

Immune exhaustion and inflammatory regulation of disease progression

Frank Tacke

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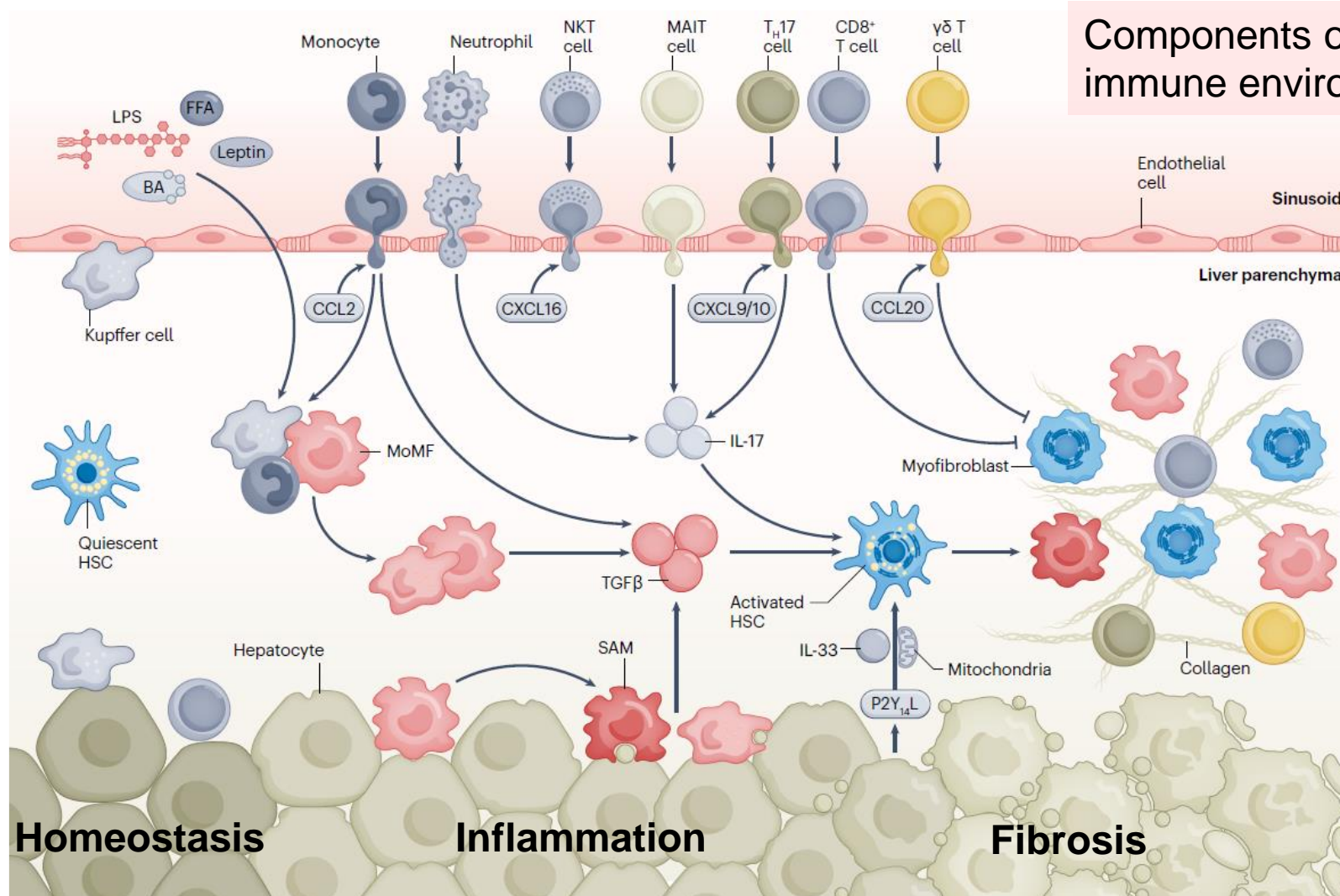
Paris MASH Meeting
Paris, France, Sept 5, 2024

Disclosures Frank Tacke

- *Research support:*
AstraZeneca, MSD, Gilead, Agomab
- *Speaker/Consulting:*
Novo Nordisk, AstraZeneca, Gilead, Abbvie, Alnylam, BMS, Intercept, Falk, Inventiva, MSD, Madrigal, Pfizer, Novartis, Merz, Sanofi, GSK, Orphalan
- Coordinator of the German NAFLD/MASLD guideline (DGVS),
Coordinator of the European MASLD guideline (EASL-EASD-EASO)

From chronic injury to inflammation and fibrosis

- Activation of resident (immune) cells
- Recruitment of inflammatory cells
- Intense cellular crosstalk between immune cells, parenchymal and non-parenchymal cells

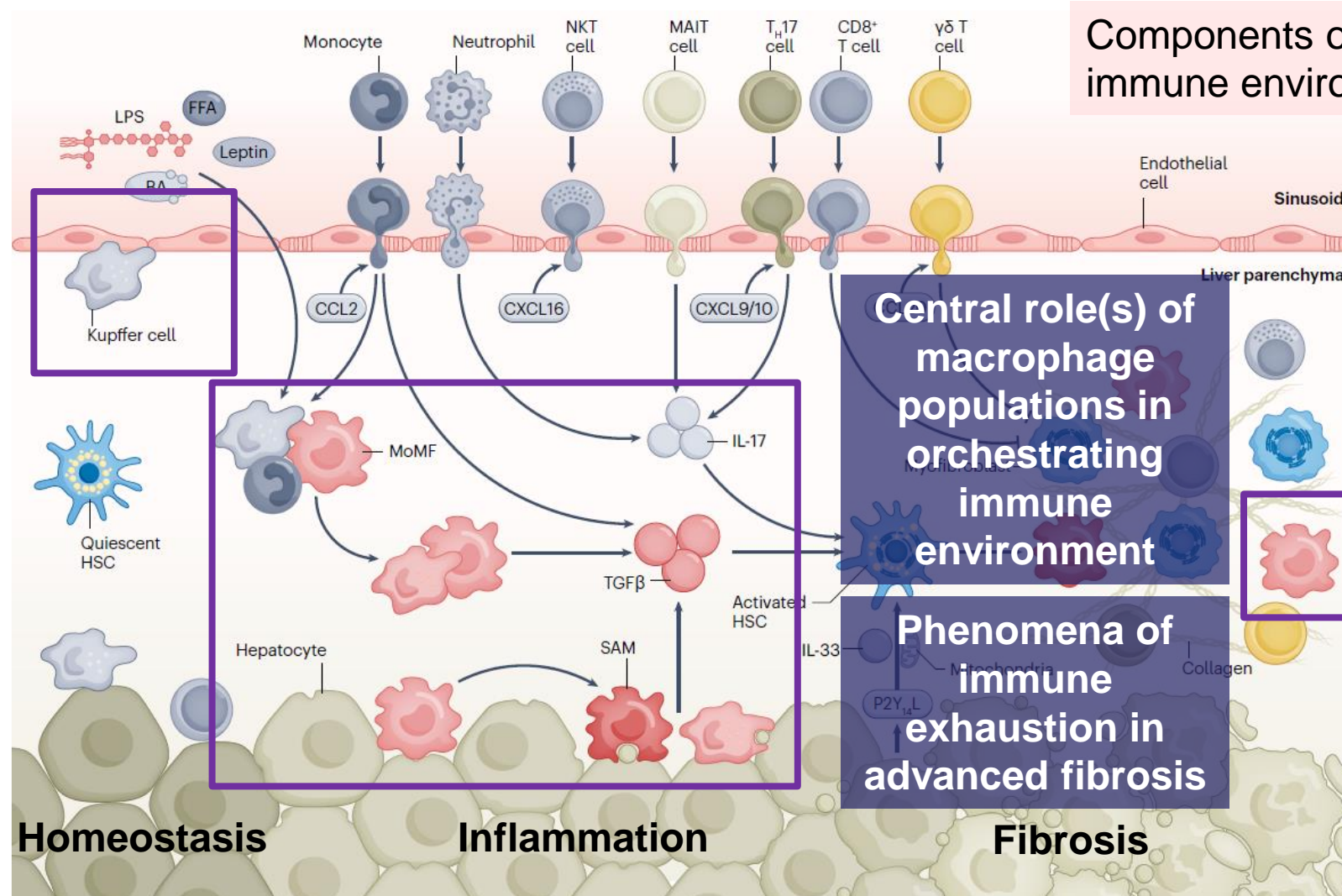


- Activation of hepatic stellate cells
- Deposition of extracellular matrix
- Deactivation of repair / regenerative pathways

Hammerich L, Tacke F. *Nat Rev Gastroenterol Hepatol.* 2023;20(10):633-646

From chronic injury to inflammation and fibrosis

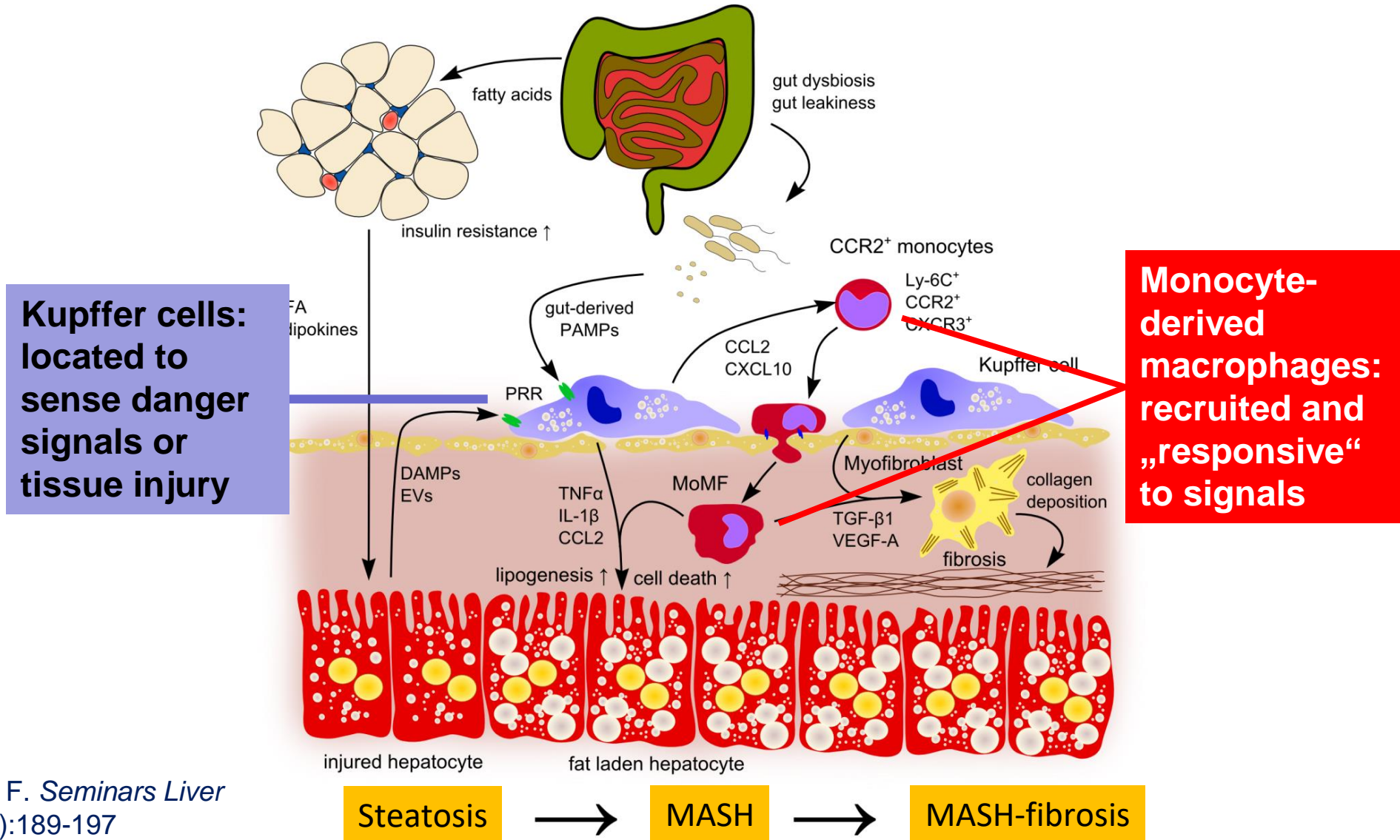
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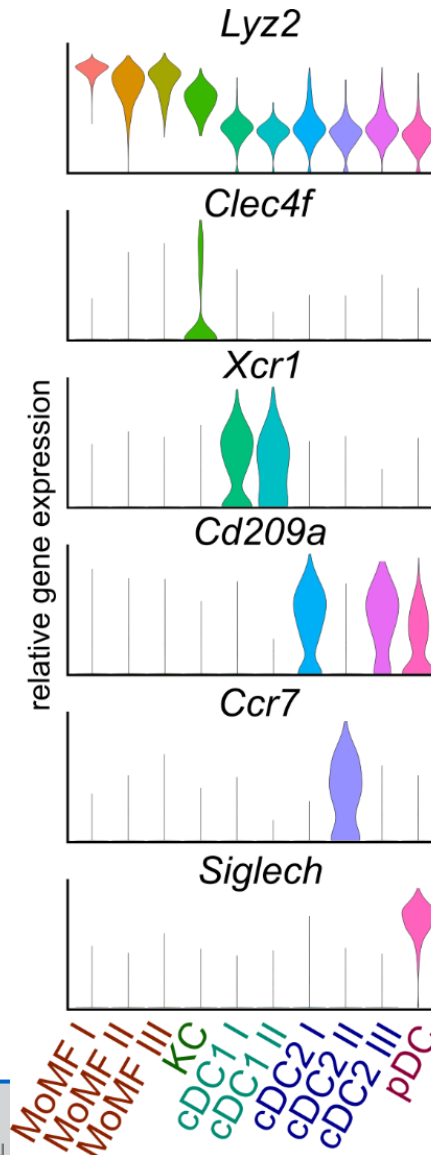
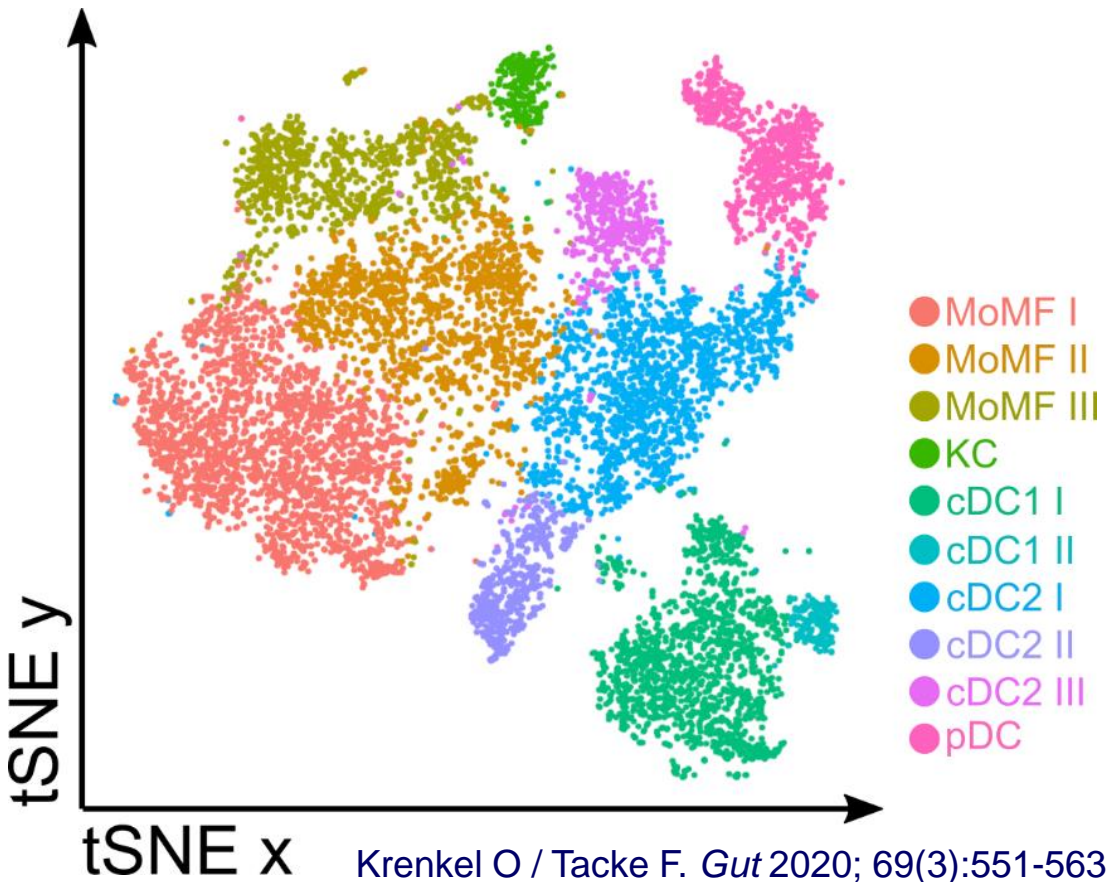
Macrophage heterogeneity in the liver (example: MASLD)



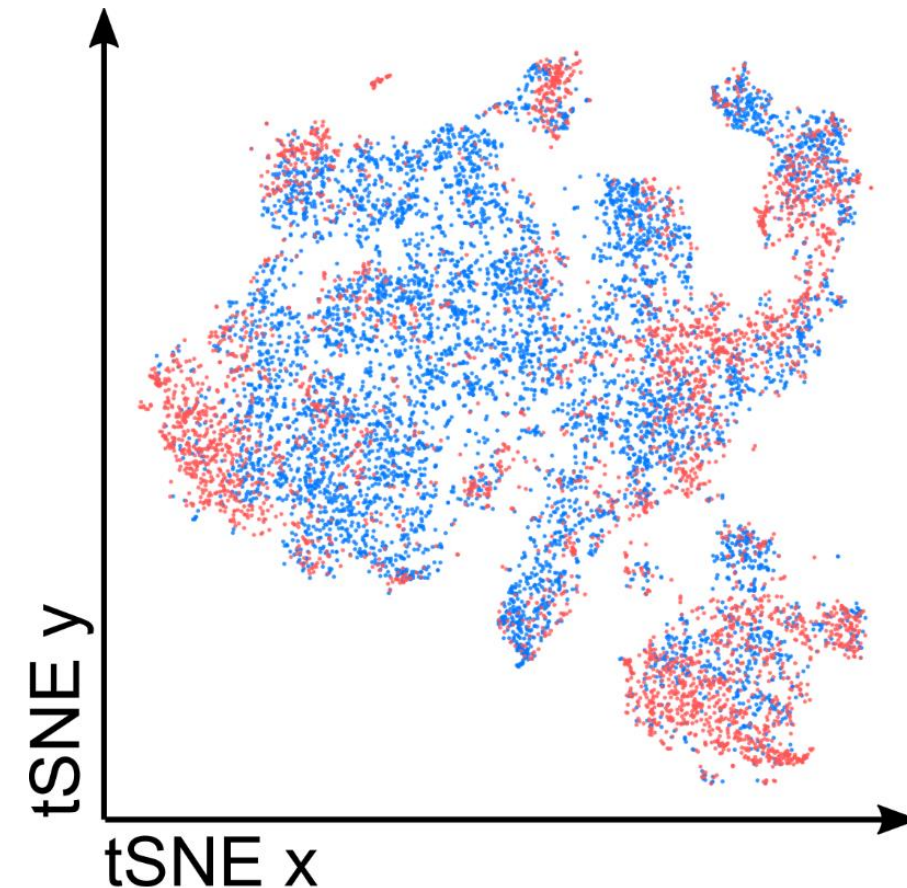
Krenkel O & Tacke F. *Seminars Liver Disease* 2017;37(3):189-197

The single-cell seq view: Liver macrophage heterogeneity

Liver non-lymphoid CD45^{pos} Ly6G^{neg}



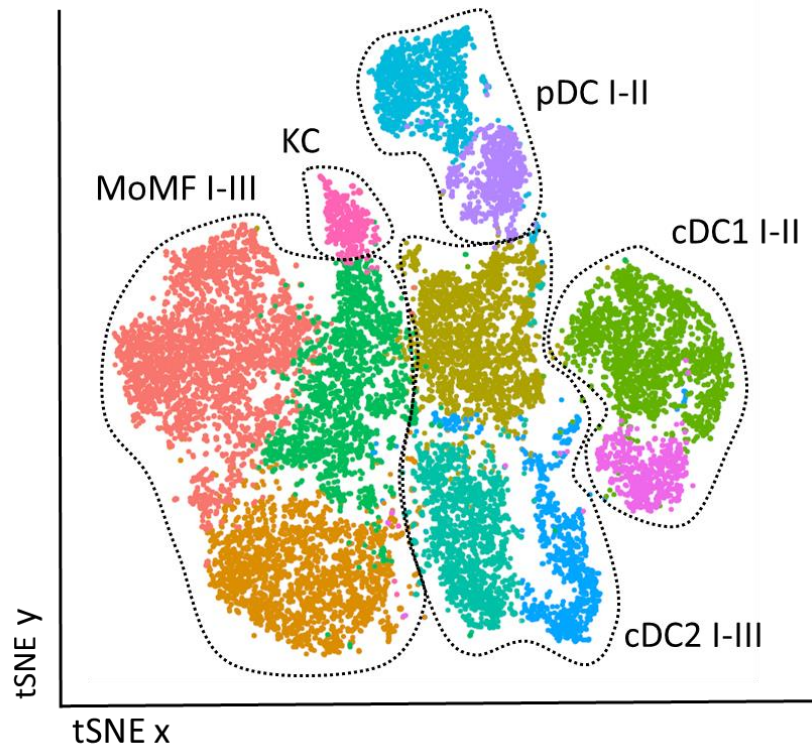
chow diet
Western diet



ScRNAseq: Liver macrophage heterogeneity across models

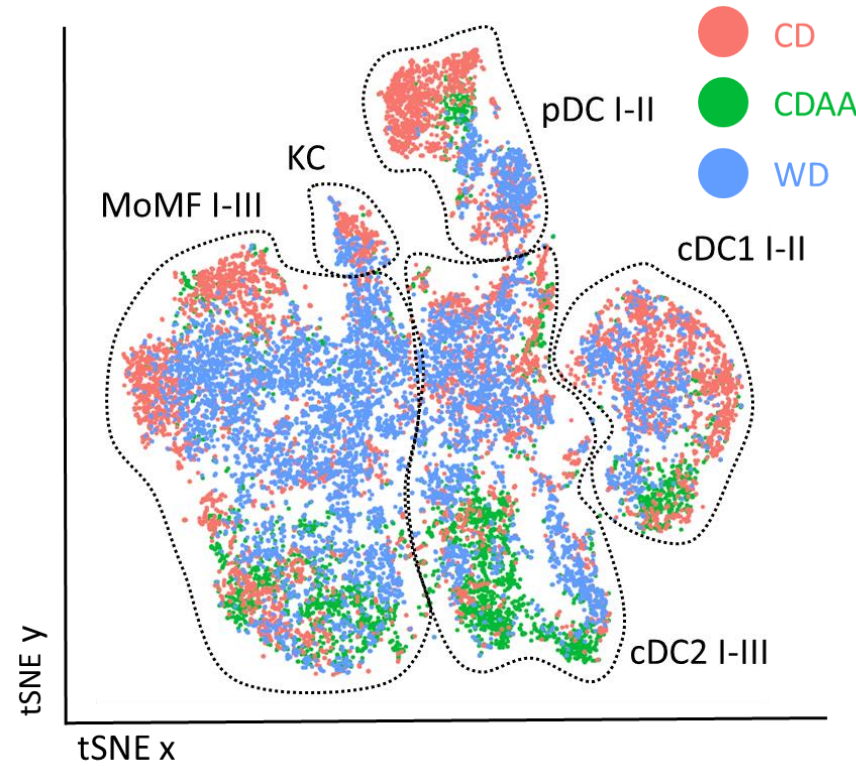
Liver

Ly6G^{neg} non-lymphoid CD45^{pos} cells

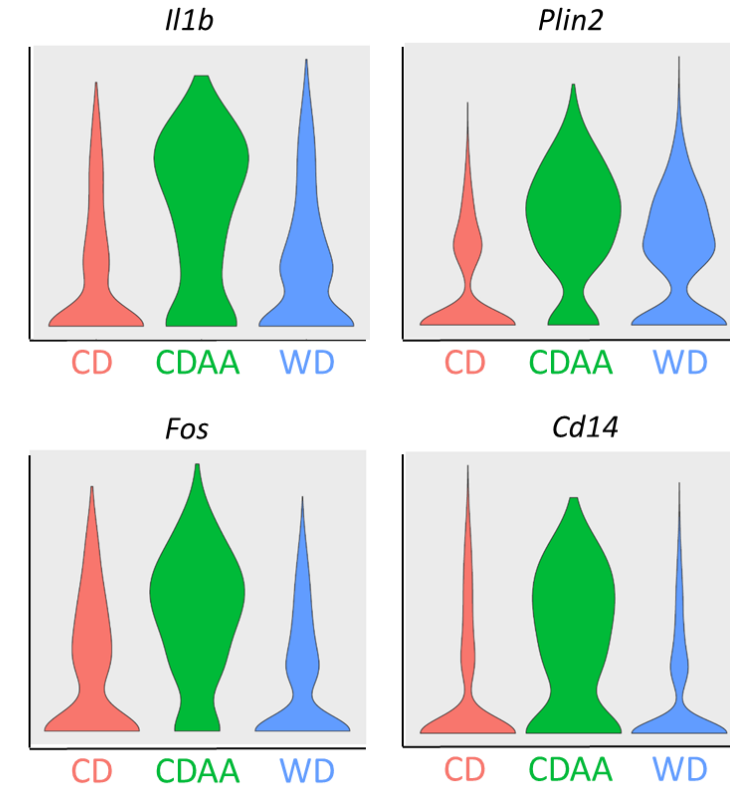


Liver

Ly6G^{neg} non-lymphoid CD45^{pos} cells



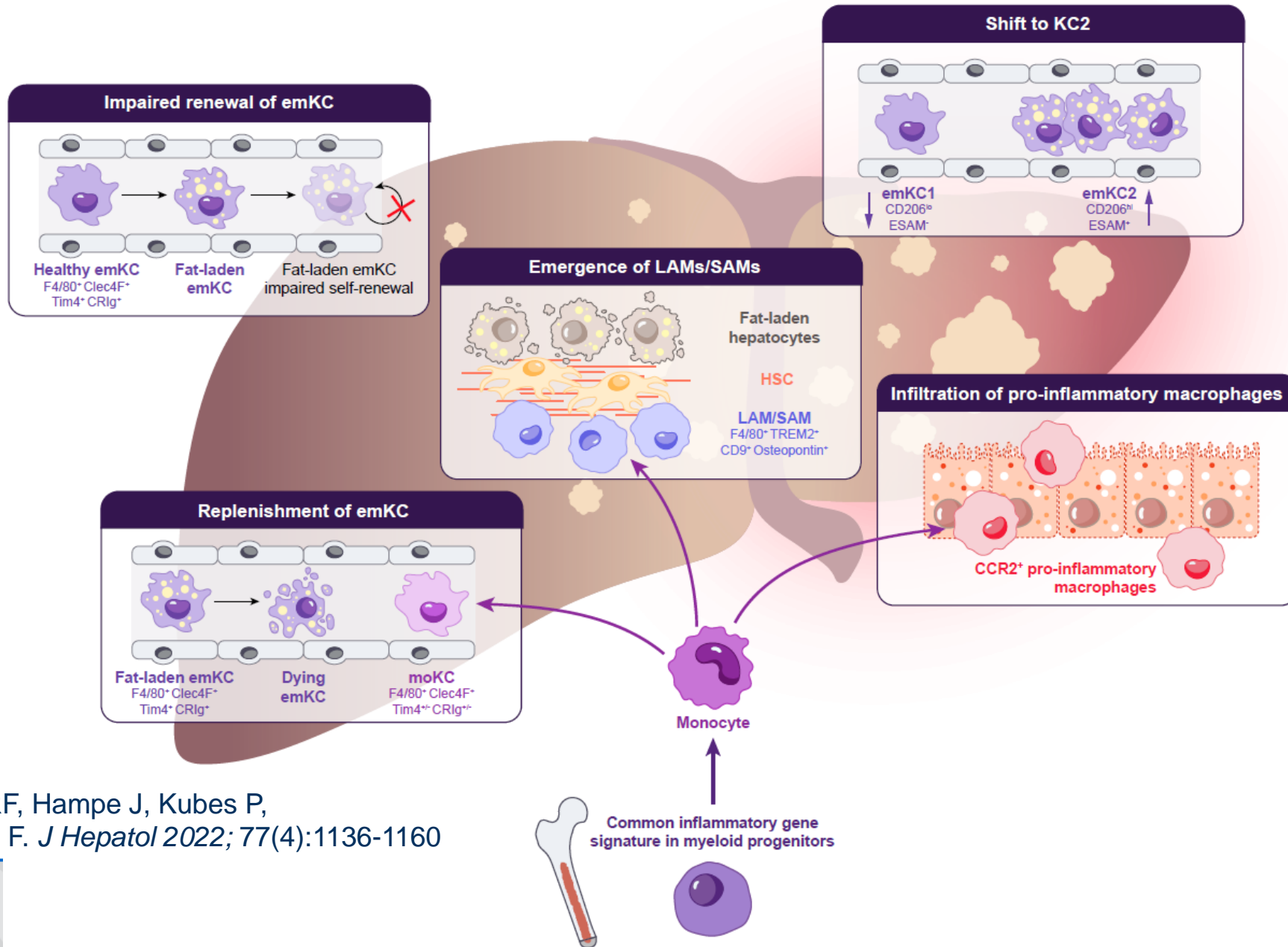
relative gene expression



Hundertmark J / Berger H / Tacke F. *unpublished data*

Macrophage diversity in MASLD & fibrosis (single-cell data)

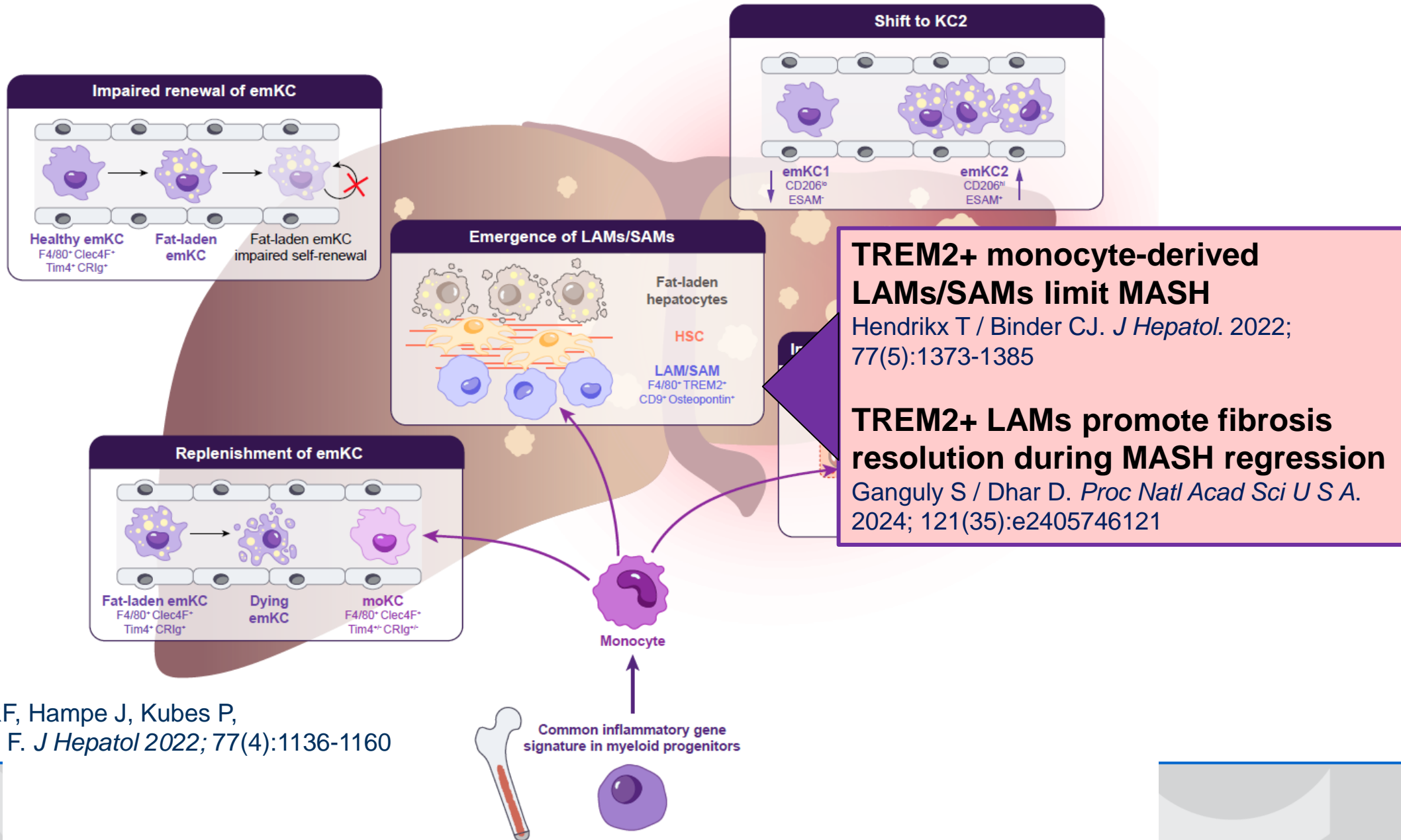
KC: Kupffer cell
LAM: lipid-associated
macrophage
SAM: scar-associated
macrophages



Peiseler M, Schwabe RF, Hampe J, Kubes P,
Heikenwälder M, Tacke F. *J Hepatol* 2022; 77(4):1136-1160

Macrophage diversity in MASLD & fibrosis (single-cell data)

KC: Kupffer cell
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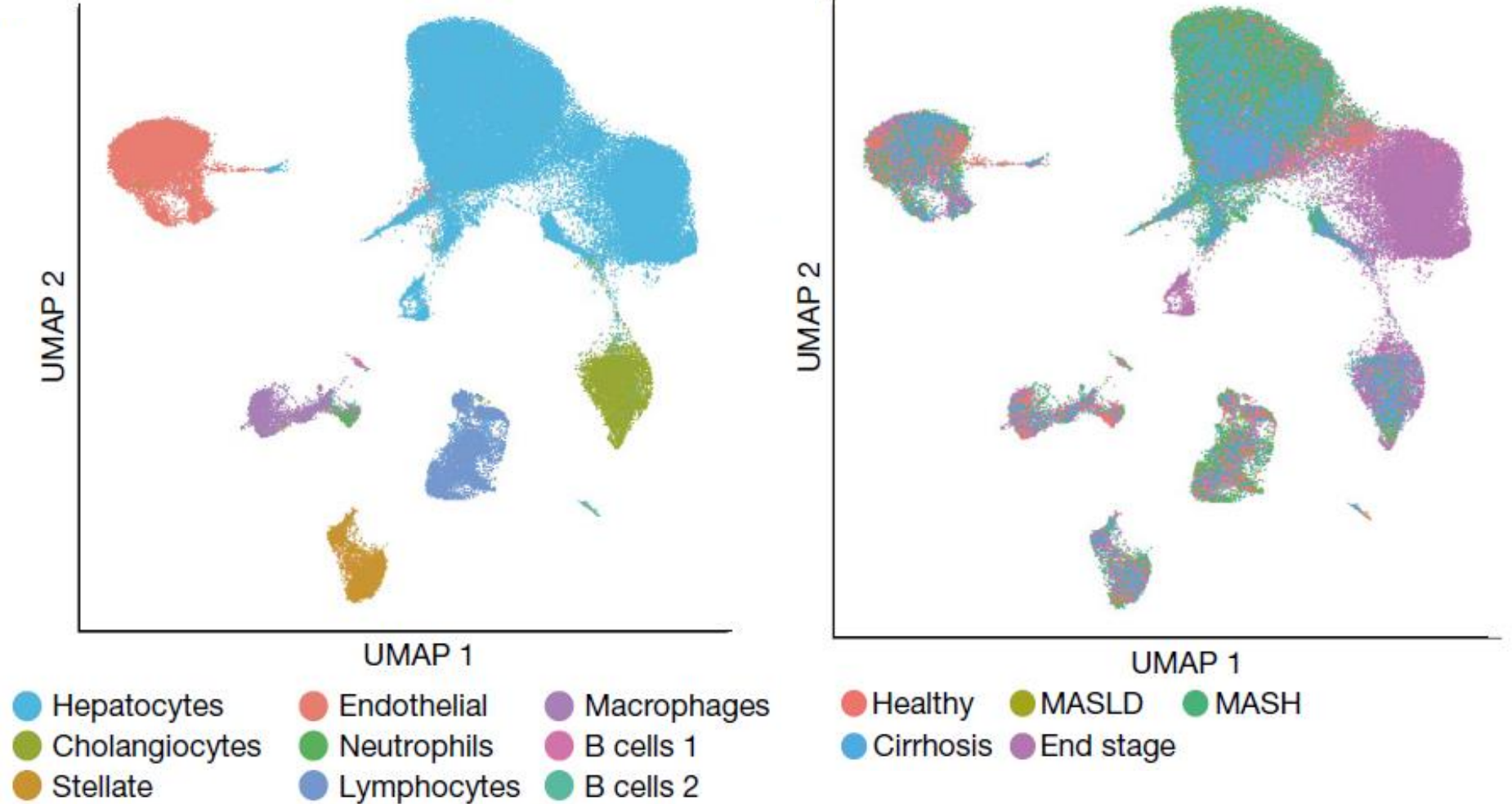
Peiseler M, Schwabe RF, Hampe J, Kubes P, Heikenwälder M, Tacke F. *J Hepatol* 2022; 77(4):1136-1160

Immune cell composition in human liver

Single nuclei RNA seq (Cambridge)

n=4 healthy
n=7 MASL
n=27 MASH
n=4 cirrhosis
n=5 decompensated

~100,000 nuclei from
n=47 liver biopsies /
resections / explants



Gribben C, ... Tacke F, ... Vallier L. *Nature*. 2024; 630(8015):166-173

Spatial immune cell composition in human liver

Multiplex immunostaining:

Multiparametric (>15) histological analyses, flexible panel design, tissue morphology, cell subpopulation location, and cell-to-cell interaction *in situ*

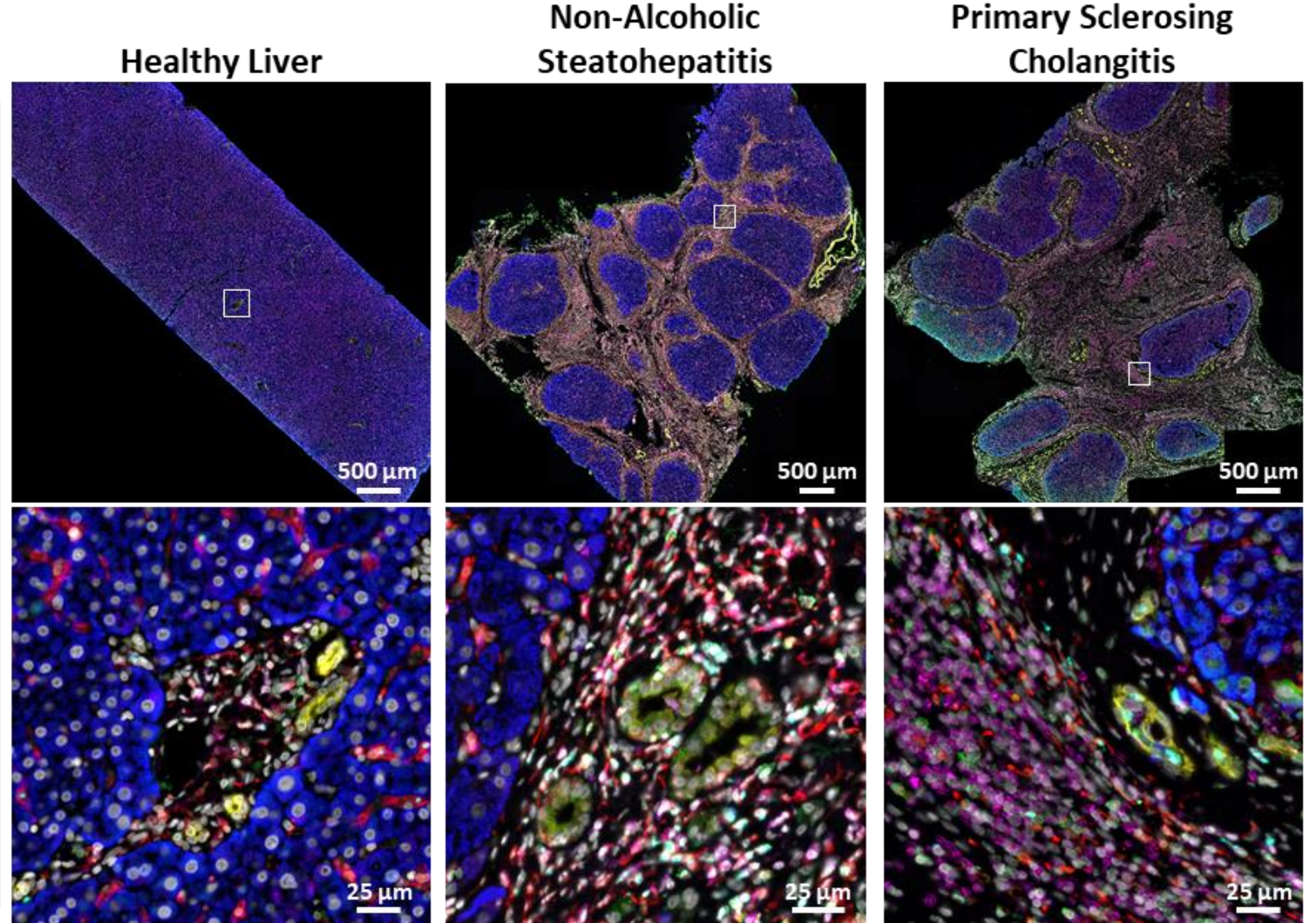
Practical applications (our collaborations):

Feng D, et al. *J Clin Invest.* 2023; 133(15):e166954
Ma J, et al. *J Clin Invest.* 2022;132(14):e157780
Dudek M, et al. *Nature.* 2021; 592(7854):444-449.
Günes Günsel G, et al. *Nat Commun.* 2022;13(1):1303
Guillot A, et al. *J Clin Invest.* 2021; 131(9):e132305.
Conlon TM, et al. *Nature.* 2020; 588(7836):151-156.
Lefere S, et al. *J Hepatol.* 2020; 73(4):757-770.

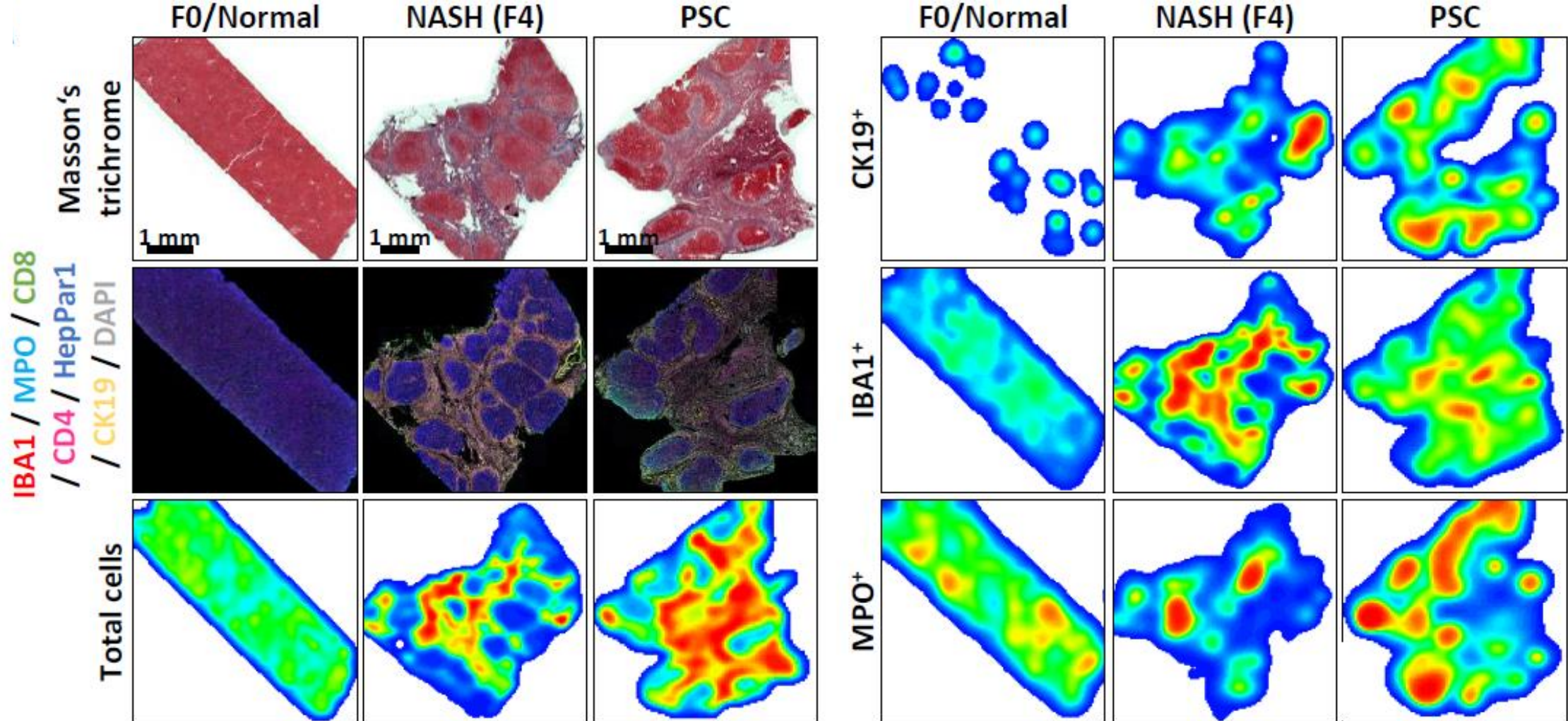
Protocol:

Guillot A, et al. *Cancers.* 2020; 12(9):2449
Guillot A, et al. *Methods Mol Biol.* 2023; 2669:245-255

IBA1 / MPO / CD8 / CD4 / HepPar1 / CK19 / DAPI

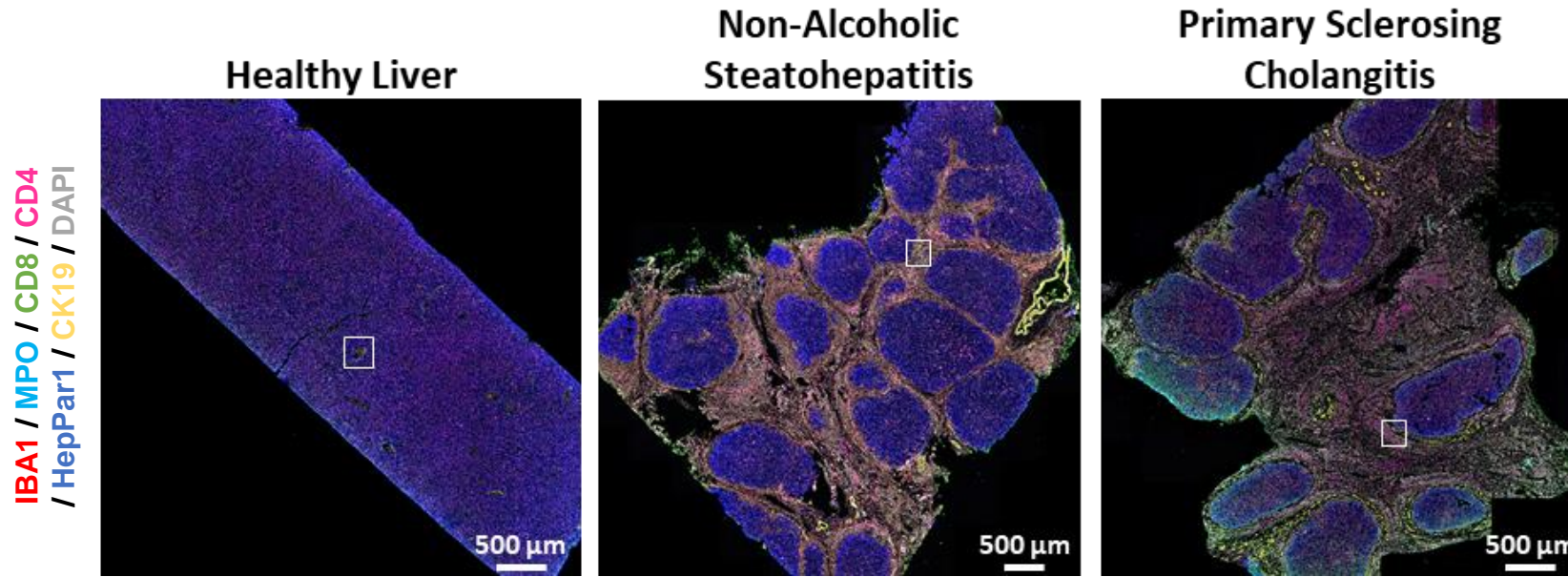


Spatial immune cell composition in human liver



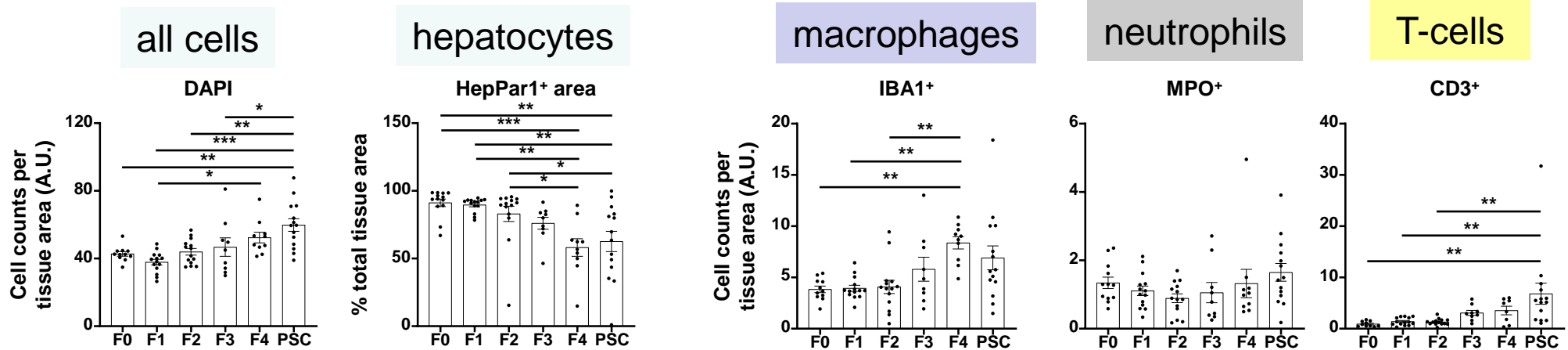
Guillot A ... Tacke F, *Hepatology* 2023; 78(1):150-166

Spatial immune cell composition in human liver

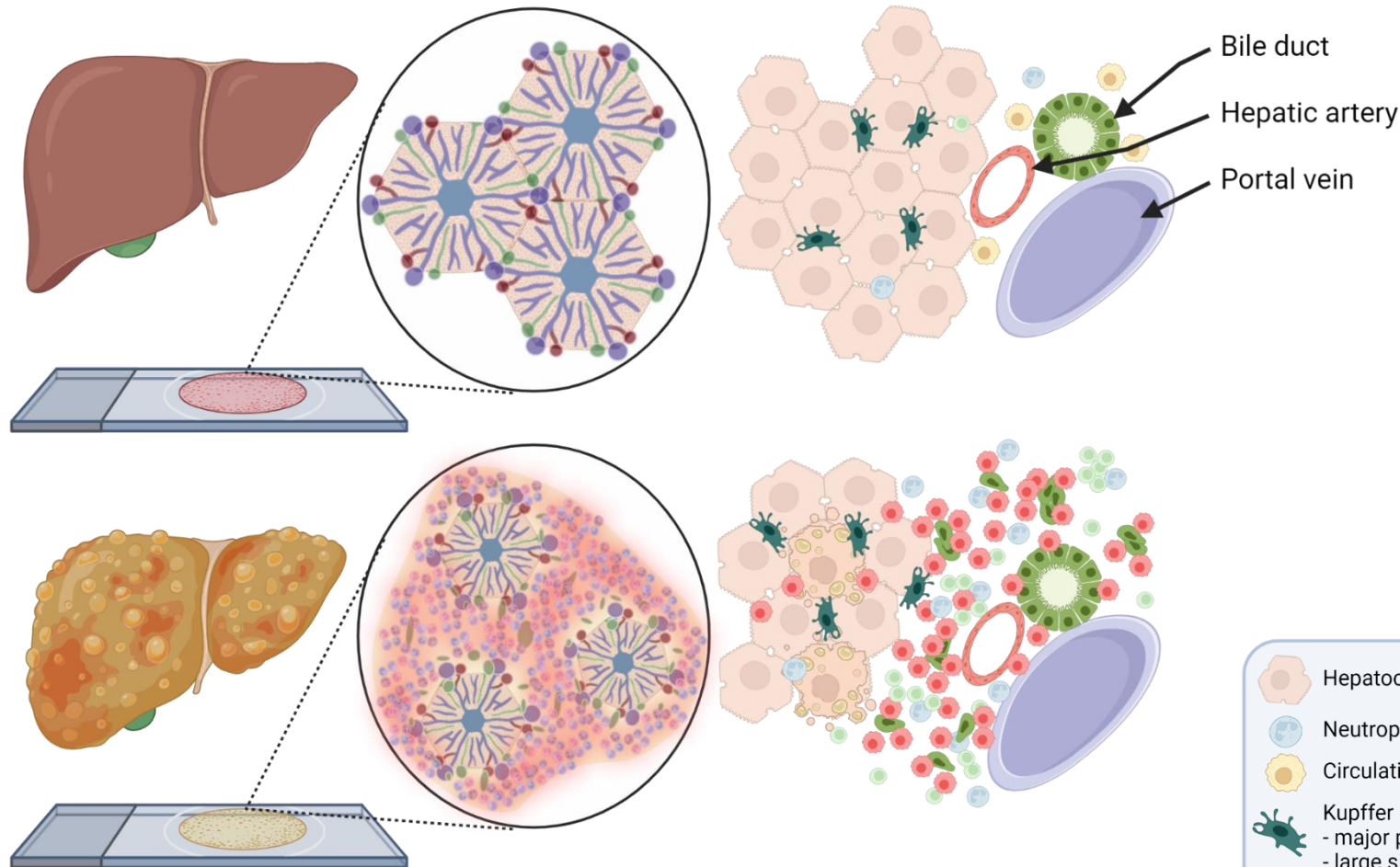


MASH:
macrophages!









PSC: T-cells!



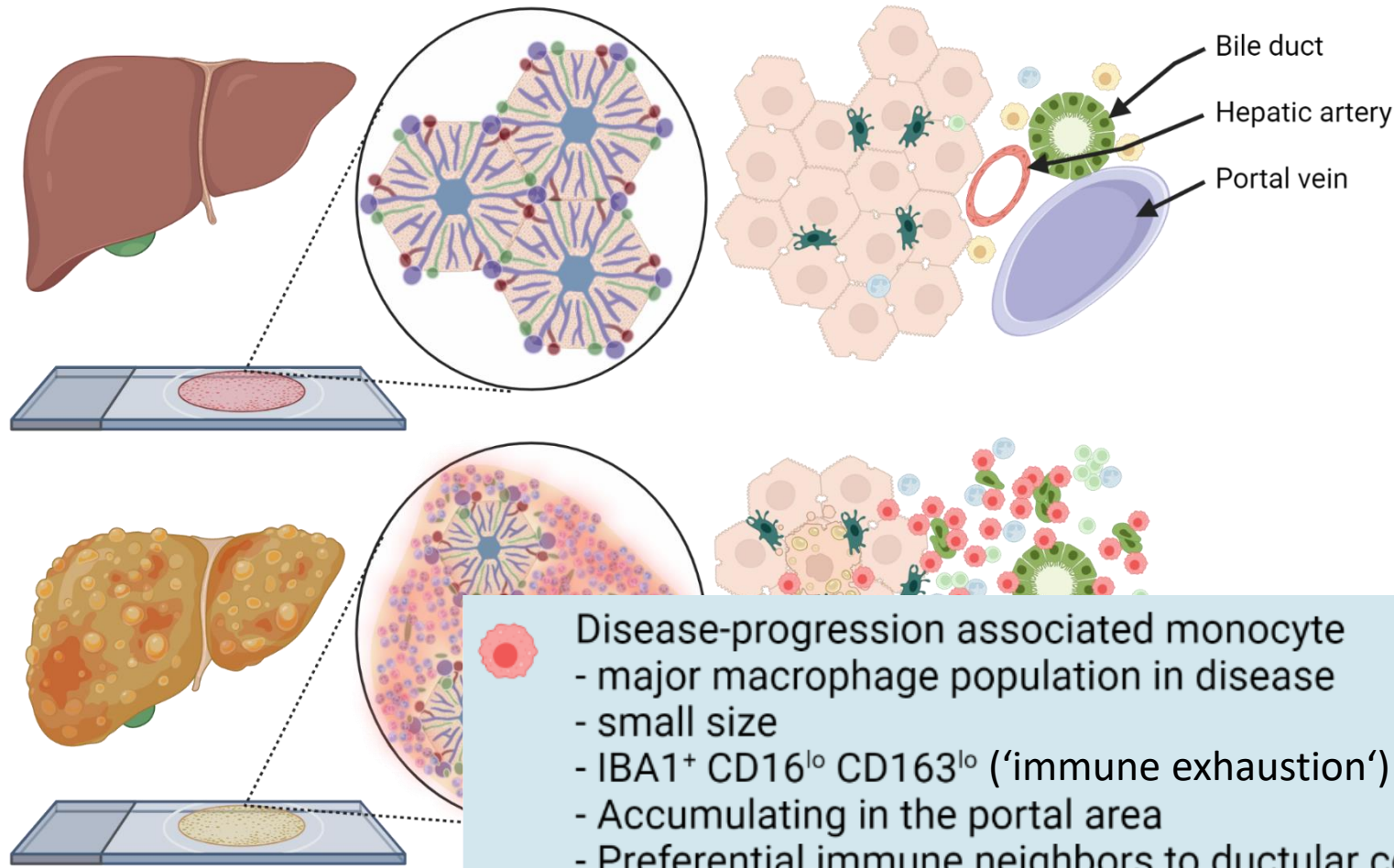
Macrophage crosstalk in the portal areas of MASH-livers



- **Macrophage accumulation is a common hallmark of chronic liver disease progression**
- **Imaging cytometry reveals distinct macrophage spatial distribution and phenotypes**
- **Proximity of accumulated macrophages (monocyte-derived) and ductular cells is associated with fibrosis severity**

	Hepatocyte		Steatotic hepatocyte
	Neutrophil		T lymphocyte
	Circulating monocyte		Ductular cell
	Kupffer cell		Disease-progression associated monocyte
	- major population in homeostasis		- major macrophage population in disease
	- large size		- small size
	- IBA1 ⁺ CD16 ^{hi} CD163 ^{hi}		- IBA1 ⁺ CD16 ^{lo} CD163 ^{lo}
	- located in the lobular areas		- Accumulating in the portal area
			- Preferential immune neighbors to ductular cells

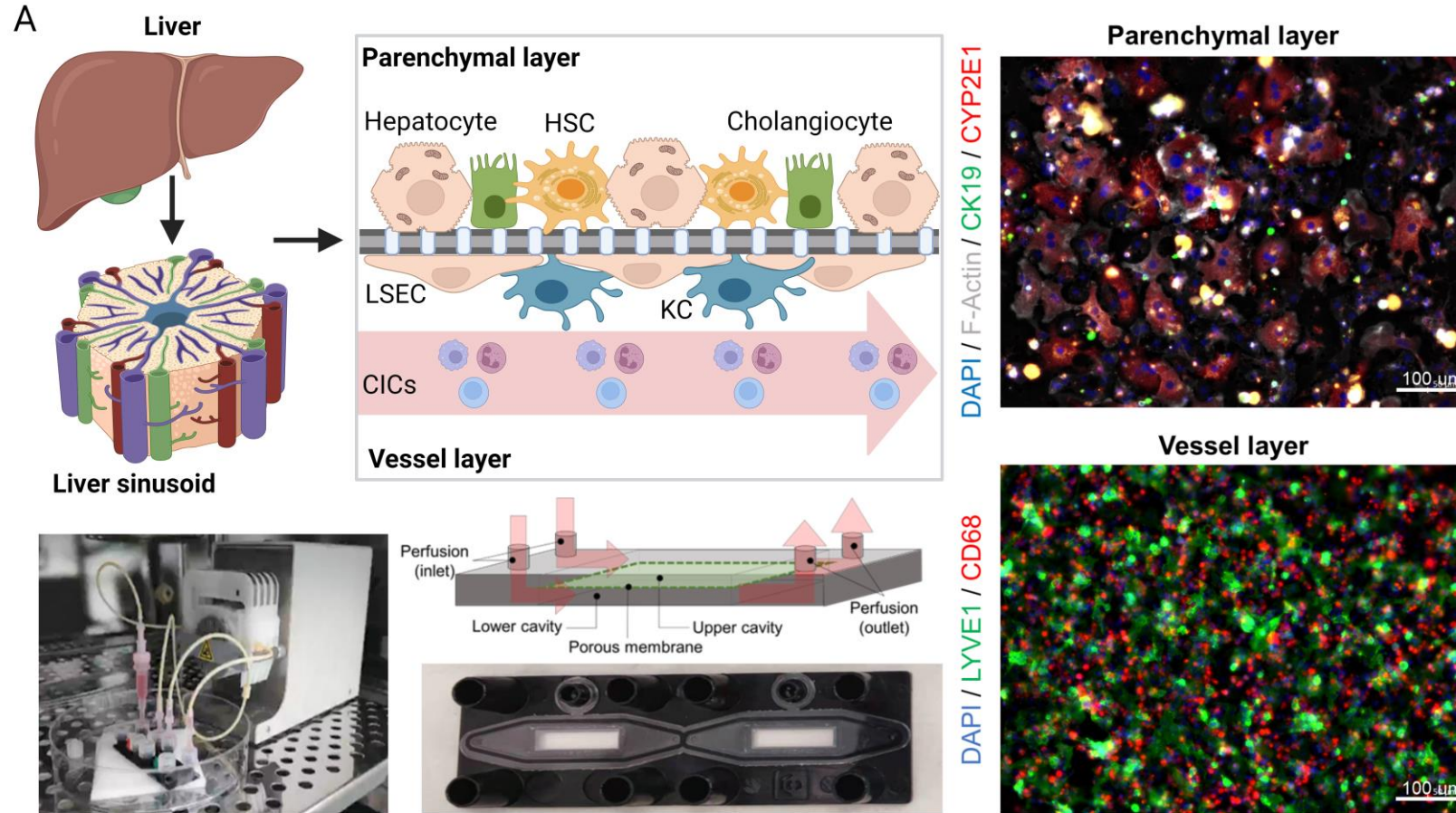
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Modelling immune cell interactions in liver disease *in vitro*

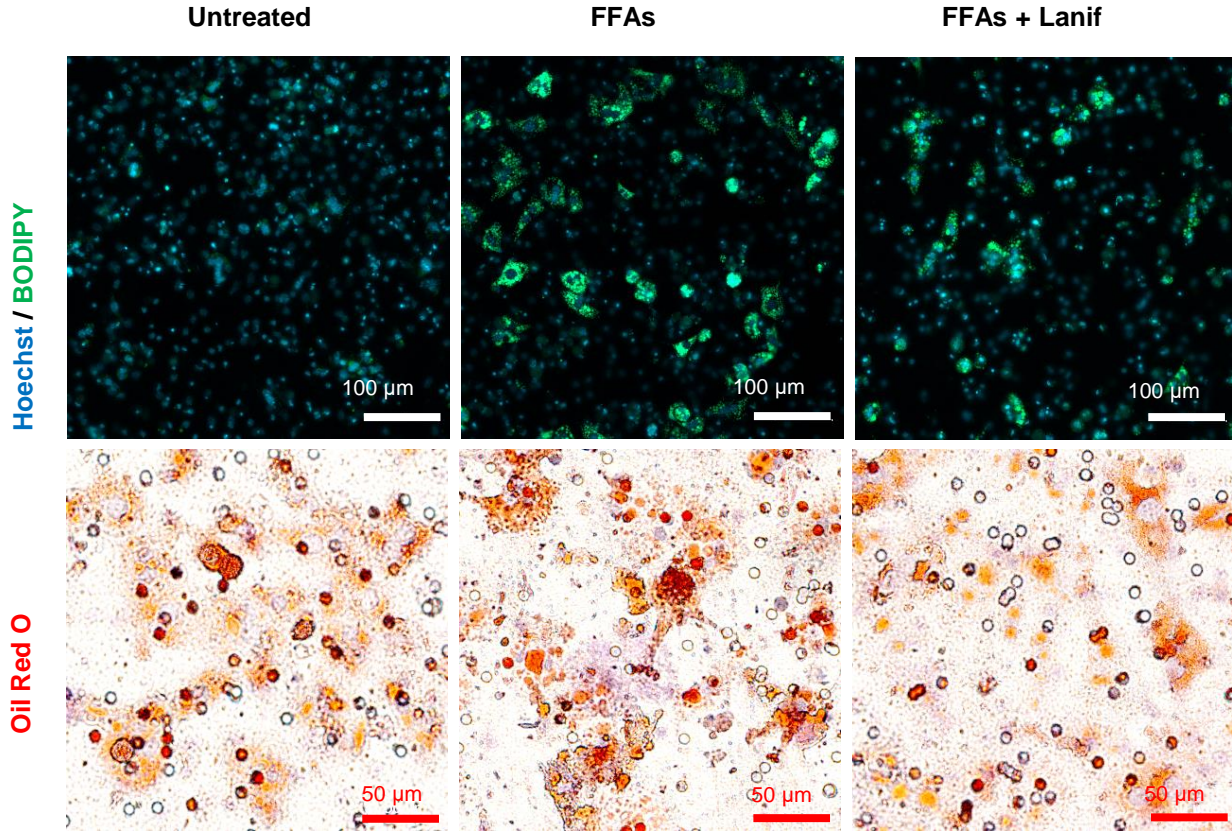
Perfusable liver-on-a-biochip with primary cell populations



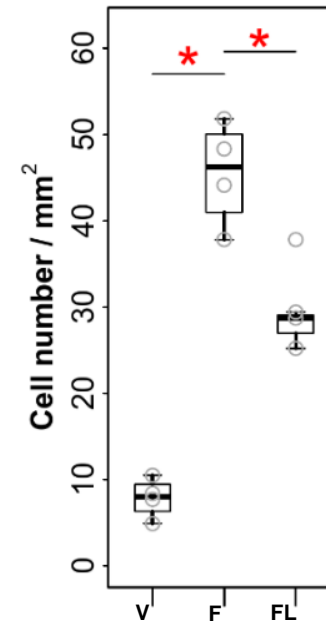
Modelling and modulating steatosis, cell injury, immune cell recruitment and fibrogenesis in MASLD

Lanifibranor (pan-PPAR-agonist) in experimental MASH

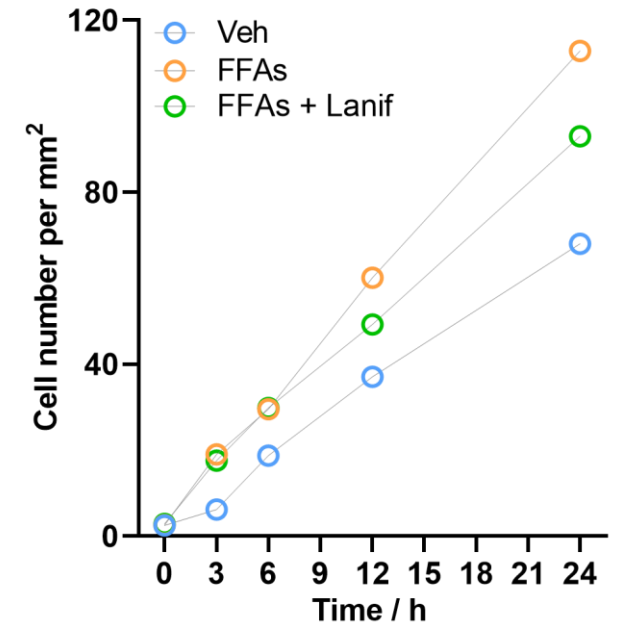
Perfusable liver-on-a-biochip with primary cell populations



CIC accumulation

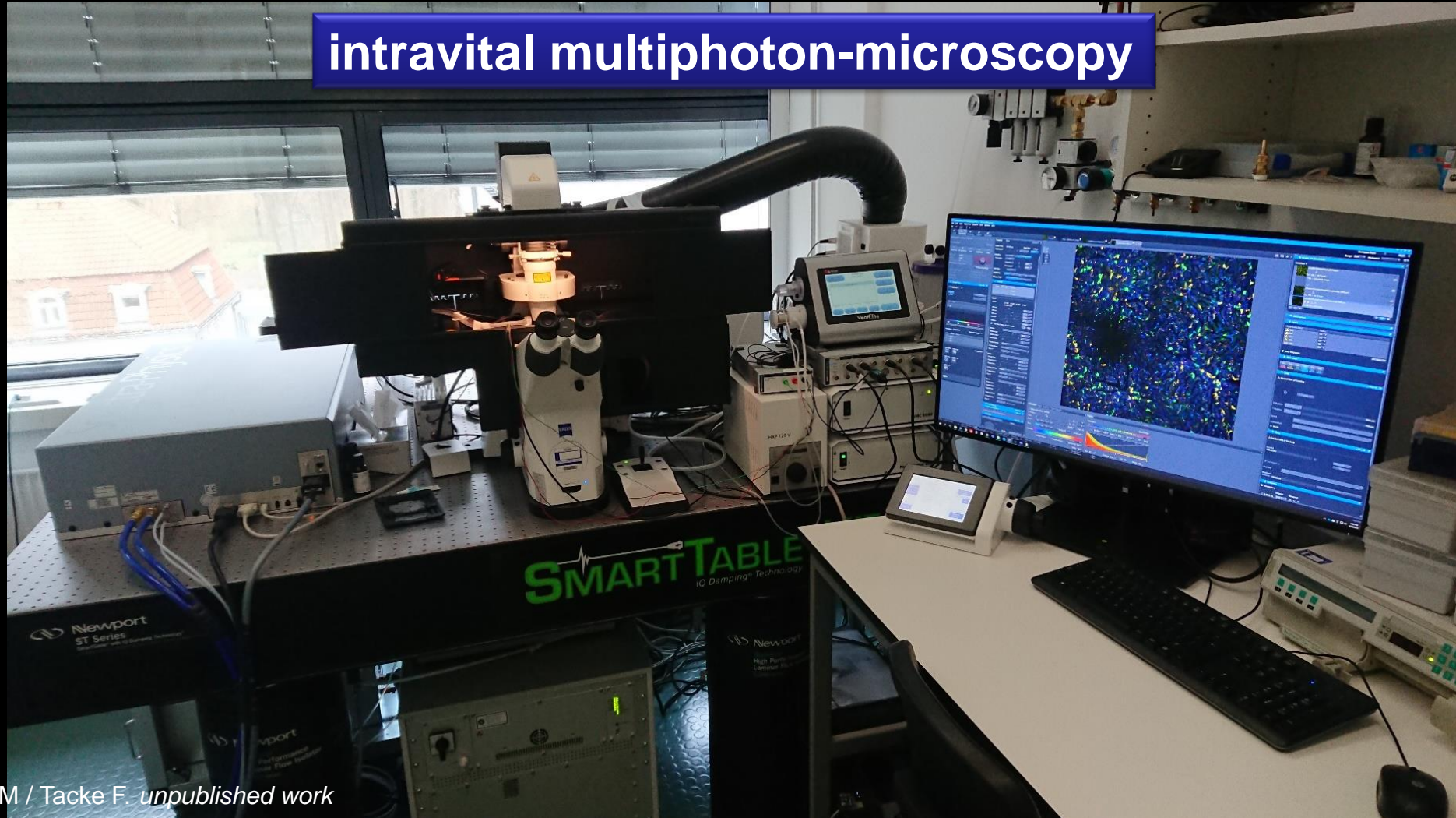


CIC accumulation



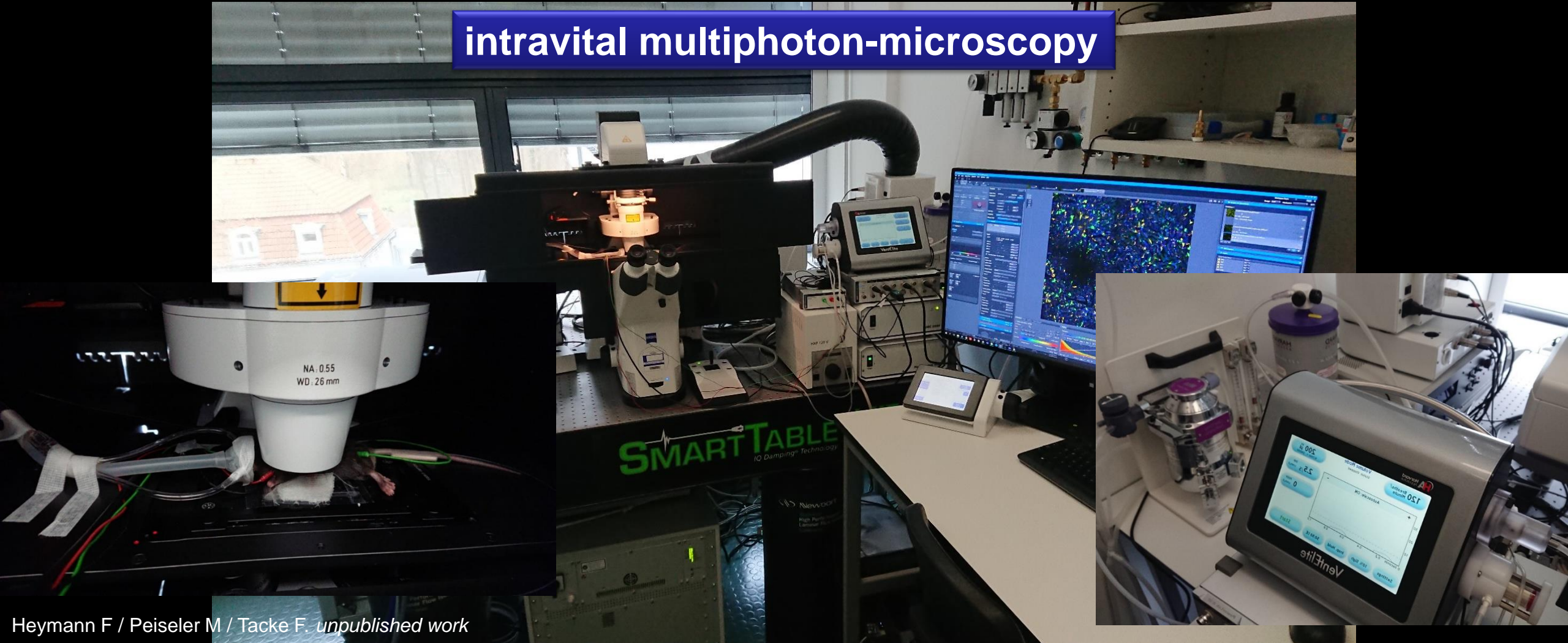
Modelling and modulating steatosis, cell injury, immune cell recruitment and fibrogenesis in MASLD

Cellular crosstalk in liver disease: the dynamic dimension



Heymann F / Peiseler M / Tacke F. *unpublished work*

Cellular crosstalk in liver disease: the dynamic dimension

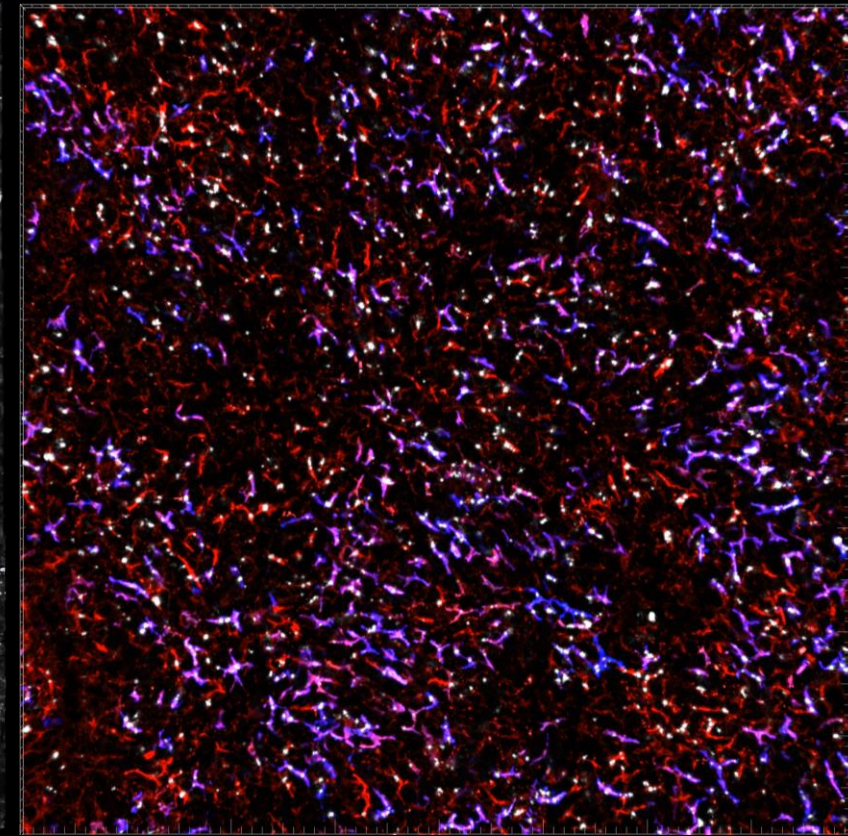
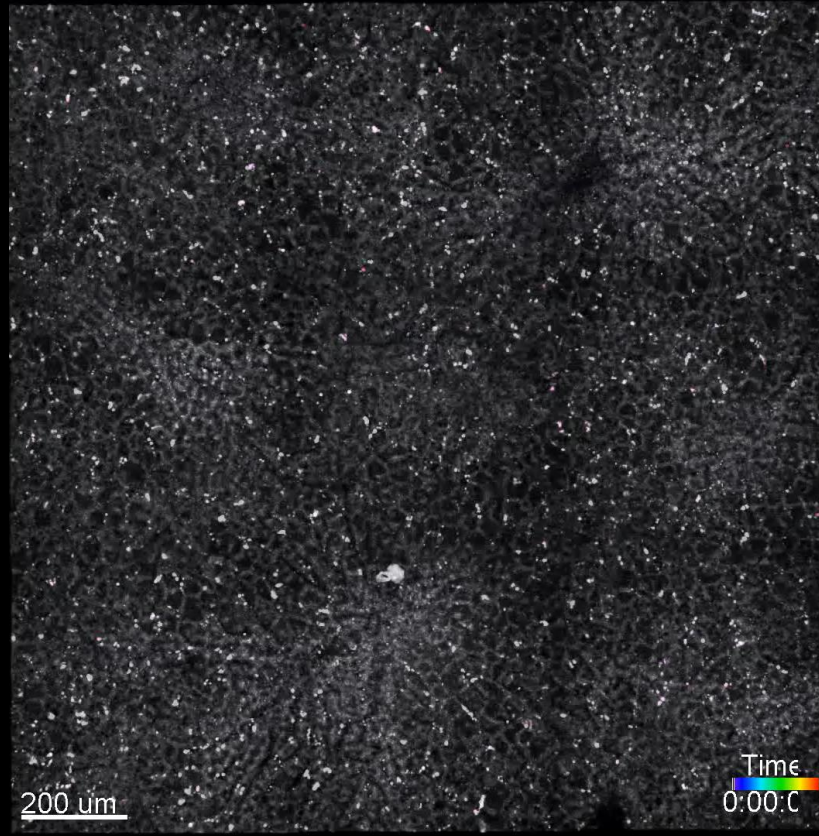
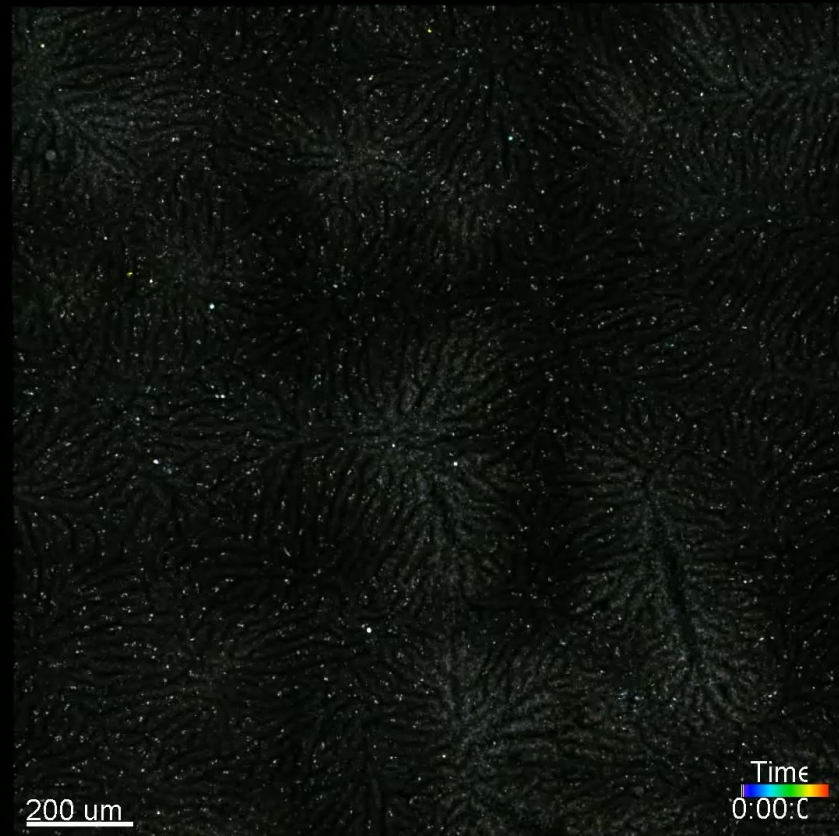


Cellular crosstalk in liver disease: the dynamic dimension

Control diet

Western diet

CDAA-HFD

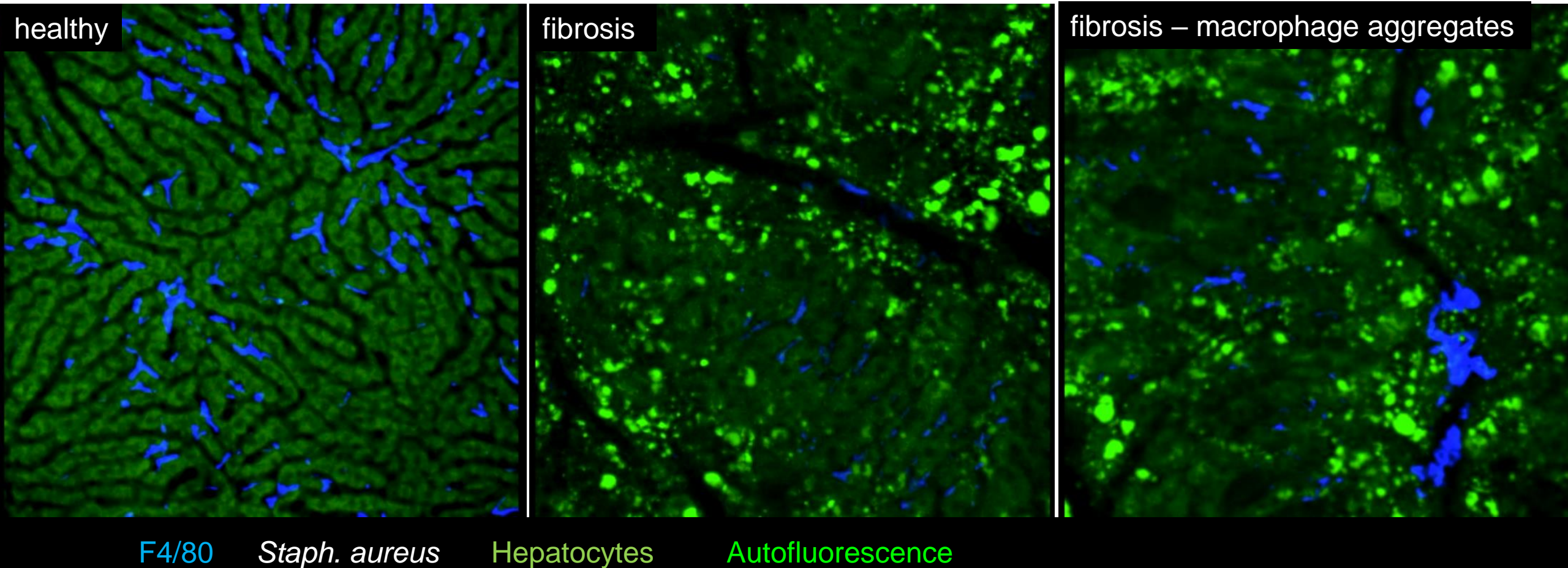


F4/80 Tim4 Beads

Nile red

Heymann F / Peiseler M / Tacke F. *unpublished work*

Monocyte-derived macrophages form clusters to compensate Kupffer cell loss in liver fibrosis

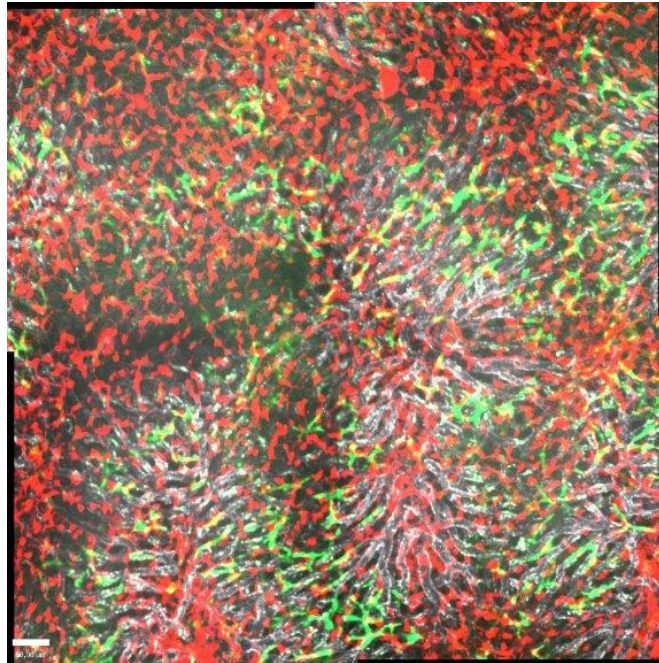


Peiseler M, ... Tacke F, Kubes P. *Science*. 2023; 381(6662):eabq5202

Dynamic changes of macrophage compartment in fibrosis

intravital microscopy (mice)

Control

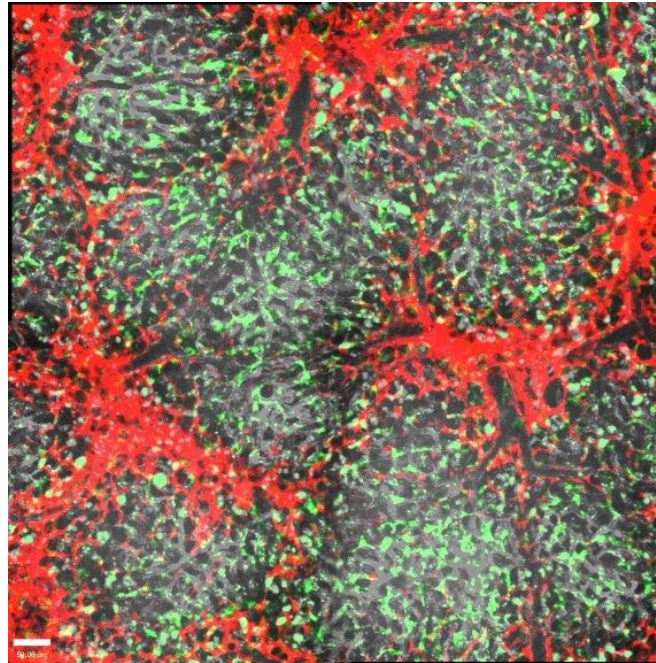


F4/80

Lrat

CD31

Fibrosis

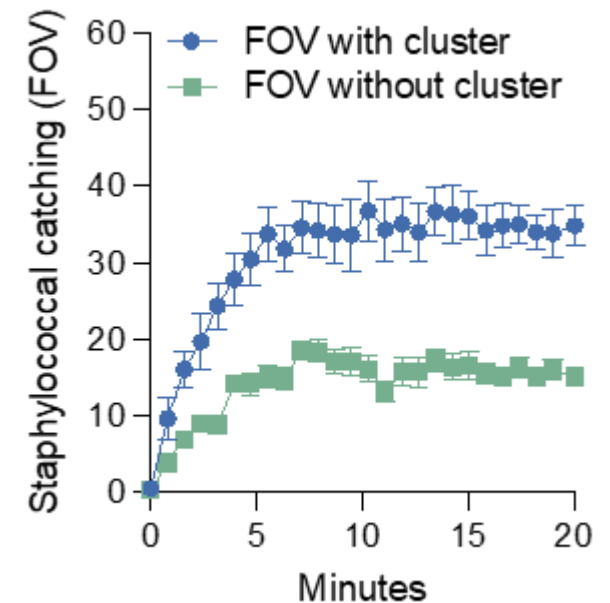
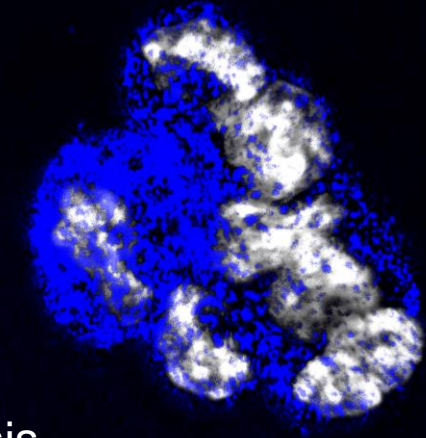


- Loss of KC / HSC contact in sinusoids
- Kupffer cell numbers are reduced
- Giant macrophages → catch bacteria

Hoechst

F4/80

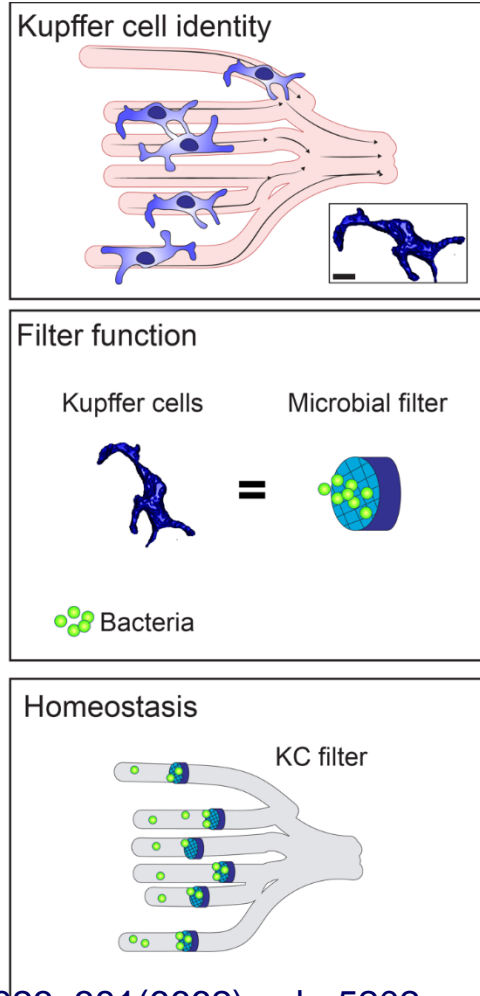
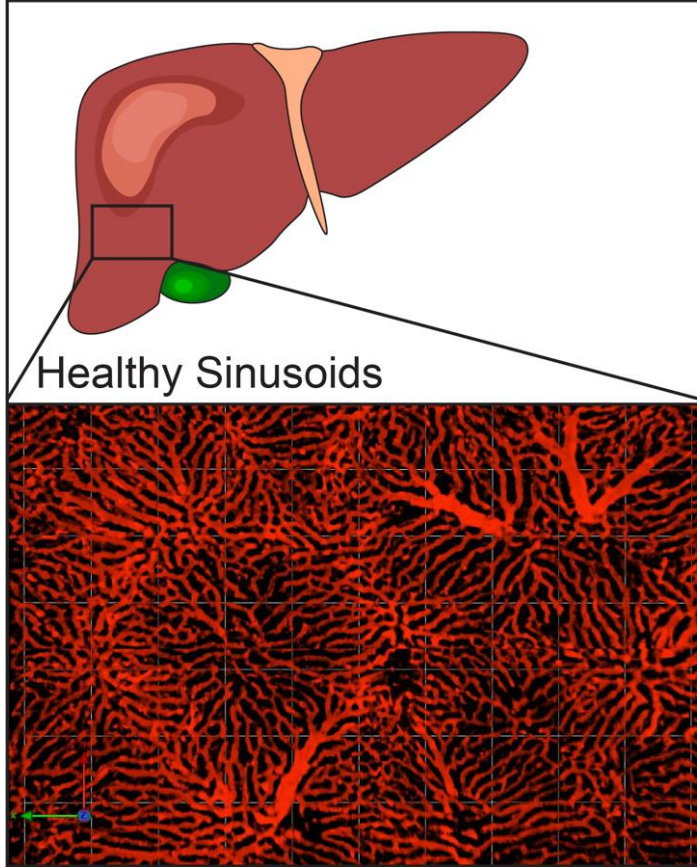
CCl₄ fibrosis



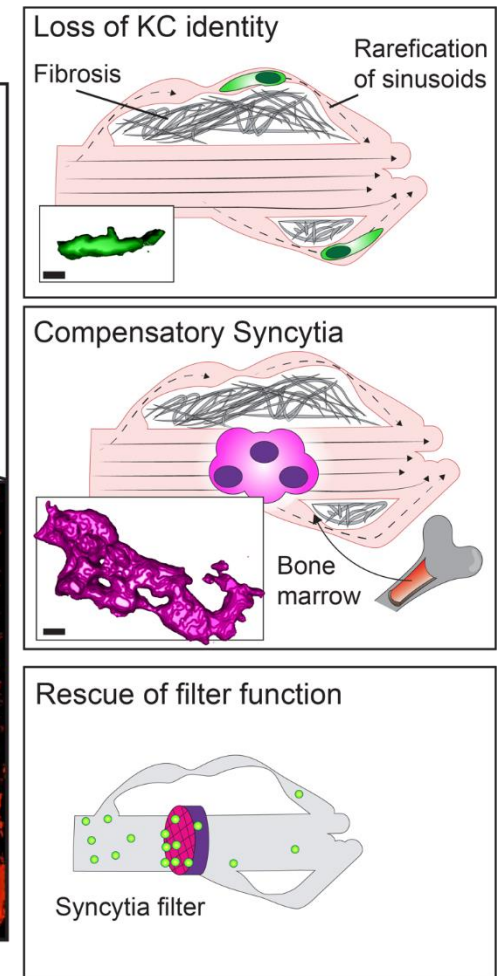
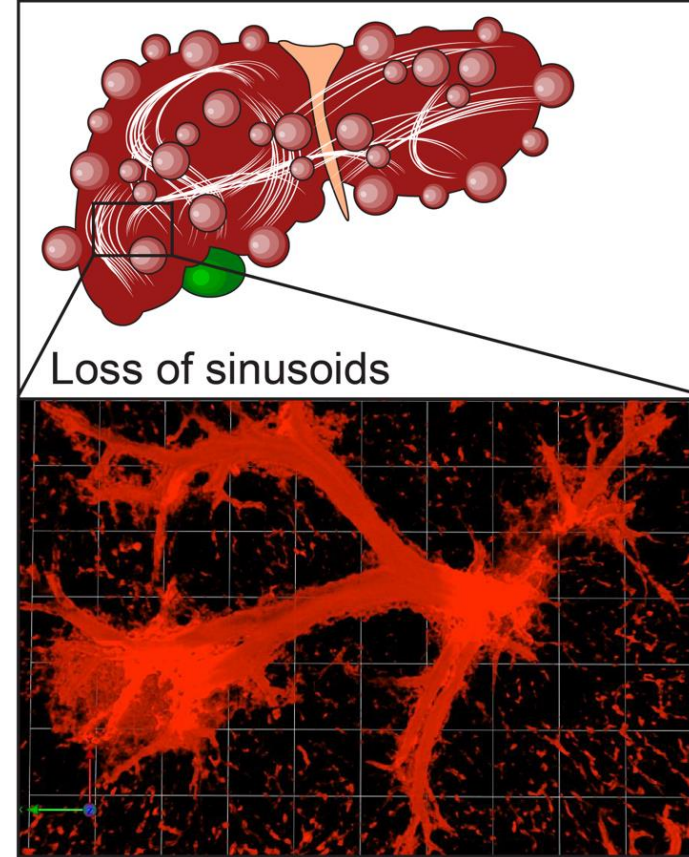
Peiseler M, ... Tacke F, Kubes P. *Science*. 2023; 381(6662):eabq5202

Monocyte-derived macrophages form clusters to compensate Kupffer cell loss in liver fibrosis

Homeostasis



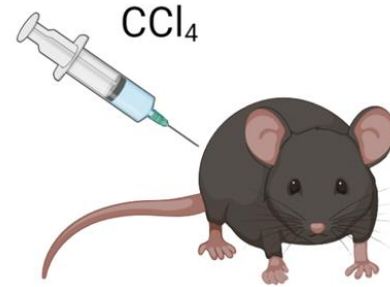
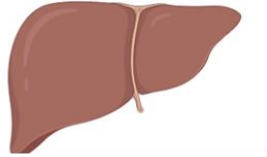
Liver Fibrosis



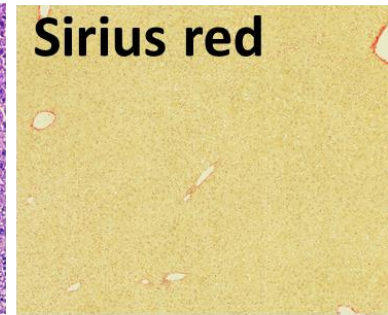
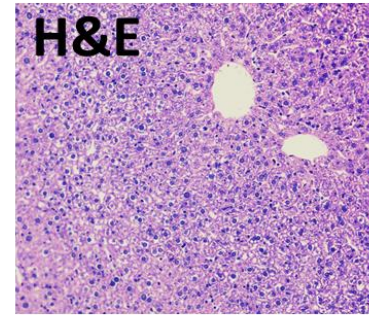
Peiseler M, ... Tacke F, Kubes P. *Science*. 2023; 381(6662):eabq5202

Impact of fibrosis progression or regression on liver macrophages

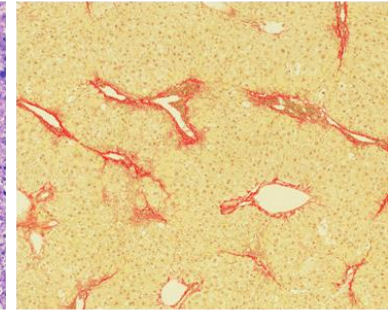
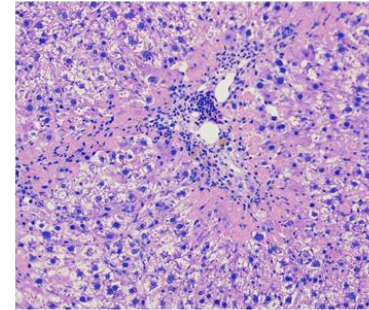
Homeostasis



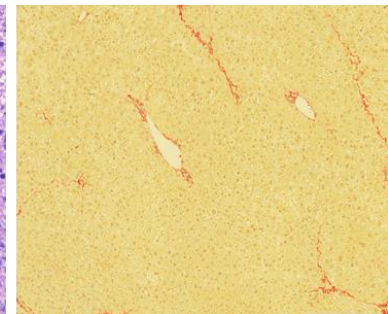
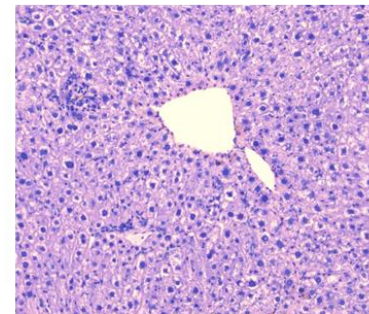
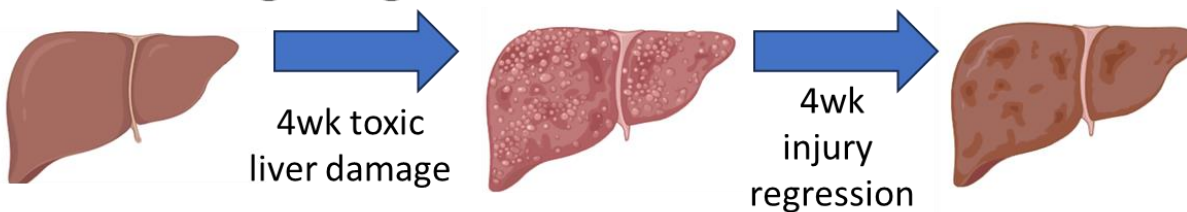
CX3CR1.gfp



Chronic liver damage

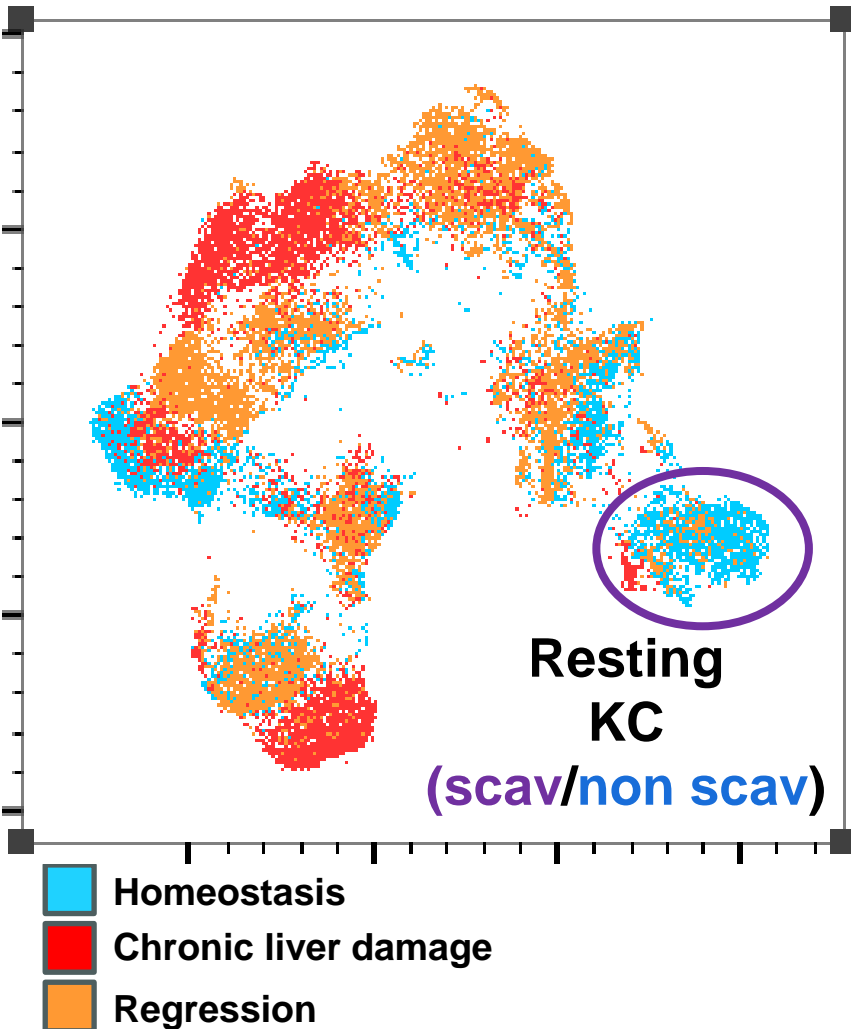


Liver damage regression

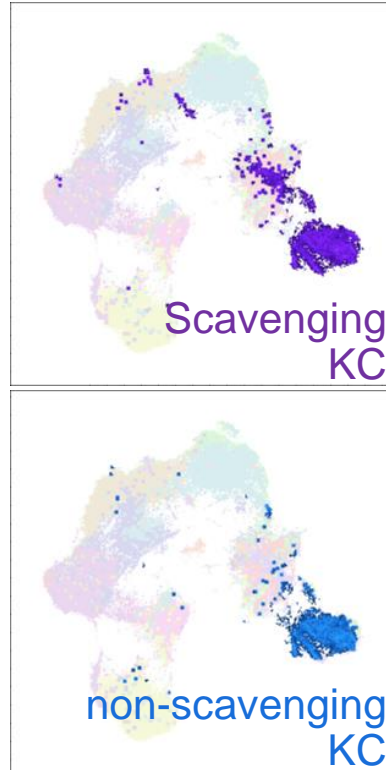


Heymann F / Peiseler M / Horn P / Tacke F (*unpublished data*)

Impact of fibrosis progression or regression on liver macrophages



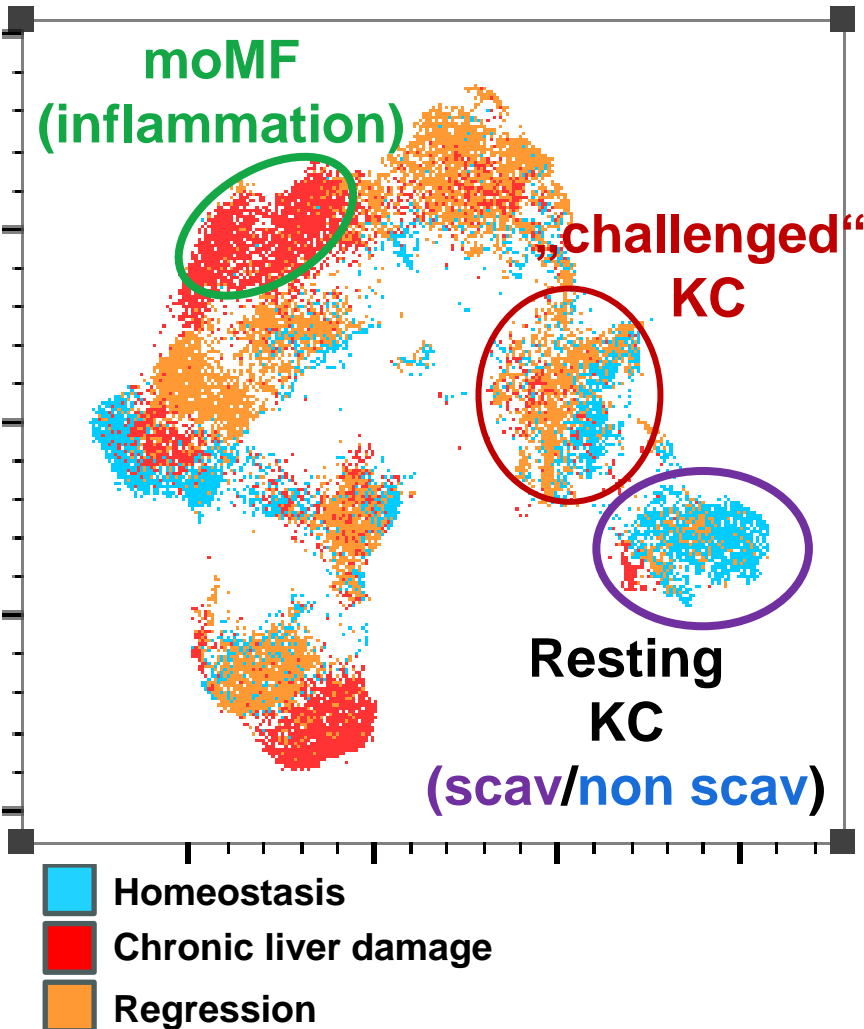
Homeostasis



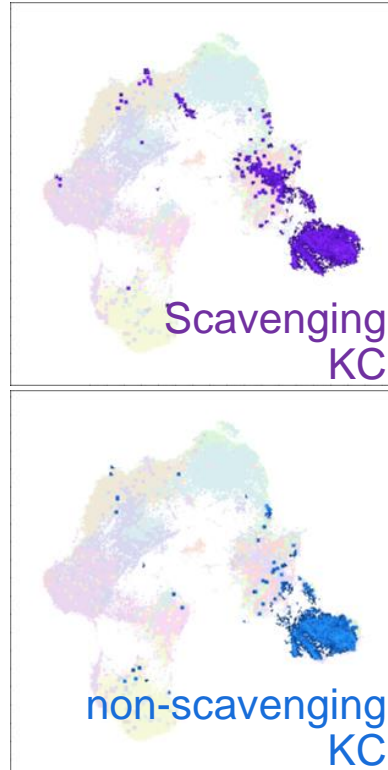
- Kupffer cell populations in homeostasis

Heymann F / Peiseler M / Horn P / Tacke F (*unpublished data*)

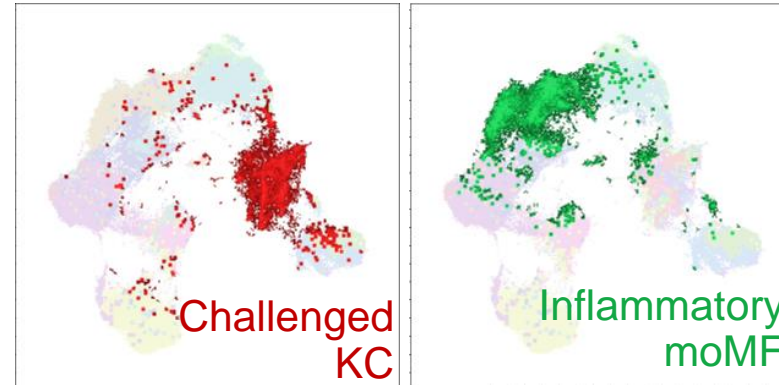
Impact of fibrosis progression or regression on liver macrophages



Homeostasis



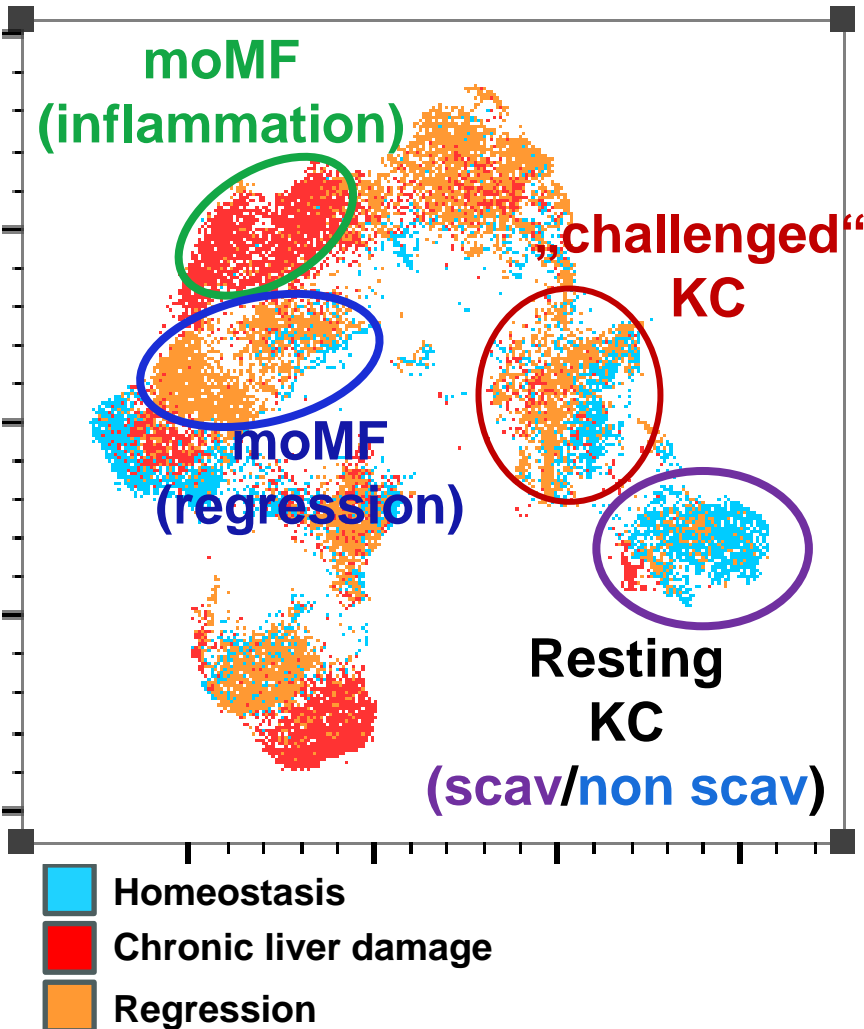
Inflammation / Regression



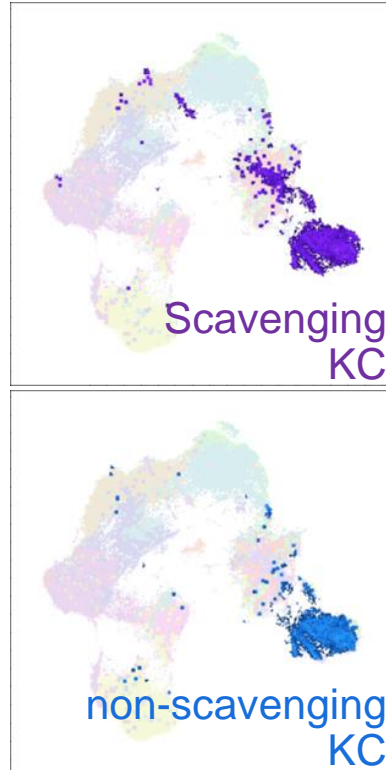
- Kupffer cell populations in homeostasis
- Recruitment of inflammatory monocyte-derived macrophages and „activation“ of Kupffer cells in injury

Heymann F / Peiseler M / Horn P / Tacke F (*unpublished data*)

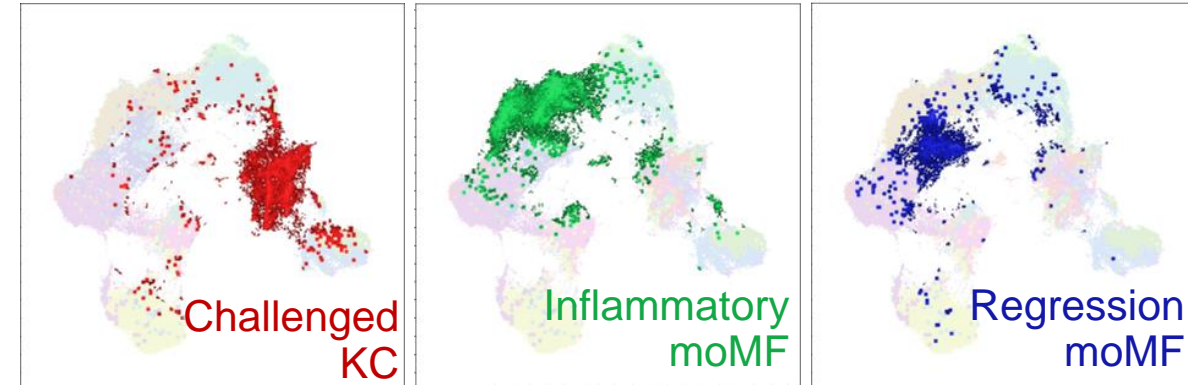
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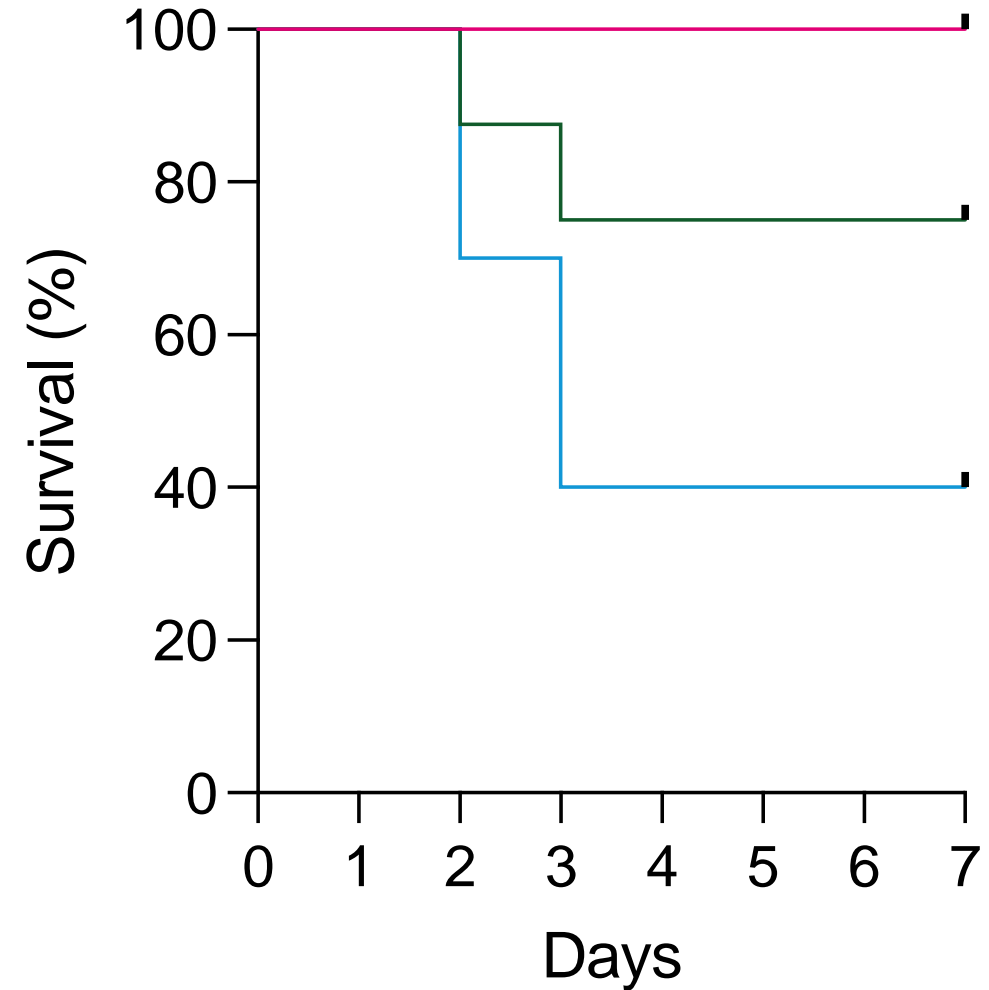
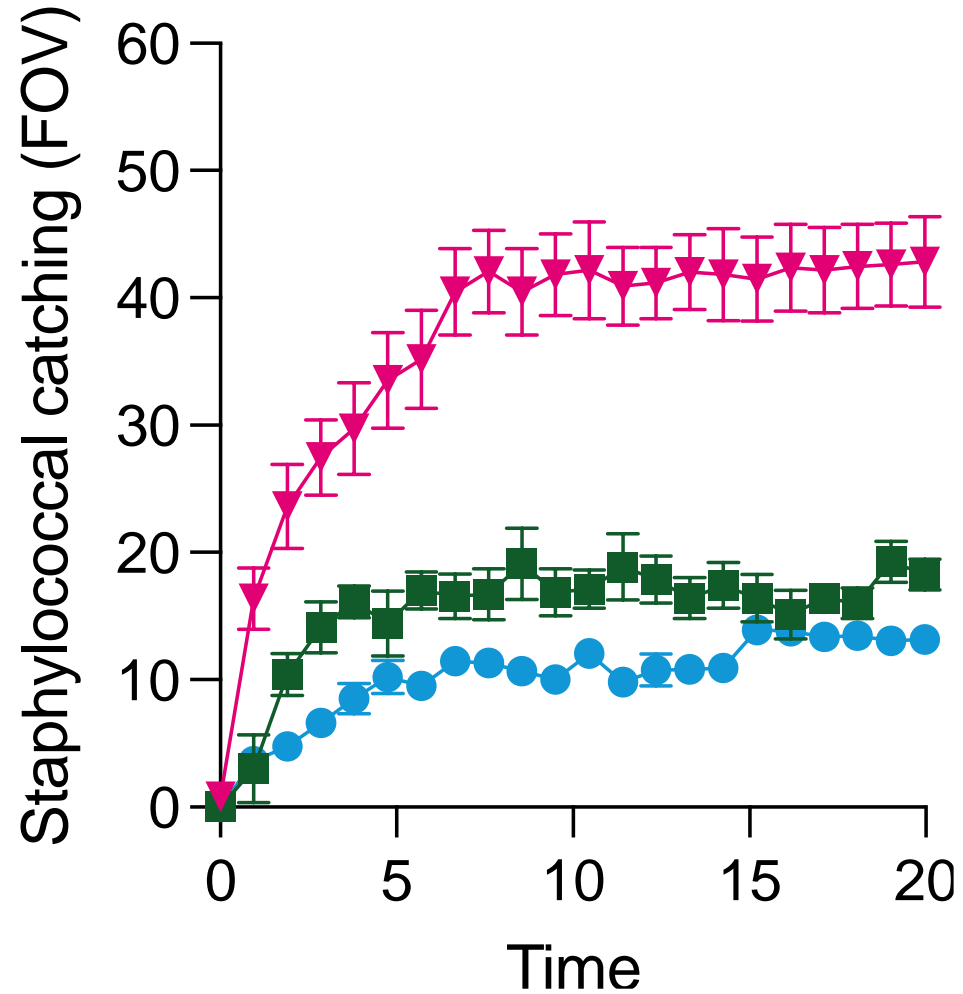
Inflammation / Regression



- Kupffer cell populations in homeostasis
- Recruitment of inflammatory monocyte-derived macrophages and „activation“ of Kupffer cells in injury
- Long-lasting altered macrophages (with specific „regression MoMF“) after full histological regression

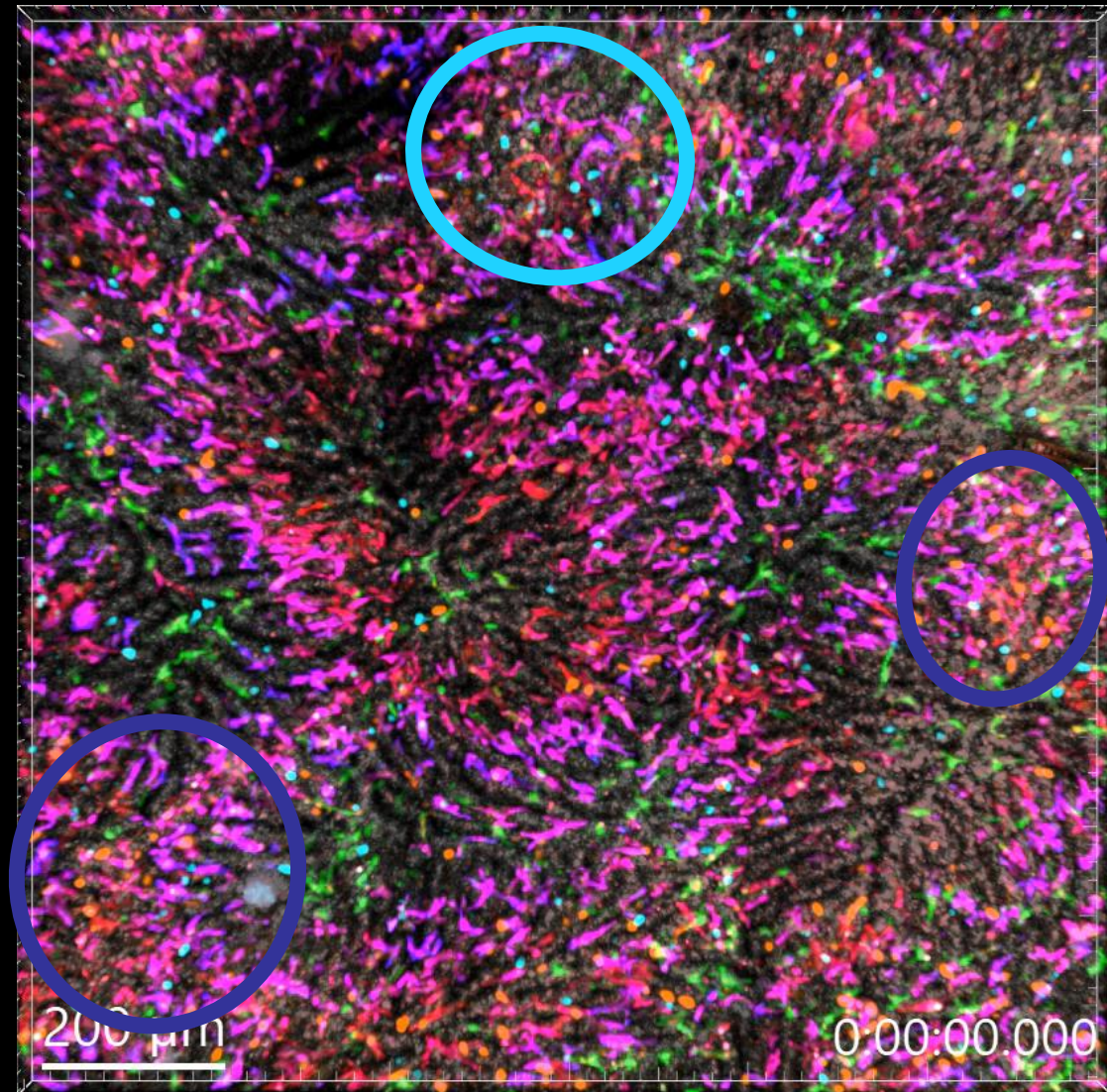
Heymann F / Peiseler M / Horn P / Tacke F (*unpublished data*)

Less efficient antibacterial defense after fibrosis regression

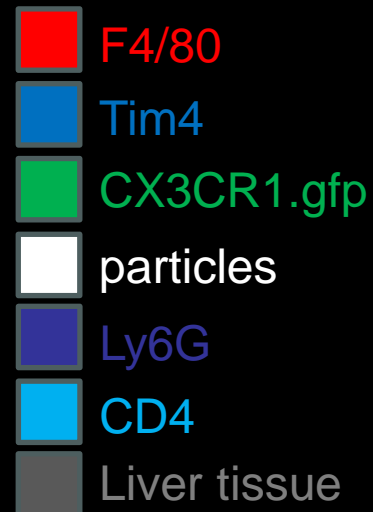


Peiseler M, Araujo David B, Kubes P, et al (*unpublished data*)

Cell-cell communication with liver macrophages

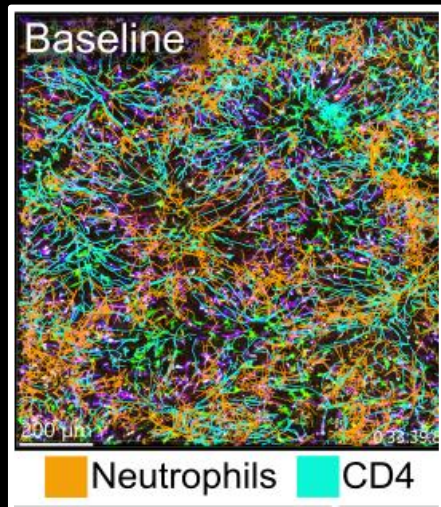
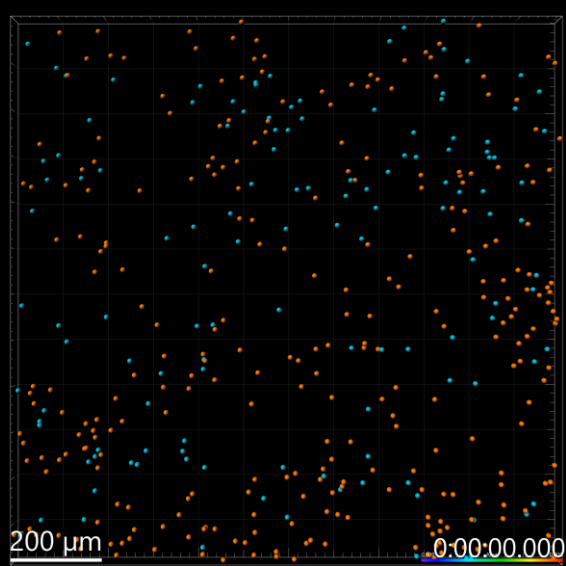


- Transient contact between passenger leukocytes and resident liver macrophages
- Kupffer cells as main interaction hubs with both **CD4⁺** and **Ly6⁺** cells

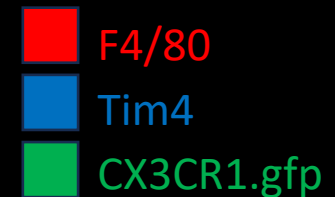


Heymann F / Peiseler M / Horn P / Tacke F (unpublished data)

Cell-cell communication with liver macrophages

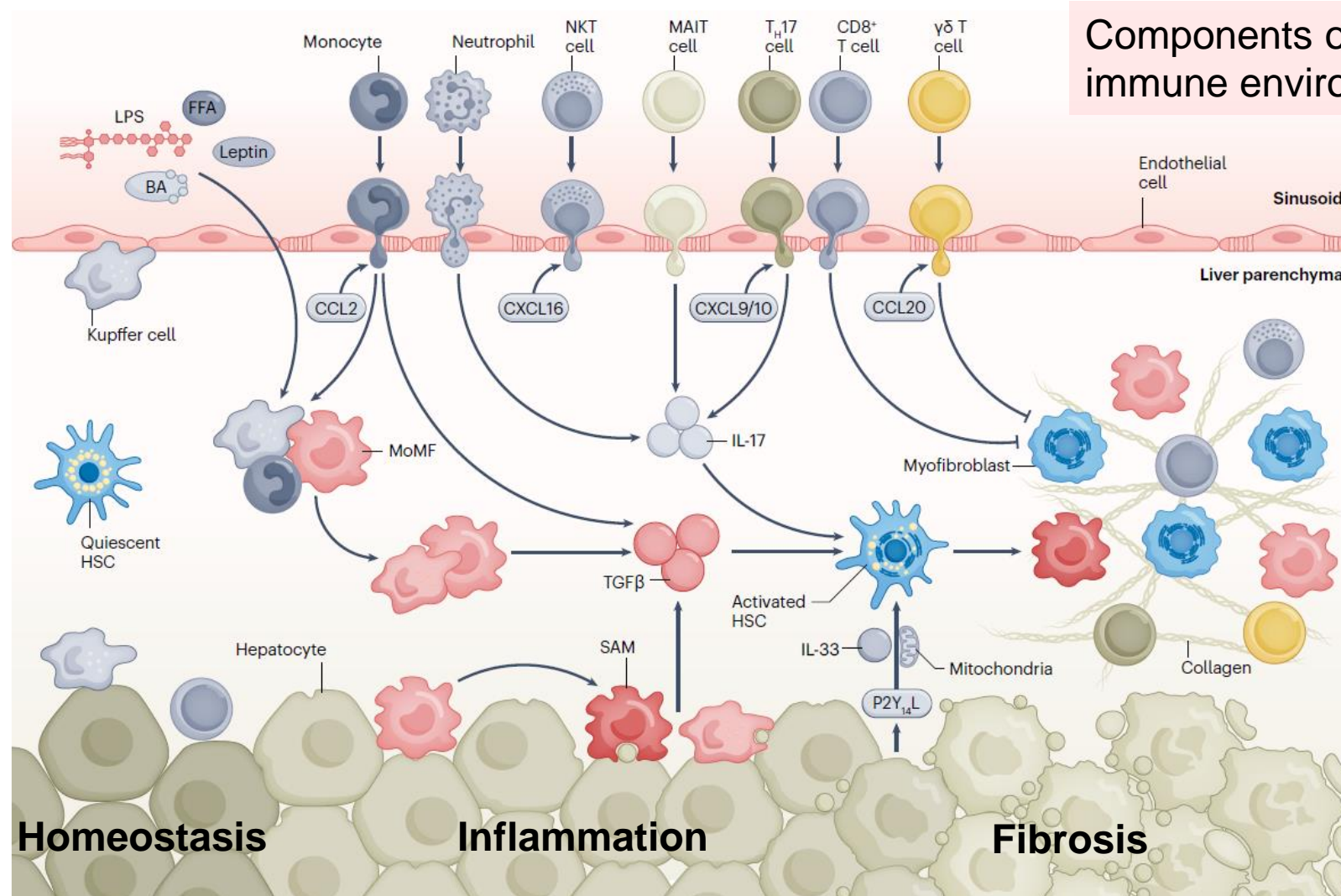


- Altered interactions following inflammation
- Differential homing of CD4 T cells and neutrophils
- Hot spots following liver injury and regression



From metabolic injury to inflammation and fibrosis

- Activation of resident (immune) cells
- Recruitment of inflammatory cells
- Intense cellular crosstalk between immune cells, parenchymal and non-parenchymal cells



- Activation of hepatic stellate cells
- Deposition of extracellular matrix
- Deactivation of repair / regenerative pathways

Hammerich L, Tacke F. *Nat Rev Gastroenterol Hepatol.* 2023;20(10):633-646

From metabolic injury to inflammation and fibrosis

- Activation of resident (immune) cells
- Recruitment of immune cells
- Interactions between immune cells, parenchymal and non-parenchymal cells

Metabolic injury triggers auto-aggressive CXCR6+ CD8+ T cells
Dudek M / Knolle PA. *Nature*. 2021; 592(7854):444-449

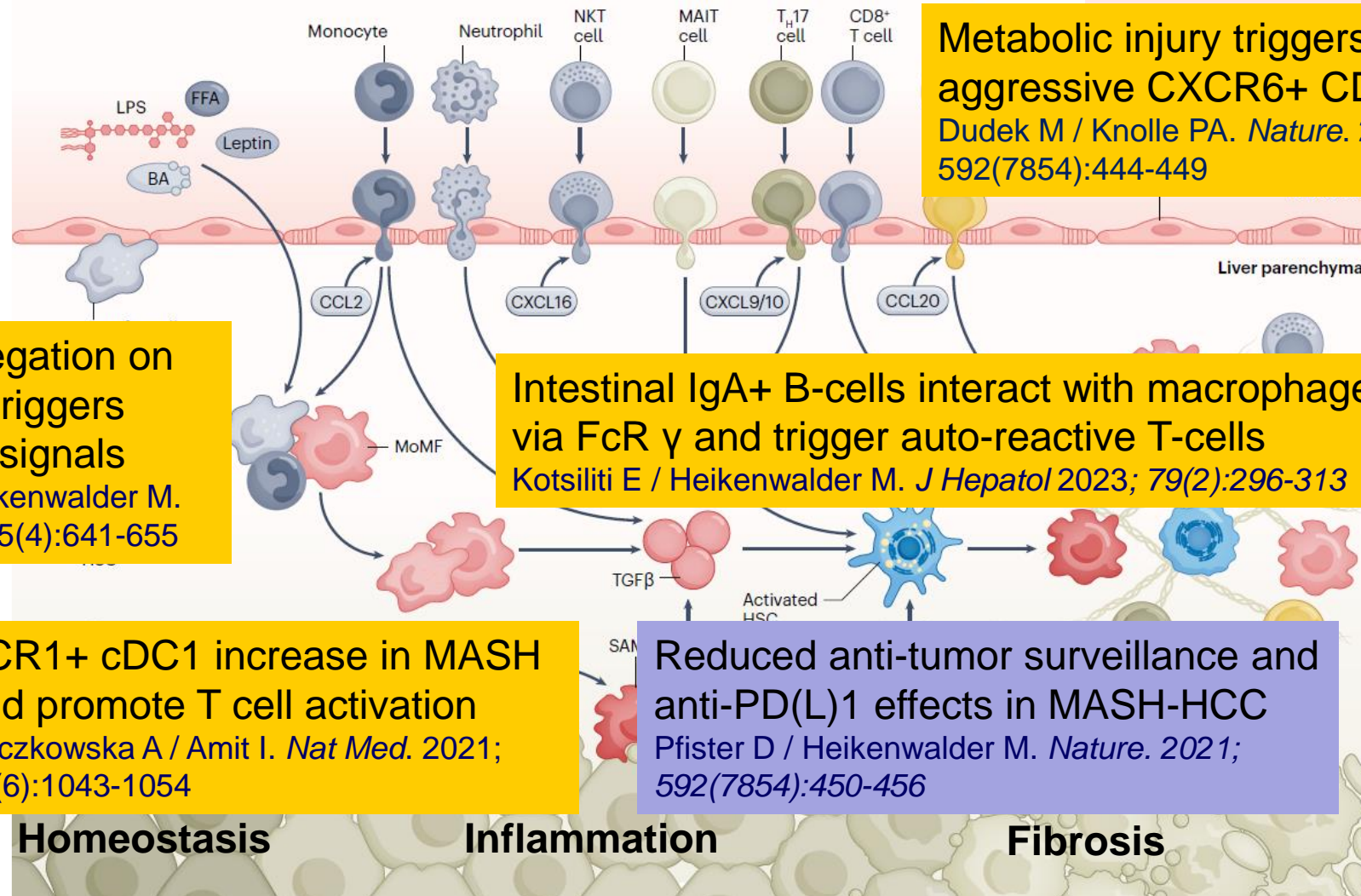
Platelet aggregation on Kupffer cells triggers inflammatory signals
Malehmir M / Heikenwalder M. *Nat Med*. 2019; 25(4):641-655

Intestinal IgA+ B-cells interact with macrophages via FcR γ and trigger auto-reactive T-cells
Kotsiliti E / Heikenwalder M. *J Hepatol* 2023; 79(2):296-313

XCR1+ cDC1 increase in MASH and promote T cell activation
Deczkowska A / Amit I. *Nat Med*. 2021; 27(6):1043-1054

Reduced anti-tumor surveillance and anti-PD(L)1 effects in MASH-HCC
Pfister D / Heikenwalder M. *Nature*. 2021; 592(7854):450-456

- Activation of hepatic stellate cells
- Deposition of extracellular matrix
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Immune exhaustion and inflammatory regulation of disease progression

- Metabolic injury to the liver causes immune adaptation processes driving progression of inflammation, fibrosis and cancer, but has also consequences on immune functions ('immune exhaustion')
- Single-cell RNA sequencing and accompanied imaging techniques (spatial proteo-transcriptomics, dynamic microscopy) help to dissect functionally distinct immune cell subsets and their interactions
- Macrophage populations are key orchestrators during chronic liver disease – their diversity includes different ontogeny, heterogeneous subtypes and versatile phenotype adaptation
- The hepatic macrophage pool changes during inflammation and does not fully revert to “normal” after regression - monocyte-derived macrophages closely interact with cholangiocytes in the portal area (ductular reaction) and cannot fully compensate physiological functions of Kupffer cells
- Rapid advances in basic science will likely identify new targets (or bring new insights on known targets) – an integrated view is needed, translation into clinical success remains challenging

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Thank you for your attention!

Tacke Lab

Adrien Guillot
Moritz Peiseler
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