Supplementary Fig. S1 Platelet viability, as indicated by the level of platelet adenosine triphosphate (ATP), is not altered by platelet isolation procedure via gel filtration (A); or over the storage time up to 6 hours following platelet isolation (B). Platelet count in platelet-rich plasma (PRP) and gel-filtered platelets was adjusted with modified Tyrode’s buffer. ATP concentration indicating cell metabolic activity was measured using luminescent cell viability assay. Residual ATP level detected in plasma was subtracted from total ATP in PRP to quantify actual platelet ATP (A, “PRP” group). In Fig. 1B, ATP level of $1 \times 10^6$ platelets is reported. In graphs, data of 4 independent experiments with different donors are summarized, mean ± standard deviation (SD) as error bars are plotted. p-Values were calculated vs. “0 hours” group by one-way analysis of variance (ANOVA): no asterisk $p > 0.05$.

Supplementary Fig. S2 Representative curves of platelet aggregation induced by biochemical agonists in platelet-rich plasma (PRP) and gel-filtered platelets (GFP) of the same donor. Platelet concentration in PRP and GFP was adjusted to 250,000 platelets/µL and 150,000 platelets/µL, respectively. PRP and GFP samples were recalcified with 1 mM CaCl$_2$, and 3 mg/mL fibrinogen was added to GFP. No fibrinogen was added to GFP in case of thrombin-mediated platelet aggregation to prevent clotting. The following agonist concentrations were used to induce platelet aggregation: 10 µM adenosine diphosphate (ADP), 32 µM thrombin receptor-activating peptide 6 (TRAP-6), 10 µg/mL collagen, 1 mM arachidonic acid, 10 µg/mL epinephrine, and 1 U/mL thrombin.
Supplementary Fig. S3 Biochemical agonists induce P-selectin exposure on the platelet surface. The following agonist concentrations were used to induce platelet activation: 10 µM adenosine diphosphate (ADP), 10 µg/mL epinephrine, 100 µg/mL collagen, 32 µM thrombin receptor activating peptide 6 (TRAP-6), 1 U/mL thrombin, and 1 mM arachidonic acid. Recalci

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gel-filtered platelets (GFP) (20,000 platelets/µL, 2.5 mM CaCl₂) were incubated with agonists for 10 minutes at room temperature. P-selectin exposure was detected using anti-CD62P-APC (clone Psel. KO2.3, eBioscience); the number of CD62P-positive platelets (P6-population) were measured by flow cytometry. Representative diagrams showing APC fluorescence intensity versus particle count for every agonist are reported from the left. In a bar graph, data of 4 independent experiments with different donors are summarized, mean (M) ± standard deviation (SD) as error bars are plotted. p-Values were calculated as compared with group “intact platelets” by one-way analysis of variance (ANOVA): **p ≤ 0.01.
Supplementary Fig. S4 Biochemical agonists, adenosine diphosphate (ADP), and thrombin, promote αIIbβ3 integrin activation. Recalciﬁed gel-ﬁltered platelets (GFP) (20,000 cells/µL, 2.5 mM CaCl2) were incubated with agonists undisturbed for 10 minutes at room temperature. αIIbβ3 expression and activation were detected using dual immunostaining with anti-CD41/CD61-ﬂuorescein isothiocyanate (FITC) (clone PAC-1, BioLegend) and anti-CD41-APC (clone MEM-06, Thermo Scientiﬁc). Percentage of CD41/CD61-positive platelets (marked as P3-population) among CD41-positive population were quantiﬁed. Representative diagrams showing FITC ﬂuorescence intensity vs. particle count are reported from the left. In a bar graph data of 6 independent experiments with different donors are summarized, mean (M) ± standard deviation (SD) as error bars are plotted. p-Values were calculated vs. intact platelets by one-way analysis of variance (ANOVA): **p < 0.01.