RILP interacts with HOPS complex via VPS41 subunit to regulate endocytic trafficking

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Supplemental data

Figure s1

Hela cells were co-transfected with GFP-Rab7 and myc-tagged VPS11, VPS16, VPS18, VPS33, VPS39 and VPS41, respectively. Immunofluorescence microscopy revealed that Rab7 does not recuit HOPS subunits efficiently. Bar=20μm

Figure s2

Hela cells were co-transfected with GFP-RILP and myc-tagged human VPS11, VPS16, VPS18, VPS39 and VPS41, respectively. The cells were processed for Immunofuorescence microscopy. Anti-CD63 was used to label the late endosomes/lysosomes followed by Cy5 conjugated secondary antibody, VPS proteins are immuno-labeled by 9E10 antibody followed by Texas-red conjugated secondary antibody and viewed along with GFP signal. The results demonstrated that RILP can recruit HOPS subunits to the membrane of late endosomes/lysosomes. Bar=20μm.

Figure s3

Since there are not good commercial antibodies against HOPS subunits, we detect the knockdown efficiency of shRNA-VPS by examining the mRNA level of the indicated genes. mRNA was extracted using Trizol reagent (Invitrogen, California, USA), and converted to single strain cDNA according to HiFi-MMLV cDNA Kit (CWBIO, Beijing, China). The primer1 (5'-ATCTTTGCCAATAACCCG-3') and primer 2 (5'-ACCATCCTGGAAGTCGTG-3'), primer 3 (5'-TGCTGTTCGGGACTA TCAC-3') and primer 4 (5'-TCCTTCTGTTGCACCTTGT-3'), primer 5 (5'-CTTACCACTGTCAGCACGAGG-3') and primer 6 (5'-ACAGCAGGTAGTTGTGGATGG-3'), primer 7 (5'-ACATTACGCCTCTG GATGG-3') and primer 8 (5'-TTCTCCCGGTT GACGTTTC-3'), primer 9 (5'-AGCACCAGCCTCCCTACAT-3') and primer 10(5'-GCAGAGCCAATTCAAACT-3'), primer 11(5’- GACATCATCCAGCCAC TT-3’) and primer 12(5’-CTGCCATCAATGCTTCT -3’), were used for PCR to detect the lever of transcript of VPS11, VPS16, VPS18, VPS33, VPS39 and VPS41, respectively. primer 13 (5’-ACCACAGTCCATGCCATCAC-3’) and primer 14(5’-TCCACCACCCTGTTGCTGTA-3’) were used for detect the level of G3PDH for control. The results indicated that the mRNA level of the correspondent HOPS subunit was significantly reduced.

Figure s4

Immunofluorescence microscopy revealed that sequentially depleting HOPS subunit, VPS11, VPS16, VPS18 or VPS39, does not interfere the co-localization of RILP with other HOPS subunits, respectively. Bar=20μm

Figure s5

Immunofluorescence microscopy revealed the signals of EGF-Rhodamine haven’t much difference in control cells, VPS41-knockdown cells and VPS41(428-855) over-expressing cells after short time of endocytosis (5 min). Bar=20μm

Figure s6

A. MCF cells stably expressing shRNA-VPS41 were transfected with pCMV-myc-VPS11, VPS18, VPS33, VPS39 and VPS35, respectively. The resulted cell lysates were subjected to immuno-precipitaion assay using anti-VPS16 antibody, the results demonstrated that loss of VPS41 inhibits the interaction of VPS16 with VPS11 and VPS39, suggesting that loss of VPS41 potentially affects the stability of HOPS complex. B. the relative loading amount of corresponding proteins in A. VPS35 serves as control.

Figure s7

A. Hela cell lysates derived from cells expressing myc-tagged HOPS subunits, VPS11, VPS16, VPS18, VPS33, VPS39 and VPS41, respectively, were subjected to GST-pulldown assay using immobilized GST-RILP. 9E10 antibody was used for western-blotting to detect the protein bound to GST-RILP. The results revealed that RILP binds to all HOPS subunits except for VPS33, suggesting RILP interacts with HOPS complex. B. GST-pulldown assay using GST-RILP, GST-RILP (1-198) and GST-RILP(199-401) demonstrated that N-terminal region (1-198aa) of RILP interacts with HOPS complex.

Figure s8

A. Hela cells were transfected with pSuper.GFP-scramble-shRNA(lane 1) or shRNA-Rab7. 48h later, knocked-down cells were transfected with myc-tagged VPS11 (lane 2), VPS16 (lane 3), VPS18 (lane 4), VPS39 (lane 5) and VPS41 (lane 6), respectively. 72h later, cells were harvested and processed for detection of the knockdown efficiency. The results revealed that Rab7 was depleted efficiently in the cells expressing HOPS subunits. B. Cell lysates described above were subjected for GST-pulldown assay using GST-RILP, the results demonstrated that RILP can still bind to HOPS subunits when Rab7 was effectively depleted, suggesting RILP interacts with HOPS complex independent of Rab7. C. Hela lysates containing myc-tagged VPS11, VPS16, VPS18, VPS39 and VPS41 were subjected for GST-pulldown assay using GST-RILP and GST-RILP304AAA306 mutant (not interacting with Rab7), the data showed that this RILP mutant can still bind to HOPS sununits, although no longer interacting with Rab7 (bottom panel). D. Hela lysates containing myc-tagged VPS11, VPS16, VPS18, VPS33, VPS39 and VPS41 were subjected for GST-pulldown assay using GST-Rab7 or GST-Rab7L8A mutant (not interacting with RILP showed in bottom panel). The results indicating, like wildtype Rab7, Rab7L8A can still weakly interacts with HOPS complex, suggesting Rab7 interacts with HOPS complex independent of RILP.

Figure s9

GST-pulldown experiments demonstrated that depletion of VPS41 decreases the amount of HOPS subunits bound to RILP.

Figure s10

A. Pulldown experiments showed that RILP specifically binds to VPS41 through its C-terminal region(428-855aa). B. Purified His-VPS41 or His-Vps33 recombinant protein was incubated with immobilized GST-RILP to show RILP directly interacts with VPS41, but not VPS33.

Figure s11

MCF7 cells were transfected with pSuper.GFP-scramble-shRNA or shRNA-VPS41, 48h later, the cells were starved for overnight, then stimulated with EGF for the indicated time. The protein level of EGFR was examined by western-blot using mAb against EGFR. The results demonstrated EGFR decreased quickly in scramble knocked-down cells, but the degradation of EGFR is compromised in shRNA-VPS41 knocked-down cells.

Figure s1

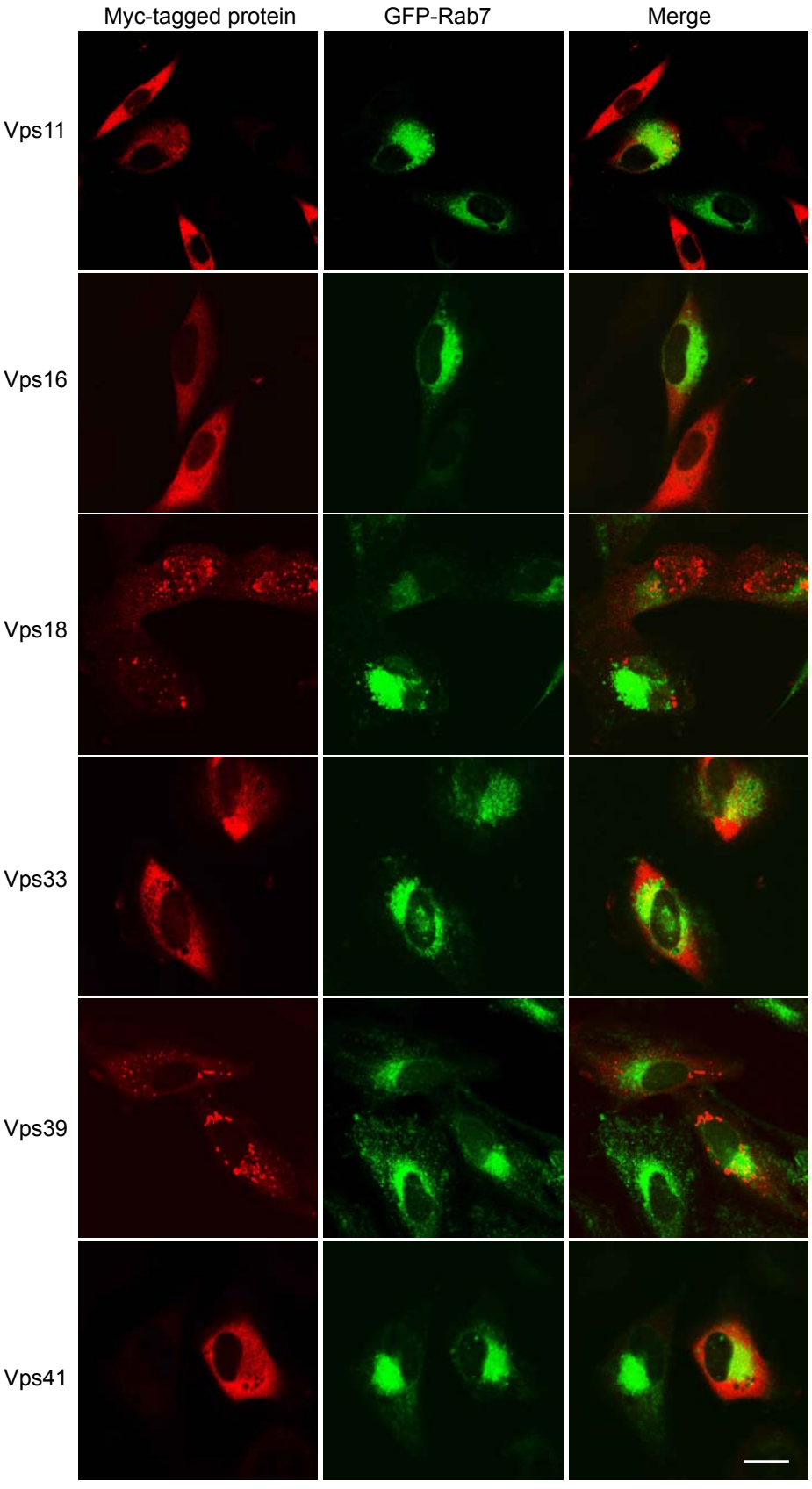

Myc-tagged protein
GFP-Rab7
Merge
Vps11
Vps16
Vps18
Vps33
Vps39
Vps41

Figure s2

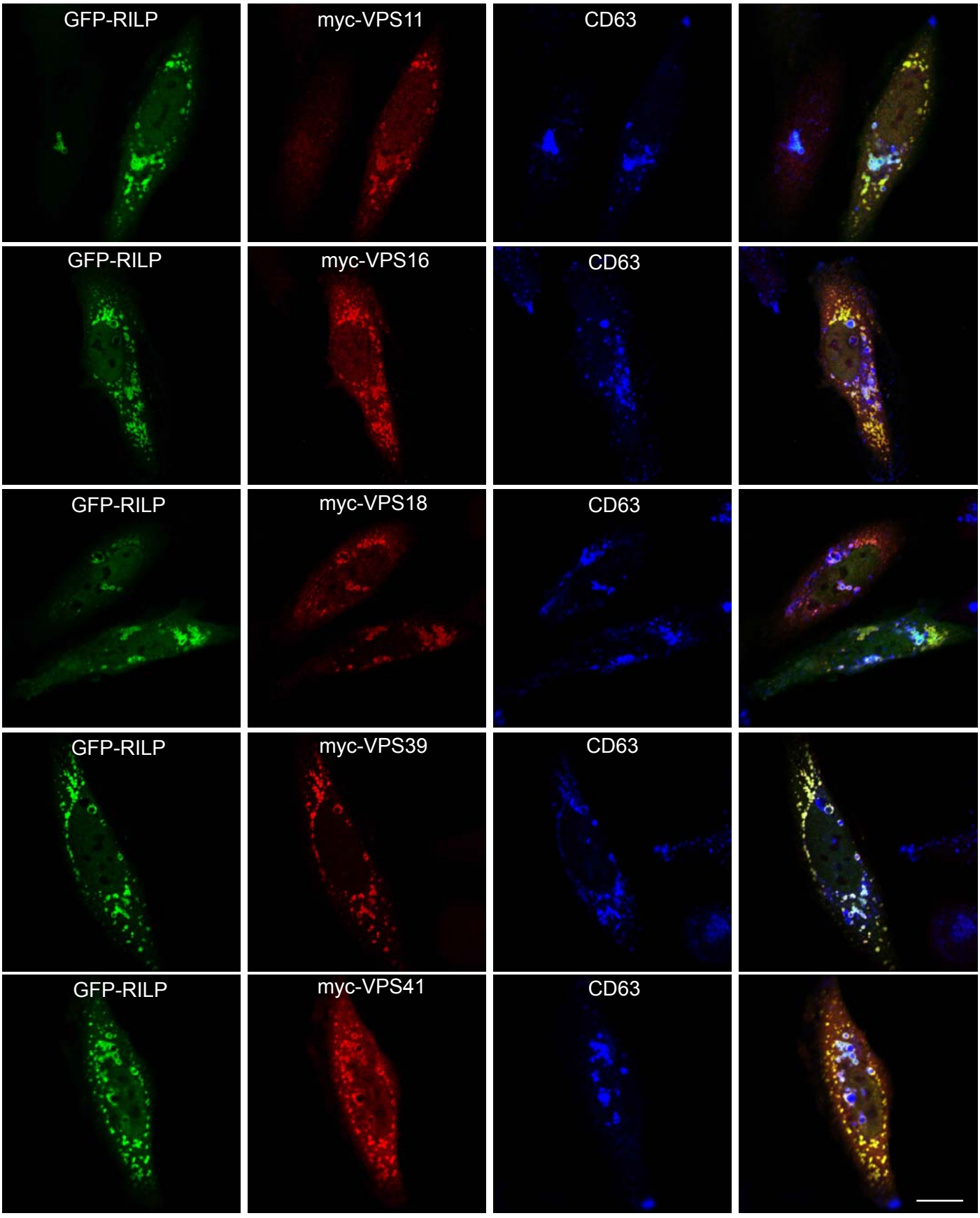

GFP-RILP
myc-VPS11
CD63
GFP-RILP
myc-VPS16
CD63
GFP-RILP
myc-VPS18
CD63
GFP-RILP
myc-VPS39
CD63
GFP-RILP
myc-VPS41
CD63

Figure s3

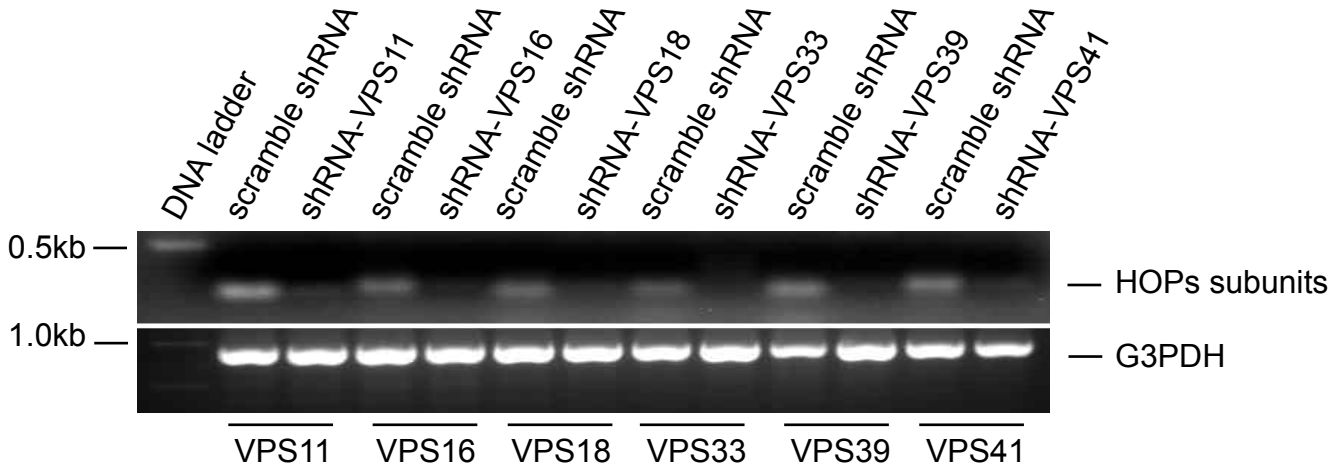

DNA ladder
scramble shRNA
shRNA-VPS11
scramble shRNA
shRNA-VPS16
scramble shRNA
shRNA-VPS18
scramble shRNA
shRNA-VPS33
scramble shRNA
shRNA-VPS39
scramble shRNA
shRNA-VPS41
0.5kb
1.0kb
HOPs subunits
G3PDH
VPS11
VPS16
VPS18
VPS33
VPS39
VPS41

Figure s4

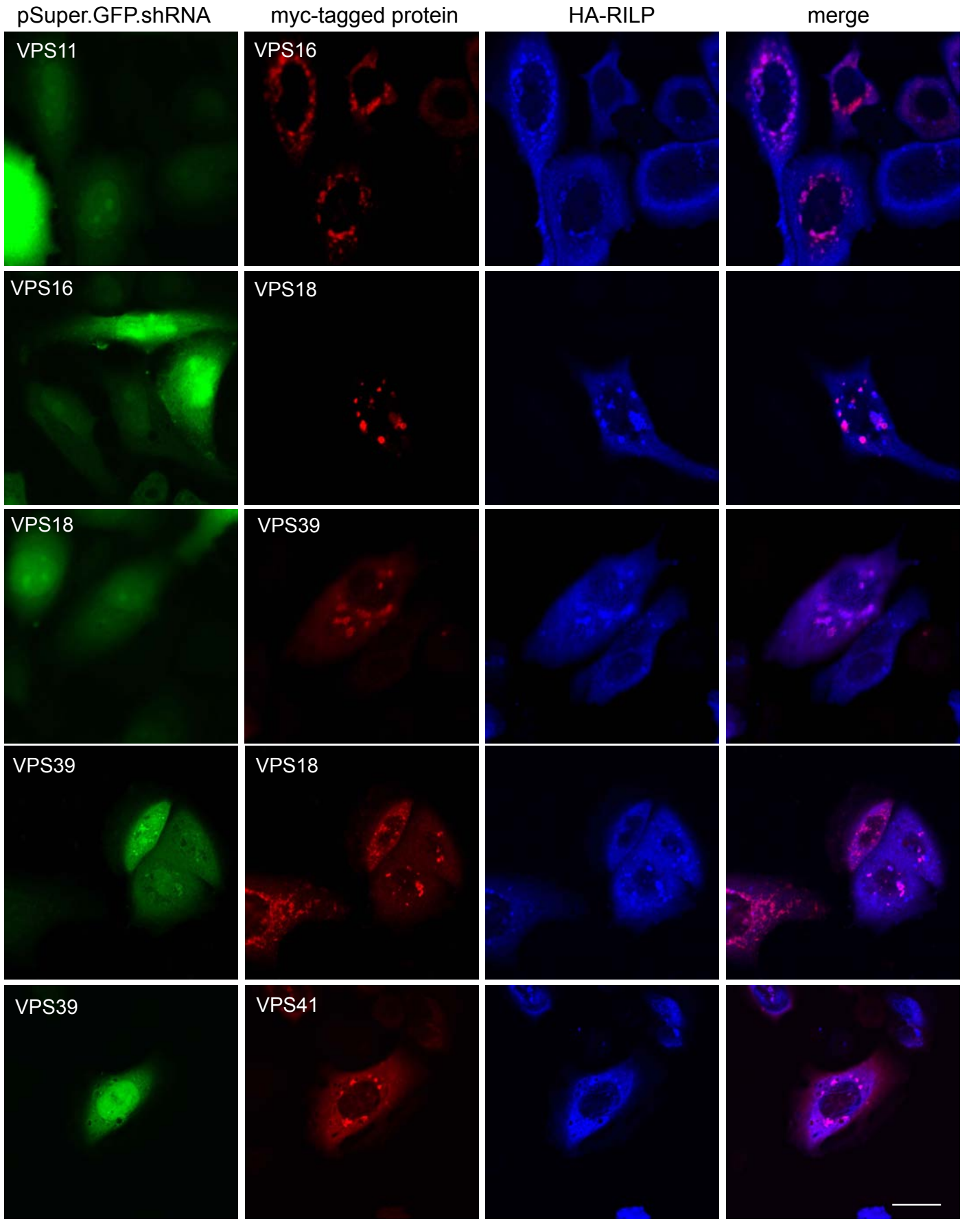

pSuper.GFP.shRNA
myc-tagged protein
HA-RILP
merge
VPS11
VPS16
VPS16
VPS18
VPS18
VPS39
VPS39
VPS18
VPS39
VPS41

Figure s5

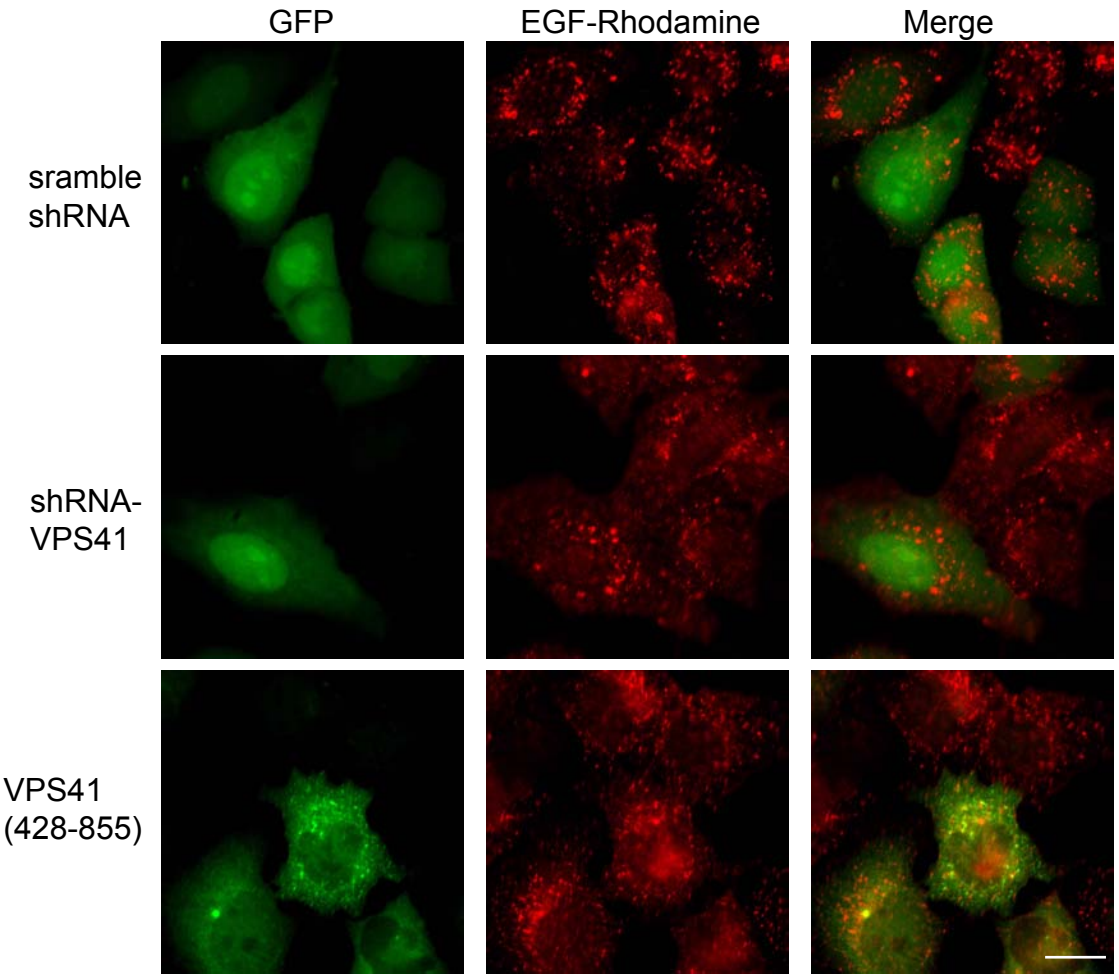

GFP
EGF-Rhodamine
Merge
sramble shRNA
shRNA-VPS41
VPS41 (428-855)

Figure s6

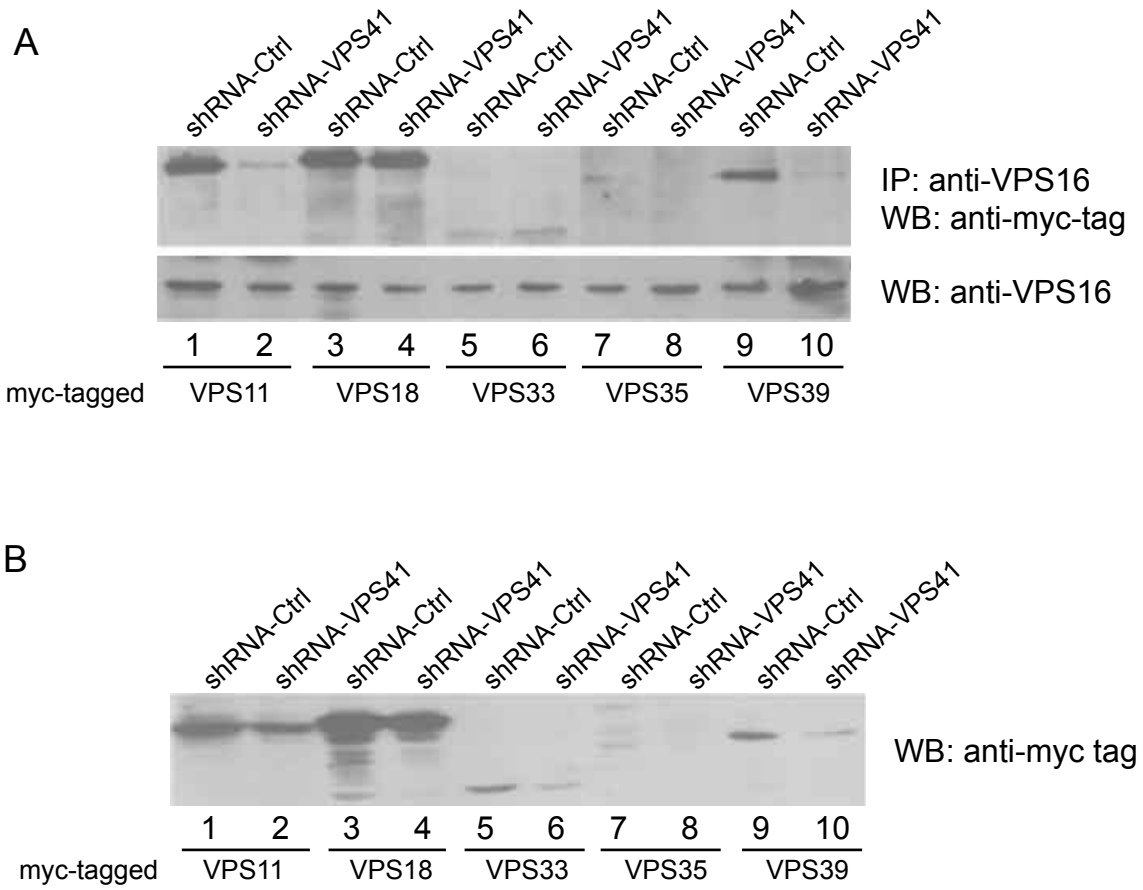

A
shRNA-Ctrl
shRNA-VPS41
IP: anti-VPS16
WB: anti-myc-tag
WB: anti-VPS16
1 2 3 4 5 6 7 8 9 10
myc-tagged
VPS11
VPS18
VPS33
VPS35
VPS39
B
shRNA-Ctrl
shRNA-VPS41
WB: anti-myc tag
1 2 3 4 5 6 7 8 9 10
myc-tagged
VPS11
VPS18
VPS33
VPS35
VPS39

Figure s7

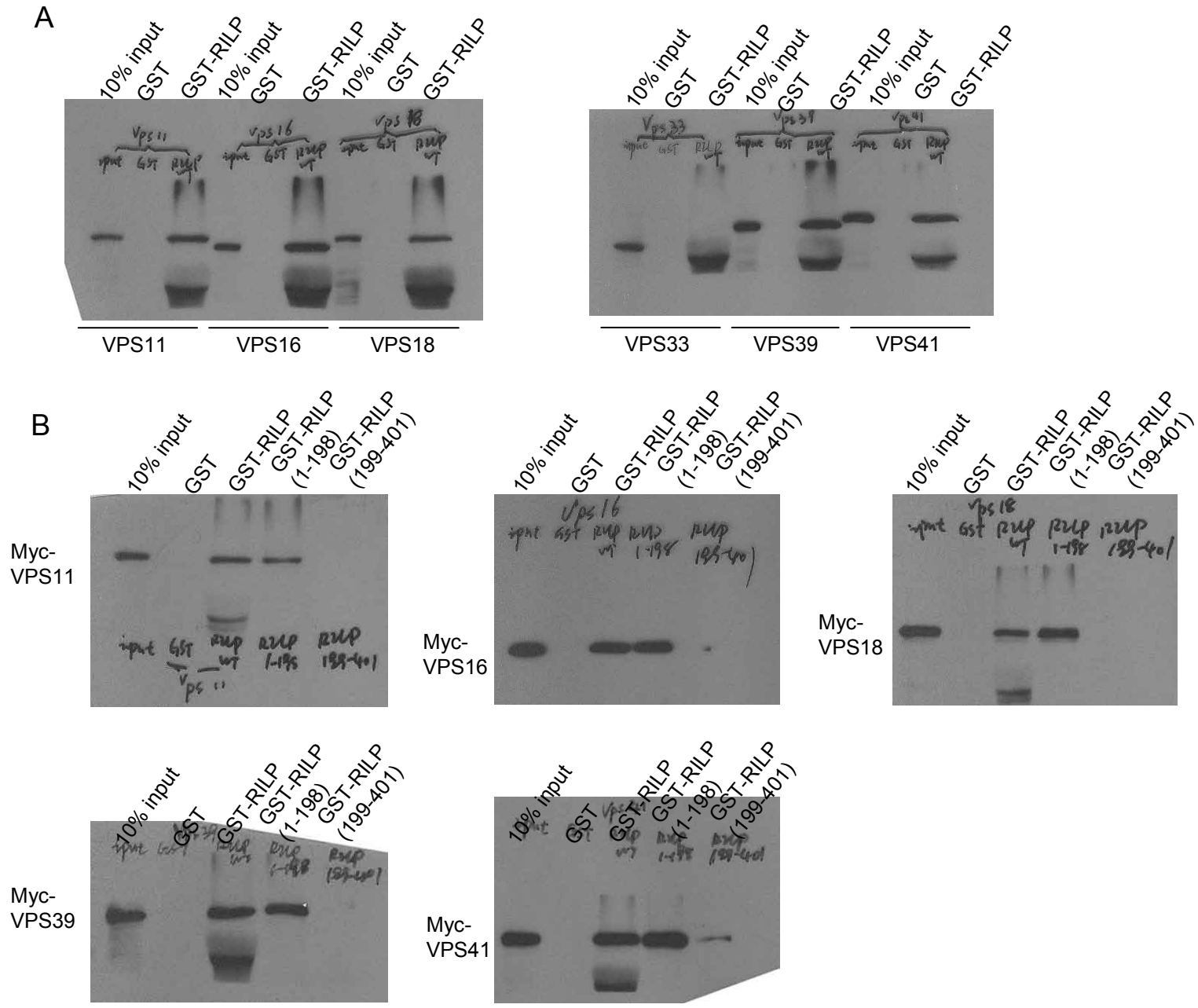

A
10% input
GST
GST-RILP
VPS11
VPS16
VPS18
VPS33
VPS39
VPS41
B
Myc-VPS11
Myc-VPS16
Myc-VPS18
Myc-VPS39
Myc-VPS41
GST-RILP (1-198)
GST-RILP (199-401)

Figure s8

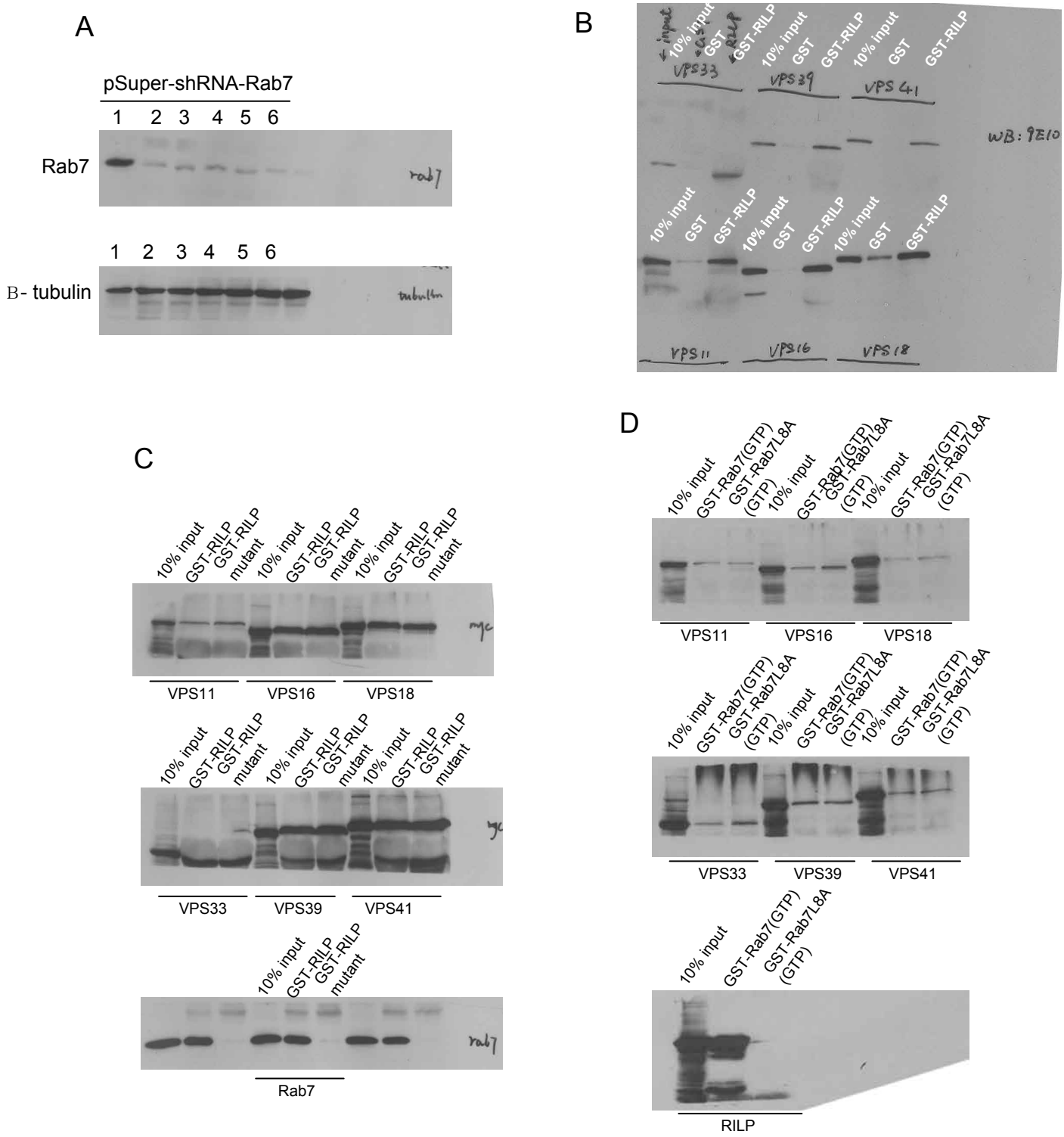
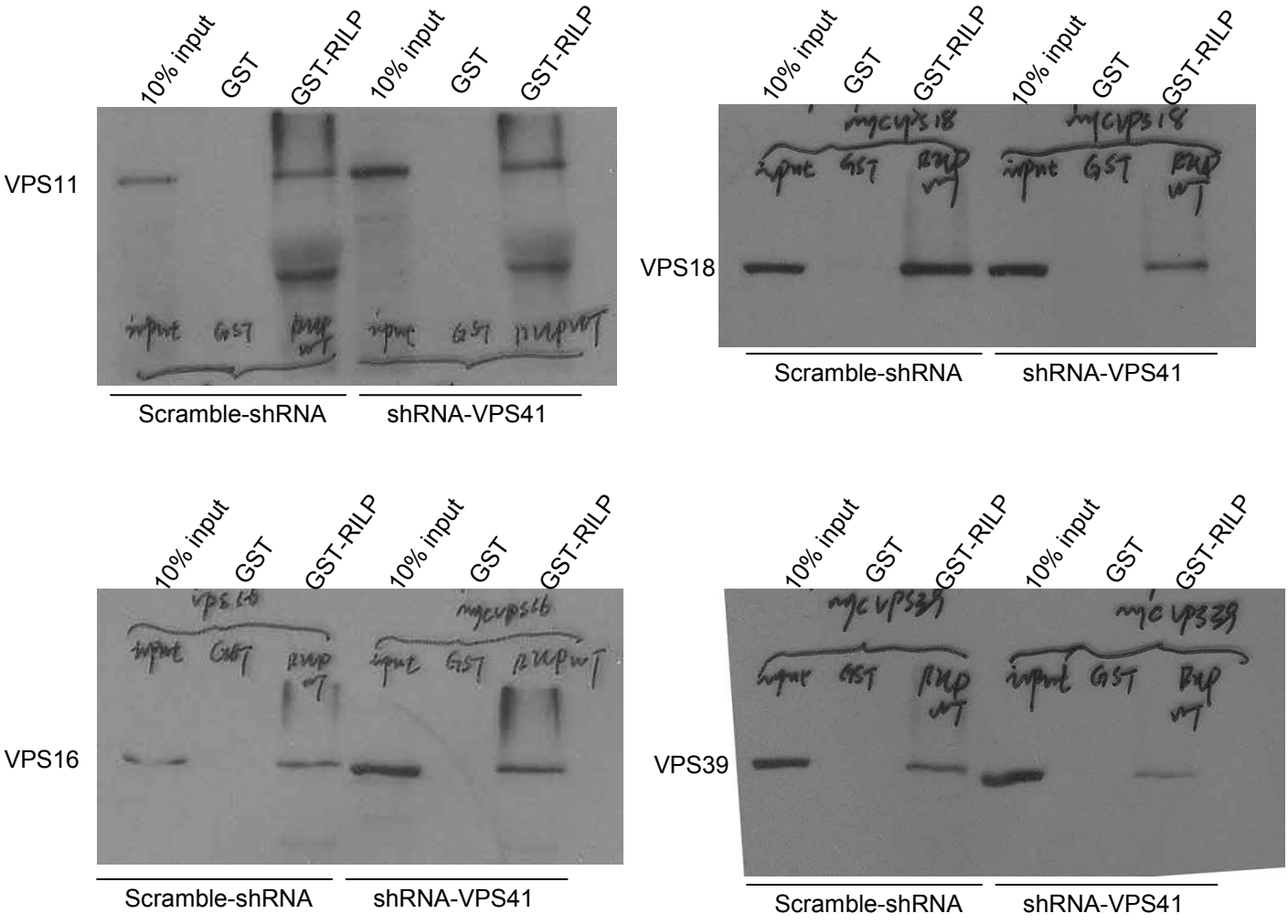

A
pSuper-shRNA-Rab7
1 2 3 4 5 6
Rab7
1 2 3 4 5 6
B- tubulin
B
10% input
GST
GST-RILP
VPS33
VPS39
VPS41
WB: 9E10
VPS11
VPS16
VPS18
C
10% input
GST-RILP
GST-RILP mutant
VPS11
VPS16
VPS18
VPS33
VPS39
VPS41
Rab7
D
10% input
GST-Rab7(GTP)
GST-Rab7L8A (GTP)
VPS11
VPS16
VPS18
VPS33
VPS39
VPS41
RILP

Figure s9


10% input
GST
GST-RILP
10% input
GST
GST-RILP
VPS11
Scramble-shRNA
shRNA-VPS41
10% input
GST
GST-RILP
10% input
GST
GST-RILP
VPS18
Scramble-shRNA
shRNA-VPS41
10% input
GST
GST-RILP
10% input
GST
GST-RILP
VPS16
Scramble-shRNA
shRNA-VPS41
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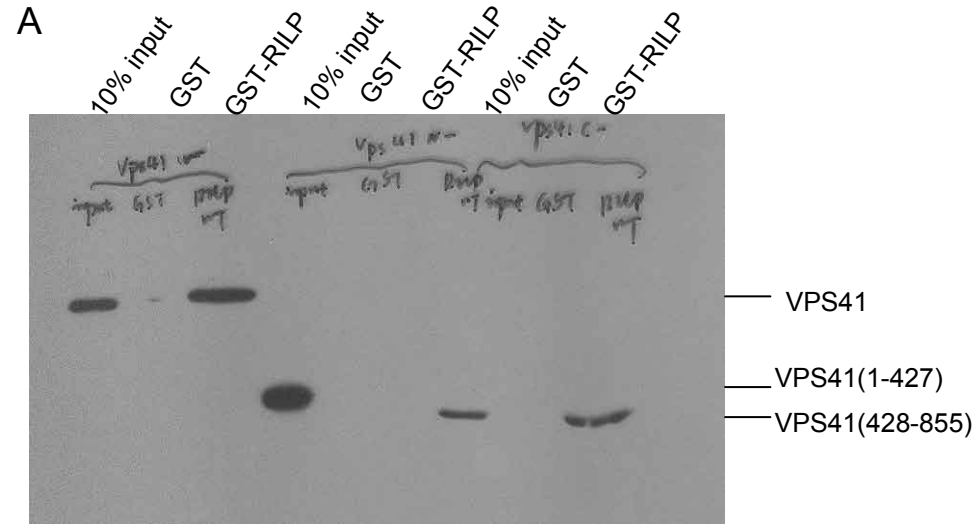
10% input
GST
GST-RILP
VPS39
Scramble-shRNA
shRNA-VPS41

Figure s10


A
10% input
GST
GST-RILP
10% input
GST
GST-RILP
10% input
GST
GST-RILP
VPS41
VPS41(1-427)
VPS41(428-855)

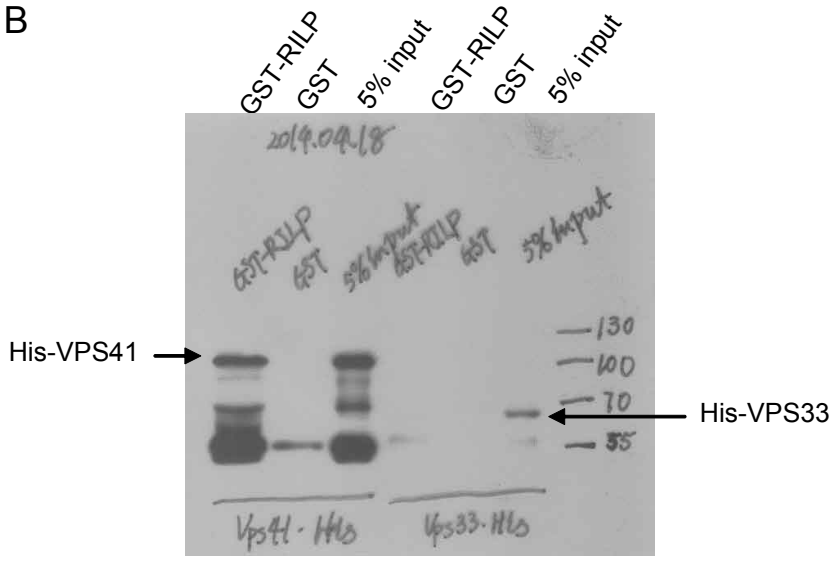
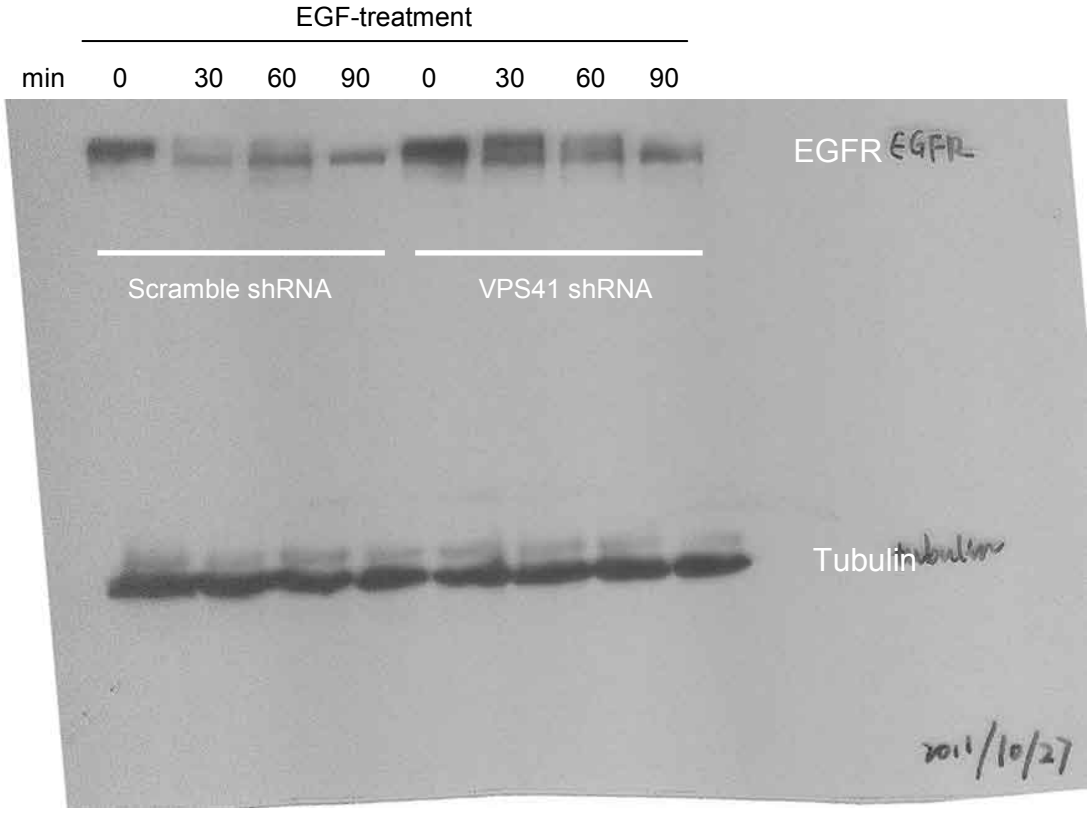

B
GST-RILP
GST
5% input
GST-RILP
GST
5% input
2014.04.18
His-VPS41
130
100
70
55
His-VPS33

Figure s11


EGF-treatment
min 0 30 60 90 0 30 60 90
EGFR
Scramble shRNA
VPS41 shRNA
Tubulin
2011/10/27