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|---|---|
| Title | Characterization of intracellular dynamics of inoculated PrP-res and newly generated PrPSc during early stage prion infection in Neuro2a cells |
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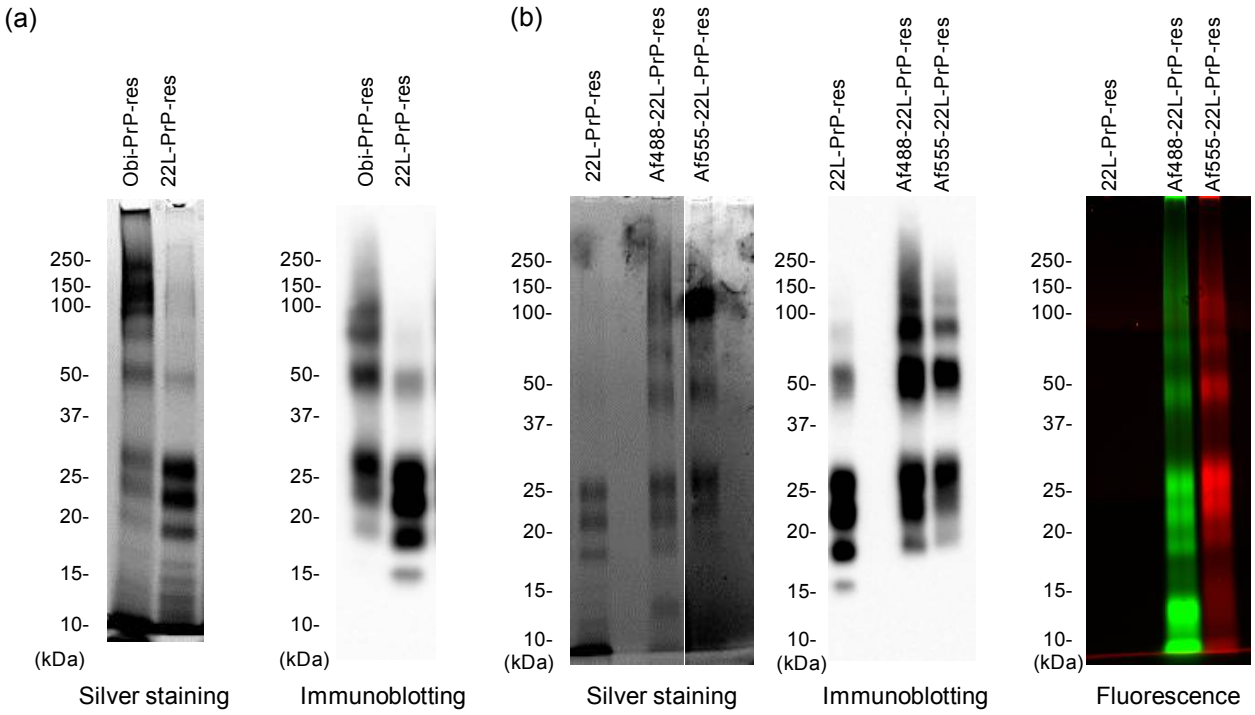
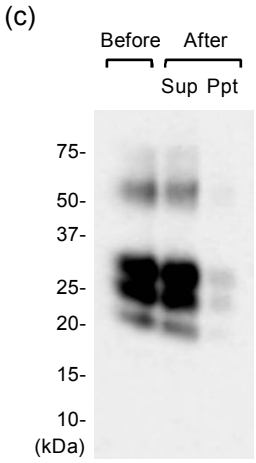
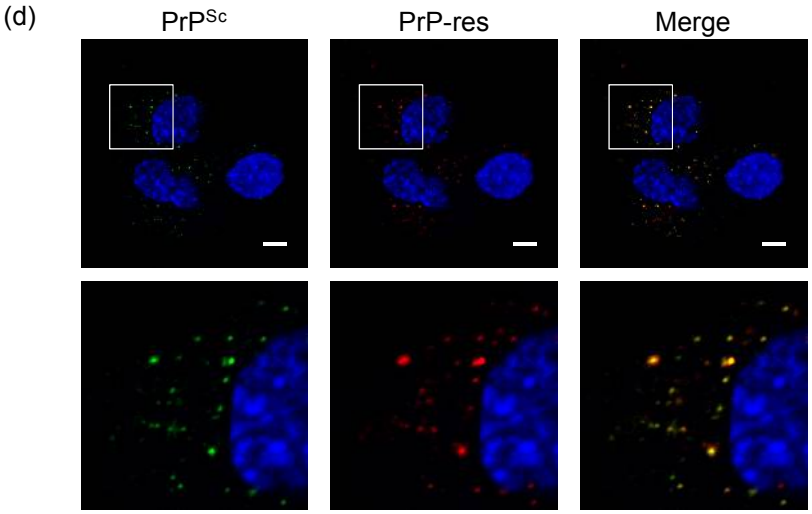
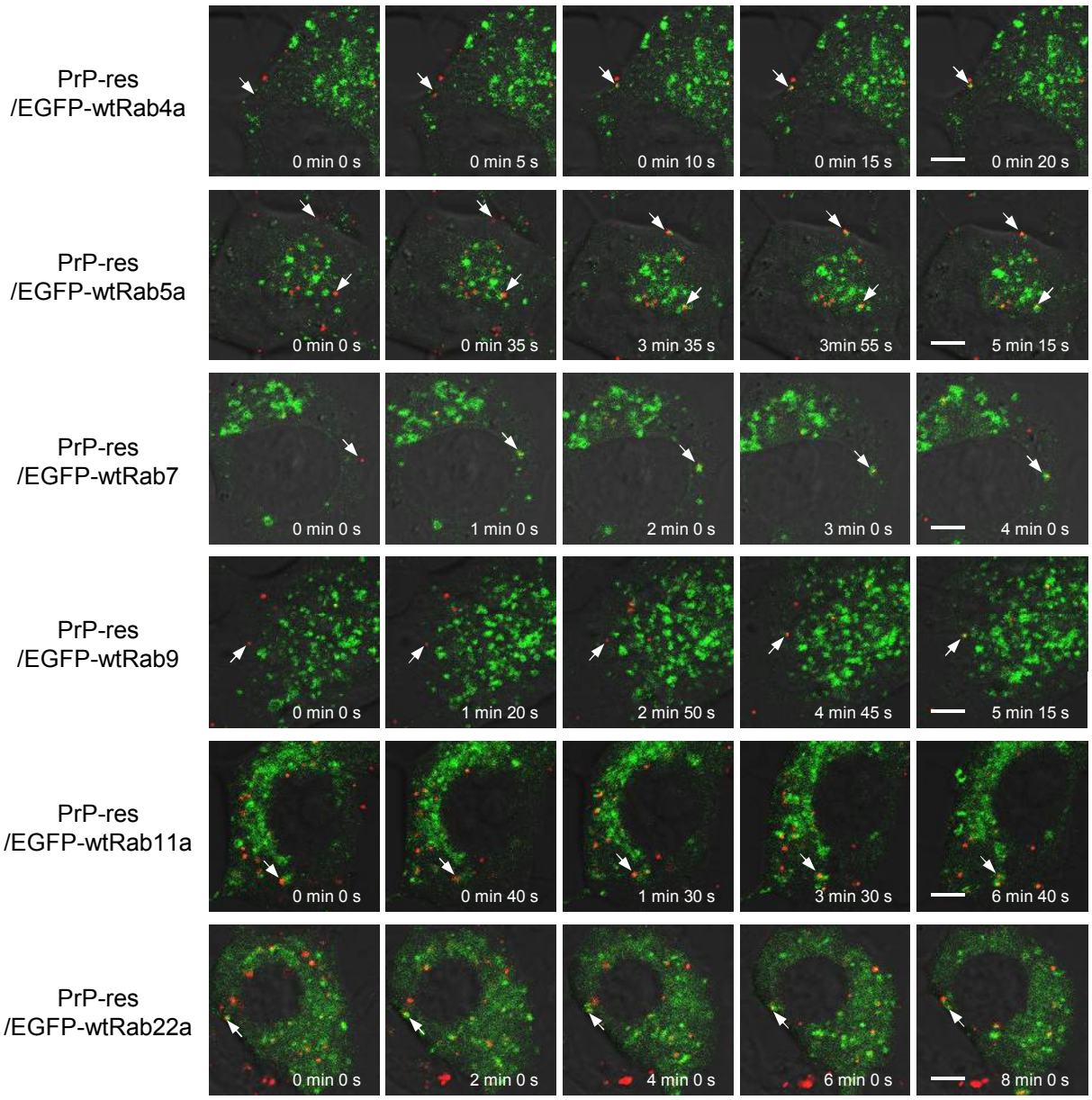
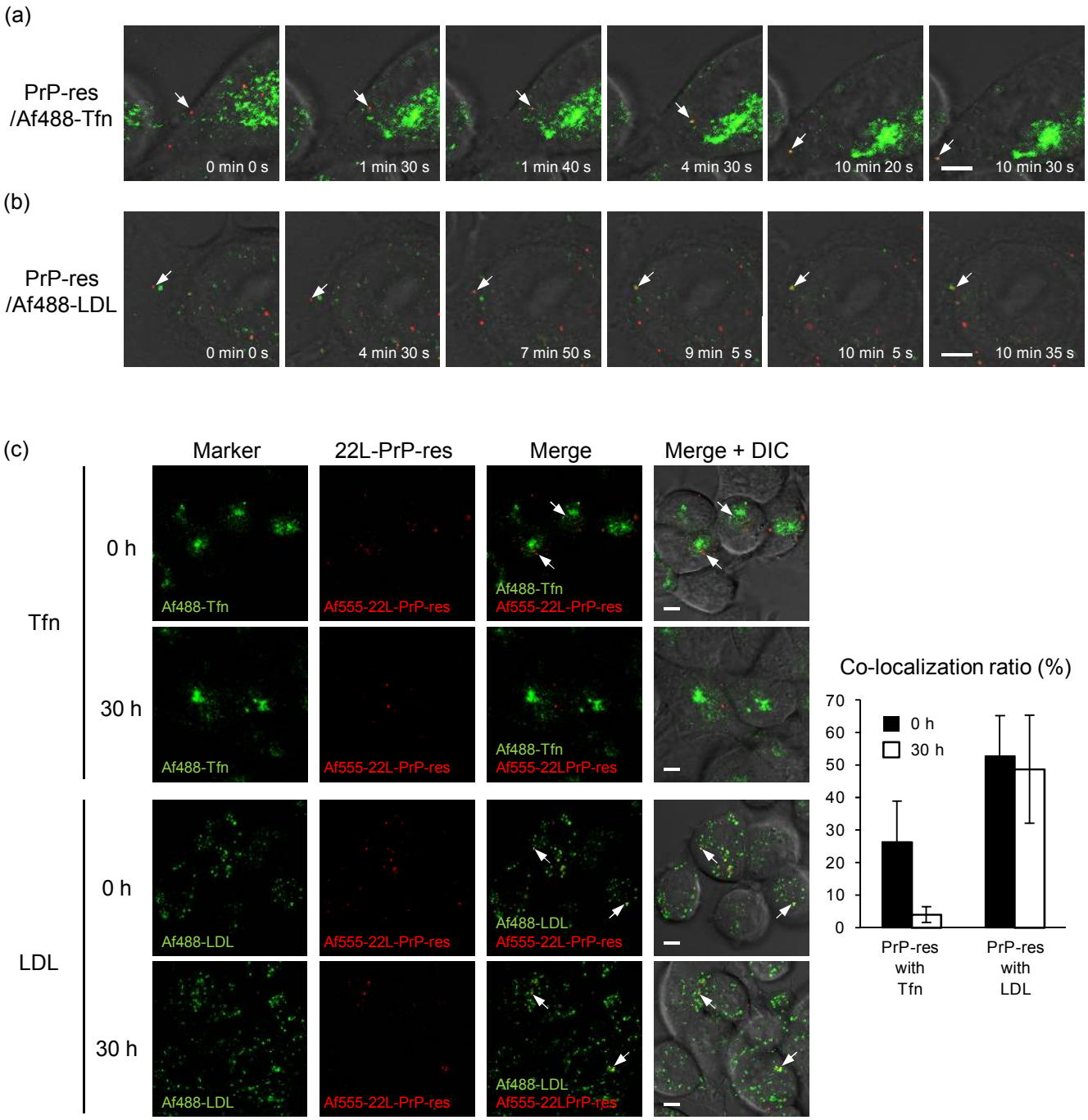
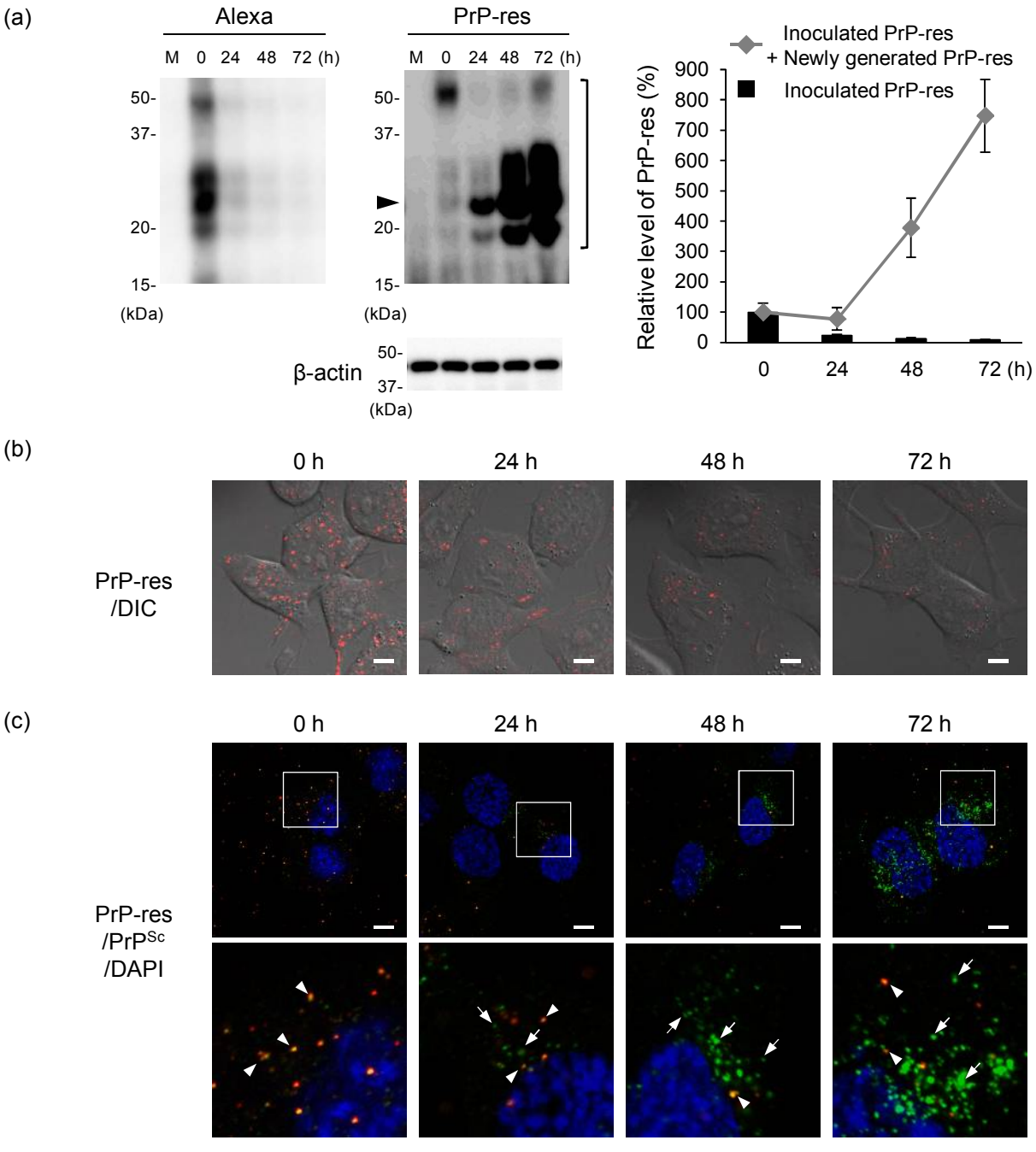

(a)
Obi-PrP-res
22L-PrP-res
250-
150-
100-
50-
37-
25-
20-
15-
10-
(kDa)
Silver staining
Immunoblotting
(b)
22L-PrP-res
Af488-22L-PrP-res
Af555-22L-PrP-res
Silver staining
Immunoblotting
Fluorescence

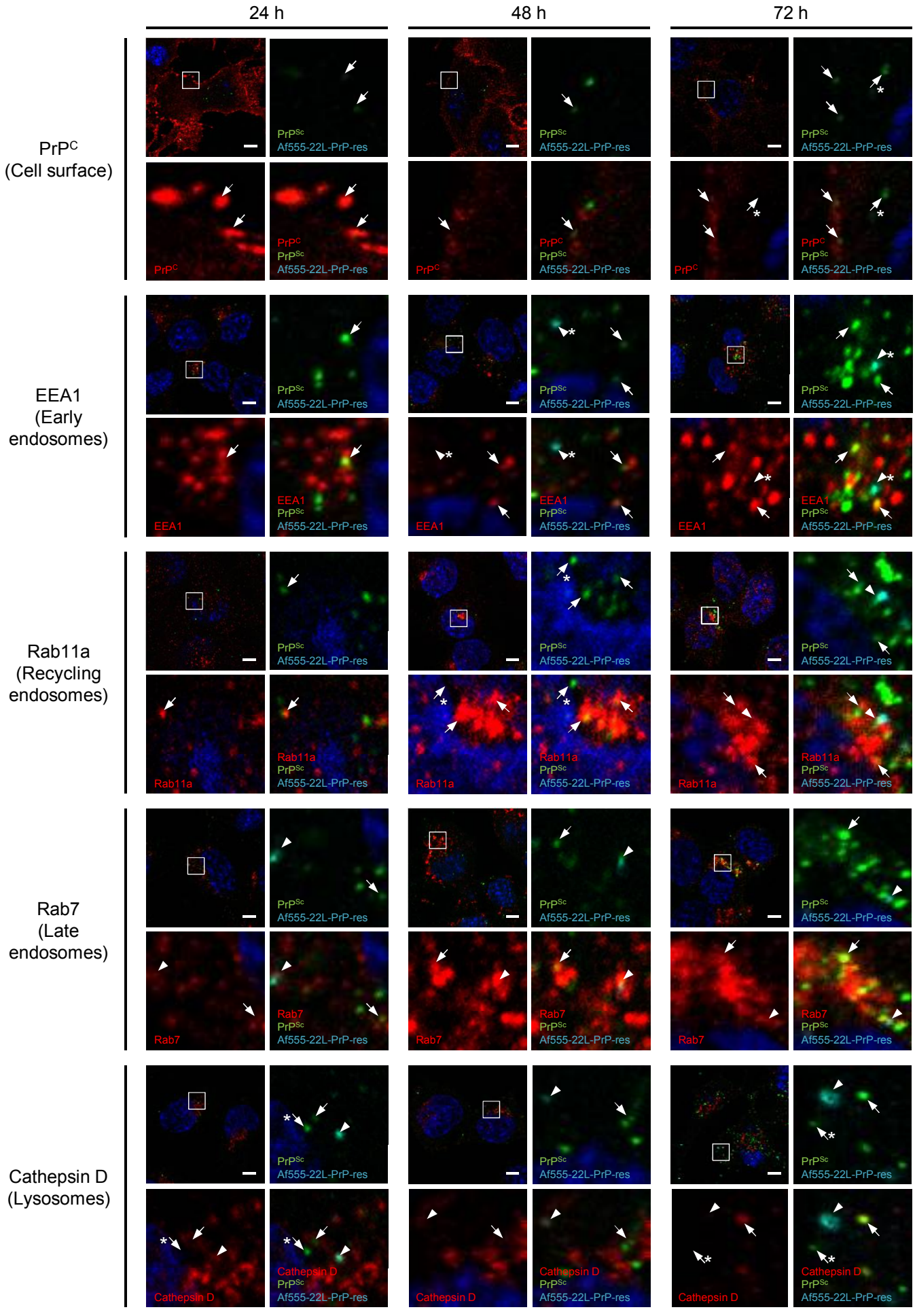

(c)
Before
After
Sup
Ppt
75-
50-
37-
25-
20-
15-
10-
(kDa)


(d)
PrPSc
PrP-res
Merge

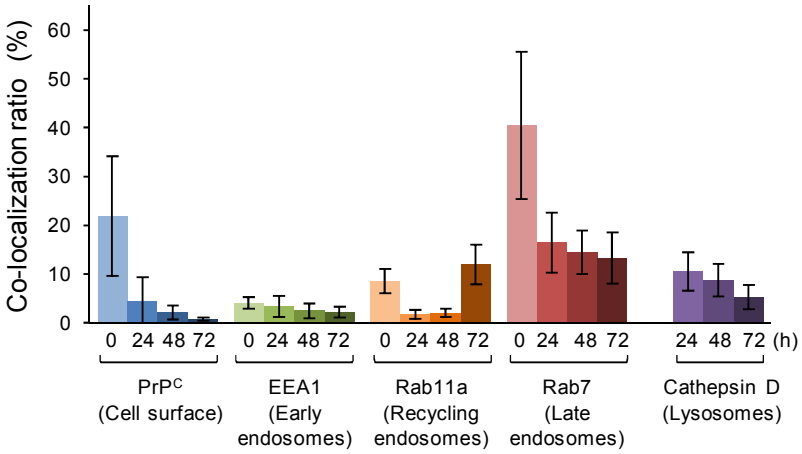

PrP-res /EGFP-wtRab4a
0 min 0 s
0 min 5 s
0 min 10 s
0 min 15 s
0 min 20 s
PrP-res /EGFP-wtRab5a
0 min 0 s
0 min 35 s
3 min 35 s
3min 55 s
5 min 15 s
PrP-res /EGFP-wtRab7
0 min 0 s
1 min 0 s
2 min 0 s
3 min 0 s
4 min 0 s
PrP-res /EGFP-wtRab9
0 min 0 s
1 min 20 s
2 min 50 s
4 min 45 s
5 min 15 s
PrP-res /EGFP-wtRab11a
0 min 0 s
0 min 40 s
1 min 30 s
3 min 30 s
6 min 40 s
PrP-res /EGFP-wtRab22a
0 min 0 s
2 min 0 s
4 min 0 s
6 min 0 s
8 min 0 s


(a)
PrP-res
/Af488-Tfn
0 min 0 s
1 min 30 s
1 min 40 s
4 min 30 s
10 min 20 s
10 min 30 s
(b)
PrP-res
/Af488-LDL
0 min 0 s
4 min 30 s
7 min 50 s
9 min 5 s
10 min 5 s
10 min 35 s
(c)
Marker
22L-PrP-res
Merge
Merge + DIC
Tfn
0 h
30 h
LDL
0 h
30 h
Af488-Tfn
Af555-22L-PrP-res
Af488-Tfn
Af555-22L-PrP-res
Af488-Tfn
Af555-22LPrP-res
Af488-LDL
Af555-22L-PrP-res
Af488-LDL
Af555-22L-PrP-res
Af488-LDL
Af555-22LPrP-res
Co-localization ratio (%)
70
60
50
40
30
20
10
0
0 h
30 h
PrP-res
with
Tfn
PrP-res
with
LDL

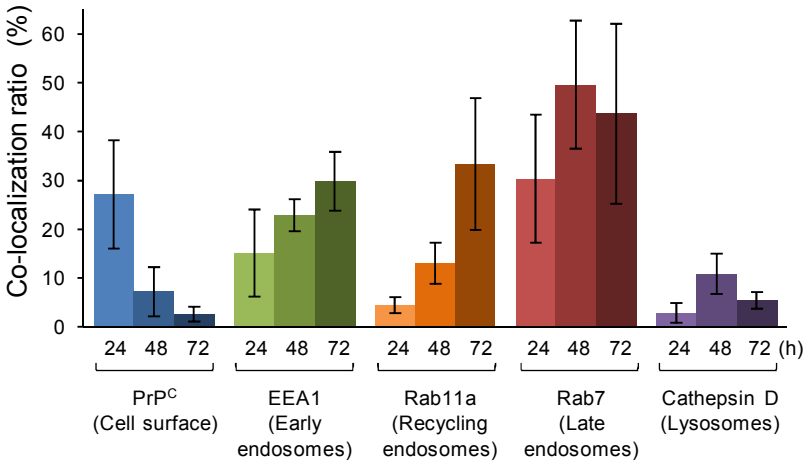

(a)
Alexa
PrP-res
M 0 24 48 72 (h)
50-
37-
20-
15-
(kDa)
β-actin
Inoculated PrP-res
+ Newly generated PrP-res
Inoculated PrP-res
Relative level of PrP-res (%)
900
800
700
600
500
400
300
200
100
0
0 24 48 72 (h)
(b)
0 h
24 h
48 h
72 h
PrP-res
/DIC
(c)
PrP-res
/PrP^Sc
/DAPI

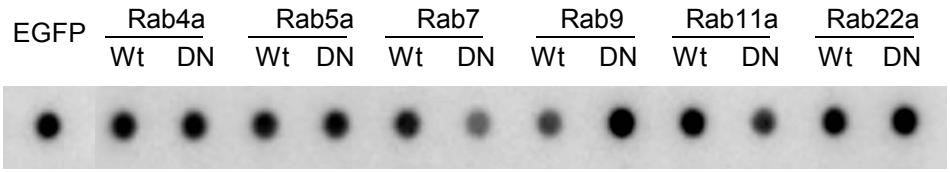

24 h
48 h
72 h
PrP^C
(Cell surface)
EEA1
(Early
endosomes)
Rab11a
(Recycling
endosomes)
Rab7
(Late
endosomes)
Cathepsin D
(Lysosomes)
PrP^Sc
Af555-22L-PrP-res
PrP^C
EEA1
Rab11a
Rab7
Cathepsin D

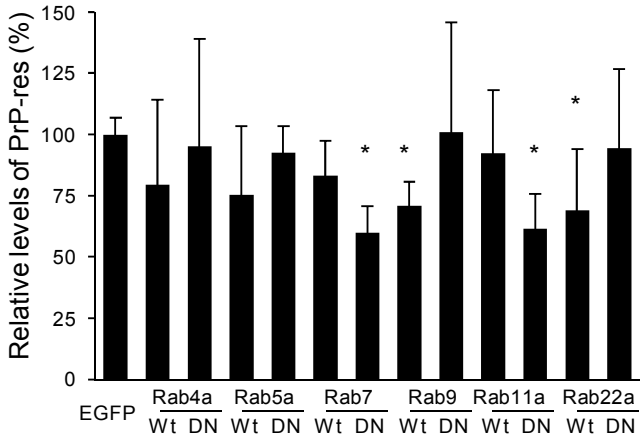
(a) Co-localization of inoculated PrP-res with markers




(b) Co-localization of newly generated PrP^Sc with markers




EGFP
Rab4a
Rab5a
Rab7
Rab9
Rab11a
Rab22a
Wt
DN


Relative levels of PrP-res (%)
150
125
100
75
50
25
0
*
EGFP
Rab4a
Rab5a
Rab7
Rab9
Rab11a
Rab22a
Wt
DN