Supplementary Information
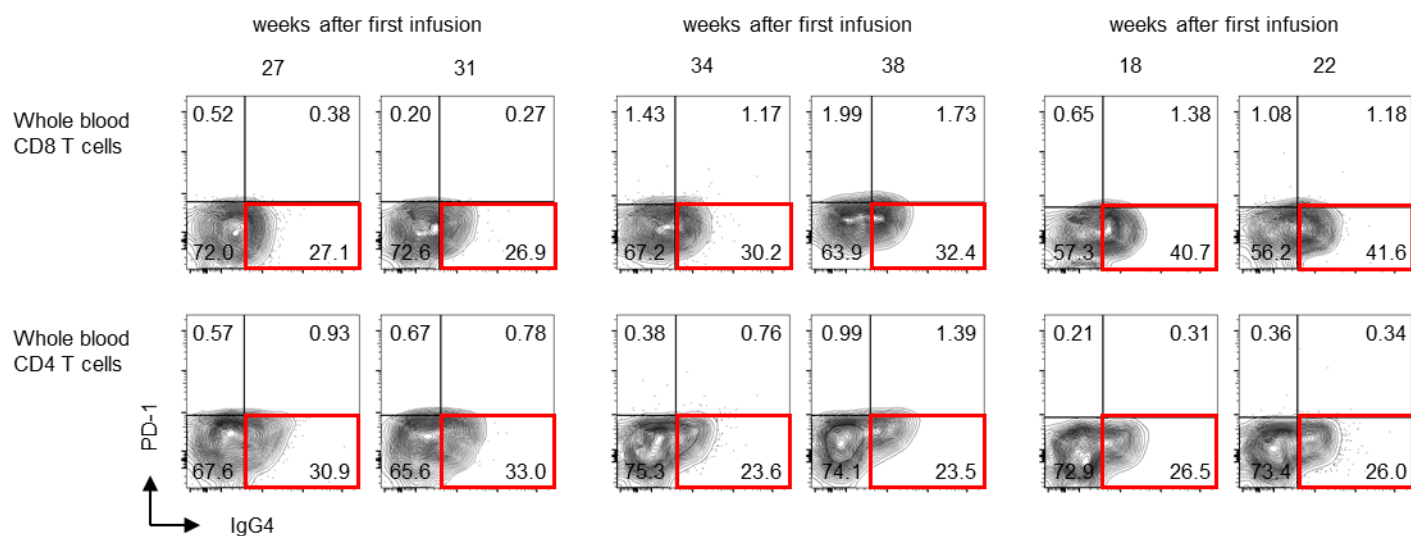
Clinical implications of monitoring nivolumab immunokinetics in previously treated non–small cell lung cancer patients
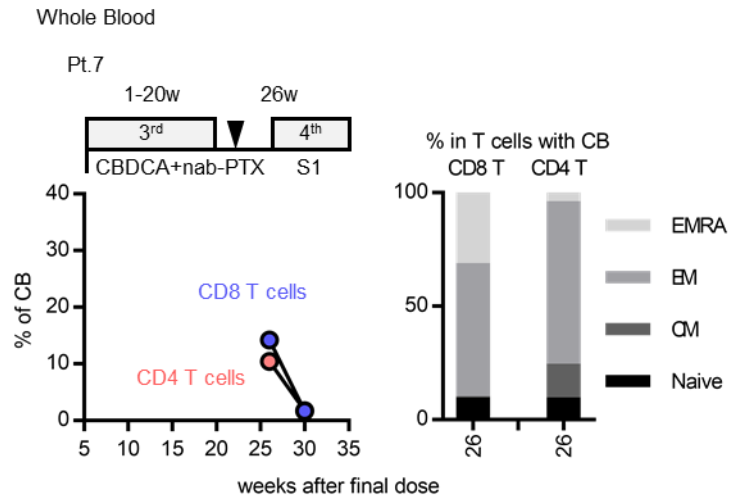
Akio Osa, Takeshi Uenami, Shohei Koyama, Kosuke Fujimoto, Daisuke Okuzaki, Takayuki Takimoto, Haruhiko Hirata, Yukihiro Yano, Soichiro Yokota, Yuhei Kinehara, Yujiro Naito, Tomoyuki Otsuka, Masaki Kanazu, Muneyoshi Kuroyama, Masanari Hamaguchi, Taro Koba, Yu Futami, Mikako Ishijima, Yasuhiko Suga, Yuki Akazawa, Hirotomo Machiyama, Kota Iwahori, Hyota Takamatsu, Izumi Nagatomo, Yoshito Takeda, Hiroshi Kida, Esra A. Akbay, Peter S. Hammerman, Kwok-kin Wong, Glenn Dranoff, Masahide Mori, Takashi Kijima, Atsushi Kumanogoh

Supplemental Figures 1 – 8

weeks after first infusion | weeks after first infusion | weeks after first infusion

27 | 31 | 34 | 38 | 18 | 22

Whole blood CD8 T cells

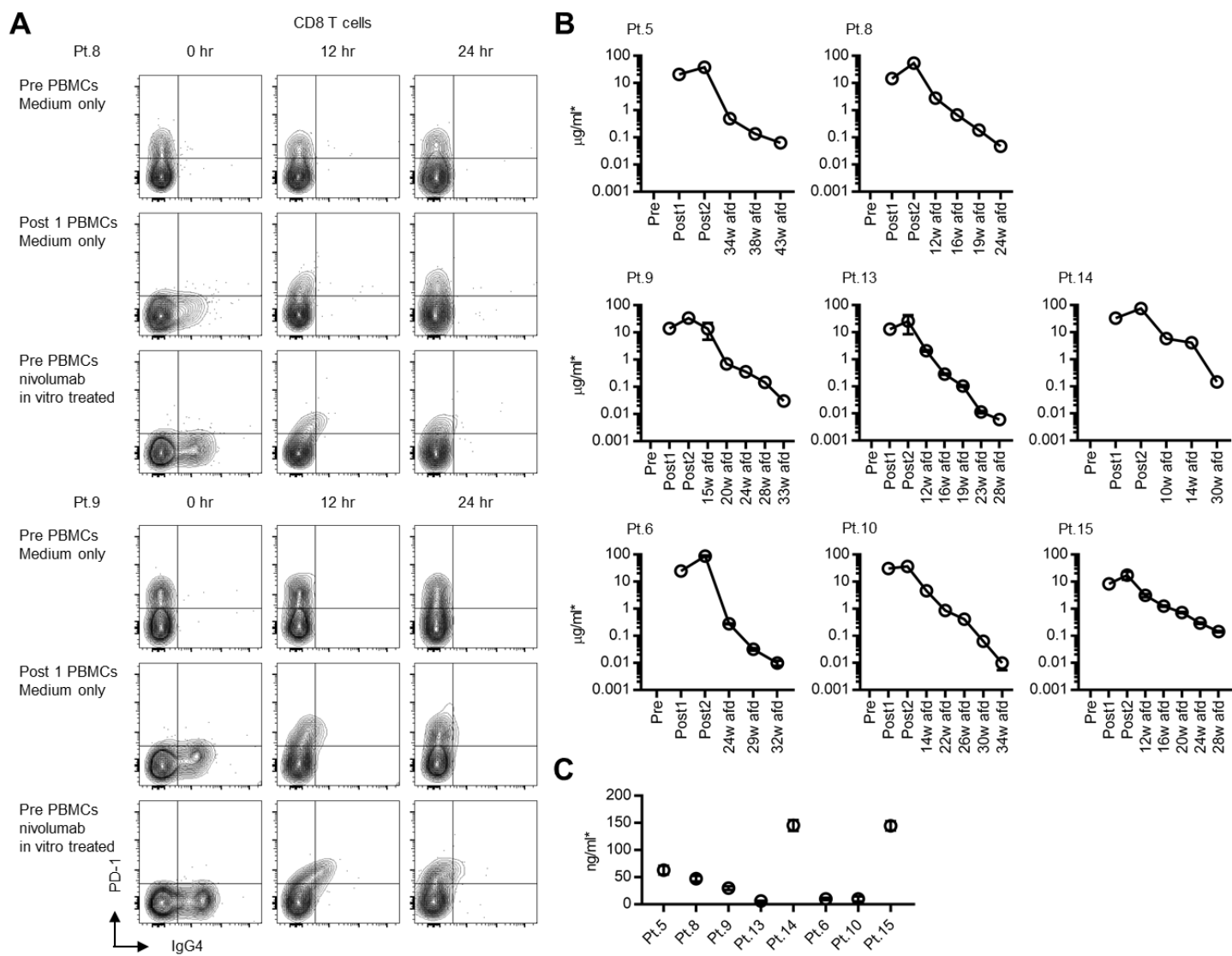Whole blood CD4 T cells

PD-1

IgG4

Supplemental Figure 1. The frequency of nivolumab-bound T cells was maintained in patients who continued treatment.

Nivolumab binding in CD8 and CD4 T cells was analyzed at two follow-up points, as indicated, in fresh peripheral blood from three representative cases from protocol 1 that continued treatment.
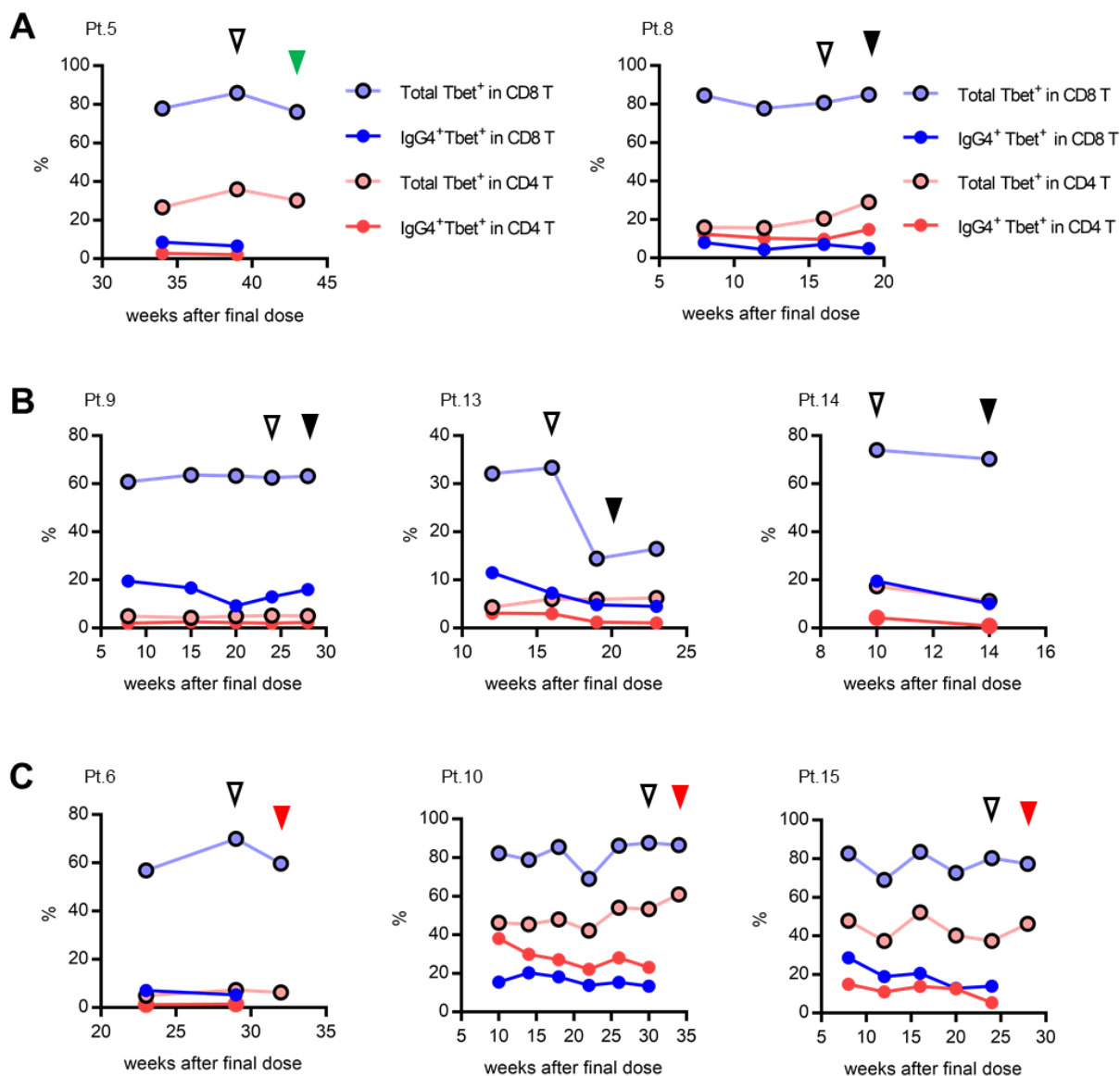
Whole Blood

Pt.7

Supplemental Figure 2. Long-term follow-up of nivolumab binding to T cells from fresh whole blood.

Nivolumab binding was followed up in fresh peripheral blood from an additional case, Pt.7.
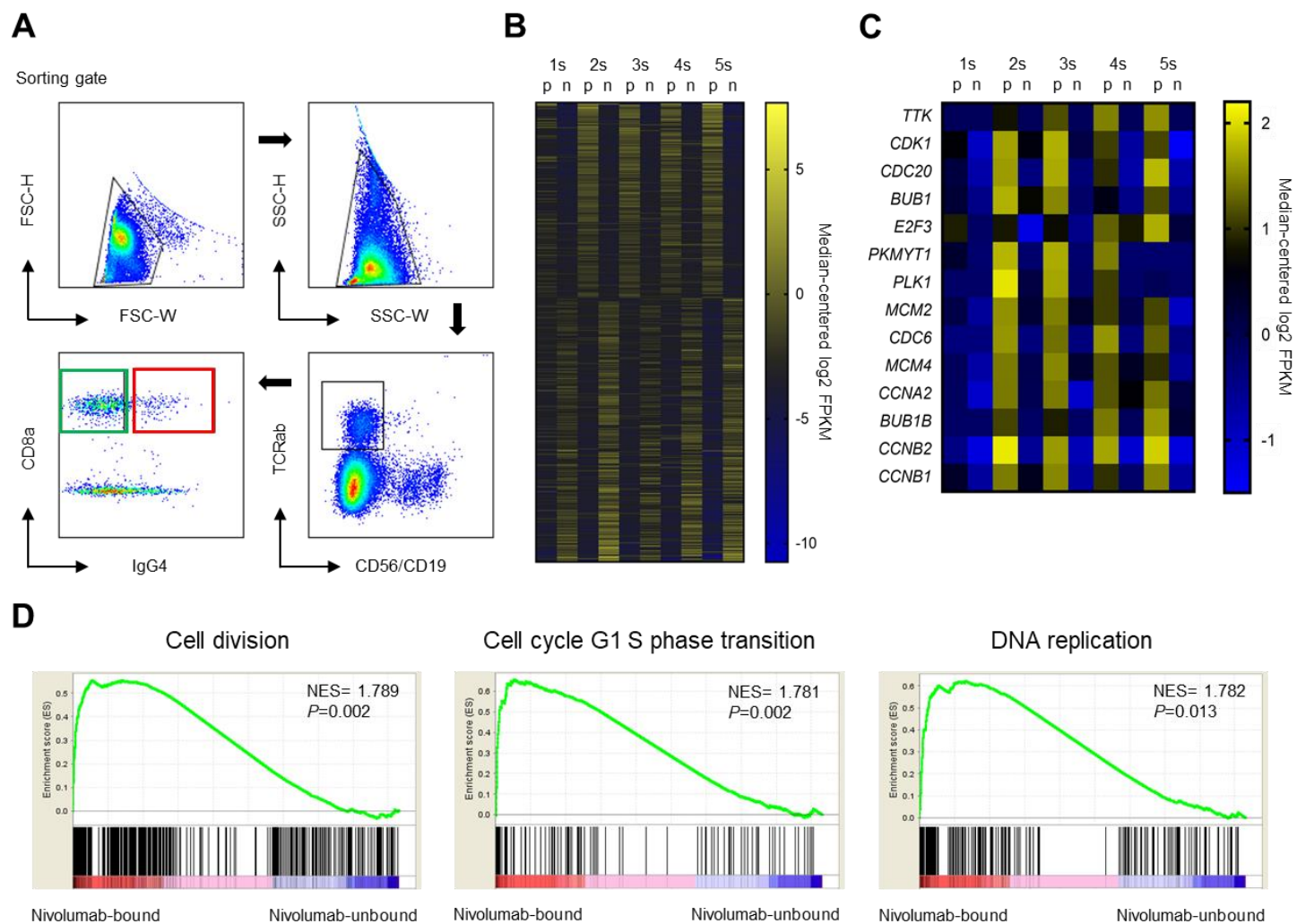
Supplemental Figure 3. Long-term duration of nivolumab binding is due to sustained circulation of residual nivolumab in plasma.

(**A**) PBMCs acquired from Pt.8 and 9 at pretreatment (pre PBMCs) and after a single dose (post 1 PBMCs) were cultured in regular medium without nivolumab (top and middle). Pre PBMCs were also incubated with 10 μg/ml nivolumab in vitro before the cultures were started (bottom). Nivolumab binding status was monitored at the indicated time points. (**B**) Concentrations of nivolumab in plasma from patients at the indicated time points when binding on T cells was analyzed (afd: after final dose). Data indicate three replicate wells and are representative of three independent experiments. (**C**) Plasma level of nivolumab at the time of absolute loss of CB (last follow-up point). *Units are calculated based on the concentration of nivolumab in PBS, as described in Methods (**B,C**).

Supplemental Figure 4. Tbet positivity in total and nivolumab-bound CD8 and CD4 T cells in patients who underwent sequential treatment.

A, B, C. Tbet positivity in total and nivolumab-bound CD8 and CD4 T cells was followed up in fresh whole blood samples from eight patients with non–small cell lung cancer. Display order of patients is the same as in Figure 4. Black and green arrows show points of progressive disease and tumor marker re-elevation without increase in the size of the targeted tumor (as determined by CT scan), respectively.

Supplemental Figure 5. Sorted nivolumab-bound CD8 T cells used for transcriptome profiling.

(**A**) Gating strategy for sorting CD45^{+}CD56^{-}CD19^{-}TCRab^{+}CD8a^{+}IgG4[+ or -] T cells. (**B**) A total of 206 genes were significantly upregulated, and 279 were significantly downregulated, in nivolumab-bound CD8 T cells (IgG4^{+}) vs. nivolumab-unbound cells (IgG4^{-}). Statistical significance was determined by Mann–Whitney U test (p<0.05). (**C**) Fourteen representative cell cycle–related genes were upregulated in nivolumab-bound cells. Cell cycle–related genes were identified based on the "cell cycle process" Gene Ontology annotation in MSigDB. (**D**) Enrichment plots from GSEA conducted on RNA-Seq expression datasets. Significant enrichment scores (NES) and low p-values were observed for genes related to "Cell division," "Cell cycle G1 S phase transition," and "DNA replication" in nivolumab-bound vs. nivolumab-unbound cells.
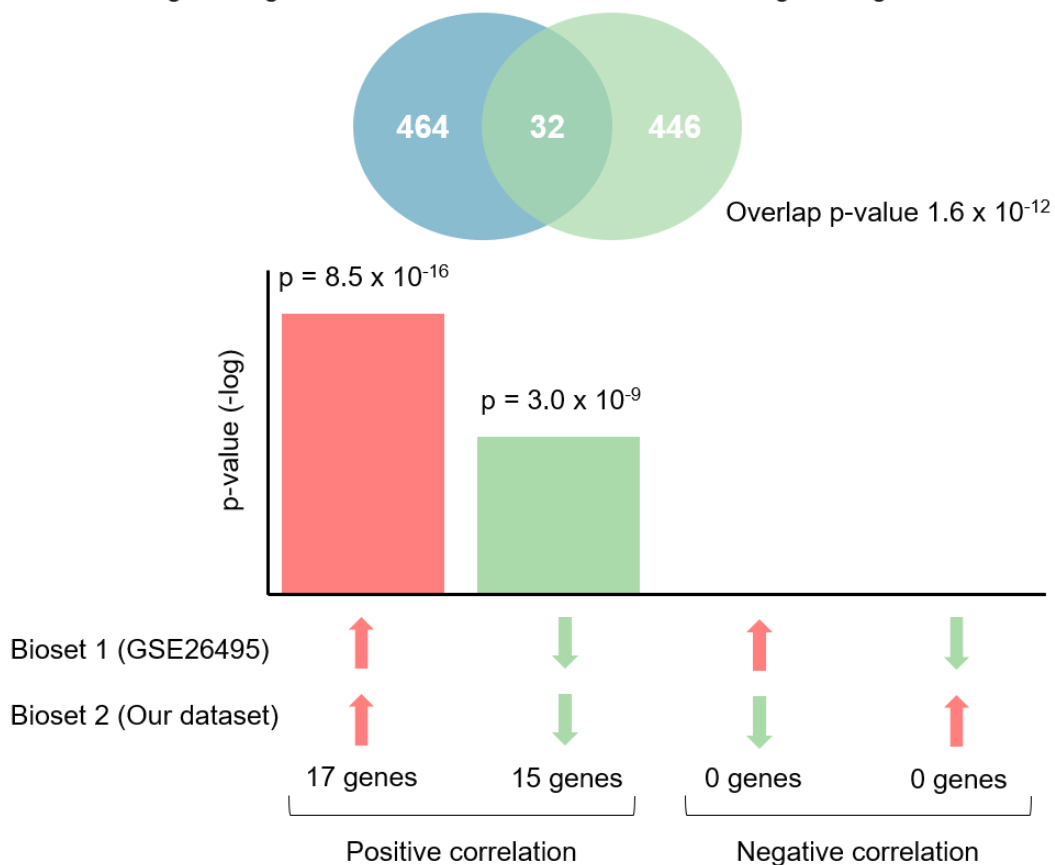
6

Duraiswamy J, et al.

PD-1^{hi} vs PD-1^{lo} CD8$^+$CD3$^+$ T cells from peripheral blood of healthy adults (GSE26495)

↑ Up-regulated genes: 198
↓ Down-regulated genes: 299

Our dataset

IgG4$^+$CD8 T cells vs IgG4$^-$ CD8 T cells (> 2 fold change)

↑ Up-regulated genes: 205
↓ Down-regulated genes: 273

464   32   446

Overlap p-value 1.6 x 10^{-12}

p = 8.5 x 10^{-16}

p = 3.0 x 10^{-9}

p-value (-log)

Bioset 1 (GSE26495)

Bioset 2 (Our dataset)

17 genes   15 genes   0 genes   0 genes

Positive correlation   Negative correlation

Supplemental Figure 6. NextBio analysis of RNA-Seq data from nivolumab-bound vs. unbound CD8 T cells.

Using NextBio analysis, genes differentially expressed in nivolumab-bound (IgG4$^+$) and unbound (IgG4$^-$) CD8 T cells were compared with those in PD-1^{hi} and PD-1^{lo} CD8 T cells from healthy donors. Duraiswamy J, et al. (2011): data obtained from a public repository (GSE26495). Our dataset included RNA-seq data from this study, in which 478 of the 485 genes up- or down-regulated > 2.0-fold in IgG4-negative vs. IgG4-positive CD8 T cells were recognized and could be interpreted by NextBio. Venn diagram shows the number of common and unique genes in both sets. Bar graphs show overlap p-values for genes up-regulated (red arrows) or down-regulated (blue arrows) under each condition. The y-axis scale represents −log (p-value).
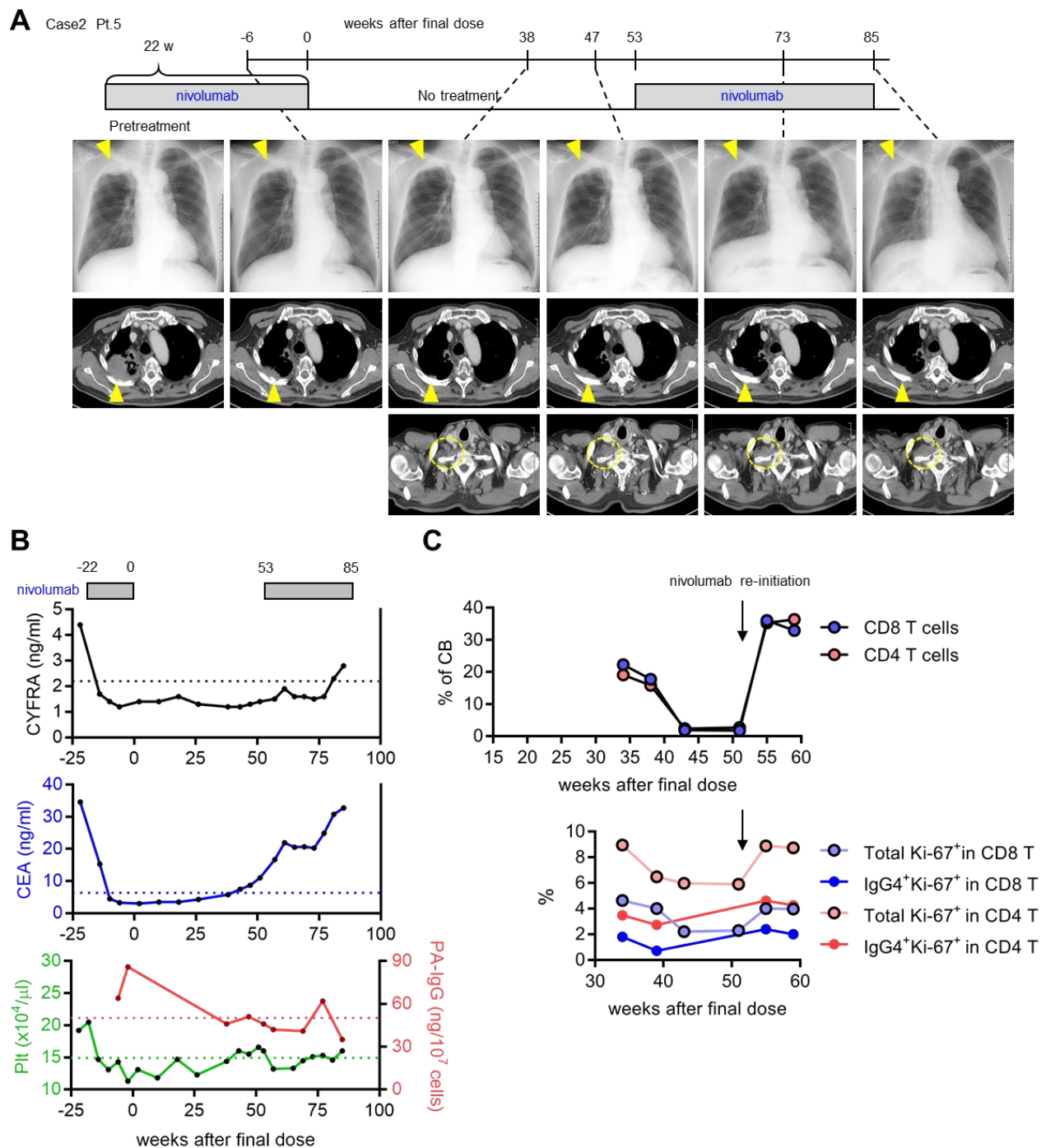
Upstream regulator	Predicted activation state	Activation z-score	p-value of overlap	Target molecules in dataset
PTGER2	Activated	5.19	1.67E-23	*ADGRE1,ASPM,BUB1B,CCNA2,CCNB2,CCR7,CDH1,CDKN3,CENPF,CEP55, CKAP2L,DEPDC1,ECT2,IL10,KIF11,KIF15,KIF20A,KIF2C,MELK,MKI67,NEK2, NUF2,NUSAP1,PBK,PLK1,TPX2,TROAP,TTK*
TBX2	Activated	4.24	1.42E-14	*ANLN,ASF1B,AURKB,BUB1,CCNA2,CCNB1,CDC6,CDCA3,CDCA5,CDH1,CD K1,CDT1,E2F7,FOXM1,MCM2,MCM4,PKMYT1,PLK1*
FOXM1	Activated	4.17	3.37E-18	*AURKB,AXIN2,BIRC5,BUB1B,CCNA2,CCNB1,CCNB2,CDC20,CDC25A,CDCA 2,CDH1,CDK1,CDKN3,CENPF,ESR1,FOXM1,KIF20A,LEF1,MKI67,MYC,NEK2 ,PLK1,PLK4,STMN1,TOP2A*
AREG	Activated	3.99	3.18E-11	*AURKB,BIRC5,CCNA2,CCNB1,CCNB2,CDC20,CENPF,FOXM1,HJURP,KIF14 ,KIF20A,MKI67,MYBL2,PLK1,RRM2,TOP2A*
CSF2	Activated	3.88	1.28E-20	*ANLN,BIRC5,BUB1,BUB1B,CCL4,CCNA2,CCNB1,CCR5,CCR7,CD38,CD74,C DC20,CDCA2,CDCA3,CDCA5,CDH1,CDK1,CISH,CTLA4,DSCC1,E2F7,EXO1, FCGR2B,FOXM1,HJURP,IL10,IL2RA,IL5RA,KIF11,MKI67,MYC,NEK2,NUSAP 1,OSM,PLK1,PTGER2,RRM2,SKA1,SOCS2,STMN1,TLR5,TNFRSF11A,TNFR SF4,TNFRSF9,TOP2A,TPX2,UBE2C,UHRF1*
HGF	Activated	3.88	7.89E-13	*AKAP12,AURKB,BIRC5,BUB1,BUB1B,CCL4,CDC20,CDC25A,CDC6,CDH1,C DK1,CDKN3,CENPF,DUSP4,EDA,FOXM1,HMMR,HMOX1,IL10,IL5RA,KIF11,K IF15,KIF2C,MCM2,MELK,MKI67,MYC,NDC80,NEK2,PKMYT1,PLK1,PLK4,RU NX2,SOCS2,SPRY2,TTK,UBE2C,VCAM1*
RABL6	Activated	3.80	1.21E-25	*AURKB,BUB1,BUB1B,CCNA2,CCNB1,CDC25A,CENPF,HMMR,HMOX1,KIF23 ,MCM10,MCM2,MELK,MYC,NCAPG,NDC80,NEK2,PBK,PLK1,PMAIP1,POLE2 ,TOP2A,TPX2,TTK,UBE2C*
EP400	Activated	3.72	1.08E-15	*CCNA2,CDC20,CDC25A,CDC6,CDCA3,CENPF,E2F7,FOXM1,MAPK12,MCM4 ,MYBL2,NEK2,PLK1,SKA1,UHRF1*
MITF	Activated	3.70	5.02E-09	*AURKB,CCNB1,CDCA3,CDT1,CENPF,CEP55,ECT2,ESPL1,KIF20A,KIF4A,KI FC1,LYST,MCM2,MCM4,NGFR,NUF2,PLK1,POLE2,SPC24,STXBP1,TPX2,UB E2C*
MYC	Activated	3.61	8.55E-05	*ABCB9,ACTN1,ACVR2A,AKAP12,AURKB,BCAT1,BIRC5,BUB1,BUB1B,CCNA 2,CCNB1,CCNB2,CDC20,CDC25A,CDH1,CDK1,DUSP4,E2F3,ERBB2,FABP5, FOXM1,GLS2,HMOX1,IL10,IL5RA,JAG2,LIMS1,MKI67,MYC,PLK1,PMAIP1,RA RG,RRM2,SCAMP5,SEMA4A,SMS,STMN1,TF,TLE1,TYMS,UBE2C,VCAM1*
E2f	Activated	3.39	7.78E-10	*CCNA2,CCNB1,CDC25A,CDC6,CDK1,GINS2,MCM10,MCM2,MCM4,MYBL2, MYC,NUSAP1,RRM2,TPX2,TTK,TYMS,UBE2C*
ERBB2	Activated	3.25	3.96E-18	*ASPM,BIRC5,BUB1,BUB1B,CAVIN3,CCL4,CCNA2,CCNB1,CCNB2,CDC20,C DC25A,CDC6,CDCA2,CDCA3,CDCA5,CDH1,CDK1,CDKN3,CDT1,CENPF,CH D7,CLEC11A,COL6A1,CYP2J2,DLL1,E2F3,E2F7,EPHX2,ERBB2,ESPL1,ESR 1,ETV1,FZD4,GINS1,GINS2,JAG2,MCM10,MCM2,MCM4,MKI67,MYBL2,MYC, NCAPG,NDC80,NEK2,PFN2,POLE2,POLQ,RAD51AP1,RARG,RRM2,SOCS2, SOWAHC,SPINT2,TOP2A,TYMS,UBE2C,ZWINT*
CD40	Activated	3.24	7.71E-07	*BIRC5,CCL4,CCNA2,CD27,CD38,CD70,CR2,CTLA4,FCGR2B,IL10,IL2RA,IL5 RA,MYC,PDCD1,PTGER2,RGS13,TNFRSF9,VCAM1*
E2F3	Activated	3.21	8.34E-10	*BAIAP2L1,BIRC5,CCNA2,CCNB1,CCNB2,CDC25A,CDC6,CDCA3,CDK1,E2F3 ,MCM10,MCM2,MCM4,MYBL2,MYC,PCLAF,PLK1,RRM2,TPX2,UBE2C*
CCND1	Activated	3.02	1.34E-17	*ASPM,BIRC5,CCNA2,CDC6,CDCA2,CDH1,CENPF,CEP55,CLSPN,DDIAS,DE PDC1,DTL,E2F7,EPGN,EPS8,ERBB2,FAM83D,FOXM1,HJURP,KIF11,KIF20A ,KIF2C,KIF4A,KNL1,KRT1,MCM10,MCM4,MELK,MYC,NT5E,PCLAF,PLK1,RR M2,SATB1,STXBP1,TPX2,TYMS,UHRF1*
ELAVL1	Activated	2.97	4.65E-05	*CDC6,HMOX1,KIF11,MYBL2,MYC,NCAPG,NEK2,NUF2,PBK,TOP2A*
FOXO1	Activated	2.94	6.86E-14	*AFF3,ANLN,ASPM,ATP6V0A1,BACH2,BIRC5,CCL4,CCNA2,CCNB1,CCNB2, CCR7,CD70,CDK1,CENPF,DEPDC1,DLGAP5,EFHD1,FOXM1,GZMA,HMOX1, IRS2,KIF11,KLF7,MYC,NCAPG,NEK2,NUSAP1,PBK,PMAIP1,RUNX2,SCD,SP RY2,TNFRSF11A,TNFRSF9,TNFSF9,VCAM1*
TICAM1	Activated	2.75	0.00878	*CCL4,CD38,CXCL13,IL10,MCM2,MKI67,TLR3,VCAM1*
RARA	Activated	2.69	3.58E-05	*AASS,ASPM,BIRC5,CCNA2,CD38,CDK1,CDKN3,CENPF,CXCR5,E2F7,IL5RA ,KIF23,KNL1,MND1,MYC,NCAPG,PBK,PLK4,RARG*
Vegf	Activated	2.66	3.26E-15	*AKAP12,AURKB,AXIN2,BIRC5,BUB1,BUB1B,CDC20,CDC25A,CDC6,CDH1,C DK1,CDKN3,CENPF,CSGALNACT1,DUSP4,EDA,FOXM1,HMMR,HMOX1,IL5 RA,KIF11,KIF15,KIF2C,LEF1,MCM2,MELK,MKI67,MYC,NDC80,NEK2,P2RY1 4,PKMYT1,PLK1,PLK4,PMAIP1,SOCS2,TLR3,TNFRSF11A,TNFRSF9,TPX2,T TK,UBE2C,VCAM1*
SMOC2	Activated	2.63	1.76E-10	*AURKB,CCNB1,CDH1,CDK1,CENPF,MCM10,MCM4*
YAP1	Activated	2.62	1.67E-07	*ANLN,AURKB,BIRC5,CDH1,CDK1,FOXM1,MYC,NEK2,PDGFB,PLK1,PMAIP1, UHRF1,VCAM1*
MTOR	Activated	2.59	0.0157	*BIRC5,CCR7,CDH1,FABP5,IL10,IRS2,MYC,PRX,RUNX2,TLR5,UBE2C,VCAM 1*
VEGFA	Activated	2.55	0.00516	*BIRC5,CTLA4,DLL1,DUSP4,E2F3,HMOX1,MKI67,PDCD1,PDGFB,RUNX2,TN FRSF9,UNC5B,VCAM1*

S100A6	Activated	2.45	1.63E-06	*CDH1,CDK1,CDKN3,DEPDC1,NEK2,PBK,TYMS*
E2F2	Activated	2.45	2.14E-10	*BIRC5,CCNA2,CCNB1,CCNB2,CDC25A,CDC6,CDK1,E2F3,MCM10,MCM2,MCM4,MYBL2,MYC,RRM2*
E2F1	Activated	2.42	9.45E-10	*AURKB,BIRC5,CCNA2,CCNB1,CCNB2,CDC20,CDC25A,CDC6,CDK1,DUSP4,E2F3,ECT2,FOXM1,GINS1,IRS2,JAG2,KIF23,KRT1,MCM10,MCM2,MCM4,MYBL2,MYC,NDC80,NUSAP1,PCLAF,RRM2,SOAT2,STMN1,TNFSF9,TOP2A,TYMS,UHRF1,VCAM1*
RAF1	Activated	2.42	2.34E-06	*AKAP12,CD74,CDC25A,CDC6,CHN1,CXCR5,DUSP4,ERBB2,ESR1,IL10,MKI67,MYBL2,MYC,RGS1,RRM2,SH2D1A,SPRY2*
TNFSF12	Activated	2.39	0.0229	*CCR5,CXCR5,DLL1,JAG2,MEF2B,VCAM1*
PI3K(family)	Activated	2.39	0.0202	*BIRC5,CDH1,ESR1,HMOX1,IL10,PDGFB*
EGR1	Activated	2.39	0.036	*CCL4,CDC20,CDH1,CHGB,DUSP4,HMOX1,MYC,VCAM1*
TRAF2	Activated	2.24	0.0276	*AURKB,CCNB1,CDK1,CR2,PLK1*
ATF6	Activated	2.24	0.00742	*BUB1,CDKN3,HMMR,TROAP,UBE2C*
OGT	Activated	2.24	0.000211	*BIRC5,CDH1,FOXM1,MYC,NEK2,PLK1*
OSM	Activated	2.24	0.00771	*ATP9A,C1R,CA6,CDH1,CHI3L2,COL6A1,CXCL13,DENND5A,EPHA1,HMOX1,IL10,MYC,OSM,PLLP,RARG,TLR3,TNFRSF11A,TOP2A,TPX2,VCAM1*
CCNE1	Activated	2.22	0.00017	*CCNA2,CCNB1,CDC6,MCM2,MCM4*
IFNB1	Activated	2.19	0.00531	*CCL4,CCR5,CD38,DKK3,DLL1,IL10,MCM10,MYC,PDCD1,PMAIP1,SPRY2,TLR3,TNFRSF4*
MYD88	Activated	2.19	0.0448	*CCL4,CD38,CISH,CXCL13,HMOX1,IL10,MCM2,MKI67,MYC,VCAM1*
MOG	Activated	2.18	0.000932	*CCR5,CTLA4,IL10,MKI67,PDCD1,VCAM1*
NFkB (complex)	Activated	2.02	0.00564	*ADGRE1,BIRC5,C1R,CCL4,CCNB1,CCNB2,CCR7,CD74,CDH1,E2F7,ERBB2,FABP5,GZMK,HLA-DMB,HMOX1,IL10,IL2RA,MYC,PLK1,PMAIP1,TNFRSF4,TNFRSF9,VCAM1*
Nfat (family)	Activated	2.00	0.12	*DKK3,IL10,PDGFB,VCAM1*
DOCK8	Activated	2.00	0.0546	*CCL4,CD70,RGS1,TLR3*
NELFCD	Activated	2.00	1.18E-05	*CCNB2,DLGAP5,MYBL2,TOP2A*
FGFR3	Activated	2.00	0.000628	*CCL4,CDH1,CR2,DLL1,NOG*
NELFA	Activated	2.00	0.000021	*CCNB2,DLGAP5,MYBL2,TOP2A*
Hbb-b2	Activated	2.00	0.00134	*CCR5,CD74,CXCR5,CXCR6,TF*
FLT1	Activated	2.00	0.0754	*BCAT1,BIRC5,HMOX1,LIMS1*
NELFE	Activated	2.00	0.000021	*CCNB2,DLGAP5,MYBL2,TOP2A*
Sod	Inhibited	-2.00	9.37E-06	*CCL4,HLA-DRA,HMOX1,PMAIP1,VCAM1*
HCAR2	Inhibited	-2.00	0.000347	*KIF14,KIF20A,PLK1,PLK4*
TNFSF13	Inhibited	-2.00	0.00134	*CDC6,MCM2,MCM4,MYC,POLE2*
TOB1	Inhibited	-2.00	0.016	*CCNA2,CDT1,HJURP,RPRM*
ATF3	Inhibited	-2.00	0.113	*AURKB,CDK1,NEK2,PLK1*
E2F6	Inhibited	-2.00	0.00004	*CDC25A,CDC6,GINS2,MCM2,MYC,PCLAF,RAD51AP1,RRM2*
TP63	Inhibited	-2.03	0.0063	*CCNA2,CCNB1,CDH1,CDK1,DLL1,GLS2,JAG2,KIF23,KRT1,MCM10,MYC,NT5E,PMAIP1,RGS13,RUNX2,UPK2*
Rb	Inhibited	-2.04	3.15E-10	*AURKB,BIRC5,CCNA2,CDC6,CDH1,CDK1,CDT1,ECT2,FOXM1,MYBL2,MYC,PLK1,TOP2A,TYMS*
IL31	Inhibited	-2.20	3.39E-05	*CCNB1,CDK1,CISH,MCM4,SOCS2*

CAT	Inhibited	-2.20	0.00398	*HLA-DRA,HMOX1,IL2RA,PMAIP1,VCAM1*
CREM	Inhibited	-2.21	0.207	*CCNB1,DUSP4,HLA-DRA,IRS2,TSHR*
CTLA4	Inhibited	-2.23	0.000505	*CCNA2,IL10,IL2RA,MKI67,PDCD1,TOP2A,TPX2*
TP73	Inhibited	-2.30	7.53E-05	*ADAM23,BIRC5,C1R,CCNB1,CDH1,CDK1,CNN3,DAB1,EDA,FOXJ1,HIST1H2 BH,JAG2,KCNQ1,LZTS1,PDGFB,PMAIP1,PROCR,RPRM,STMN1*
ESR2	Inhibited	-2.31	0.00156	*ACER1,AKAP12,ANK1,ASB2,BCAT1,BIRC5,CCNA2,CDC25A,CDC42BPA,CD H1,ESR1,FOXM1,MKI67,MYC,PMAIP1,PTGDS,RUNX2,SOCS2,SUSD4*
RBL1	Inhibited	-2.32	4.21E-09	*AURKB,CCNA2,CDC25A,CDC6,CDK1,MCM10,MCM2,MYBL2,MYC,NEK2,PL K1,RRM2,TYMS*
BNIP3L	Inhibited	-2.33	1.75E-06	*ANK1,CCNA2,CDKN3,CENPF,KIF11,MYBL2,NUF2,RRM2,TOP2A*
DYRK1A	Inhibited	-2.44	4.26E-06	*CCNB1,CDC6,CDK1,MCM4,MYBL2,UBE2C*
WNT3A	Inhibited	-2.57	0.0367	*AXIN2,C2orf40,CLEC11A,FHL2,IRS2,LEF1,MYC,NGFR,RUNX2,VCAM1*
ZFP36	Inhibited	-2.78	1.79E-06	*CDC6,IL10,KIF11,KIF14,NEK2,NHSL2,NUF2,OSM,PBK,TOP2A,VCAM1*
RBL2	Inhibited	-2.89	2.78E-11	*AURKB,BUB1,BUB1B,CCNA2,CCNB1,CCNB2,CDC25A,CDC6,CDK1,KIFC1,M YBL2,MYC,NEK2,PLK1,RRM2,STMN1,TYMS*
TP53	Inhibited	-2.90	9.2E-23	*ACTN1,AKAP12,ANK1,ANLN,ASF1B,ASPM,AURKB,AXIN2,BIRC5,BUB1,BUB 1B,CCL4,CCNA2,CCNB1,CCNB2,CD70,CDC20,CDC25A,CDC42BPA,CDC6,C DH1,CDK1,CDKN3,CDT1,CENPF,CEP55,CNN3,CRACR2B,DDIAS,DKK3,DLG AP5,DPP4,DUSP4,EPS8,ERBB2,ESPL1,ESR1,EXO1,FAM83D,FHL2,FOXM1, GAL3ST4,GLS2,HJURP,HMMR,HMOX1,IL10,IL2RA,IL5RA,KIF23,KIFC1,MAP K12,MCM2,MCM4,MELK,MKI67,MYBL2,MYC,NCAPG,NDC80,NEK2,NUSAP1, PACSIN1,PBK,PCLAF,PDGFB,PDIA5,PLK1,PMAIP1,PODXL,POLE2,PRKN,P TGDS,PTPRO,RAD51AP1,RGS13,RPRM,RRM2,RUNX2,SALL2,SH2D1A,SOC S2,STMN1,TCEA3,TNFRSF9,TNFSF9,TOP2A,TPX2,TSPAN6,TTK,TYMS,TYM SOS,UBE2C,UHRF1,UNC5B*
RB1	Inhibited	-2.95	5.33E-07	*ASF1B,AURKB,BIRC5,CCNA2,CCNB1,CDC25A,CDC6,CDCA5,CDH1,CDK1,C DT1,E2F3,EPS8,MCM10,MCM2,MCM4,MELK,MYBL2,MYC,PCLAF,PLK1,RAD 51AP1,RRM2,RYR1,TYMS*
SMARCB1	Inhibited	-2.97	2.48E-06	*CCNA2,CDC6,CDH1,CDK1,CDT1,ERBB2,KIF11,KIF23,MCM10,MCM2,MCM4, MYBL2,MYC,PLK4,RAD51AP1*
CDKN1A	Inhibited	-3.09	2.79E-25	*ANLN,ASPM,AURKB,BIRC5,BUB1,BUB1B,CCNA2,CCNB1,CDC20,CDC25A,C DC6,CDK1,CDKN3,CENPF,CEP55,DLGAP5,DTL,EXO1,FOXM1,HJURP,KIF20 A,KIF2C,MCM2,MCM4,MKI67,MYBL2,MYC,NUSAP1,PBK,PCLAF,PLK1,STMN 1,TOP2A,TPX2,TTK,TYMS,UBE2C,UHRF1,ZNF629*
CDKN2A	Inhibited	-3.30	3.58E-12	*ASF1B,AURKB,BIRC5,BUB1B,CCL4,CCNA2,CCNB1,CCR7,CDC25A,CDCA5, CDK1,CXCL13,CXCR5,E2F3,EPS8,FHL2,IL5RA,KIFC1,LEF1,MCM4,MELK,M KI67,MS4A1,MYBL2,MYC,PLK1,PMAIP1,RAD51AP1,RRM2*
KDM5B	Inhibited	-3.48	4.43E-10	*BUB1B,CCNB1,CDCA3,CDK1,DLGAP5,ECT2,ERBB2,ESR1,FABP5,HACD1,H MMR,KIF2C,MCM2,NDC80,NEFL,PBK,TOP2A,TTK*
Irgm1	Inhibited	-3.73	9.71E-14	*AURKB,BUB1,CCNA2,CCNB1,CCNB2,CDCA3,DTL,GINS1,KIF20A,MKI67,NC APG,NEK2,RRM2,UBE2C*
TCF3	Inhibited	-3.80	5.51E-22	*ANLN,ASF1B,AXIN2,BACH2,BIRC5,BUB1,CCNA2,CCNB1,CCNB2,CDC20,CD CA3,CDH1,CDK1,CDKN3,CEP55,CR2,ECT2,HMMR,IL2RA,KIF11,KIF2C,KIF4 A,LEF1,MAL,MEF2B,MKI67,MYC,NCAPG,NDC80,NEK2,NUF2,NUSAP1,PLK4 ,RRM2,SERINC5,SPC24,TF,TOP2A,TOX,TTK,TYMS,ZP1,ZP3*
NUPR1	Inhibited	-4.32	1.67E-12	*AKAP12,ANK1,ASIC1,ASPM,ATP6V0A1,BUB1,BUB1B,CCNA2,CCNB2,CDCA 2,CDCA3,CKAP2L,EPGN,ESPL1,ETV1,EXO1,FAM111B,FHL2,GINS1,HJURP, IRS2,KIF11,KIF20A,KIF23,KIF2C,KIFC1,KNL1,MCM10,MKI67,MYC,NGFR,PL K1,POLE2,POLQ,SHCBP1,SLC16A10,SPATS2L,SPC24,UNC5B*

Supplemental Figure 7. Ingenuity pathway analysis for RNA sequence data from nivolumab-bound vs. unbound CD8 T cells.

Upstream Regulator Analysis in QIAGEN's Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, CA, USA; www.qiagen.com/ingenuityIPA) was performed to predict the activation or inhibition of transcription factors (TFs) based on relationships with genes in the dataset and the direction of changes in expression in nivolumab-bound vs. unbound CD8 T cells (IgG4^{+} vs. IgG4^{-} CD8 T cells). The z-score for each TF indicated that strongest predicted activation corresponds to z-score ≥ 2, and the strongest predicted inhibition corresponds to z-score ≤ −2. Significance was calculated using the right-tailed Fisher's exact test.

Supplemental Figure 8. A case in which monitoring of nivolumab binding was important for evaluating residual efficacy and toxicity.

(**A**) Case 2 (Pt. 5) discontinued nivolumab treatment after 22 weeks due to thrombocytopenia and fever. Chest X-ray and CT images were acquired pretreatment (left), 16 weeks after initiation of nivolumab (second column), and 38, 47, 73, and 85 weeks after the final dose (third to sixth

columns). Yellow arrow and yellow dotted circle show tumor in the right upper lung. (**B**) Follow-up data: CYFRA (ng/ml), CEA (ng/ml), platelet counts ($\times 10^4$/μl), and platelet-associated IgG (ng/10^7 cells) at the indicated time points. (**C**) Percent of complete nivolumab binding in CD8 and CD4 T cells (upper) and Ki-67 positivity in total and nivolumab-bound CD8 and CD4 T cells (lower). Black arrow shows the time point of re-initiation of nivolumab (53 weeks after final dose).

Supplementary Information

Clinical implications of monitoring nivolumab immunokinetics in previously treated non–small cell lung cancer patients

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Supplementary Methods

Patient eligibility criteria

Key inclusion criteria for patient enrollment are listed below.

1. Pathologically confirmed non–small cell lung cancer.

2. Stage VI or recurrent diseases (without any indications for operation or radiotherapy) with peripheral blood and/or pleural effusion that can be taken.

3. Have measurable lesion in lung using the RECIST ver1.1 (Response Evaluation Criteria In Solid Tumors) criteria.

4. At least 20 years old at the time of informed consent.

5. Have adequate organ function within 14 days before enrollment.

6. Estimated life expectancy of at least 12 weeks.

7. Have signed an informed consent document.

Key exclusion criteria for patient enrollment are listed below.

1. Interstitial pneumonia or pulmonary fibrosis on chest X-ray.

2. Clinically significant drug allergy.

3. Presence of other active malignancy.

4. Pericardial effusion, pleural effusion, or ascites in need of drainage.

5. Positive serum hepatitis B virus antigen.

6. Severe infection, cardiac diseases, hypertension, or other severe complication.

RNA sequence data analysis

The Illumina Casava1.8.2 software was used for base calling. Sequenced reads were mapped to the human reference genome sequences (hg19) using TopHat v2.0.13 in combination with Bowtie2 ver. 2.2.3 and SAMtools ver. 0.1.19. Fragments per kilobase of exon per million mapped fragments (FPKM) was calculated using Cuffnorm version 2.2.1. After calculating differential expression of genes for IgG4-negative vs. IgG4-positive CD8 T cells in Pt.1s–5s, 485 genes were up- or down-regulated > 2.0-fold in IgG4-negative vs. IgG4-positive CD8 T cells (see Supplemental Figure 5 and Supplemental Table 3). Fold changes were evaluated using the Mann-Whitney U test ($p < 0.05$) using the Subio Platform and Subio Basic Plug-in (v1.20; Subio Inc., Amami, Japan). The raw data have been deposited in the NCBI Gene Expression Omnibus database (GEO accession GSE100860).

To determine whether cell cycle–related gene sets of interest were statistically enriched among up- and downregulated genes, we analyzed our non-redundant list of genes using GSEA 3.0. The gene sets used in this study (e.g., 'C5 GO biological process collection') were obtained from the Broad Molecular Signatures Database (MSigDB).

The Ingenuity Pathway Analysis (IPA) regulation z-score algorithm (Ingenuity Systems, www.ingenuity.com) was used to identify upstream pathways that were significantly differentially regulated in IgG4-positive vs. IgG4-negative CD8 T cells. We considered only functions with z-score > 2. P-values were calculated using the right-tailed Fisher's exact test to determining the probability that each functional network or pathway was assigned to that data due to chance alone. To define the similarity of differentially expressed genes, we used the Systems Biology analysis tool NextBio. Gene overlap p-values by NextBio were calculated using Fisher's exact test.

Supplementary Material

Antibody list

Antigen	Clone name	Fluorescence	Manufacturer
CD3e	OKT3	BV 510	BioLegend
	UCHT1	Pacific Blue	BD Biosciences
CD8a	HIT8a	PE Cy7	BioLegend
	HIT8a	APC	BioLegend
	RPA-T8	BV 510	BioLegend
CD4	RPA-T4	APC Cy7	BioLegend
	OKT4	PE Cy7	BioLegend
CCR7	G043H7	PerCP Cy5.5	BioLegend
CD45RA	HI100	FITC	BioLegend
CD19	HIB19	FITC	BioLegend
CD56	HCD56	FITC	BioLegend
TCRab	IP26	PerCP Cy5.5	BioLegend
IgG4 Fc / Isotype	HP6025/ ab81200	PE	Abcam
Tbet / Isotype	4B10/ MOPC-21	APC	BioLegend
Ki-67 / Isotype	Ki-67/ MOPC-21	BV 421	BioLegend
CTLA-4 / Isotype	L3D10 / MOPC-21	APC	BioLegend
CD73	AD2 / MOPC-21	BV 421	BioLegend
CXCR6	K041E5/ MOPC-21	BV 421	BioLegend
PD-1 / Isotype	EH12.1/ MOPC-12	PE Cy7	BD Biosciences

Supplementary Information

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Supplemental Tables 1–3

Supplemental Table 1

Follow-up study design and schedule after nivolumab discontinuation

Follow-up study design

| | Jan 2016 | | Nov 2016 | May 2017 | | May 2018 |

Enrollment (n=60) — Period 1 (n=42) — Period 2 (n=18)

Follow-up study (n=11 #)

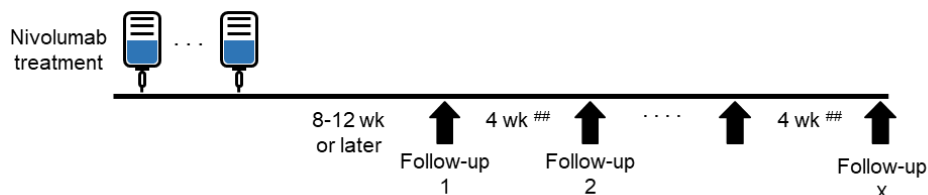# 2 patients discontinued follow-up due to the events as shown below

Follow-up protocol
Sample collection was performed at follow-up time points later than 8wk (black arrows).
4 wk ± 1wk permitted due to outpatient schedule

Nivolumab treatment · · ·

8-12 wk or later
Follow-up 1
4 wk ## — Follow-up 2
· · · ·
4 wk ## — Follow-up X

Follow-up schedule

Patient No	Started period and dose of nivolumab	Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4	Follow-up 5	Follow-up 6	Follow-up 7
Pt.5	Period 1 12 doses	34w	38w	43w				
Pt.6	Period 1 5 doses	24w	29w	32w				
Pt.7	Period 1 2 doses	26w	30w					
Pt.8	Period 1 15 doses	8w	12w	16w	19w	24w		
Pt.9	Period 1 13 doses	8w	#1	15w	20w	24w	28w	33w
Pt.10	Period 1 7 doses	10w	14w	18w	22w	26w	30w	34w
Pt.11	Period 1 5 doses	10w	14w	#2				
Pt.12	Period 1 3 doses	8w	11w	#3				

Patient No	Started period and dose of nivolumab	Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4	Follow-up 5	Follow-up 6	Follow-up 7
Pt.13	Period 2 8 doses	12w	16w	19w	23w	28w		
Pt.14	Period 2 3 doses	10w	14w	#4	#4	#4	30w	
Pt.15	Period 2 14 doses	8w	12w	16w	20w	24w	28w	

#1 Sample collection failed
#2 Nivolumab was discontinued due to irAE, and best supportive care was chosen
#3 Nivolumab was restarted,
#4 Sample collection failed due to follow-up by different department during this period

Supplemental Table 2

Patient characteristics

Case	Age, Gender	Histology	Prior regimens	Duration of nivolumab treatment
Pt.1	62 M	Adeno	1	2 doses x 2 wk
Pt.2	67 M	Adeno	1	10 doses x 2 wk
Pt.3	67 M	Adeno	2	8 doses x 2 wk
Pt.4	62 M	Squamous	5	1 dose x 2 wk
Case	Age, Gender	Histology	Prior regimens	Duration of nivolumab treatment
Pt.5	67 M	Squamous	2	12 doses x 2 wk
Pt.6	72 M	Adeno	3	5 doses x 2 wk
Pt.7	68 M	Squamous	1	2 doses x 2 wk
Pt.8	76 M	Adeno	8	15 doses x 2 wk
Pt.9	69 M	Squamous	1	13 doses x 2 wk
Pt.10	68 F	Adeno	5	7 doses x 2 wk
Pt.11	76 M	Adeno	2	5 doses x 2 wk
Pt.12	87 M	Squamous	1	3 doses x 2 wk
Pt.13	67 M	Squamous	2	8 doses x 2 wk
Pt.14	68 M	Adeno	1	3 doses x 2 wk
Pt.15	75 M	Adeno	5	14 doses x 2 wk

Case	Age, Gender	Histology	Prior regimens	Duration of nivolumab treatment
1s	66 M	Adeno	1	> 10 doses x 2 wk
2s Pt.13	67 M	Squamous	1	8 doses x 2 wk
3s	81 M	Squamous	2	5 doses x 2 wk
4s	61 F	Adeno	5	3 doses x 2 wk
5s	68 M	Adeno	8	5 doses x 2 wk

Supplemental Table 3

Significantly differentially regulated genes in the IgG4⁺ nivolumab-bound population vs. the IgG4⁻ nivolumab-unbound population

Up-regulated				Down-regulated		
Gene name	fold change ratio	p-value		Gene name	fold change ratio	p-value
TMEM155	58.10	0.009		SNORA7B	0.03	0.034
SNORA16B	54.72	0.034		ADAM23	0.07	0.026
PDCD1	27.02	0.009		ANKRD36BP2	0.07	0.005
PACSIN1	12.87	0.009		SCARNA16	0.08	0.016
PP12613	11.66	0.008		EDN3	0.08	0.028
ETV1	10.69	0.009		NEFL	0.08	0.028
TNFRSF9	8.05	0.009		MIR1248	0.08	0.028
CCNB2	7.84	0.009		RMRP	0.09	0.047
DLGAP5	7.80	0.028		SUSD4	0.09	0.016
HJURP	7.56	0.009		CA6	0.10	0.009
KIAA0101	7.00	0.009		MXRA8	0.11	0.047
MND1	6.60	0.047		SFRP5	0.11	0.005
TYMS	6.39	0.047		AEBP1	0.12	0.009
FAM111B	6.35	0.016		PLXNA4	0.12	0.005
SGPP2	6.21	0.016		TNFRSF11A	0.12	0.016
CXCR5	6.04	0.009		TMPRSS11E	0.12	0.005
KIF20A	5.98	0.008		PCSK5	0.12	0.009
CXCL13	5.91	0.034		SLC1A7	0.13	0.028
UBE2C	5.80	0.009		EDA	0.13	0.009
HMMR	5.66	0.028		ISM1	0.13	0.016
HIST1H2BH	5.63	0.016		TCEA3	0.14	0.028
GZMK	5.61	0.009		FLJ21408	0.15	0.034
TOX2	5.54	0.016		ADTRP	0.16	0.026
ARHGAP19-SLIT1	5.43	0.041		EFHC2	0.16	0.009
CDC20	5.39	0.009		TM4SF19	0.16	0.016
CHN1	5.38	0.009		KANK1	0.16	0.009
TMEM171	5.33	0.047		EPHA1	0.16	0.009
MYBL2	5.33	0.009		RFPL2	0.17	0.016
DTL	5.29	0.009		AK5	0.17	0.016

IL26	5.18	0.024		*LOC285965*	0.17	0.009
DEPDC1	5.16	0.009		*CRB3*	0.17	0.009
EXO1	5.12	0.009		*MAGI2-AS3*	0.17	0.008
CDCA5	5.04	0.016		*PLLP*	0.18	0.005
NCAPG	5.02	0.016		*MB21D2*	0.18	0.009
IL10	4.95	0.009		*HDGFRP3*	0.18	0.009
CXCR6	4.94	0.009		*CCL27*	0.18	0.019
TOP2A	4.71	0.009		*KLF7*	0.19	0.009
LOC100131234	4.70	0.047		*SCML1*	0.19	0.009
CEP55	4.64	0.009		*CR2*	0.19	0.005
CDC6	4.64	0.028		*SLC22A17*	0.19	0.009
MKI67	4.60	0.047		*EFHA2*	0.19	0.016
ASB2	4.57	0.009		*TNFRSF4*	0.19	0.028
NEK2	4.54	0.009		*CNN3*	0.20	0.016
SPRY2	4.50	0.047		*LOC729041*	0.20	0.009
C18orf56	4.48	0.005		*LINC00092*	0.20	0.009
CCR5	4.47	0.016		*C2orf40*	0.20	0.028
UHRF1	4.44	0.028		*PRSS30P*	0.20	0.009
BIRC5	4.39	0.028		*CLEC11A*	0.20	0.028
SLCO4A1	4.36	0.009		*IL2RA*	0.21	0.016
SHCBP1	4.28	0.028		*MDS2*	0.21	0.016
DUSP4	4.21	0.016		*LOC389493*	0.21	0.019
MCM10	4.20	0.028		*TGIF2-C20ORF24*	0.22	0.047
SPC24	4.16	0.016		*SLC16A10*	0.22	0.009
KIFC1	4.15	0.047		*FAM63A*	0.22	0.009
KIF23	4.15	0.016		*TXNDC3*	0.22	0.047
LOC728978	4.14	0.034		*C17orf67*	0.22	0.009
PBK	4.12	0.016		*GPC2*	0.23	0.013
VCAM1	4.12	0.009		*MAL*	0.23	0.009
RGS13	4.11	0.028		*ANK1*	0.23	0.047
CCL23	4.04	0.034		*STXBP1*	0.23	0.009
FBXO43	4.02	0.007		*RAB43*	0.24	0.009
MS4A1	3.94	0.047		*P2RY14*	0.24	0.028
RBM20	3.90	0.013		*SHC2*	0.24	0.019
TROAP	3.90	0.016		*ACTN1*	0.24	0.047

KIF15	3.85	0.016	*EDAR*	0.24	0.034
RRM2	3.80	0.047	*TCEAL2*	0.24	0.009
CHI3L2	3.79	0.009	*CYP2J2*	0.25	0.013
AURKB	3.76	0.028	*NOG*	0.25	0.005
ADAM29	3.74	0.005	*EEF1DP3*	0.25	0.047
KIF4A	3.71	0.028	*NT5E*	0.25	0.009
ANLN	3.70	0.028	*PRRT1*	0.25	0.008
E2F7	3.68	0.015	*NPAS2*	0.25	0.009
CDCA2	3.64	0.028	*PTGDS*	0.25	0.047
ASPM	3.57	0.016	*SOX8*	0.25	0.008
PLK1	3.56	0.047	*KRT72*	0.25	0.047
TTK	3.56	0.016	*KRT1*	0.25	0.007
CTLA4	3.47	0.009	*CDH1*	0.26	0.047
CDT1	3.45	0.016	*ZP1*	0.26	0.028
CKAP2L	3.42	0.028	*ZP3*	0.26	0.016
DSCC1	3.42	0.016	*EFHD1*	0.26	0.041
PHEX	3.38	0.005	*IL5RA*	0.26	0.028
CDC25A	3.38	0.028	*CNKSR2*	0.26	0.047
KIF14	3.34	0.034	*FOXJ1*	0.27	0.026
ZWINT	3.34	0.016	*NGFR*	0.27	0.015
TNFSF9	3.34	0.028	*CYP4F12*	0.27	0.024
CD38	3.31	0.028	*GAL3ST4*	0.27	0.028
TSHR	3.30	0.028	*C1orf177*	0.27	0.016
ADAM28	3.28	0.009	*HPCAL4*	0.27	0.047
PKMYT1	3.27	0.009	*DNAH10*	0.27	0.045
DEPDC1B	3.21	0.028	*PCSK4*	0.27	0.016
FABP5	3.21	0.009	*SNED1*	0.28	0.028
KIF11	3.21	0.016	*VIPR1*	0.28	0.009
CENPF	3.20	0.047	*BAIAP2L1*	0.28	0.009
UPK2	3.20	0.034	*ACVR2A*	0.28	0.009
KIF2C	3.17	0.028	*BACH2*	0.28	0.009
REREP3	3.15	0.016	*AMN*	0.28	0.009
TPX2	3.13	0.028	*ZFR2*	0.28	0.045
TOX	3.12	0.009	*CBR3-AS1*	0.28	0.028
RAD51AP1	3.10	0.028	*CSNK1G2-AS1*	0.28	0.045

POLQ	3.10	0.009		LINC00299	0.28	0.028
CASC5	3.09	0.009		EMR1	0.28	0.028
DKK3	3.09	0.028		LAPTM4B	0.29	0.047
AGAP1	3.08	0.028		GNLY	0.29	0.028
CDKN3	3.07	0.009		RASL11B	0.29	0.016
FCRL3	3.05	0.009		ALPK1	0.29	0.028
CDK1	3.05	0.009		LGALS17A	0.29	0.041
CDCA3	3.04	0.016		IRS2	0.29	0.028
NUSAP1	3.03	0.016		PTPRO	0.29	0.009
BUB1	3.02	0.028		CNKSR1	0.29	0.009
GINS2	2.99	0.028		DSEL	0.30	0.009
GINS1	2.99	0.047		PSPN	0.30	0.045
KCNK5	2.98	0.028		CISH	0.30	0.016
ESPL1	2.96	0.047		CD248	0.30	0.047
BUB1B	2.94	0.028		PROCR	0.30	0.047
HNRPLL	2.91	0.009		FXYD7	0.30	0.028
AFF3	2.89	0.007		GP5	0.30	0.009
ATP9A	2.89	0.028		COL9A2	0.30	0.009
DFNB31	2.85	0.009		RPRM	0.30	0.041
BCAT1	2.84	0.028		CMKLR1	0.30	0.028
RGS1	2.83	0.009		LOC100128252	0.31	0.047
CCNB1	2.83	0.016		C17orf51	0.31	0.009
SEMA4A	2.81	0.016		RGMB	0.31	0.009
ST6GALNAC3	2.80	0.047		DLL1	0.32	0.009
PRKCDBP	2.79	0.019		CCR7	0.32	0.016
GJD3	2.77	0.016		C1orf162	0.32	0.009
PMAIP1	2.77	0.047		HOXC5	0.32	0.013
DTHD1	2.75	0.047		TXK	0.32	0.009
PTPLA	2.73	0.045		ADAMTS10	0.32	0.028
NUF2	2.72	0.009		ACCN2	0.32	0.028
C7orf61	2.71	0.016		HS3ST3B1	0.32	0.016
MELK	2.70	0.047		PLAG1	0.32	0.028
BREA2	2.69	0.009		GRASP	0.33	0.047
LANCL3	2.68	0.028		BDAG1	0.33	0.047
CD27	2.66	0.028		ZNF516	0.33	0.028

HLF	2.66	0.028		*PRPF40B*	0.33	0.009
C12orf42	2.61	0.028		*GREM2*	0.33	0.047
SCD	2.58	0.009		*GPR55*	0.33	0.028
CLSPN	2.57	0.047		*FRY*	0.33	0.016
RYR1	2.51	0.009		*ALS2CL*	0.33	0.009
SAPCD2	2.51	0.028		*SPTB*	0.34	0.047
TNIP3	2.50	0.016		*PARK2*	0.34	0.047
LIMS1	2.49	0.009		*LOC100506046*	0.34	0.047
LOC153469	2.48	0.013		*LINC00085*	0.34	0.016
ESR1	2.47	0.034		*GNAL*	0.34	0.047
FCGR2B	2.47	0.028		*AGPAT4-IT1*	0.34	0.047
NDC80	2.47	0.009		*RAB25*	0.34	0.019
HLA-DMA	2.46	0.009		*TRPC1*	0.34	0.009
ZBTB32	2.46	0.047		*TLR5*	0.34	0.016
CCNA2	2.46	0.047		*GDPD5*	0.34	0.016
MAPK12	2.46	0.041		*DENND5A*	0.35	0.009
LYST	2.45	0.047		*AXIN2*	0.35	0.047
CPNE2	2.43	0.009		*ACER1*	0.35	0.047
CRTAM	2.42	0.016		*C1orf228*	0.35	0.047
ITM2A	2.38	0.047		*SPINT2*	0.35	0.028
SPATS2L	2.38	0.009		*PDIA5*	0.35	0.028
TRPS1	2.35	0.016		*EPHX2*	0.35	0.009
MYO1E	2.32	0.009		*LOC100506746*	0.35	0.008
ZNF532	2.30	0.028		*IQCA1*	0.35	0.028
MCM2	2.30	0.047		*C4orf32*	0.35	0.009
GLDC	2.27	0.034		*COL6A1*	0.35	0.009
BATF3	2.25	0.016		*PFN2*	0.36	0.028
CCL4	2.24	0.016		*C2orf89*	0.36	0.009
C11orf82	2.23	0.047		*GNA11*	0.36	0.028
NINJ2	2.23	0.016		*ATP6V0A1*	0.36	0.047
AGAP7	2.23	0.047		*PLEKHG3*	0.36	0.028
CD70	2.22	0.047		*SATB1*	0.36	0.009
CYTH3	2.20	0.016		*HEMGN*	0.36	0.028
FAM83D	2.20	0.009		*DLEC1*	0.36	0.009
E2F3	2.19	0.047		*MYC*	0.36	0.009

LOC79015	2.17	0.047	EIF4EBP3	0.36	0.028
FOXM1	2.17	0.047	LOC641518	0.37	0.028
SCAMP5	2.16	0.028	TRIM2	0.37	0.047
MCM4	2.15	0.047	AIM1L	0.37	0.047
POC1A	2.15	0.028	DAB1	0.37	0.019
C1R	2.13	0.009	GPR150	0.37	0.047
PLK4	2.13	0.016	ARHGAP32	0.37	0.028
SH2D1A	2.13	0.009	PIP5KL1	0.37	0.045
ECT2	2.12	0.028	MEF2B	0.37	0.047
SH2B2	2.11	0.009	PRX	0.37	0.047
CLDND1	2.11	0.028	ARHGEF4	0.38	0.009
HMOX1	2.10	0.047	EBF4	0.38	0.047
CMC1	2.10	0.009	FAM178B	0.38	0.047
TIGIT	2.10	0.016	RUNX2	0.38	0.009
POLE2	2.10	0.047	MTUS2	0.38	0.019
CD74	2.10	0.009	CHGB	0.38	0.019
SMS	2.09	0.009	STK32C	0.39	0.028
HLA-DMB	2.09	0.047	LEF1	0.39	0.009
STMN1	2.08	0.016	SOAT2	0.39	0.047
HLA-DRA	2.07	0.028	GSDMA	0.39	0.047
AASS	2.07	0.047	CARNS1	0.39	0.009
EFCAB4A	2.07	0.009	EPS8	0.39	0.015
CIB2	2.07	0.047	FPGT-TNNI3K	0.39	0.047
GALM	2.05	0.047	DPP4	0.40	0.016
LOC643733	2.05	0.009	RHOBTB3	0.40	0.047
EMID1	2.05	0.019	AGBL3	0.40	0.009
UNC5B	2.05	0.019	SDK2	0.40	0.019
PDGFB	2.04	0.016	TSPAN6	0.40	0.028
ZNF80	2.03	0.047	ABCB9	0.40	0.016
PEX11A	2.03	0.047	SOWAHC	0.40	0.024
TLR3	2.03	0.016	PLD6	0.40	0.016
CDYL2	2.03	0.028	JAG2	0.41	0.016
WDR34	2.02	0.028	TLE1	0.41	0.047
FHL2	2.02	0.028	KLHL6	0.41	0.009
ASF1B	2.02	0.047	FZD4	0.41	0.047

GZMA	2.01	0.028		*PLEKHG4*	0.42	0.016
SKA1	2.00	0.016		*EPGN*	0.42	0.005
				SOCS2	0.42	0.009
				SIGLEC6	0.42	0.034
				NRIP3	0.42	0.047
				FLJ34208	0.42	0.047
				ERBB2	0.42	0.028
				ASB13	0.42	0.047
				SPIN3	0.43	0.047
				CHD7	0.43	0.016
				BAI2	0.43	0.047
				TTC8	0.43	0.047
				TTC23	0.43	0.034
				LOC100289509	0.43	0.019
				SALL2	0.43	0.028
				GOLM1	0.43	0.047
				LOC100506134	0.43	0.008
				ZNF502	0.44	0.047
				CDC42BPA	0.44	0.034
				ADD2	0.44	0.019
				LZTS1	0.44	0.034
				OSM	0.44	0.016
				MAST4	0.44	0.009
				FNIP2	0.45	0.016
				RNF24	0.45	0.009
				LONRF3	0.45	0.009
				TTC26	0.45	0.028
				ATG9B	0.45	0.028
				SMARCD3	0.45	0.016
				C14orf132	0.45	0.005
				ZNF425	0.45	0.047
				PTGER2	0.45	0.016
				LINC00511	0.46	0.041
				C1orf101	0.46	0.028
				HTR7P1	0.46	0.041

				TF	0.46	0.013
				TMEM30B	0.46	0.016
				NUDT12	0.46	0.015
				ANKK1	0.46	0.019
				KCNQ1	0.46	0.047
				NPB	0.46	0.047
				LOC100507392	0.46	0.047
				TSPAN2	0.46	0.016
				REEP6	0.46	0.047
				CSGALNACT1	0.47	0.047
				A1BG-AS1	0.47	0.028
				AKAP12	0.47	0.028
				HYI	0.47	0.047
				DIRC3	0.47	0.016
				SPON1	0.47	0.028
				FLJ35946	0.48	0.019
				MBOAT2	0.48	0.016
				RPS6KL1	0.48	0.045
				GLS2	0.48	0.028
				DCDC2B	0.48	0.047
				GPR152	0.48	0.047
				TNK1	0.48	0.047
				ACSS2	0.48	0.047
				TAS2R5	0.48	0.047
				WWC2	0.48	0.028
				DTWD2	0.48	0.016
				PLEKHA4	0.48	0.047
				CCDC13	0.48	0.015
				MSX2P1	0.49	0.009
				ZNF629	0.49	0.034
				NHSL2	0.49	0.047
				C1orf115	0.49	0.047
				SERINC5	0.49	0.028
				LOC285359	0.49	0.047
				RARG	0.49	0.028

			NEFM	0.49	0.008
			PODXL	0.50	0.028
			RNF165	0.50	0.034
			NMUR1	0.50	0.047
			RNF144A	0.50	0.009