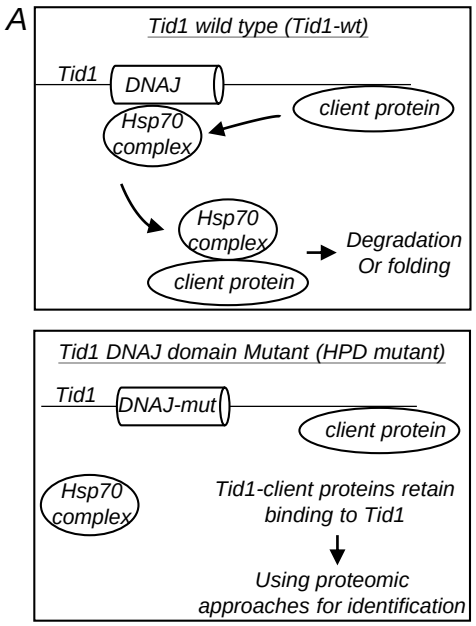
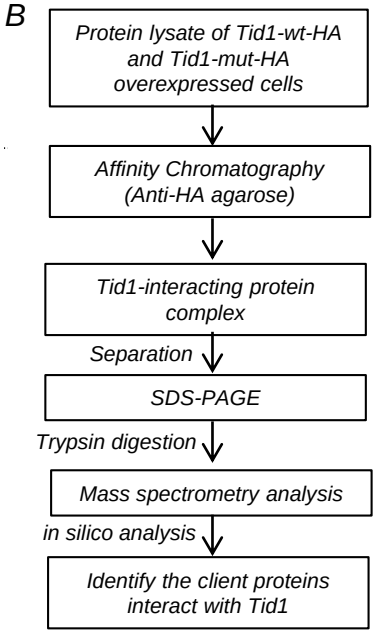
Supplementary Figure 1.

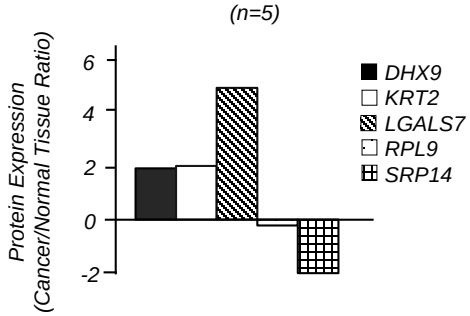




C

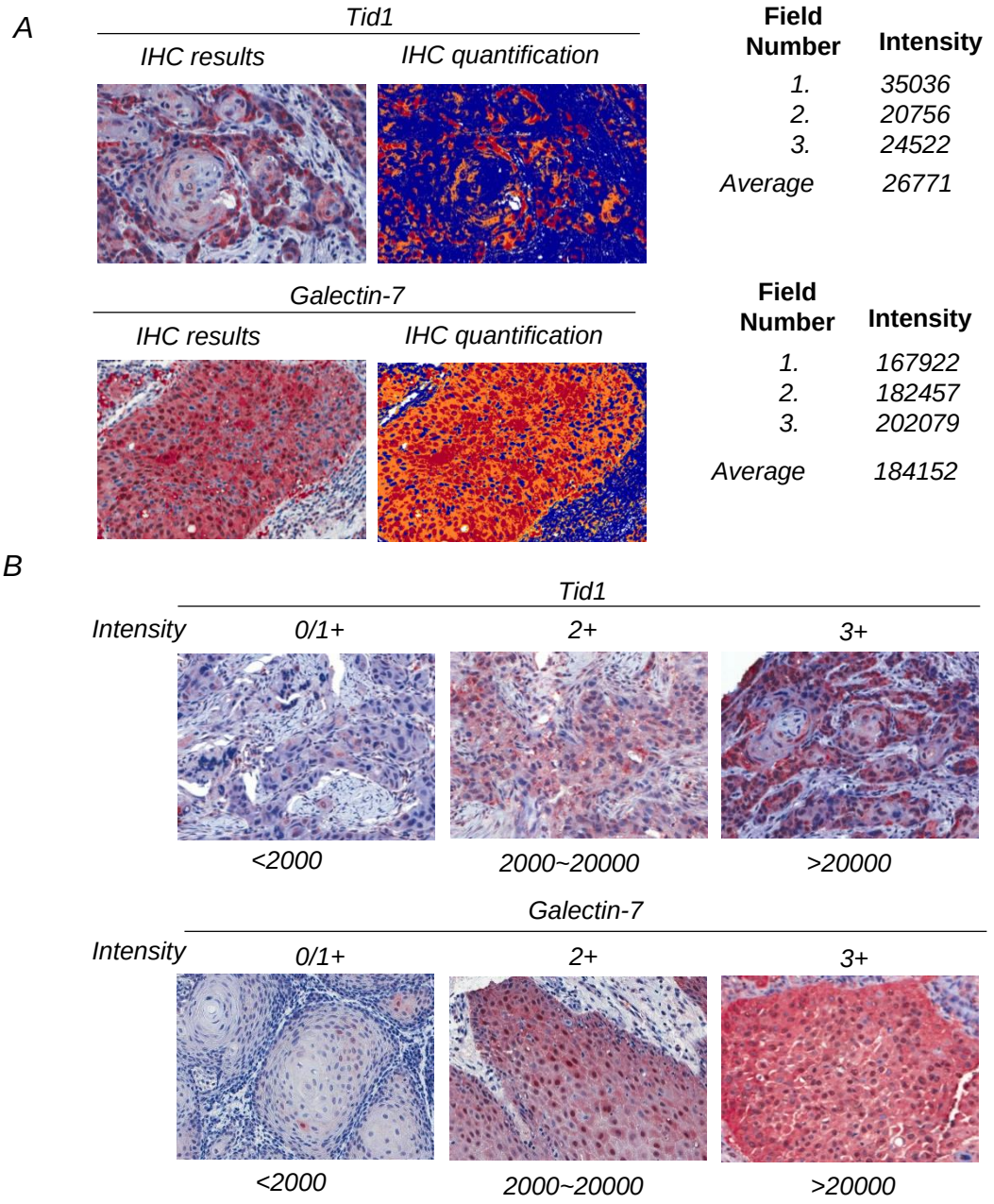
Tid1-L-mut interacting protein	Tid1-L-wt interacting protein	Tid1-S-mut interacting protein	Tid1-S-wt interacting protein
DHX9	HIST1H2AE	ATP5C1	ANXA1
HNRNPUL1	HNRNPUL1	BAG2	ATP1A1
HSPA9	HSP90AA1	COX5B	ATP1A1
IGF2BP1	HSPA8	DHX9	HSP90AA1
KRT2	HSPA9	EMG1	HSPA5
LGALS7	PABPC1	HIST2H2AA4	HSPA8
RPL9	RPL18A	HNRNPD	HSPA9
SRP14	RPL21	HNRNPDL	KRT9
STAU1	RPS10	HNRNPUL1	PARP1
TNRC6B	RPS12	HNRPU	YMHAZ
	RPS16	ILF3	
	RPS18	PHB2	
	RPS24	PHF6	
	SLC25A11	RPL9	
	TTN	RPLP0	
		RPLP1	
		RPS5	
		TUBB4A	

D *Significance level change from various cancer types and corresponding normal tissues (n=5)*



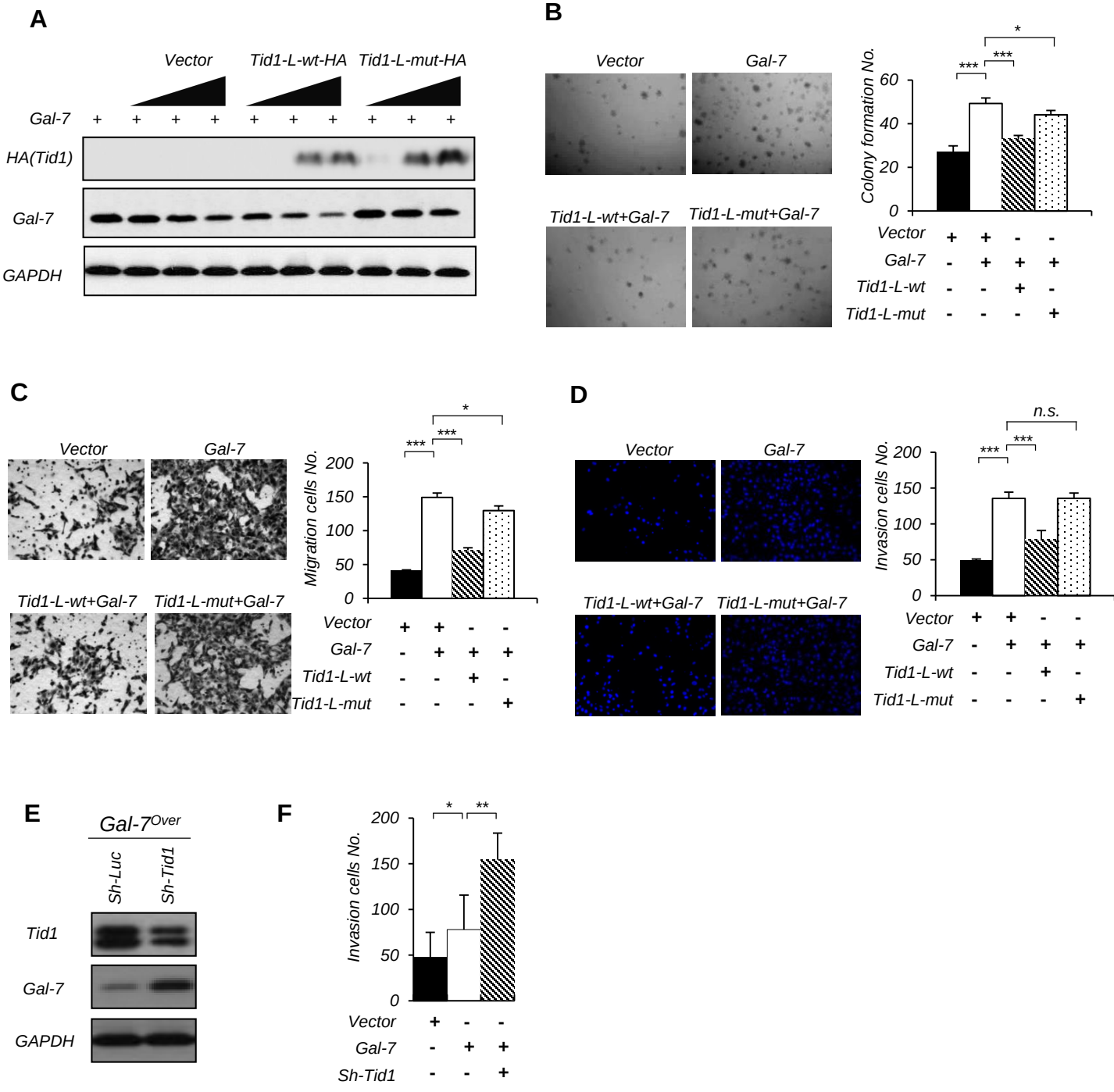
Supplementary Figure 1. Purification and identification of the Tid1-interacting protein complex. *(A) The strategy for identification of Tid1-interacting client proteins by using a DNAJ mutant of Tid1. (B) Schematic for identification of the Tid1 binding partners by affinity chromatography and systemic proteomic analyses. 293T cells were transfected with the HA-tagged WT Tid1 (Tid1-wt-HA) or DNAJ domain mutant Tid1 (Tid1-mut-HA) expression plasmid, respectively. Total protein lysates of the transfected cell, respectively, were immunoprecipitated (IP) with anti-HA antibody, separated by SDS-PAGE, and then analyzed by mass spectrometry as described in Materials and Methods. (C) The list of different Tid1-interacting proteins with wild type- or DNAJ mutant- Tid1 by further mass spectroscopy analyses. (D) Ratio of differential Tid1-L-mut interacting protein levels between tissues derived from HNSCC cancer and corresponding normal tissues were determined using the dbDEPC 2.0 proteomics database.*

Supplementary Figure 2.



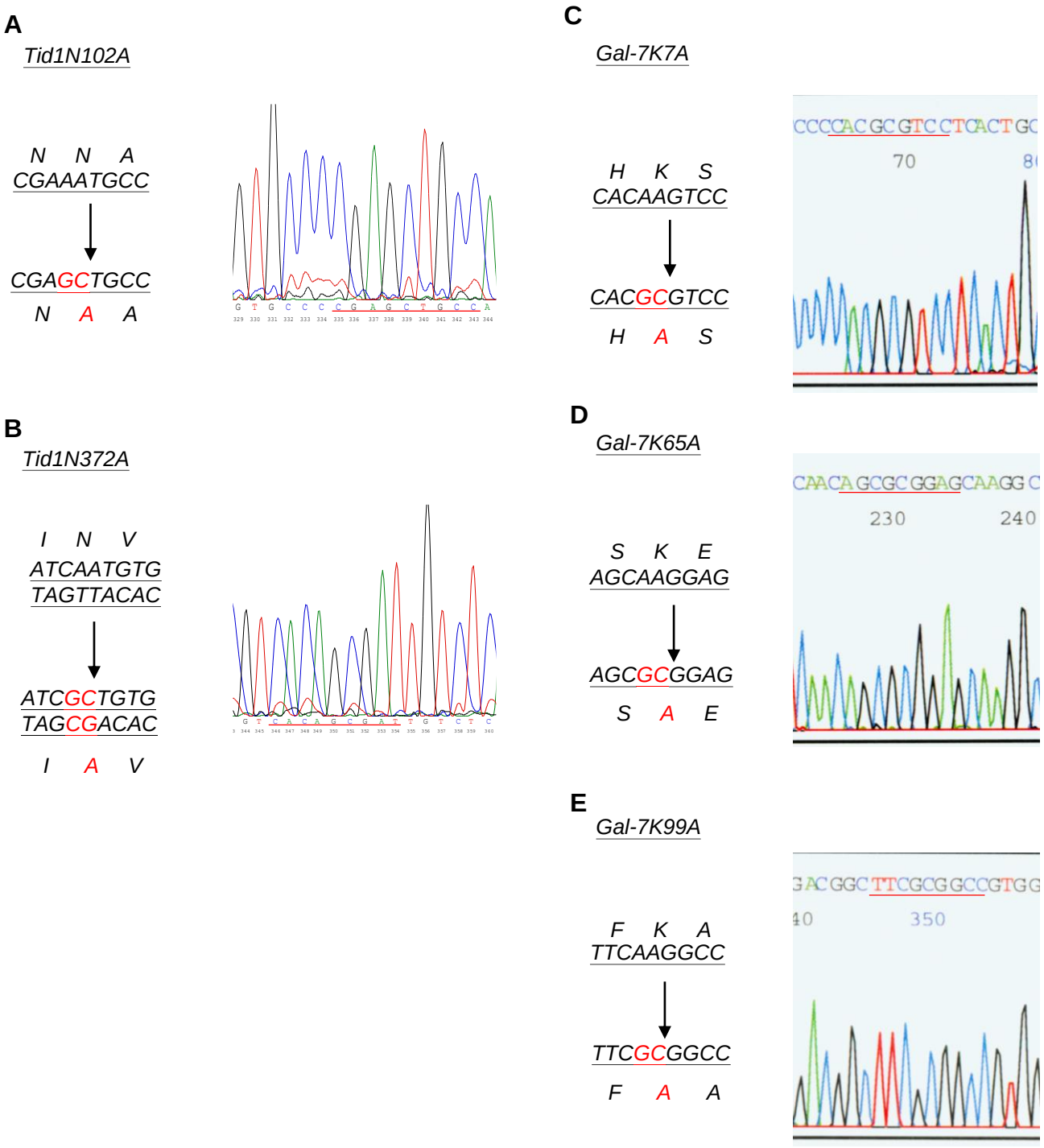
Supplementary Figure 2. IHC scoring of Tid1 and Galectin-7 intensity in tissue sections. *(A) Left panels: Identification of Tid1 or Galectin-7 positive staining by Aperio's Image Scope software. Right panel: Numeric of intensity in three representative fields and the average of these intensities for Tid1 or Galetin-7 staining. (B) IHC of Tid1 score of 0/1+, 2+ and 3+(upper panels). IHC of Galectin-7 score of 0/1+, 2+ and 3+((lower panels) (C) Survival curves of Tid1$^{f/f}$, and K5-Tid1$^{f/f}$ mice treated with 4NQO, respectively, were plotted according to body weight loss more than 30% of total body weight or the sacrifice of animal at end point.*

Supplementary Figure 3.



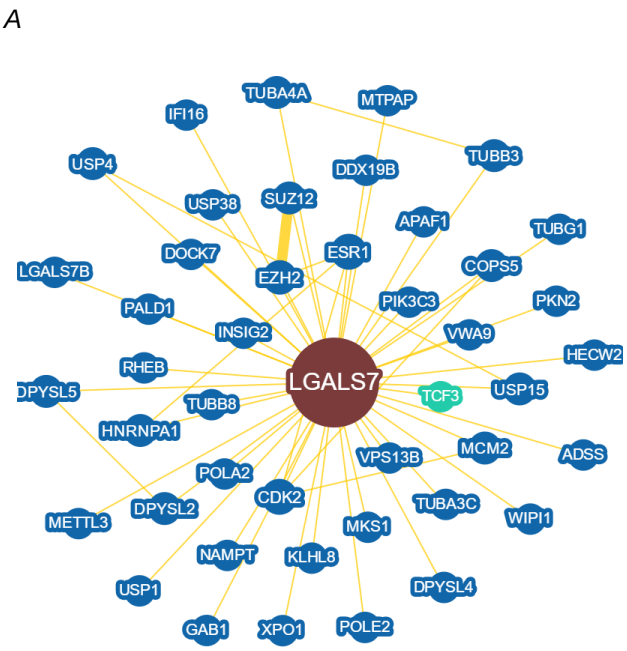
Supplementary Figure 3. Tid1 decreases exogenous Galectin-7, and inhibits Galectin-7 induced malignancy in HNSCC. *(A) Increasing amounts of control (0.5, 1 and 2 μg), Tid1-L-wt-HA (0.5, 1 and 2 μg) or Tid1-L-mut-HA (0.5, 1 and 2 μg) plasmids, respectively, were co-transfected with Gal-7-myc plasmids into 293T cells. The expression level of Tid1-L and Gal-7 proteins was determined by western blot analyses. (B-D) SAS cells were either singly or combinatorically transfected in accordance with plasmids including control, Gal-7-Myc, Tid1-L-wt-HA, or Tid1-L-mut-HA. The transfected cells were examined for (B) anchorage-independent growth, (C) Transwell® migration ability and (D) matrix gel invasion ability. (E-F) The effect of Tid1 depletion on Gal-7 expression and function was elucidated. Sh-Luc or sh-Tid1-L vector was co-transfected with Gal-7-myc into SAS cells. The expression of Tid1-L and Galectin-7 in the transfected SAS cells was determined by immunoblot (E), and the matrix gel invasion ability of transfected cells were examined (F). The histograms shown are the means ± SD from three independent experiments (∗p < 0.01; ∗∗p < 0.005).*

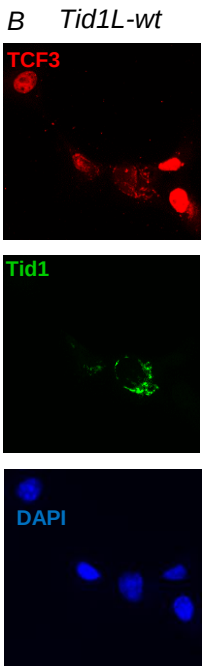
Supplementary Figure 4.



Supplementary Figure 4. The mutants of Tid1 N-link glycosylation sites and Galectin-7ubiquitination sites were confirmed by sequencing. *(A-B) DNA sequencing data of Tid1N102A and Tid1N372A (C-E) DNA sequencing data of Gal-7K7A, Gal-7K65A and Gal-7K99A.*

Supplementary Figure 5.





Supplementary Figure 5. Tid1 modulates TCF3 activity by regulating Gal-7 expression. *(A) Protein–protein interaction network of Gal-7 generated using BioGRID (https://thebiogrid.org). (B) SAS-M5 cells were transfected with Tid1L-wt and then stained with Gal-7 (red) and Tid1(green). Samples were then analyzed by confocal microscopy.*

Supplementary Materials and Methods

Mass spectrometry analysis

Tid1-HA-interacting complex proteins were purified from transfected 293T cells by affinity chromatography using anti-HA–agarose beads (Sigma–Aldrich). Tid1-interacting proteins were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS analysis was performed as described in a previous study.[2]

Plasmids

The J-domain mutants of Tid1 (Tid1-L-mut and Tid1-S-mut) plasmids were generated by replacing the 121H122P123D amino acid residues in the J-domain region with 121Q122N123A residues by using the site-direct mutagenesis method as previously described.[1] A plasmid pcDNA3.1-Tid1-HA containing full-length Tid1 cDNA was used to produce the Tid1-N-glycosylation mutants. The codon for asparagine in the potential N-glycosylation sites (Asn-103 and Asn-373; Tid1N103A and Tid1N373A) was mutated to a codon for Alanine, respectively. Full-length Galectin-7 cDNA was cloned into a pcDNA4/Myc plasmid and pcDNA3.1/HA plasmid for transient ectopic expression. The Galectin-7 mutants (K7A, K65A, and K99A) were mutated in the predicted ubiquitination sites, again, by site-directed mutagenesis.

Metastatic xenograft mouse model

In brief, SASM-1 or SASM-5 cells were suspended in PBS at a density of 10^5 cells/ 100 μl and then injected into BALB/c nude mice via the tail vein. The mice were euthanized 65 days after injection, and metastatic lung foci were counted by gross examination.

Co-immunoprecipitation assay

Total protein lysates were extracted with cell lysis buffer (50 mM Tris-HCl at pH 8, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, and 0.1% SDS). The lysates and anti-HA antibody-conjugated beads or anti-Myc antibody-conjugated beads were mixed and incubated in a rotary device for overnight at 4ºC. The beads were then washed gently three times with PBS buffer for 10 minutes each. The precipitated proteins were boiled in the loading buffer (4% SDS, 20% glycerol, 0.004% bromophenol blue and 0.125M Tris-HCl (pH 6.8) before immunoblotting.

Immunofluorescence staining.

Cells were plated onto poly-L-ornithine coated glass coverslips, fixed with 4% paraformaldehyde, and then washed with PBS buffer. Cells were permeabilized with 0.1% Triton X-100/PBS buffer for 10 minutes, then washed twice with PBS buffer. Consequently, the cells were blocked with blocking solution and incubated with primary antibodies (HA, Myc or TCF3). After PBS buffer washing (three times), cells were further probed with FITC or PE-tagged secondary antibodies. Fluorescence images were visualized with a fluorescence microscope. The fluorescence intensity was recorded by inverted fluorescence microscope equipped with CCD camera, and the percentage of fluorescence signal per photographed field was analyzed by Image process software (Image Pro-Plus).

Fractionations of nuclear and cytosolic lysates

Transfected 293T cells (293T-Gal-7-Myc, 293T-Tid1L-wt-HA, 293T-Tid1L-mut-HA and their corresponding vector controls) were incubated in cytosolic lysis buffer containing 20 mM Hepes pH 7.9, 10 mM KCl, 10 mM NaF, 0.5% NP-40, 2 mM $MgCl_2$ and a protease inhibitor tablet on ice for 10 min. Total protein lysates were then centrifuged at 4000 rpm for 5 min at 4 °C to separate cytosolic and nuclear fractions. The supernatant was used as a cytoplasmic fraction, and the pellet was resuspended in nuclear lysis buffer containing 150 mM NaCl, 1 mM EDTA, 20mM Tris-HCl, 0.5% NP-40, 10mM NaF and a protease inhibitor tablet. Nuclear fraction was obtained from the supernatant after centrifuging at 1, 2000 rpm.

Cell invasion and migration assays

Cell migration assay was analyzed using an 8-μm filter membrane containing upper chamber. Cell invasion assay was analyzed using BioCoat Matrigel invasion chamber (Becton-Dickinson, Bedford, MA) according to the protocol provided by the manufacturer. Briefly, 2×10^5 cells were resuspended in 300 μl of culture medium containing 0.5% FBS and then plated to the upper chamber. Subsequently, 600 μL of medium containing 10% FBS was added to the lower chamber. After 24 hours of incubation at 37°C, the non-invading cells on the upper surface of the membrane were removed from the chamber, and the invading cells on the lower surface of the membrane were fixed with 4% paraformaldehyde for 20 minutes, and then stained with 0.005% crystal violet. After two washes with water, the chambers were allowed to air

dry. The numbers of invading cells were counted using a phase-contrast microscope.

Cell anchorage-independent growth assay

Anchorage-independent growth assay was performed as followings: each well of a 6-well culture dish was coated with 1 mL bottom agar mixture (DMEM, 10% [vol/vol] FBS, 0.6% [wt/vol] agar, 1% [vol/vol] penicillin-streptomycin). After the bottom layer was solidified, 1×10^4 virus-infected cells were plated and cultured within 1 ml of top agar-medium mixture (DMEM, 10% [vol/vol] FBS, 0.3% [wt/vol] agar, 1% [vol/vol] penicillin-streptomycin), and the dishes were incubated at 37°C for 2 weeks. Cells grown on the plates were stained with 0.005% crystal violet for 1 hour, and then the cell colonies were quantitated.

Downregulation by small hairpin RNA interference (shRNAi)

The lentiviral shRNA plasmids for the knockdown of Galectin-7 protein level were obtained from the National RNAi Core Facility (Academia Sinica, Taiwan). A shRNA vector against luciferase (shLuc: 5'-GCGGTTGCCAAGAGGTTCCAT-3') was used as a negative control. The method of lentivirus production and cell infection was described in the manufacturer's protocol. Stable shRNA-expressing cells were selected by puromycin (1-2 μg/mL) treatment. The detailed target sequences are provided in below. Sh-Gal-7-1: TGCCAGCAGGTTCCATGTAAA (Clone ID: TRCN0000057393), Sh-Gal-7-2: GCTCATCATCGCGTCAGACGA (Clone ID: TRCN0000057396).

Reporter assay

The MMP-9 promoter region (–640 to +133 base pairs) was cloned by PCR amplification of genomic DNA from 293T cells and inserted into the *Sac1*/*HindIII* sites of the pGL3-Enhancer vector. Reporter constructs were co-transfected with indicated expression constructs into 293T for 24 hours before the luciferase activity was measured.

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed using EZ-ChIP^{TM} (Millipore, Billerica, MA) according to the manufacturer's instructions. In brief, the transfected 293T cells were fixed in 1% formaldehyde for 10 min and then sonicated to shear DNA. Upon sonication, 1% of these collected supernatants were used as input samples. Consequently, the supernatants were immunoprecipitated with either control IgG, or monoclonal antibody against HA-tag. The protein/DNA complexes were collected by using anti-HA antibody-conjugated agarose beads and then eluted and examined by using 2% agarose gel electrophoresis. For immunoprecipitations, the following antibodies will be used: anti-HA and anti-IgG. PCR determined the presence of an amplified MMP9 promoter sequence. The primer sets used for the PCR were listed as following: hMMP-9-1: Forward: 5'GGAGGTGGTGTAAGCCCTTT3'; Reverse: 5' AGGGC-AGAGGTGTCTGACTG3'; hMMP-9-2: Forward: 5'TAAGACATTTGCCCG-AGGTC3'; Reverse: 5'CCTCTTTTTCCCTCCCTGAC3'; hMMP-9-3: Forward: 5'TAATTGGGGCTGGAGATTTG3'; Reverse: 5'CCTGCCAAAAGACCATG-ATT3',

References

1 Chen CY, Chiou SH, Huang CY, Jan CI, Lin SC, Hu WY *et al*. Tid1 functions as a tumour suppressor in head and neck squamous cell carcinoma. The Journal of pathology 2009; 219: 347-355.

2 Liao KA, Tsay YG, Huang LC, Huang HY, Li CF, Wu TF. Search for the tumor-associated proteins of oral squamous cell carcinoma collected in Taiwan using proteomics strategy. J Proteome Res 2011; 10: 2347-2358.