

## **Supplementary Tables and Figures**

**Somogyi et al: Peptide vaccine candidate mimics the heterogeneity of natural SARS-CoV-2 immunity in convalescent humans and induces broad T cell responses in mice models**

**Supplementary Table 1. Donor baseline and demographic information.** All donors were caucasoid, with mild/asymptomatic disease and no hospitalization (except one, marked with \*). S/Co, sample/control ratio; values were determined according to the manufacturer's instructions, and test results are interpreted as negative in S/Co <0.9, not conclusive if S/CO = 0.9–1.1, and positive if S/Co >1.1. COI, cut-off index; values were determined according to the manufacturer's instructions, and test results are interpreted as negative in COI <0.9, inconclusive with COI 0.9–1.1, and positive if COI >1.1. NA, data not available. *Italic, negative or inconclusive values.* \*\* Complaints: a, cough; b, sore throat; c, fever; d, short of breath; e, stomach/intestinal complaints; f, chest pain; g, sore eyes; h, odor or taste loss; i, headache; j, fatigue; k, other complaints (pulmonary embolism and cardiac arrest for IMXP00759; leg pain, arm pain, muscle pain, pain in the eyes).

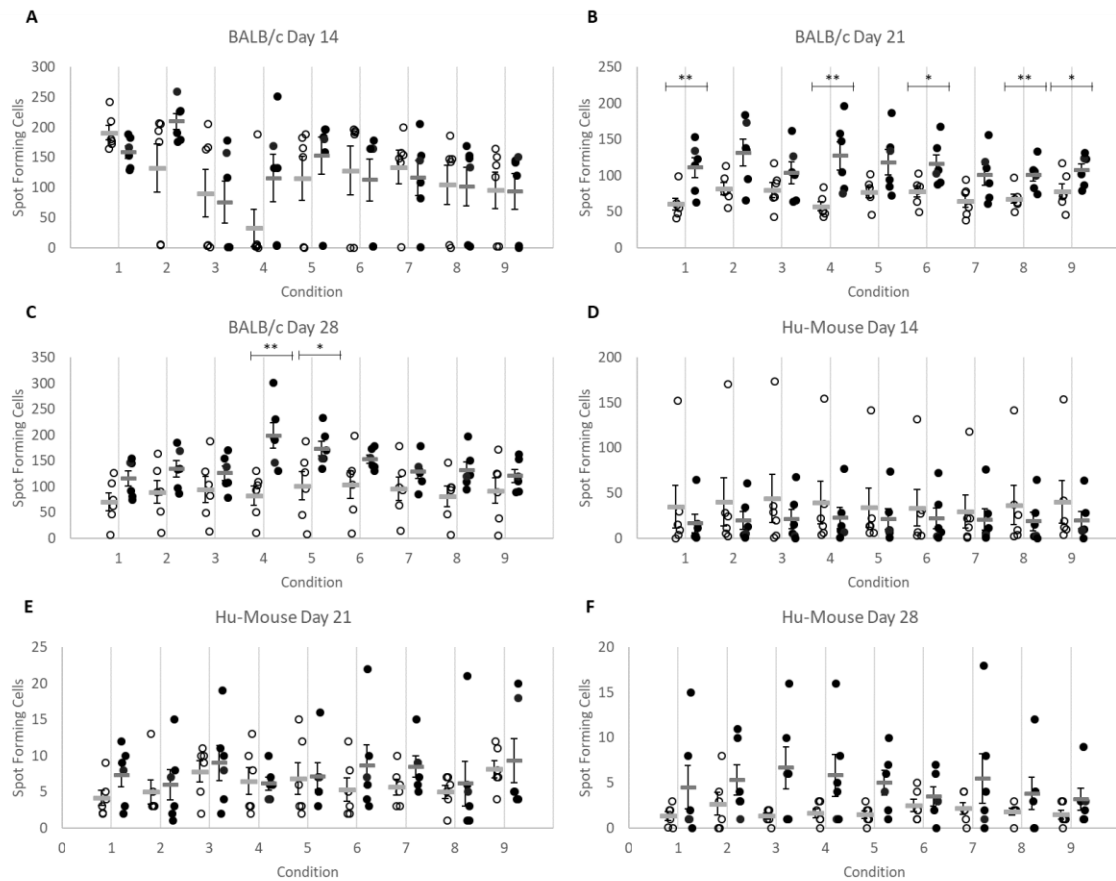
Donor ID	Gender	Complaints from/ to: (as reported by the donors)	Complaints**	Blood collection date	Time from first symptom to blood collection	IgA	IgM	IgG	IgG-S1	IgG-N
						DiaPro ELISA			EUROIMMUN	ROCHE
						S/Co	S/Co	S/Co	S/Co	COI
IMXP00394	Female	30 March 2020 - 20 April 2020	a,b,c,d,h,i,j	4-Aug-20	126 days	<i>0.36</i>	5.065	5.752	4,48	54.38
IMXP00714	Male	1 May 2020 – 15 May 2020	a,b,c,h,i,j,k	27-Jul-20	87 days	1.324	8.524	11.524	5,35	73.06
IMXP00739	Female	30 April 2020	j	2-Jun-20	63 days	<i>0.929</i>	8.841	11.967	4,54	77.61
IMXP00756	Female	2 April 2020 - 12 April 2020	b,c,d,f,i,j	9-Jun-20	68 days	<i>0.989</i>	4.606	12.193	3,56	78.47
IMXP00757	Female	29February 2020 - 14 April 2020	a,b,c,d,e,h,i,j	9-Jun-20	101 days	1.154	5.847	8.701	7,62	29.47
IMXP00758	Female	2 April 2020 - 30 arpril 2020	c,d,h,i,j	15-Jun-20	74 days	1.356	7.757	11.774	5,79	121.9
IMXP00759*	Male	13 March 2020 - 28 March 2020	a,c,d,f,h,i,j,k	15-Jun-20	94 days	6.307	10.666	13.838	9,27	87.09
IMXP00762	Female	15 March 2020 – 19 March 2020	b,c,j	29-Jun-20	106 days	1.251	7.314	4.46	7,25	131.5
IMXP00764	Female	16 March 2020 – 2 April 2020	a,b,e,h,i,j,k	6-Jul-20	115 days	5.161	9.739	11.677	1,32	46.59
IMXP00765	Female	29 March 2020 - 15 May 2020	a,d,e,h,i,j,k	7-Jul-20	100 days	<i>0.565</i>	2.948	1.54	1,32	13.4
IMXP00766	Female	20 June 2020 - 23 June 2020	b,c,h,j	7-Jul-20	17 days	<i>0.771</i>	4.648	3.973	4,14	6.25
IMXP00767	Female	10 April 2020 - 10 May 2020	d,e,f,j,k	7-Jul-20	88 days	<i>0.88</i>	5.402	3.459	2,37	52.29
IMXP00771	Female	18 March 2020 – 1 April 2020	a,d,i,j	28-Jul-20	131 days	<i>0.791</i>	7.775	8.322	4,04	119.4
IMXP00772	Female	30 March 2020 - 30 April 2020	g,k	28-Jul-20	120 days	1.105	4.256	2.54	1,26	10.87
IMXP00776	Female	9 March 2020 - 14 March 2020	c,e,i,j,k	4-Aug-20	148 days	<i>1.012</i>	9.196	10.887	2,26	88.64
PTC1	Male	15 April 2020	e	13-Jul-20	89 days	<i>0.53</i>	<i>0.41</i>	2.63	NA	18.96
PTC2	Female	15 April 2020	e	13-Jul-20	89 days	<i>0.45</i>	<i>0.35</i>	1.49	NA	26.09

**Supplementary Table 2. Sequence alignment results between PolyPEPI-SCoV-2 and other coronavirus strains.** Sequence comparison was made with 8-mer long peptide matching between the aligned protein sequence pairs, defined as the minimum length requirement for a CD8<sup>+</sup> T cell epitope. Max AA matching: the longest identical amino acid sequence length. Highlighted grey values represent identical sequences of at least eight amino acids.

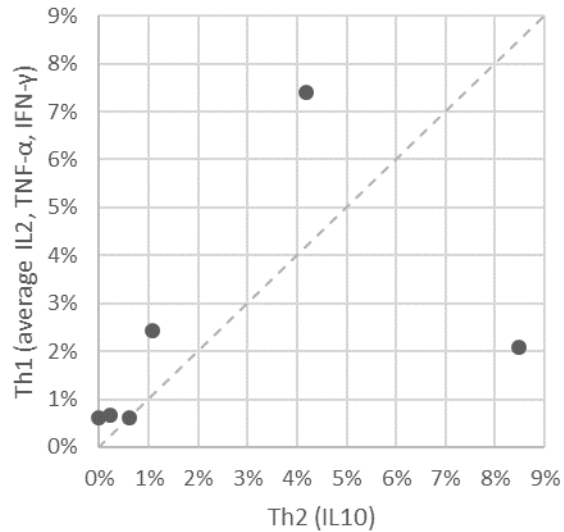
Target Protein	PolyPEPI-SCoV-2 vaccine peptide sequences		Common 8-mer % / Max AA matching					
			229E	NL63	OC43	HKU1	MERS	SARS
Spike	S2	GVYYDPKVFRRSSVLHSTQDLFLPFFSNVTW	0% / 4	0% / 4	0% / 4	0% / 3	0% / 3	4% / 8
	S5	DSSSGWTAGAAAYVGYLQPRTFLLKYNN	0% / 3	0% / 4	0% / 4	0% / 4	0% / 5	0% / 4
	S9	ALQIPFAMQMAYRFNGIGVTQNVLYENQKL	0% / 4	0% / 4	0% / 4	0% / 4	0% / 5	96% / 29
Nucleoprotein	N1	RSKQRRPQGLPNNTASWFTALTQHGEDLK	0% / 3	0% / 3	0% / 3	0% / 3	0% / 6	78% / 25
	N2	SKKPRQKRTATKAYNVTQAFGRRGPEQTQG	0% / 4	0% / 4	0% / 6	0% / 7	0% / 4	65% / 17
	N3	ELIRQGTDYKHWPQIAQFAPSASAFFGMSR	0% / 3	0% / 4	0% / 3	0% / 4	0% / 5	96% / 29
	N4	QRQKKQQTVTLLPAADLDDFSKQLQSSMSS	0% / 5	0% / 3	0% / 4	0% / 3	0% / 3	9% / 9
Membrane	M1	LSYFIASFRLFARTRSMWSEFNPETNILLNV	0% / 5	0% / 6	4% / 8	4% / 8	4% / 8	78% / 25
Envelope	E1	NIVNVSLVKPSFYVYSRVKLNLSRVPDLL	0% / 4	0% / 4	0% / 3	0% / 5	0% / 4	35% / 12

**Supplementary Table 3. Response rate of COVID-19 convalescent donor patients to one, two, three, or all four viral antigens targeted by the PolyPEPI-SCoV-2 vaccine, as measured by *ex vivo* FluoroSpot assay.** Nine-mers are the hotspot HLA class I PEPIs embedded within each 30-mer vaccine peptide corresponding to the four structural proteins: S, Spike; N, Nucleoprotein; M, membrane; E, envelope proteins.

Number of reactive antigens (S, N, M, E)	Percentage of subjects responsive to 30-mer peptides (N=17)	Percentage of subjects responsive to 9-mer peptides (N=17)
1	94%	100%
2	82%	53%
3	59%	18%
4	18%	6%

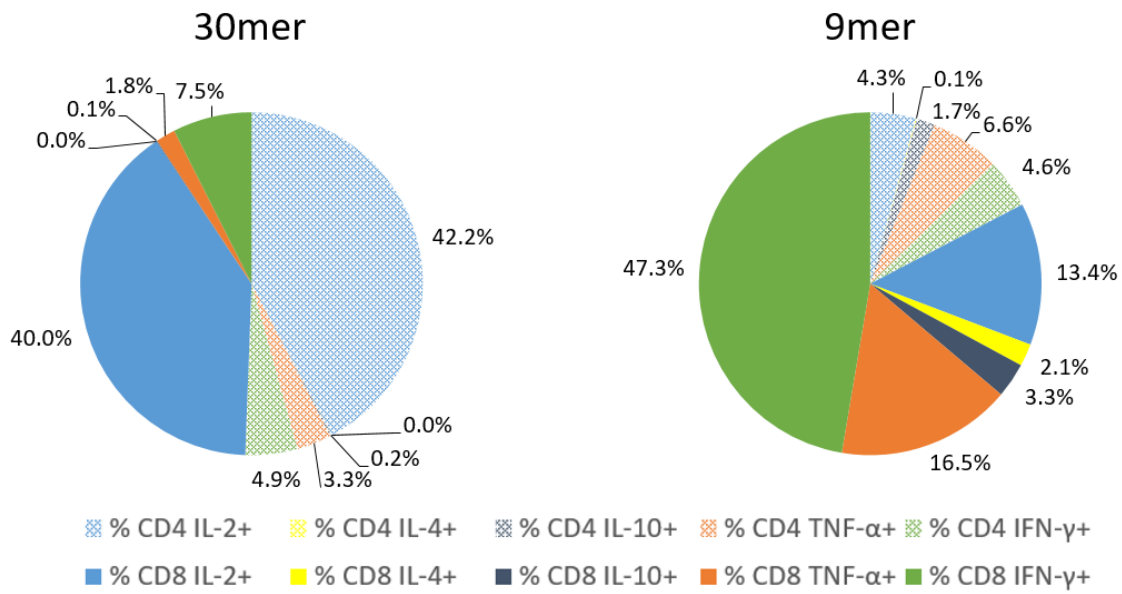


**Supplementary Figure 1. The PolyPEPI-SCoV-2 treatment increases IFN- $\gamma$ -producing T cells in mice.** PolyPEPI-SCoV-2 vaccinated mice are shown with black dots, and compared to Vehicle (DMSO/Water emulsified with Montanide) control animals shown in white dots. IFN- $\gamma$  production was analyzed by *ex vivo* ELISpot in the spleen after re-stimulation with peptides at day 14 (**A**, BALB/c; and **D**, Hu-mice), day 21 (**B**, BALB/c; and **E**, Hu-mice), and day 28 (**C**, BALB/c; and **F**, Hu-mice). Condition 1, S-pool; Spike-specific 30-mer pool of S2, S5, and S9 peptides. Condition 2, N-pool; Nucleoprotein-specific 30-mer pool of N1, N2, N3, and N4 peptides. Condition 3, M1 Membrane-specific 30-mer peptide. Condition 4, E1 Envelope-specific 30-mer peptide. Condition 5, S-pool; Spike-specific 9-mer pool of s2, s5, and s9 HLA class I PEPI hotspot fragment of the corresponding 30-mers. Condition 6, N-pool; Nucleoprotein-specific 9-mer pool of n1, n2, n3, and n4 HLA class I PEPI hotspot fragment of the corresponding 30-mers. Condition 7, m1 Membrane-specific 9-mer HLA class I PEPI hotspot fragment of the corresponding 30-mer. Condition 8, e1 Envelope-specific 9-mer HLA class I PEPI hotspot fragment of the corresponding 30-mer. Condition 9, unstimulated control. Individual spot forming cell (SFC) values and means are shown and represent spots per  $2 \times 10^5$  splenocytes.  $n=6$  mice per group were analyzed. Statistical analysis was performed by Mann-Whitney test. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

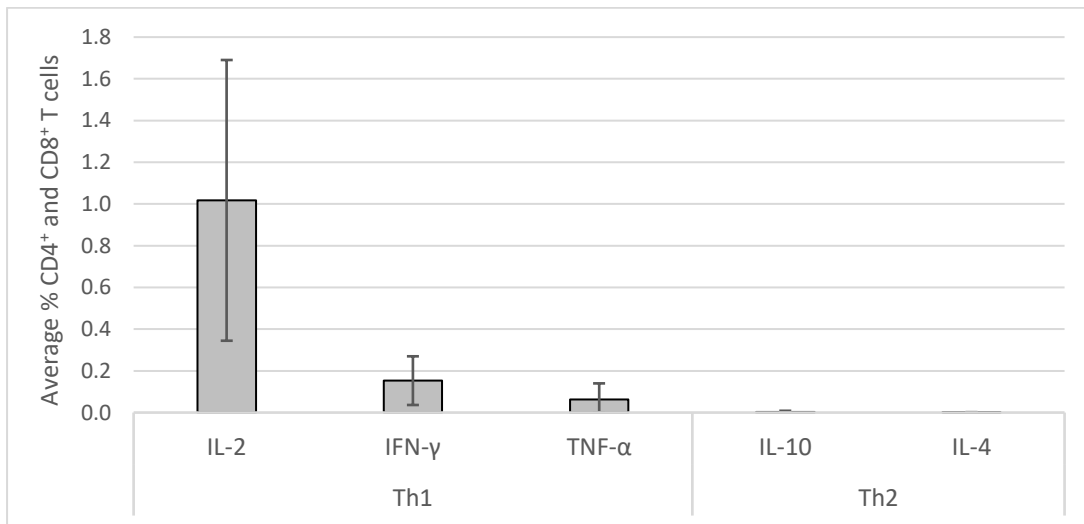


**Supplementary Figure 2. Th1/Th2 balance for T cells detected with PolyPEPI-SCoV-2 vaccine in BALB/c mice at day 28.** Average CD4<sup>+</sup> and CD8<sup>+</sup> T cells producing IL2, TNF- $\alpha$ , IFN- $\gamma$  (Th1 cytokines) and IL10 (Th2 cytokine) for each immunized mice (n=6) using ICS assay.  $2 \times 10^5$  cells were analyzed, gated for CD45<sup>+</sup> cells, CD3<sup>+</sup> T cells, CD4<sup>+</sup> or CD8<sup>+</sup> T cells. The average percent was obtained by pooling the background subtracted values of the 4 stimulation conditions (30-mer S-pool, N-pool, E1 and M1 peptides) for each cytokine for CD4<sup>+</sup> and CD8<sup>+</sup> T cells

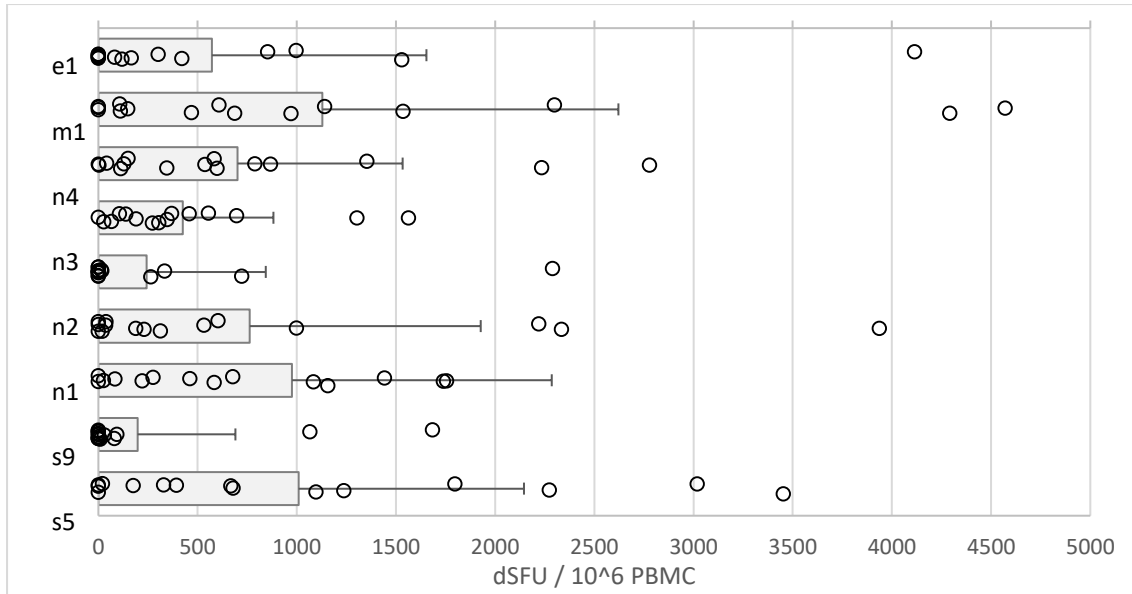
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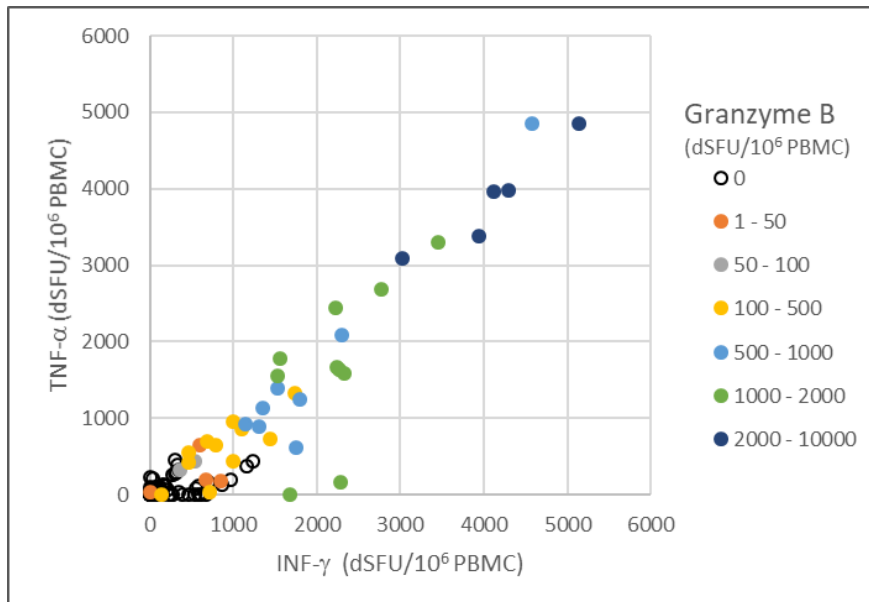
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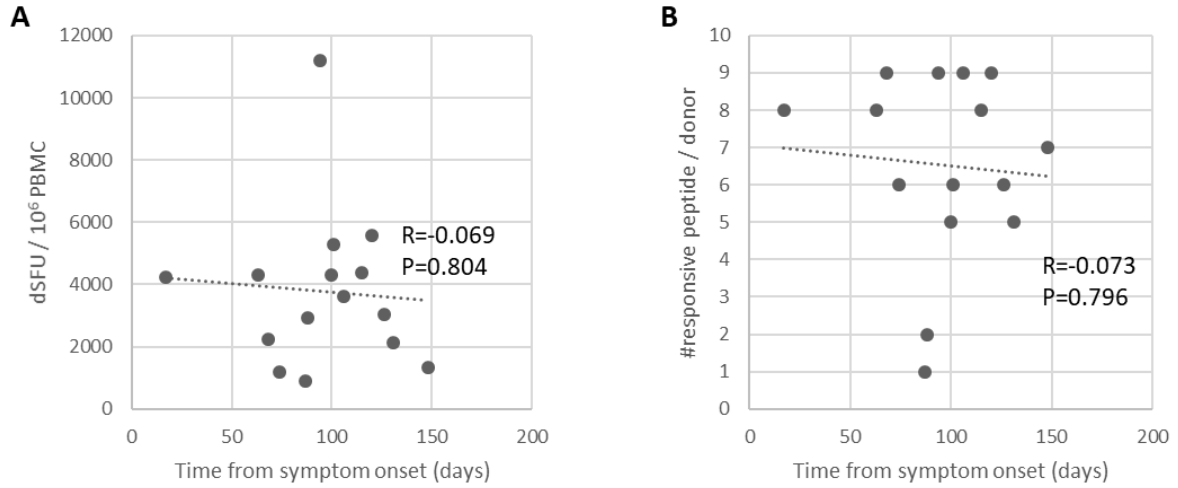
**Supplementary Figure 3. Cytokine production by COVID-19 convalescents' T cells reactive to PolyPEPI-SCoV-2 peptides determined *ex vivo* from their PBMC by intracellular staining assay. A) Cytokine profile of CD4<sup>+</sup> and CD8<sup>+</sup> T cells+ obtained by stimulations with 9-mer and 30-mer peptides (n=17). B) Th1 dominance in vaccine-specific T cells stimulated with 30-mer peptides.**



**Supplementary Figure 4. IFN- $\gamma$  + T cell responses detected for COVID-19 convalescent donors against the 9-mer peptides (PEPI hotspots) of PolyPEPI-SCoV-2 vaccine measured by enriched FluoroSpot assay.** s2, s5, and s9 are the three S-specific 9-mer peptide sequences derived from the Spike-specific vaccine 30-mers. n1–n4 are the four Nucleoprotein-specific 9-mer peptide sequences derived from the N-specific vaccine 30-mers. e1 and m1 are Envelope and Membrane-specific 9-mer peptide sequences derived from the E or M-specific vaccine 30-mers, respectively (Table 1 Bold). dSFU, delta spot forming units calculated as non-stimulated background corrected spot counts per  $10^6$  PBMC. Average and individual data for each subject are presented. PBMC, peripheral blood mononuclear cells.



**Supplementary Figure 5. PolyPEPI-SCoV-2-specific polyfunctional T cells detected in COVID-19 convalescents' blood.** IFN- $\gamma$  and/or TNF- $\alpha$  and/or Granzyme-B positive T cell responses detected for each patient with individual 9-mer peptide stimulations using enriched FluoroSpot assay. dSFU stands for delta spot forming units, calculated as non-stimulated background corrected spot counts per  $10^6$  PBMC.



**Supplementary Figure 6. Magnitude and breadth of COVID-19 convalescent donors' T cell responses relative to time from symptom onset.** **A)** Magnitude of PolyPEPI-SCoV-2-reactive T cell responses **B)** Breadth of vaccine peptide-reactive CD8<sup>+</sup> T cell responses from convalescent donors, detected with enriched ELISpot assay. dSFU stands for delta spot forming units, calculated as non-stimulated background corrected spot counts per 10<sup>6</sup> PBMC. R- Pearson correlation coefficient.