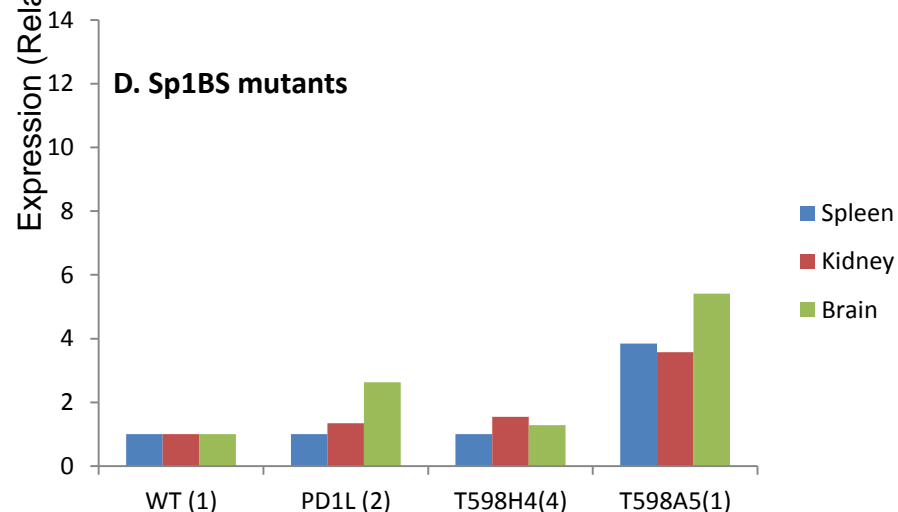
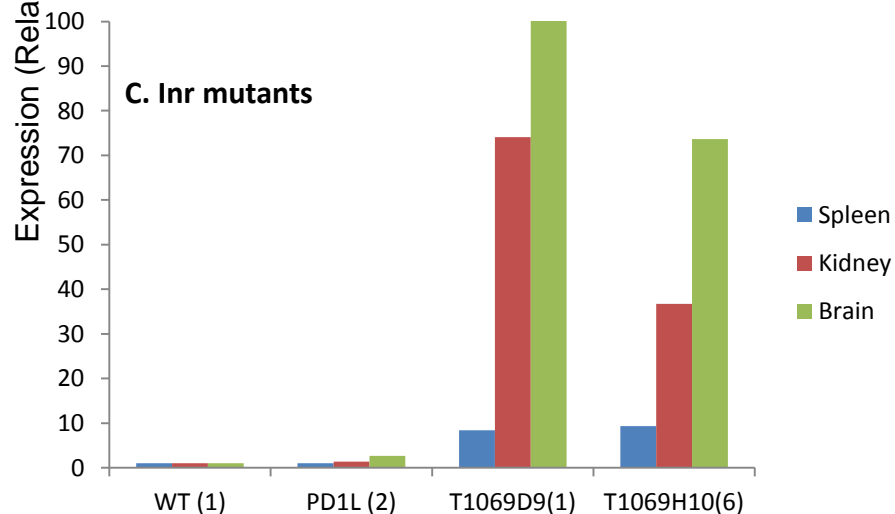
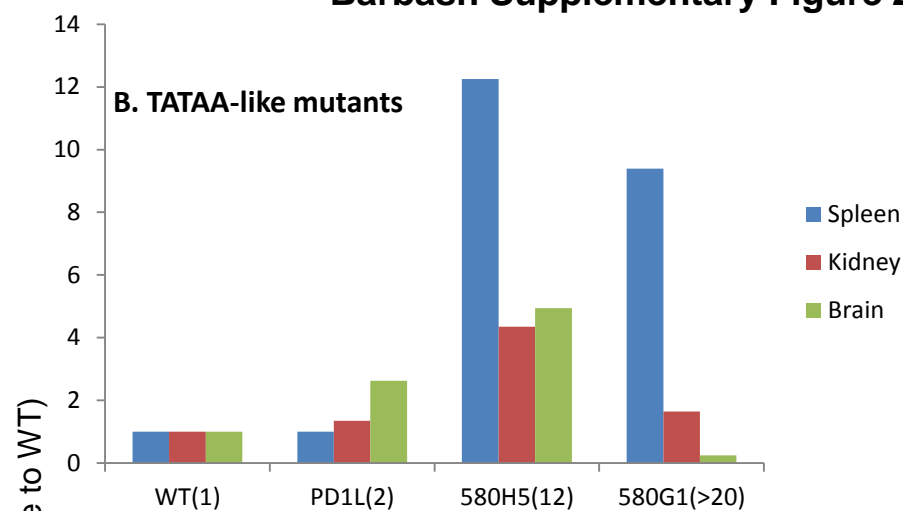
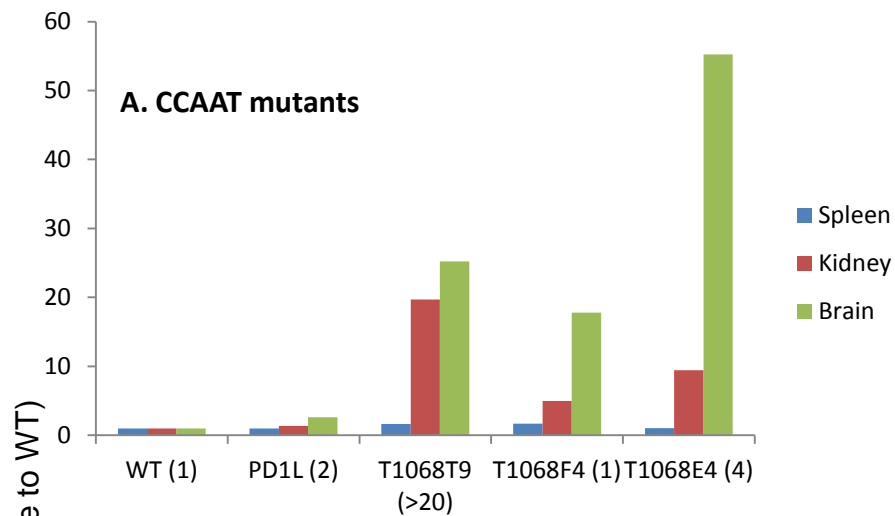
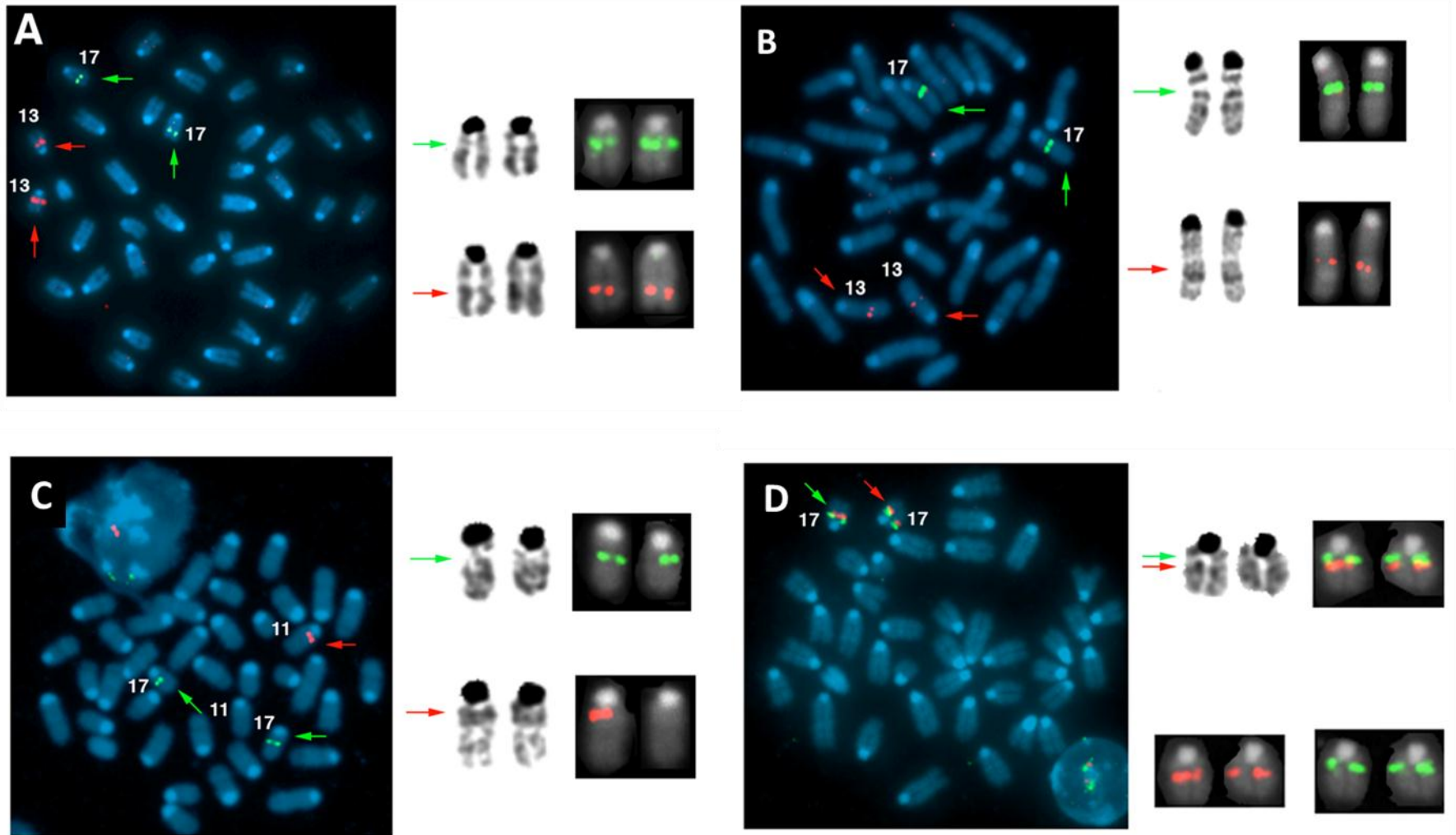




## Barbash Supplementary Figure 2

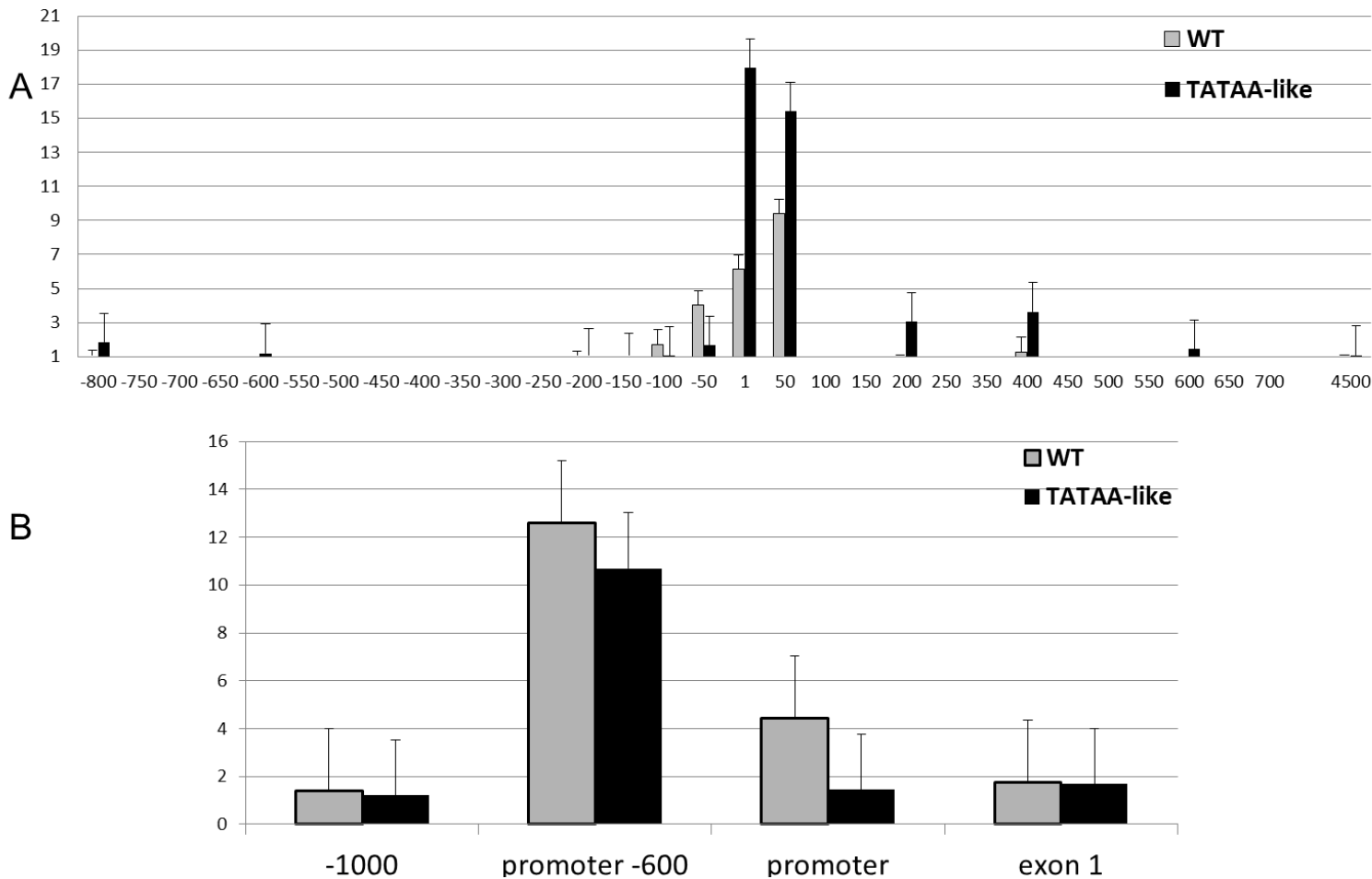


**Supplementary Figure 2:** Core Promoter Mutant Transgenes Display Distinct Patterns of PD1 RNA expression. PD1 RNA levels in spleen, kidney and brain were determined in independently-derived lines of CCAAT mutants (A), TATAA-like mutants (B), Inr mutants (C) and Sp1BS mutants (D) and compared to WT 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.



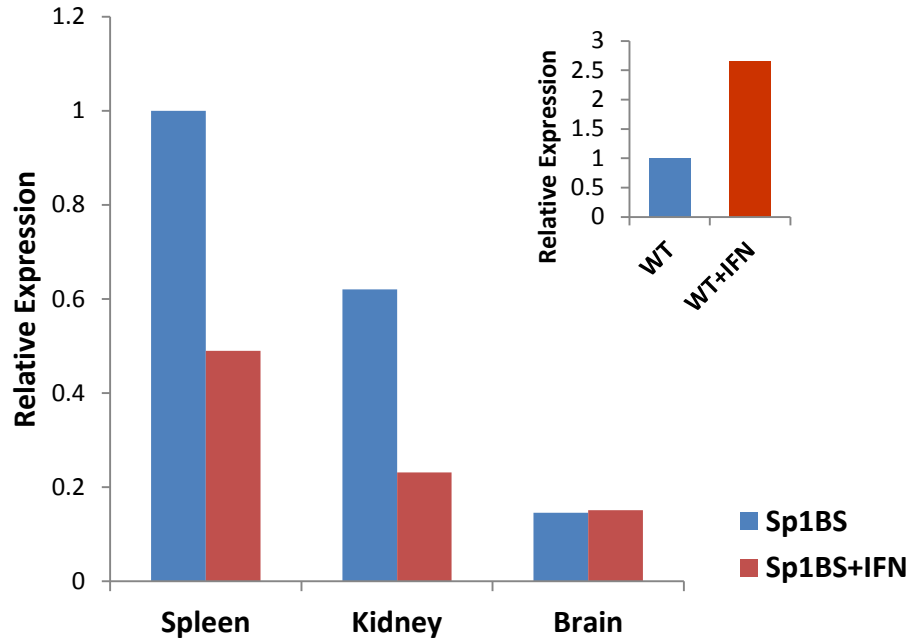
**Supplementary Figure 3: In situ hybridization of PD1 transgene and H2-K<sup>D</sup> endogenous gene in metaphase spreads of spleen cells from the different transgenic strains.** Spleen cells from transgenic mice were plated and hybridized as described in supplementary material and methods. **A)** Hybridization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

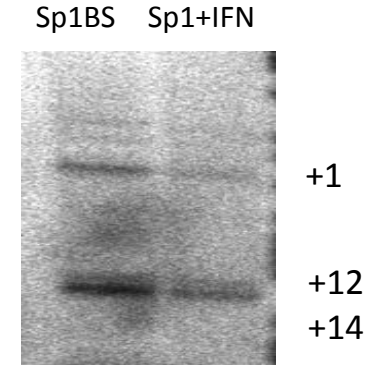


**Supplementary Figure 4: TBP binding to chromatin of TATAA-like and WT transgenes. A)** ChIP analysis of TBP binding across the PD1 transgene of WT and TATAA-like [T580H5, T580G1] core promoter mutant mice. The ChIP was performed on chromatin extracted from spleen. X axis denotes location relative to the TSS and is not to scale. **B)** ChIP analysis of TBP binding across the endogenous mouse MHC class I gene H2K<sup>b</sup>. 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].

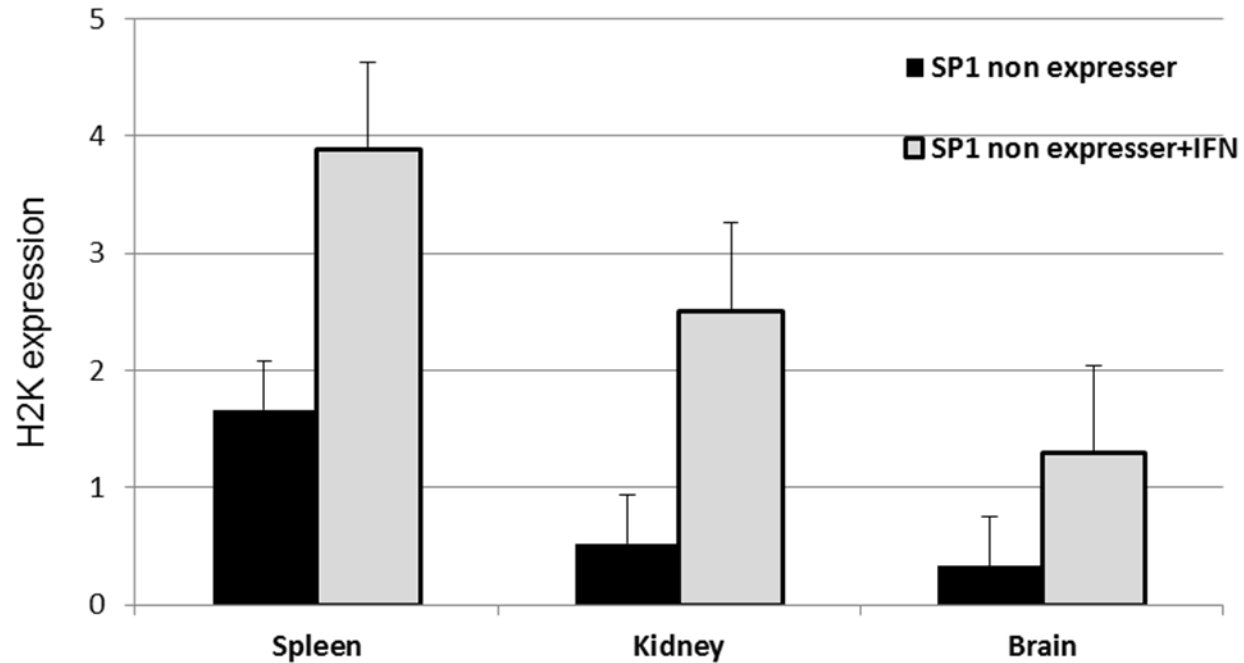
**A.**



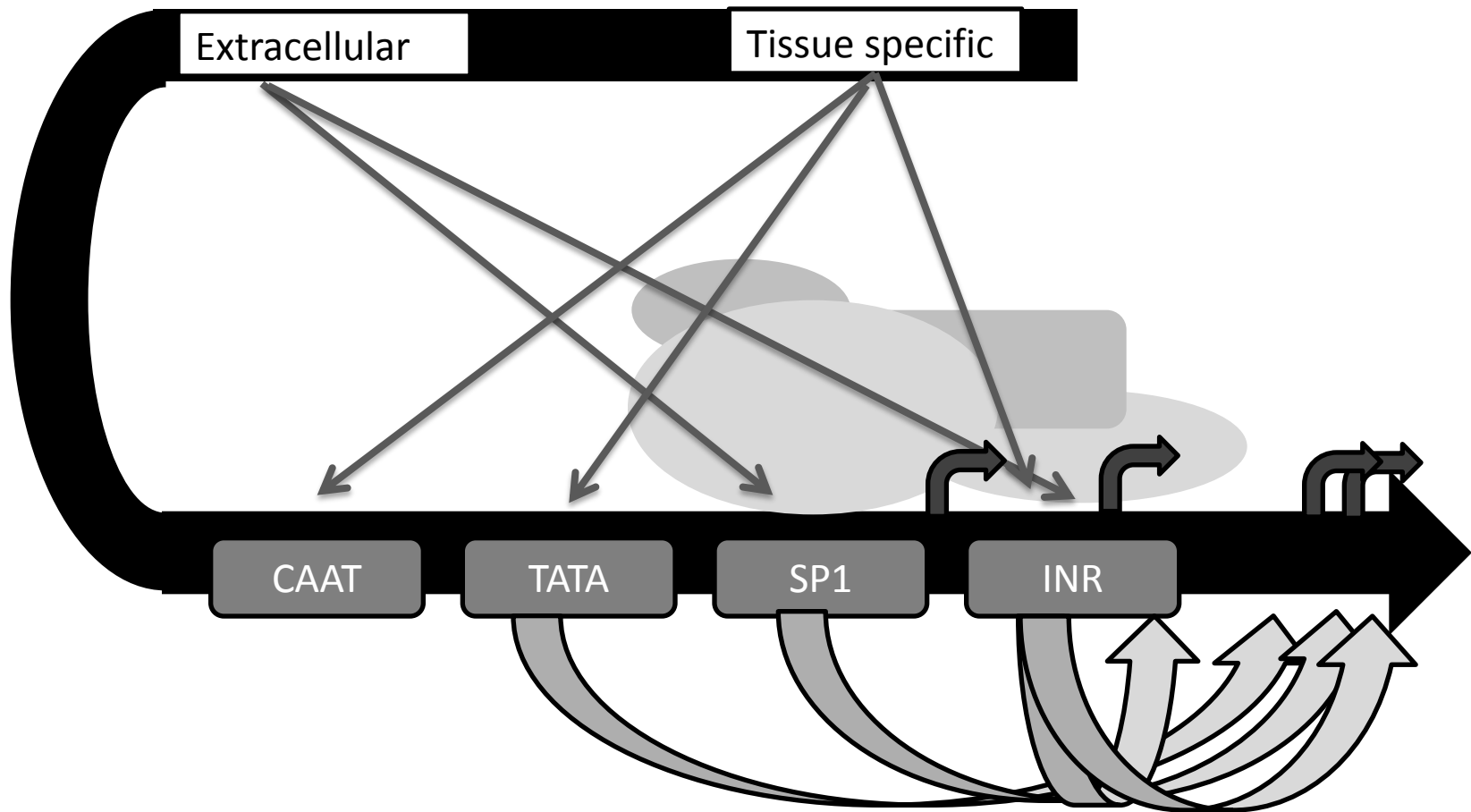
**B.**



**Supplementary Figure 5. Sp1BS core promoter element mutation modulates the IFN $\gamma$  response.** **A.** Sp1BS (T598H4) mice were treated with IFN $\gamma$ , 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 IFN $\gamma$  in parallel and subjected to RT PCR, as a control for the IFN $\gamma$  injection. Real time PCR was relative to an 18S standard. **B.** Primer extension analysis of splenic RNA from Sp1BS (T598H4) mice treated with IFN $\gamma$ .



**Supplementary Figure 6. Response to IFN $\gamma$  treatment of non-expresser SP1BS [T598J5] mice.** Mice were treated with IFN $\gamma$  as described in Methods. The level of endogenous H2K<sup>b</sup> 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  $\text{IFN}\gamma$ , are regulated by Sp1BS and Inr elements. All four elements contribute to the overall transcription level of the gene.