Suppl. Methods section

Patient data

The patient was treated at the university hospital LMU Munich. The patient consented to taking part in this study. The study was approved by local ethics committee of Munich. Data on presentation, treatment and outcome are depicted in Fig 1a-b, Suppl. Fig 1a and the laboratory values table (Suppl Table 1-3). A HIT-IL-Acustar assay and a HYPHEN BioMed HIT-ELISA Test deemed sensitive for TTS returned negative for this patient [5]. PF4 induced platelet activation(PIPA) and heparin induced platelet activation (HIPA) tests were performed as previously reported[24].

Suppl. Table 1

Laboratory values at admission			
Parameter		Unit	Range
INR	1.1	-	0.8-1.2
aPTT	31	sec	22-34
CRP	0.2	mg/dl	≤0.5
D-Dimer	1.6	µg/ml	≤0.5
Leukocytes	8.68	G/l	3.9-9.8
Hemoglobin	16.5	g/dl	13.5-17.5
Platelets	118	G/l	146-328
Thrombophilia screening			
Factor-V-Leiden mutation	1691G>A not detected		
Prothrombin mutation	20210G>A not detected		
Lupus anticoagulant	not detected		
APC screening	4,4 (range >=2,5)		
Protein C	78% (range 68-150)		
Protein S	91% (range 74-136)		

Suppl. Table 2

HIT tests	
Heparin/PF4 Ab Screening Test	**positive**
HIT-IL-Acustar-Assay	negative
HIT-ELISA-Test (HYPHEN Biomed)	negative
HIPA test	negative
PIPA test	negative

Suppl. Table 3

Platelet auto-antibody panel	
PakAuto® Test*	**positive**
GPIb/IX Auto-antibodies*	negative

GPIa/IIa Auto-antibodies*	negative
GPIIb/IIIa Auto-antibodies*	**positive**

*diagnostics performed after IVIG treatment initiation

Mouse Strains

C57BL/6 mice were purchased from The Jackson Laboratory. $AID^{-/-}$ $sIgM^{-/-}$ mice were bred and maintained at our animal facility. Mice were aged between 12-16 weeks for the experiments and were age and sex-matched for inter-group analyses. All mice live in standardized conditions where temperature, humidity, and hours of light and darkness are maintained at a constant level and provided water and food ad libitum. All animal experiments were performed in compliance with the relevant ethical regulations and were approved by the local administrative authority for the protection of animals (*Regierung von Oberbayern, München*).

Vaccine acquisition

Only residual content of discarded BNT162b2 (Pfizer-BioNTech) and ChAdOx1 nCov-19 (AstraZeneca) vials, that, according to the supervising physicians' body (*Kassenärztliche Vereinigung Bayerns*), could not be used for human application anymore, were used for this study. For ChAdOx1 nCov-19 this meant a storage period greater than 48h after first withdrawal of a dose from a multi-dose vial, for BNT162b2 it meant a storage period greater than 24h after first withdrawal of a dose from a multi-dose vial. Both vaccines were stored continuously at 4°C. Experiments were conducted as soon as possible after vaccine acquisition.

Human platelet isolation

Human blood for *in-vitro* assays was obtained after informed consent from healthy male and female donors aged 20-35. Blood was collected with a syringe containing 1/7 of the blood volume citrate (39 mM citric acid, 75 mM sodium citrate, 135 mM dextrose; ACD). The blood was further diluted 1:1 with Tyrode's buffer Tyrode's buffer (137 mM NaCl, 2.8 mM KCl, 12 mM $NaHCO_3$, 5.5 mM glucose, 10 mM HEPES, pH = 6.5), centrifuged at 70g for 35min, and the supernatant platelet-rich-plasma (PRP) was taken. PRP was further spun down after adding 0.1mg/ml PGI2 at 1200g for 10min. The resulting washed platelet pellet was resuspended in 7.2pH Tyrode's buffer and used for subsequent experiments.

Mouse platelet isolation

Mouse blood was obtained via retro-orbital blood collection, after anesthesia with medetomidine, midazolam and fentanyl (MMF). Blood was collected via a capillary and mixed with 1/7 of the blood volume citrate. The blood was further diluted 1:1 with Tyrode's buffer (137 mM NaCl, 2.8 mM KCl, 12 mM $NaHCO_3$, 5.5 mM glucose, 10 mM HEPES, pH = 6.5), centrifuged at 70g for 10min, and the supernatant platelet-rich-plasma (PRP) was taken. PRP was further spun at 1200g for 10min. The resulting washed platelet pellet was resuspended in 7.2pH Tyrode's buffer and used for subsequent experiments.

Platelet incubation with vaccines

Ca. $1x10^8$ washed platelets were incubated with ~$5x10^7$ ChAdOx1 nCov-19 viral particles (100µl), or 100µl BNT162b2 or PBS for 20min. After this 1:200 X649 (mouse, emfret Analytics) or CD41 (human, Biolegend) was added and further incubated for 10min and then analyzed via flow-cytometry. For transfusion, after

separate vaccine incubation with ChAdOx1 nCov-19 and BNT162b2, platelets were stained with 1:200 X649 or X488 (emfret Analytics) respectively for 10min. X488 and X649 labelling was switched for half of the mice to ensure no color bias. The platelets were spun down at 1200g for 10min, resuspended in 200µl 7.2pH Tyrode's buffer and immediately injected separately via tail vein injection.

Mouse vaccine injection and blood collection

5µl (low dose) or 50µl of ChAdOx1 nCov-19, BNT162b2, PBS, or heat inactivated ChAdOx1 nCov-19 were used for injections. Heat inactivation of ChAdOx1 nCov-19 was achieved by heating the vaccine to 99°C for 30min, then spinning the resulting protein precipitate down at 10,000g for 3min and taking the supernatant. Injection was performed either via tail vein or in the medial aspect of the thigh of isoflurane anesthetized mice. At specified time points, vaccine injected, and transfused mice were briefly anesthetized with isoflurane and blood was obtained via facial vein puncture and collected in EDTA capillaries. Blood counts were measured with the Sysmex XN-V Series XN-1000V cell counter, only blood counts that passed internal quality control were used.

Platelet bound immunoglobulin detection

Mouse washed platelets were incubated with 1:4 plasma of mice 6d post-inoculation (p.i.) and control mice for 40min. After adding 1:100 CD41 PB, IgG Cy3 and IgM FITC and 20min incubation washed platelets were fixed, diluted and analyzed via flow cytometry.

Intravital imaging of the spleen

Co-injection of platelets was performed as previously described[25]. After injection of X488 or X649 labelled platelets incubated with ChAdOx1 nCov-19 or PBS, mice were also injected with 15µl each of CD169 PE and F4/80 BV421 (Biolegend), anesthetized with MMF, shaven, and the spleen exposed via a small lateral cut. Subsequently the spleen was mobilized and imaged with a Zeiss LSM 880 confocal microscope (x20 obj.) in airyscan mode. Regular blood flow in the spleen was observed throughout imaging, and z-Stack images (1µm slice thickness) as well as videos were taken.

Flow cytometry

Flow cytometry to characterize platelet profiles was performed as previously described [26]. Briefly, incubation with panels at 1:200 per antibody of either washed platelets or whole blood, at least 5x the amount of FACS Lysing Solution (BD) was added to fixate platelets and lyse blood. Flow cytometry was performed on a BD LSRFortessa and BD FACSCanto. Flowjo (BD) was used for flowcytometric analysis, gating strategies are shown in Supplemental Figure 2. For adenovirus binding, as well as IgM and IgG binding a positive gate was used, for MFIs the MFI of each marker per data point was normalized by dividing it by the highest MFI of that marker and experimental run.

Antibodies used for Flow Cytometry:

Color	Target	Species reactivity	Catalogue # and company
AF700	CD44	Mouse	#103026, biolegend
APC	GPIb	Mouse	X649, emfret Analytics
APC/Fire 750	CD31	Mouse	#102433, biolegend

BV421	CD41	Mouse	#133932, biolegend
BV510	AnV	Mouse	#640937, biolegend
BV711	CD107a	Mouse	#121631, biolegend
PE-Cy7	CD62p	Mouse	#148309, biolegend
PE-Dazzle	CD9	Mouse	#124821, biolegend
PerCP-Cy5.5	CD154	Mouse	#106513, biolegend
Cy3	IgG	Mouse	#M30010, ThermoFisher
PE	GP2b3a	Mouse	M023-2, emfret Analytics
FITC	IgM	Mouse	A21042, life technologies
FITC	GPIb	Mouse	X488, emfret Analytics
FITC	Hexon	Adenovirus	ab87333, abcam
BV650	CD63	Human	#353026, biolegend
FITC	CLEC-2	Human	#372007, biolegend
PE	CD162/PSGL1	Human	#328805, biolegend
PERCP-Cy5.5	CD36	Human	#336224, biolegend
AF 647	PAC-1	Human	#362806, biolegend
APC-Cy7	CD184 (CXCR4)	Human	#306528, biolegend
BV510	CD42b	Human	#303933, biolegend
BV 711	CD154 (CD40L)	Human	#310837, biolegend
AF 700	CD41	Human	#133926, biolegend
BV421	CD62P	Human	#304926, biolegend
PE-Dazzle	CD31 (PECAM)	Human	#303130, biolegend
PE-Cy7	CD284 (TLR4)	Human	#312805, biolegend
BV650	CD63	Human	#353026, biolegend

Histology

Organs were harvested directly after sacrifice, fixed at 4%PFA for 1h and subsequently 30% sucrose overnight, then embedded in OTC and stored at -80°C. Sections were stained with antibodies against F4/80 (Biolegend, Cat. No. 123110), CD45R/B220 (Biolegend, Cat. No. 103212), ki67 (Abcam, Cat. No. ab15580), Lectin PNA (Thermofisher, Cat. No. L32458). Secondary antibodies and nucleic acid stain

included FITC goat anti mouse (Thermofisher, Cat. No. A11029) and Cy3 goat anti rabbit (Jackson Immunoresearch, Cat. No. 111-165-003) and Hoechst33342. Micrographs were taken on a Zeiss LSM 880 confocal microscope in airyscan mode and a Leica LAS X epifluorescence microscope.

Data analysis and statistics

Analysis of histology and intravital imaging was done using ImageJ v2.1 or Imaris (Bitplane). Intravital imaging cell tracking was done manually on randomly selected cells which could be traced for at least 5 frames. Statistics were computed with Imaris, Meandering Index was defined as Net displacement/Total track length. Unpaired t-tests with Welch´s correction was used for all cell tracking analyses. For area quantification, thresholds were taken with ImageJ and overlap quantified by percent area overlapping between thresholds. For all statistical tests Prism (GraphPad) was used. Unpaired t-test were used unless otherwise noted. All data are shown as mean ± standard error of the mean (s.e.m.). When more than three t-tests were applied on the same data set, multiple testing correction was done.

Suppl. Figure Legends

Supplementary Figure 1 | a, Time course of hemoglobin, leukocyte and platelet counts of SVT patient. **b,** Platelet surface marker expression of human platelets incubated with ChAdOx1 nCov-19 or BNT162b2. Normalized MFIs. Multiple t-tests with Holm-Sidak correction, non-significant. n=8 donors per group. **c,** Quantification of adenovirus platelet binding to mouse platelets**.** One-way ANOVA with post-hoc Tukey´s test. Comparison of ChAdOx1 nCov-19 to both controls. n=4 per group. **d,** Cell count parameters of blood taken at 24h p.i. with either ChAdOx1 nCov-19 i.v. or i.m., BNT162b2 i.v. or PBS i.m.. Two-way ANOVA with post-hoc Tukey´s test, all nonsignificant. n≥5 per group. **e,** Comparison of platelet count at 24h p.i. with either ChAdOx1 nCov-19 i.v. or i.m. (same data as in Figure 1f) or low-dose ChAdOx1 nCov-19 i.v. administration. Unpaired t-tests, n≥4 per group. **f,** Time course of platelet counts right before and after ChAdOx1 nCov-19 i.v. administration of $AID^{-/-}sIgM^{-/-}$ mice. Unpaired t-tests, n=4 per time point. **g,** Zoom-in of the crop outs in Figure 2a. Scale bars are 5µm. **h,** Illustration of intravital splenic imaging with transfused platelets and representative overview image of intravital microscopy. Scale bar 10µm. **i,** Z-projection of a 3D-stack intravital splenic microscopic image showing phagocytosed ChAdOx1 nCov-19 pretreated transfused platelets (white, arrow) in comparison to PBS treated platelets (green). Scale bar 5µm. **j,** Series of images from an intravital splenic microscopic video showing phagocytosis (upper arrow) and interactions (lower arrow) of ChAdOx1 nCov-19 pretreated transfused platelets. Time is shown on the upper left, scale bars 5µm. **k,** Platelet activation marker expression after plasma incubation of mice with i.v. or i.m. ChAdOx1 nCov-19 administration. Normalized MFIs. Unpaired t-tests, non-significant. n=4 mice per group. Error bars are mean ±s.e.m. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 2 | a, Gating strategy for human in-vitro platelet-adenovirus binding. Last gate was used to quantify platelet-adenovirus binding. **b,** Gating strategy for murine in-vitro platelet-adenovirus binding. Last gate was used to quantify platelet-adenovirus binding. **c,** Gating strategy for murine in-vivo platelet-adenovirus binding and platelet surface marker expression. Second gate was used to derive MFIs for platelet surface marker expression, last gate was used to quantify platelet-adenovirus binding. **d,** Gating strategy for transfused platelet tracking. Both of the last gates were used to quantify ChAdOx1 nCov-19 and BNT162b2 incubated transfused platelet fraction. **e,** Gating strategy for immunoglobulin binding of platelets incubated with plasma from vaccinated mice. Last gate was used to quantify either IgG (shown) or IgM binding. All gating is shown as contour plots with 2% counter and outliers shown.

Supplementary Figure 3 | Graphical abstract: Thrombocytopenia and splenic platelet directed immune responses after intravenous ChAdOx1 nCov-19 administration. Intramuscular injection of ChAdOx1 nCov-19 leads to normal vaccine response without platelet involvement. Accidental intravascular injection of ChAdOx1 nCov-19 leads to adenovirus-platelet binding and platelet activation. Platelets are cleared by professional phagocytes, particularly in the spleen. Trafficking and processing of platelet adenovirus-aggregates from the red pulp to splenic follicles leads to a B cell response with the emergence of platelet binding antibodies.