processed for luciferase assay.

b HeLa cells were co-transfected with SRE-Luc (200ng), β-gal (100ng), and SHKBP1 or its mutants plasmids (300ng). Thirty-six hours post-transfection cells were starved in serum free DMEM for 12 hours. The cells were then stimulated with EGF for additional 12 hours and processed for luciferase assay. The luciferase activity was measured in triplicated samples and expressed as the mean ± s.d. Student's t-test was performed to compare the statistical difference between each group to pCMV control group; '**' indicates $p<0.01$, '*' indicates $p<0.05$.

Table 1. Yeast two-hybrid experiment examining the interaction of SHKBP1 or its mutants with CIN85.

MAV203 yeast strain was co-transformed with SHKBP1/pDBLeu or its mutants plasmids and CIN85/pPC86 plasmids. SHKBP1 or its mutants co-transformed with empty pPC86 and CIN85 co-transformed with empty pDBLeu were used as control. And pPC86 and pDBLeu vectors were used as negative control. Transformed yeasts were selected in SC-Leu-Trp plates, and a LacZ assay was performed with colonies grown on the SC-Leu-Trp plates and control yeast strains: a strong positive control A, a weak positive control C and a negative control supplied by manufacturer. ('+++' indicates a strong positive signal, '+' indicates a weak positive signal and '–' indicates a negative signal).

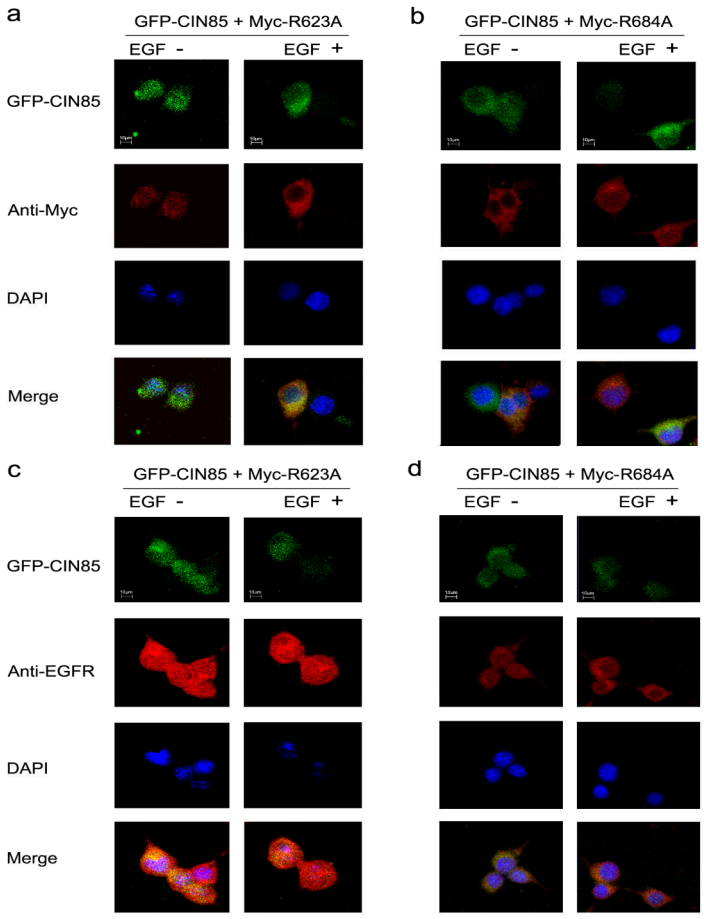
Supplement Figure 1. SHKBP1-R623A and SHKBP1-R684A disturbed the translocation of CIN85 to EGFR degradation vesicles

HEK293T cells were co-transfected with GFP-CIN85 plasmid and Myc-SHKBP1-R623A (a, c) or Myc-SHKBP1-R684A (b, d). Thirty-six hours post-transfection cells were starved in serum free DMEM for 12 hours. The cells were left unstimulated or stimulated with hEGF for 10 minutes. Immuno-fluorescence studies on cells were performed with antibodies against Myc tag (a, b) or EGFR (c, d), and the nucleus were stained with DAPI. The merged pictures were shown in the bottom panel, and the staffs (10μm) were shown in the upper panel.

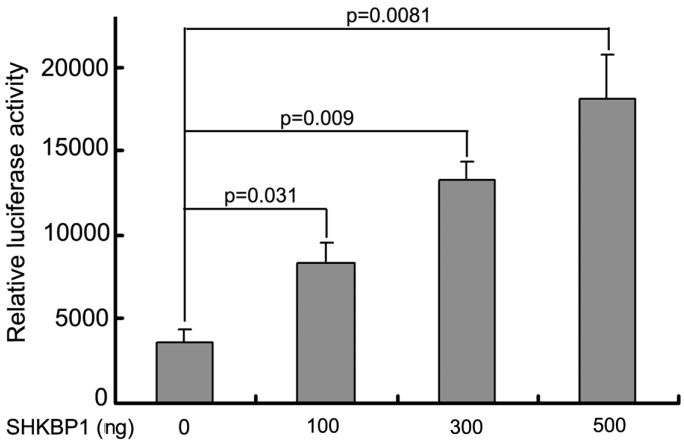
Supplement Figure 2. Dose-dependent effect of SHKBP1 on SRE transcription activity.

HEK293T cells were co-transfected with EGFR (100ng), c-Cbl (100ng), CIN85 (100ng), SRE-Luc (100ng), β-gal (50ng), and SHKBP1 plasmids (0/100/300/500ng). Thirty-six hours post-transfection cells were starved in serum free DMEM for 12 hours. The cells were then stimulated with hEGF for another 12 hours and processed for luciferase assay. The luciferase activity was measured in triplicated samples and expressed as the mean ± s.d. Student's t-test was performed to compare the statistical difference between each group.

Supplemental Figure 1. SHKBP1-R623A and SHKBP1-R684A disturbed the translocation of CIN85.



Supplemental figure 2. Dose-dependent effect of SHKBP1 on SRE transcription activity.



Supplemental table 1: List of primers used for construction.

Primer Name	Sequences (5'-3')
SHKBP1-F	GTATGAATTCATGGCCGTCGCGACGACT
SHKBP1-R	GCGCCTCGAGAAAGGAAGTTTCGTTAAG
CIN85-F	CATACGGAATTCATGGTGGAGGCCATAGTG
CIN85-R	CGCCCCCTCGAGTTTTGATTGTAGAGCTTT
CIN85-3SH3-R	GCGCGCTCGAGGATGAGAGTGACTATATC
CIN85-PCC-F	GCGCGCGAATTCAATAAGGACTGCATCGAC
c-Cbl-F	TATACTGCAGGCCATGGGCAACGTGAAG
c-Cbl-R	TGAAATCTCGAGGGTAGCTACATGGGCAGG
R623A-F	GGGTGAGGGGCTGGGGAAAGAGCC
R623A-R	GCGACCTCTCTTACCAGTCTCCACTCAGCCTC
R684A-F	GCACCCTCCACCAGTCTTGGGAATCCC
R684A-R	AGGGGCTGGTGTTGGCGGCCATCT
SHKBP1-YTH-F	TATAGTCGACAATGGCCGTCGCGACGACT
SHKBP1-YTH-R	GAGAGCGGCCGCAAAGGAAGTTTCGTTAAG
CIN85-YTH-F	AATTGTCGACAATGGTGGAGGCCATAGTG
CIN85-YTH-R	GGTAGCGGCCGCTTTTGATTGTAGAGCTTT
CIN85-GFP-F	ATCTCGAGTGGTGGAGGCCATAGTGGAG
CIN85-GFP-R	CGGGATCCTCATTTTGATTGTAGAGCTTTC

Type of file:	figure
Label:	Supplentary Figure 1
Filename:	Supplemental Fig 1-R623A and R684A on the translocation of to EGFR degradation vesicles-1.jpg

a

GFP-CIN85 + Myc-R623A

EGF -

EGF +

GFP-CIN85

Anti-Myc

DAPI

Merge

b

GFP-CIN85 + Myc-R684A

EGF -

EGF +

c

GFP-CIN85 + Myc-R623A

EGF -

EGF +

GFP-CIN85

Anti-EGFR

DAPI

Merge

d

GFP-CIN85 + Myc-R684A

EGF -

EGF +