

Supplemental files

Profiling of microglia nodules in multiple sclerosis reveals propensity for lesion formation

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Supplementary table 1: Outliers removed from RNA sequencing analysis

Sequence ID		Diagnosis	Tissue	Age	Sex
s104343_001_003	94-116	Stroke	Microglia nodules	72	F
s104343_001_004	94-116	Stroke	NAWM	72	F
s104343_001_028	17-100	MS	NAWM	66	M

Supplementary table 2: Primers used for RT-qPCR on nodule tissue

Gene name	Forward sequence (5'-3')	Reverse sequence (5'-3')
EEF1A1	AAGCTGGAAGATGGCCCTAAA	AAGCGACCCAAAGGTGGAT
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGA
IGKC	CCATCTGTCTTCATCTTCCCG	ATCCACCTTCCACTGTACTTTG
IGHG1	GGTCAAAGGCTTCTATCCCAG	AGGCGTGGTCTTGTAGTTG
IGHG2	GGAGATGACCAAGAACCAGG	GGAGGTGTGGTCTTGTAGTTG
IGKV3-15	CCTCATCTATGGTGCATCCAC	TGCTGATGGTGAGAGTGAAC

Supplementary table 3: Top 50 upregulated DE genes in MS nodules vs MS nnNAWM. Differential expression was assessed using an empirical Bayes moderated t-test within limma's linear model framework including the precision weights estimated by voom and the consensus correlation between samples of the same donor. The differential expression analysis was performed both with and without a covariate for the estimated microglia content (in percent). Resulting p values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate.

Gene symbol	Gene name	logFC	P. Adj.	LogFC cor.	P. Adj. Cor.
RGS16	regulator of G protein signaling 16	10.186	2.27e-5	9.149	0.015
CXCL16	C-X-C motif chemokine ligand 16	10.074	2.11e-6	9.666	0.004
TNFRSF1B	TNF receptor superfamily member 1B	9.578	3.36e-6	9.079	0.006
C1QA	complement C1q A chain	9.451	1.40e-5	8.344	0.022
CTSC	cathepsin C	9.025	1.40e-5	7.611	0.033
MX1	MX dynamin like GTPase 1	8.935	1.40e-5	8.283	0.022
RGS1	regulator of G protein signaling 1	8.869	0.001	7.142	0.162
EML4-AS1	EML4 antisense RNA 1	8.846	1.21e-5	8.333	0.015
IL18	interleukin 18	8.773	1.21e-5	8.771	0.008
NCKAP1L	NCK associated protein 1 like	8.491	1.46e-5	7.309	0.033
SRP68	signal recognition particle 68	8.479	0.001	9.201	0.026
SLC7A5	solute carrier family 7 member 5	8.454	0.002	9.215	0.026
LCP1	lymphocyte cytosolic protein 1	8.352	0.001	7.297	0.162
APBB1IP	amyloid beta precursor protein binding family B member 1 interacting protein	8.350	0.003	7.614	0.147
C1QB	complement C1q B chain	8.315	0.001	7.831	0.037
ERCC5	ERCC excision repair 5, endonuclease	8.267	1.85e-4	8.427	0.026
SLCO2B1	solute carrier organic anion transporter family member 2B1	8.257	0.002	7.878	0.051
IGSF6	immunoglobulin superfamily member 6	8.171	0.001	7.135	0.111
CD84	CD84 molecule	8.169	0.001	5.701	0.342
WARS1	tryptophanyl-tRNA synthetase 1	8.121	0.002	7.574	0.156
VAMP8	vesicle associated membrane protein 8	8.111	0.001	6.343	0.270
XAF1	XIAP associated factor 1	8.090	0.001	7.407	0.156
SAT2	spermidine/spermine N1-acetyltransferase family member 2	8.049	0.006	8.251	0.153
SCIN	scinderin	7.971	0.019	6.159	0.402
GPRIN3	GPRIN family member 3	7.928	0.001	7.268	0.147
MED11	mediator complex subunit 11	7.922	0.001	7.171	0.170

LPL	lipoprotein lipase	7.916	0.002	5.355	0.395
FCER1G	Fc fragment of IgE receptor Ig	7.897	0.013	6.426	0.347
CAMKK2	calcium/calmodulin dependent protein kinase kinase 2	7.877	0.002	6.950	0.200
RPGR	retinitis pigmentosa GTPase regulator	7.790	1.85e-4	7.929	0.029
ARHGAP24	Rho GTPase activating protein 24	7.787	0.002	6.099	0.321
ARHGAP18	Rho GTPase activating protein 18	7.783	0.001	6.861	0.147
MRPL55	mitochondrial ribosomal protein L55	7.750	0.011	6.202	0.453
HLA-DPB1	major histocompatibility complex, class II, DP beta 1	7.735	0.008	7.106	0.203
PLEK	pleckstrin	7.697	0.003	5.824	0.321
CD14	CD14 molecule	7.690	0.002	6.245	0.231
LPAR6	lysophosphatidic acid receptor 6	7.646	0.001	7.031	0.051
EML4	EMAP like 4	7.638	0.004	7.184	0.147
TRIM52	tripartite motif containing 52	7.613	0.002	8.153	0.070
SERPINA1	serpin family A member 1	7.551	0.006	6.582	0.353
SLC2A5	solute carrier family 2 member 5	7.542	0.002	5.592	0.373
MPPE1	metallophosphoesterase 1	7.492	0.002	7.091	0.164
ETV5	ETS variant transcription factor 5	7.488	0.003	7.152	0.188
TANC1	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	7.484	0.009	7.899	0.093
DAGLB	diacylglycerol lipase beta	7.432	0.001	7.622	0.053
ITGAX	integrin subunit alpha X	7.412	0.006	6.118	0.352
LAPTM5	lysosomal protein transmembrane 5	7.410	0.029	6.363	0.334
RCOR1	REST corepressor 1	7.404	0.006	5.679	0.407
AXL	AXL receptor tyrosine kinase	7.396	0.003	6.047	0.342

Supplementary table 4: Upregulated DE genes in stroke nodules vs stroke nnNAWM. Differential expression was assessed using an empirical Bayes moderated t-test within limma's linear model framework including the precision weights estimated by voom and the consensus correlation between samples of the same donor. The differential expression analysis was performed both with and without a covariate for the estimated microglia content (in percent). Resulting p values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate.

Gene symbol	description	logFC	P. Adj.	LogFC cor.	P. Adj. Cor.
BICD2	BICD cargo adaptor 2	10.061	1.97e-5	9.803	2.85e-4
NEB	nebulin	9.979	3.37e-5	9.785	4.50e-4
HLA-B	major histocompatibility complex, class I, B	9.439	0.005	9.061	0.010
C1QB	complement C1q B chain	9.042	0.001	8.891	0.002
APOC1	apolipoprotein C1	8.731	0.005	8.497	0.009
SLC11A1	solute carrier family 11 member 1	8.227	0.005	7.755	0.010
ASCC3	activating signal cointegrator 1 complex subunit 3	7.989	0.006	8.115	0.013
DIS3L2	DIS3 like 3'-5' exoribonuclease 2	7.840	0.044	8.301	0.072
WASL	WASP like actin nucleation promoting factor	7.779	0.018	7.885	0.033
RPGR	retinitis pigmentosa GTPase regulator	6.599	0.037	6.644	0.073

Supplementary table 5: Top 50 upregulated DE genes in MS nodules vs stroke nodules. Differential expression was assessed using an empirical Bayes moderated t-test within limma's linear model framework including the precision weights estimated by voom and the consensus correlation between samples of the same donor. The differential expression analysis was performed both with and without a covariate for the estimated microglia content (in percent). Resulting p values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate.

Gene symbol	Gene name	logFC	P. Adj.	LogFC cor.	P. Adj. Cor.
MRPS34	mitochondrial ribosomal protein S34	8.639	0.002	8.948	0.031
ABCF3	ATP binding cassette subfamily F member 3	8.317	0.002	8.480	0.034
GTPBP6	GTP binding protein 6 (putative)	8.294	0.004	8.396	0.055
SEPTIN4-AS1	SEPTIN4 antisense RNA 1	8.016	0.002	7.928	0.031
KIN	Kin17 DNA and RNA binding protein	7.841	0.004	8.281	0.056
CYB5B	cytochrome b5 type B	7.780	0.004	7.880	0.056
CNPY3	canopy FGF signaling regulator 3	7.730	0.005	7.352	0.068
MFN2	mitofusin 2	7.693	0.010	7.812	0.068
NBR1	NBR1 autophagy cargo receptor	7.668	0.003	7.710	0.031
HLA-DRB5	major histocompatibility complex, class II, DR beta 5	7.643	0.002	7.632	0.037
COPG1	COPI coat complex subunit gamma 1	7.614	0.004	7.119	0.080
FABP5	fatty acid binding protein 5	7.601	0.002	6.942	0.056
ACBD5	acyl-CoA binding domain containing 5	7.540	0.004	7.955	0.055
TMEM147	transmembrane protein 147	7.528	0.013	7.198	0.110
FXD5	FXD domain containing ion transport regulator 5	7.504	0.004	7.183	0.068
CHMP1A	charged multivesicular body protein 1A	7.495	0.006	7.510	0.056
ISG15	ISG15 ubiquitin like modifier	7.460	0.004	6.667	0.098
TRMT6	tRNA methyltransferase 6	7.428	0.005	7.560	0.068
UFC1	ubiquitin-fold modifier conjugating enzyme 1	7.396	0.015	7.066	0.080
HYI	hydroxypyruvate isomerase (putative)	7.341	0.005	7.286	0.078
PARP9	poly(ADP-ribose) polymerase family member 9	7.279	0.013	7.503	0.080
TRAPPC4	trafficking protein particle complex 4	7.252	0.004	6.605	0.090
SNX24	sorting nexin 24	7.245	0.015	6.995	0.145
RAB33A	RAB33A, member RAS oncogene family	7.244	0.013	7.560	0.085
PPME1	protein phosphatase methylesterase 1	7.242	0.013	7.017	0.123
TAX1BP1-AS1	TAX1BP1 antisense RNA 1	7.226	0.004	7.011	0.068
COMMD1	copper metabolism domain containing 1	7.195	0.015	6.955	0.116

UNC5C	unc-5 netrin receptor C	7.194	0.016	7.622	0.080
NOP16	NOP16 nucleolar protein	7.180	0.013	7.176	0.116
H2AJ	H2A.J histone	7.152	0.015	7.098	0.097
APBB1IP	amyloid beta precursor protein binding family B member 1 interacting protein	7.131	0.031	6.736	0.110
DPF2	double PHD fingers 2	7.131	0.013	7.098	0.120
EXOC3	exocyst complex component 3	7.121	0.014	6.854	0.123
CHKA	choline kinase alpha	7.084	0.008	7.747	0.055
SS18	SS18 subunit of BAF chromatin remodeling complex	7.075	0.009	7.463	0.079
IAH1	isoamyl acetate hydrolyzing esterase 1 (putative)	7.047	0.008	7.222	0.056
UTP18	UTP18 small subunit processome component	7.029	0.008	6.589	0.118
TXN	thioredoxin	6.987	0.020	6.673	0.123
TMEM222	transmembrane protein 222	6.974	0.020	8.081	0.068
ZFAND2B	zinc finger AN1-type containing 2B	6.948	0.014	7.055	0.084
PHACTR2	phosphatase and actin regulator 2	6.940	0.013	6.893	0.120
HIKESHI	heat shock protein nuclear import factor hikeshi	6.924	0.008	7.451	0.068
CENPO	centromere protein O	6.910	0.005	6.822	0.078
ARHGDIA	Rho GDP dissociation inhibitor alpha [Source:HGNC Symbol;Acc:HGNC:678]	6.887	0.040	6.659	0.125
UROD	uroporphyrinogen decarboxylase [Source:HGNC Symbol;Acc:HGNC:12591]	6.878	0.010	6.396	0.123
CLINT1	clathrin interactor 1 [Source:HGNC Symbol;Acc:HGNC:23186]	6.860	0.015	6.709	0.107
ATP6V0E1	ATPase H+ transporting V0 subunit e1 [Source:HGNC Symbol;Acc:HGNC:863]	6.855	0.024	6.489	0.118
EML4-AS1	EML4 antisense RNA 1 [Source:HGNC Symbol;Acc:HGNC:40112]	6.837	0.004	6.506	0.068
SNX32	sorting nexin 32 [Source:HGNC Symbol;Acc:HGNC:26423]	6.831	0.026	6.178	0.209

Supplementary table 6: Upregulated DE genes in MS nnNAWM vs stroke nnNAWM. Differential expression was assessed using an empirical Bayes moderated t-test within limma's linear model framework including the precision weights estimated by voom and the consensus correlation between samples of the same donor. The differential expression analysis was performed both with and without a covariate for the estimated microglia content (in percent). Resulting p values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate.

Gene symbol	Gene name	logFC	P. Adj.	LogFC cor.	P. Adj. Cor.
RAD23A	RAD23 homolog A, nucleotide excision repair protein	9.132	2.92e-5	9.139	3.78e-5
NSUN5	NOP2/Sun RNA methyltransferase 5	8.827	1.61e-5	8.825	2.75e-5
COPA	COPI coat complex subunit alpha	8.435	0.006	8.435	0.008
NKAP	NFKB activating protein	8.405	0.006	8.401	0.008
ARFGAP2	ADP ribosylation factor GTPase activating protein 2	8.202	0.006	8.219	0.008
ATF7IP	activating transcription factor 7 interacting protein	8.135	0.026	8.179	0.021
GPAA1	glycosylphosphatidylinositol anchor attachment 1	8.007	0.006	8.030	0.008
KBTBD11	kelch repeat and BTB domain containing 11	7.922	0.015	7.877	0.018
HINT1	histidine triad nucleotide binding protein 1	7.832	0.026	7.846	0.027
ACO2	aconitase 2	7.799	0.013	7.817	0.016
EIF3G	eukaryotic translation initiation factor 3 subunit G	7.759	0.010	7.764	0.012
CPNE3	copine 3	7.526	0.013	7.550	0.016
ABL2	ABL proto-oncogene 2, non-receptor tyrosine	7.447	0.026	7.465	0.024
ATP6V0E1	ATPase H ⁺ transporting V0 subunit e1	7.407	0.034	7.431	0.040
SCNM1	sodium channel modifier 1	7.364	0.046	7.398	0.045
ARSB	arylsulfatase B	7.217	0.013	7.243	0.016
ARPC5	actin related protein 2/3 complex subunit 5	7.180	0.046	7.234	0.049
POLR3H	RNA polymerase III subunit H	7.041	0.033	7.061	0.040
CAPN3	calpain 3	6.914	0.037	6.911	0.052
SF3B6	splicing factor 3b subunit 6	6.869	0.030	6.929	0.021
STRN3	striatin 3	6.729	0.047	6.755	0.060
STAM2	signal transducing adaptor molecule 2	6.554	0.047	6.573	0.060

Supplementary table 7: DE genes of interest. Genes of interest either found through literature or gene ontology analysis are shown for the various comparisons, sorted alphabetically. Differential expression was assessed using an empirical Bayes moderated t-test within limma's linear model framework including the precision weights estimated by voom and the consensus correlation between samples of the same donor. The differential expression analysis was performed both with and without a covariate for the estimated microglia content (in percent). Resulting p values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate. LogFC and adjusted p values are given without correction for microglia proportion and with correction for microglia proportion. Without correction, p values are considered significant at <0.05. With correction, p values are considered significant at <0.10.

Gene symbol	Gene name	MS nodules vs MS nnNAWM				Stroke nodules vs stroke nnNAWM				MS nodules vs stroke nodules			
		LogFC	P. Adj.	LogFC cor.	P. Adj. Cor.	LogFC	P. Adj.	LogFC cor.	P. Adj. Cor.	LogFC	P. Adj.	LogFC cor.	P. Adj. Cor.
ACLY	ATP citrate lyase	2.920	0.500	1.636	0.931	-1.410	0.880	-1.758	0.905	5.600	0.048	4.711	0.332
ACOX1	acyl-CoA oxidase 1	1.990	0.663	0.983	0.966	-2.840	0.751	-3.127	0.819	6.090	0.019	5.421	0.210
APBB1IP	amyloid beta precursor protein binding family B member 1 interacting protein	8.350	0.003	7.614	0.147	-2.776	0.848	-3.138	0.881	7.131	0.031	6.736	0.110
APOC1	apolipoprotein C1	4.670	0.172	3.538	0.715	8.730	0.005	8.497	0.009	0.268	0.929	-0.510	0.923
ASPDH	aspartate dehydrogenase domain containing	5.160	0.063	4.436	0.574	-0.417	0.968	-0.629	0.969	6.360	0.013	5.893	0.165
BID	BH3 interacting domain death agonist	3.994	0.267	3.312	0.772	1.425	0.858	1.200	0.932	6.642	0.013	6.210	0.120
C1QA	complement C1q A chain	9.450	1.40e-5	8.344	0.022	5.120	0.583	4.966	0.758	1.150	0.722	0.309	0.964
C1QB	complement C1q B chain	8.310	0.001	7.831	0.037	9.040	0.001	8.891	0.002	1.160	0.641	0.859	0.834
CASP3	caspase 3	5.850	0.030	5.359	0.436	-1.100	0.900	-1.223	0.935	6.040	0.023	5.692	0.200
CD14	CD14 molecule	7.690	0.002	6.245	0.231	2.270	0.826	1.746	0.913	5.370	0.083	4.488	0.303
CD83	CD83 molecule	7.370	0.002	4.835	0.436	4.020	0.583	3.174	0.819	2.830	0.392	1.182	0.828
CD84	CD84 molecule	8.169	0.001	5.701	0.342	3.622	0.670	2.739	0.820	4.026	0.221	2.481	0.619
CDKN1A	cyclin dependent kinase inhibitor 1A	7.150	0.004	5.255	0.404	5.960	0.202	5.364	0.417	-0.766	0.843	-2.051	0.700
CHI3L1	chitinase 3 like 1	6.900	0.050	5.630	0.515	7.540	0.140	7.100	0.274	0.748	0.845	-0.083	0.992
CHI3L2	chitinase 3 like 2	7.007	0.009	4.121	0.615	0.535	0.968	-0.464	0.988	3.747	0.269	1.913	0.716
COA4	cytochrome c oxidase assembly factor 4 homolog	3.260	0.430	3.725	0.756	-1.690	0.848	-1.525	0.918	6.300	0.021	6.595	0.131
CORO1A	coronin 1A	3.750	0.413	2.484	0.876	-0.065	0.994	-0.467	0.985	6.720	0.029	5.846	0.214
CRYZL1	crystallin zeta like 1	2.000	0.672	1.007	0.967	-0.108	0.991	-0.461	0.985	5.950	0.040	5.317	0.235
CXCL16	C-X-C motif chemokine ligand 16	10.100	2.11e-6	9.666	0.004	2.220	0.860	2.096	0.918	4.590	0.140	4.329	0.317
DAGLB	diacylglycerol lipase beta	7.430	0.001	7.622	0.053	-0.050	0.994	0.011	1.000	6.660	0.005	6.787	0.068

EGR1	early growth response 1	6.460	0.003	5.034	0.353	3.130	0.628	2.587	0.819	2.810	0.318	1.954	0.667
FABP5	fatty acid binding protein 5	-0.395	0.937	-1.280	0.931	-4.790	0.491	-5.000	0.557	7.600	0.002	6.942	0.056
FADD	Fas associated via death domain	5.300	0.036	3.033	0.736	0.248	0.968	-0.296	0.988	4.830	0.066	3.150	0.440
GPCPD1	glycerophosphocholine phosphodiesterase 1	3.100	0.439	2.102	0.887	-1.470	0.858	-1.770	0.894	6.050	0.024	5.369	0.226
GPNMB	glycoprotein nmb	7.200	0.013	6.042	0.321	6.620	0.185	6.224	0.322	2.670	0.425	1.893	0.704
GRB2	growth factor receptor bound protein 2	5.330	0.144	4.316	0.682	2.250	0.956	0.690	0.981	6.490	0.046	5.939	0.192
HLA-DMA	major histocompatibility complex, class II, DM alpha	6.570	0.009	3.456	0.679	2.670	0.759	1.613	0.900	3.900	0.209	1.900	0.685
HLA-DMB	major histocompatibility complex, class II, DM beta	5.910	0.023	3.828	0.652	1.850	0.843	1.279	0.930	3.740	0.223	2.267	0.645
HLA-DPB1	major histocompatibility complex, class II, DP beta 1	7.740	0.008	7.106	0.203	3.560	0.791	3.381	0.868	3.050	0.385	2.635	0.620
HLA-DRB1	major histocompatibility complex, class II, DR beta 1	6.310	0.042	5.426	0.453	4.250	0.660	3.980	0.819	4.200	0.204	3.623	0.444
HLA-DRB5	major histocompatibility complex, class II, DR beta 5	6.040	0.032	6.039	0.321	0.840	0.923	0.847	0.960	7.640	0.002	7.632	0.037
IAH1	isoamyl acetate hydrolyzing esterase 1 (putative)	6.480	0.024	6.752	0.196	-0.833	0.960	-0.737	0.978	7.050	0.008	7.222	0.056
IDH1	isocitrate dehydrogenase (NADP(+)) 1	3.820	0.213	2.983	0.781	-1.340	0.859	-1.586	0.900	5.090	0.047	4.505	0.299
IFI27	interferon alpha inducible protein 27	6.480	0.030	5.794	0.447	1.760	0.858	1.504	0.930	5.150	0.112	4.701	0.358
IFNAR2	interferon alpha and beta receptor subunit 2	1.050	0.851	2.011	0.894	0.015	0.997	0.305	0.989	6.190	0.016	6.851	0.085
IL18	interleukin 18	8.770	1.21e-5	8.771	0.008	3.320	0.633	3.306	0.786	4.940	0.070	4.949	0.206
IL1B	interleukin 1 beta	6.260	0.009	5.264	0.395	1.380	0.848	1.061	0.937	4.350	0.109	3.697	0.404
IL33	interleukin 33	5.550	0.073	5.429	0.509	-0.597	0.960	-0.643	0.974	6.650	0.017	6.553	0.164
IRF8	interferon regulatory factor 8	5.420	0.030	3.144	0.728	2.000	0.789	1.439	0.902	2.900	0.308	1.224	0.806
ISG15	ISG15 ubiquitin like modifier	3.660	0.346	2.536	0.847	0.248	0.968	-0.079	1.000	7.460	0.004	6.667	0.098
JAK3	Janus kinase 3	5.950	0.016	4.318	0.533	0.511	0.963	0.134	0.997	4.430	0.096	3.206	0.451
LPL	lipoprotein lipase	7.920	0.002	5.355	0.395	5.300	0.436	4.687	0.611	2.390	0.515	0.489	0.951
LRRK2	leucine rich repeat kinase 2	5.940	0.033	6.018	0.351	3.350	0.685	3.376	0.819	4.550	0.140	4.614	0.326
MAOB	monoamine oxidase B	1.965	0.654	0.757	0.975	0.284	0.978	-0.242	0.997	6.603	0.039	5.981	0.165
MERTK	MER proto-oncogene, tyrosine kinase	5.090	0.023	3.533	0.587	0.248	0.968	-0.164	0.995	4.330	0.063	3.208	0.399
MTG1	mitochondrial ribosome associated GTPase 1	6.500	0.017	8.188	0.070	-0.309	0.968	0.066	1.000	5.820	0.041	7.089	0.080
MX1	MX dynamin like GTPase 1	8.930	1.40e-5	8.283	0.022	1.330	0.870	1.072	0.939	6.420	0.013	6.033	0.101
NCF2	neutrophil cytosolic factor 2	5.820	0.033	3.204	0.743	1.720	0.830	1.094	0.937	3.590	0.224	1.637	0.747
NCKAP1L	NCK associated protein 1 like	8.490	1.46e-5	7.309	0.033	2.430	0.725	1.985	0.847	5.540	0.032	4.827	0.196
PARP9	poly(ADP-ribose) polymerase family member 9	5.970	0.098	6.246	0.445	-1.180	0.920	-1.330	0.947	7.280	0.013	7.503	0.080

PLCD3	phospholipase C delta 3	1.910	0.673	0.620	0.978	-1.370	0.880	-1.718	0.900	5.500	0.037	4.621	0.285
PPRC1	PPARG related coactivator 1	5.700	0.030	6.041	0.353	-1.270	0.880	-1.148	0.942	5.680	0.037	5.906	0.196
PSME3	proteasome activator subunit 3	3.610	0.386	2.132	0.898	-0.468	0.968	-0.853	0.964	6.100	0.037	5.028	0.303
SAMM50	SAMM50 sorting and assembly machinery component	5.857	0.027	3.988	0.634	-0.806	0.956	-1.347	0.930	5.516	0.047	4.317	0.340
SLC11A1	solute carrier family 11 member 1	7.072	0.004	5.312	0.378	8.227	0.005	7.755	0.010	-1.669	0.628	-2.926	0.544
SMAD3	SMAD family member 3	2.670	0.529	1.611	0.930	0.547	0.960	0.268	0.993	5.660	0.037	4.883	0.285
SPP1	secreted phosphoprotein 1	3.060	0.050	1.371	0.746	3.120	0.509	2.524	0.696	0.502	0.723	-0.585	0.739
STARD13	STAR related lipid transfer domain containing 13	2.490	0.569	3.656	0.744	-0.422	0.968	-0.073	1.000	6.620	0.013	7.404	0.068
TCIRG1	T cell immune regulator 1, ATPase H+ transporting V0 subunit a3	6.470	0.019	4.461	0.561	0.295	0.968	-0.391	0.987	4.860	0.112	3.609	0.437
TLR2	toll like receptor 2	5.930	0.020	4.506	0.541	0.887	0.927	0.441	0.982	3.730	0.190	2.779	0.564
TLR6	toll like receptor 6	6.780	0.006	5.903	0.342	1.180	0.875	0.897	0.952	5.090	0.062	4.507	0.321
WASL	WASP like actin nucleation promoting factor	0.444	0.941	0.719	0.976	7.780	0.018	7.885	0.033	-1.750	0.542	-1.580	0.752

Supplementary table 8: Significantly enriched GO classes in MS nodules vs MS nnNAWM. Gene ontology analysis was used to find genes of interest based on function. The column count indicates the number of genes likely associated with the GO term and the unadjusted p value is shown. P-values were obtained by a Modified Fisher Exact one-sided test, calculating the EASE score.

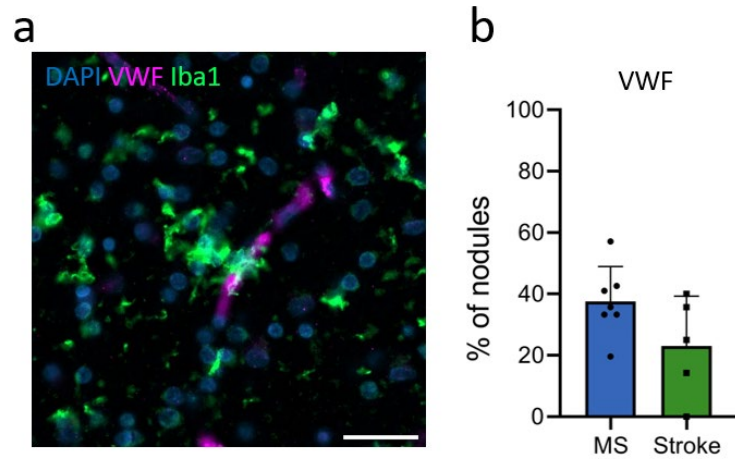
Term	GO class	Count	P
positive regulation of interferon-gamma production	GO:0032729	13	1.79e-9
positive regulation of tumor necrosis factor production	GO:0032760	13	6.65e-8
peptide antigen assembly with MHC class II protein complex	GO:0032755	6	2.58e-6
positive regulation of interleukin-6 production	GO:0032755	11	2.93e-6
immunoglobulin production involved in immunoglobulin mediated immune response	GO:0002381	6	3.61e-6
antigen processing and presentation of exogenous peptide antigen via MHC class II	GO:0019886	7	4.30e-6
positive regulation of interleukin-8 production	GO:0032757	9	4.94e-6
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	GO:0002504	6	8.71e-6
immune response	GO:0006955	22	1.43e-5
defense response to virus	GO:0051607	14	4.11e-5
negative regulation of interferon-gamma production	GO:0032689	7	4.90e-5
positive regulation of monocyte chemotactic protein-1 production	GO:0071639	5	7.33e-5
cortical actin cytoskeleton organization	GO:0030866	6	8.36e-5
erythrocyte differentiation	GO:0030218	7	1.01e-4
positive regulation of T cell activation	GO:0050870	6	1.14e-4
innate immune response	GO:0045087	22	2.22e-4
positive regulation of interleukin-1 beta production	GO:0032731	7	3.89e-4
positive regulation of T cell proliferation	GO:0042102	7	3.89e-4
intracellular signal transduction	GO:0035556	18	3.99e-4
positive regulation of interleukin-12 production	GO:0032735	6	4.10e-4
inflammatory response	GO:0006954	17	4.24e-4
positive regulation of NIK/NF-kappaB signaling	GO:1901224	7	0.001
cellular response to lipopolysaccharide	GO:0071222	11	0.001
antigen processing and presentation	GO:0019882	6	0.001
positive regulation of NF-kappaB transcription factor activity	GO:0051092	10	0.001
receptor internalization	GO:0031623	6	0.001
cell activation	GO:0001775	4	0.001
microglial cell activation	GO:0001774	5	0.001

T cell homeostasis	GO:0043029	5	0.001
dendritic cell differentiation	GO:0097028	4	0.001
response to tumor necrosis factor	GO:0034612	5	0.002
cellular response to diacyl bacterial lipopeptide	GO:0071726	3	0.002
positive regulation of I-kappaB kinase/NF-kappaB signaling	GO:0043123	10	0.002
lipopolysaccharide-mediated signaling pathway	GO:0031663	5	0.003
positive regulation of interleukin-10 production	GO:0032733	5	0.003
positive regulation of superoxide anion generation	GO:0032930	4	0.003
phagocytosis	GO:0006909	6	0.004
negative regulation of T cell proliferation	GO:0042130	5	0.005
neutrophil activation involved in immune response	GO:0002283	3	0.006
positive regulation of T-helper 1 cell cytokine production	GO:2000556	3	0.006
negative regulation of interleukin-10 production	GO:0032693	4	0.006
defense response	GO:0006952	6	0.006
positive regulation of phagocytosis	GO:0050766	5	0.006
positive regulation of interleukin-17 production	GO:0032740	4	0.006
signal transduction	GO:0007165	31	0.007
positive regulation of interleukin-4 production	GO:0032753	4	0.007
extrinsic apoptotic signaling pathway	GO:0097191	5	0.007
positive regulation of gene expression	GO:0010628	16	0.010
substrate adhesion-dependent cell spreading	GO:0034446	5	0.011
positive regulation of transcription from RNA polymerase II promoter	GO:0045944	29	0.011
calcium-mediated signaling	GO:0019722	6	0.012
positive regulation of cell migration	GO:0030335	10	0.014
extrinsic apoptotic signaling pathway in absence of ligand	GO:0097192	4	0.015
platelet activation	GO:0030168	5	0.015
organelle transport along microtubule	GO:0072384	3	0.018
positive regulation of granulocyte macrophage colony-stimulating factor production	GO:0032725	3	0.020
negative regulation of activated T cell proliferation	GO:0046007	3	0.020
regulation of tumor necrosis factor-mediated signaling pathway	GO:0010803	3	0.023
positive regulation of inflammatory response	GO:0050729	6	0.024
integrin-mediated signaling pathway	GO:0007229	6	0.024
positive regulation of neuron death	GO:1901216	4	0.025
apoptotic signaling pathway	GO:0097190	5	0.026

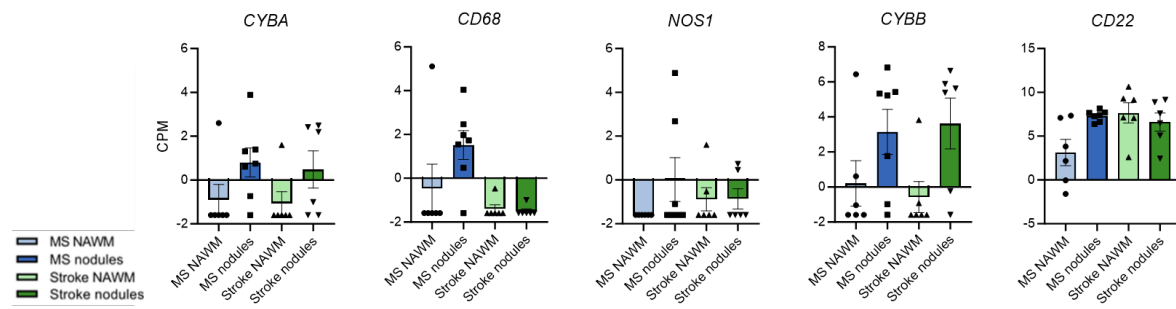
negative regulation of tumor necrosis factor production	GO:0032720	5	0.027
positive regulation of protein ubiquitination	GO:0031398	5	0.028
response to lipopolysaccharide	GO:0032496	7	0.028
cytokine-mediated signaling pathway	GO:0019221	7	0.028
late endosome to lysosome transport	GO:1902774	3	0.028
negative regulation of T cell activation	GO:0050868	3	0.028
positive regulation of nitric-oxide synthase biosynthetic process	GO:0051770	3	0.028
positive regulation of B cell proliferation	GO:0030890	4	0.029
positive regulation of chemokine production	GO:0032722	4	0.029
animal organ regeneration	GO:0031100	4	0.029
platelet aggregation	GO:0070527	4	0.029
regulation of small GTPase mediated signal transduction	GO:0051056	6	0.029
negative regulation of leukocyte apoptotic process	GO:2000107	2	0.029
toll-like receptor TLR6:TLR2 signaling pathway	GO:0038124	2	0.029
methotrexate transport	GO:0051958	2	0.029
detection of diacyl bacterial lipopeptide	GO:0042496	2	0.029
regulation of G-protein coupled receptor protein signaling pathway	GO:0008277	4	0.032
angiogenesis	GO:0001525	9	0.034
positive regulation of nitric oxide biosynthetic process	GO:0045429	4	0.036
adaptive immune response	GO:0002250	13	0.036
B cell differentiation	GO:0030183	5	0.038
MyD88-dependent toll-like receptor signaling pathway	GO:0002755	3	0.041
stimulatory C-type lectin receptor signaling pathway	GO:0002223	3	0.041
MAPK cascade	GO:0000165	6	0.041
negative regulation of lymphocyte activation	GO:0051250	2	0.044
reduced folate transmembrane transport	GO:0098838	2	0.044
nitric oxide metabolic process	GO:0046209	2	0.044
positive regulation of T cell activation via T cell receptor contact with antigen bound to MHC molecule on antigen presenting cell	GO:2001190	2	0.044
cellular response to triacyl bacterial lipopeptide	GO:0071727	2	0.044
positive regulation of glial cell proliferation	GO:0060252	3	0.045
cell projection assembly	GO:0030031	3	0.045
transmembrane receptor protein tyrosine kinase signaling pathway	GO:0007169	6	0.046
positive regulation of protein complex assembly	GO:0031334	4	0.047
toll-like receptor signaling pathway	GO:0002224	3	0.048

Supplementary table 9: Significantly enriched GO classes in MS nodules vs stroke nodules. Gene ontology analysis was used to find genes of interest based on function. The column count indicates the number of genes likely associated with the GO term and the unadjusted p value is shown. P-values were obtained by a Modified Fisher Exact one-sided test, calculating the EASE score.

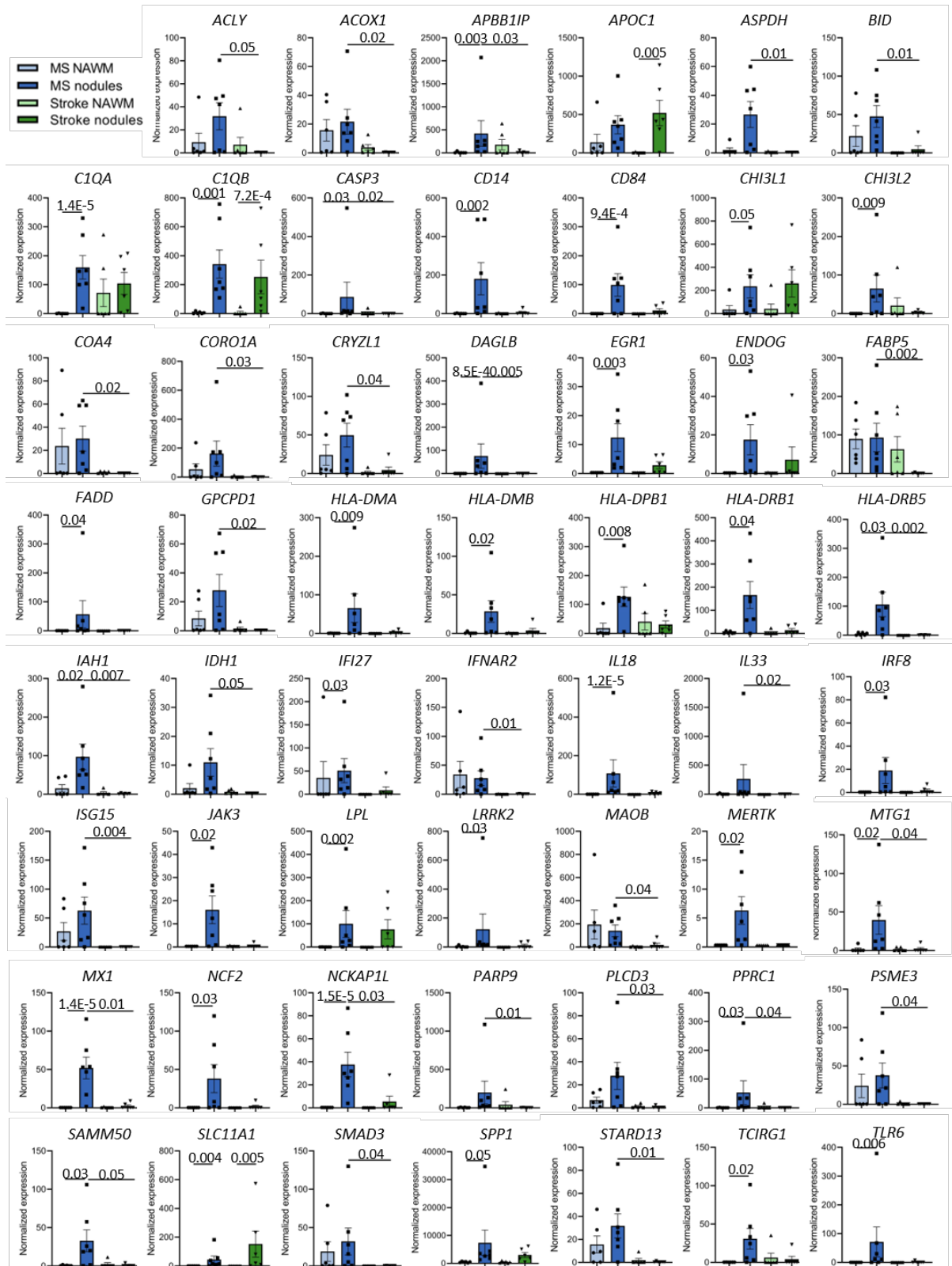
Term	GO class	Count	P
regulation of catalytic activity	GO:0050790	13	0.001
protein transport	GO:0015031	14	0.001
Golgi to plasma membrane transport	GO:0006893	4	0.003
protein targeting to mitochondrion	GO:0006626	4	0.003
regulation of small GTPase mediated signal transduction	GO:0051056	6	0.009
membrane fission	GO:0090148	4	0.009
ER to Golgi vesicle-mediated transport	GO:0006888	6	0.014
intrinsic apoptotic signaling pathway in response to DNA damage	GO:0008630	4	0.017
mitochondrial translation	GO:0032543	5	0.020
regulation of mitochondrial DNA metabolic process	GO:1901858	2	0.021
actin cytoskeleton reorganization	GO:0031532	4	0.026
lipid metabolic process	GO:0006629	7	0.026
Rho protein signal transduction	GO:0007266	4	0.027
regulation of Rho protein signal transduction	GO:0035023	3	0.037
response to aluminum ion	GO:0010044	2	0.043
glial cell apoptotic process	GO:0034349	2	0.043
T cell homeostasis	GO:0043029	3	0.047
actin cytoskeleton organization	GO:0030036	6	0.048



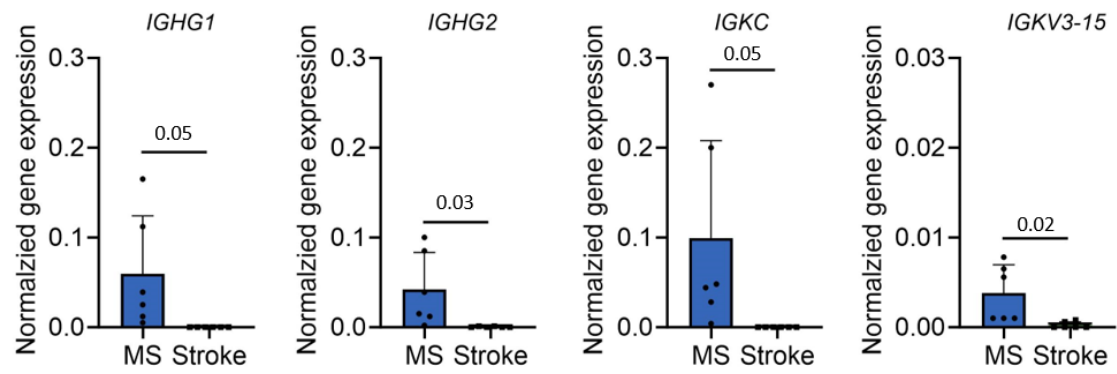
Supplementary figure 1: A) Immunohistochemistry staining of *iba1*⁺ nodule (in green) in MS in contact with a *VWF*⁺ vessel (in magenta). B) Nodules in MS and in stroke are equally often in contact with vessels, quantified in *n*=7 MS donors and *n*=5 stroke donors. Data is shown as mean $\pm$ standard deviation. Scalebar = 35 μ m. Source data are provided as a Source Data file.



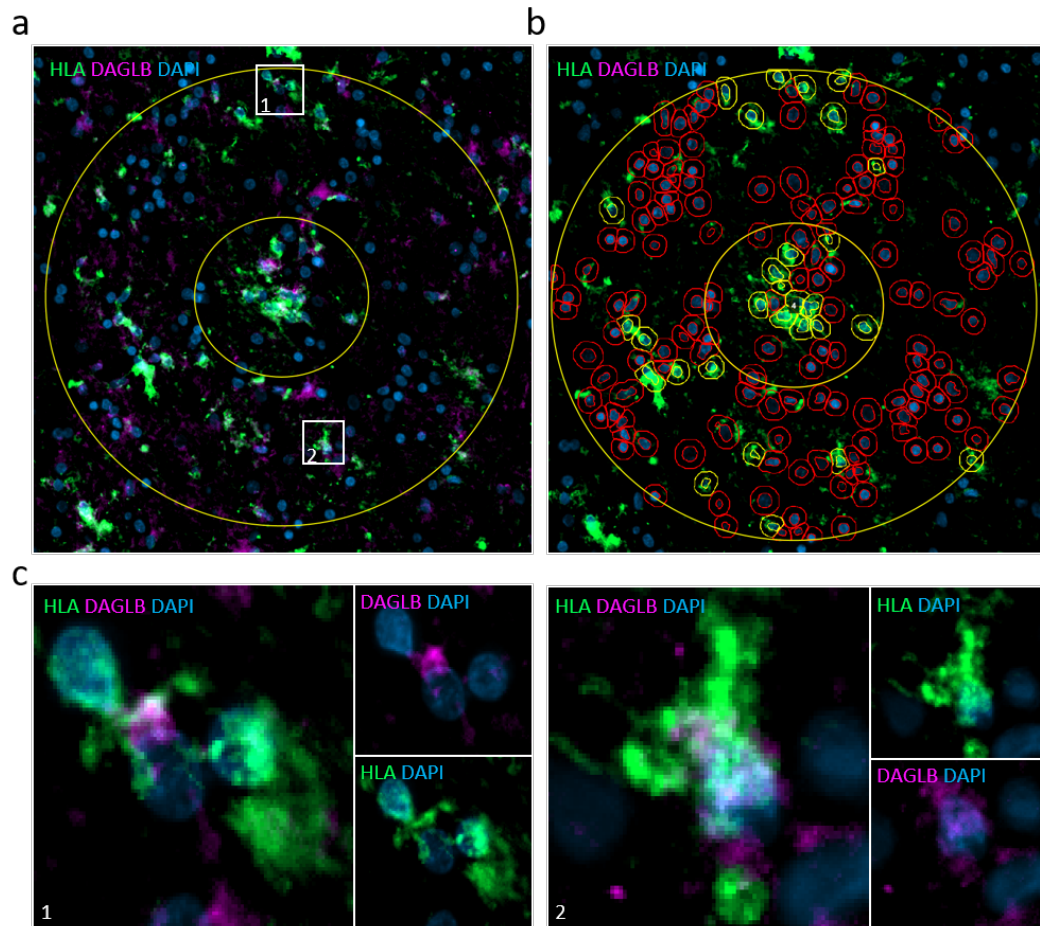
Supplementary Figure 2: Phagocytic exhaustion markers are not significantly differentially expressed in MS nodules compared to stroke nodules. Analysis performed on n=6 MS NAWM samples, n=7 MS nodule samples, n=6 stroke NAWM samples, n=6 stroke nodule samples. Data is shown as mean $\pm$ standard deviation. Source data are provided as a Source Data file.



Supplementary figure 3: Normalized expression of genes likely involved in functional pathways relevant for MS pathology. Analysis performed on n=6 MS NAWM samples, n=7 MS nodule samples, n=6 stroke NAWM samples, n=6 stroke nodule samples. Data is shown as mean $\pm$ standard deviation. Normalized gene expression is calculated as $2\log(\text{CPM})$. p value < 0.05 is indicated with *, < 0.01 is indicated with **, < 0.001 is indicated with ***. Source data are provided as a Source Data file.



Supplementary figure 4: Immunoglobulin gene expression quantified with RT-qPCR of MS (n=6) and stroke (n=6) (NA)WM tissue containing nodules showing higher expression in MS compared to stroke. Data is shown as mean $\pm$ standard deviation. Source data are provided as a Source Data file.



Supplementary figure 5: Exemplary figure of quantification of (NA)WM microglia adjacent to microglia nodules for DAGLB, FABP5, IAH1 and STARD13. A) for each nodule, an expansion of 100 μm was created of the annotation. B) Using cell-detection in QuPath, all HLA+ cells were annotated with green, all HLA- cells were annotated with red. Each HLA+ cell was annotated as C) negative (left panel) or positive (right panel) for DAGLB.