

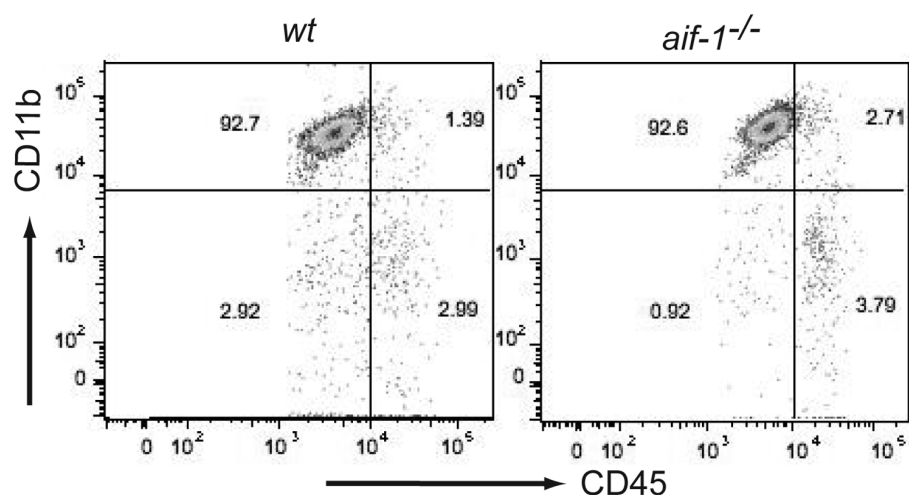
Supplemental Data

Loss of Allograft Inflammatory Factor-1 Ameliorates Experimental Autoimmune Encephalomyelitis by Limiting Encephalitogenic CD4 T-Cell Expansion

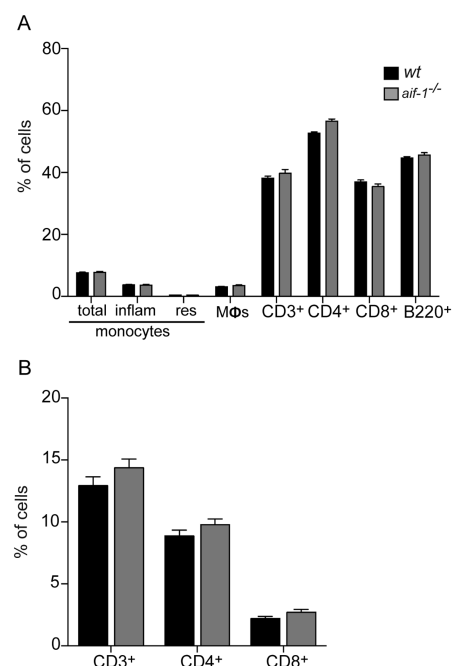
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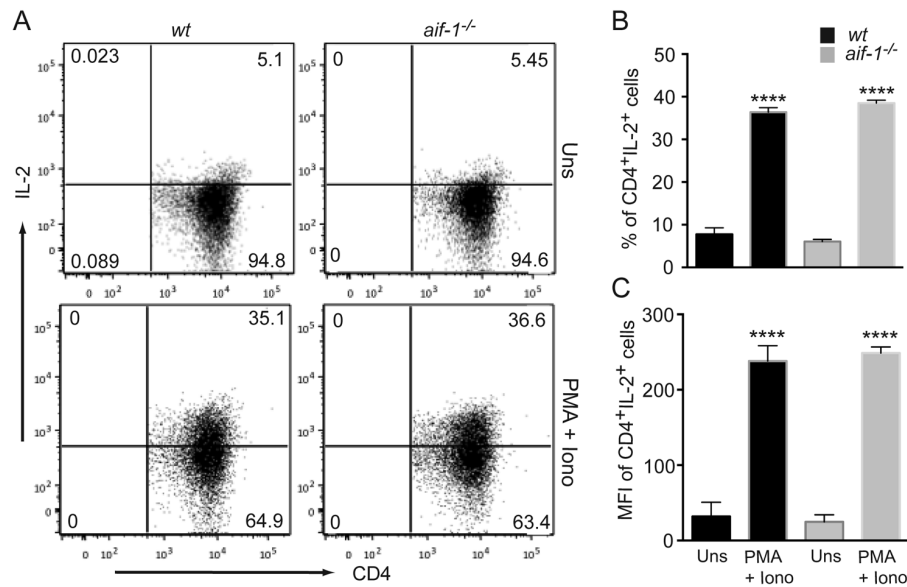
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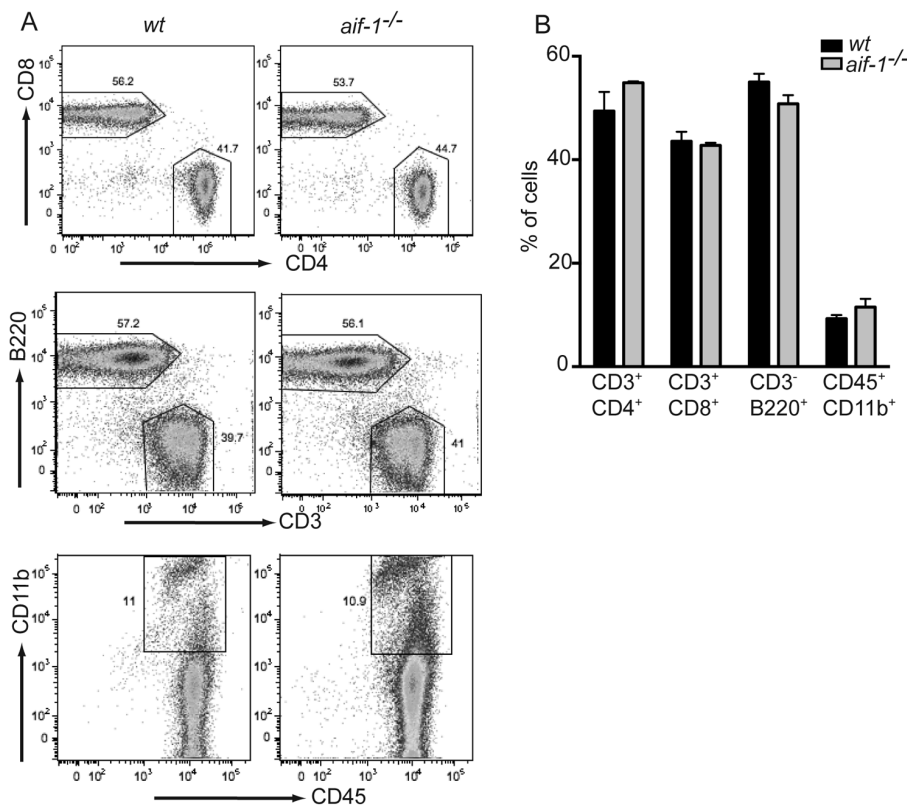
Supplementary Figure S1. Loss of Aif-1 does not affect the resident microglial population at baseline. Representative FACS data of the resident microglial population (CD45^{low}CD11b⁺) from spinal cords of *wt* and *aif-1*^{-/-} naïve mice.



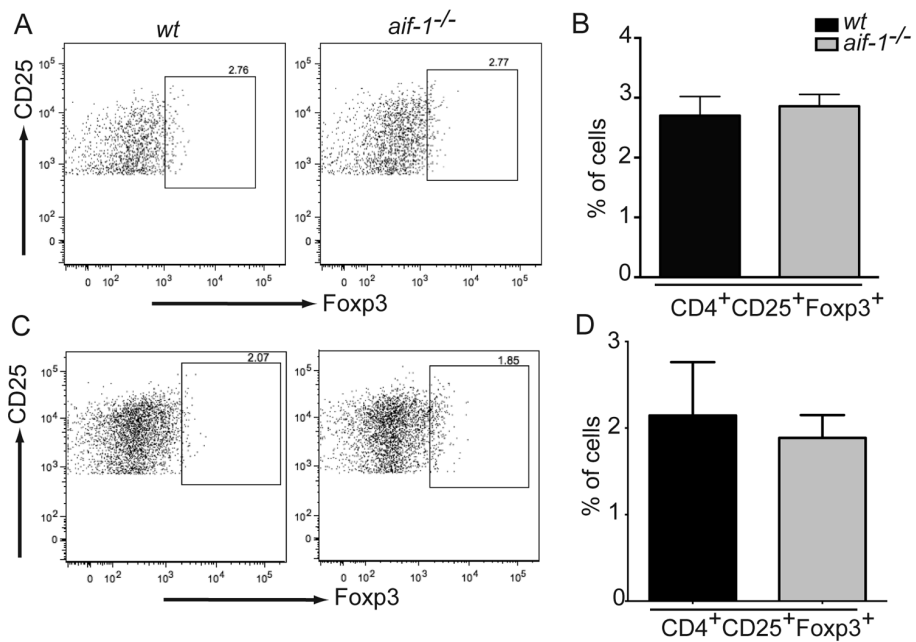
Supplementary Figure S2. *Aif-1*^{-/-} mice display normal immune cell subset populations at baseline. (A) Monocyte lineage (total (CD11b⁺); inflammatory (CD11b⁺Gr1⁺); resident (CD11b⁺Gr1⁻); and macrophage (MΦs, CD11b⁺F4/80⁺)), T cell lineage (CD3⁺; CD3⁺CD4⁺; CD3⁺CD8⁺) and B cell lineage (CD3⁻B220⁺) cells from *wt* and *aif-1*^{-/-} naïve mouse spleens. (B) T cell subsets (CD3⁺CD4⁺; CD3⁺CD8⁺) from *wt* and *aif-1*^{-/-} naïve mouse thymi (n = 4). Data are represented as mean ± SEM.



Supplementary Figure S3. *Aif-1*-deficient CD4 T cells show similar activation by Phorbol 12-myristate 13-acetate (PMA) and ionomycin (Iono). (A) Representative FACS data, (B) quantification of the percentage of CD4 T cells expressing IL-2 (CD4⁺IL-2⁺), and (C) mean fluorescent intensity (MFI) of CD4 T cells expressing IL-2 (CD4⁺IL-2⁺) from spleens of *wt* (*n* = 3) and *aif-1*^{-/-} (*n* = 4) mice. Uns, unstimulated. Data are represented as mean ± SEM. ****, *P* < 0.0001 vs. unstimulated.



Supplementary Figure S4. *Aif-1* deficiency does not affect lymph node immune cell subsets after MOG₃₅₋₅₅ immunization. (A) Representative FACS data of effector T cells (CD3⁺CD4⁺; CD3⁺CD8⁺), B cells (CD3⁺ B220⁺), and monocytes (CD45⁺CD11b⁺) from lymph nodes of d-16 EAE *wt* and *aif-1*^{-/-} mice. (B) Quantification of T cell subsets, B cells, and monocytes (*n* = 6). Data are represented as mean ± SEM.



Supplementary Figure S5. Loss of Aif-1 does not affect iTreg subsets after MOG₃₅₋₅₅ immunization. Representative FACS data for iTreg cells (CD4⁺ CD25⁺ Foxp3⁺) from spleen (A, quantified in B) and lymph nodes (C, quantified in D) (n = 6).