SI APPENDIX FIGURE LEGENDS

Figure S1: ROS regulates PD-L1 mRNA and cell surface expression in BMDM. (*A*) Representative histogram profile of ROS levels in BMDM stained with DCF-DA and left untreated (Ctrl) or treated for 24h with BSO (200μM) ± NAC (1mM). n=4/group. (*B*) *Pdl1* mRNA levels in BMDM that were treated with 20ng/ml IL-4 and 20 ng/ml M-CSF (Ctr) or BSO (200μM) ± NAC (1mM). n=3/group. (*C*) *Arg1* mRNA levels in BMDM that were left untreated or treated with IL-4 and M-CSF as in (*B*). n=3/group. (*D*) *Nqo1* and *Hmox-1* mRNA levels in BMDM that were left untreated (Ctrl) or treated for 24h with BSO (200μM) ± NAC (1mM). n=4/group. (*E*) *PD-L1* mRNA levels in human macrophages that were left untreated (Ctrl) or treated for 24h with BSO (1mM) ± NAC (1mM). n=3/group. (*F*) *NQO1* mRNA levels of human macrophages treated as in (*B*). n=3/group. (*G*) Percentage of PD-L1 positive BMDM (gated on live $CD45^+$ $CD11b^+$ $F4/80^+$) that were treated as in (*E*). n=3. (*H*) Percentage of PD-L1 positive BMDM gated on live $CD45^+$ $CD11b^+$ $F4/80^+$ within $CD206^+MHC\text{-}II^-$ or $CD206^-MHC\text{-}II^+$ populations. n=3. (*I*) Representative FACS plots of PD-L1 surface staining in $CD206^+MHC\text{-}II^-$ or $CD206^-MHC\text{-}II^+$ populations treated as in (*A*). (*J*) Fold surface expression levels of PD-L1 in $CD11b^+$ human macrophages treated as in (*B*). n=3/group. Data are normalized to values from untreated cells (Ctrl). Data in B-H and J are presented as mean ± S.E.M of biological replicates. *P≤0.05, **P≤0.01, ***P≤0.001.

Figure S2: Paclitaxel-induced ROS regulates PD-L1 mRNA and cell surface expression in BMDM. (*A*) Left, representative histogram of intracellular ROS levels in BMDM as measured by DCF-DA staining. Cells were treated with DMSO (Ctrl), paclitaxel (100nM), olaparib (0.5μM) or cisplatin (2μM) for 24h. Right, quantification (n=4/group). (*B*) Left, representative histogram of DNA damage in BMDM stained for phosphorylated H2AX (γ-H2AX) and treated as in (*A*). Right, quantification (n=4/group). (*C*) SRB assay in BMDM treated for 5 days with DMSO (Ctrl) and paclitaxel (100nM). (*D*) Representative histogram profile of ROS levels in BMDM stained with DCF-DA and left untreated (Ctrl) or treated for 24h with Paclitaxel (100nM) ± NAC (1mM). n=4/group. (*E*) *Pdl1* mRNA levels in BMDM that were treated with 20ng/ml IL-4 and 20 ng/ml M-CSF (Ctr) or BSO (200μM)

± NAC (1mM). n=3/group. (*F*) *Hmox-1* mRNA levels in BMDM treated with DMSO (Ctrl) or paclitaxel (100nM) ± NAC (1mM). n=4/group. (*G*) Representative FACS plots of PD-L1 surface staining in $CD206^{+}MHC\text{-}II^{-}$ or $CD206^{-}MHC\text{-}II^{+}$ populations treated as in (*D*). (*H*) *PD-L1* mRNA levels in human macrophages that were treated for 24h with DMSO (Ctrl) or paclitaxel (100nM) ± NAC (1mM). n=3. (*I*) Surface expression levels of PD-L1 in $CD11b^{+}$ human macrophages (n=3/group). Cells were treated as in (*H*) and values normalized to control (DMSO-treated cells). (*J*) *NQO1* mRNA levels in human macrophages that were treated as in (*H*). n=3. Data in A, B, E, F and H-J are presented as mean ± S.E.M of biological replicates. *P≤0.05, **P≤0.01, ***P≤0.001.

Figure S3: ROS-regulated PD-L1 expression depends on NF-kB transcriptional activity. (*A*) Representative images of BMDM positive for S536 phosphorylated-p65 (P-p65) when treated with DMSO (Ctrl) or BSO (200mM) and paclitaxel (100nM) ± SC514 for 3h. Stimulation of BMDM with LPS for 30min was used as positive control for phosphorylated p65 (S536). The use of isotype control antibody was included as negative control. (*B*) Representative image of the analysis of P-p65 signal intensity performed by ImageJ. A mask defining the nuclear region (based on DAPI staining) was overlaid on FITC-positive image. The mean FITC signal intensity was then calculated in the defined area. (*C*) *IkBa*, *Vegfa* and *Pdl1* mRNA levels in BMDM left untreated (Ctrl) or treated with BSO (200mM) and paclitaxel (100nM)±CH-223191 (10μM) AhR inhibitor for 24h. n=3. (*D*) Representative FACS plot of PD-L1 surface expression in $CD206^{+}MHCII^{-}$ BMDM. (*E*) Spearman's correlation coefficients between mRNA expression levels of *RelA*/*p50*, *Nfkb1*/*p65* and *Pdl1* in GSE27112 dataset from BMDM that were stimulated with LPS at different time points. (*F*) ChIP-qPCR of p65 at *Il6* promoter region in BMDM treated with BSO±NAC for 1h. n=3. Data in C and F are presented as mean ± S.E.M of biological replicates. *P≤0.05, **P≤0.01, ***P≤0.001,ns=not significant.

Figure S4: Paclitaxel increases PD-L1 expression in tumor-associated macrophages in vivo. (*A*) Positive correlation between tumor-infiltration of monocytic lineage cells (monocyes and macrophages) and *PD-L1* expression in the TCGA human basal-like and HR-defective BC datasets. See Materials and Methods for additional

details. (*B*) Representative FACS plots of PD-L1 surface staining in TAM ($CD49f^{low/-}CD45^+$ $CD11b^+$ $F4/80^+$ $CD206^+MHC\text{-}II^{low}$) in tumors from mice treated with vehicle (saline) or paclitaxel (20mg/kg) for 24h and 5 days. (*C*,*D*) *Pdl1* and *arginase1* mRNA levels in BMDM that were cultured alone or sorted after co-culture with KBP mammary tumor cells for 24h in vitro. n=3. (*E*) Representative FACS plot of PD-L1 staining in $CD11b^+$ cells from peripheral blood in tumour-bearing mice treated vehicle and paclitaxel for 24h and 5 days. (*F*) Representative FACS plot of PD-L1 staining in $CD45^-CD49f^+$ cells in tumors from mice treated vehicle and paclitaxel for 24h and 5 days. (*G*) Representative histogram of PD-L1 surface expression in KBP cells treated with the indicated doses of paclitaxel for 24h. (*H*) Representative FACS plot of IL-10, IL-17 and IL-12 in CD11b+F4/80+ cells isolated from vehicle- and paclitaxel-treated tumors at 5 days post-treatment. (*I*) Representative histogram of phospho-p65 levels in TAM ($CD49f^{low/-}CD45^+$ $CD11b^+$ $F4/80^+$ $CD206^+$ $PD\text{-}L1^+$) isolated from KBP tumors 5 days after treatment with paclitaxel or vehicle. n=6/group. (*J*) Positive correlation between M1 or M2 gene expression signatures (as determined by Chung et al, 2018 and Azizi et al., 2018) and the expression levels of *PD-L1*, *p65*/*NFKB1* and the "Chuang oxidative stress response" gene signature in the TCGA human HR-defective BC cohort. See Materials and Methods for details. Data in C and D are presented as mean ± S.E.M of biological replicates. *P≤0.05, **P≤0.01, ***P≤0.001.

Figure S5: Paclitaxel and PD-L1 blockade affects pathological features of KBP mammary tumors. (*A*) SRB cell viability assay in BMDM that were treated for 5 days with isotype control (10μg/ml) or paclitaxel (100nM)±αPD-L1 (10μg/ml). (*B, C*) Percentage of CD206 (*B*) and MHC-II (*C*) positive cells within BMDM ($CD49f^{low/-}CD45^+$ $CD11b^+$ $F4/80^+$) treated as in (*A*) for 24h. (*D*) *Pdl1* mRNA levels in BMDM treated as in (*A*). (*E*) Left, representative images of CD31-positive areas in tumors from mice treated with vehicle, isotype control, paclitaxel and αPD-L1 as indicated. Slides were counterstained with H&E. Right, quantification. n=5. (*F*) Representative images of P-p65 positive cells in tumor tissues from mice treated as in (*E*). Slides were counterstained

with H&E. (G) Representative images of cleaved caspase 3 immunostaining in tumors treated as in (*E*). Data in B-E are presented as mean ± S.E.M of biological replicates.

Figure S6: Paclitaxel and PD-L1 blockade affects the immune profile of KBP mammary tumors. (*A*) Percentage of $CD4^+$ T cells gated on live $CD49f^-$ $CD45^+$ $CD3^+$cells and isolated from KBP tumors at day 14 in mice treated with vehicle, isotype control paclitaxel and αPD-L1 as indicated. n=10-15/group. (*B*) Representative FACS plot of $CD4^+$ and $CD8^+$ population in tumors from mice treated as in (*A*). (*C*) Percentage of $FoxP3^+$ cells gated on $CD49f^-$ $CD45^+$ $CD3^+$ $CD4^+$ $CD25^+$ cells and isolated from KBP tumors at day 14 in mice treated as in (*A*). n=10-15/group. (*D*) Percentage of $CD8^+$ T cells gated on live $CD49f^-$ $CD45^+$ $CD3^+$cells isolated from KBP tumors at day 14 in mice treated as in (*A*). n=10-15/group. (*E*) Representative FACS plot of $CD8^+$ population stained for CD44 and CD62L to define naïve, memory and effector T cell subpopulations in tumors from mice treated as in (*A*). (*F-I*) Representative FACS plots showing the levels of IFN-γ, Granzyme-B, PD-1 and CD107a in $CD8^+$ population in tumors from mice treated as in (*A*). Data in A, C and D are presented as mean ± S.E.M of biological replicates. *P≤0.05, **P≤0.01, ***P≤0.001.

Figure S1

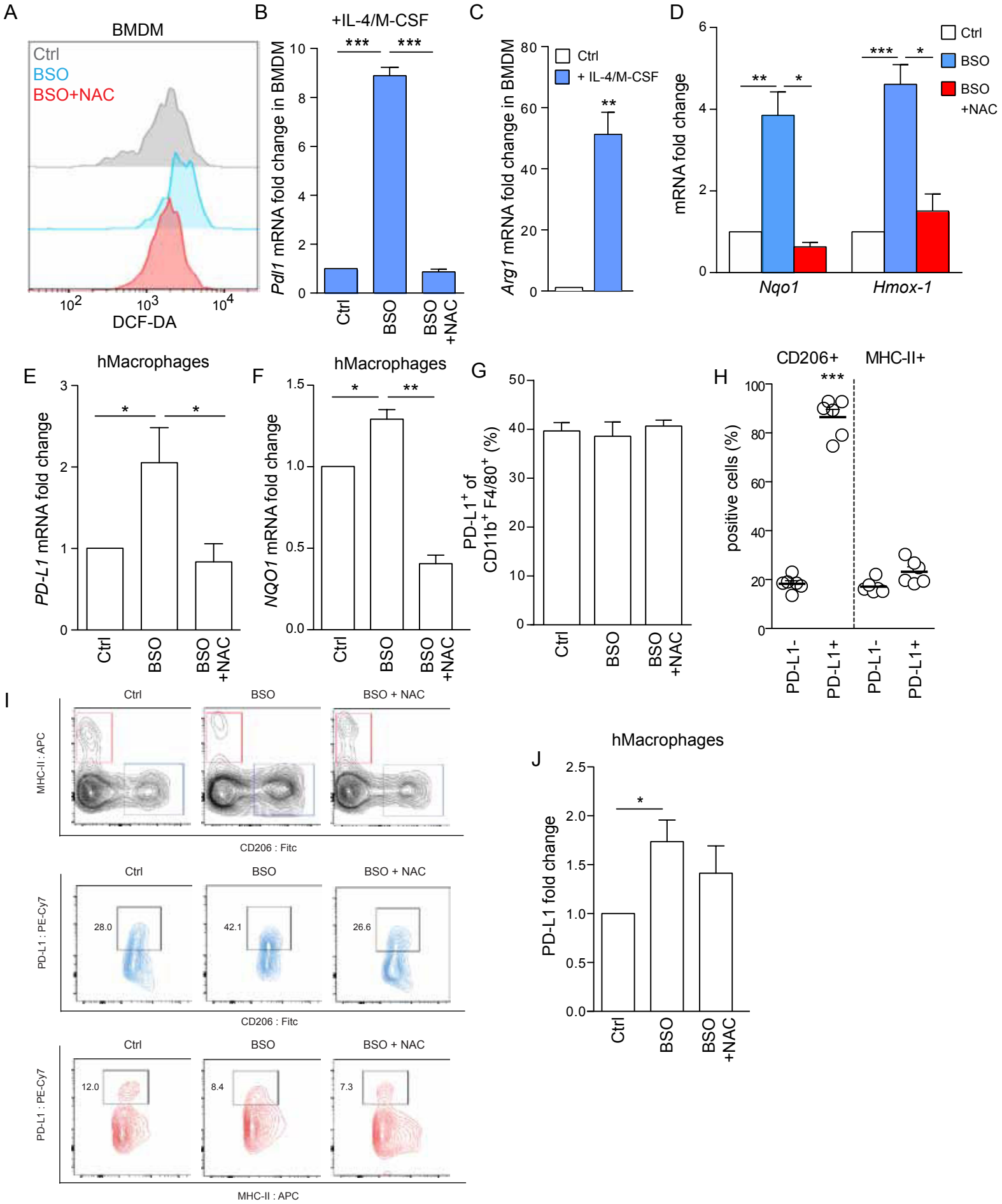

A
BMDM
Ctrl
BSO
BSO+NAC
DCF-DA
B
+IL-4/M-CSF
Pdl1 mRNA fold change in BMDM
Ctrl
BSO
BSO +NAC
C
Arg1 mRNA fold change in BMDM
Ctrl
+ IL-4/M-CSF
D
mRNA fold change
Ctrl
BSO
BSO +NAC
Nqo1
Hmox-1
E
hMacrophages
PD-L1 mRNA fold change
F
hMacrophages
NQO1 mRNA fold change
G
PD-L1+ of CD11b+ F4/80+ (%)
H
CD206+
MHC-II+
positive cells (%)
PD-L1-
PD-L1+
I
Ctrl
BSO
BSO + NAC
MHC-II : APC
CD206 : Fitc
PD-L1 : PE-Cy7
28.0
42.1
26.6
12.0
8.4
7.3
J
hMacrophages
PD-L1 fold change

Figure S2

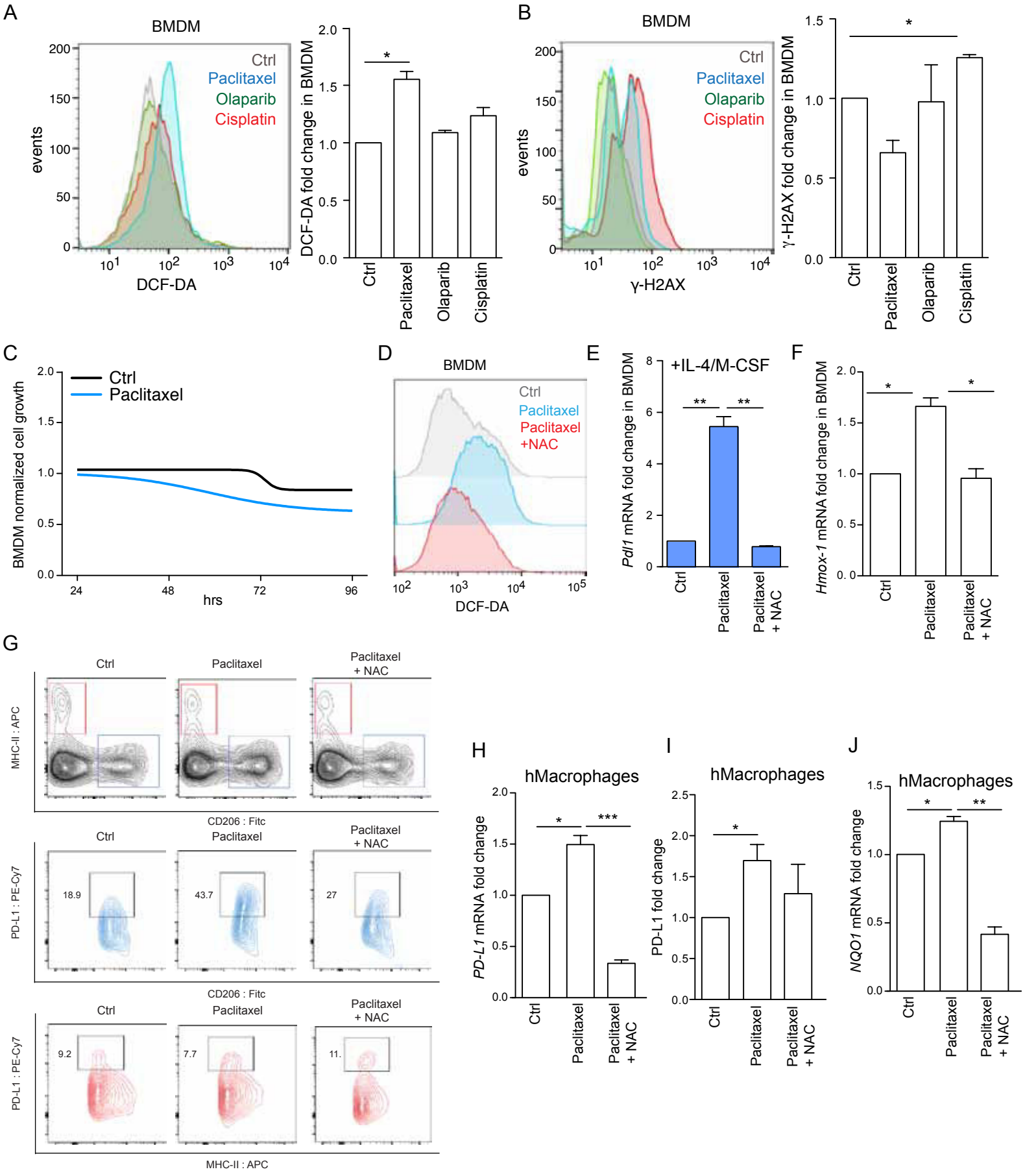
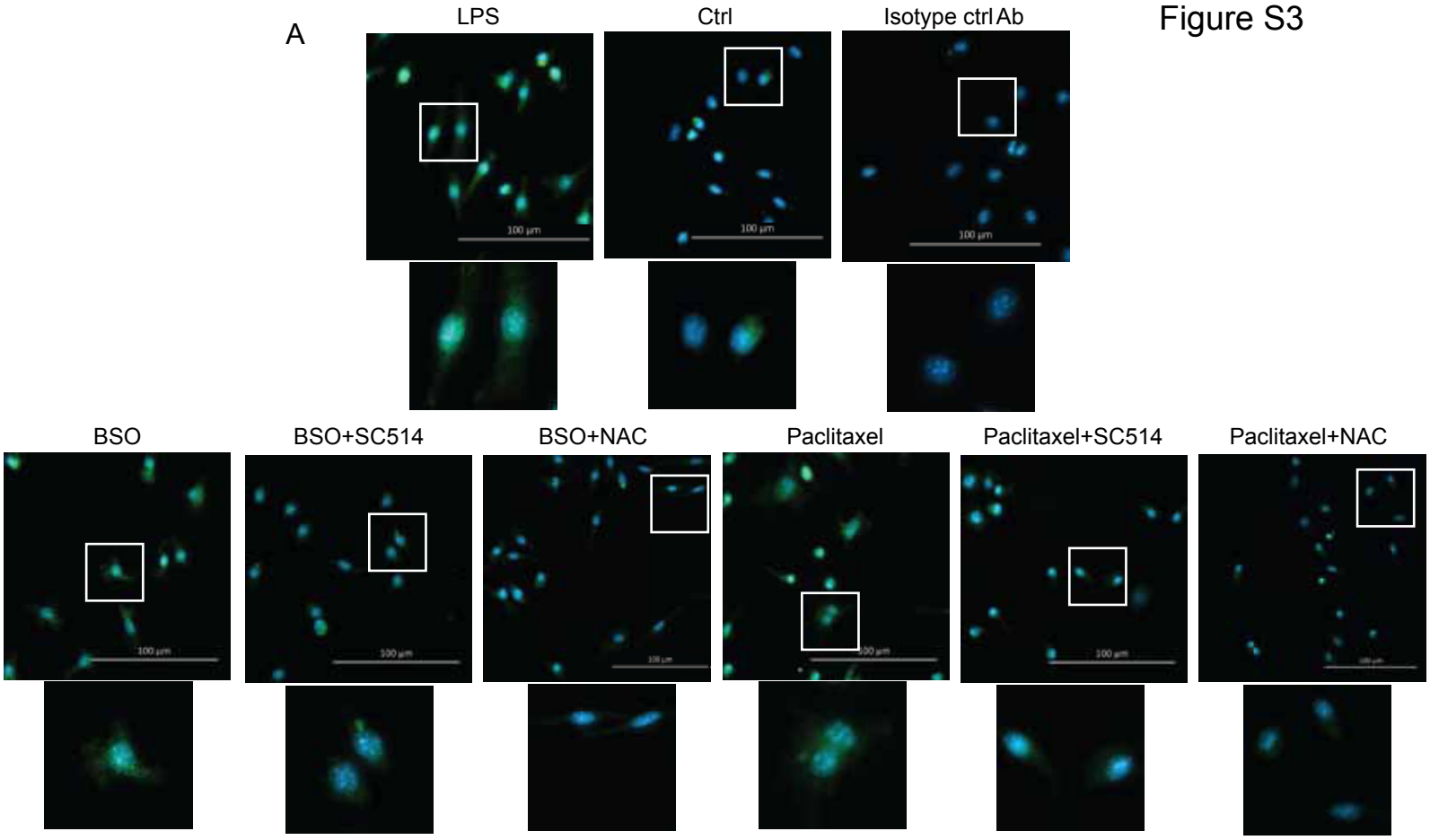
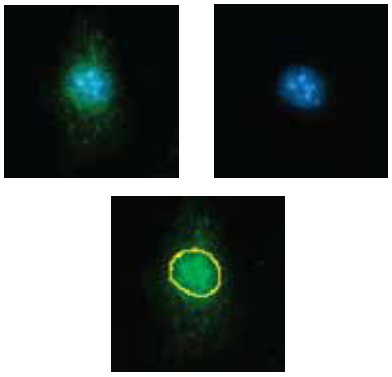
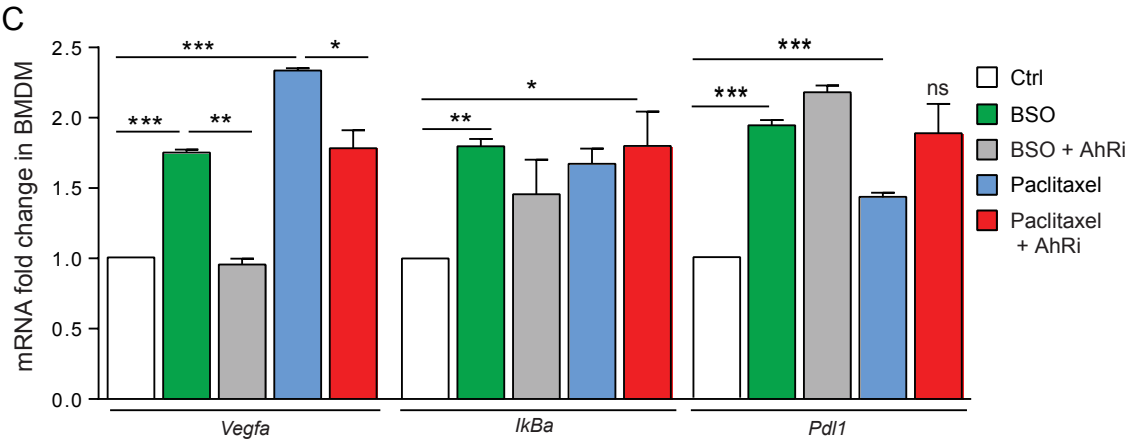
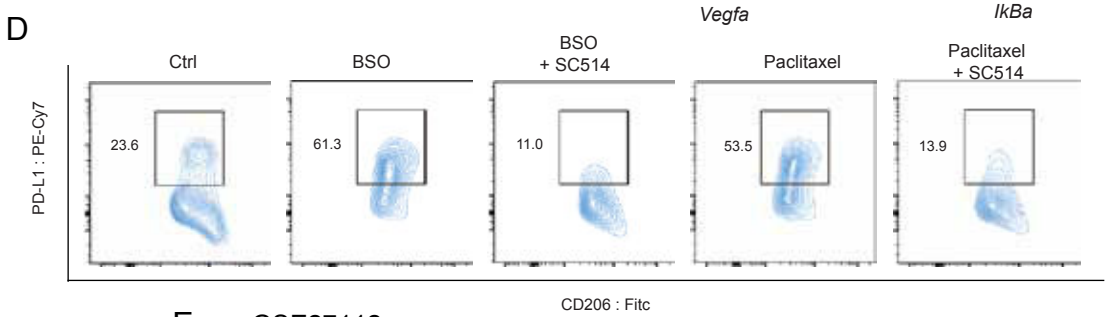

A
BMDM
Ctrl
Paclitaxel
Olaparib
Cisplatin
events
DCF-DA
DCF-DA fold change in BMDM
B
BMDM
γ-H2AX
γ-H2AX fold change in BMDM
C
Ctrl
Paclitaxel
BMDM normalized cell growth
hrs
D
BMDM
Paclitaxel +NAC
DCF-DA
E
+IL-4/M-CSF
Pdl1 mRNA fold change in BMDM
Paclitaxel + NAC
F
Hmox-1 mRNA fold change in BMDM
G
MHC-II : APC
PD-L1 : PE-Cy7
CD206 : Fitc
18.9
43.7
27
9.2
7.7
11.
H
hMacrophages
PD-L1 mRNA fold change
I
PD-L1 fold change
J
NQO1 mRNA fold change

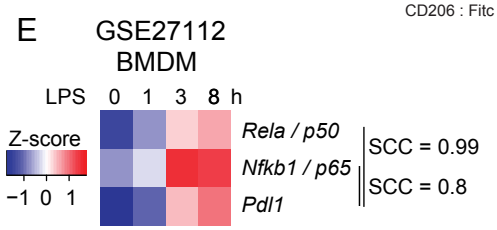
Figure S3


A
LPS
Ctrl
Isotype ctrl Ab
BSO
BSO+SC514
BSO+NAC
Paclitaxel
Paclitaxel+SC514
Paclitaxel+NAC


B


C
mRNA fold change in BMDM
2.5
2.0
1.5
1.0
0.5
0.0
***
*
**
ns
Vegfa
IkBa
Pdl1
Ctrl
BSO
BSO + AhRi
Paclitaxel
Paclitaxel + AhRi


D
Ctrl
BSO
BSO + SC514
Paclitaxel
Paclitaxel + SC514
23.6
61.3
11.0
53.5
13.9
PD-L1 : PE-Cy7
CD206 : Fitc


E
GSE27112
BMDM
LPS 0 1 3 8 h
Z-score
−1 0 1
Rela / p50
Nfkb1 / p65
Pdl1
SCC = 0.99
SCC = 0.8

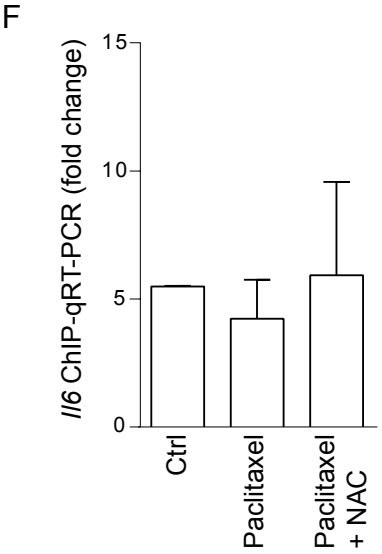

F
Il6 ChIP-qRT-PCR (fold change)
15
10
5
0
Ctrl
Paclitaxel
Paclitaxel + NAC

Figure S4

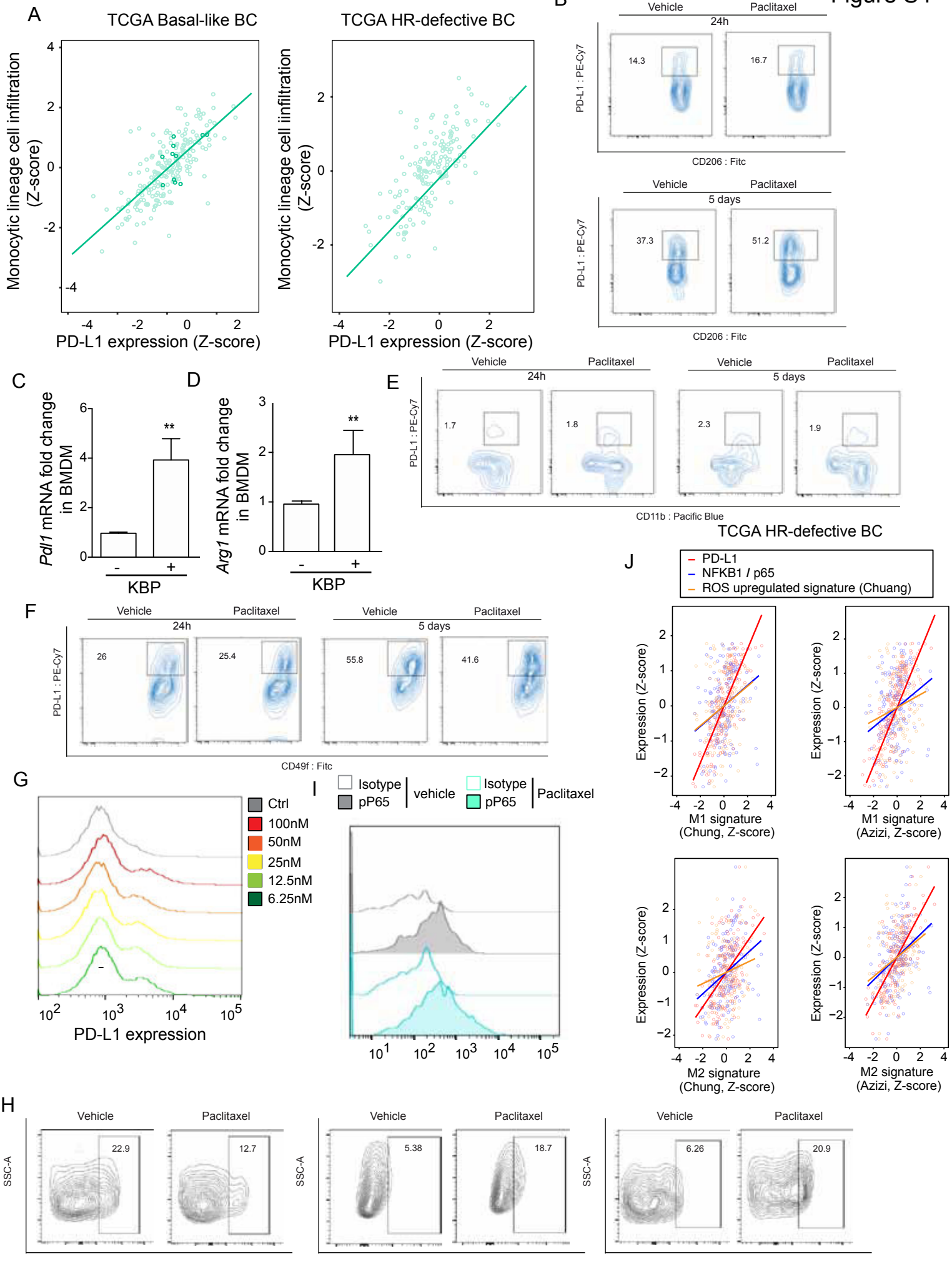

A
TCGA Basal-like BC
TCGA HR-defective BC
Monocytic lineage cell infiltration (Z-score)
PD-L1 expression (Z-score)
B
Vehicle
Paclitaxel
24h
5 days
PD-L1 : PE-Cy7
CD206 : Fitc
14.3
16.7
37.3
51.2
C
Pdl1 mRNA fold change in BMDM
KBP
D
Arg1 mRNA fold change in BMDM
E
CD11b : Pacific Blue
1.7
1.8
2.3
1.9
F
CD49f : Fitc
26
25.4
55.8
41.6
G
Ctrl
100nM
50nM
25nM
12.5nM
6.25nM
PD-L1 expression
I
Isotype
pP65
vehicle
Paclitaxel
J
TCGA HR-defective BC
PD-L1
NFKB1 / p65
ROS upregulated signature (Chuang)
M1 signature (Chung, Z-score)
M1 signature (Azizi, Z-score)
M2 signature (Chung, Z-score)
M2 signature (Azizi, Z-score)
Expression (Z-score)
H
SSC-A
IL-12 : PE
IL-17 : PE
IL-10 : APC
22.9
12.7
5.38
18.7
6.26
20.9

Figure S5

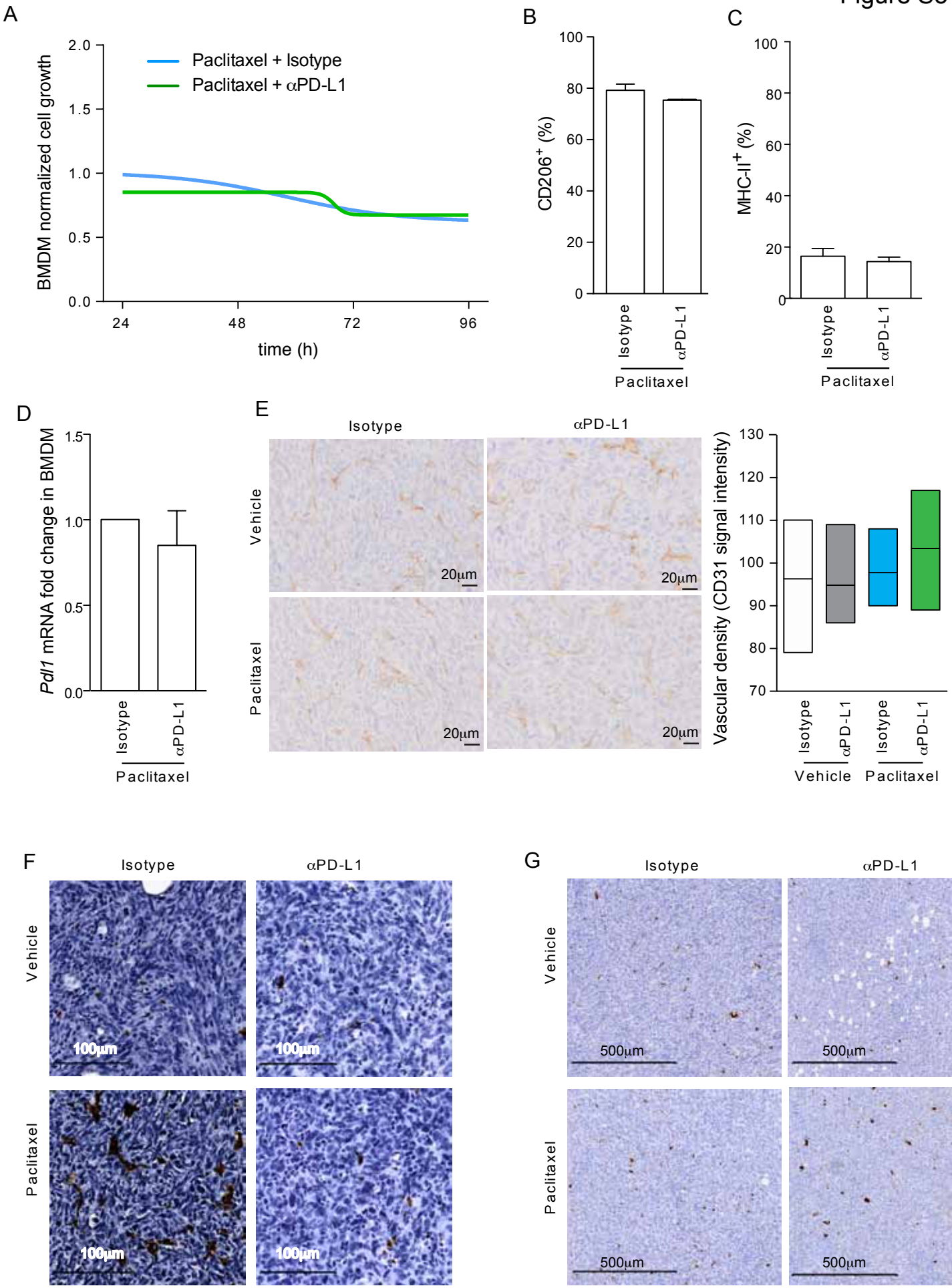
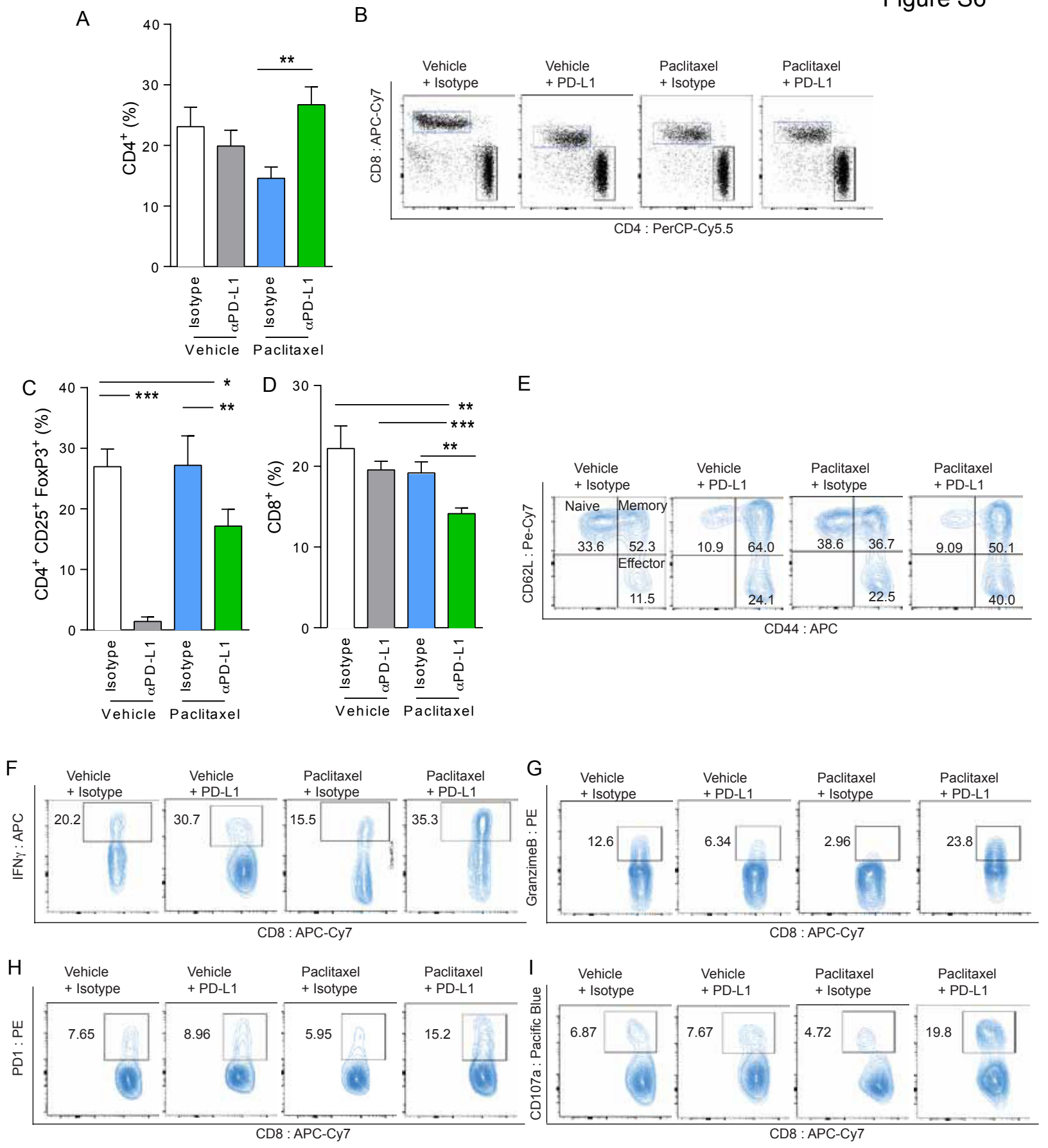

A
BMDM normalized cell growth
Paclitaxel + Isotype
Paclitaxel + αPD-L1
time (h)
B
CD206+ (%)
Isotype
αPD-L1
Paclitaxel
C
MHC-II+ (%)
Isotype
αPD-L1
Paclitaxel
D
Pdl1 mRNA fold change in BMDM
Isotype
αPD-L1
Paclitaxel
E
Isotype
αPD-L1
Vehicle
Paclitaxel
20μm
Vascular density (CD31 signal intensity)
Isotype
αPD-L1
Isotype
αPD-L1
Vehicle
Paclitaxel
F
Isotype
αPD-L1
Vehicle
Paclitaxel
100μm
G
Isotype
αPD-L1
Vehicle
Paclitaxel
500μm

Figure S6


A
B
C
D
E
F
G
H
I
CD4+ (%)
Isotype
αPD-L1
Vehicle
Paclitaxel
Vehicle + Isotype
Vehicle + PD-L1
Paclitaxel + Isotype
Paclitaxel + PD-L1
CD8 : APC-Cy7
CD4 : PerCP-Cy5.5
CD4+ CD25+ FoxP3+ (%)
CD8+ (%)
CD62L : Pe-Cy7
CD44 : APC
Naive
Memory
Effector
33.6
52.3
11.5
10.9
64.0
24.1
38.6
36.7
22.5
9.09
50.1
40.0
IFNγ : APC
20.2
30.7
15.5
35.3
GranzimeB : PE
12.6
6.34
2.96
23.8
PD1 : PE
7.65
8.96
5.95
15.2
CD107a : Pacific Blue
6.87
7.67
4.72
19.8

Supplementary Table 1: Primers used for qRT-PCR and ChIP-qPCR (m=mouse) (h=human).

Gene	Primer Sequence
mRsp9 F	GCAAGATGAAGCTGGATTAC
mRps9 R	GGGATGTTCACCACCTG
mNqo1 F	AGGATGGGAGGTACTCGAATC
mNqo1 R	AGGCGTCCTTCCTTATATGCTA
mHo-1 F	AAGCCGAGAATGCTGAGTTCA
mHo-1 R	GCCGTGTAGATAATGGTACAAGGA
mGclc F	GGCTCTCTGCACC
mGclc R	GTTAGAGTACCGA
mGclm F	AAGTTAACCTGGC
mGclm R	GAGAGCAGTTCTT
mPdl1 F	CAGCAACTTCAGGGGGAGAG
mPdl1 R	TTTGCGGTATGGGGCATTGA
mIkBa F	AACCTGCAGCAGACTCCACT
mIkBa R	GACACGTGTGGCCATTGTAG
mVegfa F	CCGGGCCTCGGTT
mVegfa R	GGGACCACTTGGC
hRsp9 F	GTTTGCTTAGGCGCAGACG
hRps9 R	CCATACTCGCCGATCAGCTT
hPdl1 F	AAATGGAACCTGGCGAAAGC
hPdl1 R	GATGAGCCCCTCAGGCATTT
hNqo1 F	TCCCCCTGCAGTGGTTTGGAGT
hNqo1 R	ACTGCCTTCTTACTCCGGAAGGGT
mIL-6 F (ChIP)	CACTTCACAAGTCGGAGGCT
mIL-6 R (ChIP)	AATGAATGGACGCCCAGACT
mPdl1-I1551 F (ChIP)	GCCAGGCAGAACTAAAGTGG
mPdl1-I1551 R (ChIP)	GGTTCCTCAGGGTGACTCAG