SUPPLEMENTARY MATERIALS & DATA

Supplementary materials

Magnetic Resonance Imaging (MRI) protocol

The protocol consisted of a T2 and T2*-weighted imaging sequences. For T2-weighted, repetition time was 2650 ms, with an echo time of 42 ms. With a 256x256 matrix, a field of view of 22x22 mm was scanned with 19 contiguous slices of 0.8-mm thickness. For T2*-weighted, slices were imaged with repetition time/echo time=600/8 ms, flip angle of 30°, field of view=22x22 mm and matrix=128x128.

Blood sampling

Blood was collected before and one day after surgery (CVST or sham). Blood sample was withdrawn from the retro-orbital venous sinus using capillary tubes with an appropriate anticoagulant (EDTA, heparin or sodium citrate). EDTA-anticoagulated whole blood count was carried out using a Scil VetABC animal blood analyzer (Viernheim, Germany). To obtain poor-platelet-plasma (PPP), citrated samples were centrifuged twice at 2500g for 15 min at 18°C. PPP samples were immediately frozen and stored at -80°C until use.

Immunohistochemical analyses

Organs were embedded in paraffin, sliced into 10-µm sections, conducted with hematoxylin-eosin (HE) and Nissl staining to assess haemorrhagic and ischemic brain lesions. For immunofluorescence, brain sections were deparaffinized, rinsed in PBS before permeabilization (0.05% Triton X-100 in PBS, 5 minutes). Sections were blocked with 3% bovine serum albumin (BSA) in PBS for 1 hour. Then, slides were incubated 2 hours at room temperature with various combinations of appropriate primary antibodies to stain neutrophils, platelets, endothelium and immunoglobulin G through injury area (Table S1). Brain sections were rinsed before incubation with corresponding secondary antibodies, under the same conditions as previously mentioned. DNA was finally counterstained using 6-diamidino-2-phenylindole. Red blood cells (RBC) were visualized via their inherent auto-fluorescence. Negative controls of the immunofluorescent staining were achieved by omission of the primary antibody on control slices. Images were acquired using a laser Axiovert Zeiss fluorescence microscope (Oberkochen, Germany) and processed using ImageJ

software (National Institutes of Health). Quantitative assessment of neutrophil and intravascular fibrin deposits was performed in non-haemorrhagic cortical parenchyma using ImageJ software (National Institutes of Health).

Table S1: List of antibodies used for immunofluorescence studies related to experimental procedure

Target	Marker	Company (Cat♯)	Species	Conjugate	Dilution
MPO	Neutrophils	Dako (A0398)	Rabbit	-	1: 330
GPIX	Platelets	Emfret (M-051-0)	Rat	-	1: 50
GLUT-1	Cerebral Endothelium	Merck millipore, (07-1401)	Rabbit	-	1: 1000
Fibrin	Fibrin	Merck millipore, (59D8)	Mouse	AF555	1:400
DAPI	DNA	Invitrogen	-	-	1: 1000
Goat IgG	Secondary antibody	Invitrogen (A-32732)	Rabbit	AF555	1: 500
Donkey IgG	Secondary antibody	Jackson (712-607-003)	Rat	AF647	1: 500
Sheep IgG	Secondary antibody	Jackson (313-607-003)	Rabbit	AF647	1: 500
Goat IgG	IgG	ThermoFisher (A-21237)	Mouse	AF647	1: 500
AF: Alexafluor					

Supplemental data

Table S2: Hematological parameters in *Jak2*^{WT} and *Jak2*^{V617F} mice at baseline and at day 1 (D1) after CVST.

Results are expressed as mean ±SD (n=20). Statistical analysis was performed using student t-test. Data were compared (*a)* between *Jak2*^{WT} and *Jak2*^{V617F} mice at baseline, (*b)* between baseline and D1 in each group. ns: not significant. SII: Systemic Immune Inflammation Index (Platelet (G/L) × neutrophil (G/L) / Lymphocyte (G/L)).

	Baseline			***CVST D1***		
	***Jak2*^{WT}**	***Jak2*^{V617F}**	*p value* [a]	***Jak2*^{WT}**	***Jak2*^{V617F}**	*p value* [b]
White Blood Cells, x10^9/L	10.5±3.5	15±3.3	*<0.01*	*7.2*±1.9	21±9.7	*ns*
Neutrophils, x10^9/L	2.2±0.7	4.8±1	*< 0.0001*	1.5±0.8	8±3.4	*ns*
Haemoglobin, g/dL	13.5±1.1	18.4±1.9	*< 0.0001*	11.5±2.4	17.2±1.8	*ns*
Haematocrit, %	43±5.6	59.7±8.6	*< 0.0001*	*38*±10	*58.6*±6.7	*ns*
Platelets, x10^9/L	914±171	1014±106	*ns*	*984*±180	*1201*±221	*ns*
SII index	72±37	400±83	*<0.01*	163±51	802±185	*<0.05*

Table S3: Characteristics of patients with CVST and *JAK2*^{V617F} mutation.

Patient	Gender	Age (years)	Hb, g/dL	Ht, %	WBC, x10^9/L	Neutrophils, x10^9/L	PLT, x10^9/L	*JAK2*^{V617F} allelic burden (%)
1	F	73	16.7	53	24.7	20	155	48
2	F	74	21.5	63	23.4	21.5	133	81
3	F	24	13.1	41	7.2	4.4	643	15
4	F	33	14.2	44	10.1	6.8	389	4
5	F	76	14.1	42	13.1	11.1	326	9
6	F	26	13.6	41	9.6	6.8	497	11
7	F	42	14.1	40	15.4	11.1	297	7
8	F	79	14	43	11.3	9.1	319	12
9	F	20	12.5	37	6.5	4.8	290	4

F : Female , Hb : Haemoglobin, Ht : Haematocrit, WBC : White blood cells, PLT : Platelets

Figure S1: SSS thrombosis induces higher thrombotic expansion at D1 in *Jak2*^{V617F} mice.

(A) Representative images of the cortical parenchyma at day 1 after CVST showing more fibrin (red) in the brain vasculature from *Jak2*^{V617F} mice than in *Jak2*^{WT} mice. Scale bar: 100 µm. (B) The number of fibrin deposits was measured in cortical area of *Jak2*^{WT} and *Jak2*^{V617F} mice. Results are expressed as mean±SD (n=4-6 per group). For each mouse, six images taken at x20 magnification were analysed. Data were analysed using Mann–Whitney's t test. *:p<0.05.

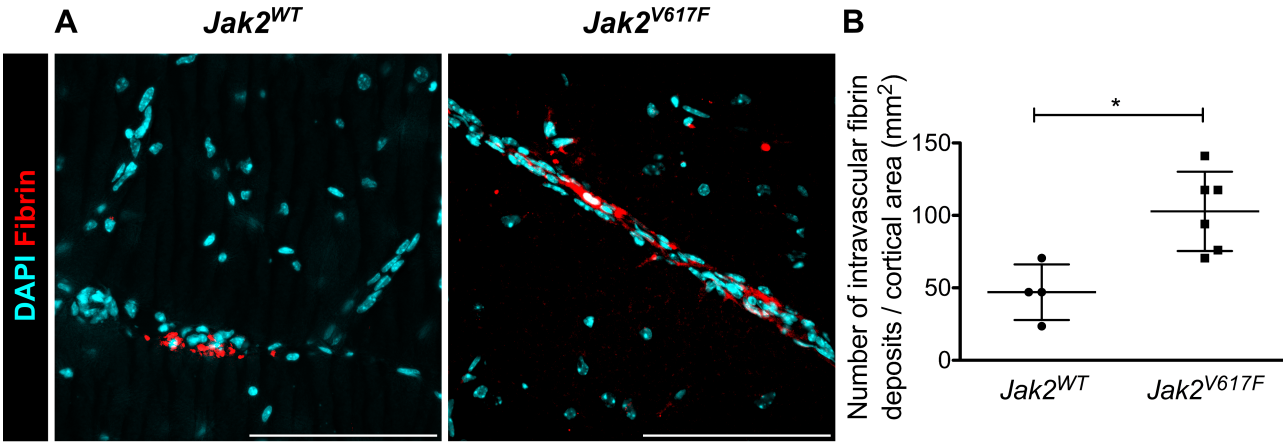
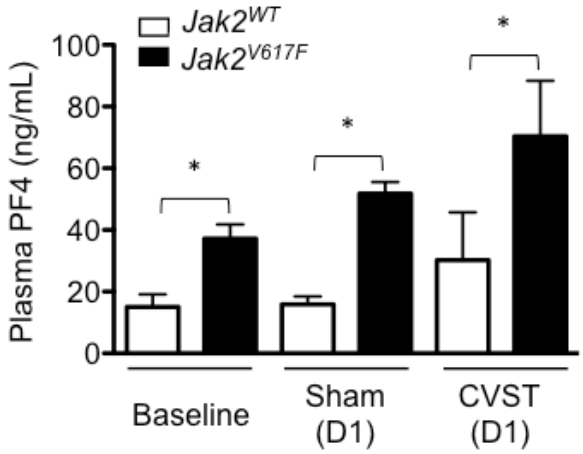


Figure S2: Plasma levels of platelet factor 4 (PF4) in *Jak2*^{WT} and Jak2^{V617F} mice at baseline and at day 1 (D1) after sham-surgery or CVST.

Results are expressed as mean±SD, n=7-8 per group. Data are analysed using Kruskal-Wallis test followed by post hoc Dunn's test. *: p<0.05.



Supplemental Video 1:

Intravital imaging of rhodamine-labeled leukocytes and platelets in $Jak2^{WT}$ and $Jak2^{V617F}$ mice after CVST. Note the important margination of leukocytes in venules of $Jak2^{V617F}$ mice compared to $Jak2^{WT}$ mice. SSS: Superior sagittal sinus, V: Veins.