SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. (A) HCC827 TKI resistant cells, ERC4 cells, up-regulate mesenchymal markers including N-cadherin and vimentin while TKI-sensitive HCC827 cells maintain epithelial markers, E-cadherin. CDH1: E-cadherin, CDH2: N-cadherin, VIM: vimentin, ACTB: β-actin. A representative immunoblot from 3 independent experiments. (B) Representative IHC analyses from HCC827 and ERC4 tumors using E-cadherin, CD44, N-cadherin and vimentin antibodies. Scale 100 μM. Table: Table: Scoring by board-certified pathologists, -, negative; +, weak; ++, moderate; +++, strong.

Supplementary Figure 2. (A) A heatmap showing angiogenic factor and growth factor secretion from epithelial (HCC4006, HCC827) and mesenchymal (HCC4006Ge-R, HCC4006O-R and ERC4) cell lines. MFIs from Luminex multiplex assay analysis were $\log_2$ transformed and the differences in expression are displayed for each analyte independently. (B) HCC4006Ge-R cells show up-regulation of mesenchymal markers, N-cadherin and vimentin. CDH1: E-cadherin, CDH2: N-cadherin, VIM: vimentin, ACTB: β-actin. A representative immunoblot from 3 independent experiments. (C) HCC4006O-R cells also show up-regulation of mesenchymal markers, N-cadherin and vimentin. CDH1: E-cadherin, CDH2: N-cadherin, VIM: vimentin, ACTB: β-actin. A representative immunoblot from 3 independent experiments. (D) HCC827 with stable knockdown of E-cadherin (ΔCDH1) up-regulate mesenchymal markers N-cadherin and vimentin compared to control (ΔNT). CDH1: E-cadherin, CDH2: N-cadherin, VIM: vimentin, ACTB: β-actin. A representative immunoblot from 3 independent experiments. (E) VEGF-A and

EDN1 secretion normalized with protein concentration. Error bars: S.D. t-test *** p<0.001, **** p<0.0001. (F) VEGF-A and EDN1 secretion normalized with protein concentration. Error bars: S.D. t-test *** p<0.001, **** p<0.0001. (G) VEGF-A and EDN1 secretion normalized with cell count in HCC827 and ERC4 cells under hypoxic conditions (1%O_2) for 72 hours. Error bars: S.D. t-test ** p<0.01 *** p<0.001, ****p<0.0001. (H) VEGF-A and EDN1 secretion normalized with cell count. The indicated cells were grown in normoxic or hypoxic conditions and treated with vehicle or gefitinib for 72 hours (100 nmol/L). Error bars: S.D. t-test ****p<0.0001.

Supplementary Figure 3. (A) Colorimetric *in vitro* proliferation assay for 72 hours with gefitinib of HCC827 EDN1 overexpressing cells. N=3. Error bars: S.D. (B) EDN1 secretion from HCC827Flag-V5 and HCC827EDN1-V5 cell xenografts following 6 days of vehicle or gefitinib (50 mg/kg) treatment normalized with protein concentration. Error bars: S.D. One-way ANOVA. N.S. Not significant, ** p<0.01, **** p<0.0001. (C) Mass spectrometry-based quantification of gefitinib in HCC827Flag-V5 and HCC827EDN1-V5 cell xenografts, normalized with total protein. The tumor bearing mice were treated with gefitinib (20 mg/kg) for 1 hour prior to sacrifice and harvesting tumors. Error bars: S.D. t-test. *** p<0.001. (D) Residual tumor (%) after 4 weeks of growth and 6 days of gefitinib treatment. Error bars: S.D. t-test *p<0.05. (E) Exposure of HCC827 cells for 72 hours to rEDN1 does not decrease epithelial marker, CDH1 and does not increase the expression of mesenchymal markers, CDH2 and VIM. CDH1: E-cadherin, CDH2: N-cadherin, VIM: vimentin, ACTB: β-actin. A representative immunoblot from 3 independent experiments.

(F) Colorimetric *in vitro* proliferation assay for 72 hours with osimertinib in the absence (HCC827) or presence of rEDN1 (100 nmol/L, HCC827+rEDN1). N=3. Error bars: S.D. (G) Colorimetric *in vitro* proliferation assay for 72 hours with gefitinib of HCC4006Ge-R EDN1 repressing cells. N=3. Error bars: S.D. (H) Canonical epithelial and mesenchymal markers of HCC827 EDN1-V5 overexpressing cells and HCC4006Ge-R EDN1 repressing cells. A representative immunoblot from 3 independent experiments.

Supplementary Figure 4. (A) (Left) Relative blood flow in the tumors captured using a Laser Doppler flowmeter. Error bars: S.D. t-test. ** p<0,01. (Center) Microvessel density of CD31 stained sections of HCC827 and ERC4 xenografts. (Right) Representative IHC analyses from HCC827 and ERC4 tumor xenografts using anti-ACTA2 (αSMA) antibody. Scale 100 μM. (B) Mass spectrometry-based quantification of gefitinib in HCC827 and ERC4 tumor xenografts normalized with total protein. The tumor bearing mice were treated with gefitinib (20 mg/kg) for 1 hour prior to sacrifice and harvesting tumors. Error bars: S.D. t-test. **** p<0,0001. (C) $5x10^6$ of the HCC4006 cells were grafted on flanks of mice and allowed to grow for 14 days prior to treatments with vehicle, gefitinib (20 mg/kg), bosentan (200 mg/kg) or combination treatment. Tumor volumes were measured weekly. Error bars: S.D. t-test. * p<0,05. (D) Body weight (gr) of the mice was measured weekly. (E) EDN1 secretion from the HCC4006 tumor xenografts treated with vehicle or gefitinib (20 mg/kg) was quantified normalized with total protein. Error bars: SEM. t-test. * p<0,05. (F) The quantification of allelic prevalence of EGFR T790M *vs.* total EGFR in tumors from vehicle, gefitinib and gefitinib and bosentan groups, using ddPCR. Error bars: SD. One-way ANOVA. N.S. Not significant. (G) Tumors from vehicle, acute gefitinib (1-hour

treatment), chronic gefitinib and chronic gefitinib and bosentan treated mice were homogenized and pEGFR (Y1068), tEGFR and vinculin were analyzed in the lysates.

Supplemental Figure 5. (A) Graphical summary depicting the impact of EDN1 secretion from tumors on blood vessels and rationale for EDNR antagonist and TKI combination therapy.