Interleukin-19 alleviates experimental autoimmune encephalomyelitis by attenuating antigen-presenting cell activation

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Abstract

Interleukin-19 (IL-19) acts as an anti-inflammatory cytokine in various inflammatory diseases. Multiple sclerosis (MS) is a major neuroinflammatory disease in the central nervous system, but it remains uncertain how IL-19 contributes to MS pathogenesis. Here, we demonstrate that IL-19 deficiency aggravates experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, by promoting IL-17–producing helper T cell (Th17 cell) infiltration into the central nervous system. In addition, IL-19–deficient splenic macrophages expressed elevated levels of major histocompatibility complex class II, co-stimulatory molecules, and Th17 cell differentiation–associated cytokines such as IL-1β, IL-6, IL-23, TGF-β1, and TNF-α. These observations indicated that IL-19 plays a critical role in suppression of MS pathogenesis by inhibiting macrophage antigen presentation, Th17 cell expansion, and subsequent inflammatory responses. Furthermore, treatment with IL-19 significantly abrogated EAE. Our data suggest that IL-19 could provide significant therapeutic benefits in patients with MS.

38 **Introduction**

39 Multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), a
40 mouse model of MS, are major autoimmune demyelinating diseases of the central nervous
41 system (CNS)[1,2]. Various types of immune cells and soluble mediators contribute to the
42 complex mechanisms underlying the onset and progression of both MS and EAE, and
43 recent studies have shown that type 1 helper T (Th1) cells and interleukin-17–producing
44 helper T (Th17) cells play pivotal roles in their pathogenesis[3, 4, 5]. In these diseases,
45 autoreactive Th17 cells primed in the lymph nodes infiltrate the CNS and activate
46 microglia/macrophages that induce inflammatory demyelination and subsequent neuronal
47 damage, resulting in a wide range of clinical features, including sensory and motor
48 paralysis, blindness, pain, incontinence, and dementia[1, 2].

49 Interleukin-19 (IL-19) is an IL-10 family cytokine that is homologous and highly
50 similar to IL-20 and IL-24[6, 7]. IL-19 binds to the heterodimeric receptor consisting of IL-
51 20Rα and IL-20Rβ, and its downstream signaling is mediated by STAT3 phosphorylation[8,
52 9]. IL-19 is mainly produced by activated macrophages and microglia[10, 11, 12, 13]. Recent
53 studies showed that IL-19 exerts anti-inflammatory effects on macrophages by inhibiting
54 inflammatory cytokine production, downregulating antigen-presenting capacity, and
55 enhancing M2 phenotype polarization, which promotes type 2 helper T (Th2) cell
56 differentiation and suppresses Th1 and Th17 cell differentiation[11, 12, 14, 15, 16, 17]. In fact, IL-
57 19 plays a critical role in development of various autoimmune diseases, including
58 asthma[18, 19], psoriasis[20, 21, 22], inflammatory bowel disease[11, 23], rheumatoid arthritis[24], and
59 Type I diabetes[25]. However, it remains to be elucidated how IL-19 contributes to MS

60 pathogenesis.

61 Here, we examined the pathological role of IL-19 in EAE using IL-19–deficient
62 (IL-19$^{-/-}$) mice. IL-19 deficiency markedly exacerbated EAE, and treatment with IL-19
63 effectively suppressed EAE accompanied by inhibiting macrophage antigen presentation
64 and subsequent expansion of Th17 cells. Our findings suggest that IL-19 may provide
65 significant therapeutic benefits for treating MS.

66

67 **Results**

68

69 **IL-19 deficiency exacerbates EAE**

70 We generated myelin oligodendrocyte glycoprotein (MOG)-induced EAE in C57BL/6J
71 wild-type (WT) and IL-19–deficient (IL-19$^{-/-}$) mice. IL-19$^{-/-}$ mice exhibited earlier disease
72 onset and more severe symptoms than WT mice (Fig. 1A). Histological analysis of the
73 lumbar spinal cords revealed more inflammatory cell infiltration in IL-19$^{-/-}$ mice than in
74 WT mice (Fig. 1B). Flow cytometric analysis also disclosed that IL-19$^{-/-}$ EAE mice had
75 more CNS-infiltrating cells than WT EAE mice (Fig. 1C). We then chronologically
76 evaluated IL-19 expression levels in the spleen and lumbar spinal cord of WT mice at
77 pre-immunization, disease onset, and disease peak. Splenic IL-19 mRNA expression was
78 upregulated at disease onset, but was strongly suppressed at the disease peak (Fig. 1D).
79 By contrast, upregulation of IL-19 mRNA in the lumbar spinal cord was observed at
80 disease peak (Fig. 1E). These results suggest that endogenous IL-19 serves as a negative
81 regulator of EAE pathogenesis at both the induction and effector phases.

82

83 **IL-19 deficiency increases Th17 cell infiltration into the CNS**

84 Because EAE is a Th1 and Th17 cell–mediated autoimmune disease, we next assessed
85 whether IL-19 deficiency would increase CNS infiltration of Th1 and Th17 cells. Flow
86 cytometric analysis revealed that at disease peak, IL-19$^{-/-}$ mice exhibited more infiltration
87 of Th17 cells in the spinal cord than WT mice (Fig. 2A, B). By contrast, no difference
88 was observed in Th1 cell infiltration between IL-19$^{-/-}$ and WT mice (Fig. 2C). These

89 results indicate that IL-19 deficiency mediates elevated CNS infiltration by Th17 cells,
90 but not Th1 cells.
91
92 **IL-19 deficiency expands Th17 cell population**
93 To determine whether IL-19 contributes to the expansion of Th17 cells during the
94 induction phase of EAE, we assessed the antigen-specific expansion of Th17 cells *ex vivo*.
95 Splenic $CD4^+$ T cells isolated from WT and IL-$19^{-/-}$ mice at MOG-EAE onset were
96 stimulated with MOG peptide for 3 days *in vitro*. IL-19 deficiency significantly
97 upregulated *Il17a* mRNA and downregulated *Foxp3* mRNA, but it did not affect the level
98 of *Ifng* mRNA (Fig. 3A). Flow cytometric analysis also revealed that IL-19 deficiency
99 expanded the Th17 cell population (Fig. 3B, C). These results indicate that IL-19
100 deficiency mediates expansion of Th17 cells in the peripheral lymphoid tissues.
101
102 **IL-19 deficiency skews cytokine expression profiles toward Th17 cell expansion in**
103 **macrophages**
104 We then examined how IL-19 deficiency expands Th17 cells in the induction phase of
105 EAE. First, we evaluated the mRNA expression level of IL-19 receptor (heterodimer of
106 IL-20Rα and IL-20Rβ subunits) in the splenic immune cells such as macrophage,
107 dendritic cell (DC), and $CD4^+$ helper T cell. We found that both the IL-20Rα and IL-20Rβ
108 subunits were more highly expressed in $CD11b^+$ macrophages and $CD4^+$ helper T cells
109 than in $CD11c^+$ DCs (Fig. S1). These results suggest that IL-19 mainly affects
110 macrophages and $CD4^+$ helper T cells.

111 Next, we assessed whether IL-19 directly differentiates naïve $CD4^+$ T cells into
112 Th17 cells. Naïve T cells were polarized using immobilized CD3 and CD28 antibodies in
113 the presence of IL-6 and transforming growth factor β1 (TGF-β1), with or without IL-19.
114 Quantitative PCR (qPCR) and flow cytometry revealed that IL-19 did not alter the
115 differentiation of naïve T cells into Th17 cells (Fig. S2).

116 Because antigen-presenting cells (APCs) are crucial for differentiation of naïve
117 T cells into effector T cells, we evaluated the expression levels of cytokines required for
118 Th17 cell expansion in splenic macrophages and DCs. Interestingly, IL-$19^{-/-}$ macrophages
119 exhibited a significant increase in mRNA levels of the genes encoding IL-1β, IL-6, TGF-
120 β, IL-12 p40, IL-23 p19, and tumor necrosis factor α (TNF-α), which play pivotal roles
121 in Th17 cell differentiation and expansion (Fig. 4). Although a previous study showed
122 that IL-19 increases IL-10 expression [26], our data showed that IL-19 deficiency did not
123 alter the *Il10* mRNA level in macrophages (Fig. 4). By contrast, IL-$19^{-/-}$ DCs did not
124 exhibit a significant alteration in the expression levels of these cytokines (Fig. S3). These
125 findings indicated that IL-19 deficiency skews the cytokine expression profiles toward
126 Th17 cell differentiation and expansion in macrophages. Conversely, our data suggested
127 that IL-19 suppresses Th17-skewed condition by activating macrophages.

128

129 **IL-19 deficiency promotes MHC class II expression in macrophages**

130 To determine whether IL-19 signaling contributes to antigen presentation by macrophages,
131 we assessed the expression of major histocompatibility complex (MHC) class II (H2-Ab)
132 and co-stimulatory molecules (CD80 and CD86) in splenic $CD11b^+$ macrophages from

133 WT and IL-19$^{-/-}$ EAE mice. (Fig. 5A). IL-19 deficiency significantly enhanced expression
134 of the gene encoding MHC class II, whereas the genes encoding co-stimulatory molecules
135 CD80 and CD86 were not affected (Fig. 5A). Flow cytometric data corroborated the
136 enhanced presentation of MHC class II in IL-19$^{-/-}$ splenic macrophages (Fig. 5B). These
137 observations suggested that IL-19 also suppresses the antigen-presenting activity of
138 macrophages.
139
140 **Treatment with IL-19 abrogates EAE**
141 To determine whether exogenous IL-19 abolishes the effect of IL-19 deficiency in EAE,
142 we treated IL-19$^{-/-}$ EAE mice with recombinant mouse IL-19 protein (20 ng/g of body
143 weight) by intraperitoneal injection every other day, starting on day 2 post-immunization.
144 As shown in Figure 6A, administration of IL-19 to IL-19$^{-/-}$ mice abolished the
145 exacerbation of EAE (Fig. 6A, IL-19$^{-/-}$ + IL-19). We then investigated the therapeutic
146 effect of IL-19 on EAE. When we treated WT EAE mice with recombinant mouse IL-19
147 protein in the same manner, we found that IL-19 treatment almost completely inhibited
148 EAE (Fig. 6B). These results indicated that IL-19 represents a potential target for MS
149 therapy.
150

151 **Discussion**

152 In this study, we demonstrated that endogenous IL-19 negatively regulates development
153 of EAE by inhibiting macrophage activation, and that IL-19 treatment effectively
154 abrogates EAE. As shown in Fig. 1D and 1E, endogenous *Il19* mRNA expression was
155 upregulated at EAE onset and downregulated at EAE peak in the spleen, whereas it was
156 elevated at EAE peak in the CNS. We have previously identified IL-19 as a negative-
157 feedback regulator to limit proinflammatory response of macrophages and microglia in
158 autocrine/paracrine manners[11, 12]. From this point of view, these data imply that
159 endogenous IL-19 increases to suppress inflammation accompanied by
160 macrophage/microglia activation as disease progresses from the periphery to CNS,
161 although it is insufficient to halt EAE progression.

162 Th17 cell infiltration in the CNS is considered critical for the development of
163 EAE[5, 27]. Our data revealed that IL-19 deficiency increased CNS infiltration of Th17 cells,
164 but not Th1 cells (Fig. 2). In addition, IL-19 deficiency enhanced the peripheral expansion
165 of Th17 cells in the induction phase of EAE (Fig. 3). Conversely, our data indicated that
166 IL-19 negatively regulates Th17 cell differentiation and expansion, which is critical for
167 EAE development.

168 However, IL-19 did not directly affect differentiation of Th17 cells from naïve T
169 cells (Fig. S2). APCs such as macrophages, microglia, and DC are also essential for
170 effector T cell differentiation and expansion in EAE[28]. Specifically, IL-1β, IL-6, IL-23,
171 TGF-β1, and TNF-α released by APCs play pivotal roles in Th17 cell differentiation and
172 expansion[5, 29, 30, 31 32, 33]. In this study, we revealed that IL-19 deficiency significantly

173 upregulated these Th17 cell differentiation–associated cytokines in macrophages, but not
174 in DCs (Fig. 4 and Fig. S3). These phenomena were correlated with the expression level
175 of IL-19 receptor (IL-20Rα and IL-20Rβ heterodimer), which was highly expressed in
176 macrophages, but not in DCs or helper T cells (Fig. S1). Thus, IL-19 might negatively
177 regulate Th17 cell differentiation and expansion through inhibition of cytokine release
178 from macrophages.

179 Moreover, activated APCs highly express MHC class II and co-stimulatory
180 molecules (CD80 and CD86) on the cell surface and acquire the ability to prime T cells[34].
181 As shown in Fig. 5, IL-19 deficiency further enhanced MHC class II expression in
182 macrophages. Taken together, our findings suggest that IL-19 suppresses Th17 cell
183 differentiation and expansion by suppressing cytokine production and antigen
184 presentation in macrophages. Interestingly, a previous study reported that IL-17A induces
185 IL-19 production[19]. Thus, IL-19 also might serve as a negative regulator of further Th17
186 cell polarization.

187 In addition, activated macrophages and microglia directly contribute to
188 neuroinflammation-induced demyelination by releasing proinflammatory cytokines such
189 as IL-1β, IL-6, and TNF-α[28, 35, 36, 37]. In our study, IL-19 deficiency increased the levels
190 of these proinflammatory cytokines in macrophages and exacerbated EAE until the late
191 phase of the disease. We previously revealed that IL-19 secreted from activated
192 macrophages and microglia suppressed their proinflammatory responses in an
193 autocrine/paracrine manner[11, 12]. Therefore, IL-19 might suppress development of EAE
194 by dual inhibition of both autoreactive Th17 cell expansion and macrophage/microglia-

195 mediated CNS neuroinflammation.

196 Although the IL-19 signaling pathway has not been fully elucidated, IL-19
197 mediates its downstream signaling at least by STAT3 activation[8, 9, 12, 38]. However, it
198 remains controversial whether STAT3 activation is beneficial or harmful with regard to
199 autoimmune-mediated neuroinflammation. Previous studies also reported that STAT3
200 activation in myeloid cells (including macrophages and microglia) exacerbates EAE[39, 40].
201 By contrast, STAT3 ablation worsens neuroinflammation in mice with spinal cord injury[41],
202 and STAT3 activation alleviates cuprizone-induced CNS demyelination[42]. This
203 discordancy may depend on spatiotemporally specific activation of STAT3[43]. Indeed, in
204 contrast to macrophages, IL-19 deficiency did not affect activation of DCs. Recent
205 clinical trials of JAK/STAT inhibitors for autoimmune diseases have revealed a
206 complicated signal network of cytokine/STAT axes in multiple cell types[44]. Further
207 studies are needed to elucidate the precise IL-19 signaling pathway in each cell type.

208 In summary, we revealed that IL-19 deficiency exacerbated EAE by upregulating
209 Th17 cell differentiation–associated cytokines and enhancing antigen presentation in
210 macrophages, followed by Th17 cell expansion and infiltration in the CNS. We also
211 demonstrated that IL-19 administration potently prevented development of EAE.
212 Therefore, enhancement of IL-19 signaling represents a promising therapeutic strategy
213 against MS and other Th17-mediated autoimmune diseases.

214

215 **Material and Methods**

216

217 **Reagents**

218 MOG peptide 35–55 ($MOG_{35–55}$; MEVGWYRSPFSRVVHLYRNGK) was synthesized
219 and purified by Operon Biotechnologies (Tokyo, Japan). Incomplete Freund's adjuvant
220 was obtained from Sigma-Aldrich (St. Louis, MO, USA). Heat-killed *Mycobacterium*
221 *tuberculosis* H37Ra was obtained from Difco (Detroit, MI, USA), and pertussis toxin was
222 obtained from List Biological Laboratories (Campbell, CA, USA). Recombinant mouse
223 IL-6, IL-19, and TGF-β1 were obtained from R&D Systems (Minneapolis, MN, USA).

224

225 **Animals**

226 All animal experiments were conducted under protocols approved by the Animal
227 Experiment Committee of Nagoya University (approved numbers: 15017 and 15018) and
228 Yokohama City University (approved number: F-A-19-036). C57BL/6J (B6) mice were
229 purchased from Japan SLC (Hamamatsu, Japan). IL-$19^{-/-}$ mice (B6 background) [11, 12] were
230 obtained from Regeneron Pharmaceuticals (Tarrytown, NY, USA).

231

232 **EAE induction and treatment studies**

233 MOG-EAE was induced as previously described [45, 46, 47]. In brief, 8-week-old female mice
234 were immunized subcutaneously at the base of the tail with 0.2 ml of emulsion containing
235 200 μg $MOG_{35–55}$ in saline, combined with an equal volume of complete Freund's
236 adjuvant containing 300 mg heat-killed *Mycobacterium tuberculosis* H37Ra. The mice

237 were intraperitoneally injected with 200 ng pertussis toxin on days 0 and 2 post-
238 immunization. To investigate the effect of IL-19, EAE mice were treated with mouse
239 recombinant IL-19 protein (20 ng/g of body weight) by intraperitoneal injection every
240 other day starting on day 2 post-immunization, according to a modification of a
241 previously reported method [48, 49]. The mice were assessed daily for clinical signs of EAE,
242 according to the following grading system: 0, normal; 1 - limp tail or mild hind limb
243 weakness; 2 - moderate hind limb weakness or mild ataxia; 3 - moderate to severe hind
244 limb weakness; 4 - severe hind limb weakness, mild forelimb weakness or moderate
245 ataxia; 5 - paraplegia with moderate forelimb weakness; and 6 - paraplegia with severe
246 forelimb weakness, severe ataxia, or moribundity.
247
248 **Isolation of cells from spleen and lumbar spinal cord**
249 Mononuclear cells were collected from the spleen and lumbar spinal cord as described
250 previously [45, 46, 47]. $CD4^+$, $CD11b^+$, and $CD11c^+$ cells were isolated using the MACS
251 system (Miltenyi Biotec, Bergisch Gladbach, Germany). Helper T cell differentiation was
252 induced as described previously [5, 32, 47]. For flow cytometric analysis, cells were stained
253 with PerCP/Cy5.5 or BV421-conjugated anti–mouse CD4 rat monoclonal antibody
254 (RM4-5; BD Biosciences, Franklin Lakes, NJ, USA). The cells were then fixed and
255 permeabilized with Cytofix/Cytoperm reagent (BD Biosciences) and stained with PE-
256 conjugated anti–mouse IFN-γ rat monoclonal antibody (XMG1.2; BD Biosciences) and
257 APC- or PE-conjugated anti–mouse IL-17A rat monoclonal antibody (TC11-18H10; BD
258 Biosciences). The samples were analyzed using a FACS Aria III system (BD Biosciences)

259 and the FlowJo software (FlowJo, Ashland, OR, USA).

260

261 **Histological analysis**

262 Histological analysis was performed as previously described [45]. Mice with peak EAE
263 were anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M PBS.
264 Lumbosacral spinal cords were immediately removed, postfixed in 4% paraformaldehyde,
265 and embedded in paraffin. Eight-micron-thick sections were stained with hematoxylin
266 and eosin. Stained sections were analyzed on a NanoZoomer 2.0-RS slide scanner
267 (Hamamatsu Photonics, Hamamatsu, Japan).

268

269 **RNA extraction and reverse-transcription polymerase chain reactions (RT-PCRs)**

270 We evaluated the expression levels of proinflammatory factors in the lumbar spinal cords
271 and spleens by qPCR as described previously [12, 45]. In brief, lumbar spinal cords and
272 spleens were collected from EAE mice at pre-immunization, EAE onset, and EAE peak
273 (approximately on days 0, 10, 16 post-immunization, respectively). Total RNA was
274 isolated with an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and reverse transcribed
275 with SuperScript III (Life Technologies, Carlsbad, CA, USA). Expression levels of
276 mRNAs were evaluated by qPCR using SYBR Select Master Mix (Applied Biosystems,
277 Foster City, CA, USA) on a Rotor-Gene Q (Qiagen) or LightCycler 96 (Roche). Mouse
278 gene–specific primers were obtained from Life Technologies (Table 1). Gene-expression
279 values were determined using the $\Delta\Delta C_T$ method. Levels of mRNAs of interest were
280 standardized to the geometric mean of the level of hypoxanthine

281 phosphoribosyltransferase 1 (*Hprt1*). Assays were carried out in three independent trials.

282

283 **Statistical analysis**

284 Statistical significance was analyzed using Student's t-test or one-way analysis of
285 variance (ANOVA) followed by post hoc Tukey's test in GraphPad Prism version 8
286 (GraphPad Software, La Jolla, CA, USA).

287

288 **Data availability**

289 The datasets generated and analyzed during the study are available from the
290 corresponding author upon reasonable request.

291

292

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Author contributions

H.H., B.P., A.S., and H.T. designed the research; H.H., B.P., H.K., Y.O., J.S., K.T., Y.A., and H.T. performed the research; H.H., B.P., H.K., F.T., A.S., and H.T. analyzed the data; and H.H., B.P., and H.T. wrote the paper.

Competing interests

The authors declare no competing interests.

483 **Figure Legends**

484

485 **Figure 1. IL-19 deficiency aggravates EAE.**

486 (A) EAE clinical scores for WT (black) and IL-19$^{-/-}$ (red) mice. Data represent means ±
487 SD (n = 10). ***, $p < 0.0001$. (B) Micrographs of hematoxylin/eosin staining of L5
488 lumbar spinal cords at the peak EAE of WT and IL-19$^{-/-}$ mice. The right panels show
489 enlargements of the boxed areas in the left panels. Scale bars: 500 μm (left), 100 μm
490 (right). (C) Flow cytometric analysis of cell infiltration in the CNS at the peak EAE (n =
491 5). Data represent means ± SD. **, $p < 0.01$. (D and E) chronological qPCR data for IL-
492 19 mRNA expression level in the spleen (D) and the lumbar spinal cord (E) of WT EAE
493 mice (n = 5). Pre, pre-immunization; onset, EAE onset; peak, EAE peak. Data represent
494 means ± SD. **, $p < 0.01$.

495

496 **Figure 2. IL-19 deficiency increases CNS infiltration of Th17 cells, but not Th1 cells.**

497 (A) Representative flow-cytometric data for IL-17– and IFN-γ–producing CD4^{+} T cells
498 in the CNS at the peak EAE. (B) Percentage of IL-17–producing CD4^{+} T cells. (C)
499 Percentage of IFN-γ–producing CD4^{+} T cells. Data represent means ± SD (n = 3). *, $p <$
500 0.05.

501

502 **Figure 3. IL-19 deficiency expands the Th17 cell population.**

503 (A) qPCR data for levels of mRNAs encoding IL-17A, IFN-γ, and FoxP3 in splenic CD4^{+}
504 T cells. (B) Representative flow cytometric data for IL-17–producing CD4^{+} T cells in the

505 spleens of EAE mice. (C) Percentage of IL-17–producing CD4+ T cells in the spleens of
506 EAE mice. Data represent means ± SD (n = 3). *, $p < 0.05$.
507
508 **Figure 4. IL-19 deficiency upregulates Th17 cell differentiation–associated cytokines**
509 **in macrophages.**
510 (A) qPCR data for mRNAs encoding IL-1β, IL-6, TGF-β1, IL-12 p40, IL-23 p19, IL-10,
511 and TNF-α in splenic macrophages of EAE mice. Assessments were performed 7 days
512 after immunization. Data represent means ± SD. ***, $p < 0.0001$ (n = 6).
513
514 **Figure 5. IL-19 deficiency enhances antigen-presenting activity in macrophages.**
515 (A) qPCR data for mRNAs encoding MHC class II (H2-Ab), CD80, and CD86 in the
516 splenic macrophages on day 7 after immunization. Data represent means ± SD. *, $p <$
517 0.05 (n = 6). (B) Representative flow cytometric data for MHC class II (H2-Ab)
518 presentation in splenic macrophages.
519
520 **Figure 6. Treatment with recombinant IL-19 alleviates EAE.**
521 (A) EAE clinical score. WT + PBS (black): WT EAE mice treated with PBS; IL-19$^{-/-}$ +
522 PBS (red): IL-19$^{-/-}$ EAE mice treated with PBS; IL-19$^{-/-}$ + IL-19 (blue): IL-19$^{-/-}$ EAE mice
523 treated with IL-19. (B) EAE clinical score. WT + PBS (black), WT EAE mice treated
524 with PBS; WT + IL-19 (blue), WT EAE mice treated with IL-19. Data represent means ±
525 SD. *, $p < 0.05$ (n = 5).
526

527 **Table 1. Primers for qPCR**

Gene	Primer sequence
mouse *Il19* sense	CAACCTGCTGACATTCTACAGAG
mouse *Il19* antisense	CCTGACATCGCTCCAGAGATTT
mouse *Il17a* sense	TCATCTGTGTCTCTGATGCTGTTG
mouse *Il17a* antisense	TCGCTGCCTTCACTGT
mouse *Ifng* sense	TGGCATAGATGTGGAAGAAAAGAG
mouse *Ifng* antisense	TGCAGGATTTTCATGTCACCAT
mouse *Foxp3* sense	TTCATGCATCAGCTCTCCAC
mouse *Foxp3* antisense	CTGGACACCCATTCCAGACT
mouse *Il1b* sense	GAAATGCCACCTTTTGACAGTG
mouse *Il1b* antisense	TGGATGCTCTCATCAGGACAG
mouse *Il6* sense	TCTATACCACTTCACAAGTCGGA
mouse *Il6* antisense	GAATTGCCATTGCACAACTCTTT
mouse *Tgfb1* sense	CGAAGCGGACTACTATGCTAAAGA
mouse *Tgfb1* antisense	GTTTTCTCATAGATGGCGTTGTTG
mouse *Il10* sense	GAGAAGCATGGCCCAGAAATC
mouse *Il10* antisense	CGCATCCTGAGGGTCTTCA
mouse *Il12p40* sense	GGTGCAAAGAAACATGGACTTG
mouse *Il12p40* antisense	CACATGTCACTGCCCGAGAGT
mouse *Il23p19* sense	GCACCAGCGGGACATATGA
mouse *Il23p19* antisense	CCTTGTGGGTCACAACCATCT
mouse *Tnfa* sense	GACCCTCACACTCAGATCATCTTCT
mouse *Tnfa* antisense	CCACTTGGTGGTTTGCTACGA
mouse *Il20ra* sense	GGAAACTCAAGTCAGCCCAC
mouse *Il20ra* antisense	AGATGGACTTCTCGCCAGTT
mouse *Il20rb* sense	CCGAAATGCAACTGTCCTCAC
mouse *Il20rb* antisense	AATAACCAGATGCAGCCCATGT
mouse *Rorgt* sense	GCGACTGGAGGACCTTCTAC
mouse *Rorgt* antisense	TCCCACATTGACTTCCTCTG
mouse *H2ab1* sense	AGACGCCGAGTACTGGAACAGCCAGC
mouse *H2ab1* sense	CAGAGTGTTGTGGTGGTTGAGGGCCTC
mouse *Cd80* sense	CATCAAAGCTGACTTCTCTACCC
mouse *Cd80* antisense	GGGTTTTTCCCAGGTGAAGT
mouse *Cd86* sense	TCAGTGATCGCCAACTTCAG
mouse *Cd86* sense	GAAACTCTTGAGTGAAATTGAGAGG
mouse *Hprt1* sense	CAGTCAACGGGGACATAAA
mouse *Hprt1* antisense	GGGGCTGTACTGCTTAACCAG

528

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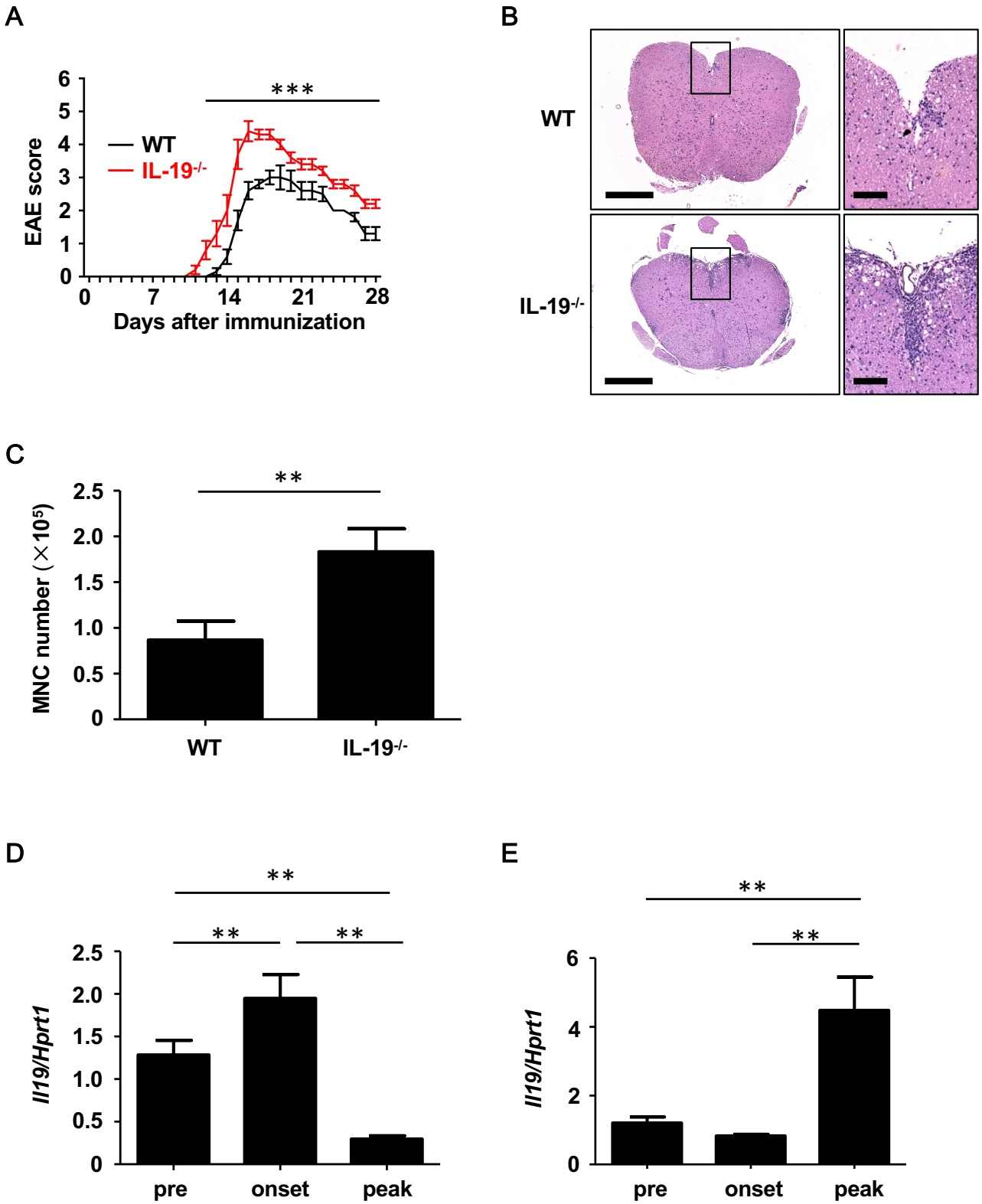
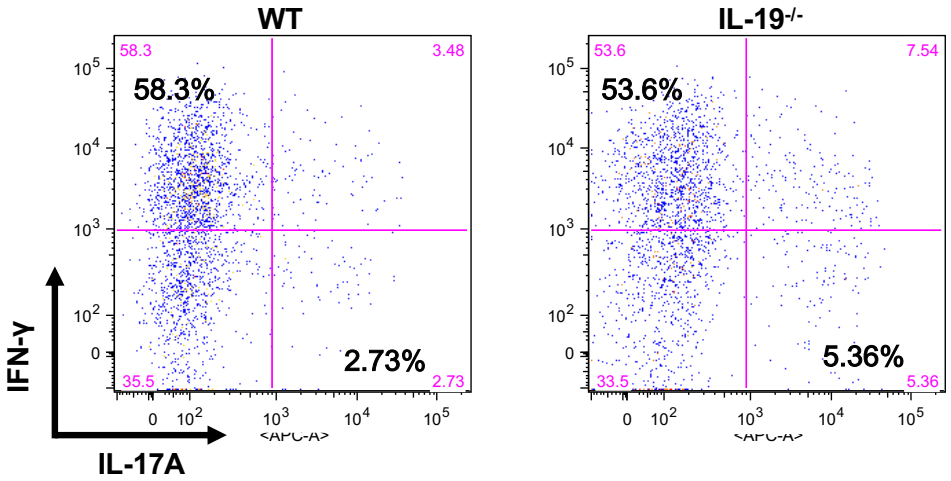


Horiuchi et al, Figure. 1

A



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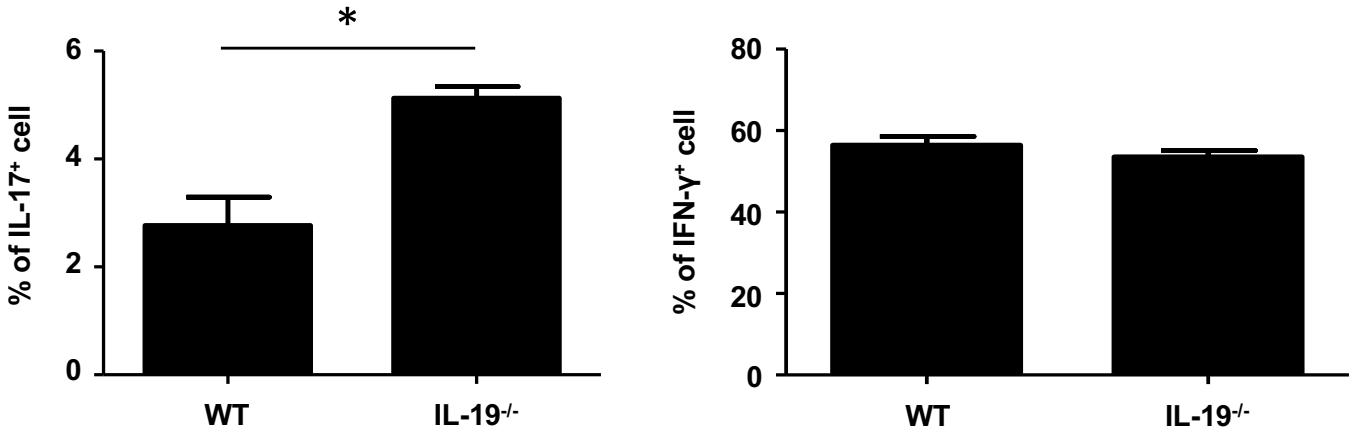

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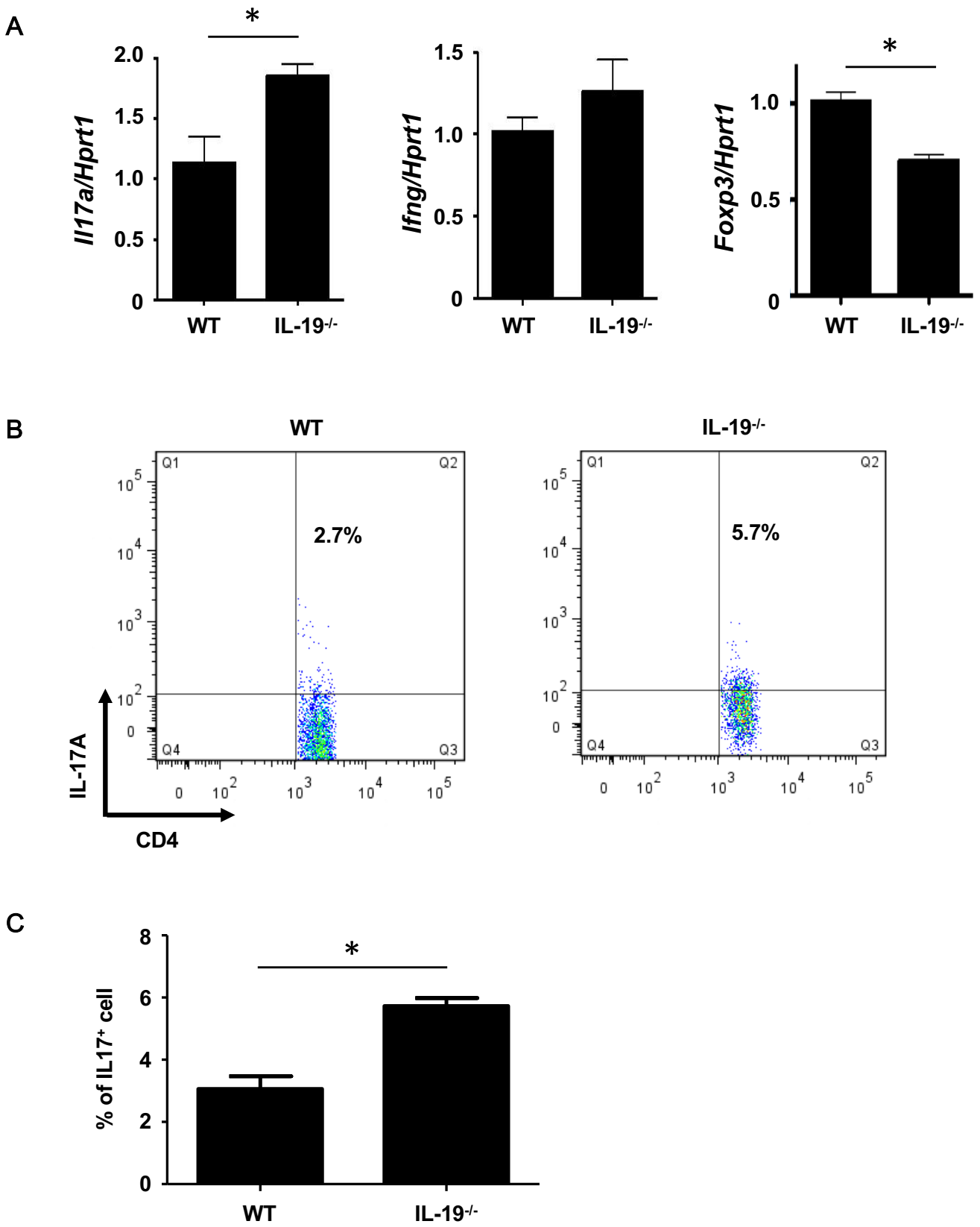

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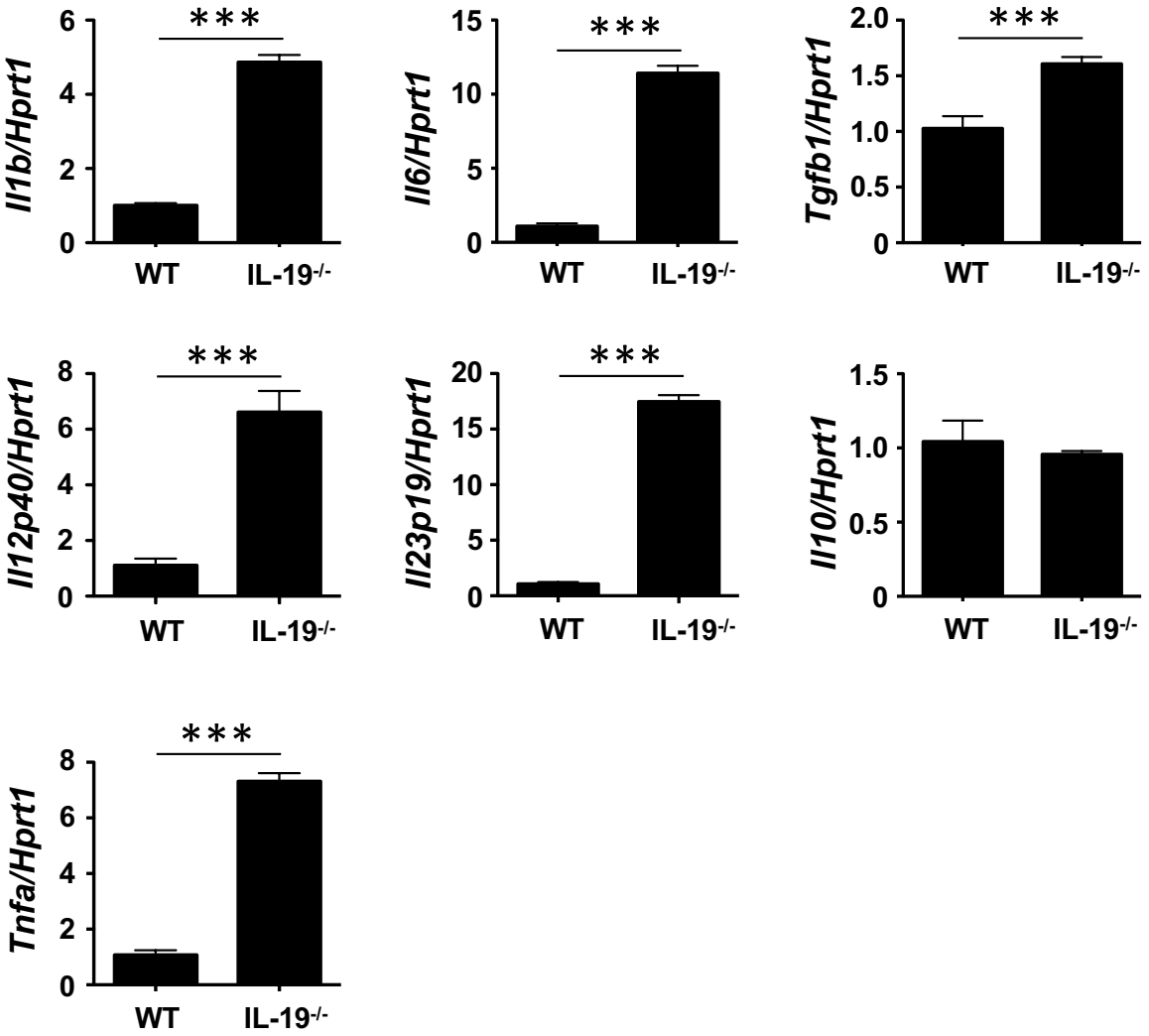

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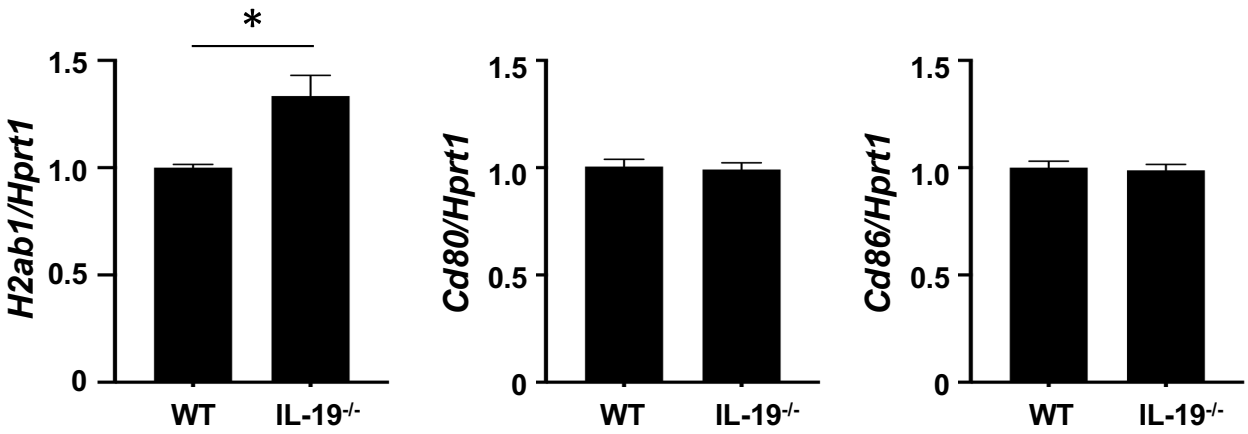

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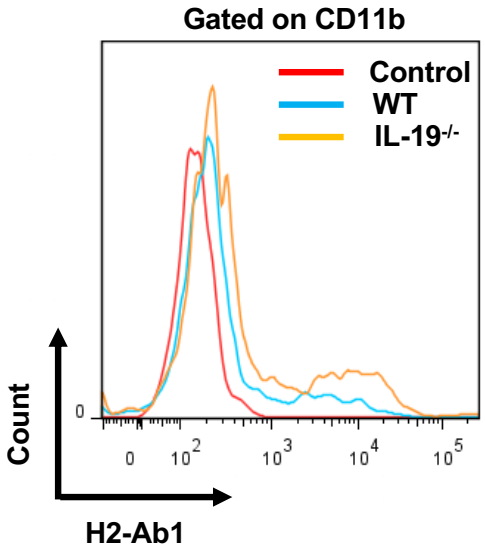

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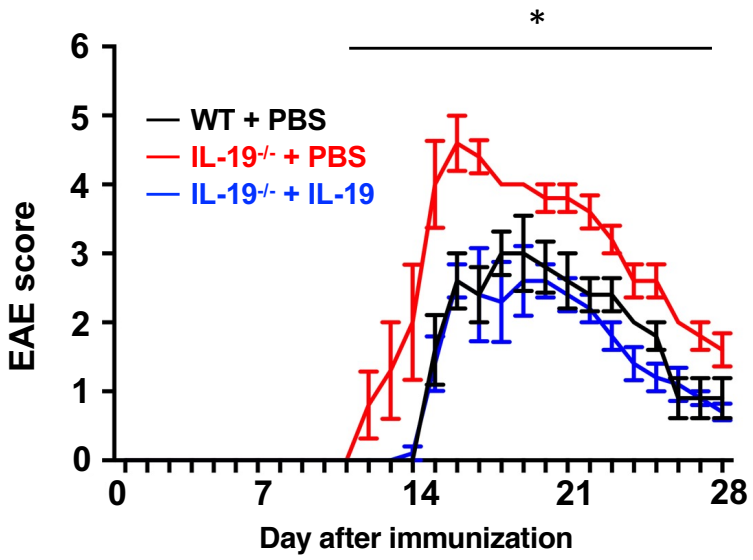

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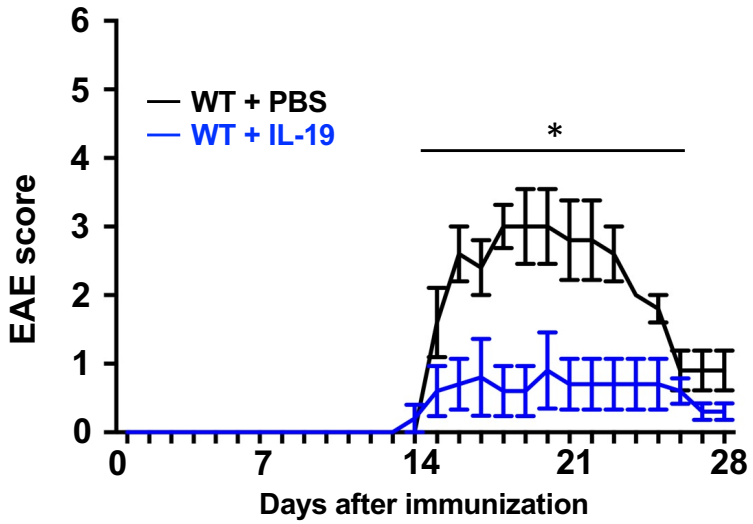

Horiuchi et al, Figure. 6