Detailed Materials and Methods – Strell et al.

Patient samples

The population-based DCIS cohort included 458 women in Uppland and Västmanland counties in Sweden diagnosed with primary DCIS between 1986 and 2004. The construction and quality control of the tissue micro arrays (TMA), including 2 cores per case, has been previously described [1]. Cases with primary DCIS showing signs of microinvasion (invasive focus of <2 mm) were excluded. The analyzed endpoint was development local recurrence (*in situ* or invasive; n=102) or metastasis (n=14). The TMA of the DCIS_Nation cohort [2,3] , composed of 2 cores per case, was used for validation of findings from the population-based cohort and included samples from patients with primary DCIS, who all developed recurrences. Overlapping cases with the population-based cohort were excluded. Analysis of TMAs was approved by the Ethics Committee Uppsala University Hospital (Dnr. 99422) and the Local Ethical Review Boards Uppsala (Dnr. 2005:118) and Umeå (Dnr. 2014-230-321M), no written informed consent was needed. Sections from FFPE core needle biopsies of normal breast tissue were provided within the KARMA (The Karolinska Mammography Project for Risk Prediction of Breast Cancer) study [4] and approved by the ethical review board of Karolinska Institutet (Dnr. 2011/1464-31/1). The invasive breast cancer cohort including staining and analysis is described in [5].

The results shown in Supplementary Fig. 3A are based upon data from the TCGA Research Network: http://cancergenome.nih.gov/ and were generated using the TCGA Firebrowse Version 1.1.35 from the Broad Institute of MIT & Harvard (https://www.broadinstitute.org/).

Immunohistochemistry (IHC) and scoring

Human DCIS TMA and sections

FFPE sections were de-paraffinized and the antigen retrieval was performed at 110° C for 5 minutes in pH 10 Tris retrieval buffer (SigmaAldrich, Taufkirchen, Germany). The staining for PDGFRα and -β was performed using the DAKO Techmate Horizon 30 autostainer system (DAKO) and the Dako REAL EnVision Detection System (K500711-2, DAKO, Glostrup, Danmark). The automated protocol included inactivation of

endogenous peroxidases (DAKO REAL peroxidase-blocking solution) for 5 min and blocking with 1% bovine serum albumin (BSA) for 5 min. Incubation with the primary antibodies was performed for 30 min. The human-specific PDGFRα antibody (#5241, clone D13C6) was diluted 1:150, and the PDGFRβ (#3169, clone 28E1) (both Cell Signaling Technology, Danvers, MA) 1:75 in DAKO REAL antibody diluent. For detection Dako Rabbit Envision/HRP was applied for 10 min and developed with DAKO REAL DAB reagents for 3 min. Slides were counterstained with Mayer's HTX Hematoxylin (Histolab, Goteborg, Sweden). Staining of PAE/PDGFRα and PAE/PDGFRβ cells demonstrated that the PDGFR antibodies have high affinity for their corresponding receptor (Supplementary Fig. 1A).

The PDGFR scoring approach was guided an experienced pathologist for diseases of the mammary gland. The PDGFRα and –β staining was originally scored as the positive stroma fraction (0/negative, 1/low, 2/moderate or 3/high) as previously described [5] and dichotomized by defining scores 0 and 1 as low and scores 2 and 3 as high positive stroma fraction (Supplementary Figure 1B). Scoring was performed independently by three individuals blinded to survival data. In cases of disagreement, which was in 13.9% of cases, a consensus score was discussed.

For Laminin-γ2 staining de-paraffinized sections were treated with 0.01% protease XXIV (SigmaAldrich) for 15 min. Sections were blocked with 1% BSA for 15 min and incubated with the mouse monoclonal Laminin-γ2 (clone D4B5, Merck Milipore, Darmstadt, Germany) at 1:1000 dilution in 1% BSA ON at 4°C. ImmPRESS HRP Anti-Mouse IgG (VectorLaboratories, Burlingame, CA, US) was applied for 30 min and visualized using the DAB substrate kit (VectorLaboratories). For presentation images were adjusted in brightness and contrast within the ImageScope v. 11.1.2.752 program (Aperio Technologies, Inc.). Laminin- γ2 was scored as present or absent.

Sections from mouse tumor models

PDGFR stainings of sections from mouse tumor models were performed on the Ventana Discovery Autostainer System (Ventana, Rche, Basel, Switzerland) using the mouse reactive PDGFRα antibody (#3174, clone D1E1E) at a dilution of 1:100 and the PDGFRβ (#3169, clone 28E1) (both Cell Signaling Technology) at 1:100 in Discovery antibody diluent (Ventana, Roche). Extensive antigen retrieval was performed with Discovery Cell

Conditioning buffer 1 (CC1, Ventana, Roche) (for mouse PDGFRα) and pH 10 Tris retrieval buffer (SigmaAldrich) for PDGFRβ staining. Antibodies were incubated for 8h at room temperature. Detection was performed using the OmniMap DAB anti-rabbit kit (Ventana, Roche). Mouse PDGFR staining was scored as high or low.

Digital analysis of IHC stainings

Digital analysis of PDGFR stainings was performed using the FIJI image analysis software (http://rsb.info.nih.gov/ij). A script for automated analysis was established including H DAB color deconvolution as first step. Obtained image were converted to 8-bit and inverted.

For analysis of the DCIS Nation TMA the positive fraction of the whole core and the mean intensity within this fraction were threshold appropriately and measured. For further survival analysis the product of positive fraction and mean intensity was calculated and the resulting data was dichotomized in a way leading to similar sized groups as within the histological soring groups.

For analysis of xenograft tumor sections, the tumor-associated stroma was outlined prior to the automated script-based analysis and the analysis was restricted to measurement of the mean intensity after thresholding. Median cut-off was applied to dichotomize the data into low and high PDGFRα groups.

Triple immunofluorescence (IF) staining of PDGFRα, Hes1, laminin-γ2 and of PDGFRα, PDGFRβ, Cytokeratin (CK)14

Sections were de-paraffinized, followed by antigen-retrieval for 20 min with BOND TM *ER2* solution (pH 9.0) using the BOND MAX autostainer (Leica Biosystems Nussloch, Germany). Peroxidase-blocking solution (DAKO REAL) was applied for 15 min, followed by incubation with serum-free protein blocking solution (DAKO) for 30 min and with rabbit monoclonal Hes1 antibody (#11988, clone D6P2U, Cell Signaling Technology,) diluted 1:1000 in DAKO REAL antibody diluent overnight at 4°C. The sections were treated with TNB blocking buffer (FP1012, PerkinElmer Life Sciences, Waltham, MA, US) for 15 min, thereafter with ImmPRESS HRP Anti-Rabbit IgG (VectorLaboratories) for 30 min and the TSA Plus Cyanine 3.5 diluted 1:150 in amplification buffer (NEL763001KT, PerkinElmer) for 15 min. In order to avoid cross-

detection the sections were heated as before using the BOND MAX autostainer. The staining procedure was repeated with the human-specific PDGFRα antibody diluted 1:200, but this time the TSA Plus Cyanine 5 system (NEL745001KT, PerkinElmer) was applied. For following Laminin-γ2 staining the sections were processed with protease XXIV and incubated with the antibody as described previously. Secondary AlexaFluor488 Donkey Anti-Mouse IgG (JacksonImmunoResearch Laboratories, West Grove, PA, US) diluted 1:100 in 0.5% TNB blocking buffer was applied for 2 h. Autofluorescence was blocked with a 1% Sudan Black solution for 5 min. Sections were scanned at 40x magnification on the Metasystems Vslide scanning microscope (Metasystems, Alltlussheim, Germany). For presentation, images were adjusted in brightness and contrast within the Metaviewer program. Semi-quantitative scoring was performed on the basis of ten randomly selected lesions from four different DCIS sections. Tumor-associated stromal PDGFRα staining was scored as described for IHC. Laminin-γ2 was scored as negative/low and high. Hes1 was scored as high if more than one quarter of the tumor-associatedl stroma cells showed nuclear positivity.

Triple stainings for PDGFRα, PDGFRβ and Cytokeratin (CK)14 were performed in same way with minor modifications. PDGFRβ (#3169, clone 28E1, Cell Signaling Technology) staining was performed first. After heating using the BOND MAX autostainer to avoid cross-detection between the two rabbit derived-antibodies, PDGFRα staining was performed. Residual HRP activity was inactivated through incubation of the slides in 0.1% (w/v) NaN3 in PBS for 20 min at room temperature prior to the CK14 staining using the mouse monoclonal anti CK14 antibody (#NCL-L-LL002, clone LL002, Novocastra, Leica Biosystems, Newcastle, UK) at 1:100 dilution.

Digital analysis of immunofluorescence images

Digital analysis of fluorescent stainings was performed using the FIJI image analysis software.

The mean fluorescent intensity of Laminin- γ2 and the corresponding stromal PDGFRα staining was measured without threshold. Data was log2 transformed for representation.

Concerning Hes1 expression, total nuclei number within the tumor associated stroma region was determined based on thresholding of the DAPI image after Gaussian blur and the fraction of Hes1 positive nuclei was determined by adjustment and application a cut-

off value for positive Hes1 staining. Hes1 was defined as high if more than one quarter of the tumor-associatedl stroma cells showed nuclear positivity. For the correlation analysis between Hes1 and stromal PDGFRα, the PDGFRα data was dichotomized based on a histogram-guided cut-off value.

Cell culture

The human MCF10A.DCIS.com cell line was directly purchased from Asterand (Asterand, Detroit, MI, USA) and maintained in DMEM:F12 GlutaMax containing 5% donor horse serum, 100 units (U)/ml penicillin and streptomycin (P/S) (all from Hyclone, GE Healthcare, South Logan, Utah, USA). Immortalized normal human mammary fibroblast cells (HMF) are described in [6]. HMF cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 100 U/ml P/S and 2 mM glutamine. All cell lines were screened for mycoplasma regularly (MycoAlert™, Lonza, Amboise, France) and not used for more than 5 passages.

Co-culture assays

Tumor cell–conditioned media was generated by incubation of MCF10DCIS cells for 24h in DMEM with 1% FBS. Medium was collected, centrifuged at 4000 rpm for 10 min at 4°C to remove cell debris. Four different co-culture set-ups were established. (i) For treatment of HMF with conditioned media, 200.000 HMF cell were seeded into 6-well plates (Corning) and cultured for 24h. HMF were starved for 6 h with DMEM containing 1% FBS before incubation with conditioned medium for 48 h. (ii) 200.000 MCF10DCIS cells were seeded into 0.4 mm cell culture inserts and 200.000 HMF were seeded into 6-well plates and cultured for 24h. All cultures were starved for 6 h with DMEM containing 1% FBS before the inserts were added onto the HMF cultures without any media changes. Co-culture was performed for 48 h. (iii) For direct co-cultures, MCF10DCIS and HMF-gfp cells were mixed in a 1:1 ratio with 100.000 cells/cell type. Cells were cultured for 24h in DMEM with 10% FBS. Thereafter, media was changed to DMEM with 1% FBS and culture continued for 48h. DAPT (Selleck, #S2215; Selleck, Munich, Germany) was used at final 5 uM. (iv) 200.000 MCF10DCIS cells or 200.000 HMF-gfp cell were seeded in 6-well plates and cultured for 48h. Cells were fixed with 2%

Formaldehyde (Sigma-Aldrich) for 15 min at 4°C. 200.000 HMF-gfp cells were seeded in DMEM with 10% FBS on top of the fixed cultures. After 24h media was changed to DMEM with 1% FBS and culture was continued for further 48h. At the end of every co-culture experiment, cultures were washed once with *Dulbecco's Phosphate-Buffered Saline (DPBS)* (Hyclone) and lysed for subsequent RNA or protein analysis.

Cell culture on Jagged-1 coated plates

Recombinant human Jagged-1 Fc chimera protein (#P78504, R&DSystems, Abingdon, UK) was applied at 5 ug/ml in DPBS + 1% BSA on 6-Well plates over night at 4°C. 200.000 HMF cells were seeded in DMEM with 10% FBS. After 24h media was changed to DMEM with 1% FBS and culture continued for 48h.

Viral transduction

50000 HMF were seeded in a 12 well. 50 ul of eGFP-Lentivirus (pLV.ExSi.P/Neo-EF1α-eGFP; Cyagen, Biosciences Inc., Santa Clara, CA, US) were added to 1 ml culture media containing 25 ug/ml proteamin sulphate (Sigma-Aldrich) and incubated for 24 h. GFP positive cells were enriched by FACS sorting.

FACS sorting

Cells were trypsinized and collected by centrifugation at 1500 rpm for 5 min at 4°C. The pellet was resuspended in sorting buffer (DPBS plus 1.5mM EDTA, 1% FCS, 25mM Hepes), filtered through a 40 μm cell strainer and adjusted to $5x10^6$ cells/ml. The sort was performed at 4°C using a BD FACS Aria III Cell Sorter B5/R3/V3 in 4-Way Purity mode. Sorted cells were collected by centrifugation.

For the sorting of co-cultures of HMF and MCF10DCIS, these cells were seeded with $1.5x10^6$ cells each in T-160 flasks (Sarstedt, Nuembrecht, Germany) in DMEM media with 10% FBS. Control mono-cultures were seeded at equal total density. After 24h, the culture media was changed to DMEM media with 1% FBS. The gfp-positive HMF cells were separated from the gfp-negative MCF10DCIS cells and both cell types were collected. Duplexes were excluded. Control mono-cultures were sorted with the same settings and gates as the co-cultures to compensate for a possible sort-specific enrichment

of subpopulations. Sorted cells were collected by centrifugation at 1500 rpm for10 min at 4°C and cell pellets were further used for RNA isolation.

Transient transfection

HMF and MCF10DCIS cells were transfected using the Nucleofector™II (Lonza) according to the manufacturer's instructions. For HMF Nucleofector® Solution V and for MCF10DCIS Solution T was used. (i) For experiments with siRNA modified HMF-gfp cells, 50 ng of specific or scrambled siRNA (Qiagen, Hilden, Germany) were added (Supplementary table 3). (ii) For reporter gene assays, 1 µg of either pGL3-(CAGA)12-Luc [7] or pGL3-BRE2-Luc [8] (both generous gift of Aristidis Moustakas, Ludwig Institute for Cancer Research, Uppsala University, Uppsala, Sweden) or 12xCSL-luc reporter vector [9] together with 50 ng of the renilla luciferase control pRL-TK vector (Promega Corporation, Madison, Wisconsin, US) were added to the corresponding sample. (iii) For the reintroduction of Notch2 into HMF cells after CRISPR-Cas9 mediated depletion of *NOTCH2*, cells were transfected with 1 µg of pcDNA3.1 with human full length *NOTCH2* [10]. Electroporation was performed with program A-23 for HMF-gfp or T-16 for MCF10DCIS.

CRISPR-Cas9 gRNA targeting gene inactivation

The optimized single guide RNA (sgRNA) targeting *NOTCH2* (5′-GGTGGAGCCTGGAGTACAGG-3′) and *JAG1* (5′-CTTACGAACGGTGTCATTAC-3′) was cloned and ligated to the guide RNA vector (Addgene pX459, Cambridge, MA, US). Cells were transfected with the gRNA vector, puromycin dihydrochloride (Sigma-Aldrich) at 1µg/ml was used for selection. Single cell colonies were isolated and subjected to Western blot and DNA sequencing analysis as previously described [11]. Potential off-target effects were controlled and excluded through co-culture experiments with transient reintroduction of *NOTCH2* in HMF-*NOTCH2* knock-out cells and reintroduction of Jagged-1 through plate coating respectively (Supplementary Fig 5 E and F).

Immunoblot analysis

Cultures were lysed with 250 µl NuPAGE LDS sample buffer (ThermoFisherScientific) containing 1% 2-Mercaptoethanol (Sigma-Aldrich) and 2µg/ml aprotinin (Sigma-Aldrich). Celllysates were heated prior to use and 15 µl cell lysate was loaded on a 1mm NuPage 4-12% Bis-Tris Gel (ThermoFisherScientific). Wet-transfer was performed onto Immobilon-P Membrane (Merck Millipore) using the Mini Trans-Blot Cell (Bio Rad) with 10mM sodiumtetraborate decahydrate (Sigma-Aldrich) as running buffer. The membrane was blocked with 5% non-fat milk powder and incubated with primary antibody diluted in 1% BSA in TBS at 4°C over night. Incubation with ECL™ HRP-linked secondary anti-mouse or –rabbit antibody (GE Healthcare, Little Chalfont, UK) diluted 1:10000 in TBS/Tween buffer was done for 1 h. Signals were detected using Amersham ECL™ Prime Reagent (GE Healthcare) and the ImageQuant LAS 4000 digital imaging system (GE Healthcare). Densitometric analysis of the obtained bands was performed with the FIJI image analysis software (http://rsb.info.nih.gov/ij). Results were normalized to beta-actin or gfp respectively. Immunoblots were sequential stripped with 0.4 M NaOH solution for 15 min at RT and reprobed. The following primary antibodies were used: PDGFRα and PDGFRβ as above; Notch2 (#5732, clone D76A6, Cell Signaling Technology); gfp (#2555, Cell Signaling Technology); Jagged1 (ab109536, clone EPR4290Abcam); beta-actin (A5441, clone AC-15 Sigma Aldrich). Antibodies were diluted 1:1000 except beta-actin with 1:5000.

RNA isolation, cDNA synthesis, RT² Profiler PCR Arrays and validation qRT-PCR

RNA was isolated with GenElute™ Mammalian Total RNA Miniprep Kit (Sigma-Aldrich) according to manufacturer's instructions, including DNaseI treatment. RNA concentration was measured with a NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, USA). CDNA synthesis from 500 ng RNA was performed with the SuperScript™ III Reverse Transcriptase (ThermoFisherScientific) kit using oligo dT primer according to manufacturer's instructions. Two different quantitative real time (qRT) PCR arrays (RT² Profiler PCR Arrays PAHS-035Z and PAHS-235Z, Qiagen, Hilden, Germany) were performed and analyzed according to the manufacturer's instructions in order to screen for changes in signaling pathways between sorted co-

cultured HMF and mono-cultured HMF. For validation of differentially expressed genes, independent qRT-PCR experiments were performed using the 2x Power SYBR Green PCR Master Mix (ThermoFisherScientific), 200 nM as final primer concentration and 10 ng of cDNA per 10 µl reaction on the Applied Biosystems 7900HT Fast Real-Time PCR system. For reactions with Quantitec primer stocks from Qiagen (Hilden, Germany), the stock was used at a final dilution of 1:20. The expression values were calculated by the comparative $\Delta\Delta C_T$-method for the gene-of-interest relative to the expression level of the housekeeping gene *GAPDH* or to *GFP*. Thereafter, the expression-values were normalized to the mean value of the control group for representation. All primers are listed in Supplementary table 1.

Reporter gene assay

Luciferase activity was determined using the DualLuciferase® Reporter Assay System (Promega) according to the manufacturer's instructions and the Centro LB 960 Microplate Luminometer (Berthold Technologies, Bad Wildbad, Germany). Firefly luciferase activity values were normalized to Renilla luciferase activity values in order to consider possible differences in transfection efficiency. TGF-beta signaling is measured with the pGL3-(CAGA)12-Luciferase vector carrying the Smad3/Smad4 binding element, the BMP signaling activity is measured with the pGL3-BRE2-Luciferase vector carrying the BMP responsive element (BRE) and Notch signaling activity is measured with the 12xCSL-Luciferase vector**.** For further vector description see also Transient Trasfection.

Animal studies

Seven- to eight-week-old female CB17/Icr-Prkdc(scid)/IcrIcoCrl mice (Scanbur, Karlslunde, Denmark) were injected into the mammary fat pad with either 250000 MCF10DCIS cells alone or together with 500000 HMF. Ten animals were included in each injection group. Mice were euthanized after 3 weeks. The mammary glands were collected**.** The genetic MMTV-PyMT model was used to collected mammary glands with tumors at 6 and 12 weeks of age. Control normal mammary glands were collected from

wild type FVB mice of similar age. Animal experiments were approved by Jordbruksverket (N220/14; N96/11).

In situ sequencing for detection of RNA transcripts of *PDGFRA, PDGFRB and HES1*

In situ sequencing was performed as described previously [12] using the padlock-probes and LNA primers listed in Supplementary table 1. Images were acquired with an automated Zeiss Axioplan II epifluorescence microscope (Zeiss, Oberkochen, Germany). The images from the respective sequencing cycles were aligned and decoded using Cellprofiler v.2.1.1 (Broad Institute, MA, USA) and Matlab (v.8.5.1, Mathworks, Sweden). DAB-based pan-Cytokeratin (#M3515, clone AE1/AE3, DAKO) staining was done to enable discrimination between epithelial and stromal fraction using the DAB deconvolution in the FIJI image analysis software (http://rsb.info.nih.gov/ij). For the analysis of overlap between areas of high transcript density, the analysis was restricted to the tumor-associated stroma area. Kernel density estimation plots were generated as described in [13] with a bandwidth of 200 and their overlap was calculated with FIJI.

Cluster and gene ontology analysis

Publically available normalised gene expression data from Ma *et al.* [14] (GEO14548) was used to perform hierarchical clustering analysis of 14 normal and 11 DCIS stroma samples in order to validate patient relevance of the genes of interest, which were selected on basis of the gene expression profiling of fibroblasts co-cultured with DCIS cells (Supp. Table 4). Genes of interest, were extracted from the dataset and used to cluster samples (median centred by feature/gene, Pearson correlation, average linkage) in a semi-supervised manner with the *heatmap3* package in R version 3.4.0. Analysis of the corresponding pathways was performed using the *GAGE* (PMID: 19473525) package. The 5% false discovery rate was calculated with Benjamini and Hochberg correction and corresponding q-values are indicated.

Statistical analysis

Associations between stromal PDGFR protein and clinico-pathological parameters were analyzed with contingency tables and Fishers' exact test, two-sided. The Kaplan-Meier and log rank test were used to compare risk to develop local recurrence (*in situ* or invasive) or metastasis. A Cox proportional hazards model was used for estimation of hazard ratios (HR) in uni- as well as multivariable analyses including relevant risk factors as age, tumor size, estrogen receptor (ER) status, EORTC grade[15], radiotherapy and surgical treatment in the model. The P value for cox regression is based on Wald test. For Cox regression analysis the assumption of proportional hazard was verified graphically through evaluation of parallelism of the log(-log(S(t))) versus time plot as well as statistically through the Schoenfeld Residuals Test. A weak interaction with time was observed after 240 months of follow-up, when patient numbers became very low. This interaction did completely disappear when dropping the last follow-up times from analysis. Calculations were performed with the statistical package IBM SPSS Statistics Version 22 (SPSS Inc.).

The relationship between two continuous variables was assessed through the Spearman rank correlation method, two-sided, stating the correlation coefficient *rho* as well as the p-value. Group differences were evaluated by using two-sided Student's t-test for two-group comparisons and one-way ANOVA with Bonferroni *post hoc* test for multiple group comparisons (GraphPadPrism 7.0b, GraphPad Software Inc.). P-values derived from multiple two-sided t-test comparisons were adjusted by 5% false discovery rate with Benjamini & Hochberg correction. P-values <0.05 were considered statistically significant.

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Supplementary table 1: List of primers, siRNA and padlock probes.

Product	Vendor	Cat.-Nr.	Sequence
Primer			
BMP1	Qiagen*, QuantiTec	QT00000819	-
BMP4	Qiagen*, QuantiTec	QT00012033	-
CHDR	Qiagen*, QuantiTec	QT00032599	-
DAND5	Qiagen*, QuantiTec	QT00214291	-
DLL1	Qiagen*, QuantiTec	QT00057631	-
DLL3	Qiagen*, QuantiTec	QT00021791	-
DLL4	Qiagen*, QuantiTec	QT00081004	-
GAPDH	Qiagen*, QuantiTec	QT00079247	-
GFP	Sigma-Aldrich†	-	Fwd: 5'-GCAACATCCTGGGCAATAAGATG-3' Rev: 5'-GGCGGATCTTGAAGTTCACC-3'
GREM1	Qiagen*, QuantiTec	QT00202062	-
HES1	Qiagen*, QuantiTec	QT00039648	-
HEY1	Qiagen*, QuantiTec	QT00035644	-
ITGA11	Qiagen*, QuantiTec	QT00068362	-
JAG1	Qiagen*, QuantiTec	QT00031948	-
JAG2	Qiagen*, QuantiTec	QT01003163	-
MMP9	Qiagen*, QuantiTec	QT00040040	-
NOTCH1	Qiagen*, QuantiTec	QT00231056	-
NOTCH2	Qiagen*, QuantiTec	QT00072212	-
NOTCH3	Qiagen*, QuantiTec	QT00003374	-
NOTCH4	Qiagen*, QuantiTec	QT00065023	-
PDGFRA	Sigma-Aldrich†	-	Fwd: 5'-GGTCTGCGAGCTGTGTCTGTT-3' Rev: 5'-CATTCCTCTGCCTGACATTGAC3'
PDGFRB	Sigma-Aldrich†	-	Fwd: 5'-GGATGGCTGGGTGGTCACT-3' Rev: 5'-GAGACTGTTGGGCGAAGGTTA-3'
RUNX	Qiagen*, QuantiTec	QT00026712	-
TGFB1	Qiagen*, QuantiTec	QT00000728	-
TGFB3	Qiagen*, QuantiTec	QT00001302	-
PDGFRA (LNA)	Qiagen* (Exiqon)	-	CAGC+TCTG+AT+GT+TGCT+TTTA
PDGFRB (LNA)	Qiagen* (Exiqon)	-	+CC+ATGG+AA+CCCA+GG+CAAGC
siRNA			
scrambled	Qiagen*, Gene Solution	SI03650325	-
si*NOTCH2* nr3	Qiagen*, Gene Solution	SI00136206	-
si*NOTCH2* nr5	Qiagen*, Gene Solution	SI03067526	-
si*NOTCH3*	Qiagen*, Gene Solution	SI00009499	-
Padlock probes			
PDGFRA	IDT‡	5Phos/GAGTGACCACCCAGCCATTCCTCTATGATTACTGACT GCGTCTATTTAGTGGAGCCAGCGCTATCTTCTTTGGATGAG GAGTTTCTGAG	
PDGFRB	IDT‡	5Phos/CCATTTAATGTTTATGCTTTTCCTCTATGATTACTGAC TGCGTCTATTTAGTGGAGCCCAGACTATCTTCTTTGAAAGAA GTTCCAGACCATC	
HES1	IDT‡	5Phos/CCGGATAAACCAAAGACAGCTCCTCTATGATTACTGA CTGCGTCTATTTAGTGGAGCCCGCCCTATCTTTCAGCCAGT GTCAACACGACA	

(*) Hilden, Germany; (†) Taufkirchen, Germany; (‡) Leuven, Belgium;
LNA=Locked Nucleic Acid

Supplementary table 2. Associations between PDGFRα and PDGFRβ expression and clinicopathological parameters.

[Clinicopathological parameter]	PDGFR expression, No. (%)*					
	PDGFRα (n = 351)			PDGFRβ (n = 360)		
PDGFR expression	**low**	**high**	**p value†**	**low**	**high**	**p value†**
Total	283	68	-	158	202	-
Age, y:						
<50	76 (26.9)	25 (36.8)	0.28	38 (24.0)	66 (32.7)	0.21
50-64	116 (41.0)	23 (33.8)		66 (41.8)	76 (37.6)	
≥65	91 (32.1)	20 (29.4)		54 (34.2)	60 (29.7)	
Size, mm:						
<15	119 (42.1)	31 (45.6)	0.48	68 (43.0)	84 (41.6)	0.96
≥15	103 (36.4)	20 (29.4)		54 (34.2)	72 (35.6)	
multifocal	27 (9.5)	10 (14.7)		17 (10.8)	24 (11.9)	
missing	34 (12.0)	7 (10.3)		19 (12.0)	22 (10.9)	
ER:						
ER-negative	81 (28.6)	12 (17.7)	0.005	40 (25.3)	65 (32.2)	0.007
ER-positive	192 (67.9)	47 (69.1)		114 (72.2)	119 (58.9)	
missing	10 (3.5)	9 (13.2)		4 (2.5)	18 (8.9)	
EORTC grade:						
I	18 (6.3)	9 (13.2)	0.18	10 (6.3)	16 (7.9)	0.03
II	124 (5.9)	32 (47.1)		83 (52.6)	77 (38.1)	
III	139 (49.1)	27 (39.7)		65 (41.1)	108 (53.5)	
missing	2 (0.7)	0 (0.0)		0 (0.0)	1 (0.5)	
Surgery:						
Mastectomy	64 (22.6)	15 (22.1)	1.00	38 (24.0)	43 (21.3)	0.56
Breast conserving	219 (77.4)	53 (77.9)		118 (74.7)	158 (78.2)	
missing	0 (0.0)	0 (0.0)		2 (1.3)	1 (0.5)	
Postoperative radiotherapy:						
no	106 (37.5)	24 (35.3)	0.78	57 (36.1)	80 (39.6)	0.51
yes	177 (62.5)	44 (64.7)		101 (63.9)	122 (60.4)	
PDGFRα:						
low	-	-	-	140 (88.6)	121 (59.9)	<0.001
high	-	-		6 (3.8)	61 (30.2)	
missing	-	-		12 (7.6)	20 (9.9)	

* Percentages are calculated within columns.
†Associations were calculated, with two-sided Fisher's Exact test.

y = years; ER = estrogen receptor alpha

Supplementary table 3: Multivariable analysis of risk for local recurrence (*in situ* or invasive) and metastasis.

[Clinicopathological parameter]		including PDGFRα (289*)		including PDGFRβ (297*)		including PDGFRα and –β (272 *)	
	No. events/total	HR (95% CI) †	P-value	HR (95% CI) †	P-value	HR (95% CI) †	P-value
Age:							
<50	37/121	1 (Reference)	0.06	1 (Reference)	0.14	1 (Reference)	0.11
50-64	39/187	0.55 (0.31 to 0.95)		0.60 (0.35 to 1.02)		0.58 (0.33 to 1.02)	
≥65	40/150	0.56 (0.30 to 1.04)		0.64 (0.36 to 1.16)		0.57 (0.30 to 1.09)	
Size §:							
< 15 mm	52/197	1 (Reference)	0.07	1 (Reference)	0.23	1 (Reference)	0.08
≥ 15 mm	52/158	0.90 (0.49 to 1.68)		0.95 (0.53 to 1.70)		0.95 (0.51 to 1.77)	
Multifocal	24/49	1.96 (1.02 to 3.75)		1.64 (0.88 to 3.08)		2.01 (1.03 to 3.94)	
ER status ‖:							
negative	31/123	1 (Reference)	0.76	1 (Reference)	0.79	1 (Reference)	0.83
positive	75/292	0.91 (0.51 to 1.62)		0.93 (0.55 to 1.57)		1.06 (0.59 to 1.91)	
EORTC grade ¶:							
I	11/37	1 (Reference)	0.99	1 (Reference)	0.99	1 (Reference)	0.90
II	58/203	0.98 (0.43 to 2.22)		1.04 (0.47 to 2.30)		0.96 (0.40 to 2.33)	
III	47/215	0.95 (0.41 to 2.19)		1.00 (0.44 to 2.28)		0.85 (0.35 to 2.08)	
Surgery #:							
Mastectomy	11/104	1 (Reference)	0.02	1 (Reference)	0.004	1 (Reference)	0.02
Breast conserving	103/350	2.90 (1.20 to 7.02)		3.65 (1.52 to 8.78)		2.91 (1.21 to 7.01)	
Postoperative radiotherapy:							
No	84/297	1 (Reference)	0.01	1 (Reference)	0.005	1 (Reference)	0.009
Yes	32/161	0.48 (0.27 to 0.85)		0.46 (0.27 to 0.79)		0.46 (0.26 to 0.83)	
PDGFRα **:							
low	71/283	1 (Reference)	0.87	-	-	1 (Reference)	0.21
high	15/68	0.95 (0.51 to 1.77)		-		0.65 (0.33 to 1.28)	
PDGFRβ ††:							
low	36/158	-	-	1 (Reference)	0.15	1 (Reference)	0.02
high	57/202	-		1.41 (0.88 to 2.24)		1.95 (1.12 to 3.40)	
Total case number:	116/458						

* No of patients included in Cox proportional hazards model.
† Hazard ratios are estimated using proportional hazards regression with event defined as local recurrence or metastasis. All variables were included in the regression model.
‡ P values are based on a two-sided Wald test.
§ Data missing for 54 patients. ‖ Data missing for 43 patients. (¶) Data missing for 3 patients. (#) Data missing for 4 patients. (**) Data missing for 107 patients.
(††) Data missing for 98 patients.

HR = Hazard Ratio; CI = confidence interval

Supplementary Table 4. Co-culture mediated gene expression changes in fibroblasts.

Gene	fold change co-cultured HMF versus mono-culture HMF (n=1)	Comment	Validation presented in manuscript	Pathway	Gene	fold change co-cultured HMF versus mono-culture HMF (n=1)	Comment	Validation presented in manuscript	Pathway
HES1	209.10	†	x	Notch signaling	*RARA*	1.24			
TGFBR3	133.73	†			*GDF6*	1.18			
SNAI1	41.53				*TGFB1*	1.17		x	TGF ligand
HEY1	23.11		x	Notch signaling	*GDF2*	1.17	‡		
*IFRD1**	22.23	†			*DCN*	1.12			
*IFRD1**	1.29				*MYC**	1.09			
BMPR2	16.37				*MYC**	-1.73			
TGFB3	13.62		x	TGF ligand	*TGFBRAP1*	1.08			
NOG	9.14		x	BMP Inhibitor	*BMP5*	1.04	§		
BMPR1B	7.24	†			*GDF3*	1.04	§		
BMP1	7.00		x	BMP ligand	*AIPL1*	1.04	§		
IL6	6.91	‡			*IL10*	1.04	§		
*SERPINE1**	6.83				*MSX2*	1.04	§		
*SERPINE1**	-1.13				*MYOD1*	1.04	§		
NOTCH1	6.59				*PTGS2*	1.02			
CHRD	6.02		x	BMP Inhibitor	*THBS1**	1.01			
PDGFA	6.01				*THBS1**	-1.01			
VEGFA	4.95				*TGFB111*	1.01			
SMAD7	4.43				*MBD1*	1.01			
SHH	4.13	‡			*TGIF1*	1.00	‡		
MECOM	3.80				*PTK2*	-1.01			
BMP2	3.76	‡			*EMP1**	-1.08			
*FOS**	3.54	‡			*EMP1**	-1.25			
*FOS**	2.40				*RHOA*	-1.10			
ACTA2	3.37				*JUN*	-1.11			
*RUNX1**	3.30		x	BMP Inhibitor	*BGLAP*	-1.12			
*RUNX1**	1.35				*SMAD6*	-1.12	‡		
LEFTY1	3.20				*SMAD1**	-1.14			
CEBPB	3.11				*SMAD1**	-1.48			
PTHLH	3.11				*LTBP1*	-1.14			
*TNFSF10**	2.96				*PLG*	-1.16			
*TNFSF10**	1.59				*CTNNB1*	-1.19			
BHLHE40	2.81				*RHOB*	-1.19			
*TGFB2**	2.78		x	TGF ligand	*IGF1*	-1.21			
*TGFB2**	1.15				*INHBB*	-1.21			
ID3	2.75				*ACVRL1**	-1.22	‡		
COL1A1	2.45				*ACVRL1**	-1.74	‡		
*SMAD3**	2.45				*BAMBI*	-1.22			
*SMAD3**	-4.66				*CREBBP*	-1.22			
KLF10	2.37				*PPARA*	-1.25			
SMAD4	2.33				*SMAD2*	-1.30			
INHBA	2.27	‡			*MAP3K7*	-1.33			
AGT	2.23	‡			*RYBP*	-1.36			
*SOX4**	2.06				*NODAL*	-1.36			
*SOX4**	-2.11				*BRD2*	-1.38			
*ATF4**	1.98				*JUNB*	-1.38			
*ATF4**	1.25				*IGFBP3*	-1.40			
*ACVR1**	1.97				*BMP7*	-1.40	‡		
*ACVR1**	1.61				*GLI2*	-1.43	‡		
*ID2**	1.95	†			*HIPK2*	-1.50			
*ID2**	-3.14				*BCL2L1*	-1.52			
STAT1	1.94	†			*TGFBR2**	-1.54			
ACVR2A	1.93				*TGFBR2**	-13.24			
S100A8	1.89	‡			*DNAJA1*	-1.57			
BMP3	1.85	‡			*BMPR1A*	-1.57			
GDF5	1.84				*E2F4*	-1.59			
*GADD45B**	1.81	‡			*CREB1*	-1.72			
*GADD45B**	1.46				*EP300*	-1.76			
PTK2B	1.80				*TXNIP*	-1.81			
PDGFB	1.71				*SMURF1*	-1.86	‡		
EPHB2	1.66				*GTF2I*	-1.91			
AMHR2	1.66	‡			*FN1*	-1.94			
BMP6	1.64	‡			*CDC6*	-1.95			
PLAU	1.63				*MAPK8*	-2.07			
*ENG**	1.62	‡			*RBL1*	-2.07			
*ENG**	1.26				*RAD21*	-2.21			
CDKN2B	1.58	‡			*CRYAB*	-2.25	†		
ATF3	1.55				*CDKN1A*	-2.32			
GSC	1.54				*NFIB*	-2.35			
DLX2	1.53				*SREBF2*	-2.35			
TSC22D1	1.52				*MAPK14*	-2.42			
*ID1**	57.08				*AR*	-2.54			
*ID1**	1.47				*GDF7*	-2.82			
NFKBIA	1.45				*COL1A2*	-2.85			
TGFBI	1.42	‡			*CDKN1B**	4.52			
TGFBR1	1.40				*CDKN1B**	-3.09			
HMOX1	1.37				*SP1*	-3.28			
LTBP2	1.33				*BDNF*	-3.70			
FURIN	1.33				*MMP2*	-4.11		x	matrix remodeling
*HERPUD1**	1.33				*BACH1*	-4.33			
*HERPUD1**	1.04	§			*INHA*	-5.06	‡		
AMH	1.31	‡			*BMPER*	-5.94			
*SMAD5**	1.31				*FST*	-8.77	†		
*SMAD5**	-1.14				*BMP4*	-9.79	†	x	BMP ligand

qRT-PCR array (PAHS-035Z and PAHS-235Z, Qiagen, Hilden, Germany) of sorted HMF after 48h of co-culture with MCF10DCIS versus mono-cultured HMF. Both arrays were performed once. Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in the Test Sample divided the normalized gene.

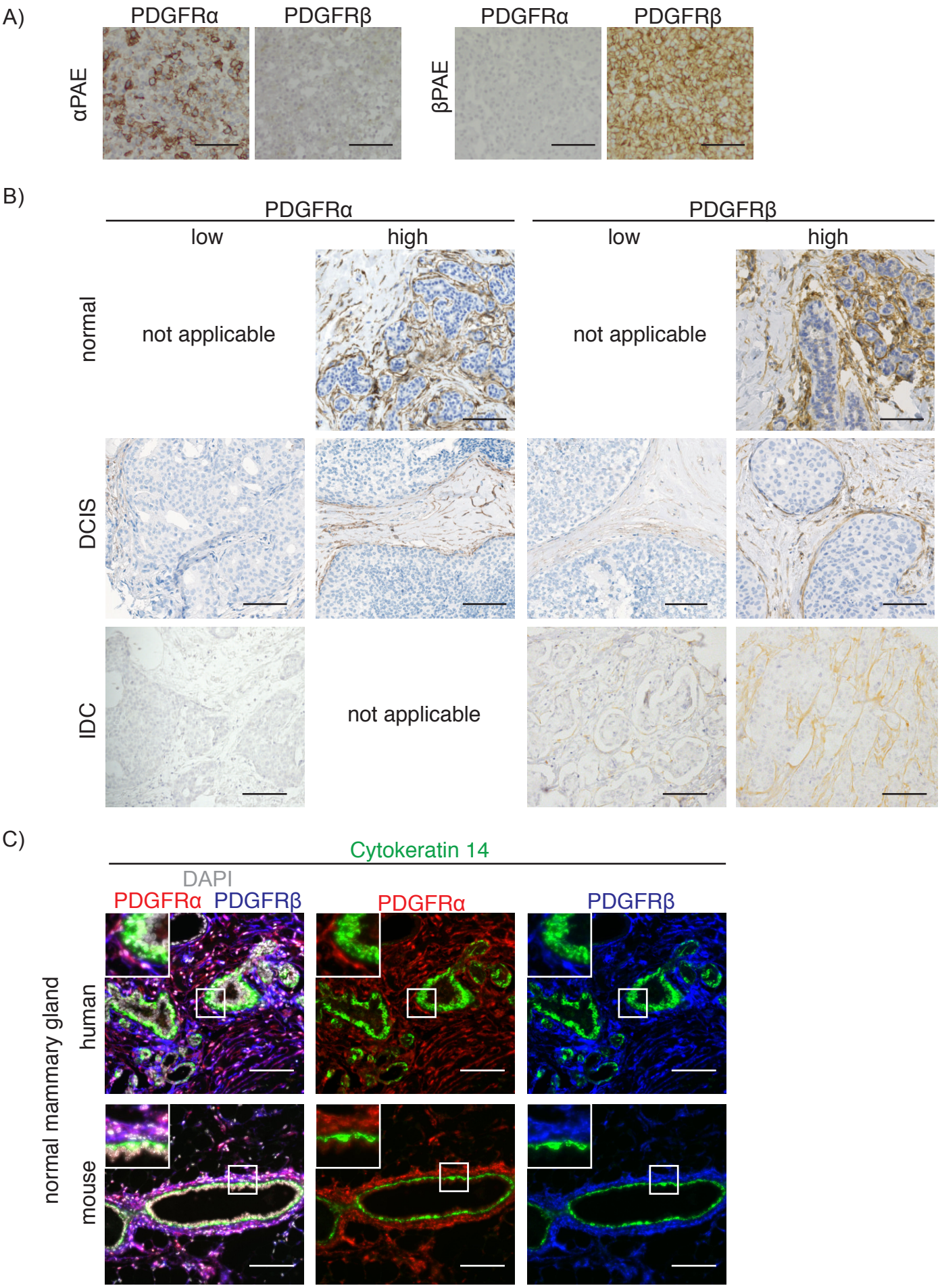
* Genes overlapping between the arrays.

†: This gene's threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other

‡: This gene's threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test

§: This gene's threshold cycle is greater than the defined cut-off value (default 35).

Supplementary Figure 1



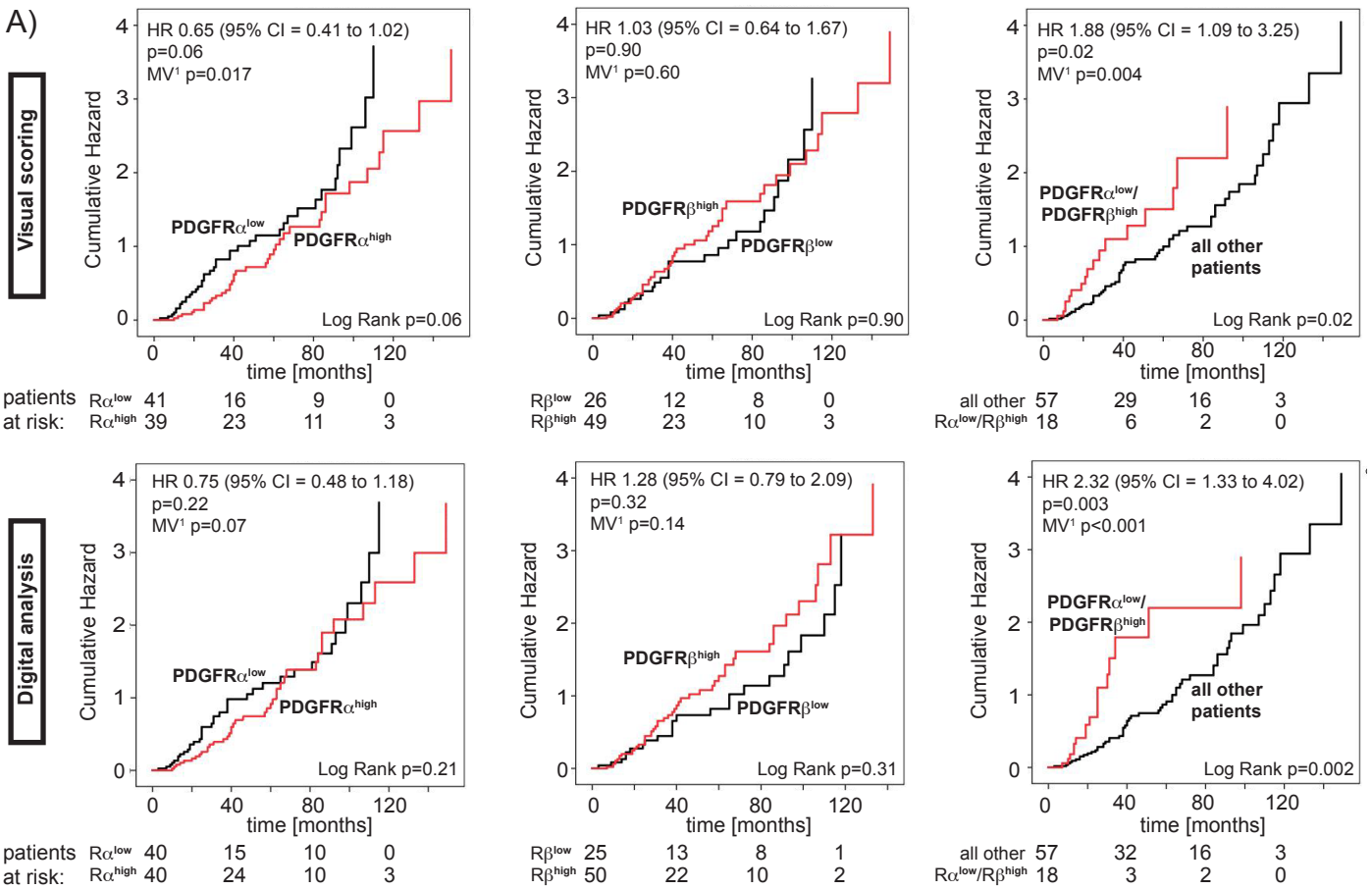
Supplementary figure 1. PDGFR staining evaluation, scoring scheme and expression pattern in human and murine mammary gland.

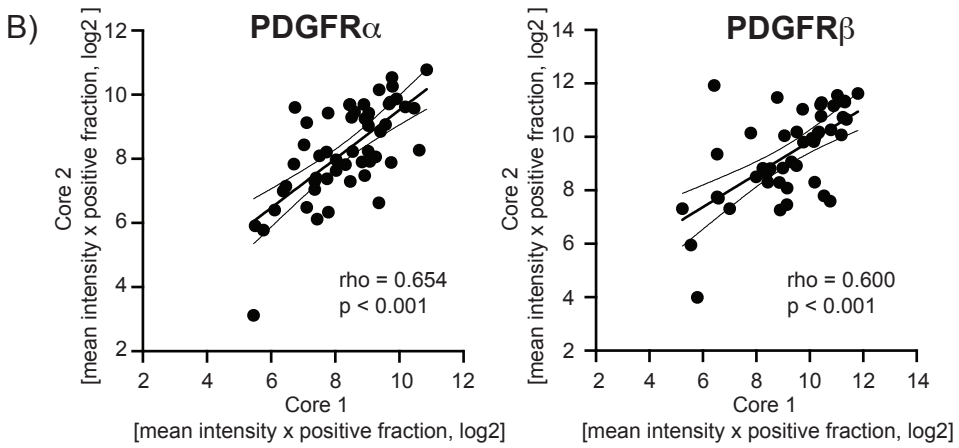
(A) PDGFR antibody validation was performed on FFPE reparations of PAE cells stably expressing PDGFRα or PDGFRβ. Sections were stained with the PDGFRα and PDGFRβ-antibodies to analyze antibody specificity.
(B) Microphotographs of representative cases of human normal breast tissue, DCIS and invasive carcinoma cases scored as high or low regarding PDGFRα and PDGFRβ expression. For further information on scoring of the IDC cohort please see Paulsson et al, AJP 2009[1].
(C) Microphotographs of representative immunofluorescence triple stainings of human and murine normal breast tissue for the myoepithelial marker Cytokeratin 14 (green) together with PDGFR α (red) and PDGFRβ (blue). Nuclei were stained with DAPI (white). Size-bars are 100 micrometer.

FFPE = formalin-fixed paraffin embedded; PAE = porcine aortic endothelial cells; DCIS = ductal carcinoma *in situ*; IDC = invasive ductal carcinoma

1. Paulsson J, Sjöblom T, Micke P, et al. Prognostic Significance of Stromal Platelet-Derived Growth Factor β-Receptor Expression in Human Breast Cancer. *Am J Pathol*. 2009;175(1):334. doi:10.2353/ajpath.2009.081030

Supplementary Figure 2





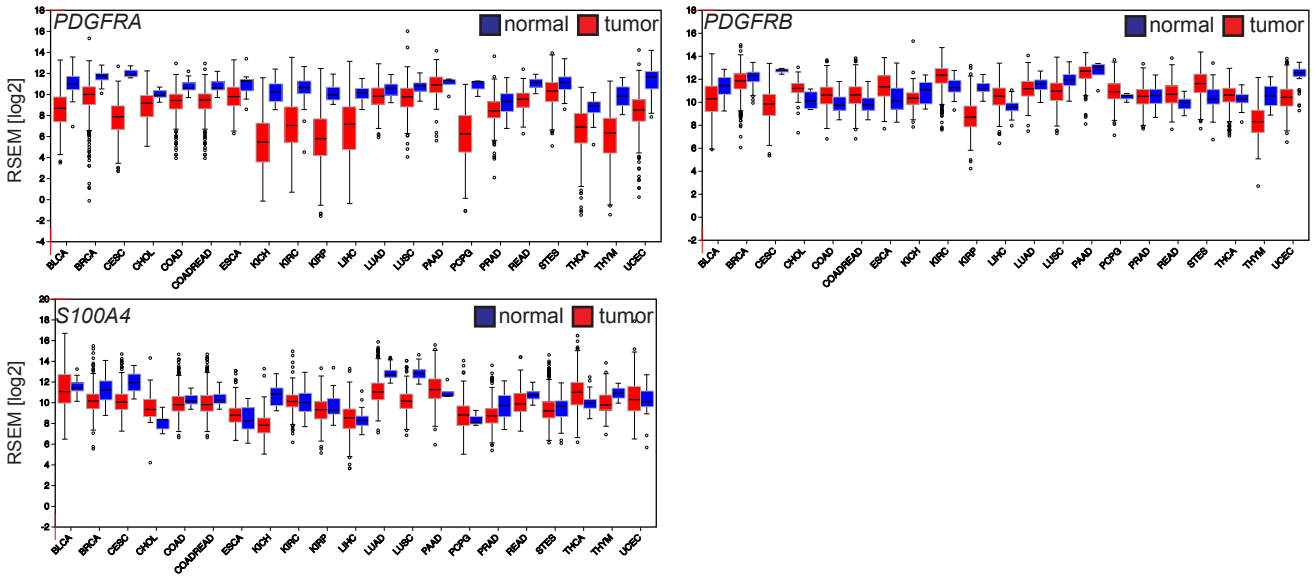
Supplementary figure 2. Kaplan-Meier plots of DCIS_Nation cohort and evaluation of intratumoral heterogeneity.

(A) PDGFR status of the DCIS_Nation cohort was evaluated through combined visual scoring of the staining intensity and positive fraction *(upper panel)* as well as digital analysis of the mean staining intensity multiplied by the positive area fraction *(lower panel).* The product of the mean staining intensity and positive area fraction was calculated for the digital data and dichotomization was performed in a way aiming for similar group sizes as obtained with the histological scoring. Kaplan-Meier plots show the relationships between PDGFR status and risk for local recurrence (*in situ* or invasive) in DCIS with P-values derived from Log Rank test. Graphs also present HR, including CIs as determined by univariate Cox proportional hazards regression analyses with P-values derived from Wald test. MV of risk for local recurrence was performed using a Cox proportional hazards model including age, size, ER-status, surgery, radiotherapy ([1]note, in contrast to the MV on the population based analysis in the main manuscript, EORTC grade was not included in the MV for DCIS_Nation due to incomplete data). MV P-values are based on Wald test. Corresponding tables indicate the number of patients at risk.
(B) Potential intratumoral heterogeneity in PDGFR expression per patient was investigated through correlation analysis between the two cores per patient that were included in the DCIS_Nation cohort using the data of the digital scoring. The digital data (product of mean intensity and positive area fraction) was log2-transformed for presentation. Rho- and p-value are derived from Spearman rank correlation, two-sided. The linear regression line with 95% confidence interval is indicated.

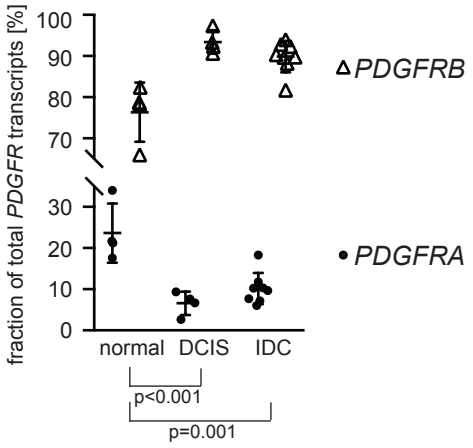
DCIS = ductal carcinoma *in situ*; HR = Hazard Ratios; CI = confidence interval; MV = Multivariable analysis; ER = estrogen receptor alpha

Supplementary Figure 3

A)

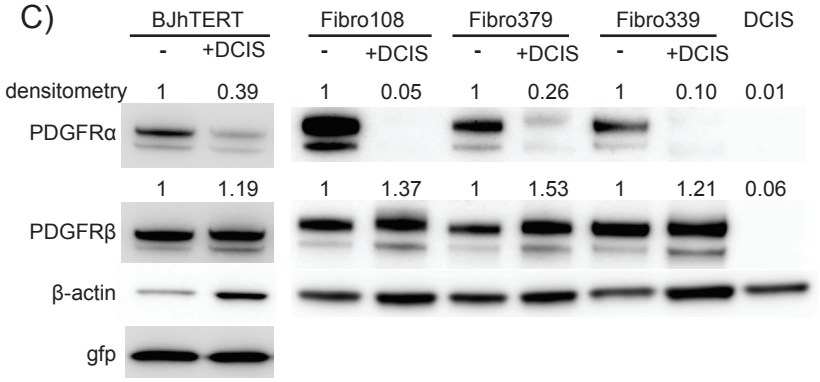


B)

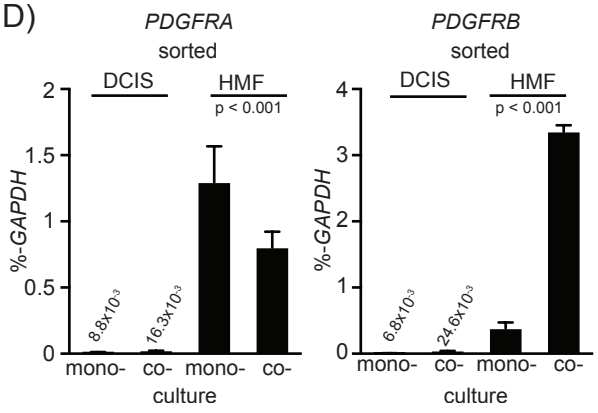


	number of transcripts			number of transcripts	
Stroma of:	***PDGFRA***	***PDGFRB***	**Stroma of:**	***PDGFRA***	***PDGFRB***
normal 1	709	3320	**IDC 1**	673	10465
normal 2	1051	2037	**IDC 2**	672	5840
normal 3	371	1378	**IDC 3**	362	3371
normal 4	226	816	**IDC 4**	965	8460
DCIS 1	16	582	**IDC 5**	476	6135
DCIS 2	84	1179	**IDC 6**	567	4252
DCIS 3	253	2447	**IDC 7**	2103	25320
DCIS 4	116	1398	**IDC 8**	3105	13875

C)



D)

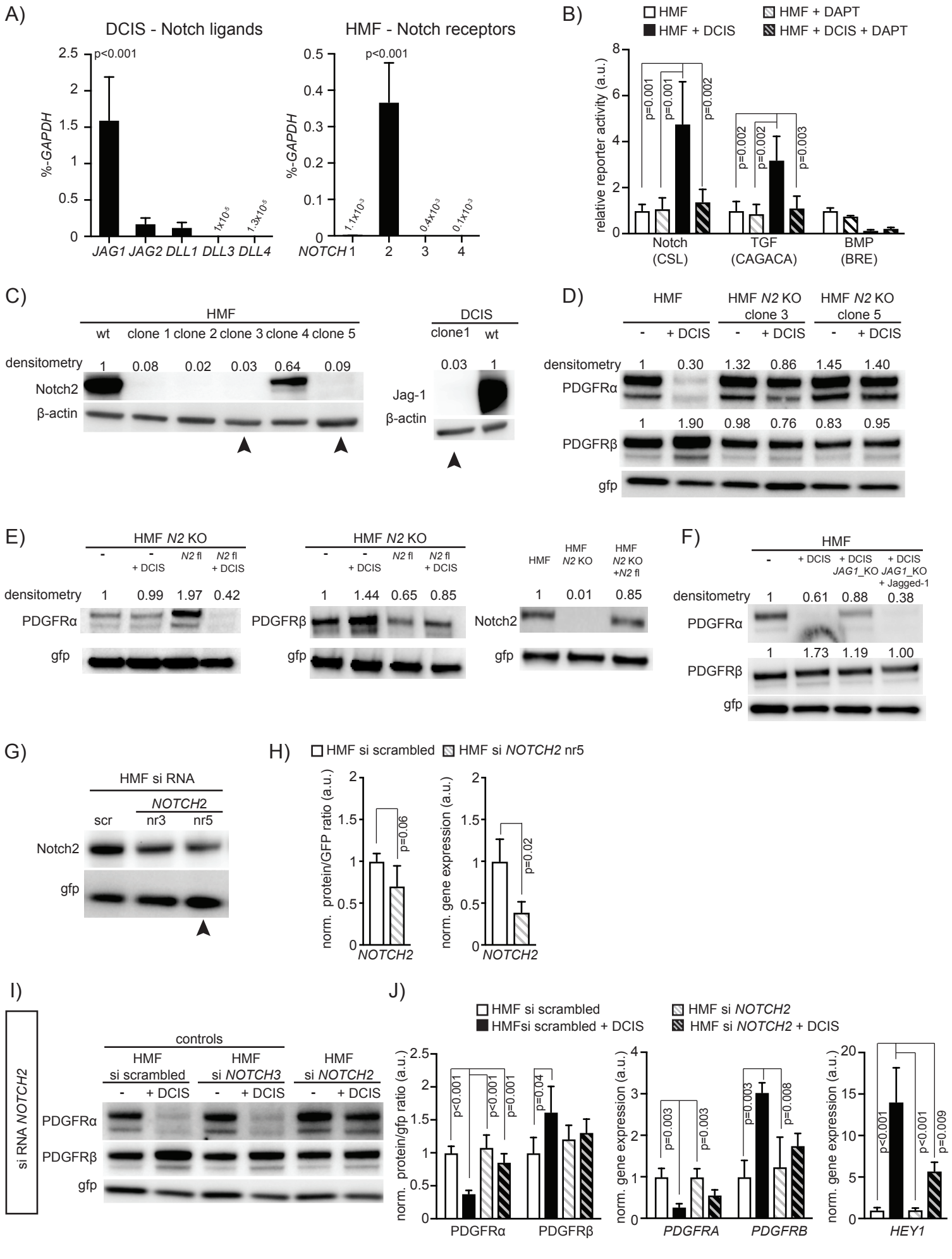


Supplementary figure 3. Changes of PDGFRα protein and mRNA level during tumor progression and upon co-culture with tumor cells

(A) Gene-expression of *PDGFRA* and *PDGFRB* is represented based on RSEM values from RNA sequencing data of various solid tumor types and in corresponding normal tissue. Gene-expression data of *S100A4* is also presented to allow evaluation of overall stroma abundance in the tumor and normal tissue. Box-plots present the median and the whiskers indicate the 25-75 percentile. Outliners are presented as circles and are defined as values that lie more than 1.5 times of the IQR above or below the upper or lower quartile respectively. Data are derived from the TCGA Research Network: http://cancergenome.nih.gov/ and were generated using the TCGA Firebrowse Version 1.1.35 (https://www.broadinstitute.org/).
(B) The padlock-probe based in situ sequencing technique was used to investigate the abundance of transcripts for *PDGFRA* (dots) and *PDGFRB* (triangles) in stroma regions of four sections of normal breast tissue, four sections of DCIS lesions and eight sections of invasive cancers. The fraction of *PDGFRA* or respectively *PDGFRB* on total *PDGFR* transcript abundance is presented in percentage. Mean value with standard deviation is indicated and p-values are ANOVA with Bonferroni *post hoc* test. The table presents raw transcript counts.
(C) Direct co-culture with DCIS cells impacts PDGFR expression in fibroblast. Representative immunoblots of PDGFR expression in fibroblasts under conditions of mono-culture or in co-culture with MCF10DCIS cells using immortalized human foreskin fibroblasts (BJhTert) or three different cultures of primary human mammary fibroblasts (Fibro108, Fibro379 and Fibro339). Last lane demonstrates absence of PDGFR protein in DCIS mono-cultures. For PDGFR α and - β detection the membranes were stripped and reprobed. Beta-actin or gfp respectively were detected on the same membrane. Densitometric values for BJhTERT cells were determined using gfp as loading control. No loading control could be applied for the primary fibroblasts as these cells were not transfected with an internal reference. Densitometric values were further normalized to the control group and are presented as arbitrary unit.
(D) QRT-PCR analysis was performed to analyze *PDGFR* expression in FACS sorted DCIS and HMF cells derived from mono-cultures or co-cultures. Expression is presented as the percentage of *GAPDH*. Quantifications are based on at least three experiments. The indicated p-values are derived from group comparison by ANOVA with Bonferroni *post hoc* test and indicate the difference between each HMF subgroup (mono- and co-culture) to each DCIS subgroup (mono- and co-culture).

IQR = interquartile range; TCGA = The Cancer Genome Atlas; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; COADREAD: Colorectal adenocarcinoma; ESCA: Esophageal carcinoma; KICH: Kidney Chromophobe; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; STES: Stomach and Esophageal carcinoma; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; DCIS = ductal carcinoma *in situ*; IDC = invasive ductal carcinoma; gfp = green fluorescent protein; HMF = human mammary fibroblasts

Supplementary Figure 4



Supplementary figure 4. Notch signaling dependent regulation of PDGFR expression in fibroblasts

(A) Profiling for the expression of Notch-ligands in breast DCIS cells and Notch receptors in human mammary fibroblasts was performed by qRT-PCR analyses of MCF10DCIS cells and HMF for indicated genes. Expression is presented as the percentage of *GAPDH*. Quantifications are based on at least three experiments. P-values are derived from ANOVA with Bonferroni *post hoc* test.
(B) Co-culture induced modulation of promoter-activity in fibroblasts is sensitive to DAPT. Activity from indicated promoter-reporter constructs was determined in HMF under conditions of monoculture or co-culture with MCF10DCIS cells, in the absence or presence of the gamma-secretase-inhibitor DAPT. Notch signaling is measured with the 12xCSL-Luciferase vector, TGF-beta signaling with the pGL3-(CAGA)12-Luciferase and BMP signaling with the pGL3-BRE2-Luciferase vector. Quantifications are based on four experiments. Data was normalized to the mean of the control group (set to 1.0 a.u.) and is presented as average with standard deviation. P-values are derived from ANOVA with Bonferroni *post hoc* test.
(C-F) Show the identification and characterization of HMF and breast MCF10DCIS cells with CRISPR/Cas9-mediated inactivation of *NOTCH2* and *JAG1*, respectively.
(C) Identification of fibroblasts and MCF10DCIS cells with CRISPR/Cas9-mediated inactivation of *NOTCH2* and *JAG1*, respectively. Immunoblot-analyses of target gene status in HMF cells subjected to CRISPR/Cas9-targeting of *NOTCH2* (left) and breast DCIS cells subjected to CRISPR/Cas-targeting of *JAG1* (right). Corresponding wt cells were included as positive controls. Selected clones are marked by arrowheads.
(D) Notch2-dependent regulation of PDGFR expression in fibroblasts during co-culture with MCF10DCIS cells. The representative immunoblot demonstrates, that the two selected clones of HMF with CRISPR/Cas9-mediated inactivation of *NOTCH2* perform equally in the experimental setting. For PDGFRα and -β detection the membranes were stripped and reprobed. Gfp was detected on the same membrane.
Densitometric values were determined using β-actin (C) or gfp (D) as loading control, normalized to the control monoculture and are presented as arbitrary unit.
(E) Western Blot analysis to test for off-target effects of *NOTCH2* inactivation in HMF cells. Co-culture assay was performed with HMF *NOTCH2* knock-out cells and MCF10DCIS. Notch2 was reintroduced through transfection of HMF *NOTCH2* knock-out with a vector construct for full-length *NOTCH2*. Notch2 reintroduction is demonstrated in the right panel. Densitometric values were determined using gfp as loading control. The experiment was performed with the two different *NOTCH2* knock-out clones showing similar result.
(F) Western Blot analysis to test for off-target effects of *JAG1* inactivation in MCF10DCIS cells. Co-culture assay was performed with HMF and MCF10DCIS *JAG1* knock-out cells. Jagged-1 was reintroduced through coating of the culture dish with Jagged-1 protein. Densitometric values were determined using gfp as loading control. The experiment was performed twice with similar result.
(G-J) Notch2-dependent regulation PDGFR expression in fibroblasts is investigated by siRNA-mediated down-regulation of *NOTCH2*.

(G) Demonstration of siRNA-mediated down-regulation of Notch2 in HMF. Immunoblot-analyses of Notch2 in HMF transfected with control siRNA or two different siRNAs against Notch2. (H) Quantification of Notch2 protein and mRNA in fibroblasts transfected with control siRNA or *NOTCH2*-targeting siRNA.
(I) Representative immunoblots of PDGFR expression in HMF transfected with control siRNA, *NOTCH2*-targeting siRNA or *NOTCH3*-targeting siRNA under condition of mono-culture or in co-culture with MCF10DCIS cells.
(J) Corresponding quantification of PDGFR protein (left panel), *PDGFR* mRNA (middle panel) and *HEY1* mRNA (right panel).
Quantifications in H and J are based on three experiments. Protein levels (Western Blot data) were determined using gfp as loading control and further normalized to the mean of the control group (set to 1.0 a.u.). For PDGFRα and -β detection the membranes were stripped and reprobed. Gfp was detected on the same membrane. Changes in geneexpression (qRT-PCR data) were calculated by the comparative $\Delta\Delta C_T$-method with *GFP* as housekeeping gene for input control. Expression values were further normalized to the mean of the control (set to 1.0 a.u.). Data are presented as average with standard deviation. P-values are derived from ANOVA with Bonferroni *post hoc* test.

DCIS = ductal carcinoma *in situ*; HMF = human mammary fibroblasts; a.u. = arbitrary units; wt = wildtype; gfp/*GFP* = green fluorescent protein; N2 = Notch2; fl = full-length; KO = knock-out; scr = scrambled