Supplemental Materials: Reduction of serum IgE in mice and in human cells by depletion of the IgE B cell lineage

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Supplemental Methods

PCR genotype assay for M1' knockin mice. A PCR-based genotyping assay for the presence of the M1' knockin allele was developed. The sequence of the forward primer is 5'-gggctggctggcggctccgc and the sequence of the reverse primer is 5'-ctatgccctggtctggaagatg, yielding a PCR product from the wildtype allele of 668 base pairs and the PCR product from the M1' knockin allele of 457 base pairs.

Southern blot verification of M1' knockin mice. 5 µg of genomic DNA from wild type or heterozygous knockin mice was digested with HindIII for Left Arm (LA) analysis or BamHI for Right Arm (RA) analysis. Probes were generated by PCR using the GC RICH PCR System (Roche). Primer sequences for generating the LA and RA probes are as follows: LA forward primer 5'-tgtctggtggtggacctggaaagcg; LA reverse primer 5'-tcctcgctctcctcctctggtggtg; RA forward primer 5'-ccatgcaacctagtatcctattctc; RA reverse primer 5'-ctttatacaggagaacctagcccag. Probes were detected with anti-digoxigenin-AP antibody and DIG wash and buffer set (Roche). The expected sizes of the wildtype and knockin mouse alleles are 7.4 kB (WT) and 3 kB (KI) for the LA probe; and 14.1 kB (WT) and 18.1 kB (KI) for the RA probe.

Apoptosis crosslinking assay. Daudi cells transfected with human membrane IgE were cultured in triplicate in cell culture medium, as recommended by ATCC, with 10 µg/ml anti-M1' antibody or isotype control antibody for 3 days in 24 well plates. For crosslinking conditions, 50 µg/ml anti-mouse IgG F(ab')2 (Jackson Immunoresearch) was also added to the cell culture medium. Cells were harvested and analyzed by FACS for levels of apoptosis using the Annexin V-FITC Apoptosis Detection Kit I

(BD Biosciences). Data was analyzed using FlowJo FACs analysis software (Tree Star).

Calcium flux assay. Daudi cell transfected with human membrane IgE labeled with 3 mM Fura-2 (Molecular Probes) at 37°C for 20 minutes. Cells were washed and resuspended in ice-cold calcium buffer (HEPES saline, 50 mM $MgCl_2$, 100mM $CaCl_2$, 1 mg/ml BSA). Prior to measurements, cells were warmed for 2 minutes at 37°C. Intracellular calcium flux was measured using a Hitachi F-4500 fluorimeter, and cells were stimulated with 20 μg/ml anti-M1' 47H4 mIgG1 antibody, goat anti-human IgM antibody (Jackson Immunoresearch), control mIgG1 antibody, or 5 μM ionomycin, after establishment of a baseline signal for one minute. Maximum and minimum values for each sample were determined by treatment with 0.05% Triton-X100 or 2 mM EGTA, respectively. Results are reported as a ratio of fluorescence signal at 340nm vs. 380nm, normalized to the average baseline signal between 45 and 60 seconds.

U266 apoptosis assay and IgE production. U266 cells were cultured in triplicate (10^6/mL) in cell culture medium, as recommended by ATCC, with anti-M1' antibody or isotype control antibody for 96 hours in 24 well plates. Cells were harvested and analyzed by FACS for levels of apoptosis using the Annexin V-FITC Apoptosis Detection Kit I (BD Biosciences). Data was analyzed using FlowJo FACs analysis software (Tree Star). Human IgE was measured in U266 culture supernatants using a human IgE ELISA (Genentech).

U266 calcium flux assay. U266 cells were labeled with 5 μM Indo-1 (Molecular Probes) at $5x10^6$/mL in complete media at 37°C for 30 minutes. Cells were washed and resuspended in complete media at $1x10^6$/mL. Prior to measurements, cells were warmed for 2 minutes at 37°C. Intracellular calcium flux was measured using an Aria Flow Cytometer (BD), and cells were stimulated with 1 μg/mL anti-M1' 47H4 mIgG1 antibody or 5 μM ionomycin for 5 minutes, after establishment of a baseline signal for one minute. Results are reported as a ratio of unbound and bound Indo-1 dye. Data was analyzed using FlowJo FACs analysis software (Tree Star).

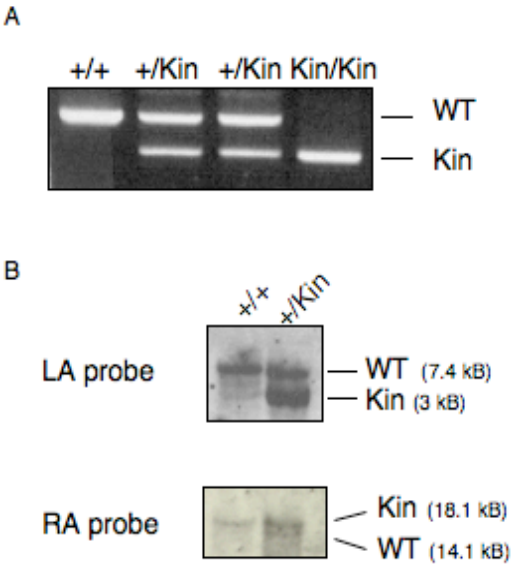
Supplemental Table 1. Data collection and refinement for Fab-h47H4 / M1' peptide.

Data collection	
source	ESRF ID29
space group	P212121
cell parameters (Å,˚)	***a***=64.76, ***b***=183.8, ***c***=194.9
Resolution (Å)	50 – 2.4 (2.49 – 2.40)
Rsyma,b	0.080 (0.508)
Number of observations	325504
Unique reflections	81785
Completeness (%)b	88.9 (72.3)
I/sIb	15 (2.0)
Refinement	
Resolution (Å)	50 – 2.4
Number of reflections	81785
Final Rc, RFREE	0.221, 0.261
number of residues	1806
number solvent molecules	276
number of atomsd	14071 (439)
Mean B-factor (Å2)	40
Rmsd bonds (Å)	0.010
Rmsd angles (˚)	1.4
Rmsd bonded Bs (Å2)	2.2/2.8
Number of TLS groups	8
RMSD NCS (Å)	0.7
Ramachandran plot (%)	89, 9.7, 0.8, 0.5

a Rsym = S||I| - |<I>||/S|<I>|, where I is the intensity of a single observation and <I> the average intensity for symmetry equivalent observations.
b In parenthesis, for the highest resolution shell.
c R = S|Fo-Fc|/S|Fo|, where Fo and Fc are observed and calculated structure factor amplitudes, respectively. RFREE is calculated as R for 1015 reflections (2.6%) sequestered from refinement.
d In parenthesis, the number of atoms assigned less than unit occupancy.

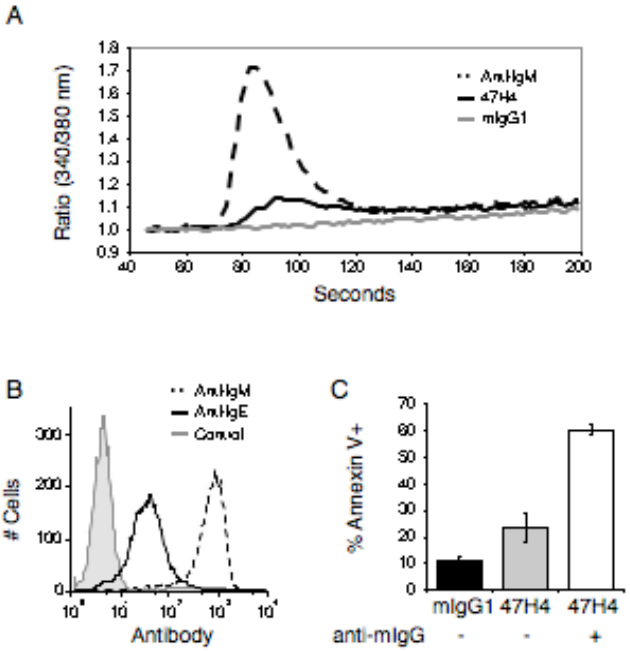
Supplemental Figure 1

Genotyping of the human M1' knockin mouse. (**A**) PCR genotyping assay for human M1' knockin mouse. The forward primer anneals upstream of the M1 exon splice acceptor site, and the reverse primer anneals in the M2 exon. The expected PCR product sizes are 688 base pairs for the wildtype allele and 457 base pairs for the M1' knockin allele, reflecting the insertion of the human M1' sequence. (**B**) Southern blot verification of human M1' knockin mouse. Genomic DNA was digested with HindIII for the left arm or BamHI for the right arm. The left arm probes anneals within the region encoding the CH3 domain of mouse IgE, and the right arm probe anneals approximately 7 kB downstream of the M2 exon of mouse IgE. The expected sizes of the wildtype and knockin mouse alleles are 7.4 kB (WT) and 3 kB (KI) for the left arm probe, and 14.1 kB (WT) and 18.1 kB (KI) for the right arm probe.


A
+/+
+/Kin
+/Kin
Kin/Kin
WT
Kin
B
+/+
+/Kin
LA probe
WT (7.4 kB)
Kin (3 kB)
RA probe
Kin (18.1 kB)
WT (14.1 kB)

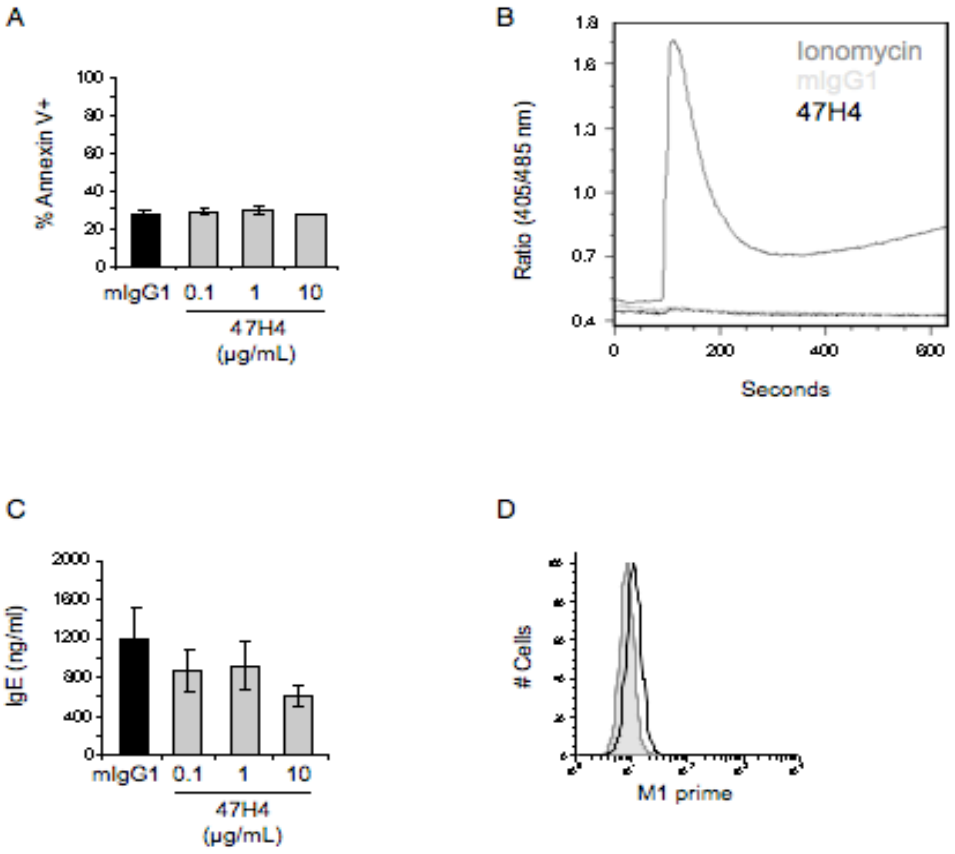
Supplemental Figure 2

Characterization of human membrane IgE-transfected Daudi cells and assessment of anti-M1' antibody-induced apoptosis and intracellular calcium signaling. (**A**) Anti-M1' 47H4 antibody induces intracellular calcium signaling in human membrane IgE-transfected Daudi B cells, as detected by the ratio of fluorescence at 340 nM vs. 380 nM in Fura-2-labeled cells. (**B**) Flow cytometry analysis of human membrane IgE-transfected Daudi cells indicates a low level of human membrane IgE expression. Daudi cells express endogenous human membrane IgM. (**C**) Crosslinking of anti-M1' 47H4 antibody with anti-mouse IgG F(ab')2 enhances the magnitude of anti-M1' 47H4 antibody-induced apoptosis.


A
Ratio (340/380 nm)
Seconds
Ant-IgM
47H4
mIgG1
B
Cells
Antibody
Ant-IgM
Ant-IgE
Control
C
% Annexin V+
mIgG1
47H4
47H4
anti-mIgG
-
-
+

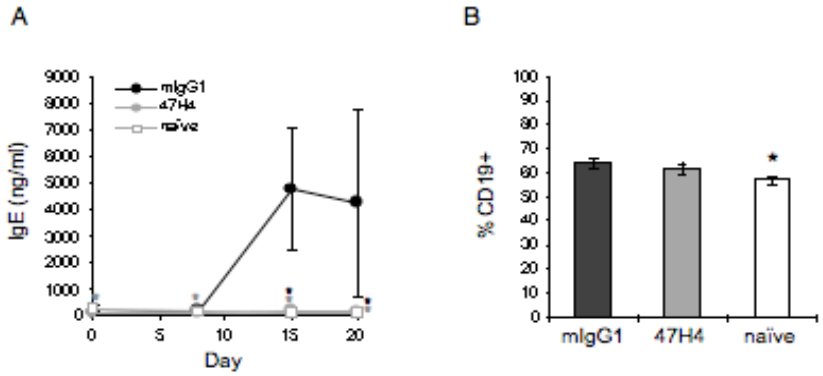
Supplemental Figure 3

Assessment of anti-M1' antibody effects on U266 cells. (**A**) Anti-M1' 47H4 antibody does not induce apoptosis of U266 cells. Mouse IgG1 control antibody is at 10 µg/mL. (**B**) Anti-M1' 47H4 antibody does not induce an intracellular calcium signal in U266 cells, as detected by the ratio of fluorescence at 405 nM vs. 485 nM in Indo-1-labeled cells. (**C**) Anti-M1' 47H4 antibody does not affect the production of soluble IgE from U266 cells. Mouse IgG1 control antibody is at 1 µg/mL. (**D**) Flow cytometry analysis indicates a very low level of human membrane IgE expression on U266 cells.


A
% Annexin V+
100
80
60
40
20
0
mIgG1
0.1
1
10
47H4
(µg/mL)
B
Ratio (405/485 nm)
1.8
1.6
1.3
1.0
0.7
0.4
0
200
400
600
Seconds
Ionomycin
mIgG1
47H4
C
IgE (ng/ml)
2000
1600
1200
800
400
0
mIgG1
0.1
1
10
47H4
(µg/mL)
D
Cells
M1 prime

Supplemental Figure 4

Characterization of total spleen B cells and serum IgE levels upon treatment of *N. brasiliensis* infection of M1' knockin mice with anti-M1' antibody. (**A**) Anti-M1' 47H4 antibody inhibits *N. brasiliensis*-induced serum IgE. Results are mean ± SD. **P* < 0.05. Data corresponds to the same study animals in which anti-M1' 47H4 antibody treatment reduced IgE-switched GFP+ splenic B cells (Figure 6H). (**B**) Flow cytometry analysis of *N. brasiliensis*-infected mice indicates no significant difference in the percentage of total splenic CD19+ B cells between anti-M1' 47H4 antibody and control antibody treated groups on day 21. Total splenic B cells are increased in *N. brasiliensis*-infected mice on day 21, as compared to uninfected mice. Results are mean ± SD. **P* <0.001. Data corresponds to the same study animals in which anti-M1' 47H4 antibody treatment reduced IgE-switched GFP+ splenic B cells (Figure 6H).


A
B
IgE (ng/ml)
Day
mIgG1
47H4
naïve
% CD19+
mIgG1
47H4
naïve