Supplementary materials

Kinome reprogramming is a targetable vulnerability in ESR1 fusion-driven breast cancer

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List of Supplementary Materials

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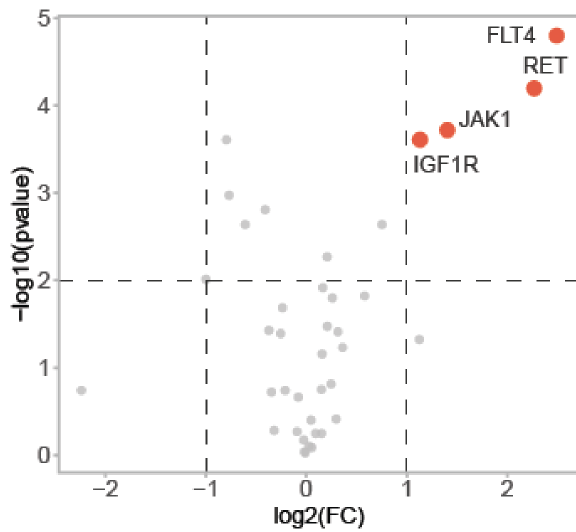
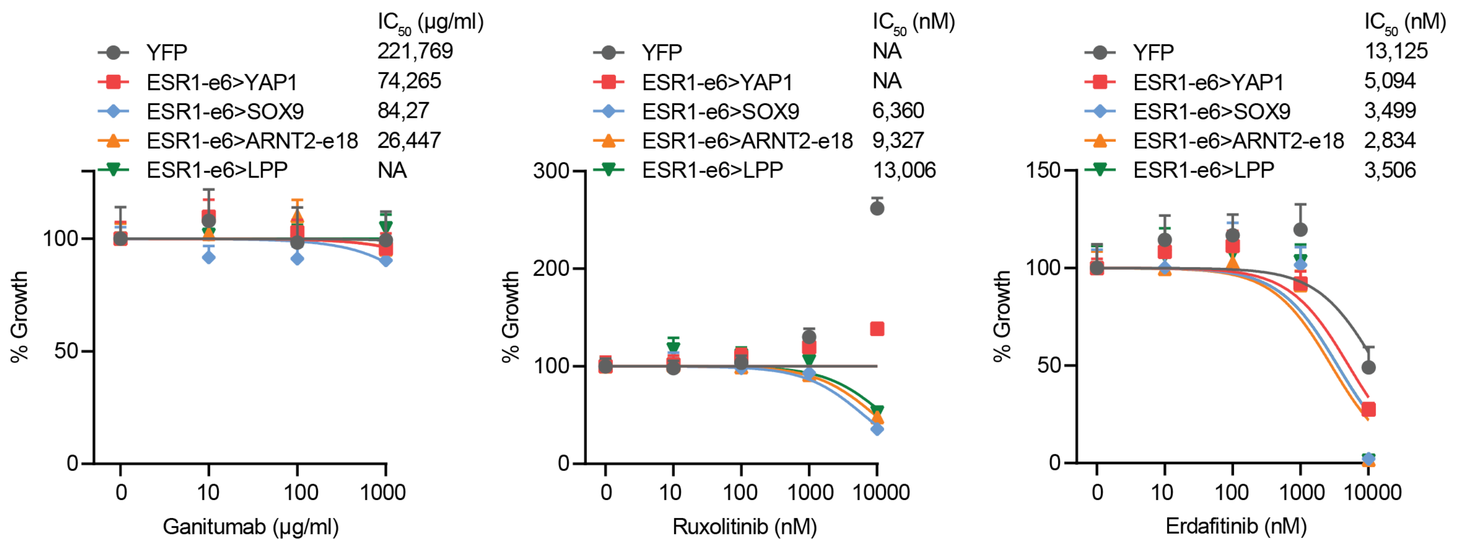
Supplementary Figures

Supplementary figure 1 (related to Figure 1)



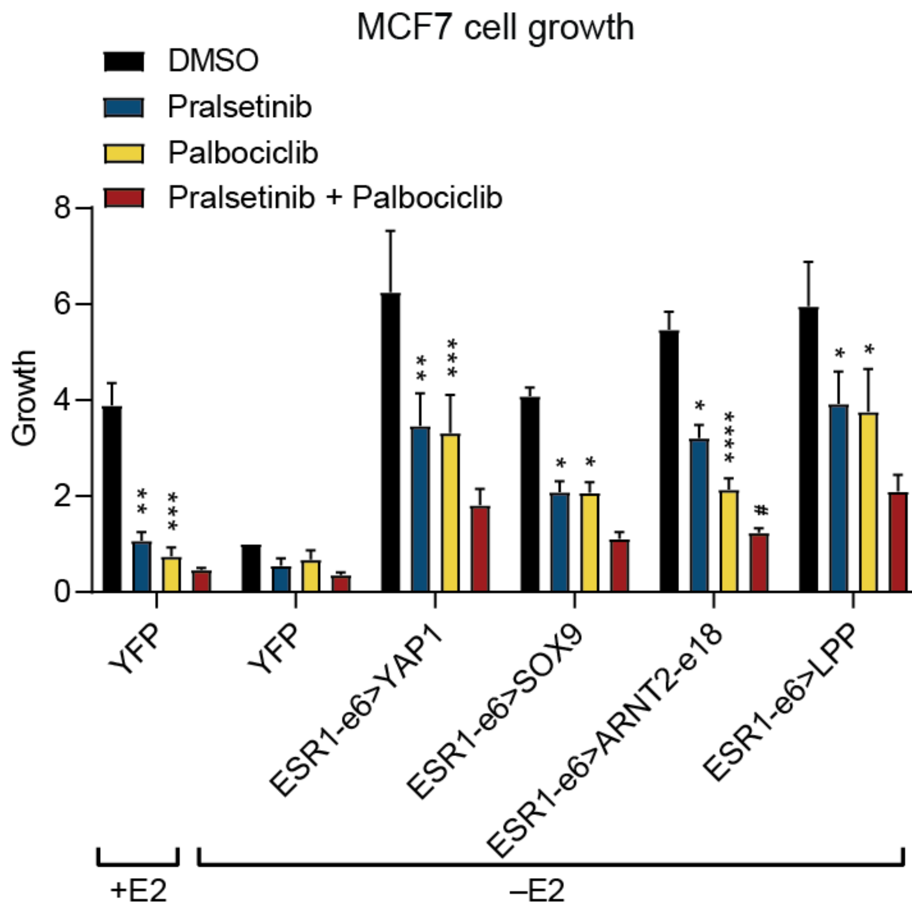
Supplementary figure 1 (related to Figure 1). Volcano plot showing the most significantly upregulated kinases in T47D cells expressing active ESR1 fusions and point mutations compared to those expressing inactive fusions and controls ($p<0.01$, fold change>2).

Supplementary figure 2 (related to Figure 2)



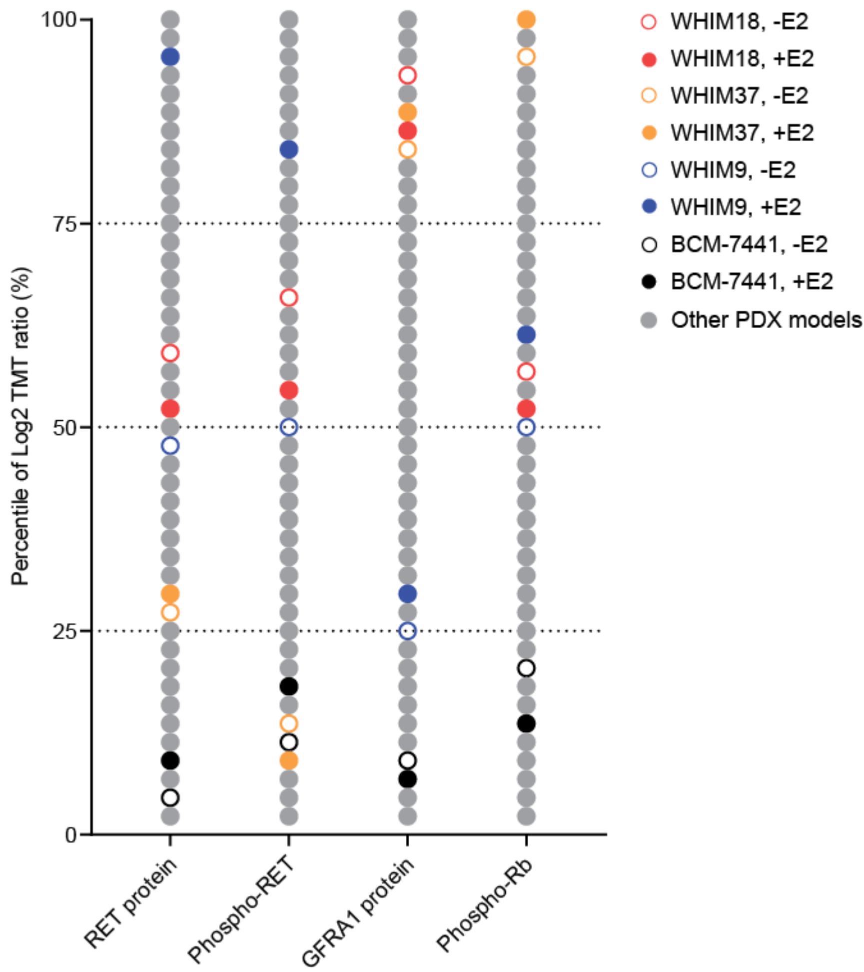
Supplementary figure 2 (related to Figure 2). IC_{50} determination of small molecule inhibitors targeting kinases reprogrammed in T47D cells expressing active ESR1 fusion proteins. T47D cell growth driven by active ESR1 fusions was not suppressed by anti-IGF1R antibody (ganitumab), pan-JAK inhibitor (ruxolitinib), or a pan-FGFR inhibitor (erdafitinib). YFP cells or ESR1 fusion expressing cells were grown in charcoal stripped serum-containing phenol red-free, RPMI media (CSS) media for 7 days. DMSO (vehicle control) or increasing doses of ganitumab, ruxolitinib, or erdafitinib were then added to all the cell lines, and after eight days, cell viability was measured using an alamarBlue assay.

Supplementary figure 3 (related to Figure 2)



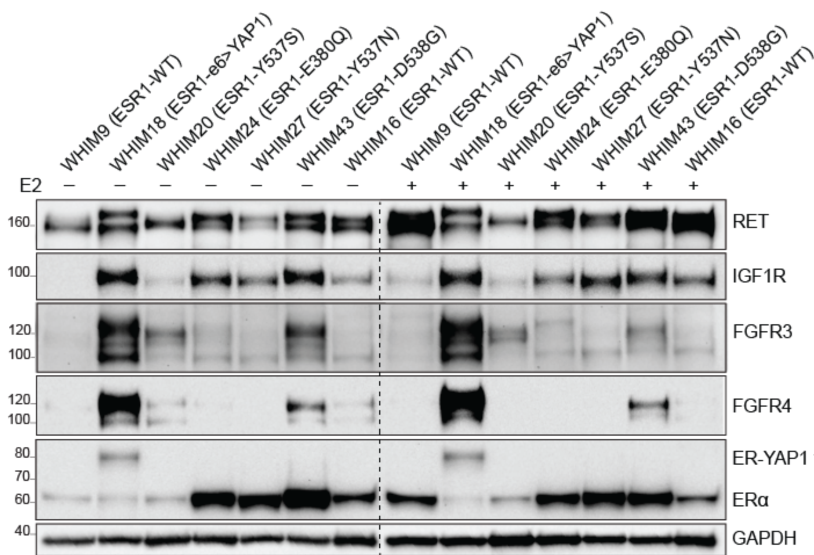
Supplementary figure 3 (related to Figure 2). RET and CDK4/6 inhibitors suppress MCF7 cell growth driven by active ESR1 fusions. Cell growth was assayed in E2-deprived stable cells (mean ± SEM, n=3). Growth reading values were normalized to that of hormone-deprived YFP control cells in the vehicle (+DMSO) group. Two-way ANOVA followed by Tukey's test was used for multiple comparisons for each stable cell line after 500 nM pralsetinib, 100 nM palbociclib, or the combination treatment in the presence or absence of 10 nM E2. Single agent alone compared to DMSO: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Combinatorial treatment compared to pralsetinib alone: $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, and $^{\#\#\#\#}$p<0.0001.

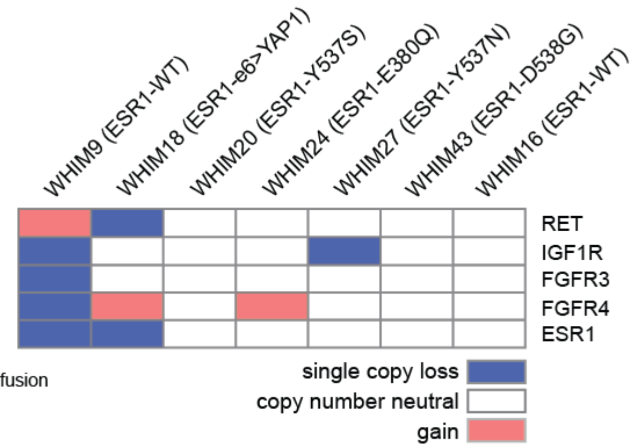
Supplementary figure 4 (related to Figure 3)



Supplementary figure 4 (related to Figure 3). Distribution plot showing the percentiles of RET, phospho-RET, GFRA1, and phospho-Rb proteins among 22 ERα+ PDX models.

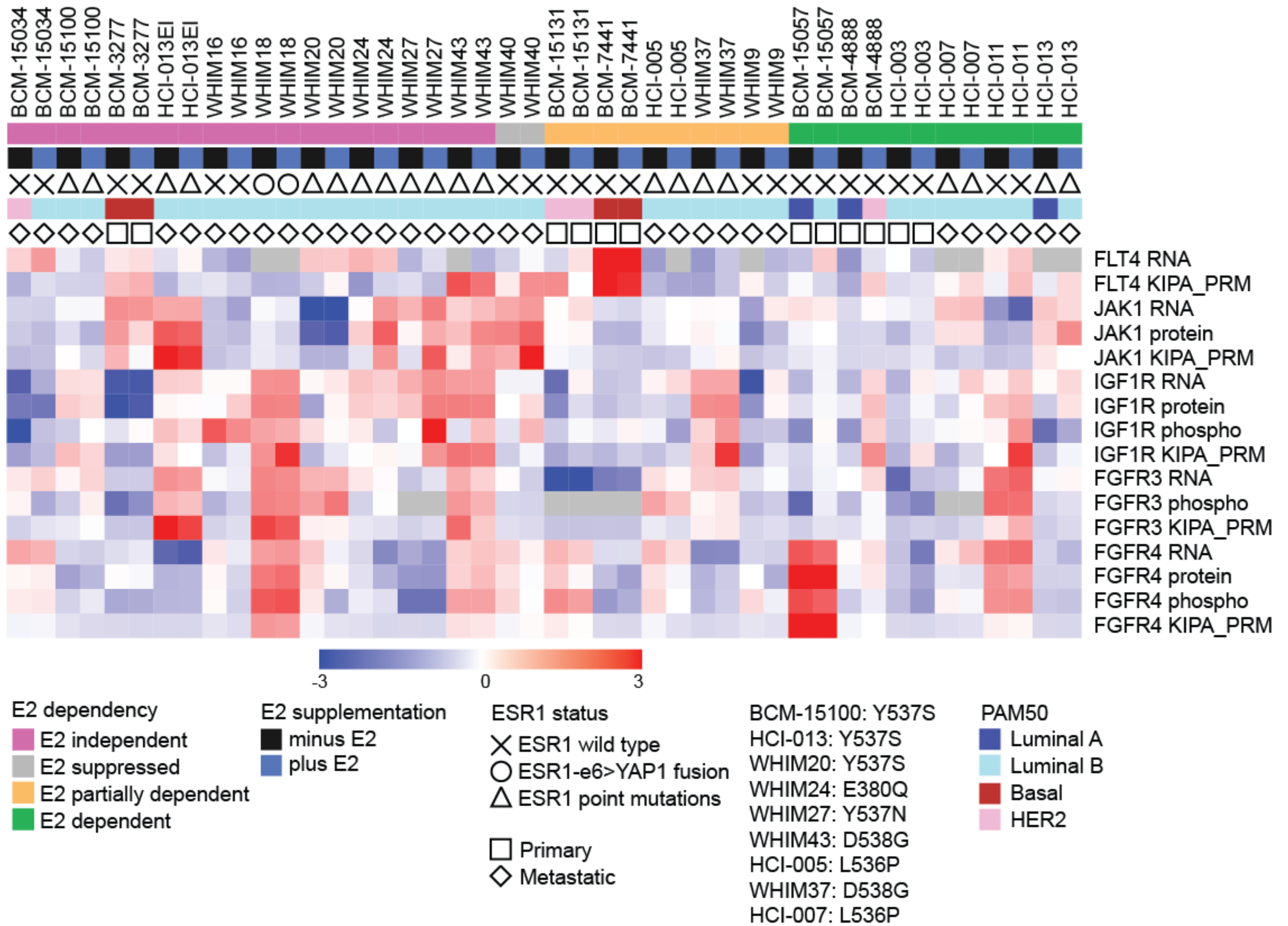
Supplementary figure 5 (related to Figure 3)





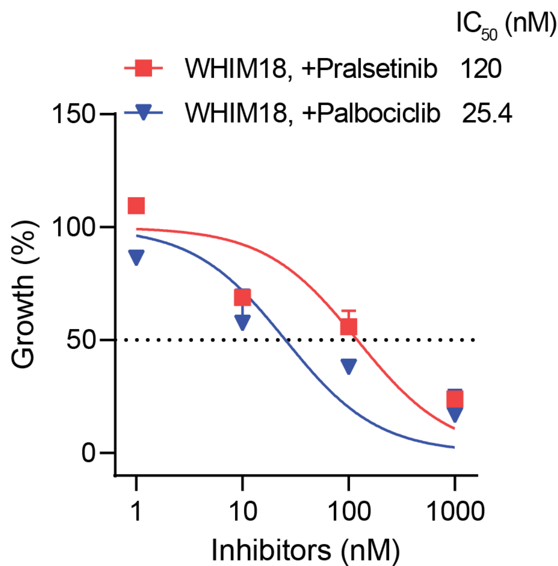
Supplementary figure 5 (related to Figure 3). Validation of kinome expression reprogrammed in an active ESR1-YAP1 fusion expressing PDX (WHIM 18) versus other ERα+ PDX tumors. (A) Immunoblotting of four reprogrammed kinases and ERα proteins in lysates made from 7 ERα+ PDX tumors. GAPDH serves as a loading control. The dashed line indicates two separate blots that were conducted at the same time. **(B)** Elevated RET level in WHIM18 PDX tumors is not due to a gain in genomic copy number. Copy number variation of other kinases in ERα+ PDX tumors is also depicted.

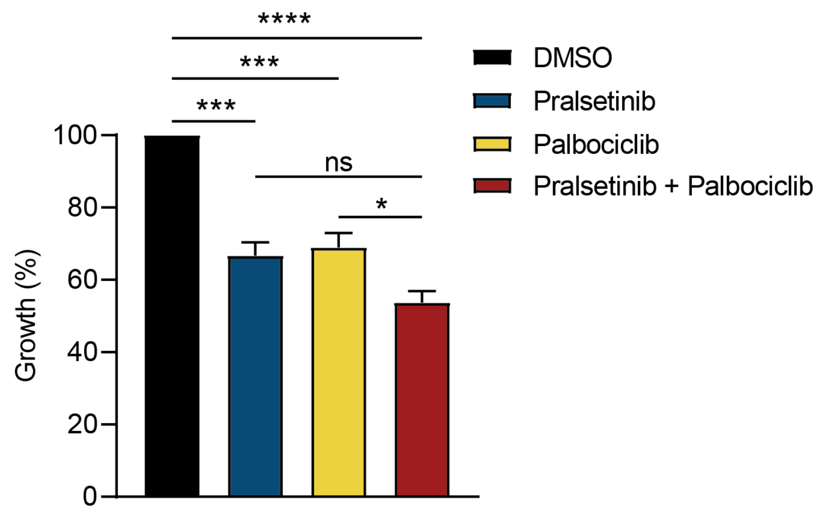
Supplementary figure 6 (related to Figure 3)



Supplementary figure 6 (related to Figure 3). Heatmap showing proteogenomic characterization of additional active ESR1 fusion reprogrammed kinase targets (FLT4, JAK1, IGF1R, FGFR3 and FGFR4) in 22 ERα+ PDX models. Heatmap was depicted in same format as **Figure 3**. Scale bar indicates row z scores of RNA (based on RNA-seq), protein and phospho-protein (based on TMT labeled MS (-/+) IMAC enrichment as log_2 ratios) or KIPA data (log_{10} ratio).

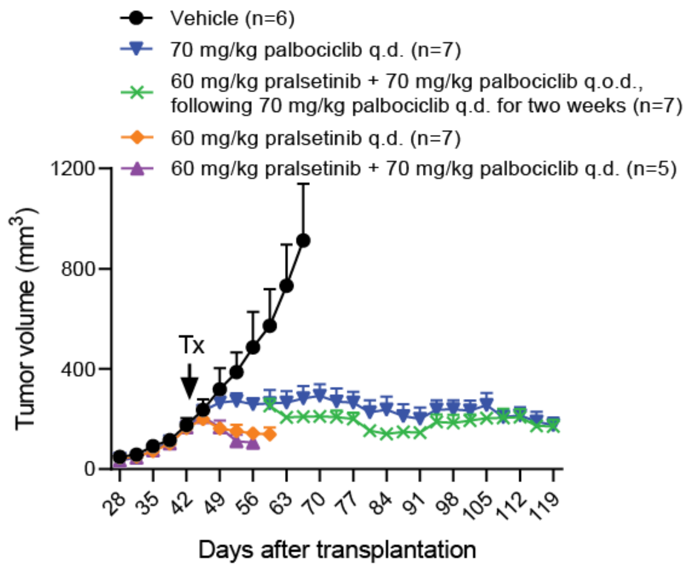
Supplementary figure 7 (related to Figure 5)

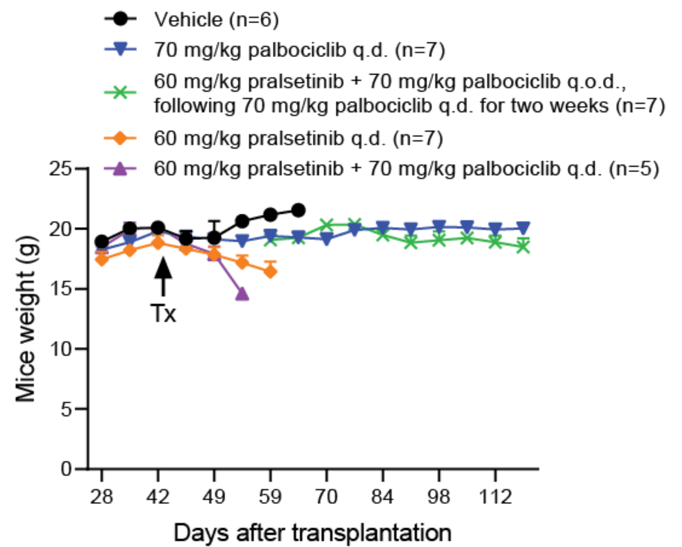


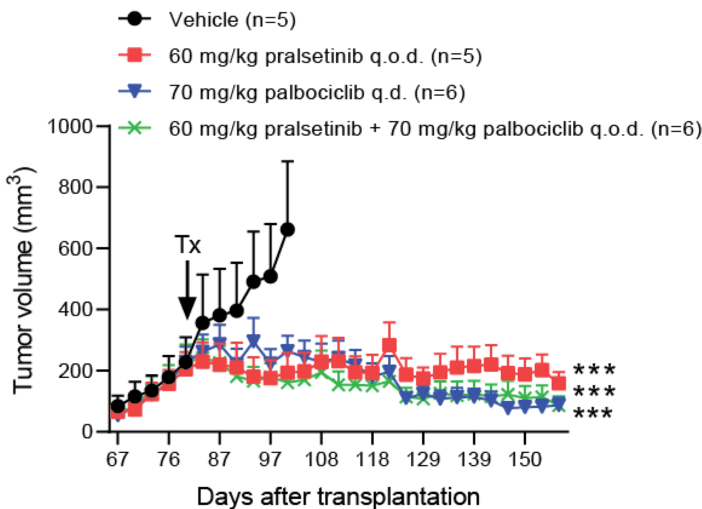


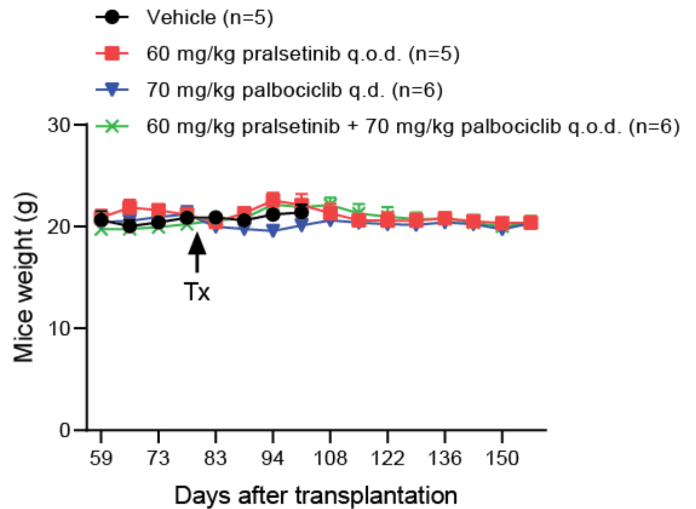
Supplementary figure 7 (related to Figure 5). RET and CDK4/6 inhibitors reduce luciferase-tagged WHIM18 PDxO growth, but the combination of the two inhibitors does not confer much added benefit versus the single agents in untagged WHIM18 PDxOs. (A) WHIM18 PDX cells expressing a luciferase reporter were plated as organoid domes and treated by DMSO or increasing doses of pralsetinib and palbociclib (1 – 1000 nM). After a 14-day treatment, luciferin was added to the domes and the inhibition was quantified by counting photons produced by the luciferase enzyme (mean ± SEM, n=2). **(B)** WHIM18 PDxOs were treated with DMSO, 20 nM pralsetinib, 15 nM palbociclib, or the combination for two weeks. Growth was quantified by a CellTiter-Glo 3D cell viability assay and presented as mean ± SEM, n=3. One-way ANOVA followed by Tukey's test was used for multiple comparisons between each condition. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. ns: not significant.

Supplementary figure 8 (related to Figure 6)









Supplementary figure 8 (related to Figure 6). RET and CDK4/6 inhibitors suppress PDX tumor growth in WHIM18 and WHIM9 mice. All mice in this experiment had intact ovaries and thus would produce endogenous E2, which allows for higher RET expression in the WHIM9 model. The main purpose of this experiment was to test efficacy and safety of different dosings of the RET inhibitor pralsetinib as a single agent or in combination with the CDK4/6 inhibitor palbociclib. **(A)** Tumor volumes of WHIM18 PDX (mean ± SEM, n in each arm is indicated). Arrow indicates the start of treatments (Tx), including vehicle, pralsetinib (60 mg/kg once a day (q.d.) or every other day (q.o.d.)), palbociclib (70 mg/kg), or combination drug containing chow diets. **(B)** Mice body weight of WHIM18 PDX. Apparent toxicity (decreased body weight) was observed with 60 mg/kg pralsetinib given q.d. as a single agent and in combination with palbociclib. **(C)** Tumor volumes of WHIM9 PDX (mean ± SEM, n in each arm is indicated). Arrow indicates the start of treatments (Tx), including vehicle, pralsetinib (60 mg/kg given q.o.d. only), palbociclib, or combination drug containing chow diets. **(D)** Mice body weight of WHIM9 PDX revealed no toxicity using these drug dosings. One-way ANOVA followed by Tukey's test was used to compare each treatment to vehicle arm (*$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$). P-value described significance of tumor growth rates (slopes) derived from tumor volumes on the day of randomization to endpoint.