

## Supplementary materials:

**Table S1.** MFI of P2X7, CD11b, and CD11c on leukocyte subtypes in the discovery cohort (Model 1)<sup>1</sup>.

Variable	Discovery cohort (Model 1)		
	Control ( $\leq 25$ CL <sup>2</sup> )	Case ( $> 25$ CL)	Effect size of Case ( $> 25$ CL)
P2X7 MFI on total lymphocytes	16.9 $\pm$ 4.8	14.1 $\pm$ 3.3	-0.572
P2X7 MFI on NK cells (CD14 <sup>-</sup> CD16 <sup>+</sup> )	20.1 $\pm$ 7.4	16.3 $\pm$ 5.3	-0.509
P2X7 MFI on B&T cells (CD14 <sup>-</sup> CD16 <sup>-</sup> )	16.3 $\pm$ 4.5	13.8 $\pm$ 3.0	-0.559
P2X7 MFI on total monocytes	68.9 $\pm$ 37.0	50.4 $\pm$ 28.4	-0.501
P2X7 MFI on non-classical monocytes (CD14 <sup>dim</sup> CD16 <sup>+</sup> )	43.2 $\pm$ 22.2	32.0 $\pm$ 17.9	-0.503
P2X7 MFI on intermediate monocytes (CD14 <sup>+</sup> CD16 <sup>+</sup> )	82.5 $\pm$ 49.0	57.3 $\pm$ 34.1	-0.514
P2X7 MFI on classical monocytes (CD14 <sup>+</sup> CD16 <sup>-</sup> )	72.0 $\pm$ 39.7	52.7 $\pm$ 29.6	-0.487
P2X7 MFI on total neutrophils	35.1 $\pm$ 16.0	25.8 $\pm$ 7.9	-0.581
P2X7 MFI on CD16 <sup>++</sup> neutrophils	33.3 $\pm$ 13.2	25.0 $\pm$ 7.7	-0.625
CD11c MFI on total lymphocytes	9.5 $\pm$ 2.6	7.7 $\pm$ 2.5	-0.701
CD11c MFI on total monocytes	87.5 $\pm$ 38.4	62.9 $\pm$ 26.6	-0.639
CD11c MFI on total neutrophils	24.9 $\pm$ 10.8	18.6 $\pm$ 6.0	-0.580
CD11b MFI on total lymphocytes	48.3 $\pm$ 23.6	35.5 $\pm$ 18.8	-0.541
CD11b MFI on total monocytes	458.9 $\pm$ 214.4	319.2 $\pm$ 137.8	-0.651
CD11b MFI on total neutrophils	397.8 $\pm$ 182.9	296.1 $\pm$ 130.1	-0.556

<sup>1</sup> Data was calculated as mean  $\pm$  standard deviation. Same for Table S2, S3, and S4.

<sup>2</sup> CL: Centiloid – the scale of PET-A $\beta$  burden in the brain.

**Table S2.** MFI of P2X7, CD11b, and CD11c on leukocyte subtypes in the discovery cohort (Model 2).

Variable	Discovery cohort (Model 2)				
	CN ( $\leq 25$ CL)	CN ( $> 25$ CL)	Dementia <sup>3</sup>	Effect size of CN ( $> 25$ CL)	Effect size of Dementia <sup>4</sup>
P2X7 MFI on total lymphocytes	17.1 $\pm$ 4.7	14.7 $\pm$ 3.9	13.4 $\pm$ 2.5	-0.601	-0.938
P2X7 MFI on NK cells (CD14 <sup>-</sup> CD16 <sup>+</sup> )	20.4 $\pm$ 7.4	16.9 $\pm$ 5.1	15.6 $\pm$ 4.0	-0.550	-0.758
P2X7 MFI on B&T cells (CD14 <sup>-</sup> CD16 <sup>-</sup> )	16.5 $\pm$ 4.5	14.4 $\pm$ 3.3	13.1 $\pm$ 2.3	-0.615	-0.991
P2X7 MFI on total monocytes	70.6 $\pm$ 36.6	53.2 $\pm$ 28.9	46.6 $\pm$ 21.8	-0.499	-0.689
P2X7 MFI on non-classical monocytes (CD14 <sup>dim</sup> CD16 <sup>+</sup> )	44.1 $\pm$ 22.1	32.9 $\pm$ 17.2	30.6 $\pm$ 15.8	-0.560	-0.673
P2X7 MFI on intermediate monocytes (CD14 <sup>+</sup> CD16 <sup>+</sup> )	84.6 $\pm$ 48.7	58.4 $\pm$ 34.5	54.9 $\pm$ 29.1	-0.668	-0.758
P2X7 MFI on classical monocytes (CD14 <sup>+</sup> CD16 <sup>-</sup> )	73.8 $\pm$ 39.3	53.9 $\pm$ 29.0	50.1 $\pm$ 23.7	-0.564	-0.672
P2X7 MFI on total neutrophils	35.6 $\pm$ 16.0	27.9 $\pm$ 7.1	23.6 $\pm$ 3.4	-0.746	-1.171
P2X7 MFI on CD16 <sup>++</sup> neutrophils	33.7 $\pm$ 13.2	27.0 $\pm$ 7.3	23.0 $\pm$ 3.3	-0.670	-1.067
CD11c MFI on total lymphocytes	9.6 $\pm$ 2.6	7.9 $\pm$ 2.5	7.5 $\pm$ 2.4	-0.672	-0.831
CD11c MFI on total monocytes	88.5 $\pm$ 38.9	65.1 $\pm$ 26.5	60.8 $\pm$ 29.9	-1.054	-1.249
CD11c MFI on total neutrophils	24.9 $\pm$ 11.0	20.3 $\pm$ 6.9	17.3 $\pm$ 6.3	-0.845	-1.405
CD11b MFI on total lymphocytes	49.2 $\pm$ 23.5	35.1 $\pm$ 18.3	35.3 $\pm$ 19.3	-0.767	-0.755
CD11b MFI on total monocytes	465.0 $\pm$ 216.2	326.7 $\pm$ 137.9	312.2 $\pm$ 152.7	-1.186	-1.311
CD11b MFI on total neutrophils	396.4 $\pm$ 186.3	302.8 $\pm$ 130.8	296.7 $\pm$ 143.6	-0.808	-0.861

<sup>3</sup> Dementia: patients classified with MCI +ve and AD.<sup>4</sup> Effect size of dementia compared with CN (CL  $\leq 25$ ).

**Table S3.** MFI of P2X7 on leukocyte subtypes in the validation cohort A (Model 1).

Variable	Validation A (Model 1)		
	Control ( $\leq 25$ CL)	Case ( $> 25$ CL)	Effect size of Case ( $> 25$ CL)
P2X7 MFI on total lymphocytes	$4.4 \pm 1.4$	$4 \pm 0.8$	-0.381
P2X7 MFI on NK cells (CD14 <sup>-</sup> CD16 <sup>+</sup> )	$13.0 \pm 21.6$	$8.6 \pm 8.3$	-0.535
P2X7 MFI on B&T cells (CD14 <sup>-</sup> CD16 <sup>-</sup> )	$3.7 \pm 0.5$	$3.6 \pm 0.4$	-0.319
P2X7 MFI on total monocytes	$13.7 \pm 10.3$	$11 \pm 4.5$	-0.573
P2X7 MFI on intermediate monocytes (CD14 <sup>+</sup> CD16 <sup>+</sup> )	$12.8 \pm 4.9$	$11.9 \pm 5.0$	-0.174
P2X7 MFI on classical monocytes (CD14 <sup>+</sup> CD16 <sup>-</sup> )	$10.1 \pm 2.5$	$9.4 \pm 2.5$	-0.285
P2X7 MFI on non-classical monocytes (CD14 <sup>dim</sup> CD16 <sup>+</sup> )	$14.7 \pm 13.6$	$9.1 \pm 4.5$	-1.233
P2X7 MFI on total neutrophils	$9.3 \pm 4.0$	$8 \pm 0.7$	-0.788
P2X7 MFI on CD16 <sup>++</sup> neutrophils	$9.3 \pm 4.9$	$8.4 \pm 3.1$	-0.188

**Table S4.** MFI of P2X7 on leukocyte subtypes in the validation cohort A (Model 2).

Variable	Validation cohort (Model 2)					
	CN ( $\leq 25$ CL)	CN ( $> 25$ CL)	Effect size of CN ( $> 25$ CL) <sup>5</sup>	MCI ( $\leq 25$ CL)	Dementia <sup>6</sup>	Effect size of Dementia <sup>7</sup>
P2X7 MFI on total lymphocytes	4.4 $\pm$ 1.5	3.8 $\pm$ 0.6	-0.997	4.6 $\pm$ 0.9	4.1 $\pm$ 0.9	-0.566
P2X7 MFI on NK cells (CD14 <sup>-</sup> CD16 <sup>+</sup> )	13.8 $\pm$ 23.0	6.2 $\pm$ 6.1	-1.234	10.0 $\pm$ 7.5	9.4 $\pm$ 8.8	-0.082
P2X7 MFI on B&T cells (CD14 <sup>-</sup> CD16 <sup>-</sup> )	3.7 $\pm$ 0.5	3.6 $\pm$ 0.5	-0.228	4.0 $\pm$ 0.4	3.5 $\pm$ 0.4	-1.175
P2X7 MFI on total monocytes	14.0 $\pm$ 10.8	11.1 $\pm$ 5.6	-0.522	14.2 $\pm$ 6.7	10.6 $\pm$ 3.7	-0.530
P2X7 MFI on intermediate monocytes (CD14 <sup>+</sup> CD16 <sup>+</sup> )	13.0 $\pm$ 5.2	11.5 $\pm$ 3.2	-0.480	12.3 $\pm$ 1.6	12.0 $\pm$ 5.7	-0.250
P2X7 MFI on classical monocytes (CD14 <sup>+</sup> CD16 <sup>-</sup> )	10.3 $\pm$ 2.5	10.0 $\pm$ 2.8	-0.093	10.0 $\pm$ 1.2	8.9 $\pm$ 2.2	-0.933
P2X7 MFI on non-classical monocytes (CD14 <sup>+</sup> CD16 <sup>+</sup> )	15.1 $\pm$ 14.1	8.9 $\pm$ 5.3	-1.183	14.8 $\pm$ 12.3	9.0 $\pm$ 3.8	-0.473
P2X7 MFI on total neutrophils	9.3 $\pm$ 4.0	7.6 $\pm$ 1.4	-1.204	8.2 $\pm$ 1.6	8.6 $\pm$ 3.2	0.224
P2X7 MFI on CD16 <sup>++</sup> neutrophils	9.3 $\pm$ 5.0	7.3 $\pm$ 1.4	-1.477	8.1 $\pm$ 1.5	8.5 $\pm$ 3.4	0.262

<sup>5</sup> Effect size of CN ( $> 25$  CL) compared with CN ( $\leq 25$  CL).<sup>6</sup> Dementia: patients classified with MCI +ve and AD.<sup>7</sup> Effect size of dementia compared with MCI (CL  $\leq 25$ ).

**Table S5.** 12 P2X7 and P2X4 SNPs and their corresponding functional scores.

CHR	SNP	AA	Gene	function	PRS-pore <sup>8</sup>			PRS-phago <sup>9</sup>				Reference
					0EA	1EA	2EA	function	0EA	1EA	2EA	
12	rs17525809	V76A	P2X7	LOF	0	-0.5	-1	No change	0	0	0	[43]
12	rs28360447	G150R	P2X7	LOF	0	-0.5	-1	LOF	0	-0.25	-0.5	[44]; [45]
12	rs208294	H155Y <sub>10</sub>	P2X7	GOF	0	0.5	1	No change	0	0	0	[45]; [46]
12	rs7958311	R270H	P2X7	LOF	0	-0.25	-0.5	No change	0	0	0	[43]; [45]
12	rs7958316	R276H	P2X7	LOF	0	-0.5	-1	No change	0	0	0	[43]; [45]
12	rs28360457	R307Q	P2X7	LOF	0	-1	-1	Change	0	0	0	[45]; [47]
12	rs1718119	A348T <sup>11</sup>	P2X7	GOF	0	0.5	1	No change	0	0	0	[45]; [46]
12	rs2230911	T357S	P2X7	LOF	0	-0.5	-1	No change	0	0	0	[43]
12	rs2230912	Q460R	P2X7	No Change	0	0	0	No change	0	0	0	[45]; [46]
12	rs3751143	E496A	P2X7	LOF	0	-0.5	-1	No change	0	0	0	[48]; [49]
12	rs1653624	I568N	P2X7	LOF	0	-0.75	-1	LOF	0	-0.5	-1	[45]; [50]
12	rs28360472	Y315C	P2X4	No change	0	0	0	LOF	0	-0.8	-1	[44]

<sup>8</sup> To assess the pore formation function of P2X7<sup>9</sup> To assess the innage phagocytic function of P2X7<sup>10</sup> If both H155Y and A348T were heterozygous, PRS-pore added 0.5; if one was germline and another was homozygous, PRS-pore added 0.75; if both were homozygous, PRS-pore added 1.<sup>11</sup> If both H155Y and A348T were heterozygous, PRS-pore added 0.5; if one was germline and another was homozygous, PRS-pore added 0.75; if both were homozygous, PRS-pore added 1.

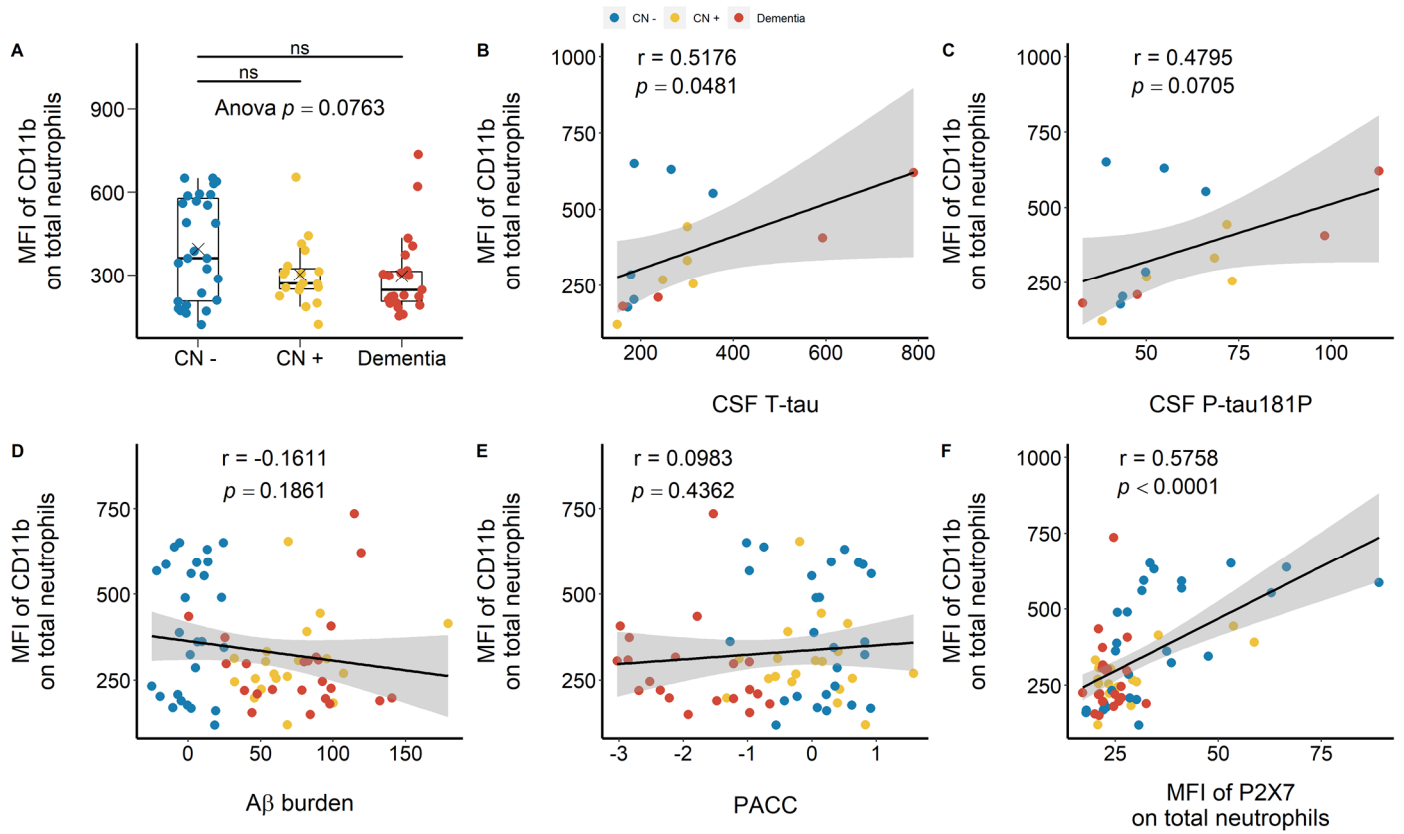
**Table S6.** 12 P2X7 and P2X4 SNPs in the AIBL cohort (647 CN, 77 MCI, and 176 AD).

CHR	SNP	AA	Gene	A1	A2	F_A	F_U	CHISQ	P	OR	MAF_CN <sup>12</sup>	MAF_MCI <sup>13</sup>	MAF_AD <sup>14</sup>
12	rs17525809	<b>V76A</b>	P2X7	C	T	0.072	0.099	3.858	<b>0.050</b>	0.702	0.072	0.087	0.072
12	rs28360447	G150R	P2X7	A	G	0.014	0.025	2.760	0.097	0.548	0.014	0.040	0.014
12	rs208294	H155Y	P2X7	T	C	0.434	0.440	0.070	0.791	0.973	0.434	0.427	0.434
12	rs7958311	R270H	P2X7	A	G	0.274	0.260	0.403	0.525	1.077	0.274	0.286	0.274
12	rs7958316	R276H	P2X7	A	G	0.027	0.025	0.092	0.761	1.105	0.027	0.048	0.027
12	rs28360457	R307Q	P2X7	A	G	0.017	0.019	0.126	0.723	0.873	0.017	0.024	0.017
12	rs1718119	A348T	P2X7	T	C	0.389	0.414	1.010	0.315	0.900	0.389	0.389	0.389
12	rs2230911	T357S	P2X7	G	C	0.075	0.073	0.047	0.829	1.044	0.075	0.063	0.075
12	rs2230912	Q460R	P2X7	G	A	0.177	0.164	0.451	0.502	1.097	0.177	0.175	0.161
12	rs3751143	E496A	P2X7	G	T	0.170	0.172	0.009	0.925	0.987	0.170	0.167	0.170
12	rs1653624	I568N	P2X7	A	T	0.029	0.021	0.894	0.344	1.386	0.029	0.032	0.029
12	rs28360472	Y315C	P2X4	G	A	0.013	0.015	0.150	0.698	0.846	0.013	0.024	0.013

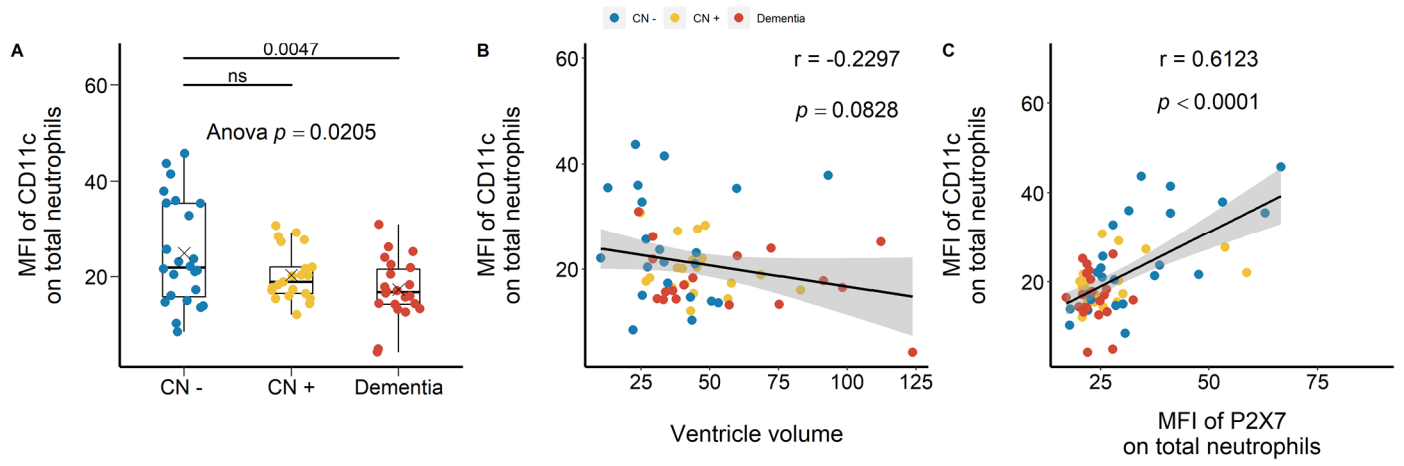
<sup>12</sup> Minor allele frequency (MAF) in the CN cohort.<sup>13</sup> MAF in the MCI cohort.<sup>14</sup> MAF in the AD cohort.

**Table S7.** 12 P2X7 and P2X4 SNPs in the ADNI cohort (255 CN, 379 MCI, and 152 AD).

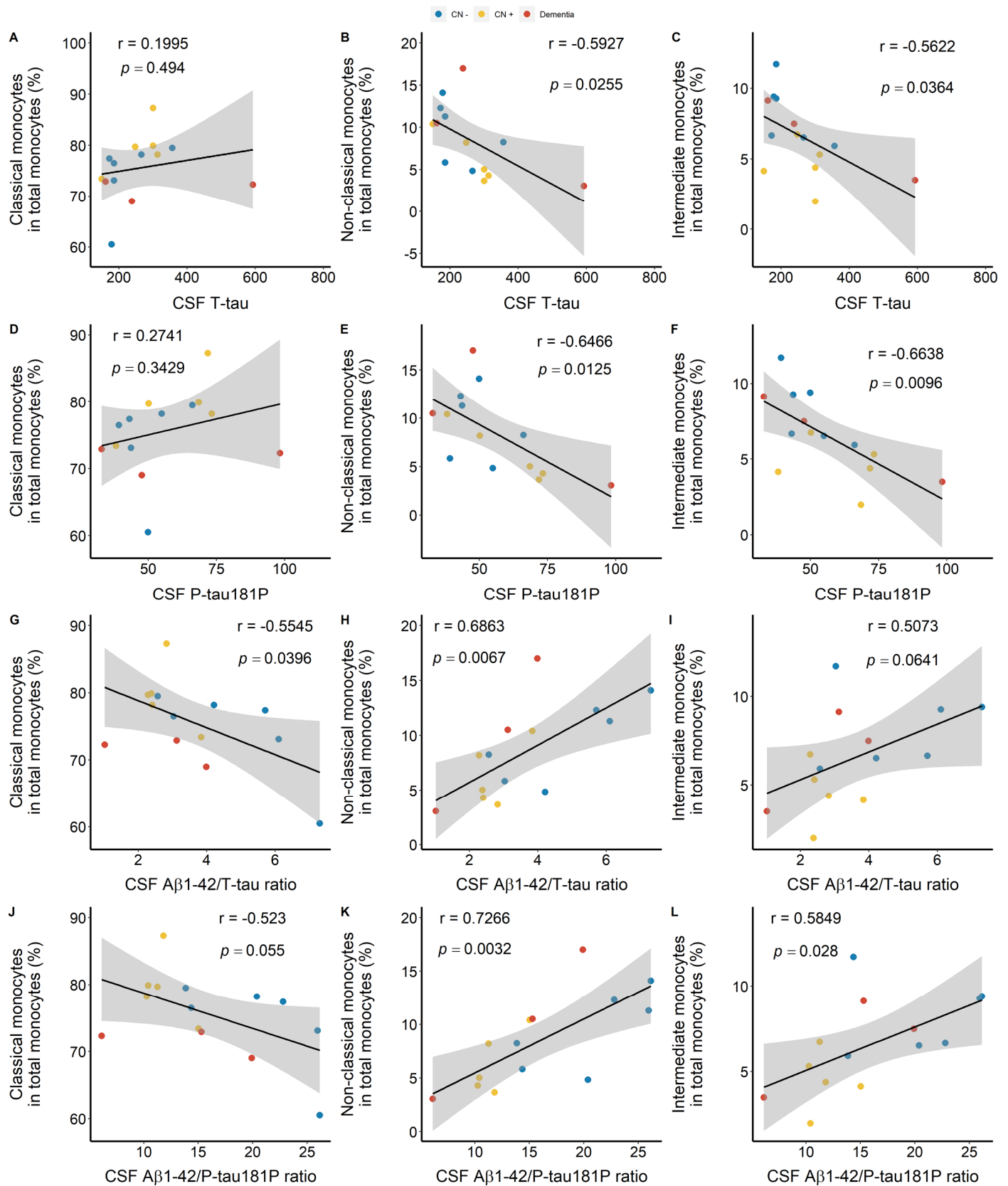
CHR	SNP	AA	Gene	A1	A2	F_A	F_U	CHISQ	P	OR	MAF_CN	MAF_MCI	MAF_AD
12	rs17525809	V76A	P2X7	C	T	0.081	0.073	0.193	0.660	1.123	0.073	0.065	0.081
12	rs28360447	G150R	P2X7	A	G	0.016	0.019	0.145	0.704	0.811	0.019	0.014	0.016
12	rs208294	H155Y	P2X7	T	C	0.438	0.437	0.001	0.980	1.004	0.437	0.447	0.438
12	rs7958311	R270H	P2X7	A	G	0.255	0.286	1.001	0.317	0.852	0.286	0.245	0.255
12	rs7958316	R276H	P2X7	A	G	0.016	0.023	0.551	0.458	0.673	0.023	0.017	0.016
12	rs28360457	R307Q	P2X7	A	G	0.025	0.021	0.135	0.714	1.188	0.021	0.009	0.025
12	rs1718119	A348T	P2X7	T	C	0.370	0.361	0.068	0.794	1.039	0.361	0.372	0.370
12	rs2230911	T357S	P2X7	G	C	0.087	0.082	0.062	0.803	1.065	0.082	0.076	0.087
12	rs2230912	Q460R	P2X7	G	A	0.154	0.170	0.911	0.34	0.891	0.153	0.140	0.146
12	rs3751143	E496A	P2X7	C	A	0.186	0.174	0.218	0.640	1.090	0.174	0.188	0.186
12	rs1653624	I568N	P2X7	A	T	0.034	0.021	1.366	0.243	1.650	0.021	0.025	0.034
12	rs28360472	Y315C	P2X4	G	A	0.016	0.011	0.258	0.611	1.362	0.011	0.014	0.016



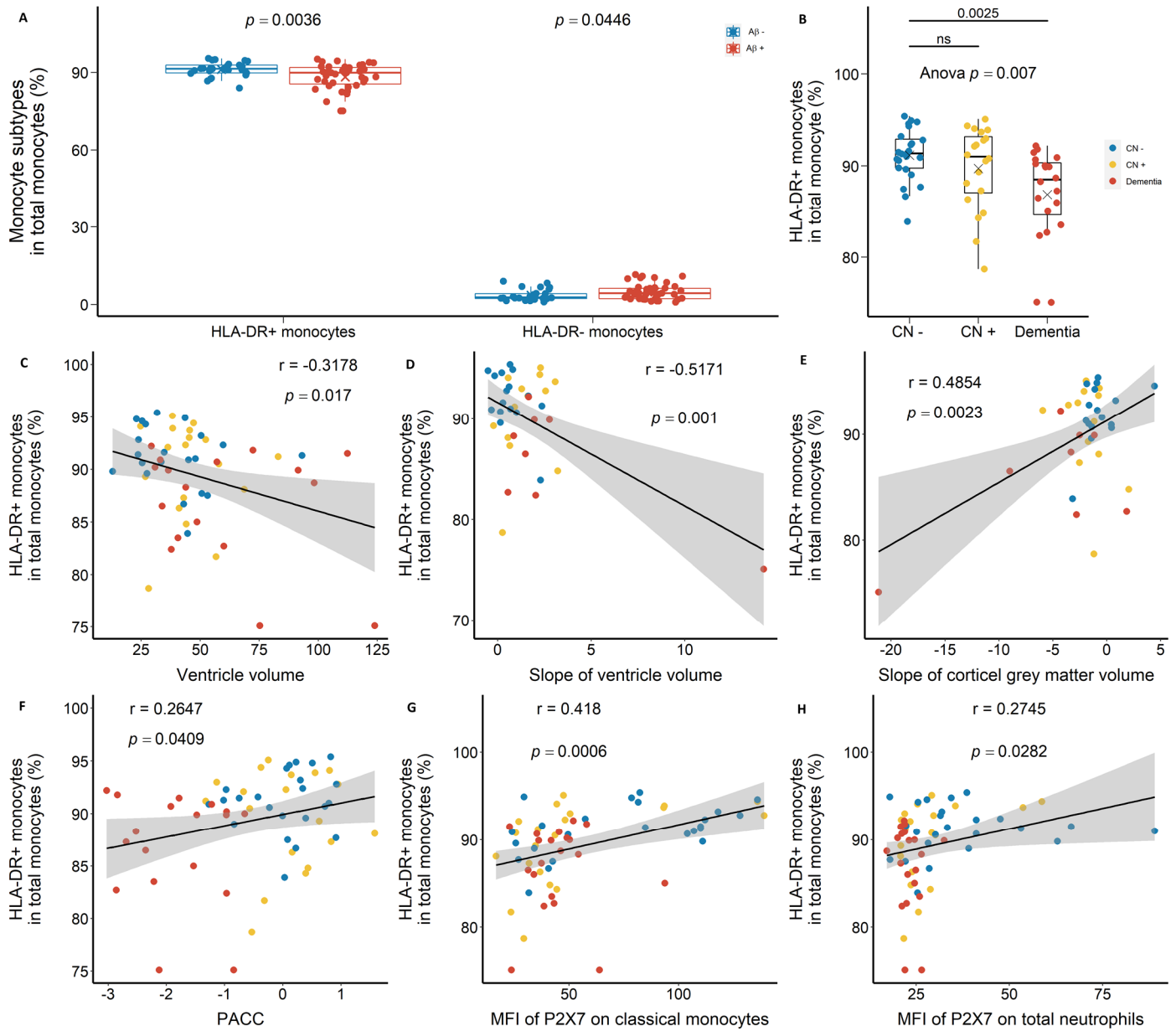
**Figure S1.** CD11b expression on peripheral neutrophils in the discovery cohort. **A.** CD11b expressions on total neutrophils between CN (CN -: CN -ve), pre-clinical (CN +: CN +ve), and AD with dementia (dementia: MCI +ve and AD) individuals. Bar graphs illustrated the boxplot distribution of individual measurements with “x” denoting the mean. Three-group comparison was determined by one-way ANOVA followed by multiple comparison using Dunnett’s post-hoc test (solid line). **B-E.** The associations between neutrophils CD11b expressions, CSF biomarkers ( $\mu\text{g/L}$ ), A $\beta$  burden (CL) measured by PET, and PACC. **F.** Association between neutrophil CD11b expression and neutrophil P2X7 expression. Correlation  $r$  and  $P$  values were calculated by Pearson product-moment correlational analysis. The “grey” band indicated the 95% confidence interval of the black linear regression line. ns: no significance.



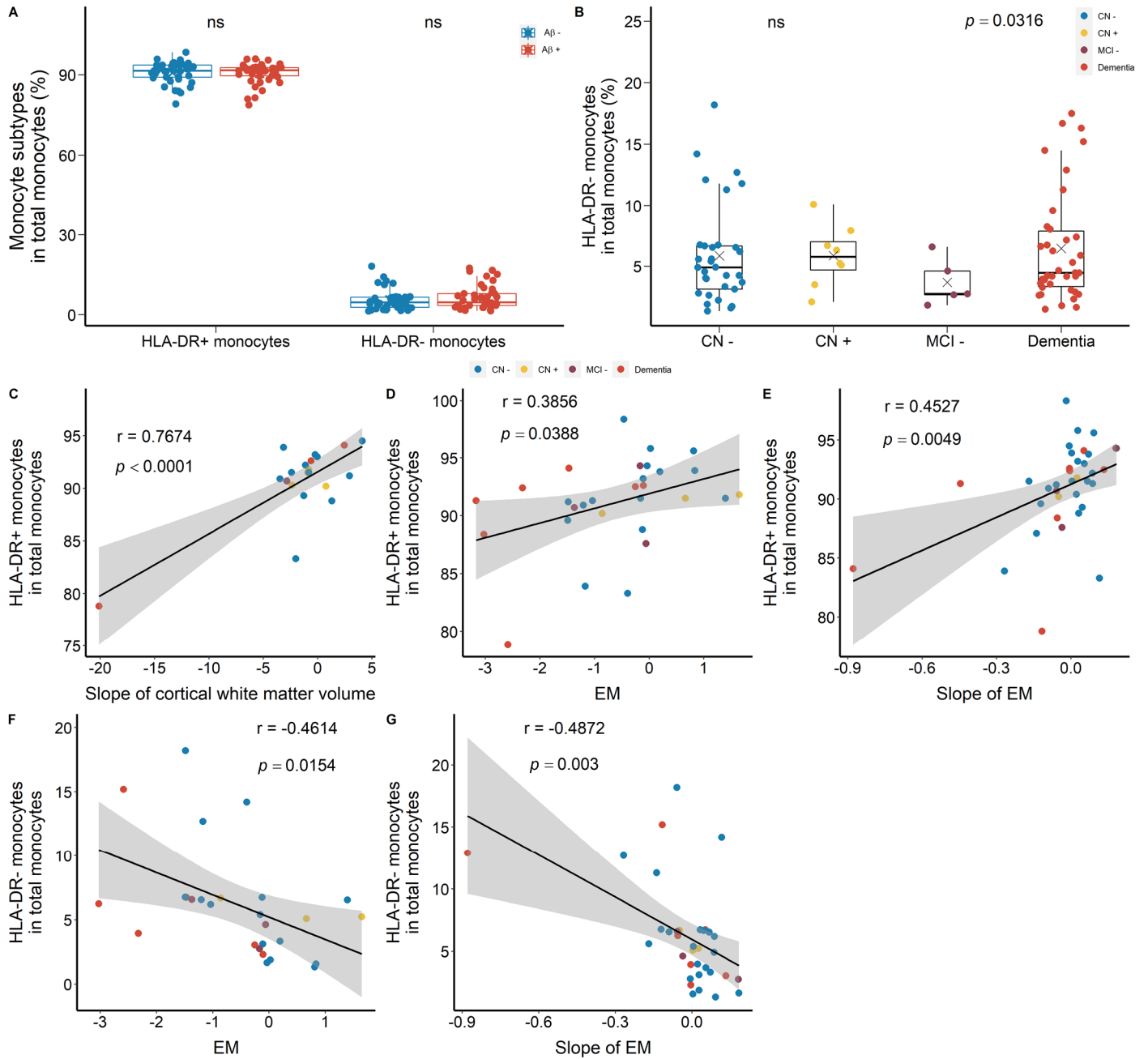
**Figure S2.** CD11c expression on peripheral neutrophils in the discovery cohort. **A.** CD11c expressions on total neutrophils between CN (CN -: CN -ve), pre-clinical (CN +: CN +ve), and AD with dementia (dementia: MCI +ve and AD) individuals. Bar graphs illustrated the boxplot distribution of individual measurements with “x” denoting the mean. Three-group comparison was determined by one-way ANOVA followed by multiple comparison using Dunnett’s post-hoc test (solid line). **B-C.** The associations between neutrophil CD11c expressions, ventricle volume, and neutrophil P2X7 expression. Correlation  $r$  and  $P$  values were calculated by Pearson product-moment correlational analysis. The “grey” band indicated the 95% confidence interval of the black linear regression line. ns: no significance.



**Figure S3.** Monocyte subpopulations associated with CSF biomarkers in the discovery cohort. **A-C.** Associations with CSF T-tau concentration (μg/L). **D-F.** Associations with CSF P-tau181P concentration (μg/L). **G-I.** Associations with CSF Aβ<sub>1-42</sub>/T-tau ratio. **J-L.** Associations with CSF Aβ<sub>1-42</sub>/P-tau181P ratio. Correlation  $r$  and  $P$  values were calculated by Pearson product-moment correlational analysis. The “grey” band indicated the 95% confidence interval of the black linear regression line.



**Figure S4.** Relative percentages of HLA-DR<sup>+</sup> and HLA-DR<sup>-</sup> monocytes in total monocytes in the discovery cohort. **A.** Comparisons of HLA-DR<sup>+</sup> and HLA-DR<sup>-</sup> monocytes between  $A\beta$  +ve cases ( $A\beta^+$ ) and  $A\beta$  -ve controls ( $A\beta^-$ ). Bar graphs illustrated the boxplot distribution of individual measurements with “x” denoting the mean. Two-group comparison was determined by *t*-test. **B.** Comparison of HLA-DR<sup>+</sup> monocytes between CN (CN<sup>-</sup>: CN -ve), pre-clinical (CN<sup>+</sup>: CN +ve), and AD with dementia individuals (dementia: MCI +ve and AD). Bar graphs illustrated the boxplot distribution of individual measurements with “x” denoting the mean. Three-group comparison was determined by one-way ANOVA followed by multiple comparison using Dunnett’s post-hoc test (solid line). **C-H.** Associations between HLA-DR<sup>+</sup> monocytes, brain atrophy, PACC, and P2X7 expressions. Correlation *r* and *P* values were calculated by Pearson product-moment correlational analysis. The “grey” band indicated the 95% confidence interval of the black linear regression line. ns: no significance.



**Figure S5.** Relative percentages of HLA-DR<sup>+</sup> and HLA-DR<sup>-</sup> monocytes in total monocytes in the validation cohort B. **A.** Comparisons of HLA-DR<sup>+</sup> and HLA-DR<sup>-</sup> monocytes between A $\beta$  +ve cases (A $\beta$  +) and A $\beta$  -ve controls (A $\beta$  -). **B.** Comparison of HLA-DR<sup>-</sup> monocytes between CN (CN -: CN -ve) and pre-clinical (CN +: CN +ve) individuals. Comparison of HLA-DR<sup>-</sup> monocytes between prodromal (MCI -: MCI -ve) and AD with dementia individuals (dementia: MCI +ve and AD). Bar graphs illustrated the boxplot distribution of individual measurements with “x” denoting the mean. Two-group comparison was determined by *t*-test. **C-G.** Associations between HLA-DR<sup>+</sup> monocytes, brain atrophy and EM. Correlation *r* and *P* values were calculated by Pearson product-moment correlational analysis. The “grey” band indicated the 95% confidence interval of the black linear regression line. ns: no significance.