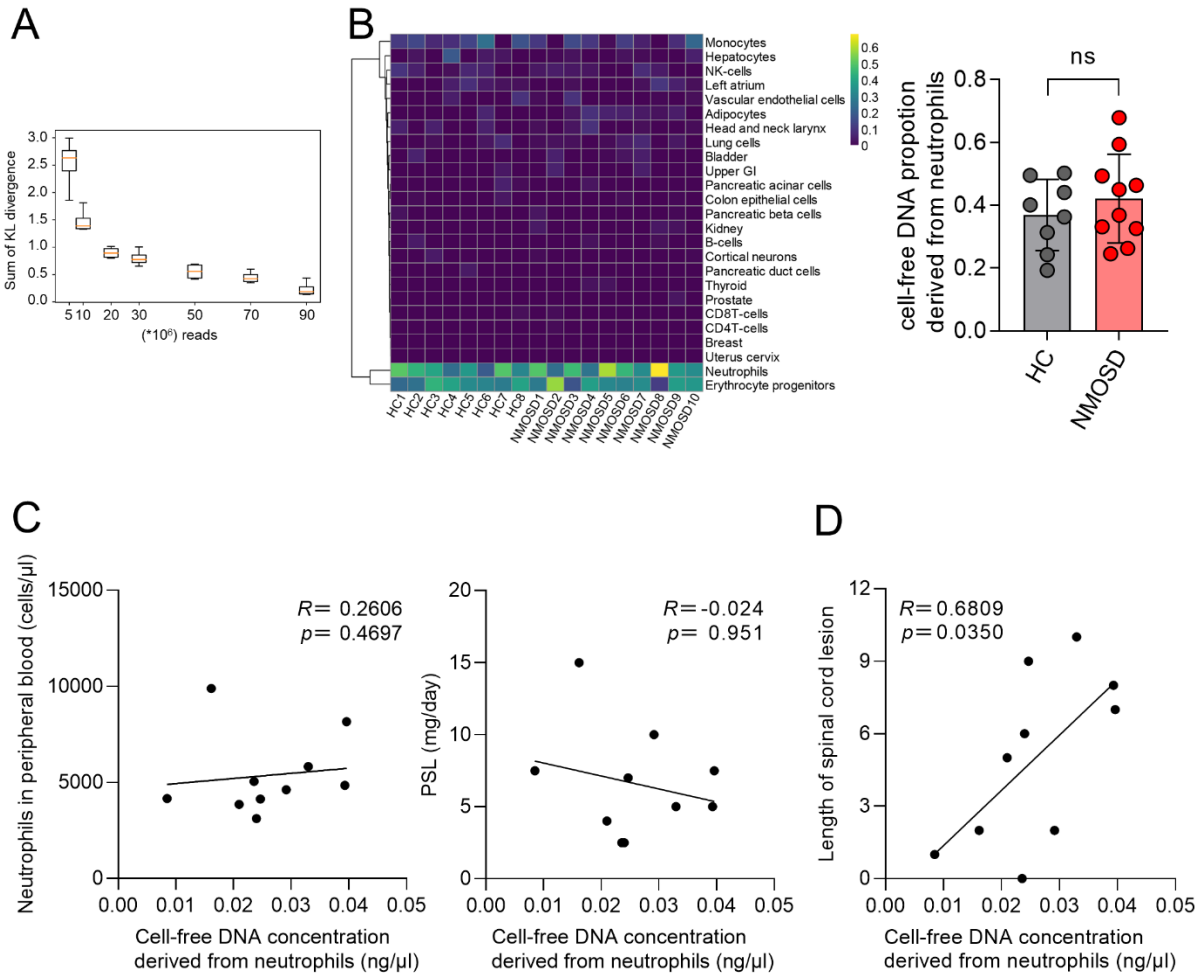
Supplementary Material

Cell-free DNA Derived from Neutrophils Triggers Type1 Interferon Signature in NMOSD

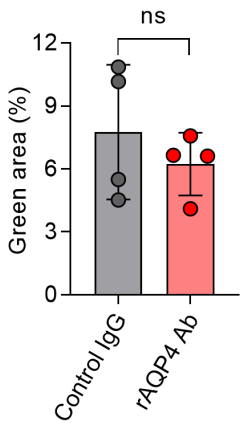
eMethods

Recombinant AQP4 antibody assay.

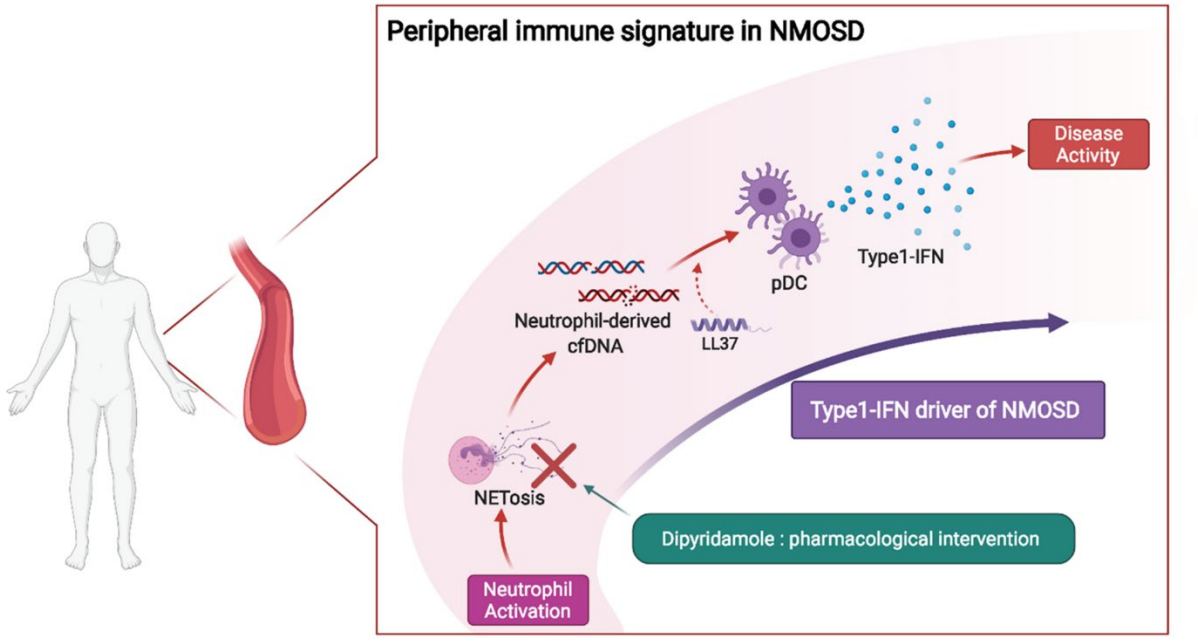
Neutrophils were seeded on Iwaki chamber slides at a density of 10^6 cells/ml. Cells were incubated for 1 hour in a CO2 incubator at 37°C and then stimulated with isotype control IgG (200µg/ml) or recombinant AQP4 antibody[e1] (200µg/ml). After stimulation for 4 hours, each well was washed with PBS, then SYTOX™ green stain (Invitrogen™, Waltham, MA) was added to non-fixed live cells so that only extracellular DNA would be detected. Immunofluorescence confocal microscopy was performed by use of a BZ-X710 All-In-One Fluorescence Microscope (Keyence, Osaka, Japan), and the images were analyzed with ImageJ software (National Institutes of Health, Bethesda, MD).



eFigure 1. Next-generation sequencing analysis of cfDNA derived from neuromyelitis optica spectrum disorder (NMOSD) patients. (**A**) KL divergence for estimating the required number of reads. (**B**) Heat map visualization of cfDNA proportion from different sources. (**C**) Correlation between cell-free DNA concentration derived from neutrophils (ng/μl) and neutrophils in peripheral blood (cells/μl) or the dosage of prednisolone (PSL) (mg/day). Spearman′s correlation coefficient was used to determine statistical significance. (**D**) The correlation between cell-free DNA concentration derived from neutrophils (ng/μl) and length of spinal cord lesion. Spearman's correlation coefficient was used to determine statistical significance.



eFigure 2. The influence of recombinant AQP4 antibody on NETosis in neutrophils. Quantification of NET formation in neutrophils stimulated with recombinant AQP4 antibody or isotype control IgG. Data are expressed as total SYTOX™ green-positive extracellular DNA area. The Mann-Whitney U test was used to determine statistical significance.



eFigure 3. Schematic model in which neutrophil activation serves as IFN-1 driver in dysregulated immune signature of NMOSD.

eTable 1. Demographic and Clinical Characteristics of patients with NMOSD and HC		
	NMOSD (n=32)	**HC (n=23)**
Age (years): median (IQR)	46.5 (42.25-62.5)	42 (34-46)
Sex (female): n (%)	30/32 (93.8)	15/23 (65.2)
Duration of disease (years): median (IQR)	5 (3-9.75)	N/A
Annualized relapse rate: median (IQR)	0.33 (0.21-0.65)	N/A
EDSS: median (IQR)	3 (1-6)	N/A
Brain and spinal cord lesions: n (%)	26/32 (81.3)	N/A
Optic neuritis lesion: n (%)	16/32 (50)	N/A
Treatment		
PSL (mg/day): median (IQR)	6 (4.25-10)	N/A
Oral immunosuppressive agents: n (%)	23/32 (71.88)	N/A
Eculizumab: n (%)	1/32 (3.1)	N/A
Satralizumab: n (%)	3/32 (9.4)	N/A
Rituximab: n (%)	0/32 (0)	N/A
Serological profile		
AQP4 antibody (IU/mL): median (IQR)	11.55 (2.03-40)	N/A
ANA prevalence: n (%)	17/30 (56.7)	N/A
Anti-SSA prevalence: n (%)	12/29 (41.4)	N/A
CSF oligoclonal bands positive: n (%)	6/26 (23.1)	N/A
Data are presented as n (%) or median (IQR)		
Abbreviations: ANA = antinuclear antibodies; CSF = cerebrospinal fluid; EDSS = Expanded Disability Status Scale; HC = healthy control; IQR = interquartile range; PSL = prednisolone.		

eTable 1. Characteristics of patients with NMOSD and HC collected at Osaka University Hospital.

eReferences

e1. Shimizu M, Okuno T, Kinoshita M, et al. Mitochondrial DNA enhance innate immune responses in neuromyelitis optica by monocyte recruitment and activation. *Sci Rep*. 2020;10(1):13274; doi:10.1038/s41598-020-70203-x