bioRxiv preprint doi: https://doi.org/10.1101/2024.10.25.620274; this version posted October 29, 2024. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Table S2: **The c259 TCR affinities to the positional scanning peptide library.** Geometric mean, geometric standard deviation across N experiments is reported. K_D values have been excluded if pMHC was unstable (indicated as N/A) or no TCR binding response was observed (indicated as NB).

Position 1					Position 2					Position 3				
Abbr	Sequence	Geo. mean	Geo. SD	N	Abbr	Sequence	Geo. mean	Geo. SD	N	Abbr	Sequence	Geo. mean	Geo. SD	N
1A	ALLMWITQV	2.606	2.781	2	**2A**	SALMWITQV	N/A			**3A**	SLAMWITQV	0.596	1.057	2
1D	DLLMWITQV	18.846	1	1	**2D**	SDLMWITQV	N/A			**3D**	SLDMWITQV	N/A		
1E	ELLMWITQV	N/A			**2E**	SELMWITQV	N/A			**3E**	SLEMWITQV	N/A		
1F	FLLMWITQV	57.473	5.281	2	**2F**	SFLMWITQV	N/A			**3F**	SLFMWITQV	13.441	3.048	3
1G	GLLMWITQV	3.478	3.361	3	**2G**	SGLMWITQV	N/A			**3G**	SLGMWITQV	N/A		
1H	HLLMWITQV	21.339	10.308	3	**2H**	SHLMWITQV	N/A			**3H**	SLHMWITQV	17.216	18.519	2
1I	ILLMWITQV	2.787	6.294	3	**2I**	SILMWITQV	5.397	63.1	2	**3I**	SLIMWITQV	6.639	11.558	3
1K	KLLMWITQV	1.444	8.647	3	**2K**	SKLMWITQV	N/A			**3K**	SLKMWITQV	N/A		
1L	LLLMWITQV	3.7	9.695	3	**2L**	SLLMWITQV	0.701	1.966	3	**3L**	SLLMWITQV	0.701	1.966	3
1M	MLLMWITQV	N/A			**2M**	SMLMWITQV	6.684	23.015	2	**3M**	SLMMWITQV	2.885	3.632	3
1N	NLLMWITQV	0.409	1	1	**2N**	SNLMWITQV	N/A			**3N**	SLNMWITQV	3.951	19.691	2
1P	PLLMWITQV	N/A			**2P**	SPLMWITQV	N/A			**3P**	SLPMWITQV	N/A		
1Q	QLLMWITQV	0.309	1.403	2	**2Q**	SQLMWITQV	2.241	14.094	2	**3Q**	SLQMWITQV	0.688	6.074	2
1R	RLLMWITQV	0.226	1.082	2	**2R**	SRLMWITQV	N/A			**3R**	SLRMWITQV	N/A		
1S	SLLMWITQV	0.701	1.966	3	**2S**	SSLMWITQV	N/A			**3S**	SLSMWITQV	1.82	2.026	3
1T	TLLMWITQV	1.046	3.568	2	**2T**	STLMWITQV	N/A			**3T**	SLTMWITQV	6.228	1	1
1V	VLLMWITQV	0.91	1.746	3	**2V**	SVLMWITQV	50.287	1	1	**3V**	SLVMWITQV	2.657	1	1
1W	WLLMWITQV	11.631	1.329	3	**2W**	SWLMWITQV	33.447	3.481	3	**3W**	SLWMWITQV	9.861	1.691	3
1Y	YLLMWITQV	3.993	1.292	3	**2Y**	SYLMWITQV	18.095	1.288	3	**3Y**	SLYMWITQV	15.39	1.34	3
Position 4					**Position 5**					**Position 6**				
Abbr	Sequence	Geo. mean	Geo. SD	N	Abbr	Sequence	Geo. mean	Geo. SD	N	Abbr	Sequence	Geo. mean	Geo. SD	N
4A	SLLAWITQV	431.199	19.596	2	**5A**	SLLMAITQV	450.833	2.219	2	**6A**	SLLMWATQV	46.1	1.912	3
4D	SLLDWITQV	55.894	4.407	3	**5D**	SLLMDITQV	1779.52	1	1	**6D**	SLLMWDTQV	226.549	1.541	2
4E	SLLEWITQV	335.407	3.105	3	**5E**	SLLMEITQV	406.72	2.54	3	**6E**	SLLMWETQV	971.245	1.531	2
4F	SLLFWITQV	248.389	1.439	3	**5F**	SLLMFITQV	730.463	1.622	3	**6F**	SLLMWFTQV	16.258	4.03	3
4G	SLLGWITQV	111.398	2.492	3	**5G**	SLLMGITQV	658.784	1.293	2	**6G**	SLLMWGTQV	118.893	1	1
4H	SLLHWITQV	161.301	1.704	3	**5H**	SLLMHITQV	1436.123	1	1	**6H**	SLLMWHTQV	61.979	1.624	3
4I	SLLIWITQV	85.441	1.52	3	**5I**	SLLMIITQV	220.473	1	1	**6I**	SLLMWITQV	0.701	1.966	3
4K	SLLKWITQV	22.035	4.9	3	**5K**	SLLMKITQV	654.509	2.109	2	**6K**	SLLMWKTQV	476.51	1.479	3
4L	SLLLWITQV	2.392	17.993	3	**5L**	SLLMLITQV	815.925	1.446	3	**6L**	SLLMWLTQV	5.596	5.279	3
4M	SLLMWITQV	0.701	1.966	3	**5M**	SLLMMITQV	670.428	1.447	2	**6M**	SLLMWMTQV	16.572	16.638	3
4N	SLLNWITQV	26.905	2.998	3	**5N**	SLLMNITQV	758.195	1	1	**6N**	SLLMWNTQV	40.021	2.134	3
4P	SLLPWITQV	241.083	2.6	3	**5P**	SLLMPITQV	N/A			**6P**	SLLMWPTQV	41.366	1	1
4Q	SLLQWITQV	0.927	1.821	3	**5Q**	SLLMQITQV	154.879	1	1	**6Q**	SLLMWQTQV	38.707	1.705	3
4R	SLLRWITQV	604.902	1.555	2	**5R**	SLLMRITQV	4576.089	1	1	**6R**	SLLMWRTQV	389.248	1.019	2
4S	SLLSWITQV	48.243	1.186	3	**5S**	SLLMSITQV	289.89	1.941	3	**6S**	SLLMWSTQV	12.323	2.107	3
4T	SLLTWITQV	2.985	1.985	3	**5T**	SLLMTITQV	584.582	1.56	3	**6T**	SLLMWTTQV	15.427	1.101	3
4V	SLLVWITQV	30.833	1.08	2	**5V**	SLLMVITQV	1048.028	1.686	2	**6V**	SLLMWVTQV	1.796	1.671	3
4W	SLLWWITQV	146.053	1.285	3	**5W**	SLLMWITQV	0.701	1.966	3	**6W**	SLLMWWTQV	9.808	1.354	3
4Y	SLLYWITQV	208.894	1.518	3	**5Y**	SLLMYITQV	243.945	1.92	3	**6Y**	SLLMWYTQV	12.347	1.439	3
Position 7					**Position 8**					**Position 9**				
Abbr	Sequence	Geo. mean	Geo. SD	N	Abbr	Sequence	Geo. mean	Geo. SD	N	Abbr	Sequence	Geo. mean	Geo. SD	N
7A	SLLMWIAQV	15.201	3.121	3	**8A**	SLLMWITAV	126.651	3.221	3	**9A**	SLLMWITQA	6.31	18.975	3
7D	SLLMWIDQV	6.163	9.833	3	**8D**	SLLMWITDV	440.519	1.688	2	**9D**	SLLMWITQD	N/A		
7E	SLLMWIEQV	85.179	1.266	3	**8E**	SLLMWITEV	1758.587	2.616	3	**9E**	SLLMWITQE	N/A		
7F	SLLMWIFQV	408.909	1.802	3	**8F**	SLLMWITFV	961.039	1	1	**9F**	SLLMWITQF	1773.142	1	1
7G	SLLMWIGQV	65.685	9.19	2	**8G**	SLLMWITGV	45.631	3.139	3	**9G**	SLLMWITQG	N/A		
7H	SLLMWIHQV	45.044	4.503	3	**8H**	SLLMWITHV	99.112	1.372	3	**9H**	SLLMWITQH	N/A		
7I	SLLMWIIQV	168.64	2.155	3	**8I**	SLLMWITIV	267.092	1.481	2	**9I**	SLLMWITQI	51.817	1	1
7K	SLLMWIKQV	259.139	1	1	**8K**	SLLMWITKV	305.156	1.099	2	**9K**	SLLMWITQK	N/A		
7L	SLLMWILQV	321.614	1.129	2	**8L**	SLLMWITLV	357.078	2.024	2	**9L**	SLLMWITQL	3.39	1.913	2
7M	SLLMWIMQV	127.392	2.346	3	**8M**	SLLMWITMV	536.124	1.767	3	**9M**	SLLMWITQM	N/A		
7N	SLLMWINQV	20.053	7.134	3	**8N**	SLLMWITNV	N/A			**9N**	SLLMWITQN	N/A		
7P	SLLMWIPQV	83.799	4.303	3	**8P**	SLLMWITPV	N/A			**9P**	SLLMWITQP	N/A		
7Q	SLLMWIQQV	58.27	1.297	3	**8P**	SLLMWITQV	0.701	1.966	3	**9Q**	SLLMWITQQ	4607.455	1	1
7R	SLLMWIRQV	738.23	1.616	2	**8R**	SLLMWITRV	109.572	1.42	3	**9R**	SLLMWITQR	N/A		
7S	SLLMWISQV	1.199	1.839	3	**8S**	SLLMWITSV	122.849	1.544	3	**9S**	SLLMWITQS	N/A		
7T	SLLMWITQV	0.701	1.966	3	**8T**	SLLMWITTV	420.7	2.238	3	**9T**	SLLMWITQT	N/A		
7V	SLLMWIVQV	196.983	2.279	3	**8V**	SLLMWITVV	330.714	5.072	2	**9V**	SLLMWITQV	0.701	1.966	3
7W	SLLMWIWQV	1497.217	4.298	2	**8W**	SLLMWITWV	677.351	3.87	3	**9W**	SLLMWITQW	88.229	1	1
7Y	SLLMWIYQV	1190.897	2.148	3	**8Y**	SLLMWITYV	939.924	3.947	2	**9Y**	SLLMWITQY	N/A		