Supplementary Figure legends

Supplementary Figure S1. TGF-β stimulates conversion of LC3-I to LC3-II in HepG2 hepatocellular carcinoma cells. HepG2 cells (from American Type Culture Collection) expressing GFP-LC3 were treated with TGF-β1 (1 ng/mL) for 24 h and subjected to immunoblotting with anti-LC3 antibody.

Supplementary Figure S2. Dose-dependent effect of TGF-β1 on GFP-LC3 dot formation in HuH7 cells. The area of GFP-LC3 dots was also quantified by ImageJ 1.36b after treatment with various doses of TGF-β1 for 12 h in HuH7 cells. ***$p<0.001$.

Supplementary Figure S3. Confirmation of knockdown of BECLIN1, ATG5, ATG7, and DAPK by siRNA transfection. ***A,*** levels of mRNA expression of BECLIN1, ATG5, ATG7, and DAPK were determined by quantitative RT-PCR analysis after transfection of control siRNA or corresponding siRNAs in HuH7 cells. Values were normalized to the expression levels of HPRT1. *Columns*, mean of triplicate determinations; *bars*, SD. ***B,*** levels of protein expression of BECLIN1, ATG5, ATG7, and DAPK were determined by immunoblot analysis after transfection of control siRNA or corresponding siRNAs in HuH7 cells.

Supplementary Figure S4. Effect of dominant negative mutant of TβRII (dnTβRII) on autophagy activation induced by TGF-β. dnTβRII was introduced into HuH7 cells by lentiviral-mediated gene transfer. After transduction of dnTβRII, GFP-LC3 dot formation was analyzed after 12 h of treatment with TGF-β1. **$p<0.01$.

Supplementary Figure S5. Effects of Smad2/3 knockdown and inhibition of mRNA synthesis on TGF-β-mediated autophagy activation. ***A,*** effect of Smad2/3 knockdown on GFP-LC3 dot formation by TGF-β in HuH7 cells. HuH7 cells were transfected with control siRNA or siRNA targeting Smad2 and Smad3 (Invitrogen, Stealth RNAi) before 24-48 h of TGF-β1 treatment. The area of GFP-LC3 dots was quantified after 12 h of treatment with TGF-β1. *$p<0.05$. ***B,*** effect of mRNA synthesis inhibition on GFP-LC3 dot formation by TGF-β in HuH7 cells. HuH7 cells were treated with actinomycin D (Sigma-Aldrich, 1 μg/mL) before 1 h of TGF-β1 treatment. The area of GFP-LC3 dots was quantified after 12 h of treatment with TGF-β1. **$p<0.01$.

Supplementary Figure S6. Knockdown of JNK1/2 by siRNA transfection. ***A,*** confirmation of knockdown of JNK1/2 by siRNA transfection. Levels of mRNA expression of JNK1 and JNK2 were determined by quantitative RT-PCR analysis after transfection of control siRNA or siRNA targeting JNK1/2 in HuH7 cells. Values were normalized to the expression levels of HPRT1. ***B,*** effect of JNK1/2 knockdown on the upregulation of ATG5 by TGF-β was evaluated by quantitative RT-PCR analysis. *Columns*, mean of triplicate determinations; *bars*, SD. **$p<0.01$.

Supplementary Figure S7. Induction of PARP cleavage by TGF-β in HuH7 cells. HuH7 cells were treated with TGF-β1 (1 ng/mL) for the indicated times and subjected to immunoblotting with anti-PARP antibody.

Supplementary Figure S8. Requirement of Bim for TGF-β-mediated apoptosis induction. HuH7 cells were transfected with control siRNA or siRNA targeting Bim (Invitrogen, Stealth RNAi) before 48 h of TGF-β1 treatment. After the treatment with TGF-β1 (5 ng/mL) for 24 h, assessment of apoptosis was performed as in Fig. 5*B*. *p<0.05.

Supplementary Figure S9. TGF-β stimulates LC3 conversion in breast cancer cells. ***A,*** dose-dependent effect of TGF-β1 on GFP-LC3 conversion in MDA-MB-231 cells. GFP-LC3 conversion was analyzed by immunoblot analysis of the cell lysates after treatment with various doses of TGF-β1 for 12 h in MDA-MB-231 cells. ***B,*** TGF-β stimulates conversion of LC3-I to LC3-II in JygMC(A), mouse mammary carcinoma cells. JygMC(A) cells were maintained as described previously (25). JygMC(A) cells expressing GFP-LC3 were treated with TGF-β1 (12 h) and subjected to immunoblotting with anti-LC3 antibody.

Supplementary Figure S10. Effect of autophagy inhibition on TGF-β-mediated growth inhibitory function in MDA-MB-231 cells. Cells were seeded at a density of 3 $x10^3$ cells/well in 96-well plates, transfected with control siRNA or siRNAs targeting ATG5 or ATG7, and treated with various doses of TGF-β1 48 h after transfection. MDA-MB-231 cells were resistant to growth inhibition by TGF-β1, and a higher concentration of TGF-β1 (5 ng/mL) was needed to detect the modest growth inhibitory effect of TGF-β1. Cell viability was determined by WST-8 assay in triplicate after 48 h. *Columns*, mean of triplicate determinations; *bars*, SE. **p<0.01.

Supplementary Figure S11. mTOR activation by TGF-β. HuH7 cells were treated with TGF-β1 (1 ng/mL) for the indicated times and subjected to immunoblotting with rabbit polyclonal anti-phospho-mTOR (Ser2448) antibody (#2971, Cell Signaling) and rabbit polyclonal anti-total-mTOR antibody (#2972, Cell Signaling). Transient activation of mTOR was induced by TGF-β.

Supplementary Figure S12. BECLIN1 suppresses the proliferation of HuH7 cells. Hemagglutinin(HA)-tagged BECLIN1 cDNA was introduced into HuH7 cells by lentiviral-mediated gene transfer. ***A,*** immunoblot assay. After transduction of BECLIN1, the cell lysates were subjected to immunoblotting with rat monoclonal anti-HA antibody (3F10, Roche Diagnostics). ***B, C,*** **and** ***D,*** cell viability assay (*B*), apoptosis assay (*C*), and cell cycle analysis (*D*). Cell viability assay (WST-8 assay), apoptosis assay, and cell cycle analysis were performed as in Fig. 5. While BECLIN1 expression reduced the proliferation of HuH7 cells, the effects on cell cycle and apoptosis were not significant. *$p<0.05$. n.s., not significant.