**Supplemental data**

## Materials and Methods

### *2.1. Microarray and data analysis*

Microarray was conducted on Illumina Human Ref-6 Version 2 Expression Chip (Illumina, San Diego, CA). T47D cells (si-Luc and si-CypB cells) were cultured in the growth medium for three days followed by 24 hours arrest prior to PRL treatment (100 ng/ml) for 2 hours. Three independent cultures were used for RNA isolation with RNeasy plus mini kit (Qiagen, Valencia, CA). RNA quality was checked by Agilent Bioanalyzer (Agilent, Santa Clara, CA). An Ambion labeling kit was used for labeling cDNA followed by hybridization to Illumina chips. ChIP scan data was extracted by Illumina Beadstudio and subsequently analyzed using Bioconductor lumi package [1]. The data was first variance stabilization transformed [2] and then normalized by quantile normalization method. In order to reduce false positives, probes with measurement value below the background level (detection p-value < 0.01) in all hybridizations were filtered out. 17901 probes were kept for subsequent statistical analysis. Genes with significant differential expression levels were identified using ANOVA with Bayes adjustment of the variance implemented in Bioconductor limma package [3]. To control the effects of multiple testing and reduce false positives, the gene selection of differentially expressed genes is based on: the false discovery rate p-value (FDR) < 0.05, and p-value < 0.05. The identified genes were used for the outlined studies. Microarray data were deposited in the Gene Expression Omnibus database with accession number GSE15505 (GEO, http://www.ncbi.nlm.nih.gov/geo/).

### *2.2. Ingenuity Pathway Analysis (IPA)*

Ingenuity Pathway Analysis was used to identify gene networks according to biological functions and/or diseases in the Ingenuity Pathways Knowledge Base (Ingenuity Systems, Redwood City, CA). The transcripts with known gene identifiers (HUGO gene symbols) and expression levels (fold change ≥1.3 up or down, $p<0.05$, FDR<0.05) were filtered and entered into the Ingenuity Pathways Knowledge Base IPA 4.0. Each gene identifier mapped in the Ingenuity Pathways Knowledge Base was termed as a focus gene, which was overlaid into a global molecular network established from the information in the Ingenuity Pathways Knowledge Base. Each network contained maximum of 35 focus genes. Genes were represented as nodes with different shapes and colors (see figure legends), and biological relationships were represented by edges (different lines). All edges were supported by at least one reference as interaction or action [4]. The nodes and the edges were described in the figures and figure legends.

*2.3. RT-PCR and real-time PCR*

Two micrograms of RNA was used for cDNA synthesis in 20 μl reaction volume using Superscript III first strand synthesis kit (Invitrogen). 4 μl cDNA (2.5 ng/μl), 1 μl primers (2 μM each) and 5 μl of 2× Power SYBR Mastermix (Applied Biosystems, Foster city, CA) was used for real-time PCR in 10 μl reaction volume performed on 384-well plate. Real-time PCR was conducted on ABI 7900HT Thermocycler (Applied Biosystems). All real-time PCR reactions were run in triplicate. Data were normalized to 18S rRNA and /or GAPDH, and fold change was represented as $2^{-\Delta\Delta Ct}$ ($2^{-((Ct\ target-Ct\ 18S)siCypB\ -\ (Ct\ target-Ct\ 18S)siLuc)}$). Primers for real-time PCR are listed in Table 1 and from our published papers [5,6].

*2.4. Western blot*

Fifty microgram of lysates from cells were subject to 10% SDS-PAGE gel, transferred onto PVDF membrane and immunoblotted. Images were captured using a Fujifilm LAS-3000 image analyzer (Fuji, Japan) [7].

*2.5. Soft agar growth*

Cells were plated to 30% confluency in the puromycin-free growth medium for two days. Cells were then trypsinized, counted, and plated on soft agar (10,000 cells per well in 6-well plate). The colonies were grown in the growth medium supplied with PRL (200ng/ml). After two weeks, the images of colonies were captured under phase-contrast microscope and analyzed with ImageJ (NIH, Bethesda, MD) to calculate colony number and colony areas [8].

*2.6. Cell motility assay*

Cell motility was assayed using Boyden chambers (12 uM 12-well, polycarbonate membrane transwell, Corning), as previously described [9]. To enhance motility non-occlusively, the lower surface of Boyden chamber inserts were coated with 200 ul collagen I (25 ng/ul, 5 ug total in 1×PBS). T47D cells were arrested in defined (FBS-free) medium for 24 hours, then detached with Versene (Invitrogen). $2\times10^5$ detached cells were resuspended in the 500 μl of defined medium and added into the upper Boyden chamber. One ml of defined medium with PRL (500 ng/ml) was placed in the lower chambers. Cells were cultured for 20 hours and the cells that underwent migration were counted under microscope [8].

**Results**

PRL action is mediated in the cells through distinct signaling pathways including the Jak2/Stat5 signaling pathway. In this pathway, the intranuclear PRL/CypB complex acts as a Stat5 transcriptional inducer to regulate gene expression (24, 25). To initially evaluate the expression of CypB in breast cancer cells, CypB mRNA and protein expression were measured in the several representative breast cancer cell lines. These breast cancer cell lines and MCF10A have a different expression of estrogen receptors (ER) and prolactin receptors (PRLr) (For ER, T47D≈MCF7>MDA231 ≈MCF10A; For PRLr, T47D>MCF7>MDA231≈MCF10A). Real-time PCR (Fig S1A) and Western blot analysis (Fig S1B) showed that MCF7 cells had a highest CypB expression, and T47D cells had a modest CypB expression. Since T47D cells have the highest level of PRLr expression and are most responsive to PRL stimulation acutely this line was selected to examine the role of CypB in PRL/Stat5 signaling pathway.

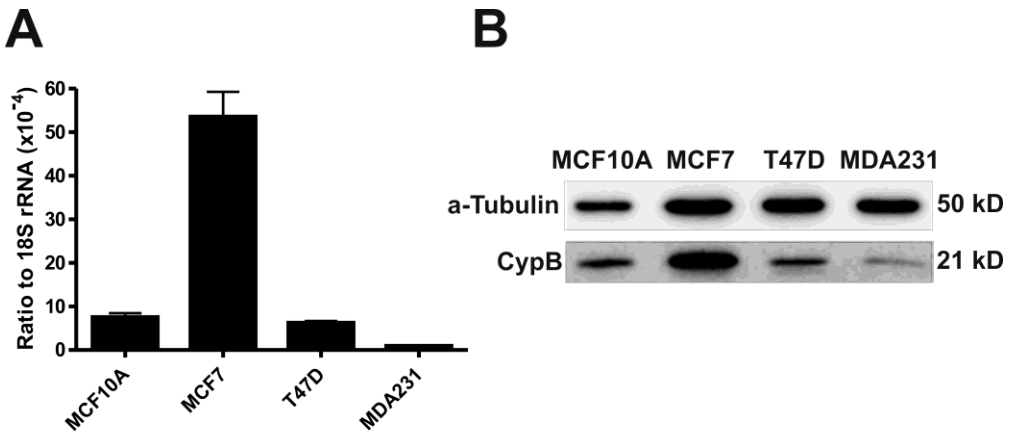


**Figure legends**

Fig. S1. The expression of CypB in MCF10A and selective breast cancer cell lines assessed by real-time PCR (A) and Western blot (B). Same cell number were counted and lysed in the RIPA buffer, and total protein was used for Western blot with anti-tubulin and anti-CypB antibodies.

**Table S1.** Primers used for real-time PCR. Primers were obtained using Primer Express 3.0 software, from Primerbank or from published sequences (CEBPB [12], BCL6 [13]). All primer pairs span two exons, except the intronless 18S rRNA and CEBPβ. Primers were tested by dissociation curve analysis to assure only single amplicon. The base pairs that the forward primer starts and reverse primer ends are based on mRNA sequence. All primers are located inside the coding sequence.

| Gene name | Primer sequences | Starts | Ends | mRNA accession number |
|---|---|---|---|---|
| 18S rRNA | olg200 5'-CCCCATGAACGAGGGAATT-3'<br>olg201 5'-GGGACTTAATCAACGCAAGCTT-3' | 1626 | 1686 | NR_003286 |
| GAPDH | olg74 5'-CATGAGAAGTATGACAACAGCCT-3'<br>olg75 5'-AGTCCTTCCACGATACCAAAGT-3' | 511 | 623 | NM_002046 |
| Cyclin D1 (BCL1) | olg310 5'- CCGTCCATGCGGAAGATC<br>olg311 5'-GAAGACCTCCTCCTCGCACTT | 369 | 443 | NM_053056 |
| BCL3 (BCL4) | olg304 5'- GACATCGACGCAGTGGACATT-3'<br>olg305 5'- ACCATGCTAAGGCTGTTGTTTTC-3' | 867 | 943 | NM_005178 |
| BCL6 (BCL5) | olg286 5'-CTGCAGATGGAGCATGTTGT-3'<br>olg287 5'-TCTTCACGAGGAGGCTTGAT-3' | 661 | 752 | NM_001706 |
| CEBPβ | olg284 5'- AGAACGAGCGGCTGCAGAAGA-3'<br>olg285 5'-CAAGTTCCGCAGGGTGCTGA-3 | 1121 | 1185 | NM_005194 |
| CISH | olg133 5'-AGAGGAGGATCTGCTGTGCAT-3'<br>olg134 5'-GGAACCCCAATACCAGCCAG-3' | 311 | 380 | NM_145071 |
| c-Myc | olg314 5'- GGATTTTTTTCGGGTAGTGGAA<br>olg315 5'- TTCCTGTTGGTGAAGCTAACGTT | 527 | 601 | NM_002467 |

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