**Supplementary Note 2.** Exon 5B sequence is not required for inclusion or suppression of E5B-GFP.

In order to investigate whether authentic exon 5B is required for alternative selection of E5A-RFP and E5B-GFP, we deleted E5B sequences in the mini-genes below. Out of 300-bp of the authentic exon 5B sequence used in the BGAR mini-gene ([Fig. 1a](#)), 94-bp and 6-bp of 3’ portions were left in Δ200BGAR and Δ300BGAR, respectively (a). These mini-genes were expressed under the unc-51 promoter. Expression of E5B-GFP was not affected in either of the reporters, while the expression of E5A-RFP was diminished in the Δ300BGAR reporter worm (b-c). Three/two forms of mRNAs were predominantly generated from each mini-gene (a). These results suggested that selection of E5A and E5B was not affected by the deletion of E5B. E4I4E5BE5A from the Δ300BGAR reporter, in which intron 4 and E5B-GFP were retained, was considered to correspond to the selection of E5A; deletion near the splice acceptor site for E5A may have prevented the authentic splicing to E5A, resulting in the retention of intron 4.

**a.** Schematic representation of egl-15 reporters Δ200BGAR (top) and Δ300BGAR (bottom), and schematic representation of major forms of mRNAs derived from each reporter under the unc-51 promoter. Boxes indicate exons. Predicted ORFs are colored in magenta for E5A-RFP, in green for E5B-GFP and in blue for others. *E5A denotes cryptic 3’-splice site within E5A. b-c.** Transgenic worms expressing Δ200BGAR (b) and Δ300BGAR (c) reporters under the unc-51 promoter. Projection images of E5A-RFP and E5B-GFP, merged and DIC images of the same fields are shown. L2-L3 larvae. Scale bar, 100 µm.