SUPPLEMENTARY figure legends

Loss of Par3 promotes breast cancer metastasis by compromising cell-cell cohesion

Bin Xue, Kannan Krishnamurthy, D. Craig Allred and Senthil K. Muthuswamy

Figure S1 Loss of Par3 cooperates with ErbB2 activation to induce invasive behaviour. DIC images of day 16 acini from shGFP or shPar3 cells in the presence of ErbB2 activation for 8 days forming invasive protrusions.

Figure S2 Downregulation of Par3 promotes ErbB2 tumour metastasis. (a) Representative tumours of mice transplanted with MMTV-NDL-tumour-derived cells infected with shGFP or mPar3 shRNA lentivirus 12 weeks after transplantation. The arrows point to the local metastatic nodules growing on the abdominal wall. (b) The number of metastatic nodules was plotted.

Figure S3 Loss of Par3 weakens E-cadherin-mediated cell-cell adhesion. (a) Phase contrast images of 10A.B2 shGFP or shPar3 cells growing on plastic dishes at low density without (-) or with (+) ErbB2 stimulation for 24 hours. (b) Phase contrast images of 10A.B2 cells cultured in the presence of a function-neutralizing antibody against E-cadherin (HECD-1) or control mIgG for 24 hours. (Scale bar = 100 μm)

Figure S4 Loss of Par3-induced invasive behaviour is mediated by aberrant activation of Rac. (a) CFP and FRET of Confluent 10A.B2 cells expressing Raichu-Rac untreated or treated with ErbB2 dimerizer. (b) Cell lysates from 10A.B2 cells overexpressing control or myc-Tiam1 PHn-CC-Ex were analysed for myc-tag expression (upper) and α-tubulin as loading control. (c) These cells were grown in 3D culture for 12 days with ErbB2 stimulation for the last four days and imaged by phase contrast and fluorescent microscopy. Morphology score was determined by scoring 144 acini for shPar3 and 187 acini for shPar3/Tiam1-PHnCC-Ex for each condition. (d) The cells expressing control or Tiam1 PHn-CC-Ex were subjected to transwell invasion assay. Data are presented as mean ± SEM collected from three independent experiments, $^{**}P=0.005$ by Student's *t*-test. (e) 10A.B2 shPar3 or shGFP cells were seeded in plastic

dishes at low density under the indicated conditions. The cell morphology was monitored using light microscopy.

Figure S5 Loss of Par3 induces changes in organization and dynamics of actin at cell-cell junctions. (a) T47D cells expressing shGFP or shPar3 were stained with phalloidin for actin cytoskeleton (green) and nuclei (DAPI, blue). (b) Low-magnification images of tissue sections of tumours and lung metastasis from transplantation experiments immunostaind for actin cytoskeleton (b-actin) and ARPC2. (Scale bar = 50 μm) (c) Tissue sections from paired primary tumour and spontaneous lung metastasis in an MMTV-NDL mouse were immunostained for actin cytoskeleton (β-actin) and ARPC2. (Scale bar = 20 μm) (d) Cell lysates from confluent monolayers of 10A.B2 cells expressing control vector, Flag-Par3-FL and Par3-ΔC were immunoprecipitated using anti-Flag and immunoblotted as indicated. (e) 10A.B2 shPar3 cells untreated or treated with 25 μM NSC23766 compound in the presence of ErbB2 stimulation for 12 hours were stained with phalloidin for F-actin and nuclei were stained with DAPI. (Scale bar = 20 μm)

Figure S6 Mislocalization of Par3 in human breast cancer. The image shows a representative example of overexpressed and mislocalized Par3. The insert is the zoom in Par3 staining of the boxed area.

Figure S7 Uncropped image blots

Supplementary Video 1 Actin and E-cadherin dynamics at cell-cell junctions. 10A.control or 10A.shPar3 cells expressing E-cad-GFP and Lifeact-RFP were imaged in the middle of the cell (1.5 mm above the bottom) at the rate of 12 frames per minute for 10 minutes.

Supplementary Table 1 Relationship between membrane Par3 and tumour characteristics. Histological grade, ER and HER2 oncogene expression were evaluated as described in Method. Membrane-localized Par3 was evaluated by immunofluorescence and quantified using the Allred score. The mean value of membrane-localized Par3 in each subgroup was circulated and the association was determined by Student's *t*-test.

Supplementary Table 2: Information of antibodies used in the experiments.