

Supplementary figures and tables for:

**Invasive lobular and ductal breast carcinoma differ in immune response, protein translation efficiency and metabolism**

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## Supplementary Figures

### Fig. S1

#### **Differentially expressed pathways between LumA ILC and LumA IDC**

(a)(b) Top 15 activated (a) or inhibited (b) pathways in ILC (ranked by adjusted p-value). Fisher's exact test was performed on the 853 up-regulated and 602 down-regulated DE genes in Fig. 1a.  $-\log_{10}(0.05)$  is marked with red line. Gene set enrichment analysis (GSEA) were also conducted as a validation of fisher's exact test (Supplementary table 5).

### Fig. S2

#### **LumA ILCs have higher immune phenotypes because of higher GSVA scores for nearly all cell types**

(a) Increased proportion of higher immune phenotype class for LumA ILC. LumA ILC n=157, LumA IDC n=303. Tumors were classified into 6 immune-phenotypes (immune-phenotype 1-6) using hierarchical agglomerative clustering by Tamborero et al.<sup>33</sup>. Immune infiltration and cytotoxic component increased from immune-phenotype 1 to immune-phenotype 6. (b) LumA ILC and LumA IDC have similar GSVA score for all immune cell types, but 13/16 are higher in ILC vs IDC. Lines for a median GSVA difference >0.2 are shown. (c) Immune cell type comparison between high immune LumA ILC vs high immune LumA IDC. Immune cell signatures were from Tamborero et al.<sup>33</sup>. Mann-Whitney U tests were performed, and q-values represent multiple comparisons correction using Benjamini-Hochberg method.

### Fig. S3

#### **Immune expression was increased in ILC vs. IDC for the majority of immune cell types using 3 different gene signatures for immune cell types.**

(a) Immune cell type expression from Tamborero et al.<sup>33</sup>. (b) Immune cell type expression from Davoli et al.<sup>35</sup>. (c) Infiltration score for specific immune cell lineages from Li et al.<sup>36</sup>. Immune cell infiltration scores for each patient were

calculated in Li et al.<sup>36</sup> and directly used in our study. For a, b, and c, Mann-Whitney U tests were performed, and q-values represent multiple comparisons correction using Benjamini-Hochberg method.

#### **Fig. S4**

##### **Expression of immune-checkpoint genes in LumA ILC (n=157) and LumA IDC (n=303).**

Gene expression data were obtained from TCGA without CPE correction. Two-way anova for the effect of histological subtype on immune checkpoint gene expression, \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ . The effect of immune phenotype on immune checkpoint gene expression,  $p < 0.0005$  for all genes. No significant interaction ( $p > 0.05$ ) between histology and immune phenotype.

#### **Fig. S5**

##### **Differentially expressed genes and pathways with CPE correction.**

(a) With tumor purity (CPE) correction, 789 and 571 genes (marked in red) were up- and down-regulated in both TCGA and METABRIC (LumA ILCs,  $n=157$  vs LumA IDCs,  $n=307$ ,  $p\text{-value} < 0.05$ ). (b) DE gene number changes after CPE correction. Blue: CPE not corrected (LumA ILC,  $n=159$ ; LumA IDC,  $n=311$ ); Red: CPE corrected (LumA ILC,  $n=157$ ; LumA IDC,  $n=307$ ). (c) DE gene number changes influenced by the change of the number of patients. Blue: LumA ILC,  $n=159$  vs LumA IDC,  $n=311$ ; Red: LumA ILC,  $n=157$  vs LumA IDC,  $n=307$ . The sets of patients were identical to the sets in Fig. S2b. All DE analyses were performed without CPE correction. (d) Top 15 activated pathways in LumA ILCs with CPE correction.  $-\log_{10}(0.05)$  is marked with red line.

#### **Fig. S6**

##### **LumA ILC has lower protein/mRNA ratio than LumA IDC.**

Slope of linear regression in LumA ILC ( $n=8$ ) and LumA IDC ( $n=16$ ) using TCGA mass spectrometry data. For each patient, slope of linear regression was calculated with protein expression level ( $\log_2$  iTRAQ ratios) as y-axis and TCGA mRNA level ( $\log_2$  TPM) as x-axis. Protein expression data were obtained from Mertins 2015. We selected 9117 protein/genes based on data availability from

the gene list used by Mertins et al <sup>44</sup> in their calculation of protein-mRNA correlations.

## **Supplementary tables**

### **Supplementary table 1**

Patient summary in TCGA, METABRIC, and Ciriello et al, Cell 2015 datasets.

### **Supplementary table 2**

TCGA tumor histology and PAM50 assignment

### **Supplementary table 3**

METABRIC validated TCGA DE genes before and after CPE correction

### **Supplementary table 4**

Adjusted p-value of selected DE genes in survival analysis

### **Supplementary table 5**

Pathway analysis before and after CPE correction (Fisher's exact test and GSEA)

### **Supplementary table 6**

Genes in immune cell signatures

### **Supplementary table 7**

METABRIC validated TCGA DE genes in protein translation/regulation pathways.

### **Supplementary table 8**

Alteration of proteins in PI3K/AKT/mTOR pathway (CPE corrected).

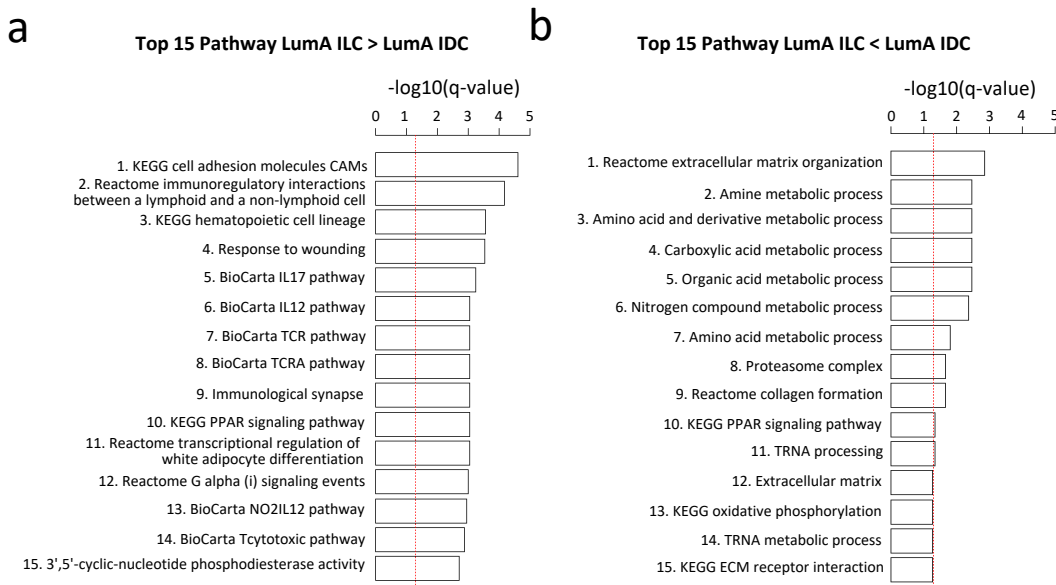
### **Supplementary table 9**

DE proteins in LumA ILC vs LumA IDC and ER+ ILC vs ER+ IDC (CPE corrected)

### **Supplementary table 10**

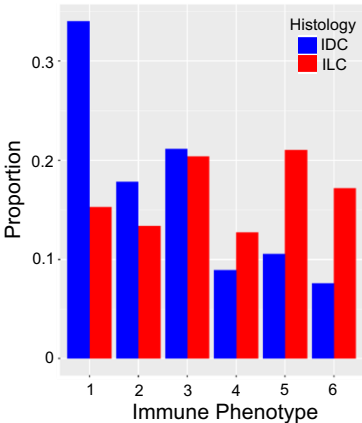
The list of 156 proteins used in the calculation of protein/mRNA ratio

Figure S1: Differentially expressed pathways between LumA ILC and LumA IDC

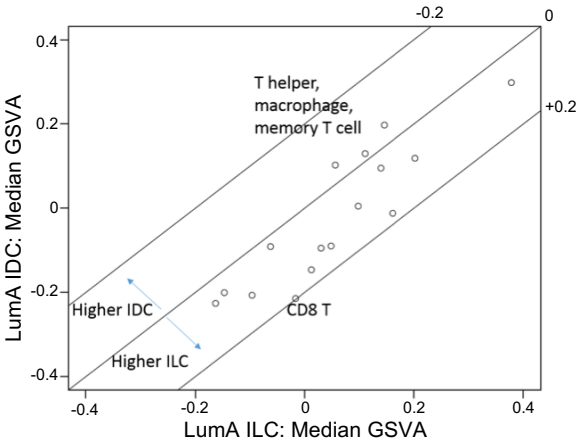


**Figure S2: LumA ILCs have higher immune phenotypes because of higher GSVA scores for nearly all cell types**

a

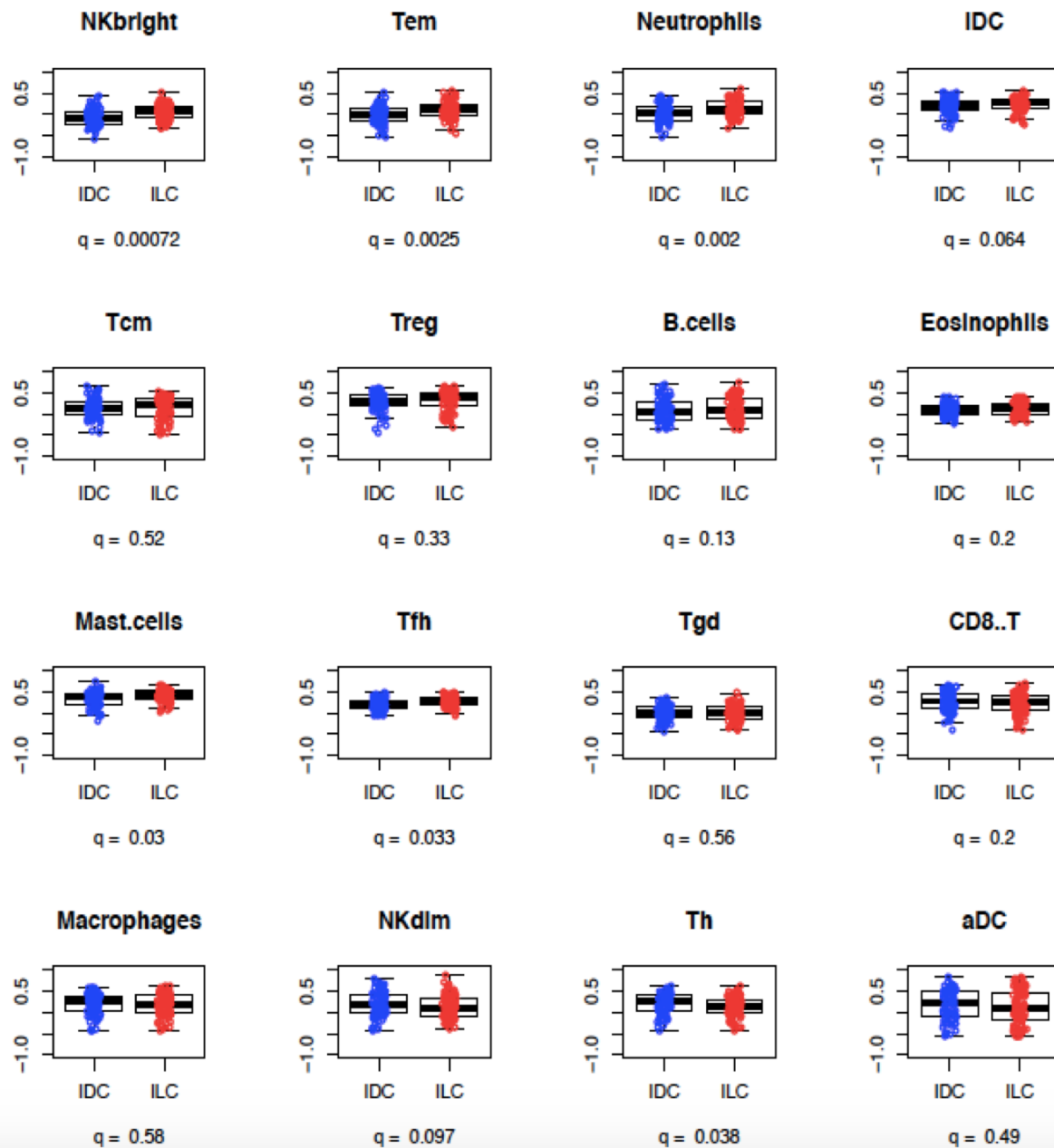


b

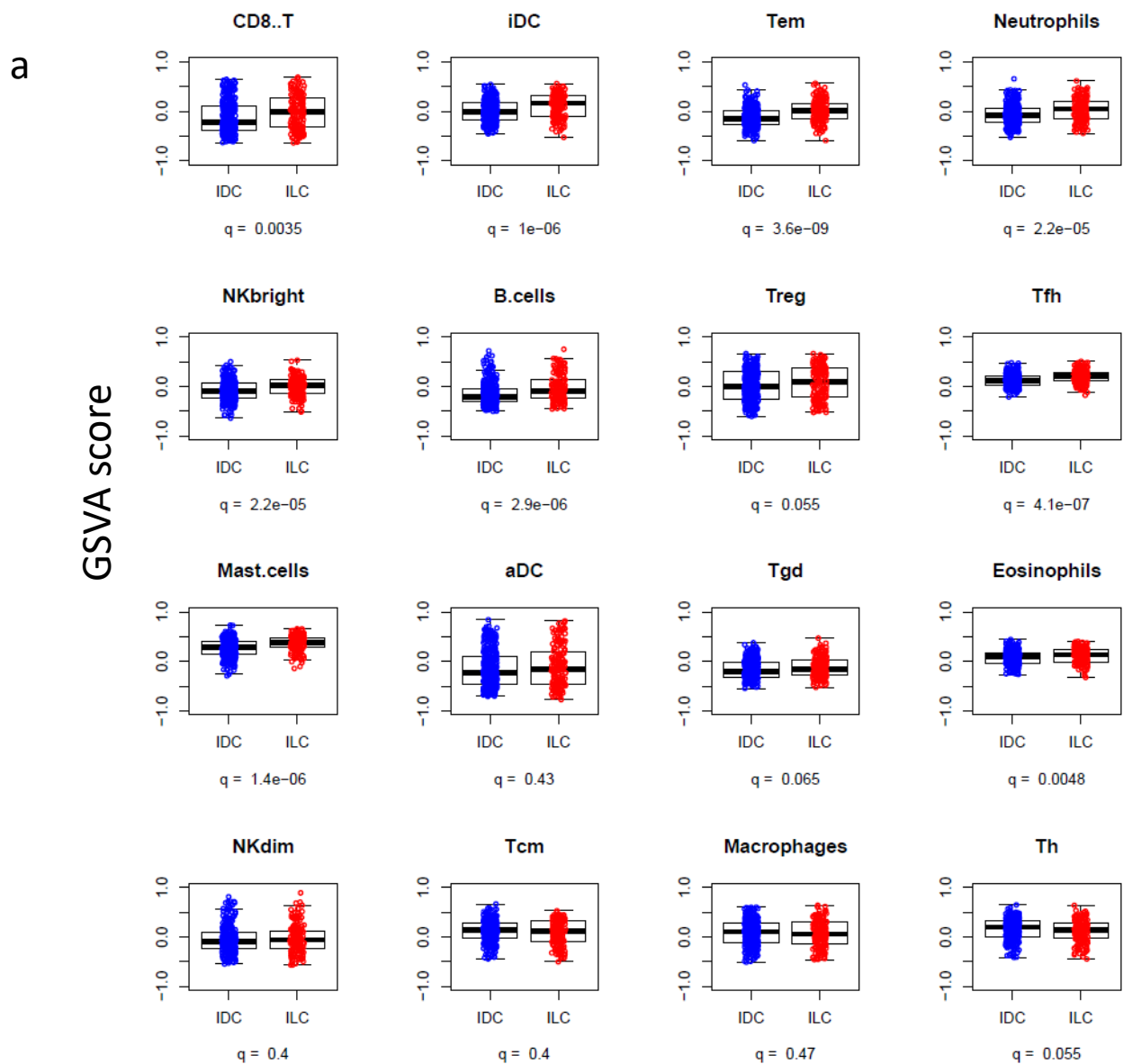


C

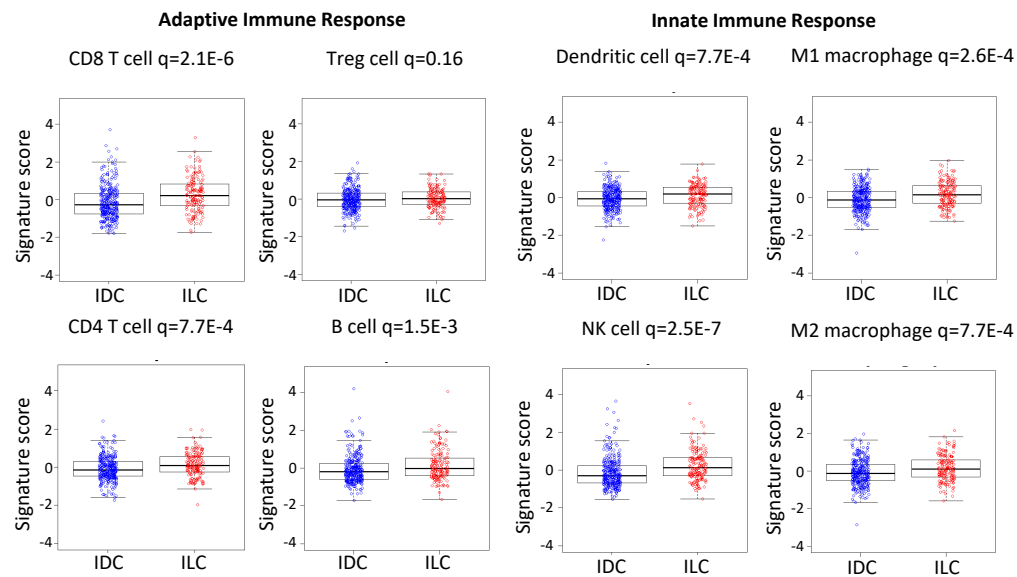
GSVA score



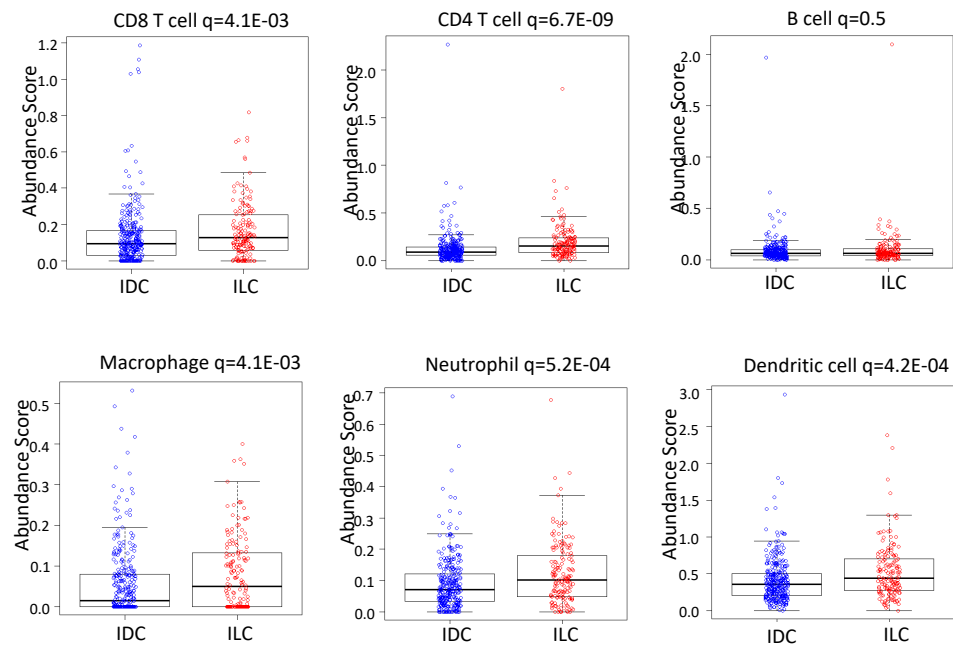
**Figure S3: Immune expression was increased in ILC vs. IDC for the majority of immune cell types using 3 different gene signatures for immune cell types.**



b



c



**Figure S4: Expression of additional immune-checkpoint genes in LumA ILC (n=157) and LumA IDC (n=303).**

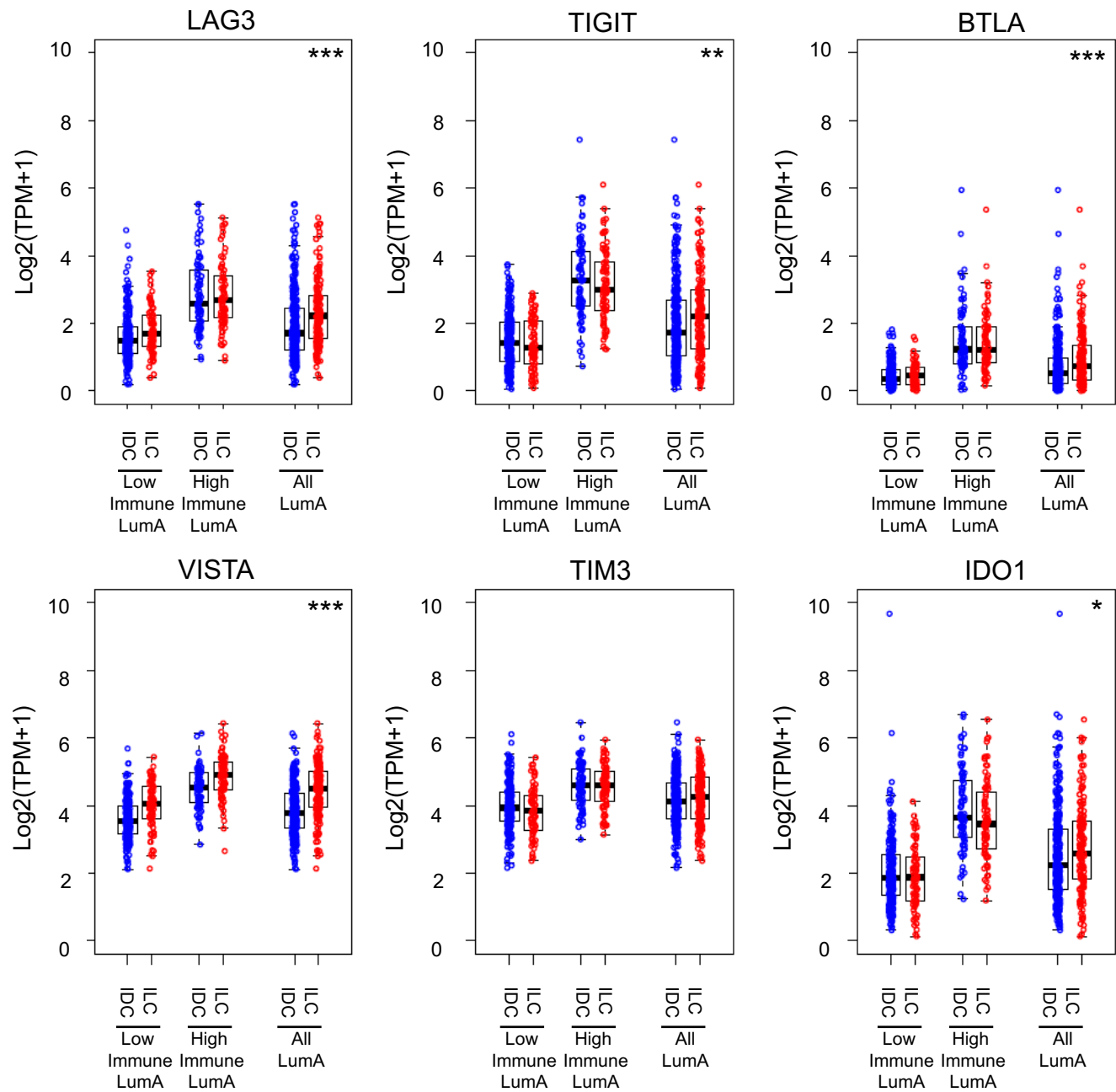
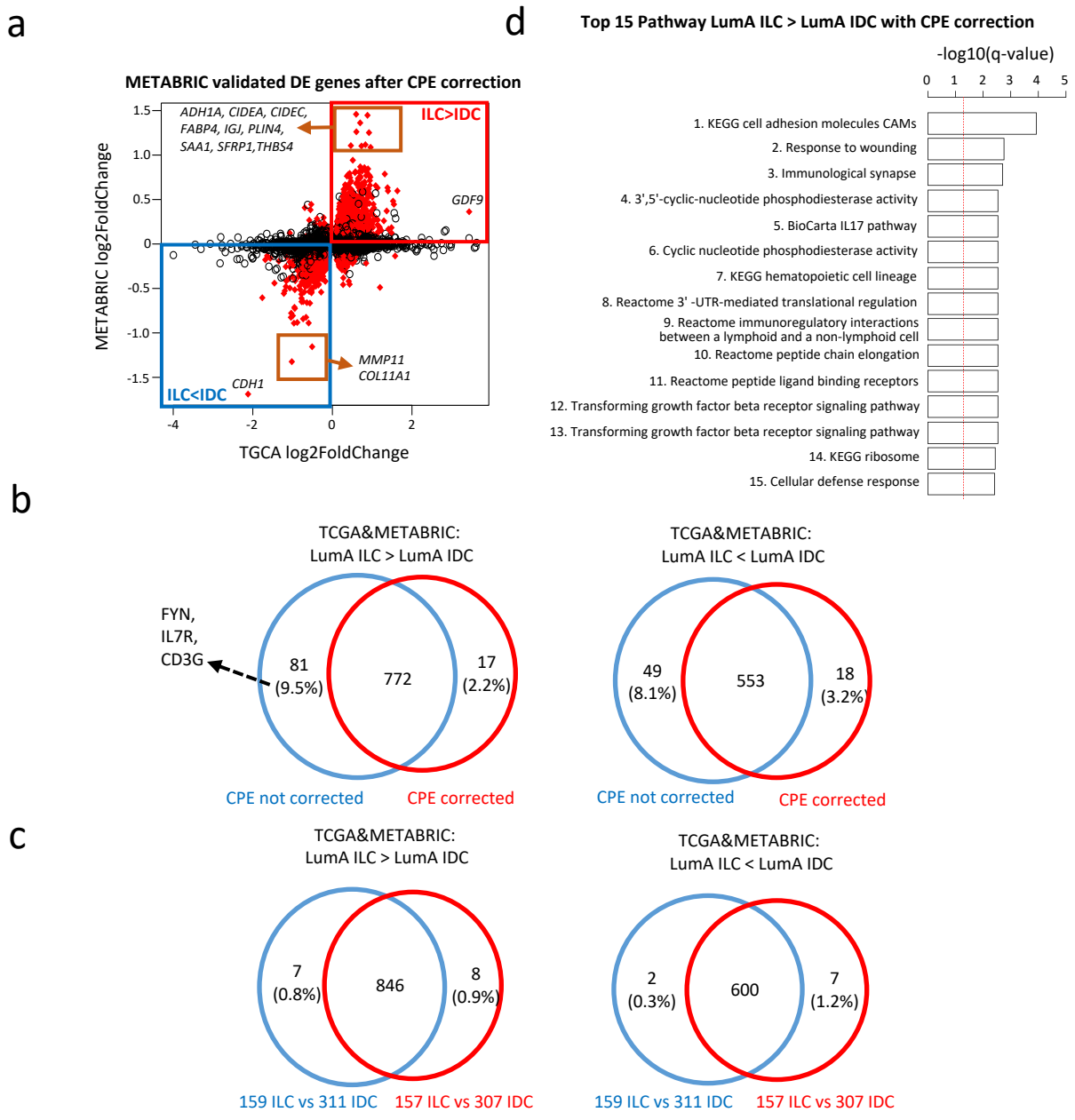
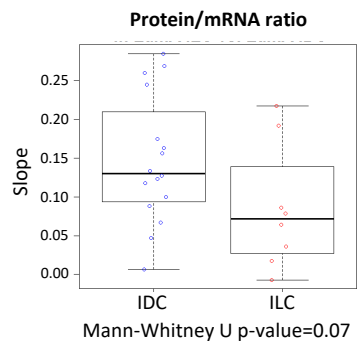


Figure S5: Differentially expressed genes and pathways with CPE correction.



**Figure S6: LumA ILC has lower protein/mRNA ratio than LumA IDC**



**The following tables are available in additional excel files.**

**Supplementary table 2**

TCGA tumor histology and PAM50 assignment

**Supplementary table 3**

METABRIC validated TCGA DE genes before and after CPE correction

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Pathway analysis before and after CPE correction (Fisher's exact test and GSEA)

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**Supplementary table 9**

DE proteins in LumA ILC vs LumA IDC and ER+ ILC vs ER+ IDC (CPE corrected)

**Supplementary table 10**

The list of 156 proteins used in the calculation of protein/mRNA ratio

# Supplementary table 1

## Patient summary in TCGA, METABRIC, and Ciriello et al, Cell 2015 datasets.

|                  | TCGA  |        | METABRIC |        | Ciriello et al, Cell 2015 |        |
|------------------|-------|--------|----------|--------|---------------------------|--------|
|                  | N=971 | %      | N=1695   | %      | N=617                     | %      |
| <b>Histology</b> |       |        |          |        |                           |        |
| ILC              | 197   | 20.29% | 147      | 8.67%  | 127                       | 20.58% |
| IDC              | 774   | 79.71% | 1548     | 91.33% | 490                       | 79.42% |
| <b>ER</b>        |       |        |          |        |                           |        |
| Positive         | 718   | 73.94% | 1282     | 75.63% | 440                       | 71.31% |
| Negative         | 210   | 21.63% | 392      | 23.12% | 140                       | 22.69% |
| <b>ILC PAM50</b> |       |        |          |        |                           |        |
| LumA             | 159   | 80.71% | 65       | 44.22% | 106                       | 83.46% |
| LumB             | 18    | 9.14%  | 29       | 19.73% | 6                         | 4.72%  |
| Normal           | 13    | 6.60%  | 38       | 25.85% | 12                        | 9.45%  |
| Basal            | 2     | 1.02%  | 5        | 3.40%  | 1                         | 0.79%  |
| Her2             | 5     | 2.54%  | 9        | 6.12%  | 2                         | 1.57%  |
| <b>IDC PAM50</b> |       |        |          |        |                           |        |
| LumA             | 311   | 40.18% | 533      | 34.43% | 201                       | 41.02% |
| LumB             | 202   | 26.10% | 401      | 25.90% | 122                       | 24.90% |
| Normal           | 18    | 2.33%  | 120      | 7.75%  | 9                         | 1.84%  |
| Basal            | 162   | 20.93% | 278      | 17.96% | 107                       | 21.84% |
| Her2             | 81    | 10.47% | 212      | 13.70% | 51                        | 10.41% |

*Note:* The percentage of each intrinsic subtypes in our assignment is similar to what's defined in Ciriello et al. <sup>2</sup>.

# Supplementary table 4

## Adjusted p-value of selected DE genes in survival analysis

|         | Log2FoldChange<br>in TCGA | Log2FoldChange<br>in METABRIC | LumA ILC<br>P-value | LumA IDC<br>P-value |
|---------|---------------------------|-------------------------------|---------------------|---------------------|
| ADH1A   | 0.7099                    | 1.4590                        | 0.9155              | 0.7336              |
| CD36    | 0.3933                    | 1.0495                        | 0.8498              | 0.7336              |
| CDH1    | -2.1789                   | -1.6887                       | 0.8498              | 0.7863              |
| CIDEA   | 1.0873                    | 1.0869                        | 0.8498              | 0.9420              |
| CIDEA   | 1.0578                    | 1.4471                        | 0.8498              | 0.7336              |
| COL11A1 | -0.9868                   | -1.3236                       | 0.9155              | 0.5502              |
| FABP4   | 0.7739                    | 1.2617                        | 0.9155              | 0.7336              |
| GDF9    | 2.0855                    | 0.3627                        | 0.8498              | 0.7336              |
| GRIA2   | -0.7157                   | -1.0851                       | 0.8498              | 0.7336              |
| IGJ     | 0.6413                    | 1.1060                        | 0.8498              | 0.9420              |
| KLK11   | 1.8255                    | 1.1339                        | 0.8498              | 0.5502              |
| MMP11   | -0.4760                   | -1.1558                       | 0.9155              | 0.5502              |
| PLIN4   | 0.9942                    | 1.1162                        | 0.9155              | 0.7336              |
| SAA1    | 1.2003                    | 1.3621                        | 0.9155              | 0.7336              |
| SFRP1   | 0.8354                    | 1.2520                        | 0.8498              | 0.2062              |
| THBS4   | 0.8108                    | 1.1017                        | 0.9155              | 0.7336              |

*Note:* METABRIC survival analysis using median gene expression as cutoff. Log rank test p-values were adjusted by Benjamini-Hochberg method. All log2FoldChanges were not corrected with CPE.

Supplementary table 7

METABRIC validated TCGA DE genes in protein translation/regulation pathways.

| Pathways                                           | DE genes                                                                                                                                                                        |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reactome 3' -UTR-mediated translational regulation | EIF3G<br>EIF3K<br><b>RPL12</b><br><b>RPL13A</b><br><b>RPL18A</b><br><b>RPL31</b><br><b>RPL35A</b><br>RPL4<br>RPL5<br>RPL7A<br>RPS13<br>RPS27<br>RPS27A<br>RPS3<br>RPS3A<br>RPS6 |
| Reactome peptide Chain elongation                  | <b>RPL12</b><br><b>RPL13A</b><br><b>RPL18A</b><br><b>RPL31</b><br><b>RPL35A</b><br>RPL4<br>RPL5<br>RPL7A<br>RPS13<br>RPS27<br>RPS27A<br>RPS3<br>RPS3A<br>RPS6                   |

Note: Ribosome protein synthesis genes were marked in bold

Supplementary table 8

Alteration of proteins in PI3K/AKT/mTOR pathway (CPE corrected).

|                         | LumA ILC vs LumA IDC |                  | ER+ ILC vs ER+ IDC |                  |
|-------------------------|----------------------|------------------|--------------------|------------------|
| Protein                 | Log2FoldChange       | Adjusted p-value | Log2FoldChange     | Adjusted p-value |
| AKT                     | -0.077               | 0.18             | -0.092             | 0.044            |
| Phospho-AKT (Ser473)    | 0.094                | 0.38             | 0.2                | 0.014            |
| Phospho-AKT (Thr308)    | 0.11                 | 0.19             | 0.20               | 0.0053           |
| AMPKα                   | 0.0064               | 0.89             | 0.052              | 0.16             |
| Phospho-AMPKα (The 172) | 0.046                | 0.54             | 0.070              | 0.25             |
| PI3K (p110α)            | 0.0038               | 0.90             | 0.0053             | 0.86             |
| PI3K (p85)              | 0.054                | 0.17             | 0.079              | 0.015            |
| mTOR                    | -0.063               | 0.12             | -0.069             | 0.037            |
| Phospho-mTOR (Ser 2448) | -0.089               | 0.00035          | -0.082             | 0.00029          |

Note: LumA ILC (n=113), LumA IDC (n=242); ER+ ILC (n=135), ER+ IDC (n=411). P-values were adjusted by Benjamini-Hochberg method