Supplementary

## PLEKHA7, An Apical Adherens Junction Protein, Suppresses Inflammatory Breast Cancer in the Context of High E-cadherin and p120-catenin Expression

Lindy J. Pence <sup>1</sup>, Antonis Kourtidis <sup>2</sup>, Ryan W. Feathers <sup>1</sup>, Mary T. Haddad <sup>1</sup>, Sotiris Sotiriou <sup>3</sup>, Paul A. Decker <sup>4</sup>, Aziza Nassar <sup>5</sup>, Idris T. Ocal <sup>6</sup>, Sejal S. Shah <sup>7</sup> and Panos Z. Anastasiadis <sup>1,\*</sup>

- <sup>1</sup> Department of Cancer Biology, Mayo Clinic, Jacksonville, FL 32224, USA; emails: pence.lindy@mayo.edu (L.J.P.); feathers.ryan@mayo.edu (R.W.F.); haddad.mary@mayo.edu (M.T.H.); panos@mayo.edu (P.Z.A.)
- <sup>2</sup> Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC 29425, USA; <u>kourtidi@musc.edu</u>
- <sup>3</sup> Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55901, USA; sotiriou.sotiris@mayo.edu
- <sup>4</sup> Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55901, USA; decker.paul@mayo.edu
- <sup>5</sup> Department of Laboratory Medicine and Pathology, Mayo Clinic, Jacksonville, FL 32224, USA; nassar.aziza@mayo.edu
- <sup>6</sup> Department of Laboratory Medicine and Pathology, Mayo Clinic, Phoenix, AZ 85054, USA; ocal.toljay@mayo.edu
- <sup>7</sup> Department of Pathology, Kaiser Permanente, Irvine, CA 92618, USA; sejal.s.shah@kp.org
  - Correspondence: panos@mayo.edu; Tel.: 904-953-2609

B

A



Solid Pattern

**Glandular Pattern** 

**Figure S1.** Tumor patterns observed in IBC patient samples. **A)** Example of H&E from IBC tumor demonstrating solid cellular pattern. **B)** Example of H&E from IBC tumor demonstrating glandular cellular pattern. Scale bar represents 100µm.



**Figure S2.** PLEKHA7 expression and localization. **A**) Example of PLEKHA7 staining in a normal breast sample by immunohistochemistry. Scale bar is 100 $\mu$ m. **B**) Immunofluorescence staining of PLEKHA7, p120-catenin and DAPI in MCF12A cell line (scale bar = 10 $\mu$ m) and in Caco2 cell line (scale bar = 20 $\mu$ m). Note: Comparison of intensity is not possible as laser power was different across the two cell lines. **C**). Protein levels of PLEKHA7, E-cadherin, p120-catenin across Caco2, MCF12A, SUM149, and SUM190 cell lines by Western blot.  $\beta$ -actin is used as the loading control.





**Figure S3:** Effects of PLEKHA7 re-expression in SUM149 cell growth in 2D culture. **A**) A representative graph displaying cell growth over time, as measured by the MTT cell metabolic assay, between SUM149 LZRS ms neo (control) and SUM149 LZRS PLEKHA7 cells cultured in 2D.



**Figure S4**. Expression of Cyclin-D1, Snail, and c-Myc in xenograft tumors. **A**) Fraction of Cyclin-D1 positive cells from SUM149 LZRS ms neo (control) or SUM149 LZRS PLEKHA7 xenografts at the 8-week end-point. **B**) Fraction of Snail positive cells from SUM149 LZRS ms neo or SUM149 LZRS PLEKHA7 xenografts at 8-weeks. **C**) Fraction of c-Myc positive cells from SUM149 LZRS ms neo or SUM149 LZRS ms neo or SUM149 LZRS PLEKHA7 xenografts at 8-weeks. *n=*6 for control group, *n=* 8 for PLEKHA7 group.

## A Cyclin D1 Expression in SUM149 Xenografts

	Solid/Tubu	Nuclear	% of SOLID	pattern for	% of overall tumor	0/ ( D=	<b>P</b> 7	% of overall tumor	PLEKHA
Locati	lar	pleomorph	i tumor that is	SOLID	expressing basal	% of P7	pattern	that is apical	7
on	formation	sm	P7+	areas	PLEKHA7	in gland	in gland	PLEKHA7+	intensity
Dermis	97/3	2	90	basal	87.30%	90	apical	2.70%	3
Lymph pode	99/1	3	70	basal	69.30%	50	apical	0.50%	2+3
Dermis	99/1	3	60	hasal	59 40%	50	anical	0 50%	2+3
Lymph	<i>))</i> /1	5	00	Dasai	57.4070	50	apical	0.5078	210
node	98/2	3	20	basal	19.60%	5	apical	0.10%	1
Breast	97/3	3	90	basal	87.30%	40	apical	1.20%	3
Dermis	100/0	3	50	basal	50%	no glands	none	0	2+3
Dermis	95/5	3	70	basal	66.50%	60	apical	3%	2+3
Dermis	95/5	3	60	basal	57%	0	none	0	2
Lymph node	90/10	3	90	basal	81%	50	apical	5%	2+3
Lymph node	98/2	3	70	cytoplasmic	68.6% (CYTOPLASMIC)	0	none	0	2
Breast	75/25		0		0	40	apical	10%	2
Breast	90/10	2	70	basal	63%	60	apical	6%	2+3
Dermis	60/40	3	50	basal	30%	50	apical	20%	2
Dermis	96/4	3	70	basal	67.20%	70	apical	2.80%	2+3
Dermis	96/4	3	10	basal	9.60%	0	none	0	2
Dermis	97/3	3	10	basal	9.70%	0	none	0	2
Dermis	97/3	3	10	basal	9.70%	0	none	0	2
Lymph pode	95/5	3	80	basal	76%	30	apical	1.50%	3
Dermis	97/3	2	50	basal	48.50%	0	none	0	1
Dermis	99/1	2	90	cytoplasmic	89.1% (CYTOPLASMIC)	0	none	0	2
Dermsi	98/2	3	20	basal	19.60%	0	none	0	1
Lymph node	98/2	3	40	basal	39.20%	0	none	0	1+2
Breast	99/1	3	20	basal	19.80%	0	none	0	1+2
Dermis	75/25	3	0		0	80	apical	20%	3
Breast	80/20	3	70	basal	56%	30	apical	6%	3
Dermis	95/5	2	90	basal	85.50%	80	apical	4%	3
Dermis	100/0	2	40	basal	40%	0	none	0	2
Lymph node	90/10	3	0		0	10	apical	1%	2
Lymph node	85/15	1	0		0	0	none	0	1
Lymph node	95/5	3	50	basal	47.50%	40	apical	2%	2
Lymph node	95/5	2	90	basal	85.50%	80	apical	4%	3
Lymph node	90/10	3	30	basal	27%	30	apical	3%	2
Dermis	90/10	3	0		0	70	apical	7%	2
Dermis	100/0	3	10	basal	10%	0	none	0	1
Dermis	100/0	3	30	basal	30%	0	none	0	2

Table S1. Characterization of IBC Patient Samples.

Breast	60/40	3	0		0	70	apical	28%	3
Breast	50/50	2	40	basal	20%	40	apical	20%	2+3
Breast	95/5	3	90	basal	85.50%	90	apical	4.50%	3
Breast	45/55	2	70	basal	31.50%	40	apical	22%	2+3
Breast	20/80	2	40	basal	8%	0	none	0	2+3
Breast	99/1	3	30	basal	29.70%	0	none	0	2
Breast	98/2	3	40	basal	39.20%	0	none	0	2
Breast	95/5	2	90	basal	85.50%	0	none	0	2
Breast	99/1	3	80	basal	79.20%	80	apical	0.80%	2+3
Breast	95/5	3	70	basal	66.50%	80	apical	4%	2+3
Breast	100/0	2	30	basal	30%	0	none	0	1+2
Breast	99/1	2	10	basal	9.90%	0	none	0	1