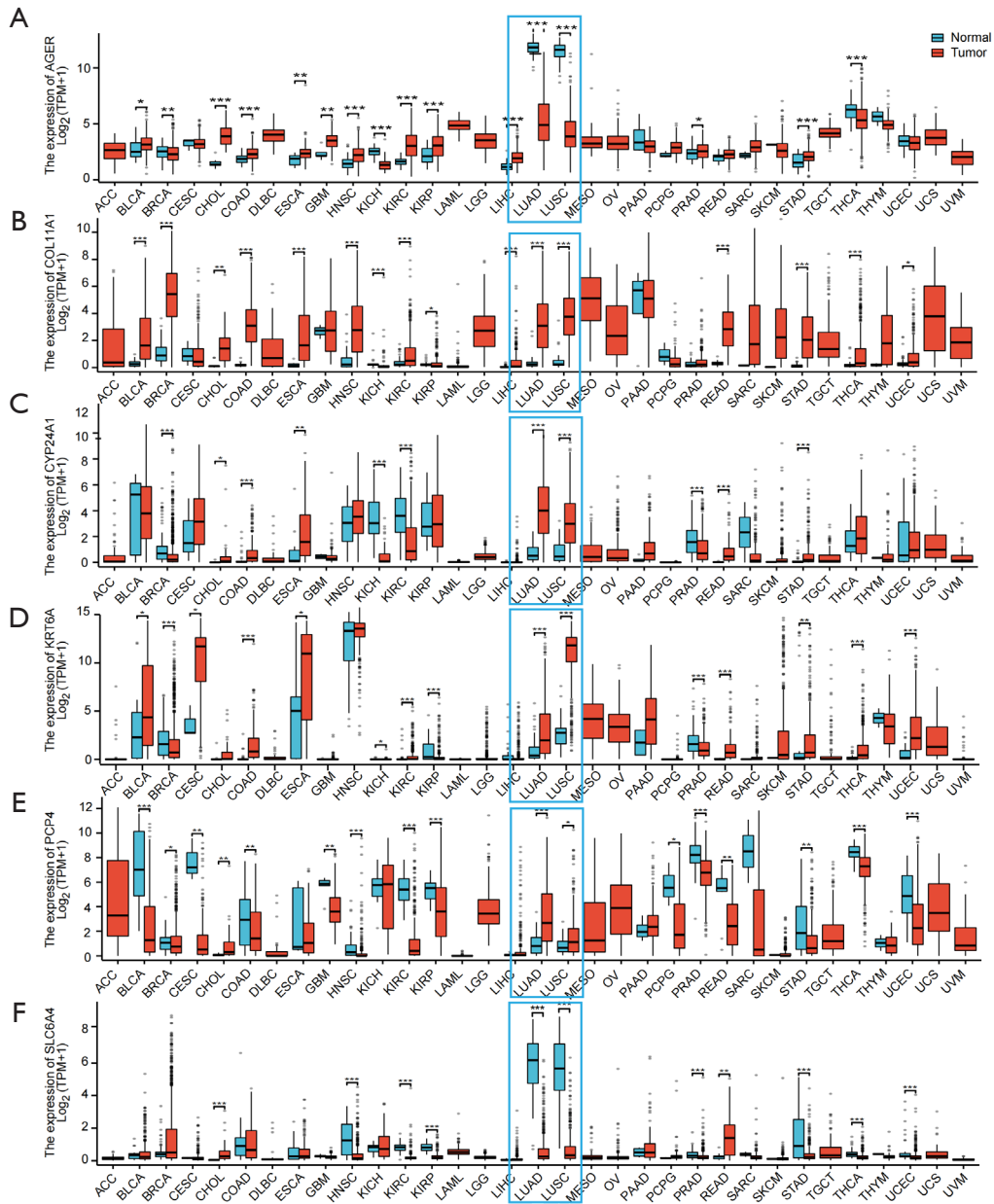
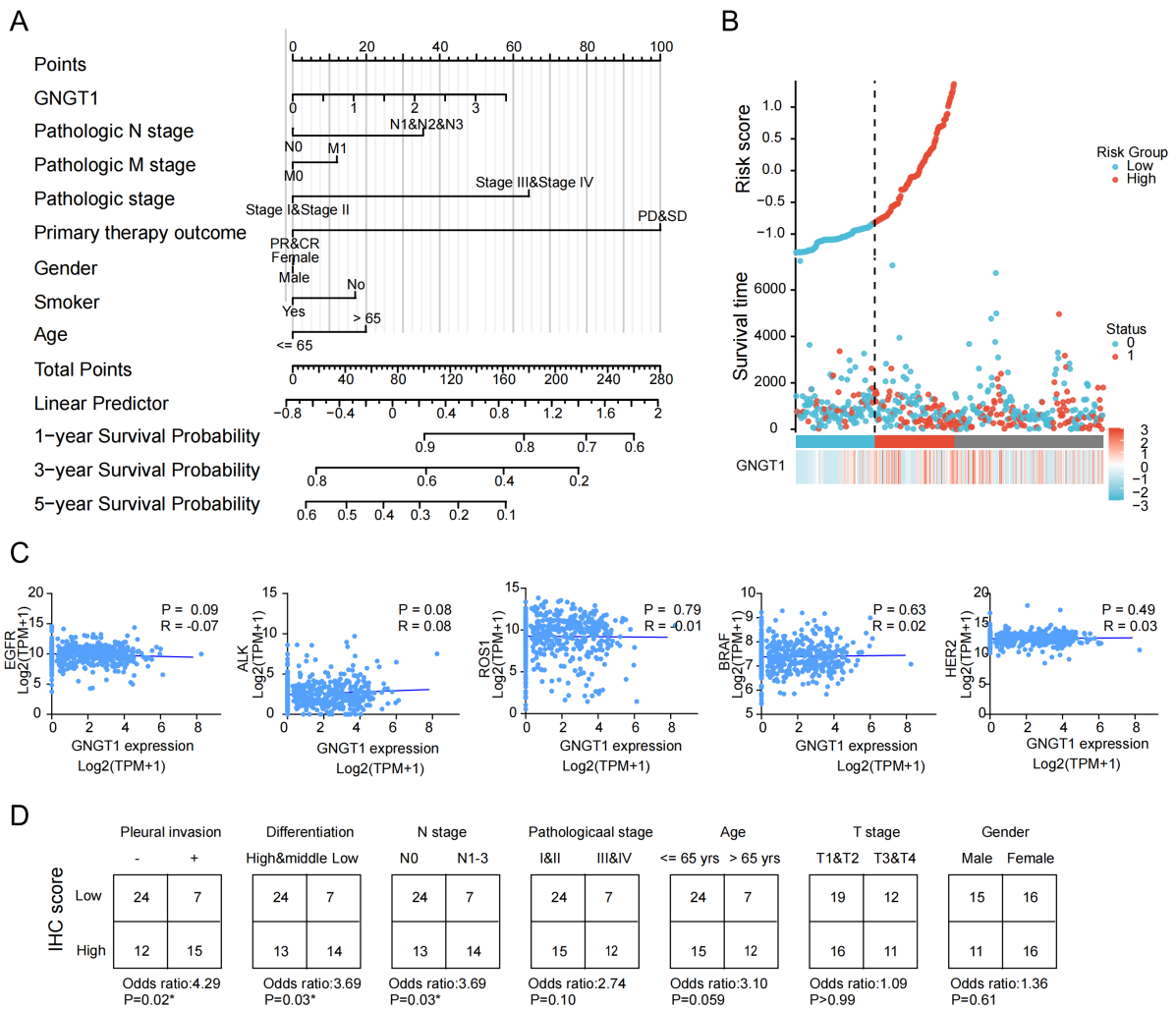


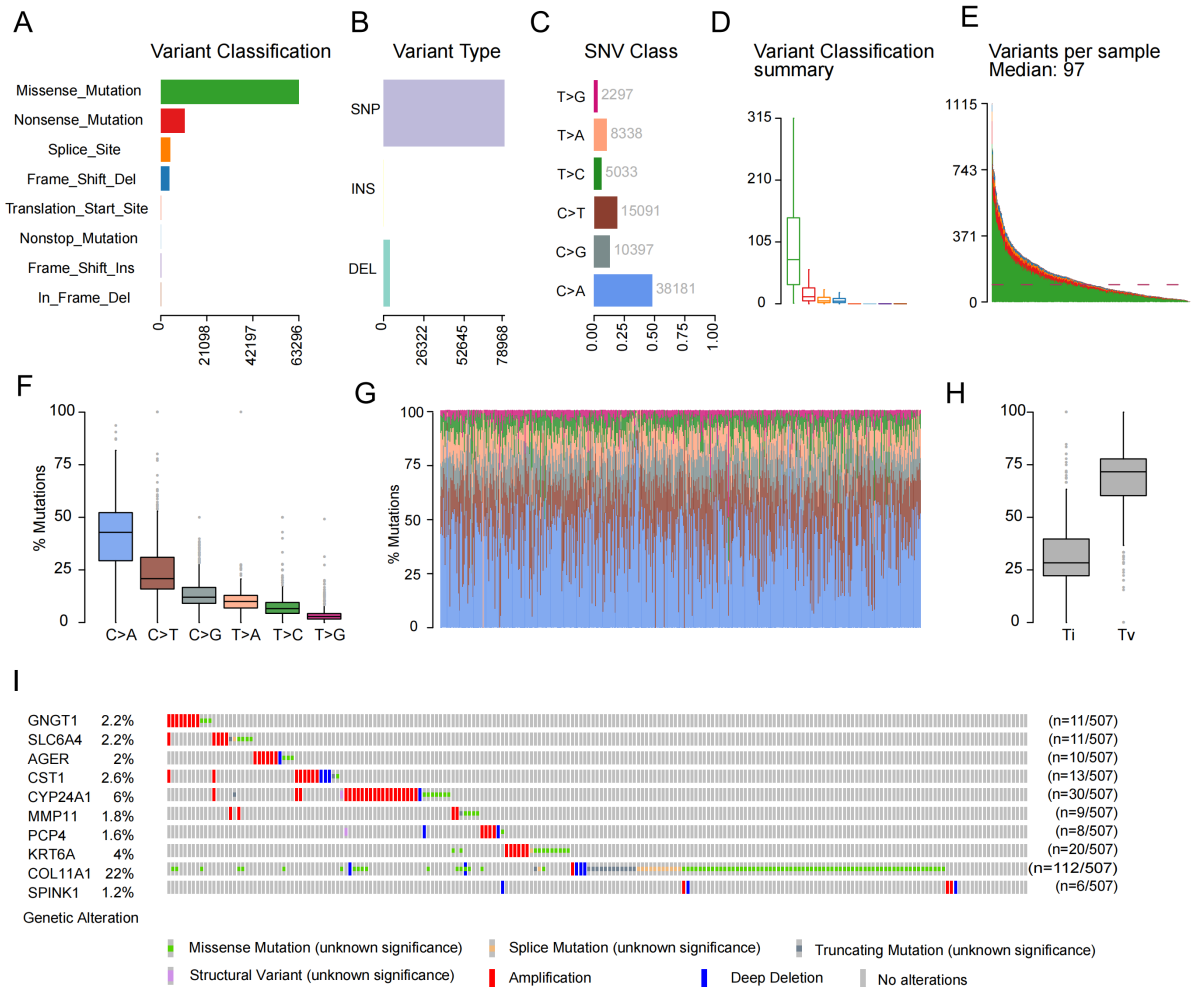
**Figure S1** Kaplan-Meier analysis and expression of partial hub genes in LUAD. Three genes (CST1, MMP11, SPINK1) related with LUAD but without impact on survival. (B) Expression of CST1, MMP11, SPINK1 in LUAD. \*,  $0.01 < P < 0.05$ . LUAD, lung adenocarcinoma.



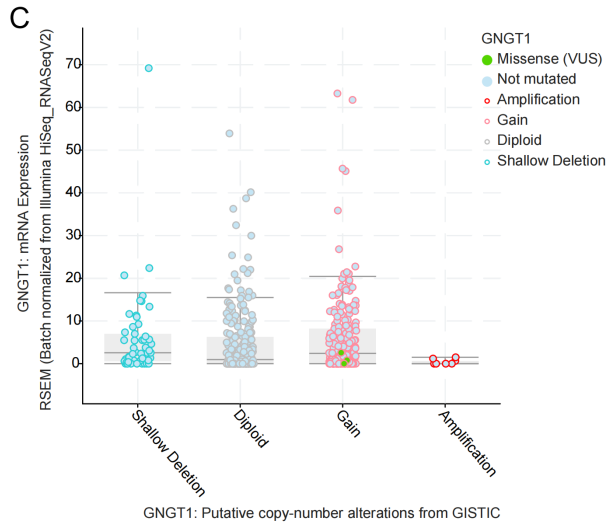
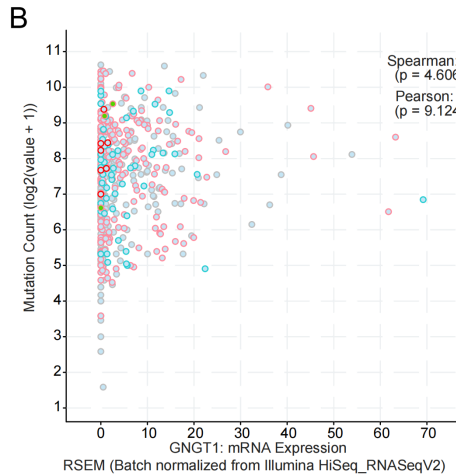
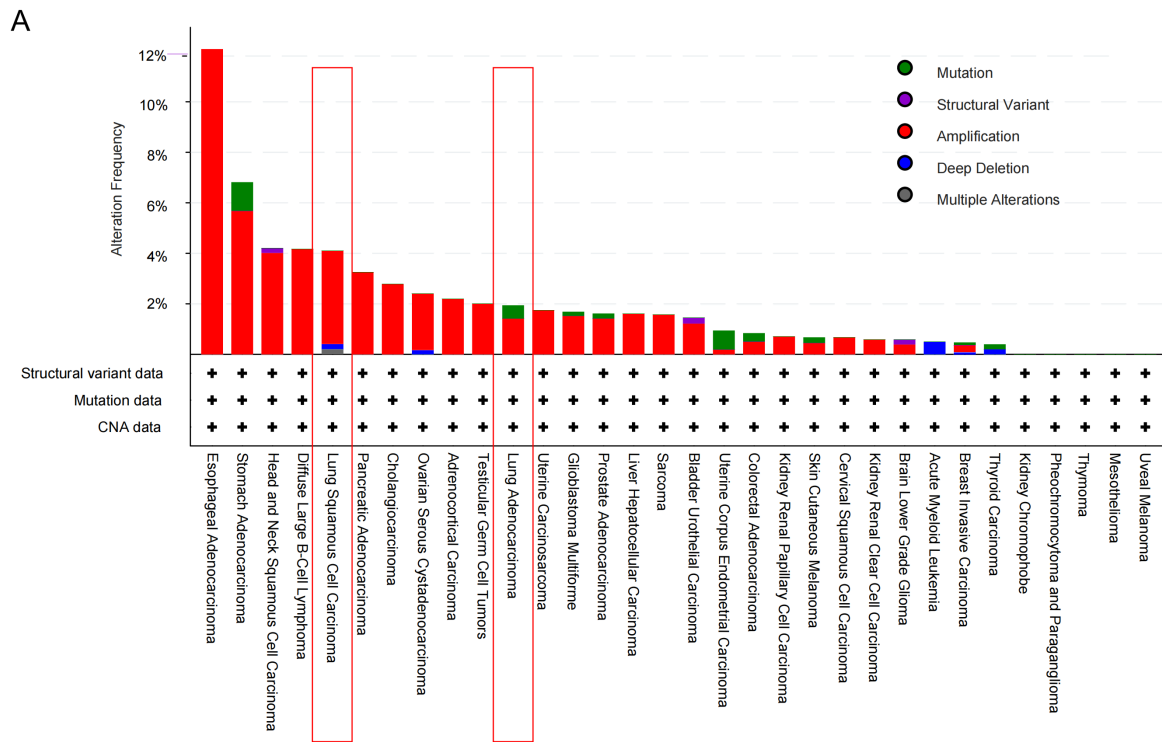
**Figure S2** Expression levels of various cancers of other six genes in TCGA database. (A) AGER. (B) COL11A1. (C) CYP24A1. (D) KRT6A. (E) PCP4. (F) SLC6A4. TCGA, The Cancer Genome Atlas.



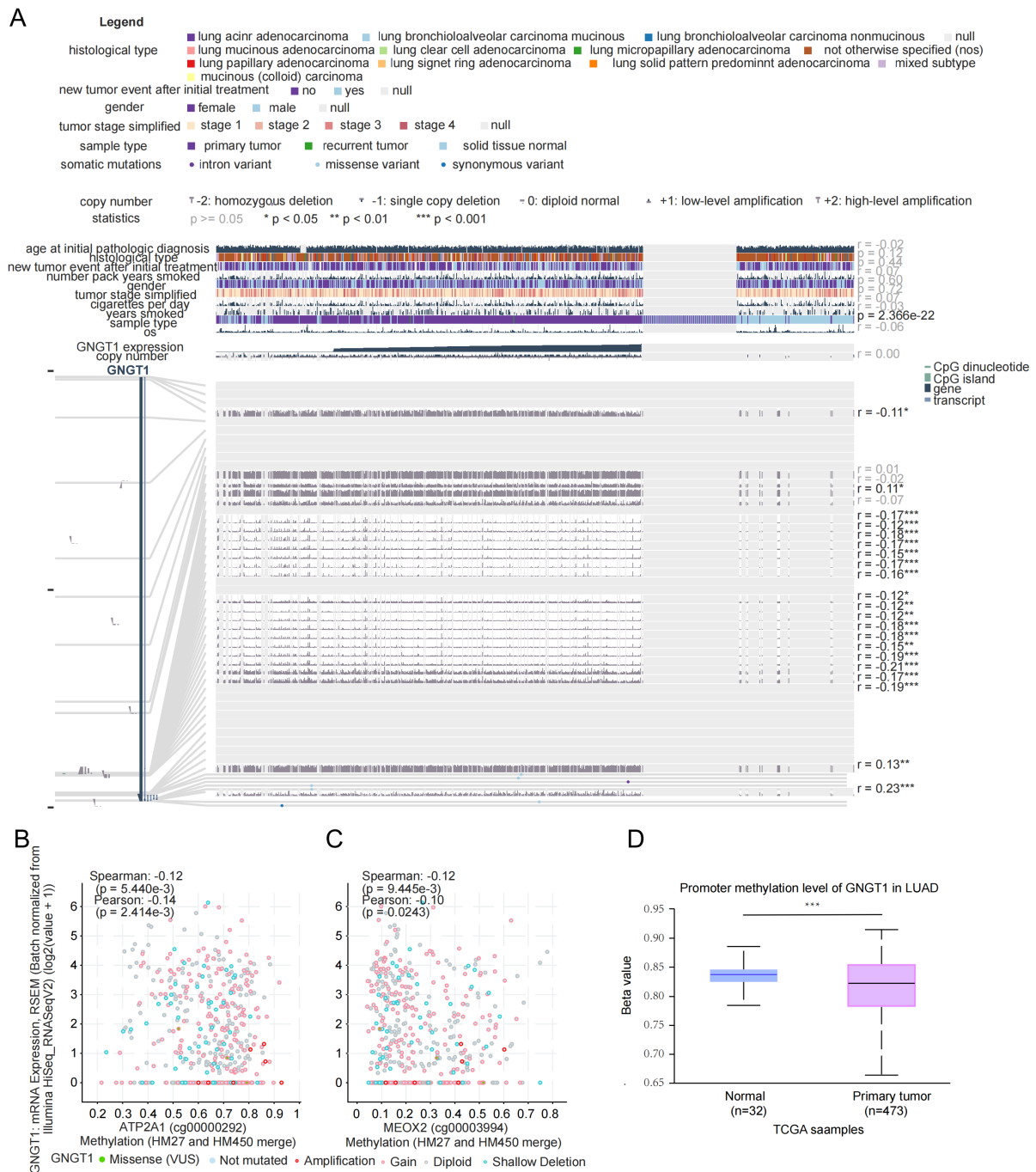
**Figure S3** The correlation between GNGT1 expression and clinical pathological features and standard biomarkers. (A) Nomographic chart of GNGT1 and clinical pathological features in TCGA. (B) Risk factor map of different GNGT1 gene expression in LUAD patients in the TCGA database. (C) The relationship of GNGT1 and standard biomarkers. (D) Analysis data sheet of GNGT1 IHC grade and clinical pathological features. TCGA, The Cancer Genome Atlas; IHC, immunohistochemistry.



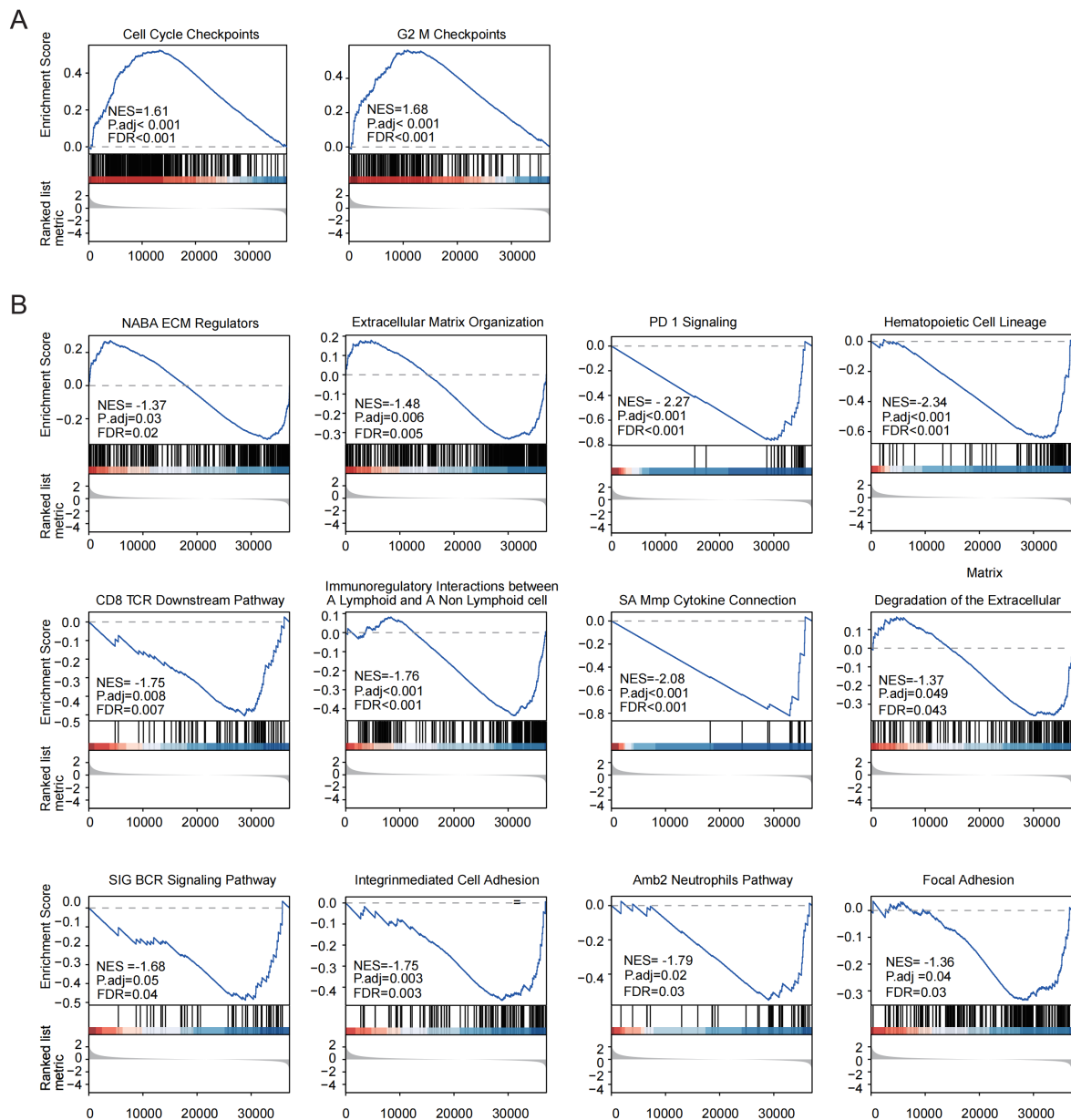
**Figure S4** Genetic alteration and mutation analyses in LUAD. (A-E) Gene alterations were identified through OncoVar. (F,G) Mutated bases in LUAD were visualized by OncoVar. (H) Ti and Tv were identified in LUAD. (I) Gene alterations burden of ten hubgenes were identified through cBioportal in LUAD. Ti, transitions; Tv, transversions; LUAD, lung adenocarcinoma.



**Figure S5** Gene alteration analysis of GNGT1. (A) The genetic alteration status of GNGT1 detected in various type of cancer. (B) The mRNA expression of GNGT1 is positive correlation with mutation count. (C) The relationship between putative copy-number alterations and mRNA expression of GNGT1.

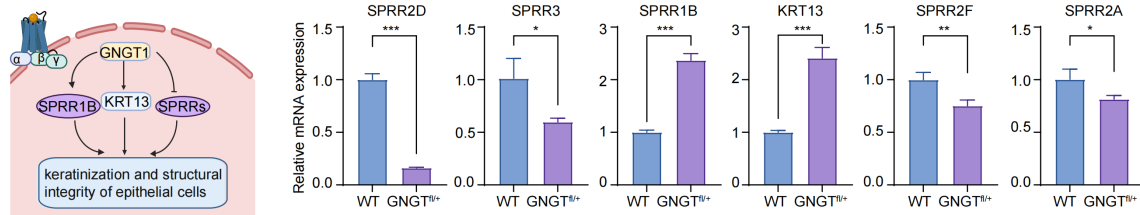


**Figure S6** Methylation levels analysis of GNGT1 in LUAD. (A) The methylation sites in GNGT1 promoters were evaluated by MEXPRESS. (B,C) The relationship between GNGT1 expression and methylation in (B) ATP2A1 (cg00000292) and (C) MEOX2 (cg00003994) sites. (D) GNGT1 promoter methylation level is decreased in LUAD compare with normal tissues. LUAD, lung adenocarcinoma.

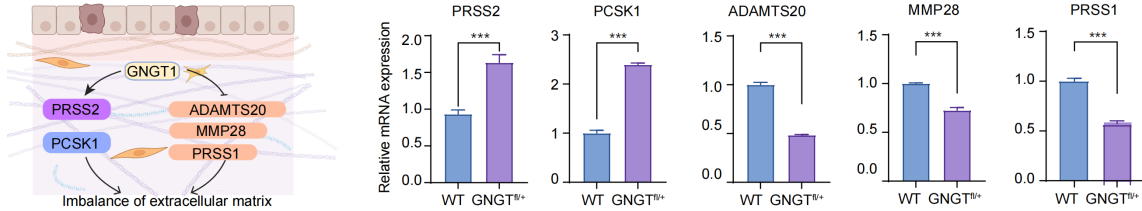


**Figure S7** Part of gene set enrichment analysis of GNGT1 high group versus low group in LUAD. (A) Up-regulated gene enrichment in part of the pathway. (B) Down-regulated gene enrichment in part of the pathway. LUAD, lung adenocarcinoma.

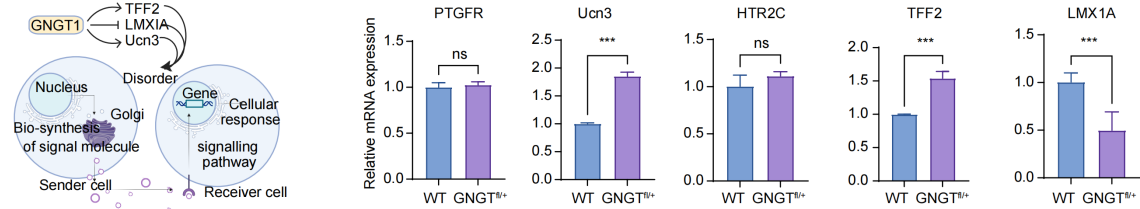
## A Structural integrity of epithelial cells



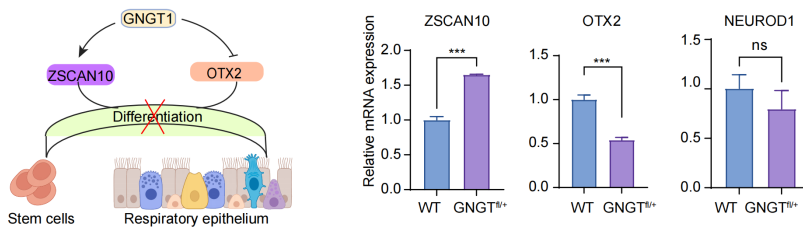
## B Extracellular matrix organization



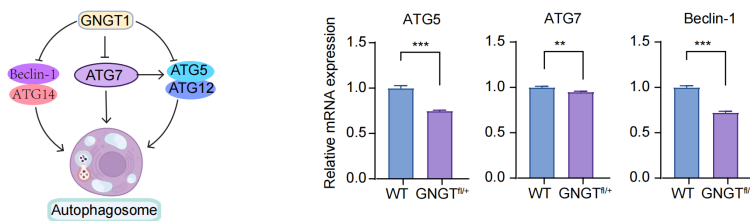
## C Cell-Cell communication



## D Transcriptional regulation and differentiation of pluripotent stem cells



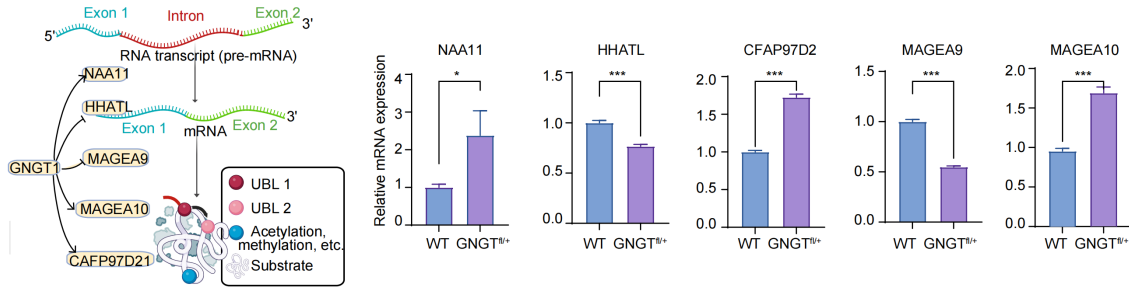
## E Autophagy



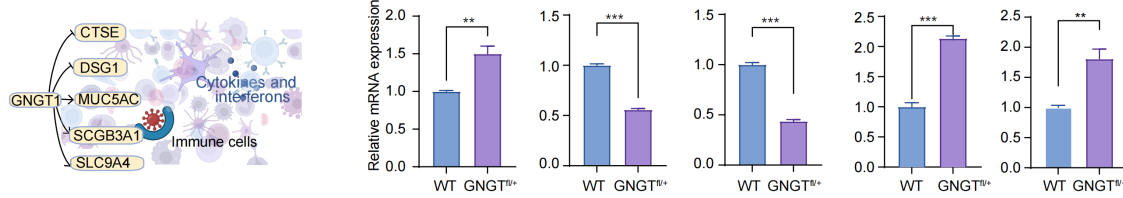
**Figure S8** RT-qPCR validation of DEGs level in lung tissues of wild type and GNGT1<sup>fl/+</sup> mouse models. (A) DEGs related to structural integrity of epithelial cells. (B) Extracellular matrix organization. (C) Cell-Cell communication. (D) Transcriptional regulation and differentiation of pluripotent stem cells. (E) Regulation of autophagy. RT-qPCR, real time quantitative polymerase chain reaction; DEGs, differentially expressed genes.



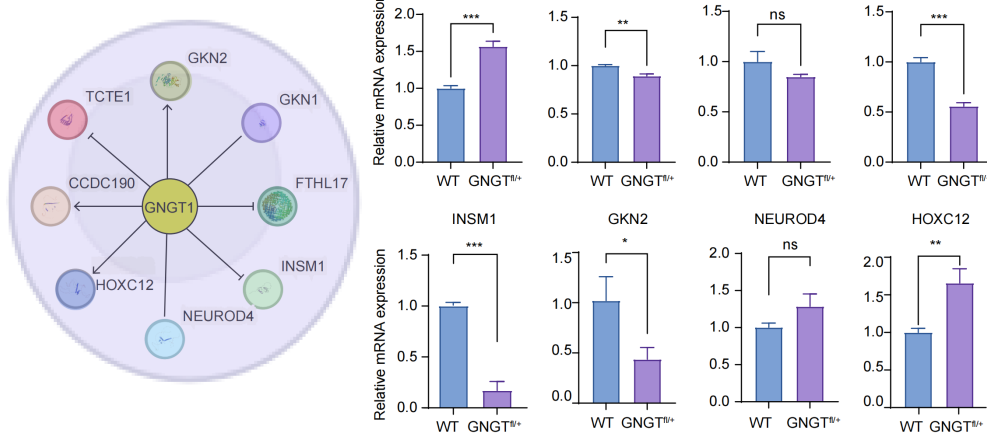
## A Transcriptional regulation and posttranscriptional modification



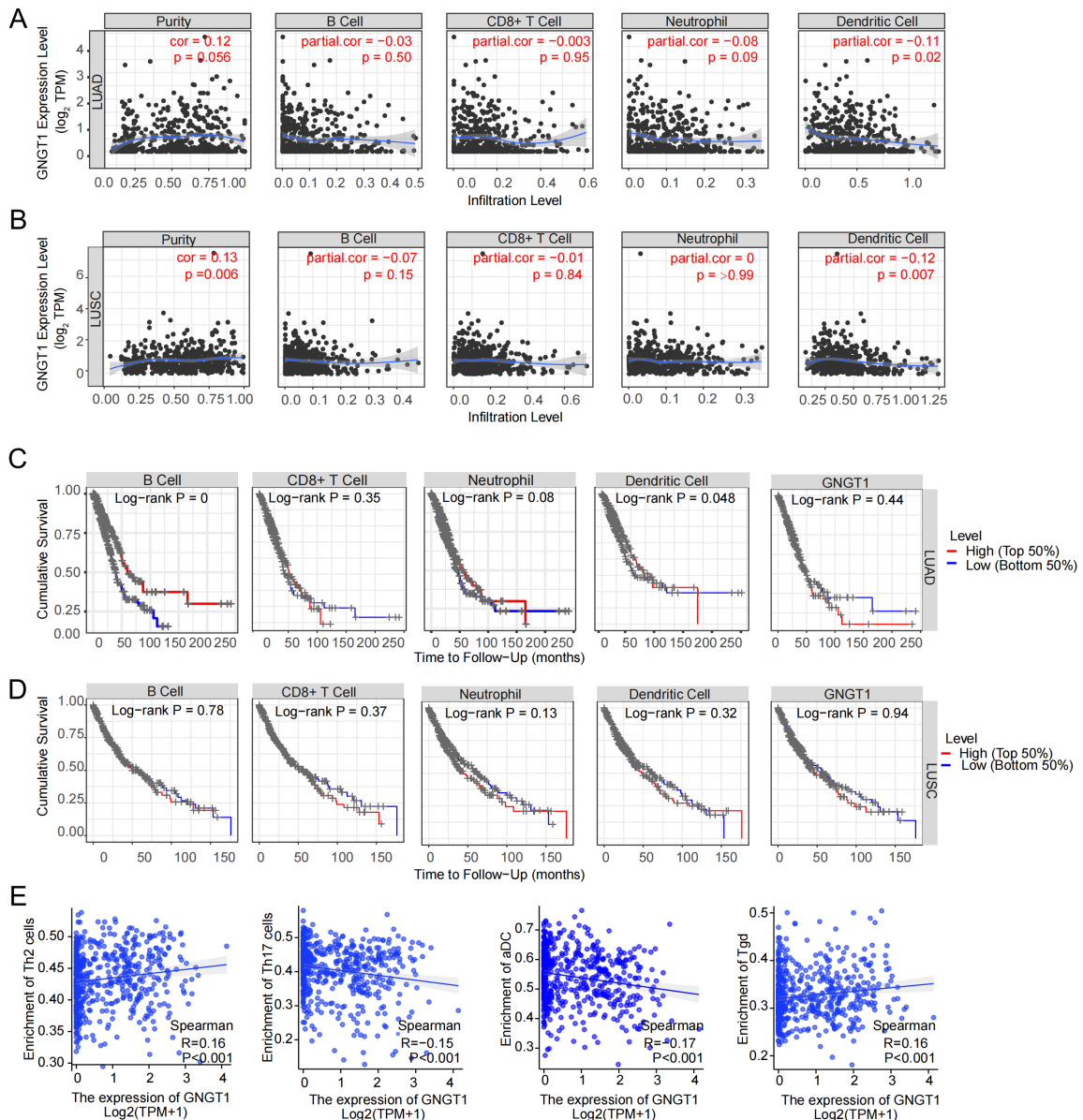
## B Immune regulation



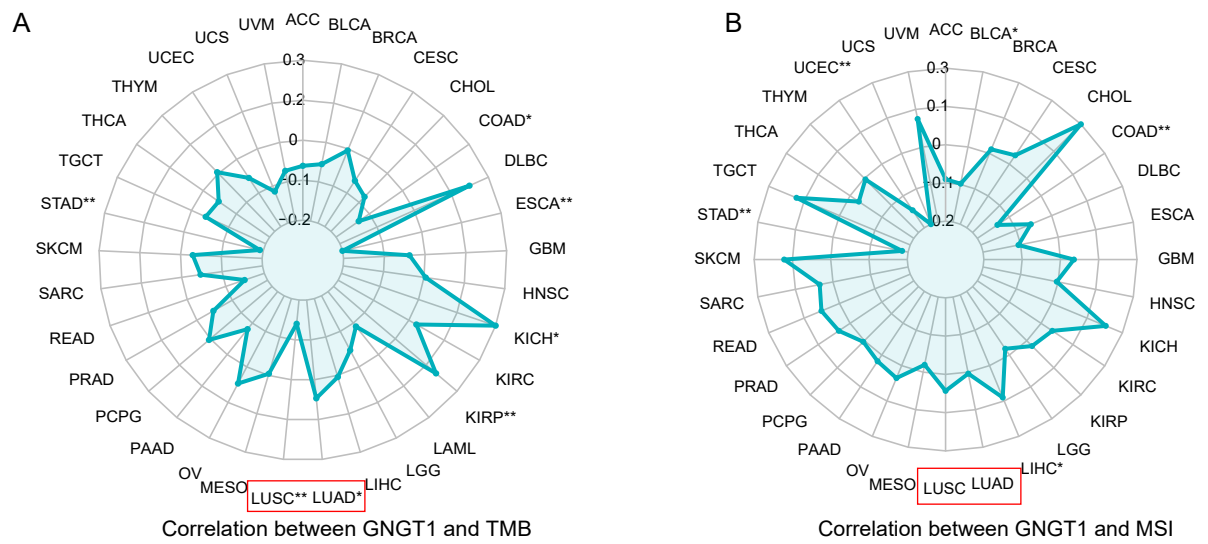
## C Other DEGs expression



**Figure S9** RT-qPCR validation of DEGs level in lung tissues of wild type and GNGT1<sup>fl/+</sup> mouse models. (A) DEGs related to transcriptional regulation and posttranscriptional modification. (B) Immune regulation. (C) Other top DEGs mRNA expression. RT-qPCR, real time quantitative polymerase chain reaction; DEGs, differentially expressed genes.



**Figure S10** Immune infiltration analysis in LUAD and the association with GNGT1 expression. (A,B) The correlation between GNGT1 expression and different immune cell types by TIMER database in LUAD (A) and LUSC (B) by TIMER. (C,D) Immune cell-related survival analysis by TIMER in LUAD (C) and LUSC (D). (E) The correlation between GNGT1 expression and different immune cell types by R software. Th, T helper; aDC, activated dendritic cells; Tgd, T gamma delta cells; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.



**Figure S11** TMB and MSI analysis in pan-cancer and the association with GNGT1 expression. (A) The correlation between GNGT1 expression and TMB. (B) The correlation between GNGT1 expression and MSI. TMB, tumor mutation burden; MSI, microsatellite instability.

**Table S1** The baseline characteristics and GNGT1 IHC expression score of enrolled patients

IHC of GNGT1	T stage	Location	Age (years)	Gender	Final patient stage
Low	T1	Upper lobe of right lung	60	Male	pT1aN0M0 IA stage
Low	T1	Upper lobe of right lung	60	Female	pT1bN0M0 IA2 stage
Low	T1	Upper lobe of right lung	70	Male	pT1aN0M0 IA2 stage
Low	T1	Upper lobe of right lung	61	Male	pT1aN0M0 IA stage
Low	T1	Upper lobe of right lung	49	Female	pT1bN0M0 IA2 stage
Low	T1	Upper lobe of right lung	63	Male	pT1bN0M0 IA2 stage
High	T1	Upper lobe of right lung	57	Female	pT1bN0M0 IA2 stage
Low	T1	Upper lobe of right lung	52	Male	pT1aN0M0 IA stage
Low	T1	Upper lobe of right lung	46	Female	pT1aN0M0 IA stage
Low	T1	Middle lobe of right lung	61	Female	T1aN0M0 IA stage
Low	T1	Upper lobe of right lung	57	Male	pT1bN0M0 IA2 stage
High	T1	Inferior lobe of right lung	57	Male	pT1bN0M0 IA2 stage
High	T1	Upper lobe of right lung	43	Female	pT1aN0M0 IA stage
Low	T1	Upper lobe of left lung	62	Female	pT1bN0M0 IA2 stage
Low	T1	Inferior lobe of left lung	54	Female	pT1bN0M0 IA2 stage
Low	T1	Upper lobe of right lung	49	Female	pT1bN0M0 IA2 stage
Low	T2	Inferior lobe of right lung	63	Female	pT2bN2M0 IIIA stage
High	T2	Upper lobe of right lung	69	Female	T2aN2M0 IIIA stage
High	T2	Inferior lobe of right lung	82	Female	pT2bN1M0 IIB stage
High	T2	Upper lobe of right lung	54	Female	pT2aN0M0 IB stage
Low	T2	Inferior lobe of right lung	61	Female	pT2N0M0 IB stage
High	T2	Upper lobe of left lung	76	Male	pT2aN0M0 IB stage
Low	T2	Upper lobe of left lung	59	Female	pT2aN0M0 IB stage
Low	T2	Upper lobe of left lung	59	Female	pT2aN0M0 IB stage
High	T2	Upper lobe of left lung	70	Male	pT2aN0M0 IB stage
High	T2	Upper lobe of right lung	62	Male	pT2aN0M0 IB stage
High	T2	Inferior lobe of right lung	54	Male	pT2aN0M0 IB stage
High	T2	Inferior lobe of left lung	88	Male	pT2aN0M0 IB stage
Low	T2	Upper lobe of right lung	79	Male	pT2bN1M0 IIB stage
High	T2	Middle lobe of right lung	51	Female	pT2aN2M0 IIIA stage
High	T2	Upper lobe of left lung	72	Female	pT2aN0M0 IB stage
High	T2	Upper lobe of left lung	67	Female	pT2bN2M0 IIIA stage
High	T2	Inferior lobe of left lung	69	Male	pT2bN0M0 IIA stage
High	T2	Inferior lobe of left lung	77	Female	pT2aN1M0 IIB stage
High	T2	Inferior lobe of left lung	51	Female	pT2aN2M0 IIIA stage
Low	T3	Inferior lobe of right lung	65	Female	pT3N0M0 IIB stage
Low	T3	Upper lobe of right lung	55	Male	pT3N0M1b IV stage
Low	T3	Inferior lobe of right lung	50	Female	pT3N2M0 IIIB stage
Low	T3	Upper lobe of right lung	67	Male	pT3N0M0 IIB stage
Low	T3	Inferior lobe of left lung	55	Female	pT3N1M0 IIIA stage
High	T3	Inferior lobe of right lung	68	Female	pT3N0M0 IIB stage
High	T3	Upper lobe of left lung	47	Male	pT3N2M0 IIIB stage
High	T3	Inferior lobe of right lung	64	Female	pT3N2M0 IIIB stage
High	T3	Inferior lobe of right lung	69	Female	pT3N0M0 IIB stage
High	T3	Inferior lobe of right lung	52	Female	pT3N1M0 IIIA stage
High	T3	Inferior lobe of left lung	66	Male	pT3N0M0 IIB stage
Low	T3	Upper lobe of right lung	64	Male	pT3N0M0 IIB stage
Low	T3	Inferior lobe of right lung	58	Female	pT3NxM1a IVA stage
Low	T3	Upper lobe of right lung	68	Male	pT3N0M0 IIB stage
Low	T3	Upper lobe of right lung	68	Male	pT3N0M0 IIB stage
Low	T3	Upper lobe of left lung	64	Male	pT3N0M0 IIB stage
High	T3	Inferior lobe of left lung	58	Female	pT3N1M0 IIIA stage
High	T3	Inferior lobe of right lung	66	Female	pT3N1M0 IIIA stage
Low	T4	Upper lobe of right lung	72	Male	pT4N2M0 IIIB stage
High	T4	Upper lobe of right lung	67	Male	pT4N1M0 IIIA stage
Low	T4	Upper and inferior lobe of right lung	69	Male	pT4NxM0 IIIA stage
High	T4	Inferior lobe of left lung	48	Male	pT4N2M0 IIIB stage
High	T4	Inferior lobe of right lung	40	Female	pT4N1M0 IIIA stage

IHC, immunohistochemistry.

**Table S2** The primer sequence of RT-qPCR

Gene	Primer	Sequence (5'-3')
hGAPDH	Forward	GACAGTCAGCCGCATCTTCT;
	Reverse	GCGCCCAATACGACCAAATC;
hNGT1	Forward	GTTTCCAAATGTTGTGAAGAAGTA;
	Reverse	GAAGGGATTTTTGTCCTCTG;
mGAPDH	Forward	GAAGGTCGGTGTGAACGGAT;
	Reverse	ACTGTGCCGTTGAATTTGCC;
mNGT1	Forward	TGTCTTCTGGAATCCCCTTCAC;
	Reverse	AGAAGGAAGTGACTGAGAG;
mFGB	Forward	ATGGCTGCTGCTGCTATTGTG;
	Reverse	CGTCGGTTCATCGTAGTCATCATC;
mALB	Forward	AAGACGTGTGTTGCCGATGA;
	Reverse	GGCCTTCAAATGGTGGCAG;
mCTAG2	Forward	TGAAGGCAGCTCGCATCTTA;
	Reverse	ACAGGGACCCCTTGAGTTGAG;
mFGF4	Forward	CCGGTATGTTGATGGCCCTC;
	Reverse	ACCTTCATGGTAGGCGACAC;
mAFP	Forward	TGCGTGACGGAGAAGAATGT;
	Reverse	ACACCCATCGCCAGAGTTTT;
mHP	Forward	ATCGCTGCCGACAGTTCTAC;
	Reverse	ATCTTGGCCTGCCTCACATT;
mCDH2	Forward	TCATTGTAGCCAACCTAACTGTCAC;
	Reverse	GTCTCCACCACTGATTCTGTATGC;
mE-cadherin	Forward	TCTGATCCTGCTGCTCCTACTG;
	Reverse	CTTCTTCTCCACCTCCTTCTTCATC;
mATG7	Forward	CGGTGGCTTCTACTGTTATTGC;
	Reverse	CGGCTCCCTGCTGCTTGG;
mATG5	Forward	AAGCAGCTCTGGATGGGACTG;
	Reverse	CCGCTCCGTCGTGGTCTG;
mBecn1	Forward	CAGTACCAGCGGGAGTATAGTGAG;
	Reverse	TGGAAGGTGGCATTGAAGACATTG;
mADAMTS20	Forward	GGGTCCCTGGGAAGTTCGTTT;
	Reverse	GTCACCACTTCGTAGGAGGC;
mC-MYC	Forward	TCTATCACCAGCAACAGCAGAGC;
	Reverse	CGACCGCAACATAGGATGGAGAG;
mKLF4	Forward	CAAGTTTGTGCTGAAGGCGTCTC;
	Reverse	TGCTAACACTGATGACCGAAGGG;
mCD44	Forward	TCAAGTGCGAACCAGGACAGTG;
	Reverse	GAATCAGAGCCAGTGCCAGGAG;
mCD133	Forward	CAACAAATGCGGCGGAGAGATG;
	Reverse	CAGACAAATCACCAGGAGGGAGAG;
mNanog	Forward	GGCTCAGCACCAGTGGAGTATC;
	Reverse	TCCAGATGCGTTCACCAGATAGC;
mOCT4	Forward	ACTAGCATTGAGAACCGTGTGAGG;
	Reverse	CAAGCTGATTGGCGATGTGAGTG;
mSOX2	Forward	CATGACCAGCTCGCAGACCTAC;
	Reverse	GCCTCGGACTTGACCACAGAG;
mPADI4	Forward	GTCCCATCTCTGTGTGTCCTC;
	Reverse	TGTGGAACCTCCGTGGTAACT;
mMPO	Forward	AACACATACCCCGAGACTT;
mMPO R	Reverse	GGCAGACTCCCAACCTCTA;
mElane F	Forward	ATTCCATTATCCGAAGCCATA;
mElane R	Reverse	CAGACAGGTCCTAGTTGGTCC;
mHMGB1	Forward	AGGCTGACAAGGCTCGTTATGAAAG;
	Reverse	GGGCGGTACTCAGAACAGAACAAAG;
mCXCL15	Forward	TCCGTCCCTGTGACTCAAGAG;
	Reverse	GCCAACAGTAGCCTTCACCCATG;
mIL-6	Forward	TCTTGGGACTGATGCTGGTGAC;
	Reverse	TCTGTTGGGAGTGGTATCCTCTGTG;
mMMP9	Forward	ACGGCAACGGAGAAGGCAAAC;
	Reverse	GTCCACTCGGTTAGGGCAGAAG;
mCXCL4	Forward	CTGGTCCCGAAGAAAGCGATGG;
	Reverse	AGGCTGGTGTGCTTAAGATGG;
mKRAS	Forward	TCTGAAGATGTGCCTATGGTCCTG;
	Reverse	CCCCTAACTCCTTGCTAACTCCTG;
mKEAP1	Forward	GCTCAACCGCTTGTGTATGC;
	Reverse	CATCCGCCACTCATTCTCTCTG;
mNFE2L2	Forward	GTTGCCACCGCCAGGACTAC;
	Reverse	AACTTGTACCGCCTCGTCTGG;
mSTK11	Forward	ACACCTTCATCCACCGCATCG;
	Reverse	GTCCAGCACCTCCTTCACCTTG;
mB2M	Forward	GCTCGGTGACCCTGGTCTTTC;
	Reverse	AGTATGTTCCGGCTTCCCATTCTCC;
mPTEN	Forward	TGAAGACCATAACCCACCACAGC;
	Reverse	TCATTACACCAGTCCGTCCCTTTC;

RT-qPCR, real time quantitative polymerase chain reaction.

**Table S3** The function of seven hub genes by online database Metascape

Gene symbol	Description	Biological process (GO)	Protein function (protein atlas)	Canonical pathways	Hallmark gene sets
GNGT1	G-protein subunit gamma transducin 1	GO:0010659 cardiac muscle cell apoptotic process; GO:0010658 striated muscle cell apoptotic process; GO:0010657 muscle cell apoptotic process	RAS pathway related proteins; Predicted intracellular proteins	(M204) PID RHODOPSIN PATHWAY	(M5923) PI3K; AKT; mTOR SIGNALING
KRT6A	keratin 6A	GO:2000536 negative regulation of entry of bacterium into host cell; GO:2000535 regulation of entry of bacterium into host cell; GO:0052372 modulation by symbiont of entry into host	Human disease related genes; Congenital malformations: Congenital malformations of skin; Predicted intracellular proteins; Disease related genes		
CYP24A1	cytochrome P450 family 24 subfamily A member 1	GO:0070561 vitamin D receptor signaling pathway; GO:0042369 vitaminD catabolic process; GO:0042363 fat-soluble vitamin catabolic process	Disease related genes; Enzymes; Potential drug targets; Predicted intracellular proteins; ENZYME proteins: Oxidoreductases; Human disease related genes: Congenital disorders of metabolism; Other congenital disorders of metabolism		
COL11A1	collagen type XI alpha 1 chain	GO:0035989 tendon development; GO:0050910 detection of mechanical stimulus involved in sensory perception of sound	Disease related genes; Human disease related genes: Congenital malformations; Other congenital malformations; Human disease related genes: Congenital malformations: Congenital malformations of the musculoskeletal system; Cancer-related genes: Candidate cancer biomarkers; Predicted secreted proteins; Predicted intracellular proteins; Human disease related genes: Nervous system diseases; Eye disease	(M3005) NABA COLLAGENS; (M198) PID SYNDECAN 1 PATHWAY; (M18) PID INTEGRIN1 PATHWAY	(M5942) HALLMARK UV RESPONSE DN; (M5930) HALLMARK EPITHELIAL MESENCHYMAL TRANSITION
PCP4	Purkinje cell protein 4	GO:0099004 calmodulin dependent kinase signaling pathway; GO:0045666 positive regulation of neuron differentiation; GO:0045664 regulation of neuron differentiation	Predicted intracellular proteins		(M5907) ESTROGEN RESPONSE LATE; (M5953) KRAS SIGNALING UP
AGER	advanced glycosylation end-product specific receptor	GO:1904470 regulation of endothelin production; GO:1904472 positive regulation of endothelin production; GO:1900453 negative regulation of long-term synaptic depression	Transporters: Accessory Factors Involved in Transport; Predicted secreted proteins; Cancer-related genes: Candidate cancer biomarkers	(M159) PID AMB2 NEUTROPHILS PATHWAY	(M5947) HALLMARK IL2 STAT5 SIGNALING
SLC6A4	solute carrier family6 member 4	GO:0014064 positive regulation of serotonin secretion; GO:0090067 regulation of thalamus size; GO:0032227 negative regulation of synaptic transmission, dopaminergic	FDA approved drug targets: Small molecule drugs; Human disease related genes: Other diseases: Mental and behavioural disorders; Transporters: Electrochemical potential-driven transporters		

RAS, rat sarcoma; FDA, Food and Drug Administration.

**Table S4** Multivariate Cox regression analysis of GNGT1 in TCGA LUAD cohort

Factor	Coef	Exp(coef)	Se(coef)	z	Pr(> z )	Lower 0.95	Upper 0.95
GNGT1	0.387	1.47	0.16	2.33	0.02*	1.06	2.02
T	0.15	1.16	0.10	1.48	0.14	0.95	1.42
N	0.08	1.09	0.01	0.85	0.39	0.90	1.31
M	-0.07	0.93	0.01	-0.74	0.46	0.76	1.13
Stage	0.41	1.50	0.01	4.09	<0.001***	1.24	1.83
Radiation therapy	-0.44	0.65	0.21	-2.108	0.04*	0.43	0.97

\*, P<0.05; \*\*\*, P<0.01. TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma.

**Table S5** Univariable Cox regression analysis of GNGT1 in TCGA LUAD cohort

Factor	Coef	Exp(coef)	Se(coef)	z	Pr(> z )	Lower 0.95	Upper 0.95
GNGT1	0.33	1.38	0.15	2.17	0.03*	1.03	1.87
T	0.43	1.53	0.09	4.83	<0.001***	1.29	1.83
N	0.35	1.42	0.07	5.21	<0.001***	1.24	1.61
M	-0.02	0.98	0.09	-0.27	0.79	0.82	1.16
Stage	0.53	1.70	0.07	7.38	<0.001***	1.47	1.95
Radiation therapy	-0.73	0.48	0.19	-3.77	<0.001***	0.33	0.70

\*, P<0.05; \*\*\*, P<0.01. TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma.