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Supplementary Materials for

A defect in KCa3.1 channel activity limits the ability of CD8$^+$ T cells from cancer patients to infiltrate an adenosine-rich microenvironment

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Supplementary Materials

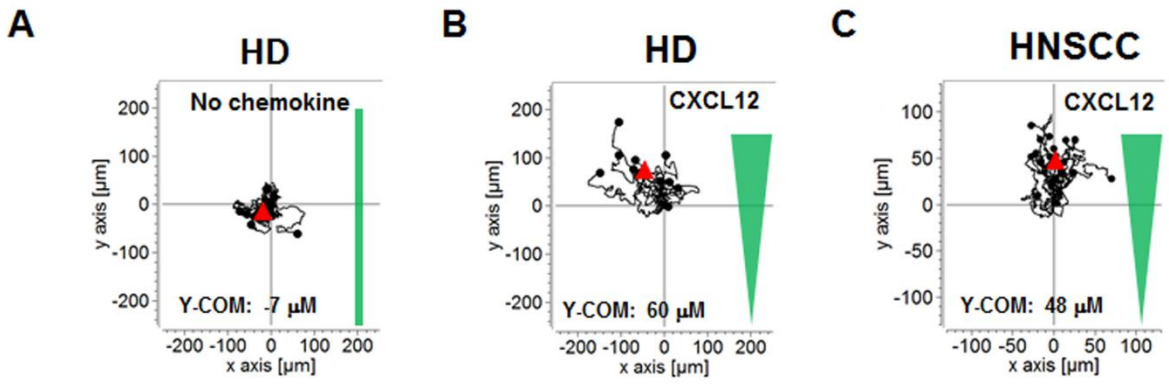

Fig. S1. HD and HNSCC CD8$^+$ T cells chemotax toward CXCL12. (A) Trajectories of HD CD8$^+$ T cells migrating in the absence of a chemokine. (B and C) Trajectories of HD (B) and HNSCC (C) CD8$^+$ T cells migrating towards CXCL12. Trajectories of at least 15-20 cells are shown for each condition and the starting point of each cell trajectory is artificially set to the same origin. Data are representative of 5 HD and 6 HNSCC patients. The red triangles represent Y-COM values.

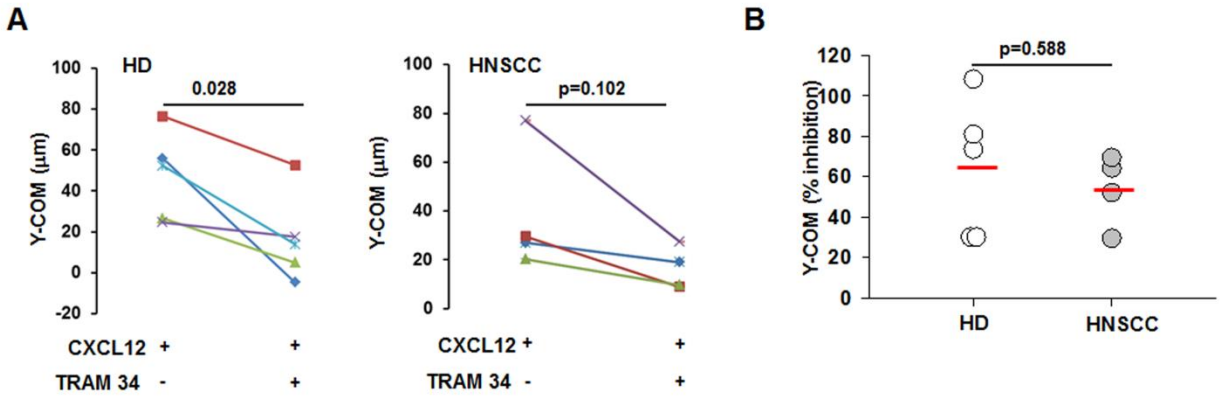

Fig. S2. KCa3.1 channel blockade inhibits the chemotaxis of HD and HNSCC CD8$^+$ T cells. (A) The Y-COM values for CD8$^+$ T cells from HD and HNSCC patients migrating towards CXCL12 or CXCL12 and TRAM-34 (1 μM, n=5 HD and n=4 HNSCC patients). (B) Percentage inhibition in the Y-COM values in the presence of 1μM TRAM-34. Horizontal red line represents mean values for each group. Statistical significance was determined by Student's t-test.

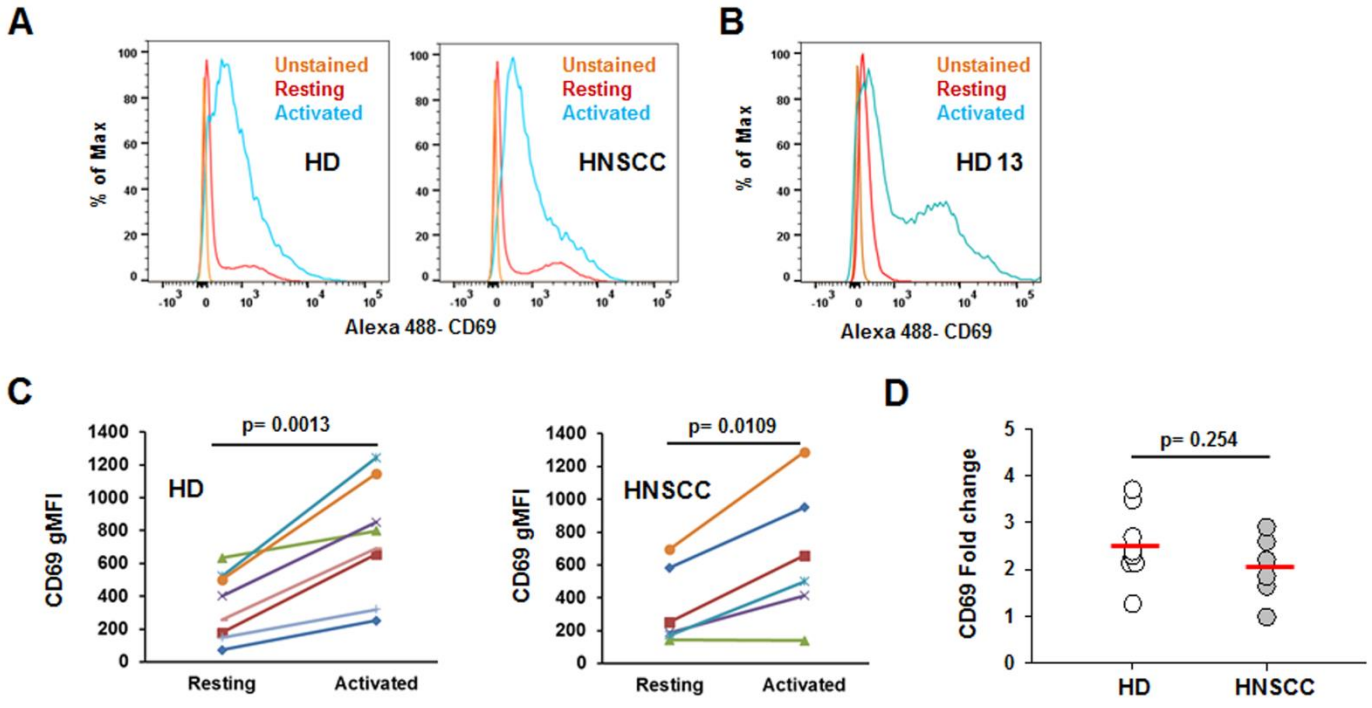

Fig. S3. Activation of CD8$^+$ T cells from HD and HNSCC patients. (A and B) Representative flow cytometry histograms showing CD69 expression in resting and activated CD8$^+$ T cells from a representative HD and HNSCC patient. (B) The histogram of one healthy donor (HD-13) showed a bi-modal distribution in the CD69 fluorescence. All of the other HD's as well as HNSCC patients showed a normal distribution in the CD69 fluorescence histograms. (C) To account for this non-normal distribution, the geometric mean of the CD69 fluorescence intensity (CD69 gMFI) was calculated for all resting and activated CD8$^+$ T cells from HD (n=8 donors) and HNSCC patients (n=6 patients). (D) Fold change in CD69 expression (ratio between resting and activated CD69 gMFI for the same individual) in CD8$^+$ T cells from HD (n=8) and HNSCC (n=6) from data shown in C. Horizontal red line represents mean values for each group. Data in C were analyzed by paired Student's t-test and data in D were analyzed by unpaired Students' t-test.

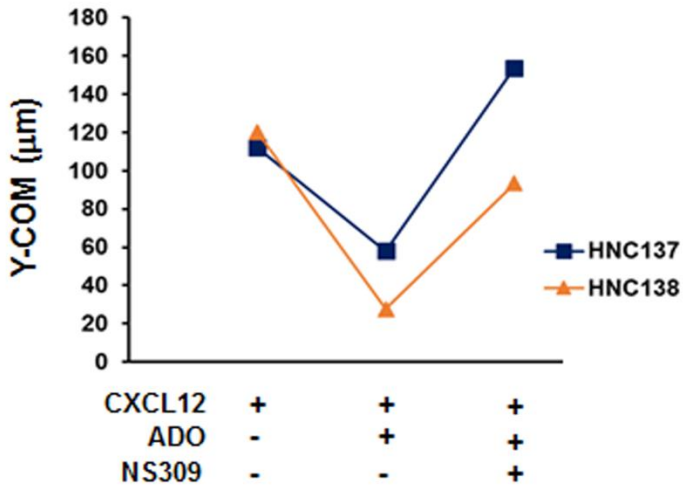

Fig. S4. Activation of KCa3.1 channels by NS309 restores the chemotaxis of HNSCC CD8$^+$ T cells in the presence of adenosine. The Y-COM values calculated for HNSCC CD8$^+$ T cells migrating towards CXCL12 or CXCL12 and adenosine, with or without preincubation with 1 µM NS309. Chemotaxis was analyzed in 15 cells per condition in 2 HNSCC patients.

Table S1. Clinicopathologic characteristics of individual HNSCC patients. The tumor location, clinical staging, HPV status (p16 status), tumor differentiation, and disease progression information for the 39 HNSCC patients used in this study. Time to median follow up for HNSCC patients was 9.5 months (Range 2-58 months). CD73 expression and $CD8^+$ T cell infiltration in tumor biopsy specimens are determined by immunohistochemistry. N/A= data not available.

	Patient ID	Head and Neck Disease Site	Clinical Stage	p16 Status	Differentiation	Disease Progression	CD73 expression		CD8 infiltration
							Tumor	Stroma	
1	HNC-01	Oropharynx	T1N1M0	Pos	Poorly differentiated	N	Low	High	Low
2	HNC-03	Oropharynx	T2N2BM0	Pos	Moderately differentiated	N	Low	High	High
3	HNC-05	Oral Cavity	T4bN3M0	Neg	Moderately differentiated	Y	Low	Low	N/A
4	HNC-06	Larynx	T1N0M0	Neg	Moderately differentiated	N	Low	High	Low
5	HNC-12	Oropharynx	T3N2aM0	Neg	Poorly to moderately differentiated	Y	N/A	N/A	N/A
6	HNC-32	Larynx	T3N0M0	Neg	Poorly differentiated	N	N/A	N/A	N/A
7	HNC-37	Larynx	T4N2cM0	Neg	Poorly to moderately differentiated	N	Low	Low	Low
8	HNC-39	Oral Cavity	T4aN0M0	Neg	Moderately differentiated	Y	N/A	N/A	N/A
9	HNC-42	Oral Cavity	T4bN2bM0	Neg	Moderately differentiated	Y	N/A	N/A	N/A
10	HNC-43	Larynx	T2N1M0	N/A	Moderately differentiated	N	N/A	N/A	N/A

11	HNC-47	Oral Cavity	T3N0M0	N/A	Moderately differentiated	N	Low	N/A	High
12	HNC-48	Larynx	T4N2bM0	Neg	Poorly to moderately differentiated	Y	N/A	N/A	N/A
13	HNC-49	Oral Cavity	T2N0M0	N/A	N/A	N	Low	Low	Low
14	HNC-52	Larynx	T2N0	N/A	N/A	Y	N/A	N/A	Low
15	HNC-53	Oropharynx	T4aN2cM1	Pos	N/A	Y	N/A	N/A	N/A
16	HNC-54	Oral Cavity	T3N2bM0	N/A	Moderately differentiated	N	Low	Low	High
17	HNC-61	Hypopharynx	T2N2bM0	N/A	Poorly differentiated	N	N/A	N/A	N/A
18	HNC-62	Nasopharynx	T4N2M0	Neg	Poorly differentiated	N	N/A	N/A	N/A
19	HNC-63	Oropharynx	T1N2bM0	Pos	N/A	N	N/A	N/A	N/A
20	HNC-64	Oropharynx	T4N2cM0	Pos	Undifferentiated	Y	Low	High	High
21	HNC-65	Oral Cavity	TxN2cM0	Neg	Moderately differentiated	Y	High	Low	High
22	HNC-66	Oropharynx	T3N2bM0	Neg	Poorly differentiated	N	Low	High	High
23	HNC-67	Oral Cavity	T3N0M0	N/A	Moderately differentiated	N	High	Low	High
24	HNC-70	Oropharynx	T2N2bM0	Pos	Poorly differentiated	N	Low	Low	High
25	HNC-72	Oropharynx	T2N2bM0	Pos	Poorly differentiated	N	Low	High	High
26	HNC-73	Oropharynx	T1N2bM0	Pos	Poorly differentiated	N	N/A	N/A	N/A
27	HNC-74	Oropharynx	T4aN2bM0	Pos	Moderately differentiated	N	N/A	N/A	N/A
28	HNC-78	Oral Cavity	T4aN2bM0	N/A	Poorly to moderately differentiated	Y	N/A	N/A	N/A
29	HNC-79	Oral Cavity	T2N2cM0	N/A	Moderately differentiated	N	N/A	N/A	N/A
30	HNC-83	Oropharynx	T1N2bM0	Pos	Poorly differentiated	N	N/A	N/A	High
31	HNC-84	Oropharynx	T1N2bM0	Pos	N/A	N	N/A	N/A	N/A

32	HNC-85	Larynx	T3N2cM0	N/A	Poorly differentiated	N	N/A	N/A	N/A
33	HNC-88	Oral Cavity	T3N0M0	Neg	Poorly to moderately differentiated	N	N/A	N/A	N/A
34	HNC-89	Oropharynx	T2N2bM0	Pos	Poorly differentiated	N	N/A	N/A	N/A
35	HNC-90	Oropharynx	T4aN2bM0	Pos	Moderately differentiated	N	N/A	N/A	N/A
36	HNC-98	Oral Cavity	N/A	Pos	N/A	N	N/A	N/A	N/A
37	HNC-103	Oropharynx	T2NxMx	Pos	Poorly differentiated	Y	Low	High	Low
38	HNC-105	Oropharynx	T1N1M0	Pos	Moderately differentiated	N	High	High	N/A
39	HNC-108	Larynx	T3N0M0	Neg	Moderately differentiated	N	N/A	N/A	N/A

Table S2. $CD8^+$ T cells from HDs and HNSCC patients chemotax toward CXCL12 similarly. Activated $CD8^+$ T cells from HDs (n = 5 donors) and HNSCC patients (n = 6 patients) were exposed to a CXCL12 chemokine gradient and the indicated values were measured. Data are means ± SEM. Y-COM, center of mass along the Y-axis, along the chemokine gradient; X-COM, center of mass along the X-axis, perpendicular to the chemokine gradient; FMI^Y, forward migration index in the direction of the y-axis (it represents the efficiency of forward migration towards the chemokine gradient); FMI^X, forward migration index in the direction of the x-axis (represents the efficiency of migration perpendicular to the chemokine gradient respectively); directness, the cells' tendency to migrate along a straight line; Euclidian distance, the linear distance between the starting point and ending point of a cell; Accumulated distance, total distance travelled by the cell during the course of the entire microscopy recording.

Parameter	HD (n=5)	HNSCC (n=6)	p-value (HD vs HNSCC)
X-COM (μm)	5.660 ± 4.739	-7.125 ± 9.596	0.293
Y-COM (μm)	43.279 ± 9.221	46.773 ± 18.178	0.876
FMI^x	0.016 ± 0.019	-0.030 ± 0.023	0.179
FMI^y	0.141 ± 0.031	0.191 ± 0.039	0.362
Directness	0.203 ± 0.024	0.256 ± 0.040	0.317
Velocity (μm/min)	0.210 ± 0.021	0.146 ± 0.042	0.232
Accumulated distance (μm)	315.462 ± 31.352	211.297 ± 62.163	0.195
Euclidean distance (μm)	62.122 ± 6.277	38.126 ± 8.524	0.057

Table S3. Electrophysiological parameters of resting and activated CD8$^+$ T cells isolated from HD and HNSCC patients. The cell capacitance, KCa3.1 conductance (normalized to cell capacitance, conductance/capacitance), and Kv1.3 current density (peak current/capacitance) information for resting and activated CD8$^+$ T cells from HD and HNSCC patients. Data are means ± SEM. [a] $P<0.001$, [b] $P<0.001$ [c] $P=0.332$ vs. activated HD,[d] $P<0.001$, [e] $P=0.207$, [f] $P=0.557$ vs. activated HNSCC, [g] $P<0.001$, [h] $P=0.011$, [i] $P=0.299$ vs. activated HNSCC; [j] $P<0.001$, [k] $P<0.001$, [l] $P=0.675$ vs. activated HNSCC. Statistical significance in [d] and [j] determined by Student's t-test, all other p-values measured by Mann-Whitney rank sum test.

		HD		HNSCC	
Parameter	**Unit**	**Resting**	**Activated**	**Resting**	**Activated**
Capacitance	pF	2.1±0.2[a,g] (n=35 cells, 6 donors)	6.2±0.3[j] (n=30 cells, 6 donors)	2.2±0.3[d] (n=17 cells, 4 donors)	4.4±0.3 (n=21cells, 4 donors)
KCa3.1 Conductance/capacitance	nS/pF	0.02±0.02[b,h] (n=34 cells, 6 donors)	0.17±0.02[k] (n=30 cells, 6 donors)	0.04±0.02[e] (n=17 cells, 4 donors)	0.07±0.02 (n=21 cells, 4 donors)
Kv1.3 current density Peak current/capacitance	pA/pF	127.7±13.2[c,i] (n=27 cells, 5 donors)	140.0±36.2[l] (n=25 cells, 5 donors)	114.9±13.9[f] (n=17 cells, 4 donors)	139.3±36.9 (n=21 cells, 4 donors)