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Supplementary Materials for

Adenoviral-based vaccine promotes neoantigen-specific CD8$^+$ T cell stemness and tumor rejection

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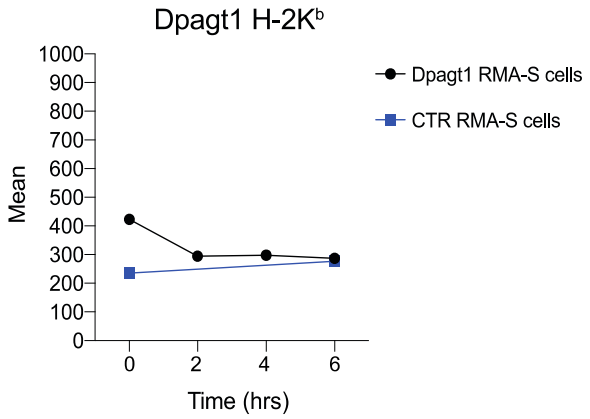
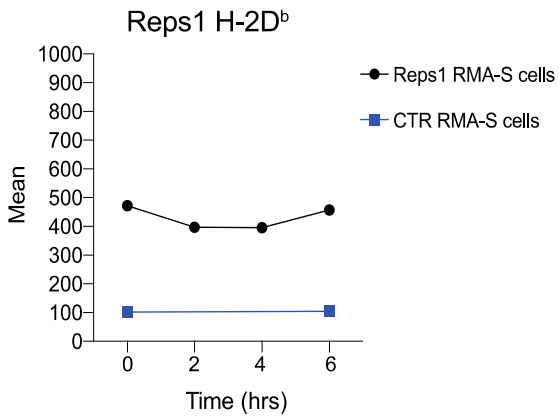
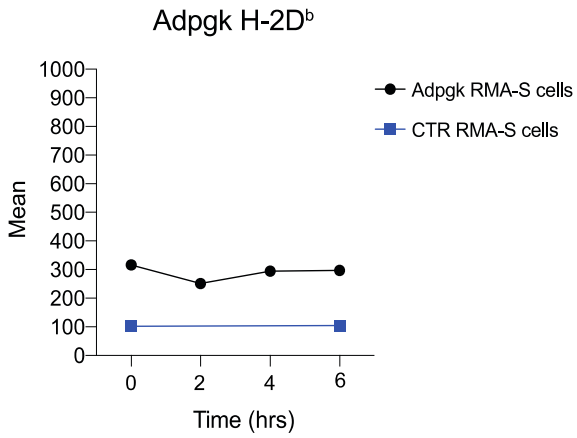
The PDF file includes:

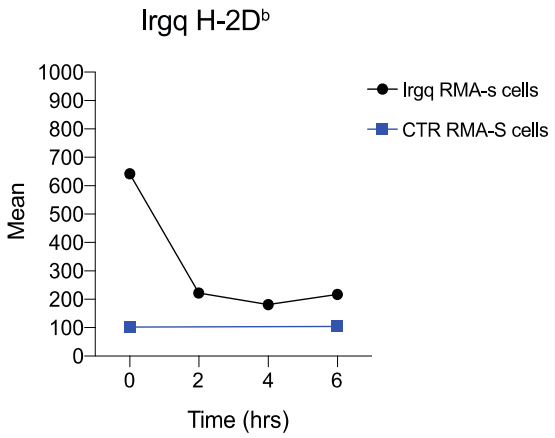
Other Supplementary Material for this manuscript includes the following:

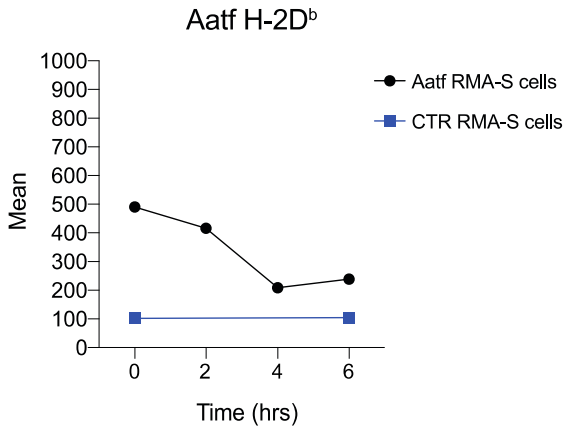
Supplementary figures











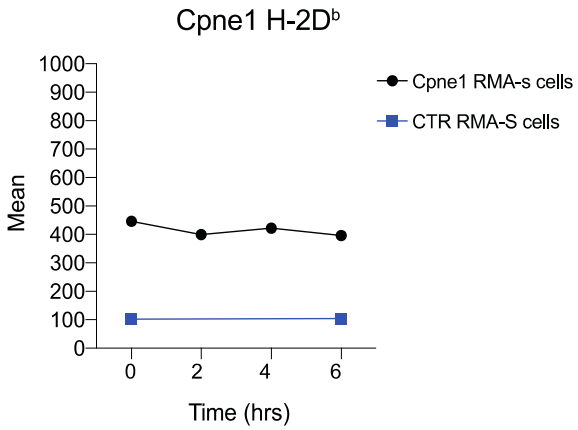


Fig. S1. Kinetics of MHC-I (major histocompatibility complex I)-peptide affinity and stability were evaluated over time. Dpagt1, Reps1, Adpgk, Irgq, Aatf and Cpne1 were analyzed. Mean of two independent experiments is shown.

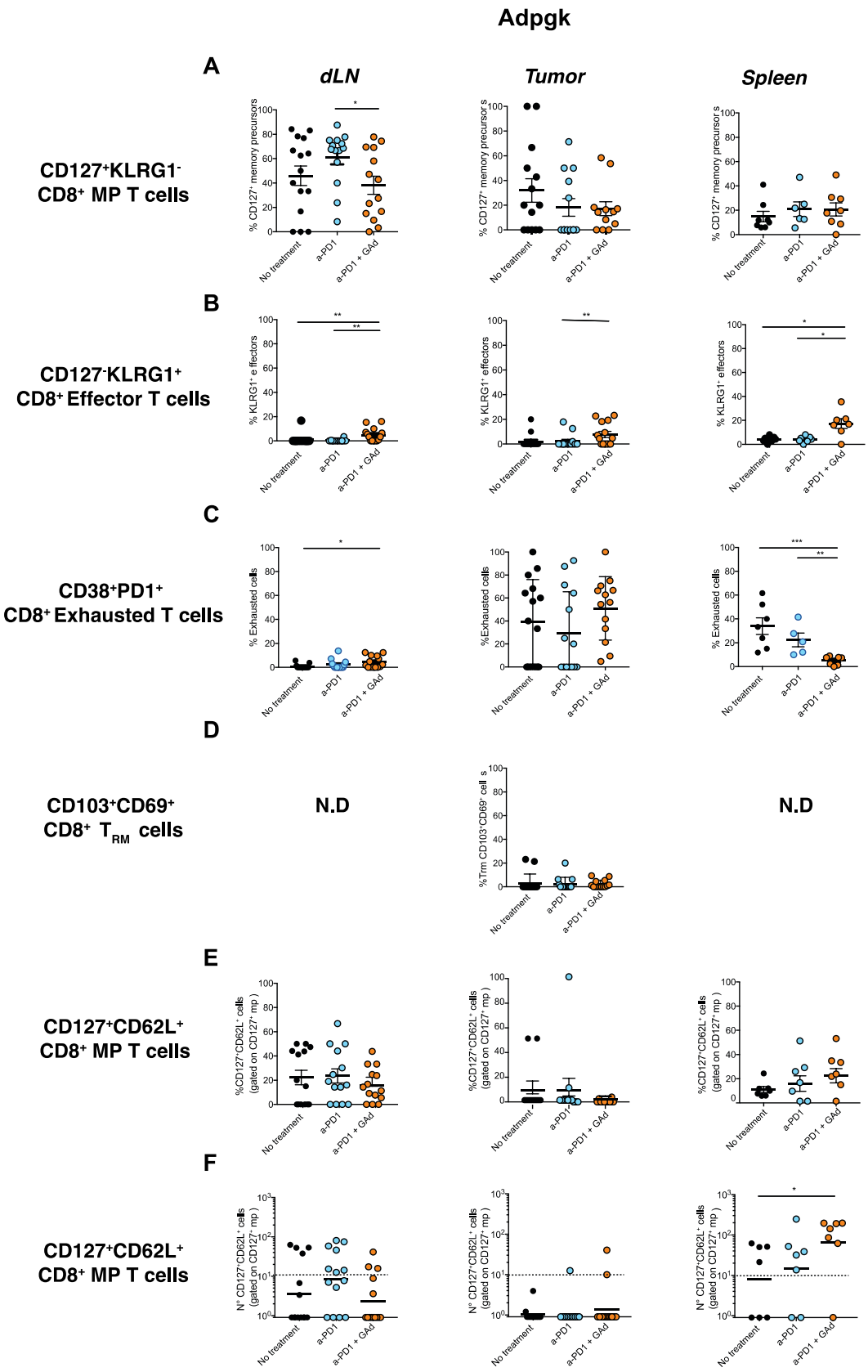

Adpgk
A
dLN
Tumor
Spleen
CD127+KLRG1-
CD8+ MP T cells
% CD127+ memory precursors
B
CD127-KLRG1+
CD8+ Effector T cells
% KLRG1+ effectors
C
CD38+PD1+
CD8+ Exhausted T cells
% Exhausted cells
%Exhausted cells
D
CD103+CD69+
CD8+ TRM cells
N.D
%Trm CD103+CD69+ cells
E
CD127+CD62L+
CD8+ MP T cells
%CD127+CD62L+ cells (gated on CD127+ mp)
F
CD127+CD62L+
CD8+ MP T cells
N° CD127+CD62L+ cells (gated on CD127+ mp)
No treatment
a-PD1
a-PD1 + GAd

Fig. S2. Analysis of neoepitope specific D^b-Adpgk$^+$ $CD8^+$ T cell differentiation following GAd combined with αPD-1 treatment in tumor-bearing mice.

(**A**-**E**) Percentages of memory precursor (**A**), effector (**B**), exhausted (**C**), T_{RM} (**D**) (not determined = N.D) in draining lymph nodes and spleens, $CD127^+CD62L^+$ memory precursors cells (**E**) gated on D^b-Adpgk$^+$ $CD8^+$ T cells were measured in draining lymph nodes, tumors and spleens of tumor-bearing mice. **(F)** Numbers of $CD127^+CD62L^+$ memory precursors on gated D^b-Adpgk$^+$ $CD8^+$ T cells were measured in draining lymph nodes, tumors and spleens of tumor-bearing mice. Data are shown as mean with SEM in (**A**-**E**) and as geometric mean in (**F**). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ (Mann-Whitney test). Graphs are representative of six experiments with seven mice per group.

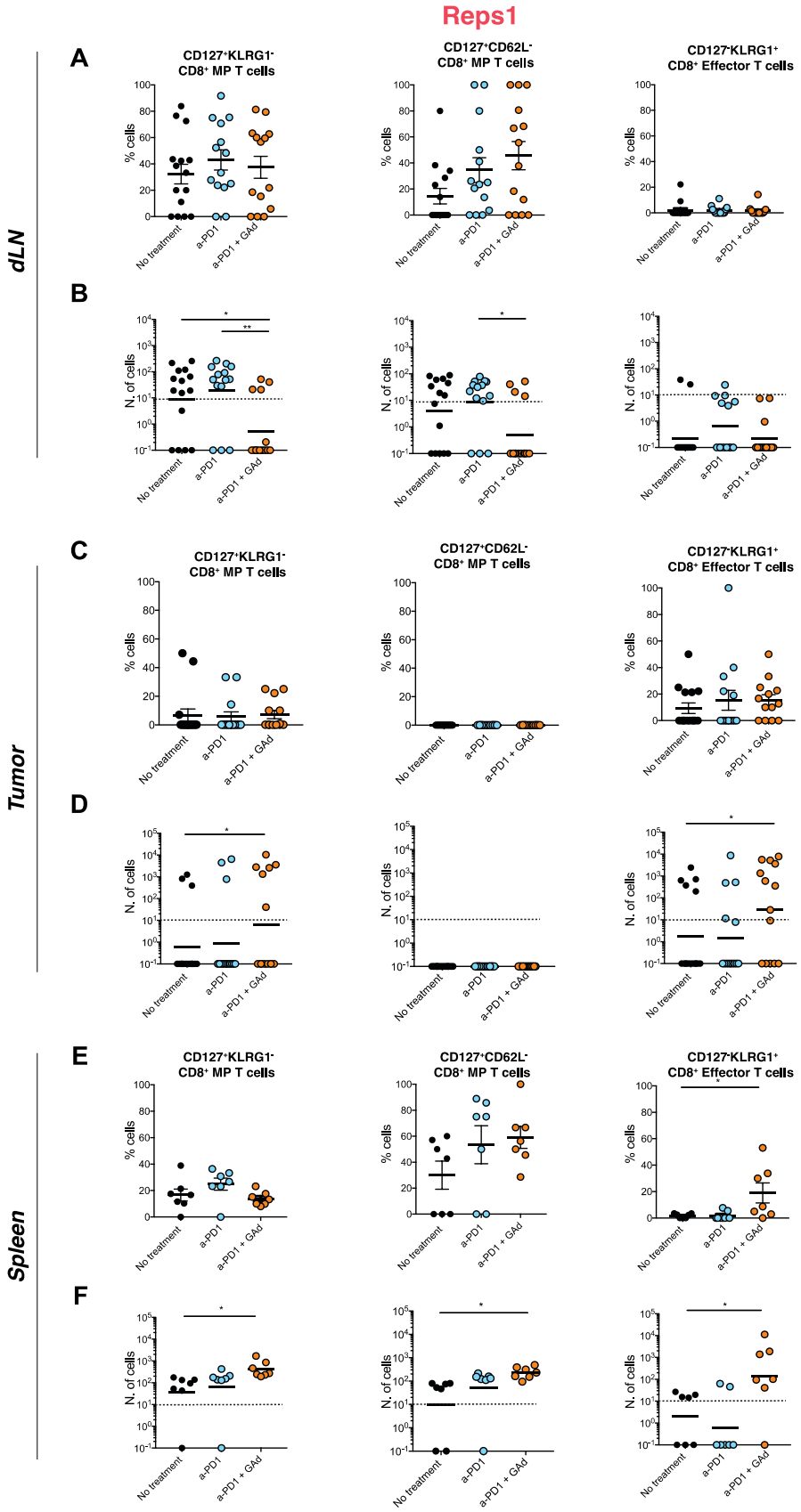

Reps1
dLN
Tumor
Spleen
A
B
C
D
E
F
CD127+KLRG1-
CD8+ MP T cells
CD127+CD62L-
CD8+ MP T cells
CD127-KLRG1+
CD8+ Effector T cells
% cells
N. of cells
No treatment
a-PD1
a-PD1 + GAd

Fig. S3. Analysis of neoepitope specific D^b-Reps1$^+$ CD8$^+$ T cell differentiation following GAd combined with αPD-1 treatment in tumor-bearing mice.

Percentages (**A**) and numbers (N.) (**B**) of CD127$^+$ memory precursor, CD127$^+$CD62L$^+$ central memory precursor, and KLRG1$^+$ effector cells gated on D^b-Reps1$^+$ CD8$^+$ T cells were measured in draining lymph node of tumor-bearing mice. Percentages (**C**) and numbers (**D**) of CD127$^+$ memory precursor, CD127$^+$CD62L$^+$ central memory precursor, and KLRG1$^+$effector cells gated on D^b-Reps1$^+$ CD8$^+$ T cells were measured in tumors. Percentages (**E**) and numbers (**F**) of CD127$^+$ memory precursors, CD127$^+$CD62L$^+$ central memory precursors, and KLRG1$^+$effectors on gated D^b-Reps1$^+$ CD8$^+$ T cells were measured in spleen of tumor-bearing mice. Percentages are shown as mean with SEM and cell numbers are shown as geometric mean .*= $P < 0.05$, **= $P < 0.01$, *** = $P < 0.001$ (Mann-Whitney test). Not detected (N.D.). Graphs are representative of six experiments with seven mice per group.

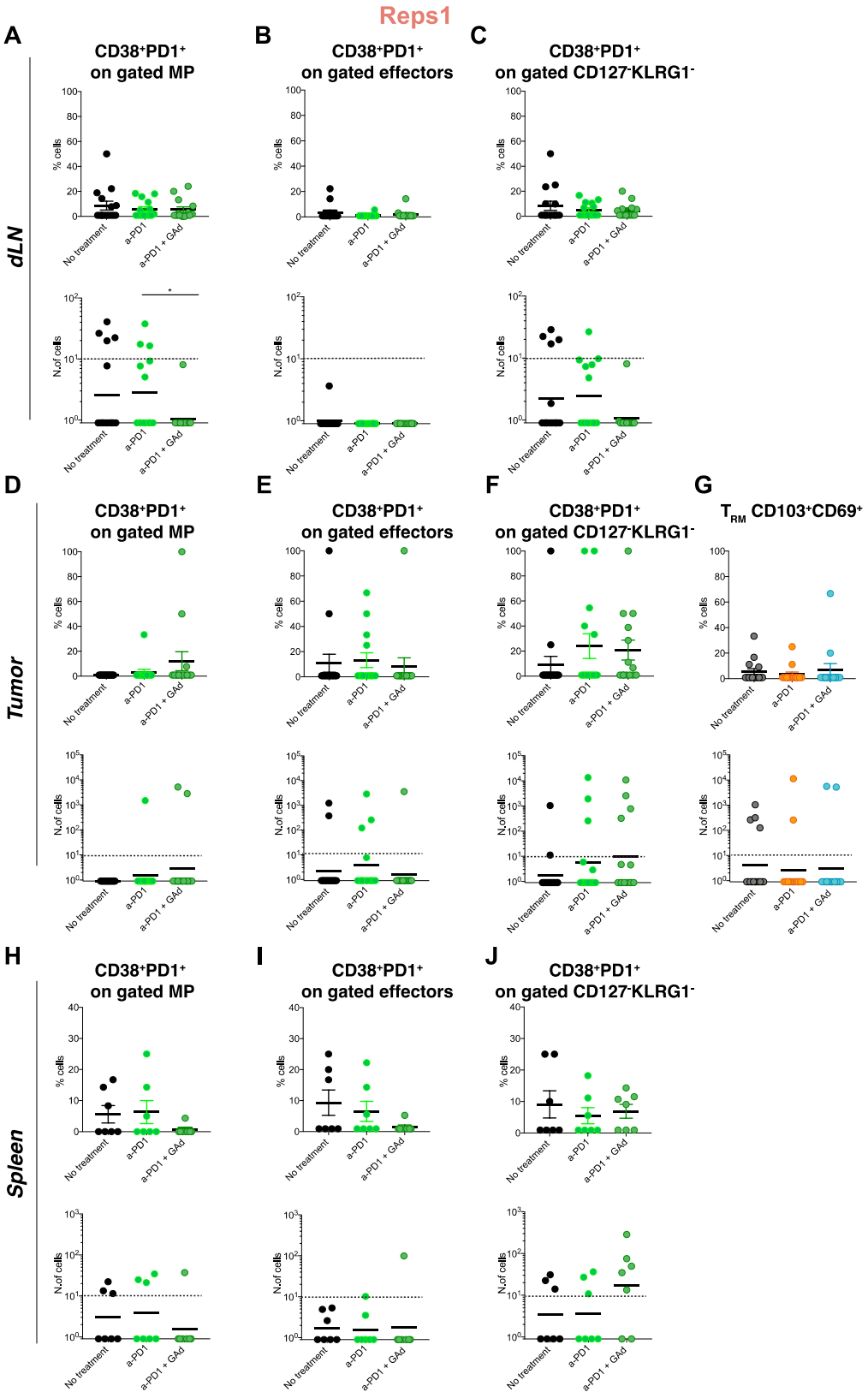

Reps1
A
CD38+PD1+
on gated MP
B
CD38+PD1+
on gated effectors
C
CD38+PD1+
on gated CD127-KLRG1-
dLN
D
CD38+PD1+
on gated MP
E
CD38+PD1+
on gated effectors
F
CD38+PD1+
on gated CD127-KLRG1-
G
TRM CD103+CD69+
Tumor
H
CD38+PD1+
on gated MP
I
CD38+PD1+
on gated effectors
J
CD38+PD1+
on gated CD127-KLRG1-
Spleen
% cells
N.of cells
No treatment
a-PD1
a-PD1 + GAd
*

Fig. S4. Analysis of neoepitope specific D^b-Reps1$^+$ $CD8^+$ T cell differentiation following GAd combined with αPD-1 treatment in tumor-bearing mice.

(**A-C**) Percentages (top row) and numbers (N.) (bottom row) of $CD38^+PD1^+$ cells gated on memory precursor (**A**), on effector (**B**) and on $CD127^-KLRG1^-$ (**C**) D^b-Reps1$^+$ $CD8^+$ T cells were measured in draining lymph node. (**D-F**) Percentages (top row) and numbers (bottom row) of $CD38^+PD1^+$ cells gated on memory precursor (**D**), on effector (**E**) and on $CD127^-KLRG1^-$ (**F**) D^b-Reps1$^+$ $CD8^+$ T cells were measured in tumors. (**G**) Percentages (top row) and numbers (bottom row) of $CD103^+CD69^+$ D^b-Reps1$^+$ $CD8^+$ T_{RM} T cells were measured in tumors. (**H-J**) Percentages (top row) and numbers (bottom row) of $CD38^+PD1^+$ cells gated on memory precursor (**H**), on effector (**I**) and on $CD127^-KLRG1^-$ **(J)** D^b-Reps1$^+$ $CD8^+$ T cells were measured in spleen. Percentages are shown as mean with SEM and cell numbers are shown as geometric mean. *= $P < 0.05$, **= $P < 0.01$, *** = $P < 0.001$ (Mann-Whitney test). Graphs are representative of six experiments with seven mice per group.

D'Alise et al., Supplementary Figure S5

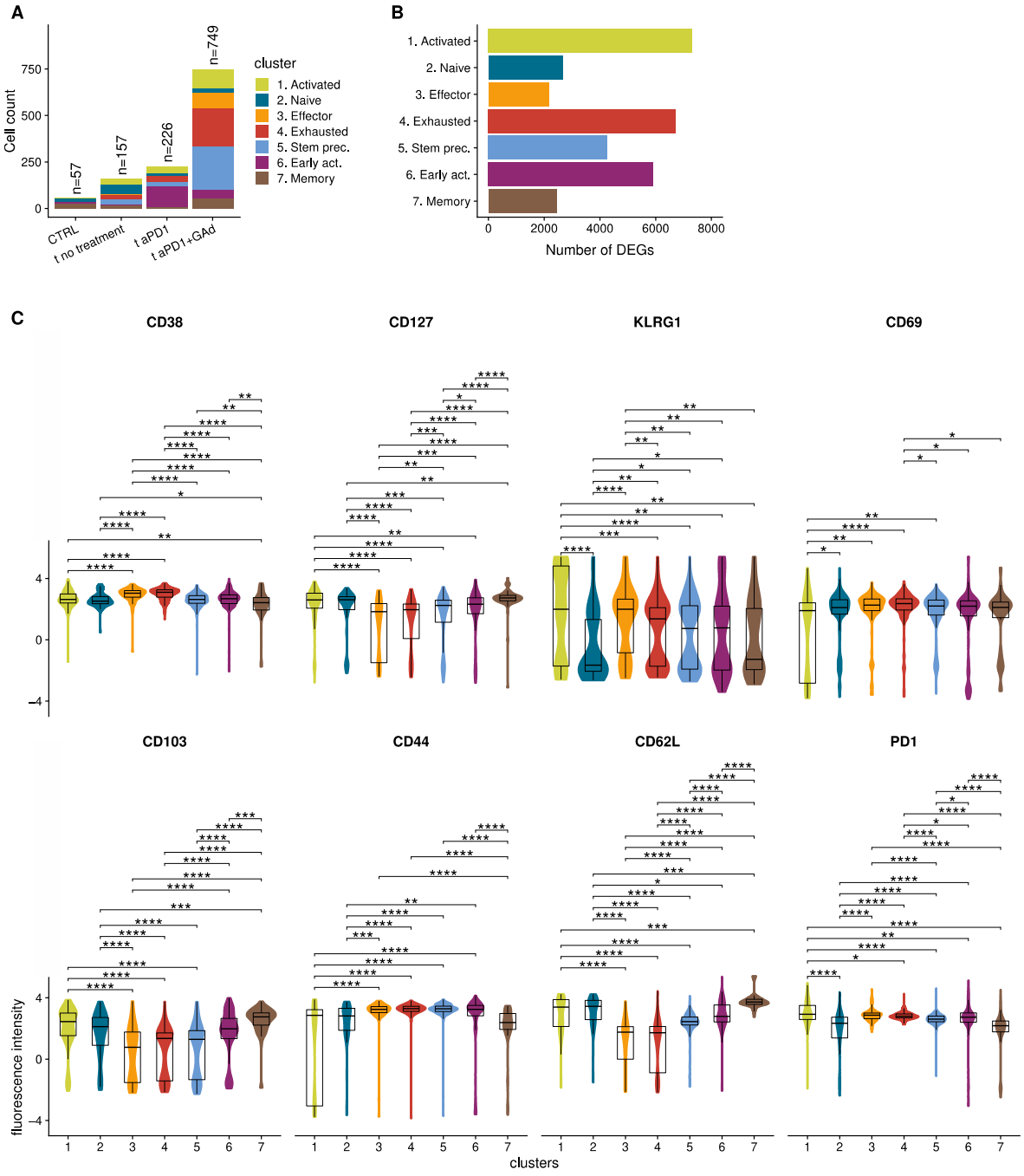


Fig. S5. scRNA-seq analysis of Adpgk specific CD8$^+$ T cells from treated mice. (**A**) Distribution of D^b-Adpgk$^+$ CD8$^+$ T cells in each experimental condition (CTRL= mice without tumor and without treatment, T no treatment = untreated tumor-bearing mice, T αPD1= tumor-bearing mice treated with αPD1 and T αPD1+GAd= tumor-bearing mice treated with αPD1+ GAd vaccine) color coded by cluster. (**B**) Differentially expressed genes for each cluster. (**C**) The geometric Mean Fluorescence Intensity (gMFI) values obtained from FACS sorting were projected onto cluster data, shown as $\log_{10}(x)$ and $-\log_{10}(x)$ of raw positive and negative values, respectively.

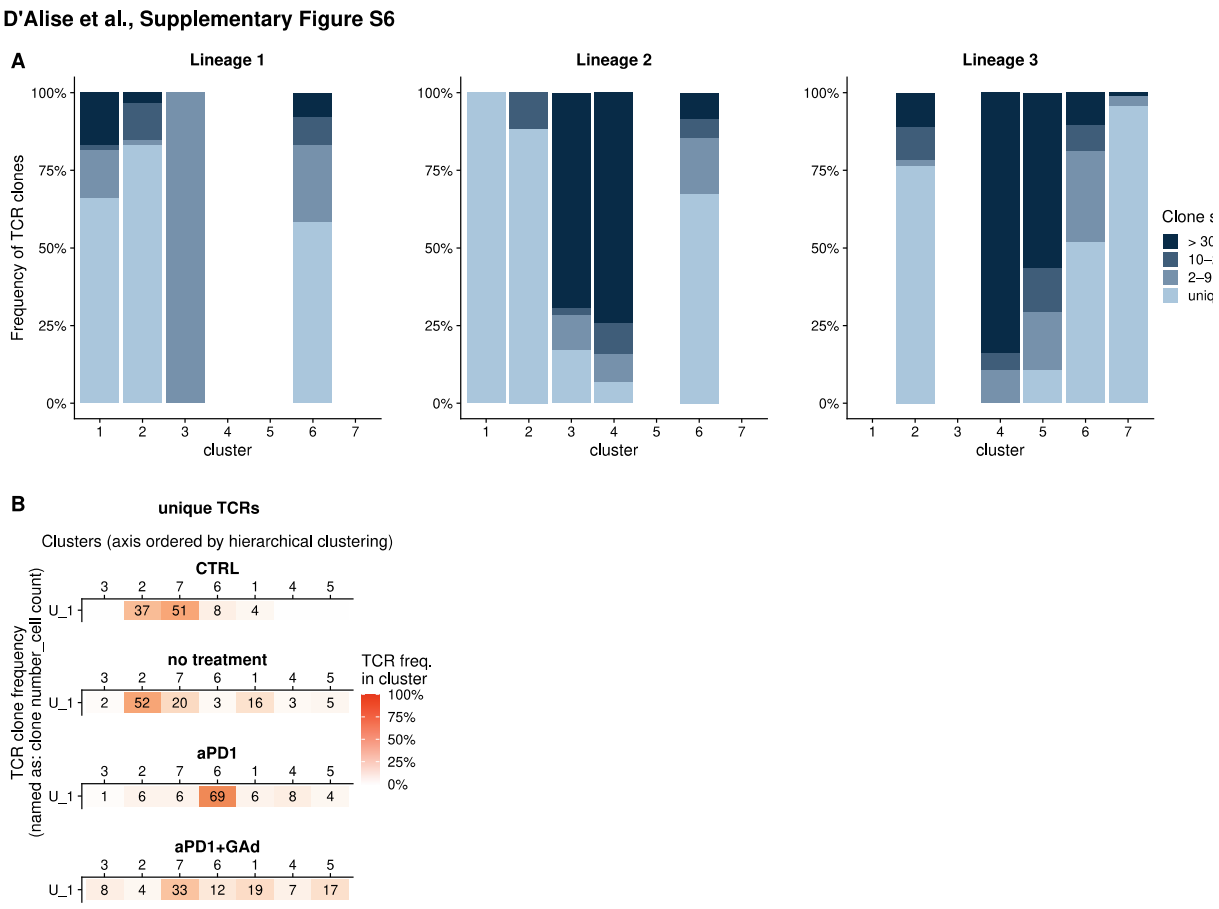


Fig. S6. scTCR analysis of Adpgk specific CD8^{+} T cells. (**A**) Distribution of TCR clonotypes in the different lineages. (**B**) Frequencies of unique clonotypes across clusters.

Table S1: Baseline demographics and disease characteristics.

	Dose 1 (N=3)	**Dose 2 (N=9)**	**Total (N=12)**
Male, N (%)	3 (100)	5 (56)	8 (67)
Age mean (range)	53.7 (31-74)	57.7 (27-79)	56.7 (27-79)
ECOG PS, N (%)			
0	2 (67)	3 (33)	5 (42)
1	1 (33)	6 (67)	7 (58)
Cancer Type, N (%)			
Colorectal	2 (67)	6 (67)	8 (67)
Gastric	0 (0)	3 (33)	3 (25)
Gastro-esophageal junction	1 (33)	0 (0)	1 (8)
Line of therapy, N (%)			
1L	0 (0)	6 (67)	6 (50)
2L	3 (100)	3 (33)	6 (50)
Metastases			
Liver	2 (67)	2 (22)	4 (33)
Peritoneal	0 (0)	6 (67)	6 (50)
Genetic alterations			
Lynch syndrome carrier, N (%)	0 (0)	2 (22)	2 (17)
Tumor burden			
Target lesion sum at baseline, mm, mean (range)	67.3 (44-111)	65.4 (26-108)	65.9 (26-111)

Table S2: List of FSPs detected in patient 1 (Excel file)