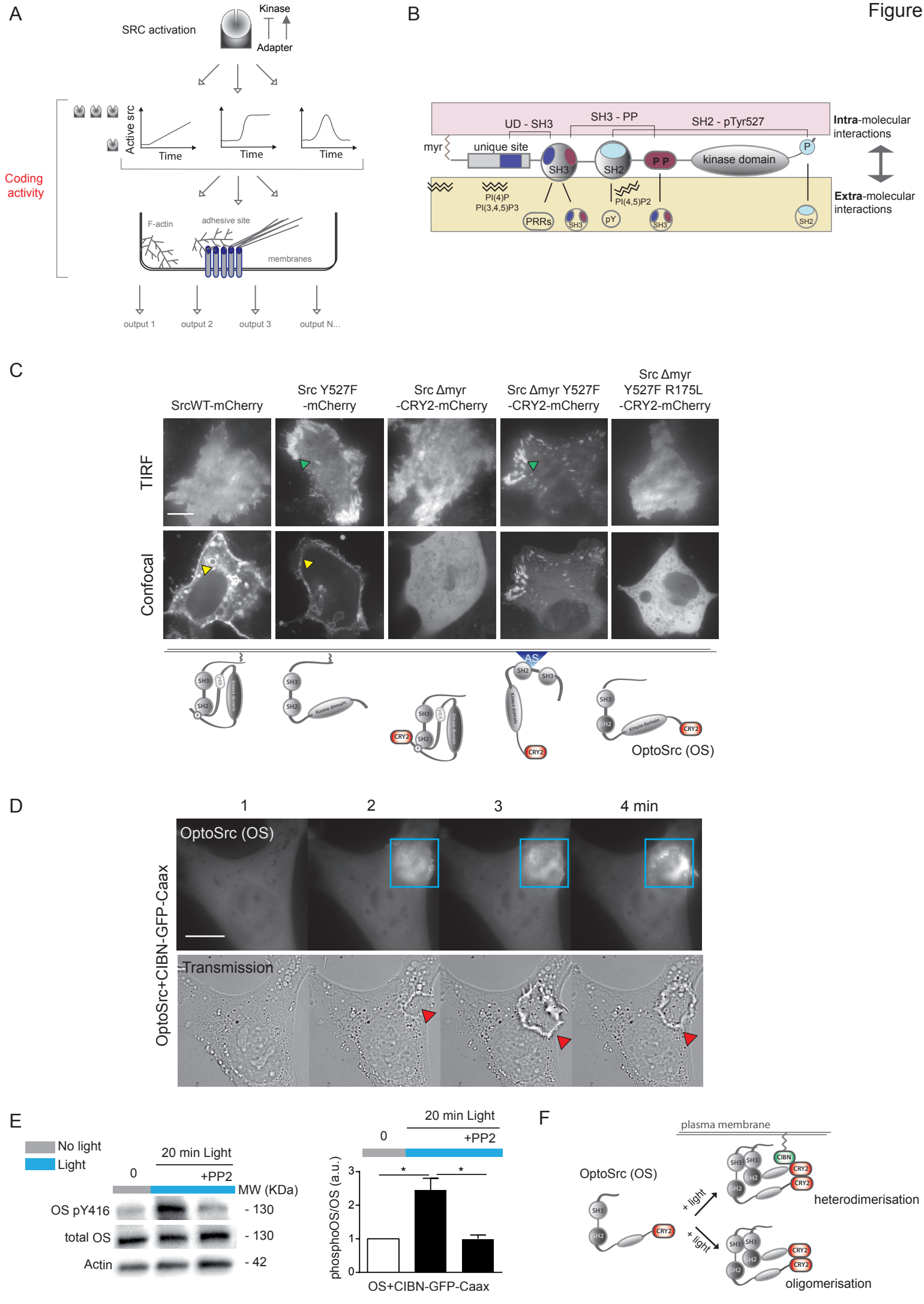
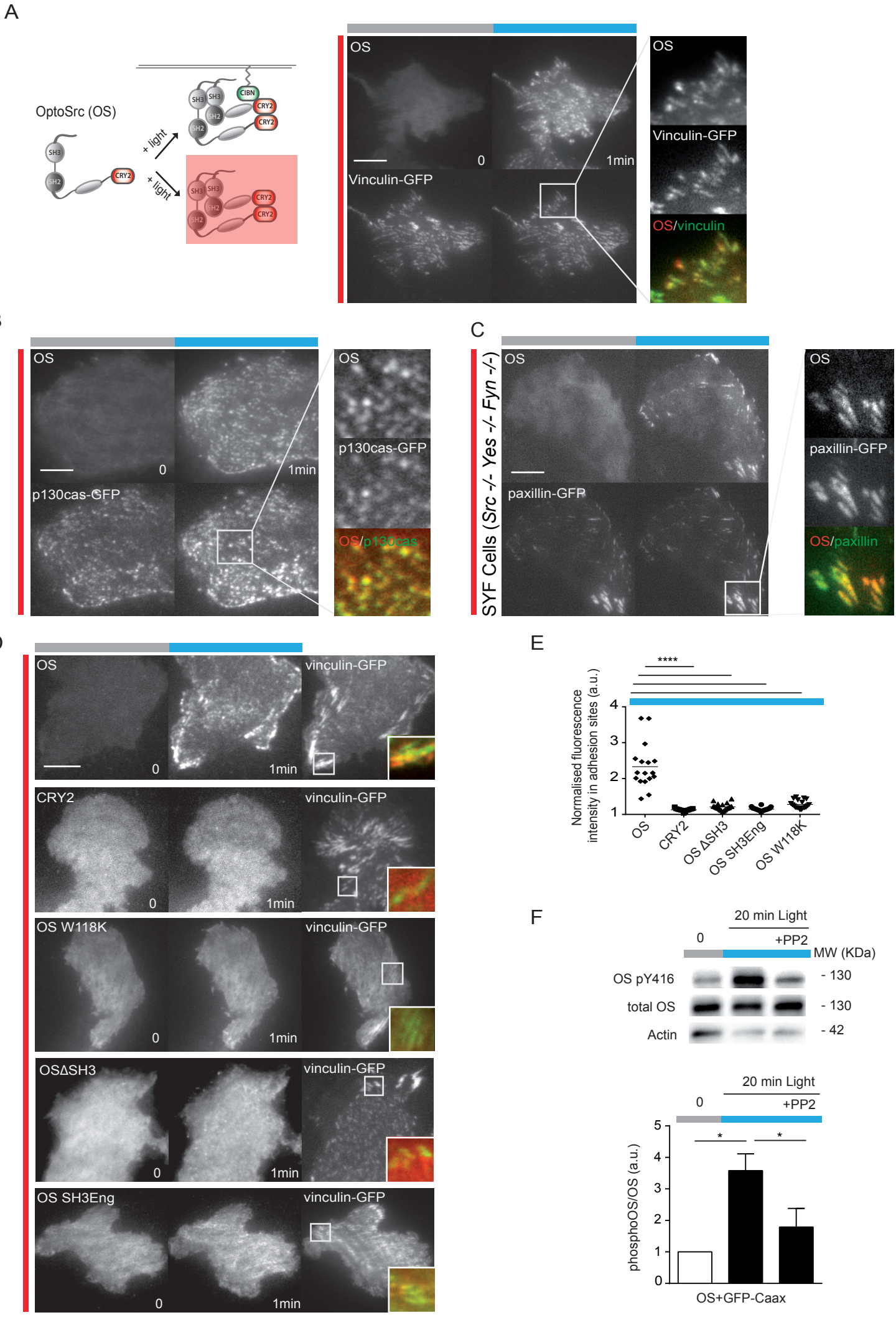
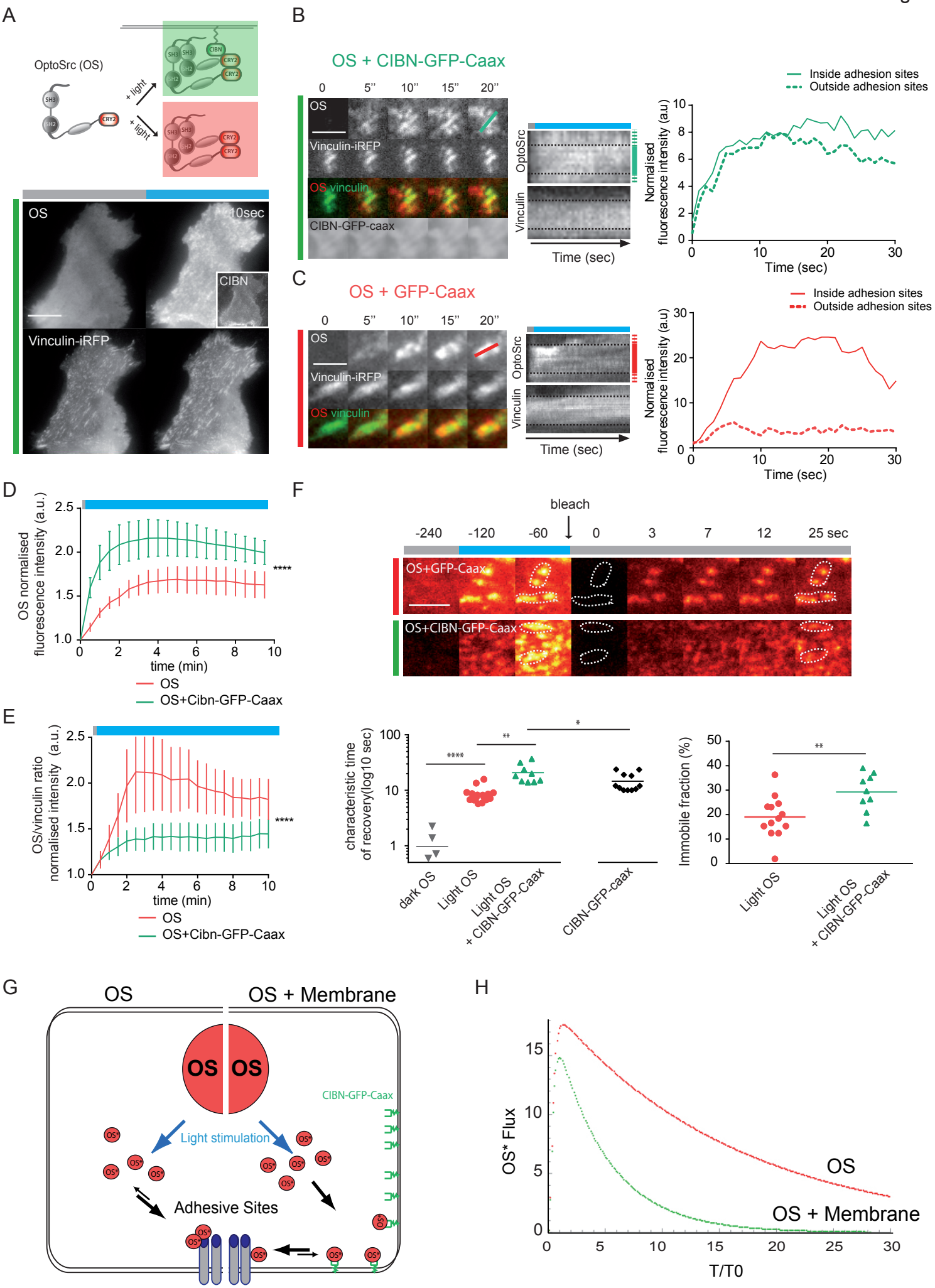
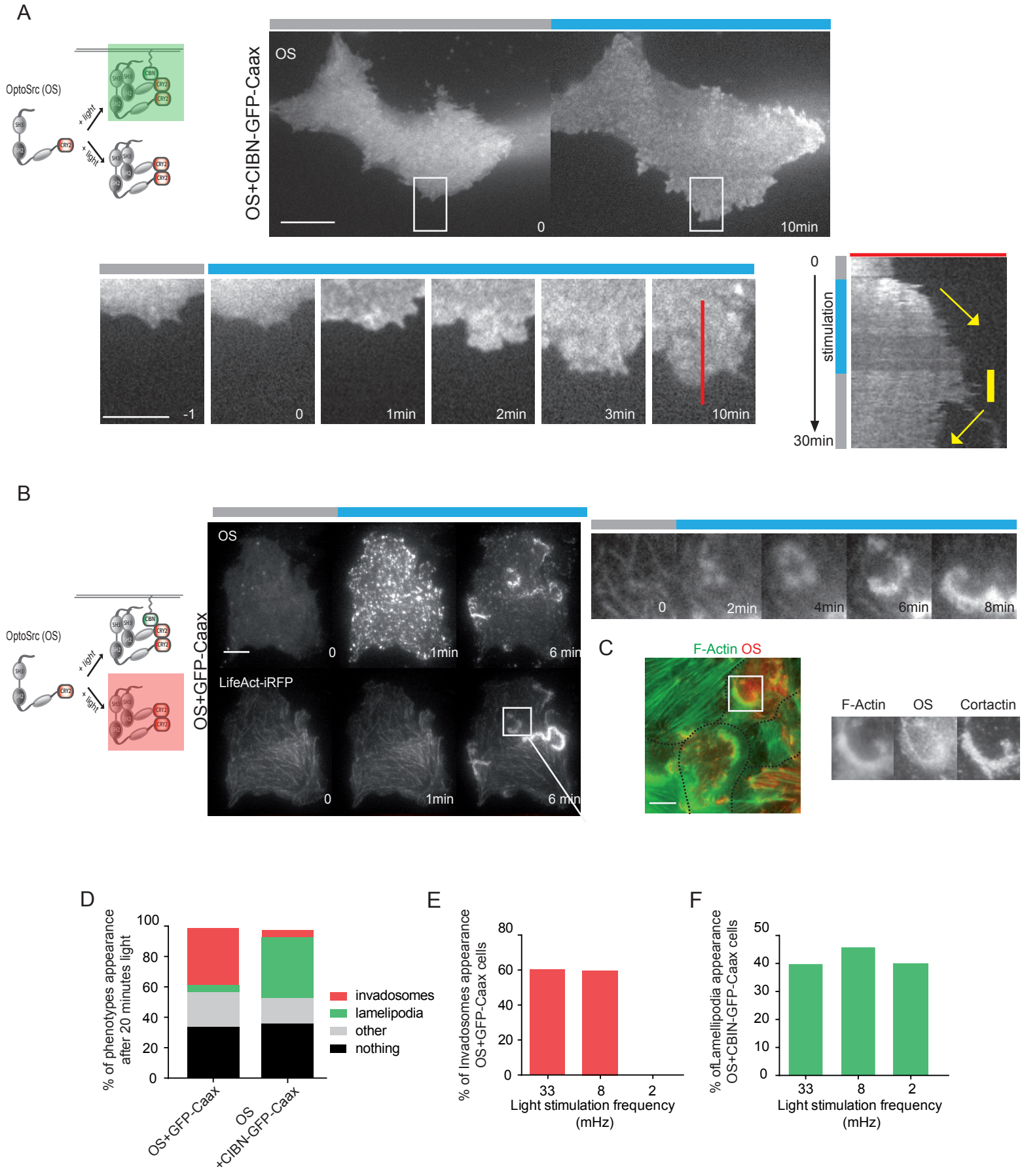
Figure 1


A
SRC activation
Kinase
Adapter
Active src
Time
Time
Time
Coding activity
F-actin
adhesive site
membranes
output 1
output 2
output 3
output N...
B
UD - SH3
SH3 - PP
SH2 - pTyr527
Intra-molecular interactions
myr
unique site
SH3
SH2
P P
kinase domain
P
PI(4)P
PI(3,4,5)P3
PI(4,5)P2
Extra-molecular interactions
PRRs
SH3
pY
SH3
SH2
C
SrcWT-mCherry
Src Y527F -mCherry
Src Δmyr -CRY2-mCherry
Src Δmyr Y527F -CRY2-mCherry
Src Δmyr Y527F R175L -CRY2-mCherry
TIRF
Confocal
AS
CRY2
OptoSrc (OS)
D
1
2
3
4 min
OptoSrc (OS)
OptoSrc+CIBN-GFP-Caax
Transmission
E
No light
Light
20 min Light
0
+PP2
MW (KDa)
OS pY416
- 130
total OS
- 130
Actin
- 42
20 min Light
0
+PP2
phosphoOS/OS (a.u.)
OS+CIBN-GFP-Caax
F
plasma membrane
OptoSrc (OS)
+ light
+ light
CIBN
CRY2
heterodimerisation
oligomerisation


A
OptoSrc (OS)
SH3
SH2
CRY2
CIBN
+ light
+ light
OS
0
1min
Vinculin-GFP
OS
Vinculin-GFP
OS/vinculin
B
OS
0
1min
p130cas-GFP
OS
p130cas-GFP
OS/p130cas
C
SYF Cells (Src -/- Yes -/- Fyn -/-)
OS
paxillin-GFP
OS
paxillin-GFP
OS/paxillin
D
OS
vinculin-GFP
CRY2
OS W118K
OSΔSH3
OS SH3Eng
0
1min
E
Normalised fluorescence intensity in adhesion sites (a.u.)
****
OS
CRY2
OS ΔSH3
OS SH3Eng
OS W118K
F
20 min Light
0
+PP2
MW (KDa)
OS pY416
total OS
Actin
- 130
- 130
- 42
phosphoOS/OS (a.u.)
OS+GFP-Caax


A
OptoSrc (OS)
+ light
+ light
OS
10sec
CIBN
Vinculin-iRFP
B
OS + CIBN-GFP-Caax
0
5"
10"
15"
20"
OS
Vinculin-iRFP
OS vinculin
CIBN-GFP-caax
OptoSrc
Vinculin
Time (sec)
Inside adhesion sites
Outside adhesion sites
Normalised fluorescence intensity (a.u)
Time (sec)
C
OS + GFP-Caax
0
5"
10"
15"
20"
OS
Vinculin-iRFP
OS vinculin
OptoSrc
Vinculin
Time (sec)
Inside adhesion sites
Outside adhesion sites
Normalised fluorescence intensity (a.u)
Time (sec)
D
OS normalised fluorescence intensity (a.u.)
time (min)
****
OS
OS+Cibn-GFP-Caax
E
OS/vinculin ratio normalised intensity (a.u.)
time (min)
****
OS
OS+Cibn-GFP-Caax
F
bleach
-240
-120
-60
0
3
7
12
25 sec
OS+GFP-Caax
OS+CIBN-GFP-Caax
characteristic time of recovery(log10 sec)
****
**
*
dark OS
Light OS
Light OS + CIBN-GFP-Caax
CIBN-GFP-caax
Immobile fraction (%)
**
Light OS
Light OS + CIBN-GFP-Caax
G
OS
OS + Membrane
OS
OS
CIBN-GFP-Caax
Light stimulation
Adhesive Sites
H
OS* Flux
OS
OS + Membrane
T/T0


A
OptoSrc (OS)
+ light
- light
OS+CIBN-GFP-Caax
OS
0
10min
-1
0
1min
2min
3min
10min
0
stimulation
30min
B
OptoSrc (OS)
+ light
- light
OS+GFP-Caax
OS
0
1min
6 min
LifeAct-iRFP
0
1min
6 min
0
2min
4min
6min
8min
C
F-Actin OS
F-Actin
OS
Cortactin
D
% of phenotypes appearance after 20 minutes light
100
80
60
40
20
0
OS+GFP-Caax
OS +CIBN-GFP-Caax
invadosomes
lamelipodia
other
nothing
E
% of Invadosomes appearance OS+GFP-Caax cells
80
60
40
20
0
33
8
2
Light stimulation frequency (mHz)
F
% ofLamellipodia appearance OS+CBIN-GFP-Caax cells
50
40
30
20
10
0
33
8
2
Light stimulation frequency (mHz)

A

OS + GFP-Caax

PY-proteins at steady state (dark)

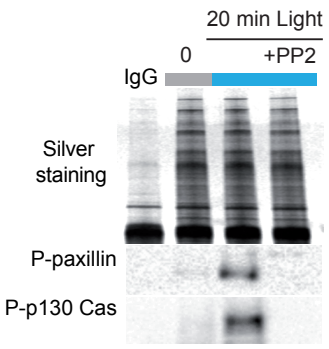
PY-proteins 5min → PY-proteins 20min

OS + CIBN-GFP-Caax

PY-proteins 5min → PY-proteins 20min

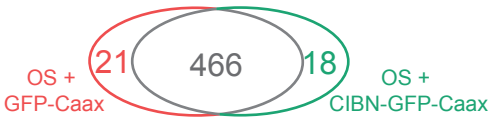
B

OS + GFP-Caax



C

PY-proteins at non stimulated state (Dark)



Total PY-proteins identified

506

90

OS-sensitive PY-proteins

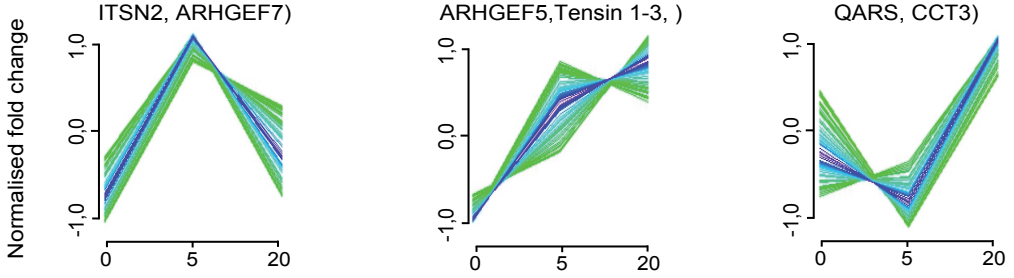
D

OS + GFP-Caax

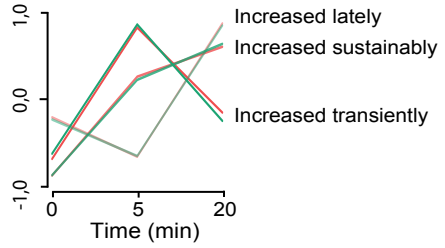
Increased transiently
(MAP3K7, Sgk269 ITSN2, ARHGEF7)

Increased sustainably
(OS, p130Cas, paxillin, Sgk223, ARHGEF5,Tensin 1-3,)

Increased lately
(PPP2R3A, EF-Tu QARS, CCT3)



Averaged tendency curves

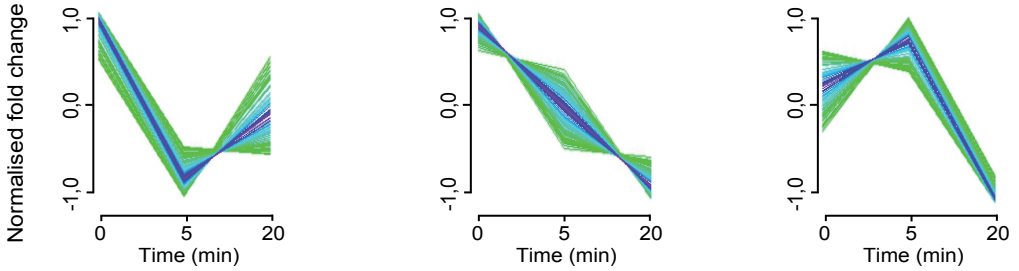


OS + GFP-Caax
OS + CIBN-GFP-Caax

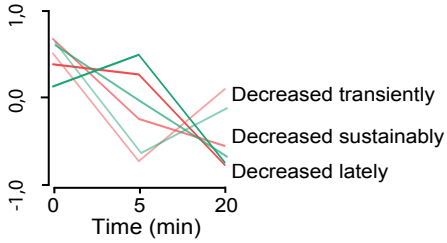
Decreased transiently
(SH3BP4, ARHGAP31, SIPA1L3, PPP2R1A, RAPGEF6)

Decreased sustainably
(p120Cas, GMPR2, KARS)

Decreased lately
(PPP1C, EPHB2, EIF5B, COPG1, EndophilinA2)



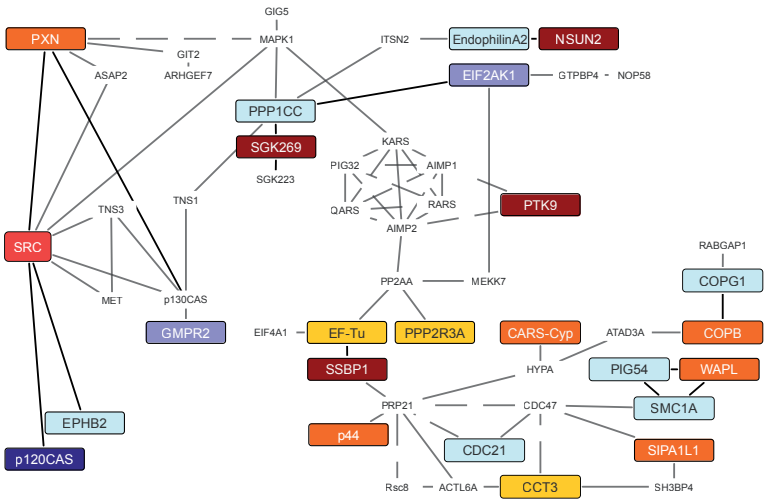
Averaged tendency curves



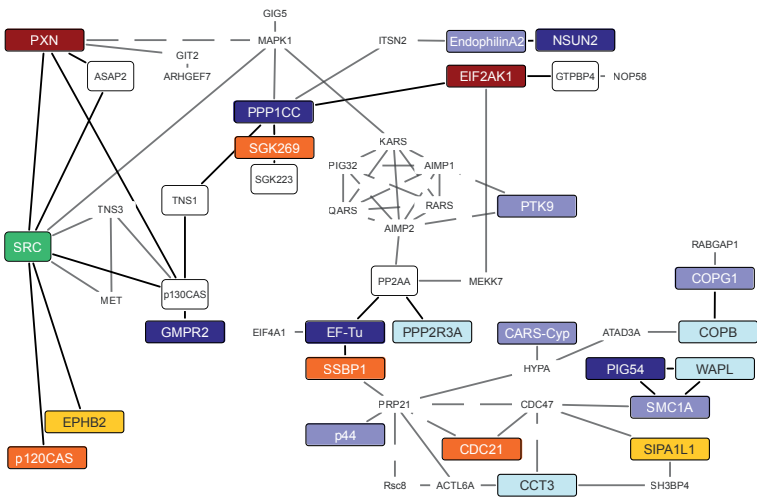
E

Network and changed behaviors of OS-sensitive proteins

OS + GFP-Caax



OS + CIBN-GFP-Caax



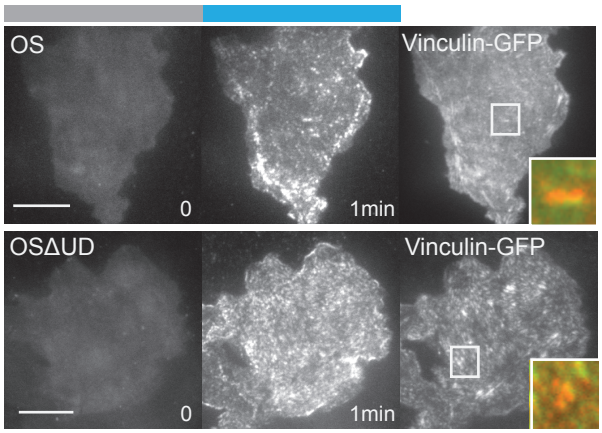
Behaviors of modulated nodes

- Decrease transiently
- Increase transiently
- Decrease sustainably
- Increase sustainably
- Decrease lately
- Increase lately

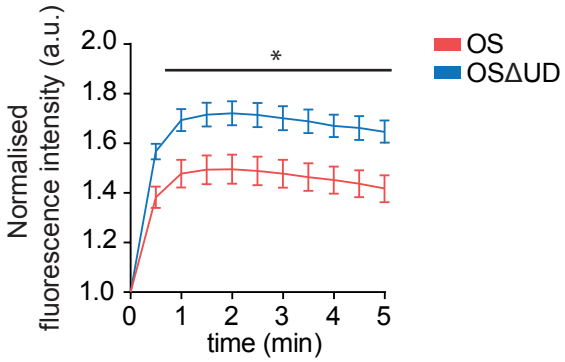
Increase of amplitude

X

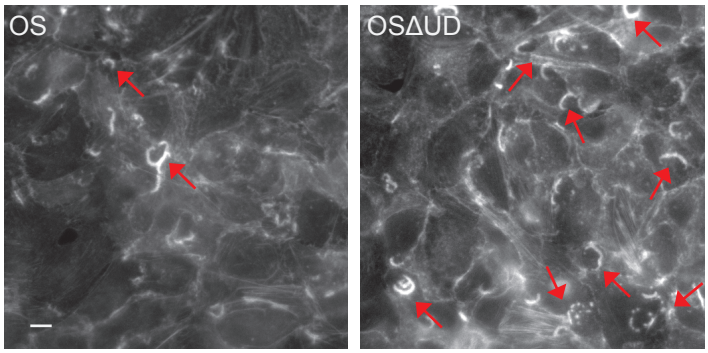
A



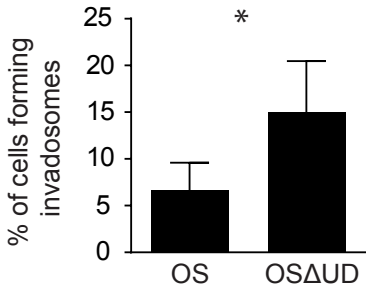
B

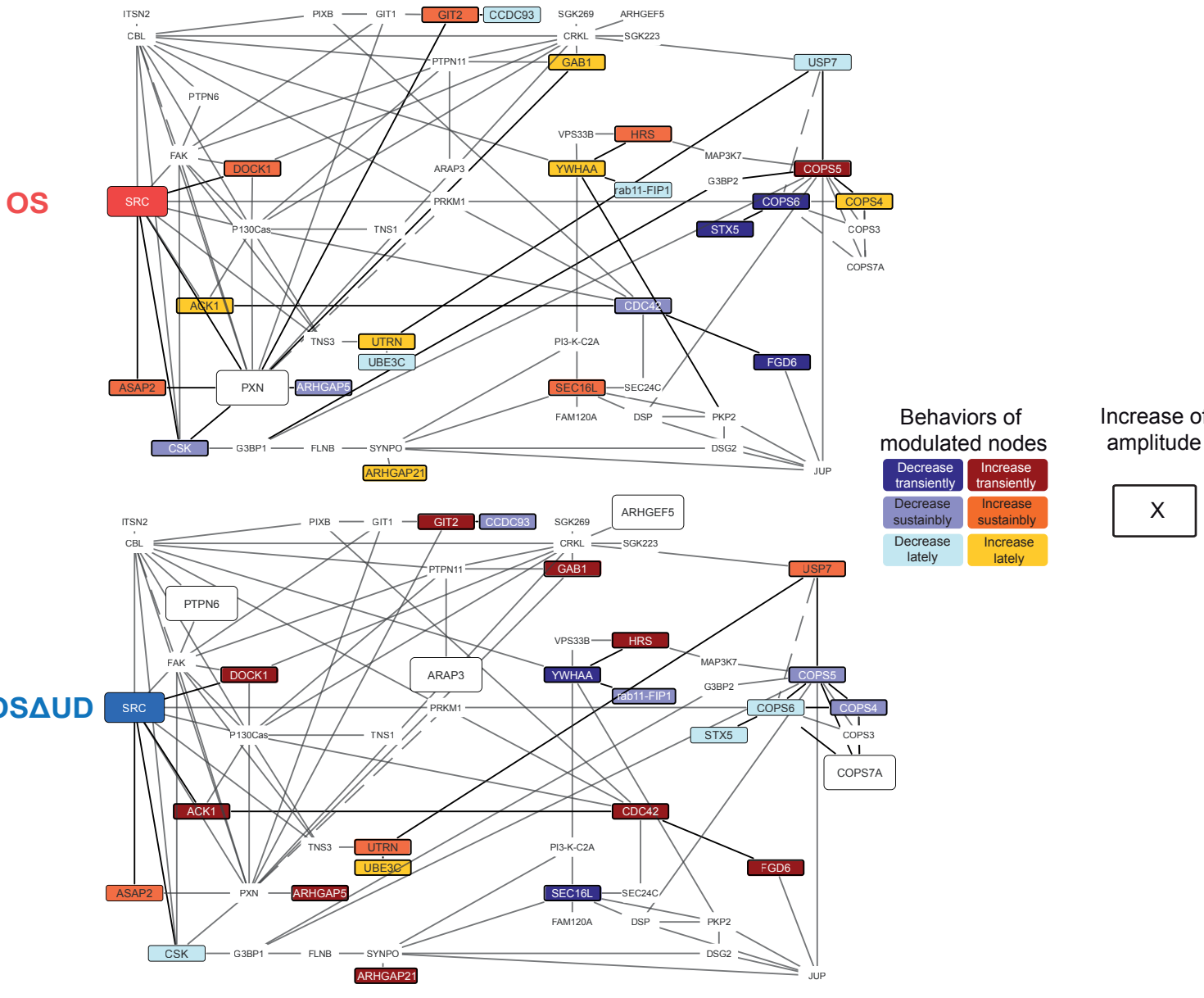


C



D



E

Network and changed behaviors of OS-sensitive proteins