Supplemental Materials

Molecular Biology of the Cell

Xia et al.

Supplementary Figure Legends

Figure S1. Atlas of PACF system

Schematic illustration of PACF design and construction maps.

Figure S2. Characterization of EB1-PACF expression and photoactive properties

A. Cell lysates expressing EB1-nPACF (lane 1), EB1-cPACF (lane 2) and co-expressing EB1-n/cPACF (lane 3) were subjected to SDS-PAGE and transferred onto nitrocellulose membrane. Proteins were probed by EB1 antibody.

B. Native PAGE gel was stained Coomassie blue. From left to right recombinant PACF and EB1-PACF.

C. The Native PAGE gel was first irradiated with a 413 nm light followed by excitation at 488 nm and detected at 530 nm. From left to right recombinant PACF and EB1-PACF.

D. Western blot analysis of EB1-PACF using an anti-EB1 mouse antibody (BD Biosciences).

Figure S3. Analysis of photobleach of EB1-PACF and EB1-PAGFP

A. After 24 hrs transfection of EB1-PACF and EB1-PAGFP, cells were scanned with cycles of 488 nm laser. For each cycle, cells were exposed under 100% power 488 nm laser for 50 ms. Photoactivation (PA) was performed with 50% power 405 nm laser for 2 second after the first cycle of 488 nm laser exposure. The scale bars are 5 μm.

B. Statistics analysis of photobleach of EB1-PACF and EB1-PAGFP, each data point was calculated with more than 100 cells in three independent experiments. The results show that photobleach characters of EB1-PACF and EB1-PAGFP are comparative.

C. Schematic drawing of the inducible dimerization construct containing EB1. The C-terminus of EB1 (EBC) was replaced by the homodimerization domain FKBP, which becomes dimerized upon the addition of homodimerizer AP20187.

D. Cells expressing the inducible EB1 dimerization system were exposed to 488 nm laser before and after photoactivation with 405 nm laser. AP20187 (100 nM) was utilized to induce the dimerization of

$EB1^{CH\text{-}Linker}$-FKBP. Control cells were treated with equal volume of vehicle. The scale bars are 5 μm.

E. Statistical analyses of intensity ratios (fluorescent intensity in cells divided by background) in each group illustrated in (D). Data were collected from more than 30 cells from 3 separated experiments.

Figure S4. Statistical analyses of the distribution of localization precisions

A. The distribution of localization precisions of fixed EB1-PACF proteins (Related to Figure 1E).

B. The distribution of localization precisions of live EB1-PACF and EB1-PAGFP proteins (Related to Figure 1 F and G).

C. The distribution of localization precisions of live EB1-PACF dimerized proteins in every time point (Related to Figure 2).

Figure S5. Real-time imaging of a directional migrating cell

A. MCF7 cells expressing EB1-eGFP was pretreated with EGF at 30 ºC.

B. In-frame images of 5 x15 μm rectangular regions of (A) which representing “Trailing Edge (1)”, “Cell Body (2)”, and “Leading edge (3)”, were exhibited with a frame rate of 2 seconds. Note that trailing edge has much less +TIPs comparing with cell body and leading edge, and the +TIPs seems winding but not docking/tracking/catastrophe when touching the cell cortex. Many of +TIPs at leading edge will dock to the pseudopodia, while most of them at cell body keep tracking forward. The arrows point to microtubule plus-ends.

Figure S6. EB1-PACF exhibits different localization patterns at cell body and leading edge

A. PALM images of EB1-PACF at leading edge from the 100 consecutive exposure frames over time were assembled into two stacks that contains first 50 exposures (snapshot #1-50) and the later 50 exposures (snapshot #51-100). Related to Figure 3C. Bar: 200 nm.

B. PALM images of EB1-PACF at cell body from the 100 exposure frames were separated into 1-50 and 51-100 frames. Related to Figure 3A. Bar: 200 nm.

C. Fixed cells expressing EB1-PACF were co-stained with α-tubulin antibody. Diffraction limited

TIRFM images of microtubule (indicated by immunofluorescence of alpha-tubulin antibody) were overlaid with PALM images of EB1-PACF. Label “1” refers to plus-end at leading edge, while label “2” refers to plus-end at cell body. Bar: 10 μm in image of whole cell; 500 nm in in-frame.

D. Fixed cells expressing EB1-PACF were co-stained with EB1 antibody. Diffraction limited TIRFM images of EB1 were overlaid with PALM images of EB1-PACF. Label “1” refers to plus-end at leading edge, while label “2” refers to plus-end at cell body. Bar: 10 μm in image of whole cell; 500 nm in in-frame.

E. The microtubule plus-ends distribution of EB1-PACF relative to microtubules or EB1 was defined as Type A and Type B as in figure 3E. The percentage of these microtubule plus-ends were plotted for leading edge and cell body. Data were collected from 6 separated experiments with more than 300 plus-ends were analyzed in each group.

Figure S7. Sequence alignment of full-length human and mouse EB1 and EB3

Figure S8. Insertion of fluorescent proteins adjacent to the unconserved flexible linker was found to perturb the microtubule plus-end-tracking of EB1

A. Schematic model and the microtubule plus-end-tracking of full-length EB1 with GFP fused in its end. Bar: 10 μm.

B. Schematic model and the microtubule plus-end-tracking of full-length EB1 with GFP fused between the linker and the EBC (C-terminal domain of EB1). Bar: 10 μm.

C. Schematic model and the microtubule plus-end-tracking of full-length EB1 with GFP fused between the CH (Calponin homology) domain and the linker. Bar: 10 μm.

Figure S9. Characterization of the expression of PACF fused EB1 WT/WT, KR5Q/KR5Q and WT/KR5Q by western blot

Cell lysates expressing PACF fused EB1 WT/WT (lane 1), KR5Q/KR5Q (lane 2), and WT/KR5Q (lane 3) were subjected to SDS-PAGE and transferred onto nitrocellulose membrane (GE Healthcare). Proteins

were probed by an anti-EB1 antibody (BD Bioscience).

Figure S10. The distribution of localization precisions of dimerized PACF-EB1 proteins (WT/WT, KR5Q/KR5Q, and WT/KR5Q). (Related to Figure 4B)

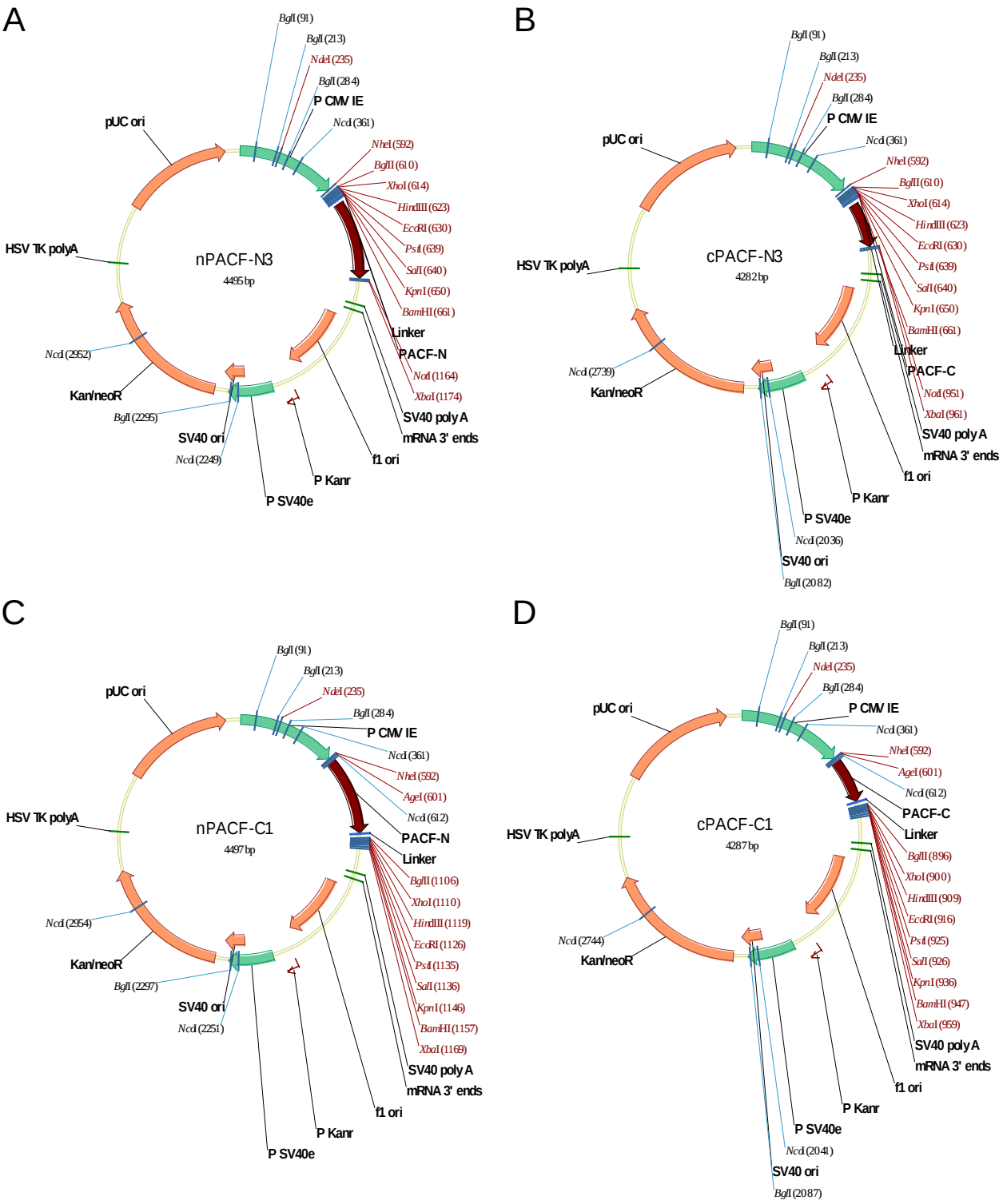
Figure S11. SDS-PAGE analyses of the expression and purification of the FRET sensors (Relative to Figure 4 D-F).

Figure S12. Surface charge analysis of EB1 CH and EBC domain

A. Electrostatic surface of EB1 CH domain (PDB 1PA7) is depicted (Red= negatively charged atoms; blue= positively charged atoms; white= no charge).

B. Electrostatic surface of EB1 EBC domain (PDB 1WU9) is depicted (Red=negatively charged atoms; blue=positively charged atoms; white=no charge).

Figure S1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nPACF-N3
4495 bp
pUC ori
HSV TK polyA
Kan/neoR
SV40 ori
P SV40e
P Kanr
f1 ori
SV40 poly A
mRNA 3' ends
Linker
PACF-N
P CMV IE
BglII (91)
BglII (213)
NdeI (235)
BglII (284)
NcoI (361)
NheI (592)
BglII (610)
XhoI (614)
HindIII (623)
EcoRI (630)
PstI (639)
SalI (640)
KpnI (650)
BamHI (661)
NotI (1164)
XbaI (1174)
NcoI (2952)
BglII (2295)
NcoI (2249)
B
cPACF-N3
4282 bp
pUC ori
HSV TK polyA
Kan/neoR
SV40 ori
P SV40e
P Kanr
f1 ori
SV40 poly A
mRNA 3' ends
Linker
PACF-C
P CMV IE
BglII (91)
BglII (213)
NdeI (235)
BglII (284)
NcoI (361)
NheI (592)
BglII (610)
XhoI (614)
HindIII (623)
EcoRI (630)
PstI (639)
SalI (640)
KpnI (650)
BamHI (661)
NotI (951)
XbaI (961)
NcoI (2739)
NcoI (2036)
BglII (2082)
C
nPACF-C1
4497 bp
pUC ori
HSV TK polyA
Kan/neoR
SV40 ori
P SV40e
P Kanr
f1 ori
SV40 poly A
mRNA 3' ends
Linker
PACF-N
P CMV IE
BglII (91)
BglII (213)
NdeI (235)
BglII (284)
NcoI (361)
NheI (592)
AgeI (601)
NcoI (612)
BglII (1106)
XhoI (1110)
HindIII (1119)
EcoRI (1126)
PstI (1135)
SalI (1136)
KpnI (1146)
BamHI (1157)
XbaI (1169)
NcoI (2954)
BglII (2297)
NcoI (2251)
D
cPACF-C1
4287 bp
pUC ori
HSV TK polyA
Kan/neoR
SV40 ori
P SV40e
P Kanr
f1 ori
SV40 poly A
mRNA 3' ends
Linker
PACF-C
P CMV IE
BglII (91)
BglII (213)
NdeI (235)
BglII (284)
NcoI (361)
NheI (592)
AgeI (601)
NcoI (612)
BglII (896)
XhoI (900)
HindIII (909)
EcoRI (916)
PstI (925)
SalI (926)
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XbaI (959)
NcoI (2744)
NcoI (2041)
BglII (2087)

Figure S2

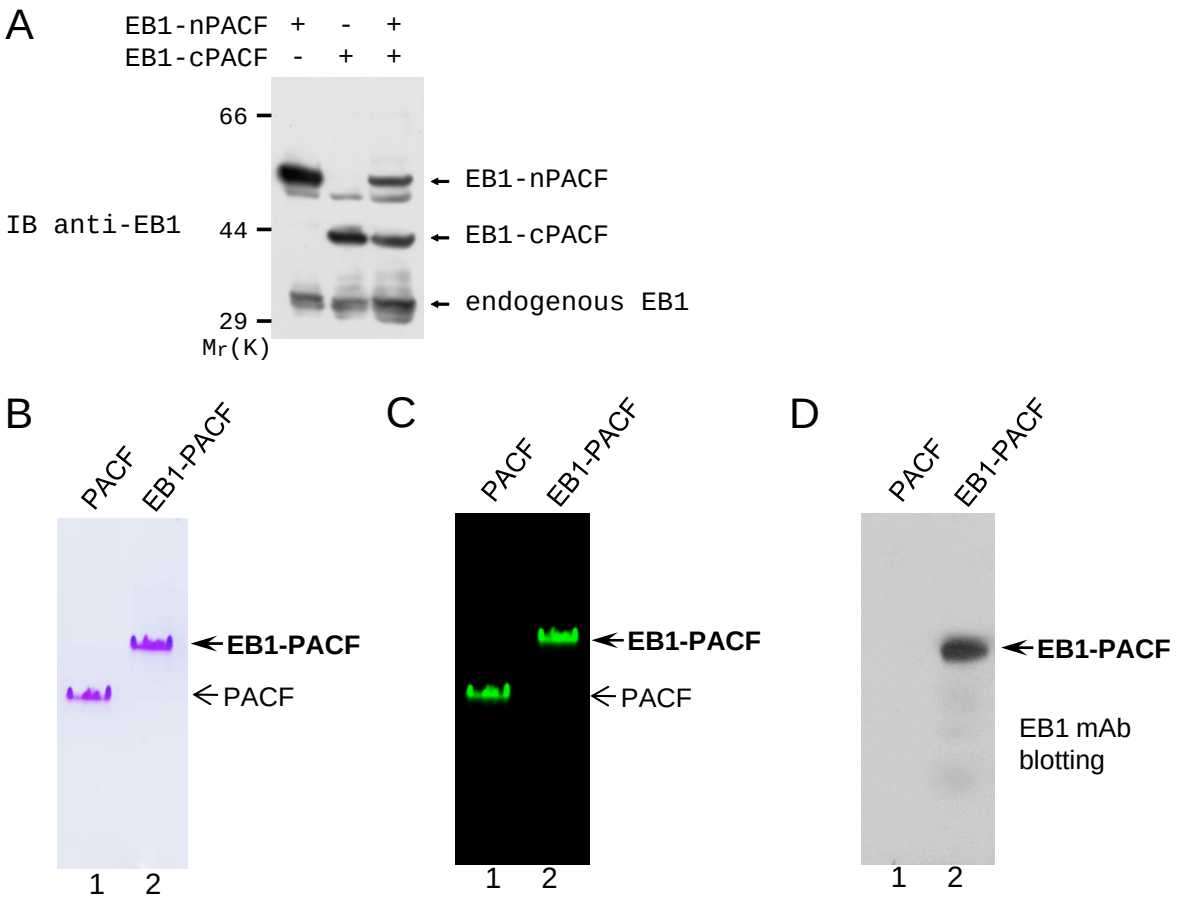

A
EB1-nPACF + - +
EB1-cPACF - + +
66
44
29
Mr(K)
IB anti-EB1
EB1-nPACF
EB1-cPACF
endogenous EB1
B
PACF
EB1-PACF
EB1-PACF
PACF
1 2
C
PACF
EB1-PACF
EB1-PACF
PACF
1 2
D
PACF
EB1-PACF
EB1-PACF
EB1 mAb
blotting
1 2

Figure S3

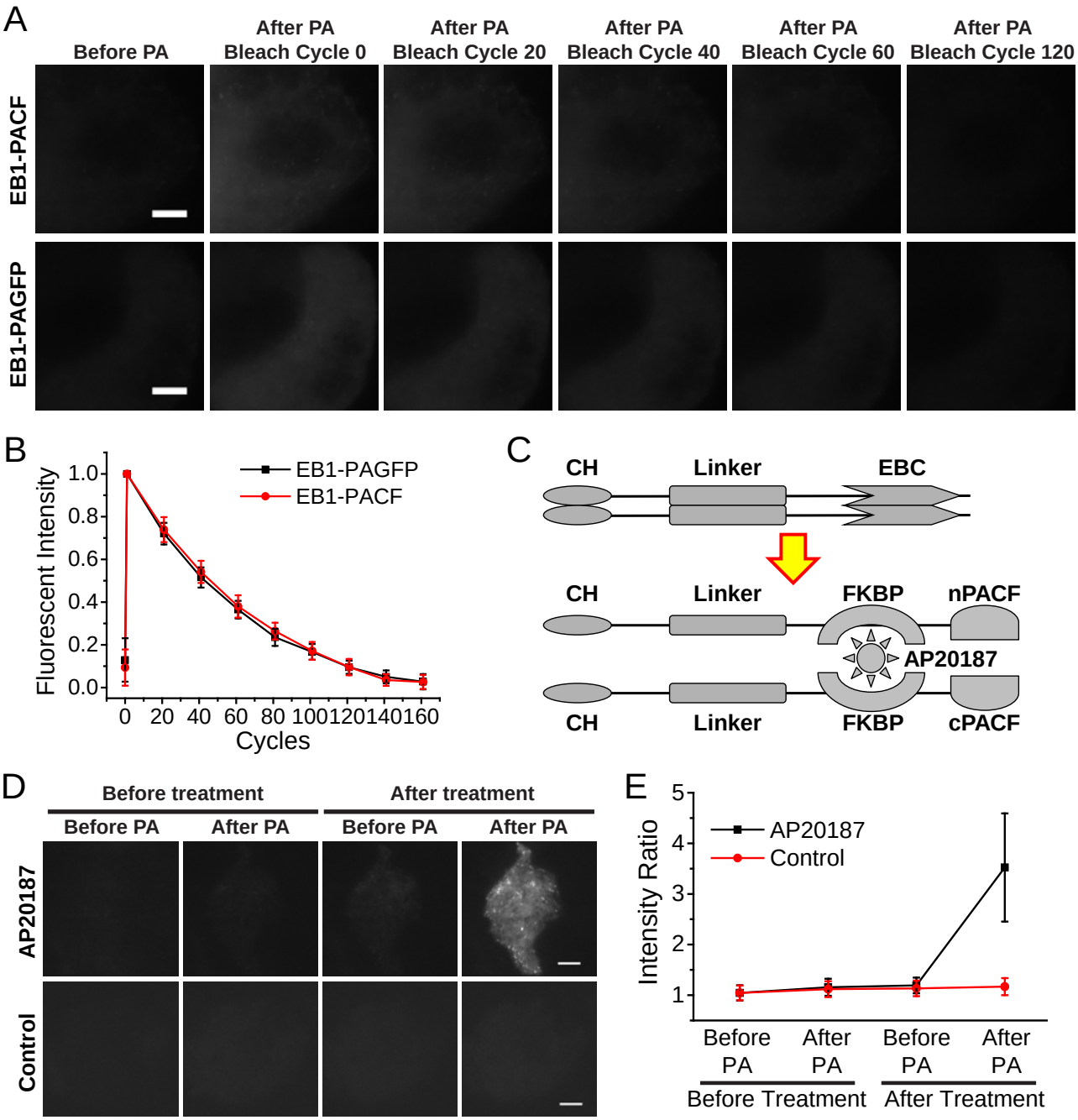

A
Before PA
After PA Bleach Cycle 0
After PA Bleach Cycle 20
After PA Bleach Cycle 40
After PA Bleach Cycle 60
After PA Bleach Cycle 120
EB1-PACF
EB1-PAGFP
B
Fluorescent Intensity
1.0
0.8
0.6
0.4
0.2
0.0
0 20 40 60 80 100 120 140 160
Cycles
EB1-PAGFP
EB1-PACF
C
CH
Linker
EBC
CH
Linker
FKBP
nPACF
AP20187
CH
Linker
FKBP
cPACF
D
Before treatment
After treatment
Before PA
After PA
Before PA
After PA
AP20187
Control
E
Intensity Ratio
5
4
3
2
1
AP20187
Control
Before PA
After PA
Before PA
After PA
Before Treatment
After Treatment

Figure S4

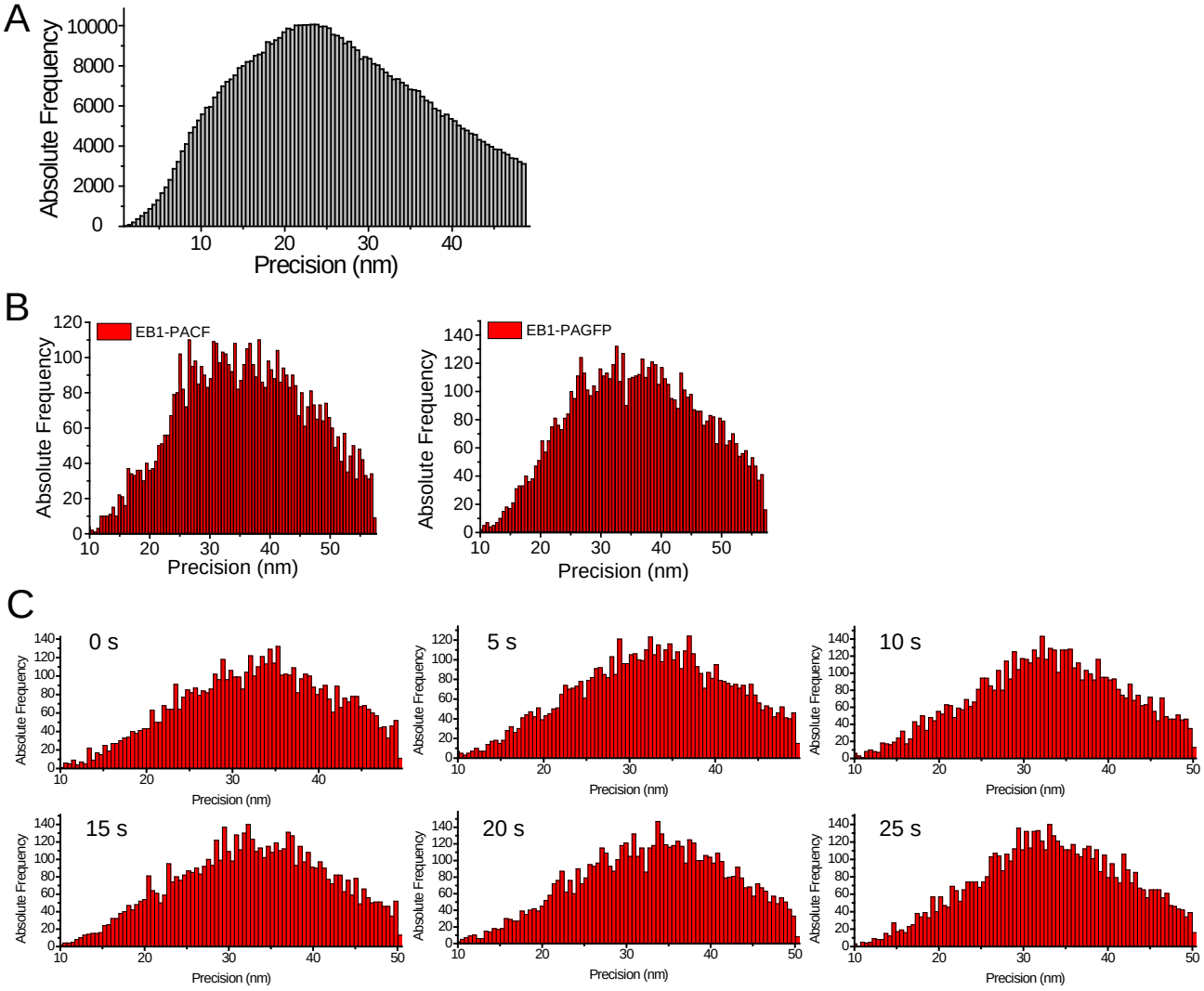
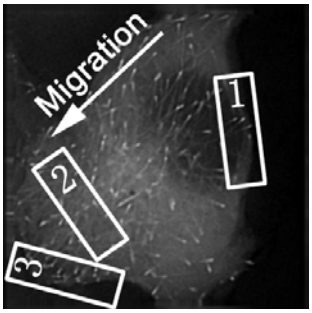

A
Absolute Frequency
Precision (nm)
B
EB1-PACF
EB1-PAGFP
Absolute Frequency
Precision (nm)
C
0 s
5 s
10 s
15 s
20 s
25 s
Absolute Frequency
Precision (nm)

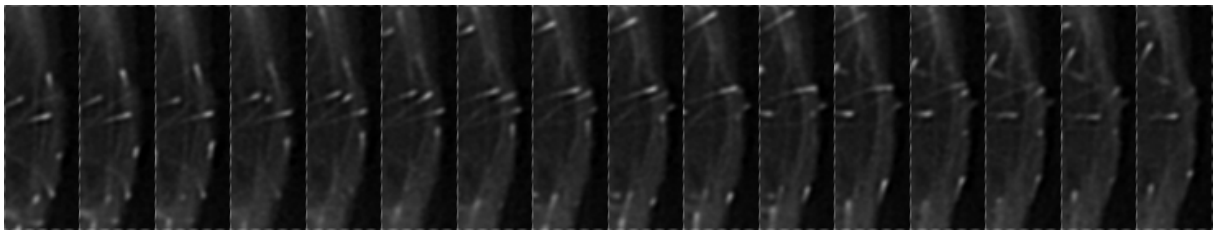
Figure S5

A

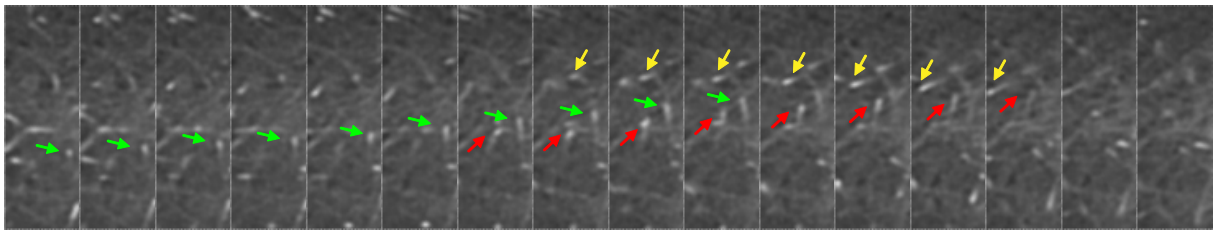


B

1. Trailing Edge



2. Cell Body



3. Leading Edge

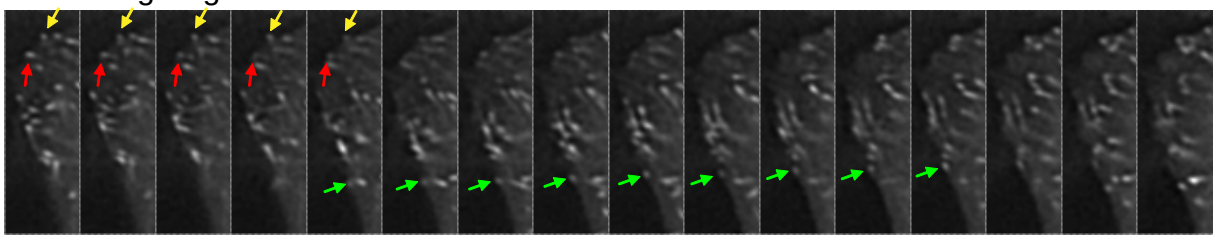


Figure S6

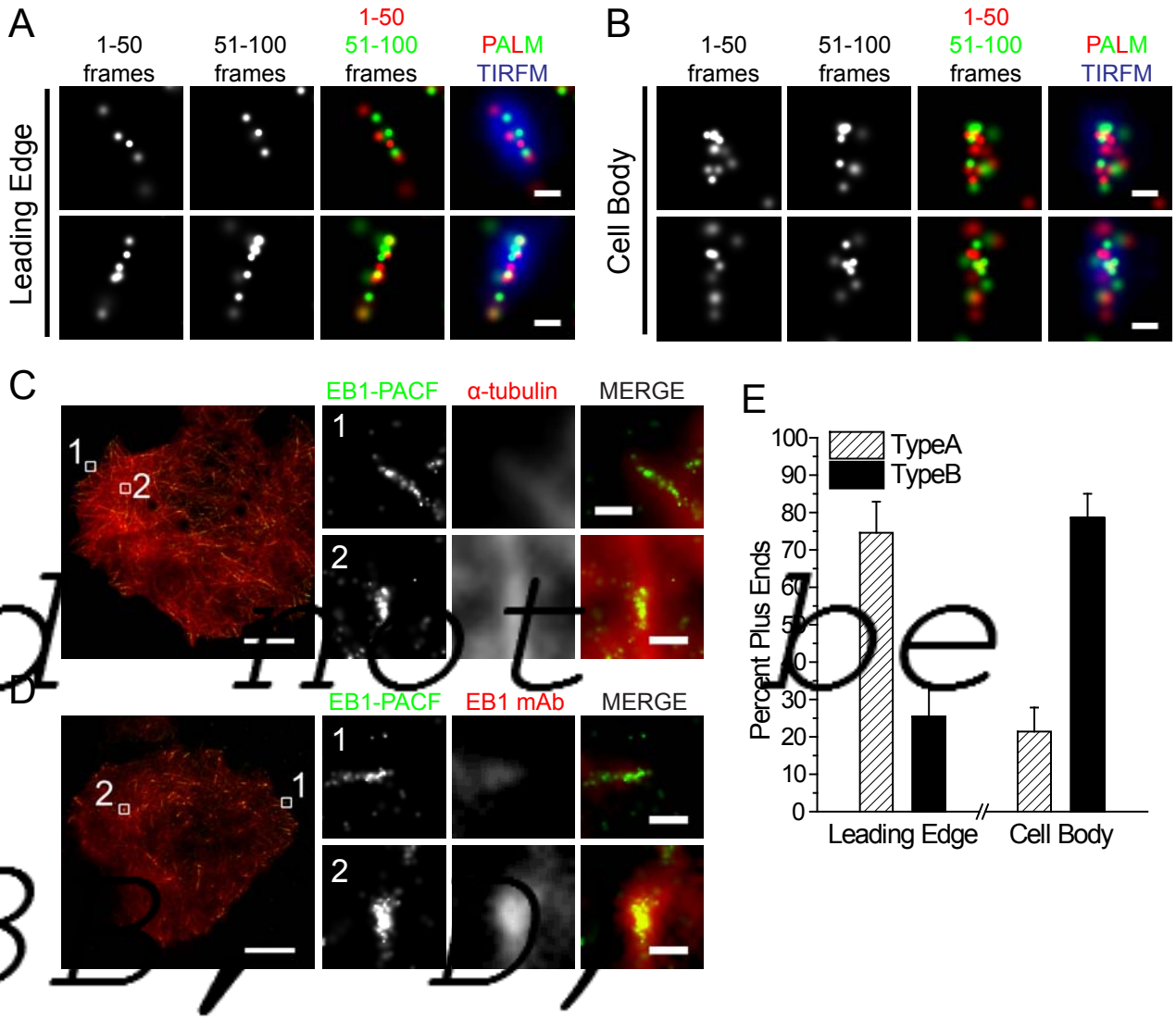

A
1-50 frames
51-100 frames
1-50 51-100 frames
PALM TIRFM
Leading Edge
B
1-50 frames
51-100 frames
1-50 51-100 frames
PALM TIRFM
Cell Body
C
EB1-PACF
α-tubulin
MERGE
1
2
D
EB1-PACF
EB1 mAb
MERGE
1
2
E
TypeA
TypeB
Percent Plus Ends
100
90
80
70
60
50
40
30
20
10
0
Leading Edge
Cell Body

Figure S7

```
1    MAVNVYSTSVTSDNLSRHDMLAWINESLQLNLTKIEQLCSGAAYCQFMDMLFPGSIALKK   60   EB1_HUMAN
1    MAVNVYSTSVTSDNLSRHDMLAWINESLQLNLTKIEQLCSGAAYCQFMDMLFPGSIALKK   60   EB1_MOUSE
1    MAVNVYSTSVTSENLSRHDMLAWVNDSLHLNYTKIEQLCSGAAYCQFMDMLFPGCVHLRK   60   EB3_HUMAN
1    MAVNVYSTSVTSENLSRHDMLAWVNDSLHLNYTKIEQLCSGAAYCQFMDMLFPGCVHLRK   60   EB3_MOUSE
     ************:**********:*:**:** **********************.: *:*

61   VKFQAKLEHEYIQNFKILQAGFKRMGVDKIIPVDKLVKGKFQDNFEFVQWFKKFFDANYD  120   EB1_HUMAN
61   VKFQAKLEHEYIQNFKILQAGFKRMGVDKIIPVDKLVKGKFQDNFEFVQWFKKFFDANYD  120   EB1_MOUSE
61   VKFQAKLEHEYIHNFKVLQAAFKKMGVDKIIPVEKLVKGKFQDNFEFIQWFKKFFDANYD  120   EB3_HUMAN
61   VKFQAKLEHEYIHNFKVLQAAFKKMGVDKIIPVEKLVKGKFQDNFEFIQWFKKFFDANYD  120   EB3_MOUSE
     ************:***:***.**:*********:*************:************

121  GKDYDPVAARQGQETAVAPSLVAPALNKPKKPLTSSSAA---PQRPISTQRTAA-APKAG  176   EB1_HUMAN
121  GKEYDPVAARQGQETAVAPSLVAPALSKPKKPLGSSTAA---PQRPIATQRTTA-APKAG  176   EB1_MOUSE
121  GKDYNPLLARQGQDVAPPPNPGDQIFNKSKKLIGTAVPQRTSPTGPKNMQTSGRLSNVAP  180   EB3_HUMAN
121  GKDYNPLLARQGQDVAPPPNPGDQIFNKSKKLIGTAVPQRTSPTGPKNMQTSGRLSNVAP  180   EB3_MOUSE
     **:*:*: *****:.*  *.     :.* ** : ::      *  *   * :   :  *

177  PGVVRKNPGVG-N----GDDEAAELMQQVNVLKLTVEDLEKERDFYFGKLRNIELICQEN  231   EB1_HUMAN
177  PGMVRKNPGVG-N----GDDEAAELMQQVKVLKLTVEDLEKERDFYFGKLRNIELICQEN  231   EB1_MOUSE
181  PCILRKNPPSARNGGHETDAQILELNQQLVDLKLTVDGLEKERDFYFSKLRDIELICQEH  240   EB3_HUMAN
181  PCILRKNPPSARNGGHEADAQILELNQQLLDLKLTVDGLEKERDFYFSKLRDIELICQEH  240   EB3_MOUSE
     * ::****  . *     * :  ** **:  *****: *********.***:*******.

232  EGENDPVLQRIVDILYATDEGFVIPDEGGPQEEQ----EEY  268   EB1_HUMAN
232  EGENDPVLQRIVDILYATDEGFVIPDEGGPQEEQ----EEY  268   EB1_MOUSE
241  ESENSPVISGIIGILYATEEGFAPPEDDEIEEHQQEDQDEY  281   EB3_HUMAN
241  ESENSPVISGIIGILYATEEGFAPPEDDEIEEHQQEDQDEY  281   EB3_MOUSE
     *.**.**:. *: *****:***. *::   :*.*    :**
```

Figure S8

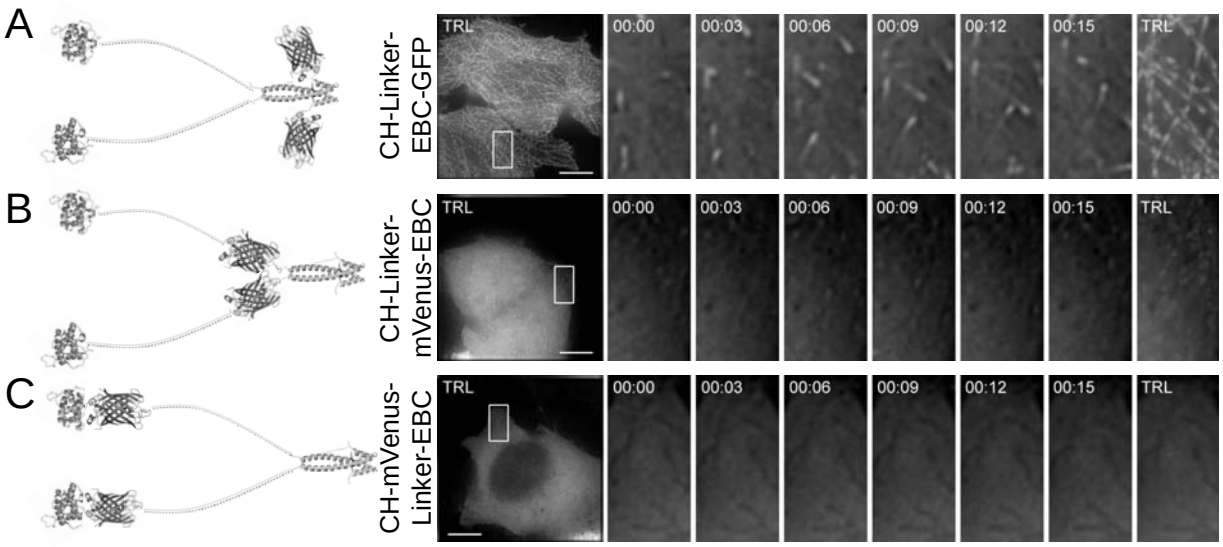

A
B
C
CH-Linker-EBC-GFP
CH-Linker-mVenus-EBC
CH-mVenus-Linker-EBC
TRL
00:00
00:03
00:06
00:09
00:12
00:15
TRL

Figure S9

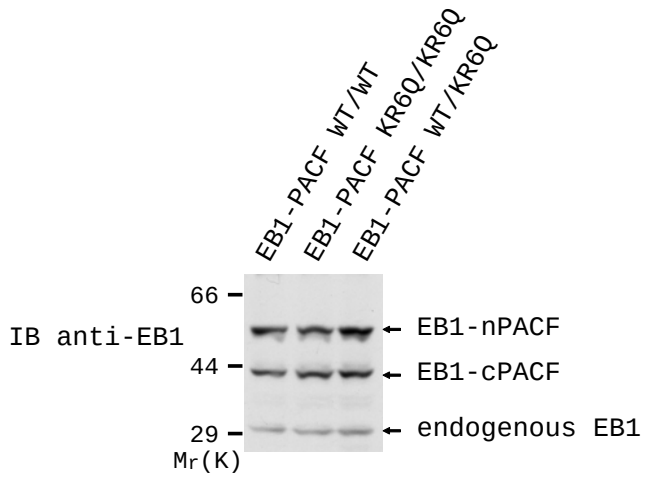

EB1-PACF WT/WT
EB1-PACF KR6Q/KR6Q
EB1-PACF WT/KR6Q
66
44
29
Mr(K)
IB anti-EB1
EB1-nPACF
EB1-cPACF
endogenous EB1

Figure S10

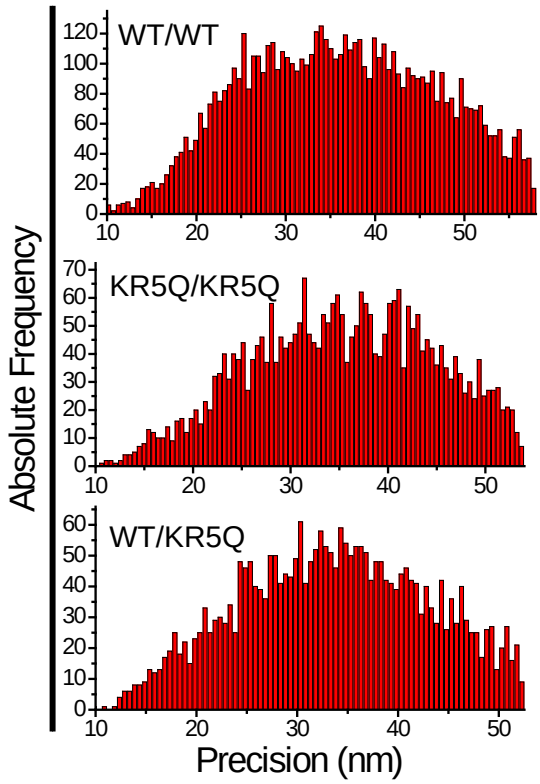


Figure S11

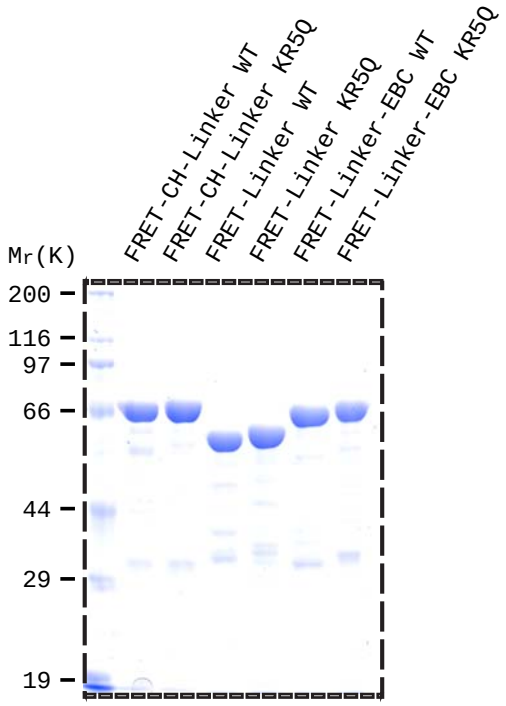

FRET-CH-Linker WT
FRET-CH-Linker KR5Q
FRET-Linker WT
FRET-Linker KR5Q
FRET-Linker-EBC WT
FRET-Linker-EBC KR5Q
Mr(K)
200
116
97
66
44
29
19

Figure S12