Supplementary Methods and Materials

Natural Product Library Generation. The library of microbial natural product fractions was derived from marine-derived Actinomycetes, Firmicutes, a-proteobacteria and the remainder from other genera. These bacteria were cultivated from marine sediment samples collected in the Gulf of Mexico (Texas, Louisiana), estuaries in South Carolina and the Bahamas. A variety of techniques were utilized to isolate strains, including use of small-molecule signaling compounds (*N*-acylhomoserine lactones, siderophores, surfactins and others) that mimic the natural environment of the bacteria of interest. Use of nutrient-limited isolation media, such as those composed of only humic or fulvic acid was used for isolation of actinomycetes and a-proteobacteria from mangrove and estuary sediments. Universal 16S rRNA primers FC27 and RC 1492 were used for the majority of the phylogentic analysis. 16S rRNA sequences were compared to sequences in available databases using the Basic Local Alignment Search Tool. This collection of ~4000 pools was used to discover new biological activity of previously reported compounds as well as novel chemistry with biological activity.

To generate the fraction library, bacterial strains were fermented in 5 × 2.8 L Fernbach flasks each containing 1 L of a seawater based medium (10 g starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40 mg $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg KBr) and shaken at 200 rpm for seven days at 27 ºC. After seven days of cultivation, XAD-7 resin (20 g/L) was added to adsorb the organic products, and the culture and resin were shaken at 200 rpm for 2 hours. The resin was filtered through cheesecloth, washed with deionized water, and eluted with acetone to give a crude extract. Further fractionation of the bacterial crude extracts was accomplished using an Isco medium pressure

automatic purification system using reversed phase C_{18} chromatography (gradient from 90:10 H_2O:CH_3CN to 0:100 H_2O:CH_3CN over 25 minutes).

Natural Product Cytotoxicity Screens. The following 25 NSCLC were used: HCC366, H1993, H2009, H2122, HCC15, HCC827, H2073, HCC44, H2887, HCC193, H1819, HCC515, HCC95, H2250, H1437, HCC122, H2126, H2347, H1693, HCC4017, H2087, H2052, H1395, HCC4018, and H2882. HBEC30KT, a normal immortalized cell line was also used. NSCLC cell lines and HBEC30KT were grown and maintained in 384-well microtiter assay plates (Bio-one; Greiner) in growth medium (NSCLC cell lines in 5% Fetal Bovine Serum/RPMI/L-glutamine medium from Invitrogen, Inc.; HBEC30KT was grown in ACL4 medium). On day 1, cell lines were plated at an appropriate cell density per well such that they reached ~70-80% confluence after 5 days of incubation at 37°C. Following cell plating, assay plates were incubated overnight at 37°C and 5% CO2. On day 2, natural product fractions were added to each plate at 4 doses (0.18 µg/mL, 0.55 µg/mL, 1.65 µg/mL and 5 µg/mL) using a BioMekFx liquid handler (Beckman, Inc.). The cell lines were incubated for 96 hours at 37°C and 5% CO2. CellTiter-Glo (15 µl, Promega, Inc.) was then added to each well and plates were incubated for 15 min prior to reading. Luminescence was determined at room temperature for each plate on an EnVision multi-label plate reader. For these experiments, psymberin was used as inhibitory control (column 1 of each 384 well assay plate) and DMSO (final concentration 0.5%) was used as the vehicle control (columns 2 and 23 of each 384 well assay plate).

Purification of piericidin A from SNB-051. The marine-derived bacterium strain SNB-051 was isolated from a sediment sample collected from Sweetings Cay, Bahamas in

2008. Briefly, 1 mL of sediment was diluted in 5 mL of sterile seawater, vortexed for 10 minutes then centrifuged. 10 µL of the solution was added to rabbit manure (RM) agar media (10 g rabbit manure, 1L sterile seawater, 8 g agar) and allowed to incubate at 27ºC for 60 days. Colonies were selected and streaked on fresh RM plates. A pure colony was selected for 16S rRNA phylogenetic analysis and revealed to have 99% identity to *Streptomyces variabilis* (GenBank accession number MH231205).

For activity-guided isolation of PA, a 20L fermentation of SNB-051 in A1BFe+C media (10 g starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40 mg $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg KBr, 1L 75% sH_2O) was placed on shakers at 200 rpm at 27 ºC for 7 days, then extracted with XAD-7 resin, the resin was collected and subsequently washed with acetone (2 L) to give 6.5 g of crude extract. The crude extract was then fractionated using reversed-phase chromatography (C18) flash column and a step gradient from 20-100% methanol in water resulting in 37 fractions. These fractions were combined based on similarity by LC-MS to five total fractions which were tested for viability defects against HCC44 and H2122 NSCLC cell lines identifying the active fraction, SNB-051-F36 (HCC44 EC_{50} = 2.7 µg/mL; H2122 EC_{50} = 0.004 µg/mL). Fraction F36 was further purified by reversed phase HPLC (Phenomenex Luna, C18(2), 250x10 mm, 5 µm) using a linear gradient solvent system from 40% to 100% acetonitrile in water (0.1% formic acid). And monitoring by 254 nm UV wavelength (Supplementary Fig. S1B), generating seven fractions. These seven fractions were again tested for toxicity against HCC44 and H2122 NSCLC cell lines to determine which fraction was responsible for activity. The peak that maintained the greatest selective toxicity when screened against HCC44 and H2122 cells (F36-H7) was later identified as being the pure compound, piericidin A.

NMR Characterization. HRMS analysis of SNB-051-F36-H7 fraction gave a *m/z* 416.2794 $[M+H]^+$ as the major metabolite (>95%), which was consistent with the molecular formula $C_{25}H_{37}NO_4$. The molecular formula of $C_{25}H_{37}NO_4$, along with the proton NMR and UV chromophore were all indications the active molecule was piericidin A. Downfield olefin ^{1}H NMR signals at 5.43 (dd, *J* = 13.0, 6.9 Hz), 5.52 (dd, *J* = 15.5, 6.9 Hz), 6.07 (d, *J* = 15.5 Hz), 5.27 (d, *J* = 9.5 Hz) and 5.30 (m) correspond to H-2, H-5, H-6, H-8 and H-12 respectively. Proton signals for 6 methyl groups at 1.61 (d, *J* = 7.5 Hz), 1.73 (s), 1.73 (s), 0.79 (d, *J* = 6.9 Hz), 1.59 (s) and 2.03 (s) corresponding to H-13, H-14, H-15, H-16, H-17 and H-6' as well as ^{1}H signals for 2 methoxy groups at 3.73 (s) and 3.89 (s) were consistent with piericidin A. Additionally two methylene 1H signals at 3.34 (d, J = 6.9 Hz) and 2.76 (d, J = 6.9 Hz) corresponding to H-1 and H-4 and two methine protons, one of which was adjacent to a heteroatom at 3.68 (d, J = 8.3 Hz) with the other at 2.66 (m) for H-10 and H-9 respectively account for all proton signals of piericidin A.