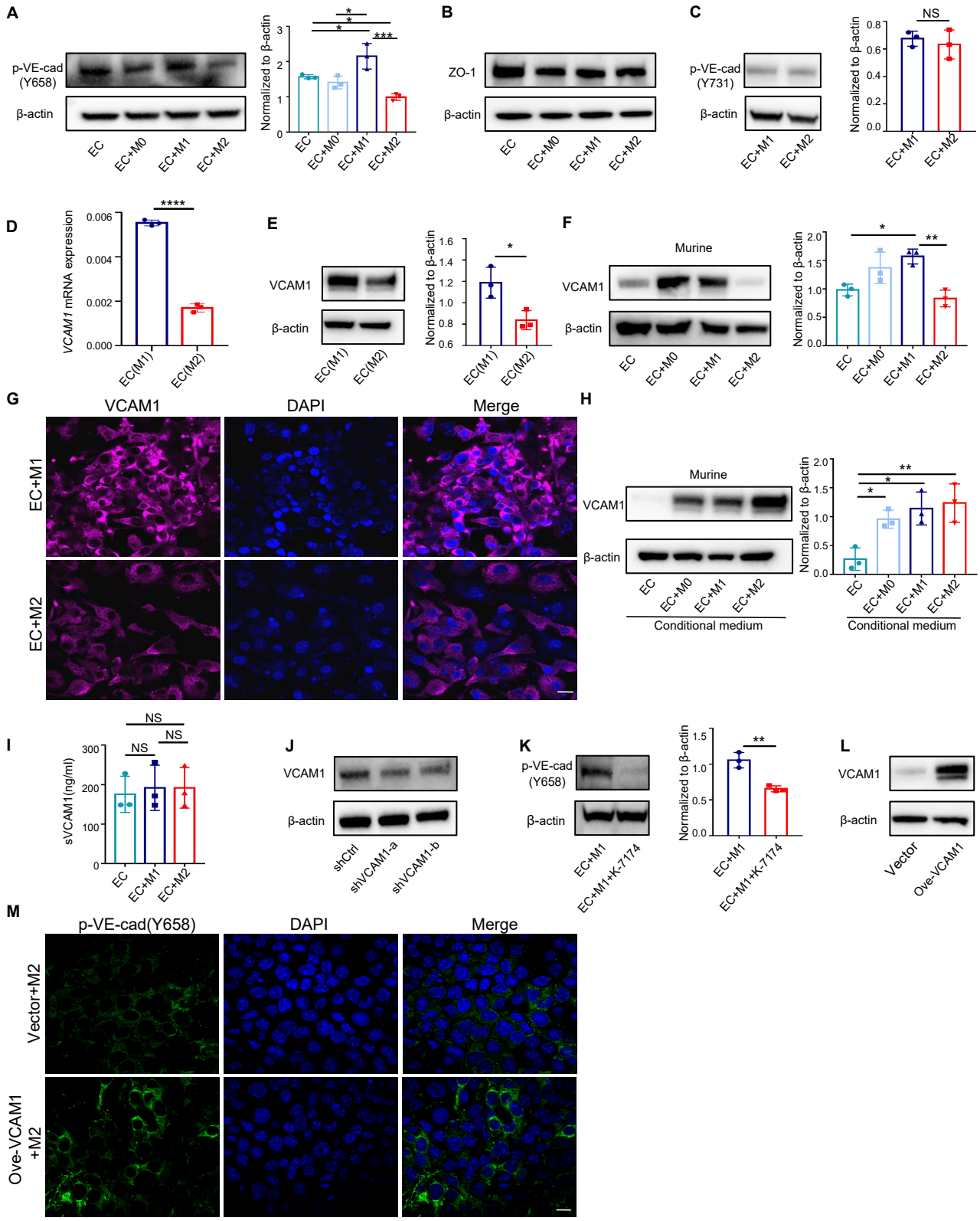
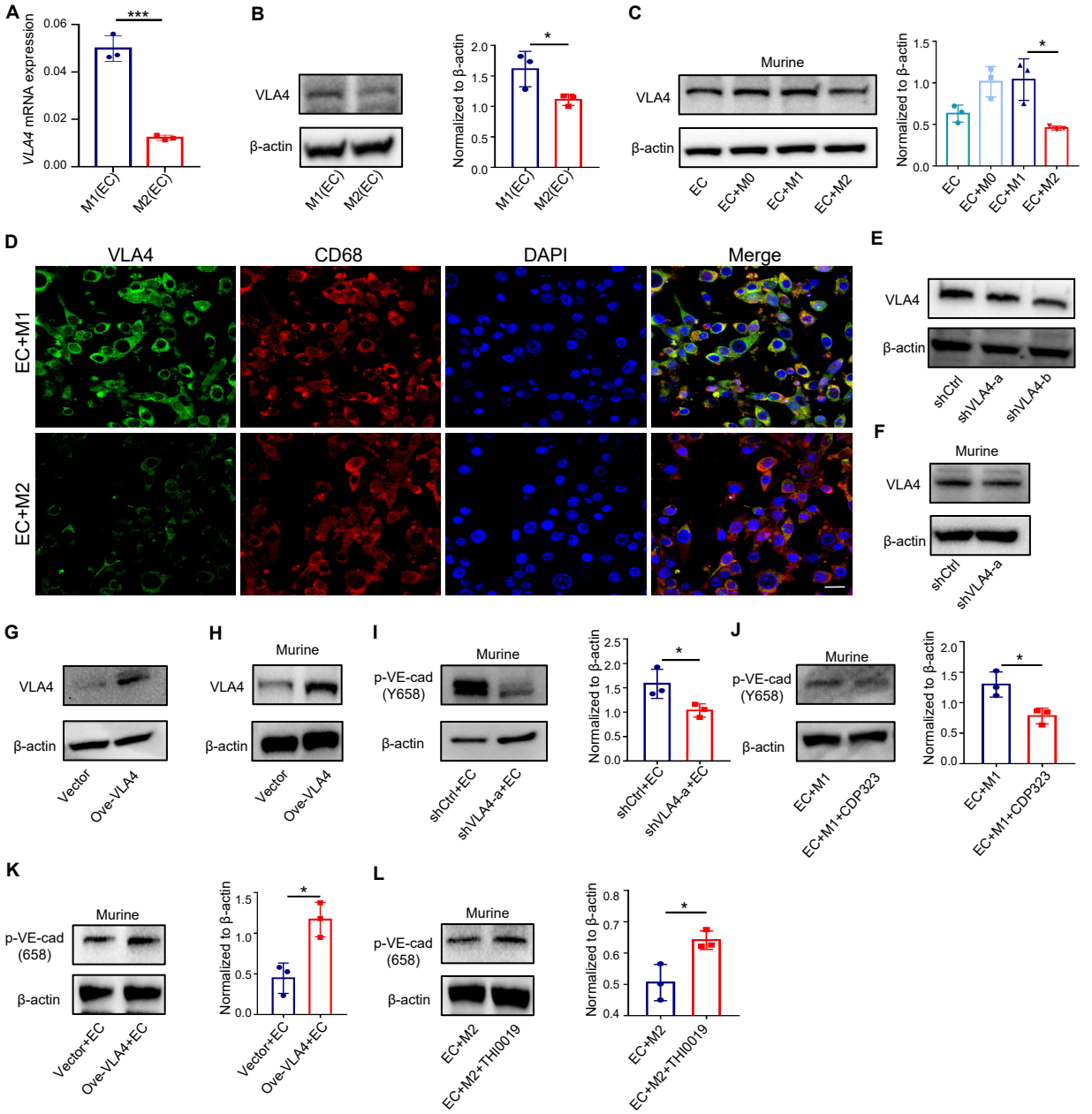
Supplemental Figure 1



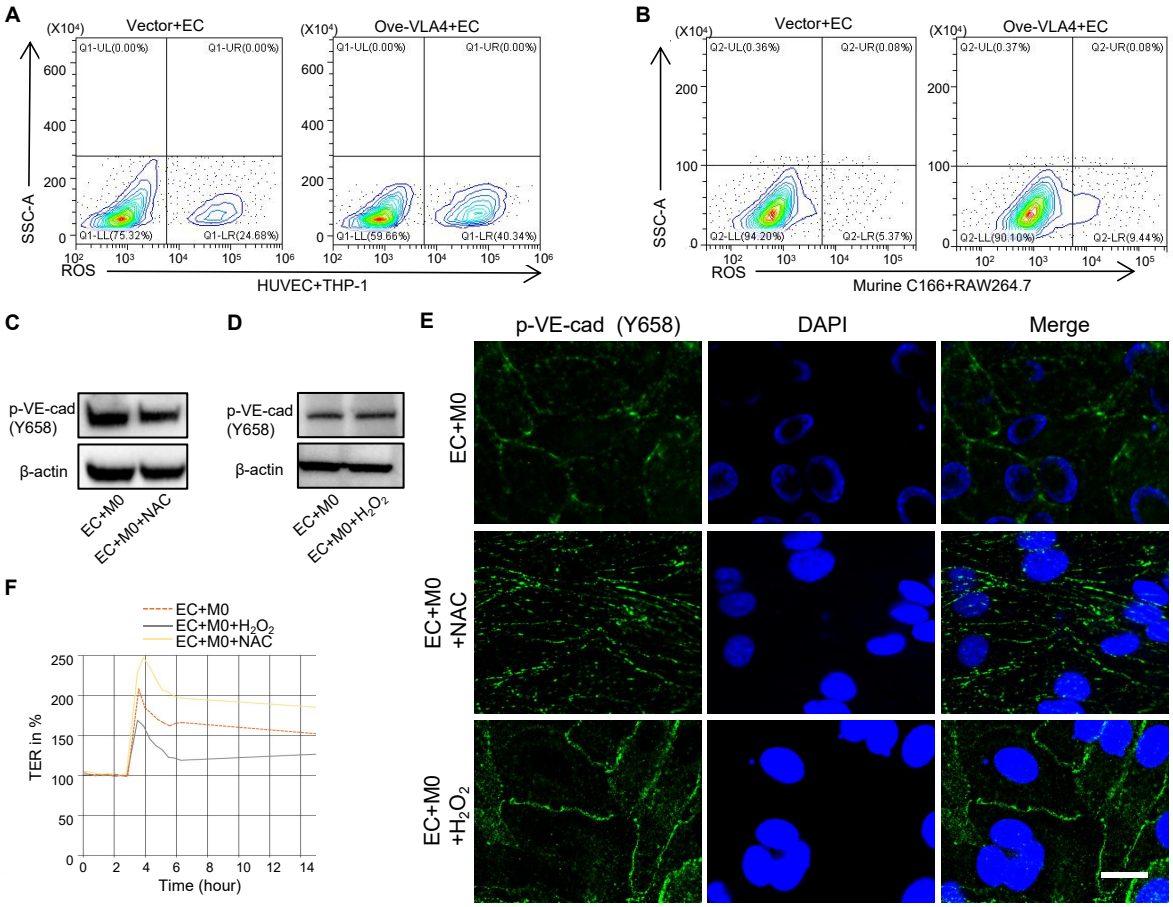
Supplemental Figure 1. Decreased VCAM1 expression in ECs cocultured with M2 macrophages. (A, B) The expression of the phosphorylation of VE-cadherin (p-VE-cad) at tyrosine 658 (A) and ZO-1 (B) in HUVECs cocultured with different subtypes of macrophages detected by western blot. A, n=3. (C) p-VE-cad at tyrosine 731 in HUVECs in different direct cocultures detected by western blot (n=3). (D, E) mRNA (D) and protein (E) levels of VCAM1 in HUVECs isolated from different direct cocultures (n=3). (F) The expression of VCAM1 in C166 ECs in different cocultures detected by western blot (n=3). (G) Localization of VCAM1 protein (purple) in C166 ECs from different cocultures by immunofluorescence analysis. DAPI stains cell nucleus. Scale bar: 20 μm. (H) Expression of VCAM1 in C166 ECs in different transwell cocultures detected by western blot (n=3). (I) Soluble VCAM1 protein expression in the supernatant from different cocultures (n=3). (J) VCAM1 protein expression in HUVECs that were transiently transfected with VCAM1-specific shRNAs (shVCAM1-a, shVCAM1-b) or a control shRNA (shCtrl). (K) p-VE-cad expression in M1 macrophage-cocultured HUVECs treated with K-7174 (n=3). (L) VCAM1 protein expression in HUVECs that were transiently transfected with a VCAM1-specific vector (Ove-VCAM1) or a control vector. (M) Immunofluorescence analysis of p-VE-cad in M2 macrophage-cocultured C166 ECs with VCAM1 overexpressed. Scale bar: 20 μm. Results represent three separate experiments. The results are shown as the mean ± SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ and NS, not significant, as assessed by one-way ANOVA (A, F, H, I) and Student's t-test (C-E, K).

Supplemental Figure 2



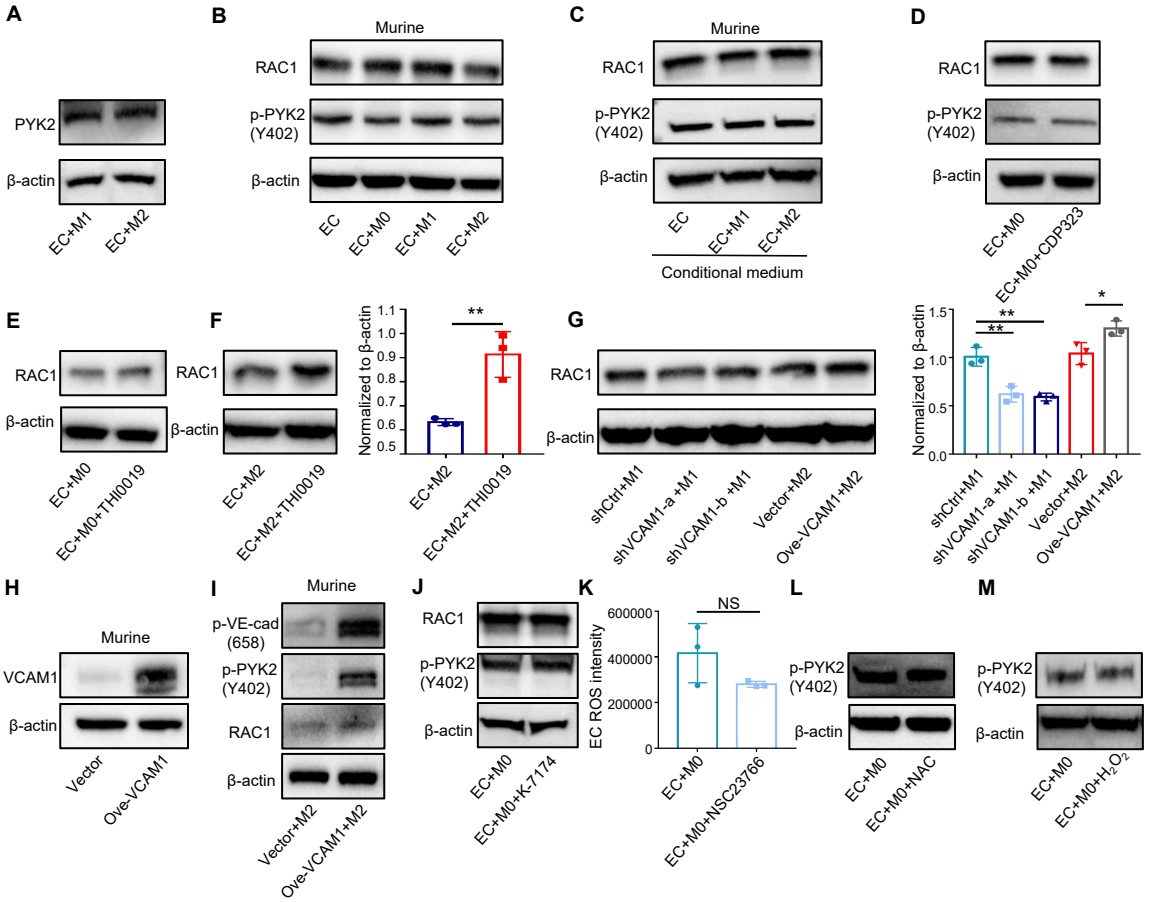
Supplemental Figure 2. VLA4 dictates macrophage-mediated vascular permeability. (A, B) mRNA (A) and protein (B) levels of VLA4 in macrophages isolated from different direct cocultures (n=3). (C) The expression of VLA4 in RAW264.7 detected by western blot in different cocultures (n=3). (D) Immunofluorescent analysis of VLA4 protein (green) in RAW264.7 macrophages (detected by CD68, red) from different cocultures. DAPI stains cell nucleus. Scale bar, 20 μm. (E) VLA4 protein expression in THP-1 macrophages that were transiently transfected with VLA4-specific shRNAs (shVLA4-a, shVLA4-b) or a control shRNA (shCtrl). (F) VLA4 protein expression in RAW264.7 that were transiently transfected with a VLA4-specific shRNA (shVLA4-a) or a control shRNA (shCtrl). (G, H) VLA4 protein expression in THP-1 (G) or RAW264.7 (H) that were transiently transfected with a VLA4-specific vector (Ove-VLA4) or a control vector. (I, J) p-VE-cad expression in C166 ECs cocultured with RAW264.7 that were transiently transfected with a VLA4-specific shRNA (shVLA4-a) or a control shRNA (shCtrl) (I), or with M1 macrophages treated with CDP323 (J) (n=3). (K, L) p-VE-cad expression in C166 ECs cocultured with macrophages that were transiently transfected with a VLA4-specific vector (Ove-VLA4) or a control vector (K), or with M2 macrophages treated with THI0019 (L) (n=3). Results represent three separate experiments. The results are shown as the mean ± SD, * $p< 0.05$, *** $p < 0.001$, as assessed by one-way ANOVA (C) and Student's t-test (A, B, I-L).

Supplemental Figure 3



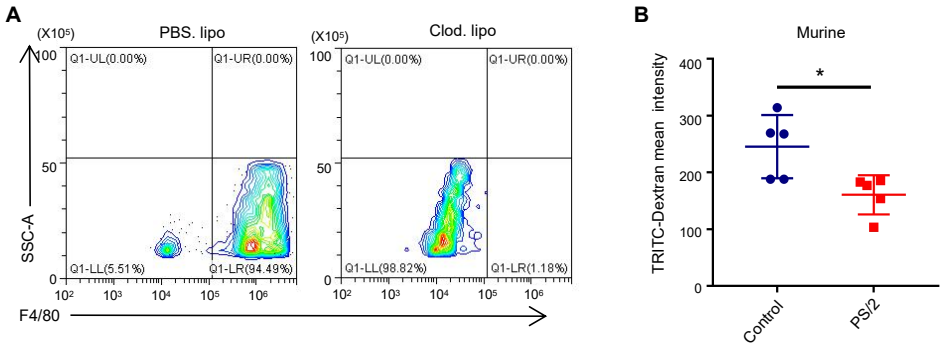
Supplemental Figure 3. ROS functions downstream of VLA4 to regulate macrophages-mediated permeability. (A, B) Flow cytometric analysis of ROS levels in HUVECs (A) or C166 ECs (B) cocultured with macrophages that were transiently transfected with a VLA4-specific vector (Ove-VLA4) or a control vector. (C, D) Immunoblot analysis of p-VE-cad in HUVECs from M0 macrophage-cocultured system treated with NAC (C) or H_2O_2 (D). (E) Immunofluorescence staining of p-VE-cad in HUVECs from M0 macrophage-cocultured system treated with NAC and H_2O_2. Scale bar, 20 μm. (F) Effects of NAC or H_2O_2 treatment on HUVEC barrier cocultured with M0 macrophages measured in real-time using the automated system (CellZscope®). Results represent three separate experiments.

Supplemental Figure 4



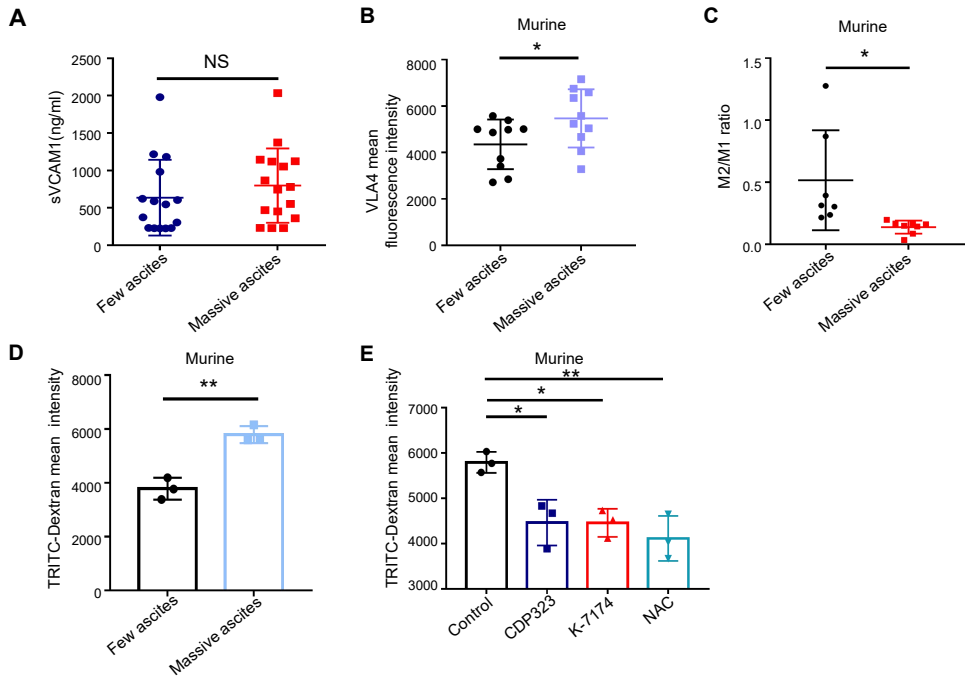
Supplemental Figure 4. RAC1 expression and phosphorylated PYK2 in different cocultures. (A) Protein levels of total PYK2 in HUVECs from different cocultures examined by western blot. (B) Protein levels of murine RAC1 and phosphorylated PYK2 (p-PYK2) in C166 ECs from different cocultures examined by western blot. (C) Protein levels of p-PYK2 and RAC1 in C166 ECs from different transwell cocultures examined by western blot. (D) RAC1 and p-PYK2 protein expression in HUVECs from M0 macrophage-cocultured system treated with CDP323. (E, F) RAC1 protein expression in HUVECs from M0 (E) or M2 (F) macrophage-cocultured system treated with THI0019. F, n=3. (G) RAC1 protein expression in M1 macrophages cocultured HUVECs that were transiently transfected with VCAM1-specific shRNAs (shVCAM1-a, shVCAM1-b) or a control shRNA (shCtrl) and in M2 macrophages cocultured HUVECs that were transiently transfected with a VCAM1-specific vector (Ove-VCAM1) or a control vector (n=3). (H) Expression of murine VCAM1 in C166 ECs that were transiently transfected with a VCAM1-specific vector (Ove-VCAM1) or a control vector. (I) Murine p-VE-cad, p-PYK2 and RAC1 protein expression in C166 ECs overexpressing VCAM1 cocultured with M2 macrophages. (J) RAC1 and p-PYK2 expression in HUVECs from M0 macrophage-cocultured system treated with K-7174. (K) Flow cytometric analysis of ROS levels in HUVECs from M0 macrophage-cocultured system treated with RAC1 inhibitor, NSC23766 (n=3). (L, M) p-PYK2 expression in HUVECs from M0 macrophage-cocultured system treated with NAC (L) or from M2 macrophage-cocultured system treated with H_2O_2 (M). Results represent three separate experiments. The results are shown as the mean ± SD, * $p< 0.05$, ** $p < 0.01$, and NS, not significant, as assessed by one-way ANOVA (G) and Student's t-test (F, K).

Supplemental Figure 5



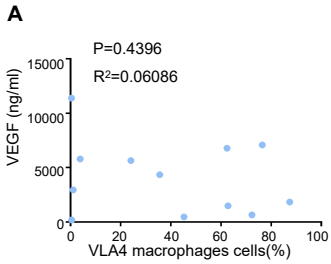
Supplemental Figure 5. Validation of macrophage depletion and peritoneal permeability treated with PS/2. (A) Representative graphs demonstrating the efficiency of macrophages depletion assessed by flow cytometry. Results represent three separate experiments. (B) Peritoneal permeability 48 hours after injection of PS/2-treated or control antibody IgG2b-treated macrophages in mice peritoneum (n=5). The results are shown as the mean ± SD, * $p < 0.05$, as assessed by Student's t-test (B).

Supplemental Figure 6



Supplemental Figure 6. VLA4 expression is correlated with ascites volume. (A) Soluble VCAM1 expression in different ovarian cancer ascites (n=15 for Few ascites, n=16 for Massive ascites). (B, C) VLA4 expression (B) and M2/M1 ratio (C) in murine peritoneal macrophages isolated from ascites. B, n=10. C, n=7 for Few ascites (less than 2 ml), n=8 for Massive ascites (more than 2 ml). (D) TRITC-Dextran permeability in C166 ECs cocultured with macrophages isolated from different ovarian cancer ascites (n=3). (E) TRITC-Dextran permeability in C166 ECs cocultured with macrophages isolated from ovarian cancer ascites treated with CDP323, K-7174, and NAC (n=3). The results are shown as the mean ± SD, *p< 0.05, ** $p < 0.01$, and NS, not significant, as assessed by one-way ANOVA (E) and Student's t-test (A-D).

Supplemental Figure 7



Supplemental Figure 7. VLA4 expression has no correlation with VEGF expression in ascites. (A) Correlation between VEGF and VLA4 expression in macrophages of OC patients' ascites (n=12). R^2 value and P value were calculated based on the analysis of Pearson's correlation.

Supplemental Table 1

Supplemental Table 1. Clinical characteristics of the 32 OC patients

Patient characteristics	Cohort 1	Cohort 2
No. of patients	20	12
Age, years (median, range)	53, 42-68	51, 45-63
Tumor size, cm (≤ 5/> 5)	14/6	10/2
TNM stage (I+II/III+IV)	9/11	5/7
metastasis (no/yes)	12/8	11/1
Ascites volume （Few/Moderate/Massive）	10/0/10	4/4/4

Note: Patients in ovarian cancer cohort 1 contributed fresh samples for FACS that were used in analyses of cell ratio, and correlation; patients in ovarian cancer cohort 2 contributed to the paraffin-embedded samples for IF that were used in analyses of VLA4 expression.

Supplemental Table 2

Supplemental Table 2. Antibodies used in experiments

Antibody	Catalog/Clone #	Supplier
	Immunoblotting	
Rabbit anti-Integrin alpha 4/CD49D antibody	EPR1355Y	abcam
Rabbit anti-VCAM1 antibody	EPR5047	abcam
Anti-phospho-pyk2 Polyclonal Antibody	bs-3400R	Bioss

Rabbit Anti-PYK2 antibody	YE353	abcam
Phospho-VE-cadherin (Tyr731) Polyclonal Antibody	44-1145G	Invitrogen
Phospho-VE-cadherin (Tyr658) Polyclonal Antibody	44-1144G	Invitrogen
Anti-ZO1 tight junction antibody	EPR19945-296	abcam
Purified Mouse Anti-Rac1 antibody	102/Rac1	BD Transduction Laboratories
Mouse experiments		
InVivoMAb anti-mouse/human VLA4 (CD49d)	PS/2	Bio X Cell
InVivoMAb rat IgG2b isotype control	LTF-2	Bio X Cell
Flow cytometry		
PE/Cy7 anti-mouse CD45	HI30	biolegend
APC/Cyanine7 anti-mouse F4/80 Antibody	BM8	biolegend
APC anti-mouse CD86 Antibody	GL-1	biolegend
PE anti-mouse CD206 (MMR) Antibody	C068C2	biolegend
Brilliant Violet 421™ anti-human CD206 (MMR)	15-2	biolegend

ITGA4 monoclonal antibody (44H6),FITC	44H6	Invitrogen
PerCP/Cy5.5 anti-mouse/human CD11b	M1/70	biolegend
PerCP/Cy5.5 anti-human CD11b	ICRF44	biolegend
PE/Cyanine7 anti-human CD45 Antibody	2D1	biolegend
Brilliant Violet 605 anti-human CD49d Antibody	9F10	biolegend
APC anti-human CD14 Antibody	63D3	biolegend
PE Mouse Anti-Human CD29	MAR4	BD Transduction Laboratories
PerCP/Cyanine5.5 anti-human CD86 Antibody	BU63	biolegend