Table S1. Primers used to generate constructs

Constructs	Primers
pB42AD-Tmp21 (aa 108-208):	
Forward:	ccggaattctgttttgagagcaagggaacaggg
Reverse:	ccgctcgagcaggtagaagacctgccag
pB42ADTmp21 (aa108-185):	
Forward:	ccggaattctgttttgagagcaagggaacaggg
Reverse:	ccgctcgagccgagtgtttgttgactcgttgg
pB42ADTmp21 (aa183-211):	
Forward:	ggaattcgtcctatacttcagcatcttttcaatgttctgtctcattggactagctacct ggcaggtcttctacctgctcgagcgg
Reverse:	ccttaagcaggatatgaagtcgtagaaaagttacaagacagagtaacctgatcgatgga ccgtccagaagatggacgagctcgcc
pEBG-Tmp21/p23 (aa 1-219)	
Forward:	ccgctcgaggatccatgtctggtttgtctggccc
Reverse:	gactagtctactcaatcaatttcttggcc
pEBG-Tmp21/p23 (aa 108-219)	
Forward:	gaagatctctcgaggatccatgtgttttgagagcaaggg
Reverse:	gactagtctactcaatcaatttcttggcc
pEBG-Tmp21/p23 (aa 1-208)	
Forward:	ccgctcgaggatccatgtctggtttgtctggccc
Reverse:	gactagtctagcgtcgcaggtagaagacc
pEBG-Tmp21/p23 (aa 1-185)	
Forward:	ccgctcgaggatccatgtctggtttgtctggccc
Reverse:	gactagtctaccgagtgtttgttgactcg
PKCαC1a-b	
Forward	ccggaattcatggctgacgttttcccgggcaacga
Reverse	cgcggatccctaaacctcagcctttaggtaaatcc
PKCε C1a-b	
Forward	ccggaattctcgtcgggtgaagcccctaaagacaatg
Reverse	cgcggatccctaggtaacgcccaggtcggccagtactttg
PKCδ C1a-b	
Forward	ccggaattcgaggacgtggattgcaaacagtctatg
Reverse	cgcggatccctatctctgggtgacttggttcaaggcct
PKCζ C1	
Forward	ccggaattcgttttcccgagcacccctgagcagcctg
Reverse	cgcggatccctaagactctgccccagggctaagcaaatc
RasGRP1 C1	
Forward	ccggaattcttctgtgtgatggacaaagatagg
Reverse	cgcggatcccacagagctgatgttttctgtgg
β2-chimaerin C1	
Forward	ccggaattcaaaacaaacgtcacacatgaagaacacacagc
Reverse	cgcggatccctatgttgtgaggtcacaacagtacact
PKCε C1a	
Forward	gaattcaacggccacaagttcatg
Reverse	ggatcccccagcacactttgtgatta
PKCε C1b	
Forward	gaattcatgccccacaagttcggtat
Reverse	ggatcccactccacagttgggag

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. *Binding of C1 domains to p23/Tmp21.* EGY48 yeast (containing 8op-LacZ vector) was co-transformed with pLexA-fused C1 domains from PKCα (C1αa-b), PKCε (C1εa-b), PKCδ (C1δa-b), PKCζ (C1ζ), RasGRP1 (C1RasGRP1), or β2-chimaerin (C1β2-ch), and pB42AD-HA-tagged p23/Tmp21 (aa 108-208). **A**. Alignment of PKCα, PKCδ, PKCε C1b, RasGRP1, and β2-chimaerin C1 domains. Positions 15 and 36 in C1 domains are indicated with arrows. **B**. Schematic representation of C1 domains fused to pLexA. **C.** Assay of β-galactosidase activity on induction (*upper panel*) or no-induction (*lower panel*) plates, carried out 72 h after transformation. *Gal/Raf*, galactosidase/raffinose. Two additional experiments gave similar results. **D**. HeLa cells were co-transfected with plasmids encoding GFP-PKCα, GFP-PKCδ, GFP-PKCε, GFP-RasGRP1 or GFP-β2-chimaerin, and pcDNA3.1-V5-p23/Tmp21. Forty-eight h later, cells were fixed and co-localization examined by confocal microscopy. *Bar*, 10 μm. All experiments have been performed at least three times with similar results.

Figure S2. *FBS and EGF enhance the association of β2-chimaerin with p23/Tmp21.* COS-1 cells were co-transfected with pEBG-p23/Tmp21 and GFP-β2-chimaerin, and 48 h later either serum-starved for 18 h and stimulated with EGF (100 ng/ml) or left in 10% FBS. GST-p23/Tmp21 was precipitated with glutathione Sepharose 4B beads and associated GFP-β2-chimaerin detected by Western blot using an anti-GFP antibody. A representative example is shown together with a densitometric analysis of 3 independent experiments. *P<0.05 between control (0 min.) *vs.* treatments.

Figure S3. *Co-localization of β2-chimaerin with a Golgi marker*. HeLa (CTL), HeLa (shRNA#2) or HeLa (shRNA#3) cells were transfected with pEGFP-β2-chimaerin and 48 h later treated with vehicle or PMA (3 μM) in the presence of GF109203X (5 μM). After 30 min., cells were fixed, stained with anti-GS28 cis-Golgi marker and examined by confocal microscopy. Co-localization images and Pearson's correlation coefficient (Rr) were generated by Image J. Similar results were observed in 3 separate experiments. *Bar*, 10 μm.

Figure S4. *PKCε (Asp257/Met278) does not co-localize with p23/Tmp21*. HeLa cells were co-transfected with either pEGFP-PKCε (wt) or pEGFP-PKCε (Asp257/Met278) and pcDNA3.1/V5-p23/Tmp21 (full-length). Forty-eight h later, cells were treated with PMA (1 μM) or vehicle for 30 min, fixed, and visualized by confocal microscopy. *Upper panels*, green fluorescence from GFP-PKCε or GFP-PKCε (Asp257/Met278); *middle panels*, red fluorescence from pcDNA3.1/V5-p23/Tmp21; *lower panels*, overlapped images. *Bar*, 10 μm. Similar results were obtained in 3 independent experiments. Peripheral localization is marked with arrows.

Figure S5. *Videos for translocation experiments in Fig. 5B.* Time-lapse images of translocation of GFP-β2-chimaerin or its mutants in living cells were captured by fluorescence microscopy at different time points after PMA treatment. AVI files (movies) were made using Northern Eclipse software (version 6.0).

Figure S1

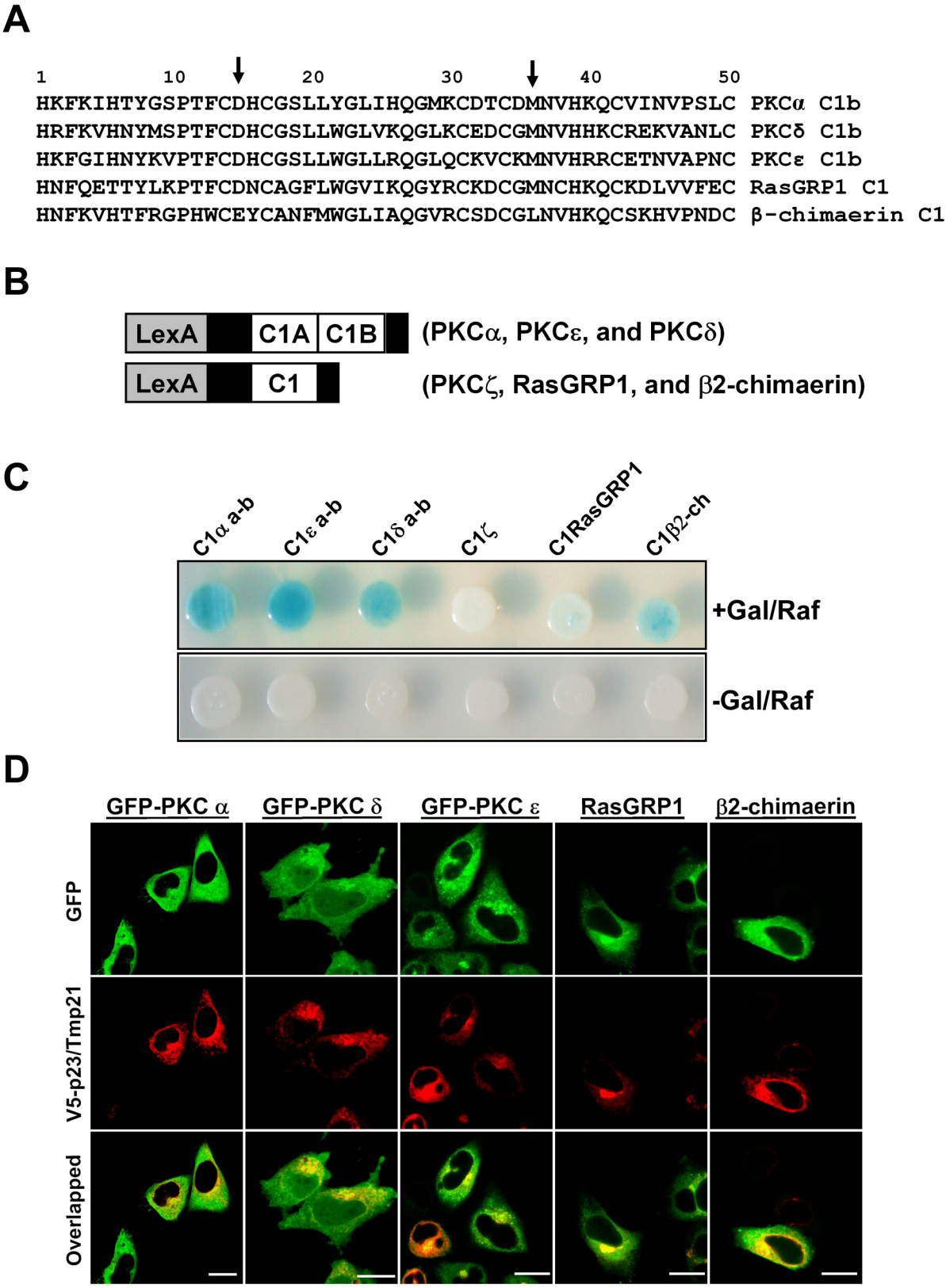

A
1 10 20 30 40 50
HKFKIHTYGSPTFCDHCGSLLYGLIHQGMKCDTCDMNVHKQCVINVPSLC PKCα C1b
HRFKVHNYMSPTFCDHCGSLLWGLVKQGLKCEDCGMNVHHKCREKVANLC PKCδ C1b
HKFGIHNYKVPTFCDHCGSLLWGLLRQGLQCKVCKMNVHRRCETNVAPNC PKCε C1b
HNFQETTYLKPTFCDNCAGFLWGVIKQGYRCKDCGMNCHKQCKDLVVFEC RasGRP1 C1
HNFKVHTFRGPHWCEYCANFMWGLIAQGVRCSDCGLNVHKQCSKHVPNDC β-chimaerin C1
B
LexA
C1A
C1B
(PKCα, PKCε, and PKCδ)
LexA
C1
(PKCζ, RasGRP1, and β2-chimaerin)
C
C1α a-b
C1ε a-b
C1δ a-b
C1ζ
C1RasGRP1
C1β2-ch
+Gal/Raf
-Gal/Raf
D
GFP-PKC α
GFP-PKC δ
GFP-PKC ε
RasGRP1
β2-chimaerin
GFP
V5-p23/Tmp21
Overlapped

Figure S2

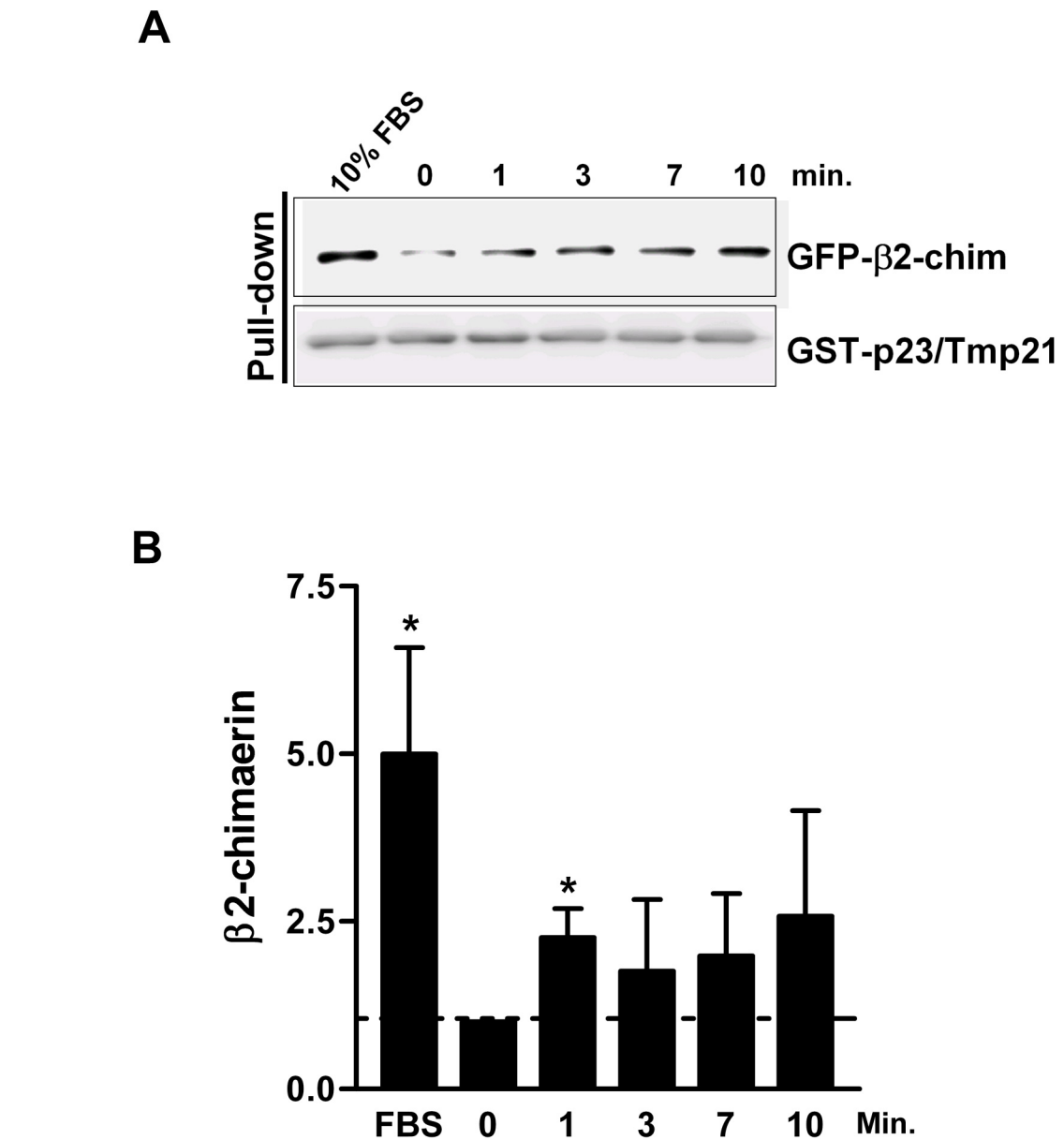

A
10% FBS
0
1
3
7
10
min.
Pull-down
GFP-β2-chim
GST-p23/Tmp21
B
β2-chimaerin
7.5
5.0
2.5
0.0
*
*
FBS
0
1
3
7
10
Min.

Figure S3

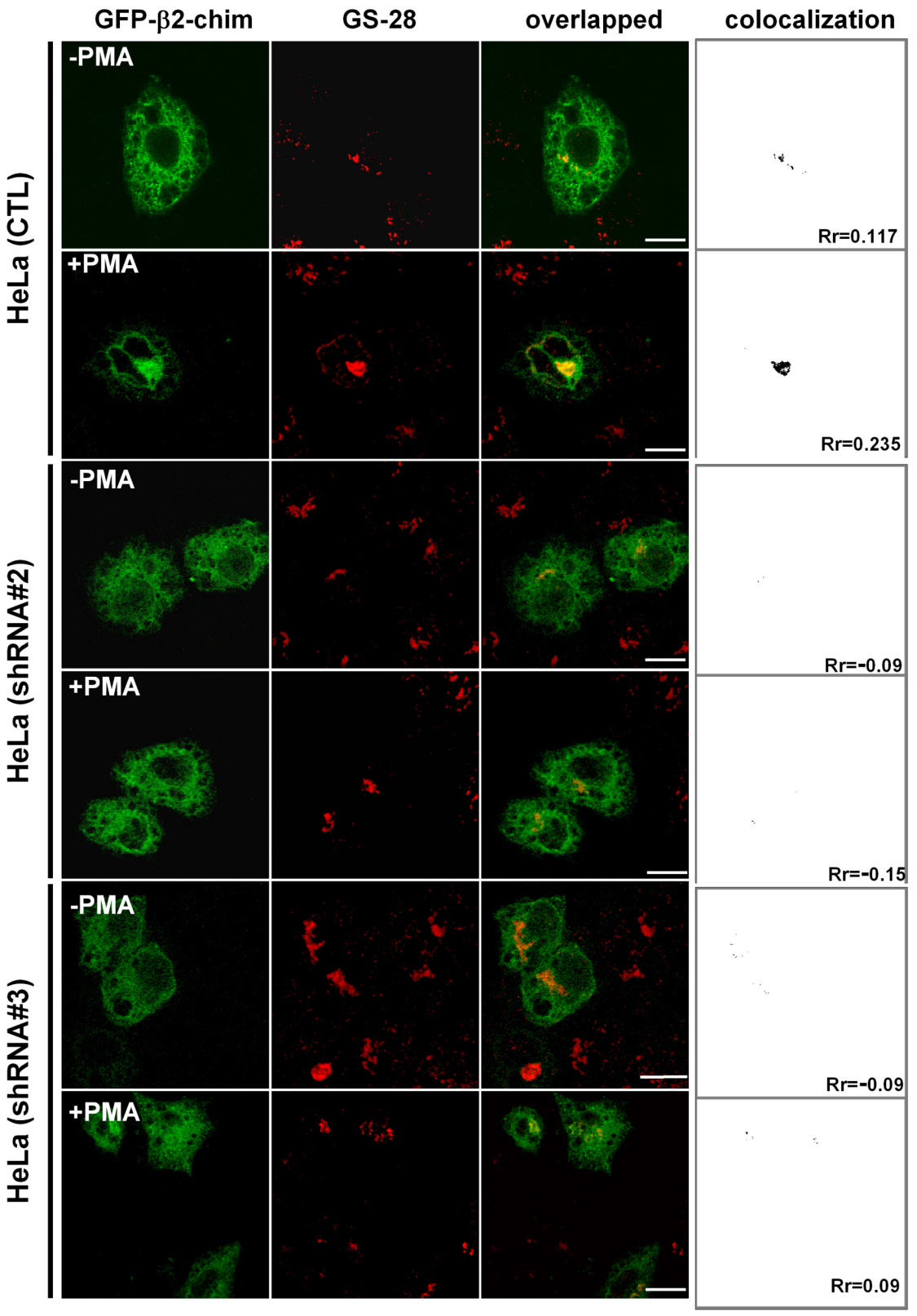
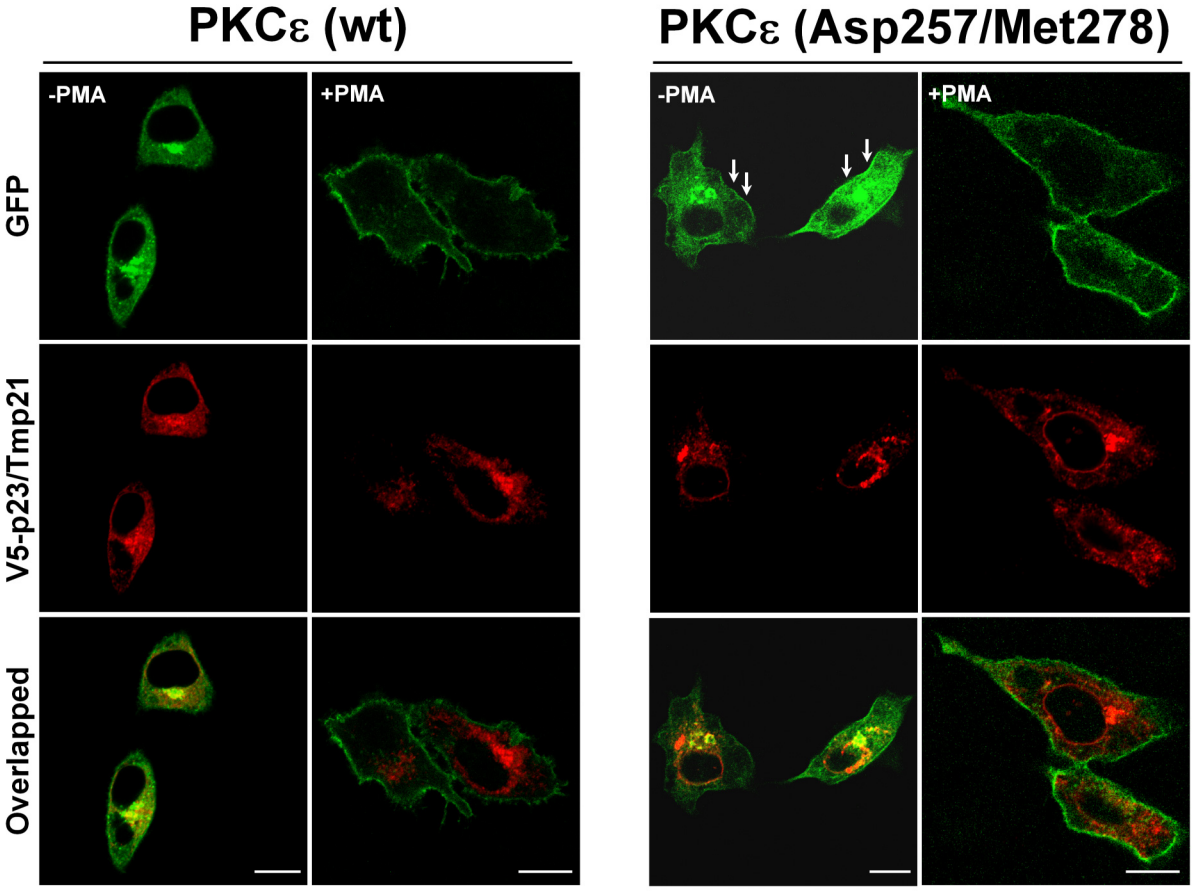

GFP-β2-chim
GS-28
overlapped
colocalization
HeLa (CTL)
-PMA
+PMA
Rr=0.117
Rr=0.235
HeLa (shRNA#2)
-PMA
+PMA
Rr=-0.09
Rr=-0.15
HeLa (shRNA#3)
-PMA
+PMA
Rr=-0.09
Rr=0.09

Figure S4


PKCε (wt)
PKCε (Asp257/Met278)
-PMA
+PMA
-PMA
+PMA
GFP
V5-p23/Tmp21
Overlapped