Supplementary Material

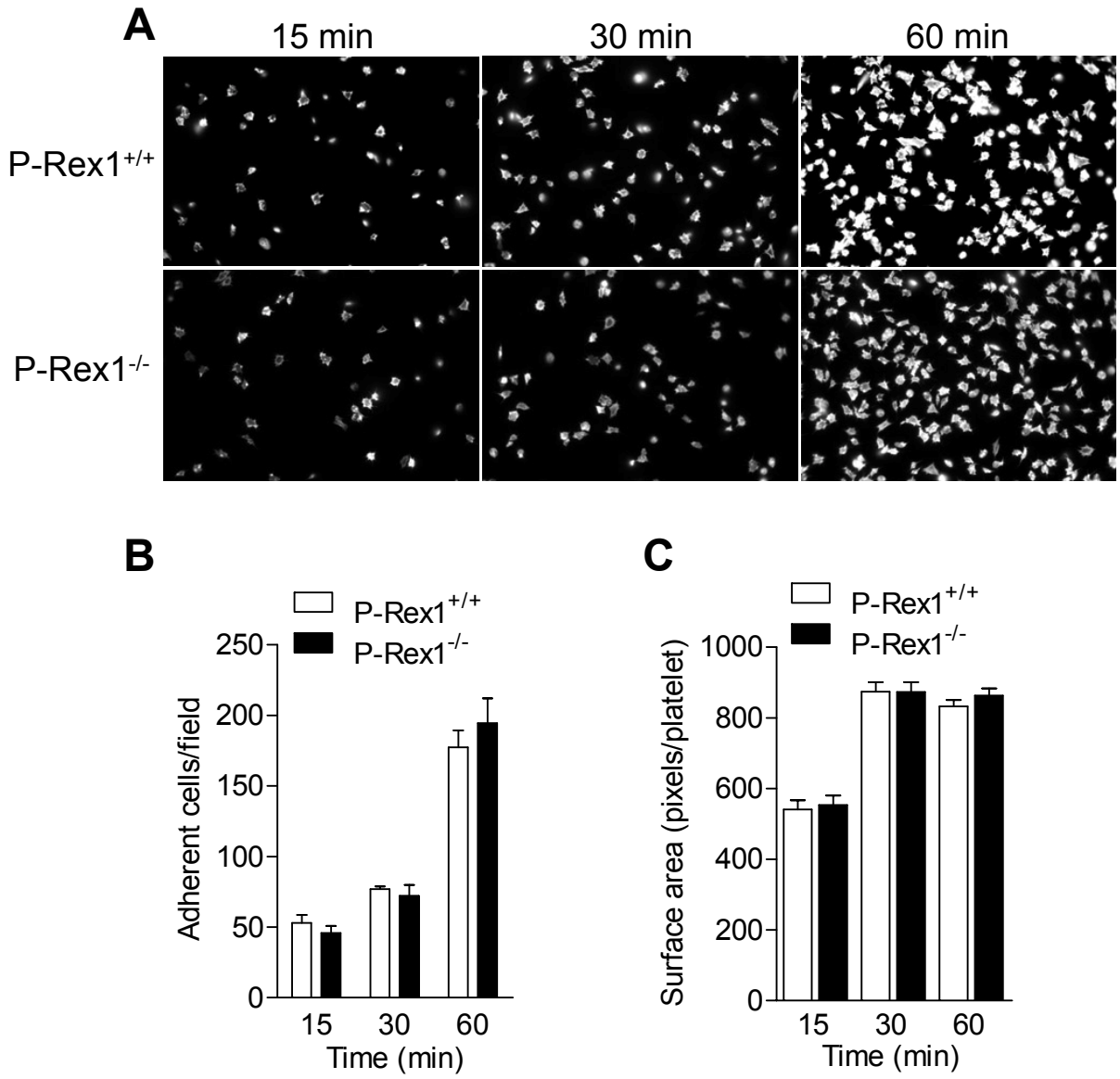

Figure I. P-Rex1 deficiency does not affect platelet adhesion and spreading on fibrinogen. (A) Chamber slides were coated with 100 μg/ml fibrinogen. Washed WT and P-Rex1-/- platelets were allowed to adhere and spread for 15, 30, and 60 min. Platelets were subsequently stained with PE-conjugated phalloidin and photographed using a fluorescence microscope. Shown in the figure are representative pictures from one of 3 experiments with similar results. Quantitative analysis of numbers of platelet (B) and platelet surface area (C) in 3 random fields (mean ± s.e.m.). Statistical analysis performed using Student *t* test. At all 3 time points, no statistical difference between WT and P-Rex1$^{-/-}$ groups

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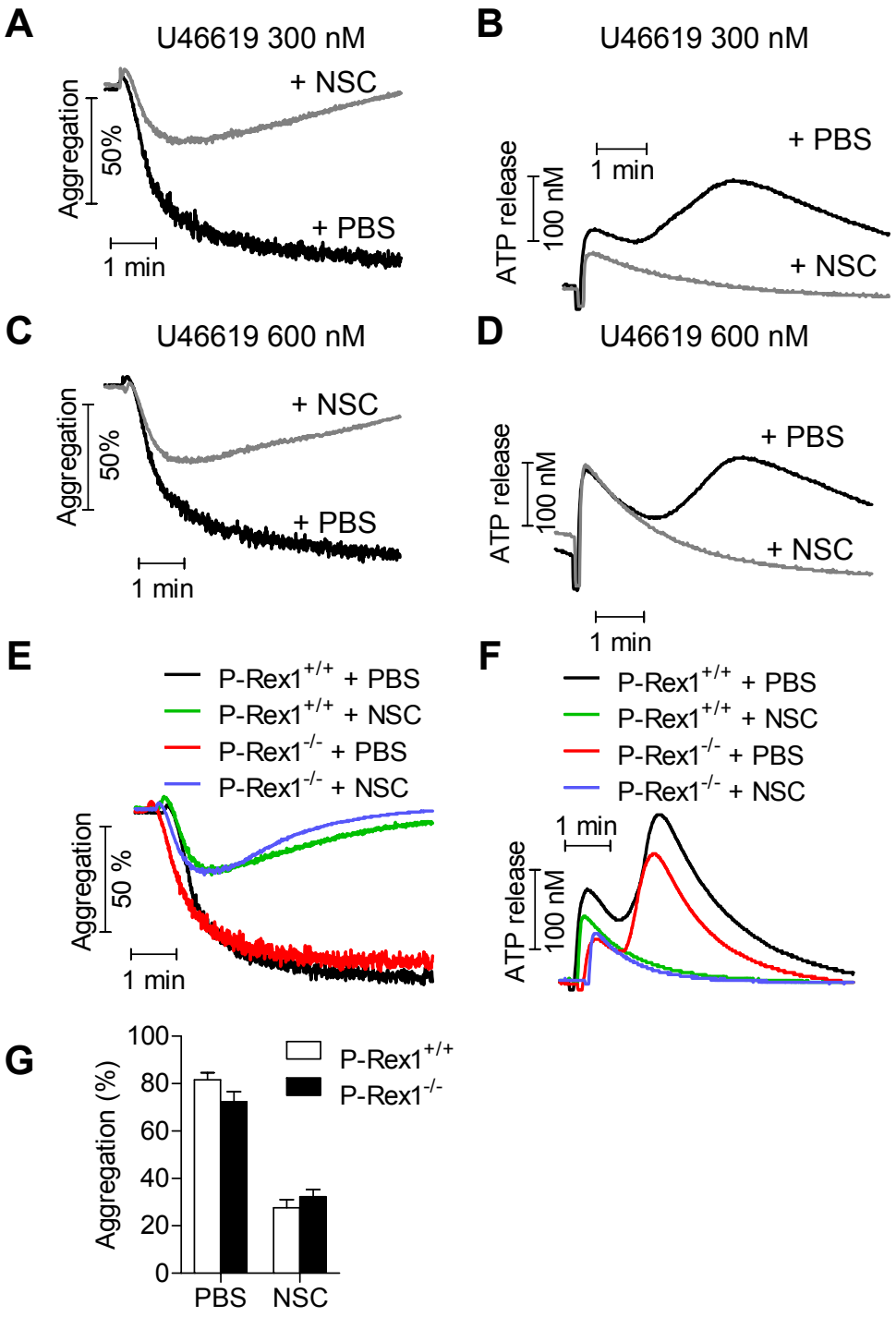

Figure II. NSC23766 inhibits platelet aggregation and secretion. Washed platelets from WT mice were pre-treated with Rac inhibitor NSC23766 at 1 μM for 1 min and stimulated with U46619 at the concentrations of 300 nM (A and B) and 600 nM (C and D). Platelet aggregation (A and C) and ATP release (B and D) were recorded in an aggregometer at 37°C. The combined effects of P-Rex1 gene deletion and NSC23766 were shown in (E) and (F). Platelets from WT and P-Rex1 deficient mice were similarly treated with NSC23766 and then stimulated with 300 nM U46619. Platelet aggregation (E) and ATP release (F) were recorded, and aggregation data were quantified in (G). Means ± SEM were shown based on 3 experiments.

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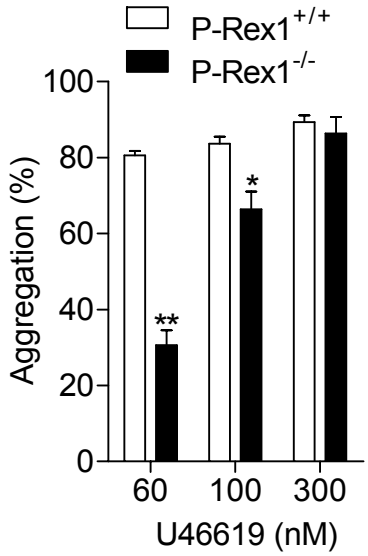

Figure III. The summary of aggregation induced with U46619. Washed WT and P-Rex1$^{-/-}$ platelets were stimulated with U46619 at indicated concentration. The bar graph shown the percentage of maximum aggregation after U46619 stimulation (means ± SEM based on 3 experiments; *, $p<0.05$ and **, $p<0.01$).

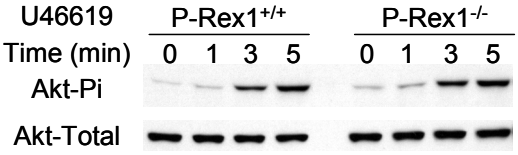

Figure IV. U46619-induced Akt activation. Washed platelets from WT and P-Rex1 KO mice were stimulated with 300 nM U46619 at 37ºC in the aggregometer. Western blotting was performed to detect Akt phosphorylation. The total Akt were detected as loading controls. Shown in the figure is a set of Western blots representative of 3 experiments with similar results.