# OCA-B does not act as a transcriptional co-activator in T cells

Running title: Extrinsic role for *Pou2af1* in T cells

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**Abstract**

*Pou2af1* encodes for OCA-B, a coactivator of OCT-1/2 transcription factors, which plays a key role in B cell maturation. The function of OCA-B has also been studied in T cells, where T cells from *Pou2af1*$^{-/-}$ mice have impaired functions, such as cytokine production and T follicular helper (Tfh) differentiation. Arguably, some of these T cell phenotypes may result from impaired T-B interactions, secondary to the well-documented B cell defects in *Pou2af1*$^{-/-}$ mice. Yet, *Pou2af1* is actively transcribed in activated T cells, suggesting a T cell-intrinsic role. To isolate the T cell-intrinsic impact of *Pou2af1*, we generated *Pou2af1*$^{fl/fl}$ mice with specific genetic disruption of *Pou2af1* either in all hematopoietic cells or exclusively in T cells. While we confirm that *Pou2af1* is expressed in activated T cells, we surprisingly find that T cell cytokine production is not impaired in *Pou2af1*-deficient T cells. Moreover, *Pou2af1*-sufficient and -deficient T cells have comparable transcriptome profiles, arguing against a T cell-intrinsic role for *Pou2af1*. In line with these observations, we demonstrate that Tfh maturation is influenced by T cell-extrinsic deletion of *Pou2af1*, as observed both in competitive bone marrow chimeras and in *Pou2af1*$^{fl/fl}$ mice with specific deletion in B cells. Overall, this study provides strong evidence that *Pou2af1* does not act as a transcriptional coactivator in T cells, and conclusively demonstrates that loss of OCA-B in B cells indirectly impacts Tfh differentiation, clarifying the role of OCA-B in the immune system.

Keywords: B cells / OCA-B / Pou2af1 / T cells / germinal centers/ Tfh

## INTRODUCTION

OCA-B (Oct-coactivator from B cells, also known as OBF-1 or BOB.1) is encoded by the *Pou2af1* gene and was first identified in B cell extracts on the basis of its ability to promote immunoglobulin (Ig) gene transcription [1]. It is a transcriptional coactivator that interacts with the ubiquitously expressed OCT-1 and the B cell-specific OCT-2 transcription factors [1, 2]. OCA-B expression is restricted to the hematopoietic system, with strong expression in the spleen, lymph nodes (LN), bone marrow and blood [2, 3].

The first *in vitro* studies of OCA-B function suggested that OCA-B was one of the main drivers of Ig gene transcription in B cells [1, 2]. However, the generation of the B6.129S-*Pou2af1*$^{-/-}$ mouse, hereafter referred to as *Pou2af1*$^{-/-}$ mice, in which expression of OCA-B is disrupted in all cell types, revealed that OCA-B is dispensable for Ig transcription [4, 5]. *Pou2af1* mRNA is detectable in B cells at all stages of maturation [6], and *Pou2af1* deficiency impairs many aspects of B cell biology including B cell development [7-10], germinal center (GC) induction [4, 5, 11], IgG production [4, 5], B cell receptor signaling [12] and plasma cell differentiation [13]. The target genes of OCA-B/OCT-1/2 complexes remain mostly unknown but candidate genes include *Spib* and *Bcl6* [14, 15], both of which contribute to GC B cell differentiation [16, 17], such that this could explain the reduced ability of *Pou2af1*$^{-/-}$ B cells to differentiate into GC B cells.

In addition to B cells, the function of OCA-B has been studied in T cells. Indeed, *Pou2af1* is transcribed in T cells following *in vitro* activation [18, 19]. As OCT-1 is ubiquitously expressed, OCA-B in T cells could act through OCT-1 coactivation. Notably, T follicular helper (Tfh) and T helper

17 (Th17) differentiation as well as $CD4^+$ T cell cytokine production are impaired in *Pou2af1*$^{-/-}$ mice [18, 20, 21]. Still, due to the germline disruption of *Pou2af1* in these mice, one cannot conclude whether the T cell phenotypes are due to a cell-intrinsic loss of *Pou2af1* expression or are indirectly attributable to the loss of *Pou2af1* expression in other hematopoietic cells.

To determine the T cell-intrinsic roles for OCA-B, we generated mouse models in which we performed conditional deletion of *Pou2af1* specifically in hematopoietic cells or in T cells. Using these models, we provide strong evidence that *Pou2af1* does not impact T cell cytokine production or Th17 differentiation, and does not act as a transcriptional coactivator in T cells. Rather, we demonstrate that *Pou2af1* regulates Tfh differentiation in a T cell-extrinsic manner, specifically via *Pou2af1*-expressing B cells.

## RESULTS

### Generation of a *Pou2af1*$^{fl/fl}$ mouse for cell-specific *Pou2af1* deletion

In order to generate a mouse strain with cell-specific disruption of the *Pou2af1* gene, we obtained *Pou2af1*$^{+/LacZ}$ mice (*Pou2af1*$^{tm1a(KOMP)Wtsi}$) from the KOMP Repository [22]. In this mouse strain, the *Pou2af1* locus has been targeted to generate a *knockout-first* allele which can be converted to a conditional (Fl, floxed) allele through FLP-mediated recombination (Supplementary figure 1**a**). Proper targeting of the locus was confirmed through 5' and 3' long range PCR performed on genomic DNA isolated from mouse tails (Supplementary figure 1**b**). Genotyping PCR using internal primers also confirmed the generation of *Pou2af1*$^{+/LacZ}$ mice (Supplementary figure 1**c**). These animals were next crossed with ACTBFLPe transgenic mice, which expressed the FLPe recombinase gene under the direction of the human ACTB promoter [23], to excise the LacZ and Neomycin (Neo) cassettes. The resulting ACTBFLPe.*Pou2af1*$^{+/fl}$ mice were then crossed with C57BL/6J mice to isolate the floxed allele from the ACTBFLPe transgene and proper recombination at the *Pou2af1* locus was confirmed in the progeny of these mice using specific genotyping PCR (Supplementary figure 1**d**). *Pou2af1*$^{+/fl}$ mice were intercrossed to obtain *Pou2af1*$^{fl/fl}$ mice.

To disrupt the *Pou2af1* gene in all hematopoietic cells, *Pou2af1*$^{fl/fl}$ mice were crossed to Vav-Cre$^{+}$ mice [24, 25]. Complete absence of *Pou2af1* mRNA expression was confirmed by RT-qPCR on spleen cells from Vav-Cre$^{+}$ and Vav-Cre$^{-}$ littermates (deletion > 99%) (Supplementary figure 1**e-f**). As for *Pou2af1*$^{-/-}$ mice [4, 7, 20], B cell proportion and numbers were reduced in the spleen, Peyer's patches (PP) and mesenteric LNs (mLNs) of Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice relative to Vav-Cre$^{-}$ littermates (Figure 1**a, b**). Similar to *Pou2af1*$^{-/-}$ mice [20], Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice also

showed a reduction of GC B cells (GL-7$^{+}$FAS$^{+}$) in both PP and mLNs relative to littermate control mice (Figure 1**c, d**). In addition, most of the remaining GL-7$^{+}$FAS$^{+}$ GC B cells in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice expressed a CD86$^{+}$CXCR4$^{Low}$ phenotype (Figure 1**e, f**). The low number of GC B cells in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice are thus skewed towards a phenotype associated with light zone B cells, suggesting that GC B cell differentiation is perturbed in the absence of OCA-B.

## *Pou2af1* is expressed in T cells

Before investigating the effect of a T cell-specific deletion of *Pou2af1*, we validated that OCA-B was expressed in T cells. With the anti-OCA-B 6F10 monoclonal antibody yielding non-specific binding (Supplementary figure 2**a, b**), we quantified *Pou2af1* transcription by measuring mRNA levels in *Pou2af1*-sufficient mice. *Pou2af1* mRNA was expectedly abundant in total splenocytes, whereas mRNA levels were barely detectable in T cells (Figure 2**a**). As previously reported [26], *in vitro* activation of T cells induced a small but significant increase in *Pou2af1* mRNA levels (Figure 2**a**). These data show that OCA-B is expressed at low levels in total T cells and its expression can be induced upon activation. OCA-B expression was specific, as *Pou2af1* mRNA was undetectable in both unactivated and activated T cells isolated from either Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ or CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (Figure 2**a**). Note that CD4-Cre is expressed in the early CD4$^{+}$CD8$^{+}$thymic differentiation stage, such that genetic deletion is transmitted to all daughter cells, therefore resulting in targeted gene deletion in both CD4$^{+}$ and CD8$^{+}$ T cells (Supplementary figure 2**c, d**) [27]. As previously reported for *Pou2af1*$^{-/-}$ mice [5, 10, 20], deletion of *Pou2af1* in both Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice did not affect CD4$^{+}$ and CD8$^{+}$ T cell numbers in secondary lymphoid organs (Supplementary figure 2**e, f**). Taken

together, these data show that *Pou2af1* deletion is efficient in both Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. Indeed, B cell phenotypes in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice recapitulate those observed in *Pou2af1*$^{-/-}$ mice, demonstrating that *Pou2af1* is efficiently deleted in this model. In addition, *Pou2af1* mRNA is undetectable in T cells from both Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. More importantly, these data confirm that OCA-B is expressed, all be it at low levels, in T cells.

**_Pou2af1_ is dispensable for previously reported T cell phenotypes**

We next sought to investigate whether T cell phenotypes previously reported in *Pou2af1*$^{-/-}$ mice were T cell-intrinsic or extrinsic. One of the reported T cell phenotypes in *Pou2af1*$^{-/-}$ mice is impaired cytokine production [18]. Specifically, IL-2 cytokine production was quantified in CD4$^{+}$ T cells that had been stimulated with anti-CD3 and anti-CD28 antibodies, rested for 8 days, and restimulated with anti-CD3 and anti-CD28 antibodies for 6 hours. Under these conditions, IL-2 cytokine production by CD4$^{+}$ T cells from *Pou2af1*$^{-/-}$ mice was shown to be significantly decreased when compared to B6 mice [18]. Here, we quantified IL-2, TNF-α and IFN-γ cytokine production in CD4$^{+}$ T cells from CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ relative to Cre$^{-}$.*Pou2af1*$^{fl/fl}$ littermate controls, using a similar CD4$^{+}$ T cell activation protocol, with an 8 day rest period prior to a 6h restimulation with anti-CD3 and anti-CD28 antibodies. We found that *Pou2af1*-sufficient and -deficient CD4$^{+}$ T cells both produced similar levels of IL-2, TNF-α and IFN-γ (Figure 2**b, c**), suggesting that *Pou2af1* plays a cell-extrinsic role in facilitating CD4$^{+}$ T cell cytokine production. To validate the cell-extrinsic role of *Pou2af1*, we also assessed IL-2, TNF-α and IFN-γ cytokine production in CD4$^{+}$ T cells from Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ relative to Cre$^{-}$.*Pou2af1*$^{fl/fl}$ littermate controls. In this model, with the deletion of *Pou2af1* in all hematopoietic cells, we expected that

143 CD4$^+$ T cell cytokine production would be impaired in *Pou2af1*-deficient T cells, similar to what
144 had been observed in *Pou2af1*$^{-/-}$ mice [18]. However, IL-2, TNF-α and IFN-γ cytokine production
145 was similar in CD4$^+$ T cells from Vav-Cre$^+$.*Pou2af1*$^{fl/fl}$ and Cre$^-$.*Pou2af1*$^{fl/fl}$ mice (Figure 2**d**). This
146 was observed through a wide range of anti-CD3 concentration, in the presence or absence of
147 co-stimulation (Figure 2**e, f**). These results thus contrast with previous findings [18] and suggest
148 that *Pou2af1* does not impact CD4$^+$ T cell cytokine production neither directly, nor indirectly.
149
150 Due to the discrepancy between the CD4$^+$ T cell cytokine production of *Pou2af1*$^{-/-}$ mice and of
151 our newly generated *Pou2af1*$^{fl/fl}$ mice, we considered potential differences in the genetic make-
152 up of the strains. The same exons, namely 2, 3 and 4, are targeted in both models ([5] and
153 Supplementary figure 1). The main difference is genetic background. *Pou2af1* gene targeting in
154 the *Pou2af1*$^{-/-}$ mice was initially performed in 129S embryonic stem cells and the resulting mice
155 were kept on a mixed B6 and 129S genetic background [5]. In contrast, gene targeting in the
156 *Pou2af1*$^{fl/fl}$ mice described here was performed directly on C57BL/6N-A$^{tm1Brd}$ background and
157 *Pou2af1*$^{fl/fl}$ mice were maintained by backcrossing to B6 mice. As the level of CD4$^+$ T cell
158 cytokine production from *Pou2af1*$^{-/-}$ mice on a mixed genetic background was compared to that
159 of CD4$^+$ T cells from B6 mice [18], we questioned whether genetic variants from the 129S
160 background could explain the difference in phenotype. We quantified IL-2 cytokine production in
161 CD4$^+$ T cell from B6 and 129S mice and found that IL-2 production was significantly reduced in
162 CD4$^+$ T cell from 129S relative to B6 mice (Figure 2**g, h**). Next, we acquired *Pou2af1*$^{-/-}$ mice with
163 a mixed genetic background and tested IL-2 production from CD4$^+$ T cells. When compared
164 among littermates (i.e. with similar mixed genetic background), IL-2 production was not affected
165 by the *Pou2af1* genotype (Figure 2**i**). Altogether, these data suggest that genetic variants from

the 129S background in *Pou2af1*$^{-/-}$ mice likely affect CD4$^{+}$ T cell cytokine production and that *Pou2af1* expression is dispensable for optimal cytokine production by CD4$^{+}$ T cells.

The observation that potential 129S carryover genes could affect T cell phenotypes prompted us to revisit the impact of *Pou2af1* on Th17 differentiation [21] and memory CD4$^{+}$ T cells [18]. When subjected to Th17 differentiation conditions, T cells from Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ littermates showed similar levels of RORγt induction and IL-17 production, demonstrating that Th17 differentiation is not affected by the genetic deletion of *Pou2af1* (Figure 3**a-c**). Th17 differentiation and IL-17 production was also similar for CD4$^{+}$ T cells from *Pou2af1*$^{-/-}$ mice and the littermate controls (Figure 3**d, e**). CD4$^{+}$ T cells with a memory phenotype can be distinguished from naïve cells based on a CD62L$^{Lo}$CD44$^{Hi}$CD45RB$^{Lo}$ phenotype. The absence of CD25 expression also discriminates memory CD4$^{+}$ T cells from early effector T cells [28]. No difference in the percentage or number of CD4$^{+}$ T cells with a memory phenotype was observed in the spleen, bone marrow and LN for both Vav- and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice relative to their littermate controls (Figure 3**f, g** and Supplementary figure 3). Altogether, these results argue against an effect of *Pou2af1* on Th17 differentiation and CD4$^{+}$ T cell memory phenotype.

**T cell-extrinsic impact of *Pou2af1* deletion on Tfh and GC development**

*Pou2af1*$^{-/-}$ mice also display a strong reduction of Tfh in PP at steady state, relative to wild type (WT) mice [20]. In contrast to the other T cell traits investigated above, we find a clear reduction in the percentage and number of Tfh in both the PP and mLN of Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (Figure 4**a, b** and Supplementary figure 4**a, e**). *Pou2af1* expression in hematopoietic cells is therefore necessary for optimal Tfh differentiation. Interestingly, while Tfh are considerably

189 reduced in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, early Tfh (CXCR5$^{Low}$PD-1$^{Low}$) are present in similar
190 proportions in PP and mLN (Figure 4**c** and Supplementary figure 4**b**) and are increased numbers
191 in PP (Supplementary figure 4**f**), relative to Cre$^{-}$ littermate controls. When gated on total CD4$^{+}$ T
192 cells expressing CXCR5 and PD-1, to include both early and mature Tfh, we observe a severe
193 decrease in the expression level of CXCR5 and PD-1 (Figure 4**d**). These data suggest that Tfh
194 differentiation is blocked at the early Tfh maturation stage in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice.
195
196 To determine if *Pou2af1* plays a T cell-intrinsic role in Tfh differentiation, we studied Tfh
197 differentiation in PP and mLN from CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. In contrast to *Pou2af1*$^{-/-}$ mice and
198 Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, the proportion and number of Tfh and of early Tfh was not affected
199 by the specific deletion of *Pou2af1* in T cells from both PP and mLN (Figure 4**e-g** and
200 Supplementary figure 4**a, b, g, h**). In addition, specific disruption of *Pou2af1* in T cells did not
201 affect the percentage and number of GC B cells in PP and in mLN (Figure 4**h** and Supplementary
202 figure 4**c, i**). The GC B cell bias towards a light zone phenotype observed in the Vav-
203 Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (Figure 1**e, f**) was also not observed in CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (Figure
204 4**i** and Supplementary figure 4**d**). These results suggest that T cell-intrinsic expression of
205 *Pou2af1* is not necessary for the development of both Tfh and GCs.
206
207 As *Pou2af1* mRNA levels are low in naïve T cells and induced upon activation (Figure 2**a**), we
208 reasoned that Tfh differentiation in CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice may only be impaired upon
209 immunization rather than steady state conditions. To test this possibility, we immunized Vav-
210 and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice as well as Cre$^{-}$.*Pou2af1*$^{fl/fl}$ littermate controls with sheep red
211 blood cells, as they induce robust GC formation in the spleen [29]. Ten days post-immunization,

we quantified GC B cells and Tfh in the spleen of these mice. Expectedly, the proportion of PD-1$^{+}$CXCR5$^{+}$ Tfh (Figure 4**j, k**) and GL-7$^{+}$FAS$^{+}$ GC B cells (Figure 4**l**) were low in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. However, both CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ and littermate controls had similar levels of Tfh and GC B cells in the spleens (Figure 4**j-l**). Consistent with our data in the PPs and mLNs at steady state, we observed a reduction of PD-1 and CXCR5 RFI in Tfh from Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice relative to both CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice and littermate controls (Supplementary figure 5**a**). Altogether, these results strongly point to a T cell-extrinsic role of *Pou2af1* in regulating Tfh differentiation in *Pou2af1*$^{-/-}$ mice.

**OCA-B is not an active transcriptional coactivator in T cells**

As we observed no T cell-intrinsic phenotypes in the absence of *Pou2af1*, we sought to investigate whether the increase in *Pou2af1* mRNA in activated T cells has an impact on the T cell transcriptome. OCA-B associates with the OCT-1 and OCT-2 transcription factors to regulate transcription of their target genes [1, 2]. As OCT-1 is expressed in both CD4$^{+}$ and CD8$^{+}$ T cells [30], OCA-B may act as a transcriptional cofactor in activated T cells. To identify the potential consequence of *Pou2af1* induction on the T cell transcriptome, we sorted naïve CD4$^{+}$ and CD8$^{+}$ T cells from the spleen of CD4-Cre$^{-}$.*Pou2af1*$^{fl/fl}$, CD4-Cre$^{+}$.*Pou2af1*$^{fl/+}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ littermates. Sorted cells were stimulated *in vitro* with anti-CD3 and anti-CD28 for 48h hours, RNA was isolated and subjected to RNA-Seq analysis. Principal component analysis (PCA) applied to the 500 most differentially expressed genes did not segregate T cells on the basis of their genotype (Figure 5**a**), suggesting an absence of transcriptomic regulation by *Pou2af1*. *Pou2af1* mRNA expression was significantly decreased in T cells from CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice relative to T cells from CD4-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ mice, confirming that *Pou2af1* mRNA is expressed in

activated CD4$^+$ and CD8$^+$ T cells (Figure 5**b** and Supplementary figure 6**a**). Apart from *Pou2af1*, only four genes were significantly differentially expressed when comparing CD4$^+$ or CD8$^+$ cells from CD4-Cre$^-$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^+$.*Pou2af1*$^{fl/fl}$ littermates (Figure 5**b** and Supplementary figure 6**a**). Among those four genes, only two were observed in both CD4$^+$ and CD8$^+$ T cells: *Rps3a1* and *Tpm3-rs7*. Of note, these two genes were also differentially expressed in T cells from CD4-Cre$^+$.*Pou2af1*$^{fl/+}$ heterozygous mice when compared to CD4-Cre$^-$.*Pou2af1*$^{fl/fl}$ mice (Figure 5**b**), suggesting that the expression of the CD4-Cre transgene may be responsible for these differences, rather than an effect of the *Pou2af1* deletion. Together, these data demonstrate that, although *Pou2af1* is expressed in T cells following *in vitro* stimulation, it does not act as an important coactivator of transcription.

### *Pou2af1* expression in B cells is necessary for adequate Tfh maturation

Notwithstanding the absence of a T cell-intrinsic role for *Pou2af1*, our data point to a T cell-extrinsic role in Tfh differentiation. To specifically test if the Tfh maturation block observed in Vav-Cre$^+$.*Pou2af1*$^{fl/fl}$ mice is caused by defective crosstalk between T cells and *Pou2af1*$^{-/-}$ hematopoietic cells *in vivo*, we generated competitive bone marrow chimeras. B6.SJL (CD45.1) and Vav-Cre$^+$.*Pou2af1*$^{fl/fl}$ (CD45.2) bone marrow were mixed at 1:1 ratio and injected in lethally irradiated B6 x B6.SJL F1 (CD45.1 and CD45.2 co-dominant expression) (Supplementary figure 6**b**). Expectedly, WT B cells (CD45.1) showed a growth advantage over *Pou2af1*-deficient B cells differentiated from Vav-Cre$^+$.*Pou2af1*$^{fl/fl}$ (CD45.2) bone marrow (Figure 5**c, d**). In contrast, and consistent with our observations in both Vav- and CD4-Cre$^+$.*Pou2af1*$^{fl/fl}$ mice (Supplementary figure 2**e, f**), T cells from WT and Vav-Cre$^+$.*Pou2af1*$^{fl/fl}$ precursors were present in equal proportions (Figure 5**c, d**), confirming an absence of competitive disadvantage for

*Pou2af1* targeted T cells. We also observed a severe reduction of GC B cells of Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ origin (Supplementary figure 6**c, d**), confirming the B cell-intrinsic defect of GC induction in the absence of *Pou2af1*. As opposed to the observations made in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, CD4$^{+}$ T cells were able to fully mature into Tfh in the spleen, PP and mLN of the chimeras, regardless of their genotype (Figure 5**e, f**). This is likely due to the presence of a sufficient number of OCA-B expressing B cells in this competitive bone marrow chimera setting (Figure 5**c**), suggesting a T cell extrinsic role for OCA-B in Tfh differentiation. These results are consistent with a previous report also demonstrating a T cell-extrinsic role for OCA-B in Tfh development and function using mixed bone marrow chimeras [31].

Next, to determine if loss of OCA-B in B cells is sufficient to impede Tfh differentiation, we crossed *Pou2af1*$^{fl/fl}$ mice to CD19-Cre$^{+}$ mice to disrupt *Pou2af1* specifically in B cells. The percentage of B cells were reduced in the lymphoid organs of CD19-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, in line with effective *Pou2af1* disruption (Figure 5**g**). Compared to CD19-Cre$^{-}$ littermates, CD19-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice showed a significant reduction in Tfh and a reduced expression of CXCR5 and PD-1 on CD4$^{+}$ T cells from the PP and mLN (Figure 5**h-j**). These results show that specific loss of OCA-B expression in B cells is sufficient to impair Tfh differentiation. Altogether, our data conclusively demonstrate that *Pou2af1* plays a T cell-extrinsic role in Tfh differentiation.

## DISCUSSION

The levels of expression of *Pou2af1* are clearly more prominent in B cells than in T cells. The Immunological Genome Project database shows that *Pou2af1* mRNA is detectable in all B cell stages, from B cell precursors to plasma cells [6]. Multiple transcriptomic and proteomic databases also show that, among immune cell types, *Pou2af1* expression is undetectable in non-B cells, in both mice and humans [3, 6, 32]. Still, we and others report that *Pou2af1* expression can be induced in T cells [19, 26](and this manuscript), and the biological impact of *Pou2af1* in T cells has been investigated for more than two decades [19, 33]. By generating a new mouse strain in which *Pou2af1* can be conditionally deleted, we provide evidence that, as opposed to previous reports [18, 21, 34], *Pou2af1* is dispensable for $CD4^+$ T cell cytokine production, Th17 differentiation and does not affect the T cell pool. Using an unbiased approach to determine the impact of *Pou2af1* in regulating the transcriptional profile of $CD4^+$ and $CD8^+$ T cells, we find that *Pou2af1* does not act as an effective transcriptional coactivator in activated T cells. We also report that *Pou2af1* indirectly promotes the differentiation of $CD4^+$ T cells into mature Tfh, via *Pou2af1*-expressing B cells.

As mentioned, $CD4^+$ T cells isolated from B6.129S-*Pou2af1*$^{-/-}$ show defects in cytokine production, immunological memory as well as in Th17 and Tfh differentiation [18, 20, 21]. Moreover, *Pou2af1* mRNA is induced in activated T cells [18, 19]. With this growing literature on the role of OCA-B in T cells, we expected to find that T cell specific deletion of *Pou2af1* expression would alter T cell functions. With the two mouse models that specifically target deletion in either all hematopoietic cells or in all T cells, we set out to determine the T cell specific role of OCA-B in transcriptional regulation. Instead, we were taken aback and noted that T cell specific deletion

of *Pou2af1* expression does not influence T cell phenotypes and does not significantly affect the T cell transcriptome. We provide evidence to suggest that the difference between our results and that of others appear to stem from the differences in genetic background; whereas our floxed model was generated on the C57BL/6N-A$^{tm1Brd}$ background, the B6.129S-*Pou2af1*$^{-/-}$ model is held on a mixed B6 and 129S genetic background. Notably, we find that cytokine production in T cells from 129S mice is reduced relative to that of B6 mice. These observations are in line with previous reports demonstrating that carryover genes influence the phenotype of knock-out and congenic mice [35, 36].

Notwithstanding the lack of impact of *Pou2af1* in various T cell phenotypes, hematopoietic deletion of *Pou2af1* expression resulted in a significant decrease in Tfh. Characterization of mature Tfh in CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice and in competitive bone marrow experiments confirmed a T cell-extrinsic role of *Pou2af1* in Tfh differentiation. Notably, the proportion and absolute numbers of early Tfh cells were unaffected in Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice whereas late Tfh were significantly reduced, suggesting that lack of OCA-B predominantly affects the transition from early Tfh to late Tfh. This is not surprising considering that early Tfh differentiation is highly dependent on DCs [37], which do not express *Pou2af1*. IL-6 and IL-21 produced by DCs during priming of CD4$^{+}$ T cells induce changes in chemokine receptors expression, resulting in migration of early Tfh to the B cell follicle border [38]. At this step, early Tfh express low levels of BCL6, CXCR5 and PD-1 and require additional signals to further differentiate into mature Tfh [39]. At the T-B border, B cells provide crucial signals to developing Tfh, such as ICOS-L, antigens, and cytokines [38, 40]. As B cells express high levels of OCA-B, *Pou2af1* disruption strongly affects their differentiation and functions [7, 8, 11](and this manuscript). The reduction of total B cells and

GC B cells in *Pou2af1*$^{-/-}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice likely impairs crosstalk between B cells and early Tfh, thereby limiting early Tfh maturation. Using CD19-Cre mice to specifically disrupt *Pou2af1* in B cells, we show that crosstalk between B cells and early Tfh requires OCA-B expression in B cells to promote efficient Tfh maturation. This crosstalk might be impaired in OCA-B-deficient mice through reduced expression of ICOSL, highly expressed by GC B cells [41], or by impaired cytokine production by B cells. Indeed, OCA-B promotes IL-6 production in activated B cells during infection, and B cell-derived IL-6 can induce Tfh development [31]. CXCR5$^{+}$PD-1$^{+}$ CD4$^{+}$ T cells from *Pou2af1*$^{-/-}$ mice also display lower BCL6 levels relative to B6 mice [20]. As such, it was suggested that *Pou2af1* directly promotes Tfh differentiation by regulating BCL6 [20]. However, considering that *Pou2af1* indirectly regulates Tfh differentiation, the reduction of BCL6 expression in Tfh from *Pou2af1*$^{-/-}$ mice is most likely caused by the maturation block at the early Tfh stage, which express lower BCL6 levels than mature Tfh [39].

It is interesting to note that *Pou2af1*$^{-/-}$ mice show a severe reduction in serum IgG, IgA and IgE, while IgM is not affected [4, 5, 10]. This feature of the *Pou2af1*$^{-/-}$ mouse does not seem to be attributed to a B cell-intrinsic class switching defect, as *Pou2af1*$^{-/-}$ B cells are able to class switch *in vitro* as efficiently as WT B cells [5]. Since Tfh directly promote B cell class switching from IgM to other isotypes [42, 43], the lack of mature Tfh indirectly caused by *Pou2af1* deletion in B cells may explain the reduction of IgG, IgA and IgE in the *Pou2af1*$^{-/-}$ mouse. Alternatively, a B cell-intrinsic role of OCA-B in plasma cell differentiation may also contribute to the reduction of isotype switched serum antibody levels [13].

345 Although *Pou2af1* expression is induced in T cells following activation, we did not identify any T
346 cell intrinsic function. By comparing the mRNA transcriptome of activated T cells isolated from
347 CD4-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, we further show that OCA-B does not seem
348 to exhibit significant transcriptional activity in T cells. The functional impact, if any, of the low
349 *Pou2af1* mRNA expression levels in activated T cells remains to be determined.
350
351 In conclusion, this study shows that *Pou2af1* is dispensable for most of the T cell-associated
352 phenotypes previously identified in the *Pou2af1*$^{-/-}$ mouse, with the exception of the T cell-extrinsic
353 effect on Tfh differentiation; *Pou2af1* expression in B cells promotes maturation of early Tfh into
354 Tfh *in vivo*. Overall, this study clarifies the role of OCA-B in the immune system, by conclusively
355 demonstrating that *Pou2af1* does not act as a coactivator of transcription in T cells, and therefore
356 does not bear T cell-intrinsic activities. Furthermore, we demonstrate that OCA-B expression in
357 B cells facilitates Tfh differentiation, explaining the T cell-extrinsic role of *Pou2af1*.
358

## METHODS

### Mice

B6 mice (#000664, Jax labs, Bar Harbor, United States) were crossed to B6.SJL mice (#002014, Jax labs) to generate F1 mice (B6.1.2), with co-dominant expression of CD45.1 and CD45.2 on all hematopoietic cells, were used as bone marrow chimera recipients. *Pou2af1*$^{+/LacZ}$ mice (*Pou2af1*$^{tm1a(KOMP)Wtsi}$) were obtained from the KOMP Repository (#049152-UCD, Davis, United States) [22], for which embryonic stem cells were of C57BL/6N-A$^{tm1Brd}$ background. Proper targeting of the locus was confirmed by 5' and 3' long range PCR performed on genomic DNA isolated from mouse tails using a combination of two primers located respectively inside and outside the targeting construct. Genotyping PCR using internal primers also confirmed the generation of *Pou2af1*$^{+/LacZ}$ mice. The genotyping primers are: P1, 5'-TACAGAGAGACTAGACACGGTCTGC-3'; P2, 5'-AGAAGGCCTCGTTACACTCCTATGC-3'; P3, 5'-GAGATGGCGCAACGCAATTAATG-3'; P4, 5'-GATGAGGACTCTGGGTTCAGAGAGG-3'; P5, 5'-GGGATCTCATGCTGGAGTTCTTCG-3'. These mice were crossed with ACTBFLPe Tg mice (#005703, Jax labs) to excise the LacZ and Neo cassettes and generate *Pou2af1*$^{+/fl}$ mice (*Pou2af1*$^{tm1c}$; see Supplementary figure 1) in which exon 2, 3 and 4 are flanked by loxP sites. The ACTBFLPe transgene was removed by subsequent breeding to B6 mice. *Pou2af1*$^{+/fl}$ mice were intercrossed to generate *Pou2af1*$^{fl/fl}$ mice. Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$, CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ and CD19-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (#008610 and #022071, #006785, Jax labs) were generated by crossing Cre$^{+}$.*Pou2af1*$^{fl/+}$ mice to *Pou2af1*$^{fl/fl}$, to avoid germline deletion [44]. ROSA-YFP mice (#007903, Jax labs) were crossed to CD4-Cre$^{+}$ mice to validate genetic deletion in T cells. 129S and *Pou2af1*$^{-/-}$ mice (#002448, #007596, Jax labs) were used to test the influence of the genetic background on the phenotypes. Genotype of all transgenic mice was verified by PCR.

Transgenic positive and negative littermates were used in every experiment. Male and female mice were used in this study and no phenotypic difference was observed based on sex. All of the mouse strains were maintained at the Maisonneuve-Rosemont Hospital animal facility (Montreal, Canada). The Maisonneuve-Rosemont Hospital ethics committee, overseen by the Canadian Council for Animal Protection, approved the experimental procedures.

**Generation of bone marrow chimeras**

Prior to irradiation, recipient mice were injected intraperitoneally with 1 mL of PBS to avoid dehydration. Bone marrow cell suspensions were prepared from the tibia and femur of 10-week-old mice in sterile RPMI. The red blood cells were lysed using $NH_4Cl$ and the bone marrow single cell suspension were prepared in sterile PBS. A 1:1 bone marrow ratio was confirmed by flow cytometry (with anti-CD45.1 and anti-CD45.2 antibodies) prior to injection. Eight-week-old recipient mice were irradiated at 11Gy, using linac X-ray source and, subsequently, injected intravenously with $2x10^6$ bone marrow cells.

**Sheep red blood cell immunization**

Mice were injected intra-peritoneally with $5x10^8$ sheep red blood cells (Innovative Research, Novi, United States) diluted in PBS. Ten days after injection, the spleens were collected for flow cytometry analysis.

**Flow cytometry and cell sorting**

Spleen, bone marrow, PP and LNs were pressed through a 70-µm cell strainer (Thermo Fisher Scientific, Waltham, United States). Spleen cell suspensions were treated with $NH_4Cl$ to lyse red

blood cells. Single-cell suspensions were stained for 30 minutes at 4°C with different combinations of antibodies listed as target (clone): From BioLegend (San Diego, United States), B220 (RA3-6B2), CD4 (GK1.5), CD8a (53-6.7), CD19 (6D5), CD25 (PC61.5.3), CD44 (IM7), CD45.1 (A20), CD45.2 (104), CD45RB (C363-16A), CD62L (MEL-14), CD69 (H1.2F3), CD86 (GL-1), CXCR4 (L276F12), CXCR5 (L138D7), GL-7 (GL7), IFN-γ (XMG1.2), IL-2 (JES6-5H4), PD-1 (RMP1-30), TCRβ (H57-597), TNF-α (MP6-XT22); From BD Biosciences (New Jersey, United States), CD95 (Jo2); From Thermo Fisher, CD8b (H35-17.2), FoxP3 (FJK-16s), IL-17 (eBio1787), RORγt (B2D); From Santa Cruz Biotechnology (Dallas, United States), OCA-B (6F10). Biotin-labelled antibodies were revealed with fluorescently-coupled streptavidins from BioLegend. Viable cells were stained using LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit (Thermo Fisher). Before staining with 6F10 antibodies, cells were fixed and permeabilized using BD Cytofix/Cytoperm™ kit, as published [18]. Cells were stained in 100µl of buffer plus 7.5µl of PE-conjugated 6F10 antibodies [18]. For transcription factor staining, cells were treated with FOXP3 Transcription Factor Staining Buffer Set (Thermo Fischer). Data were collected on an LSRFortessaX20 (BD Biosciences), and analyzed with FlowJo software (BD Biosciences). For cell sorting, spleen cell suspensions were stained with antibodies against TCRβ and B220, in sterile conditions. T cells were sorted on a FACS Aria II (BD Biosciences) as $TCR\beta^{+}B220^{-}$ single cells (purity > 99%).

### *In vitro* T cell stimulation

As described in Shakya *et al.*[18], *in vitro* T cell stimulation was performed in flat bottom 96 well plates (Sarstedt, Nümbrecht, Germany) coated with anti-CD3 (either 1 µg $mL^{-1}$ or graded concentrations as indicated, 145-2C11) and anti-CD28 (10 µg $mL^{-1}$, 37.51) antibodies in PBS

overnight at 4°C. Plates were washed in serum-containing media prior to adding the cells. For primary stimulation, spleen cells ($5x10^5$ $mL^{-1}$) were stimulated for 2 days in complete RPMI-1640 medium. In some experiments, cells were analyzed after primary stimulation. Otherwise, cells were rested for 8 days, in complete RPMI-1640 medium with IL-2 (30 U $mL^{-1}$). For secondary stimulation, rested cells were activated for 6 hours with plate bound anti-CD3 and anti-CD28 antibodies, at 1 and 10 μg $mL^{-1}$ respectively, in the presence of Brefeldin A (10 μg $mL^{-1}$). Cells were then harvested and stained with antibodies prior to analysis by flow cytometry.

**RT-qPCR**

5-10 x $10^6$ sorted T cells were stored in Trizol while the remaining T cells (2.5-5 x $10^5$) were activated *in vitro* for 2 days using anti-CD3 and anti-CD28 antibodies. RNA extraction for all samples was conducted as indicated by the manufacturer (Trizol, Thermo Fisher Scientific). Mouse *Pou2af1* mRNA levels were measured using primers targeting exons 4 and 5 of *Pou2af1* (Pou2af1-F: 5'-CCTGCCTTGACATGGAGGTT-3' and Pou2af1-R: 5'-AGTGCTTCTTGGCGTGACAT-3'), *Gapdh* (Gapdh-F:5'-TCAACGGCACAGTCAAGG-3' and Gapdh-R: 5'-ACTCCACGACATACTCAGC-3') and *Actb* (Actb-F:5'-GAAATCGTGCGTGACATCAAAG-3' and Actb-R: 5'-TGTAGTTTCATGGATGCCACAG-3') mRNA levels were used as loading controls and ddCT variations calculated in all cases.

**Th17 differentiation**

To differentiate T cells into either Th0 or Th17 profiles, LN cells were activated with plate bound anti-CD3 (2 μg $mL^{-1}$) and anti-CD28 (2 μg $mL^{-1}$) antibodies. While Th0 differentiation proceeded in the absence of cytokines, Th17 was induced in the presence of 2 ng $mL^{-1}$ rhTGF-β1 and 25

ng $mL^{-1}$ rmIL-6 (both cytokines from Miltenyi Biotec, Bergisch Gladbach, Germany), as described in Yosef *et al*. [21]. Cells were cultured for 3 days prior to analysis. T cell supernatants were collected at the end of the 3-day culture. Quantification of IL-17A in the media was performed by ELISA as indicated by the manufacturer (Mouse Il-17 Quantikine ELISA Kit, R&D Systems, Minneapolis, United States). For quantifying IL-2 and IL-17A production by flow cytometry, cells were incubated with phorbol 12-myristate 13-acetate (50 ng $mL^{-1}$), ionomycin (500 ng $mL^{-1}$) and Brefeldin A (10 $\mu$g $mL^{-1}$) for 4h prior to staining.

**RNA sequencing**

Naïve $CD4^+$ and $CD8^+$ T cells were sorted from the spleen as $CD19^-TCR_\beta^+CD4^+CD62L^+$ and $CD19^-TCR_\beta^+CD8^+CD62L^+$ single cells, respectively (purity > 96%). T cells were activated *in vitro* for two days using anti-CD3 and anti-CD28 antibodies. RNA was isolated using Trizol and sent to the IRIC genomic platform for processing. Libraries were prepared using the KAPA mRNA stranded Hyperprep Kit. Libraries were sequenced using the Illumina NextSeq 500 FASTQ files were trimmed for sequencing adapters and low quality 3' bases using Trimmomatic version 0.35 [45] and aligned to the reference mouse genome version GRCm38 (gene annotation from Gencode version M25, based on Ensembl 100) using STAR version 2.7.1a [46]. Read counts were extracted directly from STAR at the gene level. DESeq2 (R; version 1.26) was then used to normalize gene read counts. Batch correction was added to the statistical model for differential expression to adjust for samples sorted on 2 separate days. Log normalized counts were batch corrected using the removeBatchEffect function from the limma R package (v 3.42.2) [47] and used as input for PCA and heatmap visualizations. The raw RNAseq data files have been uploaded into the GEO database (accession number: GSE171544).

## Statistical analyses

Data were tested for significance using a nonparametric Mann-Whitney *U*-test or a one-way ANOVA, where appropriate. Numbers of animal used per group are indicated in the figure legends. The minimal significance threshold was set at 0.05 for all tests.

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**Conflict of interest:** The authors declare that they have no conflicts of interest.

**Data availability**: The raw RNAseq data files have been uploaded into the GEO database (accession number: GSE171544).

**Author contributions:** F.L.-V. designed and conducted most of the experiments, prepared the figures, and wrote the manuscript. J.L. generated and validated the *Pou2af1*$^{fl/fl}$ mouse and designed *Pou2af1* RT-qPCR primers. G.C.-R. performed the flow cytometry analysis of CD4-Cre$^{+}$.ROSA-YFP$^{+}$ mice. M.F. contributed to the generation of the *Pou2af1*$^{fl/fl}$ mouse and to revisions of the manuscript. S.L. supervised the study, wrote and revised the manuscript.

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## Figures

Figure 1. **B cell phenotypes following hematopoietic cell-specific deletion of *Pou2af1.***

**(a)** Percentage and **(b)** number of B cells in the spleen, PP and mLN of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 8, collected in six independent experiments). **(c)** Representative flow cytometry profiles of GL-7 and FAS expression on total B cells gated as B220$^{+}$ cells from the mLN of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ littermate controls. The gate selects for GL-7$^{+}$FAS$^{+}$ GC B cells. **(d)** Percentage of GC B cells from the PP and mLN of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ littermate controls (n = 8, collected in six independent experiments). **(e)** Representative flow cytometry profiles of CXCR4 and CD86 expression by GC B cells from the mLN of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ littermate controls. **(f)** Ratio of dark zone (CXCR4$^{Hi}$CD86$^{-}$) over light zone (CXCR4$^{Low}$CD86$^{+}$) GC B cells from the PP and mLN of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$ (n = 8, collected in six independent experiments). Data information: Mann Whitney *U*-test, *P*-values * < 0.05; ** < 0.01; *** < 0.001.

Figure 2. ***Pou2af1* is expressed in activated T cells but does not affect T cell cytokine production.**

**(a)** *Pou2af1* relative mRNA expression in spleen cells as well as in unactivated and activated T cells from *Pou2af1*$^{fl/fl}$ (Cre-), Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ (Vav-Cre+) and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ (CD4-Cre+) mice (n = 3-8, collected in three to four independent experiments), measured by RT-qPCR. *Actb* was used as control. **(b)** Representative flow cytometry profiles of IL-2 expression by rested and re-stimulated CD4$^{+}$ T cells from the spleens of CD4-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. **(c, d)** Percentage of IL-2-, TNFα- and IFNγ-producing CD4$^{+}$ T cells after re-stimulation of T cells from **(c)** CD4-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice and **(d)**

Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 6, collected in three independent experiments). **(e)** Percentage of IL-2-producing CD4$^{+}$ T cells after re-stimulation of T cells from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ at the indicated anti-CD3 concentrations in the presence of anti-CD28 (n = 3, collected in two independent experiments). **(f)** Representative flow cytometry profiles of IL-2 expression by re-stimulated CD4$^{+}$ T cells from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, in absence of anti-CD28 antibodies. Representative of two experiments. **(g)** Representative flow cytometry profiles of IL-2 expression by re-stimulated CD4$^{+}$ T cells from the spleens of B6 and 129S mice. **(h)** IL-2 relative fluorescence intensity (RFI) of IL-2-producing CD4$^{+}$ T cells after re-stimulation of T cells from B6 and 129S mice (n = 6, collected in three independent experiments). **(i)** IL-2 RFI of IL-2-producing CD4$^{+}$ T cells after re-stimulation of T cells from B6.129S-*Pou2af1*$^{-/-}$ littermates carrying *Pou2af1* +/+, +/- and -/- genotypes (n = 6, collected in three independent experiments). NS, non-significant, *P*-value > 0.05, ***, *P*-value < 0.001.

Figure 3. ***Pou2af1* does not affect Th17 differentiation and CD4$^{+}$ T cell memory phenotype.** **(a)** Representative flow cytometry profiles of RORγt expression by CD4$^{+}$ T cells from the spleen of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice under Th0 and Th17 differentiation conditions. MFI are indicated for each population. **(b)** IL-17A concentration in the supernatants of T cell cultures from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice under Th0 and Th17 differentiation conditions, measured by ELISA (n = 3, collected in three independent experiments). **(c)** Representative flow cytometry profiles of IL-2 and IL-17A expression by CD4$^{+}$ T cells from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice under Th0 and Th17 differentiation conditions. **(d)** Representative flow cytometry profiles of RORγt expression by

652 $CD4^+$ T cells from *Pou2af1*$^{-/-}$ mice and control littermates under Th0 and Th17 differentiation
653 conditions. MFI of RORγt are indicated for each population. **(e)** Representative flow cytometry
654 profiles of IL-2 and IL-17A expression by $CD4^+$ T cells from the same mice as in **(d)** under Th17
655 differentiation conditions. **(f, g)** Percentage (left panels) and absolute numbers (right panels) of
656 $CD62L^-CD44^{Hi}CD45RB^-CD25^-$ $CD4^+$ T cells from the spleen, bone marrow (BM) and of pool of
657 axial, brachial and inguinal LN of (**f)** Vav-$Cre^-$.*Pou2af1*$^{fl/fl}$ and Vav-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice and **(g)**
658 CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 6, collected in three independent
659 experiments). Data information: Mann Whitney *U*-test, NS, non-significant, *P*-value > 0.05.
660
661 Figure 4. **OCA-B expression in non-T cells is necessary for Tfh maturation. (a)**
662 Representative flow cytometry profiles of CXCR5 and PD-1 expression on $CD4^+$ T cells from the
663 PP of Vav-$Cre^-$.*Pou2af1*$^{fl/fl}$ and Vav-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice. **(b, c)** Percentage of Tfh ($CXCR5^{Hi}$PD-
664 $1^{Hi}$) and early Tfh ($CXCR5^{Low}$PD-$1^{Low}$) in the PP of Vav-$Cre^-$.*Pou2af1*$^{fl/fl}$ and Vav-$Cre^+$.*Pou2af1*$^{fl/fl}$
665 mice (n = 8, collected in three independent experiments). **(d)** PD-1 and CXCR5 relative
666 fluorescence intensity (RFI) on $CXCR5^+$PD-$1^+$ $CD4^+$ T cells from the PP of Vav-$Cre^-$.*Pou2af1*$^{fl/fl}$
667 and Vav-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 8, collected in three independent experiments). **(e)**
668 Representative flow cytometry profiles of CXCR5 and PD-1 expression on $CD4^+$ T cells from the
669 PP of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice. **(f, g)** Percentage of Tfh and early
670 Tfh in the PP of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three
671 independent experiments). **(h)** Percentage of GC B cells in the PP of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and
672 CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three independent experiments). **(i)** Ratio of dark
673 zone ($CXCR4^{Hi}CD86^-$) over light zone ($CXCR4^{Low}CD86^+$) GC B cells from the PP of CD4-$Cre^-$
674 .*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three independent experiments).

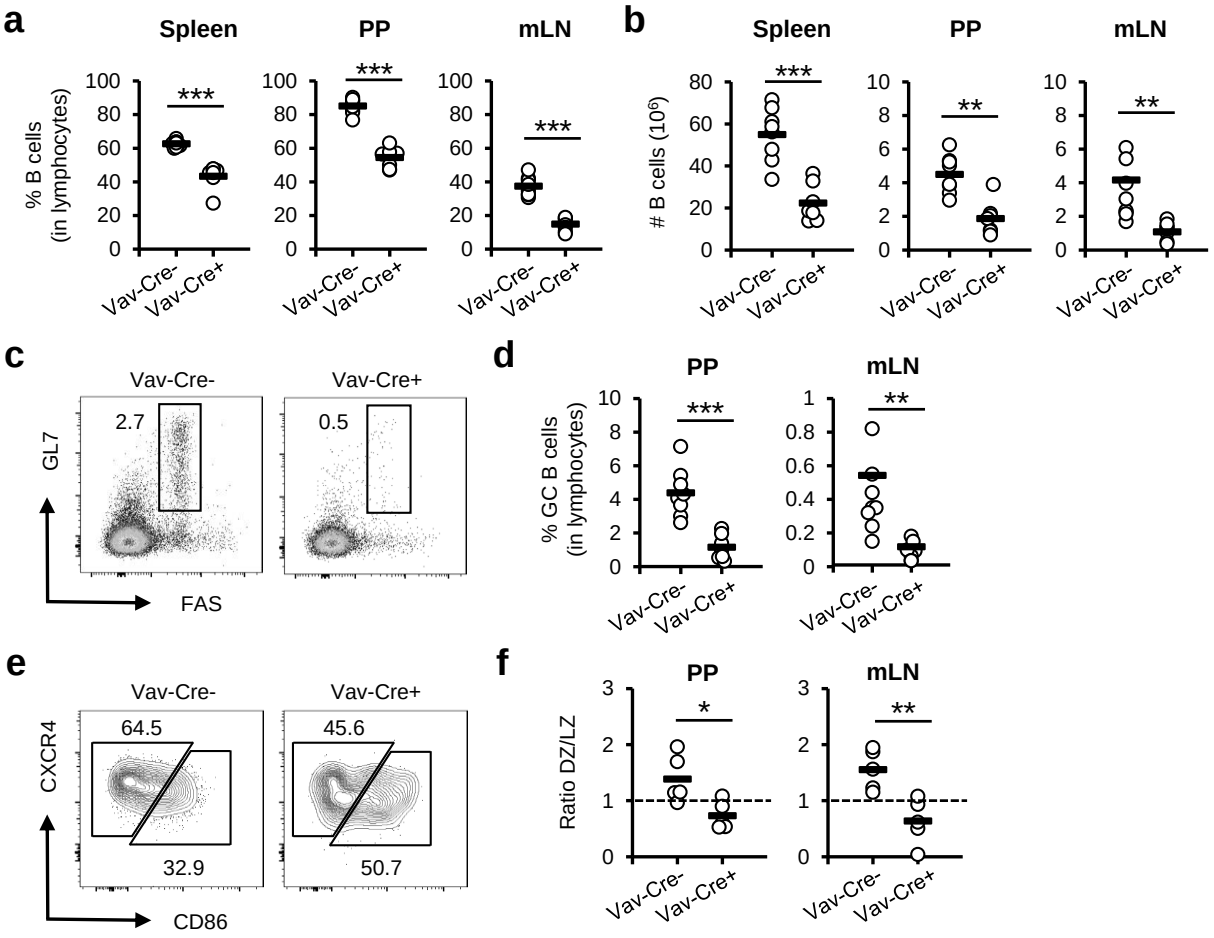
**(j)** Representative flow cytometry profiles of Tfh ($CXCR5^{Hi}PD\text{-}1^{Hi}$) from the spleen of immunized $Cre^{-}$.*Pou2af1*$^{fl/fl}$, Vav-$Cre^{+}$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^{+}$.*Pou2af1*$^{fl/fl}$ mice. **(k)** Percentage of Tfh cells in the spleen of the same groups of mice listed in **(j)** (n > 5, collected in four independent experiments)**. (l)** Percentage of GL-$7^{+}FAS^{+}$ GC B cells in the spleen, assessed by flow cytometry, of the same groups of mice listed in **(j)** (n > 5, collected in four independent experiments). Data information: Mann Whitney *U*-test for figures (**b-d, f-i)**. One-way ANOVA for figures **(k-l)**, NS, non-significant *P*-value > 0.05; *P*-values ** < 0.01; *** < 0.001.

Figure 5. **OCA-B is not an active transcriptional coactivator in $CD4^{+}$ and $CD8^{+}$ T cells and is required in B cells to facilitate Tfh differentiation.**

**(a)** Principal Component Analysis of top 500 most variable genes from $CD4^{+}$ and $CD8^{+}$ T cell RNA-Seq samples from the spleen of CD4-$Cre^{-}$.*Pou2af1*$^{fl/fl}$ (WT), CD4-$Cre^{+}$.*Pou2af1*$^{fl/+}$ (Het) and CD4-$Cre^{+}$.*Pou2af1*$^{fl/fl}$ (KO) mice. **(b)** Row-normalized (z-score) expression heatmap of selected DEGs, where each row represents a gene and columns represent individual samples. Red represents a higher relative expression for a given gene, while blue denotes a lower relative expression. Column annotation tracks represent sample cell type and genotype. **(c)** Representative flow cytometry profiles of CD45.1 and CD45.2 expression on B (left panel) and T (right panel) cells from the spleen of the chimeras. **(d)** Ratio of $CD45.1^{+}$ (WT genotype) over $CD45.2^{+}$ (KO genotype) cells in the spleen of the chimeras (n = 5, collected in two independent experiments). **(e)** Representative flow cytometry profiles of Tfh from the PP of Vav-$Cre^{-}$.*Pou2af1*$^{fl/fl}$, Vav-$Cre^{+}$.*Pou2af1*$^{fl/fl}$ and the bone marrow competitive chimeras, for which the profiles are separated based on $CD45.1^{+}$ and $CD45.2^{+}$ expression. **(f)** Percentage of Tfh in the spleen, PP and mLN of the chimeras, gated based on the expression of CD45.1 (B6.SJL) and

CD45.2 (Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$) to identify the origin and, thus the genotype, of the donor cells (n = 5, collected in two independent experiments). **(g)** Percentage of B cells in the PP and mLN of CD19-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD19-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, **(h)** percentage of Tfh in the PP and mLN of CD19-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD19-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice, and **(i-j)** CXCR5 and PD-1 RFI on CXCR5$^{+}$PD-1$^{+}$ CD4$^{+}$ T cells from the PP of CD19-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD19-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 7-10, collected in five independent experiments). Data information: Mann Whitney *U*-test, NS, non-significant, *P*-value > 0.05; *P*-values ** < 0.01; *** < 0.001.

Figure 1


a
Spleen
PP
mLN
% B cells (in lymphocytes)
***
Vav-Cre-
Vav-Cre+
b
# B cells (10^6)
**
c
Vav-Cre-
Vav-Cre+
2.7
0.5
GL7
FAS
d
% GC B cells (in lymphocytes)
e
64.5
45.6
32.9
50.7
CXCR4
CD86
f
Ratio DZ/LZ
*

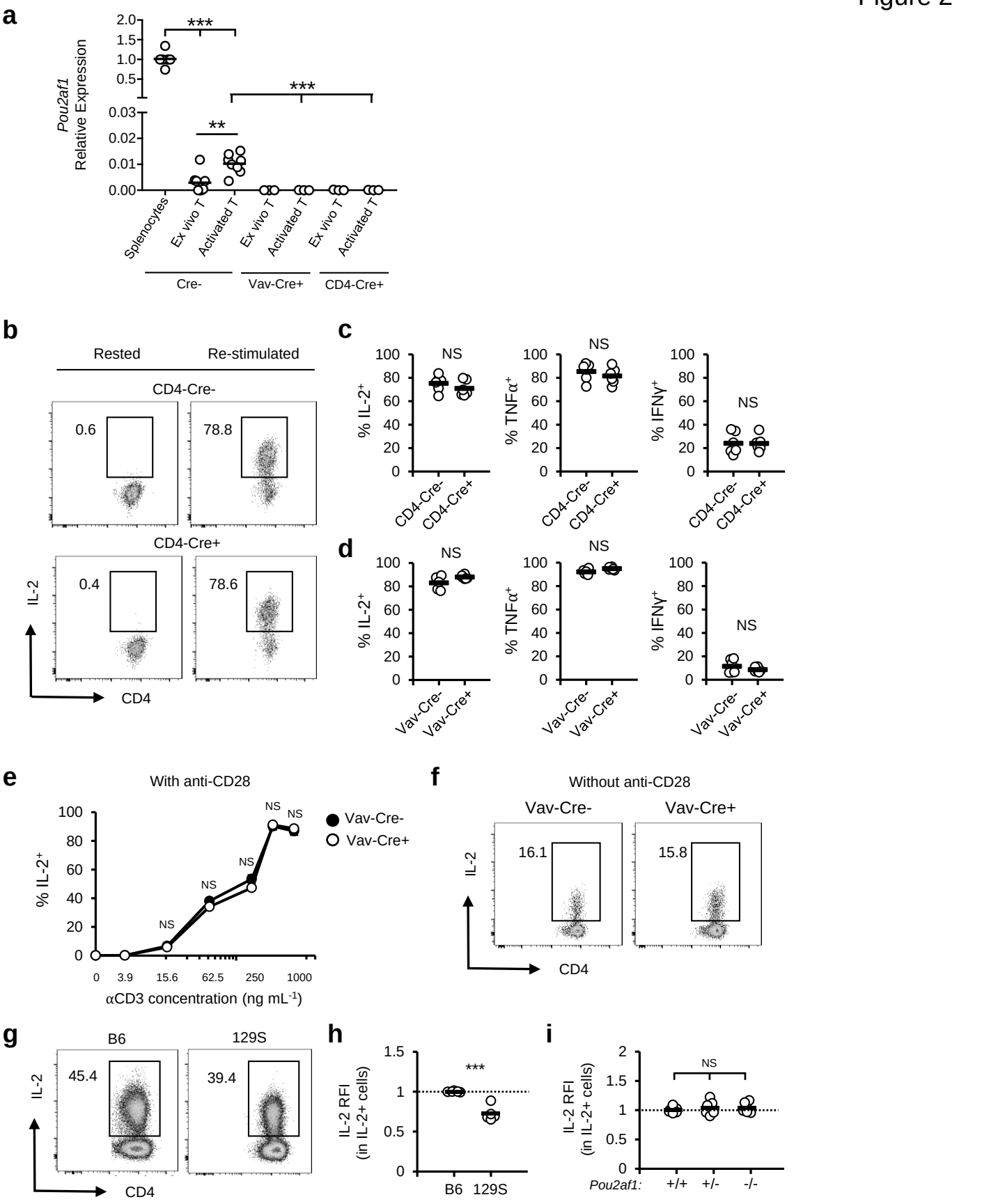

a
Pou2af1 Relative Expression
2.0
1.5
1.0
0.5
0.03
0.02
0.01
0.00
***
***
**
Splenocytes
Ex vivo T
Activated T
Ex vivo T
Activated T
Ex vivo T
Activated T
Cre-
Vav-Cre+
CD4-Cre+
b
Rested
Re-stimulated
CD4-Cre-
0.6
78.8
CD4-Cre+
0.4
78.6
IL-2
CD4
c
% IL-2+
% TNFα+
% IFNγ+
NS
CD4-Cre-
CD4-Cre+
d
% IL-2+
% TNFα+
% IFNγ+
NS
Vav-Cre-
Vav-Cre+
e
With anti-CD28
% IL-2+
NS
Vav-Cre-
Vav-Cre+
0
3.9
15.6
62.5
250
1000
αCD3 concentration (ng mL-1)
f
Without anti-CD28
Vav-Cre-
Vav-Cre+
IL-2
16.1
15.8
CD4
g
B6
129S
IL-2
45.4
39.4
CD4
h
IL-2 RFI (in IL-2+ cells)
***
B6
129S
i
IL-2 RFI (in IL-2+ cells)
NS
Pou2af1:
+/+
+/-
-/-

Figure 3

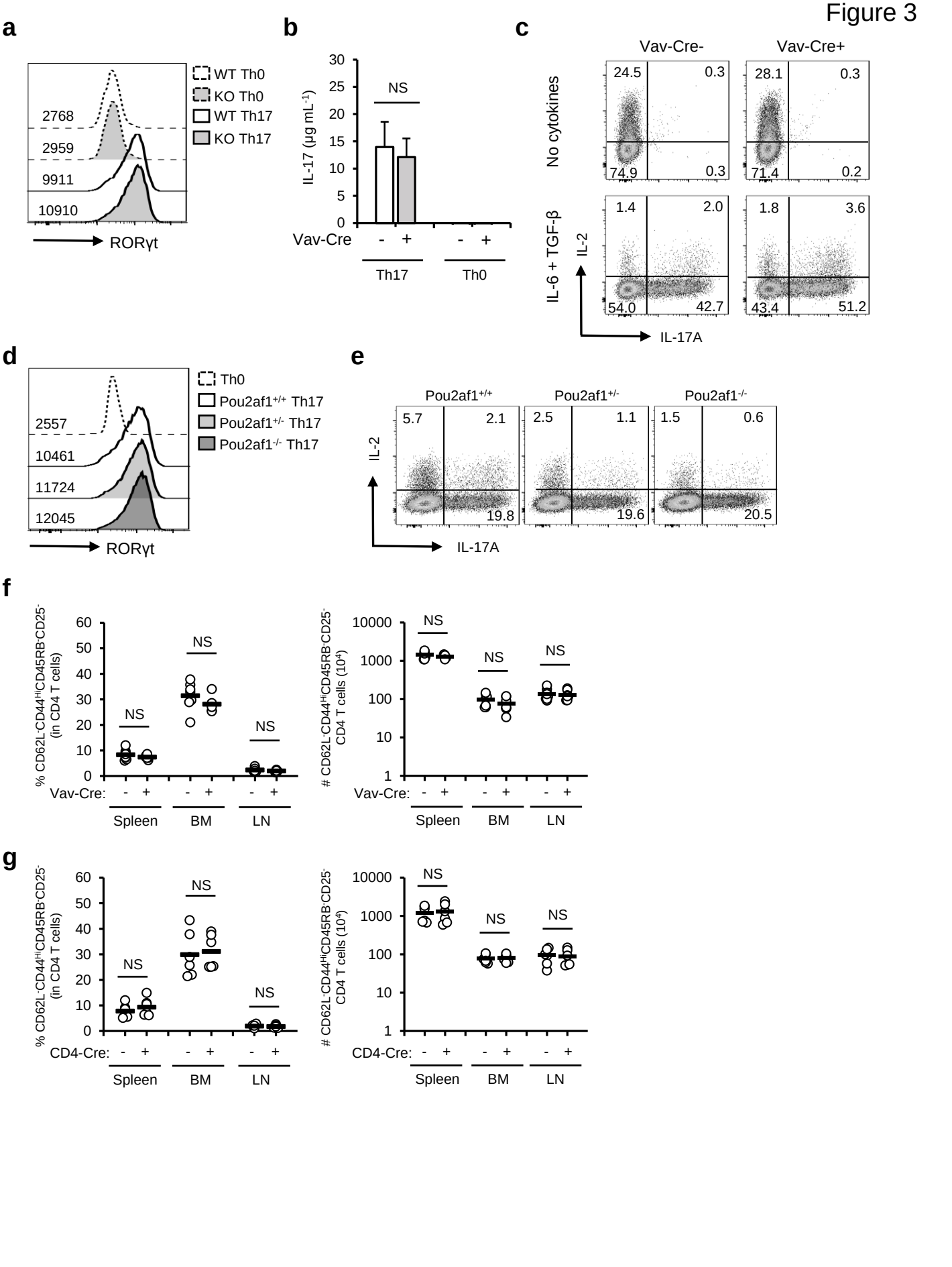

a
WT Th0
KO Th0
WT Th17
KO Th17
2768
2959
9911
10910
RORγt
b
IL-17 (μg mL-1)
NS
Vav-Cre
Th17
Th0
c
Vav-Cre-
Vav-Cre+
No cytokines
IL-6 + TGF-β
IL-2
IL-17A
24.5
0.3
74.9
0.3
28.1
0.3
71.4
0.2
1.4
2.0
54.0
42.7
1.8
3.6
43.4
51.2
d
Th0
Pou2af1+/+ Th17
Pou2af1+/- Th17
Pou2af1-/- Th17
2557
10461
11724
12045
RORγt
e
Pou2af1+/+
Pou2af1+/-
Pou2af1-/-
IL-2
IL-17A
5.7
2.1
19.8
2.5
1.1
19.6
1.5
0.6
20.5
f
% CD62L-CD44HiCD45RB-CD25- (in CD4 T cells)
# CD62L-CD44HiCD45RB-CD25- CD4 T cells (10⁴)
NS
Vav-Cre:
Spleen
BM
LN
g
% CD62L-CD44HiCD45RB-CD25- (in CD4 T cells)
# CD62L-CD44HiCD45RB-CD25- CD4 T cells (10⁴)
NS
CD4-Cre:
Spleen
BM
LN

Figure 4

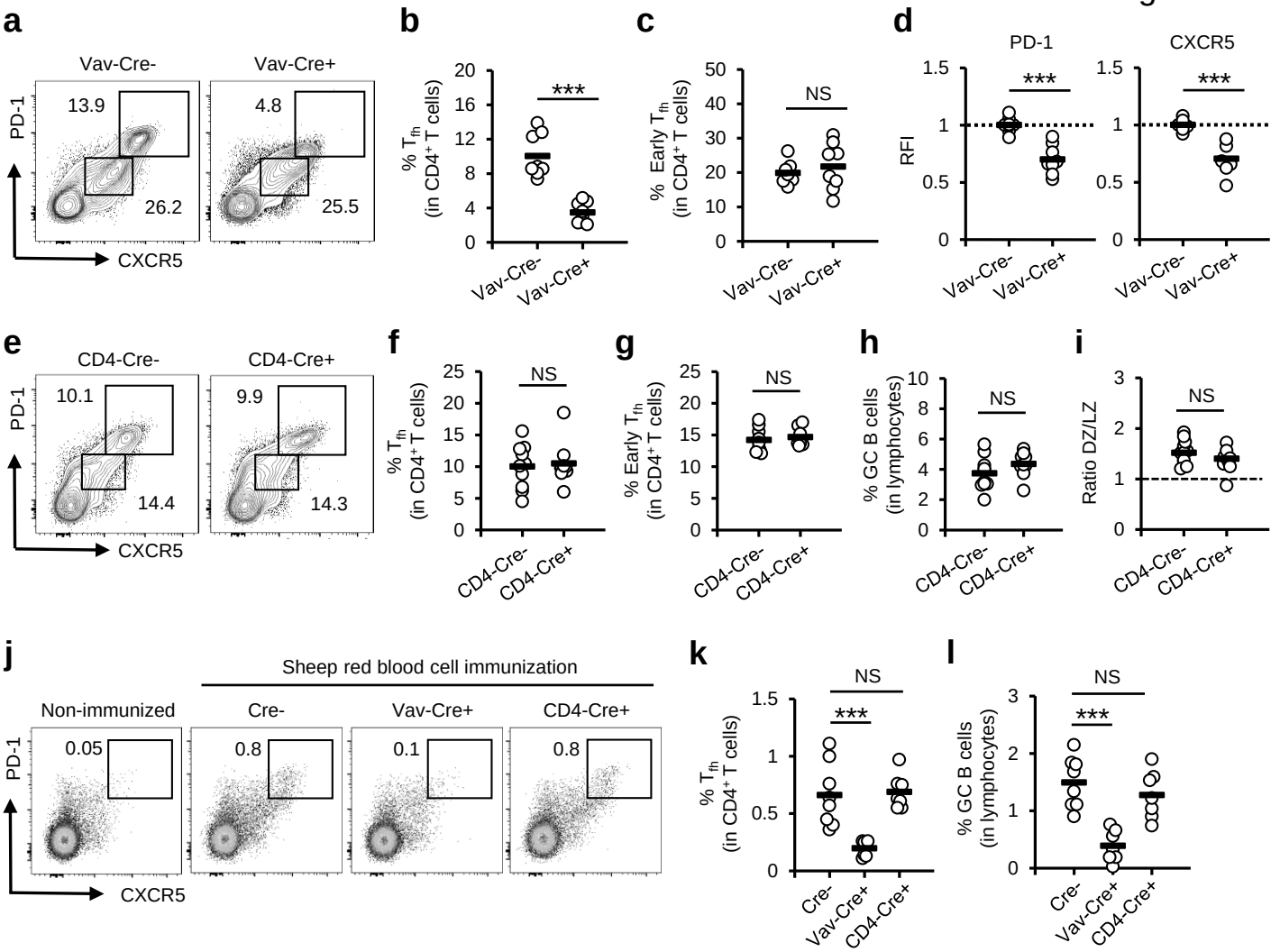
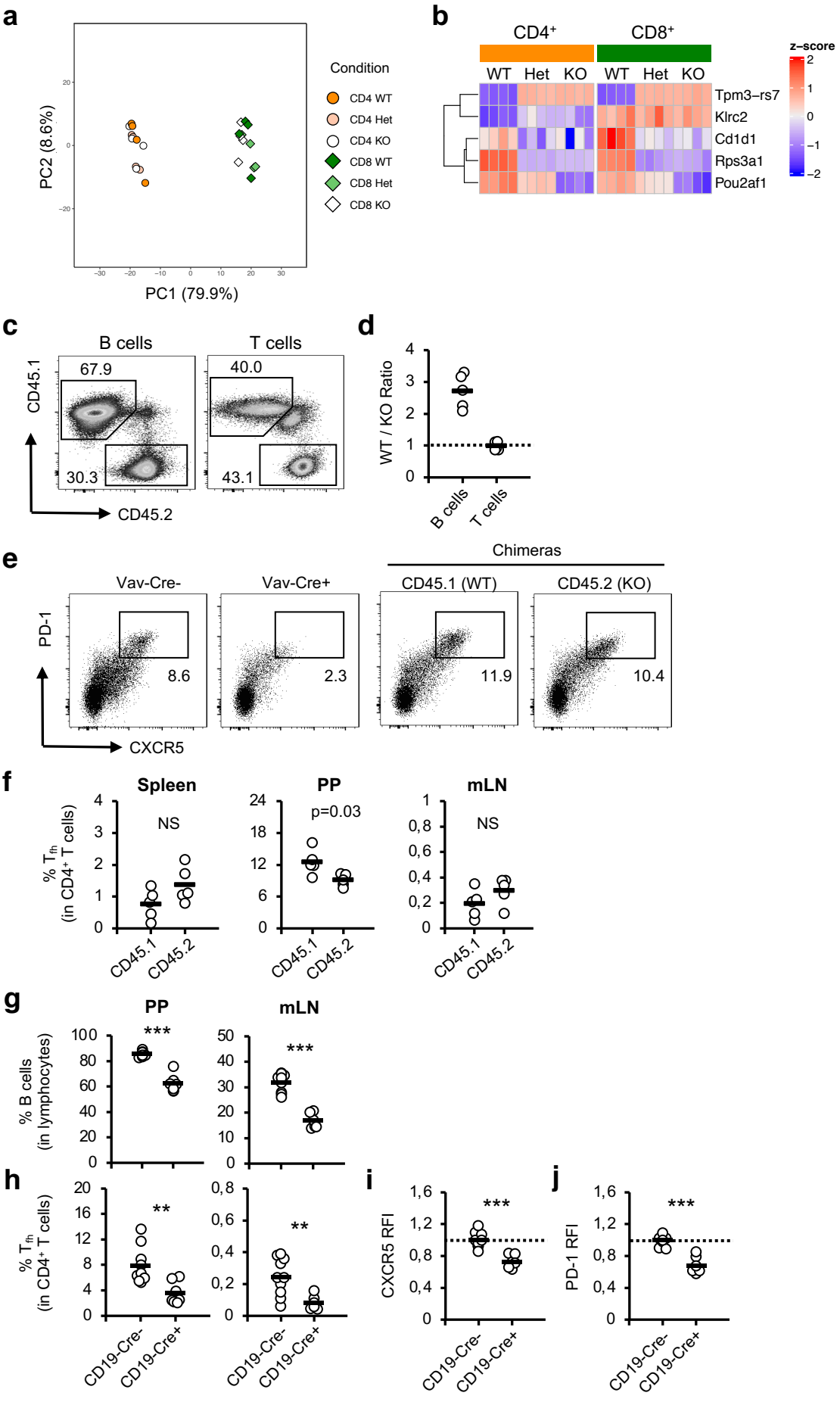
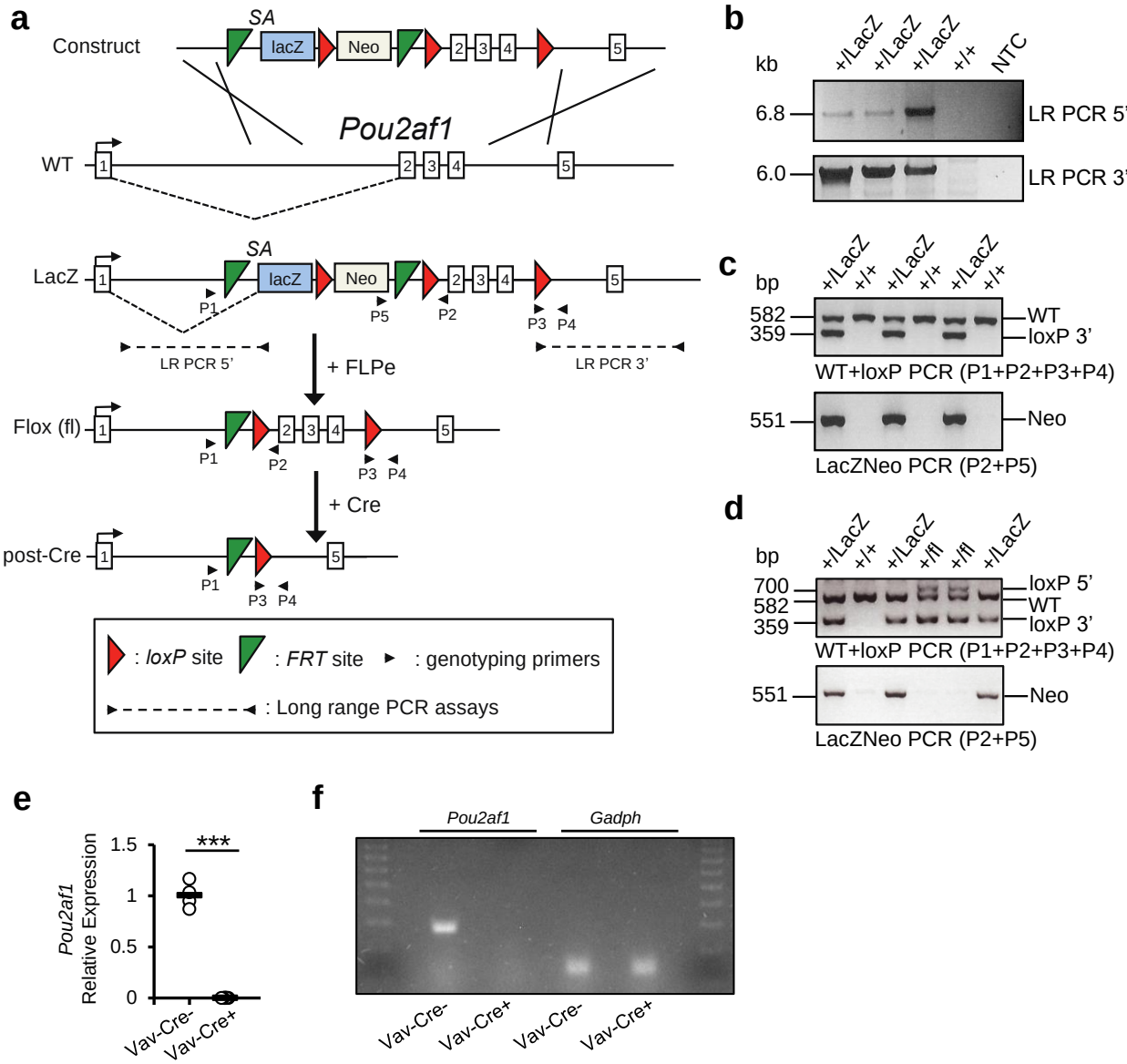
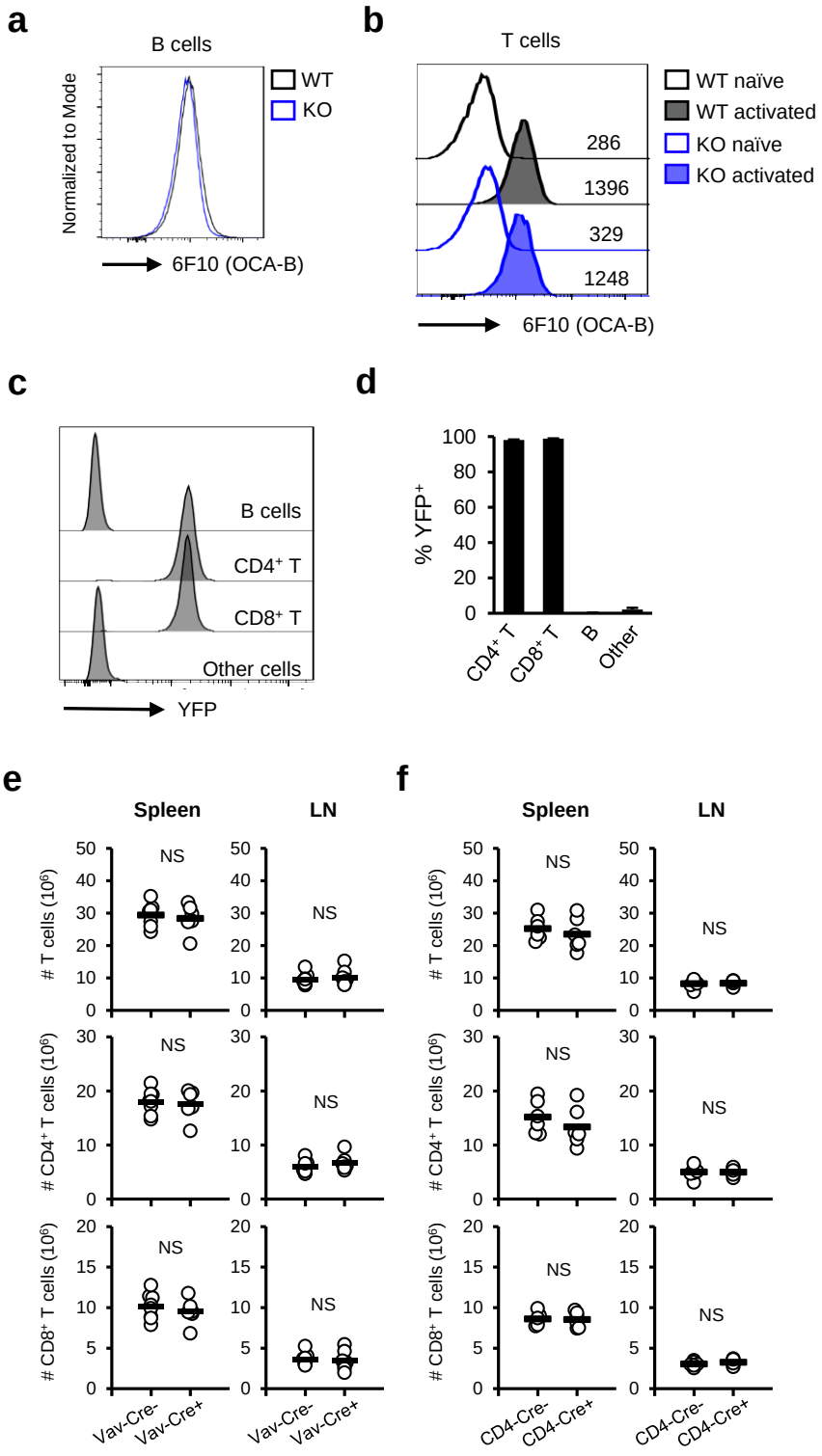

a
Vav-Cre-
Vav-Cre+
PD-1
CXCR5
13.9
26.2
4.8
25.5
b
% Tfh (in CD4+ T cells)
***
Vav-Cre-
Vav-Cre+
c
% Early Tfh (in CD4+ T cells)
NS
d
PD-1
CXCR5
RFI
***
e
CD4-Cre-
CD4-Cre+
10.1
14.4
9.9
14.3
f
g
h
% GC B cells (in lymphocytes)
i
Ratio DZ/LZ
j
Sheep red blood cell immunization
Non-immunized
Cre-
Vav-Cre+
CD4-Cre+
0.05
0.8
0.1
k
l

Figure 5

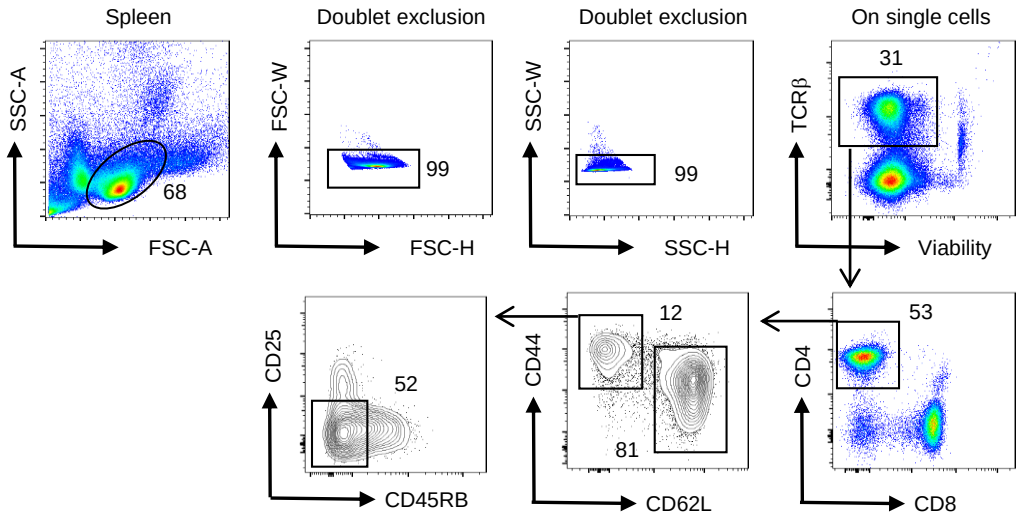

a
PC2 (8.6%)
PC1 (79.9%)
Condition
CD4 WT
CD4 Het
CD4 KO
CD8 WT
CD8 Het
CD8 KO
b
CD4+
CD8+
WT Het KO WT Het KO
Tpm3−rs7
Klrc2
Cd1d1
Rps3a1
Pou2af1
z−score
c
B cells
T cells
CD45.1
CD45.2
67.9
30.3
40.0
43.1
d
WT / KO Ratio
B cells
T cells
e
Chimeras
Vav-Cre-
Vav-Cre+
CD45.1 (WT)
CD45.2 (KO)
PD-1
CXCR5
8.6
2.3
11.9
10.4
f
Spleen
PP
mLN
% Tfh (in CD4+ T cells)
NS
p=0.03
NS
CD45.1
CD45.2
g
PP
mLN
% B cells (in lymphocytes)
***
h
% Tfh (in CD4+ T cells)
**
CD19-Cre-
CD19-Cre+
i
CXCR5 RFI
***
j
PD-1 RFI
***



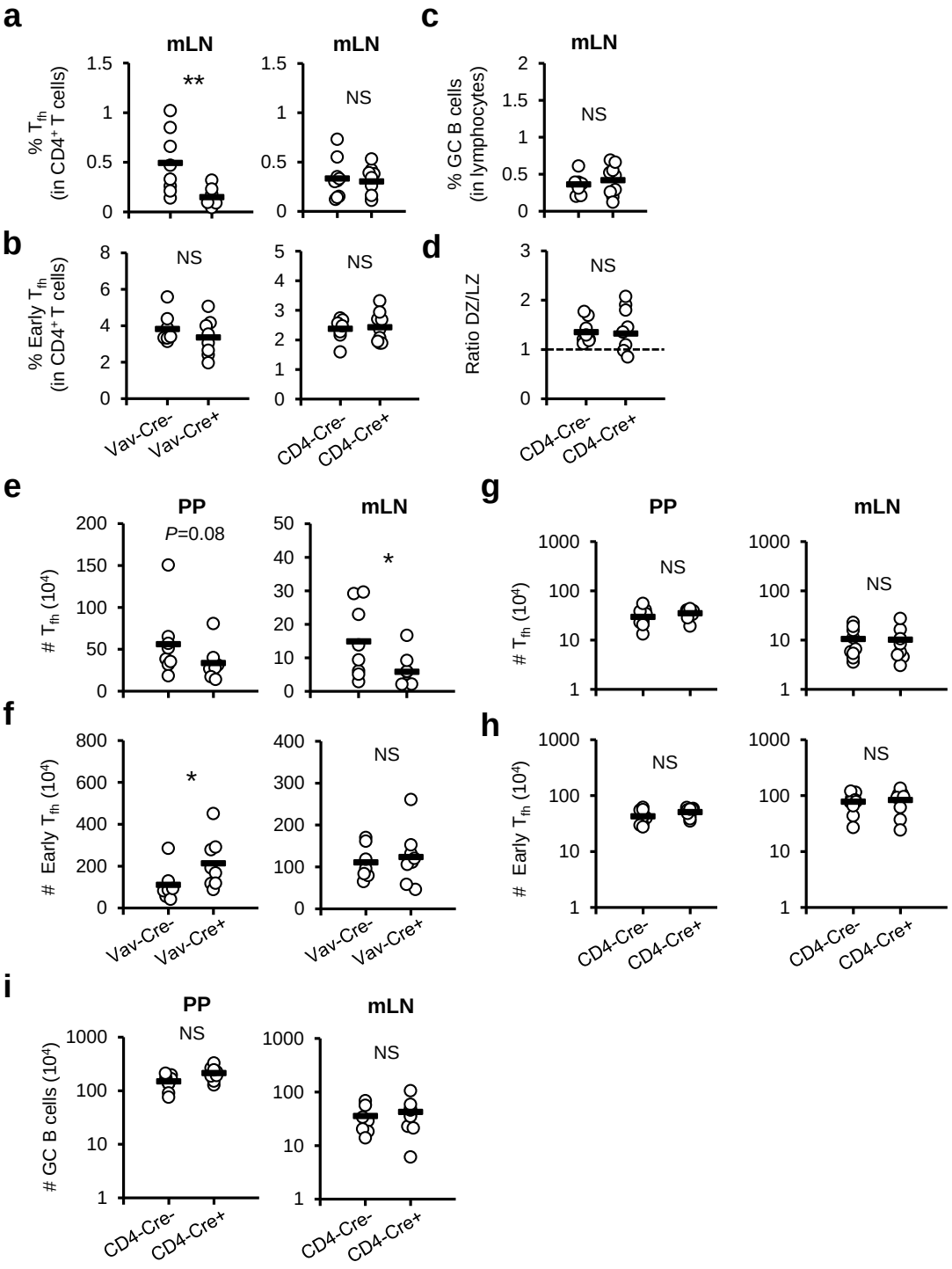

Supplementary figure 1. **Generation of a *Pou2af1*$^{fl/fl}$ mouse for cell specific deletion of *Pou2af1***. (**a**) Targeting strategy used to generate Pou2af1$^{+/LacZ}$ and Pou2af1$^{+/fl}$ mice. The position of the primers and long-range PCR assays used for genotyping are indicated. *SA:* splice acceptor site located in front of the lacZ cassette. (**b**) Long range PCR on mouse tail DNA confirming the proper targeting of the *Pou2af1* locus. (**c**) Genotyping PCR demonstrating the generation of *Pou2af1*$^{+/LacZ}$ mice. (**d**) Genotyping PCR demonstrating the generation of *Pou2af1*$^{+/fl}$ mice following breeding with *ACTBFLPe* mice. (**e**) *Pou2af1* relative expression in splenocytes from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 4, collected in two independent experiments), measured by RT-qPCR. *Gapdh* mRNA levels were used as loading control. (**f**) *Pou2af1* and *Gadph* RT-qPCR products were migrated on agarose gel for mice of the indicated genotypes. ***, *P*-value < 0.001.




Supplementary figure 2. **Validation of tools to the study of OCA-B expression and function in T cells. (a)** Representative flow cytometry profiles of 6F10 staining on B cells from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. **(b)** Representative flow cytometry profiles of 6F10 staining on naïve and activated T cells from Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice. MFI are indicated for each population. **(c)** Representative flow cytometry profiles of YFP expression on cells from the spleen of CD4-Cre$^{+}$.ROSA-YFP$^{+}$ mice. **(d)** Percentage of YFP$^{+}$ cells from the spleen of CD4-Cre$^{+}$.ROSA-YFP$^{+}$ mice (n = 3, collected in one experiment). **(e)** Numbers of total, CD4$^{+}$ and CD8$^{+}$ T cells from the spleen and a pool of inguinal, axial and brachial LN of Vav-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and Vav-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 6, collected in three independent experiments), and **(f)** CD4-Cre$^{-}$.*Pou2af1*$^{fl/fl}$ and CD4-Cre$^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 6, collected in three independent experiments). NS, non-significant, *P*-value > 0.05

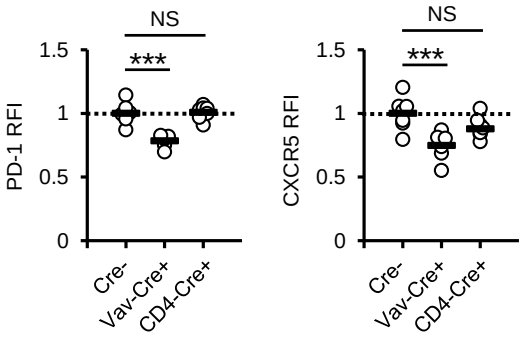



Supplementary figure 3. **Gating strategy for the analysis of CD4$^+$ T cells with a memory phenotype.** Representative flow cytometry profiles of cells from the spleen of Vav-Cre$^-$.*Pou2af1*$^{fl/fl}$ mice.

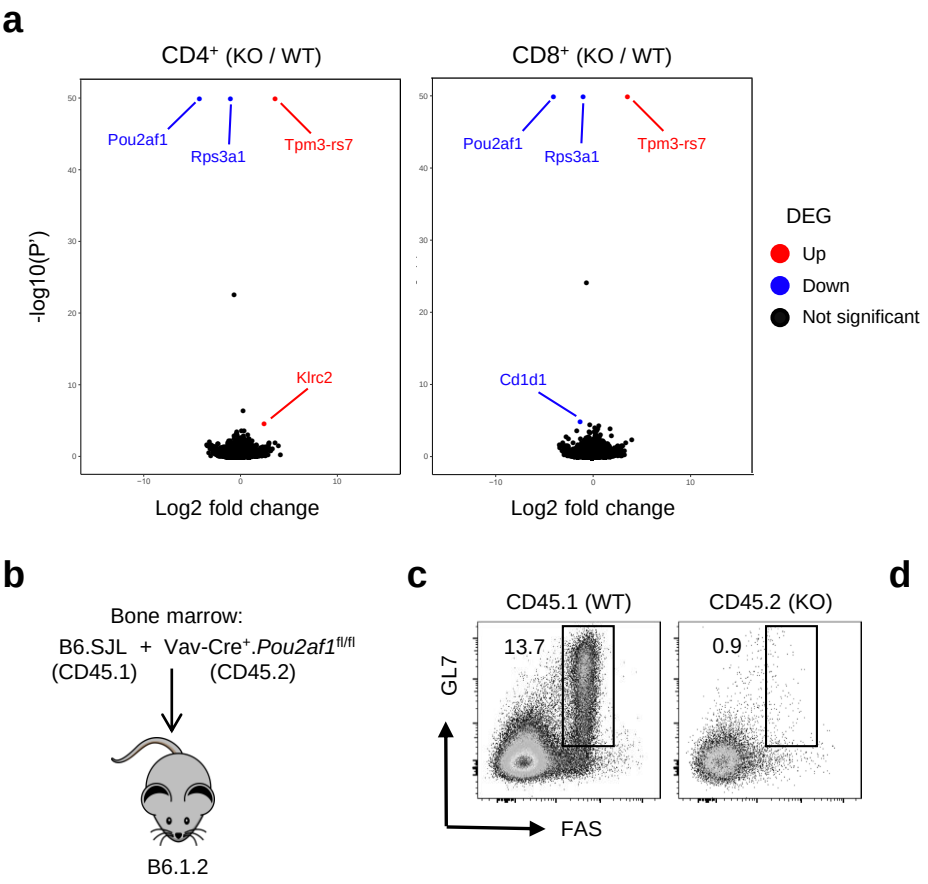



Supplementary figure 4. **Hematopoietic cell-specific but not T cell-specific *Pou2af1* deletion limits Tfh maturation. (a, b)** Percentage of **(a)** Tfh ($CXCR5^{Hi}PD\text{-}1^{Hi}$) and **(b)** early Tfh ($CXCR5^{Low}PD\text{-}1^{Low}$) in the mLN of Vav-$Cre^-$.*Pou2af1*$^{fl/fl}$, Vav-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice, CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 8-10, collected in three independent experiments). **(c)** Percentage of GC B cells in the mLN of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three independent experiments). **(d)** Ratio of dark zone ($CXCR4^{Hi}CD86^-$) over light zone ($CXCR4^{Low}CD86^+$) GC B cells from the mLN of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three independent experiments). **(e-h)** Absolute numbers of Tfh and early Tfh cells from the PP and mLN of **(e, f)** Vav-$Cre^-$.*Pou2af1*$^{fl/fl}$ and Vav-$Cre^+$.*Pou2af1*$^{fl/fl}$, and **(g, h)** of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three independent experiments). **(i)** Absolute numbers of GC B cells from the PP and mLN of CD4-$Cre^-$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^+$.*Pou2af1*$^{fl/fl}$ mice (n = 10, collected in three independent experiments). NS, non-significant, *P*-value > 0.05; *P*-values * < 0.05, ** <0.01.

**a**




Supplementary figure 5. ***Pou2af1*** **deletion impacts Tfh maturation and GC formation in a T cell-extrinsic manner. (a)** PD-1 and CXCR5 RFI on $CXCR5^{+}PD\text{-}1^{+}$ $CD4^{+}$ T cells from the spleen of the same groups of mice listed in Figure 4j, namely sheep red blood cell immunized $Cre^{-}$.*Pou2af1*$^{fl/fl}$, Vav-$Cre^{+}$.*Pou2af1*$^{fl/fl}$ and CD4-$Cre^{+}$.*Pou2af1*$^{fl/fl}$ mice (n = 6-8, collected in four independent experiments). NS, non-significant, *P*-value > 0.05; ***, *P*-value < 0.001.




Supplementary figure 6. **OCA-B is not an active transcriptional coactivator in CD4⁺ and CD8⁺ T cells.** **(a)** Volcano Plots of Differentially Expressed Genes (DEG) from CD4⁺ (left) and CD8⁺ (right) T cells of CD4-Cre⁻.*Pou2af1*$^{fl/fl}$ and CD4-Cre⁺.*Pou2af1*$^{fl/fl}$ mice at FDR <0.05 and Fold Change > 1.5. Genes upregulated in the KO are in red, downregulated in blue, and black if not significantly differentially expressed. **(b)** Schematic representation of the competitive bone marrow chimeras. **(c)** Representative flow cytometry profiles of GL7⁺FAS⁺ GC cells in B220⁺ B cells from the PP of the chimeras. **(d)** Percentage of GC B cells in the PP of the chimeras (n = 5, collected in two independent experiments). ***, $P < 0.001$.