Supporting Information Table S1: Details of materials and methods for each of the three participating laboratories according to the MIATA guideline

Module 1, the cell samples

	Center 1	Center 2	Center 3
Samples			
PBMC samples			
Donors	Healthy volunteers from blood bank	Healthy volunteers from blood bank	Healthy volunteers from blood bank
Informed consent	Yes	Yes	Yes
Source of PBMC	Venipuncture, leukaphereses and buffy coats	Venipuncture, leukaphereses and buffy coats	Venipuncture, leukaphereses
Anticoagulant	Citrat-Phosphat-Dextrose	Citrat-Phosphat-Dextrose	Citrat-Phosphat-Dextrose
Method for HLA typing	Genomic DNA typing	Genomic DNA typing	Genomic DNA typing
CMV seropositivity	Known	Known	Unknown
Transportation and storage	RT	RT	RT
Cell processing method	Density gradient separation	Density gradient separation	Density gradient separation
Dilution and washing buffer	Sterile PBS	Sterile PBS	Sterile PBS
Time between blood collection and end of sample processing	< 6 hrs	< 6 hrs	< 6 hrs
Viability after PBMC isolation	> 90%	> 90%	> 90%
Freezing and storage	90% HI-FBS (Hyclone, Perbio Science, Bonn, Germany), 10% DMSO, freezing container. Long term storage: nitrogen tank	Non-commercial, serum-free freezing medium. Freezing container. Long term storage: nitrogen tank	90% HI-FBS (Gibco, Naerum, Denmark) 10% DMSO. Freezing container. Long term storage: -150°C freezer
N° of PBMC frozen per vial	15 - 20 $x10^6$	20 x 10^6	5 - 20 $x10^6$
TIL samples			
Donors			HLA-A*02 cancer patients
Informed consent			Yes
Source of cells			Melanoma lesions
Transportation and storage			Culture medium
Cell processing method			Mechanical disruption
Time between tissue collection and end of sample processing			Within 2 hours
Cell culture			CD3 mAb + IL-2 + allogenic feeders
Freezing and storage			See PBMC
N° of TIL frozen per vial			15 - 50 $x10^6$

Cell thawing and counting			
Medium and procedure used for thawing	IMDM 2.5% HI-HS, Pen/Strep, β-mercaptoethanol 0.05mM, DNAse 3µg/ml (Sigma) Thaw vial in water bath, transfer to warm medium and centrifuge	CTL-Wash, L-Glutamine, Benzonase 5000 U/ml (Merck, Darmstadt, Germany) Thaw vial in water bath, transfer to medium and centrifuge	RPMI, 10% HI FBS Optional addition: 0.025 mg/ ml Pulmozyme (Roche, Hvidovre, Denmark) and 2.5 mM $MgCl_2$ Thaw the content of the vial through dilution with preheated (37°C) media and centrifuge
Resting phase before assay	No	No	No
Serum pretested	Yes	n.a.	No
Viability after thawing	> 80%	> 80%	>80% for PBMC, >60% for TIL
Cell counting method	trypan blue	trypan blue + Turk´s solution	trypan blue

Module 2, the assay

	Center 1	Center 2	Center 3
Assay			
HLA-monomer production and storage			
Peptide source	Kindly provided by S. Stevanović	Kindly provided by P. Lewandrowski	Purchased from Pepscan,Lelystad, NL
Method	Refolding according to Altman et al.	Refolding according to Altman et al.	Refolding with a conditional ligand
Final concentration (µg/ml)	2000	2000	2000-3000
Buffer (excl. further additives NaN3, EDTA,or protease inhibitors)	20 mM Tris pH=8	20 mM Tris pH=8	PBS
Storage t°	-80°C	-80°C	-80°C with 16% glycerol
MHC multimer production and storage			
Ratio Streptavidin-fluorochrome / HLA-monomer	4/1 for Strep-PE and –APC 1/30 for Strep-QD (all Invitrogen Darmstadt, Germany)	4/1 for Strep-PE and –APC 1/30 for Strep-QD (all Invitrogen Darmstadt, Germany)	4/1 for Strep-PE and –APC 1/30 for Strep-QD (Strep-PE, -APC, PE-Cy7, BioLegend, Copenhagen, Denmark, Strept-QD Invitrogen)
Add biotin for blocking free Streptavidin	Only for Strep-QD multimers D-biotin (Sigma) 28 µM final	D-Biotin 25µM final (Sigma)	D-Biotin 25µM final (Sigma)
MHC multimer concentration before cryopreservation (µg/ml based on MHC concentration)	Multimer-PE 480; Multimer-APC 730 Multimer-QD 100	Mult-PE 497; Mult-APC 760 Mult-QD 836	Single color MHC multimer analyses 10; Combinatorial encoded MHC multimer panels 100
Final glycerol for freezing	16%	range: 0 -16%	range: 0 -16%

Others reagents contained in the HLA-multimer freezing buffer	0.02% NaN_3 0.5% HSA (Biotest, Dreieich, Germany) 1X Complete Protease inhibitor (Roche, Grenzach-Wyhlen, Germany)	0.02%NaN_3 0.5% BSA (Sigma) 1X Complete Protease inhibitors (Roche, Grenzach-Wyhlen, Germany)	0.02%NaN_3 0.5% BSA (Sigma)
Frozen MHC multimer concentration (µg/ml based on HLA concentration)	Mult-PE 321; mult-APC 488 Mult-QD 66	Mult-PE 367; mult-APC 591 Mult-QD 650	10 for single color staining; 100 for combinatorial staining;
Procedure for freezing /thawing multimers	Add concentrated freezing solution, mix gently and aliquot on ice. Transfer aliquots to -80°C. Thaw aliquots on ice without direct light exposure.	Add concentrated freezing solution mix gently and aliquot on ice. Transfer aliquots to -80°C. Thaw aliquots on ice without direct light exposure.	Add concentrated freezing solution mix gently and aliquot on ice. Transfer aliquots to -80°C. Thaw aliquots on ice without direct light exposure.
Storage t°	-80°C	-80°C	-20°C or -80°C
Staining			
Number of cells stained	1.5 x 10^6 /test	0.4 - 5 x 10^6 /test	0.5 - 3 x 10^6 /test PBMC 0.2 - 1 x 10^6 /test TIL
Staining volume	50 µl	50 – 100 µl	50 – 100 µl
MHC multimer concentration (based on HLA concentration)	5 µg/ml	5 µg/ml for single color staining 5 - 10 µg/ml for combinatorial staining.	1 µg/ml for single color staining 2 µg/ml for combinatorial staining
MHC multimer combinations	1 fluorochrome per multimer	1 fluorochrome per multimer or combinatorial staining	1 fluorochrome per multimer or combinatorial staining
MHC multimer dilution buffer	PBS + 50% HI FBS + 2 mM EDTA + 0.02% NaN_3	PBS + 50% HI FBS+ 2 mM EDTA + 0.02% NaN_3	PBS + 2% FBS + 2 mM EDTA + 0.02% NaN_3
Centrifugation of diluted MHC multimer	14000 g, 5 min, 4°C	13000 g, 2 hrs, 4°C	3300g, 5 min, RT
Incubation conditions	1st step, 30 min, RT	2nd step, 30 min, 4°C	1st step, 15 min, 37°C
mAbs	CD8-PE-Cy7 SFCI21Thy2D3 (Beckman Coulter, Krefeld, Germany)	In combination with dump channel: CD8-AF700 HIT-8a, (BD Biosciences, Heidelberg, Germany) without dump channel: CD8 FITC SK1, CD3 Pacific Blue UCHT1 (BD Biosciences, Heidelberg, Germany)	CD8-Alexa Fluor F700 HIT8a (Biolegend) or CD8-PerCP SK1 (Biolegend)
Dump channel	CD4-FITC HP2/6 (homemade)	If used: CD4-FITC SK3, CD14-FITC MφP9, CD16-FITC NKP15, CD19-FITC 4G7, CD40-FITC LOB7/6 (BD Biosciences)	CD4-FITC SK3, CD14-FITC MφP9, CD16-FITC NKP15, CD19-FITC 4G7, CD40-FITC LOB7/6 (BD Biosciences, Albertslund, Denmark)
mAb dilution buffer	PBS + 2% HI FBS + 2 mM EDTA +	PBS + 2% HI FBS + 2mM EDTA +	PBS 1X + 2% FBS + 2mM EDTA +

	0.02% NaN_3	0.02% NaN_3	0.02% NaN_3
Incubation condition	2^{nd} step, 20 min, 4°C	3^{rd} step, 20 min, 4°C	2^{nd} step, 30 min, 4°C
Dead cell dye	LIVE/DEAD aqua (Invitrogen)	LIVE/DEAD fixable near-IR or aqua dead cell stain kit (Invitrogen)	LIVE/DEAD fixable near-IR dead cell stain kit (Invitrogen)
Dilution buffer	PBS	PBS	PBS 1X + 2% FBS + 2mM EDTA + 0.02% NaN_3
Incubation step	3^{rd} step alone, 30 min, 4°C	1^{st} step alone, 20 min, 4°C	2^{nd} step together with mAb, 30 min, 4°C
Sample fixation	Formaldehyde 1%, no following wash	BD FACS permeabilizing Solution 2 RT for 10 min, then wash with mAb dilution buffer	No fixation
Staining with spiked glycerol			
Final concentrations	16 - 0.5%	16 - 0.5%	16 - 0.5%
Staining step	1^{st} step, 30 min, RT together with multimer, before mAb	1^{st} step, 30 min, 4°C, before staining procedure	1^{st} step, 15 min, 37°C together with multimer and 2^{nd} step together with mAb

Module 3, data acquisition

	Center 1	Center 2	Center 3
Data acquisition			
Flow cytometer	BD Canto II	BD LSR II SORP	BD LSR II SORP
Software for acquisition	Diva	Diva	Diva
Instrument settings and performance control	CS&T beads, compensation with beads and cells	CS&T beads, daily performance check, compensation with beads	CS&T beads, daily performance check, compensation with beads
Number of cells acquired	All in the test tube	Up to 5 x 10^6	Up to 1 x 10^6 lymphocytes or all in the tube

Module 4, data processing

	Center 1	Center 2	Center 3
Data processing			
Software for analysis	Flow Jo 7.2 (Treestar, Ashland, OR)	Flow Jo 9.5.2 (Treestar)	Diva Software v6.1.3
Gating strategy for measurements of viability and $CD8^+$ cells	Time histogram, singlets FSC-A/H, lymphos FSC-A/SSC-A, living	Time histogram, singlets FSC-A/H, lymphos FSC-A/SSC-A, living	Time histogram, singlets FSC-A/H, lymphos FSC-A/SSC-A, dead cell dye

	lymphos FSC-A/dead cell dye, CD8 histogram (marker $CD8^+$)	lymphos FSC-A/dead cell dye, CD8/dump channel (gate $CD8^+$)	(histogram), CD8/dump channel (gate $CD8^+$)
Gating strategy for measurements of MHC multimer+ CD8 T cells	Time, singlets, lymphos, CD4/CD8, CD8/MHC multimer quadrants or gates	Time, singlets, lymphos, CD8/dump channel, CD8/MHC multimer quadrants or gates	Time, singlets, lymphos, living cells, CD8/dump channel (CD4, CD14, CD16, CD19, CD40), CD8/MHC multimer quadrants or gates. For combinatorial staining, see (16,18)
Gating between experiments	Gates and quadrants had to be slightly adapted	Gates and quadrants had to be slightly adapted	Gates and quadrants had to be slightly adapted
Any data excluded	No	No	No
Positivity criteria	n.a.	n.a.	n.a.
Dot-plots provided on demand	Yes	Yes	Yes

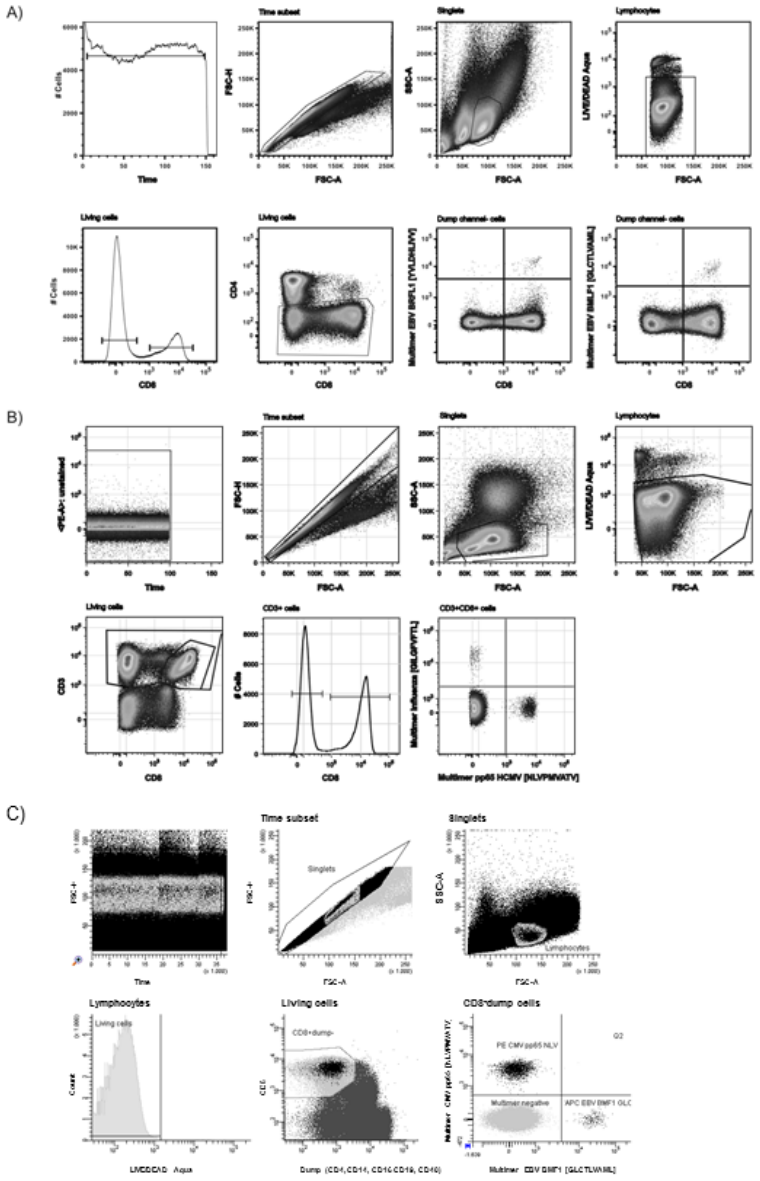
Module 5, general lab operation

	Center 1	Center 2	Center 3
Lab conditions			
Guidance of lab operations	Exploratory research	Exploratory research	Exploratory research
Trained personal	Yes	Yes	Yes
Accreditation of the lab	No	No	No
Participation to proficiency panels	Yes CIP and CIC	Yes, CIP and CIC	Yes, CIP
Status of protocols	Established lab protocols	SOPs	Established lab protocols
Status of assays	Qualified	Qualified	Qualified

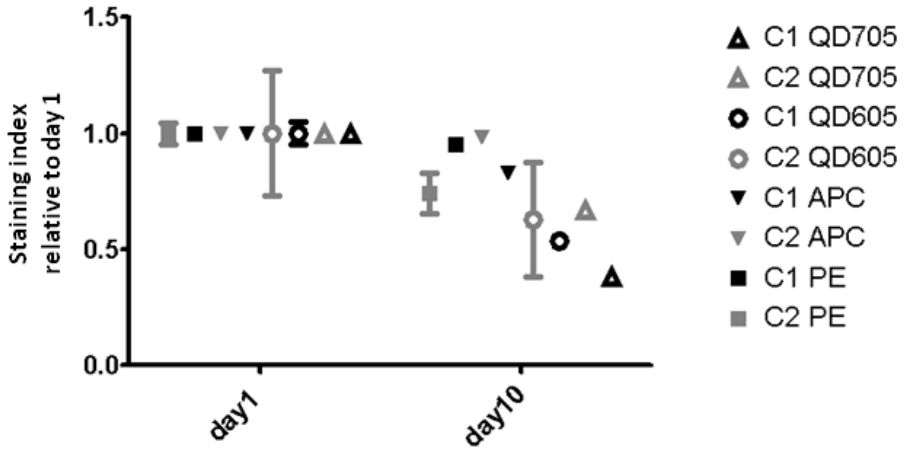
FBS = fetal bovine serum; HI = heat- inactivated; mAb = monoclonal antibody; Strep = streptavidin; Lymphos = lymphocytes

n.a. = not applicable; RT = room temperature;

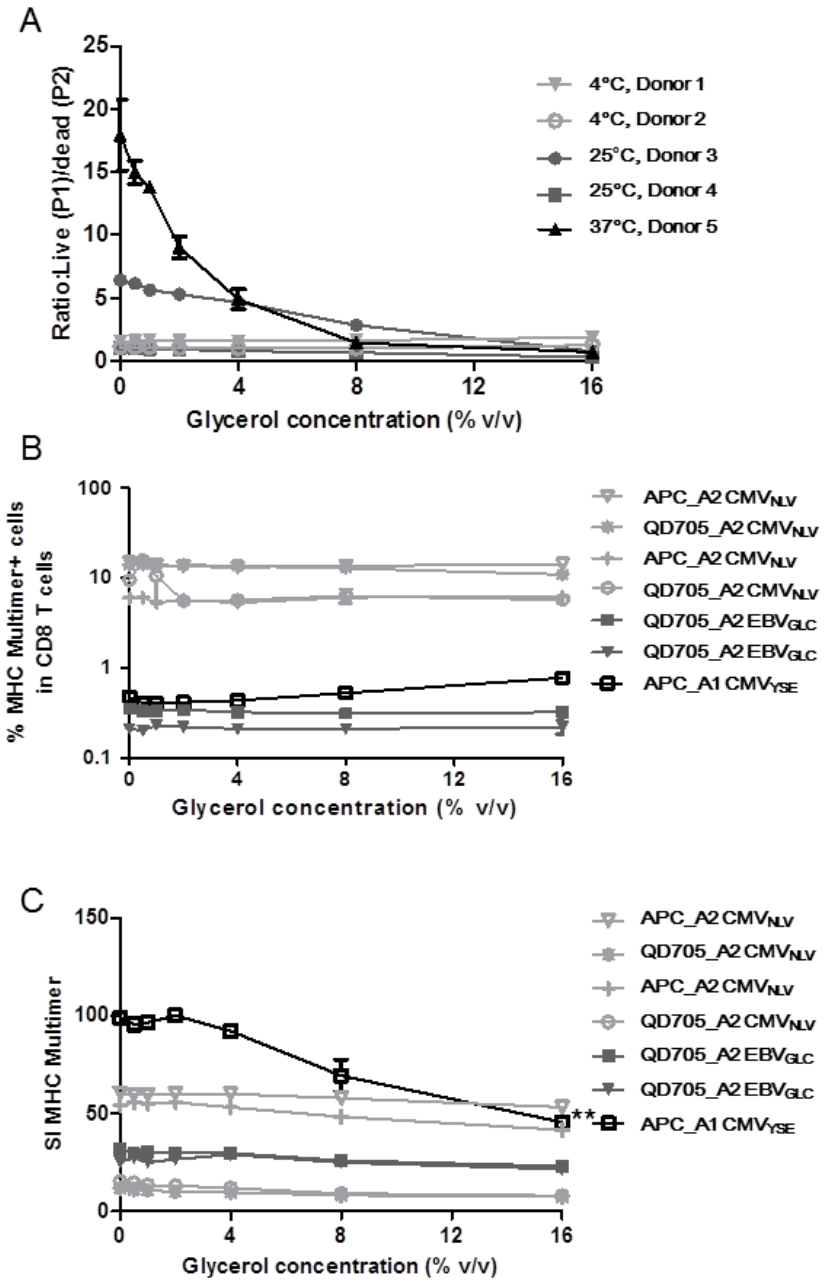
Supporting Information Figure S1.



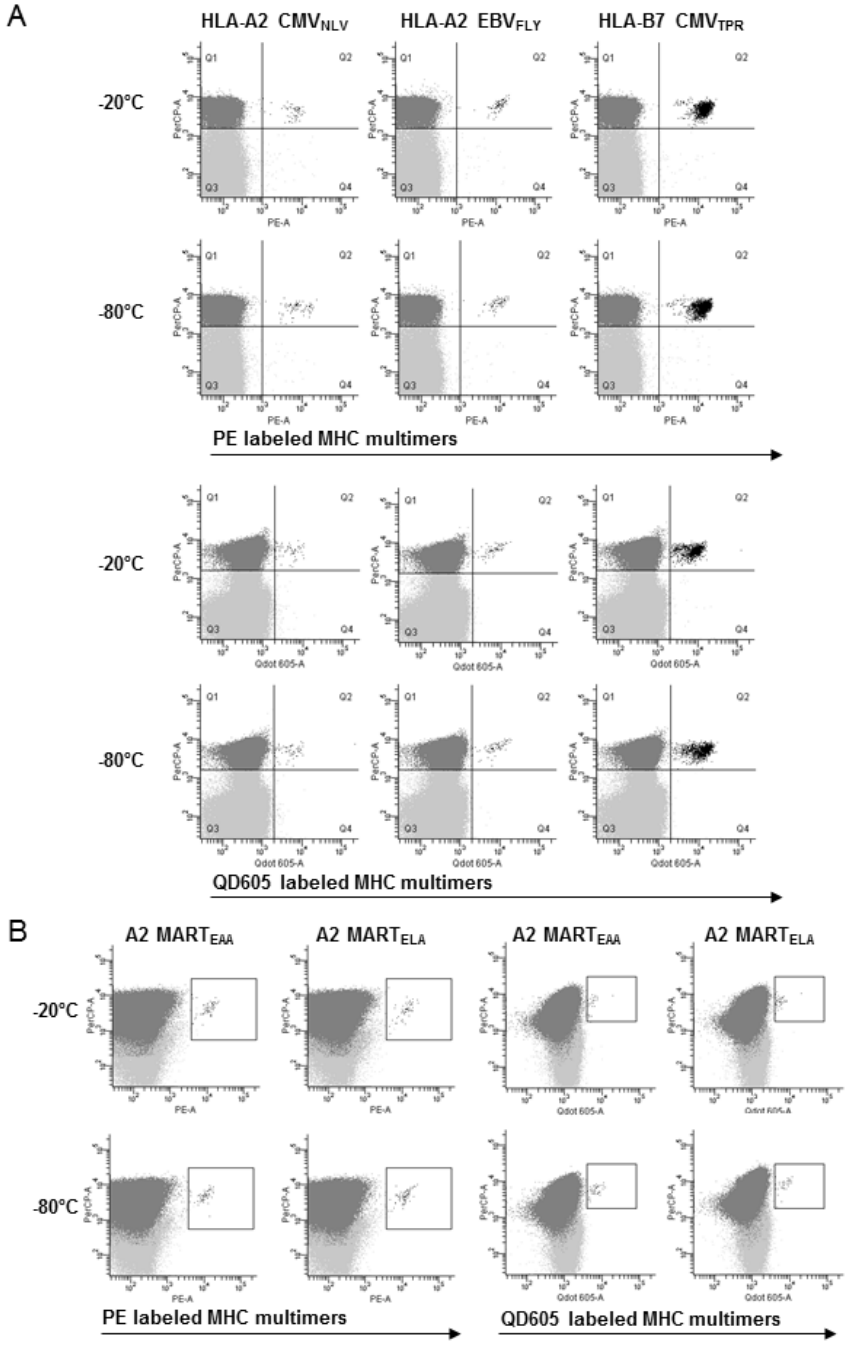
Supporting Information Figure S2.



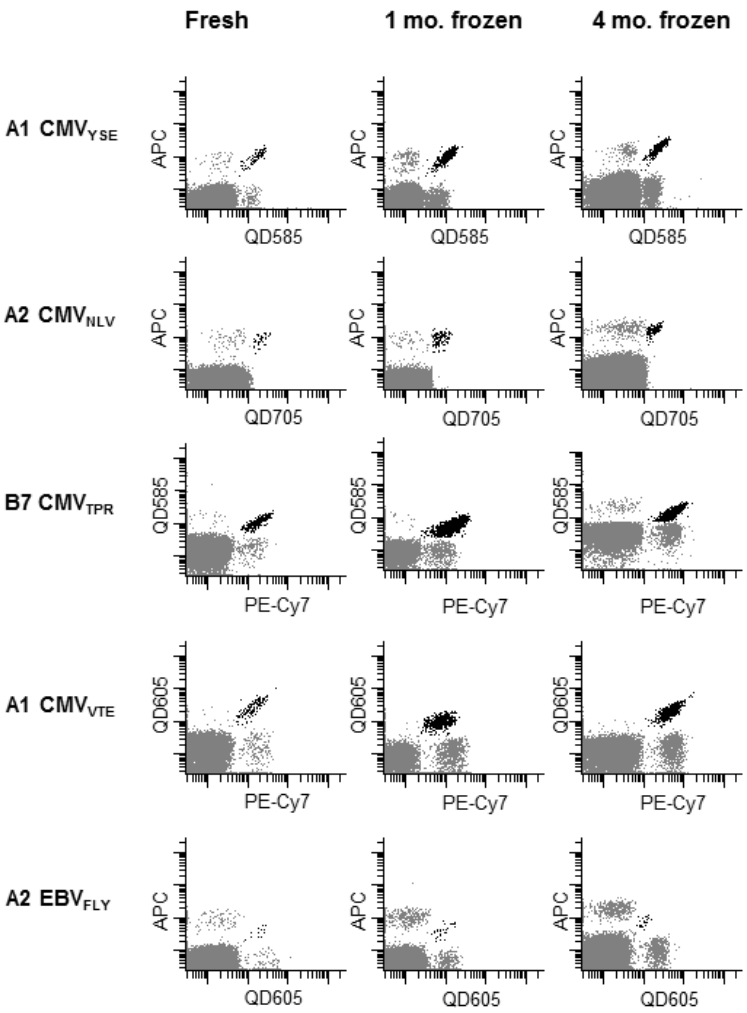
Supporting Information Figure S3.



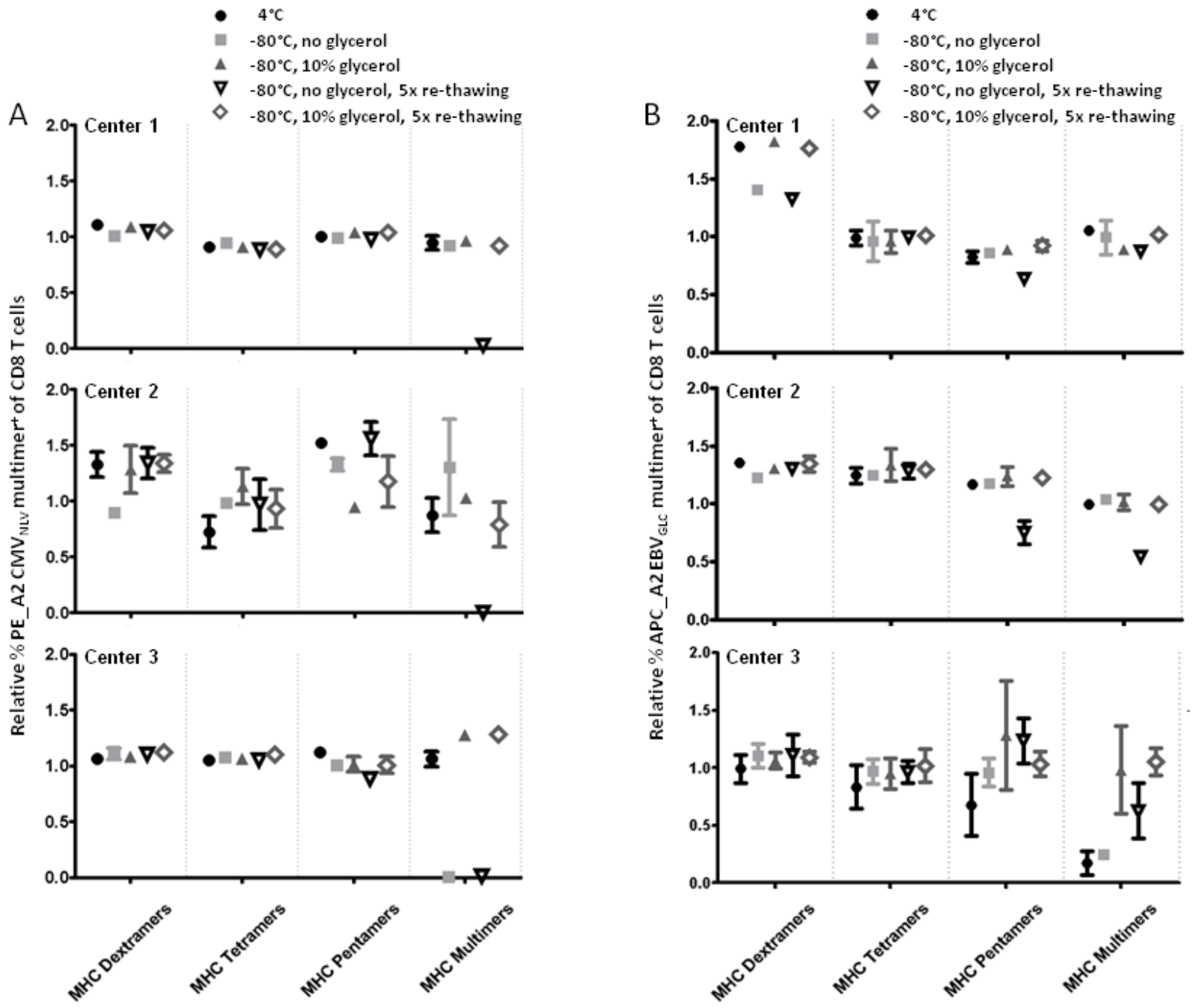
Supporting Information Figure S4.



Supporting Information Figure S5.



Supporting Information Figure S6.



Supporting Information figure legends:

Figure S1. **Gating strategies** of the three centers for identification of CD8 T cells and MHC multimer+ T cells.

Figure S2: **MHC multimer staining after 4°C storage.** MHC multimers, HLA-A2 CMV_{NLV} coupled to Streptavidin PE or QD605 and HLA-A2 EBV_{GLC} coupled to Streptavidin APC or QD705 were stored at 4°C for 10 days without glycerol. The effect of storage time is depicted as the average staining index for the multimer specific T-cell population calculated relative to the average staining index at day 1 after multimerisation. These tests were performed as two independent experiments at two different Centers (C1, C2). Error bars show range of duplicates.

Figure S3: **Effect of glycerol on cell viability and cell staining.** Increasing concentrations of glycerol (0, 0.5, 1, 2, 4, 8, 16%) were added to the first step of the pMHC multimer staining. pMHC multimers were freshly prepared and contained no glycerol themselves. The effect of glycerol addition was tested on five different donors (Center 2: light grey, two donors, Center 1: dark grey, two donors, Center 3: black, one donor). A) Cell viability under different staining conditions. The ratio between P1 (living cell numbers) and P2 (dead cell numbers) when separated according to their FSC/SSC profile, without the use of a dead-cell marker. The majority of P2 would normally fall out of the lymphocytes gate as given in Fig. 2. Three different incubation temperatures were used for the staining step with multimers, as per local protocols. B and C) MHC multimer staining in terms of percentage and intensity (SI) for seven different virus-specific T-cell populations. All data points are the average of duplicates, error bars indicate range (often not visible).

Figure S4: **Dot plots** relating to the experiment plotted in Figure 4C showing MHC multimer+ T cell populations after staining of PBMC or TIL with MHC multimers cryopreserved at -20°C or -80°C for 6 months. A) Dot plots are depicted for one donor, showing T cells binding to A2 CMV_{NLV}, A2 EBV_{FLY} or B7 CMV_{TPR} multimers labeled with PE or QD605. B) Dot plots are depicted for one TIL culture, showing T cells binding to A2 $MART_{EAA}$ or A2 $MART_{ELA}$ multimers labeled with PE or QD605.

Figure S5: **Dot plots** relating to the experiment plotted in Figure 5. Representative dot plots for the 5 different MHC multimer+ T-cell populations detected in Donor 2: APC/QD605_A2 EBV_{FLY}, APC/QD705_A2 CMV_{NLV}, APC/QD585_A1 CMV_{YSE}, QD605/PE-Cy7_A1 CMV_{VTE}, QD585/PE-Cy7_B7 CMV_{TPR}.

Figure S6. **Testing of different storage conditions for commercially available and in-house generated MHC-multimers.** We measured the frequency of the MHC multimer^{+} T cell populations in total CD8 T cells after staining using three different commercial available MHC multimers: MHC dextramers (Immudex), MHC tetramers (TCMetrix), and MHC pentamers (ProImmune), in parallel with in-house produced MHC multimers from the three different centers. MHC multimers PE_A2 CMV_{NLV} and APC A2_EBV_{GLC} were included and tested shortly after purchase and following10 days storage under the indicated conditions. A) The frequency of PE_A2 CMV_{NLV} positive T cells at day 10 calculated relative to the SI at day 1, respectively shown for the different storage conditions, separately for each of the three centers. B) The frequency of APC A2_EBV_{GLC} positive T cells at day 10 calculated relative to the SI at day 1, respectively shown for the different storage conditions, separately for each of the three centers. Performed as three

independent, but parallel experiments at Centers 1-3. Each dot is the average of duplicates except staining with the MHC dextramers for Center 1, and error bars indicate range (often not visible).

Cytometry Part A
Author Checklist: MIFlowCyt-Compliant Items
Hadrup et al. Cryopreservation of MHC multimers
- Recommendations for quality assurance in detection of antigen specific T cells

Requirement	**Please Include Requested Information**
1.1. Purpose	Purpose of the study was to establish optimal freezing conditions for long-term storage of peptide-MHC class I multimers. Hypothesis was that HLA-multimers are unstable when stored for longer period of time at 4°C and that albumin and glycerol can be used as cryoprotectors.
1.2. Keywords	MHC multimer; Long term storage; Cryopreservation; quality assurance; glycerol
1.3. Experiment variables	Experiments were performed at 3 different labs using local reagents, protocols and cytometers. 1. Cells were stained with a combination of monoclonal antibodies, dead cell marker and fluorescent HLA-multimers for detecting antigen-specific CD8 T cells. Both % and staining indexes of HLA-multimer binding CD8 cells were determined. In some experiments, cell viability and % and staining indexes of CD8 cells were assessed • HLA-multimers specificities: altogether 13 different multimers were included, refolded with 13 different epitopes derived from viral proteins (CMV, EBV, Flu) or tumor-associated antigens (MelanA, Tyrosinase) and restricted by 3 HLA-class I alleles (Table S2). • HLA-multimer labelling: altogether 10 different fluorochromes were tested • Antibodies/markers: at least a CD8 antibody was systematically included, together with a dump channel and a dead cell dye (Table S1) 2. HLA-multimers freezing conditions • Temperature: -20°C and –80°C • Glycerol concentration in the freezing buffer: serial dilutions ranging from min 0% to max. 16% glycerol. • Freezing duration: time points ranging from 1 day up to 12 months
1.4. Organization name and address	1. Center for Cancer Immune Therapy (CCIT), Dept. of Hematology, University Hospital Herlev, Denmark 2 Immatics Biotechnologies GmbH, Tübingen, Germany 3 Institute for Cell Biology, Dept. of Immunology, University of Tübingen, Germany 4 Translational Oncology, University Medical Center, Johannes Gutenberg-University gGmbH, Mainz, Germany 5 Department of Clinical Oncology, Leiden University Medical Center, Leiden, The Netherlands
1.5. Primary contact name and email address	Sine Reker Hadrup, email: Sine.Reker.Hadrup@regionh.dk
1.6. Date or time period of experiment	From October 2010 to July 2014
1.7. Conclusions	Long term storage of HLA-multimers is possible when using albumin and glycerol and should contribute to quality assurance of long-term immunomonitoring studies. Recommendations for cryopreservation were formulated (Table 1)
1.8. Quality control measures	Labs operate with qualified assays using established protocols or SOP Series of experiments were performed at least at two different centers (except for Fig 5). Each figure is based on

	results obtained with at least in 2 donors and includes different multimers (specificities and fluorescent labelling). Most tests were performed in duplicates. Details are given in the respective figures´ legends. Results are reported according to the framework for Minimal Information About T-cell Assays. Statistical analyses were performed when relevant.
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	Samples were human peripheral blood mononuclear cells from healthy volunteers and tumor infiltrating lymphocytes from melanoma patients
2.1.1.2. Biological sample source description	PBMC were isolated by density gradient from leukaphereses of buffy coats and frozen. TILs were obtained after mechanical tissue disruption, in vitro culture including a rapid expansion step (see Materials and Methods), and freezing. All donors were HLA-typed. All human biological material used in relation to this study was collected for research purposes under informed written consent and approval by the local ethics committee.
2.1.1.3. Biological sample source organism description	Human
2.1.2.2. Environmental sample location	Samples were obtained from the Center for Clinical Transfusion Medicine of the Tübingen University Hospital (Germany) and at the Department of Clinical Immunology and Blood Bank of Herlev Hospital (Denmark), as well as from internally produced tumor infiltrating lymphocyte cultures (IM Svane, Herlev Hospital, Denmark).
2.3. Sample treatment description	Cell samples were thawed and directly used for staining according to given protocols (Table S1). HLA-multimers were aliquoted and either preserved at 4°C (fridge) or frozen at -20°C or -80°C with or without albumin glycerol at tested concentrations. For stability experiments, aliquots were subjected to successive cycles of thawing (on ice) and freezing, at a min. of 1 day interval.
2.4. Fluorescence reagent(s) description	All reagents i.e. monoclonal antibodies, HLA-multimer and dead cell dyes are described in details in Table S1 CD8 T cells were identified as: • CD4 neg (HP2/6 FITC) CD8 pos (CD8 SFCI21Thy2D3 -PE-Cy7) or • CD3 pos (UCHT1 Pacific Blue) CD8 pos SK1 FITC) • Dump CD4(SK3) CD14 (MϕP9) CD16 (NKP15) CD19 (4G7) CD40 (LOB7/6) neg (all FITC) CD8 pos (HIT-8a AF700) • Dump CD4(SK3) CD14(MϕP9), CD16(NKP15), CD19(4G7), CD40(LOB7/6) neg (all FITC) CD8 pos (HIT8a AF700 or SK1 PerCP) 1. Antigen-specific CD8 cells were stained with HLA-multimers labelled with PE, APC, PE-Cy7, QD565, QD585, QD605, QD625, QD655, QD705 and-QD800. Single or dual-color labelling was used. 2. Viability was assessed by exclusion of cells labelled with AquaLive/Dead or near IR
3.1. Instrument manufacturer	BD Biosciences
3.2. Instrument model	Canto II or LSR-II SORP
3.3. Instrument configuration and settings	• Configuration of the Canto II, Center 1

Detector channel	Long pass	Band pass	Fluorochrome

	filter	filter	
Violet laser (405 nm, 30mV)			
A	502LP	510/50	LIVE⁄DEAD aqua
A Alternative	595LP	605/15	QD605
B	-	450/50	-
B Alternative	502LP	510/50	LIVE⁄DEAD Aqua
C	-	-	-
Blue laser (488 nm, 20mV)			
A	735LP	780/60	PE-Cy7
B	655LP	670LP	-
B Alternative	655LP	710/40	QD705
C	-	610LP	-
D	556LP	585/42	PE
E	502LP	530/30	FITC
F	-	488/10	SSC
G	-	-	-
Red laser (633 nm, 20mV)			
A	735LP	780/60	-
B	-	685LP	-
C	-	660/20	APC

- Configuration of the SORP LSR II, Center 2

Violet laser (405 nm, 100mV)			
A	735LP	780/60	QD800
B	670LP	710/50	QD705
C	635LP	660/20	QD655
D	615LP	625/15	QD625
E	595LP	605/40	QD605
F	570LP	585/15	QD585
G	557LP	560/40	QD565
H	-	450/50	Pacific Blue
Blue laser (488 nm, 100mV)			
A	635LP	685/35	-
B	505LP	510/20	FITC
C	-	488/10	SSC
Green laser (532 nm, 150mV)			
A	735LP	780/60	-
B	685LP	710/50	-
C	635LP	670/14	-
D	600LP	610/20	-
E	-	575/26	PE

Red laser (633 nm, 20mV)			
A	755LP	780/60	Near-IR
B	690LP	710/50	Alexa Fluor 700
C	-	670/14	APC
Alternative C	-	670/30	APC

- Configuration of SORP LSR II, Center 3

Detector channel	**Long pass filter**	**Band pass filter**	**Fluorochrome**
UV laser (355 nm, 60mV)			
A	770LP	800/30	Qdot800
B	680LP	710/50	Qdot705
C	595LP	605/12	Qdot605
D	535LP	580/30	Qdot585
Violet laser (405 nm, 100mV)			
A	635LP	655/8	Qdot655
B	610LP	625/20	Qdot625
C	-	450/50	-
Blue laser (488, 100mV)			
A	660LP	695/40	PerCP
B	505LP	525/50	FITC

	C	-	488/10	SSC
	Yellow-green laser (561 nm, 50mV)			
	A	735LP	780/60	PE-Cy7
	B	685LP	710/50	-
	C	635LP	670/30	-
	D	600LP	610/20	-
	E	570LP	585/15	PE
	Red laser (640 nm, 40mV)			
	A	735LP	780/60	Near-IR
		710LP	725/50	Alexa Fluor 700
	C	-	660/20	APC

All: FSC /SSC linear, all fluorescence log amplification
Rectangular Quartz Flow Cell

4.1. List-mode data files

*We recommend all authors to submit their data files to http://flowrepository.org and to make them available for the peer-review process. If you have done so, please let us know by inserting the following codes (replace the red text):

1) The link for peer-review process:

http://flowrepository.org/id/RvFr5O8CfOtHSXyn9CYCoZnOhj77ltqgU1hFeJ8vIaYLeB7mOmN3fEXFCuwo2qwX

2) The repository identifier:
http://flowrepository.org/id/http://flowrepository.org/id/FR-FCM-ZZEM

4.2. Compensation description

PMT voltages were adjusted for each fluorescence channel using unstained cells, and compensations set with compensation beads (BD Biosciences or Invitrogen) labeled with antibodies. ArC Amine reactive compensation bead kit (Invitrogen) or dead cells (after exposure to 65°C for 20min) stained with the LIVE/DEAD dye were used for

	compensating the relevant channel. Hardware compensation was exclusively applied (Center 2 and 3) or mostly applied (Center 1).
4.3. Data transformation details	Analysis of FCS files was performed with the software BD FACSDiva or Flow Jo. If used, FCS file format was FCS 3.0. Bi-exponential transformation was used when appropriate.
4.4.1. Gate description	Lymphocytes were identified by time histogram, singlets FSC-A/FSC-H and FSC/SSC. Further gating was adapted to the staining mix, including dead cell exclusion (Table S1 and figure legends). Gates or quadrants were used as per lab experience, and could be slightly adapted between experiments and/or samples. Examples are shown in Fig S1.
4.4.2. Gate statistics	Gate statistics from Flow Jo or Diva were % of dead lymphocytes (Fig2) or dead cells (Fig S2), % of CD8 cells (Fig 2), and % HLA-multimer binding cells within the CD8 subset as well as median fluorescence of multimer positive and multimer neg CD8 cells for calculating staining indexes (Fig1, Fig2, Fig 3, Fig 4, Fig5, Fig S2)
4.4.3. Gate boundaries	Example of gate boundaries are shown in Fig1, FigS1, S3 and S4.