Method.

To make GloPLP, ApaI/BglII DNA fragment containing PLP exon 3B, the upstream 5 nucleotides in exon 3A (in order to preserve the natural DM20 5' splice site) and the first 100 nucleotides of intron 3 (forward primer: ApaI (lower case): 5'-AAAGCTgggcccGAGCGCAACGGTAACAG-3' and reverse primer: BglII (lower case): 5'-AAAGCTagatctCCCCTAGAGAGGACCCAGCCT-3') was cloned into DUP4-1 (kind gift of Dr. Black), 97 nucleotides from the fifth base preceding the 5' site of globin exon 1 through the ApaI I in intron 1 were deleted, forward primer, 5'-GATGAAGTTGGTGGTGAGGCCCTGCAACGGTAACAGGGGGCCAGAAGG-3' (PLP underlined), reverse primer: 5'-CCTTCTGGCCCCCTGTTACCGTTGCAGGGCCTCACCACCAACTTCATC-3' (PLP underlined). In the Glo-PLP construct, sequences necessary for 3' splice site recognition are provided by the heterologous β-globin gene. All constructs were sequenced.

PLP and DM20 products derived from the GloPLP were amplified with forward primer (5'-AGTTGGTGGTGAGGCCCTGCAACG-3') and reverse primer DUPRT3 (5'-AACAGCATCAGGAGTGGACAGATCCC-3') (Fig 1A). PCR reactions were performed with 1 tenth (50 ng original RNA input) of the RT cDNA in ABI3000 (Applied Biosystems, Foster City, CA). After agarose gel electrophoresis, band intensities were quantified with Kodak 440CF Digital Image Station using 1D analysis software. The data are expressed as ratio of the exon3B+ PCR product/exon3B- PCR product.

Legend:

Figure S1: *M2 enhances DM20 5' splice site in a minimal globin PLP construct.* **A**. Diagram of the GloPLP: β-globin sequences are in bold lines, PLP exon 3B with the natural DM20 5' splice site including 5 nucleotides of exon 3A (shown in brackets) and 100 nucleotides of intron 3 are in shaded lines. 349 indicates the G of the invariant GT at

the DM20 5' splice site in exon 3B. The arrows show the position of the primers used for PCR amplification. **B**. GloPLP transcripts with exon 3B present and absent were amplified from RNA extracted from transfected Oli-neu cells (30 PCR cycles). The ratio of exon 3B+/exon 3B- product±SD is calculated based on 3 independent transfections. The increase in the ratio is statistically significant ($p=0.01$)