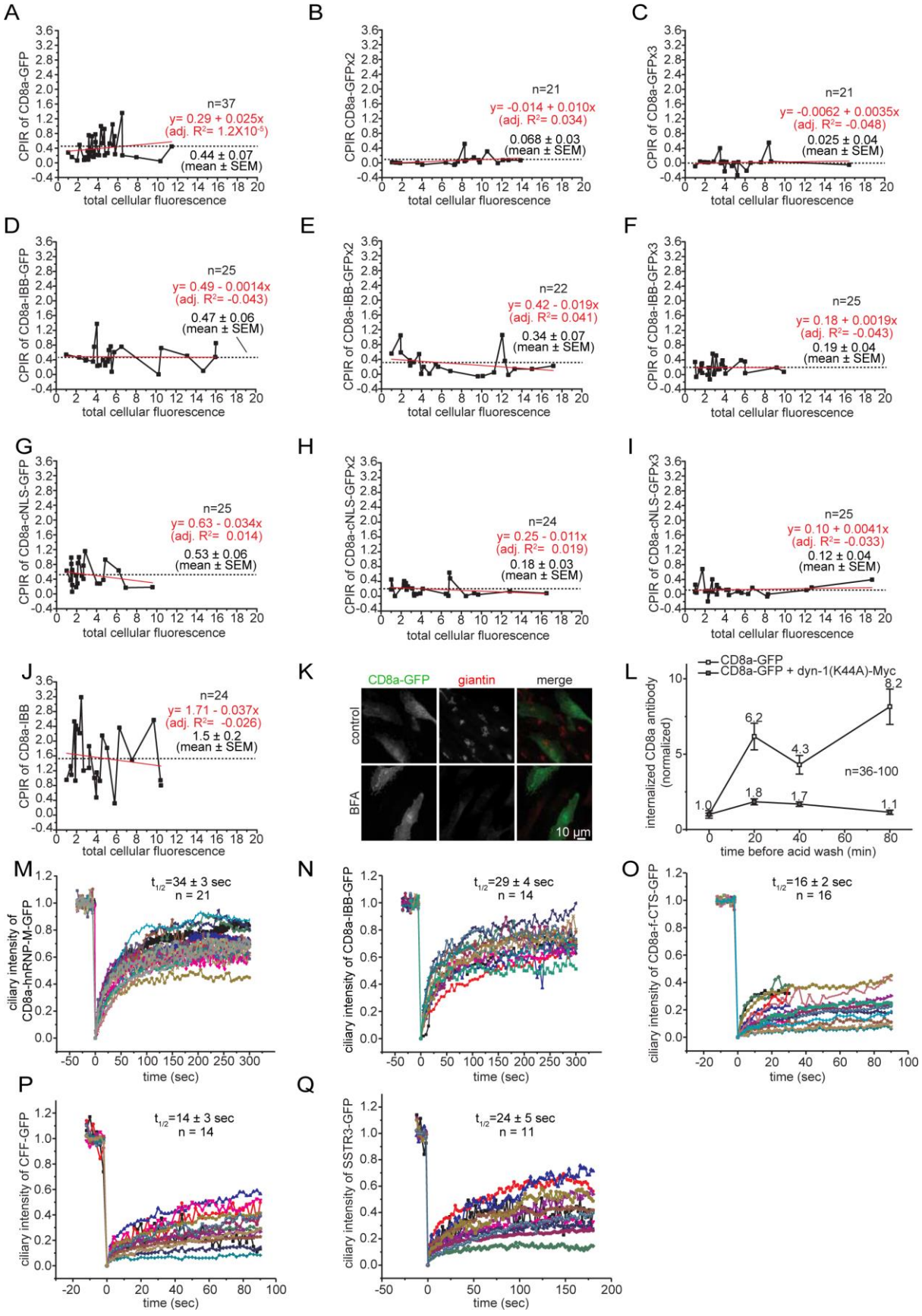
A
CPIR of CD8a-GFP
n=37
y= 0.29 + 0.025x
(adj. R²= 1.2X10⁻⁵)
0.44 ± 0.07
(mean ± SEM)
total cellular fluorescence
B
CPIR CD8a-GFPx2
n=21
y= -0.014 + 0.010x
(adj. R²= 0.034)
0.068 ± 0.03
(mean ± SEM)
total cellular fluorescence
C
CPIR of CD8a-GFPx3
n=21
y= -0.0062 + 0.0035x
(adj. R²= -0.048)
0.025 ± 0.04
(mean ± SEM)
total cellular fluorescence
D
CPIR of CD8a-IBB-GFP
n=25
y= 0.49 - 0.0014x
(adj. R²= -0.043)
0.47 ± 0.06
(mean ± SEM)
total cellular fluorescence
E
CPIR of CD8a-IBB-GFPx2
n=22
y= 0.42 - 0.019x
(adj. R²= 0.041)
0.34 ± 0.07
(mean ± SEM)
total cellular fluorescence
F
CPIR of CD8a-IBB-GFPx3
n=25
y= 0.18 + 0.0019x
(adj. R²= -0.043)
0.19 ± 0.04
(mean ± SEM)
total cellular fluorescence
G
CPIR of CD8a-cNLS-GFP
n=25
y= 0.63 - 0.034x
(adj. R²= 0.014)
0.53 ± 0.06
(mean ± SEM)
total cellular fluorescence
H
CPIR of CD8a-cNLS-GFPx2
n=24
y= 0.25 - 0.011x
(adj. R²= 0.019)
0.18 ± 0.03
(mean ± SEM)
total cellular fluorescence
I
CPIR of CD8a-cNLS-GFPx3
n=25
y= 0.10 + 0.0041x
(adj. R²= -0.033)
0.12 ± 0.04
(mean ± SEM)
total cellular fluorescence
J
CPIR of CD8a-IBB
n=24
y= 1.71 - 0.037x
(adj. R²= -0.026)
1.5 ± 0.2
(mean ± SEM)
total cellular fluorescence
K
CD8a-GFP
giantin
merge
control
BFA
10 µm
L
CD8a-GFP
CD8a-GFP + dyn-1(K44A)-Myc
internalized CD8a antibody (normalized)
n=36-100
time before acid wash (min)
M
t1/2=34 ± 3 sec
n = 21
ciliary intensity of CD8a-hnRNP-M-GFP
time (sec)
N
t1/2=29 ± 4 sec
n = 14
ciliary intensity of CD8a-IBB-GFP
time (sec)
O
t1/2=16 ± 2 sec
n = 16
ciliary intensity of CD8a-f-CTS-GFP
time (sec)
P
t1/2=14 ± 3 sec
n = 14
ciliary intensity of CFF-GFP
time (sec)
Q
t1/2=24 ± 5 sec
n = 11
ciliary intensity of SSTR3-GFP
time (sec)

Fig. S1.CPIRs of CD8a chimeras vs their expression levels, effect of BFA treatment on the Golgi, effect of Dynamin-1(K44A) expression on endocytosis and whole cilium FRAP analysis of CD8a-GFP chimeras and SSTR3-GFP. (A-J) Ciliated RPE1 cells expressing different CD8a chimeras were analyzed for CPIRs and the total cellular intensity of CD8a chimeras. CPIRs are plotted against the total cellular intensity of CD8a chimera (arbitrary unit). Plots are organized similarly to Figure 1C. In each plot, the trend of data is represented by a linear regression fitting line, which, together with its formula and adjusted R^2 (adj. R^2), is shown in red. (K) RPE1 cells expressing CD8a-GFP were treated with 5 μM BFA or DMSO (control) for 30 min and processed for immunofluorescence labeling of endogenous giantin (a Golgi marker). Scale bar, 10 μm. (L) Dynamin-1(K44A) inhibited the endocytosis of CD8a-GFP. RPE1 cells expressing CD8a-GFP alone or together with dyn-1(K44A)-Myc were incubated with anti-CD8a antibody on ice for 60 min. After washing away unbound antibody, cells were warmed up to 37 °C for indicated time before treatment with ice cold acid to remove surface-bound antibody (acid wash). The internalized antibody was fluorescence stained. The total intensity of internalized antibody was divided by the total intensity of CD8a-GFP and plotted against the incubation time before acid wash. Error bar, SEM. Mean values are indicated. (M-Q) Whole cilium FRAP traces of CD8a-GFP chimeras and SSTR3-GFP. Experiments correspond to Figure 2E-G.

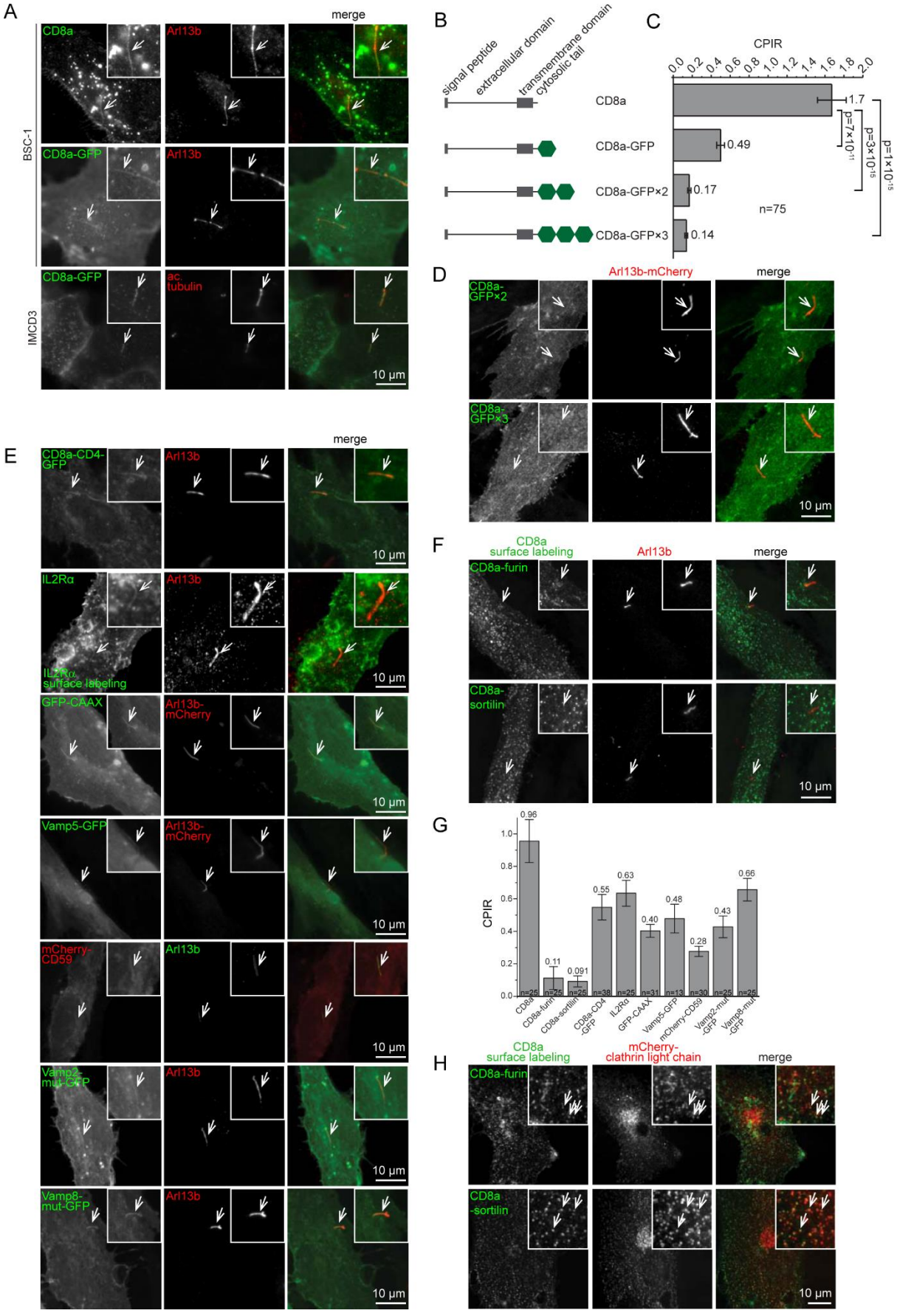

Fig. S2.PM-localized membrane proteins can be found at cilia. Cells were transiently transfected by various DNA plasmids and subsequently subjected to induction for ciliogenesis. (A) The ciliary localization of CD8a or CD8a-GFP in BSC-1 or IMCD3 cells. (B) A schematic diagram showing CD8a chimeras tagged by 0-3 copies of GFP. (C) The ciliary localization of CD8a chimera is dependent on its cytosolic size. The bar graph shows CPIRs of CD8a chimeras tagged by 0-3 copies of GFP. Error bar, SEM. n=75 cells. The mean value is indicated at the right of each column. p values (t-test) of selected pairs are denoted. (D) Images of CD8a-GFP×2 and CD8a-GFP×3 showing that they are virtually devoid of ciliary localization. (E) CD8a-CD4-GFP, IL2Rα, GFP-CAAX, Vamp5-GFP, mCherry-CD59, Vamp2-mut-GFP, Vamp8-mut-GFP localized to cilia in ciliated RPE1 cells. (F) CD8a-furin and –sortilin did not localize to cilia. Cells expressing CD8a fusion chimeras were surface-labeled by anti-CD8a antibody. (G) CPIRs of CD8a, CD8a-furin, CD8a-sortilin, CD8a-CD4-GFP, IL2Rα, GFP-CAAX, Vamp5-GFP, mCherry-CD59, Vamp2-mut-GFP and Vamp8-mut-GFP in ciliated RPE1 cells. Error bar, SEM. The mean and number of cells, n, are indicated. The experiment was performed together with Figure 1E. Therefore, both share the same CPIR of CD8a. (H) CD8a-furin and –sortilin localized to clathrin-coated pits at the PM. RPE1 cells co-expressing mCherry-clathrin light chain and CD8a-furin or –sortilin were subjected to surface labeling by anti-CD8a antibody. Three examples of colocalization spots are indicated by arrows in each panel. For all panels, cilia were identified by endogenous Arl13b, acetylated tubulin (ac. tubulin) or co-expressed Arl13b-mCherry. In each image, an area of interest was enlarged and boxed at the upper right corner to show the positive or negative ciliary colocalization. The cilium of interest is indicated by an arrow. Scale bar, 10 μm.

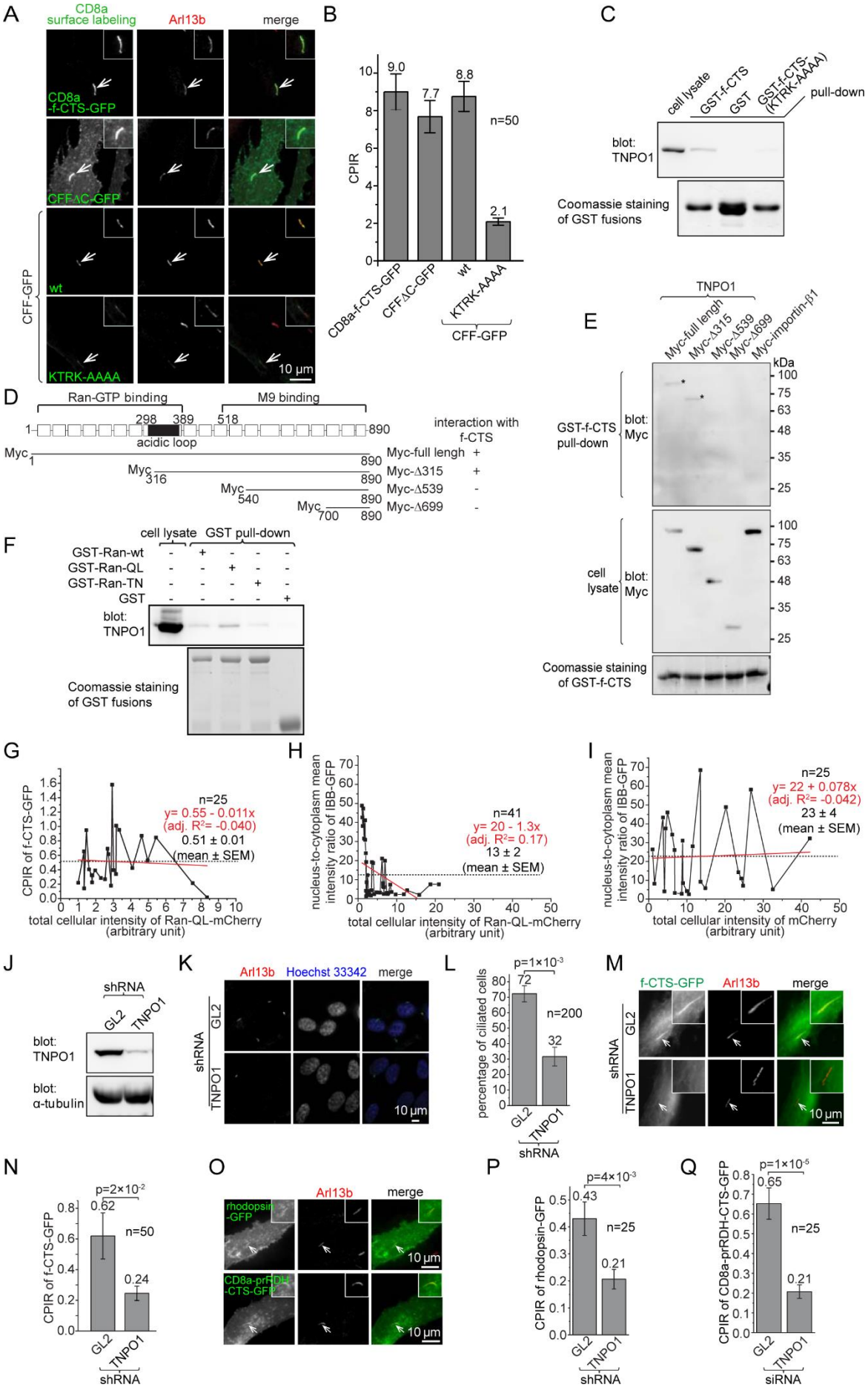

Fig. S3.Characterizing the interaction between f-CTS and TNPO1 and the effect of cellular depletion of TNPO1 on ciliary targeting of reporters. (A and B) Images and CPIRs of CD8a-fused fibrocystin chimeras in ciliated RPE1 cells. The cilium of interest is indicated by an arrow, enlarged and boxed at the upper right corner of each image. To acquire the CPIR, CD8a chimeras were revealed by surface labeling. Scale bar, 10 μm. Error bar, SEM. n=50 cells. The mean value is indicated at the top of each column. (C) KTRK to AAAA mutation (KTRK-AAAA) greatly reduced the interaction between f-CTS and TNPO1. Bead-immobilized GST-f-CTS or GST-f-CTS-(KTRK-AAAA) were incubated with HEK293T cell lysate and the material pulled down was blotted for TNPO1. (D) A schematic diagram showing the domain organization and truncation clones of TNPO1 used in this study. (E) The interaction between f-CTS and TNPO1 requires the central region (amino acid 316-539) of TNPO1. Bead-immobilized GST-f-CTS was incubated with HEK293T cell lysate expressing Myc-tagged truncations of TNPO1 and the protein pulled down was blotted for Myc-tag. "∗" denotes the band for full-length or Myc-Δ315 protein. In selected gel blots, numbers at the right indicate the molecular weight markers in kDa. (F) Bead-immobilized GST-Ran-QL pulled down significantly more endogenous TNPO1 from HEK293T cell lysate than corresponding TN and wild type GST-Ran. (G) The ciliary localization of f-CTS-GFP was not affected by the overexpression of Ran-QL-mCherry. Ciliated RPE1 cells co-expressing f-CTS-GFP and Ran-QL-mCherry were analyzed to plot the CPIR of f-CTS-GFP against the total cellular intensity of Ran-QL-mCherry. Data points are connected by lines from low to high total cellular intensity. (H and I) The nucleus-to-cytoplasm mean intensity ratio of IBB-GFP decreased when the cellular amount of Ran-QL-mCherry increased, demonstrating the functionality of our Ran-QL-mCherry. RPE1 cells co-expressing IBB-GFP and Ran-QL-mCherry (H) or mCherry (I, as a negative control) were analyzed to plot the nucleus-to-cytoplasm mean intensity ratio of IBB-GFP against the total cellular intensity of Ran-QL-mCherry or mCherry. Data points are connected by lines from low to high total cellular intensity. In (G, H and I), the trend of data is represented by a linear regression fitting line, which, together with its formula and adjusted R^2 (adj. R^2), is shown in red. (J) Endogenous TNPO1 was significantly depleted by shRNA-mediated knockdown of endogenous TNPO1. (K and L) The depletion of TNPO1 reduced the percentage of ciliated cells. TNPO1-depleted RPE1 cells were induced for ciliogenesis and ciliated cells were counted. Images and percentages are shown in (K) and (L) respectively. In (L), n=200 cells. (M and N) TNPO1-

depleted RPE1 cells were transfected to express f-CTS-GFP and induced for ciliogenesis. Images and CPIRs are shown in (M) and (N) respectively. In (N), n=50 cells. (O) GFP-tagged full-length rhodopsin and CD8a-prRDH-CTS localized to cilia. (P and Q) TNPO1-depleted RPE1 cells were transfected to express GFP-tagged rhodopsin or CD8a-prRDH-CTS and induced for ciliogenesis. CPIRs are plotted. In (M and O), the cilium of interest is indicated by an arrow, enlarged and boxed at the upper right corner of each image. Scale bar, 10 μm. In (L, N, P and Q), p values (t-test) between GL2 and TNPO1 and the number of cells, n, are indicated. The mean value is denoted at the top of each column. Error bar, SEM.

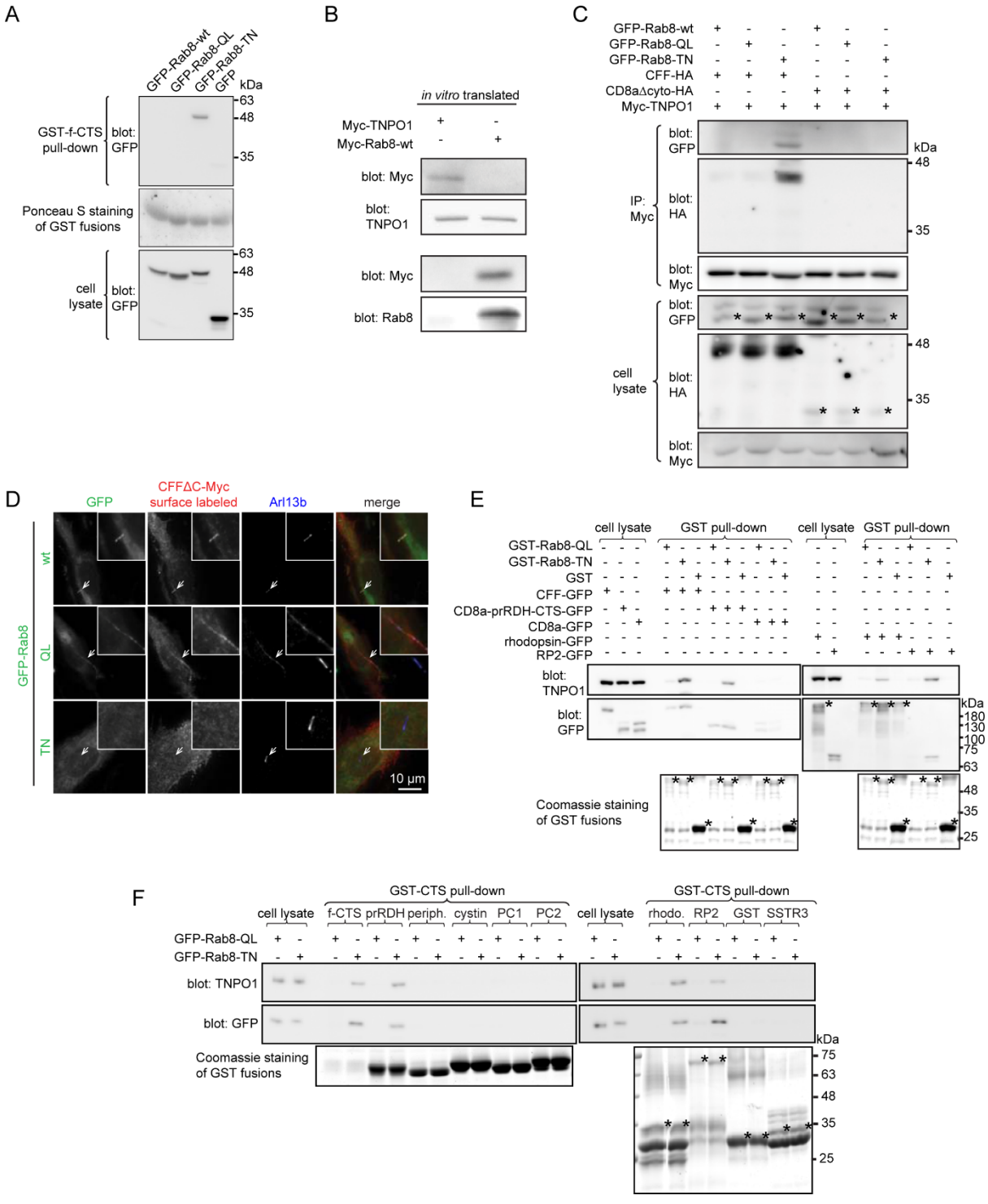
A
GFP-Rab8-wt
GFP-Rab8-QL
GFP-Rab8-TN
GFP
kDa
63
48
35
GST-f-CTS pull-down
blot: GFP
Ponceau S staining of GST fusions
cell lysate
blot: GFP
B
in vitro translated
Myc-TNPO1
Myc-Rab8-wt
blot: Myc
blot: TNPO1
blot: Myc
blot: Rab8
C
GFP-Rab8-wt
GFP-Rab8-QL
GFP-Rab8-TN
CFF-HA
CD8aΔcyto-HA
Myc-TNPO1
IP: Myc
blot: GFP
blot: HA
blot: Myc
cell lysate
blot: GFP
blot: HA
blot: Myc
kDa
48
35
D
GFP
CFFΔC-Myc surface labeled
Arl13b
merge
GFP-Rab8
wt
QL
TN
10 µm
E
cell lysate
GST pull-down
cell lysate
GST pull-down
GST-Rab8-QL
GST-Rab8-TN
GST
CFF-GFP
CD8a-prRDH-CTS-GFP
CD8a-GFP
rhodopsin-GFP
RP2-GFP
blot: TNPO1
blot: GFP
Coomassie staining of GST fusions
kDa
180
130
100
75
63
48
35
25
F
GST-CTS pull-down
GST-CTS pull-down
cell lysate
f-CTS
prRDH
periph.
cystin
PC1
PC2
cell lysate
rhodo.
RP2
GST
SSTR3
GFP-Rab8-QL
GFP-Rab8-TN
blot: TNPO1
blot: GFP
Coomassie staining of GST fusions
kDa
75
63
48
35
25

Fig. S4.The interaction among CTSs, Rab8 and TNPO1. (A) Bead-immobilized GST-f-CTS specifically pulled down GFP-Rab8-TN but not -QL and -wt from the cell lysate expressing respective GFP chimeras. (B) Rabbit reticulocyte lysate from *in vitro* transcription and translation system contained endogenous TNPO1 but not Rab8. Rabbit reticulocyte lysate was mixed with methionine and Myc-tagged TNPO1 or Rab8 expression DNA plasmids that contain T7 promoters for 1 hour at 30 °C. The lysate was subsequently separated by electrophoresis and blotted for Myc-tag, TNPO1 and Rab8. The Rab8 antibody used here should cross-react with rabbit Rab8 as the identity of antigen regions between human and rabbit is as high as 97%. (C) TNPO1 indirectly interacted with Rab8-TN via f-CTS. The experiment also provided further evidence showing that Rab8-TN increases the interaction between TNPO1 and f-CTS. HEK293T cell lysate triply co-expressing Myc-TNPO1, one of the GFP-Rab8 mutants (wt, QL and TN) and HA-tagged CFF or CD8aΔcyto was subjected to IP using anti-Myc antibody and co-IPed proteins were blotted for HA-tag and GFP. HA was blotted by HRP-conjugated anti-HA antibody while GFP was blotted by anti-GFP primary antibody followed by HRP-conjugated protein A. (D) The effect of Rab8 guanine nucleotide binding mutants on the ciliary localization of fibrocystin. Ciliated RPE1 cells co-expressing CFFΔC-Myc and GFP, GFP-Rab8-wt, -QL or –TN were subjected to surface labeling by CD8a antibody to reveal the ciliary localization of CFFΔC-Myc. Cilia were identified by endogenous Arl13b staining. The cilium of interest is indicated by an arrow, enlarged and boxed at the upper right corner of each image. Scale bar, 10 μm. (E) RP2, rhodopsin or the CTS of prRDH assembled a ternary complex with Rab8-TN and TNPO1. Bead-immobilized GST or GST-Rab8 mutants were incubated with HEK293T cell lysate expressing GFP-tagged CFF (positive control), CD8a-prRDH-CTS, CD8a (negative control), rhodopsin or RP2. The bound GFP fusion proteins and endogenous TNPO1 were subsequently blotted. Note the high apparent molecular weight of full-length rhodopsin-GFP in our experimental condition (marked by "∗"). (F) The screen showing that RP2 and CTSs of fibrocystin, prRDH and rhodopsin, but not peripherin, cystin, PC1, PC2, and SSTR3, demonstrate enhanced interaction with endogenous TNPO1 in the presence of Rab8-GDP mutant form. GST-CTSs immobilized on beads were incubated with HEK293T cell lysate expressing GFP-Rab8-QL or -TN. The bound GFP-Rab8 and endogenous TNPO1 were subsequently blotted. CTSs of fibrocystin and prRDH were positive controls. Periph., peripherin. rhodo.,

rhodopsin. “∗” denotes the specific band. In selected gel blots, numbers at the right indicate the molecular weight markers in kDa.

Table S1

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