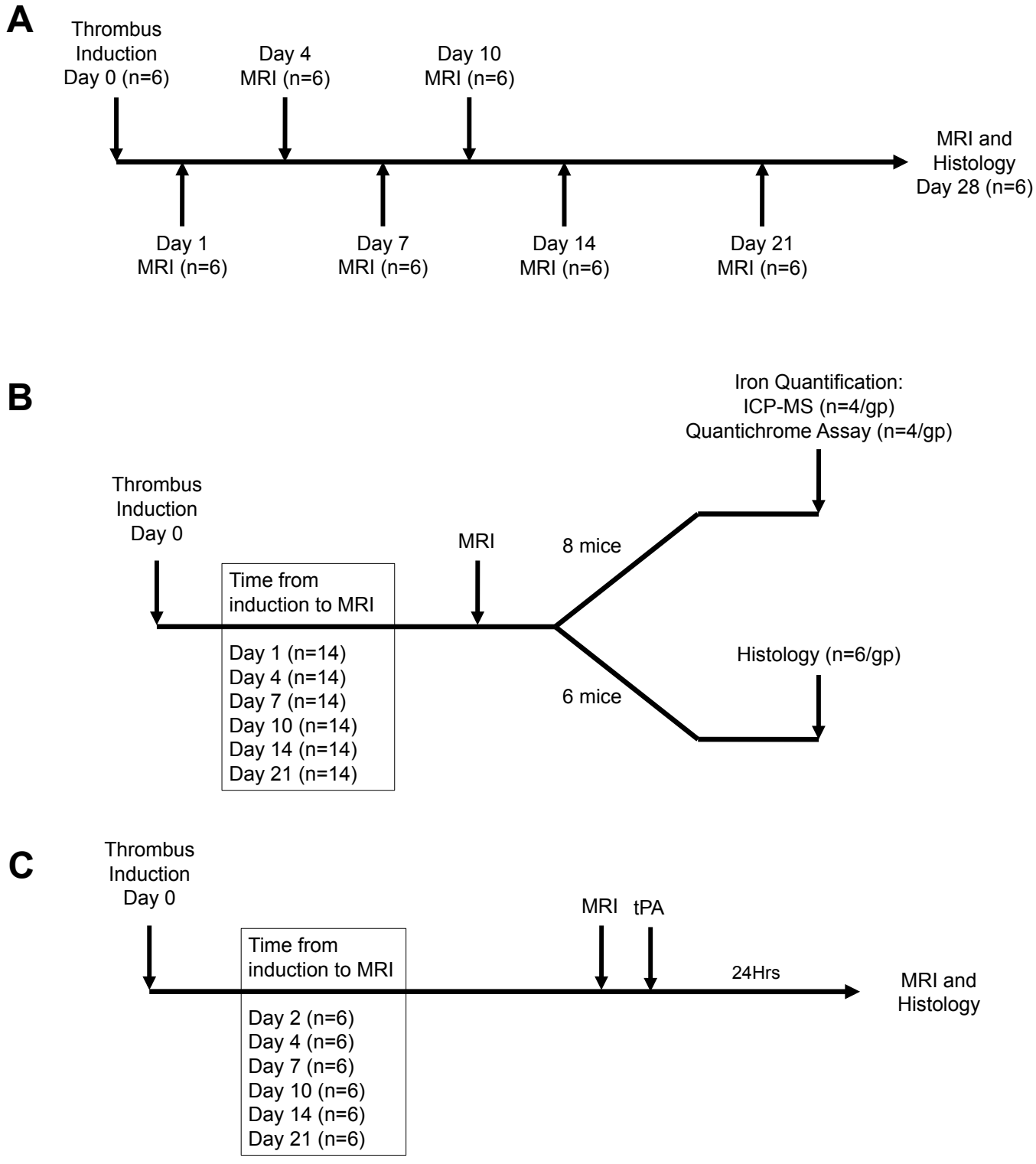
SUPPLEMENTAL MATERIAL



Imaging protocols used in this study

a) Longitudinal protocol used to image BALB/C, iNOS$^{-/-}$, CCR2$^{-/-}$ and wild-type controls mice. Each mouse was imaged consecutively between day 1 and 28 before thrombus was harvested and processed for histology; b) Cross-sectional protocol used to image BALB/C mice. Thrombus was induced at day 0 and imaged between days 1 and 21 before being harvested for analysis at each time point; c) Thrombolysis protocol used to image BALB/C mice. Thrombus was induced as before and scanned between day 2 and 21. After each scan, tissue plasminogen activator (t-PA) was infused into the tail vein. Mice were scanned 24hours after lysis to check for a response and the thrombus was harvested for analysis

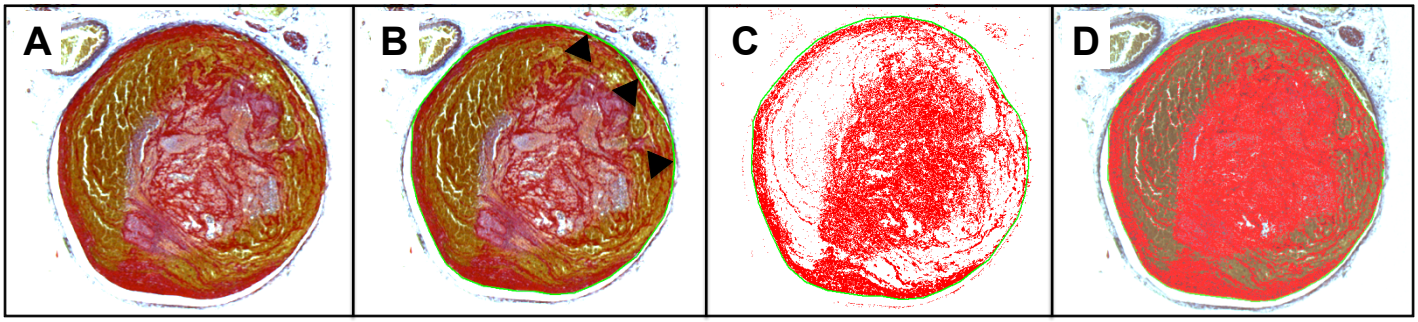
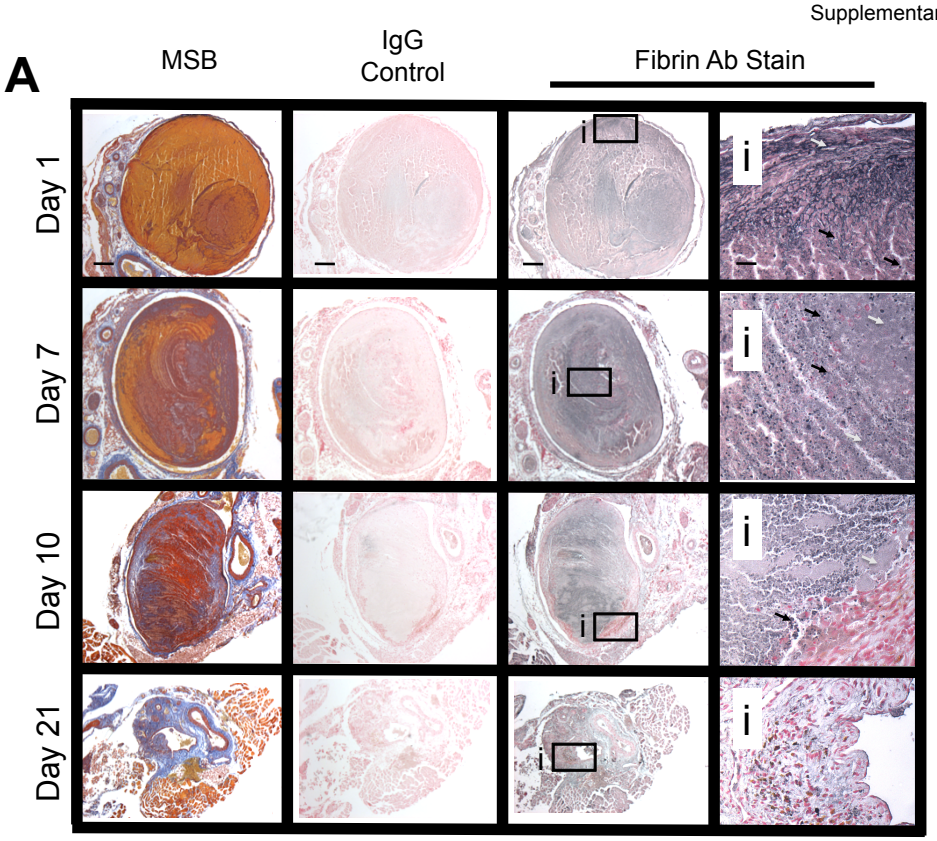
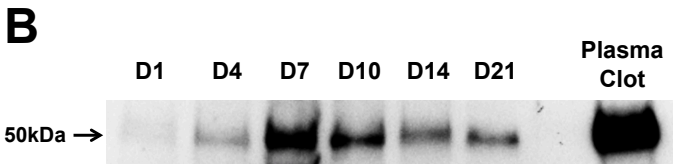
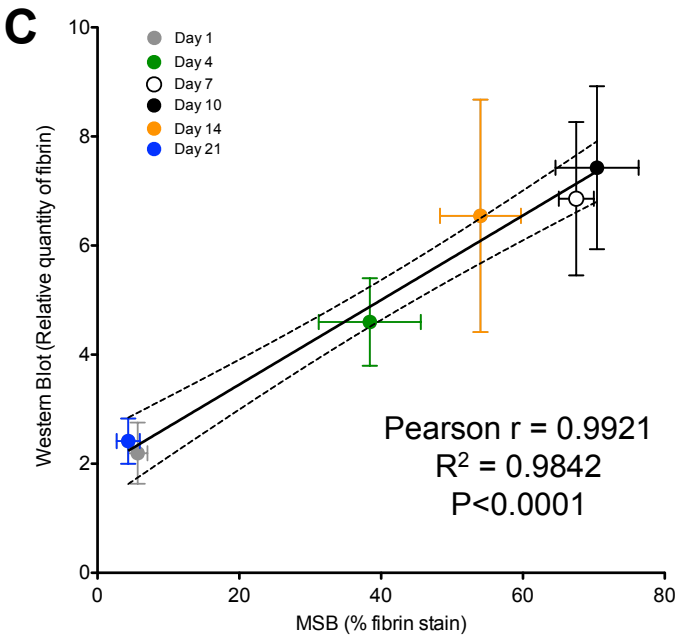


Image analysis of MSB sections of venous thrombi

A) Bitmap images of histological sections of experimental venous thrombi were imported into Image Pro-plus 7. B) A region of interest (ROI) was drawn around the thrombus (green line) as shown with black arrow heads to calculate the area of thrombus. C) Images were segmented based on their colour intensity histogram to identify the fibrin stain (red). D) The area of the segmented region within the ROI was then calculated and used for analysis. The same segmentation parameters of colour intensity was applied to all histological sections.



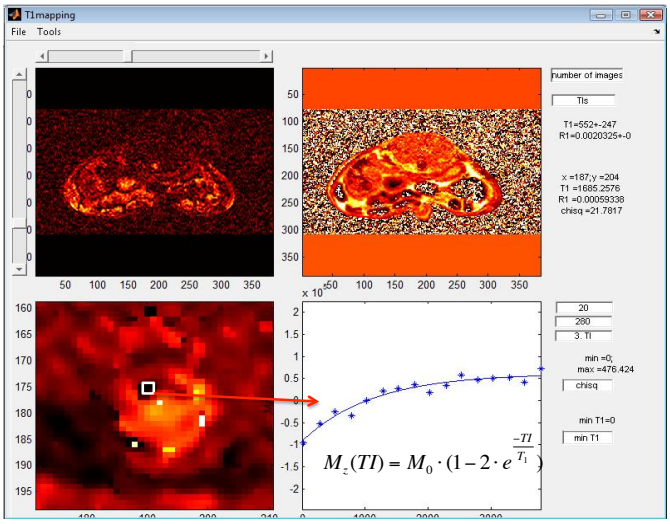




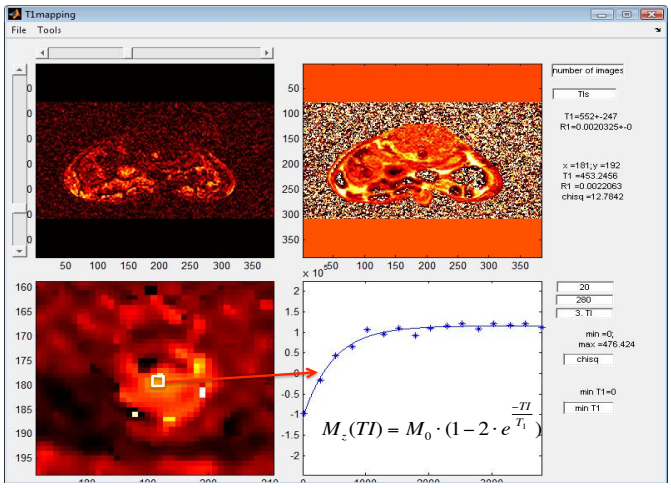
Spatial and temporal distribution of fibrin in experimental venous thrombi

A) The spatial distribution of fibrin appears similar with MSB sections and immunohistochemistry (5F3 clone) during thrombus resolution (bar=200μm). High magnification images are shown in (i). Black arrows indicate platelets, white arrows show fibrin (bar=25μm). B) Representative western blot analysis of fibrin during thrombus resolution (anti fibrin Ab, 59D8 clone). C) Comparison of fibrin quantification using western blot and MSB sections (n=6 per group).

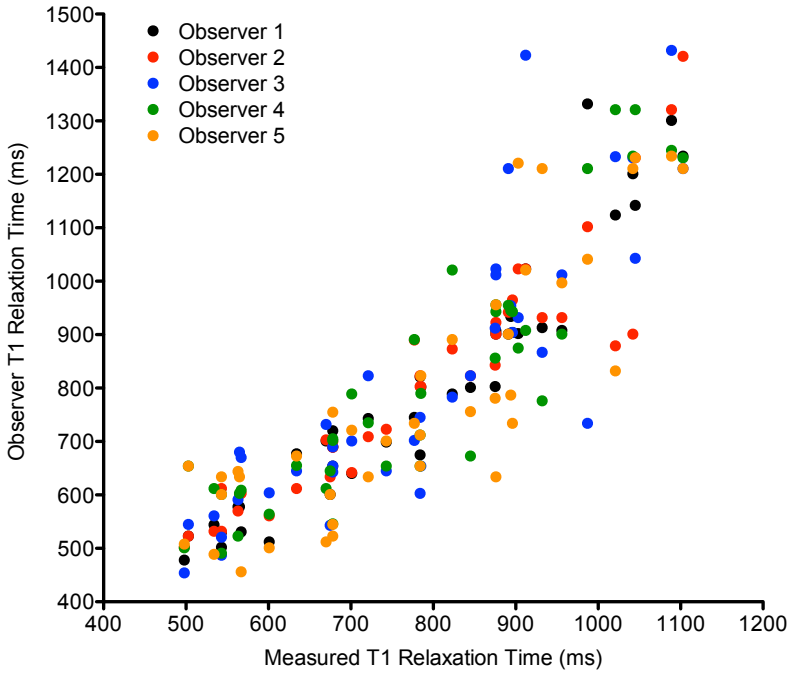
A



B



C

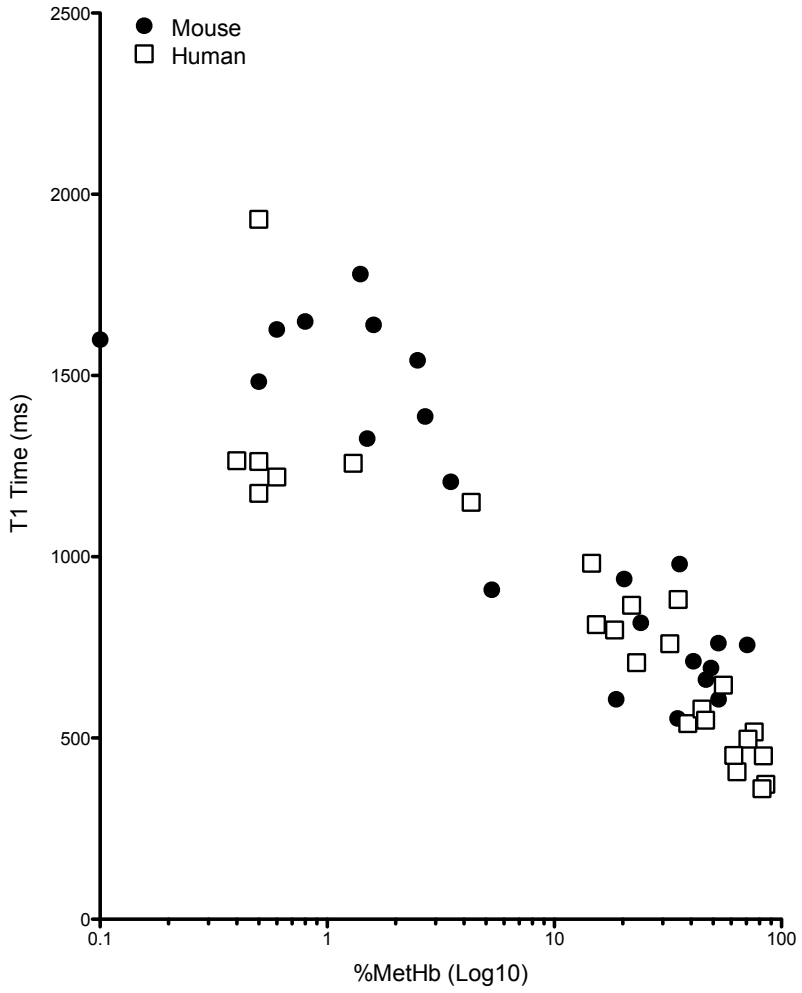


D

Observer	Pearson r	R^2	P value
1	0.95	0.90	<0.0001
2	0.93	0.87	<0.0001
3	0.85	0.73	<0.0001
4	0.89	0.81	<0.0001
5	0.85	0.72	<0.0001

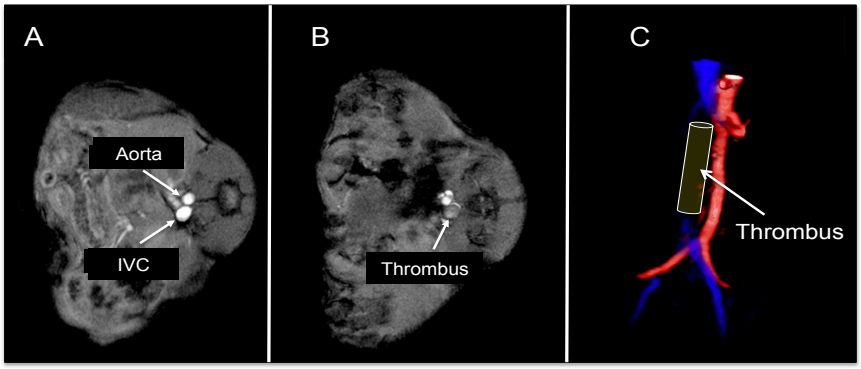
T1 quantification and inter-observer analysis

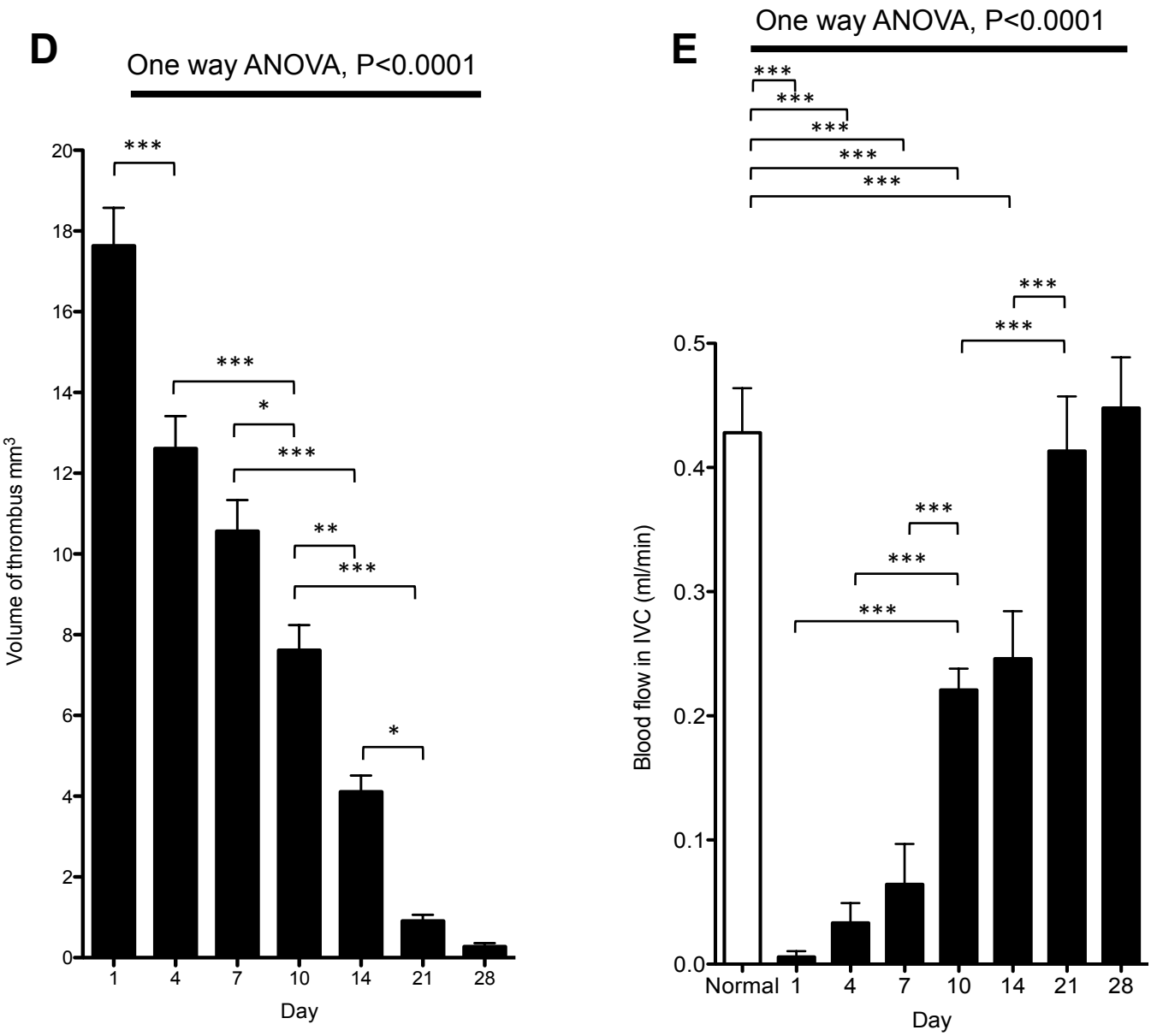
Acquired images were imported into MATLAB as individual slices and analysed using a bespoke programme to quantify T1 relaxation time on a pixel by pixel basis. An example of a region with a long T1 relaxation time is shown in A) and short T1 relaxation time in B). T1 relaxation across the whole cross sectional area of thrombus were averaged for each slice and mean thrombus T1 relaxation time calculated across the length of the thrombus. C) 5 independent observers were asked to analyse 40 slices to identify thrombus and calculate mean T1 relaxation time. Correlation between observers and the authors' measured T1 relaxation time is shown in D).



T_1 relaxation time of blood *in vitro* with different percentages of Methaemoglobin

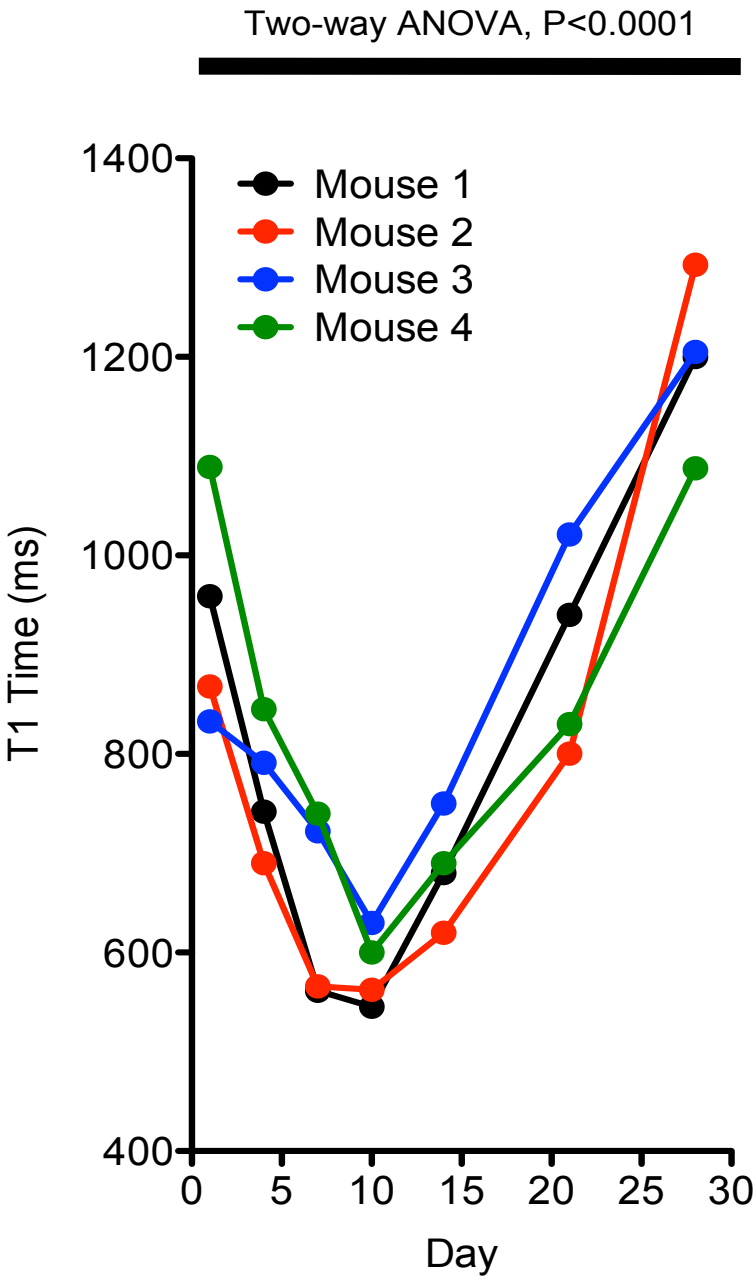
Human blood (squares) and murine blood (circles) exert a similar shortening of T_1 relaxation time *in vitro* as percentage of methaemoglobin (metHb) increases (n=25/gp).





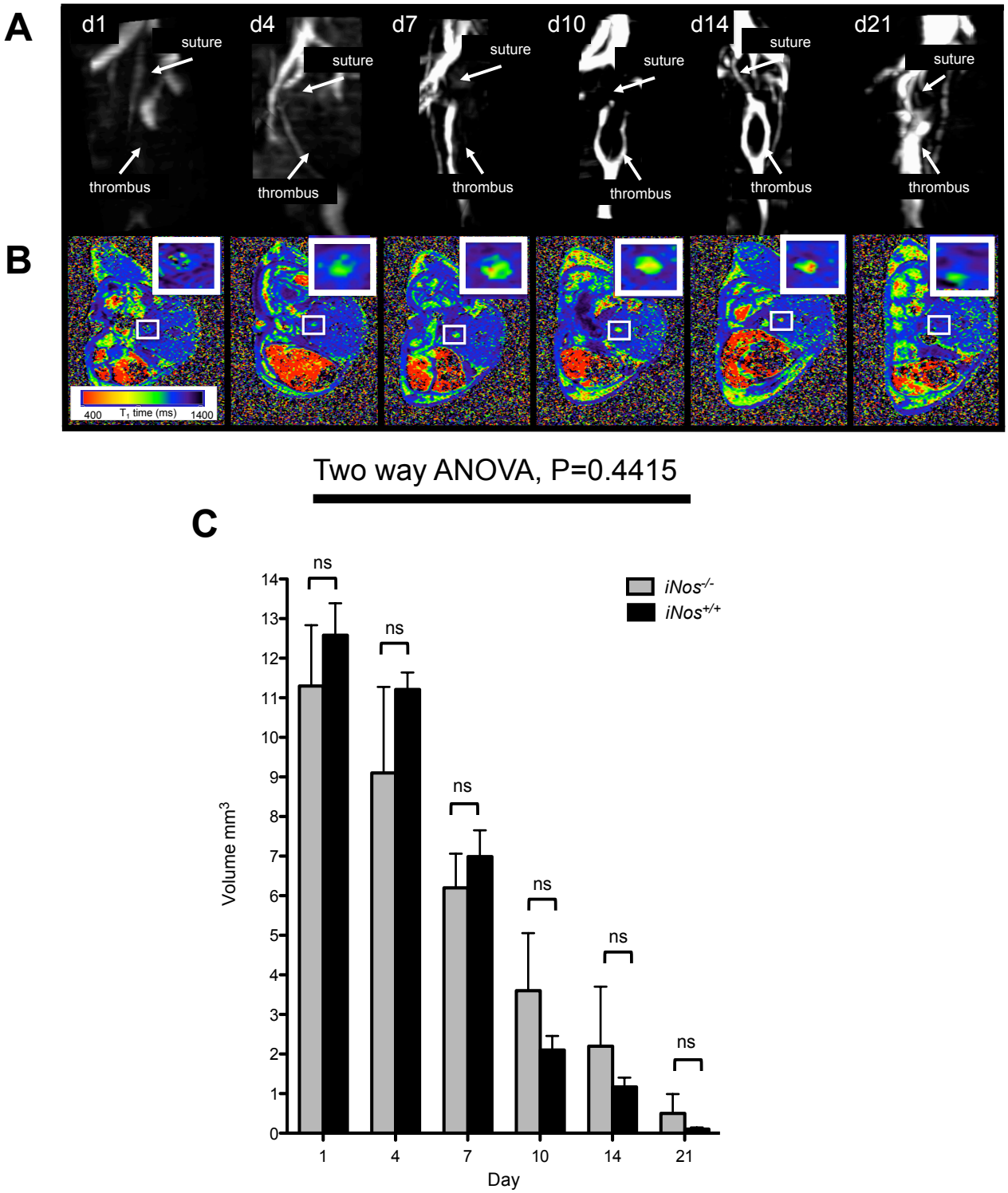
Natural resolution of murine venous thrombosis

The velocity of blood was measured across the IVC. (A) In the image white indicates normal blood flow, which is disturbed by the presence of a thrombus (B). Time of flight images demonstrate a filling defect in the IVC (blue) where thrombus is present (C). The aorta (red) represents a fixed anatomical landmark for reference. (D) Volume (mm^3) of the thrombus as it resolves with time (E) Flow (ml/min) across the IVC during thrombus resolution Mean±SEM shown. One way ANOVA with Bonferroni post test analysis. (*=P<0.05, **=P<0.01, ***=P<0.001, n=18/gp)



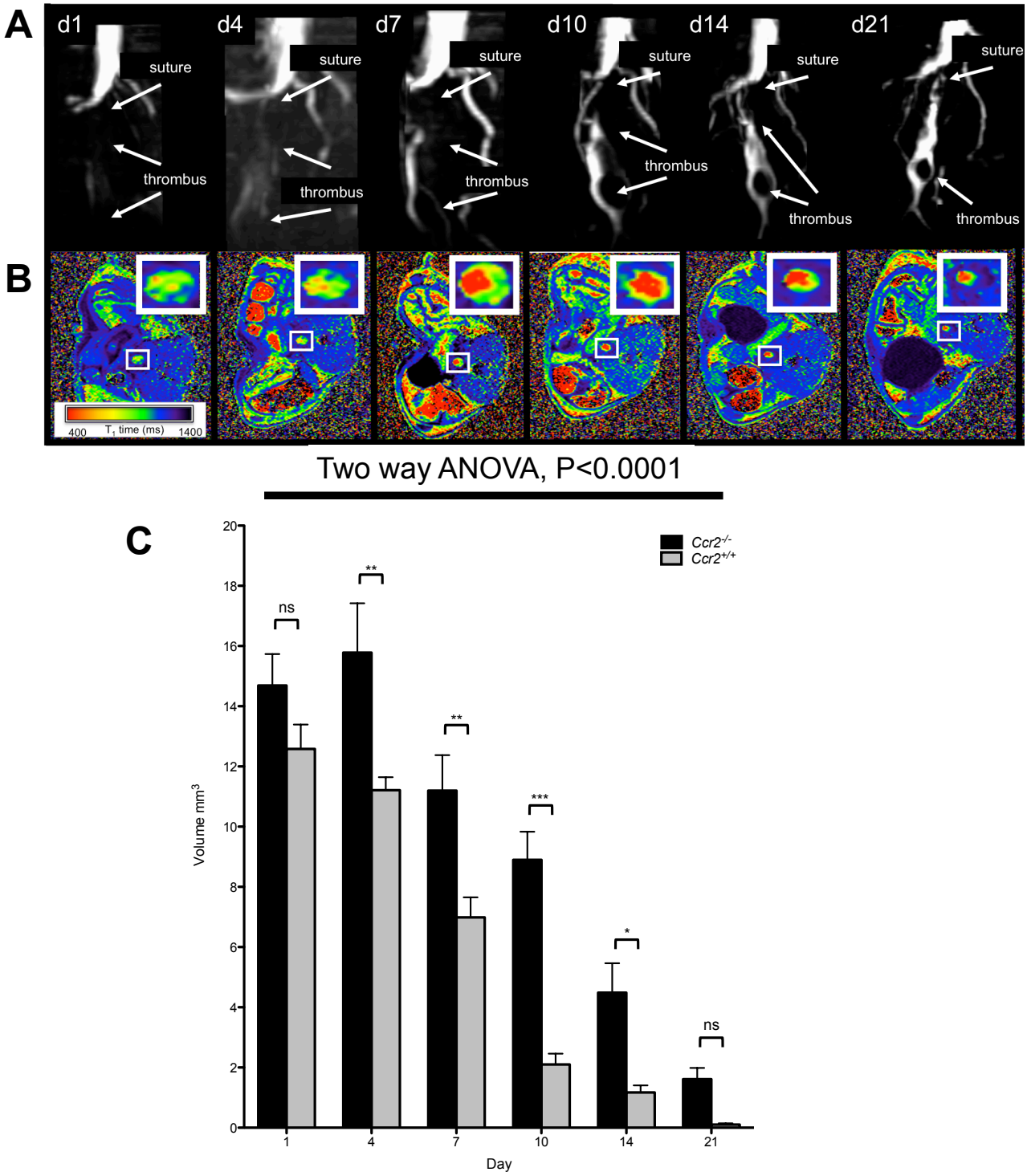
Longitudinal changes in T1 relaxation times (ms) during resolution

T1 maps were quantified of individual mice that were sequentially scanned between day 1 and 28. Mean T1 relaxation time shown. Two way ANOVA over time (P<0.0001). 2 mice which died during this experiment have been excluded from analysis.



Venous thrombi in *iNos*$^{-/-}$ mice

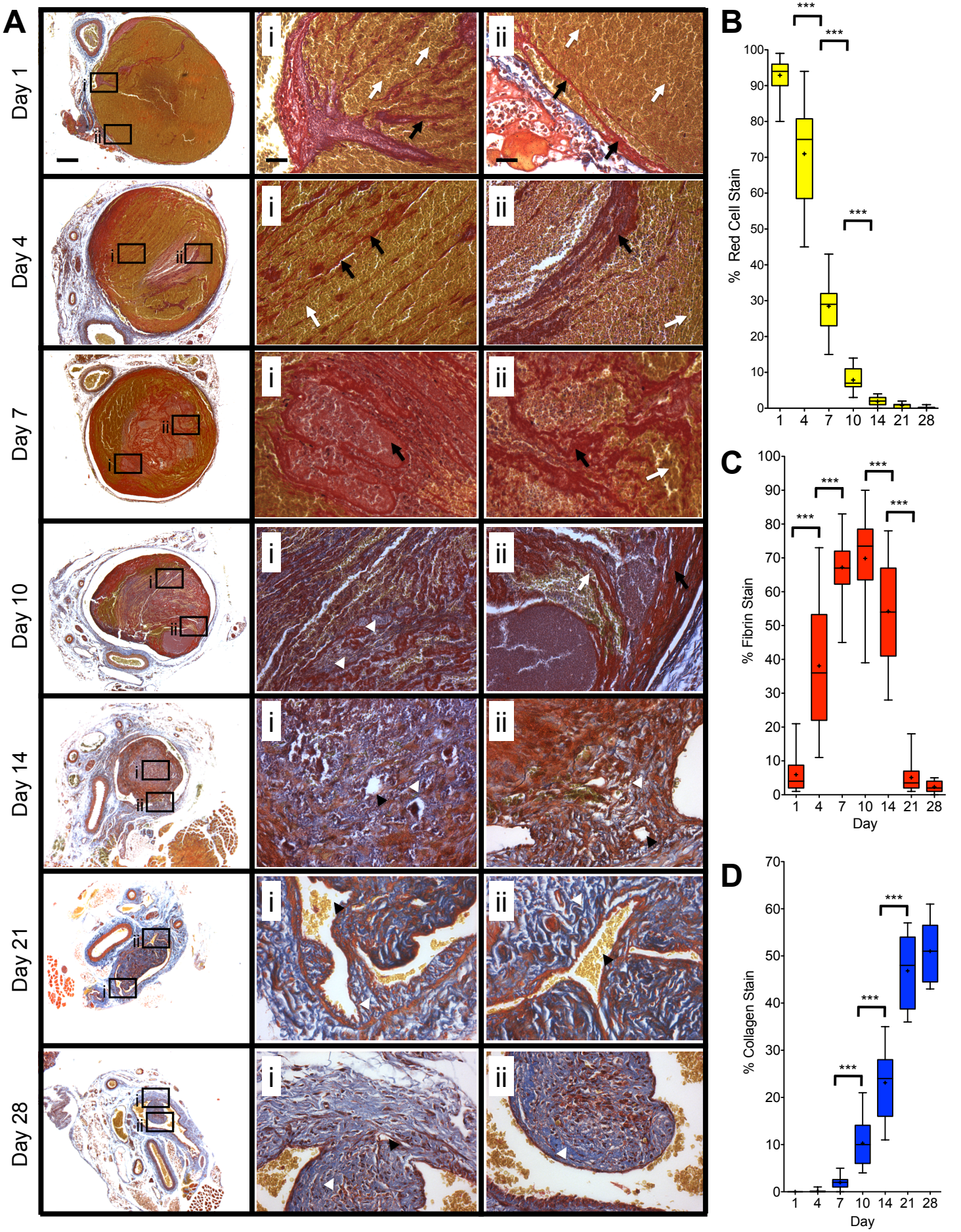
a) Venogram of the murine IVC in *iNos*$^{-/-}$ mice following thrombus induction. b) Corresponding T_1 maps of thrombi in *iNos*$^{-/-}$ mice. c) Volume of thrombus (mm^3) in *iNos*$^{-/-}$ and *iNos*$^{+/+}$ mice over time measured using TOF sequences. Mean+SEM of thrombus volume (mm^3) of *iNos*$^{-/-}$ (grey bar) and *iNos*$^{+/+}$ (black bar) mice are shown over time (n=6/gp). Two way ANOVA comparing differences in groups over time is used for analysis with Bonferroni post test for each time point.



Venous thrombi in *Ccr2*$^{-/-}$ mice

a) Venogram of the murine IVC in *Ccr2*$^{-/-}$ mice following thrombus induction. b) Corresponding T_1 maps of thrombi in *Ccr2*$^{-/-}$ mice. c) Volume of thrombus (mm^3) in *Ccr2*$^{-/-}$ and *Ccr2*$^{+/+}$ mice over time measured using TOF sequences in during resolution. Mean+SEM of thrombus volume (mm^3) of *Ccr2*$^{-/-}$ (black bar) and *Ccr2*$^{+/+}$ (grey bar) mice are shown over time (n=6/gp). Two way ANOVA comparing differences in groups over time is used for analysis with Bonferroni post test for each time point (*=P<0.05, **=P<0.01, ***=P<0.001).

Supplementary Figure 10. Saha & Andia et al.



MSB sections of experimental venous thrombi during its resolution

A) Examples of histological sections of venous thrombi during its resolution are shown (yellow=red cells, red=fibrin, blue=collagen, bar=200μm). High magnification images of the boxes are shown in i) and ii), bar=25μm. White arrows indicate red cells, black arrows indicate fibrin, white arrow heads indicate collagen and black arrow heads regions of neovascular channels. Box and whisker quantification of MSB sections for red cell (B), fibrin (C) and collagen (D) is shown for each level of individual sections across the whole thrombus at each time point (n=6 per group, range: minimum to maximum, median line is shown and mean represented by '+', ***=P<0.001).