Supplementary Material

Targeting FGFR1 with aloperine suppresses angiogenesis, vasculogenic mimicry, and metastasis in triple-negative breast cancer

Tianci Tang[1#], Luisa Müller[1#], Yixuan Xu[1], Matthias W. Laschke[1], Yuan Gu[1]*

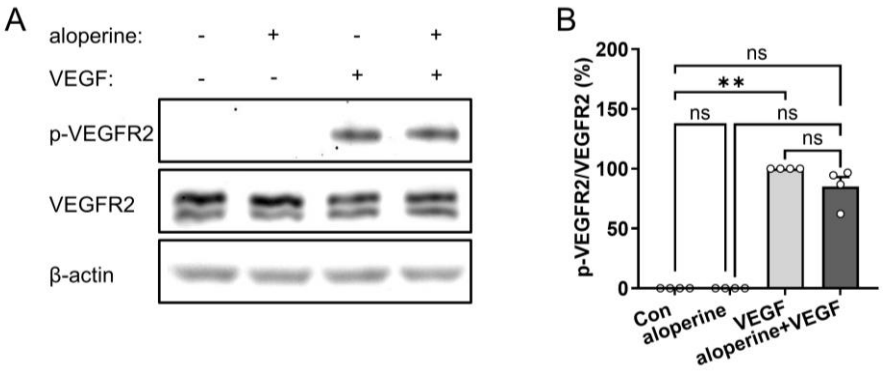
[1]Institute for Clinical and Experimental Surgery, Saarland University, PhramaScienceHub (PSH), 66421 Homburg, Germany

[#] These authors contributed equally.

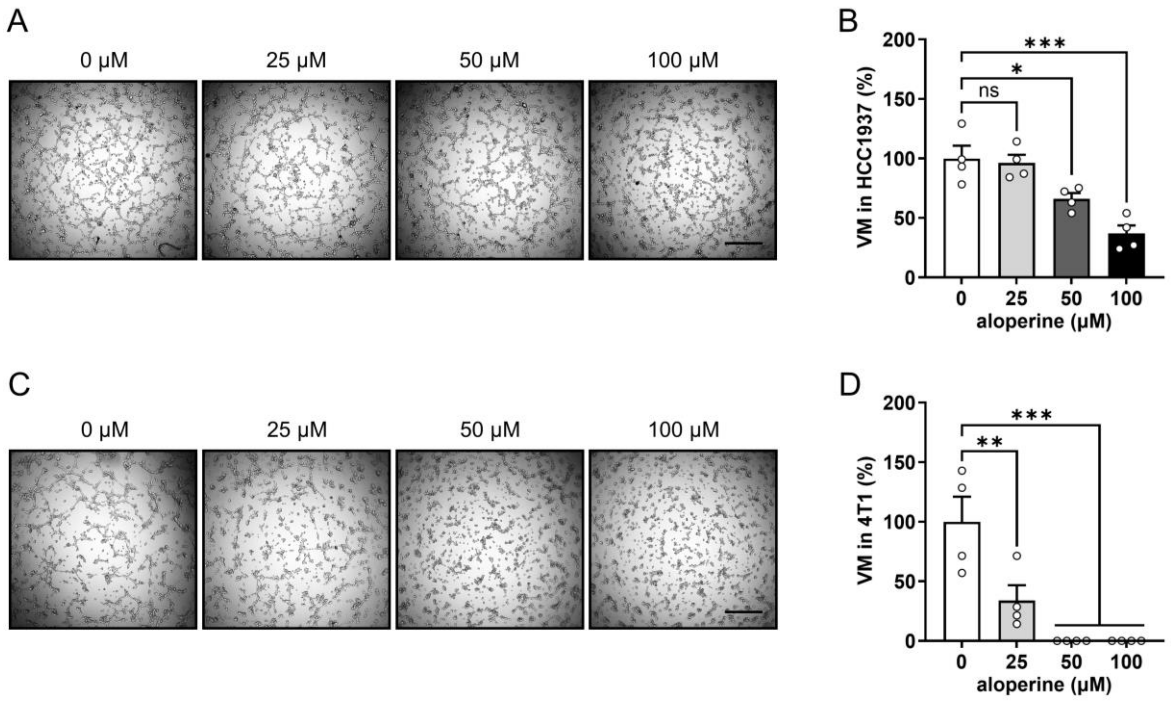
***Corresponding author:**

Yuan Gu, Ph.D.
Institute for Clinical and Experimental Surgery
Saarland University
66421 Homburg
Germany

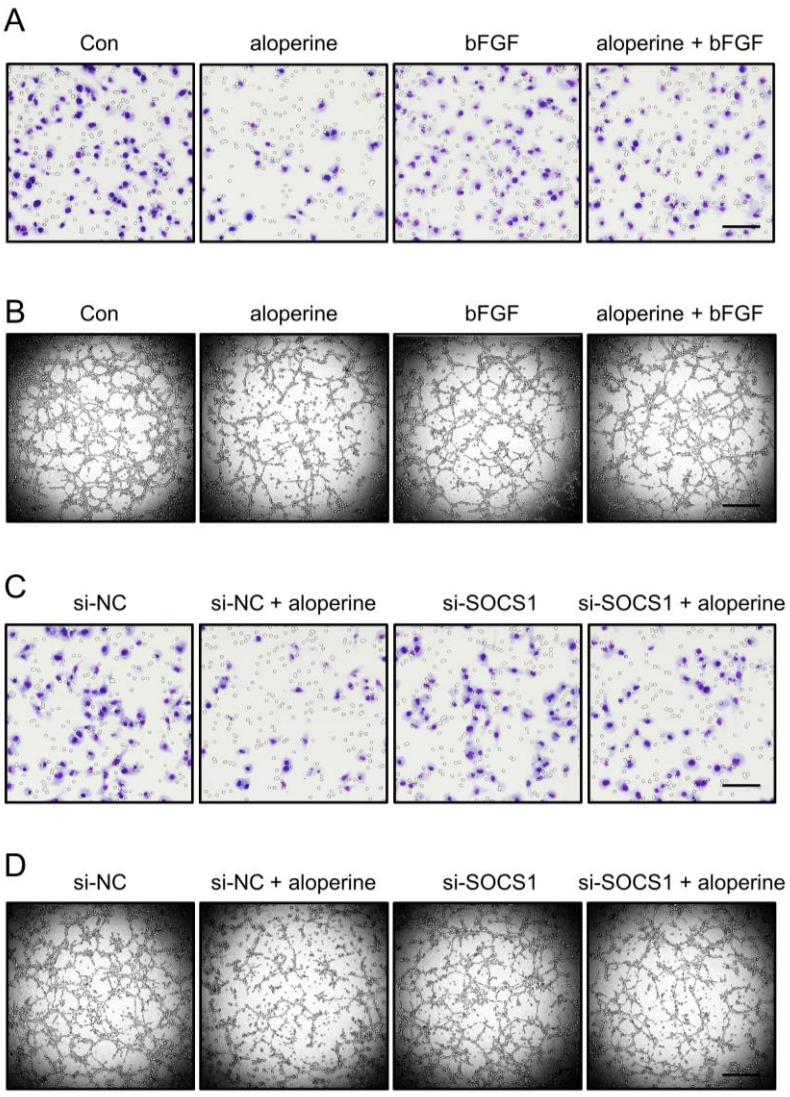
phone: +49 6841 162 6368
fax: +49 6841 162 6553
e-mail: yuan.gu@uks.eu



Supplementary Fig. 1 Effects of aloperine on the protein levels of p-VEGFR2 and VEGFR2 in HUVECs. **A:** Representative Western blots showing p-VEGFR2, VEGFR2, and β-actin expression in HUVECs that were treated with 0.1% DMSO (Con) or 100 μM aloperine for 4 h and then stimulated with or without 50 ng/mL VEGF for another 7 min. **B:** Expression levels (% of control) of p-VEGFR2/VEGFR2 in treated HUVECs depicted in (A), as assessed by Western blotting (n = 4 independent experiments). Data are presented as Means ± SEM. ***$P < 0.001$; ns, not significant.



Supplementary Fig. 2 Aloperine inhibits VM in HCC1937 and 4T1 cells. **A:** Representative images of tube-forming HCC1937 cells that were treated for 18 h with 0, 25, 50, and 100 μM aloperine. Scale bar: 500 μm. **B:** VM (% of 0 μM) in treated HCC1937 cells depicted in (A), as assessed by tube formation assay (n = 4). **C:** Representative images of tube-forming 4T1 cells that were treated for 18 h with 0, 25, 50, and 100 μM aloperine. Scale bar: 500 μm. **D:** VM (% of 0 μM) in treated 4T1 cells depicted in (C), as assessed by tube formation assay (n = 4). Data are presented as Means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.



Supplementary Fig. 3 Aloperine inhibits TNBC cell migration and VM formation by suppressing the FGFR1/JAK2/STAT3 signaling pathway. **A:** Representative images of migrated MDA-MB-231 cells. The cells were treated with 0.1% DMSO (vehicle) or 100 μM aloperine for 18 h, then seeded into Transwell inserts and incubated with DMSO or aloperine in the absence or presence of 150 ng/mL bFGF for an additional 5 h. Scale bar: 100 μm. **B:** Representative images of tube-forming MDA-MB-231 cells that were treated for 18 h with 0.1% DMSO (Con) or 50 μM aloperine in the absence or presence of 150 ng/mL bFGF. Scale bar: 500 μm. **C:** Representative images of migrated MDA-MB-231 cells. The cells were transfected with si-NC or si-SOCS1 for 30 h followed by treatment with 0.1% DMSO (vehicle) or 100 μM aloperine for 18 h. Cells were then seeded into Transwell inserts and incubated with DMSO or aloperine for an additional 5 h. Scale bar: 100 μm. **D:** Representative images of tube-forming MDA-MB-231 cells. The cells were transfected with si-NC or si-SOCS1 for 48 h, then seeded onto Matrigel-coated plates and treated with 0.1% DMSO (vehicle) or 50 μM aloperine for an additional 18 h. Scale bar: 500 μm.