Supplemental Figure S1 shows the differential expression of PKD3 in invasive ductal carcinoma (IDC) by HER2 status.

Supplemental Figure S1 (Supplemental Material to Figure 1): PKD3 intensity in HER2+ and HER- IDC was determined using Aperio positive pixel count algorithm in the ImageScope software (Aperio, Vista, CA). P values were acquired with the student’s t-test using Prism v5 software. There was no statistically-significant difference between samples.
Supplemental Figure S2 shows the status of PKD1, PKD2 and PKD3 expression in invasive breast cancer cell lines.

Supplemental Figure S2 (Supplemental Material to Figure 3): MCF10A, MDA-MB-231, BT20, MDA-MB-468, HCC1954 and MCF7 cell lysates were analyzed by Western blot for the expression of PKD1 (anti-PKD1), PKD2 (anti-PKD2) and PKD3 (anti-PKD3), or β-actin (anti-β-actin) as a loading control.
Supplemental Figure S3 depicts the effect of PKD3 knockdown on MDA-MB-231 cell proliferation

Supplemental Figure S3 (Supplemental Material to Figure 3): MDA-MB-231 cells were transfected with lentivirus harboring control shRNA (scr-shRNA) or two different shRNAs specifically-targeting PKD3 (PKD3-shRNA#1 and PKD3-shRNA#2) (Western blot control shown in Fig. 3A). 48 hours after initial infection, cell proliferation was measured by MTT assay for 72 h. The data represent the mean ± SEM derived from three independent experiments. The asterisks indicate statistical significance.
Supplemental Figure S4 depicts the effect of PKD3 knockdown on MDA-MB-231 cell migration

Supplemental Figure S4 (Supplemental Material to Figure 3): MDA-MB-231 cells were transfected with lentivirus harboring control shRNA (scr-shRNA) or two different shRNAs specifically-targeting PKD3 (PKD3-shRNA#1 and PKD3-shRNA#2) (Western blot control shown in Fig. 3A). 48 hours after initial infection, cells were seeded on transwell filters and Transwell migration assays were performed as described in Materials & Methods. The asterisks indicate statistical significance.
Supplemental Figure S5 shows the effect of PKD3 knockdown on tumor growth (A-C) and local invasion (D).

Supplemental Figure S5 (Supplemental Material to Figure 3): MDA-MB-231 cells stably expressing control shRNA (scr-shRNA) or two different shRNAs specifically-targeting PKD3 (PKD3-shRNA#1 and PKD3-shRNA#2) were injected into the mfp of female NOD scid mice. A: Tumor growth was continuously monitored with caliper measurement. At the end point, primary tumors were stained by IHC for the expression of PKD3. Scale bar is 50 μm. B: Primary tumors of different groups were stained by IHC for the expression of Ki67 and cleaved-caspase 3. The bars represent 100 μm. C: Statistical analysis was performed using Aperio positive pixel count algorithm in the Imagescope software. P values were acquired with the student’s t-test. The asterisk indicates statistical significance. D: Samples of primary tumor were stained with H&E. Areas where tumors connect with mouse mammary gland tissue were enhanced.
Supplemental Figure S6 depicts the effect of CRT0066101 on MDA-MB-231 cell proliferation

Figure S6 (Supplemental Material to Figure 4): MDA-MB-231 cells were seeded in 96-well plates and treated with 2.5 μM CRT0066101 or DMSO (control). Cell proliferation was measured by MTT assay for 48 hours. The data represent the mean ± SEM derived from three independent experiments. The asterisks indicate statistical significance.
Supplemental Figure S7 depicts the effect of CRT0066101 on MDA-MB-231 cell migration.

Supplemental Figure S7 (Supplemental Material to Figure 4): MDA-MB-231 cells were seeded on transwell filters in media containing 2.5 μM CRT0066101 or DMSO (control). and Transwell migration assays were performed as described in Materials & Methods. The asterisks indicate statistical significance.
Supplemental Figure S8 shows the impact of PKD2 and PKD3 knockdown alone or in combination on MDA-MB-231 cell migration.

Supplemental Figure S8 (Supplemental Material to Figure 4): MDA-MB-231 cells infected with control shRNA (scr-shRNA) or PKD3-shRNA#1 were transfected with control vector (Control) or a siRNAs specifically-targeting PKD2 (PKD2-siRNA). A: 48 hours after initial transfection, a fraction of the cells was lysed and PKD2 and PKD3 knockdown was verified by Western blot. B, C: 48 hours after initial transfection, cells were seeded directly on transwell filters (B, migration assay) or on Matrigel-coated transwell filters (C, invasion assay) and Transwell assays were performed as described in Materials & Methods. The asterisks indicate statistical significance.
Supplemental Figure S9 depicts the effect of CRT0066101 on tumor growth over time in orthotopic xenografts.

Supplemental Figure S9 (Supplemental Material to Figure 5): MDA-MB-231 cells expressing luciferase were injected into the mfp of female NOD scid mice. After establishment of primary tumors, mice were treated orally with 80 mg/kg CRT0066101 or vehicle every other day. Tumor volume (caliper measurement) was measured every week. Asterisks indicate statistic significance with p ≤ 0.05.