Targeted isoform-specific analysis of *Csf3r* alternative splicing in splicing factor mutant myeloid cells

Clara H. Ambrose*, Abigail L. Rich, and Sandra S. Zinkel

SUPPORTING INFORMATION.

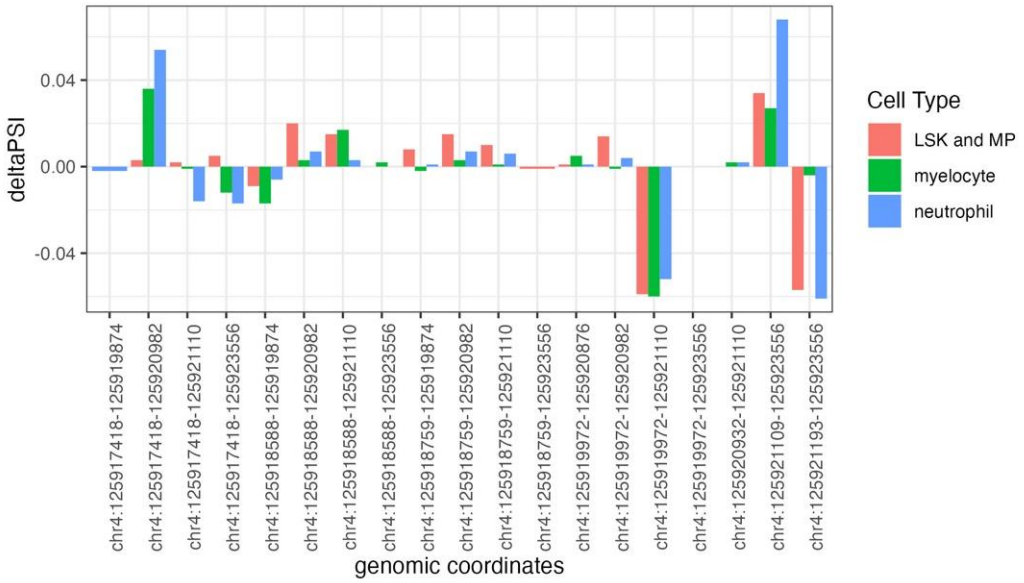

Figure S1. RNA Sequencing Data: A cluster of 16 differentially spliced introns with genomic coordinates from Gencode m29 (coord) was identified in P95H mutant hematopoietic stem and progenitor cells (LSK and MP), myelocytes, or neutrophils in *Csf3r*. deltaPSI or "delta Percent Spliced In" describes the difference in the frequency of a splicing event when comparing P95H – WT. chr4:125919972-125921110 coordinates refer to the exon 2 – exon 3 junction.

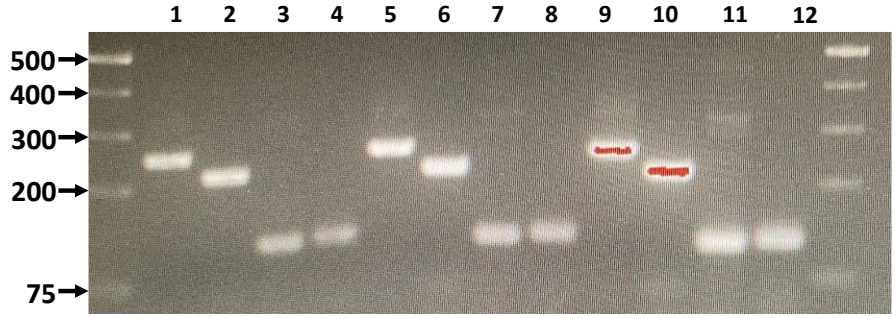

Figure S2. Primer Verification: Three primers (I1 e2-e3 A, I1 e2-e3 B, I1 e2-e3 C) targeting normal exon 2-3 junction were successfully verified using PCR and agarose gel electrophoresis. Lanes 1, 5, and 9 are the *Csf3r* control which was designed to produce an amplicon of 243 base pairs. Lanes 3, 7, and 11 are I1 e2-e3 A which was designed to produce an amplicon of 113 base pairs. Lanes 4, 8, and 12 are I1 e2-e3 B which was designed to produce an amplicon of 117 base pairs. Lanes 2, 6, and 10 is I1 e2-e3 C which was designed to produce an amplicon of 215 base pairs. Primers were loaded into well at concentrations of 1 µL (Lanes 1, 2, 3, 4), 2 µL (Lanes 5, 6, 7, 8), and 3 µL (Lanes 9, 10, 11, 12) from left to right. Brightness increases as concentration increases demonstrating efficacy of primers. Red pixels (lanes 9-10) represent oversaturated pixels with very strong signal.

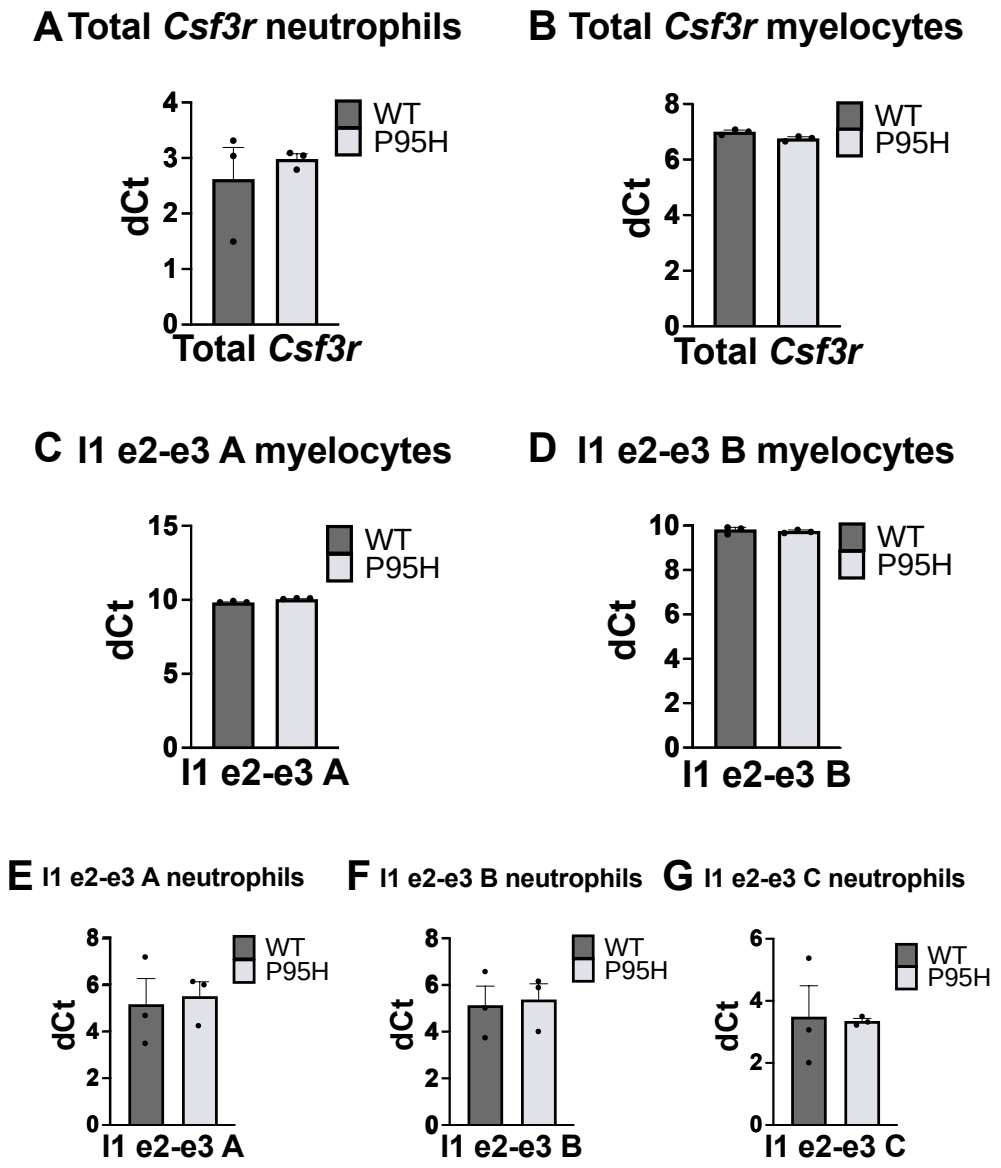

Figure S3. RT-qPCR Delta Ct Results for Exon 2-3: Total *Csf3r* targeting splicing of exon 2-3 of *Csf3r* across three custom primer pairs was measured by the delta Ct method. The lower the dCt, the more mRNA amplification.