

GTSE1, CDC20, PCNA and MCM6 Synergistically Effect in Cell Cycle and Indicate Poor Prognosis in Liver Cancer

Supplementary Materials

YONGCHANG ZHENG^{1,*}, YUE SHI^{1,*}, SI YU^{1,*}, YUANYUAN HAN², KAI KANG¹, SHUNDA DU¹, HUAJIAN GU³, XINTING SANG¹, YANG CHEN^{4,#} AND JINGYU WANG^{4,5}
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¹Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China;

²Center of Tree Shrew Germplasm Resources, Institute of Medical Biology, The Chinese Academy of Medical Sciences and Peking Union Medical College, Yunnan Key Laboratory of Vaccine Research and Development on Severe Infectious Diseases, Kunming, Yunnan 650118, P.R. China.

³Department of Hepatobiliary Surgery, Affiliated Hospital of Guizhou Medical University, No. 28, Guiyi Street, Yunyan District, 550004 Guiyang P. R. China.

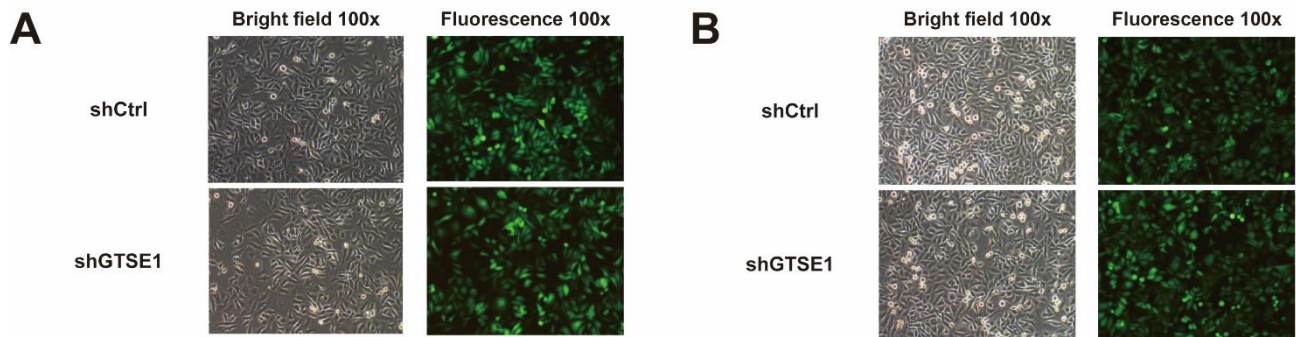
⁴MOE Key Laboratory of Bioinformatics, Center for Synthetic and Systems Biology, Bioinformatics Division, BNRist, Department of Automation, Tsinghua University, Beijing 100084, China.

⁵Department of Basic Medical Science, Tsinghua-Peking Joint Center for Life Sciences, School of Medicine, Tsinghua University, Beijing 100084, China.

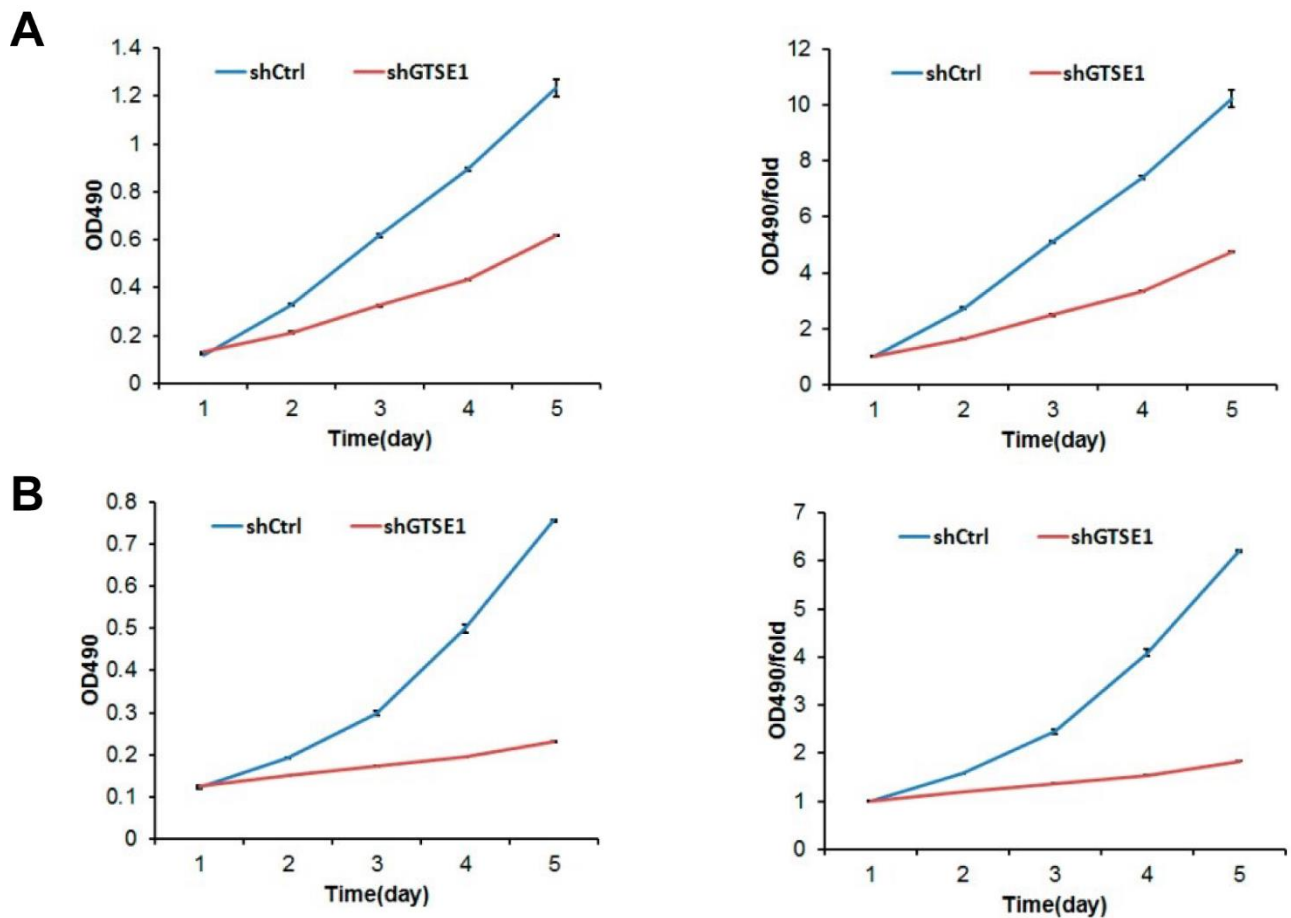
*These authors contributed equally to this work.

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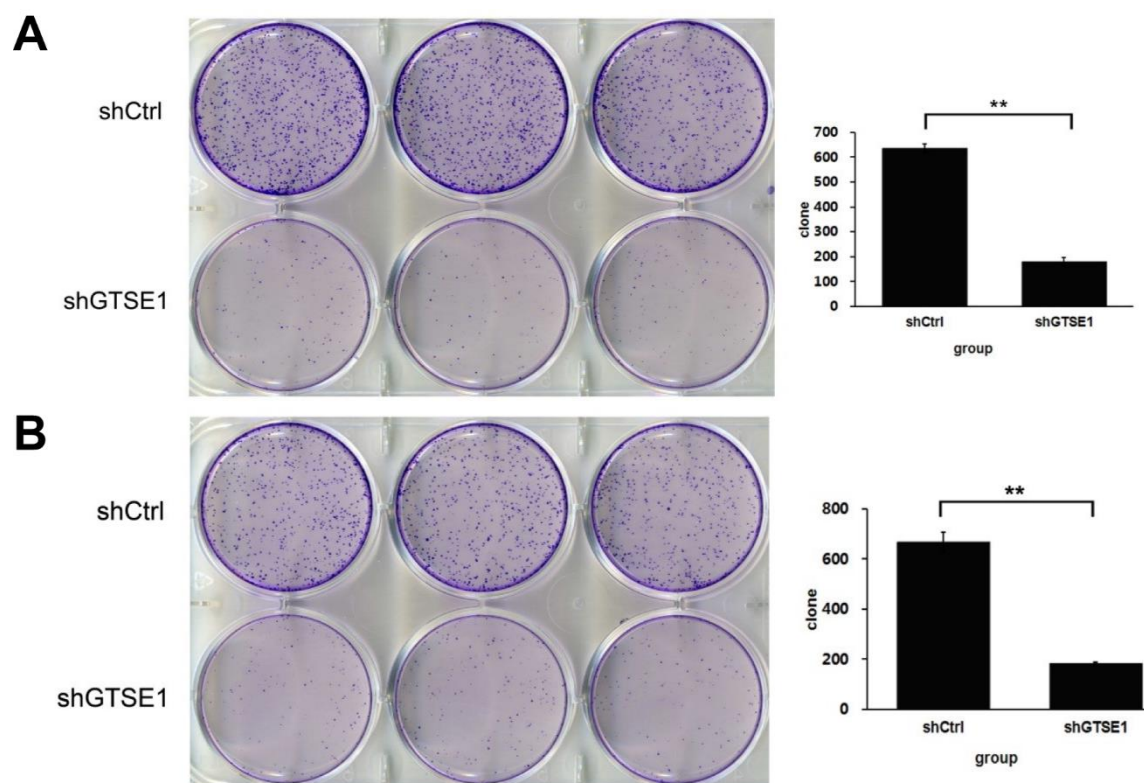
Correspondence and requests for materials should be addressed to Y.C. (email: yc@tsinghua.edu.cn) or to J.Y.W. (email: wangjingyu@tsinghua.edu.cn)



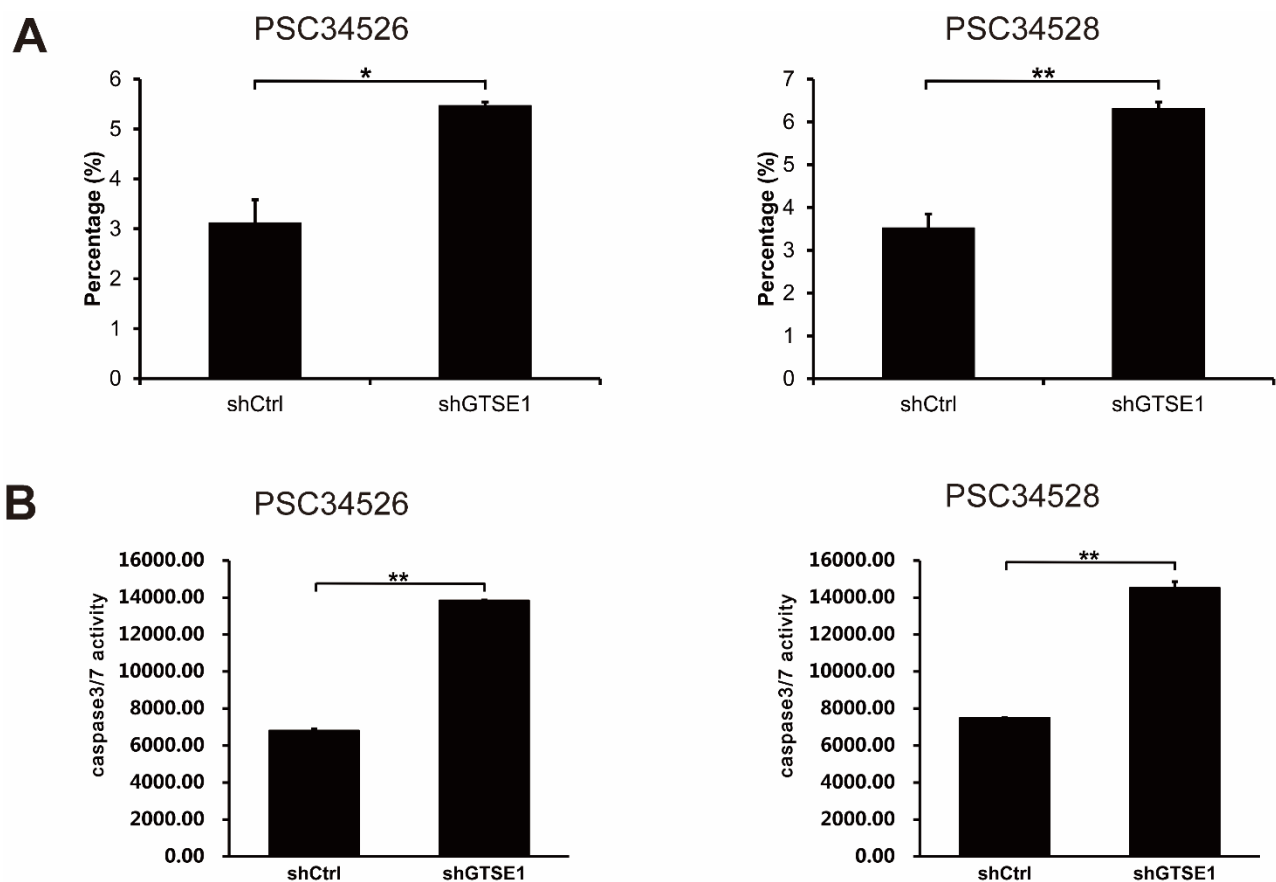
Supplementary Figure 1. Transfection images of BEL-7404 cells transfected by shRNA lentivirus against GTSE1. **(A)** Transfection image of psc34526. **(B)** Transfection image of psc34528. Over 80% cells in both shGTSE1 (psc34526) and shGTSE1 (psc34528) group were infected after 72 hours after transfection.



Supplementary Figure 2. Cell growth rates measured by methyl-thiazolyl-tetrazolium (MTT) assay. **(A)** Absorption and absorption fold changes of shCtrl and shGTSE1 (psc34526) at 490 nm. **(B)** Absorption and absorption fold changes of shCtrl and shGTSE1 (psc34528) at 490 nm. For **A** and **B**, OD 490 represents the number of living cells, data are presented as the mean \pm SD. GAPDH was used as the endogenous control.



Supplementary Figure 3. Colony formation of BEL-7404 cells with GTSE1 knockdown. **(A)** Image and colony number evaluation of shGTSE1 (psc34526). **(B)** Image and colony number evaluation of shGTSE1 (psc34528). For **A** and **B**, data are presented as the mean \pm SD. ** $p < 0.01$ vs. shCtrl. GAPDH was used as the endogenous control.



Supplementary Figure 4. Results of apoptosis assay and caspase3-7 assay. **(A)** Apoptosis percentage of shGTSE1 (psc34526), shGTSE1 (psc34526) and shCtrl 5 days after RNAi transfection. **(B)** Caspase3/7 activity between shGTSE1 (psc34526), shGTSE1 (psc34526) and shCtrl 5 days after RNAi transfection. For **A** and **B**, data are presented as the mean \pm SD. * $p < 0.05$ and ** $p < 0.01$ vs. shCtrl. GAPDH was used as the endogenous control.

Supplementary Table 1. Sequences of shRNA against GTSE1

Sequence number	5' additional bases	STEM	Loop	STEM	3' additional bases
psc34526-1	CCGG	GCCTACTCCTA CAAATCAATT	CTCGAG	AATTGATTTGT AGGAGTAGGC	TTTTTG
psc34526-2	AATTCAAA AA	GCCTACTCCTA CAAATCAATT	CTCGAG	AATTGATTTGT AGGAGTAGGC	
psc34528-1	CCGG	CGGCGAGATT CCTGTCTAAAT	CTCGAG	ATTTAGACAGG AATCTCGCCG	TTTTTG
psc34528-2	AATTCAAA AA	CGGCGAGATT CCTGTCTAAAT	CTCGAG	ATTTAGACAGG AATCTCGCCG	

Supplementary Table 2. Efficiency of shRNA knockdown against GTSE1

Sample	Average ($2^{-\Delta\Delta Ct}$)	STDEV	P-value
shCtrl	1.018	0.241	
shGTSE1(PSC34526)	0.122	0.001	0.023
shGTSE1(PSC34528)	0.118	0.005	0.023

Supplementary Table 3. Cell counts of transwell assay (200×)

200X Cell counts	shCtrl			shGTSE1		
	Hole 1	Hole 2	Hole 3	Hole 1	Hole 2	Hole 3
field 1	198	217	207	102	112	108
field 2	186	210	194	98	108	107
field 3	211	197	208	115	119	112
field 4	217	219	202	99	111	99
field 5	227	199	215	90	109	118
field 6	201	187	210	97	108	102
field 7	209	196	226	107	98	120
field 8	229	227	219	108	118	119
field 9	216	192	206	106	101	91
Migratory cells per field	210	205	210	102	109	108
Average	208			107		
Stdev.	3.01			3.75		
P VALUE	3.32026E-06					
Migration Fold Change	1.01	0.98	1.01	0.49	0.52	0.52
Average	1.00			0.51		
Stdev.	0.01			0.02		
P VALUE	3.32026E-06					

Supplementary Table 4. Cell counts of invasion assay (200×)

	shCtrl	shGTSE1
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200X Cell counts	Hole 1	Hole 2	Hole 3	Hole 1	Hole 2	Hole 3
field 1	164	164	147	117	101	101
field 2	172	177	151	124	98	87
field 3	182	172	158	117	98	97
field 4	166	181	150	120	87	103
field 5	168	188	155	112	107	111
field 6	152	172	147	101	104	102
field 7	170	171	141	121	97	121
field 8	155	173	160	107	103	107
field 9	161	169	139	107	105	96
Migratory cells per field	166	174	150	114	100	103
Average	163			106		
Stdev.	12.34			3.75		
P VALUE	0.002284129					
Migration Fold Change	1.01	1.07	0.92	0.70	0.61	0.63
Average	1.00			0.65		
Stdev.	0.08			0.05		
P VALUE	0.002284129					

Supplementary Table 5. Data of Cell Cycle analysis

PSC34526				PSC34528			
Group	G1	S	G2/M	Group	G1	S	G2/M
shCtrl	48.92	48.4	2.68	shCtrl	51.8	31.86	16.34
	50.6	47.41	2		52.39	30.69	16.92
	49.79	47.31	2.9		52.1	31.4	16.5
shGTSE1	60.67	34.15	5.17	shGTSE1	52.83	26.98	20.19
	60.27	33.64	6.09		53.13	25.58	21.29
	62.12	31.96	5.92		53.1	26.42	20.48
Average	G1	S	G2/M	Average	G1	S	G2/M
shCtrl	49.77	47.71	2.53	shCtrl	52.1	31.32	16.59
shGTSE1	61.02	33.25	5.73	shGTSE1	53.02	26.33	20.65
STDEV	ST.G1	ST.S	ST.G2/M	STDEV	ST.G1	ST.S	ST.G2/M

shCtrl	0.8402	0.6025	0.4692	shCtrl	0.295	0.5894	0.2996
shGTSE1	0.9734	1.1459	0.4895	shGTSE1	0.1652	0.7047	0.5701
T-Test	P Value			T-Test	P Value		
	G1	S	G2/M		G1	S	G2/M
shCtrl vs shGTSE1	0.00011	0.00004	0.00122	shCtrl vs shGTSE1	0.00911	0.00071	0.0004