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## APPENDICES

**Appendix A. The Allele frequency of the selected HLA alleles used in the study.** The HLA alleles of A, B, and DRB1 haplotypes with frequencies >0.05 were selected for epitope selection. The frequencies presented in range represent the frequencies of the allele in different populations.

HLA Alleles	Allele Frequency (%)
HLA-A*02:01	6.6 - 7.5
HLA-A*02:03	6.9
HLA-A*11:01	13.9 - 16.4
HLA-A*24:02	13.9 - 14.4
HLA-A*24:07	20.7 - 26.4
HLA-A*33:03	8.3 - 16.9
HLA-A*34:01	6.7 - 8.3
HLA-B*15:02	10.7 - 16.7
HLA-B*15:13	11.0 - 12.5
HLA-B*15:21	6.2 - 11.1
HLA-B*18:01	6.4 - 7
HLA-B*35:05	5.6 - 9.0
HLA-B*38:02	5.4 - 6.0
HLA-B*44:03	7.0 - 9.3
HLA-B*58:01	5.9 - 6.0
HLA-DRB1*07:01	7.5 - 13.7
HLA-DRB1*11:01	7.5 - 8.9
HLA-DRB1*12:02	12.5 - 53.4
HLA-DRB1*15:01	6.3 - 12.0
HLA-DRB1*15:02	14.6 - 47.9
HLA-DRB1*16:02	5.0

**Appendix B. The chosen class I epitopes of NY-ESO-1 and MAGE-A10 for vaccine construct.** The class I epitopes were chosen from predicted binders in the 1% percentile rank, with positive immunogenicity value and IC50 of <500 nM. None of the selected class I epitopes was homologous with gut microbiome proteomes.

Protein	Epitope	Allele	Immunogenicity	Binding Affinity (IC50) (nM)	Gut Microbiome Homology
MAGE-A10	ILILSIVFI	HLA-A*02:01	0.09946	107.5	-
	ILILILSIV	HLA-A*02:03	0.08934	61.5	-
	WVQENYLEY	HLA-B*15:02	0.10251	57.8	-
		HLA-B*35:05		27.9	
		HLA-B*15:21		455.2	
	YEDHFPLLF	HLA-B*44:03 HLA-B*18:01	0.12747	209.5 48	-
	YEFLWGPRA	HLA-B*18:01	0.29532	152.7	-
	FPLWYEEAL	HLA-B*35:05	0.4173	18.3	-
	LSIVFIEGY	HLA-B*58:01	0.42832	167	-
	NY-ESO-1	RLLEFYLAM	HLA-A*02:01	0.22117	14.3
LMWITQCFL		HLA-A*02:01	0.15748	38.2	-
LAMPFATPM		HLA-B*15:02	0.10895	16.3	-
		HLA-B*15:21		120.4	
		HLA-B*15:13		273.8	
		HLA-B*58:01		102.3	
LLMWITQCF		HLA-B*35:05	0.20277	474.5	-
		HLA-B*15:02		173.7	
MPFATPMEA	HLA-B*35:05	0.01503	23.9	-	
ITQCFLPVF	HLA-B*58:01	0.02647	148.5	-	

**Appendix C. The selected HLA class II epitopes of NY-ESO-1 and MAGE-A10 for vaccine construct.**

The class II epitopes were predicted binders in 5% percentile rank, with IC50 <1000 nM and able to induce IFN $\gamma$  response. All of the epitopes were non-homologous to gut microbiome proteome.

Peptide	Core	Allele	Binding Affinity (IC50) (nM)	IFN $\gamma$ release	Gut Microbiome Homology
MAGE-A10					
PTGHSFVLVTSLSGLT	FVLVTSLSGL	HLA-DRB1*16:02	62.07	+	-
		HLA-DRB1*07:01	43.19		
		HLA-DRB1*15:02	175.32		
TGHSFVLVTSLSGLTY	FVLVTSLSGL	HLA-DRB1*16:02	58.85	+	-
		HLA-DRB1*07:01	35.68		
		HLA-DRB1*15:02	127.87		
GHSFVLVTSLSGLTYD	FVLVTSLSGL	HLA-DRB1*07:01	32.59	+	-
		HLA-DRB1*16:02	46.63		
		HLA-DRB1*15:01	93.86		
		HLA-DRB1*15:02	101.15		
PARYEFLWGPRAHAE	YEFLWGPRA FLWGPRAHA	HLA-DRB1*11:01,	50.52	+	-
		HLA-DRB1*16:02	71.55		
		HLA-DRB1*15:02	507.96		
		HLA-DRB1*15:01	291.35		
		HLA-DRB1*12:02	811.25		
RYEFLWGPRAHAEIR	FLWGPRAHA	HLA-DRB1*11:01	39.64	+	-
RKMSLLKFLAKVNGS	LLKFLAKVN	HLA-DRB1*11:01	28.2	+	-
KMSLLKFLAKVNGSD	LLKFLAKVN	HLA-DRB1*11:01	30.61	+	-
		HLA-DRB1*12:02	134.74		
MSLLKFLAKVNGSDP	LKFLAKVNG	HLA-DRB1*12:02	217.66	+	-
		HLA-DRB1*11:01	31.44		
SLLKFLAKVNGSDPR	LKFLAKVNG	HLA-DRB1*12:02	317.3	+	-
		HLA-DRB1*11:01	35.28		
RAHAEIRKMSLLKFL	IRKMSLLKF	HLA-DRB1*12:02	80.63	+	-
AHAEIRKMSLLKFLA	IRKMSLLKF	HLA-DRB1*12:02	64.7	+	-
HAEIRKMSLLKFLAK	IRKMSLLKF	HLA-DRB1*12:02	40.18	+	-
AEIRKMSLLKFLAKV	IRKMSLLKF	HLA-DRB1*12:02	43.97	+	-
LESVIRNYEDHFPLL	IRNYEDHFP	HLA-DRB1*15:02	801.56	+	-

		HLA-DRB1*15:01	210.78		
ESVIRNYEDHFPLLF	IRNYEDHFP	HLA-DRB1*15:02 HLA-DRB1*15:01	547.61 147.43	+	-
DPARYEFLWGPRAHA	YEFLWGPRA	HLA-DRB1*15:02 HLA-DRB1*16:02 HLA-DRB1*11:01	475.05 64.95 74.14	+	-
HSFVLVTSLSGLTYDG	FVLVTSLSGL	HLA-DRB1*16:02, HLA-DRB1*07:01, HLA-DRB1*15:02	72.33 42.1 147.76	+	-
SDPARYEFLWGPRAH	YEFLWGPRA	HLA-DRB1*15:02 HLA-DRB1*16:02	707.11 90.4	+	-
ARYEFLWGPRAHAEI	YEFLWGPRA FLWGPRAHA	HLA-DRB1*11:01 HLA-DRB1*16:02 HLA-DRB1*15:02	83.48 43.49 536.81	+	-
SCYPLIPSTPEEVSA	LIPSTPEEV	HLA-DRB1*07:01	345.43	+	-
CYPLIPSTPEEVSAD	LIPSTPEEV	HLA-DRB1*07:01	552.63	+	-
YPLIPSTPEEVSADD	LIPSTPEEV	HLA-DRB1*07:01	582.46	+	-
DPTGHSFVLVTSLSGL	FVLVTSLSGL	HLA-DRB1*07:01	37.97	+	-
QFLLFKYQMKEPITK	FKYQMKEPI	HLA-DRB1*11:01	42.68	+	-
FLLFKYQMKEPITKA	FKYQMKEPI	HLA-DRB1*11:01	35.35	+	-
KAEILESVIRNYEDH	LESVIRNYE	HLA-DRB1*11:01	813.49	+	-
AEILESVIRNYEDHF	LESVIRNYE	HLA-DRB1*11:01	698.1	+	-
YEFLWGPRAHAEIRK	FLWGPRAHA	HLA-DRB1*11:01	64.49	+	-
LLKFLAKVNGSDPRS	FLAKVNGSD	HLA-DRB1*11:01	51.02	+	-
LKFLAKVNGSDPRSF	FLAKVNGSD	HLA-DRB1*11:01	160.02	+	-
PRAHAEIRKMSLLKF	IRKMSLLKF	HLA-DRB1*12:02	104	+	-
ILESVIRNYEDHFPL	IRNYEDHFP	HLA-DRB1*15:01	327.4	+	-
SVIRNYEDHFPLIFS	IRNYEDHFP	HLA-DRB1*15:01	185.6	+	-
NY-ESO-1					
LLKEFTVSGNILTIR	FTVSGNILT	HLA-DRB1*07:01	26.59	+	-
SGNILTIRLTAADHR	ILTIRLTAA	HLA-DRB1*12:02	374.37	+	-
GNILTIRLTAADHRQ	ILTIRLTAA	HLA-DRB1*12:02	408.28	+	-

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ESRLLEFYLAMPFAT	LEFYLAMPF	HLA-DRB1*15:01	39.75	+	-
SRLLEFYLAMPFATP	LEFYLAMPF	HLA-DRB1*15:02	84.8	+	-
		HLA-DRB1*15:01	32.24		

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**Appendix D. The clustered epitopes sequences of NY-ESO-1 and MAGE-A10 with IEDB cluster analysis tool.** The epitopes were aligned together to form sequences from the overlapping sequences.

Protein	Sequence	Epitope										
MAGE-A10	PRAHAEIRKMSLLKFLAKVNGSDPRSF	PRAHAEIRKMSLLKF										
		RAHAEIRKMSLLKFL										
		AHAEIRKMSLLKFLA										
		HAEIRKMSLLKFLAK										
		AEIRKMSLLKFLAKV										
		RKMSLLKFLAKVNGS										
		KMSLLKFLAKVNGSD										
		MSLLKFLAKVNGSDP										
		SLLKFLAKVNGSDPR										
		LLKFLAKVNGSDPRS										
LKFLAKVNGSDPRSF												
MAGE-A10	SDPARYEFLWGPRAHAEIRK	SDPARYEFLWGPRAH										
		DPARYEFLWGPRAHA										
		PARYEFLWGPRAHAE										
		ARYEFLWGPRAHAEI										
		RYEFLWGPRAHAEIR										
		YEFLWGPRAHAEIRK										
		YEFLWGPR										
		MAGE-A10	KAEILES VIRNYEDHFPLLFS	KAEILES VIRNYEDH								
				AEILES VIRNYEDHF								
				ILES VIRNYEDHFPL								
LES VIRNYEDHFPLL												
ES VIRNYEDHFPLLF												
SVIRNYEDHFPLLFS												
YEDHFPLLF												
MAGE-A10	DPTGHSFVLVTSGLTYDG			DPTGHSFVLVTSGL								
				PTGHSFVLVTSGLT								
				TGHSFVLVTSGLTY								
		GHSFVLVTSGLTYD										
		HSFVLVTSGLTYDG										
		MAGE-A10	SCYPLIPSTPEEVSADD	SCYPLIPSTPEEVS								
				CYPLIPSTPEEVSAD								
				YPLIPSTPEEVSADD								
				MAGE-A10	QFLLFKYQMKEPITKA	QFLLFKYQMKEPITK						
						FLLFKYQMKEPITKA						
MAGE-A10	ILILISIVFI					ILILISIV						
						ILILISIVFI						
						MAGE-A10	FPLWYEEAL	FPLWYEEAL				
								MAGE-A10	LSIVFIEGY	LSIVFIEGY		
										MAGE-A10	LSIVFIEGY	LSIVFIEGY
		MAGE-A10	WVQENYLEY									WVQENYLEY

	ESRLLLEFYLAMPFATPMEA	ESRLLLEFYLAMPFAT SRLLLEFYLAMPFATP RLLLEFYLAM LAMPFATPM MPFATPMEA
NY-ESO-1	LLMWITQCFL	LLMWITQCF LMWITQCFL
	SGNILTIRLTAADHRQ	SGNILTIRLTAADHR GNILTIRLTAADHRQ
	ITQCFLPVF	ITQCFLPVF
	LLKEFTVSGNILTIR	LLKEFTVSGNILTIR

**Appendix E. Affirmation of the vaccine HLA class I and II epitopes presentation.** The vaccine was checked for its epitope ability to be presented. NB represents non-binder while B represents binder in a determined threshold of 1% and 5% for class I and II epitopes respectively.

Peptide	Allele	HLA binder in vaccine	Binding Affinity (IC50) in vaccine (nM)	Native Protein Binding affinity (IC50) (nM)	Note
MAGE-A10					
ILILSIVFI	HLA-A*02:01	NB	45	107.5	%rank in native protein: 0.8 %rank in vaccine: 1.00
ILILILSIV	HLA-A*02:03	NB	25.3	61.5	%rank in native protein: 0.8 %rank in vaccine: 1.50
WVQENYLEY	HLA-B*15:02	B	57.8	57.8	-
	HLA-B*15:21	B	455.2	455.2	
	HLA-B*35:05	B	27.9	27.9	
YEDHFPLLF	HLA-B*18:01	B	48	48	-
	HLA-B*44:03	B	209.5	209.5	
YEFLWGPRA	HLA-B*18:01	B	152.7	152.7	-
FPLWYEEAL	HLA-B*35:05	B	18.3	18.3	-
LSIVFIEGY	HLA-B*58:01	B	167	167	-
PTGHSFVLVTSGLT	HLA-DRB1*07:01	B	43.19	43.19	-
	HLA-DRB1*16:02	B	62.07	62.07	
	HLA-DRB1*15:02	B	175.32	175.32	
TGHSFVLVTSGLTY	HLA-DRB1*07:01	B	35.68	35.68	-
	HLA-DRB1*16:02	B	58.85	58.85	
	HLA-DRB1*15:02	B	127.87	127.87	
GHSFVLVTSGLTYD	HLA-DRB1*07:01	B	32.59	32.59	-
	HLA-DRB1*16:02	B	46.63	46.63	
	HLA-DRB1*15:02	B	101.15	101.15	
	HLA-DRB1*15:01	B	93.86	93.86	

	HLA-DRB1*11:01	B	50.52	50.52	
	HLA-DRB1*16:02	B	71.55	71.55	
PARYEFLWGPRAHAE	HLA-DRB1*15:02	B	507.96	507.96	-
	HLA-DRB1*15:01	B	291.35	291.35	
	HLA-DRB1*12:02	B	811.25	811.25	
RYEFLWGPRAHAEIR	HLA-DRB1*11:01	B	39.64	39.64	-
RKMSLLKFLAKVNGS	HLA-DRB1*11:01	B	28.2	28.2	-
KMSLLKFLAKVNGSD	HLA-DRB1*11:01	B	30.61	30.61	-
	HLA-DRB1*12:02	B	134.74	134.74	
MSSLLKFLAKVNGSDP	HLA-DRB1*11:01	B	31.44	31.44	-
	HLA-DRB1*12:02	B	217.66	217.66	
SLLKFLAKVNGSDPR	HLA-DRB1*11:01	B	35.28	35.28	-
	HLA-DRB1*12:02	B	317.3	317.3	
RAHAEIRKMSLLKFL	HLA-DRB1*12:02	B	80.63	80.63	-
AHAEIRKMSLLKFLA	HLA-DRB1*12:02	B	64.7	64.7	-
HAEIRKMSLLKFLAK	HLA-DRB1*12:02	B	40.18	40.18	-
AEIRKMSLLKFLAKV	HLA-DRB1*12:02	B	43.97	43.97	-
LESVIRNYEDHFPLL	HLA-DRB1*15:01	B	210.78	210.78	-
	HLA-DRB1*15:02	B	801.56	801.56	
ESVIRNYEDHFPLLF	HLA-DRB1*15:01	B	147.43	147.43	-
	HLA-DRB1*15:02	B	547.61	547.61	
DPARYEFLWGPRAHA	HLA-DRB1*15:02	B	475.05	475.05	-
	HLA-DRB1*16:02	B	64.95	64.95	
	HLA-DRB1*11:01	B	74.14	74.14	
HSFVLVTSLGLTYDG	HLA-DRB1*16:02	B	72.33	72.33	-
	HLA-DRB1*07:01	B	42.1	42.1	
	HLA-DRB1*15:02	B	147.76	147.76	
SDPARYEFLWGPRAH	HLA-DRB1*16:02	B	90.4	90.4	-
	HLA-DRB1*15:02	B	707.11	707.11	
ARYEFLWGPRAHAEI	HLA-DRB1*16:02	B	83.48	83.48	-
	HLA-DRB1*11:01	B	43.49	43.49	
	HLA-DRB1*15:02	B	536.81	536.81	
SCYPLIPSTPEEVSA	HLA-DRB1*07:01	B	345.43	345.43	-
CYPLIPSTPEEVSAD	HLA-DRB1*07:01	B	552.63	552.63	-
YPLIPSTPEEVSADD	HLA-DRB1*07:01	B	582.46	582.46	-

DPTGHSFVLVTSLGL	HLA-DRB1*07:01	B	37.97	37.97	-
QFLLFKYQMKEPITK	HLA-DRB1*11:01	B	42.68	42.68	-
FLLFKYQMKEPITKA	HLA-DRB1*11:01	B	35.35	35.35	-
KAEILESVIRNYEDH	HLA-DRB1*11:01	B	813.49	813.49	-
AEILESVIRNYEDHF	HLA-DRB1*11:01	B	698.1	698.1	-
YEFLWGPRAHAEIRK	HLA-DRB1*11:01	B	64.49	64.49	-
LLKFLAKVNGSDPRS	HLA-DRB1*11:01	B	51.02	51.02	-
LKFLAKVNGSDPRSF	HLA-DRB1*11:01	B	160.02	160.02	-
PRAHAEIRKMSLLKF	HLA-DRB1*12:02	B	104	104	-
ILESVIRNYEDHFPL	HLA-DRB1*15:01	B	327.4	327.4	-
SVIRNYEDHFPLLFS	HLA-DRB1*15:01	B	185.6	185.6	-
NY-ESO-1					
RLLEFYLAM	HLA-A*02:01	B	14.3	14.3	-
LMWITQCFL	HLA-A*02:01	B	38.2	38.2	-
LAMPFATPM	HLA-B*15:02	B	16.3	16.3	-
	HLA-B*15:13	B	273.8	273.8	
	HLA-B*15:21	B	120.4	120.4	
	HLA-B*58:01	B	102.3	102.3	
LLMWITQCF	HLA-B*35:05	B	474.5	474.5	-
	HLA-B*15:02	B	173.7	173.7	
MPFATPMEA	HLA-B*35:05	B	23.9	23.9	-
ITQCFLPVF	HLA-B*58:01	B	148.5	148.5	-
LLKLEFTVSGNILTIR	HLA-DRB1*07:01	B	26.59	26.59	-
SGNILTIRLTAADHR	HLA-DRB1*12:02	B	374.37	374.37	-
GNILTIRLTAADHRQ	HLA-DRB1*12:02	B	408.28	408.28	-
ESRLLLEFYLAMPFAT	HLA-DRB1*15:01	B	39.75	39.75	-
SRLLLEFYLAMPFATP	HLA-DRB1*15:02	B	84.8	84.8	-
	HLA-DRB1*15:01	B	32.24	32.24	

**Appendix F. MolProbity results vaccine model assessed with SWISS-MODEL.** The protein model predicted by I-TASSER had unsatisfactory structure assessment results.

	Score	Note
Clash Score	12.02	(A498 TYR-A516 PHE), (A494 PHE-A516 PHE), (A417 TYR-A479 TYR), (A273 TYR-A277 PHE), (A314 TYR-A318 PRO), (A482 TYR-A483 ALA)
Ramachandran Outliers	11.20%	A317 ILE, A318 PRO, A192 ALA, A205 PRO, A61 GLN, A246 TRP, A432 GLY, A196 VAL, A305 ASP, A499 GLY, A365 ALA, A501 GLU, A126 VAL, A398 ARG, A36 ARG, A467 ILE, A500 ALA, A464 ILE, A439 ALA, A293 SER, A39 ALA, A84 ALA, A110 ARG, A294 PHE, A327 ASP, A408 PHE, A463 SER, A520 GLY
Rotamer Outliers	14.39%	A377 LEU, A429 HIS, A38 ARG, A480 LEU, A316 LEU, A249 ARG, A348 LYS, A443 LEU, A288 ASP, A438 ARG, A320 THR, A155 VAL, A40 GLU, A518 GLU, A6 ASN, A142 SER, A108 ARG, A327 ASP, A376 LEU, A131 GLU, A495 LEU, A89 GLU, A255 ARG, A489 TRP, A42 THR, A189 LYS, A246 TRP, A3 GLU, A147 VAL, A475 VAL, A482 TYR, A230 SER, A334 GLN, A392 PRO, A167 LYS, A410 THR, A174 LYS, A361 ILE, A368 HIS, A404 LEU, A487 LEU, A420 ILE, A508 ILE, A341 GLN, A210 GLU, A277 PHE, A445 TYR, A37 GLU, A422 ILE, A494 PHE, A328 ASP, A390 ARG, A481 GLU, A68 LEU, A229 ARG, A222 LYS, A256 LYS, A300 LEU
C-Beta Deviations	59	A406 MET, A36 ARG, A378 LYS, A281 PHE, A408 PHE, A415 ALA, A416 ALA, A360 THR, A277 PHE, A320 THR, A390 ARG, A145 ARG, A418 ILE, A241 ARG, A368 HIS, A195 LYS, A400 LEU, A409 ALA, A200 GLU, A246 TRP, A60 LEU, A443 LEU, A463 SER, A394 PRO, A199 LYS, A61 GLN, A292 HIS, A407 PRO, A35 LEU, A223 VAL, A338 PHE, A163 ALA, A302 LEU, A6 ASN, A343 LYS, A196 VAL, A211 ILE, A229 ARG, A42 THR, A46 THR, A410 THR, A316 LEU, A344 GLU, A367 ASP, A251 HIS, A317 ILE, A110 ARG, A358 ILE, A10 ILE, A254 ILE, A67 GLN, A318 PRO, A412 MET, A305 ASP, A392 PRO, A428 ILE, A106 LEU, A28 VAL, A490 ILE