Supplement

Aging drives organ-specific alterations of the inflammatory microenvironment guided by immunomodulatory mediators in mice

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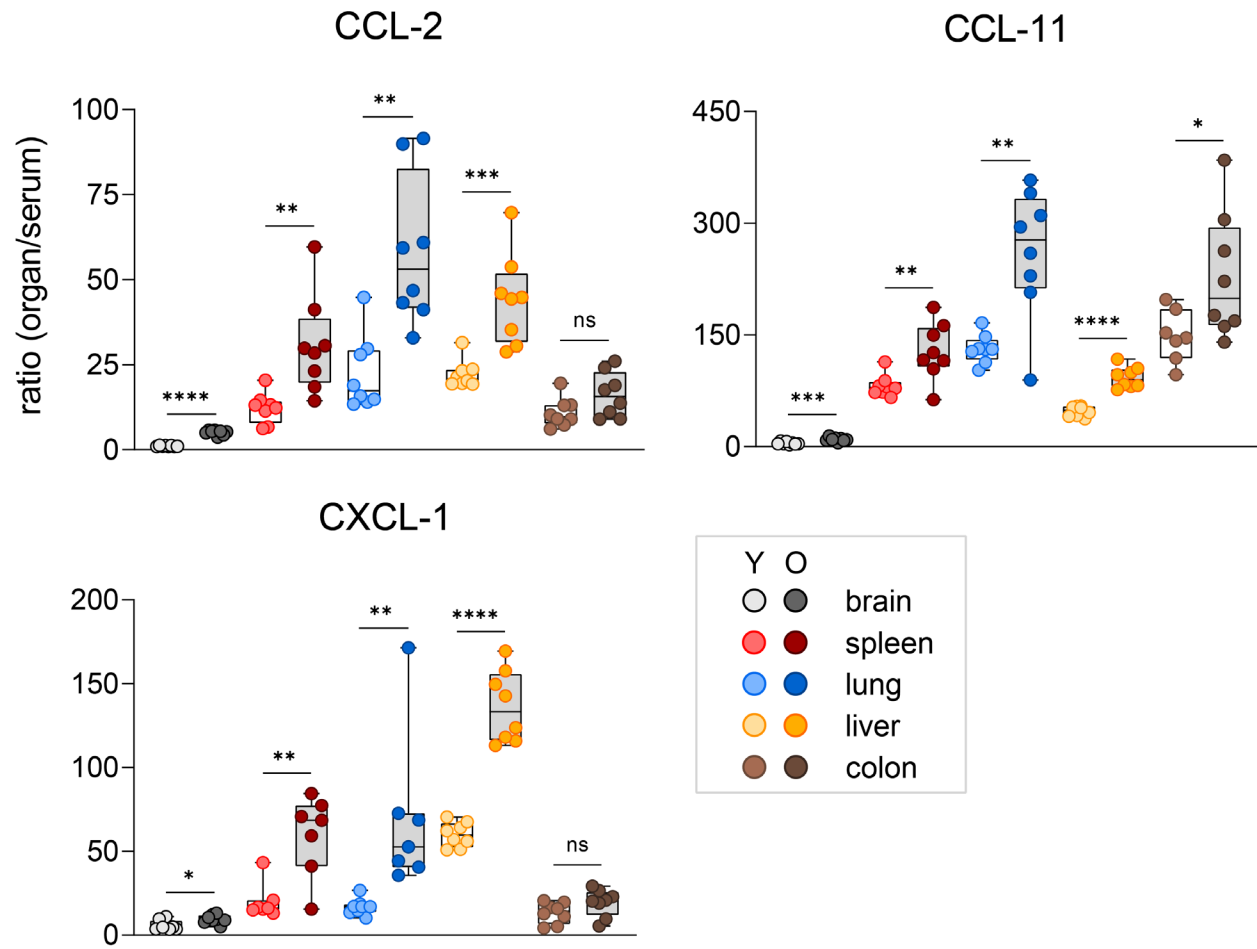
Figure S1 – Effect of aging on chemokine ratio between serum and organs

Ratio of organ and serum levels of MCP-1/CCL-2, eotaxin/CCL-11 and NAP-3/CXCL-1 were calculated for brain, spleen, lung, liver and colon from young and old C57BL/6 mice. Results are given as individual values and presented in a box and whisker plot. Number of biological replicates: n = 8. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001, ns = not significant.
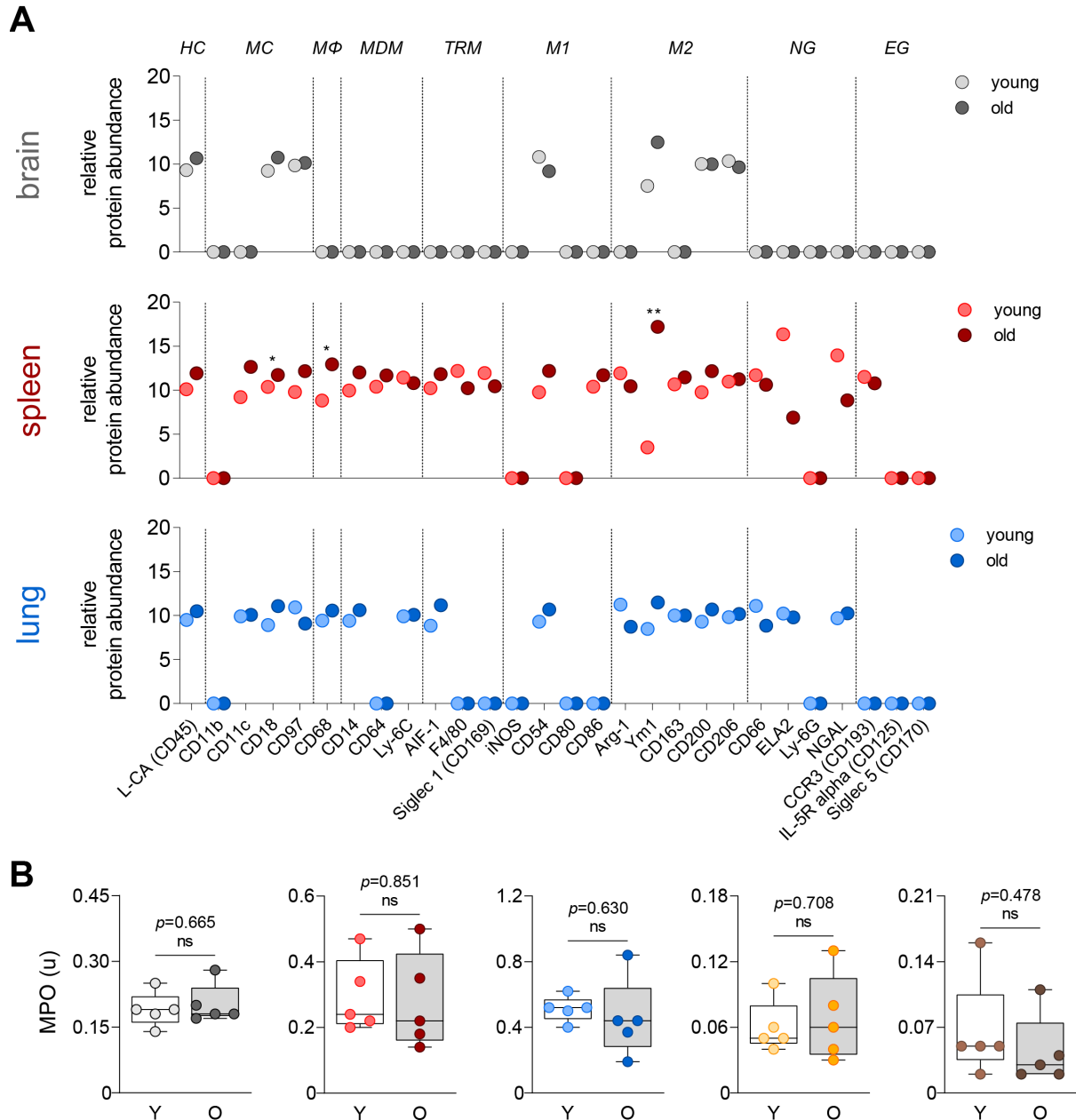
A

B

Figure S2 – Aging changes immune cell populations within organs
A) Marker proteins of different immune cell populations were analyzed and clustered based on proteome data for brain, spleen and lung samples of young (4 months) and old mice (18 months). Original data was gathered by Yu *et al.* (2020).[1] All values are given as means of scaled abundance. Values equal to zero mean that proteins were not detected. Markers were clustered as follows: HC – hematopoietic cells, MC – myeloid cells, MΦ – macrophages, MDM – monocyte-derived macrophages, TRM – tissue-resident macrophages, M1 – classically-activated (M1) macrophages, M2 – alternatively-activated (M2) macrophages, NG – neutrophilic granulocytes and EG – eosinophilic granulocytes. **B)** Activity of myeloperoxidase (MPO) was assessed in brain, spleen, lung, liver, and colon of young and old mice. Results (units) are given as individual values and presented in a box and whisker plot. Number of biological replicates: n = 5 (except for spleen (young): n=4). * p ≤ 0.05, ** p ≤ 0.01, ns = not significant.

References

1. Yu Q, Xiao H, Jedrychowski M, *et al.* Sample multiplexing for targeted pathway proteomics in aging mice. *Proc. Natl. Acad. Sci. U. S. A.* 2020; **117**, 9723–9732-.
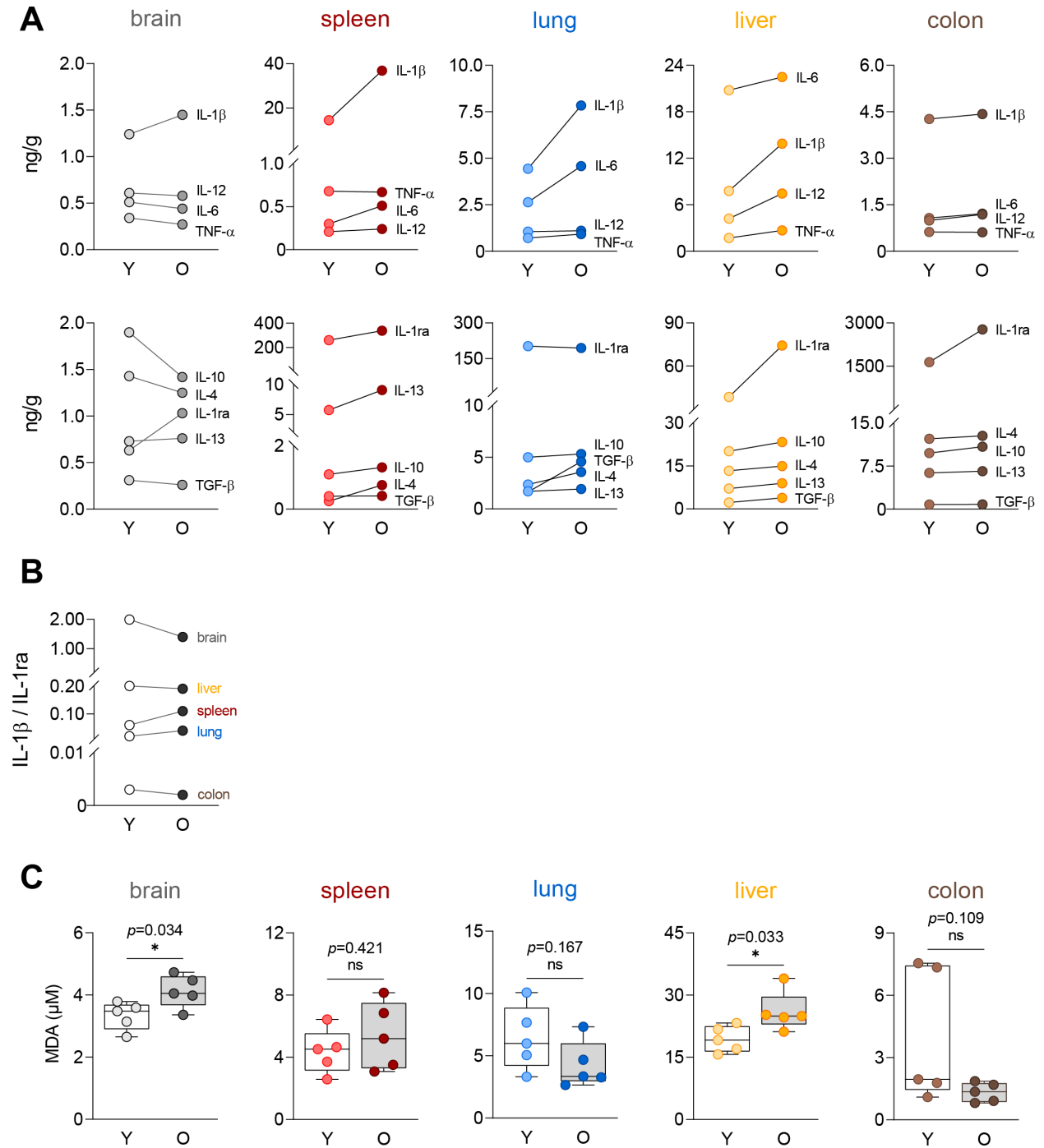
Figure S3 – Aging effects pro- or anti-inflammatory cytokine release depending on the type of organ

A) Levels of pro-inflammatory (upper row) and anti-inflammatory cytokines (lower row) are presented as means (ng/g of organ) and age-related trend between young (Y) and old (O) is indicated. **B)** Ratio of IL-1β and IL-1ra was calculated for each organ, and the change from mean of young (Y) to mean of old (O) is shown. Number of biological replicates: n = 8. **C)** Level of malondialdehyde as marker for lipid peroxidation. Number of biological replicates: n = 5. * p ≤ 0.05, ns = not significant.

		brain (Y)			brain (O)			spleen (Y)			spleen (O)			lung (Y)			lung (O)		
COX	PGD$_2$	4.4	±	0.3	5.5	±	0.3	751.4	±	42.1	580.3	±	39.5	17.1	±	2.1	25.7	±	3.0
	PGE$_2$	6.8	±	0.2	10.4	±	0.6	674.1	±	46.0	528.8	±	44.2	295.2	±	58.7	189.3	±	38.0
	PGF$_{2\alpha}$	24.6	±	1.2	30.3	±	1.7	134.2	±	7.7	195.1	±	12.0	217.8	±	28.0	188.9	±	24.9
	TXB$_2$	16.9	±	1.2	18.1	±	1.3	649.4	±	89.7	630.7	±	59.3	142.3	±	24.5	153.1	±	24.5
5-LOX	LTB$_4$	6.6	±	0.5	8.9	±	0.7	6.0	±	1.2	7.9	±	1.1	9.2	±	0.7	12.7	±	1.3
	t-LTB$_4$	5.1	±	0.7	7.0	±	0.7	19.4	±	0.5	33.0	±	3.7	13.2	±	1.1	23.1	±	2.9
	5-HEPE	1.7	±	0.3	2.1	±	0.3	2.5	±	0.3	1.3	±	0.2	6.9	±	0.4	5.7	±	0.9
	5-HETE	117.1	±	9.4	134.6	±	12.7	40.8	±	3.2	35.0	±	4.5	360.8	±	47.9	316.6	±	69.7
SPM	LXA$_4$	0.26	±	0.03	0.39	±	0.04	5.15	±	1.32	1.69	±	0.23		n.d.			n.d.	
	AT-LXA$_4$	1.54	±	0.12	2.24	±	0.21	5.10	±	1.04	3.96	±	0.86	2.82	±	0.57	3.33	±	1.16
	LXB$_4$	0.38	±	0.04	0.56	±	0.05		n.d.			n.d.			n.d.			n.d.	
	MaR1	0.43	±	0.07	0.60	±	0.08	1.19	±	0.09	2.73	±	0.37	0.93	±	0.18	1.33	±	0.24
	MaR2	1.38	±	0.16	1.88	±	0.21		n.d.			n.d.		1.96	±	0.28	2.39	±	0.32
	PD1	0.36	±	0.10	2.06	±	0.27	1.52	±	0.13	1.71	±	0.23	0.94	±	0.07	1.19	±	0.11
	AT-PD1	1.98	±	0.31	2.86	±	0.37	1.40	±	0.11	1.66	±	0.19	1.01	±	0.13	1.02	±	0.09
	PDX	5.25	±	0.75	7.92	±	1.00	3.51	±	0.25	3.94	±	0.47	1.47	±	0.20	1.60	±	0.13
	RvD1	0.86	±	0.10	1.37	±	0.21	0.95	±	0.10	0.69	±	0.07	1.24	±	0.28	0.77	±	0.11
	RvD2	0.22	±	0.03	0.33	±	0.04		n.d.			n.d.			n.d.			n.d.	
	RvD4	0.27	±	0.06	1.49	±	0.22	1.87	±	0.40	0.25	±	0.10	3.57	±	1.03	2.88	±	0.76
	RvD5	3.99	±	0.57	5.47	±	0.65	2.21	±	0.09	2.31	±	0.24	0.59	±	0.09	0.89	±	0.08
12/15-LOX	14-HDHA	74.8	±	11.8	109.8	±	14.9	847.0	±	41.3	1448.4	±	106.5	233.1	±	16.6	354.2	±	42.3
	17-HDHA	150.5	±	27.6	215.2	±	33.6	86.1	±	5.5	104.8	±	11.1	107.7	±	11.0	92.3	±	13.3
	12-HEPE	1.8	±	0.4	3.0	±	0.4	429.9	±	28.1	560.0	±	34.6	88.8	±	6.6	164.1	±	31.2
	15-HEPE	1.2	±	0.3	1.9	±	0.4	15.5	±	1.4	11.1	±	1.2	9.6	±	1.0	8.4	±	0.9
	12-HETE	126.7	±	7.0	199.1	±	21.9	2081.3	±	97.0	2589.2	±	161.2	1394.5	±	81.1	2225.6	±	518.6
	15-HETE	258.3	±	13.2	351.7	±	48.2	882.5	±	71.5	950.4	±	83.9	654.3	±	76.7	540.7	±	107.0
monohydroxylated FA	4-HDHA	26.1	±	2.1	34.7	±	4.1	5.3	±	0.5	5.5	±	0.2	107.4	±	15.3	81.0	±	18.1
	7-HDHA	13.1	±	1.6	16.0	±	2.0	6.6	±	0.4	6.9	±	0.5	23.9	±	3.0	21.3	±	4.0
	10-HDHA	23.5	±	3.2	31.6	±	4.6		n.d.			n.d.		29.0	±	3.1	24.2	±	2.7
	13-HDHA	49.2	±	8.0	67.7	±	9.9	52.3	±	4.2	59.7	±	4.0	78.7	±	9.2	60.5	±	6.2
	11-HEPE	0.9	±	0.2	1.4	±	0.2	10.7	±	1.1	7.8	±	0.4	10.0	±	1.5	7.2	±	0.6
	18-HEPE	3.6	±	1.0	5.3	±	1.0	7.3	±	1.5	3.6	±	0.4	17.4	±	3.0	12.5	±	2.9
	8-HETE	1.9	±	0.6	2.8	±	0.5	62.1	±	2.4	82.5	±	7.4	147.0	±	18.2	131.4	±	18.5
	11-HETE	96.3	±	3.1	120.5	±	19.7	430.7	±	24.1	481.0	±	29.1	392.5	±	33.3	362.5	±	38.4
	9-HODE	53.5	±	8.0	51.6	±	6.4		n.d.			n.d.		1207.2	±	154.0	893.9	±	131.9
	13-HODE	95.3	±	7.7	114.7	±	15.8		n.d.			n.d.		1565.2	±	139.1	1297.6	±	131.9
PUFA	AA	128373	±	11285	124336	±	8396	13054	±	1505	14600	±	1557	130638	±	26112	181062	±	36178
	EPA	6670	±	899	4833	±	289	2021	±	819	745	±	102	12708	±	1629	17457	±	3458
	DHA	23671	±	2706	22304	±	1850	7375	±	433	9207	±	604	6639	±	2261	11877	±	4557

Figure S4 – Lipid mediator signature profiles of brain, spleen and lung

Eicosanoid, docosanoid and PUFA levels were measured in whole lysates of brain, spleen and lung by UPLC-MS/MS (see methods). Values are given as means ± S.E.M. and represent raw data for **Fig. 4** (all values in ng of LM per g of organ). Number of biological replicates: n = 10, except spleen (Y/O): n = 7; n.d. = not detectable at a detection limit of 0.5 pg LM.

		liver (Y)			liver (O)			colon (Y)			colon (O)		
COX	PGD$_2$	7.1	±	1.1	7.4	±	1.7	37.6	±	6.7	50.5	±	10.7
	PGE$_2$	7.1	±	1.7	7.2	±	1.2	284.1	±	29.5	218.6	±	30.6
	PGF$_{2\alpha}$	13.7	±	1.7	14.8	±	2.5	228.4	±	20.6	194.4	±	21.3
	TXB$_2$	16.3	±	2.3	32.4	±	8.1	168.4	±	21.4	112.0	±	16.8
5-LOX	LTB$_4$	3.0	±	0.7	2.0	±	0.4	4.8	±	0.4	3.7	±	0.4
	t-LTB$_4$	3.8	±	0.8	2.3	±	0.5	7.3	±	1.5	4.8	±	0.5
	5-HEPE	13.5	±	2.9	7.0	±	0.9	8.5	±	1.0	5.7	±	0.7
	5-HETE	124.6	±	22.0	83.1	±	12.4	163.3	±	16.4	103.3	±	3.4
SPM	LXA$_4$		n.d.			n.d.		0.47	±	0.12	0.20	±	0.03
	AT-LXA$_4$	1.00	±	0.16	0.68	±	0.14	1.66	±	0.40	0.73	±	0.06
	LXB$_4$		n.d.			n.d.		1.30	±	0.16	1.03	±	0.14
	MaR1	0.51	±	0.15	0.12	±	0.05		n.d.			n.d.	
	MaR2	0.75	±	0.18	0.22	±	0.06	1.61	±	0.16	1.00	±	0.07
	PD1	0.77	±	0.20	0.29	±	0.05	1.20	±	0.34	0.78	±	0.04
	AT-PD1	1.70	±	0.35	0.84	±	0.18	0.79	±	0.08	0.56	±	0.04
	PDX	2.16	±	0.51	0.87	±	0.17	2.05	±	0.27	1.43	±	0.24
	RvD1	0.82	±	0.12	0.42	±	0.09	1.15	±	0.28	0.70	±	0.07
	RvD2		n.d.			n.d.			n.d.			n.d.	
	RvD4	1.03	±	0.20	0.34	±	0.08	0.92	±	0.20	0.59	±	0.04
	RvD5	0.93	±	0.12	0.70	±	0.15	1.56	±	0.16	0.80	±	0.04
12/15-LOX	14-HDHA	56.3	±	4.5	49.5	±	8.9	73.2	±	3.7	82.5	±	12.7
	17-HDHA	48.1	±	4.6	35.1	±	6.0	88.0	±	6.9	71.8	±	7.9
	12-HEPE	8.4	±	1.7	10.4	±	2.5	25.7	±	4.6	31.5	±	3.8
	15-HEPE	3.7	±	0.4	2.2	±	0.3	12.0	±	1.1	7.8	±	0.7
	12-HETE	85.1	±	15.1	92.8	±	18.3	296.8	±	24.9	393.0	±	43.1
	15-HETE	132.5	±	9.7	98.3	±	15.5	490.3	±	33.3	387.6	±	31.8
monohydroxylated FA	4-HDHA	42.8	±	9.3	19.7	±	3.1	19.7	±	2.0	12.4	±	1.2
	7-HDHA	20.8	±	2.4	19.3	±	2.9	12.5	±	0.9	9.2	±	0.9
	10-HDHA	22.0	±	1.9	17.1	±	3.0	14.7	±	1.3	11.0	±	1.1
	13-HDHA	35.9	±	2.6	26.0	±	4.5	61.3	±	5.5	47.8	±	5.0
	11-HEPE	4.1	±	0.4	2.8	±	0.4	12.5	±	0.8	8.4	±	0.8
	18-HEPE	8.1	±	0.8	4.4	±	0.6	19.1	±	2.4	10.8	±	1.0
	8-HETE	41.1	±	6.9	32.4	±	5.0	69.6	±	6.9	51.3	±	5.8
	11-HETE	67.4	±	10.6	54.0	±	7.6	353.1	±	21.8	278.3	±	22.3
	9-HODE	625.0	±	89.0	360.2	±	48.6	1240.5	±	334.0	990.6	±	85.8
	13-HODE	956.8	±	130.5	575.9	±	74.8	1538.2	±	215.9	990.6	±	85.8
PUFA	AA	103489	±	7830	136343	±	9775	290485	±	24794	278340	±	35439
	EPA	25329	±	2448	25692	±	2659	45141	±	5689	44854	±	6985
	DHA	24643	±	1873	27822	±	2376	42501	±	4441	42799	±	6011

Figure S5 – Lipid mediator signature profiles of liver and colon
Eicosanoid, docosanoid and PUFA levels were measured in liver and colon by UPLC-MS/MS (see methods). Values are given as means ± S.E.M. and represent raw data for **Fig. 4** (all values in ng of LM per g of organ). Number of biological replicates: n = 10, except liver (Y): n = 9; n.d. = not detectable at a detection limit of 0.5 pg LM.

lipid mediator class	brain (Y)		brain (O)		spleen (Y)		spleen (O)		lung (Y)		lung (O)	
	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%
prostaglandins	35.74	44.2	46.19	43.2	1559.71	69.1	1304.20	65.4	530.06	74.7	404.01	66.4
thromboxane	16.93	20.9	18.08	16.9	649.40	28.8	630.68	31.6	142.33	20.1	153.09	25.2
leukotrienes	11.65	14.4	15.97	15.0	25.39	1.1	40.88	2.0	22.39	3.2	35.86	5.9
lipoxins	1.80	2.2	2.63	2.5	10.25	0.5	5.66	0.3	2.82	0.4	3.33	0.5
maresins	1.81	2.2	2.48	2.3	1.19	0.1	2.73	0.1	2.88	0.4	3.72	0.6
protectins	7.59	9.4	12.83	12.0	6.43	0.3	7.32	0.4	3.42	0.5	3.80	0.6
D-series resolvins	5.35	6.6	8.65	8.1	5.03	0.2	3.25	0.2	5.40	0.8	4.55	0.7
SPMs	16.55	20.5	26.59	24.9	22.91	1.0	18.96	1.0	14.52	2.0	15.40	2.5

lipid mediator class	liver (Y)		liver (O)		colon (Y)		colon (O)	
	ng/g	%	ng/g	%	ng/g	%	ng/g	%
prostaglandins	27.88	46.0	29.42	41.7	550.20	74.1	463.44	78.4
thromboxane	16.30	26.9	32.43	45.9	168.39	22.7	112.04	19.0
leukotrienes	6.82	11.2	4.29	6.1	12.10	1.6	8.49	1.4
lipoxins	1.00	1.6	0.68	1.0	2.13	0.3	0.93	0.2
maresins	1.26	2.1	0.34	0.5	1.61	0.2	1.00	0.2
protectins	4.63	7.6	2.01	2.8	4.04	0.5	2.76	0.5
D-series resolvins	2.79	4.6	1.46	2.1	3.63	0.5	2.09	0.4
SPMs	9.67	15.9	4.48	6.3	11.41	1.5	6.79	1.1

Figure S6 – Levels of bioactive lipid mediators in organs of young and old mice
Levels of prostaglandins, thromboxane, leukotrienes, and SPM (lipoxins, maresins, protectins, D-series resolvins) in brain, spleen, lung, liver and colon were measured by UPLC-MS/MS. Mean values for each group of LM are presented in ng per g of organ and as percentage of all bioactive LM in the respective organ (values are basis for Fig. 4E). Number of biological replicates: n = 10, except spleen (Y/O): n = 7 / liver (Y): n = 9.
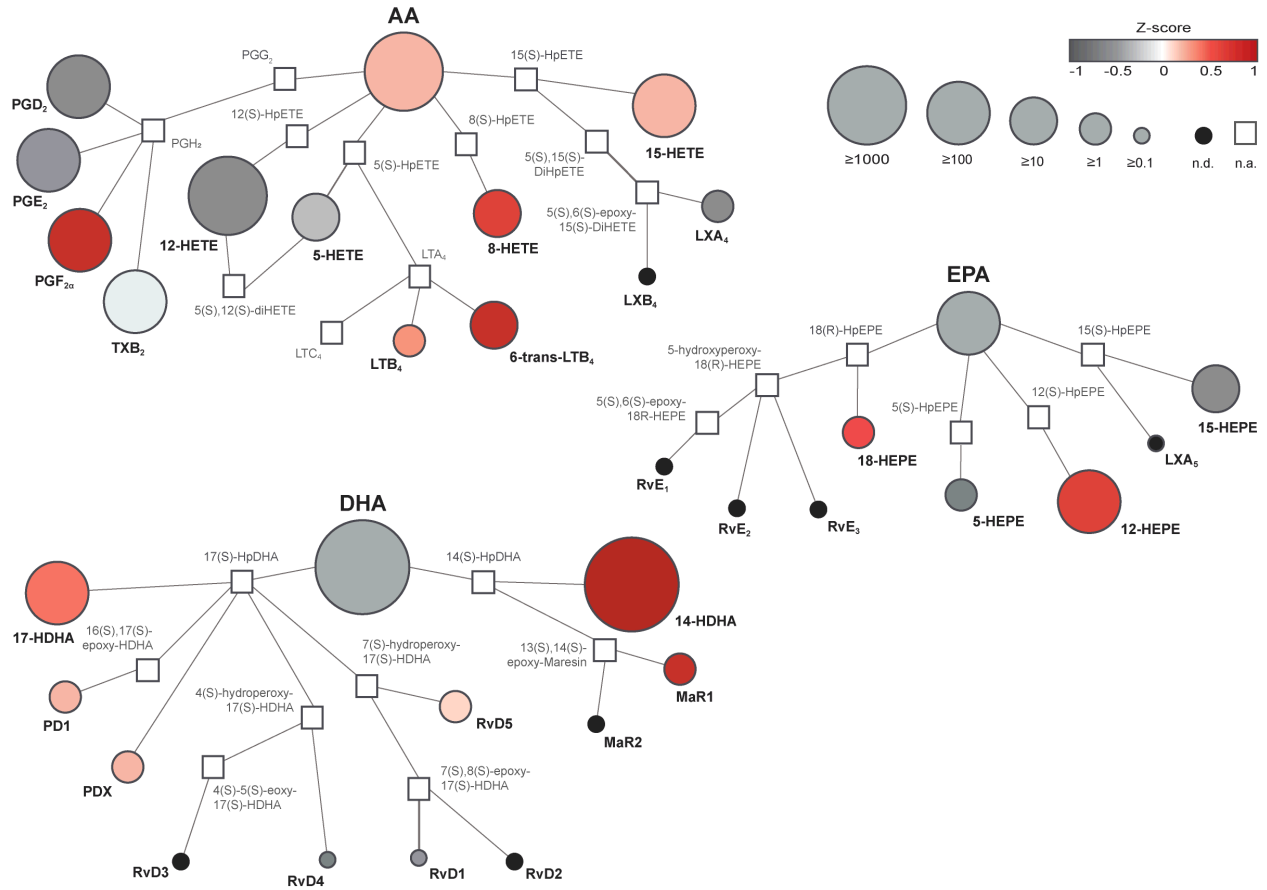
Figure S7 – Pathway analysis of PUFA-derived lipid mediators in the spleen
Network pathway analysis of AA, DHA and EPA and their corresponding LM was performed for spleen of young and old mice. In the network the age-mediated change in LM abundance from young to old is represented by color (Z-score) and absolute abundance of the respective LM in spleen from old mice is visualized (size of circle). Intermediate products that are not included in the analysis are marked with white squares, while LM below the limit of detection are shown in black. Number of biological replicates: n=7.
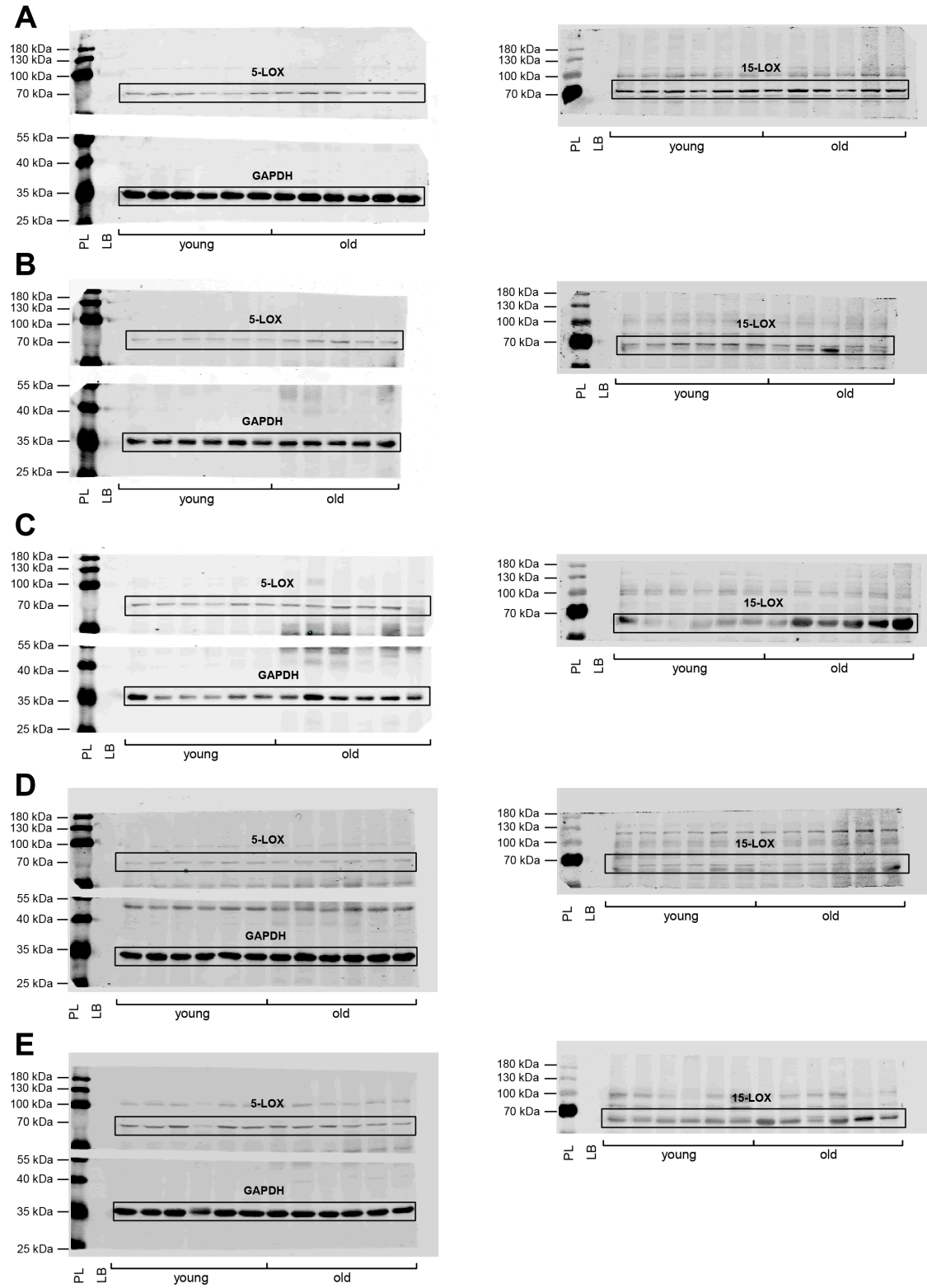
Schädel et al. (2021)

Figure S8 – Effect of aging on the expression of lipid mediator-biosynthetic enzymes
Representative full scans of Western blots of 5-LOX and 15-LOX in whole organ lysates of **A)** brain, **B)** spleen, **C)** lung, **D)** liver and **E)** colon from young and old mice. Molecular weight markers are indicated on the left and lanes containing protein ladder (PL) or loading buffer (LB) are marked. Black boxes highlight the bands that were used for quantification in **Fig. 5B, C.**