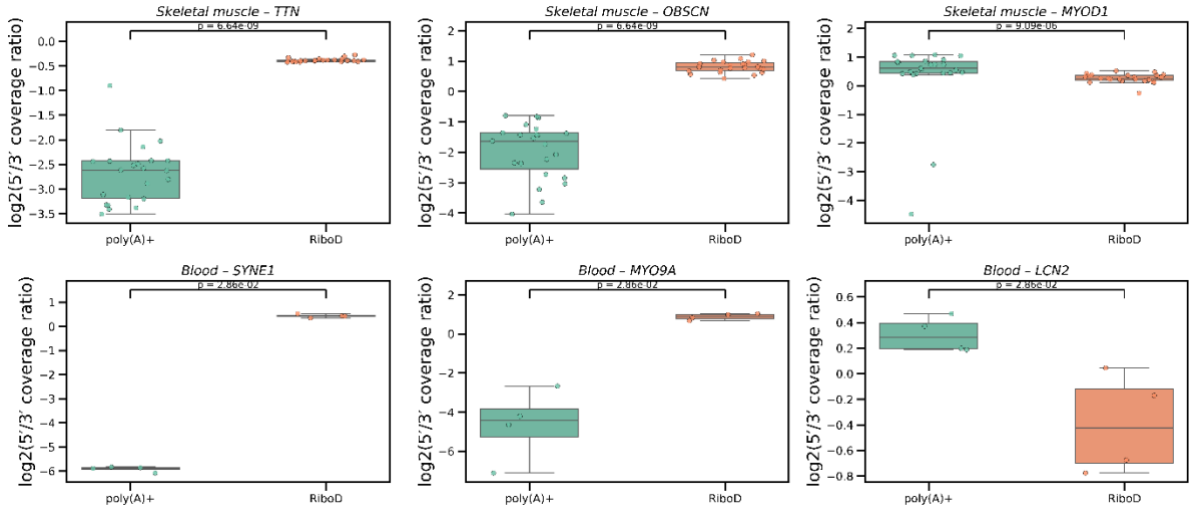
Supplementary 1

A. 5'-3' end coverage assessment

Skeletal muscle: *TTN* (>100kb) , *OBSCN* (~39kb), *MYOD1* (~2kb)

Blood: *SYNE1* (~47 kb), *MYO9A* (~20 kb), *LCN2* (~1kb)



B. Titinopathy patient information with IGV explanation

Patient A: TTN(NM_001267550.2):c.4646-1G >A

An intronic variant was identified in intron 26 of 362 in both poly(A)+ (supported by 2 reads) and rRNA depleted (supported by 322 reads) RNA-Seq. Consistent with the acceptor loss outcome, only rRNA depleted RNA revealed multiple aberrant splice events. These included the combined skipping of exons 26 and 27 (supported by 24 reads), occurrence of a cryptic acceptor site resulting in the loss of first 14 nucleotides in exon 27, supported by 1195 reads), and the skipping of exon 26 (43 supporting reads).

Patient B: TTN(NM_001267550.2):c.25063+1G>A

An intronic variant was identified in intron 86 of 362 in both poly(A)+ (supported by 2 reads) and rRNA depleted (supported by 8109 reads) RNA-Seq. The variant causes the loss of the

donor site and only rRNA depleted RNA-Seq revealed clear evidence of aberrant splicing junctions, including the skipping of exon 86 (1363 supporting reads) and the combined skipping of exons 85 and 86 (1538 supporting reads).

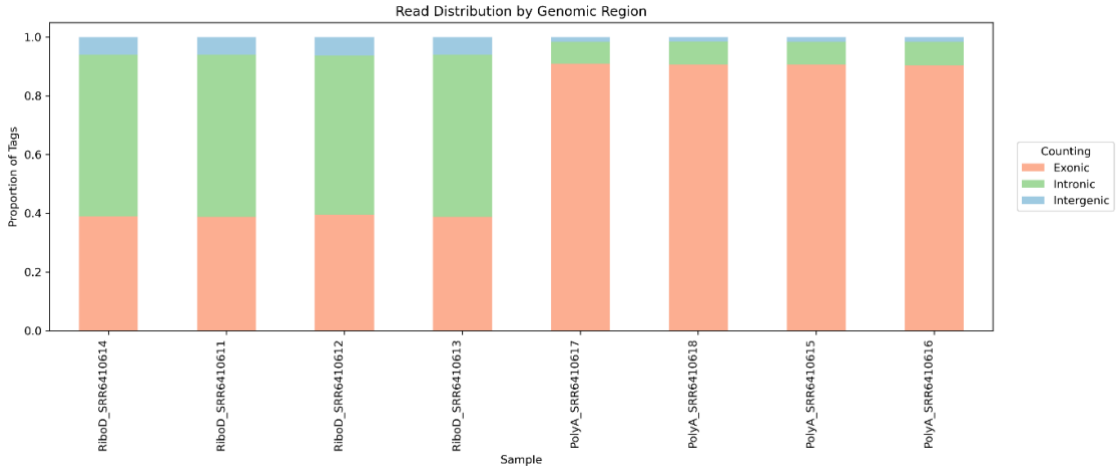
Patient C: TTN(NM_001267550.2):c.19426+2T>A

An intronic variant was detected in intron 66 of 362 in both poly(A)+ (supported by 5 reads) and rRNA depleted (supported by 8297 reads) RNA-Seq. Although the intron retention is observed in both RNA libraries, there are more reads supporting the intronic region in rRNA depleted RNA-Seq compared to ploy(A)+. Furthermore, only the rRNA depleted RNA-Seq demonstrated clear aberrant splicing junctions in IGV. Specifically, the skipping of exon 66 was supported by 14969 reads, whereas there are only 3 reads supporting that aberrant junction in poly(A)+ RNA-Seq data. In addition, rRNA depleted RNA-Seq revealed an alternative aberrant junction supporting the skipping of exons 66 and 67 by 1994 reads.

Patient D: TTN(NM_001267550.2):c.15776-1G>T

An intronic variant was identified in intron 53 of 362 in both poly(A)+ (supported by 6 reads) and rRNA depleted (supported by 19376 reads) RNA-Seq data. The variant causes the loss of the acceptor site and only rRNA depleted RNA-Seq clearly indicated the alternative splicing events (***Figure 2 D)***, including the skipping of exons 53 and 54 (664 supporting reads) and the skipping of exons 54 and 55 (1891 supporting reads).

C. Public Blood RNA-Seq sample annotation



D. Skeletal muscle RNA-Seq sample annotation