
Supplementary information

Neoantigen-specific cytotoxic Tr1 CD4 T cells suppress cancer immunotherapy

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Neoantigen-Specific Cytotoxic Tr1 CD4 T Cells Suppress Cancer Immunotherapy

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Supplemental Table 1*.

Sarcoma line	Mouse strain	Sex	MHC allele	Gene	SLP used in the vaccine
T3	129S6	Male	MHC-I (H-2K ^b)	<i>Lama4</i> <i>Alg8</i>	KISFFDGF <u>EVGFNFRTL</u> QPNGLLFYYT AVGITYTW <u>TRLY</u> ASVLTGSL
			MHC-II (I-A ^b)	<i>Itgb1</i>	DDCWFYFTYSVNGY <u>NEAIVHVV</u> ETPDCP
F244	129S6	Male	MHC-I (H-2K ^b)	<i>Pex14</i>	YGALAIIMAGI <u>AFAFHQL</u> YKRYLLPLIL
			MHC-II (I-A ^b)	<i>Plec</i>	LFQAMKKDLIVRYHGVRLLEAQIAT
1956	C57BL/6N	Female	MHC-I (H-2K ^b)	<i>Psm6</i>	AVRENNI <u>APYYEAL</u> CKSLDWQMDVDLL
			MHC-II (I-A ^b)	<i>Cs</i>	TEEQVSWLSREWAKSAALPSHVVTMLDN

Supplemntal Table 1. The synthetic long peptide sequences used in the vaccination studies. The binding core is underlined.

Supplemental Table 2:

gene_symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
Gzmb	7.79577848	6.20656977	38.3530939	6.70E-17	4.35E-13	28.6459567
Ccl5	6.37668419	10.4779208	37.4823444	9.56E-17	4.35E-13	28.0303607
Ccr2	5.36149305	5.37582485	31.7108801	1.27E-15	3.46E-12	25.9475391
Ccl4	5.9192673	4.39843145	30.5417271	2.26E-15	5.15E-12	25.252574
Cxcr6	4.16665655	9.31136991	27.5823146	1.08E-14	1.81E-11	23.9127833
Cx3cr1	5.58745227	3.45148977	27.41215	1.19E-14	1.81E-11	23.495452
Ccr5	4.95203584	4.11218566	26.863922	1.62E-14	2.22E-11	23.4080236
Sema4a	4.01485796	4.96365667	25.5906748	3.42E-14	3.83E-11	22.8320927
Lilr4b	3.65168793	5.09377661	23.648104	1.14E-13	1.04E-10	21.6832626
Chsy1	3.33859925	5.52356244	23.5023243	1.25E-13	1.07E-10	21.6066952
Lilrb4a	3.22837184	5.23095709	23.4027229	1.34E-13	1.08E-10	21.5348027
Serpina3f	4.11857904	4.77752504	22.6056464	2.27E-13	1.72E-10	21.002497
Prdm1	3.84723076	4.05593633	22.5221114	2.40E-13	1.73E-10	20.8891294
Adam8	3.68530006	5.25672282	22.2418791	2.90E-13	1.78E-10	20.780104
Gas7	4.17706937	4.74310449	22.2378928	2.91E-13	1.78E-10	20.7589356
Matn2	3.34521324	4.93090018	22.1934226	3.00E-13	1.78E-10	20.7376913
Csf1	4.52456914	4.1236649	21.946946	3.56E-13	2.02E-10	20.521677
Fgl2	5.59858592	2.08122184	21.532432	4.75E-13	2.53E-10	19.737406
F2r	3.07983266	5.36997056	21.4437754	5.05E-13	2.53E-10	20.2367955
Sytl2	4.50806623	3.68753785	20.85665	7.70E-13	3.63E-10	19.736619
Entpd1	4.51005832	4.66211172	20.7379865	8.39E-13	3.82E-10	19.7231286
Il10	5.96755197	3.29378388	20.6189685	9.16E-13	3.98E-10	19.513229
Tmprss13	4.04427117	3.44690461	20.5842537	9.39E-13	3.98E-10	19.5145997
Prfl	4.21509335	4.67904196	20.5538026	9.60E-13	3.98E-10	19.5914019
Ifngr1	2.95875415	6.7046412	20.4130737	1.07E-12	4.14E-10	19.4988427
Serpina3g	2.82024228	6.61015104	20.341787	1.12E-12	4.14E-10	19.4460221
Ccl3	5.9559615	1.71363753	20.3302471	1.13E-12	4.14E-10	18.8289384
Pik3ap1	5.54598274	2.35476735	20.3073509	1.15E-12	4.14E-10	19.0669082
Tmem37	4.01322046	3.70537292	20.0802304	1.37E-12	4.78E-10	19.1909706
Metnl	5.81401777	1.53743197	19.7873414	1.70E-12	5.82E-10	18.4000307
Kcnk5	3.08996338	5.37159179	18.820205	3.62E-12	1.18E-09	18.278
Pvrig	4.33963367	2.63656695	18.6489051	4.16E-12	1.32E-09	17.9736953
F2rl2	6.50675757	1.88093549	18.5839318	4.38E-12	1.34E-09	17.7210582
Il12rb2	3.83534147	3.00201045	18.1273427	6.36E-12	1.81E-09	17.6372865
Tm6sf1	5.00916387	1.89459447	17.5486841	1.03E-11	2.77E-09	16.9572333
Il2ra	4.21092001	2.67994769	17.039517	1.60E-11	4.06E-09	16.7150539
Ifi214	2.37222728	5.87277348	16.5124884	2.56E-11	6.03E-09	16.2977329
Asb2	2.67313816	6.08816559	16.4001768	2.83E-11	6.55E-09	16.1918711
Arnt2	3.41618314	2.78405558	16.2166394	3.35E-11	7.62E-09	16.025623
Plek	2.68841103	6.26756989	15.911122	4.43E-11	9.61E-09	15.7334148

Slamf1	2.26907491	6.09963603	15.7190605	5.30E-11	1.13E-08	15.5518882
Cish	3.01034924	4.89205644	15.6271994	5.78E-11	1.22E-08	15.4972224
Acsbg1	2.25338654	5.10886351	15.5405172	6.28E-11	1.28E-08	15.4058874
1700017B05Rik	2.40360946	5.03614851	15.4245069	7.01E-11	1.39E-08	15.2964154
L1cam	2.3762347	5.1563049	15.2853618	8.01E-11	1.52E-08	15.1561173
Myadm	2.52370697	4.40047225	15.2651379	8.17E-11	1.53E-08	15.1664084
Plac8	4.69400442	1.97578018	15.2269626	8.48E-11	1.56E-08	15.0363852
Gpld1	4.41018084	1.42172622	14.9952823	1.06E-10	1.91E-08	14.7161919
Foxp3	6.67593825	0.49768022	14.95615	1.10E-10	1.93E-08	14.3576579
Agpat4	2.59736274	5.30698847	14.8835062	1.19E-10	2.03E-08	14.7513372
Mmp9	7.88491289	-1.4754909	14.7845823	1.31E-10	2.18E-08	12.7964156
Ppp3ca	3.22688679	3.23006369	14.7500551	1.35E-10	2.23E-08	14.6857181
Padi2	4.10411701	2.21076553	14.7279411	1.38E-10	2.25E-08	14.6056779
Ifi206	2.71685655	4.29827892	14.7094669	1.41E-10	2.26E-08	14.6206359
Lag3	2.12640653	7.93236832	14.5975333	1.58E-10	2.42E-08	14.4526452
Nkg7	2.44658558	10.5249798	14.5545576	1.64E-10	2.50E-08	14.4526172
St6galnac2	1.85273843	6.65264226	14.3306706	2.06E-10	3.06E-08	14.1588807
Rnase4	2.18340807	5.80110574	14.3140802	2.10E-10	3.08E-08	14.1507536
Cdkn1a	2.98174651	3.36231689	14.2605001	2.22E-10	3.22E-08	14.1956247
Osgin1	2.62082498	3.65248109	14.1305943	2.53E-10	3.64E-08	14.0552096
Arsb	2.59730032	4.24929628	14.100811	2.61E-10	3.72E-08	13.9981948
Eif4e3	2.21941598	4.75603422	14.0596736	2.73E-10	3.73E-08	13.9280537
Fasl	2.25849689	5.22992118	14.0457367	2.77E-10	3.73E-08	13.8891954
Gm6637	3.87506899	2.88140981	13.9499856	3.05E-10	4.01E-08	13.8777268
Heatr9	6.11502225	1.26886884	13.6707745	4.10E-10	5.13E-08	13.4399031
Ctla2a	2.48041252	3.46835009	13.627414	4.29E-10	5.28E-08	13.5330789
Adgrg1	7.43120285	-1.1598239	13.5129827	4.85E-10	5.86E-08	12.1394623
Slc52a3	3.43453099	2.32292711	13.463362	5.11E-10	6.07E-08	13.3545326
Lgals3	2.05311507	8.0858388	13.2632554	6.35E-10	7.30E-08	13.0268422
Aopep	1.99637339	5.5489608	13.2435467	6.49E-10	7.38E-08	13.0004254
Ctla4	1.72929148	7.18086381	13.1459332	7.22E-10	8.08E-08	12.8762072
Slc16a6	3.08902575	3.23107822	13.0455683	8.06E-10	8.88E-08	12.9097536
Kctd12	4.65935369	0.89277497	13.0332062	8.17E-10	8.93E-08	12.721717
Tnfrsf1b	1.94785825	7.11238332	12.8195184	1.04E-09	1.08E-07	12.5023408
Ifit1b1	2.0679745	4.95813864	12.8007924	1.06E-09	1.09E-07	12.5284189
Lamb3	2.12328366	3.99149237	12.7947653	1.07E-09	1.09E-07	12.5864887
Coro2a	2.00191487	5.22396112	12.7606232	1.11E-09	1.13E-07	12.4663744
Laptn4b	2.92868671	2.68975774	12.6867476	1.20E-09	1.22E-07	12.5221371
Jaml	1.97582318	4.7304303	12.5852296	1.35E-09	1.36E-07	12.2936186
Atp2b4	2.35854992	5.09596689	12.5796325	1.36E-09	1.36E-07	12.2632733
Sell	2.09085667	6.19663872	12.4697445	1.54E-09	1.51E-07	12.0915001
Gimap7	1.86134353	8.69090189	12.3962776	1.68E-09	1.62E-07	12.0429238

Dapk2	2.83508611	2.72070164	12.3895716	1.69E-09	1.63E-07	12.1852494
5830411N06Rik	7.34001258	-1.2393906	12.3655955	1.74E-09	1.66E-07	11.1204772
Hip1	2.08800673	5.09132639	12.2676039	1.95E-09	1.83E-07	11.8945554
Glrx	1.81415972	8.25504068	12.2654803	1.95E-09	1.83E-07	11.8762404
Epas1	3.18367635	2.2555568	12.085397	2.41E-09	2.24E-07	11.8367463
Gbp3	1.59837363	6.35826962	12.0632141	2.47E-09	2.28E-07	11.6018118
Ube2l6	2.00172614	5.50857661	11.9866932	2.71E-09	2.48E-07	11.531642
Pglyrp1	1.7387295	6.88566864	11.8914206	3.03E-09	2.72E-07	11.3922948
1500009L16Rik	4.15813747	2.31181096	11.8573016	3.16E-09	2.80E-07	11.570444
Galnt2	1.84386554	5.75993558	11.686182	3.88E-09	3.37E-07	11.1495837
Hid1	2.50403793	2.73649457	11.6773057	3.92E-09	3.39E-07	11.346532
Gbp6	1.68667811	6.15187152	11.5901075	4.36E-09	3.70E-07	11.0187326
Cyth4	1.67284533	7.88273532	11.5823088	4.40E-09	3.71E-07	11.0280033
Ldlr	2.23392071	3.77479499	11.5654981	4.49E-09	3.76E-07	11.139762
Neb	5.78675614	0.53728125	11.4784502	4.99E-09	4.13E-07	10.9773699
Pdgfb	2.61650138	2.36649227	11.4464772	5.19E-09	4.27E-07	11.076962
Ddx28	1.71284923	5.8825719	11.4020162	5.48E-09	4.49E-07	10.7867988
Gm33782	4.13286685	0.25987338	11.3942992	5.54E-09	4.50E-07	10.8174969
Dennd5a	2.31601839	3.15477414	11.3787728	5.64E-09	4.56E-07	10.9565307
D16Ertd472e	1.5483859	5.93489197	11.2870295	6.32E-09	5.02E-07	10.6379733
Txndc5	1.74811471	5.1898307	11.2554215	6.58E-09	5.19E-07	10.6332021
Aplp2	1.67128104	6.17984202	11.1917759	7.12E-09	5.56E-07	10.5096174
Clic4	2.22282807	4.16472221	11.1081218	7.91E-09	6.07E-07	10.5262928
Zyx	1.62175759	8.2360799	11.0660567	8.34E-09	6.33E-07	10.3764716
Gm8818	3.21864796	1.51605073	11.0198082	8.84E-09	6.65E-07	10.5469281
Ccnd3	1.5478576	7.65806898	11.017863	8.87E-09	6.65E-07	10.2971441
Gbp7	1.62813918	7.95329967	10.8756099	1.06E-08	7.89E-07	10.117991
Rab11fip4	1.62105125	6.20768803	10.8283498	1.13E-08	8.34E-07	10.0321098
Rxra	2.37059352	2.83655458	10.7829924	1.20E-08	8.79E-07	10.220406
Klrg1	4.94500112	1.7453238	10.7690739	1.22E-08	8.90E-07	10.2389434
Pea15a	1.83382064	6.80231456	10.6857628	1.36E-08	9.76E-07	9.83969379
Ly6c2	1.71765452	6.57934924	10.6615462	1.40E-08	1.00E-06	9.80604623
Snx9	3.61740626	0.52666925	10.6492949	1.42E-08	1.01E-06	10.0021028
Car5b	1.71920874	4.65201192	10.6340526	1.45E-08	1.02E-06	9.85844117
Lamc1	3.20591411	1.32113242	10.6250011	1.47E-08	1.03E-06	10.046725
Cd38	1.59022386	4.76581943	10.6087905	1.50E-08	1.05E-06	9.81462268
Ctsd	1.70140575	9.81492049	10.5932456	1.53E-08	1.06E-06	9.79366479
Rrm2	1.58636617	4.8318105	10.533385	1.66E-08	1.13E-06	9.70722197
Mxd1	1.63760339	4.83635574	10.5036373	1.72E-08	1.17E-06	9.66651061
Adgre5	1.44680293	8.53755382	10.5015373	1.73E-08	1.17E-06	9.63313407
Ifitm10	1.74830658	4.64325378	10.4831672	1.77E-08	1.19E-06	9.65549839
Ptpv	2.13133601	4.18809862	10.4405811	1.87E-08	1.24E-06	9.6403602

Adam19	1.72708458	6.40806364	10.4389488	1.87E-08	1.24E-06	9.50424373
Socs2	3.70829629	1.16059287	10.3093718	2.23E-08	1.43E-06	9.6367016
Sdcbp2	1.5249421	7.02834811	10.3084703	2.23E-08	1.43E-06	9.3280418
Tmem154	1.35612508	6.43495579	10.2575671	2.39E-08	1.52E-06	9.25396104
Icam1	1.39177897	6.50777695	10.2091924	2.55E-08	1.60E-06	9.18634386
Ccr8	2.35478347	2.48911133	10.1620355	2.71E-08	1.67E-06	9.41994798
Ildr1	1.66738688	4.4205191	10.143168	2.78E-08	1.68E-06	9.20893167
Abi3	1.916699	5.02808665	10.1306898	2.83E-08	1.70E-06	9.13714479
Ranbp10	1.48243416	5.04071783	10.1170202	2.88E-08	1.73E-06	9.11702792
Plekhg3	4.09439108	0.75996467	10.1098293	2.91E-08	1.74E-06	9.35317654
Mcoln2	1.69731968	4.57349816	10.0669969	3.09E-08	1.82E-06	9.08794653
Il12rb1	1.52121892	5.17466414	10.0318099	3.24E-08	1.91E-06	8.9872116
Ifitm3	2.06822463	3.29981446	10.0256963	3.26E-08	1.91E-06	9.16252579
Hopx	1.56494859	4.66259242	9.98830902	3.44E-08	2.01E-06	8.96899547
Bcl2l1	1.36131885	6.70052957	9.95865	3.58E-08	2.08E-06	8.8341646
Slamf7	1.36446995	5.46535279	9.94925401	3.62E-08	2.09E-06	8.85109259
Selplg	1.5905908	10.0312646	9.94714884	3.63E-08	2.09E-06	8.90776429
Rexo5	2.04822137	3.76135919	9.87152272	4.03E-08	2.30E-06	8.89639882
Zfp652	1.60995923	4.35211005	9.81705617	4.35E-08	2.46E-06	8.75634364
Ccrl2	2.17295319	2.59972518	9.76776834	4.65E-08	2.60E-06	8.86849348
Lair1	2.01146762	3.53007623	9.7674206	4.66E-08	2.60E-06	8.77470353
Dusp5	1.72282003	3.99619273	9.76603082	4.67E-08	2.60E-06	8.72087326
Rcbtb2	1.33266803	6.53674914	9.74304049	4.82E-08	2.65E-06	8.52512234
Gbp4	1.42512312	7.02466253	9.73839628	4.85E-08	2.65E-06	8.522451
Sla2	1.3776351	6.44677381	9.72555098	4.94E-08	2.69E-06	8.5002064
Zbtb38	1.33368153	7.11766445	9.6737793	5.31E-08	2.85E-06	8.43057954
Nedd4	3.1604269	0.87563883	9.65223632	5.47E-08	2.93E-06	8.75553863
Lrrc8c	1.29133067	6.82420011	9.61947444	5.73E-08	3.04E-06	8.34725243
Plxdc1	2.62899486	3.00937964	9.60465264	5.85E-08	3.10E-06	8.59946964
Snai3	2.1697131	3.01946256	9.59362147	5.94E-08	3.13E-06	8.58264077
Peak1	1.6076486	4.39650419	9.59180277	5.95E-08	3.13E-06	8.42775649
AA467197	3.19670827	0.5719742	9.56936662	6.14E-08	3.22E-06	8.62471278
Gm33104	3.56864462	0.06774774	9.5091285	6.69E-08	3.46E-06	8.4861011
Cd200r4	2.9166401	1.14251758	9.50042264	6.77E-08	3.49E-06	8.55475537
Ehd1	1.33582648	5.54683121	9.47475293	7.02E-08	3.61E-06	8.1605501
Ppp1r3b	3.10794533	0.93463474	9.44798481	7.30E-08	3.73E-06	8.47855455
Nccrp1	3.81140746	-0.0985932	9.43753973	7.41E-08	3.77E-06	8.36494639
Lrrn4	4.20169257	1.13294329	9.4271785	7.52E-08	3.82E-06	8.45300309
Arl5a	1.46125335	5.32186457	9.39180266	7.91E-08	3.98E-06	8.05231597
Cdk6	1.4602242	7.32295825	9.37164584	8.14E-08	4.09E-06	7.99155084
Ltb4r1	3.97912143	0.27855556	9.35509999	8.33E-08	4.17E-06	8.30769141
Runx3	1.29043763	6.52088719	9.33851629	8.53E-08	4.24E-06	7.93203982

Trex1	1.44893298	5.8317959	9.31430205	8.83E-08	4.37E-06	7.90918595
Gzmc	3.14942775	0.82090464	9.29160857	9.13E-08	4.50E-06	8.25818927
Crybg1	1.30915189	5.69904331	9.23805283	9.86E-08	4.79E-06	7.80092862
Alpk2	3.30779633	0.65964244	9.23669756	9.88E-08	4.79E-06	8.17509153
Apbb1	1.76742466	4.19166459	9.1731394	1.08E-07	5.19E-06	7.83229002
Sord	1.5079561	4.34231327	9.16621684	1.09E-07	5.21E-06	7.80540318
St6galnac1	1.26424391	7.18757645	9.09124913	1.22E-07	5.73E-06	7.56757395
Pitpnc1	1.3245096	7.9755474	9.01388717	1.37E-07	6.29E-06	7.47235715
Cd86	2.43428728	2.63465	9.00356487	1.39E-07	6.34E-06	7.75976484
Srebf2	1.24964124	6.69252382	9.00113646	1.39E-07	6.34E-06	7.42329893
Slc2a3	1.86471357	5.24333794	8.99460707	1.41E-07	6.38E-06	7.46079232
Itgae	2.86049028	1.12398225	8.99123451	1.41E-07	6.39E-06	7.83433713
Podnl1	1.26682102	8.57629266	8.98845886	1.42E-07	6.40E-06	7.45330578
Ccr9	3.79379864	0.21636623	8.98164939	1.43E-07	6.43E-06	7.78788319
Misp3	2.45462653	2.20224892	8.98090314	1.44E-07	6.43E-06	7.76567878
Cela1	1.91214433	3.22817308	8.96681605	1.47E-07	6.53E-06	7.63756046
Nxpe4	3.53606978	0.09491598	8.96119218	1.48E-07	6.55E-06	7.74713955
Prr5l	1.69189572	4.12643371	8.94872894	1.51E-07	6.65E-06	7.50061121
Racgap1	1.30572327	5.65070638	8.91747986	1.58E-07	6.92E-06	7.3165518
Gm20696	2.53198916	1.45075843	8.91215108	1.59E-07	6.94E-06	7.71038455
Capn2	1.29466956	6.70866127	8.91157521	1.59E-07	6.94E-06	7.28600246
Rhoc	1.95057062	3.04430405	8.87039094	1.69E-07	7.33E-06	7.51382039
Serpini1	1.59717548	3.90806752	8.86253147	1.71E-07	7.39E-06	7.39459342
Ptpn4	1.82829389	3.78728731	8.85909516	1.72E-07	7.41E-06	7.4040317
Tapbpl	1.28742457	7.41959106	8.84648365	1.75E-07	7.53E-06	7.19820324
Gm47586	3.26027948	2.56524663	8.81887419	1.83E-07	7.77E-06	7.48918166
AU020206	1.24896849	7.63687516	8.81176991	1.85E-07	7.83E-06	7.15070255
Atf6	1.3801587	4.35835179	8.749243	2.03E-07	8.52E-06	7.16768901
Usp20	1.59911839	4.20295418	8.74643516	2.04E-07	8.53E-06	7.18095742
Lztfl1	1.51318445	3.72647883	8.72327813	2.11E-07	8.80E-06	7.2026268
Fam20a	1.61775108	3.75272469	8.71754909	2.13E-07	8.85E-06	7.19044235
Anxa4	1.42758062	4.42934033	8.70122534	2.18E-07	8.99E-06	7.0852812
Itih5	2.04905098	2.90977882	8.69090392	2.21E-07	9.10E-06	7.25568787

Supplemental Table 2. The top 200 highly expressed genes in cluster-3 cells were derived from bulk RNAseq data in **Fig.3 d**.