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The GPIbα intracellular tail - role in transducing VWF- and collagen/GPVI-mediated signaling

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ABSTRACT

The GPIbα-VWF A1 domain interaction is essential for platelet tethering under high shear. Synergy between GPIbα and GPVI signaling machineries has been suggested previously, however its molecular mechanism remains unclear. We generated a novel GPIbα transgenic mouse (*GpIbα*$^{\Delta sig/\Delta sig}$) by CRISPR-Cas9 technology to delete the last 24 residues of the GPIbα intracellular tail that harbors the 14-3-3 and phosphoinositide-3 kinase binding sites. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets bound von Willebrand factor (VWF) normally under flow. However, they formed fewer filopodia on VWF/botrocetin in the presence of a αIIbβ3 blocker, demonstrating that despite normal ligand binding, VWF-dependent signaling is diminished. Activation of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets with ADP and thrombin was normal, but *GpIbα*$^{\Delta sig/\Delta sig}$ platelets stimulated with collagen-related-peptide (CRP) exhibited markedly decreased P-selectin exposure and αIIbβ3 activation, suggesting a role for the GpIbα intracellular tail in GPVI-mediated signaling. Consistent with this, while hemostasis was normal in *GpIbα*$^{\Delta sig/\Delta sig}$ mice, diminished tyrosine-phosphorylation, (particularly pSYK) was detected in CRP-stimulated *GpIbα*$^{\Delta sig/\Delta sig}$ platelets as well as reduced platelet spreading on CRP. Platelet responses to rhodocytin were also affected in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets but to a lesser extent than those with CRP. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets formed smaller aggregates than wild-type platelets on collagen-coated microchannels at low, medium and high shear. In response to both VWF and collagen binding, flow assays performed with plasma-free blood or in the presence of αIIbβ3- or GPVI-blockers suggested reduced αIIbβ3 activation contributes to the phenotype of the *GpIbα*$^{\Delta sig/\Delta sig}$ platelets. Together, these results reveal a new role for the intracellular tail of GPIbα in transducing both VWF-GPIbα and collagen-GPVI signaling events in platelets.

Introduction

In order to fulfil their hemostatic function, platelets are recruited to sites of vessel damage by von Willebrand factor (VWF), which interacts with exposed collagen and, thereafter, to glycoprotein (GP) Ibα on the platelet via its A1 domain. VWF-mediated platelet tethering facilitates platelet capture.[1] Subsequent interaction of platelets with additional ligands (e.g., αIIbβ3-fibrinogen, collagen-GPVI, collagen-α2β1) and changes in platelet phenotype are required to stabilize the platelet plug. Although the VWF-GPIbα interaction primarily facilitates platelet recruitment, it also transduces a signal that causes intraplatelet Ca^{2+} release and activation of the platelet integrin, αIIbβ3.[2-5] These signaling events are highly dependent upon flow as shear forces induce unfolding of the GPIbα mechanosensitive juxtamembrane region that translates the mechanical signal into intracellular biochemical events.[6,7] Signaling is dependent upon the binding of adaptor and signaling molecules (e.g., Src kinases, Lyn and c-Src, 14-3-3 isoforms and phosphoinositide-3 kinase [PI3K]) that can associate with the GPIbα intracellular tail.[8-12] Downstream activation of phospholipase Cγ2

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(PLCγ2), PI3K-Akt, cGMP-PKG, mitogen activated kinase and LIM kinase 1 pathways have also been reported.[13-19] By comparison to other platelet agonists (e.g., collagen, thrombin, ADP, thromboxane A2), signaling through GPIbα is considered weak. VWF-GPIbα signaling, which we term platelet 'priming' rather than activation, does not induce appreciable degranulation.[5] Therefore, the contribution of platelet 'priming' to normal hemostasis remains unclear as the effects of the other platelet agonists have the potential to mask those of GPIbα. However, in scenarios where other platelet agonists are either absent or in low abundance (e.g., platelet recruitment to endothelial or bacterial surfaces), the effects/importance of GPIbα signaling may become more prominent.[5]

GPVI is a collagen/fibrin receptor on the platelet surface that non-covalently associates with Fc receptor γ-chain (FcRγ) and signals via immunoreceptor tyrosine-based activation motifs (ITAM).[20-22] Collagen binding to platelets induces clustering of GPVI, which results in the phosphorylation of FcRγ by Src family kinases, Lyn and Fyn, that associate with the intracellular domain of GPVI.[23,24] This causes the recruitment and phosphorylation of Syk tyrosine kinase, and formation of a LAT-based signaling complex that can activate PLCγ2 and lead to release of intraplatelet Ca^{2+} stores, activation of protein kinase (PK) C, and ultimately αIIbβ3 activation and both α- and dense-granule release.[21]

Previous studies have suggested functional associations between GPIbα and GPVI and/or its co-receptor FcRγ.[13,25,26] For example, VWF-GPIbα-mediated platelet responses are reportedly impaired in GPVI/FcRγ deficiencies in both mice and humans.[13,27] There is also evidence that VWF can potentiate responses after collagen mediated responses in human platelets.[28] However, the molecular basis of GPIbα and GPVI receptor crosstalk has not been elucidated. Using a novel GPIbα transgenic mouse in which the last 24 amino acids (a.a.) of the GPIbα intracellular tail were deleted, we demonstrate the importance of this region not only to VWF-dependent signaling in platelets, but also reveal a major contribution in augmenting GPVI-mediated platelet signaling.

Methods

Mice

All procedures were performed with the United Kingdom Home Office approval in accordance with the Animals (Scientific Procedures) Act of 1986. *Gplbα*$^{\Delta sig/\Delta sig}$ mice were generated in-house by the Medical Research Council transgenic group at Imperial College using CRISPR-Cas9 technology (Figure 1). Briefly, pronuclear injections (CBAB6F1) were performed with Cas9 mRNA (75 ng/μL), guide RNA (gRNA; 25-50 ng/μL) and single-strand oligo donor DNA (25-50 ng/μL). The donor DNA (GGTAAGGCCTAATGGGCGAGTGGGGCCTCTGGTAGCAGGACGGCGACCCTGAGCTCTGAGTCAGGGTCGTGGTCAGGACCTATTGGGCACAGTGGGCATTA) had 50 bp homology arms at the 5' and 3' ends (Integrated DNA Technologies). Embryos were transferred to pseudo-pregnant CBAB6F1 female mice. Two founder mice originated from the same gRNA (CGACCCTGACTCAGAGCTGAGGG) were bred with C57BL/6 mice. F1 *Gplbα*$^{\Delta sig/+}$ mice were bred to obtain *Gplbα*$^{\Delta sig/\Delta sig}$ mice, and *Gplbα*$^{+/+}$ littermates were used as controls. Genotyping was performed by polymerase chain reaction (PCR) amplification of a *Gplbα* fragment (551 bp) using primers: AAGCACTCACACCACAAGCC and AGTATGAATGAGCGGGAGCC and subsequent Sanger sequencing (Genewiz).

Experimental procedures were performed as previously described.[29,30] Additional details are included in the *Online Supplementary Appendix*.

Results

Generation of *Gplbα*$^{\Delta sig/\Delta sig}$ mice

Sequence identity between human and murine GPIbα intracellular region is very high, supporting the contention that their functions are well conserved (Figure 1A). In order to evaluate the role of the GPIbα intracellular tail upon both VWF- and collagen/GPVI-mediated signaling, we generated a novel transgenic mouse (*Gplbα*$^{\Delta sig/\Delta sig}$) using CRISPR-Cas9 technology. We introduced a point mutation (Ser695Stop) that resulted in a premature stop codon that deletes the last 24 a.a. of the GPIbα intracellular tail (a.a. 695-718) containing the entire 14-3-3 isoform and PI3K binding region,[10,12] but maintains the upstream filamin binding site in GPIbα (residues 668-681 in murine GPIbα)[31] (Figure 1A and B). Introduction of the mutation was confirmed by sequencing and by western blotting using an anti-GPIbα antibody that recognizes the terminal region of the intracellular tail (Figure 1C to E). *Gplbα*$^{\Delta sig/\Delta sig}$ mice were viable and born with the expected Mendelian frequencies.

Gplbα$^{\Delta sig/\Delta sig}$ mice platelet count, platelet size and hemostatic function

Gplbα$^{\Delta sig/\Delta sig}$ mice had mildly reduced (~20%) platelet counts and slightly larger platelet size (Figure 2A and B), but other hematological parameters were unaffected (*Online Supplementary Table S1*). This is in contrast to the severe thrombocytopenia and giant platelets observed in complete GPIbα deficiency in mice or Bernard-Soulier patients.[32,33] Expression of the major platelet receptors, GPVI, αIIbβ3, GPIbα, and the extracellular region of GPIbα was unaltered on *Gplbα*$^{\Delta sig/\Delta sig}$ platelet surfaces (Figure 2C).

In order to assess hemostatic function in *Gplbα*$^{\Delta sig/\Delta sig}$ mice, we performed tail bleeding assays. Unlike *Vwf*$^{-/-}$ mice or mice lacking the extracellular domains of GPIbα,[32,34,35] *Gplbα*$^{\Delta sig/\Delta sig}$ mice displayed normal blood loss following tail transection (Figure 2D), suggesting that *Gplbα*$^{\Delta sig/\Delta sig}$ platelets can be recruited to sites of vessel damage similar to wild-type mice.

There was no difference between *Gplbα*$^{\Delta sig/\Delta sig}$ mice and wild-type littermates in a non-ablative laser-induced thrombus formation, as measured by the kinetics and extent, of both platelet accumulation and fibrin deposition (Figure 2E to G; *Online Supplementary Figure S1*; *Online Supplementary Video S1*).[29,30,36] These results support the contention that deletion of the GPIbα does not appreciably influence either platelet recruitment or their ability to support thrombin generation. In this model, platelet accumulation requires both VWF and thrombin but has less dependency upon collagen exposure or GPVI signaling due to the non-ablative injury.[37,38]

Gplbα$^{\Delta sig/\Delta sig}$ platelets bind von Willebrand factor (VWF) normally, but exhibit decreased VWF-mediated signaling

In order to specifically examine the effect of the GPIbα intracellular tail truncation upon VWF-dependent platelet capture, we coated microchannels with murine VWF over

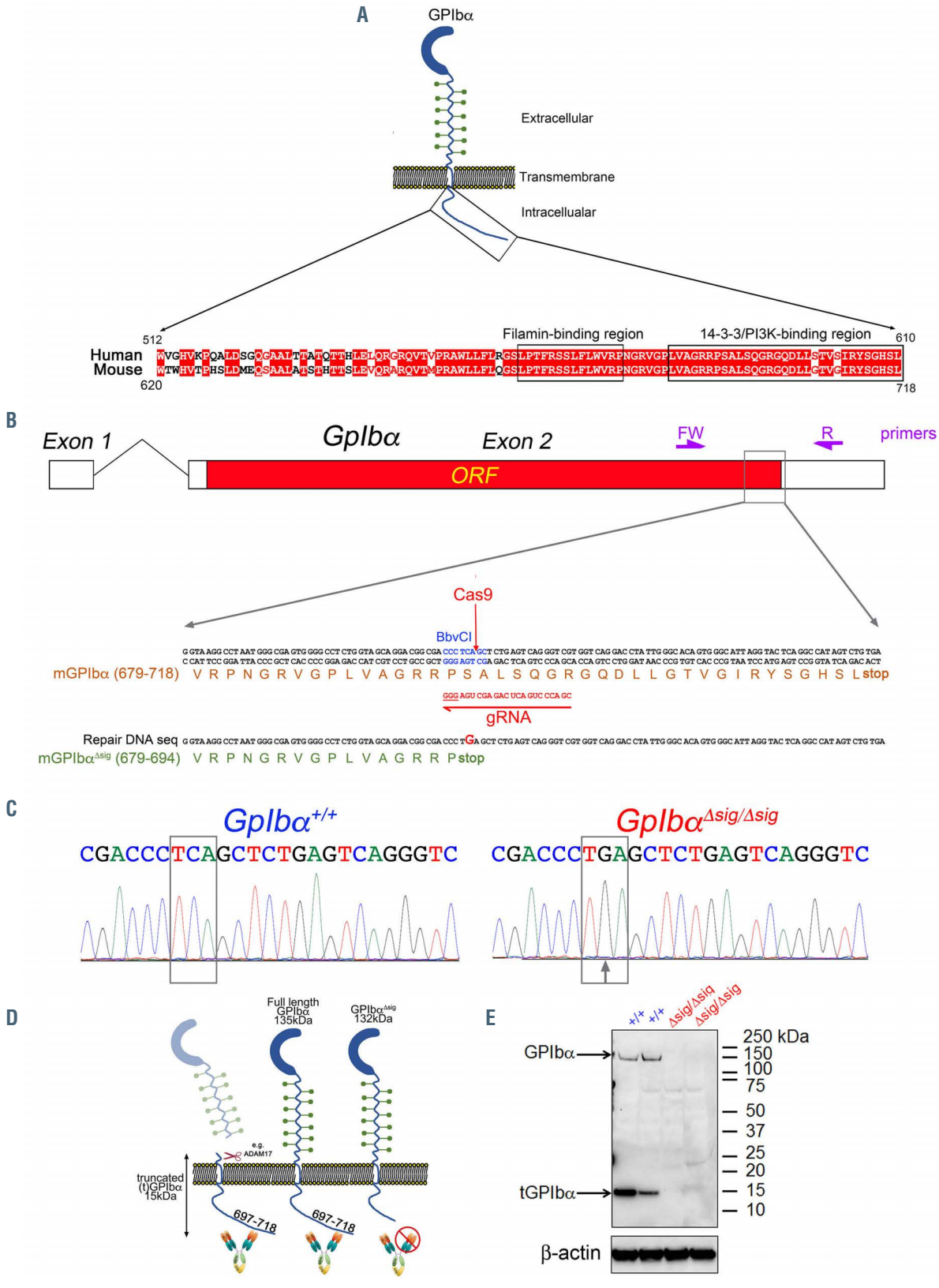

Figure 1. Generation and characterization of *Gplbα*$^{\Delta sig/\Delta sig}$ mice. (A) Sequence alignment of the last 100 amino acids (a.a.) of human and mouse GPIbα. Sequence identities are highlighted in red. Filamin binding region: (a.a. 560-573) and (a.a. 668-681) for human and mouse GPIbα; PI3K/14-3-3 binding region: (a.a. 580-610) and (a.a. 688-718) for human and mouse GPIbα. (B) Schematic representation of the *Gplbα* gene with CRISPR guide target site, gRNA sequence, BbvCI restriction enzyme site and Cas9 predicted cut site. Primers used to amplify the *Gplbα* allele from genomic DNA are indicated in purple. Design of the 101 bp single stranded DNA repair template with the point mutation to introduce a codon stop eliminating the BbvCI restriction enzyme site and removing the last 24 a.a. of GPIbα is also shown. The resulting truncated a.a. sequence from *Gplbα*$^{\Delta sig/\Delta sig}$ mice is indicated in green. (C) Genomic DNA sequences from *Gplbα*$^{+/+}$ and *Gplbα*$^{\Delta sig/\Delta sig}$ mice. Successful substitution is indicated with an arrow. (D) Diagram showing the binding of the anti-GPIbα tail Ab (Biorbyt; orb 215471). (E) Platelet lysates from *Gplbα*$^{+/+}$ and *Gplbα*$^{\Delta sig/\Delta sig}$ mice were probed with the anti-GPIbα tail and β-actin antibodies. Absence of band in the GPIbα western-blot confirms the successful truncation of the GPIbα intracellular tail in *Gplbα*$^{\Delta sig/\Delta sig}$ mice.

which we perfused plasma-free blood (to remove fibrinogen and outside-in activation αIIbβ3) at 1,000s^{-1}. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets were recruited normally to murine VWF-coated surfaces with rolling velocities, surface coverage and platelet accumulation unaltered compared to *GPIbα*$^{+/+}$ platelets (Figure 3A to D; *Online Supplementary Video S2*).

In order to investigate the impact of the deletion of the last 24 a.a. of GPIbα on VWF signaling, we performed platelet spreading assays on murine VWF, which rely upon VWF-GPIbα signaling. On VWF alone, very few *GPIbα*$^{+/+}$ or *GpIbα*$^{\Delta sig/\Delta sig}$ platelets bound to VWF and only very few exhibited filopodia (Figure 3E and F). When these experiments were repeated in the presence of botrocetin (a snake venom that increases the affinity of VWF A1 domain for GPIbα)[39] a large proportion (90±2.8%) of *GPIbα*$^{+/+}$ platelets underwent shape changes and developed filopodia (Figure 3E and G; *Online Supplementary Figure S2A* and *B*), a well-described consequence of VWF-GPIbα signaling.[9,19] This process was significantly diminished in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets with only 46±2.6% platelets exhibiting filopodia (Figure 3E

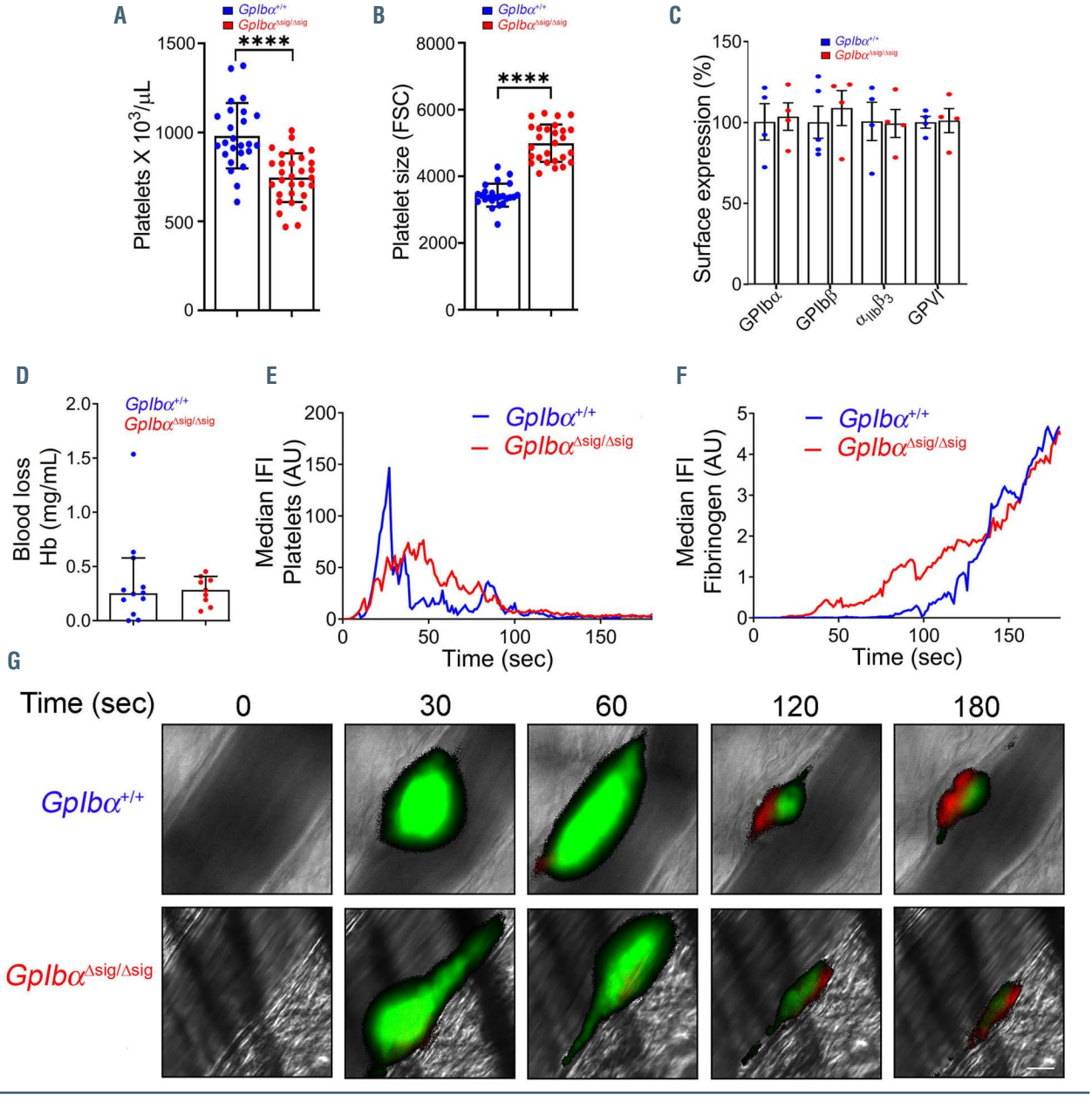

Figure 2. *GpIbα*$^{\Delta sig/\Delta sig}$ mice display normal bleeding loss and platelet and fibrin accumulation in the laser-induced thrombosis model. A) Platelet counts and (B) platelet size in *GpIbα*$^{+/+}$ (n=25) and *GpIbα*$^{\Delta sig/\Delta sig}$ mice (n=30) as determined by flow cytometry. (C) Surface expression of platelet receptors GPIbα, GPIbβ, αIIbβ3 and GPVI in *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice (n=4 for each genotype) determined by flow cytometry and expressed as % of control. (D) Bar graph analyzing blood loss after 10 minutes following tail transection in *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice (n=9 for each genotype). (E-G) Mice cremaster muscle arterioles were subjected to the laser-induced thrombosis model as described in the *Online Supplementary Appendix*. Curves represent median integrated fluorescence intensity (IFI) from platelets (arbitrary units: AU) (E) or fibrin(ogen) (F) as a function of time after the injury (20 thrombi in 3 *GpIbα*$^{+/+}$ and 34 thrombi in 4 *GpIbα*$^{\Delta sig/\Delta sig}$ mice). (G) Representative composite fluorescence images of platelets (green) and fibrin (red) with bright field images after laser-induced injury of the endothelium of *GpIbα*$^{+/+}$ (top panels) *vs.* *GpIbα*$^{\Delta sig/\Delta sig}$ mice (bottom panels). Scale bar represents 10 μm. Each symbol represents one thrombus. Horizontal lines intersecting the data set represent the median. Data was analyzed using Mann Whitney test; ns: *P*>0.05. Also see the *Online Supplementary Video S1* and the *Online Supplementary Figure S1*. FSC: foward scatter; Hb: hemoglobin.

and G; *Online Supplementary Figure S2C* to *D*).[9,19] When experiments were performed in the presence of both botrocetin and GR144053, which competitively inhibits the interaction of αIIbβ3 with VWF and/or fibrinogen, the number of *GPIbα*$^{+/+}$ platelets forming filopodia was not appreciably influenced (*Online Supplementary Figure S2B*), but the proportion of that formed >3 filopodia was significantly reduced (37±6.7% *vs.* 74±6.9%) (*Online Supplementary Figure S2A*), revealing the contribution of outside-in signaling to filopodia formation. Under these conditions, here again although *GpIbα*$^{\Delta sig/\Delta sig}$ platelets bound VWF surfaces, they had a significantly diminished ability to form filopodia (Figure 3E and H). Moreover, GR144053 had no effect upon filopodia formation in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets (*Online Supplementary Figure S2C*), suggesting that the reduced filopodia formation in these platelets was likely due to a defect in VWF-GPIbα signaling manifest by a lack of activation of αIIbβ3 in response to VWF-GPIbα binding. Taken together, these results indicate that deletion of the last 24 a.a. of the intracellular tail of GPIbα does not influence platelet binding to VWF, but significantly reduces VWF-GPIbα downstream signaling response including αIIbβ3 activation.

The intracellular tail of GPIbα is important for GPVI signaling

We next evaluated agonist-induced platelet activation in *GpIbα*$^{\Delta sig/\Delta sig}$ mice. In response to ADP, washed *GpIbα*$^{\Delta sig/\Delta sig}$ platelets exhibited normal αIIbβ3 activation and P-selectin exposure and normal platelet aggregation (Figure 4A to D). Responses to thrombin were also normal except for a slight significant decrease in P-selectin exposure with the lowest

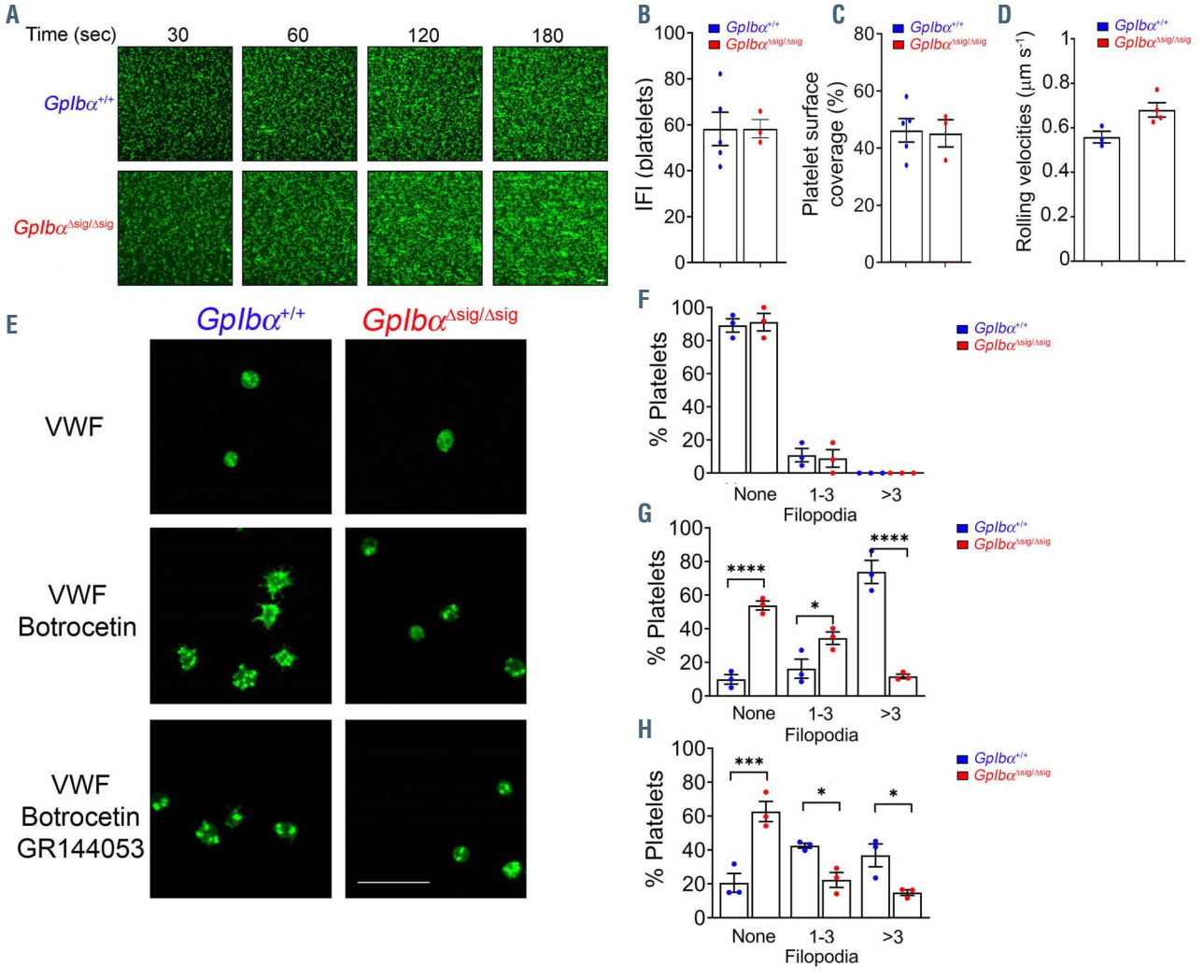

Figure 3. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets exhibit normal binding to von Willebrand factor but disrupted GPIbα-mediated signaling. (A-D) Plasma-free blood from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice supplemented with anti-GPIbb-DyLight488 anitbody was perfused over murine VWF at a shear rate of 1,000 s^{-1}. (A) Representative fluorescence images (n≥3; scale bar 10 μm) and bar graphs analyzing the integrated fluorescence intensity (IFI) (B) and the surface coverage (C) of *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ platelets captured by murine von Willebrand factor (VWF) after 3.5 minutes of flow. (D) Rolling velocities (median ± confidence interval [CI]) were calculated from (approximately 10,000) platelets rolling/adhering to murine VWF within the first 30 seconds (n≥3) (E) Representative confocal images of *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ platelets (n=3 for each genotype) spread on mVWF and stained with Phalloidin-Alexa 488, in the absence or presence of Botrocetin or Botrocetin and GR144053 (scale bar 10 μm). (F to H) Percentage of platelets from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice (individual data points representing the average of 3-6 fields of view) with no filopodia, 1-3 filopodia or >3 filopodia formed on murine VWF in the absence (F; 129 *GpIbα*$^{+/+}$ platelets and 115 *GpIbα*$^{\Delta sig/\Delta sig}$ platelets analysed) or presence of Botrocetin (G; 511 *GpIbα*$^{+/+}$ platelets and 547 *GpIbα*$^{\Delta sig/\Delta sig}$ platelets analysed), or Botrocetin and GR144053 (H; 359 *GpIbα*$^{+/+}$ platelets and 480 *GpIbα*$^{\Delta sig/\Delta sig}$ platelets analysed). Data represents mean ± standard error of the mean (B, C, F to H) or median ± CI (D) and was analyzed using unpaired two-tailed Student's *t*-test (B and C), unpaired Mann Whitney test (D) or using two-way ANOVA followed by Sidak's multiple comparison test (F to H); **P*<0.05, ****P*<0.001, *****P*<0.0001. Also see the *Online Supplementary Figure S2* and the *Online Supplementary Video S2*.

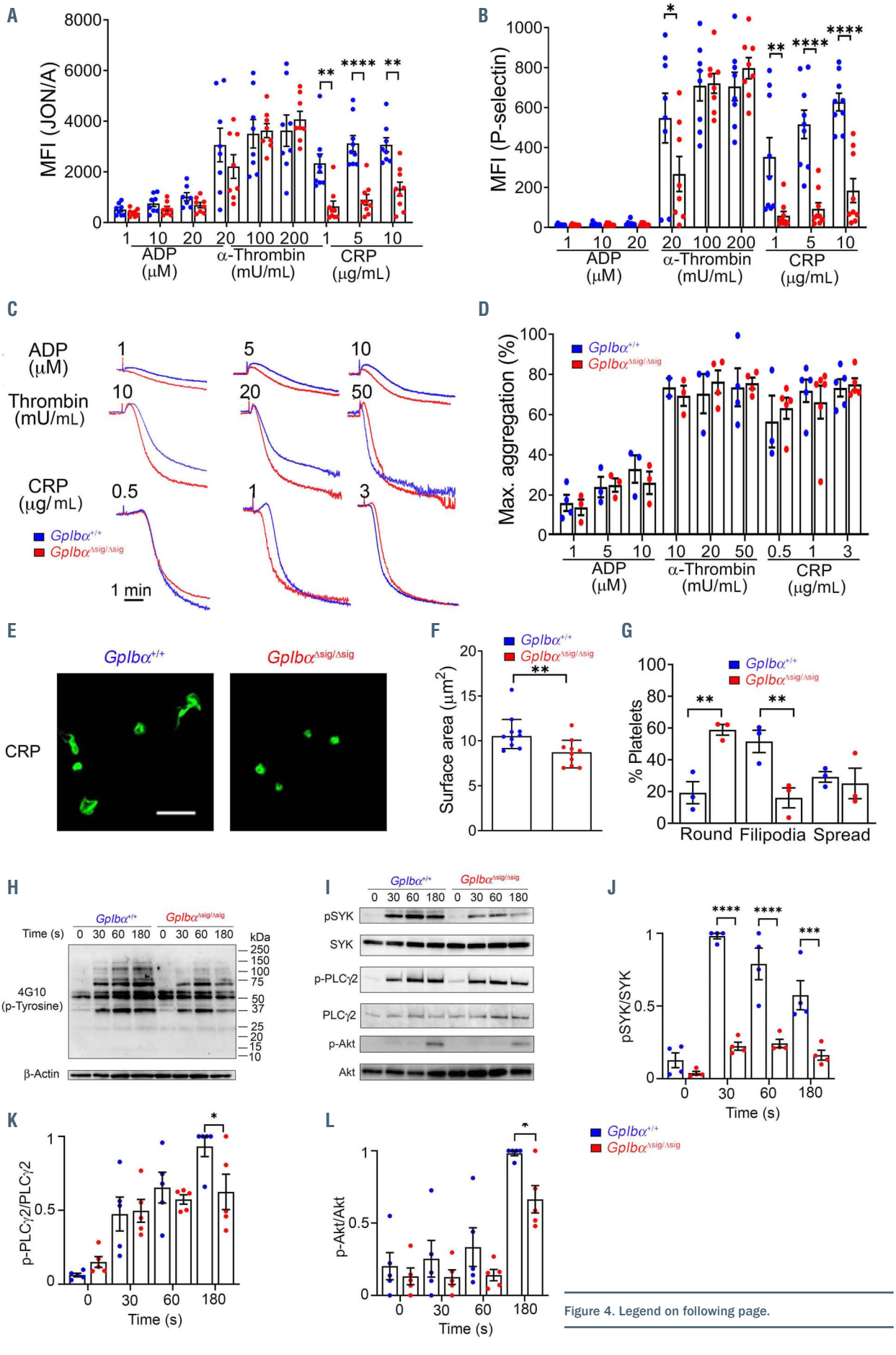

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Figure 4. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets exhibit altered GPVI-mediated signaling. (A and B) Flow cytometric analysis of surface expression of activated αIIbβ3 (A) and P-selectin (B) in *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ platelets (n=8) in response to ADP (1-20 μM), α-thrombin (20-200 mU/mL), or CRP (1-10 μg/mL). MFI: geometric mean fluorescence intensity (C) Representative aggregation traces (n=3-6) of washed platelets isolated from *GpIbα*$^{+/+}$ (blue) or *GpIbα*$^{\Delta sig/\Delta sig}$ (red) mice and stimulated with ADP (1-10 μM), α-thrombin (20-50 mU/mL) or CRP (0.5-3 μg/mL). Aggregation was monitored using a Chronolog aggregometer over 6 minutes. (D) Bar graph analysing the maximum aggregation (%) obtained in the conditions presented in (C). (E) Representative micrographs (n=3 for each genotype; 3 fields of view analyzed per condition; scale bar 10 μm) of 454 *GpIbα*$^{+/+}$ and 420 *GpIbα*$^{\Delta sig/\Delta sig}$ platelets spread on CRP and stained with Phalloidin-Alexa 488. Bar graphs quantifying the surface area (F) and percentages (G) of platelets that remained round, formed filopodia or spread on CRP. (H) Western blot analyzing tyrosine kinase phosphorylation in platelets from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice, following stimulation with 3 μg/mL CRP for 0-180 seconds (s), using β-actin as a loading control (representative of n=3). (I) Western blots analyzing the levels of phosphorylated and non-phosphorylated SYK, PLCγ2 and Akt in platelets from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice, after 0-180 s stimulation with CRP (representative of n=3). (J-L) Bar graphs displaying the levels of phosphorylated SYK, PLCγ2 and Akt in platelets from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice, after 0-180 s stimulation with CRP and normalizing the intensity according to the non-phosphorylated levels of SYK, PLCγ2 and Akt. For the surface area (F), the data represent the median ± confidence interval (CI) and was analyzed using the unpaired Mann Whitney test. All other data is displayed as mean ± standard error of the mean and was analyzed using two-way ANOVA followed by Sidak's multiple comparison test. **P*<0.05, ***P*<0.01, ****P*<0.001, ****P<0.0001. Also see the *Online Supplementary Figures S2* and *S3*.

thrombin concentration (Figure 4A and B) but this did not influence thrombin-induced platelet aggregation (Figure 4C and D). How this reduced P-selectin exposure in response to low thrombin concentration is manifest remains unclear, but may reflect the findings of a previous study that suggested the importance of 14-3-3ζ binding to GPIbα specifically for low-dose thrombin responses.[40] Despite largely unaffected responses to ADP and thrombin, in response to collagen-related peptide (CRP), *GpIbα*$^{\Delta sig/\Delta sig}$ platelets exhibited markedly reduced αIIbβ3 activation and P-selectin exposure (Figure 4A and B). Interestingly, *GpIbα*$^{\Delta sig/\Delta sig}$ platelet aggregation following CRP stimulation appeared normal (Figure 4C and D).

Next, we evaluated the ability of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets to spread on fibrinogen surfaces with and without prior stimulation with thrombin. Without platelet stimulation, similar to wild-type platelets, most *GpIbα*$^{\Delta sig/\Delta sig}$ platelets remained round while upon stimulation with thrombin approximately 80% platelets spread fully with no difference observed in the spread platelet area (*Online Supplementary Figure S3A* to *E*). As full spreading is highly dependent upon outside-in signaling through αIIbβ3,[41] this suggests that this signaling pathway is unaffected in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets. We then explored the ability of platelets to spread on CRP-coated surfaces. Consistent with diminished platelet activation in response to CRP, *GpIbα*$^{\Delta sig/\Delta sig}$ platelets remained round in contrast to wild-type platelets (59±3.4% *vs.* 19±7%; Figure 4E and G). This effect was also quantified by a 20% reduction in bound platelet area (Figure 4F) and in the reduced incidence of filopodia formation – 16±6.3% for *GpIbα*$^{\Delta sig/\Delta sig}$ *versus* 52±7.1% for *GPIbα*$^{+/+}$ (Figure 4E and G). Collectively, these results reveal an appreciable defect in GPVI-mediated signaling in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets.

There was an overall reduction in tyrosine phosphorylation after CRP stimulation in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets compared to wild-type platelets (Figure 4H). Further analysis revealed appreciably reduced Syk kinase activation in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets, as measured by phosphorylation of Syk on Tyr525 and Tyr526 in response to CRP and lower phosphorylation levels of its downstream target pPLCγ2 (p-Tyr 1217), although this was less marked than for those observed with pSyk (Figure 4I to K). In addition, phosphorylation levels of Akt (p-Ser 473), a known substrate of PI3K were also appreciably diminished in *GpIbα*$^{\Delta sig/\Delta sig}$ *versus* *GpIbα*$^{\Delta sig/\Delta sig}$ (Figure 4I and L). In order to assess whether the effect of truncation of GPIbα was specific for GPVI-mediated platelet responses, or whether other tyrosine-mediated signaling pathways might also be affected, we stimulated *GpIbα*$^{\Delta sig/\Delta sig}$ and wild-type platelets with rhodocytin (C-type lectin receptor 2 [CLEC-2] agonist). Tyrosine-phosphorylation profile of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets in response to rhodocytin was similar to that of *GPIbα*$^{+/+}$ platelets, with slightly reduced phosphorylation of Syk (approximately 20%) (*Online Supplementary Figure S4A* to *C*). P-selectin exposure in response to rhodocytin was reduced in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets while αIIbβ3 activation was only diminished for the lowest concentration of the toxin without reaching statistical significance (*Online Supplementary Figure S4D* to *E*). These results suggest that the GPIbα tail may also influence CLEC-2 ITAM-mediated signaling, but perhaps with reduced dependency.

The role of the GPIbα intracellular tail in platelet recruitment and aggregation under flow

In order to examine the consequences of the combined effects of disrupted VWF-GPIbα signaling and diminished GPVI signaling in platelets in more physiological assays, we quantified platelet recruitment and aggregate formation on collagen-coated microchannels under flow. Experiments were performed at high (3,000 s^{-1}), medium (1,000 s^{-1}) and low (200 s^{-1}) shear, as platelet recruitment is increasingly dependent on VWF-GPIbα as shear increases while subsequent platelet aggregate formation on collagen surfaces becomes more dependent upon GPVI signaling.[42-44]

Perfusing whole blood at 3,000 s^{-1} and 1,000 s^{-1} over collagen, we observed a marked reduction in surface coverage of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets when compared to *GPIbα*$^{+/+}$ platelets (Figure 5A and B; Figure 6A and B; *Online Supplementary Videos S3 and S4*). *GpIbα*$^{\Delta sig/\Delta sig}$ platelets that bound to collagen also formed smaller aggregates than *GPIbα*$^{+/+}$ platelets (Figures 5C and 6C), likely reflecting the subsequent effect of diminished collagen-GPVI signaling. Perfusing wild-type plasma-free blood (to remove soluble VWF and fibrinogen) in collagen-coated microchannels revealed a significant reduction of both platelet adhesion and thrombus growth to similar levels observed in *GpIbα*$^{\Delta sig/\Delta sig}$ samples (Figure 6A, B, D and E) showing that a small amount of VWF-independent binding to collagen occurs at 1,000 s^{-1}. When whole blood experiments were performed in the presence of GR144053, to block αIIbβ3, *GPIbα*$^{+/+}$ platelets were recruited to the collagen surface as a monolayer. However, additional platelet-platelet recruitment was abolished and therefore there was limited thrombus growth in 3D. This was measured by an increase in surface coverage with a decrease in thrombus formation (i.e., total platelet fluorescence; Figure 6A, B and D).[45] Surface coverage as well as platelet accumulation of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets was similar in both the absence and presence of GR144053 (Figure 6A, B and E), suggesting that lack of active αIIbβ3 is part of the platelet phenotype. In order to more specifically examine the role of GPVI in this system, we performed experiments in the presence of JAQ1, an anti-murine GPVI blocking antibody. Blocking GPVI significantly reduced surface coverage and platelet accumulation in *GpIbα*$^{\Delta sig/\Delta sig}$ and *GPIbα*$^{+/+}$

platelets, revealing the important contribution of GPVI signaling at 1,000 s^{-1} (Figure 6F to I), in stabilizing platelet recruitment and their subsequent aggregation.

At venous shear rates (200 s^{-1}) where the dependencies on VWF and collagen are slightly different to 1,000 s^{-1}, surface coverage of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets was slightly reduced compared to *GPIbα*$^{+/+}$ platelets although it did not reach significance. However, thrombus growth was significantly diminished (Figure 7A to C; *Online Supplementary Video S5*). Using plasma-free blood, the surface coverage was similar for *GpIbα*$^{\Delta sig/\Delta sig}$ and *GPIbα*$^{+/+}$ platelets, mediated by direct (VWF-independent) interaction with collagen (Figure 7A and B). Similar to high-shear conditions, platelet accumulation under plasma-free conditions of *GPIbα*$^{+/+}$ platelets was significantly reduced compared to whole blood (Figure 7D) similar to those observed with *GpIbα*$^{\Delta sig/\Delta sig}$ platelets (Figure 7E). In the presence of GR144053, we saw the same increase in surface coverage of *GPIbα*$^{+/+}$ platelets with reduced localized 3D-platelet thrombi (Figure 7A and B) although the platelet accumulation was not significantly different to *GPIbα*$^{+/+}$ whole blood (Figure 7D) likely due to the increased platelet coverage. Consistent with the results obtained under high-shear conditions, the effect of increased surface coverage in the presence of GR144053 was not observed with *GpIbα*$^{\Delta sig/\Delta sig}$ platelets, nor was platelet accumulation appreciably further diminished (Figure 7A, B and E). Finally, similar to results obtained under arterial shear conditions, blocking GPVI significantly reduced surface coverage and platelet accumulation in both *GpIbα*$^{\Delta sig/\Delta sig}$ and *GPIbα*$^{+/+}$ platelets (Figure 7F to I). As removal of either VWF or blocking of GPVI had very similar effects, this suggests that VWF-GPIbα and GPVI-collagen binding may act synergistically to recruit platelets at low shear.

Discussion

The ability of platelet GPIbα binding to VWF to transduce intraplatelet signaling is well-known, but the hemostatic role of the platelet 'priming' that follows has frequently been perceived as redundant due to the comparatively mild phenotypic changes in platelets that ensue when compared to other platelet agonists (e.g., thrombin, collagen). Using a novel *GpIbα*$^{\Delta sig/\Delta sig}$ mouse, we now demonstrate that the intracellular tail of GPIbα is important not only for transduction of VWF-GPIbα signaling, but also collagen-GPVI-mediated responses in platelets (Figure 8).

The binding of GPIbα to VWF, and of GPVI to collagen, are critical events for platelet plug formation.[42,46,47] Previous studies reported associations between GPIbα and GPVI, or its co-receptor FcRγ suggesting potential interplay between these signaling pathways.[25,26,28] Functional crosstalk between these signaling pathways is supported by the diminished VWF-GPIbα-dependent responses in platelets deficient in GPVI and by the ability of VWF to further potentiate platelet secretion in response to CRP.[13,27,28]

In order to explore GPIbα signaling function and its influence upon GPVI signaling, we generated *GpIbα*$^{\Delta sig/\Delta sig}$ mice by introduction of a stop codon downstream of the main filamin binding site (a.a. 668-681), but upstream of the 14-3-3 isoforms and PI3K binding regions that are important for VWF-GPIbα signaling.[8-12,48] (Figure 1) This resulted in uniform production of platelets that express GPIbα with truncated intracellular tail. This circumvented the limitations associated with studying/expressing platelet receptor complexes in heterologous cellular systems. Previously generated full knockout (*GPIbα*$^{-/-}$) and also GPIbα/IL4Rα-tg mice that lack the extracellular region of GPIbα do not enable analysis of VWF signaling *per se*, as they lack the ability to bind VWF, meaning that one cannot dissociate the effects of loss of VWF binding and/or VWF signaling upon functional effects upon the platelets.[32,35] Transgenic mice (hTg^{Y605X}) that express human GPIbα that lacks the terminal 6 a.a. of the intracellular tail displayed reduced megakaryocyte recovery following induced thrombocytopenia,[49] but more recent *in vitro* studies have revealed that these mice do not lack the entire 14-3-3/PI3K binding region,[9,10,12] suggesting that their VWF signaling function may not be fully disrupted making interpretation of the mouse phenotype difficult.

GpIbα$^{\Delta sig/\Delta sig}$ mice had a modest reduction in platelet counts compared to *GPIbα*$^{+/+}$ littermates that is likely be attributable to the small increase in platelet size (Figure 2A and B). Interestingly, platelet size is also moderately increased in the GPIbα/IL4Rα-tg mice,[35] but, again, this is modest compared to the size observed in *GPIbα*$^{-/-}$ or in Bernard-Soulier platelets.[32,33] Although the major filamin binding site remains intact in *GpIbα*$^{\Delta sig/\Delta sig}$ mice, our findings may be consistent with CHO cell studies that suggested the presence of additional or extended filamin binding regions within the intracellular tail of GPIbα.[48] By themselves, the 20% reduction in platelet count and slight increase in platelet size would not impart a hemostatic defect.[50]

GpIbα$^{\Delta sig/\Delta sig}$ mice exhibited normal hemostatic responses to tail transection, and normal thrombus formation following mild laser-induced thrombosis (Figure 2D to G). We used a non-perforating endothelial cell injury that does not induce collagen exposure. Therefore, this non-ablasive model is independent of collagen-mediated signaling pathways.[36,38] However, both the tail transection and laser-induced models are sensitive to VWF function.[34,37] Our results reveal the normal VWF-binding function of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets. Normal bleeding times were also reported in *hTg*^{Y605X} transgenic mice with no overt effect on platelet or coagulation functions.[49]

Truncation of the intracellular tail of GPIbα did not alter expression of its extracellular domain (nor influence surface expression of GPIbα, GPVI or αIIbβ3) (Figure 2C). Consequently, *GpIbα*$^{\Delta sig/\Delta sig}$ platelet capture to mouse VWF-coated surfaces was unaffected as well their rolling velocities (Figure 3A to D). Despite normal VWF binding, deletion of the PI3K and 14-3-3 binding region in GPIbα[9,10,12] significantly decreased filopodia extension upon stimulation of VWF binding with botrocetin but also in the presence of an αIIbβ3 antagonist that prevent outside-in signaling induced by the VWF C4 domain binding to activated αIIbβ3 (Figure 3E, G to H). Normal VWF-platelet binding in *GpIbα*$^{\Delta sig/\Delta sig}$ mice is in line with previous studies showing that deletion of the 14-3-3ζ binding site in human GPIbα in GPIb-IX CHO cells does not influence VWF binding, but does reduce their ability to spread.[9,51] Other studies showed that a membrane-permeable inhibitor of the 14-3-3ζ-GPIbα interaction (MP-αC) inhibited GPIbα-dependent platelet agglutination and was protective in murine thrombosis models.[11,52] However, although this peptide disrupts the interaction between 14-3-3ζ and GPIbα, it may also influence 14-3-3ζ function

independent of GPIbα binding. This contention is perhaps supported by a recent study revealing that 14-3-3ζ deficient mice are protected against arterial thrombosis with normal VWF-GPIbα-mediated platelet function.[53]

In addition to defective VWF-mediated signaling, *GpIbα*$^{\Delta sig/\Delta sig}$ platelets exhibited markedly diminished collagen-mediated signaling through GPVI evidenced by reduced surface expression of P-selectin and activation of αIIbβ3, fewer filopodia upon CRP stimulation (Figure 4A, B, E to G), and severely diminished platelet aggregate formation on collagen under venous and arterial shears (Figures 5 to 7). Bernard-Soulier patient platelets have historically been reported to respond normally to collagen in aggregation assays.[54] However, the thrombocytopenia and giant platelets associated with full GPIbα deficiency combined with the loss of VWF-dependent platelet recruitment on collagen impair full analysis of other platelet signaling pathways under physiological flow conditions. Interestingly, although early studies on Bernard-Soulier patients reported that platelet aggregation in response to collagen was normal, their transformation into procoagulant platelets was specifically impaired in response to collagen (but not other agonists).[55] More recently, a Bernard-Soulier patient with mutations in both GPIbα and filamin A was also reported to exhibit defects in GPVI-mediated signaling responses.[56] Although the authors contended that this defect might be due to the filamin A mutation, this may warrant some reappraisal in light of the data presented herein. Like Bernard-Soulier platelets, we found that *GpIbα*$^{\Delta sig/\Delta sig}$ platelets aggregated normally in response to CRP (Figure 4C to D). The signaling deficit presumably allows sufficient activation of αIIbβ3 for the platelets to aggregate. This is perhaps unsurprising given that *Gp6*$^{+/-}$ platelet aggregation is only affected at low collagen concentrations.[57,58] Taken together, previous studies support the contention that Bernard-Soulier patient platelets exhibit a partial deficit in GPVI signaling that resembles the deficit in *GpIbα*$^{\Delta sig/\Delta sig}$ mouse platelets.

Platelets can interact with collagen directly through GPVI and α2β1, and indirectly via GPIbα binding to VWF, the latter being increasingly important as shear rates rise to first capture the platelets and enable the aforementioned direct interactions to take place.[42,59] This is demonstrated in wild-type mice, similar to previous reports,[43,60] by the markedly reduced binding of platelets to collagen in the absence of plasma (and therefore VWF) at medium shear rates (Figure 6A, B and D). Although we demonstrated that *GpIbα*$^{\Delta sig/\Delta sig}$ platelets bind VWF normally, we saw the largest defect in platelet coverage/accumulation when compared to wild-type mice at 3,000 s^{-1} (Figure 5). Based on these results, it seems likely that VWF-GPIbα signaling is also important at these high shear rates, similar to the importance of GPIbα binding to VWF for platelet tethering. We therefore contend that under medium/high shear conditions, VWF-GPIbα platelet priming induces some rapid activation of αIIbβ3, which enable the platelets to better withstand the higher shear rates, prior to their interaction/activation by collagen (Figure 8). Although most evident at the highest shear rates, *GpIbα*$^{\Delta sig/\Delta sig}$ platelets exhibited reduced accumulation at venous shear rates (Figure 7C). Given that the surface coverage on collagen was not significantly altered at 200 s^{-1} in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets compared to wild-type platelets (Figure 7A and B), the deficit in subsequent platelet accumulation must be due to reduced reactivity of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets. This is supported by the clear importance of αIIbβ3 activation to this assay, demonstrated by the effects of GR144053 in preventing 3D accumulation of platelets at both 200 s^{-1} and 1,000 s^{-1} in wild-

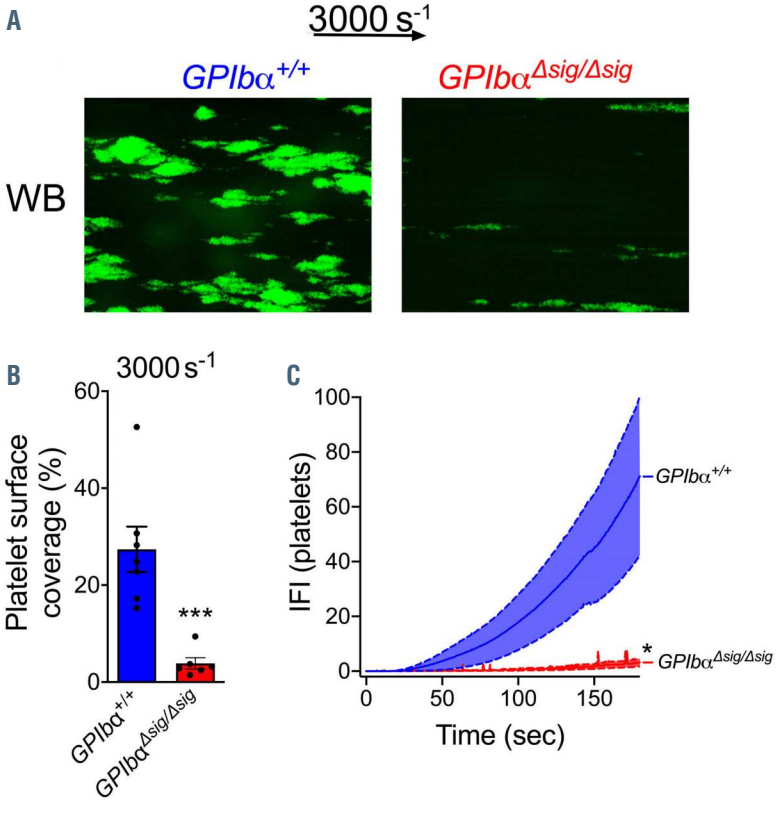

Figure 5. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets have a reduced ability to bind to collagen and form microthrombi at 3,000 s^{-1}. Hirudin anticoagulated whole blood from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice was labeled with anti-GPIbα-DyLight488 antibody and perfused over fibrillar collagen type I (0.2 mg/mL) at a shear rate of 3,000 s^{-1} for 3 minutes. (A) Representative fluorescence images (n=6) after 3 minutes of perfusion in whole blood (WB) from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice at 3,000 s^{-1}. Platelet deposition (B) and thrombus build-up measured as integrated fluorescence intensity (IFI) (C). All data is shown as mean ± standard error of the mean and analyzed using unpaired two-tailed student's *t*-test The maximal platelet IFI was used to compare the thrombus build up data. **P*<0.05, ****P*<0.001. Scale bar 100 μm. Also see the *Online Supplementary Video S3*.

type platelets (Figure 6A to D; Figure 7A to D). We also observed an increase in the platelet coverage in wild-type platelets in the presence of the αIIbβ3 blocker. This is in line with our previous study and others showing that αIIbβ3 blockade allows the formation of a platelet monolayer, but prevents thrombus growth in 3D and also lateral platelet-platelet aggregation (Figure 6B; Figure 7B).[5,45,61,62] This underscores the importance in quantifying both platelet coverage and accumulation in flow assays when studying platelet signaling defects.[45,61] Importantly, GR144053 did not alter these parameters when added to *GpIbα*$^{\Delta sig/\Delta sig}$ platelets (Figure 6A, B, D and E; Figure 7A, B, D and E), demonstrating a lack of αIIbβ3 activation that would be consistent with a diminished GPVI-mediated signaling response. It is important to note that this response is diminished, rather than ablated as the addition of JAQ1 led to a marked decrease in both platelet tethering and accumulation at both 1,000 s^{-1} and 200 s^{-1} shear rates (Figure 6F to I; Figure 7F to I). The question remains open as to the precise contribution of VWF-GPIbα *versus* collagen-GPVI signaling deficits to the phenotype of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets. Our data suggest that both signaling pathways likely con-

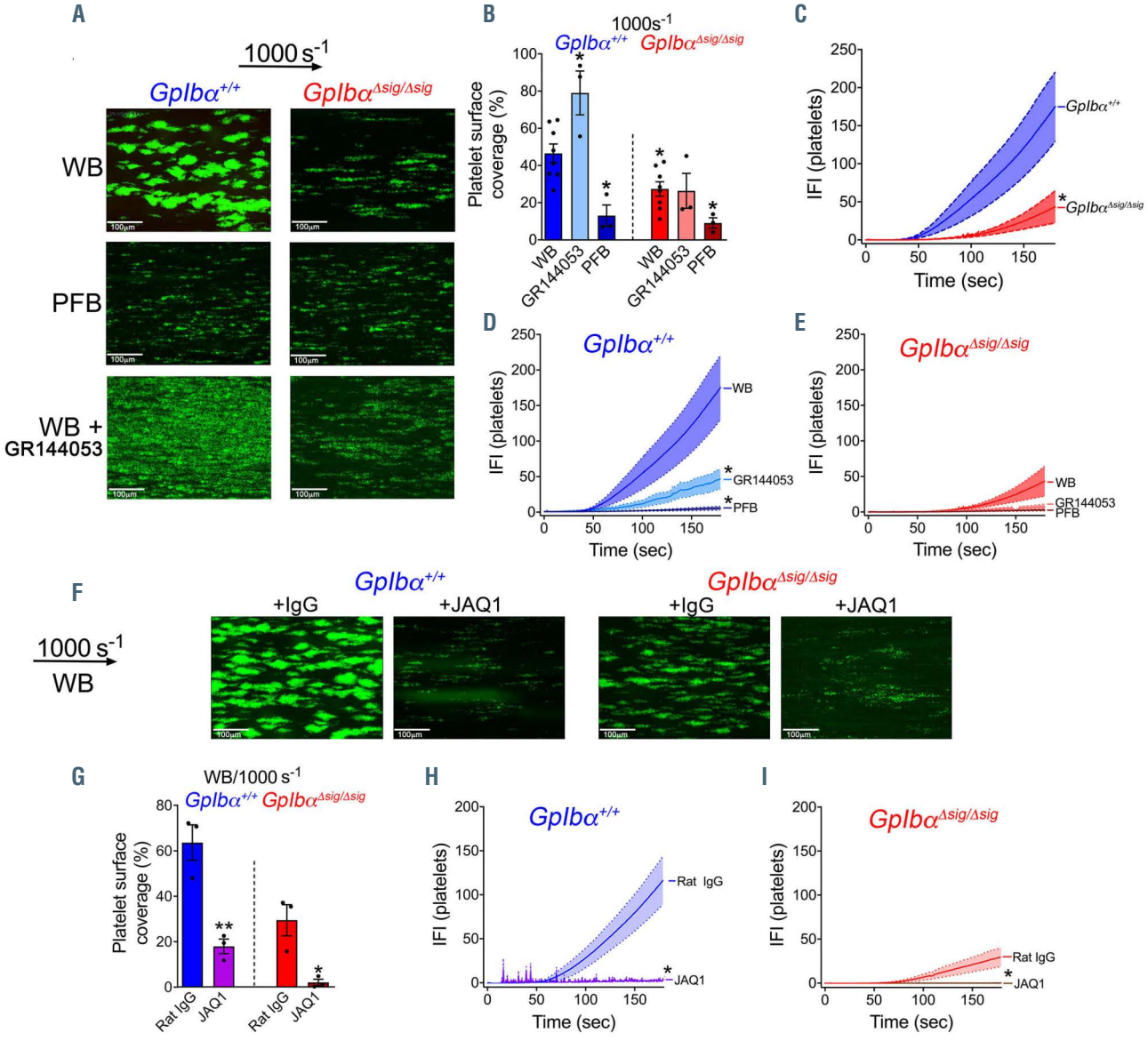

Figure 6. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets have a reduced ability to bind to collagen and form microthrombi at 1,000 s^{-1}. (A to E) Hirudin anticoagulated whole blood supplemented or not with GR144053 or plasma-free blood from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice was labeled with anti-GPIbα-DyLight488 antibody (Ab) and perfused over fibrillar collagen type I (0.2 mg/mL) at a shear rate of 1,000 s^{-1} for 3 minutes (min). (A) Representative fluorescence images (n≥3) after 3 min of perfusion in whole blood (WB), plasma-free blood (PFB) or WB + GR144053 from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice. Platelet deposition (B) and thrombus build-up measured as integrated fluorescence intensity (IFI) (C to E). All data is shown as mean ± standard error of the mean and analyzed using unpaired two-tailed student's *t*-test (C) or one-way ANOVA followed by Dunnett's multiple comparison test (B, D to E). Data is compared to means from *GpIbα*$^{+/+}$ WB (B and D) or *GpIbα*$^{\Delta sig/\Delta sig}$ WB (E). The maximal platelet integrated fluorescence intensity (IFI) was used to compare the thrombus build up data. *P<0.05. Scale bar 100 µm. Also see the *Online Supplementary Video S4*. (F to I) Hirudin anticoagulated whole blood from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice supplemented with JAQ1 or Rat-IgG control Ab (20 µg/mL) was labeled with anti-GPIbα-DyLight488 Ab and perfused over fibrillar collagen type I (0.2 mg/mL) at a shear rate of 1,000 s^{-1} for 3 min. (F) Representative fluorescence images (n=3) after 3 min of perfusion. Platelet deposition (G) and thrombus build-up measured as IFI (H and I). All data is shown as mean ± standard error of the mean and analyzed using unpaired two-tailed student's *t*-test. The maximal platelet IFI was used to compare the thrombus build up data. *P<0.05, **P<0.01. Scale bar 100 µm.

tribute to this, as disruption of either interaction causes a major reduction in platelet accumulation in wild-type platelets under both venous and arterial shear rates.

GPVI belongs to the immunoglobulin superfamily and signals via tyrosine kinase phosphorylation pathways. In order to further investigate the defect in GPVI signaling in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets, analysis of tyrosine phosphorylation downstream of GPVI revealed that SYK and PLCγ2 phosphorylation was reduced in *GpIbα*$^{\Delta sig/\Delta sig}$ platelets (Figure 4H to K). Interestingly, the diminished phosphorylation was more pronounced for SYK than for PLCγ2 perhaps highlighting the existence of LAT-independent mechanisms of PLCγ2 phosphorylation.[63] Interestingly, activation of *GpIbα*$^{\Delta sig/\Delta sig}$ platelets via CLEC-2, another receptor that signals via an ITAM motif,[64] was also affected, but perhaps to a lesser extent than those mediated by GPVI (*Online Supplementary Figure S4*) suggesting that the function of the GPIbα intracellular tail is more important for GPVI mediated responses. Based on these findings, we hypothesize that the tail of GPIbα may be important for the docking of signaling molecules such as SYK, LAT and PLCγ2 that are downstream of GPVI and CLEC-2 on ITAM phosphorylated motif of the FcRγ and CLEC-2 receptors and warrant further investigation. It would also be of interest to determine if the reduction in PI3K signaling in response to CRP stimulation (Figure 4I to L) is due to the lack of binding of

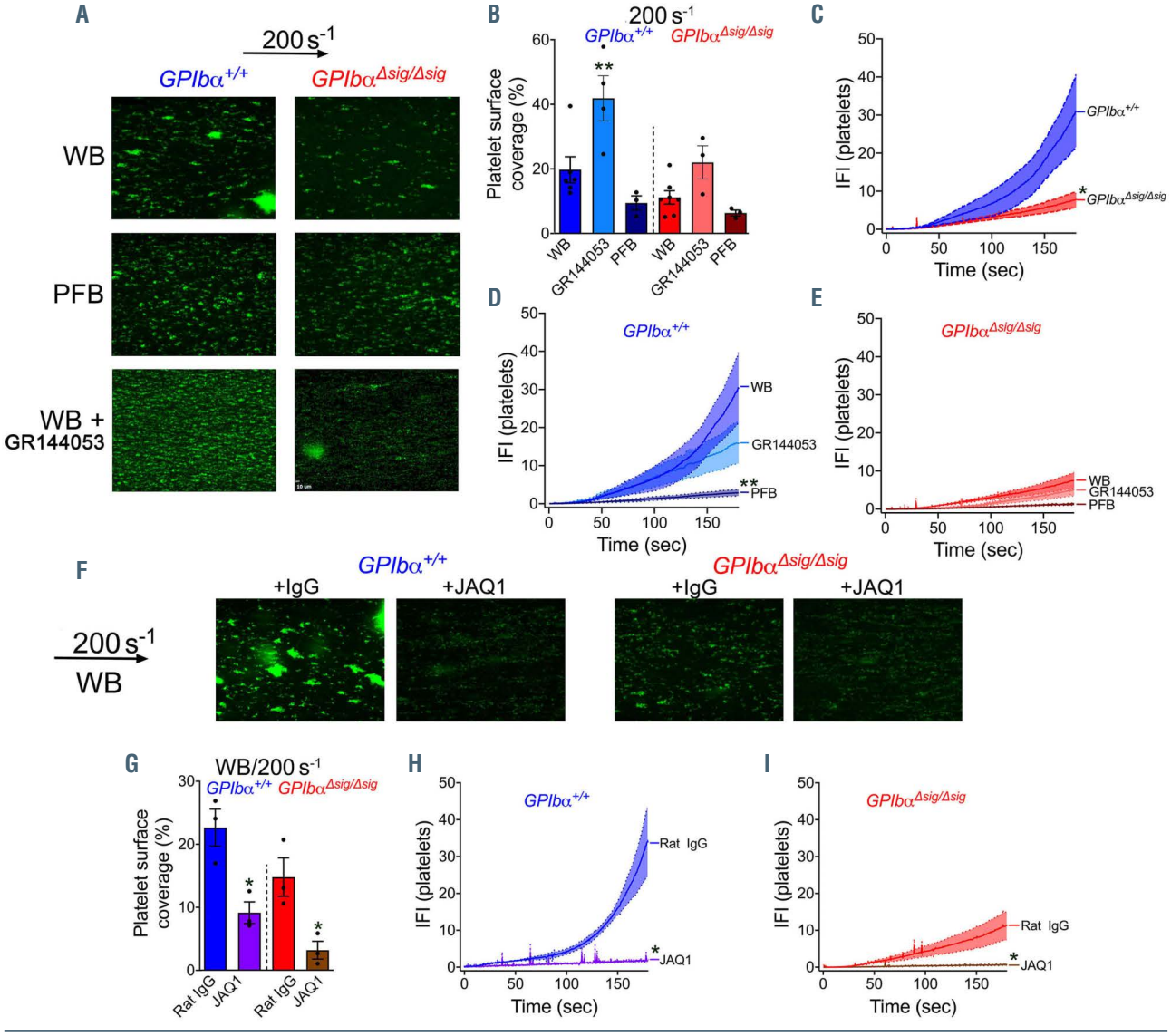

Figure 7. *GpIbα*$^{\Delta sig/\Delta sig}$ platelets have a reduced ability to bind to collagen and form microthrombi at 200 s^{-1}. (A to E) Hirudin anticoagulated whole blood supplemented or not with GR144053 or plasma-free blood from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice was labeled with anti-GPIbα-DyLight488 Ab and perfused over fibrillar collagen type I (0.2 mg/mL) at a shear rate of 200 s^{-1} for 3 minutes (min). (A) Representative fluorescence images (n≥3) after 3 min of perfusion in whole blood (WB), plasma-free blood (PFB) or WB + GR144053 from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice. Platelet deposition (B) and thrombus build-up measured as integrated fluorescence intensity (IFI) (C to E). All data is shown as mean ± standard error of the mean and analyzed using unpaired two-tailed student's *t*-test (C) or one-way ANOVA followed by Dunnett's multiple comparison test (B, D to E). Data is compared to means from *GpIbα*$^{+/+}$ WB (B and D) or *GpIbα*$^{\Delta sig/\Delta sig}$ WB (E). The maximal platelet integrated fluorescence intensity (IFI) was used to compare the thrombus build-up data. *P<0.05, **P<0.01. Scale bar 100 μm. Also see the *Online Supplementary Video S5*. (F to I) Hirudin anticoagulated whole blood from *GpIbα*$^{+/+}$ and *GpIbα*$^{\Delta sig/\Delta sig}$ mice supplemented with JAQ1 or Rat-IgG control antobodies (20 μg/mL) was labeled with anti-GPIbα-DyLight488 Ab and perfused over fibrillar collagen type I (0.2 mg/mL) at a shear rate of 200 s^{-1} for 3 min. (F) Representative fluorescence images (n=3) after 3 min of perfusion. Platelet deposition (G) and thrombus build-up measured as IFI (H and I). All data is shown as mean ± standard error of the mean and analyzed using unpaired two-tailed student's *t*-test. The maximal platelet IFI was used to compare the thrombus build up data. *P<0.05. Scale bar 10 μm.

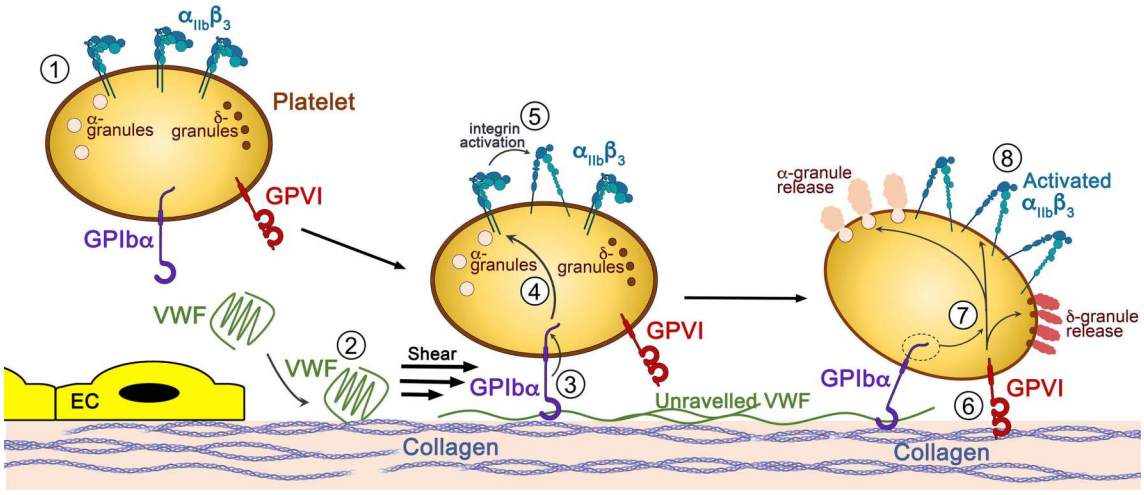

Figure 8. Proposed model for GPIbα-GPVI cross talk. Under normal conditions, resting/circulating platelets (1) present αIIbβ3 on their surface in its closed conformation. Plasma von Willebrand factor (VWF) (2) circulates in its globular conformation with its A1 domain hidden, preventing interaction with platelet GPIbα. Upon vascular injury, the subendothelial extracellular matrix containing collagen becomes exposed to the blood. VWF, via its A3 domain, binds to collagen and, due to shear forces, unravels to expose its A1 domain to which platelet GPIbα binds (3). Next, mechanosensitive signaling events downstream of VWF A1-GPIbα that require the intracellular tail of GPIbα take place leading to some activation of surface αIIbβ3 (4) while the deceleration of platelets allows for the subsequent binding of platelets to collagen via several collagen receptors including GPVI (6). The intracellular tail of GPIbα is also crucial for optimal collagen/GPVI signaling that lead to platelet activation, shape change and granule release (7). Ultimately, additional circulating platelets will be recruited at the site of injury to form the hemostatic plug (8).

PI3K to the intracellular tail of GPIbα or it is a consequence of diminished SYK phosphorylation.[65]

In summary, we generated a novel GPIbα transgenic mouse in which their platelets bind VWF normally, but the subsequent VWF-GPIbα signaling is disrupted. Intriguingly, these mice clearly reveal the molecular link between GPIbα- and GPVI-mediated signaling in platelets and underscore the cooperative functions of these two major platelet receptors.[45] Platelets in addition to their important role in thrombosis and hemostasis contribute to the host response to infection and inflammation.[66-69] Our recent work suggests that VWF-GPIbα-dependent platelet priming potentiates the recruitment of neutrophils, which may represent a key early event in the targeting of pathogens, but also in the development of deep vein thrombosis.[5] The *GpIbα*$^{\Delta sig/\Delta sig}$ mice now provide an invaluable tool to probe the importance of the GPIbα-mediated signaling in inflammatory diseases such as atherosclerosis and deep vein thrombosis, as well as in the host response to infection but also to fully decipher the molecular dependency of GPVI signaling upon GPIbα.

Disclosures

No conflicts of interest to disclose.

Contributions

AC-B designed and performed experiments, analyzed data and wrote the manuscript; YAW performed experiments and revised the manuscript; KJW designed and performed experiments and revised the manuscript; PM and KV provided critical reagents and revised the manuscript; JTBC designed experiments, prepared the figures and wrote the manuscript; IIS-C designed and performed experiments, analyzed data, prepared the figures and wrote the manuscript.

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Data sharing statement

Additional information on original data and protocols will be available upon request via email i.salles@imperial.ac.uk.

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