and emerging data illustrates that environmental factors

disparate.[37]

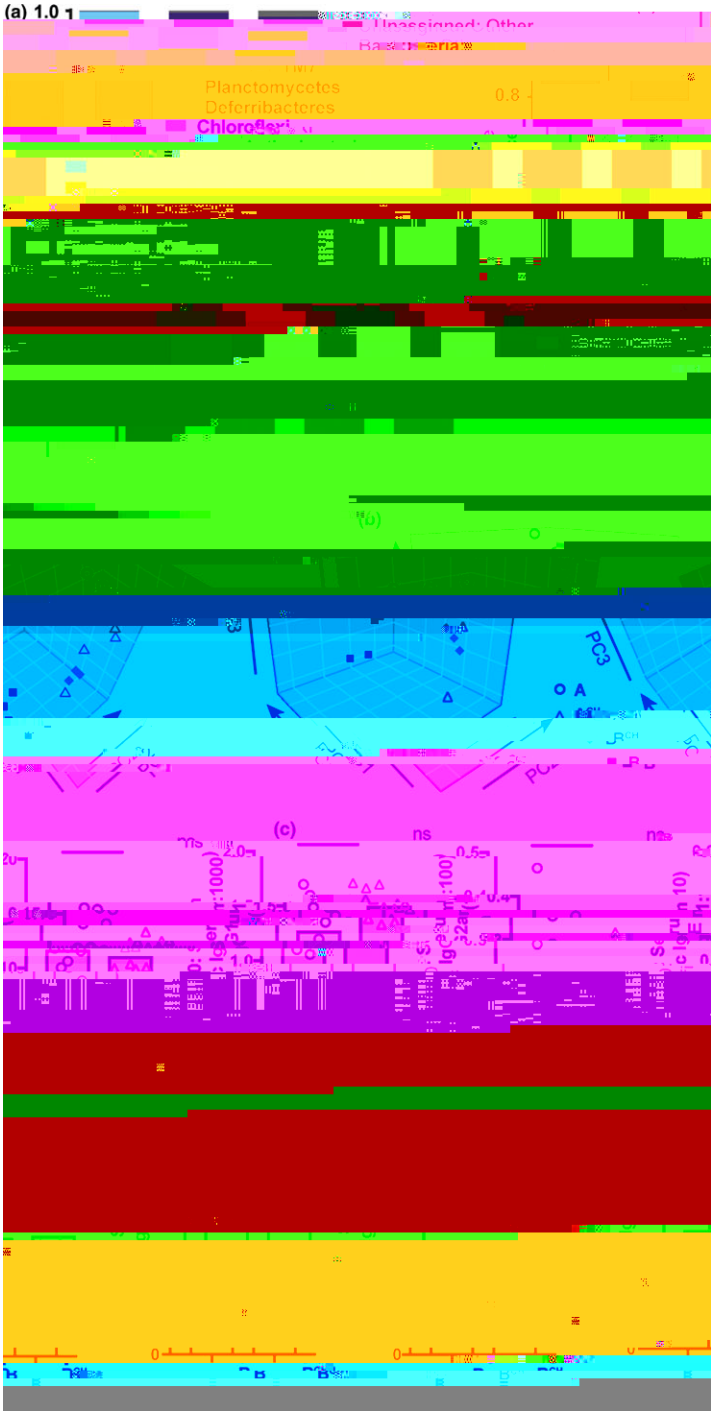


Figure 2. Microbiota composition does not regulate altered antibody responsiveness in BALB/c A and BALB/c B mice. BALB/c A and BALB/c B mice were cohoused to promote microbiota transfer and 4 weeks later vaccinated with NP-OVA in IFA; cohoused BALB/c A (A^{CH}), BALB/c B (B^{CH}), BALB/c A and BALB/c B. Gut microbiota composition was determined with taxonomic profiling of fecal bacterial communities from 16s rRNA sequencing. (a) Relative abundance at the phyla level with key populations-of-interest highlighted at the order, family or genus level and (b) principal component analysis of fecal microbiota composition; left and right plot provide two views of the taxonomic diversity. Samples taken 1 day prior to immunization (= 5 per group). NP-specific IgG1, IgG2a and IgE titers in serum of (c) A^{CH} and BALB/c A and NP-specific IgG1, IgG2a and IgE titers in serum of (d) B^{CH} and BALB/c B 14 days after immunization with NP-OVA in IFA. Data points represent individual mice and heights of the bar represent the median. Dashed lines represent lower limit of sensitivity, set at blank OD. Data are representative of at least two experiments.

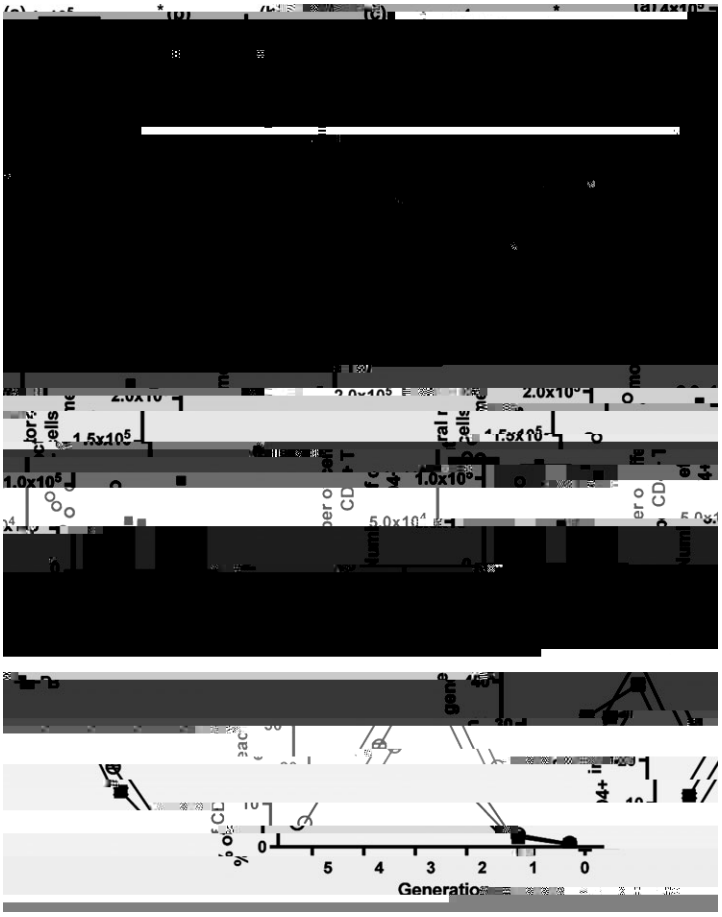
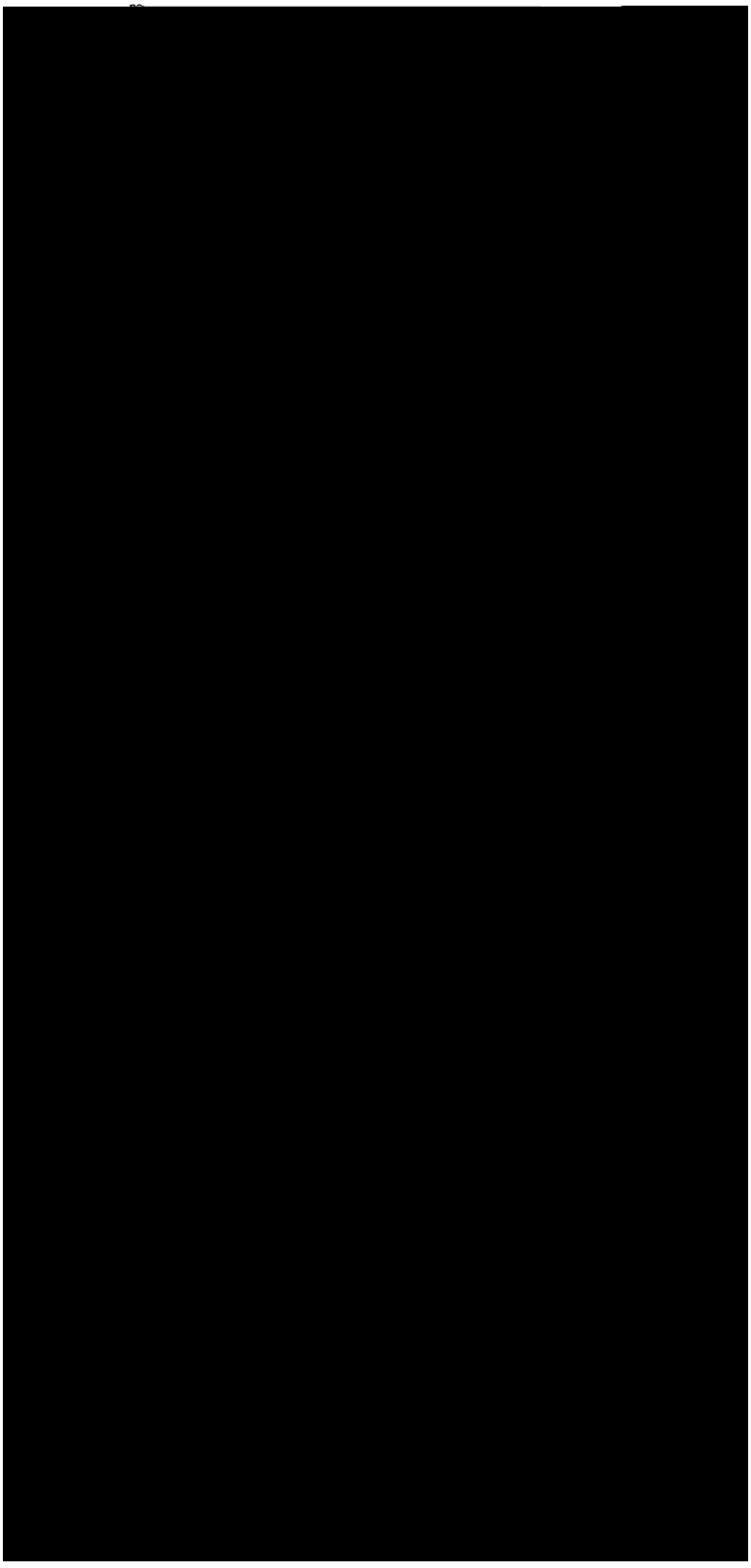


Figure 4. Tfh response capability is altered in low-responder BALB/c A. (a) The number of Tfh cells within draining lymph nodes 14 days after NP-OVA + IFA immunization was determined by flow cytometry. Tfh gated on size, viability $CD3^+CD4^+CXCR5^+PD\text{-}1^+FOXP3^-$. (b) Tfh cells were sorted from draining lymph nodes 7 days after immunization and expression of *B 6*, *40*, *21* and *4* determined by qRT-PCR. The relative expression of each target to 18S is presented, calculated relative to BALB/c A responsiveness that is set at 1. (c) Number of Tfr cells and (d) central memory and effector memory $CD4^+$ T cells within draining lymph nodes 14 days after NP-OVA + IFA immunization was determined by flow cytometry. $CD4^+$ T cells gated on size, viability $CD3^+CD4^+$; Tfr: $CXCR5^+PD\text{-}1^+FOXP3^+$, central memory: $CD62L^+CD127^+CD44^+$ and effector memory: $CD62L^-CD127^+CD44^+$. (e) proliferation of naïve $CD4^+$ T cells in response to aCD3, aCD28 and IL-2 stimulation. Data points represent individual mice and heights of the bar represent the median. Data are representative of at least three experiments.

(Figure 4b). These data suggest Tfh-cell activity may also be diminished in low-responder BALB/c A mice. T follicular regulatory (Tfr) cells have previously been shown to control the Tfh-cell response.[40] However, numbers of Tfr cells were significantly reduced in low-responder BALB/c A mice, indicating Tfr cells were unlikely to be causative of the attenuated Tfh-cell frequency in this substrain (Figure 4c). To ascertain whether the difference in the Tfh-cell response was specific to this $CD4^+$ T-cell subset, the differentiation of central and effector memory $CD4^+$ T-cell subsets induced in response to NP-OVA in IFA immunization was assessed and found to be comparable between the substrains (Figure 4d). Numbers of $CD4^+$ T cells in the lymph nodes and spleen were also equivalent under homeostatic conditions (Supplementary figure 3). Furthermore, naïve $CD4^+$ T cells were equally able to respond to polyclonal activation in vitro (Figure 4e). These results suggest the difference in the Tfh-cell response is specific to this $CD4^+$ T-cell subset and not due to dysfunction of the $CD4^+$ T-cell compartment as a whole.

Diminished capability of low-responder BALB/c A B cells to class switch when stimulated through TLR or CD40 *in vitro*

We performed quantitative analysis of naïve B cells stimulated in vitro to establish whether the altered CSR



binding site, possibly upregulating its transcription. A deletion in the guanine nucleotide-binding protein (G protein), gamma 4 (Gng4) regulatory region, was predicted to result in the loss of a Trf1 and RREB1 transcription factor binding site, which may reduce Gng4 transcription. Fifteen further intergenic variants were identified but were

genetic dysfunction afflicts a B-cell intrinsic regulator or mediator of CSR. Low-responder BALB/c A mice were able to mount a sufficient GC B-cell response and appropriate IgM production. However, subsequent isotype class-switch responses were skewed, leading to significantly reduced IgG1, IgG2a and IgE responses in BALB/c A. As there are no known markers for selection of GC B cells in this transition state, we are unable to characterize the isotype expression profile of this responding population. The Tfh response was also reduced in low-responder BALB/c A mice indicating that the pathways in the generation of the antibody response, in addition to CSR, are also deficient in BALB/c A. Further investigation is required to determine whether perturbation in Tfh responsiveness and CSR capability are regulated by shared or distinct polymorphisms, and indeed if other immunological mechanisms are also affected. Toward the development of immune-adjuvant therapeutics, determining the relative contribution that CSR efficiency and Tfh responsiveness impart to the magnitude of antibody response capability could provide valuable insight to whether therapeutic targeting of just one dominant pathway would be sufficient to boost responsiveness, or whether multiple pathways in antibody generation need to be targeted in unison.

The observation that at least some B cells from low-responder BALB/c A mice retained the capability to class switch suggests the genetic dysfunction afflicts protein activity of a C-S regulator. Moreover, altered C-S capability after stimulation through both TLR and CD40 demonstrates the C-S regulator lies downstream of

the expected antibody response capability of BALB/c mice.[53] Four candidates with the greatest potential to confer the perturbed C-S response capability of low-responder BALB/c A mice were identified. Altered Ets1 activity has the potential to regulate C-S to all antibody

support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf. Amplicon sequences were processed using Qiime 1.8. Paired end reads were quality filtered using a Q30 cutoff, and chimeric sequences identified using the USEARCH method against the Greengenes alignment (version 13_8) were removed. OTUs were picked at 97% similarity using the UCLUST method, and representative sequences were assigned taxonomies using the RDP classifier. Principle component analysis of the relative abundances of taxa identified up to the genus level for Balb/c, Balb/c A^{ch}, Balb/c B and Balb/c B^{ch}.

Whole genome sequencing and analysis

For whole genome sequencing, genomic DNA was extracted from 100 mg of fresh liver tissue using the DNeasy Tissue kit (Qiagen) following the manufacturer instructions. Briefly, each liver tissue sample was lysed with proteinase K and lysis buffer and incubated over night at 55°C, the lysed tissue was then loaded onto a DNeasy spin columns with a silica-gel membrane, washed and eluted in 100 lL elution buffer. DNA

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