Supplemental Materials

Molecular Biology of the Cell

Xu et al.

Supplementary information

Figure S1. Consistency of mRNA and protein expression levels in both Raw264.7 and HL60 cells. A. mRNA levels of PKD1-3 in both Raw264.7 and HL60 cells. **B.** Protein expression profile of PKD1-3 in both Raw264.7 and HL60 cells.

Figure S2. PKD inhibitors impair chemotaxis of HL60 cells. Montage shows images of EZ-TaxiScan chemotaxis assay to examine the inhibitory effects of PKD-specific inhibitors on chemotaxis at times of 0 and 10 min after applying the gradient. Chemotactic HL60 cells were pre-treated with two PKD inhibitors of 1μM CID755673 or 100 nM Gö 6976 for 30 min. HL60 cells in the presence or absence of fMLP gradient with or without inhibitors were allowed to chemotax in 100 nM fMLP gradients for 10 min.

Figure S3. PKD and PKC inhibitors do not abolish calcium response upon 1 μM fMLP stimulation in HL60 cells. A. Montage showing the calcium response of HL60 cells before (-fMLP) or after (+fMLP) fMLP stimulation while the maximum calcium response was monitored by the intensity increase of green fluorescent calcium indicator fluo-4 upon 1 μM fMLP stimulation (**Figure S3 Supplemental Movies 1-6)**. Chemotactic HL60 cells were pretreated with the indicated inhibitors of Gαi (PTX), PLC (U73122), PKC (Gö 6983) of PKD (Gö 6976) or CID755673) at indicated concentration for 30 min and then subjected to calcium response experiments. 1 μg/mL Fluo4 (Molecular Probe) was used to stain the cells for 30 min prior to the experiment. Intensity of Fluo4 was measured and normalized as 1 right before the addition of fMLP at time 0. The quantitative measurements of calcium response under different conditions are presented in **B**.

Figure S4. mRNA of PKD family members and expression of receptor CXCR4 in both CTL and *pkd*^{kd} Raw264.7 cells. A. Quantification of PKD1-3 mRNA levels in both CTL and *pkd*^{kd} Raw264.7 cells. **B.** A normal level expression of receptor CXCR4 in both CTL and *pkd*^{kd} cells.

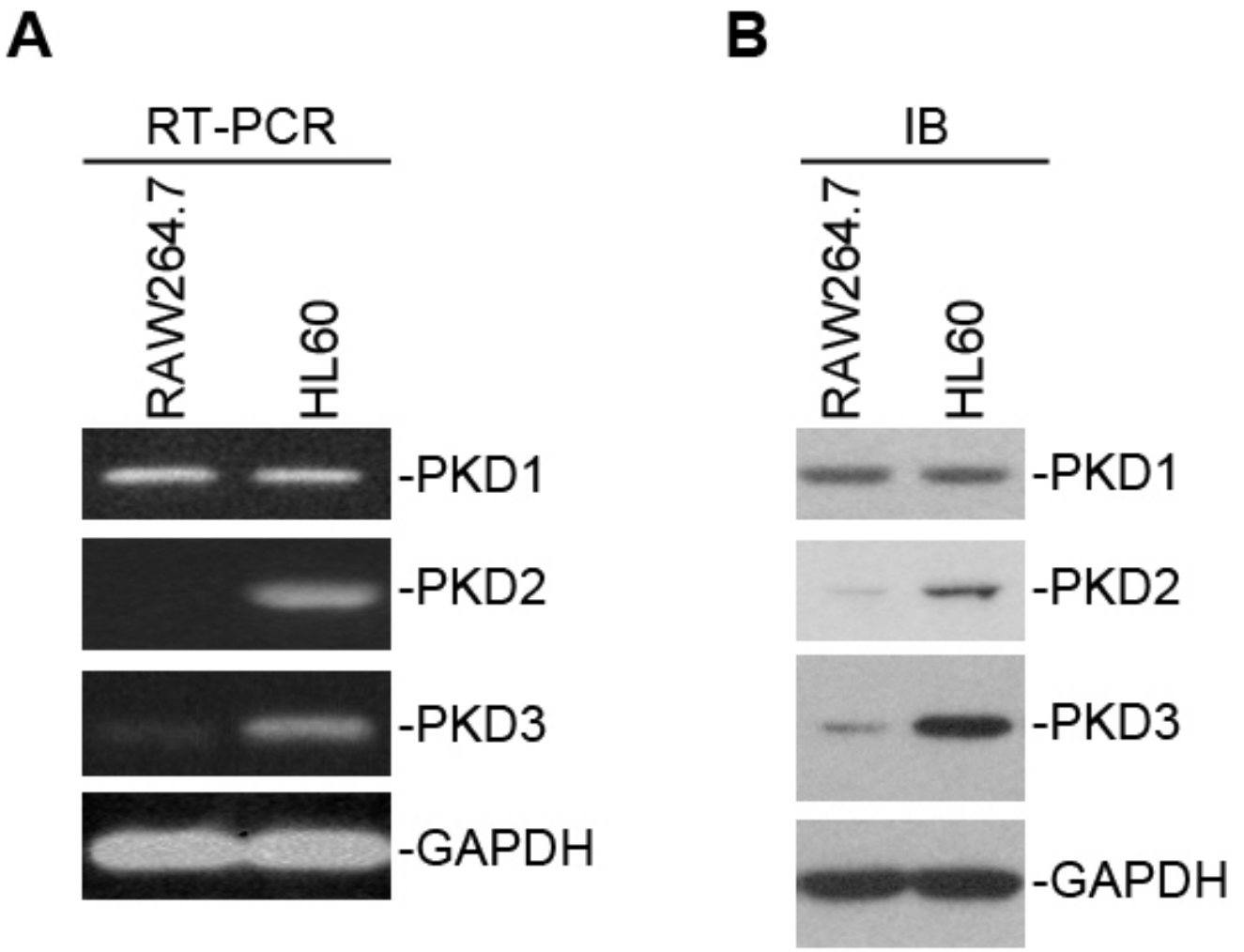
Figure S5. Identification of SDF-1α concentration for a robust transwell chemotaxis assay. A. Chemotaxis capability of Raw 264.7 cells in response to a dilution series of SDF1α was examined by transwell chemotaxis assay.

Figure S6. Dynamic membrane translocation of PKD family member upon SDF1α stimulation. A. Membrane translocation of PKD members (Green) upon uniformly applied 100 ng/mL SDF-1α (Red) stimulation (**Figure S6 Supplemental Movies 1-4)**. In **A.** and **C.**, Alexa 594 (Red) was mixed with 500nM or 100nM SDF-1α, respectively, to visualize homogeneously applied uniform stimulation or gradient stimulation of SDF-1α. Scale bar = 10 μm. Normalized membrane translocation of PKD isoforms in response to uniform stimulation of fMLP was normalized and presented in **B**. **C**. Montage shows the leading edge localization of PKD members in 100 ng/mL SDF-1α gradient (**Figure S6 Supplemental Movies 5-8)**.

Figure S7. PLC inhibitor impairs chemotaxis. A. Montage shows the images of EZ-TaxiScan chemotaxis assay at times of 0 and 10 min after applying the gradient to HL60 cells. **B**. PLC inhibitor (U73122) also inhibits Raw 264.7 cell chemotaxis. HL60 and Raw 264.7 cells were allowed to chemotax in indicated chemoattractant gradients for 30 min or 90 min in EX-TaxiScan or transwell chemotaxis assays, respectively.

Figure S8. Membrane translocation of PKCβI and PKCβII upon fMLP stimulation. A. Montage showing the membrane translocation of GFP (Green)-tagged PKCβI and βII upon 1 μM fMLP stimulation (Red) (**Figure S8 Supplemental Movie 1-3)**. Presence of Red (Alex 594 mixed with fMLP) in lower panel indicates uniformly applied fMLP stimulation. Quantitative measurement of membrane translocation of PKCβI and PKCβII is presented in **B.**

Figure S9. Abrogation of PKCβ function impairs chemotaxis of HL60 cells. A. Montage showing the images of EZ-TaxiScan chemotaxis assay at times of 0 and 10 min after gradient is applied to cells expressing endogenous PKCβII or epigenetically expressing PKCβII-CA or PKCβII-DN or cells pre-treated with PKC inhibitor Gö 6983. HL60 cells were pre-treated with the indicated concentration of 100 nM Gö 6983 for 30 min before the experiment and allowed to chemotax in indicated chemoattractant gradients for 30 min.


A
RT-PCR
RAW264.7
HL60
-PKD1
-PKD2
-PKD3
-GAPDH
B
IB
RAW264.7
HL60
-PKD1
-PKD2
-PKD3
-GAPDH

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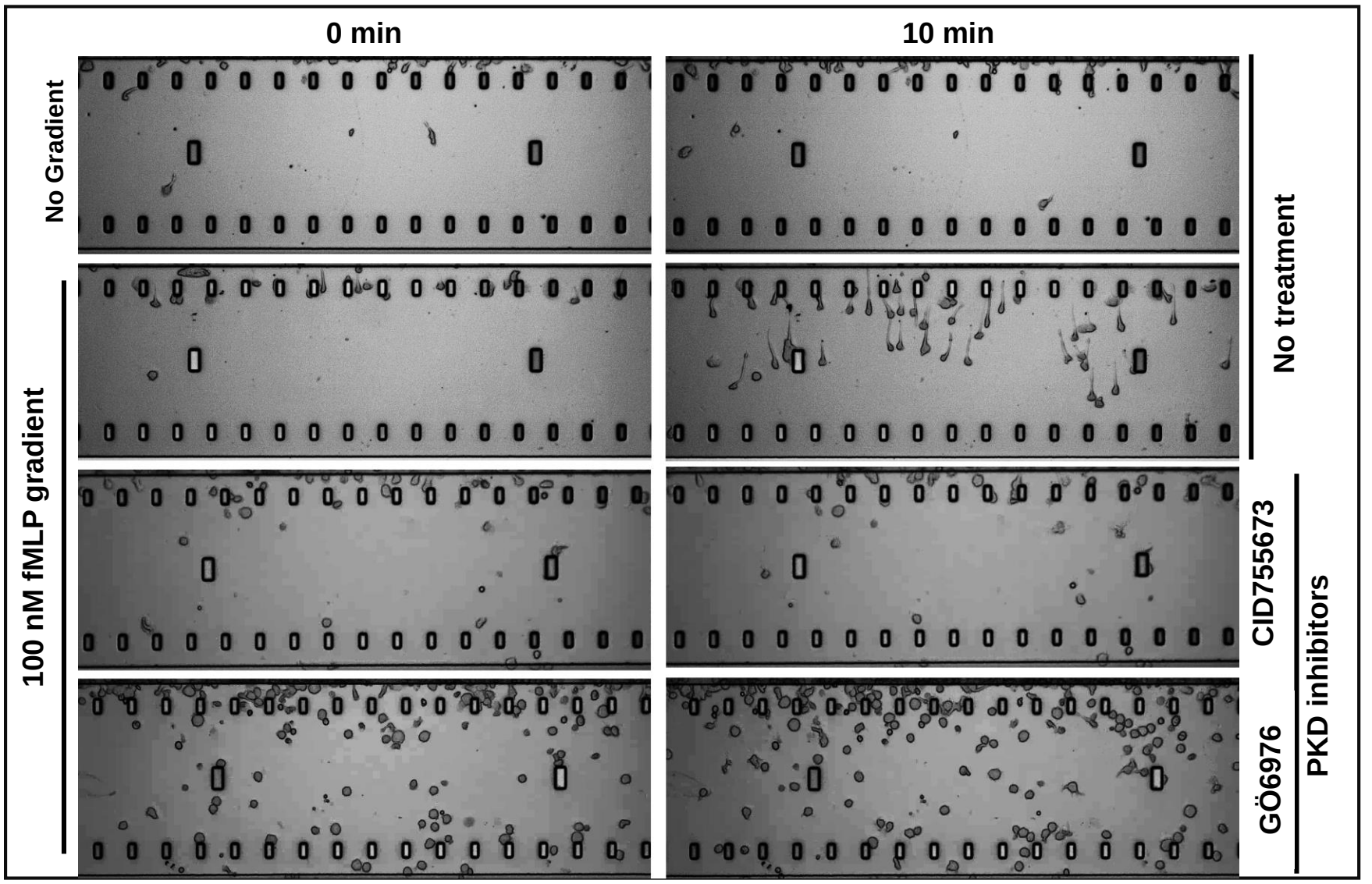

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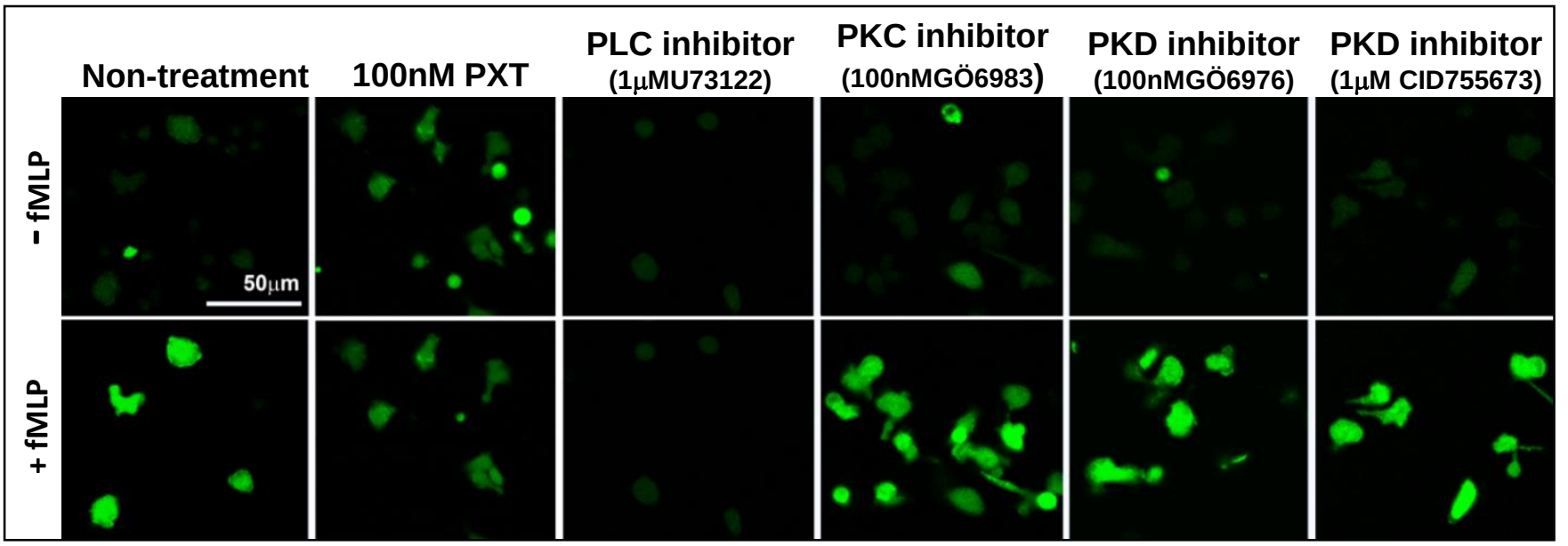
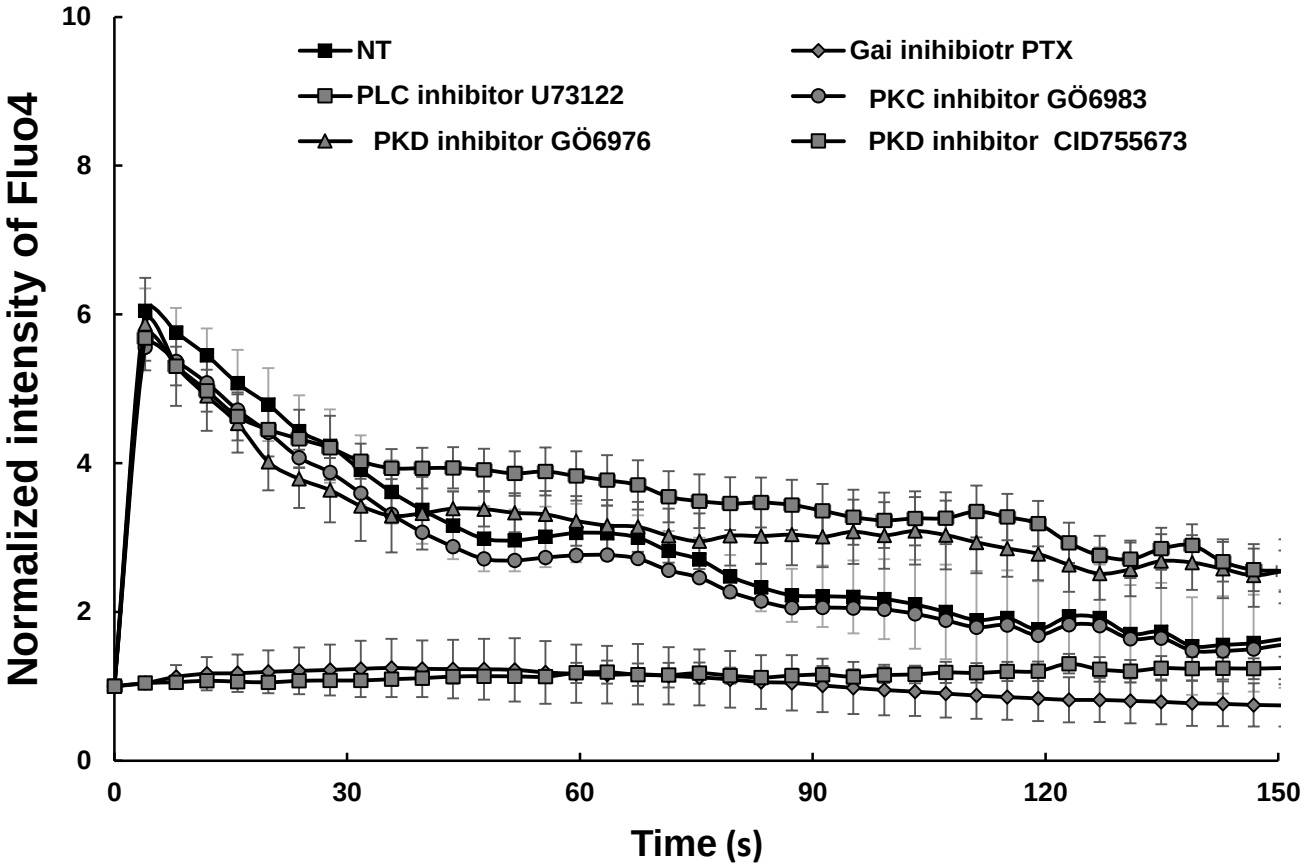
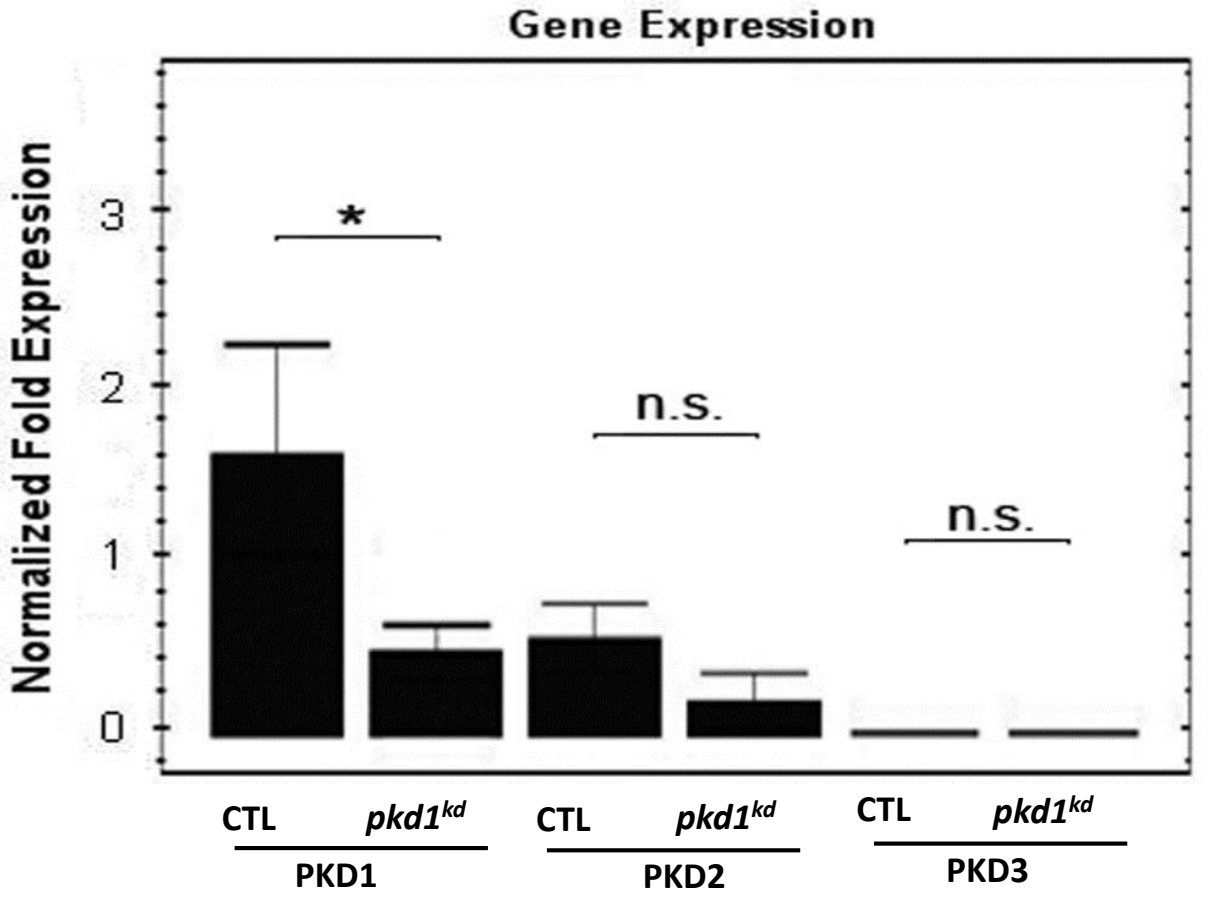
A



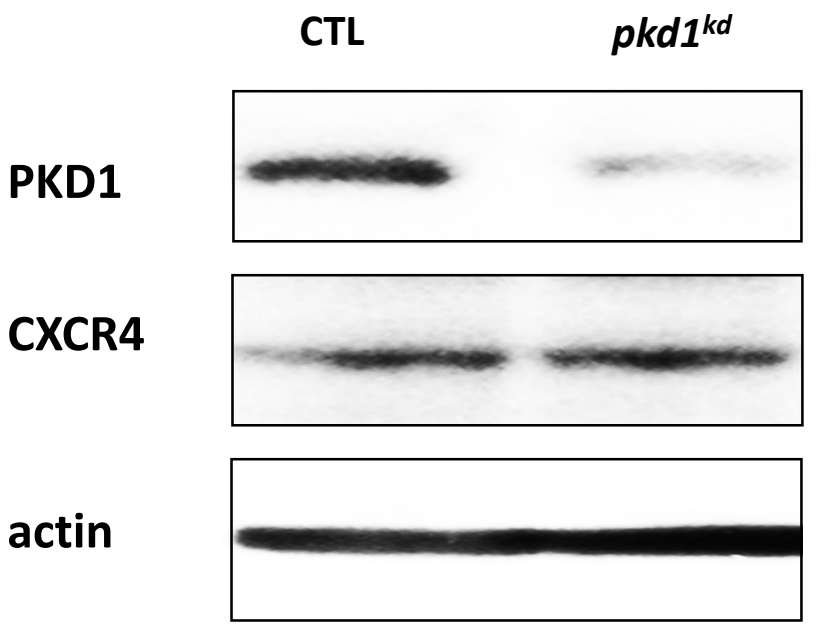
B



A


Gene Expression
Normalized Fold Expression
3
2
1
0
*
n.s.
n.s.
CTL
pkd1kd
CTL
pkd1kd
CTL
pkd1kd
PKD1
PKD2
PKD3

B


CTL
pkd1kd
PKD1
CXCR4
actin

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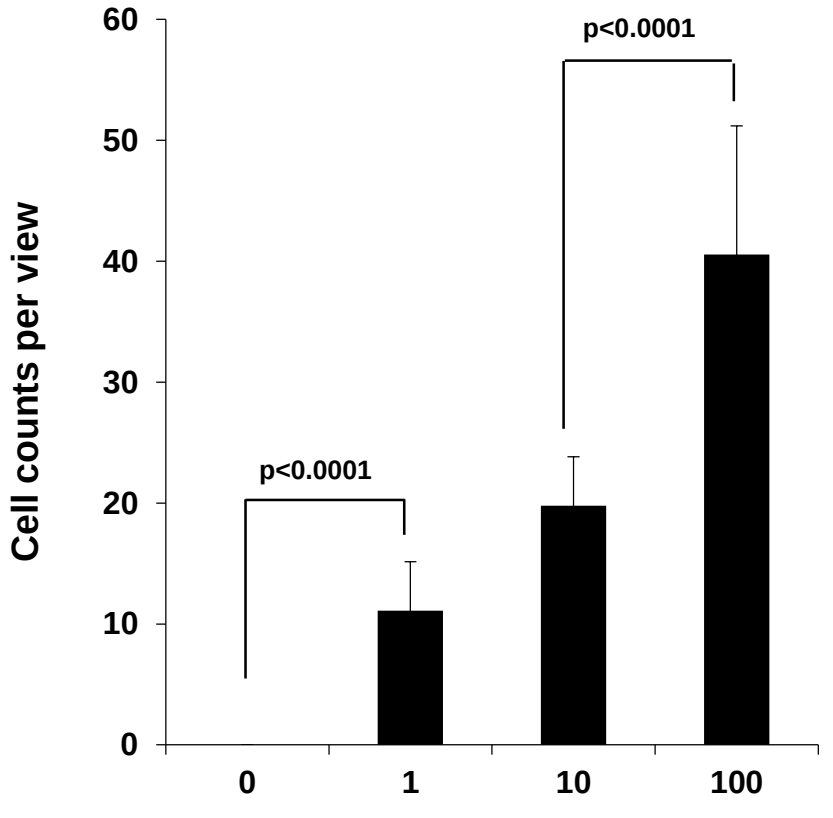
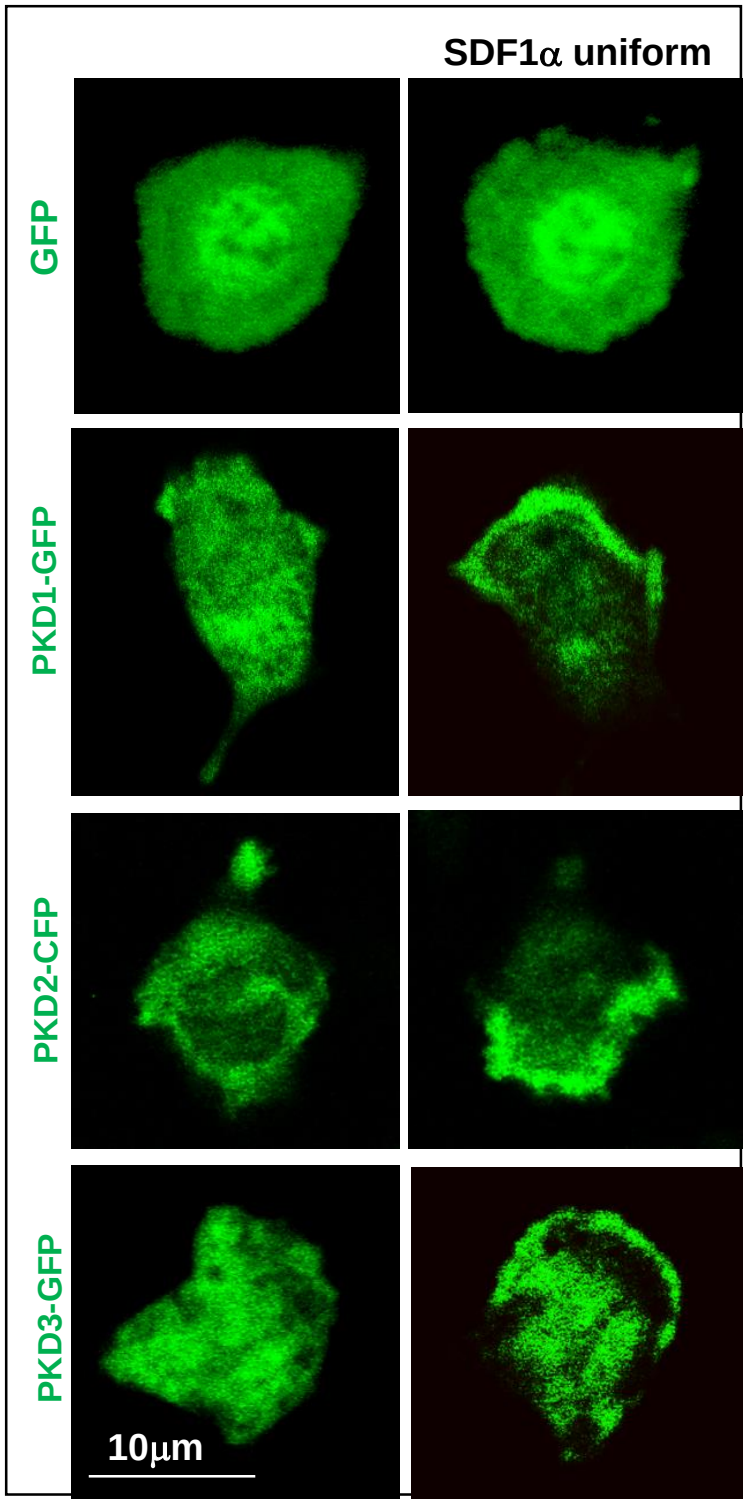
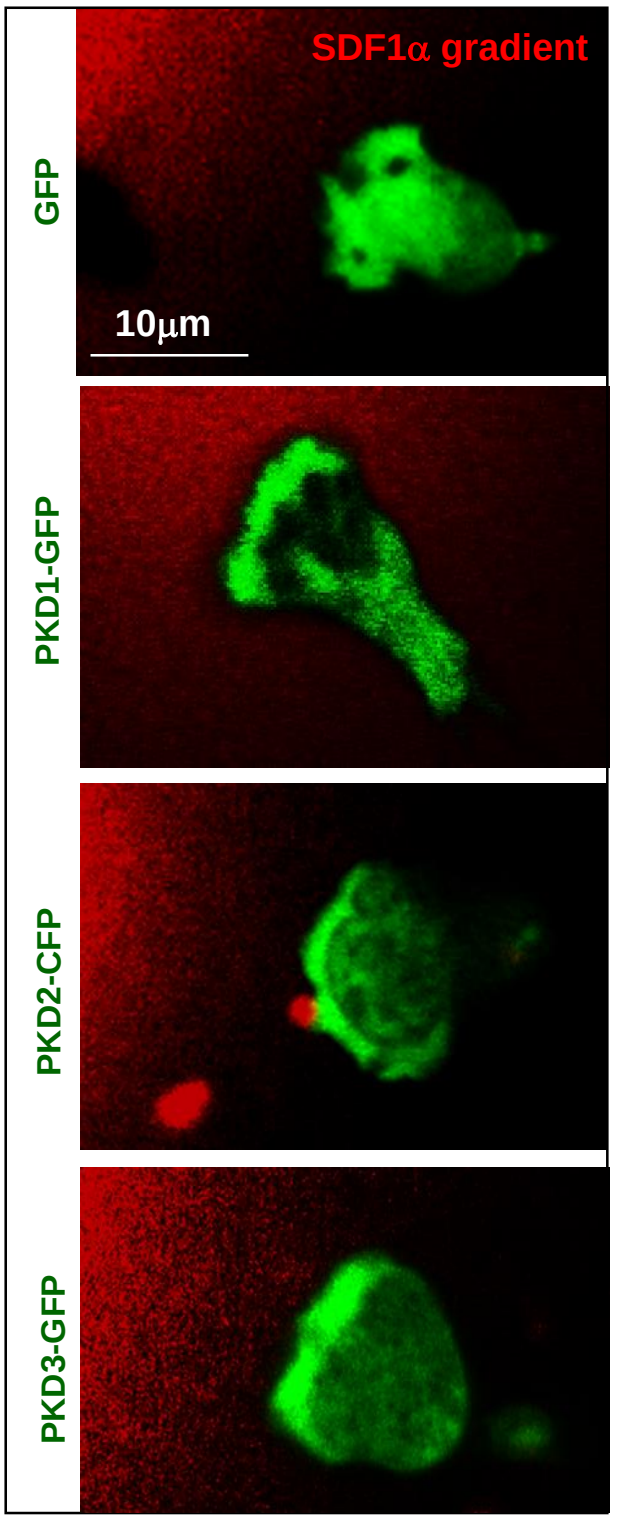


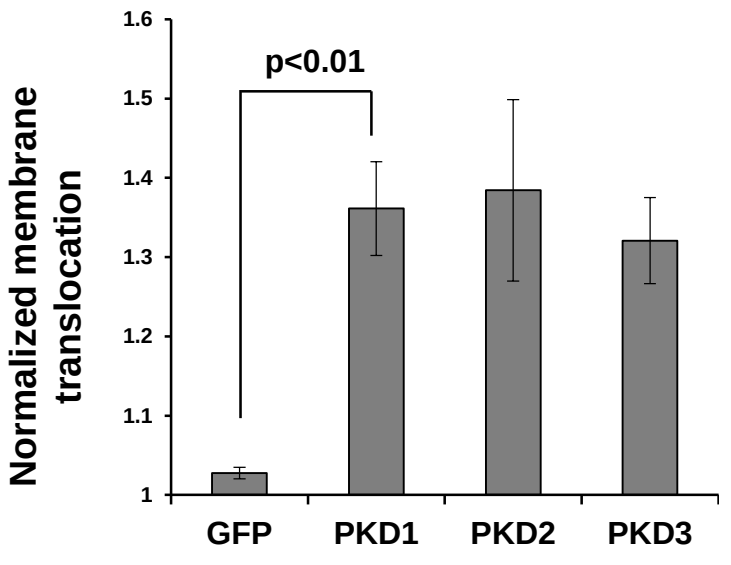
A


SDF1α uniform
GFP
PKD1-GFP
PKD2-CFP
PKD3-GFP
10μm

C


SDF1α gradient
GFP
10μm
PKD1-GFP
PKD2-CFP
PKD3-GFP

B


Normalized membrane translocation
1.6
1.5
1.4
1.3
1.2
1.1
1
p<0.01
GFP
PKD1
PKD2
PKD3

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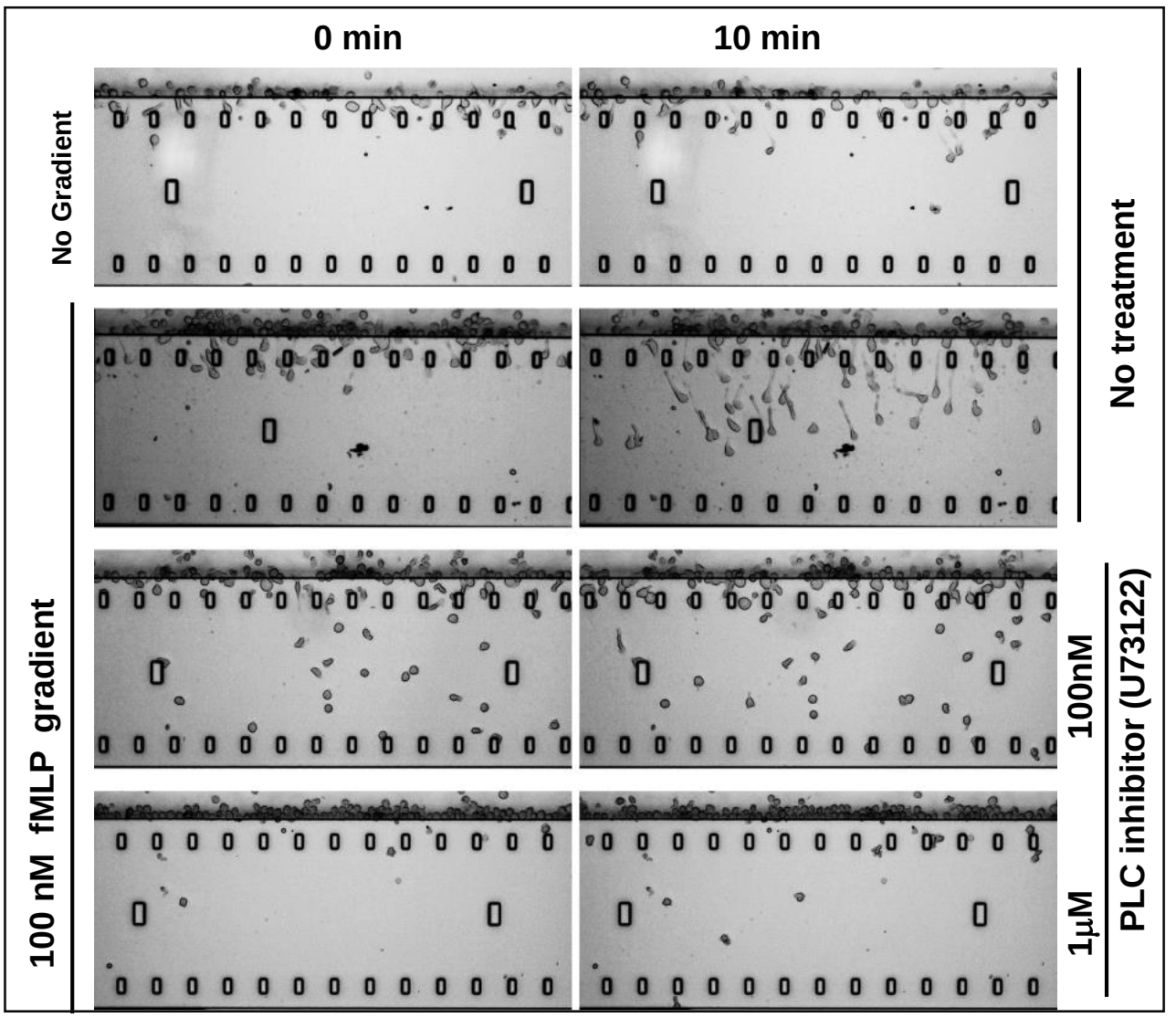
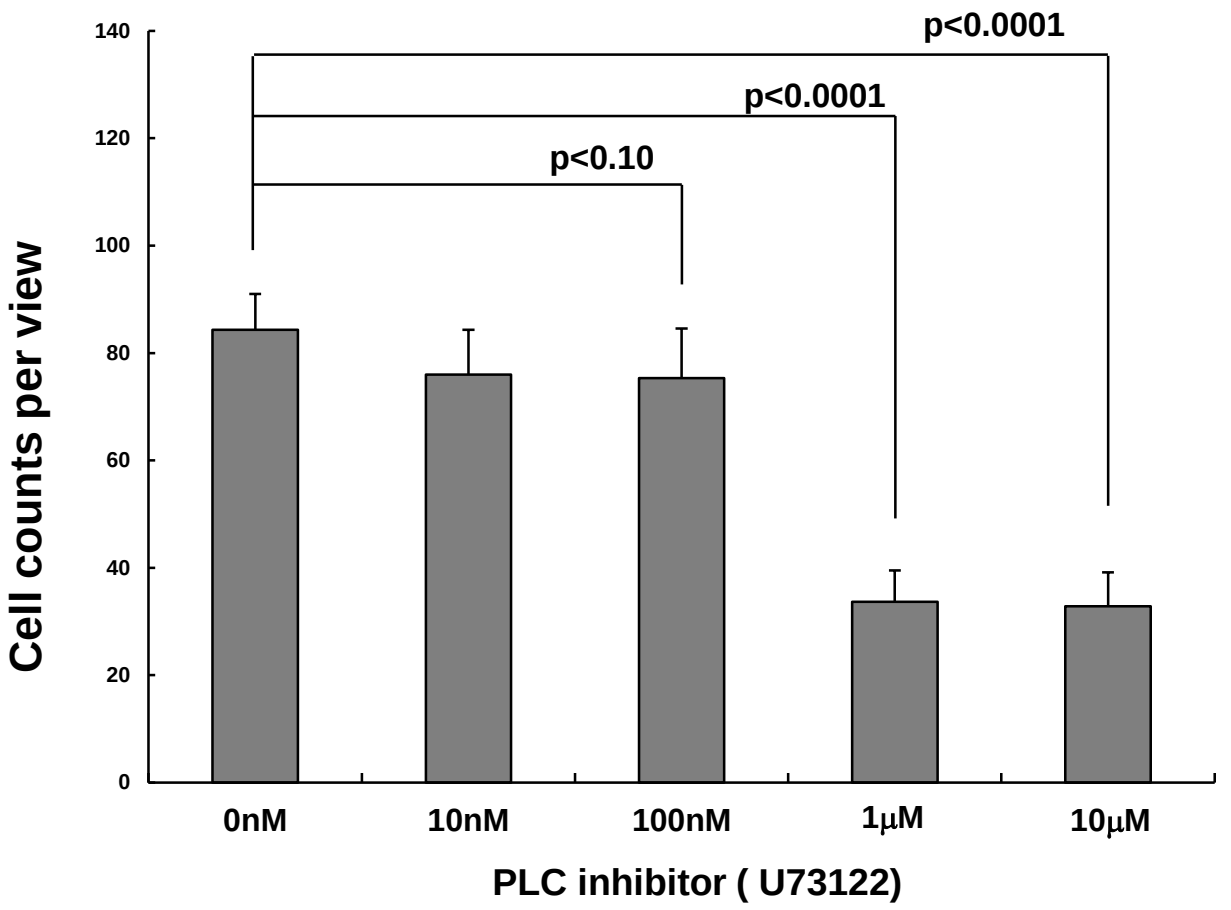
A



B



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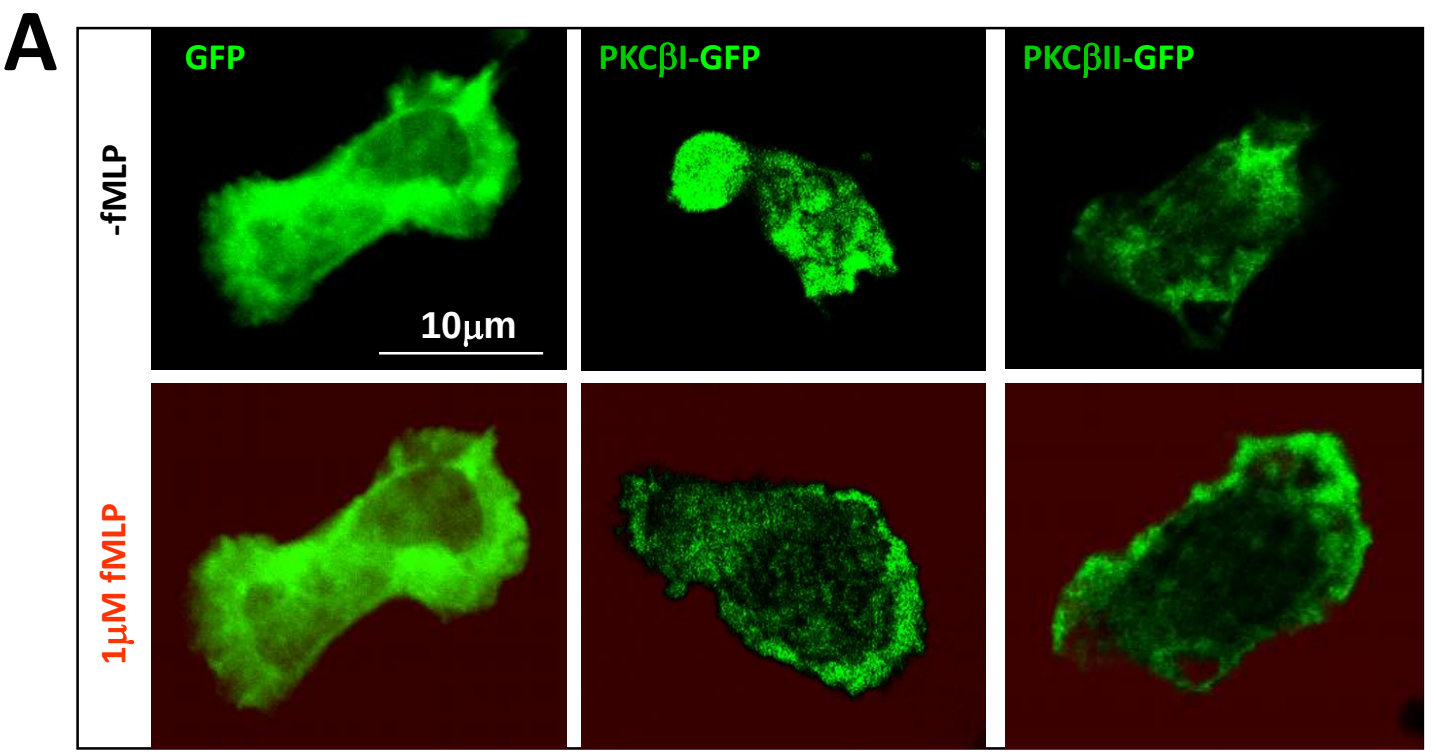
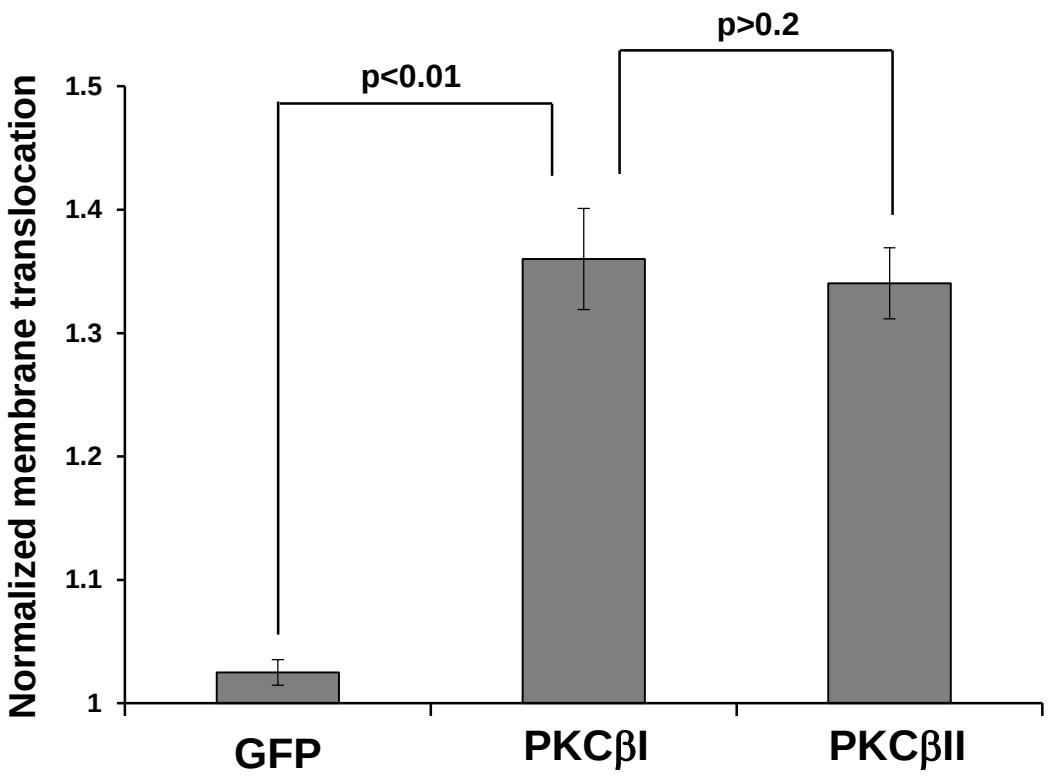
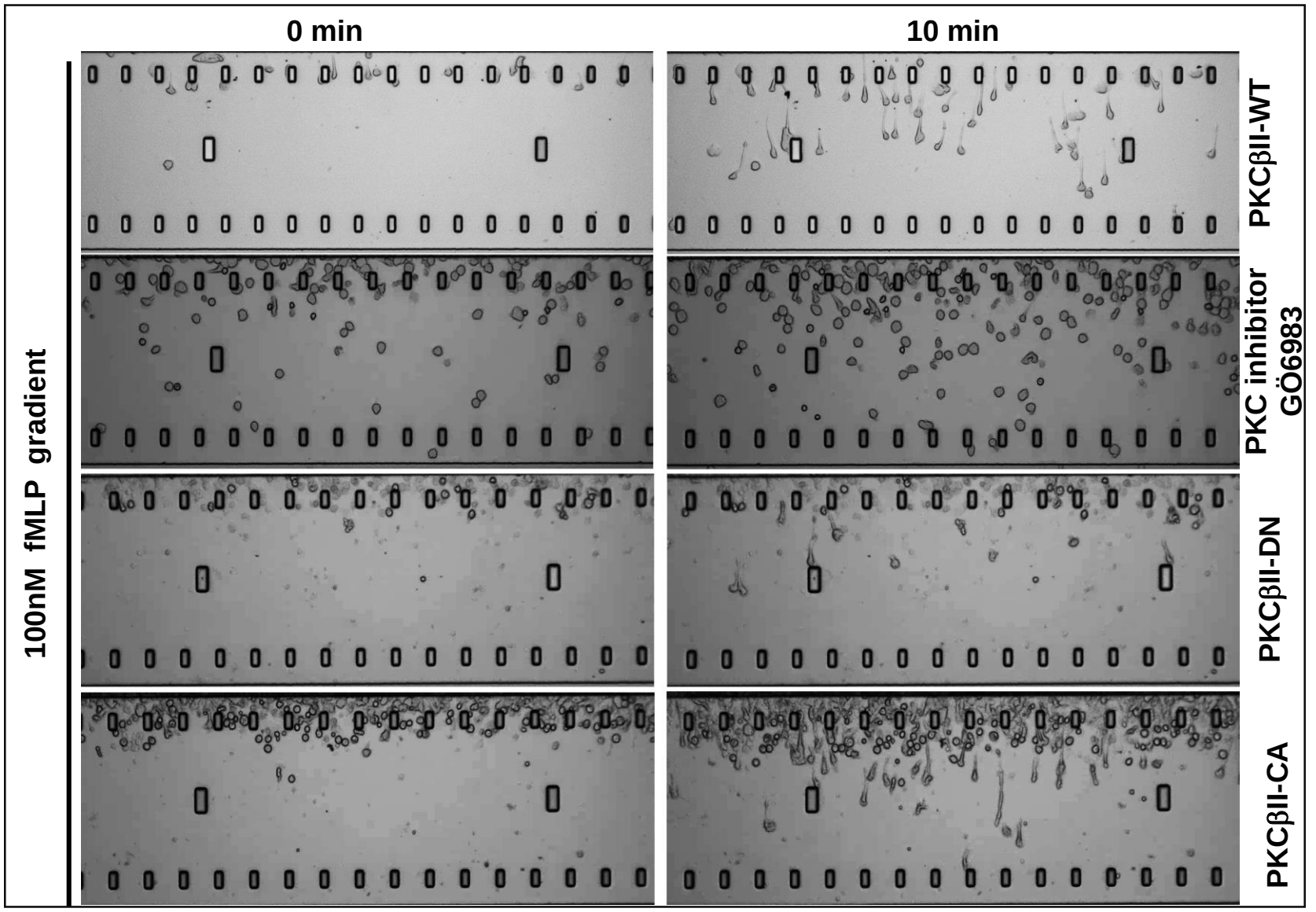




A


0 min
10 min
100nM fMLP gradient
PKCβII-WT
PKC inhibitor GÖ6983
PKCβII-DN
PKCβII-CA