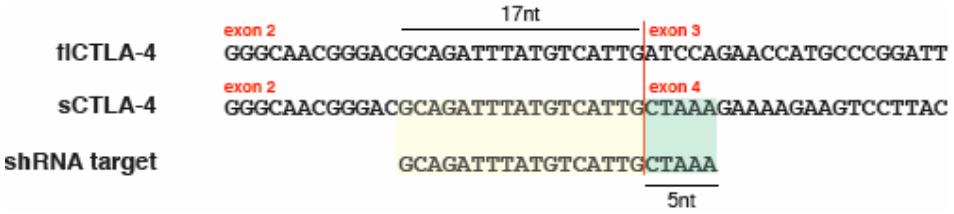
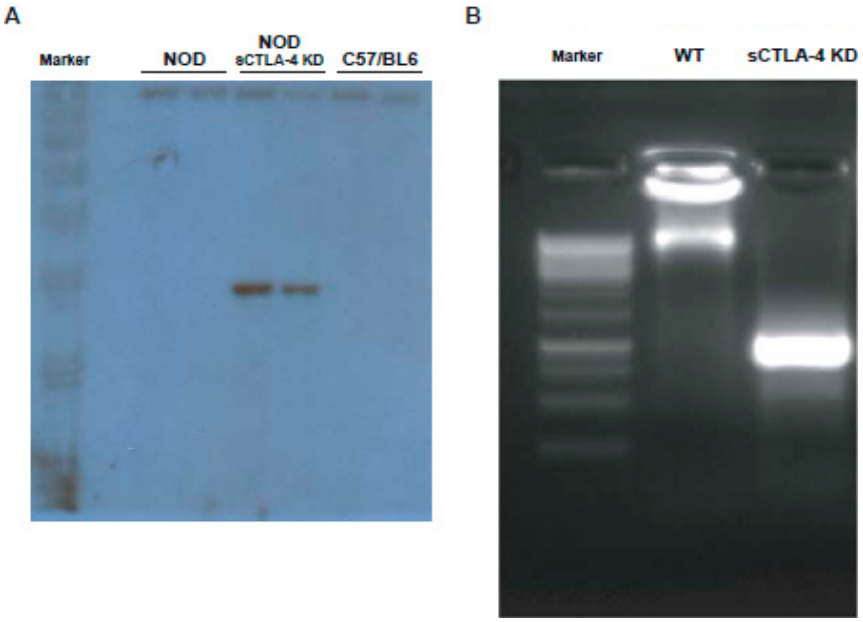
SUPPLEMENTARY DATA

Supplementary Figure. 1. High degree of sequence complementarity (nucleotides 1-17, 19 and 22 of the shRNA, i.e. 86%) **between flCTLA-4 mRNA and the shRNA sequence targeting sCTLA-4 mRNA does not hinder splice variant specificity**. A BLAST search identified no off-target transcript with a higher sequence identity with the shRNA than flCTLA-4. Since flCTLA-4 levels are not affected by shRNA expression (see Figure 1), the risk of the sCTLA-4 KD phenotype being caused by off-target effects is minimal. The relevant parts of the different sequences are shown.



Supplementary Figure 2. sCTLA-4 KD mice contain a single copy of the lentiviral transgene (A) Southern blot analysis of genomic DNA from sCTLA-4 KD mice using a GFP-sequence probe. (B) Genomic localization of the lentiviral insertion site. Genomic DNA was digested with EcoRI (unique in the lentiviral insert) and DNA fragments were self-ligated. A portion of the insert together with the endogenous flanking region was amplified by nested PCR. The PCR product (shown) was sequenced. sCTLA-4 KD mice have a single insertion on chromosome 4, outside of any known gene sequence.



SUPPLEMENTARY DATA

Supplementary Figure 3. sCTLA-4 silencing impairs the suppressive function of Treg cells (A) sCTLA-4 KD $CD4^+CD25^-$ Teff cells and wt or sCTLA-4 KD $CD4^+CD25^+$ cells were stimulated with irradiated splenocytes and anti-CD3 antibody at the indicated cell ratios. Proliferation was measured after 72 hours. (B) $CD4^+CD62L^+$ T cells from wt or sCTLA-4 KD mice were stimulated with anti-CD3 and anti-CD28 antibody in the presence of TGF-β (2ng/ml) for 96 hours. Freshly isolated sCTLA-4 KD $CD4^+CD25^-$ Teff cells and TGF-β treated cells (iTreg) were then stimulated with irradiated splenocytes and anti-CD3 antibody at the indicated cell ratios, and proliferation was measured at 72 hours. (C) wt $CD4^+CD25^-$ Teff cells (stained with SNARF-1 carboxylic acid acetate succinimidyl ester - a red fluorescent dye) and wt or sCTLA-4 KD $CD4^+CD25^+$ Treg cells (stained with CFSE, carboxyfluorescein diacetate succinimidyl ester - a green fluorescent dye) were stimulated with irradiated splenocytes and anti-CD3 antibody at the indicated Teff:Treg cell ratios. Cell division was measured by SNARF-1 dye-dilution (gated on CD4+ CFSE- cells) after 72 hours. Because the use of a PE-labelled antibody during magnetic isolation of $CD4^+CD25^+$ cells could cause Treg cells to appear as $CD4^+SNARF\text{-}1^+$ (overlapping emission spectra) during analysis, these cells were additionally labelled with CFSE (regardless of GFP expression, that would be usable for the same purpose but only for transgenic and not wt populations), allowing the complete exclusion of Treg cells from analysis. Black line: Teff cells alone. Grey line: Teff cells + sCTLA-4 KD Treg cells. Filled histogram: Teff cells + wt Treg cells.