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Supplemental information

Targeted knockdown of the adenosine A_{2A} receptor by lipid NPs rescues the chemotaxis of head and neck cancer memory T cells

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Supplemental Material

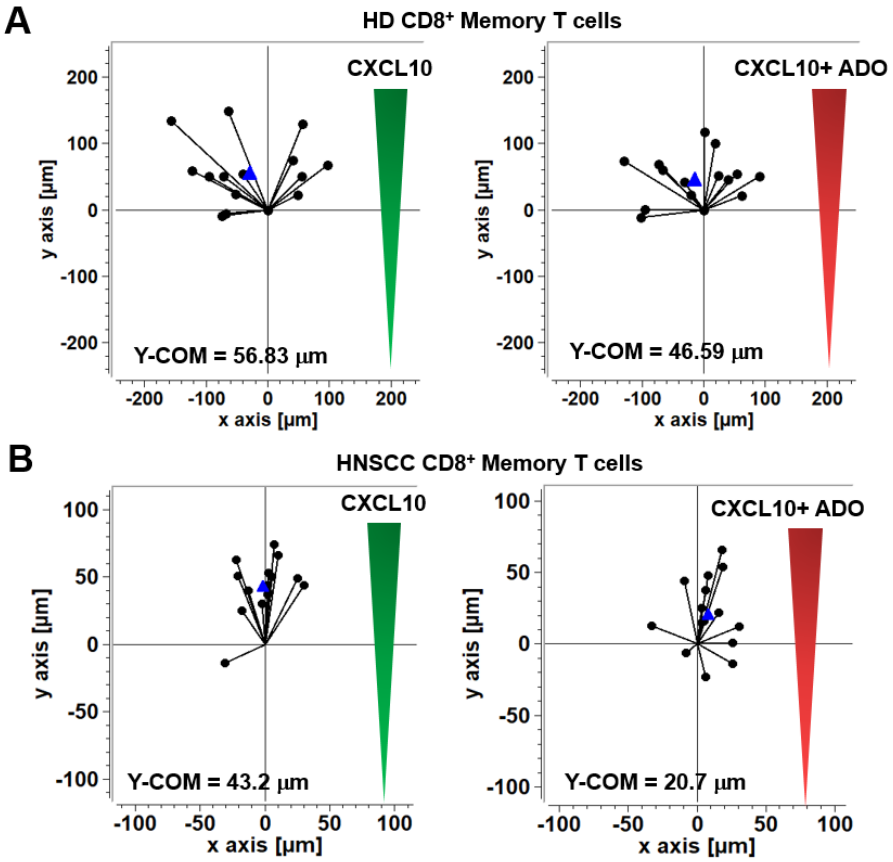
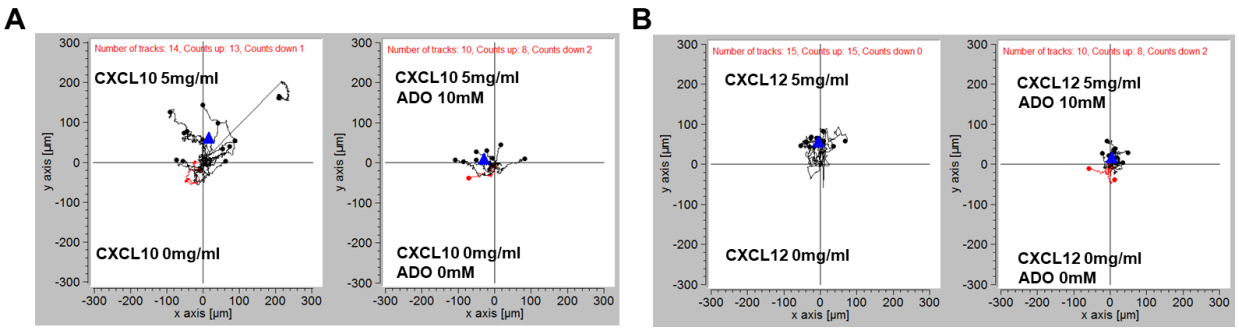


Figure S1. Adenosine does not inhibit chemotaxis in HD CD8$^+$ memory T cells but does inhibit chemotaxis in HNSCC CD8$^+$ memory T cells. (A and B) Trajectories of CD8$^+$ memory T cells migrating along the CXCL10 gradient (green triangle) or CXCL10+adenosine (ADO) gradient (blue triangle) in (A) HD and (B) HNSCC samples. Trajectories are artificially set to start at the origin with the blue triangle representing the Y-COM. The same experiment was performed in CD8$^+$ memory T cells isolated from two independent HDs and two independent HNSCC patients. Shown here are the trajectories of migrating cells from a representative single HD and representative single HNSCC patient. Trajectories of 10-15 cells were calculated for each condition for each experiment.



C

	CXCL10	CXCL10 + ADO	CXCL12	CXCL12 + ADO
Y-COM	62.38	10.47	58.36	18.74
FMI^x	0.036	-0.13	-0.05	-0.02
FMI^y	0.23	0.08	0.36	0.09
Directness	0.21	0.24	0.39	0.2
Velocity (mm/min ± SD)	0.09 ± 0.041	0.06 ± 0.027	0.043 ± 0.027	0.047 ± 0.012
p-value (Rayleigh test)	0.028	0.055	8.55E-07	0.069
Accumulated distance (mm)	476	256	211	235
Euclidean distance (mm)	99	58	65	41

Figure S2. The effect of adenosine is the same on T cell chemotaxis towards CXCL10 and CXCL12. (A) Representative trajectories of T cells migrating along the CXCL10 gradient or CXCL10+adenosine (ADO) gradient in HD $CD3^+$ T cells. Trajectories are artificially set to start at the origin with the blue triangle representing the Y-COM. **(B)** Representative trajectories of T cells migrating along the CXCL12 gradient or CXCL12+adenosine (ADO) gradient in HD $CD3^+$ T cells. Trajectories are artificially set to start at the origin with the blue triangle representing the Y-COM. **(C)** Chemotactic parameters for HD $CD3^+$ T cells in migration media with CXCL10±ADO versus CXCL12±ADO. (n = 1 patient; 10-15 cells per condition).

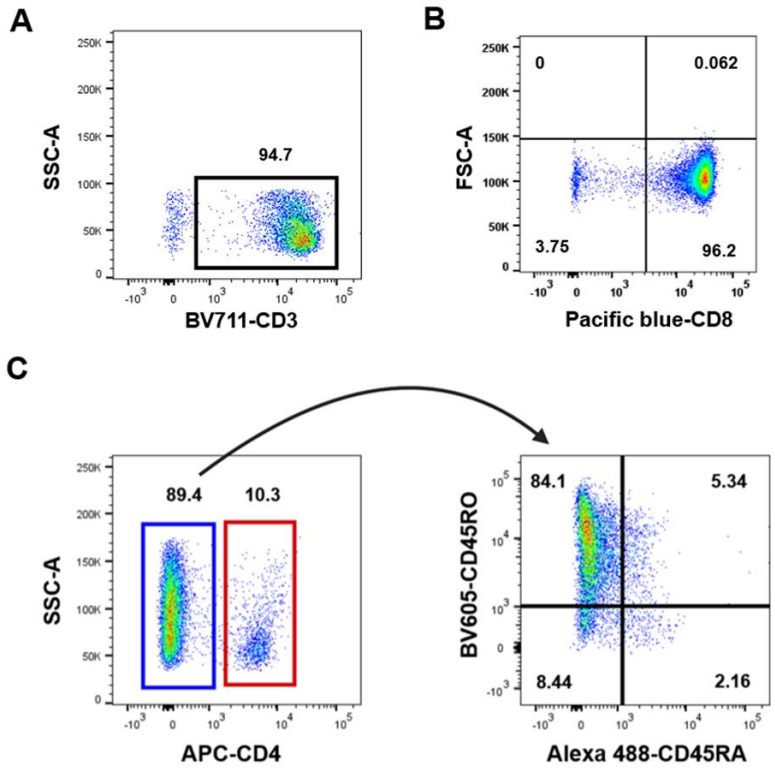


Figure S3. Purity of T cell populations used in the study. (A and B) $CD3^+$ and $CD8^+$ T cell populations were enriched by negative selection from HD PBMCs using EasySep Human T cell and Human $CD8^+$ T cell enrichment kits respectively (StemCell Technologies) as per the manufacturer's instructions. The purity of the enriched cell $CD3^+$ (A) and $CD8^+$ (B) T cell fractions were assessed by flow cytometry. **(C)** $CD8^+$ memory T cell population was enriched using the EasySep Human Memory $CD8^+$ T Cell Enrichment Kit (StemCell Technologies) as per the manufacturer's instructions. This kit enriches $CD8^+$ memory T cells ($CD8^+CD45RA^-CD45RO^+$) from human PBMCs by targeting non-memory $CD8^+$ T cells for removal with antibodies recognizing specific cell surface markers. The $CD8^+$ memory T cell content in the enriched fraction was evaluated by flow cytometry and was defined as cells that were $CD8^+CD45RO^+CD45RA^-$. In panel (C), the $CD4^-$ population from the enriched cells were defined as the $CD8^+$ population. All experiments were performed in two independent donors and shown here are representative scatter plots showing the purity of the enriched $CD3^+$, $CD8^+$ and $CD8^+$ memory T cells from one HD.