**Background** Effective co-stimulation of CD8 T cells is critical for anti-tumor effector responses and maintenance of antigen-specific TCF-1+ memory CD8 T cells necessary for long-term tumor control. NKG2D co-stimulation of CD8 T cells via binding of stress ligands such as membrane-bound MIC enhances CD8 T cell effector functions and is important for promoting memory development. Cancer cells often shed MIC into a soluble form (sMIC) that inhibits CD8 T cell activation. Humanized TRAMP/MICB prostate tumor mice treated with anti-sMIC/MIC targeting antibody develop potent anti-tumor responses by CD8 T cells by 1) sequestering sMIC and 2) inducing NKG2D pathway signaling, thereby re-invigorating CD8 T cell immunity. However, the mechanistic underpinnings of CD8 T cell reprogramming by NKG2D co-stimulation in the tumor microenvironment are poorly understood. In this preliminary study, we used scRNA-seq of CD8 TILs from TRAMP/MICB mice treated or untreated with the anti-sMIC/MIC targeting antibody B10G5 to investigate the hypothesis that B10G5 differentially reprograms CD8 T cells at the transcriptional and epigenetic level via sustained NKG2D pathway co-stimulation, thereby optimizing TCR-dependent effector and memory responses.

**Methods** Adult TRAMP/MICB mice were treated with 200 μg B10G5 intraperitoneally twice per week for 4 weeks. Prostate tumors from B10G5-treated mice, untreated mice with well-differentiated tumors, and untreated mice with poorly differentiated tumors were collected, and their CD45+ cells isolated via FACS for scRNA-seq. Subclusters specific to CD8 T cells were the focus of subsequent transcriptomic analyses.

**Results** Prostate tumors from mice treated with anti-sMIC/MIC antibody B10G5 revealed enhanced functional heterogeneity of CD8 T cell subtypes compared to tumors from untreated mice. In contrast to tumors from untreated mice that were populated with primarily effector CD8 T cells expressing CD226hi, CXCR3hi, GZMBhi, and/or NKG7, tumors from B10G5-treated mice were enriched in TCF7hi IL7R+ EOMEShi stem-like memory CD8 T cells and EOMEShi CD27hi PD-1+ effector memory CD8 T cells. These memory populations upregulated epigenetic modifiers (KMT2A/E) and transcription factors (NR4A1/2/3) important in metabolic reprogramming and memory CD8 T cell differentiation.

**Conclusions** These results establish a groundwork for identifying targets for epigenetic and metabolic alteration of CD8 T cells via NKG2D co-stimulation within the tumor microenvironment.

**Ethics Approval** All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) committee of Northwestern University.