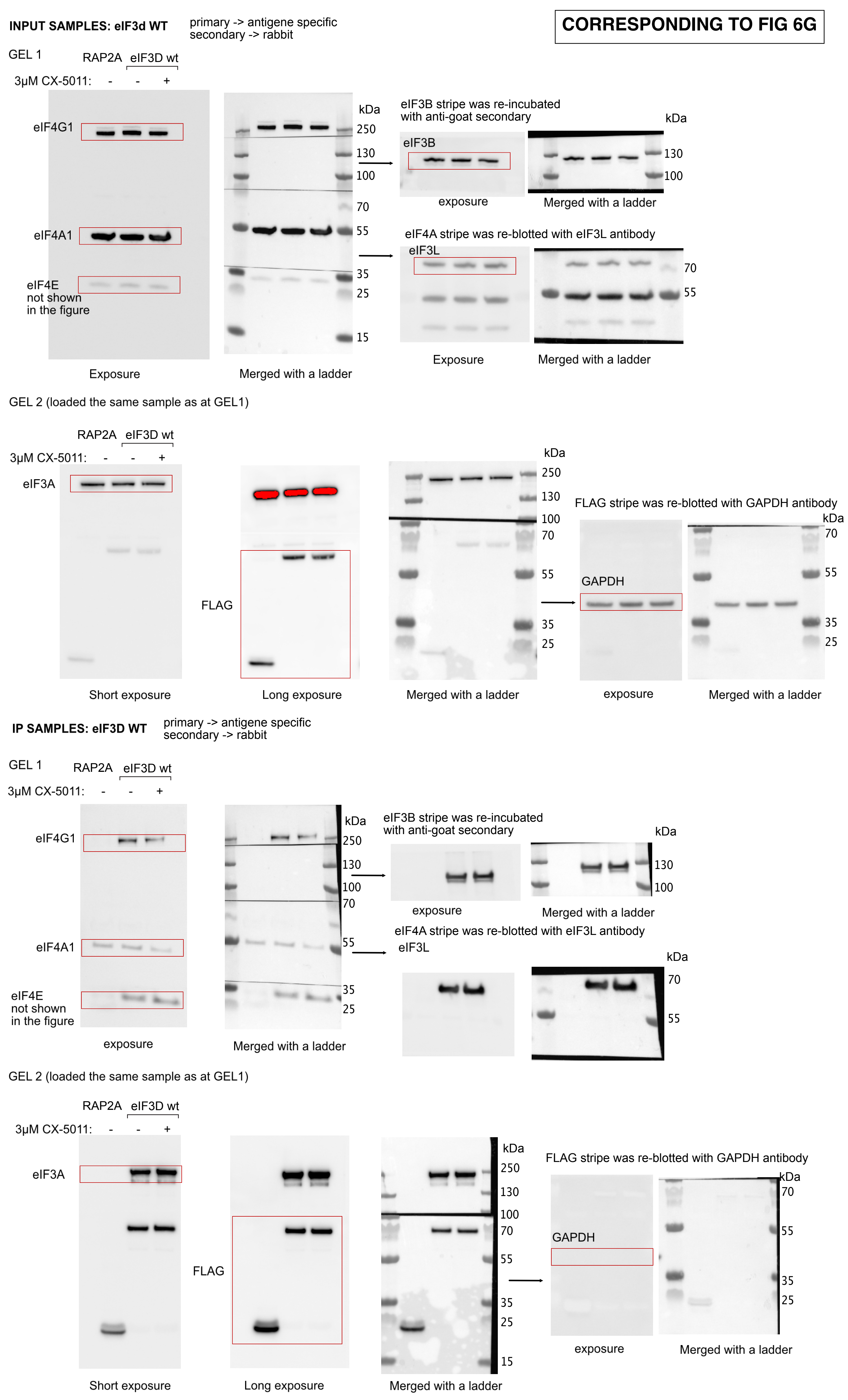
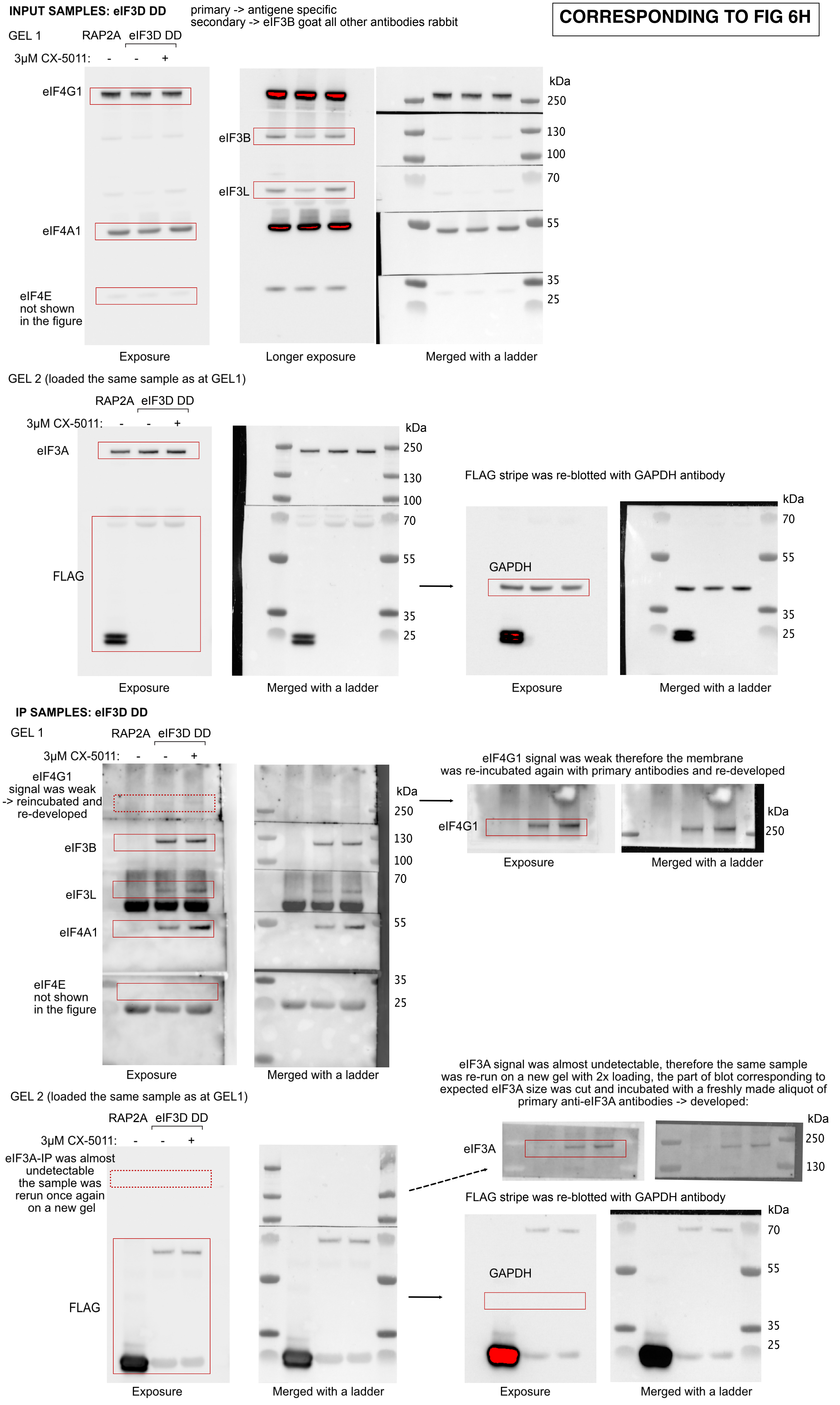


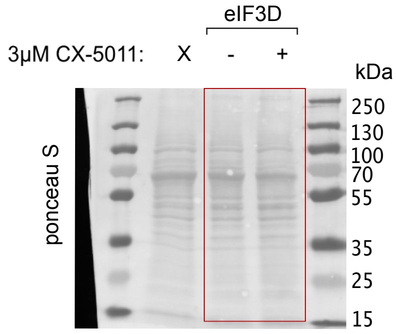
CORRESPONDING TO FIG 6G
INPUT SAMPLES: eIF3d WT
primary -> antigene specific
secondary -> rabbit
GEL 1
RAP2A
eIF3D wt
3µM CX-5011: - - +
eIF4G1
eIF4A1
eIF4E
not shown
in the figure
Exposure
Merged with a ladder
kDa
250
130
100
70
55
35
25
15
eIF3B stripe was re-incubated
with anti-goat secondary
eIF3B
exposure
Merged with a ladder
eIF4A stripe was re-blotted with eIF3L antibody
eIF3L
Exposure
Merged with a ladder
GEL 2 (loaded the same sample as at GEL1)
RAP2A
eIF3D wt
3µM CX-5011: - - +
eIF3A
FLAG
Short exposure
Long exposure
Merged with a ladder
FLAG stripe was re-blotted with GAPDH antibody
GAPDH
exposure
Merged with a ladder
IP SAMPLES: eIF3D WT
primary -> antigene specific
secondary -> rabbit
GEL 1
RAP2A
eIF3D wt
3µM CX-5011: - - +
eIF4G1
eIF4A1
eIF4E
not shown
in the figure
exposure
Merged with a ladder
eIF3B stripe was re-incubated
with anti-goat secondary
exposure
Merged with a ladder
eIF4A stripe was re-blotted with eIF3L antibody
eIF3L
GEL 2 (loaded the same sample as at GEL1)
RAP2A
eIF3D wt
3µM CX-5011: - - +
eIF3A
FLAG
Short exposure
Long exposure
Merged with a ladder
FLAG stripe was re-blotted with GAPDH antibody
GAPDH
exposure
Merged with a ladder



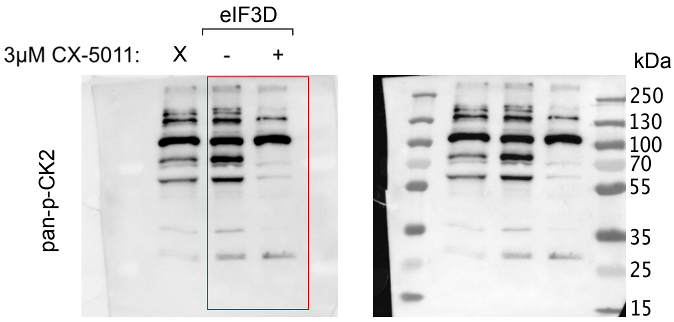
INPUT SAMPLES: eIF3D DD
primary -> antigene specific
secondary -> eIF3B goat all other antibodies rabbit
CORRESPONDING TO FIG 6H
GEL 1
RAP2A eIF3D DD
3µM CX-5011: - - +
eIF4G1
eIF3B
eIF3L
eIF4A1
eIF4E
not shown
in the figure
kDa
250
130
100
70
55
35
25
Exposure
Longer exposure
Merged with a ladder
GEL 2 (loaded the same sample as at GEL1)
RAP2A eIF3D DD
3µM CX-5011: - - +
eIF3A
FLAG
kDa
250
130
100
70
55
35
25
FLAG stripe was re-blotted with GAPDH antibody
GAPDH
kDa
70
55
35
25
Exposure
Merged with a ladder
Exposure
Merged with a ladder
IP SAMPLES: eIF3D DD
GEL 1
RAP2A eIF3D DD
3µM CX-5011: - - +
eIF4G1
signal was weak
-> reincubated and
re-developed
eIF3B
eIF3L
eIF4A1
eIF4E
not shown
in the figure
kDa
250
130
100
70
55
35
25
eIF4G1 signal was weak therefore the membrane
was re-incubated again with primary antibodies and re-developed
eIF4G1
kDa
250
Exposure
Merged with a ladder
Exposure
Merged with a ladder
eIF3A signal was almost undetectable, therefore the same sample
was re-run on a new gel with 2x loading, the part of blot corresponding to
expected eIF3A size was cut and incubated with a freshly made aliquot of
primary anti-eIF3A antibodies -> developed:
GEL 2 (loaded the same sample as at GEL1)
RAP2A eIF3D DD
3µM CX-5011: - - +
eIF3A-IP was almost
undetectable
the sample was
rerun once again
on a new gel
eIF3A
kDa
250
130
FLAG stripe was re-blotted with GAPDH antibody
FLAG
GAPDH
kDa
70
55
35
25
Exposure
Merged with a ladder
Exposure
Merged with a ladder

CORRESPONDING TO FIG S7E

Membrane directly after the western blot transfer was stained with ponceau S, and the image was acquired:



the same membrane was washed from ponceau S in PBS-T, the membrane was blocked and incubated with pan-pCK2 antibodies, developed:



Exposure

Merged with ladder

X-line → one more negative control (was not included in the final figure)