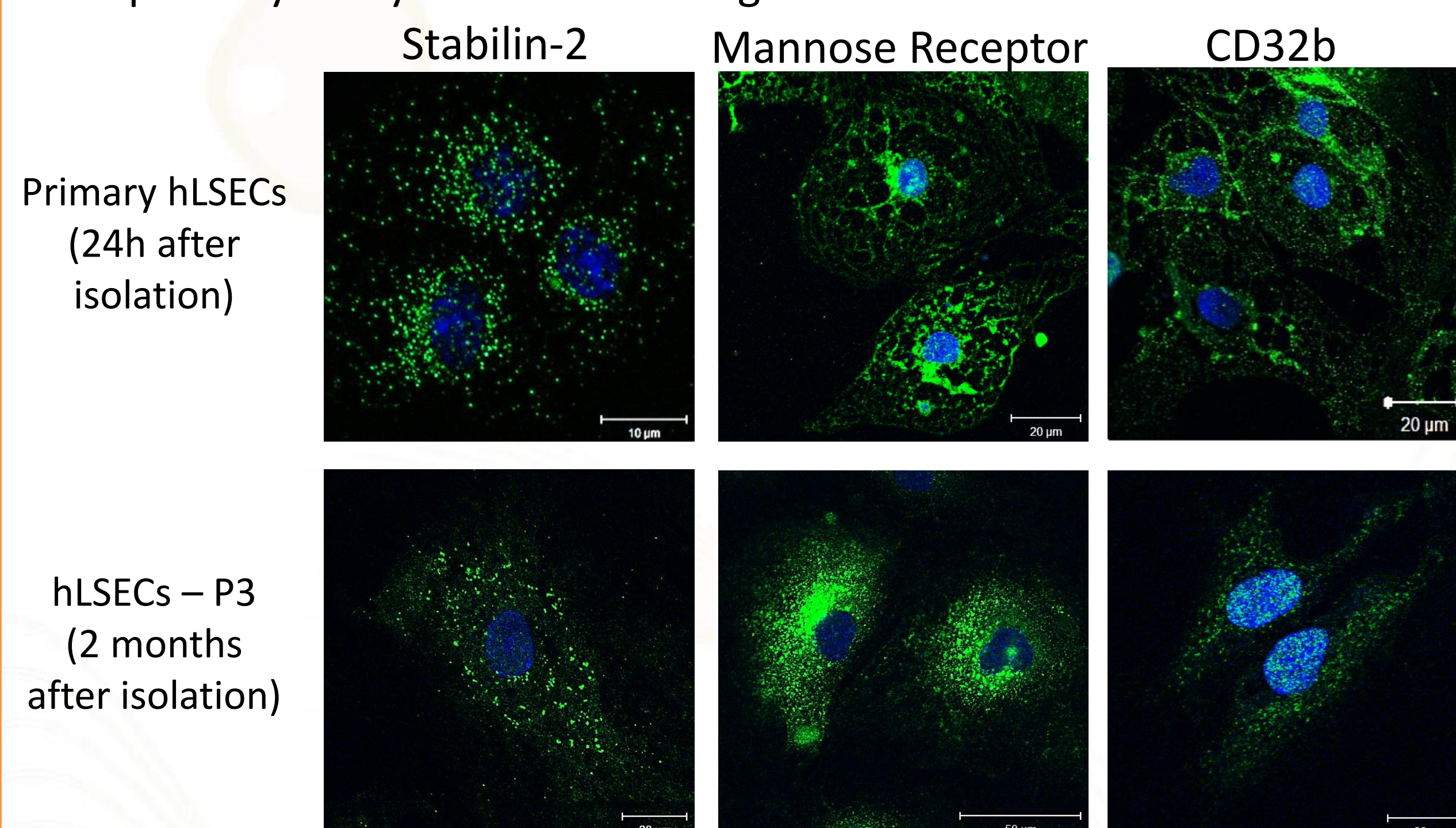


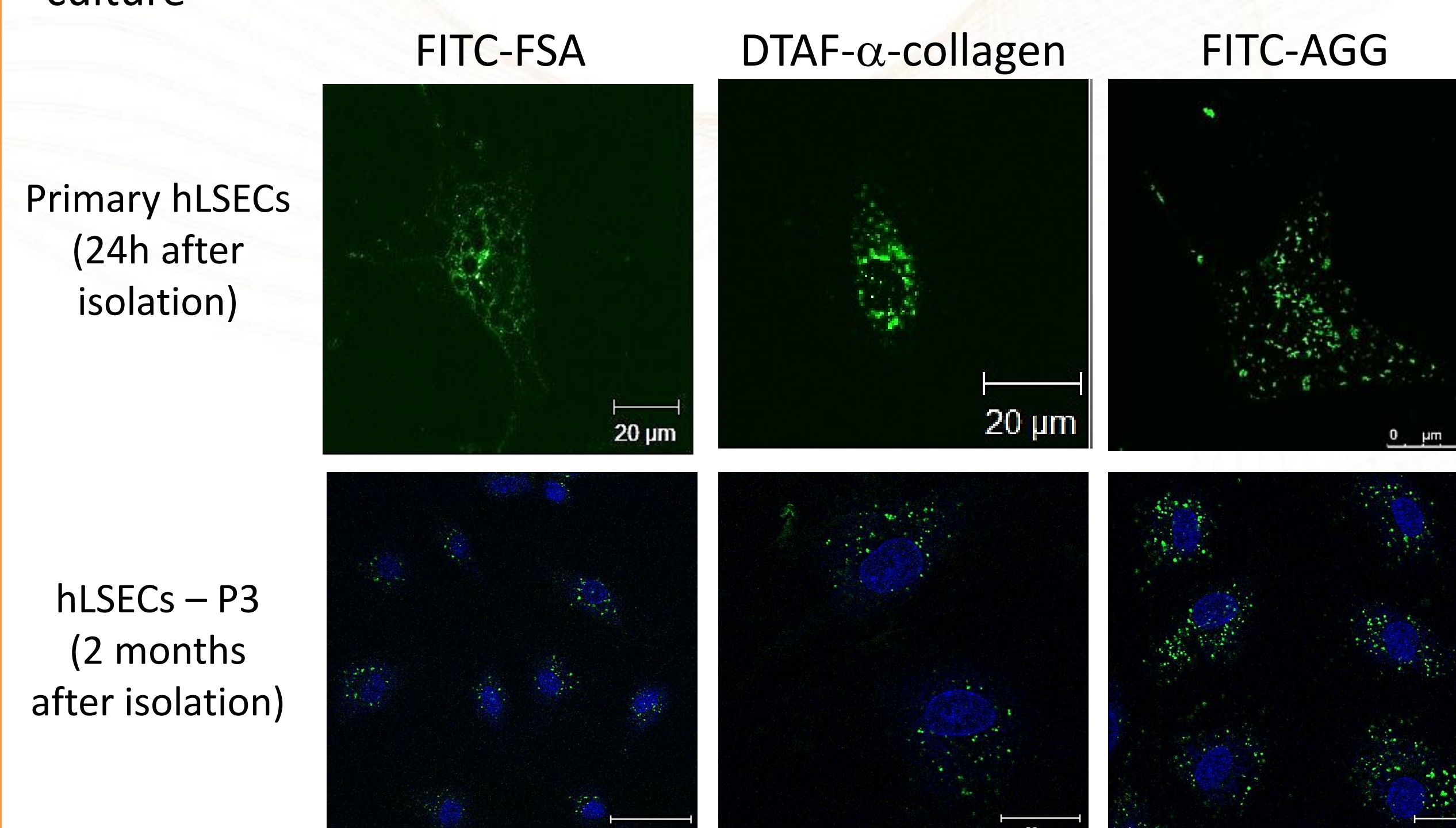
## INTRODUCTION

The liver is the central organ of drug metabolism and consequently most prone to toxicity. Human hepatocytes and hepatocyte-like cells are generally used for toxicity assessment. However, a more complex system mimicking the intact human liver would be a more relevant device for such tests. We aim to create a hepatic microfluidic bioreactor (HeMiBio), a 3D liver-simulating device that will incorporate both hepatocytes and non-parenchymal cells. Our group, one of the 12 partners involved in the HeMiBio project, works towards establishing the methods for isolation and characterization of primary human liver sinusoidal endothelial cells (hLSECs). These cells represent a specialized type of scavenger endothelium lining the liver sinusoids that use receptor-mediated endocytosis to remove numerous physiological and non-physiological soluble macromolecules and colloids from the blood circulation (1).

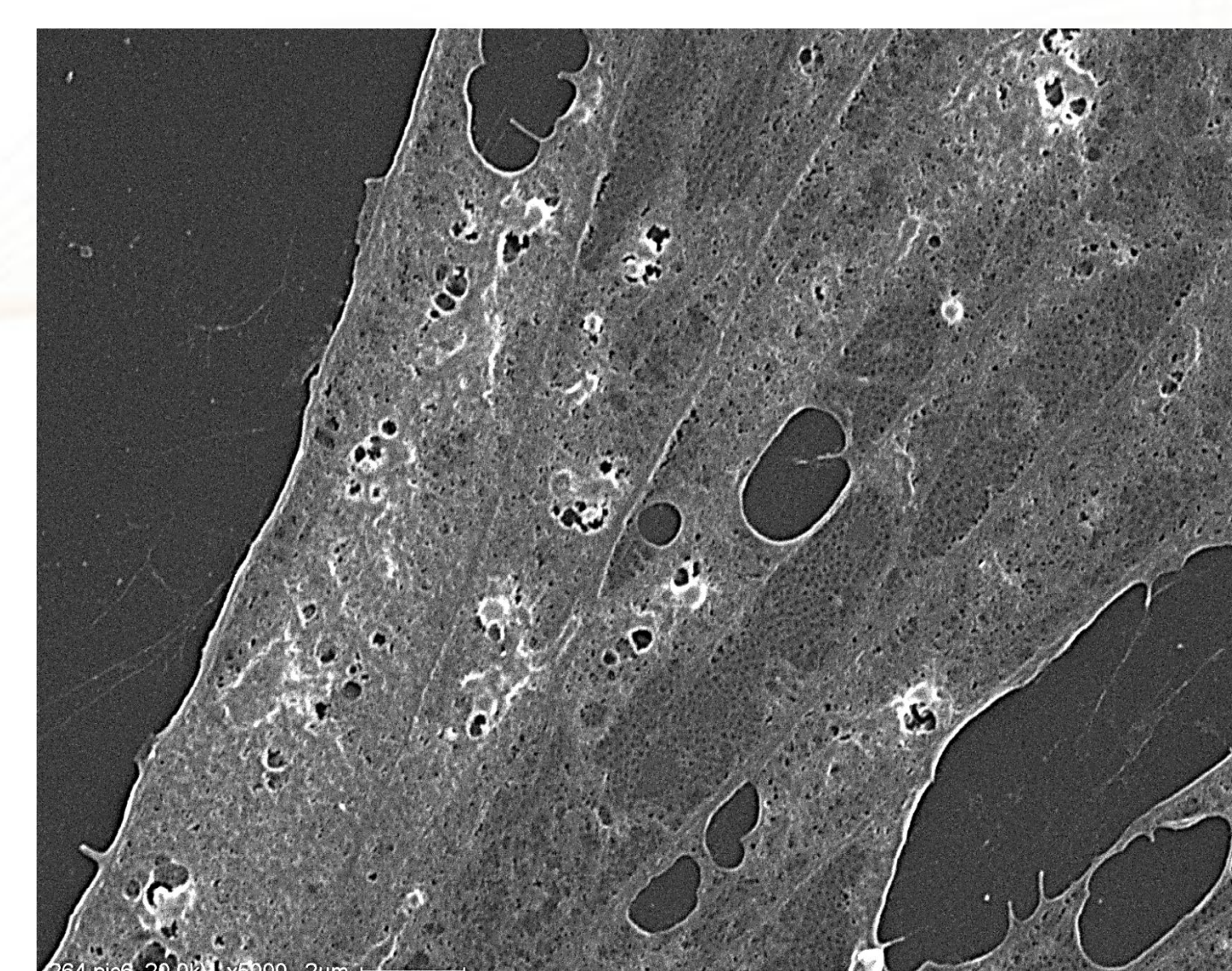
**Figure 3.** Immunofluorescence shows expression of scavenger receptors by freshly isolated and long-term cultivated hLSECs



**Figure 4.** The scavenger function of hLSECs as monitored by incubation with specific ligands is preserved in cells up to 2 months in culture



**Figure 5.** The signature feature of LSECs (fenestrations) is preserved in human LSECs for up to 1 month in culture, as shown by scanning EM



## CONCLUSION

We purified and characterized the hLSECs with regard to their specific morphological features and scavenger functions for up to 1 month in culture. This provides a good basis for further testing in 2D and 3D co-cultures with hepatocytes and stellate cells.

## REFERENCES

1. Seternes T et al., Proc Natl Acad Sci USA, 2002.99:7594-7

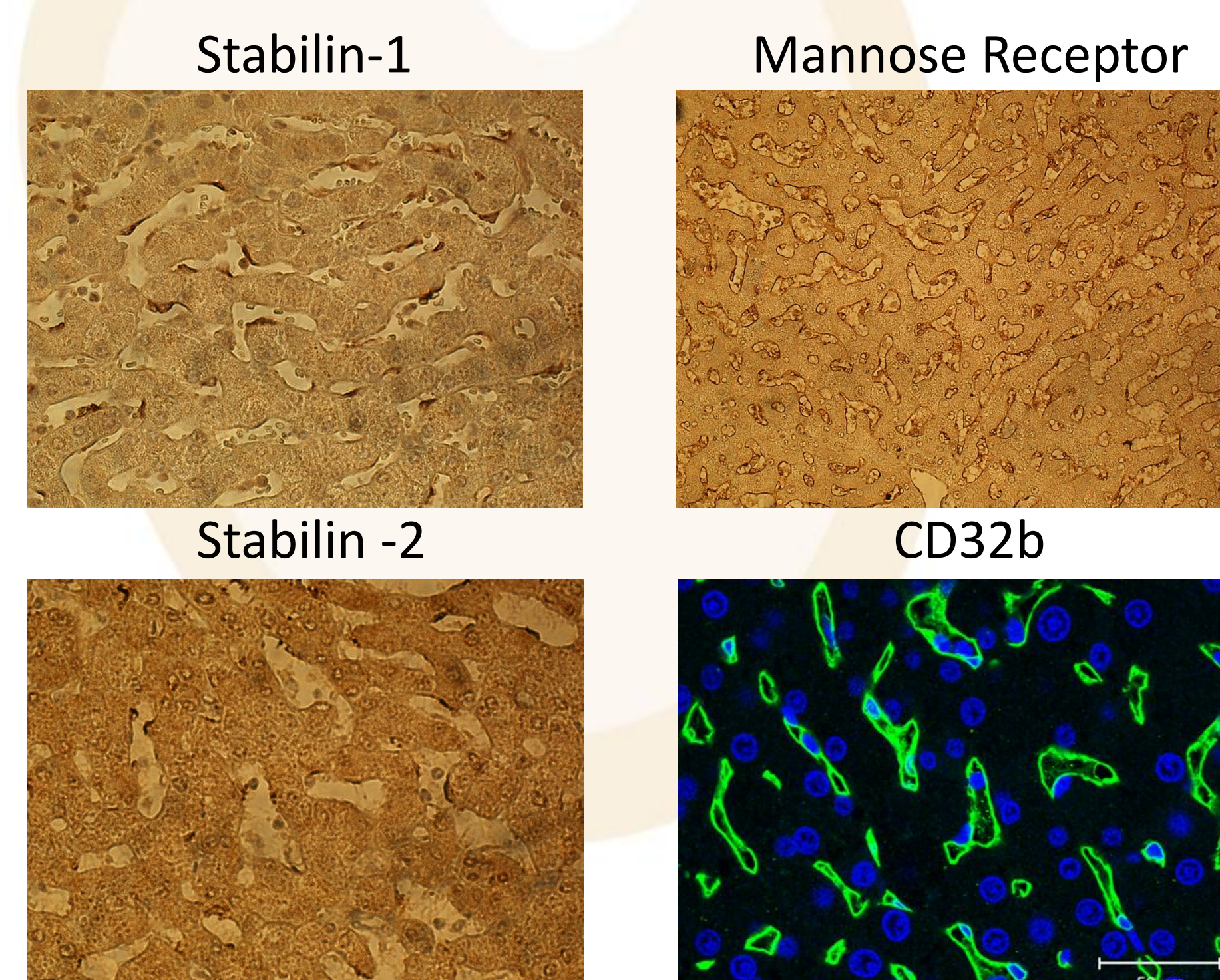
HeMiBio is jointly funded by the European Commission within its FP7 Programme and COLIPA, the European Cosmetics Association, as part of the SEURAT cluster Contract number HEALTH-F5-2010-266777

## MATERIALS & METHODS

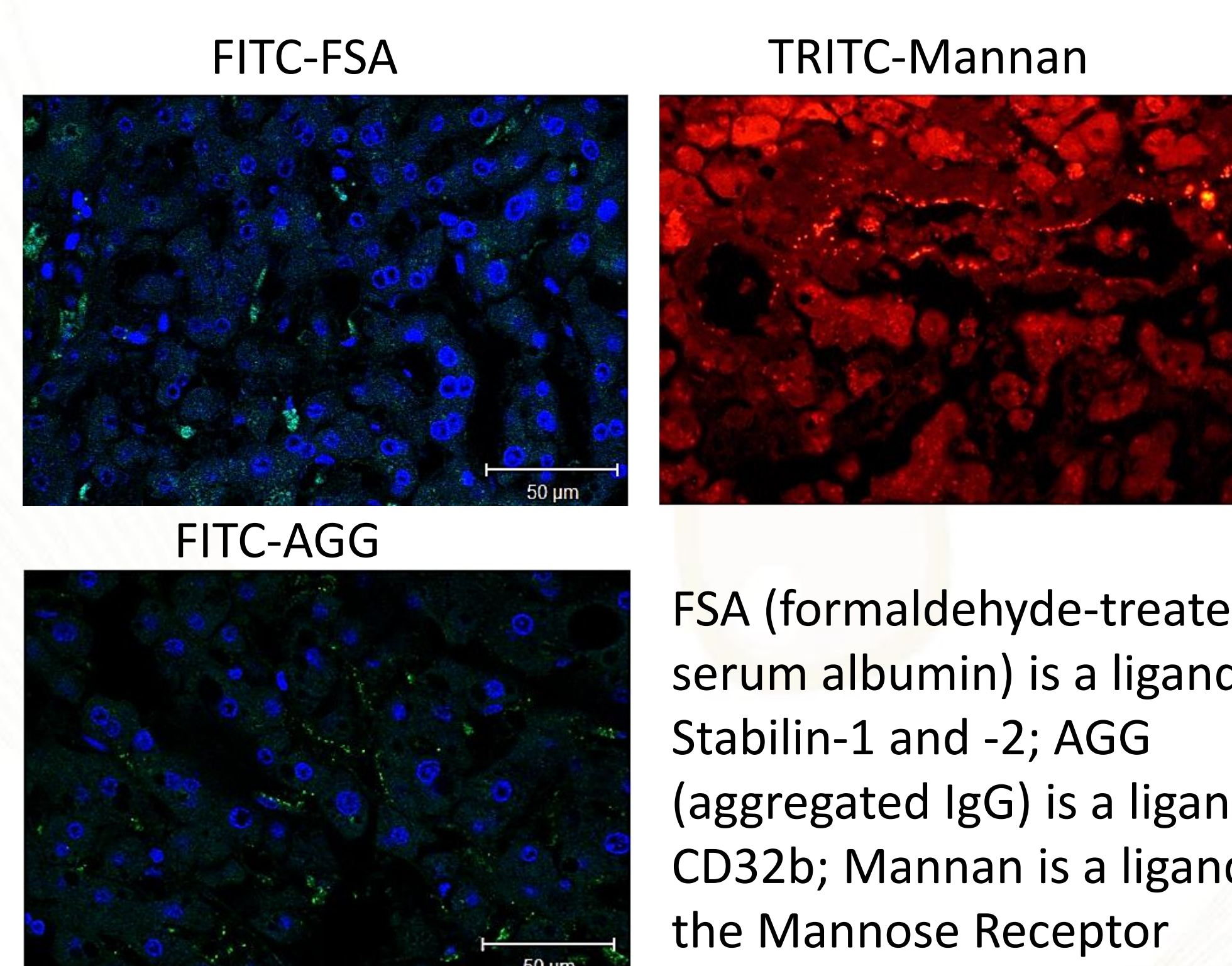
Primary hLSECs were isolated from human liver resections obtained from the University Hospital of Northern Norway (Tromsø) and the National Hospital (Oslo). Collagenase digestion, density gradient centrifugation, and CD32b positive immunomagnetic selection were used for purification of the cells. hLSECs were propagated on collagen-coated dishes in culture medium containing foetal bovine serum, HGF and VEGF. Immunohistochemistry, immunofluorescence, *ex vivo* and *in vitro* endocytosis of specific scavenger receptor ligands, and scanning electron microscopy (SEM) were used for morphological and functional characterization of the cells.

## RESULTS

**Figure 1.** Human liver sinusoids showed positive staining for LSEC-specific scavenger receptors



**Figure 2.** *Ex vivo* i.v. injection of ligands for LSECs endocytosis receptors accumulated exclusively in cells lining the liver sinusoids



FSA (formaldehyde-treated serum albumin) is a ligand for Stabilin-1 and -2; AGG (aggregated IgG) is a ligand for CD32b; Mannan is a ligand for the Mannose Receptor