

## SUPPLEMENTARY FIGURES

### **Acquired mutations and transcriptional remodeling in long-term estrogen-deprived locoregional breast cancer recurrences**

Nolan Priedigkeit, Kai Ding, William Horne, Jay K. Kolls, Tian Du, Peter C. Lucas, Jens-Uwe Blohmer, Carsten Denkert, Anna Machleidt, Barbara Ingold Heppner, Steffi Oesterreich, Adrian V. Lee

**Supplementary Figure 1.** DNA-seq target interval coverages

**Supplementary Figure 2.** Copy number call distribution

**Supplementary Figure 3.** tumorMatch: Proportion of shared variants (POSV) between samples in patient-matched cohort

**Supplementary Figure 4.** Local recurrence CNA Correlation Matrix

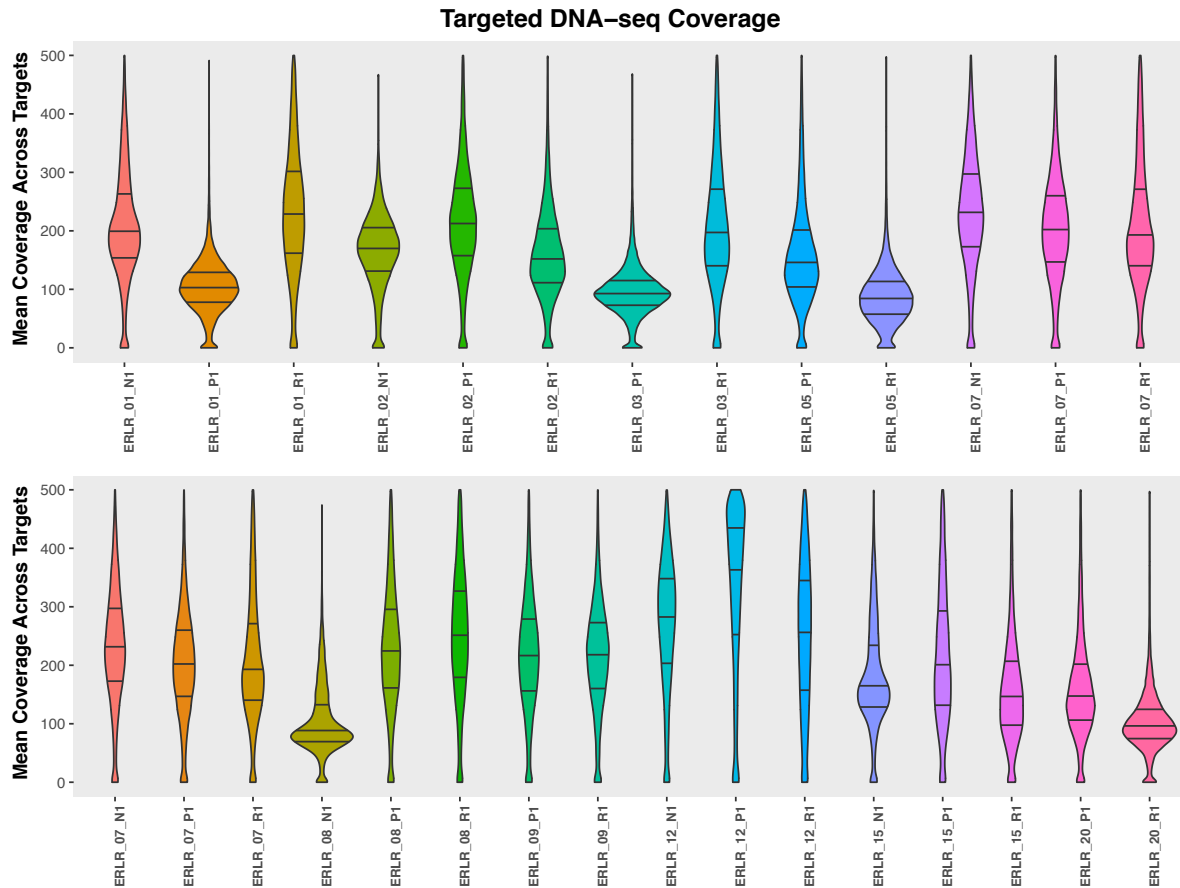
**Supplementary Figure 5.** Allele frequency of SNVs called from RNA-seq vs DNA-seq

**Supplementary Figure 6.** Overlap of differentially expressed genes between local recurrences and ER+ LTED lines

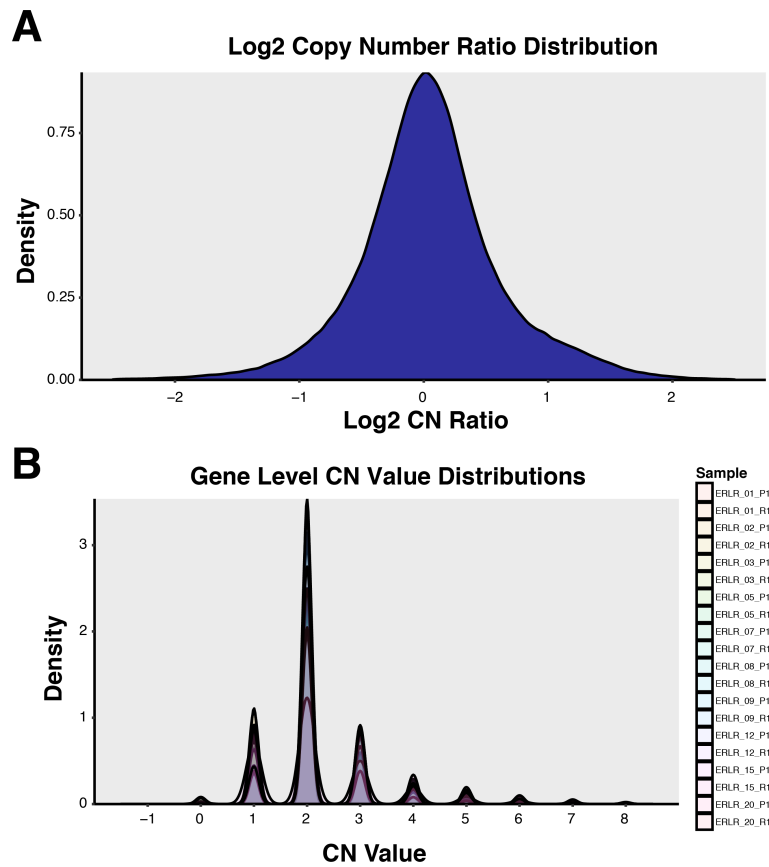
**Supplementary Figure 7.** *KLK7* and *PROM1* basal breast carcinoma expression and E2 regulation.

**Supplementary Figure 8.** NDRG1 expression in PAM50 subtypes and survival influence in ER-positive breast cancer.

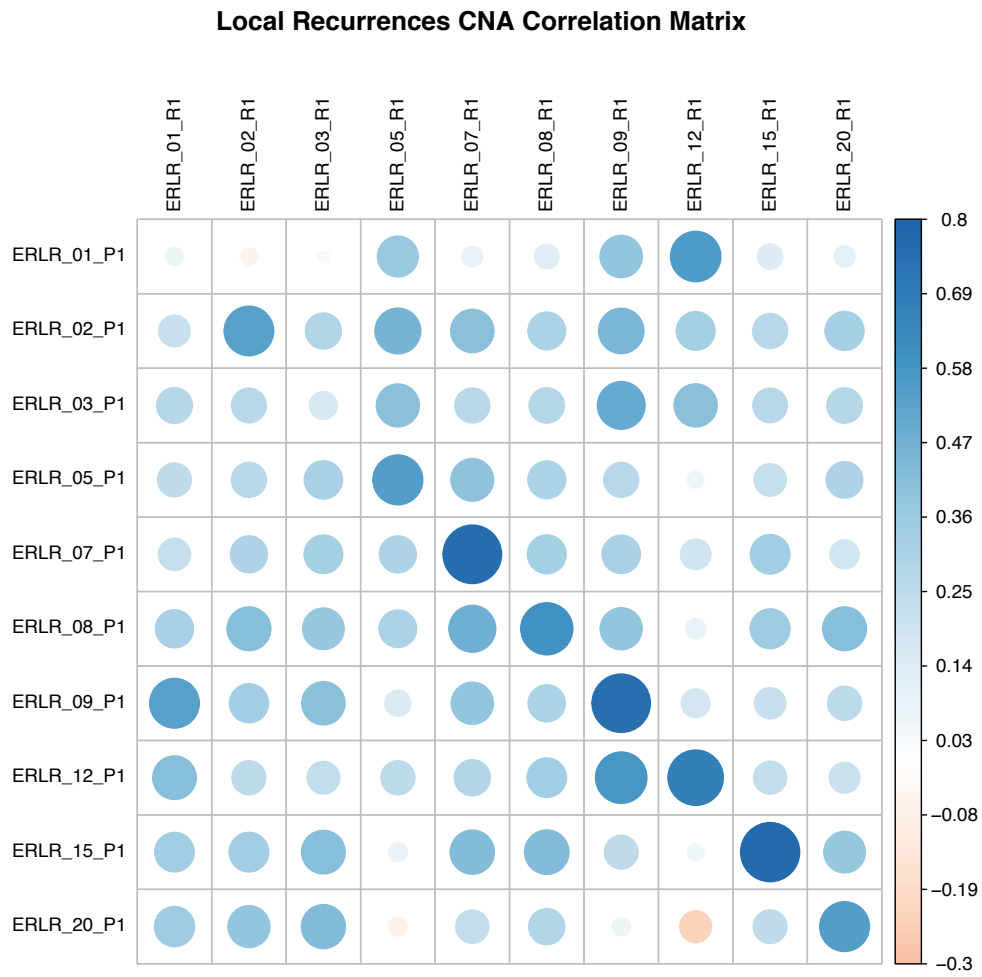
**Supplementary Figure 9:** Top five up and downregulated ESR1-depleted gene expression changes in ESR1 non-depleted cases.



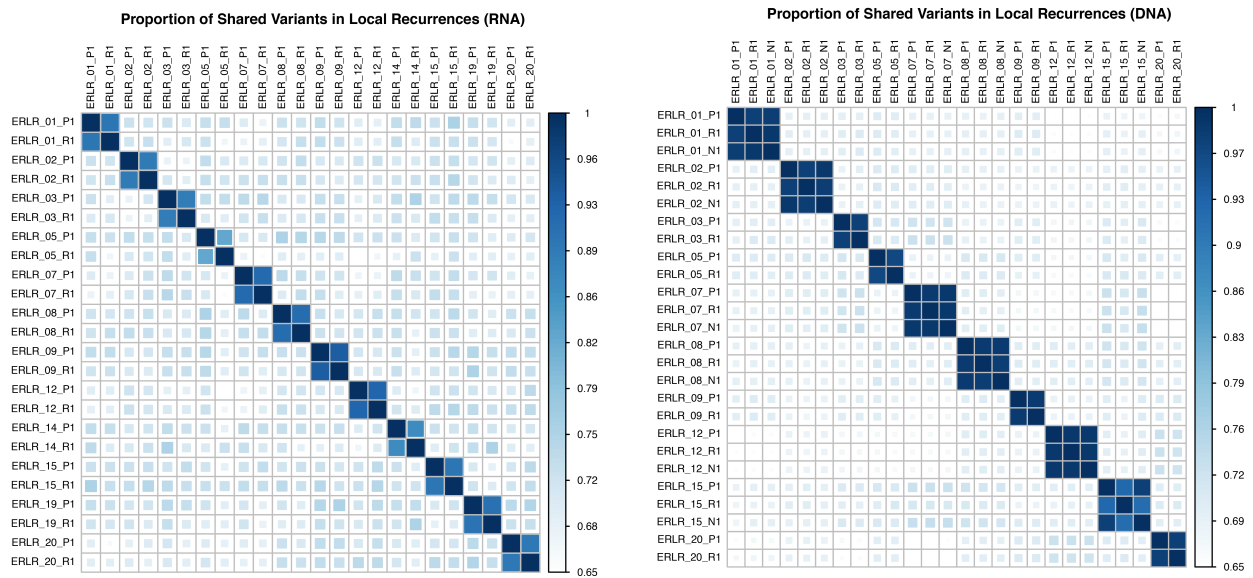
**Supplementary Figure 1: DNA-seq target interval coverages.** Violin plots showing the distribution of mean DNA-sequencing coverage across all targeted intervals. 25th, 50th and 75th percentiles are indicated with horizontal black lines. To better visualize distributions, y-axis limit was set at 500.



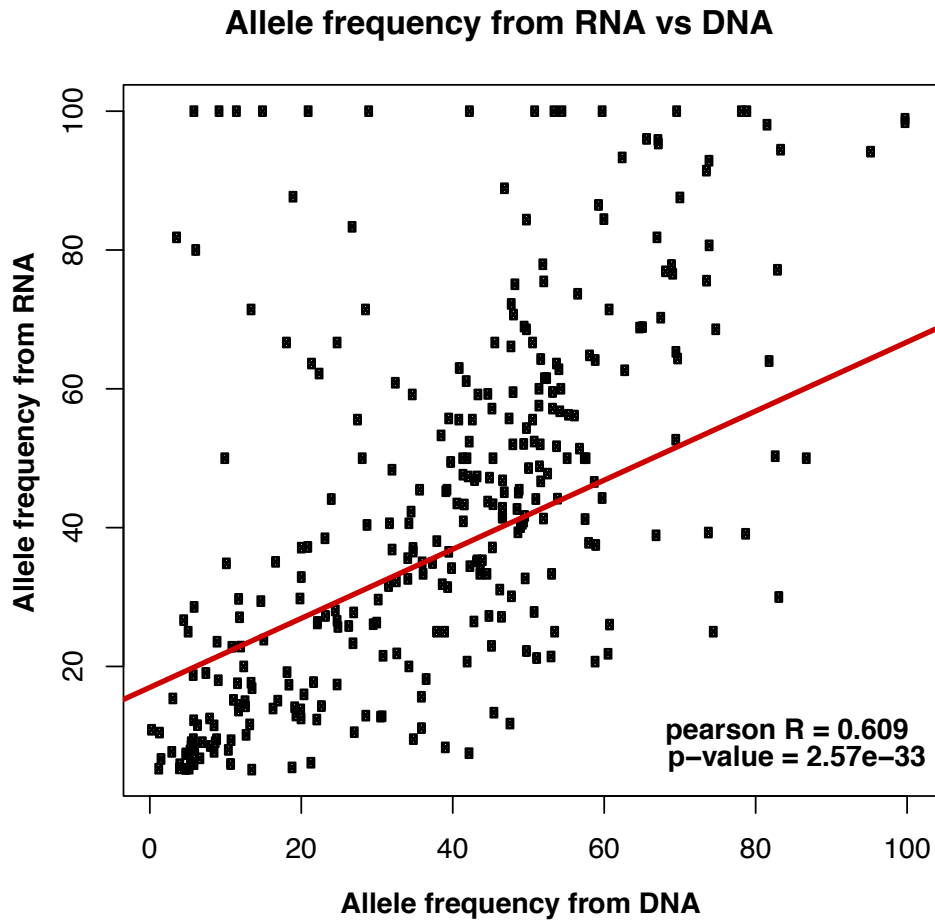
**Supplementary Figure 2: Copy number call distribution.** (A) Log2 copy number ratio distribution, derived from *CNVkit*, for all samples in cohort. (B) Distribution of discrete, gene-level copy number calls with gene-level values representing the mean of discrete calls across all probed regions covering the gene.



**Supplementary Figure 3: CNA Correlation Matrix.** Correlation matrix of Pearson R values from log<sub>2</sub>CN values between all samples.

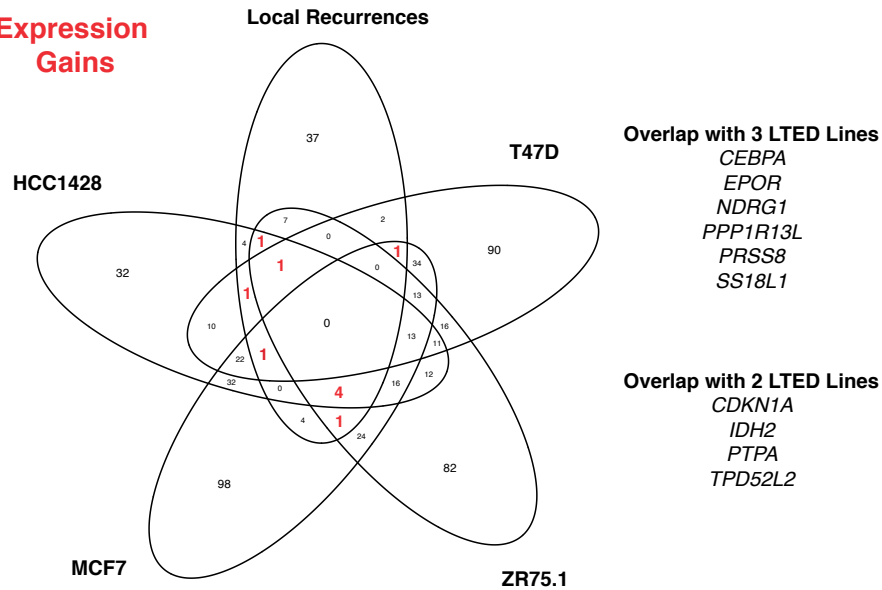
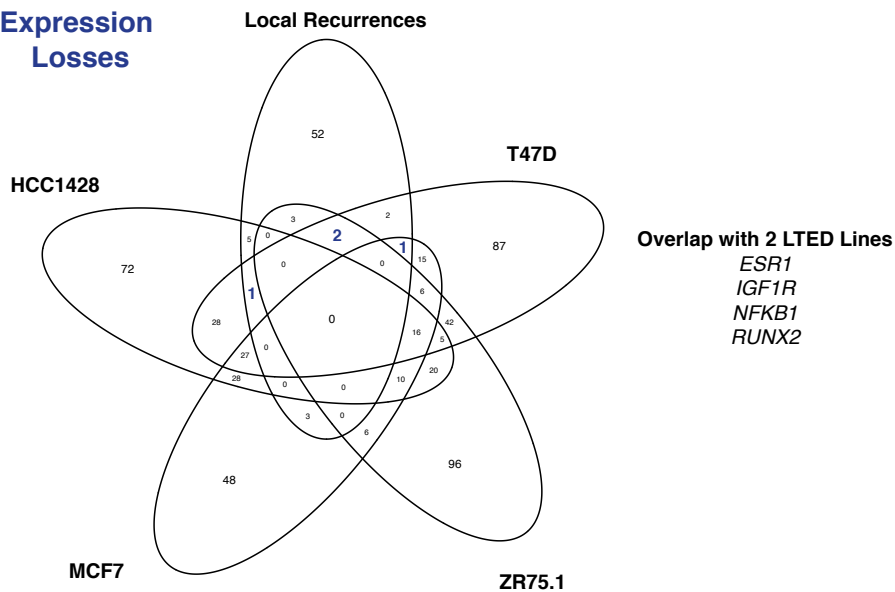
**tumorMatch in local recurrence cohort**

**Supplementary Figure 4: tumorMatch proportion of shared variants (POSV) between samples in patient-matched cohort. tumorMatch plots for both RNA- (left) and DNA-sequencing, showing all paired specimens, including trios that include normal, are patient-matched.**



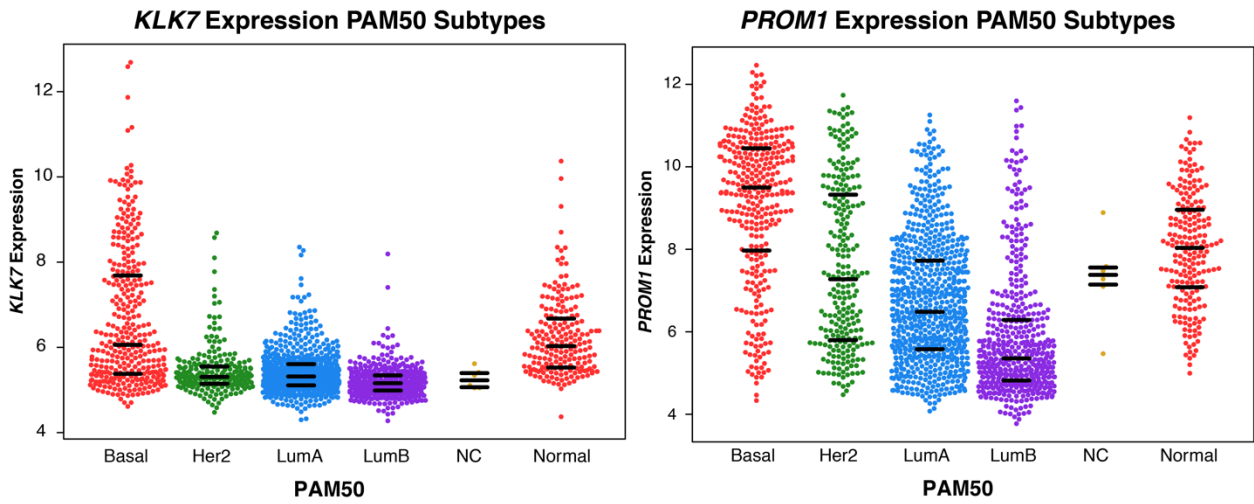
**Supplementary Figure 5: Allele frequency of SNVs called from RNA-seq vs DNA-seq.**

Allele frequency calls of 633 total somatic, nonsynonymous nucleotide variants from RNA-seq and DNA-seq (pearson R = 0.609).

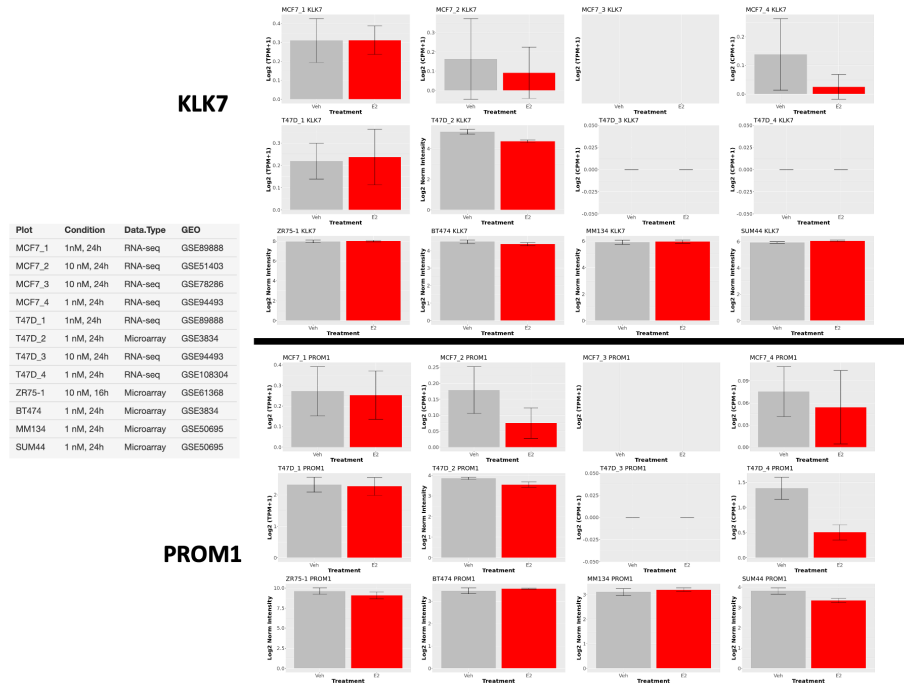
**A****Expression Gains****B****Expression Losses****Supplementary Figure 6: Overlap of differentially expressed genes between local****recurrences and ER+ LTED lines. (A)** Genes significantly upregulated in both localrecurrences vs. primaries and LTED vs. parental lines. **(B)** Genes significantly downregulated in

both local recurrences vs. primaries and LTED vs. parental lines.

**A**

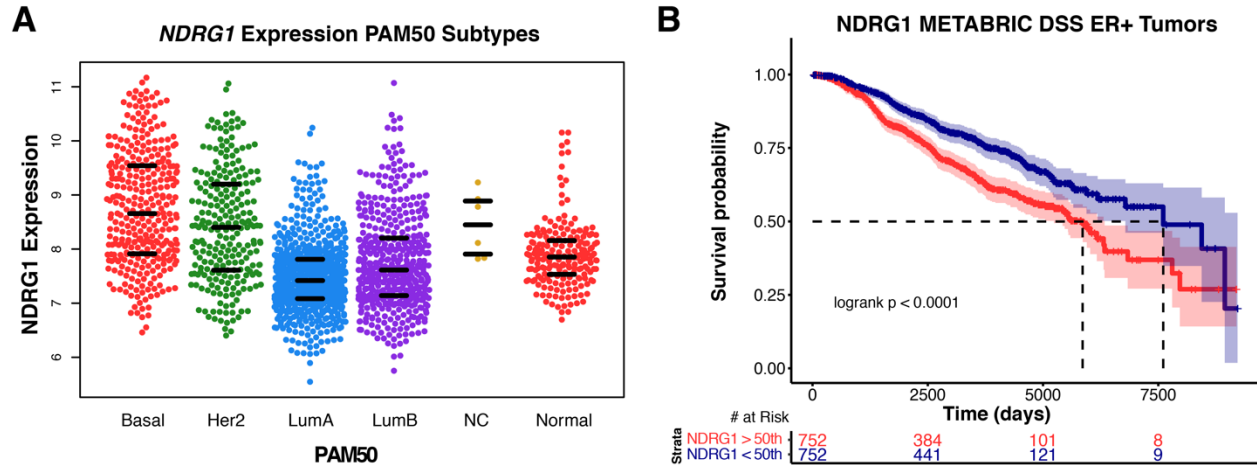


**B**

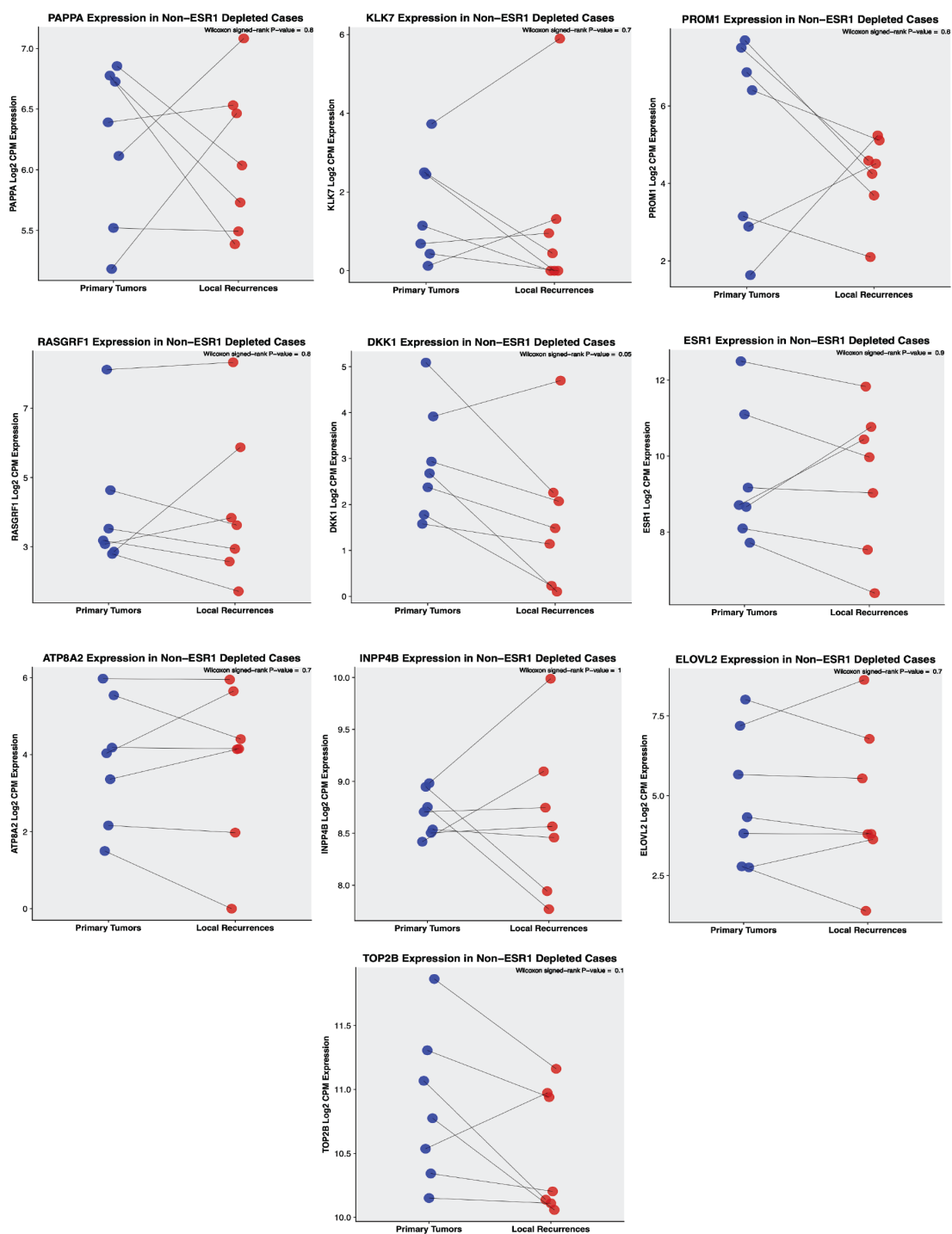


**Supplementary Figure 7: KLK7 and PROM1 basal breast carcinoma expression and E2 regulation. (A)** Normalized microarray expression values (METABRIC) of *KLK7* and *PROM1*, segregated by PAM50 subtypes. Horizontal black bars indicate 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentile values. **(B)** Relative expression of *KLK7* and *PROM1* with E2 induction in various conditions and breast cancer cell lines.





**Supplementary Figure 8: *NDRG1* expression in PAM50 subtypes and survival influence in ER-positive breast cancer. (A) *NDRG1* expression in PAM50 subtypes. (B) Disease-specific survival in METABRIC of patients with ER-positive primary tumors that express *NDRG1* highly (>50<sup>th</sup> percentile, red) or lowly (<50<sup>th</sup> percentile, blue).**



**Supplementary Figure 9:** Top five up and downregulated ESR1-depleted gene expression changes in ESR1 non-depleted cases. Plots of log<sub>2</sub>CPM expression values of primary (blue) and recurrent (red) tumors from non-ESR1 depleted cases and associated Wilcoxon signed-rank P-values.