SUPPLEMENTARY APPENDIX

Post-translational polymodification of β1-tubulin regulates motor protein localization in platelet production and function

Abdullah O. Khan,[1] Alexandre Slater,[1] Annabel Maclachlan,[1] Phillip L.R. Nicolson,[1] Jeremy A. Pike,[1,2] Jasmeet S. Reyat,[1] Jack Yule,[1,2] Rachel Stapley,[1] Julie Rayes,[1] Steven G. Thomas,[1,2] and Neil V. Morgan[1]

[1]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham and [2]The Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham and University of Nottingham, Midlands, UK

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Correspondence: *ABDULLAH O. KHAN* - a.khan.4@bham.ac.uk

NEIL V. MORGAN - N.V.Morgan@bham.ac.uk

Post-translational polymodification of β1 tubulin regulates motor protein localisation in platelet production and function

Abdullah O. Khan[1]✉, Alexandre Slater[1], Annabel Maclachlan[1], Phillip L.R. Nicolson[1], Jeremy A. Pike[1,2], Jasmeet S. Reyat[1], Jack Yule[2], Rachel Stapley[1], Julie Rayes[1], Steven G. Thomas[1,2], and Neil V. Morgan[1]✉

[1]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, UK, B15 2TT
[2]Centre of Membrane and Protein and Receptors (COMPARE), University of Birmingham and University of Nottingham, Midlands, UK

Supplementary Materials and Methods

Whole exome sequencing (WES).

To identify the causative mutation in these families we sequenced the whole exome of the affected individuals with the SureSelect human AllExon 50 Mb kit (Agilent Technologies) and sequenced on the HiSeq 2500 (Illumina) with 100 bp paired-end reads. The sequences were aligned to the reference genome (hg19 build), with Novoalign (Novocraft Technologies Sdn Bhd). Duplicate reads, resulting from PCR clonality or optical duplicates, and reads mapping to multiple locations were excluded from downstream analysis. Depth and breadth of sequence coverage was calculated with custom scripts and the BedTools package. Single nucleotide substitutions and small insertion deletions were identified and quality filtered within the SamTools software package and in-house software tools. All calls with a read coverage <4 and a phred-scaled SNP quality of <20 were filtered out. Genetic variants were annotated with respect to genes and transcripts with the Annovar tool. Candidate variants were prioritised according to their frequency in the latest population databases where a rare variant was defined as <0.01.

Platelet preparation.

25 mL volumes of blood were drawn from volunteers into sodium citrate. PRP (platelet-rich plasma) was generated by centrifugation of samples for 20 minutes at 200 g. PRP was further spun to isolate platelets by centrifugation at 1,000 g for 10 minutes with prostacyclin (0.1 μg/mL) and ACD. The resulting pellet was suspended in Tyrode's buffer prepared fresh, and pre-warmed at 37°C (5 mM glucose, 1 mM $MgCl_2$, 20 mM HEPES, 12 mM $NaHCO_3$, 2.9 mM KCL, 0.34 mM Na_2HpO_4, 129 mM NaCl to a pH of 7.3). This suspension was spun again at 1,000 g with prostacyclin at the same concentration before being re-suspended to a final concentration of 2 x 10^8/mL. Platelets were left to rest for 30 minutes at room temperature before any further processing or treatment.

Platelet spreading.

Resting platelets were fixed by preparing platelets at a concentration of 4 x 10^7/mL and mixing with equal volumes of 10% neutral buffered Formalin in a 15 mL falcon tube. This mixture was inverted gently to mix the sample, and left to incubate for 5 minutes before subsequently adding 300 μL of the resulting fixed, resting platelets to coverslips coated in Poly-L-Lysine (Sigma). Cells were then spun down at 200 g for 10 minutes.

Spreading was performed on human fibrinogen (Plasminogen, von Willebrand Factor and Fibronectin depleted - Enzyme Research Laboratories) and Horm collagen (Takeda). Coverslips were coated overnight at a concentration of 100 μg and 10 μg/mL (fibrinogen and collagen) respectively, before blocking for 1 hour in denatured fatty acid free 1% BSA (Life Technologies). Finally coverslips were washed once with PBS before the addition of platelets. Unless otherwise stated (as in the time course experiment), platelets were spread for 45 minutes at 37°C. Fixation for spread platelets was performed in formalin as for resting platelets for 10 minutes.

Construct cloning and transfection.

β1-tubulin WT construct was generated by the gibson assembly (HiFi Kit, New England Biolabs) of a TubB1 sequence fragment synthesised as a gBlock by Integrated DNA Technologies (IDT) and a C-terminal mApple empty backbone (mApple-C1 was a gift from Michael Davidson (Addgene plasmid #54631; http://n2t.net/addgene:54631 ; RRID:Addgene_54631)[41]. All mutagenesis experiments were performed using the Q5 site directed mutagenesis kit from New England Biolabs following their supplied protocol. The table in supplementary figure S2 provides the primers and annealing temperatures used to produce the various mutants using the mAPPLE TubB1 plasmid as a template. All primers were designed using the online NEBase changer tool. Transfection was performed using a standard Lipofectamine 3000 protocol in Hek293T cells maintained in complete DMEM as described in the author's previous work[42,43].

Immunofluorescence.

After fixation platelets were washed twice with PBS before incubation in 0.1% Triton X-100 for 5 minutes. The subsequently permeabilised cells were washed twice with PBS before blocking in 2% Goat serum (Life Technologies) and 1% BSA (Sigma). Fixed, permeablised, and blocked cells were then incubated with primary antibodies at a concentration of 1:500 unless other-wise stated. The following antibodies were used for experiments in this work: polyglutamylated tubulin (mouse monoclonal antibody, clone B3 T9822, Sigma), pan-polyglycylated antibody (mouse monoclonal antibody, AXO49, MABS276 Millipore), monoglycylated antibody (AXO 962 mouse monoclonal MABS277, EMD Millipore), kinesin-1 (rabbit monoclonal to KIF1B ab 167429, abcam), axonemal dynein, β1-tubulin (Rabbit polyclonal PA5-16863), tyrosinated tubulin (rabbit monoclonal antibody, clone YL1/2, MAB1864, EMD Millipore), acetylated tubulin (Lys40, 6-11B-1, mouse monoclonal antibody, Cell Signalling Technology) and DNAI1 antibody (PA5-30643 Invitrogen).

After a 1 hour incubation in the relevant mix of primary antibodies. Cells were washed twice with PBS before incubation in secondary antibodies (Alexa-568-phalloidin, anti-rabbit Alexa-647, anti-mouse Alexa-588 (Life Technologies) for one hour at a dilution of 1:300 in PBS.

Stem Cell Culture.

Gibco human episomal induced pluripotent stem cell line was purchased from Thermo Scientific and cultured on Geltrex basement membrane in StemFlex medium (Thermo Scientific). Routine passaging was performed using EDTA (Sigma), with single cell seeding performed for transfection and attempted clonal isolation through the use of TrypLE (Thermo Scientific). Briefly, cells were washed twice with PBS and once with either EDTA (for clump passaging) or TrypLE (for single cell) before incubation in 1 mL relevant detachment media for 3 minutes at 37°C. For clump passaging, EDTA was removed and 1 mL of StemFlex added. Cells were detached by triturating media onto the bottom of the well and subsequently adding the required volume to fresh media (in a new, Geltrex coated plate). For single cell seeding, TrypLE was diluted in 2 mL StemFlex and the solution added to a 15 mL falcon tube for centrifugation at 200 g for 4 minutes. The supernatant was then discarded and the cell pellet resuspended in the required volume.

iPSC MK differentiation.

iPSC differentiation to mature, proplatelet forming megakaryocytes was performed using a protocol based on work published by Feng et al.[23]. To summarise, cells were detached by clump passaging and seeded on dishes coated with Collagen Type IV (Advanced Biomatrix) at 5 μg. Cells were seeded overnight with RevitaCell (Life Technologies) to support survival on the new basement substrate. To begin the protocol cells were washed twice and incubated in phase I medium comprised of APELII medium (Stem Cell Technologies) supplemented with BMP-4 (Thermo Scientific), FGF2-β, and VEGF (Stem Cell Technologies) at 50 ng/mL each. Cells were incubated at 5% oxygen for the first four days of the protocol before being placed in a standard cell culture incubator for a further two days in freshly made phase I medium. At day 6 of the protocol cells were incubated in phase II media comprised of APELII, TPO (25 ng/mL), SCF (25 ng/mL), Flt-3 (25 ng/mL), Interleukin-3 (10 ng/mL), Interleukin-6 (10 ng/mL) and Heparin (5 U/mL) (all supplied by Stem Cell Technologies). Each day in phase II media suspension cells were spun down at 400 g for 5 minutes and frozen in 10% FBS/DMSO. After 5 days of collection, all frozen cells were thawed for terminal differentiation. Terminal differentiation was performed by incubating cells in StemSpan II with heparin (5 U/mL) and Stem Cell Technologies Megakaryocyte Expansion supplement on low attachment dishes (Corning).

RNP Complexes.

The IDT Alt-R®RNP system was used to target and knock-out TUBB1. crRNAs were ordered at 2 nmol and resuspended in 20 μL TE buffer (IDT) for a final concentration of 100 μM. Atto-555 labelled tracrRNAs were ordered at 5 nmol and resuspended in a volume of 50 μL for a final concentration of 100 μM. To prepare small guide RNAs (sgRNA), equimolar ratios of both crRNA and tracrRNA were mixed with Nuclease Free Duplex Buffer (IDT). This mix was then incubated at 95˚C for 5 minutes before allowing the reaction mix to cool at -1˚C/second to 25˚C. This mix was then spun down and complexed with HiFi Cas9 V3 (1081058 - IDT) purified Alt-R®. Cas9 protein was diluted to 6 μg per transfection and incubated with an equal volume of annealed sgRNA. This mix was left for 30 minutes at room temperature to form complete and stable RNP complexes.

Stem Cell Transfection.

iPSC transfection was performed using Lipofectamine Stem (Life Technologies) according to manufacturer instructions. Briefly, iPSC were seeded on 24 well dishes coated with Geltrex at 50,000 cells per well. After an overnight incubation in StemFlex with RevitaCell, cells were washed twice with PBS and once with OptiMem before incubation in OptiMem with RevitaCell. RNP complexes were prepared as described in section and resuspended in 25 μL OptiMem per reaction. A Lipofectamine Stem master mix was prepared using 25 μL OptiMem and 2 μL Lipofectamine STEM per reaction (4 μL if a donor template is included). Equal volumes of both Lipofectamine and RNP mix were incubated to form lipofection complexes over a 10 minute incubation at room temperature. The final transfection mix was added to cells in OptiMem and left for 4 hours before the addition of StemFlex medium (and any relevant small molecules).

Measurement of iPSC transfection efficiency after treatment with Lipofectamine STEM and IDT RNP complexes was performed using manual cell counting in Evos acquired images (Phase contrast and fluorescence).

Microscopy.

Images were acquired using an Axio Observer 7 inverted epifluorescence microscope (Carl Zeiss) with Definite Focus 2 autofocus, 63x 1.4 NA oil immersion objective lens, Colibri 7 LED illumination source, Hammamatsu Flash 4 V2 sCMOS camera, Filter sets 38, 45HQ and 50 for Alexa488, Alexa568 and Alexa647 respectively and DIC optics. LED power and exposure time were chosen as appropriate for each set of samples but kept the same within each experiment. Using Zen 2.3 Pro software, five images were taken per replicate, either as individual planes (spread platelets) or representative Z-stacks (resting platelets). Images were prepared for presentation using Fiji (ImageJ). LUTs were adjusted for presentation purposes, and a rolling ball background subtraction applied. Where Z-stacks are taken, images are presented as a maximum intensity projection. Hek293T cells were imaged using an AiryScan confocal microscope as detailed in Smith et al.[44] dSTORM imaging of TTLL10 patient and control samples was performed as described by Khan et al.[42,43] applying dSTORM and ThunderSTORM reconstruction to samples labelled with AlexaFluor 647 in the presence of a blinking buffer.

Image analysis.

Image analysis of resting and spread platelets was performed using a customised workflow published by Pike et al[45]. Briefly, the actin channel from resting and spread platelet images was used to train Ilastik pixel classifiers (approximately 6 images per condition) for segmentation based on this channel as described by Pike et al.. This was incorporated into a KNIME workflow which would run images through the classifier to generated segmented binaries in which co-localisation and fluorescence intensity statistics were calculated[46–48]. For the data presented in this manuscript, $M1_{diff}$ (a corrected Mander's co-efficient to channel 1) was used to determine the co-localisation of PTMs to tubulin, and an $M2_{dif\ f}$ value (corrected Mander's co-efficient to channel 2) was used to calculate the co-localisation of motor proteins to PTMs of interest[49]. MKs and Hek293T were segmented manually by drawing a region of interest around cells in a projected Z-stack image. For co-localisation, these images were then fed through the same automated analysis pipeline as the platelet data[45] for co-localisation values. For measures of intensity in Hek293T images, cells were manually segmented (an ROI drawn) and fluorescence values in polyglutamylated and polyglycylated channels were subsequently measured and normalised to account for variation in transfection efficiency.

Quantitative Real Time PCR (qRT-PCR).

To determine whether the 13 mammalian TTLLs and 6 CCPs were expressed in iPSC-MKs at the different stages of differentiation (day 1, day 5 and day 5 +heparin) a qRT-PCR panel was developed using TaqMan technology and an ABI 7900 HT analyser (Applied Biosystems, Warrington, UK). RNA samples were isolated and reverse-transcribed and amplified with the relevant primers using SYBR-Green based technology (Power SYBR(r) Master Mix, Life Technologies). Total RNA was extracted from iPSC cells using the NucleoSpin RNA kit (Machery-

Nagel) and cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). qRT-PCR was performed on all the TTLL/CCP fragments generated from primers designed in supplementary figure 5 and the housekeeping control GAPDH (GAPDHFOR 5'-GAAGGTGAAGGTCGGAGT-3' and GAPDHREV 5'-GAAGATGGTGATGGGATTTC-3'). Each reaction was set up in triplicate including a non-template control. Expression was analysed using the CT method using D1 undifferentiated cells as a control. A full list of primer sequences can be found in figure S8.

Supplementary Figures and Tables

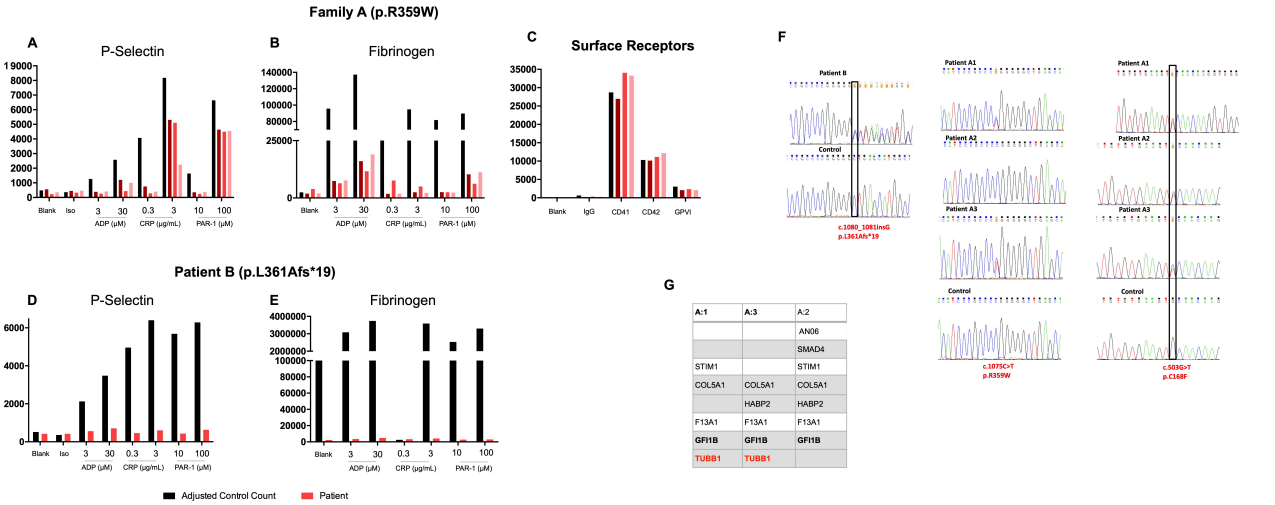
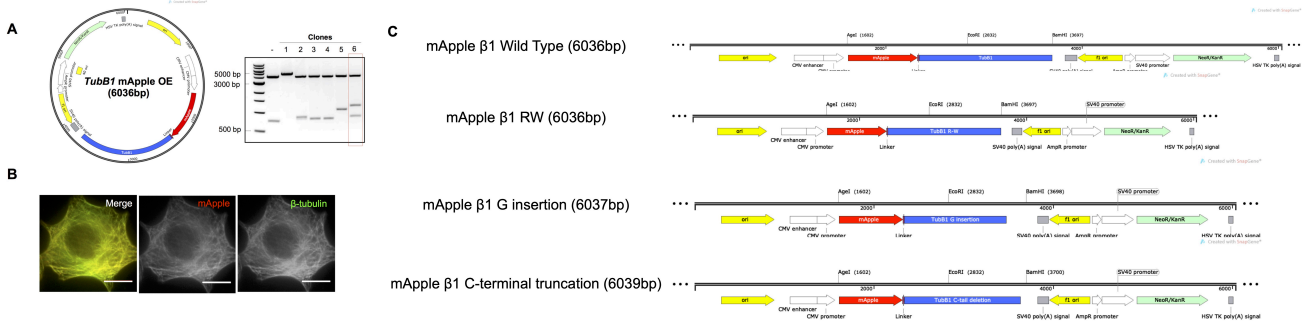


Fig. S1. Patient flow cytometry data reveals secondary defects. The GAPP project collects phenotypic data on patient recruitment, allowing for the assessment of secondary defects through FACS screening. (A) Individuals from family A show a reduction in P-selectin at both concentrations of CRP, and low concentration PAR-1. (B) Patients similarly show a reduction in fibrinogen uptake compared to controls, but show no difference in (C) surface marker expression. (D) Patient B shows a marked reduction in P-selectin surface expression and (E) fibrinogen uptake compared to controls. Platelet surface receptor normal range values (from healthy volunteers) - Blank –190 (SD 27); isotype control - 637 (SD 208); CD41 – 28727 (15378); CD42b – 10318 (SD 4257); GPVI – 5693 (SD 2447). P-selectin mean responses (Neat PRP) : Blank – 485 (SD 132); ADP 3 µm – 1261 (SD 558); ADP 30 µm – 2575 (SD 742). CRP 0.3 µg/mL– 4076 (SD 2297); CRP 3.0 µg/mL – 8177 (SD 1781). PAR-1 10 µM – 1636 (SD 1118); PAR-1 100 µM – 6642 (SD 1211). Fibrinogen mean responses (Neat PRP) : Blank – 2442 (SD 1253); ADP 3 µm – 95756 (SD 57434); ADP 30 µm – 137242 (SD 99347). CRP 0.3 µg/mL– 35095 (SD 62454); CRP 3.0 µg/mL – 94905 (SD 63054). PAR-1 10 µM – 81794 (SD 75990); PAR-1 100 µM – 89744 (SD 54673). (F) Sanger sequencing of patient samples from Family A. (G) WES analysis and subsequent genetic variant interpretation in Family A. This was performed using the clinical variant interpretation platform Congenica https://www.congenica.com) and candidate genes were filtered and selected based on an Inherited bleed-ing Disorder (IBD)-specific panel of 119 genes[29]. The following rare variants were found collectively within the different individuals of family A (AN06 - ENST00000435642.1:c.1165+21delT, COL5A1 - NM_000093.4:c.4760T>G, NP_000084.3:p.Ile1587Ser, F13A1 - NM_000129.3:c.-18-7dupT, GFI1B - NM_004188.4:c.503G>T, NP_004179.3:p.Cys168Phe, HABP2 - NM_004132.3:c.1046G>A, NP_004123.1:p.Gly349Glu, TUBB1 - NM_030773.3:c.1075C>T, NP_110400.1:p.Arg359Trp, SMAD4 - NM_005359.5:c.-127-1G>T, STIM1 - NM_003156.3:c.974G>A, NP_003147.2:p.Arg325Gln). Individuals and their variants with a low platelet count are shown in red font where only GFI1B and TUBB1 variants are common to both individuals.(H) Clinical and laboratory interpretation of the GFI1B and TUBB1 variants found in individuals of family A. In silico prediction tools tools used were as follows (POLYPHEN http://genetics.bwh.harvard.edu/pph2/; SIFT https://sift.bii.a-star.edu.sg; Align GVGD http://agvgd.hci.utah.edu/agvgd_input.php). Population frequencies were obtained from the genome aggregation database (Gnomad https://gnomad.broadinstitute.org). Current ACMG guidelines are used as supporting evidence; PP1: co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease; PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)



D

	Primers	Annealing temperatures (°C)
RW	F: 5' CATCCCGCCCtGGGGGCTGAG 3' R: 5' TCGCAGACAGCCACCTTGACG 3'	72
G insertion	F: 5' GCTGAGCATGGCCGCCACCTTC 3' R: 5' CCCCCGGGGCGGGATGTC 3'	72
C tail truncation	F: 5' TAAGAGGAAGATGAAGAGGTC 3' R: 5' TAGAACTGCTTTGGCATC 3'	59

Fig. S2. Generation of WT and mutated mApple-β1-tubulin plasmids. (A) An N-terminal mApple-β1 tubulin over expression vector was designed and cloned through the gibson assembly of the β1 tubulin sequence into a C-terminal mApple construct (mApple-C1 was a gift from Michael Davidson (Addgene plasmid # 54631 ; http://n2t.net/addgene:54631 ; RRID:Addgene_54631). Of the 6 selected clones presented, clone 6 demonstrated cleavage bands of the predicted molecular weight, and was subsequently cloned. (B) The correctly assembled sequence was then transfected to and co-stained with a β-tubulin antibody to confirm the correct expression and fold of this tubulin construct. (C) Mutants of the WT construct were generated through a Q5 site directed mutagenesis kit to generate constructs harbouring patient RW and G insertion mutants, as well as an artificially designed C-terminus truncation of the protein. (D) Primers used for the site directed mutagenesis are listed with their respective annealing temperatures.

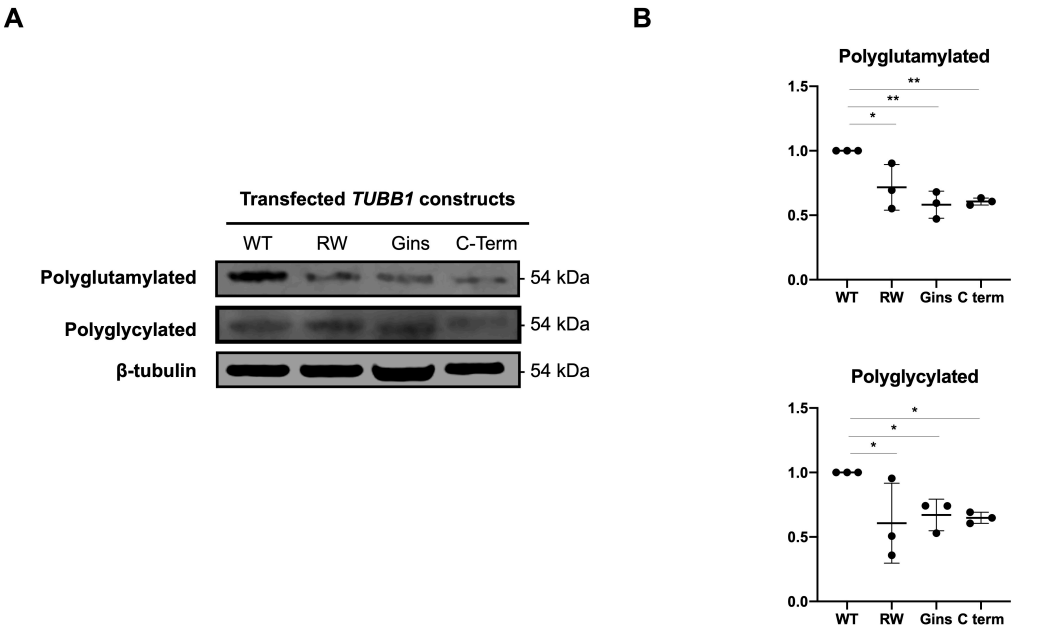


Fig. S3. Western blotting of TubB1 mutant constructs. Mutant TubB1 constructs were re-cloned without the mApple label and transfected into Hek293T. Transfected cells were then collected and lysed for western blotting. (A) A reduction in polyglutamylation and polyglycylation is evident when compared to wild type TubB1 constructs. (B) Polyglutamylation was significantly reduced in RW, G insert, and C terminal truncations (* p = 0.0102, ** p = 0.0011, ** p = 0.0017), as was polyglycylation (* p = 0.0210, 0.0434, p = 0.0338.).

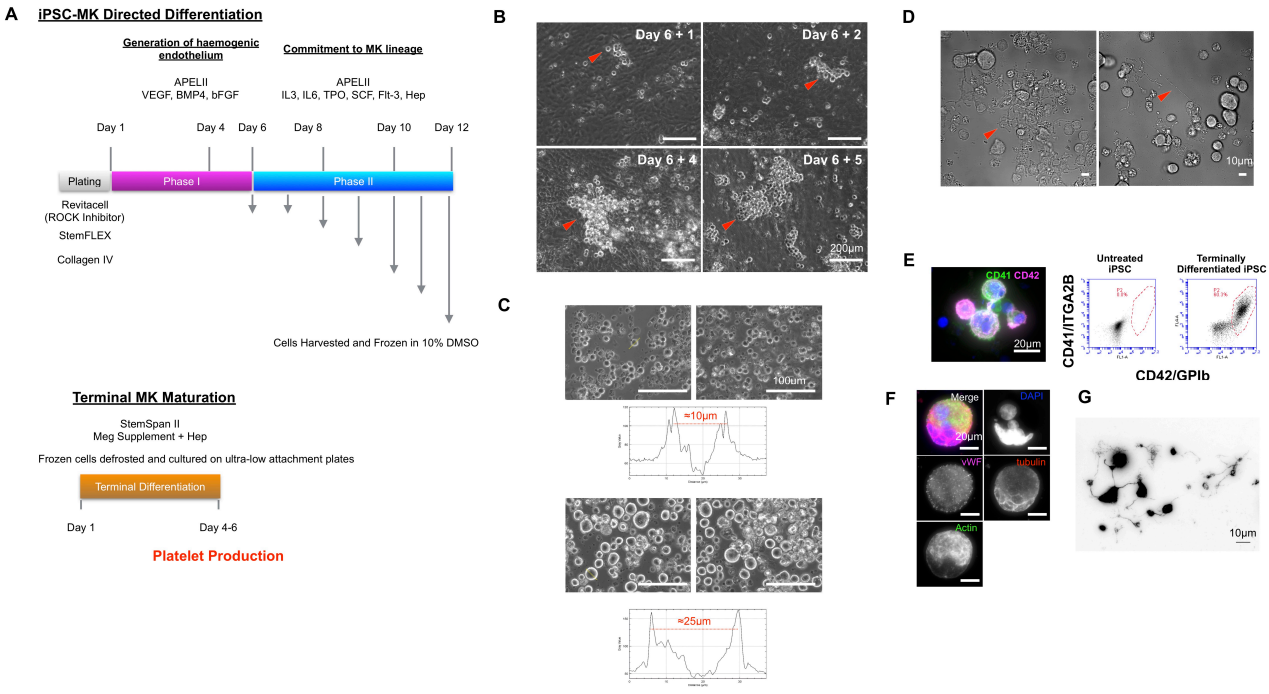


Fig. S4. Directed differentiation of iPSC to proplatelet forming MKs. (A) A 3 stage protocol was adapted from a method previously published by Feng *et al.*. Briefly, iPSC were clump passaged on to collagen IV coated plates and incubated in RevitaCell overnight before beginning Phase I of the differentiation. Phase I involves a 4 day incubation at 5% O_2 in APEL2 media supplemented with 50ng of BMP4, VEGF, and FGF2, after which fresh media was added and cells were incubated for 2 more days at normoxic conditions. Phase II of the protocol involved incubation in APEL2 supplemented with IL3, IL6, Flt-3, hSCF, TPO, heparin, during which time cells were harvested and frozen every 48 hours and fresh media added. Finally, harvested cells were thawed and incubated in StemSpan II medium with MK supplement for 5 days before samples were prepared for downstream assays (immunofluorescence, RT-PCR etc.). (B) During Phase II of the differentiation, progressively larger numbers of blast like cells are observed emerging from a layer of haemogenic endothelium. (C) During Phase III of the differentiation, cells grow from progenitors and blast like cells approximately 10 μm in size to large, mature MKs ranging in 25-40 μm in size. (D) At day 5, on treatment with Y-27632 and heparin, cells form elaborate proplatelet networks. (E) 60% of terminally (Phase III) differentiated cells are CD41 and CD42 double positive and on staining demonstrate (F,G) a mix of ploidies and proplatelet networks consistent with mature platelet producing MKs.

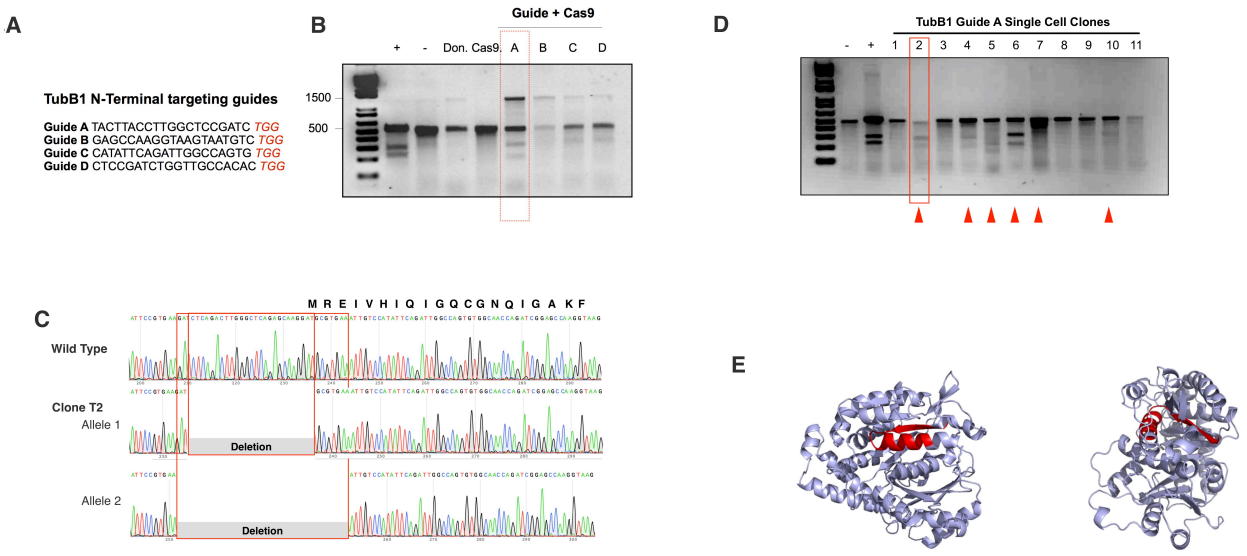


Fig. S5. CRISPR bi-allelic knock-out of β1 tubulin. (A) Guides targeting exon 1 of the *TUBB1* gene were designed and (B) tested for efficiency using a T7EI cleavage assay. The population evidencing the most cleavage (and hence most efficient guide (guide A)) was taken forward to generate *TUBB1* knock-out clones, through single cell clonal isolation. (C) Cells positive for cleavage on single cell expansion (identified by the red arrows) were taken forward for sequencing. (D) Clone T2 revealed a bi allelic loss of the start codon, (E) resuling in a deletion of a significant portion of the N-terminus and as evidenced in the main text, a loss of *TUBB1* expression.

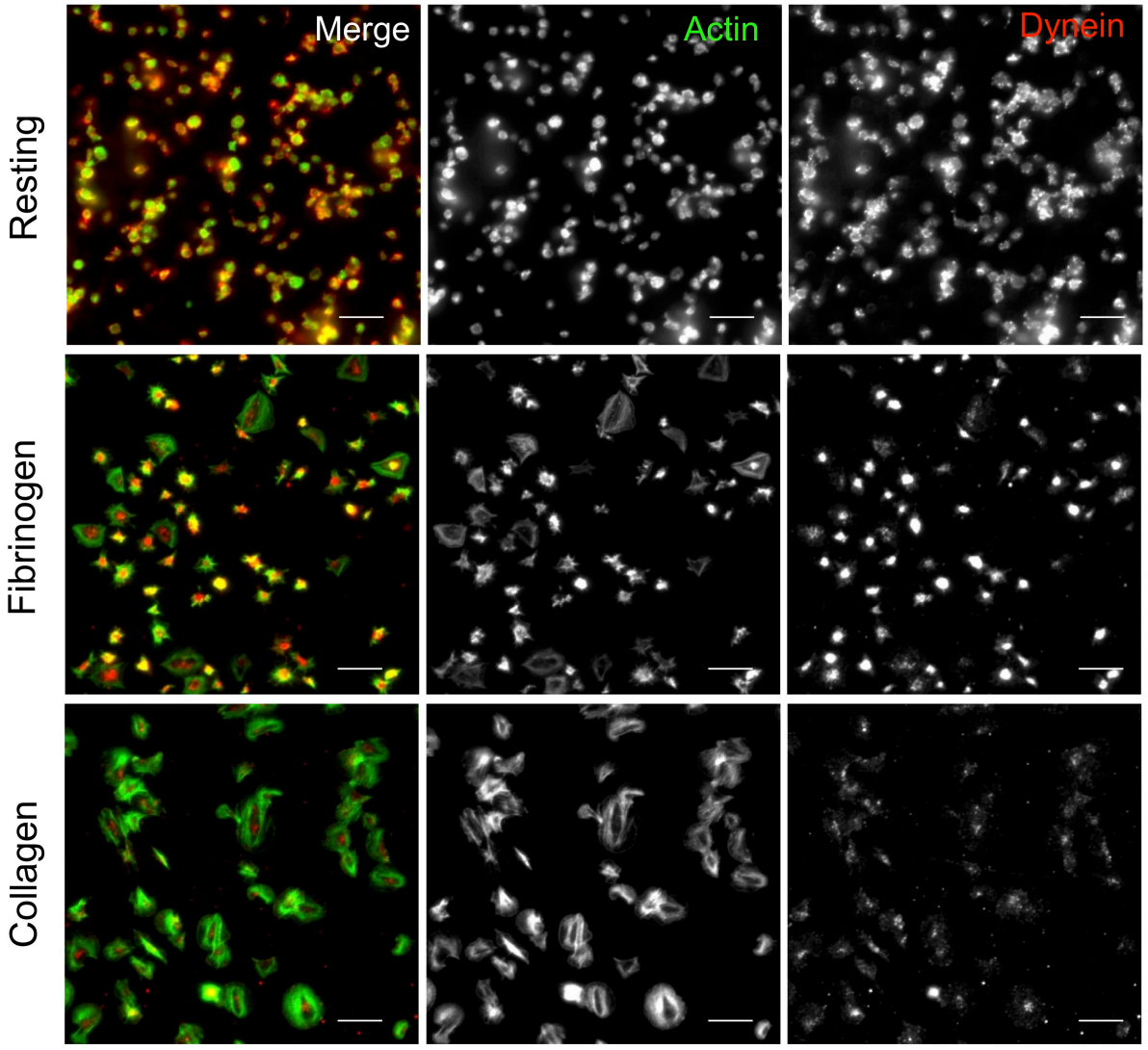


Fig. S6. Staining of Cytoplasmic Dynein in resting and Spread Platelets. Resting and spread human donor platelets were stained for cytoplasmic dynein to compare the distribution of this isoform of the motor protein to axonemal dynein. While axonemal dynein is primarily found on the edge of spreading cells, cytoplasmic dynein is found at the centre of spread cells, suggesting that the axonemal variant is involved in platelet spreading and activation.

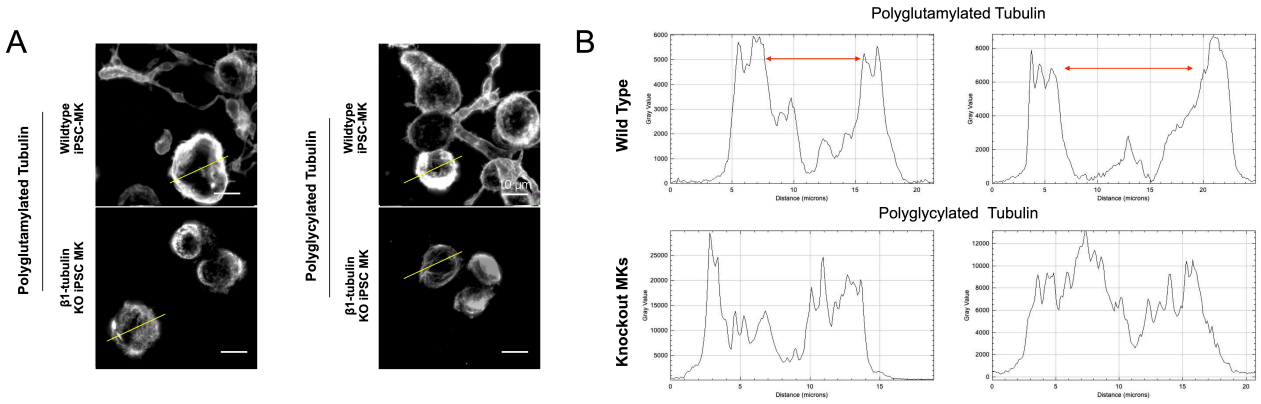


Fig. S7. Line profiles demonstrating difference in distribution of polymodified tubulin in knock-out cells. Line profiles demonstrating the different distribution of polymodified residues in wild type vs. knock-out iPSC MK. In wild type cells, both polymodifications accumulate around the periphery of mature cells forming a dense microtubule band indicated by distinctive peaks on their respective line profiles (B). In contrast, knock-out cells have a disordered and diffuse distribution of tubulin indicated by their line profiles.

Name	Sequence : (5' to 3')	Fragment Size	Name	Sequence : (5' to 3')	Fragment Size
FH1_TTLL1	AGTCAACCATTTTCCAAACC	143 bp	FH1_TTLL11	ATTTGTTTATCCGGTTCCTG	76 bp
RH1_TTLL1	AGTCCAGATAGAGGTATTTTCC		RH1_TTLL11	CTCCTTATGAAGGTACGAAAG	
FH1_TTLL2	GCCTTTACCCTTAACATTCC	138 bp	FH1_TTLL12	CATTCTGGAGGAAAACAAGG	84 bp
RH1_TTLL2	TTTCTTCTTCTCCAGTGTTG		RH1_TTLL12	GTGTAGACCTTGAAGATGTG	
FH1_TTLL3	AAGCCTTCATAGAGGACTTC	95 bp	FH1_TTLL13	ACCTGACCAACTATGCTATC	477 bp
RH1_TTLL3	TACTGCCTGAATAGGGTATG		RH1_TTLL13	TGGTTTTGATGATGATGTCC	
FH1_TTLL4	GAAGCTAAACCATTTCCCAG	106 bp	FH1_AGTPBP1 (CCP1)	AAAAACAAATGCCAGGAGAG	100 bp
RH1_TTLL4	GAAACTGAACTCCTTCTTGC		FH1_AGTPBP1 (CCP1)	CATGTTTCTATGCCGGTTATC	
FH1_TTLL5	AATTCATATTCGAAGGACCG	85 bp	FH1_AGBL2 (CCP2)	GGCCTATCAGTTTATCTTCAG	170 bp
RH1_TTLL5	GATTGTTGATCAGGTAGACG		RH1_AGBL2 (CCP2)	ATCTGTAATCCCAGCTACTC	
FH1_TTLL6	AAGCCCTTTATCATTGATGG	87 bp	FH1_AGBL3 (CCP3)	GAAGAGCAAAGAAGGAACAG	102 bp
RH1_TTLL6	GTACACAAAAATCCTGAGAGG		RH1_AGBL3 (CCP3)	TTGTTACCCAGAGTAGATCC	
FH1_TTLL7	CAGAATTGGTGGTAAAGACC	152 bp	FH1_AGBL1 (CCP4)	AGATGATGACTTGGAAACAG	111 bp
RH1_TTLL7	CCATGGCTTTAGTTTTCTATCC		RH1_AGBL1 (CCP4)	CTATAGGAGAGCTCAAGACAC	
FH1_TTLL8	AACAAGGAATTTCCCAAGAC	174 bp	FH1_AGBL5 (CCP5)	CTATATCCAAAGCTCATCTCC	178 bp
RH1_TTLL8	AGTGGAACTTCTTCTCTACC		RH1_AGBL5 (CCP5)	AGTTGCATTCAAGTGTGTAG	
FH1_TTLL9	ATCATGAAGCCTGTAGCC	158 bp	FH1_AGBL4 (CCP6)	AAATGATGATGCCATTGGAG	114 bp
RH1_TTLL9	GGATTTTCAATGTAACGCTG		RH1_AGBL4 (CCP6)	TTACCACTTTCAAAGCAAGC	
FH1_TTLL10	GAAGAGTTTTTCCCAGAGAC	90 bp			
RH1_TTLL10	GATCCATATCTGGGTTTCATC				

Fig. S8. Primers and predicted fragment lengths for qRT-PCR screen of TTLL and CCP expression. Exon overlapping primers were disgned for a qRT-PCR screen of TTLL and CCP expression. Forward and reverse primer sequences are listed, along with predicted fragment length.

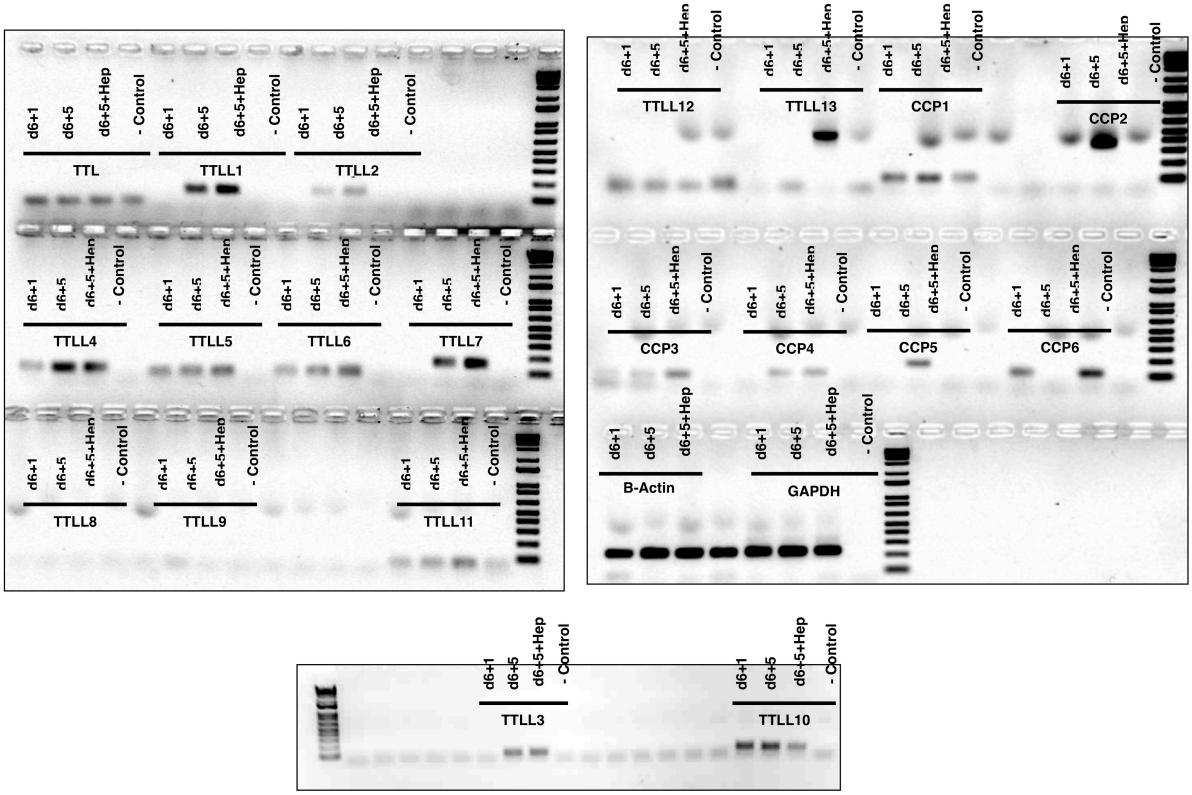


Fig. S9. Whole gel for TTLL and CCP RT-PCR screen in iPSC-MKs. Complete gels used in figure 6.

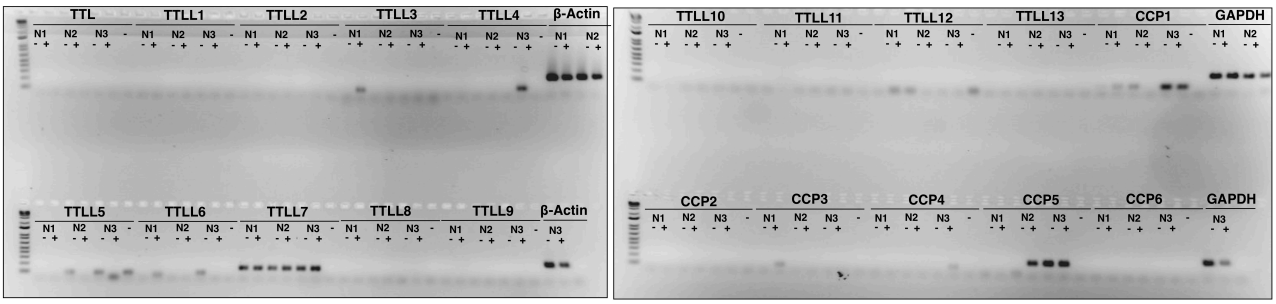


Fig. S10. Whole gel for TTLL10 and CCP RT-PCR screen in human peripheral blood platelets. Complete platelet gel used in figure 6.

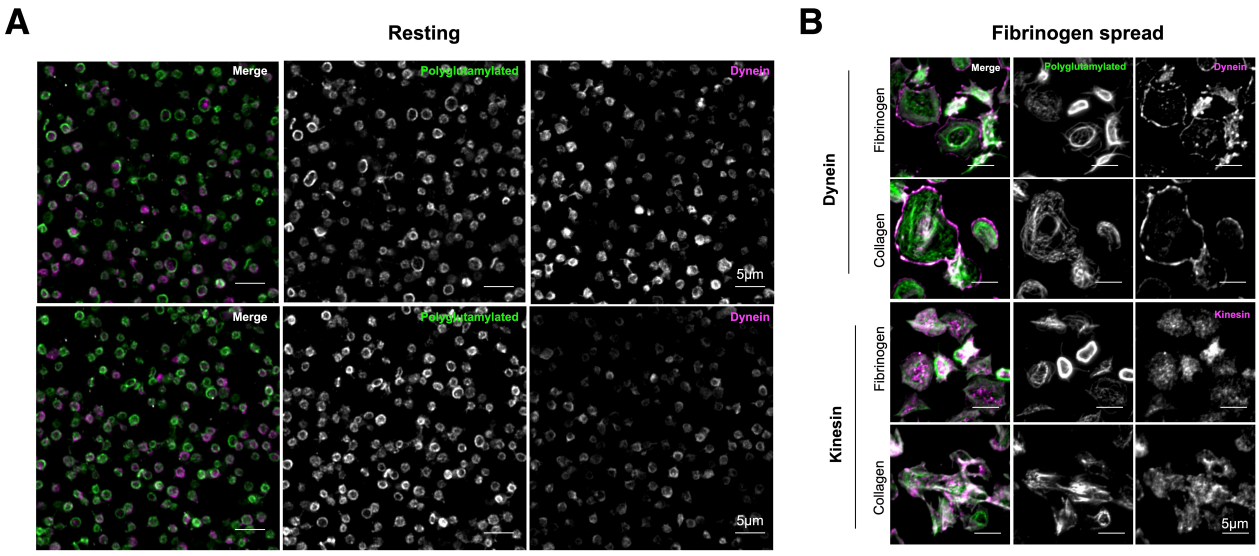


Fig. S11. Resting platelets co-stained for dynein and kinesin. Resting platelets co-stained for kinesin and dynein to supplement spread figures.

	Family A		Family B	
	II:2	II:3	II:2	III:1
Total number of variants identified by WES	26,302	26,379	26,456	27,018
Total number of variants (excluding synonymous) with a MAF ≤ 0.01	2,282	2,277	2,378	2,601
Shared significant variants from the panel of platelet and endothelial cell genes with a MAF ≤ 0.01	11		11	
Total number of novel variants	159	167	129	140
Total number of shared novel variants	51		53	
Total number of shared genes predicted to be pathogenic using all bioinformatics tools	9		11	

Family A, II:2 & II:3							
Gene	**Genomic variation**	**Protein effect**	**Variation type**	**Prevalence**	**Mutation taster**	**ACMG criteria**	**Classification**
TTLL10	c.462delG	p.P154Rfs*38	Frameshift deletion	Novel	Disease causing	PM2 PP3 PM4	Uncertain significance
Family B, II:2 & III:1							
Gene	**Genomic variation**	**Protein effect**	**Variation type**	**Prevalence**	**Mutation taster**	**ACMG criteria**	**Classification**
TTLL10	c.745_746insG	p.V249Gfs*57	Frameshift insertion	2.15×10^{-3}	Disease causing	PM2 PP3 PM4	Uncertain significance

Fig. S12. Rare and novel TTLL10 genetic variants identified in WES data from affected individuals. Variants were filtered; synonymous variants and variants with a minor allele frequency (MAF) were excluded, and non-shared variants. The pathogenicity of the remaining variants was predicted using tools (Mutation Taster, Phastcons, SIFT, Provean etc.) and American College of Medical Genetics and Genomics (ACMG) classification Candidate variants are selected on the basis of a pathogenic prediction. Supporting evidence is given where PM2 is “Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium”, PP3 is “Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)” and PM4 is “Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants”.

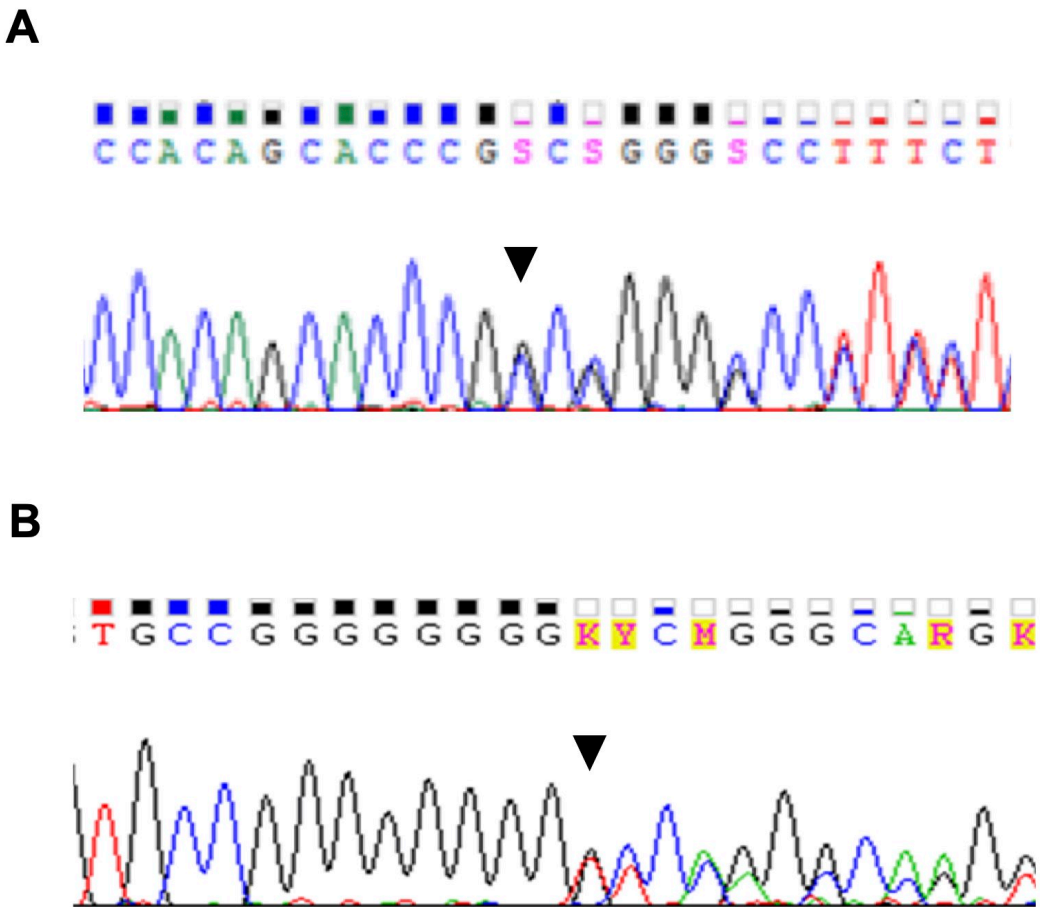


Fig. S13. Supplementary figure 13 Sanger sequencing of the TTLL10 variants for confirmation following WES. Representative confirmative Sanger sequencing electropherograms are shown. Black arrow shows sites of genomic variation. A) c.462delG. B) c.745_746insG. Reference sequence- NM_001130045.