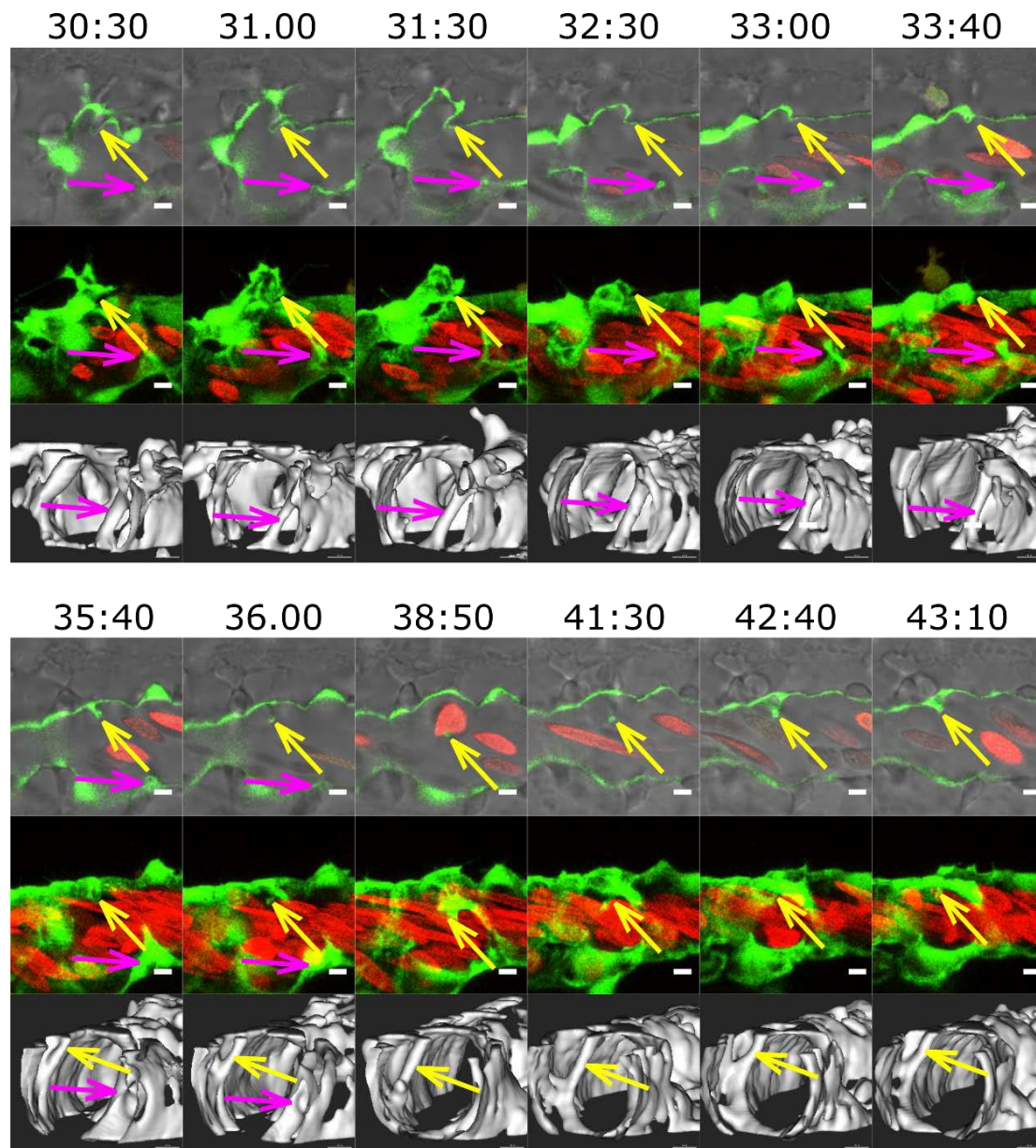


Suppl. Fig. S1: Formation of a pillar and subsequent rupture.

Mechanism of pillar formation (magenta arrows): lumen expansion/sprouting angiogenesis. Initially, the vessel is only partially lumenised, the pillar emerges as the lumen fully inflates and the vessel is perfused. The pillar first appears in the lumen as a fold (31:40-32:00). The pillar core is thin but present (31:40, 36:00), seems to disappear later (37:30) which weakens the pillar, and it ruptures within next 10 minutes (until 37:40).

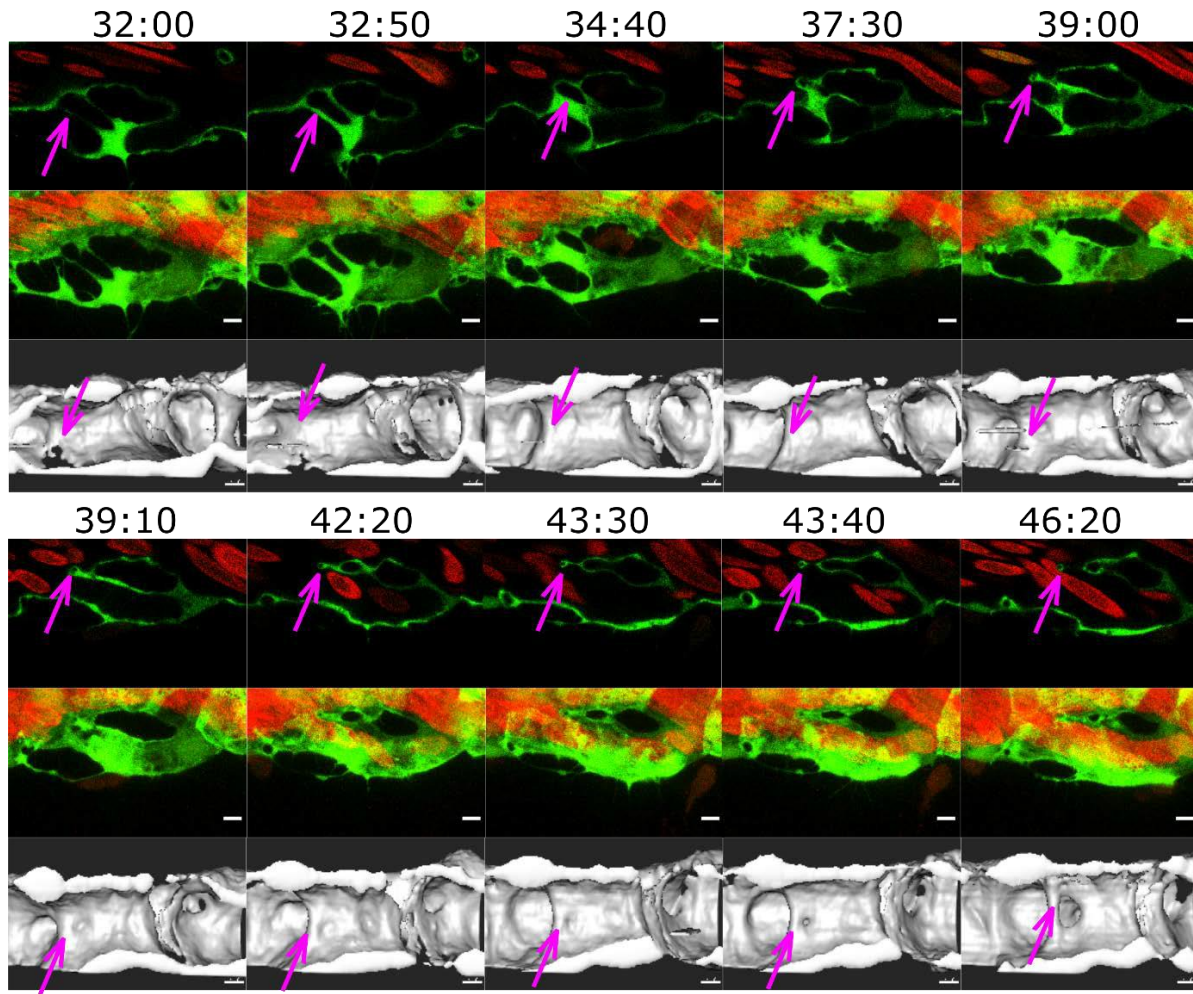
Endothelium in green (eGFP), erythrocytes in red (dsRed), bright field in grey. First row: single slice (5µm thick); second row: maximum intensity projection (only green and red channel); third row: surface model in grey (based on the GFP channel). Time points (hh:mm post fertilization) valid for each column, scale bar: 5µm.



Suppl. Fig. S2: Formation of a pillar and its disappearance by merging with the vessel wall.

Mechanism of pillar formation (yellow arrows): capillary wall folding. At the beginning, the vessel is already lumenised and there is active sprouting. The pillar forms later when the outer vessel surface looks smooth, not sprouting anymore. First, a fold appears (32:30). As the blood cells enter the vessel and its diameter expands, the fold closes around the pillar core (33:40-35:40), and eventually the pillar enters the lumen (36:00). Later the pillar moves back to the vessel wall where it originated from and fuses with it again (43:10). Another pillar (magenta arrows), that has formed before 30:30, also disappears via this mechanism (36:00).

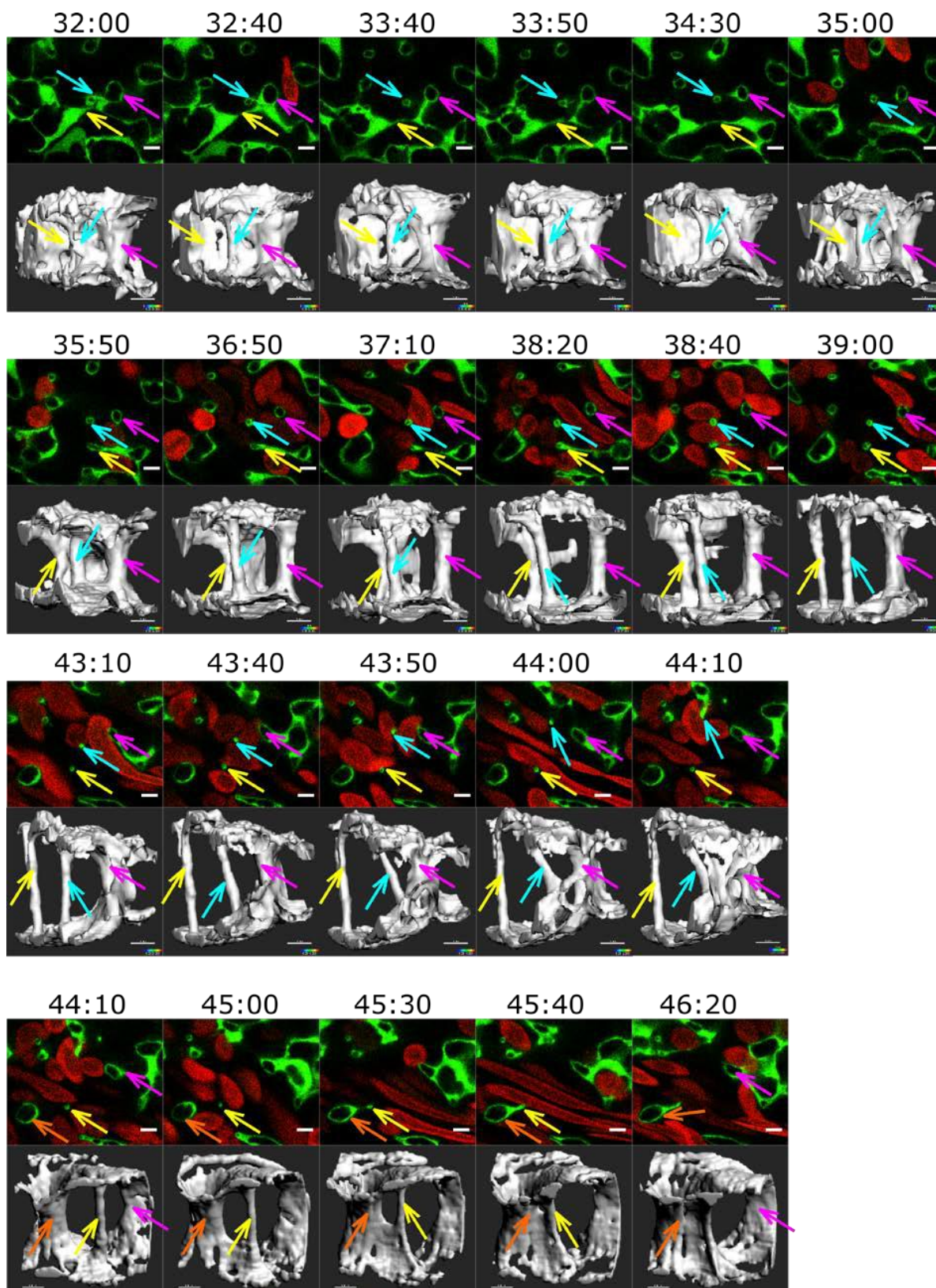
Endothelium in green (eGFP), erythrocytes in red (dsRed), bright field in grey. First row: single slice (5µm thick); second row: maximum intensity projection (only green and red channel); third row: surface model in grey (based on the GFP channel). Time points (hh:mm post fertilization) valid for each column, scale bar: 5µm.



Suppl. Fig. S3: Formation of a pillar by sprouting angiogenesis.

At 32:00, numerous filopodia are present and via their contact, meshes form (32:50-37:30). One of them gives rise to an oval-shape structure that eventually divides the vessel into two branches (39:00-42:00), the second one disappears again, the retraction of the filopodia is visible (37:30-42:20). A pillar splits off the oval mesh (43:30-46:20). The origins of the pillar core can be tracked back to the sprouting phase – the future core is delineated by the filopodia.

Endothelium in green (eGFP), erythrocytes in red (dsRed), bright field in grey. First row: single slice (5µm thick); second row: maximum intensity projection (only green and red channel); third row: surface model in grey (based on the GFP channel). Time points (hh:mm post fertilization) valid for each column, scale bar: 5µm.



Suppl. Fig. S4: Pillar behaviour.

Mechanism of formation: possibly by lumen expansion/sprouting (yellow, magenta and cyan arrows, not tracked from the very beginning). At 32:00, the future cores of two pillars (magenta and cyan) are clearly visible. At this time point the vessel is already partially lumenised and there is active sprouting that possibly formed the basis of those future pillars. However, the major force in pillar formation is lumen expansion. The pillars appear as the connection between their folds is perforated (32:40 -35:00). The pillar marked with the yellow arrow splits from a long intraluminal fold (32:00-34:30) that evolved into a longitudinal mesh (35:00-37:10), with a transient presence of an endothelial bridge connecting the yellow pillar with the other half of the mesh (38:20-38:40). The pillar marked with magenta partially merges with a neighbouring mesh (43:10 until 46:20) but they do not form a single large mesh. The pillar marked with cyan gets thinner (it seems as if its core has disappeared), moves away (43:10-44:00) and eventually merges with a nearby mesh (44:10). The pillar marked with yellow follows the same fate (41:00- 46:20, mesh marked with an orange arrow).

Endothelium in green (eGFP), erythrocytes in red (dsRed). First row: single slice (5 μ m thick); second row: surface model in grey (based on the GFP channel). Time points (hh:mm post fertilization) valid for each column, scale bar: 5 μ m.