UNFOLDED PROTEIN RESPONSE AND KDELR SIGNALLING

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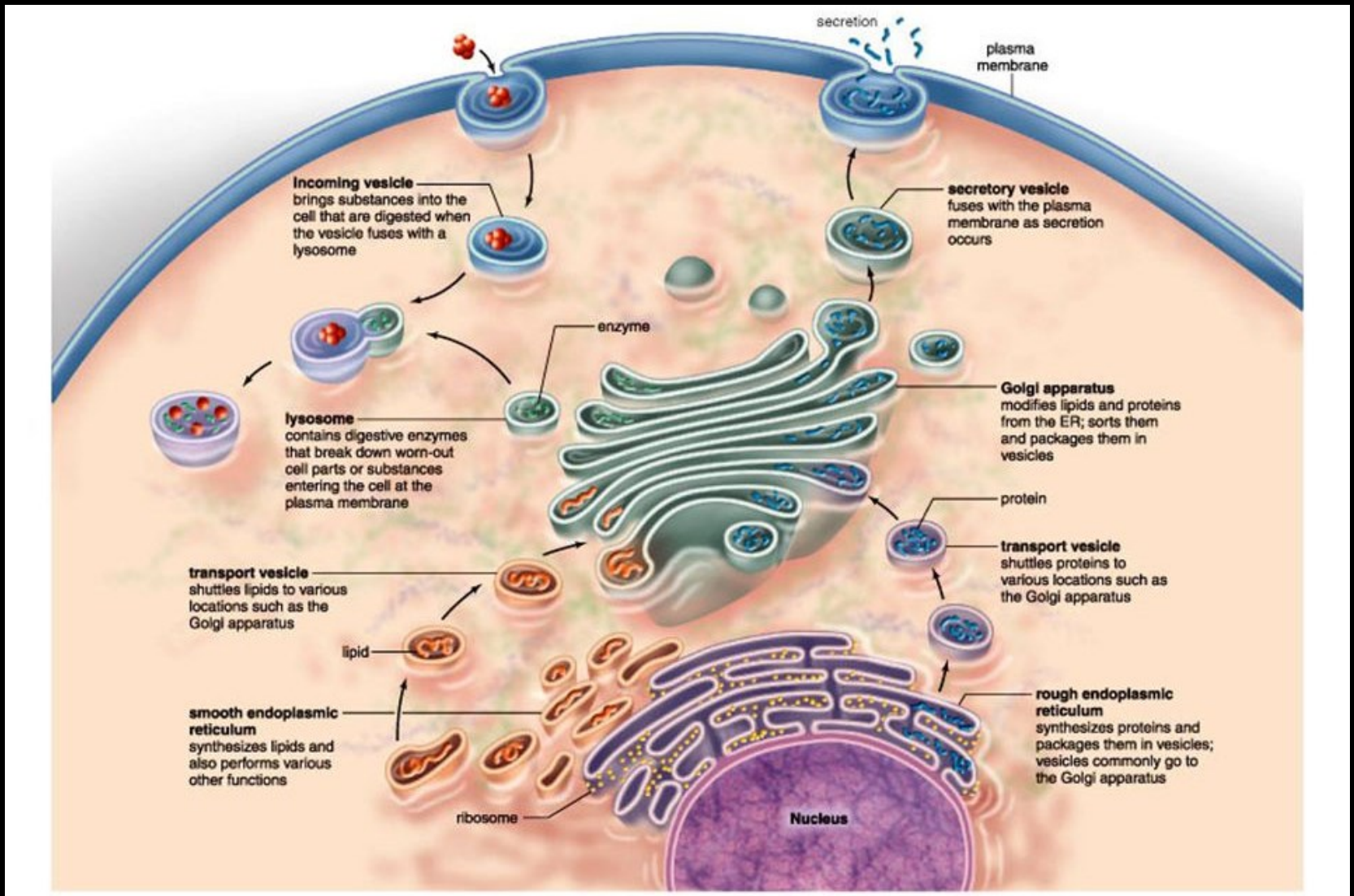
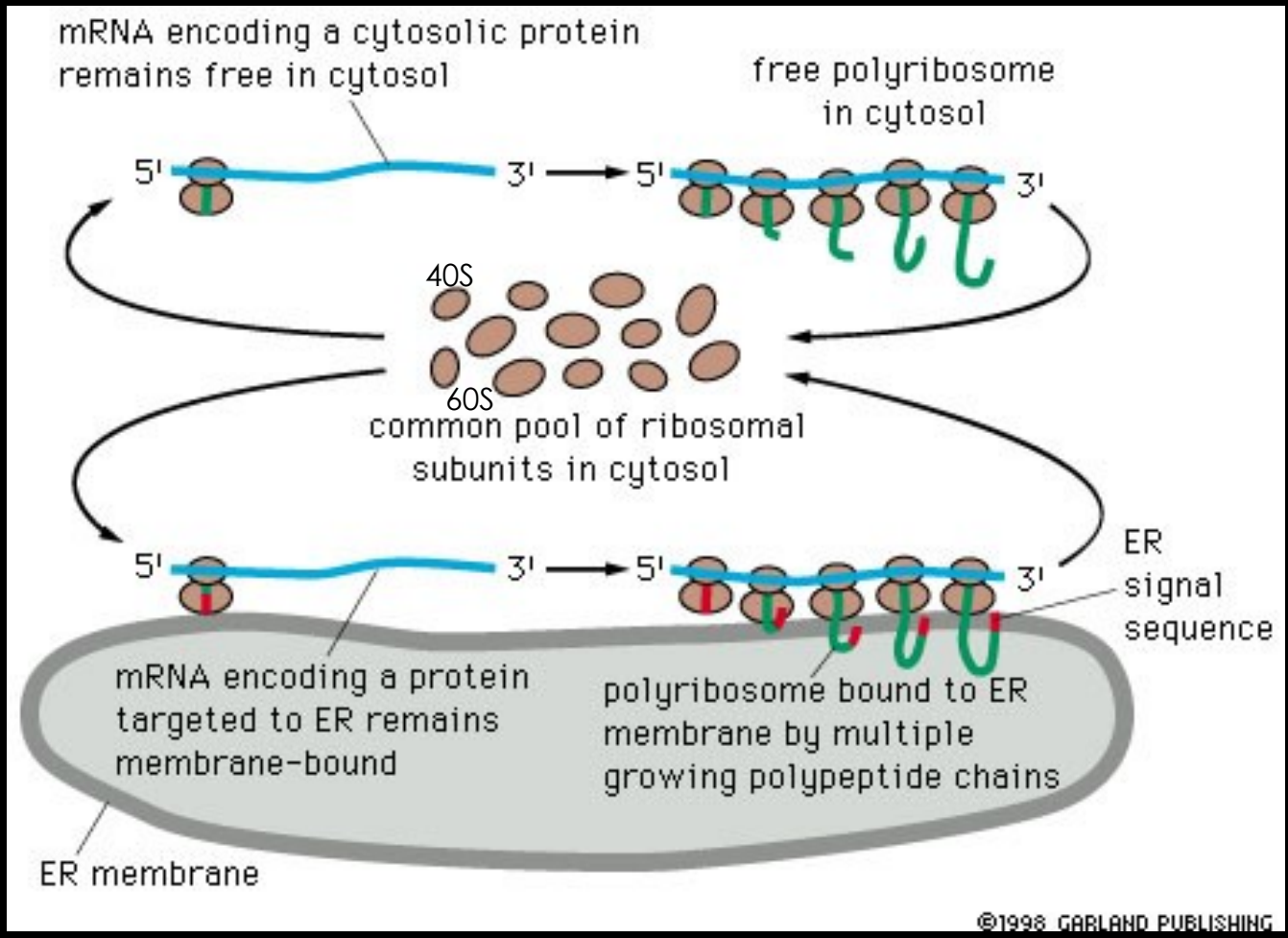
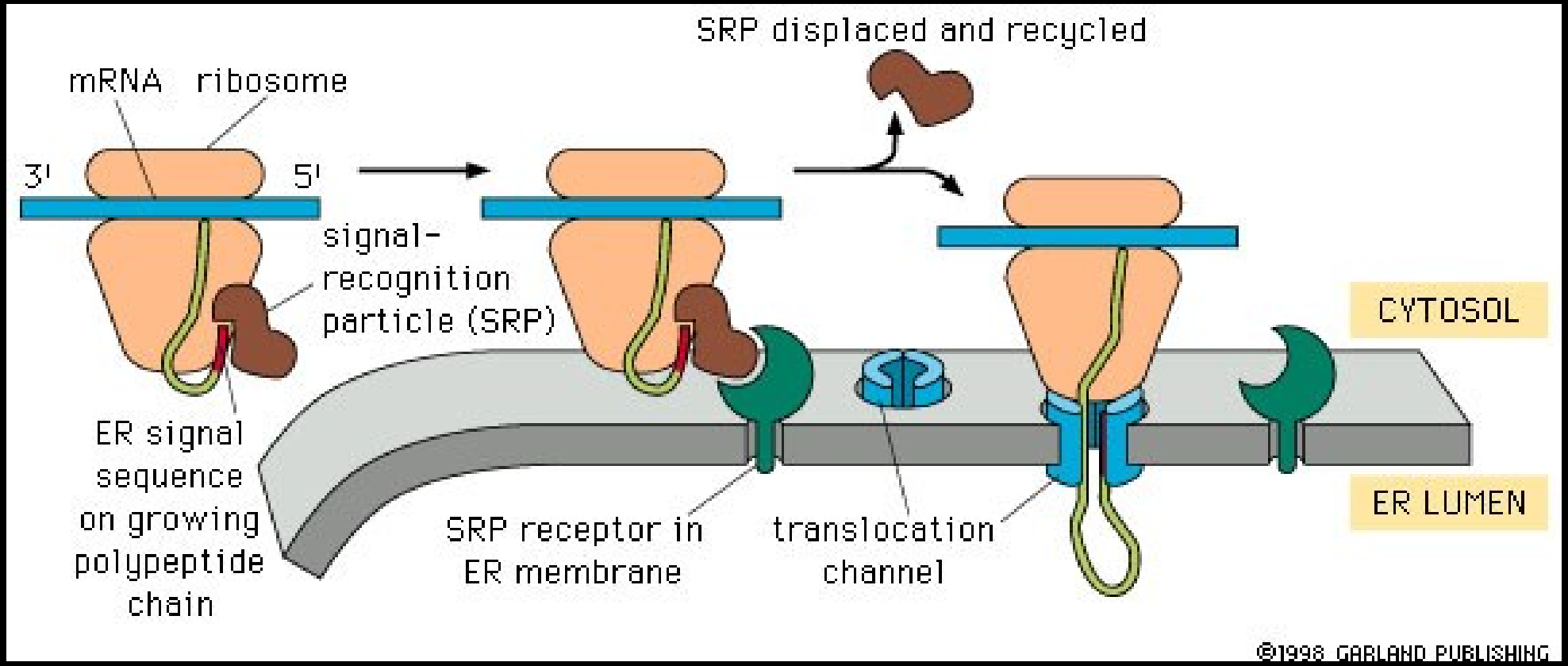
ENDOMEMBRANE SYSTEM



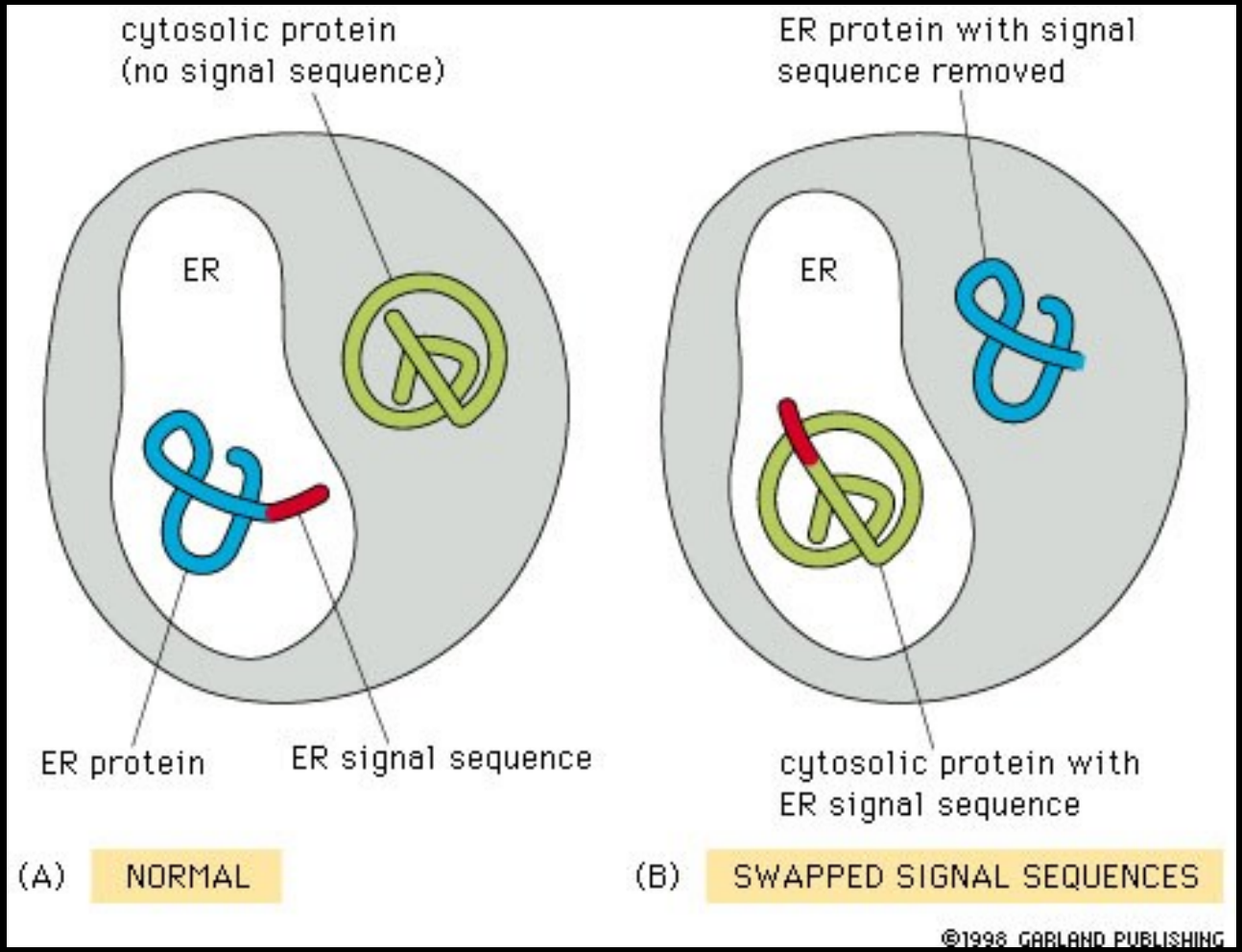
PROTEIN SYNTHESIS



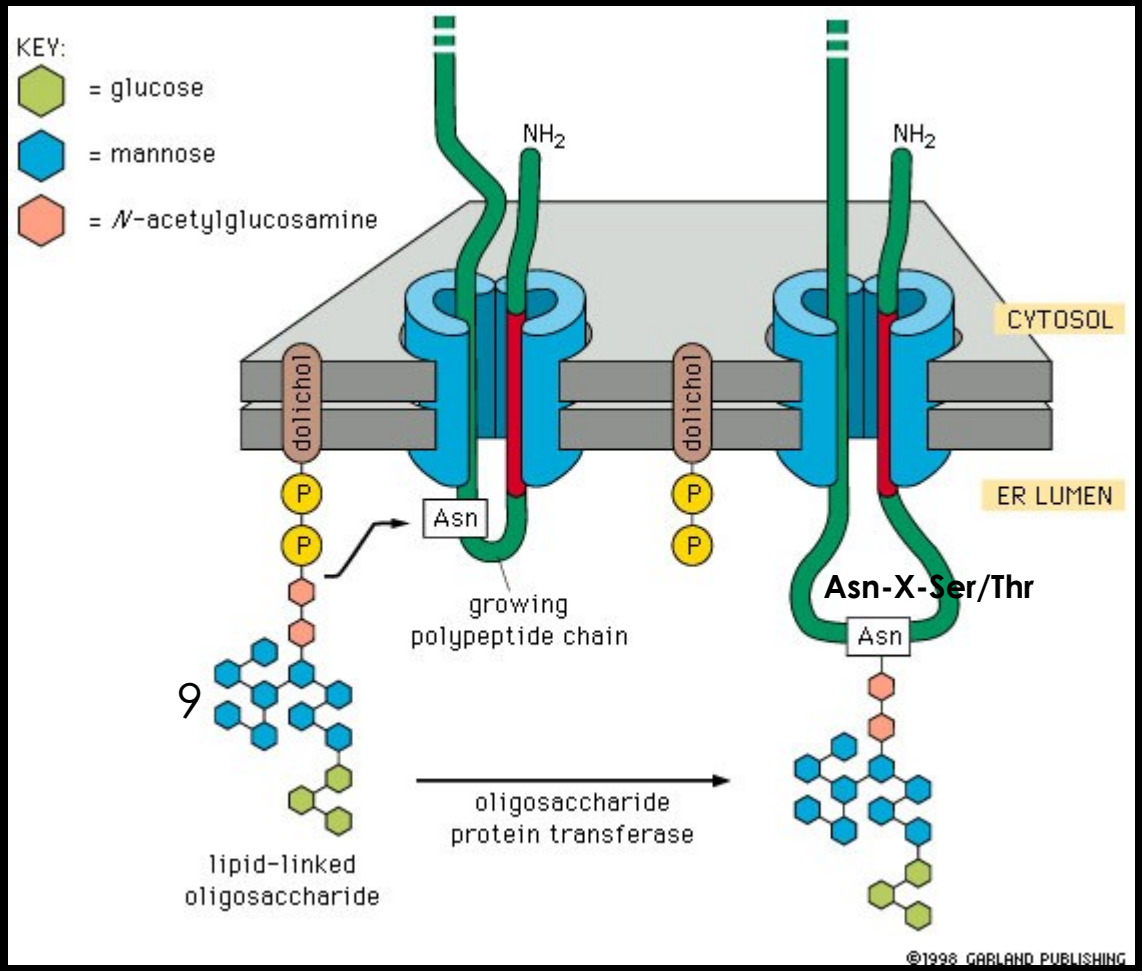
THE SIGNAL RECOGNITION PARTICLE: SRP

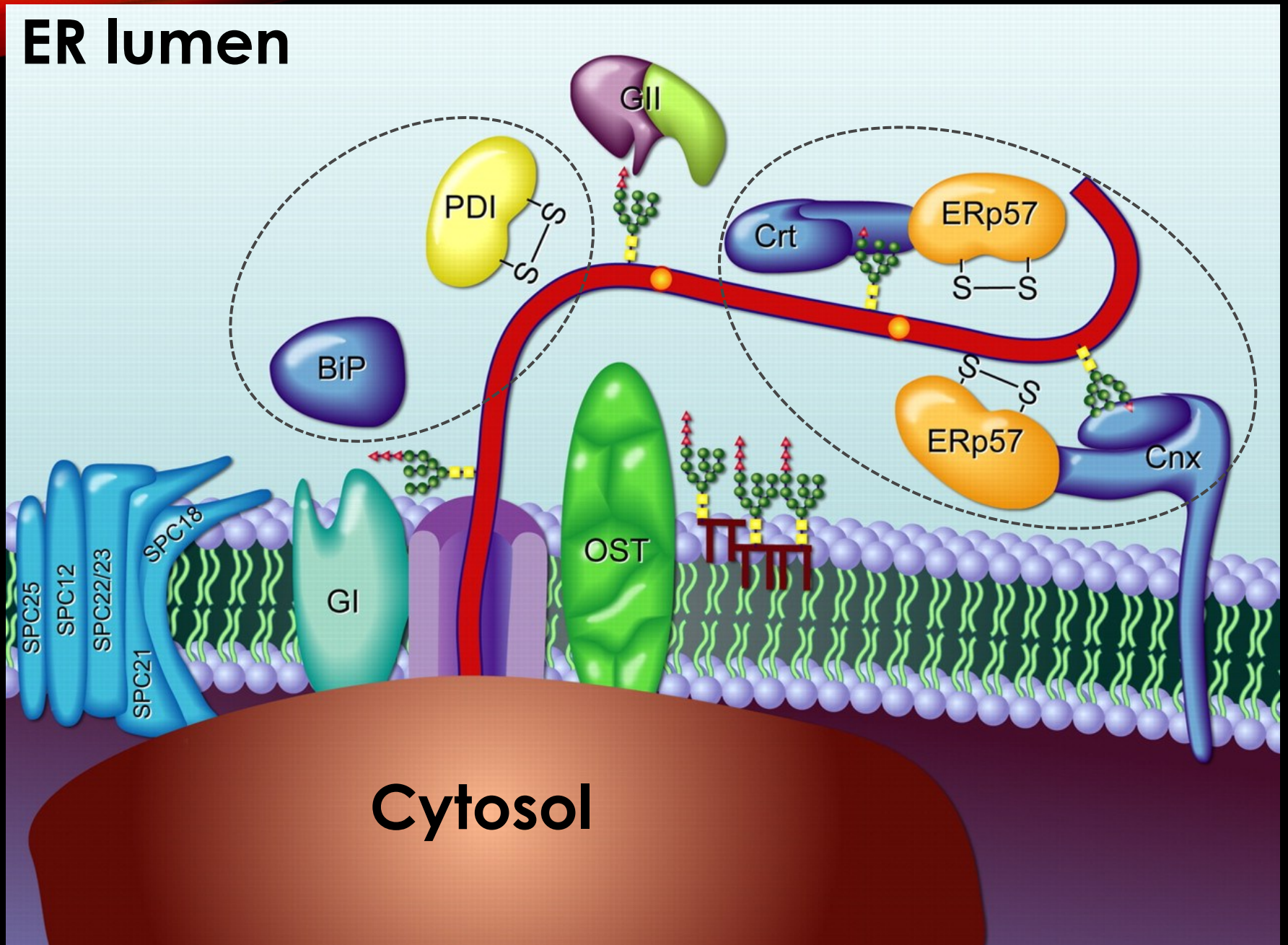


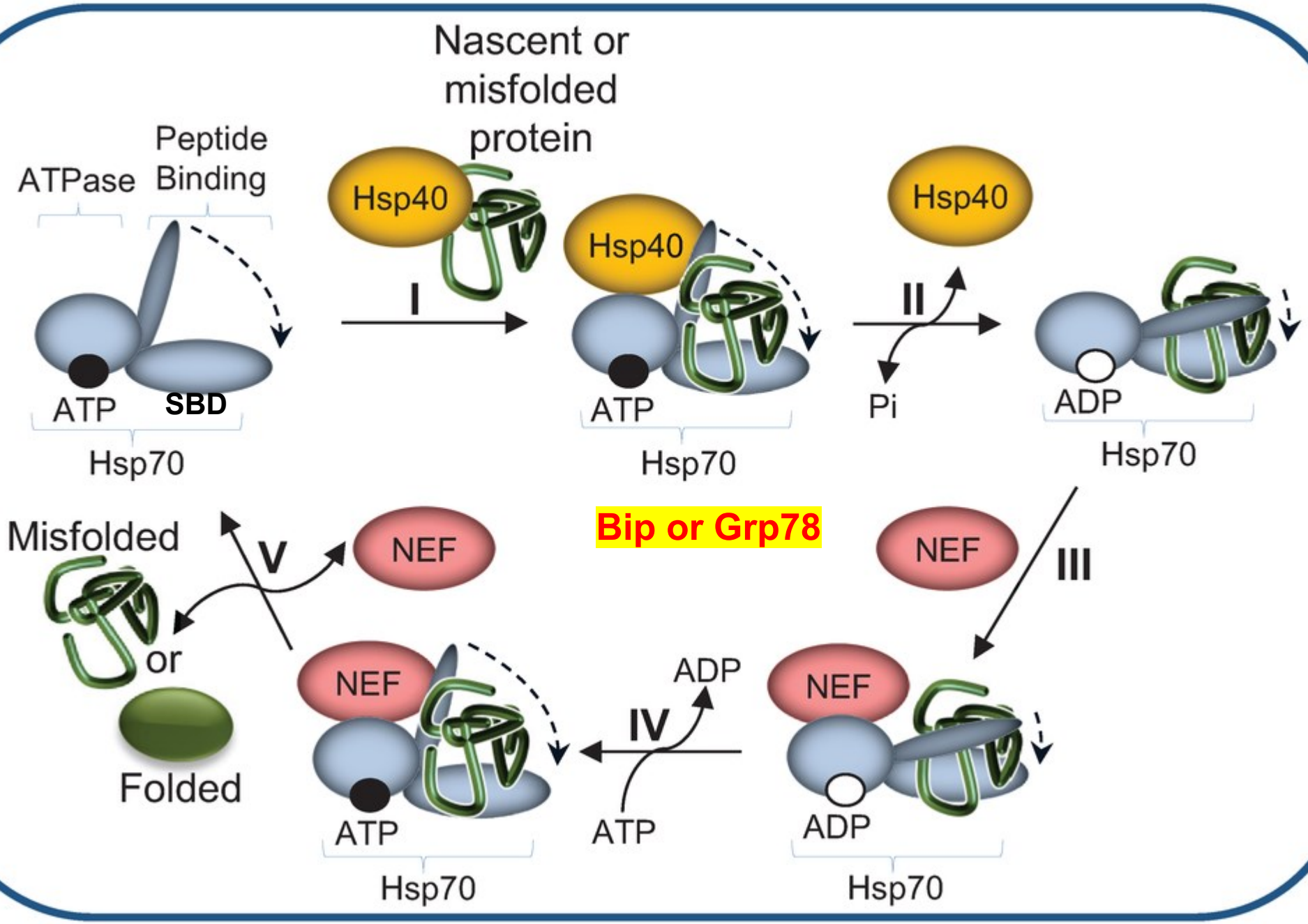
EXPERIMENTAL TEST SHOWING THAT ER TARGETING SIGNAL IS BOTH NECESSARY AND SUFFICIENT TO BRING ABOUT TARGETING

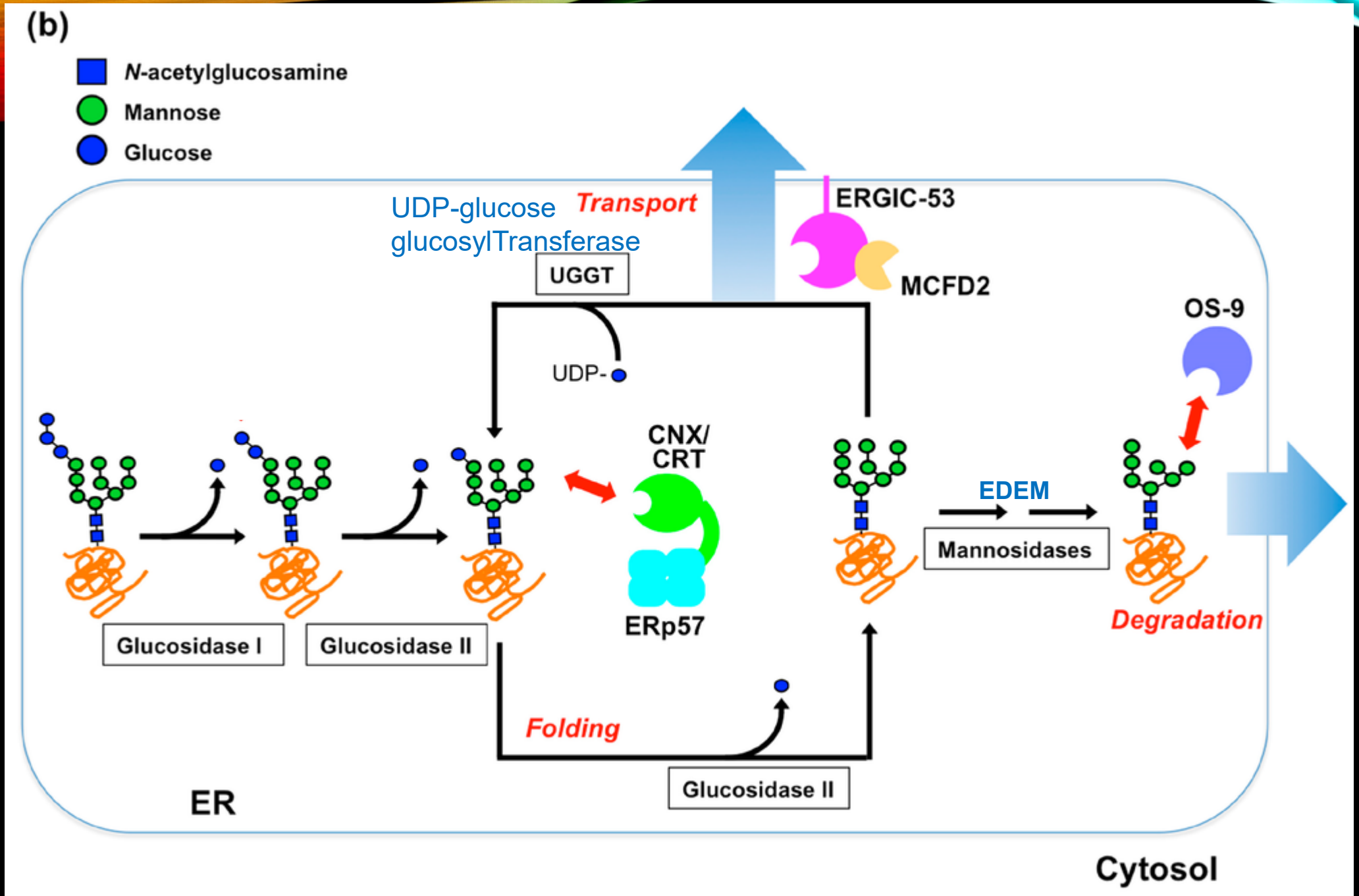


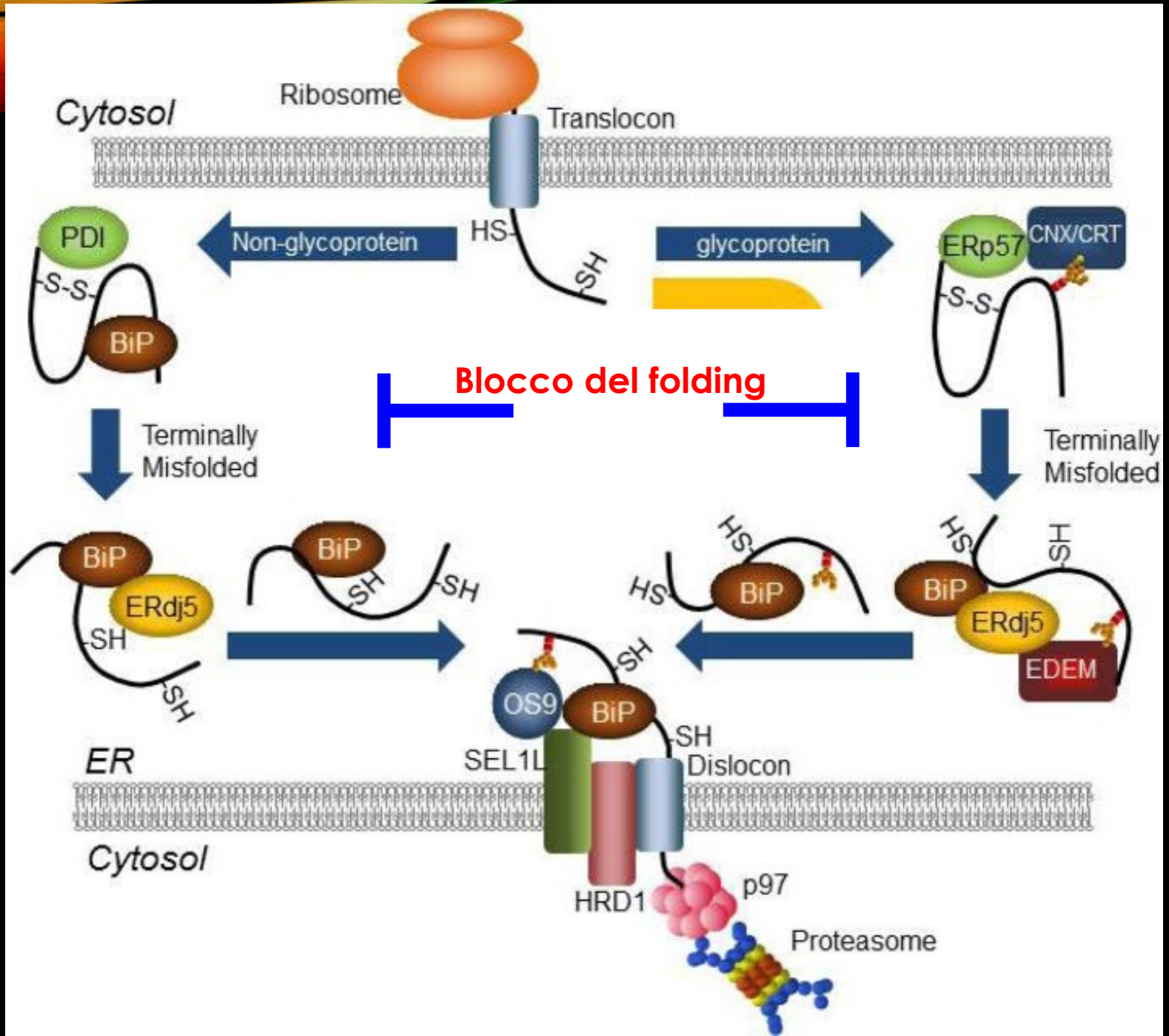
N-GLYCOSYLATION: A CRITICAL STEP IN PROTEIN FOLDING




ER lumen
GII
PDI
S
S
Crt
ERp57
S—S
BiP
S—S
ERp57
Cnx
SPC18
SPC25
SPC12
SPC22/23
SPC21
GI
OST
Cytosol


Nascent or misfolded protein
Peptide Binding
ATPase
Hsp40
I
Hsp40
ATP
SBD
Hsp70
ATP
Hsp70
II
Hsp40
Pi
ADP
Hsp70
Bip or Grp78
Misfolded
or
Folded
V
NEF
NEF
III
NEF
ADP
IV
ATP
ATP
ADP
Hsp70
Hsp70


(b)
N-acetylglucosamine
Mannose
Glucose
UDP-glucose glucosylTransferase
Transport
ERGIC-53
MCFD2
UGGT
OS-9
UDP-
CNX/ CRT
EDEM
Mannosidases
Glucosidase I
Glucosidase II
ERp57
Degradation
Folding
Glucosidase II
ER
Cytosol

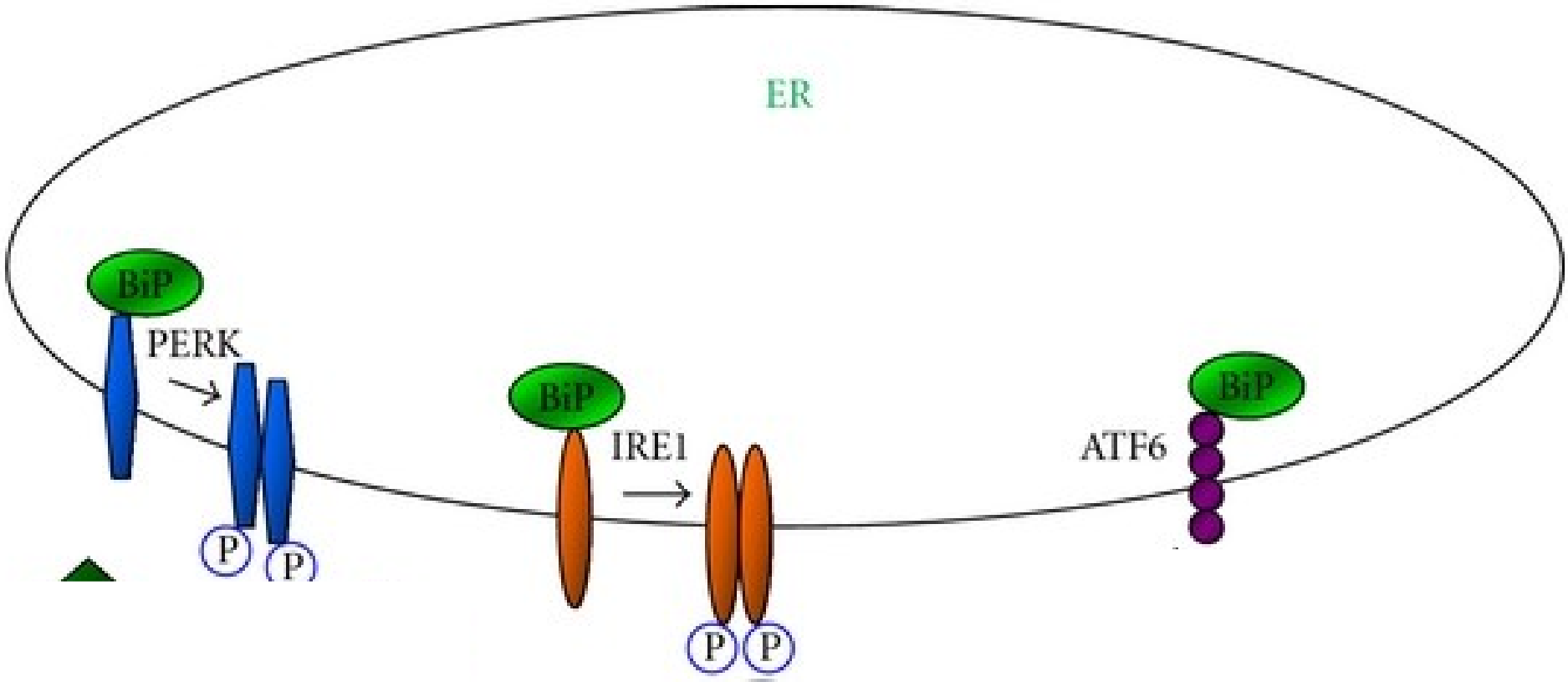

Cytosol
Ribosome
Translocon
PDI
S-S
BiP
Non-glycoprotein
HS
SH
glycoprotein
ERp57
CNX/CRT
S-S
Blocco del folding
Terminally
Misfolded
Terminally
Misfolded
BiP
ERdj5
SH
SH
BiP
SH
SH
HS
HS
BiP
HS
BiP
ERdj5
EDEM
SH
OS9
BiP
SH
ER
SEL1L
Dislocon
Cytosol
HRD1
p97
Proteasome

ALTERAZIONI E CONSEQUENZE DEL PROESSO DI FOLDING

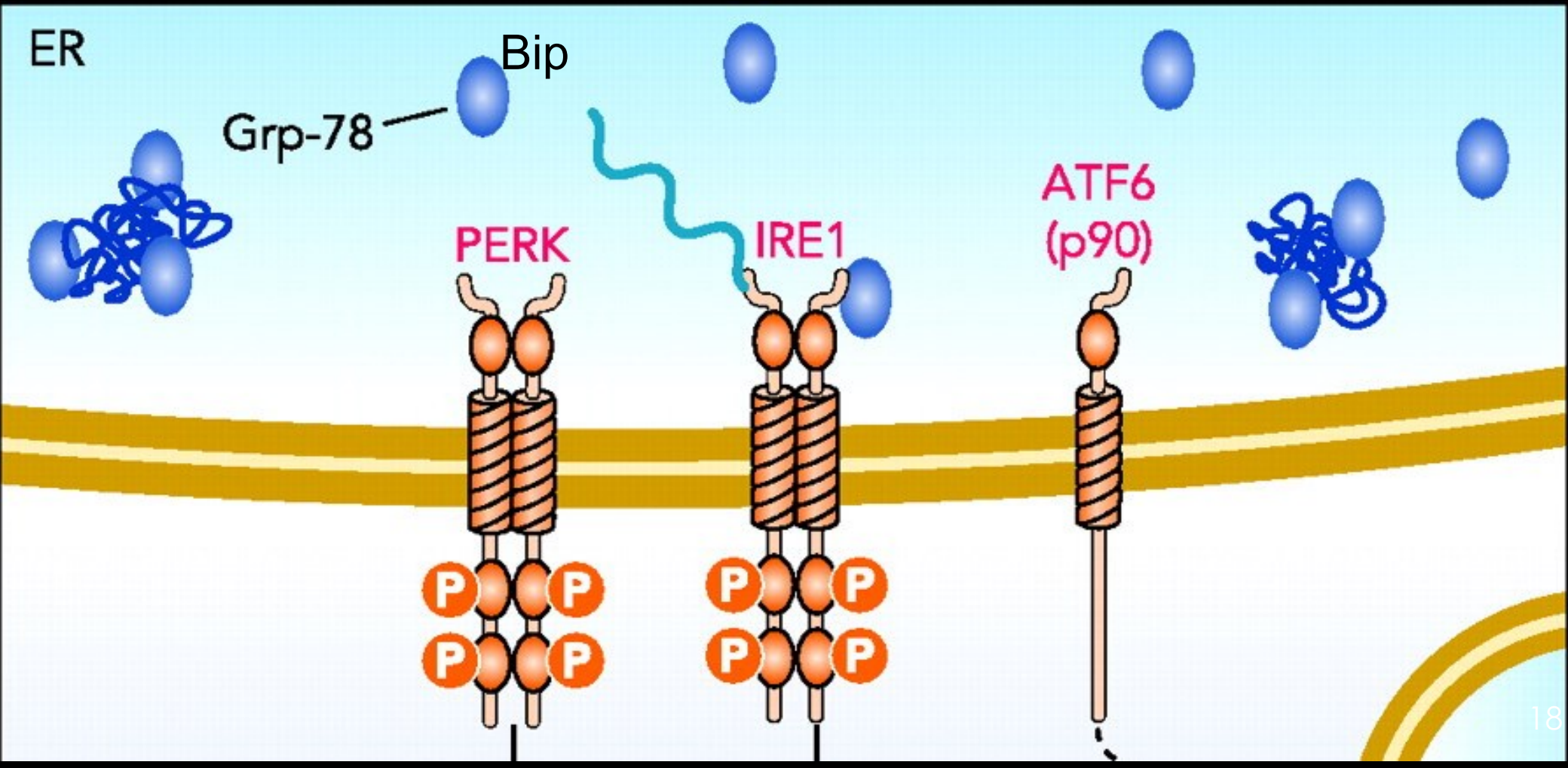
TRIGGERS OF ER STRESS

--Development of secretory cells
  --pancreatic β cells
  --plasma cells
--Altered metabolism
  --glucose deprivation
  --hyperhomocysteinemia
--Genetic mutation
  --DNA damage
  --mutated secretory proteins
--Pathogenesis
  --polyglutamine neuropathies
  --viral infection
--Chemical insult
  --inhibition of *N*-linked glycosylation
  --disruption of Ca^{2+} homeostasis

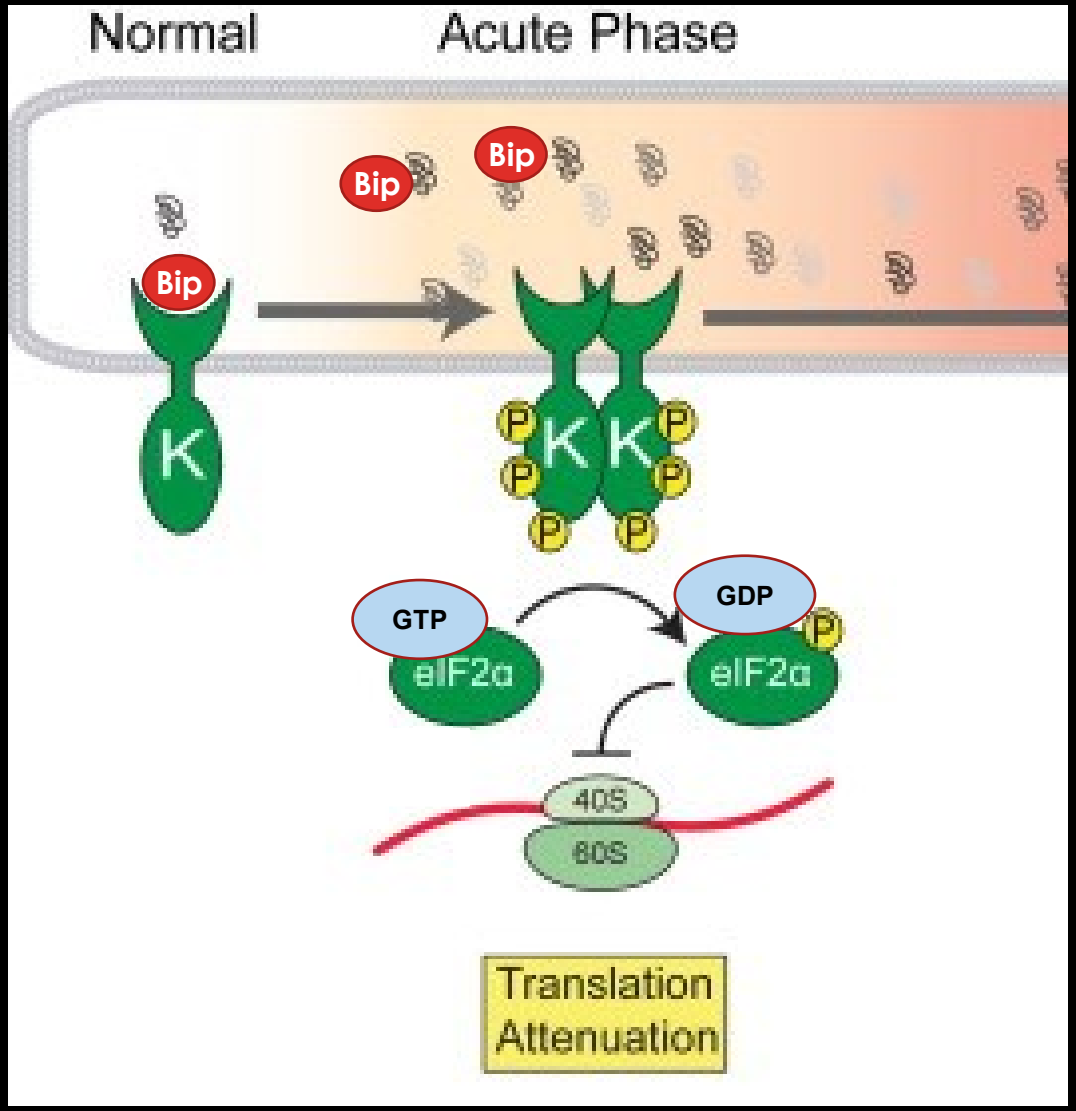
THE UNFOLDED PROTEIN RESPONSE (UPR)

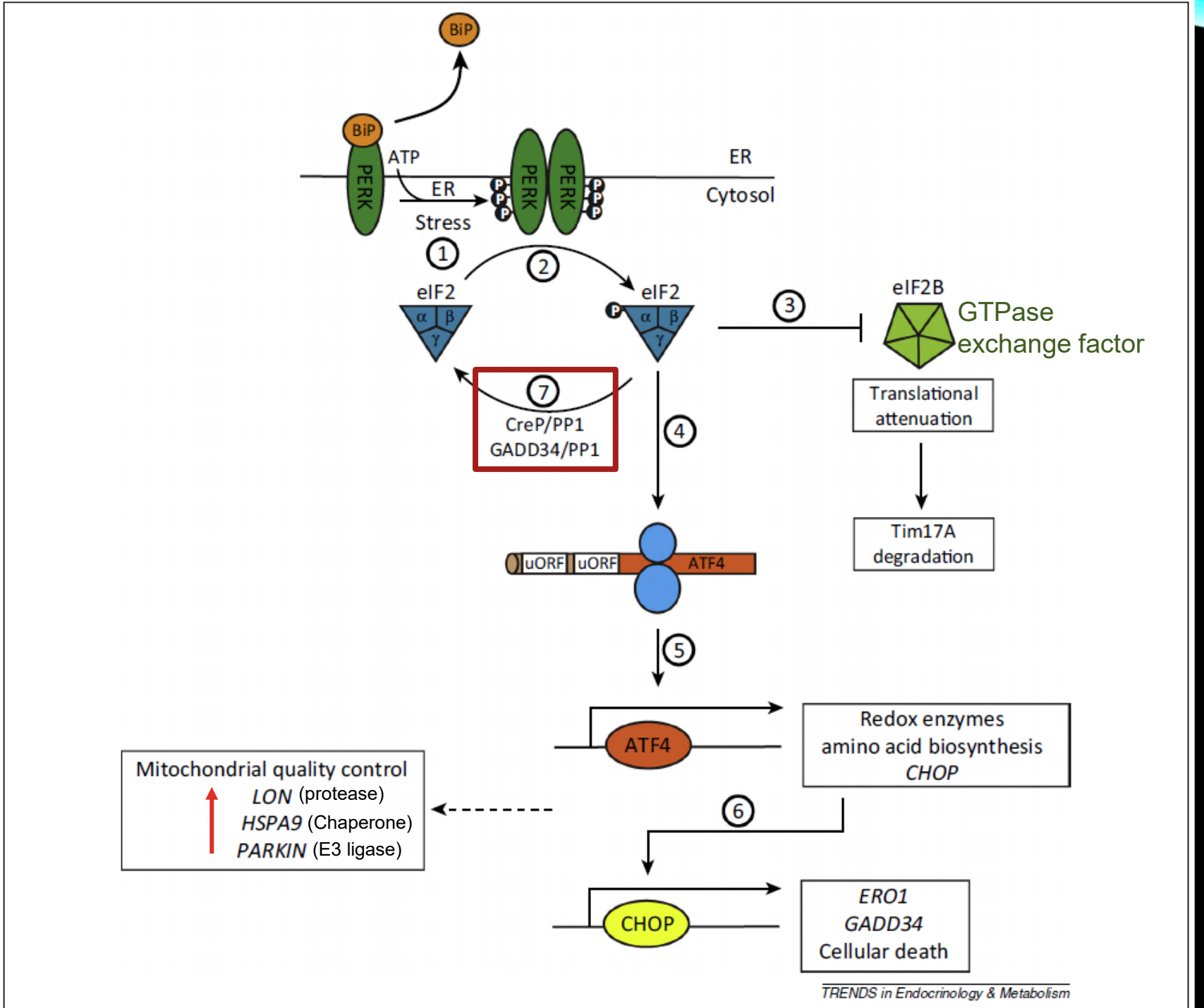


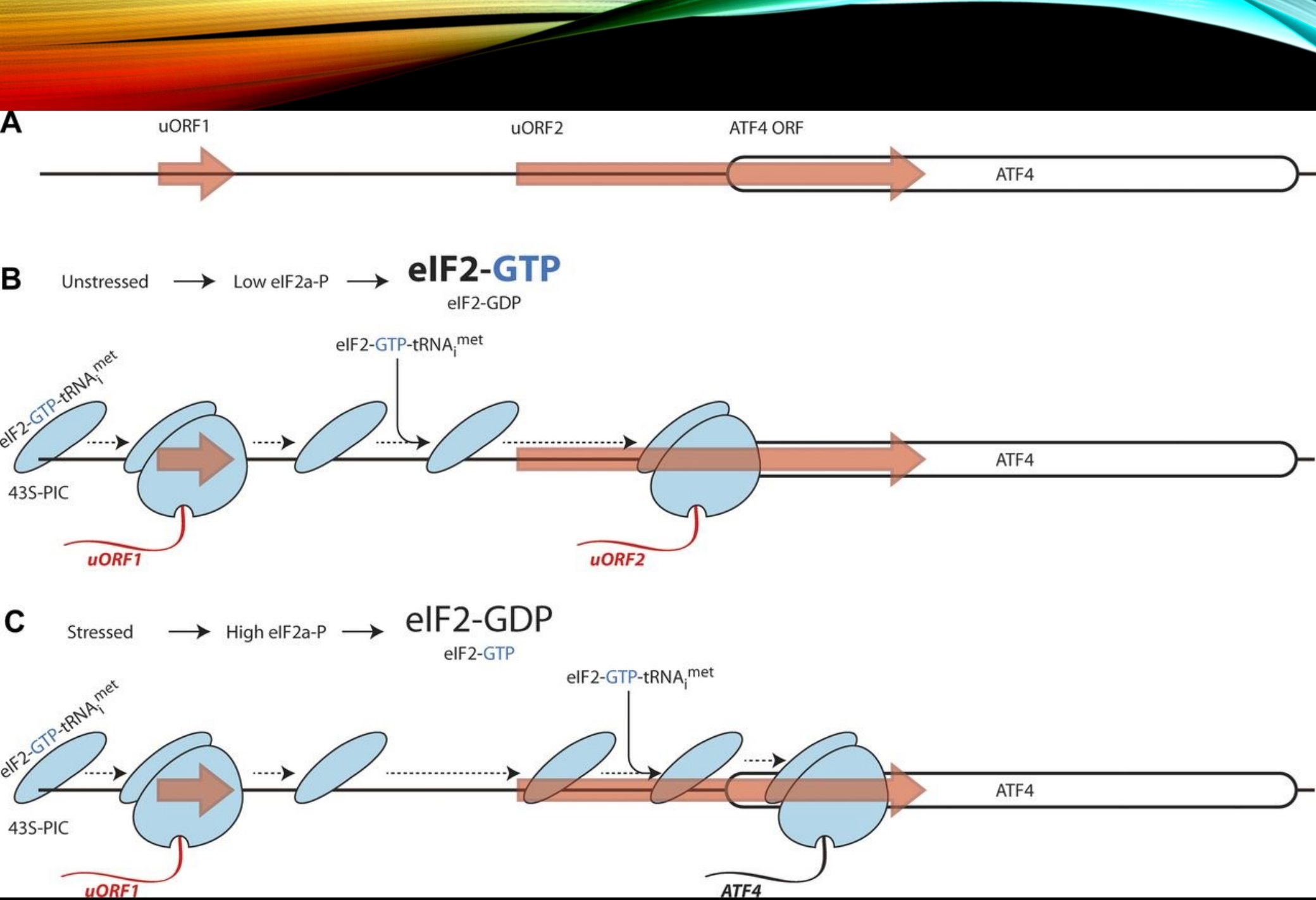
THE UNFOLDED PROTEIN RESPONSE (UPR)



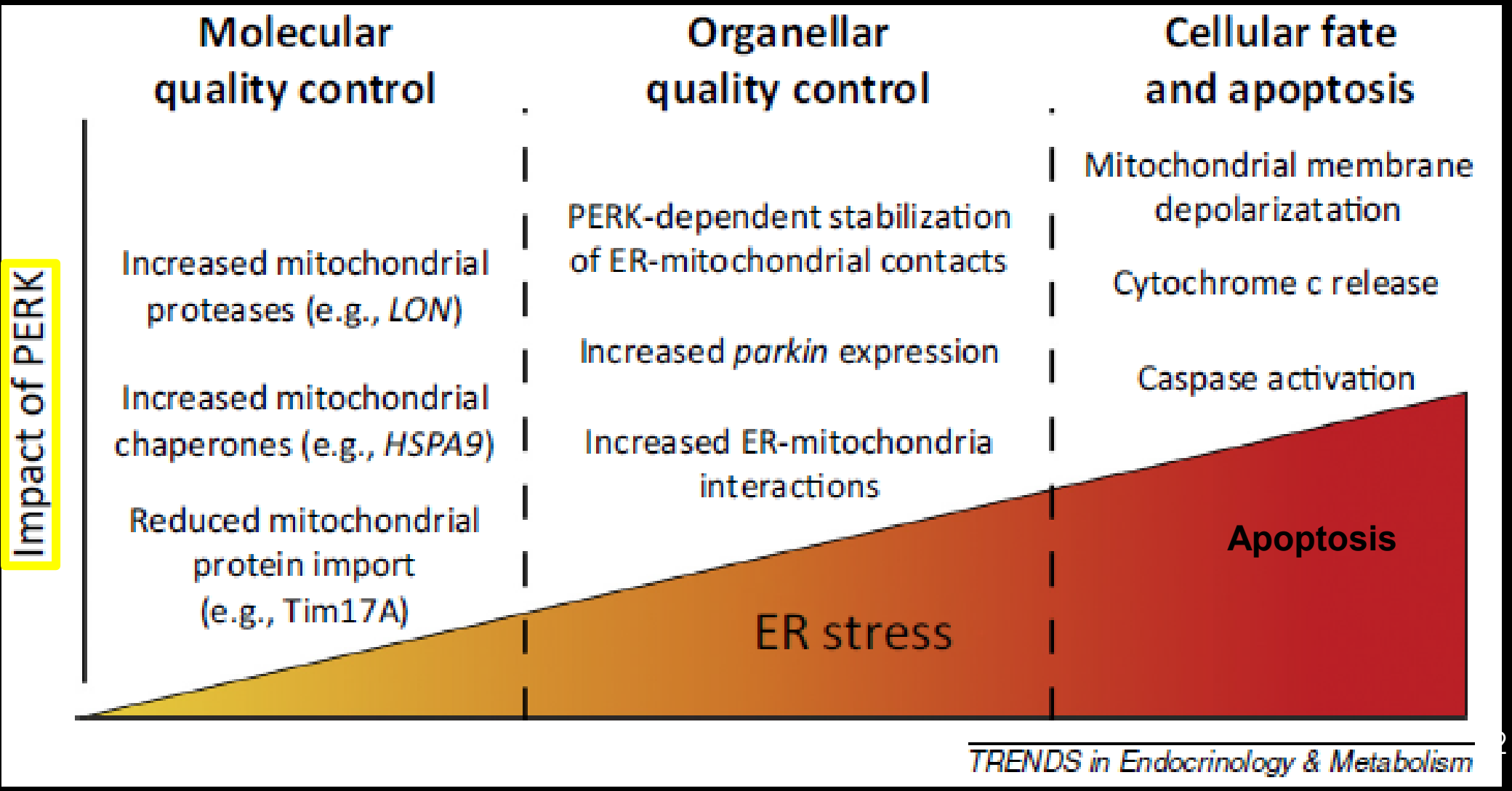
PERK PATHWAY



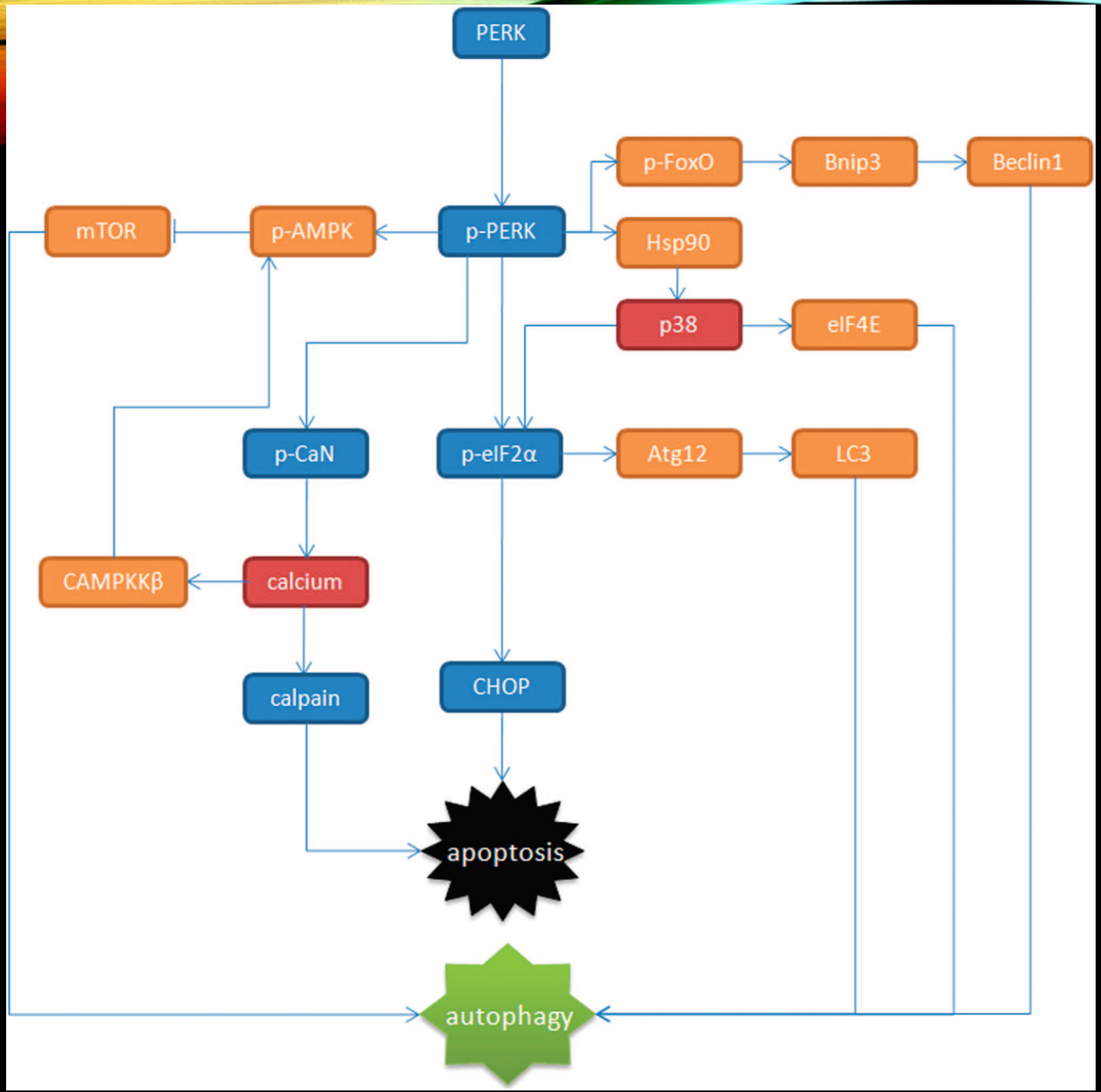

BiP
BiP
ATP
ER
Stress
PERK
PERK
PERK
ER
Cytosol
1
2
eIF2
eIF2
3
eIF2B
GTPase
exchange factor
Translational
attenuation
Tim17A
degradation
7
CreP/PP1
GADD34/PP1
4
uORF
uORF
ATF4
5
ATF4
Redox enzymes
amino acid biosynthesis
CHOP
Mitochondrial quality control
LON (protease)
HSPA9 (Chaperone)
PARKIN (E3 ligase)
6
CHOP
ERO1
GADD34
Cellular death
TRENDS in Endocrinology & Metabolism


A
uORF1
uORF2
ATF4 ORF
ATF4
B
Unstressed
Low eIF2a-P
eIF2-GTP
eIF2-GDP
eIF2-GTP-tRNA$_i^{met}$
eIF2-GTP-tRNA$_i^{met}$
43S-PIC
uORF1
uORF2
ATF4
C
Stressed
High eIF2a-P
eIF2-GDP
eIF2-GTP
eIF2-GTP-tRNA$_i^{met}$
eIF2-GTP-tRNA$_i^{met}$
43S-PIC
uORF1
ATF4
ATF4

IMPACT OF PERK SIGNALLING ON MITOCHONDRIA



TRENDS in Endocrinology & Metabolism


PERK
p-FoxO
Bnip3
Beclin1
mTOR
p-AMPK
p-PERK
Hsp90
p38
eIF4E
p-CaN
p-eIF2α
Atg12
LC3
CAMPKKβ
calcium
calpain
CHOP
apoptosis
autophagy

AUTOPHAGY

- An intracellular homeostatic mechanism important for degradation of cytoplasmic components in acidic lysosomal compartment

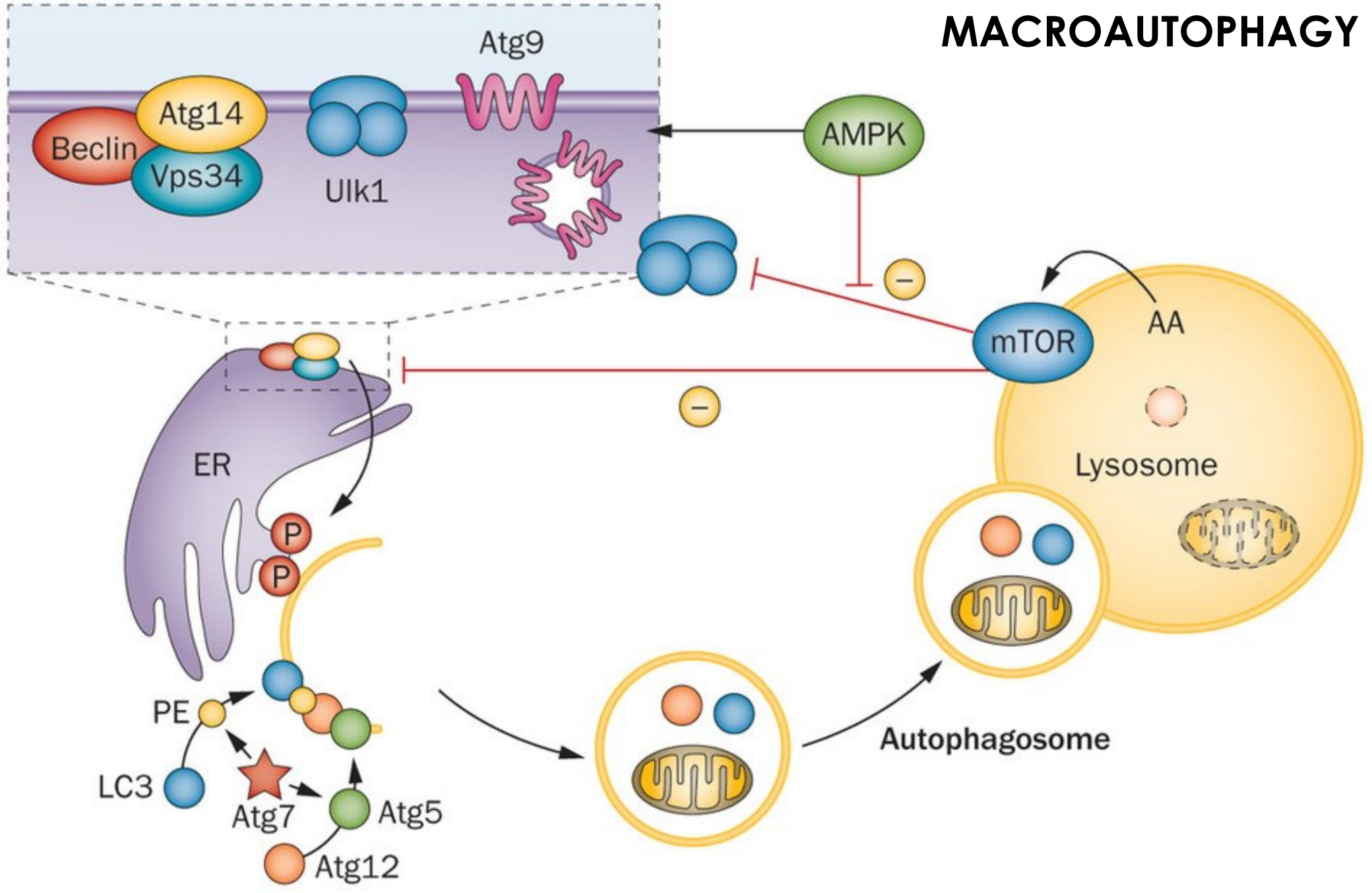
- Targets:
  - Intracellular organelle
  - Misfolded proteins

- Main aims:
  1. It disassembles unnecessary or dysfunctional components
  2. It promotes cellular survival by maintaining cellular energy levels during starvation
  3. adaptive response to stress, this promotes cellular survival in cancer

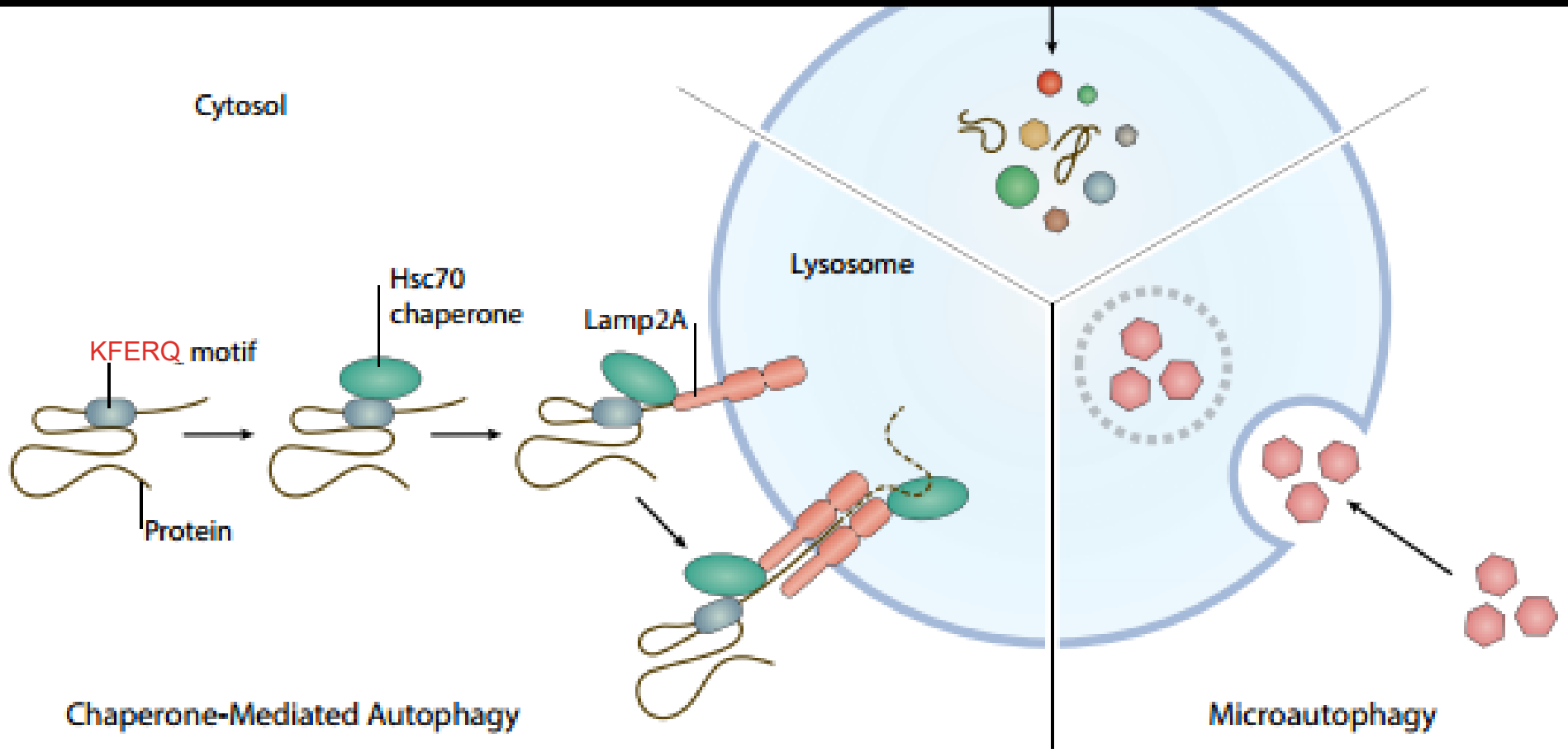
AUTOPHAGY

- Macroautophagy
  - it is used primarily to waste damaged cell organelles or unused proteins. This involves the formation of a double membrane known as an autophagosome, around the organelle marked for destruction.
- Microautophagy
  - It involves the direct engulfment of cytoplasmic material into the lysosome. This occurs by inward folding of the lysosomal membrane.
- Chaperone-mediated autophagy
  - It is a protein specific pathway which involves the recognition of KFERQ sequence by the hsc70-containing complex and translocation through the lysosomal membrane.

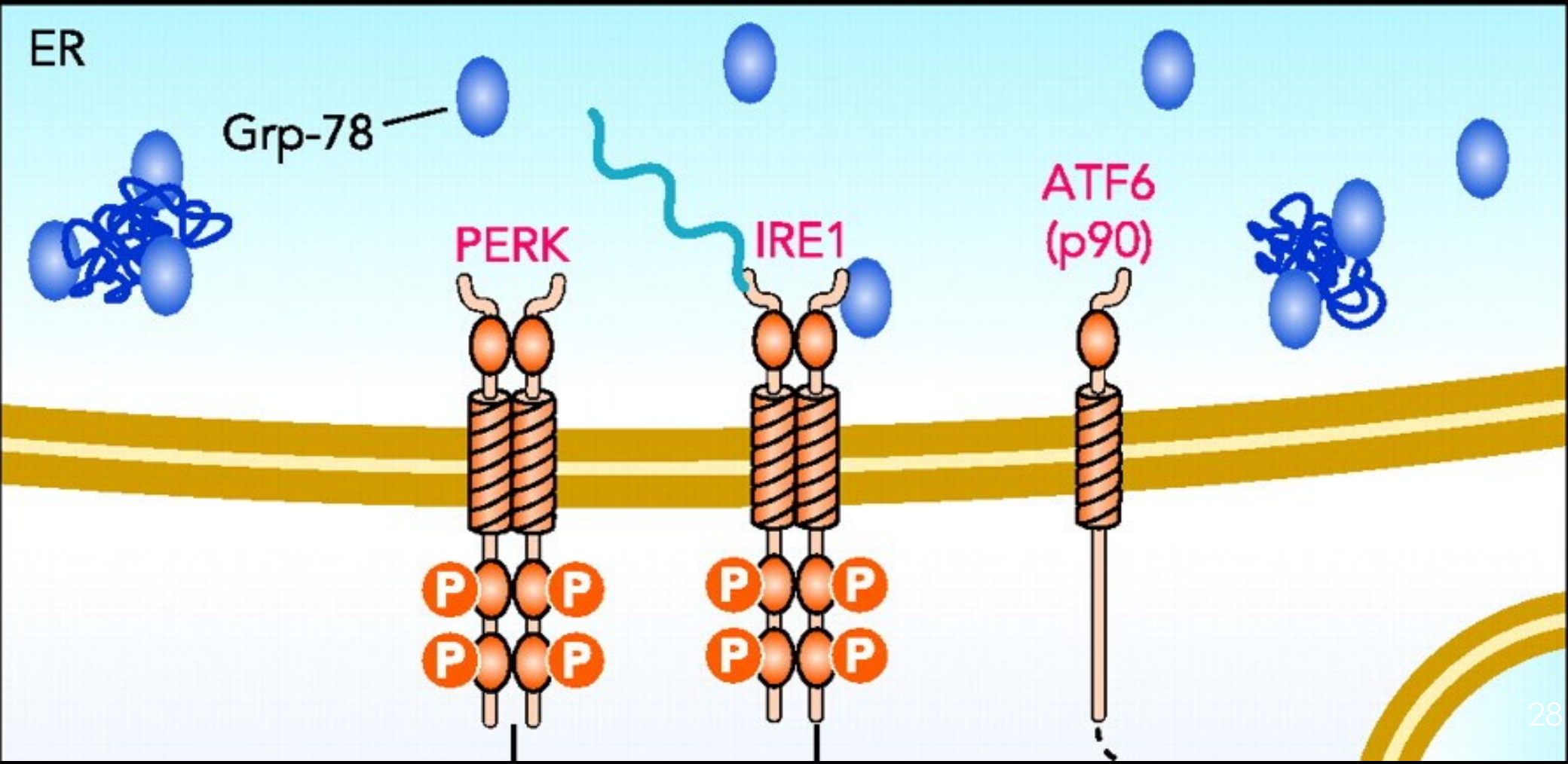
MACROAUTOPHAGY

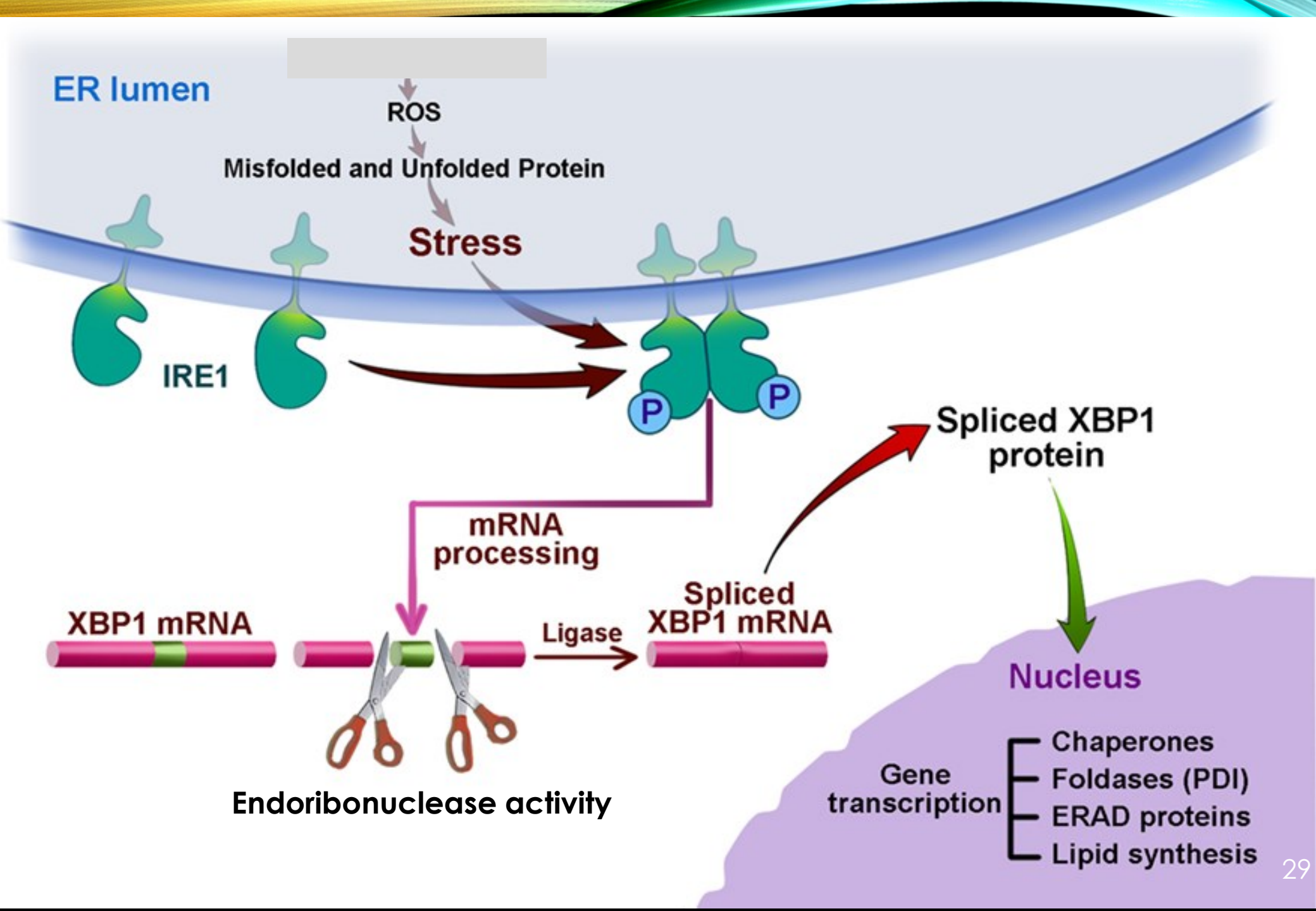


CHAPERONE-MEDIATED AUTOPHAGY AND MICROAUTOPHAGY

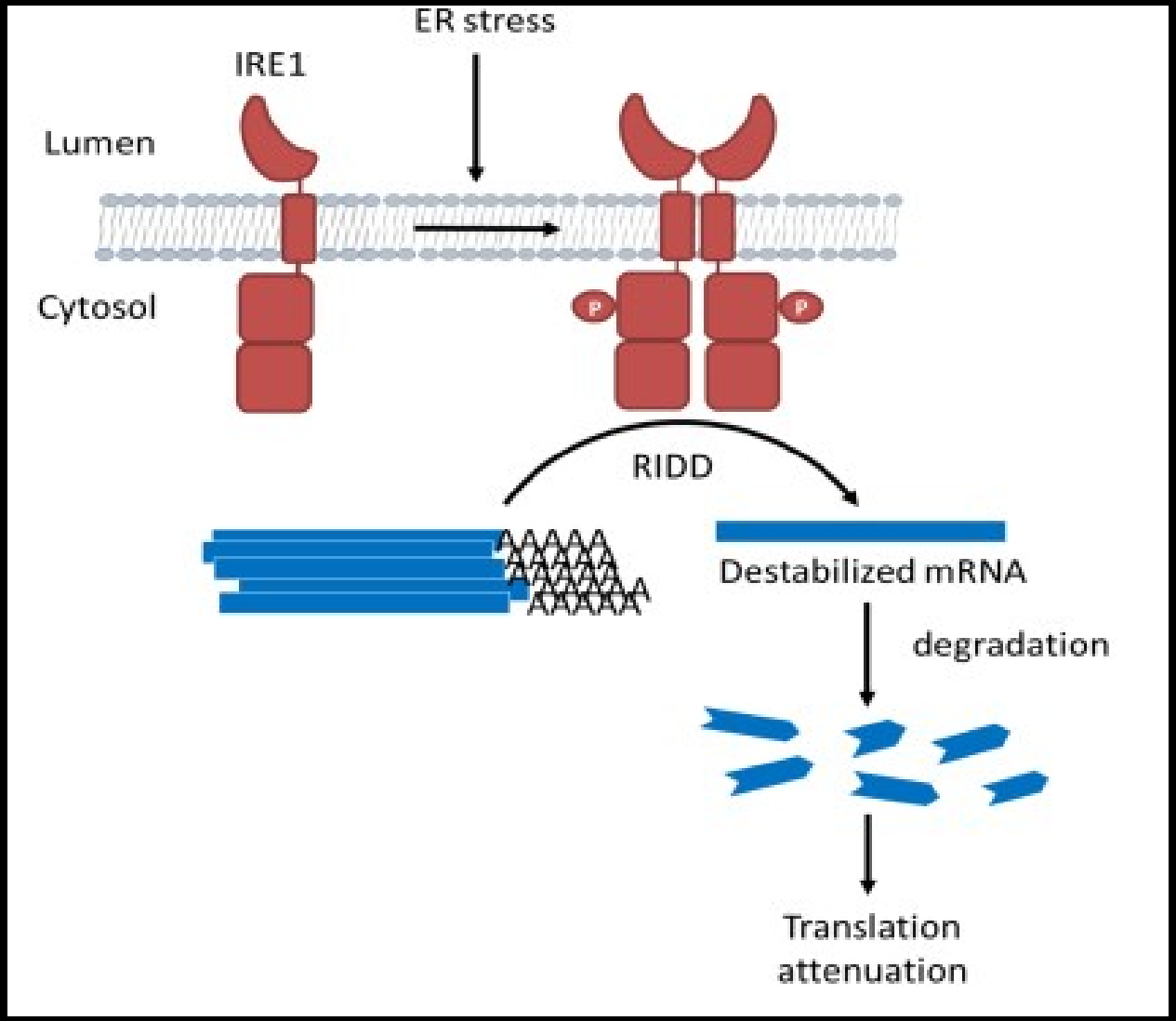


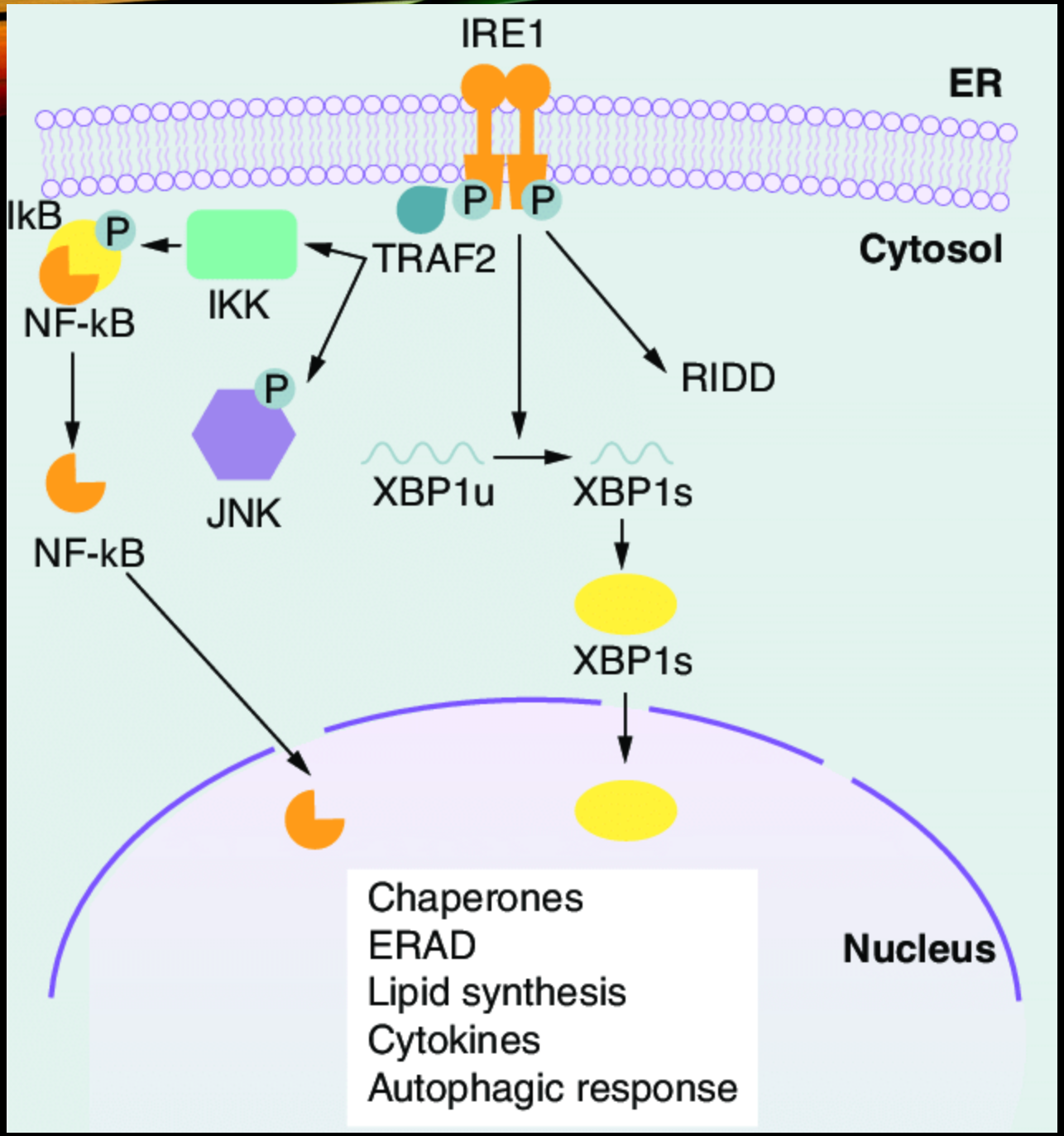
UPR MACHINERY

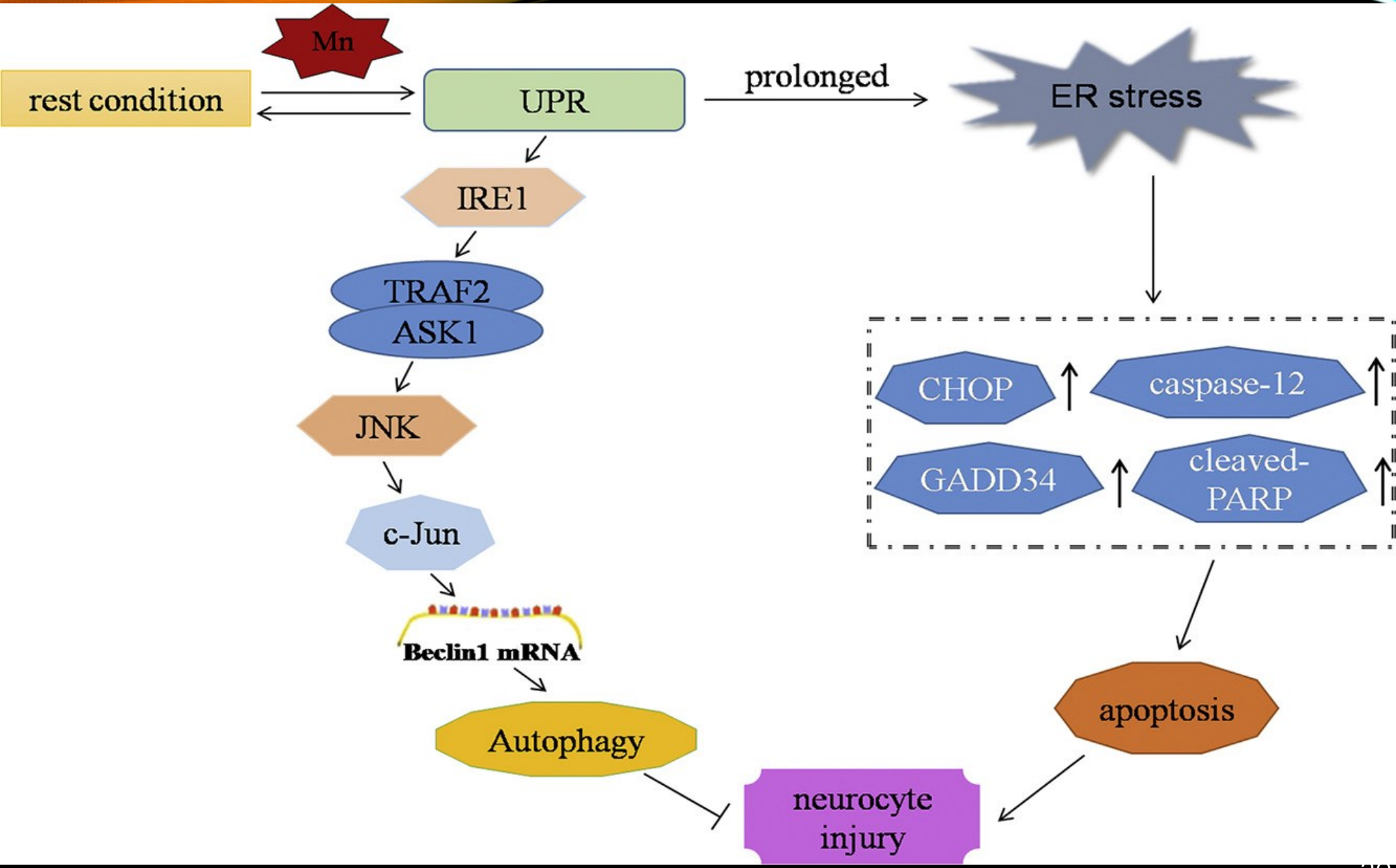


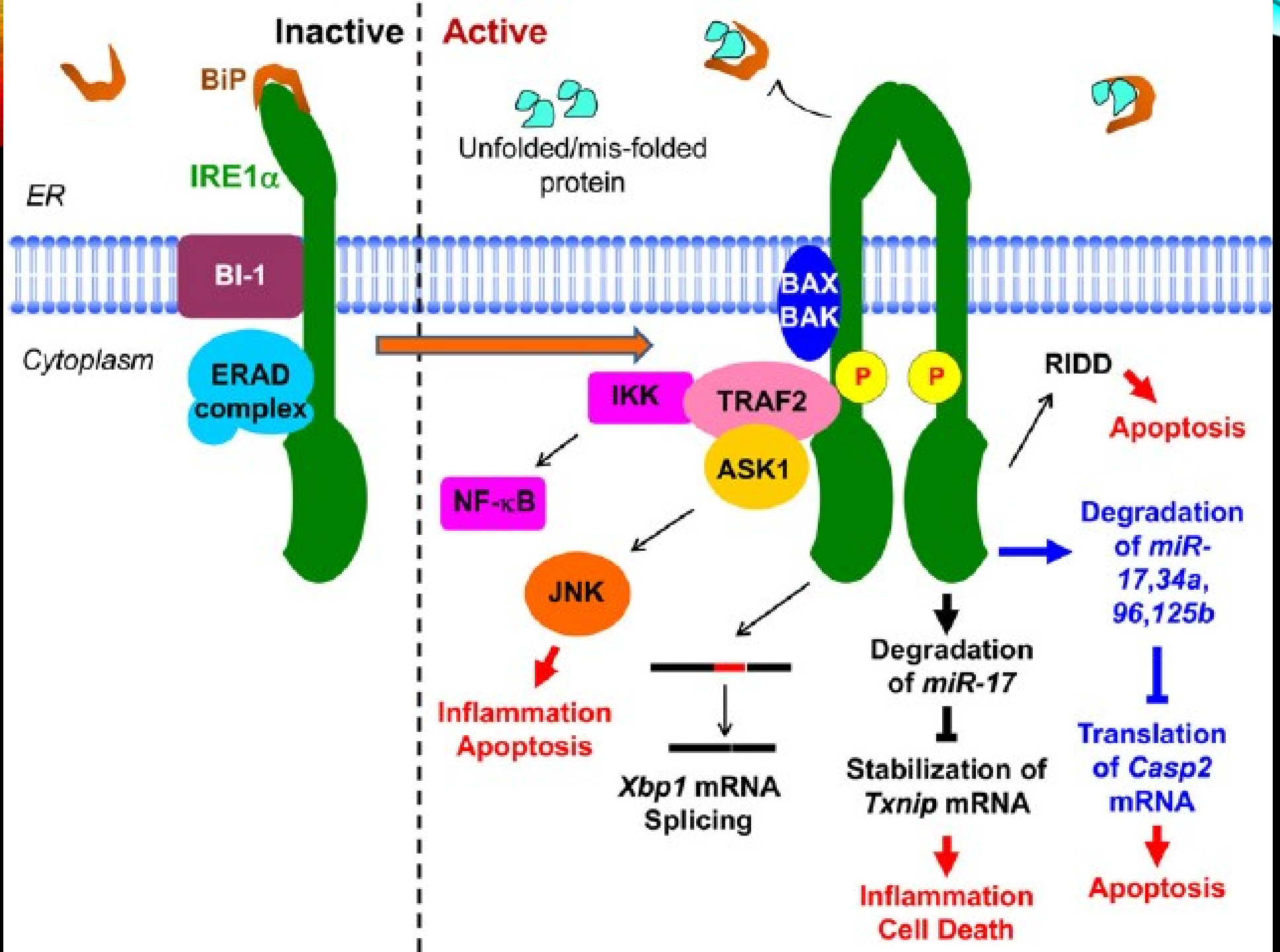

ER lumen
ROS
Misfolded and Unfolded Protein
Stress
IRE1
P
P
Spliced XBP1 protein
mRNA processing
XBP1 mRNA
Ligase
Spliced XBP1 mRNA
Nucleus
Gene transcription
Chaperones
Foldases (PDI)
ERAD proteins
Lipid synthesis
Endoribonuclease activity

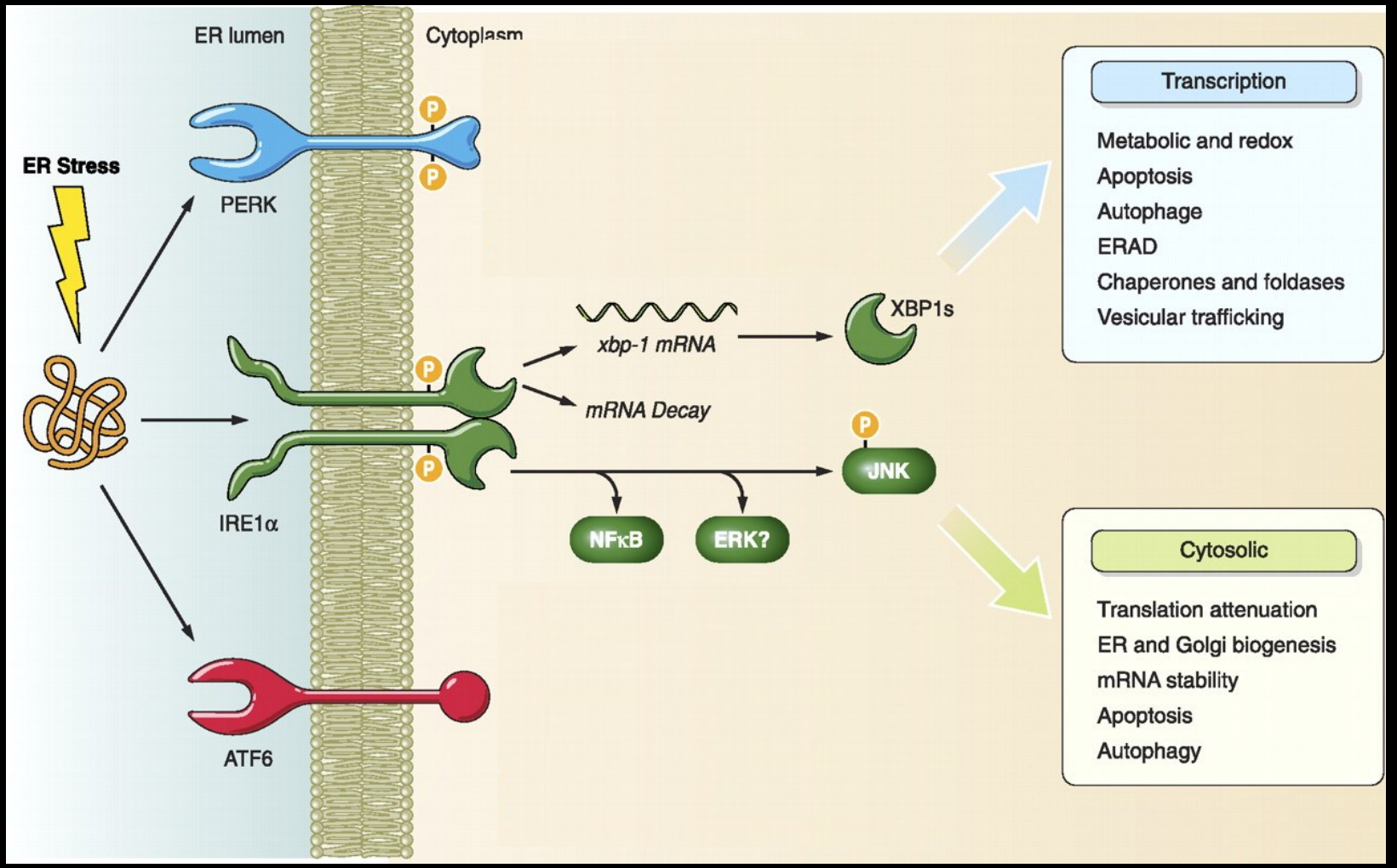
REGULATED IRE1 DEPENDENT DECAY (RIDD)



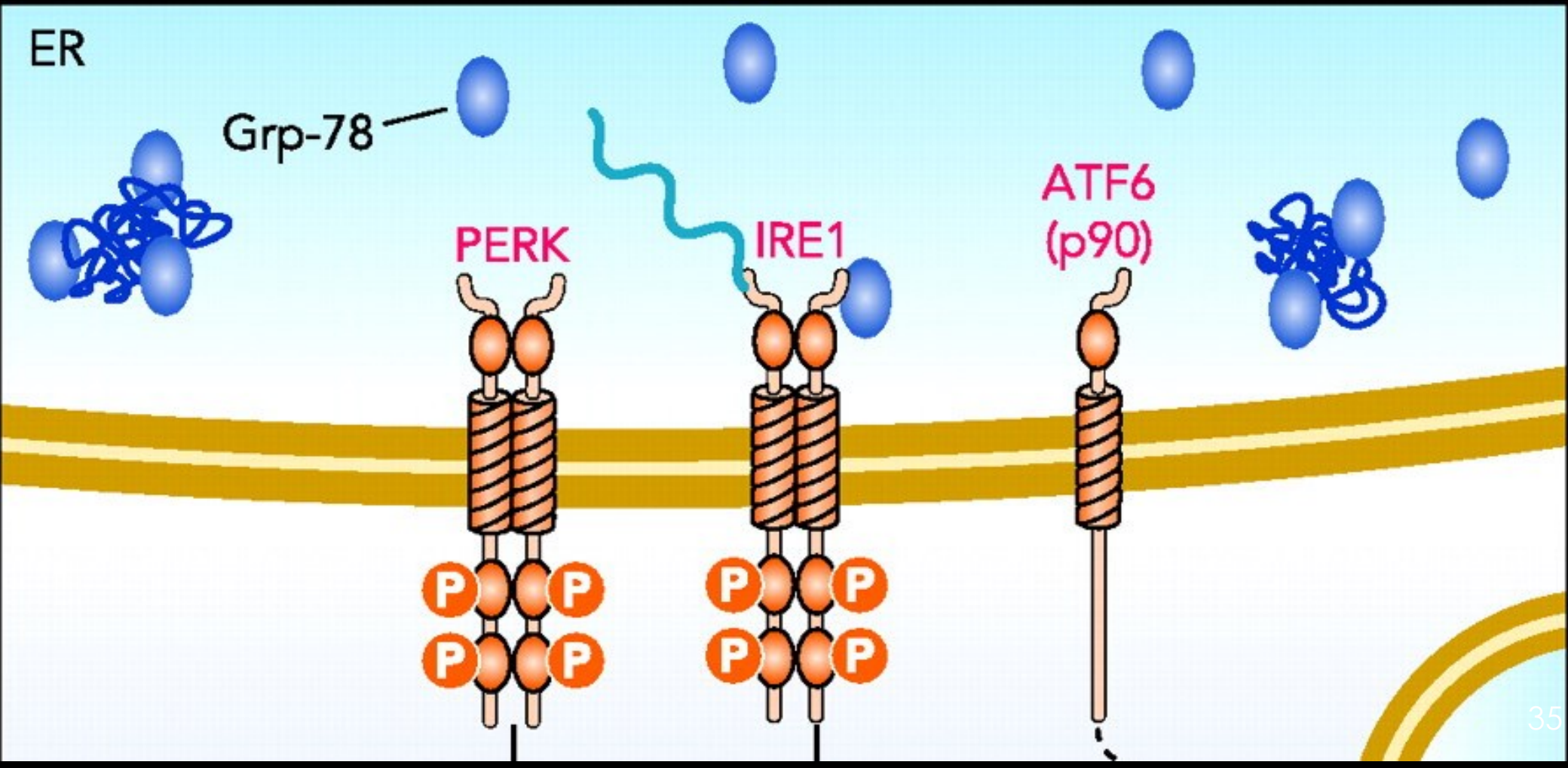

IRE1
ER
Cytosol
IkB
P
NF-kB
IKK
TRAF2
P
P
RIDD
P
JNK
XBP1u
XBP1s
NF-kB
XBP1s
Chaperones
ERAD
Lipid synthesis
Cytokines
Autophagic response
Nucleus

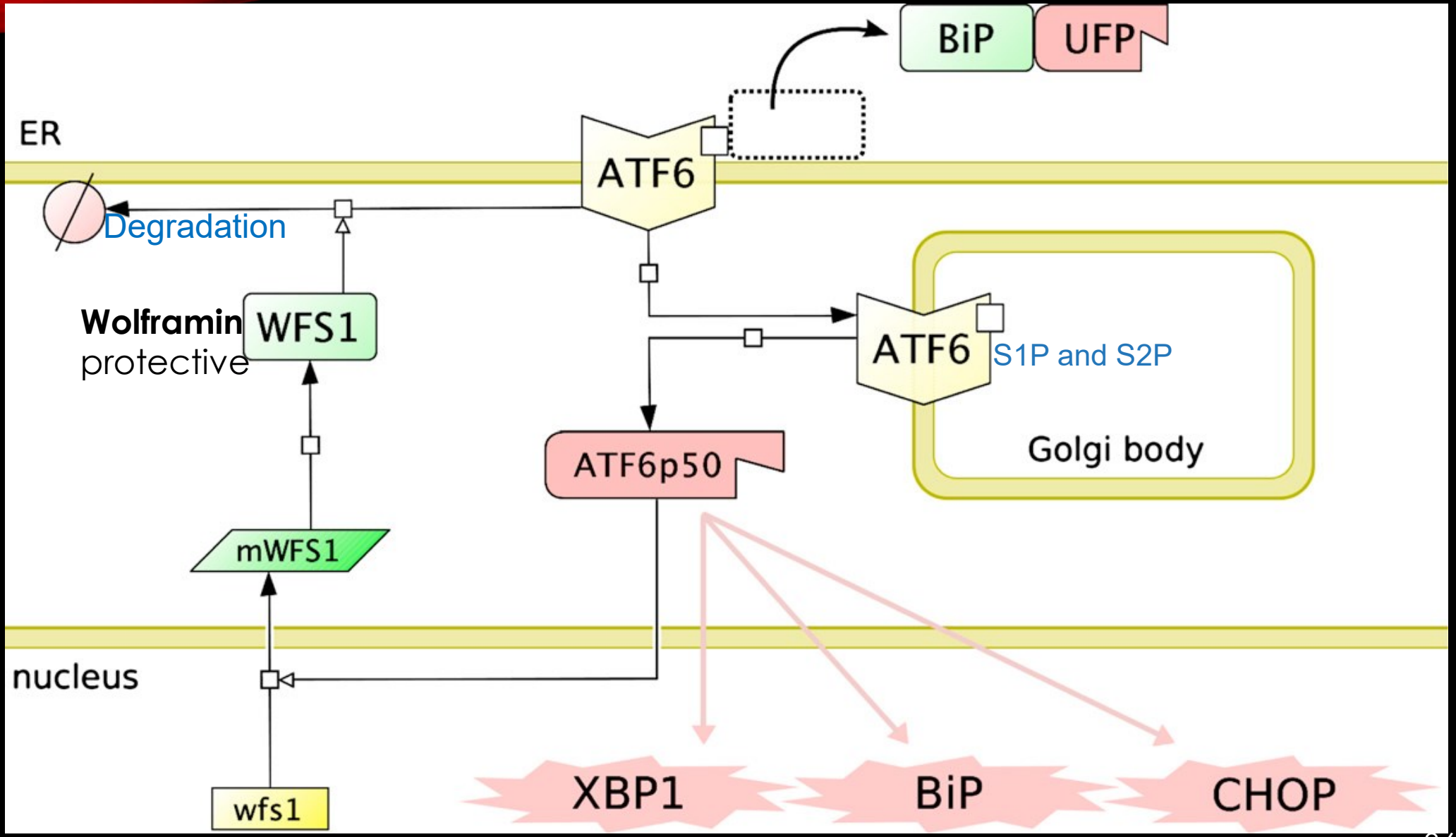

Mn
rest condition
UPR
prolonged
ER stress
IRE1
TRAF2
ASK1
JNK
c-Jun
Beclin1 mRNA
Autophagy
CHOP
caspase-12
GADD34
cleaved-PARP
apoptosis
neurocyte injury

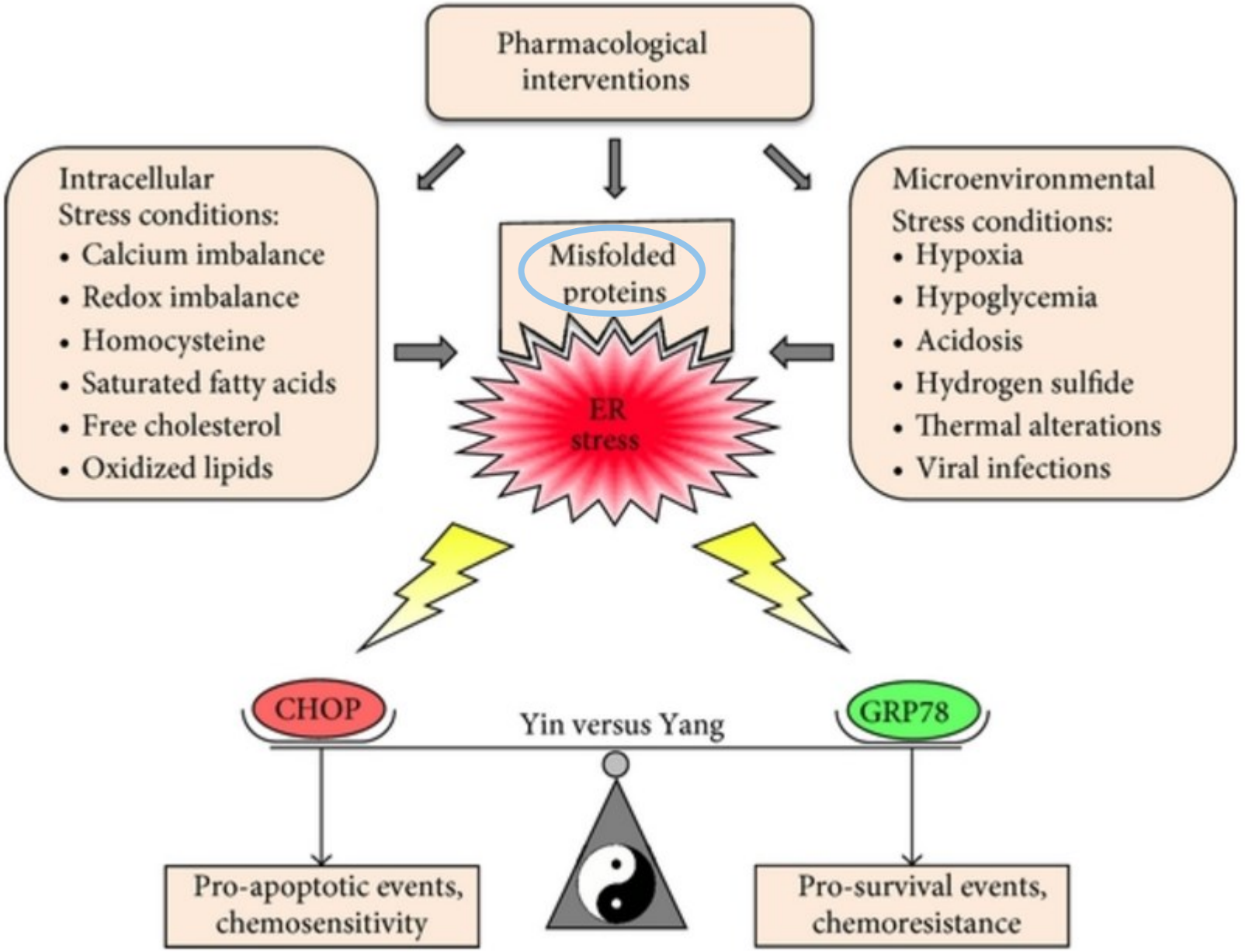

Inactive
Active
BiP
IRE1α
ER
Unfolded/mis-folded protein
BI-1
Cytoplasm
ERAD complex
BAX
BAK
P
P
IKK
TRAF2
ASK1
NF-κB
JNK
RIDD
Apoptosis
Degradation of miR-17,34a, 96,125b
Translation of Casp2 mRNA
Apoptosis
Inflammation Apoptosis
Xbp1 mRNA Splicing
Degradation of miR-17
Stabilization of Txnip mRNA
Inflammation Cell Death


ER lumen
Cytoplasm
ER Stress
PERK
IRE1α
ATF6
xbp-1 mRNA
XBP1s
mRNA Decay
JNK
NFκB
ERK?
Transcription
Metabolic and redox
Apoptosis
Autophage
ERAD
Chaperones and foldases
Vesicular trafficking
Cytosolic
Translation attenuation
ER and Golgi biogenesis
mRNA stability
Apoptosis
Autophagy

UPR MACHINERY

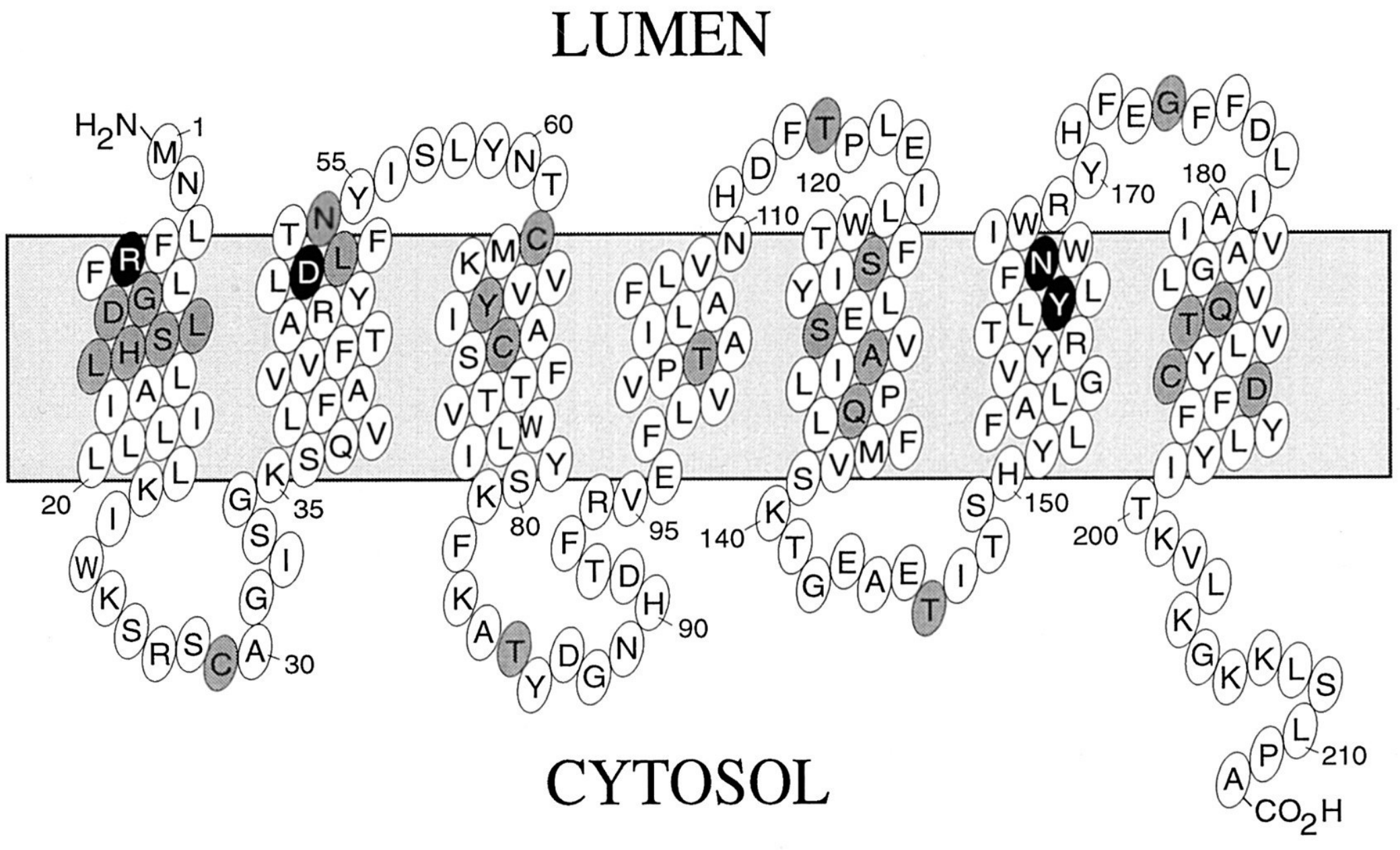



BiP
UFP
ER
ATF6
Degradation
Wolframin
protective
WFS1
ATF6
S1P and S2P
Golgi body
ATF6p50
mWFS1
nucleus
wfs1
XBP1
BiP
CHOP

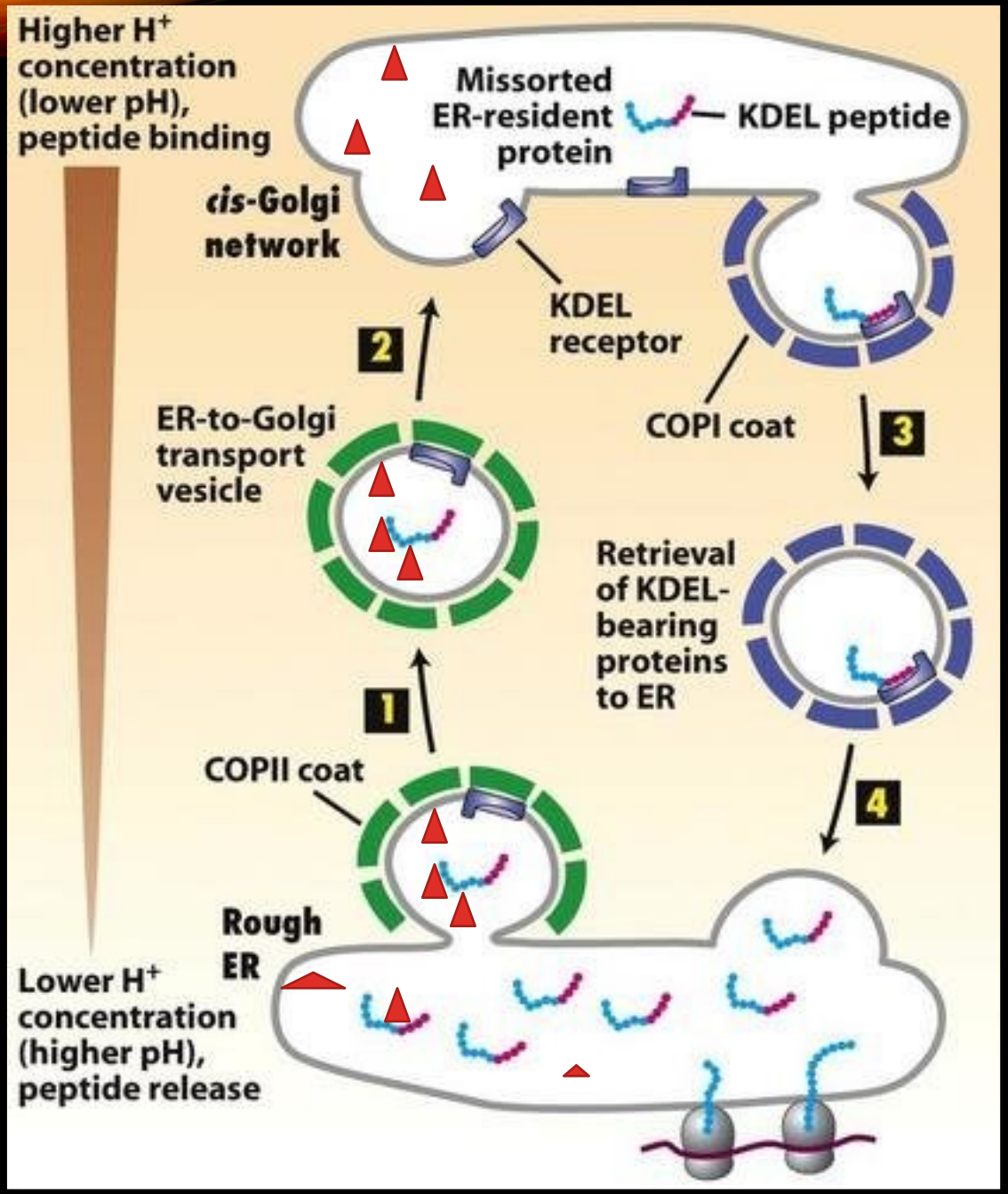

Pharmacological interventions
Intracellular Stress conditions:
• Calcium imbalance
• Redox imbalance
• Homocysteine
• Saturated fatty acids
• Free cholesterol
• Oxidized lipids
Misfolded proteins
ER stress
Microenvironmental Stress conditions:
• Hypoxia
• Hypoglycemia
• Acidosis
• Hydrogen sulfide
• Thermal alterations
• Viral infections
CHOP
Yin versus Yang
GRP78
Pro-apoptotic events, chemosensitivity
Pro-survival events, chemoresistance

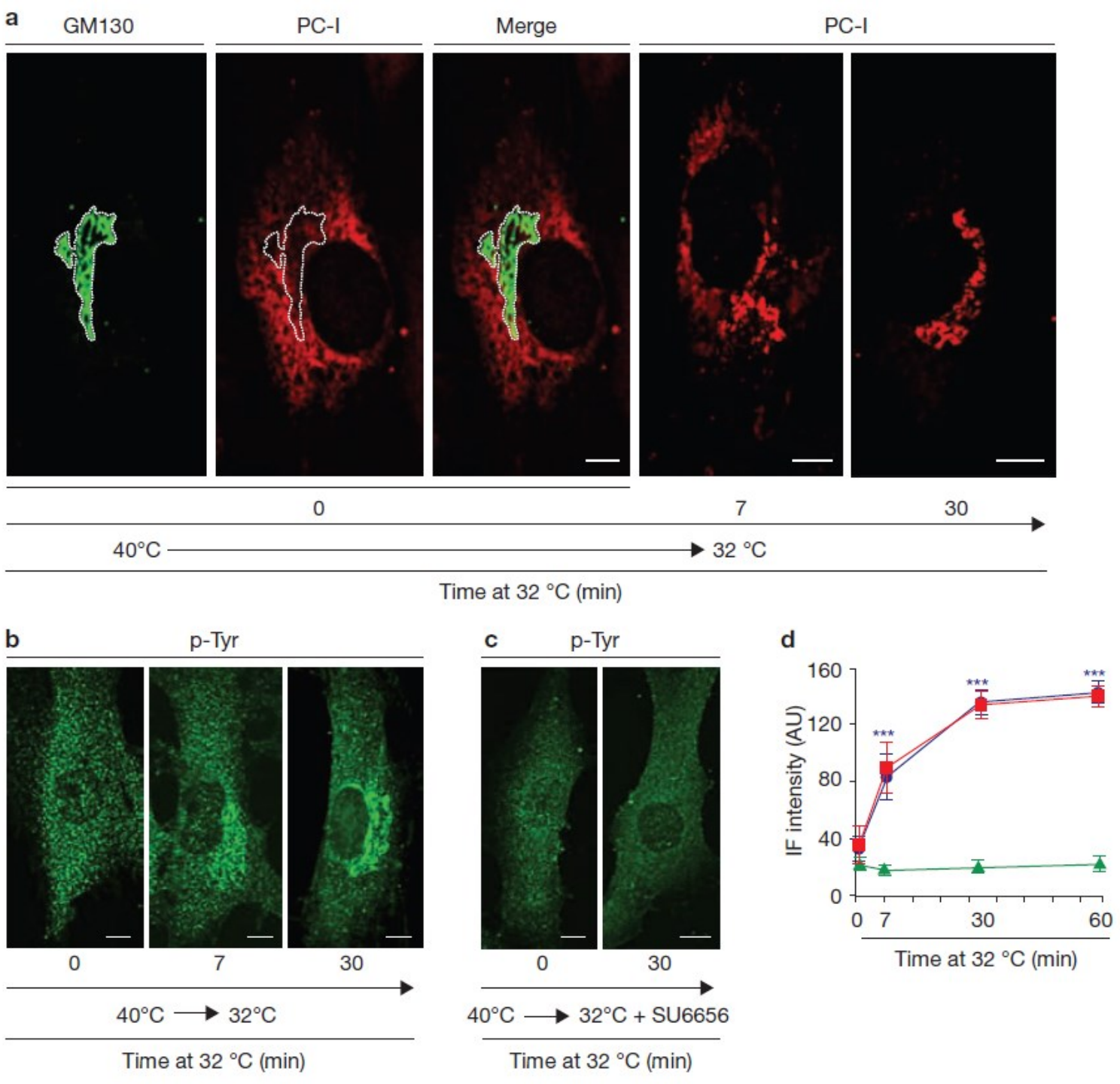
IL RECETTORE KDEL

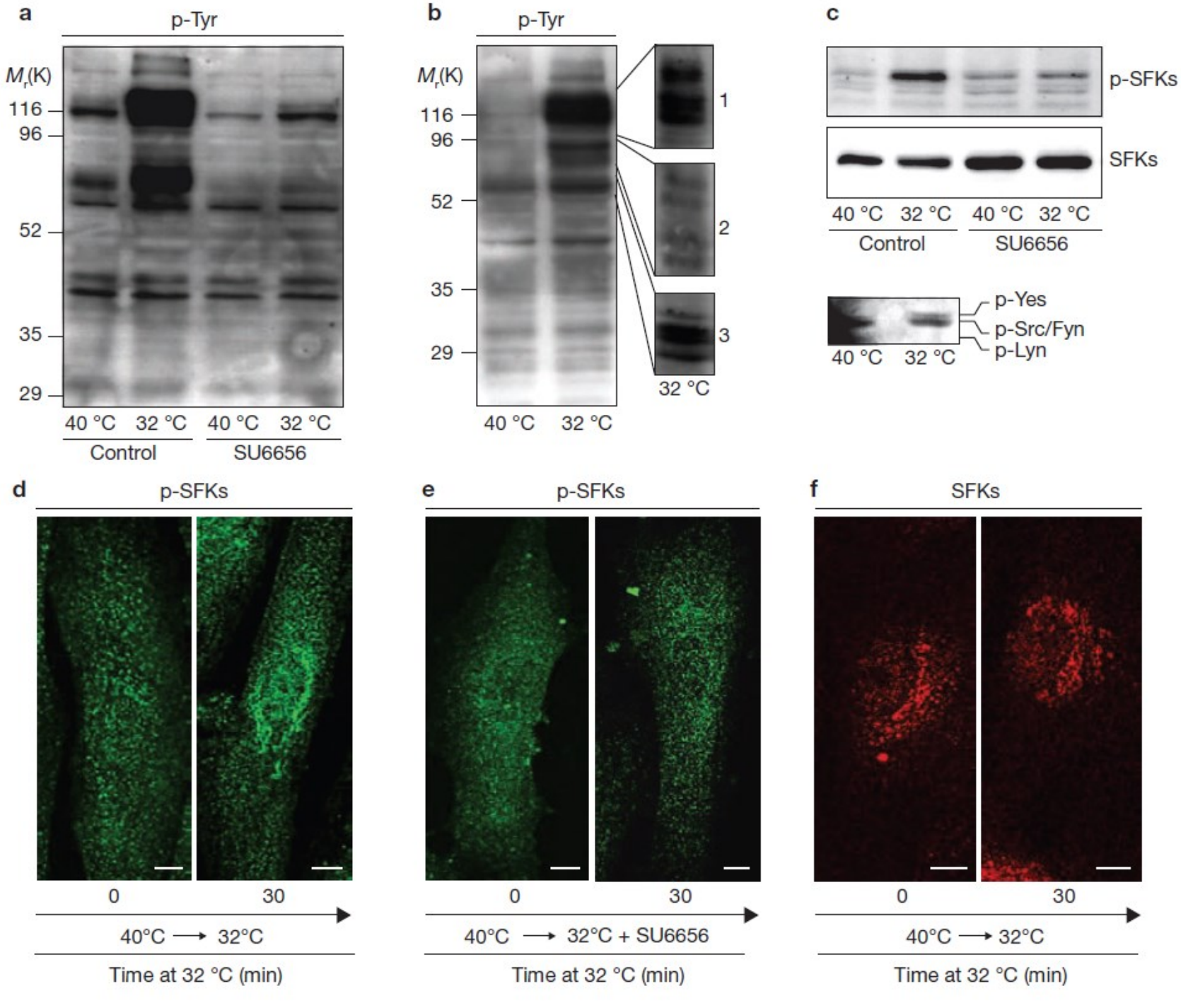
KDELR

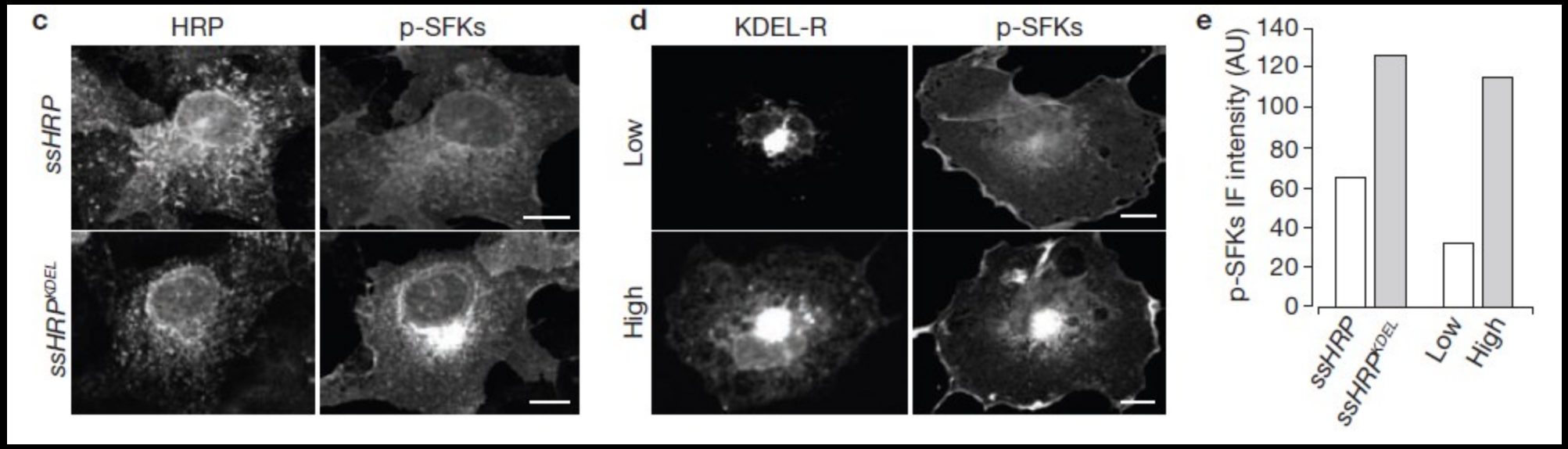

LUMEN
H_2N
1
20
30
35
55
60
80
90
95
110
120
140
150
170
180
200
210
CO_2H
CYTOSOL

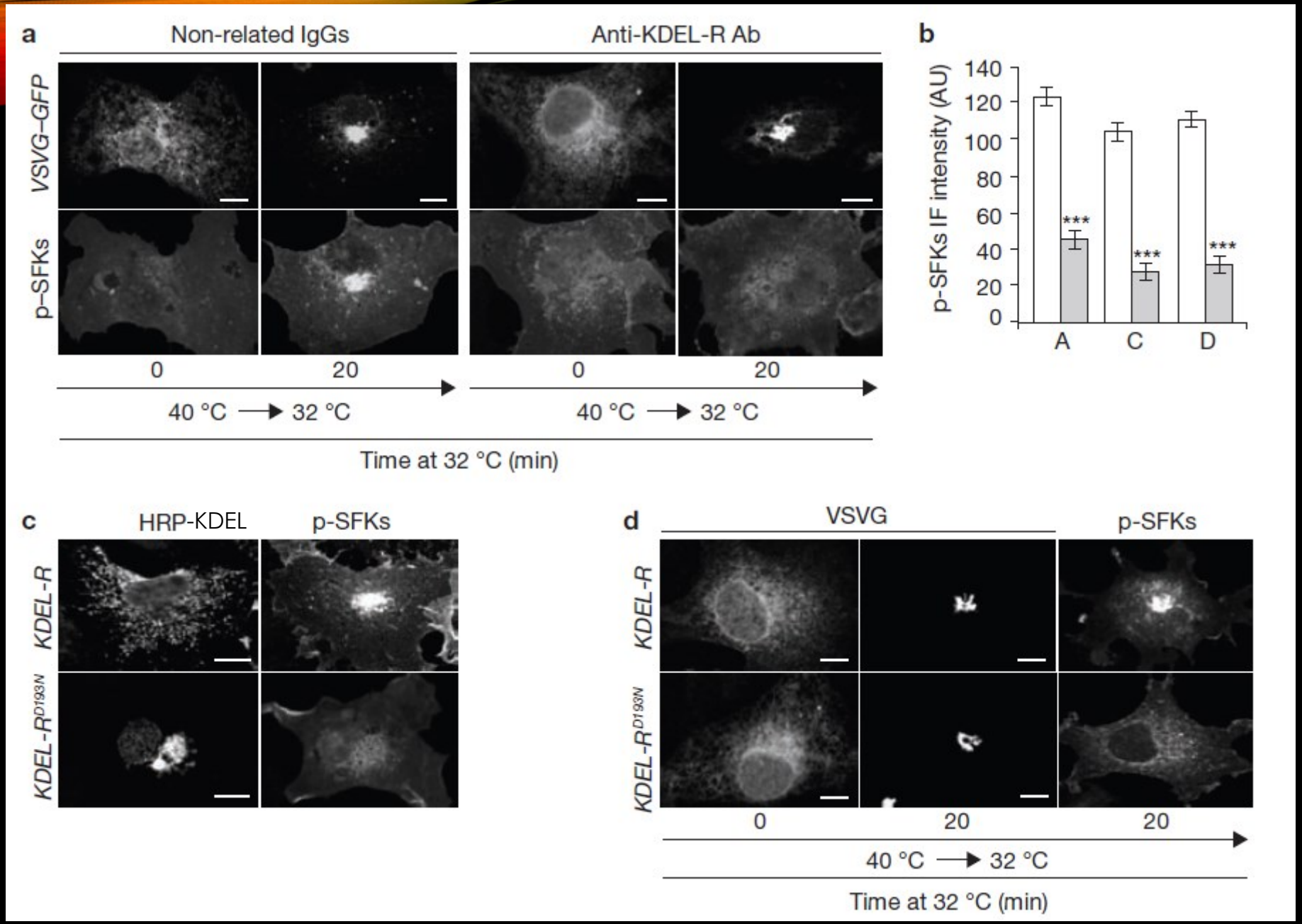
	HDEL receptor	KDEL receptor 1	KDEL receptor 2	KDEL receptor 3a	KDEL receptor 3b
HDEL receptor (Ac. No. CAA84860)		50,2% / 70,3%	49,5% / 68,0%	47,5% / 69,7%	43,9% / 65,2%
KDEL receptor 1 (Ac. No. NP_006792)	50,2% / 70,3%		83,5% / 93,9%	72,0% / 86,0%	65,5% / 79,5%
KDEL receptor 2 (Ac. No. NP_006845)	49,5% / 68,0%	83,5% / 93,9%		75,7% / 87,9%	69,1% / 81,4%
KDEL receptor 3a (Ac. No.NP_006846)	47,5% / 69,7%	72,0% / 86,0%	75,7% / 87,9%		91,4% / 92,3%
KDEL receptor 3b (Ac. No. NP_057839)	43,9% / 65,2%	65,5% / 79,5%	69,1% / 81,4%	91,4% / 92,3%	

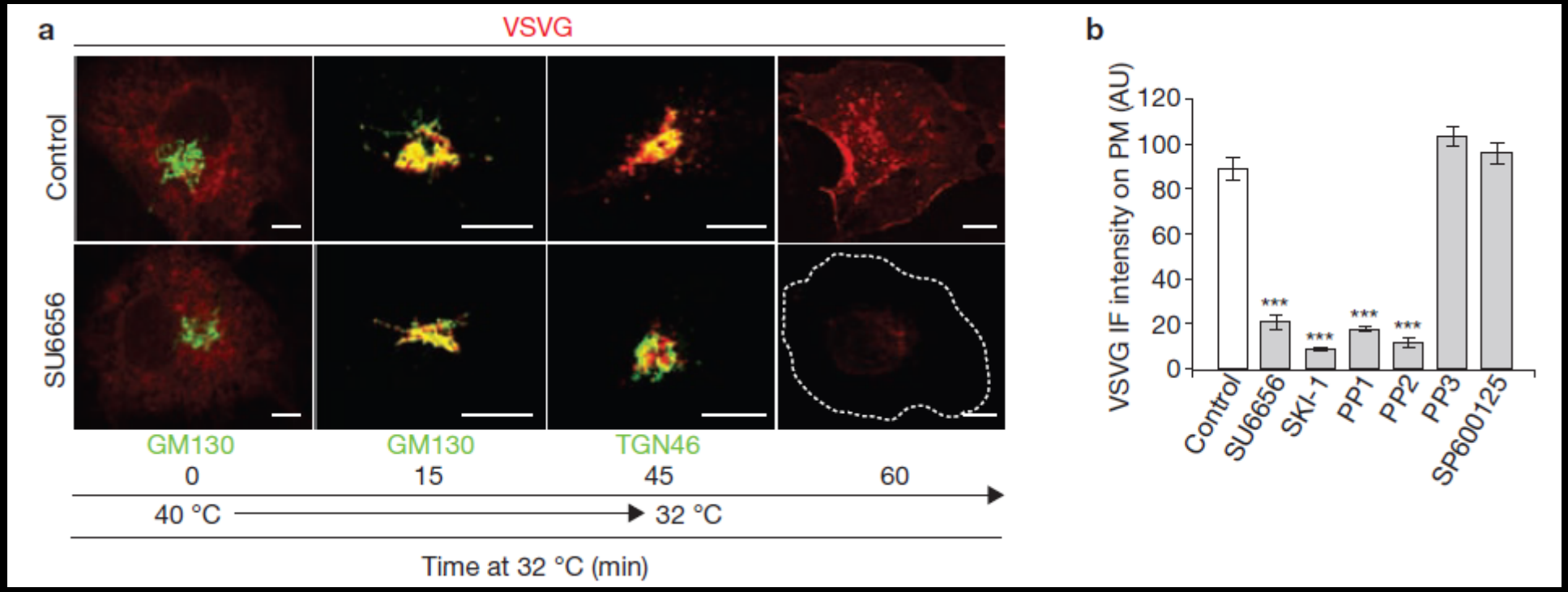

Higher H+ concentration (lower pH), peptide binding
Missorted ER-resident protein
KDEL peptide
cis-Golgi network
KDEL receptor
2
ER-to-Golgi transport vesicle
COPI coat
3
Retrieval of KDEL-bearing proteins to ER
1
COPII coat
4
Rough ER
Lower H+ concentration (higher pH), peptide release

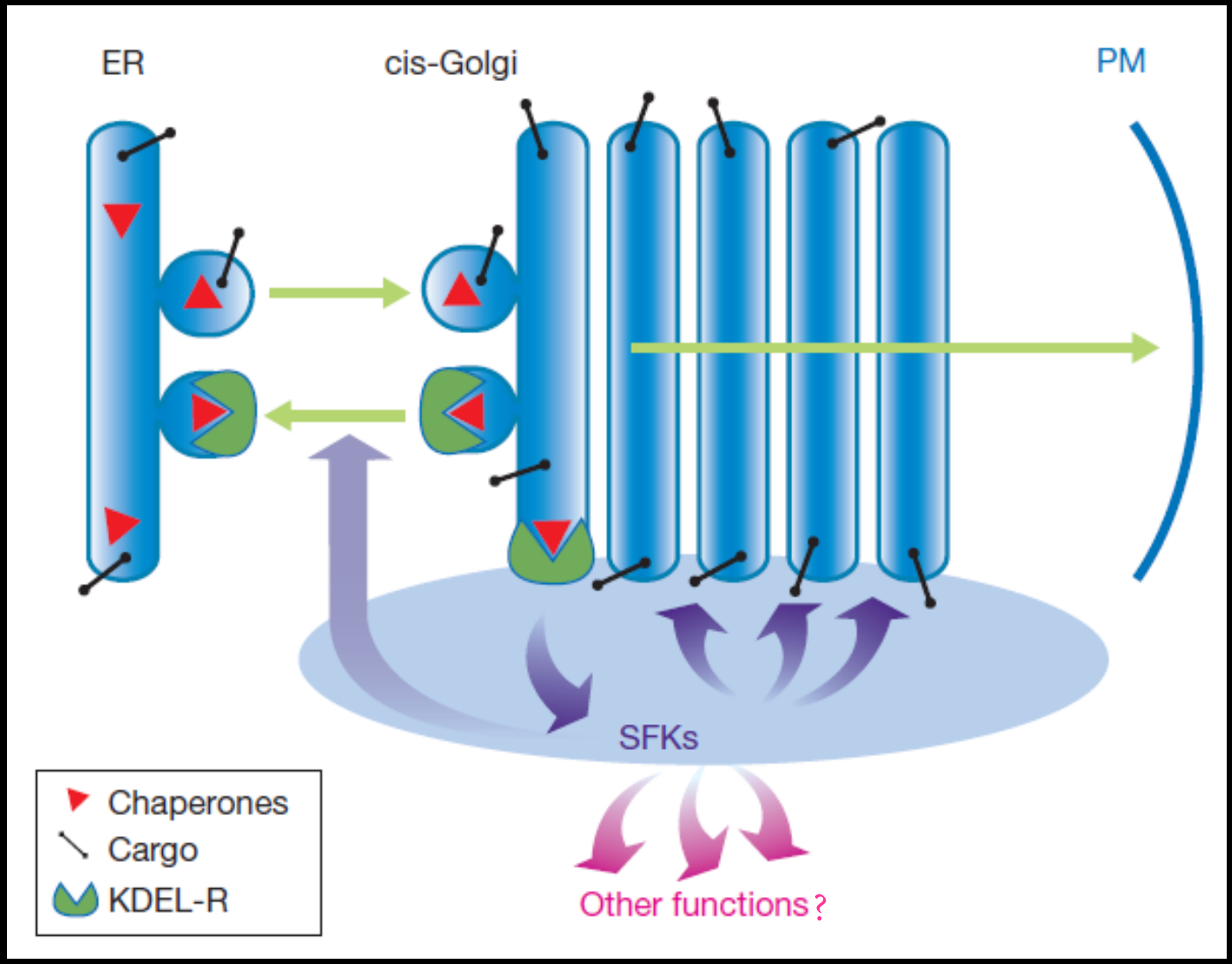

a
GM130
PC-I
Merge
PC-I
0
7
30
40°C
32 °C
Time at 32 °C (min)
b
p-Tyr
0
7
30
40°C → 32°C
Time at 32 °C (min)
c
p-Tyr
0
30
40°C → 32°C + SU6656
Time at 32 °C (min)
d
IF intensity (AU)
160
120
80
40
0
***
***
***
0
7
30
60
Time at 32 °C (min)

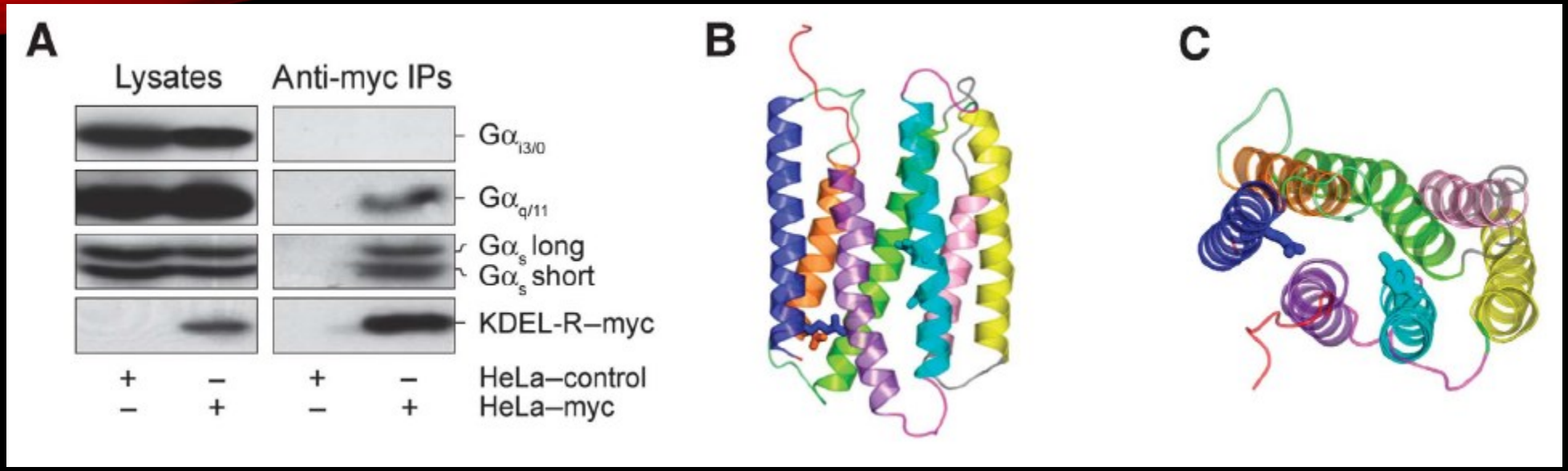

a
p-Tyr
M_r(K)
116
96
52
35
29
40 °C
32 °C
40 °C
32 °C
Control
SU6656
b
p-Tyr
M_r(K)
116
96
52
35
29
1
2
3
32 °C
40 °C
32 °C
c
p-SFKs
SFKs
40 °C
32 °C
40 °C
32 °C
Control
SU6656
p-Yes
p-Src/Fyn
p-Lyn
40 °C
32 °C
d
p-SFKs
0
30
40°C → 32°C
Time at 32 °C (min)
e
p-SFKs
0
30
40°C → 32°C + SU6656
Time at 32 °C (min)
f
SFKs
0
30
40°C → 32°C
Time at 32 °C (min)

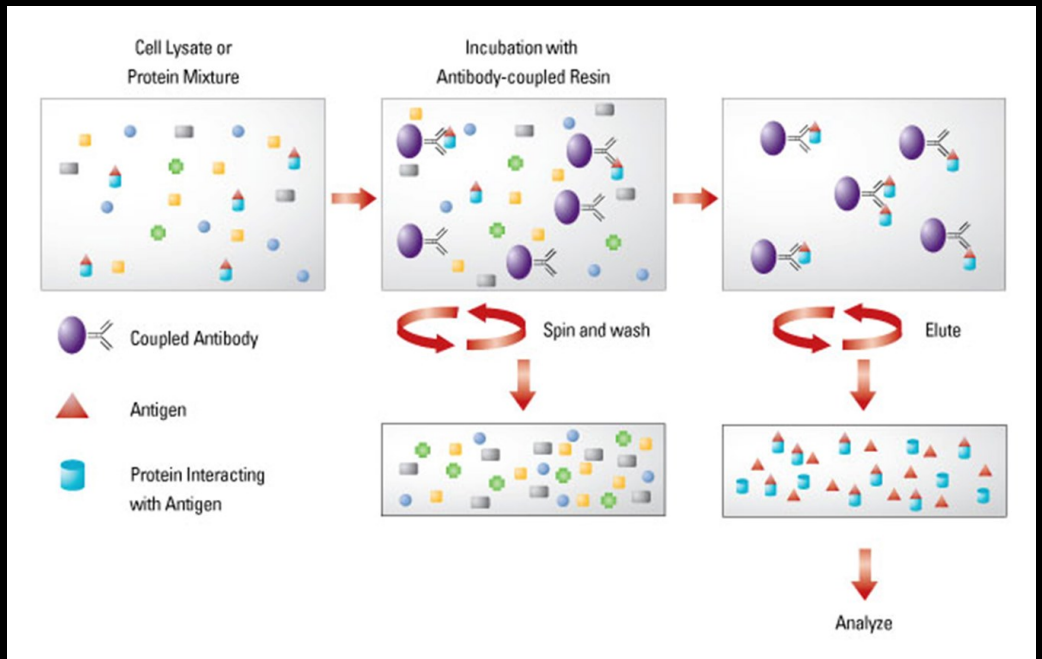

c
HRP
p-SFKs
ssHRP
ssHRPKDEL
d
KDEL-R
p-SFKs
Low
High
e
p-SFKs IF intensity (AU)
140
120
100
80
60
40
20
0
ssHRP
ssHRPKDEL
Low
High

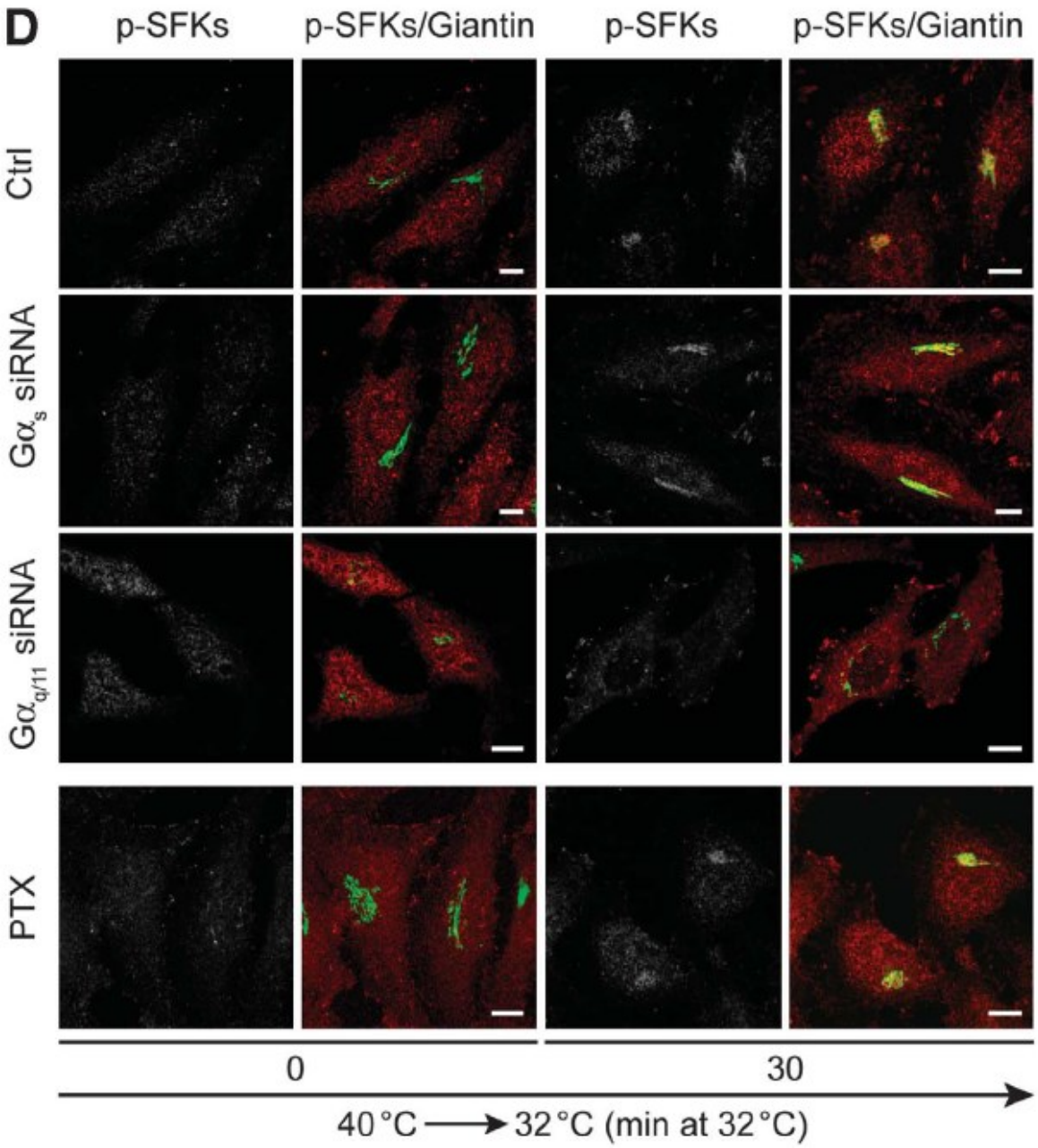

a
Non-related IgGs
Anti-KDEL-R Ab
VSVG–GFP
p–SFKs
0
20
0
20
40 °C → 32 °C
40 °C → 32 °C
Time at 32 °C (min)
b
p-SFKs IF intensity (AU)
140
120
100
80
60
40
20
0
***
***
***
A
C
D
c
HRP-KDEL
p-SFKs
KDEL-R
KDEL-R^D193N
d
VSVG
p-SFKs
KDEL-R
KDEL-R^D193N
0
20
20
40 °C → 32 °C
Time at 32 °C (min)

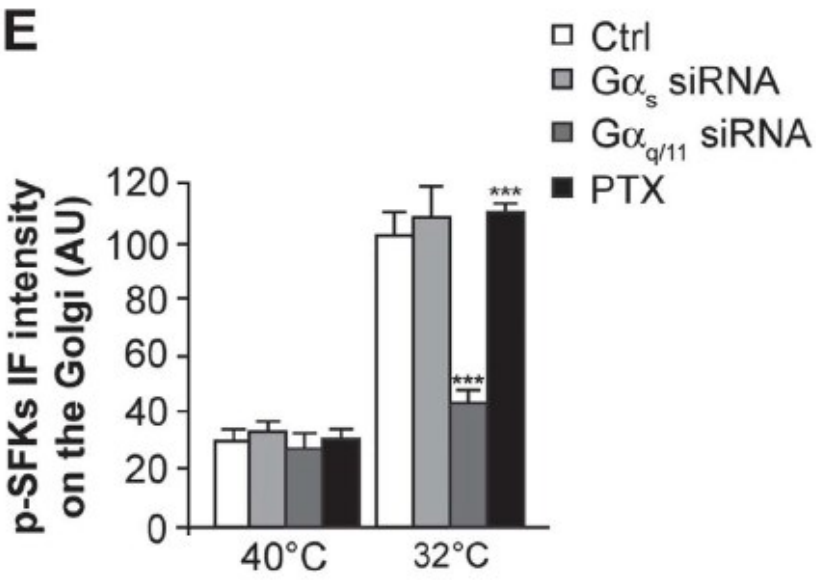

a
VSVG
Control
SU6656
GM130
GM130
TGN46
0
15
45
60
40 °C
32 °C
Time at 32 °C (min)
b
VSVG IF intensity on PM (AU)
120
100
80
60
40
20
0
***
Control
SU6656
SKI-1
PP1
PP2
PP3
SP600125

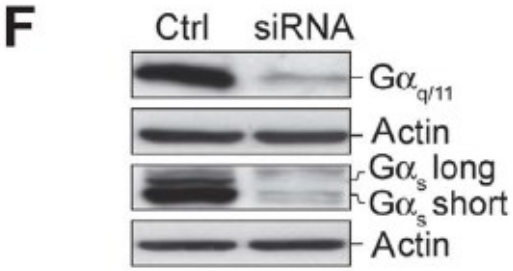

ER
cis-Golgi
PM
SFKs
Chaperones
Cargo
KDEL-R
Other functions?

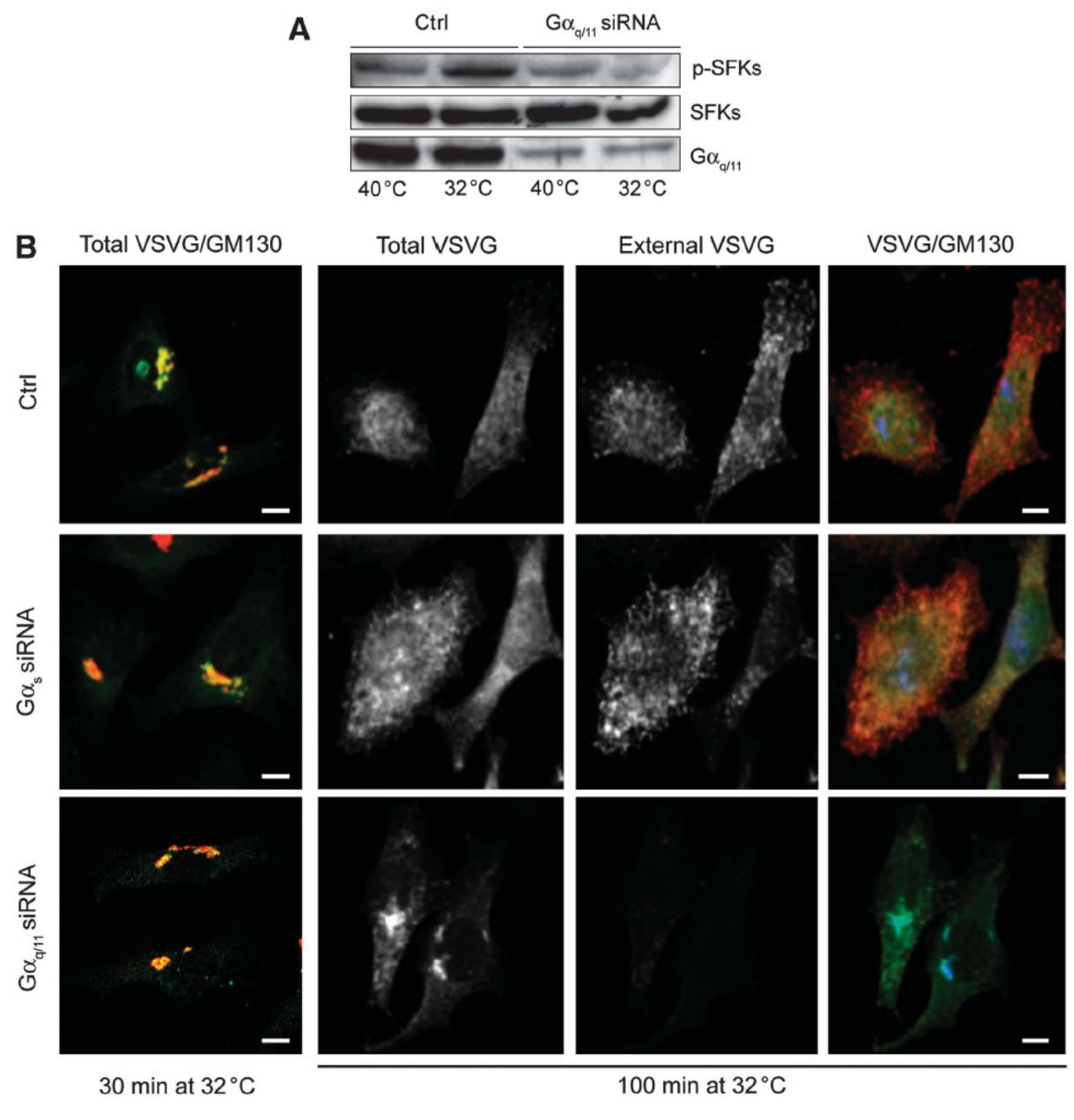

A
Lysates
Anti-myc IPs
$G\alpha_{i3/0}$
$G\alpha_{q/11}$
$G\alpha_s$ long
$G\alpha_s$ short
KDEL-R–myc
+ – + – HeLa–control
– + – + HeLa–myc
B
C

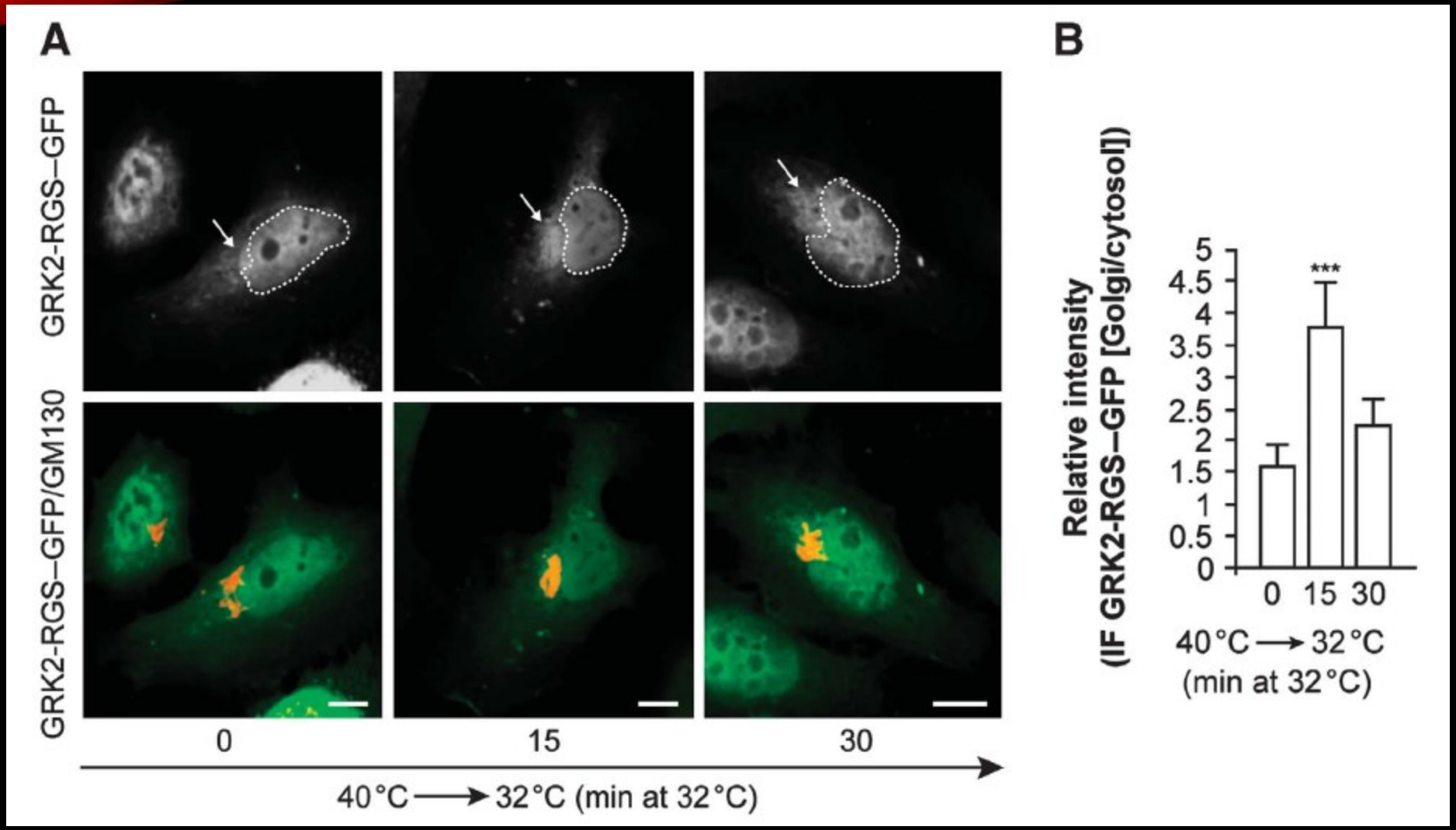

Cell Lysate or
Protein Mixture
Incubation with
Antibody-coupled Resin
Coupled Antibody
Antigen
Protein Interacting
with Antigen
Spin and wash
Elute
Analyze

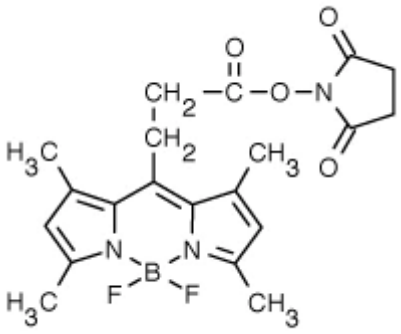

D
p-SFKs
p-SFKs/Giantin
p-SFKs
p-SFKs/Giantin
Ctrl
Gαs siRNA
Gαq/11 siRNA
PTX
0
30
40 °C → 32 °C (min at 32 °C)

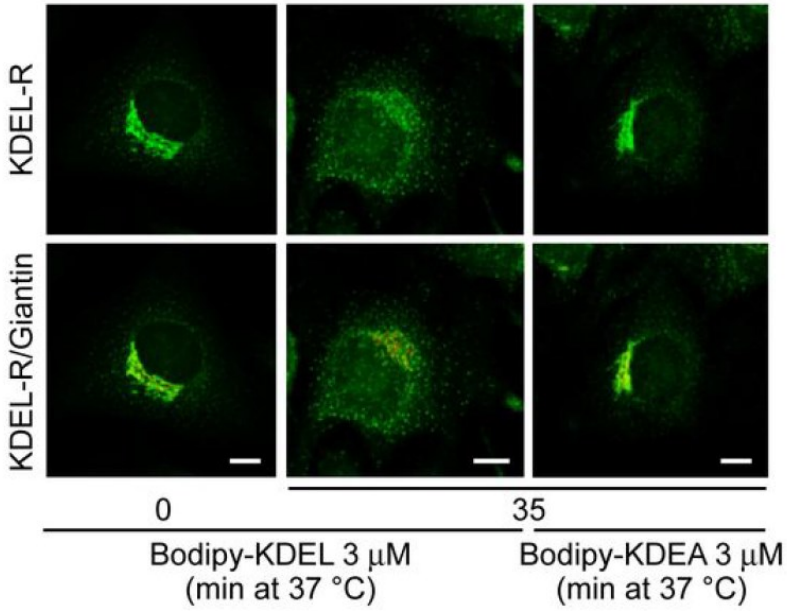

E
Ctrl
Gαs siRNA
Gαq/11 siRNA
PTX
p-SFKs IF intensity on the Golgi (AU)
120
100
80
60
40
20
0
***
***
40°C
32°C


F
Ctrl
siRNA
Gαq/11
Actin
Gαs long
Gαs short
Actin

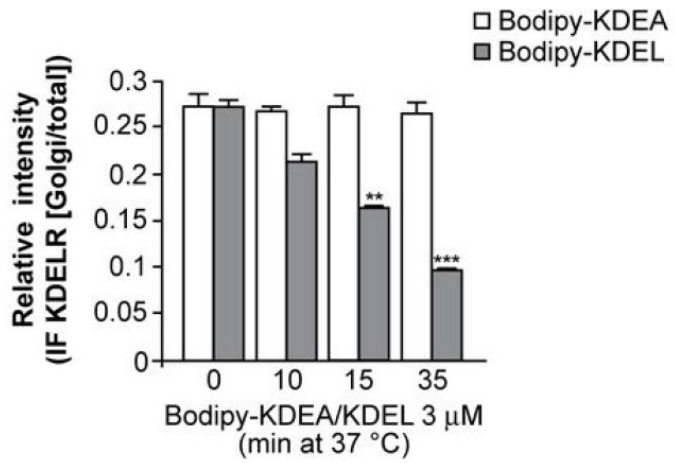

A
Ctrl
Gα$_{q/11}$ siRNA
p-SFKs
SFKs
Gα$_{q/11}$
40 °C
32 °C
40 °C
32 °C
B
Total VSVG/GM130
Total VSVG
External VSVG
VSVG/GM130
Ctrl
Gα$_s$ siRNA
Gα$_{q/11}$ siRNA
30 min at 32 °C
100 min at 32 °C


A
GRK2-RGS–GFP
GRK2-RGS–GFP/GM130
0
15
30
40 °C → 32 °C (min at 32 °C)
B
Relative intensity
(IF GRK2-RGS–GFP [Golgi/cytosol])
***
0 15 30
40 °C → 32 °C
(min at 32 °C)

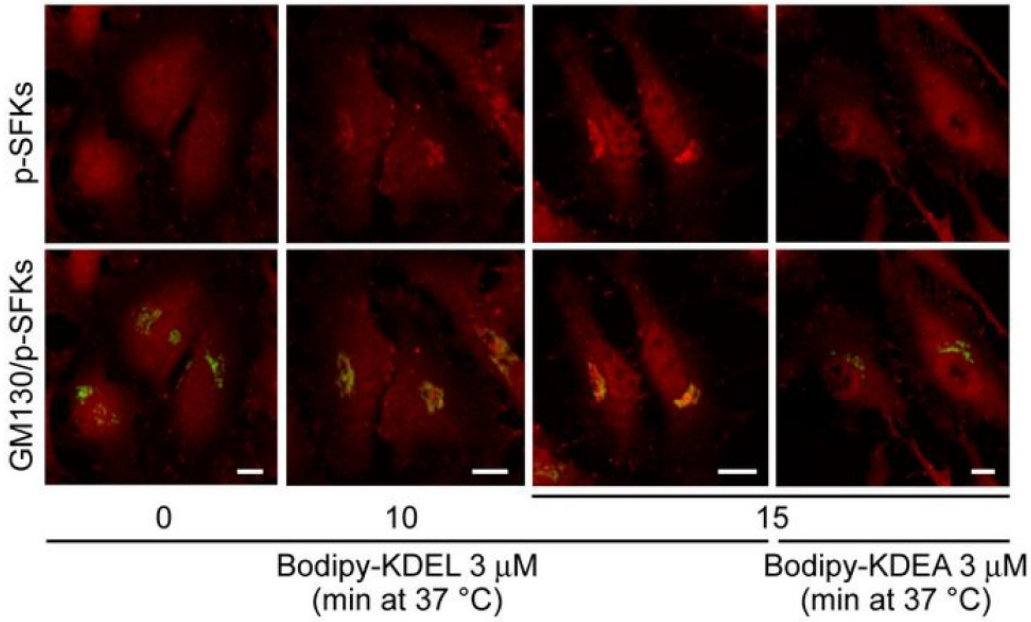



KDEL-R
KDEL-R/Giantin
0
35
Bodipy-KDEL 3 μM
(min at 37 °C)
Bodipy-KDEA 3 μM
(min at 37 °C)

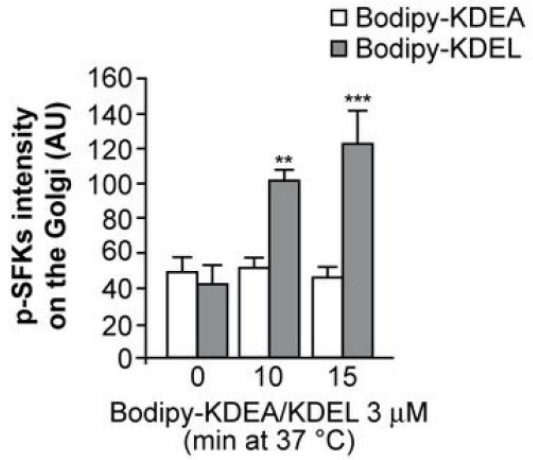
B

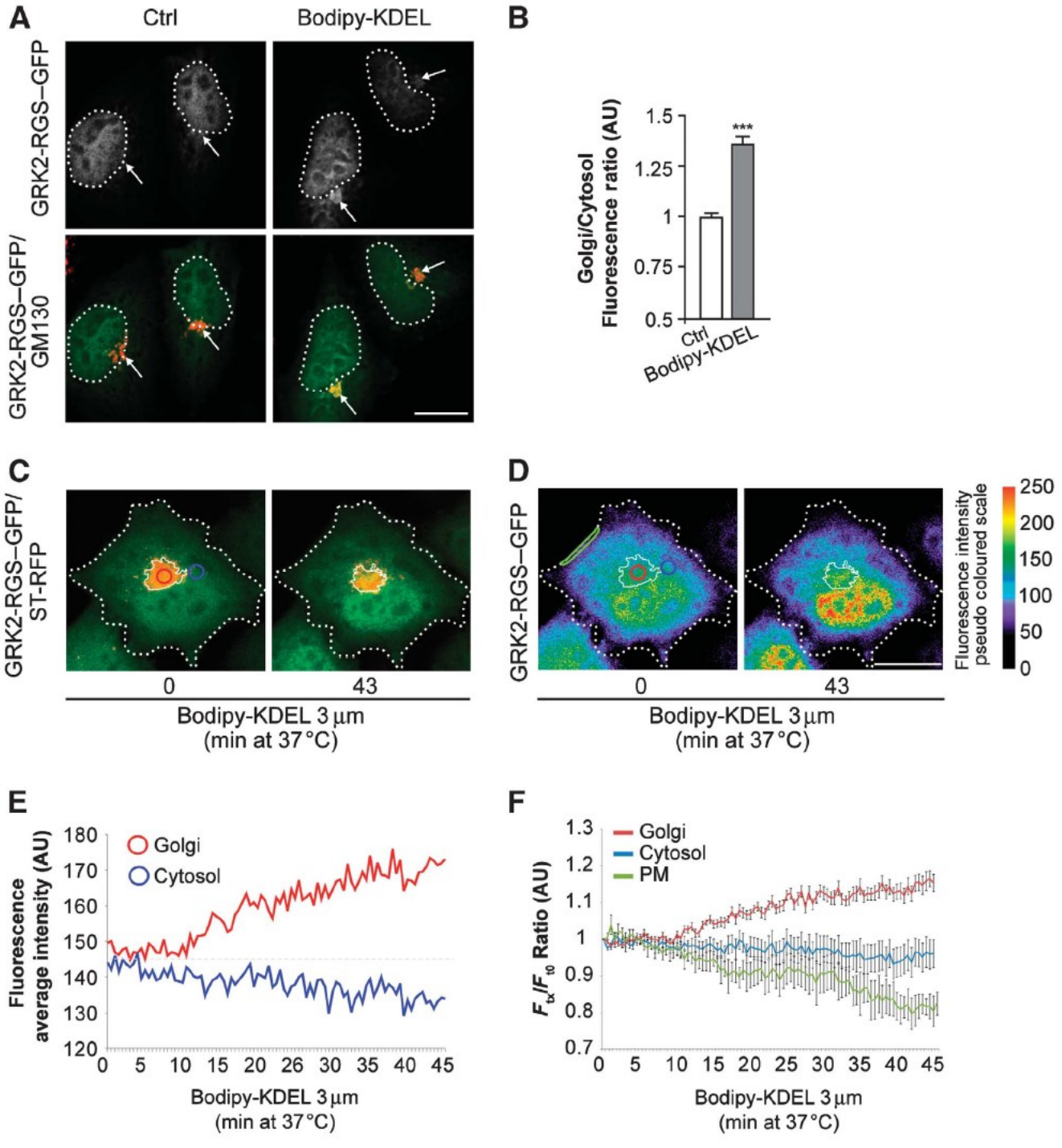

Bodipy-KDEA
Bodipy-KDEL
Relative intensity
(IF KDELR [Golgi/total])
0.3
0.25
0.2
0.15
0.1
0.05
0
**
***
0
10
15
35
Bodipy-KDEA/KDEL 3 μM
(min at 37 °C)

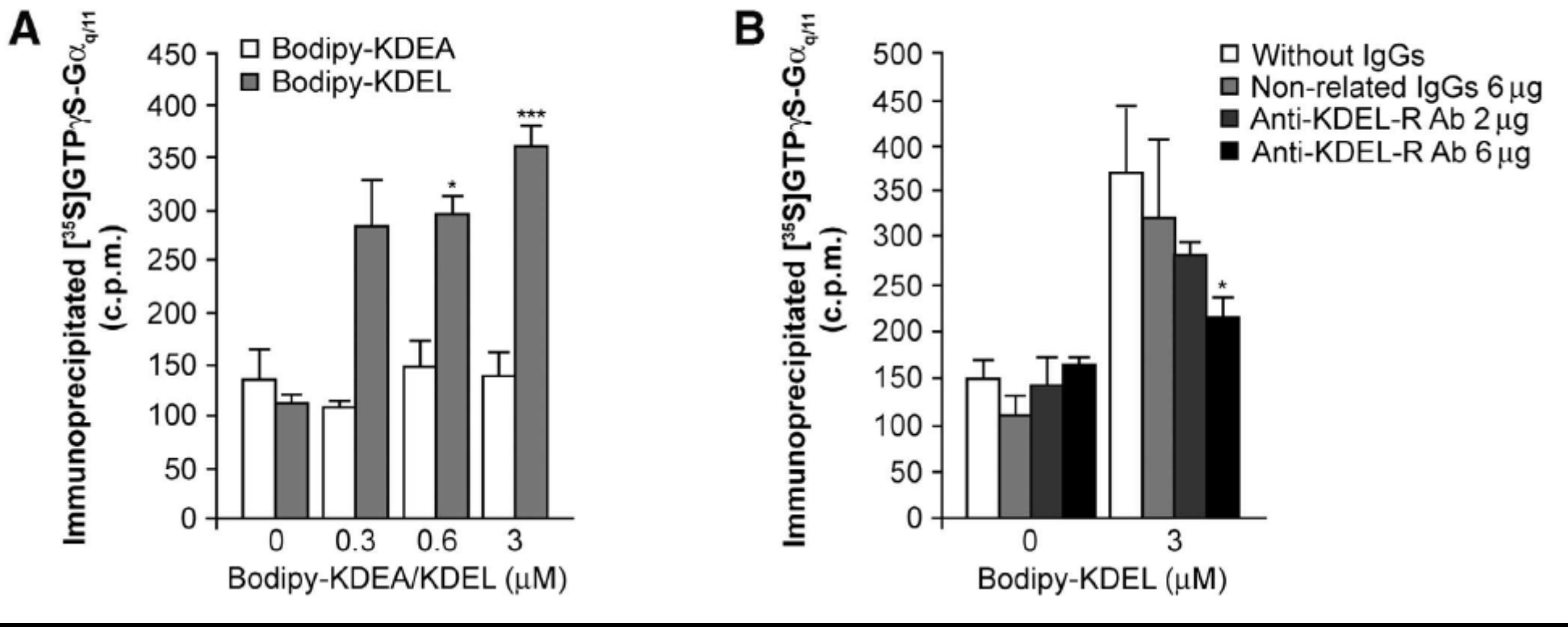
C

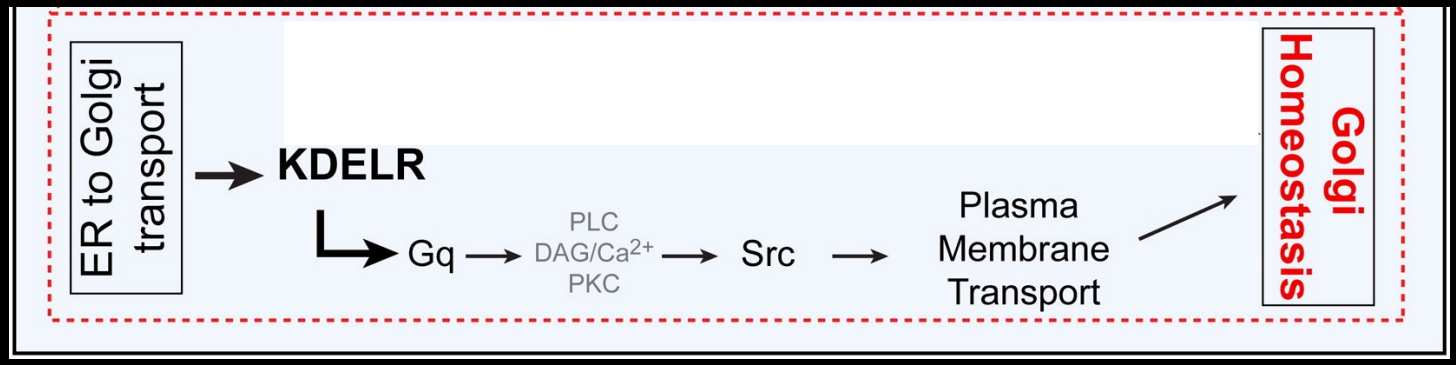

p-SFKs
GM130/p-SFKs
0
10
15
Bodipy-KDEL 3 μM
(min at 37 °C)
Bodipy-KDEA 3 μM
(min at 37 °C)

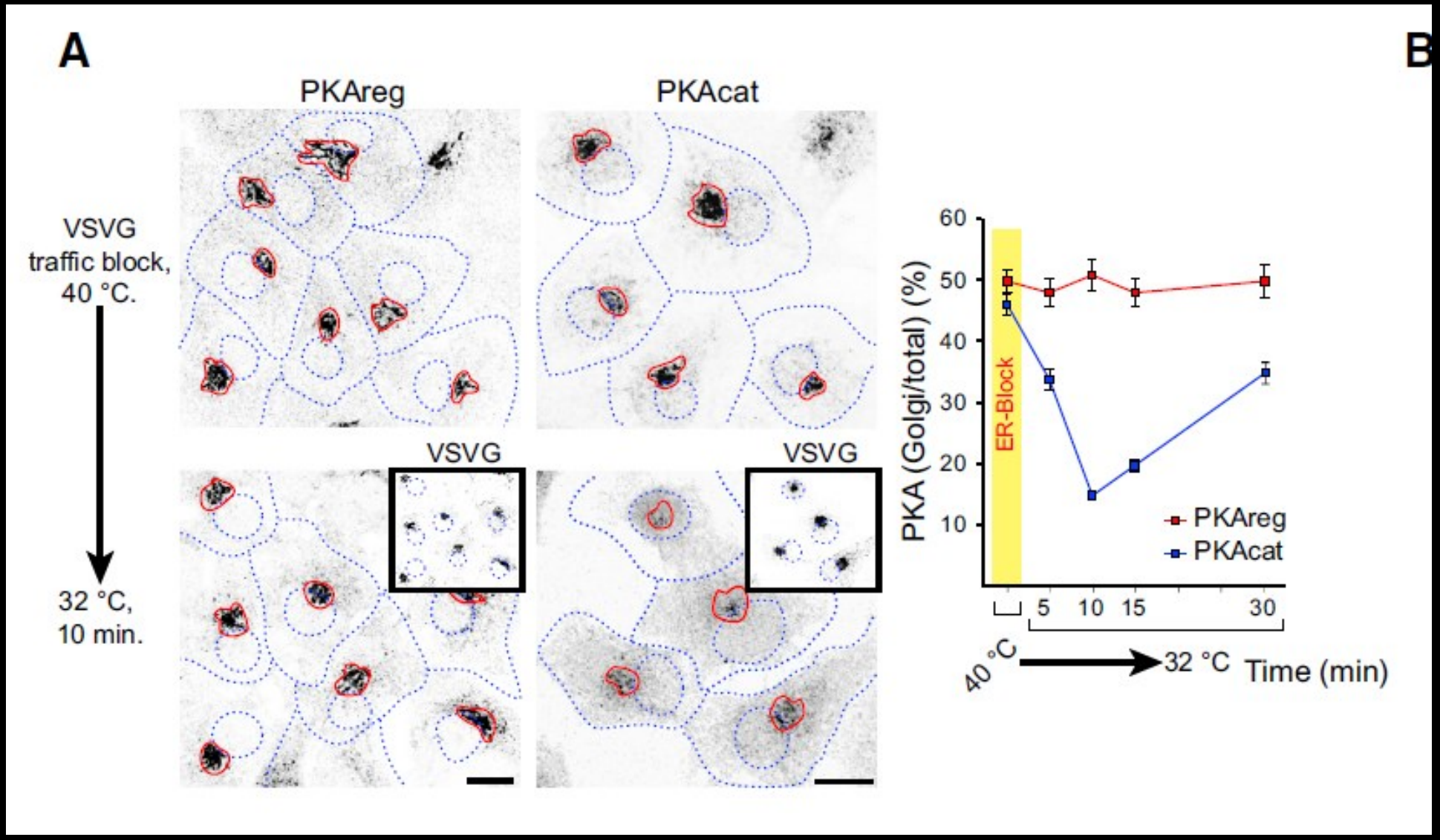
D

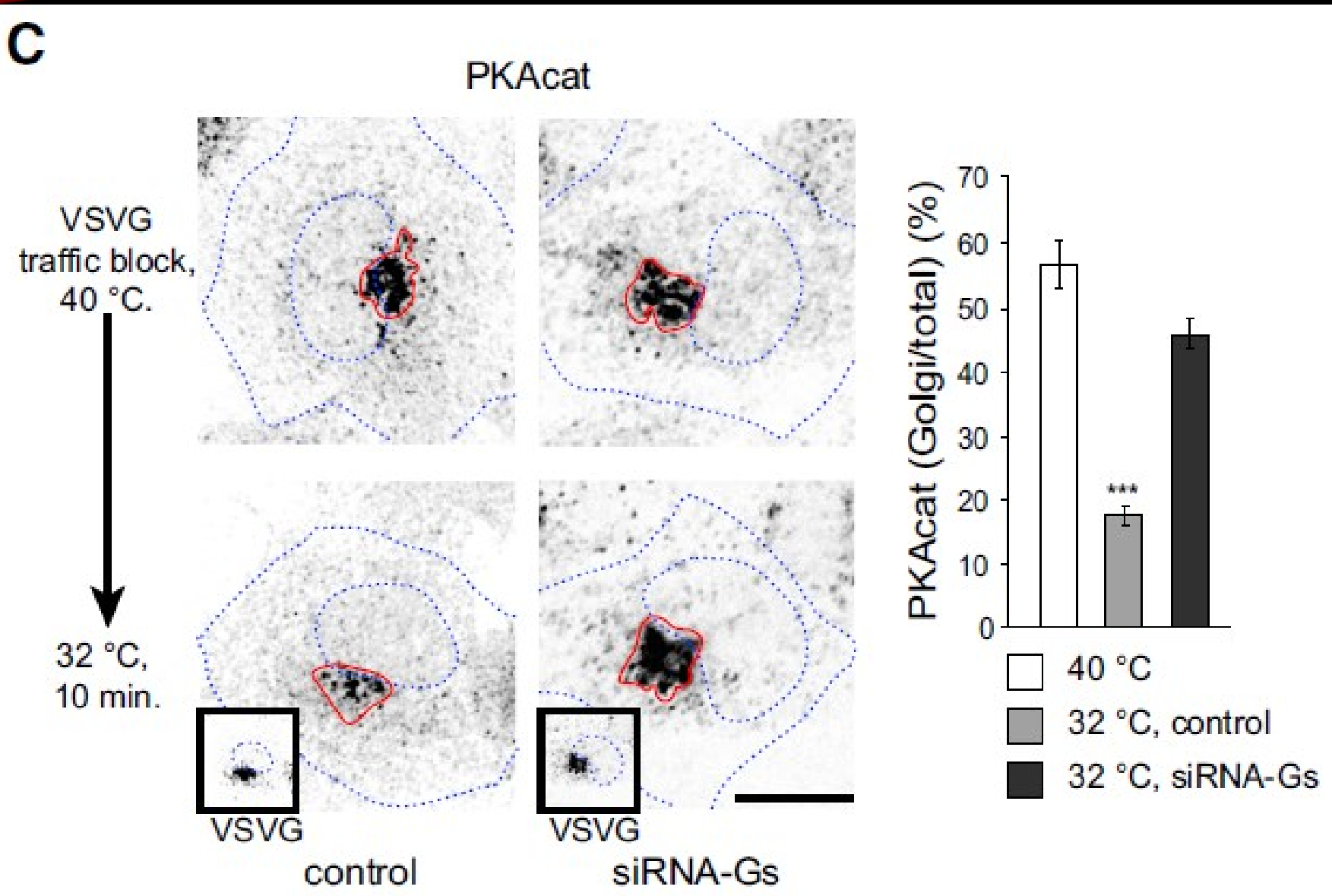

Bodipy-KDEA
Bodipy-KDEL
p-SFKs intensity
on the Golgi (AU)
160
140
120
100
80
60
40
20
0
**
***
0
10
15
Bodipy-KDEA/KDEL 3 μM
(min at 37 °C)

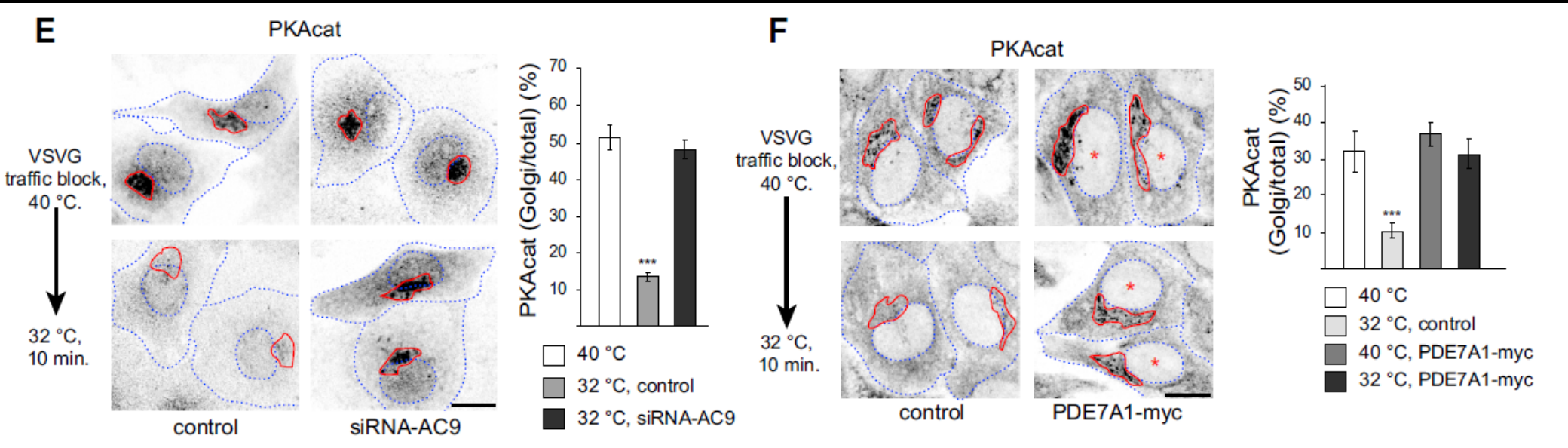

A
Ctrl
Bodipy-KDEL
GRK2-RGS–GFP
GRK2-RGS–GFP/
GM130
B
Golgi/Cytosol
Fluorescence ratio (AU)
1.5
1.25
1
0.75
0.5
***
Ctrl
Bodipy-KDEL
C
GRK2-RGS–GFP/
ST-RFP
0
43
Bodipy-KDEL 3 µm
(min at 37 °C)
D
GRK2-RGS–GFP
0
43
Bodipy-KDEL 3 µm
(min at 37 °C)
Fluorescence intensity
pseudo coloured scale
250
200
150
100
50
0
E
Fluorescence
average intensity (AU)
180
170
160
150
140
130
120
Golgi
Cytosol
0 5 10 15 20 26 30 35 40 45
Bodipy-KDEL 3 µm
(min at 37 °C)
F
F_{tx}/F_{t0} Ratio (AU)
1.3
1.2
1.1
1
0.9
0.8
0.7
Golgi
Cytosol
PM
0 5 10 15 20 25 30 35 40 45
Bodipy-KDEL 3 µm
(min at 37 °C)

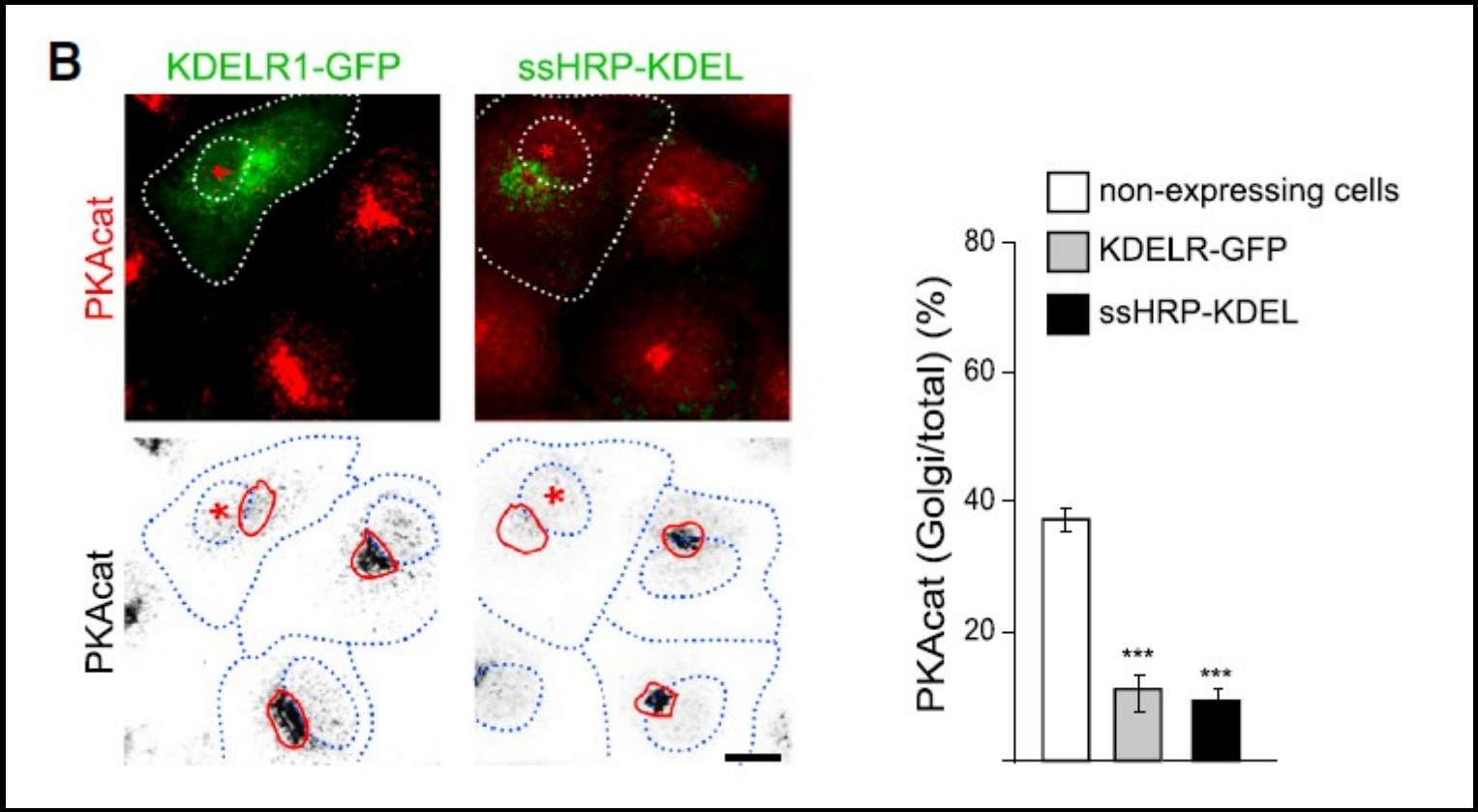

A
Immunoprecipitated [35S]GTPγS-Gαq/11 (c.p.m.)
Bodipy-KDEA
Bodipy-KDEL
450
400
350
300
250
200
150
100
50
0
*
***
0
0.3
0.6
3
Bodipy-KDEA/KDEL (μM)
B
Immunoprecipitated [35S]GTPγS-Gαq/11 (c.p.m.)
Without IgGs
Non-related IgGs 6 μg
Anti-KDEL-R Ab 2 μg
Anti-KDEL-R Ab 6 μg
500
450
400
350
300
250
200
150
100
50
0
*
0
3
Bodipy-KDEL (μM)

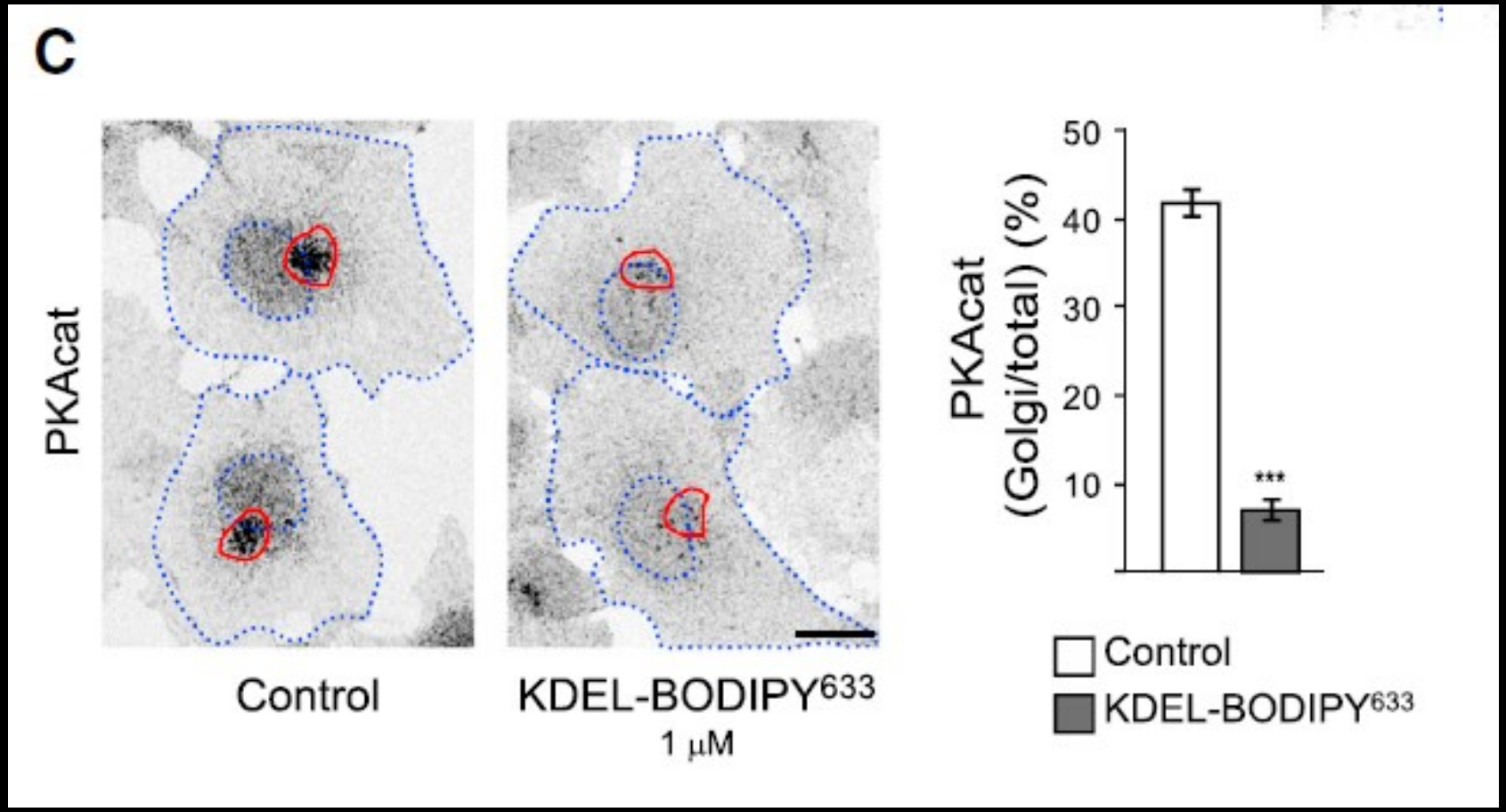

ER to Golgi transport
KDELR
Gq
PLC
DAG/Ca2+
PKC
Src
Plasma Membrane Transport
Golgi Homeostasis

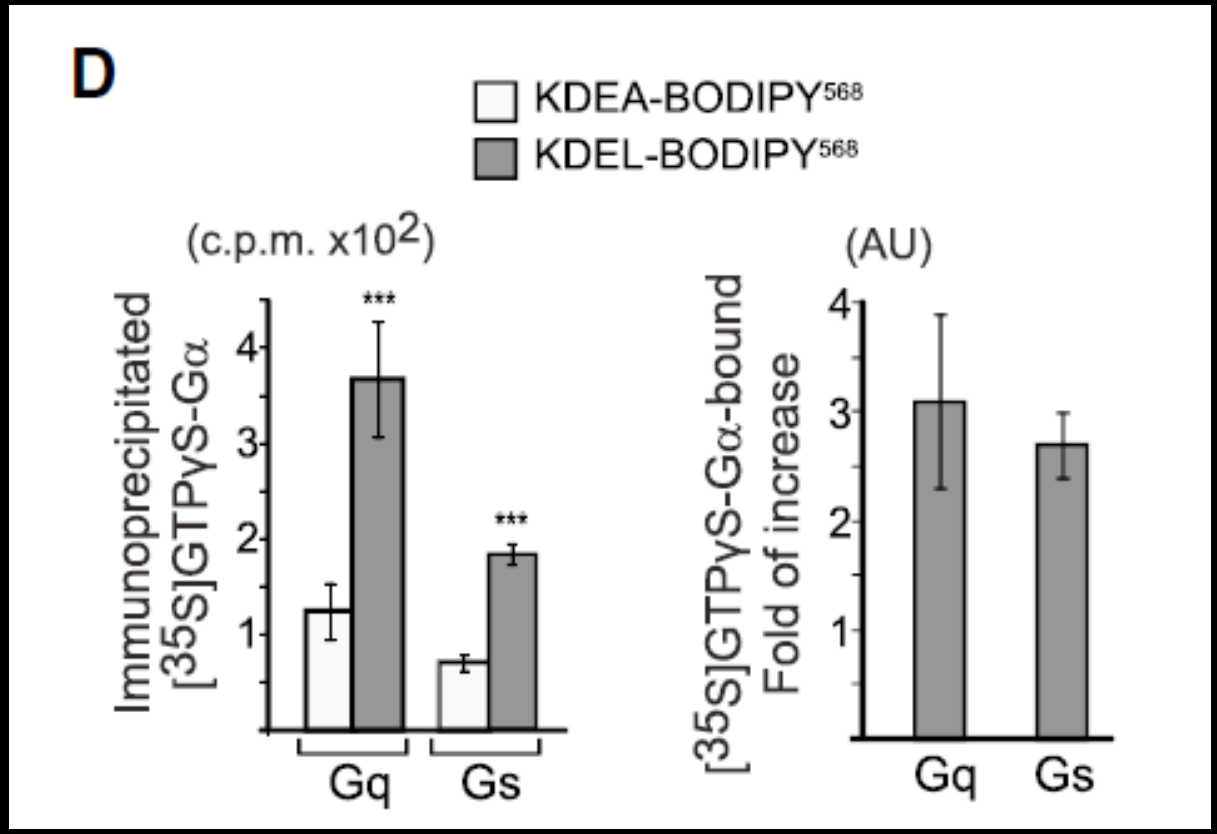

A
PKAreg
PKAcat
VSVG
traffic block,
40 °C.
32 °C,
10 min.
VSVG
VSVG
B
PKA (Golgi/total) (%)
60
50
40
30
20
10
ER-Block
PKAreg
PKAcat
5
10
15
30
40 °C
32 °C
Time (min)


C
PKAcat
VSVG
traffic block,
40 °C.
32 °C,
10 min.
VSVG
control
VSVG
siRNA-Gs
PKAcat (Golgi/total) (%)
70
60
50
40
30
20
10
0
***
40 °C
32 °C, control
32 °C, siRNA-Gs

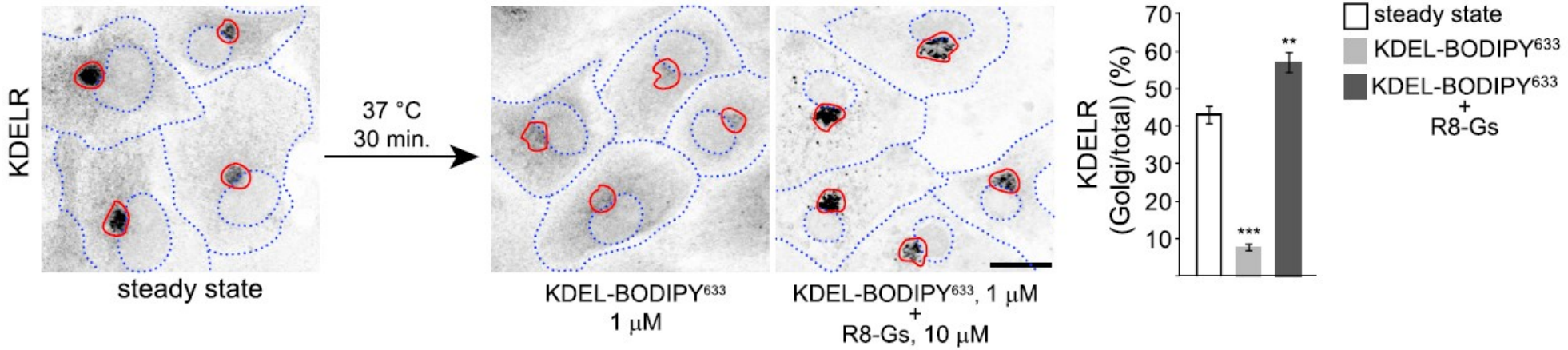

E
PKAcat
VSVG
traffic block,
40 °C.
32 °C,
10 min.
control
siRNA-AC9
PKAcat (Golgi/total) (%)
70
60
50
40
30
20
10
***
40 °C
32 °C, control
32 °C, siRNA-AC9
F
PKAcat
VSVG
traffic block,
40 °C.
32 °C,
10 min.
control
PDE7A1-myc
PKAcat
(Golgi/total) (%)
50
40
30
20
10
***
40 °C
32 °C, control
40 °C, PDE7A1-myc
32 °C, PDE7A1-myc

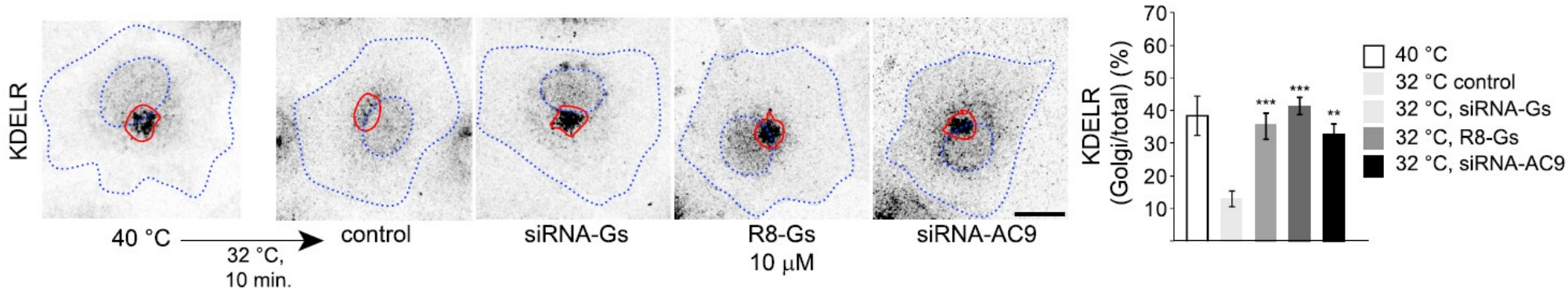

B
KDELR1-GFP
ssHRP-KDEL
PKAcat
PKAcat
non-expressing cells
KDELR-GFP
ssHRP-KDEL
PKAcat (Golgi/total) (%)
80
60
40
20
***
***

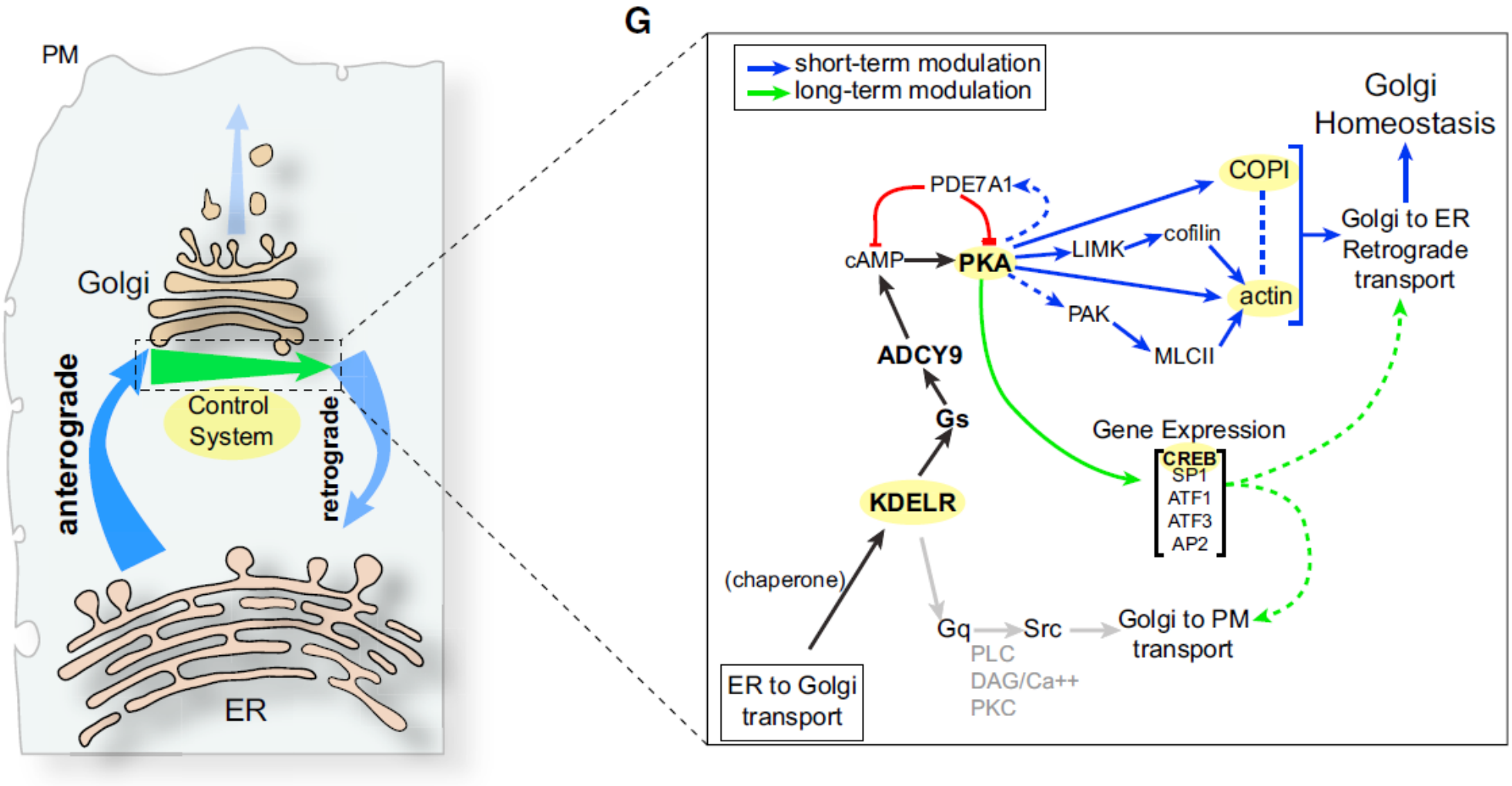

C
PKAcat
Control
KDEL-BODIPY633
1 μM
PKAcat
(Golgi/total) (%)
50
40
30
20
10
***
Control
KDEL-BODIPY633


D
KDEA-BODIPY568
KDEL-BODIPY568
(c.p.m. x10²)
Immunoprecipitated [35S]GTPγS-Gα
***
***
Gq
Gs
(AU)
[35S]GTPγS-Gα-bound Fold of increase
Gq
Gs

RUOLO DEL SIGNALING KDELR-$G_{\alpha s}$


KDELR
37 °C
30 min.
steady state
KDEL-BODIPY633
1 μM
KDEL-BODIPY633, 1 μM
+
R8-Gs, 10 μM
KDELR
(Golgi/total) (%)
70
60
50
40
30
20
10
**
***
steady state
KDEL-BODIPY633
KDEL-BODIPY633
+
R8-Gs


KDELR
40 °C
32 °C,
10 min.
control
siRNA-Gs
R8-Gs
10 μM
siRNA-AC9
KDELR
(Golgi/total) (%)
70
60
50
40
30
20
10
***
***
**
40 °C
32 °C control
32 °C, siRNA-Gs
32 °C, R8-Gs
32 °C, siRNA-AC9


PM
Golgi
anterograde
Control System
retrograde
ER
G
short-term modulation
long-term modulation
Golgi Homeostasis
PDE7A1
COPI
cAMP
PKA
LIMK
cofilin
actin
Golgi to ER Retrograde transport
PAK
MLCII
ADCY9
Gs
Gene Expression
CREB
SP1
ATF1
ATF3
AP2
KDELR
(chaperone)
Gq
Src
PLC
DAG/Ca++
PKC
Golgi to PM transport
ER to Golgi transport

Grazie per l'attenzione