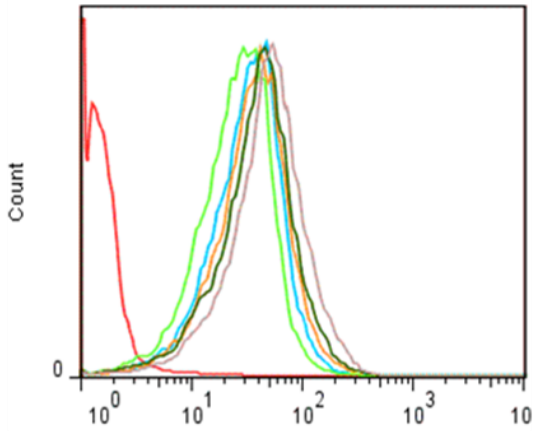
Barbash Supplementary Figure 1

A

B

	-58	-30	-16	+1
WT	GAAGCCAATCGGCGCCACTGCGGTTCCCGGTTCTAAACTCTCCACCCACCCGGCTCTGCTCAGCTTCTCCCCAGACTCCGAGGCTGAGGATCATGGGGCC			
INR	GAAGCCAATCGGCGCCACTGCGGTTCCCGGTTCTAAACTCTCCACCCACCCGGCTCTGggCAGgacgaCCCCAGACTCCGAGGCTGAGGATCATGGGGCC			
Sp1BS	GAAGCCAATCGGCGCCACTGCGGTTCCCGGTTCTAAACTCTCCACggAtCCGGCTCTGCTCAGCTTCTCCCCAGACTCCGAGGCTGAGGATCATGGGGCC			
TATA	GAAGCCAATCGGCGCCACTGCGGTTCCCGGTggTtgACTCTCCACCCACCCGGCTCTGCTCAGCTTCTCCCCAGACTCCGAGGCTGAGGATCATGGGGCC			
CAAT	GAAGatctTCGGCGCCACTGCGGTTCCCGGTTCTAAACTCTCCACCCACCCGGCTCTGCTCAGCTTCTCCCCAGACTCCGAGGCTGAGGATCATGGGGCC			

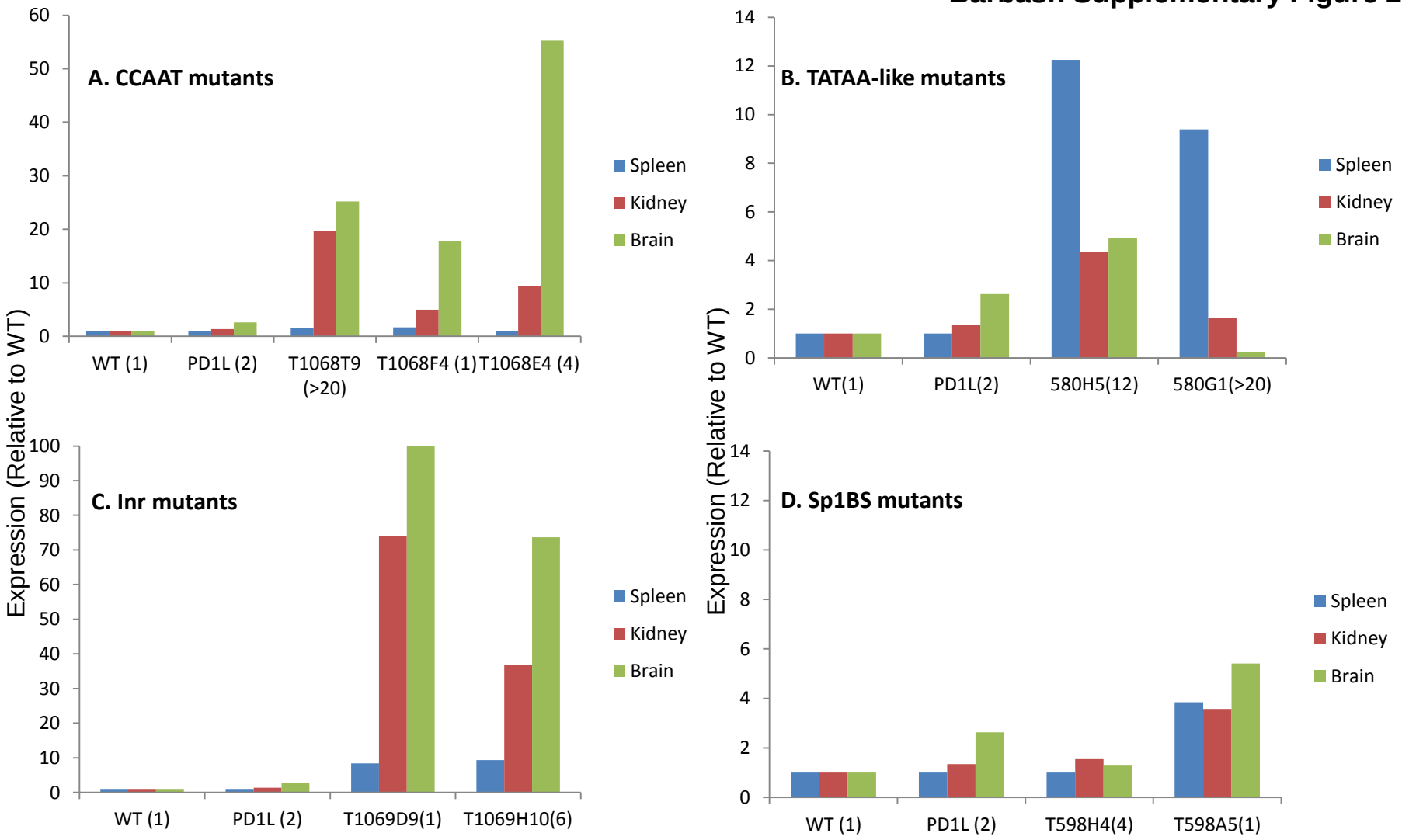
C



	Population Name	Median , FL1-H
	CAAT	50.2
	TATA	40.9
	SP1 BS	26.7
	INR	37.2
	WT	33.8
	NC	1.33

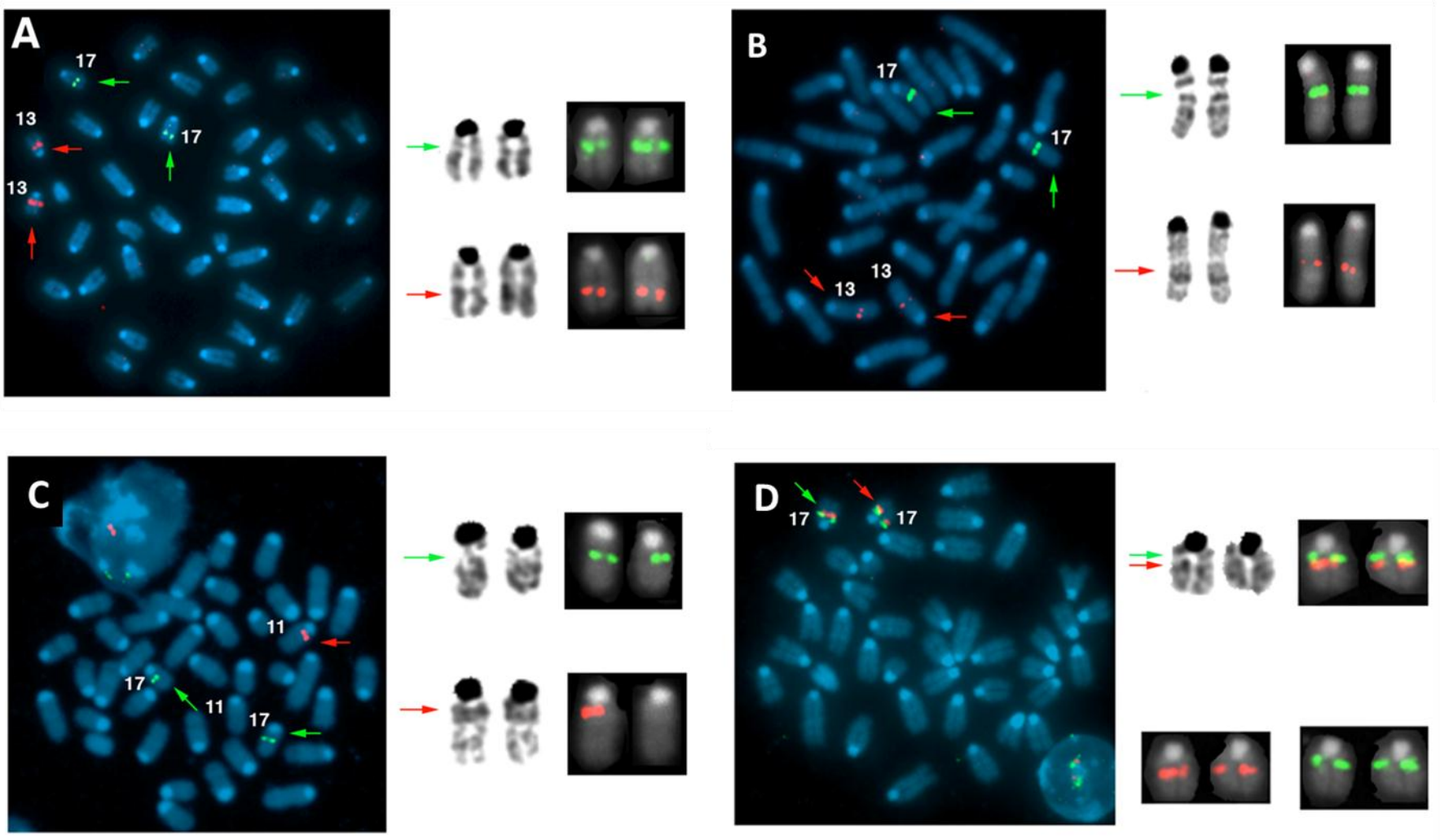
Supplementary Figure 1: Expression of the PD1 gene is not abrogated by mutations in the core promoter. A) Diagrammatic representation of the PD1 core promoter upstream of PD1 coding sequences. B) Sequences of the wild type (WT) core promoter and core promoter element mutations used in the study. C) HeLa 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.)

Barbash Supplementary Figure 2



Supplementary Figure 2: Core Promoter Mutant Transgenes Display Distinct Patterns of PD1 RNA expression. PD1 RNA levels in spleen, kidney and brain were determined in independently-derived lines of CCAAT mutants (A), TATAA-like mutants (B), Inr mutants (C) and Sp1BS mutants (D) and compared to WT and with published data from a second transgenic line, PD1L, that has a wild type promoter (34). Numbers in parentheses next to the transgene name indicate transgene copy number. Note that in each tissue, the data are expressed relative to the wild type level in that tissue.

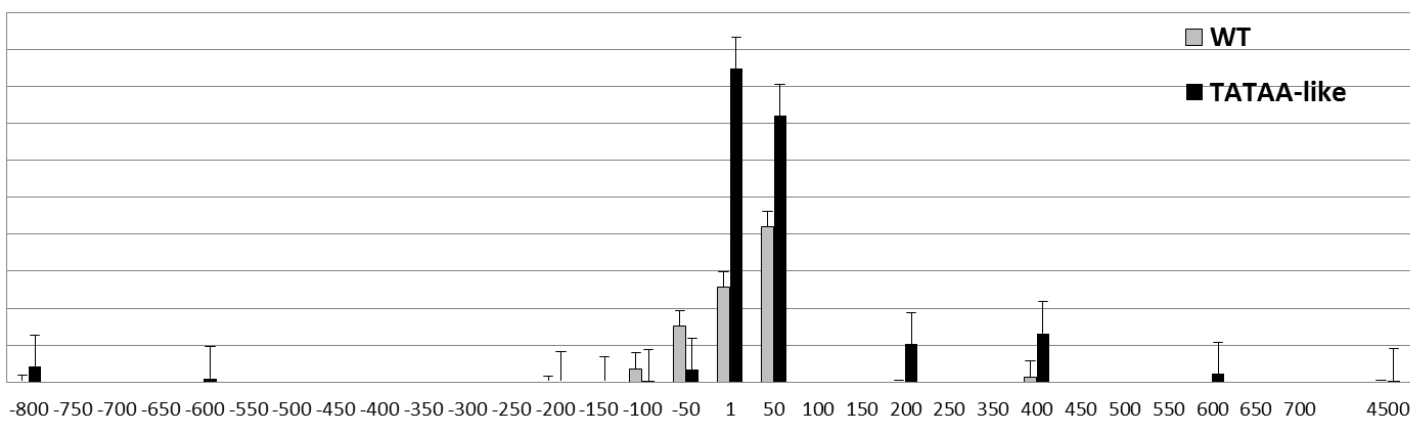
Barbash Supplementary Figure 3



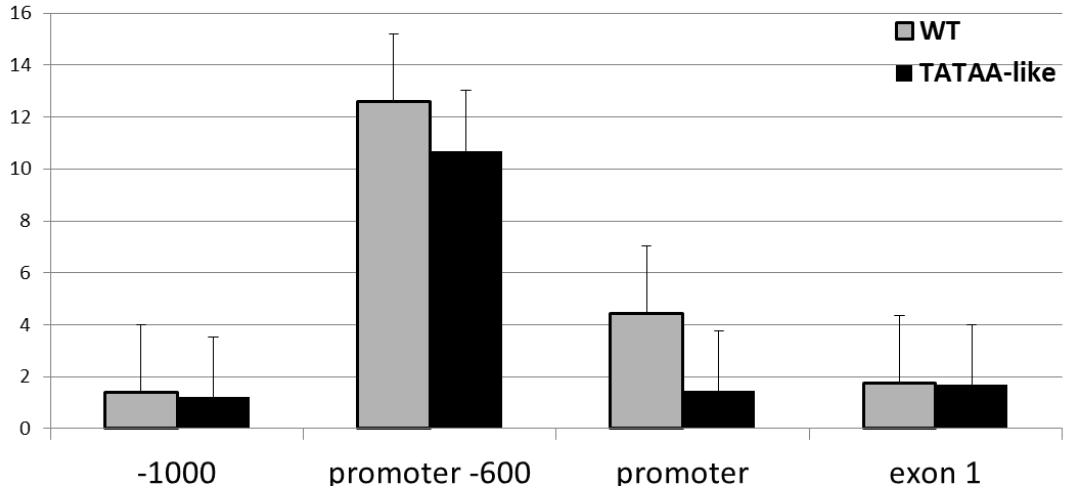
Supplementary Figure 3: In situ hybridization of PD1 transgene and H2-K^{D} endogenous gene in metaphase spreads of spleen cells from the different transgenic strains. Spleen cells from transgenic mice were plated and hybridized as described in supplementary material and methods. **A)** Hybridization of PD1 probe (red) and of H-2K^{D} 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.

Barbash Supplementary Figure 4

A

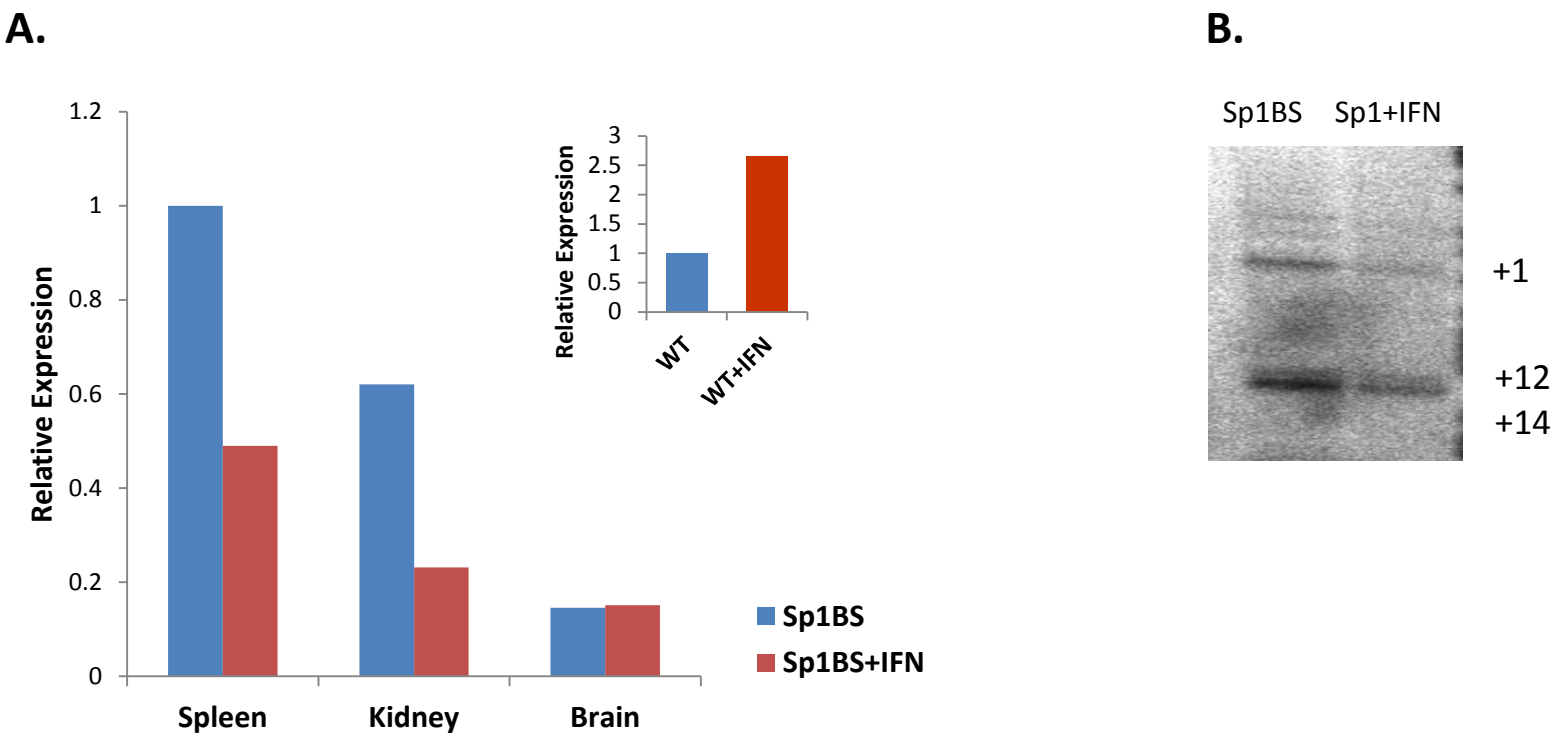


B



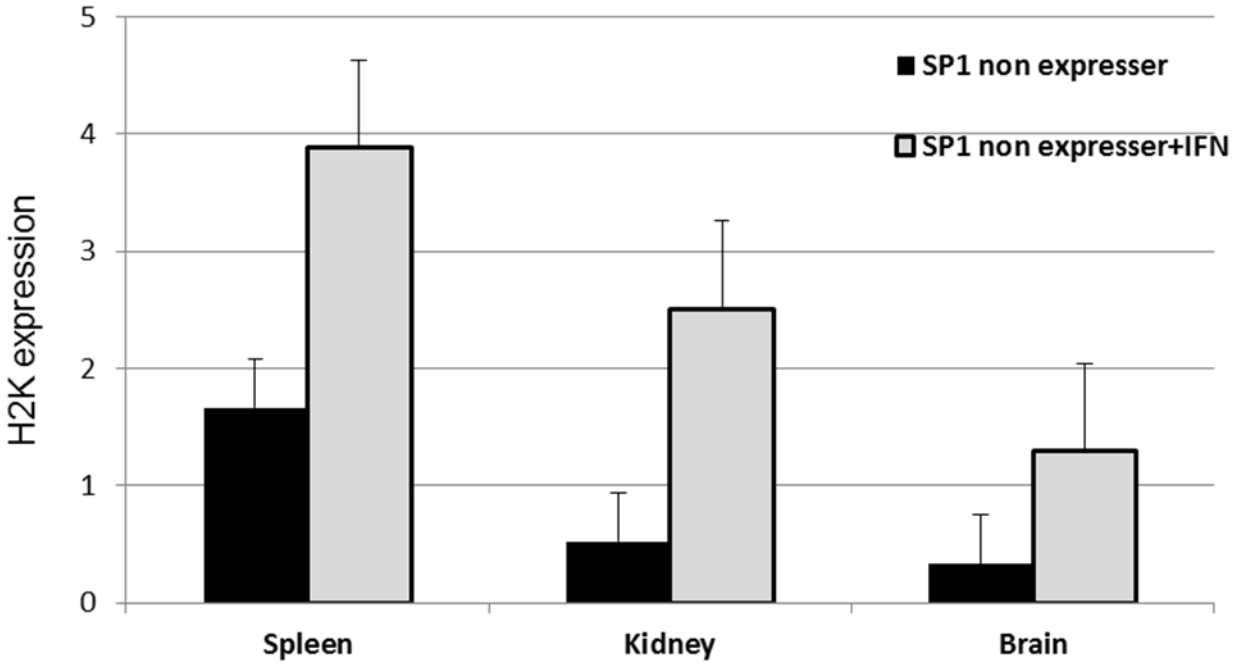
Supplementary Figure 4: TBP binding to chromatin of TATAA-like and WT transgenes. A) ChIP analysis of TBP binding across the PD1 transgene of WT and TATAA-like [T580H5, T580G1] core promoter mutant mice. The ChIP was performed on chromatin extracted from spleen. X axis denotes location relative to the TSS and is not to scale. **B)** ChIP analysis of TBP binding across the endogenous mouse MHC class 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].

Barbash Supplementary Figure 5



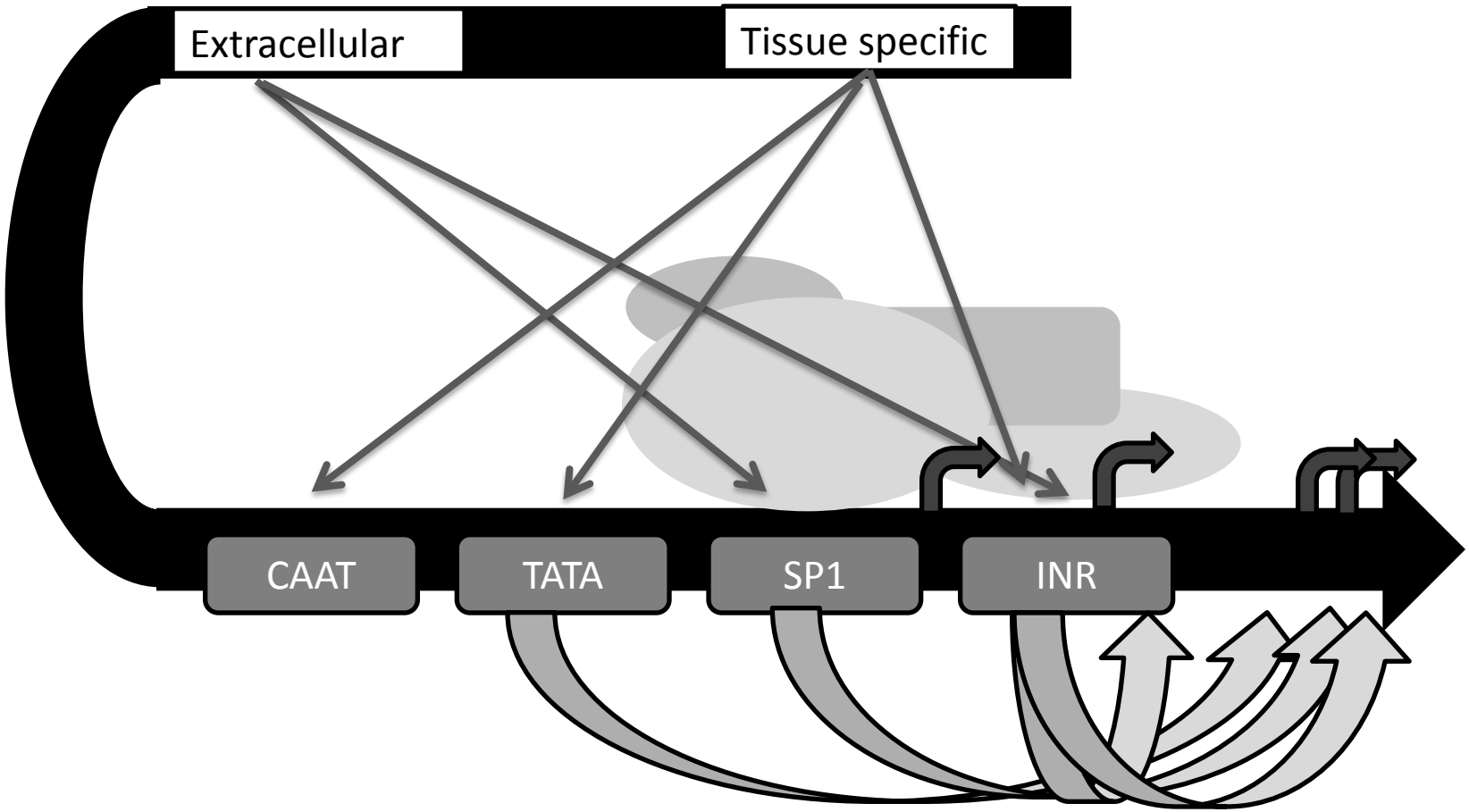
Supplementary Figure 5. **Sp1BS core promoter element mutation modulates the IFNϒ response**. **A**. Sp1BS (T598H4) mice were treated with IFNϒ, as described in Methods. RNA was extracted from spleen, kidney and brain and subjected to RT PCR. Inset, Splenic RNA from WT mice treated with IFNϒ in parallel 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.

Barbash Supplementary Figure 6



Supplementary Figure 6. Response to IFNγ treatment of 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.

Barbash Supplementary Figure 7



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.