nature portfolio

Corresponding author(s): Ching-Ping Tseng

Last updated by author(s): Sep 11, 2024

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	BD Accuri C6 Software 1.0.264.21 was used for data acquisition of flow cytometry .
Data analysis	The following software was used for data analysis (as referenced in the manuscript): GraphPad Prism 5, GraphPad Prism 8, Image J 1.48v, BD Accuri C6 Software 1.0.264.21 and Proteome Discoverer 1.4 Software.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are available in the main text or in the Supplementary Information. The proteomic result is available in the dataset of ProteomeXchange with the identifier of PXD048490. Source data are provided with this paper. For original data and other information, please contact ctseng@mail.cgu.edu.tw.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Both female and male participants were used in this study. Sex of healthy participants was not reported in the manuscript. Sex of patients with thrombocytopenia was reported in the manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings of participants have not been reported in the manuscript.
Population characteristics	Participants were healthy donor with age above 20 years old and body weight above 50 kg without anti-platelet drug administration. A total of 11 patients with thrombocytopenia (3 males and 8 females with the age of 7-54) undergoing treatment were enrolled.
Recruitment	Participants were recruited from healthy volunteers at Chang Gung University, Taoyuan 333, Taiwan, Republic of China. Patients with thrombocytopenia were recruited at Taichung Veterans General Hospital, Taichung 407, Taiwan, Republic of China. Patients were recruited into the study when they visited clinics and agreed to sign informed consent. A consecutive 11 patients with thrombocytopenia were recruited without self-selection bias or other biases.
Ethics oversight	Written informed consent was obtained from all participants or their legally authorized representatives, in line with approval from the Chang Gung Memorial Hospital Institutional Review Board with the approval ID of 202300838A3 and the Institutional Review Board of Taichung Veterans General Hospital (IRB No. CF23098C).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Power analysis was not performed to determine sample sizes, rather prior experience and pilot studies informed sample size. Sufficient number of biological replicates that will yield statistical power were chosen as mentioned in figures. Most of the in vitro experiments are representative of a minimum of 3 independent experiments, while for in vivo survival analysis, a minimum of 5 animals per group was chosen for statistical analysis. In vivo experiments were also independently repeated at least two times.
Data exclusions	No data was excluded from analysis.
Replication	Most of the in vitro experiments were repeated at least 3 times as stated in each figure and all attempts at replication were successful. At least 3 biological replicates but not technical replicate were used for statistics analysis in the revised manuscript. For qualitative experiment, at least 2 biological replicates were performed. In vivo experiments were independently repeated at least two times,.
Randomization	In vitro experiments: Platelets were randomly assigned to control or treatment groups and a minimum of 3 independent experiments or biological replicates were performed to ensure reporoducibility. In vivo work: Cages of mice were randomly assigned to different groups and equal distributions of starting weights for each group were confirmed.
Blinding	No blinding. Data presented is quantitative and did not rely on qualitative assessments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

All antibodies used in this study are listed.

Antibodies for Western blotting: (Antibody, Clone number (if applicable), Cat no, Supplier)
Actin (AC-15), NB600-501, Novus Biologicals
Dab2 (p96), 610465, BD Transduction Laboratories
Dab2 (EP2297Y), ab76253, Abcam
Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, ab172730, Abcam
HA-Tag (6E2), 2367S, Cell Signaling
p-AKT (Ser473), AP0637, ABclonal
AKT (pan) (C67E7), 4691X, Cell Signaling
AKT (pan) (40D4), 2920S, Cell Signaling
p-mTOR (Ser2481), 2974S, Cell Signaling
Total mTOR, 2972S, Cell Signaling
p-PLD1 (Thr147), 3831S, Cell Signaling
Total PLD1, 3832, Cell Signaling
GST (B-14), sc-138, Santa Cruz Biotechnology

Antibodies for flow cytometry: (Antibody, Clone number (if applicable), Cat no, Supplier)
PE-conjugated anti-CD62P antibody (Psel.KO2.3), 12-0626-82, eBioscience
PE-conjugated mouse IgG1 kappa Isotype Control (P3.6.2.8.1), 12-4714-82, eBioscience
Alexa Fluor 488-conjugated fibrinogen, F13191, Invitrogen
FITC-conjugated anti-TP receptor antibody, 10012559, Cayman Chemicals Co.
FITC-conjugated Normal Rabbit IgG, 10010322, Cayman Chemicals Co.

Validation

All antibodies used in this study are commercially available and were validated by the manufacturer. Links to the product page for all the antibodies are provided below.

Antibodies for Western blotting:
Actin (AC-15), NB600-501, Novus Biologicals (https://www.novusbio.com/products/beta-actin-antibody-ac-15_nb600-501)
Dab2 (p96), 610465, BD Transduction Laboratories (https://www.bdbiosciences.com/en-au/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-disabled-2-p96.610465)
Dab2 (EP2297Y), ab76253, Abcam (https://www.abcam.com/products/primary-antibodies/dab2-antibody-ep2297y-ab76253.html)
Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, ab172730, Abcam (https://www.abcam.com/en-tw/products/primary-antibodies/rabbit-igg-monoclonal-epr25a-isotype-control-ab172730)
HA-Tag (6E2), 2367S, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/ha-tag-6e2-mouse-mab/2367)
p-AKT (Ser473), AP0637, ABclonal (https://abclonal.com/catalog-antibodies/Phospho-Akt1-S473mAb/AP0637)
AKT (pan) (C67E7), 4691X, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/akt-pan-c67e7-rabbit-mab/4691)
AKT (pan) (40D4), 2920S, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/akt-pan-40d4-mouse-mab/2920)
p-mTOR (Ser2481), 2974S, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/phospho-mtor-ser2481-antibody/2974)
Total mTOR, 2972S, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/mtor-antibody/2972)
p-PLD1 (Thr147), 3831S, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/phospho-pld1-thr147-antibody/3831)
Total PLD1, 3832, Cell Signaling (https://www.cellsignal.com/products/primary-antibodies/pld1-antibody/3832)
GST (B-14), sc-138, Santa Cruz Biotechnology (https://www.scbt.com/p/gst-antibody-b-14)

Antibodies for flow cytometry:
PE-conjugated anti-CD62P antibody (Psel.KO2.3), 12-0626-82, eBioscience (https://www.thermofisher.com/antibody/product/CD62P-P-Selectin-Antibody-clone-Psel-KO2-3-Monoclonal/12-0626-82)
PE-conjugated mouse IgG1 kappa Isotype Control (P3.6.2.8.1), 12-4714-82, eBioscience (https://www.thermofisher.com/antibody/product/Mouse-IgG1-kappa-clone-P3-6-2-8-1-Monoclonal/12-4714-82)
Alexa Fluor 488-conjugated fibrinogen, F13191, Invitrogen (https://www.thermofisher.com/order/catalog/product/F13191)
FITC-conjugated anti-TP receptor antibody, 10012559, Cayman Chemicals Co. (https://www.caymanchem.com/product/10012559/

tp-receptor-(human)-polyclonal-fitc-antibody)
FITC-conjugated Normal Rabbit IgG, 10010322, Cayman Chemicals Co. (https://www.caymanchem.com/product/10010322/normal-rabbit-igg-fitc)

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) with the Approval ID of CGU110-122 and were performed in accordance with the guidelines set by the IACUC. Mice were housed in filter top cages in Tecniplast units plus paper bedding within the Chang Gung University Animal Facility, with a 12 h light cycle from 7:00 am-7:00 pm, at temperature of 22 ± 1 °C and a relative humidity of 45%-55%. The generation of Rosa26fl/fl mice was described in Fig. 1a-c. The megakaryocyte lineage-restricted hDAB2-KI mice were generated by cross-breeding Rosa26fl/fl mice with the PF4-Cre mice. Both female and male mice at the age of eight- to twelve-week old were used in the study.
Wild animals	No wild animals were used in the study.
Reporting on sex	Sex and/or gender is not related with our study purpose. Therefore, sex and/or gender was not considered in the study design. The information of sex was not collected. Both female and male mice were used in this study.
Field-collected samples	Peripheral blood from patients with thrombocytopenia was collected and used in this study.
Ethics oversight	The animal work was reviewed and approved by the Institutional Animal Care and Use Committee (Approval ID: CGU110-122). Written informed consent was obtained from all human participants, in line with approval from the Chang Gung Memorial Hospital Institutional Review Board with the approval ID of 202300838A3 and the Institutional Review Board of Taichung Veterans General Hospital (IRB No. CF23098C).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	No plants were used in this study.
Novel plant genotypes	n/a
Authentication	n/a

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	As described in methods, resting or agonist-stimulated platelets (6x107/ml, 25 µl) were incubated with either 5 µl of PE-conjugated anti-CD62P antibody (0.05 µg/µl, eBioscience, San Diego, CA, USA), 5 µl of Alexa Fluor 488-conjugated fibrinogen (20 µg/µl, Invitrogen, Waltham, Massachusetts, USA) or 5 µl of FITC-conjugated anti-TP receptor antibody (1 µg/µl, Cayman Chemicals Co., Ann Arbor, Michigan, USA) for 20 min at room temperature in the dark. The reactions were stop by adding cold 1X PBS and the samples were analyzed within 30 min using an Accuri C6 Flow Cytometer with CFlow® Software (BD Biosciences, Franklin Lakes, NJ, USA). For analysis of hDab2 expression in platelets, the whole blood with EDTA anticoagulant was incubated with the 1-step Fix/Lyse Solution (eBioscience, San Diego, CA, USA) for 15 min at room temperature for fixation of leukocytes and platelets and lysis of red blood cells. The pellets containing platelets and leukocytes were collected by centrifugation at 2,700 g for 5 min and then washed twice with 1X PBS. After removal of supernatants, the pellets were resuspended in 100 µl of 1X PBS and then permeabilized with 1 ml of 100% methanol for 30 min at 4 °C. After washing twice with 1X PBS, the pellets were

	resuspended with 3% BSA in 1X PBS. Portion of the platelet/leukocyte suspension was subject to flow cytometry analysis to estimate the platelet number in the suspension. The area in the forward scatter (FSC) vs side scatter (SSC) plot where platelets were normally resided was gated. The number of particles in the area was counted for estimation of the platelet number in the sample. For immunofluorescent staining, the recombinant anti-Dab2 antibody [EP2297Y] (Abcam, Boston, MA, USA) and the rabbit isotype IgG antibody were labeled with FlexAble FITC Plus Antibody Labeling Kit (KFA008) according to the manufacturer's instruction (Proteintech, Rosemont, IL, USA). Then platelets (2.5 x 105) were stained with FITC-conjugated recombinant anti-Dab2 antibody and PE-conjugated anti-CD62P antibody (eBioscience, San Diego, CA, USA) for 1 h at room temperature in the dark. Staining with the respective isotype control antibodies was performed simultaneously as a control of background fluorescent signal. After 1 h of incubation, samples were washed twice with 1X PBS and resuspended with 100 µl of 1X PBS for analysis of hDab2 expression in platelets using an Accuri C6 Flow Cytometer with CFlow® Software (BD Biosciences, Franklin Lakes, NJ, USA).
Instrument	BD Accuri C6 Flow Cytometer.
Software	BD Accuri C6 Software was used for data acquisition and data analysis.
Cell population abundance	Platelet abundance was determined after collection of a minimum of 10,000 singlets for each sample.
Gating strategy	Washed mouse platelets were gated using SSC-A/FSC-A. For analysis of hDab2 expression in human platelets, platelets were gated according to the FSC/SSC scatter plot (P1, shown in Supplementary Fig. 5) and PE-CD62P fluorescence (M1, shown in Supplementary Fig. 5). Then FITC-hDab2 fluorescence (M2, shown in Supplementary Fig. 5) in platelets (M1 in P1) was gated. The percent positive (%) and mean fluorescence for Dab2 was recorded. The relative levels of hDab2 expression in platelets was calculated by multiplying the values of percent positive (%) with the mean fluorescence.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.