Supplementary Methods

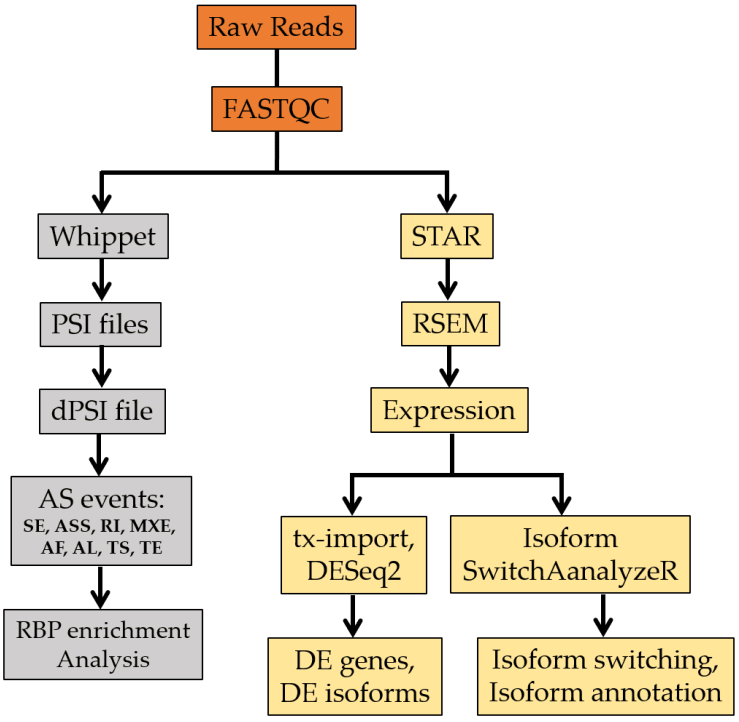

Figure S1: Flow chart showing the different bioinformatic tools and steps used in this study. The FASTQC utility was used for quality check of raw RNA-seq reads. Two pipelines were then applied: (i) shown on the left is the pipeline used for the analysis of alternative splicing changes upon DSCAM-AS1 silencing, using whippet and (ii) on the right is shown the pipeline and tools used for performing differential expression analysis at both gene and isoform levels, as well as isoform switching analysis.

Table S1: Bioinformatic programs used in this study. Their versions and references are reported.

Program	Version	Reference
FASTQC	0.11.9	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
STAR	2.5.1b	[56]
RSEM	1.3.0	[57]
tximport	1.14.0	[59]
DESeq2	1.26.0	[58]
ggplot2	3.2.1	[60]
IsoformSwitchAnalyzer	1.9.1	[27]
Whippet	0.11.1	[28]

Table S2: Alignment summary statistics of RNA-seq reads reported by STAR. The total number of reads and the mapping rates are given for each sample.

Condition	**LNA-CTRL**			**LNA-DSCAM-AS1**		
Replicate	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 1**	**Sample 2**	**Sample 3**
Total reads	36026970	29319986	40069022	30597833	24551251	40369663
uniquely mapped	32692276 (90.74%)	26479032 (90.31%)	36251447 (90.47%)	27657411 (90.39%)	22220099 (90.50%)	36776609 (91.10%)
multi-mapped	1861674 (5.17%)	1550351 (5.29%)	2113054 (5.27%)	1641748 (5.37%)	1285383 (5.24%)	1925797 (4.77%)
mapped to too many loci	16528 (0.05%)	12689 (0.04%)	20313 (0.05%)	15581 (0.05%)	11865 (0.05%)	20441 (0.05%)

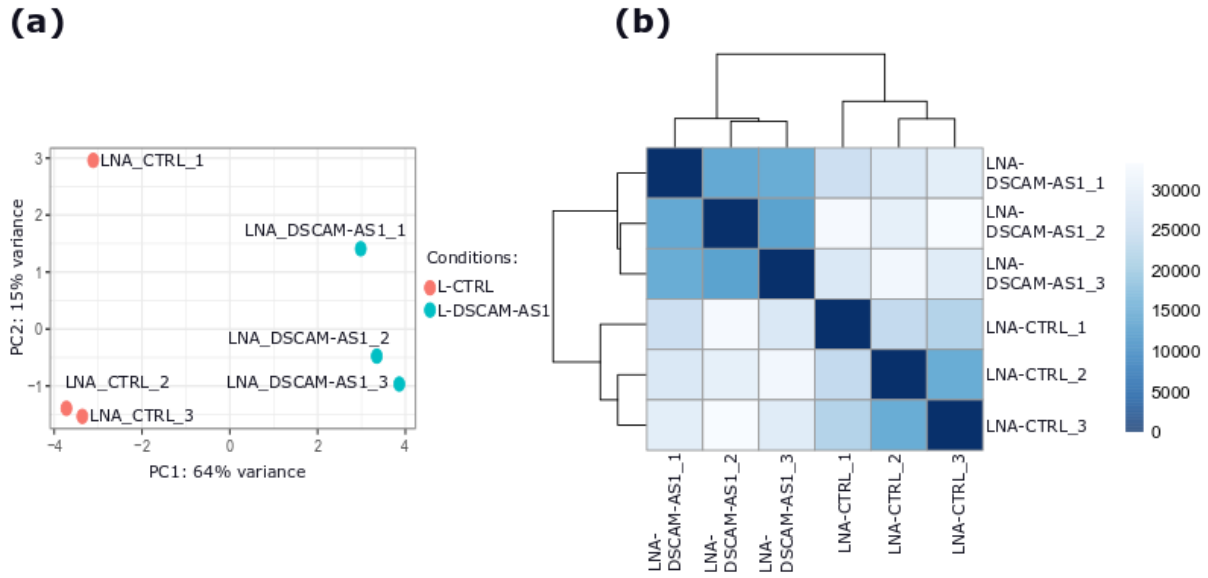

Figure S2: Quality control check of the RNA-seq dataset. **(a)**, a PCA plot showing the separation of replicates based on gene normalized read counts. **(b)**, heat map showing dissimilarity matrix between replicates using gene normalized read counts.

References:

27. Vitting-Seerup, K.; Sandelin, A. IsoformSwitchAnalyzeR: analysis of changes in genome-wide patterns of alternative splicing and its functional consequences. *Bioinformatics* **2019**, *35*, 4469–4471.
28. Sterne-Weiler, T.; Weatheritt, R.J.; Best, A.; Ha, K.C.H.; Blencowe, B.J. Whippet: an efficient method for the detection and quantification of alternative splicing reveals extensive transcriptomic complexity.
56. Dobin, A.; Davis, C.A.; Schlesinger, F.; Drenkow, J.; Zaleski, C.; Jha, S.; Batut, P.; Chaisson, M.; Gingeras, T.R. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **2013**, *29*, 15–21.
57. Li, B.; Dewey, C.N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011, *12*.
58. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550.
59. Soneson, C.; Love, M.I.; Robinson, M.D. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Res.* **2015**, *4*, 1521.
60. Wilkinson, L. ggplot2: Elegant Graphics for Data Analysis by WICKHAM, H. *Biometrics* 2011, *67*, 678–679.