**SUPPLEMENTARY INFORMATION**

# Katacine is a new ligand of CLEC-2 that acts as a platelet agonist

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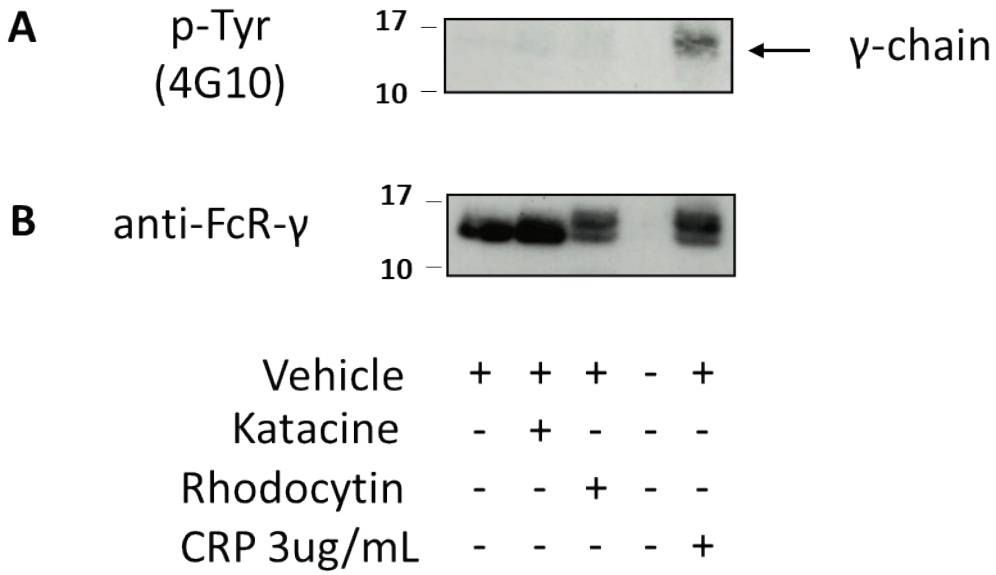
## Supplementary Methods

### Mouse platelet isolation

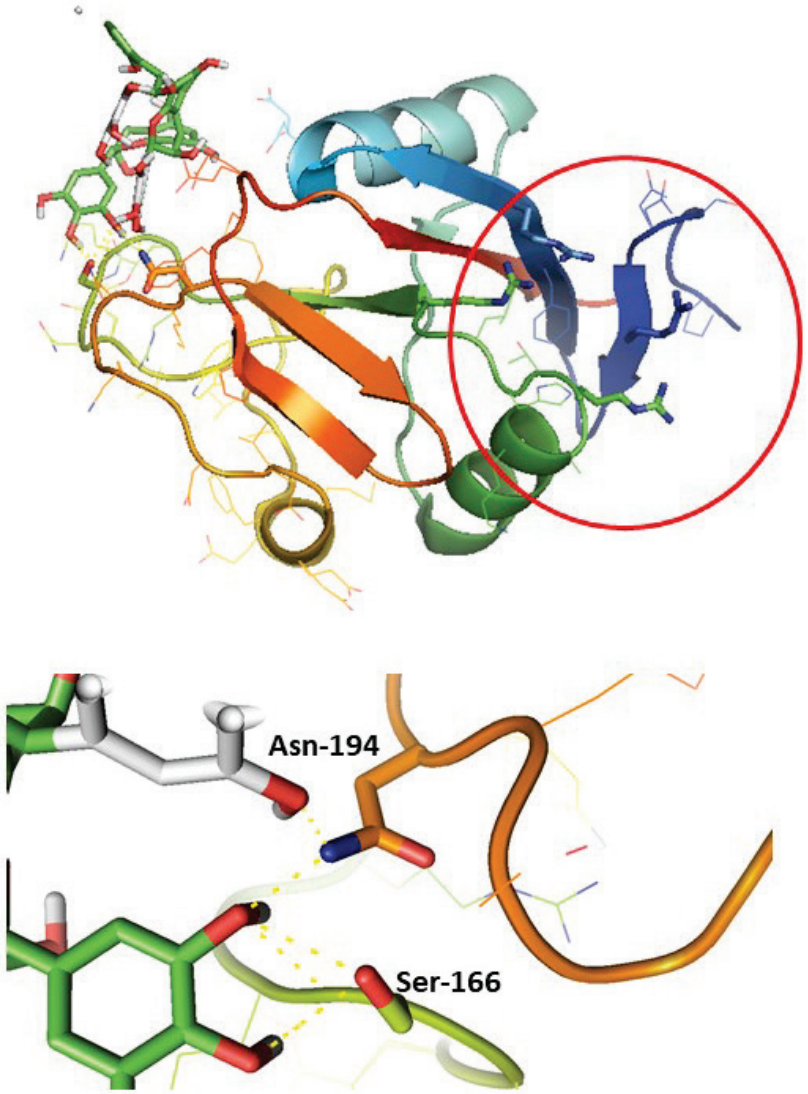
Blood was taken from anaesthetised wild type (WT) C57BL/6 mice into 10% ACD washed platelets, PRP was obtained by centrifugation at 200 x g for 20 minutes, followed by addition of 0.2 µg/ml prostacyclin and centriguation at 1000 x g for 10 min. Platelets were resuspended in Tyrode's buffer (134 mM NaCl, 0.34 mM $Na_2HPO_4$, 2.9 mM KCl, 12 mM $NaHCO_3$, 20 mM HEPES, 5mM glucose, 1 mM $MgCl_2$, pH 7.3), followed by an additional centrifugation step as above. Platelets were resuspended and allowed to rest for 30 min at room temperature.

**Supplementary Table 1.** Detailed information of top compounds identified during ALPHA screening as a disruptor of CLEC-2 and podoplanin interaction and ranked according to their potency showed on the concentration-response curves on Figure 1.

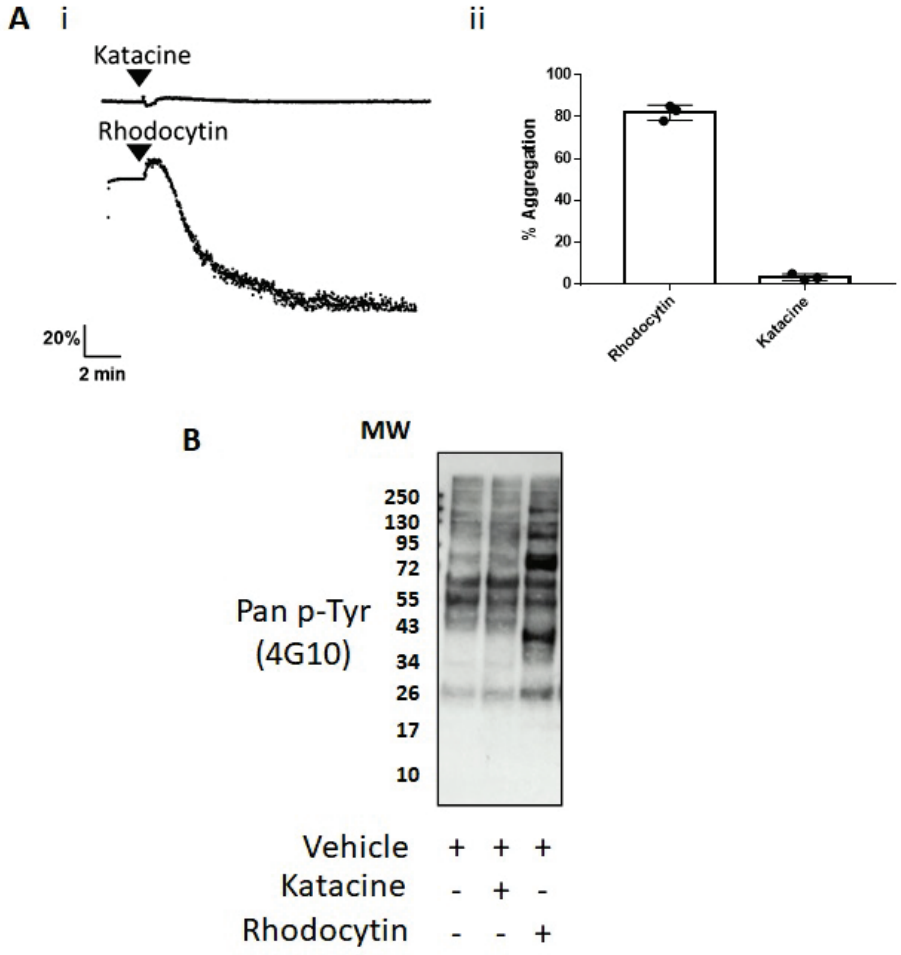
| Label | Name | MW | IC50 (μM) | SMILE CODE |
|---|---|---|---|---|
| **A** | **Sennoside A** | 862.74 | 1.3 | OC[C@H]([C@H]([C@@H]([C@H]1O)O)O)O[C@@H]1Oc1cccc([C@@H]([C@@H](c2cccc(O[C@H]([C@@H]([C@H]3O)O)O[C@H](CO)[C@H]3O)c22)c3cc(C(O)=O)cc(O)c3C2=O)c2c3c(O)cc(C(O)=O)c2)c1C3=O |
| **B** | **Katacine** | 914.77 | 2.7 | O[C@H]1[C@@H](c(cc2O)cc(O)c2O)Oc2c([C@H]([C@H]3O)c(c(O)cc(O)c4[C@H]([C@H]5O)c(c(O)cc(O)c6)c6O[C@@H]5c(cc5O)cc(O)c5O)c4O[C@@H]3c(cc3O)cc(O)c3O)c(O)cc(O)c2C1 |
| **C** | **4-{[4-(pyridin-2-yl)-1,3-thiazol-2-yl]amino}phenol** | 269.32 | 2.9 | Oc(cc1)ccc1Nc1nc(-c2ncccc2)cs1 |
| **D** | **6-dipyridin-2-ylpyrimidin-4-amine** | 357.41 | 5.3 | C(CNc1cc(-c2ncccc2)nc(-c2ncccc2)n1)Cn1cncc1 |
| **E** | **N-allyl-6-phenyl-2-pyridin-2-ylpyrimidin-4-amine** | 288.35 | 6.3 | C=CCNc1cc(-c2ccccc2)nc(-c2ncccc2)n1 |
| **F** | **N'-[(E)-(3-ethoxy-2-hydroxyphenyl)methylidene]pyridine-2-carbohydrazide** | 285.30 | 6.7 | CCOc1cccc(/C=N/NC(c2ncccc2)=O)c1O |




**Supplementary Figure 1. Katacine does not induce an increase in tyrosine phosphorylation levels of the FcR γ-chain in human platelets**. Representative immunoblots of platelet lysates ($4x10^8$) stimulated with DMSO 0.1% (negative control), 100 nM rhodocytin, 10 µM katacine, or 3µg/mL CRP (positive control). Lysates were run by SDS-PAGE and transferred to a PDVF membrane and incubated against the anti-tyrosine antibody (4G10) (A). The same membrane was incubated against the anti-FcR antibody (B).




**Supplementary Figure 2.** Molecular docking suggested an additional highly scored binding site on CLEC-2 for katacine interaction on the opposite site to the canonical binding site reported for other ligands (enclosed in red circle). Autodock vina has predicted a binding energy of -6.9 kcal/mol for katacine binding on Ser-166 and Asn-194 This finding may indicate a potential allosteric binding site for CLEC-2, previously unreported.




**Supplementary Figure 3. Katacine does not induce platelet aggregation or protein tyrosine phosphorylation in mouse platelets.** Ai) Representative traces of mouse washed platelets ($2x10^8$ platelets/mL) stimulated with 10 µM katacine or 100nM rhodocytin. Aii) Column chart representing the mean aggregation ± SD of three independent experiments. B) Representative immunoblots of mouse platelet lysates ($4x10^8$ platelets/mL) stimulated with 0.1% DMSO (negative control), 100 nM rhodocytin (positive control) or 10 µM katacine.