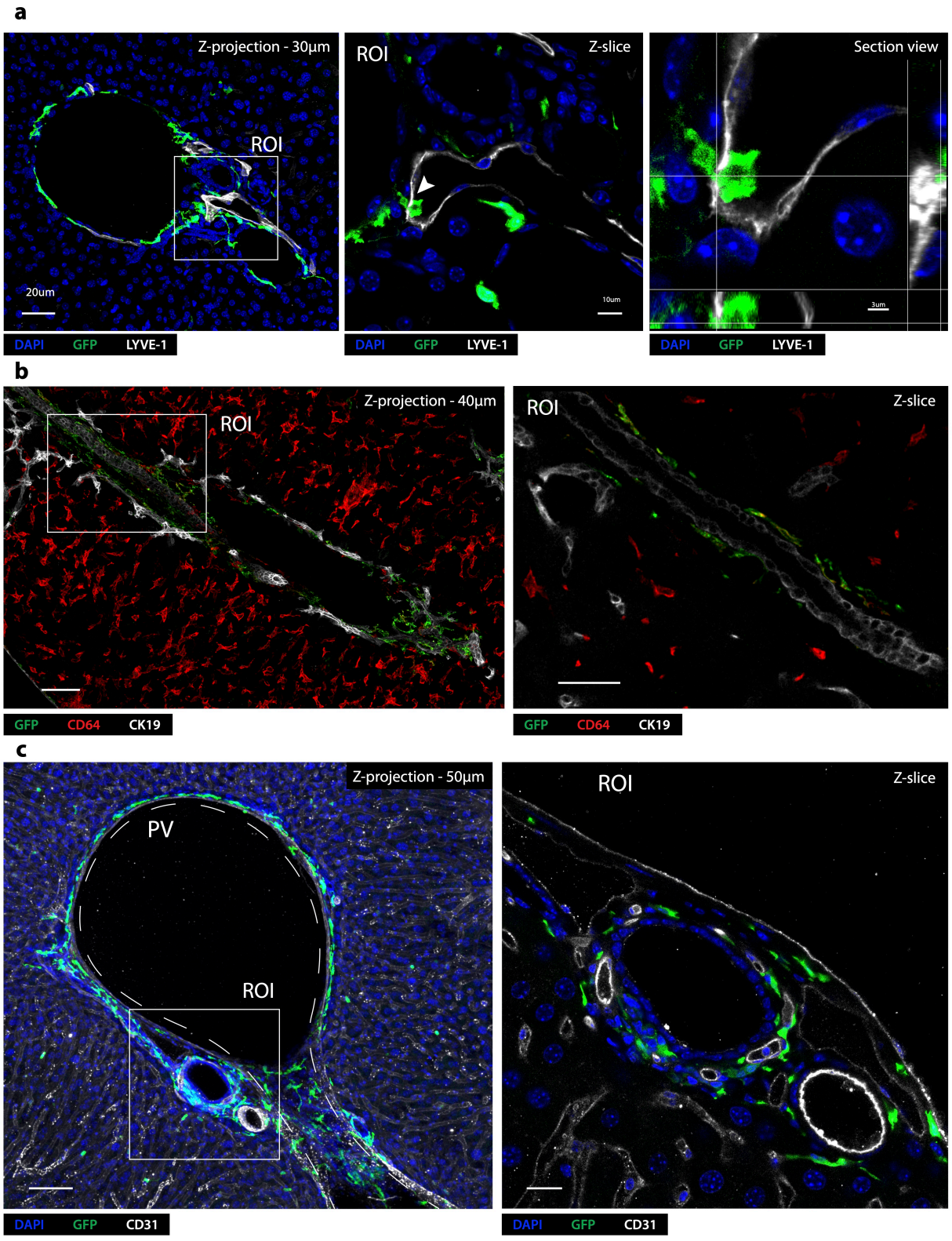
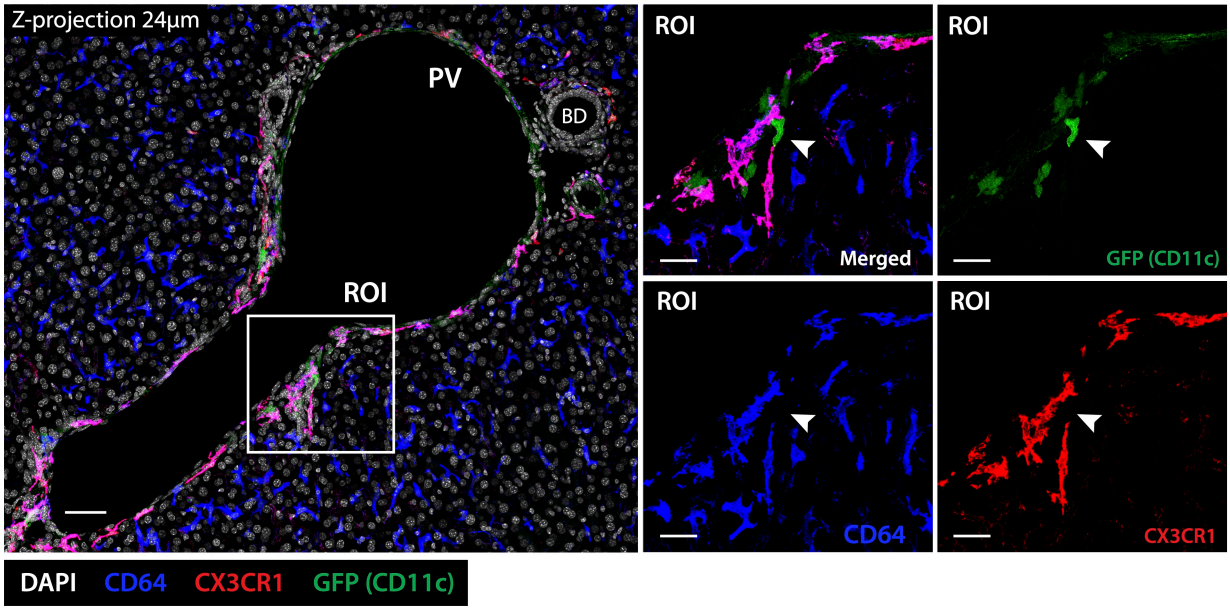
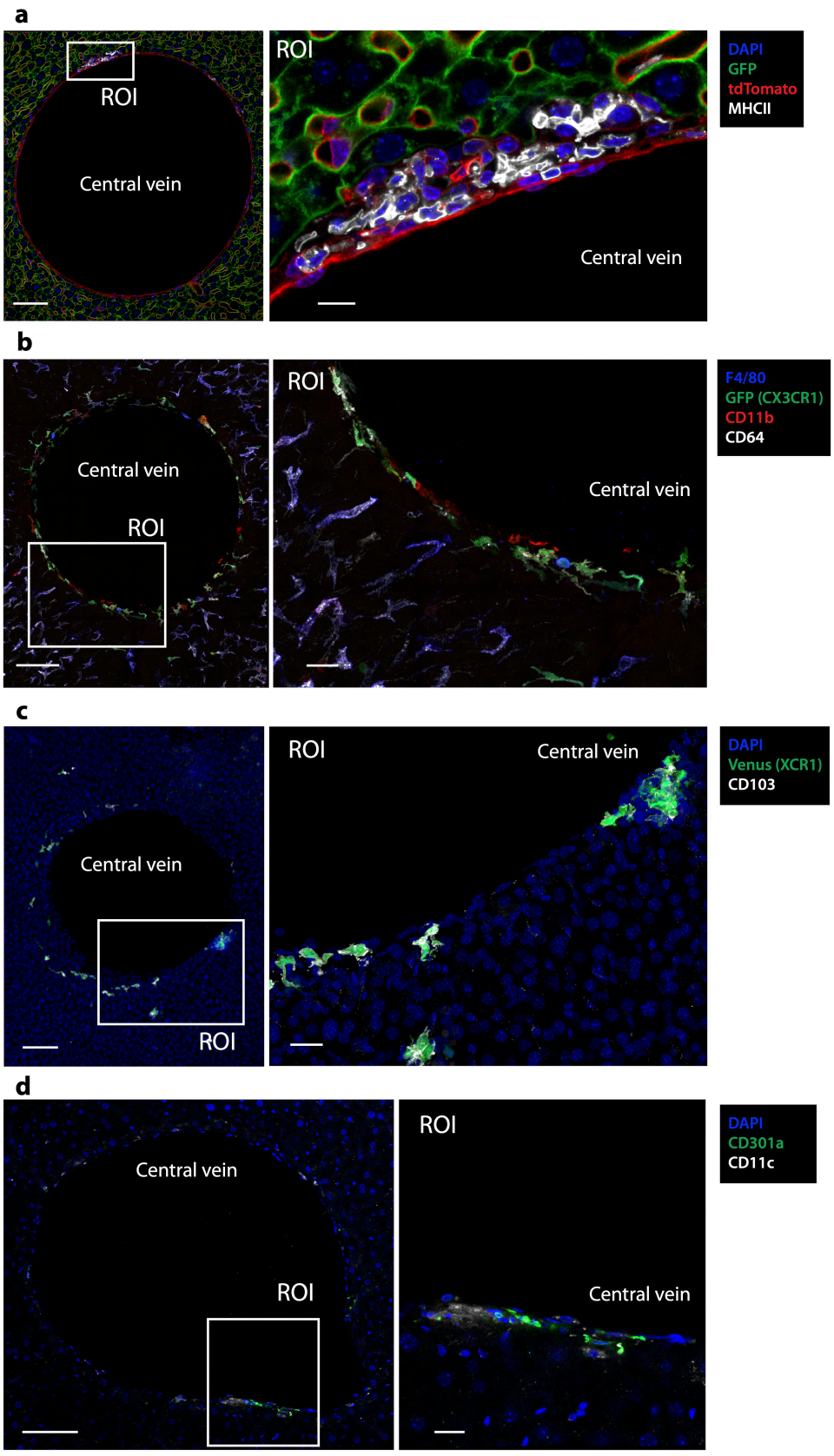


Supplementary figure 1: CX3CR1^{+} macrophages are lined up along the edge of the portal tract area and also surround the portal vein branch and the bile duct. **(a)** Left panel: Z-projection of a portal tract cross section of a *Cx3cr1*$^{+/gfp}$ reporter mouse liver, stained for LYVE-1 showing CX3CR1^{+} macrophages and the lymphatic vasculature (DAPI – blue; GFP – green; LYVE-1 – white; scale bar 20µm). Middle panel: higher magnification image of ROI in left panel, shown in a single Z-slice, illustrates a CX3CR1^{+} macrophage protruding from the portal interstitium into the lymphatic lumen; scale bar 10µm. Right panel shows a higher magnification section view of this interaction; scale bar 3µm. **(b)** Left panel: Z-projection of a portal tract cross section from a *Cx3cr1*$^{+/gfp}$ mouse liver in the steady state, stained to identify CX3CR1^{+} macrophages and the biliary tree (GFP – green; CD64 – red; CK19 – white; scale bar 100µm). Right panel: Higher magnification view of a single Z-slice of the ROI indicated in the left panel; scale bar 70 µm, illustrating CX3CR1^{+} macrophages closely interacting with the CK19^{+} biliary tree. **(c)** Left image: Z-projection of a portal tract cross section from a *Cx3cr1*$^{+/gfp}$ mouse liver in the steady state, stained to identify CX3CR1^{+} macro-phages and vascular endothelium (DAPI – blue; GFP – green; CD31 – white; scale bar 100µm, showing that CX3CR1^{+} form a network lining the portal vein (PV), dotted line. Right panel: magnified view of single Z-slice of ROI in the left panel; scale bar 20µm, illustrating CX3CR1^{+} macrophages interacting with biliary capillaries, and to a lesser extent with the hepatic artery. Images are representative of n = 4 mice from 2 independent experiments.

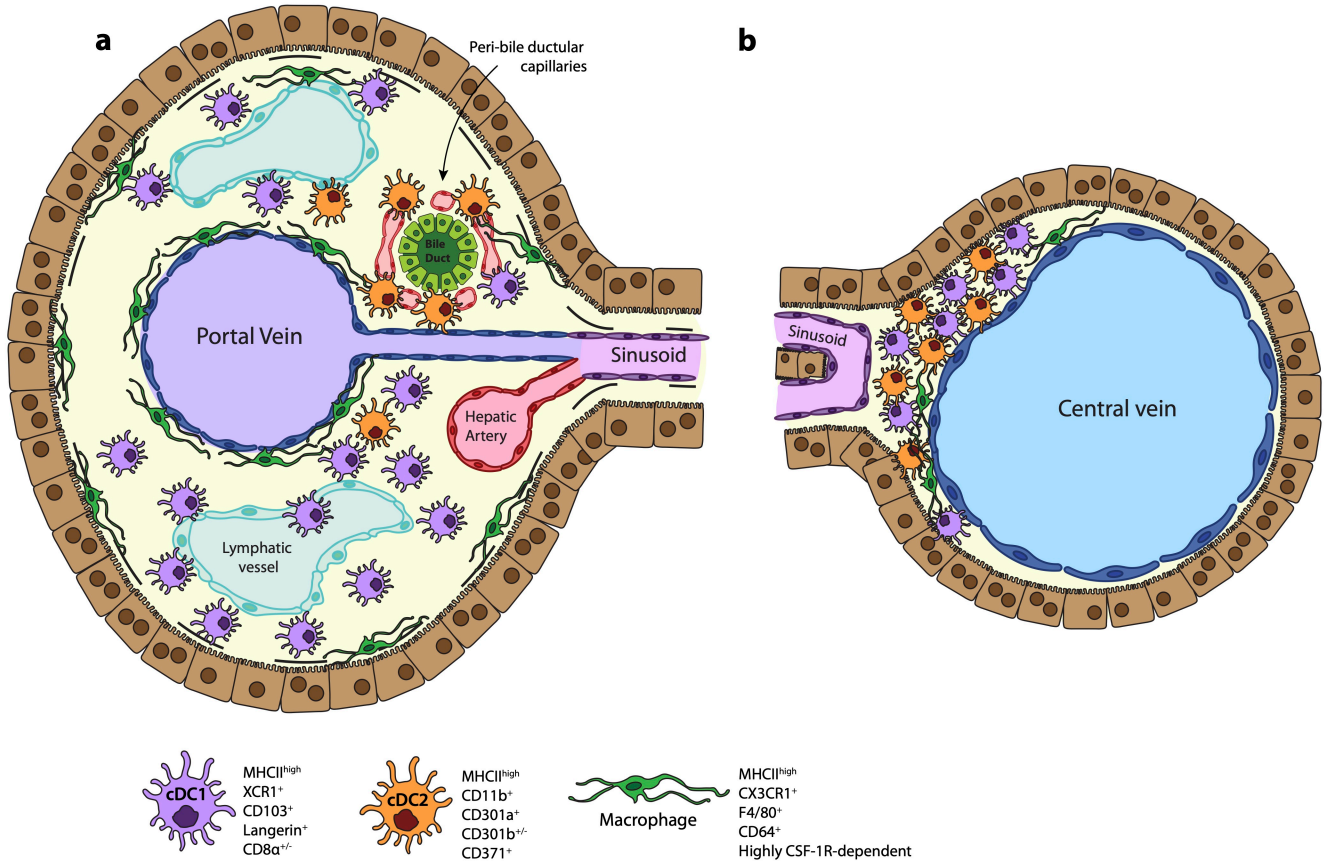
a



Supplementary figure 2: Steady state portal tracts contain cells with dendritic cell phenotype.
(a) Left panel: Z-projection of a portal tract cross section from an *Itgax*$^{cre\text{-}gfp}$ mouse liver in the steady state, stained to identify Kupffer cells, portal tract macrophages, and dendritic cells; (DAPI – grey; EGFP – green; CD64 – blue; CX3CR1 – red; scale bar 50µm). PV indicates portal vein; BD indicates bile duct. Right panels: magnified images of ROI from left panel, showing CD64^{+}CX3CR1^{-} Kupffer cells, CD64^{+}CX3CR1^{+} portal tract macrophages and CD11c^{+}CD64^{-}CX3CR1^{-} dendritic cells (green – arrowhead) contained within the portal tract; scale bar 25µm. Images are representative of at least n = 4 mice from 2 independent experiments.



Supplementary figure 3: Clusters of MHCII^{high} cells composed of CX3CR1^{+} macrophages, XCR1^{+} cDC1s and CD301a^{+} cDC2s are located adjacent to the central veins in the steady state liver.
(a) Left panel: central vein cross section from an Alb-Cre x *ROSA*$^{mT/mG}$ mouse liver in the steady state, stained to identify MHCII^{+} cells (DAPI – blue; GFP – green; tdTomato – red; MHCII – white; scale bar 100μm). Right panel: Magnified view of the ROI indicated in the left panel, illustrating a cluster of MHCII^{high} cells between the final layer of hepatocytes and the central vein endothelium; scale bar 25μm. **(b-d)** Left panels: central vein cross section from *Cx3cr1*$^{+/gfp}$ **(b)**, *Xcr1*$^{+/venus}$ **(c)**, and C57BL/6 (stained for CD301a) **(d)** mouse livers in the steady state; scale bars 100 μm. Right panels: higher magnification images of ROIs shown in left panels, illustrating CX3CR1^{+} macrophages **(b)**, XCR1^{+} cDC1s **(c)** and CD301a^{+} cDC2s **(d)** closely associated with the same central vein; scale bar 25μm. Images are representative of n = 4 mice from 2 independent experiments.



Supplementary Figure 4: Study summary showing the distinct myeloid cell subsets detected in portal tracts and their distribution throughout this compartment.
(a) Our findings suggest that portal tracts represent a unique immune environment packed with a dense and hetero-geneous population of MHCII^{high} cells with potential APC function, composed of at least 3 distinct subsets occupying distinct niches: i) a CSF1R-dependent subset of F4/80^{+} CD64^{+} CD11c^{-} CD11b^{+} CX3CR1^{high} macrophages, which are positioned along the portal vein branch, the bile duct and the edge of the portal tract; ii) a CSF1R-independent subset of F4/80^{-} CD64^{-} CD11c^{+} CD11b^{-} XCR1^{+} cells, identified as cross-presenting cDC1s preferentially associated with lymphatic vessels (while most of these cDC1s express CD103, a minority co-express CD8α); and iii) a CSF1R-independent subset of CD11c^{+} CD11b^{+} CD103^{-} F4/80^{-} cells identified as type B cDC2s, characterised by their high expression of CD301a and Clec12A and positioned in the portal interstitium close to the bile duct and hepatic artery. These myeloid subsets are arranged in an extended interconnected cellular network within the portal tracts and interact with each other. **(b)** Our findings also demonstrate that CX3CR1^{+} macrophages, XCR1^{+} cDC1s, and CD301a^{+} cDC2s form isolated clusters in the region between the final layer of hepatocytes and the endothelium of large central veins.