Supplementary Methods

Immunoprecipitation and proteomic analysis

Human platelets were isolated from fresh venous blood donated by healthy donors who have all given the signed informed consent. All experimental procedures were approved by the Chang Gung Memorial Hospital Institutional Review Board with the approval ID of 202300838A3 and performed according to the guidelines set by the Committee. Human washed platelets were incubated with 0.5 mM RGDS peptide for 30 min and then stimulated with the vehicle control or 2 μM U46619 for 2 min. After 2 min, platelets were lysed with 5X lysis buffer (50 mM Tris-HCl (pH 7.4), 500 mM NaCl, 2.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 5% Triton X-100, 50 μg/ml aprotinin, 50 μg/ml leupeptin, 5 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 5 mM EGTA) on ice for 30 min. The platelet lysates (2 mg) were subject to immunoprecipitation overnight at 4 °C with 2 μg of antibody and protein G agarose (20 μl). Each sample was washed five times with 1 ml of 1X lysis buffer. The immunoprecipitated proteins were eluted five times by adding 20 μl of 50% acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA) at room temperature for 2 min. The extracted proteins were dried, incubated with 5 mM tris (2-Carboxyethyl) phosphine (TCEP) at 65 °C for 30 min for reduction and 10 mM methyl methanethiosulfonate (MMTS) at room temperature for 20 min for alkylation. The proteins were digested with trypsin (1:50, w/w) overnight at 37 °C. After drying the peptides under vacuum, the samples were acidified with 0.1% formic acid. Samples were analyzed by LC-MS/MS and LTQ orbitrap for generating a list of identified proteins. Relative quantification of the identified proteins was performed in Proteome Discoverer 1.4 Software (Thermo Fisher Scientific). The following procedures were then performed to filter for hDab2-interacting proteins with high confidence. Keratin- and immunoglobulin-related proteins were removed from the list of identified proteins. The proteins with unique peptide number < 2 and the number of peptide-spectrum match < 4 were also removed. The proteins that were present in the samples immunoprecipitated with anti-Dab2 antibody but not in the samples immunoprecipitated with control rabbit IgG antibody were considered as the hDab2-interacting proteins with high confidence. On the other hand, the ratio for the peak area of the proteins identified in the samples immunoprecipitated with anti-Dab2 antibody and the peak area for the corresponding proteins in the sample immunoprecipitated with the control rabbit IgG antibody was calculated. The identified proteins were considered as the Dab2-interacting proteins with high confidence when the ratio for the peak area was > 2. The proteomic result is available in the dataset of ProteomeXchange with the identifier of

PXD048490.

Supplementary Tables

Supplementary Table 1. The complete blood count of *Rosa26*$^{fl/fl}$ and *hDAB2-KI* mice

Parameters	*Rosa26*$^{fl/fl}$ (n = 19)	*hDAB2-KI* (n = 16)
Erythrocytes		
Red blood cells (x10^6 cells/μl)	11.2 ± 0.1	11.2 ± 0.1
Hemoglobin (g/dl)	14.8 ± 0.1	14.6 ± 0.1
Hematocrit (%)	55.3 ± 0.3	54.2 ± 0.4
Mean corpuscular volume (fl)	49.2 ± 0.2	48.4 ± 0.2
Mean corpuscular hemoglobin (pg)	13.2 ± 0.1	13.0 ± 0.1
Mean corpuscular hemoglobin concentration (g/dl)	26.8 ± 0.1	26.9 ± 0.1
RBC distribution width (%)	21.5 ± 0.2	20.6 ± 0.2
Leukocytes		
White blood cells (x10^3 cells/μl)	7.8 ± 0.6	8.1 ± 0.5
Neutrophils (x10^3 cells/μl)	0.7 ± 0.0	0.7 ± 0.1
Lymphocytes (x10^3 cells/μl)	6.5 ± 0.5	6.8 ± 0.5
Monocytes (x10^3 cells/μl)	0.4 ± 0.0	0.3 ± 0.0
Eosinophils (x10^3 cells/μl)	0.2 ± 0.0	0.2 ± 0.0
Basophils (x10^3 cells/μl)	0.0 ± 0.0	0.0 ± 0.0
Thrombocytes		
Platelets (x10^3 cells/μl)	1883 ± 73	1782 ± 60
Mean platelet volume (fl)	6.4 ± 0.0	6.4 ± 0.1

Unpaired two-sided Student's *t* test was used for analysis of all parameters. Data represent the mean ± SEM. No biological significance for all parameters of complete blood count between *Rosa26*$^{fl/fl}$ and *hDAB2-KI* mice. Exact *p* values for each group and Source data are provided as a Source Data file.

Supplementary Table 2. Basic characteristics and laboratory analysis of platelet hDab2 expression and U46619-stimulated ATP release of ITP patients.

Case no.[a]	Gender	Bleeding score	Platelet number ($x10^3$/μl)	hDab2 expression	ATP release ($x10^{-8}$ nmole/platelet)
01	F	15	27	2328.4	52.5
02	M	6	70	2165.6	85.4
03	M	3	105	2705.4	358.7
04	F	8	42	2225.5	82.3
05	F	18	35	2754.9	24.3
06	M	9	56	3514.1	239.9
07	F	6	192	4860.0	578.1
08	F	15	211	538.1	53.7
09	F	10	28	2503.4	43.2
10	F	7	48	4208.7	192.5
11	F	12	21	1054.2	111.6

[a]This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB No. CF23098C). A total of 11 patients with ITP that were undergoing treatment (age range 7-54) were enrolled.

Supplementary Table 3. Agonist-stimulated ATP release in human platelet-rich plasma with different concentrations of platelet

Agonist	Platelet concentration[a]	Donor no.	The amount of ATP release (nmole)			Ratio (Max/Min)	CV[c] (%)
			Median[b]	Max[b]	Min[b]		
Thrombin (1 U/ml)	$3x10^8$/ml	5	184.9	195.7	126.9	1.5	18.2
	$0.5x10^8$/ml	5	27.1	34.0	21.5	1.6	17.6
	$0.1x10^8$/ml	5	12.5	16.5	9.5	1.7	23.6
Collagen (10 µg/ml)	$3x10^8$/ml	5	116.1	264.1	100.7	2.6	44.8
	$0.5x10^8$/ml	5	22.8	32.7	12.2	2.7	40.9
	$0.1x10^8$/ml	5	4.0	7.0	2.8	2.5	42.2
U46619 (10 µM)	$3x10^8$/ml	8	80.9	162.2	27.7	5.8	54.2
	$0.5x10^8$/ml	8	4.1	17.6	1.9	9.1	87.1
	$0.1x10^8$/ml	8	0.7	3.8	0.4	9.5	88.9

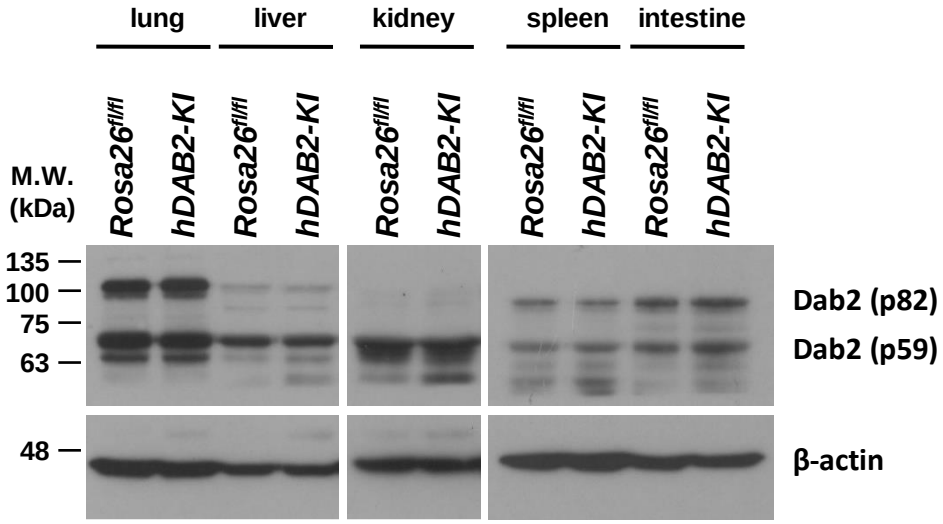
[a]This study was approved by the Chang Gung Memorial Hospital Institutional Review Board with the approval ID of 202300838A3.
[b]The number represents the median, maximal (Max), and minimal (Min) amount of agonists-stimulated ATP release among the donors.
[c]Coefficient of variation.
Source data are provided as a Source Data file.

Supplementary Table 4. The lists of TxA_2-regulated hDab2-interacting proteins involved in the regulation of actin cytoskeleton and platelet activation

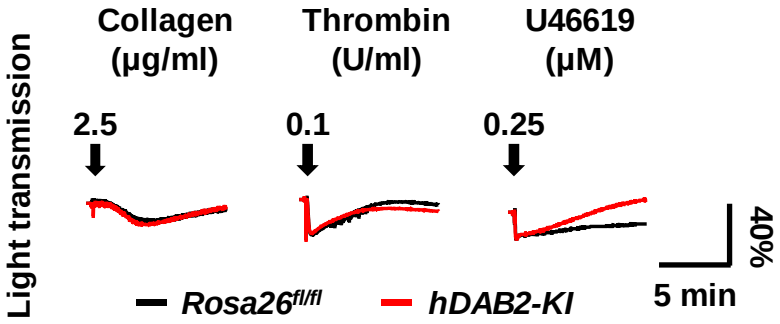
Regulation of actin cytoskeleton
Actin-related protein 2/3 complex, alpha-actinin, ARF GTPase-activating protein GIT1, cofilin-1, cytoplasmic FMR1-interacting protein 1, fibronectin, gelsolin, kininogen-1, myosin light chain kinase, smooth muscle, myosin regulatory light chain, myosin heavy chain 9, phosphatidylinositol 5-phosphate 4-kinase type-2 alpha, protein phosphatase 1 regulatory subunit 12, Ras GTPase-activating-like protein IQGAP2, serine/threonine-protein phosphatase PP1-alpha catalytic subunit and vinculin.
Platelet activation
Fermitin family homolog 3, fibrinogen alpha chain, fibrinogen beta chain, guanine nucleotide-binding protein G(i) subunit alpha-2, myosin regulatory light chain, platelet glycoprotein IX, platelet glycoprotein, protein phosphatase 1 regulatory subunit 12, RAS guanyl-releasing protein 2, Ras-related protein Rap-1b, serine/threonine-protein phosphatase PP1-alpha catalytic subunit, stromal interaction molecule 1 and Talin-1.

This study was approved by the Chang Gung Memorial Hospital Institutional Review Board with the approval ID of 202300838A3.

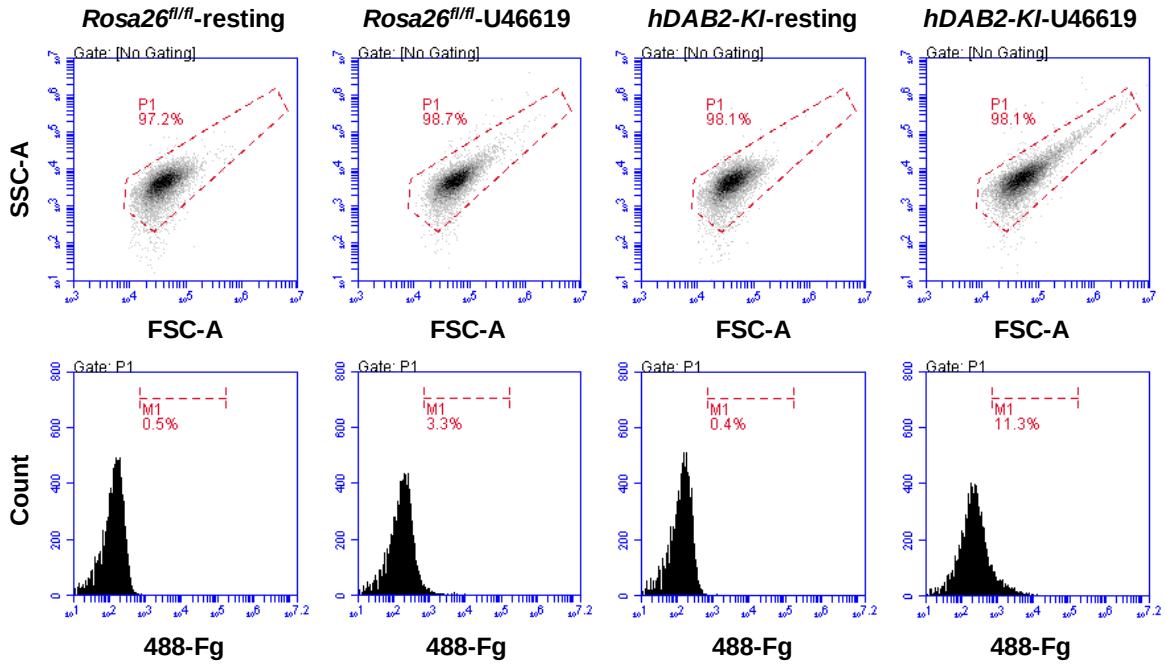
Supplementary Figures



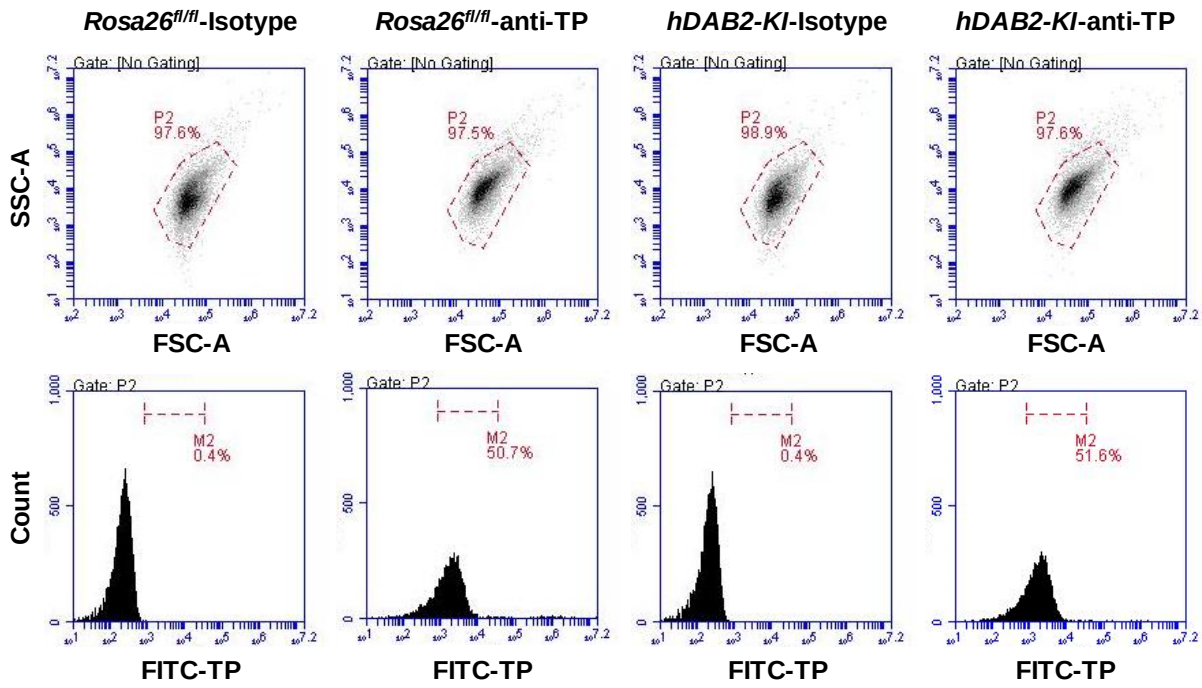
Supplementary Figure 1. The expression level of Dab2 in various organs of *Rosa26$^{fl/fl}$* and *hDAB2-KI* mice. Lysates from the indicated organs of *Rosa26$^{fl/fl}$* and *hDAB2-KI* mice were collected and analyzed by Western blot using an anti-Dab2 antibody. The expression of β-actin was used as a control of equal protein loading. Representative data for at least 2 independent experiments are shown.



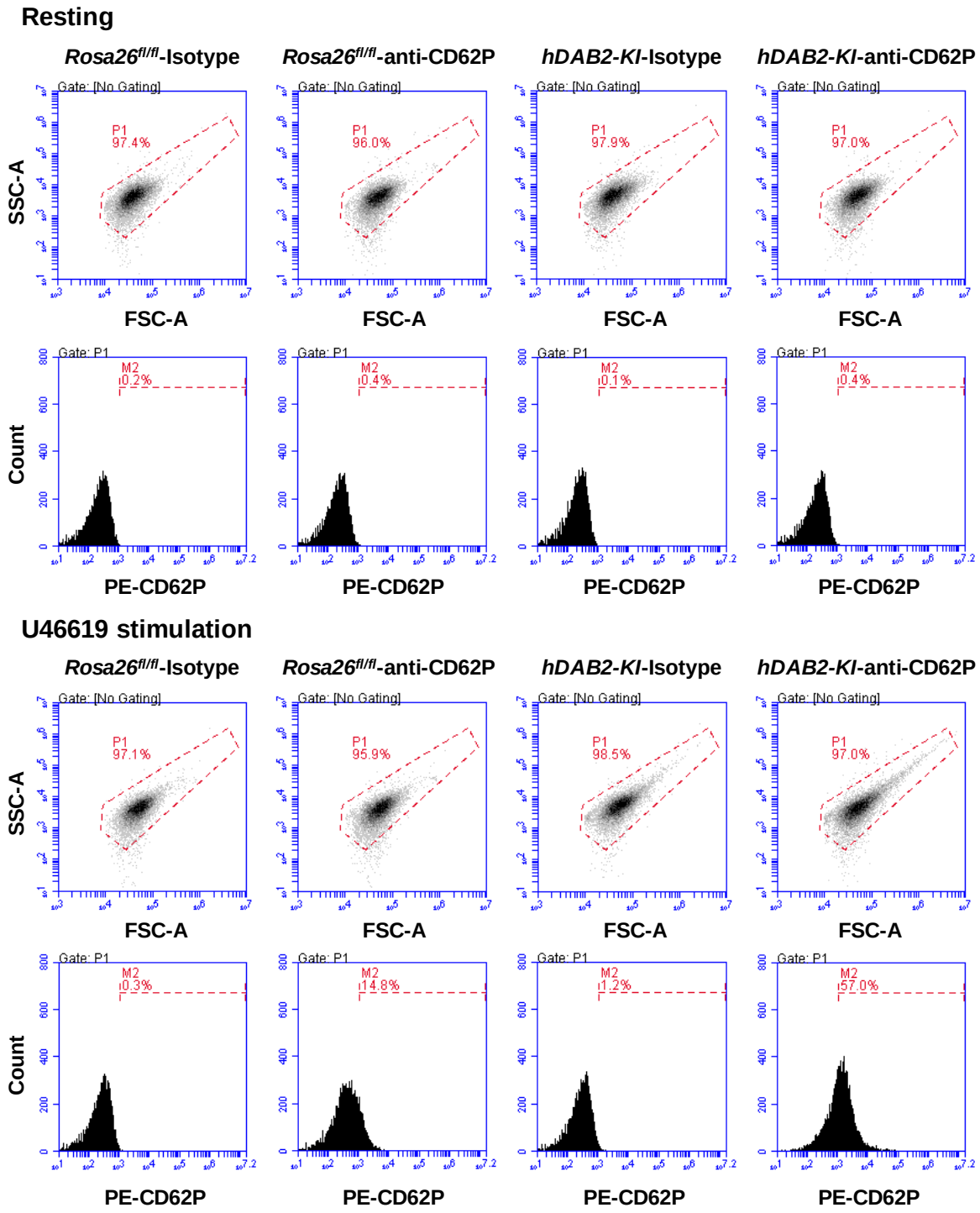
Supplementary Figure 2. No aggregation was observed with low concentrations of agonists at the platelet concentration of 1.2x10^8/ml. Washed platelets (1.2x10^8/ml) were stimulated by the indicated soluble agonists and platelet aggregation was recorded by an AggRAMTM system (Helena Laboratories). Representative aggregation traces in response to 2.5 µg/ml collagen (n = 2 biological replicates), 0.1 U/ml thrombin (n = 2 biological replicates) and 0.25 µM U46619 (n = 2 biological replicates) are shown. Arrows indicate the point of adding the agonist into the reaction.



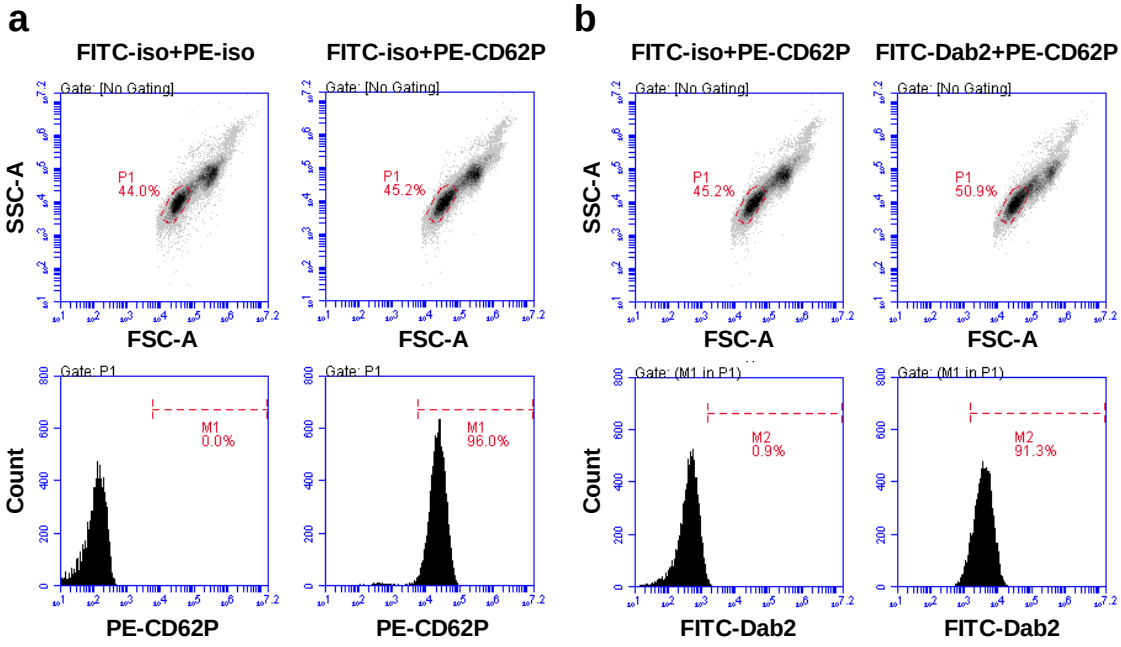
Supplementary Figure 3. Representative flow cytometry and gating strategy for figure 4c in analyzing fibrinogen binding of *Rosa26$^{fl/fl}$* and *hDAB2-KI* platelets. Resting (n = 3 biological replicates) or U46619-stimulated (0.25 μM, n = 3 biological replicates) platelets were incubated with Alexa Fluor 488-conjugated fibrinogen (488-Fg) followed by flow cytometry analysis.



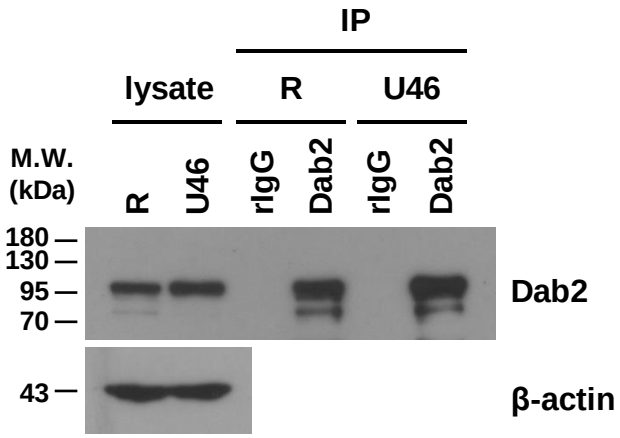
Supplementary Figure 4. Representative flow cytometry and gating strategy for figure 5f in analyzing TP expression of *Rosa26*$^{fl/fl}$ and *hDAB2-KI* platelets. Washed platelets (n = 4 biological replicates) were incubated with the isotype antibody or the FITC-conjugated anti-TP receptor antibody (FITC-TP) and analyzed by flow cytometry.



Supplementary Figure 5. Representative flow cytometry and gating strategy for figure 6a in analyzing CD62P expression of *Rosa26*$^{fl/fl}$ and *hDAB2-KI* platelets. Resting (n = 6 biological replicates) and U46619-stimulated (0.25 μM, n = 3 biological replicates) platelets were incubated with the isotype antibody or PE-conjugated anti-CD62P antibody (PE-CD62P) followed by flow cytometry analysis.



Supplementary Figure 6. Representative flow cytometry and gating strategy for figure 7 in analyzing platelet Dab2 expression for ITP patients. (a-b) The platelet/leukocyte suspension from ITP patients (n = 11) was incubated with FITC-conjugated isotype antibody (FITC-iso) and PE-conjugated isotype antibody (PE-iso) (group 1), FITC-iso and PE-conjugated anti-CD62P antibody (PE-CD62P) (group 2), FITC-iso and FITC-conjugated anti-Dab2 antibody (FITC-Dab2) (group 3) or FITC-Dab2 and PE-CD62P (group 4) followed by flow cytometry analysis. Group 1 was used to set as double negative control. Group 2 and group 3 were used to set compensation for FITC and PE signals, respectively. Group 1 and group 2 were used to determine CD62P expression. Group 2 and group 4 were used to determine Dab2 expression. The particles in the P1 region of the FSC/SSC scatter plot with positive signal of PE-CD62P fluorescence (M1) were considered as platelets (M1 in P1). Then the particles with positive signal of FITC-Dab2 fluorescence (M2) in platelet fractions were selected and counted. The percent positive (%) and mean fluorescence for FITC-Dab2 was recorded. The relative levels of hDab2 expression in platelets was calculated by multiplying the values of percent positive (%) with the mean fluorescence.



Supplementary Figure 7. Dab2-interacting proteins in resting and U46619-stimulated human platelets were pulled down by immunoprecipitation. Human washed platelets were pre-incubated with 0.5 mM RGDS peptide at room temperature for 30 min and then stimulated with U46619 or not. The lysates (2 mg) from the resting (R) or U46619-stimulated (U46, 2 μM) human platelets were immunoprecipitated by the control rabbit IgG (rIgG) or by the anti-Dab2 (EP2297Y) antibody. The immunoprecipitated proteins (IP) were analyzed by Western blotting using the indicated antibodies.