WIP and WICH/WIRE co-ordinately control invadopodium formation and maturation in human breast cancer cell invasion

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SUPPLEMENTARY INFORMATION

SUPPLEMENTAL TABLES

Table S1. Characteristics of BCC cell lines

Cell line	Gene cluster	ER/PR/HER2 expression	Tumor type	Culture medium
BT474	luminal	+ / + / +	IDC	RPMI, FBS, In, $NaHCO_3$
HS578T	basal B	- / - / -	IDC	DMEM, FBS, In
MCF7	luminal	+ / + / -	IDC	DMEM, FBS
MDA-MB-157	basal B	- / - / -	MC	DMEM, FBS
MDA-MB-231	basal B	- / - / -	AC	DMEM, FBS
T47D	luminal	+ / + / -	IDC	RPMI, FBS, SP, $NaHCO_3$
SKBR3	luminal	- / - / +	AC	McCoys 5A, FBS

Tumor type: IDC, invasive ductal carcinoma; F, fibrocystic disease; MC, metaplastic carcinoma; AC, adenocarcinoma. Culture conditions: RPMI, Roswell Park Memorial Institute 1640 medium; DMEM, Dulbecco's modified Eagle's medium (both from Sigma); F-12, Ham's F12 nutrient mixture (Gibco); FBS, fetal bovine serum 10%; HS, horse serum 5% (both from Sigma); HC, hydrocortisone 0.5 mg/ml (Calbiochem); EGF, epidermal growth factor 20 ng/ml (Peprotech); In, insulin (10 μg/ml, Gibco); $NaHCO_3$ 0.075% (Sigma); SP, sodium pyruvate (1 mM, Sigma)

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Expression of WIP family proteins in BCC and WIP- and WIRE-deficient cells. (a) Relationship between WIP mRNA levels and BCC invasive behavior (1). (b) WIP mRNA levels in basal-B, basal-A and luminal BCC (2). (c) MDA-MB-231 cells were transduced with lentiviral particles containing different shRNA-coding plasmids that target WIP or WIRE. Representative WB in which cell lysates (30 μg/lane) were examined using anti-WIP, -WIRE and -GAPDH antibodies. (d) Quantification of WIP and WIRE expression after depletion. Data shown as mean ± SD of at least three independent experiments. ** $p < 0.01$, *** $p < 0.001$ by 1-way ANOVA and Tukey's post-hoc test.

Figure S2. Disruption of Nck binding to WIP leads to opposite phenotypes in control and WIRE-deficient cells. Orthogonal views of cells shown in Figure 5. (a) MDA-MB-231 cells overexpressing WIP or WIPΔNBD (eGFP, green) were plated on rhodamine-gelatin (red, 5 h), fixed and stained for cortactin (cyan). (b) shWIRE cells overexpressing WIP, WIP-ΔCBD, WIP-ΔNBD or WIP-ΔWBD (eGFP, green). Arrows indicate invadopodia.

REFERENCES

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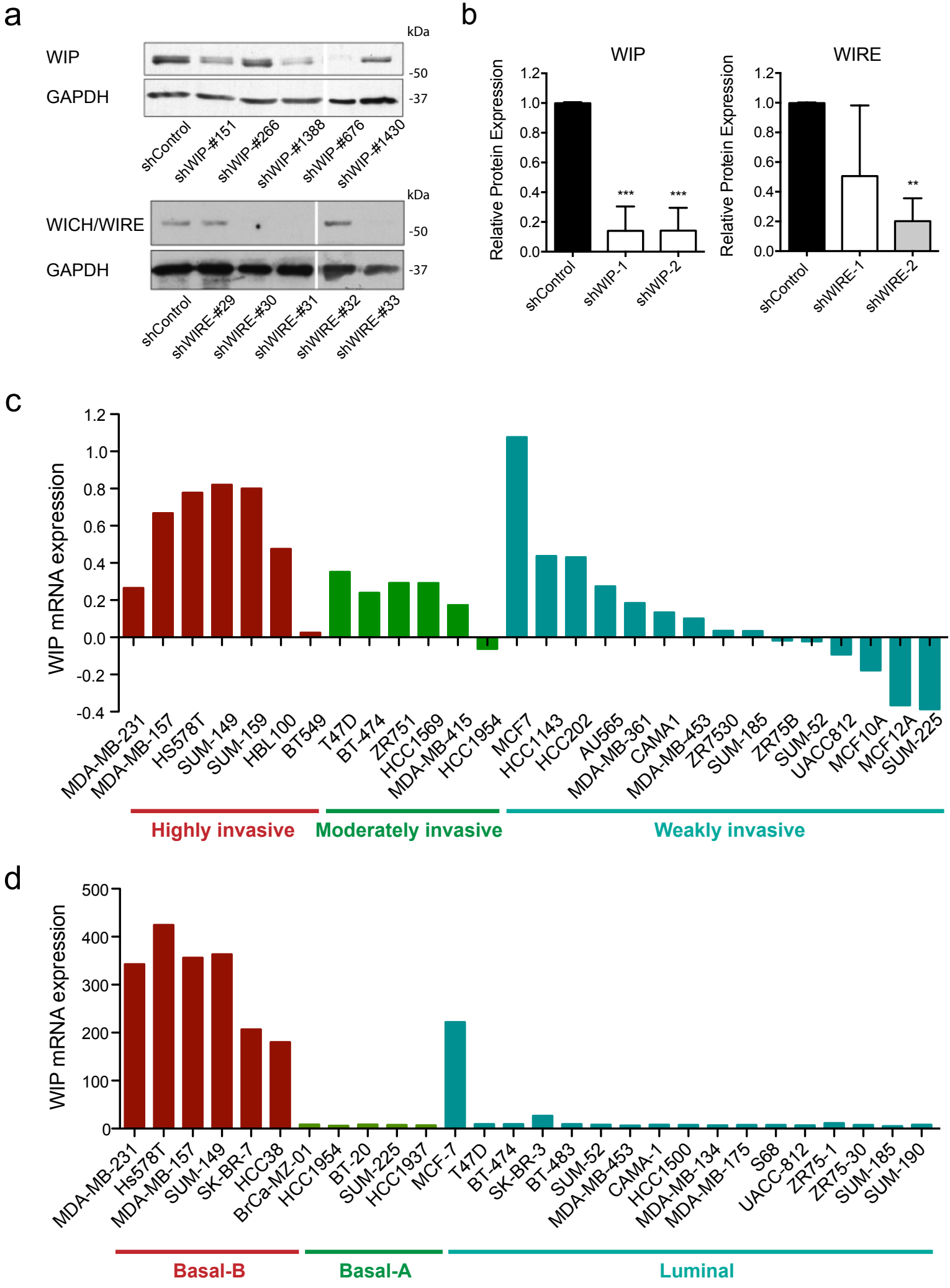
2. Charafe-Jauffret, E. et al. Gene expression profiling of breast cell lines identifies potential new basal markers. *Oncogene* **25**, 2273-2284 (2006).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WIP
GAPDH
kDa
-50
-37
shControl
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shWIP-#266
shWIP-#1388
shWIP-#676
shWIP-#1430
WICH/WIRE
GAPDH
shControl
shWIRE-#29
shWIRE-#30
shWIRE-#31
shWIRE-#32
shWIRE-#33
b
WIP
WIRE
Relative Protein Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
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shControl
shWIP-1
shWIP-2
shWIRE-1
shWIRE-2
c
WIP mRNA expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
MDA-MB-231
MDA-MB-157
HS578T
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SUM-159
HBL100
BT549
T47D
BT-474
ZR751
HCC1569
MDA-MB-415
HCC1954
MCF7
HCC1143
HCC202
AU565
MDA-MB-361
CAMA1
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ZR7530
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SUM-52
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Highly invasive
Moderately invasive
Weakly invasive
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WIP mRNA expression
500
400
300
200
100
0
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MDA-MB-157
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BrCa-MZ-01
HCC1954
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UACC-812
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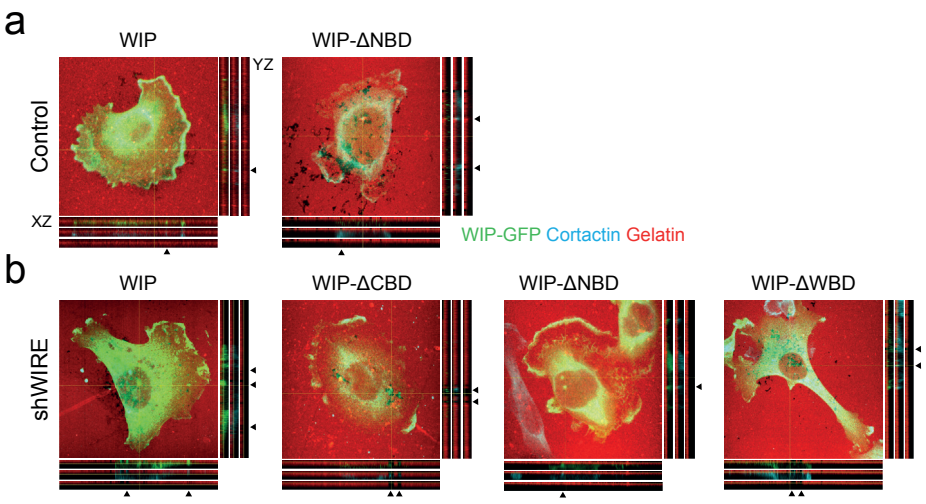
SUM-190
Basal-B
Basal-A
Luminal


a
WIP
WIP-ΔNBD
Control
YZ
XZ
WIP-GFP Cortactin Gelatin
b
WIP
WIP-ΔCBD
WIP-ΔNBD
WIP-ΔWBD
shWIRE