SUPPLEMENTAL MATERIAL

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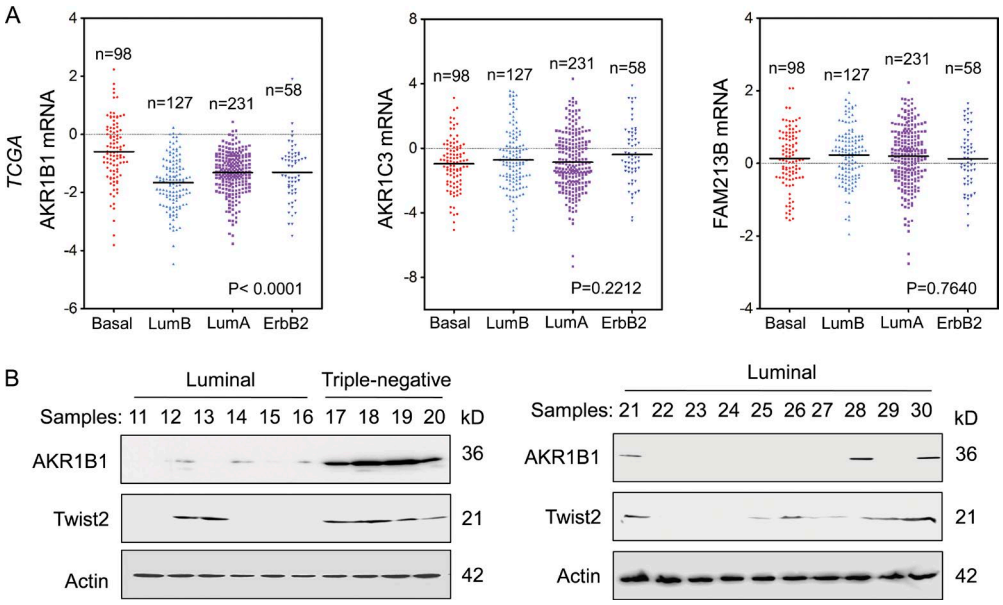

Figure S1. **AKR1B1 is up-regulated in BLBC.** (A) Dot plots indicate the expression of *AKR1B1*, *AKR1C3*, and *FAM213B* in different subtypes of breast cancer from microarray datasets from TCGA. Comparisons were analyzed by one-way ANOVA. (B) Lysates of fresh frozen tumor samples from 16 cases of luminal breast cancer and 4 cases of triple-negative breast cancer were analyzed for the expression of AKR1B1 and Twist2 by Western blotting. Actin was used as a loading control.

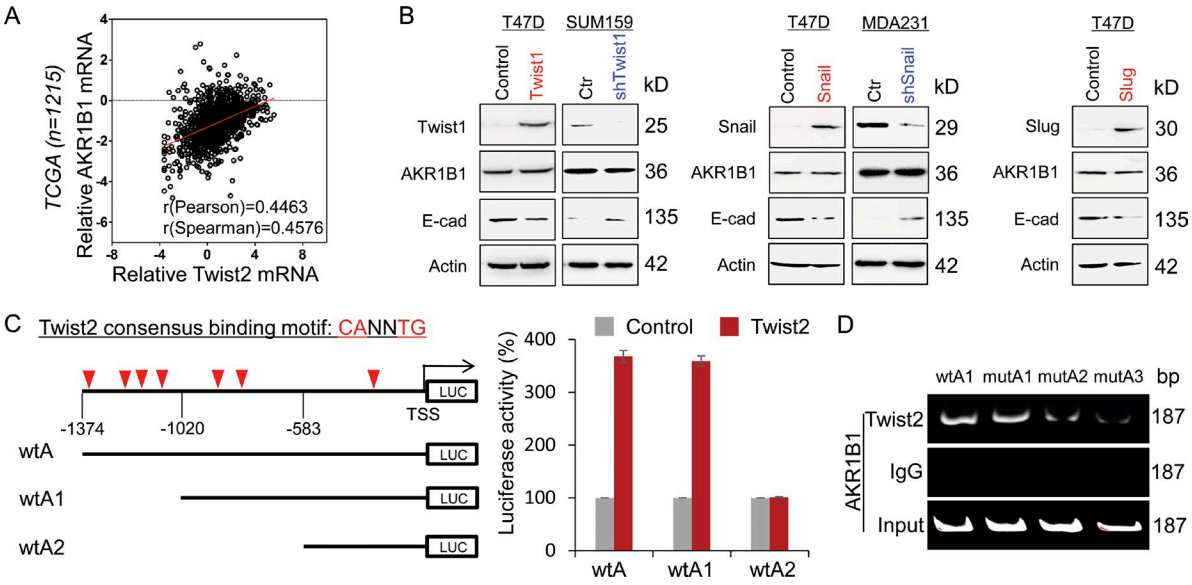

Figure S2. **AKR1B1 is a target of Twist2.** (A) Analysis of a public dataset (TCGA) for the expression of *AKR1B1* and *Twist2*. The relative level of AKR1B1 was plotted against that of Twist2. Correlations were analyzed using Pearson's correlation method and Spearman's rank correlation test. (B) Expression of AKR1B1 and E-cadherin (E-cad) was analyzed by Western blotting in T47D cells infected with empty vector (Ctr) or Twist1-, Snail-, or Slug-expressing vector, as well as MDA-MB231 cells or SUM159 cells with empty vector or knockdown of Twist1 or Snail expression. (C) Schematic diagram showing seven positions of potential Twist2-binding E-boxes in the AKR1B1 promoter. (Left) Deletion constructs of AKR1B1 promoter luciferase (LUC) are also shown. AKR1B1 promoter luciferase constructs (wtA, wtA1, and wtA2) were coexpressed with vector or Twist2 in T47D cells. (Right) Luciferase activities were determined and normalized. Data are mean ± SD in three separate experiments. (D) After a wild-type construct (wtA1) as well as its E-box mutants (mutA1, mutA2, and mutA3) were coexpressed with empty vector or Twist2 in HEK293 cells, ChIP analysis for binding of Twist2 to the AKR1B1 promoter was performed.

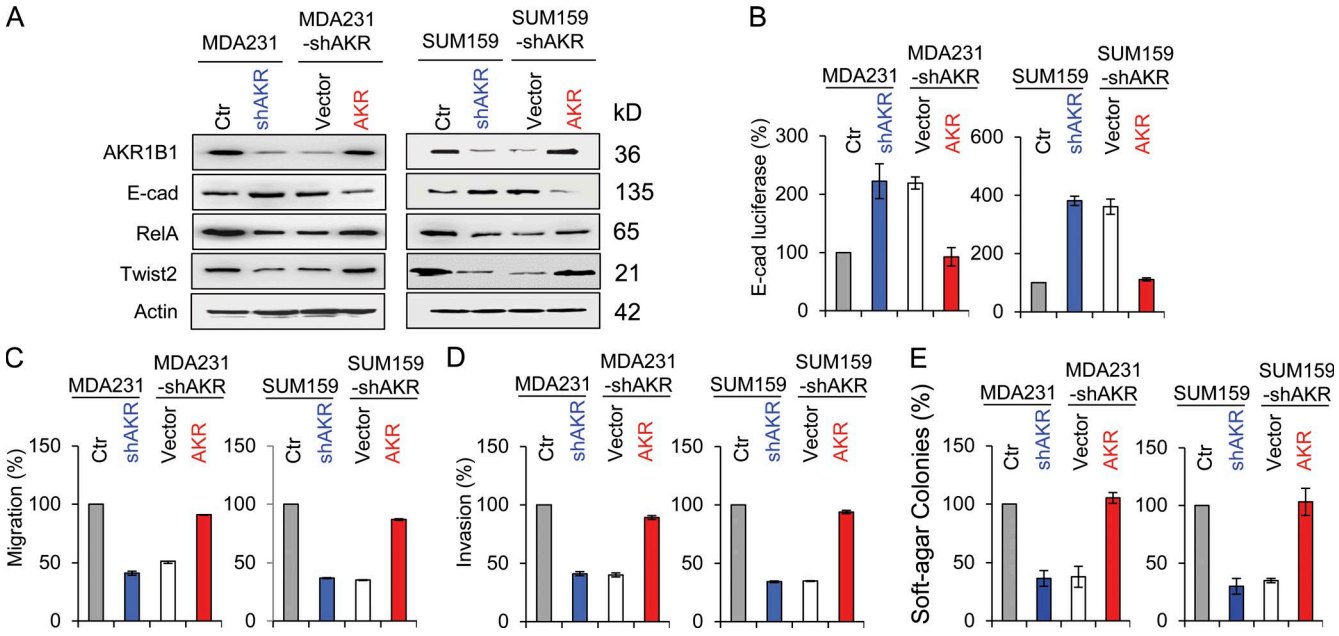

Figure S3. **AKR1B1 is associated with migration, invasion, and colony formation of breast cancer cells.** (A) Expression of AKR1B1, E-cadherin (E-cad), RelA, and Twist2 was analyzed by Western blotting in MDA-MB231 and SUM159 cells with stable empty vector (Ctr) or knockdown of AKR1B1 expression, as well as shAKR1B1-expressing MDA-MB231 and SUM159 cells infected with empty pLenti6.3/V5 vector (Vector) or pLenti6.3/V5-AKR1B1 (AKR). (B) The E-cadherin reporter activity was examined in MDA-MB231 and SUM159 cells with empty vector or knockdown of AKR1B1 expression, as well as shAKR1B1-expressing MDA-MB231 and SUM159 cells infected with pLenti6.3/V5 vector or pLenti6.3/V5-AKR1B1. After 48 h, luciferase activities were normalized and determined. (C–E) Migratory ability (C), invasiveness (D), and formation of colonies (E) of MDA-MB231 and SUM159 cells with stable empty vector or knockdown of AKR1B1 expression, as well as shAKR1B1-expressing MDA-MB231 and SUM159 cells infected with empty pLenti6.3/V5 vector or pLenti6.3/V5-AKR1B1, were analyzed. Data are presented in the bar graphs. Data are mean ± SD in three separate experiments.

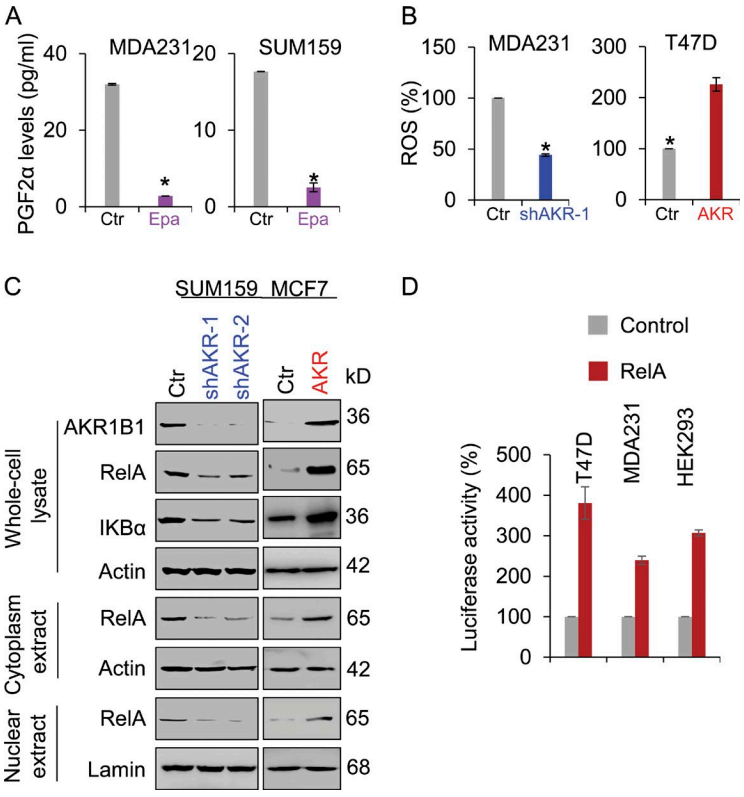

Figure S4. **AKR1B1-mediated NF-κB activation induces Twist2 expression.** (A) The level of PGF2α was measured in MDA-MB231 and SUM159 cells treated with or without 20 µM epalrestat (Epa). The level of PGF2α is shown in the bar graph. Ctr, stable empty vector. (B) ROS generation was analyzed in MDA-MB231 cells with stable empty vector or knockdown of AKR1B1 expression, as well as T47D cells with stable empty vector or AKR1B1 expression. (C) Expression of AKR1B1, RelA, and IKBα was analyzed by Western blotting in SUM159 cells with stable empty vector or knockdown of AKR1B1 expression (left), as well as MCF7 cells with stable empty vector or AKR1B1 expression (right). (D) The Twist2 promoter luciferase construct (wtT) was coexpressed with empty vector or RelA in T47D, MDA-MB231, and HEK293 cells. After 48 h, luciferase activities were determined and normalized. Data are mean ± SD in three separate experiments. *, $P < 0.01$ by Student's *t* test.

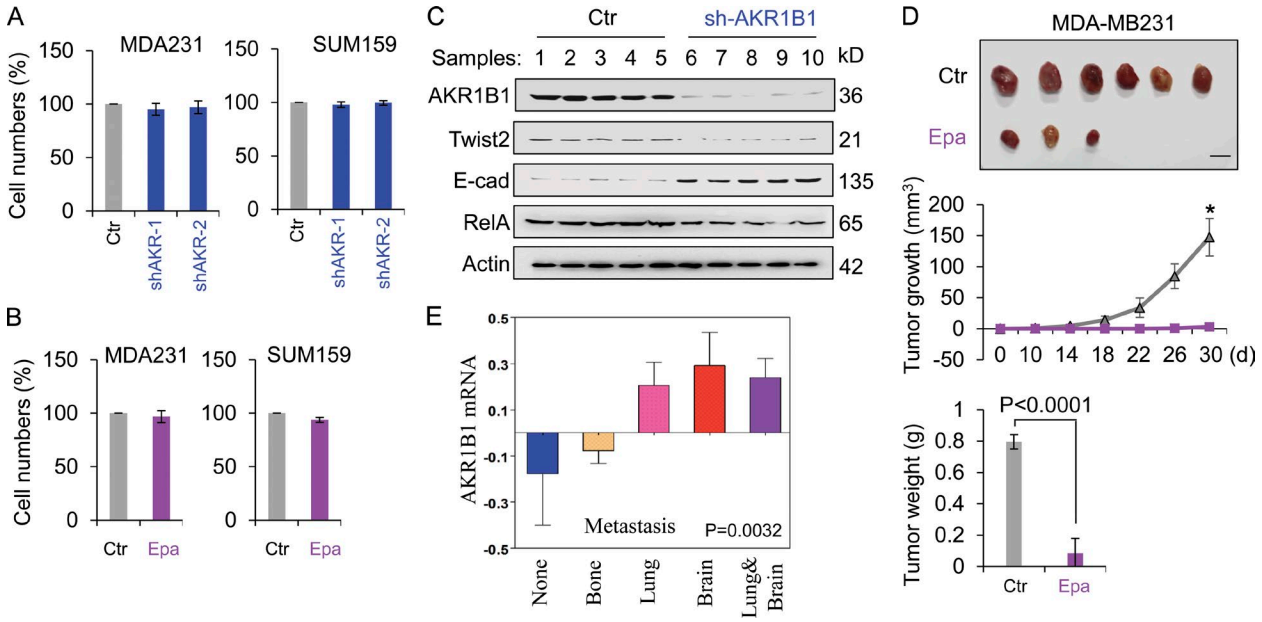

Figure S5. **Silencing of AKR1B1 inhibits breast tumor growth and metastasis.** (A and B) Cell growth for MDA-MB231 and SUM159 cells with stable empty vector (Ctr) or knockdown of AKR1B1 expression (A), as well as MDA-MB231 and SUM159 cells treated with or without 20 µM epalrestat (Epa; B), was measured by cell-count assay for a period of 2 d. Data are shown as a percentage over control cells (mean ± SD in two independent experiments), and no significant change was observed by Student's *t* test. (C) Expression of AKR1B1, RelA, Twist2, and E-cadherin (E-cad) was analyzed by Western blotting in tumor samples removed from the two groups of mice in Fig. 7 D. (D) For evaluation of epalrestat, after injection of MDA-MB231 cells, mice were divided into two group (six mice per group) and intragastrically treated with 50 mg/kg/d epalrestat or sterile water. The growth of breast tumors was monitored every 2 d. Tumor size and weight were measured. Bar, 1 cm. Data are presented as mean ± SEM from six mice. *, $P < 0.001$. (E) Analysis of a dataset (GSE12276) for the relationship between AKR1B1 expression and metastatic tendency of primary tumors. Comparisons were analyzed using one-way ANOVA. Data are mean ± SEM. None, $n = 19$; bone, $n = 111$; lung, $n = 45$; brain, $n = 16$; lung and brain, $n = 66$.