SUPPLEMENTARY DATA

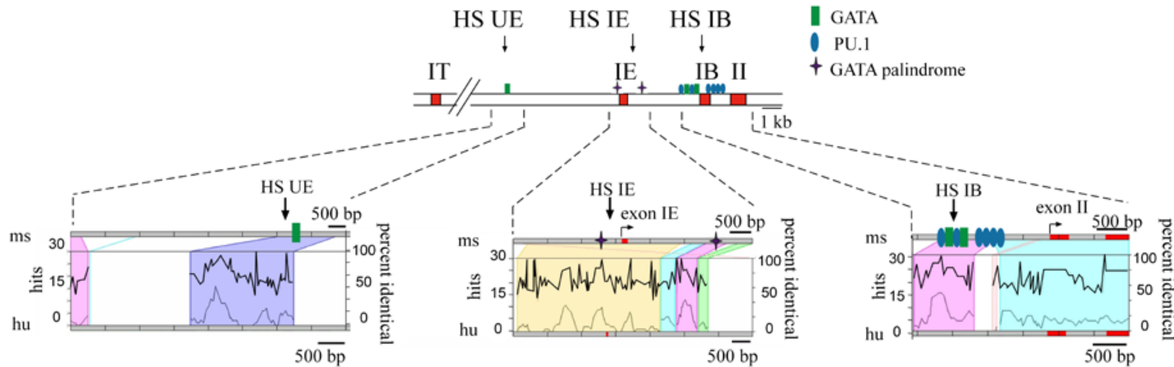
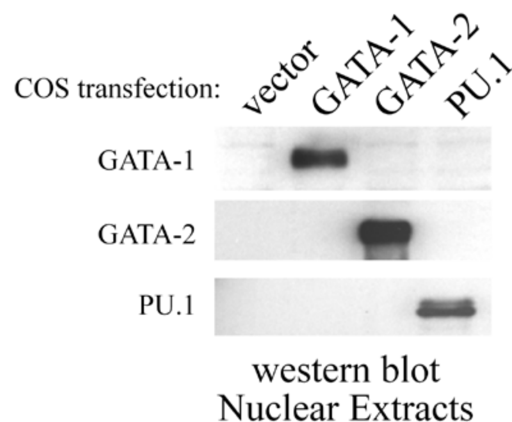


Figure S1: Phylogenetic sequence comparison analysis to identify conserved transcription factor binding sites. Three genomic regions conserved between the mouse (ms) and human (hu) GATA-1 gene were determined with the TRAFAC software program: The upstream enhancer, the exon IE promoter, the GATA palindrome within the first intron, and the hypersensitive site in proximity to exon IB. These sites correspond to previously identified DNaseI hypersensitive sites (labeled HS UE, HS IE, HS IB, see text for details). Conserved blocks are denoted by colored quadrilateral; percent sequence similarity (percent identical) is shown by the dark line. The number of transcription factor binding sites within conserved regions (hits) is depicted by the lighter line. Putative GATA sites are denoted by green rectangles and PU.1 sites are denoted by blue ovals. The GATA palindromic sites are depicted by purple stars.

Figure S2: Protein expression of GATA-1 and GATA-2 and PU.1 in nuclear extracts from transfected COS cells. Western blot analysis for GATA-1, GATA-2 and PU.1 of nuclear extracts from COS transfected cells demonstrate expression of protein.



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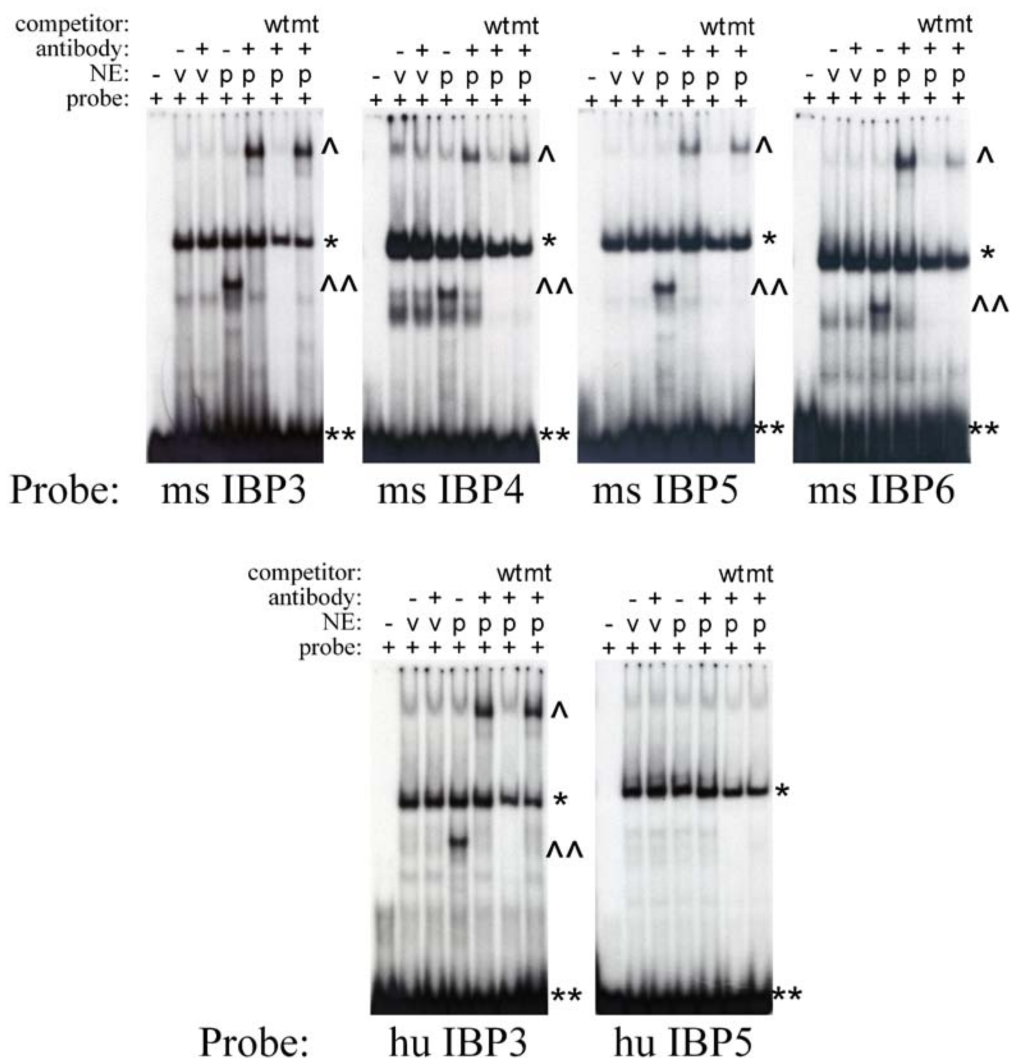

Figure S3: Specificity of PU.1 binding to sequences in the GATA-1 gene. EMSA with the probes containing the GGAA binding motifs from the mouse GATA-1 gene (ms IBP3, ms IBP4, ms IBP5 and ms IBP6) and the human GATA-1 gene (hu IBP3 and hu IBP5). Nuclear extracts (NE) for COS cells transfected with PU.1 (p) show DNA:protein complexes (denoted by ^^) with ms IBP3, ms IBP4, ms IBP5, ms IBP6 and hu IBP3. Vector transfected COS cells nuclear extracts (v) do not show these complexes. These complexes can be supershifted with a PU.1 specific antibody (denoted by ^). Unlabeled wild type probe (wt) competes for binding of the radiolabeled probe, while unlabeled probe with the GGAA core mutated (mt) does not compete for binding. * denotes background DNA:protein complex and ** denotes free probe.

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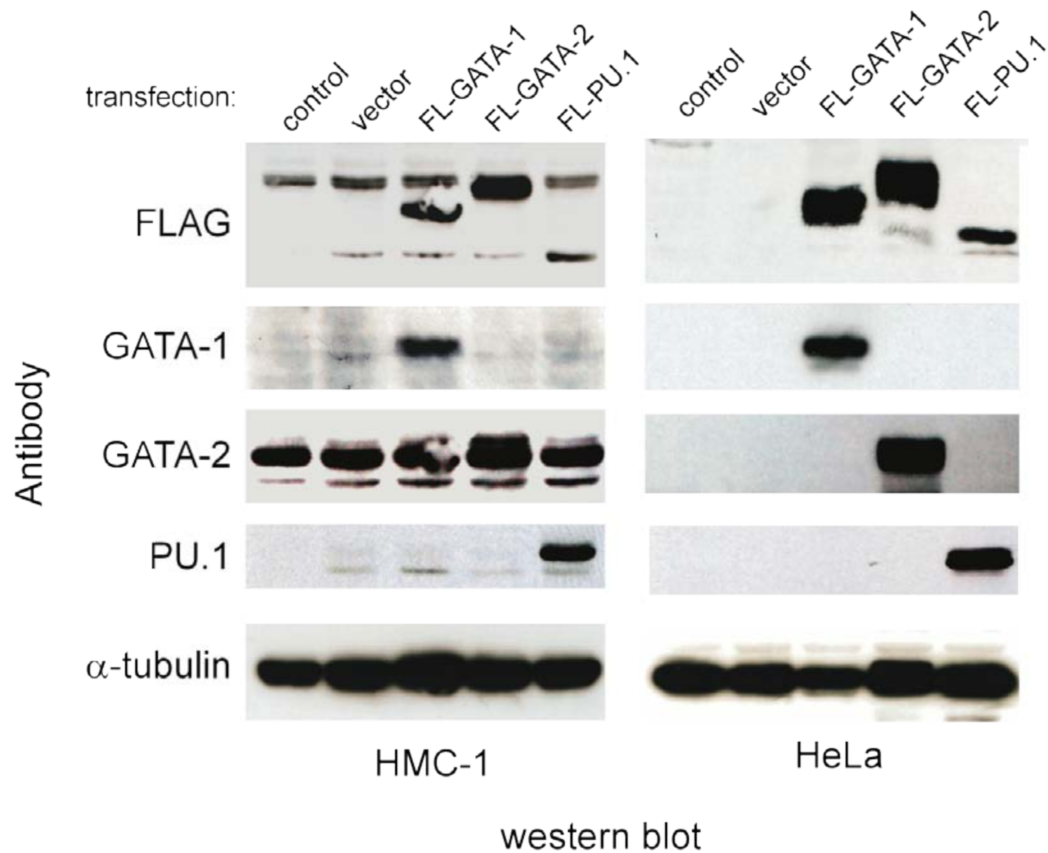

Figure S4: Overexpression of transfected transcription factors in HMC-1 and HeLa cells. The GATA-1, GATA-2 and PU.1 expression constructs contain a FLAG epitope. An antibody to the FLAG epitope was used for western blotting and demonstrates expression of the transfected protein in both HMC-1 cells and HeLa cells. Antibodies specific for GATA-1, GATA-2 and PU.1 were also used to detect endogenous and overexpressed protein. HeLa does not express appreciable amounts of endogenous GATA-1, GATA-2 and PU.1. HMC-1 expresses endogenous GATA-2, but relative little GATA-1 and PU.1. α-tubulin blot demonstrates equivalent loading between samples.

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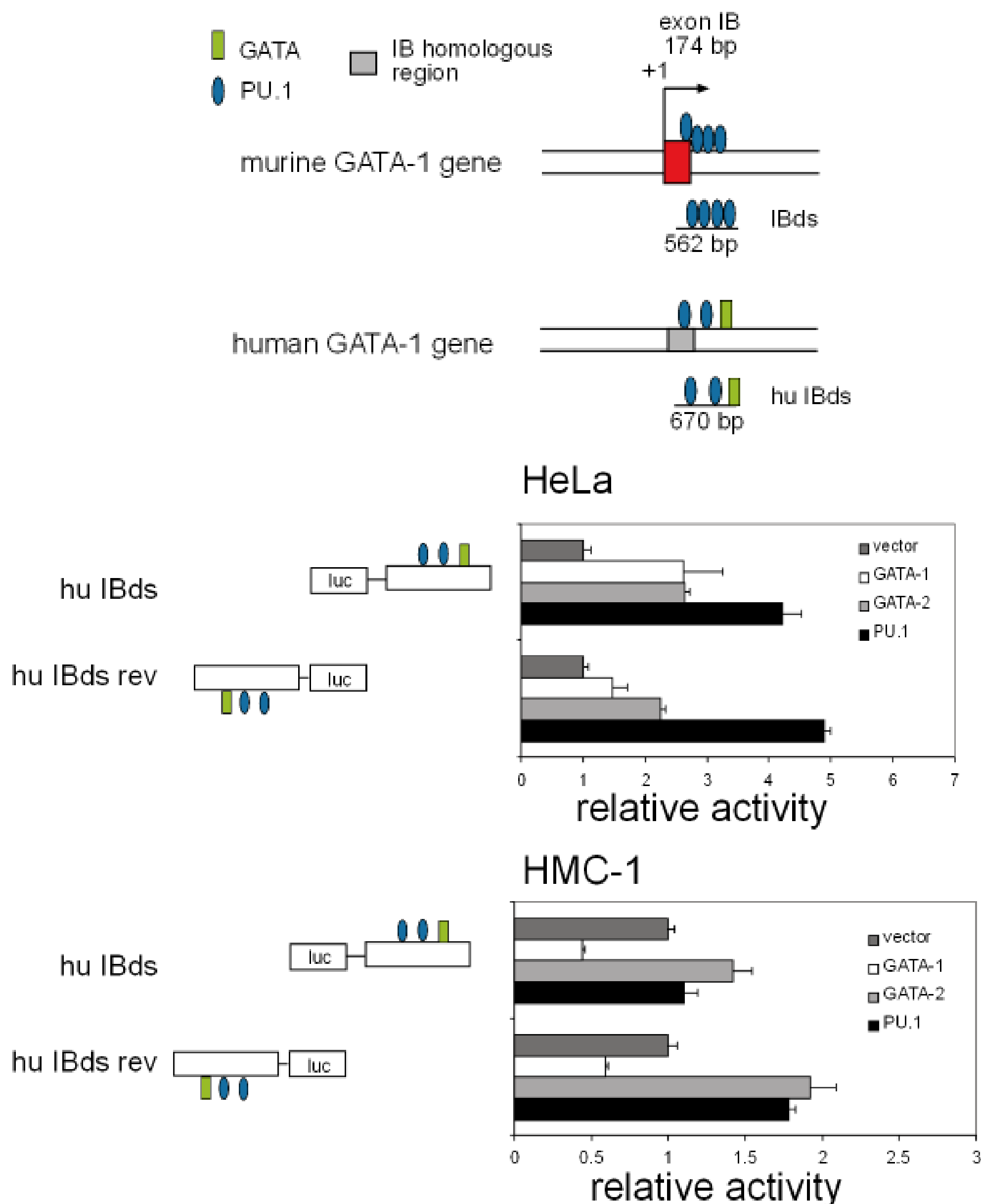

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Figure S5: PU.1 activates the conserved human cis-element that is homologous to the murine IB region. The human genomic fragment (grey box) homologous to the murine IB region (red box) contains two conserved PU.1 binding sites (one site is bound with high affinity by EMSA, see figure 4E and S3). The murine region contains four sites that bind PU.1 by EMSA (blue ovals). There is also a GATA binding site in the human sequence (green rectangle) that is not present in the murine sequence. The pGL2 SV40 luciferase reporter construct (hu IBds) contains this human genomic region and is activated by PU.1 in HeLa cells when placed upstream or downstream of luciferase. In HMC-1, this element is significantly activated by PU.1 when placed upstream of the luciferase gene. The human genomic element is also transactivated by GATA-1 and GATA-2 in HeLa cells and by GATA-2 in HMC-1 mast cells.

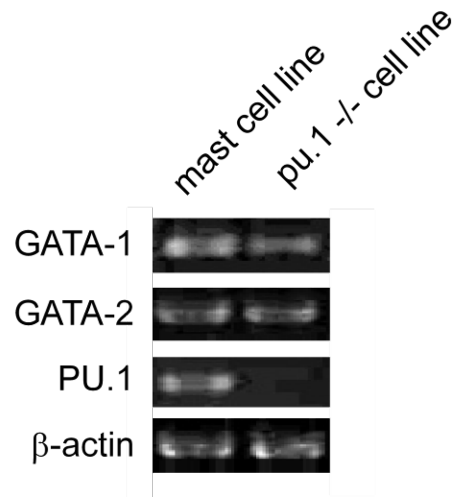

Figure S6: Expression of GATA-1 and GATA-2 in PU.1 -/- cells and mast cells. RT-PCR analysis show expression of the GATA factors from the C57 mast cell line and PU.1 -/- fetal liver cells. PU.1 is detected only from the mast cell line.

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