François-Xavier Hubert,[1,2] Sarah A. Kinkel,[3,6] (ois-XaD12 (foK5079 670.7386 Tm Gayle.)-2493 (MA.)-249.4 Davey)6739 (l,) TJ6 0 0 63054.878 673.4887 Tm,2

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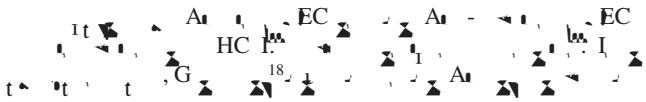
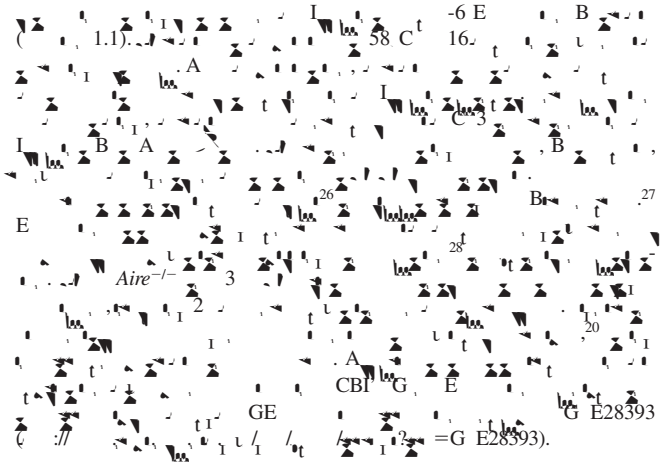
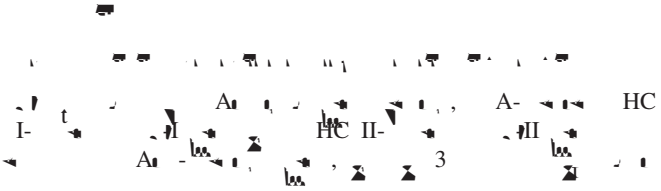
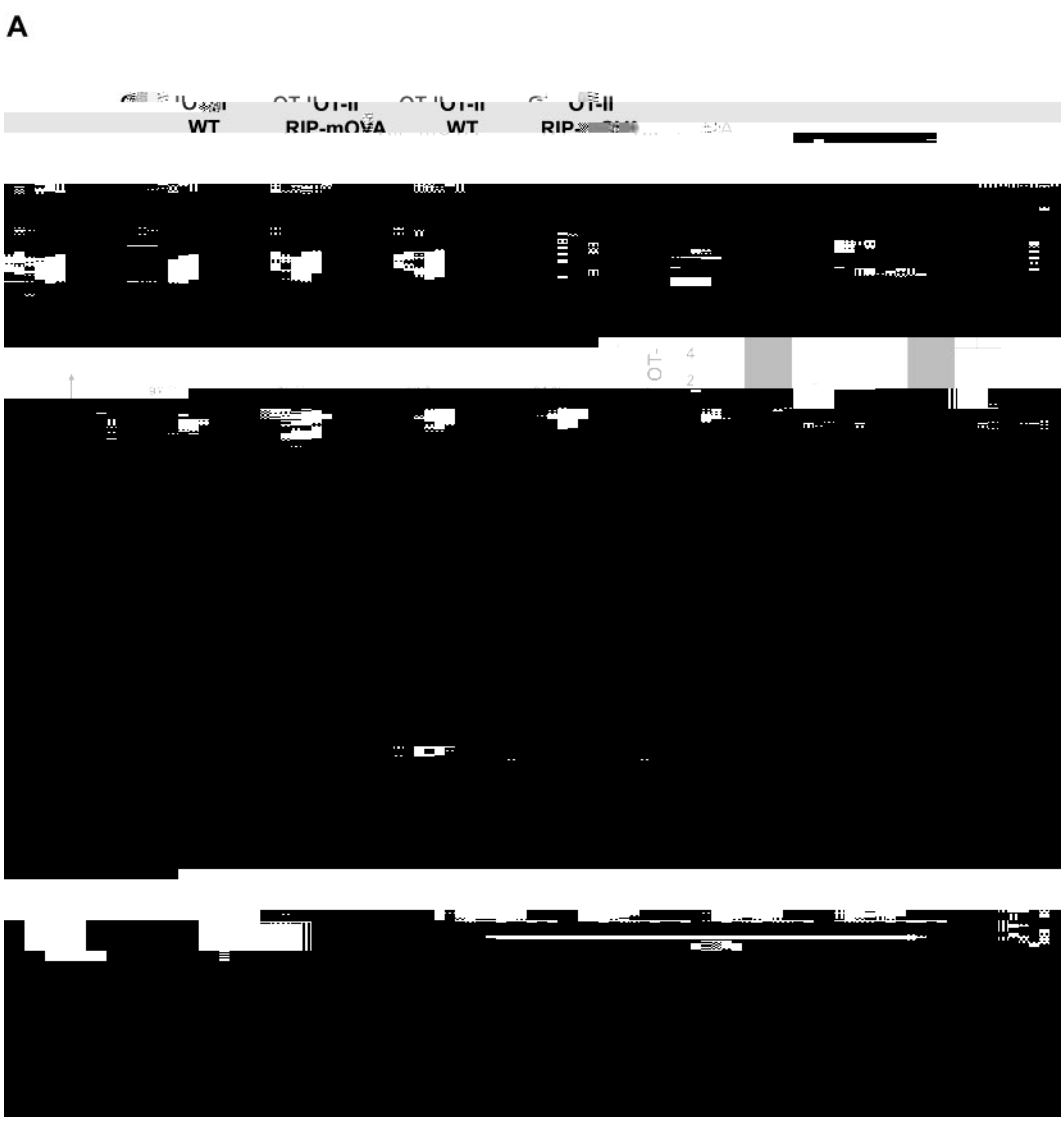
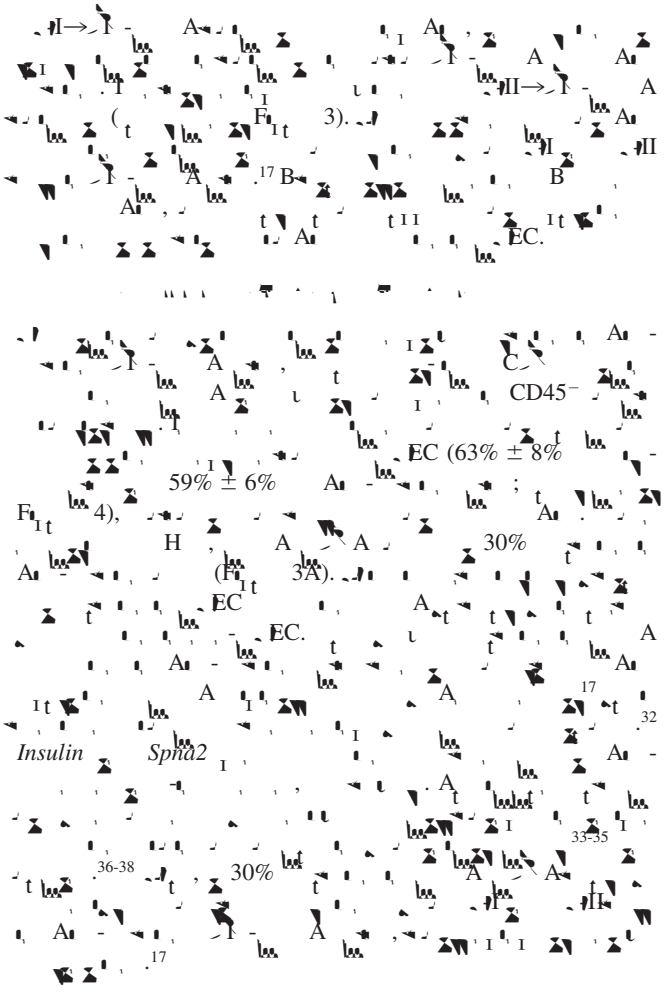


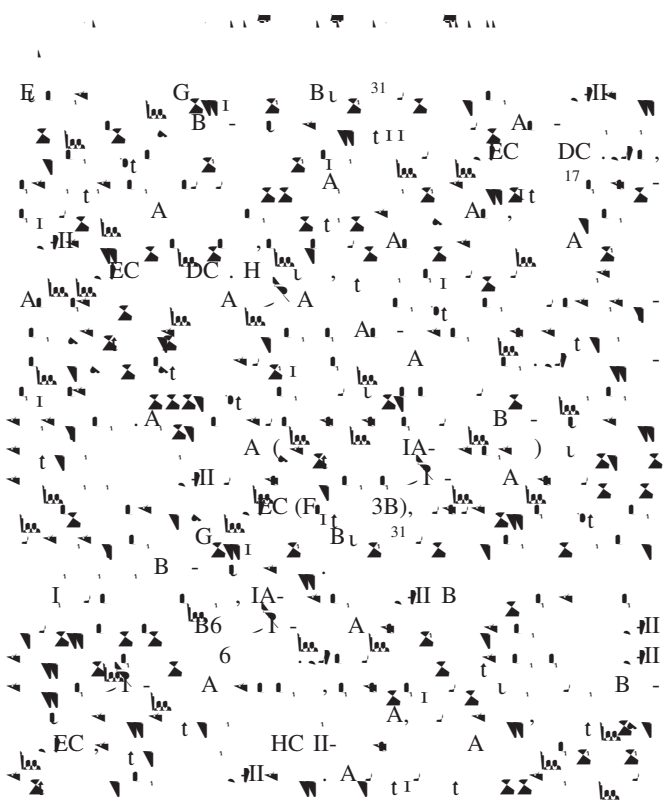


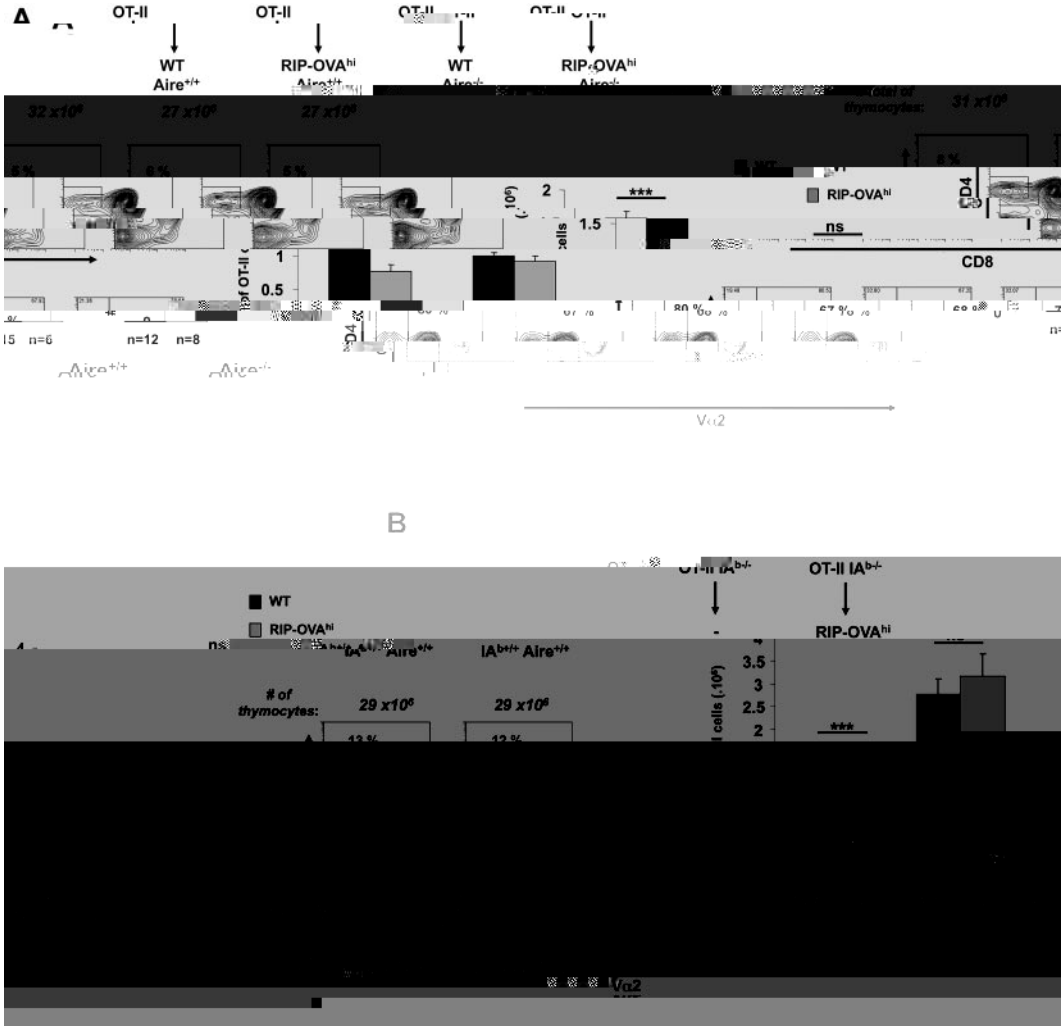




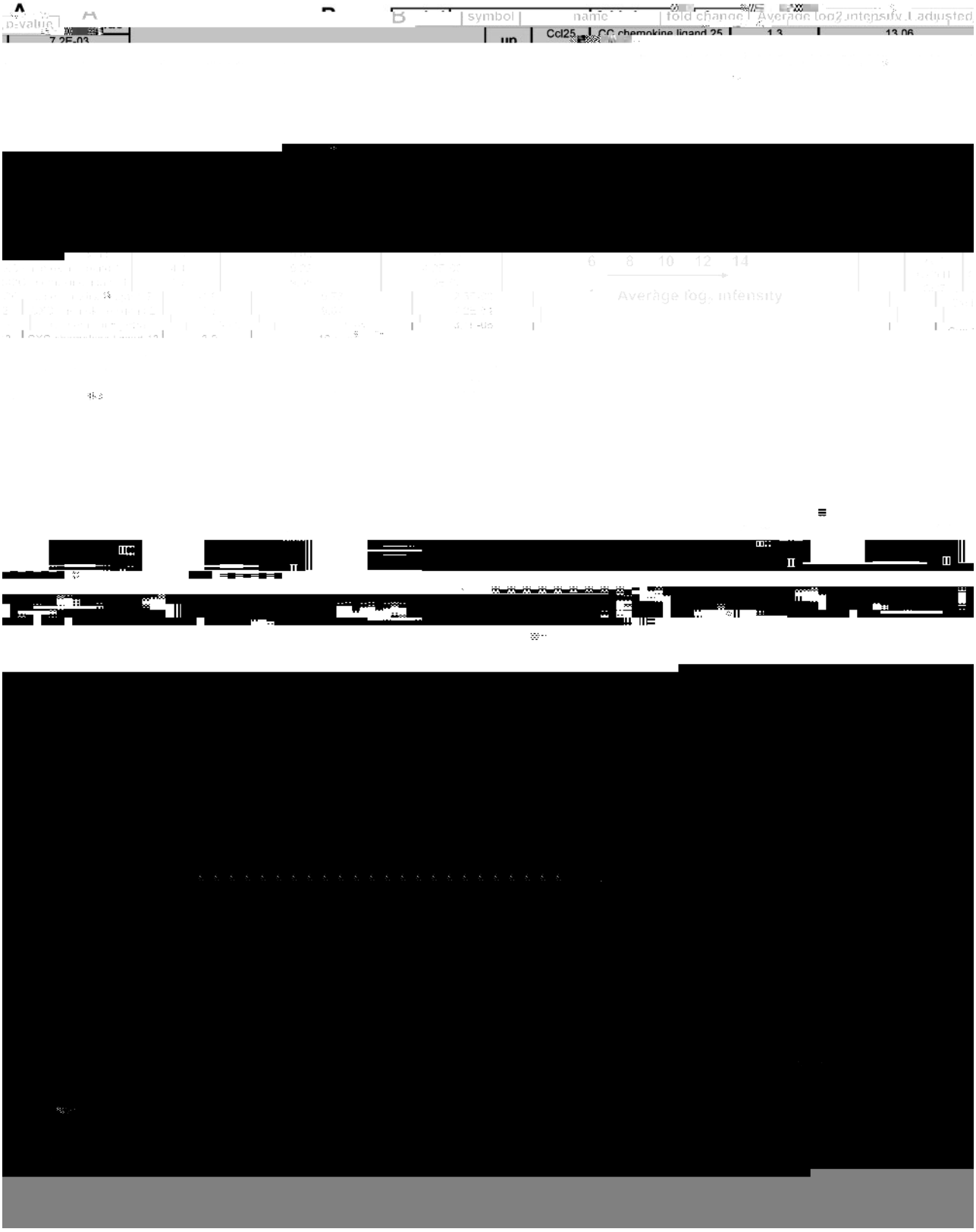
(A) Thymocytes (3-week-old mice) generated by crossing OT-II.Aire$^{+/-}$ to RIP-mOVA.Aire$^{+/-}$ mice were analyzed by flow cytometry for expression of CD4, CD8, and Vα2 (representative contour plots, left) and enumerated for SP thymocytes expressing high levels of TCR (CD4^{+}CD8^{-}Vα2^{+} cells, right). (B) Thymocytes (6 weeks postreconstitution) from chimeric mice generated by reconstituting wt.Aire$^{+/+}$, wt.Aire$^{-/-}$, RIP-mOVA.Aire$^{+/+}$, or RIP-mOVA.Aire$^{-/-}$ mice with OT-I BM were analyzed by flow cytometry for expression of CD4, CD8, and Vα2 (representative contour plots, left) and enumerated for SP thymocytes expressing high levels of TCR (CD4^{-}CD8^{+}Vα2^{+} cells, right). Representative contour plots were not all from the same experiment: Aire$^{+/+}$ groups are matched, as are Aire$^{-/-}$ groups. Histograms show the mean ± SEM for each group. n = number of mice pooled from several experiments. Figures 2B and 6 share the same groups lacking expression of the RIP transgene. Significance relative to WT: ***$P \leq .001$; ns indicates not significant.



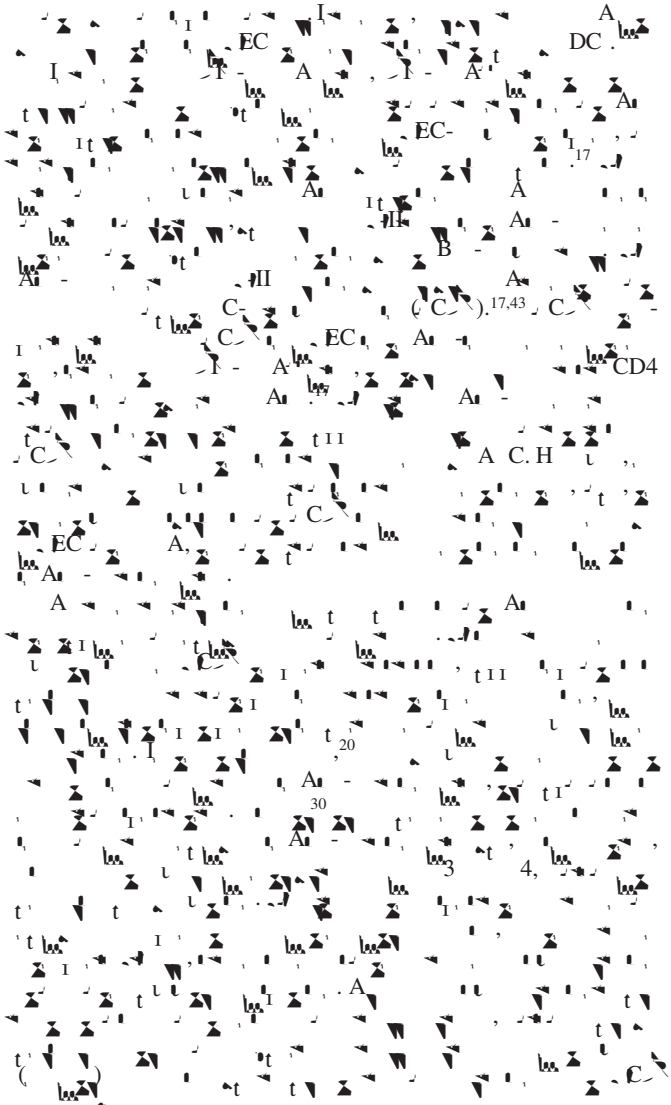


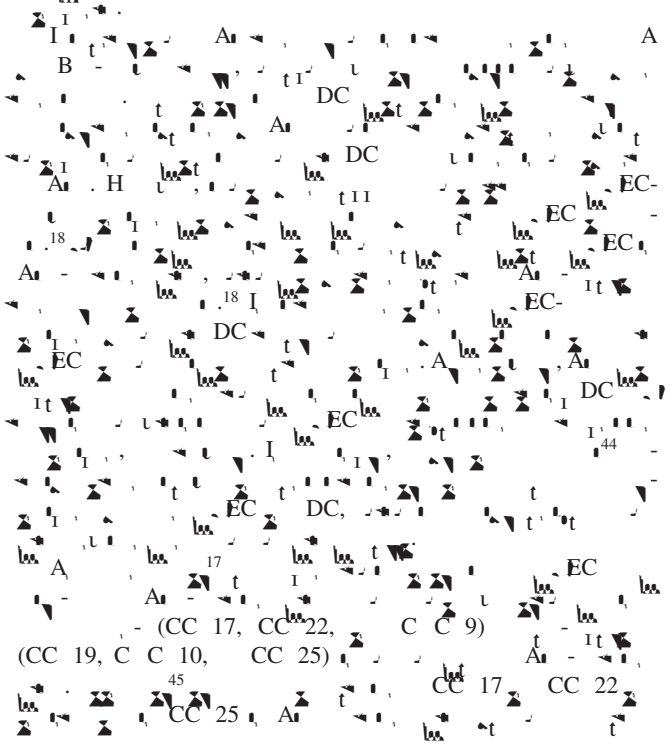


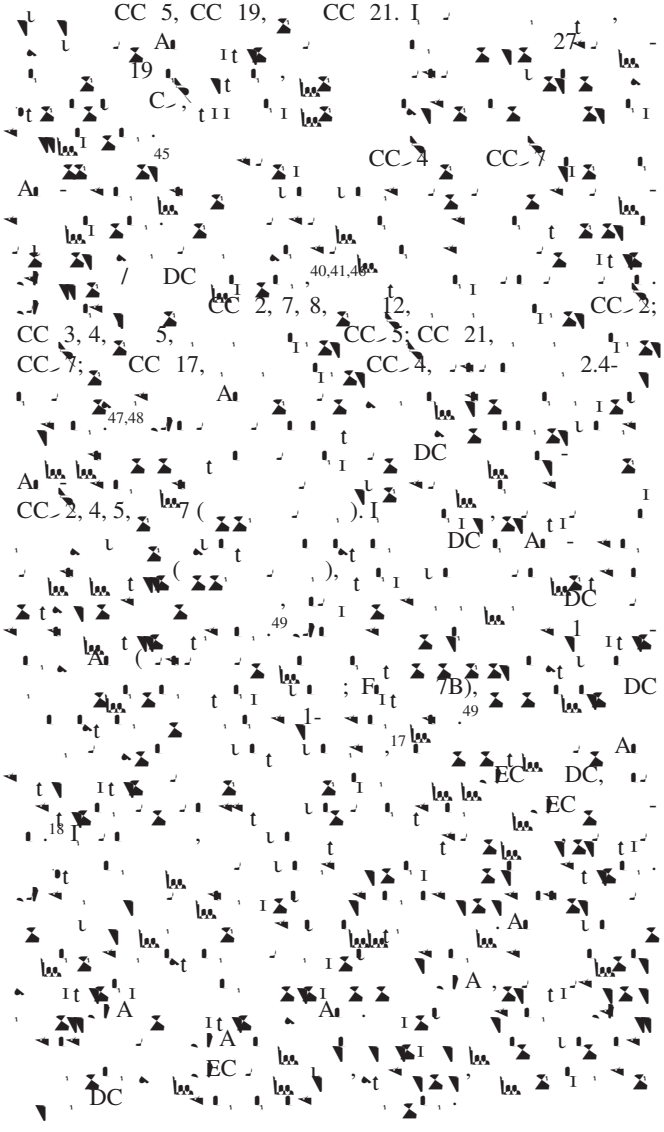
(A) Thymocytes (6 weeks postreconstitution) from chimeric mice generated by reconstituting wt.Aire$^{+/+}$, wt.Aire$^{-/-}$, RIP-OVA^{hi}.Aire$^{+/+}$, or RIP-OVA^{hi}.Aire$^{-/-}$ mice with OT-II BM were analyzed by flow cytometry for expression of CD4, CD8, and Vα2 (representative contour plots, left) and enumerated for SP thymocytes expressing high levels of TCR (CD4^{+}CD8^{-}Vα2^{+} cells, right). (B) mTECs are not able to present OVA on MHC II. Lethally irradiated 8-week-old B6 or RIP-OVA^{hi} mice were grafted with IA^{b}-deficient OT-II BM. At 6 weeks after reconstitution, thymocytes from the indicated mice were analyzed by flow cytometry for expression of CD4, CD8, and Vα2 (representative contour plots, left) and enumerated for SP thymocytes expressing high levels of TCR (CD4^{+}CD8^{-}Vα2^{+} cells, right). Contour plots show a representative experiment. Histogram shows the mean ± SEM for each group. n = number of mice pooled from several experiments. Figure 5A and supplemental Figure 3 as well as Figures 5B and 3B share, respectively, the same groups lacking expression of the RIP transgene. Significance relative to WT: ***$P \leq .001$; ns indicates not significant.



(A) The Fitted model MA plot representing different genes (4002 down and 4366 up) regulated by Aire (gray dots) according to the fold change and average intensity at a 5% FDR using Illumina beadchip. (B) List of chemokine genes whose expression levels differ significantly between Aire$^{+/+}$ and Aire$^{-/-}$ mTEC according to the Illumina beadchip analysis presented with fold change, average of the intensity, and adjusted *P* value. (C) Relative expression of Ccl1, Ccl3, Ccl6, Ccl7, Ccl8, Ccl9, Ccl11, Ccl17, Cxcl4, Cxcl13, Cxcl15, and Ccl25 was determined by quantitative real-time PCR on cDNA prepared from thymic CD45^{-}MHC II^{hi} Ly51^{-} cells. Expression values are shown in relative to WT after normalization to Hprt. Data shown are the mean ± SEM of 3 independent experiments. A total of 6-8 individual thymi were pooled per experiment. Significance relative to WT: $*P \leq .05$, $**P \leq .01$; ns indicates not significant. (D) List of cytokine genes whose expression levels differ significantly between Aire$^{+/+}$ and Aire$^{-/-}$ mTEC according to the Illumina beadchip analysis presented with fold change, average of the intensity, and adjusted *P* value.

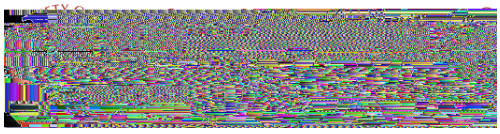






. G , . E. C , . H , A G F. H C F (F) 6 F E , C 040998 (F- .H.); A A (.A. .); H C (171601, 461202, 461204); H C (257501, 264573, 406700, 454465); E E A ; 6 F E ; A EHI (H. . .).

C : F- .H., .A. ., G. D, . . ., A. ., A.I. ., . .F.C., .F. ; B. . G. . . ; C.C.G., . ., F. .C.



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Aire regulates the transfer of antigen from mTECs to dendritic cells for induction of thymic tolerance

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