

The epidermal growth factor receptor regulates cofilin activity and promotes transmissible gastroenteritis virus entry into intestinal epithelial cells

Supplementary Material

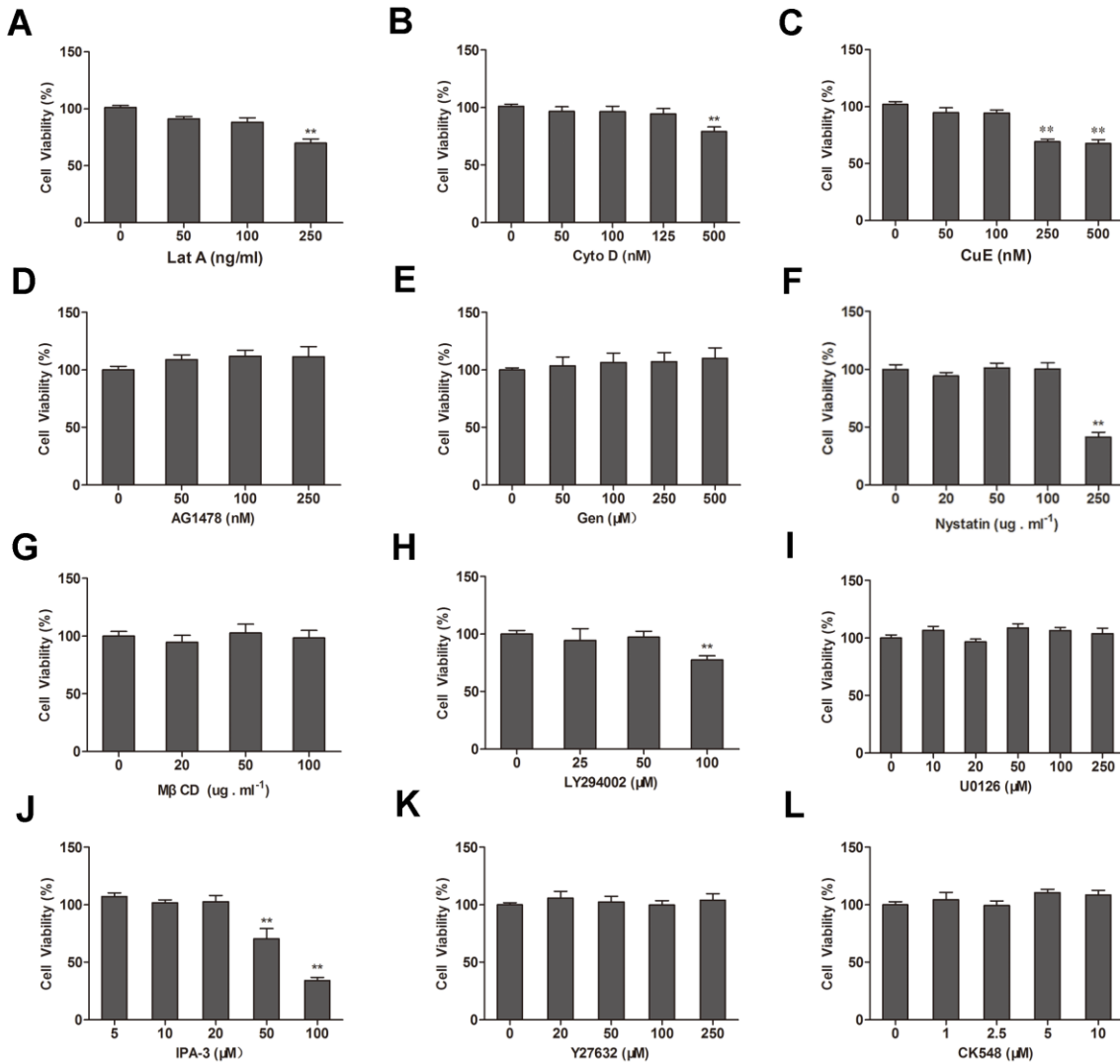


Figure S1: Cytotoxicity of chemical inhibitors. The cytotoxicity of chemical inhibitors were determined with a MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay. Statistical significance was assessed by student's t-test (*) $0.01 < p < 0.05$, (**) $p < 0.01$, all of the experiments were performed for three separate experiments.

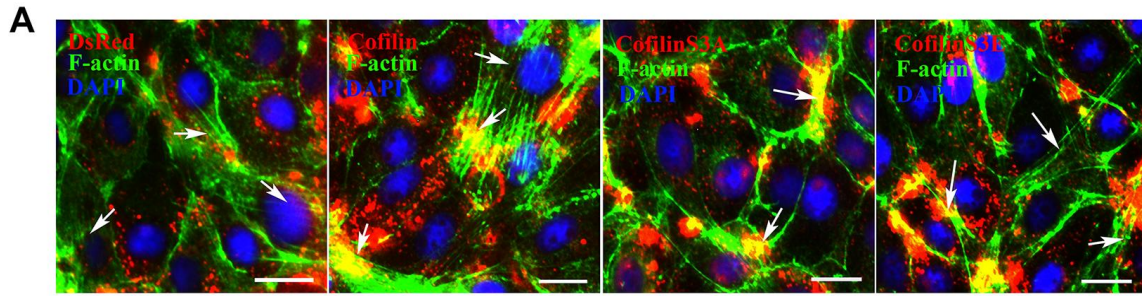


Figure S2: WT cofilin and mutants cofilins (S3A and S3E) destroy the arrangement of actin. Cells were transfected with lentiviral constructs expressing WT cofilin and mutant cofilins S3A and S3E. After incubation for 48 h, actin was stained with phalloidin-TRITC (Green).

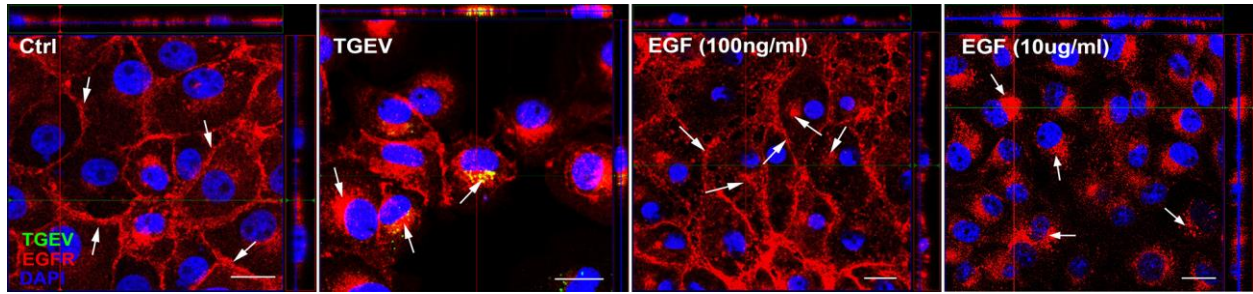


Figure S3: TGEV infection causes EGFR endocytosis. IPEC-J2 cells were infected with TGEV (MOI=2) or were stimulated with EGF (100ng/ml) at 4 °C for 1h then 37 °C for 30 min. The cells were fixed and stained with mouse anti-TGEV mAb followed by DyLight 488-conjugated goat anti-mouse IgG and rabbit anti-EGFR pAb followed by DyLight 594-conjugated goat anti-rabbit IgG, cells were examined by confocal microscope.

Table S1: List of pharmacological inhibitors.

Inhibitor	Specificity Functions	Supplier
Latrunculin A	sequesters actin monomers	Life
Cytochalasin D	actin depolymerizing agent	Life
Cucurbitacin E	stabilizes actin polymers	Sigma
AG1478	EGFR specific inhibitor	Sigma
Genistein	Receptor tyrosine kinase inhibitor	Sigma
Nystatin	sequesters and blocks cholesterol	Sigma
MβCD	Deplete cholesterol	Sigma
LY294002	Specific PI3K inhibitor	Sigma
U0126	MEK1/2 inhibitor	Sigma
IPA-3	PAK-1 inhibitor	Sigma
Y27632	ROCK-1 inhibitor	Sigma
CK548	Arp2/3 complex inhibitor	Sigma

Table S2: List of antibodies.

Name	Antibody information	Supplier	Catalog Number
EGFR	EGF Receptor (D38B1) XP® Rabbit mAb	CST	4267
p-EGFR	Phospho-EGF Receptor (Tyr1068) (D7A5) XP ® Rabbit mAb	CST	3777
p-LIMK1	Phospho-LIMK1 (Thr508) Rabbit mAb	CST	3841
Akt	Akt1 (C73H10) Rabbit mAb	CST	2938
p-Akt	Phospho-Akt (Ser473) (193H12) Rabbit mAb	CST	4058
ERK1/2	p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb	CST	4695
p-ERK1/2	Phospho-p44/42 MAPK (Thr202/Tyr204) (D13.14.4E) XP™ Rabbit mAb	CST	4370
Cofilin	Cofilin Rabbit pAb	CST	3312
p-Cofilin	Phospho-Cofilin (Ser3) (77G2) Rabbit mAb	CST	3313
HA	Mouse monoclonal Antibody to HA	CMCTAG	AT0024
TGEV	TGE Virus Mouse mAb	Santa Cruz	sc-52436
Dylight488	Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 488 conjugate	Thermo	35502
	Goat anti-Mouse IgG(H+L) Secondary Antibody, DyLight 594 conjugate	Thermo	35510
Dylight594	Goat anti-Rabbit IgG (H+L) Secondary Antibody, DyLight 594 conjugate	Thermo	35560

TableS3: List of primer sequences used in PCR

Name	Primer sequence(5'-3')	Vector
Cofilin	F: ACCCAAGCTGgctagcATGGCCTCCGGTGTG (NheI) R: GCTCGGTACCaagcttCAAAGGCTTGCCCTCCAG (HindIII)	pcDNA3.1
Cofilin	F: CGAGCTCAAGCTTCgaattcATGGCCTCCGGTGTGGCT (EcoRI) R: GTGGCGACCGGTggatccCAAAGGCTTGCCCTCCAG (BamHI)	pLVX-DsRed
CofilinS3A	F: CGAGCTCAAGCTTCgaattcATGGCCGCAGGTGTGGCT (EcoRI) R: GTGGCGACCGGTggatccCAAAGGCTTGCCCTCCAG (BamHI)	pLVX-DsRed
CofilinS3E	F: CGAGCTCAAGCTTCgaattcATGGCCGAAGGTGTGGCT (EcoRI) R: GTGGCGACCGGTggatccCAAAGGCTTGCCCTCCAG (BamHI)	pLVX-DsRed
RhoA	F: ACCCAAGCTGgctagcATGGCTGCCATCAGGAAG (NheI) R: GCTCGGTACCaagcttCAAGACAAGGCACCCAGAT (HindIII)	pcDNA3.1
RhoA	F: CGAGCTCAAGCTTCgaattcATGGCTGCCATCAGGAAG (EcoRI) R: GTGGCGACCGGTggatccCAAGACAAGGCACCCAGAT (BamHI)	pLVX-DsRed
N19RhoA	F: CGAGCTGAAGCTTCgaattcATGGCTGCCATCAGGAAG (EcoRI) R: GCTCGGTACCaagcttCAAGACAAGGCACCCAGAT (HindIII)	pLVX-DsRed
L63RhoA	F: CGAGCTCAAGCTTCgaattcATGGCTGCCATCAGGAAG (EcoRI) R: GTGGCGACCGGTggatccCAAGACAAGGCACCCAGAT (BamHI)	pLVX-DsRed
Rac1	F: ACCCAAGCTGgctagcATGCAGGCCATCAAGTGTGT (NheI) R: GCTCGGTACCaagcttCAACAGCAGGCACTTCCTCT (HindIII)	pcDNA3.1
Rac1	F: CGAGCTCAAGCTTCgaattcATGCAGGCCATCAAGTGT (EcoRI) R: GTGGCGACCGGTggatccCAACAGCAGGCACTTCCT (BamHI)	pLVX-DsRed
N17Rac1	F: CGAGCTGAAGCTTCgaattcATGCAGGCCATCAAGTGT (EcoRI) R: GTGGCGACCGGTggatccCAACAGCAGGCACTTCCT (BamHI)	pLVX-DsRed
L61Rac1	F: CGAGCTCAAGCTTCgaattcATGCAGGCCATCAAGTGT (EcoRI) R: GTGGCGACCGGTggatccCAACAGCAGGCACTTCCT (BamHI)	pLVX-DsRed
Cdc42	F: ACCCAAGCTGgctagcATGCAGACGATTAAGTGTGTTG (NheI) R: GCTCGGTACCaagcttAATATACAGCACTTCCTTTTG (HindIII)	pcDNA3.1
Cdc42	F: CGAGCTCAAGCTTCgaattcATGCAGACGATTAAGTGTGTTG (EcoRI) R: GTGGCGACCGGTggatccAATATACAGCACTTCCTTTTG (BamHI)	pLVX-DsRed
N17Cdc42	F: CGAGCTCAAGCTTCgaattcATGCAGACGATTAAGTGTGTTG (NheI) R: GTGGCGACCGGTggatccAATATACAGCACTTCCTTTTG (BamHI)	pLVX-DsRed
L61Cdc42	F: CGAGCTCAAGCTTCgaattcATGCAGACGATTAAGTGTGTTG (EcoRI) R: GTGGCGACCGGTggatccAATATACAGCACTTCCTTTTG (BamHI)	pLVX-DsRed
TGEV-S1	F: TCTAGCCCGGGCggatccTGTGCTAGTTATGTGGCT (BamHI) R: ATCGTATGGGTAtctagaATTTGTATAATTATATATAGAG (XbaI)	pLVX-HA
EGFR	F: CTCAAGCTTCgaattcATGCGACGCTCCTGGGCG (EcoRI) R: GTGGCGACCGGTggatccTCATGCCCCAGTAAGG (BamHI)	pLVX-DsRed

Table S4: List of primer sequences used in the site-directed mutagenesis

Name	Primer sequence(5'-3')
CofilinS3A	F: TAGCATGGCC GCA GGTGTGGCTGTCTCTGACGGGGTCATCAAAG R: CAGCCACACCT TGC GGCCATGCTAGCCAGCTTGGGTCTCCCTAT
CofilinS3E	F: TAGCATGGCC GAA GGTGTGGCTGTCTCTGACGGGGTCATCAAAG R: CAGCCACACCT TTC GGCCATGCTAGCCAGCTTGGGTCTCCCTAT
N19RhoA	F: TGTGGCAAGA ATT GTTTGCTCATTGTCTTAGCAAGGACCAAT R: GAGCAAACA AT TCTTGCCACAGGCTCCATCACCAACAATCAC
L63RhoA	F: ACAGCAGGACT TGGA AAGATTATGATCGTTTGAGGCCCCCT R: ATAATCTTCC AGT CCTGCTGTGTCCCACAAAGCCAACCTCA
N17Rac1	F: GTGGGTAAAA ATT GCCTGATCAGTTACACGACCAATGC R: CAGGAGGCA ATTTT TACCCACAGCTCCGTCTCCCACCACCA
L61Rac1	F: ACGGCTGGACT AGA AAGATTACGACCGGTTACGTCCCCTGTC R: GTAATCTTCT AGT CCAGCCGTATCCATAAGCCCAGATTCA
N17Cdc42	F: GTTGGTAAAA ACT GTCTCCTGATATCCTACACGACAAACAAT R: TCAGGAGACAG TTTTT TACCAACGGCACCATCGCCCACAACAAC
L61Cdc42	F: ACTGCAGGG CT AGAGGATTATGACAGATTACGACCGCTGAG R: ATAATCCTCT AGC CTGCAGTATCAAAAAGTCCAAGAGTAT

TableS 5: List of RNA interferences.

Name	mRNA accession	Targeting sequence
Cofilin1	NM_001004043	shCof1: GGGTCATCAAAGTGTTCAATG shCof2: GGTCATCAAAGTGTTCAATGA shCof3: GCGAGGACAAGAAGAACATCA shCof ctrl: CAAAGTGTTCAATGAGGTCAT
EGFR	NM_214007.1	shEGFR1: GCAGATCATCCGAGGAAATG shEGFR2: GCCATGGGCCACATCTGTAAC shEGFR3: GGCATGAACTATCTGGAAGA shEGFR ctrl: GCCACATCTGTAACGCCATGG