

# *Farnesoid X Receptor and its ligands inhibit the function of platelets*

Article

Supplemental Material

Supplementary figures

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## Supplemental Figures

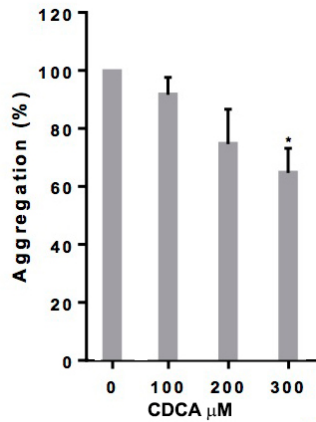


Figure I – FXR ligands inhibit platelet aggregation induced by collagen. Washed platelets were incubated with increasing concentrations of CDCA (100 - 300  $\mu\text{M}$ ) or vehicle (containing DMSO (0.1% (v/v))) prior to stimulation for 180 s with Collagen (0.5  $\mu\text{g mL}^{-1}$ ) and aggregation measured at 37°C under constant stirring conditions. Numerical data represent the percentage compared with control, mean  $\pm$  SD (n=4). t-test \* $p \leq 0.05$ , \*\*  $p \leq 0.01$ .

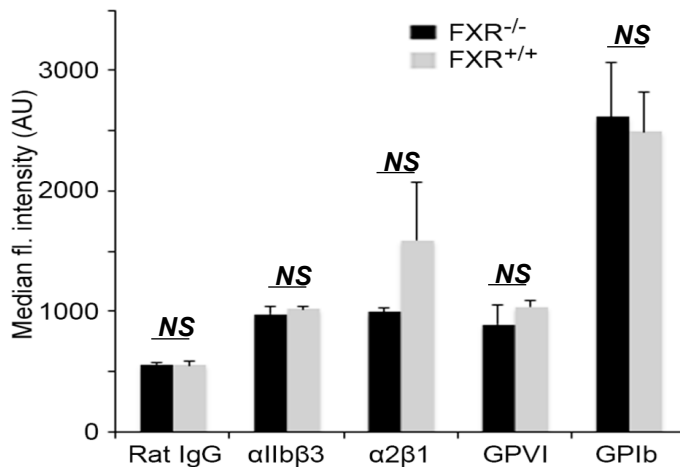


Figure II – Characterization of FXR-deficient platelets. The expression levels of  $\alpha\text{IIb}\beta\text{3}$ ,  $\alpha\text{2}\beta\text{1}$ ,

GPVI and GPIb were analyzed on FXR<sup>-/-</sup> and FXR<sup>+/+</sup> platelets by flow cytometry (A). Data represent mean (of median fluorescence intensity)  $\pm$  SD (n=4). t-test  $P > 0.05$  (non-significant - NS).

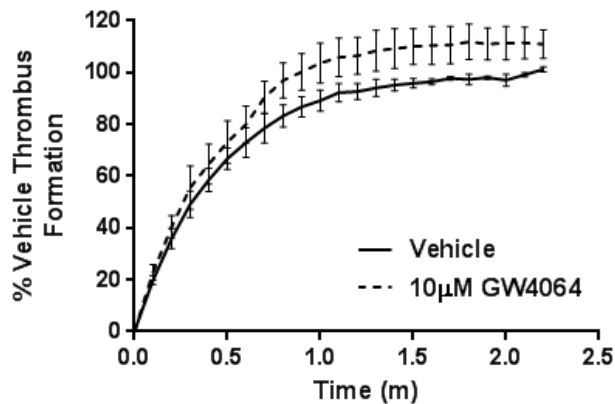


Figure III –GW4064 does not inhibit the adhesion of platelets to collagen under arterial flow conditions. Human whole blood was loaded with DiOC6 and incubated with vehicle (containing DMSO (0.1% (v/v))) (black) or 10  $\mu$ M GW4064 (dashed line) in the presence of integrilin (10  $\mu$ M), to prevent platelet aggregation, for 10 minutes before perfusion through collagen coated (100  $\mu$ g/mL) Vena8Biochips at a shear rate of 20 dyn/cm<sup>2</sup> for 2.5 minutes. Following confocal microscopy using an A1R system (Nikon Instruments, UK) platelet adhesion was determined by comparing fluorescence intensity in the vehicle and treated samples. Data represent mean  $\pm$  SD (n=3). 2-way ANOVA with Bonferroni post test  $p > 0.05$  (non-significant).

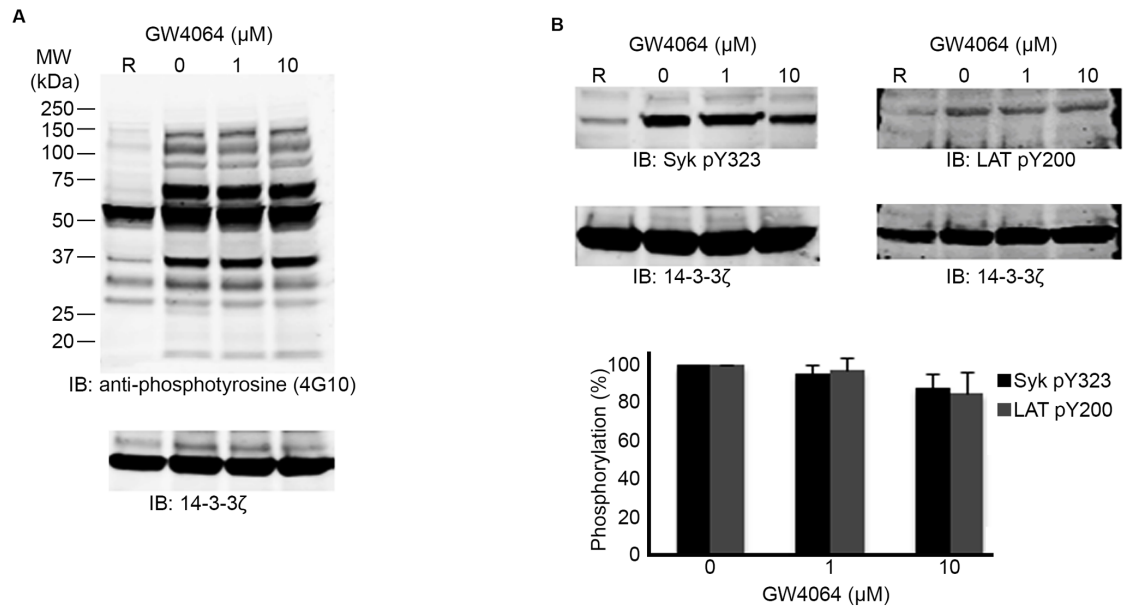


Figure IV – FXR agonists do not alter platelet protein tyrosine phosphorylation levels. (A) Washed human platelets were stimulated with CRP-XL (1 $\mu\text{g}/\text{mL}$ ) in the absence or presence of GW4064. Whole-cell protein tyrosine phosphorylation levels were assessed by immunoblot analysis. Data are representative of 4 experiments. R represents untreated resting platelets. (B) Platelet lysates were subjected to immunoblot analysis using phospho-specific antibodies for the tyrosine kinase Syk (Y323) and the adapter protein LAT (Y200). Fluorescence images were visualised using a fluorimager and analysed using Imagequant software.  $P > 0.05$ ,  $n = 4$ , T-test (non-significant).