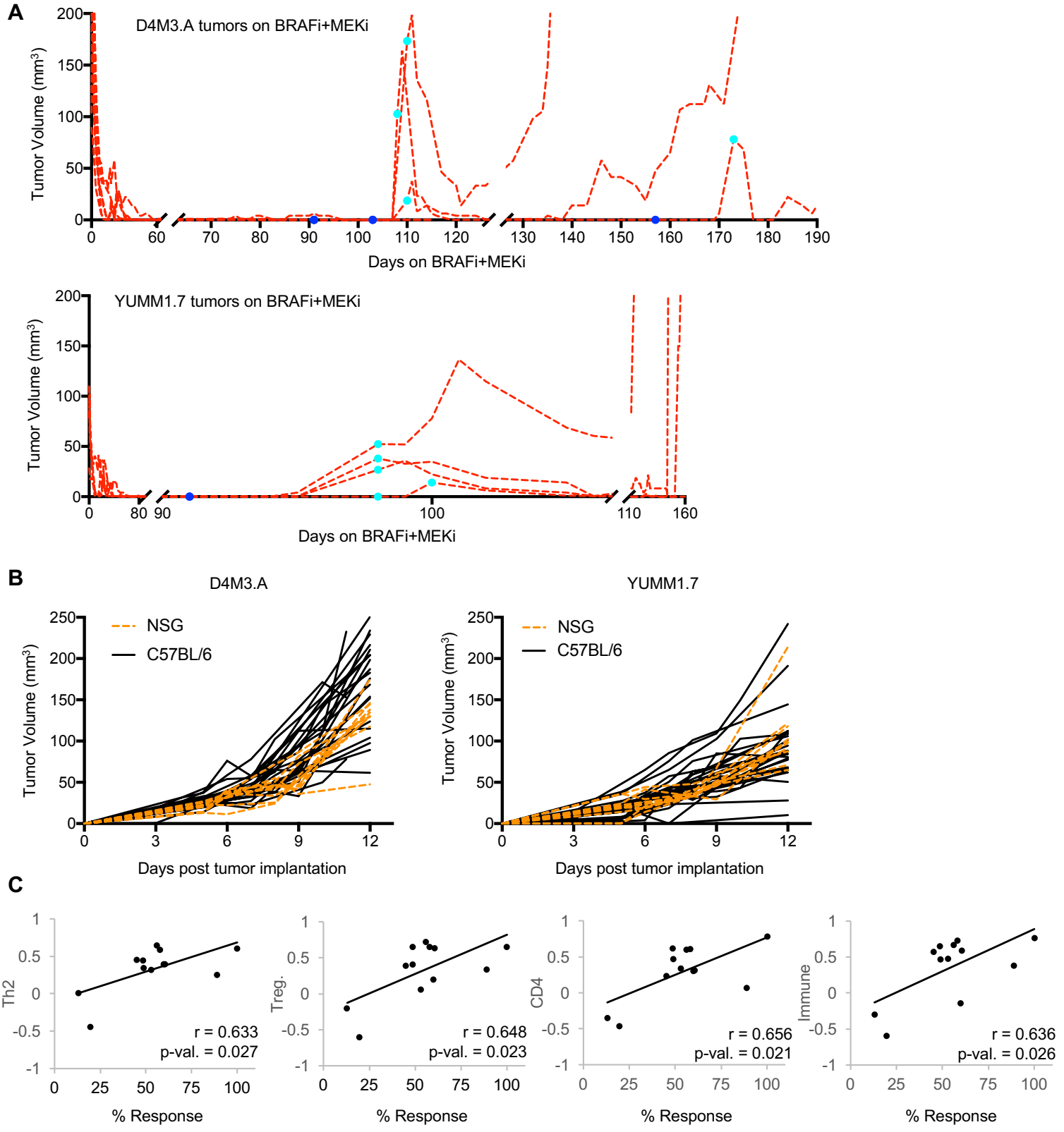
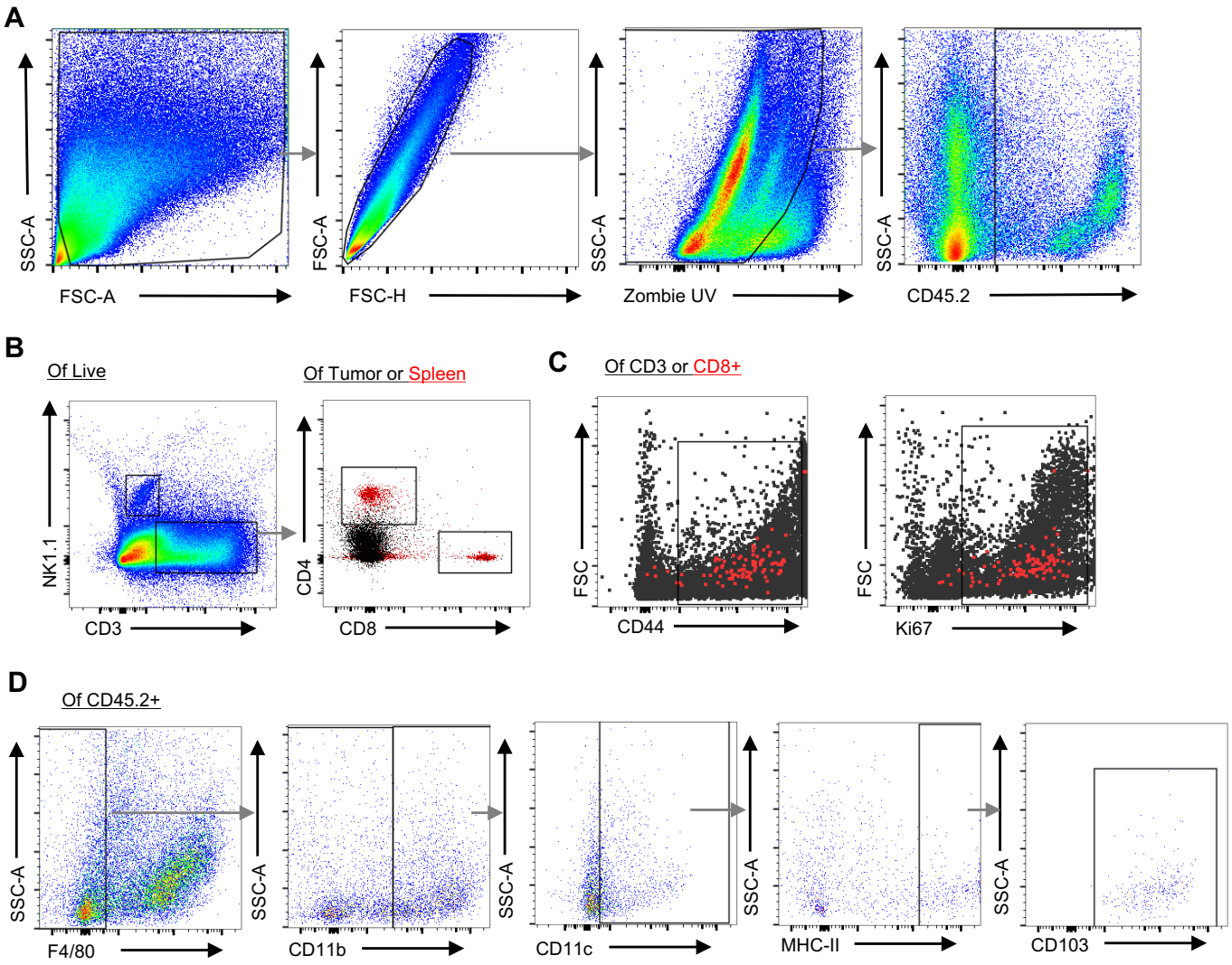
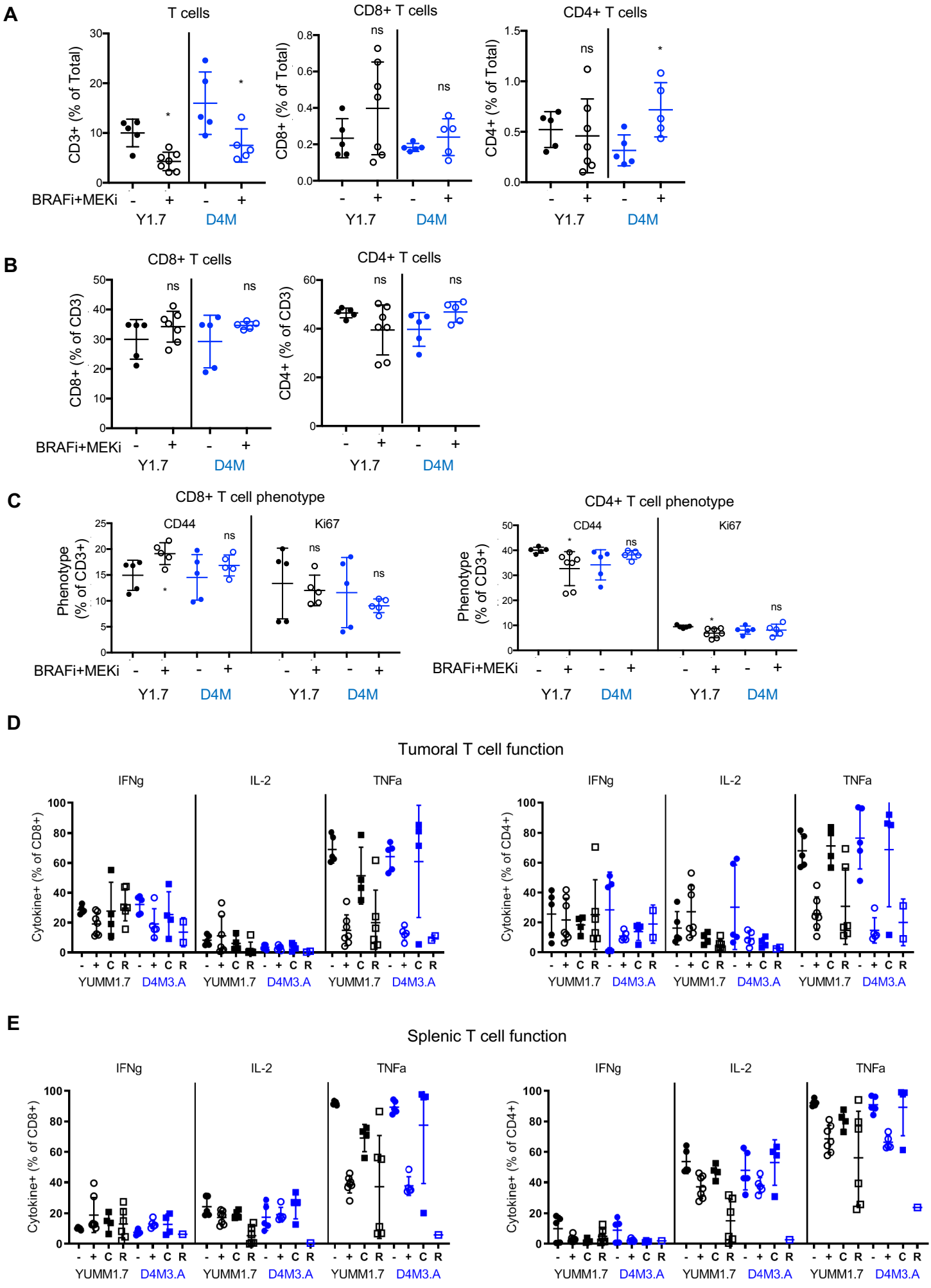
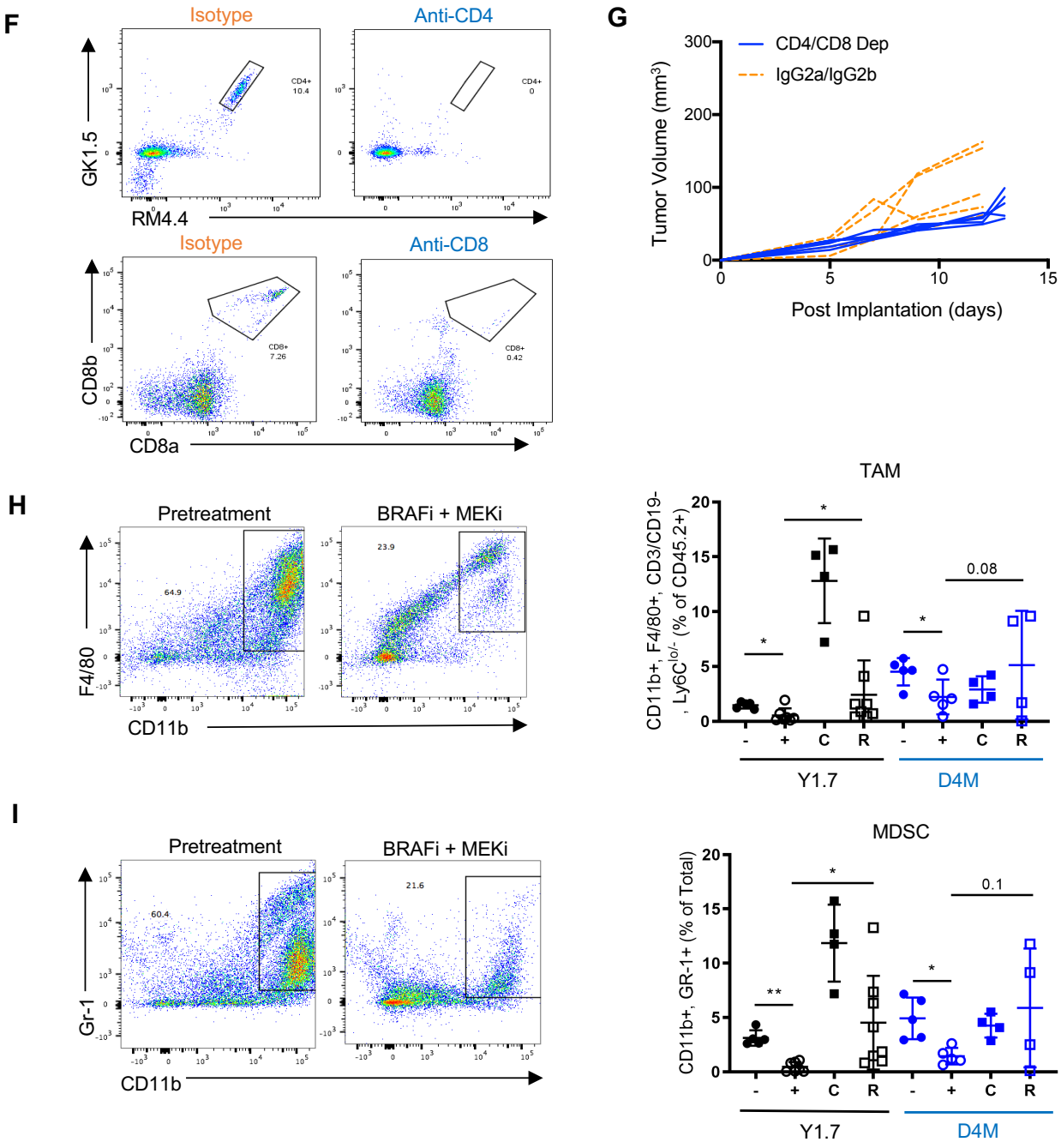


Supplemental Figure 1: D4M3.A and YUMM1.7 tumor growth following targeted inhibitor treatment in C57BL/6 and NSG mice. A) Altered scaling of growth curves of D4M3.A and YUMM1.7 tumors treated with BRAFi + MEKi from Figure 1A, in order to clearly visualize tumor kinetics when mice were removed from drug (dark blue dots) and then re-administered drug (cyan dots). **B)** D4M3.A ($3x10^5$) or YUMM1.7 ($2.5x10^5$) cells were injected into either C57BL/6 or NSG mice and allowed to form tumors. Tumor volumes (mm^3) following treatment with BRAFi + MEKi is represented over time. **C)** Scatter plots of GSVA scores and percent tumor regression data for on-treatment samples. Pearson correlation coefficient (r) and p-values are displayed.

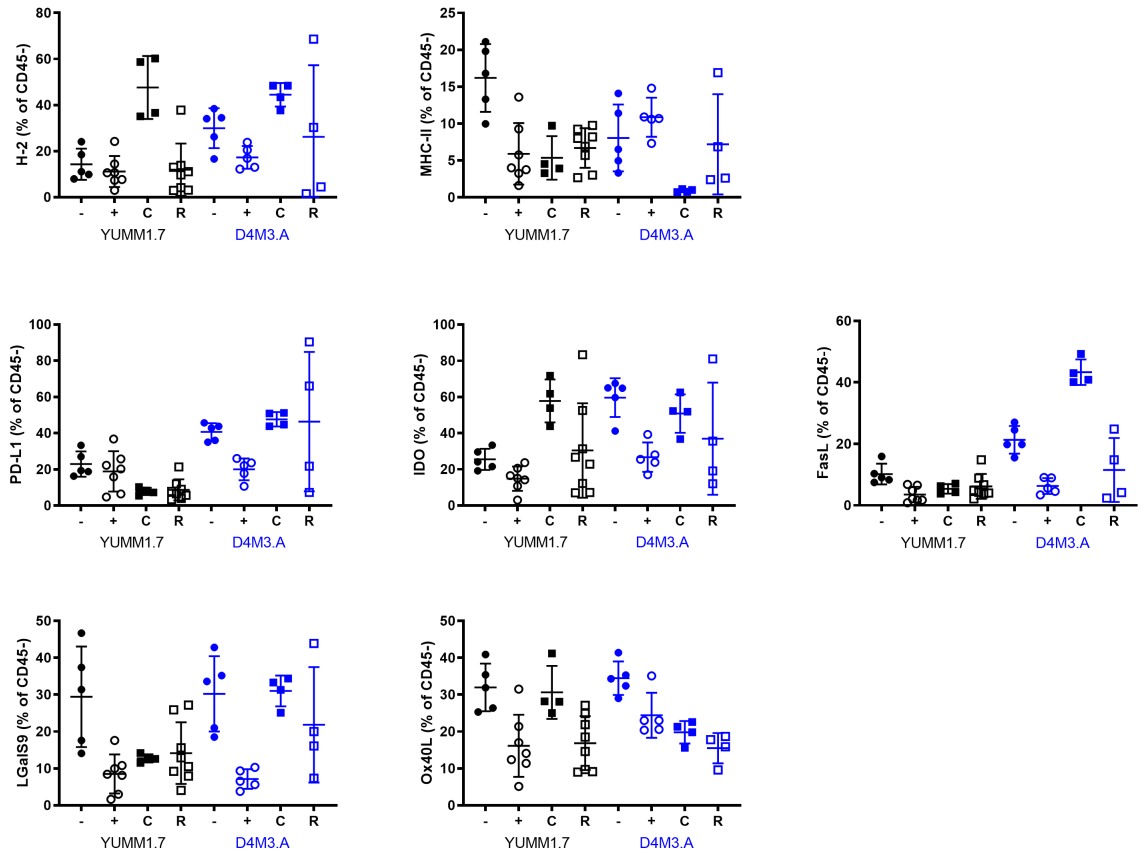


Supplemental Figure 2: Representative flow cytometry plots for TIL. Representative gating strategies for immune cell analysis from a YUMM1.7 tumor pre-treatment or four days on treatment with BRAFi + MEKi. **A)** Identification of live (Zombie UV-), CD45.2+ cell populations. **B)** Gating strategy for CD4+ cells (CD4+, CD8- of CD3+, NK1.1-) and CD8+ cells (CD4-, CD8+ of CD3+, NK1.1-). **C)** Representative gating of CD44+ or Ki67+ CD8+ T cells. **D)** Identification of dendritic cell populations (F4/80-, CD11b+/-, CD11c+, MHC-II^{hi}, CD103+).

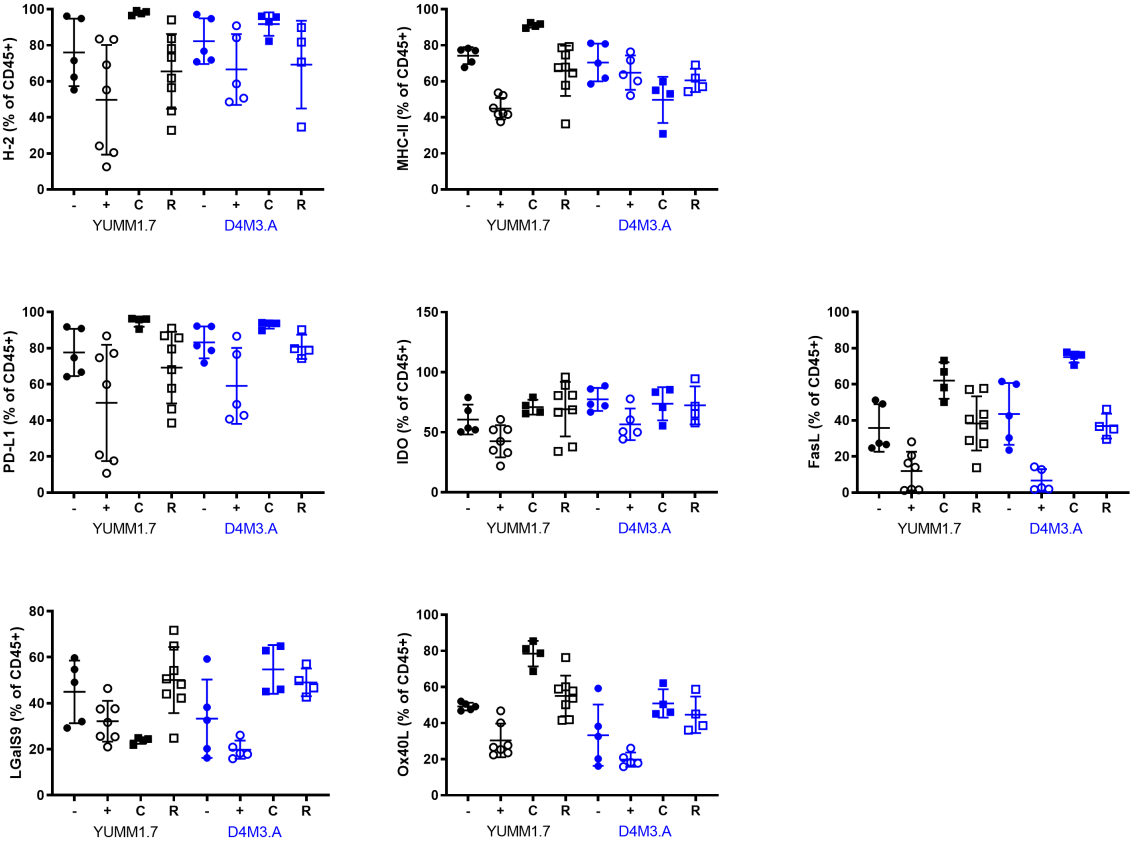

A
T cells
CD8+ T cells
CD4+ T cells
CD3+ (% of Total)
CD8+ (% of Total)
CD4+ (% of Total)
ns
*
BRAFi+MEKi
Y1.7
D4M
B
CD8+ T cells
CD4+ T cells
CD8+ (% of CD3)
CD4+ (% of CD3)
C
CD8+ T cell phenotype
CD4+ T cell phenotype
CD44
Ki67
Phenotype (% of CD3+)
D
Tumoral T cell function
IFNg
IL-2
TNFa
Cytokine+ (% of CD8+)
Cytokine+ (% of CD4+)
YUMM1.7
D4M3.A
E
Splenic T cell function


F
Isotype
Anti-CD4
GK1.5
RM4.4
CD4+
10.4
CD4+
0
Isotype
Anti-CD8
CD8b
CD8a
CD8+
7.26
CD8+
0.42
G
CD4/CD8 Dep
IgG2a/IgG2b
Tumor Volume (mm3)
Post Implantation (days)
H
Pretreatment
BRAFi + MEKi
F4/80
CD11b
64.9
23.9
TAM
CD11b+, F4/80+, CD3/CD19-, Ly6C lo/- (% of CD45.2+)
0.08
Y1.7
D4M
I
Pretreatment
BRAFi + MEKi
Gr-1
CD11b
60.4
21.6
MDSC
CD11b+, GR-1+ (% of Total)
0.1
Y1.7
D4M

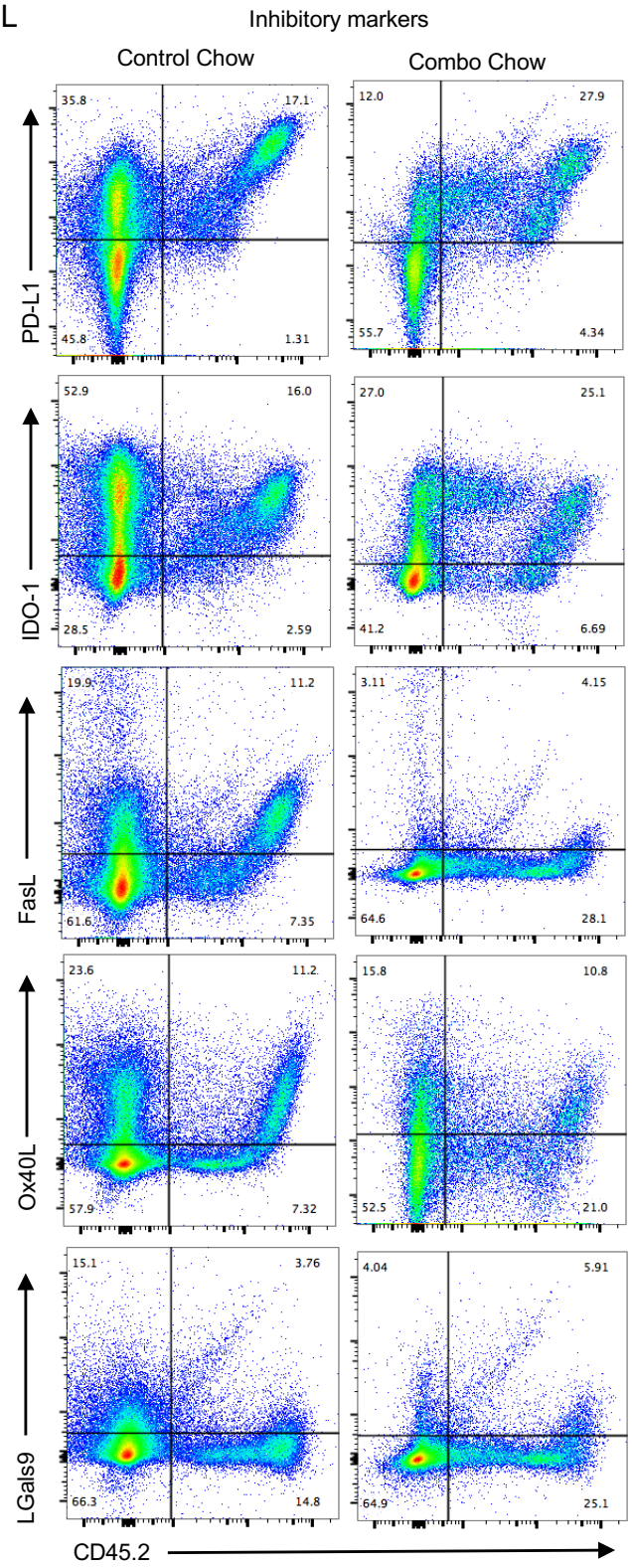
J


H-2 (% of CD45-)
MHC-II (% of CD45-)
PD-L1 (% of CD45-)
IDO (% of CD45-)
FasL (% of CD45-)
LGalS9 (% of CD45-)
Ox40L (% of CD45-)
- + C R - + C R
YUMM1.7
D4M3.A

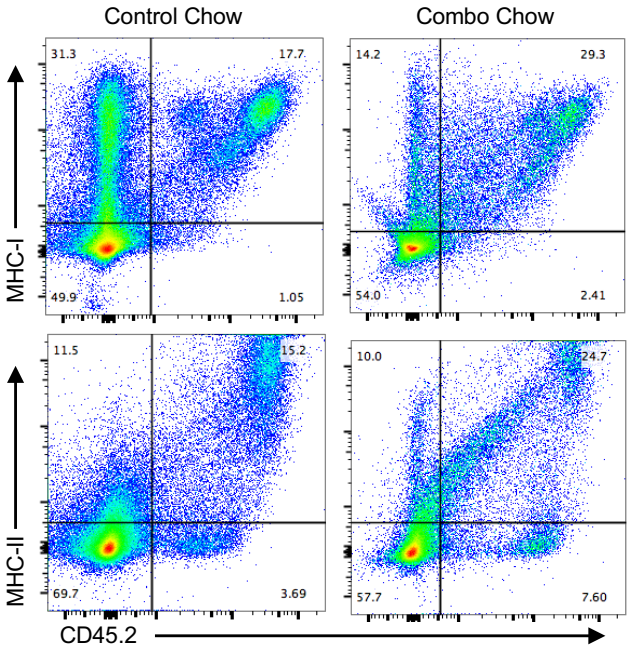
K


H-2 (% of CD45+)
MHC-II (% of CD45+)
PD-L1 (% of CD45+)
IDO (% of CD45+)
FasL (% of CD45+)
LGalS9 (% of CD45+)
Ox40L (% of CD45+)
- + C R - + C R
YUMM1.7
D4M3.A

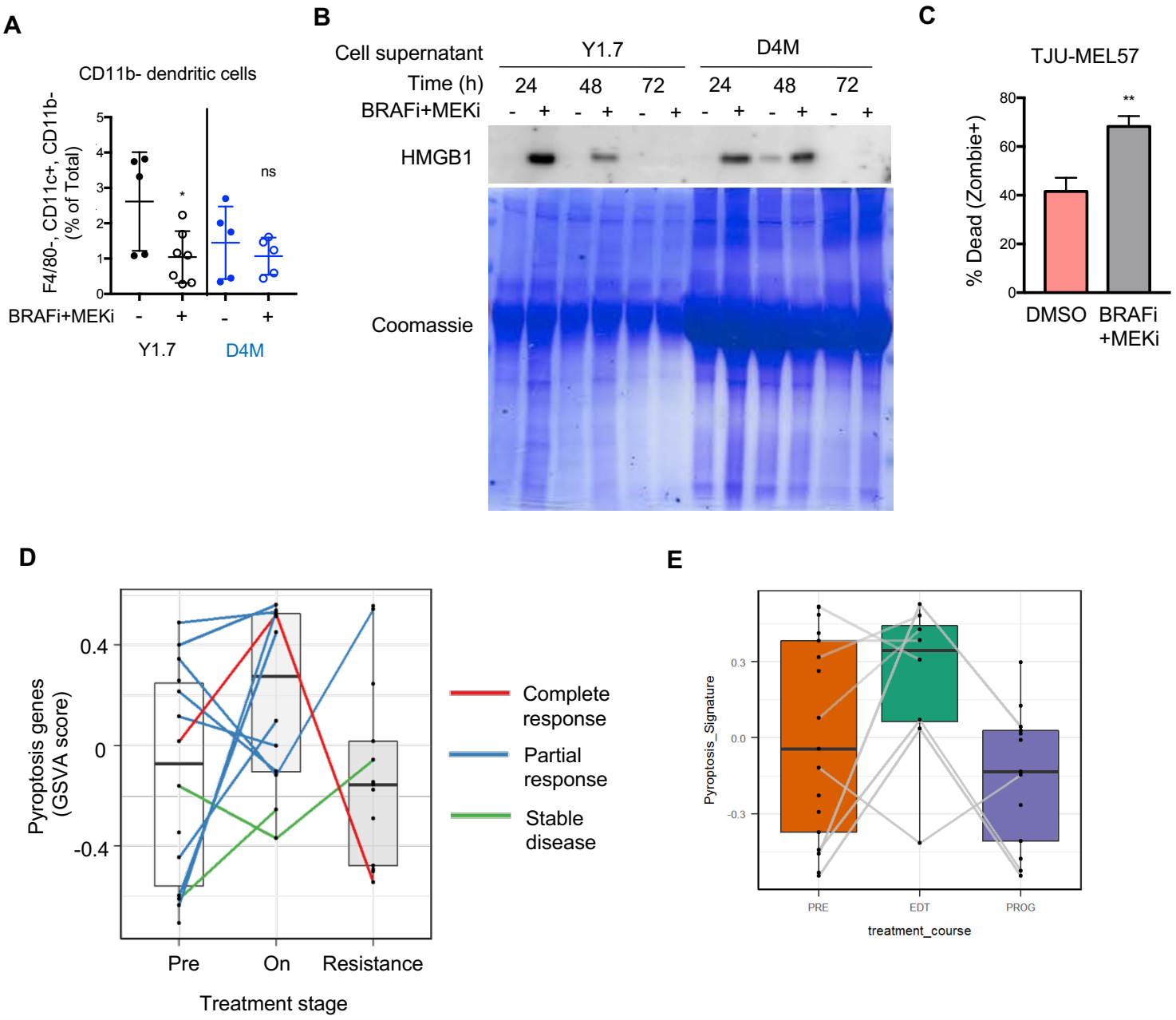
L


Inhibitory markers
Control Chow
Combo Chow
PD-L1
35.8
17.1
45.8
1.31
12.0
27.9
55.7
4.34
IDO-1
52.9
16.0
28.5
2.59
27.0
25.1
41.2
6.69
FasL
19.9
11.2
61.6
7.35
3.11
4.15
64.6
28.1
Ox40L
23.6
11.2
57.9
7.32
15.8
10.8
52.5
21.0
LGals9
15.1
3.76
66.3
14.8
4.04
5.91
64.9
25.1
CD45.2

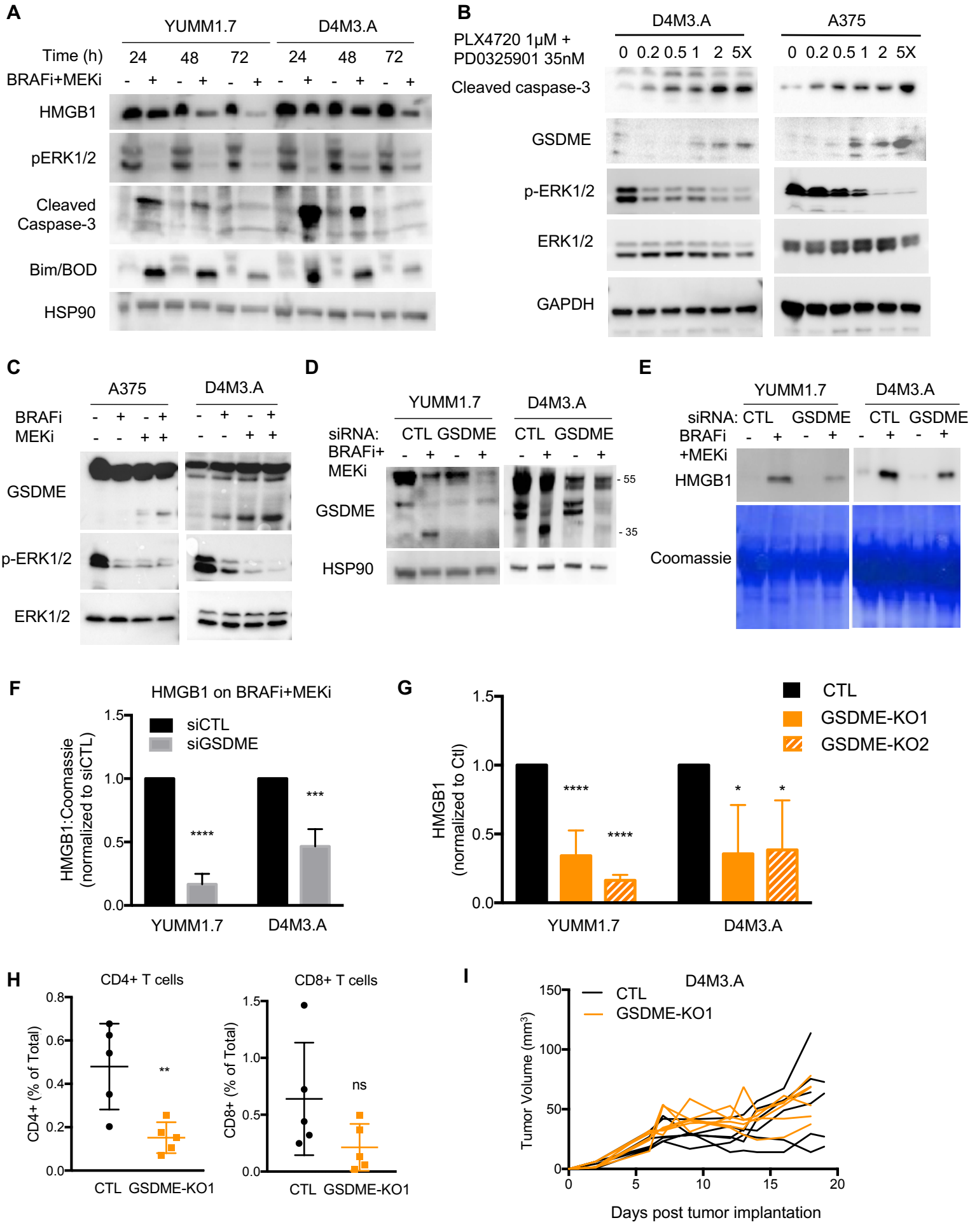
M


Stimulatory markers
Control Chow
Combo Chow
MHC-I
31.3
17.7
49.9
1.05
14.2
29.3
54.0
2.41
MHC-II
11.5
15.2
69.7
3.69
10.0
24.7
57.7
7.60
CD45.2

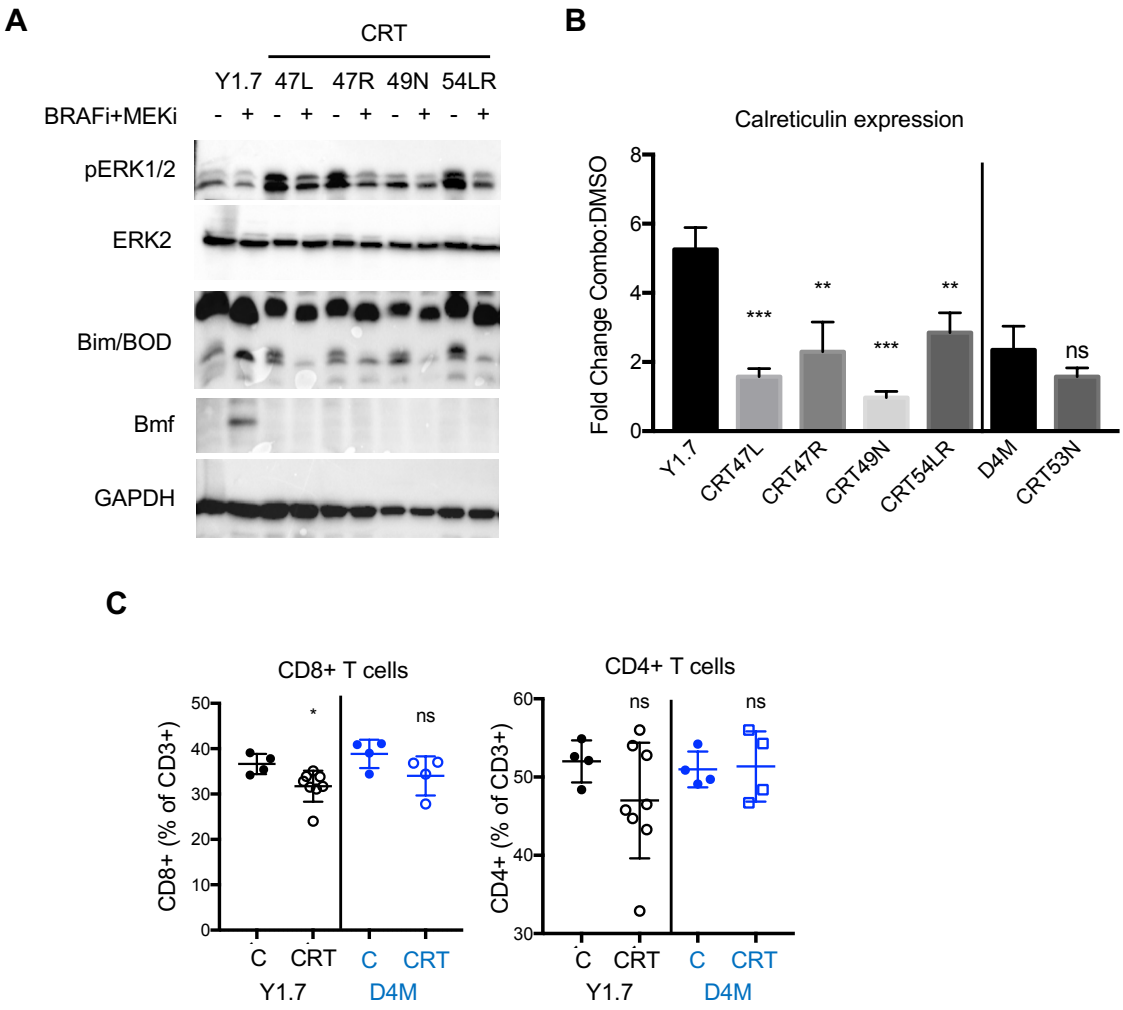
Supplemental Figure 3: Alterations in immune cells in tumors and spleens during BRAFi + MEKi. YUMM1.7 (Y1.7, black) or D4M3.A (D4M, blue) tumor-bearing mice were treated 200 ppm PLX4720 and 7 ppm PD0325901. Tumors were harvested pre-treatment or after four days of BRAFi + MEKi treatment and intra-tumoral T cells were accessed by flow cytometry. **A)** T cells (CD3+ or CD8+, CD3+, or CD4+, CD3) in tumors displayed as a percentage of all cells analyzed in tumors. **B)** Spleen-associated CD8+ and CD4+ T cells displayed as a percentage of CD3+ cells. **C)** Phenotype of spleen-associated T cells, CD44 = activated, Ki67 = proliferating. **D)** YUMM1.7 (Y1.7, Black) or D4M3.A (D4M, Blue) were treated with BRAF/MEKi (combo) as in Figure 1. Cohorts of mice were sacrificed pretreatment (-), after four days of BRAFi + MEKi (+), untreated at sacrifice (C), or after resistance formation (R). The functionality of T cells was determined using *ex vivo* PMA/ionomycin stimulation. Functional capacity as measured by IFNγ, IL-2, and TNFα release from intra-tumoral CD8 and CD4+ T cells. **E)** Same as D, but from the spleen. **F)** Representative FACS plots of CD4+ and CD8+ T cells in the blood from the mice in Figure 2F-G (day 10 post treatment). **G)** Tumor growth pre-BRAFi + MEKi treatment from the mice in Figure 2F-G. **H)** YUMM1.7 (Y1.7, Black) or D4M3.A (D4M, Blue) were treated with BRAFi + MEKi. Mice were sacrificed pretreatment (-), after four days of BRAFi + MEKi (+), untreated at sacrifice (C), or after resistance formation (R). The immune inhibitory microenvironment of tumors was accessed by FACS. Representative gating of tumor associated macrophages (CD11b+, F4/80+, TAM). Graphed is the quantification of TAM (CD11b+, F4/80+, CD3/CD19-, Ly6C$^{lo/-}$). **I)** Representative gating of myeloid derived suppressor cells (CD11b+, Gr-1+, MDSC). Graphed is the quantification of MDSC in tumors. Statistics were determined via student's T-test, * $p<0.05$, ** $p<0.01$. **J)** BRAFi+MEKi effects on the immunogenicity of melanoma tumor microenvironment from the same tumors used in Supp. Fig. S3H and I. Quantification of immunogenic markers on CD45.2- cells in tumors. **K)** As in J, but quantification of immunogenic markers on CD45.2+ cells in tumors. **L)** Representative gating strategies for inhibitory markers from immune cell analysis from YUMM1.7 tumors either pre-treated or treated tumors 4 days post treatment. **M)** As for L, but stimulatory markers were analyzed.



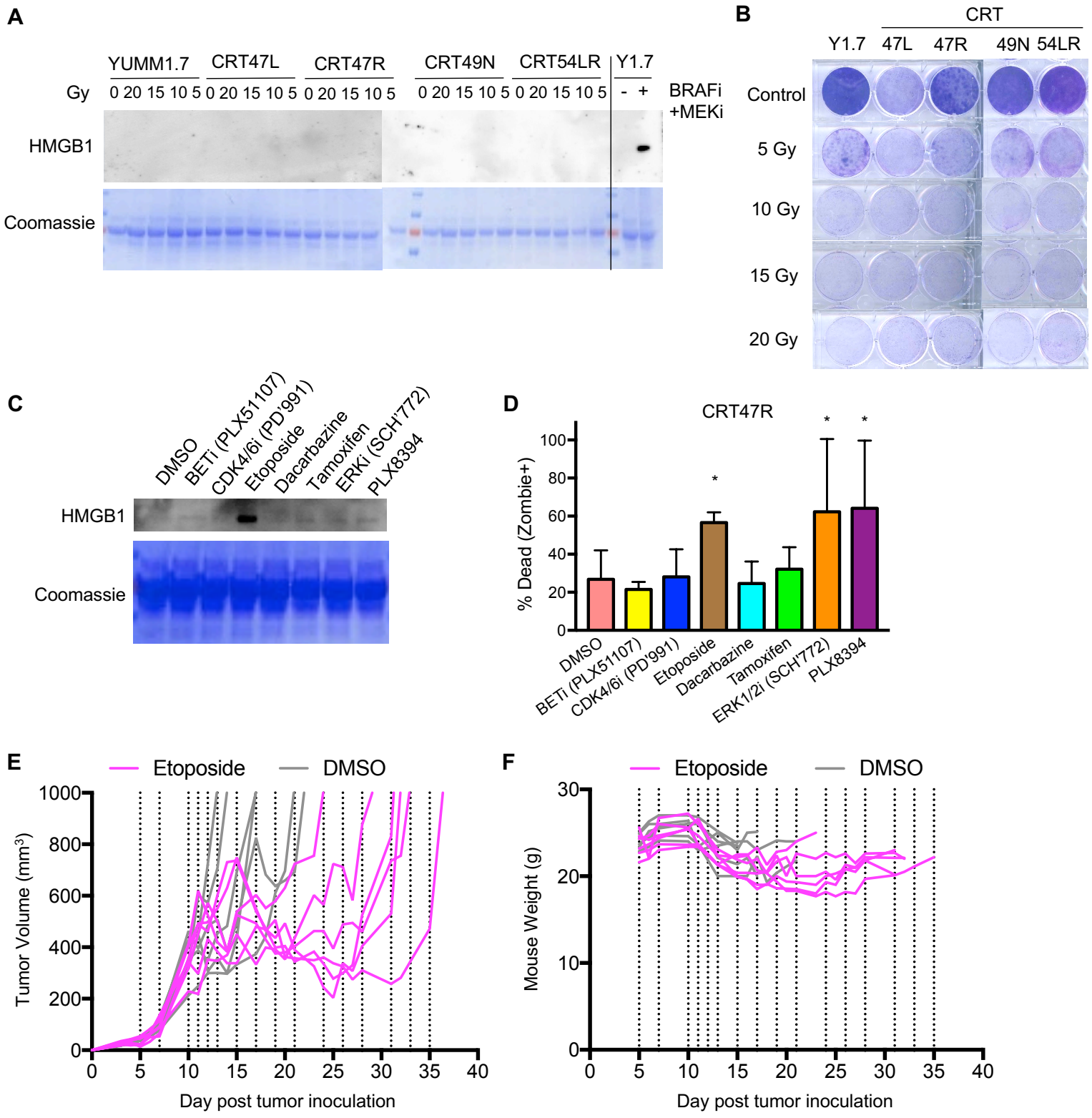
Supplemental Figure 4: BRAFi + MEKi-induces immune stimulatory cell death. A) TIL analysis from BRAFi + MEKi treated tumors. Percent of tumor-associated CD11b- dendritic cells (F4/80-, CD11b-, CD11c+). **B)** Levels of HMGB1 in the supernatant of mouse melanoma YUMM1.7 or D4M3.A cells treated with PLX4720 (1 μM) and PD0325901 (3.5 nM) for 24, 48, or 72 hours. Coomassie stained gel for loading control. **C)** Relative cell death (Zombie NIR+, right) of short-term cultures of patient derived tumor, TJUMEL57, from Figure 3E-F. **D)** RNA-seq data were collected from Kwong et al. 2015 Ref 39. Gene set scores were calculated using the GSVA package (version 1.28.0) in R (version 3.5.1). Box plot of pre-treatment (Pre), on-treatment (On) and resistant (Resist.) patient tumor data for pyroptosis signature expression. A line represents a patient with both pre- and on- treatment samples or pre, on and resistant samples. Line colors represent response to treatment. **E)** Analysis of pyroptosis signature in GSE99898 data containing patient-matched pre-treatment, on-treatment and progression samples (Kakavand et al., 2017; Ref 43). Box plots of pre-treatment, on-treatment and resistant patient tumor data for pyroptosis signature expression. Each line represents a patient with pre, on and/or resistant samples. No treatment response data are associated with this dataset.


A
YUMM1.7
D4M3.A
Time (h)
24 48 72 24 48 72
BRAFi+MEKi
- + - + - + - + - + - +
HMGB1
pERK1/2
Cleaved Caspase-3
Bim/BOD
HSP90
B
D4M3.A
A375
PLX4720 1μM + PD0325901 35nM
0 0.2 0.5 1 2 5X
0 0.2 0.5 1 2 5X
Cleaved caspase-3
GSDME
p-ERK1/2
ERK1/2
GAPDH
C
A375
D4M3.A
BRAFi
MEKi
GSDME
p-ERK1/2
ERK1/2
D
YUMM1.7
D4M3.A
siRNA:
CTL GSDME CTL GSDME
BRAFi+ MEKi
GSDME
HSP90
55
35
E
YUMM1.7
D4M3.A
siRNA: CTL GSDME CTL GSDME
BRAFi
+MEKi
HMGB1
Coomassie
F
HMGB1 on BRAFi+MEKi
siCTL
siGSDME
HMGB1:Coomassie (normalized to siCTL)
1.5
1.0
0.5
0.0
****
***
YUMM1.7
D4M3.A
G
CTL
GSDME-KO1
GSDME-KO2
HMGB1 (normalized to Ctl)
1.5
1.0
0.5
0.0
****
****
*
*
YUMM1.7
D4M3.A
H
CD4+ T cells
CD4+ (% of Total)
0.8
0.6
0.4
0.2
0.0
**
CTL GSDME-KO1
CD8+ T cells
CD8+ (% of Total)
1.5
1.0
0.5
0.0
ns
CTL GSDME-KO1
I
D4M3.A
CTL
GSDME-KO1
Tumor Volume (mm3)
150
100
50
0
0 5 10 15 20
Days post tumor implantation

Supplemental Figure 5: Pyroptosis time course and GSDME knockdown confirmation. A) YUMM1.7 or D4M3.A cells were treated with PLX4720 (1 μM) and PD0325901 (3.5 nM). Levels of HMGB1, cleaved caspase 3 and Bim/BOD in cell lysates after 24, 48, and 72 hours of treatment. HSP90 serves as loading control. **B)** A375 or D4M3.A cells were treated with increasing concentrations of PLX4720 and PD0325901 (0, 0.2= 0.2 μM/7 nM; 0.5= 0.5 μM/17.5 nM; 1= 1 μM/35 nM; 2= 2 μM/70nM; 5X= 5 μM/175 nM) for 24 hours. Cell lysates were analyzed by Western blot with antibodies to cleaved caspase 3, GSDME, phospho-ERK1/2, total ERK1/2 and GAPDH. **C)** A375 and D4M3.A cells were treated with individually or combined with PLX4720 (1 μM) and PD0325901 (3.5 nM). Cell lysates were analyzed by Western blotting with the indicated antibodies. **D)** YUMM1.7 and D4M3.A cells were transiently transfected with siRNA specific to GSDME and then treated with PLX4720 (1 μM) and PD0325901 (35 nM) for 24 hours. Cell lysates were analyzed by Western blotting for levels of GSDME. Full-length GSDME runs at 55 kDa and cleaved GSDME runs at 35 kDa. HSP90 serves as loading control. **E)** Levels of HMGB1 in supernatants from B by Western blotting. Coomassie stained gel as loading control. **F)** Quantification of HMGB1 from C. **G)** Quantification of HMGB1 from Figure 4D from 3 independent experiments. Significance was determined by a t-test of control to drug treated groups, *$p<0.05$, ***$p<0.001$, ****$p<0.0001$. **H)** T cells (CD8+, CD3+ or CD4+, CD3+) in CTL or GSDME-KO1 YUMM1.7 tumors. **I)** Tumor growth of CTL or GSDME-KO1 D4M3.A tumors prior to beginning BRAFi + MEKi treatment.



Supplemental Figure 6: BRAFi + MEKi combination resistant cell lines (CRTs) do not undergo pyroptosis. YUMM1.7 CRT cells (CRT47L, 47R, 49N, and 54LR) or D4M3.A CRT cells (CRT53L) were treated with PLX4720 (1 μM) and PD0325901 (35 nM) for 24 hours. **A)** Lysates from YUMM1.7 and YUMM1.7-derived CRT cells (CRT47L, 47R, 49N, and 54LR) were analyzed by Western blotting with indicated antibodies. **B)** Calreticulin surface expression of CRT cells treated with PLX4720 (1 μM) or PD0325901 (35 nM) for 24 hours. Graphed is the fold change of BRAFi+MEKi:DMSO treated cells. **C)** Spleen-associated CD8+ and CD4+ T cells (of CD3+ cells) from Figure 5H-I.


A
YUMM1.7
CRT47L
CRT47R
CRT49N
CRT54LR
Y1.7
Gy
0 20 15 10 5 0 20 15 10 5 0 20 15 10 5 0 20 15 10 5 0 20 15 10 5
- +
BRAFi +MEKi
HMGB1
Coomassie
B
CRT
Y1.7
47L
47R
49N
54LR
Control
5 Gy
10 Gy
15 Gy
20 Gy
C
DMSO
BETi (PLX51107)
CDK4/6i (PD'991)
Etoposide
Dacarbazine
Tamoxifen
ERKi (SCH'772)
PLX8394
HMGB1
Coomassie
D
CRT47R
% Dead (Zombie+)
100
80
60
40
20
0
*
*
*
DMSO
BETi (PLX51107)
CDK4/6i (PD'991)
Etoposide
Dacarbazine
Tamoxifen
ERK1/2i (SCH'772)
PLX8394
E
Etoposide
DMSO
Tumor Volume (mm3)
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40
Day post tumor inoculation
F
Etoposide
DMSO
Mouse Weight (g)
30
20
10
0
0 5 10 15 20 25 30 35 40
Day post tumor inoculation

Supplemental Figure 7: Etoposide-induces HMGB1 release and cell death of CRTs. A-B) Parental YUMM1.7 and YUMM1.7 CRT cell lines were exposed to 5, 10, 15, and 20 gray (Gy, n=3). **A)** Level of HMGB1 in the supernatant 24 hours after radiation. Coomassie stained gel as a loading control. **B)** Representative crystal violet growth assay of radiotherapy treated cells five days post radiation. **C)** CRT47R cells were treated with DMSO, BET inhibitor (2 μM PLX51107), CDK4/6 inhibitor (1 μM PD'991), etoposide (37.5 μM), dacarbazine (20 μM), tamoxifen (1 μM), ERK1/2 inhibitor (1 μM SCH'772984), or paradox-breaking BRAF inhibitor (500 nM PLX8394) for 24 hours (n=3). Levels of HMGB1 in the supernatant were detected by Western blotting. Coomassie stained gel as loading control. **D)** As in C, except cells were stained for Zombie NIR. Dead cells indicated by % zombie positive. **E)** Mice from Figure 6E, showing tumor growth and etoposide treatment schedule (vertical dotted lines) from tumor implantation. **F)** Mouse weights during treatment.