Supplementary Methods:

Cell isolation. Spleen and lymph nodes (axillary, inguinal) were removed from mice and gently meshed in DMEM containing 10% FBS to prepare for single cell suspensions. $CD4^+CD25^+$Tregs and $CD4^+CD25^-$ T cells were isolated by the $CD4^+CD25^+$ regulatory T cell isolation kit (Miltenyi Biotec) according to manufacturer's instruction. The purity of $CD4^+CD25^-$and $CD4^+CD25^+$ subpopulations were around 90% and 80-85%, respectively. Splenic macrophages and DCs were isolated through positive selection by CD11b and CD11c microbeads kit (Miltenyi Biotec), respectively.

Cell culture experiments, $CD4^+CD25^-$ naïve T cells were cultured with soluble anti-CD3 antibody (0.5 μg/ml) and T-cell-depleted splenocytes as antigen presenting cells (APCs) or with plated bound anti-CD3 and soluble anti-CD28 antibodies in the presence or absence of TGFβ1 at indicated concentrations for indicated time points. In some experiments, macrophages and DCs were used as APCs for induction of $Foxp3^+$ T_{reg} cells in culture. The cells were then analyzed by real-time PCR for Foxp3, TβR I and TβRII mRNA expression or Foxp3, IL17, Annixin V, 7-AAd, and Ki-67 expressions by flow cytometric analysis. In some experiments, TGF-β1 and IL-6 were added into the T cell cultures and cells were collected for IL-17 and RORγt mRNA expression. IL-17 protein in $CD4^+$ T cells were determined by intracellular IL-17 staining by flow cytometry and ELISA analysis for IL17 cytokine in the culture supernatants.

Mixed bone marrow chimeras. Bone marrow was isolated from 6 to 8 weeks old PARP-1$^{-/-}$or PARP-1 $^{+/+}$ mice (CD45.2^{+}, C57BL/6), or age-matched C57BL/6 CD45.1^{+} wild type mice. Bone marrow was depleted of T cells by mouse CD90.2 microbeads. Rag1$^{-/-}$ recipient mice were sublethally irradiated (4.5G) and reconstituted with a mixture of PARP-1$^{+/+}$ or PARP-1$^{-/-}$ bone marrow (1×10^{6} cells) and CD45.1 bone marrow (1×10^{6} cells) in 1:1 ratio. Five weeks after reconstitution, the cells from thymus and spleen were collected and analyzed by flow cytometry.

Flow cytometric analysis*.* Flow cytometric analysis was performed as described before (5). Briefly, thymus, spleen and lymph node were gently meshed in complete DMEM containing 10% FBS (BioWhittaker) for single cell suspension. The cells were washed using PBS containing 0.5% BSA for 10 min at 300g, and then resuspended in staining buffer for cytometric analysis with antibodies against CD4, CD8, CD25 and intracellular Foxp3, IL17 and RORγt. The stained cells were analyzed on FACSCalibur flow cytometry.

Real-time PCR. Real-time RT-PCR was done as described [2]. Mouse *Foxp3*, *Tgfbr1, Tgfbr2* and *Hprt* and human *Foxp3*, *Tgfbr1, Tgfbr2, Rorc* and GAPDH were analyzed with TaqMan gene expression assay kit. The following primers were from Applied Biosystems: mouse *foxp3*, Mm00475156; *Tgfβr1*,Mm03024015; *Tgfβr2*, Mm03024091; *Hprt*, Mm00446968. Human *Tgfβr1*, Hs00610320; *Tgfβr2*, Hs00234253; Rorc Mm01261019; *GAPDH*, Hs99999905.

***Chromatin immunoprecipitation (ChIP) assay*.** ChIP assay was done as described (5). Freshly isolated $CD4^+CD25^-$ cells or cells stimulated with plate-bounded anti-CD3 and soluble anti-CD28 with TGF-β 1 for 30 min were used. Processed chromatin was incubated with 4μg mouse PARP-1 or Smad3 antibodies or their respective isotype-matched control antibody. Immunoprecipitated DNA was analyzed by SYBR Green Real-time PCR detection system. The results were normalized with chromatin that was not treated with antibodies as the input control. PCR primers for the detection of regions were as follows: Foxp3 5'-CATGTTGGCTTCCAGTCTCCTT-3' (forward) and 5'-CCACACTTTTGACCTCTGCAGTA-3' (reverse). TβRI and TβRII primers were listed in supplementary figure 5a.

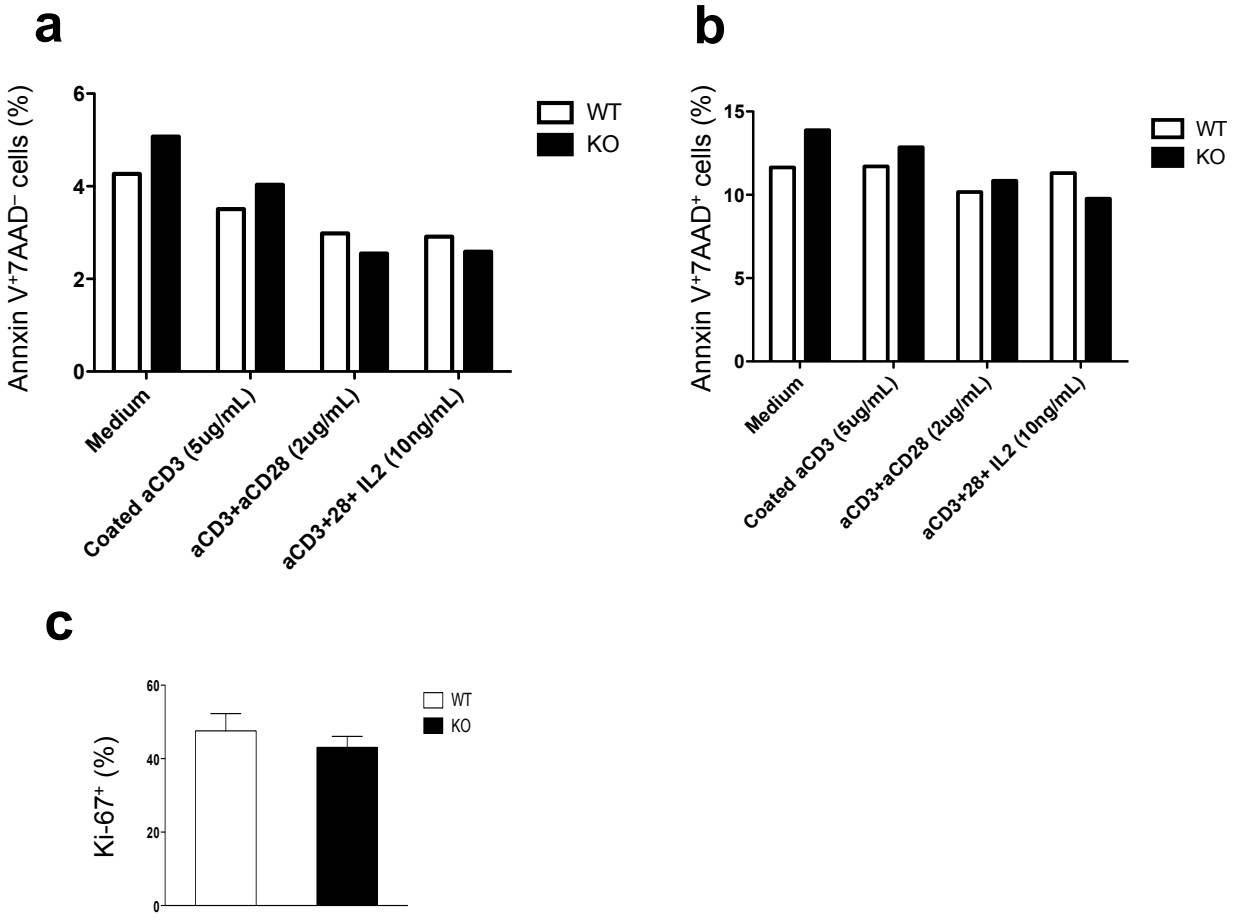
Luciferase assay. The mouse TβRI (+1 to -1183) and TβRII (+101 to -1023) promoter were synthesized (GenScript) and inserted into pGL4 basic vector. EL4/LAF cells were transfected with reporter construct together with PARP-1 expressing pCMV-SPORT6 vector (Thermo Scientific) or control pCMV-SPORT6 vector by using Nucleofector L kit (LONZA). After 18 hr, the cells were stimulated with anti-CD3 (1 μg/mL) and anti-CD28 (1 μg/mL) in the presence or absence of TGF-β1 (2 ng/mL) for 24h. The TβRI or TβRII promoter activity was determined by a dual-luciferase assay system (Promega) according to manufacturer's instruction. Transfections were normalized with the pRL-TK vector (Promega) as an internal

control and the results were shown as (firefly luminescence - background) / (renilla luminescence - background).

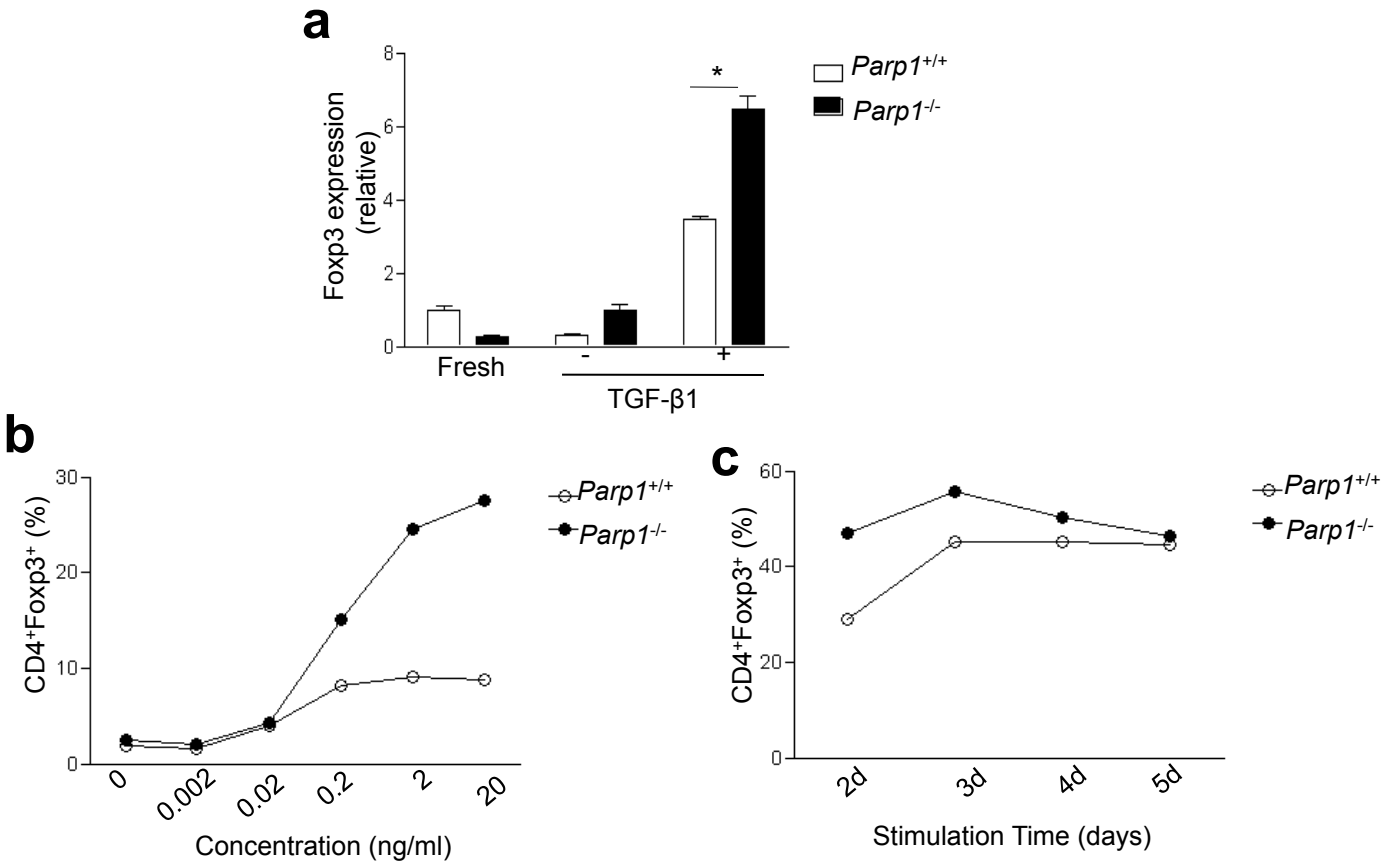
Oral tolerance. OTII transgenic PARP-1$^{+/+}$ or PARP-1$^{-/-}$CD4^{+}CD25^{-} T cells (CD45.1^{+}) were adoptively transferred to C57BL/6 mice (CD45.2^{+}). Recipient mice were fed with1.5% OVA protein in drinking water for 5 consecutive days. The drinking water was replaced every 48 hours. On day 6, spleen, lymph nodes (axillary, brachial, mesenteric, and peyer's patches), *lamina propria* lymphocytes (LPLs) were collected, and Foxp3 expression was assessed in transferred cells by flow cytometric analysis.

Immunoblot analysis. Immunoblot analysis was done as described [2].The following antibodies were used: antibodies to phospho-Smad2 (S465/467), Smad2 (L16D3) and GAPDH.

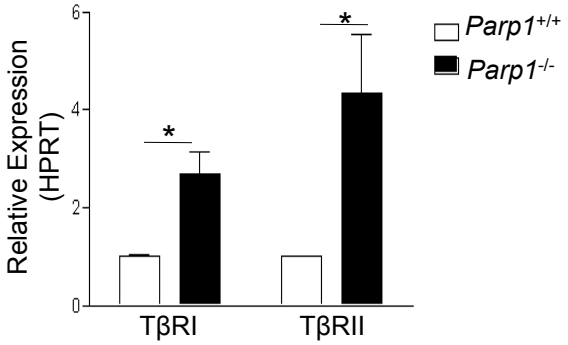
Isolation of subsets of human CD4^{+} T cells and cell culture Human peripheral blood mononuclear cells (PBMC) were obtained by leukopheresis of normal volunteers at the Department of Transfusion Medicine at the National Institutes of Health (NIH, Bethesda, MD). The CD4^{+}CD25^{-} T cells were isolated using the human T regulatory isolation kit .[18] Purified cells were pre-incubated with 5-AIQ for 20 mins, cells were stimulated with human anti-CD3 and CD28 antibodies in X-VIVO 20 medium for the indicated time for real-time PCR analysis.



Supplementary Fig. 1 Analysis of Tregs apoptosis and proliferation. (**a-b**) Staining of 7AAD versus Annexin V on 1 day cultured $CD4^+CD25^+$ Tregs of PARP-$1^{-/-}$ or PARP-$1^{+/+}$ littermate control mice at indicated conditions. Data are representative of three independent experiments. (**c**) Staining of Ki-67 on $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs in spleens of PARP-$1^{-/-}$ or PARP-$1^{+/+}$ littermate control mice. The mice shown in the figure were on sv/ 129 x C57BL6 background.

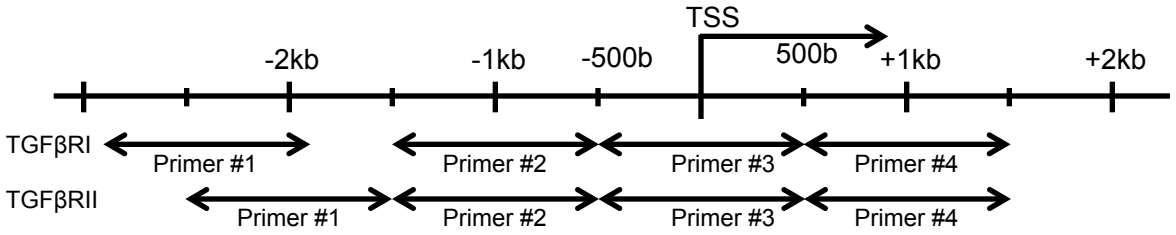


Supplementary Fig 2. Greater Foxp3 induction in PARP-1$^{-/-}$ CD4^{+} T cells *in vitro*. (**a**) Quantitative PCR analysis of *Foxp3* gene expression in CD4^{+}CD25^{-} T cells which were cultured for 24 hrs in absence or presence of TGF-β1. Data are representative of two experiments (mean±s.e.m). (**b**) The frequency of Foxp3^{+} T_{regs} analyzed by flow cytometry following culture of CD4^{+}CD25^{-} T cells with a anti-CD3 antibody, irradiated CD4^{+} depleted APCs and different dose of TGF-β1. Data are representative of more than four experiments. (**c**) The frequency of Foxp3^{+} Tregs analyzed by flow cytometry following culture of CD4^{+}CD25^{-} T cells with anti-CD3 antibody, irradiated CD4^{+} depleted APCs and TGF-β1(2ng/ml) analyzed at the indicated time points of culture. Data is representative of three experiments. The mice shown in the figure were on sv/129 x C57BL6 background. *P<0.05.



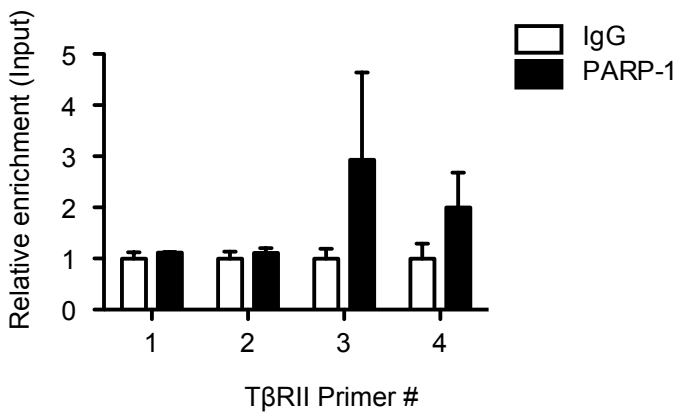
Supplementary Fig 3. Increased TβRI and TβRII expressions in $CD4^+CD25^-$ T cells of PARP-$1^{-/-}$ mice on the sv/129 x C57BL6 background. Quantitative PCR analysis of the expression of TβRI and TβRII in freshly isolated splenic $CD4^+CD25^-$ T cells of PARP-$1^{-/-}$ mice and PARP-$1^{+/+}$ littermate control mice on sv129 x C57BL6 background. **P*<0.05.

a

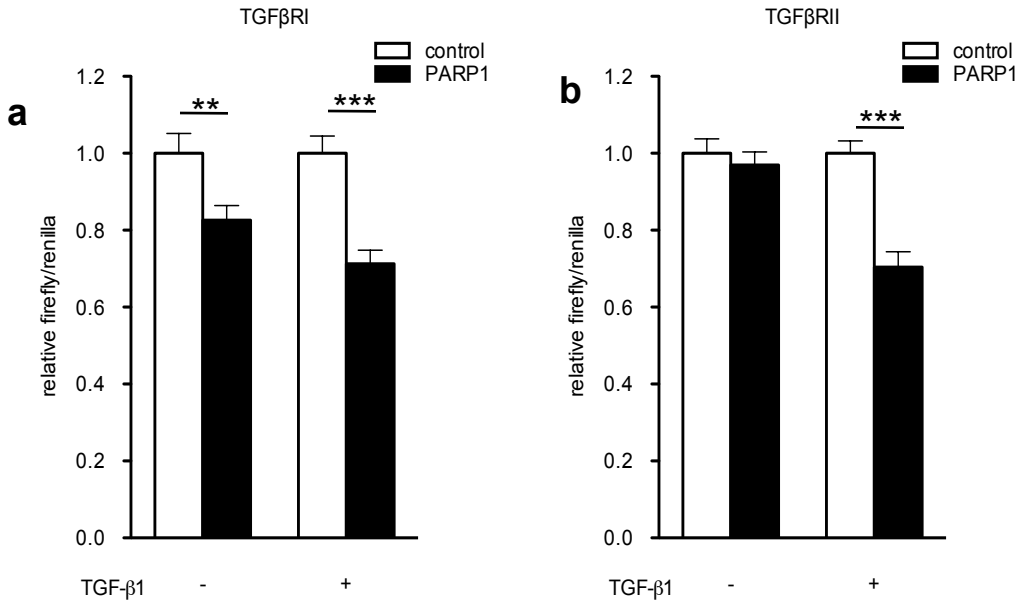


		TGFβRI (5′ --> 3′)	TGFβRII (5′ --> 3′)
#1	Forward	TGTTTTCAGATCTGTAGCTCATGG	TGAGGGAGACAGGCTGAGTT
	Reverse	TGTGTTCACTTGGAAACTGAGGA	CTCAAGGGATGGCAGTAGCC
#2	Forward	TCTGGAGCTCATTTTGGCGT	GTGGTGGAGTCTGCTTTTCAA
	Reverse	AGCGGGAGCAGTCATAGGTA	ATAGCCTTTCTACCTCCAGCC
#3 (Fig. 2f)	Forward	ACGTCGTCCGCAGCTCCTCA	TGAGCAGGAGGCTGGGGCTC
	Reverse	AAAGAGCTGAAGCGAGCCAGAG	TCTCGGCGCCCGGGTAAAGT
#4	Forward	GCAAAATACTGAGGCTCGGC	ACTGAGGCCCAGAGTCATGT
	Reverse	TGGAGAGACTTTTGTTTTGGCT	CCCATCGCAGGGGTTAGTTA

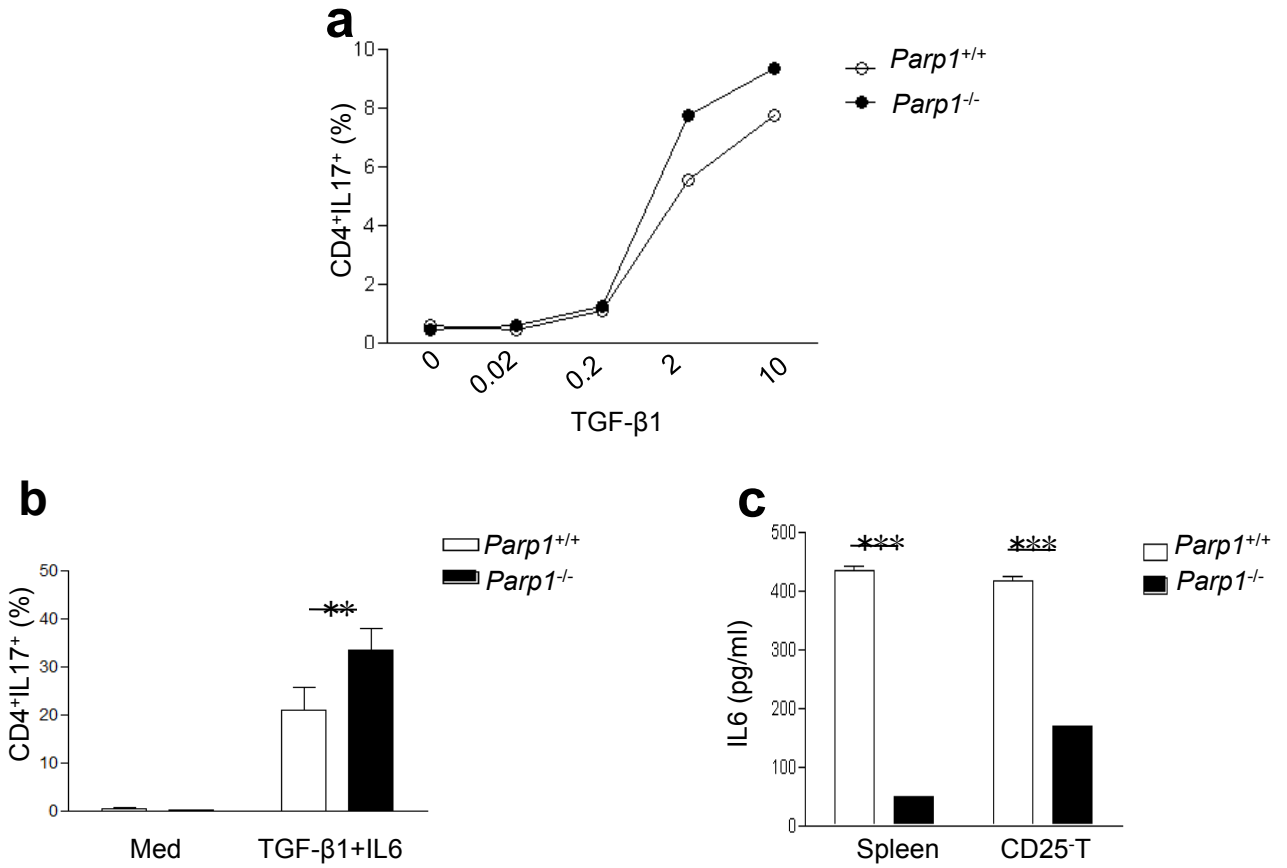
b



Supplementary Fig 4. Increased binding of PARP-1 at TβRII gene regions from -500bp to 1kb from Transcriptional Starting Site (TSS). (**a**) Illustration of the primer binding sites on TβRI and TβRII genes in the ChIP assays. (**b**) ChIP-coupled quantitative PCR analysis of PARP-1 enrichment at some regions at TβRII genes from -2 kb to +1kb from Transcriptional Starting Site (TSS). Graph shows the relative enrichment of PARP-1 binding to the regions recognized by TβRII primers 1, 2, 3, and 4.



Supplementary Fig 5. PARP-1 over expression suppresses the promoter activity of TβRI and TβRII after TGF-β1 stimulation. The mouse TβRI (+1 to -1183) and TβRII (+101 to -1023) promoter were synthesized (GenScript) and inserted into pGL4 basic vector. EL4/LAF cells were transfected with reporter construct together with PARP-1 expressing pCMV-SPORT6 vector (Thermo Scientific) or control pCMV-SPORT6 vector by using Nucleofector L kit (LONZA). After 18 hrs, the cells were stimulated with anti-CD3 (1 μg/mL) and anti-CD28 (1 μg/mL) antibodies in the presence or absence of TGF-β1 (2 ng/mL) for 24 hrs. The TβRI (**a**) or TβRII (**b**) promoter activity was determined by a dual-luciferase assay system. Transfections were normalized with the pRL-TK vector as an internal control and the results were shown as (firefly luminescence - background) / (renilla luminescence - background). Graphs show mean ± SD of triplicate wells. Data is representative of three independent experiments. **$p<0.01$, ***$p<0.002$.



Supplementary Fig 6. Greater induction of Th17 cells in PARP-1$^{-/-}$ CD4^{+} T cells *in vitro*. (a) The percentage of IL17^{+} cells in CD4^{+} population analyzed by flow cytometry. CD4^{+}CD25^{-}T from PARP-1$^{-/-}$ or PARP-1$^{+/+}$ mice on C57BL/6 background were stimulated with anti-CD3 antibody and irradiated PARP-1$^{+/+}$ CD4^{+} depleted APCs at different doses of TGFβ-1 plus IL-6 (50ng/ml). (b) The percentage of IL17^{+} cells in CD4^{+} population of PARP-1$^{-/-}$ or PARP-1$^{+/+}$ littermate controls on sv/129 x C57BL6 background which were stimulated with anti-CD3 antibody and and irradiated PARP-1$^{+/+}$ CD4^{+} depleted APCs in the absence (Med) or presence of TGFβ-1 plus IL6 (TGFβ1+IL6) for 4 days. Data are representative of more than three independent experiments. (c) IL-6 concentration determined by ELISA in supernatants from either whole splenocytes or CD4^{+}CD25^{-} T cells from PARP-1$^{-/-}$ or PARP-1$^{+/+}$ littermate controls on sv/129 x C57BL6 background which were stimulated with anti-CD3 antibody for three days. Data are representative of three to four independent experiments. ***P<0.001.