# Supplementary Information

## Inhibition of breast cancer growth and metastasis by a biomimetic peptide

Esak Lee[a], Seung Jae Lee[a], Jacob E. Koskimaki[a], Zheyi Han[a], Niranjan B. Pandey[a], and Aleksander S. Popel[a,b]

[a]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States.

[b]Department of Oncology and the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD 21231, United States.

# Contents

## Supplementary Figures

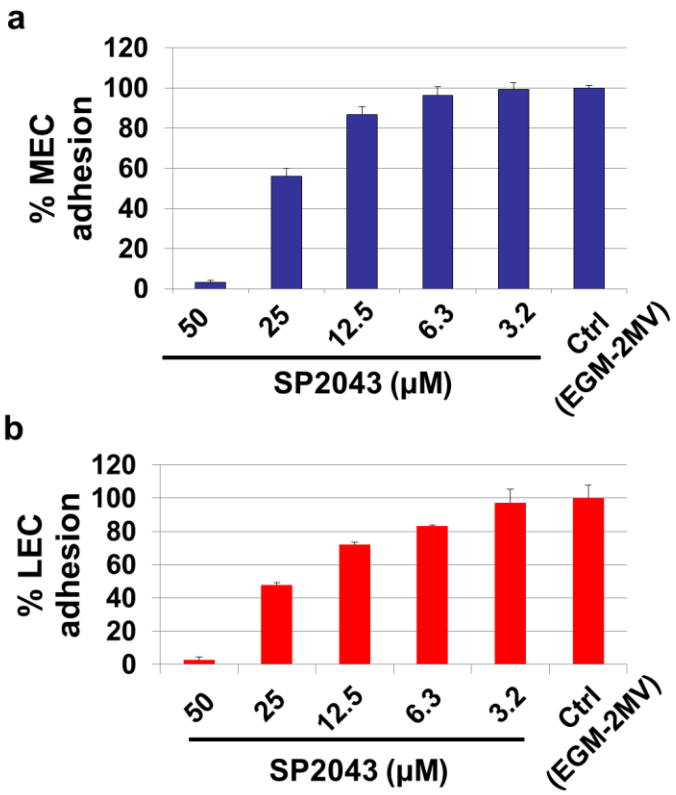



**Figure S1: In vitro activity of SP2043 on MEC and LEC.** MEC and LEC adhesion was assessed in the presence of the SP2043 peptide, using the ACEA E-plate and real time cell analysis (RTCA) system. 100 µl of 2X concentrated peptide solution was added to the appropriate wells of an E-plate (ACEA). LEC and MEC (25,000 cells/well) in EGM-2MV (LEC, MEC) were then added to each well diluting the peptides to the appropriate final concentration. After equilibrating at room temperature for 30 min, the E-plate was loaded into the RTCA system. Cell indices at 3 h were analyzed. SP2043 inhibits MEC and LEC adhesion. **(a)** Percent MEC adhesion. **(b)** Percent LEC adhesion.

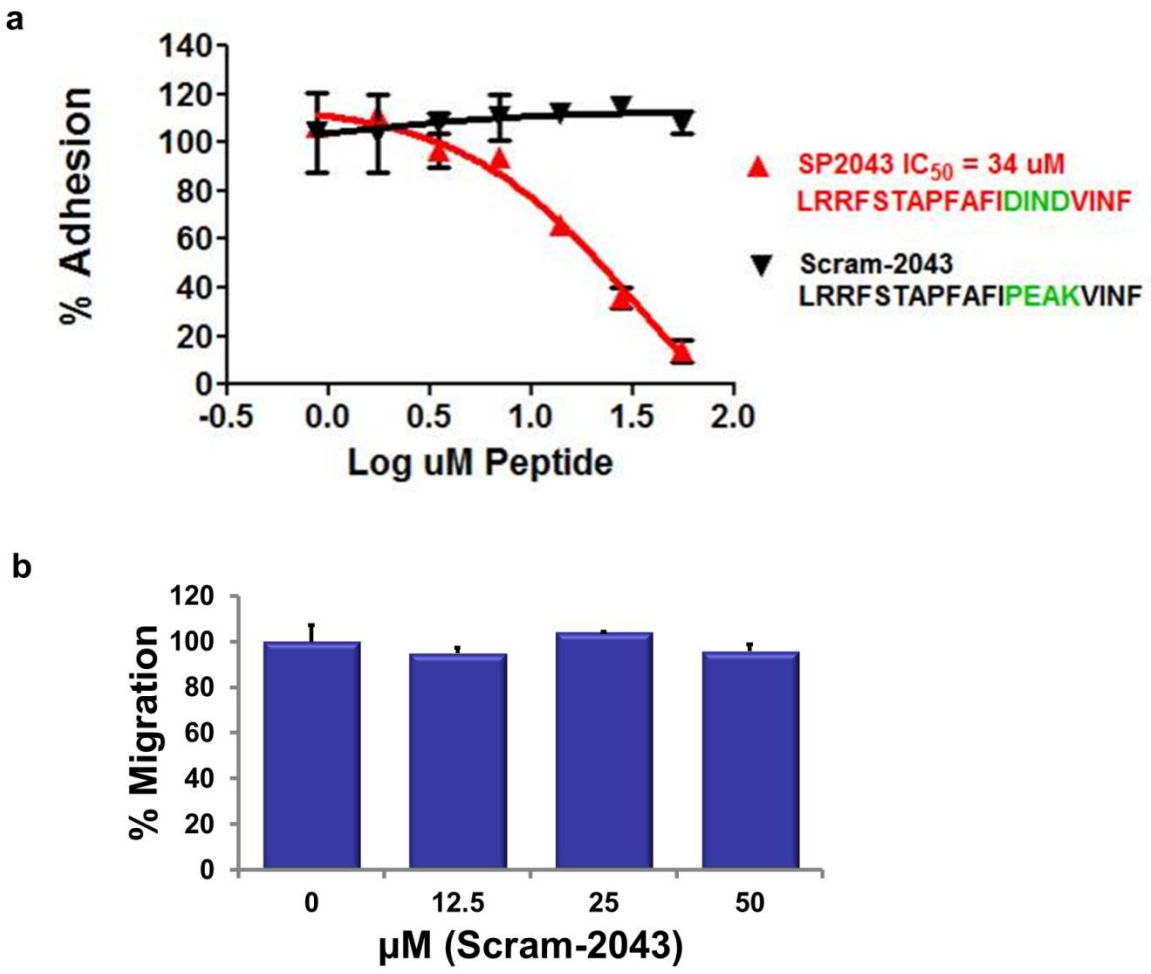



**Figure S2: Scrambled SP2043 is inactive, compared to SP2043.** Inactive peptide was prepared and tested in MEC adhesion and migration. **(a)** MEC adhesion. SP2043 inhibits MEC adhesion in dose responsive manners, but scrambled-SP2043 (Scram-2043) is inactive, demonstrating the SP2043 peptide activity is not derived from non-specific and sticky properties of peptides. **(b)** MEC migration with the Scram-2043.

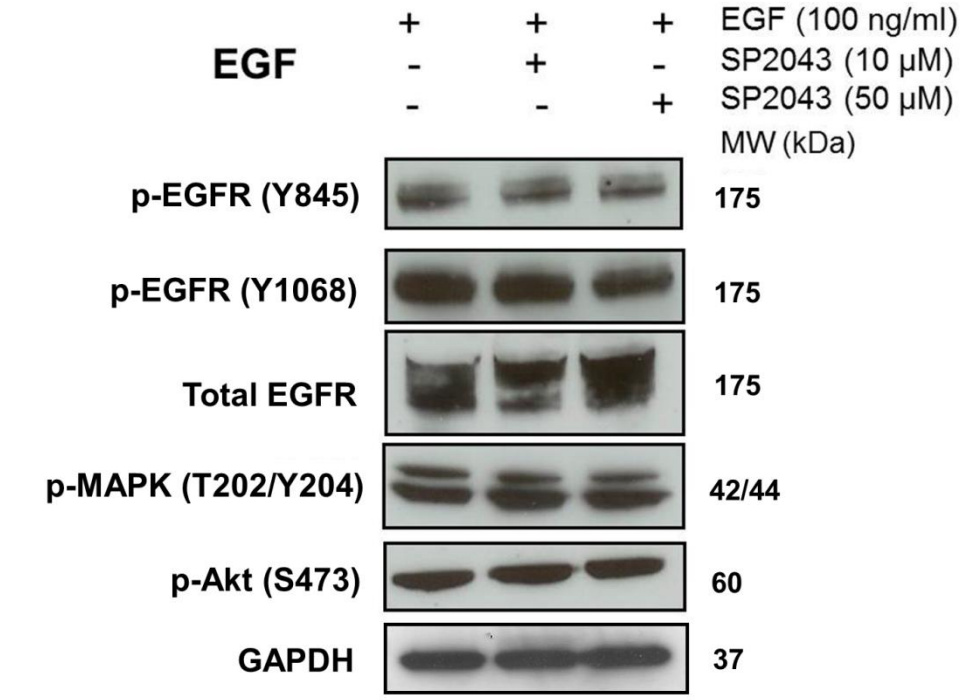



**Figure S3: SP2043 does not inhibit EGFR signaling.** MDA-MB-231 cells were treated with EGF in the presence of SP2043 and EGF. p-EGFR (Y845, Y1608), p-MAPK (T202/Y204) and p-Akt (S473) were assessed, and we observed that SP2043 does not block EGF-EGFR signaling pathways. Original gel images of data are presented in Supplementary Fig. S6.

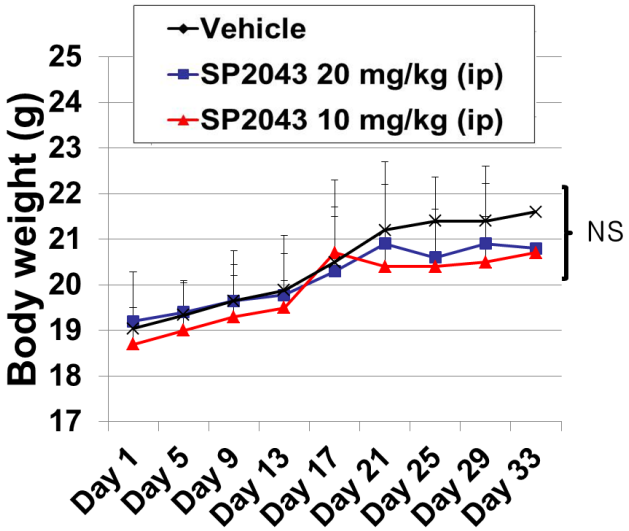



**Figure S4: Body weight of SP2043 treated animals over the course of the study.** Two million MDA-MB-231 cells were orthotopically inoculated into athymic nude mice, and the tumors were allowed to grow until they reached a size of approximately 100 $mm^3$. SP2043 (10 or 20 mg/kg) or vehicle were injected intraperitoneally (i.p.), and the body weights of the animals were measured every four days using a weighing machine for small animals. The body weights of the SP2043 treated animals were similar to that of the control animals, showing that the peptide treatment is not toxic.

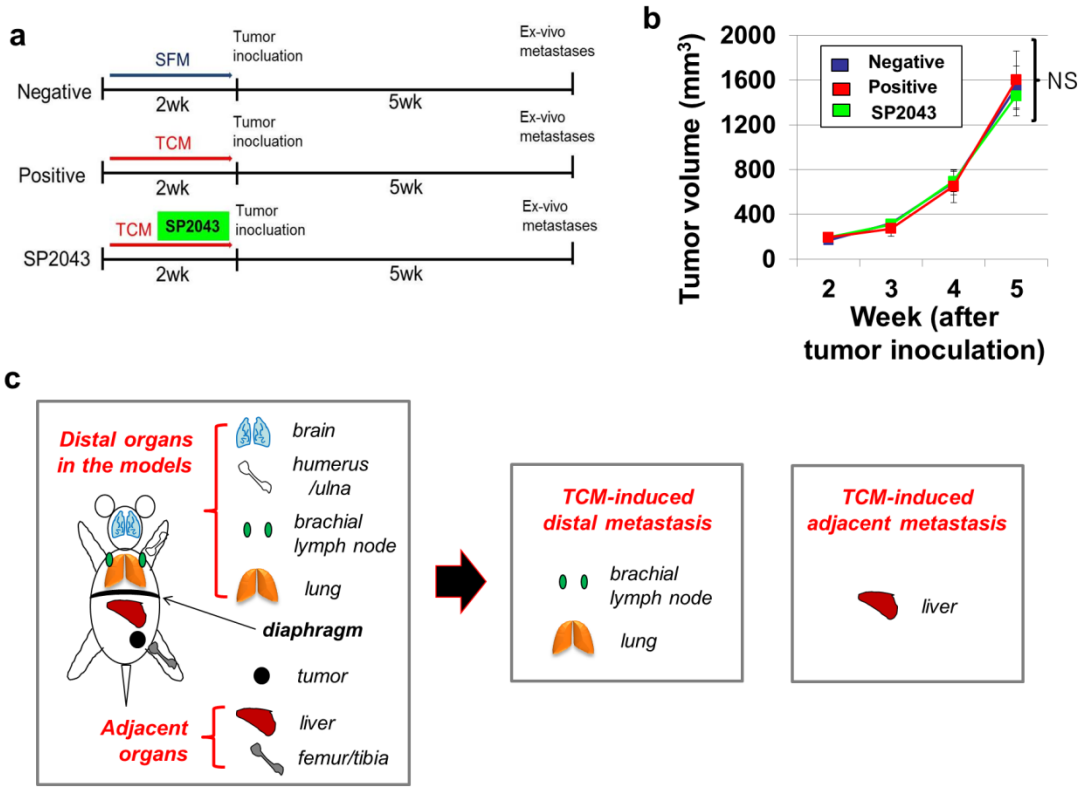



**Figure S5: Tumor-conditioned media induced spontaneous metastasis model.** Tumor-conditioned media (TCM) pre-treatment followed by tumor inoculation induce metastasis formation within a month. SP2043 was administered (10 mg/kg, i.p.) in the TCM induction phase. **(A)** Experimental group description. **(B)** Mean tumor volumes. **(C)** Description of organ metastasis. Inguinal tumors were established in mice. Only the brachial lymph node and lung metastasis are considered to constitute distant metastasis, since they are sufficiently far from the inguinal primary tumors. Liver metastases are involved with abdominal metastasis and can be regarded as adjacent metastasis.

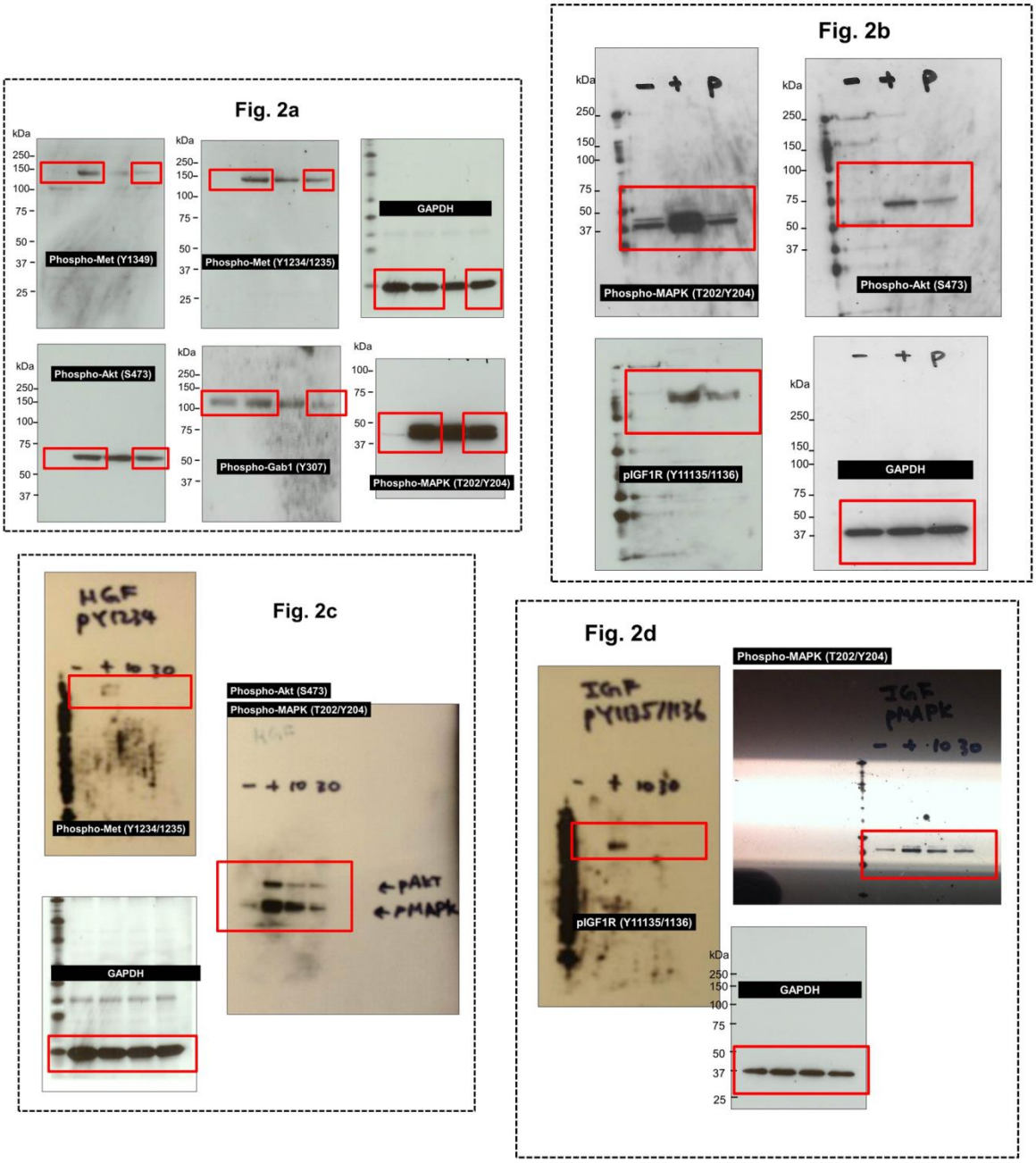



**Figure S6: Original gel images of immunoblot analysis.**

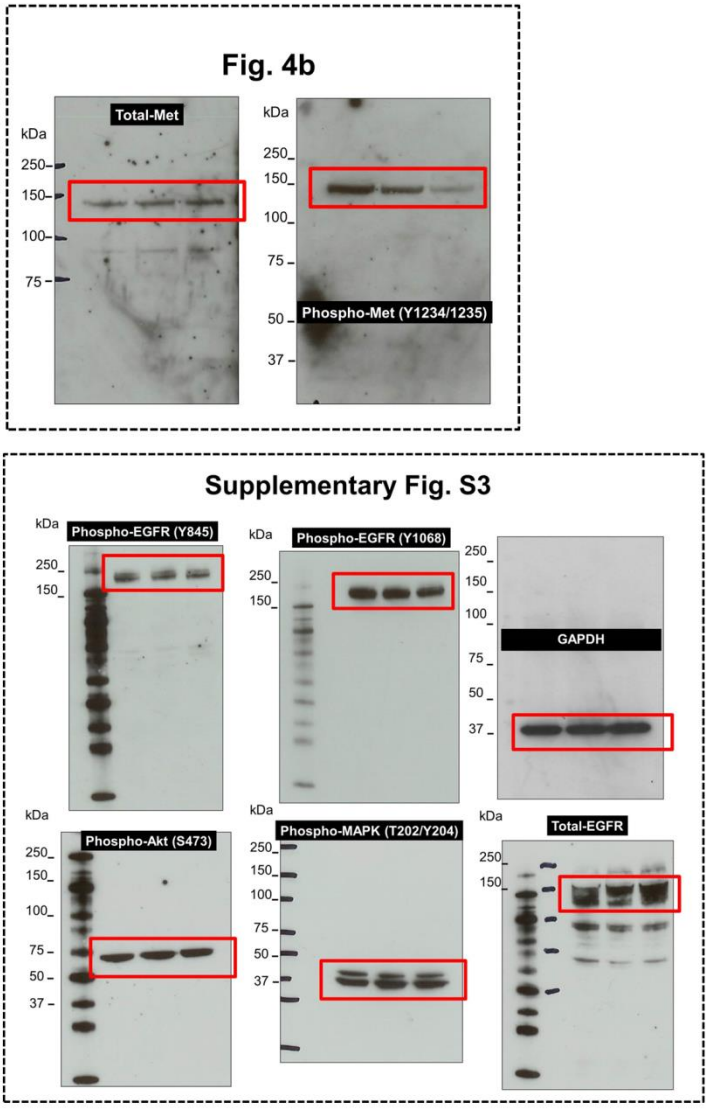



**Figure S6: Original gel images of immunoblot analysis (continued).**

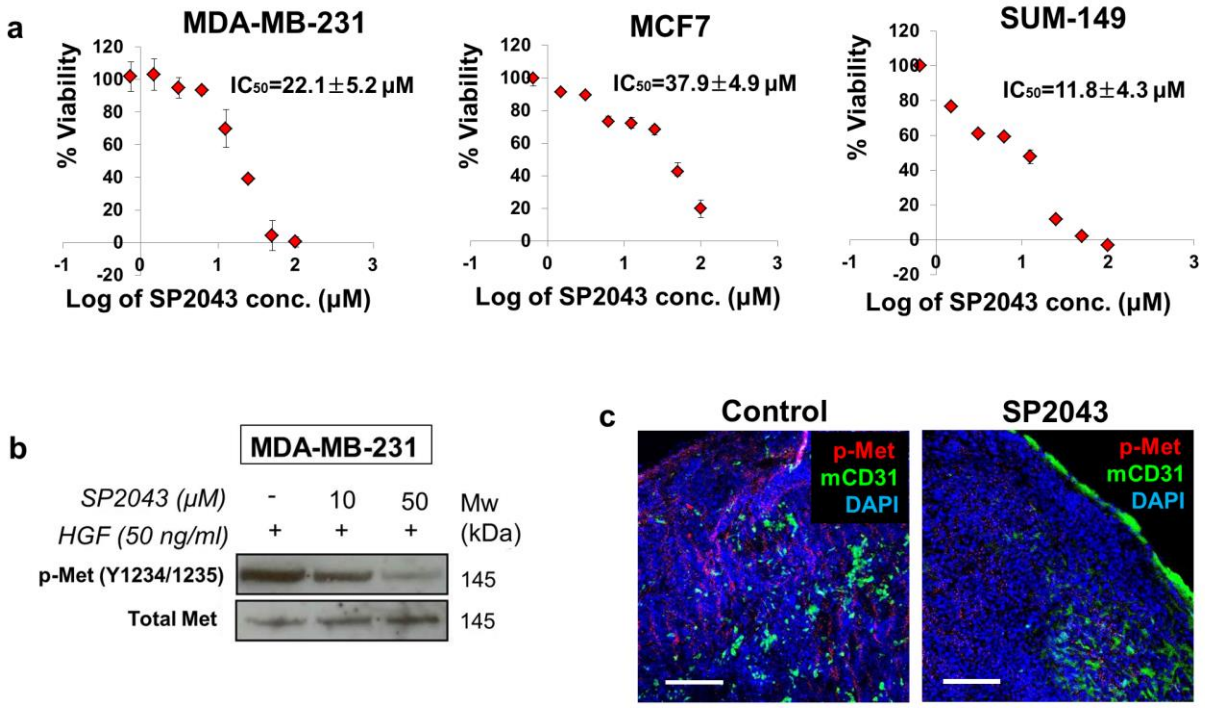
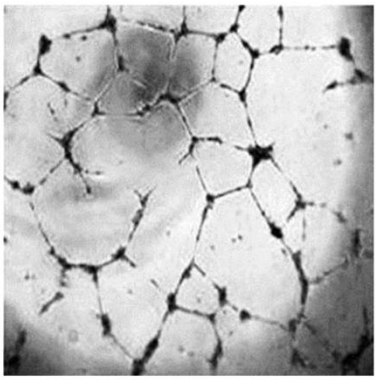


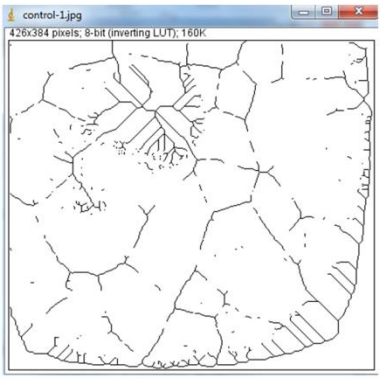

**Figure S7: Peptide activity in breast tumor cells**

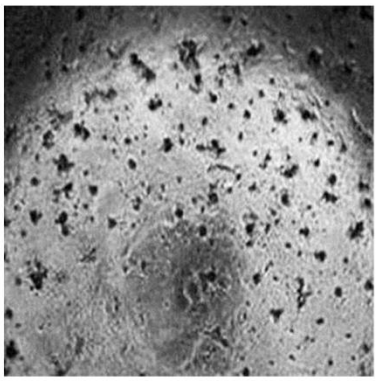
(a) Inhibition of breast cancer cell viability by SP2043. The peptide inhibited viability of MDA-MB-231, MCF-7, and SUM-149 cells. (b) In vitro western blot assays with MDA-MB-231 cells. HGF (50 ng/ml) treated MDA-MB-231 cells showed an increase in the amount of phospho-Met which was inhibited by peptide treatment. (c) Inhibition of phosphorylation of Met in vivo observed by immunostaining tumor tissues with anti-phospho-Met antibodies and mCD31 antibodies. Control tumors have ubiquitous p-Met, compared to SP2043 treated tumors. Phospho-Met is associated with the host vasculature (mCD31), the cancer cells, or the stromal cells (DAPI). Scale bars represent 200 µm. Data (a) are reported as mean ± s.e.m. Original gel images of data (b) are presented in Supplementary Fig. S6 online



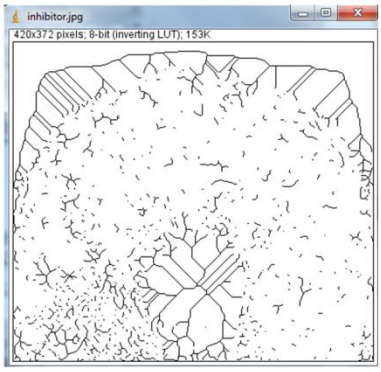
HUVEC tube (Control)

1) Making binary
2) Filling hole
3) Skeletonizing



HUVEC tube (SP2043)




Analyzing binary tree

| HUVEC | Analysed area | Nb nodes | Nb Junctions | Nb segments | Nb branches | Tot. lenght | Tot. branching lenght | Tot. segments lenght |
|---|---|---|---|---|---|---|---|---|
| control(1) | 144184 | 22 | 7 | 8 | 5 | 1075 | 1047 | 505 |
| SP2043(1) | 123840 | 15 | 3 | 3 | 4 | 640 | 360 | 100 |
| control(2) | 144184 | 55 | 17 | 22 | 7 | 1129 | 1129 | 790 |
| SP2043(2) | 138908 | 17 | 6 | 7 | 5 | 1064 | 1035 | 487 |
| control(3) | 163584 | 36 | 9 | 7 | 15 | 2096 | 1611 | 299 |
| SP2043(3) | 156240 | 30 | 8 | 6 | 13 | 1398 | 1263 | 430 |

**Figure S8: Tube formation analysis**

Tube formation was analyzed by using Angiogenesis Analyzer for ImageJ (NIH). The original images (left side, three images per group) were transformed to the binary images and the images were treated with the “fill hole” method: this minimizes the shadow effects in the image for the quantification. Then the images were skeletonized

(right side). The binary tree was analyzed and all the values were normalized with the analyzed area, making the area pixel number is 100,000. Among several categories of quantification, we chose number of nodes (Nb nodes), Nb junctions, Nb segments, Nb branches. Also we considered length values, including total length (Tot length), Tot branching length, and Tot segment length. From the analysis, we showed that SP2043 treatment inhibits HUVEC tube formation. Summary was plotted in Fig. 1e.