Supplementary Information

Proteomics-derived basal biomarker DNA-PKcs is associated with intrinsic subtype and

long-term clinical outcomes in breast cancer

Karama Asleh, Nazia Riaz, Angela S. Cheng, Dongxia Gao, Samuel C. Y. Leung, Meenakshi

Anurag, Torsten O. Nielsen

Supplementary Information includes:

- 1. Supplementary Figures (page 2-6)
- 2. Supplementary Table (pages 7-8)
- 3. Caption for Supplementary Data 1 (page 9)

а



b



Supplementary Fig. 1: Prognostic significance of DNA-PKcs expression by

immunohistochemistry in different ER subgroups of the BC Cancer series. Kaplan Meier

curves for BCSS in ER+ cases (a), and ER- cases (b). Abbreviations: BCSS, breast cancer specific survival; ER, estrogen receptor.



Supplementary Fig. 2: Prognostic stratification of core basal cases by DNA-PKcs and stromal TILs in the BC Cancer series using a higher (30%) cutpoint. Within core basal tumors, presence of sTILs (\geq 30% vs < 30%) in combination with low DNA-PKcs expression is associated with better BCSS. Abbreviations: BCSS, breast cancer specific survival; HR, hazard ratio, TIL, tumor infiltrating lymphocytes.



Supplementary Fig. 3: Boxplots showing that expression levels of *PRKDC*, as derived from the 395 ER+ breast cancers in TCGA are significantly associated with basal-like PAM50 intrinsic subtype. Centre bar denotes median, and the upper and lower box boundaries delineate the third and first quartiles.



Supplementary Fig. 4: Scatter plot showing correlation between morphologically assessed stromal TILs on full-face sections and tissue microarrays in BC Cancer series (n=317). Each data point represents one case. The Spearman correlation coefficient (*rho*) and p-values are displayed. Abbreviations: TILs, tumor infiltrating lymphocytes; TMA, tissue microarray.

Supplementary Tables:

Supplementary Table 1: Association of DNA-PKcs expression with clinicopathological

features in the UBC series

$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Clinicopathological Features	DNA-PKcs Exp	pression (IHC score)	<i>P</i> -value
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Low (< 6)	High (≥ 6)	-
Age at diagnosis0.51< 50		n = 233	n = 67	
	Age at diagnosis			0.51
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	< 50	87 (37.3)	28 (41.8)	
$\begin{array}{c cccccc} Tumor size (cm) & 0.97 \\ \leq 2 \ cm & 135 \ (57.9) & 39 \ (58.2) \\ >2 \ cm & 98 \ (42.1) & 28 \ (41.8) \end{array} & \\ \hline \\ Tumor grade & <0.001 \\ \hline \\ Grade 1 \ or 2 & 145 \ (63) & 22 \ (32.8) \\ \hline \\ Grade 3 & 85 \ (37) & 45 \ (67.2) \end{array} & \\ \hline \\ Axillary lymph node status & 0.10 \\ Positive & 76 \ (35.5) & 31 \ (48.4) \\ Negative & 138 \ (64.5) & 33 \ (51.6) \end{array} & \\ \hline \\ Lymphovascular invasion & 0.01 \\ Positive & 46 \ (20.6) & 24 \ (35.8) \\ Negative & 177 \ (79.4) & 43 \ (64.2) \end{array} & \\ \hline \\ Ki-67 \ (\%) & < & <0.001 \\ < 14 & 147 \ (64.5) & 24 \ (36.4) \\ \geq 14\% & 81 \ (35.5) & 42 \ (63.6) \end{array} & \\ \hline \\ ER expression & 0.007 \\ Negative & 189 \ (82.2) & 44 \ (66.7) \\ Progesterone receptor expression \\ Negative & 160 \ (69.9) & 38 \ (57.6) \end{array} & \\ \hline \\ HER2 expression & 0.006 \\ Negative & 160 \ (69.9) & 38 \ (57.6) \\ \hline \\ HER2 expression & 0.14 \\ Negative & 10 \ (4.4) & 8 \ (12.9) \end{array} & \\ \hline \\ EGFR expression & 0.004 \\ Negative & 211 \ (91.3) & 51 \ (78.5) \\ Positive & 20 \ (8.7) & 14 \ (21.5) \end{array}$	\geq 50	146 (62.7)	39 (58.2)	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Tumor size (cm)			0.97
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\leq 2 \text{ cm}$	135 (57.9)	39 (58.2)	
$\begin{array}{c ccccc} Tumor grade & << 0.001 \\ \hline \mbox{Grade 1 or 2} & 145 (63) & 22 (32.8) \\ \hline \mbox{Grade 3} & 85 (37) & 45 (67.2) \\ \hline \mbox{Axillary lymph node status} & 0.10 \\ \hline \mbox{Positive} & 76 (35.5) & 31 (48.4) \\ \hline \mbox{Negative} & 138 (64.5) & 33 (51.6) \\ \hline \mbox{Lymphovascular invasion} & 0.01 \\ \hline \mbox{Positive} & 46 (20.6) & 24 (35.8) \\ \hline \mbox{Negative} & 177 (79.4) & 43 (64.2) \\ \hline \mbox{Ki-67 (%)} & < 0.001 \\ < 14 & 147 (64.5) & 24 (36.4) \\ \geq 14\% & 81 (35.5) & 42 (63.6) \\ \hline \mbox{ER expression} & 0.007 \\ \hline \mbox{Negative} & 41 (17.8) & 22 (33.3) \\ \hline \mbox{Positive} & 189 (82.2) & 44 (66.7) \\ \hline \mbox{Progesterone receptor expression} & 0.06 \\ \hline \mbox{Negative} & 69 (30.1) & 28 (42.4) \\ \hline \mbox{Positive} & 160 (69.9) & 38 (57.6) \\ \hline \mbox{HER2 expression} & 0.14 \\ \hline \mbox{Negative} & 217 (95.6) & 54 (87.1) \\ \hline \mbox{Positive} & 10 (4.4) & 8 (12.9) \\ \hline \mbox{EGFR expression} & 0.004 \\ \hline \mbox{Negative} & 211 (91.3) & 51 (78.5) \\ \hline \mbox{Positive} & 20 (8.7) & 14 (21.5) \\ \hline \mbox{Positive} & 20$	>2 cm	98 (42.1)	28 (41.8)	
$\begin{array}{c cccccc} Grade 1 \mbox{ or } 2 & 145 \ (63) & 22 \ (32.8) \\ Grade 3 & 85 \ (37) & 45 \ (67.2) \\ \hline \\ \hline \\ Axillary lymph node status & 0.10 \\ Positive & 76 \ (35.5) & 31 \ (48.4) \\ Negative & 138 \ (64.5) & 33 \ (51.6) \\ \hline \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ $	Tumor grade			< 0.001
Grade 385 (37)45 (67.2)Axillary lymph node status0.10Positive76 (35.5)Negative138 (64.5)138 (64.5)33 (51.6)Lymphovascular invasion0.01Positive46 (20.6)24 (35.8)0.01Negative177 (79.4)43 (64.2)43 (64.2)Ki-67 (%)<0.001	Grade 1 or 2	145 (63)	22 (32.8)	
Axillary lymph node status0.10Positive76 (35.5)31 (48.4)Negative138 (64.5)33 (51.6)Lymphovascular invasion0.01Positive46 (20.6)24 (35.8)Negative177 (79.4)43 (64.2)Ki-67 (%)<	Grade 3	85 (37)	45 (67.2)	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Axillary lymph node status			0.10
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Positive	76 (35.5)	31 (48.4)	
$\begin{array}{c cccccc} Lymphovascular invasion & 0.01 \\ Positive & 46 (20.6) & 24 (35.8) \\ Negative & 177 (79.4) & 43 (64.2) \\ \hline Ki-67 (\%) & < 0.001 \\ < 14 & 147 (64.5) & 24 (36.4) \\ \geq 14\% & 81 (35.5) & 42 (63.6) \\ \hline ER expression & 0.007 \\ Negative & 41 (17.8) & 22 (33.3) \\ Positive & 189 (82.2) & 44 (66.7) \\ \hline Progesterone receptor expression & 0.06 \\ Negative & 69 (30.1) & 28 (42.4) \\ Positive & 160 (69.9) & 38 (57.6) \\ \hline HER2 expression & 0.14 \\ Negative & 217 (95.6) & 54 (87.1) \\ Positive & 10 (4.4) & 8 (12.9) \\ \hline EGFR expression & 0.004 \\ Negative & 20 (8.7) & 14 (21.5) \\ \hline \end{array}$	Negative	138 (64.5)	33 (51.6)	
$\begin{array}{c cccccc} Positive & 46 (20.6) & 24 (35.8) \\ Negative & 177 (79.4) & 43 (64.2) \\ \hline Ki-67 (\%) & < 0.001 \\ < 14 & 147 (64.5) & 24 (36.4) \\ \geq 14\% & 81 (35.5) & 42 (63.6) \\ \hline ER expression & & 0.007 \\ Negative & 41 (17.8) & 22 (33.3) \\ Positive & 189 (82.2) & 44 (66.7) \\ \hline Progesterone receptor expression & & 0.06 \\ Negative & 69 (30.1) & 28 (42.4) \\ Positive & 160 (69.9) & 38 (57.6) \\ \hline HER2 expression & & & 0.14 \\ Negative & 217 (95.6) & 54 (87.1) \\ Positive & 10 (4.4) & 8 (12.9) \\ \hline EGFR expression & & & 0.004 \\ Negative & 211 (91.3) & 51 (78.5) \\ Positive & 20 (8.7) & 14 (21.5) \\ \hline \end{array}$	Lymphovascular invasion			0.01
Negative $177 (79.4)$ $43 (64.2)$ Ki-67 (%)<0.001	Positive	46 (20.6)	24 (35.8)	
$ \begin{array}{c ccccc} \mbox{Ki-67 (\%)} & <& <<0.001 \\ < 14 & 147 (64.5) & 24 (36.4) \\ \ge 14\% & 81 (35.5) & 42 (63.6) \\ \hline \mbox{ER expression} & & 0.007 \\ \mbox{Negative} & 41 (17.8) & 22 (33.3) \\ \mbox{Positive} & 189 (82.2) & 44 (66.7) \\ \hline \mbox{Progesterone receptor expression} & & 0.06 \\ \mbox{Negative} & 69 (30.1) & 28 (42.4) \\ \mbox{Positive} & 160 (69.9) & 38 (57.6) \\ \hline \mbox{HER2 expression} & & 0.14 \\ \mbox{Negative} & 217 (95.6) & 54 (87.1) \\ \mbox{Positive} & 10 (4.4) & 8 (12.9) \\ \hline \mbox{EGFR expression} & & 0.004 \\ \mbox{Negative} & 211 (91.3) & 51 (78.5) \\ \mbox{Positive} & 20 (8.7) & 14 (21.5) \\ \hline \end{array} $	Negative	177 (79.4)	43 (64.2)	
	Ki-67 (%)			< 0.001
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	< 14	147 (64.5)	24 (36.4)	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\geq 14\%$	81 (35.5)	42 (63.6)	
Negative $41 (17.8)$ $22 (33.3)$ Positive $189 (82.2)$ $44 (66.7)$ Progesterone receptor expression 0.06 Negative $69 (30.1)$ $28 (42.4)$ Positive $160 (69.9)$ $38 (57.6)$ HER2 expression 0.14 Negative $217 (95.6)$ $54 (87.1)$ Positive $10 (4.4)$ $8 (12.9)$ EGFR expression 0.004 Negative $211 (91.3)$ $51 (78.5)$ Positive $20 (8.7)$ $14 (21.5)$	ER expression			0.007
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Negative	41 (17.8)	22 (33.3)	
$\begin{array}{c ccccc} Progesterone receptor expression & 0.06 \\ Negative & 69 (30.1) & 28 (42.4) \\ Positive & 160 (69.9) & 38 (57.6) \\ \hline HER2 expression & 0.14 \\ Negative & 217 (95.6) & 54 (87.1) \\ Positive & 10 (4.4) & 8 (12.9) \\ \hline EGFR expression & 0.004 \\ Negative & 211 (91.3) & 51 (78.5) \\ Positive & 20 (8.7) & 14 (21.5) \\ \hline \end{array}$	Positive	189 (82.2)	44 (66.7)	
Negative $69 (30.1)$ $28 (42.4)$ Positive $160 (69.9)$ $38 (57.6)$ HER2 expression 0.14 Negative $217 (95.6)$ $54 (87.1)$ Positive $10 (4.4)$ $8 (12.9)$ EGFR expression 0.004 Negative $211 (91.3)$ $51 (78.5)$ Positive $20 (8.7)$ $14 (21.5)$	Progesterone receptor expression			0.06
Positive 160 (69.9) 38 (57.6) HER2 expression 0.14 Negative 217 (95.6) 54 (87.1) Positive 10 (4.4) 8 (12.9) EGFR expression 0.004 Negative 211 (91.3) 51 (78.5) Positive 20 (8.7) 14 (21.5)	Negative	69 (30.1)	28 (42.4)	
HER2 expression 0.14 Negative 217 (95.6) 54 (87.1) Positive 10 (4.4) 8 (12.9) EGFR expression 0.004 Negative 211 (91.3) 51 (78.5) Positive 20 (8.7) 14 (21.5)	Positive	160 (69.9)	38 (57.6)	
Negative217 (95.6)54 (87.1)Positive10 (4.4)8 (12.9)EGFR expression0.004Negative211 (91.3)51 (78.5)Positive20 (8.7)14 (21.5)	HER2 expression			0.14
Positive 10 (4.4) 8 (12.9) EGFR expression 0.004 Negative 211 (91.3) 51 (78.5) Positive 20 (8.7) 14 (21.5)	Negative	217 (95.6)	54 (87.1)	
EGFR expression 0.004 Negative 211 (91.3) 51 (78.5) Positive 20 (8.7) 14 (21.5)	Positive	10 (4.4)	8 (12.9)	
Negative211 (91.3)51 (78.5)Positive20 (8.7)14 (21.5)	EGFR expression			0.004
Positive 20 (8.7) 14 (21.5)	Negative	211 (91.3)	51 (78.5)	
	Positive	20 (8.7)	14 (21.5)	

CK5/6 expression			0.05
Negative	217 (94.8)	57 (87.7)	
Positive	12 (5.2)	8 (12.3)	
IHC subtype			< 0.001
Luminal A ([ER+ or PR+],	132 (59.2)	18 (29.5)	
HER2-, low Ki67)			
Luminal B ([ER+ or PR+],	51 (22.9)	20 (32.8)	
HER2-, high Ki67)		. ,	
Luminal B ([ER+ or PR+],	5 (2.2)	2 (3.3)	
HER2+)			
ER-, PR-, HER2+	5 (2.2)	6 (9.8)	
ER-, PR-, HER2-	30 (13.5)	15 (24.6)	
Triple negative phenotype			0.03
Yes	30 (13.5)	15 (24.6)	
No	193 (86.5)	46 (75.4)	
Core basal subtype			0.02
Yes	17 (7.3)	11 (16.4)	
No	216 (92.7)	56 (83.6)	
Stromal TILs (H&E)			0.11
< 10%	191 (82)	49 (73.1)	
$\geq 10\%$	42 (18)	18 (26.9)	
CD8 iTIL count			0.006
< 1	93 (49.2)	14 (27.5)	
≥ 1	96 (50.8)	37 (72.5)	

Abbreviations: IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; CK, cytokeratin; H&E, hematoxylin and eosin; CD, cluster of differentiation.

Supplementary Data 1 (appears as an Excel file): Characteristics of the BC Cancer series including pathological features for the whole cohort and genomic *PRKDC* mutation data for the ER+ subset.