# Supplementary information

## iFRAP analysis

All iFRAP results were corrected and normalized using the equation described in (Dundr et al., 2002), with slight modifications:

$$\mathbf{I_{rel} = (I_t / I_o)*(T_{mean} / T_t)}$$

where I(t) is the average intensity of the unbleached region of interest at time point t, $I_o$ is the average pre-bleach intensity of the region of interest and $T_{mean}$ and $T_t$ are the total mean cell intensity of the whole post-bleach period or average total cell intensity at each post-bleach point in time, respectively.

For fitting the iFRAP curves, we used the Graphpad Prism v.3.0 programme (Graphpad software, San Diego, CA). iFRAP curves were modeled equally well with a two-phase exponential decay equation (also see Supp. Fig. 1).

$$\mathbf{Y\ (fluorescence\ decay) = Span1 \cdot exp(-K1 \cdot X) + Span2 \cdot exp(-K2 \cdot X) + Plateau}$$

**Supplemental Figure 1** Goodness-of-fit for iFRAP curves. Examples of average residuals between fit and the experimental data are shown over time for single and bi-exponential decay fits. Bi-exponential fits of Lamp1 alone (***A***) or co-transfected with Rab8 and Rab8 Q67L (***B***), MPR or co-transfected with siCN or siHTT (***C***), MPR co-transfected with FL-17Q-htt (***D***), LcB (***E***) and VSV-G (***F***) in either wt or mhtt cells correct the systematic deviations observed with single exponential fits.

**Supplemental Figure 2.** Full length mhtt reduces localization of optineurin in the Golgi apparatus of M213 cells. (A) Double immunocytochemistry for optineurin and GM130 showed localization of optineurin in the Golgi complex of M213 cells. (B) Optineurin colocalized with htt at the Golgi complex (arrowheads). Pink arrowheads indicate vesicles containing both proteins (enlarged region). Colocalization of optineurin with GT-YFP showed reduced localization of optineurin in the Golgi apparatus in M213 cells transfected with FL-75Q-htt and GT-YFP compared to those transfected with FL-17Q-htt and GT-YFP or GT-YFP alone (CN). Images represent the projection of the two slices containing the maximal cross-section of the cell nucleus. Bar, 8 μm.

**Supplemental Figure 3.** Expression of Rab8 Q67L delocalizes γ-adaptin from the Golgi apparatus in wt cells. Wt cells expressing Rab8 Q67L showed reduced colocalization between γ-adaptin and GT-CFP. Images represent the projection of the two slices containing the maximal cross-section of the cell nucleus. Bar, 8 μm.

**Supplemental Figure 4.** iFRAP analysis showed impaired post-Golgi transport of VSV-G (A) and LcB (B) in mhtt cells compared to wt cells. Representative Golgi apparatus fluorescence for each condition is shown on the right. Results are represented as a mean ± s.e.m. determined from the analysis of three independent experiments.

**Supplemental Figure 5.** Exon1-103Q-htt stimulates post-Golgi transport of Lamp-1 and also increases the labeling of cathepsin D in lysotracker positive structures. iFRAP analysis showed increased post-Golgi transport of Lamp-1 in wt cells expressing exon1-103Q-htt (A). Colocalization studies showed increased labelling of cathepsin D in lysotracker positive structures in wt cells expressing exon1-103Q-htt respect to those

expressing exon1-25Q-htt. Pink arrowheads show colocalization between cathepsin D and Lysotracker positive estructures(enlarged region). (*$p<0.05$) Images represent the projection of the two slices containing the maximal cross-section of the cell nucleus. Bar, 8 μm.

**Movie 1.** Movie of a representative iFRAP experiment of wt and mhtt cells expressing VSV-G. Note the lower Golgi fluorescence decay of VSV-G-expressing mhtt cells compared to wt cells.Time: 20 min; pre-bleach: 4 pictures taken every 5 s; post-bleach: 241 pictures taken every 5 s.

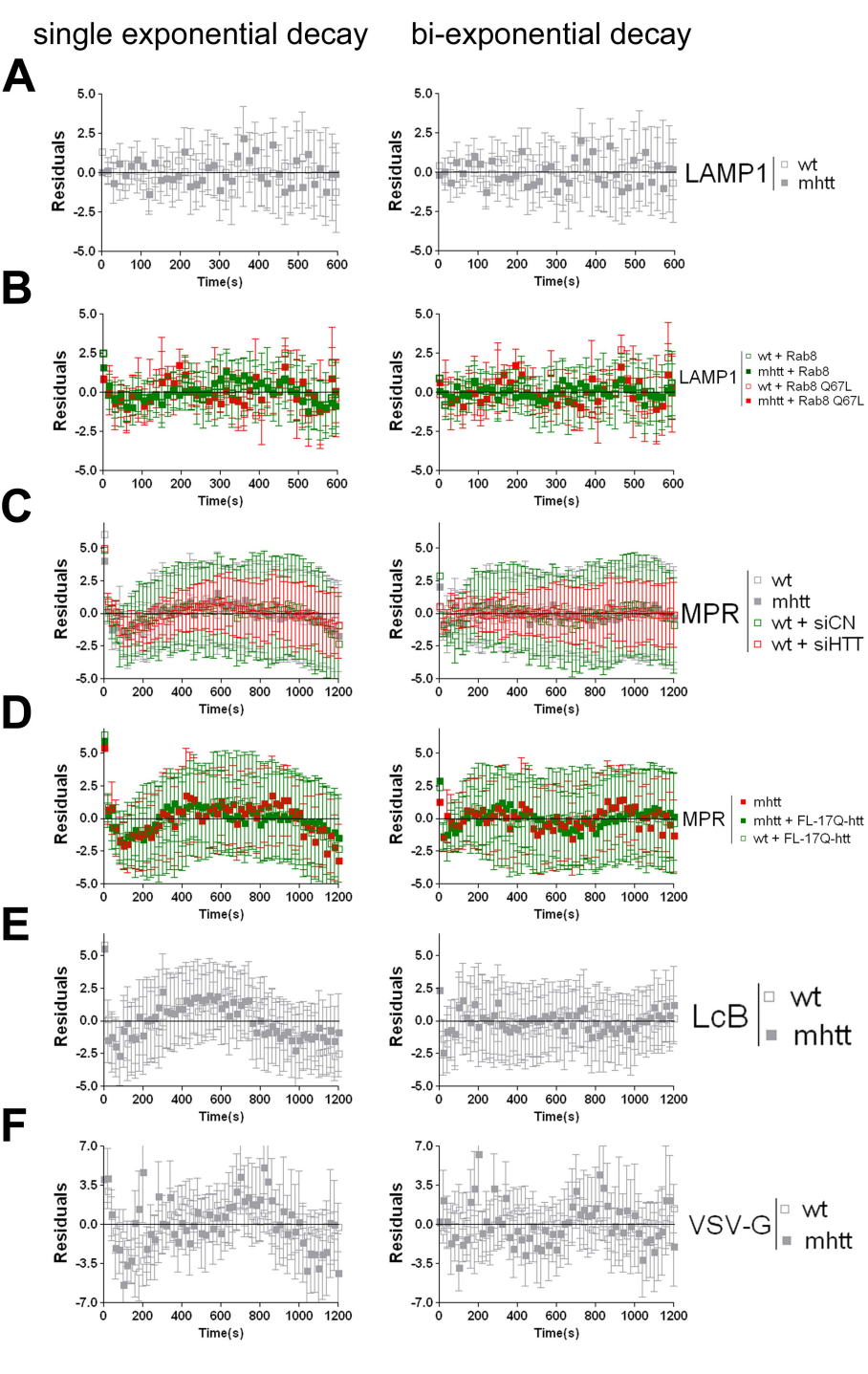
**Movie 2.** Movie of a representative iFRAP experiment of wt and mhtt cells expressing Lamp1. Note the lower Golgi fluorescence decay of Lamp1-expressing mhtt cells compared to wt cells. In contrast, wt and mhtt cells co-expressing Lamp1 and Rab8Q67L showed similar Golgi fluorescence decay of Lamp1. Time: 10 min; pre-bleach: 4 pictures taken every 5 s; post-bleach: 120 pictures taken every 5 s.

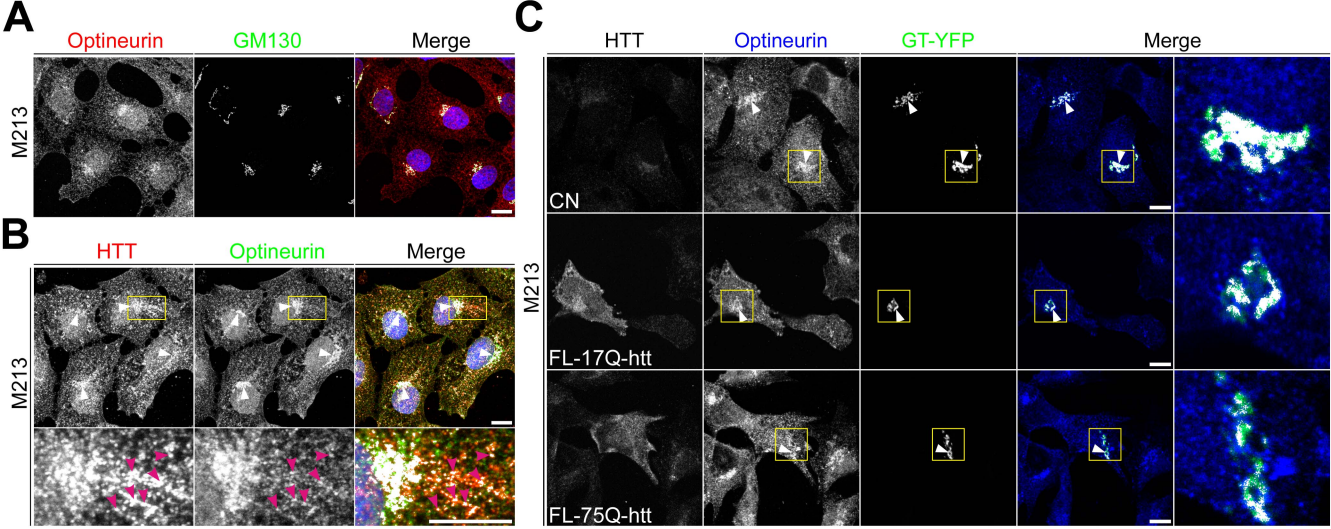
**Movie 3.** Movie of a representative iFRAP experiment of wt and mhtt cells expressing LcB. Note the lower Golgi fluorescence decay of LcB-expressing mhtt cells respect to wt cells.Time: 20 min; pre-bleach: 4 pictures taken every 5 s; post-bleach: 241 pictures taken every 5 s.

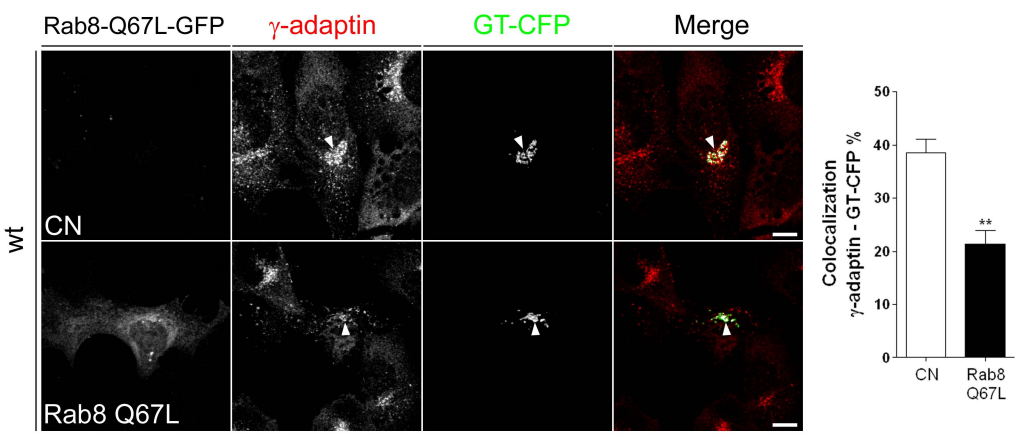
**Movie 4.** Movie of a representative iFRAP experiment of wt and mhtt cells expressing MPR. Note the lower Golgi fluorescence decay of MPR-expressing mhtt cells respect to wt cells.Time: 20 min; pre-bleach: 4 pictures taken every 5 s; post-bleach: 241 pictures taken every 5 s.

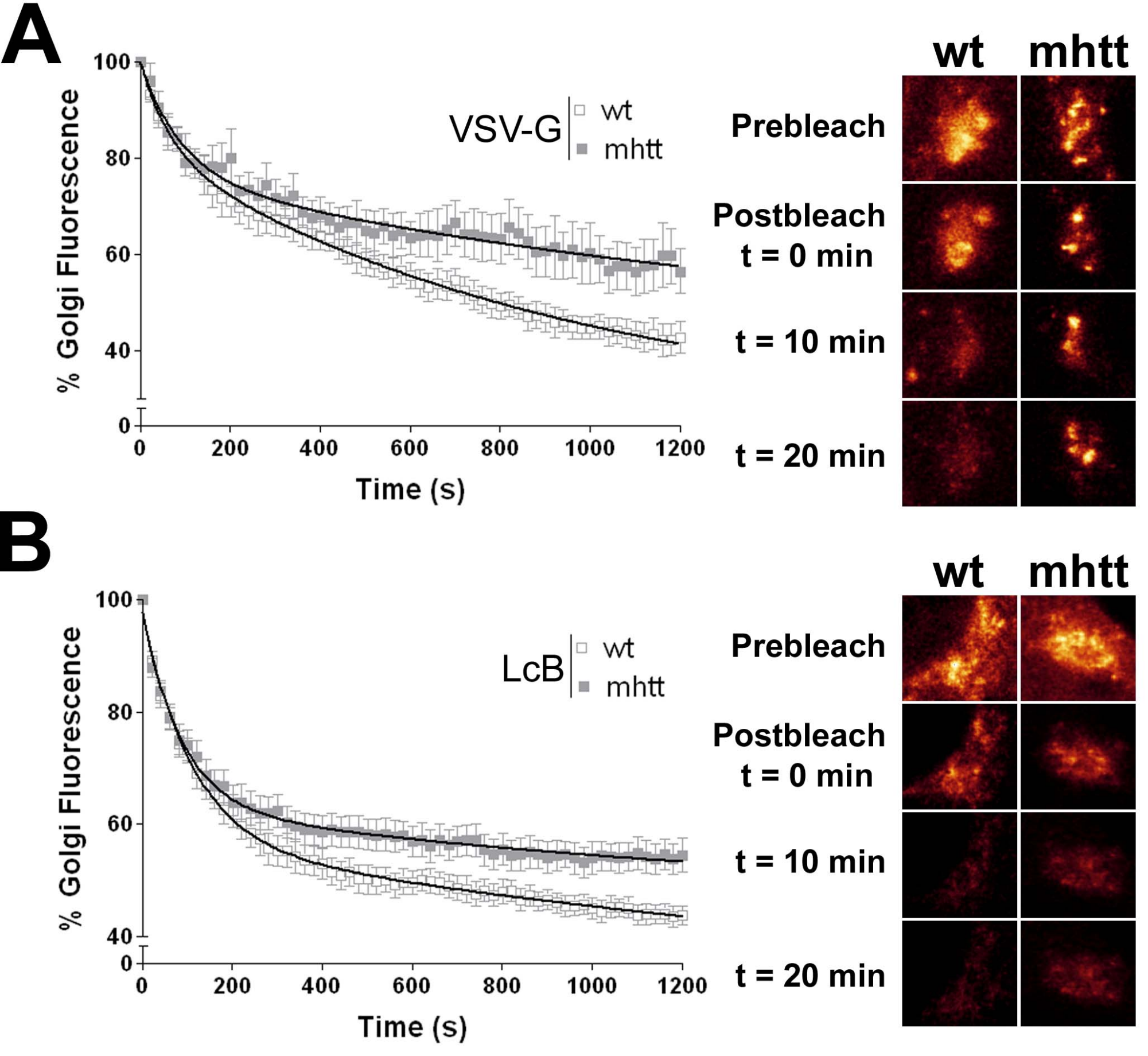
**Movie 5.** Movie of a representative iFRAP experiment of wt and mhtt cells expressing MPR and FL-17Q-htt. Note the lower Golgi fluorescence decay of MPR-expressing mhtt cells respect to those co-expressing FL-17Q-htt. Time: 20 min; pre-bleach: 4 pictures taken every 5 s; post-bleach: 241 pictures taken every 5 s.

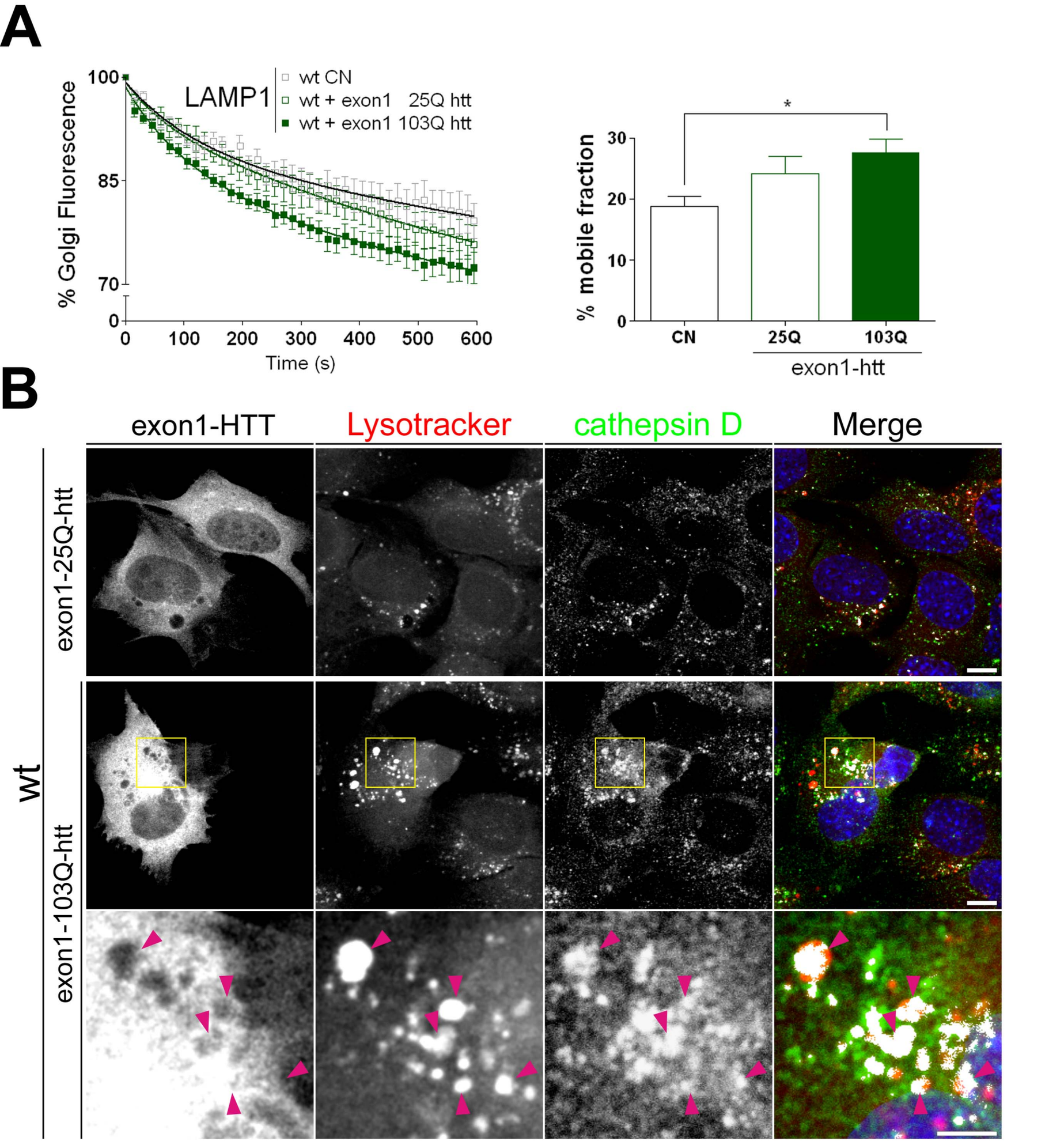
**Movie 6.** Movie of a representative iFRAP experiment of wt cells expressing MPR and siCN or siHTT. Note the lower Golgi fluorescence decay of wt cells expressing MPR and siHTT respect to those expressing siCN RNA. Time: 20 min; pre-bleach: 4 pictures taken every 5 s; post-bleach: 241 pictures taken every 5 s.


single exponential decay
bi-exponential decay
A
B
C
D
E
F
Residuals
Time(s)
LAMP1
wt
mhtt
wt + Rab8
mhtt + Rab8
wt + Rab8 Q67L
mhtt + Rab8 Q67L
MPR
wt + siCN
wt + siHTT
mhtt + FL-17Q-htt
wt + FL-17Q-htt
LcB
VSV-G


A
Optineurin
GM130
Merge
M213
B
HTT
Optineurin
Merge
M213
C
HTT
Optineurin
GT-YFP
Merge
M213
CN
FL-17Q-htt
FL-75Q-htt


Rab8-Q67L-GFP
γ-adaptin
GT-CFP
Merge
wt
CN
Rab8 Q67L
Colocalization
γ-adaptin - GT-CFP %
50
40
30
20
10
0
**
CN
Rab8
Q67L


A
100
80
60
40
0
% Golgi Fluorescence
VSV-G
wt
mhtt
0
200
400
600
800
1000
1200
Time (s)
wt
mhtt
Prebleach
Postbleach
t = 0 min
t = 10 min
t = 20 min
B
100
80
60
40
0
% Golgi Fluorescence
LcB
wt
mhtt
0
200
400
600
800
1000
1200
Time (s)
wt
mhtt
Prebleach
Postbleach
t = 0 min
t = 10 min
t = 20 min


A
LAMP1
wt CN
wt + exon1 25Q htt
wt + exon1 103Q htt
% Golgi Fluorescence
100
85
70
0
0
100
200
300
400
500
600
Time (s)
*
% mobile fraction
30
20
10
0
CN
25Q
103Q
exon1-htt
B
exon1-HTT
Lysotracker
cathepsin D
Merge
wt
exon1-25Q-htt
exon1-103Q-htt