

Fig S1. Cell viability, IgM production and gating strategy. Splenic mouse B cells were isolated by MACS and cultured (5x10⁵ cells/mL) for 5 days, either unstimulated or stimulated with LPS+IL-4 or anti-CD40+IL-4 in the presence of Zymosan, HKCA yeast or HKCA hyphae. (A) On day 3 and 5 of culture, viability of the cells was analysed by flow cytometry. The viability was determined by percentage of living (DAPI negative) cells among B220+ single lymphocytes. (B) Splenic mouse B cells were cultured either unstimulated or stimulated with LPS+IL-4 or anti-CD40+IL-4 in the presence of Zymosan, HKCA yeast or HKCA hyphae. The concentration of secreted IgM in cell culture supernatants was measured after 3 and 5 days of culture by ELISA. Data represent mean ± Standard error of the mean (SEM) of 4 mice, with triplicate measurements performed for each mouse. *p<0.05, **p<0.005, ***p<0.001 (C) Gating strategy for viability assessment and IgG1 class switch recombination analysis. On day 3 and 5 of culture, the cells were analysed by flow cytometry to determine the percentage of IgG1-positive cells. B cells were first gated to exclude other cells or particles (B220t). Debris and doublets were excluded in subsequent lymphocyte and single cell gates. Dead cells were excluded by positive DAPI staining and the percentage of IgG1+ cells was obtained within this gate. Representative plots show cells stimulated with anti-CD40+IL-4 in the presence of HKCA hyphae recovered after 5 days in culture. (D) Gating strategy for CD86+ B cells. Splenic mouse B cells were isolated by MACS and cultured (5x105 cells/mL) for 6h or 8h, either unstimulated or stimulated with LPS+IL-4 or anti-CD40+IL-4 in the presence of Zymosan, HKCA yeast or HKCA hyphae. After 6h and 8h, the cells were analysed by flow cytometry to determine the percentage of activated B cells. After gating for lymphocytes, dead cells were excluded by positive TOPRO staining. Within this gate, the percentage of B220+ CD86+ cells was obtained. Representative plots show cells stimulated with LPS+IL-4 in the presence of Zymosan after 6 hours in culture. (E) Isotype control for CD86* B cells. To ensure the observed activation is due to specific antibody binding to the target, experiments were performed with an additional isotype control (IgG2b kappa-PE). Representative FACS plots show activated B cells among B220⁺ cells directly after isolation (0h) and 6h stimulation with LPS+IL-4 in the presence of Zymosan.

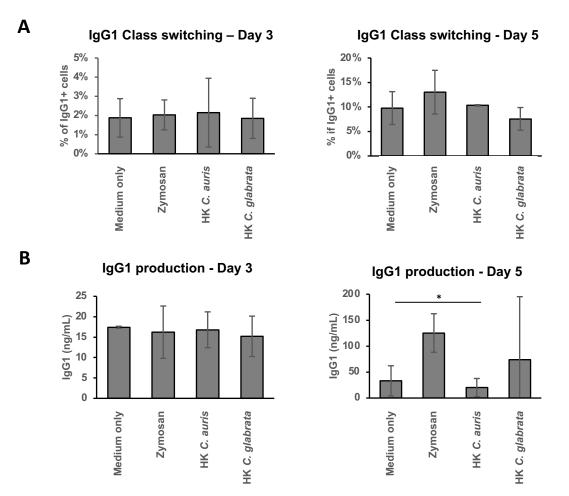


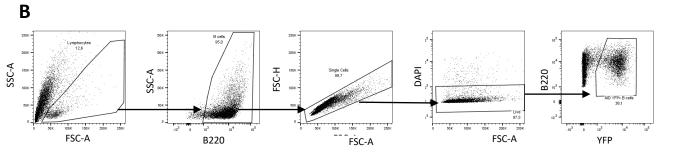
Fig. S2. B cell stimulation with heat-killed (HK) *C. auris* and *C. glabrata* yeast does not lead to increased IgG1 class switching or antibody production. Splenic mouse B cells were cultured with anti-CD40+IL-4 in the presence of Zymosan, HK *C. auris* yeast or HK *C. glabrata* yeast . (A) IgG1 class switching. Percentage of IgG1-positive cells among live B cells was measured by flow cytometry after 3 and 5 days of culture. For gating strategy see Figure S1C. (B) IgG1 production. The concentration of secreted IgG1 in cell culture supernatants was measured after 3 and 5 days of culture by ELISA. Data represent mean ± Standard error of the mean (SEM) of 3 mice, with triplicate measurements performed for each mouse. *p<0.05, **p<0.005, ***p<0.001



AID-Rosa26YFPcre/+ fl/+ mouse

Before AID activation

AID locus exons 2 - 5 Rosa26 locus AID locus exons 2 - 5 Rosa26 locus YFP loxP IoxP



After AID activation

Fig. S3. Scheme and gating strategy of AID-Rosa26YFP^{cre/+ fl/+} **mice.** (A) Schematic representation of the modified loci in AIDCre-Rosa26YFP mice before and after AID activation. Splenic B cells from both AID-Rosa26YFP^{cre/+ fl/+} and AID-Rosa26YFP^{cre/+ fl/+} mice were used. (B) Gating strategy for AID activation analysis. Splenic mouse B cells were isolated by MACS and cultured (5x10⁵ cells/mL) for 3 or 5 days, either unstimulated or stimulated with LPS+IL-4 or anti-CD40+IL-4 in the presence of Zymosan, HKCA yeast or HKCA hyphae. On day 3 and 5 of culture, the cells were analysed by flow cytometry to determine the percentage of YFP-positive cells. B cells were gated among lymphocytes. B cells were subsequently gated for single cells and dead cells were excluded by positive DAPI staining. The percentage of B220⁺ YFP⁺ cells was obtained within this gate. Representative plots show cells stimulated with LPS+IL-4 in the presence of Zymosan recovered after 5 days in culture.

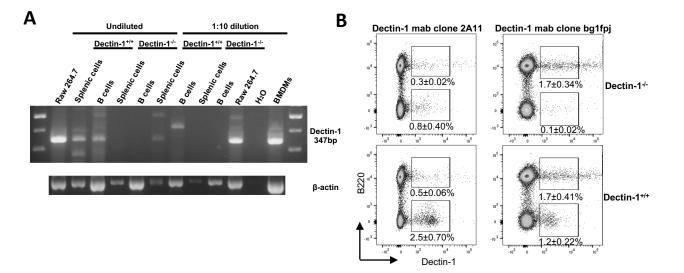


Fig S4. Dectin-1 expression in murine B cells. (A) The presence of dectin-1 mRNA was analysed by RT-PCR in splenic and B cells from Dectin-1^{-/-} and Dectin-1^{+/+} mice, the macrophage cell line RAW 264.7 and bone marrow-derived macrophages (BMDMs). β-actin was used as a loading control. (B) The expression of dectin-1 on the surface of mouse B cells was analysed by flow cytometry using two different antibody clones - bg1fpj (PE-Cy7) and 2A11 (FITC). The populations of interest were gated after exclusion of debris, dead cells and doublets. The Dectin-1 gating strategy was chosen based on the population of Dectin-1-positive cells (non-B cells) stained with the anti-Dectin-1 antibody clone 2A11. Representative dot plots are shown. Data represent mean \pm SD of 3 mice per genotype.

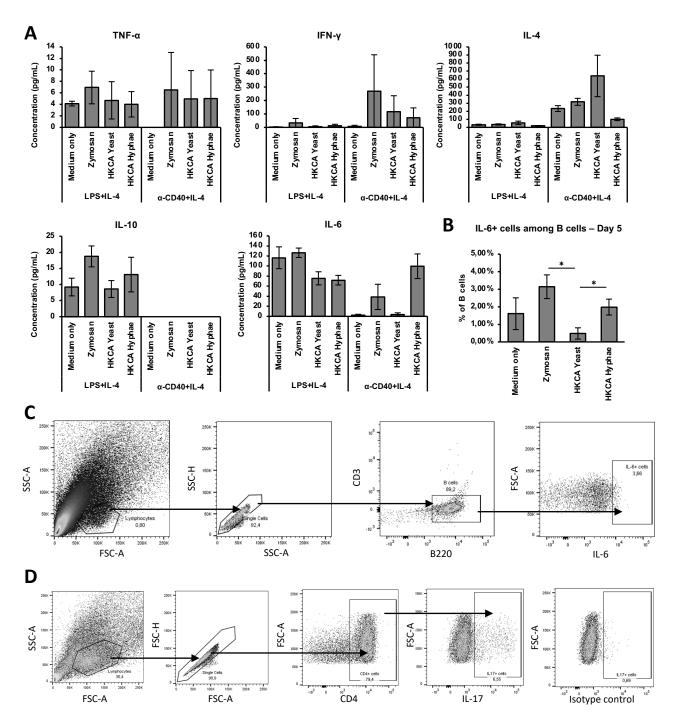


Fig S5. Screening of cytokines produced by B cells and gating strategy of T_H17 differentiation experiments. (A) Splenic B cells from wildtype mice were stimulated for 5 days with LPS+IL-4 or anti-CD40+IL-4 in the presence of Zymosan, HKCA yeast or HKCA hyphae. Supernatants recovered after 5 days of culture were screened for differences in their secreted cytokine profile using the bead-based immunoassay LEGENDplex™. Among the tested cytokines (TNF-α, IFN-γ, IL-2, IL-5, IL-4, IL-6, IL-10 and IL-13) no IL-2, 5 or 13 was detected. Data represent mean ± SEM of 3 mice, with duplicate measurements performed for each mouse. (B) Intracellular IL-6. Splenic B cells from wildtype mice were stimulated with anti-CD40+IL-4 in the presence of hyphae. Percentage of IL-6 positive cells among B cells was measured by flow cytometry after 5 days of culture in presence of Zymosan, HKCA yeast or HKCA hyphae. Data represent mean ± SEM of 3 mice, with duplicate measurements performed for each mouse. *p<0.05, **p<0.005, ***p<0.001 (C) Gating strategy for the IL-6 staining of splenic B cells. Representative FACS plots show IL-6 + cells among B220+ cells after 5 days of culture in the presence of Zymosan. (D) Splenic B cells and T cells from wildtype mice were stimulated for 4 days with anti-CD3e and anti-IgM in the presence of Zymosan, HKCA hyphae or a T_H17 differentiation inducing cocktail (anti-IL-4, anti-INF-γ, anti-IL-12, TGF-β1 and IL-6). The cells were analysed by flow cytometry to determine the percentage of IL-17 + cells. Lymphocytes were first gated to exclude debris and particles with regard to their size and granularity. Cells were subsequently gated for single cells and CD4+ cells and the percentage of IL-17+ cells was obtained within this gate. An isotype control for the IL-17 staining antibody was used to verify the analysis. Representative plots show cells after 4 days of culture in the presence of the T_H17 differentiation inducing cocktail.