Supplemental Figures

Genetic associations of T cell cancer immune response-related genes with T cell phenotypes and clinical outcomes of early-stage lung cancer

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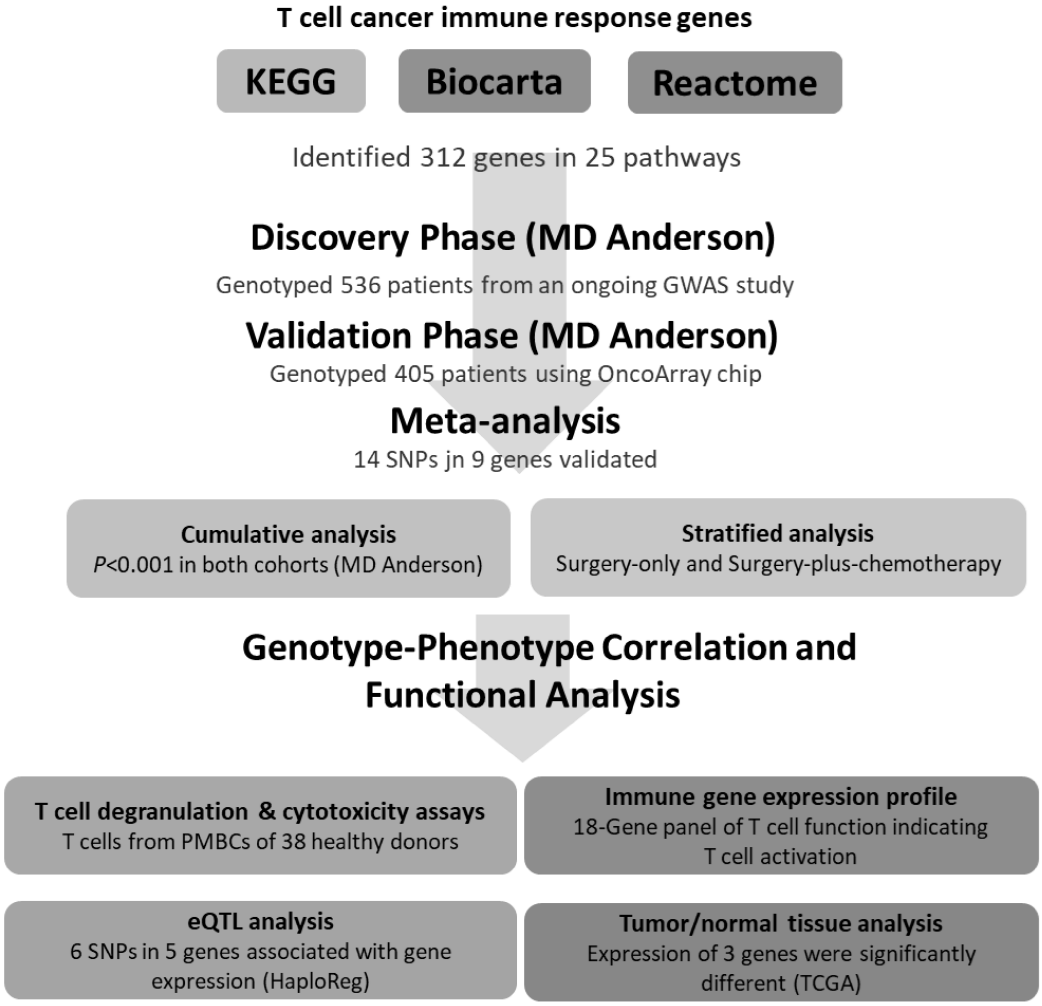
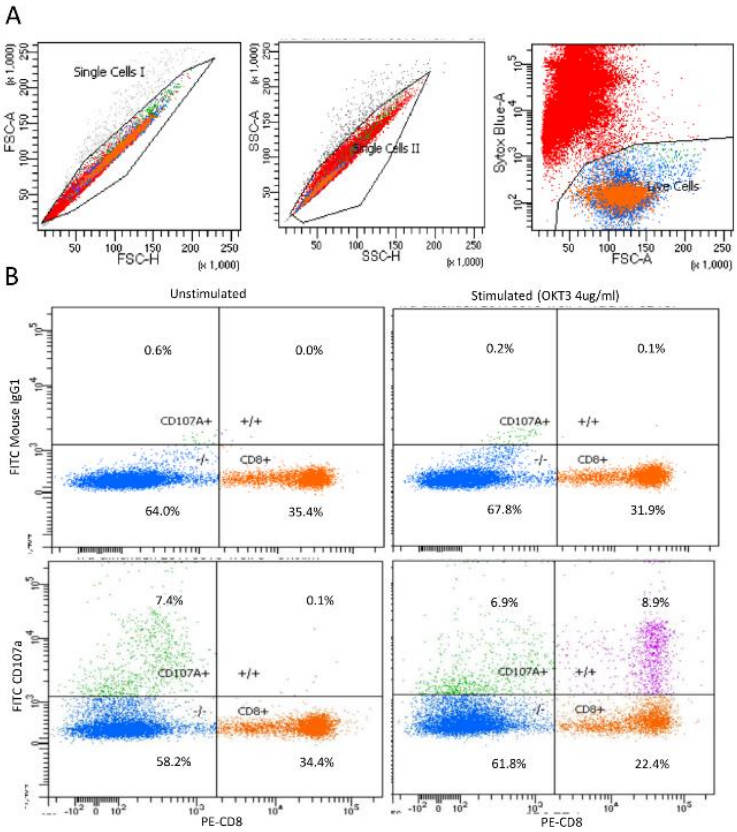
Wang Q, *et al. J Immunother Cancer* 2020; 8:e000336. doi: 10.1136/jitc-2019-000336

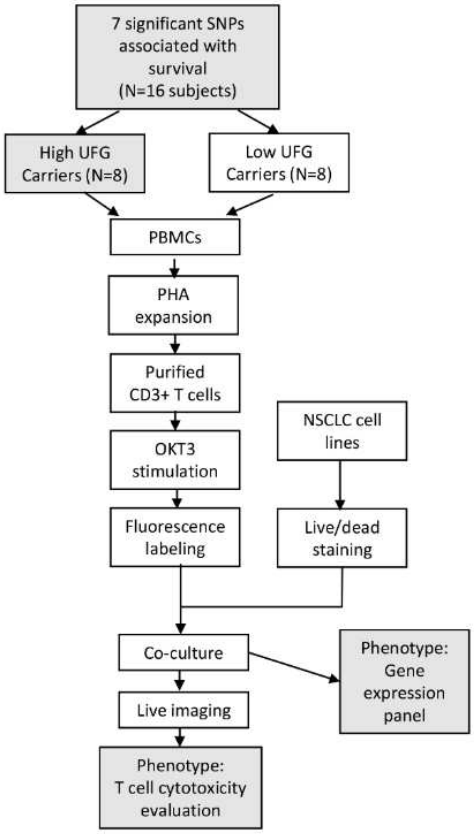


Supplemental Figure S1: Schematic flowchart of study design



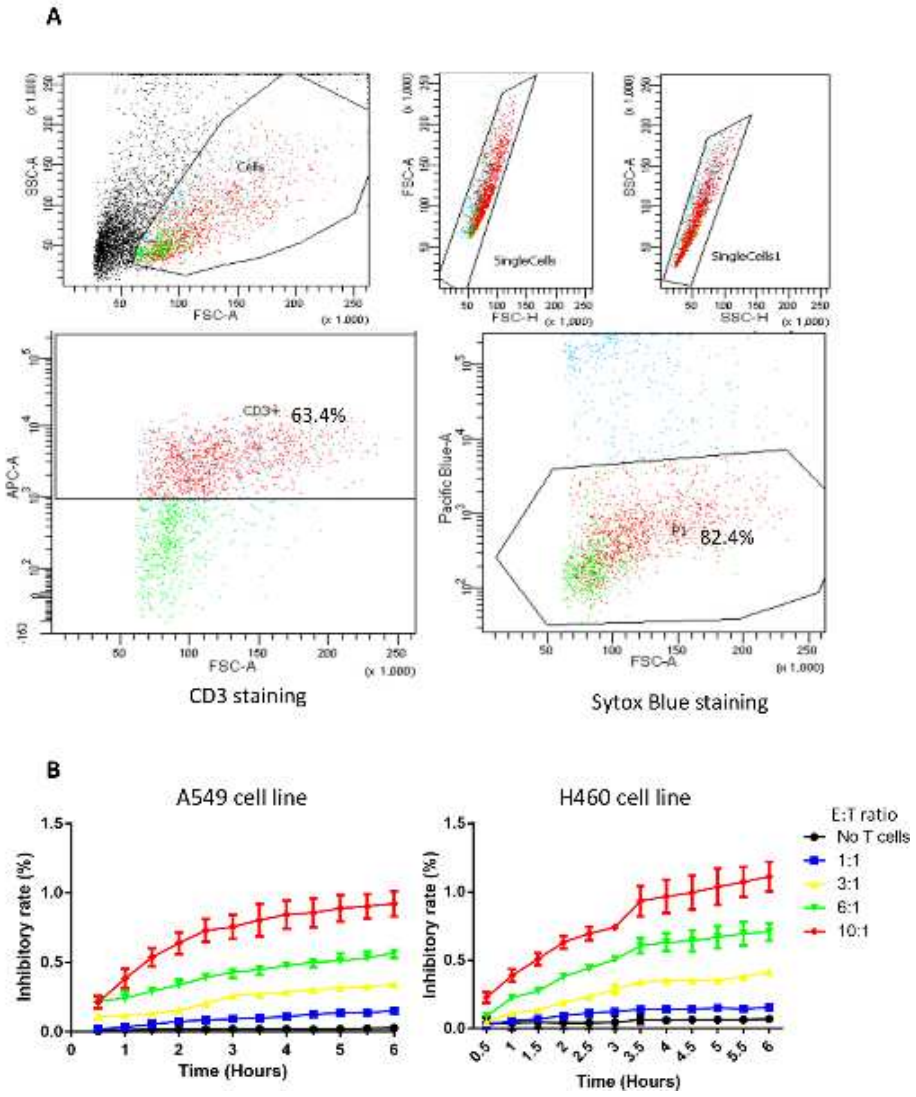
Supplemental Figure S2. Gating strategy for T cell CD107a degranulation assay

A gating strategy for flow cytometry analysis of T cell degranulation assay is shown. (**A**) Dead cells were excluded by SYTOX Blue staining. Single cells were obtained by gating on FSC-A versus FSC-H, followed by SSC-A versus SSC-H. (**B**) Representative controls of CD107a degranulation assay. CD8+CD107a+ T cells were analyzed and counted under unstimulated (PBS) and stimulated (CD3 antibody [OKT3]). Data were analyzed using FlowJo (v.10.0.8) software.



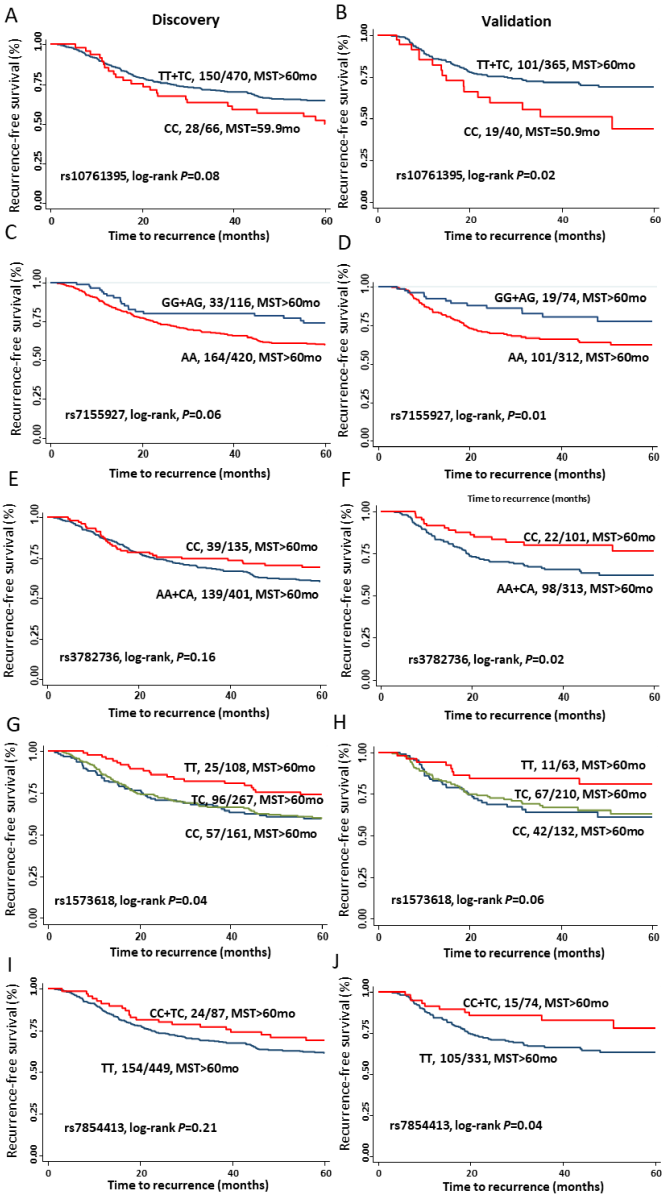
Supplemental Figure S3. Workflow of *in vitro* T cell killing assay.

Schematic flowchart of the image-based phenotypic assay to assess the level of T cell cytotoxicity in peripheral blood mononuclear cells (PBMCs) obtained from healthy low (0 or 1) and high (≥3) unfavorable genotype (UFG) carriers.



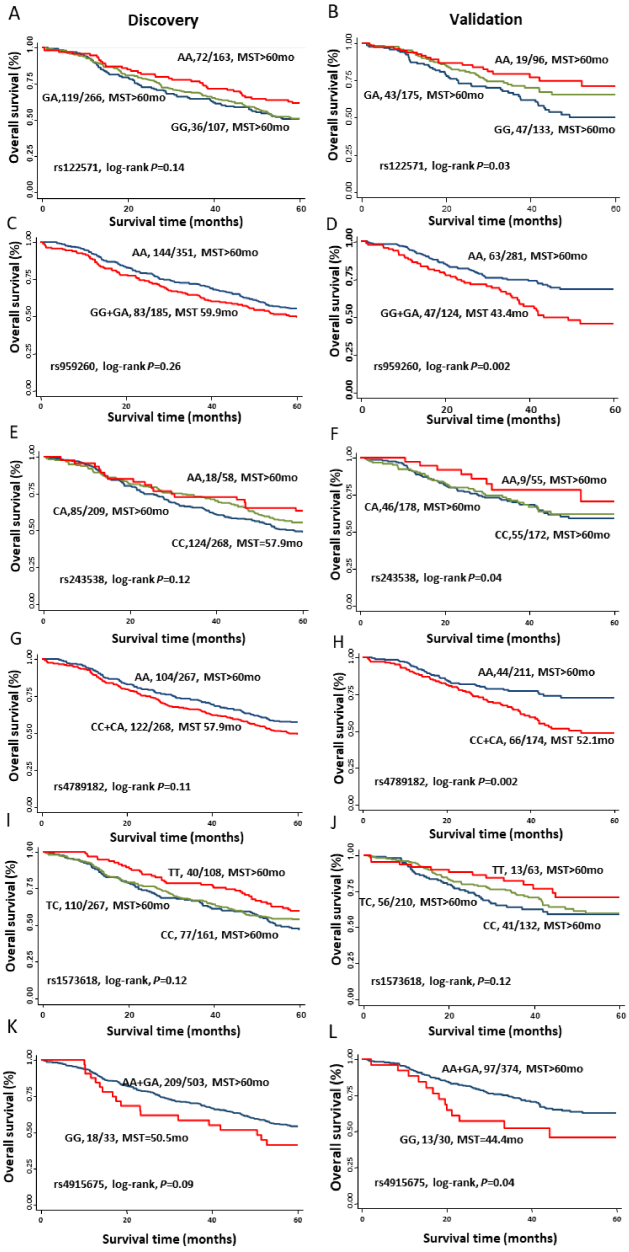
Supplemental Figure S4. Gating strategy and time course of T cell killing by effector-to-target cell ratio.

(**A**) Single leukocytes were obtained by gating on FSC-A versus FSC-H, followed by SSC-A versus SSC-H. Dead cells were excluded by SYTOX Blue staining, and CD3+ T cells were identified and sorted. Data were analyzed using FlowJo (v.10.0.8) software. (**B**) The time course of T cell cytotoxicity by effector-to-target cell (E:T) ratios for A549 and H460 cell lines indicates a dose-response trend.

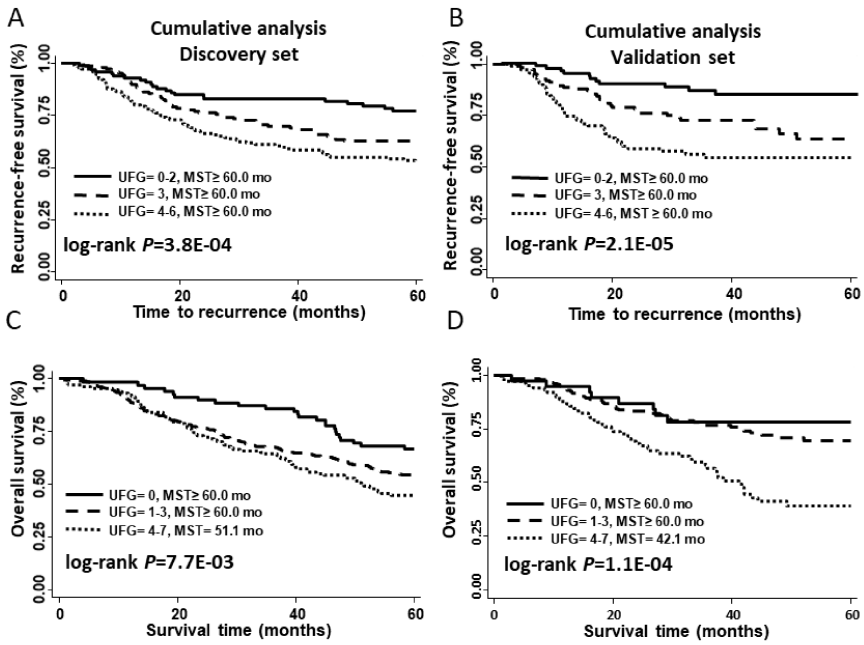


Supplemental Figure S5. Kaplan-Meier analyses of recurrence-free survival (RFS) for patients with early-stage NSCLC by genotypes of SNPs predictive of recurrence risk in the discovery and validation phases.

RFS of patients in the discovery and validation sets by genotypes of *SYK*:rs10761395 (**A** and **B**, respectively), *TRA*:rs7155927 (**C** and **D**), *CD4*:rs3782736 (**E** and **F**), *TRB*:rs1573618 (**G** and **H**), and *PDCD1LG2*:rs7854413 (**I** and **J**).). For all genetic models, blue line includes homozygous wildtype allele carriers; red line includes homozygous variant allele carriers; and green line indicates heterozygous allele carriers. MST, median recurrence-free survival time.

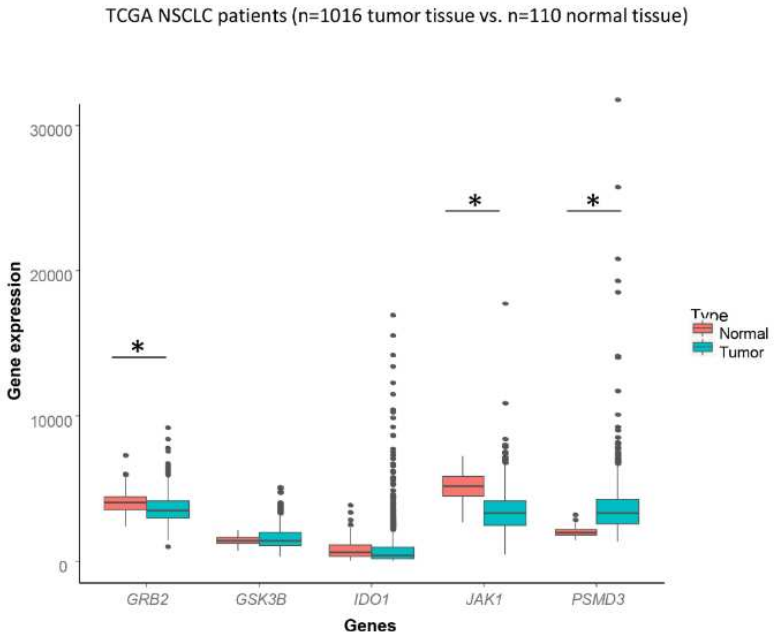


Supplemental Figure S6. Kaplan-Meier analyses of overall survival for patients with early-stage NSCLC by genotypes of SNPs predictive of death risk in the discovery and validation phases.
Overall survival of patients in the discovery and validation sets by genotypes of *CUL1*:rs122571 (**A** and **B**), *GRB2*:959260 (**C** and **D**), *CUL1*:rs243538 (**E** and **F**), *GRB2*:rs4789182 (**G** and **H**), *TRB*:1573618 (**I** and **J**), and *JAK1*:rs4915675 (**K** and **L**). For all genetic models, blue line includes homozygous wildtype allele carriers; red line includes homozygous variant allele carriers; and green line indicates heterozygous allele carriers. MST, median survival time.



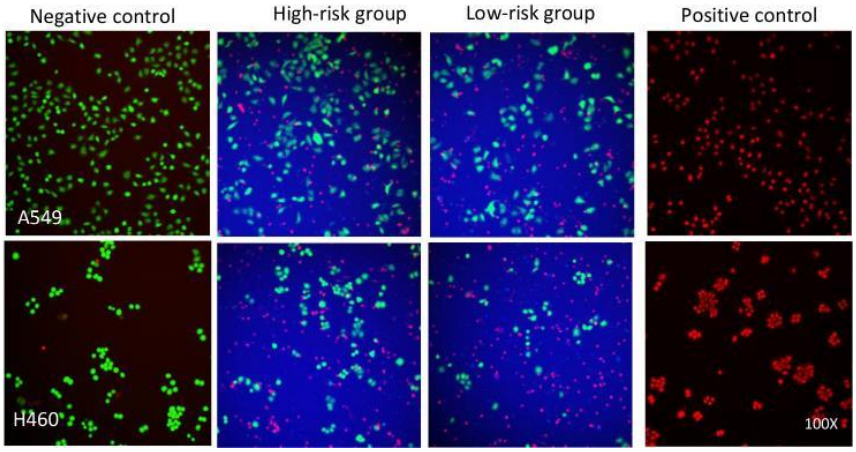
Supplemental Figure S7: Kaplan Meier analyses of combined unfavorable genotypes (UFGs) associated with recurrence and death risks

(**A** and **B**) Recurrence-free survival by number of UFGs for 7 SNPs associated with NSCLC recurrence in the discovery and validation sets. SNPs associated with recurrence are *TRB*:rs1964986, *IL2RB*:rs3218339, *SYK*:rs10761395, *TRA*:rs7155927, *CD4*:rs3782736, *TRB*: rs1573618, and *PDCD1LG2*:rs7854413. (**C** and **D**) Overall survival by number of UFGs for 7 SNPs associated with death risk in the discovery and validation sets. SNPs associated with survival are *IDO1*:rs10108662, *CUL1*:rs122571, *GRB2*:rs959260, *CUL1*:rs243538, *GRB2*:rs4789182, *TRB*:rs1573618, *JAK1*:rs4915675.



Supplemental Figure S8. Expressions of candidate T cell cancer immune response genes in NSCLC tumor and normal tissues from TCGA data.

The expressions of the identified eQTL SNP–located genes in tumor (N=1016) and normal tissues (N=110) were assessed in NSCLC (lung adenocarcinoma and squamous cell carcinoma) datasets using level 3 RSEM normalized mRNA sequencing data from The Cancer Genome Atlas (TCGA). *GRB2* and *JAK1* expressions were significantly lower in tumor tissues (P<0.01), whereas *PSMD3* expression was significantly higher in tumor tissues (P<0.01). *GSK3B* and *IDO1* expression in tumor and normal tissues did not differ significantly with the latter showing marginal significance (P=0.11). * P<0.05.



Supplemental Figure S9. Unfavorable genotypes (UFGs) in the T cell cancer immune response and T cell cytotoxicity *in vitro*.

We used an image-based *in vitro* cell-based assay to assess T cell cytotoxicity against NSCLC cells. Representative images of the two NSCLC cell lines used as cell targets (A459 [top row] and H460 [bottom row]) are shown. Green fluorescence indicates live cancer cells; red fluorescence indicates dead cancer cells; and blue fluorescence indicates T cells isolated from peripheral blood of human subjects. NSCLC cells without the addition of T cells were used as negative control (first column), and 70% ethanol–treated cancer cells were used as positive control (fourth column). Representative images of T cell cytotoxicity from high-risk group (≥3 UFGs) (second column) and low-risk group (0 or 1 UFG) (third column) are displayed.