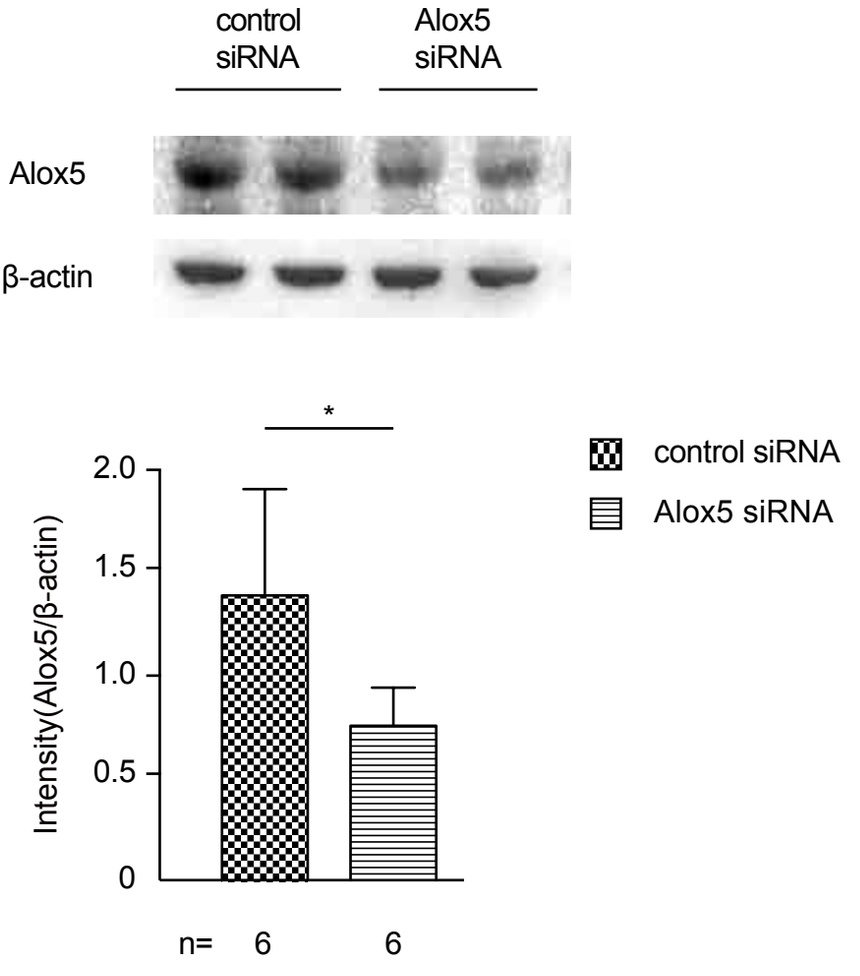
Supplemental Digital Content 1

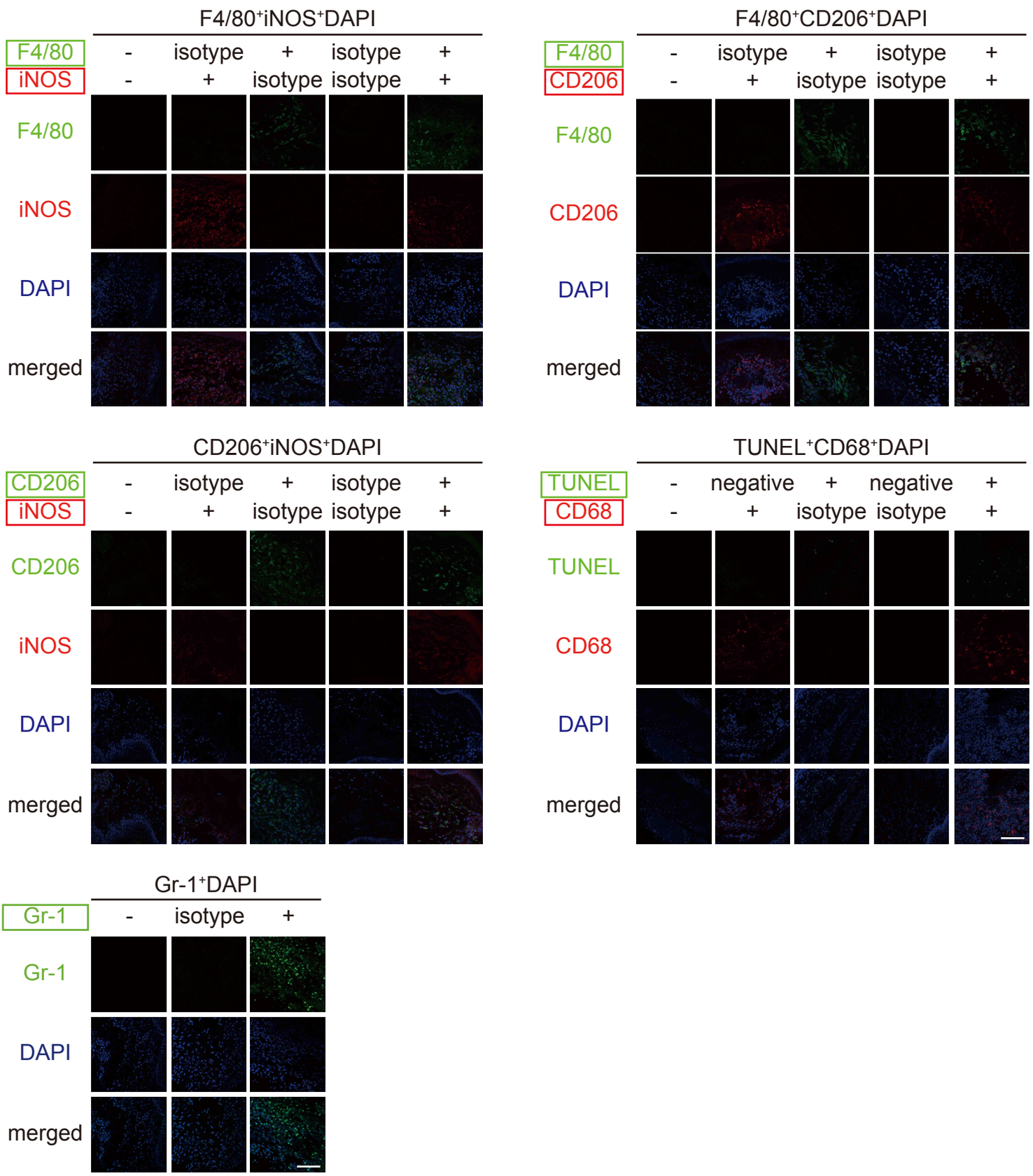
Supplementary methods

Phagocytosis assay

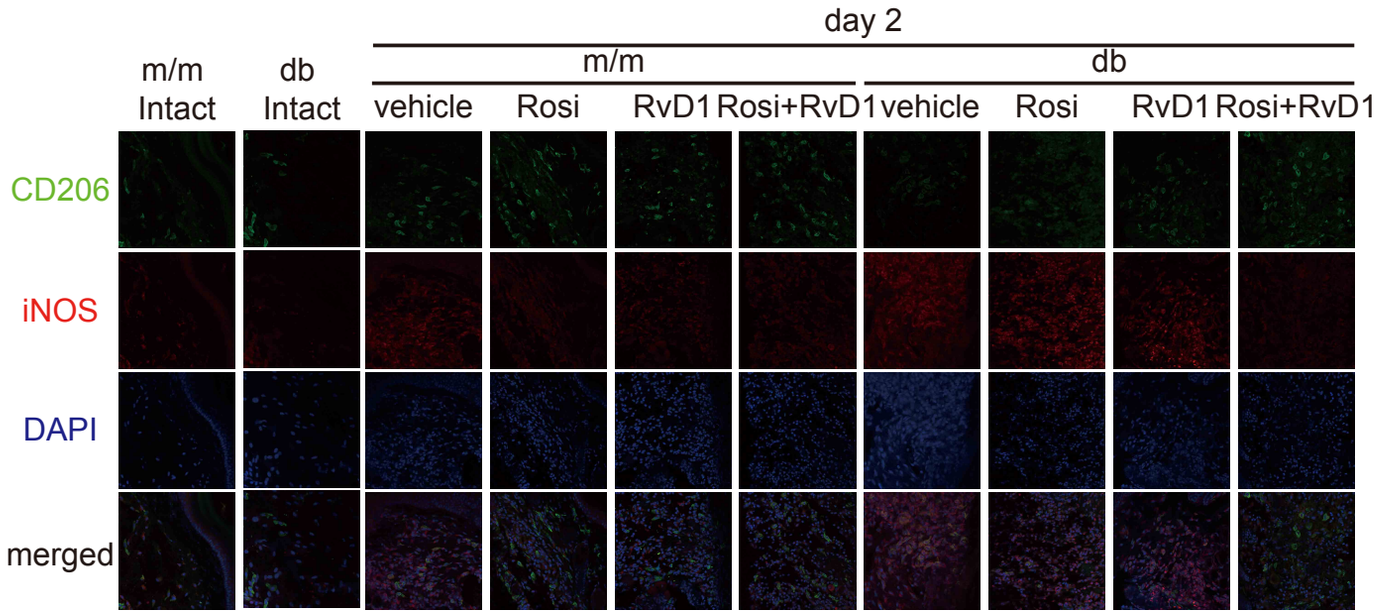
Mice were intraperitoneally administered with 3 ml of 4% thioglycollate (Sigma-Aldrich, St Louis, MO, USA). After 3 days, peritoneal macrophages were collected by peritoneal lavage with 10 ml cold phosphate buffered saline (PBS). Cells were incubated overnight with Dulbecco's modified Eagle's medium at 37°C and 5% CO_2. Non-adherent cells were removed with PBS by repeated washing. Cells were plated at 1×10^5 cells/well in 96-well flat-bottom plate and incubated with 10 ng/mL lipopolysaccharide (LPS; Sigma) and 50 μM Rosi, 10nM RvD1, 50 mM DMSO, 64.3 μM ethanol or saline. Phagocytosis of zymosan was assessed by the CytoSelect 96-well phagocytosis assay according to manufacturer's instruction (Cell Biolabs, San Diego, CA, USA). Briefly, 10 μl of zymosan suspension was added to the medium, and then phagocytosis of zymosan was stopped by the removal of supernatant. Phagocytosis was evaluated by Infinite 200 and i-control™ – Microplate Reader Software by colorimetric assay at an absorbance of 405 nm (TECAN, Kawasaki, Japan). Each experiment was performed in triplicate.

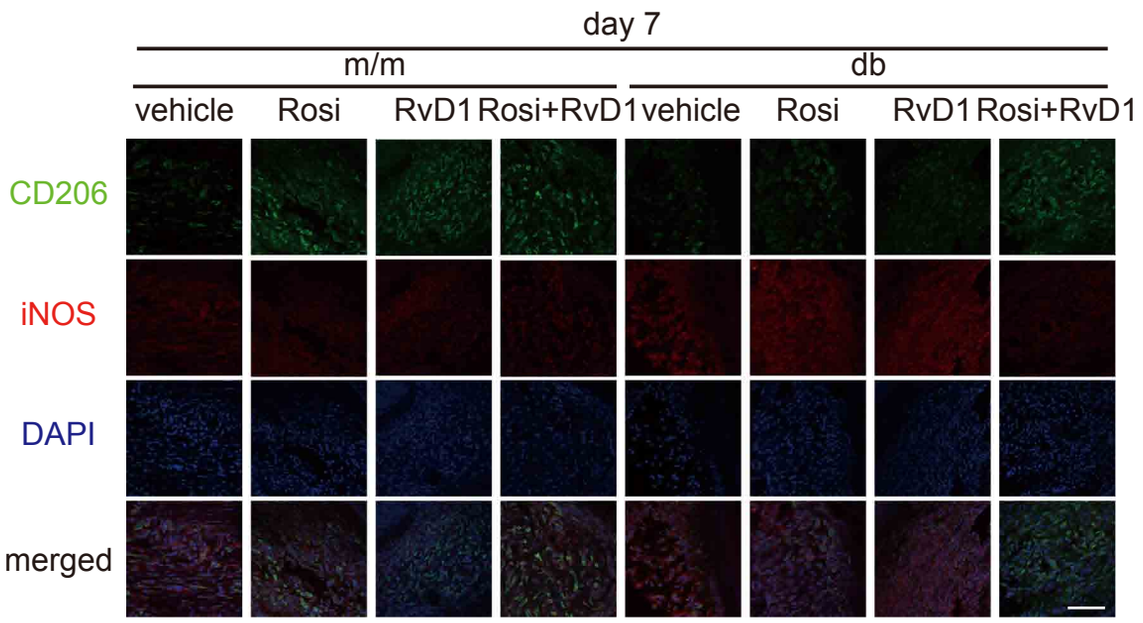


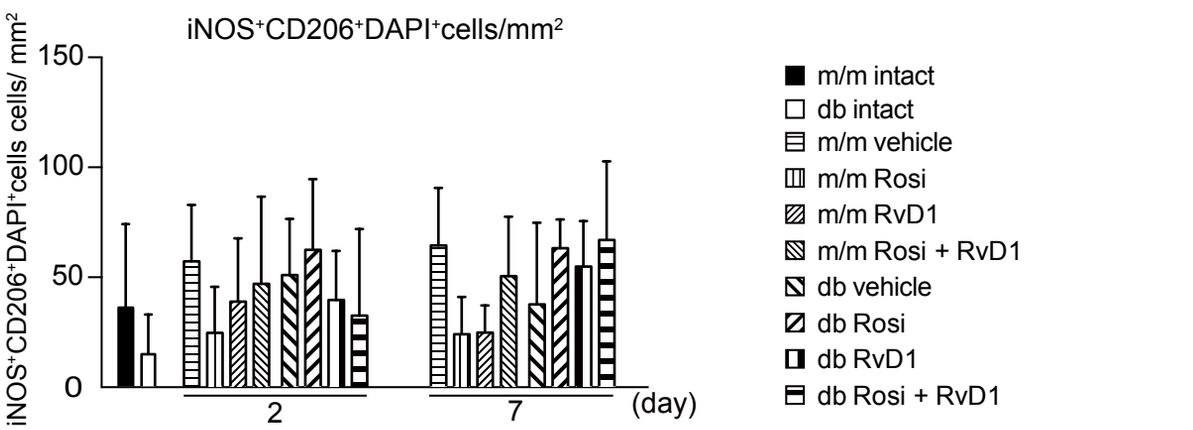
Supplementary fig. 1. Knockdown of Alox5 by siRNA reduced the expression of Alox5. Each column represents the mean ± SD. Unpaired Student's *t*-test between groups. *$P < 0.05$. Alox5; arachidonate 5-lipoxygenase.

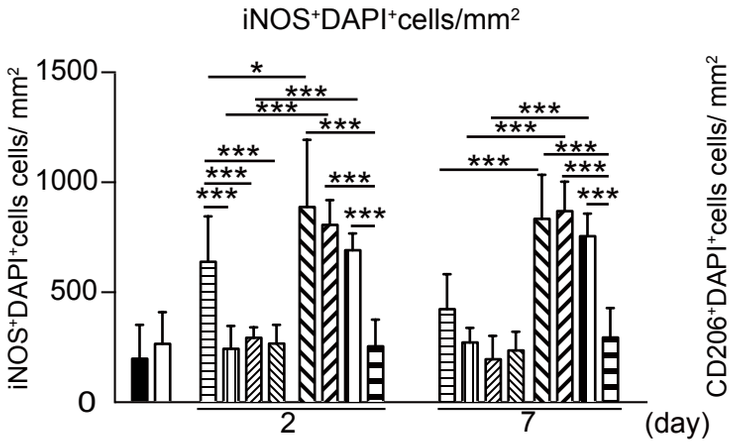


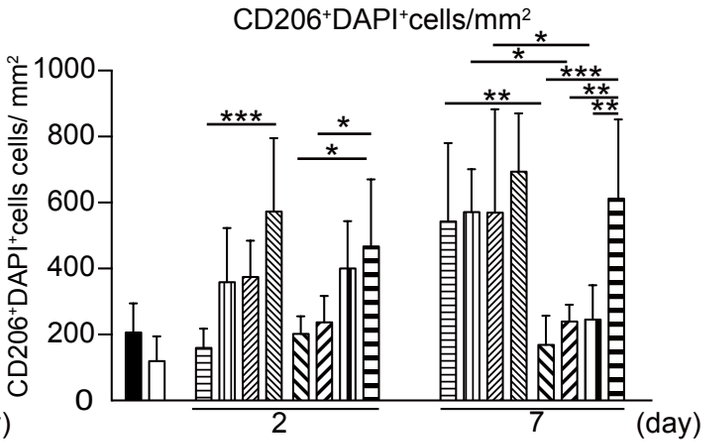
Supplementary fig. 2. Isotype control to primary antibodies for Gr-1, CD68, F4/80, iNOS, and CD206 were used to evaluate nonspecific background of immunohistochemistry. For negative control of TUNEL assay, control incubation buffer without rTdT enzyme was used. Scale bar, 100 μm.


day 2
m/m
db
m/m Intact
db Intact
vehicle
Rosi
RvD1
Rosi+RvD1
vehicle
Rosi
RvD1
Rosi+RvD1
CD206
iNOS
DAPI
merged

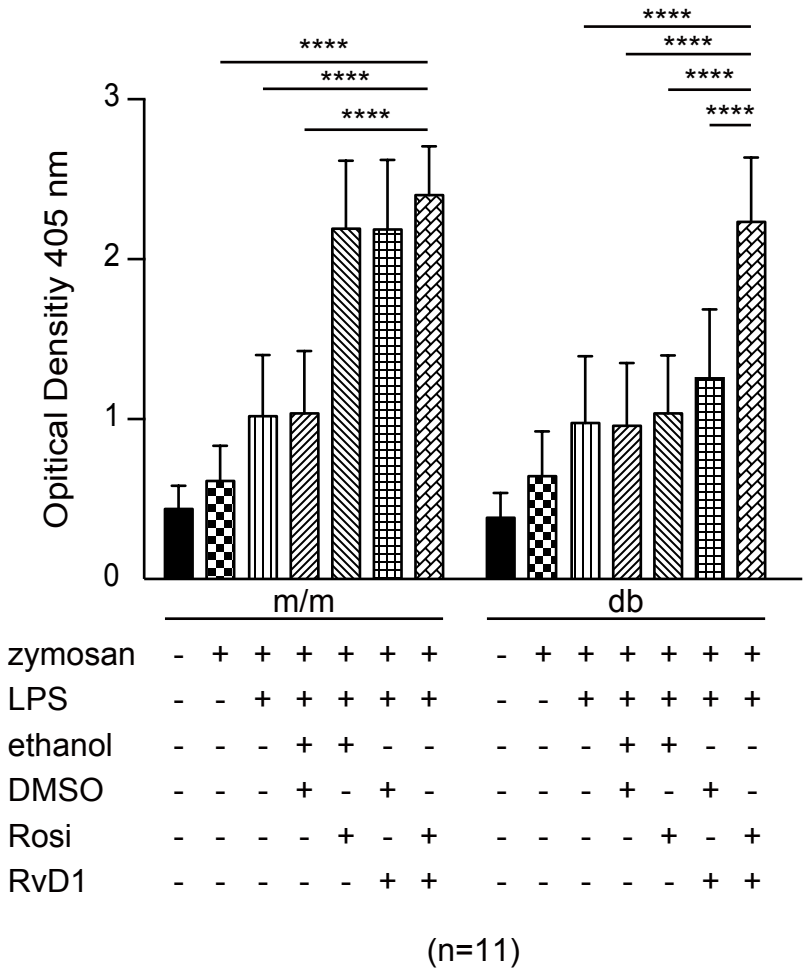

day 7
m/m
db
vehicle
Rosi
RvD1
Rosi+RvD1
vehicle
Rosi
RvD1
Rosi+RvD1
CD206
iNOS
DAPI
merged


iNOS+CD206+DAPI+cells/mm2
iNOS+CD206+DAPI+cells cells/ mm2
150
100
50
0
2
7
(day)
m/m intact
db intact
m/m vehicle
m/m Rosi
m/m RvD1
m/m Rosi + RvD1
db vehicle
db Rosi
db RvD1
db Rosi + RvD1

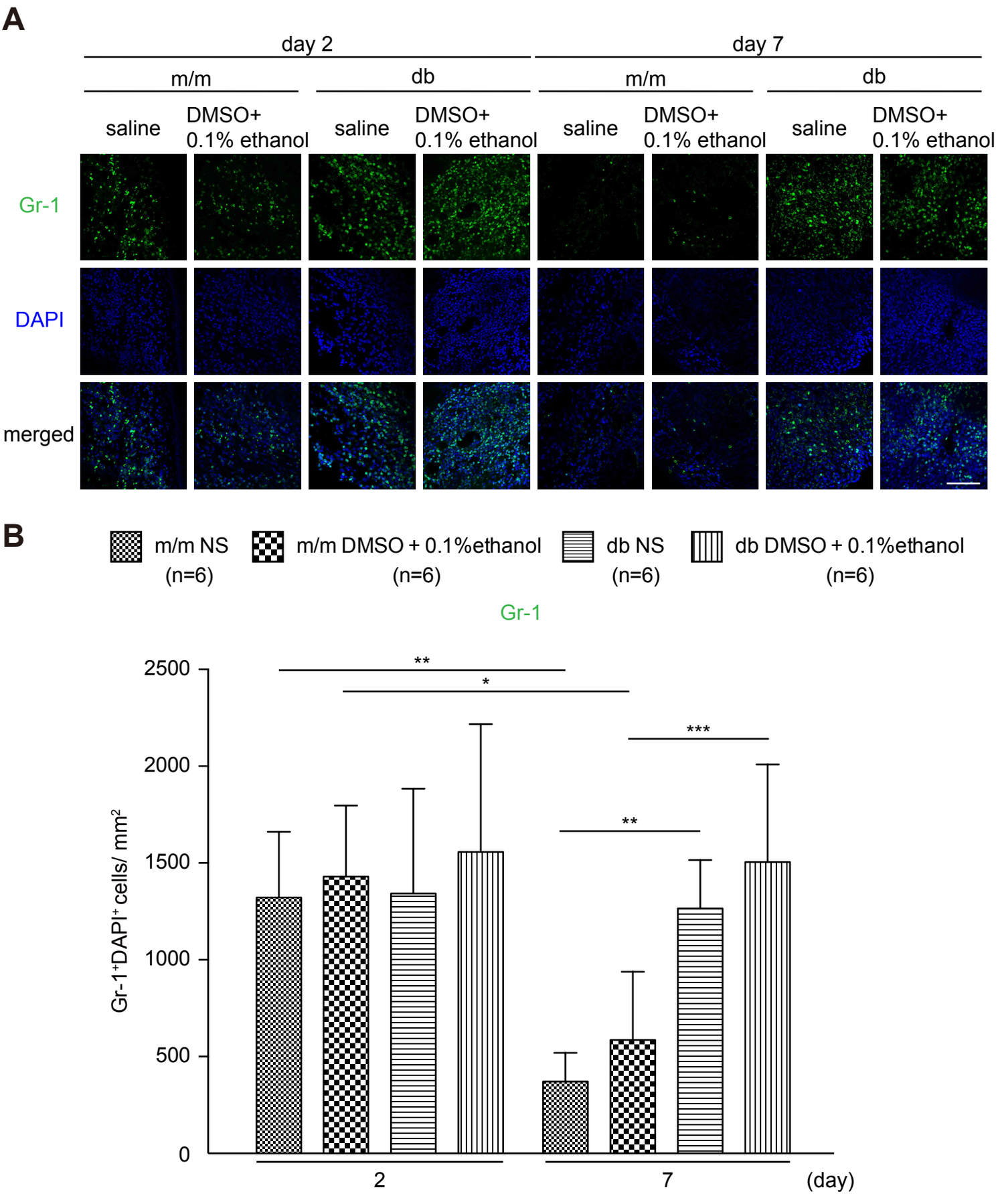

iNOS+DAPI+cells/mm2
iNOS+DAPI+cells cells/ mm2
1500
1000
500
0
2
7
(day)


CD206+DAPI+cells/mm2
CD206+DAPI+cells cells/ mm2
1000
800
600
400
200
0
2
7
(day)

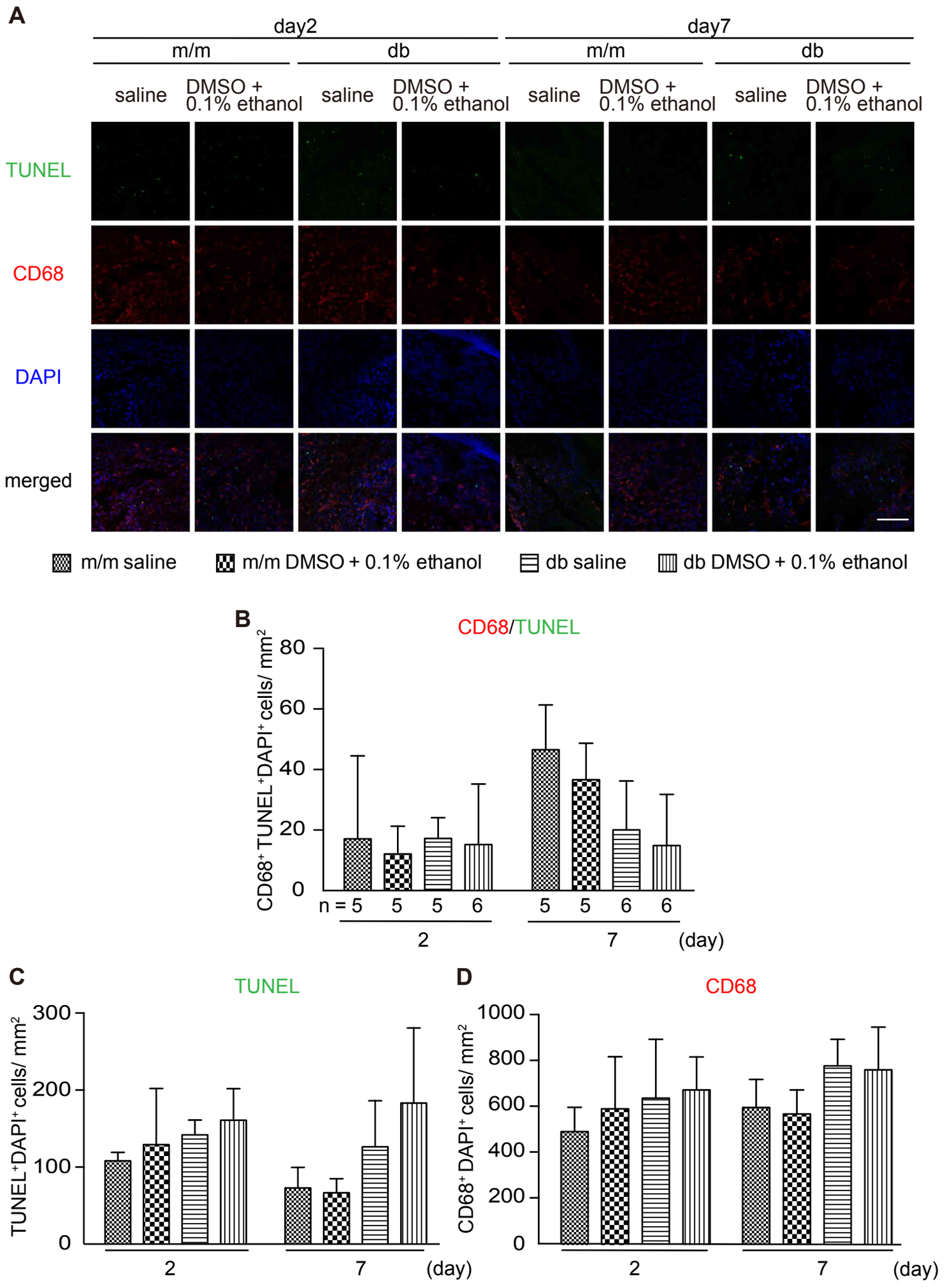
Supplementary fig. 3. Rosiglitazone coadministered with RvD1 significantly increased phagocytotic activity of db/db-derived macrophages. Two-way ANOVA (interaction factor: $F_{6,140} = 10.46$, $P < 0.0001$) followed by separate one-way ANOVA with Tukey post hoc test for comparison at each time (m/m : $F_{6,70} = 62.87$, $P < 0.0001$; db: $F_{6,70} = 29.17$, $P < 0.0001$). The results are presented as mean ± SD. **** $P < 0.0001$. LPS; lipopolysaccharide, DMSO; Dimethly sulfoxide, Rosi; Rosiglitazone. RvD1; Resolvin D1.



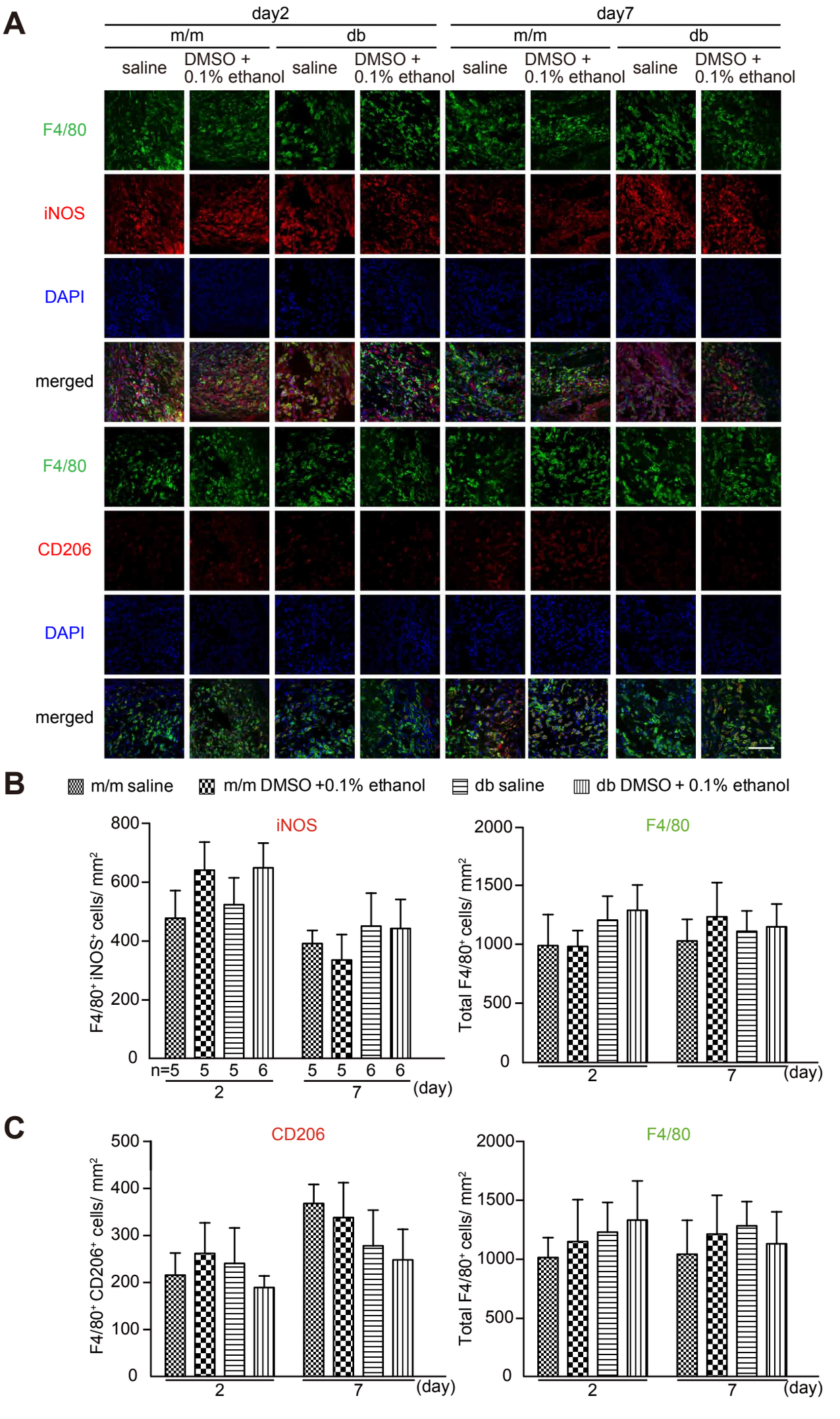
Supplementary fig. 4. The number of iNOS^{+}CD206^{+} macrophages did not differ significantly between the groups. Two-way ANOVA (iNOS^{+}CD206^{+}DAPI^{+}: interaction factor: $F_{7,107} = 1.210$, $P = 0.3037$; iNOS^{+}DAPI^{+}: interaction factor: $F_{7,107} = 1.689$, $P = 0.1192$; CD206+DAPI+: interaction factor: $F_{7,107} = 3.862$, $P = 0.0009$) followed by separate one-way ANOVA with Tukey post hoc test for comparing the mean number of CD206^{+}DAPI^{+} cells at each time (day 2 : $F_{7,55} = 7.632$, $P < 0.0001$; day 7: $F_{7,52} = 9.250$, $P < 0.0001$). The results are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Rosi; Rosiglitazone. RvD1; Resolvin D1. Green, CD206; red, iNOS, and blue; DAPI. Scale bar, 100 μm.



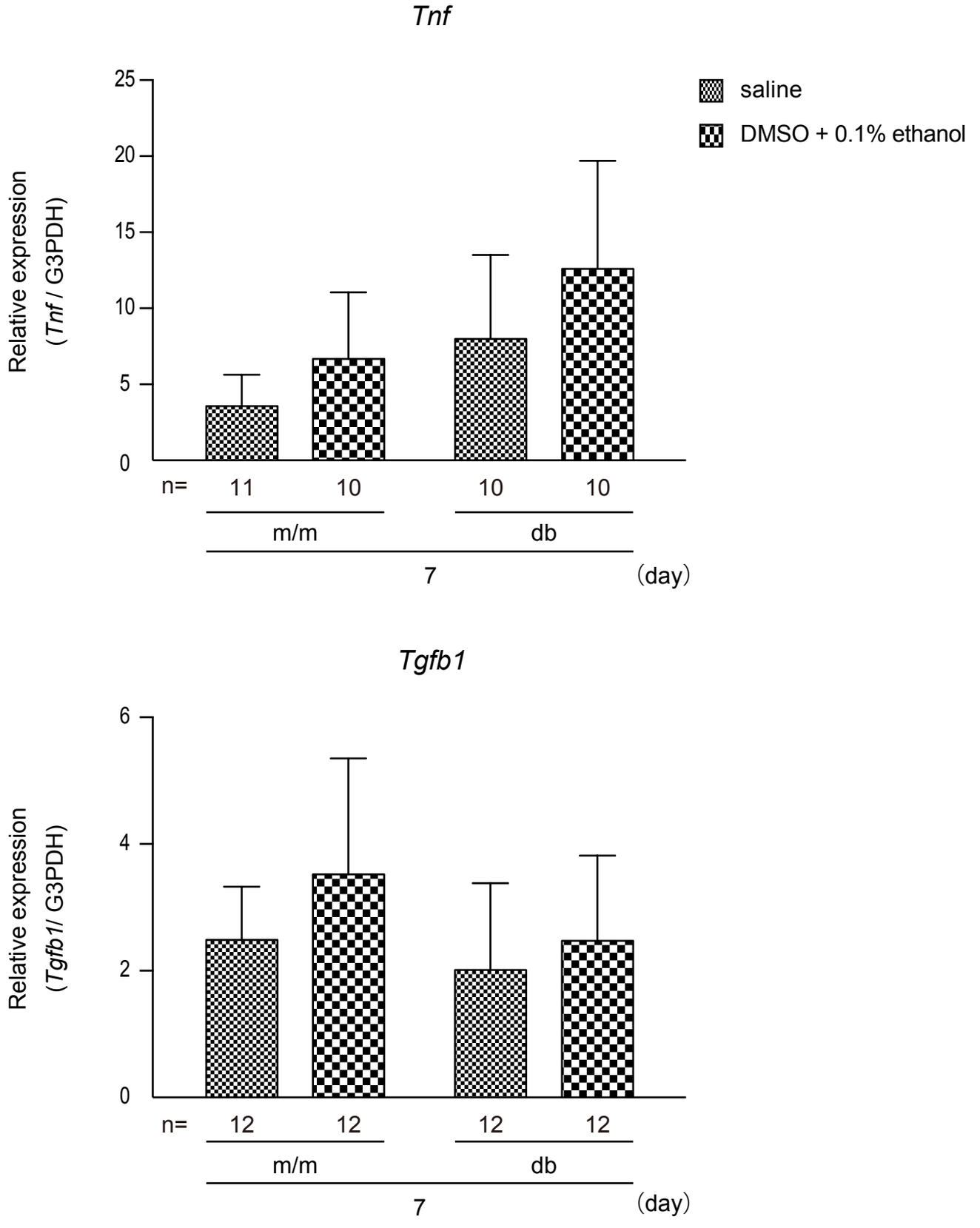
Supplementary fig. 5. Local infiltration of Gr-1⁺ PMNs was not changed by DMSO coadministered with 0.1% ethanol in both m/m and db/db mice. (A) Infiltration of Gr-1⁺ PMNs on days 2 and 7 after incision. (B) Total number of Gr-1⁺ PMNs in subcutaneous tissue was counted. Two-way ANOVA (interaction factor: $F_{3,40} = 3.871$, $P = 0.016$) followed by separate one-way ANOVA with Tukey post hoc test for comparison at each time (day 2 : $F_{3,20} = 0.2858$, $P = 0.8351$; day 7: $F_{3,20} = 15.02$, $P < 0.0001$). The results are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. DMSO; Dimethly sulfoxide. Scale bar, 100μm.


A
day2
day7
m/m
db
m/m
db
saline
DMSO + 0.1% ethanol
saline
DMSO + 0.1% ethanol
saline
DMSO + 0.1% ethanol
saline
DMSO + 0.1% ethanol
TUNEL
CD68
DAPI
merged
m/m saline
m/m DMSO + 0.1% ethanol
db saline
db DMSO + 0.1% ethanol
B
CD68/TUNEL
CD68+TUNEL+DAPI+ cells/ mm2
80
60
40
20
0
n = 5 5 5 6 5 5 6 6
2
7
(day)
C
TUNEL
TUNEL+DAPI+ cells/ mm2
300
200
100
0
2
7
(day)
D
CD68
CD68+DAPI+ cells/ mm2
1000
800
600
400
200
0
2
7
(day)

Supplementary fig. 6. Phagocytosis of apoptotic cells was not affected by injection of DMSO and 0.1% ethanol at the incision site. (A) $TUNEL^+CD68^+$, $TUNEL^+DAPI^+$, and $CD68^+DAPI^+$ cells at the incised sites. (B) The number of $CD68^+TUNEL^+DAPI^+$ per area at the incision sites. Two-way ANOVA (interaction factor: $F_{3,35} = 2.179$, $P = 0.1079$). (C) The number of $TUNEL^+DAPI^+$ cells per area at the incision sites. Two-way ANOVA (interaction factor: $F_{3,35} = 1.221$, $P = 0.3167$). (D) The number of $CD68^+DAPI^+$ cells per area at the incision sites. Two-way ANOVA (interaction factor: $F_{3,35} = 0.4546$, $P = 0.7157$). The results are presented as mean ± SD. DMSO; Dimethly sulfoxide. Green, TUNEL; red, CD68; blue, DAPI. Scale bar, 100μm.


A
day2
day7
m/m
db
saline
DMSO + 0.1% ethanol
F4/80
iNOS
DAPI
merged
CD206
B
m/m saline
m/m DMSO +0.1% ethanol
db saline
db DMSO + 0.1% ethanol
F4/80+ iNOS+ cells/ mm2
Total F4/80+ cells/ mm2
n=5 5 5 6 5 5 6 6
(day)
C
F4/80+ CD206+ cells/ mm2

Supplementary fig. 7. Phenotype shift of macrophages from M1 to M2 type was not affected by injections of DMSO and 0.1% ethanol at the incision site. (A) Infiltration of $F4/80^{+}iNOS^{+}$ M1 macrophages and $F4/80^{+}CD206^{+}$ M2 macrophages was evaluated on days 2 and 7. (B) The number of $F4/80^{+}iNOS^{+}$ and total F4/80 macrophages per area. Two-way ANOVA ($F4/80^{+}iNOS^{+}$: interaction factor: $F_{3,35} = 3.744$, $P = 0.0197$; total $F4/80^{+}$: interaction factor: $F_{3,35} = 1.852$, $P= 0.1557$) followed by separate one-way ANOVA with Tukey post hoc test for comparison of the number of $F4/80^{+}iNOS^{+}$ macrophages at each time (day 2 : $F_{3,17} = 4.655$, $P = 0.0149$; day 7: $F_{3,18} = 1.848$, $P= 0.1746$). (C) The number of $F4/80^{+}CD206^{+}$ and total $F4/80^{+}$ macrophages per area. Two-way ANOVA ($F4/80^{+}CD206^{+}$: interaction factor: $F_{3,35} = 1.830$, $P = 0.1598$; total $F4/80^{+}$: interaction factor: $F_{3,35} = 0.5955$, $P = 0.6222$). The results are presented as mean ± SD. DMSO; Dimethly sulfoxide. Green, F4/80; red, iNOS or CD206; blue, DAPI. Scale bar, 100 μm.



Supplementary fig. 8. Injections of DMSO and 0.1% ethanol did not change gene expression of TNF-α (*Tnf*) or TGF-β1 (*Tgfb1*) at the incision site on day 7. Gene expression was quantified by real-time PCR. Two-way ANOVA (tnf: interaction factor: $F_{1,37} = 0.2238$, $P = 0.6389$; *Tgfb1*: interaction factor: $F_{1,44} = 0.5068$, $P = 0.4803$). Each column represents the mean ± SD. DMSO; Dimethly sulfoxide.