The Ligase PIAS1 Restricts Natural Regulatory T Cell Differentiation by Epigenetic Repression

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ily activate ATM through the oxidation pathway. This may be due to trace amounts of Mn^{3+} in preparations of $MnCl_2$ (*30*).

To further investigate the functional effects of the C2991L mutation, we used lymphoblasts from an A-T patient lacking functional ATM and stably complemented these cells with either wild-type or C2991L alleles of ATM under the control of an inducible promoter (fig. S7). After induction, cells were exposed to a low concentration of H_2O_2 or to camptothecin, a topoisomerase poison that induces DNA breaks. Consistent with the results with purified proteins in vitro, wild-type ATM responded to both H_2O_2 and camptothecin, whereas the mutant allele only responded to camptothecin treatment (Fig. 4, A and B). To determine whether these phosphorylation events affected cell survival, we monitored both groups of cells for apoptosis, which is induced by ROS or DNA damage in lymphocytes (*31*). The cells expressing wild-type ATM showed a strong apoptotic response to both H_2O_2 and camptothecin (as measured by a fluorescent caspase 3 substrate assay), whereas cells expressing the mutant allele only underwent caspase activation in response to camptothecin (Fig. 4, C and D). Confirmation of these results was also obtained by using propidium iodide staining and annexin V to measure cleavage of nuclear DNA and loss of membrane integrity during apoptosis (figs. S8 and S9).

Considering that C2991L and wild-type heterodimers are inactive in vitro (Fig. 3C), we also overexpressed either the C2991L or a wild-type allele of ATM in cells expressing endogenous wild-type ATM (fig. S10). The cells were treated with bleomycin or H_2O_2, and phosphorylation of p53 on Ser^{15} and Chk2 on Thr^{68} was quantified. Cells overexpressing wild-type ATM showed higher phosphorylation of both p53 and Chk2 in response to H_2O_2 compared with cells overexpressing C2991L ATM; thus, ectopic expression of the C2991L mutant acted as a dominant negative and inhibited the oxidative activation of wild-type ATM in human cells.

Immortalized lymphoblasts derived from an A-T patient expressing the R3047X ATM allele were also analyzed for responses to H_2O_2 and DNA damage, which showed that R3047X ATM failed to autophosphorylate ATM or phosphorylate Chk2 after exposure to H_2O_2 but showed a response to camptothecin that was similar to that seen in cells expressing wild-type ATM (Fig. 4 and fig. S10). It is difficult to make quantitative comparisons because the WT and mutant cells were derived from different individuals. The small decrease in responsiveness of the mutant cells to campothecin could mean that the reduced response to H_2O_2 also reflects a deficit in response to DSBs, but we interpret the result to show a specific deficit in the oxidative response of the R3047X mutant.

We identified and characterized a pathway of ATM activation that is separate from the previously defined pathway that depends on MRN and DNA ends. ATM appears to act as a redox sensor in human cells, and, given the large number of substrates identified as ATM targets after DNA damage (*32*), ATM may similarly regulate global cellular responses to oxidative stress. The observation that the R3047X mutation generates an ataxia phenotype in A-T patients but retains normal activation in response to DNA damage suggests that most of the clinical manifestations of A-T may result from an inability to effectively regulate ROS, an observation that has important consequences for A-T treatment strategies.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6003/517/DC1
Materials and Methods
Figs. S1 to S12
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The Ligase PIAS1 Restricts Natural Regulatory T Cell Differentiation by Epigenetic Repression

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$CD4^+Foxp3^+$ regulatory T (T_{reg}) cells are important for maintaining immune tolerance. Understanding the molecular mechanism that regulates T_{reg} differentiation will facilitate the development of effective therapeutic strategies against autoimmune diseases. We report here that the SUMO E3 ligase PIAS1 restricts the differentiation of natural T_{reg} cells by maintaining a repressive chromatin state of the *Foxp3* promoter. PIAS1 acts by binding to the *Foxp3* promoter to recruit DNA methyltransferases and heterochromatin protein 1 for epigenetic modifications. *Pias1* deletion caused promoter demethylation, reduced histone H3 methylation at Lys^9, and enhanced promoter accessibility. Consistently, *Pias1*$^{-/-}$ mice displayed an increased natural T_{reg} cell population and were resistant to the development of experimental autoimmune encephalomyelitis. Our studies have identified an epigenetic mechanism that negatively regulates the differentiation of natural T_{reg} cells.

Naturally occurring thymus-derived regulatory T (nT_{reg}) cells play a critical role in the maintenance of self-tolerance and homeostasis within the immune system (*1*–*4*). The transcription factor Foxp3 controls the development and function of T_{reg} cells (*5*–*7*). Several regulatory DNA elements within the *Foxp3* locus have been suggested to modulate Foxp3 expression, including the promoter region and conserved noncoding sequences (*6*–*9*). Although the transcription factors that positively regulate Foxp3 expression are well characterized (*10*–*13*), little is known about the negative regulation of Foxp3.

PIAS1 (protein inhibitor of the activated signal transducer and activator of transcription STAT1)

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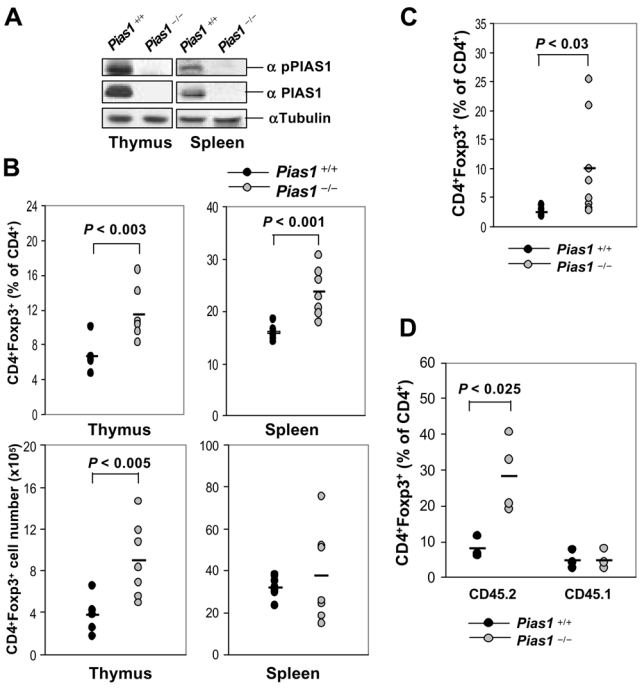

Fig. 1. Enhanced $CD4^+$ $Foxp3^+$ T_{reg} differentiation in *Pias1*$^{-/-}$ mice. (**A**) Western blot analysis of whole-cell extracts from freshly isolated thymocytes and splenocytes with an antibody specific for Ser^{90}-phosphorylated PIAS1, total PIAS1, or tubulin. (**B**) Cells from thymus or spleen of male ($n = 7$) wild-type and *Pias1*$^{-/-}$ littermates were analyzed by flow cytometry to determine the percentage and the absolute cell numbers of $Foxp3^+CD4^+$ cells (gated on a $CD4^+CD8^-$ population). Similar results were obtained with female mice. (**C**) Bone marrow was isolated from wild-type and *Pias1*$^{-/-}$ littermates and injected into the sublethally irradiated *Rag1*$^{-/-}$ recipient mice ($n = 8$). The thymic $CD4^+Foxp3^+$ population was analyzed 4 weeks after reconstitution by flow cytometry. (**D**) Same as in (C) except that *Pias1*$^{-/-}$ or wild-type bone marrow (CD45.2) was mixed with wild-type C57SJL bone marrow (CD45.1) and injected into the *Rag1*$^{-/-}$ mice ($n = 3$ for wild-type and $n = 4$ for *Pias1*$^{-/-}$). Experiments in (A) to (D) were performed at least three times ($n = 3$ to 8 for each experiment). *P* value was determined by unpaired *t* test.

is a SUMO E3 ligase that binds to chromatin to repress transcription (*14*, *15*). The recruitment of PIAS1 to chromatin requires it to be phosphorylated on Ser^{90}. This is induced by a variety of immune regulatory stimuli, including TCR (T cell receptor) activation (*16*). PIAS1 was phosphorylated on Ser^{90} in freshly isolated thymocytes and splenocytes (*17*) (Fig. 1A). There was a small increase in the percentage of thymic single-positive $CD4^+$ or $CD8^+$ T cells in *Pias1*$^{-/-}$ mice, although the total T cell number was not significantly altered (fig. S1, A and B). The frequency of both thymic and splenic $CD4^+Foxp3^+$ nT_{reg} cells was significantly increased in *Pias1*$^{-/-}$ mice (about 80%) (Fig. 1B). In addition, the number of thymic $CD4^+Foxp3^+$ nT_{reg} cells was also significantly increased in *Pias1*$^{-/-}$ mice (about 135%) (Fig. 1B). However, the mean intensity of Foxp3 expression in $Foxp3^+$ cells was not altered in *Pias1*$^{-/-}$ cells (fig. S1C). Moreover, *Pias1* disruption had no significant effect on the in vivo proliferation or survival of T_{reg} cells (fig. S2).

To test whether PIAS1 directly regulates the intrinsic differentiation potential of nT_{reg} cells, bone marrow from wild-type and *Pias1*$^{-/-}$ mice depleted of $CD4^+$ and $CD8^+$ T cells was transplanted into sublethally irradiated *Rag1*$^{-/-}$ (recombination-activating gene 1) recipient mice, which lack both T and B cells. *Pias1* disruption caused a fourfold

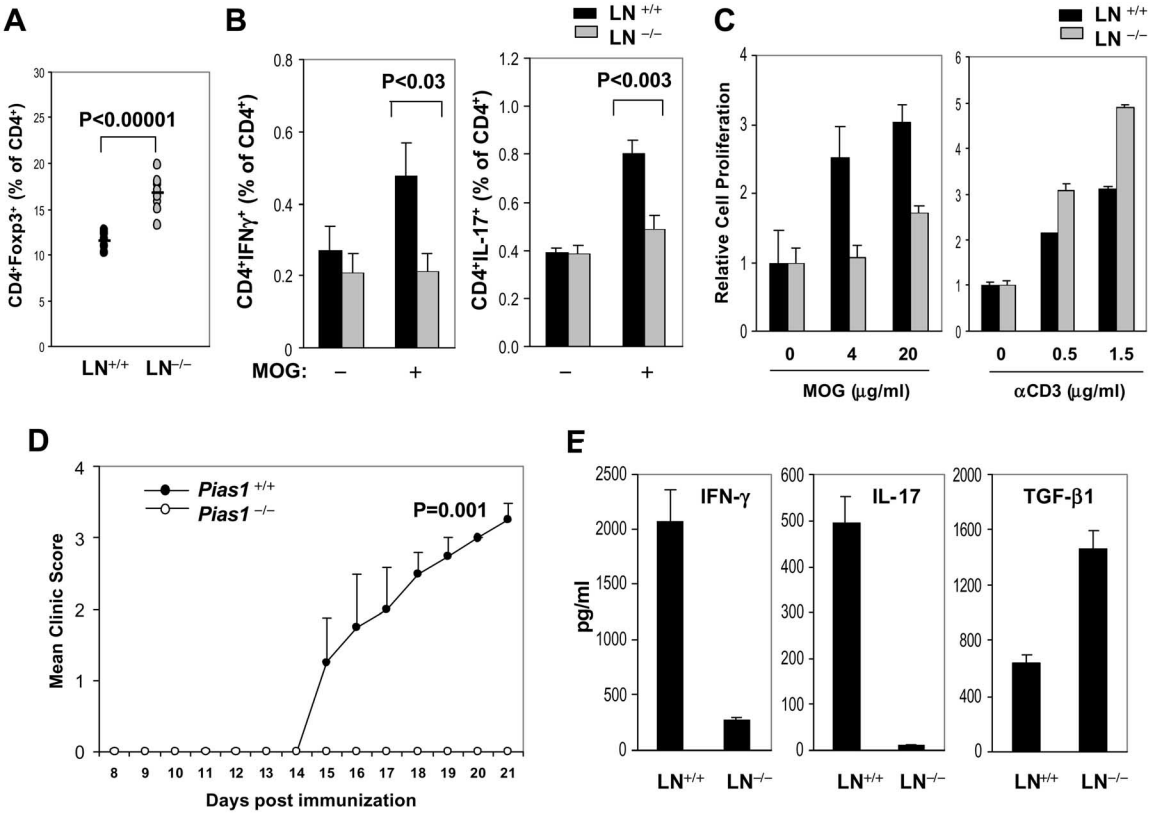

Fig. 2. *Pias1*$^{-/-}$ mice are resistant to MOG-induced EAE. (**A**) The percentage of $CD4^+Foxp3^+$ cells in wild-type ($LN^{+/+}$) and *Pias1*$^{-/-}$ ($LN^{-/-}$) lymph node cells 10 days after a single MOG_{35-55} injection emulsified in Freund's complete adjuvant ($n = 7$). (**B**) Lymphocytes from *Pias1*$^{-/-}$ mice and wild-type littermates 10 days after MOG_{35-55} injection as in (A) were either untreated or treated with 4 μg/ml of MOG_{35-55} for 3 days in the presence of brefeldin A during the last 5 hours of culture. IFN-γ– or IL-17–producing $CD4^+$ cells were assayed by intracellular staining followed by flow cytometry. (**C**) Same as in (B) except that cells were either untreated or treated with MOG_{35-55} or CD3-specific antibody for 3 days. Cell proliferation was measured by one-color cell proliferation kit. (**D**) *Pias1*$^{-/-}$ female mice and their wild-type littermates were immunized with MOG_{35-55} and pertussis toxin to induce EAE as described in (*17*). The development of EAE was scored ($n = 4$). (**E**) Wild-type and *Pias1*$^{-/-}$ littermates were immunized as in (D), and lymphocytes were isolated 21 days after the first MOG_{35-55} injection and cultured for 3 days in vitro ($n = 4$). Cytokine production in the cell supernatant was measured by enzyme-linked immunosorbent assay (ELISA). Shown in (A) to (E) is a representative of three independent experiments ($n = 3$ to 7 for each experiment). Error bars represent SEM. *P* value was determined by unpaired *t* test.

increase in the frequency of thymic $CD4^+Foxp3^+$ cells (Fig. 1C). Transplantation experiments were also performed by injecting *Rag1*$^{-/-}$ mice with a mixture of bone marrow from wild-type $CD45.1^+$ C57SJL mice and either wild-type or *Pias1*$^{-/-}$ mice (CD45.2). The percentage of thymic $CD4^+Foxp3^+$ from *Rag1*$^{-/-}$ mice reconstituted with *Pias1*$^{-/-}$ bone marrow (CD45.2) was significantly higher than that of the wild-type controls, whereas no difference was observed in thymic $CD4^+Foxp3^+$ differentiation of the $CD45.1^+$ control T cells (Fig. 1D). These studies suggest that PIAS1 negatively regulates nT_{reg} differentiation.

Peripheral naïve $CD4^+$ T cells can be differentiated into so-called induced T_{reg} cells (iT_{reg}) (*18*–*20*). In vitro differentiation studies indicate that transient TCR activation positively regulates iT_{reg} differentiation, whereas persistent TCR stimulation antagonizes Foxp3 induction (*21*). *Pias1* disruption enhanced iT_{reg} differentiation when naïve $CD4^+$ T cells were transiently stimulated by TCR alone (~70% increase), but not under the persistent TCR activation conditions (fig. S3A). In addition, *Pias1* disruption resulted in the inhibition of iT_{reg} differentiation when cells were exposed to a prolonged transforming growth factor–β (TGF-β) treatment under the persistent TCR-stimulating conditions (fig. S3B), possibly due to the aberrant up-regulation of negative-feedback molecules such as interferon-regulatory factor–1 (*22*) (fig. S3C).

To test the biological significance of PIAS1-mediated regulation of nT_{reg} in vivo, the response of *Pias1*$^{-/-}$ mice to myelin oligodendrocyte glycoprotein (MOG)–induced experimental autoimmune encephalomyelitis (EAE) was examined. Ten days after MOG immunization, an increased population of $CD4^+Foxp3^+$ T_{reg} cells in the lymph nodes of *Pias1*$^{-/-}$ mice was detected, as compared with the wild-type controls (Fig. 2A). The antigen-specific induction of the proinflammatory cytokines interferon (IFN)-γ and interleukin (IL)-17 was significantly suppressed, whereas a higher amount of TGF-β, an immunosuppressive cytokine, was detected in *Pias1*$^{-/-}$ lymphocytes (fig. S4). Consistently, intracellular staining assays showed a reduced percentage of antigen-specific IFN-γ– or IL-17–producing T cells (Fig. 2B). Furthermore, a defect in lymphocyte proliferation in response to MOG, but not polyclonal CD3-specific antibody stimulation, was observed in *Pias1*$^{-/-}$ mice (Fig. 2C). The lack of inhibition on *Pias1*$^{-/-}$ lymphocyte proliferation in response to polyclonal stimulation is likely due to the hyperproliferation of *Pias1*$^{-/-}$ non-T_{reg} lymphocytes under such conditions (fig. S5). The differentiation of T helper T_h1 or T_h17 cells was not defective in vitro in the absence of PIAS1 (fig. S6), which suggests that PIAS1 does not have an intrinsic ability to regulate the differentiation of T_h1 or T_h17 cells. *Pias1*$^{-/-}$ mice displayed resistance toward the development of EAE (Fig. 2D), and significantly reduced amounts of IFN-γ and IL-17, but increased levels of TGF-β, were detected in the lymph nodes of *Pias1*$^{-/-}$ mice (Fig. 2E). These results are consistent with the notion that the increased T_{reg} cells in *Pias1*$^{-/-}$ mice contribute to the observed resistance toward the development of EAE.

PIAS1 contains a chromatin-binding domain that targets PIAS1 to gene promoters to regulate transcription (*14*–*16*). We tested the hypothesis that *Foxp3* may be a direct PIAS1-target gene by ChIP (chromatin immunoprecipitation) assays. PIAS1 bound to the *Foxp3* promoter, but not the two conserved noncoding DNA sequences (CNS1 and CNS2), in thymic $CD4^+CD8^+$ and $CD4^+CD25^-$ T cells (Fig. 3A and fig. S7A). In contrast, the binding of PIAS1 to the *Foxp3* promoter was weak in thymic and peripheral $CD4^+CD25^+$ cells, consistent with the reduced expression of PIAS1 in these cells (fig. S7B). The *Foxp3* promoter is hypermethylated in $CD4^+CD25^-$, but hypomethylated in $CD4^+CD25^+$ cells (*6*, *7*, *23*–*25*), which inversely correlates with the amounts of PIAS1 expression

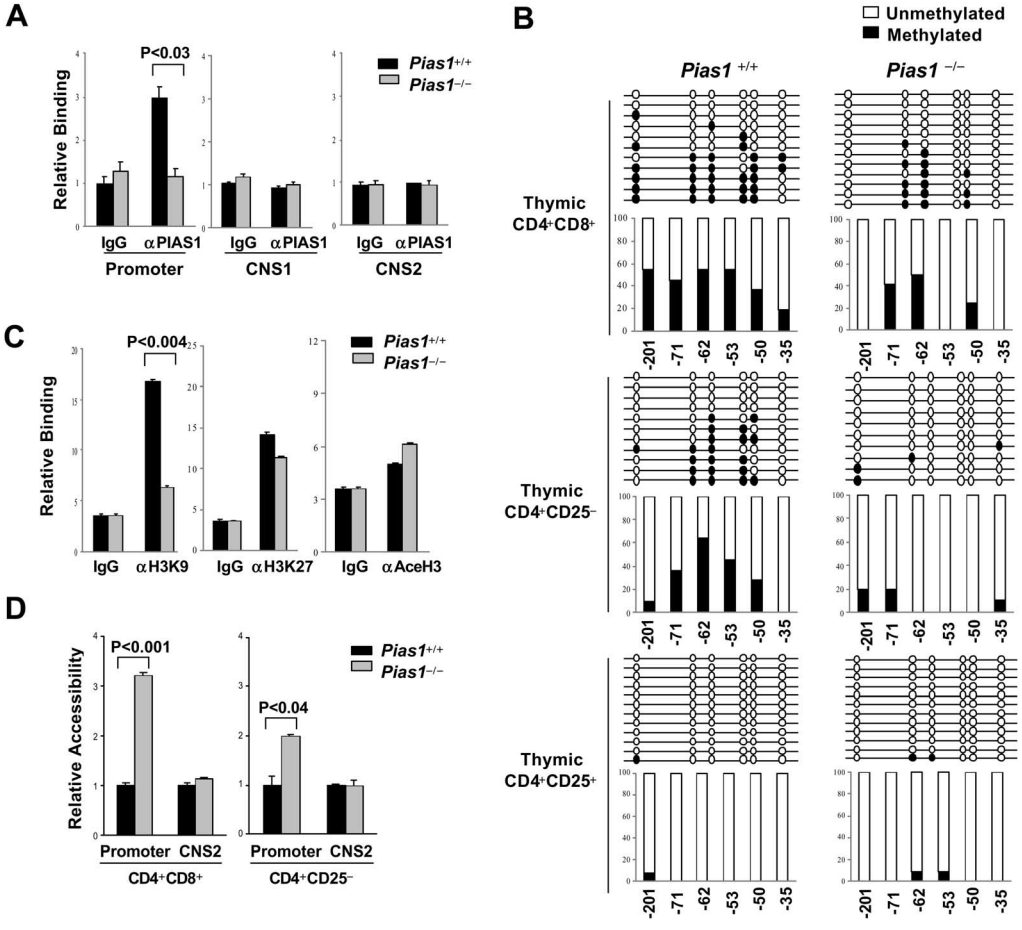

Fig. 3. PIAS1 maintains a repressive chromatin state of the *Foxp3* promoter. (**A**) ChIP assays were performed with freshly sorted $CD4^+CD8^+$ thymocytes from male *Pias1*$^{+/+}$ or *Pias1*$^{-/-}$ mice (n = 4), using PIAS1-specfic antibody or IgG. Bound DNA was quantified by quantitative real-time fluorescence polymerase chain reaction (QPCR), with specific primers against the various regions of the *Foxp3* locus, and normalized with the input DNA. (**B**) Methylation analysis of the *Foxp3* promoter was performed by bisulfite conversion of genomic DNA from various sorted T cell populations of wild-type and *Pias1* $^{-/-}$ male mice (n = 4). The x axis represents the positions of the CpG sites relative to the transcription start site (+1) in the *Foxp3* gene; the y axis represents the percentage. (**C**) ChIP assays were performed the same way as in (A) except that antibodies against trimethylated H3K9, histone H3 trimethylated at Lys^{27} (H3K27), or acetylated histone H3 (AceH3) were used, and the primers against the *Foxp3* promoter region were used. (**D**) REA assays were performed with thymic $CD4^+CD8^+$ or splenic $CD4^+CD25^-$ T cells. The data were quantified by QPCR and expressed as a ratio of digestion at the *Foxp3* promoter or CNS2 region to digestion at a nondigestible CNS1 region of the *Foxp3* locus. Shown in (A) to (D) is a representative of three independent experiments (n = 4 to 6 for each experiment). Error bars represent SEM. P value was determined by unpaired t test.

(fig. S7B). Bisulfite-sequencing analysis was performed to examine the methylation of the *Foxp3* locus in various subpopulations of wild-type and *Pias1*$^{-/-}$ cells. Several CpG sites in the *Foxp3* promoter were hypermethylated in wild-type thymic CD4$^+$CD8$^+$, CD4$^+$CD25$^-$ and splenic CD4$^+$CD25$^-$ T cells, but were hypomethylated in thymic and splenic CD4$^+$CD25$^+$ T_{reg} cells (Fig. 3B and fig. S8A). It is noteworthy that *Pias1* disruption caused a significant reduction of DNA methylation of the *Foxp3* promoter (Fig. 3B and fig. S8A). In contrast, the removal of PIAS1 showed no effect on the methylation of the heavily methylated CNS2 element (fig. S8B), which is consistent with the lack of significant PIAS1 binding to the CNS2 region (Fig. 3A). *Pias1* disruption also showed a modest effect on the demethylation of the *Foxp3* promoter in the common lymphoid progenitors and thymic CD4$^-$CD8$^-$ cells (fig. S9).

To examine whether PIAS1 also plays a role in the methylation of other gene promoters, we performed similar studies on CD25, a key regulator of T_{reg} cell differentiation (*4*, *6*). ChIP studies showed that *Cd25* is a PIAS1-target gene (fig. S10A). *Pias1* disruption resulted in the hypomethylation of the *Cd25* gene promoter in hematopoietic stem cells and thymic CD4$^-$CD8$^-$ cells (fig. S10B). Consistently, an increased frequency of CD4$^+$CD25$^+$ T cells was observed in *Pias1*$^{-/-}$ thymus and spleen (fig. S10C), as well as in transplantation studies (fig. S10, D and E).

We also examined the chromatin status of the *Foxp3* promoter by analyzing histone modifications. ChIP analysis showed a reduction of the repressive histone H3 methylation at Lys^9 (H3K9) methylation code in *Pias1*$^{-/-}$ CD4$^+$CD8$^+$ thymocytes (Fig. 3C). In addition, restriction enzyme accessibility analysis (REA) on the *Foxp3* locus (*23*) showed the enhanced accessibility of the *Foxp3* promoter, but not the PIAS1 nonbinding CNS2 region in *Pias1*$^{-/-}$ thymic CD4$^+$CD8$^+$ and splenic CD4$^+$CD25$^-$ T cells (Fig. 3D), which support a more open chromatin structure of the *Foxp3* promoter in *Pias1*$^{-/-}$ T cells. Furthermore, *Pias1* disruption resulted in the enhanced binding of STAT5, a key transcription factor involved in *Foxp3* induction (*10*–*12*), to the *Foxp3* promoter (fig. S11A). The amount of STAT5 protein or STAT5 phosphorylation was not affected by *Pias1* disruption (fig. S11, B and C). A significant increase of nuclear factor of activated T cells (NFAT) binding to the *Foxp3* promoter was also observed in *Pias1*$^{-/-}$ CD4$^+$CD8$^+$ thymocytes (fig. S11D). Consistently, *Pias1* disruption resulted in the increased frequency of Foxp3$^+$ CD4$^+$CD8$^+$ thymocytes (fig. S12).

We examined whether PIAS1 may maintain a repressive chromatin structure through the recruitment of DNA methyltransferases (DNMTs), which promote DNA methylation of chromatin (*26*). PIAS1 was shown to interact with DNMT3B in a yeast two-hybrid screen and in 293T cells upon overexpression (*27*, *28*). DNMT3A and DNMT3B, but not DNMT1, were present in PIAS1 immunoprecipitates from thymocytes or splenic CD4$^+$CD25$^-$ cells (Fig. 4A). Furthermore, sequential ChIP studies indicated that PIAS1 forms a complex with DNMT3A and DNMT3B on the *Foxp3* gene promoter (Fig. 4B). DNMT3B and DNMT3A bound to both the *Foxp3* promoter and the CNS2 region, but not the CNS1 element, in the wild-type CD4$^+$CD8$^+$ thymocytes (Fig. 4C). *Pias1* deletion completely abolished the binding of both DNMT3A and DNMT3B to the *Foxp3* promoter (Fig. 4C), but not the CNS2 region. The levels of DNMT expression were not affected by *Pias1* disruption (fig. S13). In addition, a modest binding of DNMT1 to the *Foxp3* promoter was observed, which was inhibited by

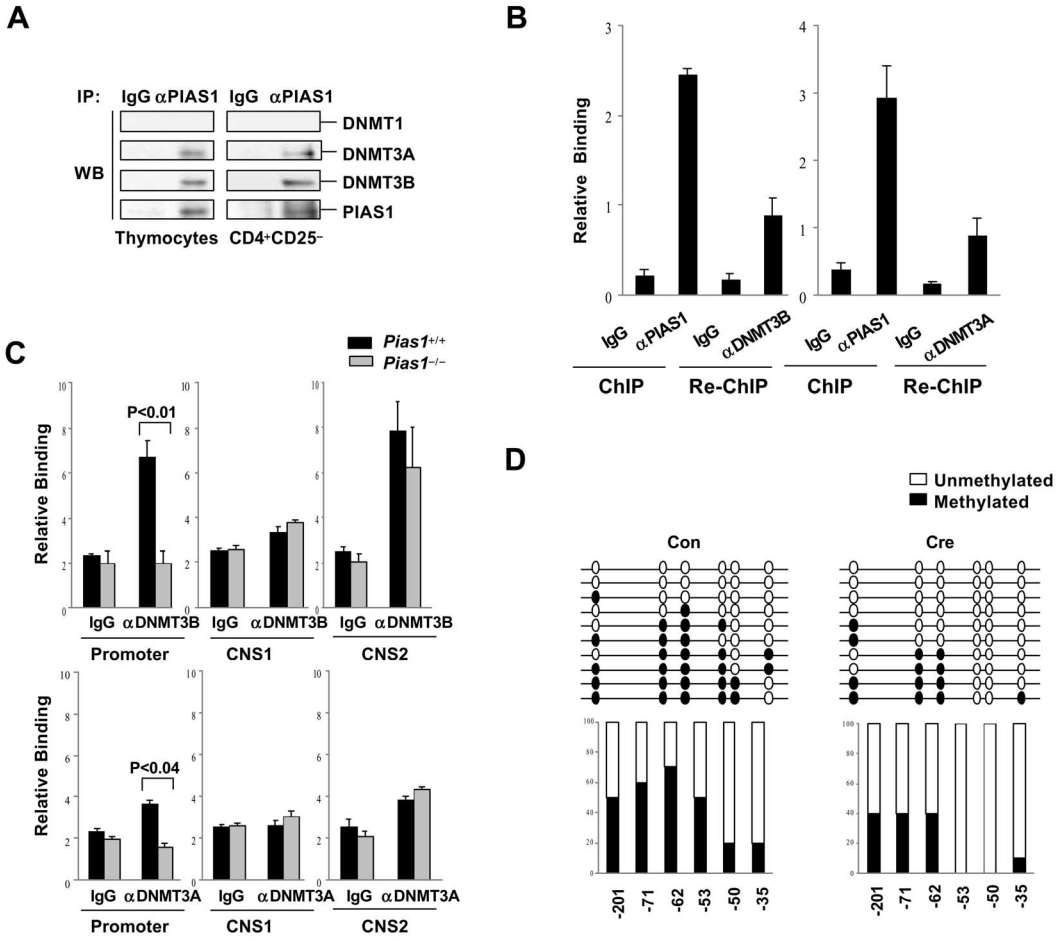

Fig. 4. PIAS1 is required for the promoter recruitment of DNMT3A and DNMT3B. (**A**) Coimmunoprecipitation assays. Protein extracts from wild-type thymocytes or splenic CD4$^+$CD25$^-$ T cells were subjected to immunoprecipitation with PIAS1-specific antibody or IgG, followed by immunoblotting with the indicated antibodies. (**B**) ChIP assays were performed with wild-type thymocytes by using PIAS1-specific antibody or IgG. The presence of the *Foxp3* promoter region in the precipitates was quantified by QPCR. In the re-ChIP experiments, PIAS1-specific antibody precipitates were released, reimmunoprecipitated with an antibody against DNMT3A or DNMT3B, and analyzed for the presence of the *Foxp3* promoter sequence. (**C**) ChIP assays were performed with freshly sorted CD4$^+$CD8$^+$ thymocytes from male *Pias1*$^{+/+}$ or *Pias1*$^{-/-}$ mice (n = 4), using an antibody against DNMT3A or DNMT3B. Bound DNA was quantified by QPCR with the specific primers against *Foxp3* promoter, CNS1 or CNS2 region. (**D**) Methylation analysis of the *Foxp3* promoter was performed by bisulfite conversion of genomic DNA from thymocytes of *Rag1*$^{-/-}$ mice reconstituted with either control GFP (Con) or Cre-GFP (Cre) retrovirus-infected *Dnmt3a*$^{2lox/2lox}$/ *Dnmt3b*$^{2lox/2lox}$ bone marrow. The x axis represents the positions of the CpG sites relative to the transcription start site (+1) in the *Foxp3* gene; the y axis represents the percentage. Shown in (A) to (D) is a representative of three independent experiments (n = 4 to 6 for each experiment). Error bars represent SEM. P value was determined by unpaired t test.

Pias1 disruption (fig. S14). The PIAS1-dependent recruitment of DNMTs to the *Foxp3* promoter was also observed in splenic $CD4^+CD25^-$ cells (fig. S15), but not in $CD4^+CD25^+$ T_{reg} cells (fig. S16). Collectively, these results indicate that PIAS1 is associated with DNMT3A and DNMT3B in T cells and is required for their recruitment to the *Foxp3* gene promoter.

To test if DNMT3A and DNMT3B play a role in the *Foxp3* promoter methylation, we performed transplantation experiments using mice in which the functional domains of both *Dnmt3a* and *Dnmt3b* genes are flanked by two loxP sites ($Dnmt3a^{2lox/2lox}$/$Dnmt3b^{2lox/2lox}$) (*29*, *30*). Bone marrow cells from $Dnmt3a^{2lox/2lox}$/$Dnmt3b^{2lox/2lox}$ mice were infected with green fluorescent protein (GFP) or Cre-GFP retrovirus, and the deletion of *Dnmt3a* and *Dnmt3b* was confirmed (fig. S17A). Sorted GFP^+ bone marrow was transplanted into sublethally irradiated $Rag1^{-/-}$ mice. Decreased *Foxp3* promoter methylation was observed in thymocytes from the $Rag1^{-/-}$ mice reconstituted with the Cre retrovirus–transduced bone marrow (Fig. 4D). ChIP assays indicated that the decreased DNMT3A and DNMT3B expression by the Cre transduction had no substantial effect on the binding of PIAS1 to the *Foxp3* promoter (fig. S17B).

Heterochromatin protein 1 (HP1) plays an important role in promoting H3K9 methylation and is known to interact with DNMTs (*31*). HP1-γ was strongly associated with the *Foxp3* promoter in wild-type, but not $Pias1^{-/-}$, thymocytes (fig. S18). The PIAS1-mediated epigenetic gene regulation is selective, because the chromatin status of genes such as *Ctla4* was not affected by *Pias1* disruption (fig. S19).

Our studies have identified an epigenetic control mechanism in the negative regulation of $Foxp3^+$ nT_{reg} differentiation. PIAS1 acts by maintaining a repressive chromatin state of the *Foxp3* promoter, at least partly through the recruitment of DNMTs and HP1 to promote epigenetic modifications (fig. S20). *Pias1* disruption results in the formation of a permissive chromatin structure of the *Foxp3* promoter and enhanced promoter accessibility to transcription factors such as STAT5 and NFAT, which lead to the increased probability that precursor cells will differentiate into $Foxp3^+$ T_{reg} cells (fig. S20). The physiological role of PIAS1 in the regulation of *Foxp3* gene is supported by the observed increase of the $Foxp3^+$ nT_{reg} population in $Pias1^{-/-}$ mice and the resistance of $Pias1^{-/-}$ mice toward the development of EAE. Thus, the PIAS1 pathway may represent a therapeutic target for the treatment of autoimmune diseases. DNMTs have no intrinsic sequence specificity (*26*). Our finding that PIAS1 regulates the binding of DNMTs to the *Foxp3* promoter, but not the CNS2 element, suggests that PIAS1 may be an important cofactor that confers specificity in the DNMT-mediated chromatin methylation. The PIAS1-mediated DNA methylation and histone modifications may serve as a fine-tuning mechanism in the control of epigenetic modifications during T cell differentiation.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S21
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