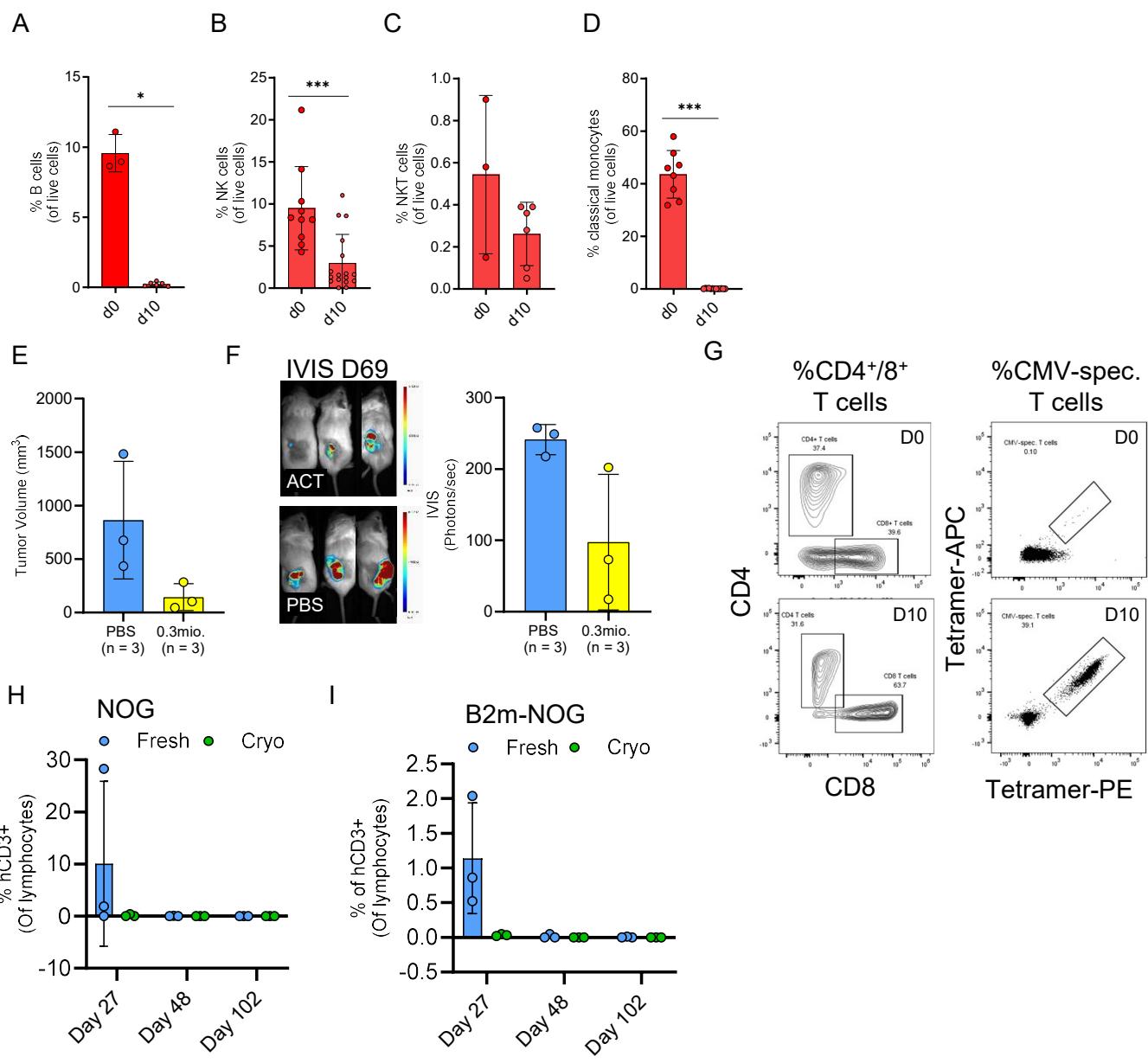
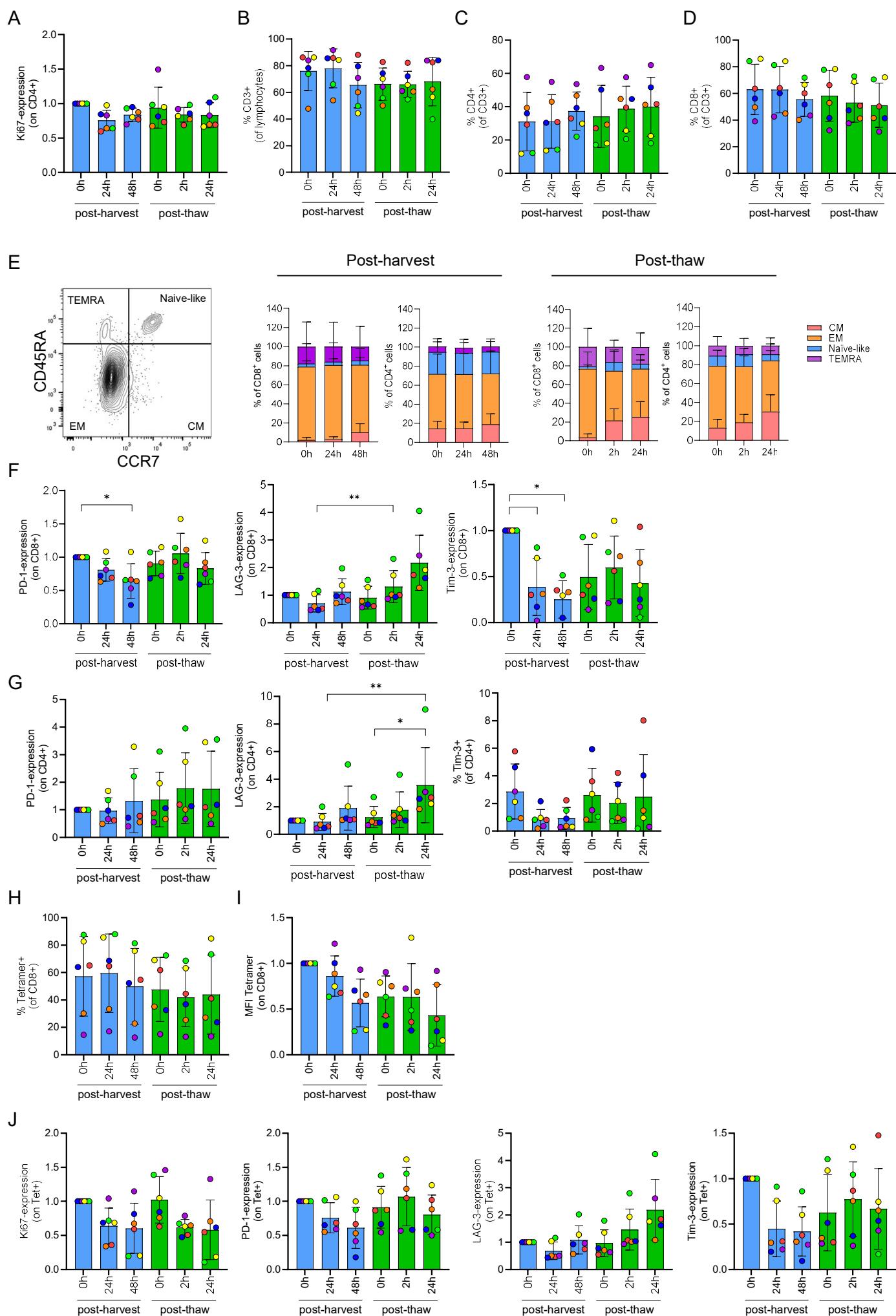


Supplementary Figure S1



Suppl. Figure S1 | Ag-scaffold-expanded T cells engraft and cause GvHD in hIL-2-NOG mice in a dose-dependent manner (A to D) Frequency of (A) B cells, (B) NK cells, (C) NKT cells, and (D) monocytes in the Ag-scaffold-expanded cell product prior to (day 0) and on the day of harvest (day 14). Data are from three to eight individual expansions. *p<0.05, ***p<0.001 (Mann-Whitney). (E and F) Tumor growth monitored over time after ACT, measured by (E) caliper measurements and (F) IVIS following i.p administration of luciferin. Graphs depict the mean+/- SD. (G) Surface expression of CD4⁺ and CD8⁺ and identification of CMV-specific cells by tetramer-staining, prior to expansion (day 0) and on the day of harvest (day 10) following expansion with Ag-scaffolds. (H and I) Human CD3⁺ T cells (hCD3⁺) in blood of (H) NOG or (I) B2m-NOG mice 27, 48 and 102 days, post ACT with (blue) fresh or (green) cryopreserved T cells, respectively.

Supplementary Figure S2

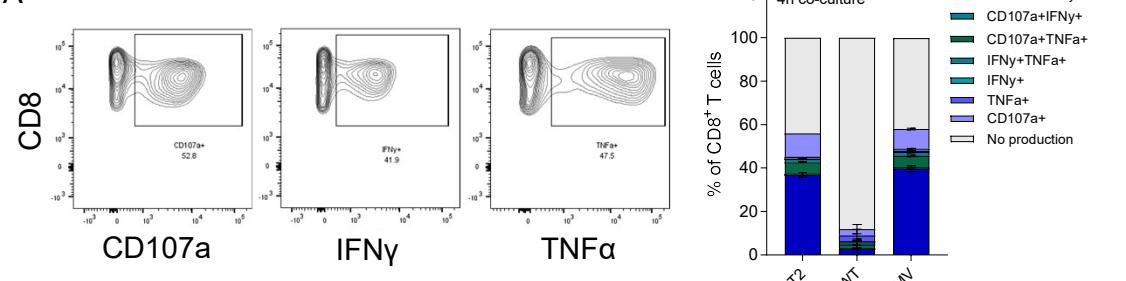


Suppl. Figure S2 | Phenotypic comparison of cryopreserved and/or rested effector T cells

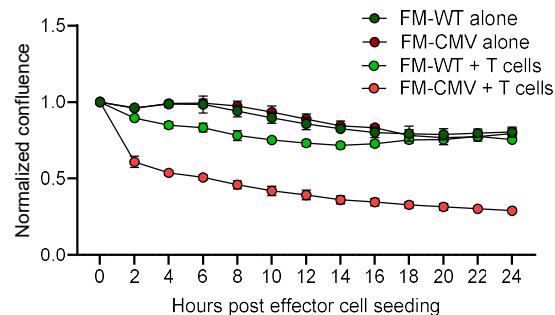
Frequency of (A) Ki67⁺ CD4⁺ T cells, and (B) CD3⁺, (C) CD4⁺, and (D) CD8⁺ T cells in the final cell product at various time points and culturing conditions. Data display the mean +/- SD frequencies of cells. (E) Characterization of the activation status of T cells after culturing under different conditions. The bars depict the mean +/- SD proportion of naïve-like, central memory (CM), effector memory (EM) and effector memory re-expressing CD45RA (TEMRA) cells out of total CD8⁺/CD4⁺ cells. (F and G) Phenotypic assessment of (F) CD8⁺ and (G) CD4⁺ T cells after culturing under different conditions. Data are the mean +/- SD of the mean fluorescence intensity of each marker after normalization to the '0h post-harvest'-sample, or the proportion of marker-positive cells (CD4⁺ cells, Tim-3). (H and I) Quantification of the (H) frequency and (I) mean fluorescent intensity of tetramer₊ antigen-specific cells in the cell product after culture under different conditions. (J) Phenotypic assessment of tetramer⁺ antigen-specific cells after culturing under different conditions. Data are the mean +/- SD of the mean fluorescence intensity of each marker after normalization to the '0h post-harvest'-sample. Each point represent one PBMC sample, analysed across two to three individual experiments. (n=4-6).

Supplementary Figure S3

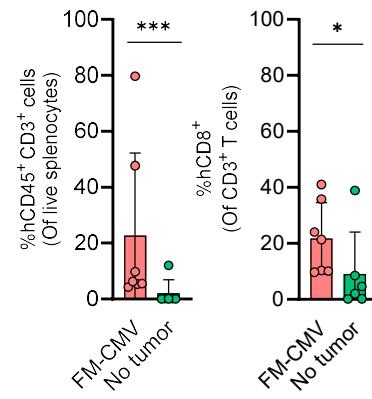
A



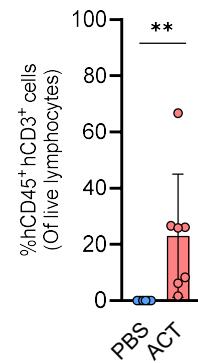
B



C



D



Suppl. Figure S3 | In vitro assessment of CMV-specific T cell functionality (A) Functional assessment by intracellular cytokine (IFNγ and TNFα) production and degranulation (surface CD107a) following co-culture of Ag-scaffold-expanded CMV-specific T cells with FM-CMV, FM-WT or T2 target cells loaded with the relevant antigenic peptide. Bars display the mean +/- SD frequency of cytokine-producing cells. (B) Tumor target killing following co-culture of Ag-scaffold-expanded CMV-specific T cells with FM-CMV, FM-WT or T2 target cells. Graph depicts the tumor target confluence at various time points, with or without addition of effector T cells normalized to the confluence upon addition of T cells. (C and D) Frequency of (C) hCD45⁺ CD3⁺ and CD8⁺ T cells in the spleen or (D) hCD45⁺ CD3⁺ T cells in the tumor, at the end of the experiment. Bars depict the mean +/- SD. *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney)

Supplementary Table S1

Marker	Conjugate	Clone	Vendor	Cat no
CD3	FITC	SK7	BD	345764
CD3	BUV737	SK7	BD	612752
CD3	BV786	SK7	BD	563800
CD8a	BV480	RPA-T8	BD	566121
CD8b	BV421	2ST8.5H7	BD	568373
CD4	BV650	SK3	BD	563875
CD4	PE-Cy7	RPA-T4	BD	560649
hCD45	BV711	HI30	BD	564357
mCD45	BUV395	30F11	BD	567249
CD45RA	BV711	HI100	BD	563733
CCR7	APC	G043H7	Biolegend	353214
Ki67	BV786	B56	BD	564071
LAG3	FITC	17B4	Adipogen	AG-20B-0012F-C100
Tim3	PE-Cy7	F38-2E2	Biolegend	345014
PD-1	BV421	EH12.1	BD	565024
CD19	FITC	4G7	BD	345776
CD16	APC	3G8	Biolegend	302012
CD14	BV480	MO-P9	BD	566141
CD56	BUV737	NCAM16.2	BD	612766
CD1d-alpha-galactosylceramide multimer	PE		TetramerShop	HCD1D-001
pan-TCRgd	BV650	B1	BD	564156
CD107a	PE	H4A3	BD	555801
TNF	PE-Cy7	MAb11	Biolegend	502930
IFNg	APC	B27	BD	554702
Streptavidin	PE		Biolegend	405204
Streptavidin	APC		Biolegend	405243