Title: Aging-related inflammation driven by cellular senescence enhances NAD consumption via activation of CD38^{+} macrophages

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Supplementary Materials:

Figure S1.
A) Flow cytometry gating strategy to identify CD45+ immune cells isolated from the stromal vascular fraction of eWAT. Cells isolated from the SVF of eWAT were first gated on forward scatter (FSCA) vs side scatter (SSCA) to discard cell debris and dead or dying cells. Next FSCH (height) vs FSCA (Area) was used to select for single cells. Single cells were then gated for auto-fluorescent using the Empty (E) BV421 vs BV711 channels (which we did not use as antibody fluorophores) to discard any cells that showed auto-fluorescence in these channels. Next, CD45+ cells were selected and analyzed for CD38 and macrophage markers (See figure 1A).
B-C) Flow cytometry quantification of CD38- (low) resident macrophages, CD38- non-resident macrophages, and CD38+ (high) non-macrophage immune cells isolated from eWAT of mice for the indicated age shown.
D) Quantification of total macrophages, and CD38+ surface expression in resident and non-resident macrophages isolated from eWAT of mice fed a HFD or control diet.
For *in vivo* experiments, data from individual mice are shown.
Statistical significance defined as **P*<0.05, ***P*<0.01, and ****P*<0.00; two-sided Student's t-test.

Figure S2
A) NADase activity measured in human PBMC derived macrophages treated with recombinant human IL-4 (M2) or LPS (M1) for 18 hours. Showing the mean of two separate experiments from different donors with 2 biological replicates for each donor.
B) mRNA expression of *CD38* in human peripheral blood monocytes -derived macrophages treated as described above. Representative data of three patient samples.
C) Schematic of the *de novo* NAD synthesis pathway.
D) mRNA expression of *de novo* NAD synthesis pathway enzymes.
E) Quantification of tryptophan metabolites measured with LC-MS in M0, M2, and M1 mouse BMDMs activated for 24 hours.
Data is showing the mean ± SEM. n=3 biological replicates except n=4 in B. Statistical significance defined as **P*<0.05, ***P*<0.01, and ****P*<0.00; two-sided Student's t-test.

Figure S3
A) mRNA levels of M2 markers in BMDMs pre-treated with or without FK866 and NR for 6 hours prior to stimulation with IL-4 for 16 hours.

B) mRNA levels of M1 markers in BMDMs pre-treated with or without FK866 and NR for 6 hours prior to stimulation with LPS for 6 hours.
Data is showing the mean ± SEM. (n=3 biological replicates). Statistical significance defined as *P<0.05, **P<0.01, and ***P<0.00; two-sided Student's t-test. All statistical comparisons are relative to M2/M1 + FK866.

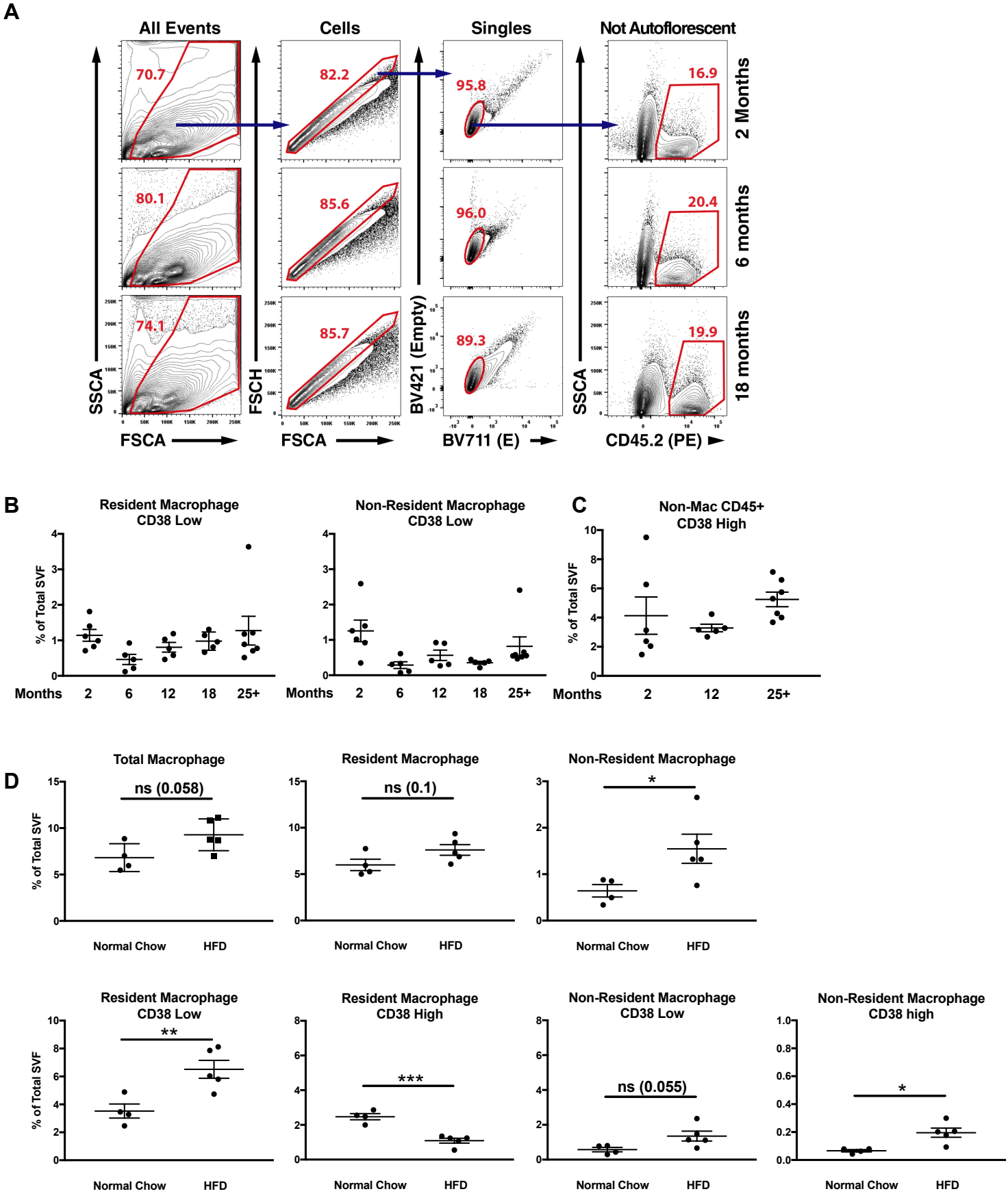
Figure S4

A) Flow cytometry results comparing CD38 surface staining in naive (M0) WT and *Cd38 KO* BMDMs or treated with IL-4 (M2) and LPS (M1) for 16 hours.
B) Western blot analysis for PARP1 and SIRT1 in WT and *Cd38 KO* BMDMs treated for the indicated times.
C) NADase activity measured in intact M0, M2, and M1 WT and *Cd38 KO* BMDMs activated for 16 hours.
D) mRNA expression of *Cd157* in M0 or M1 WT and *Cd38 KO* BMDMs for the indicated times.
E) LC-MS was used to quantify NR in M0 and M1 WT and *Cd38 KO* BMDMs treated for 16 hours.
Data is showing the mean ± SEM. (n=3 biological replicates). Statistical significance defined as *P<0.05, **P<0.01, and ***P<0.00; two-sided Student's t-test. Unless noted with a bar, all statistical comparisons are relative to untreated WT or *Cd38* KO sample.

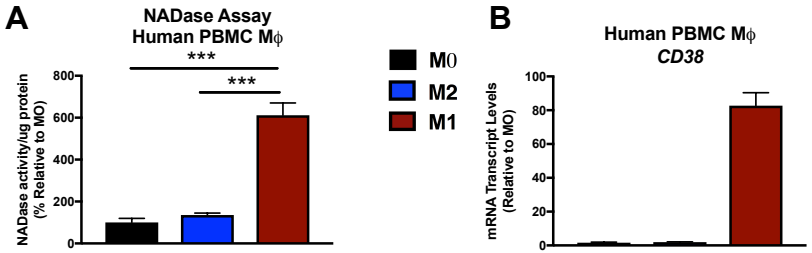
Figure S5

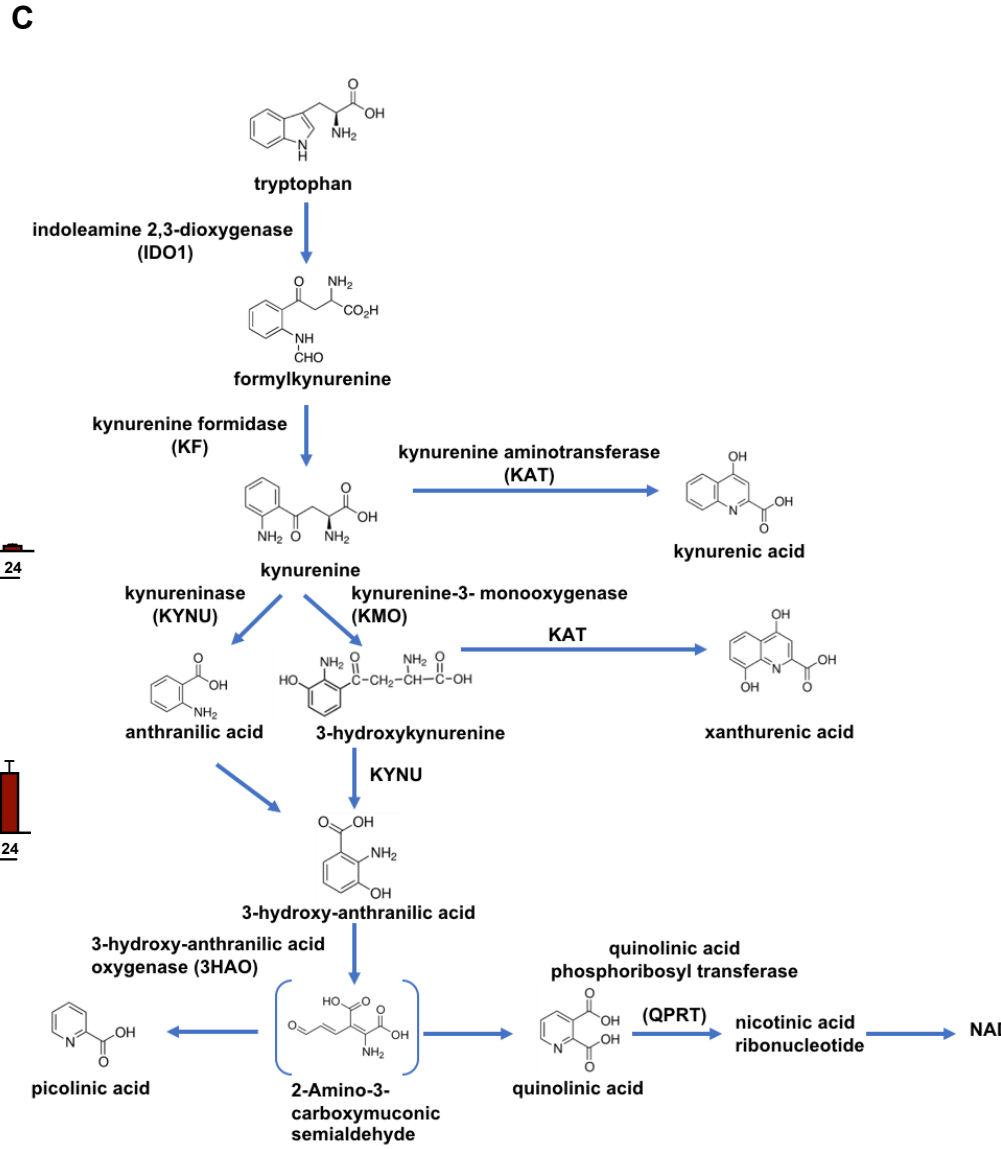
A) mRNA levels of *Il-1α*, *Cxcl1,* and *IL-10* in whole eWAT from 6 month and 25-month-old mice.
B) mRNA levels of *Il-1α*, *Cxcl1,* and *IL-10* in whole eWAT from 6-month-old mice IP injected with doxorubicin or PBS.
C) Quantification of CD38- (low) resident macrophages, and CD38- non-resident macrophages isolated from eWAT of 6-month-old mice IP injected with doxorubicin or PBS.
D) CD38 mRNA levels in WT and *Cd38 KO* BMDMs co-cultured (10:1) with senescent cells (IRR-MDFs) or untreated mouse dermal fibroblasts (MDFs) for 24 hours.
For *in vivo* experiments, data from individual mice are shown.
Data is showing the mean ± SEM. (n=4 biological replicates in D). Statistical significance defined as *P<0.05, **P<0.01, and ***P<0.00; two-sided Student's t-test except for 5a and 5b one-tailed t-test was used.

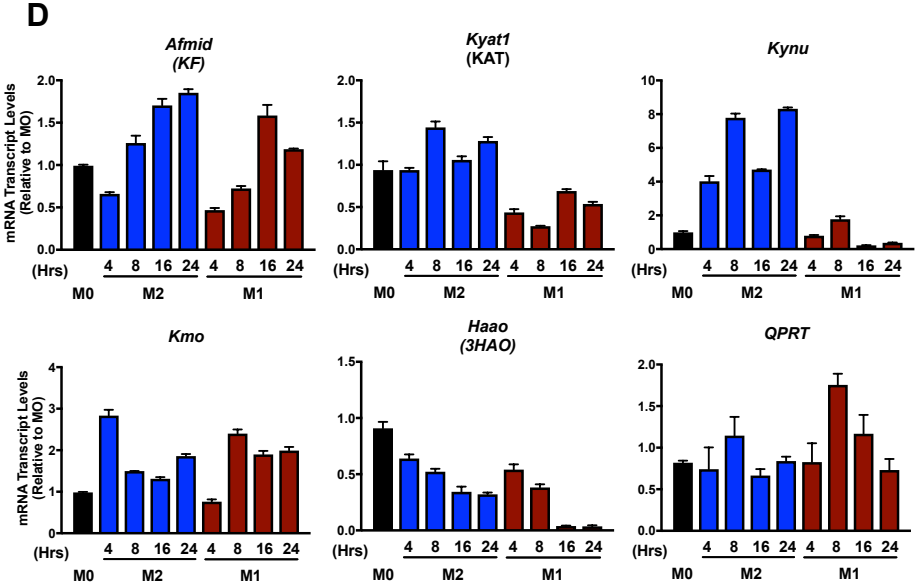
Sup Figure 1


A
All Events
Cells
Singles
Not Autoflorescent
70.7
82.2
95.8
16.9
2 Months
80.1
85.6
96.0
20.4
6 months
74.1
85.7
89.3
19.9
18 months
SSCA
FSCA
FSCH
FSCA
BV421 (Empty)
BV711 (E)
SSCA
CD45.2 (PE)
B
Resident Macrophage
CD38 Low
Non-Resident Macrophage
CD38 Low
% of Total SVF
Months 2 6 12 18 25+
C
Non-Mac CD45+
CD38 High
% of Total SVF
Months 2 12 25+
D
Total Macrophage
ns (0.058)
Resident Macrophage
ns (0.1)
Non-Resident Macrophage
*
% of Total SVF
Normal Chow
HFD
Resident Macrophage
CD38 Low
**
Resident Macrophage
CD38 High
***
Non-Resident Macrophage
CD38 Low
ns (0.055)
Non-Resident Macrophage
CD38 high
*

Sup Figure 2





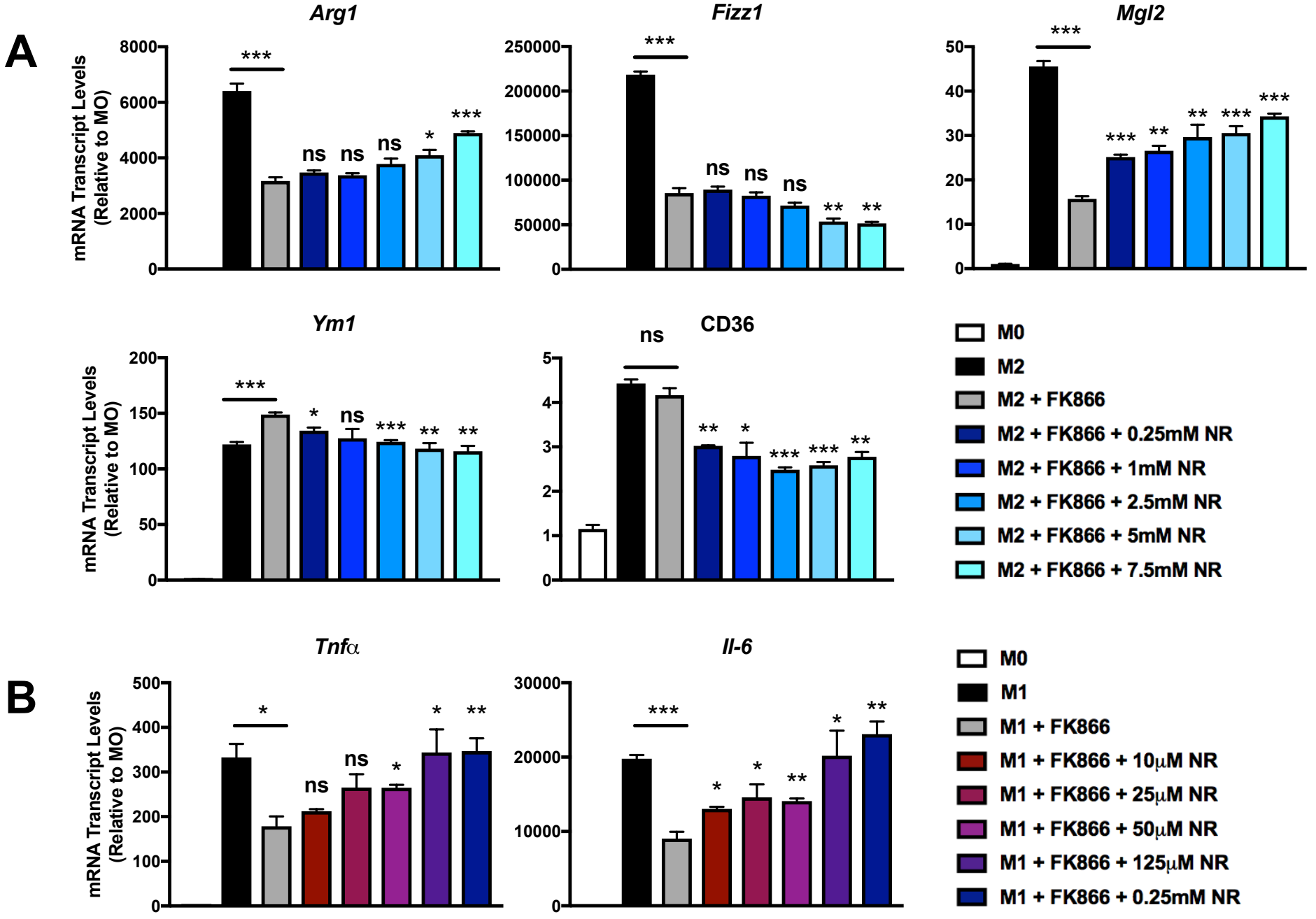


E

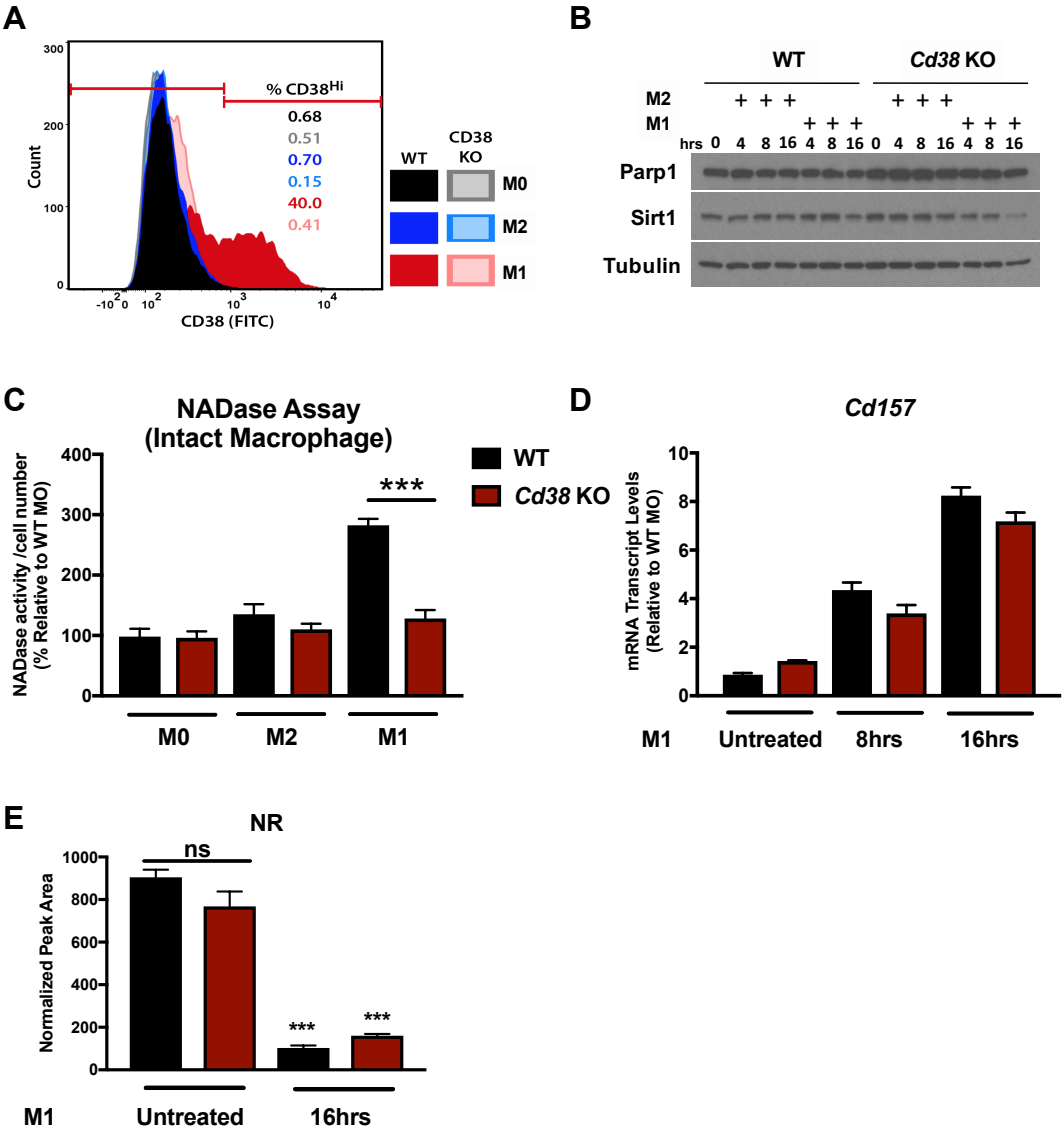
unit: pmol/mg protein

Macrophage Sample (24 hrs)	Tryptophan	Kynurenine	3-hydroxy-L-kynurenine	3-Hydroxy-anthranilic acid	Kynurenic acid	Xanthurenic acid	Anthranilic acid	Picolinic acid	Quinolinic acid
M0	25.73	0.28	ND	ND	2.21	ND	ND	ND	ND
M0	24.69	0.26	ND	ND	2.11	ND	ND	ND	ND
M0	24.57	0.25	ND	ND	2.3	ND	ND	ND	ND
M2	9.43	0.17	ND	ND	0.84	ND	ND	ND	ND
M2	11.08	0.19	ND	ND	0.96	ND	ND	ND	ND
M2	9.37	0.39	ND	ND	0.86	ND	ND	ND	ND
M1	640	10.71	ND	ND	42.89	ND	ND	ND	ND
M1	523.94	9.42	ND	ND	43.94	ND	ND	ND	ND
M1	603.24	7.01	ND	ND	51.57	ND	ND	ND	ND

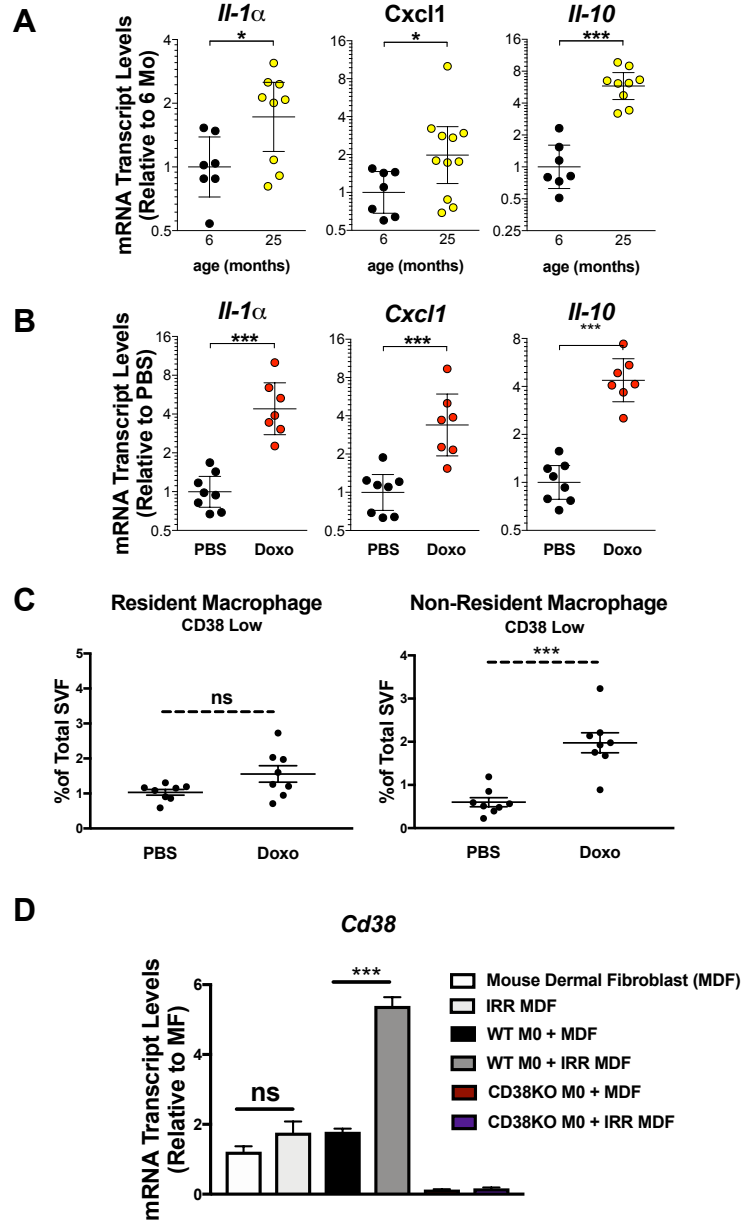
Sup Figure 3


A
Arg1
Fizz1
Mgl2
Ym1
CD36
mRNA Transcript Levels (Relative to MO)
M0
M2
M2 + FK866
M2 + FK866 + 0.25mM NR
M2 + FK866 + 1mM NR
M2 + FK866 + 2.5mM NR
M2 + FK866 + 5mM NR
M2 + FK866 + 7.5mM NR
B
Tnfα
Il-6
M0
M1
M1 + FK866
M1 + FK866 + 10μM NR
M1 + FK866 + 25μM NR
M1 + FK866 + 50μM NR
M1 + FK866 + 125μM NR
M1 + FK866 + 0.25mM NR

Sup Figure 4


A
% CD38Hi
0.68
0.51
0.70
0.15
40.0
0.41
WT
CD38 KO
M0
M2
M1
Count
CD38 (FITC)
B
WT
Cd38 KO
M2
M1
hrs 0 4 8 16 4 8 16 0 4 8 16 4 8 16
Parp1
Sirt1
Tubulin
C
NADase Assay
(Intact Macrophage)
WT
Cd38 KO
***
NADase activity /cell number
(% Relative to WT MO)
M0
M2
M1
D
Cd157
mRNA Transcript Levels
(Relative to WT MO)
M1
Untreated
8hrs
16hrs
E
NR
ns
***
***
Normalized Peak Area
M1
Untreated
16hrs

Sup Figure 5


A
Il-1α
Cxcl1
Il-10
mRNA Transcript Levels (Relative to 6 Mo)
*
*
***
age (months)
age (months)
age (months)
B
Il-1α
Cxcl1
Il-10
mRNA Transcript Levels (Relative to PBS)
***
***
***
PBS
Doxo
PBS
Doxo
PBS
Doxo
C
Resident Macrophage
CD38 Low
Non-Resident Macrophage
CD38 Low
%of Total SVF
ns
%of Total SVF
***
PBS
Doxo
PBS
Doxo
D
Cd38
mRNA Transcript Levels (Relative to MF)
***
ns
Mouse Dermal Fibroblast (MDF)
IRR MDF
WT M0 + MDF
WT M0 + IRR MDF
CD38KO M0 + MDF
CD38KO M0 + IRR MDF