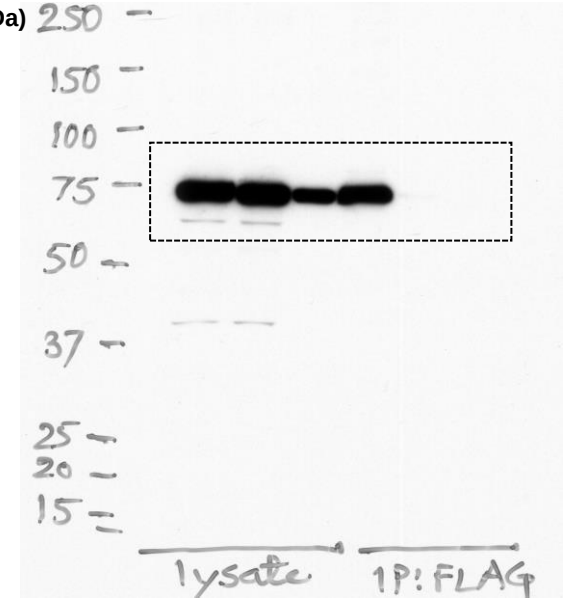


Figure 2B Upper
Myc-myoclonin1

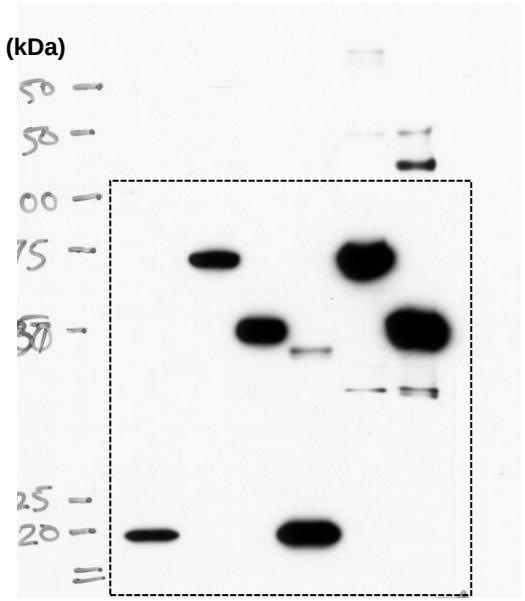


Figure 2B Lower
FLAG-IP$_3$R1/C, /N and Endophilin

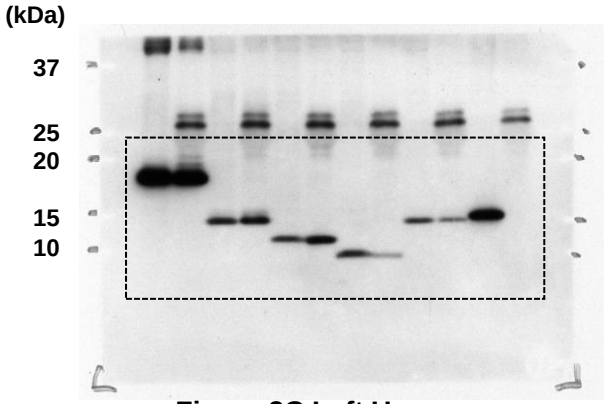


Figure 2C Left Upper
Myc-IP$_3$R1 deletion forms

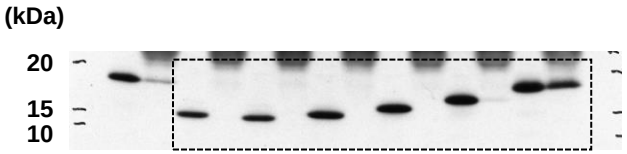


Figure 2C Right Upper
Myc-IP$_3$R1 deletion forms

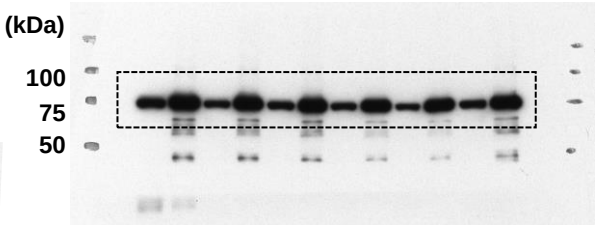


Figure 2C Left Lower
FLAG-myoclonin1

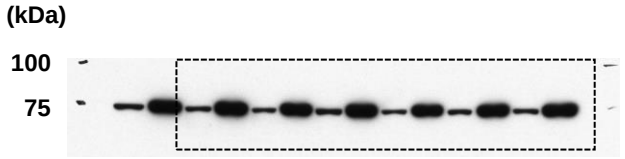


Figure 2C Right Lower
FLAG-myoclonin1

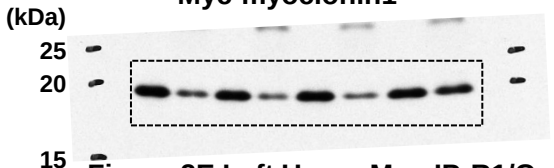


Figure 2E Left Upper Myc-IP$_3$R1/C

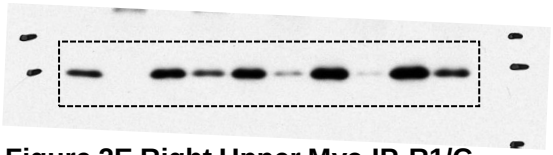


Figure 2E Right Upper Myc-IP$_3$R1/C

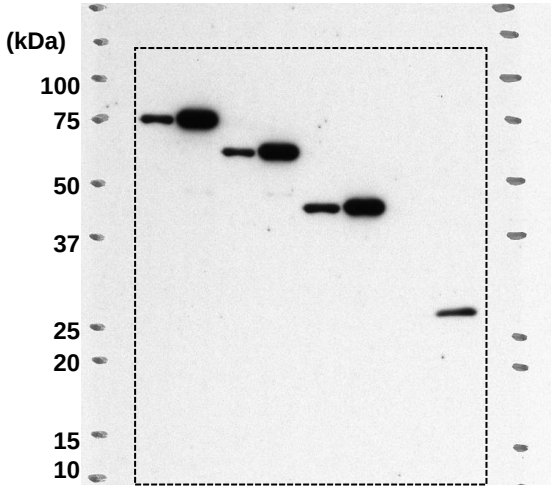


Figure 2E Left Lower
FLAG-myoclonin1 deletion forms

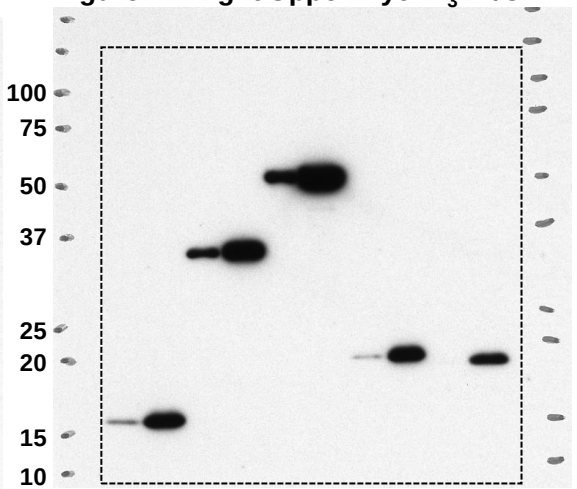


Figure 2E Right Lower
FLAG-myoclonin1 deletion forms

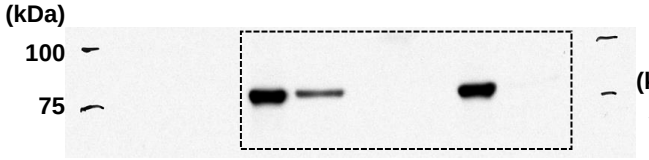


Figure 3B Upper
Myc-PRKSH (32-528)

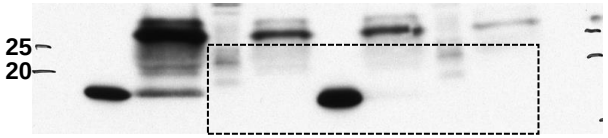


Figure 3B Middle
Myc-PRKSH (314-418)

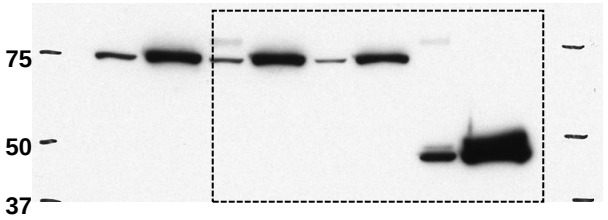


Figure 3B Lower
FLAG-myoclonin1 and Endophilin

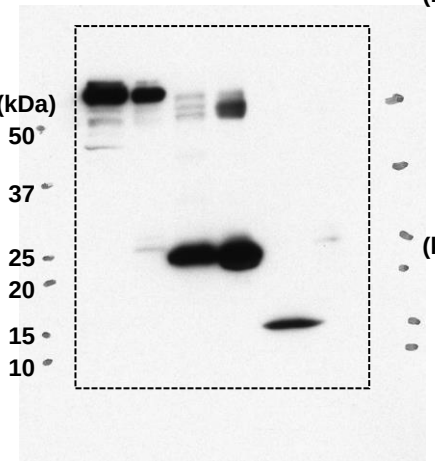


Figure 3C Upper
Myc-PRKSH deletion forms

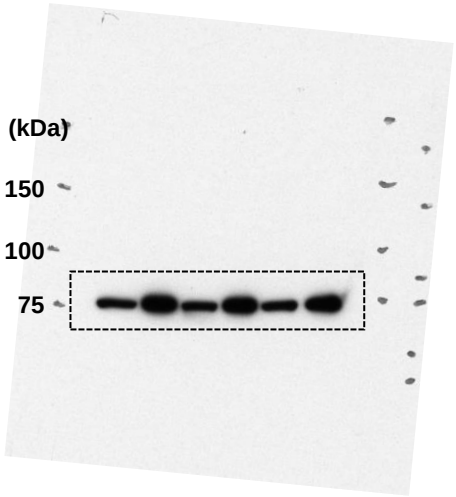


Figure 3C Lower
FLAG-myoclonin1

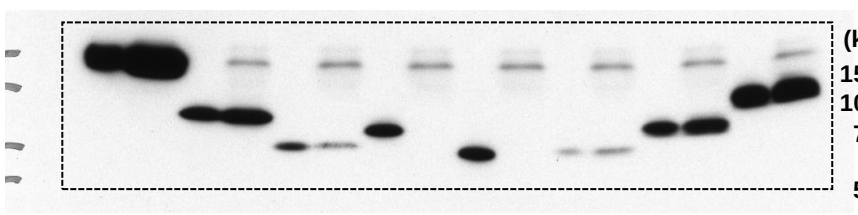


Figure 3D Upper
Myc-PRKSH deletion forms

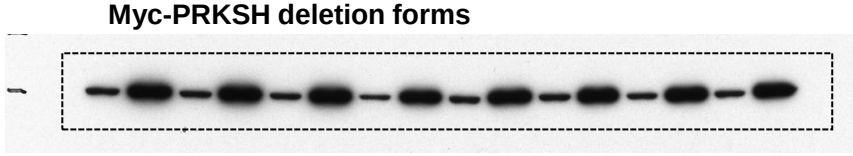


Figure 3D Lower
FLAG-myoclonin1

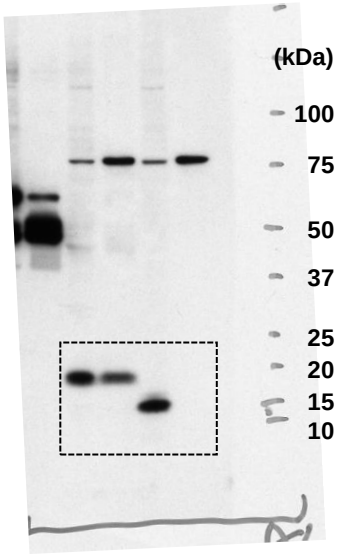


Figure 3E Upper
Myc-PRKSH deletion forms

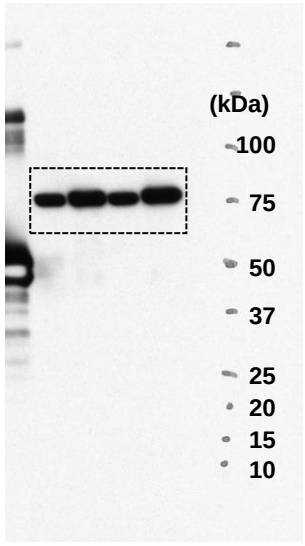


Figure 3E Lower
FLAG-myoclonin1

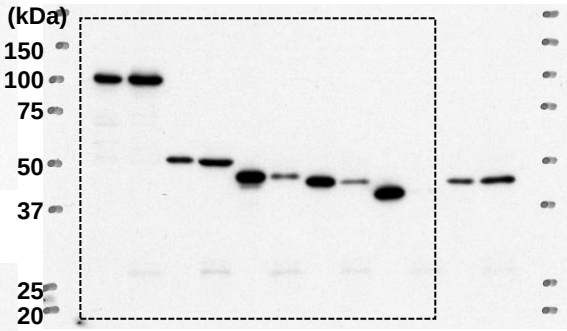


Figure 3G Left Upper
mRFP-myoclonin1 deletion forms

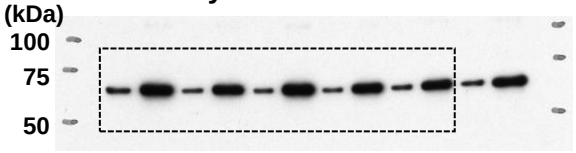


Figure 3G Left Lower
FLAG-PRKSH

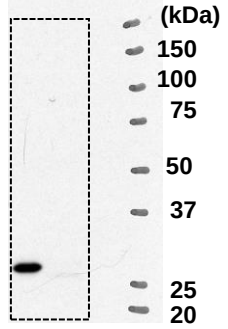


Figure 3G Right Upper
mRFP

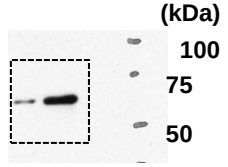
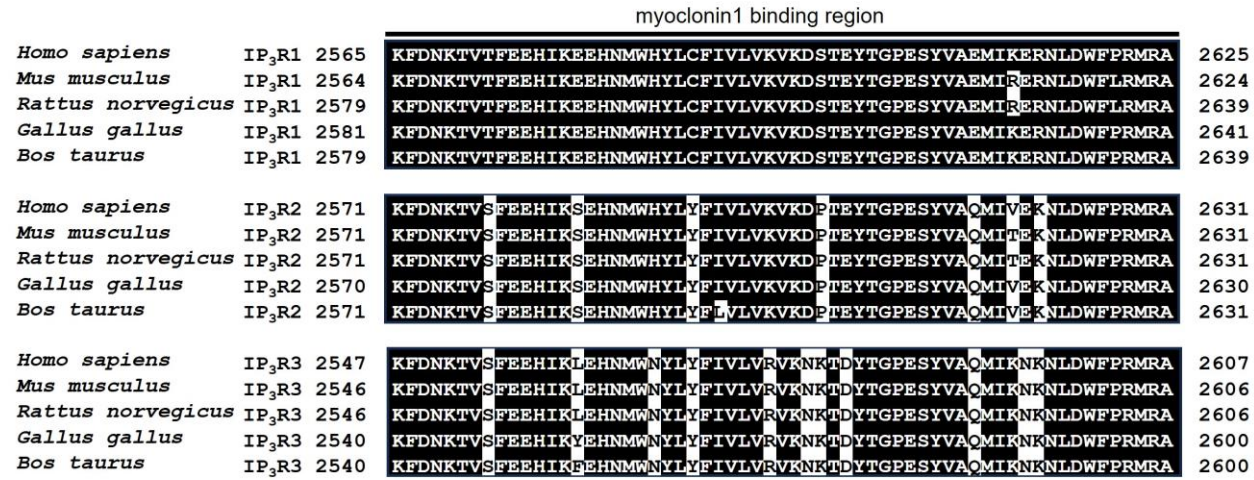


Figure 3G Right Lower
FLAG-PRKSH

Figure S1. Full size western blot images. Original scanned western blot data that were used to generate Figure 2 and 3. Dashed rectangles in the images indicate the locations of the cropped images.

A



B

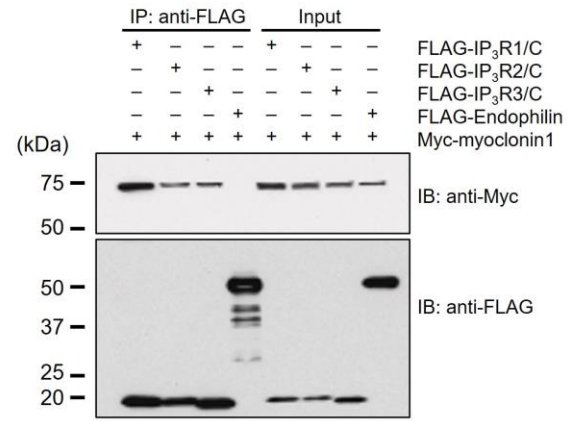


Figure S2. Myoclonin1 interacts with C–termini of all three IP_3R subtypes. (**A**) Binding regions of all three IP_3R subtypes (IP_3R1, 2, 3) to myoclonin1 are highly conserved. (**B**) IP_3R2 and IP_3R3 C-termini also interacted with myoclonin1 but not with Endophilin (negative control). IP, immunoprecipitation; IB, immunoblot; Input, 5% of cell lysate; /C, C–terminal.

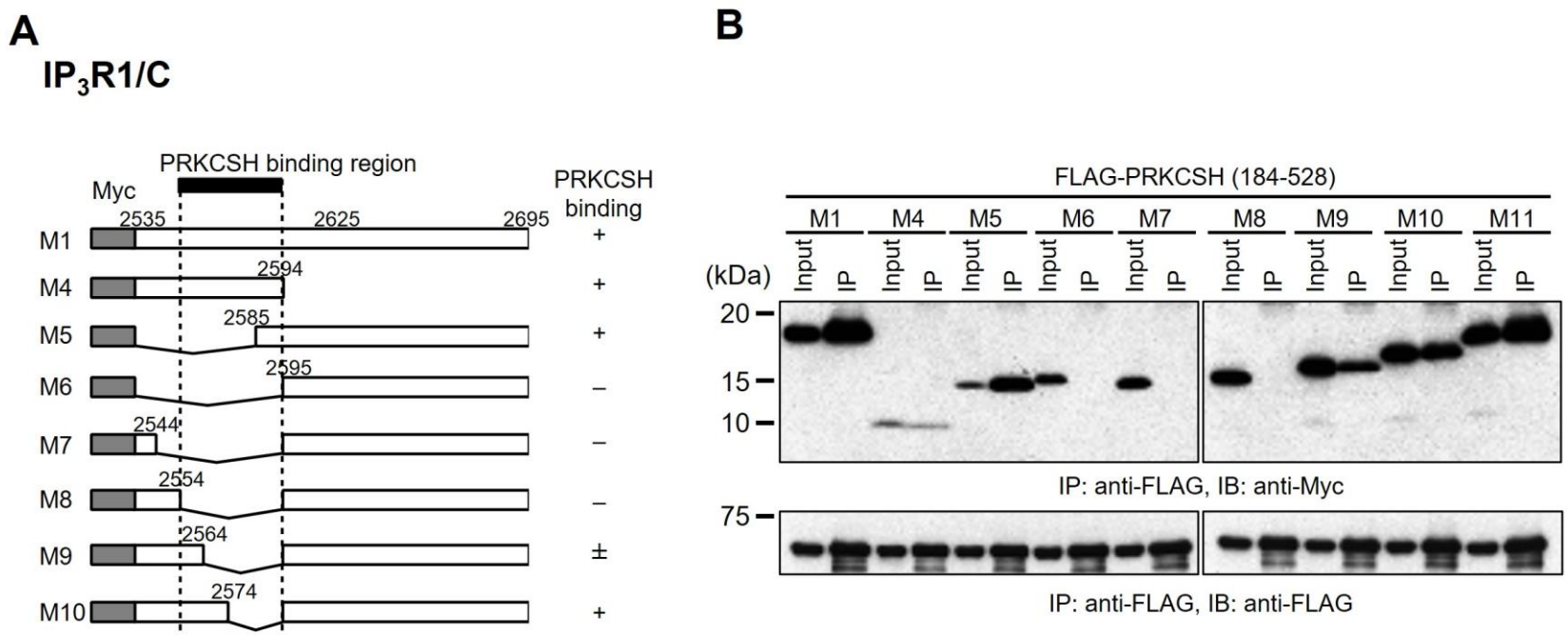


Figure S3. PRKCSH interacts with IP_3R1 C-terminus at its interaction site for myoclonin1. (**A**) Schematic diagram of C–terminal IP_3R1 deletion constructs and their binding activities to PRKCSH. A bold black bar (top) indicates a binding site to PRKCSH. Degrees of interaction are indicated by +, ± or –. (**B**) Western blots of co-IP showing that PRKCSH bound to a.a. 2555–2594 of C–terminal IP_3R1. IP, immunoprecipitation; IB, immunoblot; Input, 5% of cell lysate; /C, C–terminal.

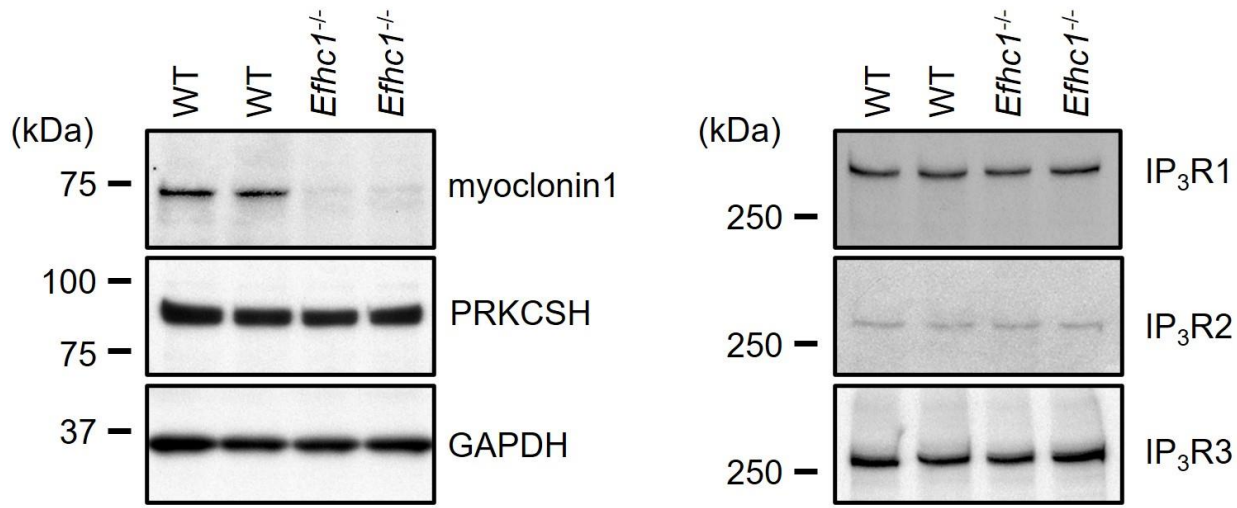


Figure S4. Expressions of IP_3Rs and PRKCSH do not change in *Efhc1*$^{-/-}$ MEFs. Western blot analyses revealed that myoclonin1 expression was abrogated in *Efhc1*$^{-/-}$ MEFs, whereas expressions of endogenous IP_3Rs and PRKCSH do not change (n=2 independent embryos per genotypes). An antibody to GAPDH was used as a control.

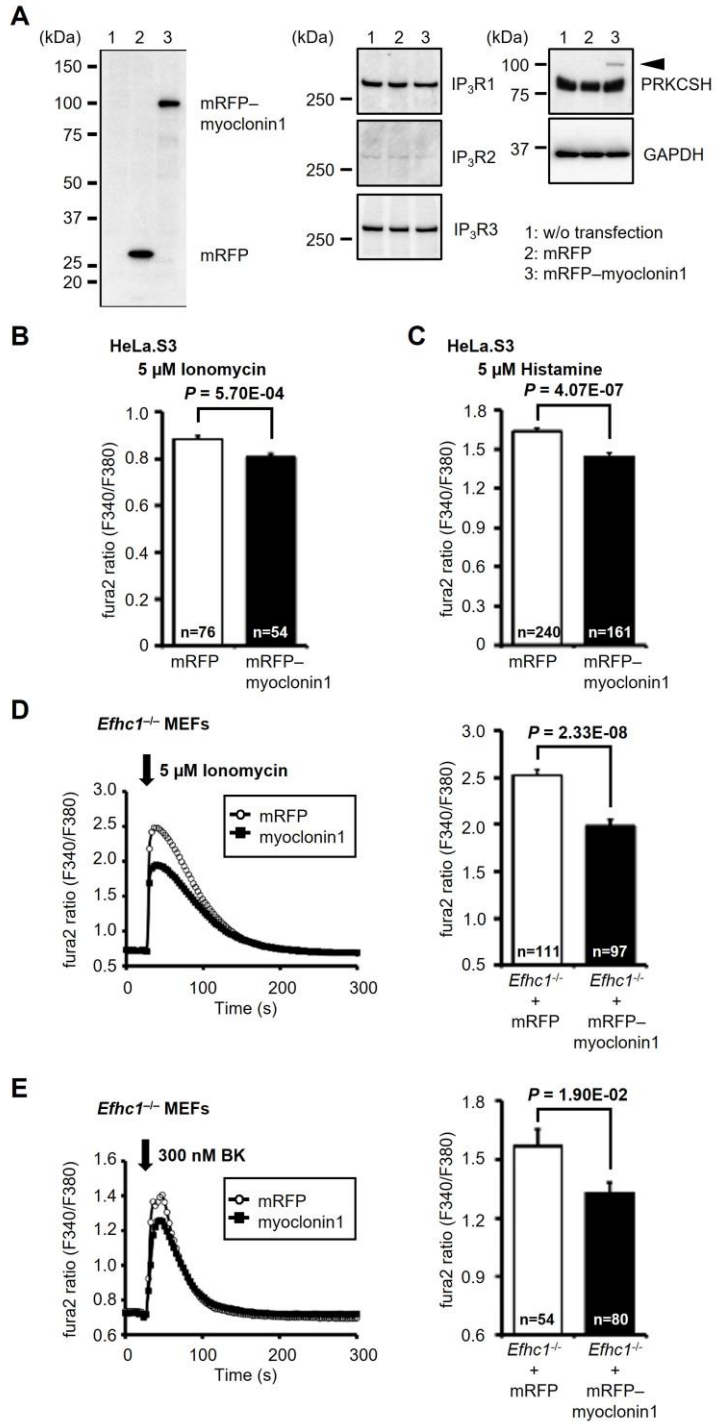

A
(kDa)
1 2 3
150
100
75
50
37
25
20
mRFP–
myoclonin1
mRFP
(kDa)
1 2 3
250
250
250
IP3R1
IP3R2
IP3R3
(kDa)
1 2 3
100
75
37
PRKCSH
GAPDH
1: w/o transfection
2: mRFP
3: mRFP–myoclonin1
B
HeLa.S3
5 μM Ionomycin
P = 5.70E-04
fura2 ratio (F340/F380)
n=76
n=54
mRFP
mRFP–
myoclonin1
C
HeLa.S3
5 μM Histamine
P = 4.07E-07
fura2 ratio (F340/F380)
n=240
n=161
mRFP
mRFP–
myoclonin1
D
Efhc1−/− MEFs
5 μM Ionomycin
mRFP
myoclonin1
fura2 ratio (F340/F380)
Time (s)
P = 2.33E-08
fura2 ratio (F340/F380)
n=111
n=97
Efhc1−/− + mRFP
Efhc1−/− + mRFP–myoclonin1
E
Efhc1−/− MEFs
300 nM BK
mRFP
myoclonin1
fura2 ratio (F340/F380)
Time (s)
P = 1.90E-02
fura2 ratio (F340/F380)
n=54
n=80
Efhc1−/− + mRFP
Efhc1−/− + mRFP–myoclonin1

Figure S5. Myoclonin1 reduces $[Ca^{2+}]_{ER}$ and IICR. (**A**) Western blot analyses [n=1 without (w/o) transfection, 1 mRFP, 1 mRFP–myoclonin1] revealed that exogenous of mRFP or mRFP–myoclonin1 proteins were detected at expected size (~30 kDa and ~100 kDa, respectively). Expression levels of endogenous IP_3Rs and PRKCSH were not affected by over–expression of myoclonin1 in the cells. An mRFP–myoclonin1 band at ~100 kDa remained weakly in blot for PRKCSH (arrowhead) even after stripping of blot. An antibody to GAPDH was used as a control. (**B, C**) Ionomycin releasable $[Ca^{2+}]_{ER}$ (B; n=76 mRFP, 54 mRFP–myoclonin1 expressing cells) and histamine evoked IICR (C; n=240 mRFP, 161 mRFP–myoclonin1 expressing cells) were significantly lower in mRFP–myoclonin1 expressing HeLa.S3 cells than mRFP expressing one. Ionomycin releasable $[Ca^{2+}]_{ER}$ (**D**, n=111 mRFP, 97 mRFP–myoclonin1) and bradykinin (BK) evoked IICR (**E**, n=54 mRFP, 80 mRFP–myoclonin1) were significantly lower in mRFP–myoclonin1 expressing *Efhc1*$^{-/-}$ MEFs than mRFP expressing one. Arrows indicate the time point of addition of ionomycin or BK. n, total number of cells measured.