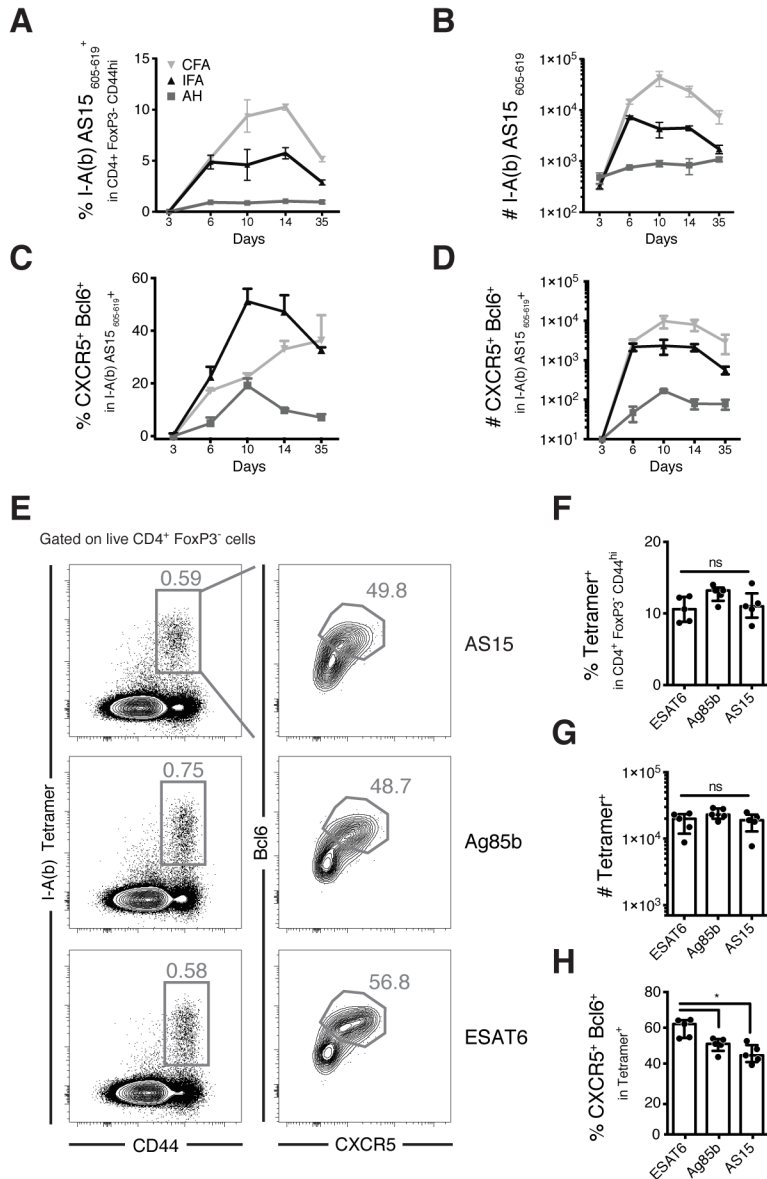


Supplementary figure 1



1

2 **Supplementary figure 1**

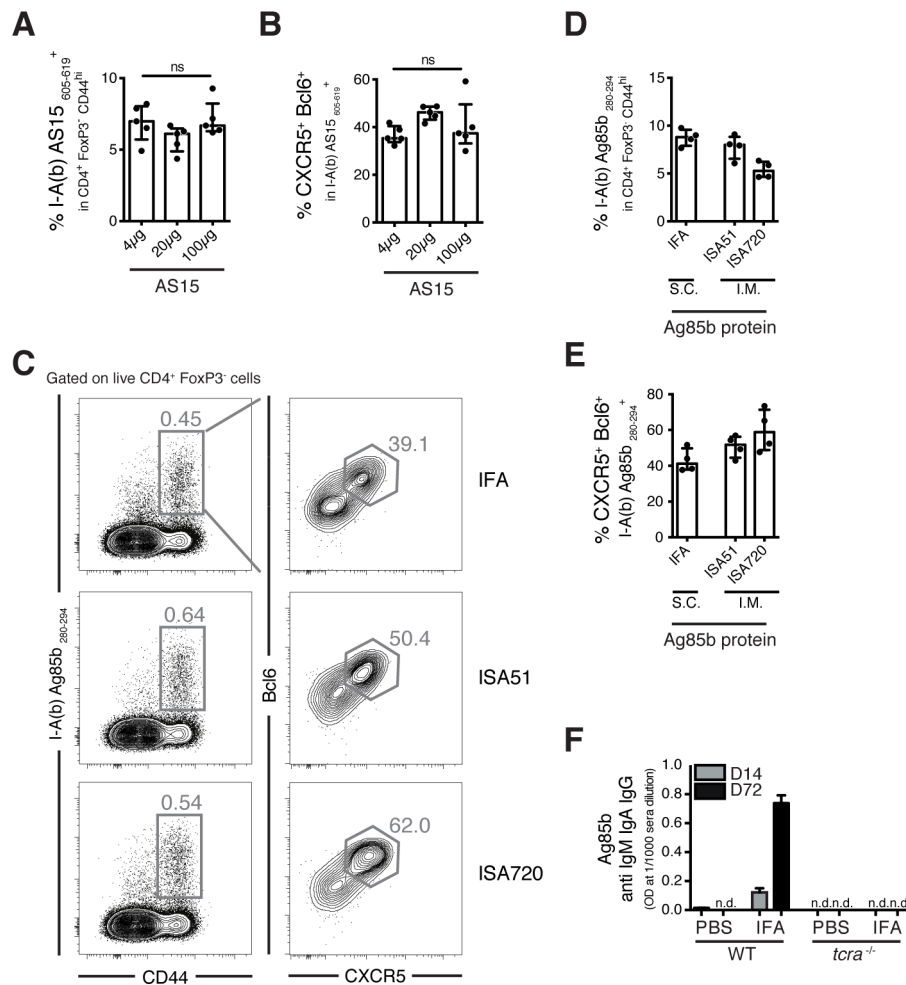
3 (A-D) Mice were immunized with AS15 peptide in Alhydrogel (AH) or emulsified in

4 IFA or CFA and draining lymph nodes (dLN) were harvested at the indicated time points.

5 (A) Frequencies and (B) total numbers of AS15 MHCII tetramer<sup>+</sup> cells within the CD4<sup>+</sup>

1 Foxp3<sup>-</sup> CD44<sup>hi</sup> population. **(C)** Frequencies and **(D)** total numbers of CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh  
2 cells within the tetramer<sup>+</sup> population cells. **(E-H)** Mice were immunized with AS15,  
3 ESAT6 or Ag85b peptides emulsified in IFA. **(E)** Representative flow cytometry plots,  
4 **(F)** frequencies and **(G)** total numbers of the respective MHCII tetramers staining. **(H)**  
5 Frequencies of CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells within the tetramer<sup>+</sup> populations. Data shown are  
6 representative of 2 experiments where each dot represents an individual mouse. Data  
7 were analysed using the non-parametric Mann–Whitney test.; ns: not significant.;  
8 \*P<0.05

## Supplementary figure 2



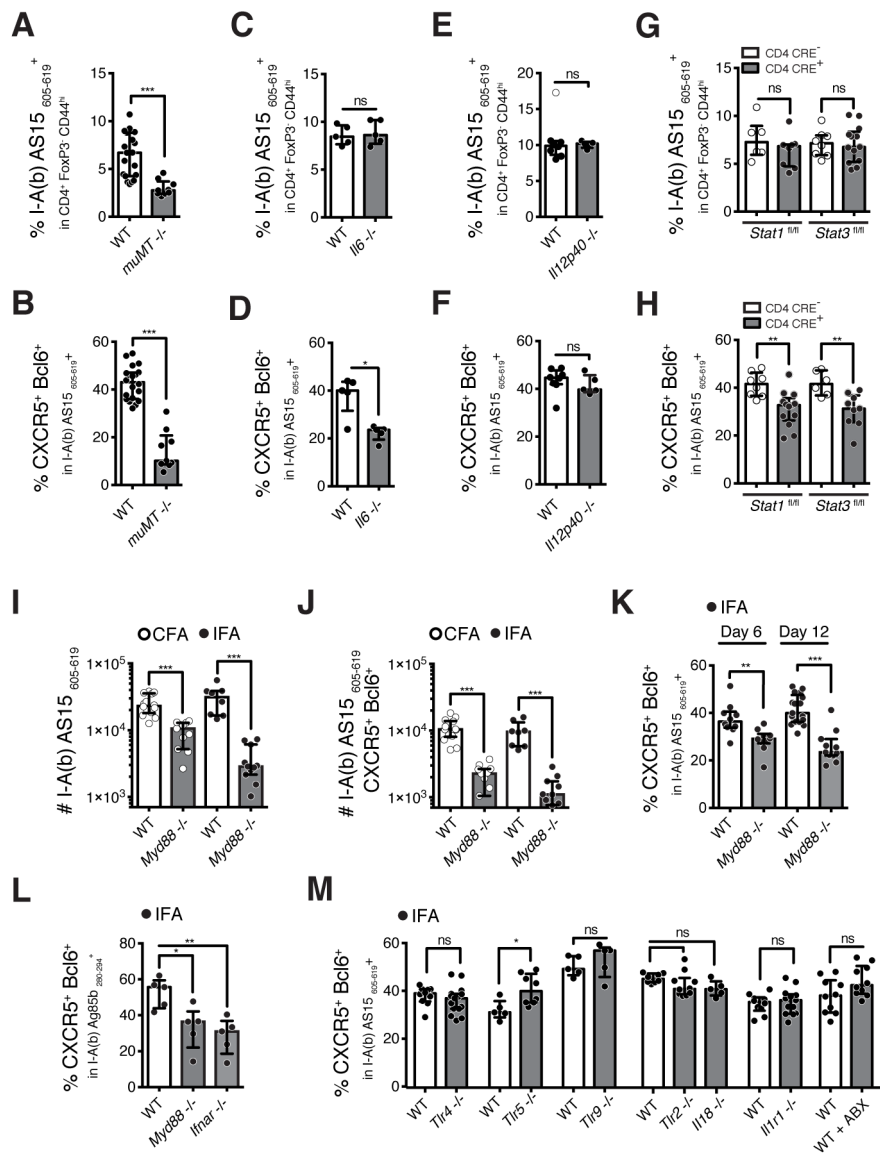
1

2 **Supplementary figure 2**

3 (A-B) WT mice were immunized with 4μg, 20μg or 100μg of AS15 peptide emulsified in  
 4 IFA. (A) Frequencies of AS15 MHCII tetramer<sup>+</sup> cells within the CD4<sup>+</sup> Foxp3<sup>-</sup> CD44<sup>hi</sup>  
 5 population and (B) frequencies of CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells within the tetramer<sup>+</sup>  
 6 population cells. (C-E) WT mice were immunized with Ag85 protein emulsified in IFA  
 7 (S.C.: sub-cutaneously), Montanide ISA 51 VG<sup>TM</sup> (ISA51) and Montanide ISA 720

1 VG<sup>TM</sup> (ISA720) (I.M.: intra-muscularly). (C) Representative flow cytometry plots,  
2 frequencies of Ag85b MHCII tetramer<sup>+</sup> cells within the CD4<sup>+</sup> Foxp3<sup>-</sup> CD44<sup>hi</sup> population  
3 and (E) frequencies of CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells within the tetramer<sup>+</sup> population cells. (F)  
4 WT and *Tcra*<sup>-/-</sup> mice were immunized S.C. with 10μg Ag85b protein emulsified in IFA  
5 and anti-Ag85b antibody were measured in the serum on day 14 (grey bars) and on day  
6 72 (black bars). For day 72 titers, mice received an I.P. immunization with 10μg Ag85b  
7 protein emulsified in IFA on Day 21. Data shown are representative of 2 experiments  
8 where each dot represents an individual mouse.

## Supplementary figure 3



1

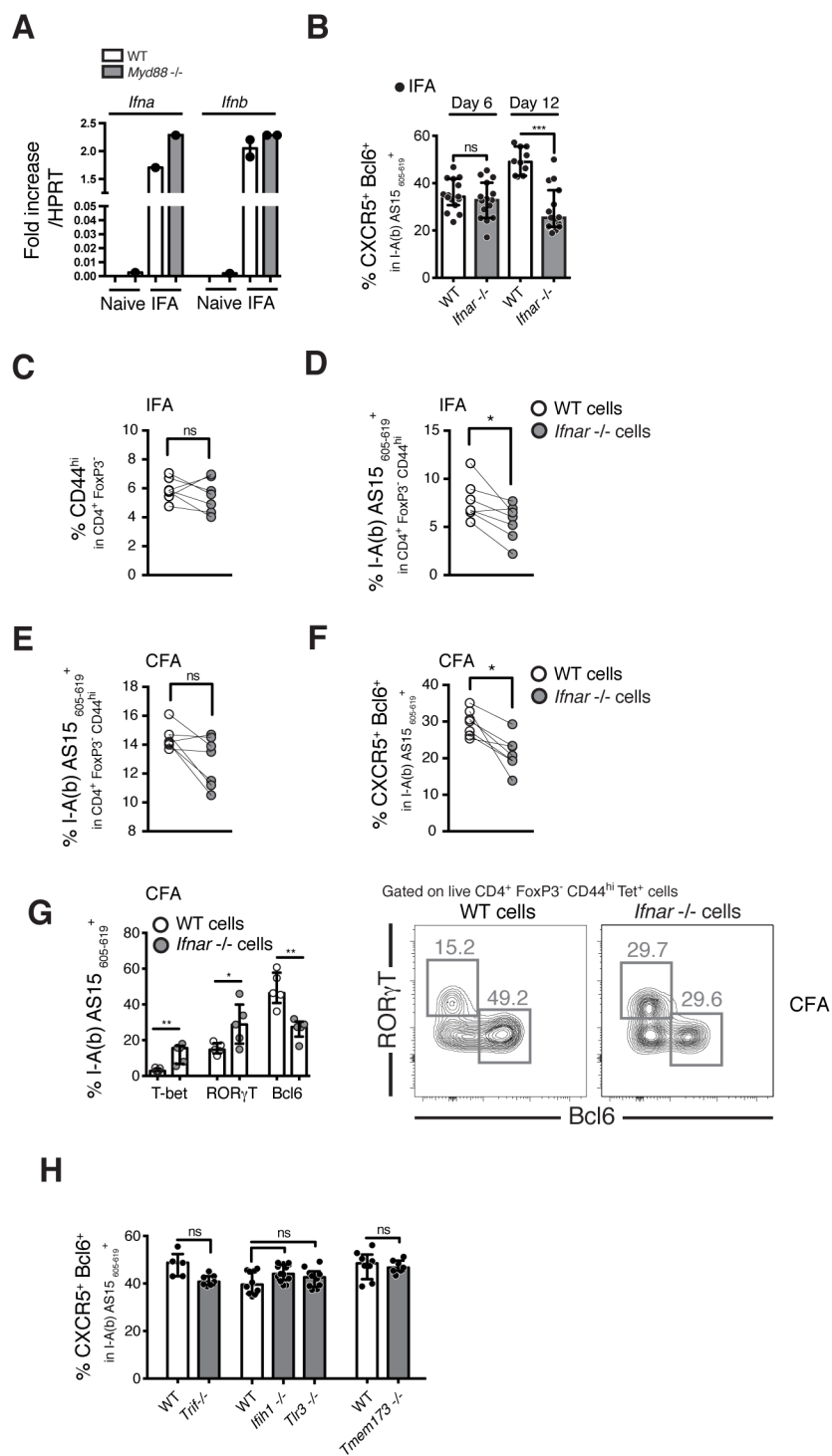
## 2 Supplementary figure 3

3 WT, mature B cell deficient *muMT*<sup>-/-</sup>, *Il6*<sup>-/-</sup>, *Il12p40*<sup>-/-</sup>, Stat1 flox/flox CD4<sup>CRE</sup> or Stat34 flox/flox CD4<sup>CRE</sup> mice were immunized with AS15 peptide emulsified in IFA.5 Frequencies of (A, C, E, G) AS15 MHCII tetramer<sup>+</sup> cells within the CD4<sup>+</sup> Foxp3<sup>-</sup> CD44<sup>hi</sup>

1 population and of **(B, D, F, H)** CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells within the tetramer<sup>+</sup> population.  
2 **(I-J)** WT and *Myd88*<sup>-/-</sup> mice were immunized with AS15 peptide emulsified in IFA or  
3 CFA and dLNs were harvested 10-14 days later **(I)** Total numbers of AS15 MHCII  
4 tetramer<sup>+</sup> cells and **(J)** of tetramer<sup>+</sup> CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells. **(K)** WT and *Myd88*<sup>-/-</sup> mice  
5 were immunized with AS15 peptide emulsified in IFA and dLNs were harvested 6 or 12  
6 days later. Histograms show the frequency of tetramer<sup>+</sup> CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells. **(L)**  
7 WT, *Myd88*<sup>-/-</sup> and *Ifnar*<sup>-/-</sup> mice were immunized with Ag85b protein emulsified in IFA  
8 and dLNs were harvested 12 days later. Histograms show the frequency of tetramer<sup>+</sup>  
9 CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells. **(M)** Frequencies of CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells within the  
10 tetramer<sup>+</sup> population from separate experiments using IFA-immunized WT, *Tlr4*<sup>-/-</sup>,  
11 *Tlr5*<sup>-/-</sup>, *Tlr9*<sup>-/-</sup>, *Tlr2*<sup>-/-</sup>, *Il18*<sup>-/-</sup> or *Il1r1*<sup>-/-</sup> mice as well as antibiotic-treated WT animals  
12 (ABX). Data shown are representative of 2-3 experiments where each dot represents an  
13 individual mouse. Data were analysed using the non-parametric Mann–Whitney test.; ns:  
14 non significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

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## Supplementary figure 4



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2

**1 Supplementary figure 4**

2 **(A)** WT or *Myd88*<sup>-/-</sup> inguinal LNs from naïve mice or 10 days after AS15 peptide  
3 emulsified in IFA were harvested and the levels of *Ifna* and *Ifnb* mRNA was quantified  
4 by qPCR and expressed as fold increase over their receptive expressions of HPRT. **(B)**  
5 WT and *Ifnar*<sup>-/-</sup> mice were immunized with AS15 peptide emulsified in IFA and dLNs  
6 were harvested 6 or 12 days later. Histograms show the frequency of tetramer<sup>+</sup> CXCR5<sup>+</sup>  
7 Bcl6<sup>+</sup> Tfh cells. **(C-G)** WT/*Ifnar*<sup>-/-</sup> mixed-bone marrow chimera mice were immunized  
8 with AS15 in IFA **(C-D)** or CFA **(E-G)** and the dLN collected after 12 days. The  
9 frequencies of WT (white dots) and *Ifnar*<sup>-/-</sup> (grey dots) of **(C)** activated CD44<sup>hi</sup> cells, **(D-**  
10 **E)** AS15 MHCII tetramer<sup>+</sup> cells within the CD4<sup>+</sup> Foxp3<sup>-</sup> CD44<sup>hi</sup> population, **(F)** tetramer<sup>+</sup>  
11 CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells or **(G)** T-bet<sup>+</sup>, RORγT<sup>+</sup> or Bcl6<sup>+</sup> within the tetramer<sup>+</sup> population  
12 (with representative flow cytometry plots) are shown. Each line represents a single  
13 recipient animal. **(H)** WT, *Trif*<sup>-/-</sup>, *Tlr3*<sup>-/-</sup>, *Ifih1*<sup>-/-</sup> (MDA5) and *Tmem173*<sup>-/-</sup> (STING)  
14 mice were immunized with AS15 peptide emulsified in IFA and the histograms show the  
15 frequencies of tetramer<sup>+</sup> CXCR5<sup>+</sup> Bcl6<sup>+</sup> Tfh cells. Data shown are representative of 2  
16 experiments where each dot represents an individual mouse. Data were analysed using  
17 the non-parametric Mann–Whitney test.; ns: non significant; \*P<0.05; \*\*P<0.01.