Supplemental Data

Supplementary Table S1.

A. Average expression value

	GABRA3	GABRD	GABRP	GABRA2	GABRE	GABRG1
Normal	0.868	18.544	16026.494	55.601	1920.895	12.215
stage I	11.830	189.756	10413.123	12.899	295.429	1.697
stage II	35.181	206.439	9723.457	20.806	370.145	2.013
stage III	35.277	214.628	5239.819	10.743	299.941	2.703
stage IV	117.567	234.122	10981.531	10.330	527.898	0.710

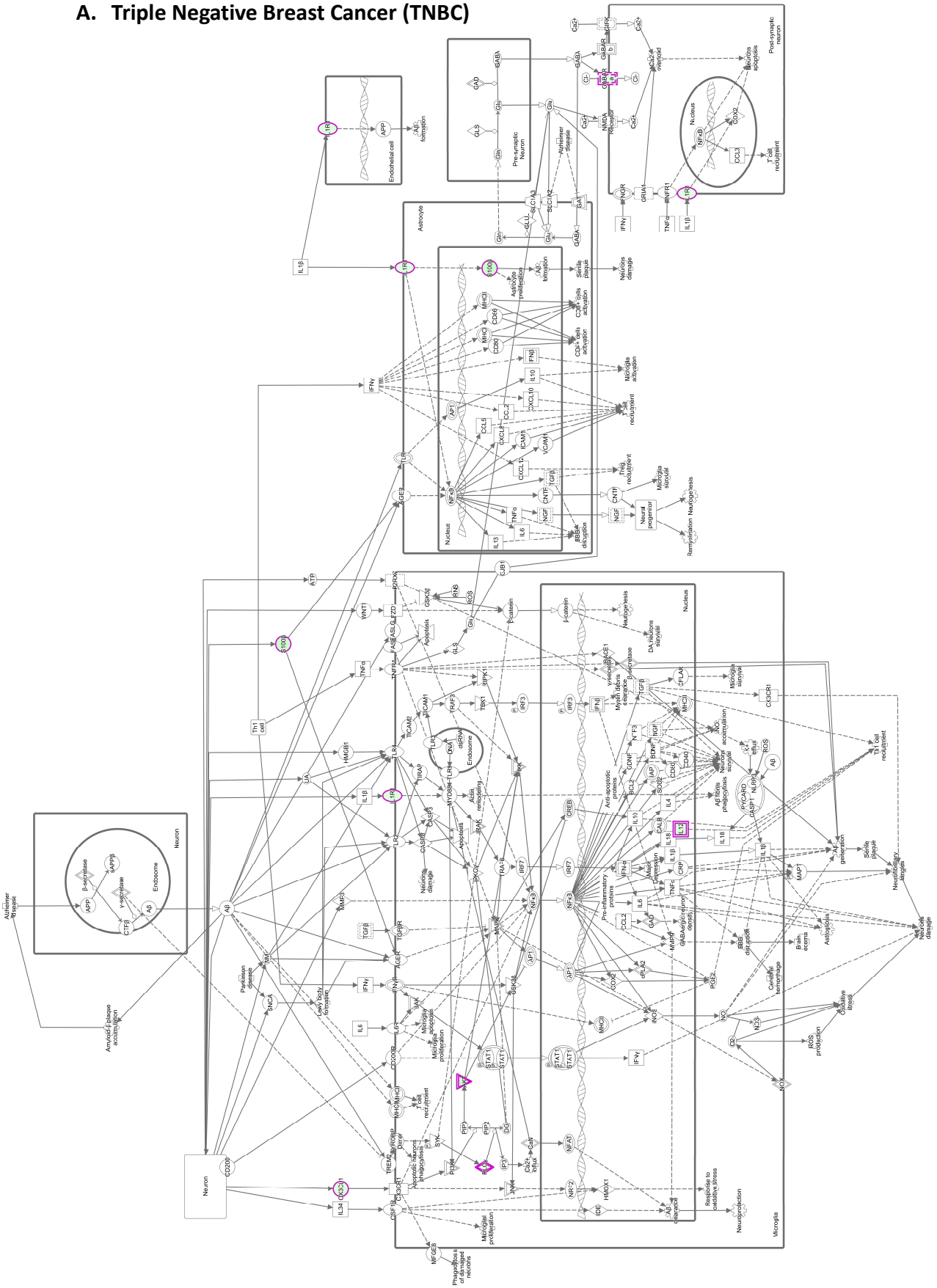
B. Standard deviation

	GABRA3	GABRD	GABRP	GABRA2	GABRE	GABRG1
Normal	2.556	25.026	11972.475	56.806	1993.472	15.594
stage I	45.530	239.893	30249.157	47.019	572.000	2.509
stage II	123.468	227.373	23894.242	94.018	1047.556	5.055
stage III	132.977	176.920	17395.268	36.214	695.032	7.479
stage IV	286.638	216.947	29677.933	24.399	1325.859	1.702

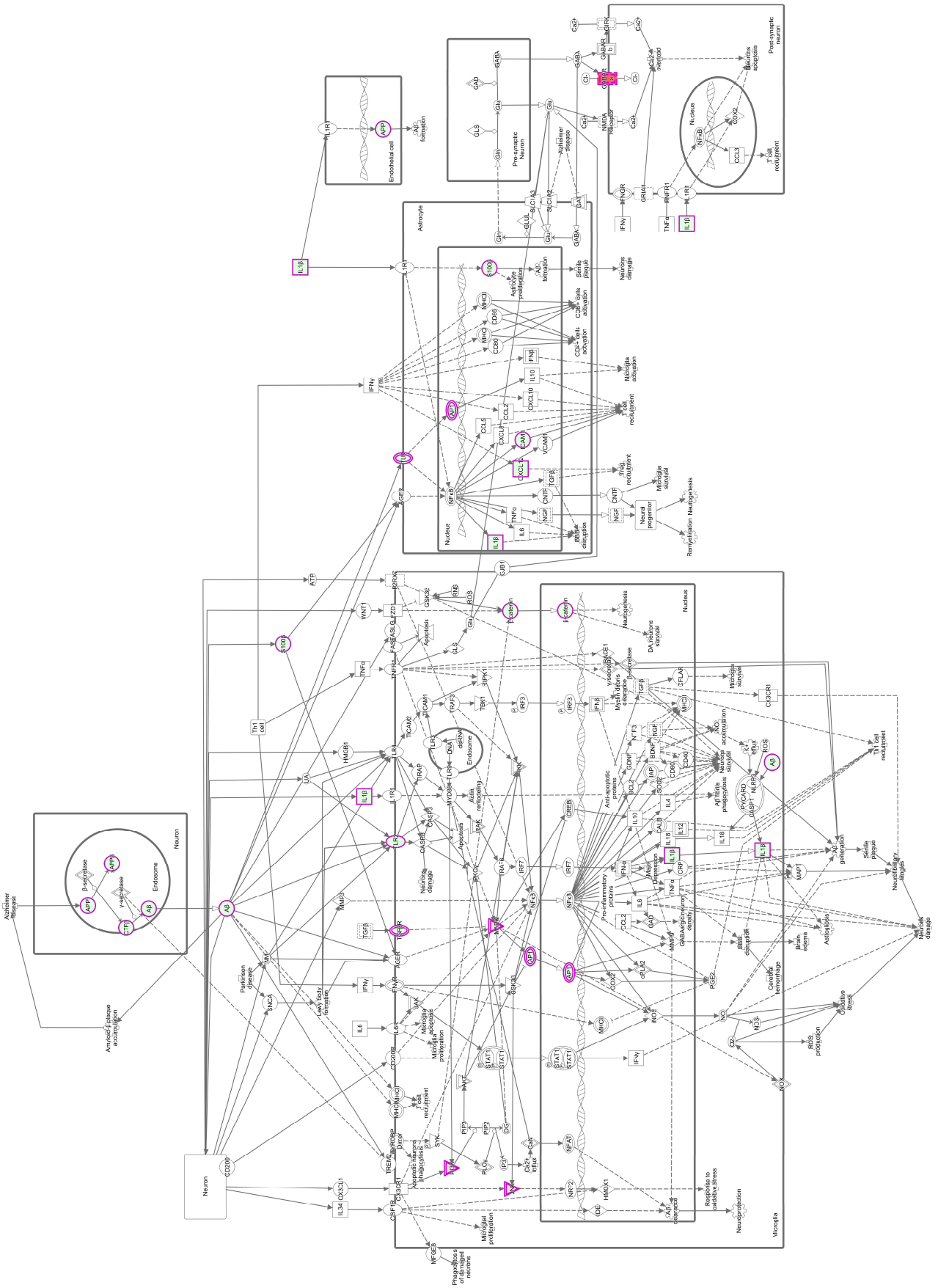
Supplementary Table S1. The expression levels of identified GABA receptors in primary BRCA tissues. To better understand the physiological relevance of GABA receptors in BRCA development and progression, we retrospectively examined expression profiles of GABA receptors from TCGA database. (**A**) shows average value and (**B**) shows standard deviation. Among 16 GABA receptors examined, 2 GABA receptors, GABRA3 and GABRD, were significantly upregulated in breast carcinoma tissues compared to normal breast tissues (* $p<0.05$; ** $p<0.01$). On the other hand, 4 GABA receptors (GABRA2, GABRE, GABRG1 and GABRP) were significantly downregulated in BRCA tissues (* $p<0.05$; ** $p<0.01$). Details of the expression levels of these GABARs are shown here.

Supplementary Figure S1

A. Triple Negative Breast Cancer (TNBC)



B. Non-Triple Negative Breast Cancer (non-TNBC)



Supplementary Figure S1. IPA analysis of GABRA3-mediated signaling and key ECM molecules in BRCA. To better understand the potential regulators involved in GABRA3 and ECM associated signalings, IPA analysis was conducted in BRCA database. Both (**A**) triple-negative BRCA and (**B**) non- triple-negative BRCA are analyzed. Several immune-modulators emerged, including pro-tumorigenic factors: IL-1R1, IL-1β, ICAM-1, AKT, CXCL1, CXCL12, PLCγ, APP, sAPPβ, CBFβ, Amyloid β, P13K, AP-1, TLR2, β-caterin, ICAM1; as well as anti-tumorigenic factors: S100B, IL-12, P-LCR, JNK, TLR.

Supplemental Experimental Procedures

Cell lines and reagents

Human breast cancer cell lines (MCF7, MDA-MB231) and a control non-carcinoma breast cell line (MCF10A) were obtained from American Type Culture Collection (ATCC). Cell lines were maintained as previously described [1]. The MCF7 and MDA-MB231 were cultured in complete DMEM medium (Gibco). MCF10A was cultured in complete DMEM/F12 medium (Gibco). Both of the complete media were supplemented with 10 % FBS (Thermo Scientific), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). Two BZDR drugs were used in this work, including 1 μM Zolpidem and 1 μM Diazepam.

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed as previously described [2]. The primers were human RSPO1 (146 bp product): sense, 5'-ACACTTCCCAGCATCTGAGACCAA-3'; antisense, 5'-TGCTGAACAGGATGGGAAGAAGGT-3'; human FGF16 (68 bp product): sense, 5'-CACGGCTTCTCCTCGTCTCT-3', antisense, 5'-AGGCGCTCGTTCAGGAAA-3'; human ADAMTS8 (138 bp product): sense, 5'-CCGCCACCCAGAGCACTA-3'; antisense, 5'-TCGATCACGGAGCAGCTTTT-3'; human COL6A6 (195 bp product): sense, 5'-CCCAGGCCACAGATTTCCAT-3', antisense, 5'-

TCCCACCCATCTGCCTGATA-3'; human VIT (105 bp product): sense, 5'-GGTCTGCGACACTGACCGCC-3', antisense, 5'-CGGAAGTTGCCCGTCCCCAC-3'; human S100B (189 bp product): sense, 5'-AGGGAGACAAGCACAAGCTG-3', antisense, 5'-CGTGGCAGGCAGTAGTAACC-3'; human FBN3 (174 bp product): sense, 5'-ACCTGGACGAATGCACCTC-3', antisense, 5'-CTGAGCTGACCAGGGTGAAG-3'; human GAPDH (131 bp product): sense, 5'-GTCTCCTCTGACTTCAACAGCG-3', antisense, 5'-ACCACCCTGTTGCTGTAGCCAA-3'. All expression values were normalized based on GAPDH as an endogenous control.

shRNA knockdown of GABRA3 in BRCA cells

GABRA3 shRNA clones (ID numbers: TRCN0000061208, TRCN0000061209, TRCN0000061210, TRCN0000061211, TRCN0000061212, TRCN0000420690 and TRCN0000426309) and control (scrambled) shRNA were purchased from (Academia Sinica, Taipei, Taiwan). The transfection experiments were performed as previously described [3]. Briefly, shRNA was transfected into MCF10A or MDA-MB231 cells (at a cell density of $5x10^5$ cells per well of a 6-well plate) using 7.5 µl TransIT-X2 Transfection Reagent (Mirus) with 25 nM of shRNA per well. Antibiotics, puromycin, was added at a concentration of 2 µg/ml to the wells 48 h after transfection to select for positively

transfected cells. Then, the cells were selected for 2 months before experiments were carried out and were continuously grown in selection media. The efficiency of RNAi knockdown was determined by real-time PCR of GABRA3 mRNA (shown in Figure 4C).

Cell growth assay

Cell growth curve was studied by Trypan Blue dye exclusion as previously described [4]. To measure the BRCA cell survival in response to presence or absence of BZDR drugs, MCF7 (or MDA-MB231 or MCF10A) cells were treated with either specific BZDR drug (Zolpidem or Diazepam) or control DMSO as time indicated.

Cell migration assay

Cell migration assay was carried out 7 days after treatment of MCF7 and MDA-MB231 cells with BZDRs or DMSO control. The study was performed as previously described [2]. Briefly, after the abovementioned treatment the cells were rinsed with PBS, and harvested using 0.05% Trypsin-EDTA (Invitrogen). The cells were then plated into a 2-well Culture-Insert (ibidi, Martinsried, Germany) according to the manufacturer's instructions. The Culture-Inserts were removed after 16 h, and cell migration was monitored over the indicated time-scale.

Cell invasion assay

Cell invasion study was performed as previously described [1]. Briefly, invasion chambers with 8-μm pores in 24-well plates (Corning, Discovery Labware, Inc., Bedford, MA, USA) were used. 192 h after BZDR treatment, the cells were plated onto the chambers. Subsequently, the cells were detached with 0.05 % Trypsin-EDTA, resuspended in conditioned medium (10 % FBS) and added to the upper compartment of the chambers, according to the manufacturer's instructions. After 20 h of incubation at 37 °C, the cells on the upper chamber were completely removed by wiping with a cotton swab, and then the filter was fixed with 100 % methanol and stained with 1% Toluidine Blue in 1% borax. Cells that had migrated from the upper to the lower side of the filter were imaged and counted with a light microscope (5 fields/filter).

Statistical Analysis

Hazard ratios (HRs) with 95% confidence intervals (CI) associated with statin use were computed using Cox proportional hazards regression in the competing risk of death after adjusting for age, CCI scores, tumor stage, and tumor size. All TMUCRD data management was performed using SAS v.9.3 software (SAS Institute Inc).

Supplementary References

1. Chang SC, Hsu W, Su EC, Hung CS, Ding JL. Human FBXL8 Is a Novel E3 Ligase Which Promotes BRCA Metastasis by Stimulating Pro-Tumorigenic Cytokines and Inhibiting Tumor Suppressors. *Cancers* 2020, **12**(8).

2. Chang SC, Hung CS, Zhang BX, Hsieh TH, Hsu W, Ding JL. A Novel Signature of CCNF-Associated E3 Ligases Collaborate and Counter Each Other in Breast Cancer. *Cancers* 2021, **13**(12).

3. Moore CB, Guthrie EH, Huang MT, Taxman DJ. Short hairpin RNA (shRNA): design, delivery, and assessment of gene knockdown. *Methods in molecular biology (Clifton, NJ)* 2010, **629:** 141-158.

4. Chang SC, Zhang BX, Su EC, Wu WC, Hsieh TH, Salazar AM*, et al.* Hiltonol Cocktail Kills Lung Cancer Cells by Activating Cancer-Suppressors, PKR/OAS, and Restraining the Tumor Microenvironment. *Int J Mol Sci* 2021, **22**(4).