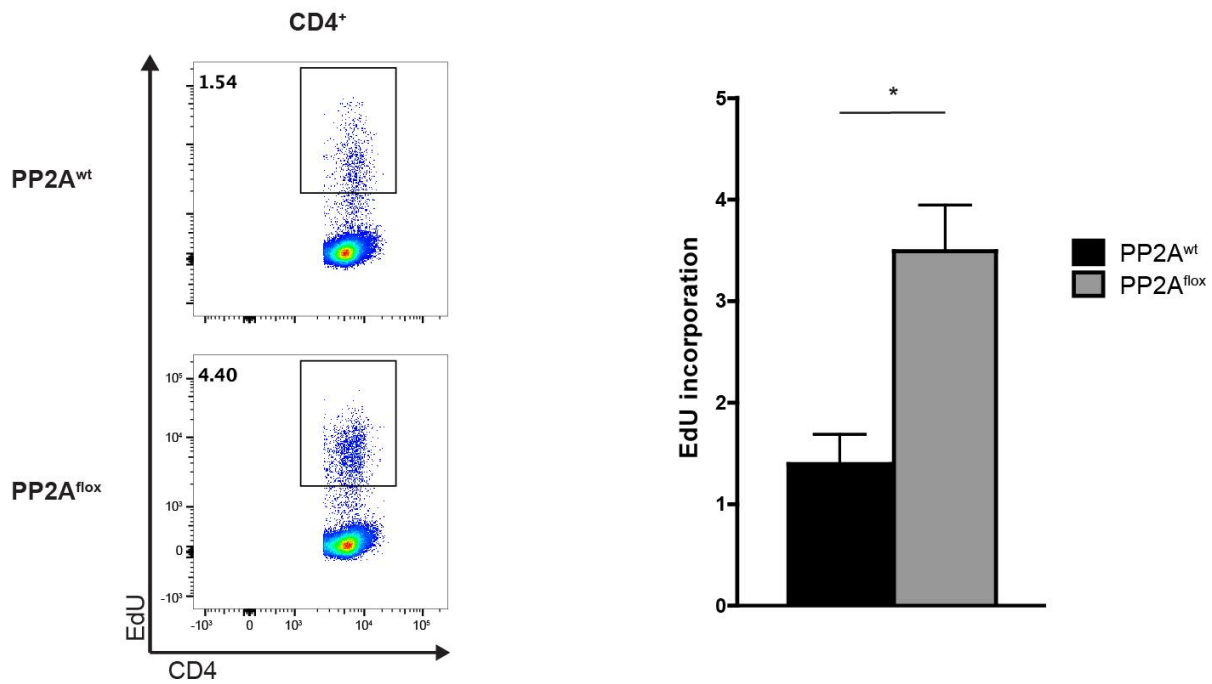


Supplementary Figure 1

T_{reg} cells exhibit greater PP2A activity than that of T_{conv} cells, and PP2A^{flox} mice develop multi-organ autoimmunity.

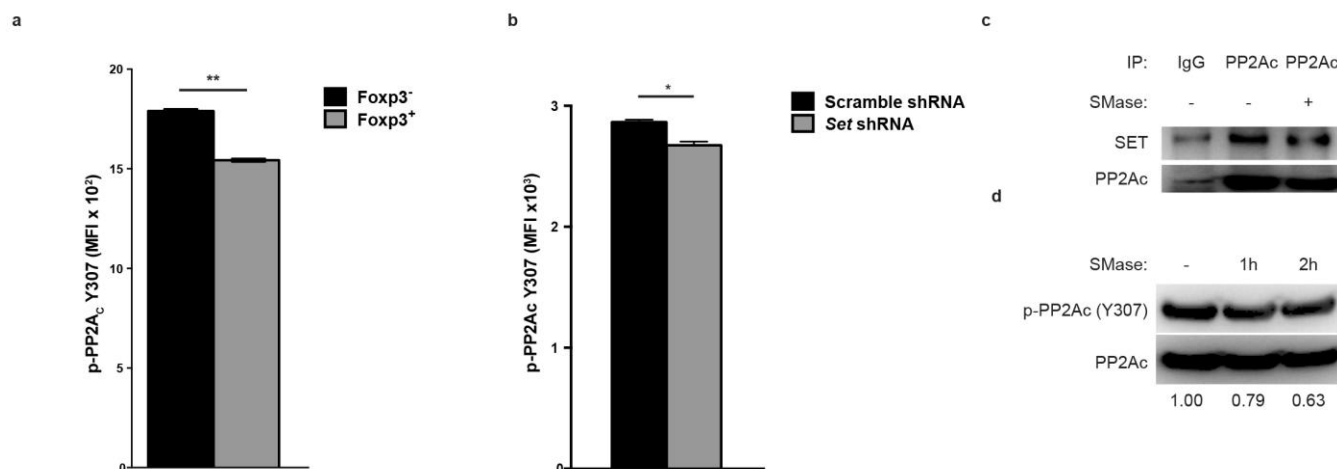
(a) The enzymatic activity of PP2A_C was quantified in CD4⁺CD25⁻ T_{conv} cells and CD4⁺CD25⁺ T_{reg} cells (n=5 per group). Data are from one experiment representative of two independent experiments with similar results. (b) *Left*: Real-time PCR of the *Ppp2r1a* and *Ppp2r1b* genes in unstimulated and stimulated naïve CD4⁺ T cells with CD3 plus CD28 antibodies (2μg/mL) for 24 hours. Each isoform was multiplexed separately in Taqman assays with the housekeeping gene *Actb* (n=3 per group). *Right*: Primer efficiency for the two isoforms with serial dilutions of the input DNA, demonstrating that the two PCRs retain efficiency over a wide range of DNA concentrations. (c) The appearance of PP2A^{wt} (left) and PP2A^{flox} (right) mice at 12 weeks of age. (d) Macroscopic and histologic image of a representative skin lesion of PP2A^{flox} mice. (e) Scaliness and crusting of the tail of PP2A^{flox} mice. (f) PP2A^{flox} mice that survived beyond 15 weeks of age (shown here at 19 weeks of age) developed significant skin disease with secondary ulceration. (g) The secondary lymphoid organs (*upper*: spleen, *lower*: peripheral lymph nodes) of PP2A^{wt} and PP2A^{flox} mice at 12 weeks of age. (h) Overt cervical lymphadenopathy in PP2A^{flox} mice. Images for (c-h) are from one experiment representative of at least three independent experiments with similar results. Mean ± s.e.m., *P<0.01 (unpaired, two-tailed t-test).



Supplementary Figure 2

Lymphoproliferation in PP2A^{flox} mice.

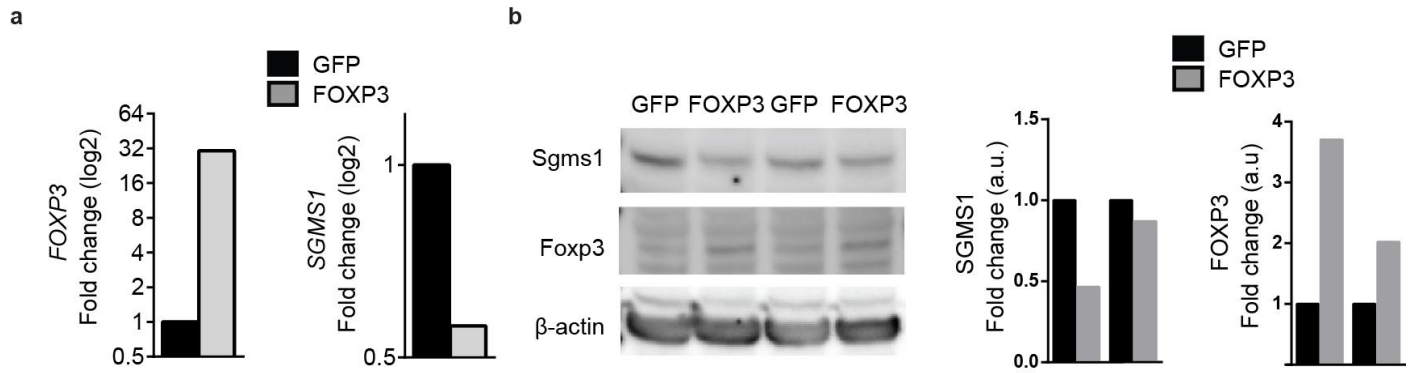
PP2A^{wt} and PP2A^{flox} mice were injected i.p. with EdU (500μg/mouse). The dividing cells were allowed to incorporate *in vivo* the EdU for 18 hours and the mice were then euthanized and the splenocytes stained for EdU. The EdU staining for the CD4⁺ T cells is shown. A representative histogram (left) and quantification of the results (right) is shown (n=3 per group). Data are from one experiment representative of two experiments with similar results. Mean ± s.e.m., **P*<0.01 (unpaired, two-tailed t-test).



Supplementary Figure 3

T_{reg} cells have decreased phosphorylation of PP2Ac at Tyr307 upon activation through modulation of the ceramide-SET-PP2A pathway.

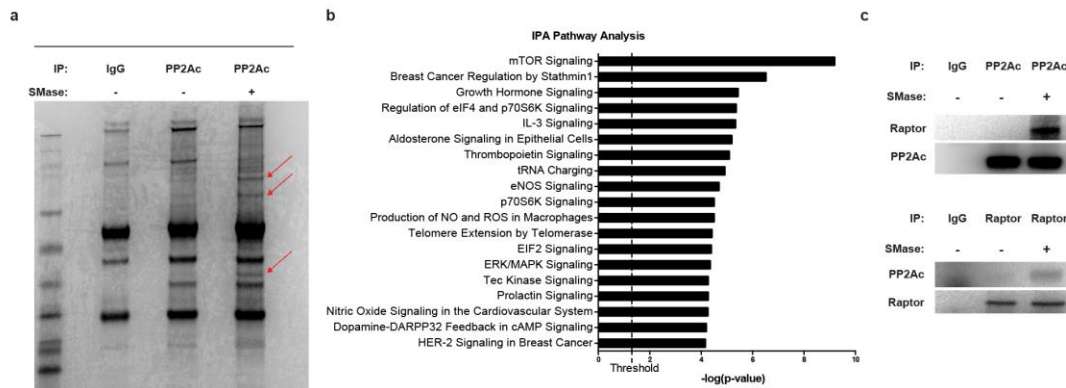
(a) Splenocytes isolated from Fcγp3^{IRES-GFP} mice were stimulated with CD3 plus CD28 (2μg/mL) antibodies for 24 hours. Intracellular staining was then performed for p-PP2Ac (Y307) in CD4⁺Fcγp3⁻ and CD4⁺Fcγp3⁺ T cells (n=3 per group). Data are from one experiment representative of three independent experiments with similar results. (b) Intracellular staining for p-PP2Ac (Y307) of naïve CD4⁺ T cells transfected with a scramble shRNA or Set-specific shRNA mCherry-expressing plasmid and then activated with CD3 plus CD28 (2μg/mL) antibodies for 24 hours. The analysis was done on mCherry⁺ T cells (n=3 per group). Data are from one experiment representative of two independent experiments with similar results. (c) Jurkat T cells were treated with SMase or vehicle for 1h and then lysed for protein immunoprecipitation with a PP2Ac-specific antibody or a mouse IgG control antibody. The blots were then probed for SET and PP2Ac. Image is from one experiment representative of two independent experiments with similar results. (d) Naïve CD4⁺ T cells were stimulated with CD3 plus CD28 (2μg/mL) antibodies for 24h and then treated with vehicle or SMase for 1 or 2 hours. The cells were then lysed for immunoblot and probed for p-PP2Ac (Y307) and total PP2Ac. The relative ratio of p-PP2Ac/total PP2Ac band densities for each lane is also shown. Image is from one experiment representative of two independent experiments with similar results. Mean ± s.e.m., MFI: Mean fluorescent intensity, *P<0.05, **P<0.01 (unpaired, two-tailed t-test).



Supplementary Figure 4

Foxp3 inhibits the expression of SMS1.

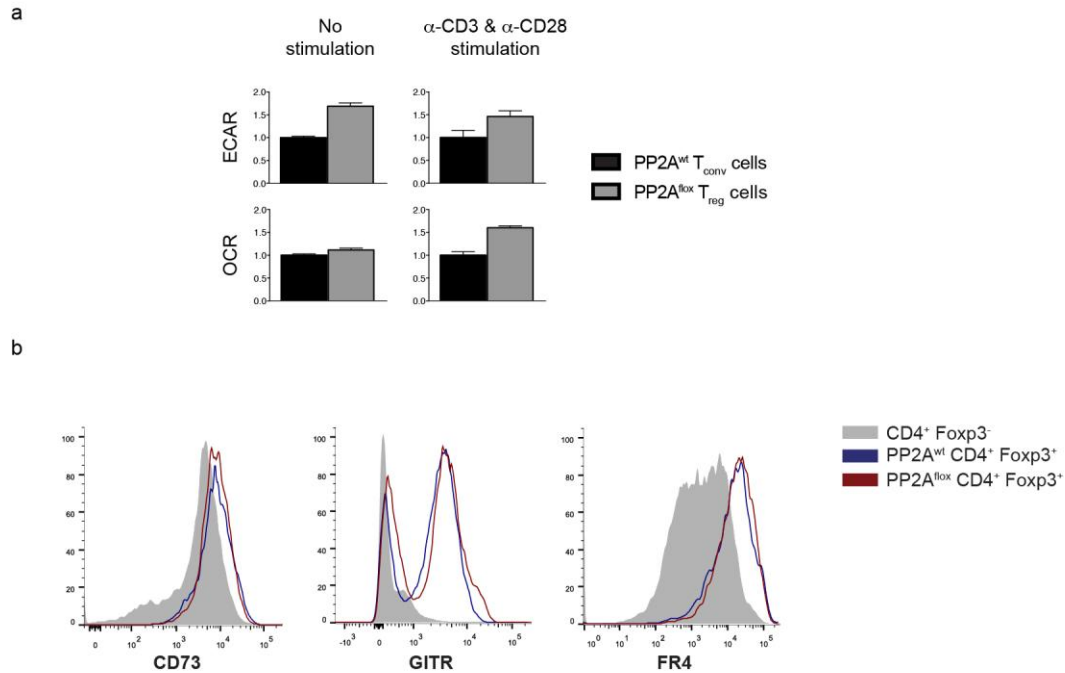
(a) Jurkat T cells were spin-infected with a Thy1.1 expressing retrovirus that harbored or not the human *FOXP3* coding sequence. Thy1.1⁺ cells were FACS-sorted 24 hours after infection and analyzed for *FOXP3* and *SGMS1* mRNA levels by real time PCR. (b) Immunoblot of protein extracts from control (*GFP*) or *FOXP3* infected Jurkat T cells were probed for *FOXP3* and *SGMS1*. Immunoblot (left) and relative quantification (right) of two distinct GFP-*FOXP3* replicate pairs are shown. Results (a-b) are from one experiment representative of three independent experiments.



Supplementary Figure 5

Activated PP2A interacts with the mTOR pathway in T_{reg} cells.

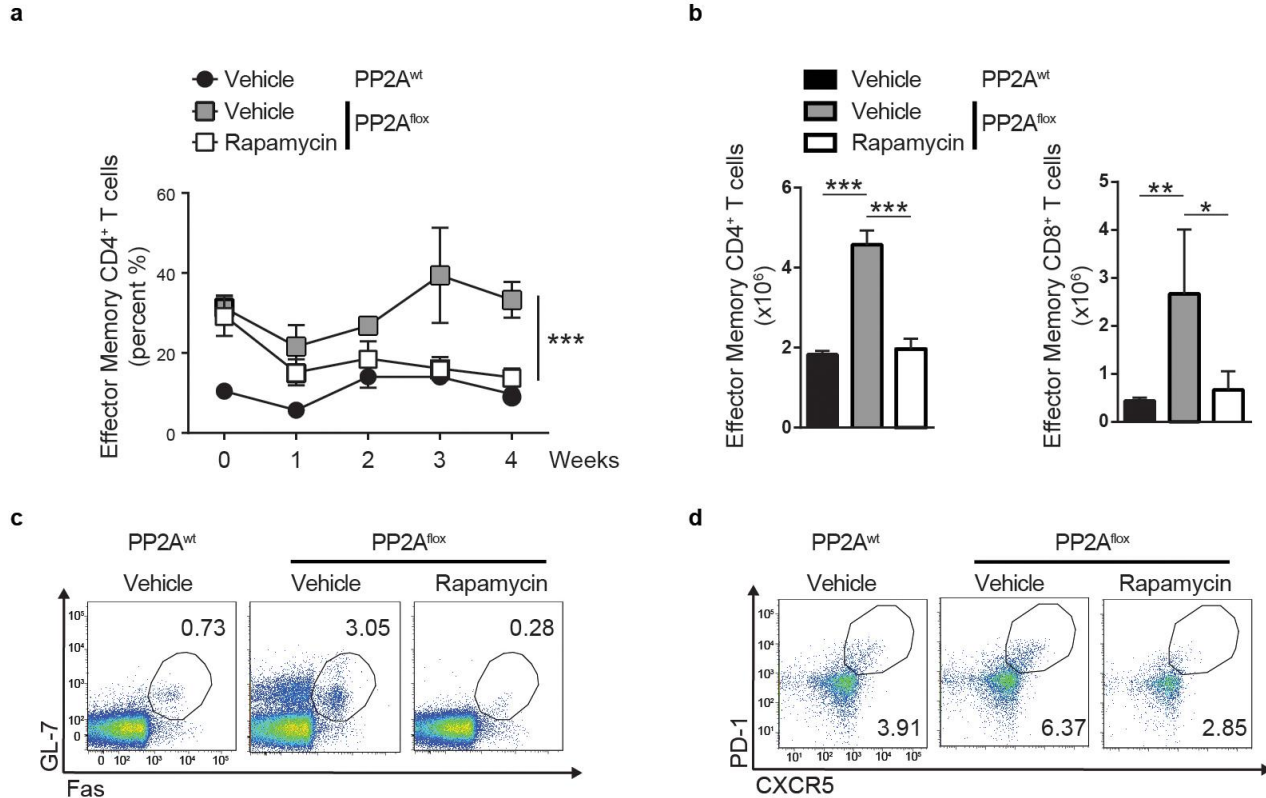
(a) Jurkat T cells were treated with SMase (0.5 units/mL) or vehicle (50% glycerol in PBS) for 1h and then lysed for protein immunoprecipitation with a PP2A_C-specific antibody. The indicated bands (arrows) were subjected to mass spectrometry. (b) The Ingenuity Pathway Analysis (IPA) of 269 proteins identified in (a) is shown. (c) Jurkat T cells treated as in (a) were lysed for protein immunoprecipitation with a PP2A_C-specific antibody (upper) or a Raptor-specific antibody (lower). The immunoblots were then probed for PP2A_C and Raptor. Image is from one experiment representative of two independent experiments with similar results.



Supplementary Figure 6

T_{reg} cell phenotype of PP2A^{wt} and PP2A^{flox} mice.

(a) The glycolytic (ECAR) and oxidative phosphorylation (OCR) rate of PP2A^{wt} T_{conv} and PP2A^{flox} T_{reg} cells were measured before and after 24 hours of stimulation with CD3 plus CD28 antibodies. Results are normalized to PP2A^{wt} T_{conv} cells for each condition. (b) PP2A^{wt} and PP2A^{flox} T_{regs} were stained for CD73, GITR and FR4. Data (a-b) are from one experiment representative of two independent experiments with similar results. Mean \pm s.e.m. is shown.



Supplementary Figure 7

Rapamycin normalizes the proportion of effector lymphocytes in PP2A^{flx} mice.

(a) The proportion of effector memory (CD44⁺CD62L⁻) CD4⁺ T cells in blood was assessed before rapamycin treatment and in a weekly basis after treatment initiation. Progression in the percentage of cells is depicted (two-way ANOVA followed by Bonferroni's test). (b) Numbers of effector memory CD4⁺ and CD8⁺ T cells in spleen from rapamycin-treated and untreated PP2A^{wt} and PP2A^{flx} mice after 4 weeks of treatment (ANOVA followed by Tukey's multiple comparison test). (c, d) Percentage of GC (GL-7⁺FAS⁺) B cells (c) and T_{fh} cells (PD-1⁺CXCR5⁺) within CD4⁺ T cells (d) in spleens from (b) are shown. Cumulative (a and b) and representative (c and d) data from two experiments with similar results are demonstrated. Mean \pm s.e.m. is shown, * P <0.05, ** P <0.01, *** P <0.001.

APEX1	CPSF3	FMR1	INTS2	NUP153	RNF10	TARS
AAK1	CPT1A	FOXK2	INTS4	NUP205	RNF40	TARS2
ABCF3	CRKL	GAPDH	INTS6	NUP210	RPAP3	TBC1D31
ABLIM1	CSPP1	GARS	INTS8	NUP214	RPL6	TCF12
ACO2	CSTF3	GFPT1	IPO11	NUP93	RPLP0	THEMIS
ACSL4	CTNNA1	GNAI2	KARS	PABPC1	RPS6KA1	THOC1
ACTB	CTTNBP2N1	GNB1	KBTBD11	PABPC4	RPSA	THOC5
ADD3	CUL2	GRIPAP1	KCNAB2	PARD3	RPTOR	TIMM50
ADNP	CUL3	GSPT1	KDM5A	PCBP2	RRM1	TMPO
ADRBK1	CUL4B	GTF3C3	KHSRP	PDIA4	SART3	TPP2
ADRBK2	DDX1	GTPBP1	KIF2C	PDS5B	SATB1	TPR
AFG3L2	DDX17	HBS1L	KIF3A	PFKP	SEC13	TRAP1
AIP	DDX26B	HCFC1	KPNB1	PHLDB3	SEC23A	TRIM56
AKR1B1	DDX50	HDGF	LASP1	PHRF1	SEC23B	TUBA1C
ANKRD13A	DDX51	HIRIP3	LETM1	PIK3C3	SEC23IP	TUBB
ANXA1	DGKA	HNRNPA0	LRCH4	PLAA	SET	TXLNA
ANXA2	DGKZ	HNRNPA1	LRRCC1	POLG	SHKBP1	UBA1
ANXA6	DHX15	HNRNPA2B	MAN1B1	POLRMT	SKIV2L	VANGL1
ARHGEF7	DNAJC2	HNRNPAB	MARCKSL1	POM121C	SKIV2L2	VAR5
ARID4B	DTL	HNRNPH3	MARK3	PPIG	SLMAP	VCP
ASUN	EEF1A1	HNRNPR	MCM5	PPP1CB	SMC5	VPS11
ATP2A3	EIF2B5	HOOK3	MCM7	PPP2CB	SMEK1	VPS35
BAHCC1	EIF3A	HP1BP3	MED23	PPP2R1A	SMEK2	WDR70
BBS2	EIF3I	HPS6	MFN2	PPP2R4	SMPD4	WRNIP1
BCL11B	EIF3M	HRNR	MLH1	PPP4R1	SORD	XRCC1
BPNT1	EIF4B	HSD17B4	MORC3	PRKCA	SPATA5L1	XRCC5
CAND2	EIF4G1	HSP90AA1	MSN	PRKCB	SRP72	XRCC6
CAPZA1	ELAVL1	HSP90AB1	MTA1	PRKCH	SSH1	ZFP91
CCDC14	ELMO1	HSPA5	MX2	PRKCQ	STAT1	ZNF217
CCDC6	ENO1	HSPA8	MYB	PRKD2	STAT3	
CCNT1	EPB41L2	HSPA9	NCBP1	PUM1	STIM1	
CD2AP	ERAP1	IDH3A	NDC1	QARS	STRBP	
CDK6	EWSR1	IDH3B	NFATC3	RALY	STRIP1	
CEP120	EXOC3	IFI16	NOP2	RANGAP1	STRIP2	
CEP135	EZR	IK	NPM1	RBM14	STRN	
CEP78	FAF1	IKZF1	NSF	RCSD1	STRN3	
CEP95	FAM160B1	IL16	NUCKS1	RFC2	STRN4	
CHUK	FBL	ILF3	NUFIP2	RFC5	SUMO1	
CLINT1	FDPS	IMMT	NUP107	RFTN1	SUN2	
COPS6	FERMT3	INTS1	NUP133	RNASEL	TALDO1	

Supplementary Table 1.

PP2A_C-interacting proteins after ceramide-mediated PP2A activation.