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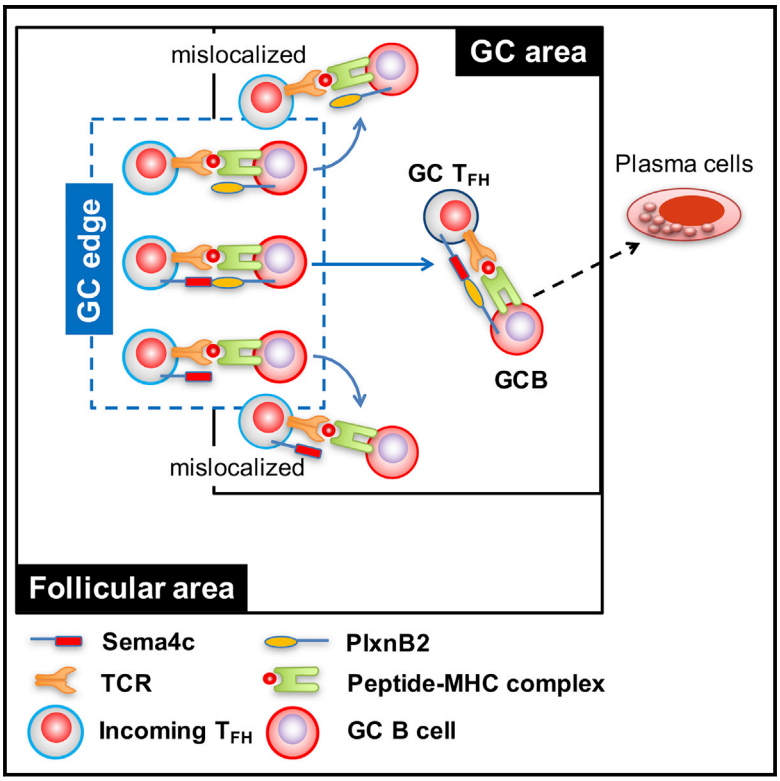
# Plexin B2 and Semaphorin 4C Guide T Cell Recruitment and Function in the Germinal Center

## Graphical Abstract

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## In Brief

Yan et al. identify Plexin B2 and Semaphorin 4C as an antigen-independent, contact-dependent guidance system that promotes GC recruitment of follicular T helper cells, a process crucial for productive antibody responses. When PlxnB2 or Sema4C is absent, follicular T helper cells are mislocalized and plasma cell generation and affinity maturation are defective.

## Highlights

- GC-expressed Plexin B2 is required for normal humoral responses
- Normal GC recruitment of follicular T helper cells requires Plexin B2
- $T_{FH}$ cells use Semaphorin 4C as a guidance receptor to sense GC-expressed Plexin B2
- Semaphorin 4C and Plexin B2 guide by regulating antigen-independent T-B adhesion

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# Plexin B2 and Semaphorin 4C Guide T Cell Recruitment and Function in the Germinal Center

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## SUMMARY

**Follicular T helper ($T_{FH}$) cells orchestrate the germinal center (GC) response locally. $T_{FH}$ localization in GCs is controlled by chemo-guidance cues and antigen-specific adhesion. Here. we define an antigen-independent, contact-dependent, adhesive guidance system for $T_{FH}$ cells. Unusual for amoeboid cell migration, the system is composed of transmembrane plexin B2 (PlxnB2) molecule, which is highly expressed by GC B cells, and its transmembrane binding partner semaphorin 4C (Sema4C), which is upregulated on $T_{FH}$ cells. Sema4C on $T_{FH}$ cells serves as a receptor to sense the GC-presented PlxnB2 cue and biases $T_{FH}$ migration inwards at the GC edge to promote GC access. The absence of PlxnB2 from the GC or Sema4C from $T_{FH}$ cells causes $T_{FH}$ accumulation along the GC border, impairs T-B cell interactions in the GC, and is associated with defective plasma cell production and affinity maturation. Therefore, Sema4C and PlxnB2 regulate GC $T_{FH}$ recruitment and function and optimize antibody responses.**

## INTRODUCTION

The germinal center (GC) is highly organized lymphoid tissue structure that supports the development of high-affinity, long-lived antibody responses (Victora and Nussenzweig, 2012). A GC is composed of densely packed yet highly mobile and interactive T and B lymphocytes enmeshed in two networks of non-hematopoietic stromal cells: the follicular dendritic cells (FDCs) in the light zone (LZ) and the CXCL12-producing reticular cells (CRCs) in the dark zone (DZ) (Bannard et al., 2013; Heesters et al., 2014). The FDCs and CRCs secrete CXCL13 and CXCL12, respectively, and guide interzonal GC B cell migration that facilitates cyclic shuffling of these cells between the site of proliferation and hypermutation in the DZ and the site of competition and selection in the LZ (Allen et al., 2007; Hauser et al., 2007; Schwickert et al., 2007; Victora et al., 2010). Help signals from follicular T helper ($T_{FH}$) cells, delivered either by surface-bound ligands or secreted cytokines, constitute one type of limiting factor that LZ B cells must compete for to continue proliferation in the DZ or to differentiate into plasma cells or memory B cells (Liu et al., 2015; Victora et al., 2010). Therefore, the number of $T_{FH}$ cells recruited and retained in individual GCs must be tightly controlled to maintain an appropriate level of GC competition and output. However, how $T_{FH}$ cells are recruited and retained in GCs is not yet fully understood.

T cells are amoeba-like migratory cells. Amoeboid migration does not require strong cell-cell or cell-matrix adhesion but rather relies on oscillatory deformation of flexible cytoskeleton and pseudopod dynamics of the cell to generate sufficient friction against surrounding tissue elements to crawl forward (Friedl, 2004; Insall, 2010; Lämmermann et al., 2008; Wolf et al., 2003). Consequently, amoeboid cells can migrate rapidly, are capable of reaching a speed of tens of microns per minute, readily move within an ensemble of other highly motile cells, and are almost exclusively guided by soluble chemo-attractive or chemo-repulsive cues that signal through G protein-coupled receptors (GPCRs) (Cyster, 2005; Renkawitz et al., 2009; Sarris and Sixt, 2015). Several GPCRs have been found to contribute collectively to follicular and GC recruitment and retention of $T_{FH}$ cells. CXCR5-mediated chemotaxis toward CXCL13 is important for drawing activated T cells into the follicular area (Breitfeld et al., 2000; Campbell et al., 2001; Förster et al., 1996). Sphingosine-1-phosphate receptor 2 (S1PR2), coupled with $G\alpha_{12/13}$ instead of $G\alpha_i$, activates the Rho small guanosine triphosphatase (GTPase), inhibits cell migration up the sphingosine-1-phosphate (S1P) gradient, and thus promotes confinement of both activated B and T cells toward the follicular center

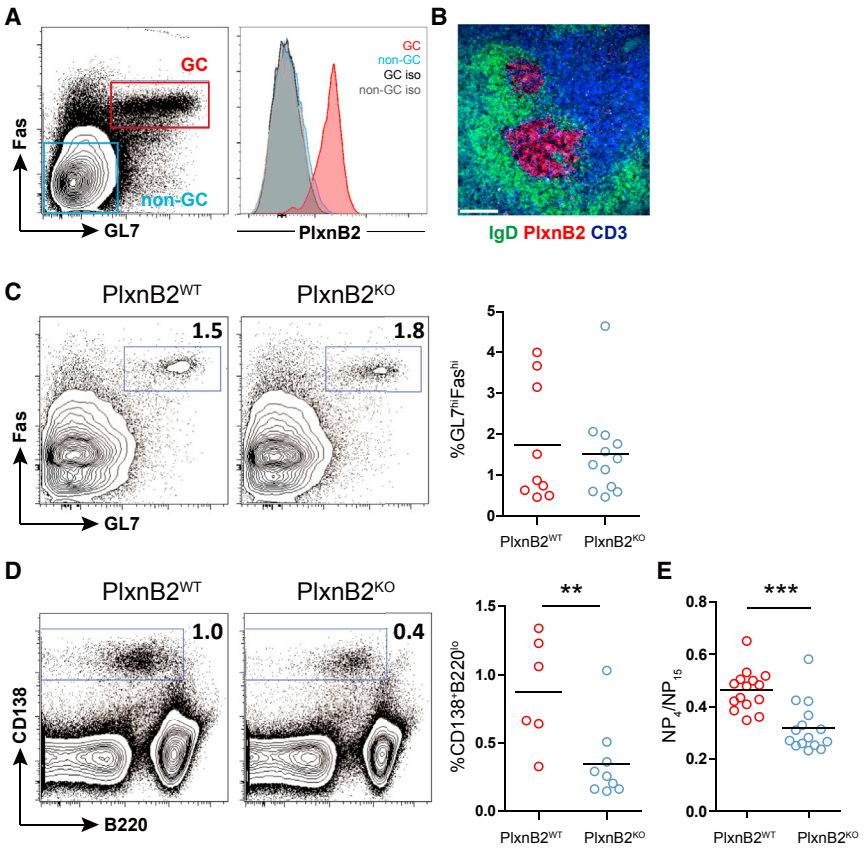


**Figure 1. PlxnB2 Is Highly Expressed by GC B Cells and Required for Normal Plasma Cell Generation and Affinity Maturation**

(A and B) PlxnB2 expression by splenic GC and non-GC B cells (A). Immunofluorescent PlxnB2 staining pattern on the splenic tissue section that were counterstained for immunoglobulin D (IgD) and CD3 (B). Data collected on day 12 post-st-NP-KLH immunization are representative of three independent experiments.

(C–E) Representative FACS profiles (C) of GC B cells (left two panels) and their fractional abundance in total B cells (right) in the spleen of indicated chimeric mice. Representative FACS profiles (D, left two panels) and abundance (right) of SPCs. Relative affinity (E) of NP-specific serum antibodies. Data collected on day 21 post-NP-KLH immunization are pooled from three independent experiments.

$^{**}p < 0.01$, $^{***}p < 0.001$.

boid cells, growth cones migrate in the mesenchymal form, characterized by strict dependence on establishing strong adhesion to immobile tissue matrices in the direction of migration. Consequently, mesenchymal migration is slower than the amoeboid type by orders of magnitude and readily steered by guidance systems that rely on cell-cell contacts. Consistent with this, plexin-mediated repulsive guidance involves inhibition of integrin-mediated adhesion (Kruger et al., 2005).

(Green et al., 2011; Moriyama et al., 2014). Downregulation of GPR183, a GPCR that promotes localization toward the outer edge of the follicle (Gatto et al., 2009; Pereira et al., 2009), also contributes to GC confinement of $T_{FH}$ cells (Suan et al., 2015). Separate from GPCR-mediated guidance, antigen-triggered T cell receptor (TCR) signaling and subsequent T-B cell adhesion play an important role in GC retention of $T_{FH}$ cells. For example, T cells deficient in SAP, an adaptor protein that promotes antigen-specific T-B interactions by protecting proximal TCR signaling from inhibitory SLAM family receptors (Chu et al., 2014; Kageyama et al., 2012), cannot be efficiently recruited to and retained in the GC (Qi et al., 2008). This occurs even though, before GC formation, SAP-deficient T cells form phenotypically normal $T_{FH}$ cells that migrate into the follicle and do not exhibit overt defects in any of the GPCR guidance systems implicated earlier (Lu et al., 2011; Qi et al., 2008).

It is not clear whether true contact-dependent guidance is involved in regulating GC recruitment of $T_{FH}$ cells. GC B cells highly express plexin B2 (PlxnB2), a bona fide guidance receptor (Yu et al., 2008). Single-pass transmembrane receptors of the plexin family bind to secreted or transmembrane semaphorin ligands, steer primordial cell migration during development of many organ systems, and regulate tissue morphogenesis (Kruger et al., 2005; Worzfeld and Offermanns, 2014). In control of axon path-finding during development of the nervous system, plexin receptors often steer growth cones away from fields rich in corresponding semaphorin ligands (Huber et al., 2003). Unlike amoe-

Given that the border between the follicular mantle and the mature GCs is well demarcated by PlxnB2 expression on the GC side, resembling separation of more permanent tissue domains, we were curious about the physiological significance of GC-restricted PlxnB2 expression and whether it regulates cellular trafficking of B and/or T cells in and around the GC. Our findings demonstrate that PlxnB2 is a cell-bound guidance cue to direct $T_{FH}$ cells arriving at the GC border to penetrate the GC territory in an adhesion-dependent manner and semaphorin 4C (Sema4C) is a $T_{FH}$-specific guidance receptor that detects the follicle-GC border demarcated by the PLXNB2 cue and effectuates steering. Our results further reveal that the PlxnB2-Sema4C system is necessary for optimal affinity maturation and plasma cell formation.

## RESULTS

### PlxnB2 Is Uniquely Expressed by GC B Cells but Non-essential for GC Formation

To better understand potential functions of PlxnB2 in regulating the GC response, we first confirmed the expression pattern of PlxnB2 in mice immunized with protein antigen in adjuvant. Similar to what was previously reported (Yu et al., 2008), a high level of PlxnB2 was expressed only by GC B cells, but not by resting non-GC B cells (Figure 1A). By histology, PlxnB2 staining

distinctively highlighted the GC and demarcated its border with the follicular mantle (Figure 1B). We went on to investigate whether and how PlxnB2 may regulate GC formation and/or GC reaction. Germline PlxnB2 deletion on the inbred B6 background causes defective neural tube closure and perinatal lethality (Deng et al., 2007; Friedel et al., 2007), but occasional live births took place and gave rise to animals that matured to grossly normal adulthood. We constructed radiation chimera using B6 recipients and donor *Plxnb2*$^{+/+}$ or *Plxnb2*$^{-/-}$ fetal liver cells (and occasionally bone-marrow cells from adult mice), with the chimera termed PlxnB2$^{WT}$ or PlxnB2$^{KO}$ mice, respectively. PlxnB2$^{WT}$ and PlxnB2$^{KO}$ mice developed GC responses of a similar magnitude, as measured either on day 14 (data not shown) or on day 21 following 4-hydroxy-3-nitrophenylacetyl-conjugated keyhole limpet haemocyanin (NP-KLH) immunization (Figure 1C). Consistent with PlxnB2 being comparably expressed in the LZ and the DZ, the DZ/LZ ratio was not skewed in the absence of PlxnB2 (data not shown). When PlxnB2$^{WT}$ and PlxnB2$^{KO}$ hen egg lysozyme (HEL)-specific SW$_{HEL}$ B cells (Phan et al., 2003) were tested in adoptive hosts immunized with HEL-conjugated sheep red blood cells (SRBCs), the mutant GC responses peaked at a similar level and around the same time but did not persist as long as the response of the wild-type (WT) (Figure S1). These data suggest that PlxnB2 is not required for GC formation per se but may play a role in regulating the ongoing GC reaction.

## PlxnB2 Is Required for Optimal Production of Plasma Cells and Antibody Affinity Maturation in a B Cell-Intrinsic Manner

To examine a potential role for PlxnB2 in regulating the GC reaction and output, we enumerated splenic plasma cells (SPCs) in PlxnB2$^{WT}$ and PlxnB2$^{KO}$ mice at day 21 post-immunization. As shown in Figure 1D, the frequency of CD138$^{hi}$B220$^{lo}$ plasma cells was reduced by ~50% in the absence of PlxnB2 ($p < 0.01$). In correlation, affinities of 4-hydroxy-3-nitrophenylacetyl (NP)-specific antibodies in the serum were significantly reduced in PlxnB2$^{KO}$ mice (Figure 1E). These data suggest that PlxnB2 may regulate processes that underlie the production of affinity-matured plasma cells. To determine whether the requirement for PlxnB2 is intrinsic to the B cell compartment, we constructed 50:50 mixed bone-marrow chimera using congenically marked WT and PlxnB2-deficient donors and compared competitive competencies of PlxnB2$^{WT}$ and PlxnB2$^{KO}$ CD45.2 cells in the two sets of chimera, as depicted in Figure 2A. Three weeks after NP-KLH immunization, PlxnB2$^{KO}$ cells comparably contributed to the GC compartment (Figure 2B), whereas their contributions to the splenic and bone marrow (BM) plasma cell compartments were significantly reduced (Figures 2C and 2D). Therefore, while PlxnB2 expression on B cells is not required for GC formation, it is essential for normal plasma cell formation and antibody affinity maturation.

## PlxnB2 Signaling-Independent Promotion of Plasma Cell Formation In Vivo

Plexin receptors function partly by signaling through intracellular C1/C2 and/or PDZ-binding domains (Tran et al., 2007). To test whether PlxnB2 directly signals to B cells to promote plasma cell differentiation, we transferred into B6 mice MD4 B cells, which recognize HEL, and OT-II T cells, which recognize the ovalbumin (OVA)-derived OVA$_{323–339}$ peptide in complex with the I-A$^{b}$ major histocompatibility complex (MHC) class II molecule, and then induced the GC response by immunization with HEL-OVA conjugate antigen. This model system produces an accelerated GC response and permits genetic manipulation of the GC-seeding B cells by retroviral infection (Pereira et al., 2009). MD4 B cells were transduced to express the full length or a truncation mutant lacking the cytoplasmic domain (PlxnB2-TT) (Figure 2E). As shown in Figure 2F, when MD4 B cells expressed an increased level of PlxnB2, they gave rise to significantly more plasma cells, consistent with the positive effect of PlxnB2 on plasma cell generation. Unexpectedly, however, the promoting effect of PlxnB2 was unchanged even when its entire cytoplasmic domain was truncated. These data suggest that direct signaling into B cells is not necessary for PlxnB2-mediated promotion of plasma cell formation, while it nonetheless operates in a B cell-intrinsic manner.

## PlxnB2 Is Required for Proper T$_{FH}$ Recruitment into the GC

The intrinsic requirement for PlxnB2 may arise from the possibility that PlxnB2 regulates how GC B cells physically access T cell help, because GC T$_{FH}$ cells play a key role in regulating B cell selection and GC output of plasma cells by providing soluble and membrane-bound helper signals such as interleukin-21 and CD40 ligand (Allen et al., 2007; Liu et al., 2015; Shulman et al., 2014; Victora et al., 2010). When PlxnB2$^{WT}$ and PlxnB2$^{KO}$ MD4 cells were tested in an OT-II co-transfer system, comparable magnitudes of the MD4 GC response and CXCR5$^{+}$PD-1$^{+}$ OT-II frequencies were observed (Figure 3A). However, PlxnB2$^{KO}$ MD4 GCs appeared to be not as densely populated by OT-II cells, particularly in the central regions (top two panels, Figure 3B). Double-blinded quantitative analyses revealed that although overall OT-II T$_{FH}$ densities in PlxnB2$^{WT}$ and PlxnB2$^{KO}$ GCs and respective encompassing mantle areas were comparable (Figure 3C), their distribution was different between the two types of GCs, with OT-II T$_{FH}$ cells concentrated along the edge of PlxnB2$^{KO}$ GCs (bottom two panels, Figure 3B). Quantitatively, approximately 60% of GC-associated OT-II T$_{FH}$ cells were at the PlxnB2$^{KO}$ GC border, as defined by a zone extending 10 μm to each side of the GC edge, whereas only 40% were at the PlxnB2$^{WT}$ GC border ($p < 0.001$) (Figure 3D). Therefore, PlxnB2 regulates proper positioning of GC T$_{FH}$ cells. Because T$_{FH}$ cells constantly migrate and exchange between the follicle and the GC territory (Moriyama et al., 2014; Qi et al., 2008; Shulman et al., 2013; Suan et al., 2015), one possibility is that when being recruited into the PlxnB2$^{KO}$ GCs, T$_{FH}$ cells reach the GC border but cannot efficiently migrate deeper into the GC proper. Alternatively, T$_{FH}$ cells may not leave the PlxnB2$^{KO}$ GC efficiently and are held at the border for prolonged period. To distinguish these possibilities, we intravitally imaged T$_{FH}$ migratory dynamics around PlxnB2$^{WT}$ and PlxnB2$^{KO}$ GCs, and we tracked individual incoming T cells that reach the GC border from the follicular mantle or outgoing T cells that reach the border from within the GC. In both types of GCs, T cells migrated with comparable speeds (data not shown). However, as exemplified in

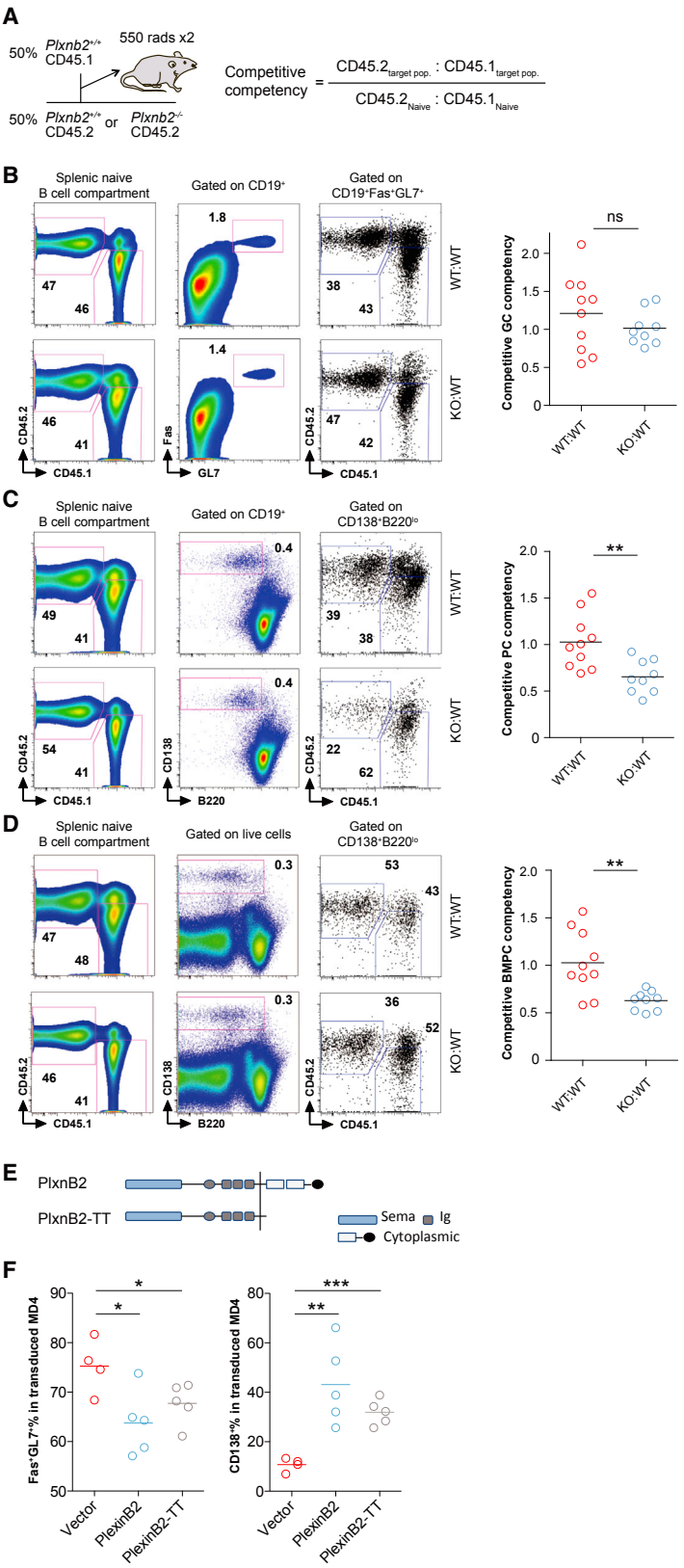


**Figure 2. PlxnB2 Promotes Plasma Cell Generation in a B Cell-Intrinsic Manner Independent of Its Cytoplasmic Domain**

(A) Schematic diagram of the protocol to make 50:50 BM chimera using CD45.1 WT BM cells mixed with CD45.2 *Plxnb2*$^{-/-}$ or *Plxnb2*$^{+/+}$ fetal liver cells (left), and the equation to quantitate competitive competency (right).

(B–D) Pseudo-color and dot plots show gating strategies and scatterplots to the right show CD45.2 competitive competency values in individual chimeric mice for the splenic Fas$^{+}$GL7$^{+}$ GC compartment (B), CD138$^{+}$B220$^{lo}$ SPC compartment (C), and bone marrow plasma cells (BMPC) compartment (D) 21 days post-NP-KLH immunization; each symbol represents one mouse, and data are pooled from three experiments.

(E) Diagrams of the full-length and truncated PlxnB2 used for retroviral transduction of MD4 cells.

(F) Percentages of Fas$^{+}$GL7$^{+}$ (left) and CD138$^{+}$ cells (right) in GFP$^{+}$ transduced MD4 cells 5 days following HEL-OVA immunization of their adoptive hosts that also received OT-II cells as the helper T cells; each symbol represents one mouse, and one of three experiments with similar results is shown.

*p < 0.05, **p < 0.01, ***p < 0.001; NS, not significant.

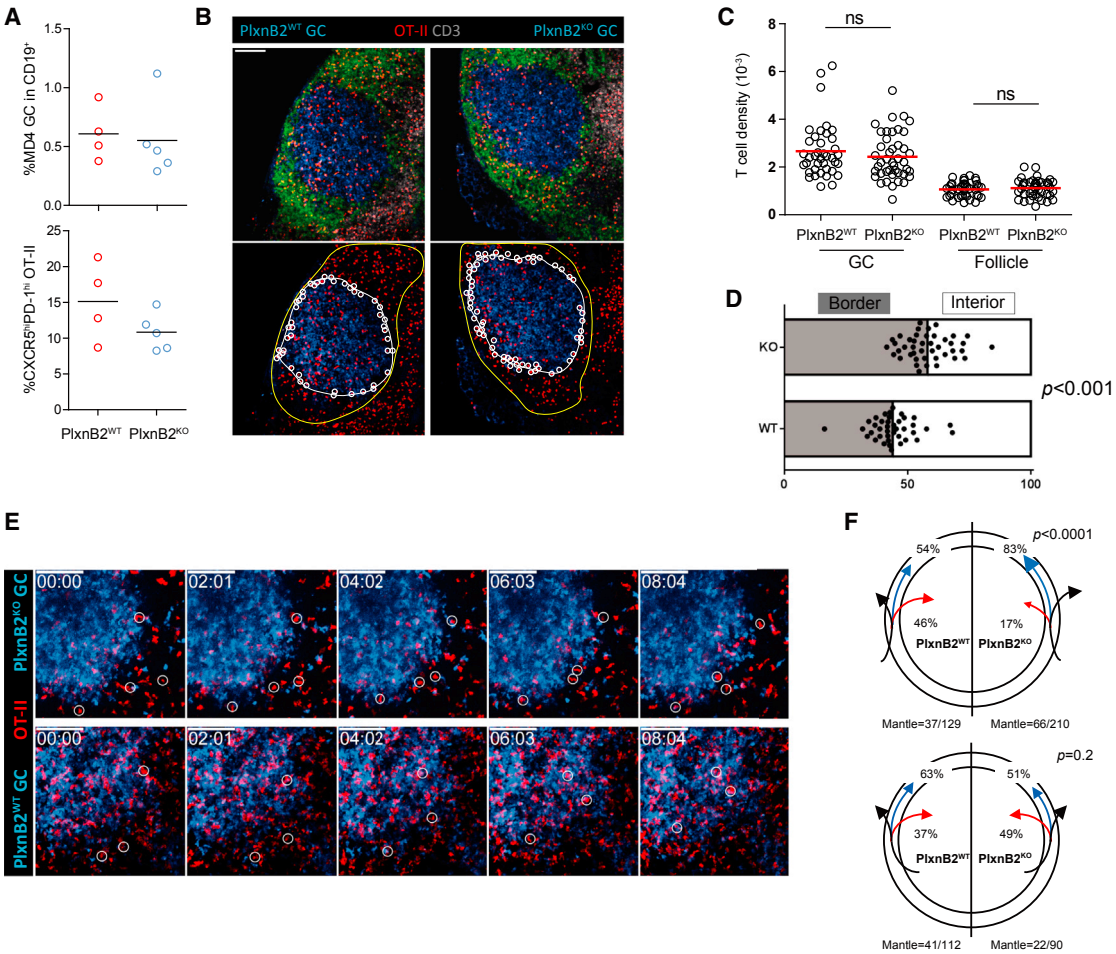


**Figure 3. PlxnB2 Is Required for Proper GC Recruitment of $T_{FH}$ Cells**

(A) MD4 GC percentage and OT-II $T_{FH}$ percentage 5 days after HEL-OVA immunization of B6 mice receiving CFP-expressing PlxnB2$^{KO}$ or PlxnB2$^{WT}$ MD4 B cells, together with dsRed-expressing OT-II T cells. Each symbol represents one mouse, and results represent three independent experiments.

(B) Typical $T_{FH}$ distribution patterns in and around PlxnB2$^{KO}$ or PlxnB2$^{WT}$ MD4 GCs. Yellow lines mark follicular and GC edges, and white circles highlight $T_{FH}$ cells within 10 μm on each side of the GC edge (border zone). Scale bar, 100 μm.

(C) Area-normalized $T_{FH}$ densities.

(D) GC $T_{FH}$ distribution between the border buffer zone and the interior. Data are pooled from three independent experiments, each involving three recipients per group.

(E) Still images from intravital imaging of OT-II $T_{FH}$ dynamics in PlxnB2$^{KO}$ or PlxnB2$^{WT}$ MD4 GCs. White circles highlighted four incoming $T_{FH}$ cells that reached the PlxnB2$^{KO}$ GC border but failed to enter (top row) and three $T_{FH}$ cells that entered the PlxnB2$^{WT}$ GC (bottom row). See also corresponding Movies S1 and S2. Scale bar, 50 μm.

(F) Incoming (top) and outgoing (bottom) OT-II migratory tracks classified as indicated. Data are pooled from three independent experiments; p values by $\chi^2$ tests. NS, not significant.

Figure 3E and Movies S1 and S2, while OT-II T cells that reached the PlxnB2$^{WT}$ GC border efficiently entered deeper into the GC proper, they could not do so as efficiently when reaching the PlxnB2$^{KO}$ GCs. Quantitatively, we defined a virtual buffer zone encompassing 10 μm on either side of the GC border, and we classified $T_{FH}$ migratory tracks according to how they behaved after arriving at the buffer zone. As shown in Figure 3F, ~70% of all OT-II $T_{FH}$ cells arriving at the buffer zone from the mantle area did not visibly leave either type of GC (PlxnB2$^{WT}$, 92/129, versus PlxnB2$^{KO}$, 144/210). Out of all these cells, ~46% were seen to move beyond the border and enter deep into the PlxnB2$^{WT}$ GC proper, whereas only ~17% were able to do so in the case of PlxnB2$^{KO}$ GCs ($p < 0.0001$ by $\chi^2$ test) (Figure 3F). In contrast, outgoing $T_{FH}$ cells that arrived at the border zone from within PlxnB2$^{WT}$ or PlxnB2$^{KO}$ GCs escaped from the GC with comparable efficiencies (PlxnB2$^{WT}$, 41/112, versus PlxnB2$^{KO}$, 22/90; $p = 0.2$ by $\chi^2$ test) (Figure 3F); for those that did not escape, comparable fractions wandered in the buffer zone or returned to the GC proper. Therefore, reduced GC penetration by incoming $T_{FH}$ cells is the reason for the concentrated distribution along the GC edge when PlxnB2 is absent. This reduced GC penetration was not accompanied by enhanced

escape of outgoing $T_{FH}$ cells, which implies a defect in directional guidance that $T_{FH}$ cells receive at the border of the PlxnB2$^{KO}$ GC.

### PlxnB2-Mediated Adhesive Guidance for $T_{FH}$ Cells

Chemokines CXCL13 and CXCL12 and lipid guidance molecules S1P and oxysterols regulate follicular migration of both B cells and $T_{FH}$ cells, influencing their distribution between the outer follicle and the follicular center (Cyster, 2005; Cyster et al., 2014; Cyster and Schwab, 2012). However, we did not detect differences in expression of *Cxcr5*, *Cxcr4*, or *Ccr7* by OT-II $T_{FH}$ cells that developed along with PlxnB2$^{WT}$ or PlxnB2$^{KO}$ GCs, and no difference in *Gpr183* and *S1pr2* mRNA expression was observed (data not shown). We also looked for signs of altered expression of other chemokines by RNA sequencing (RNA-seq) and found that PlxnB2$^{KO}$ and PlxnB2$^{WT}$ GCs exhibited similar transcriptome profiles, showing no differential expression of known guidance molecules (Figure S2). It is unlikely that a PlxnB2-dependent chemokine or chemokines produced by GC B cells are responsible for pulling $T_{FH}$ cells into the GC from its border. We thus considered the possibility that PlxnB2 directly guides $T_{FH}$ cells at the GC border.

Acute steering of lymphocytes, among the fastest-moving amoeboid cells, is mostly effectuated by soluble guidance cues in lymphoid organs, whereas plexin molecules regulate migration of primordial cells in developing nervous and cardiovascular systems as contact-dependent guidance receptors that transmit repulsive or attractive semaphorin signals (Kruger et al., 2005; Tran et al., 2007). In the immune system, the plexin A1 (PlxnA1) receptor has been found to promote dendritic cells to transmigrate across the lymphatic endothelium in response to semaphorin 3A (Sema3A) secreted by the latter cell type (Takamatsu et al., 2010). During epidermal wound healing, PlxnB2 is upregulated on keratinocytes and promotes a rounding morphological change in semaphorin 4D (Sema4D)-expressing epidermal $\gamma\delta$ T cells, a response that takes hours to develop (Witherden et al., 2012). None of these scenarios would readily explain how PlxnB2 could directly guide $T_{FH}$ recruitment into the GC. However, although amoeboid cell motility per se does not require integrin-mediated adhesion but can rely on friction forces generated by pseudopod dynamics and shape changes (Friedl, 2004; Insall, 2010; Lämmermann et al., 2008), local adhesion to other cells or the tissue matrix could in principle bias cellular protrusions and thereby steer cell migration directionally (Rørth, 2011). PlxnB2 could promote $T_{FH}$ adhesion to GC B cells.

GC B cells are antigen specific and present cognate peptide-MHC complexes to $T_{FH}$ cells. However, unlike the situation at the T-B border, where antigen-specific T-B interactions can be readily distinguished from non-specific ones because they are of longer duration (Okada et al., 2005; Qi et al., 2008), antigen-specific T-B contacts inside GCs are predominantly short lived (Allen et al., 2007), and the strength of adhesive interactions is mainly reflected in the area of contact or the entangled morphology (Liu et al., 2015; Shulman et al., 2014). To probe whether PlxnB2 promotes GC B cell-$T_{FH}$ cell adhesion, we first imaged dsRed-expressing OT-II cells interacting with GFP-expressing PlxnB2$^{WT}$ (5%) and CFP-expressing PlxnB2$^{KO}$ MD4 B cells (5%) mixed in the same GC, together with 90% non-fluorescent MD4 B cells. This allowed us to analyze individual T-B contacts in the GC. As exemplified in Figure 4A and Movie S3, contacts between OT-II $T_{FH}$ cells and PlxnB2$^{KO}$ GC B cells overall exhibited a less entangled morphology with a smaller area of contact on average. By the previously established parameter of surface engagement index (SEI) for measuring the contact extensiveness (Liu et al., 2015), $T_{FH}$ cells showed an average SEI of 0.28 ± 0.05 with PlxnB2$^{KO}$ MD4 cells, significantly reduced from the SEI of 0.34 ± 0.05 with PlxnB2$^{WT}$ counterparts ($p < 0.0001$) (Figure 4B). Additional experiments comparing OT-II interactions with GFP-expressing and CFP-expressing PlxnB2$^{WT}$ MD4 cells revealed no difference (data not shown), confirming what has previously been reported (Liu et al., 2015). These results suggest that PlxnB2 can promote $T_{FH}$ adhesion to B cells in the GC.

Next, we examined whether such an adhesion-enhancing effect of PlxnB2 at the level of individual cell-cell contacts is translated into adhesive guidance by the GC ensemble for arriving $T_{FH}$ cells, as suggested by tracking results in Figure 3. We transduced OT-II T cells with GFP-tagged utrophin, an F-actin-binding protein, to visualize the T cell uropod and axis of cell polarization and analyzed directionalities of incoming $T_{FH}$ cells at a higher magnification. As exemplified in Figure 4C and Movie S4, upon arriving at a PlxnB2$^{WT}$ GC, incoming $T_{FH}$ cells tended to exhibit a sudden increase in the area of contact with B cells at the GC border within the first 2 min or so, align their long axis of cell polarization perpendicular to the GC edge, and rapidly invade the GC toward the center. In contrast, upon arriving at a PlxnB2$^{KO}$ GC, $T_{FH}$ cells frequently failed to exhibit the increase in adhesive contact at the GC edge (Figure 4C and Movie S5). Quantitatively, more $T_{FH}$ cells maintained a positive angle of invasion (AI, defined in Figure 4D) for a longer time after reaching the PlxnB2$^{WT}$ than the PlxnB2$^{KO}$ GC edge (Figure 4E). The average AI into the PlxnB2$^{WT}$ GC was above 30 degrees for the first 2 min, whereas it decayed to zero in the same period into the PlxnB2$^{KO}$ GC (Figure 4F). These data suggest that by promoting $T_{FH}$ adhesion to GC B cells at the GC edge, PlxnB2 biases the direction of $T_{FH}$ cell migration toward the GC center.

### Sema4C Is Specifically Expressed by $T_{FH}$ Cells and Is Required for Optimal Plasma Cell Generation

Next, we sought to identify the counter-receptor that $T_{FH}$ cells use to sense PlxnB2 in the GC. In the developing nervous and cardiovascular systems, plexins serve as guidance receptors for semaphorin ligands, and class IV semaphorins are ligands for PlxnB receptors (Tran et al., 2007). Sema4D is highly expressed by CD4$^+$ T cells and B cells and promote T cell activation and antibody responses, although it mediates the latter effect by engaging the CD72 receptor (Kumanogoh et al., 2000, 2002; Shi et al., 2000). Epidermal $\gamma\delta$ T cells express a very high level of Sema4D, which can interact with PlxnB2 expressed by keratinocytes to induce morphological changes in $\gamma\delta$ T cells after wounding (Witherden et al., 2012). To test whether Sema4D is the receptor for PlxnB2 in the context of GC recruitment of $T_{FH}$ cells, we generated *Sema4d*$^{-/-}$ mice using the CRISPR/Cas9 technology (Figures S3A and S3B). Distribution of *Sema4d*$^{-/-}$ OT-II $T_{FH}$ cells was then compared to their WT counterparts around WT GCs using the protocol established in Figure 3B. As shown in Figure S3C, no appreciable difference was observed between

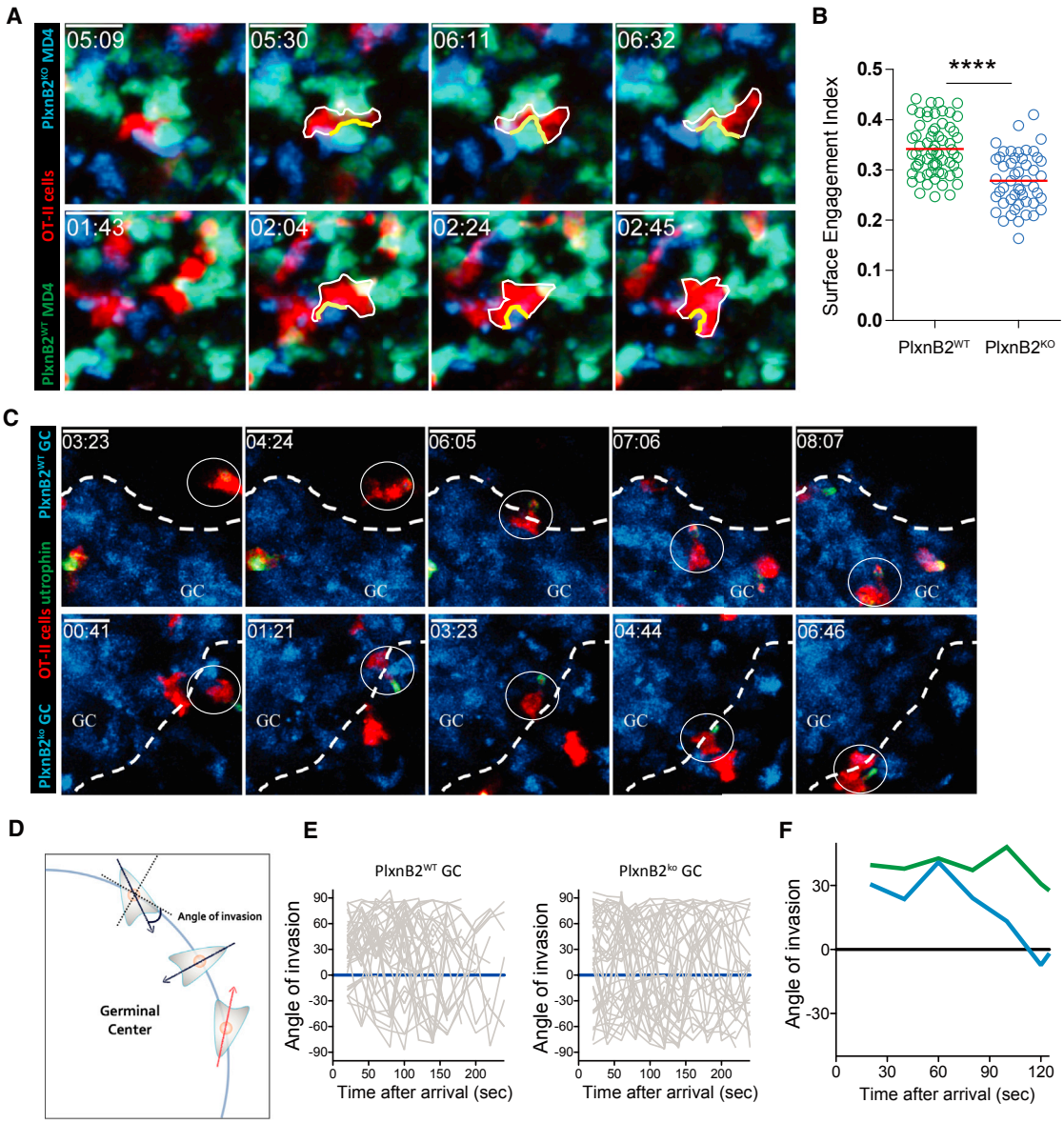


**Figure 4. PlxnB2 Biases $T_{FH}$ Migration at the GC Edge toward the Center**

(A) Examples of $T_{FH}$-GC B cell interactions visualized in a draining lymph node. White outlines mark $T_{FH}$ cells in contact with B cells, and the contact regions are highlighted with yellow lines. Scale bar, 20 μm.

(B) SEI for OT-II $T_{FH}$ cells interacting with PlxnB2$^{WT}$ or PlxnB2$^{KO}$ GC B cells. Data are pooled from three independent experiments, in each of which multiple GCs were imaged.

(C) Migratory dynamics of single incoming $T_{FH}$ cells at the edge of PlxnB2$^{WT}$ (top row) or PlxnB2$^{KO}$ (bottom row) GCs. See also corresponding Movies S4 and S5. Dashed lines demarcate the GC edge, and circles highlight incoming $T_{FH}$ cells.

(D) The method to quantitate angles of invasion for incoming $T_{FH}$ cells.

(E) Angles of invasion by individual $T_{FH}$ cells arriving at the edge (time 0) of indicated GC types, plotted over time.

(F) Average angles of invasion. Data are pooled from three independent experiments.

****$p < 0.0001$.

the two types of $T_{FH}$ cells. Thus, a T cell deficiency in Sema4D does not recapitulate the PlxnB2 deficiency on B cells, suggesting that a different receptor for PlxnB2 operates on $T_{FH}$ cells.

Among class B plexins, Sema4D interacts with plexin B1 (PlxnB1) with a high affinity (Tamagnone et al., 1999) but does not bind to PlxnB2, as measured in a cell-based assay (Deng et al., 2007). However, Sema4C binds to PlxnB2 with a high affinity, exhibiting a dissociation constant in the nanomolar range (Deng et al., 2007; Friedel et al., 2007; Perälä et al., 2011). Sema4C was found to be upregulated by CD4 T cells after activation in vitro (data not shown) and preferentially expressed by CD44$^{hi}$CXCR5$^{+}$PD-1$^{+}$ $T_{FH}$ cells in vivo (Figures 5A and 5B). In

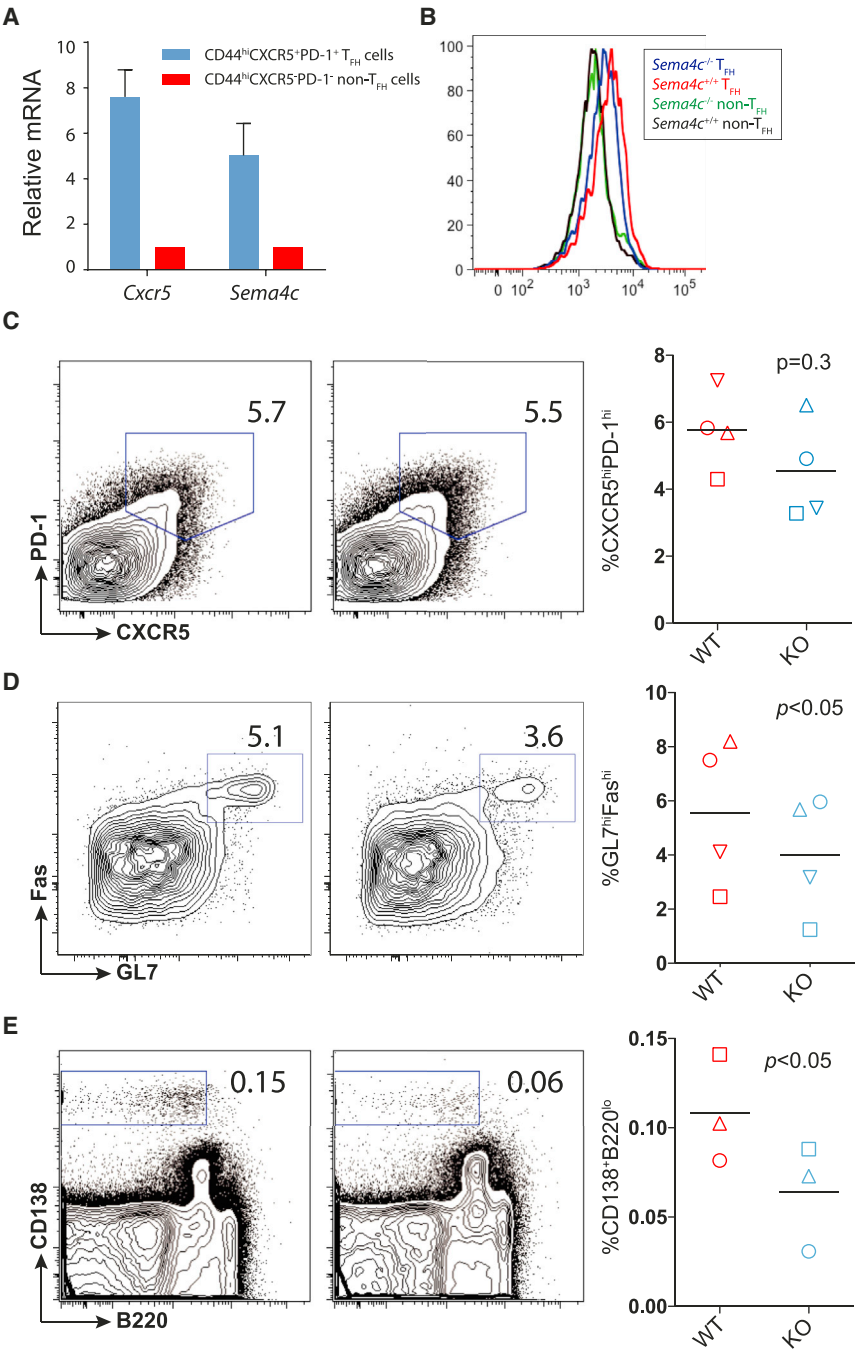


**Figure 5. Sema4C Is Expressed by $T_{FH}$ Cells and Required for Optimal Plasma Cell Generation**

(A and B) Relative Sema4C and CXCR5 expression (A) by $T_{FH}$ ($CD4^+CD44^+CXCR5^+PD\text{-}1^+$) and non-$T_{FH}$ ($CD4^+CD44^+CXCR5^-PD\text{-}1^-$) cells, with levels in non-$T_{FH}$ cells set to unit 1. Histograms of surface Sema4C staining (B) on $T_{FH}$ and non-$T_{FH}$ cells from *Sema4c*$^{+/+}$ and *Sema4c*$^{-/-}$ mice. Data represent three independent experiments with similar results. (C–E) Typical FACS profiles (left two panels) and frequencies (right) of $T_{FH}$ cells (C), GC B cells (D), and BMPCs (E) in *Sema4c*$^{+/+}$ and *Sema4c*$^{-/-}$ mice 1 week after SRBC immunization. Each shape represents one matched, independent experiment that involved at least three mice per group.
NS, not significant.

the nervous system, the Sema4C deficiency largely recapitulates the PlxnB2 null phenotype, causing similar defects in brain development. When occasional live births of either *Sema4c*$^{-/-}$ or *Plxnb2*$^{-/-}$ mice survive to fertile adulthood, they exhibit ventral pigmentation defects (Maier et al., 2011). Following immunization, comparable numbers of $CXCR5^+PD\text{-}1^+$ $T_{FH}$ cells were found in both Sema4C-deficient and WT mice (Figure 5C), whereas a significant reduction in GCs and plasma cells was observed in Sema4C-deficient mice (Figures 5D and 5E), reminiscent of findings with PlxnB2$^{KO}$ mice. The reduced magnitude of polyclonal GC responses to either NP-KLH or SRBC is seen with the Sema4C-deficient mice, but not the PlxnB2$^{KO}$ mice (Figure 1; data not shown), potentially reflecting that Sema4C is also expressed by B cells and may directly regulate their functions {Xue, 2017 #126;Xue, 2016 #125}.

## Sema4C Is Required for $T_{FH}$ Cells to Migrate Deep into the GC Proper

To test the role for Sema4C in $T_{FH}$ guidance directly, we compared positioning patterns of *Sema4c*$^{-/-}$ and *Sema4c*$^{+/+}$ OT-II $T_{FH}$ cells in the same WT MD4 GCs. As exemplified in Figure 6A and quantitated in Figure 6B, GC-associated *Sema4c*$^{-/-}$ OT-II $T_{FH}$ cells were concentrated toward the edge, with approximately 70% localized in the 20-μm border region, whereas only 45%–50% *Sema4c*$^{+/+}$ OT-II $T_{FH}$ cells were localized there ($p < 0.001$). Therefore, Sema4C-deficient $T_{FH}$ cells exhibit a mis-localization around normal GCs similar to that of $WTT_{FH}$ cells around PlxnB2$^{KO}$ GCs (Figures 3B and 3D). We further analyzed migratory dynamics of *Sema4c*$^{-/-}$ and *Sema4c*$^{+/+}$ OT-II $T_{FH}$ cells around the same GC border by intravital imaging. Although GFP-expressing *Sema4c*$^{+/+}$ and dsRed-expressing *Sema4c*$^{+/+}$ OT-II $T_{FH}$ cells were comparable in penetrating the GC territory (Figure 6C; Movie S6; data not shown), GFP-expressing *Sema4c*$^{-/-}$ $T_{FH}$ cells did not migrate as efficiently beyond the border and as deep into the GC proper as their dsRed-expressing WT counterparts (Figures 6C and 6D; Movie S7). Consistent with the observation with PlxnB2$^{KO}$ GCs (Figure 3), Sema4C mainly affects incoming $T_{FH}$ cells by directing their GC penetration from the edge (Figure 6D). Therefore, Sema4C likely serves as a guidance receptor for $T_{FH}$ cells to

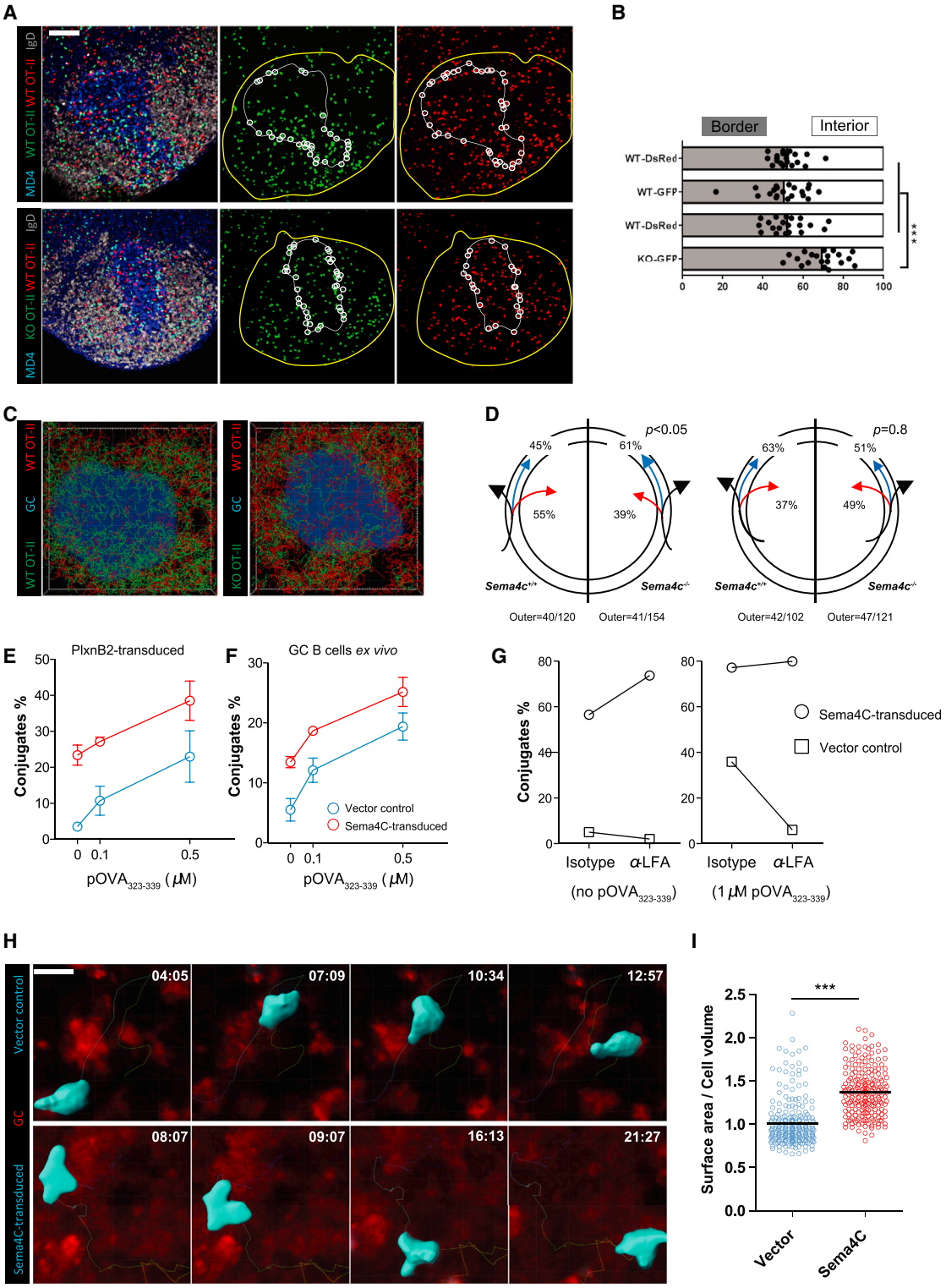


**Figure 6. Sema4C Is Required for Proper GC Recruitment of $T_{FH}$ Cells**

(A) Typical distribution patterns of *Sema4c*$^{+/+}$ (WT) or *Sema4c*$^{-/-}$ (knockout, KO) OT-II $T_{FH}$ cells in follicles containing WT MD4 GCs 5 days after HEL-OVA immunization. Yellow lines label the follicular edge, white lines label the GC edge, and white circles highlight $T_{FH}$ cells within 10 μm on each side of the GC edge. Scale bar, 100 μm.

(B) GC $T_{FH}$ distribution between the border zone and the interior. Data are pooled from two independent experiments, each involving three recipients per group.

*(legend continued on next page)*

sense PlxnB2-marked GCs. Because the strength of the migratory phenotype due to the Sema4C deficiency on $T_{FH}$ cells appeared somewhat weaker than that induced by the PlxnB2 deficiency on GC B cells, another class IV semaphorin might also be involved in the guidance regulation.

### Sema4C and PlxnB2 Promote Adhesion to B Cells and Protrusion Dynamics

To serve as an adhesive guidance receptor for GC recruitment, Sema4C should be able to mediate adhesion between T cells and GC B cells without requiring antigen and integrins. To test this, we used the fluorescence-activated cell sorting (FACS)-based cell conjugation assay (Chu et al., 2014). As shown in Figure 6E, when activated OT-II T cells were transduced with a Sema4C-expressing construct, they exhibited a strikingly increased adhesiveness toward activated B cells that were made to express PlxnB2. Sema4C-overexpressing T cells also strongly adhered to freshly isolated GC B cells, which inherently expressed a high level of PlxnB2 (Figure 6F). Sema4C and PlxnB2 promoted T-B adhesion in a manner that did not require concomitant antigen recognition, did not change the shape of the antigen dose-response curve (Figures 6E and 6F), and was not blocked by the anti-LFA-1 treatment that was sufficient to abrogate antigen-specific T-B adhesion (Figure 6G). Inside GCs, Sema4C overexpression led migratory T cells to display more extensive membrane protrusions (Figure 6H; Movies S8 and S9), leading to significantly increased surface-to-volume ratios (Figure 6I). Altogether, these data strongly indicate that Sema4C is a guidance receptor for $T_{FH}$ cells to sense the GC-marking PlxnB2 cue and that by providing adhesive guidance at the GC border, the Sema4C-PlxnB2 pair promotes arriving $T_{FH}$ cells to efficiently penetrate the GC territory.

## DISCUSSION

Cellular dynamics are crucial for how GC reactions are orchestrated to support affinity maturation and output of plasma and memory cells. In this study, we have identified the Sema4C-PlxnB2 pair as a guidance receptor-ligand system that specifically promotes GC recruitment of $T_{FH}$ cells. It is unconventional that Sema4C can serve as the guidance receptor and PlxnB2 can serve as the directional cue. Although semaphorins and plexins have been known to regulate migration-based processes in embryonic development, semaphorins predominantly act as repulsive ligands signaling through plexin receptors to orchestrate axon targeting, fasciculation, and neuronal cell-body positioning (Tran et al., 2007). In the immune system, class IV semaphorins have also been found to mediate repulsion. For example, Sema4D is abundantly expressed by all T cells (Kumanogoh and Kikutani, 2013) and, in its soluble form, can inhibit migration of immature dendritic cells (DCs) that express PlxnB1 (Chabbert-de Ponnat et al., 2005). However, in epidermal wounding healing, Sema4D on $\gamma\delta$ T cells can interact with keratinocyte-expressed PlxnB2 to orchestrate a morphological rounding process (Witherden et al., 2012), which is based on induced retraction of dendrites of $\gamma\delta$ T cells. In contrast to Sema4D, Sema4C is specifically upregulated on $T_{FH}$ cells (Figure 5), and it exhibits an affinity for PlxnB2 that is orders of magnitude higher (Deng et al., 2007). This high affinity for binding to PlxnB2, combined with a relatively low absolute level of surface expression, is likely crucial for enabling Sema4C to acutely bias the direction of fast-moving $T_{FH}$ cells in a contact-dependent manner at the GC edge. We speculate Sema4C on the T cell membrane is distributed in a polarized manner, potentially at the front of the migrating cell, creating preferential hotspots reactive to adhesive cues to generate directional biases. Consistent with this possibility, decisions of outgoing $T_{FH}$ cells reaching the GC border are not significantly affected by the presence or absence of PlxnB2 or Sema4C (Figures 3 and 6). When an excessive amount of Sema4C is made available on the cell surface of $T_{FH}$ cells, these cells extend more protrusions but migrate in a more convoluted manner inside GCs (Figures 6H and 6I; data not shown).

The contact-dependent guidance at the tissue level is effectuated by adhesive interactions between individual GC-arriving $T_{FH}$ cells and GC B cells. Migratory T cells that do not detect their cognate antigen typically move in an amoeboid manner without requiring strong integrin-mediated adhesion (Friedl, 2004; Wolf et al., 2003, 2007). However, when T cells, particularly antigen-inexperienced ones, recognize antigen on DCs, they stereotypically stop autonomous migration and form stable immunological synapses that critically depend on strong integrin-mediated adhesion triggered by inside-out signaling (Cahalan and Parker, 2008; Fooksman et al., 2010; Kinashi, 2005). Cognate interactions between antigen-specific T cells and antigen-specific B cells initially take place at the T-B border (Garside et al., 1988); they are also orchestrated in the form of long-lasting conjugates, being mobile but led by B cells (Okada et al., 2005; Qi et al., 2008). In contrast, GC $T_{FH}$-B cell adhesion is unstable and dynamic, likely because of limited antigen supply and/or heightened negative signaling in the T cells. Although constantly

(C) Tracks of GFP-expressing and dsRed-expressing OT-II cells of the indicated *Sema4c* genotypes in and around the same MD4 GCs. See also corresponding Movies S6 and S7.

(D) Incoming (left) and outgoing (right) *Sema4c*$^{+/+}$ and *Sema4c*$^{-/-}$ OT-II tracks classified as indicated. Data are pooled from three independent experiments; p values by $\chi^2$ tests.

(E and F) Frequencies of conjugation between Sema4C-transduced or vector control OT-II cells and either lipopolysaccharide (LPS)-activated, PlxnB2-transduced B cells (E) or isolated GC B cells (F) that were pulsed with $OVA_{323–339}$ at indicated concentrations. Data shown are mean ± SEM of triplicates from one of three independent experiments with similar results.

(G) Frequencies of conjugation between Sema4C-transduced or control OT-II cells and PlxnB2-transduced B cells not pulsed (left) or pulsed with indicated peptide (right) in the presence of blocking anti-LFA-1 or control antibody. One of three experiments with similar results is shown.

(H) Sema4C-transduced or control OT-II T cells migrating inside MD4 GCs. Cells are rendered as surface objects based on cytoplasmic GFP fluorescence. See also corresponding Movies S8 and S9.

(I) Volume-normalized surface areas of indicated OT-II T cells in GCs. Data are pooled from two independent experiments.

***$p < 0.001$.

migrating, T cells recognize and discriminate levels of antigen presentation on GC B cells by proportionally investing areas of the membrane surface in the cell-cell contact (Liu et al., 2015; Shulman et al., 2014), causing T cells to preferentially re-orient toward B cells presenting higher levels of TCR ligands. This process would also depend on adhesion. Therefore, when subjected to low-level TCR stimulation and associated inside-out signaling to integrins, $T_{FH}$ cells in GCs can be viewed as taking on features of mesenchymal migration, with the TCR-integrin module guiding the search for sites of highest antigen concentrations. Given the large extracellular domains of Sema4C and PlxnB2 molecules, adhesion mediated by Sema4C-PlxnB2 pairs would take place before TCR ligand recognition and may therefore facilitate ligand scanning and possibly potentiate TCR signaling and integrin-mediated adhesion.

In the absence of Sema4C or PlxnB2, reduced GC penetrance and inefficient T-B adhesion would limit the delivery of contact-dependent, antigen-specific help from $T_{FH}$ cells, which are essential for normal plasma cell formation (Victora and Nussenzweig, 2012). When mixed in the same GC, B cells lacking PlxnB2 would be at a competitive disadvantage of acquiring T cell help and would be expected to contribute less to the plasma cell compartment. GC maintenance is less affected, which is consistent with the observations that GC-derived plasma cells undergo more stringent selection in GCs than those GC B cells selected to undergo cyclic entry (Liu et al., 2015; Victora et al., 2010). While PlxnB2 signaling to the B cells does not appear to be involved in $T_{FH}$ guidance or promoting T-B adhesion, our data do not rule out that it might directly signal to GC B cells and may contribute to plasma cell programming. Curiously, however, our RNA-seq data indicate that PlxnB2$^{KO}$ GC B cells expressed a higher level of *Ztbt20* (Figure S2), the gene coding for a transcription factor that promotes the differentiation and longevity of plasma cells (Chevrier et al., 2014; Wang and Bhattacharya, 2014). Future studies are necessary to further examine the role of intrinsic PlxnB2 signaling in GC B cells.

## EXPERIMENTAL PROCEDURES

### Mice

All mice were maintained under specific pathogen-free conditions, and mice of both sexes and 6–10 weeks of age were used unless otherwise specified. All animal experiments were conducted in accordance of institutional guidelines for animal welfare and approved by the Institutional Animal Care and Use Committee (IACUC) at the Tsinghua University.

### Bone-Marrow and Fetal Liver Chimera

B6 recipients were lethally irradiated by X-ray (5.5 Gy × 2), and then intravenously transferred with $4 \times 10^6$ bone-marrow leukocytes or fetal liver cells of the indicated genotypes or combination of genotypes. Chimeras were used for experiments 8 weeks after the initial reconstitution.

### Cell Culture, Retrovirus, and In Vitro Transduction

CD4 T cells or B cells were isolated by the CD4 T Cell Isolation Kit or the Naive B Cell Isolation Kit (Miltenyi Biotec) according to the manufacturer's protocols. PlxnB2 and SEMA4C cDNA were cloned into the pIB retroviral vector that was custom made to express IRES-driven GFP or red fluorescent protein (RFP), and retrovirus used for transduction was packaged with the Plate-E system as previously described (Chu et al., 2014).

### Immunohistochemistry and T Cell Distribution

To examine T cell distribution patterns in vivo, B6 mice receiving MD4 and OT-II cells of indicated genotypes were immunized with HEL-OVA, and draining lymph nodes were harvested at indicated times to prepare 16-μm sections for immunohistochemical staining as previously described (Xu et al., 2013).

### T-B Conjugation Assay

The protocol for this assay was essentially as previously described (Chu et al., 2014). The cells were incubated for 30 min at 37°C and sheer-stressed by vortexing for 45 s before remaining T-B conjugates were enumerated by flow cytometry.

### Intravital Imaging and Analyses of Interactional and Migratory Dynamics

To discern individual $T_{FH}$-B cell interactions inside GCs, $5 \times 10^4$ CFP-expressing and $5 \times 10^4$ GFP-expressing MD4 B cells were co-transferred with $9 \times 10^5$ non-fluorescent WT MD4 B cells. Intravital imaging of the draining lymph node was performed on day 5 post-immunization as previously described (Qi et al., 2008; Xu et al., 2013).

### Statistical Analysis

Except when noted otherwise, t tests were used to compare endpoint means of different groups. Statistical tests and graphing were done with Prism (GraphPad).

## ACCESSION NUMBERS

The accession number for the RNA-seq data reported in this paper is GEO: GSE96819.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and nine movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.04.022.

## AUTHOR CONTRIBUTIONS

C.G.V. initiated the project. B.M. and R.J.R. contributed early observations. H.Q. designed and supervised most of the experiments, which were mainly performed by H.Y. and L.W. R.H.F. developed the Sema4C knockout mice. C.S. contributed to imaging experiments. S.H., J.S., and T.M. conducted RNA-seq analyses. W.C., Y.C., and H.J. also performed experiments. All authors were involved in data interpretation. H.Q. wrote the paper with contributions from H.Y., L.W., C.G.V., and R.H.F.

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## REFERENCES

Allen, C.D., Okada, T., Tang, H.L., and Cyster, J.G. (2007). Imaging of germinal center selection events during affinity maturation. Science *315*, 528–531.

Bannard, O., Horton, R.M., Allen, C.D., An, J., Nagasawa, T., and Cyster, J.G. (2013). Germinal center centroblasts transition to a centrocyte phenotype

according to a timed program and depend on the dark zone for effective selection. Immunity *39*, 912–924.

Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M., and Förster, R. (2000). Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J. Exp. Med. *192*, 1545–1552.

Cahalan, M.D., and Parker, I. (2008). Choreography of cell motility and interaction dynamics imaged by two-photon microscopy in lymphoid organs. Annu. Rev. Immunol. *26*, 585–626.

Campbell, D.J., Kim, C.H., and Butcher, E.C. (2001). Separable effector T cell populations specialized for B cell help or tissue inflammation. Nat. Immunol. *2*, 876–881.

Chabbert-de Ponnat, I., Marie-Cardine, A., Pasterkamp, R.J., Schiavon, V., Tamagnone, L., Thomasset, N., Bensussan, A., and Boumsell, L. (2005). Soluble CD100 functions on human monocytes and immature dendritic cells require plexin C1 and plexin B1, respectively. Int. Immunol. *17*, 439–447.

Chevrier, S., Emslie, D., Shi, W., Kratina, T., Wellard, C., Karnowski, A., Erikci, E., Smyth, G.K., Chowdhury, K., Tarlinton, D., and Corcoran, L.M. (2014). The BTB-ZF transcription factor Zbtb20 is driven by Irf4 to promote plasma cell differentiation and longevity. J. Exp. Med. *211*, 827–840.

Chu, C., Wang, Y., Zhang, X., Ni, X., Cao, J., Xu, W., Dong, Z., Yuan, P., Wei, W., Ma, Y., et al. (2014). SAP-regulated T Cell-APC adhesion and ligation-dependent and -independent Ly108-CD3ζ interactions. J. Immunol. *193*, 3860–3871.

Cyster, J.G. (2005). Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu. Rev. Immunol. *23*, 127–159.

Cyster, J.G., and Schwab, S.R. (2012). Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. Annu. Rev. Immunol. *30*, 69–94.

Cyster, J.G., Dang, E.V., Reboldi, A., and Yi, T. (2014). 25-Hydroxycholesterols in innate and adaptive immunity. Nat. Rev. Immunol. *14*, 731–743.

Deng, S., Hirschberg, A., Worzfeld, T., Penachioni, J.Y., Korostylev, A., Swiercz, J.M., Vodrazka, P., Mauti, O., Stoeckli, E.T., Tamagnone, L., et al. (2007). Plexin-B2, but not Plexin-B1, critically modulates neuronal migration and patterning of the developing nervous system in vivo. J. Neurosci. *27*, 6333–6347.

Fooksman, D.R., Vardhana, S., Vasiliver-Shamis, G., Liese, J., Blair, D.A., Waite, J., Sacristán, C., Victora, G.D., Zanin-Zhorov, A., and Dustin, M.L. (2010). Functional anatomy of T cell activation and synapse formation. Annu. Rev. Immunol. *28*, 79–105.

Förster, R., Mattis, A.E., Kremmer, E., Wolf, E., Brem, G., and Lipp, M. (1996). A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell *87*, 1037–1047.

Friedel, R.H., Kerjan, G., Rayburn, H., Schuller, U., Sotelo, C., Tessier-Lavigne, M., and Chedotal, A. (2007). Plexin-B2 controls the development of cerebellar granule cells. J Neurosci. *27*, 3921–3932.

Friedl, P. (2004). Prespecification and plasticity: shifting mechanisms of cell migration. Curr. Opin. Cell Biol. *16*, 14–23.

Garside, P., Ingulli, E., Merica, R.R., Johnson, J.G., Noelle, R.J., and Jenkins, M.K. (1988). Visualization of specific B and T lymphocyte interactions in the lymph node. Science *281*, 96–99.

Gatto, D., Paus, D., Basten, A., Mackay, C.R., and Brink, R. (2009). Guidance of B cells by the orphan G protein-coupled receptor EBI2 shapes humoral immune responses. Immunity *31*, 259–269.

Green, J.A., Suzuki, K., Cho, B., Willison, L.D., Palmer, D., Allen, C.D., Schmidt, T.H., Xu, Y., Proia, R.L., Coughlin, S.R., and Cyster, J.G. (2011). The sphingosine 1-phosphate receptor $S1P_2$ maintains the homeostasis of germinal center B cells and promotes niche confinement. Nat. Immunol. *12*, 672–680.

Hauser, A.E., Junt, T., Mempel, T.R., Sneddon, M.W., Kleinstein, S.H., Henrickson, S.E., von Andrian, U.H., Shlomchik, M.J., and Haberman, A.M. (2007). Definition of germinal-center B cell migration in vivo reveals predominant intrazonal circulation patterns. Immunity *26*, 655–667.

Heesters, B.A., Myers, R.C., and Carroll, M.C. (2014). Follicular dendritic cells: dynamic antigen libraries. Nat. Rev. Immunol. *14*, 495–504.

Huber, A.B., Kolodkin, A.L., Ginty, D.D., and Cloutier, J.F. (2003). Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. Annu. Rev. Neurosci. *26*, 509–563.

Insall, R.H. (2010). Understanding eukaryotic chemotaxis: a pseudopod-centred view. Nat. Rev. Mol. Cell Biol. *11*, 453–458.

Kageyama, R., Cannons, J.L., Zhao, F., Yusuf, I., Lao, C., Locci, M., Schwartzberg, P.L., and Crotty, S. (2012). The receptor Ly108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. Immunity *36*, 986–1002.

Kinashi, T. (2005). Intracellular signalling controlling integrin activation in lymphocytes. Nat. Rev. Immunol. *5*, 546–559.

Kruger, R.P., Aurandt, J., and Guan, K.L. (2005). Semaphorins command cells to move. Nat. Rev. Mol. Cell Biol. *6*, 789–800.

Kumanogoh, A., and Kikutani, H. (2013). Immunological functions of the neuropilins and plexins as receptors for semaphorins. Nat. Rev. Immunol. *13*, 802–814.

Kumanogoh, A., Watanabe, C., Lee, I., Wang, X., Shi, W., Araki, H., Hirata, H., Iwahori, K., Uchida, J., Yasui, T., et al. (2000). Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. Immunity *13*, 621–631.

Kumanogoh, A., Suzuki, K., Ch'ng, E., Watanabe, C., Marukawa, S., Takegahara, N., Ishida, I., Sato, T., Habu, S., Yoshida, K., et al. (2002). Requirement for the lymphocyte semaphorin, CD100, in the induction of antigen-specific T cells and the maturation of dendritic cells. J. Immunol. *169*, 1175–1181.

Lämmermann, T., Bader, B.L., Monkley, S.J., Worbs, T., Wedlich-Söldner, R., Hirsch, K., Keller, M., Förster, R., Critchley, D.R., Fässler, R., and Sixt, M. (2008). Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature *453*, 51–55.

Liu, D., Xu, H., Shih, C., Wan, Z., Ma, X., Ma, W., Luo, D., and Qi, H. (2015). T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. Nature *517*, 214–218.

Lu, K.T., Kanno, Y., Cannons, J.L., Handon, R., Bible, P., Elkahloun, A.G., Anderson, S.M., Wei, L., Sun, H., O'Shea, J.J., and Schwartzberg, P.L. (2011). Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. Immunity *35*, 622–632.

Maier, V., Jolicoeur, C., Rayburn, H., Takegahara, N., Kumanogoh, A., Kikutani, H., Tessier-Lavigne, M., Wurst, W., and Friedel, R.H. (2011). Semaphorin 4C and 4G are ligands of Plexin-B2 required in cerebellar development. Mol Cell Neurosci. *46*, 419–431.

Moriyama, S., Takahashi, N., Green, J.A., Hori, S., Kubo, M., Cyster, J.G., and Okada, T. (2014). Sphingosine-1-phosphate receptor 2 is critical for follicular helper T cell retention in germinal centers. J. Exp. Med. *211*, 1297–1305.

Okada, T., Miller, M.J., Parker, I., Krummel, M.F., Neighbors, M., Hartley, S.B., O'Garra, A., Cahalan, M.D., and Cyster, J.G. (2005). Antigen-engaged B cells undergo chemotaxis toward the T zone and form motile conjugates with helper T cells. PLoS Biol. *3*, e150.

Perälä, N., Jakobson, M., Ola, R., Fazzari, P., Penachioni, J.Y., Nymark, M., Tanninen, T., Immonen, T., Tamagnone, L., and Sariola, H. (2011). Sema4C-Plexin B2 signalling modulates ureteric branching in developing kidney. Differentiation *81*, 81–91.

Pereira, J.P., Kelly, L.M., Xu, Y., and Cyster, J.G. (2009). EBI2 mediates B cell segregation between the outer and centre follicle. Nature *460*, 1122–1126.

Phan, T.G., Amesbury, M., Gardam, S., Crosbie, J., Hasbold, J., Hodgkin, P.D., Basten, A., and Brink, R. (2003). B cell receptor-independent stimuli trigger immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. J Exp Med. *197*, 845–860.

Qi, H., Cannons, J.L., Klauschen, F., Schwartzberg, P.L., and Germain, R.N. (2008). SAP-controlled T-B cell interactions underlie germinal centre formation. Nature *455*, 764–769.

Renkawitz, J., Schumann, K., Weber, M., Lämmermann, T., Pflicke, H., Piel, M., Polleux, J., Spatz, J.P., and Sixt, M. (2009). Adaptive force transmission in amoeboid cell migration. Nat. Cell Biol. *11*, 1438–1443.

Rørth, P. (2011). Whence directionality: guidance mechanisms in solitary and collective cell migration. Dev. Cell *20*, 9–18.

Sarris, M., and Sixt, M. (2015). Navigating in tissue mazes: chemoattractant interpretation in complex environments. Curr. Opin. Cell Biol. *36*, 93–102.

Schwickert, T.A., Lindquist, R.L., Shakhar, G., Livshits, G., Skokos, D., Kosco-Vilbois, M.H., Dustin, M.L., and Nussenzweig, M.C. (2007). In vivo imaging of germinal centres reveals a dynamic open structure. Nature *446*, 83–87.

Shi, W., Kumanogoh, A., Watanabe, C., Uchida, J., Wang, X., Yasui, T., Yukawa, K., Ikawa, M., Okabe, M., Parnes, J.R., et al. (2000). The class IV semaphorin CD100 plays nonredundant roles in the immune system: defective B and T cell activation in CD100-deficient mice. Immunity *13*, 633–642.

Shulman, Z., Gitlin, A.D., Targ, S., Jankovic, M., Pasqual, G., Nussenzweig, M.C., and Victora, G.D. (2013). T follicular helper cell dynamics in germinal centers. Science *341*, 673–677.

Shulman, Z., Gitlin, A.D., Weinstein, J.S., Lainez, B., Esplugues, E., Flavell, R.A., Craft, J.E., and Nussenzweig, M.C. (2014). Dynamic signaling by T follicular helper cells during germinal center B cell selection. Science *345*, 1058–1062.

Suan, D., Nguyen, A., Moran, I., Bourne, K., Hermes, J.R., Arshi, M., Hampton, H.R., Tomura, M., Miwa, Y., Kelleher, A.D., et al. (2015). T follicular helper cells have distinct modes of migration and molecular signatures in naive and memory immune responses. Immunity *42*, 704–718.

Takamatsu, H., Takegahara, N., Nakagawa, Y., Tomura, M., Taniguchi, M., Friedel, R.H., Rayburn, H., Tessier-Lavigne, M., Yoshida, Y., Okuno, T., et al. (2010). Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II. Nat. Immunol. *11*, 594–600.

Tamagnone, L., Artigiani, S., Chen, H., He, Z., Ming, G.I., Song, H., Chedotal, A., Winberg, M.L., Goodman, C.S., Poo, M., et al. (1999). Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell *99*, 71–80.

Tran, T.S., Kolodkin, A.L., and Bharadwaj, R. (2007). Semaphorin regulation of cellular morphology. Annu. Rev. Cell Dev. Biol. *23*, 263–292.

Victora, G.D., and Nussenzweig, M.C. (2012). Germinal centers. Annu. Rev. Immunol. *30*, 429–457.

Victora, G.D., Schwickert, T.A., Fooksman, D.R., Kamphorst, A.O., Meyer-Hermann, M., Dustin, M.L., and Nussenzweig, M.C. (2010). Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. Cell *143*, 592–605.

Wang, Y., and Bhattacharya, D. (2014). Adjuvant-specific regulation of long-term antibody responses by ZBTB20. J. Exp. Med. *211*, 841–856.

Witherden, D.A., Watanabe, M., Garijo, O., Rieder, S.E., Sarkisyan, G., Cronin, S.J., Verdino, P., Wilson, I.A., Kumanogoh, A., Kikutani, H., et al. (2012). The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal γδ T cell function. Immunity *37*, 314–325.

Wolf, K., Müller, R., Borgmann, S., Bröcker, E.B., and Friedl, P. (2003). Amoeboid shape change and contact guidance: T-lymphocyte crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases. Blood *102*, 3262–3269.

Woolf, E., Grigorova, I., Sagiv, A., Grabovsky, V., Feigelson, S.W., Shulman, Z., Hartmann, T., Sixt, M., Cyster, J.G., and Alon, R. (2007). Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. Nat. Immunol. *8*, 1076–1085.

Worzfeld, T., and Offermanns, S. (2014). Semaphorins and plexins as therapeutic targets. Nat. Rev. Drug Discov. *13*, 603–621.

Xu, H., Li, X., Liu, D., Li, J., Zhang, X., Chen, X., Hou, S., Peng, L., Xu, C., Liu, W., et al. (2013). Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. Nature *496*, 523–527.

Yu, D., Cook, M.C., Shin, D.M., Silva, D.G., Marshall, J., Toellner, K.M., Havran, W.L., Caroni, P., Cooke, M.P., Morse, H.C., et al. (2008). Axon growth and guidance genes identify T-dependent germinal centre B cells. Immunol. Cell Biol. *86*, 3–14.