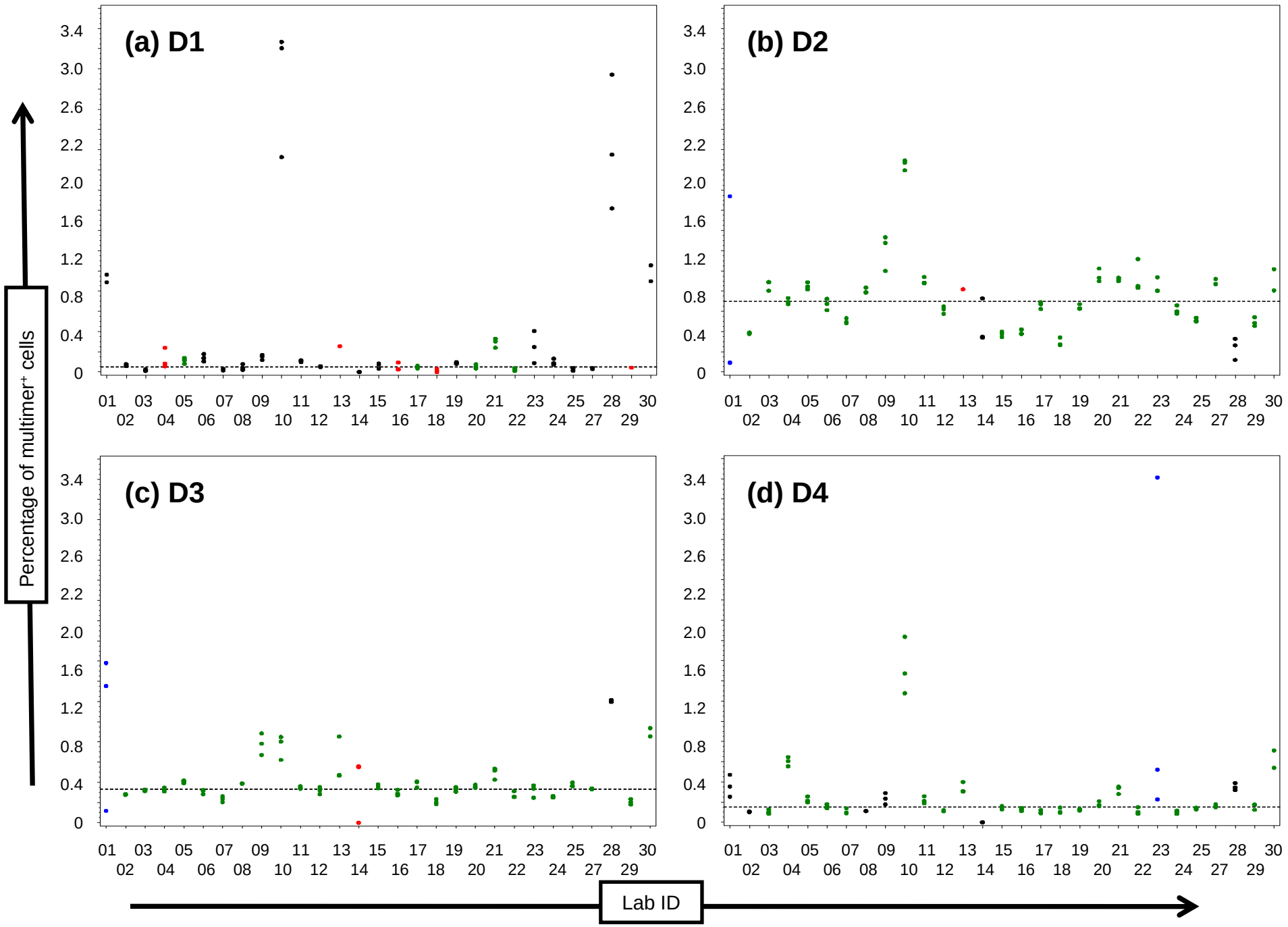
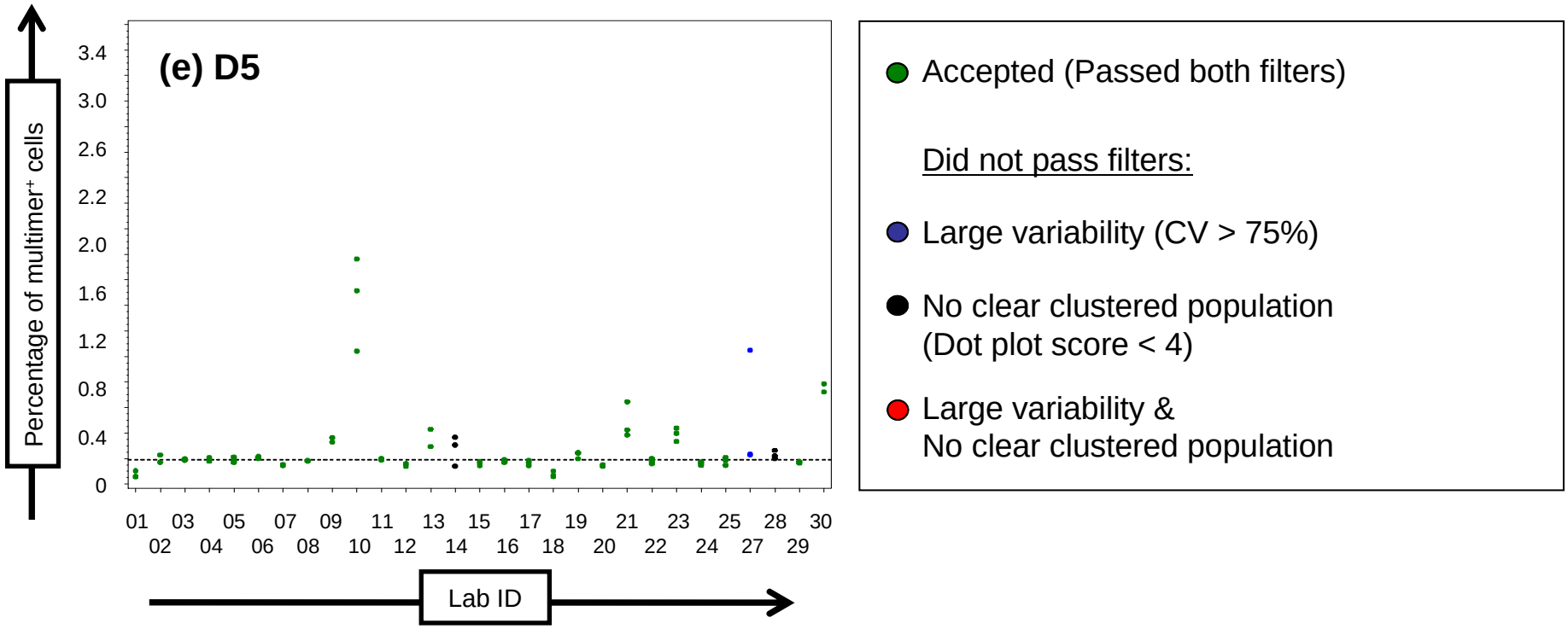
Supp. figure 1

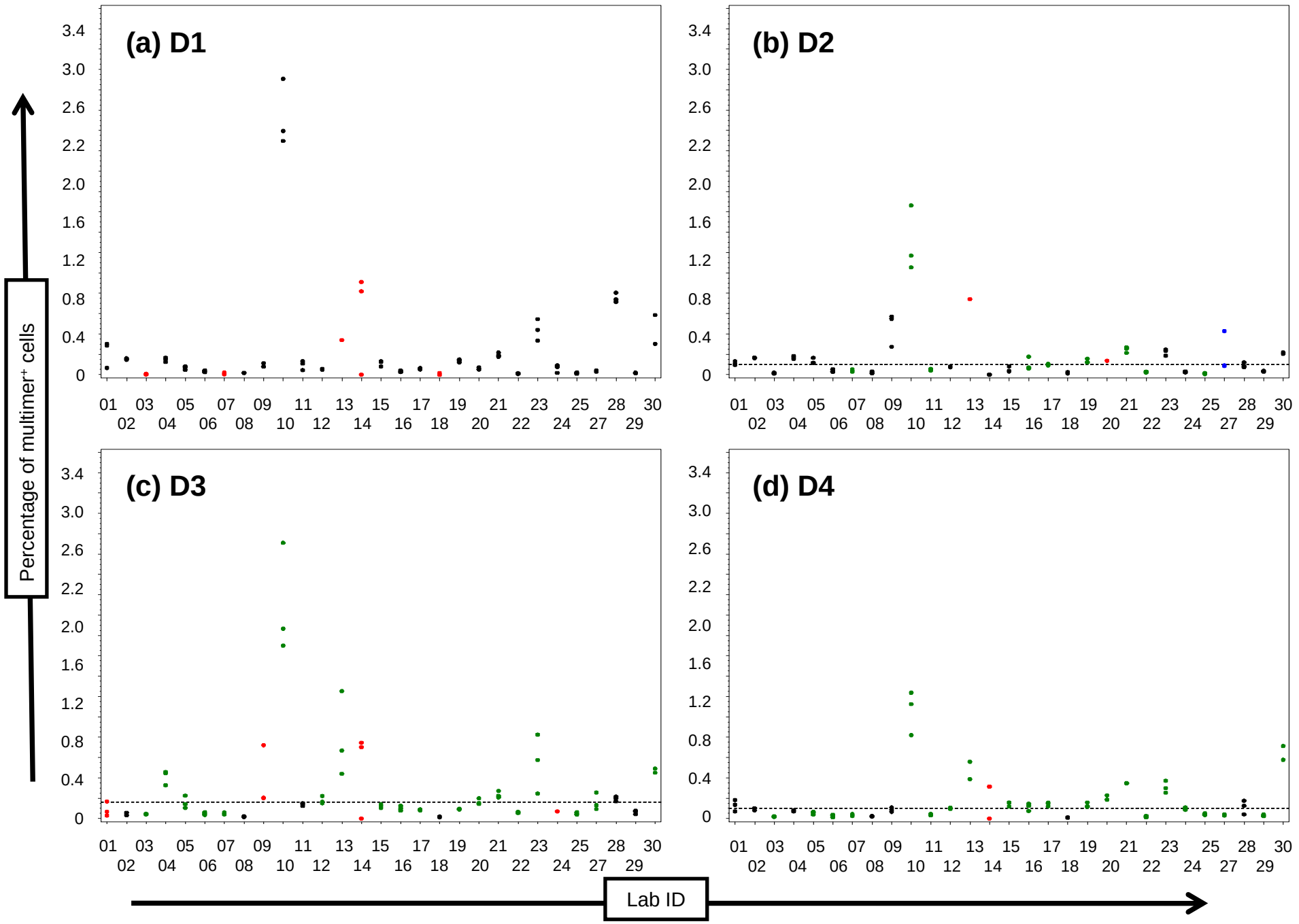


Suppl. figure 1

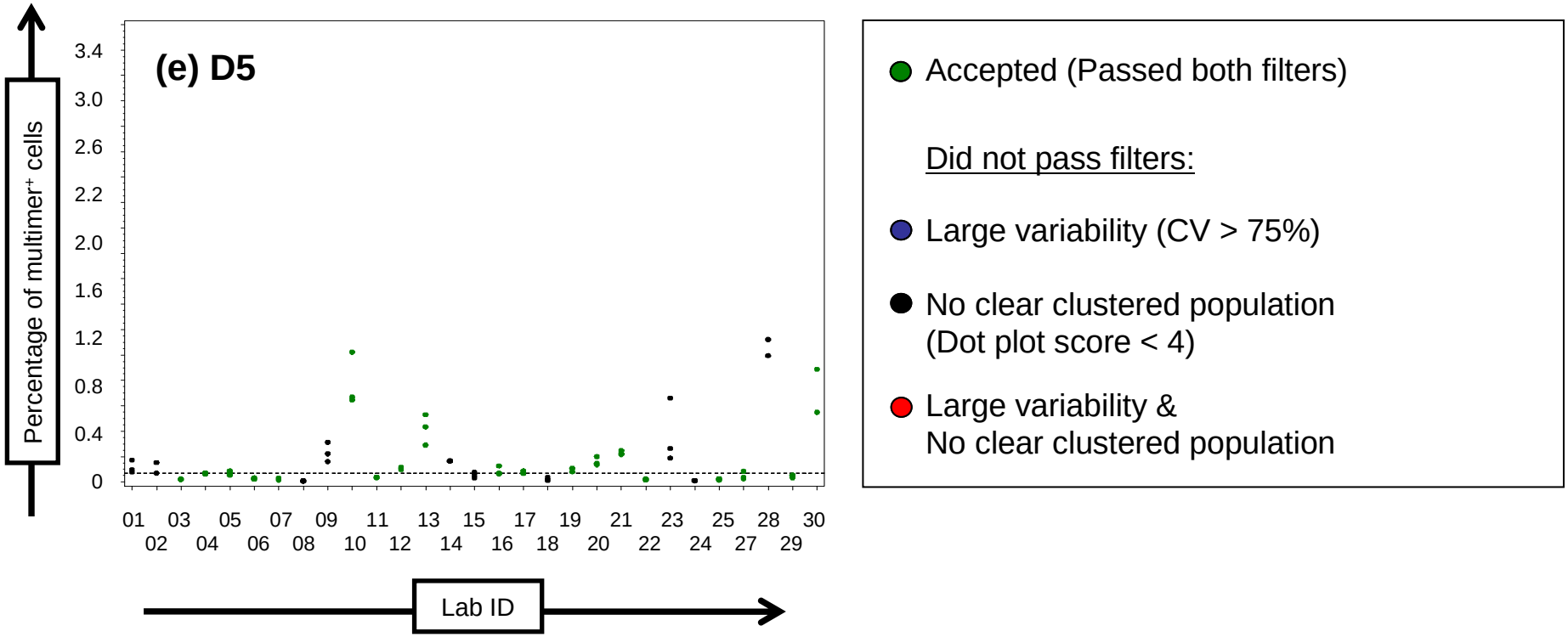


Supplementary figure 1: The figure shows a series of graphs (a-e) that display the reported percentages of multimer positive cells from each participating lab for each of the five donors. The graphs illustrate the distribution of the percentage of multimer specific CD8+ cells for the Influenza-M1 antigen for each of the five donors (D1 – D5). Labs that detected a response (passed both filters) are indicated in green. The labs that did not pass the first filter (high CV) are indicated in blue. The labs that did not pass the second filter (optical evaluation of dot plots) are indicated in black. Those labs that did not pass both filters are indicated in red. The horizontal reference line displayed in each graph represents the overall median multimer specific CD8+ binding for those labs that detected a response for that donor and antigen.

Suppl. figure 2

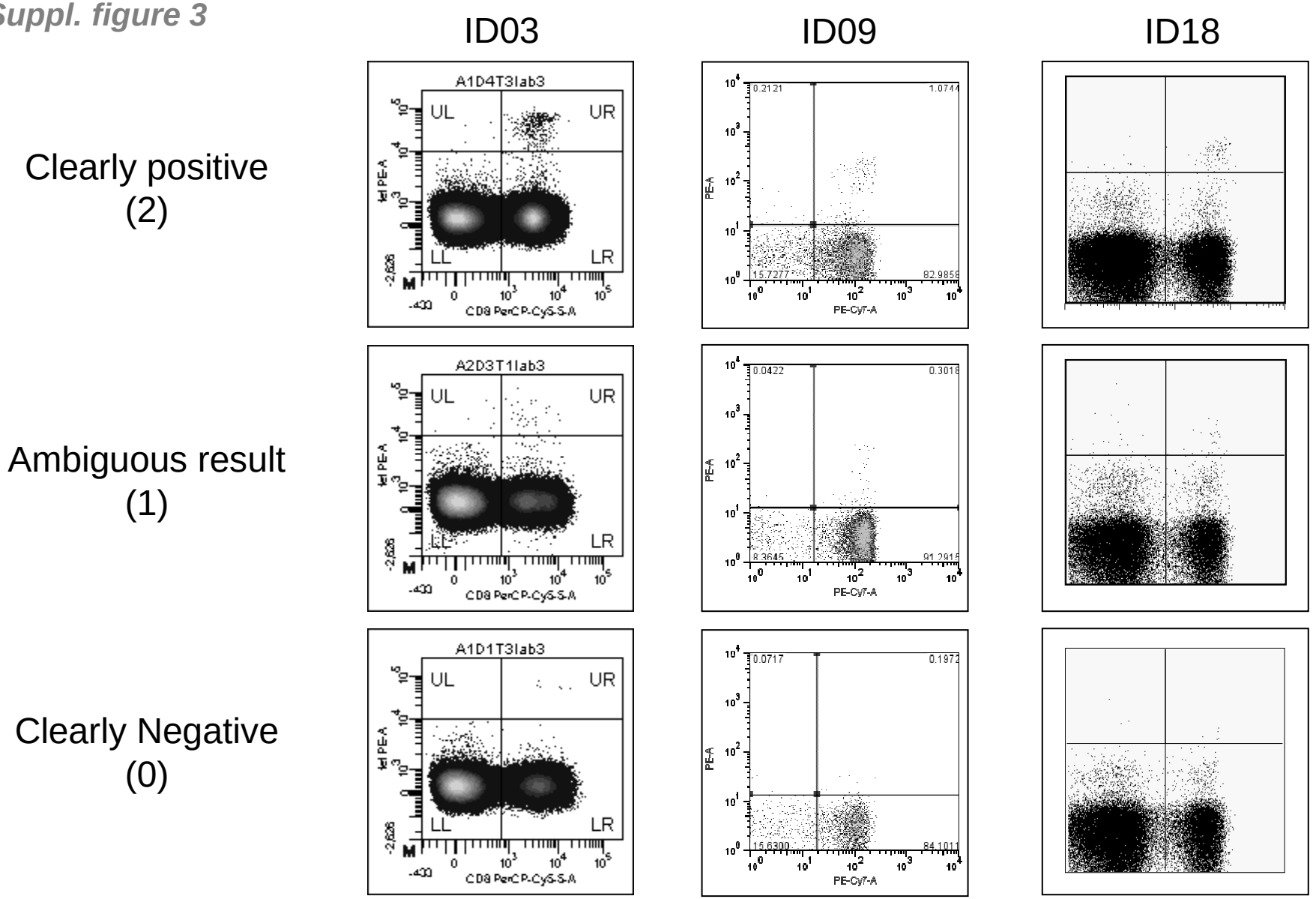


Suppl. figure 2



Supplementary figure 2: The figure shows a series of graphs (a-e) that display the reported percentages of multimer positive cells from each participating lab for each of the five donors. The graphs illustrate the distribution of the percentage of multimer specific CD8+ cells for the Melan-A/Mart-1 antigen for each of the five donors (D1 – D5). Labs that detected a response (passed both filters) are indicated in green. The labs that did not pass the first filter (high CV) are indicated in blue. The labs that did not pass the second filter (optical evaluation of dot plots) are indicated in black. Those labs that did not pass both filters are indicated in red. The horizontal reference line displayed in each graph represents the overall median multimer specific CD8+ binding for those labs that detected a response for that donor and antigen.

Suppl. figure 3



Supplementary figure 3: *Optical evaluation of dot plots.* The figure shows nine examples of dot plots from three labs (ID03, ID09 and ID18) and gives an impression on the amount of clustering that was needed for being considered as a clearly positive (2), clearly negative (0) or an ambiguous result (1).

Suppl. table 1

Variable		N	%
Multimer source	Tetramer	22	76%
	Pentamer	7	24%
DNAse	Yes	7	24%
	No	22	76%
Counting Method	Trypan Blue	17	59%
	Eosin	1	3%
	Guava cell counter	8	28%
	Beckman Coulter ViCell XR	1	3%
	Cellometer	1	3%
	NucleoCassette (Chemometec)	1	3%
Cytometer Used	BD FACSCalibur	11	38%
	BD FACSCanto	5	17%
	BD LSRII	5	17%
	BD FACScan	3	10%
	Dako Cytomation	2	7%
	BC Cytomics FC500	1	3%
	BD FACSAria	1	3%
	BD FACScan / FACSVantage	1	3%
Item used for Staining	Tube	20	69%
	Plate	9	31%
Staining Order	Multimer and then co-staining	19	66%
	Multimer and co-staining simultaneously	10	34%
Number of Colors	2	2	7%
	3	14	48%
	4	13	45%
Method for Dead Cell Exclusion	None	18	62%
	Propidium iodide	3	10%
	DAPI	2	7%
	Fixable Aqua Dead Cell Stain Kit	2	7%
	TOPRO-3 staining	2	7%
	7AAD	1	3%
	Invitrogen Live/Dead Violet Dye	1	3%
CD3 Staining	Yes	17	59%
	No	12	41%
Dump Channel	Yes	14	48%
	No	15	52%
Additional Antibodies Used	None	4	14%
	CD3 alone	11	38%
	CD19	3	10%
	CD3, CD4, CD14, CD19	3	10%
	CD3, CD19	2	7%
	CD14, CD19	2	7%
	CD14	1	3%
	CD3, CD4, CD14, CD19, CD56	1	3%
	CD4, CD13, CD19	1	3%
	CD4, CD19, CD56	1	3%

Supplementary table 1: *Questionnaire responses outlining the protocol used by each lab*. The table summarizes the questionnaire responses outlining the various protocols used by all the labs. The selected protocol variables defining subgroups of laboratories are indicated in the first column and the expression of each variable is shown in the second column. For each subgroup the number of laboratories (third column) and the percentage of centers (fourth column) are shown. Total number of analysis is 29.

Suppl. table 2

Antigen	Donor	Response	ID6	ID8	ID15	ID16	ID20	ID22	ID23	ID25	ID27	ID29
Influenza-M1	D1	<LLQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	D2	High	10.6	8.7	105.9	21.4	15.7	16.4	17.8	1.6	6.7	19.7
	D3	Moderate	62.1	25.7	26.6	15.0	1.9	5.9	111.8	4.7	4.7	32.3
	D4	Moderate	24.2	18.0	63.4	5.1	42.3	7.4	32.9	16.1	51.0	80.7
	D5	Moderate	31.3	4.3	106.2	12.5	2.5	11.9	7.5	7.0	22.1	n.d.
Melan A/Mart1	D1	Negative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	D2	Moderate	76.1	14.0	1.4	0.1	18.6	7.7	72.2	113.7	82.7	43.9
	D3	Moderate	87.1	68.8	47.3	40.4	25.9	17.7	121.8	34.1	17.4	56.4
	D4	Low	95.5	0.9	64.4	15.3	26.7	9.2	119.1	8.9	37.8	76.0
	D5	Low	87.8	35.0	14.6	42.3	10.4	19.7	113.1	51.5	51.0	n.d.
Average			59.3	21.93	53.72	19.05	18.00	10.86	59.75	29,63	34.17	51.5

Supplementary table 2: *Intra-center variability between steps 1 and 2.* The table shows the coefficient of variation for each of the 10 centers that participated in both steps. The coefficient of variation was calculated between the mean multimer-specific CD8+ T cells in the first step and the mean in the second step for each of the 10 donor antigen combinations. Results reported from donor 1 who was considered as having an extremely low response against Influenza-M1 and no detectable reactivity against Melan-A/Mart-1 were not considered. The last column shows the mean of the CV values for each of the participating laboratories as an approximate measure of the intra center variation.

Suppl. table 3

Antigen	Donor	Coefficient of Variation				
		0-24.9	25-49.9	50-74.9	75-99.9	>=100 or only 1/3 performed
Influenza-M1	D1	11	10	3	**2**	**3**
	D2	25	2	0	**0**	**2**
	D3	26	2	0	**0**	**1**
	D4	22	6	0	**1**	**0**
	D5	26	1	0	**2**	**0**
MelanA/Mart1	D1	15	7	2	**3**	**2**
	D2	16	7	3	**1**	**2**
	D3	16	6	3	**3**	**1**
	D4	19	8	1	**1**	**0**
	D5	15	10	4	**0**	**0**

Supplementary table 3: *Variation within replicates.* The table shows the number of labs with calculated CVs from their replicates. Results are shown within 5 given ranges for all 10 antigen-donor combinations. Labs with replicates that had a CV greater than 75, or labs with only one measurement for a given donor and antigen did not pass this filter. These are indicated in bold on the table.

Suppl. table 4

Antigen	Donor	Sum of Dot Plot Evaluation Score						
		0	**1**	**2**	**3**	4	5	6
Influenza-M1	D1	**12**	**3**	**3**	**6**	0	4	1
	D2	**1**	**0**	**2**	**0**	2	1	23
	D3	**2**	**0**	**0**	**4**	1	2	20
	D4	**1**	**0**	**0**	**1**	4	1	22
	D5	**1**	**0**	**1**	**0**	3	1	23
MelanA/Mart1	D1	**13**	**6**	**2**	**8**	0	0	0
	D2	**4**	**4**	**5**	**6**	3	4	3
	D3	**5**	**0**	**4**	**1**	6	3	10
	D4	**3**	**1**	**3**	**1**	7	4	10
	D5	**2**	**1**	**3**	**4**	5	2	12

Supplementary table 4: *Summary of the optical evaluation.* The table shows results from optical evaluation of all dot plots generated from the lab's analysis. The assigned score was based on whether there was a clustered population in the upper right quadrant of a dot plot showing the CD8-staining on the x-axis and the HLA-peptide multimer staining on the y-axis. A score of 0 was given when there was clearly no clustering, a score of 1 was given for ambiguous results, and a score of 2 was given when there was clearly a clustered population of dots in the upper right quadrant. The sum of each of the scores for a given donor antigen replicate are presented. Labs with replicates that had a total score of less than 4 did not pass this filter. These are indicated in bold on the table.

Suppl. table 5

Variable	Expression	N	Number of Detected Responses	Average Proportion of Detected Responses
All Labs		29	172	66%
Average viability	<75%	6	30	56%
	>75%	23	142	69%
Multimer source	Tetramer	22	121	61%
	Pentamer	7	51	81%
DNAse	Yes	7	50	79%
	No	22	122	62%
Counting Method	Manual	18	114	70%
	Machine	11	58	59%
Item used for staining	Tube	20	119	66%
	Plate	9	53	65%
Staining Order (multimer and co-staining)	Sub-sequent	19	119	70%
	simultaneous	10	53	59%
Number of fluorochromes	2	2	3	17%
	3	14	92	73%
	4	13	77	66%
Dead cell exclusion	Yes	11	66	67%
	No	18	106	65%
CD3 Staining	Yes	17	97	63%
	No	12	75	69%
Dump Channel	Yes	14	92	73%
	No	15	80	59%
Average non-specific multimer binding*	<0.03%	7	41	65%
	0.03% – 0.3%	14	85	67%
	>0.3%	6	31	57%

Supplementary table 5: *Subgroup analysis of all detected responses.* Laboratory characteristics, number of detected responses and average proportion of detected responses for each subgroup (calculated by dividing the number of detected responses by nine times the number in that subgroup). *Two labs used a gating strategy which removed non-specific CD8- cells, therefore these labs are missing in the subgroup analysis for non-specific multimer binding.