Supplemental Figures and Tables

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Figure S1. Bone marrow-derived macrophages enrich MM TICs. (A) 8-10 week old Balb/c mice were intravenously injected with bortezomib (1 mg/kg, bort) or vehicle control (veh). After 24 h, femurs were removed and flushed to obtain bone marrow cells. The indicated bone marrow cell types were identified by CyTOF mass cytometry. Data are presented as bar charts. (B-C) RPMI-8226 cells were cultured for 4 days in the presence of macrophage conditioned medium(CM). Aldehyde dehydrogenase (ALDH) activity (B) and side population assay (C) were performed using flow cytometry. Representative dot plots are shown. (D-F) Peritoneal macrophages were harvested from mice sequentially treated with thioglycollate followed by bortezomib (bort) or vehicle control (veh). The macrophages were co-cultured with RPMI-8226 cells in a 1:1 ratio for 4 days. The percentage of TICs was evaluated phenotypically (D), or by side population assay (E-F). Representative dot plots (E) and their quantification (F) are presented. (G-H) KMS-11 (G) and U266 (H) were cultured for 4 days in the presence of macrophage CM obtained from vehicle control or bortezomib-treated mice. The percentage of TICs and ALDH activity was assessed by flow cytometry. *, $p<0.05$, **, $p<0.01$, using student's t test.

Figure S2. IL-1β promotes MM-TIC enrichment. (A-B) CAG and RPMI-8226 cells were cultured for 4 days in the presence of escalating doses of TREM-1 (A) or IL-16 (B). The percentage of TICs was analyzed by flow cytometry. (C) CAG cells were cultured for 4 days in the presence of escalating doses of IL-1β. The percentage of TICs was analyzed by flow cytometry. (D) mRNA was extracted from CAG, U266 and KMS-11 cells and the level of IL-1 receptor mRNA was assessed by real-time PCR. Values were normalized to β2MG. (E) IL-1R protein expression was evaluated by Western blot on lysates of CAG and RPMI-8226 cells. Panceau was used as a loading control. (F) Peritoneal macrophages were harvested from wild-type (WT) or IL-1β-/- mice sequentially treated with thioglycollate followed by bortezomib (bort) or vehicle control (veh). Macrophages were cultured for 48 h and conditioned medium (CM) was collected. U266 and KMS-11 MM cells were cultured for 4 days with macrophage CM and the percentage of TICs was analyzed by flow cytometry.. *, $p<0.05$, **; $p<0.01$; ***, $p<0.001$ using student's t test or one way ANOVA when comparing between more than two groups.

Figure S3. Reduced survival in MM patients with high levels of M1 pro-inflammatory macrophages and TICs. BM samples were obtained from newly diagnosed MM patients (N=34) immediately before and several months after receiving bortezomib treatment. (A) Fold change in the percentage of

macrophages before and several months after receiving bortezomib therapy was calculated and plotted. Black bars represent deceased MM patients. (B-C) Patients were grouped according to the value of M1 pro-inflammatory macrophages at diagnosis (Dx; B) and post-treatment (P-Tx; C) as shown in the scatter plots. (D) Fold change in the percentage of TICs before and several months after receiving bortezomib therapy was calculated and plotted. Black bars represent deceased MM patients.

Table S1: Patient characteristics and treatment description

Characteristic	No.
Age- median (years)	64 (range, 44-79)
Sex	
Male	15 (44%)
Female	19 (56%)
International staging system	
Stage 1	10 (29%)
Stage 2	15 (44%)
Stage 3	7 (21%)
ND	2 (6%)
Type	
IgG	19(56%)
IgA	7 (20.5%)
IgD	1 (3%)
Light chain disease	7 (20.5%)
Renal involvement	11 (32%)
Bone lesions	32 (94%)
Hypercalcemia	8 (23.5%)
Time from diagnosis to BM sample (months)	8 (range, 2-22)
First line therapy	
VCD	26 (76.5%)
VTD	8 (23.5%)
Median number of cycles of VCD/VTD treatment	6 (range, 2-12)
Response to first line	
Any response	32 (94.1%)
Primary refractory	2 (5.9%)
BM result following treatment(the first time point BM was done)	
Normal	23 (67.6%)
Abnormal	11 (32.4%)
Autologous transplant	28 (82.3%)
Total regimens (from dx to follow up)	
1-2	26 (76.5%)
3-4	6 (17.6%)
5	2 (5.9%)

VCD; Velcade, cyclophosphamide, dexamethasone; VTD; Velcade, thalidomide, dexamethasone; BM; Bone marrow

Table S2. Panel of antibodies and isotype conjugates used in CyTOF.

	Cell Surface marker	Isotope
1	CD45	115Di
2	CD93	139Di
3	GR1	142Di
4	CD86	143Di
5	F4/80	144Di
6	CD4	145Di
7	CD45R	146Di
8	Ly6C	147Di
9	CD138	148Di
10	CD8	149Di
11	Ly6g	150Di
12	CD127	151Di
13	CD90	152Di
14	CD14	153Di
15	CD11c	154Di
16	IgM	155Di
17	CD49b	156Di
18	CD19	157Di
19	CD34	158Di
20	CD27	159Di
21	CD69	160Di
22	TCRb	162Di
23	CCR7	163Di
24	CD28	164Di
25	CD115	165Di
26	CD133	166Di
27	CD205	167Di
28	CD117	168Di
29	CD79b	169Di
30	CD62L	170Di
31	CD44	171Di
32	CD43	172Di
33	Sca-1	173Di
34	Ia-Ie	174Di
35	IgD	175Di
36	CD24	141Di
37	CD11b	176Di
38	SiglecH	161Di

Figure S1

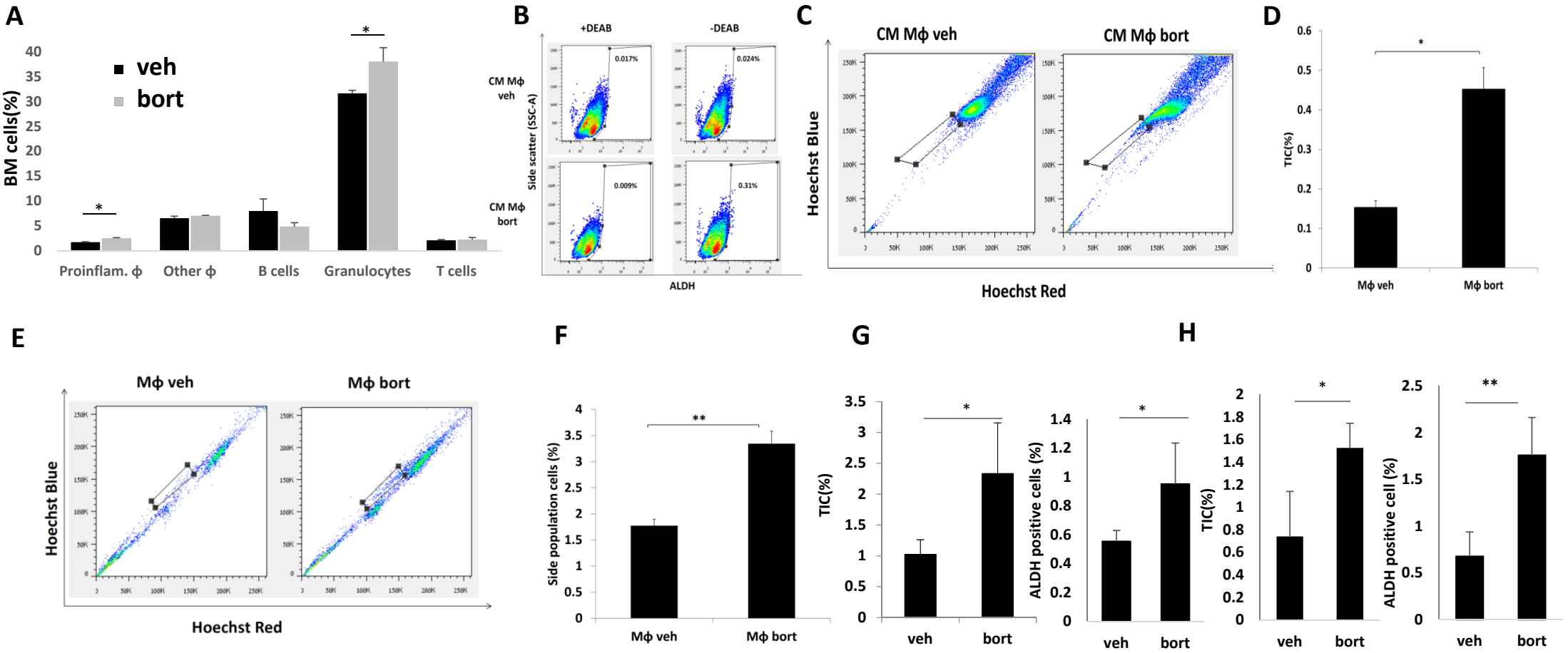

A
BM cells(%)
veh
bort
Proinflam. ϕ
Other ϕ
B cells
Granulocytes
T cells
B
+DEAB
-DEAB
CM Mϕ veh
CM Mϕ bort
Side scatter (SSC-A)
ALDH
0.017%
0.024%
0.009%
0.31%
C
CM Mϕ veh
CM Mϕ bort
Hoechst Blue
Hoechst Red
D
TIC(%)
Mϕ veh
Mϕ bort
E
Mϕ veh
Mϕ bort
Hoechst Blue
Hoechst Red
F
Side population cells (%)
Mϕ veh
Mϕ bort
G
TIC(%)
ALDH positive cells (%)
veh
bort
H
TIC(%)
ALDH positive cell (%)
veh
bort

Figure S2

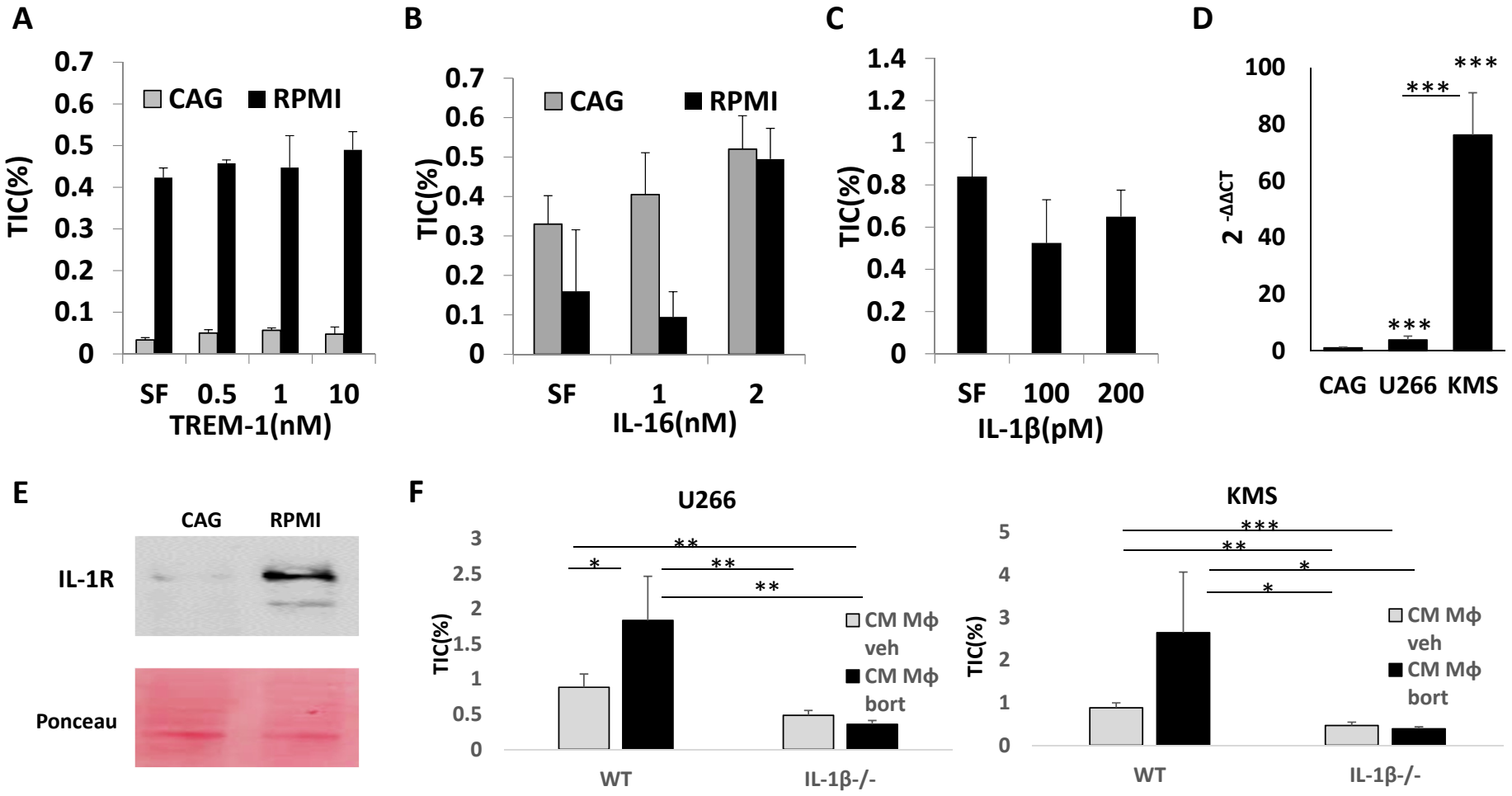
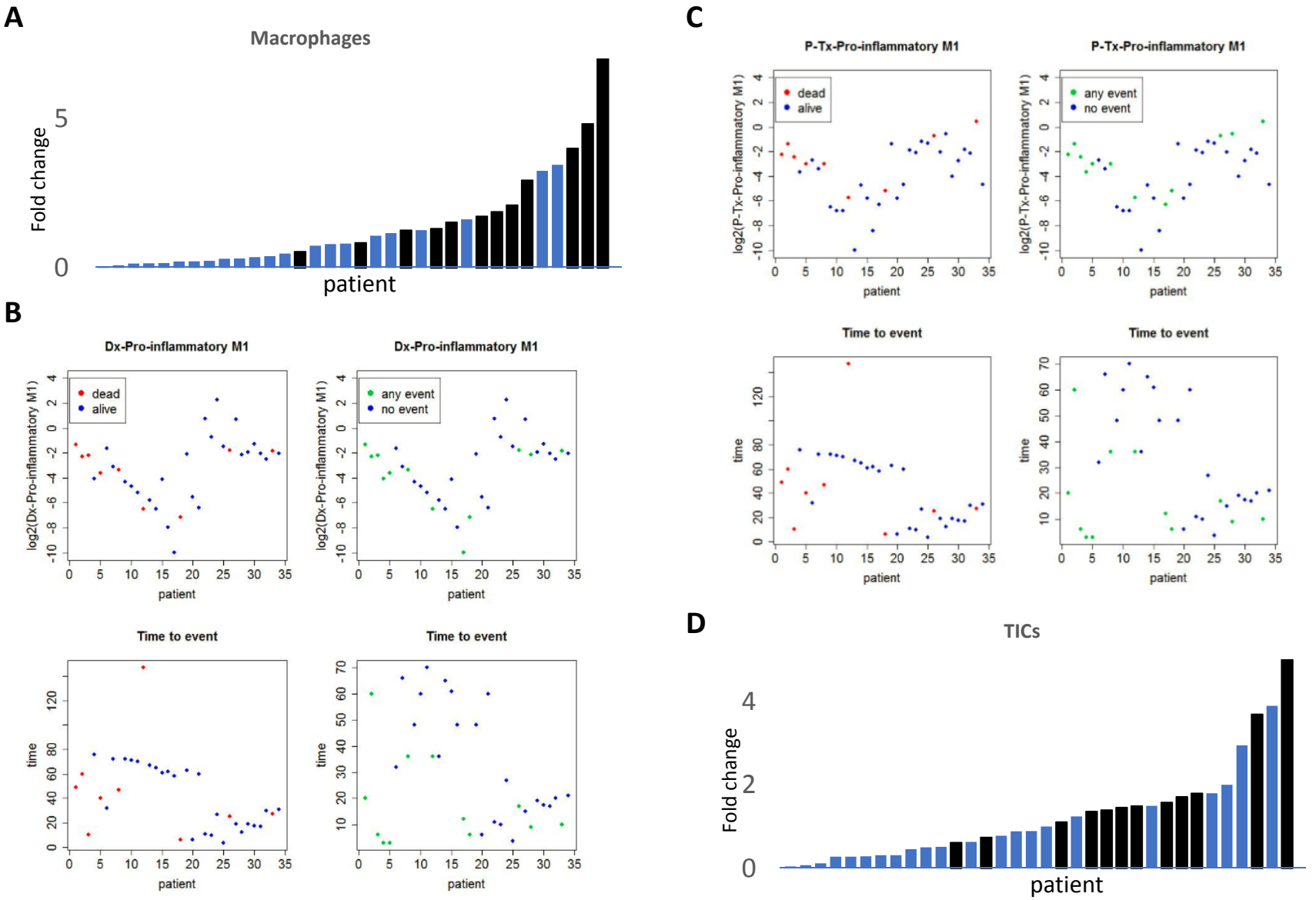

A
TIC(%)
CAG
RPMI
SF 0.5 1 10
TREM-1(nM)
B
TIC(%)
CAG
RPMI
SF 1 2
IL-16(nM)
C
TIC(%)
SF 100 200
IL-1β(pM)
D
2 -ΔΔCT
***
***
***
CAG U266 KMS
E
CAG
RPMI
IL-1R
Ponceau
F
U266
TIC(%)
CM Mϕ veh
CM Mϕ bort
WT
IL-1β-/-
KMS
TIC(%)
CM Mϕ veh
CM Mϕ bort
WT
IL-1β-/-

Figure S3


A
Macrophages
Fold change
patient
B
Dx-Pro-inflammatory M1
dead
alive
any event
no event
log2(Dx-Pro-inflammatory M1)
Time to event
time
patient
C
P-Tx-Pro-inflammatory M1
log2(P-Tx-Pro-inflammatory M1)
D
TICs