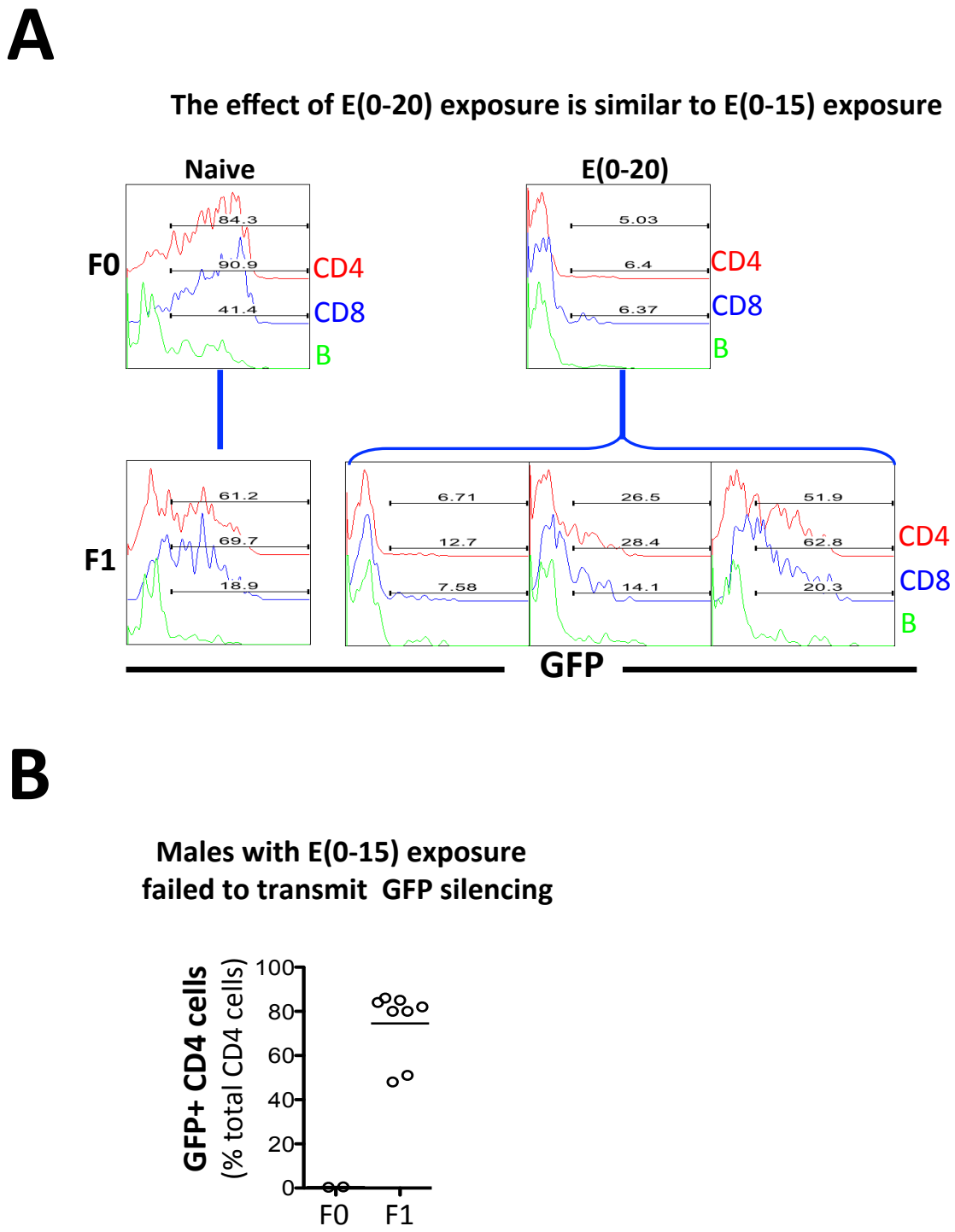

Fig. S1. GFP silencing in CMV-GFP mice. (**A**) Effect of E(0-20) exposure on GFP induction in an ensuing adult *CMV-GFP*$^{+/-}$*; rtTA*$^{+/-}$ female (F0) and her offspring. The female (on a C57/B6x129sv background) was exposed to Dox (1 mg/ml in drinking water) throughout fetal development, and then mated with a CD1 male to produce the F1 offspring. Blood was collected from the adult mice and analyzed as in Fig. 1A. Three F1 mice are shown that display severe (left), partial (middle) or no (right) GFP repression, respectively. A naïve homozygous female lacking prenatal Dox exposure and her offspring fathered by a CD1 male were used as a control (left). (**B**) Males could not transmit GFP silencing. Two *CMV-GFP*$^{+/+}$*;rtTA*$^{+/+}$ F0 mice (on a C57/B6x129sv background), pre-exposed to Dox at E(0-15), were mated with CD1 females to produce eight F1 mice. The plot displays the percentages of the CD4 cells expressing GFP following 24 hours of Dox stimulation; CD8 and B cells displayed a similar trend (not shown). The dots represent individual mice.

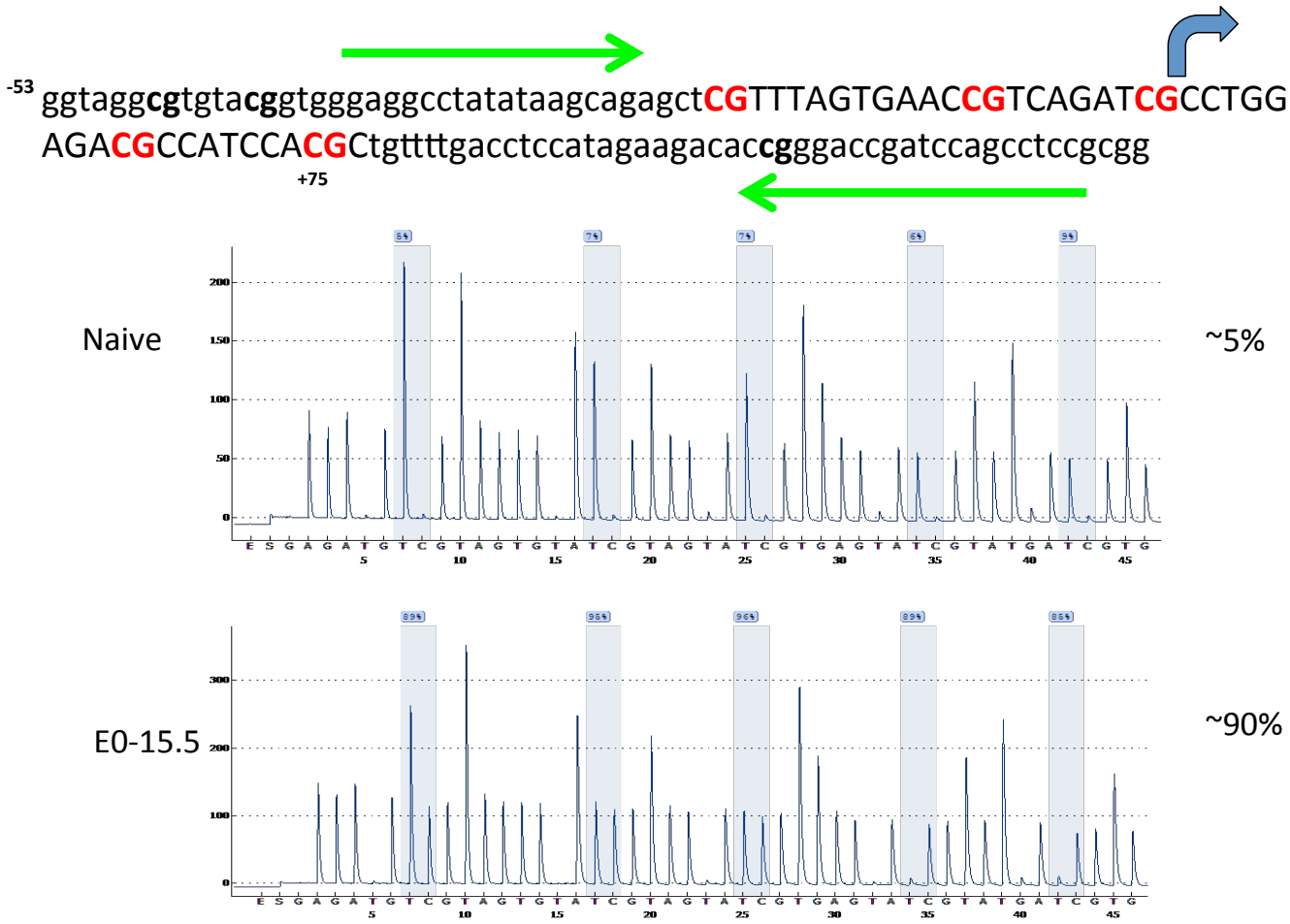

Fig. S2. An example of pyrosequencing. Gray bars mark CpG dinucleotides and the values above the bars indicate the percentage of methylation. The CMV promoter sequence is depicted at the top, with the five CpG dinucleotides highlighted in red and PCR primers indicated by green arrows.

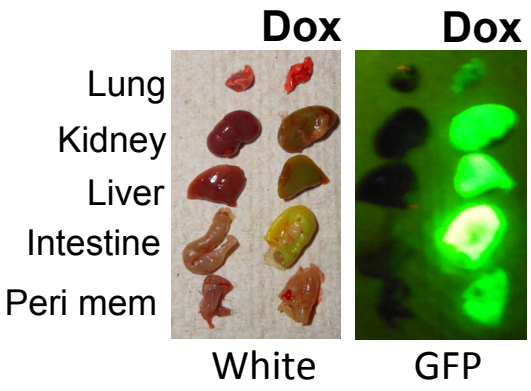

Fig. S3. Robust GFP expression in adult naïve CMV-GFP mice following 2 months of Dox administration. Dox was administered at 2 mg/ml. This demonstrates the uniqueness of ES cells, where prolonged (2 weeks) Dox treatment led to GFP silencing.

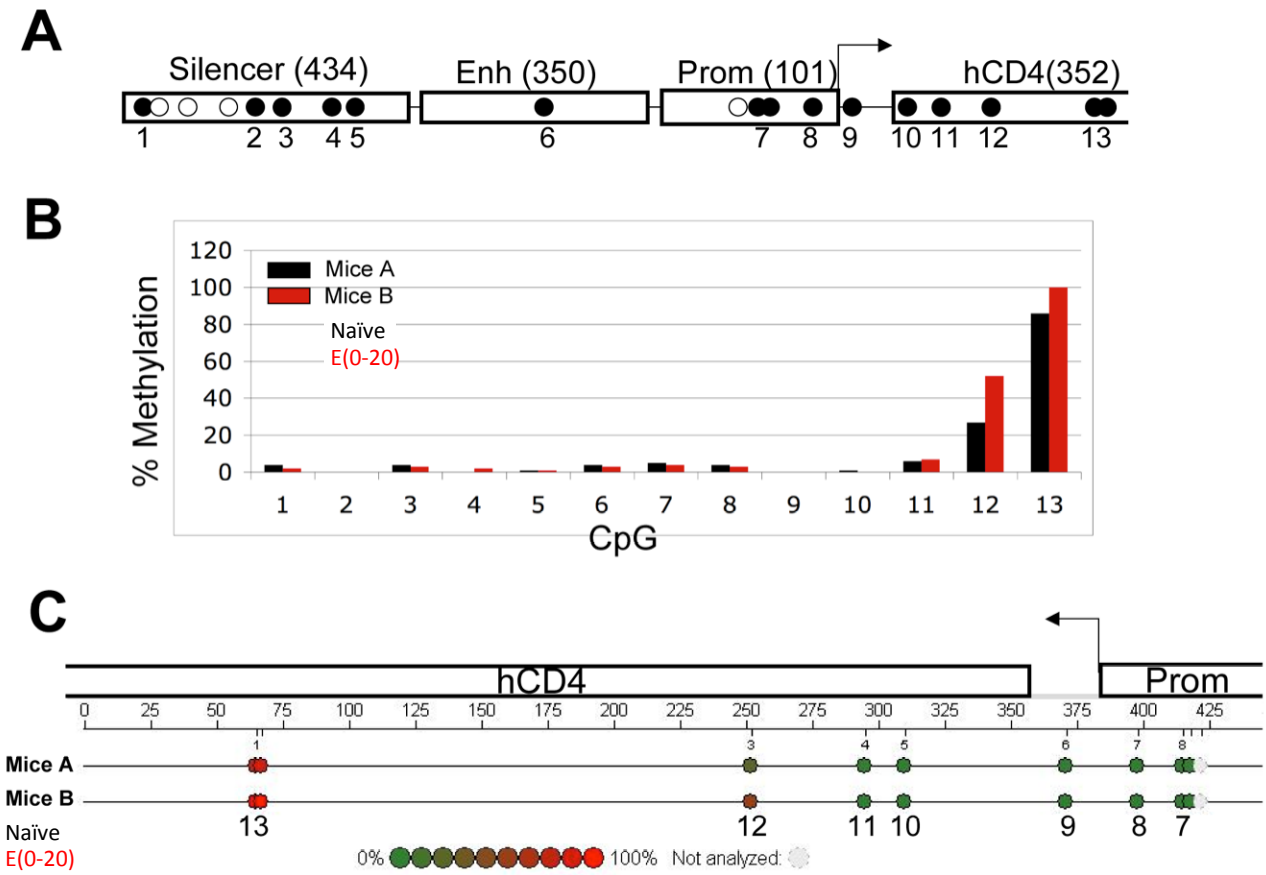

Fig. S4. CpG methylation and the effect of genetic background at the *Cd4* minigene. (A-C) Fetal Dox exposure did not significantly change CpG methylation at the *Cd4* minigene in adult CD4 cells, based on an EpiTYPER assay performed at the Yale Keck Facility. (**A**) CpG dinucleotides (dots) at the *Cd4* silencer, enhancer (Enh), promoter (Prom) and 5′ part of the human *CD4* cDNA (*hCD4*). The numbers within brackets give the length of the regulatory elements (bp). The CpGs clearly detectable by the EpiTYPER assay are filled. Note that CpG #7 consists of two adjacent CpG dinucleotides that cannot be distinguished in this assay. (**B**) The extent of methylation at various CpGs at the *Cd4* minigene in CD4 cells isolated from *Cd4 minigene*$^{+/+}$;*rtTA*$^{+/+}$ mice with or without prior Dox exposure at E(0-15.5). (**C**) Raw data (epigram) showing the methylation pattern of CpG #7 through #13 in a PCR product containing the *hCD4* and *Cd4* promoter sequences. The locations of *hCD4* and *Cd4* promoter sequences are depicted at the top, where the numbers give nucleotide positions (bp) in the PCR amplicon. The color code for percentage methylation is indicated at the bottom.

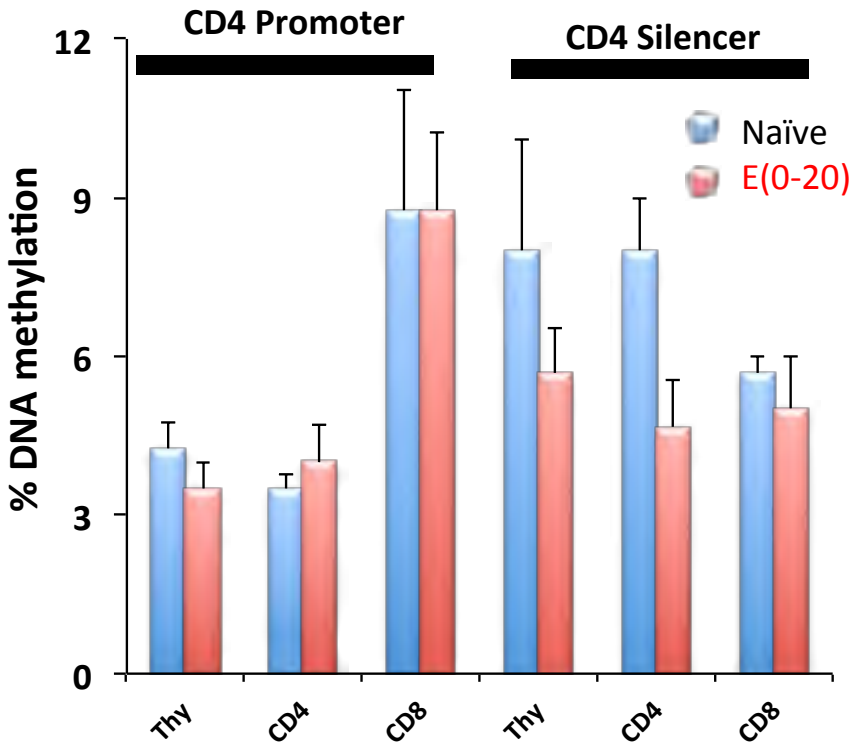

Fig. S5. Pyrosequencing assay of CpG methylation at the *Cd4* promoter and silencer in various subsets of T cells. The assay detects all four CpGs within the 101 bp *Cd4* promoter and CpGs #2-5 at the *Cd4* silencer as depicted in Fig. S3; the *Cd4* enhancer contains only one CpG and was not analyzed. Shown are averaged values for each of the CpGs at the promoter or enhancer, with the error bars indicating s.e.m. The data show that, in naïve mice, CpG methylation is <10% at the *Cd4* regulatory elements even in CD8 cells, and fetal Dox exposure could not further deplete the methylation. Our data contradict a previous observation that the *Cd4* promoter is highly methylated in both CD4 and CD8 cells (Zou et al., 2001). In that report, CpG methylation was determined by cloning and then sequencing PCR-amplified, bisulfite-converted genomic DNA. However, only six or seven individual colonies were sequenced, which could lead to sampling errors. This caveat does not apply in the current work, where ~100 ng of PCR amplicons were directly sequenced.