Supplementary Data

Title

Single-cell transcriptome analysis reveals gene signatures associated with T-cell persistence following adoptive cell therapy

Authors

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Supplementary Table S1

Persistence_Up	Persistence_Down
KLRB1	FAM3C
ZNF683	CXCR4
ITGB1	GOLIM4
C1orf162	LAIR1
IL7R	CD70
LIME1	HSPA1A
S1PR1	CD86
TIMP1	ACP5
C10orf54	FILIP1L
TBXAS1	CSF1
KLF2	CD59
LTB	CST7
UBXN11	PLEK
CD40LG	ITGAX
AMICA1	SH2D1A
FAM65B	FABP5
VCL	PGD
RASA3	FASLG
SCML4	EOMES
MYC	SLC1A5
P2RY8	AFAP1L2
CDC25B	NDFIP2
TSPAN32	SIX1
SYNJ2	GZMB
RASGRP2	NCAM1
PXN	MIR155HG
FAM102A	GZMH
ADD3	CDKN2A
TMEM63A	ACTN1
TSC22D3	LYST
AQP3	WNT11
SH3BP5	CCL3
TMEM123	LDOC1
GLUL	CD5
POU2F2	CD6
MT1F	GCNT1
C11orf21	CEBPD
BIN1	SOX4
	SKAP2
	LAIR2
	GZMK

Supplementary Table S3

9.1-NP vs 9.2-P

Gene Name	$\log_2$FC	Known Gene Function
KLRB1	3.432	A member of the C-type lectin superfamily expressed by NK cells. May be involved in the regulation of NK cell function.
ZNF683	2.712	A transcription factor that mediates a transcriptional program in tissue-resident memory T cells, natural killer cells and natural killer T cells
ITGB1	1.973	A member of integrin family involved in cell adhesion and recognition in a variety of processes including embryogenesis, hemostasis, tissue repair, immune response and metastatic diffusion of tumor cells
C1orf162	1.641	Unknown function
IL7R	1.307	A receptor for interleukin 7 (IL-7). Essential for the normal development and homeostasis of T cells
LIME1	1.257	A transmembrane adaptor protein that links the T and B-cell receptor stimulation to downstream signaling pathways via its association with the Src family kinases Lck and Lyn, respectively
S1PR1	1.224	A receptor for the lysosphingolipid sphingosine-1-phosphate (S1P). Required for the egress of T cells from the thymus or lymphoid organs
TIMP1	1.218	A member of the natural inhibitors of the matrix metalloproteinases (MMPs)
C10orf54	1.192	V-domain Ig suppressor of T cell activation (VISTA), which is an immune checkpoint molecule
TBXAS1	1.171	Thromboxane synthase, a cytochrome P450 enzyme, catalyzes the conversion of the prostaglandin endoperoxide into thromboxane A2, a potent vasoconstrictor and inducer of platelet aggregation
GZMK	-3.475	A member of related serine proteases from the cytoplasmic granules of cytotoxic lymphocytes
LAIR2	-2.411	A member of the immunoglobulin superfamily. A soluble receptor that may play roles in both inhibition of collagen-induced platelet aggregation and vessel formation during placental implantation
SKAP2	-1.949	A substrate of Src family kinases. May play a role in regulating proper activation of the immune system
CCDC50	-1.948	A soluble, cytoplasmic, tyrosine-phosphorylated protein with multiple ubiquitin-interacting domains. Mutations in this gene cause nonsyndromic, postlingual, progressive sensorineural DFNA44 hearing loss
SOX4	-1.890	A member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and in the determination of the cell fate. Required for pro-B-cell survival
CEBPD	-1.787	A bZIP transcription factor. Important in the regulation of genes involved in inflammatory responses and the activation/differentiation of macrophages
GCNT1	-1.741	A member of the beta-1,6-N-acetylglucosaminyltransferase gene family. Essential to the formation of Gal beta 1-3(GlcNAc beta 1-6)GalNAc structures and the core 2 O-glycan branch
CD6	-1.705	Cell adhesion molecule that may mediate cell-cell contacts and regulate T-cell responses
CD5	-1.696	A member of the scavenger receptor cysteine-rich (SRCR) superfamily. May negatively regulate TCR signaling
TPRG1	-1.547	Tumor Protein P63 Regulated 1. Unknown function

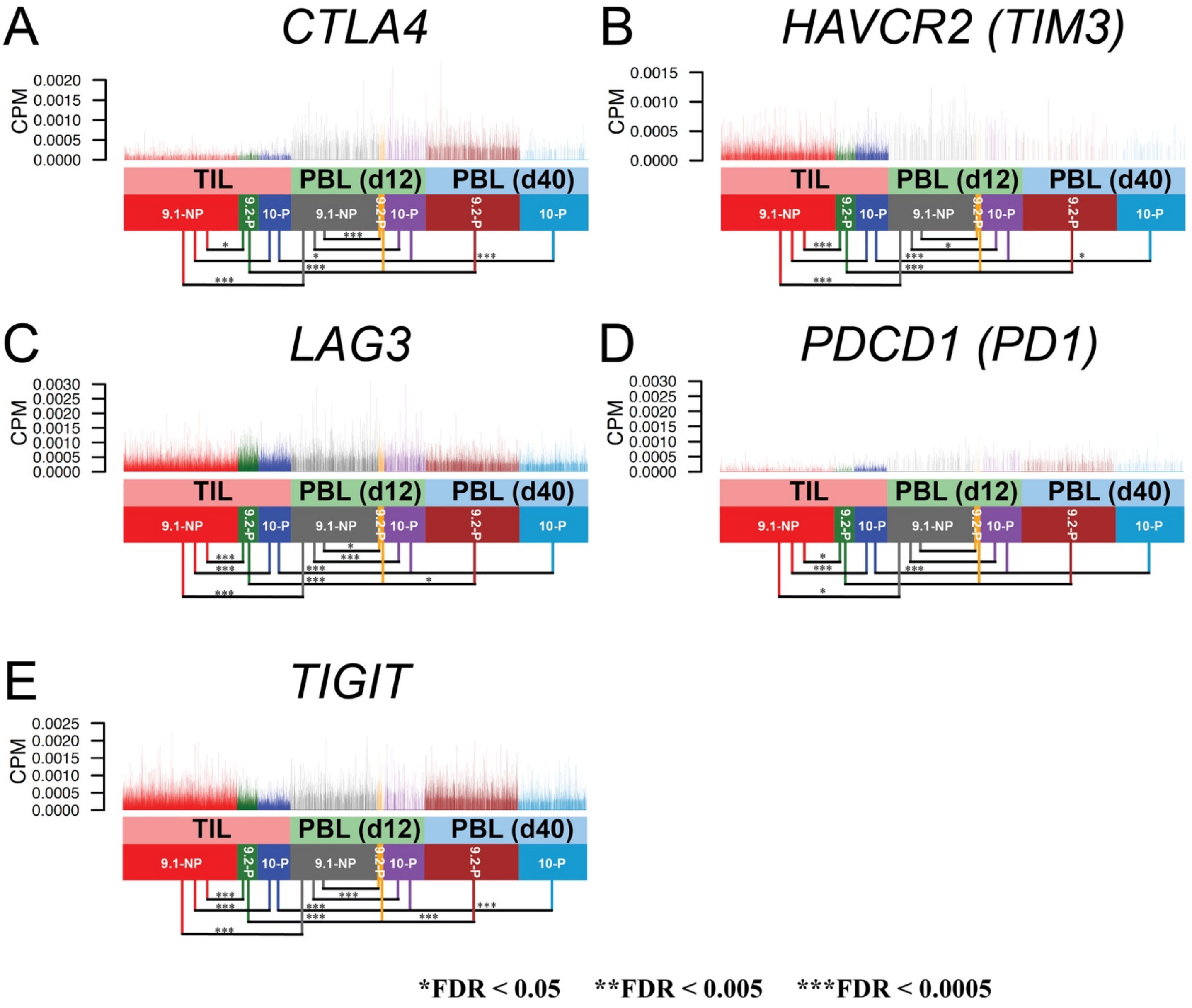
Supplementary Table S4

9.1-NP vs 10-P

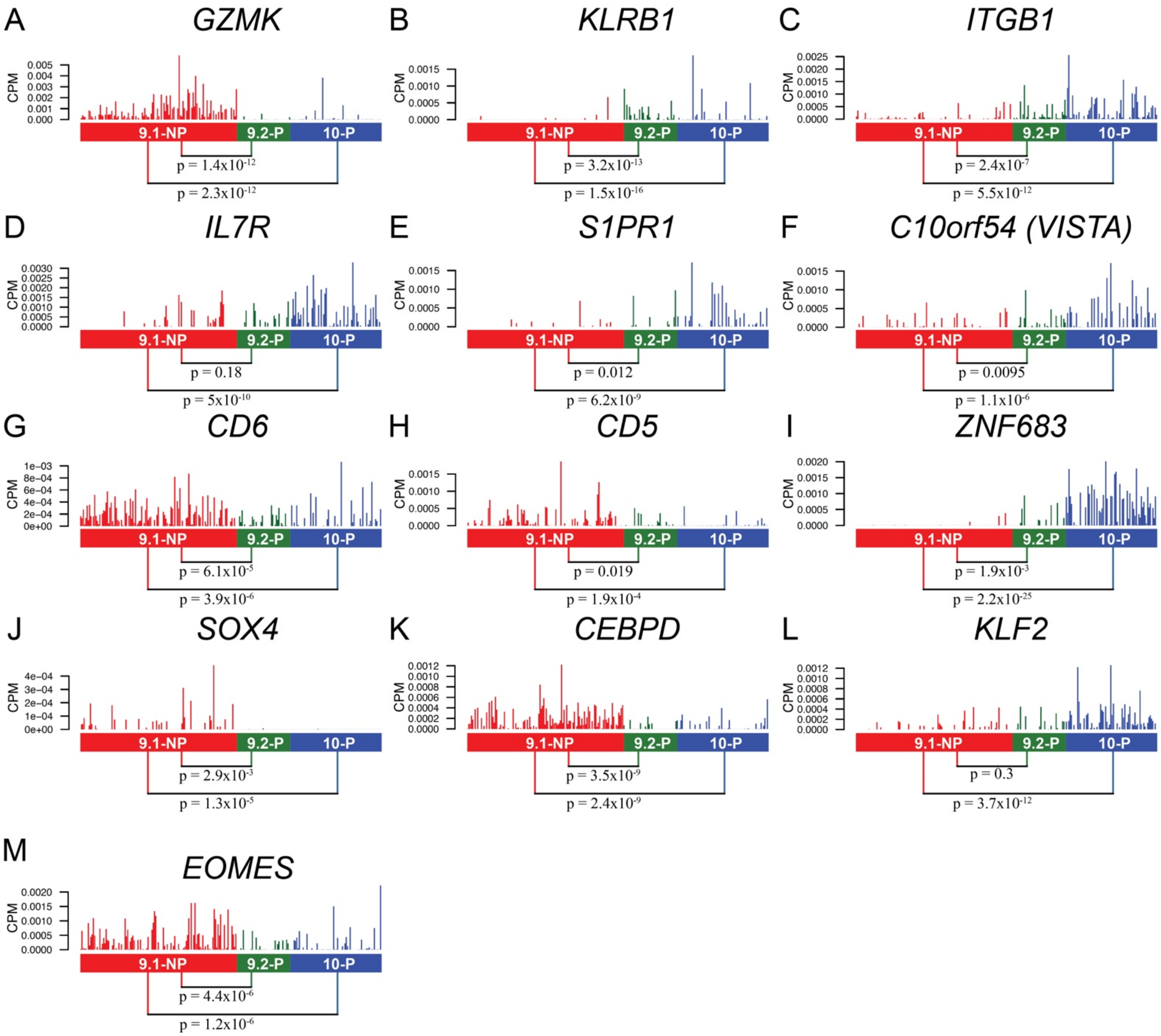
Gene Name	$\log_2$FC	Known Gene Function
ZNF683	3.967	A transcription factor that mediates a transcriptional program in tissue-resident memory T cells, natural killer cells and natural killer T cells
UBXN11	1.837	UBX Domain Protein 11. May serve a bidirectional role in the promotion and inhibition of the Rnd subfamily members of Rho GTPases through upstream signaling pathways.
ITGB1	1.832	A member of integrin family involved in cell adhesion and recognition in a variety of processes including embryogenesis, hemostasis, tissue repair, immune response and metastatic diffusion of tumor cells
ITM2C	1.818	Negative regulator of amyloid-beta peptide production. May inhibit the processing of APP by blocking its access to alpha- and beta-secretase
LIME1	1.784	A transmembrane adaptor protein that links the T and B-cell receptor stimulation to downstream signaling pathways via its association with the Src family kinases Lck and Lyn, respectively
KLRB1	1.688	A member of the C-type lectin superfamily expressed by NK cells. May be involved in the regulation of NK cell function.
KLF2	1.680	A member of Kruppel family of transcription factors. An essential regulator for T-cell migration
S1PR1	1.596	A receptor for the lysosphingolipid sphingosine-1-phosphate (S1P). Required for the egress of T cells from the thymus or lymphoid organs
RASA3	1.506	A protein that binds inositol 1,3,4,5-tetrakisphosphate and stimulates the GTPase activity of Ras p21. Functioned as a negative regulator of the Ras signaling pathway
IL7R	1.505	A receptor for interleukin 7 (IL-7). Essential for the normal development and homeostasis of T cells
GZMK	-3.554	A member of related serine proteases from the cytoplasmic granules of cytotoxic lymphocytes
CCL3	-2.572	Also known as macrophage inflammatory protein 1 alpha. Playing a role in inflammatory responses through binding to the receptors CCR1, CCR4 and CCR5
CEBPD	-2.484	A bZIP transcription factor. Important in the regulation of genes involved in inflammatory responses and the activation/differentiation of macrophages
LAIR2	-2.184	A member of the immunoglobulin superfamily. A soluble receptor that may play roles in both inhibition of collagen-induced platelet aggregation and vessel formation during placental implantation
WNT11	-2.148	A member of the WNT gene family which have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis
CPA5	-1.873	A member of the A/B subfamily of carboxypeptidases, which have functions ranging from digestion of food to selective biosynthesis of neuroendocrine peptides
SKAP2	-1.853	A substrate of Src family kinases. May play a role in regulating proper activation of the immune system
SOX4	-1.739	A member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and in the determination of the cell fate. Required for pro-B-cell survival
LYST	-1.609	A protein that regulates intracellular protein trafficking in endosomes. May be involved in pigmentation
EOMES	-1.575	A transcription factor which is crucial for embryonic development of mesoderm and the central nervous system in vertebrates. May also be necessary for the differentiation of effector CD8$^+$ T cells

Supplementary Table S6

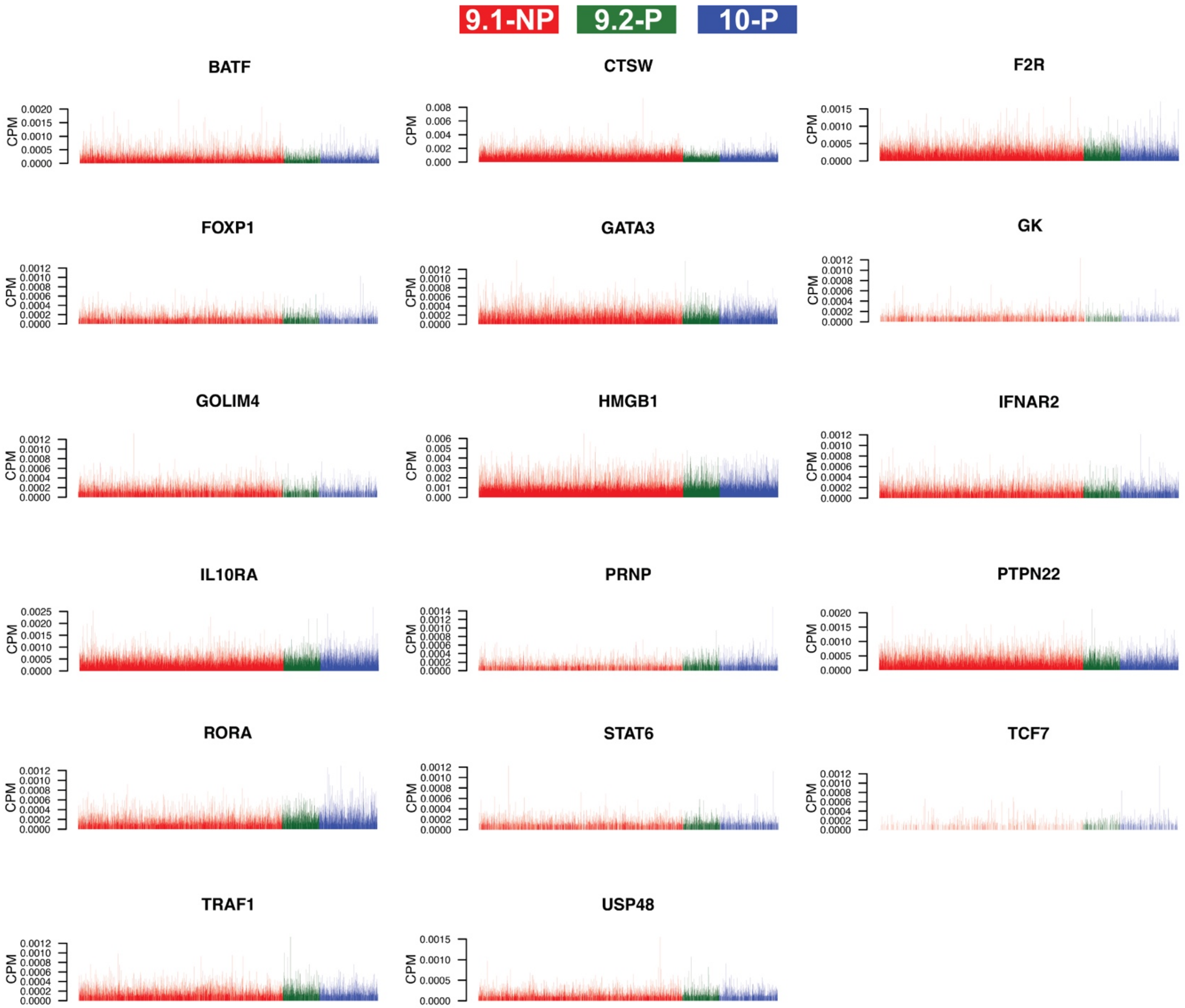
TIL ID	Targeted mutation	% in infused TILs	% in PBL at ~1 month	Ratio (% in 1m PBL / % in TIL)
4007	SKIV2L	64.4	0.93	**0.014**
4069	ZFYVE27	91.9	N.D.	**0**
4071	QSOX2	32.3	0.0014	**0.000043**
4081	ALDOC	72.1	1.40	**0.019**
4095	KRAS	49.5 / 19.1 / 6.9	N.D. / 5.3 / 2.2	**0 / 0.28 / 0.32**



Supplementary Figure S1: The expression of negative co-stimulatory molecules. The single-cell gene expression profiles of clonotypes 9.1-NP, 9.2-P and 10-P were obtained from the same experiments shown in Figure 1 for TIL4095 and Figure 4 for PBL samples. Each vertical line represents a single cell. CPM: counts per million.



Supplementary Figure S2: The gene expression profiles of single cells following Fluidigm C1 single-cell transcriptome analysis. TIL4095 T cells were enriched by KRAS-9mer or KRAS-10mer tetramer and then subjected to a Fluidigm C1 system for the single-cell transcriptome analysis, as describe in the Materials and Methods section. The gene expression of 9.1-NP, 9.2-P and 10-P single cells is shown. Each vertical line represents a single cell. The p-values were calculated by the Wilcoxon signed-rank test. CPM: counts per million.



Supplementary Figure S3: The gene expression profiles of selected memory T cell-related genes. The single-cell gene expression profiles of clonotypes 9.1-NP, 9.2-P and 10-P were obtained from the same experiment shown in Figure 1. The lists of memory T cell-related genes were obtained from Goldrath immune memory gene set and GO T-cell differentiation gene set. Only genes with detectable expression are shown. Significant differences (FDR < 0.05) were observed in CTSW, F2R, FOXP1, GATA3, GOLIM4, HMGB1, PRNP, PTPN22 and RORA genes (table S1). Each vertical line represents a single cell. CPM: counts per million.