

Mouse tissues

Control polyclonal
anti-CD31

Recombinant monoclonal
anti-CD31 AD217 (ABCD Antibodies)

CC1 pH8 64min @ 95°C
0,26µg/mL LB 60min @37°C
HQ - DAB

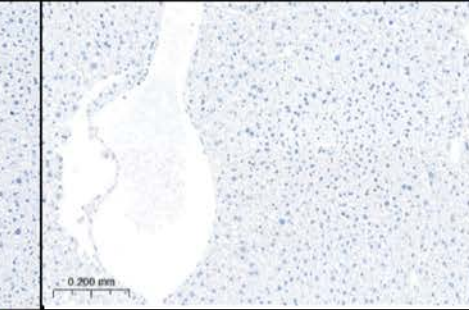
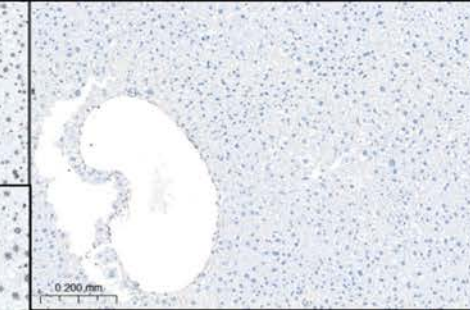
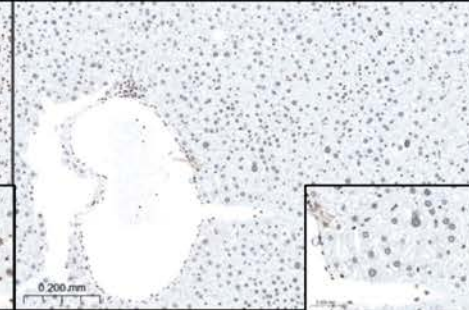
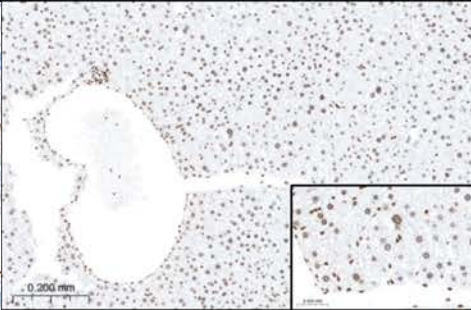
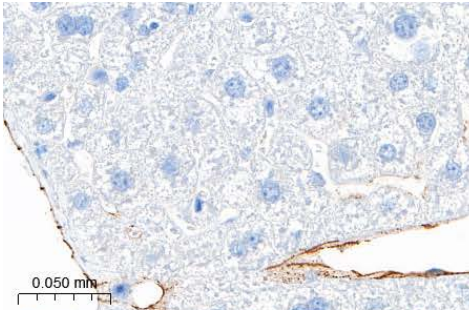
CC2 pH6 24min @ 91°C
1/100^e LB 60min @37°C
HQ - DAB

CC1 pH8 24min @ 95°C
1/100^e LB 60min @37°C
HQ - DAB

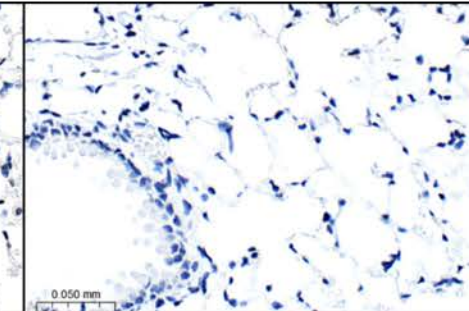
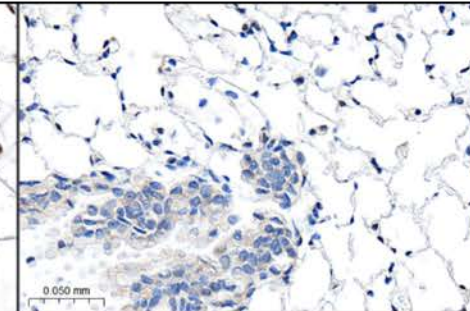
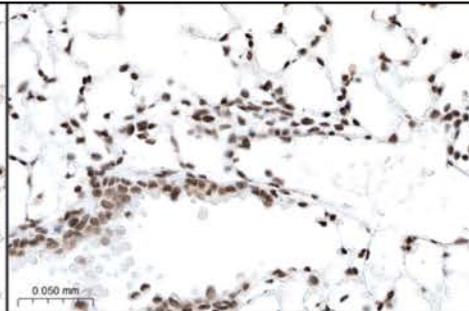
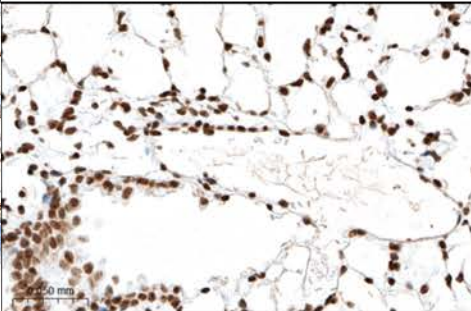
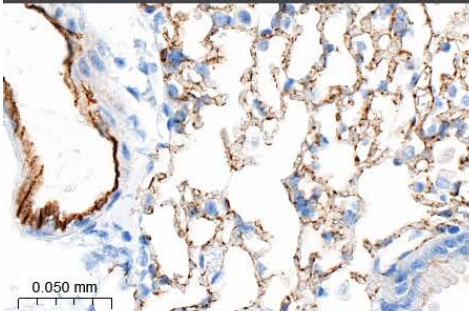
No antigen retrieval
1/100^e LB 60min @37°C
HQ - DAB

Protease 1
1/100^e LB 60min @37°C
HQ - DAB

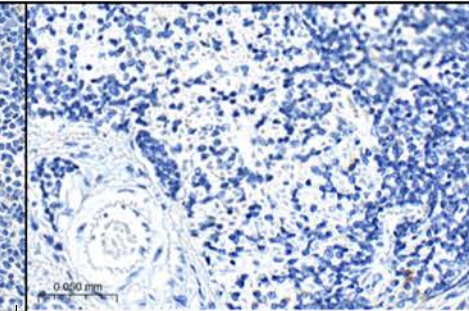
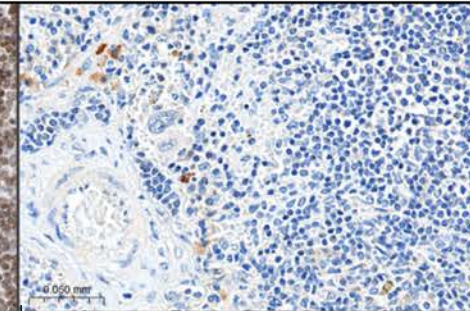
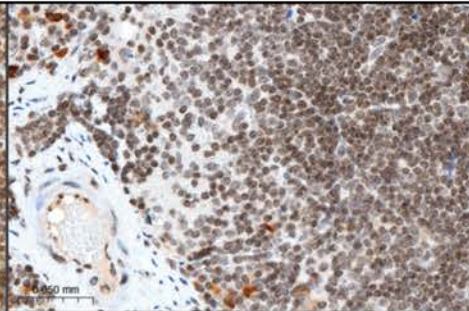
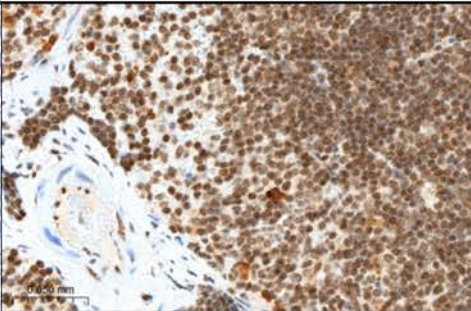
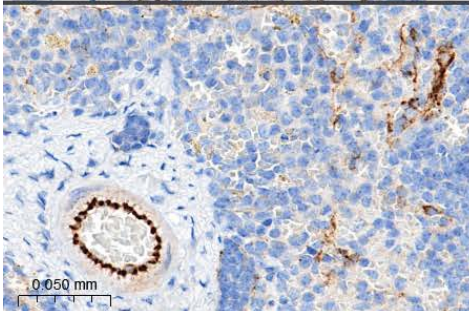
Liver



Lung



Spleen



Non-specific nuclear labelling of a majority of cells

Membrane labelling of some cells but not specific

No signal and damaged samples

Mouse tissues

Control polyclonal
anti-CD31

Recombinant monoclonal
anti-CD31 JC70 (Absolute Antibodies)

CC1 pH8 64min @ 95°C
0,26µg/mL LB 60min @37°C
HQ - DAB

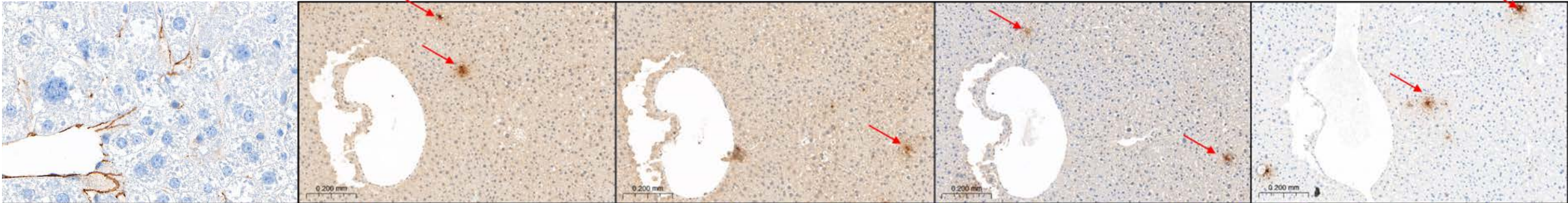
CC2 pH6 24min @ 91°C
1/100^e LB 60min @37°C
HQ - DAB

CC1 pH8 24min @ 95°C
1/100^e LB 60min @37°C
HQ - DAB

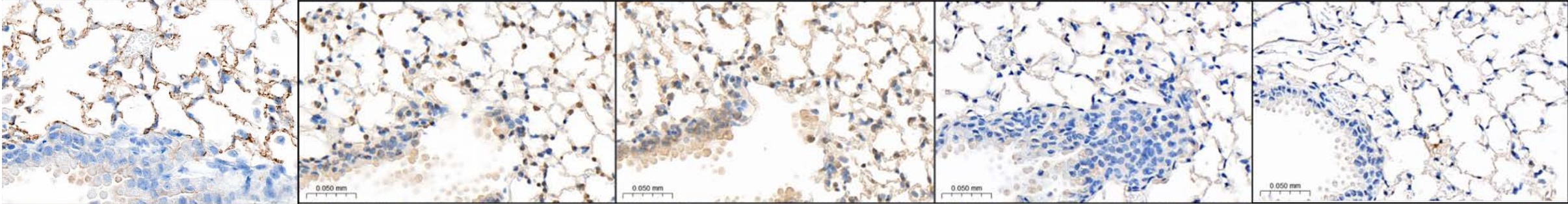
No antigen retrieval
1/100^e LB 60min @37°C
HQ - DAB

Protease 1
1/100^e LB 60min @37°C
HQ - DAB

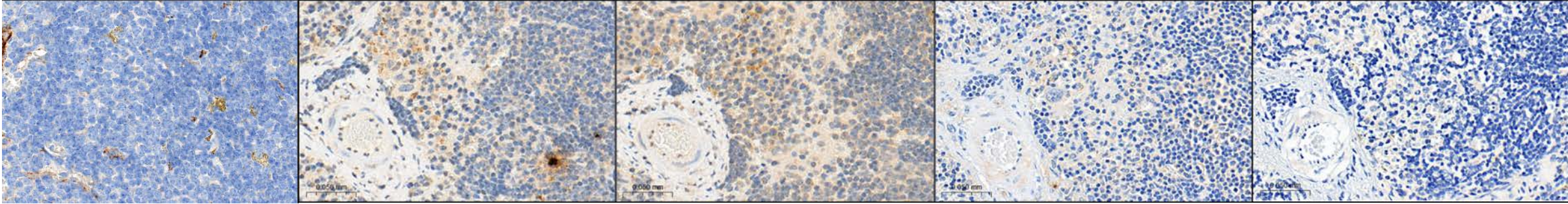
Liver



Lung

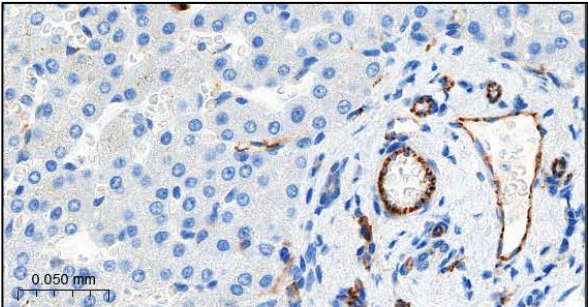
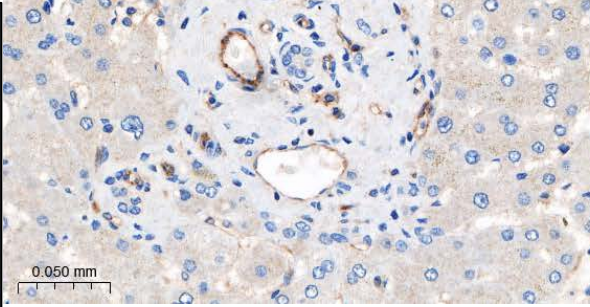
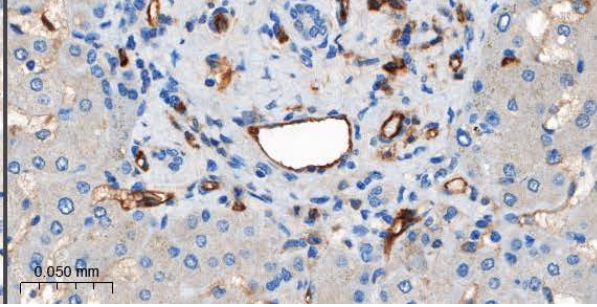
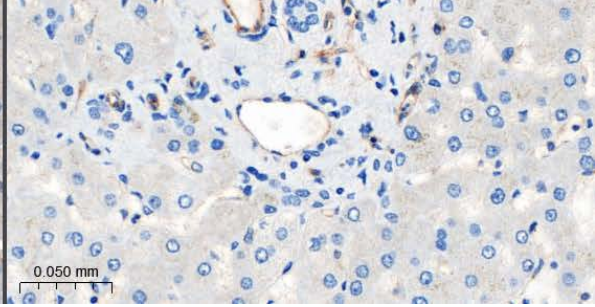
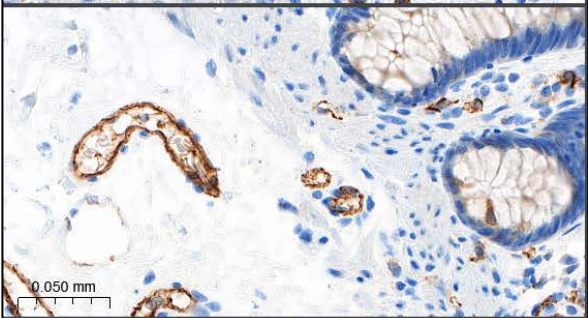
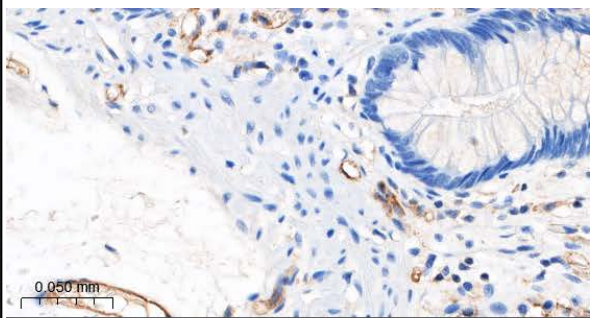
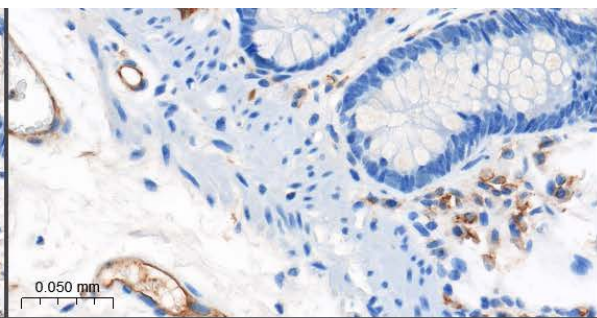
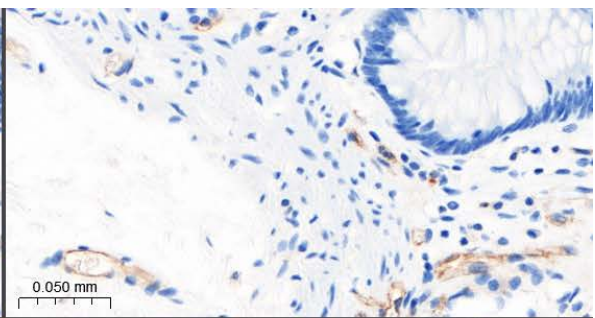
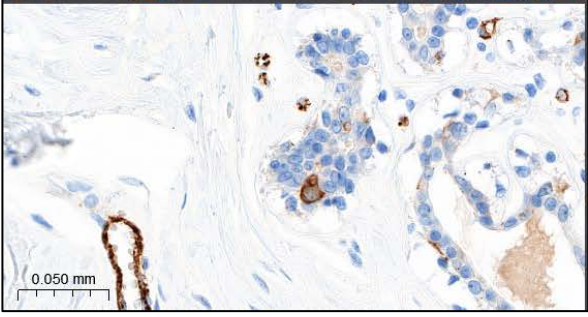
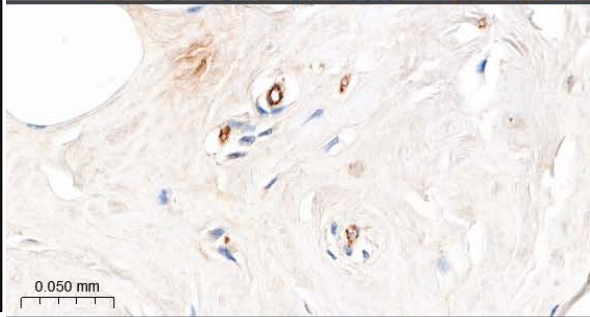
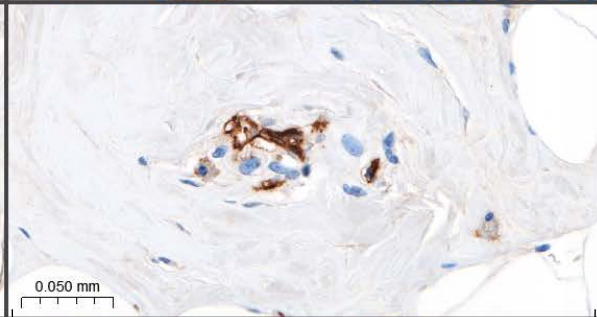
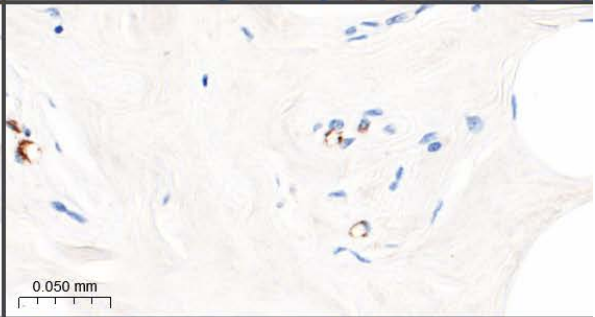


Spleen



No specific signal under any of the conditions tested. Lots of background noise, sometimes nuclear staining, spots on the slide: 

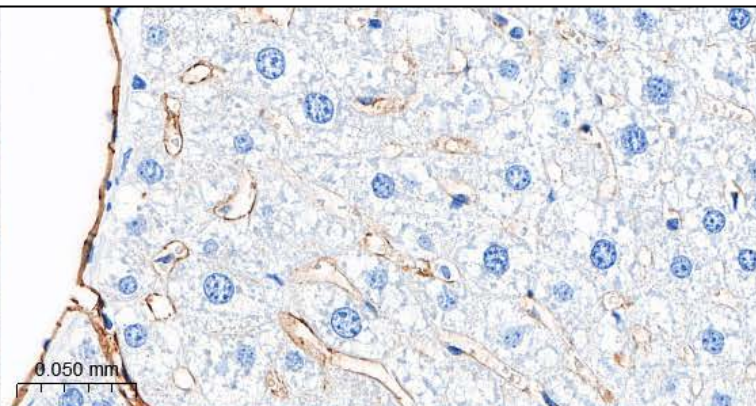
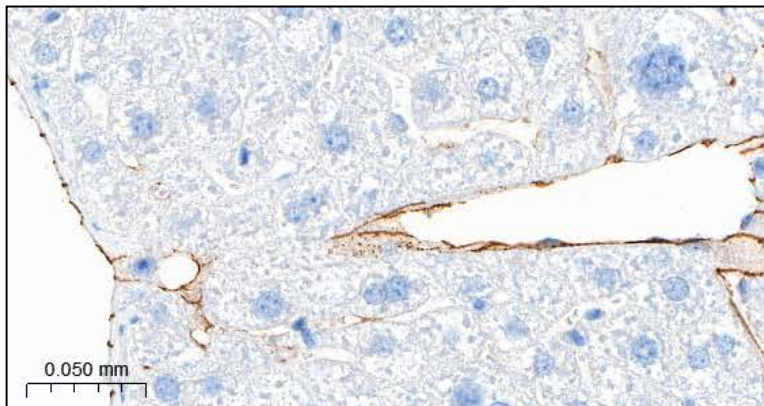
Human tissues

	Control polyclonal anti-CD31	Recombinant monoclonal anti-CD31 JC70 (Absolute Antibodies)		
	CC1 pH8 64min @ 95°C 0,26µg/mL LB 60min @37°C HQ - DAB	CC2 pH6 24min @ 91°C 1/200 ^e LB 60min @37°C HQ - DAB	CC2 pH6 24min @ 91°C 1/300 ^e LB 60min @37°C HQ - DAB	CC2 pH6 24min @ 91°C 1/400 ^e LB 60min @37°C HQ - DAB
Liver				
Colon				
Adipose tissue				
			Validated condition	

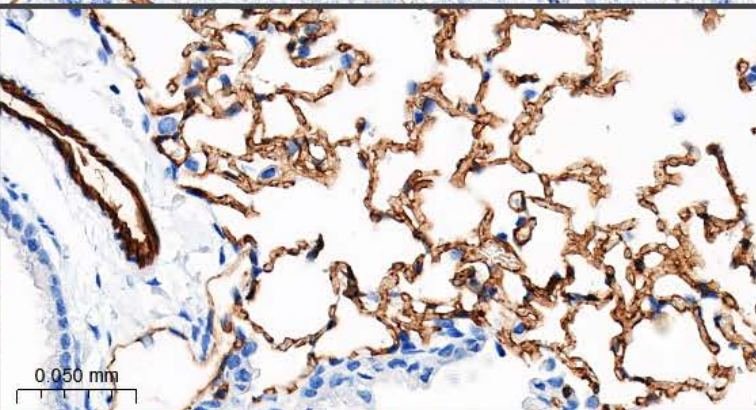
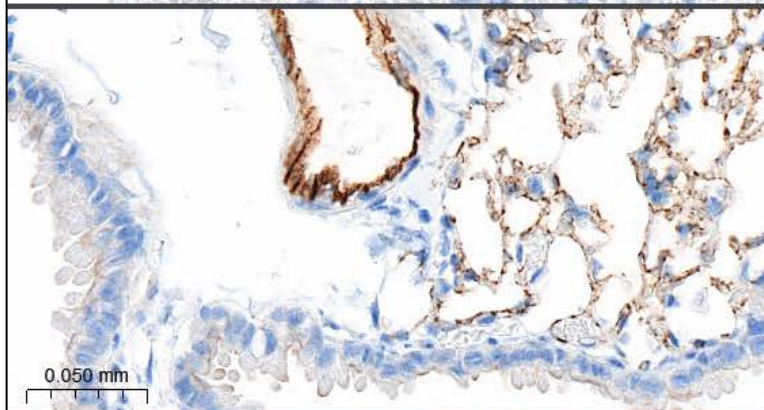
Control polyclonal anti-CD31

Recombinant monoclonal
anti-CD31 Ab182981 (Abcam)

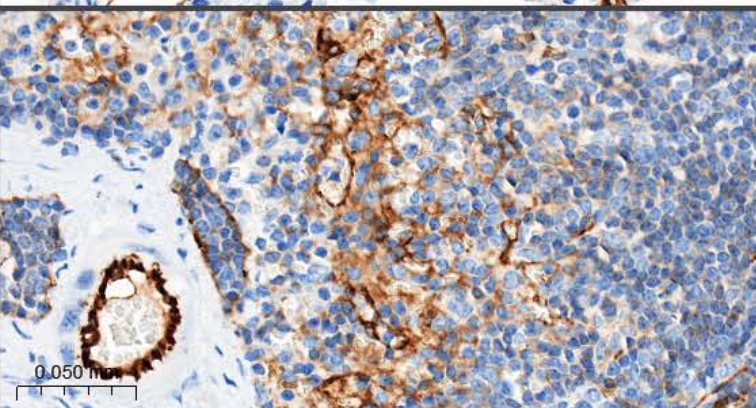
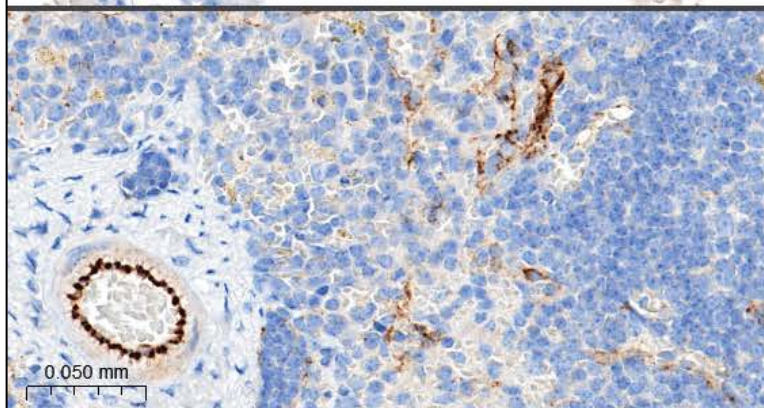
Liver



Lung

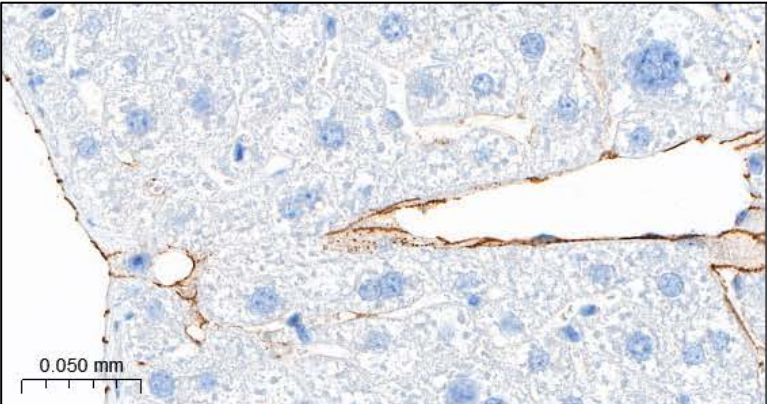
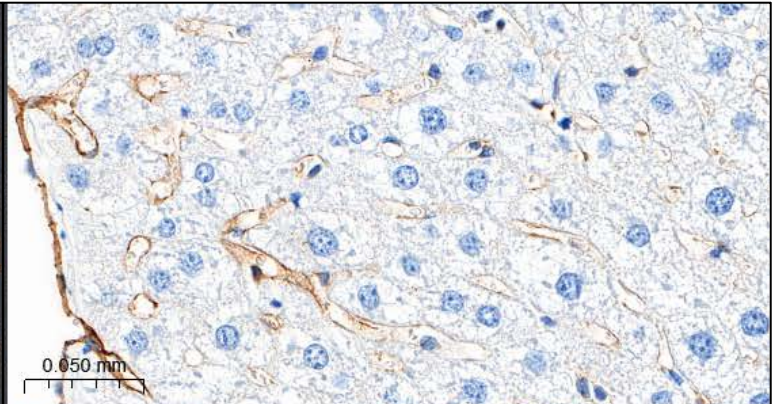
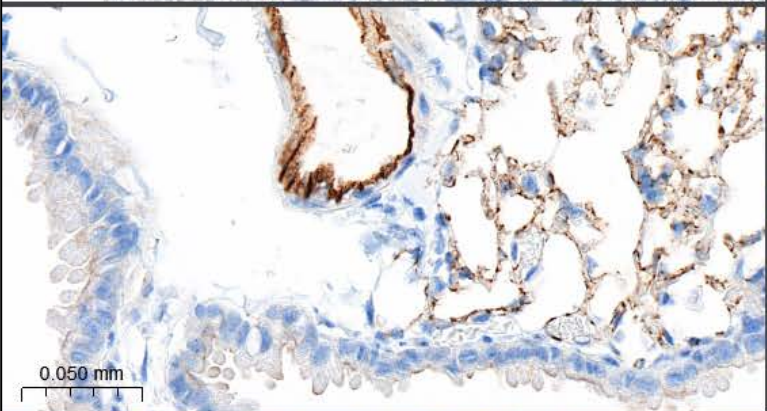
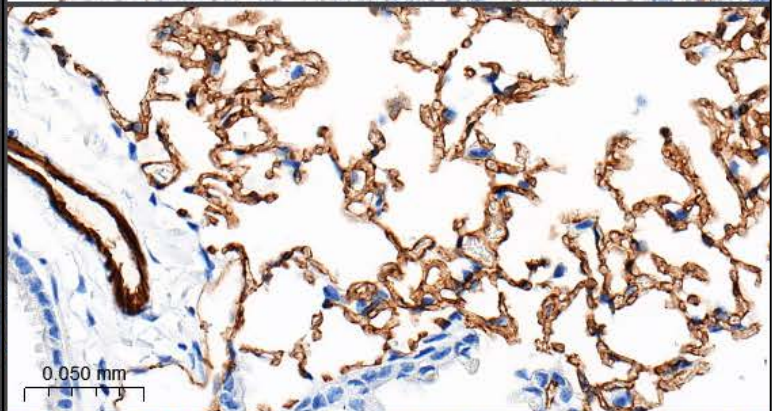
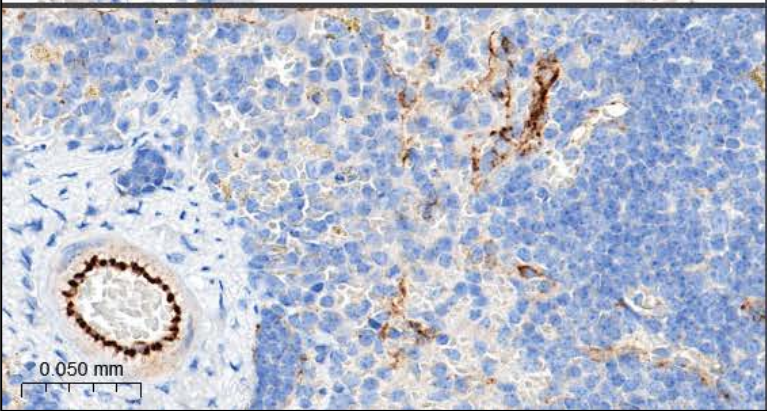
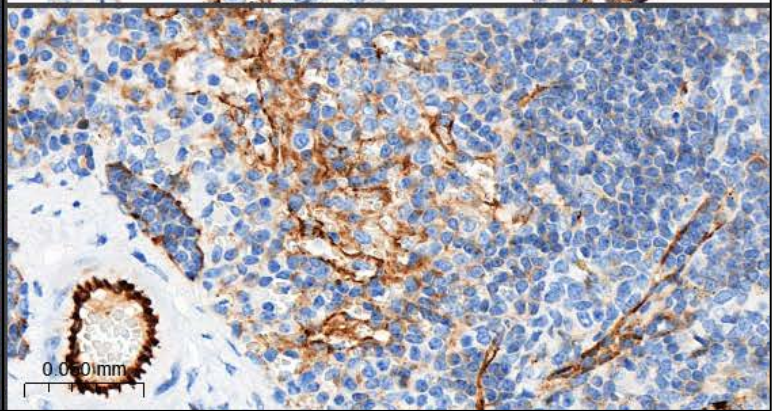


Spleen



Validated conditions :

- Antigen retrieval : CC1 pH8, 24 min @95°C
- Dilution : 1/500 (ST)
- Incubation : 60 min @37°C
- Amplification : OmniMap Rabbit

	Control polyclonal anti-CD31	Recombinant multiclonal anti-CD31 Ab281583 (Abcam)
Liver		
Lung		
Spleen		

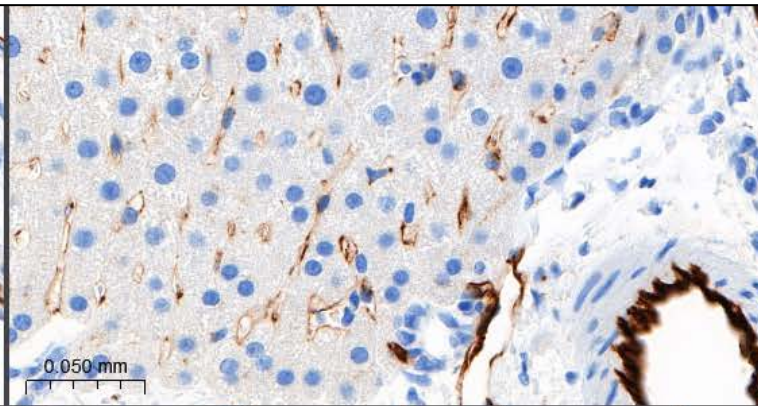
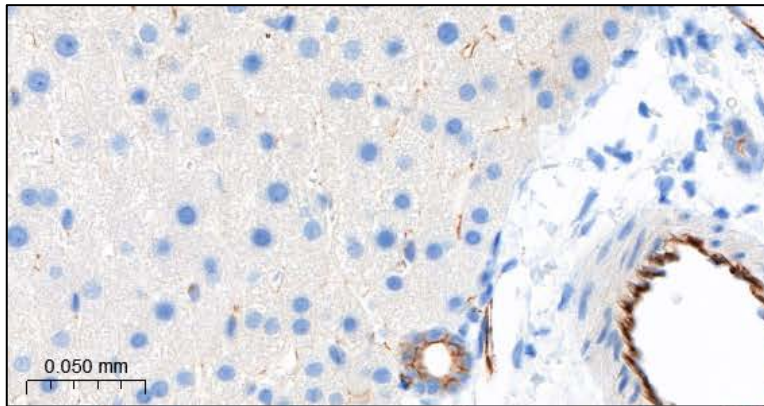
Validated conditions:

- Antigen retrieval : CC1 pH8, 24 min @95°C
- Dilution : 1/500 (ST)
- Incubation : 60 min @37°C
- Amplification : OmniMap Rabbit

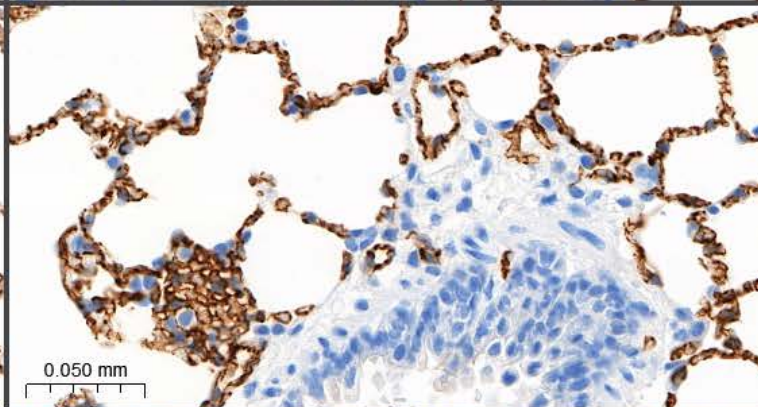
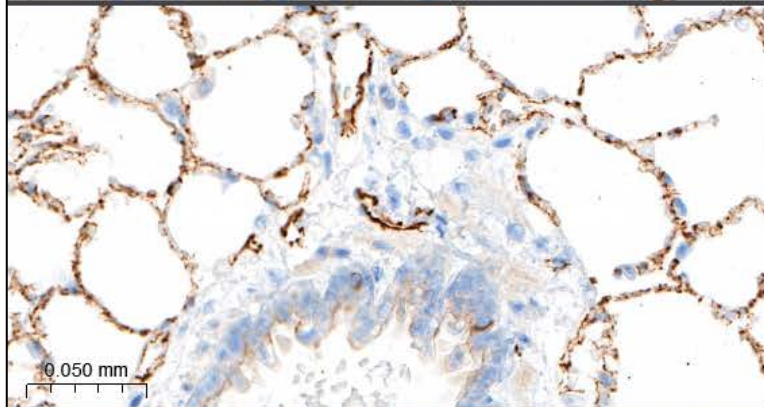
Control polyclonal anti-CD31

Recombinant monoclonal
anti-CD31 Ab182981 (Abcam)

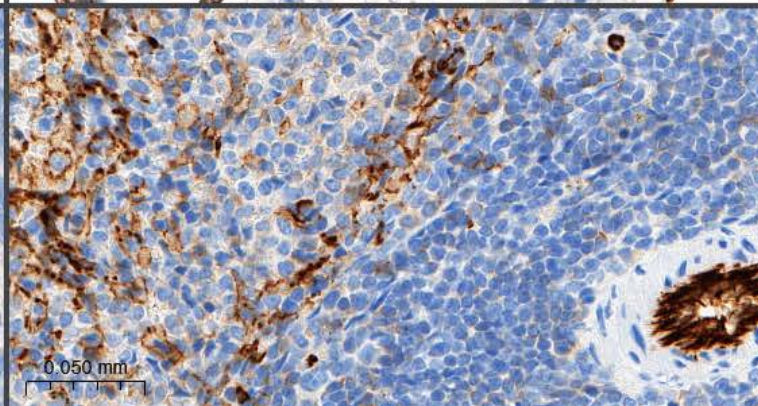
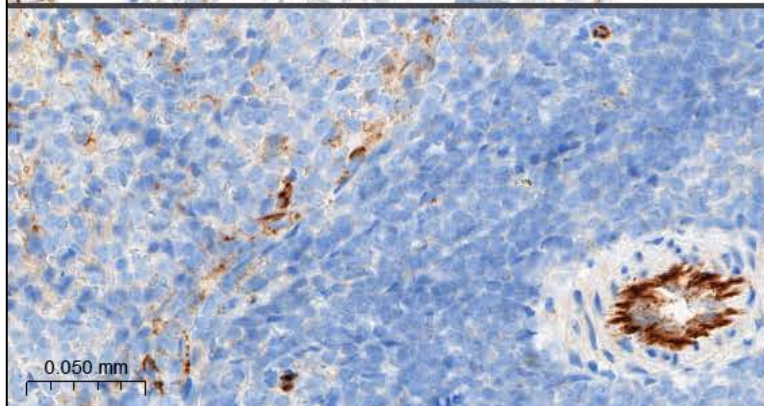
Liver



Lung



Spleen



Validated conditions:

- Antigen retrieval : CC1
pH8, 24 min @95°C

- Dilution : 1/500 (ST)

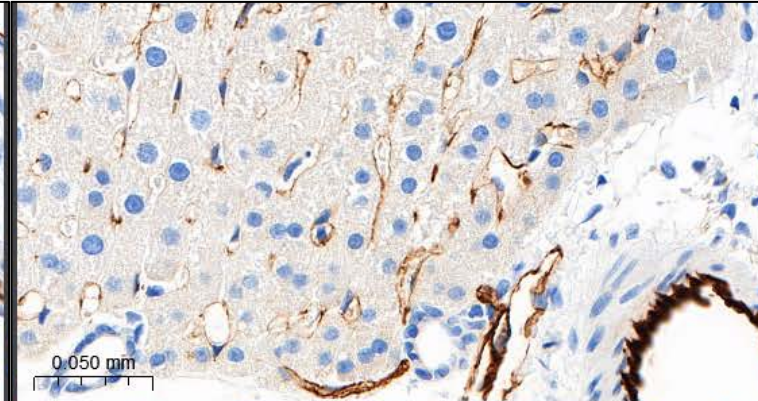
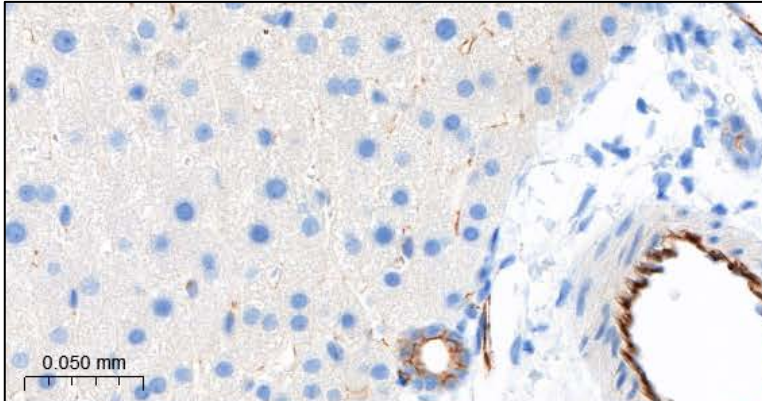
- Incubation : 60 min
@37°C

- Amplification : OmniMap
Rabbit

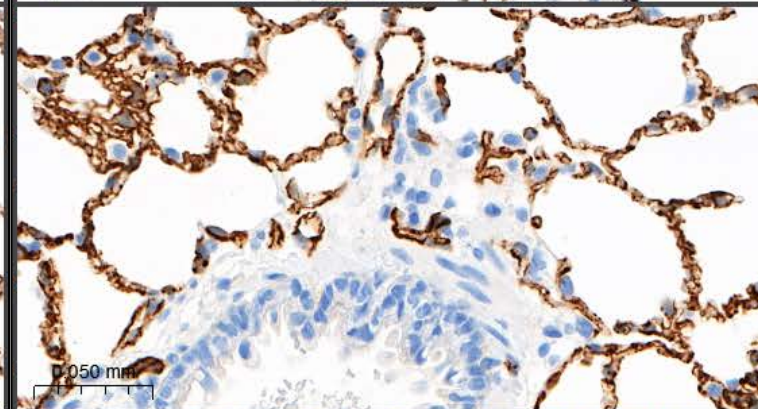
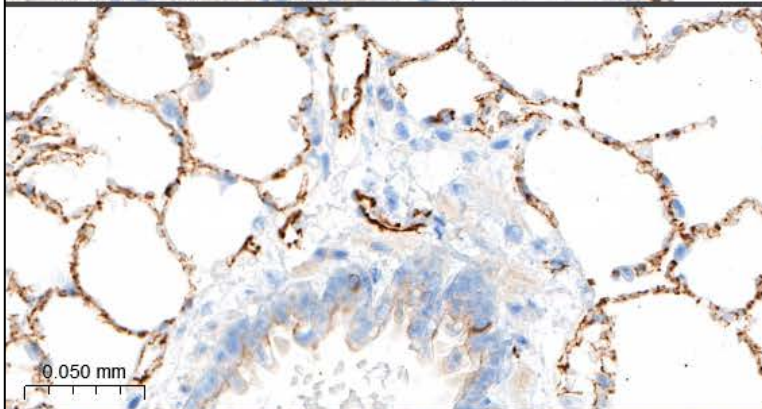
Control polyclonal anti-CD31

Recombinant multiclonal
anti-CD31 Ab281583 (Abcam)

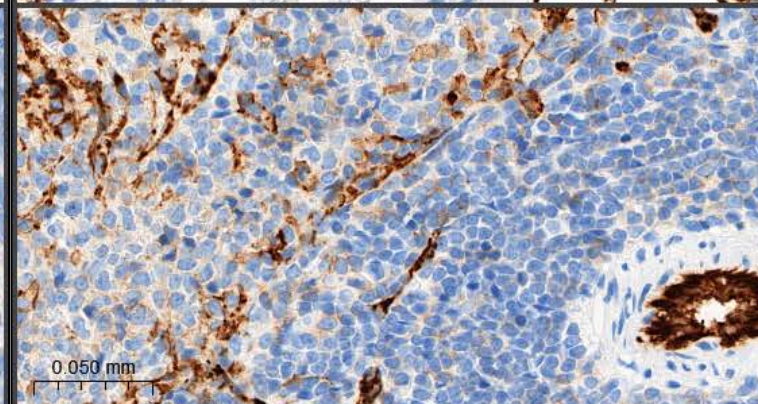
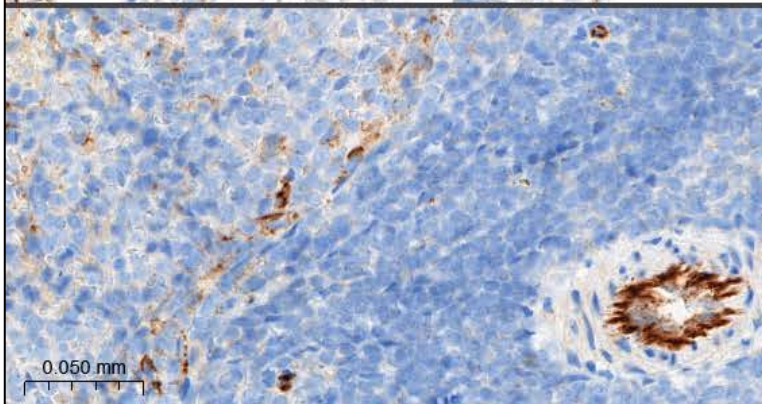
Liver



Lung



Spleen



Validated conditions:

- Antigen retrieval : CC1
pH8, 24 min @95°C

- Dilution : 1/500 (ST)

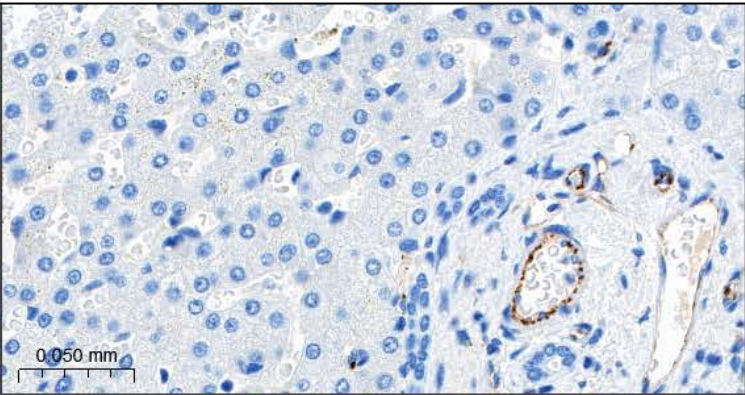
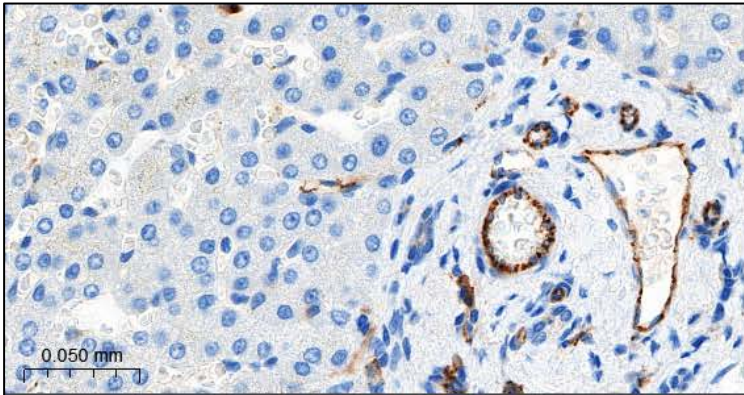
- Incubation : 60 min
@37°C

- Amplification : OmniMap
Rabbit

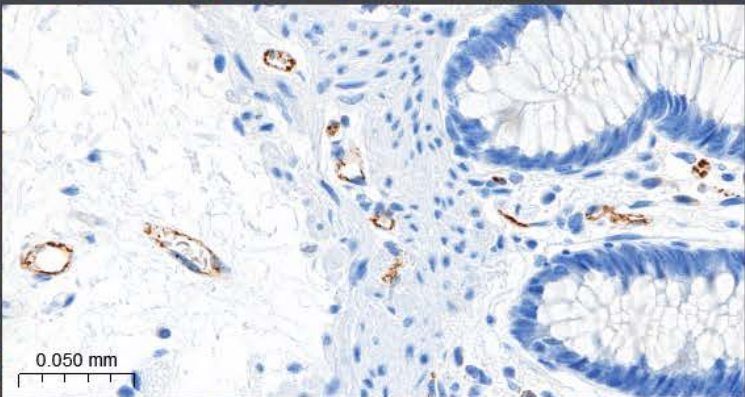
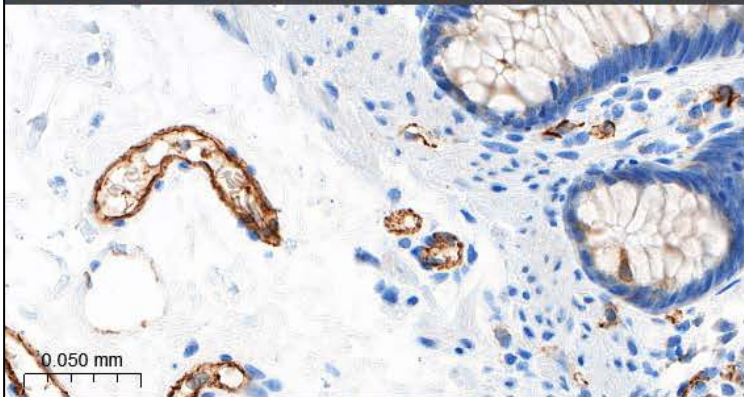
Control polyclonal anti-CD31

Recombinant monoclonal anti-CD31 Ab182981 (Abcam)

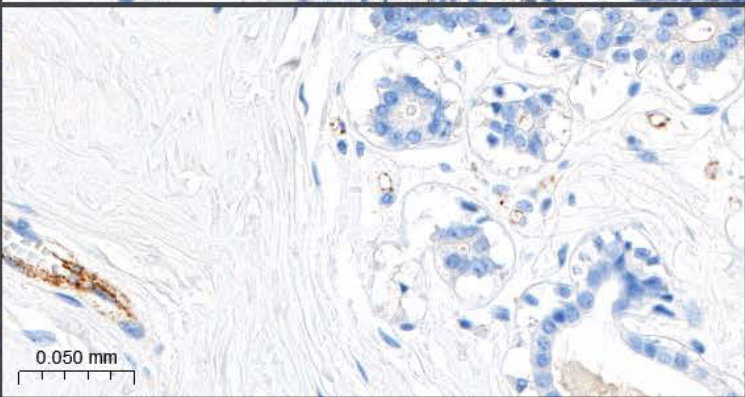
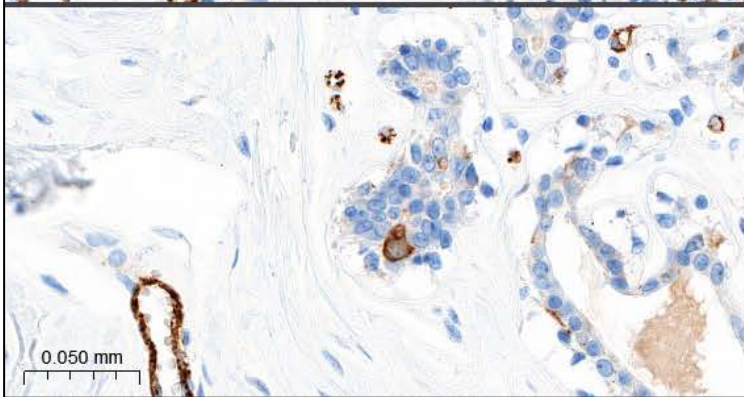
Liver



Colon

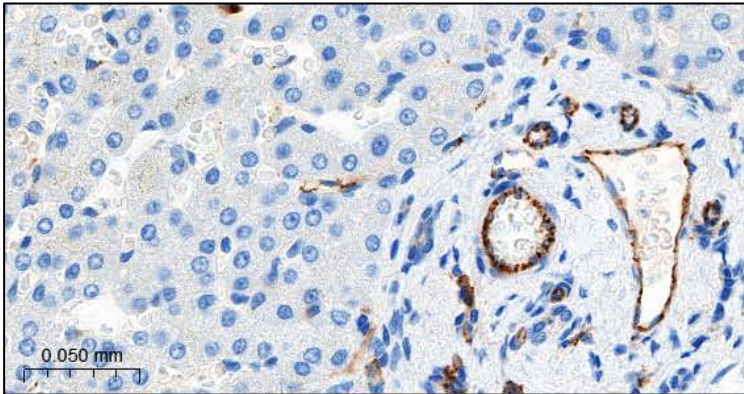
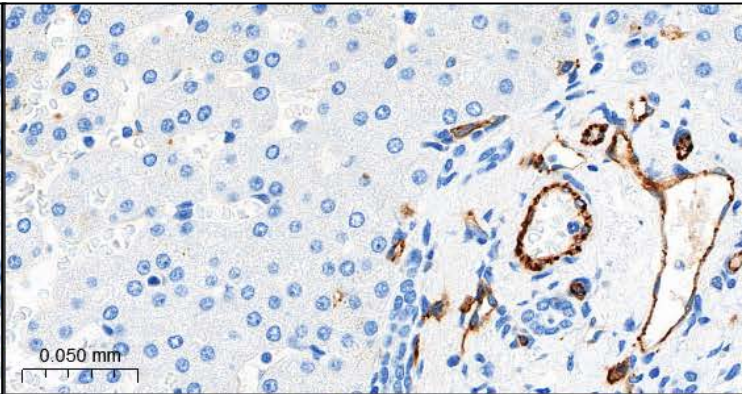
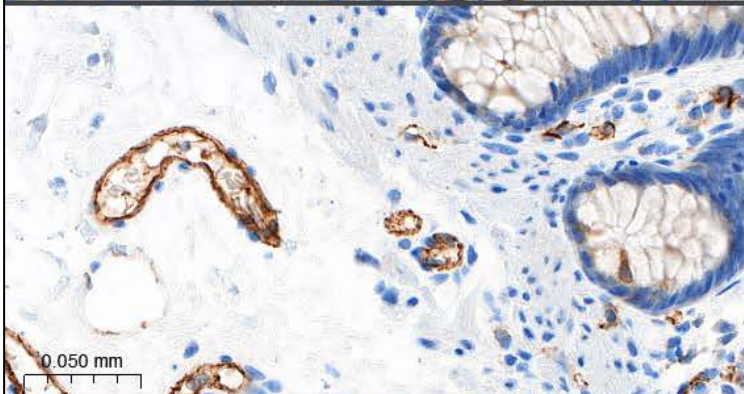
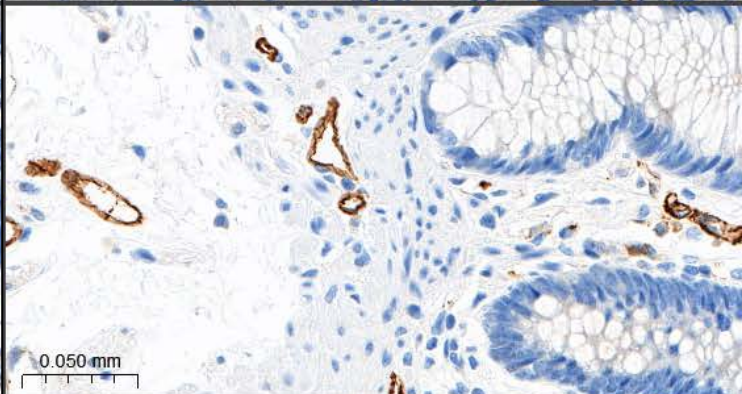
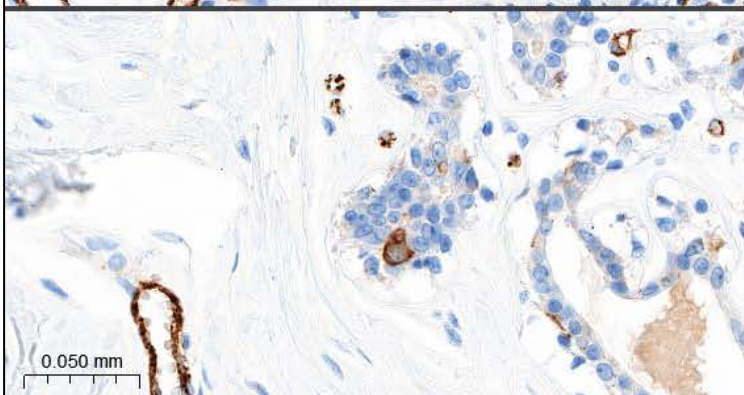
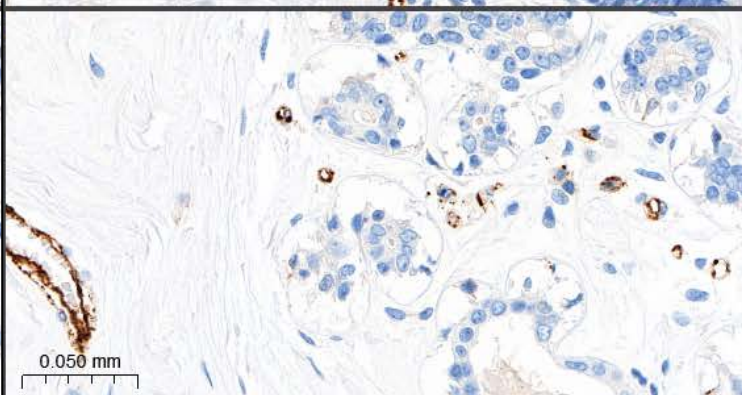


Adipose tissue



Validated conditions:

- Antigen retrieval : CC1 pH8, 24 min @95°C
- Dilution : 1/500 (ST)
- Incubation : 60 min @37°C
- Amplification : OmniMap Rabbit

	Control polyclonal anti-CD31	Recombinant multiclonal anti-CD31 Ab281583 (Abcam)
Liver		
Colon		
Adipose tissue		

Validated conditions:

- Antigen retrieval : CC1 pH8, 24 min @95°C
- Dilution : 1/500 (ST)
- Incubation : 60 min @37°C
- Amplification : OmniMap Rabbit

Materials and Methods

Experimental Strategy

The histology platform RHEM evaluated four recombinant Fc Rabbit anti-CD31 antibodies on formalin-fixed paraffin-embedded (FFPE) tissue sections from mouse (spleen, lung, liver), rat (spleen, lung, liver), and human (liver, adipose tissue, colon).

Initially, various conditions were tested on mouse tissues (liver, lung, spleen) for each antibody to determine optimal staining parameters. Once optimal conditions were established in mouse tissues, they were subsequently tested on rat and human tissues. The JC70 antibody was also tested on human tissue despite failing in mouse, as it was developed against a human antigen.

Antibody performance was assessed based on staining intensity, specificity, and background signal compared to a reference polyclonal anti-CD31 antibody used by the platform. Validation criteria included clear endothelial membrane staining with minimal background.

Biological Material

All formalin-fixed paraffin-embedded (FFPE) samples were already available on the platform. No animal has been sacrificed for these tests.

FFPE tissue sections were obtained from mouse (spleen, lung, liver), rat (spleen, lung, liver), and human (liver, adipose tissue, colon). Samples were collected and fixed 24h to 48h in neutral buffered formalin 10%, dehydrated, and embedded in paraffin. Paraffin-embedded tissue was cut into 3- μ m-thick sections, mounted on slides, then dried at 37°C overnight.

Antibody Testing

The tested rabbit recombinant monoclonal antibodies were :

- Anti-CD31 - [JC70] - Absolute Antibody - #Ab01338-23.0 - Stock concentration: 1 mg/mL
- Anti-CD31 - [AD217] - ABCD Antibodies - #ABCD_AD217 - Stock concentration: 79 μ g/mL
- Anti-CD31 - [EPR17259] - Abcam - #Ab182981 - Stock concentration: 549 μ g/mL

The tested rabbit recombinant mmulticlonal antibody was :

- Anti-CD31 - [RM1006] Abcam - #Ab281583 - Stock concentration: 505 μ g/mL

Immunohistochemistry was performed, as described previously (Rahmanzadeh, G. et al 2007), on a VENTANA Discovery Ultra automated staining instrument (Ventana Medical Systems), using VENTANA reagents, according to the manufacturer's instructions.

The recombinant anti-CD31 antibodies were tested under these conditions :

Slides were de-paraffinized. Then epitope retrieval was performed with :

1. **CC1 (pH 8)** (*Roche*, #05424569001) : Heat-induced epitope retrieval (HIER) at 95°C for 24 min,
2. **CC2 (pH 6)** (*Roche*, #05424542001) : HIER at 91°C for 24 min.

If none of these 2 conditions worked, two other conditions were tested :

3. No antigen retrieval
4. Protease 1 treatment (*Roche*, #05266688001) at 37°C for 4 min

Endogenous peroxidase was blocked with Discovery Inhibitor (*Roche*, #760-4840) for 8 min.

The antibodies were diluted at 1/100^e in Antibody Diluent (*Agilent*, *ST*, #S0809) or Antibody Diluent with Background Reducing (*Agilent*, *LB*, #S3022).

All antibodies were incubated for 60 min at 37°C.

Signal was enhanced using the OmniMap anti-rabbit detection kit (*Roche*, #05266548001) for 16 min, or for antibodies with a weak signal, Discovery HQ conjugated antibody anti-rabbit IgG (*Roche*, #07017812001) and Discovery amplification anti-HQ HRP Multimer (*Roche*, #06442544001) according to the manufacturer's instructions.

Slides were incubated with DAB (*Roche*, #05266645001) then counterstained with hematoxylin II (*Roche*, #790-2208) for 8 min, followed by Bluing reagent (*Roche*, #760-037) for 4 min. Slides were then dehydrated with Leica autostainer and coverslipped with Pertex mounting medium with CTM6 coverslipper (Microm).

A reference anti-CD31 polyclonal antibody (*Abcam* #Ab28364) was used as a positive control:

- Rabbit polyclonal
- Concentration: stock 0.013 mg/mL ; working concentrations of 0.17 µg/mL or 0.26 µg/mL (depending on the lot received)
- Validated under the following conditions: **CC1 (pH 8) 64 min at 95°C**, incubation at 37°C for 60 min, Hapten Multimer anti-Rabbit HQ and anti-HQ HRP detection (*Roche*, #7017812001 & 7017936001), DAB chromogen

Image Acquisition

Images were acquired using a scanner Panoramic MIDI II (3D Histech, MM France).

Results Summary

Two recombinant antibodies (Ab182981 and Ab281583) were validated in all three species. The JC70 antibody was validated in human tissue only. The ABCD_AD217 antibody did not produce specific staining under tested conditions.

Supplier / Reference	Clône	Epitope retrieval	Dilution	Amplification
Absolute Antibody #ab01338-23.0	JC70	CC2 – 24 min – 91°C	1/300 ^e Diluent LB	Rabbit HQ
Abcam #ab182981	EPR17259	CC1 - 24 min - 95°C	1/500 ^e Diluent ST	OmniMap Rabbit
Abcam #ab281583	RM1006	CC1 - 24 min - 95°C	1/500 ^e Diluent ST	OmniMap Rabbit

Recombinant antibodies significantly reduced the per-slide cost (1.34€/slide) compared to the polyclonal reference antibody (8.57€/slide), representing up to a six-fold cost reduction.