Supplementary materials and method

Buffers

Staining buffer for flow cytometry - PBS with 0,5%BSA and 2mM EDTA

- 1L PBS pH 7.2
- 5g bovine serum albumin (BSA)
- 4mL 0.5M EDTA

TBS wash buffer pH 7,5 (room temperature)

- *25mM TRIS-HCL*
- *150mM NaCl*

10 X Tris buffer (10X TBS):

- 24 g Tris-HCl (Tris(hydroxymethyl)aminomethane)
- 88 g NaCl (Sodium Chloride)
- 800 ml MilliQ water
- Adjust pH 7.5 with HCl.
- Adjust water up to 1L.
- Dilute 1:10 to make 1X TBS.

TBST wash buffer (room temperature)

- 25mM TRIS-HCl, PH 7.5
- 150 mM NaCl
- 0.05% Tween 20 (v/v)
- Add 500µL Tween to 1L 1X TBS to make TBST.

10X Citrate Buffer for Antigen Retrieval/Epitope retrieval

- 29,4g Tri-sodium citrate (dihydrate)
- Adjust to pH 6 with HCl, adjust volume to 1L with MilliQ water.
- Dilute 1:10 to make 1X Citrate buffer.

Supplementary Table 1: Antibodies and their dilution for antibody cocktail for flow cytometry

Panel	Marker	Fluorochrome conjugated	Clone	Product number/ Producent	Dilution in cocktail
Panel 1 and 2	CD3	BV510	145-2C11	100353/BioLegend	1:200
Panel 1 and 2	CD19	BV510	6D5	115546/ BioLegend	1:200
Panel 1 and 2	NK 1.1	BV510	PK136	108738/ BioLegend	1:200
Panel 1 and 2	F4/80	APC	BM8	123116/ BioLegend	1:100
Panel 1 and 2	CD11c	BV650	N418	117339/ BioLegend	1:200
Panel 1 and 2	CD11b	PE-Cy7	M1/70	101216/ BioLegend	1:200
Panel 1 and 2	Ly6C	BV421	HK1.4	128031/ BioLegend	1:100
Panel 1 and 2	MHC-II	PE-Dazzle 594	M5/114.15.2	107648/ BioLegend	1:300
Panel 1 and 2	CX3CR1	PerCP-Cy-5.5	SA011F11	149010/ BioLegend	1:200
Panel 1 and 2	Ly6G	AF700	1A8	127622/ BioLegend	1:50
Panel 1	CD45	APC-Fire750	30-F11	103154/ BioLegend	1:400
Panel 1	PDPN	FITC (AF488)	8.1.1	53-5381-82/Thermo scientific	1:200
Panel 1	CCR2	BV605	SA203G11	150615/BioLegend	1:400
Panel 1	Siglec F	PE	S17007L	155506/ BioLegend	1:200
Panel 1	CD115	BV711	AFS98	135515/ BioLegend	1:100
Panel 2	CD45	BV711	30-F11	103147/ BioLegend	1:200
Panel 2	CD80	BV605	16-10A1	104729/ BioLegend	1:400
Panel 2	CD86	AF488	GL-1	105018/ BioLegend	1:200
Panel 2	CD206	PE	C068C2	141706/ BioLegend	1:200
Panel 2	CD40	APC-Fire 750	3/23	124632/ BioLegend	1:100

Supplementary Table 2: Antibodies used for immunofluorescence labelling of salivary gland cryo-sections.

	Primary antibody cocktail			Corresponding secondary antibody cocktail		
	Antibody (clone)	**Product number/ Producent**	**Concentration of antibodies and incubation time**	**Antibody (AF* fluorochrome conjugate)**	**Product number/ Producent**	**Concentration of antibodies and incubation time**
F4/80, PDPN and CD206 staining	F4/80	122603/ BioLegend	1:200, 1hour	Goat anti-rat IgG (AF546)	A11081/ Invitrogen, Thermo Fisher Scientific	1:1000, 30min
	PDPN	14-5381-85/ Invitrogen, Thermo Fisher Scientific	1:75, 1hour	Goat anti-Syrian hamster IgG (AF488)	A21110/ Invitrogen, Thermo Fisher Scientific	1:1000, 30min
	CD206	AB64693/ Abcam	1:750, 1hour	Goat anti-Rabbit IgG (AF647)	A21245/ Invitrogen, Thermo Fisher Scientific	1:1000, 30min
CD45, B220 and CD3 staining	B220	MAB 1217/ RD System	1:800, 30min	Goat anti-rat IgG (AF546)	A11081/ Invitrogen, Thermo Fisher Scientific	1:1000, 30min
	CD3	A0452/ DAKO	1:400, 30min	Goat anti-Rabbit IgG (AF488)	A11034/ Invitrogen, Thermo Fisher Scientific	1:2000, 30min
	CD45-APC**	17-0451-82/ Invitrogen, Thermo Fisher Scientific	1:50, 1hour	NA	NA	NA

*AF; Alexa Fluor™

**CD45-APC; Fluorochrome direct conjugated to antibody, incubation of this antibody was performed in a separate step after B220/CD3 antibody incubation and their corresponding secondary antibody incubation.

Supplementary Table 3: Overview of antibodies, their concentration and OPAL fluorophore applied.

Staining order	Antibody	Product number/ Producent	Concentration of antibodies and incubation time	Polymer HRP detection kit	OPAL Fluorophore
1	PDPN	14-5381-85/ Invitrogen, Thermo Fisher Scientific	1:250, 30 min	Polink-2 Plus HRP Anti- Syrian Hamster DAB Detection Kit (D86-18, Golden Bridge International)	OPAL 570 (1:100) SKU FP1488001KT / Akoya Biosciences
2	F4/80	AB111101/ Abcam	1:50, Over night	Rabbit pAb Envision+ system-HRP/DAB anti rabbit detection kit (K4003, DAKO)	OPAL 520 (1:100) SKU FP1487001KT / Akoya Biosciences
3	CD206	AB64693/ Abcam	1:1000, 30min	Rabbit pAb Envision+ system-HRP/DAB anti rabbit detection kit (K4003, DAKO	OPAL 690 (1:100) SKU FP1497001KT / Akoya Biosciences
4	CX3CR1	AB217291/ Abcam	1:250, 30min	Rabbit pAb Envision+ system-HRP/DAB anti rabbit detection kit (K4003, DAKO	OPAL 780 (1:50) SKU FP1501001KT / Akoya Biosciences
5	Opal DAPI	SKU FP1490/ Akoya Biosciences	9µL DAPI + 100µL 1X TBS, 30min		

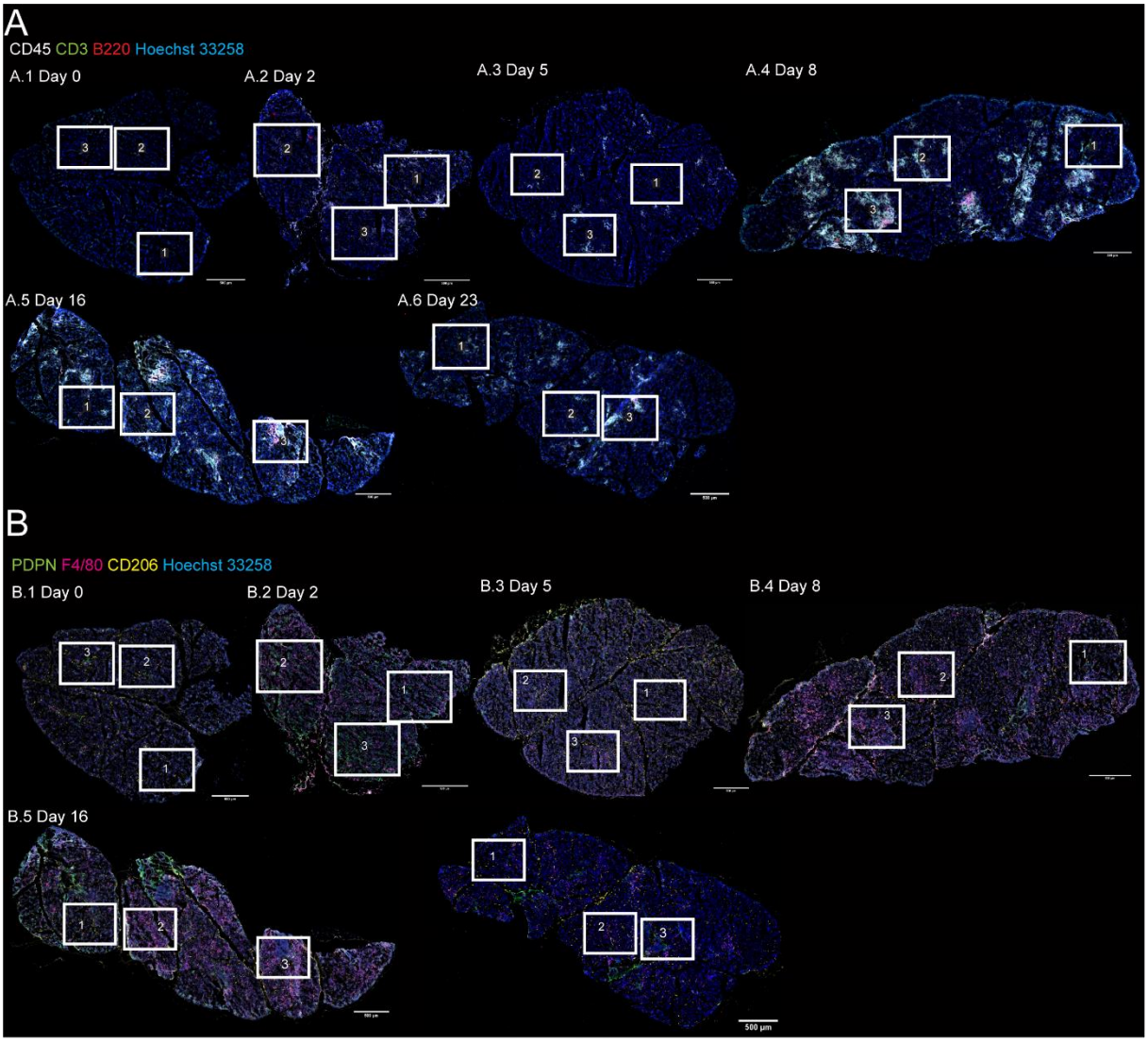
Supplementary Table 4: Overview over NZBW-F1 mice.

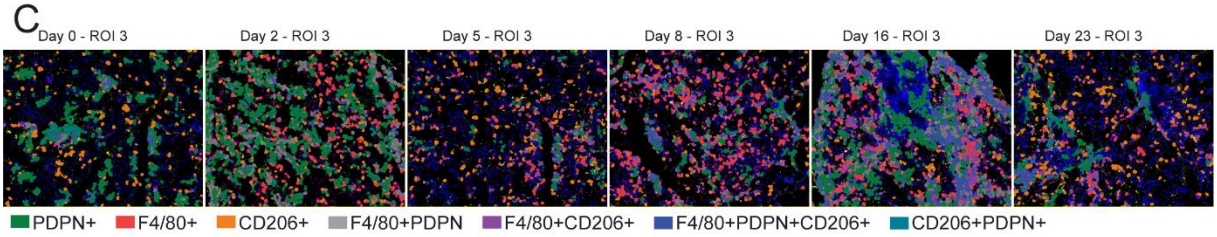
		dsDNA autoantibody	Age	Proteinuria
n(AB-)=3	J01	AB-	22wo	Neg
	J02	AB-	25 wo	Neg
	J04	AB-	25 wo	Neg
n(AB+)=4	J03	AB+	25 wo	Neg
	J11	AB+	33 wo	Neg
	J18	AB+	34 wo	Pos
	J13	AB+	27 wo	Pos

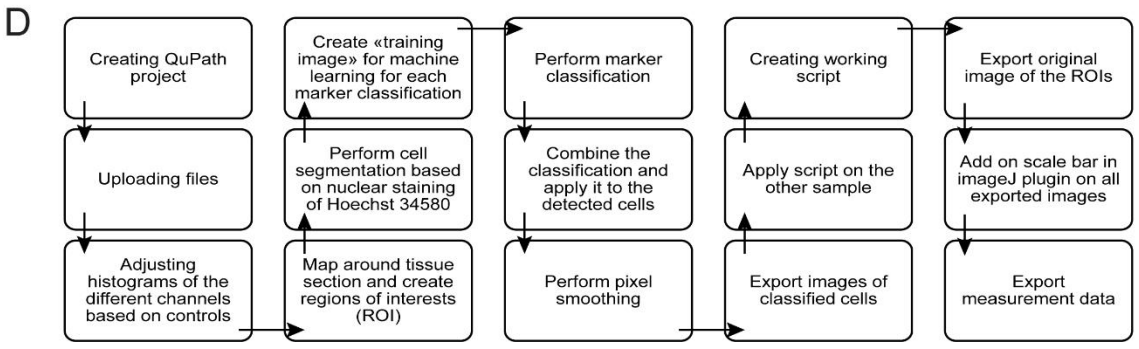
Supplementary Table 5: TaqMan probes for qPCR of mRNA from Thermo Fisher Scientific

Gene	Cat. Nr	Gene	Cat. Nr	Gene	Cat. Nr
Ccl21	Mm03646971_gh	*Ifny*	Mm99999071-m1	*Mx1*	Mm00487796-m1
Cd169	Mm00488332_m1	*Il10*	Mm99999062_m1	*Pdpn*	Mm01348912_g1
Cd206	Mm00711660_m1	*Il18*	Mm00434226-m1	*Tbp*	Mm00446973_m1
Cx3cr1	Mm02620111_s1	*Il1B*	Mm00434228-m1	*TgfB*	Mm01178820_m1
Ccxcl13	Mm00444534_m1	*LtB*	Mm00434774_g1	*Tnf*	Mm00443258-m1
Foxp3	Mm00475162-m1	*LtBr*	Mm00440235_m1		

Supplementary figures

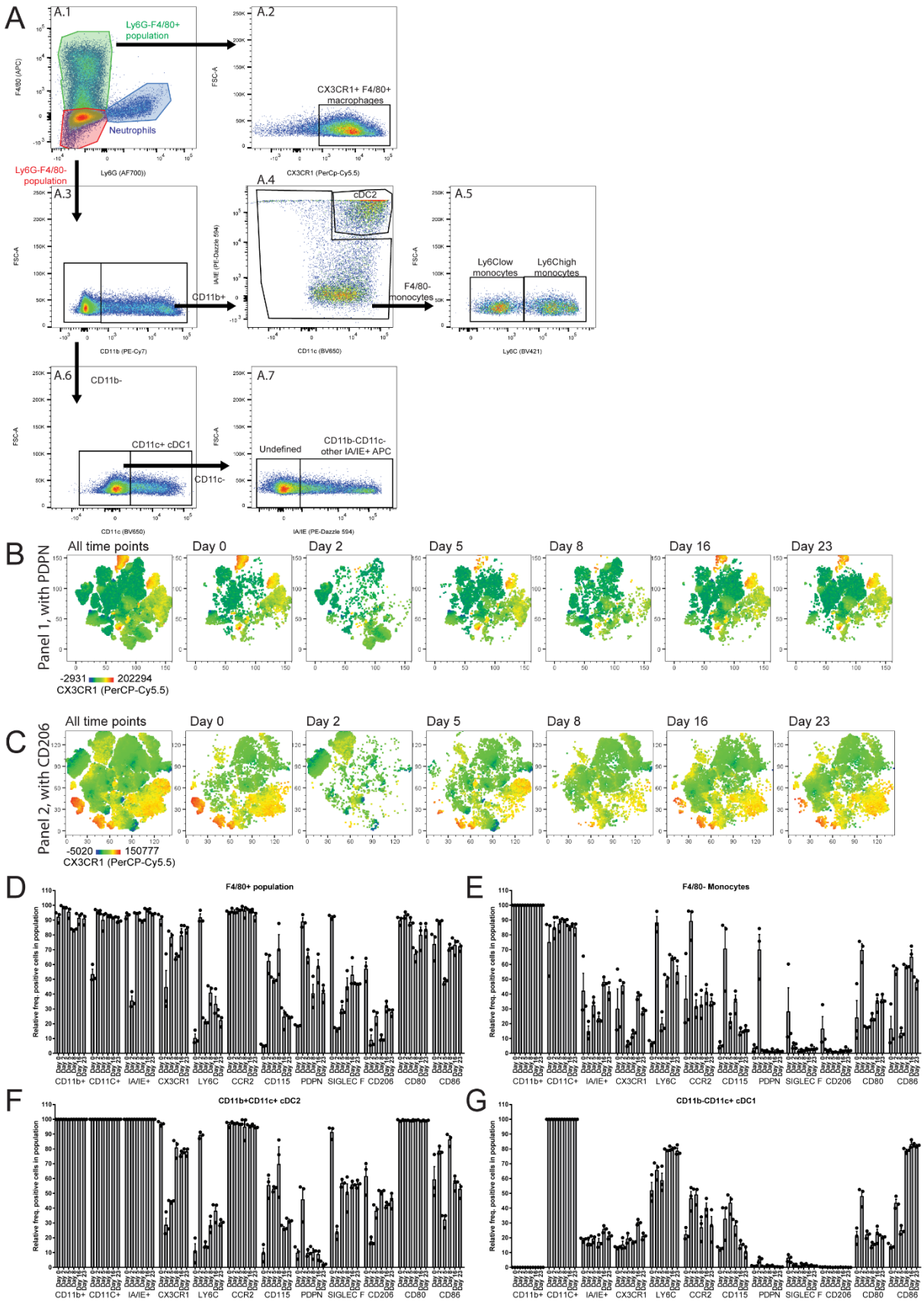






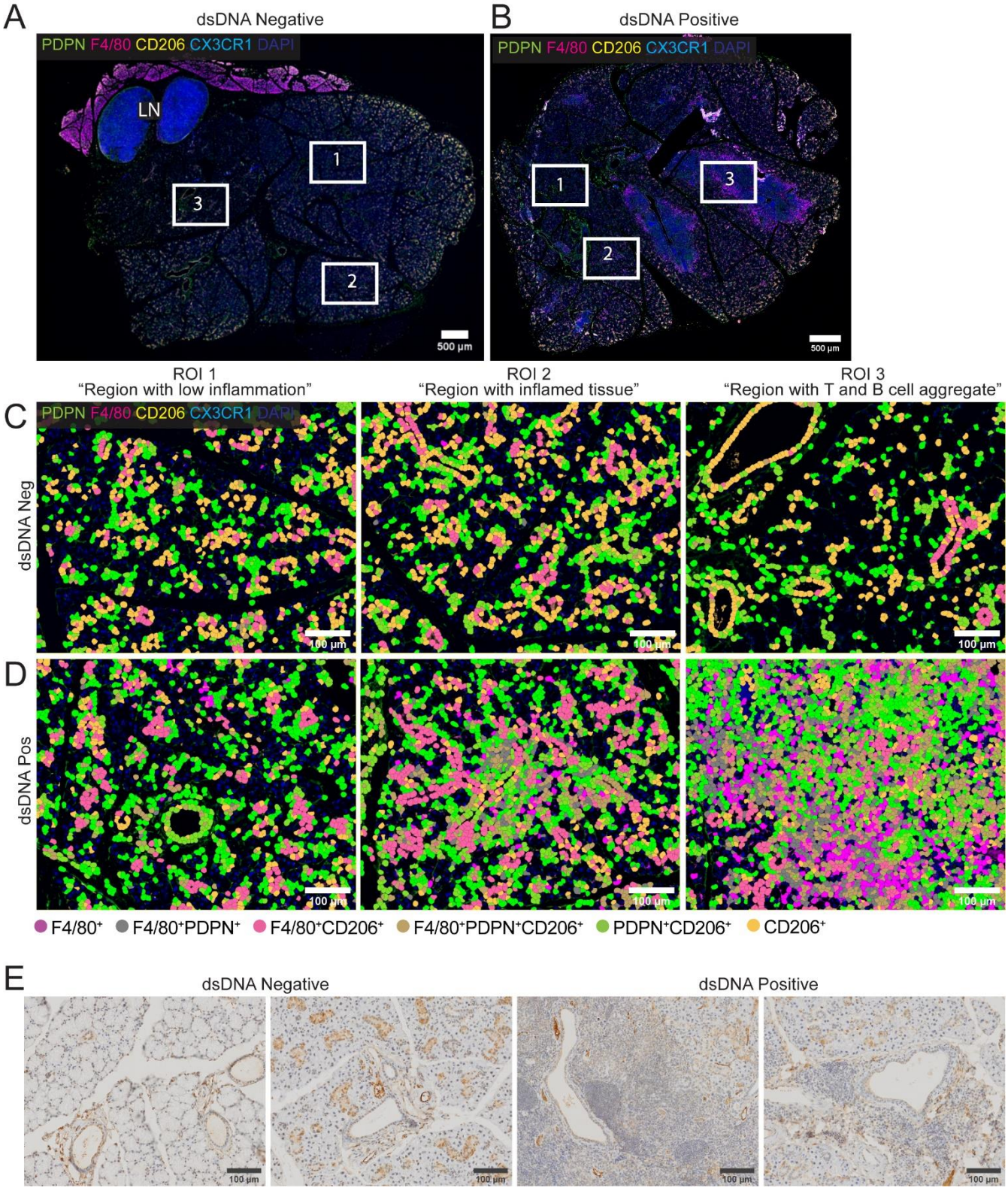
Supplementary figure 1. Immunofluorescence labelling and segmentation analysis.

Representative overview images over the A] CD45, CD3 and B220 labelling and B] PDPN, F4/80 and CD206 labelling of the salivary gland serial cryosections, including the localization of the three regions of interest (ROI). The different ROI are marked with numbered white boxes, where each number correspond to one of the three ROIs. 1 indicates ROI1 – Region with low inflammation, 2 indicates ROI2 – Region with inflammation, and 3 indicate ROI3 – Region with T and B cell aggregation. The aggregation of B and T cells, together with the infiltration of non-lymphocyte CD45+ cells, was used to allocate different ROIs. Scale bare C] Segmentation analysis of F4/80, PDPN and CD206 IF labelling. Segmentation is shown as an overlay of the original image. D] Analysis pipeline of fluorescence labelled salivary gland sections with QuPath V-0.5.1.


A
A.1
Ly6G-F4/80+ population
F4/80 (APC)
Ly6G (AF700))
Neutrophils
A.2
FSC-A
CX3CR1 (PerCp-Cy5.5)
CX3CR1+ F4/80+ macrophages
Ly6G-F4/80- population
A.3
FSC-A
CD11b (PE-Cy7)
CD11b+
A.4
IA/IE (PE-Dazzle 594)
CD11c (BV650)
cDC2
A.5
FSC-A
Ly6C (BV421)
F4/80- monocytes
Ly6Clow monocytes
Ly6Chigh monocytes
A.6
CD11b-
FSC-A
CD11c (BV650)
CD11c+ cDC1
CD11c-
A.7
FSC-A
IA/IE (PE-Dazzle 594)
Undefined
CD11b-CD11c- other IA/IE+ APC
B
All time points
Day 0
Day 2
Day 5
Day 8
Day 16
Day 23
Panel 1, with PDPN
-2931
202294
CX3CR1 (PerCP-Cy5.5)
C
All time points
Day 0
Day 2
Day 5
Day 8
Day 16
Day 23
Panel 2, with CD206
-5020
150777
CX3CR1 (PerCP-Cy5.5)
D
F4/80+ population
Relative freq. positive cells in population
CD11b+ CD11C+ IA/IE+ CX3CR1 LY6C CCR2 CD115 PDPN SIGLEC F CD206 CD80 CD86
E
F4/80- Monocytes
Relative freq. positive cells in population
CD11b+ CD11C+ IA/IE+ CX3CR1 LY6C CCR2 CD115 PDPN SIGLEC F CD206 CD80 CD86
F
CD11b+CD11c+ cDC2
Relative freq. positive cells in population
CD11b+ CD11C+ IA/IE+ CX3CR1 LY6C CCR2 CD115 PDPN SIGLEC F CD206 CD80 CD86
G
CD11b-CD11c+ cDC1
Relative freq. positive cells in population
CD11b+ CD11C+ IA/IE+ CX3CR1 LY6C CCR2 CD115 PDPN SIGLEC F CD206 CD80 CD86

Supplementary figure 2. Flow gating strategy over the myeloid cell population and tSNE plot analysis.

A] Gating strategy for identifying myeloid cell populations in the salivary gland during LucAdV5 infection. Shown is a representative gating strategy for myeloid cell populations from the salivary gland based on combining all time points. The myeloid cell population was defined as live $CD45^+$, $CD19^-$, $CD3^-$ and $NK1.1^-$ cells and were gated according to this approach: FSC-A VS. SSC-A, FSC-H VS. FSC-A, FSC-A VS CD45, FSC-A VS live/dead and dump channel (CD19, CD3, NK.1.1). The following populations are located in the respective gates; A.1] Neutrophils and $F4/80^+$ macrophage/monocyte population, A.2] $CX3CR1^+$ $F4/80^+$ macrophages/monocytes, A.4] $CD11b^+CD11c^+$ IA/IE^+DC2 and Monocytes, A.5] Ly6Chigh and Ly6Clow monocytes, A.6] $CD11b^-CD11c^+$ DC1, A.7] Other $CD11b^-IA/IE^+$ antigen-presenting cells (APC) and $CD11b^-F4/80^-CD11c^-$ IA/IE-undefined cell population. tSNE-plot with heatmap of CX3CR1 expression in B] panel 1 – containing PDPN and in C] panel 2 -containing CD206. The relative frequency of the following markers: CD11b, CD11c, IA/IE (MHC-II), CX3CR1, Ly6C, CCR2, CD115, PDPN, Siglec-F, CD206, CD80 and CD86 were analyzed by flow cytometry in the different myeloid cell populations D] $F4/80^+$ macrophages, E] $F4/80^-$ monocytes, F] $CD11b^+CD11c^+$ cDC2 and G] $CD11b^-CD11c^+$ cDC1.


A
dsDNA Negative
PDPN F4/80 CD206 CX3CR1 DAPI
LN
1
2
3
500 µm
B
dsDNA Positive
PDPN F4/80 CD206 CX3CR1 DAPI
1
2
3
500 µm
ROI 1
"Region with low inflammation"
ROI 2
"Region with inflamed tissue"
ROI 3
"Region with T and B cell aggregate"
C
dsDNA Neg
PDPN F4/80 CD206 CX3CR1 DAPI
100 µm
D
dsDNA Pos
100 µm
F4/80+
F4/80+PDPN+
F4/80+CD206+
F4/80+PDPN+CD206+
PDPN+CD206+
CD206+
E
dsDNA Negative
dsDNA Positive
100 µm

Supplementary figure 3. Immunofluorescence labelling and segmentation analysis of salivary glands from NZBW-F1.

Representative overview images over the PDPN, F4/80, CD206 and CX3CR1 labelling of the NZBW-F1 salivary gland sections from anti-dsDNA autoantibody A] negative and B] positive mice. These overview images include the localization of the three regions of interest (ROI). Segmentation analysis of F4/80, PDPN and CD206 IF labelling. Segmentation is shown as an overlay of the original image of C] anti-dsDNA autoantibody negative and D] anti-dsDNA autoantibody positive mice. Labelling of CX3CR1 was not added to the segmentation analysis. E] Representative immunohistochemistry images of CX3CR1 labelling of salivary gland tissues.