A

F-RhoU	F-actin	Overlay

LnCAP

10μm

F-RhoU	F-actin	Overlay

MDA-MB-231

10μm

B

GFP-RhoU | F-actin | Overlay

HeLa

10μm

C

α-tubulin	F-actin	RhoU	Overlay

control

5μm

Cytochalasin D

5μm

Nocodazole

5μm

supplementary figure 1, Zhang et al.

A

RhoU

| 1-49 | CDC42 homologous region (50-241) | 241-261 |

pGKBT7

| Gal4-DBD | RhoU (Q110L) |

B

CIN85(a-c) CRK-L (N) * CRK-L (C) * CRK-II (N) CRK-II (C) LCK

GRB-2 (C) * p85α * Dock Abi-I Abi-II IRSp53 ARG GST Input

34-

34-

Flag-RhoU

lysate

GST-SH3 Pull-down

52-

52-

52-

25-

GST-fusions

Ponceau S

C

15min Rh-EGF

GFP Rh-EGF GRB2 Merge

10μm

30min Rh-EGF

GFP Rh-EGF GRB2 Merge

10μm

D

pU6-shGRB2 15min Rh-EGF

Rh-EGF GRB2 Merge

10μm
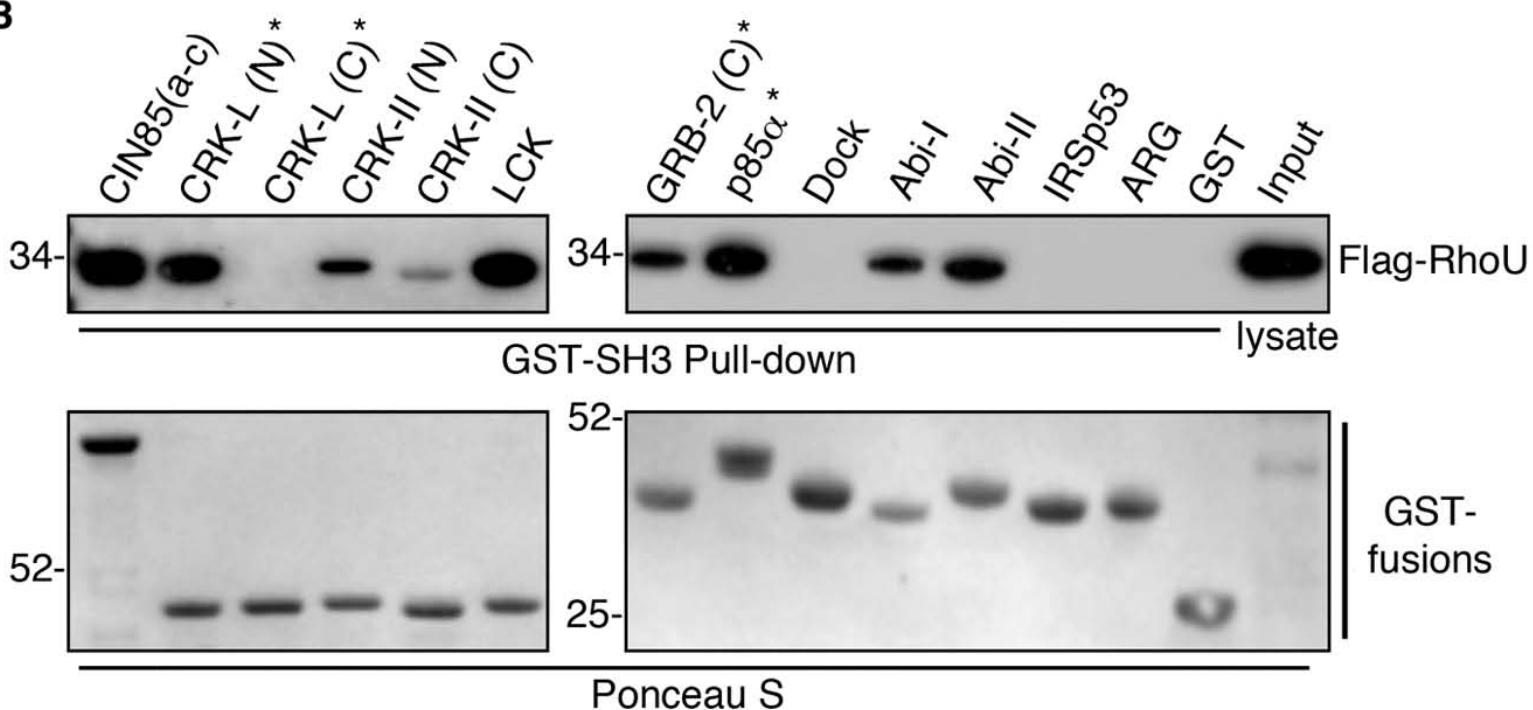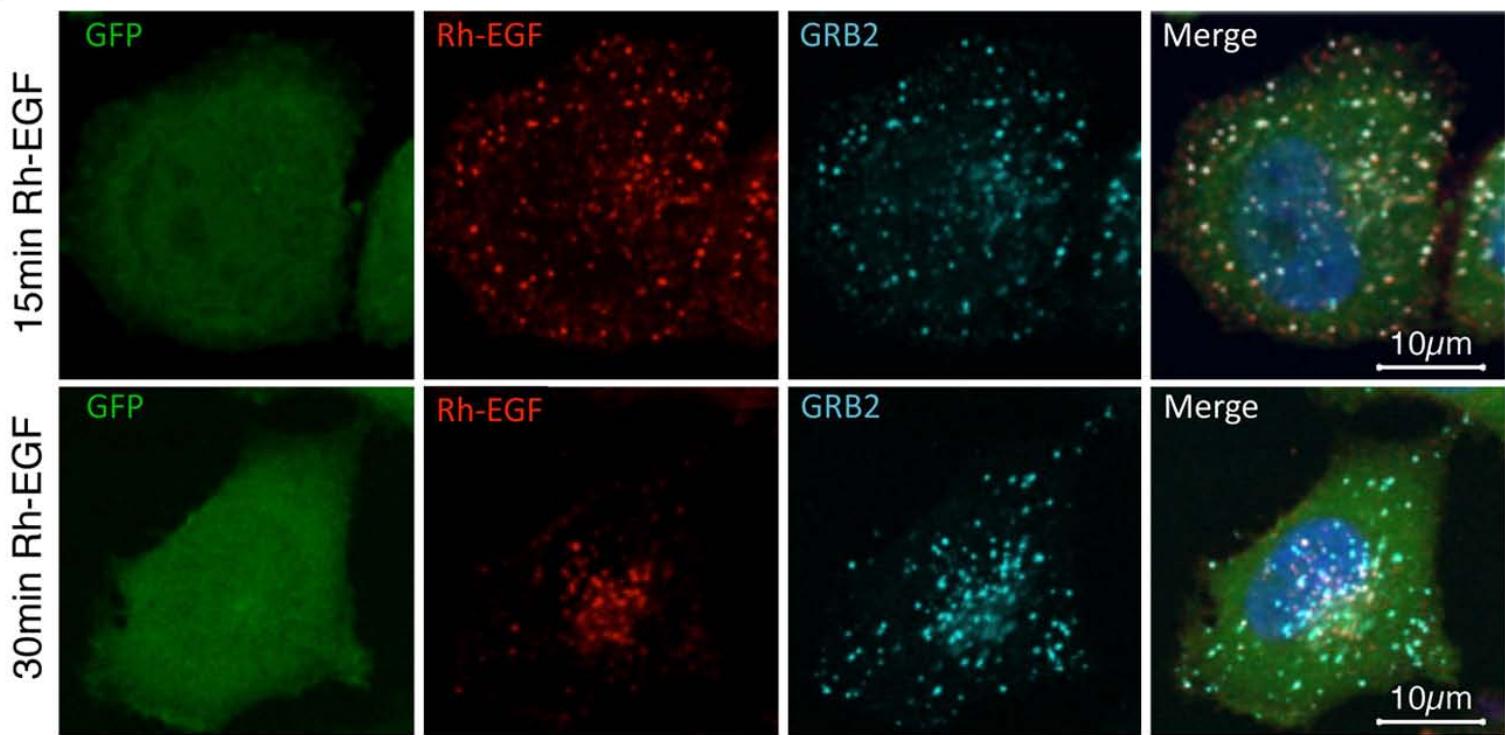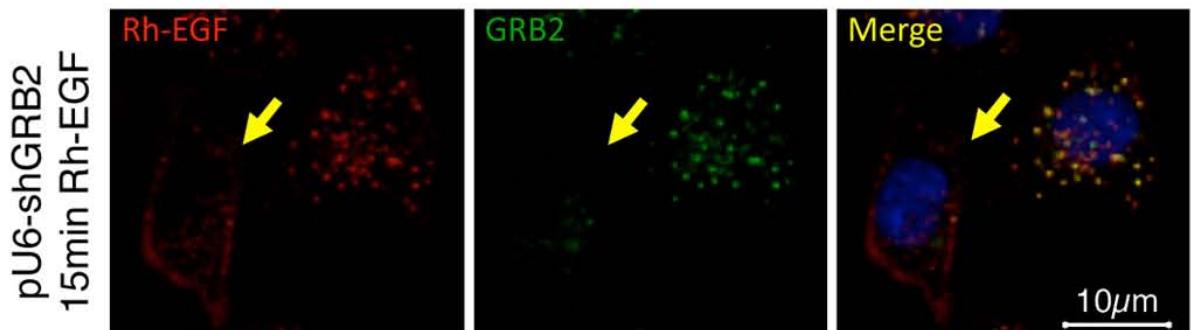
supplementary figure 2, Zhang et al.

A

RhoU Y254F

F-actin

0min EGF

EGF

15min EGF

10μm

B

Flag-RhoU

CA DN WT

GTP-bound | Flag-RhoU
GST-PBD | Ponceau S

Pull-down

Flag-RhoU

lysate

C

Flag-CDC42

CA DN

GTP-bound | Flag-CDC42
GST-PBD | Ponceau S

Pull-down

Flag-CDC42

lysate

D

percent of increase

EGF treatment in min

E

percent of increase

EGF treatment in min

F

control   Flag-RhoU

EGF 0 2 10 30   0 2 10 30 min

pPLCγ1
pAKT
β-actin

HeLa

G

percent of increase

p<0.05   p=0.01

15min EGF   30min EGF

H

xfold induction of luciferase activity

EGF - +   - +   - +   - +

control   wt RhoU   RhoU ΔC term   RhoU C256S

supplementary figure 3, Zhang et al.
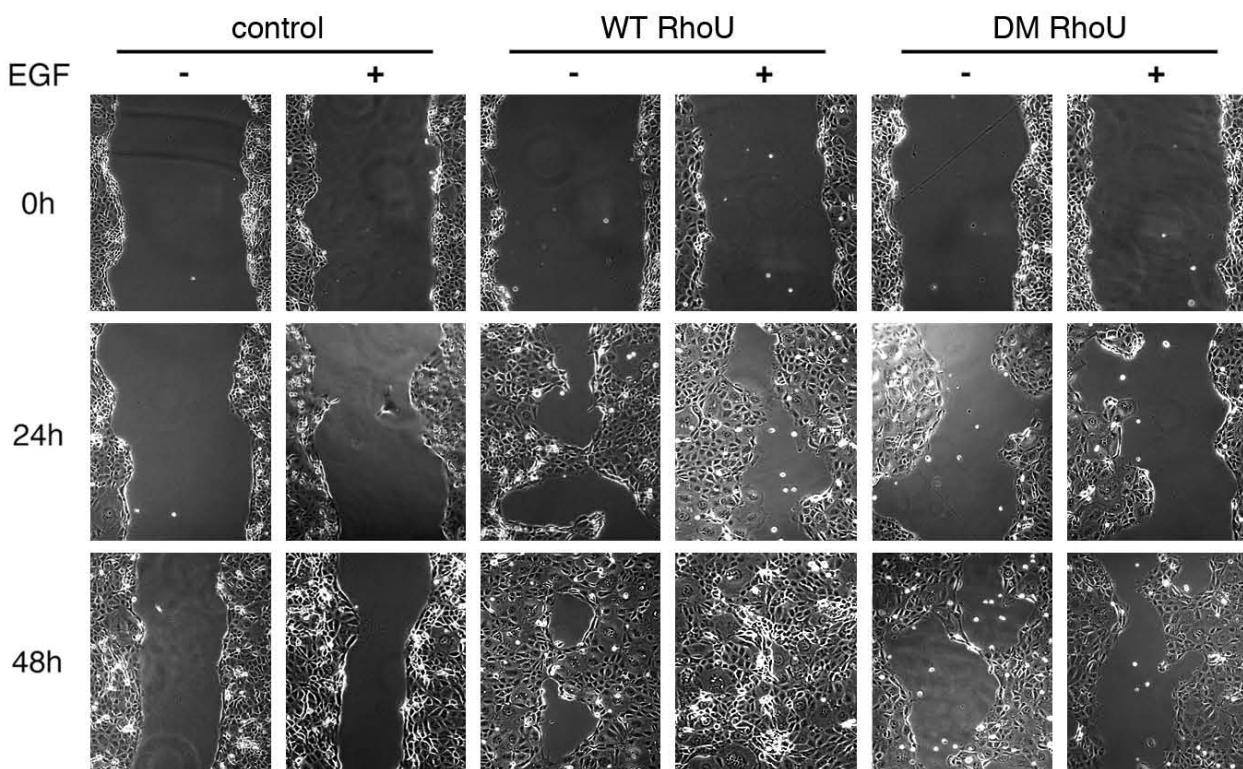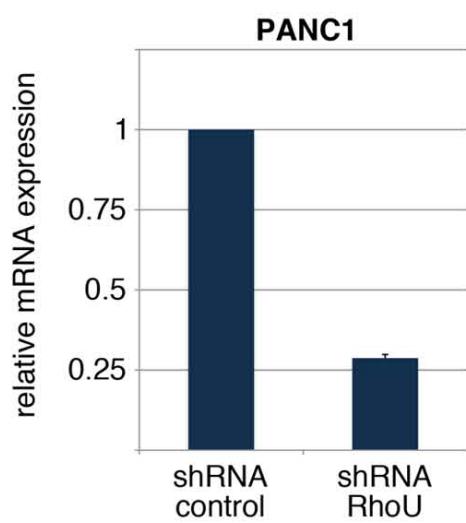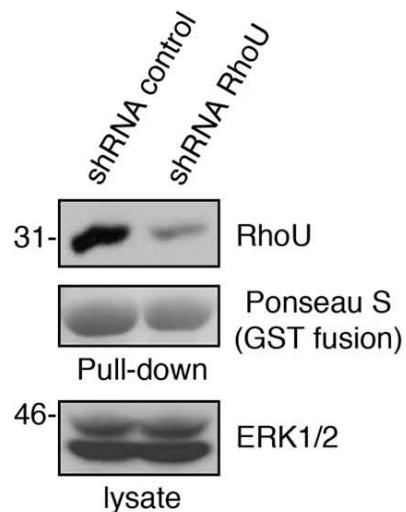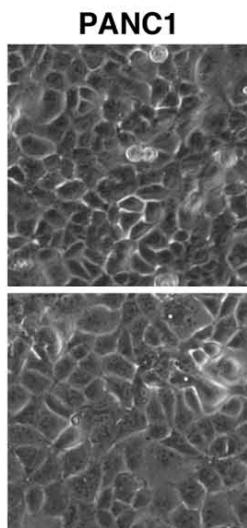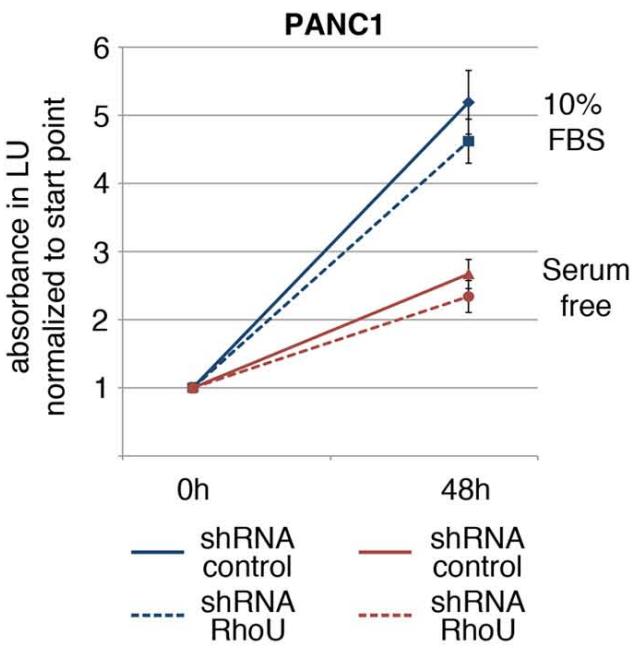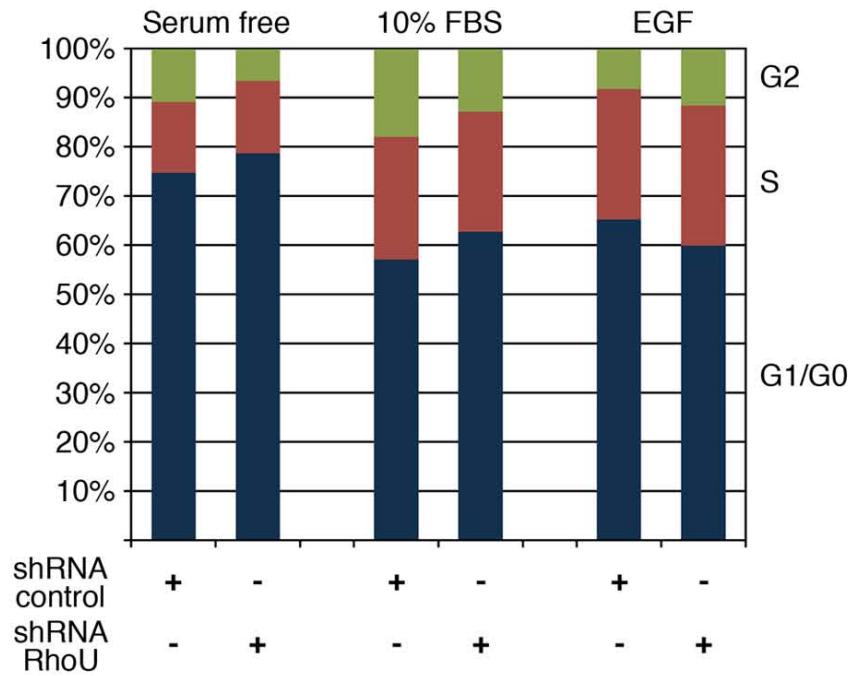
supplementary figure 4, Zhang et al.