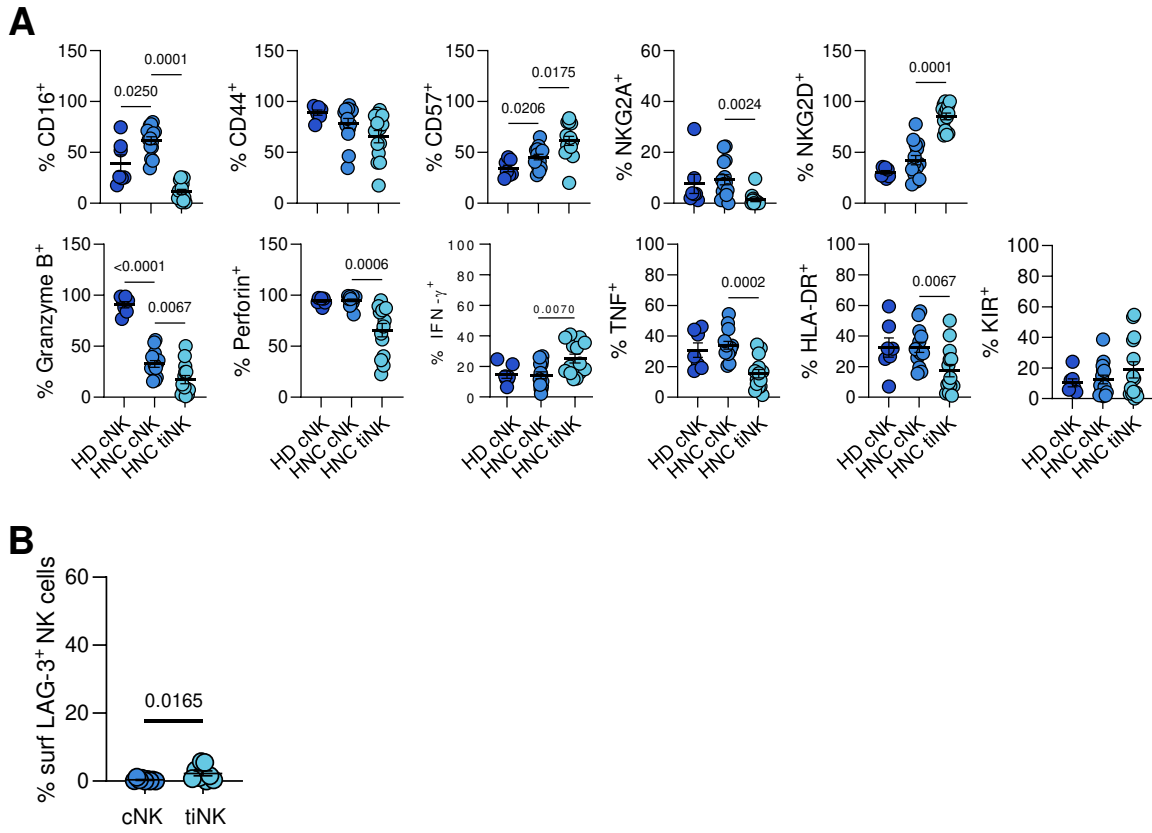


Figure S3. Activation status of NK cells is altered in TIL NK cells when compared to matching circulating NK cells. (A) Frequencies of total cNK from HD (n=7), and patient-matched cNK and tiNK (n=14) expressing CD16, CD44, CD57, CD69, NKG2A, NKG2D, granzyme B, perforin, IFN-γ, TNF and KIR2DL3 (KIR) by flow cytometry are shown. Mean values and standard errors of mean are shown. *P*-values are based on Mann-Whitney U (HD vs HNC cNK) and Wilcoxon signed-rank (HNC cNK vs tiNK) tests. (B) Surface LAG-3 expression was evaluated on the total NK cell population from patient-matched cNK and tiNK using the antibody Panel I (Table S2). Paired student *t* test was used to evaluate statistical significance.

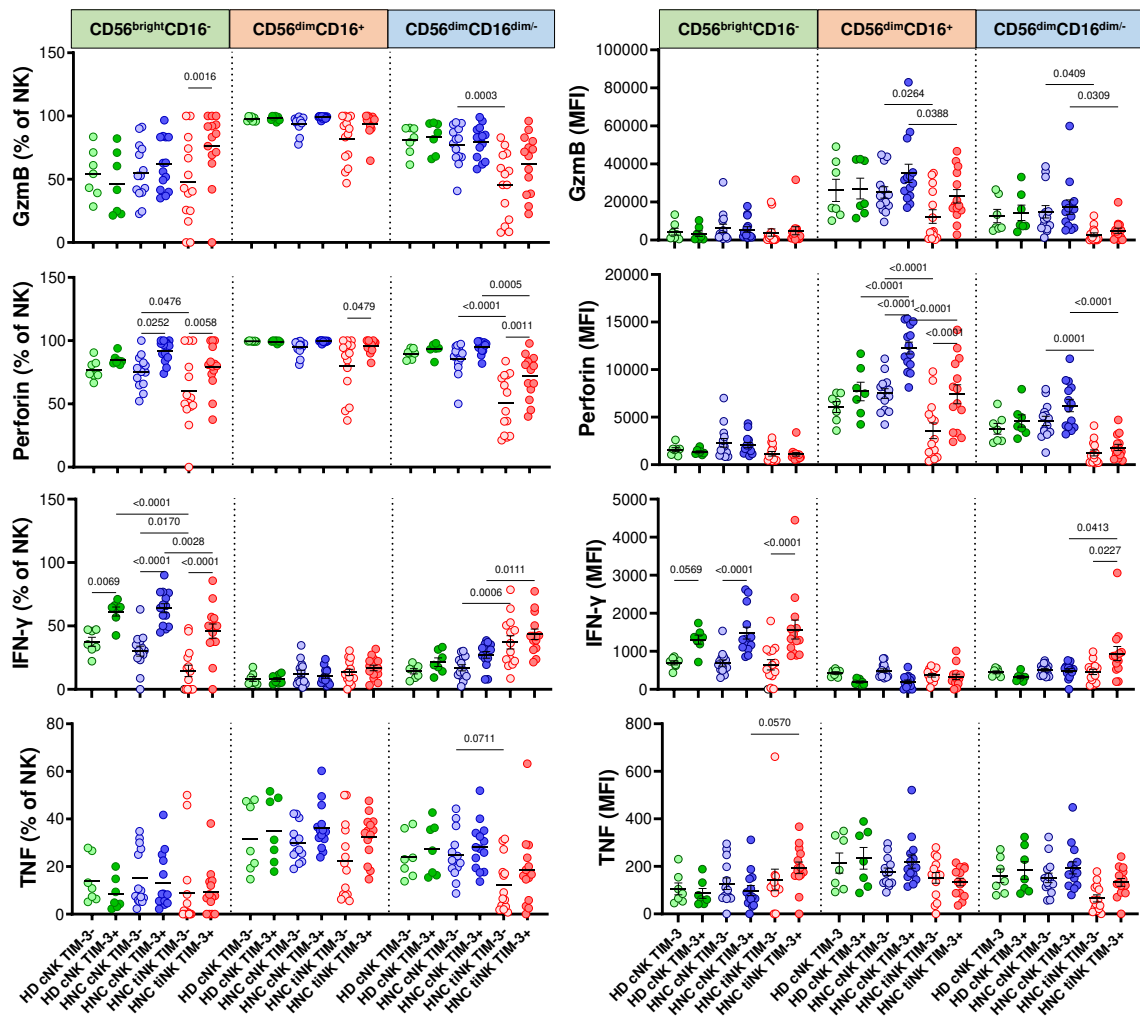


Figure S4. Activation states of cNK and tiNK subsets: cytolytic molecules and cytokines. We examined activation states of the three major NK cell subsets in PBL and TIL by flow cytometry. Donor-specific frequencies of, and mean fluorescence intensity (MFI) values for TIM-3⁻ and TIM-3⁺ NK cell subsets expressing cytolytic molecules and and cytokines are summarized. *P* values are based on the one-way ANOVA test.

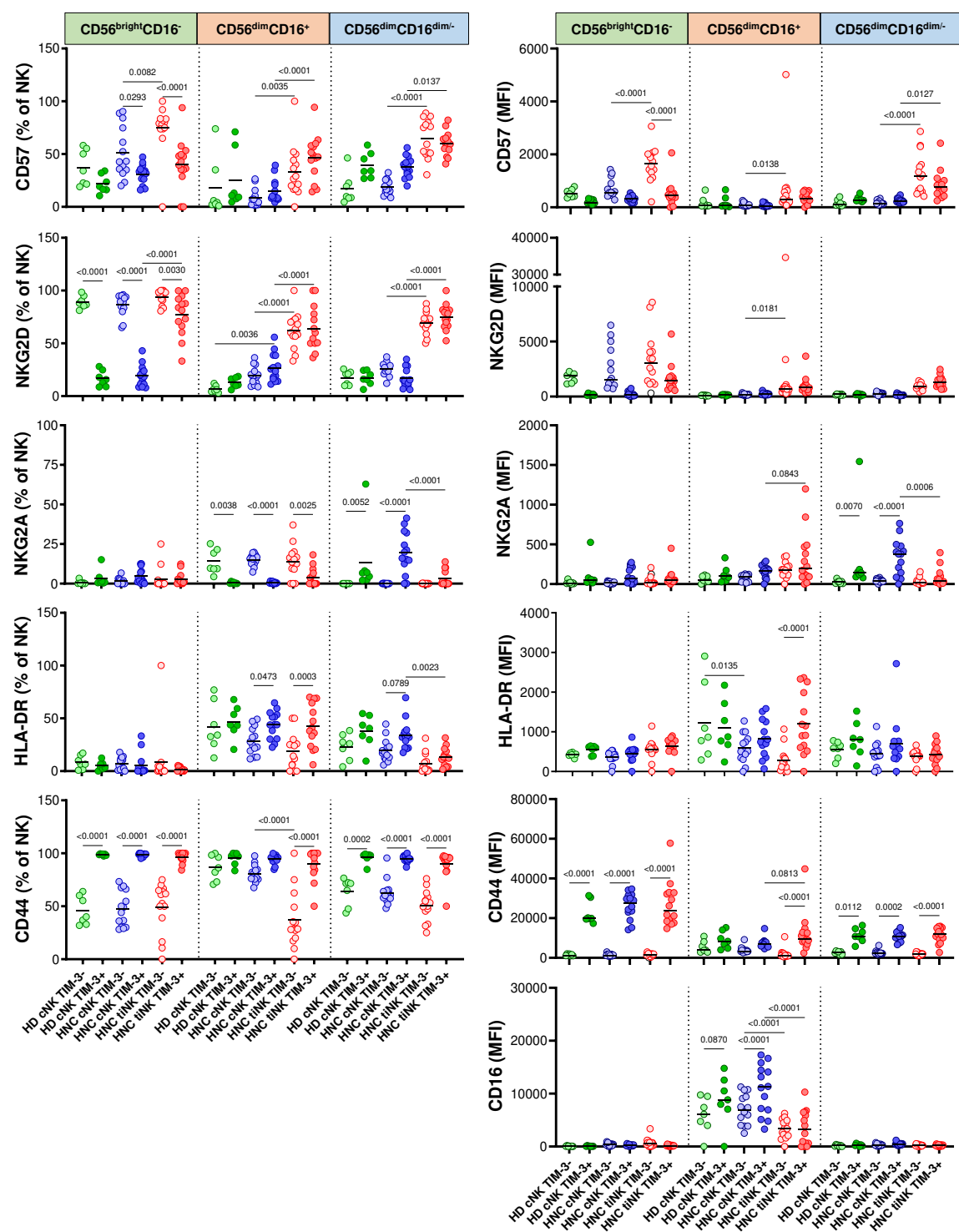


Figure S5. Activation states of cNK and tiNK subsets: activation and maturation markers. We examined activation states of the three major NK cell subsets in PBL and TIL by flow cytometry. Donor-specific frequencies of, and mean fluorescence intensity (MFI) values for TIM-3⁻ and TIM-3⁺ NK cell subsets expressing activating receptors are summarized. *P* values are based on the one-way ANOVA test.

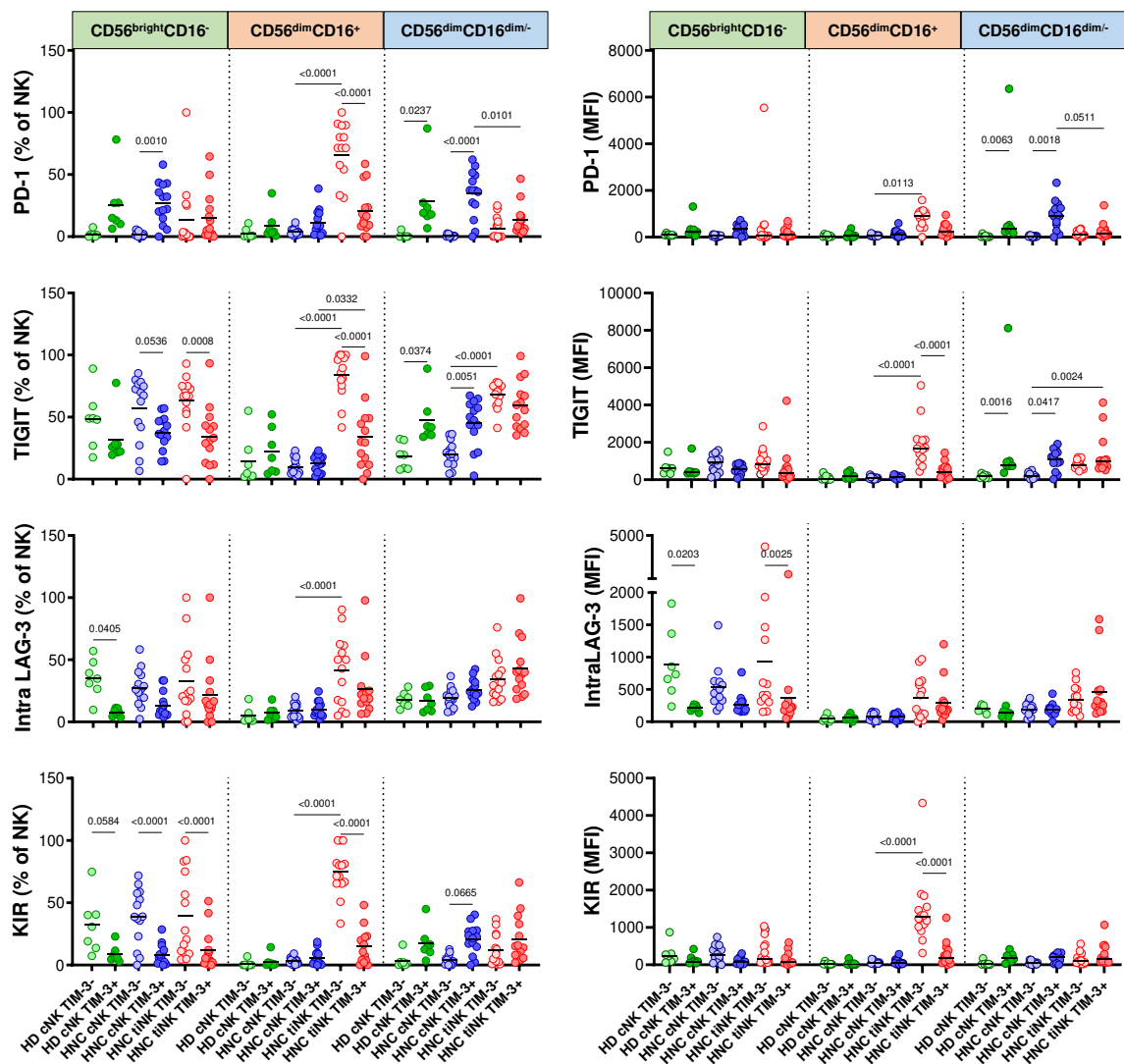


Figure S6. Activation states of cNK and tiNK subsets: inhibitory receptors. We examined inhibitory receptor profiles of the three major NK cell subsets in PBL and TIL by flow cytometry. Donor-specific frequencies of, and mean fluorescence intensity (MFI) values for TIM-3⁻ and TIM-3⁺ NK cell subsets expressing inhibitory receptors are summarized. *P* values based on the one-way ANOVA test.

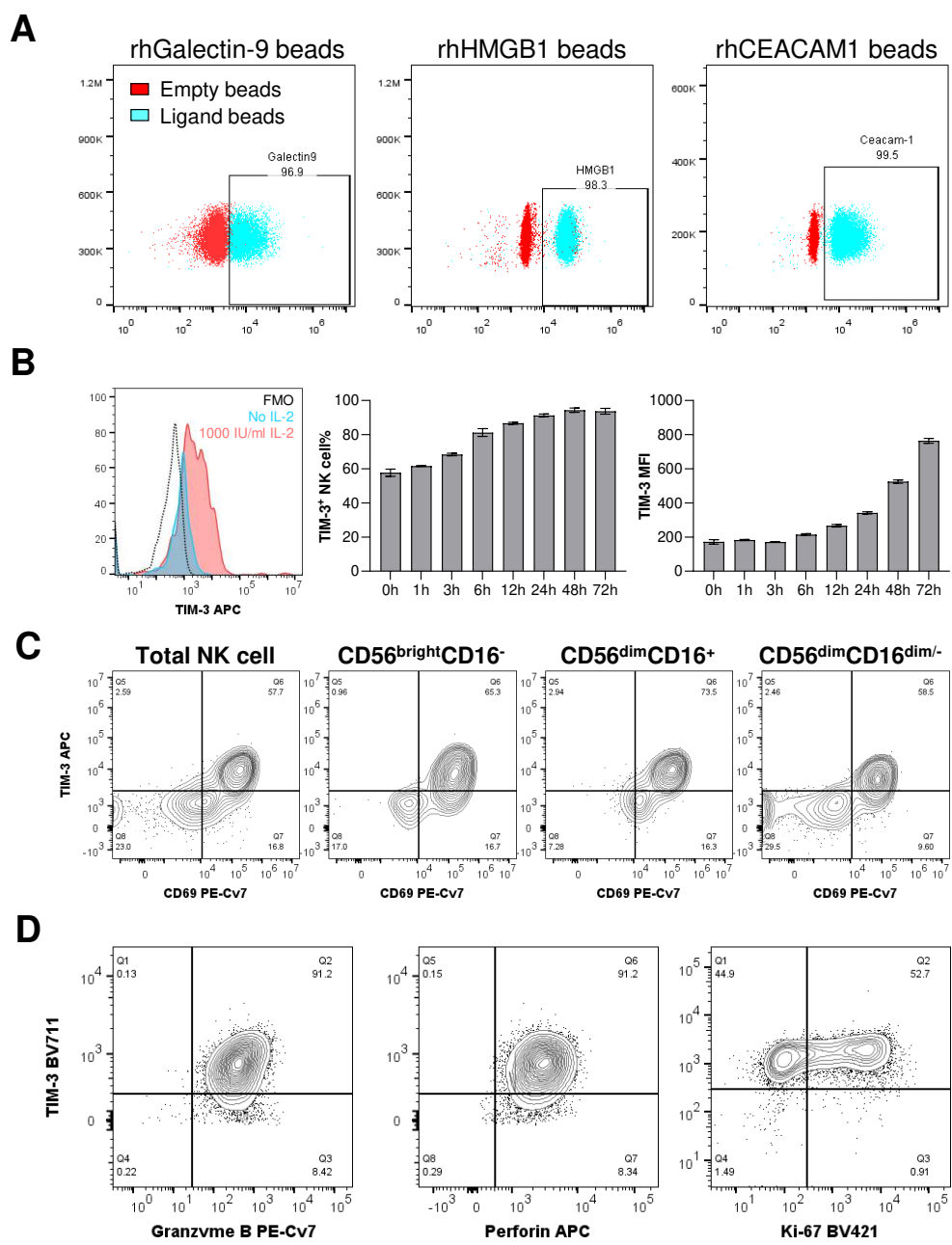


Figure S7. *In vitro* activation and treatment model for NK cells. (A) Representative flow cytometry plots showing effective ligation of recombinant human galectin-9 (rhGalectin-9), HMGB1 (rhHMGB1) and CEACAM1 (rhCEACAM1) to Epoxy beads. Empty beads are used as the negative control. (B) Peripheral HNSCC patient NK cells were isolated by MACS and treated with 1000 IU/ml IL-2 for 72 h. Time course analysis of TIM-3 expression on activated NK cells as measured by frequency and mean fluorescence intensity (MFI) is shown (n = 3). (C) Activation status of the major NK cell subsets following 48 h treatment with 1000 IU/ml IL-2 as measured by CD69 and TIM-3 expression (n = 4). (D) IL-2 activated NK cells co-express TIM-3, granzyme B, perforin and Ki-67 (n = 6).

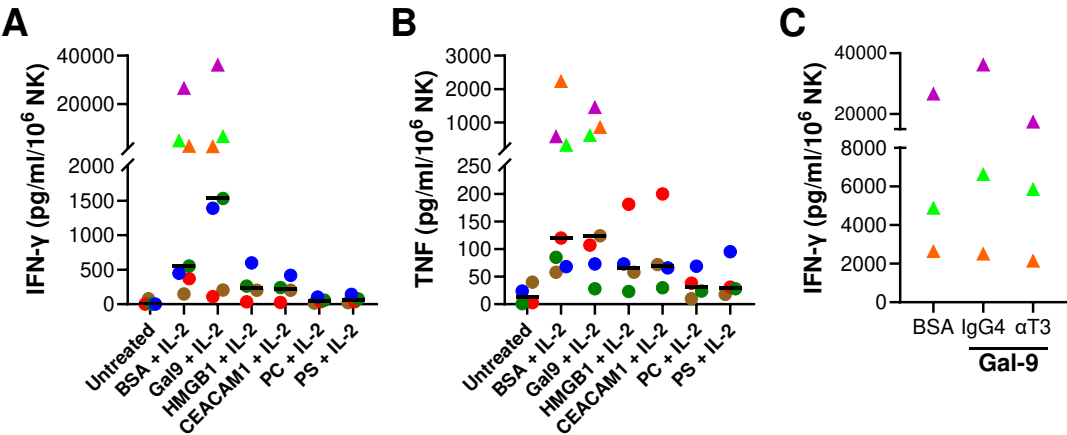


Figure S8. TIM-3 ligand treatments do not inhibit cytokine release by NK cells. Purified NK cells were activated with 1000 IU/ml IL-2. After 24h, cells were treated with BSA-, galectin-9 (Gal9)-, HMGB1-, or CEACAM1-coated beads, as well as PC or PS liposomes in the presence of 1000 IU/ml IL-2. After additional 24h, cell-free supernatants were collected and evaluated by Luminex for (A) IFN- γ and (B) TNF. Median values for each treatment condition are shown with black lines. (C) In three experiments, sabatolimab anti-TIM-3 (α T3) antibody, along with the IgG4 isotype control, were tested for their ability to block IFN- γ release due to TIM-3/galectin-9 interactions. One-way ANOVA test was used to calculate statistical significance among groups. Colored circles represent individual patients tested. n=4 for untreated, BSA, HMGB1, CEACAM1, PC and PS tests; n=7 for BSA and Gal9 tests. Circles represent the first batch of analyzed supernatants, while triangles represent the second.

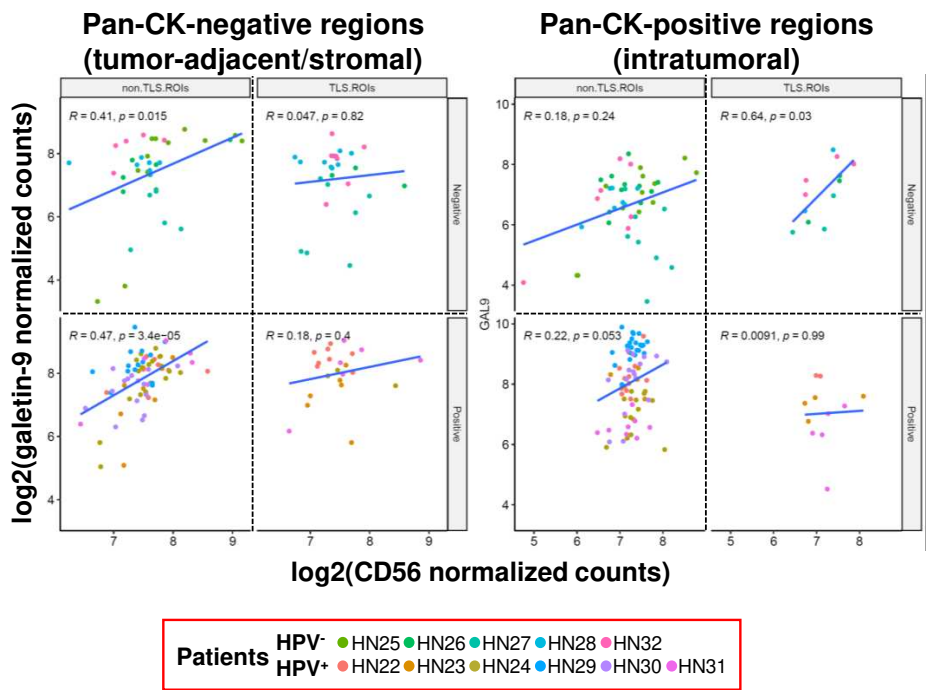


Figure S9. CD56 and galectin-9 co-expression within the HNSCC TME. TLS and non-TLS ROI from PanCK-negative (tumor-adjacent/stromal) and PanCK-positive (intratumoral) areas were evaluated for CD56 and galectin-9 co-expression. Each dot represents an individual ROI and each color represents a specific patient. The unit is log2(SNR), where SNR is the signal to noise ratio per Nanostring's DSP protocol. It is the relative abundance of galectin-9 or CD56 protein normalized against unspecific binding of the probes in DSP experiments, followed by log2 transformation. Spearman's correlation and *p*-values are shown.

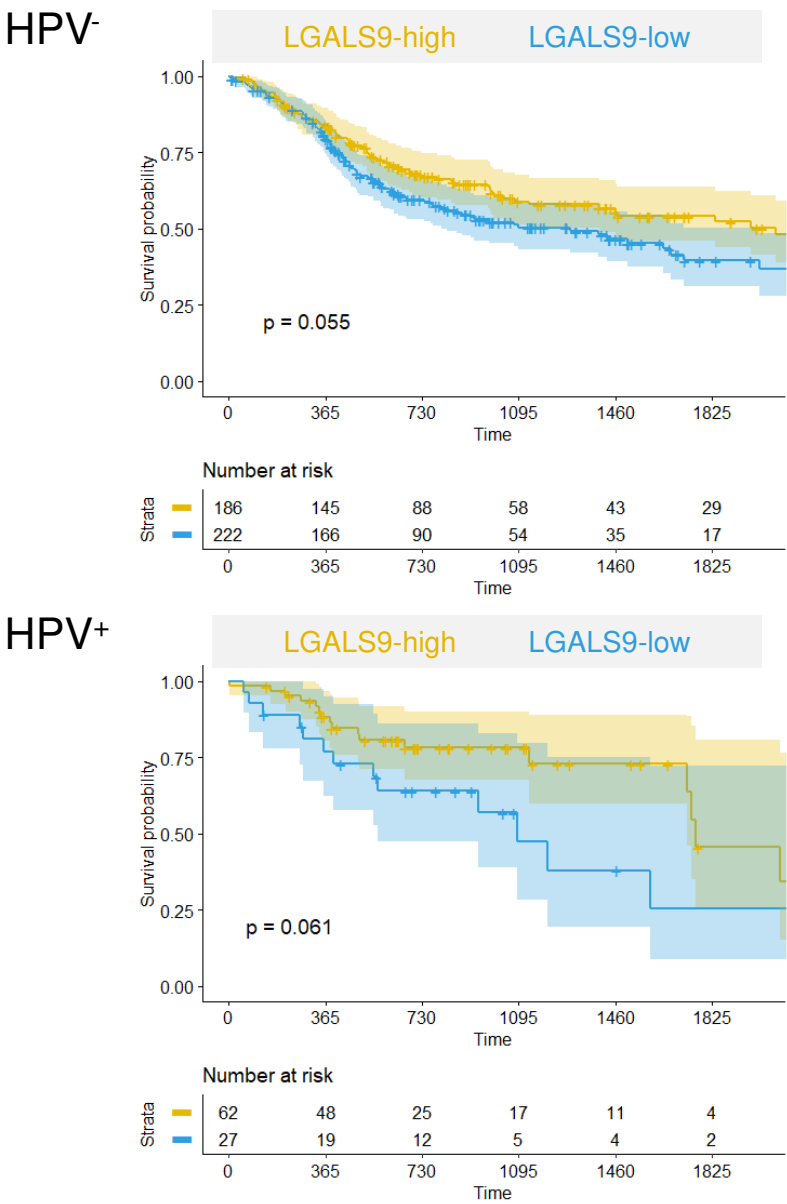


Figure S10. High *LGALS9* expression may predict better clinical outcome in and HPV- and HPV+ HNSCC patients. *LGALS9* expression was used to stratify TCGA HNSCC samples into high and low expression groups. Kaplan-Meier survival curves are shown for HPV- and HPV+ patients.