**Supplemental experimental procedures**

**Patient material**

The study population was derived from the archives of the Departments of Pathology of the University Medical Center Utrecht, Utrecht, The Netherlands and comprised 298 cases of invasive ductal carcinoma (IDC) as described (1). Histological grade was assessed according to the Nottingham scheme, and mitotic activity index (MAI) was assessed as before (2). From representative donor paraffin blocks of the primary tumors, tissue microarrays were constructed as described (1, 3). The use of anonymous or coded left over material for scientific purposes is part of the standard treatment contract with patients in The Netherlands (4). Ethical approval was not required.

**Plasmids**

For stable knockdown of p120, sequences directed against mouse (5’ GCCAGAAGTGATGGCGAATA 3’) and human (5’ GCCAGAGTTGGTTCGGATA 3’) p120 and a control siRNA sequence (5’ TTCTCGAAGCTGTACGTT 3’) were cloned into a Dox inducible lentiviral expression system as described (5). For p120 reconstitution experiments, pEGFP-C1-p120-1A (gift from Juliet Daniel, McMaster University, Hamilton, Canada) was mutated by means of QuikChange XL site directed mutagenesis (Stratagene) using primers containing three silent mutations (forward: 5’AACTCTTATTTTCAGCCAGAAGTCGTCCGATATACTACCTCTCTTAAAGG3’, reverse: 5’CTTAAGGAGTTGAATATGTATGCAGCGACTTCTGGCTGAAAATAAGGT3’). EcoRV and Nhel sites were added 5’ and 3’ of p120-1A, using Phusion PCR (Thermo Scientific) (forward: 5’AATTGATATCATGACGACTGAGGGATGGGAGTCGAC3’, reverse: 5’TAAAGCTAGCTAAATCTTCTGATCAAGGGTGCTCC3’). EcoRV-p120-1A-Nhel was subsequently cloned into the lentiviral expression vector pLV.bc.puro (a gift from Clemens Löwik, Leiden University Medical Center, Leiden, the Netherlands). pCMV-SPORT-EGF (open biosystems) was used as template to generate the DIG-labeled EGF probe by PCR. Primers: forward: 5’ GGACTTTGTGCCTGCTGCC 3’, reverse: 5’ GCGCTCGAGTGGGACCTTGGG 3’
Immunohistochemistry and fluorescence

Tissues were isolated, fixed in 4% formaldehyde for 48 hours, dehydrated, cut into 4µm sections and stained with hematoxylin and eosin. For single staining, fixed sections were rehydrated and incubated with primary antibodies. Endogenous peroxidases were blocked with 3% H₂O₂ and stained with biotin-conjugated secondary antibodies, followed by incubation with HRP-conjugated streptavidin-biotin complex (DAKO). Substrate was developed with DAB (DAKO). For immunofluorescence, fixed sections were rehydrated, boiled in citrate and incubated with primary antibodies overnight. Stainings were scored as described (1). Cells were grown on cover slips and fixed in 1% paraformaldehyde/PBS for 10 minutes. Cells were permeabilized using 0.3% Triton-X100/PBS and subsequently blocked using 5% BSA (Roche). Samples were incubated with primary antibodies in 1% BSA for 60 minutes, followed by secondary antibodies for 30 minutes. DNA was stained with DAPI for 5 minutes (Molecular Probes) and cover slips were mounted onto object glasses using vectashield mounting medium (Vector laboratories).

In situ-hybridisation–IHC double staining was performed on freshly frozen tissue as described (6). Slides were hybridized overnight with DIG-labeled EGF probe as described (6). Subsequently, the slides were incubated with primary antibody F4/80. EGF probes were detected with a sheep anti-digoxigenin antibody (Roche) followed by a secondary rabbit anti-sheep antibody (DAKO). Alexa Fluor-555-conjugated goat anti-rabbit (Invitrogen) was used to detect DIG-labeled EGF probes. Samples were analyzed using a DeltaVision RT system (Applied Precision) using a 40x, 63x and 100x lens at room temperature, equipped with a CoolSnap HQ camera and SoftWorx software. Maximum projections were taken from a stack of deconvolved images.
References


4. van Diest PJ. No consent should be needed for using leftover body material for scientific purposes. For. Bmj. 2002;325:648-51.
