**Supplemental Data**

**Supplemental Materials and Methods**

**Reagents.** The antibodies for phospho-STAT3 (#9138), and STAT3 (#9139) were purchased from Cell Signaling. The antibody for phospho-VEGFR2 was from Abcam (#ab135776). The antibody for phospho-VEGFR3 was from Cell Applications (#CY1115). The antibody for VEGFR3 was from Santa Cruz (#SC-20734).

**Sphere Formation in Three Dimensional Conditions.** Overlay model of three dimensional cultures was prepared based on previously described protocols (1). Briefly, 48-well plates were filled with 80 l/well of MatrigelTM (BD Biosciences) and polymerized at 37 ℃ for 30 min. 5000 cells per well were then seeded in 400 l 2% MatrigelTM medium, and incubated without vibration at 37 ℃ for 4 days to allow sphere formation.

**Quantification of western blot data.** Data quantification was processed with Quantity One software (Bio-Rad). The phosphorylation of RTKs were quantified relative to -tubulin and then processed for nonlinear regression fitting to generate dose curves using Prism version 6.01 (GraphPad Software). The goodness of fit was demonstrated by r squared. The IC99 values were calculated using following equation: ICF=(F/(100-F))1/H×IC50. F is 99 for IC99; H is the hillslope of the curve.

**Statistical analysis.** One-way ANOVA with Tukey post-hoc test was used for Fig. S2E and S4J. Two-way ANOVA with Tukey post-hoc test was used for Fig. S5. Statistical significance was established for *p*<0.05 (\*) and *p*<0.01 (\*\*).

**Supplemental References**

1. Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. Nat Rev Cancer. 2005;5:675-88.

**Supplemental Figure Legends**

**Supplementary Figure S1.** Multiple RTKs were activated in cancer cells.

(A-C) Frequency of activated RTK numbers in cancer cell lines (A), primary cancers (B) and mouse cancer xenografts (C).

(D and E) Frequency of activated RTK numbers in literature reported cancer cell lines (D) and primary cancers (E). See detailed sample information in Supplemental Table S2 and S3.

(F) Activation frequencies of 42 RTKs in overall 78 cancer samples. The receptors were clustered according to their RTK family classification. Red dash line indicated a frequency of 20%.

**Supplementary Figure S2.** Targeting activated RTKs was essential to inhibit cancer cell growth.

(A) H522 cells were treated with DMSO (con) or 2 M LAP for 2 h, then processed for phospho-RTK arrays. The RTK signals were normalized to Her2 phosphorylation under control treatment. Error bars represent mean±SEM.

(B) Western blot of H522 cells that were treated with different doses of LAP for 2 h.

(C) H522 cells were treated with LAP at various concentrations and cell viabilities were monitored by xCELLegence system.

(D) BEL7404 cells were treated with LAP+OSI (1:1, 500 nM or 20 M for each drug) for 72 h. Then cells were double-stained with membrane-permeable dye calcein AM and membrane-impermeable dye PI. Scale bars indicate 100 m.

(E) Photographed sphere formation of SMMC7721 cells that were treated with DMSO (con), 500 nM LAP, 500 nM OSI, 50 nM JNJ or the LAP+OSI+JNJ combination for 4 days in a three-dimensional environment (top). Scale bar represents 100 m. Spheroid numbers (bottom left) and sizes (bottom right) were quantitatively analyzed. The statistical analysis was performed by one-way ANOVA with Tukey post-hoc test. ns, not significant; \*, *p*<0.05; \*\*, *p*<0.01. Error bars represent mean±SEM.

**Supplementary Figure S3.** The RTK phosphorylation profile-based combinational treatment was cell-type independent but RTK phosphorylation pattern-dependent.

(A-C) Dose-dependent inhibitions of LAP (A), OSI (B) and JNJ (C) on their primary targets were evaluated by western blots in BEL7404, SMMC7721, WiDr and DU145 cells after a 2 h drug treatment. The RTK phosphorylation levels were quantified relative to -tubulin, and then processed for nonlinear regression fitting to generate the dose curves (right panels). The goodness of fit was demonstrated by r squared. The IC50s and IC99s for each drug on their primary targeting-RTKs in different cells were calculated. A quantitative summary of the inhibition of RTK phosphorylation in different cell lines was shown in Supplementary Tab. S4.

(D) The expressions and activations of RTK signaling molecules in nine cancer cell lines were detected by western blots. Total cell lysates with equal protein weight were loaded for each kind of cells.

(E) DU145 cells were treated with DMSO (con), single drugs or the LAP+OSI+CRI combination (top), or with LAP+OSI+CRI combinations (10:10:1) at various concentrations, which were illustrated by the doses of LAP (bottom), and cell viabilities were monitored by xCELLegence system.

(F) Re classification of cancer cells in Figure 1A according to their phospho-RTK patterns. Cell lines that were analyzed in Table 2 were highlighted in red.

**Supplementary Figure S4.** Dual inhibition of Erk and Akt phosphorylation and reduction of c-Myc protein level were essential for the RTKi combinations to block cancer cell growth**.**

(A and B) SMMC7721 (A) and WiDr (B) cells were treated with 500 nM LAP, 500 nM OSI, 50 nM JNJ, or drug combinations for the indicated time. The c-Myc, phospho-Akt and phospho-Erk levels in LAP+OSI (full lines) and LAP+OSI+JNJ (dotted lines) treatments were normalized by -tubulin, and quantified to the control treatment (right panel).

(C) DU145 cells were treated with DMSO (con), LAP+OSI (500 nM for each drugs) or LAP+OSI+JNJ (500 nM LAP and OSI, 50 nM JNJ), and cell viabilities were monitored by xCELLegence system.

(D) Phospho-RTK array of LoVo cells. Positive dots were labeled with numbers, and illustrated below the array.

(E) LoVo cells were treated with 500 nM LAP, 50 nM JNJ or combinations of them for 10 min, 1 h and 24 h. The c-Myc, phospho-Akt and phospho-Erk levels in LAP+JNJ treatment were normalized by GAPDH and quantified to control treatment (right panel).

(F) Phospho-RTK array of MDA-MB-453 cells. Positive dots were labeled with numbers, and illustrated below the array.

(G) MDA-MB-453 cells were treated with 500 nM LAP, 500 nM PD (PD173074, FGFR family inhibitor) or combinations of them for the indicated time. The c-Myc, phospho-Akt and phospho-Erk levels in LAP+PD treatment were normalized by GAPDH and quantified to the control treatment (right panel).

(H) Western blots of BEL7404 cells which were treated with 1 M U0126 (U), 0.5 M MK2206 (MK) or combinations of them (U+MK) for 2 h and 20 h.

(I) Western blots of BEL7404 cells which were treated with U (1 M) +MK (0.5 M), LAP or U+MK+LAP for 20 h.

(J) BEL7404 cell viabilities under various drug treatments were detected by 72 h MTT assays. U, 1 M; MK, 0.5 M; LAP, 0.5 M. The statistical analysis was performed by one-way ANOVA with Tukey post-hoc test. \*\*, *p*<0.01. Error bars represent mean±SEM.

**Supplementary Figure S5.** Combinations of multiple RTKis did not cause significant changes of the body weights.

Body weights of the animals in the BEL7404 xenograft models (A and B) and the SMMC7721 xenograft model (C) were shown. Error bars represent mean±SEM. Drug combination groups were compared with vehicle group by two-way ANOVA with Tukey post-hoc test, and no significant differences were found.