Supplementary Data for
Desacetylvinblastine Monohydrazide Disrupts Tumor Vessels by Promoting VE-cadherin Internalization

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25 # Supplemental methods

26 **Annexin V/PI assay**

27 The Annexin V-FITC/PI (propidium iodide) assay kit (Biouniquer Tech, Nanjing,
28 Jiangsu, China) was used to detect the cell apoptosis rate. Briefly, HUVECs treated
29 with DAVLBH (8, 16 and 32 nM) for 12 h were harvested and washed with PBS.
30 After that, the cells were stained with Annexin V-FITC and PI following the
31 manufacturer's protocol. The cells were then analyzed with flow cytometry (Guava
32 Technologies, Millipore, Billerica, MA).

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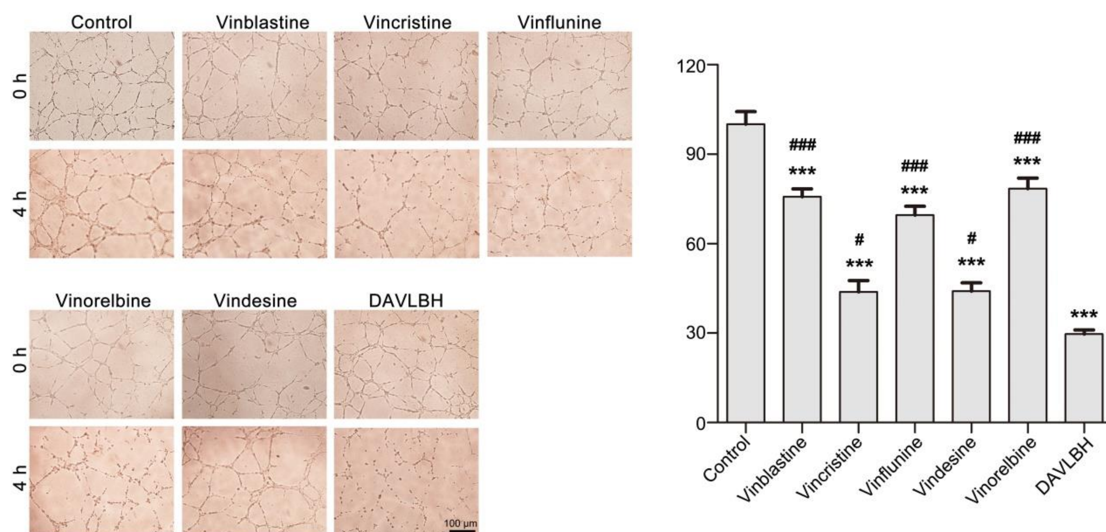
34 # Supplementary Figures



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36 **Figure S1. Capillary disruption assays with vinca alkaloids.** Newly formed
37 endothelial cell tubes were exposed to vinca alkaloids, including vinblastine,
38 vincristine, vinflunine, vinorelbine, vindesine and DAVLBH, at 16 nM for 4 h. The
39 images were taken with an inverted microscope before and after treatment. The data
40 are presented as mean ±SEM. Scale bar, 100 μm. Quantification of the results is
41 shown (n = 3). $^{***}P < 0.001$ compared with the control group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$
42 compared with the DAVLBH group (one-way ANOVA with Tukey's *post hoc*

43 comparison).

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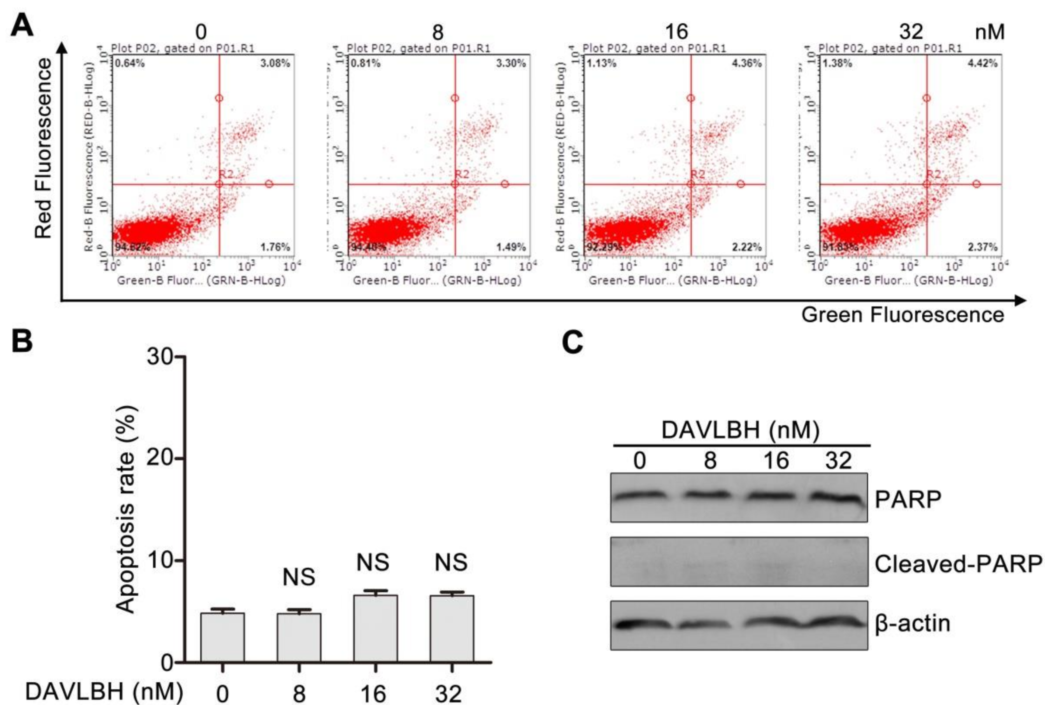

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46 **Figure S2. DAVLBH does not induce HUVEC apoptosis.** HUVECs were treated
47 with various concentration of DAVLBH (8, 16 and 32 nM) for 12 h, and the apoptotic
48 cells were detected with Annexin V/PI assay kit. The representative images and the
49 quantification of the apoptotic cells are shown in (A) and (B), respectively. The data
50 are presented as mean ±SEM (n=3). NS: no significantly difference compared
51 with control group (one-way ANOVA with Tukey's *post hoc* comparison). (C) The
52 results of western blot assay. HUVECs were treated with various concentration of
53 DAVLBH for 12 h, and the cells were harvested and lysed for western blot. The
54 β-actin was served as loading control.

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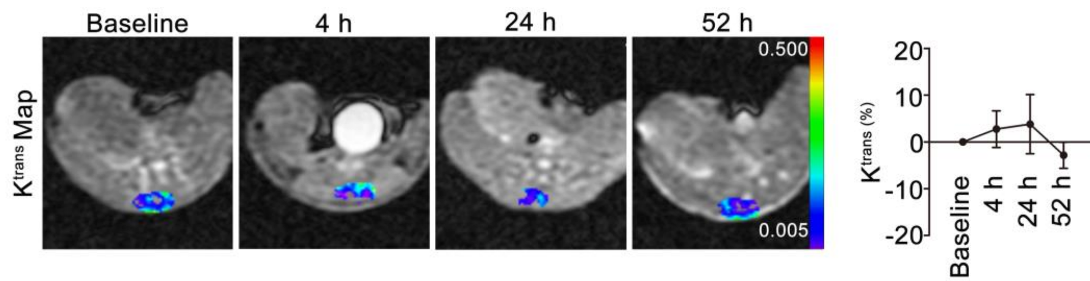

58 **Figure S3. DAVLBH has no significant effect on K^{trans} values in normal muscle**
59 **tissue.** Quantification of K^{trans} values in normal muscle is shown (right, n = 5). The
60 K^{trans} values were calculated from mice bearing HepG2 xenografts that received an i.v.
61 injection of DAVLBH (0.75 mg/kg) once every two days. The error bar represents the
62 SEM.

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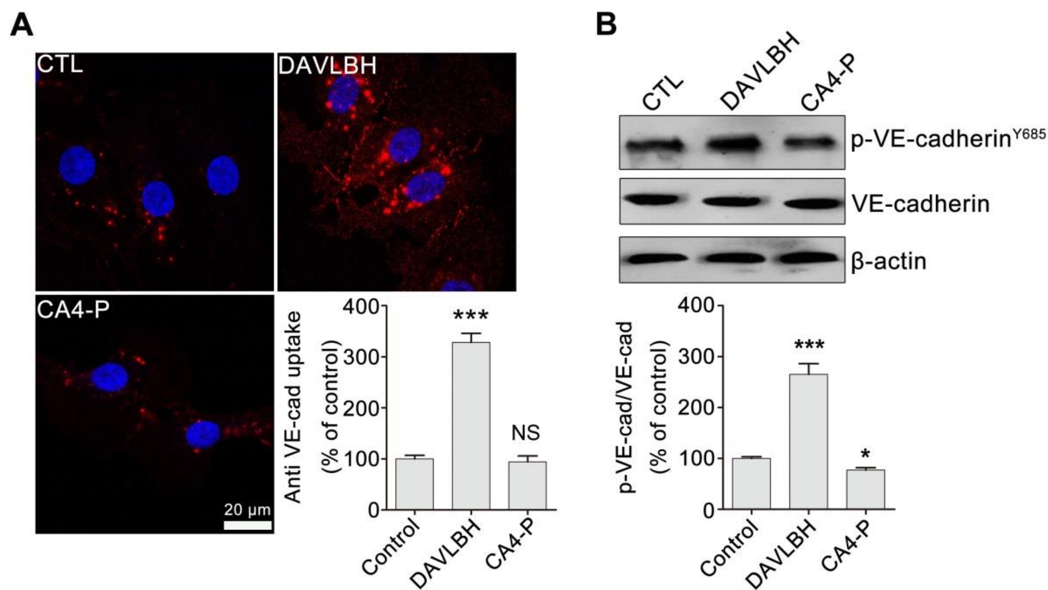

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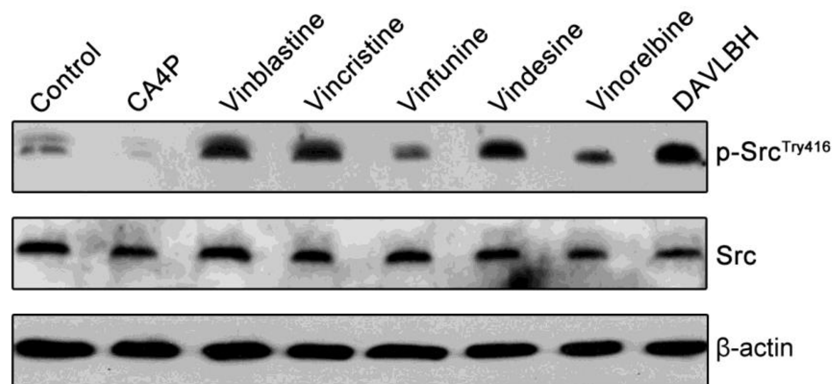
65 **Figure S4. CA4-P has no significant effect on VE-cadherin internalization. (A)**
66 CA4-P did not affect VE-cadherin internalization. HUVECs labeled with anti-BV9
67 were treated with DAVLBH (16 nM) and CA4-P (16 nM) for 4 h, washed with acid
68 PBS and labeled with an Alexa Fluor secondary antibody. Quantification of
69 internalized VE-cadherin is shown (n = 3). **(B)** CA4-P slightly suppressed the
70 phosphorylation of VE-cadherin. HUVECs were treated with DAVLBH (16 nM) or
71 CA4-P (16 nM) for 4 h, and the cells were then lysed using RIPA to determine total

72 VE-cadherin levels. The data are presented as the mean ± SEM. *$P < 0.05$, ***$P <$

73 0.001 compared with the control group. NS: no significant difference.



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75 **Figure S5. CA4-P and vinca alkaloids have different effects on Src activation.**

76 HUVECs were treated with or without CA4-P (16 nM) and vinca alkaloids (16 nM),

77 lysed in RIPA and analyzed by western blot.

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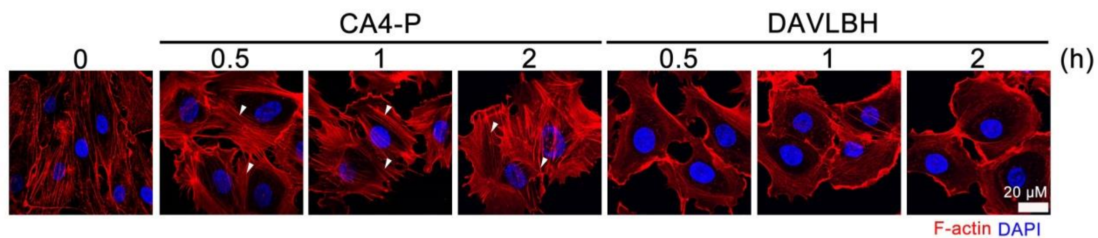

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80 **Figure S6. DAVLBH has no significant effect on the formation of actin stress**

81 **fiber.** HUVECs were treated with CA4-P (16 nM) or DAVLBH (16 nM) for various

82 time and then stained with F-actin (red) and DAPI (blue, nuclear staining). CA4-P

83 induced the formation of actin stress fiber (white arrow) in a time-dependent manner

84 whereas DAVLBH has no significant effect on the formation of actin stress fiber.

85 Scale bar, 20 μm.

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