Dextran Sulphate triggers platelet aggregation via direct activation of PEAR1

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SUPPLEMENTAL MATERIAL

Figure S1

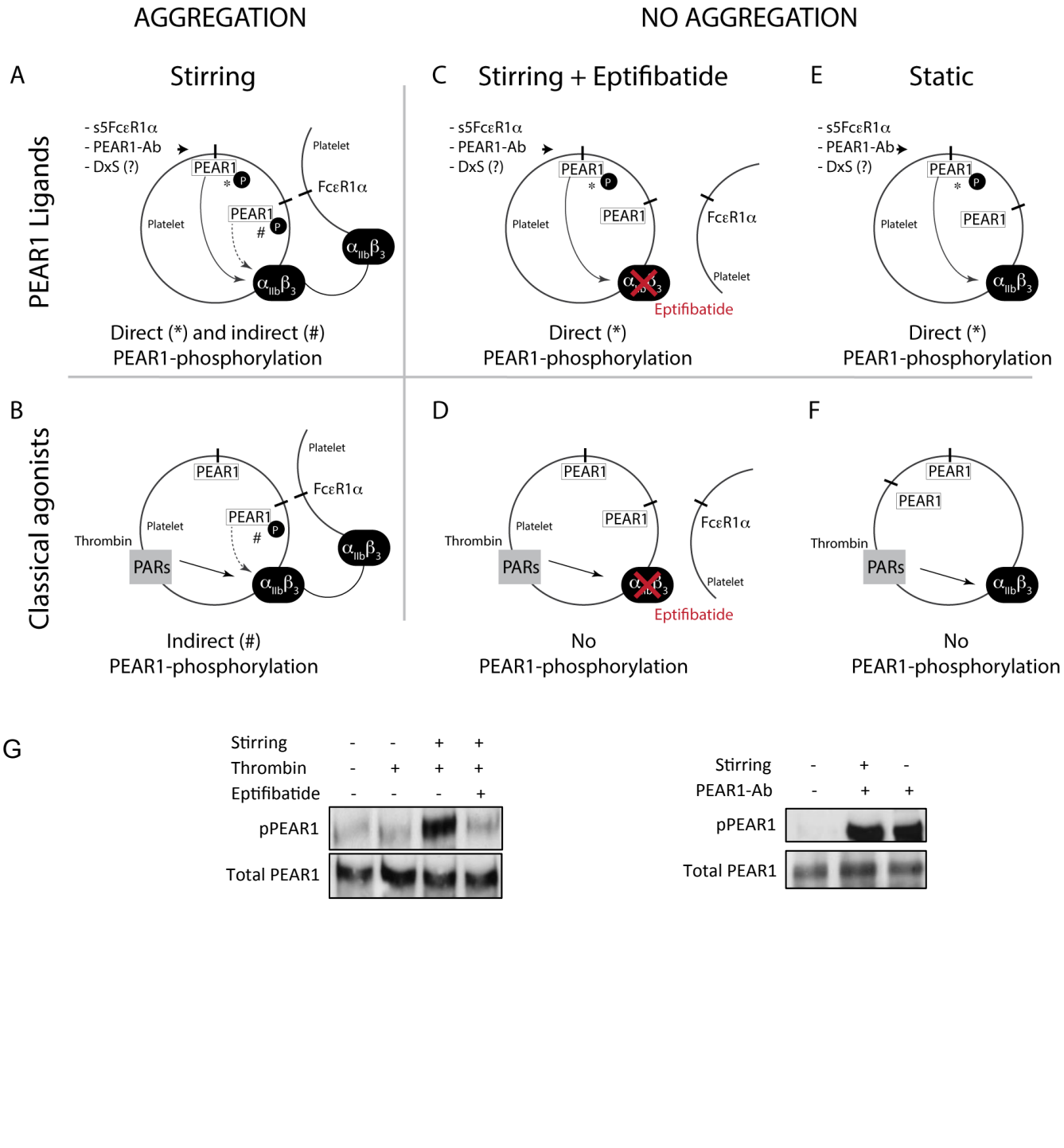
AGGREGATION
NO AGGREGATION
A
Stirring
C
Stirring + Eptifibatide
E
Static
PEAR1 Ligands
- s5FcεR1α
- PEAR1-Ab
- DxS (?)
PEAR1
Platelet
FcεR1α
Direct (*) and indirect (#)
PEAR1-phosphorylation
Eptifibatide
Direct (*)
PEAR1-phosphorylation
Direct (*)
PEAR1-phosphorylation
B
D
F
Classical agonists
Thrombin
PARs
Indirect (#)
PEAR1-phosphorylation
No
PEAR1-phosphorylation
No
PEAR1-phosphorylation
G
Stirring
Thrombin
Eptifibatide
pPEAR1
Total PEAR1
Stirring
PEAR1-Ab
pPEAR1
Total PEAR1

Figure S1 – Platelet PEAR1-phosphorylation can be triggered directly or indirectly

This figure is crucial to illustrate direct activation of PEAR1 by DxS. As previously published by our group [3], PEAR1 phosphorylation can be the result of direct PEAR1-activation (* in Figure S1) via a PEAR1-ligand (e.g. soluble recombinant pentameric FcεR1α (s5FcεR1α) or anti-PEAR1-extracellular-antibodies) interaction or can be triggered indirectly (# in Figure S1) as part of platelet amplification with traditional platelet agonists (e.g. thrombin, collagen). This has been schematically shown in Figure S1 and confirmed by western blot for pPEAR1 (PEAR1 immunoprecipitation and western blot for P-Tyr; Figure S1G). Direct activation of platelets by a specific PEAR1-ligand under stirring conditions (Figure S1A) results in direct PEAR1-phosphorylation via a PEAR1-ligand interaction and indirect PEAR1-phosphorylation via platelet-platelet contact. This is in contrast with platelet activation under stirring conditions with classical platelets agonists (Figure S1B), where only indirect PEAR1-phosphorylation occurs via platelet-platelet contact. Incubation of platelets with a specific PEAR1-ligand in non-aggregating conditions (stirring conditions in the presence of eptifibatide (Figure S1C) or static conditions (Figure S1E)) results only in direct PEAR1-phosphorylation due to the lack of platelet-platelet contact. The presence of classical platelet agonists in non-aggregating conditions does not result in phosphorylation of PEAR1 due to the lack of direct PEAR1-activation and the lack of platelet-platelet contacts.