PNAS
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Supplementary Information for

Biochemically-altered myelin triggers autoimmune demyelination

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This PDF file includes:

Figs. S1 to S5

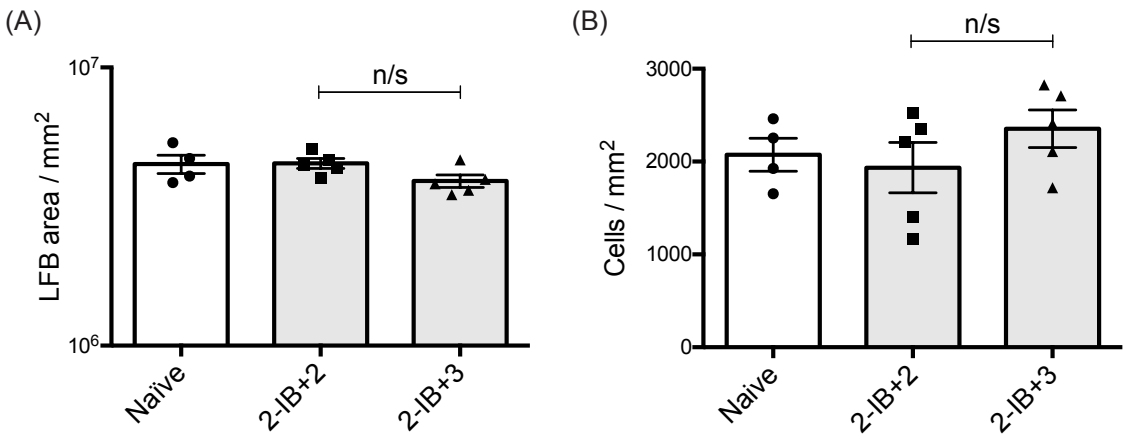

Fig. S1. Two-weeks of CPZ treatment on its own (i.e. no IB) did not result in myelin loss, even when recovery was extended by an additional week. (A) Myelin area per mm^2 was calculated in the splenium of the corpus callosum in tissue sections stained with LFB. Compared to naive controls (white bar) or animals treated with 2-weeks CPZ followed by 2 weeks on normal chow (2-IB+2, middle grey bar), myelin was not significantly altered after a third week of recovery from CPZ (2-IB+3, right grey bar). (B) Likewise, cell counts per mm^2, used as an approximation of inflammation, did not differ between control groups. We therefore concluded that CAE resulted only from a specific and sequential combination of 2-weeks CPZ then IB, rather than delayed-onset effects of CPZ alone. n = 4/5 per condition. n/s = not significant.

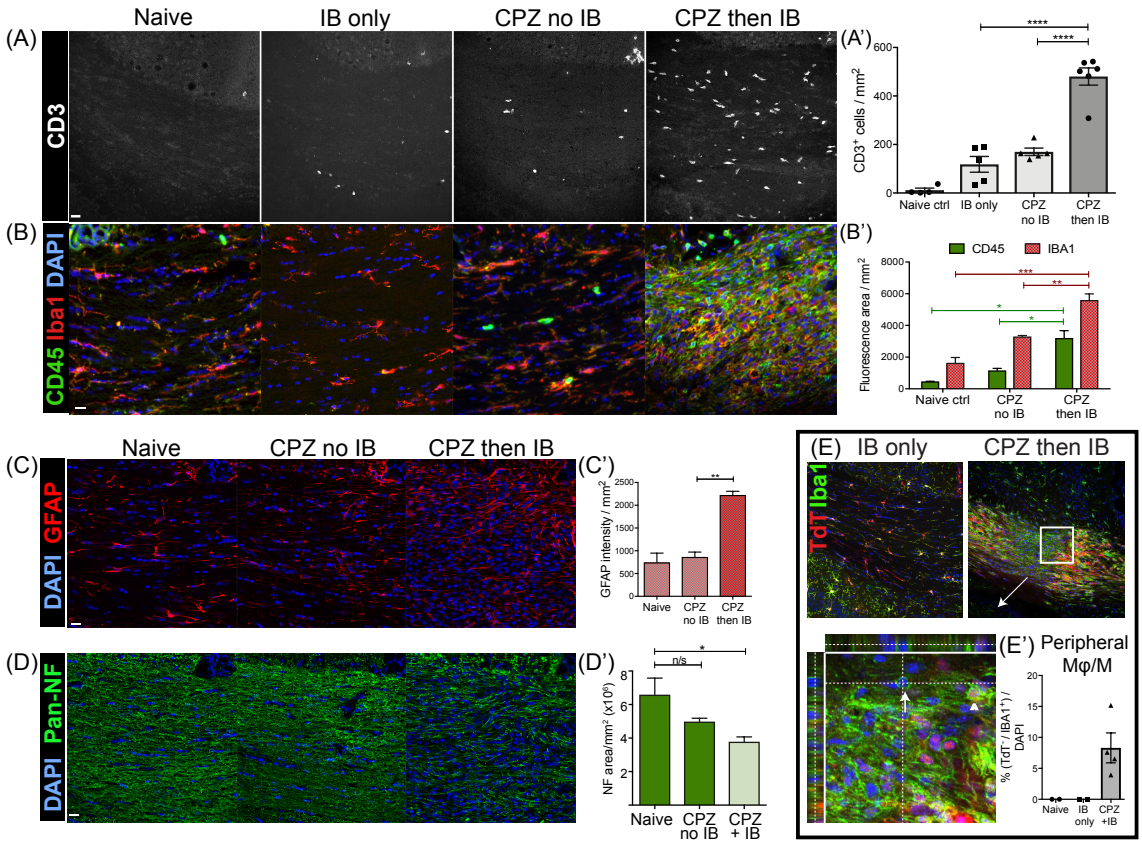

Fig. S2. CAE lesions comprised cells of both innate and adaptive immunity. (A) In contrast to a low density of $CD3^+$ T cells in control tissue sections, the combination of CPZ then IB resulted in significant T-cell infiltration. (A') Quantification of CD3 immunofluorescence. (B) $CD45^+$ leukocytes, minimal in controls, were also strongly increased in CAE lesions. An increased density of $Iba1^+$ microglia/macrophages was associated with activated, amoeboid cell morphologies. (B') Quantification of CD45/Iba1 immunofluorescence. (C) Increased GFAP in CAE lesions indicated astrogliosis, an effect quantified in (C'). (D) Inflammatory demyelination resulted in axonal dropout, as indicated by pan-neurofilament (NF) immunostaining. (D') Quantification of pan-NF immunofluorescence. (E) $CX3CR1^{CreER}$ x Ai9 ('Ai9') transgenic lines were used to quantify peripherally-derived, infiltrating macrophages/monocytes (Mφ/M) in CAE lesions. An inducible TdTomato fluorescent signal persisting only in self-renewing microglia and border-associated macrophages compared to high-turnover, signal-depleted monocytes allowed discrimination of peripheral versus central macrophages/monocytes in Ai9 mice. Compared to IB-only Ai9 controls, sections from the combination CPZ then IB contained peripheral macrophages/ monocytes (TdT^- $Iba1^+$; arrow), albeit in fewer numbers compared to resident cells (TdT^+ $Iba1^+$; arrowhead). Graphs represent the mean of 6 images from 3 sections in 4-6 separate animals. All scale bars = 10 μm. Error bars represent SEM. Significance was determined by one-way ANOVA. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; n/s., not significant.

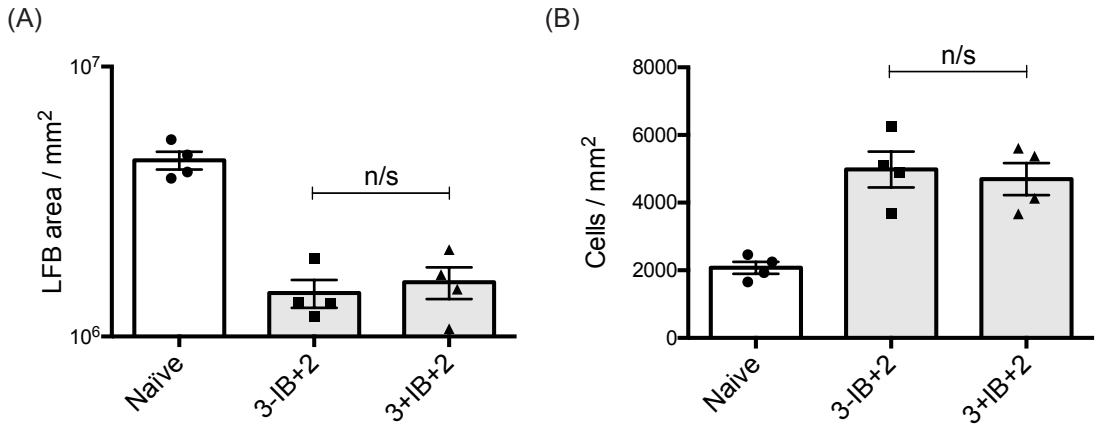

Fig. S3. IB had no discernible pathological effect after 3-weeks of cuprizone induction. (A) The amount of myelin area per mm^2 (light grey bars) and (B) cell counts per mm^2, used as a surrogate readout of inflammation, were unchanged by the addition of IB after a 3-week CPZ induction. We therefore concluded that a 3-week CPZ induction was insufficient to elicit peripheral inflammation.

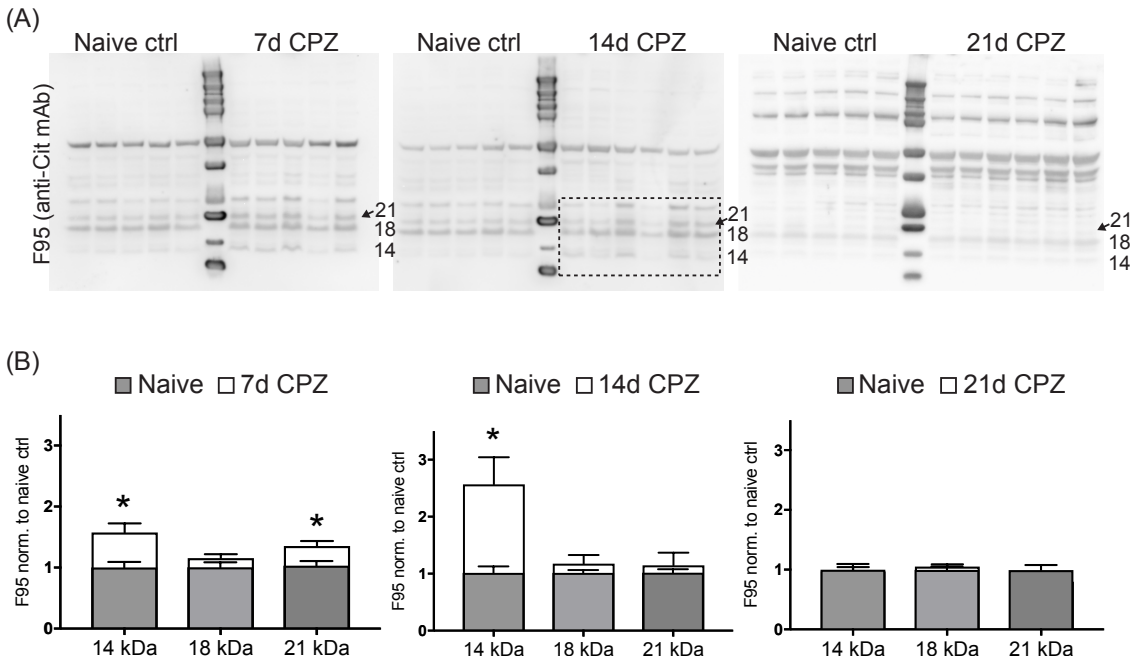

Fig. S4. White matter citrullination measured during the CPZ induction phase peaked after 2-weeks of CPZ feeding and returned to baseline after 3-weeks. Citrullination levels during the CPZ induction phase therefore mirrored their respective capacities to trigger inflammatory demyelination. (A) White matter homogenates were harvested from animals at various CPZ induction times and probed with mAb F95 by western blot. Citrullination, particularly of MBP isoforms, increased as a function of CPZ treatment during the induction phase. (B) Quantification of western blot data. Each lane is a different mouse, n = 5 per condition. Significance determined by one-way ANOVA, * P< 0.05; ** P < 0.01.

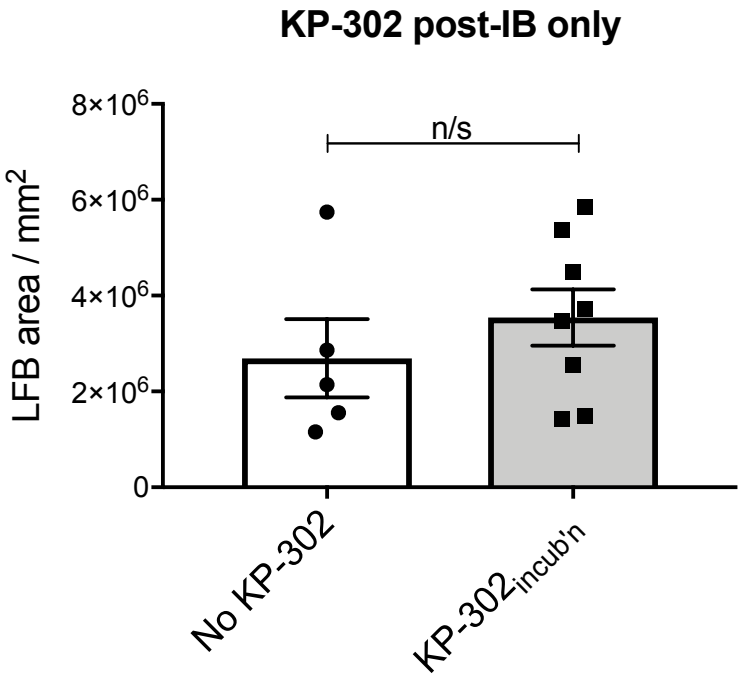

Fig. S5. KP-302 administered only during the post-IB incubation phase did not prevent inflammatory demyelination. The protection afforded by KP-302 was therefore not anti-inflammatory in nature but rather an effect exerted during the myelin-modifying CPZ induction phase. Area of LFB was measured in cryosections of the medial corpus callosum using ImageTrak analysis, as described in Figure 4. Each dot represents an average of 3 tissue sections from an individual mouse. n = 5 no KP-302; n = 8 KP-302-incubation-only. Significance determined by unpaired t-test.