

Supplementary Table 1 Differentially expressed (DE) genes (n=79) in the comparison between MUC1-Tg mice receiving anti-PDL1 and rat IgG (3 doses, bi-weekly)

Genes	lfc^{a,b} (PD-L1/IgG)	p value^b	q value^b
Fnl	-2.046974575	0.0000	0.04
Bst1	-1.845037194	0.0000	0.04
Il1r2	-1.74427792	0.0000	0.04
Fkbp5	-1.15630432	0.0000	0.04
Tfrc	-1.090664073	0.0000	0.04
Icam4	-1.268640768	0.0000	0.05
Marco	-1.323717811	0.0000	0.05
Ifitm1	-1.299836153	0.0000	0.05
Cxcr2	-1.264654671	0.0000	0.05
Card9	-1.318249112	0.0000	0.05
App	-1.082571273	0.0000	0.05
Tal1	-1.211657681	0.0000	0.05
Abcb10	-0.874191991	0.0000	0.06
Itgam	-1.139298812	0.0000	0.06
Csf2rb	-0.764665139	0.0000	0.06
Fcgr3	-0.927278192	0.0000	0.08
Il18rap	-0.678043539	0.0000	0.08
Lilrb3	-0.753196193	0.0000	0.08
Ncam1	-1.378910754	0.0100	0.08
Tirap	-0.529193679	0.0100	0.08
Tgfb1	-1.578060218	0.0100	0.08
C3	-1.237743375	0.0100	0.09
Ltb4r1	-1.313138581	0.0100	0.09
Il13ra1	-1.021445824	0.0100	0.09
Ccl9	-0.716440748	0.0100	0.09
Cfh	-1.00593644	0.0100	0.09
Nfil3	-0.768288735	0.0100	0.1
Tlr8	-0.734143561	0.0100	0.1
Ctsg	-1.910911628	0.0100	0.1
Ltbr	-0.799527059	0.0100	0.1
Ccr2	-0.68170139	0.0100	0.1
Clec5a	-1.559141406	0.0100	0.1
Fcer1g	-0.500595767	0.0100	0.11
Cd24a	-0.285166693	0.0100	0.11
Litaf	-0.562596922	0.0100	0.12
Cd34	-1.288707654	0.0100	0.12
Ceacam1	-0.468580903	0.0200	0.13
Trem1	-0.999370039	0.0200	0.13
Hlx	-0.711561271	0.0200	0.13
Plaur	-0.159309249	0.0200	0.13
Socs3	-0.415391715	0.0200	0.14
Cxcr6	2.579130943	0.0000	0.04
Cd3d	2.036329444	0.0000	0.04

Genes	lfc ^{a,b} (PD-L1/IgG)	p value ^b	q value ^b
Gzma	2.683702686	0.0000	0.04
Ccl5	2.042176015	0.0000	0.04
Sh2d1a	2.156534368	0.0000	0.04
Tcf7	2.028407776	0.0000	0.04
Lck	1.701120332	0.0000	0.05
Ccr7	1.8422903	0.0000	0.05
Cd8b1	3.923764907	0.0000	0.05
Cd3e	1.844788759	0.0000	0.05
Thy1	1.936760899	0.0000	0.06
Cd8a	1.968292738	0.0000	0.08
Tnfrsf13c	1.746390686	0.0000	0.08
Cd83	1.410641939	0.0000	0.08
Cd27	1.628021632	0.0100	0.08
Cd5	1.62282901	0.0100	0.08
Cd6	1.65196813	0.0100	0.09
Lef1	2.001314066	0.0100	0.09
Il27ra	1.570341625	0.0100	0.09
Folr4	1.632641946	0.0100	0.09
Cd2	1.454119806	0.0100	0.09
Nt5e	1.62454283	0.0100	0.09
Icos	1.596713567	0.0100	0.09
Cd247	1.598357544	0.0100	0.09
Klrd1	1.569598672	0.0100	0.1
Xcl1	1.5740809	0.0100	0.1
Bst2	1.390979019	0.0100	0.1
Cd79b	1.543251862	0.0100	0.1
Klrk1	1.508398622	0.0100	0.1
Fyn	1.251284852	0.0100	0.11
Tnfrsf4	1.684553646	0.0100	0.11
Cd40	1.318292995	0.0100	0.11
Cd226	1.484781607	0.0200	0.12
Slamf1	1.477460083	0.0200	0.12
Il2rb	1.297741658	0.0200	0.13
Stat1	1.272686962	0.0200	0.13
Prfl	2.69615605	0.0200	0.13
Ccr6	1.26768595	0.0200	0.14

^a log fold change, lfc

^b The 79 DE genes were sorted by directionality, p value and finally q value.

Negative lfc values signify genes downregulated and positive lfc denote genes upregulated in PD-L1 treated mice.

Supplementary Table 2 Differentially expressed genes (n=59) in the comparison between wild type (WT) mice receiving weekly anti-PDL1 + IFN α (n=4) and rat IgG (controls, n=4)

Genes	lfc^a (anti-PD-L1+IFNα/IgG)	p^b value	q^b value
Ppbbp	-2.758011536	0.000809859	0.113955908
Ctsg	-3.161905469	0.001588518	0.113955908
Cd14	-1.400283305	0.001725154	0.113955908
Clu	-2.615592173	0.001757661	0.113955908
Cxcl12	-3.070026088	0.00194637	0.113955908
Ltb4r1	-1.726353813	0.002369092	0.113955908
Clec5a	-1.864852796	0.002618862	0.113955908
Ifitm1	-1.563013868	0.002821524	0.113955908
Bst1	-1.606048517	0.002958401	0.113955908
Trem1	-1.937323148	0.003316109	0.118274539
App	-1.147904093	0.003886092	0.125410349
Fn1	-1.132548157	0.004211334	0.125410349
S100a9	-1.523476648	0.004325884	0.125410349
Tfrc	-0.725922818	0.005137774	0.125410349
Itgam	-1.112091048	0.005167292	0.125410349
Tgfb1	-1.495324759	0.005415536	0.125410349
C3	-1.527041927	0.005851162	0.125410349
Ifna1	-2.134444779	0.005866586	0.125410349
Fcgr3	-0.879729201	0.006578945	0.125791454
Lilrb3	-0.620066648	0.007753704	0.125791454
S100a8	-1.394647517	0.008238835	0.125791454
Gp1bb	-2.019247482	0.008557237	0.125791454
Ltf	-1.411385292	0.008899812	0.125791454
Cxcr2	-0.923296953	0.009360019	0.125791454
Marco	-1.392051162	0.00939018	0.125791454
Ccr2	-0.871990139	0.009493641	0.125791454
Il1r2	-1.555351507	0.009843984	0.125791454
Tnfrsf11a	-0.959789075	0.009863486	0.125791454
Fcgr1g	-0.656295019	0.010276379	0.125791454
Csf2rb	-0.810367195	0.010514295	0.125791454
Cd163	-1.193696033	0.010924221	0.125791454
Msr1	-1.500936281	0.011059879	0.125791454
Vcam1	-0.40894102	0.012674716	0.131609076
Camp	-1.096191098	0.012727221	0.131609076
Tal1	-0.936260041	0.012861768	0.131609076
Card9	-1.087364008	0.013037908	0.131609076
C1s	-1.626245667	0.013562829	0.132851825
Ccl24	-1.606731565	0.014851884	0.139402377
Plau	-1.620322841	0.016183581	0.146749424
Cd3d	2.696953252	0.00130855	0.113955908
Ccl5	2.919351101	0.001368791	0.113955908
Gzma	3.229433189	0.001463218	0.113955908
Klra8	3.023951	0.002604533	0.113955908

Genes	lfc (anti-PD-L1+IFNα/IgG)	p_value	q_value
Klrc1	3.901002432	0.002982024	0.113955908
Klrd1	2.437499791	0.005508951	0.125410349
Thy1	2.429916609	0.005672389	0.125410349
Tcf7	2.266255948	0.006094709	0.125410349
Cd3e	2.330146856	0.006967377	0.125791454
Cd5	2.304539409	0.008273009	0.125791454
Cd247	2.195327139	0.009332702	0.125791454
Lck	2.153690508	0.009698326	0.125791454
Cd6	2.304775351	0.010315762	0.125791454
Lef1	2.475182117	0.010367818	0.125791454
Il18r1	2.145856415	0.011080595	0.125791454
Cd96	2.671973538	0.011285962	0.125791454
Il7r	2.01294242	0.012799761	0.131609076
Cd27	2.134725049	0.013657664	0.132851825
Klra6	2.678621744	0.014852216	0.139402377
Cd8a	2.285934115	0.016026351	0.146749424

^a log fold change, lfc

^b The 59 DE genes were sorted by directionality, p value and finally q value.

Negative lfc values signify genes downregulated and positive lfc denote genes upregulated in WT mice receiving weekly doses of anti-PDL1 + IFN α .

Supplementary Table 3 Differentially expressed genes (N=39) detected among five different mouse groups

Gene symbol ^a	p value	q value
Cd3d	6.09E-05	0.012714478
Fnl1	7.13E-05	0.012714478
Gzma	6.39E-05	0.012714478
Ccl5	0.000137034	0.01466267
Tcf7	0.000127715	0.01466267
Bst1	0.000166592	0.014854425
Thy1	0.000195677	0.014955333
Ifitm1	0.000300345	0.02008555
Cd3e	0.00041132	0.020730016
Clu	0.000426225	0.020730016
Lck	0.00037148	0.020730016
App	0.00050175	0.021083641
Cd14	0.000534515	0.021083641
Cd27	0.000651301	0.021083641
Cd5	0.000657933	0.021083641
Cd8a	0.000669947	0.021083641
Lef1	0.000636758	0.021083641
Card9	0.001244526	0.022272704
Ccr7	0.001146936	0.022272704
Cd247	0.000885194	0.022272704
Cd6	0.001207783	0.022272704
Csf2rb	0.000927069	0.022272704
Cxcr6	0.00088062	0.022272704
Fcgr3	0.001332199	0.022272704
Il1r2	0.000823579	0.022272704
Itgam	0.001214113	0.022272704
Lilrb3	0.000979603	0.022272704
Nfil3	0.001303656	0.022272704
Ppbp	0.001128114	0.022272704
Tfrc	0.001297019	0.022272704
Tgfbi	0.000839307	0.022272704
Trem1	0.000999366	0.022272704
Xcl1	0.001416573	0.022965649
Ccr2	0.00152988	0.022984194
Klrd1	0.0015466	0.022984194
Ltb4r1	0.001505741	0.022984194
Cxcr2	0.00167012	0.023288167
Fkbp5	0.001697642	0.023288167
Il27ra	0.001653469	0.023288167

^a These genes are identified as differentially expressed across any two or more of the five groups. ANOVA analysis ($q < 0.025$) of Nanostring data from MUC1 Tg and WT treated biweekly with anti-PD-L1 or its isotype control IgG, and WT treated weekly with the combination of anti-PD-L1 and IFN α .

Supplementary Table 4 Differentially expressed genes (n=136) in the comparison between mice responding to anti-PDL1 treatment (n=10) and the IgG controls (n=9)

Genes	lfc (responders / nonresponders)	p value	q value
Fn1	-1.633597376	2.29E-05	0.00311044
Bst1	-1.720804803	4.63E-05	0.003545883
Tfrc	-0.934700853	5.97E-05	0.003545883
Ifitm1	-1.42352384	7.48E-05	0.003635979
App	-1.120001754	8.69E-05	0.003875182
Marco	-1.353339233	9.43E-05	0.003881124
Il1r2	-1.651691136	0.000104144	0.003960829
Card9	-1.221501838	0.000175832	0.00451726
Cxcr2	-1.116917529	0.000179487	0.00451726
Fkbp5	-0.758333434	0.000198298	0.00451726
Csf2rb	-0.786293233	0.000200325	0.00451726
Itgam	-1.126210905	0.000202643	0.00451726
Tal1	-1.08624491	0.000252703	0.00519984
Lilrb3	-0.694030051	0.000354238	0.006148735
Icam4	-1.048798439	0.000377022	0.006148735
Ltb4r1	-1.473805421	0.000396238	0.006148735
Fcgr3	-0.895773212	0.000432595	0.006428839
Ctsg	-2.423136733	0.000521177	0.006936193
Abcb10	-0.57344237	0.000524139	0.006936193
C3	-1.353629259	0.000564092	0.006936193
Clec5a	-1.680100509	0.000570453	0.006936193
Tgfb1	-1.530870624	0.000634221	0.007540185
Tirap	-0.459364265	0.000779059	0.00903749
Trem1	-1.387295778	0.000793948	0.00903749
Fcer1g	-0.566938698	0.000869804	0.009306899
Nfil3	-0.863106543	0.000971312	0.010103849
Il13ra1	-0.894372665	0.001068766	0.01039618
Tlr8	-0.67835137	0.001151401	0.010999994
Ncam1	-1.398849067	0.001326161	0.012025362
Ccr2	-0.752068859	0.001451488	0.012942439
Hlx	-0.750219483	0.001502952	0.013181632
Msr1	-1.282763827	0.001669227	0.013538975
Ltf	-1.300792049	0.001897826	0.014643895
Cd24a	-0.210600406	0.002006695	0.014909612
Ltbr	-0.926141096	0.002034396	0.014909612
Cd14	-0.870036918	0.002138714	0.01525616
Ccl9	-0.57479657	0.002203995	0.015331417

Genes	lfc (responders / nonresponders)	p value	q value
Litaf	-0.573303889	0.002206578	0.015331417
Il18rap	-0.302037128	0.002553898	0.016868339
Cfh	-0.96469238	0.002832706	0.018090464
Ppbp	-1.237932985	0.002840372	0.018090464
Csf3r	-0.404967226	0.004125321	0.024171209
Plaur	-0.056982023	0.004159708	0.024171209
Vcam1	-0.251598956	0.004201724	0.024171209
Cebpb	-0.44922141	0.004336121	0.024370271
Ceacam1	-0.334923271	0.004388288	0.024370271
Fcgr1	-0.939621636	0.004524554	0.024700372
S100a9	-1.292483543	0.005365715	0.028143702
Plau	-0.965849691	0.005532064	0.028470091
Ncf4	-0.149958926	0.005635538	0.028714407
Clu	-0.850461238	0.005960303	0.029801515
Socs3	-0.30811639	0.006195036	0.030688372
Lilrb4	-0.541717282	0.006906258	0.032410947
Cfp	-0.119654328	0.007422072	0.034231107
S100a8	-1.191400029	0.007611608	0.034805217
Cd44	-0.058787899	0.008524611	0.037661623
Cd34	-1.358898868	0.0085388	0.037661623
Myd88	-0.041802181	0.010222151	0.043061817
Cybb	-0.153310516	0.011075452	0.045309413
Cdkn1a	-0.527818433	0.011088903	0.045309413
Clec4e	-1.196071038	0.011094454	0.045309413
Traf4	-0.218220057	0.011268988	0.045673548
Prim1	-0.043325451	0.011912984	0.04687732
Cd3d	2.300977291	7.42E-06	0.00311044
Ccl5	2.399412577	1.45E-05	0.00311044
Gzma	2.87745796	2.33E-05	0.00311044
Tcf7	2.10979181	3.59E-05	0.003545883
Cd3e	2.034996587	5.03E-05	0.003545883
Lck	1.886968372	5.80E-05	0.003545883
Thy1	2.138996454	7.24E-05	0.003635979
Cd5	1.905530553	0.000111051	0.003960829
Cd27	1.829387324	0.000126201	0.004219834
Ccr7	1.875437555	0.000139152	0.00437921
Cd6	1.917539518	0.000161029	0.00451726
Cd247	1.84292355	0.000173673	0.00451726
Cxcr6	3.276546946	0.000240114	0.005138441
Cd8a	2.099597124	0.000286096	0.005668938

Genes	lfc ^a (responders / nonresponders)	p ^b value	q ^b value
Il27ra	1.646140986	0.000344809	0.006148735
Klrd1	1.94718769	0.000352082	0.006148735
Lef1	2.190682841	0.000356849	0.006148735
Cd2	1.499317129	0.000396597	0.006148735
Klra8	2.049129447	0.000402254	0.006148735
Icos	1.773177536	0.000468748	0.006777838
Cd83	1.425266976	0.000519465	0.006936193
Sh2d1a	2.856469774	0.000532228	0.006936193
Cd79b	1.583619478	0.000568545	0.006936193
Xcl1	1.637102578	0.000842394	0.009306899
Il2rb	1.500763573	0.000863525	0.009306899
Cd8b1	3.682614766	0.000982056	0.010103849
Cd4	1.466288906	0.001007106	0.010166073
Klrc1	2.409938623	0.001030218	0.01020679
Tnfrsf13c	1.639478892	0.001228424	0.011529947
Cd40	1.422377254	0.001301686	0.012006933
Btla	1.26104543	0.001571831	0.013538975
Fyn	1.27673031	0.001624588	0.013538975
Nt5e	1.584747668	0.001668785	0.013538975
Il18r1	1.531671298	0.001670229	0.013538975
Cd226	1.524836696	0.001761894	0.014068858
Ms4a1	1.496411271	0.001886788	0.014643895
Ltb	1.333790962	0.001916024	0.014643895
Cd55	1.328497896	0.001978955	0.014909612
Prfl	2.519153498	0.002113591	0.01525616
Il7r	1.430622698	0.002399344	0.016439608
Pml	1.27663676	0.002427531	0.016439608
H2-Ob	1.280490525	0.002552668	0.016868339
Klrk1	2.394933286	0.00260554	0.016999557
Il2rg	1.252660909	0.002995442	0.018707282
Ccr6	1.330072751	0.003037445	0.018707282
Cxcr3	1.585420386	0.003042119	0.018707282
Traf5	1.334703008	0.003158118	0.019133904
Ets1	1.250104557	0.003183023	0.019133904
Eomes	1.918934544	0.003536266	0.021021135
Il4	1.341746349	0.004353672	0.024370271
H2-DMb2	1.308445619	0.004418535	0.024370271
Klra4	3.259649457	0.004625813	0.024998081
Ikzf2	1.563490156	0.004908767	0.026261903
Folr4	2.40443577	0.0051026	0.027028625

Genes	lfc (responders / nonresponders)	p value	q value
Cxcr5	1.438453768	0.005534373	0.028470091
Foxp3	1.519557916	0.005822857	0.029388946
Il21r	1.221089921	0.006331539	0.031076821
Relb	1.184755987	0.006449952	0.031370219
Ctla4	1.426917576	0.006579542	0.031673278
Ly86	1.178024777	0.006630668	0.031673278
Tnfrsf9	2.469361939	0.006776018	0.032081145
Tagap	1.212651858	0.007267605	0.033810162
H2-DMa	1.168465022	0.007743029	0.035106107
H2-Aa	1.300928535	0.008469205	0.037661623
Cd79a	1.518408195	0.00859035	0.037661623
Tnfrsf4	2.189244352	0.008658654	0.037661623
Pecam1	1.130200822	0.008895125	0.038378161
Cd40lg	1.630038611	0.009127723	0.039066653
Stat4	1.301334644	0.009799318	0.041608217
Il17rb	0.986308773	0.010426031	0.043577551
Blnk	1.168468831	0.011370032	0.045736595
Cd1d1	1.278766914	0.011765974	0.04687732
Ccr9	1.632088648	0.011916478	0.04687732

^a log fold change, lfc

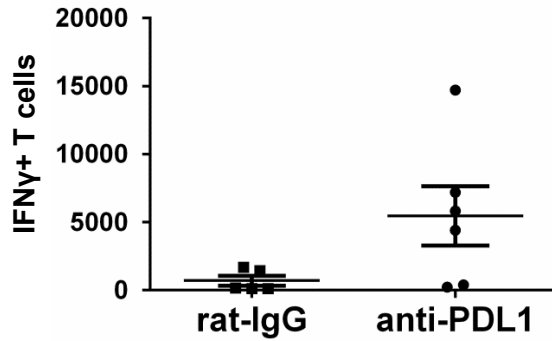
^b The 136 DE genes were sorted by directionality, p value and finally q value.

Negative lfc values signify genes downregulated and positive lfc denote genes upregulated in responder mice (includes M-tg receiving bi-weekly doses of anti-PDL1 and WT mice receiving weekly doses of anti-PDL1+ IFN α) compared to rat IgG control treated M-Tg and WT mice and non-responding WT mice treated with biweekly anti-PD-L1 antibody.

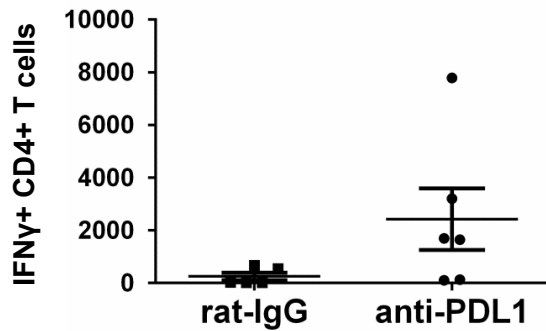
Supplementary Figure 1

MUC1 .Tg mice

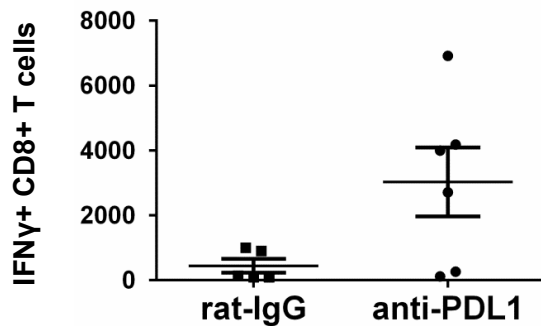
A Total T cells



B CD4+ T cells



C CD8+ T cells



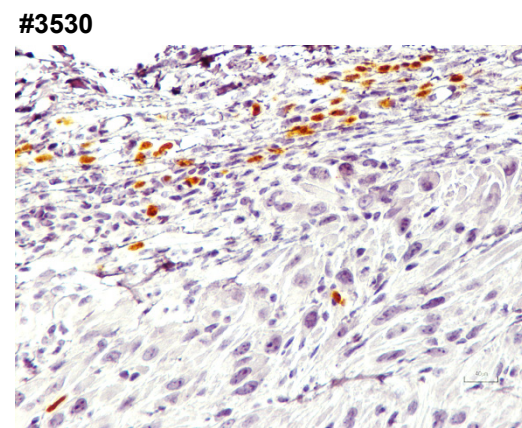
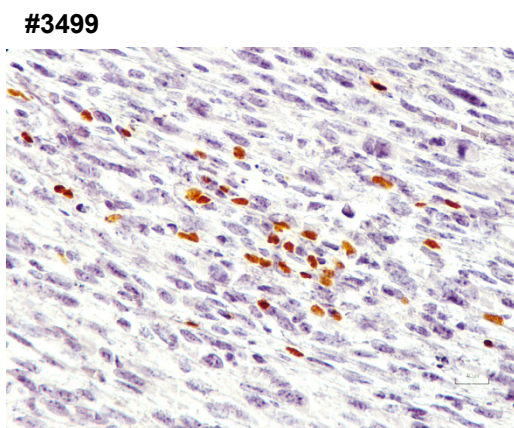
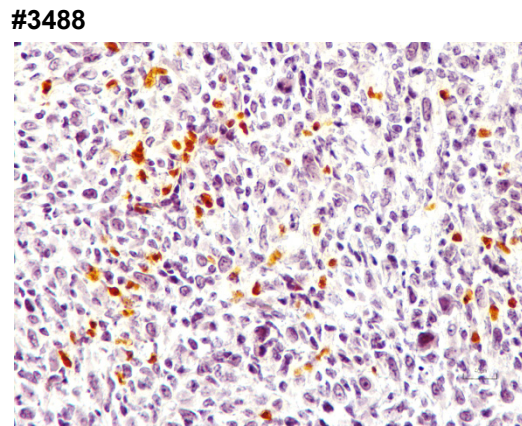
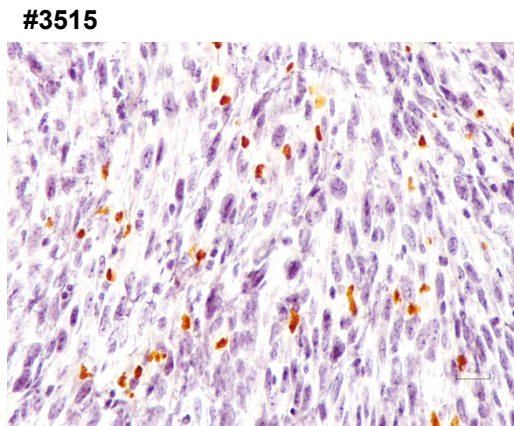
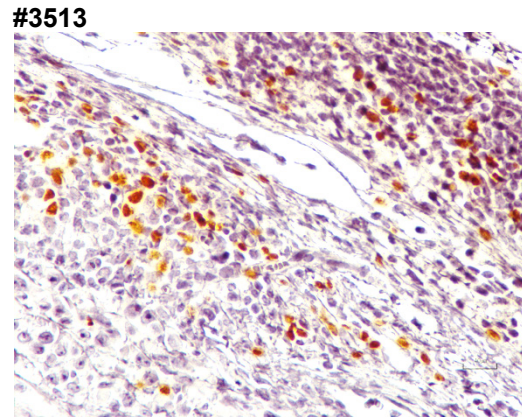
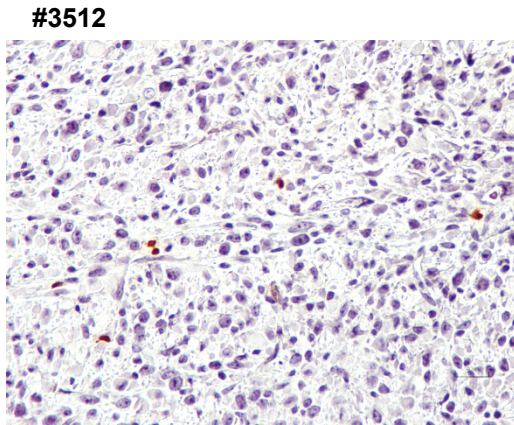
Supplementary Figure 1 Intracellular IFN γ production by CD3 + (A), CD4+ (B) and CD8+ T cells (C) in splenocytes from MUC1.Tg mice treated IP with 200 μ g anti-PD-L1 or control rat IgG, every two weeks, for a total of 3 doses, starting at day 21..

Supplementary Figure 2

Foxp3

Control IgG

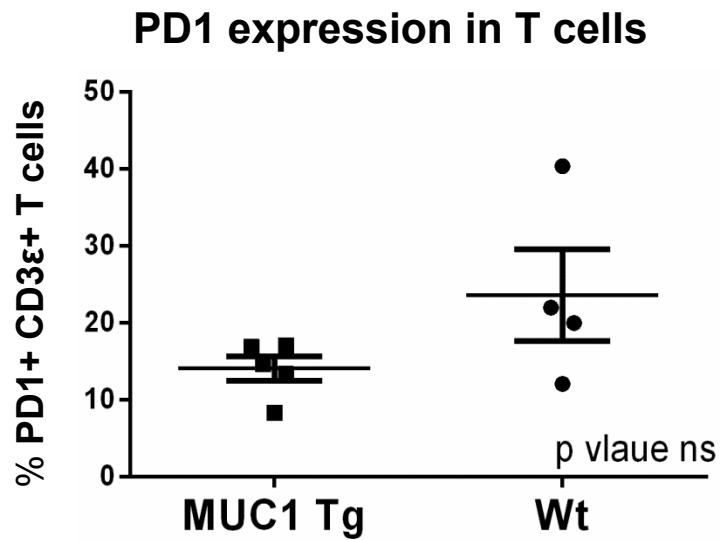
Anti-PD-L1



Supplementary Figure 2. Foxp3 expression by IHC, in tumors from anti-PD-L1-treated (right column) and control IgG-treated mice (left column). Tumors from six different mice, three representative from each group, are shown.

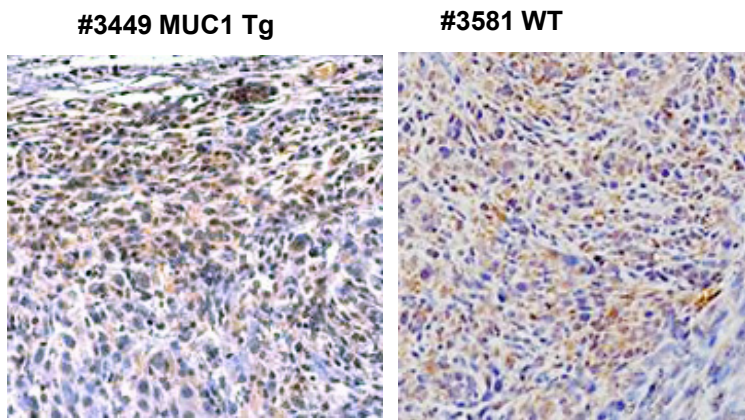
Supplementary Figure 3

A



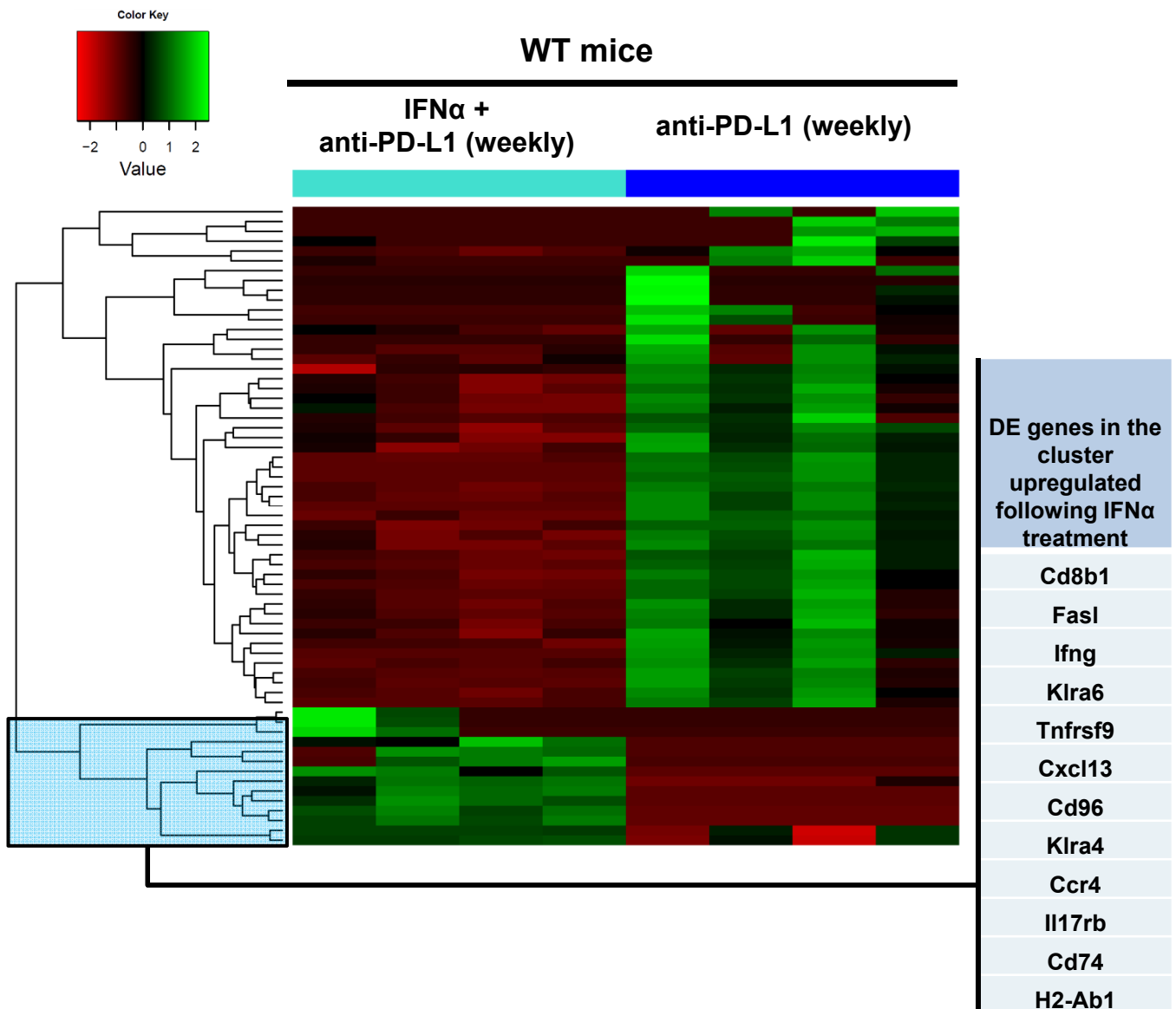
B

PD-L1 expression in 2F8 tumors



Supplementary Figure 3. A Flow cytometry of cell surface PD-1 expression by splenic T cells from MUC1Tg (n=5) and wild type (Wt, n=4) mice (ns; not significant). B Tumor PD-L1 expression by IHC in tumors from MUC1.Tg and WT mice. Representative tumors are shown.

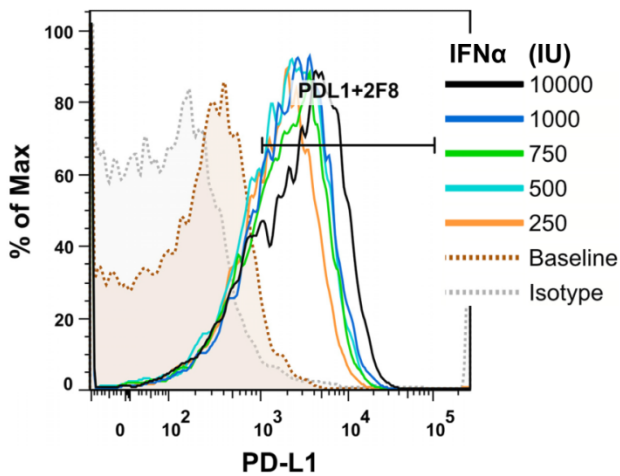
Supplementary Figure 4



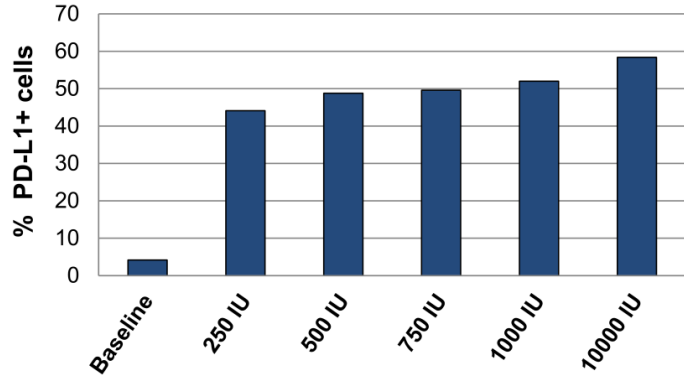
Supplementary Figure 4. Heat map of top 65 DE genes ($p < 0.05$; included in $n = 136$ genes listed in Supplementary Table 4) from the comparison of WT mice receiving weekly doses of anti-PD-L1 plus IFN α ($n = 4$) versus WT mice treated weekly with anti-PD-L1 ($n = 4$). The DE gene cluster upregulated in IFN α -treated mice (blue highlighted box) is listed in the right table inset.

Supplementary Figure 5

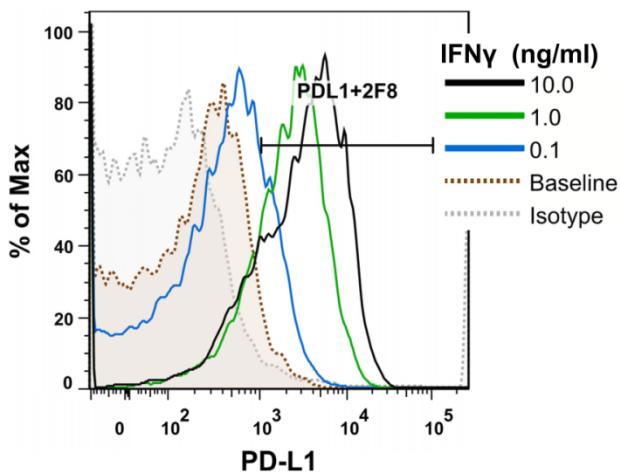
A



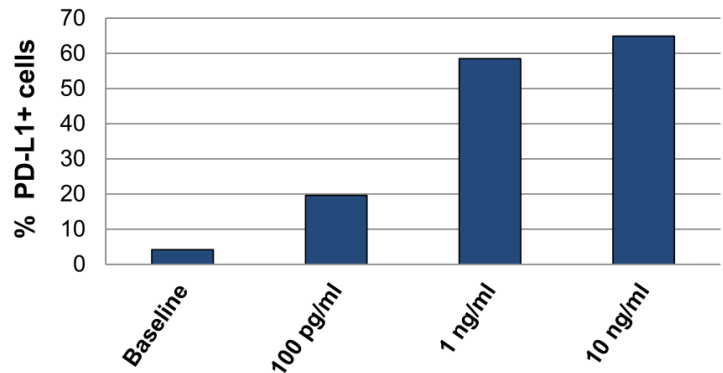
PD-L1 expression in 2F8 cells stimulated with IFN α



B



PD-L1 expression in 2F8 cells stimulated with IFN γ



Supplementary Figure 5. Tumor PD-L1 by flow cytometry in response to stimulation with IFN α and IFN γ . A 2F8 cells were cultured for 24 h in the presence of varying concentrations of IFN α (250, 500, 750, 1000 and 10,000 IU/ml;). B IFN γ was used at 10, 1.0, 0.1 ng/ml. Isotype control (grey tint) and baseline unstimulated control cells (orange tint), cultured for 24 h, were used for gating. Percentages of PD-L1 positive cells by flow cytometry are shown in the bar graphs at right.