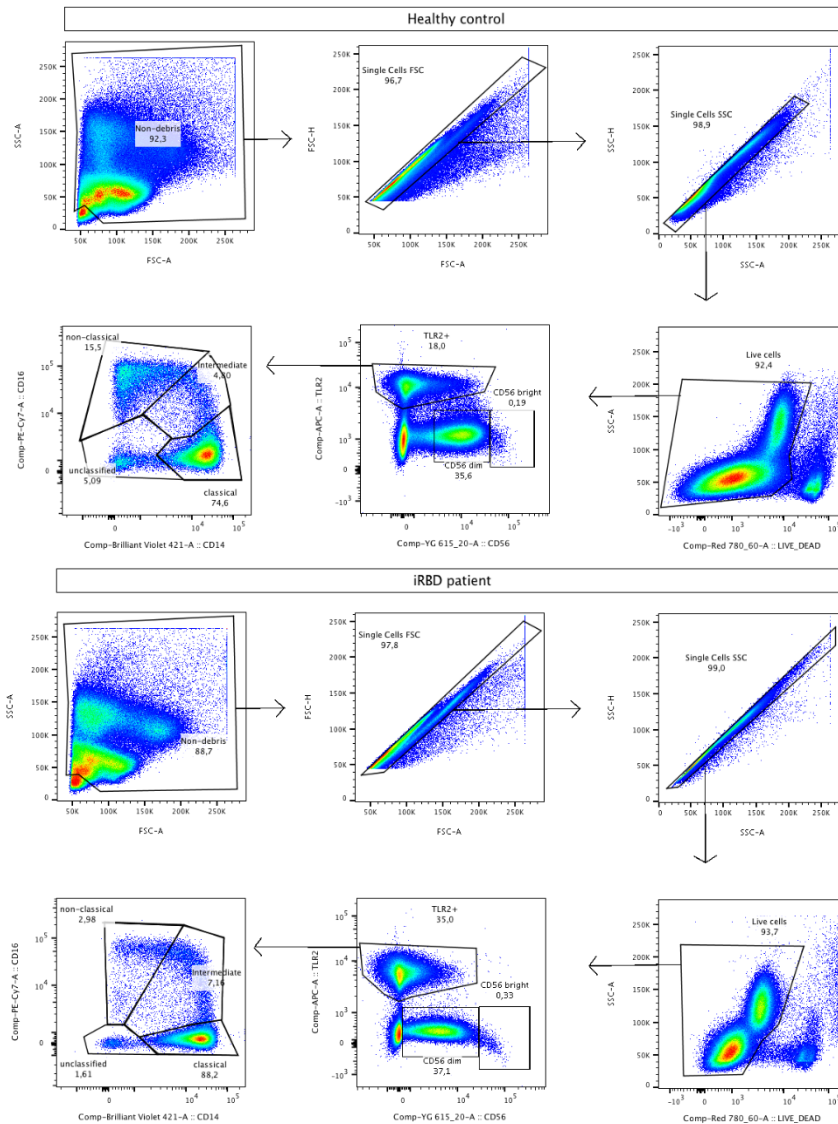


Supplementary Info:

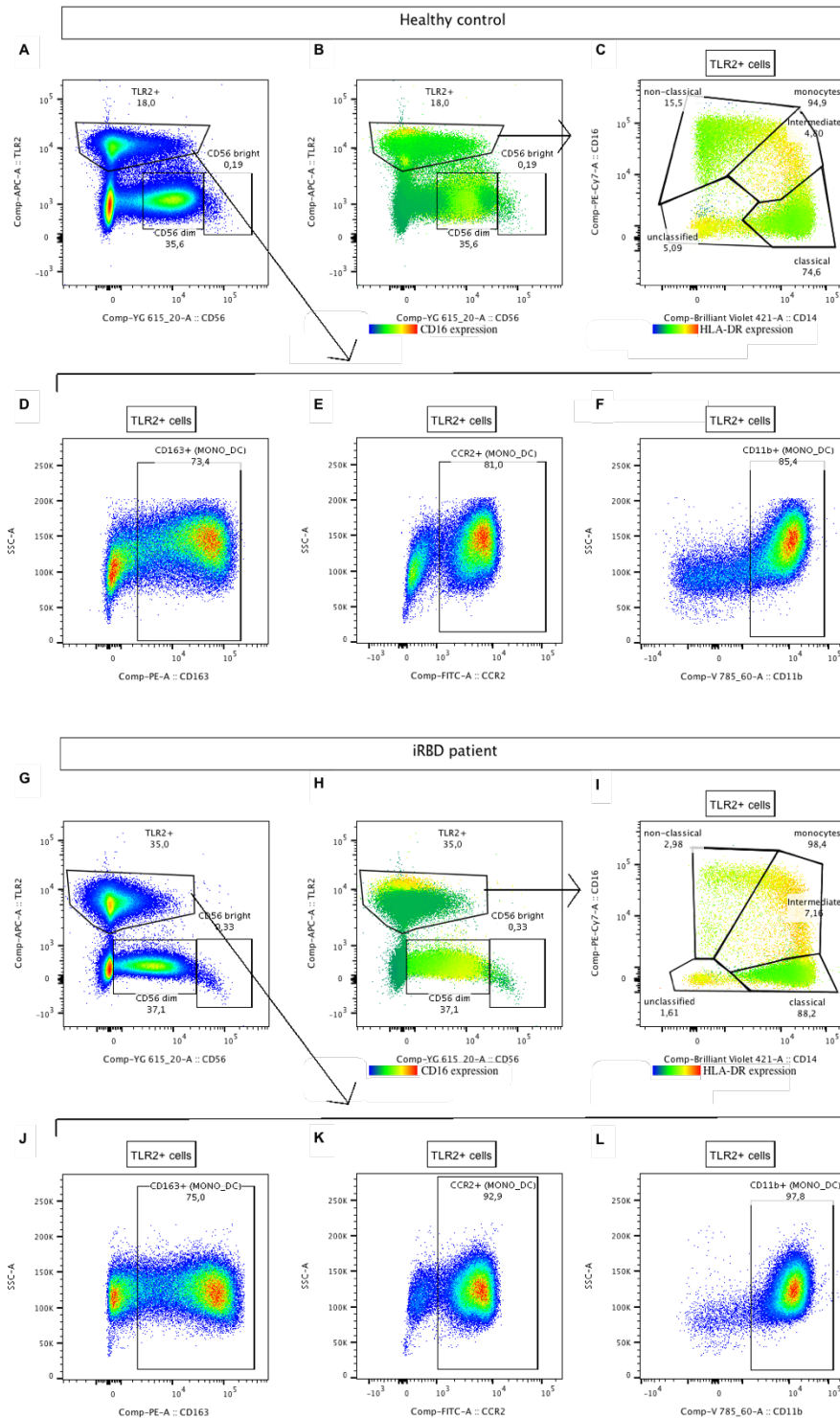
Monocyte markers correlate with immune and neuronal brain changes in REM Sleep Behaviour Disorder

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Flow cytometry gating strategy



Supplementary figure 1. Flow cytometry gating strategy used for identification of mononuclear phagocytes and natural killer cells in PBMCs. PBMCs were analysed by flow cytometry. Representative plots are shown for a healthy control (upper panel) and an iRBD patient (lower panel). Arrows indicate the pre-gated population and subsequent analysis. Debris was excluded based on FSC and SSC followed by a double exclusion of doublets by first FSC and then SSC area (A) versus height (H). Dead cells were excluded using fixable Near-IR Dead Cell Stain Kit. The natural killer (NK) cell-specific marker CD56 was used together with TLR2, a pan monocyte marker expressed in Mo&DCs, to separate the monocytes (TLR2⁺) and NK cells (TLR2⁻/CD56^{dim/bright}). The separation between the CD56 dim and bright populations were aided by CD16 expression differences (by heatmap) on these cells (Supp.Fig2A,B (HC) and Supp.Fig2G,H (iRBD)). Finally, the separation into subpopulations of Mo&DCs was done based on their different expression of the CD14 and CD16 markers. CD14⁺⁺/CD16⁻ corresponds to classical monocytes, CD14⁺⁺/CD16⁺ corresponds to intermediate monocytes, CD14^{low}/CD16⁺⁺ corresponds to non-classical monocytes and CD14^{low}/CD16⁻ corresponds to “unclassified” monocytes thus dendritic cells (DCs). The separation between monocyte subpopulations were also based on differences in HLA-DR surface expression (by heatmap) (Supp.Fig2C (HC) and Supp.Fig2I (iRBD)).



Supplementary figure 2. Gating strategy for separation of NK subpopulations, monocyte subpopulations and Mo&DCs expression of receptors. Representative plots are shown for a healthy control (upper panel A-F) and an iRBD patient (lower panel G-L). Arrows indicate the pre-gated population and subsequent analysis. **A,G** The exact separation of CD56⁺ natural killer (NK) cells into precursor (CD56 bright) and mature (CD56 dim) NK cells were based on their expression of CD56 aided by **B,H** CD16 heat-mapping (highest in the CD56dim population). Pre-gated TLR2⁺ cells were further analysed based on the **C,I** CD14 and CD16 expression and the separation of monocyte subpopulations and dendritic cells (DC) (“unclassified monocytes”) were based on their surface expression of HLA-DR evaluated by heat-mapping. In addition, pre-gated TLR2⁺ were analysed for the expression of the following immune marker (representative plots are shown for the gating): **D,J** CD163⁺, **E,K** CD11b⁺ and **F,L** CCR2⁺. The gates were set based on FMO controls.

ANALYSIS OF COVARIANCE:

Linear regression	Dependent variable	Independent variable	Estimate	Std Error	t Ratio	Prob > t	Lower 95%	Upper 95%
Simple regression	% CD163 ⁺ (live)	SN PK L	-31.86	9.366	-3.40	0.0059*	-52.47	-11.24
Simple regression	% CD163 ⁺ (live)	SN PK R	-25.37	7.381	-3.44	0.0056*	-41.61	-9.120
Simple regression	% CD163 ⁺ (live)	Putamen DOPA L	2394	949.6	2.52	0.0284*	304.1	4484
Simple regression	% CD163 ⁺ (live)	Age at visit	-0.016	0.353	-0.05	0.965	-0.794	0.762
Simple regression	% CD163 ⁺ (live)	Disease duration	-0.001	0.607	-0.00	0.999	-1.338	1,335
Multiple regression	% CD163 ⁺ (live)	SN PK L	-39.43	10.06	-3.92	0.0035*	-62.19	-16.67
		Age at visit	0.320	0.265	1.21	0.2572	-0.279	0.920
		Disease duration	0.394	0.441	0.89	0.396	-0.605	1.392
Multiple regression	% CD163 ⁺ (live)	SN PK R	-33.63	7.656	-4.39	0.0017*	-50.95	-16.31
		Age at visit	0.365	0.248	1.47	0.176	-0.197	0.926
		Disease duration	0.534	0.417	1.28	0.2331	-0.410	1.476
Multiple regression	% CD163 ⁺ (live)	Putamen DOPA L	2714	1073	2.53	0.0323*	286.2	5142
		Age at visit	-0.044	0.315	-0.14	0.893	-0.757	0.670
		Disease duration	0.494	0.574	0.86	0.4119	-0.805	1.794

Supplementary Table 1: Simple linear and multiple linear regressions were calculated to predict frequencies of CD163⁺ cells (of live cells) for iRBD patients based on BP ¹¹C-PK11195 in SN L & R or Ki ¹⁸F-DOPA in Putamen L alone or adjusted for age and disease duration.

Estimate of slope (β)/model coefficients with standard (Std) errors, the t ratio of the estimate to the std error (t Ratio), the p values (Prob >|t|), and the 95% lower and upper confidence limit for the parameter estimate are shown for simple linear regression or multiple regression analyses. Significant p values are marked with *.

Linear regression	Dependent variable	Independent variable	Estimate	Std Error	t Ratio	Prob > t	Lower 95%	Upper 95%
Simple regression	MFI TLR4 (C)	SN PK L	3821	1127	3.39	0.0048*	1387	6254
Simple regression	MFI TLR4 (C)	SN PK R	2526	975,1	2.59	0.0224*	419,1	4632
Simple regression	MFI TLR4 (C)	Putamen DOPA L	-324468	103799	-3.13	0.0080*	-548711	-100224
Simple regression	MFI TLR4 (C)	Putamen DOPA R	-244237	124837	-1.96	0.0722	-513931	25457
Simple regression	MFI TLR4 (C)	Age at visit	27.43	39.15	0.7	0.4958	-57.14	112.0
Simple regression	MFI TLR4 (C)	Disease duration	116.9	61.34	1.91	0.0791	-15.63	249.4
Simple regression	MFI TLR4 (NC)	Putamen DOPA L	-144924	50812	-2.85	0.0136*	-254697	-35152
Simple regression	MFI TLR4 (NC)	Putamen DOPA R	-140149	54551	-2.57	0.0233*	-257999	-22299
Simple regression	MFI TLR4 (NC)	Age at visit	3.838	18.78	0.2	0.841	-36.73	44.40
Simple regression	MFI TLR4 (NC)	Disease duration	52.52	29.30	1.79	0.0963	-10.77	115.8
Multiple regression	MFI TLR4 (C)	SN PK L	3598	1255	2.87	0.0153*	835.4	6362
		Age at visit	-20.37	31.84	-0.64	0.5355	-90.45	49.72
		Disease duration	75.02	53.76	1.4	0.1904	-43.30	193.3
Multiple regression	MFI TLR4 (C)	SN PK R	2723	1137	2.4	0.0338*	246.4	5200
		Age at visit	-14.25	37.76	-0.38	0.7126	-96.52	68.03
Multiple regression	MFI TLR4 (C)	SN PK R	2081	1008	2.07	0.0612	-114.1	4276
		Disease duration	76.49	58.22	1.31	0.2135	-50.36	203.3
Multiple regression	MFI TLR4 (C)	SN PK R	2344	1137	2.06	0.0637	-158.5	4846
		Age at visit	-20.83	36.90	-0.56	0.5837	-102.0	60.38
		Disease duration	81.04	60.48	1.34	0.2073	-52.09	214.2
Multiple regression	MFI TLR4 (C)	Putamen DOPA L	-278390	112783	-2.47	0.0312*	-526625	-30156
		Age at visit	12.04651	31.81	0.38	0.7121	-57.97	82.06
		Disease duration	62.02	58.93	1.05	0.3151	-67.68	191.7
Multiple regression	MFI TLR4 (NC)	Putamen DOPA L	-123369	55945	-2.21	0.0496*	-246504	-233.5
		Age at visit	-3.735	15.78	-0.24	0.8172	-38.46	30.99
		Disease duration	32.41	29.23	1.11	0.2912	-31.93	96.75
Multiple regression	MFI TLR4 (NC)	Putamen DOPA R	-142383	57635	-2.47	0.0295*	-267960	-16807
		Age at visit	-3.444	16.18	-0.21	0.835	-38.70	31.82
Multiple regression	MFI TLR4 (NC)	Putamen DOPA R	-117005	57271	-2.04	0.0637	-241789	7779
		Disease duration	32.85	27.98	1.17	0.2631	-28.10	93.80
Multiple regression	MFI TLR4 (NC)	Putamen DOPA R	-119857	59502	-2.01	0.0691	-250820	11106
		Age at visit	-7.796	16.27	-0.48	0.6413	-43.61	28.02
		Disease duration	35.98	29.65	1.21	0.2503	-29.28	101.2

Supplementary Table 2: Linear and multiple linear regressions were calculated to predict MFI TLR4 on classical and non-classical monocytes for iRBD patients based on BP 11C-PK11195 in SN L&R or Ki 18F-DOPA in Putamen L&R alone or adjusted for age and disease duration.

Estimate of slope (β)/model coefficients with standard (Std) errors, the t ratio of the estimate to the std error (t-Ratio), the p values (Prob >|t|), and the 95% lower and upper confidence limit for the parameter estimate are shown for simple linear regression or multiple regression analyses. Significant p values are marked with * p<0.05.

Linear regression	Dependent variable	Independent variable	Estimate	Std Error	t Ratio	Prob > t	Lower 95%	Upper 95%
Simple regression	MFI TLR2 Mo&DCs	UPSIT	-180.9	49.77	-3.63	0.0030**	-288.4	-73.39
Simple regression	MFI TLR2 Mo&DCs	Age at visit	37.51	96.37	0.39	0.7034	-170.7	245.7
Simple regression	MFI TLR2 Mo&DCs	Disease duration	56.66	167.9	0.34	0.7411	-306.1	419.4
Multiple regression	MFI TLR2 Mo&DCs	UPSIT	-207.9	52.27	-3.98	0.0022**	-323.0	-92.85
		Age at visit	-44.67	72.01	-0.62	0.5477	-203.2	113.8
		Disease duration	201.4	127.3	1.58	0.1418	-78.68	481.6

Supplementary table 3. Simple linear and multiple linear regressions were calculated to MFI TLR2 (Mo&DCs) for iRBD patients based on UPSIT score alone or adjusted for age and disease duration.

Estimate of slope (β)/model coefficients with standard (Std) errors, the t ratio of the estimate to the std error (t-Ratio), the p values (Prob >|t|), and the 95% lower and upper confidence limit for the parameter estimate are shown for simple linear regression or multiple regression analyses. Significant p values are marked with ** p<0.01.