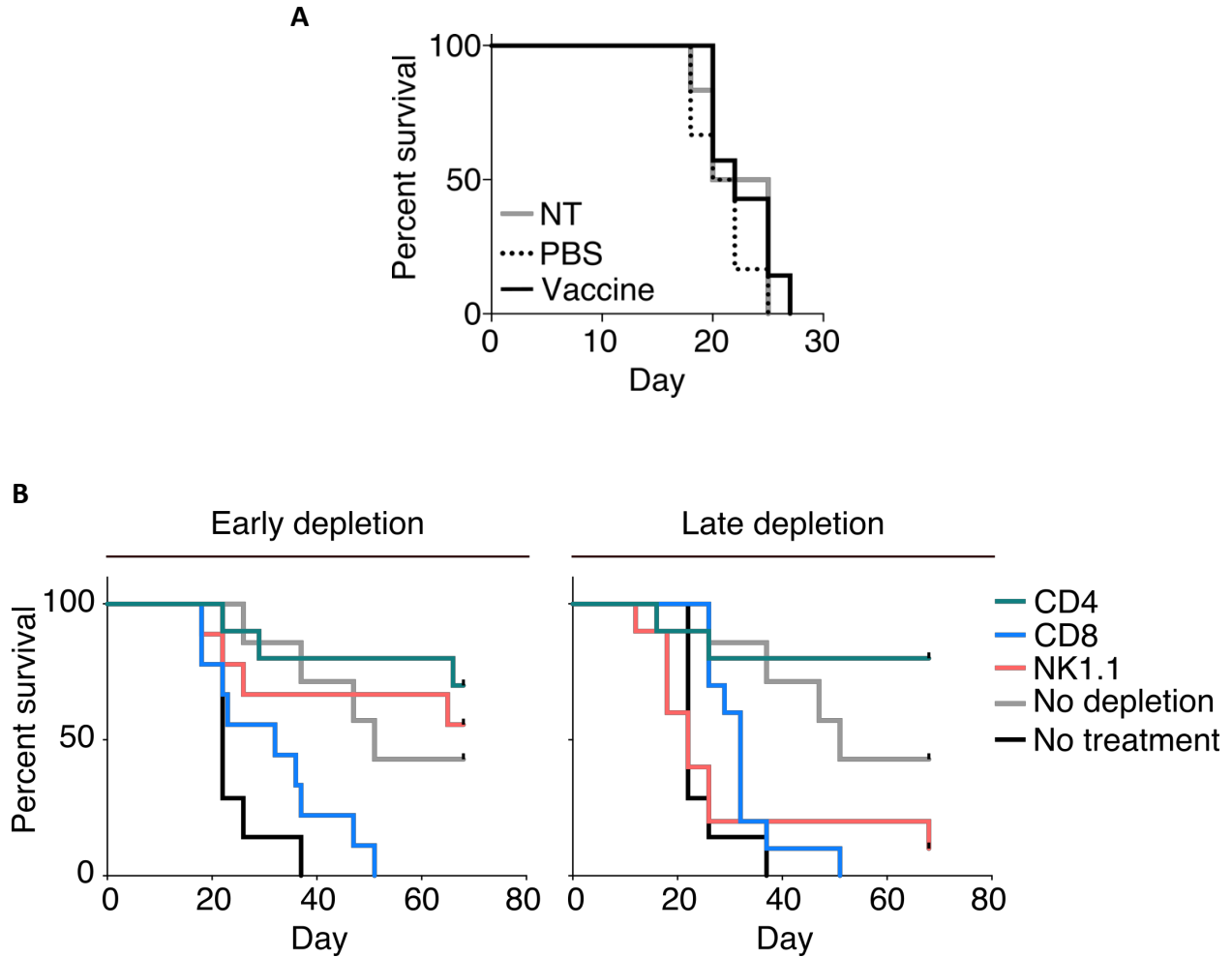


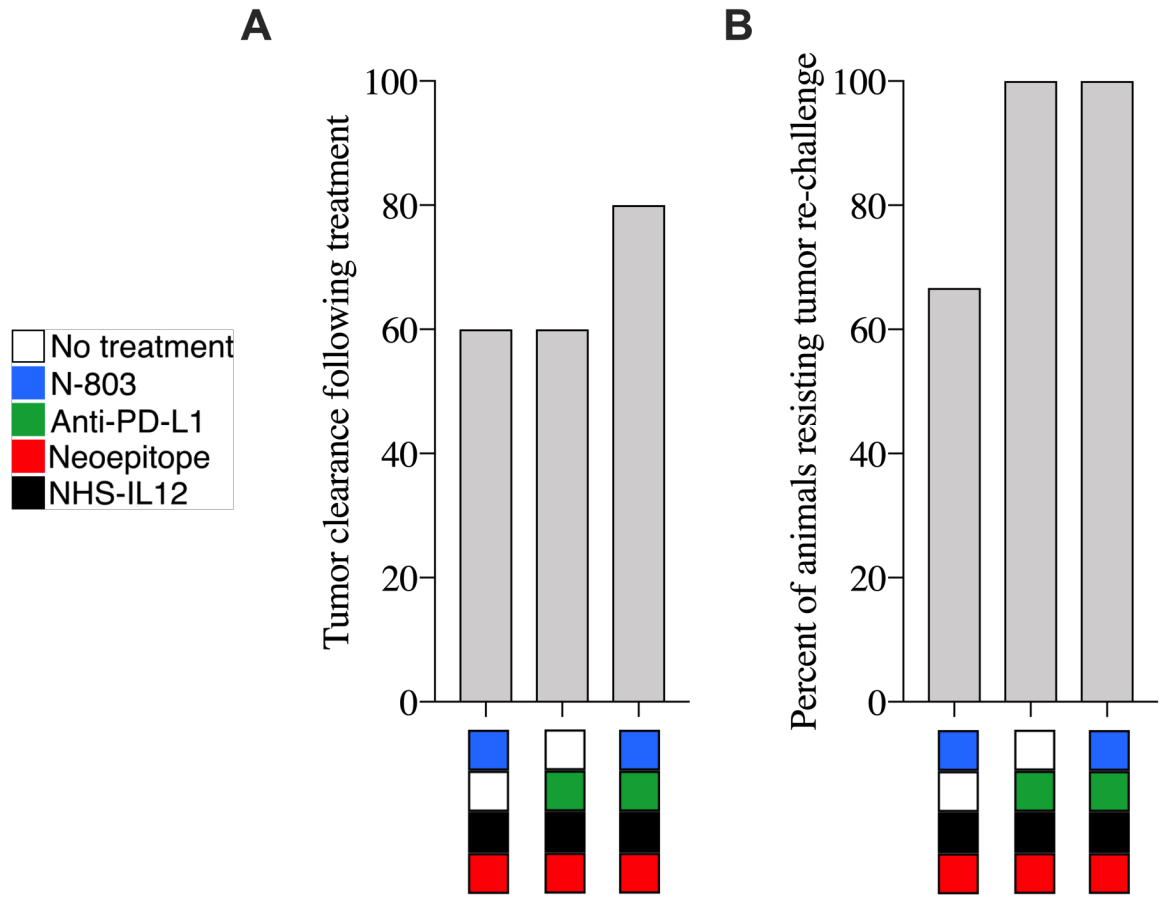
SUPPLEMENTARY FIGURES AND TABLES



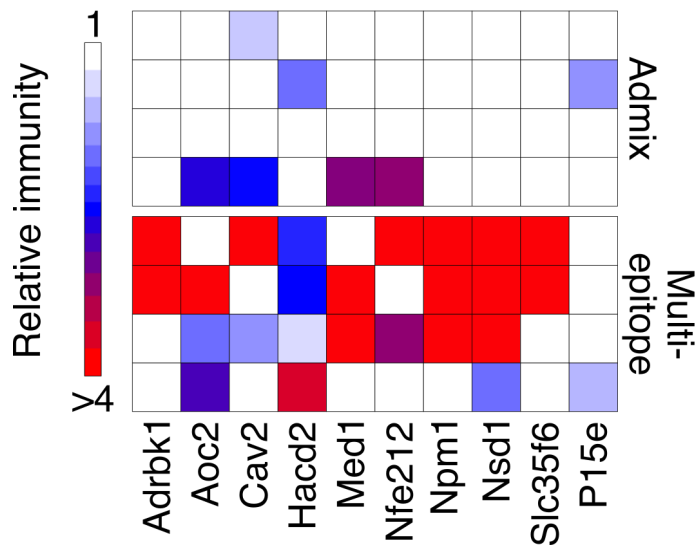
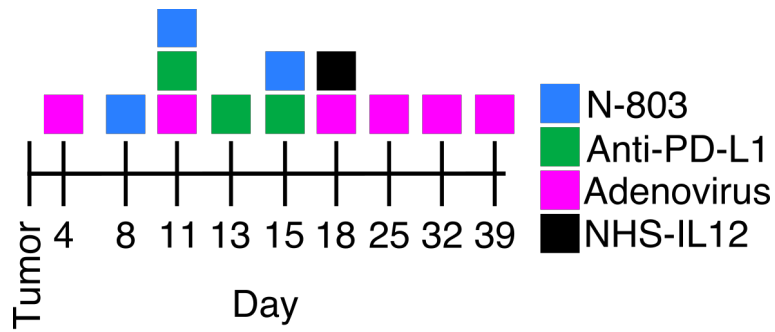
	CD4 early	CD8 early	NK early	CD4 late	CD8 late	NK late
CD8 early	***					
NK early	**	**				
CD4 late	NS	**	NS			
CD8 late	**	NS	*	**		
NK late	**	NS	*	**	NS	
No depletion	NS	*	NS	NS	*	*
No treatment	***	NS	*	**	*	NS

* < 0.05, ** < 0.01, *** < 0.001, NS = not significant

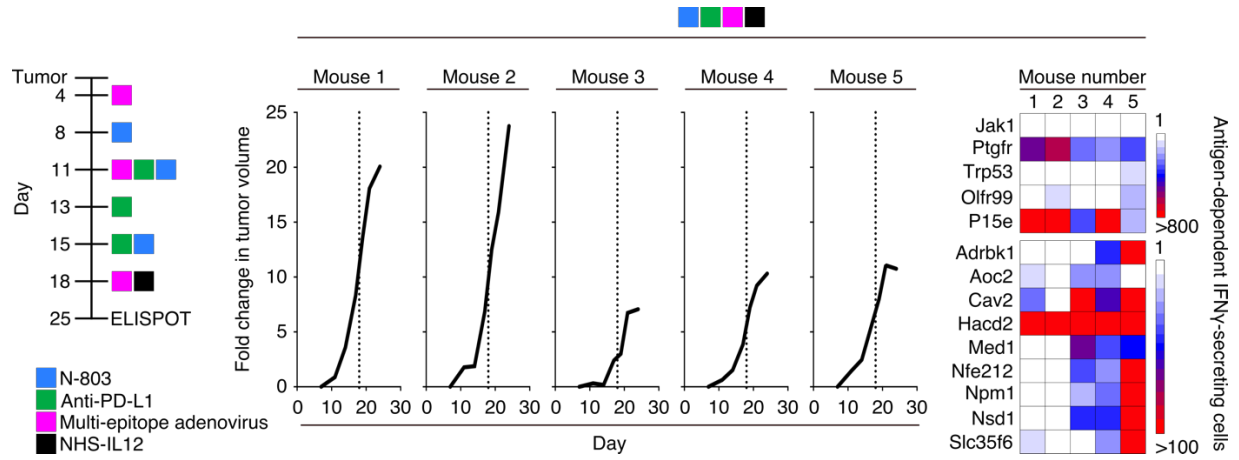
Supplementary Figure S1: A) Survival curves of animals treated with single agent neoepitope vaccine or indicated controls. B) Survival curves of animals depleted of indicated cell populations either early (day 1) or late (day 15) of tumor growth. Statistical analysis of tumor survival curves is presented within the lower table.



Supplementary Figure S2: A) Utilizing the treatment schedule presented in Figure 2A, the percentage of animals treated as indicated that were able to induce tumor regression. B) Percentage of animals that resolved their primary tumor following the indicated treatment and resisted a subsequent normally lethal challenge with the MC38 tumor.



Supplementary Figure S3: Top panel, treatment schedule. Bottom panel, relative immunity to neoepitopes expressed by MC38 tumors, but not present within the vaccine following administration of either a multi-epitope adenoviral vaccine or admixed neoepitope vaccine in which each of the four individual vaccines are mixed together prior to injection.



Supplementary Figure S4: Left panel, treatment schedule. Middle panel, fold change in tumor growth, based upon day 7 tumor measurements. Dotted line indicates the administration of NHS-IL12 on day 18 of tumor growth. Right panel, quantification of the immunity generated by animals against MC38 tumor antigens.

Target	Application	Clone	Vendor
H2K ^b	Flow Analysis	AF6-88.5	BD Biosciences
H2D ^b	Flow Analysis	KH95	BD Biosciences
CD44	Flow Analysis	IM7	BD Biosciences
CD62L	Flow Analysis	MEL-14	BD Biosciences
CD11b	Flow Analysis	M1/70	BD Biosciences
CD3 ϵ	Flow Analysis	145-2C11	BD Pharmingen
CD4	Flow Analysis	RM4-5	BD Pharmingen
CD8a	Flow Analysis	53-6.7	BD Pharmingen
CD45	Flow Analysis	30-F11	BD Pharmingen ThermoFisher Scientific
CD45.2	Flow Analysis	104	Biolegend
TCR β	Flow Analysis	H57-597	Biolegend
CD38	Flow Analysis	90	Biolegend
F4/80	Flow Analysis	BM8	Biolegend
TNF	Flow Analysis	MP6- XT22	ThermoFisher Scientific
IFN γ	Flow Analysis	XMG1.2	ThermoFisher Scientific
CD127	Flow Analysis	A7R34	ThermoFisher Scientific
CD206	Flow Analysis	MR6F3	ThermoFisher Scientific
CD4	In-vivo Depletion	GK1.5	BioXcell
CD8	In-vivo Depletion	2.43	BioXcell
NK1.1	In-vivo Depletion	PK136	BioXcell
PD-L1	In-vivo Blockade	10F.9G2	BioXcell

Supplementary Table S1: list of antibodies used in this study.

RefSeq Gene ID	Gene ID	Protein Change	Genomic Change	Normal Protein Sequence	Allele Frequency	TPM	Median TPM	Affinity (nM)	Allele	Neoepitope
NM_130863.2	Adrbk1	p.M526I	C->G	IVHGYMSKM,	0.52	53.37	3.49	107	H-2-Kb	IVHGYMSKI
NM_027406.1	Aldh1l1	p.V601L	G->T	TVVIKPAQV,	0.48	13.16	3.49	347	H-2-Kb	TVVIKPAQL
NM_178932.1	Aoc2	p.G443V	G->T	IGGHFYGGGL,	0.60	4.18	3.49	9	H-2-Kb	IVGHFYGGGL
NM_026120.4	Carnmt1	p.C391S	G->C	MMKYYYECV,	0.60	42.86	3.49	151	H-2-Kb	MMKYYYESV
NM_026120.4	Carnmt1	p.C391S	G->C	MKYYYECVL,	0.60	42.86	3.49	156	H-2-Kb	MKYYYESVL
NM_016900.4	Cav2	p.V25F	G->T	DAYSHHSGV,	0.45	66.30	3.49	70	H-2-Kb	DAYSHHSGF
NM_001081012.1	Ccdc171	p.A1169T	G->A	AATRNDFTL,	0.44	7.67	3.49	238	H-2-Db	TATRNDFTL
NM_025689.4	Ccdc51	p.A394P	G->C	VTFLATLPL,	0.39	5.45	3.49	97	H-2-Kb	VTFLPTLPL
NM_025689.4	Ccdc51	p.A394P	G->C	TCVTFLATL,	0.39	5.45	3.49	279	H-2-Kb	TCVTFLPTL
NM_008533.2	Cd180	p.P440L	C->T	SPFQNLHLL,	0.23	6.17	3.49	247	H-2-Db	SLFQNLHLL
NM_138647.3	Cers1	p.P126R	C->G	TSYPFFHDP,	0.53	5.23	3.49	284	H-2-Kb	TSYRFFHDP
NM_013581.3	Cog1	p.H151Q	C->A	QLYLCCCHL,	0.61	31.13	3.49	492	H-2-Kb	QLYLCCQQL
NM_170588.3	Cpne1	p.D302Y	C->A	SSPDSLHYL,	0.16	86.34	3.49	211	H-2-Db	SSPSLHYL
NM_170588.3	Cpne1	p.D302Y	C->A	DSLHYLSPT,	0.16	86.34	3.49	212	H-2-Kb	YSLHYLSPT
NM_172704.3	Dnajc11	p.D11N	G->A	DNEDYYSLL,	0.50	32.24	3.49	478	H-2-Kb	NNEDYYSLL
NM_007875.2	Dpagt1	p.V213L	G->T	ASIIVFNLV,	0.22	38.36	3.49	102	H-2-Kb	ASIIVFNLL
NM_010091.4	Dvl1	p.G400V	G->T	SSLTSSVPG,	0.36	27.77	3.49	136	H-2-Db	SSLTSSVPV
NM_053103.5	Entpd7	p.L385F	G->C	SCRTLLSPL,	0.73	11.61	3.49	193	H-2-Kb	SCRTEFLSPL
NM_022009.2	Flii	p.G221C	C->A	TSLEGLSNL,	0.50	99.85	3.49	259	H-2-Kb	TSLECLSNL
NM_001080746.2	Gtf2i	p.G415V	C->A	YGIPRLERI,	0.32	86.31	3.49	407	H-2-Kb	VVIPRLERI
NM_023587.2	Hacd2	p.Q231H	G->T	SYIPLFPQL,	0.51	30.08	3.49	54	H-2-Kb	SYIPLPHL
NM_023587.2	Hacd2	p.Q231H	G->T	LFPQLYFHM,	0.51	30.08	3.49	425	H-2-Kb	LFPPLYFHM
NM_146145.2	Jak1	p.G654V	C->A	IVYLYGVCV,	0.41	134.68	3.49	73	H-2-Kb	IVYLYVVCV
NM_178633.3	Klh12	p.R296L	C->A	VRTRLRTPM,	0.60	22.14	3.49	101	H-2-Db	VLTRLRTPM
NM_028233.2	Lrpprc	p.A359P	C->G	TAFQVLLAL,	0.32	69.11	3.49	258	H-2-Kb	TAFQVLLPL
NM_001081392.1	Mdn1	p.N1209T	A->C	LSRAFRRNF,	0.38	25.16	3.49	164	H-2-Kb	LSRAFRRNF
NM_001081392.1	Mdn1	p.N1209T	A->C	AFRRNFVEL,	0.38	25.16	3.49	280	H-2-Kb	AFRRNFVEL
NM_001080118.1	Med1	p.V452L	C->A	FVSFQHPV,	0.52	57.38	3.49	78	H-2-Kb	FSLSFQHPV
NM_010902.4	Nfe2l2	p.Q104L	T->A	ASYSQVAHI,	0.40	57.04	3.49	29	H-2-Kb	ASYSLVAHI
NM_008722.3	Npm1	p.V117L	C->A	ISGQHLVAV,	0.12	2280.32	3.49	260	H-2-Kb	ISGQHLAV
NM_008739.3	Nsd1	p.Q416L	A->T	KGYRHKVPQ,	0.21	9.92	3.49	39	H-2-Kb	KGYRHKVPL
NM_146515.2	Olfr99	p.Y249N	A->T	VVLLFYSTV,	0.27	7.59	3.49	37	H-2-Kb	VVLLFNSTV
NM_011151.2	Ppm1b	p.R191L	G->T	IQRVNGSLA,	0.32	58.87	3.49	142	H-2-Db	IQLVNGSLA
NM_008966.3	Ptgfr	p.D77Y	C->A	VITDFFGHL,	0.38	7.33	3.49	13	H-2-Kb	VITYFFGHL
NM_008966.3	Ptgfr	p.D77Y	C->A	ITDFFGHLI,	0.38	7.33	3.49	56	H-2-Kb	ITYFFGHLI
NM_152824.1	Rbm17	p.G90W	C->A	SAGEVLIPL,	0.36	110.07	3.49	490	H-2-Kb	SAWEVLIPL
NM_016926.1	Sart3	p.S709N	C->T	SVTVFVSNL,	0.32	47.37	3.49	107	H-2-Kb	SVTVFVNLL
NM_001081341.1	Scaper	p.T1293K	G->T	TVLQKLCQL,	0.35	13.95	3.49	330	H-2-Kb	KVLQKLCQL
NM_175675.3	Slc35f6	p.G322V	G->T	QILVFLILL,	0.40	15.14	3.49	65	H-2-Kb	QILVFLILL
NM_017407.2	Spag5	p.I699N	T->A	AQLQLTST,	0.40	60.84	3.49	443	H-2-Db	AQLQNLST
NM_011491.3	Stc2	p.I207N	A->T	SWGGLCSIL,	0.52	6.81	3.49	472	H-2-Kb	SWGGLCSNL
NM_133932.2	Tada3	p.R62L	C->A	SSASRRLRV,	0.36	33.90	3.49	206	H-2-Kb	SSASRLLV
NM_133966.2	Taf5l	p.A573S	C->A	FMACNLLLV,	0.69	29.82	3.49	79	H-2-Db	FMSCNLLLV
NM_133966.2	Taf5l	p.A573S	C->A	VQFMACNLL,	0.69	29.82	3.49	95	H-2-Kb	VQFMSCNLL
NM_201359.2	Tmem106c	p.T137S	C->G	VVLDVTATL,	0.71	7.69	3.49	348	H-2-Kb	VVLDVTASL
NM_001127233.1	Trp53	p.G242V	G->T	MGGMNRRI,	0.64	115.64	3.49	77	H-2-Db	MGVMNRRI
NM_001276465.1	Vps13d	p.W2765L	C->A	RALSGWEPF,	0.54	15.09	3.49	413	H-2-Db	RALSGLEPF
NM_026417.4	Yipf4	p.G171A	G->C	VAYGQVLGV,	0.51	38.03	3.49	106	H-2-Kb	VAYGQVLAV
NM_026417.4	Yipf4	p.G171A	G->C	LGVIIGYSLL,	0.51	38.03	3.49	241	H-2-Kb	LAVIGYSLL
NM_001309446.1	Zfand4	p.R272L	G->T	AARHRLLRV,	0.54	15.46	3.49	270	H-2-Kb	AALHRLLRV
NM_026856.2	Zfp644	p.R948L	C->A	RGHLHRAGL,	0.49	30.13	3.49	293	H-2-Kb	LGHLHRAGL

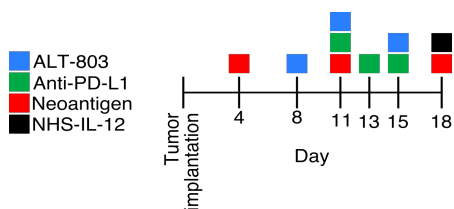
Supplementary Table S2: List of neoepitopes shared amongst both MC38 tumors analyzed.

Animal Chemistry Panels Day 33		
	ALT-803	Age-matched control
Sodium (mmol/L)	153	157
Potassium (mmol/L)	6.1	5.2
Chloride (mmol/L)	110	115
Calcium (mmol/L)	2.34	2.39
Magnesium (mmol/L)	1.22	1.18
Phosphorous, inorganic (mg/dL)	7.2	8.2
Glucose (mg/dL)	158	123
BUN (mg/dL)	18	23
Creatinine (mg/dL)	0.1	0.1
Uric Acid (mg/dL)	4	2.2
Albumin (g/dL)	3.4	3
Protein, Total (g/dL)	5.2	4.9
Cholesterol (mg/dL)	86	88
Triglycerides (mg/dL)	155	160
Alkaline Phosphatase (U/L)	98	78
ALT/GPT (Alanine Trans) (U/L)	207	28
AST/GOT (Aspartate Trans) (U/L)	291	65
Amylase (U/L)	2178	2568
Ck, Total (U/L)	318	298
LD (Lacate Dehydrogen) (U/L)	691	322

Animal Chemistry Panels Day 55		
	ALT-803	Age-matched control
Alkaline Phosphatase (U/L)	150	133
ALT/GPT (Alanine Trans) (U/L)	21	40
AST/GOT (Aspartate Trans) (U/L)	66	66
Amylase (U/L)	2962	2971
Ck, Total (U/L)	716	466
LD (Lacate Dehydrogen) (U/L)	426	289

CBC Day 33		
	ALT-803	Age-matched control
WBC Count (K/uL)	4.62	6.6
RBC Count (M/uL)	8.57	10.4
Hemoglobin (g/dL)	11.7	14.8
Hematocrit (%)	39.6	48.7
Mean corpuscular volume (fL)	46.2	46.8
Platelets (K/uL)	486	214
Polymorphonuclear leukocyte (Polys) (%)	35.8	46.5
Lymphocytes (%)	56.6	40
Monocytes (%)	4.4	7.7
Eosinophils (%)	0.4	2.4
Basophils (%)	0.2	0.2
Polys Absolute (K/uL)	1.654	3.069
Lymphocytes Absolute (K/uL)	2.615	2.64
Monocytes Absolute (K/uL)	0.203	0.508
Eosinophils Absolute (K/uL)	0.018	0.158
Basophils Absolute (K/uL)	0.009	0.013

CBC Day 55		
	ALT-803	Age-matched control
WBC Count (K/uL)	11.31	6.86
RBC Count (M/uL)	9.53	8.73
Hemoglobin (g/dL)	12.9	12.4
Hematocrit (%)	42	40.1
Mean corpuscular volume (fL)	43.9	45.9
Platelets (K/uL)	925	838
Polymorphonuclear leukocyte (Polys) (%)	7.1	9.4
Lymphocytes (%)	88.5	85.2
Monocytes (%)	0.7	1.5
Eosinophils (%)	2.2	2.3
Basophils (%)	0.4	0.2
Polys Absolute (K/uL)	0.803	0.645
Lymphocytes Absolute (K/uL)	10.009	5.845
Monocytes Absolute (K/uL)	0.079	0.103
Eosinophils Absolute (K/uL)	0.249	0.158
Basophils Absolute (K/uL)	0.045	0.014



Supplementary Table S3: Blood chemistries on days 33 and 55, and histopathological examination of tissues harvested from animals on day 33 of tumor growth. Tissues were examined by a board-certified pathologist (VitroVivo Biotech) of tumor growth. Blood and serum were analyzed by the Department of Laboratory Medicine, NIH Clinical Center.