Figure S1. GALNT6 is highly expressed in breast tumors, and GALNT6 expression is correlated with breast tumor progression. (A) *GALNT6* mRNA expression (RNA Seq V2 RSEM) in breast tumor tissues of all tumor types (n=1,100) and the ER-positive (n=594), ER-negative (n=174), HER2-positive (n=120) and triple-negative (n=82) subtypes of invasive breast carcinoma in patients in The Cancer Genome Atlas dataset shown in a box plot (*P<0.05, ***P<0.001). (B) Upregulation of *GALNT6* mRNA expression in breast tumor tissues compared with their corresponding normal controls. (C) Upregulation of *GALNT6* mRNA expression in ER-positive breast tumor tissues compared with their corresponding normal controls. (D and E) Kaplan-Meier (KM) analyses of relapse-free survival, distant metastasis-free survival and overall survival of breast cancer patients from the KM plotter database. P-values were calculated with the log-rank (Mantel-Cox) test. The set of all breast tumors (n=3,951) and the sets of the ER-positive (n=2,061), HER2-positive (n=252) and ER-negative (n=801) subtypes in breast cancer patients in the KM plotter database were stratified into 'low' and 'high' *GALNT6* mRNA expression groups (Affymetrix ID: 219956_at) based on an autoselected best cutoff value. GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.



Figure S2. (A and B) Coimmunoprecipitation of GALNT6-binding proteins in GALNT6-HA-expressing HeLa cells. Lysates of HeLa cells stably expressing mock vector or GALNT6-HA were immunoprecipitated with an anti-HA antibody. GALNT6-HA and GALNT6-HA-binding proteins were eluted for 2DICAL. Eluates were examined by immunoblotting with (A) an anti-HA antibody and (B) by silver staining. (C) *GALNT6* and *LGALS3BP* expression in breast cancer cell lines and a normal human mammary epithelial cell line. The *GALNT6* and *LGALS3BP* mRNA expression levels in human breast cancer cell lines were evaluated by reverse transcription-quantitative PCR analysis. The levels are expressed relative to those in the human mammary epithelial cell line MCF-10A, which were defined as 1.0. The data are presented as the means ± standard deviation of three independent experiments. GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; LGALS3BP, lectin galactoside-binding soluble 3 binding protein; 2DICAL, 2-dimensional image-converted analysis of liquid chromatography-mass spectrometry.



Figure S3. Loss of *GALNT6* does not inhibit *LGALS3BP* expression in ZR-75-1 cells. (A) 293T cells were cotransfected with the LGALS3BP-FLAG and GALNT6-HA (WT or the enzyme-dead H271D mutant) expression plasmids. After 72 h, cell supernatants and cell lysates were harvested and immunoprecipitated with anti-FLAG affinity gel. Then, immunoblotting analyses were performed using the indicated antibodies. The band densities on the immunoblots are expressed relative to those of cells cotransfected with the LGALS3BP-FLAG and GALNT6 WT-HA expression plasmids, which are defined as 1.0. (B) The effect of *GALNT6* knockdown on *GALNT3* expression in BT-20 cells was evaluated by quantitative (q)PCR at 72 and 96 h after transfection. The levels were expressed relative to those in EGFP siRNA-transfected cells, which were defined as 1.0. (C) ZR-75-1 cells were transfected with EGFP or GALNT6 #1 siRNA. The *GALNT6* and *LGALS3BP* mRNA expression levels at 72 h after transfection were evaluated by qPCR. The levels are expressed relative to those in EGFP siRNA-transfected cells, which were defined as 1.0. (D) LGALS3BP protein expression levels in MG132-treated ZR-75-1 cells. ZR-75-1 cells were transfected with EGFP or GALNT6 #1 siRNA. After 48 h of transfection, cells were treated with 10 μ M MG132, and harvested at 6 and 12 h after treatment. Blotting analyses were performed using the indicated antibodies. GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; LGALS3BP, lectin galactoside-binding soluble 3 binding protein; WT, wild-type; EGFP, enhanced green fluorescent protein.



Figure S4. Multiple alignment of amino acid sequence of the carboxyl-terminal regions of mammalian LGALS3BPs. *Homo sapiens* LGALS3BP (NP_005558), *Pan troglodytes* LGALS3BP (XP_001158328.1), *Canis lupus* CANT1 (XP_005624094.1), *Bos taurus* LGALS3BP (NP_001039781.1), *Mus musculus* Lgals3bp (NP_035280.1), and *Rattus norvegicus* Lgals3bp (NP_620796.1). LGALS3BP, lectin galactoside-binding soluble 3 binding protein.

Carboxyl-terminal region of mammalian LGALS3BPs

<i>H.sapiens</i> LGALS3BP (585 amino acids)	541	KAAIPSALDT NSSKSTSSFP CPAGHFNGFR TVIRPFYLTN SSGVD 585
<i>P.troglodytes</i> LGALS3BP (585 amino acids)	541	KAAIPSALDI NSSKSTSSFP CPAGHFNGFR TVIRPFYLTN SSGVD 585
<i>C.lupus</i> CANT1 (559 amino acids)	515	KALIPSALGT NSSRRPSLFP CLGGSFSSFQ VVIRPFYLTN SSDVD 559
<i>B.taurus</i> LGALS3BP (555 amino acids)	511	KALVPSALAT NSSRSTSLFP CPSGVFSRFQ VVIRPFYLTN STDMD 555
<i>M.musculus</i> Lgals3bp (577 amino acids)	533	KAPIPTALDT NSSKTPSLFP CASGAFSSFR VVIRPFYLTN STDMD 577
<i>R.norvegicus</i> Lgals3bp (574 amino acids)	530	KAPIPGTQET NSSKTPSLFP CASGAFSSFR EVIRPFYLTN STDTE 574

Figure S5. Representative data of the effects of LGALS3BP T556/T571/S582 on GALNT6-dependent LGALS3BP *O*-glycosylation and release. (A-C) HeLa cells were cotransfected with one of the LGALS3BP point mutant-FLAG [(A) T556A, T571A, T556A/T571A; (B) T579A, N580A, S581A and S582A; (C) T556A/T571A, S582A, T556A/T571A/S582A] or the LGALS3BP WT-FLAG and the GALNT6-HA expression plasmid vectors. After 72 h, cell supernatants and cell lysates were harvested and immunoprecipitated with anti-FLAG affinity gel. The band densities on the immunoblot are expressed relative to those of cells cotransfected with the LGALS3BP WT-FLAG and GALNT6 WT-HA expression plasmids, which were defined as 1.0. LGALS3BP, lectin galactoside-binding soluble 3 binding protein; WT, wild-type; GALNT6, polypeptide N-acetylgalactosaminyltransferase 6.





100-

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GALNT6

Figure S6. Full-length immune blots. WCL, whole-cell lysates; WT, wild-type; GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; LGALS3BP, lectin galactoside-binding soluble 3 binding protein; EGFP, enhanced green fluorescent protein.

Figure S6. Continued. Full-length immune blots. WCL, whole-cell lysates; WT, wild-type; GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; LGALS3BP, lectin galactoside-binding soluble 3 binding protein; EGFP, enhanced green fluorescent protein.



Figure S6. Continued. Full-length immune blots. WCL, whole-cell lysates; WT, wild-type; GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; LGALS3BP, lectin galactoside-binding soluble 3 binding protein; EGFP, enhanced green fluorescent protein.

