

## *Supplementary Information*

**Supplementary Figure S1. *DAB2IP* expression across breast cancer subtypes in ER+ TCGA and METABRIC cohorts.** (A) METABRIC breast cancer microarray gene expression matrix was downloaded to analyze *DAB2IP* expression across breast cancer molecular subtypes. (B) DESeq analysis of TCGA non-basal ER+ breast cancer RNA-seq data was performed, and DEGs were clustered across all breast cancer subtypes. Quartile-based cutoff allowed division of patients into high/low *DAB2IP*. The significance was determined at adjusted  $p < 1e-10$ . (C) Down-regulated DEGs in *DAB2IP*-low TCGA Luminal A samples were analyzed for gene enrichment using GO:Biological Processes (BP) signatures (FDR cutoff  $> 0.05$ ). (D) METABRIC breast cancer gene expression map shows clusters of ER+ DEGs based on high/low *DAB2IP* (quartile-based cutoff) across breast cancer subtypes. (E) and (F) ShinyGO platform was used to perform gene enrichment analysis using MSigDB oncogenic pathway activation gene sets and Hallmark gene sets, respectively, for upregulated DEGs in *DAB2IP*-low ER+ METABRIC samples (FDR cutoff  $> 0.05$ ). Statistical analysis were performed using Student's t-tests.

**Supplementary Figure S2. Higher grade tumors are associated with increased Ki67 Index percentage, and additional representative images showed that some high grade tumors retained *DAB2IP* expression.** (A) TMAs consisting of 116 ER+ patients were stained for *DAB2IP*. (B) Ki67 index % value available for 89 of the 116 ER+ tumors was graphed against tumor grades. (C) Additional images of Luminal A TMA consisting of 126 patients showing *DAB2IP* expression. Data was analyzed using Student's t-tests.

**Supplementary Figure S3. Effect of loss of *DAB2IP* on proliferation and tumorsphere formation in Luminal A and Luminal B cell lines.** (A) Cell cycle and proliferation genes showed increased expression in *DAB2IP* knockdown Luminal A T47D cells compared to control cells as determined by quantitative RT-PCR ( $n=3$ ). (B) Luminal B BT474 cells, after 24 hour transfection, were plated for MTS assay, and absorbance was measured after 24 and 48 hours ( $*p=0.0139$ ) ( $n=9$ ). (C) Control and *DAB2IP*-targeting shRNAs were used to transduce BT474 cells, and immunoblot was performed to determine the knockdown efficiency. (D) sh*DAB2IP* and shControl BT474 cells were plated for tumorsphere assays, and images were acquired at Day 1, Day 4, and Day 7. ( $*p=0.0147$ ,  $**p=0.0034$ ) ( $n=3$ , and each biological replicate was seeded in 2 wells). Data was analyzed using Student's t-tests.

**Supplementary Figure S4. Effect of loss of *DAB2IP* expression on the transcriptome of MCF10A cells.** (A) MCF10A cells were transfected with siRNA against *DAB2IP* or non-targeting control pool, and RNA-sequencing was performed. DESeq analysis shows distinct clusters of DEGs between knockdown and control *DAB2IP* cells. (B) and (C) Gene enrichment analysis using GO: biological processes and MSigDB Hallmark gene sets were performed, respectively, using upregulated DEGs in *DAB2IP* knockdown MCF10A cells (FDR cutoff  $> 0.05$ ). (D) Post transfection with *DAB2IP* or control siRNA, BT474 cells were transfected with either wild-type or mutant  $3 \times \kappa B$  luciferase reporter constructs along with pRL-TK Renilla luciferase construct, and a dual luciferase assay was performed ( $n=4$ ). Data was analyzed using Student's t-tests.

**Supplementary Figure S5. Loss of *DAB2IP* increases P65, SRSF1, and BIRC5 expression in matched ER+ breast cancer tissues, and loss of p38 rescues migration of T47D cells.** (A)

Matched representative images of DAB2IP, P65, SRSF1, and BIRC5 expression from 116 ER+ breast cancer specimens are shown. Arrows indicate positive cells. **(B)** Stromal P65 expression was quantified by computational measurements of staining intensity per specimen and plotted by tumor grades (\*\*\* $p=0.0002$ , \*\*\*\* $p<0.0001$ ). **(C)** SRSF1 staining intensity ratio of average nucleus to average cytoplasm was plotted against tumor grades (\* $p=0.0177$ , \*\* $p=0.0033$ , \*\*\* $p=0.0009$ ). **(D)** Ratio of average nucleus to average cytoplasm of BIRC5 staining intensity was graphed by tumor grades (\*\*\* $p=0.0008$ ). **(E)** Immunoblot was performed using siDAB2IP and siControl T47D cells to show the increase in expression of phospho-p38 in DAB2IP knockdown cells (n=3). **(F)** Post 24-hour of transfection with siControl, siDAB2IP, and/or sip T47D cells, scratch-wound assay was performed (\*\* $p=0.0071$ , \*\* $p=0.0025$ , \*\* $p=0.0032$ , \*\*\* $p=0.0001$ ) (n=3). Data was analyzed using Student's t-tests.

**Supplementary Figure S6. Impact of loss of DAB2IP on NF- $\kappa$ B target genes and neojunctions in Luminal A and Luminal B tumors and cell lines.** **(A)** *DAB2IP*-low Luminal A subtype exhibited increased expression of NF- $\kappa$ B targets *BIRC5* (adj. $p=2.2115212243978402e-7$ ), *CDK5* (adj. $p=1.0657855824892208e-8$ ), *SRSF1* (adj. $p=1.6454635733683117e-11$ ) and *TBCA* (adj. $p=5.0683220299522466e-11$ ). **(B)** Gene enrichment analysis using GO:BP was performed for upregulated NF- $\kappa$ B DEGs in Luminal A *DAB2IP*-low tumors (FDR cutoff $>0.05$ ). **(C)** Neojunction numbers mined from the TCGA breast cancer cohort were plotted by Luminal A and Luminal B subtypes (t-test  $p=3.81e-10$ ). **(D)** The number of neojunctions from the TCGA breast cancer cohort was plotted against high/low *DAB2IP* expression in Luminal B tumors. **(E)** siDAB2IP and siControl T47D cells were seeded in 96-well plates and treated with DMSO or 5  $\mu$ M Compound A for 4 hours. Expression of NF- $\kappa$ B regulated splicing genes *SRSF1*, *HNRBPA2B1*, and *HNRNPU* at the mRNA levels was analyzed by quantitative RT-PCR (n=3). Data was analyzed using Student's t-tests.

**Supplementary Figure S7. Several genes that were upregulated with low DAB2IP in Luminal A tumors are potentially regulated by the NF- $\kappa$ B signaling pathway.** **(A)** NF- $\kappa$ B transcription factor was selected for motif search, and using the online software Motifmap, (<http://motifmap.ics.uci.edu/>), upregulated genes in *DAB2IP*-low Luminal A tumors were processed to identify genes containing binding sites of NF- $\kappa$ B subunits. **(B)** Venn diagram showing the overlap between the upregulated genes in the *DAB2IP*-low Luminal A subset containing NF- $\kappa$ B binding sites and the NF- $\kappa$ B candidate gene sets that we analyzed using publicly available RelA ChIP-seq data.

**Supplementary Figure S8. Effect of loss of DAB2IP on NF- $\kappa$ B target gene BIRC5 in T47D cells.** **(A)** T47D cells transfected with siRNA specific to *BIRC5*, *DAB2IP*, or control were analyzed by immunoblotting. **(B)** IGV platform was used to analyze the ChIP-seq signal tracks for *BIRC5*. **(C)** Post transfection with siControl, siDAB2IP, and/or siBIRC5, T47D cells were subjected to MTS assay (*BIRC5*: 24hrs-\* $p=0.0181$ , \* $p=0.0270$ , \* $p=0.0120$ ; 48hrs-\* $p=0.0286$ ) (n=9). Data was analyzed using Student's t-tests.

**Supplementary Figure S9. ChIP-seq reveals differential NF- $\kappa$ B binding in DAB2IP knockdown Luminal A cell line.** **(A)** Profile heatmap around TSS of RefSeq genes with read counts spanning a region of  $\pm 1$  kb around TSS are shown for RelB, and NFKB2. Blue-to-red color

gradient shows high-to-low counts in the corresponding region. **(B)** Venn diagram showing overlap between up-peaks between shControl and shDAB2IP T47D cells for RELA, RELB and NFkB2 binding. **(C)** Venn diagrams showing the overlap of up-peak associated NFkB2 binding genes in DAB2IP knockdown T47D cells and upregulated genes in low *DAB2IP* Luminal A TCGA analyzed dataset. Common genes between TCGA RNA-seq and NFkB2 ChIP-seq were used for enrichment analysis using curated MSigDB gene sets (FDR cutoff>0.05).

Supplementary Table S1. **Proliferation score and Risk of Recurrence (ROR) scores.**

Supplementary Table S2. **Resultant gene list for all enrichment analysis**

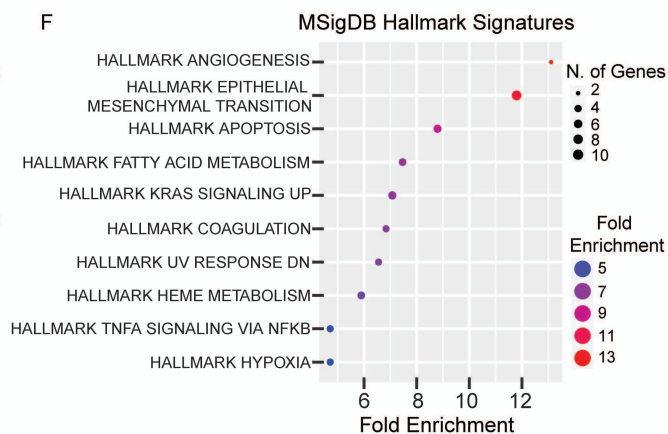
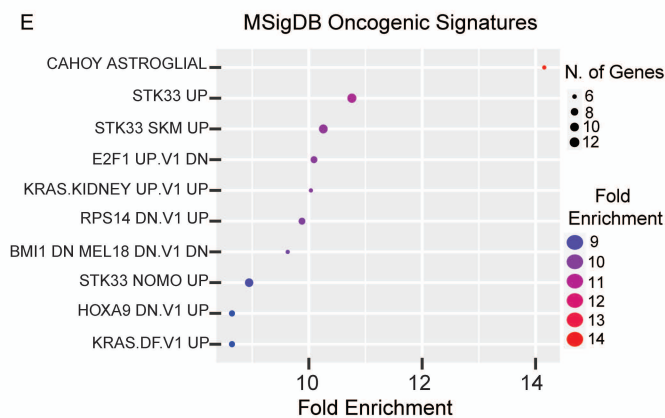
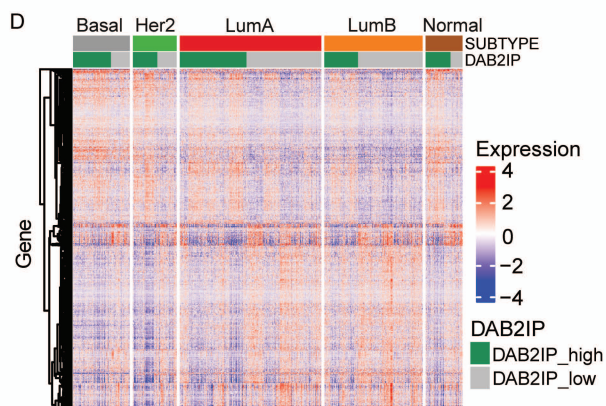
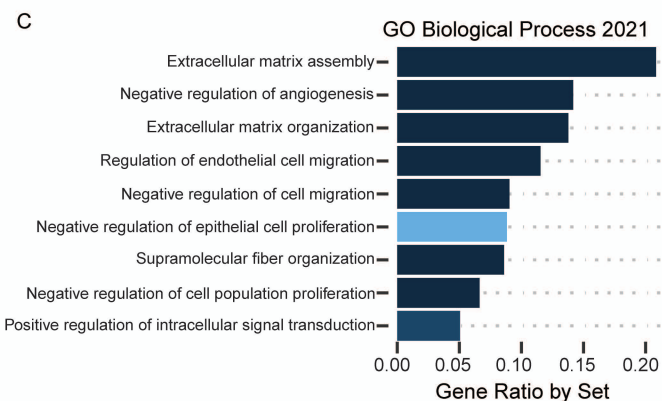
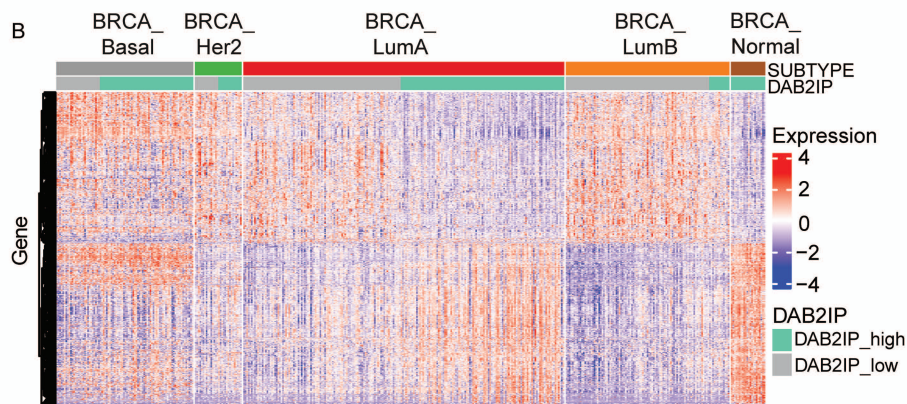
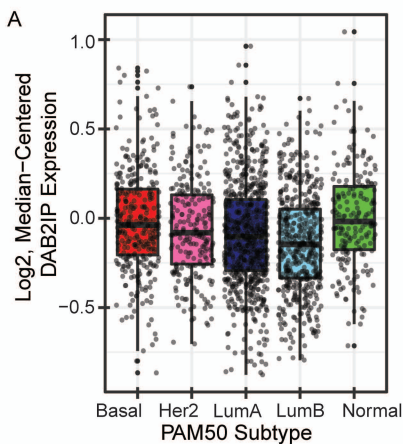
Supplementary Table S3. **Description of selected upregulated genes in low *DAB2IP* Luminal A breast cancer tumors.** Fold-changes are DAB2IP high relative to DAB2IP low, hence log<sub>2</sub>FC are negative.

Supplementary Table S4. **Breast cancer patient cohort information with different clinicopathological factors.**

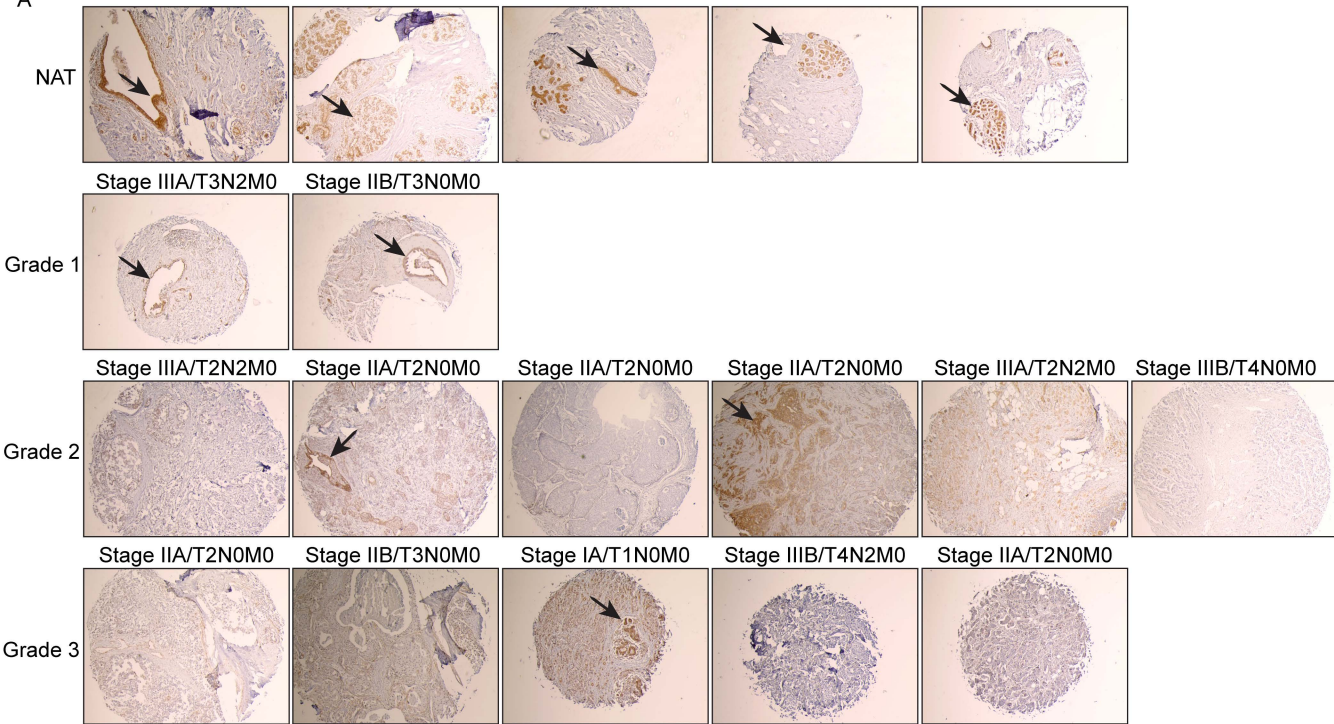
Supplementary Table S5. **Up- and down-regulated NF-κB genes in *DAB2IP*-low Luminal A tumors.**

Supplementary Table S6. **Neojunction counts in Luminal A and Luminal B tumors by *DAB2IP* high/low levels.**

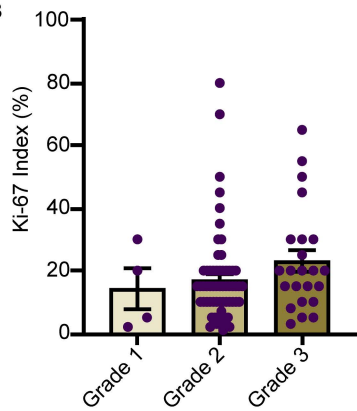
Supplementary Table S7. **Description of all NF-κB motifs analyzed for the TCGA *DAB2IP*-low Luminal A breast cancer cohort and up and down peak associated genes between shDAB2IP and shControl T47D cells for RELA, RELB, and NFkB2.**



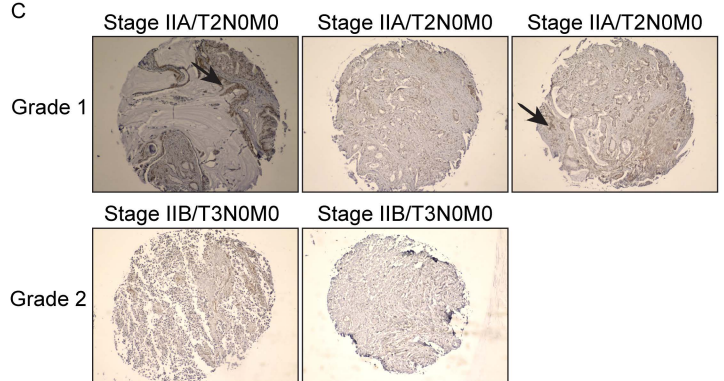
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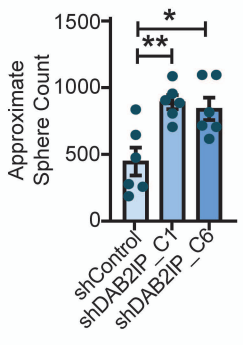
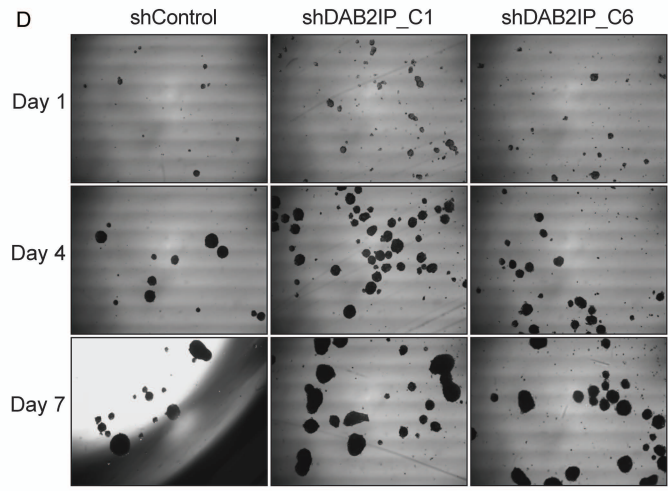
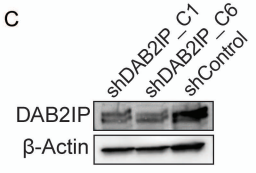
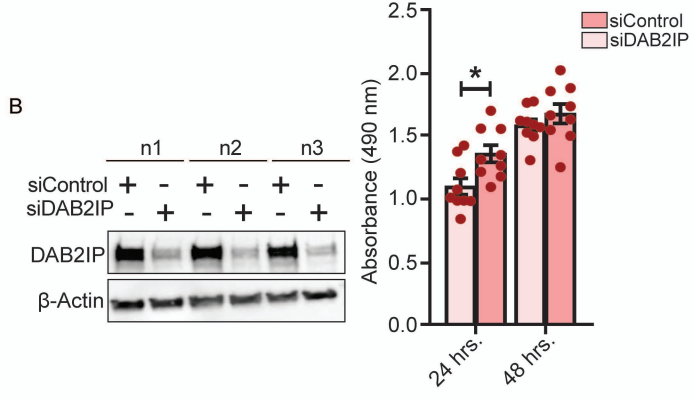
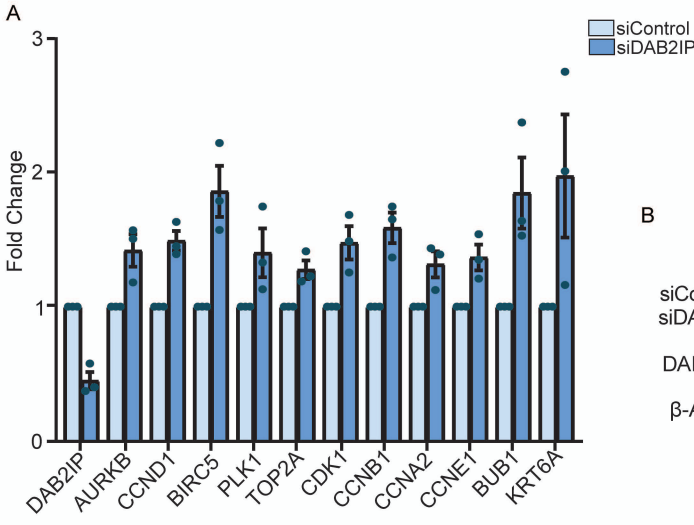


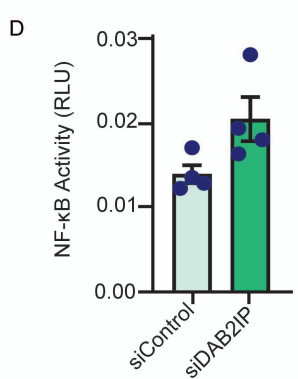
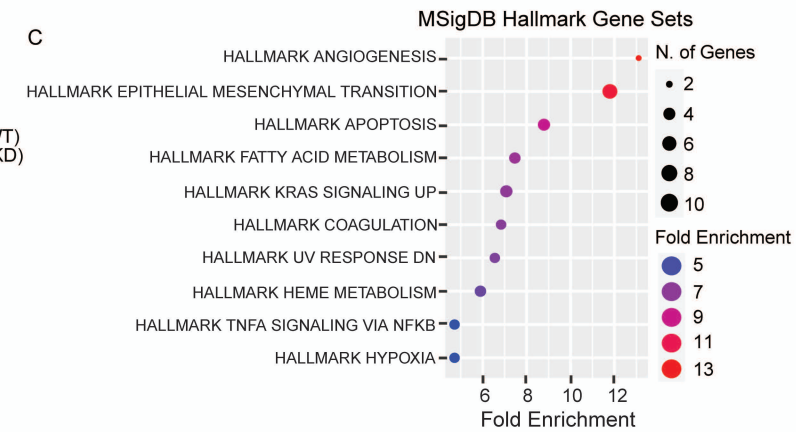
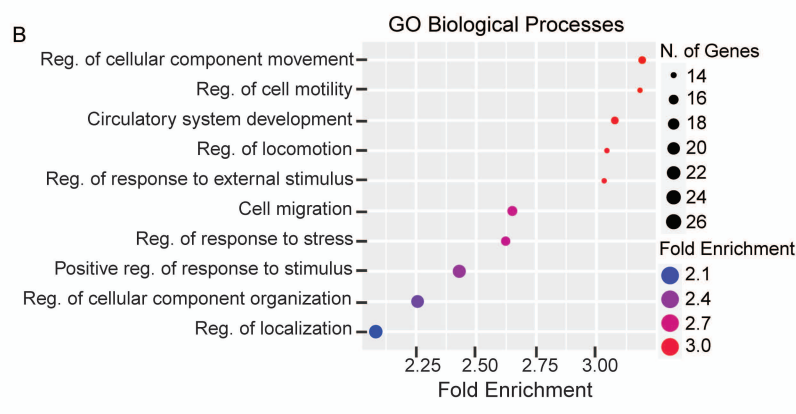
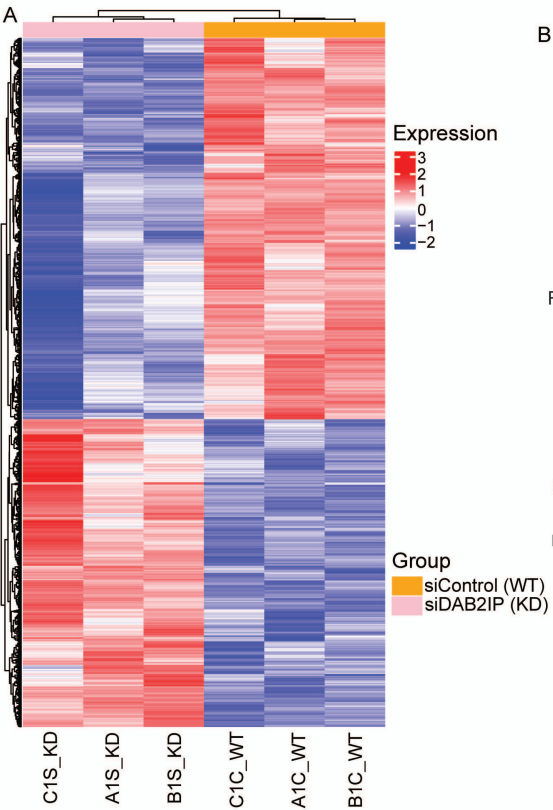
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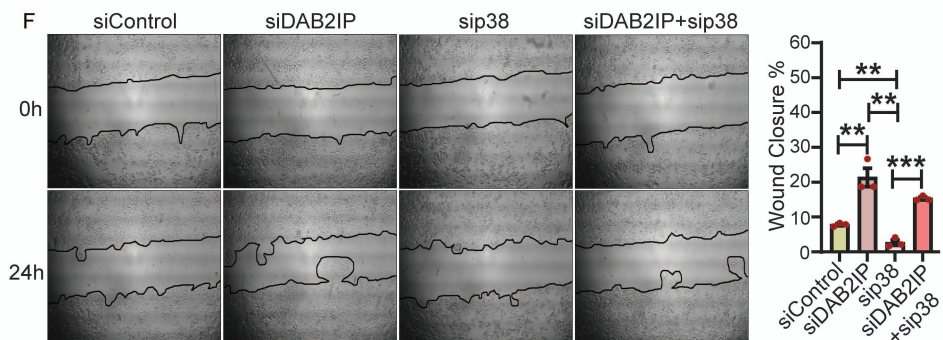
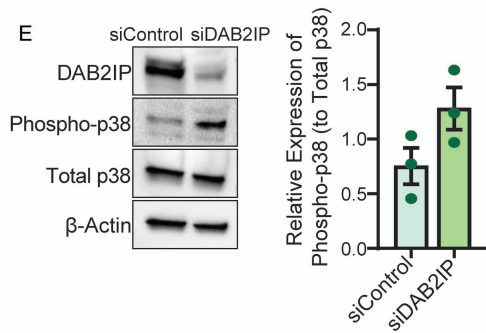
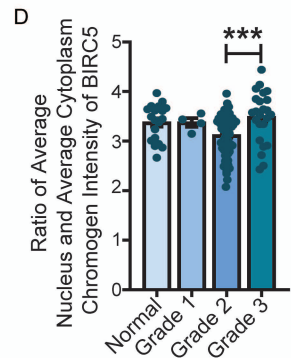
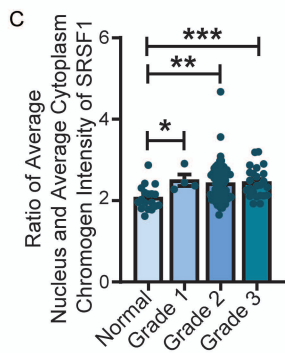
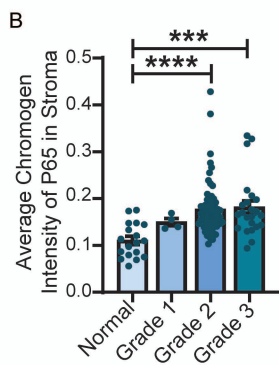
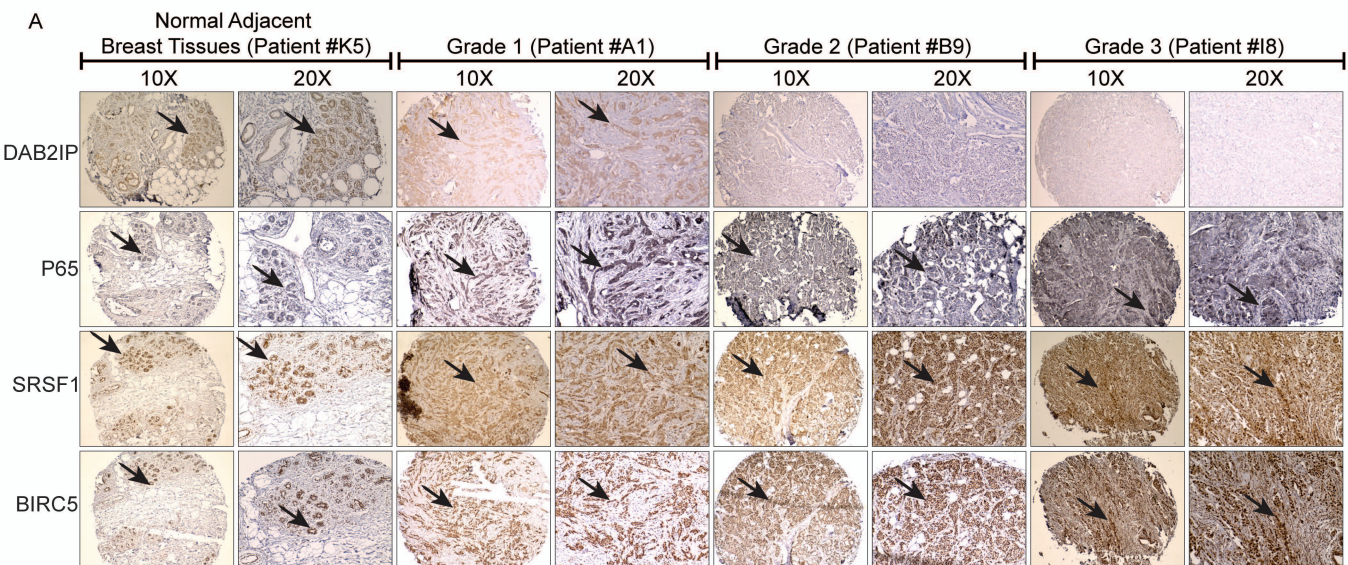


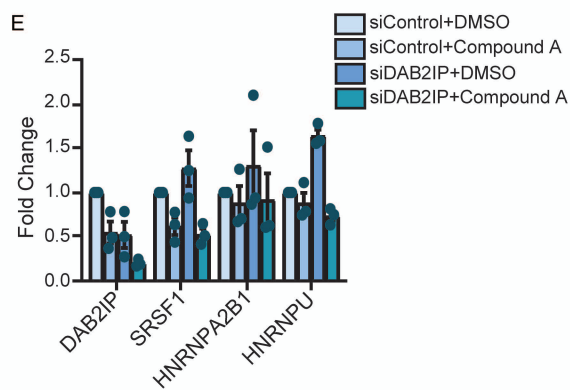
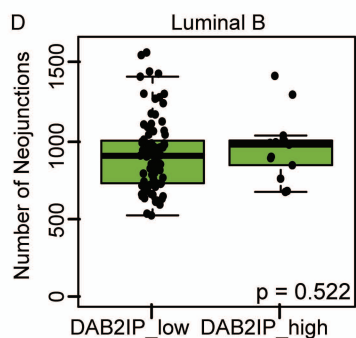
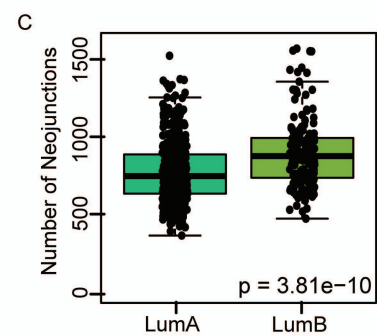
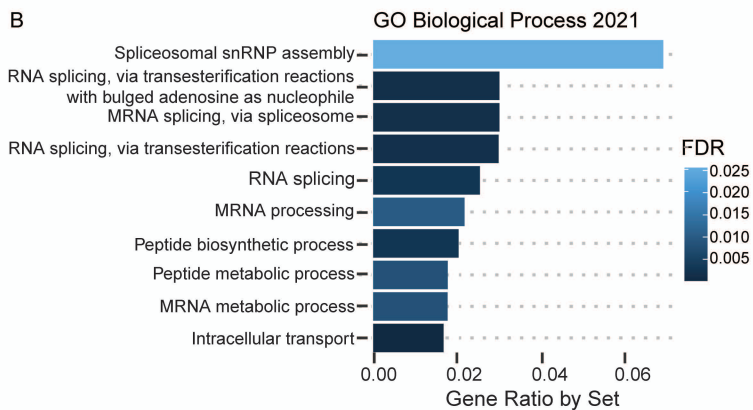
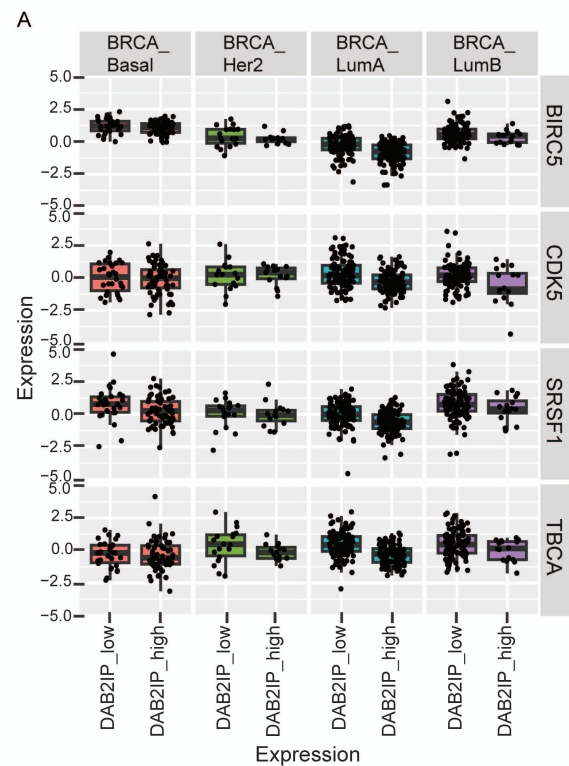
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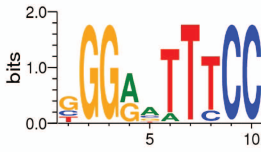






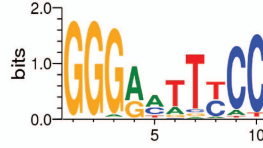
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Motif ID: M00052: NF-κB (p65)



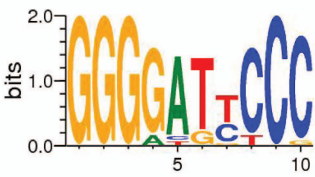
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| ATP6V1F | NIT2    | TMBIM6 |
| C2orf47 | PDE6D   | TSG101 |
| CAPNS1  | PIGC    | UBE2D3 |
| CAPZA1  | PIGM    | UBE2E1 |
| CCDC58  | POLR21  | VG     |
| CRYBA2  | PRR15L  |        |
| DLL3    | PSMA4   |        |
| EAPP    | PSMD10  |        |
| FFAR2   | PSME2   |        |
| FGF12   | RARS    |        |
| GLRX3   | RBX1    |        |
| GNG4    | RDH11   |        |
| JAGN1   | RHEB    |        |
| LSR     | SEC22A  |        |
| MANEAL  | SLC35B1 |        |
| MFSD11  | SNRPA1  |        |
| MNAT1   | STXBPL  |        |
|         | SUCLG1  |        |

Motif ID: M00054: NF-κB



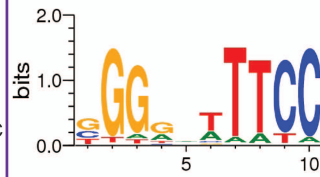
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| BLOC1S1  | POLR21 |
| C2orf47  | PSMA4  |
| CAPZA1   | PSME2  |
| CCDC58   | RBX1   |
| CRYBA2   | SEC22A |
| ELAVL1   | SMG7   |
| EPN3     | SNRPA1 |
| FFAR2    | SSSCA1 |
| FGF12    | SUCLG1 |
| GLRX3    | SUMO2  |
| GNG4     | TIMM10 |
| HIST1H4I | TMBIM6 |
| HIST1H4J | TMEM54 |
| JAGN1    | TSG101 |
| LSR      | UBE2D3 |
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Motif ID: M00051: NF-κB (p50)



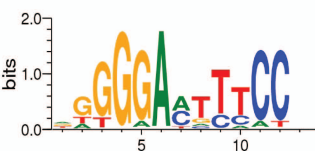
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| ARL8B   | FFAR2   | PPM1A    | SRP54    |
| ARPC5   | FGF12   | PRELID1  | SRP68    |
| ATP6V1F | FIBP    | PRR15L   | SSB      |
| BLOC1S1 | FXYD3   | PSMA4    | SSSCA1   |
| C1D     | GHITM   | PSMA5    | SUMO2    |
| C2orf47 | ILF2    | PSMB4    | TLCD1    |
| CBX4    | ING2    | PSMB6    | TMBIM6   |
| CBX8    | LAMTOR2 | PSME2    | TMEM106C |
| CCNE1   | MRPL14  | RAB1A    | TMEM165  |
| CETN2   | MTA3    | RBX1     | TMEM54   |
| CHMP5   | MTX1    | RDH11    | TP53RK   |
| COL2A1  | NDUF7   | RIAD1    | TSG101   |
| CRIP1   | NIT2    | RPL23A   | UBE2E1   |
| CRLS1   | NOL10   | RRM2     | UBE2Q1   |
| CRYBA2  | PDE6D   | SEC22A   | UBE2S    |
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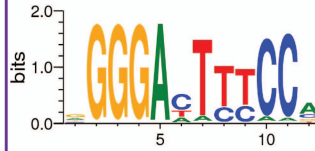
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| ATP5G1  | MNAT1   | TMBIM6 |
| ATP6V1F | MRPL24  | TSG101 |
| CAPNS1  | MRPL52  | UBB    |
| CAPZA1  | NDUFV2  | UBE2D3 |
| CCDC58  | NIT2    | UBE2E1 |
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| CRYBA2  | PIGM    |        |
| DLL3    | PSMD10  |        |
| EAPP    | PSME2   |        |
| EIF1    | RARS    |        |
| ELAVL1  | SEC22A  |        |
| FFAR2   | SLC35B1 |        |
| FGF12   | SNRPA1  |        |
| GLRX3   | SNW1    |        |
| GNG4    | SSSCA1  |        |
| IPO4    | STXBPL  |        |
| JAGN1   | SUCLG1  |        |

Motif ID: M00194: NF-κB



|         |           |        |
|---------|-----------|--------|
| ARL8B   | HNRNPA2B1 | UBE2E1 |
| BLOC1S1 | LSR       | YIPF3  |
| BTF3L4  | PAFAH1B3  |        |
| CAPZA1  | PDE6D     |        |
| CCNE1   | PIGC      |        |
| CSMD3   | SLC35B1   |        |
| DDIT3   | SLC50A1   |        |
| FFAR2   | TIMM10    |        |
| FGF12   | TIPRL     |        |

Motif ID: M00208: NF-κB



|         |          |          |        |
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| ATP5G3  | FFAR2    | RARS     | TIMM10 |
| BNIP1   | FGF12    | RFC4     | UBE2E1 |
| BTF3L4  | GNG4     | RNFT1    | VSTM2A |
| CAPZA1  | HIST1H4J | SEC22A   | YIPF3  |
| CCNE1   | JAGN1    | SLC50A1  |        |
| COX5A   | LSR      | SMG7     |        |
| CRIP1   | NOP10    | SNRPA1   |        |
| DDIT3   | PDE6D    | SNW1     |        |
| DYNLRB1 | PIGC     | SREK1IP1 |        |
|         |          | SUMO2    |        |

B  
Upregulated genes  
in Luminal A low DAB2IP tumors  
with NF-κB binding sites\_  
TCGA\_RNA-seq



Upregulated NF-κB candidate genes  
in Luminal A low DAB2IP subset\_  
TCGA\_RNA-seq

