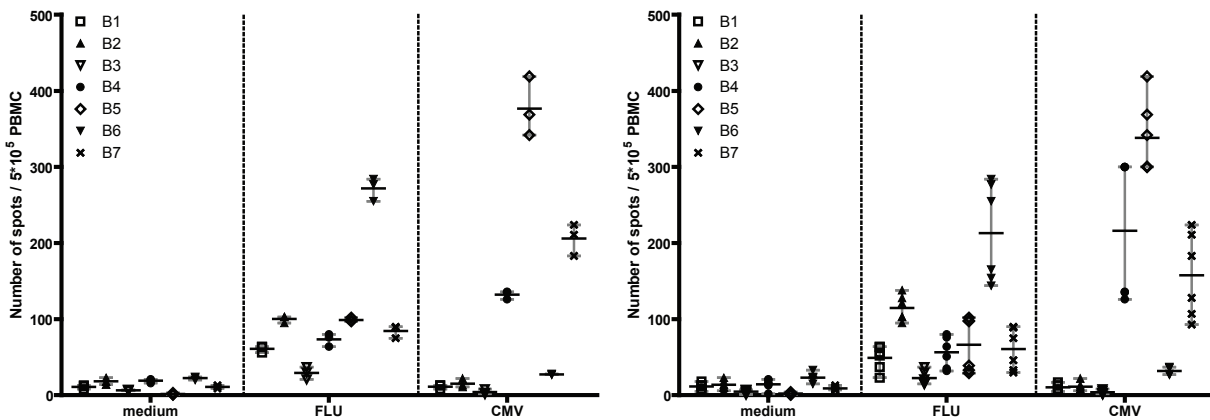
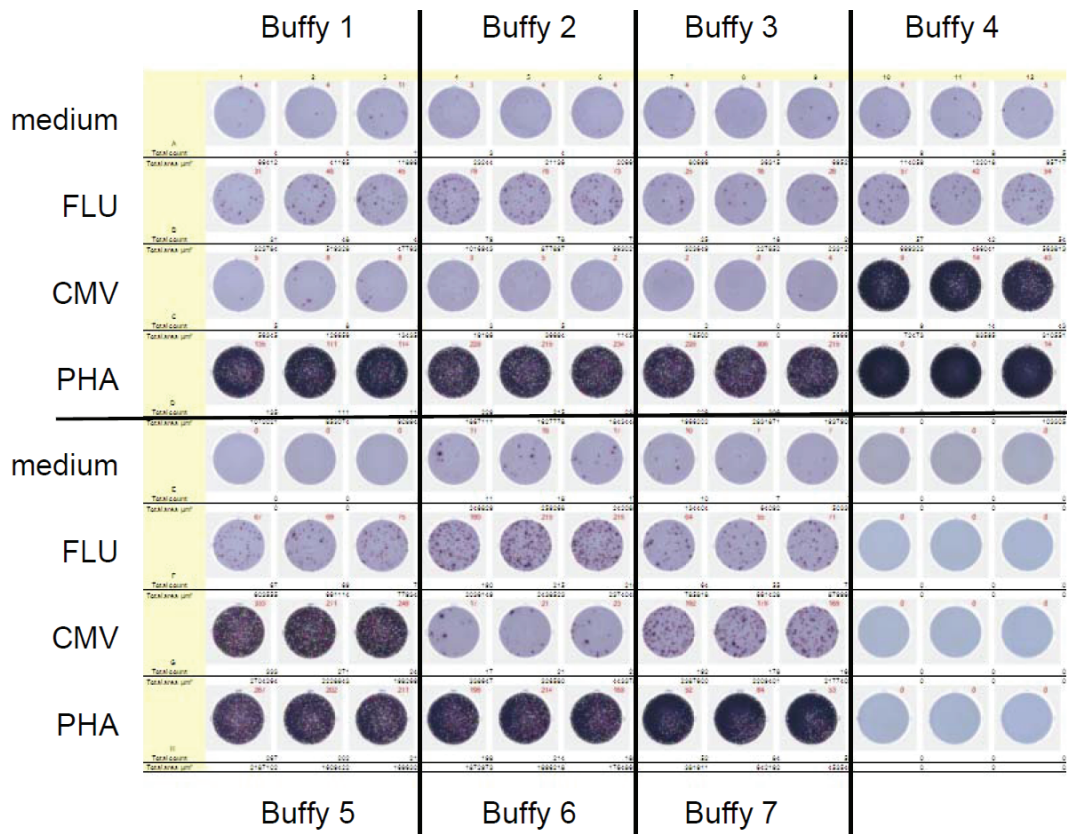


A

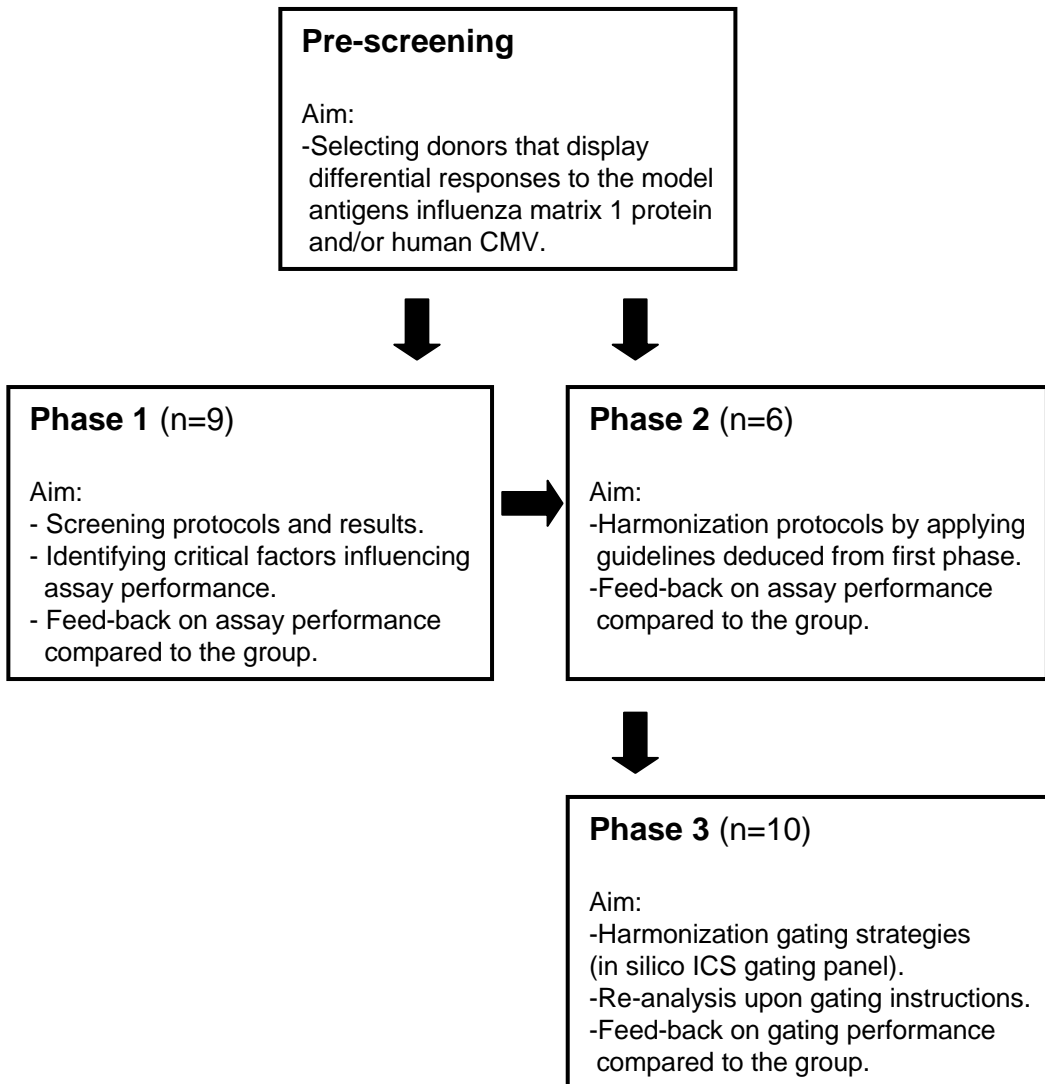


B



Online resource 1: Pre-screening of donors by IFN γ -ELISPOT

(A) The IFN γ -ELISPOT results are depicted. Left graph shows the first screening test (in triplicate wells) and the right graph the combined data of this first and a second independent screening test. PBMC isolated from 7 buffy coats (B) were analyzed for their recognition of the model antigens influenza M1 (FLU) or human pp65 CMV (CMV). (B) As an example the IFN γ -ELISPOT plate of the first pre-test is shown. PHA was taken along as a positive control.



Online resource 2: **Schematic overview of the three consecutive proficiency panels**

A

ID	Resting time (hours)	Number of cells per test	Peptide conc. (µg/ml)	Medium	Serum	CD8 antibody				CD3 antibody			
						Clone	Fluor chrome	Company	Dilution	Clone	Fluor chrome	Company	Dilution
5	16	2 x 10e6	2	RPMI	human AB	SK1	PerCP	BD	1:5	UCHT1	PE	BD	1:5
8	16	2 x 10e6	5	IMDM	human AB	SFC121 ThyD3	PE-Cy7	BC	1:120	13B8.2#	APC	BC	1:75
9	26	1-2 x 10e6	5	IMDM	human AB	SK1	APC-Cy7	BD	1:50	UCHT1	Pe-Cy7	BD	1:50
10	0	2 x 10e6	5	X-VIVO	none	SK1	APC-H7	BD	1:16	UCHT1	PE	Caltag	1:25
13	2	1 x 10e6	1	RPMI	human AB	SK1	PerCP	BD	1:10	UCHT1	PE	BD	1:10
15	18	1 x 10e6	5	RPMI	FCS	SK1	FITC	BD	1:50	SK7	PerCP-Cy5	BD	1:50
22	16	2 x 10e6	5	IMDM	human AB	SK1	APC	BD	1:10	SK7	PerCP	BD	1:5
24	2	0.25 x 10e6	5	RPMI	FCS	BW 135/80	APC	Milthenyi	1:20	UCHT1	PE	BC	1:20
26	16	0.7-1.7 x 10e6	2	IMDM	human AB	SK1	PerCP	BD	1:2.5	UCHT1	FITC	BC	1:5

ID	IFNγ antibody		Company	Dilution	Secretion blocking reagent (final conc.)	Addition of blocking reagent after	Activation time	Lyse/fix reagent	Permealization reagent	Duration of IFNγ staining
	Clone	Fluor chrome								
5	4S.B3	FITC	BD	1:25	Golgi-Stop (1 µl/ml)	1 hour	overnight	Fix (Dako)	Perm (Dako)	15 min at RT
8	B27	FITC	BD	1:200	Brefeldin A (50 µg/ml)	Immediately	5 hours	Fix (BD)	Perm (BD)	30 min at 4°C
9	4S.B3	FITC	BD	1:50	Brefeldin A (10 µg/ml)	1 hour	overnight	4% paraphormaldehyde	saponine	30 min at 4°C
10	B27	PE-Cy7	BD	1:10	Golgi-Plug (1 µg/ml)	1.5 hours	overnight	Fix (BD)	Perm (BD)	30 min at 4°C
13	4S.B3	FITC	BD	1:50	Golgi-Stop (1 µl/ml)	1 hour	overnight	Fix (Dako)	Perm (Dako)	15 min at RT
15	B27	APC	BD	n.a.	Brefeldin A (10 µg/ml)	1 hour	6 hours	1% Formaldehyd-2mM EDTA	saponine	20 min at 4°C
22	25723.11	FITC	BD	1:2.5	Brefeldin A (7.5 µg/ml)	1 hour	6 hours	Lyse (BD)	Perm (BD)	30 min at 4°C
24	25723.11	FITC	BD	1:10	Brefeldin A (10 µg/ml)	2 hours	6 hours	Fix (An der Grub)	Perm (An der Grub)	15 min at 4°C
26	45-15	APC	IQP	1:50	Brefeldin A (n.a.)	2 hours	6 hours	Lyse (BD)	Perm (BD)	30 min at 4°C

B

ID	Resting time (hours)	Number of cells per test	Peptide conc. (µg/ml)	Medium*	Serum*	CD4 antibody				CD3 antibody			
						Clone	Fluor chrome	Company	Dilution	Clone	Fluor chrome	Company	Dilution
8	16	2 x 10e6	5	X-VIVO	none	13B8.2	PE	BC	1:75	S4.1	APC	Caltag	1:75
9	24	2 x 10e6	5	X-VIVO	none	SK3	Pe-Cy7	BD	1:50	UCHT1	PB	DAKO	1:20
10	0	2 x 10e6	10	X-VIVO	none	RPA-T4	PB	Biologend	1:250	UCHT1	PE	Caltag	1:50
15	8	5 x 10e6	5	X-VIVO	none	RPA-T4	Fitc	BD	1:10	SK7	PercP	BD	1:10
22	24	3 x 10e6	5	X-VIVO	none	SK3	APC	BD	1:16	SK7	PercP	BD	1:5
24	1	1x 10e6	2	X-VIVO	none	SK3	PercP	BD	1:10	UCHT1	Fitc	BD	1:10

ID	IFNγ antibody		Company	Dilution	Secretion blocking reagent (final concentration)	Addition of blocking reagent after	Activation time	Lyse/fix reagent	Permealization reagent	Duration of IFNγ staining
	Clone	Fluor chrome								
8	B27	FITC	BD	1:200	Brefeldin A (10 µg/ml)	1 hour	6 hours	Cytofix/Cytoperm (BD)	saponine	30 min at 4°C
9	4S.B3	FITC	BD	1:50	brefeldin A (10 µg/ml)	1 hour	18 hours	4% paraphormaldehyde	saponine	30 min at 4°C
10	B27	PE-Cy7	BD	1:20	GolgiPlug (1 µg/ml)	Immediately	overnight	Cytofix/Cytoperm (BD)	Perm/Wash (BD)	30 min at 4°C
15	25723.11	APC	BD	1:33	Brefeldin A (5 µg/ml)	Immediately	12 hours	Permwash (eBioscience)	eBioscience	25 min at 4°C
22	25723.11	FITC	BD	1:2.5	Brefeldin A (7.5 µg/ml)	2 hours	18 hours	Lyse (BD)	Perm (BD)	20 min at 4°C
24	25723.11	PE	BD	1:5	Brefeldin A (10 µg/ml)	2 hours	6 hours	Lyse (BD)	Perm (An der Grub)	15 min at 4°C

Online resource 3: ICS protocol variables

The variables in the wet protocol of the ICS as conducted in proficiency panels 1 (A) and 2 (B) are given. * indicates the obligatory recommendations; # not a CD3 but CD4 antibody was used; BD, Becton Dickenson; BC, Beckman Coulter; PC, pacific blue; n.a. information not available.

Phase	Parameter	Average	Median	Range	CV (%)
1	Number of viable cells (directly after thawing)	15.5x10 ⁶	15.1 x10 ⁶	4.9 - 39.7 x10 ⁶	43.9
	% Dead cells (directly after thawing)	16.0	9.3	3.6 - 53.3	87.7
	Number of viable cells (after resting)	11.4 x10 ⁶	10.6 x10 ⁶	2.7 - 34.2 x10 ⁶	61.1
	% Dead cells (after resting)	24.6	10.7	1.5 - 82.0	97.0
	Number of lymphocytes	278521	163873	9198-1229921	114
	Number of CD8+ T-cells	63105	25703	623-335556	131
	% CD8+ T-cells	21.6	20.8	7.4-35.6	36.9
2	Number of lymphocytes	675243	715001	199922-1349327	33.7
	Number of CD3+ T-cells	591350	556426	143889-1200000	41.3
	% CD3+ T-cells	78.4	79.0	57.7-93.0	9.9
	Number of CD3+CD4- T-cells	204433	191131	52234-495797	49.4
	% CD3+CD4- T-cells	34.0	32.3	19.1-47.0	19.1
3	Number of lymphocytes	507956	505967	411364-673447	10.3
	Number of CD3+ T-cells	418646	419665	319571-559130	11.0
	% CD3+ T-cells	82.4	83.8	76.1-85.9	3.6
	Number of CD3+CD4- T-cells	160623	164364	85598-263390	26.4
	% CD3+CD4- T-cells	38.3	43.5	22.1-48.5	22.6

Online resource 4A: Parameters in the 3 phases of the ICS proficiency panels.

CMV					
ID	D1	D2	D3	D4	D5
5	0.360	3.000	1.780	0.060	0.140
8	0.010	7.590	1.620	0.010	0.070
9	0.060	4.870	1.300	0.000	0.230
10	0.000	4.696	1.771	0.013	0.105
13	1.205	8.169	13.17	0.941	0.046
15	0.000	0.000	0.330	0.050	5.680
22	0.000	5.280	5.090	0.000	0.000
24	0.000	1.540	4.020	0.000	0.040
26	0.128	0.100	0.000	0.000	0.100
Median	0.010	4.696	1.771	0.010	0.100
Average	0.196	3.916	3.231	0.119	0.712
SD	0.396	2.986	4.066	0.309	1.864
CV	202	76.3	126	259	262

FLU					
ID	D1	D2	D3	D4	D5
5	0.560	0.880	1.220	0.140	0.270
8	0.030	0.010	0.070	0.160	0.040
9	0.060	0.020	0.120	0.010	0.020
10	0.017	0.012	0.091	0.095	0.054
13	0.352	0.000	0.176	0.083	0.046
15	0.000	0.000	0.210	0.070	0.050
22	0.030	0.000	0.000	0.000	0.000
24	0.000	0.000	1.710	0.060	0.060
26	0.000	0.000	0.000	0.160	0.000
median	0.030	0.000	0.120	0.083	0.046
average	0.117	0.102	0.400	0.086	0.060
SD	0.200	0.292	0.620	0.059	0.082
CV	172	285	155	68.5	136

Online resource 4B: Percentage of IFN γ -producing CD3+CD8+ T cells as reported by participants in Phase 1 of ICS panel.

In bold the positive responses (i.e. at least twice above the negative control with a clearly visible population of events). The values are the percentages of IFN γ +CD3+CD8+ T cells after subtraction of the negative control value. The gray boxes indicate the donor (D)-antigen (CMV or FLU) combinations which were found to be negative in the pre-screening test.

CMV					
ID	D1	D2	D3	D4	D5
8	0.000	4.810	1.040	0.010	0.030
9	0.003	8.727	3.623	0.002	0.080
10	0.000	4.753	0.846	0.007	0.049
15	0.002	12.40	8.505	0.001	0.107
22	0.000	1.827	4.102	0.000	0.029
24	0.000	2.820	0.730	0.000	0.030
Median	0.000	4.781	2.332	0.002	0.040
Average	0.001	5.889	3.141	0.003	0.054
SD	0.001	3.969	3.014	0.004	0.033
CV	165	67.4	96.0	126	60.0

FLU					
ID	D1	D2	D3	D4	D5
8	0.000	0.000	0.010	0.060	0.000
9	0.015	0.027	0.115	0.133	0.027
10	0.001	0.000	0.025	0.091	0.012
15	0.033	0.026	0.145	0.209	0.015
22	0.006	0.061	0.202	0.079	0.013
24	0.030	0.010	0.100	0.020	0.010
median	0.011	0.018	0.108	0.085	0.012
average	0.014	0.021	0.099	0.099	0.013
SD	0.014	0.023	0.073	0.066	0.009
CV	101	112	73.1	66.5	67.9

Online resource 4C: Percentage of IFN γ -producing CD3+CD4-negative T cells as reported by participants in Phase 2 of ICS panel.

In bold the positive responses (i.e. at least twice above the negative control with a clearly visible population of events). The values are the percentages of IFN γ +CD3+CD4-negative T cells after subtraction of the negative control value. The gray boxes indicate the donor (D)-antigen (CMV or FLU) combinations which were found to be negative in the pre-screening test.

Lab ID	Medium			CMV			FLU		
	D1	D2	D5	D1	D2	D5	D1	D2	D5
8	0.01	0.02	0.01	0.00	10.42	0.11	0.02	0.04	0.05
9	0.01	0.02	0.01	0.00	9.52	0.10	0.03	0.03	0.04
10	0.01	0.04	0.03	0.00	11.46	0.10	0.03	0.06	0.05
11	0.13	0.21	0.10	0.06	13.09	0.12	0.01	0.16	0.08
12	0.80	0.90	0.04	0.00	10.91	0.08	0.00	0.12	0.04
14	0.01	0.02	0.01	0.00	12.48	0.14	0.04	0.04	0.07
15	0.01	0.03	0.01	0.00	11.67	0.10	0.03	0.04	0.05
16	0.01	0.05	0.09	0.00	12.55	0.11	0.03	0.05	0.05
22	0.05	0.04	0.06	0.00	12.46	0.10	0.03	0.05	0.04
24	0.30	0.20	0.10	0.00	12.80	0.20	0.00	0.10	0.10
Median	0.010	0.040	0.044	0.00	12.07	0.11	0.030	0.050	0.050
Average	0.13	0.15	0.046	0.006	11.73	0.12	0.022	0.069	0.057
SD	0.26	0.28	0.040	0.019	1.16	0.033	0.014	0.043	0.020
CV	196	185	87.6	316	9.88	28.8	63.6	62.4	35.1

Online resource 5A: Phase 3 results of in silico ICS gating panel.

In bold the positive responses (i.e. at least twice above the negative control). Note that for CMV and FLU the values are shown after subtraction of medium control.

Lab ID	Medium			CMV			FLU		
	D1	D2	D5	D1	D2	D5	D1	D2	D5
8	0.01	0.02	0.01		10.42	0.11	0.02	0.04	0.05
9	0.01	0.02	0.01		9.52	0.10	0.03	0.03	0.04
10	0.01	0.04	0.03		11.46	0.10	0.03	0.06	0.05
11	0.01	0.03	0.07		12.44	0.11	0.03	0.03	0.03
12	0	0.03	0.02		11.62	0.10	0.03	0.04	0.05
14	0.01	0.02	0.01		12.48	0.14	0.04	0.04	0.07
15	0.01	0.03	0.01		11.67	0.10	0.03	0.04	0.05
16	0.01	0.05	0.09		12.55	0.11	0.03	0.05	0.05
22	0.05	0.04	0.06		12.46	0.10	0.03	0.05	0.04
24	0.01	0.05	0.03		11.83	0.13	0.03	0.03	0.05
Median	0.010	0.030	0.025		11.75	0.11	0.030	0.040	0.050
Average	0.013	0.033	0.034		11.65	0.109	0.030	0.042	0.048
SD	0.013	0.012	0.029		0.99	0.014	0.005	0.009	0.011
CV	102.8	35.2	85.6		8.54	12.6	17.3	22.3	22.1

Online resource 5B: Re-analysis of in silico ICS gating panel after new gating instructions.

In bold the positive responses (i.e. at least twice above the negative control). Note that for CMV and FLU the values are shown after subtraction of medium control. The gray boxes indicate the 3 labs that performed the re-analysis.