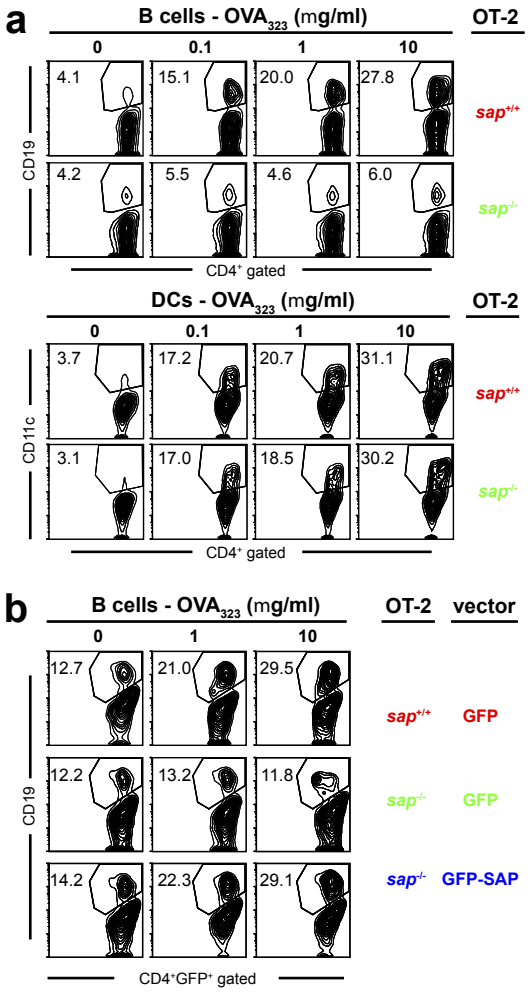
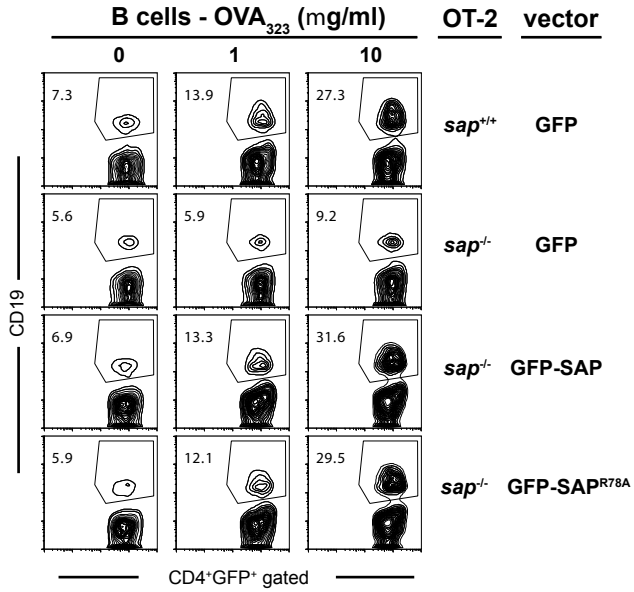
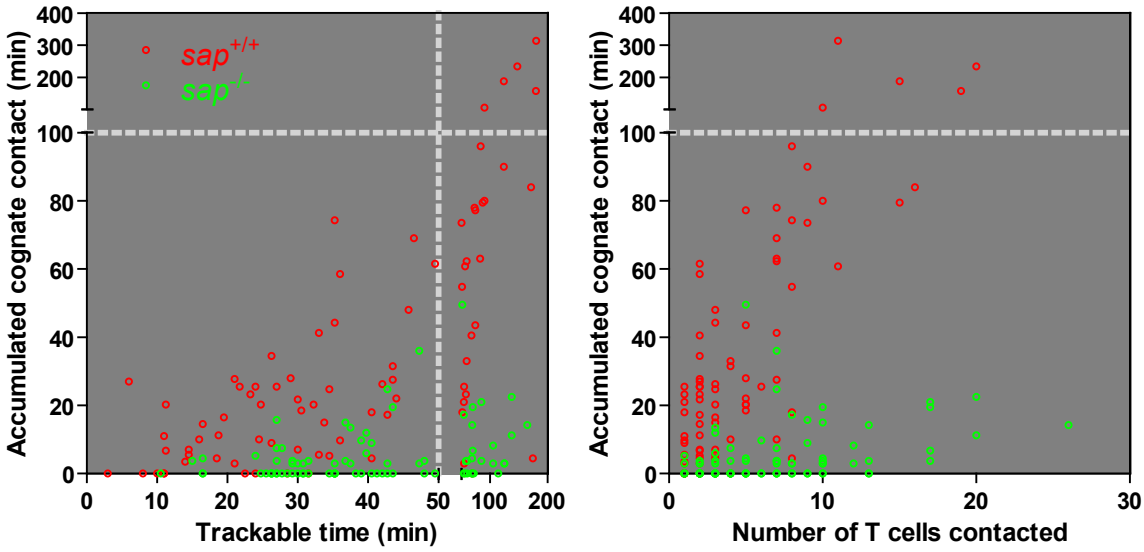


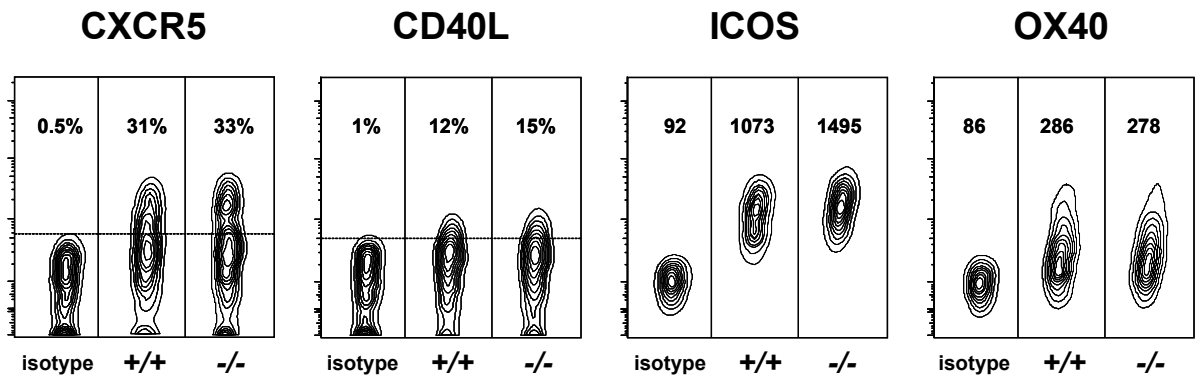
Supplementary Figure 1. Representative contour plots for T-APC conjugation assays presented in Figs. 2c and 2d. Contour plots were $CD4^{+}GFP^{+}$ events. (**a**) corresponds to Fig. 2c, and (**b**) corresponds to Fig. 2d.



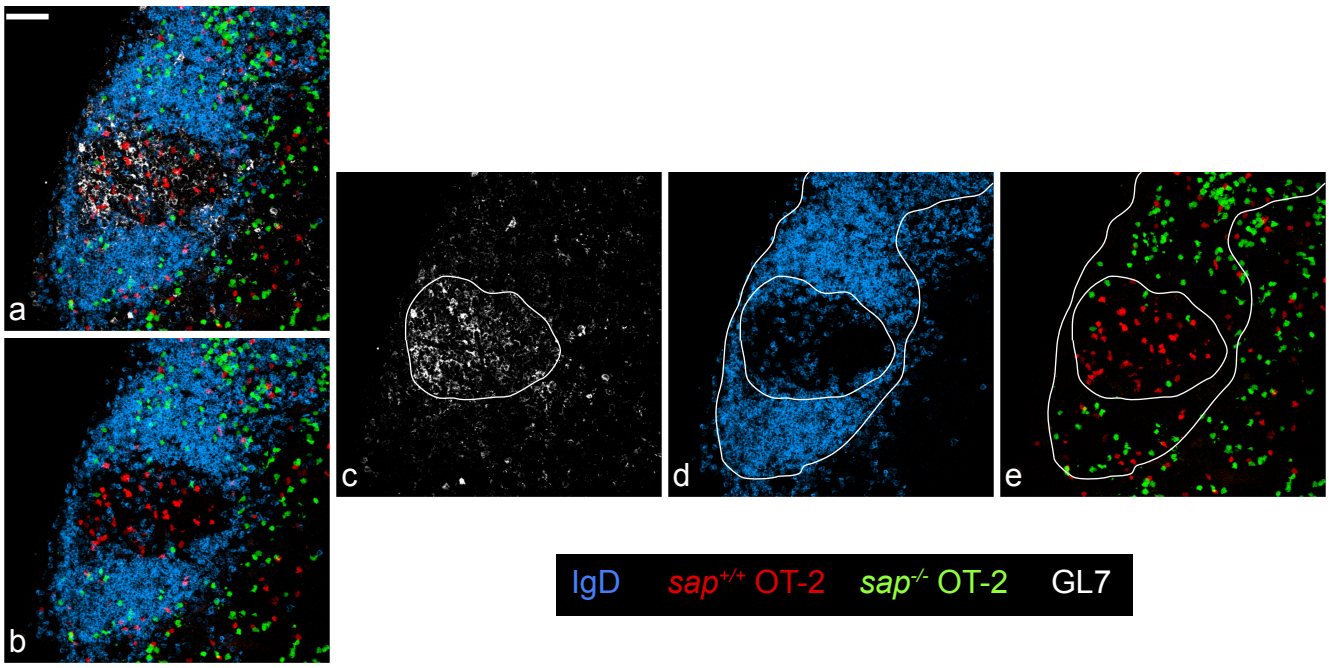
Supplementary Figure 2. Expression of Fyn-binding mutant SAP is sufficient for T-B conjugation. T-B conjugation assays similar to that used in Fig. 2e were conducted using OT-2 cells transiently transfected with DNA constructs expressing either GFP or GFP-tagged wild-type or R78A mutant SAP. Contour plots were $CD4^+GFP^+$ events. Data represent 3 independent experiments with similar results.



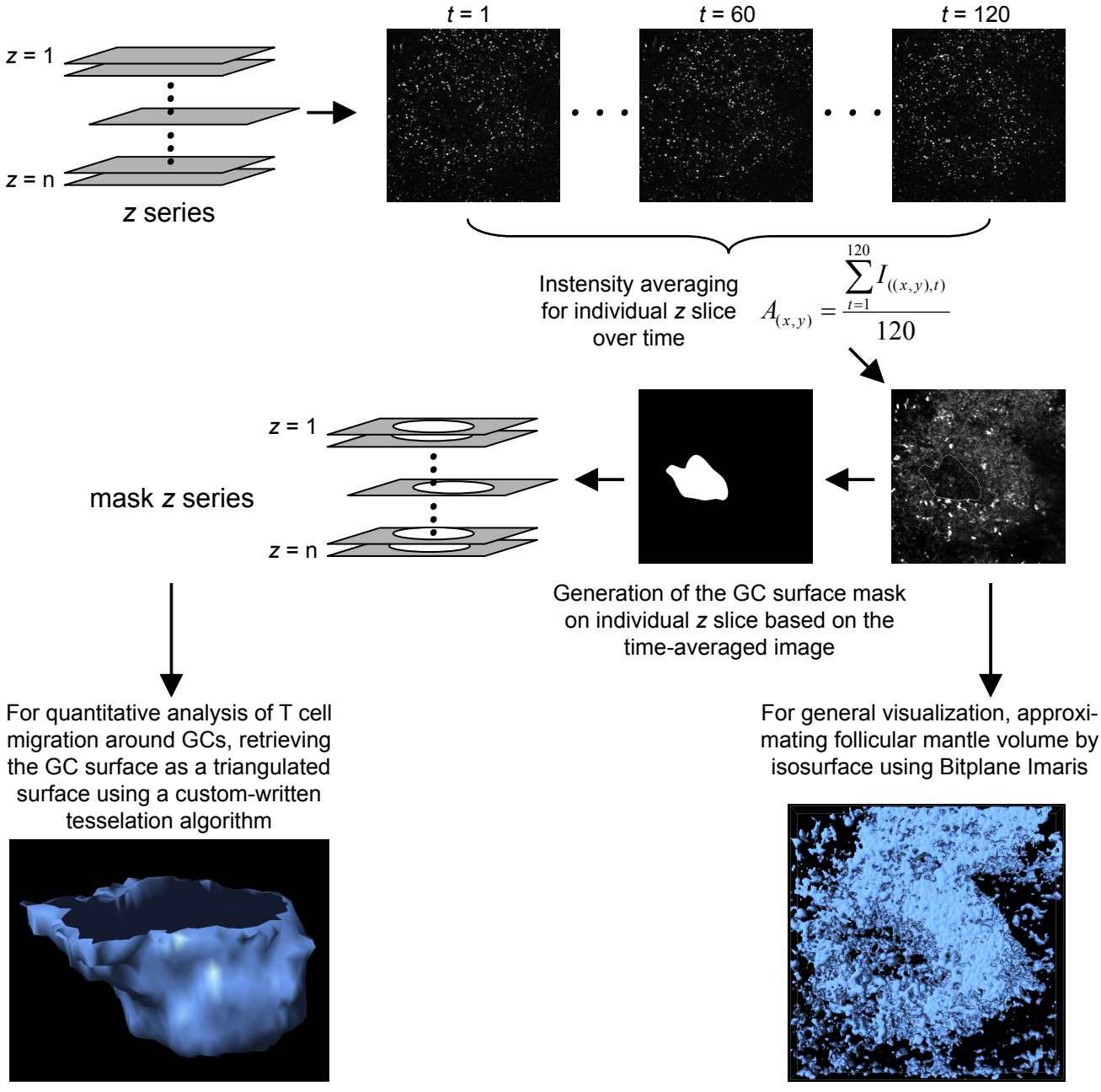
Supplementary Figure 3. Cumulative contact durations of MD4 cells and OT-2 cells normalized against trackable time of B cells or numbers of T cells contacted. Cumulative cognate contact durations for individual MD4 B cells, as presented in Fig. 3d, are plotted against their trackable time in the imaging field (left) or the number of *sap*$^{+/+}$ or *sap*$^{-/-}$ T cells they contacted (right).



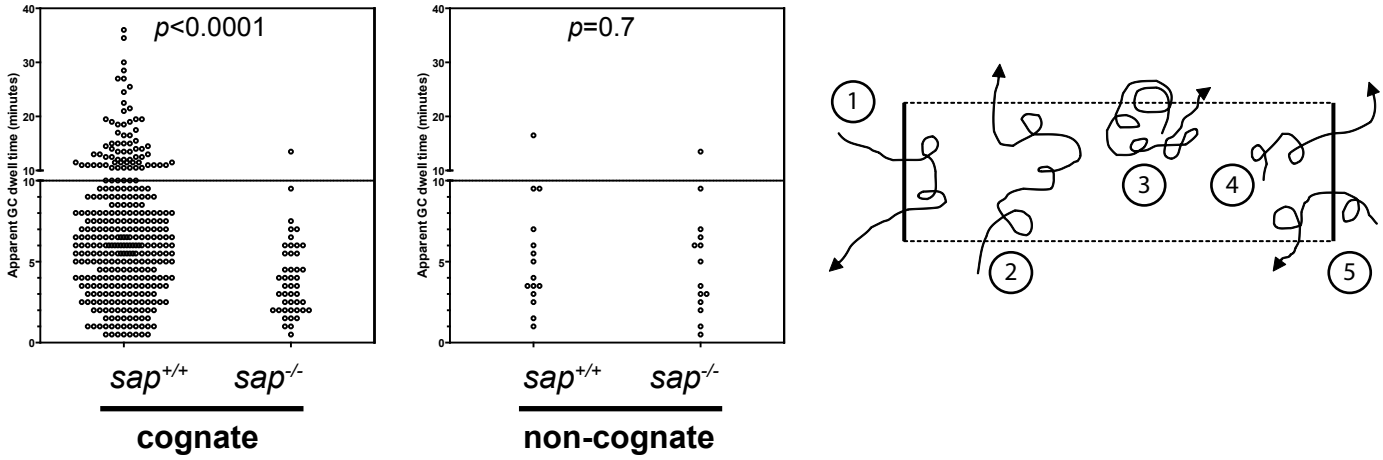
Supplementary Figure 4. T cell activation phenotypes *in vivo*. Representative contour plots of surface staining for indicated markers expressed by *sap*$^{+/+}$ or *sap*$^{-/-}$ OT-2 T cells. The number on top of each contour indicates either the percentage of positive cells or the geometric mean fluorescent intensity for the relevant parameter. Corresponding summary of quantitative data from 3 experiments is presented in Fig. 3f.



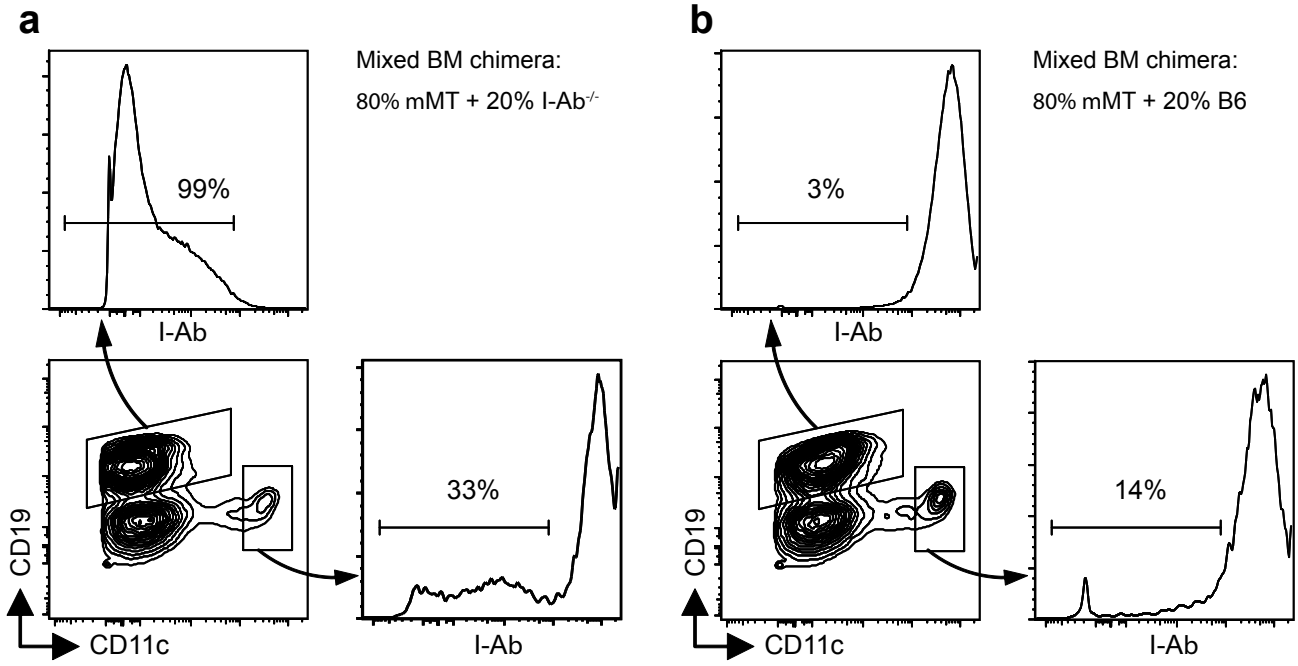
Supplementary Figure 5. Distinct intrafollicular distributions of *sap*$^{+/+}$ and *sap*$^{-/-}$ OT-2 T cells. B6 mice received an adoptive transfer of non-fluorescent MD4 B cells and CFP *sap*$^{+/+}$ and GFP *sap*$^{-/-}$ OT-2 T cells. They were then immunized with HEL-OVA. Draining LNs were taken 6-8 days post immunization and processed for immunohistochemical staining with IgD and GL7. **a, b,** A typical section of GC-containing follicles is shown as (**a**) a 4-color overlay displaying two types of T cells and IgD and GL7 staining or (**b**) a 3-color overlay without GL7. **c, d, e,** The approximate GC-mantle border and the outline of the follicle are drawn based on (**c**) GL7 staining and (**d**) IgD staining, respectively, and overlaid on (**e**), which shows the two types of T cells. Similar observations were made with multiple mice in 3 separate experiments. Scale bar = 50 mm.



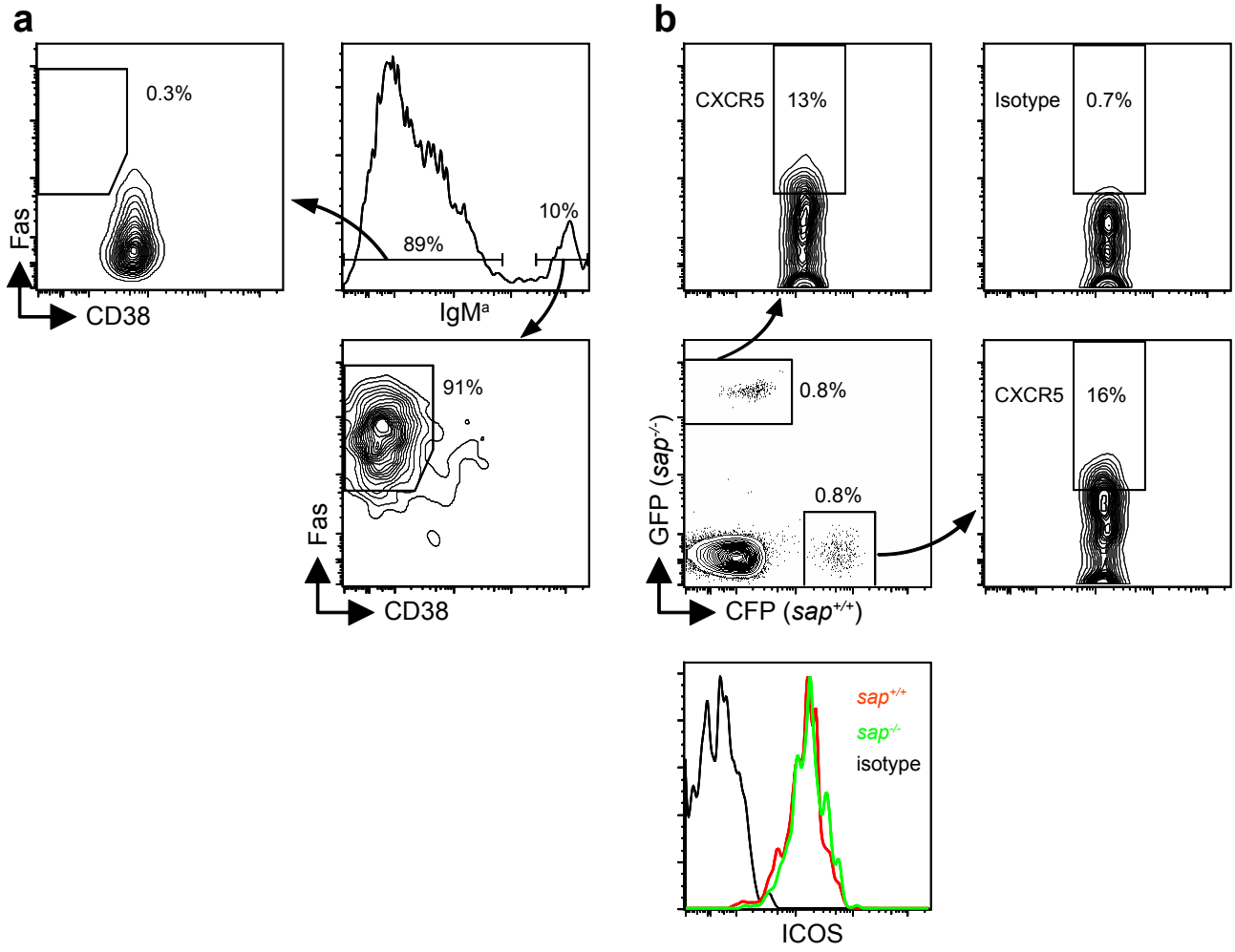
Supplementary Figure 6. A schematic diagram of data processing involved in visualization and quantitative analysis of T cell migration around GCs. Each optical (x, y) slice at a given z position is averaged over the entire time series (e.g. 120 time points), producing a corresponding time-averaged z slice. For 4D datasets in which polyclonal naïve B cells are used to mark follicles and associated GC areas, the z series composed of such time-averaged slices can be used to visualize the general configuration of the follicular mantle and GC through iso-surface generation in Imaris. The same z series is also used to generate a mask series that approximates the mantle-GC boundary. From the mask series, a custom tessellation algorithm is used to mathematically define a triangulated GC surface. The gray-scale images in the diagram are actual fluorescent images of naïve B cells on the same z slice at the indicated time points and the corresponding z slice in the time-averaged series. The black-white binary image depicts the mask generated based on the time-averaged image. The bottom-right color image is a 3D rendering of the follicular mantle by Imaris iso-surface, and the bottom-left is the corresponding triangulated GC surface.



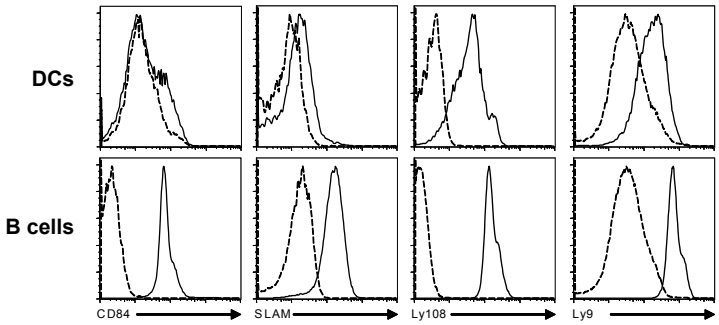
Supplementary Figure 7. Apparent residence time of OT-2 T cells within cognate GC areas. The two scatter plots show durations of residence in cognate (left) or non-cognate (right) GCs by all *sap*$^{+/+}$ and *sap*$^{-/-}$ OT-2 T cell tracks that intersected GC areas as defined by the method described in Fig. 4b and Supplementary Fig. 3. Data are pooled from 3 and 2 experiments for the cognate and non-cognate conditions, respectively. Mann-Whitney rank tests were used to calculate *p* values. Calculated in these manners, the results underestimate the amount of time a follicular helper T cell typically spends in a GC. As shown in the schematic diagram on the right, because the rectangle imaging volume typically does not contain the entire GC volume, cells of the type 2, 3, 4, 5 as depicted can come into or leave the visualized GC volume from the top or the bottom (dotted lines). Particularly, a cell of the type 3 repeatedly comes into and leaves the imaged GC volume and will be traced as multiple tracks with relatively short residence time, while in actuality it is one cell residing in the GC for a much longer period. These experimental limitations would disproportionally underestimate the residence time of *sap*$^{+/+}$ T cells as compared to that of *sap*$^{-/-}$ T cells.



Supplementary Figure 8. Verification of the status of mixed BM chimera. a, b, Mixed BM chimera were constructed by reconstituting lethally irradiated B6 or CD45.1 congenic mice with a mixture of 80% mMT and 20% I-Ab$^{-/-}$ BM cells (**a**) or, as control, 20% B6 (**b**) lineage-negative BM cells. Splenic B cells (7-AAD^{-}CD19^{+} singlet events) and DCs (7-AAD^{-}CD11c^{+}) were analyzed for I-Ab expression. Numbers in histograms indicate percentages of the I-Ab^{-} fraction. Similar data were obtained from two batches of chimeras.



Supplementary Figure 9. Activation and differentiation of MD4 B cells and OT-2 T cells in mixed BM chimeras immunized with HEL-BSA plus OVA. a, b, Mixed BM chimeras (80% mMT + 20% I-Ab$^{-/-}$) received adoptive transfer of non-fluorescent MD4 B cells and equal numbers of CFP-expressing *sap*$^{+/+}$ and GFP-expressing *sap*$^{-/-}$ OT-2 cells. Seven or 8 days after immunization of these mice with a mixture of HEL-BSA and OVA, the surface phenotypes of MD4 and OT-2 cells in the draining LN were examined by FACS. **a,** MD4 cells were identified as the IgM^{a+} fraction of B cells (7-AAD^{-}CD4^{-}D19^{+} singlet events), while endogenous B cells were IgM^{a-}. Expression of Fas and CD38 is depicted. Polygon gates indicate the Fas^{hi}CD38^{lo} GC cell phenotype. **b,** *Sap*$^{+/+}$ and *sap*$^{-/-}$ OT-2 cells were identified as CFP^{+} and GFP^{+} fractions in total CD4 T cells (7-AAD^{-}CD19^{-}CD4^{+} singlet events), respectively. Expression of CXCR5 and ICOS is depicted. Representative data from one of three similar experiments are shown.



Supplementary Figure 10. Surface expression of SLAM family proteins by B cells and DCs. Freshly isolated $CD11c^+$ Splenic DCs or $CD19^+$ B cells were stained with antibodies specific to CD84, SLAM, Ly108, and Ly9 (solid lines) or isotype control antibodies (dotted lines) and then assayed by flow cytometry. Data represent 3 independent experiments.

Supplementary movie legends

Supplementary Movie 1. Comparable interactions of *sap*$^{+/+}$ and *sap*$^{-/-}$ T cells with antigen-presenting DCs *in vivo*. Twenty-four hours following co-transfer into mice that had been injected with OVA_{323}-pulsed DCs (blue), *sap*$^{+/+}$ (red) and *sap*$^{-/-}$ (green) OT-2 T cells were visualized in draining LNs by intravital microscopy. As typified in this example, one *sap*$^{+/+}$ and 2 *sap*$^{-/-}$ OT-2 T cells were engaged in comparable, long-term interactions with the same DC. Playback speed: 450×. Also see corresponding time-lapse images in Fig. 1a.

Supplementary Movie 2. *Sap*$^{-/-}$ T cells fail to stably interact with B cells when examined together with *sap*$^{+/+}$ T cells in the same LN *in vivo*. *Sap*$^{+/+}$ OT-2 T cells (red), *sap*$^{-/-}$ OT-2 T cells (green), and MD4 B cells (blue) were co-transferred into mice that had previously been immunized with HEL-OVA conjugate antigen. Intravital imaging of the draining LNs was performed 24 to 36 hours after cell transfer. The circle at the beginning of this movie highlights a 3-cell cluster in which an MD4 B cell is engaged by both a *sap*$^{+/+}$ and a *sap*$^{-/-}$ OT-2 T cell. The *sap*$^{-/-}$ T cell then disengages quickly, while the *sap*$^{+/+}$ T cell remains in conjugation with the B cell for a much longer period of time. Playback speed: 450×. Also see corresponding time-lapse images in Fig. 2a.

Supplementary Movie 3. Another example of different B cell-interacting behaviors of *sap*$^{+/+}$ (red) and *sap*$^{-/-}$ (green) OT-2 T cells. At the beginning of this movie, three conjugate pairs of *sap*$^{+/+}$ OT-2 T cells and MD4 B cells are visible (highlighted in circles). Playback speed: 450×.

Supplementary Movie 4. *Sap*$^{-/-}$ OT-2 T cells fail to stably interact with B cells in the absence of competition from *sap*$^{+/+}$ OT-2 *in vivo*. GFP-expressing *sap*$^{+/+}$ or *sap*$^{-/-}$ OT-2 T cells (green) were co-transferred into mice together with CFP-expressing MD4 B cells (red). These mice were subsequently immunized with HEL-OVA. Intravital imaging of draining LNs was performed 64 to 72 hours post immunization. While *sap*$^{+/+}$ OT-2 T cells engaged B cells in long-lasting conjugates, *sap*$^{-/-}$ OT-2 T cells predominantly exhibited "hit-and-run" contacts with B cells. Imaging data in this movie are rendered in 3-D mode. Playback speed: 450×.

Supplementary Movie 5. B cells sequentially interact with multiple *sap*$^{-/-}$ OT-2 T cells *in vivo* but do not accumulate contact time equivalent to that with *sap*$^{+/+}$ T cells. This is the same as Movie S4 except that example MD4 tracks are shown. MD4 B cells marked by the white tracks divided during the imaging period, producing daughter cells indicated by the red tracks. Notice that, due to the "hit-and-run" nature of interactions with *sap*$^{-/-}$ OT-2 T cells, the highlighted MD4 B cells were not engaged by these T cells most of time, despite coming in contact with numerous T cells. On the other hand, MD4 B cells were in long-lasting conjugation with multiple *sap*$^{+/+}$ OT-2 T cells sequentially, accumulating large summed contact time during the 3-hour imaging period. Playback speed: 450×.

Supplementary Movie 6. *Sap*$^{-/-}$ T cells fail to be recruited and retained in nascent GC. CFP-expressing *sap*$^{+/+}$ OT-2 T cells (red), GFP-expressing *sap*$^{-/-}$ OT-2 T cells (green), and non-fluorescent MD4 B cells were co-transferred into mice that were subsequently immunized with HEL-OVA. Intravital imaging of draining LNs was performed 6 to 8 days post immunization. CMTPX-labeled polyclonal naïve B cells

(blue) were transferred 1 or 2 days prior to imaging to help demarcate follicular mantle and GC. The dotted line at the beginning of the movie approximates the GC border in this maximum intensity projection. See Fig. 4a for corresponding 3D rendering of this dataset. Playback speed: 450×.

Supplementary Movie 7. GC recruitment and retention of activated T cells depend on antigen-specific interactions with B cells. Mixed BM chimeras (80% μMT + 20% I-Aβ$^{-/-}$) received CFP-expressing *sap*$^{+/+}$ OT-2 T cells (red), GFP-expressing *sap*$^{-/-}$ OT-2 T cells (green), and non-fluorescent MD4 B cells one day before these mice were immunized with a mixture of HEL-BSA and OVA. Intravital imaging of draining LNs was performed 7 to 8 days post immunization following additional transfer of CMTPX-labeled polyclonal naïve B cells (blue). The dotted line at the beginning of the movie approximates the GC border in this maximum intensity projection. Both *sap*$^{+/+}$ and *sap*$^{-/-}$ OT-2 T cells rarely migrate into and dwell within the GC area. Note that a portion of the follicular mantle overlaying the top of this GC was captured in the z-stack, giving rise to the apparent presence of numerous naïve B cells within the GC border projected onto the XY plane. Careful analysis of x-z and y-z images confirmed that the region rarely visited by OT-2 T cells in the maximum projection movie corresponds to the GC region, as defined using the method described in Supplementary Fig. 4. Also see Fig. 4e for corresponding 3D rendering of this dataset. Playback speed: 450×.

Supplementary Movie 8. Another example of the migration pattern of *sap*$^{+/+}$ and *sap*$^{-/-}$ OT-2 T cells in and around non-cognate GCs. CFP-expressing *sap*$^{+/+}$ OT-2 T cells (red) and GFP-expressing *sap*$^{-/-}$ OT-2 T cells (green) were visualized in the context of polyclonal naïve B cells (blue) in mixed BM chimera hosts that were

constructed and immunized in the same way as for the experiment illustrated in Movie S7. Playback speed: 450×.