

Supplementary Information for
Astrocytic TRIM21 downregulation exacerbates NMOSD via potentiating
IRF3/IRF7-mediated type I interferon response

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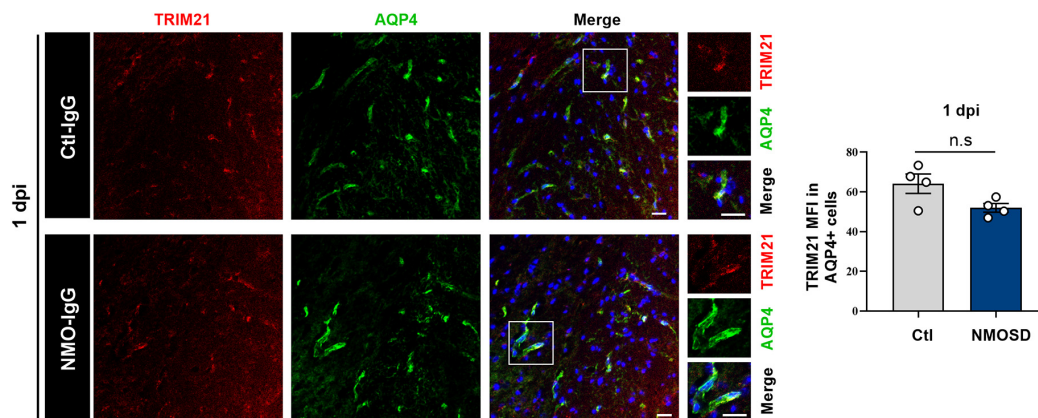


Figure S1. Representative images and quantification of TRIM21 levels in astrocytes from brain of NMOSD and control mice. Brain sections were collected 1 day after intracerebral injection of control IgG or AQP4-IgG plus human complement. Scale bar: 20 μ m. $n=4$ mice per group. For each mouse, mean fluorescence intensity (MFI) value of TRIM21 in AQP4+ cells was calculated from 8-9 regions of interest (ROIs) within peri-lesion areas at 20 $\times$ magnification. n.s, non-significant.

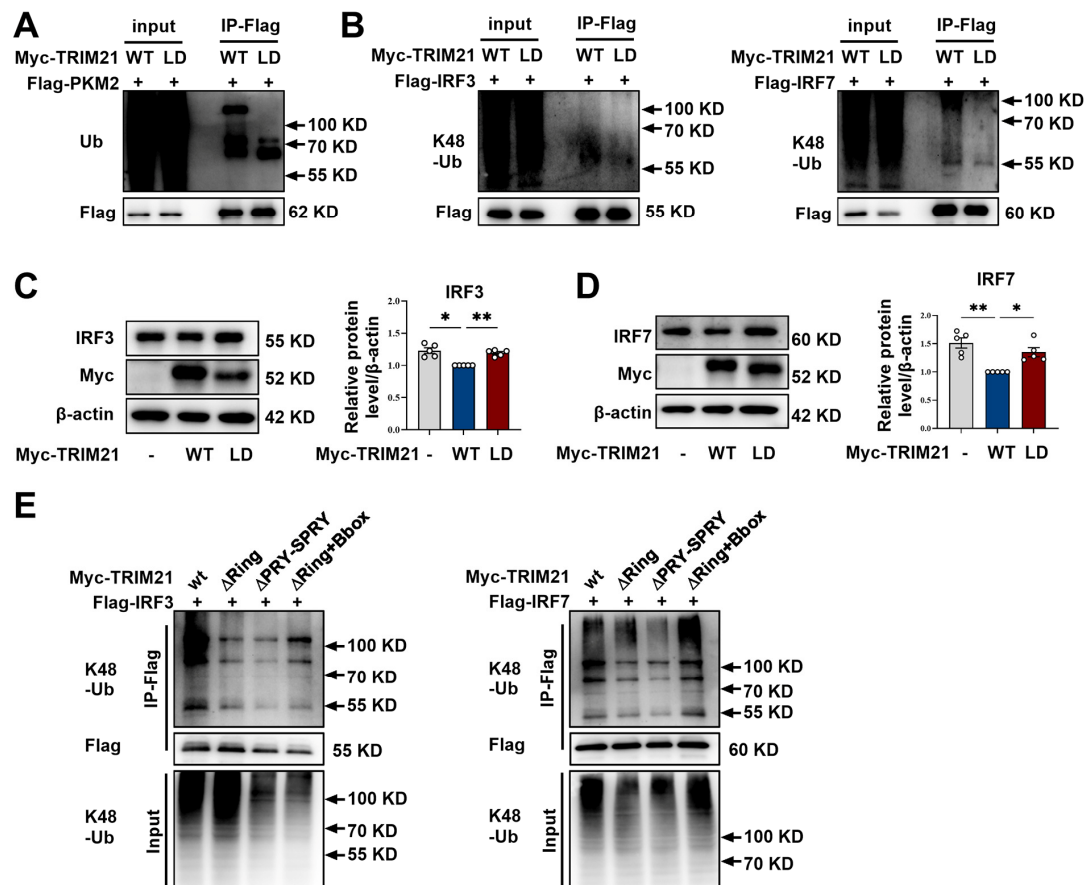


Figure S2. E3 ligase activity of TRIM21 is required for the degradation and ubiquitination of IRF3 and IRF7.

A. Ligase-dead mutant of mouse TRIM21 (C12A, C27A, H29W) lost the ability to ubiquitylate its substrate PKM2. **B.** LD mutant of TRIM21 lost the ability to catalyze K48-linked ubiquitination of IRF3 or IRF7. 293T cells were co-transfected with Flag-IRF3 (left) /Flag-IRF7 (right) and either TRIM21 WT or its ligase-dead (LD) form, following by immunoprecipitation with Flag. **C-D.** LD mutant form of TRIM21 lost the ability to degrade IRF3 or IRF7. 293T cells were transfected with empty vector, Myc-tagged WT-TRIM21 or LD-TRIM21. Immunoblots analysis and quantification of IRF3 (C) and IRF7 (D) protein levels. **E.** Deletion of RING or PRY-SPRY domain of TRIM21 abolished the TRIM21-induced K48-linked ubiquitination of IRF3 or IRF7. 293T cells were co-transfected with Flag-IRF3(left) or Flag-IRF7 (right) and WT Myc-TRIM21 or their truncation mutants for 72 h. * $P < 0.05$, ** $P < 0.01$, using one-way ANOVA analysis followed by Dunnett's multiple comparisons test.

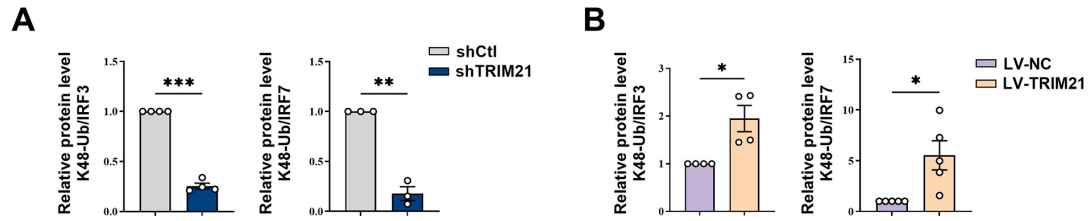


Figure S3. TRIM21 promoted the K48-linked ubiquitination of IRF3 or IRF7.

A. Quantification of K48-linked ubiquitination of IRF3 or IRF7 in TRIM21-knocked down (shTRIM21) astrocytes and controls (shCtrl) in Figure 5F. B. Quantification of K48-linked ubiquitination of IRF3 or IRF7 in TRIM21-overexpressing (LV-TRIM21) astrocytes and controls (LV-NC) in Figure 5G. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, using unpaired t-test with Welch's correction.

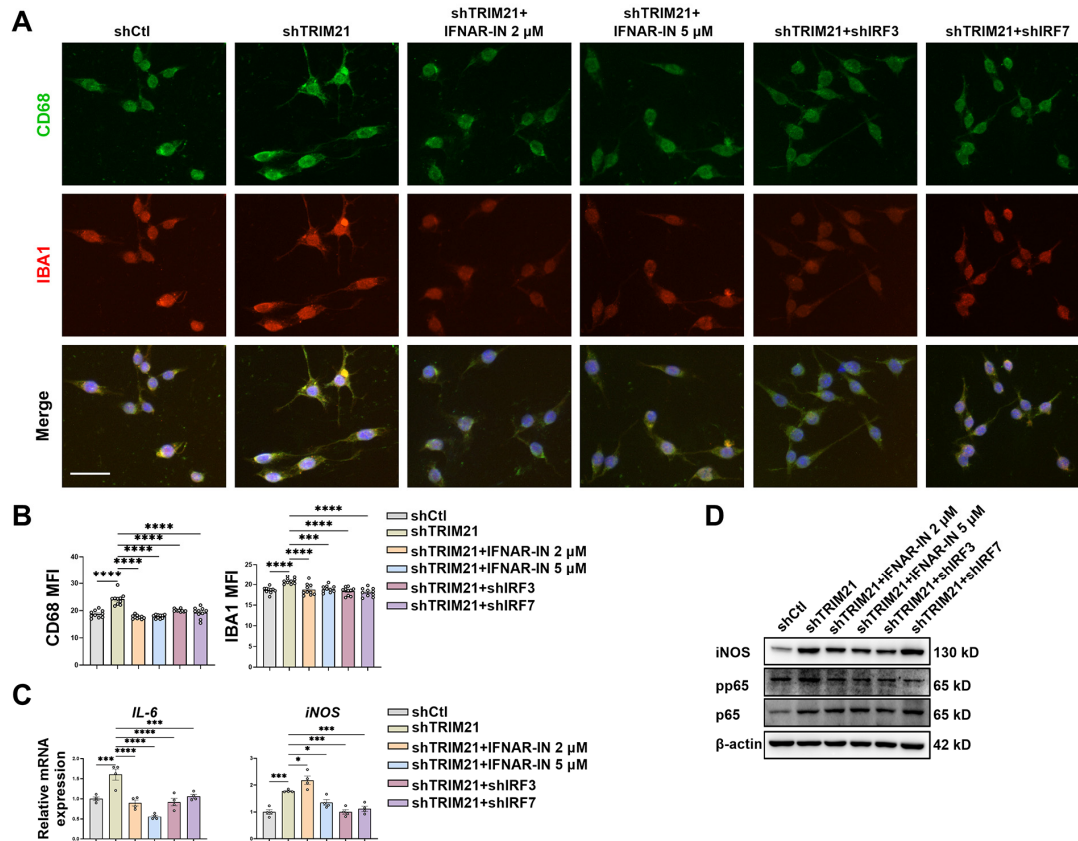


Figure S5. TRIM21 knockdown-induced microglial activation is rescued by IRF3/IRF7 knockdown or IFNAR blockade.

A. Representative fluorescence images of IBA1 staining in BV2 cells treated with ACM from the indicated group. Astrocytes were treated with NMO-IgG for 24h, followed by collection of astrocyte conditioned medium (ACM). For shTRIM21+IFNAR-IN group, BV2 cells were pretreated with IFNAR-IN (an inhibitor of IFN- α /IFN receptor interaction) at indicated concentrations for 2 h, followed by stimulation with ACM from shTRIM21-treated astrocytes. **B.** Quantification of IBA1 and CD68 intensities in each group. **C.** qRT-PCR analysis showed that upregulation of IL-6 and iNOS mRNA levels induced by ACM from shTRIM21 astrocytes was rescued by IRF3 or IRF7 knockdown, or IFNAR blockade. **D.** Immunoblotting showed that the increased levels of iNOS and phosphorylated p65 induced by ACM from shTRIM21 astrocytes were rescued by IRF3 or IRF7 knockdown, or IFNAR blockade. Scale bar: 50 μ m. * P < 0.05, *** P < 0.001, **** P < 0.0001, using one-way ANOVA analysis followed by Dunnett's multiple comparisons test.

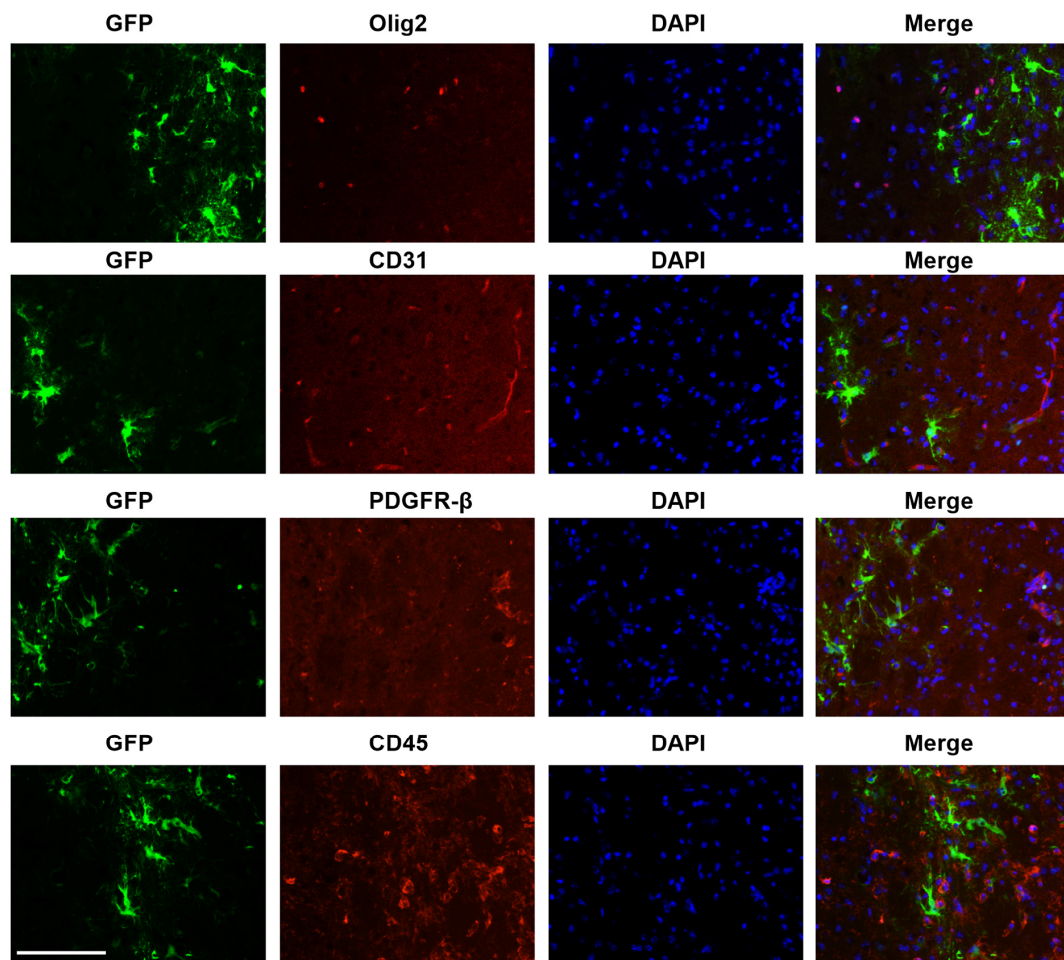


Figure S6. Immunofluorescence results confirmed astrocyte-specific lentiviral transduction, with no detectable expression in oligodendrocytes, endothelial cells, pericytes and infiltrating immune cells.

Representative immunofluorescence staining of GFP with markers of oligodendrocytes (Olig2), endothelial cells (CD31), pericytes (PDGFR-β), and infiltrating immune cells (CD45). Scale bar: 100 μm.

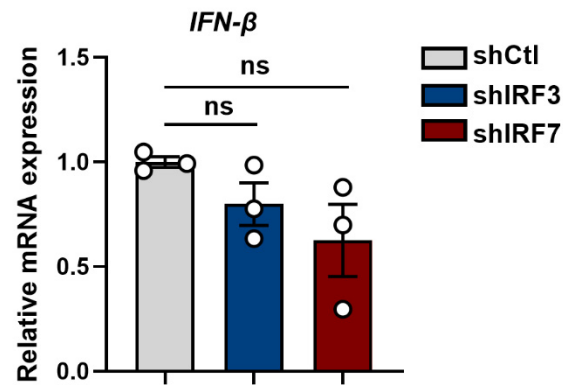


Figure S7. mRNA level of IFN- β in brain of mice. qRT-PCR analysis of IFN- β in brain of mice expressing lentivirus-shCtl, lentivirus-shIRF3 or lentivirus-shIRF7 with NMOSD induction. ns, non-significant.

Table S1. Primers used in this study.

Name	Primer sequences (5'-3' orientation)
TRIM21	Forward: GGGAGGAGGTCACCTGTTCTA Reverse: GGCACCTCGGGACATGAACTG
IL-6	Forward: GCTGGAGTCACAGAAGGAGTGGC Reverse: GGCATAACGCACTAGGTTTGCCG
iNOS	Forward: CAGCTGGGCTGTACAAACCTT Reverse: CATTGGAAGTGAAGCGTTTCG
IFN- α	Forward: GGACTTTGGATTCCCGCAGGAGAAG Reverse: GCTGCATCAGACAGCCTTGCAGGTC
IFN- β	Forward: GCTGCGTTCCTGCTGTGCT Reverse: CGCCCTGTAGGTGAGGTTGAT
GAPDH	Forward: AGGTCGGTGTGAACGGATTTG Reverse: TGTAGACCATGTAGTTGAGGTCA
β -Actin	Forward: GCTGCCTCAACACCTCAACCC Reverse: GTCCCTCACCTCCCAAAG

Table S2. Primary antibodies for immunofluorescence used in this study.

Antibodies	Source	Identifier	Concentration
Anti-TRIM21	Proteintech	Cat#67136-1-Ig	1:500
Anti-IRF3	Huabio	Cat#ET1612-14	1:100
Anti-IRF7	Huabio	Cat#ET-1610-89	1:100
Anti-GFAP	Proteintech	Cat#60190-1-Ig	1:200
Anti-AQP4	Boster	Cat#PB9475	1:200
Anti-MBP	Proteintech	Cat#10458-1-AP	1:200
Anti-Ki67	Abcam	Cat#1063774-5	1:200
Anti-Iba1	Wako	Cat#019-19741	1:200
Anti-Olig2	Oasis Biofarm	Cat#OB-PRT020	1:500
Anti-CD31	BD Biosciences	Cat#550274	1:100
Anti-PDGFR- β	R&D systems	Cat#AF1042	1:60
Anti-CD45	BD Biosciences	Cat#550539	1:200
Anti-GFP	Proteintech	Cat#50430-2-AP	1:100
Anti-Vimentin	Abmart	Cat#T55134F	1:300
	Cell Signaling		
Anti-cleaved caspase 3	Technology	Cat#9664T	1:300
	Santa Cruz		
Anti-S100A10	Biotechnology	Cat#sc-50450	1:50

Table S3. Primary antibodies for western blotting used in this study.

Antibodies	Source	Identifier	Concentration
Anti-TRIM21	Proteintech	Cat#12108-1-AP	1:1000
Anti-Actin	ServiceBio	Cat#GB12001-100	1:1000
Anti-phospho- p65(Ser536)	Bioss	Cat#bs-0982R	1:1000
Anti-p65	Huabio	Cat#ET1603-12	1:1000
Anti-TRIM21	Proteintech	Cat#67136-1-Ig	1:3000
Anti-IRF3	Huabio	Cat#ET1612-14	1:1000
Anti-IRF7	Huabio	Cat#ET-1610-89	1:3000
Anti-iNOS	Bioss	Cat#bs-0162R	1:1000
Anti-phospho- IRF3 (S386)	Huabio	Cat#ET1608-22	1:1000
Anti-PCNA	Proteintech	Cat#16286-1-AP	1:1000
Anti-Tubulin	Proteintech	Cat#11224-1-AP	1:5000
Anti-K48-ubiquitin	Abcam	Cat#ab140601	1:1000
Anti-ubiquitin	Huabio	Cat#ET1609-21	1:1000
Anti-Myc	Proteintech	Cat#16286-1-AP	1:1000
Anti-Flag	Genescript	Cat#A00187	1:1000