SUPPLEMENTAL MATERIAL

Hwang et al., http://www.jem.org/cgi/content/full/jem.20091982/DC1

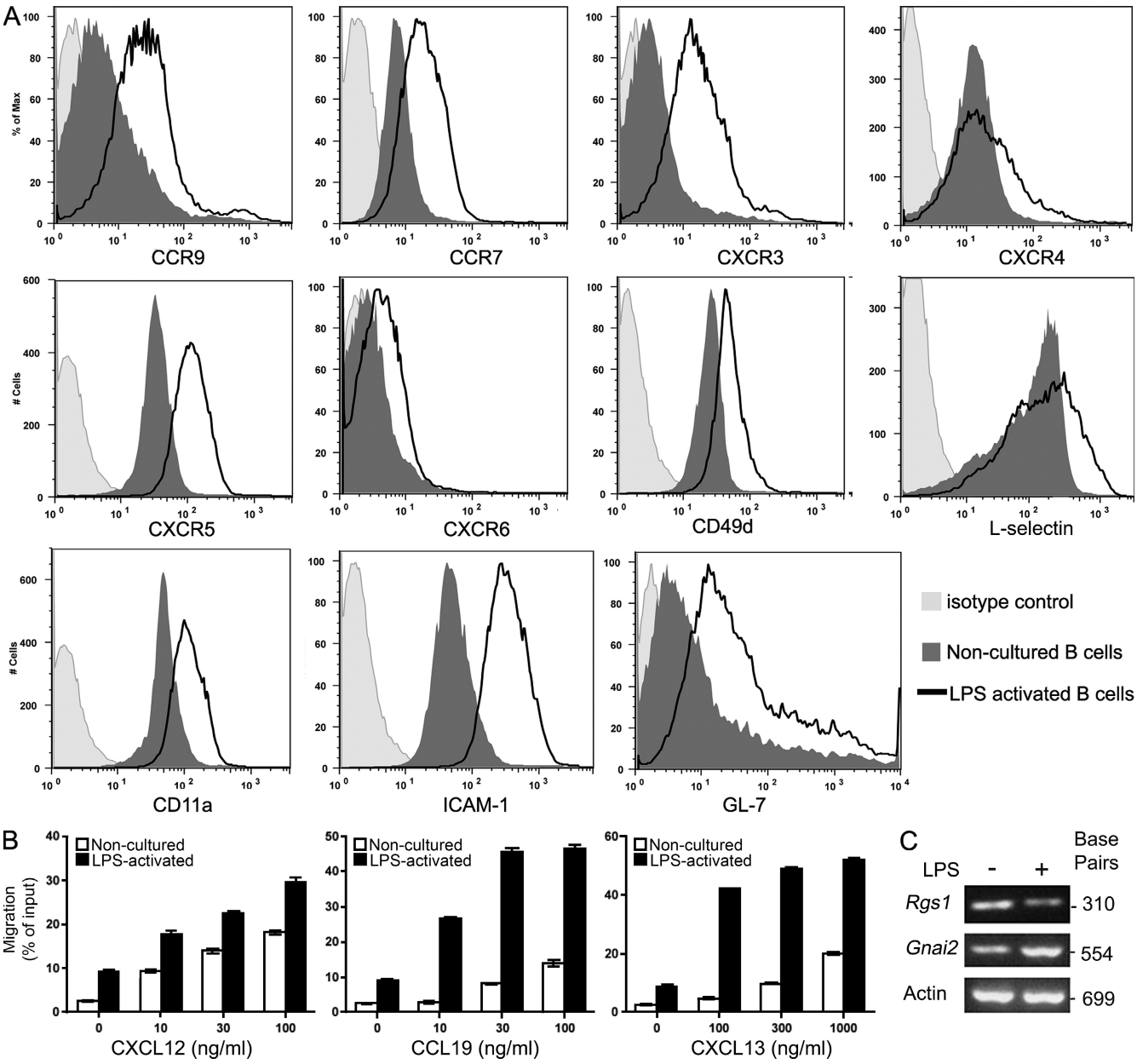


Figure S1. Comparison of freshly isolated and LPS-stimulated B cells. (A) Receptor expression. Purified splenic B cells activated with LPS or freshly isolated splenic B cells were compared. TLR4-stimulated B cell profiles are shaded in gray, nonactivated B cell profiles are nonshaded, and isotype controls are shown with a light gray line. The surface receptor recognized is indicated below each flow pattern. Representative results are from three separate experiments. (B) Chemotaxis assays. TLR4-stimulated (black bars) and nonactivated (white bars) B cells were subjected to 2 h of chemotaxis in response to CXCL12, CCL19, or CXCL13 as indicated. The percentages of cells responding to the indicated concentrations of CXCL12, CCL19, and CXCL13 are shown. Results are mean and SE of sextuplet samples from three experiments. Data are from the analysis of B cells from three mice. The experiment was performed twice with similar results. (C) Expression of *Rgs1* and *Gnai2*. RT-PCR was performed to analyze *Rgs1* and *Gnai2* messenger RNA expression in LPS-activated versus nonactivated splenic B cells. The experiment was performed three times with similar results. Actin control is shown.

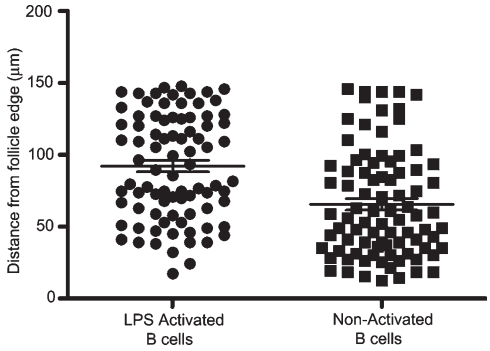


Figure S2. LPS-activated B cells tend to localize in the center of the B cell follicle versus nonactivated B cells. Shown is the distance at which transferred B cells were located from the edge of the LN follicle (center ~150 µm). Results are from the analysis of intravital microscopy of the iLN node 2 d after the transfer of differentially labeled nonactivated and LPS-activated B cells to the recipient mouse. Distances were measured using Imaris. The mean distance of the LPS-activated B cells from the follicle edge was 90.8 µm compared with the nonactivated B cell mean of 63.9 µm. Datasets were compared by the Mann Whitney *U* Test using Prism software ($P < 0.0001$). The horizontal bars indicate mean distance of the cells from the follicle edge. The error bars are vertical bar with cap. Data are from the analysis of three follicles in two mice.

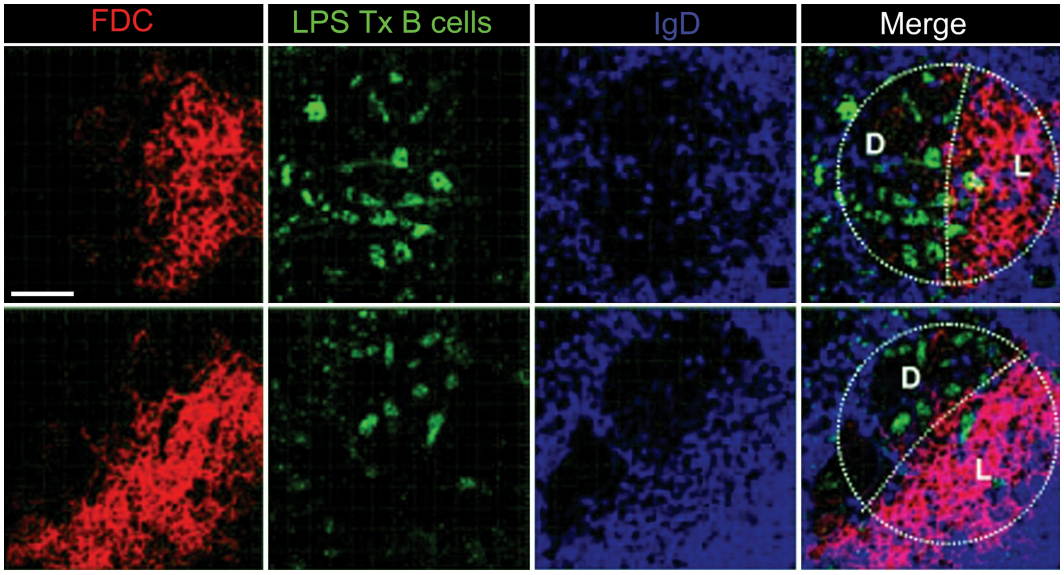


Figure S3. LPS-activated B cells enter the germinal center dark zone. CD45.2 B cells treated with LPS for 24 h in vitro were transferred into CD45.1 mice previously immunized with 100 µg HEL. 20 h later LNs were fixed and 100-µm cryostat sections were prepared. The sections were blocked overnight before immunostaining with an FDC-M2 biotin-labeled monoclonal antibody (1:200), CD45.2 Alexa Fluor 488–conjugated monoclonal antibody (1:100), and sheep anti-mouse IgD, and, finally, with donkey anti-sheep Alexa Fluor 647 and Rhodamine red X–conjugated streptavidin. The slides were examined with a confocal microscope (TCS SP1; Leica) and the imaging data was analyzed using Imaris. Light zones (L) and dark zones (D) are labeled. Two different follicles, each with a germinal center, are shown. The experiment was performed twice with similar results. Bar, 50 µm.

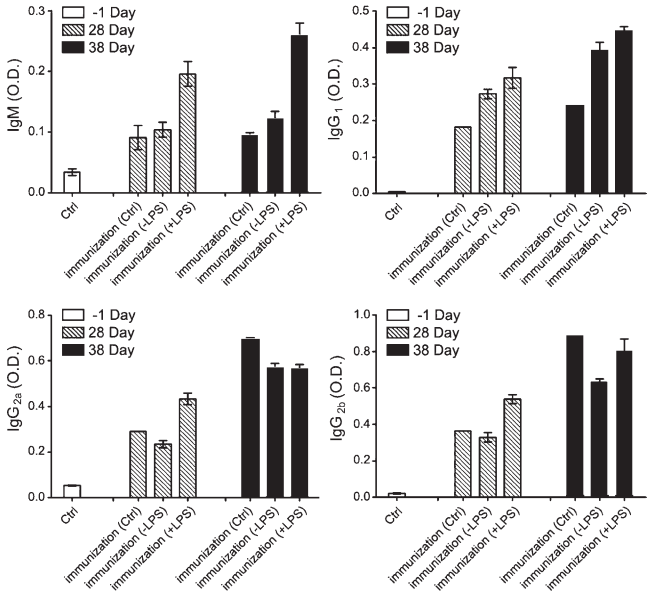


Figure S4. Transfer of TLR4 ligand–exposed B cells enhanced the HEL-specific antibody response. LPS-activated B cells or nonactivated B cells were transferred into mice immunized 1 wk previously with HEL in CFA. Similarly immunized control mice did not receive any cells. At day 28, mice were boosted with HEL in PBS. HEL-specific antibody responses were measured in sera before immunization (−1 Dy), after immunization (28 Dy), and 10 d after antigen boosting (38 Dy). The results are presented as absorbance units at $OD_{405} \pm SD$. Data are from the analysis of four mice in each group.

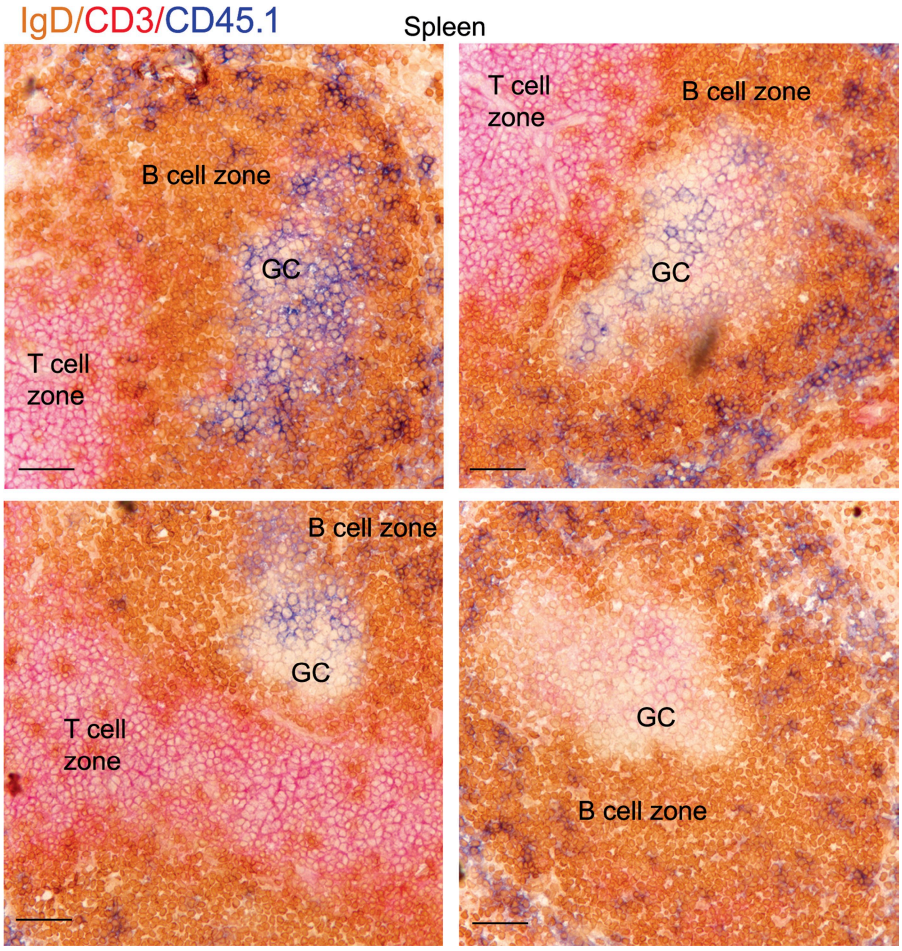
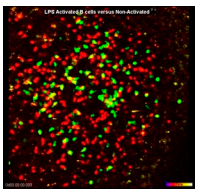
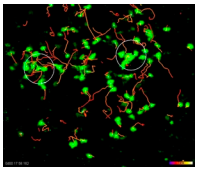


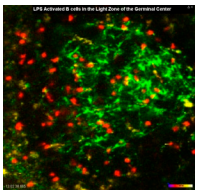
Figure S5. Transferred LPS-activated B cells are present in splenic germinal centers elicited by a secondary immunization. C57BL/6 CD45.2 mice were injected subcutaneously on day 0 with 100 µg HEL in CFA. 15 million LPS-activated B cells from C57BL/6 CD45.1 mice were injected intravenously into recipient mice on day 7. Mice were boosted on day 28 with 100 µg Hel antigen. On day 38 after immunization, the mice were sacrificed and the spleens analyzed by immunohistochemistry to detect CD3 (red), follicular B cells (IgD), and transferred CD45.2 cells (blue). Some germinal centers predominately contained CD45.2 cells, whereas others had only a few or none. Germinal centers (GC), B cell zones, and T cell zones are indicated. The experiment was performed four times with similar results. Bars, 50 µm.



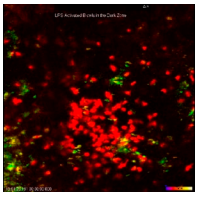
Video 1. LPS versus nonactivated B cells in the LN follicle 48 h after transfer. Splenic B cells stimulated or not with LPS were fluorescently labeled and transferred intravenously to recipient mice. 48 h later, they were imaged in the iLN by intravital microscopy and time-lapse image stacks were acquired. Nonstimulated cells are red and TLR4-stimulated cells are green.



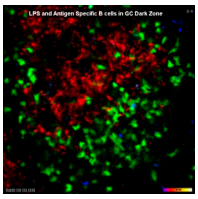
Video 2. Tracking of TLR4-stimulated B cells. Splenic B cells stimulated with LPS were fluorescently labeled and transferred intravenously to recipient mice. 48 h later, the cells were imaged in the iLN by intravital microscopy for 30 min. The imaging data were subjected to spot analysis and tracking. The individual tracks were sorted by displacement length (magnitude of a vector from the initial position to the end position). Tracks of the 40 cells with the greatest displacement during the imaging period are shown. Frame rate is 10 frames/s.



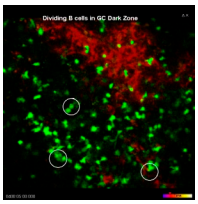
Video 3. TLR4– stimulated B cells in the light zone of a germinal center. Splenic B cells stimulated with LPS were fluorescently labeled (red) and transferred intravenously to recipient mice. Location of the TLR4-stimulated cells defined by FDC-M2–positive cells was revealed by Alexa Fluor 594–labeled FDC-M2 injected 2 h after cell transfer. Approximately 24 h later, the TLR4-stimulated B cells were imaged in the iLN by intravital microscopy. Depth of imaging is between 60 and 90 µm from the capsule. Frame rate is 10 frames/s.



Video 4. TLR4–stimulated B cell in the dark zone of a germinal center. Splenic B cells stimulated with LPS were fluorescently labeled (red) and transferred intravenously to recipient mice. Location of the TLR4-stimulated cells defined by FDC-M2–positive cells was revealed by Alexa Fluor 594–labeled FDC-M2 injected 2 h after cell transfer. Approximately 24 later, the TLR4-stimulated B cells were imaged in the iLN node by intravital microscopy. Depth of imaging is between 120 and 150 µm from the capsule. Frame rate is 10 frames/s.



Video 5. Antigen-specific versus TLR4–stimulated B cells in a germinal center. Equal numbers of LPS-stimulated (green) and HEL-transgenic (blue) B cells were transferred to recipient mice that had been immunized 10 d previously with HEL. FDC-M2 Alexa Fluor 594 (red)–labeled antibody was injected in the region of the iLN node after transfer. 2 d after transfer, the iLNs of the recipient mice were visualized by TP-LSM. Depth of imaging is between ∼120 and 150 µm from the capsule. Frame rate is 10 frames/s.



Video 6. Dividing TLR4–stimulated B cells in the germinal center dark zone. LPS-stimulated B cells were transferred into mice immunized 1 wk previously with HEL. The recipient mouse received 10 µg of conjugated FDC-M2 antibody (red) the day of the transfer. The iLN node of a recipient mouse was imaged by TP-LSM the next day. The dark zone of a germinal center was localized by the position of FDC-M2 staining. Depth of imaging is between ∼120 and 150 µm from the capsule. Frame rate is 5 frames/s.