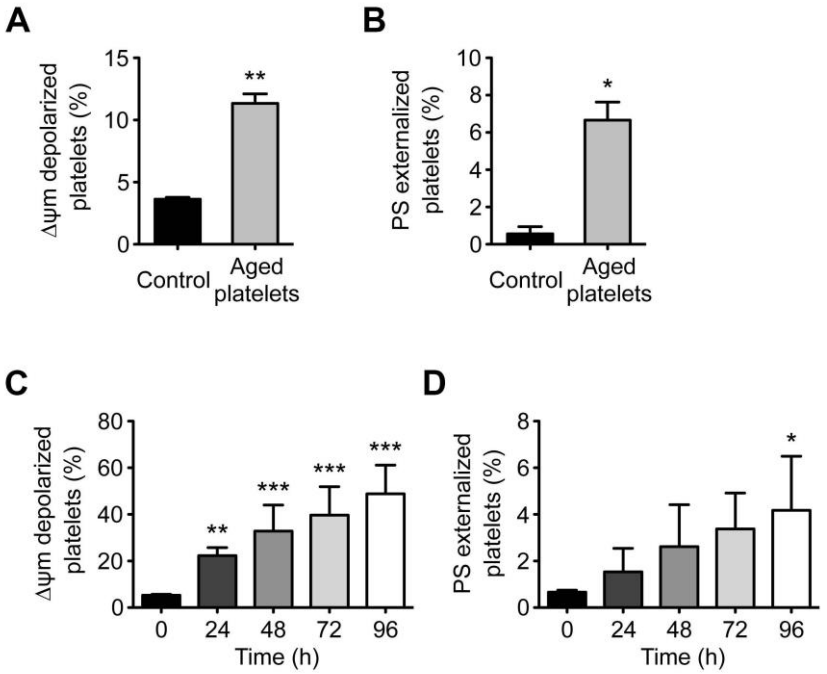
SUPPLEMENTAL DATA

Supplemental Figures

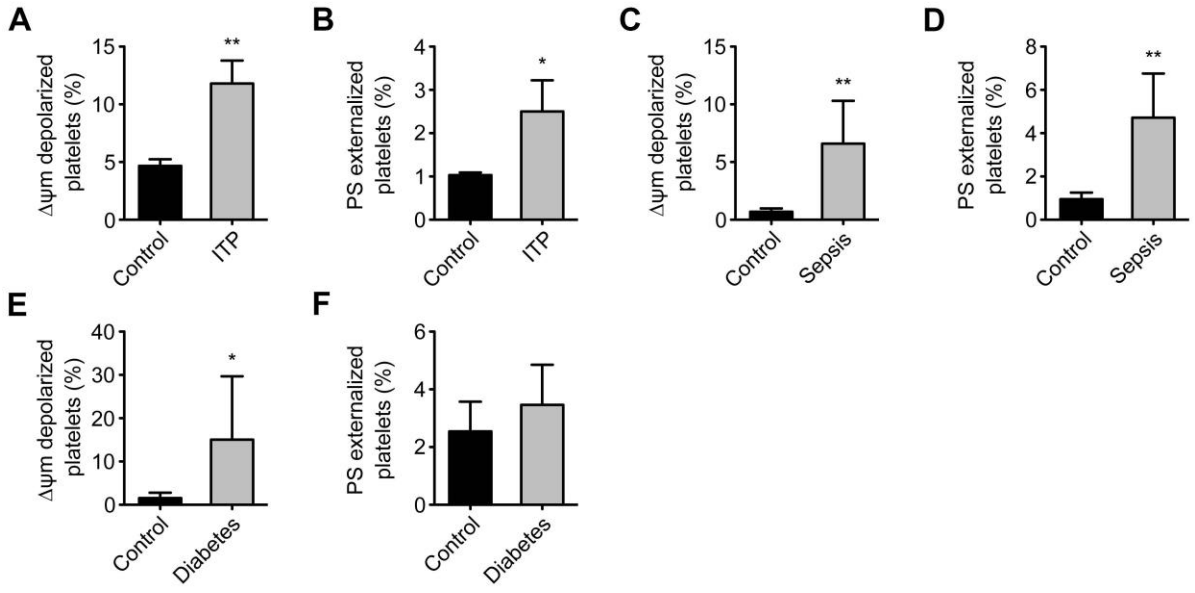
Supplemental Figure 1



Supplemental Figure 1

Apoptotic events of stored platelets. (**A** and **B**) PRP was incubated at 37℃ under agitation for 0 (control) or 16 h. The pretreated platelets were analyzed for mitochondrial membrane potential ($\Delta\psi_m$) depolarization and phosphatidylserine (PS) exposure by flow cytometry. Data are means ± SD from four independent experiments. *P < 0.05, **P < 0.01, compared with control by Student`s t-test. (**C** and **D**) Washed platelets (3×10^8/mL) were incubated at 22℃ for indicated time. $\Delta\psi_m$-depolarized platelets and PS-positive platelets were examined by flow cytometry. The results are from four independent experiments with different donors. *P < 0.05, **P < 0.01, ***P < 0.001 versus control by one-way ANOVA.

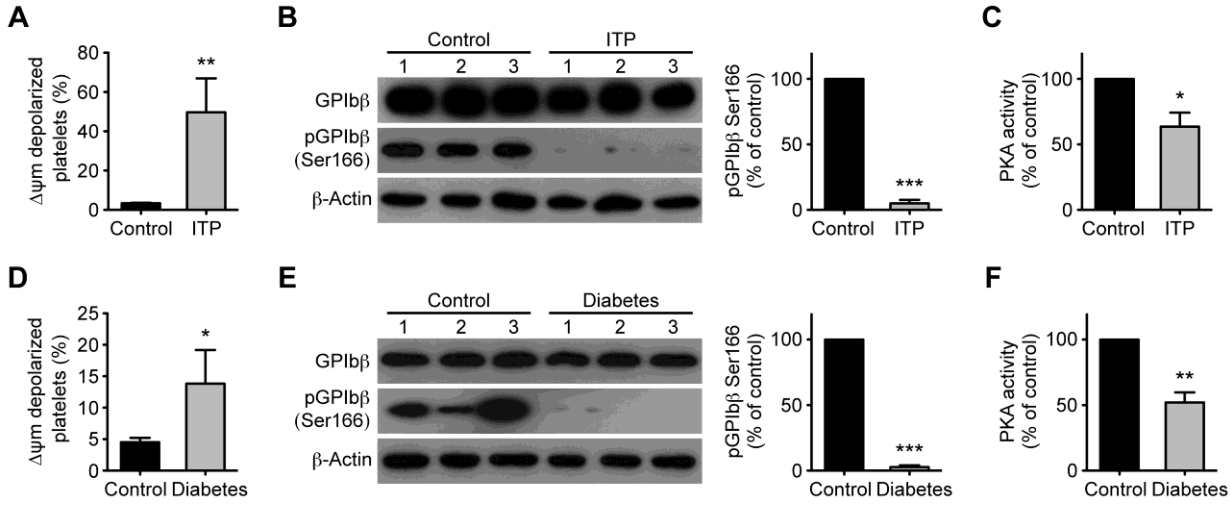
Supplemental Figure 2



Supplemental Figure 2

Apoptotic events in platelets from patients with ITP, diabetes and sepsis. PRP from patients with ITP (**A** and **B**), sepsis (**C** and **D**), and diabetes (**E** and **F**) and age- and sex-matched health controls was analyzed for $\Delta\psi_m$ depolarization and PS exposure by flow cytometry. Data are means ± SD of six patients and age- and sex-matched health controls. **P* < 0.05, ***P* < 0.01, compared with health controls by Student`s *t*-test.

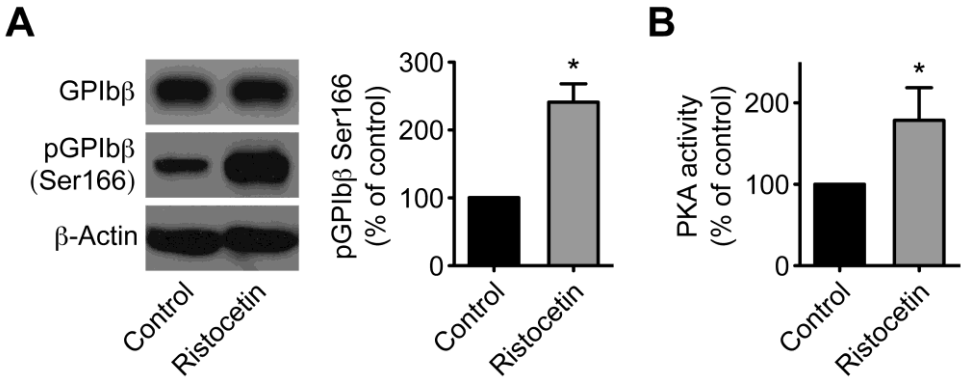
Supplemental Figure 3



Supplemental Figure 3

Plasma from patients with ITP and diabetes induces normal platelet apoptosis and reduction of PKA activity. Washed platelets (3×10^8/mL) were incubated with plasma (1:1 volume) from patients with ITP and diabetes or age- and sex-matched health controls at 37℃ for 12 h. (**A** and **D**) $\Delta\psi_m$-depolarized platelets were examined. Platelets were separated and lysed immediately. Total and phosphorylated GPIbβ was detected by western blot with anti-GPIbβ and anti-pGPIbβ Ser-166 antibodies. Representative western blots are shown. The blots were analyzed by Image J software. Densitometry of immunoblots for pGPIbβ/GPIbβ (arbitrary unit) normalized to control platelets is shown (**B** and **E**). PKA activity was examined by ELISA with PKA activity kit as described in method. PKA activity equals (sample average absorbance-blank average absorbance)/quantity of protein used per assay, normalized to control platelets (**C** and **F**). Data are means ± SD of six patients and controls. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with control by Student`s *t*-test.

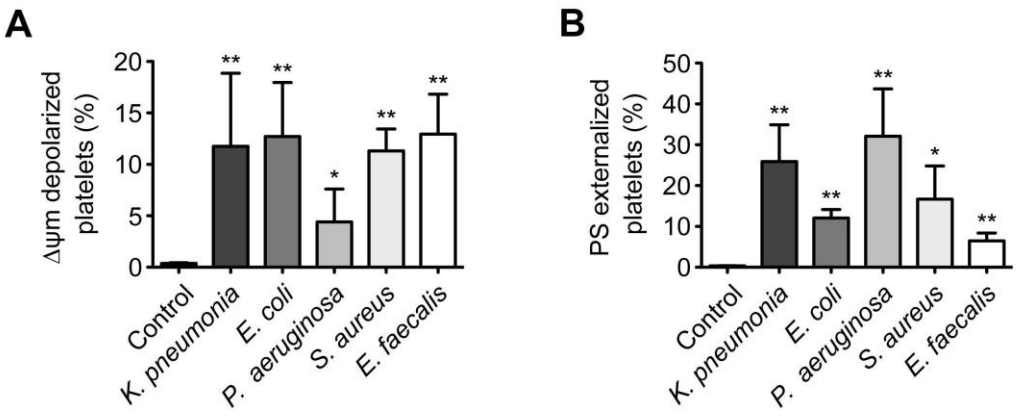
Supplemental Figure 4



Supplemental Figure 4

PKA activity is elevated in platelets stimulated with ristocetin. PRP was stimulated with ristocetin (1 mg/mL) or vehicle control at 37°C for 5 min in aggregometer. The stimulated platelets were lysed. The total and phosphorylated GPIbβ (**A**) and total PKA activity (**B**) were examined. Representative western blots are shown. The results were from three independent experiments with different donors. *$P < 0.05$, compared with control by Student`s *t*-test.

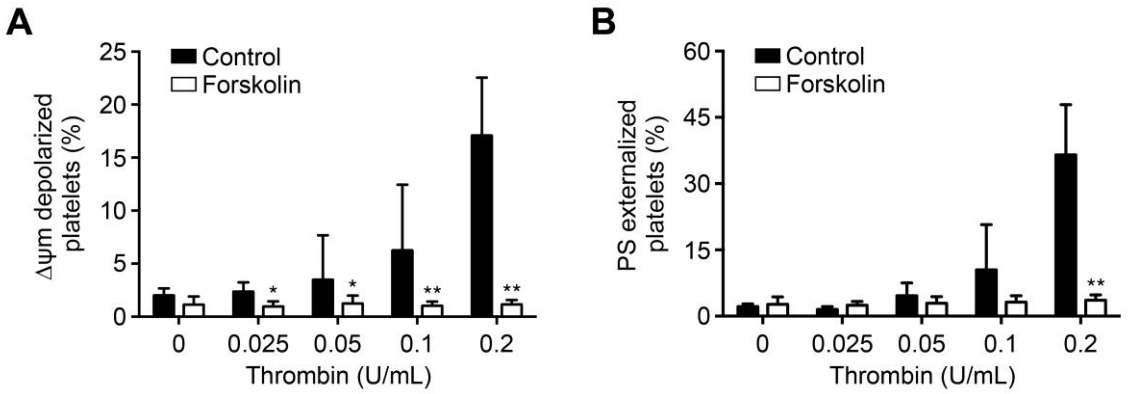
Supplemental Figure 5



Supplemental Figure 5

Bacterium induces apoptotic events in platelets. Washed platelets (1×10^7/mL) in MTB were incubated with indicated bacteria (1:20) or vehicle control at 37℃ for 90 min. $\Delta\psi_m$ depolarization and PS exposure were analyzed with flow cytometry. Means ± SD of the percentage of $\Delta\psi_m$-depolarized platelets and PS positive platelets from six independent experiments are shown. **P* < 0.05, ***P* < 0.01, compared with control platelets by Mann-Whitney test.

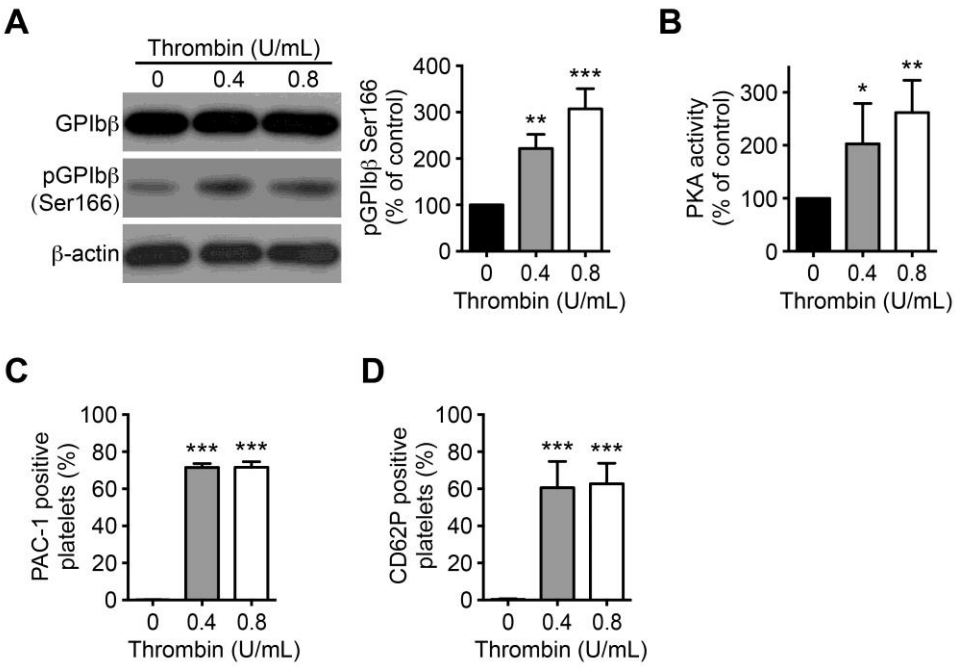
Supplemental Figure 6



Supplemental Figure 6

PKA activator (forskolin) reduces apoptotic events in platelets stimulated by thrombin. Washed human platelets were incubated with forskolin (5 μM) or vehicle (control) at 37℃ for 30 min, and further incubated with indicated concentrations of thrombin or vehicle (0) at 37℃ for 30 min. Data are means ± SD of $\Delta\psi_m$ depolarization (**A**) and PS (**B**) exposure from three independent experiments. *P < 0.05, **P < 0.01, by two-way ANOVA.

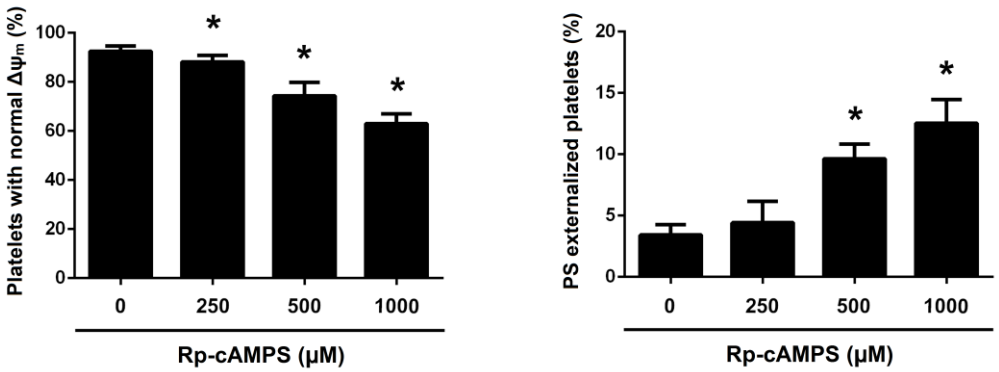
Supplemental Figure 7



Supplemental Figure 7

Higher concentration of thrombin elevated PKA activity and activated platelets. Washed platelets were incubated with indicated concentration of thrombin or vehicle at 37℃ for 30 min. (**A** and **B**) Platelets were separated and lysed immediately. Total and phosphorylated GPIbβ was detected by western blot with anti-GPIbβ and anti-pGPIbβ Ser-166 antibodies. PKA activity was examined by ELISA with PKA activity kit as described in method. Representative immunoblots and quantification for pGPIbβ (**A**) and PKA activity (**B**) are shown. Data are expressed as means ± SD from four independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with control, by one-way ANOVA. (**C** and **D**) Platelet activation was detected by P-selectin (CD62-P) surface exposure and integrin $\alpha IIb\beta_3$ activation. The treated platelets were incubated with FITC-labeled anti-CD62-P antibody or FITC-labeled PAC-1 at RT for 20 minutes in the dark and then subjected to flow cytometry analysis. Quantifications of PAC-1 (**C**) and CD62P (**D**) positive platelets were shown (mean ± SD, *n* = 6). ****P* < 0.001 compared with control, by one-way ANOVA.

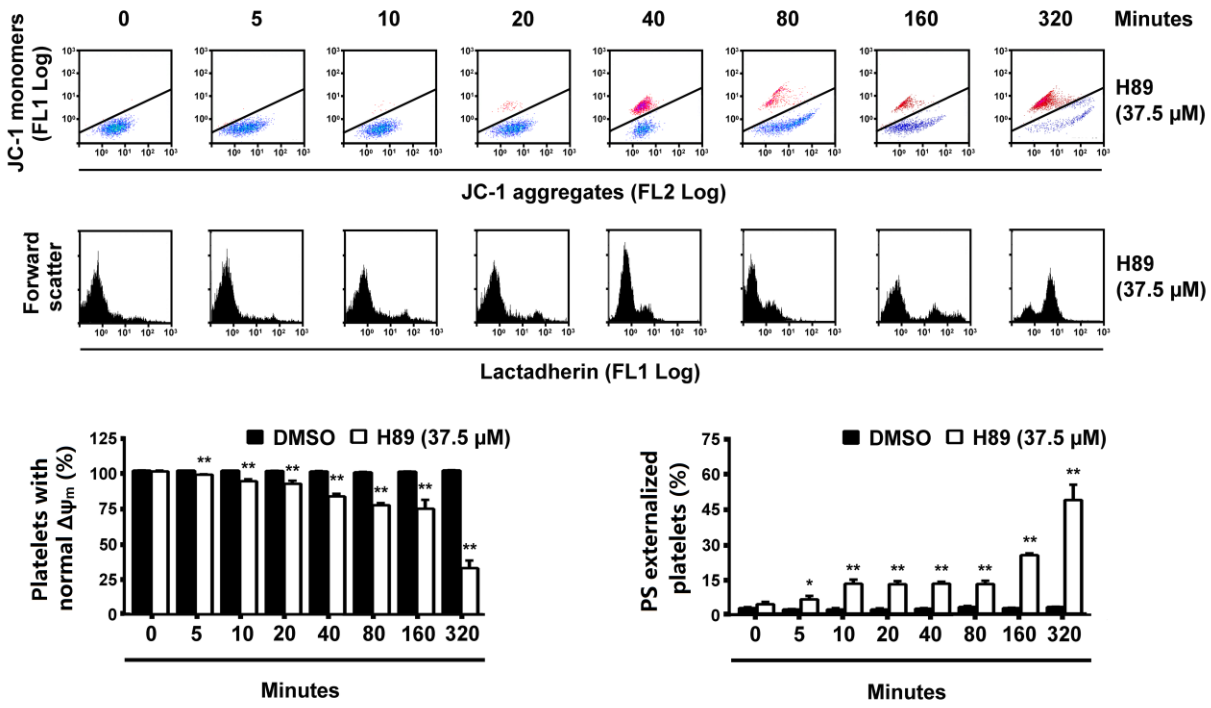
Supplemental Figure 8



Supplemental Figure 8

PKA inhibitor Rp-cAMPS induces platelet apoptosis. Washed human platelets (1×10^8/ml) were treated with Rp-cAMPS (250 μM, 500 μM, 1000 μM) or vehicle at 37°C for 24 h. TMRE (100 nM) was added into the pre-treated platelets. Then samples were further incubated in the dark at 37°C for 5 min and subjected to flow cytometry. Annexin V binding buffer was mixed with the pre-incubated platelets and FITC-annexin V at a 50:10:1 ratio. Samples were gently mixed and incubated at RT for 15 min in the dark, then subjected to flow cytometry analysis. Means ± SD of the percentage of platelets with normal $\Delta\psi_m$ and PS positive platelets from four independent experiments are shown. *P < 0.05, compared with vehicle control platelets by one-way ANOVA.

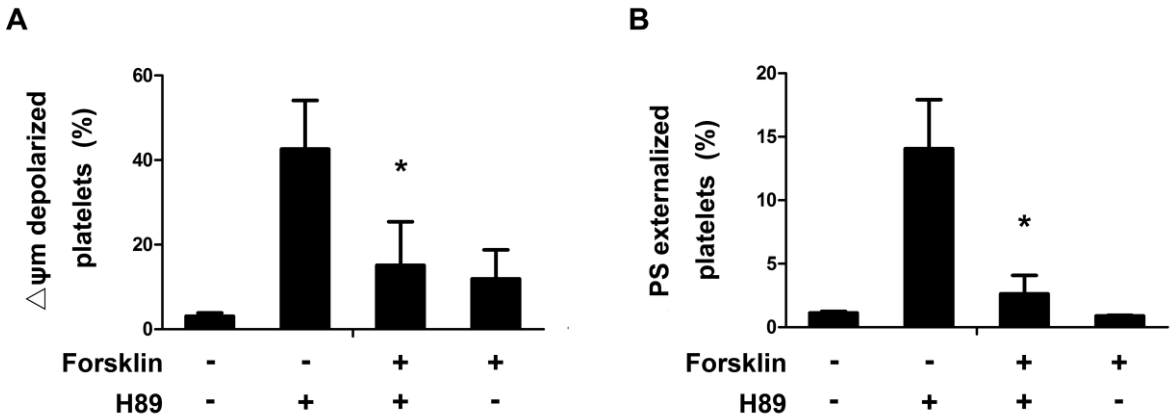
Supplemental Figure 9



Supplemental Figure 9

H89 time-dependently induced apoptotic events in platelets. Washed platelets were incubated with H89 (37.5 μM) or vehicle control at 22℃ for indicated time. $\Delta\psi_m$ depolarization (JC-1) and PS exposure were detected by flow cytometry. Representative flow cytometric figures are shown (up). Means ± SD of the percentage of platelets with normal $\Delta\psi_m$ and PS positive platelets from three independent experiments are shown. *P < 0.05, **P < 0.01, compared with control by two-way ANOVA.

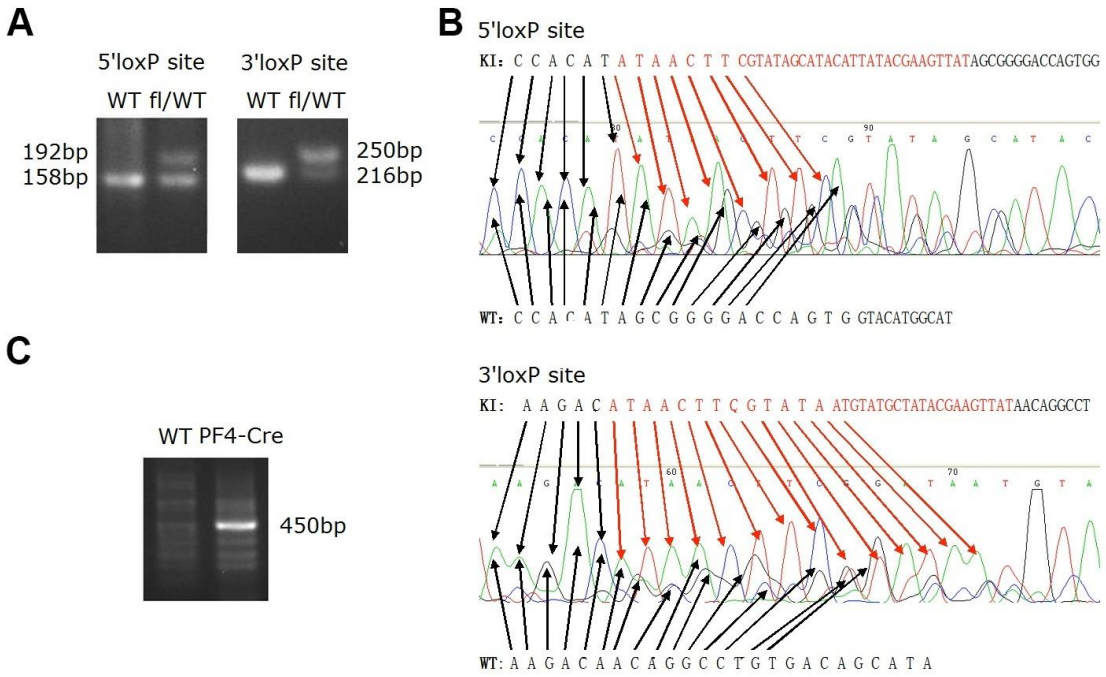
Supplemental Figure 10



Supplemental Figure 10

PKA activator forskolin reduces H89-induced apoptotic events. Washed human platelets were incubated with forskolin (10 μM) or vehicle at 22℃ for 10 min, and were further incubated with H89 (20 μM) or vehicle control at 22℃ for 320 min. $\Delta\psi_m$ depolarization (**A**) and PS exposure (**B**) were detected by flow cytometry. Means ± SD of the percentage of platelets with $\Delta\psi_m$ depolarization and PS exposure from four independent experiments are shown. *P < 0.05, compared with control by one-way ANOVA.

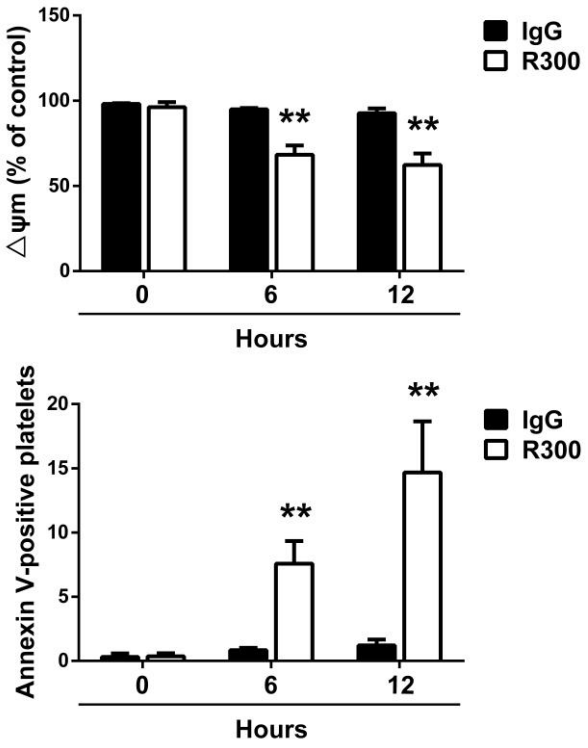
Supplemental Figure 11



Supplemental Figure 11

PCR and sequencing genotyping of mouse tail DNA. Conditional gene knockout donor DNA designed to delete the exons 6, 7 and 8 of *PKA C*α genomic DNA by insertion of loxP sites flanking the targeted exons. The donor DNA contains a 192bp short homologous arm 5' of and a 250bp short homologous arm 3' of the floxed target exons. After Cre-treatment, the floxed exons 6, 7 and 8 was removed in vivo to result in a null allele. (**A**) The WT versus floxed alleles are detected using primers against the 5' loxP site and 3' loxP site, respectively. (**B**) 5' loxP and 3' loxP sequences are detected using primers PKA Cα-F and PKA Cα-R, respectively. The black arrows indicate WT sequence; the red arrows indicate inserted sequence. (**C**) The WT versus Cre alleles (450bp) are detected using primers against Cre gene.

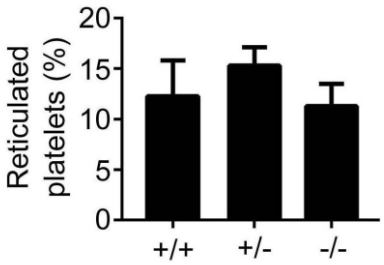
Supplemental Figure 12



Supplemental Figure 12

Anti-mouse platelet antibodies (R300) incur platelet apoptosis. Washed mouse platelets were incubated with R300 (10 μg/mL) or IgG (10 μg/mL) at 37℃ for different time, and then analyzed for $\Delta\psi_m$ depolarization and PS exposure by flow cytometry. Means ± SD from three independent experiments are shown. ***P* < 0.01, compared with IgG control by two-way ANOVA.

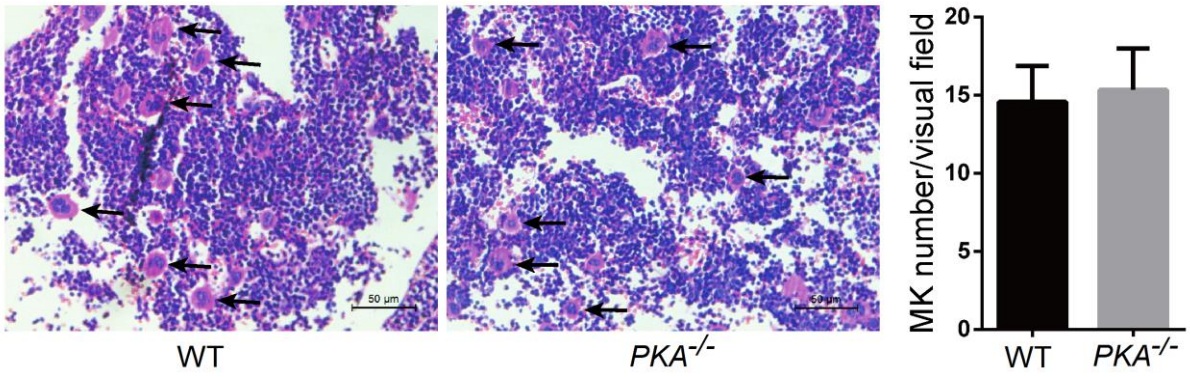
Supplemental Figure 13



Supplemental Figure 13

Flow cytometric measurement for reticulated platelets. Data are means ± SD of 7 *PKA*$^{+/+}$, 7 *PKA*$^{+/-}$ and 5 *PKA*$^{-/-}$ male mice.

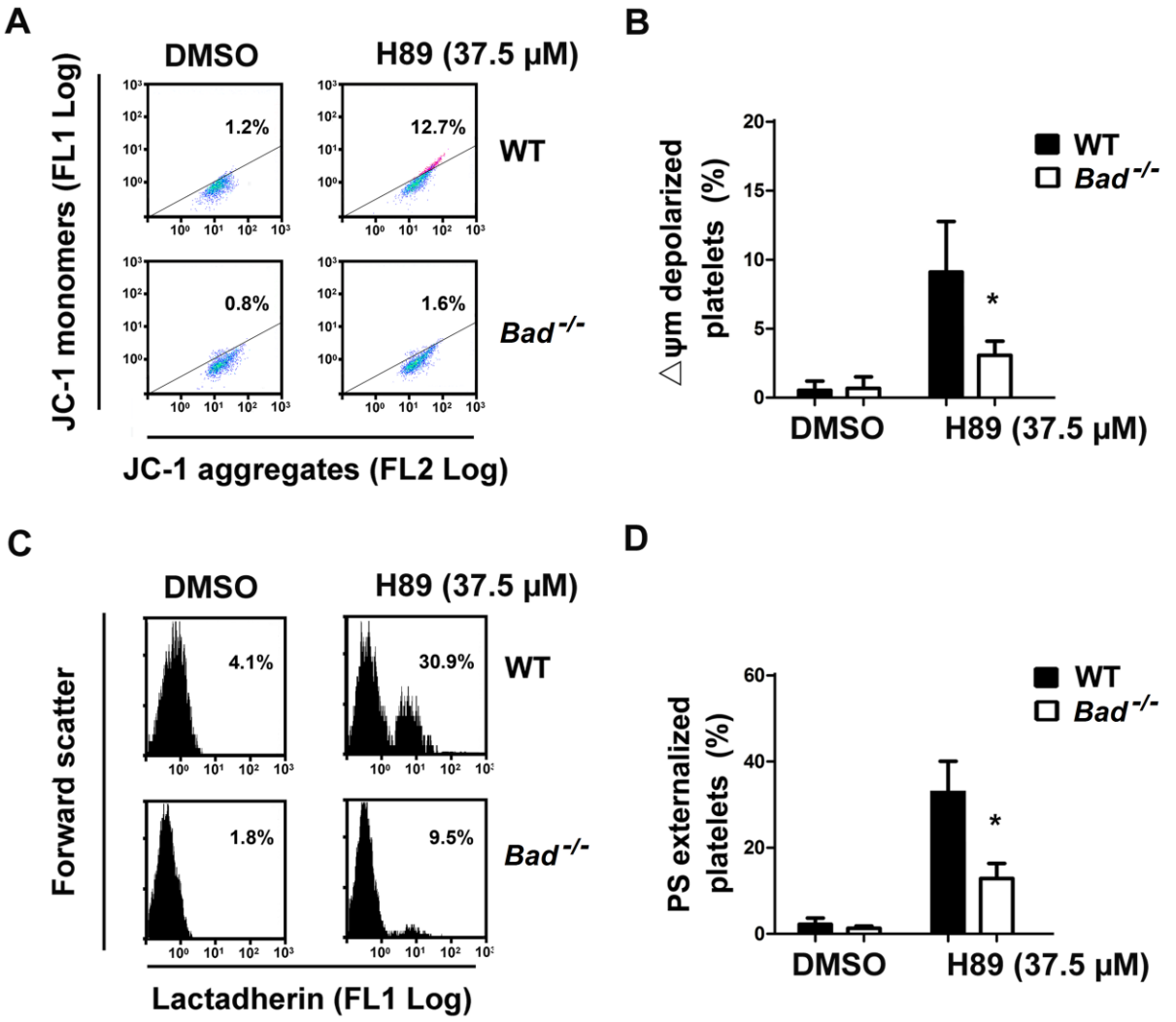
Supplemental Figure 14



Supplemental Figure 14

The number and morphology of megakaryocyte in bone marrow of the *PKA*$^{-/-}$ and WT mice (n = 5). Representative images of hematoxylin and eosin-stained sections of bone marrow from WT and *PKA*$^{-/-}$ mice are shown. Megakaryocytes (MK) are indicated by black arrows. Histograph shows mean ± SD of the MK counts in 10 random selected fields from five separated experiments. Bars = 50 μm.

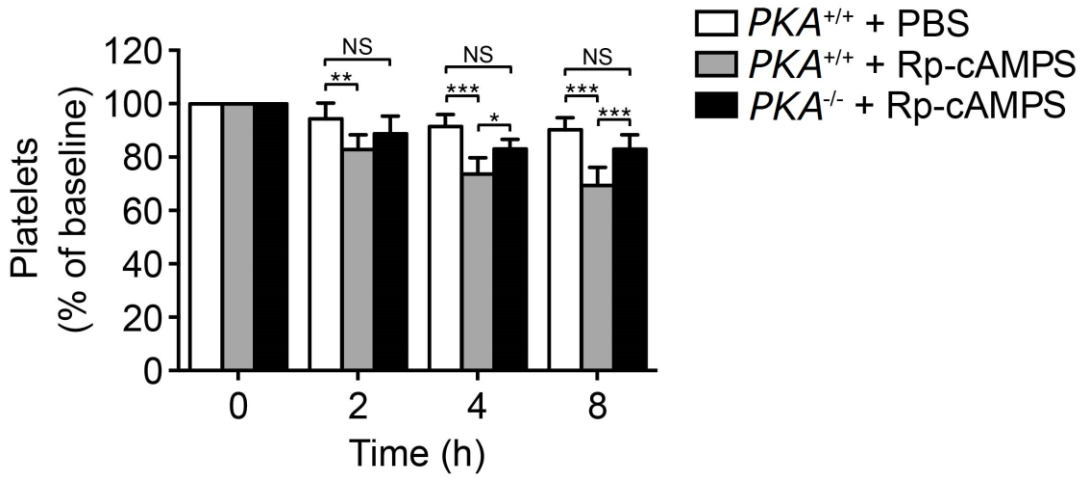
Supplemental Figure 15



Supplemental Figure 15

PKA inhibitor H89-induced apoptotic events are markedly decreased in *Bad*-deficient platelets. Washed platelets from WT and *Bad*$^{-/-}$ mice were incubated with H89 (37.5 μM) or vehicle (DMSO) control at 37℃ for 30 min. Representative flow cytometric figures (left) and quantification (right) of $\Delta\psi_m$ depolarization (**A** and **B**) and PS externalization (**C** and **D**) are shown (mean ± SD, $n = 5$). *$P < 0.05$ compared with control, by one-way ANOVA.

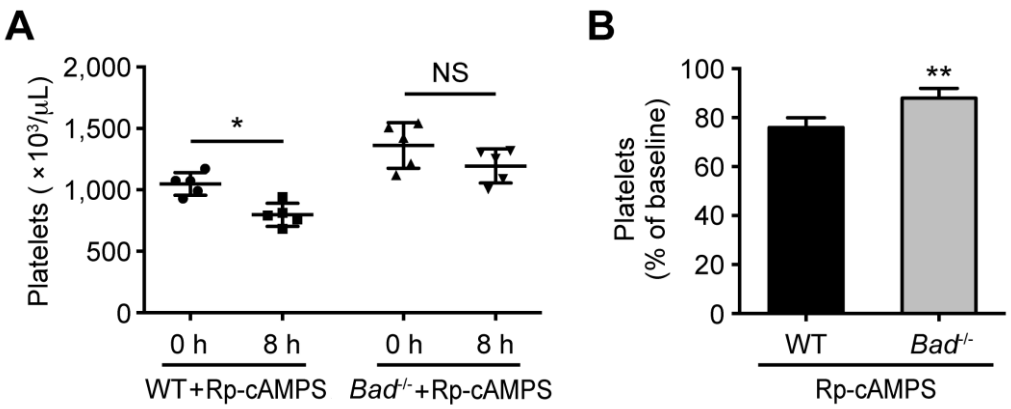
Supplemental Figure 16



Supplemental Figure 16

The *PKA*$^{-/-}$ mice are less response to PKA inhibitor-induced platelet clearance. *PKA*$^{-/-}$ and *PKA*$^{+/+}$ mice were injected with a single dose of Rp-cAMPS (50 mg/kg) or vehicle (PBS) control through tail vein. Platelet counts were determined at the indicated time points.*P< 0.05, **P< 0.01, ***P< 0.001, assessed by two-way ANOVA followed by Tukey's multiple comparison test (n = 5). NS, not significant.

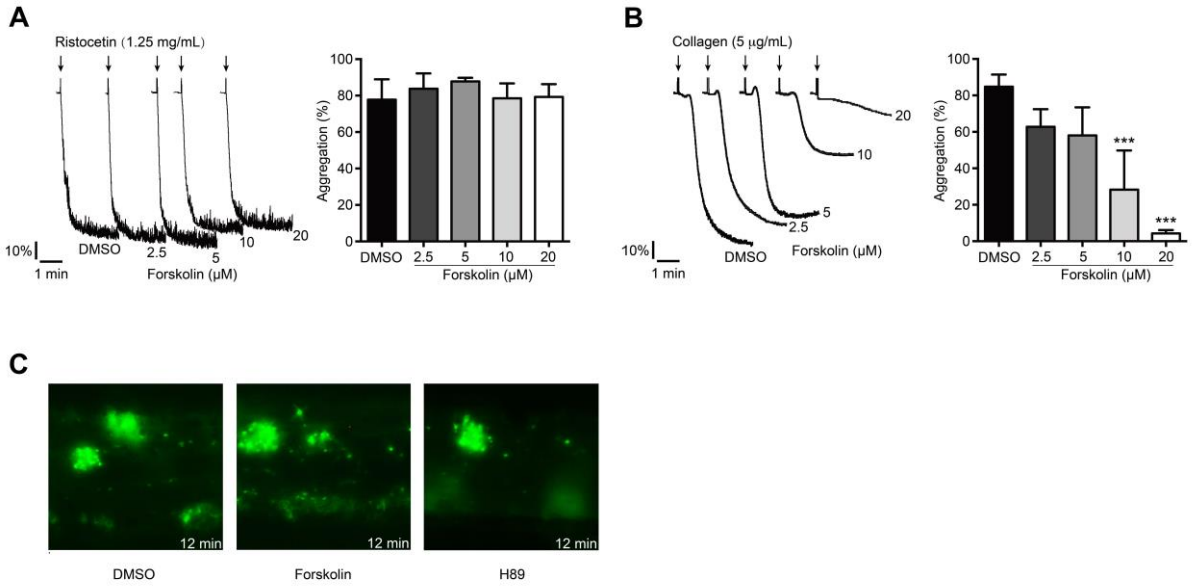
Supplemental Figure 17



Supplemental Figure 17

Rp-cAMPS-induced platelet clearance in WT and *Bad*$^{-/-}$ mice. WT and *Bad*$^{-/-}$ mice were injected with a single dose of Rp-cAMPS (4 mg/kg) through tail vein. (**A**) Platelet counts were determined before and at 8 hours after injection. Data represent absolute platelet counts of each mouse. (**B**) The platelets of baseline at 8 hours after Rp-cAMPS injection. Data are means ± SD of 5 WT, 5 *Bad*$^{-/-}$ male mice. NS, not significant; **P* < 0.05; ***P* < 0.01 compared with WT mice by Student's *t*-test.

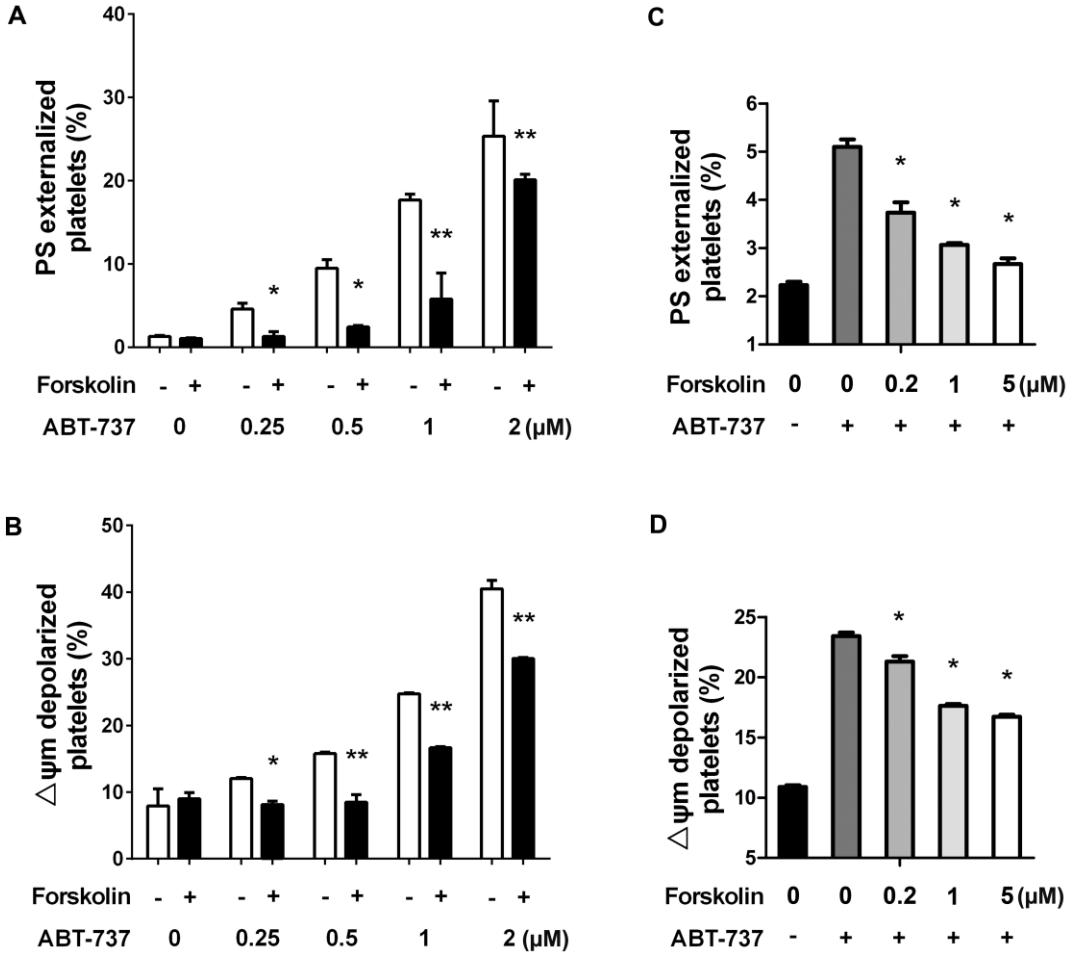
Supplemental Figure 18



Supplemental Figure 18

The effects of forskolin on platelet functions. (**A** and **B**) Human washed platelets were incubated with different concentrations of forskolin or vehicle (DMSO) at 22℃ for 30 min. The pretreated washed platelets were stimulated with ristocetin (1.25 mg/mL) plus von Willebrand factor (7.5 μg/mL) (**A**) and collagen (5 μg/mL) (**B**) at 37°C under constant stirring. Platelet aggregation was recorded in a Chrono-Log aggregometer (Havertown, PA). Histograms of maximal platelet aggregation under the indicated conditions are shown as means ± SD of 5 independent experiments (***P < 0.001, by one-way ANOVA). 5 μM forskolin did not significantly inhibit platelet aggregation induced by ristocein and collagen. (**C**) Washed mice platelets were incubated with H89 (25 μM), forskolin (5 μM) or vehicle (DMSO) at 22℃ for 30 min, and were labeled with calcein-AM (5 μg/mL). The recipient mice were injected intravenously with pretreated platelets (5 × 10^6/g). $FeCl_3$-induced mesenteric arteriole thrombosis in the mice was recorded by real time microscopy at 12 min. There is not visible difference in the presence of labeled platelets pre-treated with different reagents in the thrombus. Each image is representative of five mice.

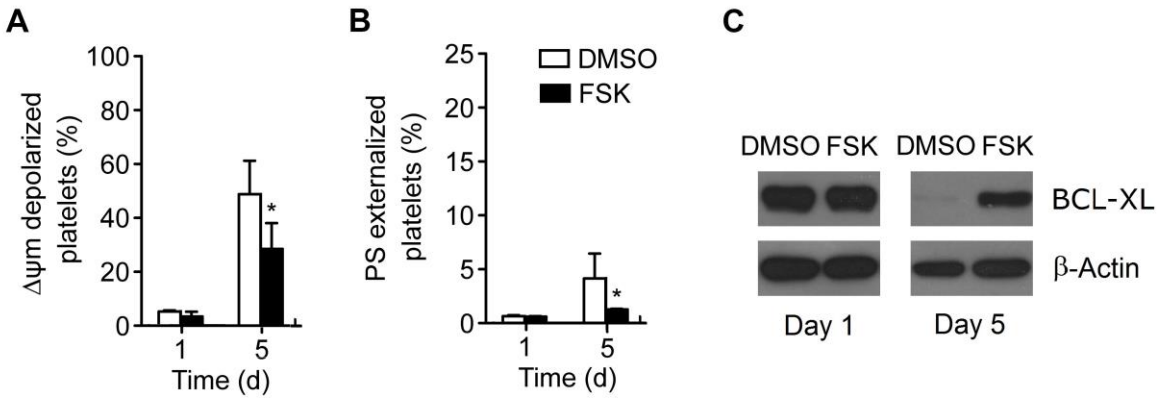
Supplemental Figure 19



Supplemental Figure 19

PKA activator protects platelets from apoptosis induced by ABT-737. (**A** and **B**) Platelets were incubated with forskolin (10 μM) or vehicle at RT for 10 min, and further incubated with different concentrations of ABT-737 at 37℃ for 90 min, $\Delta\psi_m$ depolarization and PS exposure in platelets were analyzed flow cytometry. (**C** and **D**) Platelets were incubated with different concentrations of forskolin at RT for 10 min, and further incubated with ABT-737 (1 μM) at 37℃ for 90 min, $\Delta\psi_m$-depolarization and PS exposure are shown. *P < 0.05, **P < 0.01, compared with vehicle control, by one-way ANOVA.

Supplemental Figure 20



Supplemental Figure 20

PKA activator forskolin inhibits BCL-XL degredation. Washed human platelets (3×10^8/mL) were incubated with forskolin (5 μM) or vehicle at 22℃ for indicated time. JC-1 was added into the platelets to a final concentration of 2 μg/mL and incubated in the dark for 10 min (**A**). The platelets were incubated with Lactadherin to a final concentration of 5 μg/mL in the dark for 30 min (**B**). The platelets were analyzed with flow cytometry. Means ± SD of the percentage of $\Delta\psi_m$-depolarized platelets (**A**) and PS positive platelets (**B**) from three independent experiments are shown. (**C**) The platelets were lysed with an equal volume of lysis buffer on ice for 30 min. Proteins were separated by SDS-PAGE. After blocking, membranes were incubated with primary antibodies against BCL-XL (1:1000) and ß-actin (1:1000) at 4°C overnight. The protein bands were visualized by the ECL chemiluminescence system. The figure is representative of four separate experiments with different donors. *$P < 0.05$, compared with vehicle control by Student`s *t*-test. FSK: forskolin.

Supplemental Tables

Supplemental Table 1: Clinical characteristics of patients with ITP

Donors	Age sex	Acute or chronic	PLT ($\times 10^9$/L)	Anti-Platelets antibodies	Reticulated platelet （12.5±4.15%）	PAIgG （35.6±7.4 %）
1	72 years old Male	Acute	10	GP1b + GPⅨ - GPⅡb - GPⅢa -	7.7	45.8
2	28 years old Female	Acute	20	GP1b + GPⅨ + GPⅡb - GPⅢa -	10.5	37.1
3	60 years old Male	Acute	15	GP1b + GPⅨ + GPⅡb - GPⅢa -	7.7	30
4	45 years old Female	Acute	19	GP1b + GPⅨ + GPⅡb + GPⅢa +	2.6	28.3
5	23 years old Male	Acute	21	GP1b + GPⅨ + GPⅡb + GPⅢa +	4.5	52.4
6	52 years old Female	Acute	27	GP1b + GPⅨ + GPⅡb + GPⅢa +	10.2	37.1
7	71 years old Female	chronic	24	GP1b + GPⅨ + GPⅡb + GPⅢa +	8.3	46.4

8	67 years old Female	Acute	18	GP1b + GPⅨ + GPⅡb + GPⅢa -	7.2	73.1
9	45 years old Female	chronic	31	GP1b + GPⅨ + GPⅡb + GPⅢa -	23.5	32.9
10	30 years old Female	Acute	13	GP1b + GPⅨ + GPⅡb + GPⅢa -	16.8	32.9
11	32 years old Male	chronic	13	GP1b + GPⅨ - GPⅡb - GPⅢa -	15.3	49.9
12	72 years old Male	Acute	43	GP1b - GPⅨ - GPⅡb + GPⅢa -	24.1	56
13	46 years old Female	Acute	92	GP1b + GPⅨ + GPⅡb + GPⅢa -	12.8	41.2
14	25 years old Female	Acute	10	GP1b + GPⅨ - GPⅡb - GPⅢa -	5.7	35.4
15	32 years old Female	Acute	68	GP1b + GPⅨ - GPⅡb + GPⅢa -	10.4	50.5
16	59 years old Female	Acute	21	GP1b + GPⅨ - GPⅡb - GPⅢa -	14.4	37.3

17	47 years old Female	Acute	11	GP1b + GPⅨ - GPⅡb - GPⅢa -	6.9	54.9
18	24 years old Female	Acute	218	GP1b + GPⅨ + GPⅡb + GPⅢa -	6.9	50.9
19	54 years old Female	Acute	73	GP1b + GPⅨ - GPⅡb - GPⅢa -	11.5	48.7
20	71 years old Female	Acute	Not availabl e	GP1b - GPⅨ+ GPⅡb - GPⅢa -	16.9	68.4
21	27 years old Female	Acute	7	GP1b + GPⅨ + GPⅡb + GPⅢa -	4.1	96
22	70 years old Female	Acute	9	GP1b + GPⅨ + GPⅡb + GPⅢa +	16.1	48.8
23	69 years old Female	Acute	17	GP1b - GPⅨ + GPⅡb + GPⅢa -	3.1	28.2
24	51 years old Female	Acute	2	GP1b + GPⅨ + GPⅡb + GPⅢa -	64.8	57
25	23 years old Female	Acute	5	GP1b + GPⅨ + GPⅡb + GP Ⅲa -	0.464	35.9

Accordance with the 2012 guidelines (1), ITP is an autoimmune disease characterized by isolated thrombocytopenia (platelet count < 100 × 10^9/L) due to increased platelet destruction and decreased platelet production. ITP remains a diagnosis of exclusion and presents without decrease of red and white blood cell count, normally morphological platelets on the peripheral blood smear, and normal-appearing bone marrow.

Supplemental Table 2: Clinical characteristics of patients with type 2 diabetes mellitus

Donors	Age sex	Duration of Diabetes (years)	Other clinical diagnoses	PLT ($\times 10^9$/L)	FPG (mmol/L)	HbAc1 (%)	Treatment
1	50 years old Female	4	Hypertension	284	9.3	7.5	Metformin Acarbose Rui ketone
2	56 years old Female	6	Cataract Upper respiratory infection	142	16.7	8.6	Insulin Cephalosporins
3	54 years old Female	4	Diabetic Nephropathy	260	6.1	6.5	Insulin
4	58 years old Female	10	Hypertension	187	12.4	11.7	Insulin Amlodipine Besylate
5	38 years old Male	10	Hypertension	149	4.8	6.7	Insulin Amlodipine Besylate
6	43 years old Male	10	None reported	211	9.7	8.7	Insulin Exenatide Injection
7	45 years old Male	8	Hypertension Cataract	155	10.6	9.0	Insulin Acarbose
8	46 years old Male	11	Hypertension	194	12.9	10.3	Insulin Amlodipine Besylate
9	50 years old Male	3	Hypertension Diabetic Nephropathy	184	6.6	6.0	Insulin Amlodipine Besylate
10	57 years old Male	19	Hypertension Diabetic Nephropathy	234	6.4	6.6	Insulin Amlodipine Besylate
11	66 years old Male	15	Hypertension Diabetic Nephropathy	169	5.2	5.9	Insulin Levamlodipine

Accordance with the 2002 guidelines (2), diabetes is diagnosed when fasting plasma glucose (FPG) is 7.0 mmol/L (126 mg/dL) or higher, and/or plasma glucose 2 h after 75 g glucose load

(2hPG) is 11.1 mmol/L (200 mg/dL) or higher. A casual plasma glucose (PG) > or = 11.1 mmol/L (200 mg/dL) also indicates diabetic type. Hyperglycemia is shown on two or more occasions examined on separate days. Diabetes can be diagnosed by a single PG test of Hyperglycemia if one of the following three conditions co-exists, (1) typical symptoms of diabetes mellitus; (2) HbA1c > or = 6.5% by a standardized method; or (3) unequivocal diabetic retinopathy.

Supplemental Table 3: Clinical characteristics of patients with Sepsis

Donors	Age sex	Other clinical diagnoses	cultures	PLT (×10^9/L)	WBC (×10^9/L)	Respiratory rate (breaths/min)	heart rate (beats/min)	Temperature (°C)
1	28 years old Male	Trauma	*S.haemolyticus*	176	20.6	21	134	37.2
2	82 years old Male	Pulmonary infection, cough	*K.pneumoniae*	148	9	25	87	38.4
3	63 years old Male	COPD	*P.aeruginosa*	61	14.5	16	133	37.0
4	16 years old Female	Pulmonary infection, cough	*S.haemolyticus*	27	18.6	28	144	37.6
5	82 years old Male	Pulmonary infection, Cerebral infarction	*P.aeruginosa K.pneumoniae*	210	27.8	15	102	39.4
6	69 years old Male	Biliary tract infection	*E.coli Streptococcus*	116	20.7	22	115	39.4
7	80 years old Male	Acute cholecystitis Septic shock	*E.coli*	67	26.3	24	112	39.9
8	83 years old Female	Multiple fractures	*E.coli*	6	21	30	104	39.5
9	82 years old Female	Pulmonary infection Septic shock	*E.coli*	86	49.8	21	130	39.1
10	65 years old Male	Pulmonary infection cough	*S. aureus*	91	12.3	20	105	38.5
11	80 years old Male	Intestinal obstruction	*B. Bacillus*	125	16.1	15	109	37.4
12	72 years old Female	Pancreatitis	*E.coli*	109	8.9	25	96	38.5

13	82 years old Male	Cholecystitis	*E.coli*	170	13.0	28	100	39.1
14	84 years old Male	None	*E. faecium*	228	9.8	18	114	38.0
15	78 years old Male	Cerebral infarction	*S. aureus*	113	20.7	18	85	38.0

Accordance with the 2012 guidelines (3), sepsis is defined as one or more positive cultures in blood samples from patients with clinical signs of infection at least two of the following: temperature < 36°C or > 38°C, heart rate > 90 beats/minute, respiratory rate > 20 breaths/minute, PaO_2 < 4.3 kPa (32 mmHg), or white blood cell (WBC) count < 4000 or > 12,000/mm^3.

Supplemental Table 4: Hematologic analysis of $PKA^{+/+}$, $PKA^{+/-}$and $PKA^{-/-}$ mice

Hematological parameter	$PKA^{+/+}$ n=10	$PKA^{+/-}$ n=5	$PKA^{-/-}$ n=7
WBC(10^9/L)	9.283±2.349	8.546±3.662	8.762±2.285
NEUT(10^9/L)	1.795±1.648	3.548±1.657	2.527±2.115
RBC(10^9/L)	8.937±0.744	9.290±1.097	9.560±0.589
HGB(g/L)	132.8±8.442	132.0±10.58	138.8±5.848
HCT(%)	41.17±2.074	40.93±2.650	41.94±0.782
MCV(fL)	46.21±2.209	44.96±2.302	43.98±2.245
MCH(pg)	14.90±0.542	14.46±0.513	14.52±0.376
MCHC(g/L)	322.5±5.806	322.3±5.033	330.6±9.914
RET(%)	5.007±2.005	3.403±2.377	3.764±1.262

WBC, white blood cell; NEUT, neutrophil; RBC, red blood cell; HCT, hematocrit; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RET, reticulocyte. Data are means ± SD.

Supplemental Reference

1. Chalmers S and Tarantino MD. Romiplostim as a treatment for immune thrombocytopenia: a review. *J Blood Med*. 2015;6:37-44.
2. Kuzuya T, et al. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract*. 2002;55(1):65-85.
3. Dellinger RP, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med*. 2013;41(2):580-637.