Supplementary Information

Materials
The anesthetic drugs Hypnorm (VetaPharma Ltd) and Midazolam (Roche) were used according to the regulations defined by Imperial College London and the UK Home Office. Prostaglandin E1 (PGE1), apyrase, ADP were from Sigma. U46619 was purchased from Cambridge Biosciences, human \( \alpha \)-thrombin from Enzyme Research Laboratories, and collagen related peptide (CRP) from Professor Farndale laboratory (Cambridge University). All materials related to aggregation experiments were from Labmedics. The antibodies (Abs) against \( \alpha_{\text{IIb}}\beta_3 \) (Leo.H4), GPVI (JAQ1), GPIb\( \beta \) (X488), P-selectin (Wug.E9) and the activated form of \( \alpha_{\text{IIb}}\beta_3 \) (JON/A) were all from Emfret Analytics.

Genotyping
The program used for all PCR was as follows: 2 minutes (min) at 94°C, 15 cycles of 30 seconds (sec) at 94°C, 30 sec at 62°C for the first cycle decreasing to 55°C for the last cycle, 40 sec at 72°C; 16 cycles of 30 sec at 94°C, 30 sec at 55°C, 40 sec at 72°C and followed by a final incubation of 2 min at 72°C.

RT-PCR
The following primers were used for RT-PCR analysis of Bambi: RF1 (GTGTAGGCGTTGCTCTCTGT); RR1 (CTTT-GGTGAGCAGCACAGCC); RR2 (C GTCATGCAGTCCTCGATAA); RF2 (GCGAA-GCTTGCGTCAATGGATCGCCACTCC); RR3 GCGCCTCGAGGGCGGTCATATG-ATTCCAGC; and Gapdh control: GAPDH FW (ACCACAGTCCATGCCATCAC); GAPDH R (TCCAC-CACCCTGTGGCTGTA). The following PCR program was used for the PCR reactions: 1 cycle of 94°C for 30 sec, 35 cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 68°C followed by a final extension of 5 min at 68°C.

Platelet preparation
An equal volume of modified tyrode’s buffer [134mM NaCl, 0.3mM Na\(_2\)HPO\(_4\), 2.9mM KCl, 12mM NaHCO\(_3\), 20mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 5mM Glucose, 1mM MgCl\(_2\), pH 7.3, 0.35% bovine serum albumin (BSA)] supplemented with 1µM Prostaglandin E1 (PGE1) and 20 mU/ml apyrase was added to citrated blood prior to centrifugation at 80xg for 10 min at room temperature (RT) to obtain platelet-rich plasma (PRP). For the preparation of washed platelets, PRP was centrifuged twice at 700xg for 10 min at RT, and the platelet pellet was resuspended in modified tyrode’s buffer.

Thrombin generation
PRP was prepared as described above and platelet numbers adjusted with autologous PPP to 3x10^8/ml. PPP was prepared by a two-step centrifugation at 10000xg. Single mouse PRP and PPP preparations (40 µl per well) were used with 1pM tissue factor (Dade Innovin, Dade Behring), 4µM phospholipid vesicles (1, only in PPP) and 16.6mM CaCl\(_2\). Contact activation coagulation was inhibited by adding corn
trypsin inhibitor (65 µg/ml plasma) and thrombin generation quantified by adding 0.42mM of Z-GlyArg-AMC-HCl (Bachem). Samples were run at least in duplicate with n≥3 animals per group.

**Thrombin-Antithrombin complexes (TAT) analysis**

Plasma levels of TAT were determined using a murine TAT-ELISA kit (Abcam) according to the manufacturer’s instructions. Samples were run in duplicate or triplicate with n=5 animals per group.

**Supplemental references:**


<table>
<thead>
<tr>
<th>Mouse</th>
<th>Bambi *+/+</th>
<th>Bambi *−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP</td>
<td>58.9±4.5</td>
<td>61.1±3.2</td>
</tr>
<tr>
<td>PRP</td>
<td>79.9±2.4</td>
<td>74±2.2</td>
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</table>

**TableS1: Normal thrombin generation peak heights in Bambi *−/−* mice.** Values given are mean nM Thrombin ± SEM (n≥3). Statistical analysis was performed using unpaired Student t test (P>0.05).

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Bambi *+/+</th>
<th>Bambi *−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6/µL)</td>
<td>9.3±0.7</td>
<td>10.1±0.6</td>
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<tr>
<td>WBC (10^3/µL)</td>
<td>8.6±1.1</td>
<td>8.4±1.4</td>
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<tr>
<td>Hg (g/dL)</td>
<td>13.6±1</td>
<td>14.5±1.1</td>
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<tr>
<td>Ht (%)</td>
<td>43.8±3</td>
<td>47±3.4</td>
</tr>
<tr>
<td>PLT (10^3/µL)</td>
<td>1009±54.8</td>
<td>1111±115</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>6.7±0.1</td>
<td>6.6±0.1</td>
</tr>
</tbody>
</table>

**TableS2: Hematological values in Bambi chimeric mice.** RBC, red blood cells; WBC, white blood cells; Hg, haemoglobin; Ht, haematocrit; PLT, platelets; MPV, mean platelet volume. Values given are mean ± SEM of n=4 males for each group at 7 weeks post-transplantation. Statistical analysis was performed using unpaired Student t test (P>0.05).
Figure S1. Successful ablation of the Bambi allele. RT-PCR analysis of lungs isolated from wild-type and Bambi +/- mice using primers specific to Bambi transcript (1-3) or Gapdh control (Ctrl).

Figure S2. Bambi +/- animals develop slower than wild-type littermates. (A) Males and (B) females wild-type, Bambi +/- and Bambi +/- mice weight at 6 week of age. Each symbol represents one animal. Horizontal lines intersecting datasets represent the mean. Statistical analysis was performed using Anova (** p<0.01).
Figure S3. Normal platelet receptor expression in Bambi deficient mice. Flow cytometry analysis of platelets incubated with anti-murine antibodies against GPVI, GPIbβ and αIibβ3 (all from Emfret). Results are expressed in percentage of mean fluorescence intensities in the control Bambi +/+ platelets ± SEM (n>10). Black bars: Bambi+/+; Hatched bars: Bambi +/−; White bars: Bambi −/− mice. Statistical analysis was performed using Anova (p>0.05).

Figure S4. Successful transplantation of Bambi chimeric mice. PCR was performed from BMC (10^6) isolated from Bambi +/+ mice that received Bambi −/− BMC (left) or Bambi −/− mice that received Bambi +/+ BMC (right), 7 weeks post transplantation. The Bambi deleted allele (bottom left) was clearly present in Bambi +/+ mice that received Bambi −/− BMC while the wild-type allele (top right) was detected in Bambi −/− mice that received Bambi +/+ BMC.

Supplemental video 1. FeCl3-induced thrombosis model in a Bambi +/+ mouse. Representative video of fluorescently-labeled platelets (green) accumulating in a mesenteric arteriole of a Bambi +/+ mouse after FeCl3 injury. Thrombus formation was studied until vessel was occluded or up to 40 min. A timer is shown in the bottom left corner (mm:ss).

Supplemental video 2. FeCl3-induced thrombosis model in a Bambi −/− mouse. Representative video of fluorescently-labeled platelets (green) accumulating in a mesenteric arteriole of a Bambi −/− mouse after FeCl3 injury. Thrombus formation was studied until vessel was occluded or up to 40 min. A timer is shown in the bottom left corner (mm:ss).
Supplemental video 3. FeCl₃-induced thrombosis model in a *Bambi*⁺⁻ mouse. Representative video of fluorescently-labeled platelets (green) accumulating in a mesenteric arteriole of a *Bambi*⁺⁻ mouse after FeCl₃ injury. Thrombus formation was studied until vessel was occluded or up to 40 min. A timer is shown in the bottom left corner (mm:ss).

Supplemental video 4. Laser-induced thrombus formation in a *Bambi*⁺⁻ mouse. Representative video of fluorescently-labeled platelets (green) accumulating at the site of laser-induced injury in a cremaster muscle arteriole of a *Bambi*⁺⁻ mouse. Thrombus formation was studied using a combination of brightfield and fluorescence microscopy. A timer is shown in the top left corner (hh:mm:ss:000) and a 10 μm scale bar in the bottom left corner.

Supplemental video 5. Laser-induced thrombus formation in a *Bambi*⁻⁻ mouse. Representative video of fluorescently-labeled platelets (green) accumulating at the site of laser-induced injury in a cremaster muscle arteriole of a *Bambi*⁻⁻ mouse. Thrombus formation was studied using a combination of brightfield and fluorescence microscopy. A timer is shown in the top left corner (hh:mm:ss:000) and a 10 μm scale bar in the bottom left corner.

Supplemental video 6. Laser-induced thrombus formation in a *Bambi*⁻⁻ mouse. Representative video of fluorescently-labeled platelets (green) accumulating at the site of laser-induced injury in a cremaster muscle arteriole of a *Bambi*⁻⁻ mouse. Thrombus formation was studied using a combination of brightfield and fluorescence microscopy. A timer is shown in the top left corner (hh:mm:ss:000) and a 10 μm scale bar in the bottom left corner.

Supplemental video 7. Laser-induced thrombus formation in a lethally irradiated *Bambi*⁺⁻ mouse transplanted with *Bambi*⁻⁻ bone marrow cells. Representative video of fluorescently-labeled platelets (green) accumulating at the site of laser-induced injury in a cremaster muscle arteriole of a lethally irradiated *Bambi*⁺⁻ mouse receiving bone marrow transplantation from *Bambi*⁻⁻ mice. Thrombus formation was studied using a combination of brightfield and fluorescence microscopy. A timer is shown in the top left corner (hh:mm:ss:000) and a 10 μm scale bar in the bottom left corner.

Supplemental video 8. Laser-induced thrombus formation in a lethally irradiated *Bambi*⁻⁻ mouse transplanted with *Bambi*⁺⁻ bone marrow cells. Representative video of fluorescently-labeled platelets (green) accumulating at the site of laser-induced injury in a cremaster muscle arteriole of a lethally irradiated *Bambi*⁻⁻ mouse receiving bone marrow transplantation from *Bambi*⁺⁻ mice. Thrombus formation was studied using a combination of brightfield and fluorescence microscopy. A timer is shown in the top left corner (hh:mm:ss:000) and a 10 μm scale bar in the bottom left corner.