

SUPPLEMENTAL MATERIAL

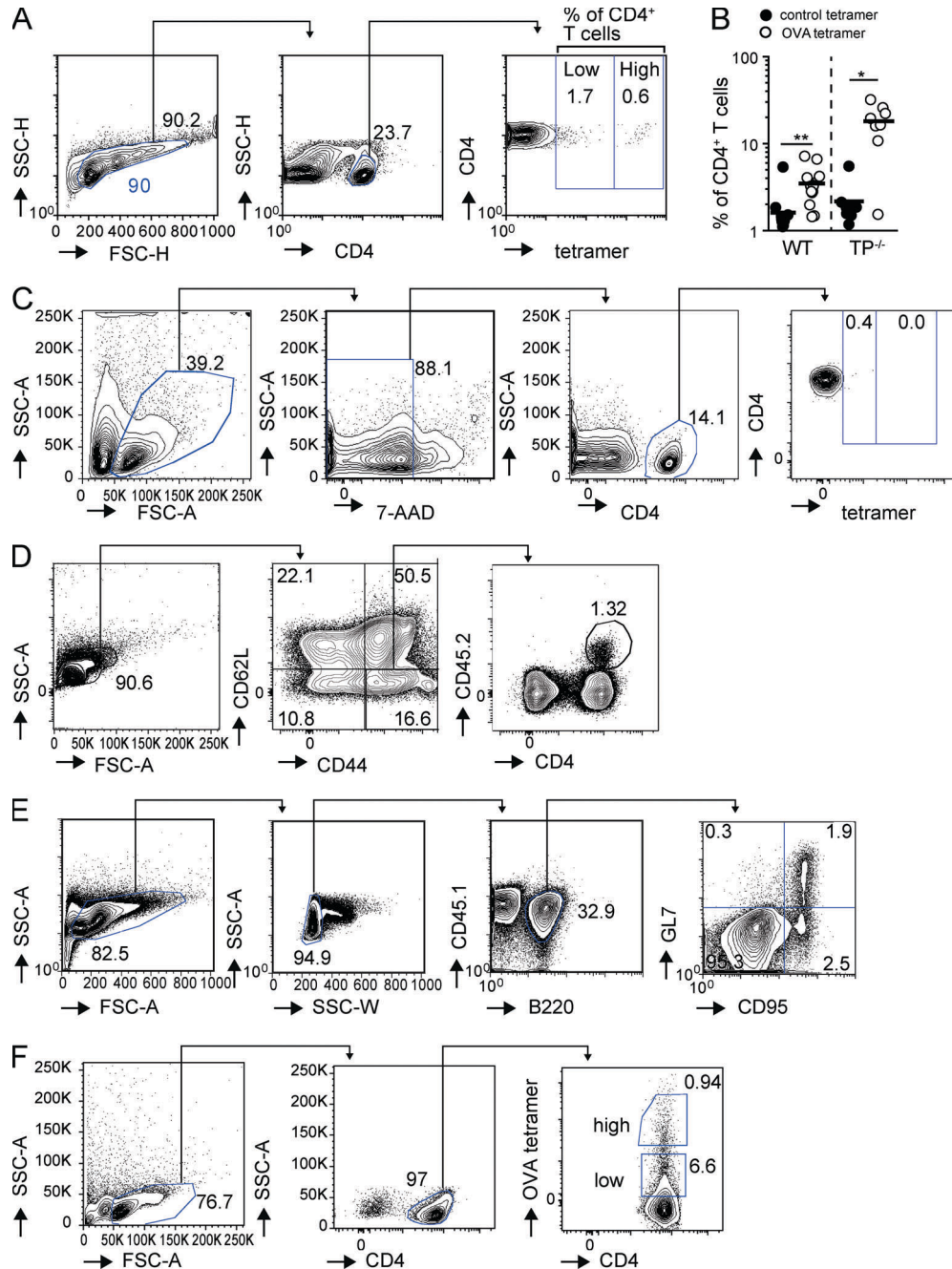
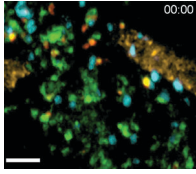
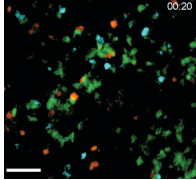
Moalli et al., <http://www.jem.org/cgi/content/full/jem.20140137/DC1>

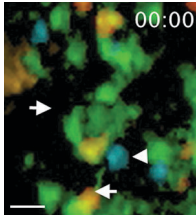
Figure S1. Gating strategies for flow cytometric analysis. (A) Gating strategy of OVA tet⁺ CD4⁺ T cells after OVA/CFA immunization. Numbers indicate percentages of gated populations. (B) Percentages of control and OVA tet⁺ CD4⁺ T cells in draining PLNs of WT and TP^{-/-} mice. (C) Gating strategy for flow cytometric analysis of gp₆₆₋₇₇ tet⁺ CD4⁺ T cells after LCMV Armstrong infections. (D) Gating strategy of adoptively transferred CD4⁺ CD44^{high} CD-62^{low} CD45.2⁺ WT or TP^{-/-} OT-II CD4⁺ T cells on day 8 after OVA/CFA immunization. (E) Gating strategy for flow cytometric analysis of germinal center B cells on day 8 after OVA/CFA immunization. (F) Sorting strategy by flow cytometry for low and high OVA tet⁺ CD4⁺ T cells on day 8 after OVA/CFA-immunization of WT and TP^{-/-} mice. Numbers indicate percentages of gated populations. Owing to low numbers of recovered high OVA tet⁺ CD4⁺ T cells, we did not transfer these cells into SMARTA mice. Data in B are pooled from two independent experiments with 4–8 mice/group and analyzed using a Kruskal-Wallis test. *, P < 0.05; **, P < 0.01.



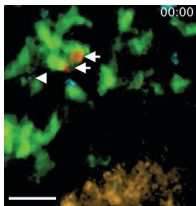
Video 1. 2PM image sequence showing WT (blue) and TP^{-/-} (red) OT-II CD4⁺ T cells and DCs (green) pulsed with 0.1 μM OVA. Selected WT and TP^{-/-} OT-II CD4⁺ T cells tracks are shown in blue and red, respectively. HEVs are labeled brown. Time is shown in minutes and seconds. Bar, 25 μm.



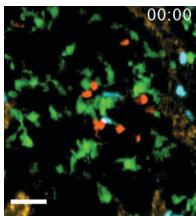
Video 2. 2PM image sequence showing WT (blue) and TP^{-/-} (red) OT-II CD4⁺ T cells and DCs (green) pulsed with 10 μM OVA. Selected WT and TP^{-/-} OT-II CD4⁺ T cells tracks are shown in blue and red, respectively. Time is shown in minutes and seconds. Bar, 30 μm.



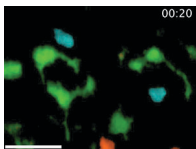
Video 3. 2PM image sequence showing WT (blue) and TP^{-/-} (red) OT-II CD4⁺ T cells interacting with DCs (green) pulsed with 0.1 μM OVA. Interactions with DCs are labeled with arrowheads for WT and arrows for TP^{-/-} OT-II CD4⁺ T cells, respectively. An HEV (brown) is seen in the top left corner. Time is shown in minutes and seconds. Bar, 15 μm.



Video 4. 2PM image sequence showing WT (blue) and TP^{-/-} (red) OT-II CD4⁺ T cells interacting with DCs (green) pulsed with 10 μM OVA. Interactions are labeled as in Video 3. An HEV (brown) is seen in the bottom. Time is shown in minutes and seconds. Bar, 15 μm.



Video 5. 2PM image sequence showing WT (blue) and TP^{-/-} (red) OT-II CD4⁺ T cells and DCs (green) pulsed with 10 μM tOVA. Selected WT and TP^{-/-} OT-II CD4⁺ T cells tracks are shown in blue and red, respectively. HEVs are labeled brown. Time is shown in minutes and seconds. Bar, 30 μm.



Video 6. 2PM image sequence showing WT (blue) and TP^{-/-} (red) OT-II CD4⁺ T cells interacting with DCs (green) pulsed with 10 μM tOVA. Interactions are labeled as in video 3. Time is shown in minutes and seconds. Bar, 10 μm.