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Supplementary Material

CD85k contributes to regulatory T cell function in chronic viral infections

Anna Estrada Brull [1,†], Felix Rost [1,†], Josua Oderbolz [2], Florian R. Kirchner [1,3], Salomé Leibundgut-Landmann [1,3], Annette Oxenius [2] and Nicole Joller [1,*]

[1] University of Zurich, Institute of Experimental Immunology, 8057 Zurich, Switzerland; anna.estradabrull@uzh.ch (A.E.B.); rost@immunology.uzh.ch (F.R.); florian.kirchner@uzh.ch (F.R.K.); salome.leibundgut-landmann@uzh.ch (S.L.-L.)
[2] ETH Zurich, Institute of Microbiology, 8093 Zurich, Switzerland; josua.oderbolz@micro.biol.ethz.ch (J.O.); aoxenius@micro.biol.ethz.ch (A.O.)
[3] University of Zurich, Section of Immunology, Vetsuisse Faculty, 8057 Zurich, Switzerland
* Correspondence: nicole.joller@uzh.ch
† These authors contributed equally.

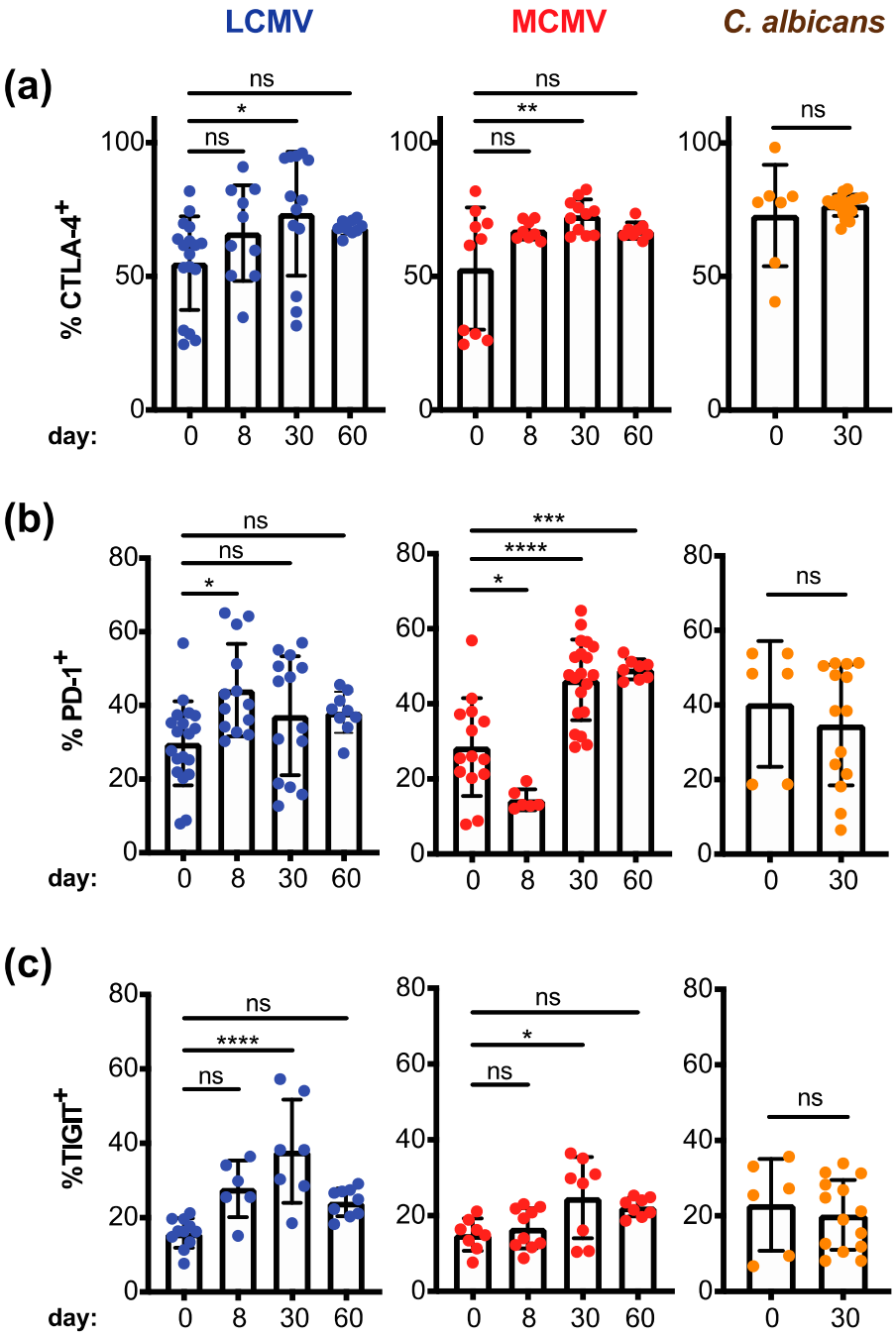

Figure S1. Treg characterization during chronic infection. C57BL/6 mice were infected with LCMV Clone 13 (blue, $2x10^6$ ffu i.v.), MCMV (red, $2x10^5$ ffu i.v.), or *C. albicans* strain 101 (orange, $2.5x10^6$ cfu sublingual), sacrificed 8, 30, and 60 days post infection. Spleens (LCMV, MCMV) or cervical lymph nodes (*C. albicans*) were harvested and directly analyzed *ex vivo* by flow cytometry. Summary graphs for frequencies of CTLA-4$^+$ (**a**), PD-1$^+$ (**b**), and TIGIT$^+$ (**c**) Tregs are depicted (Mean ± SEM; biological replicates: LCMV / MCMV / *C. albicans*: Naive n = 9-14 / 9-15 / 7; day 8 n = 8-13 / 4-10; day 30 n = 7-13 / 5-20 / 19, analyzed in 6 / 2-4 / 2-4 independent experiments). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; for comparison of two, three or six groups 2-sided t-Test, one-way ANOVA, or two-way ANOVA with multiple comparisons were used, respectively.

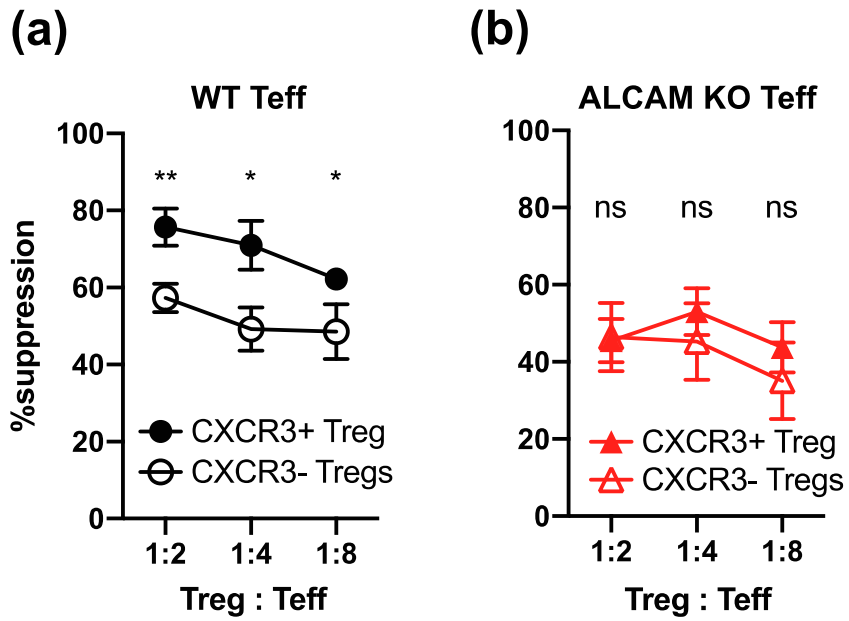

Figure S2. Suppression by type 1 Tregs is ALCAM-dependent. CXCR3^{+}CD4^{+}GFP^{+} and CXCR3^{-}CD4^{+}GFP^{+} Tregs were sorted from LCMV Clone 13 infected Foxp3-GFP reporter mice (day 25) and co-cultured with CD4^{+} Th1 effector cells sorted from C57BL/6 WT mice (**a**) or ALCAM$^{-/-}$ mice (**b**) also infected with LCMV Clone 13 (day 10). Cells were stimulated with anti-CD3 and irradiated splenic APCs for 2 days before proliferation was determined by [^{3}H]-thymidine incorporation and Treg-mediated suppression was calculated (mean ± SD; n = 3; 1 representative experiment of 2 is shown). *$p < 0.05$, **$p < 0.01$; t-Test.