Reagents and antibodies. PAR4 receptor-activating peptide H-GYPGKF-NH2 (Advanced ChemTech, Louisville, KE), annexinV-Alexa488 and calcium-sensing dye Fluo-3 (Invitrogen, Carlsbad, CA), antibodies to P-selectin, CD45.1, and B220 (BD Biosciences, Rockville, MD), and JON/A-PE (antibody against activated αIIbβ3, emfret Analytics, Wuerzburg, Germany) were purchased. Convulxin was kindly provided by Dr. K. Clemetson, Switzerland.

Generation of Orai1^{R93W} knock-in mice. Gene targeting of the *Orai1 g*ene was performed by homologous recombination in B6/3 ES cells derived from C57BL/6 mice (TaconicArtemis GmbH, Koln, Germany). To generate Orai1^{R93W} knock-in mice, codon 93 (CGG encoding R93) in exon 1 of the Orai1 gene was replaced with TGG (encoding W93). Chimeric mice with targeted *Orai1* alleles were generated by blastocyst injection of heterozygous *Orai1*$^{neo/+}$ ES cell clones. The Neo cassette was deleted by Cre expression under the control of the testis specific ACE promoter during spermatogenesis in chimeric mice. Founder *Orai1*$^{neo/+}$ chimeric mice were bred to C57BL/6 mice to establish *Orai1*$^{+/R93W}$ mice. Successful gene-targeting was confirmed by two PCR approaches, detecting (1) the remaining loxP site in the *Orai1* locus after excision of the Neo^{r}/ACE-Cre cassette and (2) the mutated codon 93, which creates a recognition site for the restriction endonuclease PvuII (CAGCTG). Primers used for detection of the loxP site were:
Forward 5′ATTTCCCAATACGTTCCACCTCCC;
Reverse 5′ TCGTACCACCTTCTTGGGACTTGA.
Primers for PCR amplification of Orai1 exon 1 followed by PvuII digest were: Forward 5′-TGGATCGGCCAGAGTTACTCC;
Reverse 5′ GATTACATGCAGGGCAGACTTCTTA.

Mice and generation of fetal liver chimeras. To generate fetal liver chimeras, fetal liver cells were obtained from E14.5 mouse embryos derived from matings of *Orai1*$^{+/R93W}$ mice on the C57BL/6 background. *Orai1*$^{R93W/R93W}$ and littermate wild-type control cells (CD45.2+) were injected intravenously into irradiated (4.5 + 4.5 Gy) *Rag2*$^{-/-}$, *gc*$^{-/-}$ mice (Taconic) or CD45.1+ wild-type C57BL/6 mice (Taconic). Reconstituted mice were sacrificed 5–6 weeks after transplantation to harvest blood, spleen, lymph nodes and thymus for isolation of T cells, B cells and platelets. All mice were maintained in specific pathogen-free barrier facilities at Harvard Medical School and NYU School of Medicine and were used in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at both institutions.

Aggregometry. Platelet-rich plasma (PRP) was obtained from heparinized whole blood by centrifugation at 100 × g for 10 minutes. Light transmission was measured by using PRP adjusted to 3×10^8 platelets per ml with modified Tyrode's buffer (137 mmol/L NaCl, 0.3 mmol/L Na_2HPO_4, 2 mmol/L KCL, 12 mmol/L $NaHCO_3$, 5 mmol/L Hepes, 5 mmol/L glucose, pH 7.3) containing 0.35% BSA and 1 mM $CaCl_2$. Agonists were added at the indicated concentrations and transmission was recorded over 10 min on a Chrono-log 4-channel optical aggregation system (Chrono-log Corp., Havertown, PA).

Flow cytometry.
Calcium flux measurement: PRP was centrifuged at 700 × g in the presence of PGI_2 (2 μg/mL) for 7 minutes at room temperature. After two washing steps, pelleted platelets were resuspended in modified Tyrode's buffer. Washed platelets were incubated with 5 μM of the calcium-sensing dye Fluo-3 for 15 minutes, activated with the indicated concentrations of PAR4p or Cvx, and

immediately analyzed on a FACScalibur (BD Biosciences). Data were analyzed with FlowJo software (Tree Star, Inc., Ashland, OR).

Platelet activation: Platelets were diluted in Tyrodes buffer containing 1 mM $CaCl_2$, activated with PAR4p and/or Cvx for 10 minutes, and stained for αIIbβ3 activation (JON/A-PE), P-selectin expression (α-Pselectin-FITC) or PS exposure (annexinV-Alexa488).

Surface Expression of CD45.1 and B220 on blood leukocytes: Whole blood was diluted 10-fold in red blood cell lysis buffer (155 mmol/L NH4Cl, 10 mmol/L KHCO3, and 0.1 mmol/L Na2EDTA, pH 7.4) and incubated for 5 minutes at room temperature (RT). After washing, leukocytes were resuspended in modified Tyrode's-HEPES buffer and stained with fluoreophore-labeled antibodies against CD45.1 and B cell marker B220.

Flow chamber studies. Whole blood was perfused over a collagen-coated surface (100 µg/ml collagen Horm) at a wall shear rate of 1000 s^{-1} for 2 minutes, followed by perfusion with Tyrodes buffer containing 2 mM $CaCl_2$, Alexa594-coupled antibodies to GPIbα, and annexinV-Alexa488. Fluorescence signals were visualized with an Axiovert 135 inverted microscope (Zeiss) equipped with a 100-W HBO fluorescent lamp source (Optiquip, Highland Mills, NY) and a silicon-intensified tube camera (C 2400; Hamamatsu, Middlesex, NJ) connected to an S-VHS video recorder (AG-6730; Panasonic, Matsushita Electric, Osaka, Japan). Images were analyzed using NIH Image 1.61 software (NIH, Bethesda, MD).

RT-PCR. Whole blood was drawn from a healthy volunteer. Platelets were purified from platelet-rich plasma by negative selection for CD45 with magnetic beads (MACS column, Miltenyi Biotec). Meg-01 cells (ATCC, Manassas, VA) were grown in RPMI-1640 medium supplemented with 10% FBS. Total RNA was isolated from highly purified platelets and Meg-01 cells using TRIZOL reagent (Invitrogen). Total RNA was reverse-transcribed with random primers using SuperScripIII cDNA synthesize kit (Invitrogen). One twentieth of the cDNA obtained was used for PCR amplification using primers specific for each of the 3 Orai transcripts or for integrin a_{IIb} (CD41) as a control. Amplification products were separated by 1% agarose gel electrophoresis and visualized by ethidium bromide staining.