Supplemental information.

Supplemental Table legends

Supplementary Table S1

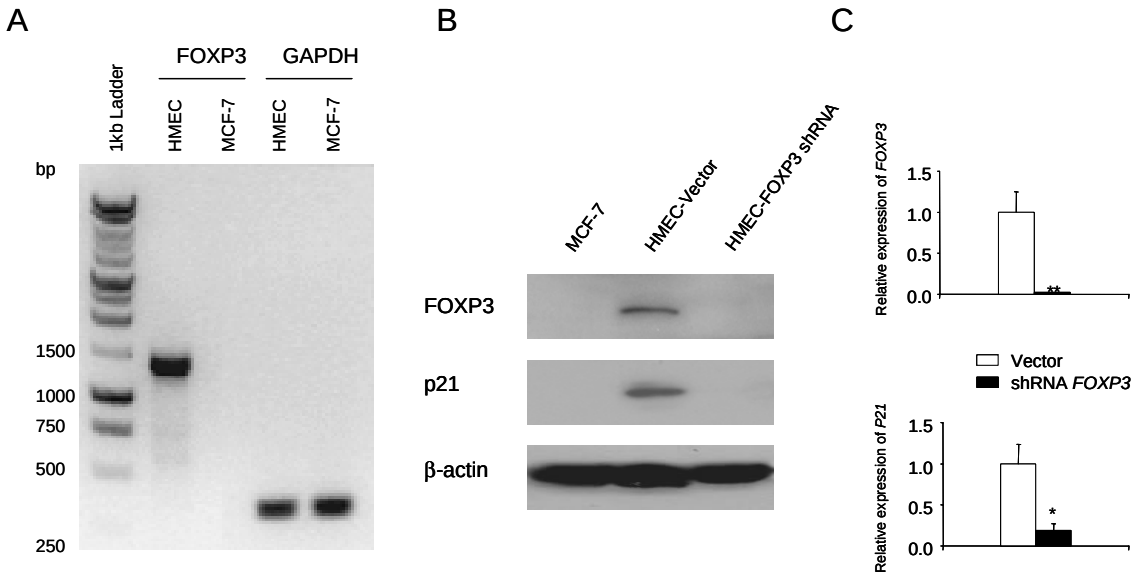
Putative FoxP3-induced genes are listed in an alphabetical order of gene names. Global mRNA expression analyses were performed before and after FoxP3 induction in the FoxP3-tet-off MCF7 cells as described in the Material and Method section. The definition of gene-induction is [average mRNA level of geneX in FoxP3(+) cells] / [average mRNA level of geneX in FoxP3(-) cells] were >2.0. *P*-values of the differences in the mRNA expressions of listed genes between FoxP3(-) and FoxP3(+) groups were calculated by Student's unpaired *t*-test.

Supplementary Table S2

Putative FoxP3-repressed genes are listed in an alphabetical order of gene names. Global mRNA expression analyses were performed before and after FoxP3 induction in the FoxP3-tet-off MCF7 cells as described in the Material and Method section. The definition of gene-repression is [average mRNA level of geneX in FoxP3(+) cells] / [average mRNA level of geneX in FoxP3(-) cells] were <0.5. *P*-values of the differences in the mRNA expressions of listed genes between FoxP3(-) and FoxP3(+) groups were calculated by Student's unpaired *t*-test.

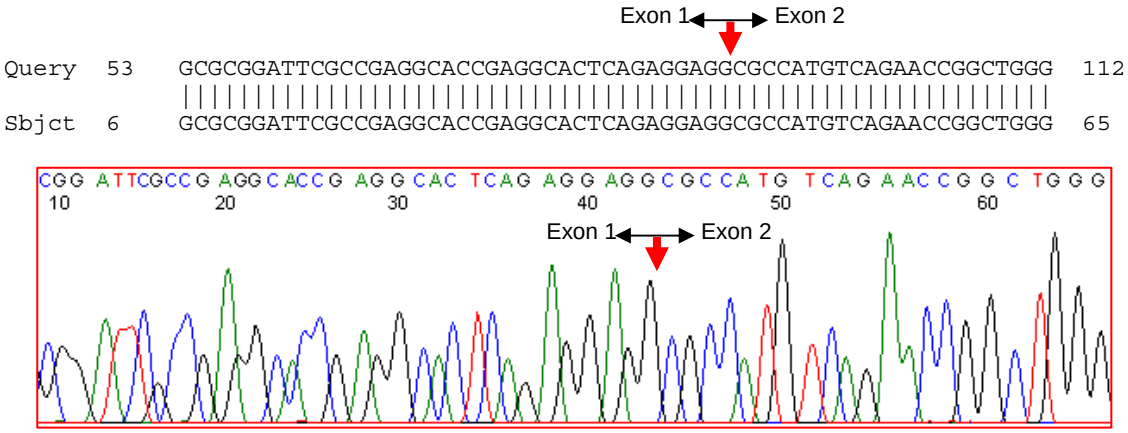
Table S3 Primer Sequence in this study

Supplemental Figures



Supplemental Fig. 1. FOXP3 controlled p21 expressions in normal human mammary cell line. (A) The expression of *FOXP3* mRNA in primary HMECs and MCF-7 cells by RT-PCR. Total RNA was reverse-transcribed, and the expression of full length mRNA was examined by PCR. *GAPDH* gene product was used to normalize the cDNA content in each sample. (B) and (C). Silencing of *FOXP3* resulted in down-regulation of the p21 protein (B) and *p21* mRNA (C) in primary HMEC. Early passage of HMEC was transfected with either control vector or FOXP3 shRNA using Nucleofection (Amaxa). The untransfected cells were removed by selection with neomycin. At 2 weeks after transfection, the protein levels were determined by Western blot, using specific anti-FOXP3, anti-p21and β-actin antibodies as loading control. The mRNA levels of the *FOXP3*

and *p21* transcripts were quantitated by real-time PCR. The RNA inputs were normalized against the housekeeping gene *GAPDH*. The vector control was defined as 1.0. Data shown are means ± SD of triplicates and represent 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ *vs* vector group.



Supplemental Fig. S2. Identification of the p21 isoform expressed after FOXP3 induction. The MCF-7 cells with Tet-off inducible expression of FOXP3 were cultured in the absence of doxycycline for 2 days. Total RNA was reverse-transcribed and the expression of *p21* mRNA was examined by PCR. The bulk PCR products were sequenced. The chromatogram showing the exon 1-exon 2 boundary is presented.

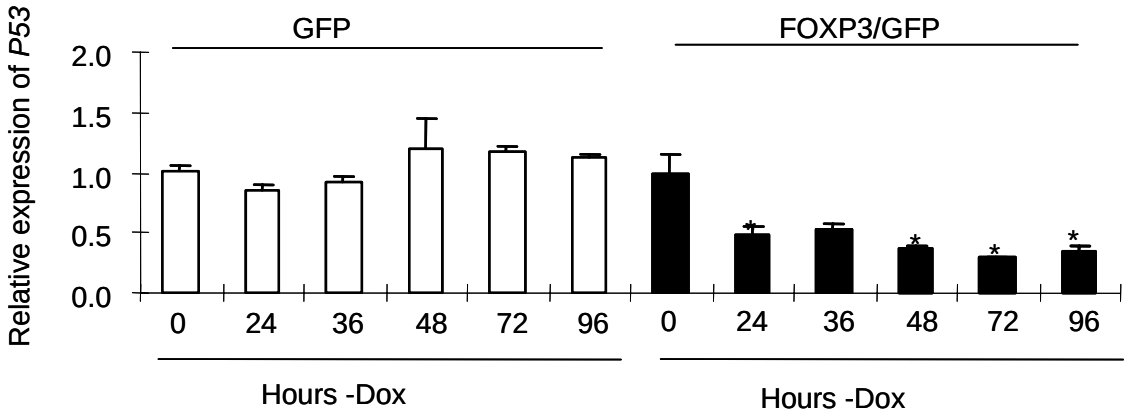
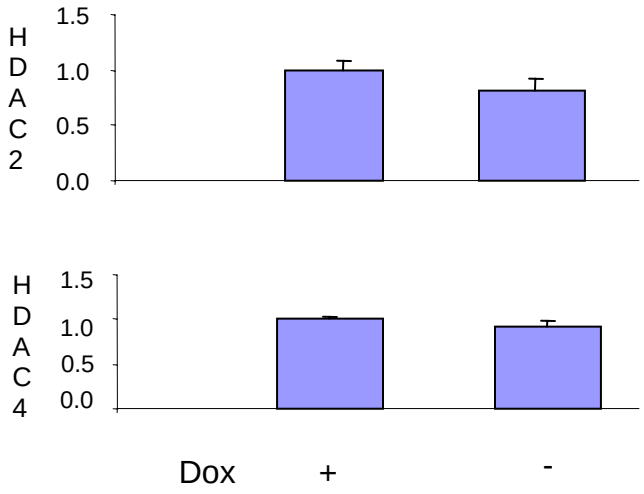


Fig. S3. FOXP3 does not induce P53 expression. MCF-7 cell line expressing either GFP or GFP and FOXP3 under the control of a tet-off system was cultured in the absence of doxycyclin for given periods of time. The mRNA transcripts of P53 were measured by real-time PCR. The means of time 0 is artificially defined as 1.0. Data shown were means and SEM of triplicates and have been repeated twice.



Supplemental Fig. S4. FOXP3 does not repress expression of *HDAC 2* and *4*. The *HDAC2* and *HDAC4* transcripts by realtime PCR in Tet-off MCF-7 cells without doxycycline from 2 days and the levels of the *HDAC* transcripts were quantitated by real-time PCR using *GAPDH* levels as internal control. Data shown are relative levels, using the means of uninduced cells as 1.0. No statistically significant difference was observed.