

1 **Infarct growth precedes cerebral thrombosis following experimental**  
2 **stroke in mice**

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7 Running head: Cerebral thrombus formation in ischemic stroke

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## 21 Supplemental Material

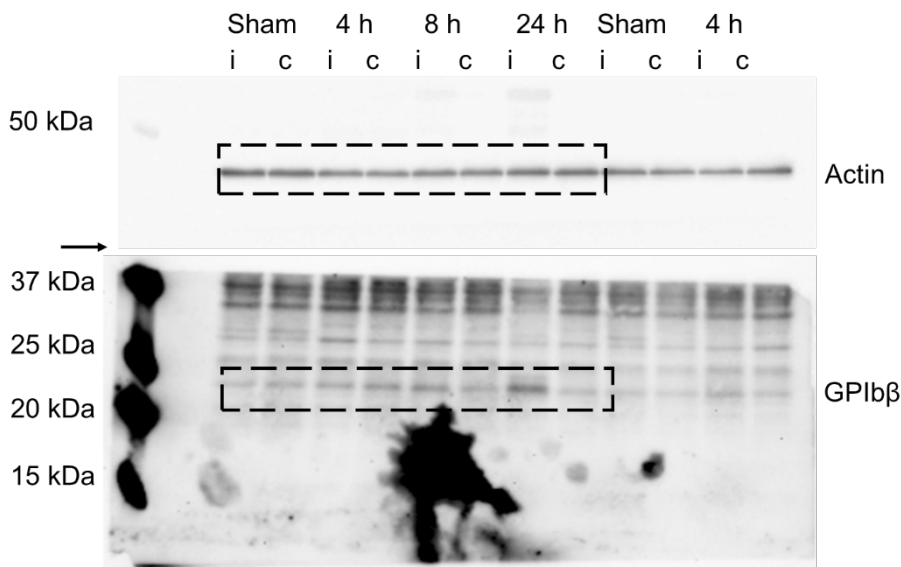
### 22 Supplemental Methods

#### 23 Analysis of brain LSFM images

24 Images acquired by LSFM were saved in TIFF format and the thrombi and vessel  
 25 channel were deconvolved using Huygens software (Scientific Volume Imaging (SVI),  
 26 Hilversum, The Netherlands; signal-to-noise ratio: 40, background subtraction with  
 27 nearest object within a 2  $\mu\text{m}$  radius and 60 iterations). For object segmentation, firstly  
 28 machine learning based pixel classification for thrombi was performed using the Ilastik  
 29 [3] software. Next, binary images were exported based on the generated probabilities  
 30 using the build-in simple segmentation option of Ilastik. These images of segmented  
 31 thrombi were further processed using "Surface module" of the 3D rendering software  
 32 Bitplane Imaris (Bitplane AG, Zürich, Switzerland) to detect platelet aggregates with a  
 33 volume above 100  $\mu\text{m}^3$  and the total number of individual aggregates per brain was  
 34 exported.

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### 36 Supplemental Figures



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38 **Supplemental Figure 1. Quantification of platelet GPIIb/IIIa protein in brain lysates**  
 39 **of the cortex.** Uncropped membrane of Western Blot shown in Figure 2D. Regions  
 40 shown in Figure 2D are indicated by the black boxes. I: Ipsilateral; c: contralateral. The  
 41 membrane was cut above the 37 kDa marker band (black arrow) before incubation with  
 42 the different antibodies.

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**45 Legends for supplemental video files**

46 **Videos: Cerebral thrombi are only detectable after 24 h of reperfusion.** Animations  
47 of 3D reconstructions of ipsilateral brain hemispheres of **(1)** sham operated mice or  
48 mice after tMCAO with 1 h occlusion and **(2)** 4 h of reperfusion, **(3)** 8 h of reperfusion  
49 or **(4)** 24 h of reperfusion. Vessels (CD105/CD31; red), platelets (GPIX, green). Grid  
50 size: 0.5 mm.

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**52 Supplemental References**

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