

LYN is a mediator of epithelial-mesenchymal transition and target of dasatinib in breast cancer

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SUPPLEMENTARY TABLES AND FIGURES

Table S1 – PCR primers and conditions for sequencing of LYN

Exon	Forward primer	Reverse primer	Anneal Temp ^a
8	5'-CGGCAGGTTGGACACTATAAG	5'-AGCTGATCGAAATTGGCTTG	57°C
9	5'-GGCAGTGGGAGGAACTCAC	5'-CCCAAAGTGCTGGGATTATAG	59°C
10	5'-ACAGAATTGCAAAGCCAATC	5'-CTCCTTCATGAATTTGACGC	57°C
11	5'-ATGGCACTTTCCAGTCACCT	5'-TCATCTTGCTGAATCTATTTGTG	57°C
12	5'-GAAAGTATGGGGTCCACATGTC	5'-TAATGCCTTCATGCAACCTG	59°C
13_1	5'-GGCATGGGTTTCTGTTCTTT	5'-GAGGGTGTTCCTCCTGAGTAATTT	57°C
13_2	5'-GGTGGCTGCCTCATTAGAG	5'-CTGCTGACTTCACCTGCAA	59°C

^aPCR was performed using 100ng DNA template, 1X Pfx buffer (Invitrogen), 1X Enhancer solution (Invitrogen), 300µM dNTPs, 1.0mM MgSO₄, 15pmol each primer, and 1.25U Platinum Pfx polymerase (Invitrogen) in a 50µl reaction. The reaction conditions were: 94°C 5min initial denaturation, followed by 35 cycles (94°C 30s; annealing temp 30s; 68°C 60s), and a final extension of 68°C 3min.

Table S2 – siRNA sequences

siRNA	Sequence
LYN #1	5'-GCGACAUGAUUAAACAUUAUU-3'
LYN #2	5'-GUGAUGUUAUUAAGCACUAUU-3'
LYN #3	5'-UUACAUCUCUCCACGAAUCUU-3'
SRC #1	5'-GCGACAUGAUUAAACAUUAUU-3'
SRC #2	5'-GCAGAGAACCCGAGAGGGA-3'
SRC #3	5'-CCAAGGGCCUCAACGUGAA-3'
SRC #4	5'-GGGAGAACCUCUAGGCACA-3'

Table S3 – LYN immunostaining in relation to other IHC markers

	LYN-	LYN+	P-value ^a
Total informative cases ^b =918	792	126	
CK5/6-	741	54	
CK5/6+	51	72	<i>P</i> <0.001
EGFR-	725	71	
EGFR+	67	55	<i>P</i> <0.001
Luminal A (ER or PR+, HER2-)	449	17	
Luminal B (ER or PR+, HER2+)	123	1	
HER2 (ER and PR-, HER2+)	104	8	
Basal-like (ER-, PR-, HER2-, CK5/6 or EGFR+) ^c	60	81	<i>P</i> <0.001
Other (ER-, PR-, HER2-, CK5/6-, EGFR-)	56	19	
Luminal A (ER or PR+, HER2-)	449	17	
Luminal B (ER or PR+, HER2+)	123	1	
HER2 (ER-, PR-, HER2+)	104	8	
Triple negative (ER-, PR-, HER2-)	116	100	<i>P</i> <0.001

^aTwo-sided Fisher's exact test; selected subtype vs. other(s)

^bScorable for all markers (LYN, ER, PR, HER2, CK5/6, EGFR)

^cSurrogate IHC subtypes, refs. (1, 2)

1. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *Jama* 2006;295:2492-502.
2. Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10:5367-74.

Figure S1. Unsupervised cluster analysis distinguishes epithelial and mesenchymal breast cancer cell lines, and normal breast fibroblasts. Clustering based on expression of 6,947 variably-expressed genes. Expression ratio (\log_2) scale shown. Annotated gene clusters (right) identify selected expression patterns (i) elevated in mesenchymal compared to epithelial breast cancer lines and normal breast fibroblasts, (ii) elevated in epithelial compared to mesenchymal breast cancer lines and normal breast fibroblasts, and (iii) shared among mesenchymal breast cancer lines and normal breast fibroblasts.

Figure S2. Cell line-derived EMT signature. Shown is a heatmap of the full 200 genes (rank ordered) comprising the EMT signature, defined as the top ranked 100 genes overexpressed in mesenchymal breast cancer cells compared to epithelial breast cancer cells and normal breast fibroblasts, and in epithelial breast cancer cells compared to mesenchymal breast cancer cells and normal breast fibroblasts. Cell line characteristics are indicated (black box = yes). Expression ratio (\log_2) scale shown.

Figure S3. EMT signature stratifies breast tumors into prognostically-relevant subgroups. In three independent breast cancer microarray datasets, **(A)** Sotirou *et al.*; **(B)** van de Vijver *et al.* (NKI cohort), and **(C)** Bild *et al.*, breast tumors were clustered (Pearson correlation; complete linkage clustering) in the space of the EMT signature genes. The resultant heatmaps are shown (*left*). For each cohort, the resultant two major sample groups were designated either “EMT+” (purple sample branches), associated more with EMT genes overexpressed in mesenchymal lines (purple gene bars); or EMT- (blue sample branches), associated more with EMT genes overexpressed in epithelial lines (blue gene bars). For each cohort, the EMT+ and EMT- groups were then compared by Kaplan-Meier analysis (*right*) (P-values shown).

Figure S4. Absence of observed morphologic changes following LYN knockdown. Shown are phase contrast micrographs of siRNA-transfected cells, photographed at 40X

magnification. Cells were re-plated 16 hours post transfection, then photographed 48 hours later. Cell line and transfected siRNA are indicated.

Figure S5. Exclusion of off-target RNAi effects. Transfection of multiple different individual siRNAs (from the LYN targeting siRNA pool) recapitulates a specific phenotype (inhibition of invasion), effectively excluding off-target RNAi effects. **(A)** Confirmation of LYN knockdown by Western blot. **(B)** Effect of LYN knockdown on tumor cell invasion, assayed by Boyden chamber. Means and standard deviations shown. *, $P < 0.05$; Student's t-test.

Figure S6. LYN (but not SRC) is overexpressed in basal-like breast lines. Heatmap shows expression levels (mean-centered log₂ ratios; fold-change indicated) of ABL and Src-family kinase genes in an expanded collection of 50 breast lines (cell line clustering based on 8,750 variably expressed genes). Microarray data from Kao *et al.*, PLoS One, 2009. LYN is significantly overexpressed in basal-like (compared to luminal) lines ($P < 0.001$; Mann-Whitney U-test).

Figure S7. LYN immunostaining patterns in breast cancers. Representative photomicrographs show the range of LYN tumor epithelia and stroma expression patterns in breast cancer cases (from the tissue microarray).

Figure S1

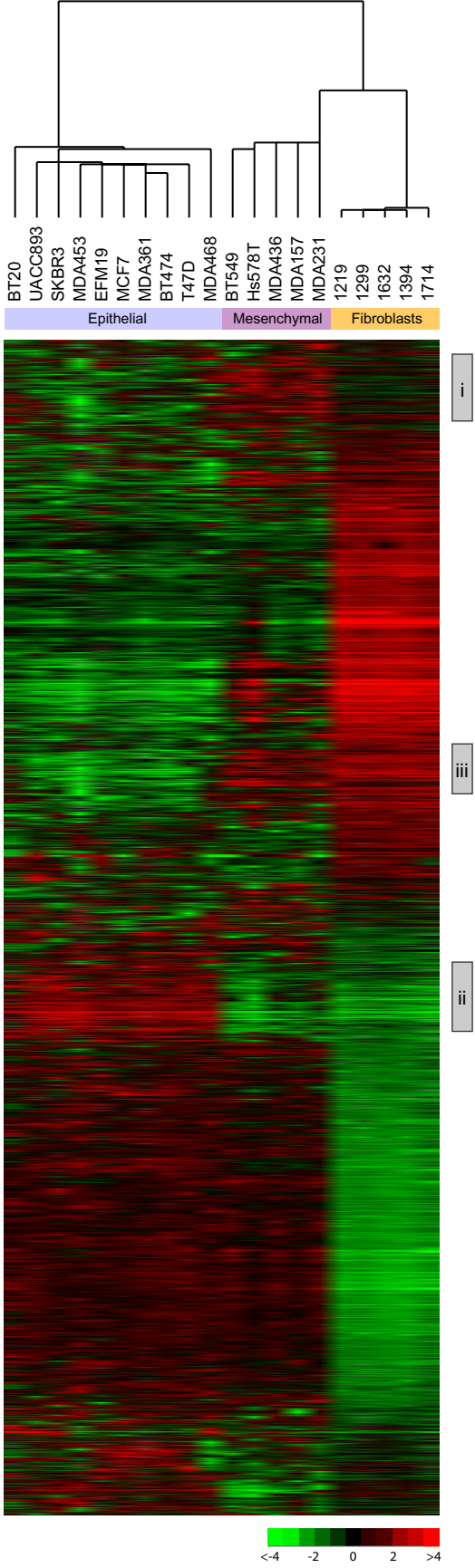


Figure S2

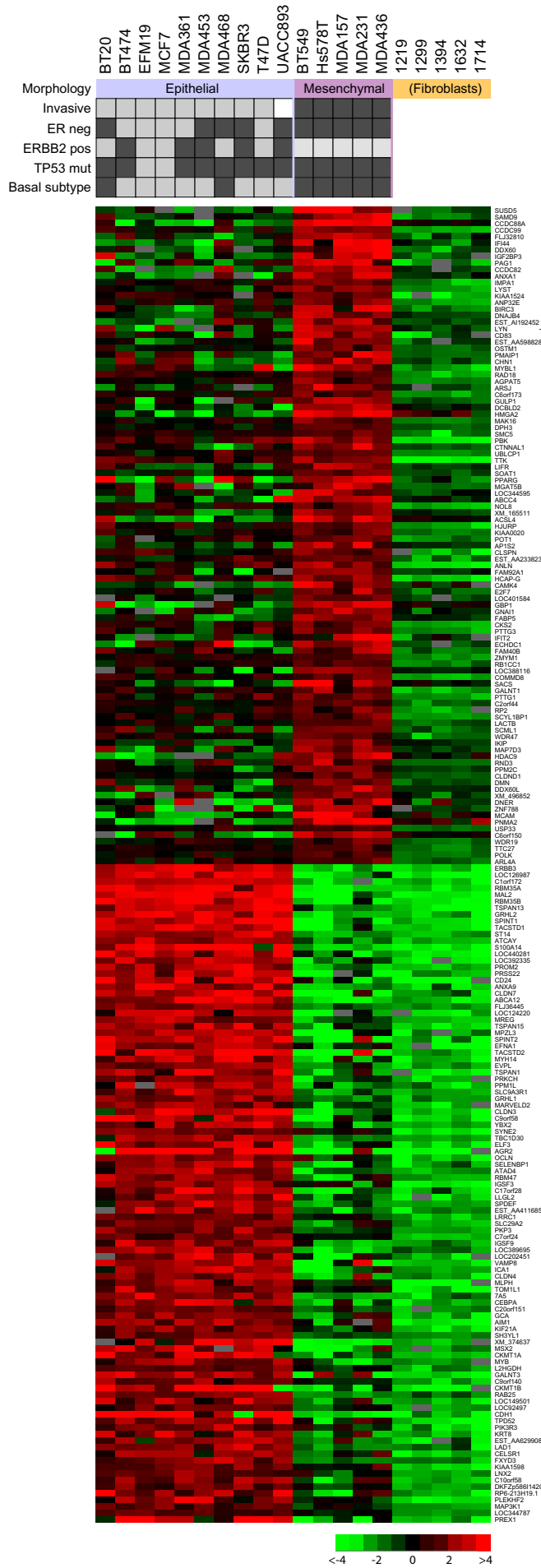


Figure S3

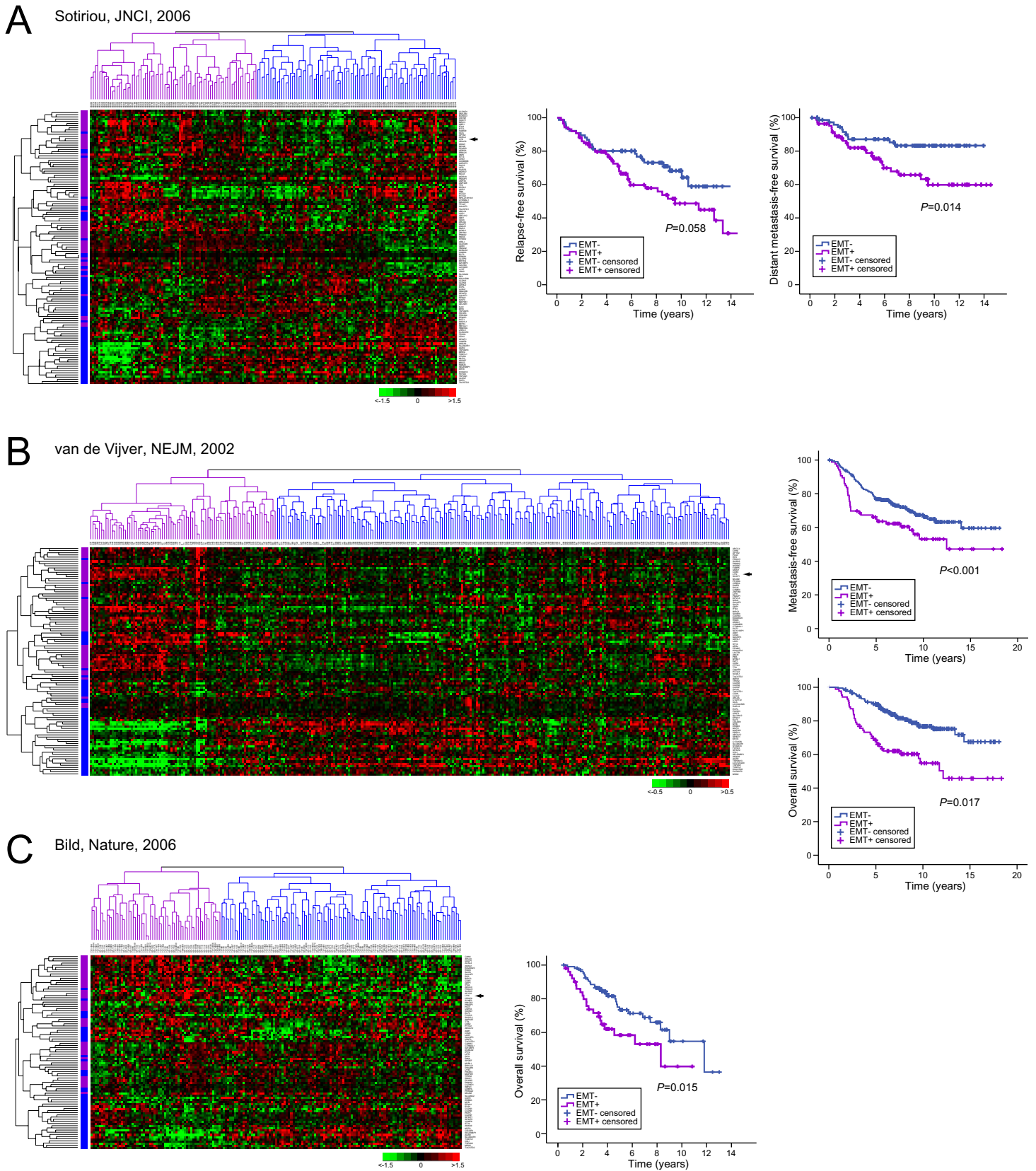
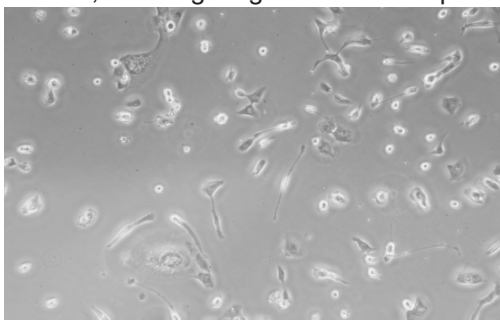
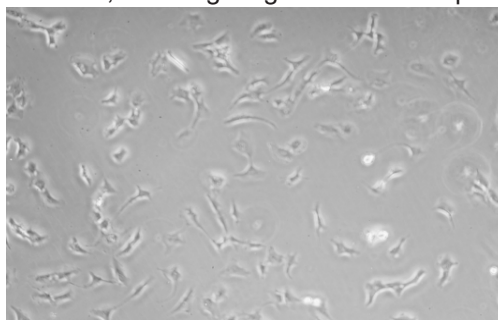


Figure S4

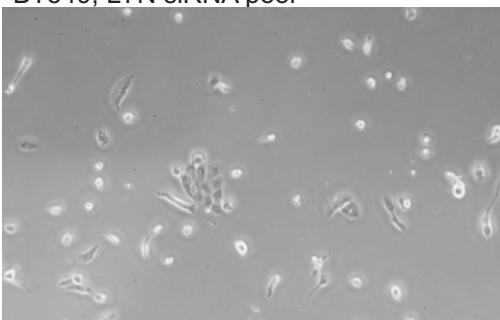
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Hs578T, non-targeting control siRNA pool



BT549, LYN siRNA pool



Hs578T, LYN siRNA pool

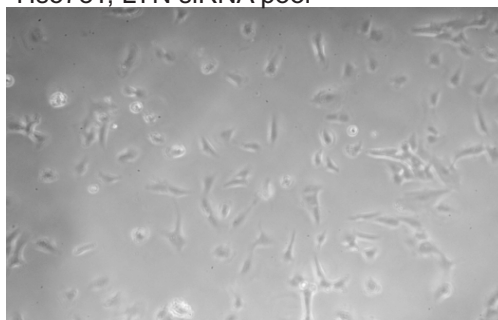


Figure S5

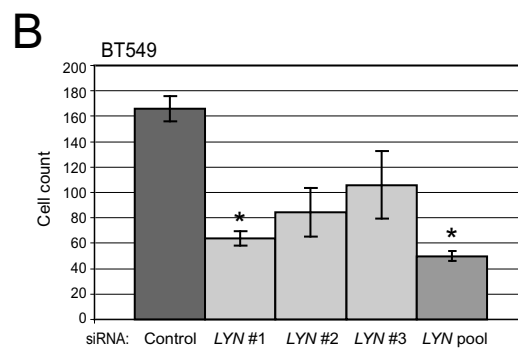
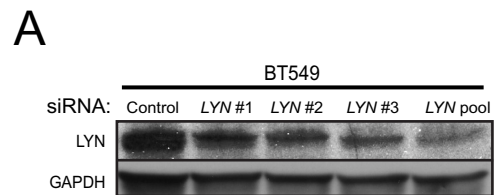


Figure S6

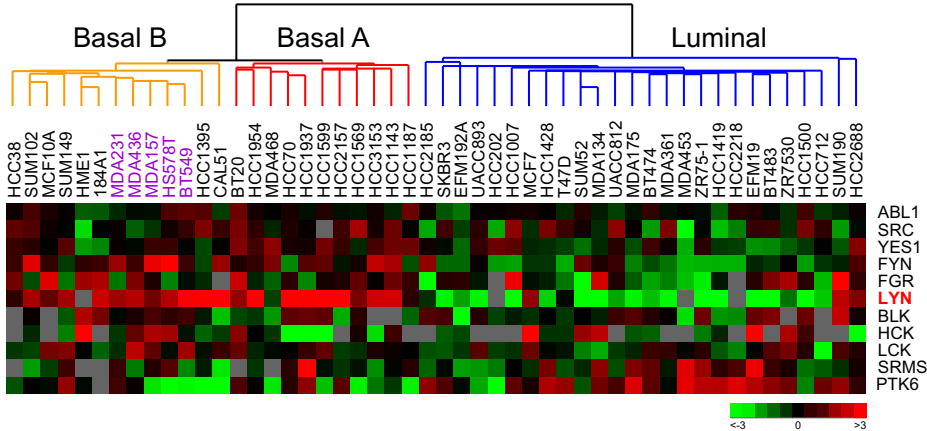
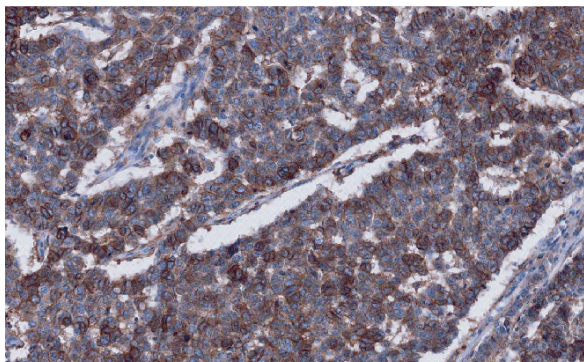
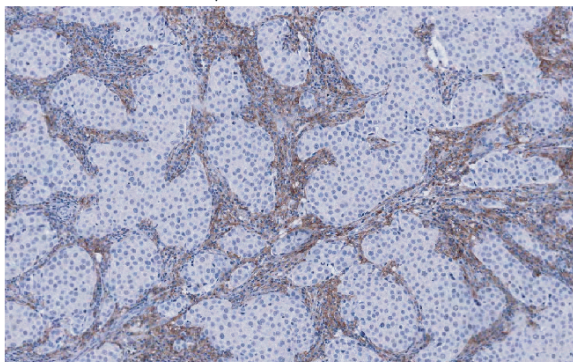


Figure S7

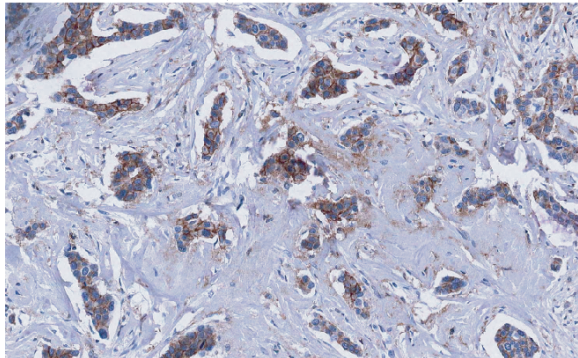
Tumor cells LYN+; stromal cells LYN+



Tumor cells LYN-; stromal cells LYN+



Tumor cells LYN+; stromal cells mostly LYN-



Tumor cells LYN-; some stromal cells LYN+

