Supplementary Materials and Methods

T cell subtypes sorting by flow cytometry

The spleens were prepared in a single-cell suspension and washed in RPMI-1640 (Gibco, Life Technologies, France). After red blood lysis, the splenocytes were washed three times in RPMI-1640.

(1) For Th1 and Th2 cell isolation, $CD4^+$ T cells were enriched by depletion ($CD4^+$ T cell isolation kit, MiltenyiBiotec, France) and were stimulated *in vitro* with a mix of PMA (50 ng/mL)/ionomycin (5 µg/mL) for 2hrs 30 min at 37°C, 5% CO_2. Then, Th1 and Th2 cells were sorted on a FACS ARIA (BD Biosciences, France) using anti-IFNγ-BV421 (clone XMG1.2) and anti-IL-4-PE (clone BVD4-1D11) antibodies (respectively BD Biosciences and MiltenyiBiotec, France) after cell permeabilization using the Cytofix/Cytoperm kit (BD Biosciences, France).

(2) $CD4^+CD25^+$ T cells were sorted directly from the splenocytes using a $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (MiltenyiBiotec, France), according to the manufacturer's instructions. Then, $CD62L^+$ Tregs and $CD62L^-$ Tregs were sorted on a FACS ARIA (BD Biosciences, France) using CD62L-PE-Cy7 (clone MEL-14) and CD44-VioBlue (clone IM7) (respectively BD Biosciences and MiltenyiBiotec, France). The cells were counted and adjusted to inject $1x10^6$ cells in 100 µL of phosphate-buffered saline intravenously into the retro-orbital plexus of the mice.

The sorting purity was ascertained by flow cytometry and was greater than 95%.

Sequencing of DNA methylation by bisulfite pyrosequencing

DNA was extracted from purified cells with the DNA blood and tissue kit (Qiagen, Hilden, Germany) using the standard protocol given by the manufacturer. The quality of extracted DNA was controlled by Nanodrop and quantified using PicoGreen (ThermoFisher Scientific, Fontenay-sous-Bois, France). Quantitative DNA methylation analysis was performed by pyrosequencing of bisulfite treated DNA(25, 26).

500ng of DNA was bisulfite converted using the EpiTect 96 Fast Bisulfite kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Regions of interest were amplified using 30 ng of bisulfite treated mouse genomic DNA and 5 to 7.5 pmol of forward and reverse primer, one of them being biotinylated. Sequences for oligonucleotides for PCR amplification and pyrosequencing, genomic coordinates of the analyzed region and number of analyzed CpGs per pyrosequencing primer are given in Supplementary Table S1. Reaction conditions were 1x HotStar Taq buffer supplemented with 1.6 mM MgCl2, 100 µM dNTPs and 2.0 U HotStar Taq polymerase (Qiagen) in a 25µl volume. The PCR program consisted of a denaturing step of 15 min at 95°C followed by 50 cycles of 30 s at 95°C, 30 s at the respective annealing temperature and 20 s at 72°C, with a final extension of 5 min at 72°C. 10 µl of PCR product were rendered single-stranded as previously described 25,26 and 4 pmol of the respective sequencing primer were used for analysis. Quantitative DNA methylation analysis was carried out on a PSQ 96MD system with the PyroGold SQA Reagent Kit (Qiagen) and results were analyzed using the PyroMark CpG software (V.1.0.11.14, Qiagen). The peak heights in the resulting output format, termed Pyrogram, were used to determine the methylation levels at individual CpGs. The methylation level was calculated as the ratio of the peaks corresponding to the methylated allele (Incorporation of C/G after bisulfite treatment) compared to the sum of the signals (peak height of the signal corresponding to the methylated alleles and the unmethylated alleles (incorporation of T/A after bisulfite treatment)) through the relative height of the peaks corresponding to the variable nucleotide positions. The average DNA methylation over all analysed CpGs in an amplification product was used for further statistical evaluation.

Measurement of Gata3 mRNA expression

mRNA expression of *Gata3* was analysed by qPCR using the following amplification primers: *Gata3* 5'-GAGGAGGAACGCTAATGG-3' and 5'-TTTCGATTTGCTAGACATCTTC-3'. *Sdha*, *Actb* and *Ppia* were selected for normalization from a selection of 12 genes included in the Genorm mouse kit (PrimerDesign Ltd, Southhampton, UK) after evaluation of the stability of expression in the analyzed sample cohort. 135 ng of input RNA was used for the cDNA synthesis by reverse

transcription using SuperscriptIII and oligodT20 (ThermoFisher). 2.5 µL of cDNA were used as input for the qPCR reaction (approximatively 2.5 ng of cDNA by qPCR essay) using the LightCycler 480 SYBR Green Master mix (Roche Molecular Diagnostics, Meylan France) on a Lightcycler 480 V2 (Roche). Amplification was performed under the following conditions: 95°C for 10 min, 45 amplification cycles at 95°C for 10s, 58°C for 10s and 72°C for 10s. The relative expression of *Gata3* was normalized to the geometric mean of the expression of the three reference genes, and calculated using the ΔΔCq method[23].

Measurement of humoral responses

Blood was collected after sensitization (day 43) and every 2 weeks during EPIT in EDTA-tubes to prepare plasma, which was subsequently used to titrate IgE, IgG1 and IgG2a specific to PPE or OVA. Specific antibodies were quantified using a semi-quantitative ELISA developed in-house(3). Microtiter plates were coated with 100 µL per well of 5 µg/mL peanut or OVA solution. Serial dilutions of 50 µL of each plasma were dispensed per well and incubated for 24 h at 4°C. A polyclonal anti-mouse IgE (STAR110A), IgG1 (STAR132A) or IgG2a (STAR82A) antibody labeled with alkaline phosphatase (Biorad, France) was used as an enzyme tracer. Reagent (pNPP, Sigma, France) was used as an enzyme substrate. Specific IgE, IgG1 and IgG2a were quantified by comparison with concentration-response curves obtained with a total IgE, IgG2a or IgG1 assay performed under identical conditions using a solid phase coated with a monoclonal anti-mouse IgE (clone LO-ME-3) or IgG2a (clone LO-MG2-9) antibody or a polyclonal anti-mouse IgG (STAR8B) antibody (Biorad).

Cellular responses in spleen

After treatment, half of the splenocytes were used for *in vitro* reactivation with PPE. Cells (2 x 10^6 cells per well) were incubated in a 24-well microtiter plate in medium alone or with PPE(12). Supernatants were harvested after 72 hrs and analyzed for the presence of cytokines (Il4, Il5, Il13, Il10, Ifnγ) using a Bio-Plex® system (Biorad, Marnes-la-Coquette, France) according to the manufacturer's instructions. The remaining splenocytes were used for the analysis of regulatory T cells just after harvesting using

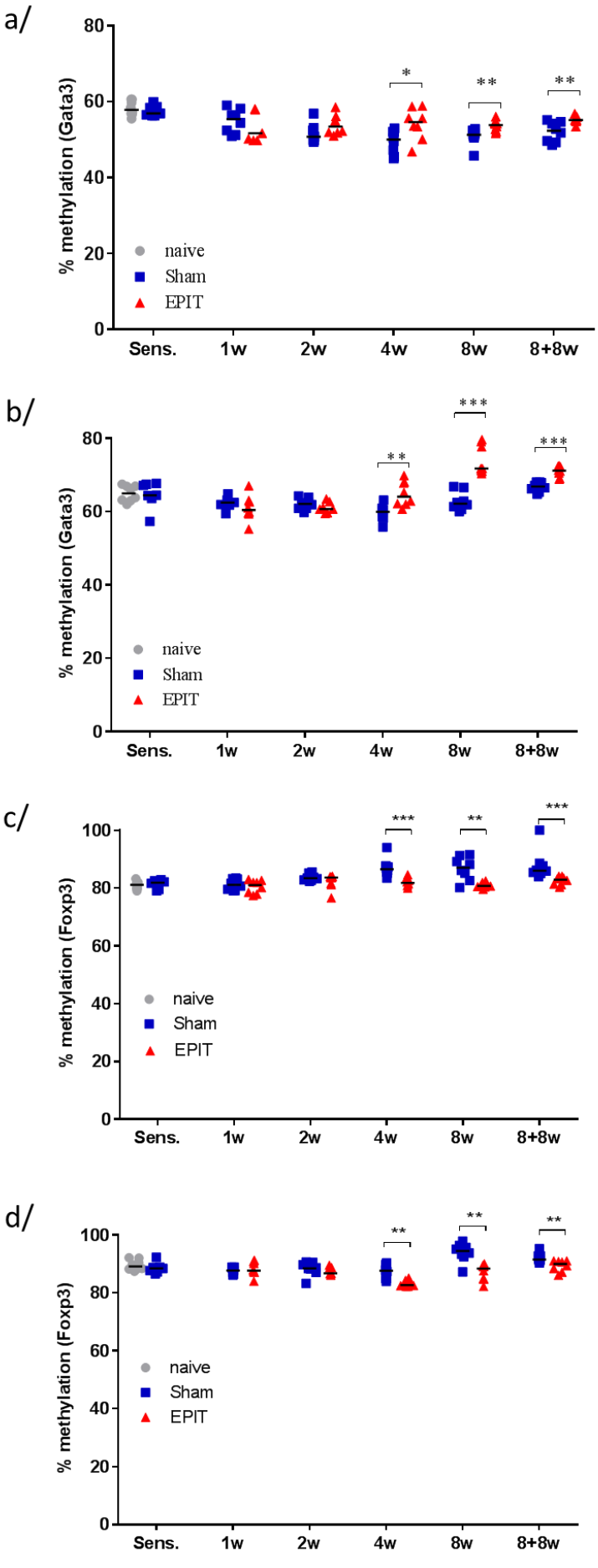
79 the combination of the following antibodies: anti-mouse CD4-PerCP-Cy5.5, CD25-FITC (all from BD
80 Biosciences, Le Pont de Claix, France) and Foxp3-APC (from e-Bioscience, San Diego, USA) or control
81 isotype(12). Flow cytometry was perfomed on Guava® easycyte (Millipore, Guyancourt, France) and
82 analysed using the FlowJo software (TreeStar Inc., Ashland, USA).

Supplementary TABLE

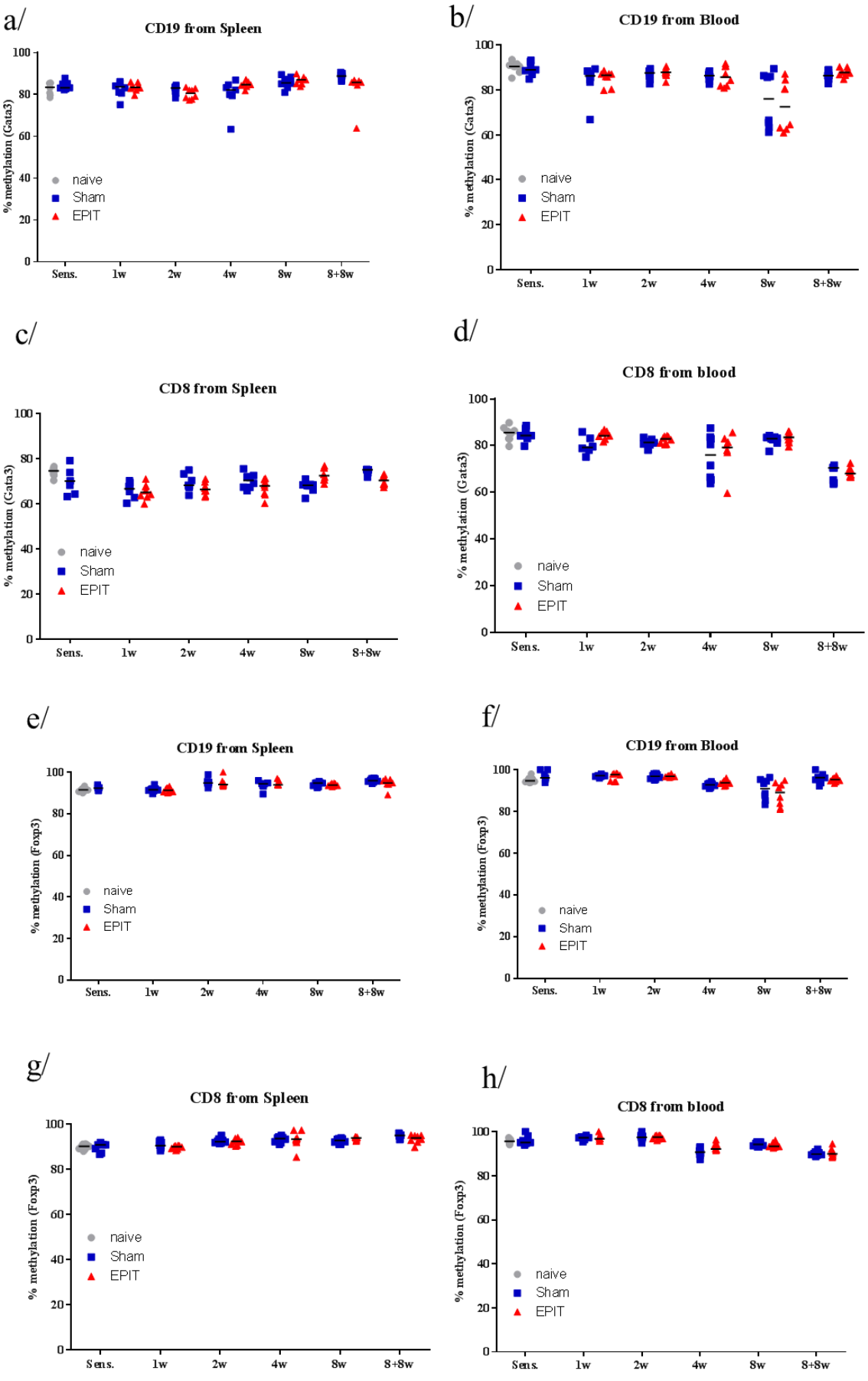
Table S1. Primer sequences used in quantitative real time-PCR assays

Gene	Size	PCR primer forward	PCR primer reverse	Pyrosequencing primer(s)	CpGs
Foxp3 (NM_054039) chrX:7,583,912-7,584,196	285	GGTTTTGTTGTTATAATTTGAATTTG	Biotin-CAACCTTAAACCCCTCTAACATC	TGTTGTTATAATTTGAATTT	1-4
				GGGGTAGATAATTTATTTTA	5-6
				GGTGATGTGGGTGTTAG	8-10
Gata3 (NM_008091) chr2:9,874,604-9,874,867	264	ATTTGATATTTGAGGTATTTTTTTT	Biotin-CAAAACCCTAAACAACCACCA	TTTTTATTTTGTTTGGT	1-10
				TGAGGATTGGAGTGGT	11-16
Gata3 (NM_008091) chr2:9,881,049-9,881,243	195	ATTGGATTTAGTTTGTAGGGGGTAT	Biotin-CCCCTCCCCTACTCTATATTTCTAA	GGGTATTTAATATGTAATTT	1-3
				GGGTAATTGGGAAAGAG	4-13
Tbx21 (NM_019507.2) chr11:97,114,687-97,114,901	215	GGTTGAATTTGGATTATAATAGGTG	Biotin-AACTCAAATAACTAACTTTCCC	GGATTATAATAGGTGGTTGT	1-8
				AGGGGTAGGGTAGTTATTTA	13-21
Rorc (NM_011281.3) chr3:94,372,824-94,373,073	250	TTTTGGGTTTTGTTTTTTGTTTT	Biotin-TACTCTATCTACCAATCCATCTCCC	TTTTTGGGTAGTTAGGGTA	2-3
				GGGGGTTTAGAGGAGG	4
Rorc (NM_011281.3) chr3:94,377,454-94,377,718	265	TTGTTTAGAAATATTGGGGGAGAG	Biotin-CTAAAACACACCCTACCAAAAAAAC	TTGTGTAGATTTAAGGGTTG	1
				AGTAAGTGAATGGGGGT	2-4

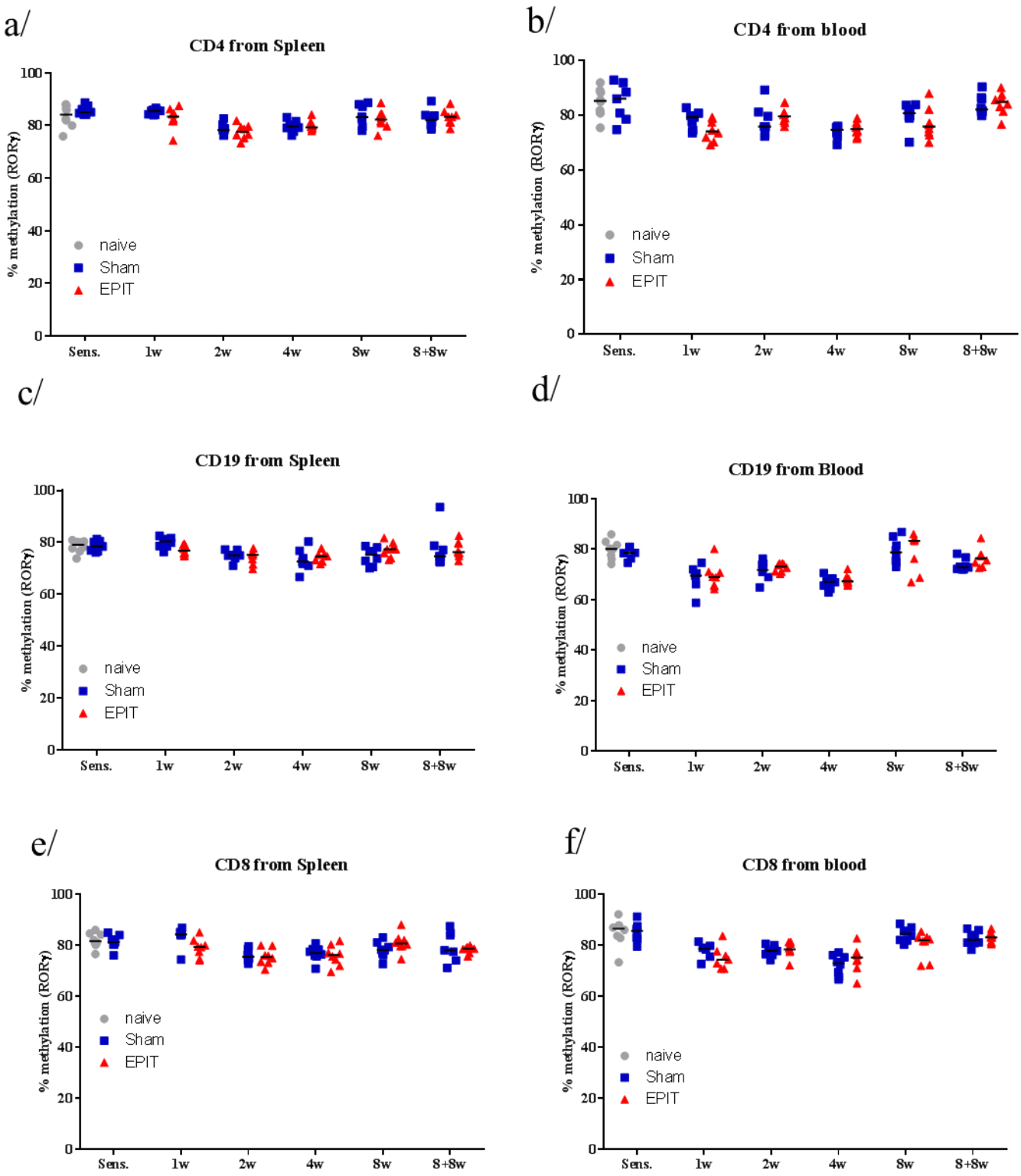
Supplementary Figure S1



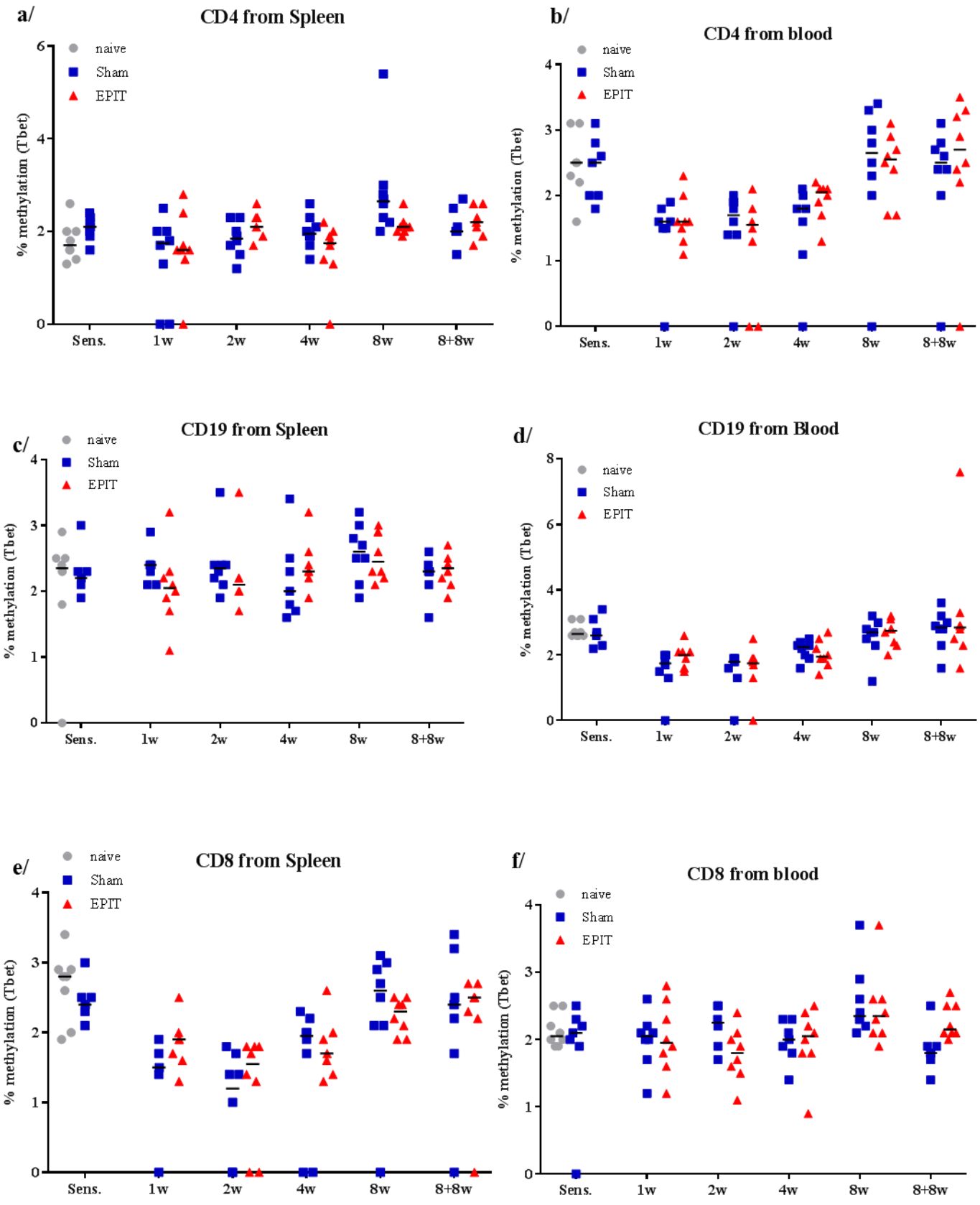
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5

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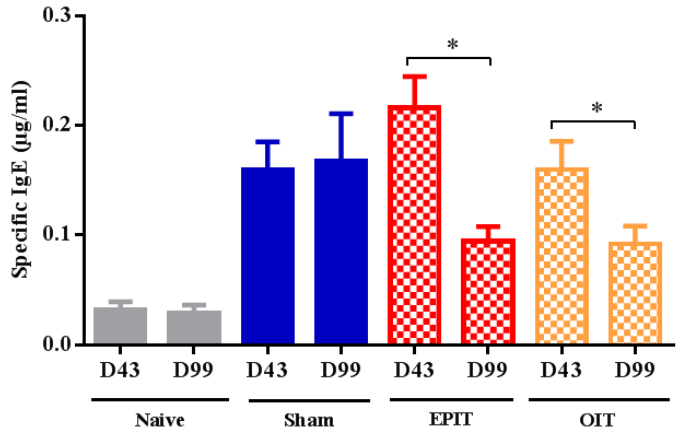

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