NineTeen Complex-subunit Salsa is required for efficient splicing of a subset of introns and dorsal-ventral patterning.

Running title: Salsa is required for dorsal-ventral patterning

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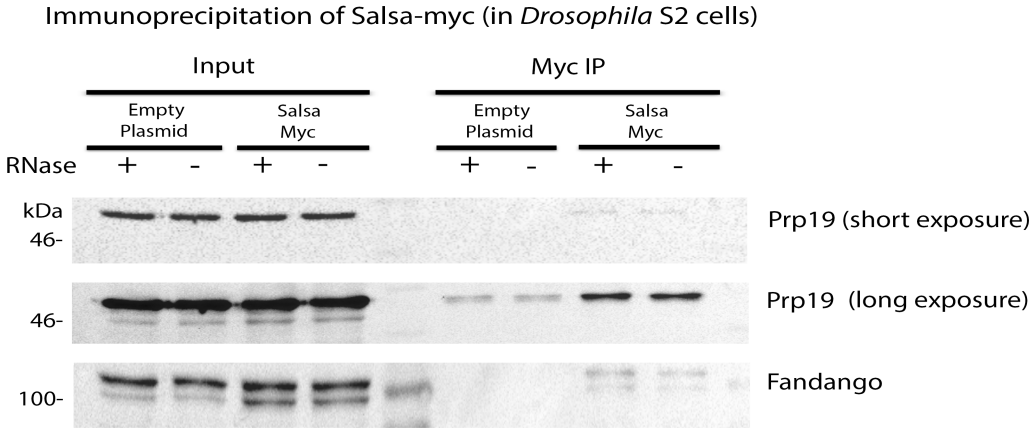
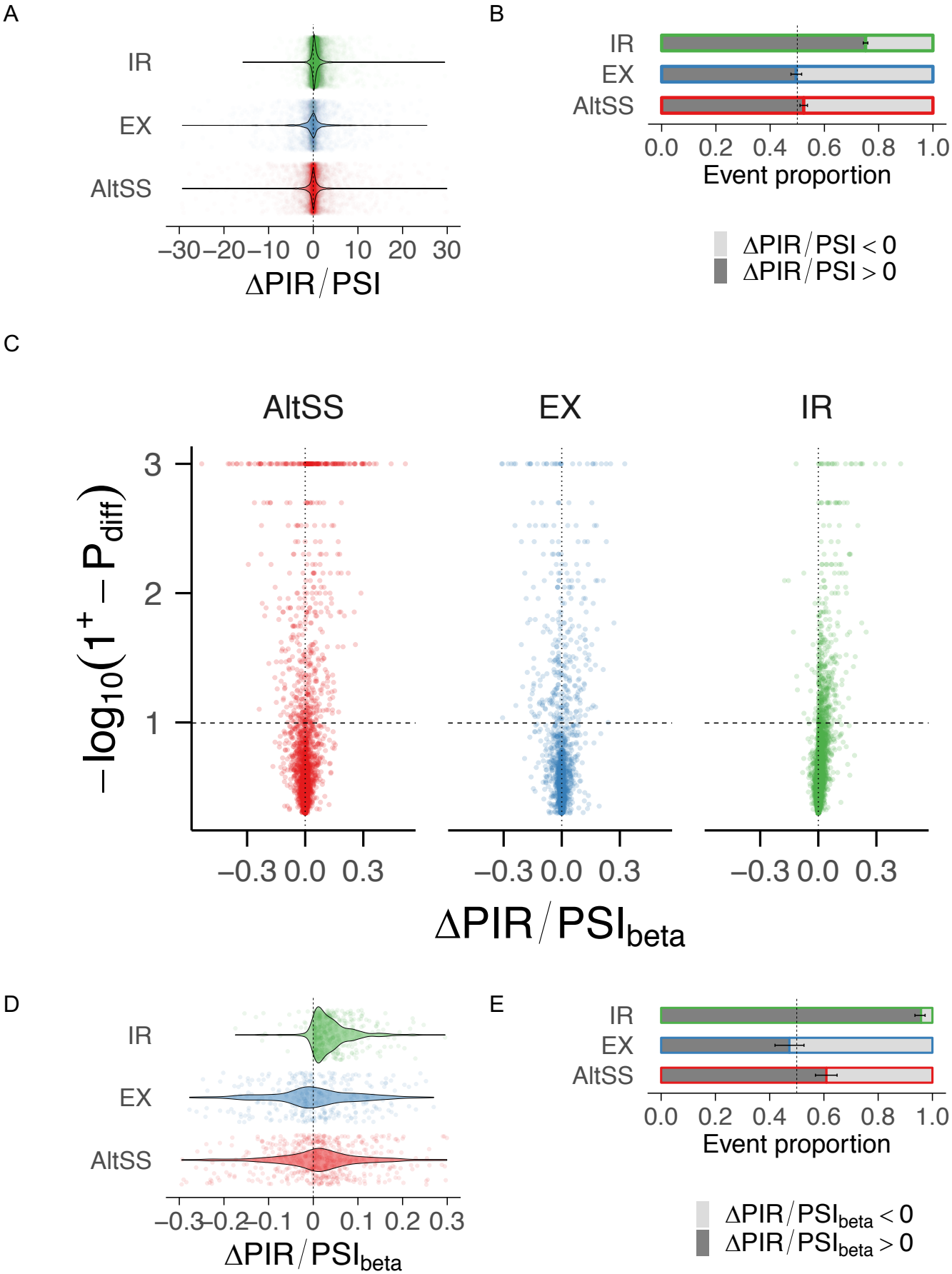
Supplementary Information

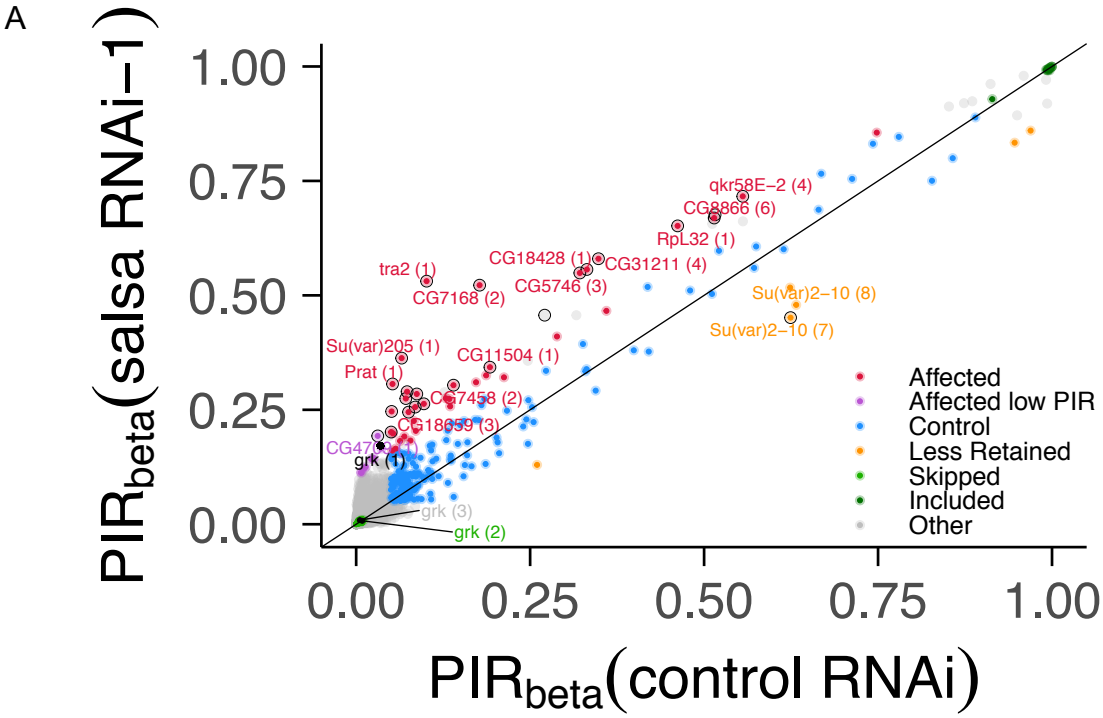
S1 Figure. Salsa interacts with spliceosome NineTeen Complex (NTC)

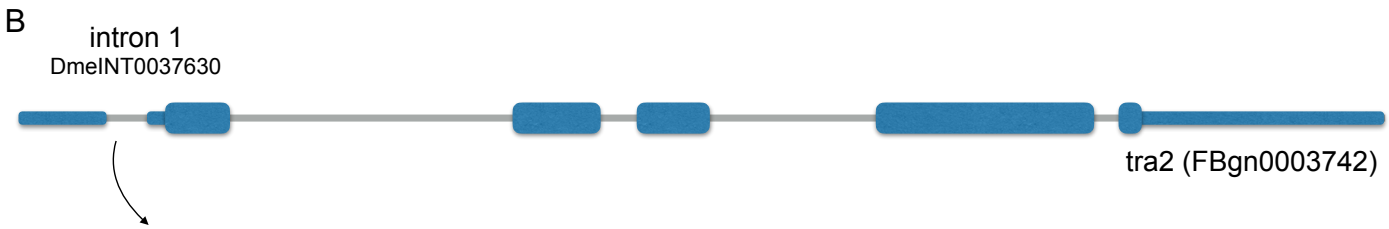


S2 Figure. The most common form of alternative splicing defects after Salsa depletion is increased levels of intron retention (IR)

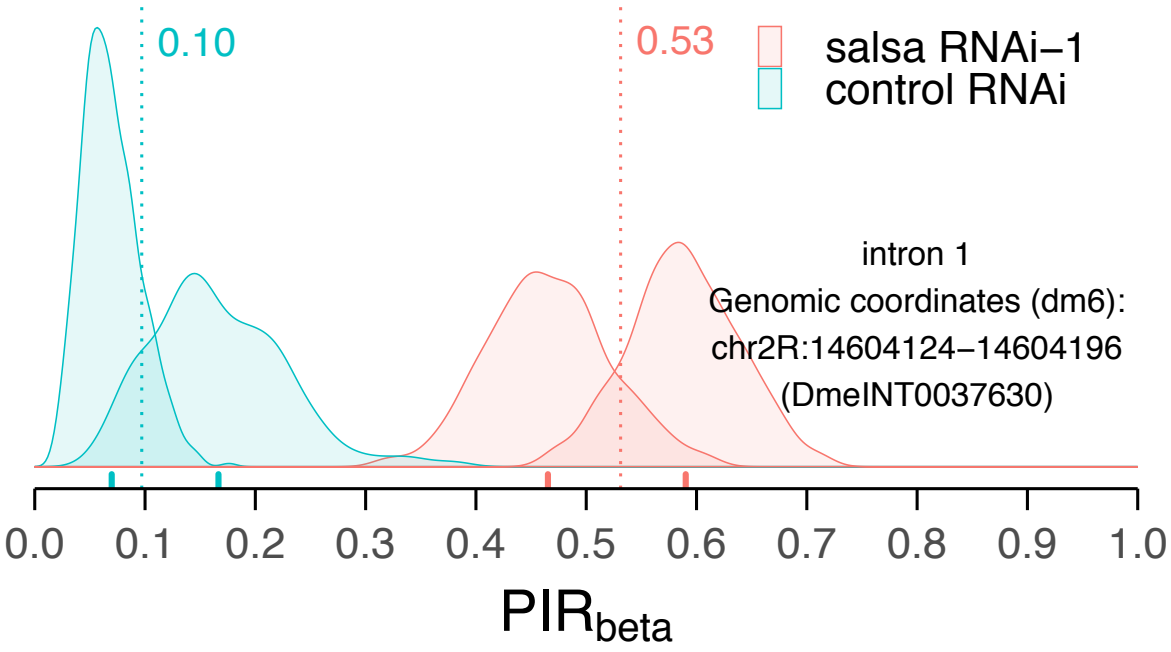


S3 Figure. A subset of introns shows increased levels of retention (IR) upon Salsa depletion. Salsa is required for splicing of the first intron of *tra2*

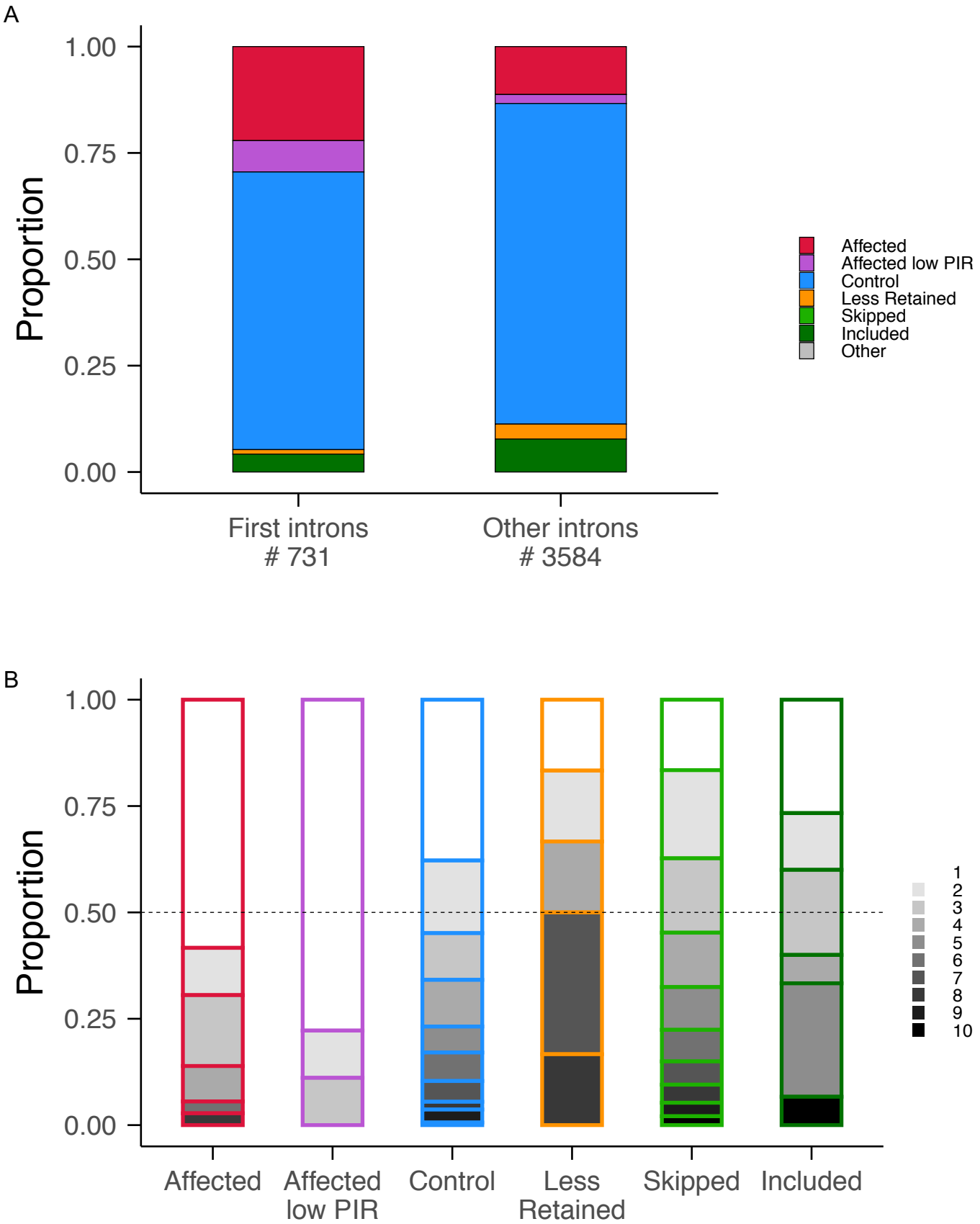




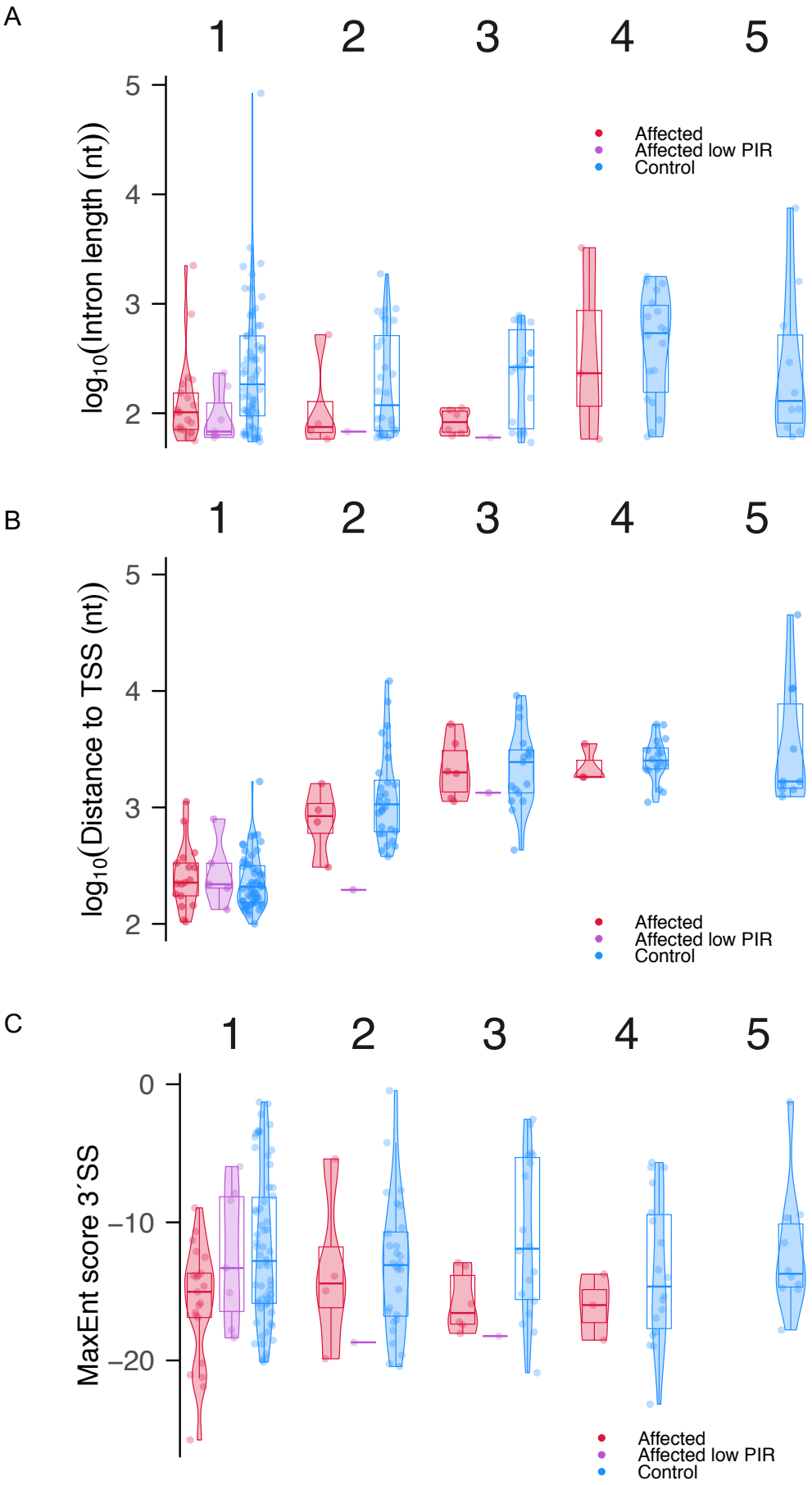
$\Delta PIR_{beta}=0.43$, $P_{diff}=1.00$



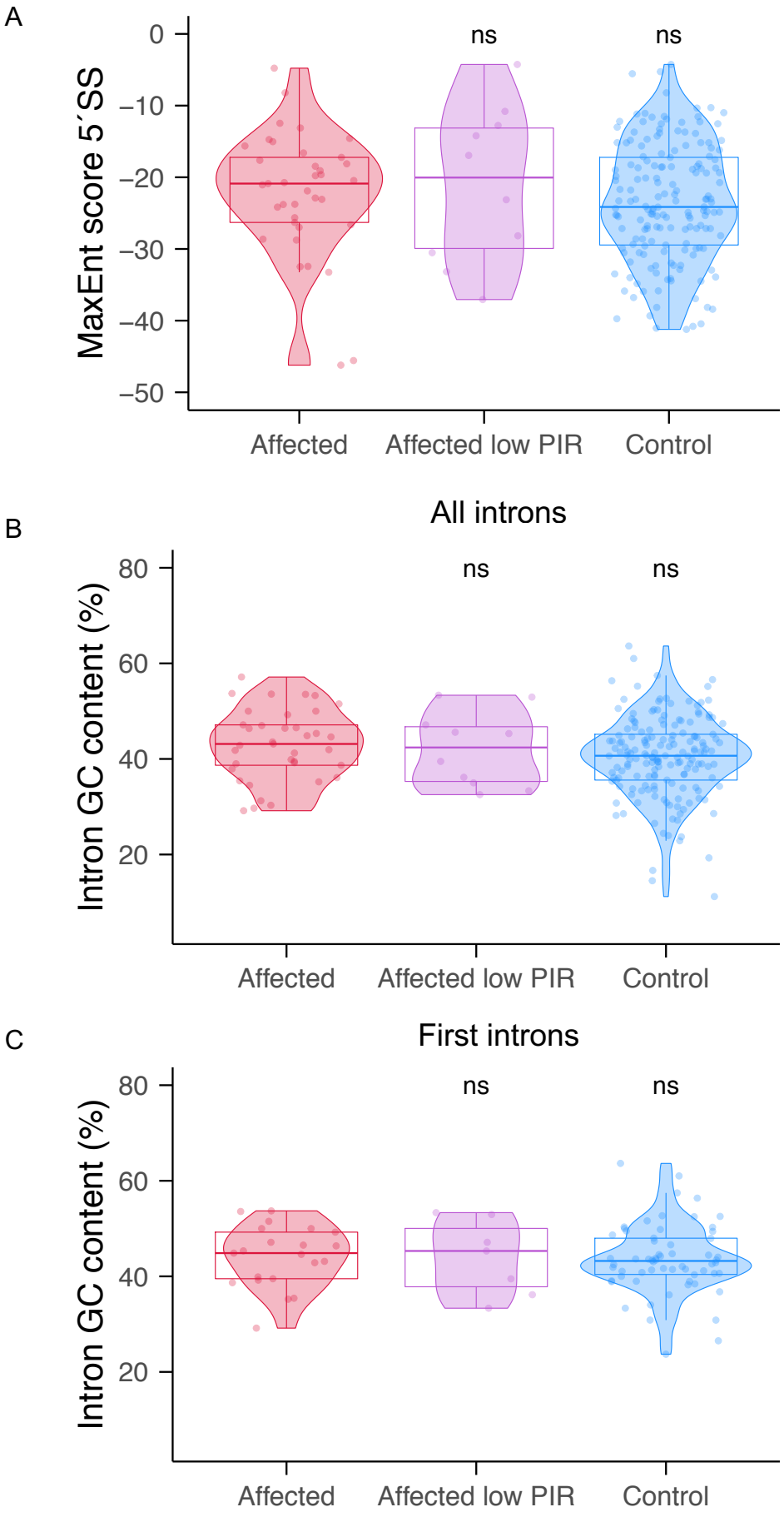
S4 Figure. First introns show a bias for increased intron retention after Salsa depletion



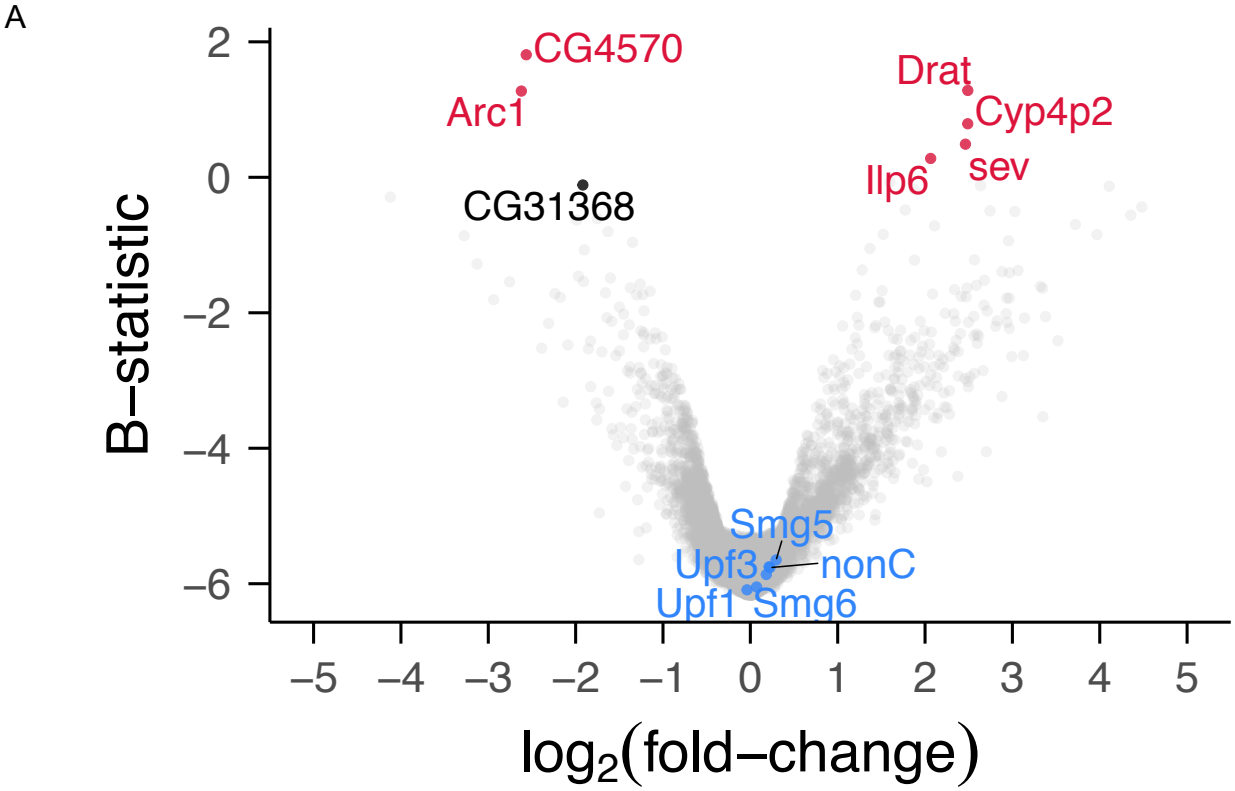
S5 Figure. Intronic features per intron position when splicing is affected by Salsa depletion

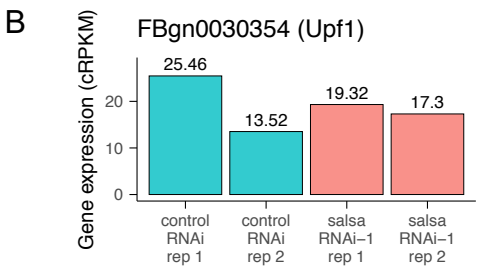


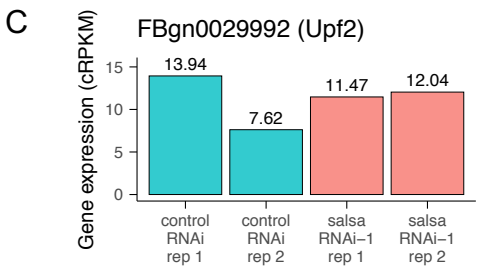
S6 Figure. No obvious 5´splice site and GC content bias in introns affected by Salsa depletion

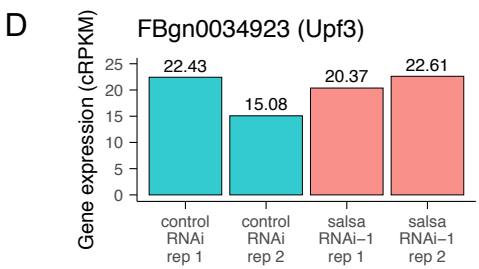


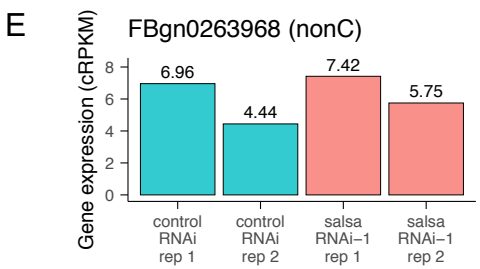
S7 Figure. Salsa regulates the expression levels of a very small number of genes. Expression of the nonsense-mediated decay pathway genes is unaffected

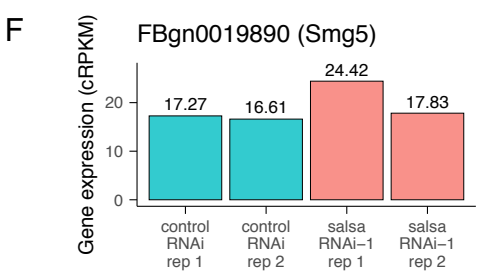


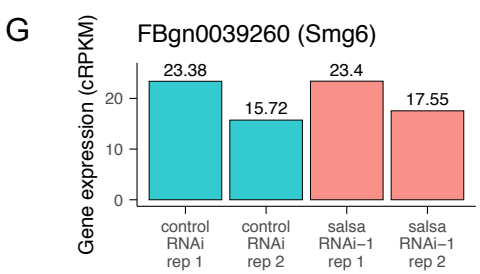




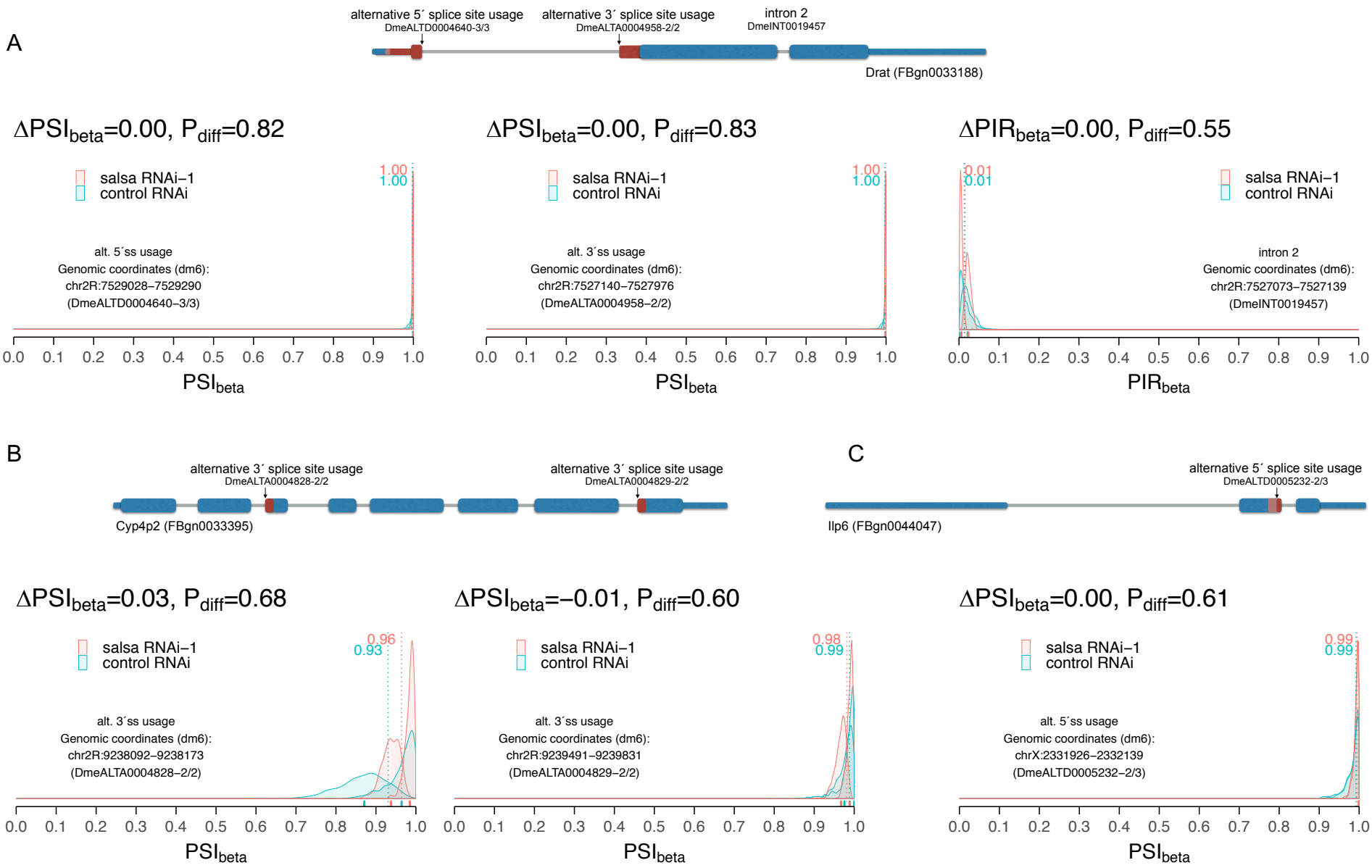




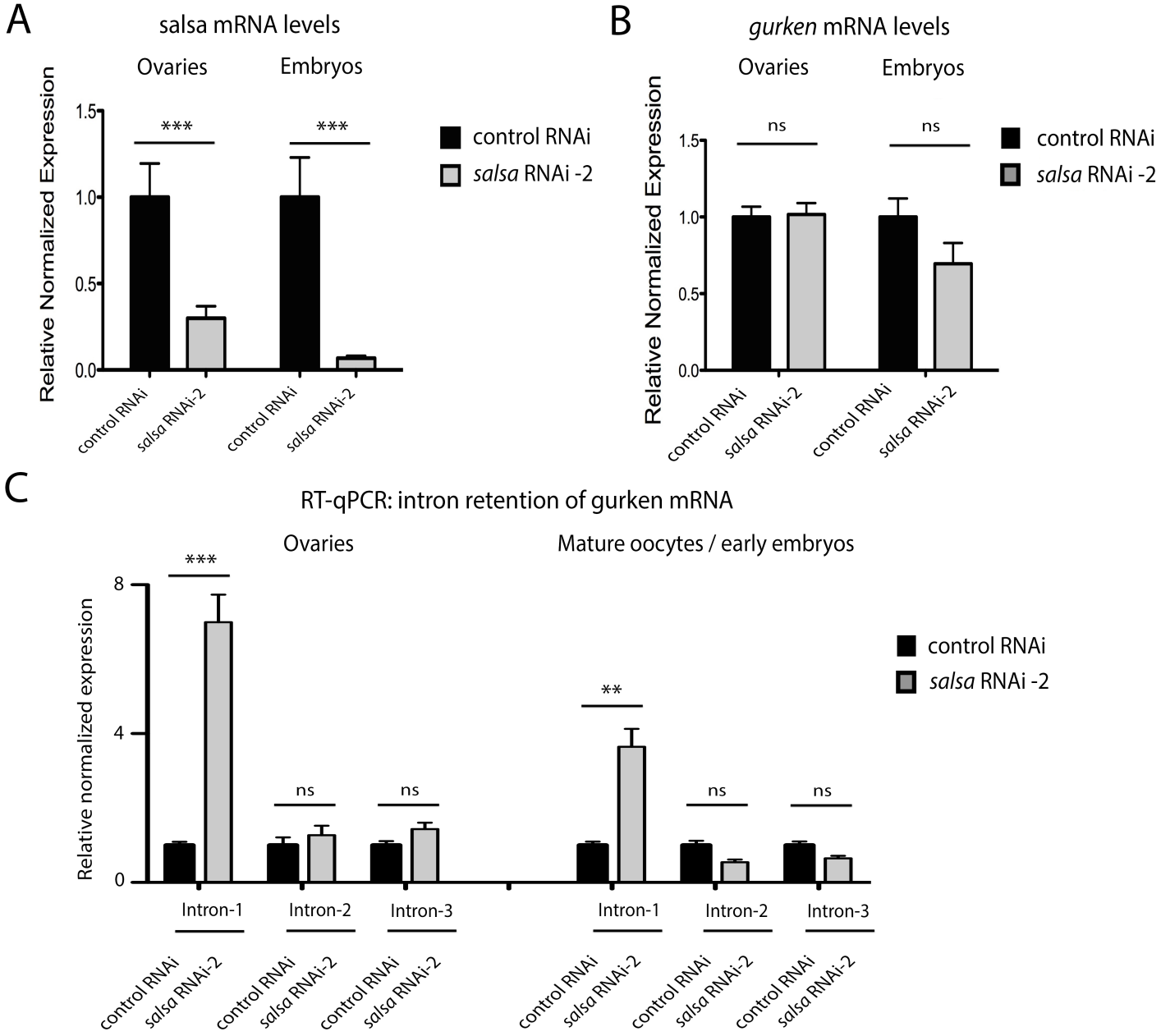




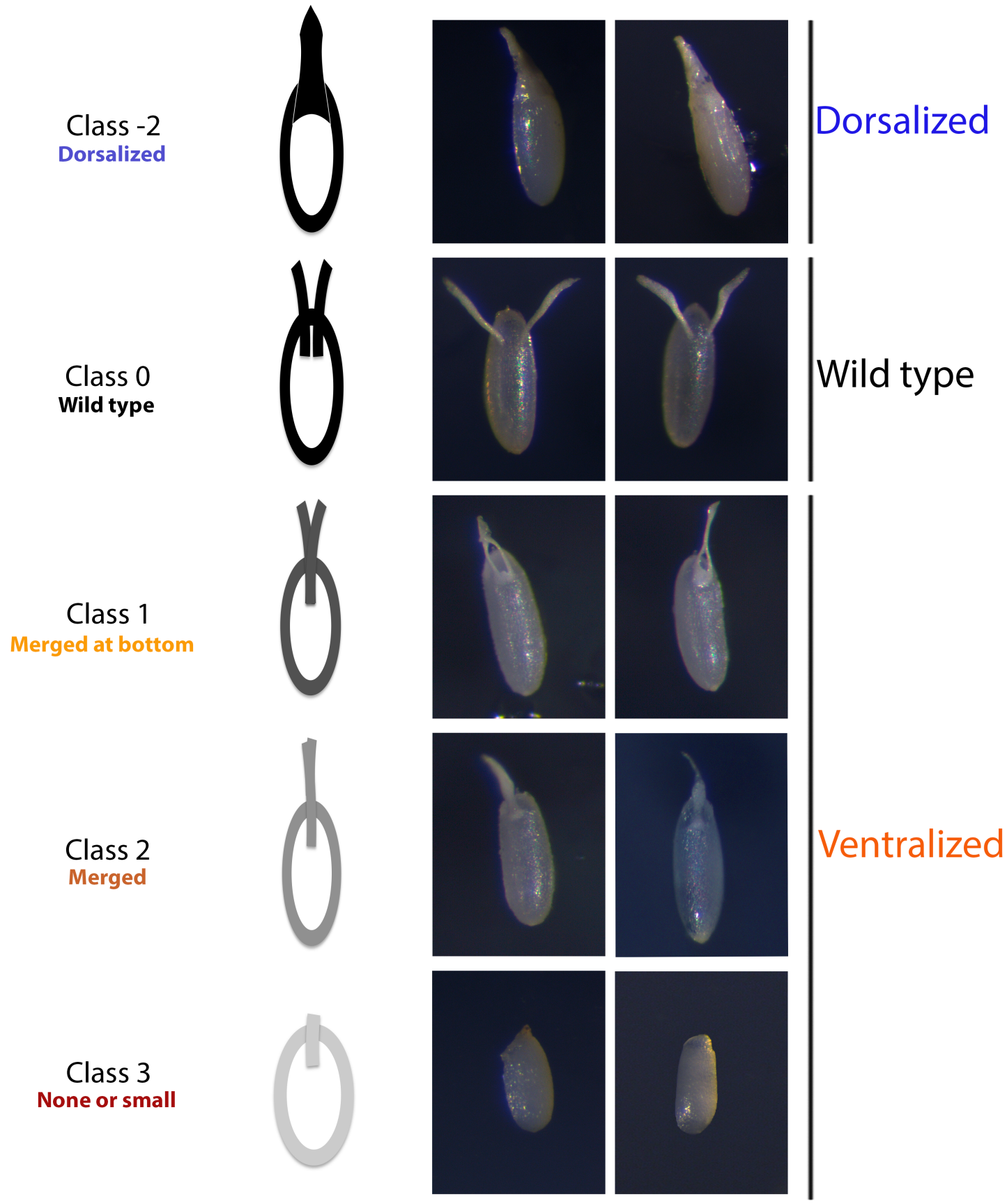
S8 Figure. Genes whose expression levels were affected by Salsa depletion show no detectable changes in alternative splicing



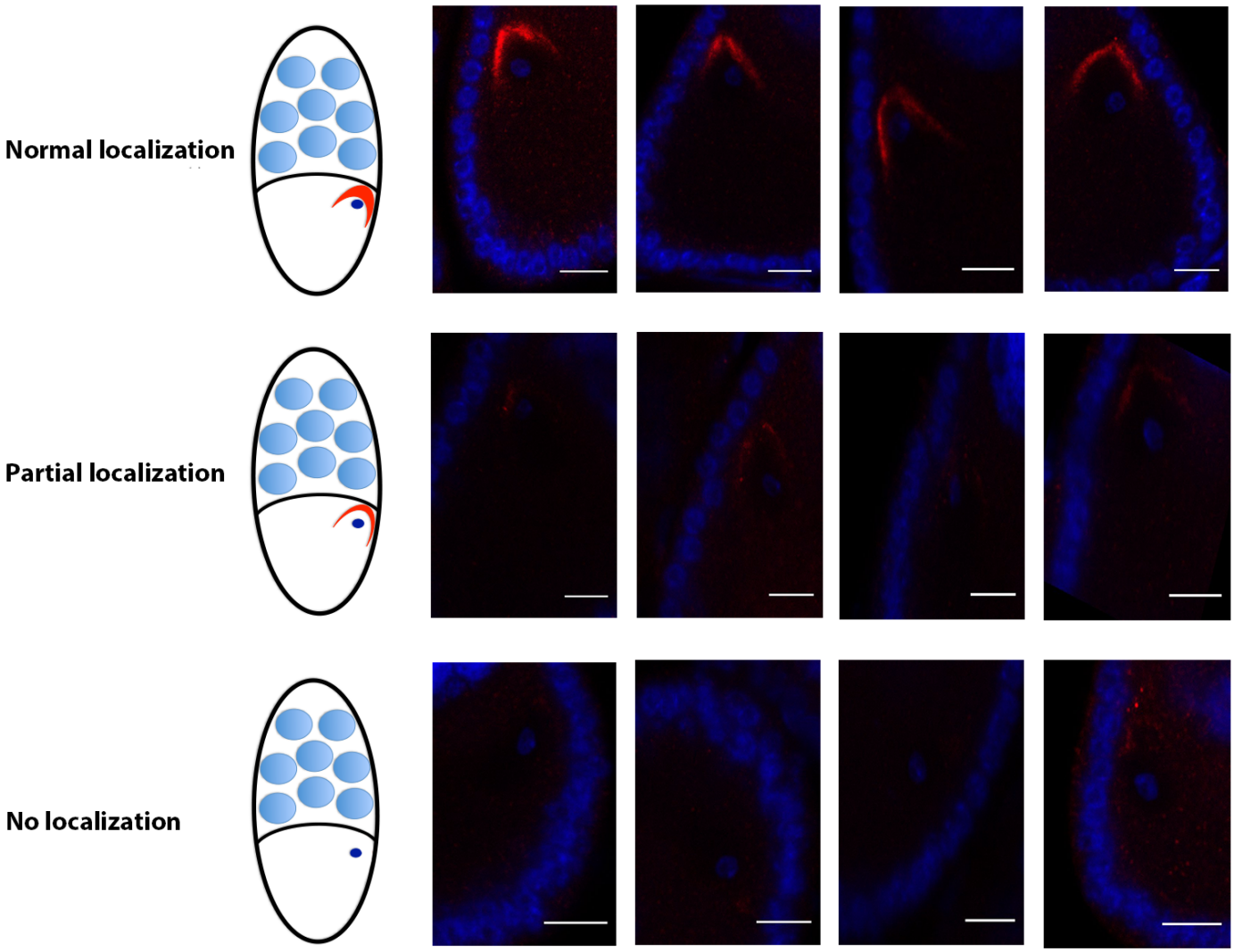
S9 Figure. Depletion of salsa impairs splicing of the first intron of *gurken* mRNA without eliciting a significant mRNA degradation



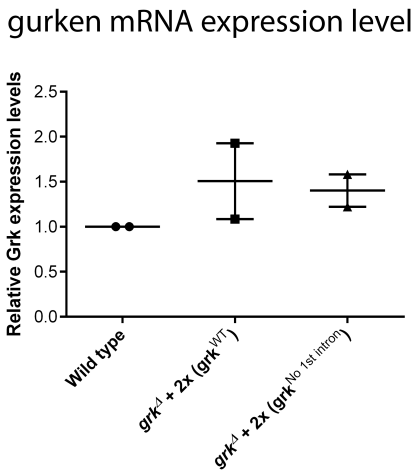
S10 Figure. Eggshell dorsal appendages defects



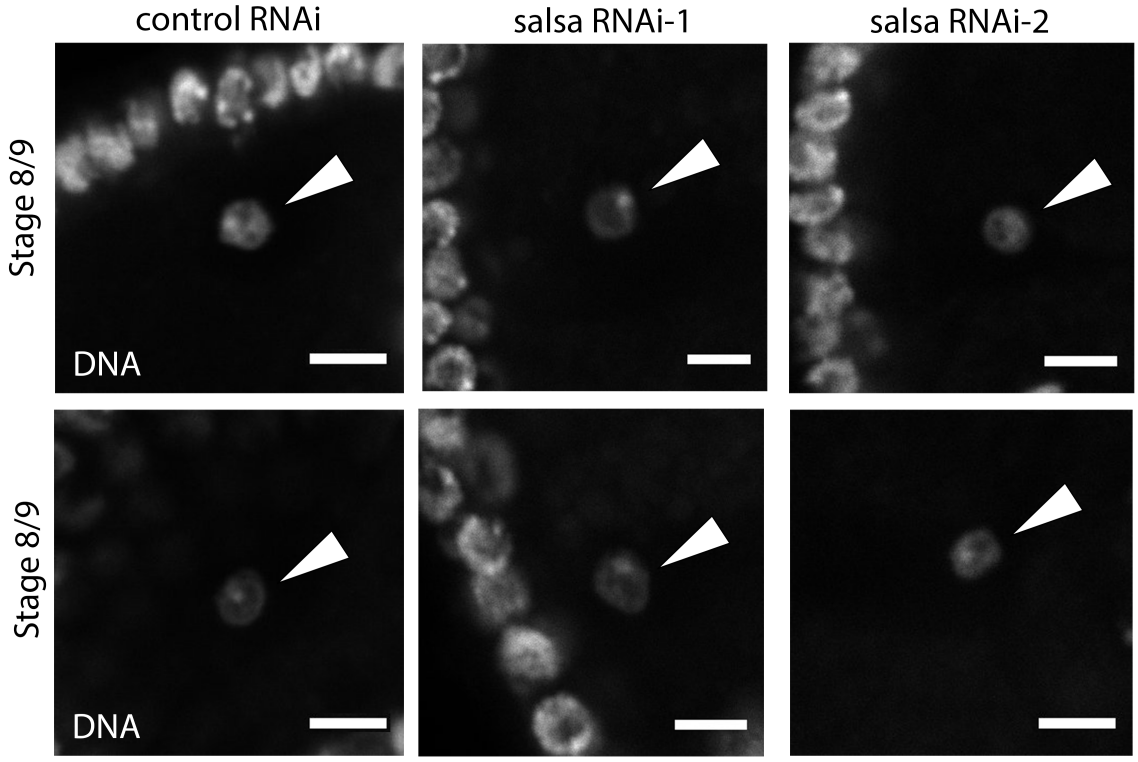
S11 Figure. *gurken* mRNA localization defects



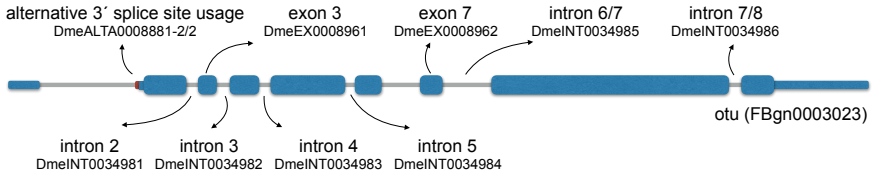
S12 Figure. Deletion of first intron does not impair expression of *gurken* mRNA



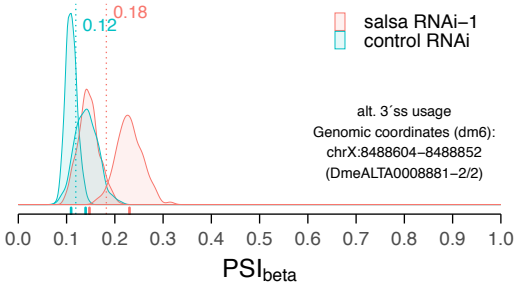
S13 Figure. Depletion of salsa does not impair the normal morphology of the oocyte karyosome (chromosomes)



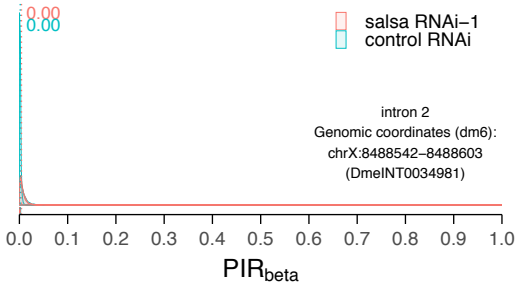
S14 Figure. Depletion of Salsa does not impair alternative splicing of *ovarian tumor* (*otu*)



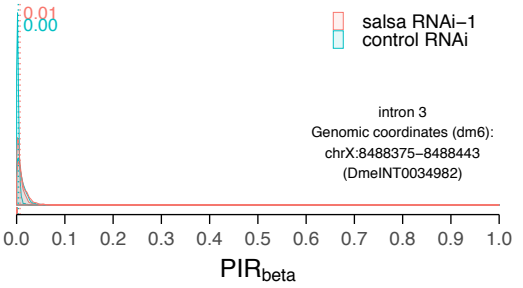
ΔPSI_{beta}=0.06, P_{diff}=0.90



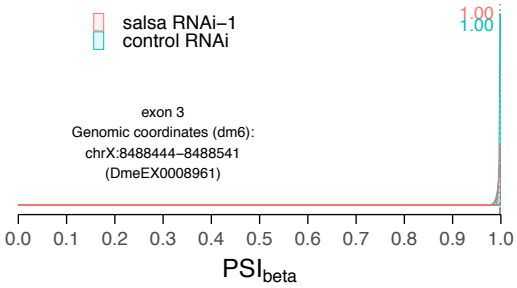
ΔPIR_{beta}=0.00, P_{diff}=0.68



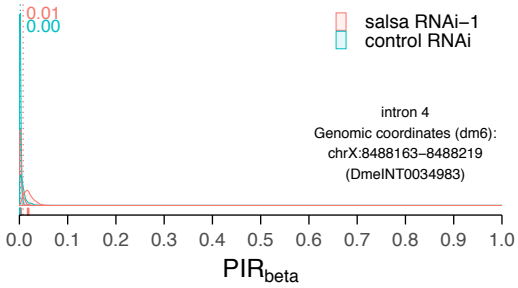
ΔPIR_{beta}=0.00, P_{diff}=0.63



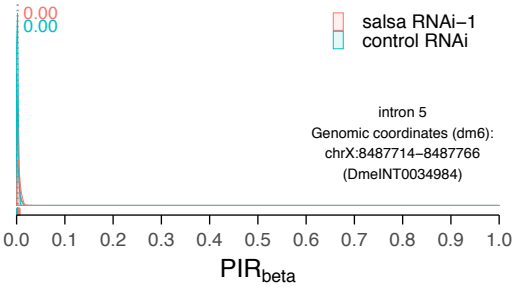
ΔPSI_{beta}=0.00, P_{diff}=0.62



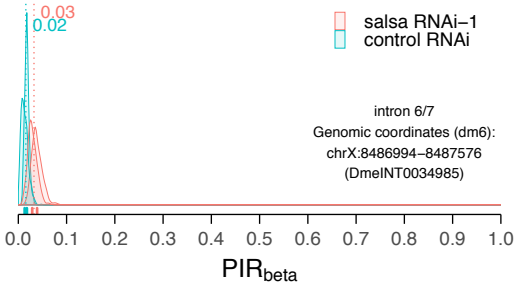
ΔPIR_{beta}=0.01, P_{diff}=0.68



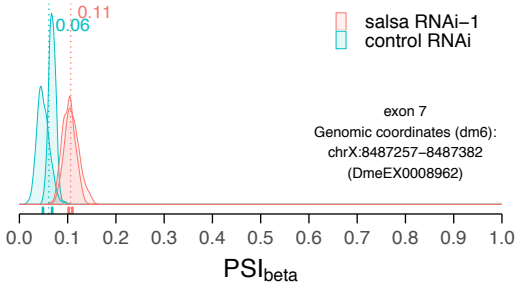
ΔPIR_{beta}=0.00, P_{diff}=0.73



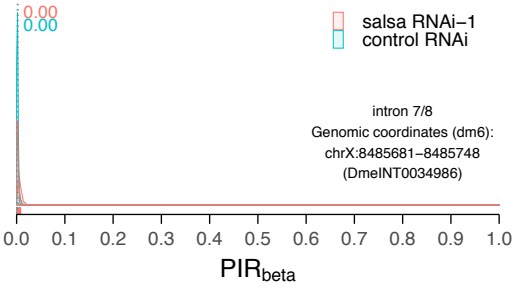
ΔPIR_{beta}=0.02, P_{diff}=0.94



ΔPSI_{beta}=0.05, P_{diff}=0.99



ΔPIR_{beta}=0.00, P_{diff}=0.70



S3 Table. List of primers

Complete list of primers	
Primer name	**Primer sequence**
Actin qRT Forward	TGGATACTCCTCCCGACACA
Actin qRT Reverse	AGTCTTTCGGTTTGGTGTCTCT
GAPDH qRT Forward	CGGCCATAGCGAAAATCGTG
GAPDH qRT Reverse	TTCTCGTGCGTCTCGTTGAT
Gurken qRT Forward	GCGCGCAACAAGACCTAAA
Gurken qRT Reverse	GTTAATCTAAAGAGCAGCAAGCG
Gurken First Intron qRT Forward	GCGTTCGTGCGACAGAAAATG
Gurken First Intron qRT Reverse	GGGGTCTAAACGATCGAGGG
Gurken Second Intron qRT Forward	AAGTTGCCGCACTAAAAACTGA
Gurken Second Intron qRT Reverse	TGTGCTGATGCTGCACAATTT
Gurken Third Intron qRT Forward	TGGAACGGATGGAACTAACGA
Gurken Third Intron qRT Reverse	CGCTGTTGGAGGCGAATAGA
Salsa qRT Forward	TATCGAAGATGCCGTCAGCC
Salsa qRT Reverse	CCACTTCGACAACGGCAAAG
Salsa_attB1_Fwd	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAAGCGAAGAAGTCAAACTAG
Salsa_ attB2_Rev (stop)	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACTAAGACTCTTCAGCTGGAGC
Salsa_ attB2_Rev (without Stop)	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGACTCTTCAGCTGGAGCC
Salsa_ RNAi -1- TS	ctagcagtCGCTTGGATATGGACGATCTAtagttatattcaagcataTAGATCGTCCATATCCAAGCGgcg
Salsa_ RNAi -1 - BS	aattcgcCGCTTGGATATGGACGATCTAtatgcttgaataataactaTAGATCGTCCATATCCAAGCGactg
Salsa_ RNAi -3- TS	ctagcagtCCACGATTATCTCCTACGCAAtagttatattcaagcataTTGCGTAGGAGATAATCGTGGgcg
Salsa_ RNAi -3 - BS	aattcgcCCACGATTATCTCCTACGCAAtatgcttgaatataactaTTGCGTAGGAGATAATCGTGGactg
pVALIUM22- Fw	GGTGATAGAGCCTGAACCAG
pVALIUM22- Rev	TAATCGTGTGTGATGCCTACC
Unspliced GRK, exon1-intron 1: forward	TATAGCAGCTCCAGTACGTC
Unspliced GRK, exon1-intron 1: reverse	CTACACACTTGCATCTCCTTG
Unspliced GRK, exon1-intron 1: probe	TTGTTTCGTGTGTGTGCGTTCGTG

spliced GRK, exon1-exon2: forward	CCATATAGCAGCTCCATAAAATCC
spliced GRK, exon1-exon2: reverse	TAAACGATCGAGGGATCGAG
spliced GRK, exon1-exon2: probe	ATGTTTCAGCTTCTGTTGCGACGC
reference GRK, exon3: forward	CGACAAGGAGACAGAGATTCAG
reference GRK, exon3: reverse	TAACGCAGAGGCAGGAATG
reference GRK, exon3: probe	TGCCCTGCAGCGAAGCTTACAATA

S4 Table. Primer efficiency and regression curve (for RT-qPCR)

No.	Name of primer	Efficiency	Regression curve
1	Actin	108	0.994
2	GAPDH	108.9	0.992
3	Gurken total	107.1	0.981
4	Gurken- First intron	109.9	0.98
5	Gurken- Second intron	105.9	0.97
6	Gurken- Third intron	99	0.98
7	Salsa	108	0.98
8	eIF4E-total	108.1	0.99
9	eIF4E-RB	108.6	0.99
10	eIF4E-RC	109.7	0.99