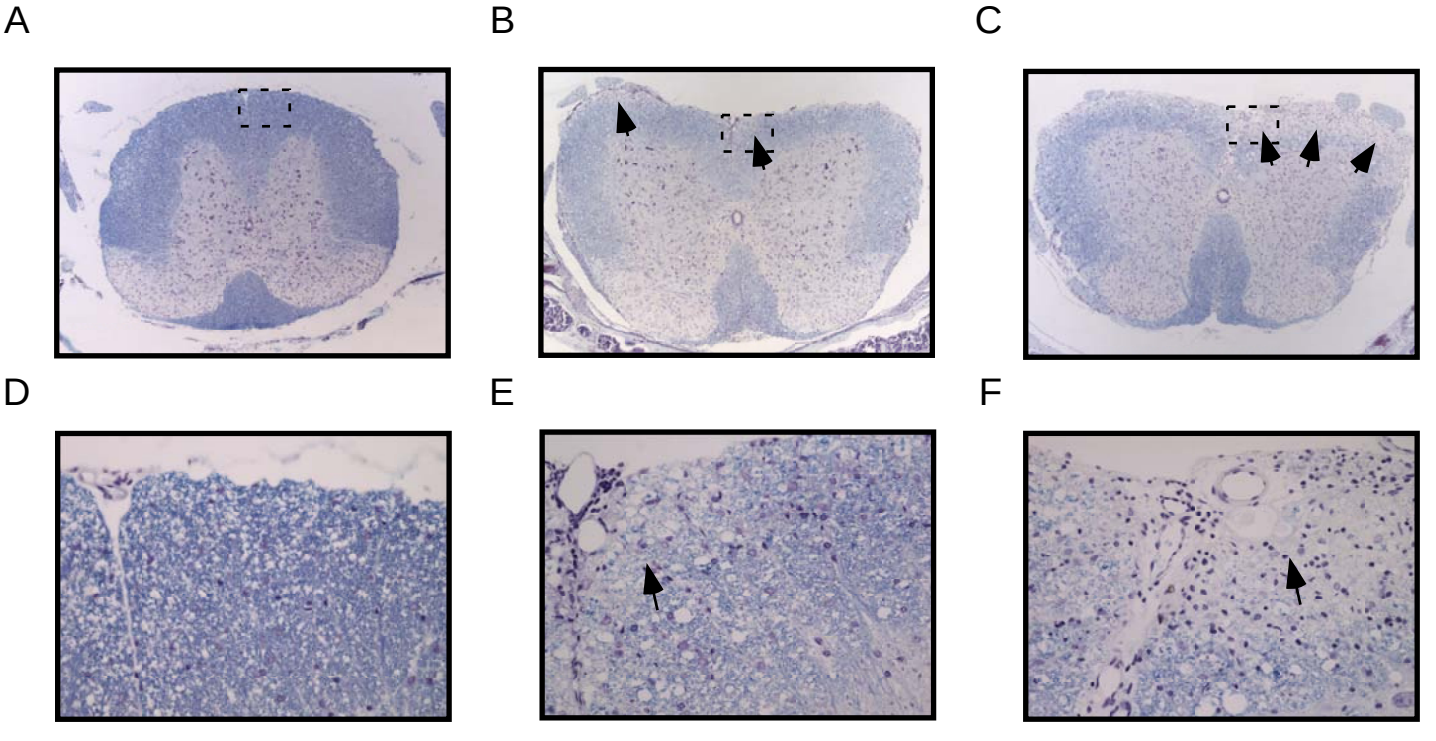
Chora et. al. Supplementary Figure 1



Chora et. al. Supplemental Figure 2

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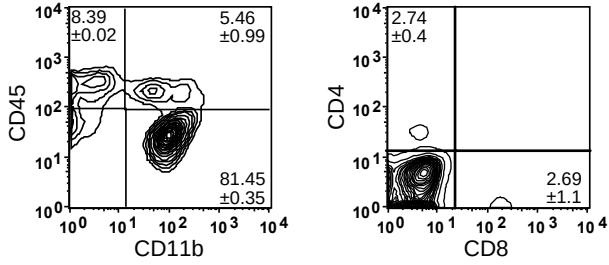

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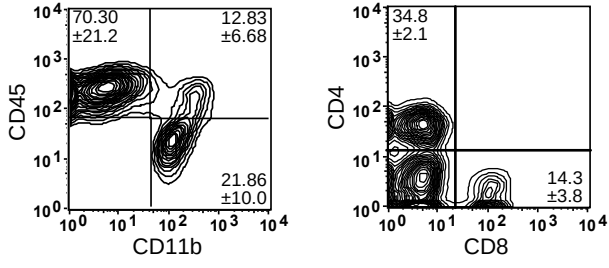
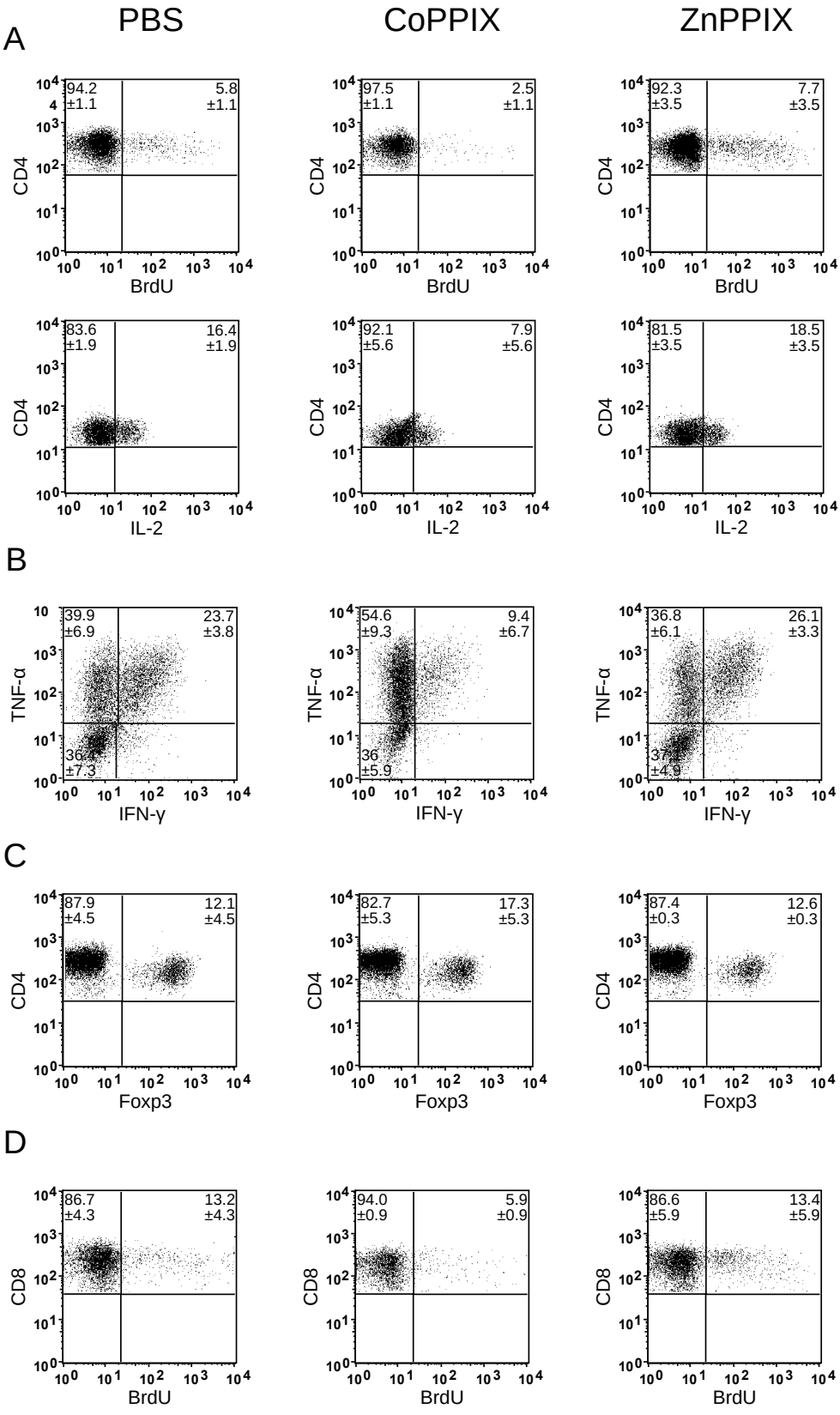
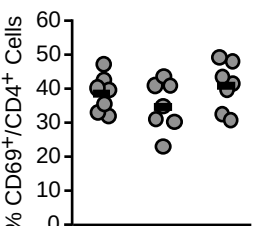
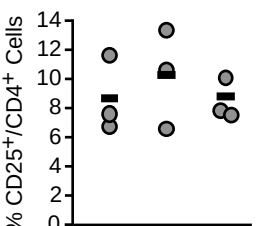


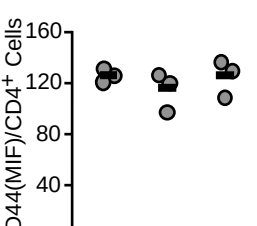
Chora et. al. Supplemental Figure 3

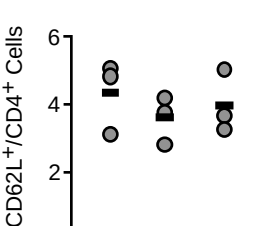


Chora et. al. Supplemental Figure 4

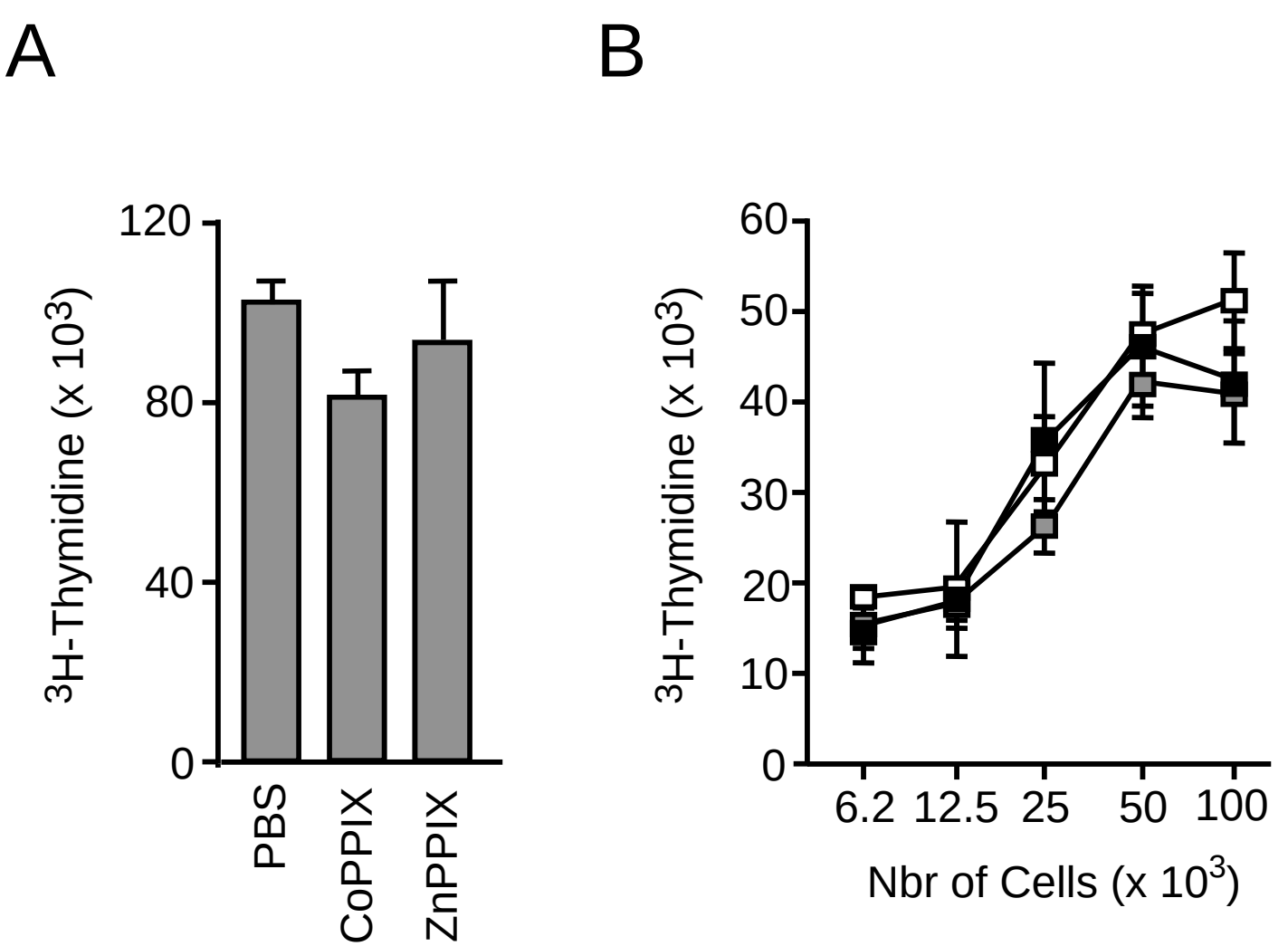






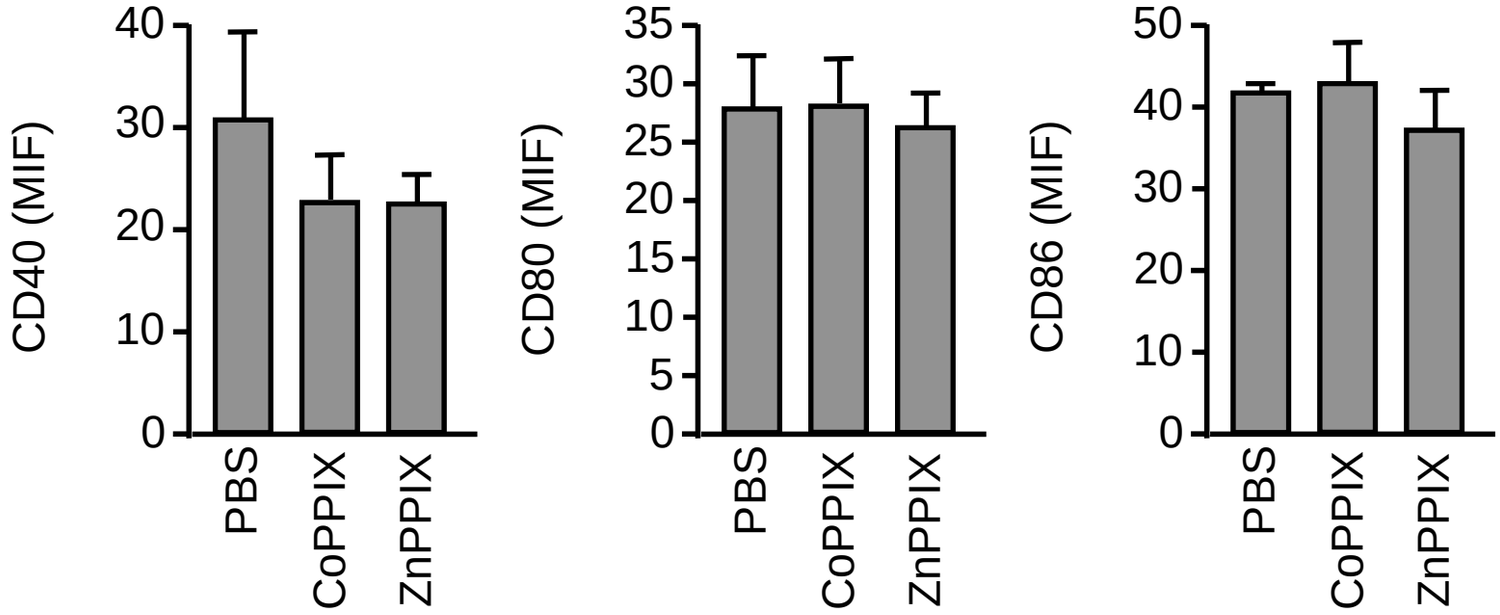


Chora et. al. Supplemental Figure 5



Chora et. al. Supplemental Figure 6

A



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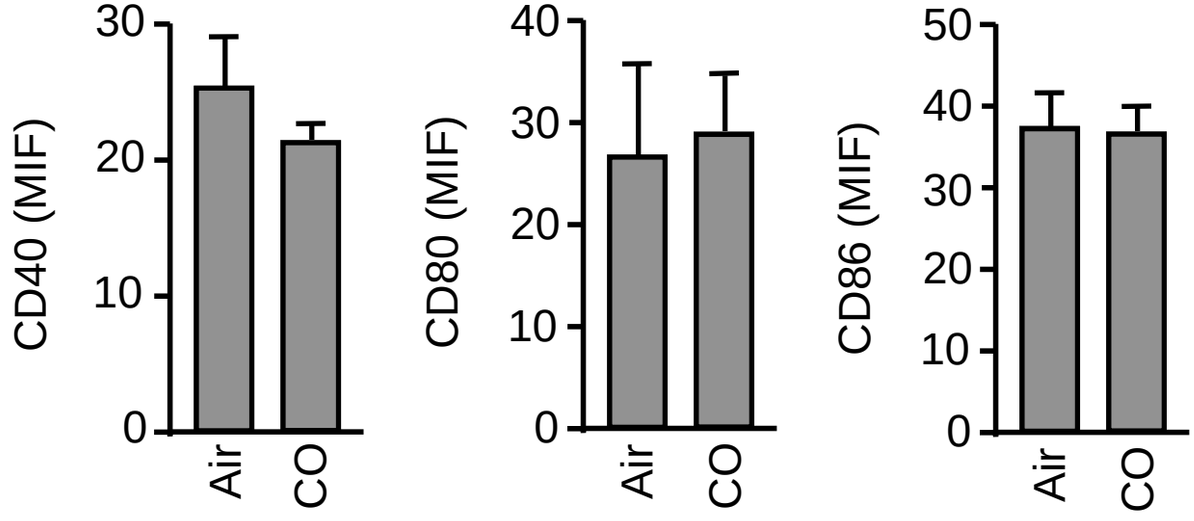
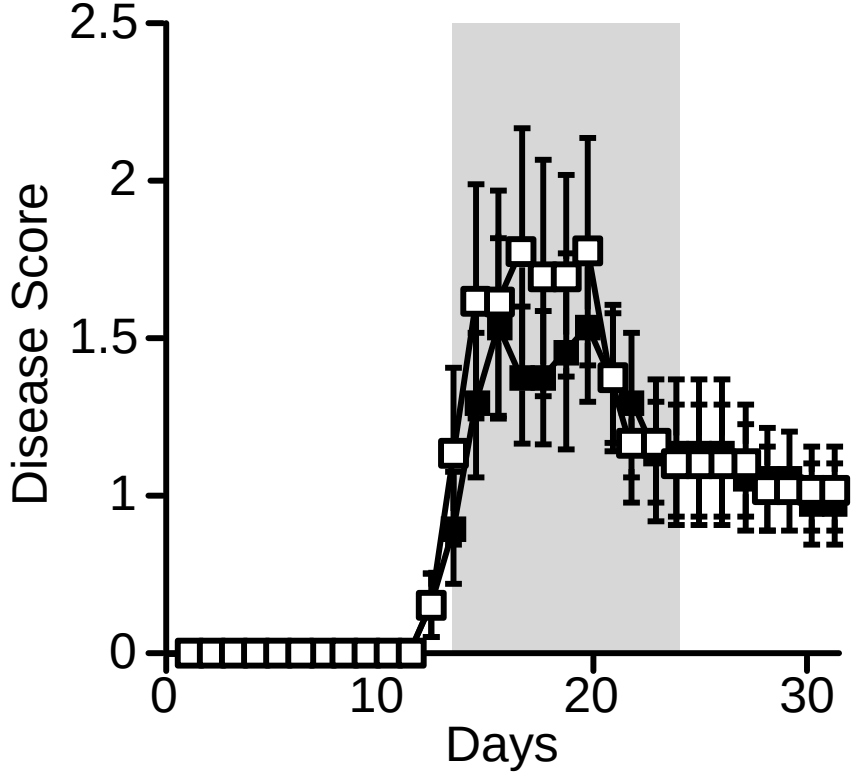


Chora et al. supplemental Figure 7

A



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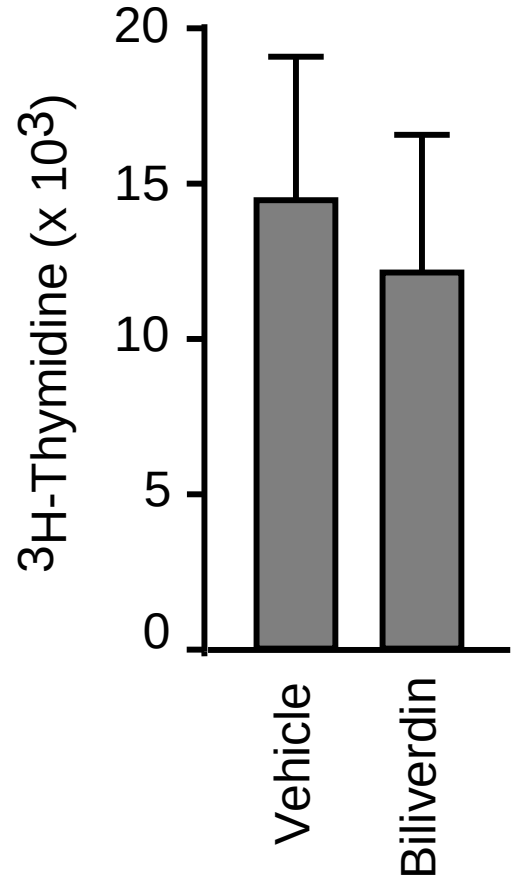

Supplemental Figure 1 - HO-1 prevents CNS demyelination. Representative Luxol fast blue staining of spinal cord sections are shown for naïve C57BL/6 mice (**A**) versus C57BL/6 *hmox-1*$^{+/+}$ (**B**) and *hmox-1*$^{-/-}$ (**C**) mice, 60 days after EAE induction. Magnifications in A-C are 40x. Dashed rectangles in (A-C) are magnified (400x) in (D-F), respectively. Arrows indicate demyelination.

Supplemental Figure 2 – CNS leukocyte infiltrates during EAE. Leukocyte infiltrates were analyzed by flow cytometry in (**A**) naïve C57BL/6 mice (n=2) or (**B**) 20 days after immunization. Representative plots are shown with relative percentages of $CD45^+$, $CD11b^+$, $CD4^+$ and $CD8^+$ cells ± standard deviation.

Supplemental Figure 3 – HO-1 modulates T_H cell effector function within the CNS. EAE was induced in C57BL/6 mice, randomized two days after disease onset and treated daily with PBS, CoPPIX or ZnPPIX,. CNS leukocyte infiltrates were analyzed by flow cytometry, 20 days post-immunization. When indicated, mice received BrdU. Representative plots are shown with mean percentages ± standard deviation (n=3-10 animals per staining). (**A**) Staining for intracellular BrdU versus surface CD4 and intracellular IL-2 versus surface CD4. (**B**) Staining for intracellular TNF-α versus intracellular IFN-γ is shown in $CD4^+$ T_H cells. (**C**) Staining for intracellular FoxP3 versus surface CD4. (**D**) Staining for intracellular BrdU versus surface CD8.

Supplemental Figure 4 – Induction of HO-1 does not modulate the expression of activation markers in T_H cells within the CNS. EAE was induced in C57BL/6 mice,

randomized two days after disease onset and treated daily with PBS, CoPPIX or ZnPPIX. Leukocyte infiltrates in the CNS were analyzed by flow cytometry, 20 days post-immunization. Each value represents an individual animal. Relative percentage of $CD69^+/CD4^+$ T_H cells, $CD25^+/CD4^+$ T_H cells and $CD62L^+/CD4^+$ T_H cells are shown. For CD44 the mean florescence intensity of CD44 is shown in $CD4^+$ T_H cells. Bars indicate mean value of all mice analyzed under each treatment.

Supplemental Figure 5 – Induction of HO-1 does not suppress naïve myelin-reactive T_H cell priming. C57BL/6 mice were immunized in the footpad with MOG_{35-55} plus CFA and treated daily with PBS (n=3), CoPPIX (n=3) or ZnPPIX (n=3). Lymph node cells were isolated 8 days post-immunization. (**A**) T_H cell proliferation was measured in vitro by ^{3}H-Thymidine incorporation, 72 hours after addition of concanavalin A (2 μg/ml). Results shown are the mean ± standard deviation of one representative assay out of five. (**B**) Increasing numbers of T_H cells (>98% $CD4^+$ T cells) from PBS- (□) (n=3), CoPPIX- (■) (n=3) or ZnPPIX- (■) (n=3) treated mice were co-cultured with T_H cell-depleted lymph node cells (<98% CD4+ T cells) from immunized but otherwise untreated mice. MOG_{35-55}-specific T_H cell proliferation was measured as in (A). Results shown are the mean ± standard deviation.

Supplemental Figure 6 – Induction of HO-1 does not modulate the expression of co-stimulatory molecules in APC. C57BL/6 mice were immunized in the footpad with MOG_{35-55} plus CFA and (**A**) treated daily with PBS (n=4), CoPPIX (n=6), ZnPPIX (n=6), (**B**) exposed to air (n=6) or CO (n=7). Draining lymph node cells were isolated 8 days

after immunization and CD40, CD80 or CD86 surface expression in DC ($CD11c^+$) was analyzed by flow cytometry. Quantifications (mean intensity of fluorescence; MIF) are shown as mean ± standard deviation.

Supplemental Figure 7 – Biliverdin does not suppress EAE progression nor does it suppress the proliferation of myelin-reactive T_H cells. (**A**) EAE was induced, mice were randomized two days after disease onset and treated daily with biliverdin (■)(n=15) or vehicle (□)(n=15) for the period indicated by the shaded area. Daily clinical scores are shown as mean ± standard error of mean. (**B**) C57BL/6 mice were immunized in the footpad with MOG_{35-55} plus CFA and treated daily with biliverdin (n=3) or vehicle (n=3), starting two days prior to immunization. Draining lymph node cells were isolated 8 days post-immunization and myelin-reactive T_H cell proliferation was assessed in vitro by ^3H-thymidine incorporation, 72 hours after addition of MOG_{35-55} (10 μg/ml). Results shown are the mean ± standard deviation from one assay with five independently treated animals per group.