Supporting Information

Mohr et al. 10.1073/pnas.1004879107

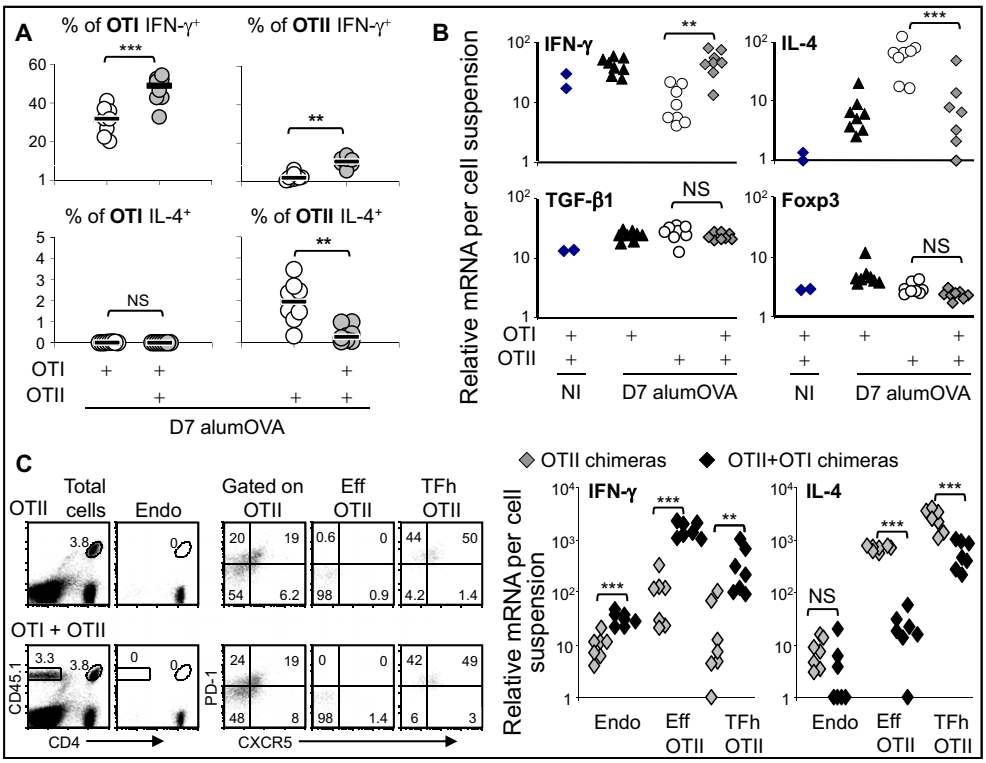

Fig. S1. CD8 OTI cells suppress Th2 features and increase Th1 features both in TFh and other Eff OTII cells, but do not change TGF-β1 and Foxp3 mRNA levels in cell suspensions of LN responding to alumOVA. Chimeras were constructed by transferring OTI or OTII cells, or both, into congenic C57BL/6 mice. The following day, the chimeras were immunized with alumOVA in both footpads or were not immunized (NI). Seven days later (D7), both popliteal LN were taken from each mouse and cell suspensions were prepared. (*A*) Percentage of OTI and OTII secreting IFN-γ (Fig. 1) and IL-4 when the cells were transferred independently or together was determined by intracellular staining and FACS analysis (*Material and Methods* and Fig. 1). (*B*) Levels of IFN-γ, IL-4, TGF-β1, and Foxp3 mRNA were assessed by duplex real-time RT-PCR and related to β-actin mRNA level. Data are derived from two independent experiments; each symbol shows results from the two pooled popliteal LN derived from 1 mouse. (*C*) LN cell suspensions were prepared from OTII alone or OTI plus OTII chimeras immunized 7 d earlier with alumOVA. As illustrated on FACS plot showing CD45.1$^+$ OTII cells, CXCR5$^+$PD-1$^+$ OTII cells representing the TFh OTII cells and CXCR5$^-$PD-1$^-$ OTII cells representing the other effector OTII cells (Eff OTII) were FACS sorted. As a control, endogenous CD4 T cells CD45.1$^-$ (Endo) were also FACS sorted and represent mostly nonresponding cells. cDNA was prepared from these different populations, and analyzed by real-time RT-PCR for relative amount of IFN-γ and IL-4 mRNA that they contained. The TFh OTII cell population was verified to contain the highest levels of BCL6, the transcription factor responsible for differentiation of TFh cells. Significance of differences between OTII and OTI plus OTII LN are indicated: NS, not significant, $^{**}P < 0.01$, $^{***}P < 0.001$.

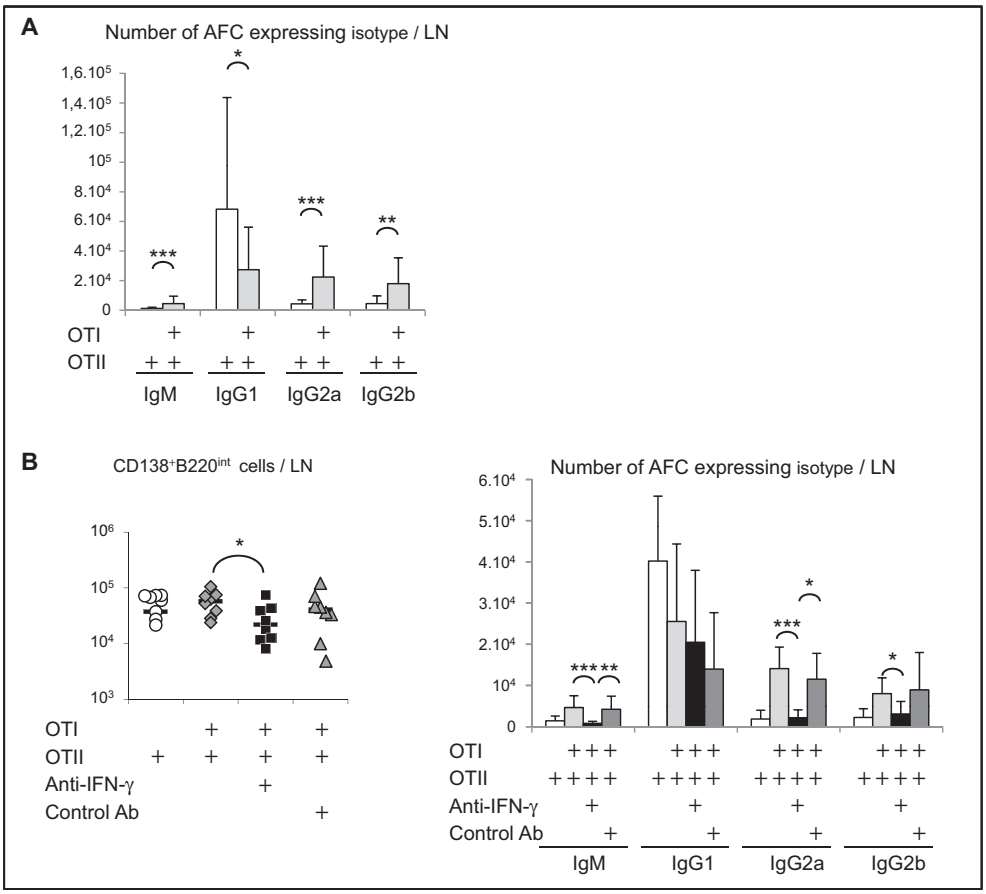

Fig. S2. Absolute number of AFC per LN that express each isotype is modified by OTI cells through IFN-γ. (*A*) Absolute number of AFC producing each isotype per LN was calculated from chimeras constructed and immunized in Fig. 2*B*. (*B*) Total number of AFC as well as the number of AFC producing each isotype per LN obtained in chimeras constructed and treated with neutralizing anti–IFN-γ Ab, or control Ab, as explained in Fig. 3.

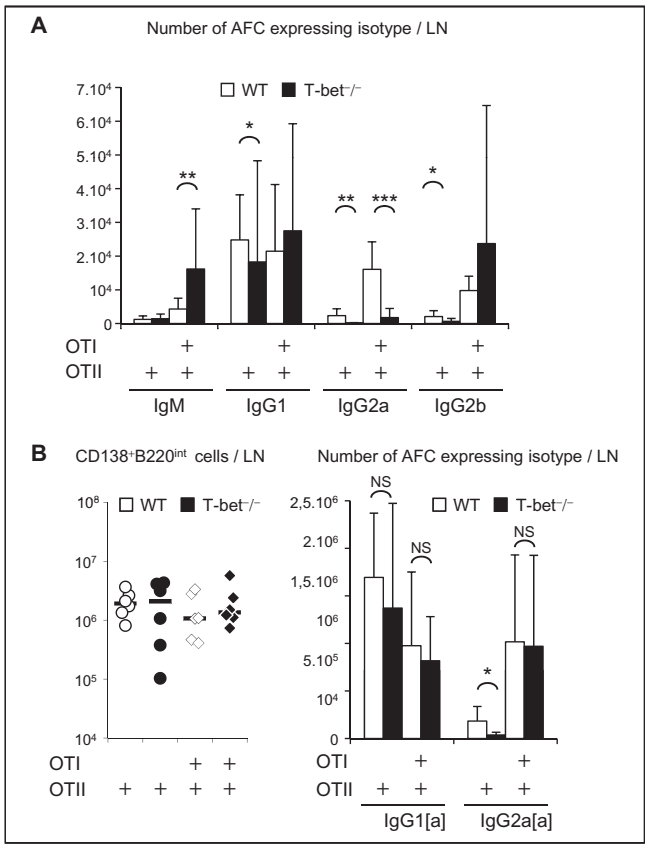

Fig. S3. Absolute number of AFC per LN in relation to T-bet knockout mice or T-bet–sufficient B cells in a T-bet–deficient environment. (*A*) Absolute number of AFC producing each isotype per LN was calculated from chimeras constructed and immunized in Fig. 4*C*. (*B*) Total number of AFC as well as number of AFC producing each isotype per LN obtained in chimeras constructed by transfer of CD45.1^{+} NP-specific B1.8^{hi} B cells and OTII cells, or both OTI plus OTII cells, either into CD45.2^{+} WT C57BL/6 or congenic T-bet$^{-/-}$ mice, as explained in Fig. 5.

Table S1. Ab and reagents used for FACS analysis

Reactivity	Isotype	Clone	Conjugate	Supplier
CD4	Rat IgG2a, κ	RM4-5	PerCP Cy5.5	BD PharMingen
CD4	Rat IgG2a, κ	RM4-5	APC	eBioscience
CD8α	Rat IgG2a	53-6.7	PerCP Cy5.5	BD PharMingen
CD45.1	Mouse IgG2a, κ	A20	FITC	eBioscience
CD45.1	Mouse IgG2a, κ	A20	PE	BD PharMingen
CD45R, B220	Rat IgG2a, κ	RA3-6B2	PerCP-Cy5.5	BD PharMingen
CD138	Rat IgG2a, κ	281-2	PE	BD PharMingen
CD138	Rat IgG2a, κ	281-2	APC	BD PharMingen
CD185 (CXCR5)	Rat IgG2a	2G8	Biotin	BD PharMingen
CD273 (PD-1)	Armenian Hamster IgG	J43	PE	eBioscience
IgM	Goat	Polyclonal	FITC	Southern Biotech
IgM	Goat	Polyclonal	Alexa 633	Molecular Probes
IgG1	Goat	Polyclonal	FITC	Southern Biotech
IgG1	Goat	Polyclonal	Alexa 633	Molecular Probes
IgG1[a]	Mouse IgG2a, κ	10.9	Biotin	BD PharMingen
IgG2a	Goat	Polyclonal	FITC	Southern Biotech
IgG2a	Goat	Polyclonal	Biotin	Southern Biotech
IgG2a[a]	Mouse IgG2a, κ	8.3	Biotin	BD PharMingen
Streptavidin			APC	BD PharMingen
IgG2b	Goat	Polyclonal	FITC	Southern Biotech
IgG2b	Goat	Polyclonal	Alexa 633	Molecular Probes

APC, allophycocyanin; PerCP, peridinin chlorophyll protein; PE, phycoerythrin.

Table S2. Primers and probes used for real-time RT-PCR

Target mRNA	Forward primer	Reverse primer	Probe	Fluorochrome
T-bet*	ATGCCAGGGAACCGCTTATA	AACTTCCTGGCGCATCCA	CCCAGACTCCCCCAACACCGGA	FAM
IL-4*	GATCATCGGCATTTTGAACGA	AGGACGTTTGGCACATCCAT	TGCATGGCGTCCCTTCTCCTGTG	FAM
IFN-γ*	TCTTCTTGGATATCTGGAGGAACTG	GAGATAATCTGGCTCTGCAGGATT	TTCATGTCACCATCCTT	FAM
Foxp3[†,‡]	Mm00475156_m1			FAM
TGF-β[†,‡]	Mm00441724_m1			FAM
β-Actin	CGTGAAAAGATGACCCAGATCA	TGGTACGACCAGAGGCATACAG	TCAACACCCCAGCCATGTACGTAGCC	Yakima Yellow
β2-Microglobulin	CTGCAGAGTTAAGCATGCCAGTAT	ATCACATGTCTCGATCCCAGTAGA	CGAGCCCAAGACC	NED

Sequences are written from 5′ to 3′ terminal.
*Run in duplex with β2-microglobulin.
[†]Run in duplex with β-actin.
[‡]Assay-on-demand (Applied Biosystems).