SUPPLEMENTARY MATERIALS

Cell cycle arrest of EriB in SMMC-7721 cells

Cells were treated with EriB at 0.5, 1, 2 and 4 µM for 24 h. Cells were harvested and washed with PBS and were fixed with 70% ethanol. Then the cells were washed with PBS and incubated with RNase A (Sigma-Aldrich) and stained with propidium iodide (Sigma-Aldrich) and were subjected to cell cycle analysis by FACSCalibur flow cytometry (BD Biosciences, Franklin Lakes, NJ). The distribution of cells of different phases was analyzed by CellQuest Pro (Figure S1).

Isolation of Eriocalyxin B and synthesis of the ABPs

Eriocalyxin B was isolated from leaves of *isodon erioclyx var. laxiflora* according to the reported method [1].

General information for chemistry: All reactions were performed under argon atmosphere using flame-dried glassware unless otherwise noted. CH2Cl2 and Et3N were distilled over CaH2. THF was distilled over sodium/benzophenone ketyl. All reagents were commercially available and used without further purification unless indicated otherwise. Thin layer chromatographies were carried out on Qing-Dao silica plates (0.25 mm layer thickness). Flash chromatography was performed with 300–400 mesh silica gels. Yields reported were for isolated, spectroscopically pure compounds. 1H and 13C NMR experiments were performed on a Bruker AM-400 and DRX-500 NMR spectrometer at ambient temperature. The residual solvent protons (1H) or the solvent carbons (13C) were used as internal standards. 1H-NMR data are presented as follows: chemical shift in ppm downfield from tetramethylsilane (multiplicity, coupling constant, integration). The following abbreviations are used in reporting NMR data: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet. EIMS and HREIMS were taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 instrument. The synthetic schemes are shown as Figure S2.

Preparation of eriocalyxin-6-yl-homo succinic ester (2): To a solution of compound 1 (69 mg) and DMAP (10 mg) in DCM (1.5 mL) at 0°C was added Et3N (101 mg) and succinic anhydride (30 mg) successivity. The resulting mixture was slowly warmed to the room temperature and stirred for 15 hours until no starting material was detected. A HCl aqueous solution (1M) was added to quench the reaction. The aqueous phase was extracted with EtOAc (15 mL×3). The combined organic layers were washed with H2O and brine successively, and purified by flash chromatography on silica gel.

Supplementary Figure S1: EriB induces hardly cell cycle arrest in SMMC-7721 cells. Experiments were done independently in triplicate, and results are reported as means and standard deviations.
dried over anhydrous Na$_2$SO$_4$ and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1: 1.5) to give compound 2 (86 mg, 97%) as white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 6.84 (d, $J = 10.1$ Hz, 1H), 5.83 (d, $J = 10.1$ Hz, 1H), 5.72 (s, 1H), 5.37 (d, $J = 8.7$ Hz, 1H), 5.29 (s, 1H), 4.94 (s, 1H), 2.99 (m, 1H), 4.27 (d, $J = 10.1$ Hz, 1H), 3.98 (d, $J = 10.1$ Hz, 1H), 1.16 (s, 3H), 1.24 (s, 3H), 2.74 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 202.2 (C), 196.8 (C), 174.7 (C), 173.8 (C), 160.6 (CH), 154.9 (C), 128.0 (CH), 114.6 (CH$_2$), 97.1 (C), 74.2 (CH), 65.7 (CH$_3$), 58.6 (C), 53.8 (CH), 48.2 (CH), 47.1 (C), 36.3 (C), 34.3 (CH), 30.5 (CH$_2$), 30.4 (CH$_3$), 30.0 (CH$_2$), 28.9 (CH$_2$), 26.5 (CH$_3$), 24.8 (CH$_2$), 19.6 (CH$_3$); ESI-MS (m/z) 467 [M+Na]$^+$. The spectra data of compound 2 is identical to that described in the literature [2].

Preparation of 2-(2-azidoethoxy) ethanol (4): To a solution of polyethylene glycol (16.52 g) in THF (100 mL) was added $\text{Et}_2\text{N}$ (15 mL) dropwise. The resulting mixture was stirred at room temperature for 0.5 hour. Then the mixture was immersed in water-ice bath. And MsCl was added to the mixture dropwise. The mixture was slowly warmed to the room temperature, and stirred overnight. Then the solvent was evaporated off. EtOH (100 mL) and NaN$_3$ (6.5 g) was added successively. The mixture was heated to reflux for 24 hours. Then the solvent was evaporated off, and saturated NaCl aqueous solution was added to dilute the mixture. The aqueous phase was extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1: 1.5) to give compound 2 (86 mg, 97%) as white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 6.84 (d, $J = 10.1$ Hz, 1H), 5.83 (d, $J = 10.1$ Hz, 1H), 5.72 (s, 1H), 5.37 (d, $J = 8.7$ Hz, 1H), 5.29 (s, 1H), 4.94 (s, 1H), 2.99 (m, 1H), 4.27 (d, $J = 10.1$ Hz, 1H), 3.98 (d, $J = 10.1$ Hz, 1H), 1.16 (s, 3H), 1.24 (s, 3H), 2.74 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 202.2 (C), 196.8 (C), 174.7 (C), 173.8 (C), 160.6 (CH), 154.9 (C), 128.0 (CH), 114.6 (CH$_2$), 97.1 (C), 74.2 (CH), 65.7 (CH$_3$), 58.6 (C), 53.8 (CH), 48.2 (CH), 47.1 (C), 36.3 (C), 34.3 (CH), 30.5 (CH$_2$), 30.4 (CH$_3$), 30.0 (CH$_2$), 28.9 (CH$_2$), 26.5 (CH$_3$), 24.8 (CH$_2$), 19.6 (CH$_3$); ESI-MS (m/z) 467 [M+Na]$^+$. The spectra data of compound 2 is identical to that described in the literature [2].

Supplementary Figure S2: Synthetic schemes of EBF 6, EBF7, EBB8.
acetate/petrol ether = 1: 3) to give compound 4 (17.02 g, 85%) as colorless oil. The structure of compound 4 was assigned by comparison the spectra data with that of the literature [3].

Preparation of 5-((dimethylamino)-N-(2-(2-hydroxyethoxy)ethyl)naphthalene-1- sulfonamide (DT5): step 1: To a solution of compound 4 (143 mg) and in MeOH (20 mL) under H₂ was added Pd/C (14 mg) in one portion. The mixture was stirred at room temperature for half an hour until no starting material was detected. The mixture was filtered through celite. The filtrate was evaporated under vacuo. The crude product was used for next step without purification.

Step 2: To a solution of the crude product (93 mg) and DMAP (5 mg) in THF (5 mL) was added Et₃N (122 mg) dropwise. The resulting mixture was stirred at 0 °C was added EDCI (8 mg) and DMAP (5 mg) in THF (5 mL) was added Et₃N (122 mg) dropwise. The resulting mixture was stirred at 0 °C in two portions. The mixture was stirred at room temperature for half an hour until no starting material was detected. H₂O was added to quench the reaction. The aqueous phase was extracted with CDCl₃ (20 mL×3). The resulting mixture was slowly warmed to the room temperature and stirred for 5 hours until no starting material was detected by TLC. HCl aqueous solution of compound 4 (17 mg, 85%) as green yellow foam.

Preparation of 6-O-[5-(dimethylamino)-naphthalene-1-sulfonamide-N-2-ethyl-1-(1-ethoxyl)-2-hydroxy]-succinic ester-yl Eriocalyxin B (EBF6): To a solution of compound 2 (11 mg) and DT5 (7 mg) in DCM (1.0 mL) at 0 °C was added EDCI (8 mg) and DMAP (5 mg) successively. The resulting mixture was slowly warmed to the room temperature and stirred for 5 hours until no starting material was detected. H₂O was added to quench the reaction. The aqueous phase was extracted with EtOAc (10 mL×3). The combined organic layers were washed with H₂O and brine successively, dried over anhydrous Na₂SO₄ and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1: 1.5) to give compound EBF6 (17 mg, 85%) as green yellow foam. ¹H NMR (400 MHz, CDCl₃) δ 8.54 (d, J = 8 Hz, 1H), 8.32 (d, J = 8 Hz, 1H), 8.24 (dd, J = 8 Hz, 4Hz, 1H), 7.57 (t, J = 8 Hz, 8Hz, 1H), 7.51 (t, J = 8 Hz, 8Hz, 1H), 7.19 (d, J = 8 Hz, 1H), 6.74 (d, J = 12 Hz, 1H), 5.85 (d, J = 8 Hz, 1H), 5.59 (t, J = 8 Hz, 4Hz, 1H), 5.37 (d, J = 8 Hz, 1H), 5.28 (s, 1H), 4.44 (s, 1H), 4.36 (d, J = 12 Hz, 1H), 4.10-4.14 (m, 1H), 4.00-4.04 (m, 2H), 3.31-3.37 (m, 4H), 3.14 (dd, J = 12 Hz, 4Hz, 2H), 2.99 (dd, J = 12 Hz, 4Hz, 1H), 2.88 (s, 6H), 2.87 (d, J = 8 Hz, 2H), 2.66-2.72 (m, 1H), 2.49 (d, J = 8 Hz, 1H), 2.29 (d, J = 12 Hz, 1H), 2.12 (d, J = 8 Hz, 1H), 2.07 (d, J = 12 Hz, 1H), 1.98 (dd, J = 8 Hz, 4Hz, 1H), 1.66 (s, 2H), 1.42 (d, J = 8 Hz, 2H), 1.40 (s, 2H), 1.37 (d, J = 8 Hz, 1H), 1.31 (d, J = 8 Hz, 1H), 1.25 (d, J = 8 Hz, 1H), 1.23 (s, 3H), 1.17 (s, 3H), 0.91 (t, J = 8 Hz, 8Hz, 1H), 0.89 (d, J = 8 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 202.4 (C), 196.5 (C), 175.1 (C), 172.1 (C), 160.1 (CH), 153.0 (C), 151.8 (C), 135.1 (C), 130.8 (CH), 130.3 (CH), 129.8 (C), 129.6 (C), 128.7 (CH), 128.3 (CH), 127.4 (CH), 123.1 (CH), 118.8 (CH), 115.6 (CH), 115.1 (CH), 96.6 (C), 74.1 (CH), 69.2 (CH₂), 68.7 (CH₃), 68.1 (C), 65.3 (CH₂), 63.2 (CH), 57.6 (C), 52.7 (CH₂), 47.1 (CH), 46.5 (C), 45.3 (CH₂), 42.8 (CH₂), 35.7 (C), 33.4 (CH), 29.6 (CH), 29.3 (CH), 29.2 (CH₂), 28.8 (CH), 25.6 (CH), 24.7 (CH), 18.8 (CH); HR-ESI-MS (m/z): 765.3044 [M+H⁺] (calcd. for C₃₀H₃₂N₂O₄S, 765.3057).

Preparation of 6-O-[5-(dimethylamino)-naphthalene-1-sulfonamide-N-2-ethyl-1-(1-ethoxyl)-2-hydroxy]-succinic ester-yl Eriocalyxin B (EBF7): To a solution of EBF6 in EtOAc (1 mL) was added Pd/C (1 mg) in one portion. The mixture was stirred at room temperature under H₂ for 3 hours until no starting material was detected by TLC. Then Pd/C was filtered off. The filtrate was evaporated under vacuo. The crude product was subjected to preparative TLC to afford EBF7 (2.9 mg) green yellow foam. ¹H NMR (600 MHz, CDCl₃) δ 8.47 (d, J = 8.5 Hz, 1H), 8.23 (d, J = 8.6 Hz, 1H), 8.18 (d, J = 7.2 Hz, 1H), 7.51 (t, J = 8.1 Hz, 1H), 7.49 – 7.42 (m, 2H), 7.13 (d, J = 7.5 Hz, 1H), 5.42 (d, J = 12.1 Hz, 3H), 5.37 (t, J = 5.9 Hz, 1H), 4.24 (t, J = 6.7 Hz, 1H), 4.19 (d, J = 10.4 Hz, 3H), 4.08 (q, J = 7.1 Hz, 2H), 4.04 – 4.00 (m, 3H), 3.99 (s, 2H), 3.92 – 3.86 (m, 3H), 3.65 (dd, J = 12.0, 8.3 Hz, 3H), 3.38 – 3.22 (m, 4H), 3.05 (dd, J = 10.6, 5.4 Hz, 2H), 2.82 (s, 6H), 2.56 (t, J = 3.2 Hz, 4H), 2.48 (t, J = 10.1 Hz, 3H), 2.45 – 2.32 (m, 4H), 2.28 – 2.25 (m, 1H), 2.25 (d, J = 1.7 Hz, 1H), 2.24 – 2.22 (m, 1H), 2.21 (s, 1H), 2.19 (s, 2H), 2.07 (d, J = 4.0 Hz, 2H), 2.05 (d, J = 4.0 Hz, 1H), 1.96 (d, J = 8.2 Hz, 3H), 1.88 – 1.82 (m, 3H), 1.78 (ddd, J = 9.4, 7.9, 2.8 Hz, 4H), 1.75 – 1.69 (m, 4H), 1.68 – 1.63 (m, 4H), 1.62 (s, 4H), 1.42 – 1.34 (m, 3H), 1.19 (s, 1H), 1.18 (s, 3H), 1.17 (s, 1H), 1.11 (s, 8H), 1.08 (s, 6H), 1.07 (s, 5H), 0.93 (s, 8H), 0.91 (s, 1H), 0.89 (t, J = 7.4 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 224.4 (C), 212.5 (C), 172.6 (C), 172.5 (C), 152.1 (C), 135.2 (C), 131.1 (C), 135.1 (C), 131.1 (C), 130.5 (C), 130.0 (CH), 129.7 (C), 129.5 (CH), 129.0 (C), 128.5 (CH), 123.4 (CH), 119.0 (CH), 115.4 (CH), 95.3 (C), 73.6 (CH), 69.4 (CH₂), 69.0 (CH₂), 65.7 (C), 65.2 (CH₂), 63.5 (CH₂),
61.1 (CH), 61.0 (CH₂), 59.8 (C), 50.2 (CH), 48.6 (C), 48.1 (CH), 45.6 (CH₂), 43.1 (CH₃), 38.4 (CH), 35.9 (CH₂), 33.0 (C), 31.2 (CH), 30.7 (C), 30.6 (CH), 29.9 (C), 29.2 (CH₂), 29.1 (CH), 27.0 (CH), 23.4 (CH), 19.3 (C), 19.0 (CH), 18.3 (CH₂), 14.3 (CH₃), 11.2 (CH₃); HR-ESI-MS (m/z): 768.3286 [M⁺] (calcd. for C₃₆H₆₂N₂O₇S, 768.3292).

Preparation of 6-O-biotinyl Eriocalyxin B (EBB8):
To a solution of biotin (18 mg) and DMAP (3 mg) in DMF (10 mL×3). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was subjected to flash chromatography on silica gel (Chloroform: MeOH = 30 : 1) to give EBB8 (20 mg, 76% brsm) as white powder.

1H NMR (400 MHz, CDCl₃) δ 6.75 (d, J = 12 Hz, 1H), 5.89 (d, J = 12 Hz, 1H), 5.86 (d, J = 4 Hz, 1H), 5.38 (d, J = 8 Hz, 1H), 5.36 (s, 1H), 5.30 (s, 1H), 4.49 (dd, J = 8 Hz, 4 Hz, 1H), 4.36 (d, J = 8 Hz, 1H), 4.31 (dd, J = 8 Hz, 4 Hz, 1H), 4.04 (d, J = 12 Hz, 1H), 3.12-3.17 (m, 1H), 2.91 (dd, J = 12 Hz, 1H), 2.61-2.64 (m, 1H), 2.50 (t, J = 8 Hz, 8 Hz, 1H), 2.48 (d, J = 12 Hz, 1H), 2.29 (d, J = 12 Hz, 1H), 2.12 (dd, J = 12 Hz, 4 Hz, 2H), 2.09 (d, J = 4 Hz, 1H), 1.78 (dd, J = 16 Hz, 8 Hz, 2H), 1.51 (m, 2H), 1.35-1.42 (m, 2H), 1.24 (s, 6H), 1.15 (s, 3H); 13C-NMR (100 MHz, CDCl₃) δ 202.6 (C), 196.5 (C), 176.6 (C), 163.6 (C), 160.1 (CH), 153.0 (C), 127.5 (CH), 115.8 (CH), 96.7 (C), 73.7 (CH), 65.3 (CH₂), 61.7 (CH), 60.1 (CH), 57.7 (C), 55.3 (CH), 52.5 (CH), 47.3 (CH), 46.5 (C), 40.4 (CH), 35.7 (C), 33.7 (CH₂), 33.4 (CH), 29.6 (C), 29.4 (CH), 27.7 (CH), 25.5 (CH₂), 24.8 (CH), 24.0 (CH), 18.8 (CH₂); HR-ESI-MS (m/z): 571.2486 [M+H⁺] (calcd. for C₃₆H₅₀N₂O₇S, 571.2477).

Dansyl tagged EriB dominantly labeled a 50-kDa protein
The cytoplasmic fraction and the nucleic fraction of SMMC-7721 cell lysates were incubated with EBF6 (5 μM), with or without the presence of EriB, followed by resolving with SDS-PAGE and imaging under UV transilluminator (Figure S3).

EriB selectively binds to p50 in HEK293T cells
HEK293T cell lysates pretreated with or without TNF-α were incubated with EBB8 in the absence or presence of EriB, or with biotin alone, followed by pull-down with streptavidin beads. The precipitates were resolved by SDS-PAGE, and the gel subjected to immuno-blotting with p50 antibody, with β-actin as the input.

Liquid chromatography-mass spectra analysis
Liquid chromatography was performed on a nano Acquity UPLC system (Waters Corporation, Milford, USA) connected to a LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an online nano-electrospray ion source (Michrom Bioresources, Auburn, USA). Peptides were re-suspended with 25 μl solvent A (5% acetonitrile, 0.1% formic acid in water). 20 μl peptide solution was loaded onto the Ctrap Peptide column (2mm x 0.5mm, Michrom Bioresources, Auburn, USA) at a 20 μl/min flow rate of solvent A for 5 min and then was separated on a Magic C18AQ reverse phase column (100 μm id×15cm, Michrom Bioresources, Auburn, USA) with a linear gradient. Starting from 5% B (90% acetonitrile, 0.1% formic acid in water) to 45% B (in other words, from 95% A to 55% A, the same below) in 70 min. The column flow rate was maintained at 500 nL/min and column temperature was maintained at 35 °C. The electrospray voltage of 1.4 kV versus the inlet of the mass spectrometer was used.

LTQ Orbitrap XL mass spectrometer was operated in the data-dependent mode to switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra with one microscan (m/z 350–1800) was acquired in the Orbitrap with a mass resolution of 100,000 at m/z 400, followed by MS/MS of the eight most-intense peptide ions in the LTQ analyzer. The automatic gain control (AGC) was set to 1000 000 ions, with maximum accumulation times of 500 ms. For MS/MS, we used an isolation window of 2 m/z and the automatic gain control (AGC) of LTQ was set to 20 000 ions, with maximum accumulation time of 120 ms. Single charge state was rejected and dynamic exclusion was used with two microscans in 15s and 30 s exclusion duration. For MS/MS, precursor ions were activated using 35% normalized collision energy at the default activation q of 0.25 and an activation time of 30 ms. The spectrum were recorded with Xcalibur (version 2.0.7) software.

The mass spectra were searched using the Mascot Daemon software (Version 2.3.0, Matrix Science, London, UK) based on the Mascot algorithm. The database used to search was the protein NFKB1 (P19838). The searching parameters were set up as follows: full trypsin (KR) cleavage with two missed cleavage was considered. Oxidation on methionine and Eriocalyxin B of the Cys...
Supplementary Figure S3: Dansyl tagged EriB dominantly labeled a 50-kDa protein.

Supplementary Figure S4: EriB selectively binds to p50 in HEK293T cells.

were set as variable modifications. The peptide mass tolerance was 20 ppm and the fragment ion tolerance was 1.0 Da. Peptides whose Mascot expected values below 0.05 were accepted as correct matches.

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