Supplementary Materials:

Supplementary Table 1. Transgene expression on PBL

Mutant founder	Number of expressers/total number of founders	Percent of expresser founders
WT	**7/7**	**100**
Inr	**16/18**	**94**
Sp1BS	**11/14**	**82**
TATAA-like	**9/9**	**100**
CAAT	**28/32**	**78**

Supplementary Table 1: Characterization of PD1 core promoter mutant founders: Several founders were established for each core promoter mutation. Tail DNA was tested for presence of transgene. PBLs from DNA positive founder mice were screened for PD1 transgene expression using FACS with relevant antibody. The table summarizes number of founders that were expressing PD1 in the PBL and the percent of expressers in each mutant.

Supplementary Table 2. Copy number and chromosomal location of insert in each strain

Mutant founder	strain	Copy number	Chromosomal location
WT	**PD1**	1	**ND**
	PD1L	**2**	**13C2**
Inr	**T1069D9**	**1**	**ND**
	T1069H10	**6**	**2B**
Sp1BS	**T598A5**	**1**	**13A1**
	T598H4	**4**	**ND**
	T598J5	**17**	**3A2**
TATAA-like	**T580H5**	**12**	**17B1**
	T580G1	**>20**	**11B**
CAAT	**T1068F4**	**1**	**ND**
	T1068T9	**>20**	**ND**
	T1068E4	**4**	**ND**

ND, not determined

Supplementary Table 2: List of the lines that were used in this study and localization of transgenes in the mouse genome: For each core promoter mutant 2 or 3 strains with differing copy number and genomic integration sites were examined. The copy number was measured using Southern blot or qPCR. The integration sites were determined by FISH as described in Supplementary Material and Methods. Each line was derived from an independent founder. The designation reflects the experiment number of the injection from which the line was derived (e.g. T1068) and the founder (e.g. F4). The PD1L transgenic mouse has been described previously (41).

Supplementary Table 3. P-value of the differences between IFNγ-treated and mock treated in each tissue of each transgenic line

		P-value
WT PD1	spleen	≤0.01
	kidney	≤0.01
	brain	≤0.01
WT H2K	spleen	≤0.01
	kidney	≤0.01
	brain	≤0.01
INR PD1	spleen	≤0.01
	kidney	N.S.
	brain	N.S.
INR H2K	spleen	≤0.01
	kidney	0.03
	brain	N.S.
Sp1BS PD1	spleen	≤0.01
	kidney	N.S.
	brain	≤0.01
Sp1BS H2K	spleen	≤0.01
	kidney	≤0.01
	brain	≤0.01
CAAT PD1	spleen	≤0.01
	kidney	0.03
	brain	0.05
CAAT H2K	spleen	≤0.01
	kidney	≤0.01
	brain	≤0.01
TATA PD1	spleen	≤0.01
	kidney	≤0.01
	brain	≤0.01
TATA H2K	spleen	≤0.01
	kidney	≤0.01
	brain	≤0.01

Supplementary Table 3: P-values of the differences between IFN treated and mock treated mice: Each experiment was repeated 3 times with the levels of PD1 mRNA measured by qPCR. The differences were calculated using student T-test, and the p-values are shown in the table. N.S. – not significant.

Supplementary Table 4. Antibodies used in this study

Antibody	Source
Anti SLA	PT85A WSU mAb Service Center, Washington State University Pullman WA
Anti SLA	74-11-10 WSU mAb Service Center, Washington State University Pullman WA
Anti mPOL-II	Sc-899 rabbit polyclonal Santa Cruz Biotechnology
Anti Trimethyl-Histone H3 (Lys4)	07-473 rabbit polyclonal, Millipore
Anti Trimethyl-Histone H3 (Lys9)	17-625 rabbit polyclonal, Millipore
Anti TBP	17-10098 rabbit polyclonal, Millipore
Anti Sp1	Sc14027 rabbit polyclonal, Santa Cruz Biotechnology

Supplementary Table 5. Primers that were used for this study:

H2-Kb	forward	reverse
Promoter	ggcgacactgattggttctt	ggatcagaactcggagacga
Promoter -600	tgcagaagtgaaactgtggag	ccggaagaggcttttcttct
Promoter -1000	aaacaccaggagagacccg	gtctctgtggggtccctgt
Exon 1	ggatgcagtggagagagagg	accatggaagtgcagacaca

Supplementary material and methods

Construction of the Promoter mutated mice;

PD1 INR and PD1 CAAT mutants: A fragment of the WT DNA construct containing the region 1-1420 was sub-cloned into the corresponding Hind III/Sac I sites in a pUC19 vector previously mutated at the Sfo I/Nar I (235) and Sap I (683) sites. PCR fragments using primers carrying the mutated sequences were made, isolated, annealed and filled by Klenow extension. These double stranded fragments were then digested at Sfo I/Sac I [INR] or Sap I/Sfo I [CAAT] sites and sub-cloned back into the pUC19 construct. The mutated 1-1420 fragments were reinserted back into the same sites in the WT construct to yield the final mutated constructs which were microinjected to generate transgenic mice.

PD1 Sp1BS and PD1 TATAA-like mutants: Duplex oligo-nucleotides containing the mutation were synthesized and inserted into the pUC19 construct noted above digested at Sfo I (-51) and Blp I (-4) sites. The mutated 1-1420 fragments were reinserted back into the same sites in the WT construct to yield the final mutated constructs which were injected.

All founders were screened by Southern blotting. Each subsequent generation was followed by PCR of genomic tail DNA. Wild type C57BL/6 or C57BL/10 mice were used as controls. Mice were euthanized using CO2 and whole body perfusion was carried out using phosphate buffered saline (PBS) so that tissues were free of contamination from circulating lymphocytes which express high levels of MHC class I. Spleens, kidneys and brains were harvested.

Flow cytometry: Flow cytometry was carried out as described elsewhere[1]. For PD1 surface expression, cells were stained with unlabeled primary Ab PT85, (WSU mAb Service Center,

Washington State University Pullman WA) followed by goat anti-mouse secondary Ab conjugated to FITC (SouthernBiotech, Birmingham, AL). For PD1 cell surface expression in the HeLa cell transfections, cells were stained with unlabeled primary Ab 74-11-10, (WSU mAb Service Center, Washington State University Pullman WA) followed by goat anti-mouse secondary Ab conjugated to FITC (SouthernBiotech, Birmingham, AL).. FACS results were analyzed using FlowJo software (Tree Star Inc.).

In situ hybridization on metaphase chromosomes of transgenic mice: Spleens were harvested from male mice carrying the transgene as verified by PCR on tail DNA. 2D FISH was performed as described [2]. To determine chromosomal integration direct-labeled FISH probes were designed using either a BAC clone spanning the H-2K gene or from the PD1 plasmid previously described in this section. The H-2K BAC clone, RP23-228K15, was identified using the BACPAC Resource Library of the Children's Hospital Oakland Research Institute. A stab culture of the BAC clone was received and was plated and propagated immediately upon arrival. Glycerol stocks were prepared. Isolation and purification of DNA was performed using the QIAGEN Plasmid Maxi Kit with only minor modifications to manufacturer’s instructions. The sequence for the BAC was obtained from the National Center for Biotechnology Information (NCBI) Web site. Primers were designed for the BAC clone and the sequence was amplified by PCR to verify the identity of the clone.

Standard Nick translation was performed to produce the fluorescence-labeled DNA probes for FISH. Gene-specific probes were labeled with Spectrum Orange-dUTP (PD1 gene) or Dy505-dUTP (H-2K gene), precipitated, and applied to metaphase cells harvested from WT and promoter mutant spleens. Chromosomal integrations were determined from the analysis of

signals from 20 metaphase cells from each of the promoter mutant mice strains. Following 2D FISH, the slides were analyzed using Leica Q-FISH image analysis software wherein metaphase cells were manually selected and centered for the camera. Two types of images were taken: pictures of the DAPI counterstained chromosomes in their metaphase state and fluorescent images of the gene-specific probes. The two images were then superimposed to generate a combined image. These images were then karyotyped using the SKYViewer software and chromosomal integration sites were determined for each of the core promoter mutant transgenic mice.

References

1. Ehrlich R, Sharrow SO, Maguire JE and Singer DS (1989) Expression of a class I MHC transgene: effects of in vivo alpha/beta-interferon treatment. Immunogenetics 30:18-26.

2. Crifasi PA, Michels VV, Driscoll DJ, Jalal SM and Dewald GW (1995) DNA fluorescent probes for diagnosis of velocardiofacial and related syndromes. Mayo Clin Proc 70:1148-53. doi: 10.1016/S0025-6196(11)63440-X

Supplementary Figures

Supplementary Figure 1: Expression of the PD1 gene is not abrogated by mutations in the core promoter. A) Diagrammatic representation of the PD1 core promoter upstream of PD1 coding sequences. B) Sequences of the wild type (WT) core promoter and core promoter element mutations used in the study. C) HeLa cells were transiently transfected with each of the individual PD1 gene constructs shown in B. NC-negative control: cells were transfected with empty vector. The levels of cell surface expression were measured by flow cytometry after staining with a PD1 specific monoclonal antibody (left panel). The median fluorescence of PD1 cell surface expression on the transfected HeLa cells is summarized in the table (right panel). (The apparent discrepancy between the FACS analysis and qPCR (e.g. TATAA-like mutant) most likely results from limiting amounts of the $\beta_2$microglobulin co-factor necessary for cell surface expression.)

Supplementary Figure 2: Detailed characterization of PD1 RNA expression levels in mice tissues. A) qPCR results of tissue expression levels in 3 CAAT mutant strains, compared to WT. **B)** qPCR results of tissue expression levels of 2 TATAA-like mutant strains, compared WT. **C)** qPCR results of tissue expression levels in 2 Sp1BS mutant strains, compared to WT. **D)** qPCR results of tissue expression levels in 2 INR mutant strains, compared to WT. Results are an average of 3 independent mice from each line. Copy number for each strain is provided in parenthesis and in Supplementary Table 2. Note that in each tissue, the data are expressed relative to the wild type level in that tissue.

Supplementary Figure 3: In situ hybridization of PD1 transgene and endogenous MHC class I gene H2-K^b in metaphase spreads of spleen cells from the different transgenic strains. Spleen cells from transgenic mice were plated and hybridized as described in supplementary material and methods. **A)** Hybridization of PD1 probe (red) and of H-2K^b probe (green) to WT transgenic cells. Arrows indicate the insertion sites; the chromosome number is indicated. Right panel shows the chromosomes identified with the transgene in SKY staining,

showing the exact chromosomal location of the insert. **B)** As in **A**, for Sp1BS [T598A5] mutated strain. **C)** As in A for TATAA-like [T580H5] mutated strain. **D)** As in A for TATAA-like [T580G1] mutated strain. Each hybridization panel shown is representative of 30 fields examined for each strain.

Supplementary Figure 4: TBP binding to chromatin of TATAA-like and WT transgenes. **A)** ChIP analysis of TBP binding across the PD1 transgene of WT and TATAA-like [T580H5, T580G1] core promoter mutant mice. The ChIP was performed on chromatin extracted from spleen. X axis denotes location relative to the TSS and is not to scale. **B)** ChIP analysis of TBP binding across endogenous mouse MHC-I gene $H2K^b$. ChIP experiments were performed as described in Methods. The experiments were performed on pools of spleens extracted from 3 mice. Every experiment was repeated 3 times in 2 different strains [T580H5, T580G1].

Supplementary Figure 5. Sp1BS core promoter element mutation modulates the IFNϒ response. A. Sp1BS (T598H4) mice were treated with IFNϒ, as described in Methods. RNA was extracted from spleen, kidney and brain and subjected to RT PCR. Inset, Splenic RNA from WT mice treated with IFNϒ in parallel and subjected to RT PCR, as a control for the IFNϒ injection. Real time PCR was relative to an 18S standard. **B.** Primer extension analysis of splenic RNA from Sp1BS (T598H4) mice treated with IFNϒ.

Supplementary Figure 6: Response to IFNγ treatment of the endogenous MHC class I gene $H2K^b$ in the non-expresser SP1BS [T598J5] mice. Mice were treated with IFNγ as described in Methods. The level of endogenous $H2K^b$ RNA was measured by qPCR. Results are expressed as relative expression.

Supplementary Figure 7: **Model of core promoter element functions**. Although none of the core promoter elements is essential for promoter activity, each contributes in a distinct way. Tissue specific patterns of expression are regulated by the combined activities of the CAAT, TATAA-like and Inr elements. Extracellular signaling responses, as exemplified by IFNγ, are regulated by Sp1BS and Inr elements. All four elements contribute to the overall transcription level of the gene.