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Supplementary Information for

Title:

aPC/PAR1 confers endothelial anti-apoptotic activity via a discrete β-arrestin-2 mediated SphK1-S1PR1-Akt signaling axis

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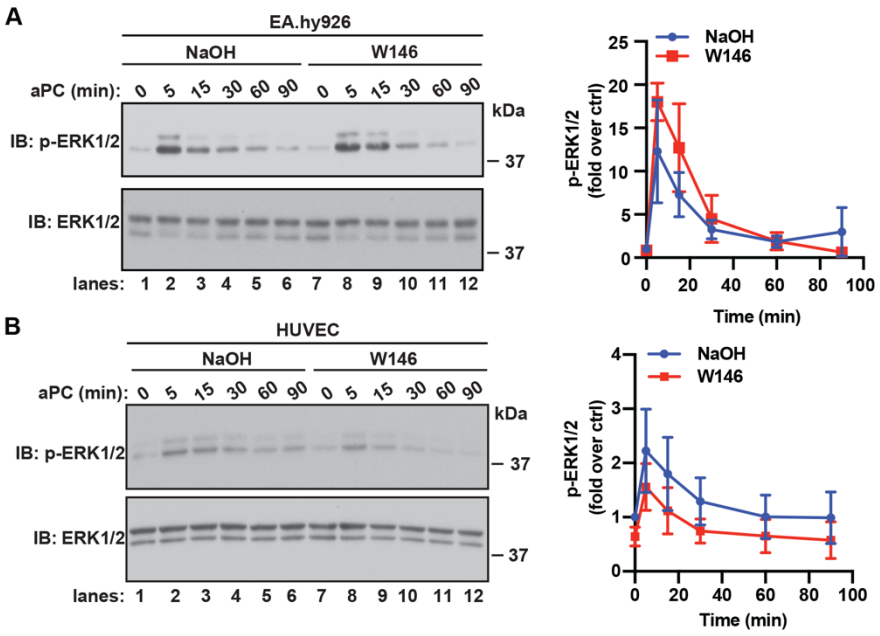
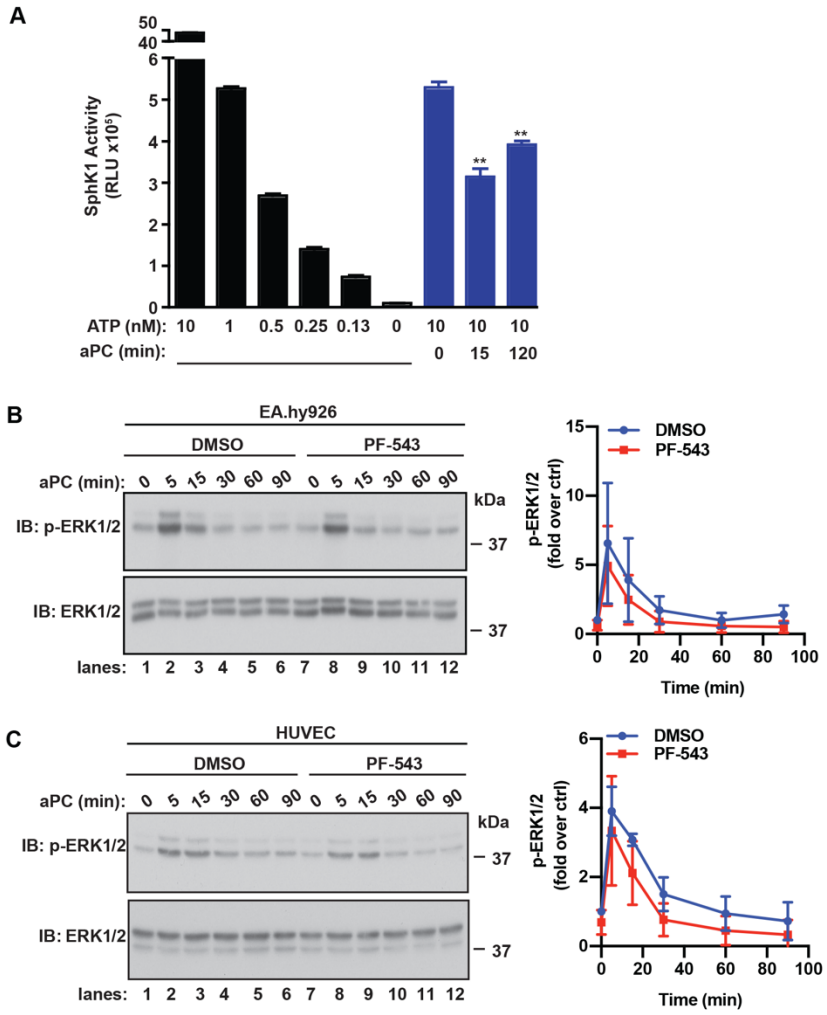
Supplemental Figure 1



Supplemental Figure 1. aPC-PAR1-induced ERK1/2 signaling is not dependent on S1PR1. (A) EA.hy926 cells were pretreated with 10 µM W146 or NaOH vehicle 30 min, stimulated with or without 20 nM of aPC for various times. ERK1/2 and Phospho-ERK1/2 was detected by immunoblotting. (B) HUVECs were pretreated with W146 or NaOH and stimulated with aPC as described in A. Data (mean ± S.D., n = 3) was analyzed by two-way ANOVA.

Supplemental Figure 2



Supplemental Figure 2. SphK1 activity ATP standard curve and SphK1 activity is not required for aPC-induced ERK1/2 activation. (A) SphK1 activity luminescence assay is a measure of ATP depletion. A standard curve of ATP concentrations on SphK1 activity is shown on the left, *black bars*. On the right, EA.hy926 cells were stimulated with 20 nM aPC for various times and SphK1 activity was measured, *blue bars*. Data (mean ± S.D., n = 3) was analyzed by Student's t-test. **, $P<0.001$. (B) EA.hy926 cells were pretreated with 100 nM PF-543 or DMSO and then stimulated with 20 nM of aPC for various times. ERK1/2 and phospho-ERK1/2 was detected by immunoblotting. Data (mean ± S.D., n = 3) was analyzed by two-way ANOVA. (C) HUVECs pretreated with PF-543, stimulated with aPC were analyze as described in B.