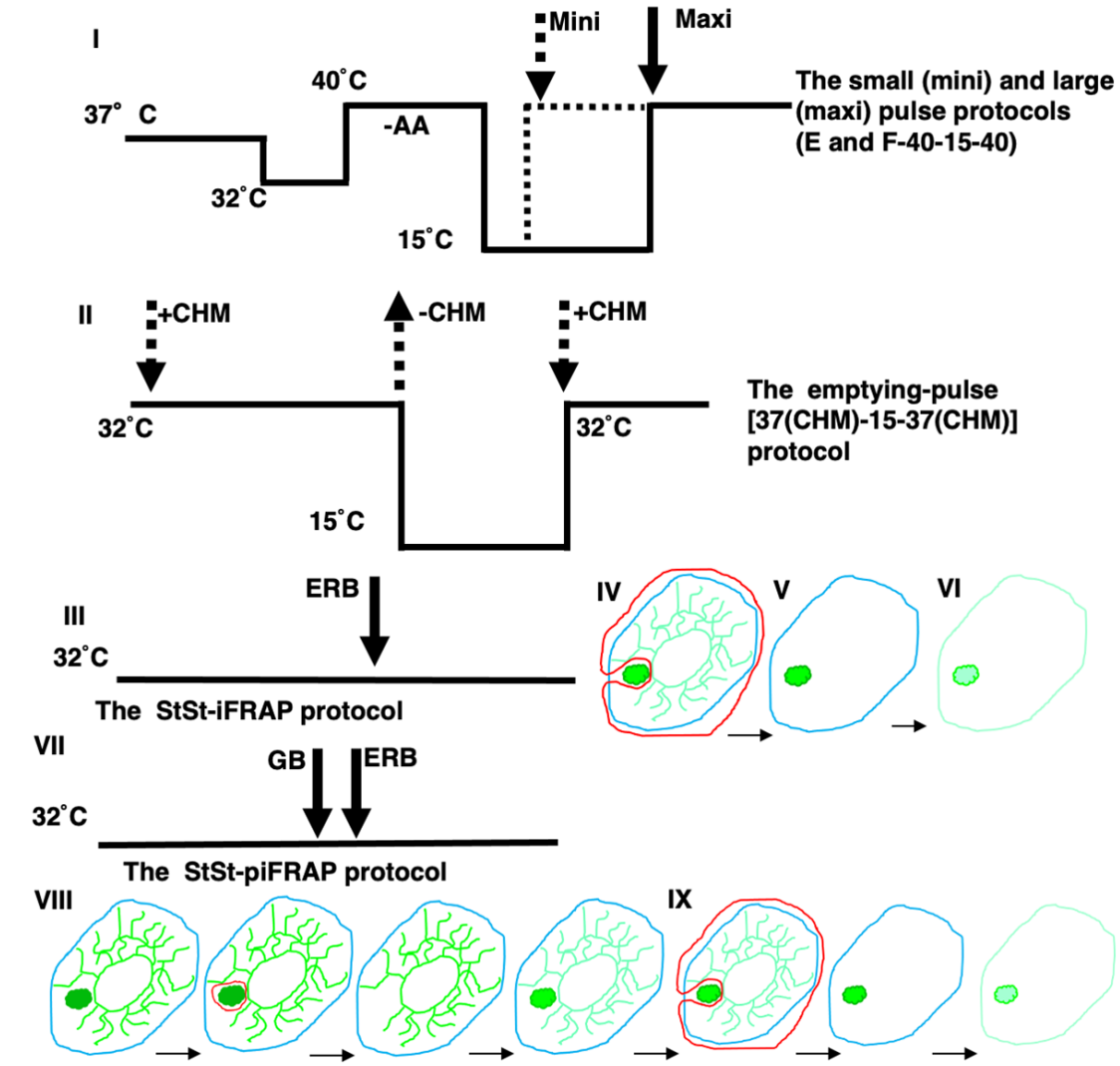

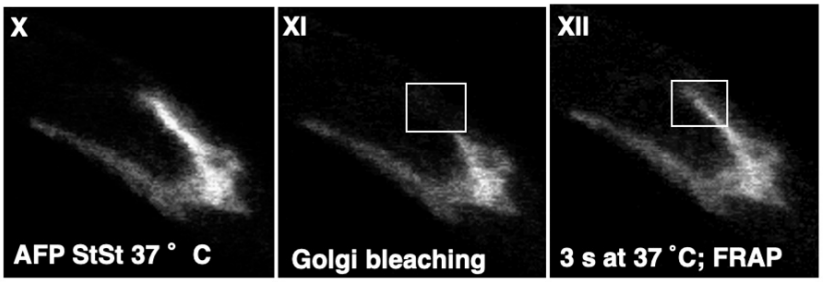

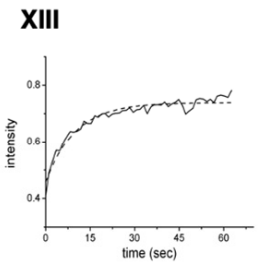

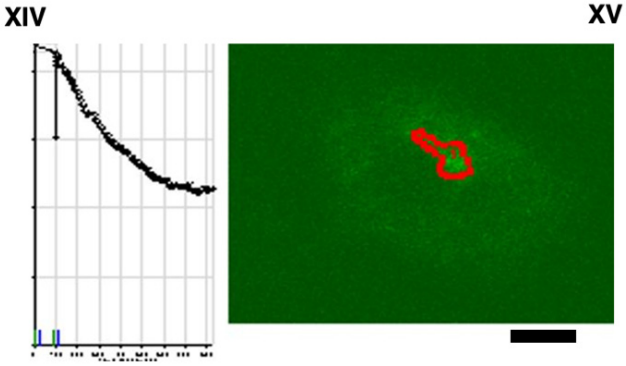

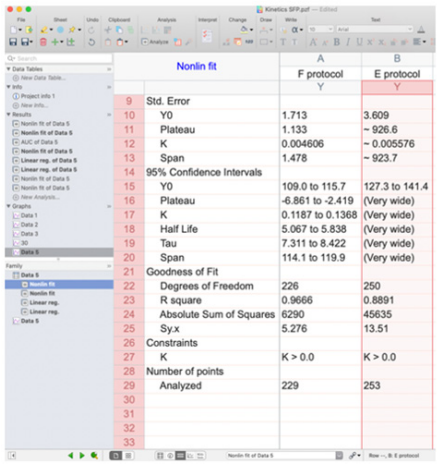

Nonlin fit

		A	B
		F protocol	E protocol
		Y	Y
9	Std. Error		
10	Y0	1.713	3.609
11	Plateau	1.133	~ 926.6
12	K	0.004606	~ 0.005576
13	Span	1.478	~ 923.7
14	95% Confidence Intervals		
15	Y0	109.0 to 115.7	127.3 to 141.4
16	Plateau	-6.861 to -2.419	(Very wide)
17	K	0.1187 to 0.1368	(Very wide)
18	Half Life	5.067 to 5.838	(Very wide)
19	Tau	7.311 to 8.422	(Very wide)
20	Span	114.1 to 119.9	(Very wide)
21	Goodness of Fit		
22	Degrees of Freedom	226	250
23	R square	0.9666	0.8891
24	Absolute Sum of Squares	6290	45635
25	Sy.x	5.276	13.51
26	Constraints		
27	K	K > 0.0	K > 0.0
28	Number of points		
29	Analyzed	229	253
30			
31			
32			
33			

Figure S1. Schemes demonstrating cargo synchronization protocols (I–IX). E-empty GC; F-filled GC; CHM, Cycloheximide; ERB, ER bleaching; GB, Golgi bleaching; FRAP, fluorescence recovery after photobleaching; arrows indicate time of the influence application (see Table 1). (I) The small (mini) and large (maxi) pulse protocols (E- and F-40-15-40). (II) The emptying-pulse [37(CHM)-15-37(CHM)] protocol. Arrows indicate the time when CHM was added and then eliminated. Horizontal line indicate temperature of the incubation medium. (III–VI) Scheme of iFRAP protocol. It includes bleaching of the whole cell less its GC (inside red curve) and measuring (arrows) of the fluorescence inside GZ (decrease of green colour intensity in green structure in [V] and [VI] and appearance of green colour in external line indicating PM). (VII–IX) Scheme of the piFRAP protocol. It includes bleaching of the Golgi area (small area inside red curve), refilling of this area bleaching of the whole cell less its GC (green structure with green borders) bleaching of the whole cell less its GC (inside red curve) and measuring (arrows) of the fluorescence inside GZ (decrease of green colour intensity in green structure in [IX] and appearance of green colour in external line indicating PM). (A–F) Analysis of the kinetics of the Golgi zone fluorescence using iFRAP at steady state (A–F) and piFRAP at steady state (G, H) synchrniozation protocols. (A) Initial position of cell. (E) Example of the image visdible on the microscopic screen. Decreasing of the GC fluorescence inside GC. (X–XII) The kymogram shows FRAP inside the white box in the cell transfected with AFP. In cells transfected with AFP FRAP is fast. (XIII) Data from plates X–XII are plotted. (XIV) Screen shot from the screen of the laser scanning confocal microscope shows how data for regression lines were acquired. Bleaching of the whole cell less its GC (outside red curve). (XV) Screen shot from the computer screen showing the estimation of the goodness-of-fit parameters. Scale bars: 1 μm (XI–XII); 5 μm (XIV).

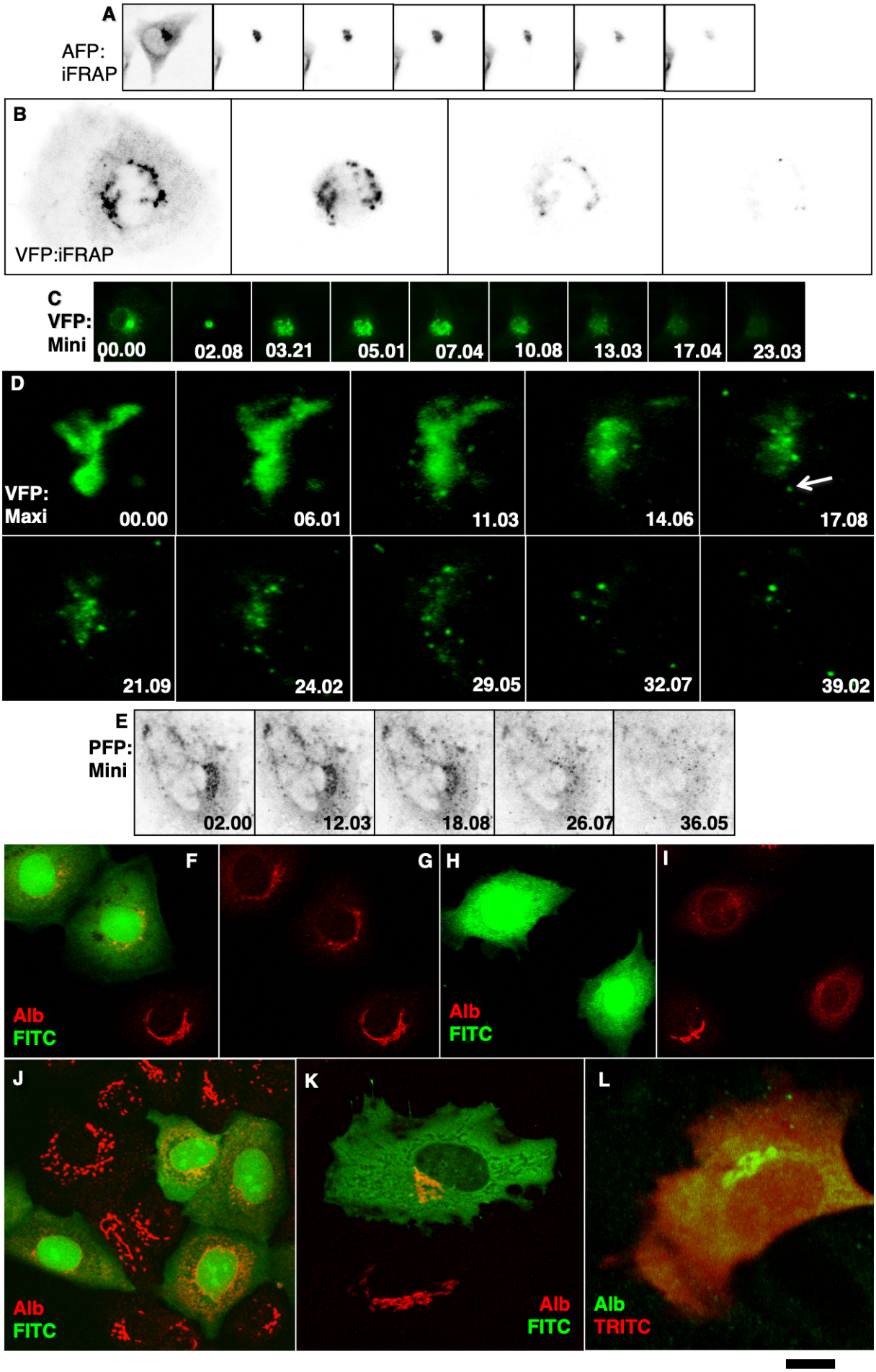
A
AFP:
iFRAP
B
VFP:iFRAP
C
VFP:
Mini
00.00
02.08
03.21
05.01
07.04
10.08
13.03
17.04
23.03
D
VFP:
Maxi
00.00
06.01
11.03
14.06
17.08
21.09
24.02
29.05
32.07
39.02
E
PFP:
Mini
02.00
12.03
18.08
26.07
36.05
F
G
H
I
Alb
FITC
Alb
FITC
J
K
L
Alb
FITC
Alb
FITC
Alb
TRITC

Figure S2. Representative additional examples of the kymograms describing the kinetic of the different cargo exit from the GZ using different synchronization protocols. (A) AFP:iFRAP. (B) VFP:iFRAP. (C) VFP:Mini-wave. (D) VFP:Maxi-wave. E); PFP:Mini-wave. The bleaching was not used (see Table 1). The names of cargo and the synchronization protocols are shown in panels. The movies were used for the measurement of the integral fluorescence within the GZ during IGT. (F, G and H, I) Two representative examples of experiments demonstrating Role of membrane fusion. Microinjection of α-SNAP mutant into HepG2 cells blocked entrance of albumin into the GC from the ER (B, D). (J) Microinjection (green cells: FITC labelling in [G–K]; red cells; TRITC labelling in [L]) of alpha-SNAP mutant into HepG2 cells inhibited exit of albumin (red in G–K) from the ER. (K, L) Microinjection the irrelevant protein (anti-albumin antibody) did not block exit of albumin (red in [K], green in [L]; TRITC red in [L]) from the ER. Scale bars: 7 μm (A,C); 2 μm (B, D); 5 μm (E–J); 2 μm (K, L).