

Dual mTOR Kinases MLN0128 Inhibitor Sensitizes HR+/HER2+ Breast Cancer Patient-derived Xenografts to Trastuzumab or Fulvestrant

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Characterization of two HR+/HER2+ breast patient-derived xenografts (PDXs), COH-SC1 and COH-SC31. (A) Representative H&E staining sections of the two PDXs and corresponding tumors. Scale bar, 100 μ m. COH-SC1 tumor was moderately differentiated and COH-SC31 was poorly differentiated. **(B)** Immunohistochemical profiles of ER, PR and HER2 expression in the two PDXs and the corresponding tumors. Scale bar, 100 μ m. **(C)** Western blot analysis of ER α expression levels in triplicate samples of COH-SC1, COH-SC31, and a positive control cell line- MCF-7. Short and long exposure were shown for better visualization of ER α expression level among the samples

Figure S2. Uterus and tumor weight of mice bearing COH-SC1 and COH-SC31 tumors in respond to estrogen treatment. COH-SC1 (left) and COH-SC31 (right) tumors were transplanted into ovariectomized mice with (E2, red) or without (CTL, blue) 1mg of E2 supplementation. Uterus **(A)** and tumor weight **(B)** were then measured upon estrogen treatment. Measurements shown as Mean \pm SEM. In COH-SC1 model (n=9), uterus weight in CTL and E2 is 0.015 \pm 0.01 g and 0.145 \pm 0.008 g, respectively; tumor

weight in CTL and E2 groups is 0.327 ± 0.099 g and 0.926 ± 0.088 g, respectively. In COH-SC31 model (n=5), uterus weight in CTL and E2 is 0.016 ± 0.001 g and 0.168 ± 0.016 g, respectively; tumor weight in CTL and E2 groups is 0.101 ± 0.031 g and 0.530 ± 0.133 g, respectively. $p < 0.01$, Student t-test.

Figure S3. Observations of body weight in response to drug treatment *in vivo*.

(A) Four-week treatment of trastuzumab (10 mg/kg/twice intraperitoneal injection per week; TRA) or sterile saline (CTRL) was performed on the intact mice bearing COH-SC1 (n=7) or E2-supplemented COH-SC31 (n=5) tumors. Body weight was monitored twice per week and summarized as Mean \pm SEM with two-way ANOVA analysis for p value. **(B)** The intact mice bearing COH-SC1 (n=7) or E2-supplemented COH-SC31 (n=5) tumors were randomized for four-week treatment of MLN0128 (0.3 mg/kg/six days of gavage per week; MLN), fulvestrant (5 mg/once subcutaneous injection per week; FUL), and combination (Comb). Body weight was monitored twice per week and summarized as Mean \pm SEM with two-way ANOVA analysis for p value.

Figure S4: Pathway analysis of up-regulated loci in COH-SC1 and COH-SC31 transcriptomes.

(A) IPA analysis of 529 common up-regulated loci in both COH-SC1 and COH-31 models and revealed that PI3K/AKT/mTOR and RTK/HER2 signaling are activated in HR+/HER2+ cancers ($p < 0.001$). Genes in PI3K/AKT/mTOR pathway were

highlighted in red; regulated genes were shown as grey nodes; non-regulated genes but those associated with the regulation of some of these genes were shown as white nodes.

(B) Pathway analysis of differential expression genes/proteins between COH-SC1 and COH-SC31 models using IPA. The expression levels of estrogen/ER α signaling components were significantly higher in COH-SC31 than in COH-SC1. Red nodes, higher expressed loci; green nodes, lower expressed loci. The molecular function of each node is denoted (see boxed inset). Lines indicate direct interaction between the products of genes. Lines with arrows indicate that the source gene “acts on” the target gene.

Figure S5: Expression patterns of up-regulated proteins in COH-SC1 and COH-SC31 PDXs assayed by RPPA. Compared to the reference RPPA data generated from an ER+/HER2- PDX, up-regulated phosphor-/pan-proteins associated with **(A)** the HER2+/ER and **(B)** the PI3K/AKT/mTOR signaling in COH-SC1 (green columns) and COH-SC31 (red columns) were individually presented in bar graphs. **(C)** Western blot analysis of activated ER and AKT signaling. Phosphorylation statuses of AKT at serine 473 and ER α at serine 118 were detected accompanied with the corresponding pan-protein levels. β -actin protein was used as loading control.

Figure S6 *In vivo* efficacy examination of trastuzumab and/or MLN0128 on

COH-SC1 PDX. Four-week treatment of trastuzumab (10 mg/kg/twice intraperitoneal injection per week; TRA) and/or MLN0128 (0.3 mg/kg/six days of gavage per week; MLN) or sterile saline (CTRL) was performed on the intact mice bearing COH-SC1 tumors (n=7). Tumor volume **(A)**, body weight **(B)**, and tumor weight **(C)** were monitored and summarized as Mean±SEM with two-way ANOVA analysis for *p* value. **(D)** Quantification of ER staining and mitoses from IHC-assayed tumor sections. Three representative examples per treatment of COH-SC1 tumors as indicated were subjected to ER IHC and HE staining. Either ER-positive scoring or mitotic counts was performed and calculated by monitoring 15 40X fields.