

## Supplementary Materials for

### **SARS-CoV-2 mutations in MHC-I–restricted epitopes evade CD8<sup>+</sup> T cell responses**

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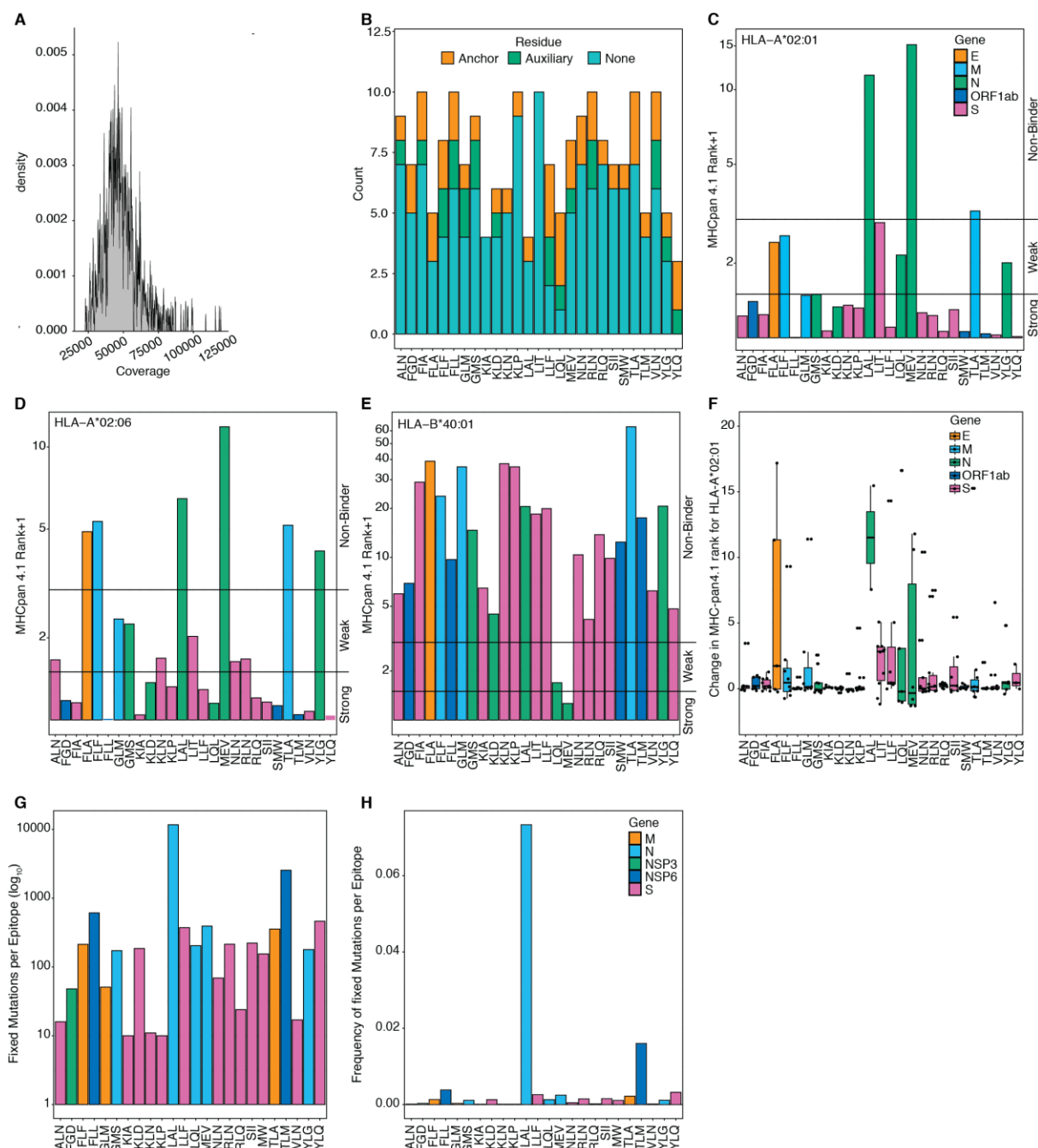
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#### **The PDF file includes:**

Figs. S1 to S5  
Tables S1, S3 to S7

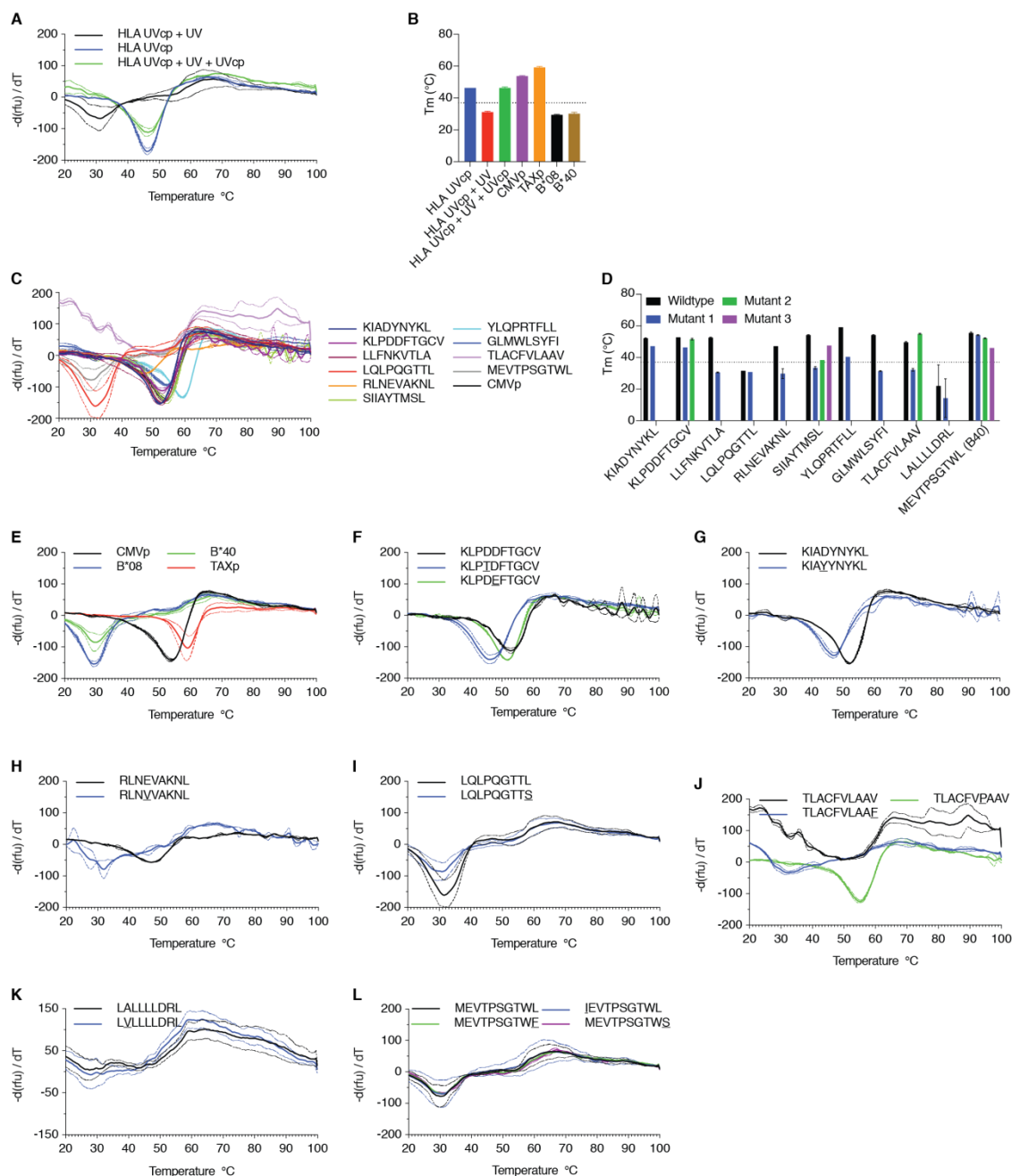
#### **Other Supplementary Material for this manuscript includes the following:**

Tables S2, S8 to S11  
Reproducibility Checklist



**Fig. S1. Supplementary figures for mutation analysis.** **A)** Coverage and read numbers of all sequenced samples. **B)** Mutation counts for specific residues of the epitopes. anchor = anchor residues, auxiliary = auxiliary residues, none = no special residue. **C-E)** netMHCpan binding predictions for the 27 wild type epitopes to HLA-A\*02:01, HLA-A\*02:06 and HLA-B\*40:01,

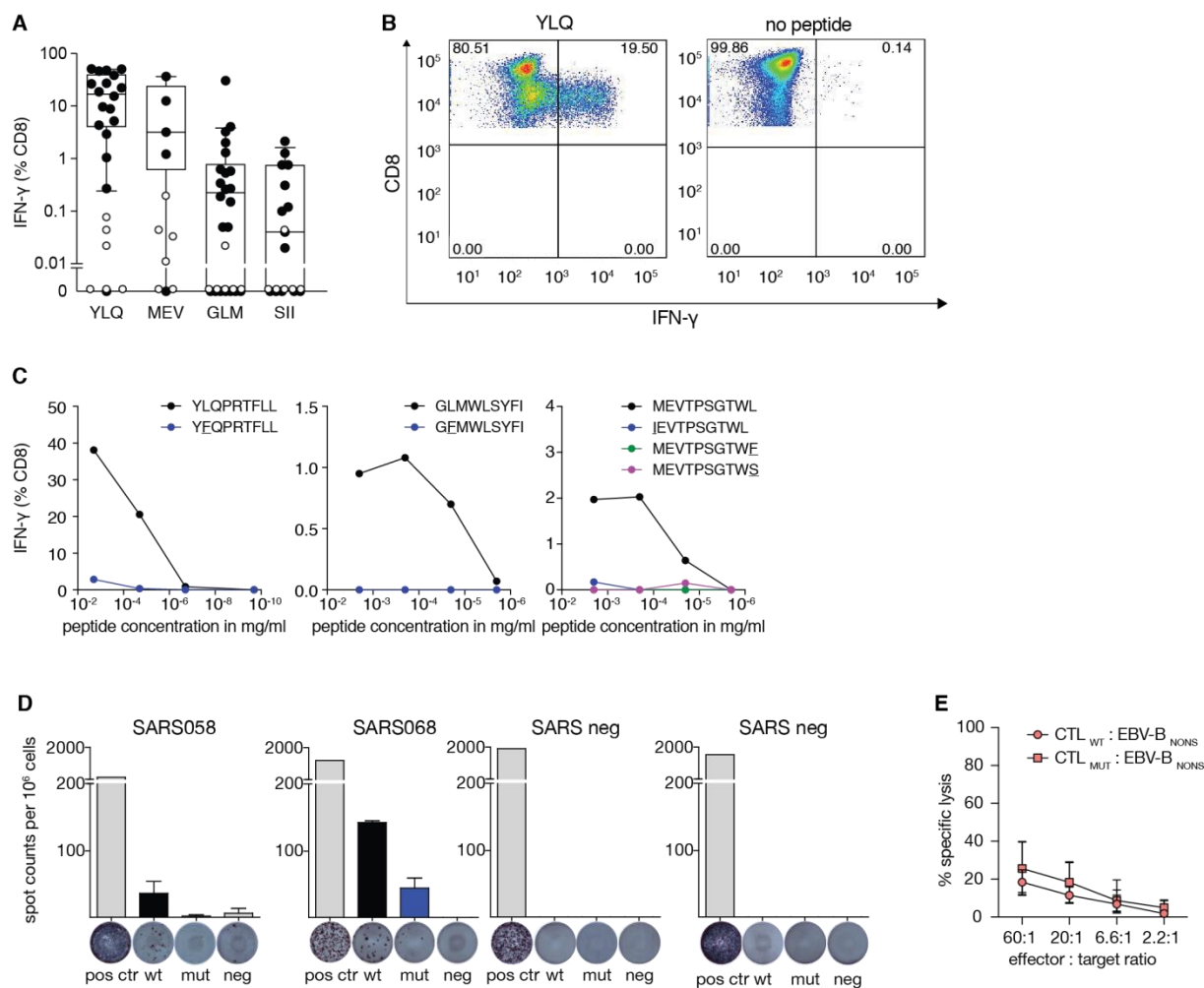
respectively. **F)** change in netMHCpan binding predictions for the mutant epitopes to HLA-A\*02:01. **G-H)** Total counts and frequency of fixed epitope mutations in the global samples.



**Fig. S2. Controls and additional DSF assay results. A)** Positive controls for the DSF assay.

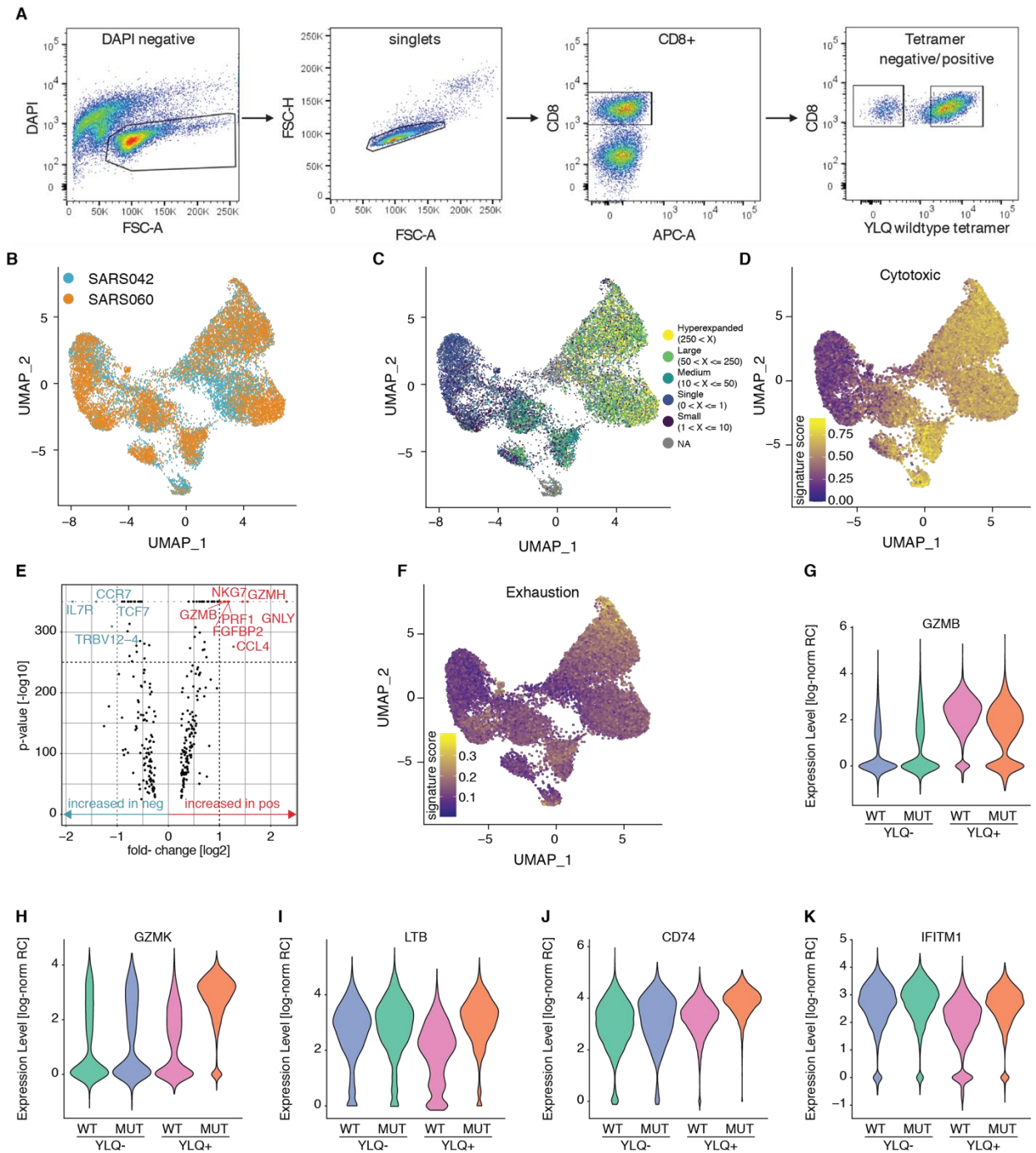
The black curve represents the complex of MHC and an UV cleavable peptide that was exposed to UV light, the blue curve represents the complex of MHC and an UV cleavable peptide not exposed to UV and the green curve represents the complex of MHC and an UV cleavable peptide exposed to UV light, prior to addition of another peptide. **B)** Bar graphs displaying  $T_m$  values of

peptide-MHC complexes for assay controls **C)** DSF curves for all wild type peptides tested. **D)** Bar graphs displaying  $T_m$  values of peptide-MHC complexes for all wild type and mutant peptides tested. **E)** Additional controls for the DSF assay. Two positive controls (CMV and TAX peptides), as well as two negative controls (HLA-B epitopes) complexed with HLA-A\*02:01. **F-K)** DSF data for additional mutant peptides tested. Curves for wild type peptides are black, mutated peptides are colored. The minimal point of the curves represents the melting temperature of peptide-MHC-I complexes. **L)** Additional negative control for the assay. A HLA-B\*40:01 epitope and its mutant forms were complexed with HLA-A\*02:01. Dashed lines for curves and error bars in bar-graphs represent mean  $\pm$  SD. n=2-3 technical replicates for all graphs.



**Fig. S3. Supplementary figures for PBMC analysis.** **A)** Intracellular cytokine staining of PBMCs from COVID-19 patients (black, n=18, 5, 22 and 15) or pre-pandemic controls with unknown HLA status (white, n=6) after 10-12 days *in vitro* expansion and restimulation with wild type peptides as indicated. Boxes show median  $\pm$  25<sup>th</sup> and 75<sup>th</sup> percentile and whiskers indicate 10<sup>th</sup> and 90<sup>th</sup> percentile **B)** Representative FACS plots of the ICS shown for the YLQ epitope and an unstimulated control. **C)** Peptide titrations for three wild type peptides and respective mutants. Peptides were tested in log<sub>10</sub> and log<sub>100</sub> dilutions in ICS assays. n=1 technical replicate. **D)** Representative *ex vivo* ELISpots for YLQ shown from two COVID-19 patients and two healthy donors. Patient IDs are as indicated in Table S6. n=2-3 technical replicates. **E)**

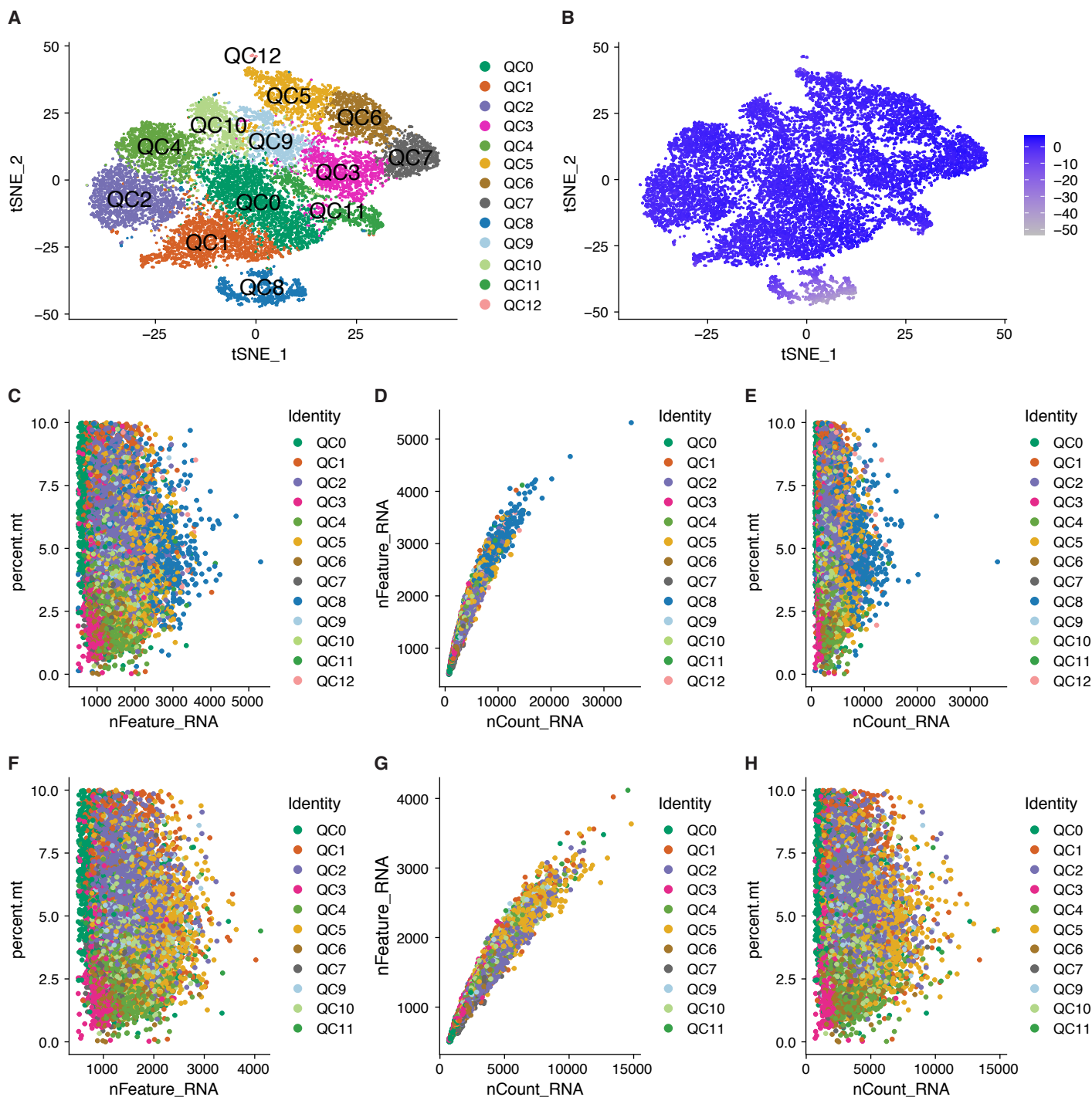
Negative controls for cytotoxicity assay. PBMCs from 4 patients were expanded with wild type or mutant YLQ peptide and co-cultured with autologous EBV<sup>+</sup> B cells that were pulsed with a nonsense peptide as negative control (n=2 technical replicates for each patient). All error bars represent mean  $\pm$  SD.



**Fig. S4. Supplementary figures for scRNA-seq experiment.** A) Gating strategy for sorting tetramer-positive and tetramer-negative CD8<sup>+</sup> T cells. B-D) UMAP plot displaying cells in 2-dimensional space. The cells are colored according to patient (B), to the number of cells with the same TCR as a measure for expansion (C), or the signature score (AUCell score) for the



cytotoxic gene signature (D). **E)** Volcano plots displaying differentially expressed genes between tetramer-positive and tetramer-negative. P-values of 0 were capped to  $10^{-350}$  (indicated by grey dotted line) **F)** UMAP plot showing the signature score (AUCell Score) for the exhaustion gene signature. **G-K)** Violin plot showing expression levels in cells expanded with mutant or wild type peptide. Expression levels given as log-normalized relative read counts (RC). All plots in B-K show combined data from both patients.



**Fig S5. Quality control plots for scRNA-seq analysis and outlier clusters.** **A)** Preliminary t-distributed Stochastic Neighbor Embedding (t-SNE) of single cells showing clusters in 2-dimensional space before removal of outliers. **B)** Differences between Cluster 8 and the rest of the cells dominate the variance in the data set, as reflected by each cell's values along the first component (PC\_1) of a Principal Component Analysis (PCA). The plot shows PC\_1 Values for each cell in the same t-SNE as in A. **C-E)** Quality control plots indicating outlier characteristics

of cluster 8 (shown in blue color). C) Number of genes per cell plotted against percentage of mitochondrial genes. D) Number of genes per cell plotted against counts per cell. E) Number of counts per cell plotted against percentage of mitochondrial genes. **F-H)** Same as C-E, but after removal of clusters 8 and 12.

**Table S1. Wild type epitopes investigated in this study.**

<b>Protein</b>	<b>Start AA</b>	<b>End AA</b>	<b>Sequence</b>	<b>Short</b>	<b>HLA</b>	<b>netMHCpan rank</b>	<b>Reference</b>
<b>ORF1ab</b>	825	833	FGDDTVIEV	FGD	HLA-A*02:01	0.401	(10)
<b>ORF1ab</b>	3639	3647	FLLPSLATV	FLL	HLA-A*02:01	0.0047	(10)
<b>ORF1ab</b>	3710	3718	TLMNVLTIV	TLM	HLA-A*02:01, HLA-A*02:06	0.0398	(12)
<b>ORF1ab</b>	3732	3740	SMWALIISV	SMW	HLA-A*02:01, HLA-A*02:06, HLA-B*52:01	0.0603	(11)
<b>S</b>	269	277	YLQPRTFLL	YLQ	HLA-A*02:01	0.0129	(12)
<b>S</b>	386	395	KLNDLCFTNV	KLN	HLA-A*02:01	0.3539	(10)
<b>S</b>	417	425	KIADYNYKL	KIA	HLA-A*02:01	0.0671	(12)
<b>S</b>	424	433	KLPDDFTGCV	KLP	HLA-A*02:01, HLA-A*02:06	0.3198	(11, 12)
<b>S</b>	691	699	SIIAYTMSL	SII	HLA-A*02:01	0.2998	(11, 12)
<b>S</b>	821	829	LLFNKVTIA	LLF	HLA-A*02:01	0.1053	(12)
<b>S</b>	958	966	ALNTLVKQL	ALN	HLA-A*02:01, HLA-A*02:06	0.2258	(11, 12)
<b>S</b>	976	984	VLNDILSRL	VLN	HLA-A*02:01, HLA-C*01:02	0.028	(11, 12)
<b>S</b>	996	1004	LITGRLQSL	LIT	HLA-A*02:01	1.9126	(11, 12)
<b>S</b>	1000	1008	RLQSLQTYV	RLQ	HLA-A*02:01	0.0622	(10, 12)
<b>S</b>	1185	1193	RLNEVAKNL	RLN	HLA-A*02:01	0.2303	(11, 12)

<b>S</b>	1192	1200	NLNESLIDL	NLN	HLA-A*02:01	0.2624	(11, 12)
<b>S</b>	1220	1228	FIAGLIAIV	FIA	HLA-A*02:01, HLA-A*02:06	0.2409	(10–12)
<b>E</b>	20	27	FLAFVVFL	FLA	HLA-A*02:01	1.4245	(10)
<b>M</b>	26	34	FLFLTWICL	F:F	HLA-A*02:01	1.5782	(10)
<b>M</b>	61	70	TLACFVLA AV	TLA	HLA-A*02:01	2.2459	(11)
<b>M</b>	89	97	GLMWLSYFI	GLM	HLA-A*02:01, HLA-A*02:06	0.4829	(11)
<b>N</b>	112	121	YLGTGPEAGL	YLG	HLA-A*02:01	1.0066	(10)
<b>N</b>	159	167	LQLPQGTTL	LQL	HLA-A*02:01	1.1552	(11)
<b>N</b>	219	227	LALLLLDRL	LAL	HLA-A*02:01	10.4342	(11)
<b>N</b>	316	324	GMSRIGMEV	GMS	HLA-A*02:01	0.4967	(11)
<b>N</b>	322	331	MEVTPSGTWL	MEV	HLA-B*40:01, HLA-B*44:03	0.2638	(4)
<b>N</b>	338	346	KLDDKDPNF	KLD	HLA-A*02:01	0.3328	(10)

**Table S3. Peptides used in the study and their % rank predicted by netMHCpan v4.1. <0.5**

strong binder, 0.5-2 weak binder, >2 non-binder.

AA sequence	Variant	Residue	A*02:01	A*02:06	B*40:01	Source
YLQPRTFLL	Wild type	NA	0.0129	0.0382	3.8259	JPT Peptide Technologies GmbH
YFQPRTFLL	Mutant	anchor	1.8892	2.3099	4.3899	JPT Peptide Technologies GmbH
KLPDDFTGCV	Wild type	NA	0.3198	0.3235	35	JPT Peptide Technologies GmbH
KLPDEFTGCV	Mutant	unknown	0.2982	0.3191	35	JPT Peptide Technologies GmbH
KLPTDFTGCV	Mutant	unknown	0.298	0.319	35	JPT Peptide Technologies GmbH
SIIAYTMSL	Wild type	NA	0.2998	0.1634	8.8673	JPT Peptide Technologies GmbH
PIIAYTMSL	Mutant	unknown	5.7413	5.9459	35	JPT Peptide Technologies GmbH
CIIAYTMSL	Mutant	unknown	2.7464	2.1016	31.75	JPT Peptide Technologies GmbH
STIAYTMSL	Mutant	unknown	1.1866	0.2433	5.6769	JPT Peptide Technologies GmbH
LLFNKVTLA	Wild type	NA	0.1053	0.2926	18.9231	JPT Peptide Technologies GmbH
LFFNKVTLA	Mutant	anchor	5.1733	7.321	23.3333	JPT Peptide Technologies GmbH
TLACFVLA AV	Wild type	NA	2.2459	4.1687	62.5	JPT Peptide Technologies GmbH
TLACFVPA AV	Mutant	none	1.6344	4.3485	49	JPT Peptide Technologies GmbH
TLACFVLA AF	Mutant	none	17.5355	19.9102	39.5	JPT Peptide Technologies GmbH
GLMWLSYFI	Wild type	NA	0.4829	1.3444	35	JPT Peptide Technologies GmbH
GFMWLSYFI	Mutant	anchor	11.8902	19.8449	33	JPT Peptide Technologies GmbH
LQLPQGTTL	Wild type	NA	1.1552	0.1519	0.6958	JPT Peptide Technologies GmbH
LQLPQGTTS	Mutant	anchor	17.8	6.8687	10.4104	JPT Peptide Technologies GmbH
LALLLLDRL	Wild type	NA	10.4342	5.4673	19.5455	In-House
LVLLLLDRL	Mutant	anchor	4.6341	2.2488	22.6667	In-House

MEVTPSGTWL	Wild type	NA	14.1747	10.8642	0.2638	JPT Peptide Technologies GmbH
IEVTPSGTWL	Mutant	unknown	12.8602	9.7767	0.2988	JPT Peptide Technologies GmbH
MEVTPSGTWS	Mutant	unknown	55.9091	42.7647	8.9259	JPT Peptide Technologies GmbH
MEVTPSGTWF	Mutant	unknown	34.7143	24.1466	0.8711	JPT Peptide Technologies GmbH
KIADYNYKL	Wild type	NA	0.0671	0.0444	5.4725	JPT Peptide Technologies GmbH
KIAYYNYKL	Mutant	none	0.9743	1.1082	13.6447	JPT Peptide Technologies GmbH
RLNEVAKNL	Wild type	NA	0.2303	0.674	3.156	JPT Peptide Technologies GmbH
RLNVVAKNL	Mutant	none	2.1559	4.3546	6.4438	JPT Peptide Technologies GmbH

**Table S4. T<sub>m</sub> values for peptides tested in DSF assay.**

<b>Peptide</b>	<b>HLA tested</b>	<b>T<sub>m</sub> (°C) Average</b>	<b>T<sub>m</sub> SD (°C)</b>
CMVp	A*02:01	53.80	0.2
KIADYNYKL	A*02:01	52.20	0.2
KIAYYNYKL	A*02:01	47.20	0
KLPDDFTGCV	A*02:01	52.80	0
KLPTDFTGCV	A*02:01	46.40	0
KLPDEFTGCV	A*02:01	51.60	0.4
LLFNKVTLA	A*02:01	52.60	0.2
LFFNKVTLA	A*02:01	30.60	0.2
LQLPQGTTL	A*02:01	31.60	0
LQLPQGTTS	A*02:01	30.80	0
MEVTPSGTWL (A2)	A*02:01	30.60	0.6
IEVTPSGTWL	A*02:01	30.40	0
MEVTPSGTWF	A*02:01	30.80	0
MEVTPSGTWS	A*02:01	31.20	0.4
RLNEVAKNL	A*02:01	47.20	0
RLNVVAKNL	A*02:01	29.80	2.2
SIIAYTMSL	A*02:01	54.20	0.2
CIIAYTMSL	A*02:01	33.40	0.6
PIIAYTMSL	A*02:01	38.40	0
STIAYTMSL	A*02:01	47.60	0
YLQPRTFLL	A*02:01	59.20	0
YFQPRTFLL	A*02:01	40.40	0
GLMWLSYFI	A*02:01	54.20	0.2
GFMWLSYFI	A*02:01	31.40	0.2
TLACFVLA AV	A*02:01	49.60	0.4
TLACFVLA AF	A*02:01	32.20	0.6
TLACFVPA AV	A*02:01	55.00	0.2
LALLLLDRL	A*02:01	22.00	10.87
LVLLLLDRL	A*02:01	14.27	9.99
MEVTPSGTWL	B*40:01	55.73	0.38
IEVTPSGTWL	B*40:01	54.53	0.5
MEVTPSGTWF	B*40:01	52.27	0.19
MEVTPSGTWS	B*40:01	46.00	0



**Table S5. Characteristics of HLA-A\*02:01/HLA-B\*40:01 positive patients.**

<b>Number of patients</b>		<b>37</b>
<b>Sex [n (%)]</b>	Female	12 (32.4)
	Male	25 (67.6)
<b>Age [years]</b>	Range	22-91
	Median	69
<b>Sample collection date</b>	03/2020-11/2020	
<b>Interval symptom onset to sample collection [weeks]</b>	Range	1-16
<b>Chronic comorbidities [n (%)]</b>	Hypertension	21 (56.8)
	Lung disease	3 (8.1)
	Diabetes	15 (40.5)
<b>Invasive ventilation [n (%)]</b>		14 (37.8)
<b>Death [n (%)]</b>		5 (13.5)

**Table S6. HLA genotypes.**

<b>Patient ID</b>	<b>HLA-A</b>	<b>HLA-A</b>	<b>HLA-B</b>	<b>HLA-B</b>	<b>HLA-C</b>	<b>HLA-C</b>
002	02:01	23:01	37:01	44:03	04:01	06:02
003	02:01	11:01	35:01	40:01	04:01	-
004	01:01	02:01	08:01	35:02	04:01	07:01
005	02:01	-	38:01	-	12:03	-
011	02:01	-	13:02	40:01	03:04	06:02
013	02:01	24:02	51:01	55:01	01:02	14:02
014	02:01	11:01	40:02	52:01	02:02	12:02
017	02:01	29:02	39:01	53:01	06:02	07:02
022	02:01	32:01	07:02	51:01	07:02	14:02
032	02:01	25:01	15:01	51:01	01:02	03:04
033	02:01	29:02	13:02	44:03	06:02	16:01
036	02:01	24:02	13:02	15:01	03:03	06:02
040	02:01	30:01	13:02	51:01	06:02	15:13
041	02:01	03:01	07:02	51:01	07:02	15:02
042	02:01	68:01	07:02	38:01	07:02	12:03
043	02:01	23:01	27:02	51:01	02:02	04:01
044	01:01	02:01	37:01	44:02	01:02	06:02
047	02:01	29:01	07:05	35:03	04:01	15:05
048	02:01	31:01	13:02	37:01	06:02	-
049	02:01	24:02	07:02	51:01	07:02	14:02
050	02:01	24:02	51:01	58:01	03:02	15:04
055	02:01	33:03	15:01	40:01	03:03	03:04
056	24:02	29:02	08:01	40:01	03:04	07:01
058	01:01	02:01	15:01	51:01	03:04	15:02
059	02:01	32:01	15:01	57:01	01:02	06:02
060	02:01	26:01	18:01	38:01	07:01	12:03
061	02:01	32:01	18:01	35:01	04:01	07:01
063	02:01	24:02	35:02	39:01	04:01	12:03
064	02:01	11:01	13:02	35:03	06:02	12:03
066	02:01	29:01	07:05	35:03	04:01	15:05
067	02:01	-	07:02	15:01	01:02	07:02
068	02:01	-	27:05	40:01	02:02	03:04
069	02:01	26:01	27:05	51:01	02:02	14:02
070*	2*	*Donor HLA-typed by flow cytometry				
081	23:01	31:01	40:01	51:01	03:04	12:03
083	02:01	30:01	08:01	13:02	06:02	07:01
085	02:01	68:01	08:01	44:05	07:01	07:02

**Table S7. Overview of ELISpot results for wild type peptides.**

<b>Protein</b>	<b>Start aa</b>	<b>End aa</b>	<b>Sequence</b>	<b>Short</b>	<b>SARS-CoV-2 patients [positive/tested (%)]</b>	<b><u>Pre-pandemic controls</u> [positive/tested (%)]</b>
S	269	277	YLQPRTFLL	YLQ	2/13 (15%)	0/5 (0%)
S	417	425	KIADYNYKL	KIA	0/10 (0%)	0/5 (0%)
S	424	433	KLPDDFTGCV	KLP	0/10 (0%)	0/5 (0%)
S	691	699	SIIAYTMSL	SII	5/13 (38%)	0/5 (0%)
S	821	829	LLFNKVTLA	LLF	0/10 (0%)	0/5 (0%)
S	1185	1193	RLNEVAKNL	RLN	0/13 (0%)	0/5 (0%)
M	61	70	TLACFVLA AV	TLA	0/12 (0%)	0/5 (0%)
M	89	97	GLMWLSYFI	GLM	1/14 (7%)	0/5 (0%)
N	322	331	MEVTPSGTWL	MEV	1/3 (33%)	0/5 (0%)