Supplemental Material

Michael Holinstat *et al*. PAR signaling in platelets activates $cPLA_2$ differently for COX-1 and 12-LOX catalysis

Legends for Supplementary Figures

Figure I: residual COX-1 activity following platelet activation with PAR-APs. Platelets were activated with PAR1-AP (20 μM), PAR4-AP (200 μM) or vehicle for 1 min. At this time [$^2H_8$] arachidonic acid (20 μM) was added. After 2 min, the reaction was stopped as described in material and methods and TxB_2 was analyzed by mass spectrometry. *: $p < 0.05$ with control (ANOVA with Dunn's multiple comparison test).

Figure II: PMA induces platelet aggregation but not release of eicosanoids. **a)** Platelets were treated with 100 nM PMA, 1000 nM PMA or vehicle and aggregation was measured for 10 minutes. PMA induced full platelet aggregation at both concentrations tested (N=7). **b)** Platelet calcium mobilization was measured following stimulation with 20 nM thrombin, 40 μM PAR1-AP, 400 μM PAR4-AP or 1000 nM PMA at the EC_{100} for calcium mobilization. Thrombin, PAR1-AP and PAR4-AP showed their characteristic curves for calcium mobilization, while PMA was unable to induce calcium mobilization even at a high concentration.

Figure I

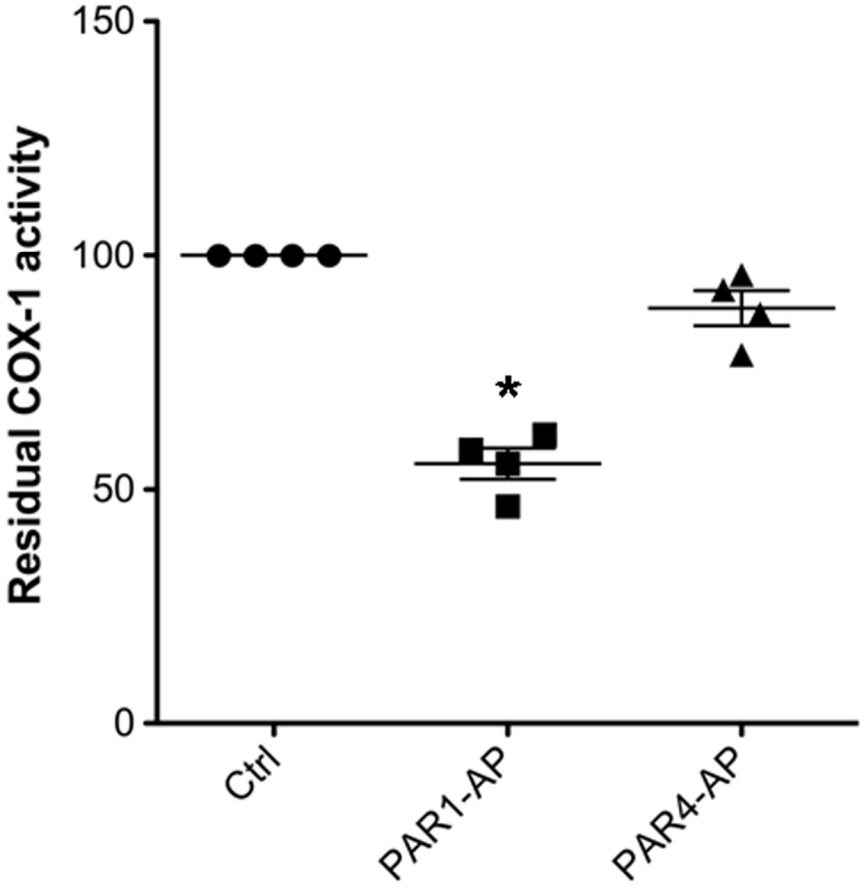
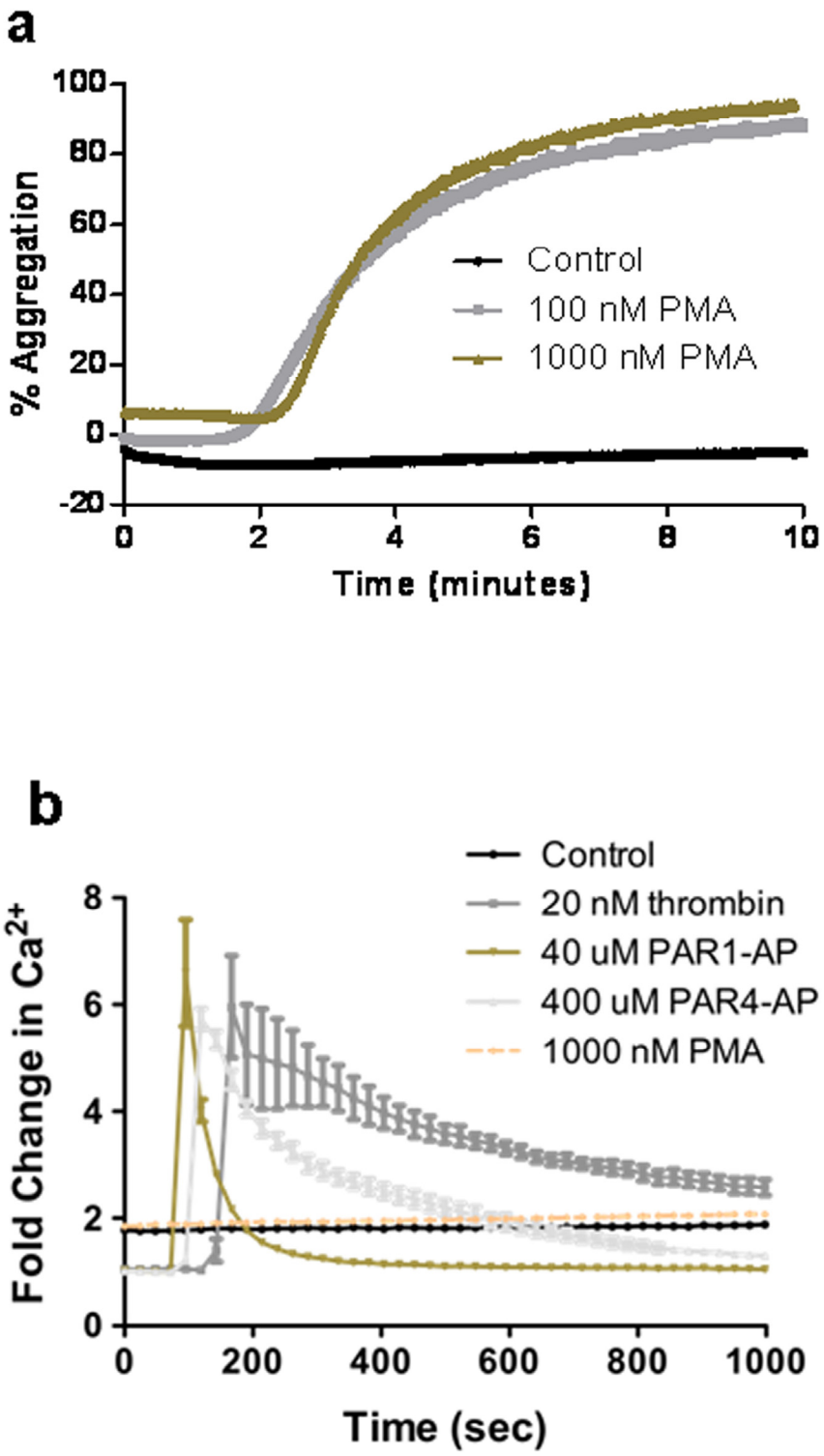


Figure II


a
100
80
60
40
20
0
-20
% Aggregation
0
2
4
6
8
10
Time (minutes)
Control
100 nM PMA
1000 nM PMA
b
Fold Change in Ca2+
8
6
4
2
0
0
200
400
600
800
1000
Time (sec)
Control
20 nM thrombin
40 uM PAR1-AP
400 uM PAR4-AP
1000 nM PMA