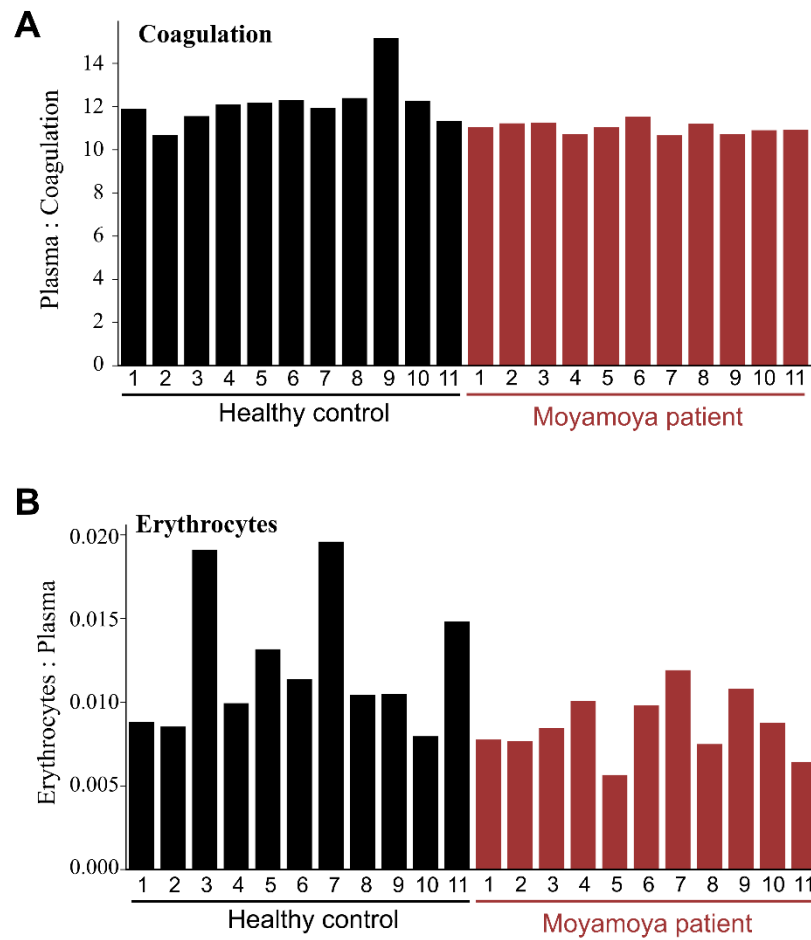


Supplementary material

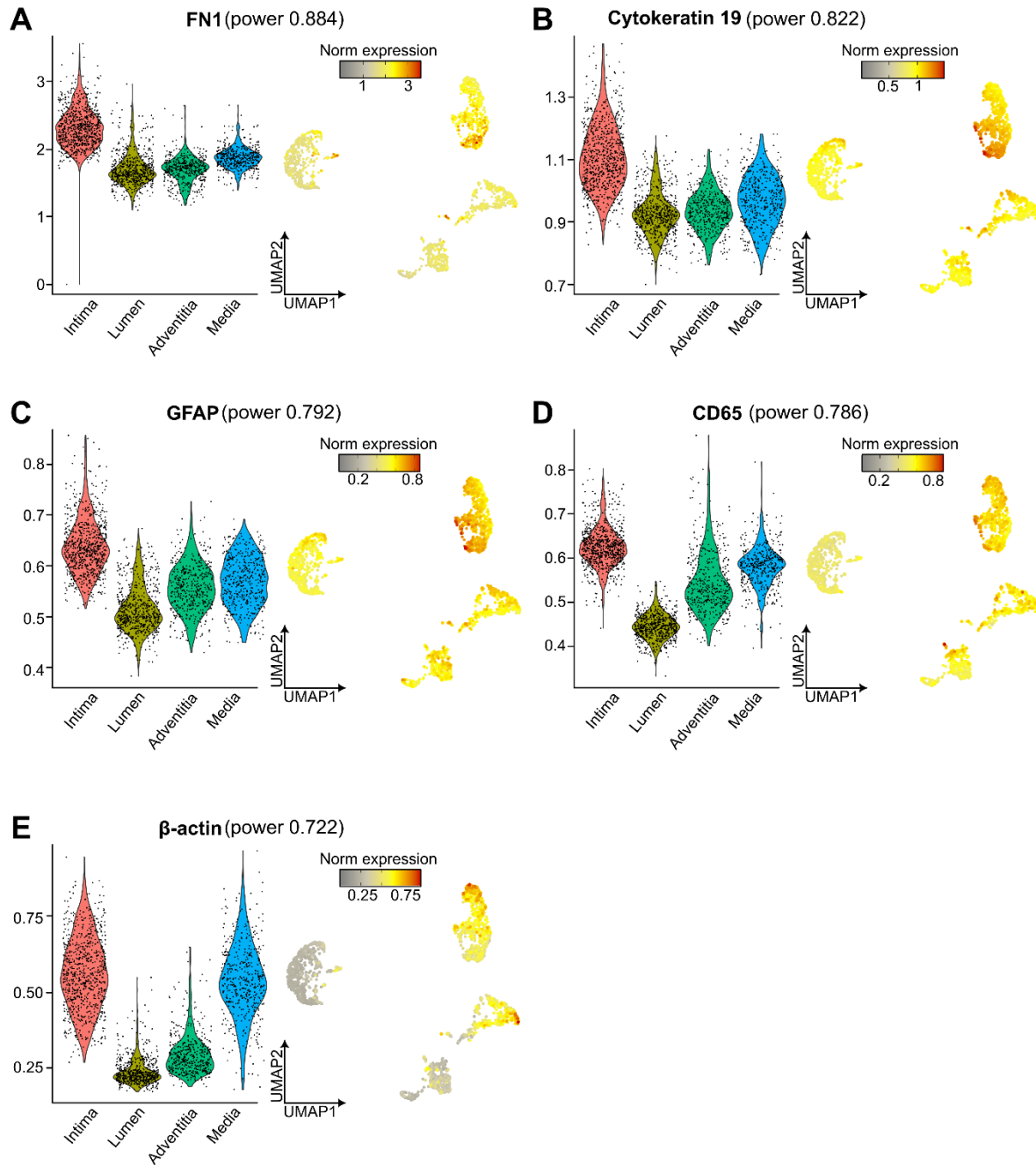
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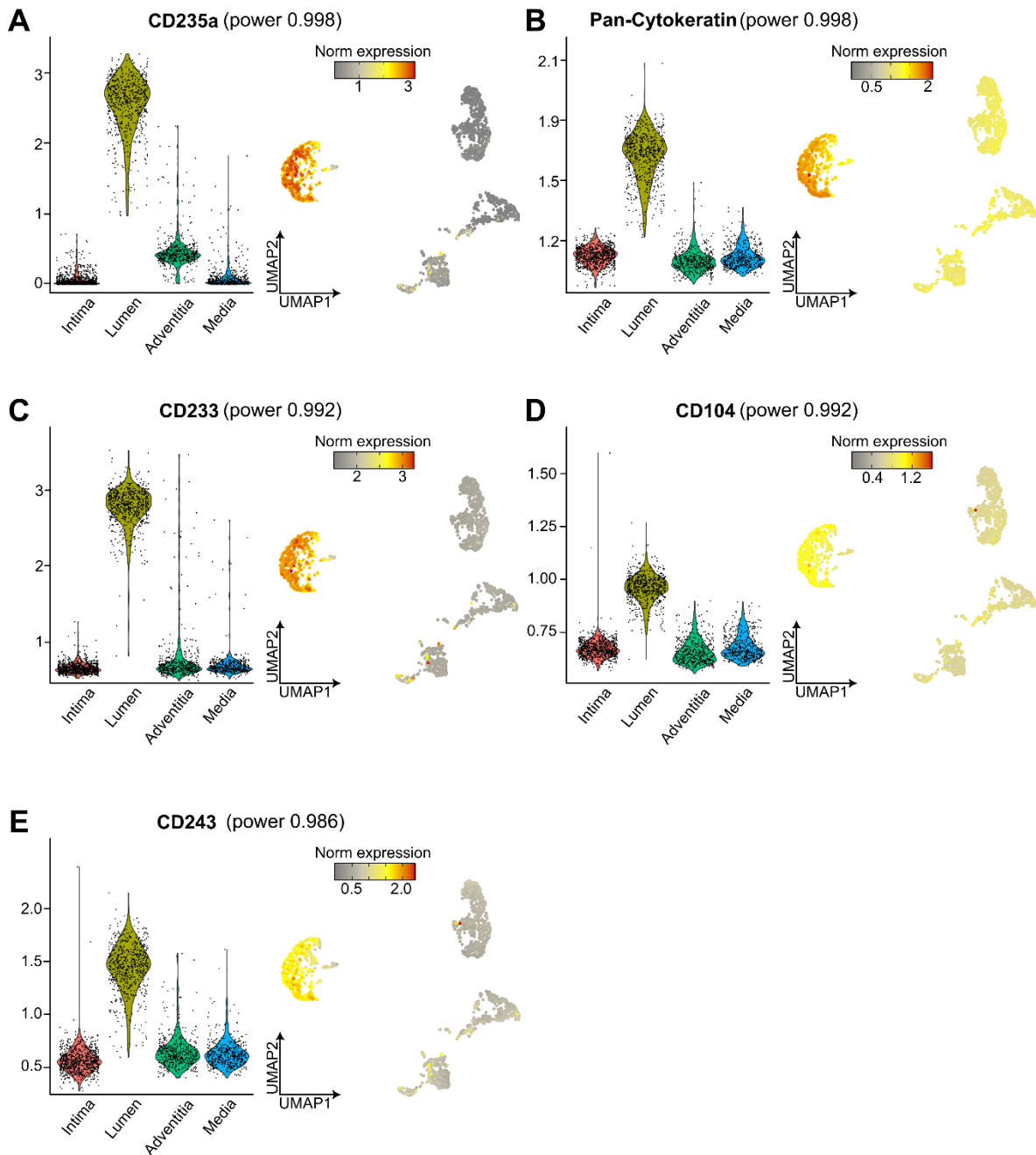
Supplementary figures



Supplementary Figure 1. Coagulation and erythrocyte cocontaminant index for each individual plasma sample calculated according to Geyer et al. (2019, *EMBO Mol Med* 11:e10427).

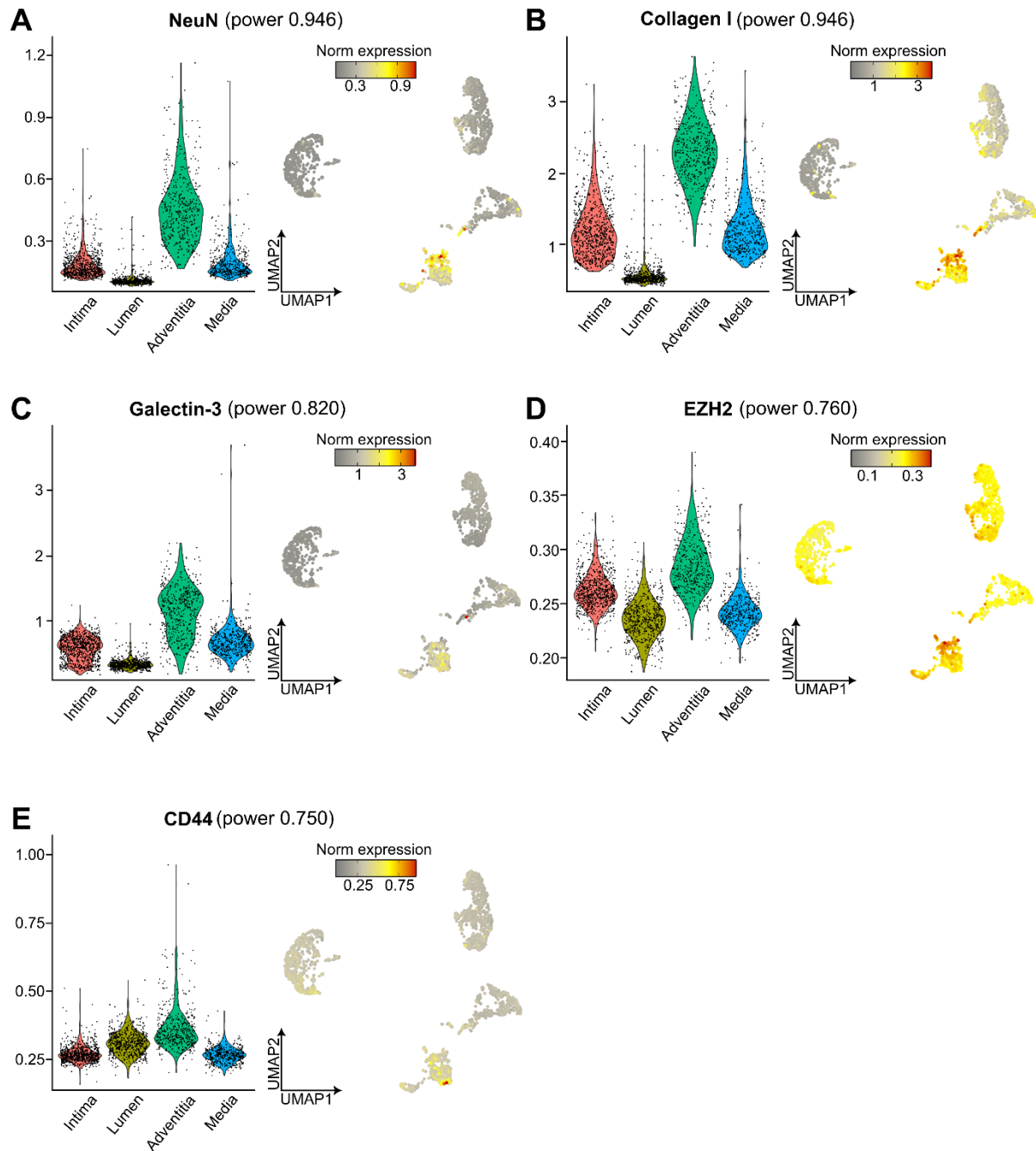


Supplementary Figure 2. Normalized expression of marker proteins of cluster 0. (A-E) Marker genes were determined using the FindAllMarkers function in Seurat using the receiver operating characteristic (roc) test and the top five features with highest classification power were displayed. For each marker normalized expression was shown in a violin plot per cluster and per cell using the Uniform Manifold Approximation and Projection (UMAP) projections. The top five markers were (A) Fibronectin1 (FN1), (B) Cytokeratin19, (C) Glial Fibrillary Acidic Protein (GFAP), (D) CD65 and (E) β -actin.

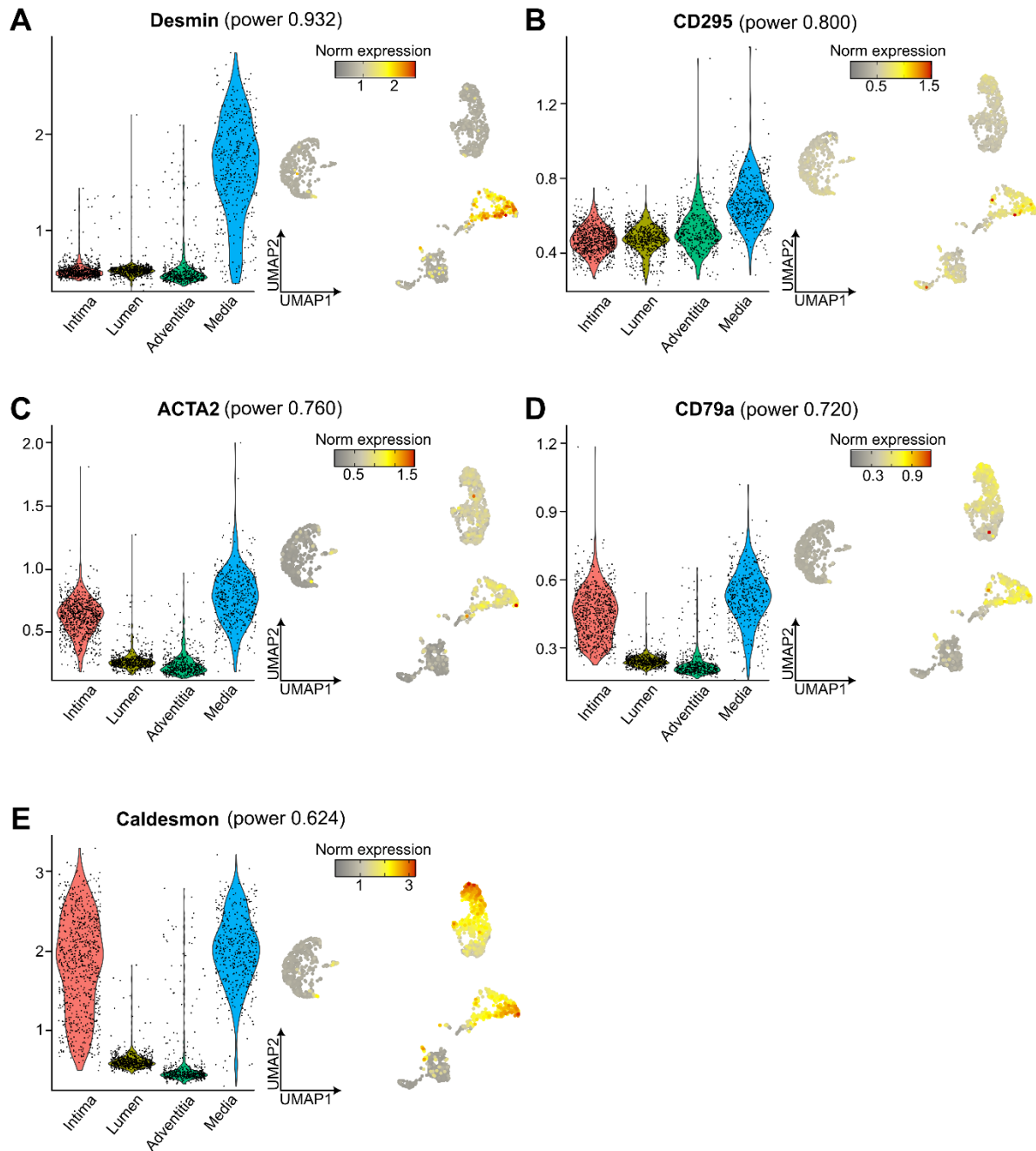


Supplementary Figure 3. Normalized expression of marker proteins of cluster 1.

(A-E) Marker genes were determined using the FindAllMarkers function in Seurat using the receiver operating characteristic (roc) test and the top five features with highest classification power were displayed. For each marker normalized expression was shown in a violin plot per cluster and per cell using the Uniform Manifold Approximation and Projection (UMAP) projections. The top five markers were (A) CD235a, (B) Cytokeratin (pan-antibody), (C) CD233, (D) CD104 and (E) CD243.

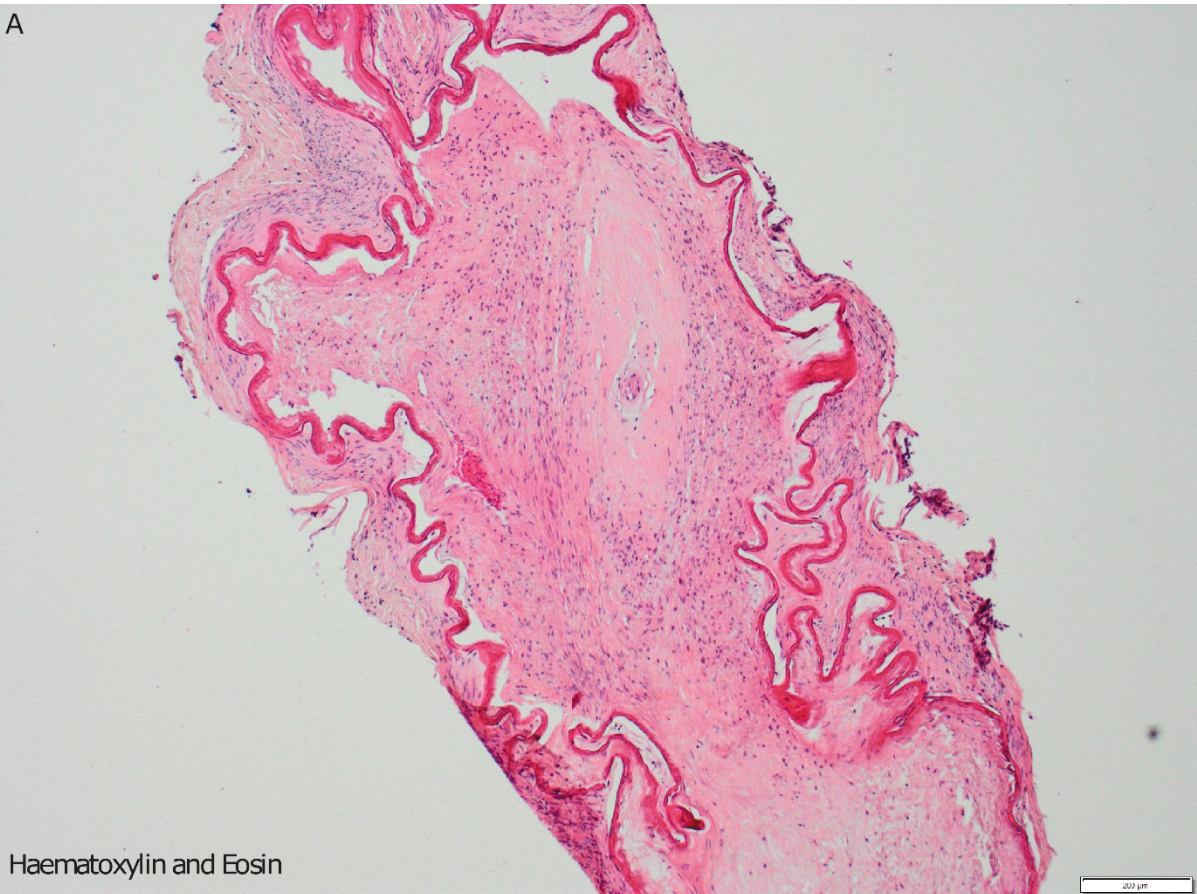


Supplementary Figure 4. Normalized expression of marker proteins of cluster 2. (A-E) Marker genes were determined using the FindAllMarkers function in Seurat using the receiver operating characteristic (roc) test and the top five features with highest classification power were displayed. For each marker normalized expression was shown in a violin plot per cluster and per cell using the Uniform Manifold Approximation and Projection (UMAP) projections. The top five markers were (A) NeuN (RNA binding fox-1 homolog 3), (B) Collagen I, (C) Galectin-3, (D) Enhancer of Zeste Homolog 2 (EZH2) and (E) CD44.

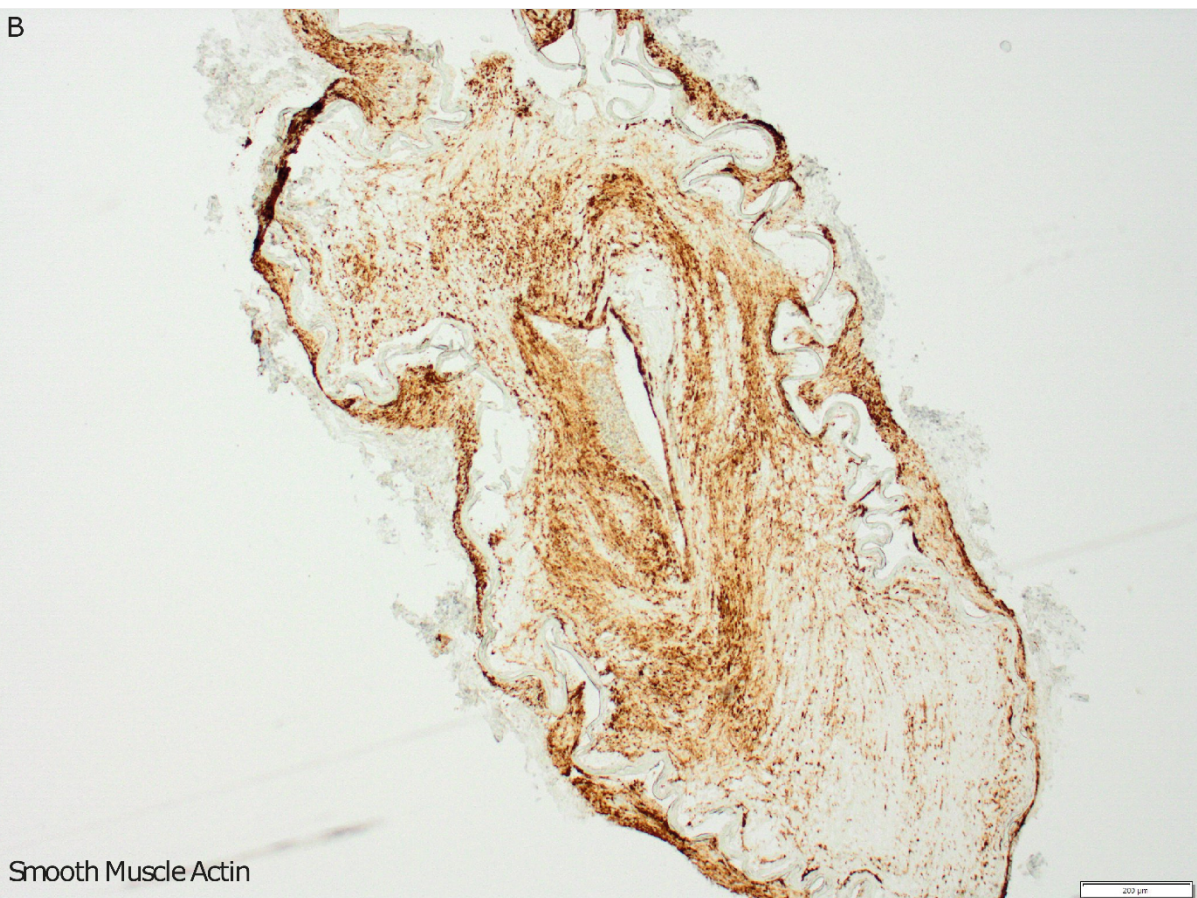


Supplementary Figure 5. Normalized expression of marker proteins of cluster 3. (A-E) Marker genes were determined using the FindAllMarkers function in Seurat using the receiver operating characteristic (roc) test and the top five features with highest classification power were displayed. For each marker normalized expression was shown in a violin plot per cluster and per cell using the Uniform Manifold Approximation and Projection (UMAP) projections. The top five markers were (A) Desmin (DES), (B) CD295, (C) Smoother muscle marker ACTA2, (D) CD79a and (E) Caldesmon.

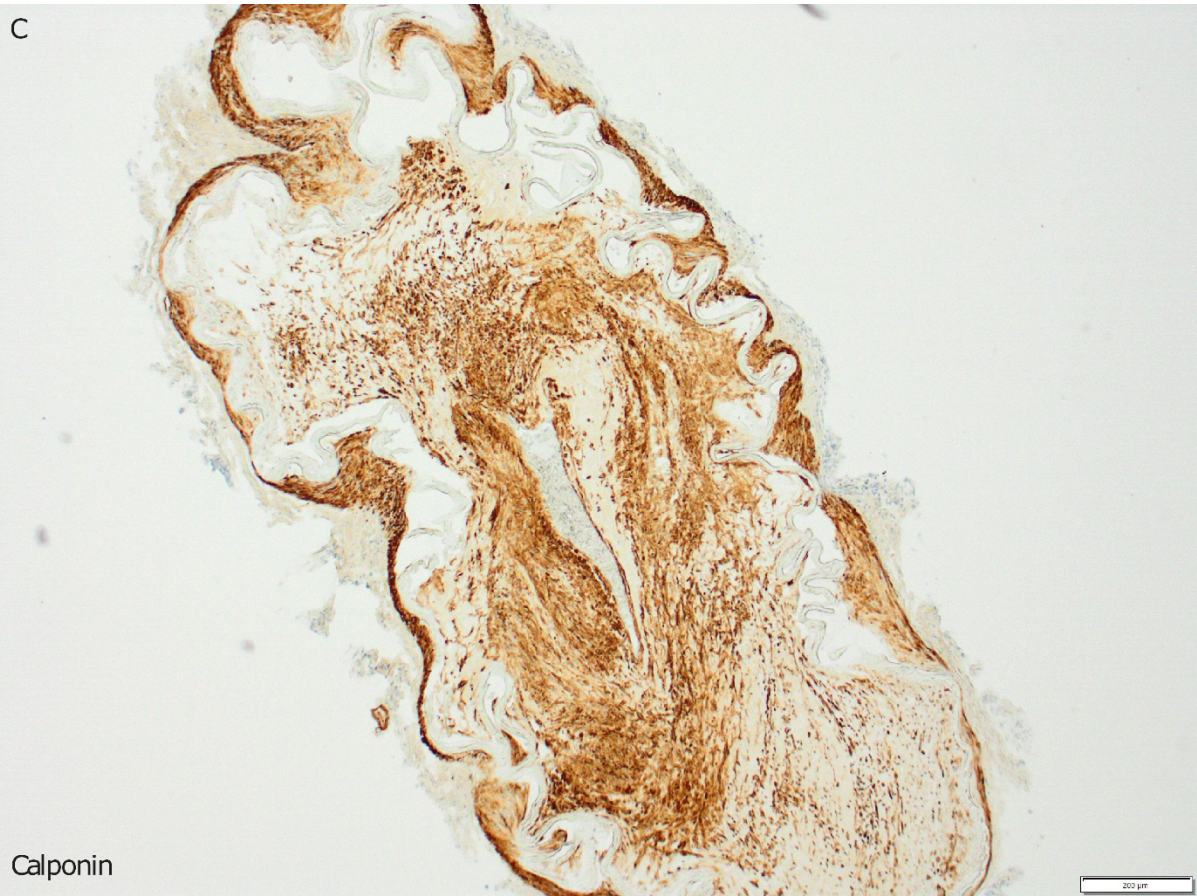
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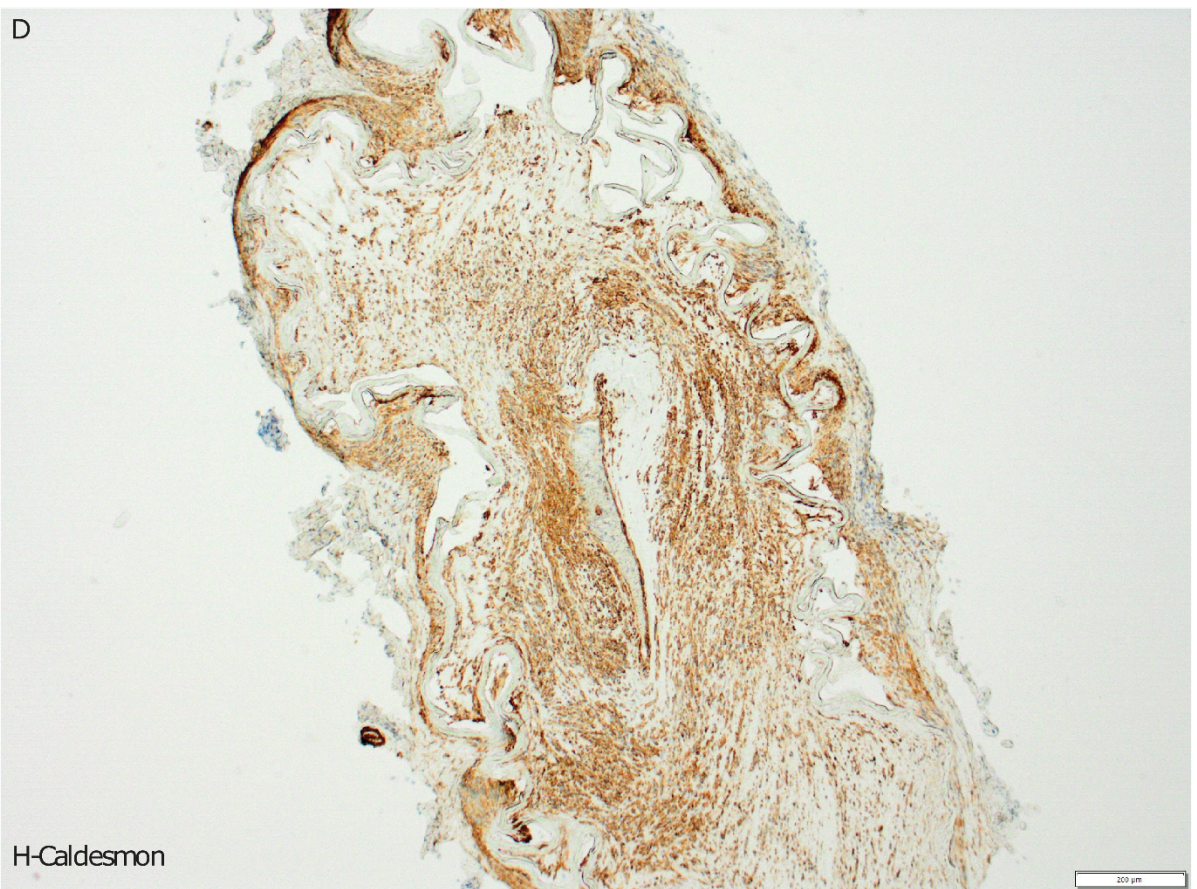
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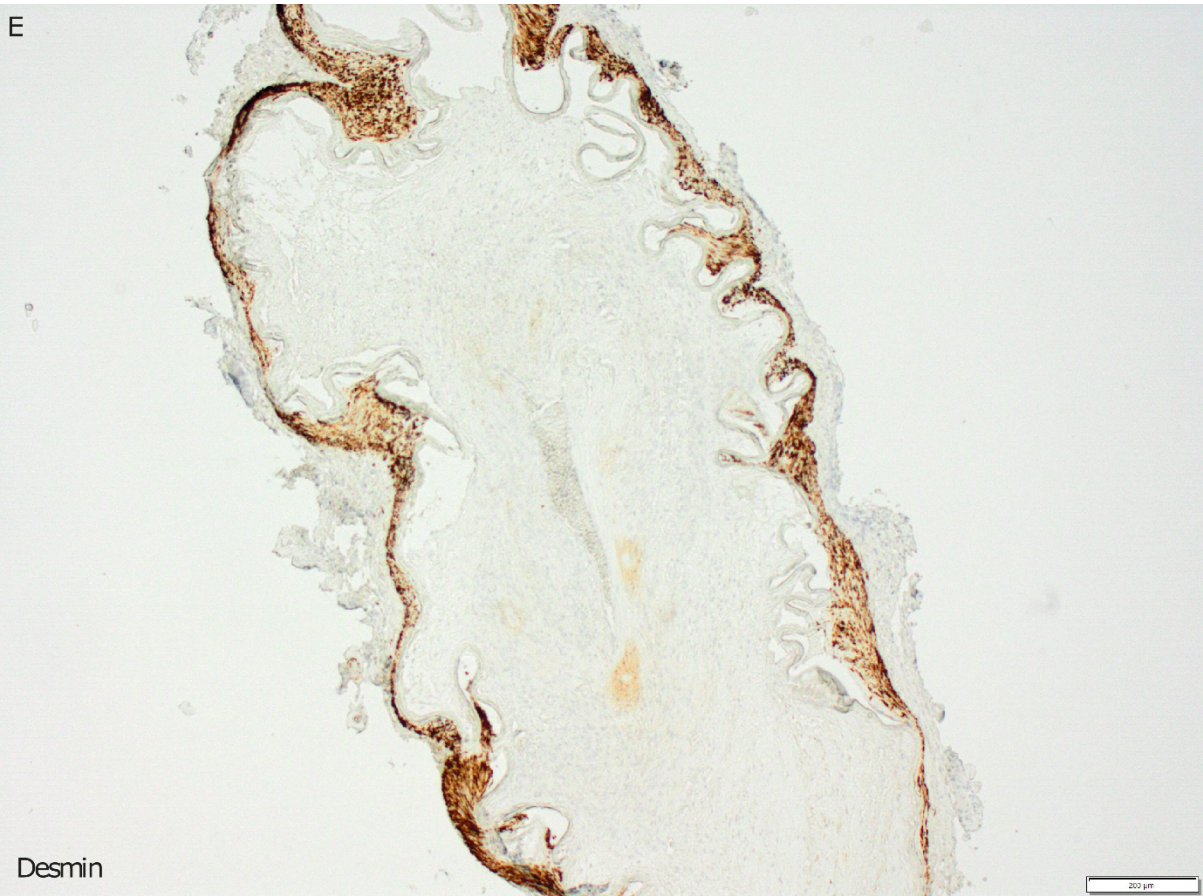
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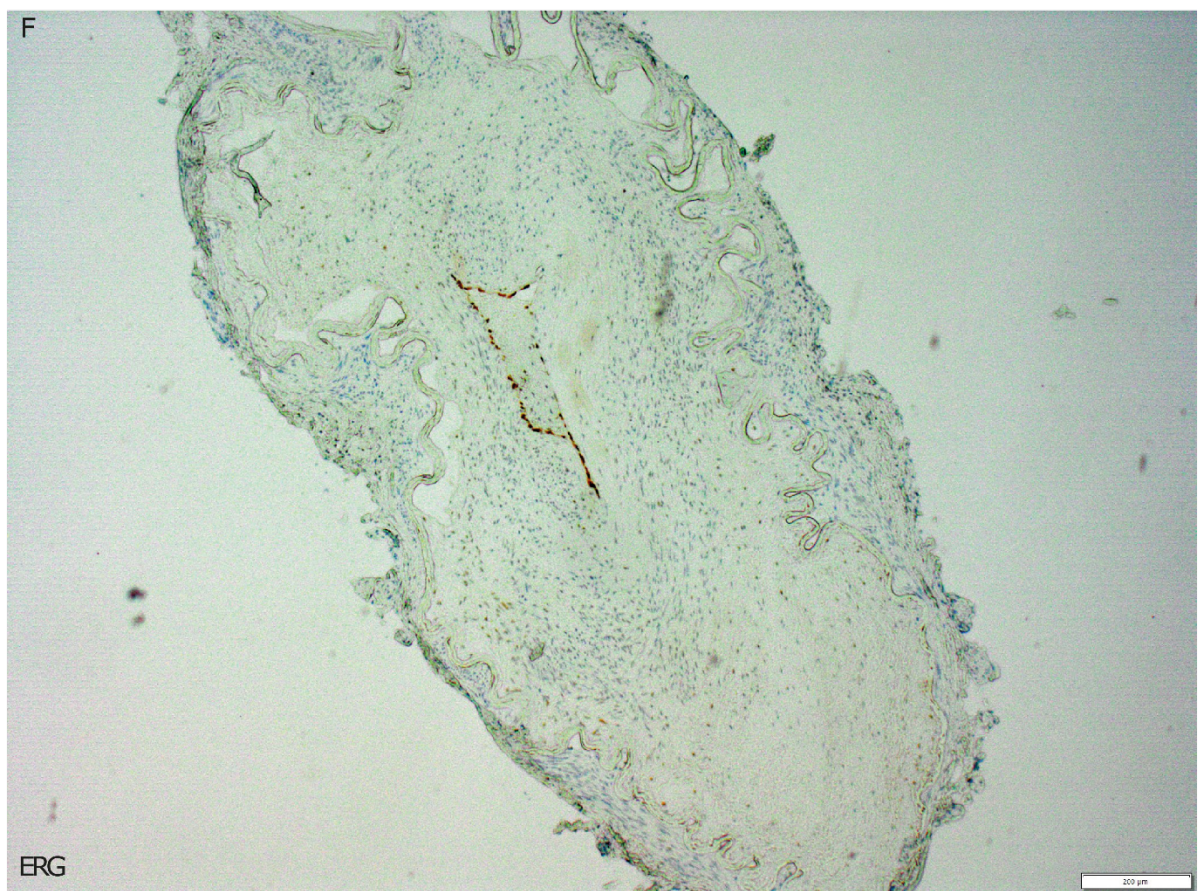
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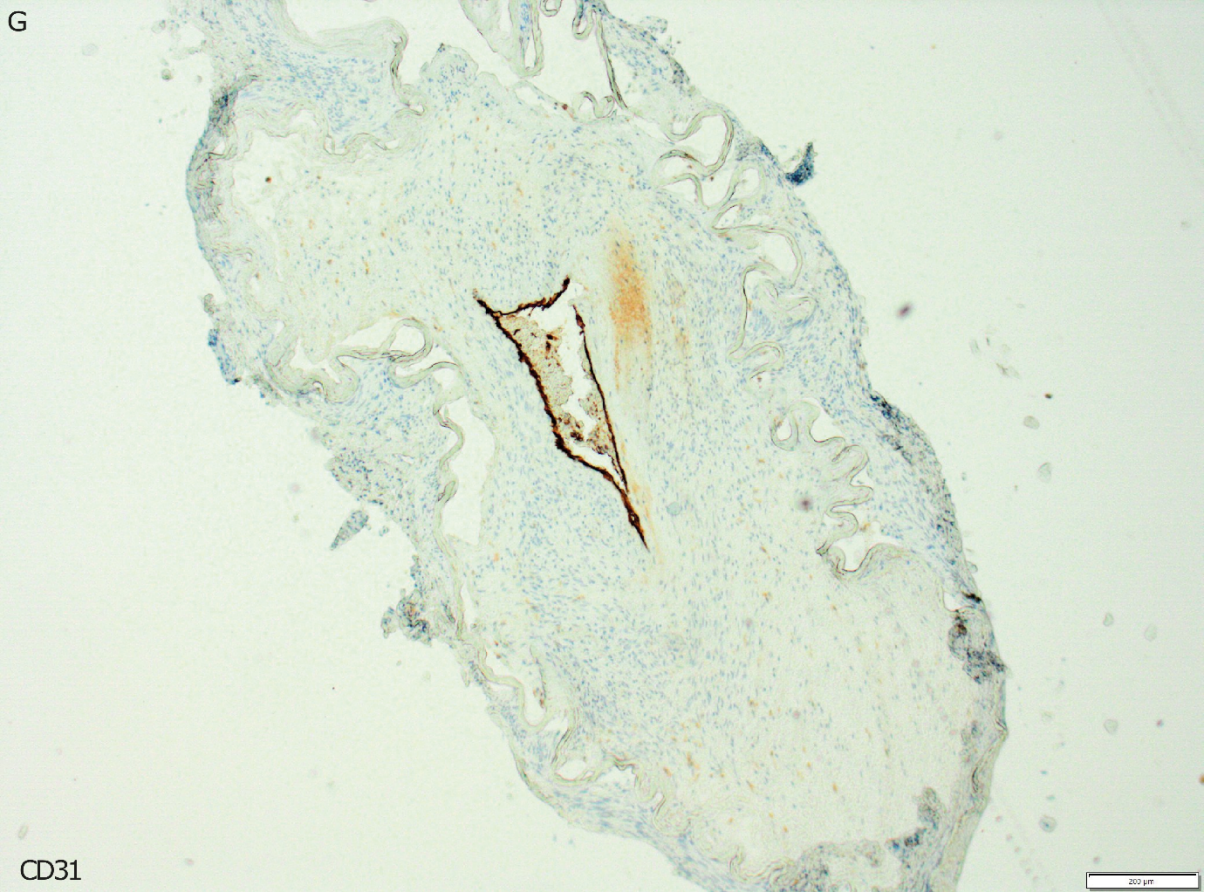
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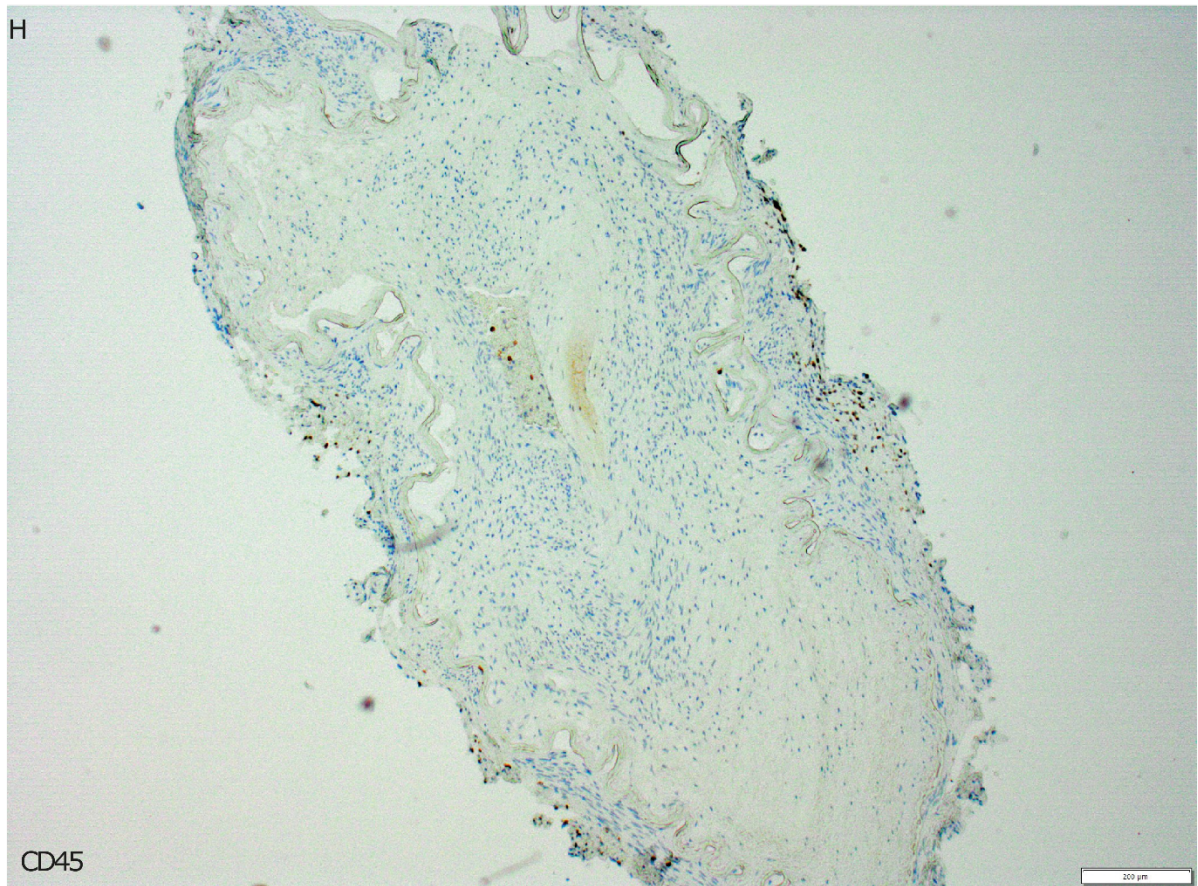
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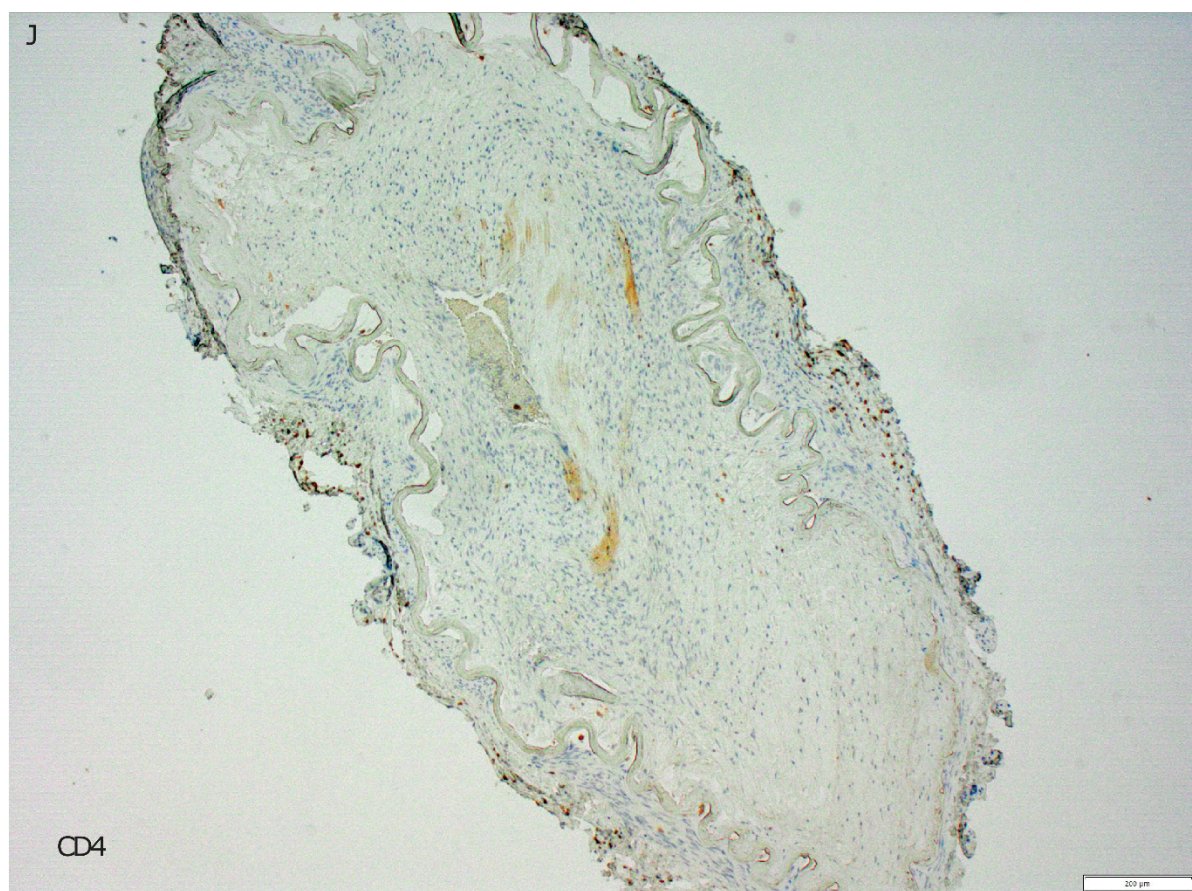
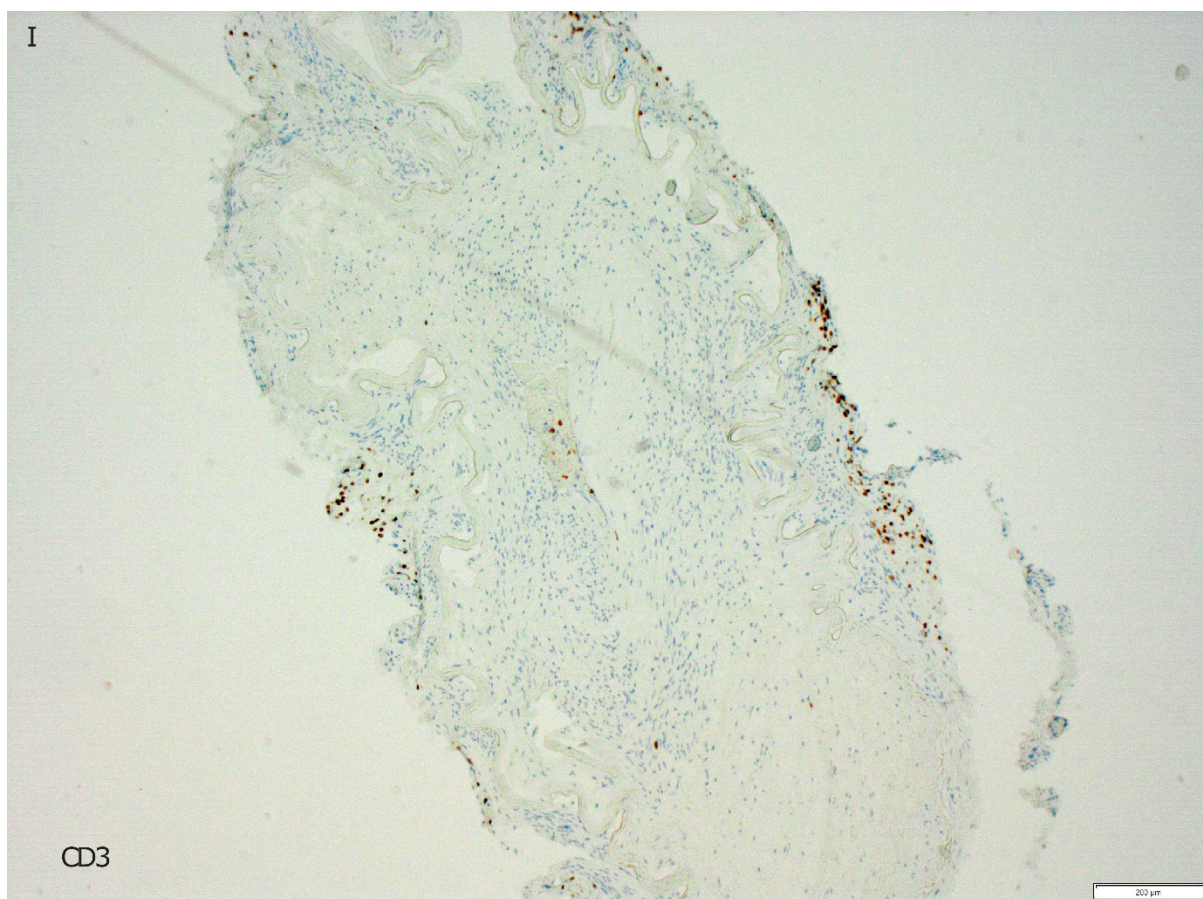


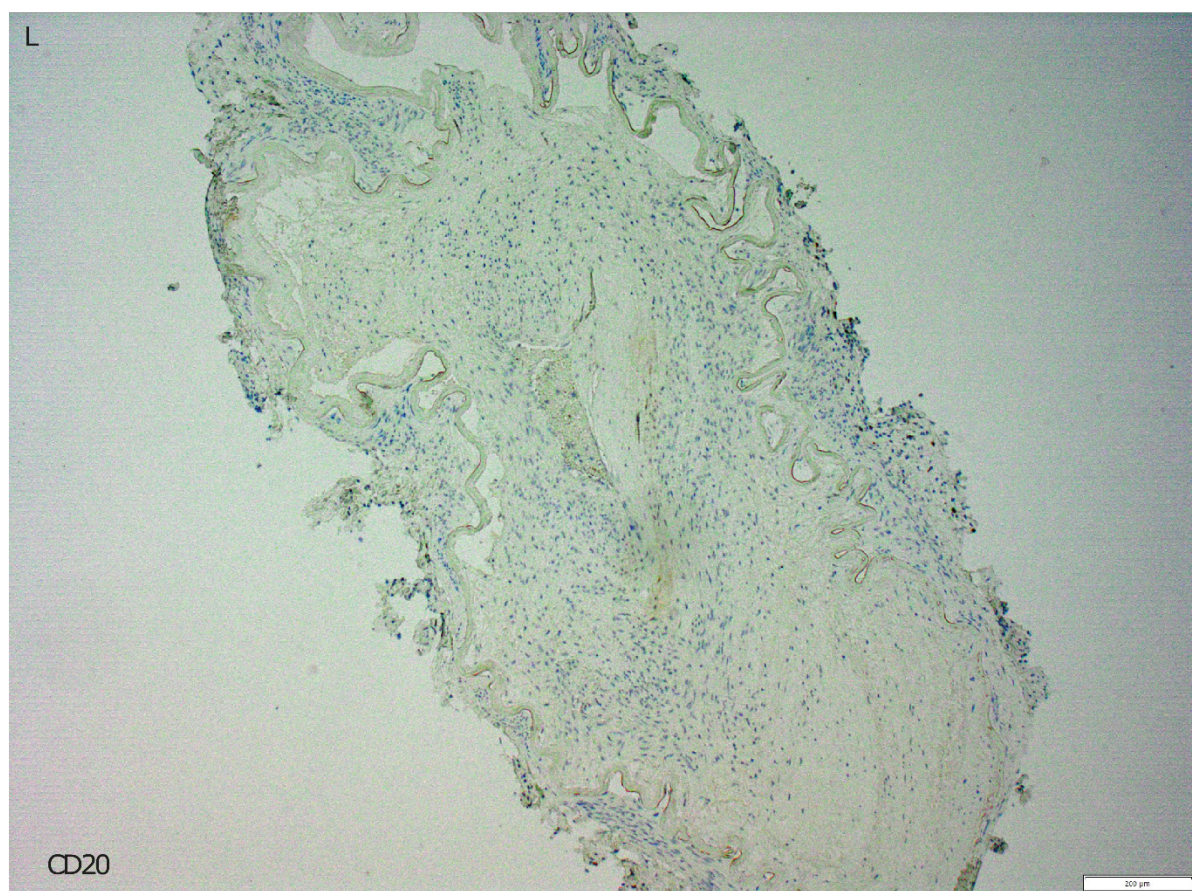
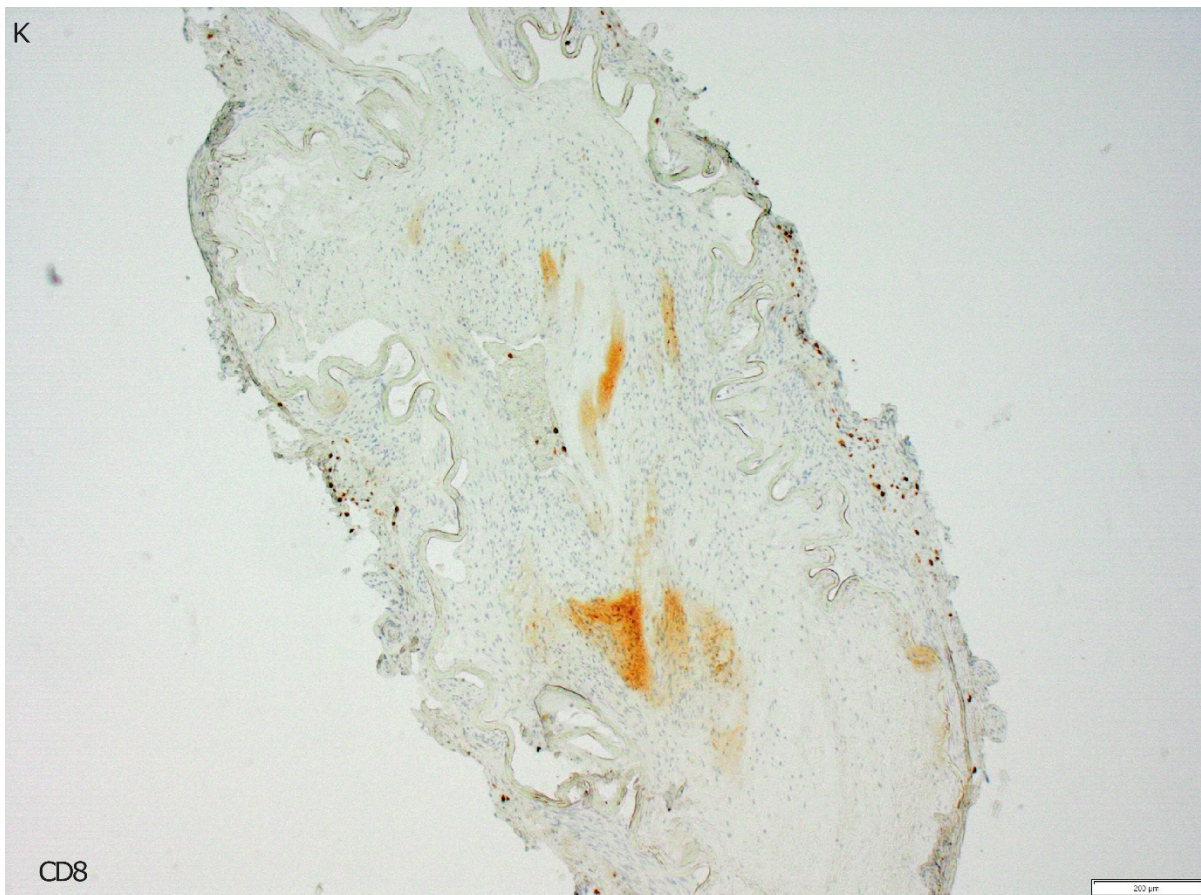
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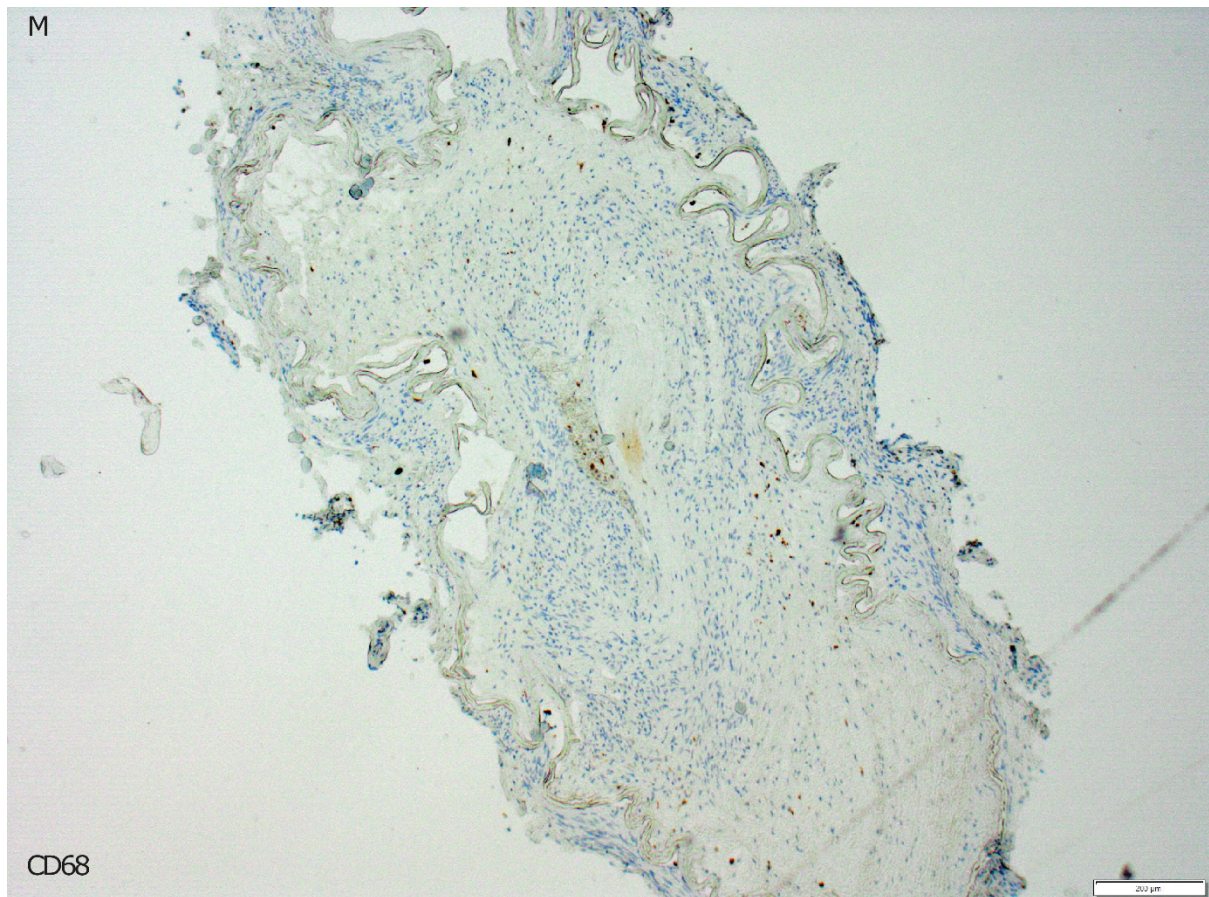


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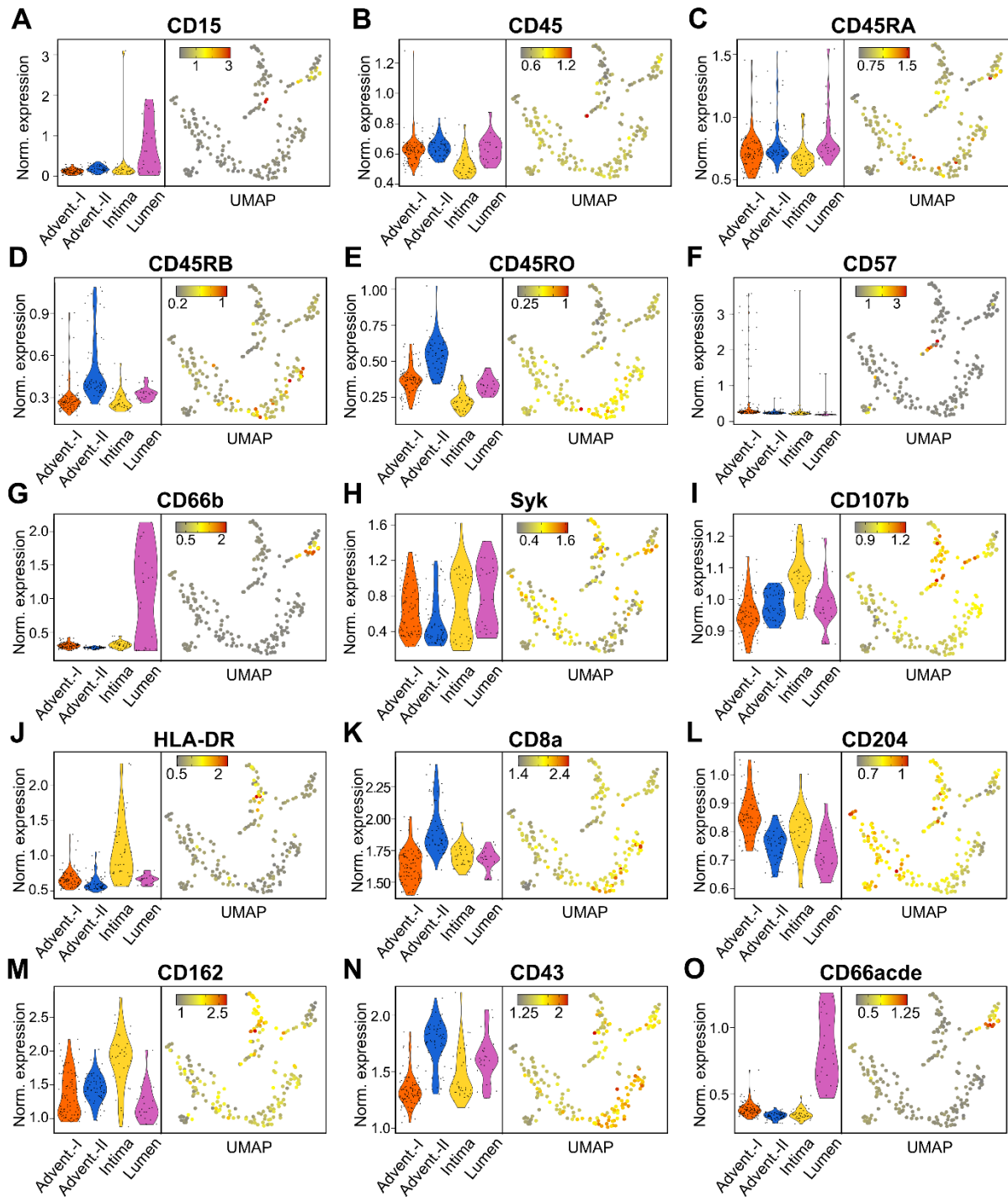




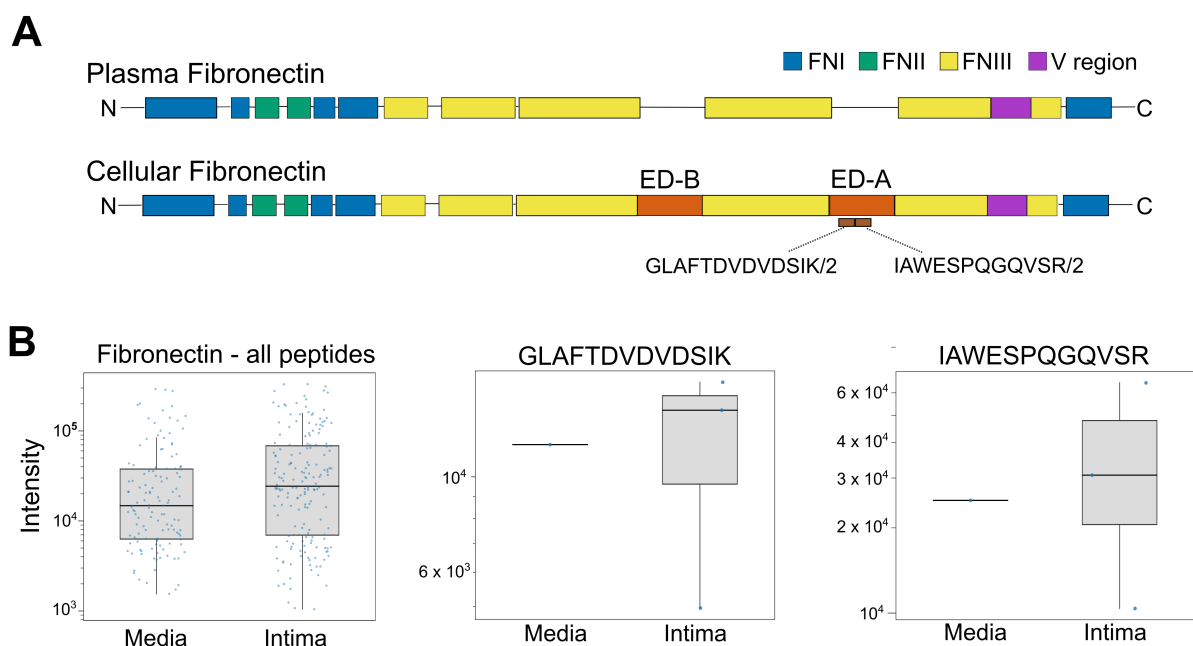




Supplementary Figure 6. Immunohistochemistry (IHC) validation stainings. (A) Hematoxylin and eosin (H&E) IHC validation staining was performed to assess the quality of the sample. (B-M) Stain for protein markers that validate cell type and immune cell annotations deducted from the spatial proteomics analysis. Stained markers included (B) smooth muscle actin (ACTA2), (C) Calponin, (D) H-Caldesmon, (E) Desmin (DES), (F) ERG, (G) CD31, (H) CD45, (I) CD3, (J) CD4, (K) CD8, (L) CD20 and (M) CD68. The white scale bar indicates 200 μm .



Supplementary Figure 7. Normalized expression of immune cell related markers. (A-O) Marker proteins included the 10 genes used for whole-cell segmentation of immune cells and additional proteins characteristic for immune cell subtypes. For each marker normalized expression was shown in a violin plot per cluster and per cell using the Uniform Manifold Approximation and Projection (UMAP) projections.



Supplementary Figure 8. Detection of cFN1 in intimal and medial artery tissue layers. (A) Schematic representation of the domain structure of plasma and cellular fibronectin (FN). Regions with FN module type I, II, and III were indicated by blue, green and yellow rectangles, respectively, while the variable region in purple. (B) Box plots showing the log₂ peptide intensity of all detected FN1 peptides (*Left*) the GLAFTDVDVDSIK peptide (*middle*) and the IAWESPQGQVSR peptide (*Right*) specific for the FN1-EDA domain, for intimal and medial tissue.

Supplementary tables

Supplementary Table 1: Antibodies used for immunofluorescence staining in MACSima™.

Custom antibodies			
Protein	Antibody name	Catalog number	Supplier
SG15	ISG15 Antibody (F-9)	sc-166755	Santa Cruz
RNF213	RNF213 Polyclonal Antibody	PA5-51902	Invitrogen
MMP2	MMP2 Polyclonal Antibody, FITC conjugated	bs-0412R-FITC	Bioss Antibodies
FN1	Fibronectin (1B9) Monoclonal Antibody , FITC Conjugated	bsm-33143M-FITC	Bioss Antibodies
IFN-γ	IFN-γ Antibody, anti-human, REAdey_lease™	130-125-275	Miltenyi biotech
ACTA2	alpha-Smooth Muscle Actin Antibody	NB300-978	Novus biologicals
REAscreen™ MAX Kit, human, FFPE, version 02			
Antigen	Clone	Fluorochrome	Antibody type
Actin (Smooth Muscle)	REAL650	FITC	REAdey_lease™
AKT Pan (PKB)	REA676	PE	REAffinity™
Amyloid A	REA931	FITC	REAffinity
AN2	REA989	PE	REAffinity
Annexin I	REA1122	FITC	REAffinity
BATF	REA486	FITC	REAffinity

Bcl-10	REA1140	PE	REAffinity
Bcl-2	REA872	FITC	REAffinity
BMI-1	REA438	FITC	REAffinity
CA-125	REAL909	PE	REAdye_lease
Caldesmon	REA1120	PE	REAffinity
Calponin	REAL635	PE	REAdye_lease
Catalase	REAL553	PE	REAdye_lease
CD100	REA316	FITC	REAffinity
CD104 (Integrin β 4)	REA236	FITC	REAffinity
CD107a (LAMP-1)	REAL653	APC	REAdye_lease
CD107b	REA1073	FITC	REAffinity
CD117	REA787	PE	REAffinity
CD123	REA918	FITC	REAffinity
CD13	REAL771	PE	REAdye_lease
CD134 (OX40)	ACT35	PE	Hybridoma
CD138	REA929	FITC	REAffinity
CD147	REA282	FITC	REAffinity
CD15	VIMC6	FITC	Hybridoma
CD158i (KIR2DS4)	JJC11.6	PE	Hybridoma
CD162	REA319	FITC	REAffinity
CD181 (CXCR1)	REA958	FITC	REAffinity
CD182 (CXCR2)	REA208	PE	REAffinity
CD183 (CXCR3)	REAL756	FITC	REAdye_lease
CD195 (CCR5)	REA245	PE	REAffinity
CD196 (CCR6)	REA190	PE	REAffinity
CD2	REA1130	FITC	REAffinity
CD20 Cytoplasmic	REAL649	APC	REAdye_lease
CD202b (TIE-2)	REA198	APC	REAffinity
CD204	REA460	PE	REAffinity
CD209 (DC-SIGN)	REAL690	APC	REAdye_lease
CD223	REA351	PE	REAffinity
CD226 (DNAM-1)	REA1040	FITC	REAffinity
CD233	REA368	FITC	REAffinity
CD234 (DARC)	REA376	PE	REAffinity
CD235a (Glycophorin A)	REAL490	FITC	REAdye_lease
CD239 (BCAM)	REA276	PE	REAffinity
CD240DCE	REA327	PE	REAffinity
CD243 (ABCB1)	REA495	PE	REAffinity
CD268	REA1115	FITC	REAffinity
CD271 (LNGFR)	REAL709	APC	REAllease®
CD279 (PD1)	REA1165	PE	REAffinity
CD295 (LEPR)	REA361	APC	REAffinity
CD2AP	REA1147	PE	REAffinity
CD3	REA1151	APC	REAffinity
CD305 (LAIR-1)	REA447	PE	REAffinity
CD317 (BST2)	REA202	FITC	REAffinity
CD334 (FGFR4)	4FR6D3	FITC	Hybridoma
CD43	REA833	FITC	REAffinity
CD44	REA690	PE	REAffinity
CD45	5B1	PE	Hybridoma
CD45RA	REAL164	APC	REAllease
CD45RB	REA119	FITC	REAffinity
CD45RO	REA611	PE	REAffinity
CD5	REAL760	PE	REAdye_lease
CD52	REA164	FITC	REAffinity
CD57	REA769	PE	REAffinity
CD65	REA1128	PE	REAffinity
CD66 (CEA)	REA1158	FITC	REAffinity

CD66abce	REA1230	PE	REAffinity
CD66acde	REA428	PE	REAffinity
CD66ace	REA889	FITC	REAffinity
CD66b	REA306	PE	REAffinity
CD66c	REA414	FITC	REAffinity
CD66e	REA876	PE	REAffinity
CD79a	REA1168	PE	REAffinity
CD88 (C5AR)	REA1213	PE	REAffinity
CD8a	REA1024	FITC	REAffinity
CD90	REAL677	PE	REAdye_lease
CD99	REAL687	FITC	REAllease
CLA	REA1101	PE	REAffinity
Collagen I	REAL958	APC	REAdye_lease
Cytokeratin	REAL648	APC	REAdye_lease
Cytokeratin 10	REA933	FITC	REAffinity
Cytokeratin 10/13	REA1138	FITC	REAffinity
Cytokeratin 14	REAL831	FITC	REAdye_lease
Cytokeratin 19	REAL822	PE	REAdye_lease
Cytokeratin 20	REAL753	APC	REAdye_lease
Cytokeratin 5/6	REAL538	PE	REAdye_lease
Cytokeratin 5/6/8/17/19	REA1141	FITC	REAffinity
Cytokeratin 7	REA935	FITC	REAffinity
Cytokeratin 8	REA885	FITC	REAffinity
Cytokeratin HMW	REAL645	FITC	REAdye_lease
Dectin-1	REA515	PE	REAffinity
Desmin	REA1134	FITC	REAffinity
Dnmt3b	REA565	APC	REAffinity
EZH2	REA907	PE	REAffinity
FcεRIα	REA758	FITC	REAffinity
Galectin-3	REA1072	PE	REAffinity
Galectin-9	REA435	FITC	REAffinity
GCDFP-15	REAL828	FITC	REAdye_lease
GFAP	REAL735	PE	REAdye_lease
Hepatocyte	REA1088	FITC	REAffinity
HLA-ABC	REAL597	APC	REAdye_lease
HLA-DR	REAL550	FITC	REAdye_lease
HLA-DR, DP, DQ	REA332	FITC	REAffinity
Hsp70	REA349	PE	REAffinity
IgD	REA740	PE	REAffinity
IRF-7	REA521	FITC	REAffinity
IRF-7 pS477/pS479	REA310	PE	REAffinity
Jak1	REA700	PE	REAffinity
JNK2 (MAPK9)	REA1153	FITC	REAffinity
Ki-67	REA1123	FITC	REAffinity
LRP-4	REA552	APC	REAffinity
MART-1	REA1093	FITC	REAffinity
Mast Cell Tryptase	REAL798	FITC	REAdye_lease
Melanocyte PMEL	REA927	PE	REAffinity
Metallothionein	REA1139	FITC	REAffinity
MLC2v	REA401	PE	REAffinity
Myogenin	REA930	FITC	REAffinity
Myosin Smooth Muscle	REA1107	FITC	REAffinity
NeuN	REA1131	PE	REAffinity
p120 Catenin pS879	REA548	PE	REAffinity
p53	REA1132	FITC	REAffinity
PAX-5 (BSAP)	REA140	PE	REAffinity
PCNA	REA858	FITC	REAffinity
PI3K p85 α	REAL589	PE	REAdye_lease

PKC α	REA156	APC	REAffinity
PLAP	REA1089	FITC	REAffinity
Plasma Cell	REA908	FITC	REAffinity
PLP	REA1155	PE	REAffinity
Podoplanin	REAL468	PE	REAffinity
Slug	REA404	PE	REAffinity
Sox2	REA320	FITC	REAffinity
SSRP1	REA555	PE	REAffinity
SUSD2	REA795	PE	REAffinity
Syk	REA111	PE	REAffinity
Synaptophysin	REA1121	FITC	REAffinity
TIM-3	REAL818	APC	REAffinity_lease
TOM22	REAL439	APC	REAffinity_lease
TTF-1	REA1090	FITC	REAffinity
Tyrosinase	REA1149	PE	REAffinity
Vimentin	REAL1008	APC	REAffinity_lease
WT1	REAL748	APC	REAffinity_lease
ZAP70	REA814	PE	REAffinity
α Tubulin	REA1136	FITC	REAffinity
α -Actinin (Sarcomeric)	REA402	PE	REAffinity
β -Actin	REAL571	PE	REAffinity_lease
β -Catenin	REA480	FITC	REAffinity
β -Tubulin 3	REA1152	PE	REAffinity
γ -Actin 1	REAL591	FITC	REAffinity_lease

Supplementary methods

Plasma proteomics

Of each sample +/- 160 μ g of protein was added to a final volume of 50 μ L S-TrapTM buffer containing 5% sodium dodecyl sulfate (SDS) and 50 mM triethylammonium bicarbonate (TEAB), pH 7.55. Proteins were reduced by addition of 5 mM tris(2-carboxyethyl)phosphine and incubation for 30 min at 55 °C and then alkylated by addition of 20 mM iodoacetamide and incubation for 15 min at room temperature in the dark. Phosphoric acid was added to a final concentration of 1.2% and subsequently samples were diluted 7-fold with binding buffer containing 90% methanol in 100 mM TEAB, pH 7.1. The samples were loaded on the 96-well S-TrapTM plate (Protifi, New York, USA), placed on top of a deepwell plate, and centrifuged for 2 min at 1,500 x g at room temperature. After protein binding, the S-TrapTM plate was washed three times by adding 400 μ L binding buffer and centrifugation for 2 min at 1,500 x g at room temperature. A new deepwell receiver plate was placed below the 96-well S-TrapTM plate and 50 mM TEAB containing trypsin (1/80, w/w) was added for digestion overnight at 37 °C. By centrifugation for 2 min at 1,500 x g, peptides were eluted in three times, first with 80 μ L 50 mM TEAB, then with 80 μ L 0.2% formic acid (FA) in water and finally with 80 μ L 0.2% FA in water/acetonitrile (ACN) (50/50, v/v). Eluted peptides were dried completely by vacuum

centrifugation and stored at -20 °C until further use. For liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, purified peptides were re-dissolved in 20 µL loading solvent A (0.1% TFA in water/ACN (98:2, v/v)) and 200 ng of each sample was loaded on an Evotip (Evosep, Denmark) according to the manufacturer's instructions. Samples were run in data-independent acquisition parallel accumulation-serial fragmentation (DIA-PASEF) mode on an Evosep One LC-system in-line connected to a timsTOF SCP mass spectrometer (Bruker). Peptides were analyzed with the 20 SPD method (72 min run length) using the endurance Evosep column (15 cm x 150 µm I.D., 1.9 µm beads, Evosep, Denmark). For elution of the peptides from the column solvent A consisted of 0.1% FA in LC-MS-grade water and solvent B consisted of 0.1% FA in ACN. Eluting peptides were measured on the timsTOF SCP in DIA-PASEF mode using a window size of 25 Da, as described before.¹

Laser capture microdissection proteomics profiling

Proteins from the excised tissue samples were extracted by ultrasonication and heating in a buffer containing 5% SDS, followed by trypsin digestion and cleanup using S-Trap™ micro columns (Protifi, New York, USA), as described in detail before.¹ Resulting peptides were loaded on Evotips (Evosep, Odense, Denmark) for LC-MS/MS analysis on an Evosep One LC-system in-line connected to a timsTOF SCP mass spectrometer (Bruker) in DIA-PASEF mode. Peptides were separated on the Evosep with the 20 SPD whisper method using a 15 cm Aurora Gen3 Elite column and measured on the timsTOF SCP in DIA-PASEF mode using a window size of 25 Da, as described before.¹

Proteomics data analysis

Raw DIA-PASEF data files were analyzed by library-free analysis using DIA-NN (version 1.9.0 for LCM, version 2.0.2 for plasma proteomics).² Firstly, a peptide precursor spectral library (peptide length 7-30) was predicted using the human UniProtKB reference proteome (20,666 proteins). Cysteine carbamidomethylation was set as a fixed modification and methionine oxidation as a variable modification, allowing one missed trypsin cleavage. All runs, of the LCM or plasma proteomics dataset, were searched against the predicted spectral library using a 15 ppm fragment and precursor ion tolerance. In case of the plasma proteomics, two samples (healthy control #8 and moyamoya disease #6) were left out due to technical considerations (suboptimal signal and identification). The outputted peptide precursor quantification matrix was used for differential protein analysis using MSqRob2 (version 1.6.1).³ Precursor intensities were log₂ transformed and subjected to sample median

normalization. Precursors were required to be quantified at least twice (LCM proteomics) or three times (plasma proteomics). Afterwards, precursor intensities were summarized at the protein level, using the aggregateFeatures function, and a MSqRob model was fitted using robust linear regression with the conditions of interest specified as covariate. In case of LCM proteomics data, contrast of occlusion to VSMC tissue regions delivered nine differentially regulated proteins ($P \leq 0.01$ and fold change ≥ 2).

For plasma proteomics, moyamoya disease plasma versus healthy, control individual identified six differentially regulated proteins (adjusted $P \leq 0.05$). The platelet contamination index was calculated using the method described by Geyer *et al.*⁴ The total intensity of platelet quality markers was divided by the total intensity per sample.

Patient vignettes

Patient 1

A woman was brought to the emergency department following symptoms suspicious for cerebrovascular involvement. A brain CT showed an extensive intraventricular haemorrhage, including both lateral third and fourth ventricles, with a discrete midline shift. CT-angiography showed an aneurysm in the choroid plexus of the left lateral ventricle, but also stenosis of the distal part of the internal carotid artery bilaterally, with collaterals, suspect for moyamoya angiopathy (MMA).

Conventional cerebral angiography confirmed an aneurysm of the left-sides choroid plexus or posterior temporal artery and MMA with occlusion of the M1-segment of the medial cerebral artery on both sides with extensive collaterals in the MCA territory via the anterior and posterior circulation and via the external carotid arteries. Neurological evaluation 4 weeks after admission revealed a right hemiparesis, ataxia, swallowing dysfunction and unilateral vocal cord paralysis. Brain MRI showed unchanged MMA characteristics.

Gradual clinical improvement occurred and she was transferred to a rehabilitation center. No follow-up data is available.

Patient 2

A woman with trisomy 21 syndrome was admitted to an emergency department following an acute ischemic stroke with left hemiparesis for which she was treated with intravenous thrombolysis with alteplase. Acute brain-CT showed no ischemic lesions. CT-perfusion images showed a small core infarct and a large penumbra in the territory of the right medial cerebral

artery (MCA). CT-angiography showed a narrow calibre of the right internal carotid artery (ICA), suspect for dissection, and a stop of the MCA, with distal filling from collateral vessels. MR-angiography 3D-TOF images showed an occlusion of the right distal ICA and proximal MCA with collateral vessel network, compatible with unilateral MMA. Cardiac evaluation revealed a patent foramen ovale (PFO) without other structural defects or arrhythmia. Conventional angiography revealed a thin calibre of the right ICA without clear stenosis, with an occlusion of the M1-segment of the right MCA, with anterograde collateralization. On the left sided there was also an occlusion of a parietal branch of the M2-segment of the MCA, also collateralized. The global perfusion of the MCA territory was disturbed with leptomeningeal collateralization coming from the anterior and posterior circulation on both sides. An irregular aspect of the A1-segment of the left anterior cerebral artery was noted. A complete neurological recovery of the left hemiparesis followed in the following weeks.

Follow-up brain imaging with MRI showed increased narrowing of the ostium of the left A1 segment, besides the already known occlusion of the right A1 segment. There was also an increased ostial narrowing of the M1-segment of the left MCA, and an unchanged ostial occlusion of the right MCA with lenticulostriatal collaterals and re-injection of the distal M1-segment without new areas of brain ischemia next to the previously documented ischemic lesions. Cortical-subcortical hemosiderine deposition was seen at the right frontal and left parietal lobes. Because of these MRI findings a control conventional angiography was done and showed an increased stenosis of the left A1 segment without other changes.

Patient 3

A woman with a medical history of mild cognitive impairment and macrocephaly was referred to a neurological department following the occurrence of sensorimotor deficit of the right arm and hand and partial status epilepticus with convulsions of the right arm and leg. Neurological work-up with brain-MRI revealed multiple ischemic lesions in the bilateral semi-oval center with aberrant course of the fornices and broadening of the lateral ventricles, with box-like broadening of the frontal ventricular horns.

Brain MR-angiography revealed high-graded stenosis of the right M1-segment and right communicating posterior artery, left A1- and M1-segment and to a lesser extend of both P1-segments. Conventional cerebral angiography confirmed stenosis of the A1-, M1- and carotid-T-segments without signs of vasculitis, suggesting beginning MMA.

In the following months and years new episodes with transient sensory focal neurological deficits occurred. Brain-MRI showed the already known MMA with high-grade stenosis of the bilateral distal ICA, the M1- and A1-segment on the left side and filiform narrowing of the right PCA, confirmed with conventional cerebral angiography that also showed the irregular high-grade M1-stenosis, clearly increased compared to prior evaluation. Upon multidisciplinary discussion, stenting of the M1-stenosis was done.

Patient 4

In the first years of life, this patient was neurologically evaluated for delayed motor development. Brain MRI revealed mild periventricular leukomalacia of unknown origin. A few years later, a couple of weeks following a respiratory infection, the patient developed neurological symptoms, more pronounced at the right side, together with ataxia and dysarthria. No brain imaging was done at that time. Another few years later, there was deterioration of the neurological problems following an episode of infection. A control brain-MRI showed new white matter lesions, atrophy and gliosis in the left frontal lobe and left insular region, bilateral putamen and periventricular white matter. MR-angiography showed significant stenosis or occlusions of the distal part of the bilateral ICA and of the proximal parts of the arteries of Willis' circle, most pronounced of the right MCA with collaterals on the right side, compatible with MMA. Further genetic analysis revealed a heterozygote pathogenic variant in ACTA2. The neurological status remained unchanged within the next years. Follow-up brain-MRI showed unchanged cerebral vasculopathy.

Patient 5

A woman was transferred to a department of neurology following an ischemic stroke with suspected cerebral vasculitis. Brain MRI showed acute ischemia in the medulla oblongata. Cardiovascular work-up showed no cardioembolic source. Secondary prevention with anti-thrombotic treatment with low-dose aspirin was started. There was no thrombophilia. A few years later, a second ischemic stroke occurred with acute ischemia in the right occipital lobe. Again, cardiovascular investigations revealed no clear source of cerebral ischemia. Some months later she developed a right-sided hemiplegia with loss of consciousness. Brain CT at the time showed multifocal ischemic lesions frontotemporoparietal on both sides, and in the left occipital lobe extending to the left basal ganglia with areas of cortical edema. Brain MRI confirmed the multifocal lesions including several cortical and subcortical acute ischemic lesions in both hemispheres. MR-angiography showed multiple stenoses and occlusions

bilaterally on the proximal circle of Willis and carotid siphon, with reinjection via collateral networks, compatible with moyamoya angiopathy or vasculitis.

Conventional cerebral angiography showed an occlusion of the right M2-segment with extensive collateralisation via perforating vessels and near-occlusion of the right distal A1 where the proximal is strongly constricted. Extensive collateral network emanating from the distal choroidea anterior artery. The PCA is occluded on the right in the P1 segment via the vertebral artery, a choroidal network can be recognised on the right that partially co-injects the posterior, forming the posterior choroidal network. On the left side, the findings are much less pronounced. The neurological symptoms worsened in the short term and after consultation with the patient's family and taking into account the patient's prior expression of will, it was decided to adopt a comfort policy. She died during the following days. An autopsy was performed.

Patient 6

This woman suffered from ischemic stroke and recurrent transient ischemic attacks. Neurovascular work-up revealed bilateral moyamoya disease for which she underwent bilateral ICA-ECA bypass surgery

A follow-up brain-MRI showed old ischemic lesions with an infarct posteriorly in the right capsula interna and capsula externa right to claustrum, an old infarct in the right middle temporal gyrus, and ischemic lesions in the left frontal white matter, together with generalized corticosubcortical atrophy. MR-angiography showed leptomeningeal collateralization in the absence of the carotid bifurcation and the A1 and M1 segments bilaterally. The posterior circulation is preserved, the right posterior is hypertrophied and presumably participates to the leptomeningeal collateralization. At the level of the right hemisphere, there are temporal-insular and lenticulostriatal ischemic lacunae, on the left there are gliotic changes in the semioval center.. No new neurological complications or symptoms developed until now.

Patient 7

This woman, underwent brain imaging for an unrelated medical work-up. This showed an aneurysm of the MCA bifurcation at the right side, partially calcified. Conventional angiography showed a broad-based aneurysm of the right MCA bifurcation, with calcifications and bulges. Both MCA branches originating from the base of the aneurysm showed caliber changes with fusiform broadening of the cranial branch and narrowing of the caudal branch. Coiling of the aneurysm was done without any complications or neurological sequelae.

Control brain MRI-MRA years following coiling of the aneurysm showed an occlusion of the aneurysm with a virtually disappeared right media and retrograde collateralization via leptomeningeal connections to the anterior and also via perforating branches, compatible with atypical moyamoya-angiopathy, without neurological symptoms. No vascular changes on the left side (ICA and MCA) were seen.

Patient 8

This woman was referred for neurological evaluation because of unexplained neurological symptoms.

Genetic work-up revealed a pathogenic R179H variant in ACTA2.

Imaging of the intracranial arteries showed moyamoya-like vasculopathy with mildly narrowing of the left MCA with dilatation and relatively straight course of the left ICA to the level the distal siphon, normal caliber and good permeability of left ACA, a gracile caliber and irregular delineation of the right carotid artery siphon and of the right MCA. The A1 segment of the right ACA, the distal segment of the basilar artery and the arteria communicans posterior bilaterally were undetectable.

Patient 9

This man with a history of migraine was diagnosed with MMA following a haemorrhagic stroke of the right internal capsula with left hemiplegia.. Follow-up brain MRI showed no diffusion restrictive lesions and excluded recent ischaemia, with remnants of the right capsulolenticular haemorrhage with precipitation, partial lacunar transformation and already known ischemic white matter lesions, mainly in the semi-oval center, more pronounced at the left side. MR-angiography revealed an overall unchanged aspect of the already known blurred delineated stenotic lesions bilaterally in the distal ICA and on the ACM-M2 and ACA-A1 segments bilaterally with diffuse lenticulostriatal collaterals.

Patient 10

This woman was admitted in a hospital with right hemisensory and other neurological symptoms. Brain-MRI showed multiple recent small infarcts in the area of the left arteria cerebri media, besides numerous presumably ischaemic white matter lesions bilaterally frontally and periventricularly arguing for a cerebral microangiopathy. MR-angiography showed pronounced wall irregularities on the course of the carotid siphon along the A1 segments of the ACA bilaterally, proximally at level of the MCA bilateral and along the P1 segments of the

ACP, suggestive for vasculitis. Thorough medical questioning revealed multiple episodes of neurological symptoms in the personal history.

Conventional angiography showed a good pass-through and normal vessel wall alignment of the left and right common carotid artery with a moderate short stenosis 1 cm post-ostial in the left ICA, vertebral arteries showed no abnormalities. Intracranial angiography revealed MMA with a narrow caliber of the right ICA and carotid siphon with filiform aspect of the proximal part of the A1 segment. There was also a narrow caliber of the M1-segment of the MCA on the right with subsequent occlusion. Collateralization of the medial and posterior region was found. On the left, a fine caliber of the ICA and carotid siphon was seen, with filiform aspect of the proximal part of the M1 segment of the left MCA. The further course of the MCA was also narrow and irregular. The left MCA region was collateralized via leptomeningeal connections from the anterior region and via fine collaterals from the lenticulostriate region. There was also collateralization of the bilateral MCA area via the PCA.

She was referred to a specialized neurosurgical department and was treated with an indirect ECA-ICA bypass at the left side with the left temporal muscle. A direct bypass was not possible due to narrow and fragile MCA vessels.

Years later, she suffered a new acute ischemic stroke. Brain-MRI showed an extensive area of recent ischemia in the right PCA and posterior watershed area. 3D TOF sequences showed a (sub)occlusion of the right PCA as well as a filiform, subocclusive appearance of the ICA on the right at the level of the carotid siphon, and a filiform aspect of the right MCA its branches, a filiform to subocclusive aspect of the M1-segment of the left MCA and filiform to subocclusive aspect of the left A1 segment. A control conventional angiography showed a complete occlusion of the right PCA, progressive MMD right-sided, with further narrowing of the ICA proximal to the para-ophthalmic segment and complete occlusion of the terminal ICA, M1 and A1 segment, with virtually disappearance of basal moyamoya collaterals and irrigation of the MCA and ACA region mainly via collaterals from the left hemisphere, as well as via transdural collaterals from ECA, with very limited leptomeningeal collateralization via the posterior circulation, without opacification of the right PCOM (Suzuki stage V right). Further a relatively stable left-sided MDD after indirect bypass was seen, with good collateralization of the MCA and ACA region via transdural collaterals and via the posterior circulation (Suzuki stage IV left).

Patient 11

The medical history of this was published earlier. We refer to the paper by Karla *et al.*⁵ for an extensive description.

Patient 12

A diagnosis of MMD was made following neurological evaluation because of intermittent pain and paresis of the left leg. Brain MRI with MRA showed fusiform widening at the transition from the A1 to the A2 segment on the right where the fusiform widening extends to the origin of the arteria fronto-orbitalis and the arteria pericalosa on the right. The origin of the right MCA showed irregular stenoses with typically localized moya moya-like collaterals from the lenticostriatal arteries, with presence of fine-grained collaterals on the course of the right anterior choroid artery. There was a preserved delineation of the intracerebral blood vessels in the left hemisphere and normal delineation of the PCA and cerebellar arteries.

She underwent following surgery: STA-MCA bypass, with EDMS and EDPS bilateral frontal

Control cerebral arteriography showed no direct visualization of a direct by-pass between the temporalis superficialis and the MCA bilaterally, with presumed occlusion. There was a significant collateralization bilaterally via the temporalis superficialis collaterals, collateralizing through the dura retrograde the ACA and MCA areas (functioning EDMS). There was a good permeability of the left vertebral and basilaris arteries and PCA at the right side with significant collateralization of the ACA and MCA on the left via leptomeningeal collaterals in the posterior area. Compared with the previous study, a significant progression of underlying stenoses and occlusions was seen.

No new neurological events occurred since the cerebrovascular bypass surgery

References

1. Maia TM, Van Haver D, Dufour S, *et al.* Proteomics-Based Analysis of Laser-Capture Micro-dissected, Formalin-Fixed Paraffin-Embedded Tissue Samples. *Methods Mol Biol.* 2025;2884:333-354. doi:10.1007/978-1-0716-4298-6_20
2. Demichev V, Szyrwił L, Yu F, *et al.* dia-PASEF data analysis using FragPipe and DIA-NN for deep proteomics of low sample amounts. *Nat Commun.* Jul 8 2022;13(1):3944. doi:10.1038/s41467-022-31492-0
3. Sticker A, Goeminne L, Martens L, Clement L. Robust Summarization and Inference in Proteome-wide Label-free Quantification. *Mol Cell Proteomics.* Jul 2020;19(7):1209-1219. doi:10.1074/mcp.RA119.001624

4. Geyer PE, Voytik E, Treit PV, *et al.* Plasma Proteome Profiling to detect and avoid sample-related biases in biomarker studies. *EMBO Mol Med.* Nov 7 2019;11(11):e10427. doi:10.15252/emmm.201910427
5. Karla AR, Pinard A, Boerio ML, *et al.* SAMHD1 compound heterozygous rare variants associated with moyamoya and mitral valve disease in the absence of other features of Aicardi-Goutieres syndrome. *Am J Med Genet A.* Apr 2024;194(4):e63486. doi:10.1002/ajmg.a.63486