Dataset S1. The role of RSK2-Y707A-RhoA signaling on U87MG cell migration and invasion *p* values

a) **Fig. 3A, B: Effects of DN-Rho GTPases on active RSK2-Y707A-induced U87MG cell migration and invasion**

Treatments		Fold Induction (Mean ± SD)	*p* value (*versus*)		
			Flag-EV+HA-EV	RSK2+HA-EV	Flag-EV+Rho-T19N
Migration (*n* = 4)					
HA-EV	Flag-EV	1			
	Flag-RSK2-Y707A	1.95 ± 0.18	3.71×10^{-5}		
HA-RhoA-T19N	Flag-EV	1.61 ± 0.19	7.05×10^{-4}		
	Flag-RSK2-Y707A	1.16 ± 0.33	0.366	5.62×10^{-3}	5.86×10^{-2}
HA-RhoB-T19N	Flag-EV	1.62 ± 0.21	1.20×10^{-3}		
	Flag-RSK2-Y707A	1.11 ± 0.09	5.67×10^{-2}	1.50×10^{-4}	4.94×10^{-3}
HA-RhoC-T19N	Flag-EV	2.73 ± 0.22	4.56×10^{-6}		
	Flag-RSK2-Y707A	1.43 ± 0.23	1.04×10^{-2}	1.12×10^{-2}	1.90×10^{-4}
Invasion (*n* = 3)					
HA-EV	Flag-EV	1			
	Flag-RSK2-Y707A	2.37 ± 0.34	2.13×10^{-3}		
HA-RhoA-T19N	Flag-EV	1.75 ± 0.36	2.24×10^{-2}		
	Flag-RSK2-Y707A	1.02 ± 0.21	0.866	4.21×10^{-3}	3.90×10^{-2}
HA-RhoB-T19N	Flag-EV	1.72 ± 0.14	9.41×10^{-4}		
	Flag-RSK2-Y707A	1.15 ± 0.07	1.61×10^{-2}	3.56×10^{-3}	3.32×10^{-3}
HA-RhoC-T19N	Flag-EV	3.66 ± 0.62	1.79×10^{-3}		
	Flag-RSK2-Y707A	1.21 ± 0.23	0.181	8.02×10^{-3}	3.11×10^{-3}

b) **Fig 3C, D: Effects of Rho GTPase silencing on active RSK2-Y707A-induced U87MG cell migration and invasion**

Treatments		Folds Induction (Mean ± SD)	*p* value (*versus*)		
			Flag-EV+shLacZ	RSK2+shLacZ	Flag-EV+shRho
Migration (*n* = 3)					
shLacZ	Flag-EV	1			
	Flag-RSK2-Y707A	1.94 ± 0.33	7.55×10^{-3}		
shRhoA	Flag-EV	1.36 ± 0.22	4.38×10^{-3}		
	Flag-RSK2-Y707A	1.05 ± 0.08	0.285	1.02×10^{-2}	8.03×10^{-2}
shRhoB	Flag-EV	1.53 ± 0.30	3.68×10^{-2}		
	Flag-RSK2-Y707A	1.31 ± 0.20	5.09×10^{-2}	4.95×10^{-2}	0.353
shRhoC	Flag-EV	2.86 ± 0.57	4.91×10^{-3}		
	Flag-RSK2-Y707A	2.09 ± 0.07	1.25×10^{-5}	0.485	8.22×10^{-2}
Invasion (*n* = 3)					
shLacZ	Flag-EV	1			
	Flag-RSK2-Y707A	2.60 ± 0.57	8.32×10^{-3}		
shRhoA	Flag-EV	1.74 ± 0.34	1.92×10^{-2}		
	Flag-RSK2-Y707A	1.35 ± 0.31	0.121	2.88×10^{-2}	0.212
shRhoB	Flag-EV	1.69 ± 0.24	7.60×10^{-3}		
	Flag-RSK2-Y707A	1.01 ± 0.15	0.876	9.70×10^{-3}	1.49×10^{-2}
shRhoC	Flag-EV	4.76 ± 1.06	3.59×10^{-3}		
	Flag-RSK2-Y707A	1.78 ± 0.15	8.53×10^{-4}	7.46×10^{-2}	8.64×10^{-3}

c) **Fig 6C: Effects of LARG-DN (ΔDH/PH) and LARG-S1288A on active RSK2-induced U87MG cell migration and invasion**

Treatments		Folds Induction (Mean ± SD)	p value (versus)	
			Flag-EV+Myc-EV	Flag-RSK2+Myc-EV
Migration (n = 3)				
Myc-EV	Flag-EV	1		
	Flag-RSK2-T577E	3.28 ± 0.85	7.24×10^{-3}	
Myc-LARG-DN	Flag-EV	1.21 ± 0.24	0.2114	
	Flag-RSK2-T577E	1.72 ± 0.52	0.0748	0.0377
Myc-LARG-S1288A	Flag-EV	1.57 ± 0.23	0.0118	
	Flag-RSK2-T577E	2.12 ± 0.69	0.0489	0.0983
Invasion (n = 3)				
Myc-EV	Flag-EV	1		
	Flag-RSK2-T577E	3.43 ± 0.54	1.44×10^{-3}	
Myc-LARG-DN	Flag-EV	1.11 ± 0.06	0.0257	
	Flag-RSK2-T577E	2.04 ± 0.28	3.10×10^{-3}	0.0164
Myc-LARG-S1288A	Flag-EV	1.51 ± 0.06	8.71×10^{-5}	
	Flag-RSK2-T577E	2.34 ± 0.42	5.18×10^{-3}	0.0499

d) Fig 7C: Effects of deletion of LARG (ARHGEF12) on active RSK2-induced U87MG cell migration and invasion

Treatments		Fold Induction (Mean ± SD)	p value (versus)		
			CRISPR-Con+Flag-EV	CRISPR-Con+RSK2	CRISPR-LARG+Flag-EV
Migration ($n = 2$)					
CRISPR-Con	Flag-EV	1			
	Flag-RSK2-T577E	1.59 ± 0.074	1.57×10^{-6}		
	Flag-RSK2-Y707A	1.71 ± 0.066	1.67×10^{-7}		
CRISPR-LARG	Flag-EV	1.25 ± 0.044	2.95×10^{-6}		
	Flag-RSK2-T577E	1.13 ± 0.12	0.0503	**2.99×10^{-5}**	0.0582
	Flag-RSK2-Y707A	1.09 ± 0.033	3.86×10^{-4}	**9.50×10^{-8}**	6.00×10^{-5}
Invasion ($n = 2$)					
CRISPR-Con	Flag-EV	1			
	Flag-RSK2-T577E	1.78 ± 0.29	0.042		
	Flag-RSK2-Y707A	1.54 ± 0.096	3.89×10^{-3}		
CRISPR-LARG	Flag-EV	0.86 ± 0.078	0.0756		
	Flag-RSK2-T577E	1.36 ± 0.12	0.1737	**0.0479**	0.0352
	Flag-RSK2-Y707A	0.82 ± 0.095	0.0661	**7.76×10^{-4}**	0.5981

e) **Fig S3F, G: The effects of WT-RSK2 and their mutants on U87MG cell migration and invasion**

Treatments	Migration (n = 3)		Invasion (n = 3)	
	Fold Induction (Mean ± SD)	*p* value (*vs* Flag-EV)	Folds Induction (Mean ± SD)	*p* value (*vs* Flag-EV)
Flag-EV	1		1	
Flag-RSK2-WT	1.34 ± 0.24	0.07012	1.21 ± 0.14	0.05384
Flag-RSK2-Y707A	1.75 ± 0.20	0.00291	2.00 ± 0.32	0.00583
Flag-RSK2-NTKD	1.67 ± 0.16	0.00188	1.39 ± 0.15	0.01203
Flag-RSK2-CTKD	1.11 ± 0.35	0.62159	1.37 ± 0.25	0.06478
Flag-RSK2-S227E	1.22 ± 0.22	0.15841	1.88 ± 0.45	0.02791
Flag-RSK2-S386E	1.25 ± 0.38	0.31266	1.42 ± 0.17	0.01298
Flag-RSK2-T577E	2.77 ± 0.33	0.00074	3.41 ± 0.57	0.00182

f) **Fig S4. A, B: The role Rho GTPases on RSK2-T577E-mediated U87MG cell migration and invasion**

Treatments		Fold Induction (Mean ± SD)	*p* value (*versus*)		
			Flag-EV+HA-EV	RSK2+HA-EV	Flag-EV+Rho-T19N
Migration (*n* = 3)					
HA-EV	Flag-EV	1			
	Flag-RSK2-T577E	3.07 ± 0.37	6.39×10^{-4}		
HA-RhoA-T19N	Flag-EV	1.44 ± 0.10	1.58×10^{-3}		
	Flag-RSK2-T577E	1.36 ± 0.29	9.51×10^{-2}	3.31×10^{-3}	0.676
HA-RhoB-T19N	Flag-EV	1.61 ± 0.07	9.14×10^{-5}		
	Flag-RSK2-T577E	1.75 ± 0.40	3.16×10^{-2}	1.40×10^{-2}	0.582
HA-RhoC-T19N	Flag-EV	2.79 ± 0.14	2.36×10^{-5}		
	Flag-RSK2-T577E	2.68 ± 0.31	7.06×10^{-4}	0.240	0.618
Invasion (*n* = 3)					
HA-EV	Flag-EV	1			
	Flag-RSK2-T577E	3.21 ± 0.22	5.89×10^{-5}		
HA-RhoA-T19N	Flag-EV	1.51 ± 0.17	7.29×10^{-3}		
	Flag-RSK2-T577E	1.61 ± 0.66	0.186	1.62×10^{-2}	0.813
HA-RhoB-T19N	Flag-EV	1.51 ± 0.19	9.14×10^{-3}		
	Flag-RSK2-T577E	1.50 ± 0.48	0.143	4.74×10^{-3}	0.970
HA-RhoC-T19N	Flag-EV	2.86 ± 0.16	4.03×10^{-5}		
	Flag-RSK2-T577E	3.06 ± 0.13	1.15×10^{-5}	0.372	0.164

Note: The *p* value was calculated using student's *t*-test. EV, empty vector; DN, dominant negative; shRhoA, small hairpin RNA against human RhoA; shRhoB, small hairpin RNA against human RhoB; shRhoC, small hairpin RNA against human RhoC; shLacZ, small hairpin RNA against β-galactosidase. *N* values are biological replicates.