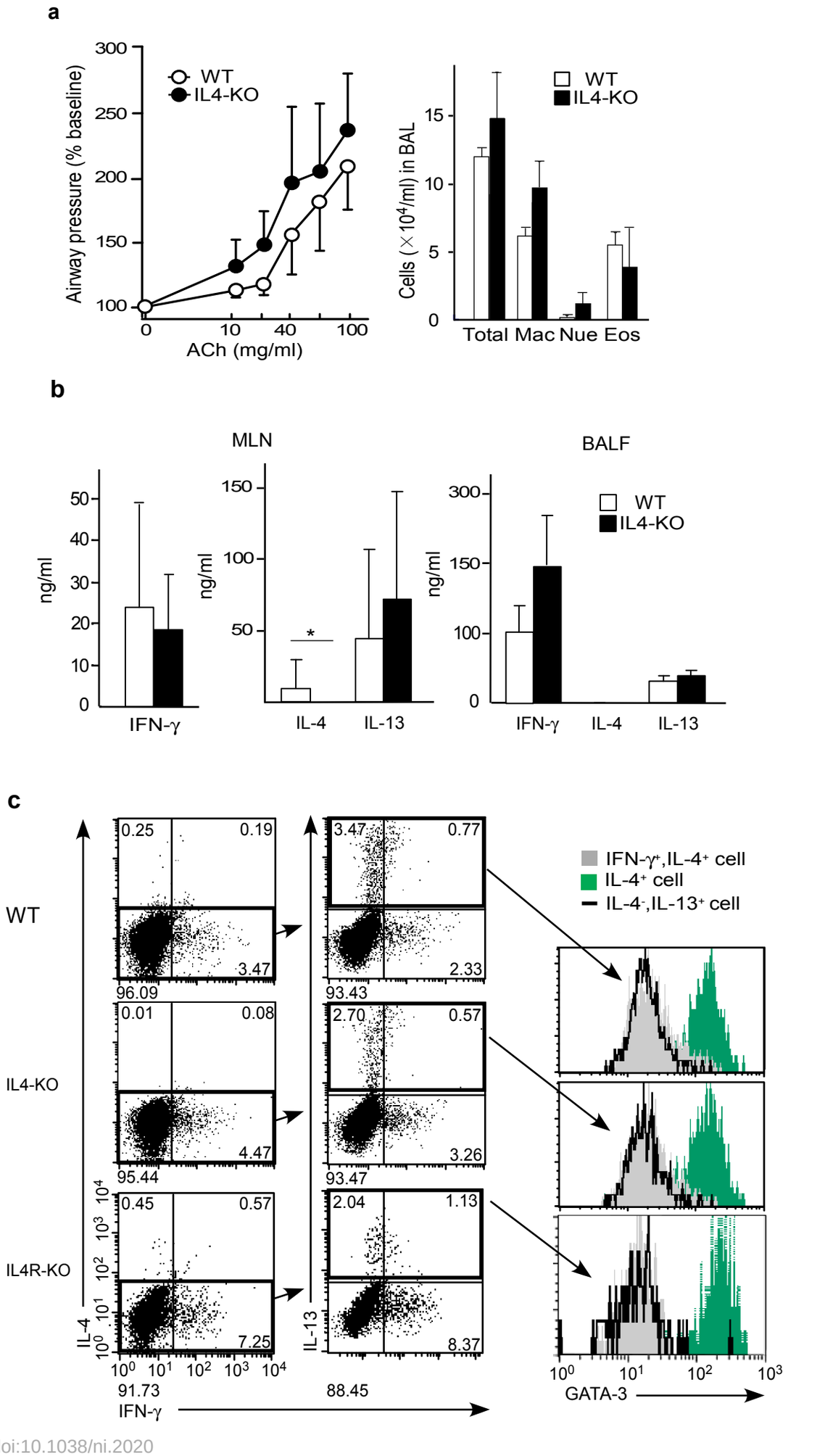
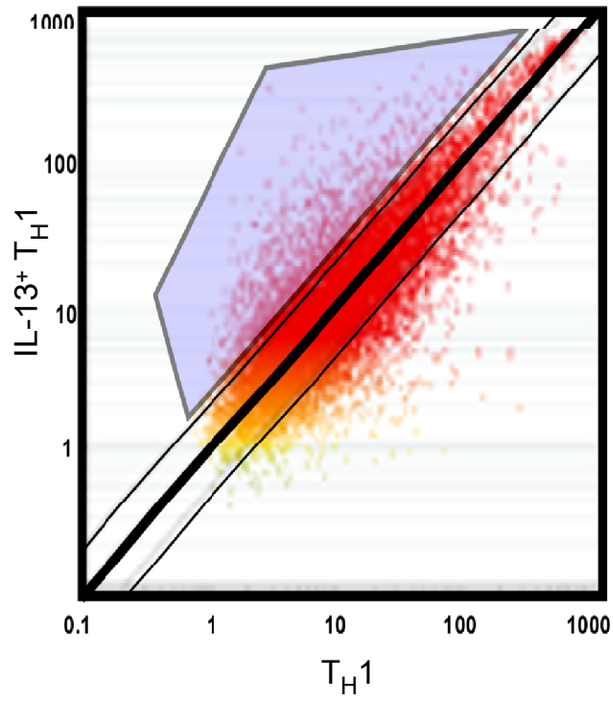
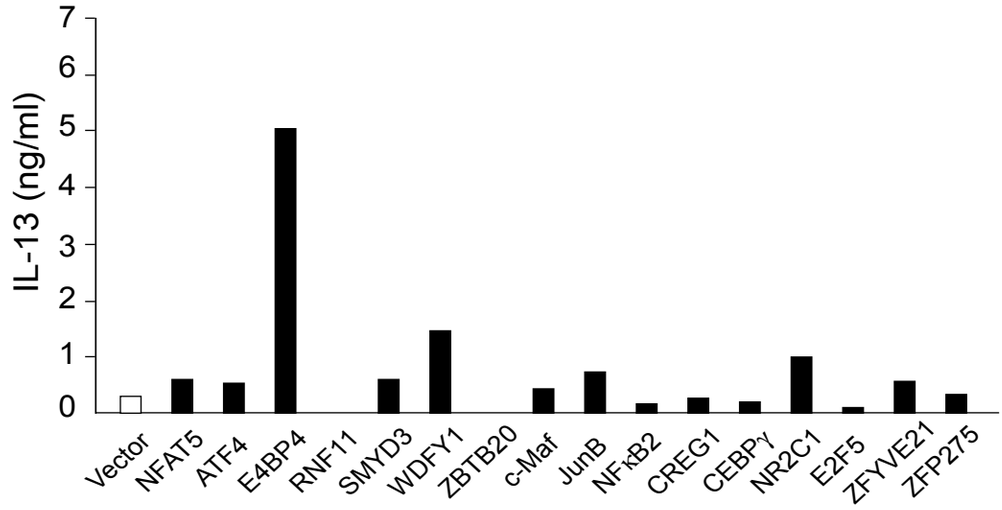
Supplementary Fig.1 Motomura et al.



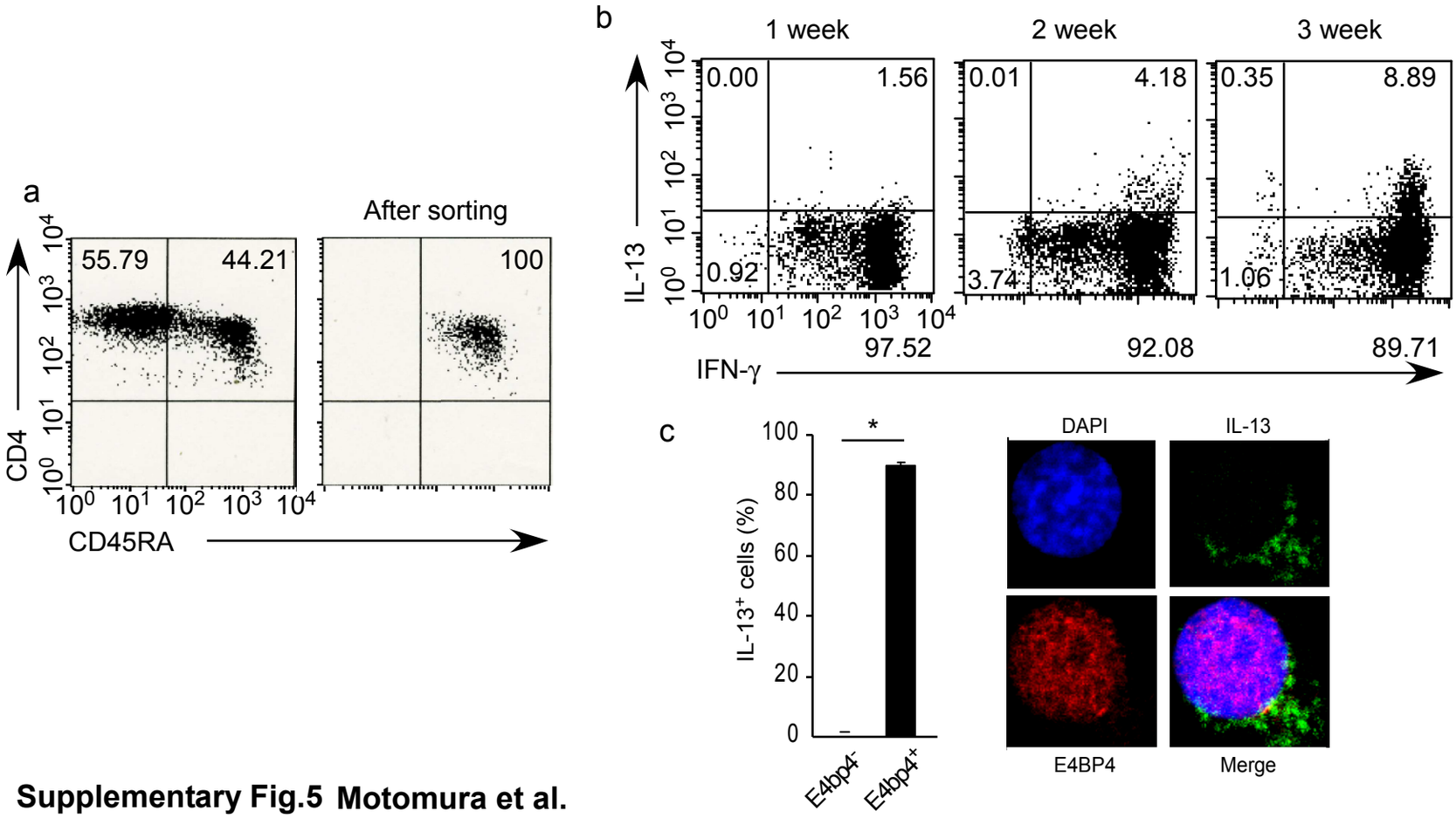
Supplementary Fig.2 Motomura et al.



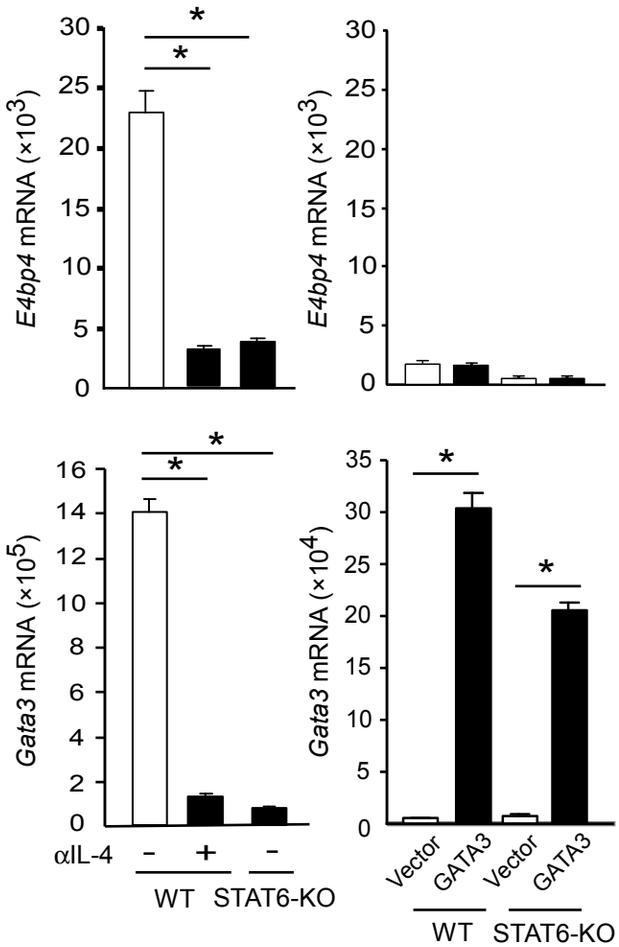
Supplementary Fig.3 Motomura et al.



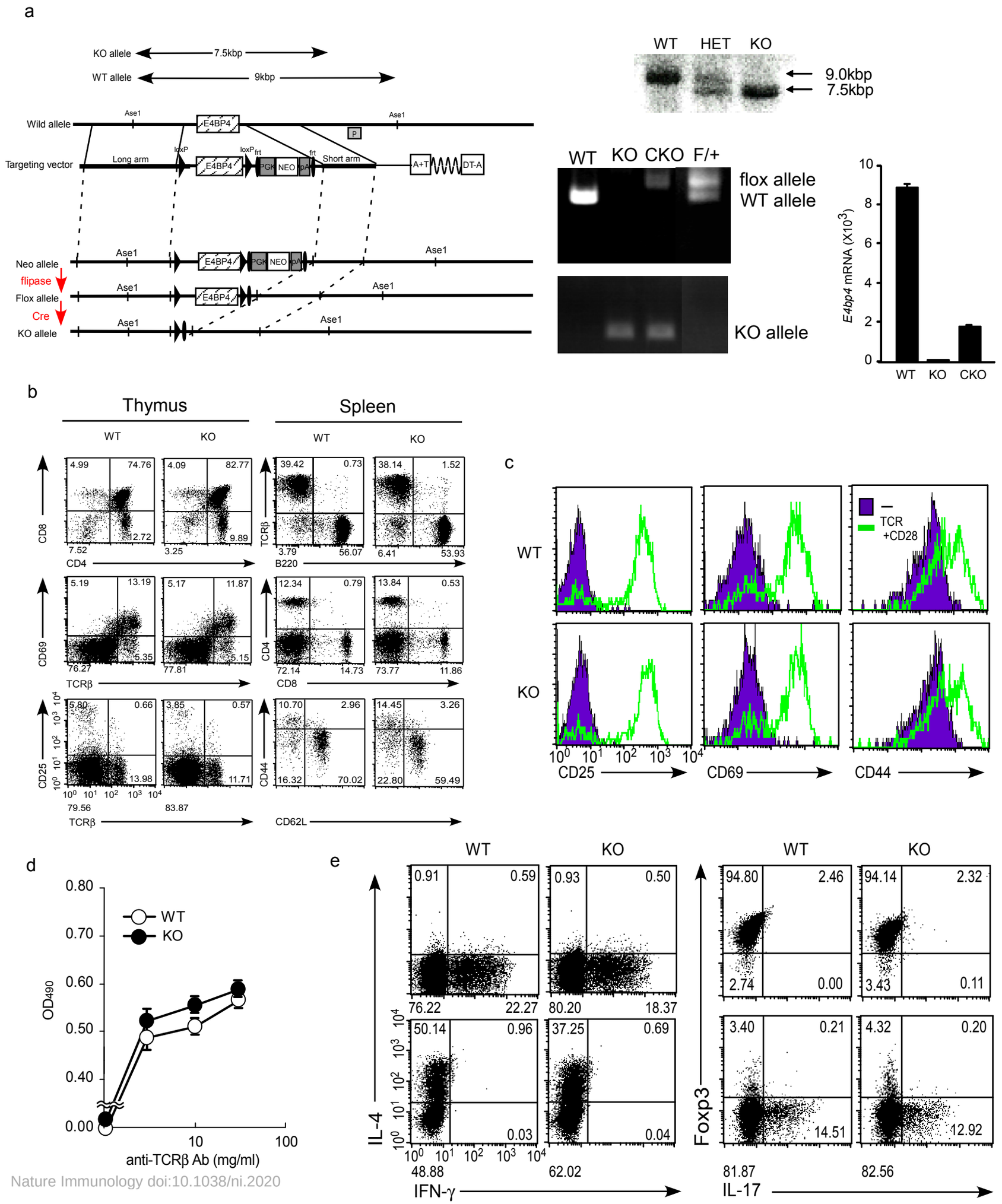
Supplementary Fig.4 Motomura et al.



Supplementary Fig.5 Motomura et al.

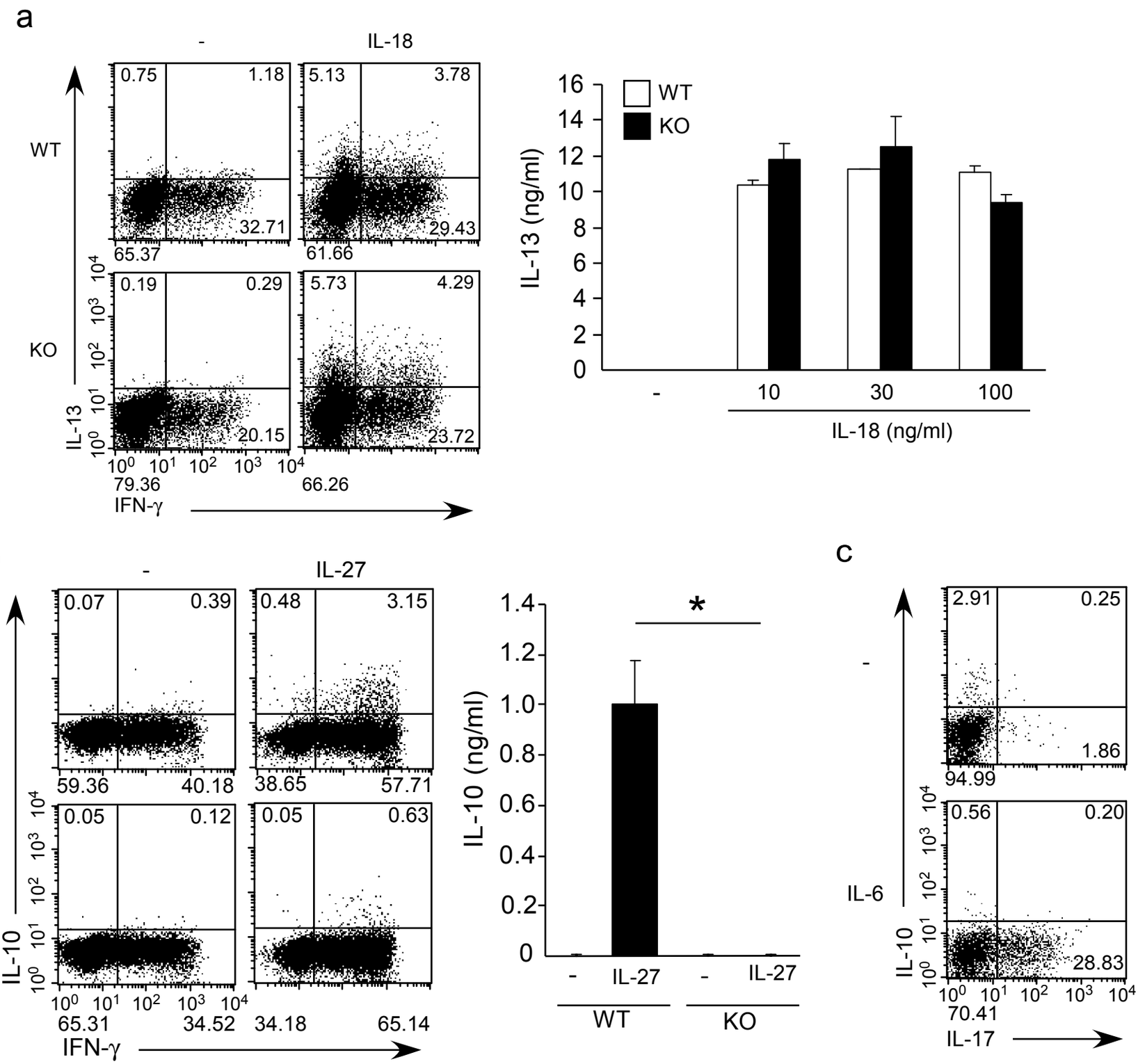


Supplementary Fig.6Motomura et al.

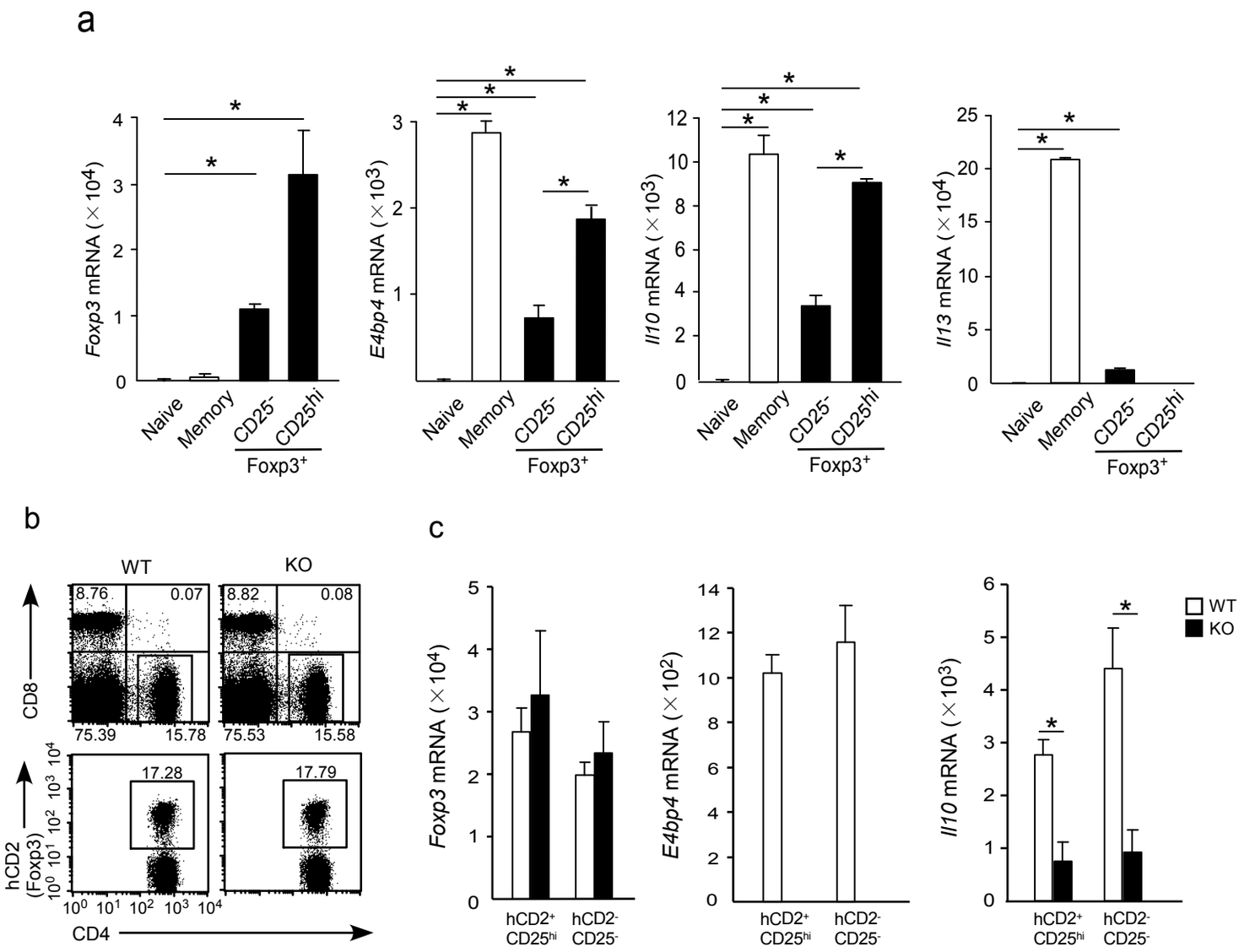


Nature Immunology doi:10.1038/ni.2020

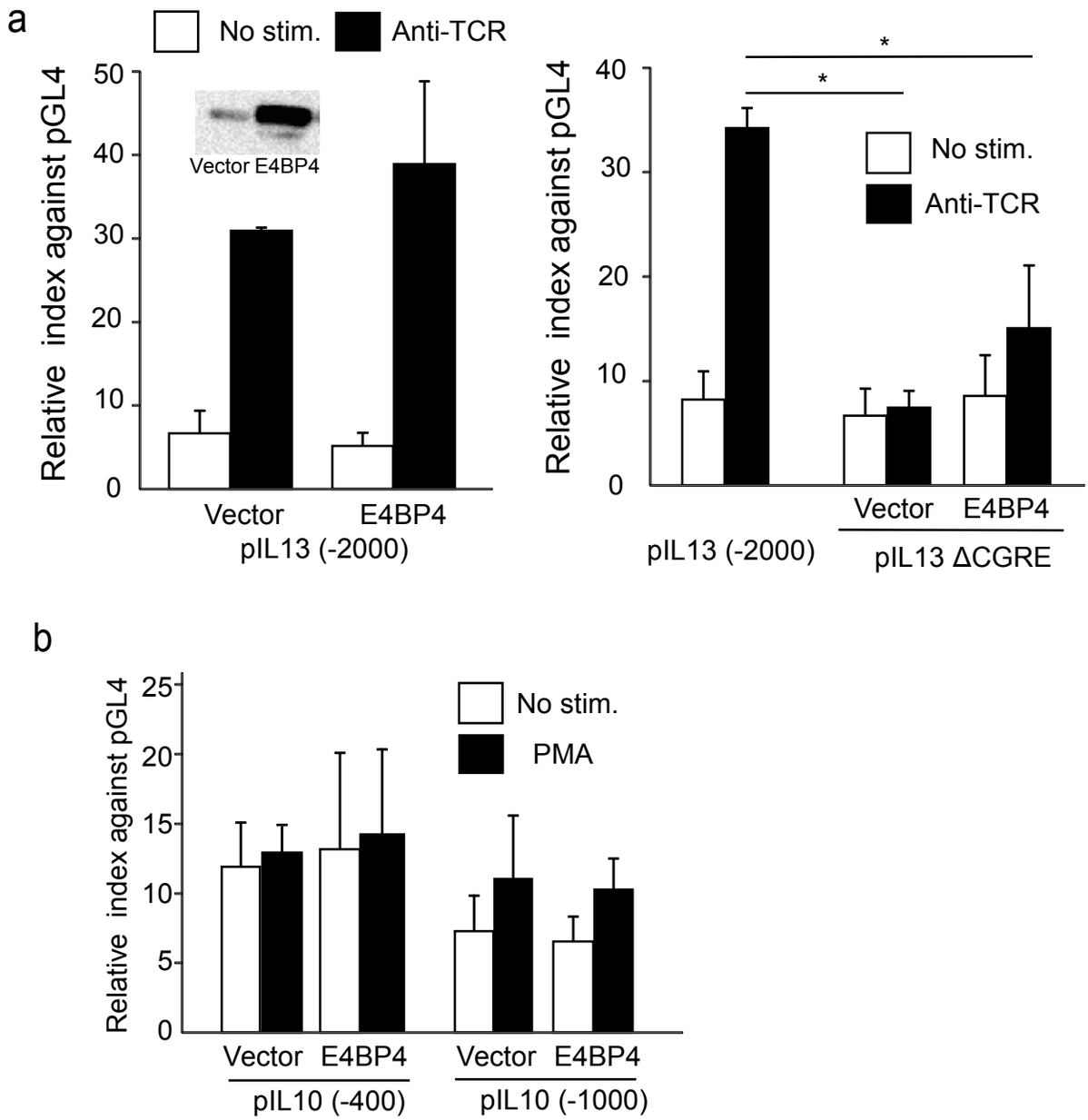
Supplementary Fig.7 Motomura et al.



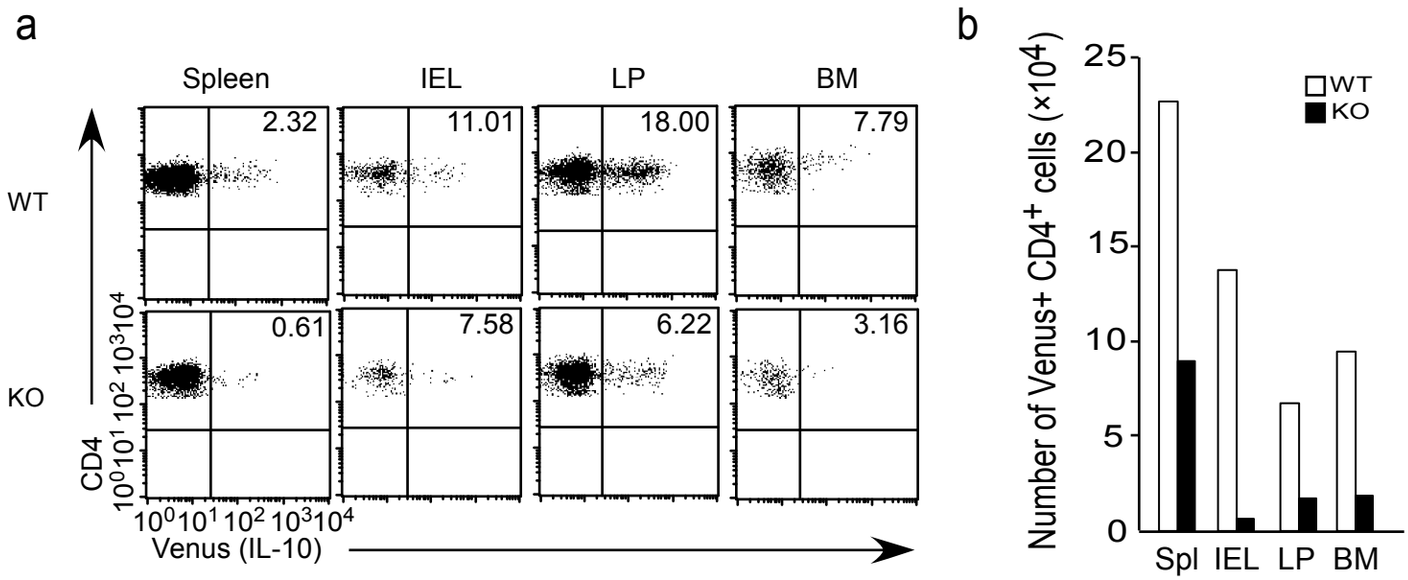
Supplementary Fig.8 Motomura et al.



Supplementary Fig.9 Motomura et al.



Supplementary Fig.10 Motomura et al.



Supplementary Fig.1

Plasticity in the regulation of IL-13 production by CD4T cells

(a) *Il4* KO mice on a B6 background were immunized with OVA (100μg) and 2.25mg of aluminium hydroxide gel (alum) intraperitoneally twice and boosted with OVA 2 times every week. Mice were further challenged with OVA by inhalation, and acetylcholine-dependent airway hyper responsiveness (AHR) was measured. Airway pressure in the response to increased concentrations of acetylcholine is indicated. Cellular infiltrates into the BAL fluid were assessed by Wright-Giemsa staining and cell counting. Data are the mean of the value of five independent mice. (b) lymph Node (LN) and BALF lymphocytes were obtained from mice immunized as in (a). Cells were stimulated with TCRβ and CD28 mAbs, and IFN-γ, IL-4 and IL-13 levels were measured by ELISA. Data are the mean of five independent mice. * $p<0.01$ (Student's t-test). (c) *Il4*$^{-/-}$ mice on a B6 background and DO11.10 BALB/c or *Il4r*$^{-/-}$ were immunized with OVA and alum followed by boosting with OVA. IFN-γ, IL-13, and IL-4 production was analyzed in CD4 T cells. The middle panel illustrates IL13 and IFN-γ production patterns in IL-4 non-producing cells. Shown on the right is *Gata3* mRNA expression in IL-13^{+} IL-4^{-} cells. Data are representative of three independent mice with similar results.

Supplementary Fig.2

Identification of transcription factors highly expressed in IL-13^{+}T$_H$1 cells

Comparative transcriptome analysis between R1T$_H$1 and R4T$_H$1 cells was performed by Affymetrix microarray. Comprehensive expression in R4T$_H$1 versus R1T$_H$1 cells was examined with Genespring software.

Supplementary Fig.3

Ectopic expression of E4bp4 induces IL-13 production in T_H1 cells

Sixteen independent candidate genes were inserted into the pMX-IRES-*Gfp* retroviral expression vector. After 7 days, retrovirally transduced CD4 T cells were sorted as GFP^+ cells and restimulated with anti-TCRβ mAb. IL-13 production was measured by ELISA.

Supplementary Fig.4

Repetitive TCR stimulation induces IL-13 production in human CD4 cells.

CD4 T cells from non-atopic healthy individuals were chronically stimulated with hCD3 and hCD28 mAbs under T_H1 skewing conditions (rIL-12 and anti-IL-4 mAb) for 3 weeks. Every week, cells were harvested and restimulated with hCD3 mAb for 6hr, and an ICS assay was performed (left). Data are representative of three independent experiments. The activated cells after three rounds of anti-hCD3 stimulation were stained with anti-IFN-γ FITC (green), anti-IL-13 alexa647 (blue) and anti-E4BP4 Alexa568 (red) and analyzed by confocal microscopy. Shown on the right is a photomicrograph of a single cell. The percentage of IL-13^+ cells was assessed in three independent viewing fields by microscopic analysis and the data are shown as the mean percentage. The data represent the subtracted background staining of unprimed human CD4 T cells with anti-IL-13 alexa647. * $p<0.01$ (Student's t-test).

Supplementary Fig.5

***E4bp4* expression in T_H2 cells is IL-4/Stat6 dependent.**

CD4T cells from *Stat6*$^{-/-}$ or C57BL/6 mice were stimulated under T_H2 conditions in the presence of anti-IL-4 mAb. *E4bp4* and *Gata3* expression was analyzed by qRT-PCR. Data are the mean and s.e.m of three independent experiments. * $p<0.01$ (Student's t-test).

Supplementary Fig.6

Generation and phenotypic analysis of *E4bp4* deficient mice

(a) Schematic representation of wild type (WT) and knock-out (KO) *E4bp4* alleles. The targeting vector contains the diphtheria toxin A gene (DT-A), loxP (P), flanked neomycin resistance gene (*Neo*), Frt (F). The *E4bp4* gene was flanked with homologous arms. Neo was excised by transient *Flp* expression and E4bp4 was excised by transient *Cre* expression (left). Southern blot analysis of WT and KO alleles was performed with genomic DNA derived from *E4bp4*$^{+/+}$, *E4bp4*$^{+/-}$, *E4bp4*$^{-/-}$mice (upper right). Genomic PCR analysis was performed with genomic DNA derived from *E4bp4*$^{+/+}$(WT), *E4bp4*$^{-/-}$ (KO) , *E4bp4*$^{f/f}$ *Cd4*cre (CKO) and *E4bp4*$^{f/+}$(lower right). RT-PCR analysis of *E4bp4* mRNA expression was performed in WT, KO, and CKO Cd4 T cells (lower right). (b) Flow cytometry analysis of thymocytes (left) and splenocytes (right) derived from *E4bp4*$^{-/-}$ and littermate mice. Analysis was performed on the CD4 gated cells. Data are representative of three independent mice. (c) Naive CD4T cells *from E4bp4*$^{+/+}$ and *E4bp4*$^{-/-}$ were stimulated with TCRβ and CD28 mAb for 24hrs. Expression of the activation markers, CD25, CD69 and CD44 was examined by flow cytometry. (d) Naive CD4T cells *from E4bp4*$^{+/+}$ and *E4bp4*$^{-/-}$ were stimulated with TCRβ and CD28 mAb for 48hrs. Proliferation was measured using an MTT assay. Data indicated OD^{490} and are the mean and SEM. (e) Helper T cell differentiation of *E4bp4*$^{+/+}$ and *E4bp4*$^{-/-}$ naïve CD4T cells. Naive CD4T cells from *E4bp4*$^{+/+}$ and *E4bp4*$^{-/-}$ mice were stimulated under

T_H1, T_H2, T_H17, and iT_{reg} skewing conditions. Cytokine production and Foxp3 expression were detected by intracellular staining. Data are representative of three independent experiments.

Supplementary Fig.7

IL-18-induced IL-13 production is independent of *E4bp4* expression, but E4bp4 is required for IL-27-induced IL-10 expression in T_H1 cells.

(a) Naive CD4T cells from WT and *E4bp4*$^{-/-}$ mice were stimulated under T_H1 skewing conditions. Cells were restimulated with anti-TCR mAb with or without rIL-18 for 24hr. IL-13 and IFN-γ production were detected by intracellular staining (left) and IL-13 levels in culture supernatants were measured by ELISA (right). (b) Naive CD4T cells from WT and *E4bp4*$^{-/-}$ mice were stimulated under T_H1 skewing conditions in the presence of rIL-27. IL-10 and IFN-γ production were detected by ICS (left) and IL-10 levels in culture supernatants were measured by ELISA (right). (c) Naive CD4T cells were stimulated with anti-TCR and anti-CD28 mAbs in the presence of rhTGFβ with or without rIL-6. After 5days, IL-10 and IL-17 production were detected by ICS. Data are the mean of three independent experiments in (a) and (b) and error bars indicate s.e.m. * $p<0.01$ (Student's t-test).

Supplementary Fig.8

E4bp4 expression is tightly correlated with *Il10* expression in Foxp3$^+$ T_{reg} cells.

Naive (Foxp3$^-$,CD62L^{hi},CD44^{lo}), memory (Foxp3$^-$,CD62L^{lo},CD44^{hi}) and Foxp3$^+$ cells (CD25$^-$ and CD25^{hi}) CD4T cells were purified from Foxp3^{hCD2}reporter mice (a) and

Foxp3^{hCD2}reporter mice on *E4bp4*$^{+/+}$ and *E4bp4*$^{-/-}$ background (b). Cells were stimulated with TCRβ mAb and CD28 mAb, and total RNA was prepared. *Foxp3*, *E4bp4*, and *Il10* expression was analyzed by qRT-PCR (a). (b) Human CD2 expression as a marker of Foxp3^{+} cells was detected by flow cytometry. (c) Foxp3^{+} CD4T cells (CD25^{-} and CD25^{hi}) were purified from Foxp3^{hCD2}reporter mice on WT and *E4bp4*KO backgrounds. Cells were stimulated with TCRβ mAb and CD28 mAb for 24h, and mRNA expression of indicated genes were measured by qRT-PCR. Data are the mean and s.e.m of three independent experiments. * p<0.01(Student's t-test).

Supplementary Fig.9

E4BP4 regulated the *Il13* promoter activity upon TCR stimulation.

(a) Luciferase reporter constructs, pGL4Basic vector (Promega) containing distal and proximal promoter of Il13 (pIL-13(-2000)) or deletion mutant of CGRE (pIL-13ΔCGRE) were transiently transfected into the 68-41 T cell hybridoma along with empty expression vector, pcDNA3, (Vector) or *E4bp4* pcDNA3. Cells were subsequently stimulated with anti-TCRβ mAb. After 6hr, cell extracts were harvested and subjected to luciferase assay by using the Luciferase Assay System (Promega). Immunoblot analysis of the expression of E4BP4 in transfected cells (upper). (b) The EL-4 T cell lymphoma line was transfected with the promoter luciferase constructs containing 400bp or 1000bp of the 5' upstream region of the *Il10* gene (pIL-10(-400) and pIL-10(-1000)) in combination with Mock or *E4bp4* pcDNA3. Cells were stimulated with PMA for 6h and luciferase activity was measured (lower). Data represent a relative index as the fold increase in luciferase activity over that obtained with the pGL4Basic vector. Data in (a) and (b) are the mean and s.e.m of three independent experiments. * p<0.01(Student's t-test).

Supplementary Fig.10

Il10^{venus} mice on *WT* and *E4bp4*$^{-/-}$ backgrounds were immunized with MOG in CFA and PTx followed 2 days later with a second dose of PTx. Cells were isolated from spleen, intestinal intraepithelial lymphocytes (IEL), lamina propria (LP) and bone marrow (BM) of immunized mice at day 35 after immunization. Venus expression in CD4^{+} cells was analyzed by flow cytometry (a) and cell numbers were calculated (b).

Supplementary Table Motomura et al

Quantitative RT-PCR		
GENE	**Forward primer**	**Reverse primer**
Atf4	gaaacctcatgggttctcca	gaaaaggcatcctccttgc
Cebpg	ggcttacagcaggttcctca	tgggtgagctctttttgctt
Creg1	tggatttgatccccagagtc	cagcgaatcccttgcatagt
E2f5	ttcaggcaccttctggtaca	aggccctgagtgactcttca
E4bp4	cagtgcaggtgacgaacatt	ttccaccacacctgttttga
Junb	atccctatcggggtctcaag	cctgtgtctgatccctgacc
c-Maf	aaagggacgcctacaaggag	tgaaaaattcgggagaggaa
Ndrg1	ctcatgaatgtgaacccctgt	ttgtgtatctcctccttgccg
Nfat5	tggtttttcgggttaatatcac	cctctcctttcactgaacagcta
Nfkb2	gatctcccgaatggacaaga	gaaccgaacctcaatgtcgt
Nr2c1	tgtgtcgtgtgtggagacaa	aggtttttccggatgcttct
Rnf11	tgcttcacgagtctcagtcc	ctggctaggtgtcggatgat
Smyd3	cccaactgctccatcgtatt	catcagcatgtccaggtagc
Wdfy1	accatccgagtatggctgaa	cattatcctggcccacaaat
Zbtb20	ccctcatccactcgacacat	agcacggaattgctgaagtt
Zfp275	tattgtaccgtggcacctca	ccaaagcaggactcacaaca
Zfyve21	cgagaggcggagttttatga	tggttgttggaaagacgaca
Gata3	agaaccggccccttatcaa	agttcgcgcaggatgtcc
T-bet	caacaacccctttgccaaag	tcccccaagcagttgacagt
beta-actin	actattggcaacgagcggttc	ggatgccacaggattccatac
BSP assay		
	Forward primer	**Reverse primer**
***pll13* CpG**	atctaccaccttaccctatcctcc	ccaacaaactaaacaaatctaacttt
EMSA		
	Forward oligonucleotide	**Reverse oligonucleotide**
E4 promoter	aaaaaatgacgtaacggaag	cttccgttacgtcatttttt
pll13	ccatctcccgttacataaggccgcgcctct	agaggcgcggccttatgtaacgggagatgg
M1	ccatctc**ggg**ttacataaggccgcgcctct	agaggcgcggccttatgtaa**ccc**gagatgg
M2	ccatctcccg**ggg**cataaggccgcgcctct	agaggcgcggccttatgc**ccc**gggagatgg
M3	ccatctcccgtta**ggg**aaggccgcgcctct	agaggcgcggcctt**ccc**taacgggagatgg
M4	ccatctcccgttacat**ggg**gccgcgcctct	agaggcgcggc**ccc**atgtaacgggagatgg
M5	ccatctcccgttacataagg**ggg**cgcctct	agaggcg**ccc**ccttatgtaacgggagatgg

ChIP assay(Il10)		
No.	**Forward primer**	**Reverse primer**
1	cttgaggggtttgatgagga	tgcccatctacgttcagttg
2	cgttgtgctttgcaacatct	cacagtgtgactggggtcat
3	ctagtccgcagaaggagcac	caaagggatcagccagattg
4	ccaaggaatgcacagactga	tttgagctgggtttcaggac
5	cgccaaacacttctcacaga	ccattggcttcaagttgttg
6	gtggctatcaccgtgcagta	tgcctctctgagctgatgttt
7	cattccctggtcaacaggac	tcagtttgggtgggaagaac
8	tgacttccgagtcagcaaga	gtggattccattcacgcact
9	ttgaagcagcaccagcatag	cacctgtgtcaacccttcct
10	aatggtgtgacctcctctgc	tgtgctcataggctgtctgg
11	tcccttcaagtcaagcgtct	gatttgtccgtctgctttgg
12	tccaaagctagggaaggtga	cggcacacactctaactgct
13	aggtctccagcctcttgaca	tccaaccctaatggctcaac
14	taccctgccctctatcctga	caccctcaattcctcttcca

ChIP assay(Il13)		
No.	**Forward primer**	**Reverse primer**
1	tcctgtgaatccttcatgtcaagc	aagtgtcatcatcgtcctggagc
2	caatcttgttcccccagtag	aagatggaaatcatgccagt
3	ggaccctcagagctacatcctcag	gagtgtgtttggatgcccagc
4	actggatgcactcccaagag	ggcatcagatcccatgaaac
5	ccagaagaagagggcatcaa	cgactgctcttccaaaggtc
6	gtcctcttatcgaccccatc	aaaggcttggggaaacac
7	gcatgccttctgcttgtcttga	gaactggaaatctgcctccgtc
8	cactggcagaattagcatcagaaga	caaagcaatgcatggtttggatac
9	gtctatatccctcccactcgt	gggcaggtgagtatcagtcta
10	tctatgctacccgagggatg	aggaagcaaagagaccgtga
11	caccctaggagggcacataa	gggattaaaggcgtgtgcta
12	tcaagcctgatgacctatgc	agggtctccagaagattagcc
13	gaatgccagtgctcttacagt	catgcccagttgagattgt
14	gcaaaagaaactctcagcaaggcc	ccgtccctctcgccttcagt
15	cgaggcctcattatcttcat	ggagatgacgaggtttgcta
16	tgcatgtactttggtagggtc	agagtggcacctgagacttag
17	tccagcctctgccttatc	ctggagcaaagaatagaccat