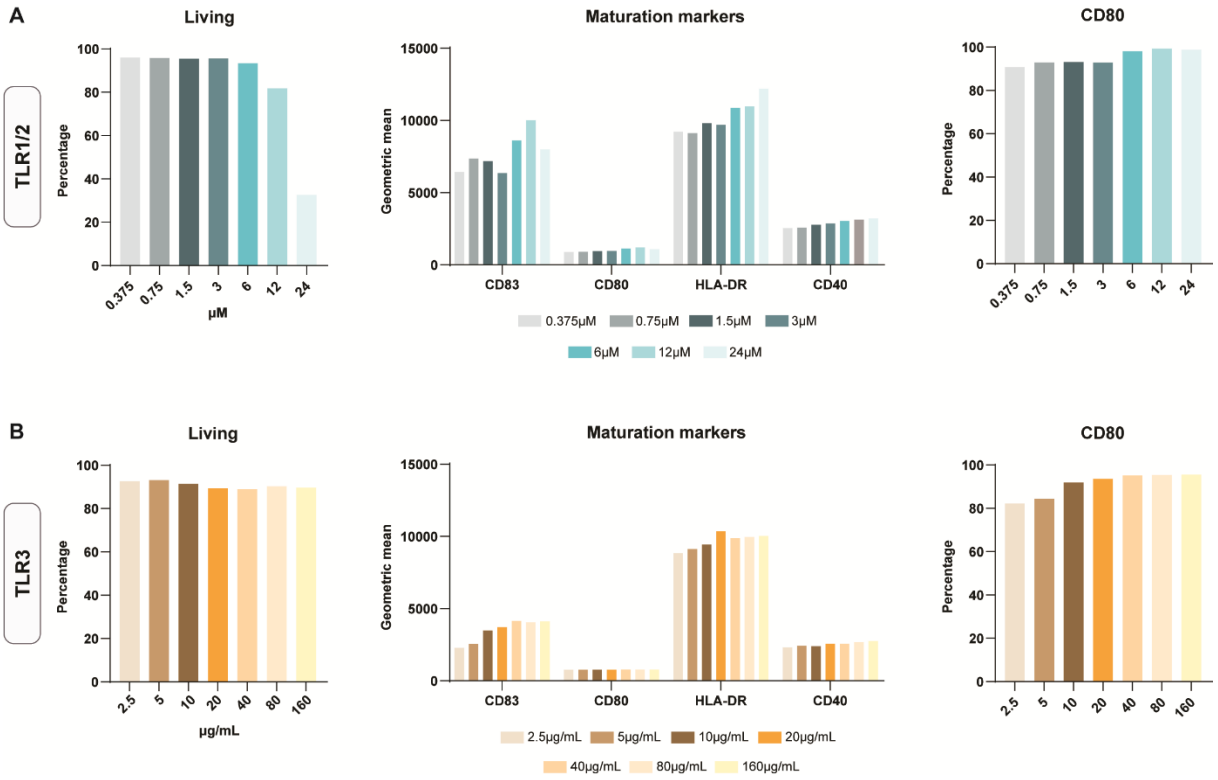
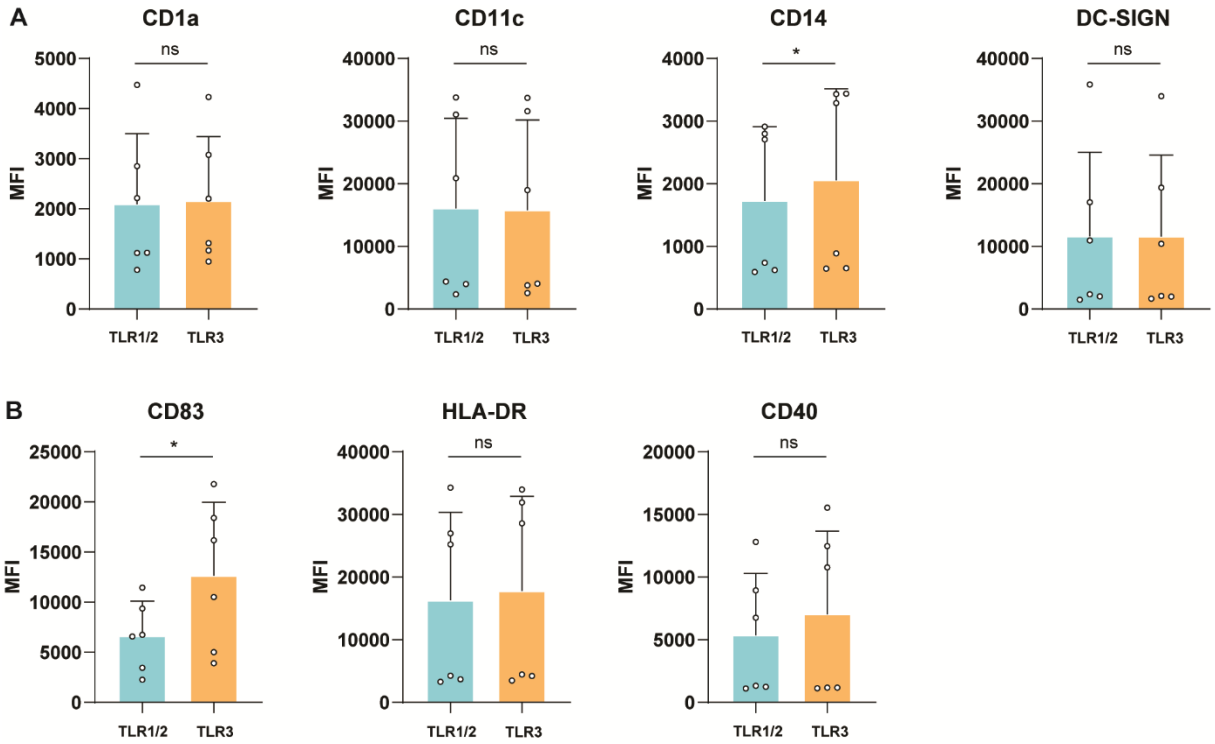
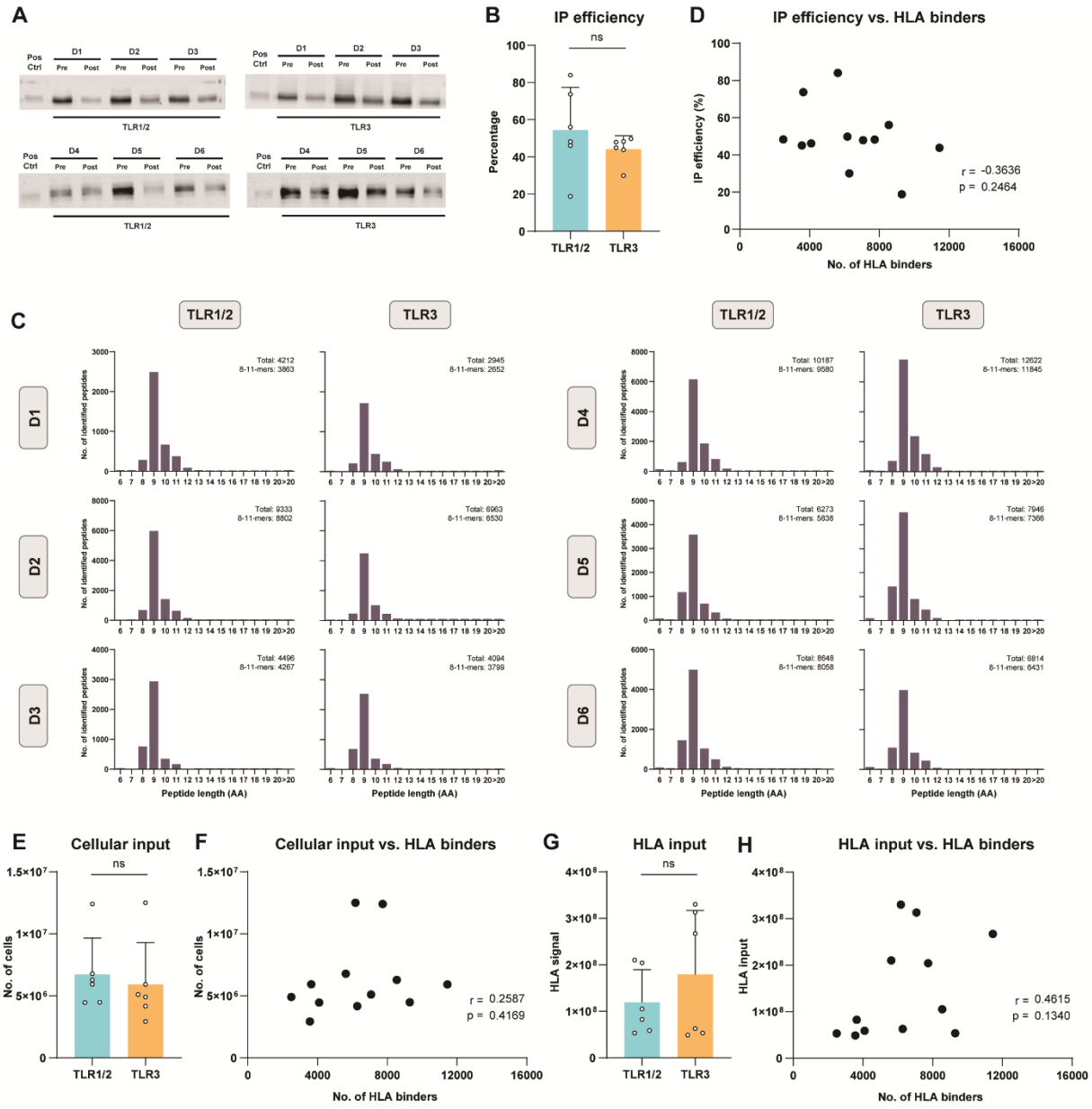
Supplementary figures



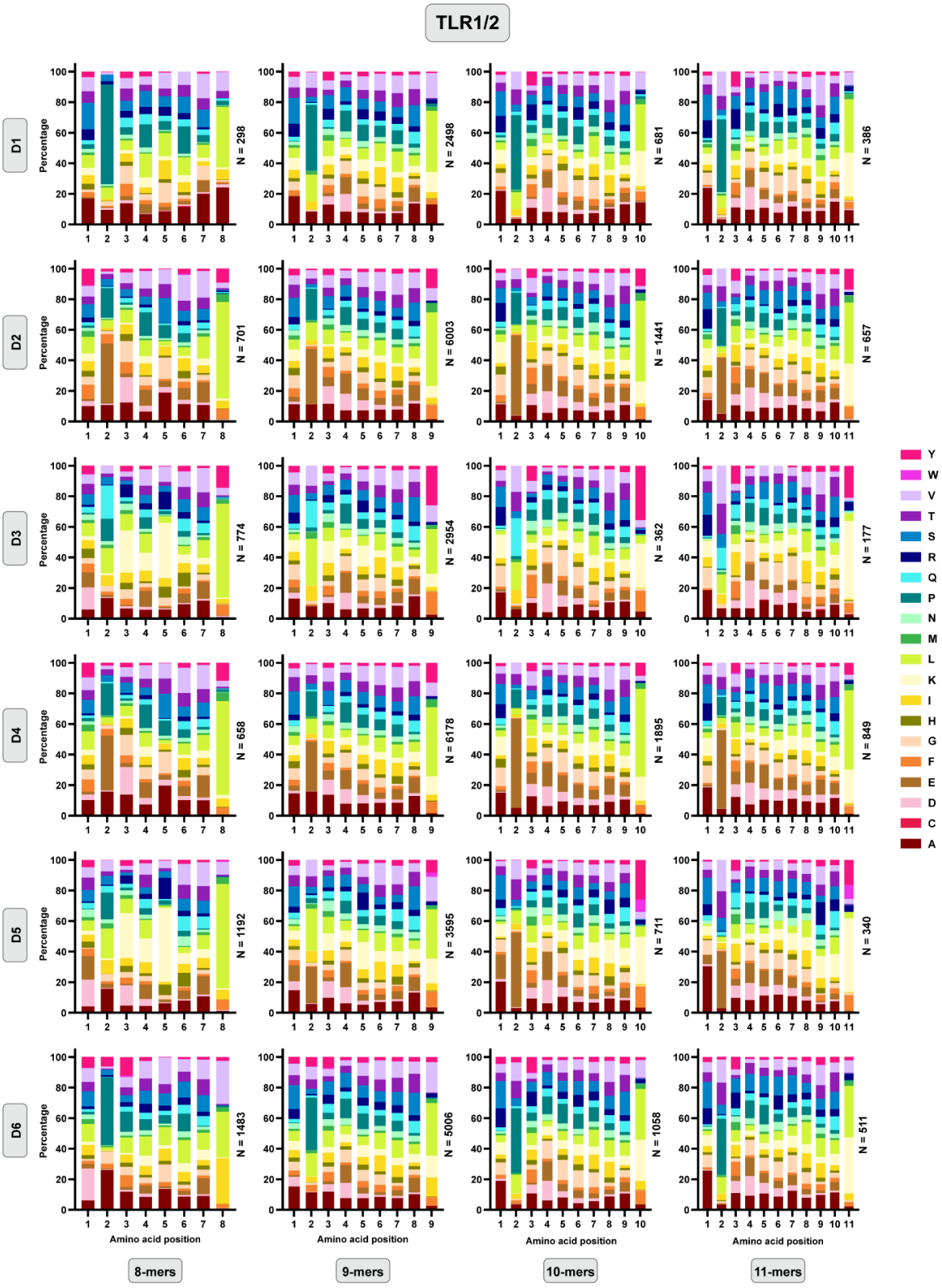
Sup. Fig. 1 | Titration of TLR1/2 ligand Amplivant and TLR3 ligand Poly I:C. Healthy donor moDCs were generated (N=1) and treated for 22 hours with two-fold increasing concentrations of either **A.** Amplivant or **B.** Poly I:C. On day 7 moDCs were harvested and phenotypically characterized by flow cytometric analysis for viability and expression of commonly used DC maturation markers. Expression is given in geometric mean values (mean fluorescence intensity, MFI). Right panels show CD80 expression as percentage of viable cells.



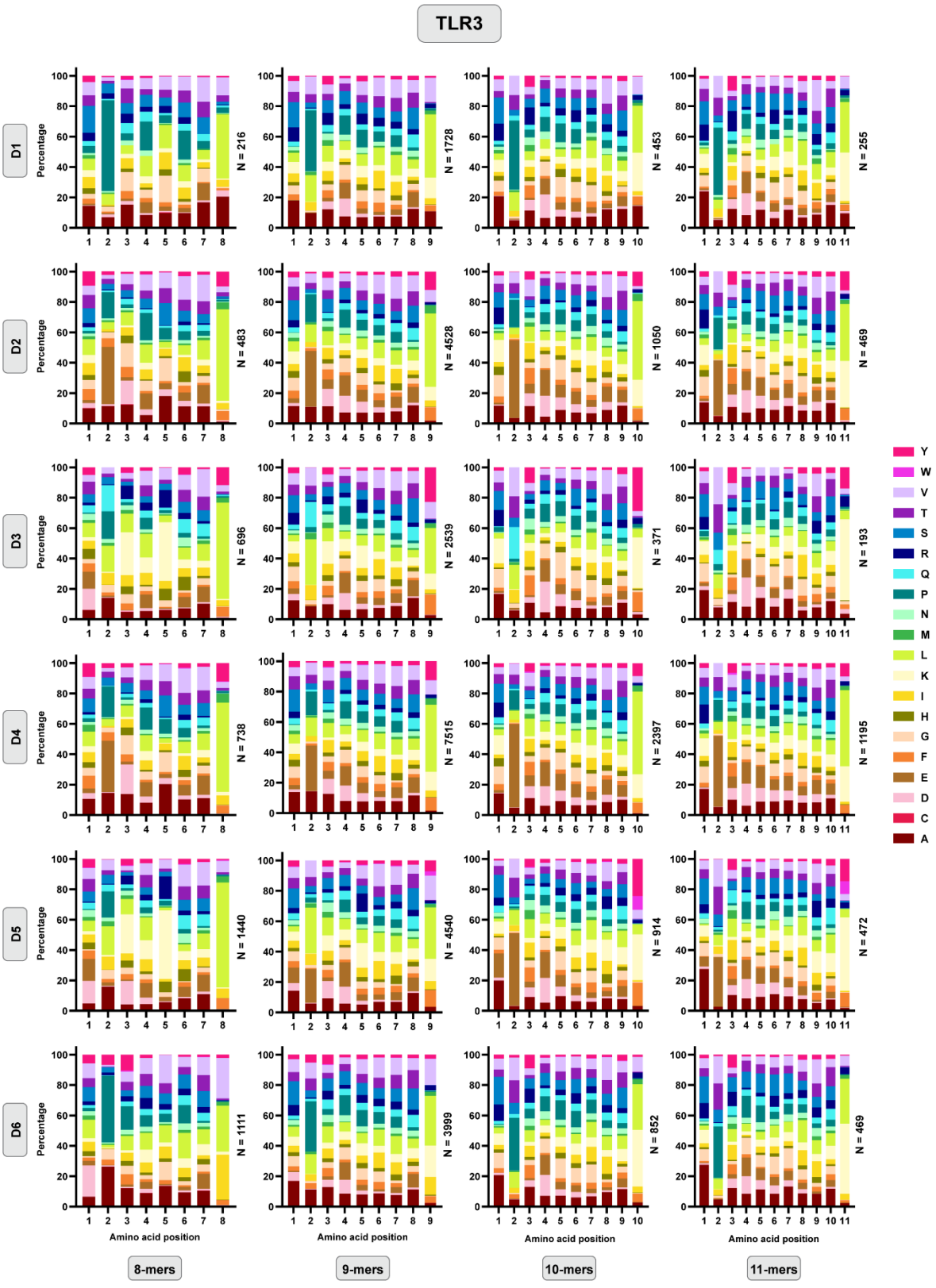
Sup. Fig. 2 | Phenotypical characterization of moDCs upon maturation and pulsing with SLPs. All moDCs were harvested on day 7 and phenotypically characterized by flow cytometric analysis. Mean fluorescent intensity (MFI) was determined for **A.** commonly used DC markers CD1a, CD11c, CD14 and DC-SIGN and **B.** DC maturation markers CD83, HLA-DR and CD40. Bars represent mean ± SD, n=6. Two-tailed paired T-test. Ns = non-significant, * = $p<0.05$.



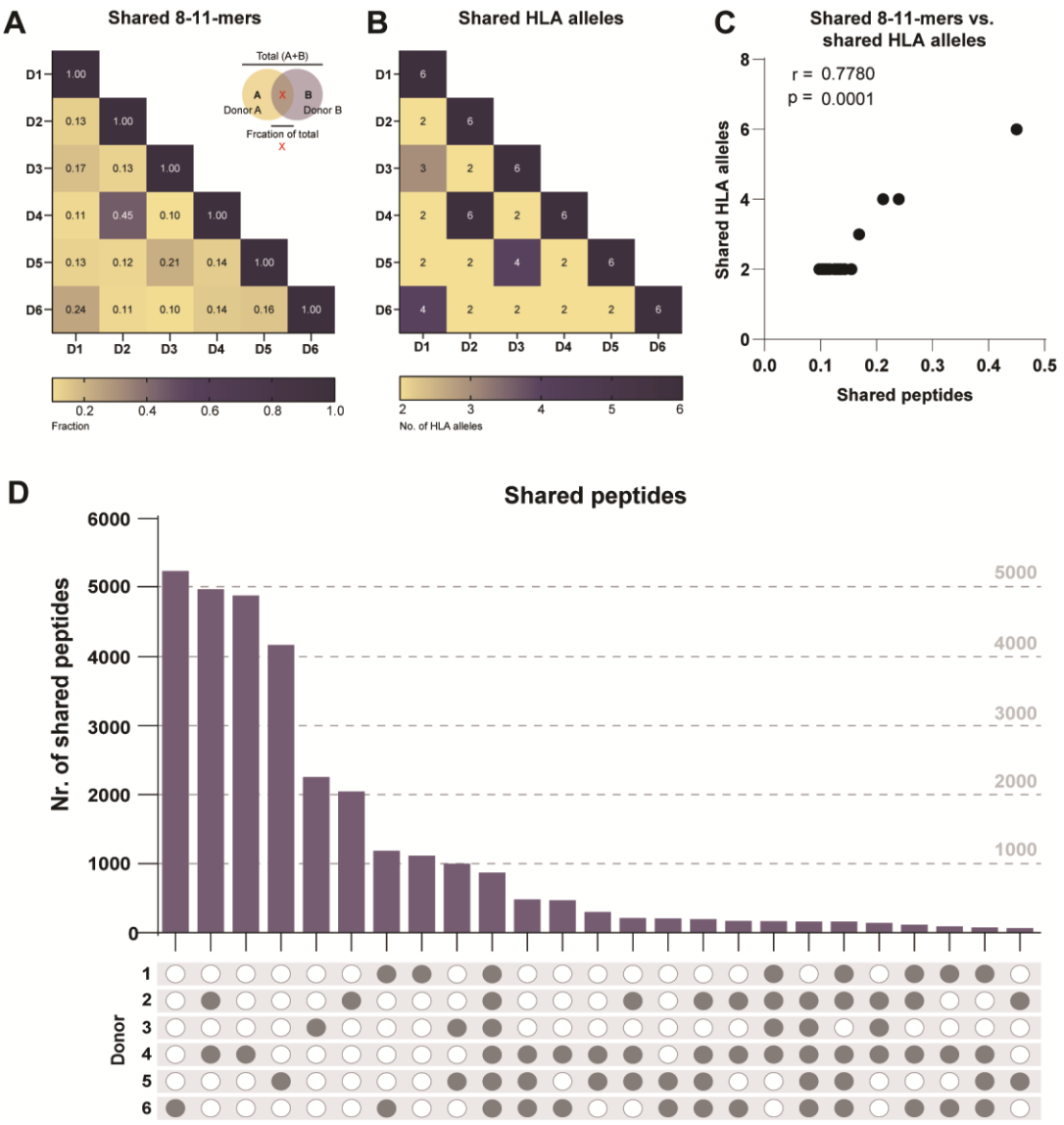
Sup. Fig. 3 | The yield of HLA binders is highly correlated with HLA input. A, B. HLA-I levels in post-nuclear supernatant (PNS) samples were quantified by Western Blot to determine HLA-I IP efficiency. Efficiency was calculated by 100-(Post/Pre*100). "Pos. ctrl"= positive control (PNS 100.000 JY cells), "pre"= pre-IP sample, "post"= post-IP sample. **C.** Peptide length distribution plots for each sample. **D.** Correlation between IP efficiency and the number of predicted HLA binders. **E.** The cellular input per sample used for LC-MS/MS analysis. Determined by correcting total moDC counts for IP efficiency as determined in panel A. **F.** Correlation between cellular input and number of predicted HLA binders. **G.** HLA input per sample for LC-MS/MS analysis. Roughly estimated by multiplying the HLA levels per moDC (blotted HLA-I signal divided by the number of moDCs in PNS) with the cellular input. **B, E, G.** Two-tailed paired T-test. ns = non-significant. **D, F, H.** Two-tailed non-parametric Spearman's rank correlation.



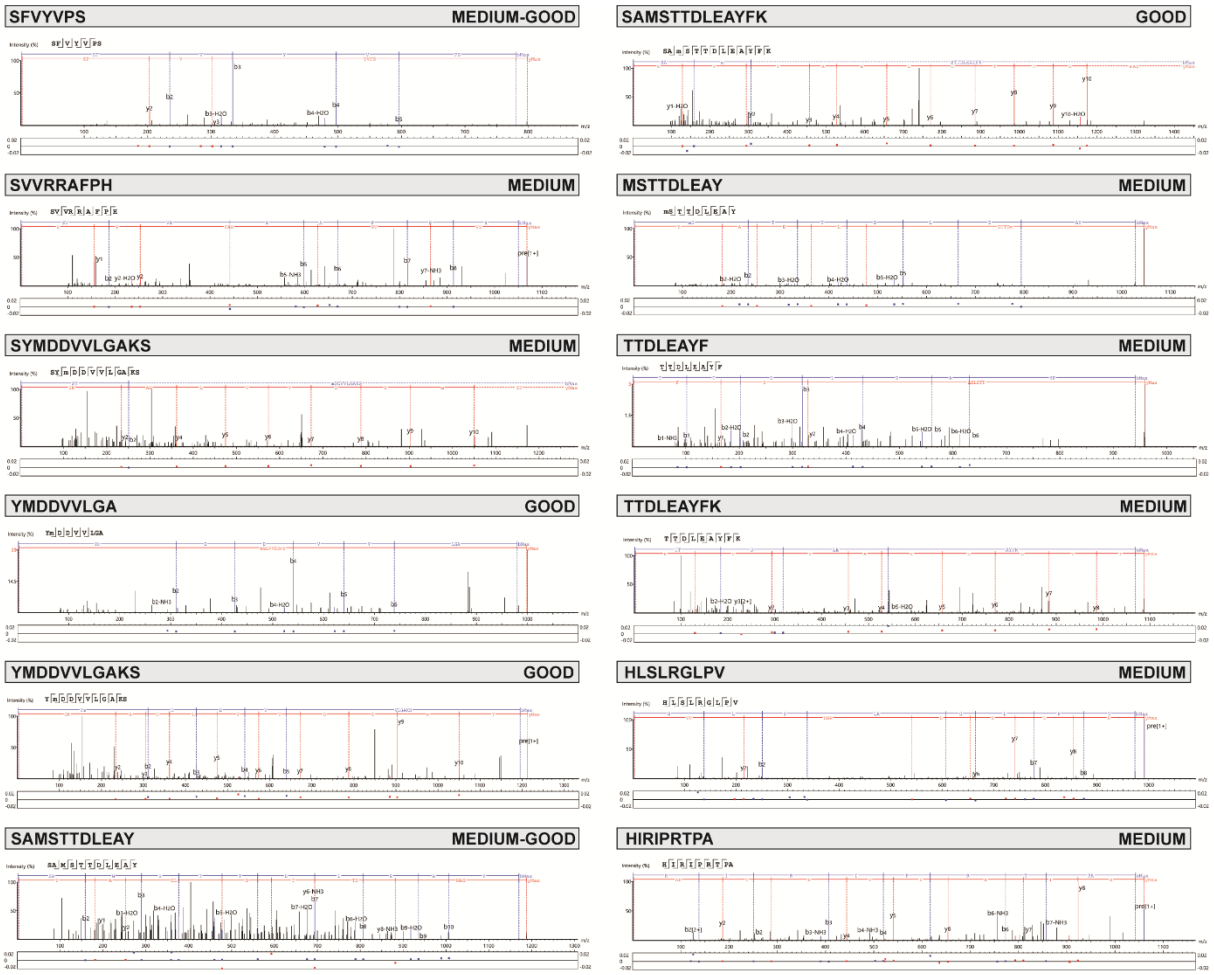
Sup. Fig. 4a | Amino acid distribution of all 8-11-mers in the TLR1/2 dataset. Each color represents 1 of the 20 amino acids. The number of peptides giving rise to each frequency plot is given.



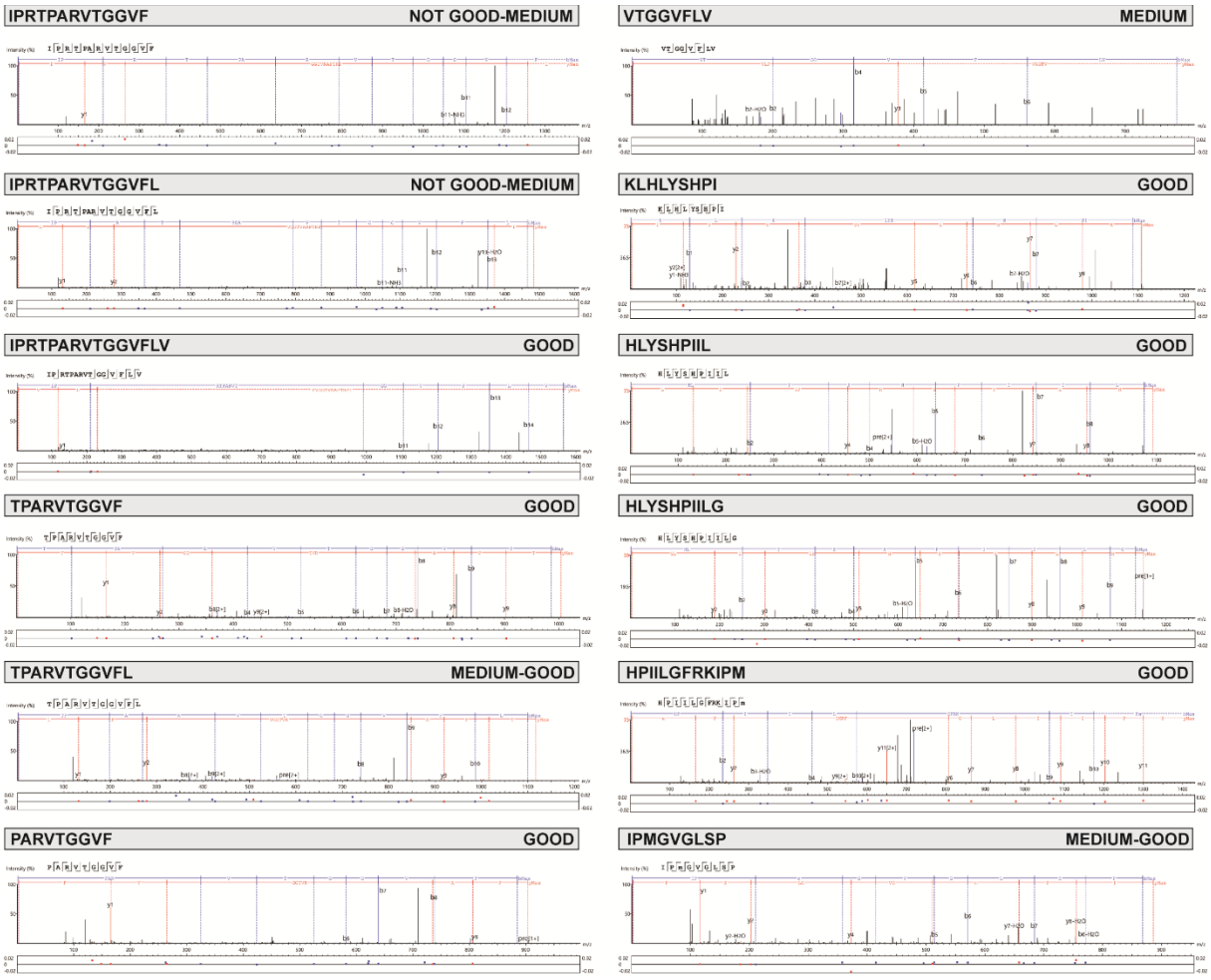
Sup. Fig. 4b | Amino acid distribution of all 8-11-mers in the TLR3 dataset. Each color represents 1 of the 20 amino acids. The number of peptides giving rise to each frequency plot is given.



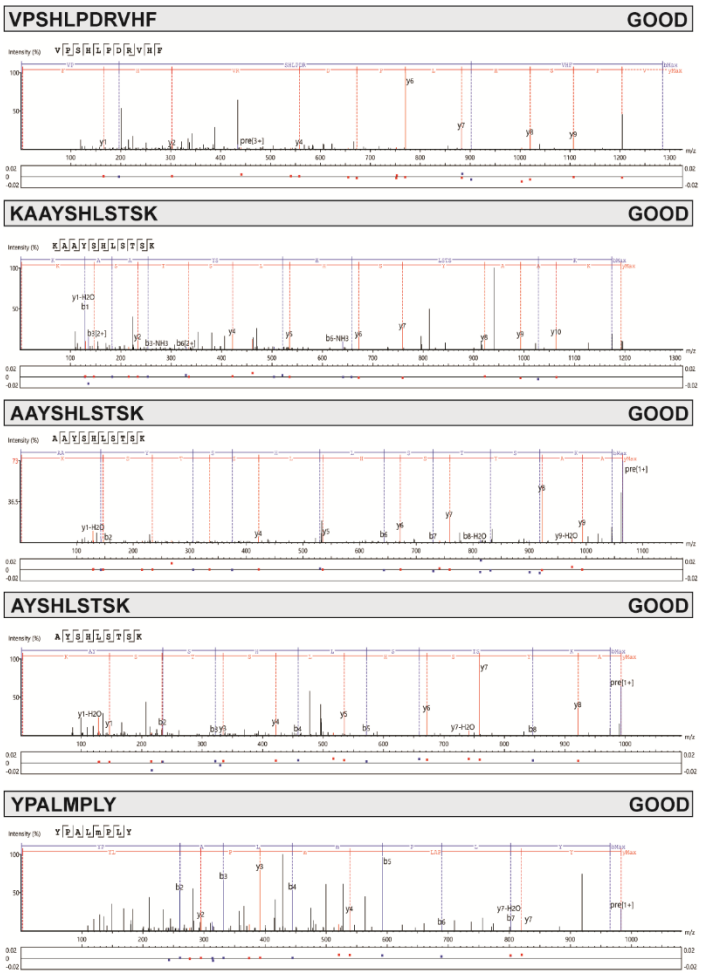
Sup. Fig. 5 | The number of shared 8-11-mers between donors relates to shared B-alleles. A. Correlation matrix of 8-11-mers shared between donors. Overlapping 8-11-mers are given as a fraction (X) of the cumulative number of unique peptide sequences identified in donor A and B. **B.** Correlation matrix of HLA alleles shared between donors (X out of 6). **C.** Correlation plot of the number of shared 8-11-mers and the number of shared HLA alleles. Two-tailed Spearman rank correlation. **D.** Number of (shared) 8-11-mers unique to donors within the dataset. Filled in dots represent peptide identifications within each corresponding donor.



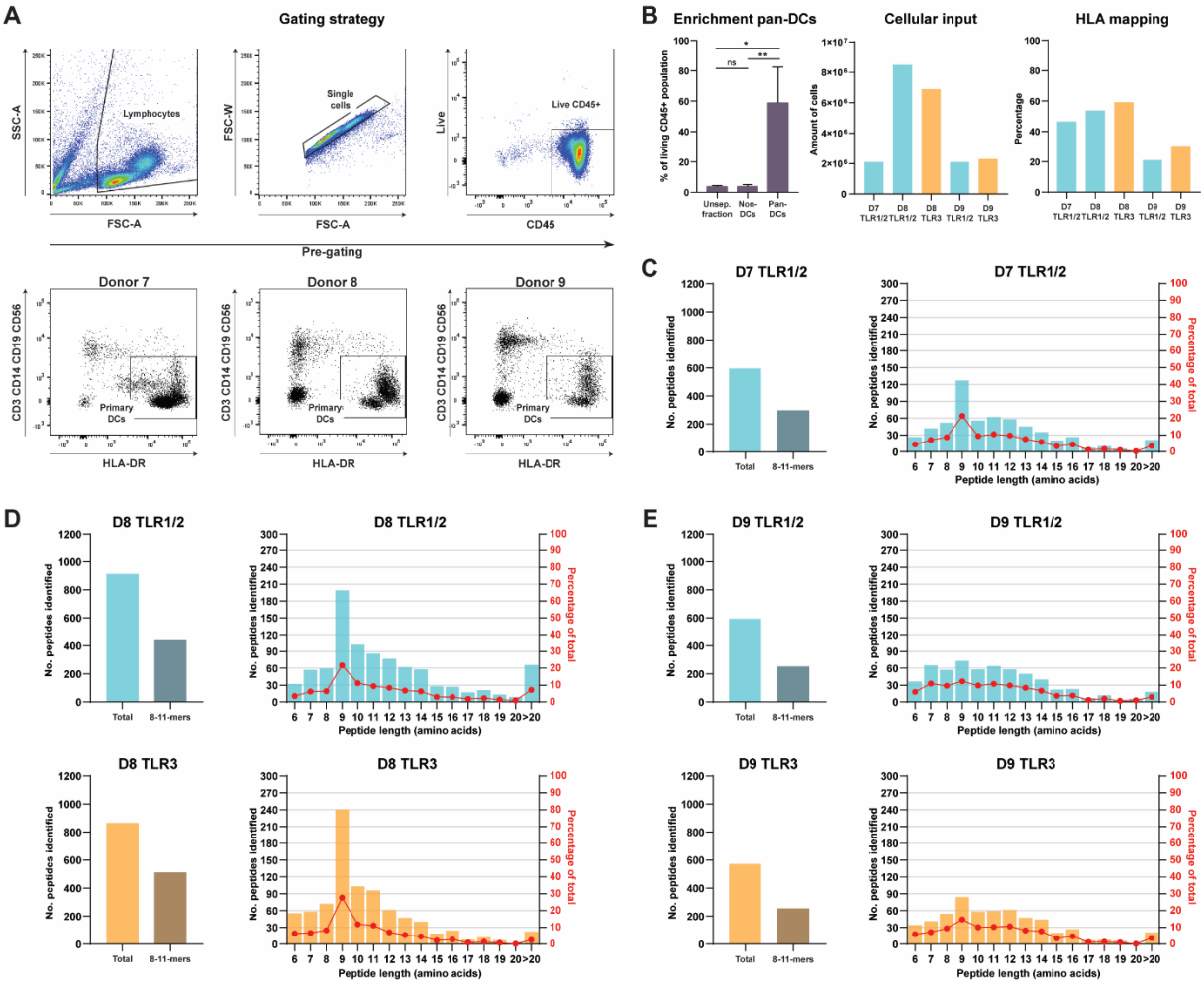
Sup. Fig. 6a | Representative spectra of all SLP-derived peptides. All spectra of SLP-derived peptides were manually checked by an in-house mass spectrometry expert. A representative spectrum was selected of each peptide of sufficient quality.



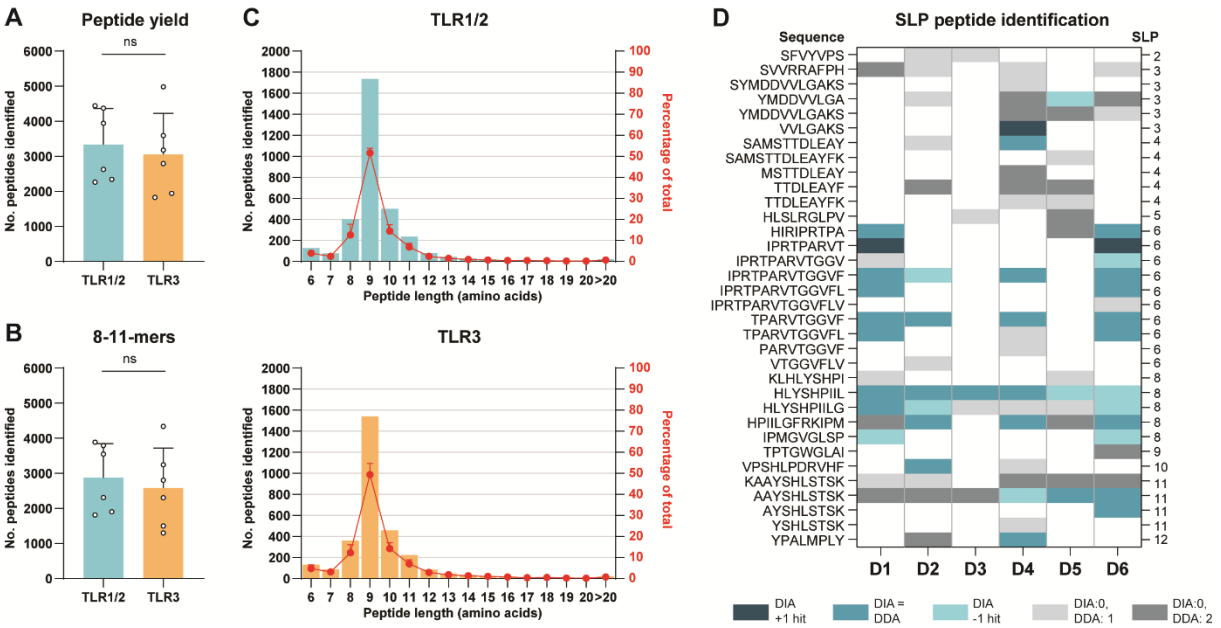
Sup. Fig. 6b | Representative spectra of all SLP-derived peptides. All spectra of SLP-derived peptides were manually checked by an in-house mass spectrometry expert. A representative spectrum was selected of each peptide of sufficient quality.



Sup. Fig. 6c | Representative spectra of all SLP-derived peptides. All spectra of SLP-derived peptides were manually checked by an in-house mass spectrometry expert. A representative spectrum was selected of each peptide of sufficient quality.



Sup. Fig. 7 | HLA-I immunopeptidomics on primary DCs yields lower amount of HLA peptides. A. Gating strategy for flow cytometric analysis of primary DC enrichment upon MACS sorting. Upper three panels are representative of n=3. **B.** The percentage of primary DCs within the alive CD45+ population in the unseparated fraction (pre-MACS), the positive fraction (non-DCs) and the unlabeled negative fraction (primary DCs) (N=3), as well as the cellular input and HLA mapping in all samples. One-way ANOVA, Friedman's post-hoc multiple comparisons test. ns = non-significant, * = p<0.05, ** = p<0.01. **C-E.** The cellular input (total primary DC count) and length distribution per sample. Note: cellular input not corrected for IP efficiency. Bars represent the mean number of peptides, red symbols represent the percentage of total peptide yield (mean ± SD).



Sup. Fig. 8 | Additional DIA revealed three more SLP identifications. A. The total peptide yield and **B.** number of 8-11-mers identified by DIA analysis. Bars represent mean ± SD. Two-tailed paired T-test. ns = non-significant. **C.** Length distributions of TLR1/2 samples (blue) and TLR3 samples (orange). Bars represent the mean number of peptides, red symbols represent the percentage of total peptide yield (mean ± SD). **D.** Overview of additional SLP peptide identifications by DIA. Given in grey are identifications previously made by DDA, but not reproduced by DIA; in light blue identifications where only 1 out of 2 hits could be reproduced by DIA; in dark blue additional identifications by DIA.

SLP sequence	Position	Length
HYFQTRHYLHTLWKAGILYKRETTR	Pol140-164	25
TSFPWLLGCAANWILRGTSFVYVPS	Pol758-782	25
SVVRRAFPHCLAFSYMDDVVLGAKS	Pol535-559	25
LSAMSTTDLEAYFKDCLFKDWEELG	X100-124	25
HLSLRGLPVCAFSSAGPCALRFTSA	X52-76	25
HHIRIPRTPARVTGGVFLVDKNPHN	Pol358-382	25
AARLCCQLDPARDVLCLRPVGAESR	X2-26	25
RKLHLYSHPIILGFRKIPMGVGLSP	Pol499-523	25
ARQRPGLCQVFADATPTGWGLAIGH	Pol688-712	25
SPSVPSHLPDRVHFASPLHVAWRPP	Pol819-843	25
ASSSSSCLHQSAVRKAAYSHLSTSK	Pol269-293	25
GFAAPFTQCGYPALMPLYACIQAKQA	Pol641-666	26

Sup. Table 1 | Overview of synthetic long peptides used in this study. SLPs were designed based on the HBV genome. Twelve SLPs derived from the polymerase and X protein were used in this study ranging between 25-26 amino acids long.

Sample		Cell harvest	IP efficiency	Normalized cellular input	HLA levels (per cell)	HLA input for MS
D1	TLR1/2	8.05E+06	73.7%	5.93E+06	13.9	8.26E+07
	TLR3	10.2E+06	48.3%	4.91E+06	10.9	5.32E+07
D2	TLR1/2	11.2E+06	56.0%	6.28E+06	16.7	10.5E+07
	TLR3	13.9E+06	30.0%	4.17E+06	15.0	6.27E+07
D3	TLR1/2	9.66E+06	46.1%	4.46E+06	13.2	5.89E+07
	TLR3	6.51E+06	45.0%	2.93E+06	16.6	4.87E+07
D4	TLR1/2	23.8E+06	18.8%	4.48E+06	11.9	5.35E+07
	TLR3	13.5E+06	43.8%	5.92E+06	45.1	26.7E+07
D5	TLR1/2	8.08E+06	84.0%	6.78E+06	30.9	21.0E+07
	TLR3	10.7E+06	47.9%	5.11E+06	61.3	31.3E+07
D6	TLR1/2	25.7E+06	48.2%	12.4E+06	16.5	20.4E+07
	TLR3	25.2E+06	49.8%	12.5E+06	26.3	3.30E+07

Sup. Table 2 | HLA-I immunoprecipitation details per sample. For each sample the cell harvest and IP efficiency is stated. The normalized cellular input used for LC-MS/MS analysis is calculated by correcting the cell harvest for the IP efficiency. HLA levels were determined by Western Blot and used to quantify the HLA input for LC-MS/MS analysis by multiplying it by the normalized cellular input.

Term description	Counts	Strength	FDR
Response to virus (GO:0009615)	18/356	0.9	3.18E-07
Defense response to virus (GO:0051607)	15/252	0.98	9.93E-07
Negative regulation of viral genome replication (GO:0045071)	9/56	1.41	1.02E-06
Negative regulation of viral process (GO:0048525)	10/94	1.23	3.17E-06
Regulation of viral life cycle (GO:1903900)	11/139	1.1	6.84E-06
Response to other organism (GO:0051707)	29/1328	0.54	8.16E-06
Response to interferon-alpha (GO:0035455)	6/22	1.64	2.33E-05
Defense response to other organism (GO:0098542)	23/989	0.57	7.50E-05
Immune system process (GO:0002376)	35/2121	0.42	7.82E-05
Immune response (GO:0006955)	26/1321	0.5	2.00E-04
Innate immune response (GO:0045087)	19/754	0.6	3.10E-04
Defense response (GO:0006952)	25/1394	0.45	1.70E-03
Response to interferon-beta (GO:0035456)	5/31	1.41	2.40E-03
Regulation of cytokine-mediated signaling pathway (GO:0001959)	8/152	0.92	5.70E-03
Response to external stimulus (GO:0009605)	33/2355	0.35	5.70E-03
Regulation of response to biotic stimulus (GO:0002831)	11/361	0.68	1.52E-02
Negative regulation of viral life cycle (GO:1903901)	4/27	1.37	2.63E-02
DNA cytosine deamination (GO:0070383)	3/9	1.72	3.16E-02
Regulation of defense response (GO:0031347)	14/638	0.54	3.53E-02
Response to stimulus (GO:0050896)	71/7835	0.16	3.82E-02

Sup. Table 3 | Overview of upregulated biological processes by Gene Ontology analysis of TLR3-specific source proteins. Given from left to right are: term description and GO ID code, source protein counts (identified / contained within category), strength (log-transformed fold increase), and the false discovery rate (FDR).

	A02:01	A11:01	B07:02	B08:01	B15:01	B35:01	B40:01	B44:02	B51:01	B55:01	C03:03	C03:04	C04:01	C05:01	C07:01	C07:02	C14:02
	D1-D6	D1-D6	D1, D6	D3, D5	D3	D2, D4	D2, D4	D5	D6	D1	D1, D3	D2, D4	D2, D4	D5	D3, D5	D1, D6	D6
SVVRRAFPH	35.31	**2.45**	13.95	15.55	7.50	13.29	32.00	31.83	52.57	17.11	18.86	18.86	38.57	45.00	15.11	12.26	12.36
SYMDDVVLGAKS	43.00	**16.48**	75.00	81.43	83.33	75.00	85.00	53.00	80.00	79.23	100.00	100.00	40.50	70.00	45.00	36.67	29.79
YMDDVVLGA	**0.09**	13.74	14.98	7.77	11.46	7.17	10.80	17.58	12.14	3.36	6.53	6.53	0.53	0.40	2.98	3.51	9.68
YMDDVVLGAKS	**4.57**	14.72	57.22	50.61	52.50	55.00	50.00	70.00	63.82	54.38	47.00	47.00	27.35	10.22	60.00	45.00	43.25
SAMSTTDLEAY	36.25	4.48	15.86	41.72	1.26	**0.37**	18.23	8.89	16.51	14.11	8.05	8.05	24.45	20.03	10.19	11.71	9.38
SAMSTTDLEAYFK	72.50	**1.11**	71.25	85.00	50.28	41.33	85.00	47.50	61.47	73.13	37.00	37.00	66.25	42.00	51.67	56.67	52.86
MSTTDLEAY	35.23	6.40	26.19	37.92	1.89	**0.34**	18.19	7.94	16.95	19.31	5.08	5.08	15.57	8.52	4.20	5.68	5.95
TTDLEAYF	29.44	21.05	17.02	17.22	17.87	7.92	16.87	20.52	13.97	54.27	6.11	6.11	0.59	**0.23**	7.01	9.95	9.48
TTDLEAYFK	38.70	**0.30**	53.08	58.79	47.20	21.61	34.67	21.55	48.00	31.64	31.67	31.67	13.60	8.66	14.39	18.78	31.64
HLSLRGLPV	3.63	29.67	4.98	**0.47**	9.39	21.71	29.00	42.00	14.14	5.01	8.76	8.76	11.67	9.84	12.93	14.34	10.00
HIRIPRTPA	18.89	14.97	0.77	2.07	8.69	16.09	37.00	49.50	20.34	**0.24**	9.63	9.63	18.54	29.70	19.53	18.66	14.87
IPRTPARVT	59.55	44.50	0.27	4.56	41.00	4.89	26.25	26.40	2.72	**0.06**	14.94	14.94	10.05	25.41	17.92	21.58	12.00
IPRTPARVTGGV	68.33	85.00	**0.92**	16.49	80.00	31.30	52.50	56.25	5.97	1.53	38.00	38.00	30.86	42.67	49.00	55.00	36.83
IPRTPARVTGGVF	85.00	67.50	**0.47**	10.96	18.54	5.05	38.00	41.00	11.00	5.20	20.46	20.46	19.71	38.67	26.86	28.25	16.20
IPRTPARVTGGVFL	61.88	85.00	**0.55**	9.27	65.00	14.89	41.00	61.67	9.05	5.39	26.60	26.60	18.65	32.67	26.86	21.79	25.13
IPRTPARVTGGVFLV	22.58	85.00	**3.29**	29.24	85.00	40.67	70.00	80.00	8.54	4.26	55.00	55.00	41.00	54.00	62.50	60.00	47.00
TPARVTGGVF	71.25	41.50	**0.19**	6.67	5.33	0.70	27.14	14.69	4.60	2.73	6.82	6.82	12.19	20.94	18.32	22.25	12.92
TPARVTGGVFL	28.89	39.00	**0.58**	9.66	38.90	2.62	12.17	20.43	3.74	3.37	11.29	11.29	8.49	13.66	3.52	5.83	19.02
PARVTGGVF	80.00	67.50	**4.80**	18.53	5.44	8.16	36.33	31.67	12.99	24.67	7.97	7.97	21.45	22.92	22.00	23.86	12.29
VTGGVFLV	10.75	22.67	59.44	47.82	54.44	63.33	60.00	66.67	**8.33**	46.64	21.83	21.83	25.58	9.73	24.44	41.50	36.67
KLHLYSHPI	**1.06**	30.67	10.08	2.97	6.80	32.38	18.77	27.33	16.49	13.33	8.21	8.21	14.06	10.97	12.13	14.03	11.86
HLYSHPIIL	**0.06**	6.31	1.47	0.08	0.64	2.53	2.47	7.53	1.73	1.95	0.21	0.21	0.37	0.68	0.16	0.16	0.53
HLYSHPIILG	**2.98**	29.33	23.56	8.10	14.69	42.33	31.75	49.50	27.05	33.59	15.86	15.86	18.00	19.98	18.27	15.31	22.64
HPIILGFRKIPM	59.55	70.00	**1.49**	3.11	50.00	3.35	53.75	54.00	12.46	4.97	29.67	29.67	39.33	55.00	49.00	51.67	33.89
IPMGVGLSP	36.92	22.61	**0.95**	9.71	29.48	1.31	19.55	26.40	1.23	0.07	14.92	14.92	11.49	21.53	26.00	25.82	18.60
TPTGWGLAI	24.85	28.29	**0.23**	4.31	35.87	0.56	12.88	15.00	0.17	0.33	3.09	3.09	2.50	6.44	8.09	12.21	8.13
VPSHLPDRVHF	31.95	25.60	**0.66**	5.14	11.02	0.71	15.57	8.46	2.21	4.79	10.81	10.81	2.97	10.56	5.05	4.97	8.91
KAAYSHLSTSK	45.33	**0.84**	33.45	68.44	23.82	46.00	62.50	44.00	57.20	36.65	34.00	34.00	30.93	32.67	26.14	20.61	22.06
AAYSHLSTSK	22.86	**0.09**	13.94	33.92	11.05	23.83	23.00	16.86	31.41	6.93	11.74	11.74	18.35	21.50	10.74	12.56	14.51
AYSHLSTSK	32.78	**2.29**	25.20	37.42	19.41	33.88	26.50	15.71	47.80	24.09	32.00	32.00	7.25	39.50	6.76	4.65	3.89
YSHLSTSK	53.67	**3.84**	38.43	37.42	19.26	26.16	52.50	44.00	30.33	14.65	22.67	22.67	18.32	19.52	25.00	27.13	17.42
YPALMPLY	54.67	19.10	3.65	8.95	8.65	**0.08**	20.21	6.50	1.19	2.23	15.55	15.55	2.36	10.95	4.42	2.71	1.93

Sup. Table 4 | HLA mapping of SLP-derived peptides. The HLA mapping of each SLP-derived peptide to all donor HLA alleles was determined with NetMHCpan4.1. A binding rank ≤0.5% is considered a strong binder, 0.5%-≤2% is considered a weak binder. Given in bold are the lowest binding ranks for each peptide (i.e. the highest affinity binding). Green values represent predicted binders, red values represent predicted non-binders.

Supplementary methods

Flow cytometric analysis. Cells were plated out into round-bottom 96-well plates (100.000 cells/well; Corning) and washed once with 200µL block buffer (PBS, 0.1% BSA, 1% human serum (Pan Biotech), 0.02% sodium azide (VWR) before labelling at 4°C for 30 min in the dark with 50µL antibody solution in block buffer. Anti-human protein antibodies used for moDC analysis were: CD45-APCeFluor780 (0.31µg/mL; HI30, Thermo Fisher), CD14-eFluor450 (2µg/mL; 61D3, Thermo Fisher), CD1a-APC (0.15µg/mL; HI149, BD Pharmingen), DC-SIGN-PerCP-Cy5.5 (0.08µg/mL; DCN46, BD Pharmingen), CD11c-FITC (5µg/mL; KB90, Dako), CD80-FITC (5µg/mL; MAB104, Thermo Fisher), CD83-APC (0.67µg/mL; HB15e, Thermo Fisher), HLA-DR-PE (0.02µg/mL; LN3, Thermo Fisher), CD40-PerCP-eFluor710 (0.08µg/mL; 5C3, Thermo Fisher), and the viability dye AQUA Live/Dead-AmCyan (1:200; Invitrogen). Anti-human protein antibodies used for primary DC analysis were: CD45-eFluor450 (0.625µg/mL, HI30, Thermo Fisher), CD19-FITC (2µg/mL, HIB19, Thermo Fisher), CD3-FITC (1.25µg/mL, UCHT1, Thermo Fisher), CD14-FITC (2µg/mL, 61D3, Thermo Fisher), CD56-FITC (0.03µg/mL, NCAM16.2, BD Biosciences), CD11c-PE-Cy7 (4µg/mL, 3.9, Thermo Fisher), HLA-DR-PerCP-Cy5.5 (0.06µg/mL, LN3, Thermo Fisher), BDCA1-APC (1 recommended test volume, AD5-8E7, Miltenyi Biotech), BDCA2-APC (1:10, AC144, Miltenyi Biotech), BDCA3-APC (1.65µg/mL, AD5-14H12, Miltenyi Biotech), and Fc Block (2.5µg/mL, BD Pharmingen). Cells were washed with 200µL block buffer after labelling and measured on the BD FACS Canto (BD Biosciences). Analysis was performed using FlowJo v10.8.1 software (FlowJo).

Generation of pan-HLA-I protein A sepharose beads. The anti-pan-HLA-I antibody W6/32 was produced in-house from hybridoma cells (HB-95, ATCC). Using a P1 peristaltic pump (GE Healthcare)-driven column set-up, the W6/32 antibody-containing medium was pumped over nProtein A sepharose Fast Flow beads (Cytiva) at a concentration of 3.2mg W6/32 antibody per mL packed beads to create pan-HLA-I affinity beads. Antibody crosslinking to beads was performed with 16mM dimethyl pimelimidate dihydrochloride (Sigma Aldrich) in 0.2 sodium borate buffer pH 9.0 for 30 minutes at RT on a rollerbank, before being quenched with 0.2M ethanolamine pH 8.0 for 1 hour at RT on a rollerbank. Conjugated pan-HLA-I affinity beads were washed twice with 0.1M glycine pH 2.5, followed by three washes with RT PBS. Affinity beads were stored as a 50% slurry in PBS + 0.1% sodium azide at 4°C until use.

Extraction of HLA peptides and liquid chromatography tandem mass spectrometry analysis. HLA peptides were eluted from the beads by adding 400µl 0.15% TFA at room temperature. This procedure was repeated 3 times to ensure collection of all peptides. To separate the HLA peptides from proteins, the

extracted HLA peptides were filtered by centrifugation on 10 kD MWCO columns (Microcon-10, MRCPRT010, Millipore). The filtered peptide fraction was desalted on an in-house made 1cc Sep-Pak column containing 10mg C18 and 10mg HLB resin. Peptides were eluted with 28% Acetonitrile with 0.1 % TFA and dried in a vacuum centrifuge. Nanoflow liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed on an EASY-nLC 1200 coupled to an Orbitrap Eclipse Tribid mass spectrometer (Thermo) operating in positive mode. Peptide mixtures were trapped on a 2cm x 100µm Pepmap C18 column (Thermo Fisher) and then separated on an in-house packed 50cm x 75µm capillary column with 2.4µm Reprosil-Pur C18 beads (Dr. Maisch) at a flowrate of 275 nL/min, using a linear gradient of 0–32% acetonitrile (in 0.1% formic acid) during 120 min. MS spectra were acquired from 375 to 1200 m/z in the Orbitrap with 120K resolution. Peptides were fragmented by HCD with a collision energy of 30% and MS/MS spectra were recorded in the Orbitrap with 30K resolution.

Western blot analysis. To determine the efficiency of HLA-I-IP, PNS samples from the equivalent of 185.000 moDCs were taken post-pre-clear and post-HLA-I-IP. Samples were denatured for 5 minutes at 95°C and run on a 10% SDS gel. Proteins were transferred to an Immobilon-FL PVDF membrane (Millipore) and after 1 hour block at RT (Licor) probed for HLA-I presence with 0.25µg/mL anti-human pan-HLA-I antibody (EMR8-5, Abcam) overnight at 4°C. After extensive washing with PBS-0.05% Tween, the membrane was incubated with 0.1µg/mL goat-anti-mouse IgG (IRDye 800CW, Licor) for 1 hour at RT. Finally, the membrane was washed five times with PBS-0.05% Tween and once with PBS, before measurement with the Odyssey DLx Imaging System (Licor). IP efficiency was calculated by dividing the HLA-I signal in the post-IP sample by the HLA-I signal in the pre-IP signal. For HLA binder correlation analysis, '*corrected cellular input'* was determined by correcting the harvest of moDCs for IP efficiency. To estimate *'HLA input',* WB-obtained HLA-I signals were normalized against moDC numbers and then multiplied by the *corrected cellular input*.