

## Supplemental Material to:

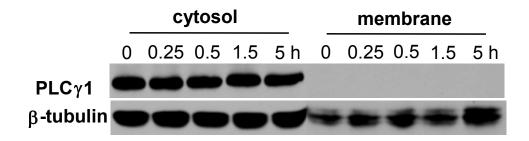
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# Cholesterol regulates HERG K+ channel activation by increasing phospholipase C β1 expression

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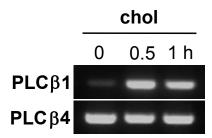
http://www.landesbioscience.com/journals/channels/article/25122/

#### Supplemental Fig. 1



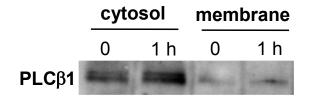
Supplemental Figure 1: The mild cholesterol enrichment did not alter the expression level of PLC $\gamma$ 1. HERG-transfected HEK293 cells were incubated with 25  $\mu$ M M $\beta$ CD-cholesterol for 0.25, 0.5, 1.5, and 5 h. Membrane and cytosol fractions were obtained as described in Materials and Methods, and monoclonal mouse antibody against PLC $\gamma$ 1 was used. Similar results were obtained from 3 different experiments. PLC $\gamma$ 1 expression was not changed by cholesterol in cytosol fraction. PLC $\gamma$ 1 expression was not observed in membrane fraction.

### **Supplemental Fig. 2**



Supplemental Figure 2: The mild cholesterol enrichment increased the expression of PLC $\beta$ 1 mRNA in HERG-transfected HEK293 cells. HERG-transfected HEK293 cells were incubated with 25  $\mu$ M M $\beta$ CD-cholesterol for 0.5 and 1 h, which was followed by isolating RNA as described in Materials and Methods. The expressions of both PLC $\beta$ 1 and PLC $\beta$ 4 mRNA were analyzed by RT-PCR. Similar results were obtained from 3 different experiments. The expression of PLC $\beta$ 1 mRNA, but not that of PLC $\beta$ 4 mRNA, was increased by cholesterol.

#### **Supplemental Fig. 3**



Supplemental Figure 3: The mild cholesterol enrichment increased PLC $\beta$ 1 expressions from myocytes. Adult mouse ventricular myocytes was isolated as described previously (Ref. 21). Cells were incubated with 25  $\mu$ M M $\beta$ CD-cholesterol for 1 h, and membrane and cytosol fractions were obtained as described in Materials and Methods. PLC $\beta$ 1 expression was increased by cholesterol from both cytosol and membrane fractions. Similar results were obtained from 3 different experiments.