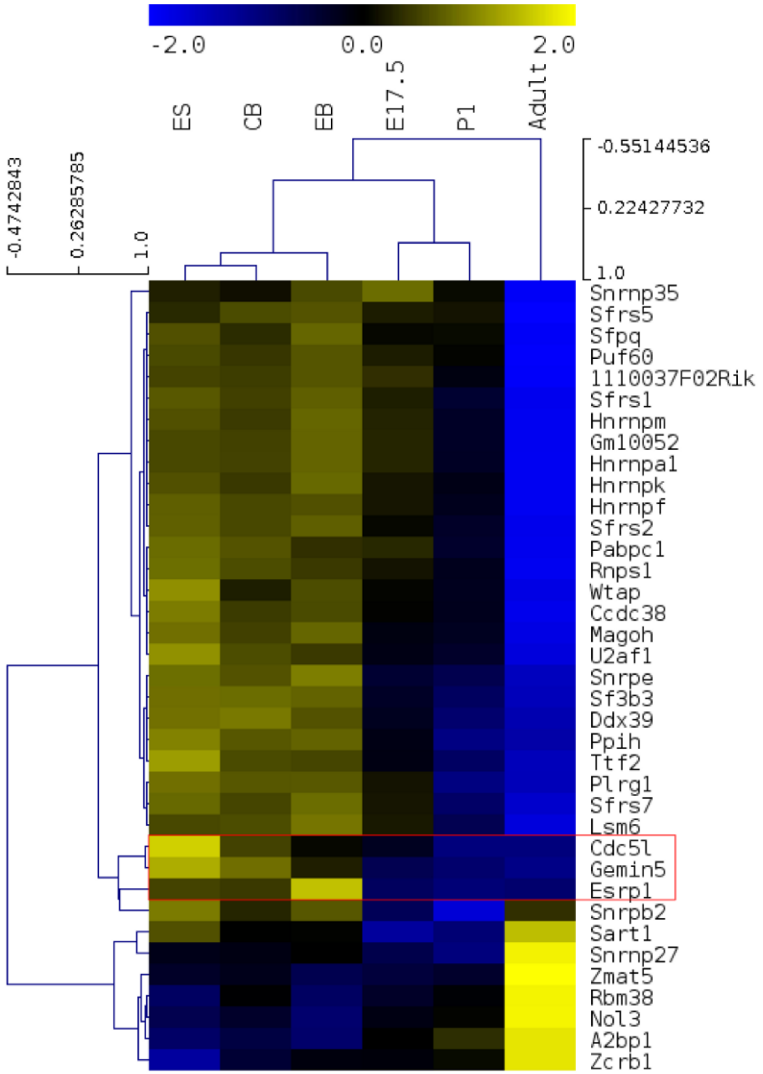
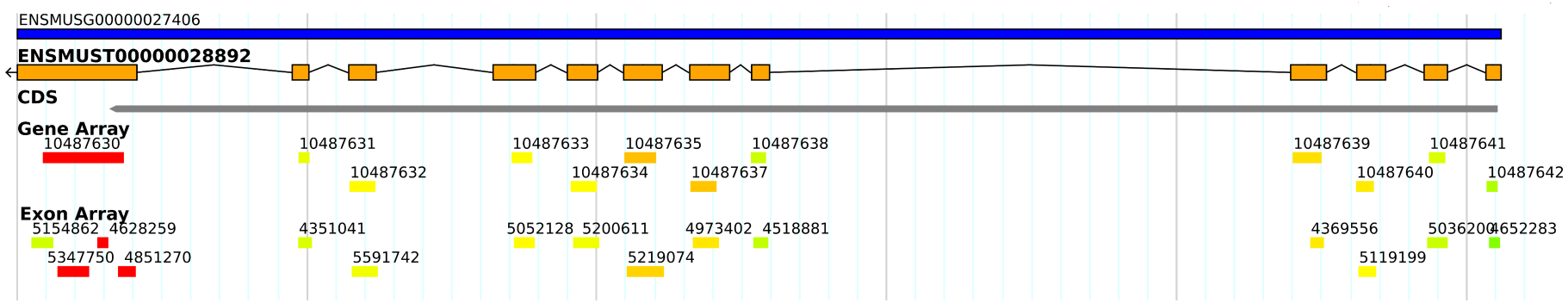


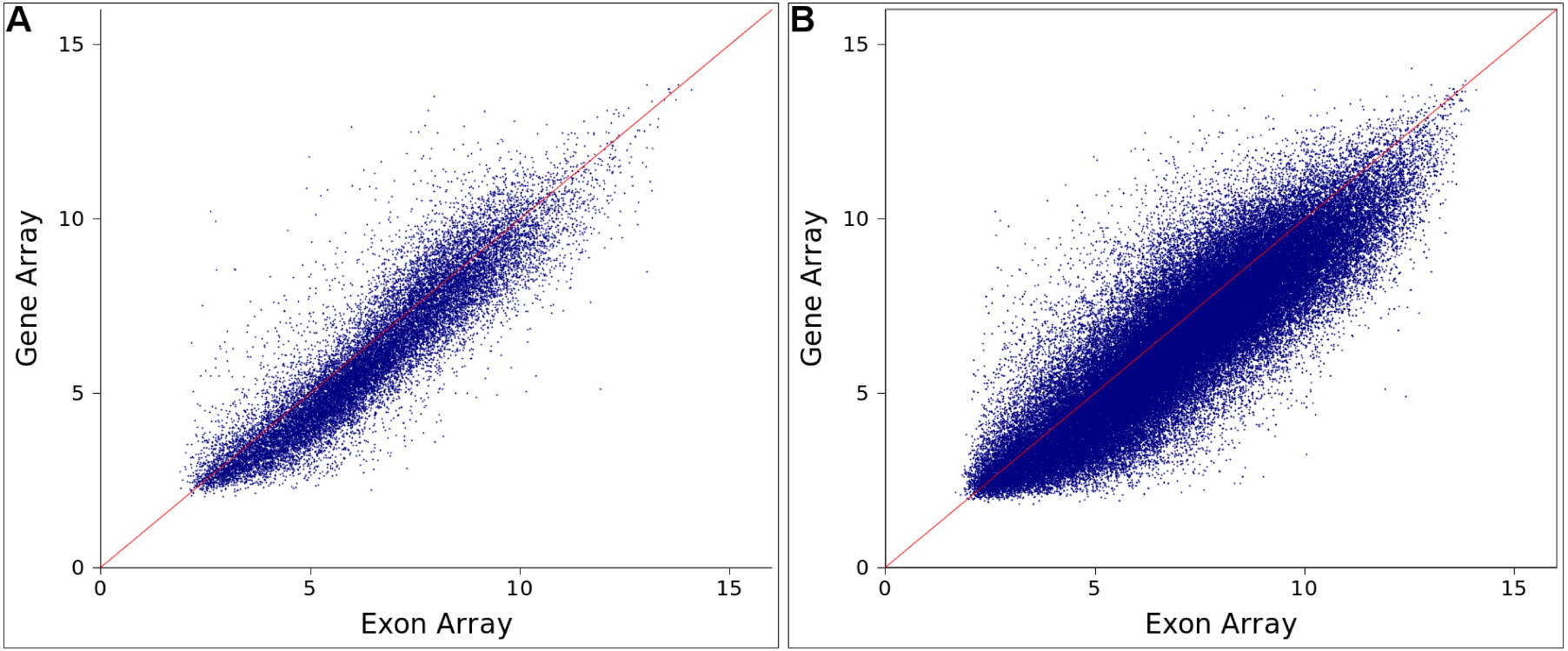
Supplementary Figure S1: Regulated splicing components

DEG (F p-value < 0.001 during the heart development or the cardiomyocyte differentiation) were selected and filtered by the Gene Ontology term GO:0008380 “RNA splicing”. We found three genes regulated during cardiomyocyte differentiation (red box) and 34 during the heart development. Gene expression signals were normalized and clustered by MeV using Pearson correlation with average linkage clustering.



Supplementary Figure S2: Probe set coverage of gene and exon arrays

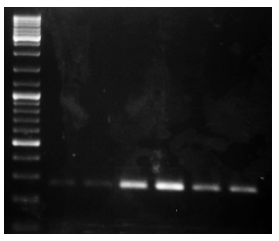
The majority of probe sets targeting well-known exons are identical on gene and exon arrays. However, in some cases, there are differences in the coverage and resolution between both array types. The lower resolution of gene arrays prevents the detection of the internal spliced exon of *Idh3b* while all other exons have similar probe sets on both arrays.

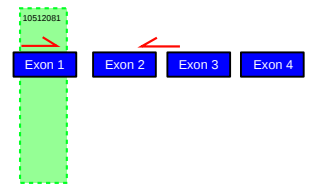


Supplementary Figure S3: Correlation between exon and gene arrays

Scatter plot of the data set for ES cells between gene and exon arrays at the gene (A) and exon (B) levels. We detected on both levels a very high correlation (0.91 and 0.87, respectively). We processed the arrays by using APT and the custom CDF files “ENSG” and “ENSE” from BrainArray (http://brainarray.mbni.med.umich.edu, release #14). These CDF files group probes in an ENSEMBL gene and ENSEMBL exon centric manner, respectively. This allows a direct comparison between the two types of arrays. Only ENSEMBL genes (n=21,094) and exons (n=125,768) present on both arrays were retained. The mean log2 values of the biological replicates were used. Red lines indicate the x-y diagonal.

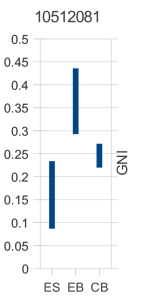
Acer2 (10505781)



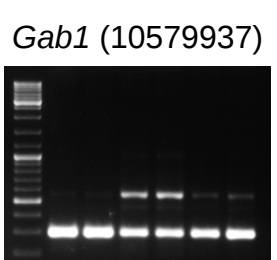


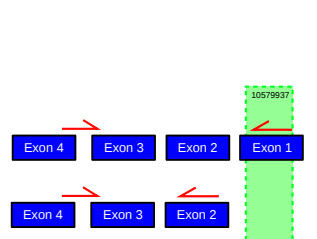
ENSMUST00000045224

Alternative promotor



Add3 (10463927)

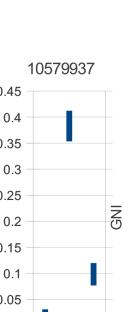




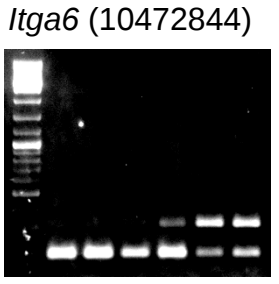
ENSMUST00000025999

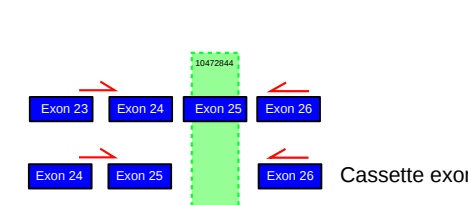
ENSMUST00000050096

Cassette exon



Arid5b (10369803)

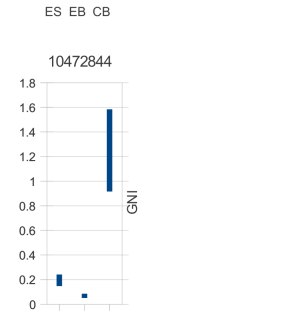




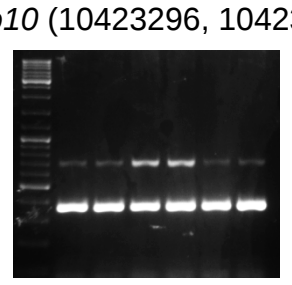
ENSMUST00000020106

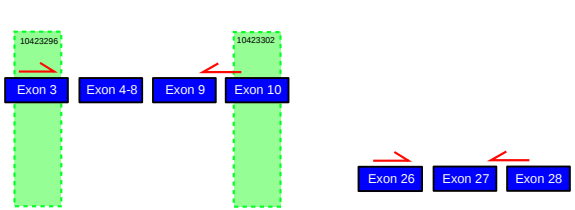
ENSMUST00000165054

Alternative promotor



Capzb (10509631)



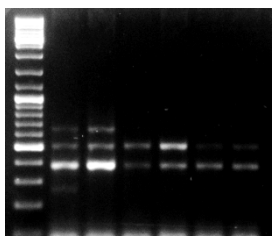


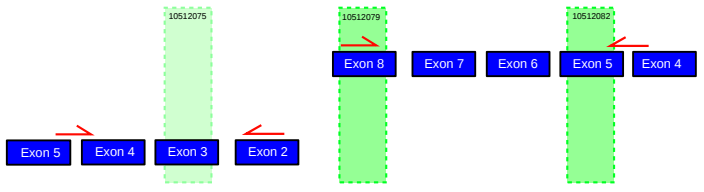
ENSMUST00000102507

ENSMUST00000042675

Cassette exon

Ddx58 (10512079, 82)





ENSMUST00000127026

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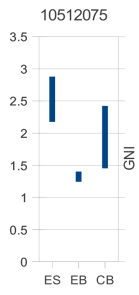
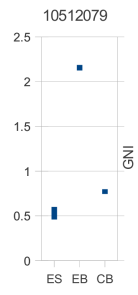
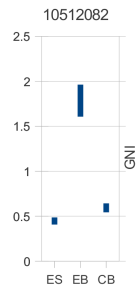






Gab1 (10579937)

ENSMUST00000034150

Alternative promotor

Itga6 (10472844)

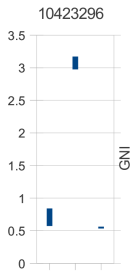
ENSMUST00000028522

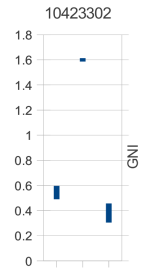
ENSMUST00000112101

Cassette exon

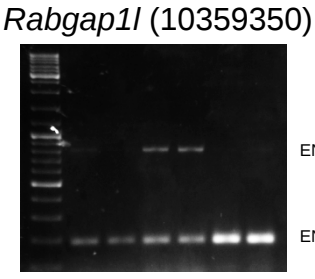
Myo10 (10423296, 10423302)

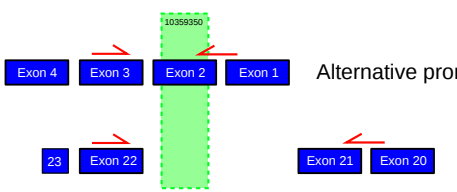
ENSMUST00000110457





Rabgap1l (10359350)

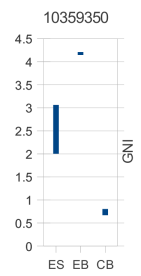




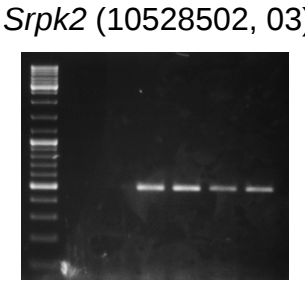
ENSMUST00000086099

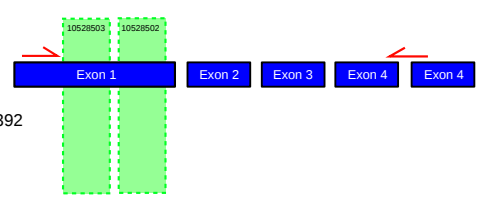
ENSMUST00000111640

Alternative promotor



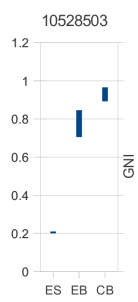
Srpk2 (10528502, 03)

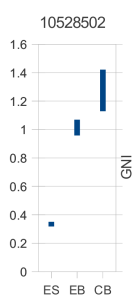




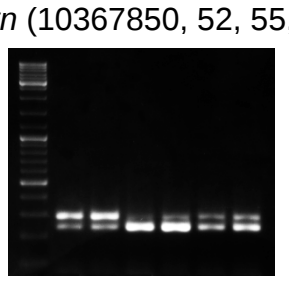
ENSMUST00000088392

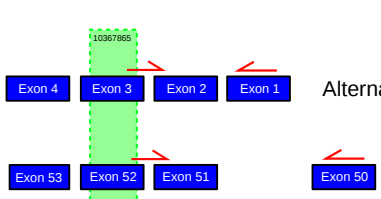
Alternative promotor





Utrn (10367850, 52, 55, 65)

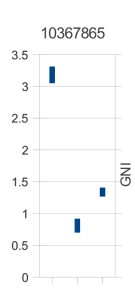




ENSMUST00000064997

ENSMUST00000076817

Alternative promotor



Supplementary Figure S4: RT-PCR results

RT-PCR results of 11 splice events. Red arraws indicate used primers, and green boxes regulated probe sets. Light green boxes represent probe sets that are not significantly regulated, but have a high SI which helped to identify the expressed isoforms. Box plots on the right show GNIs of regulated probe sets.

Supplementary Table S3: Comparison of validated exon array splice events with gene arrays.

Gene Symbol	Validated splice event identified by exon arrays	Observation with gene arrays	UCSC	AceView
Idh3b	Internal splice sites in 3' UTR	Alternative 3' exon	-	Cassette exon, alternative 3', intron retention
Kif1b	Internal 3' splice site and cassette exon	Same as exon array	Alternative 3', cassette exon	Alternative 3', cassette exon
Ldb3	Cassette exon	Gene filtered (gene not significantly expressed in non-heart group)	-	-
Mfn2	Cassette exon	Same as exon array	Cassette exon	Cassette exon
Myom1	Cassette exon	Probe set filtered (very high SI suggesting a false positive)	-	-
Slc8a1	Cassette exons	Not significant p-value, but high SI	-	Cassette exon, double cassette exon
Svil	Cassette exon	Same as exon array (only 1 probe set instead of 2)	Cassette exon	-
Trip10	Cassette exon	Same as exon array	Cassette exon	Cassette exon

Eight validated heart-specific splice events were identified by exon arrays compared to a similar analysis with gene arrays. In four cases, both chip types identified exactly the same splice events. The splice events of *Idh3b* were not detected correctly due to the lack of sufficient probe sets. Heart-enriched exons of *Slc8a1* were identified with a high SI on both chip types but no significant p-values were recorded on gene arrays. No splice events were detected for *Ldb3* and *Myom1* because both genes were filtered out by the GAA.

Supplementary Table S6: RT-PCR primer pairs

Gene	Forward primer	Reverse primer
Acer2	GAGAAGCTGCTCCGATGCT	CAGATCCAATCCCCACTACAA
Add3	CTTCGACCATGCAGTTTGAC	GGAGGTGAAGCTCTTGGAAA
Arid5b	CCACACCAAGACCAGACTCC	GCCTCTCCTTTCACCTTGCT
	GAAACCAACGTGATTGTGCT	GCCTCTCCTTTCACCTTGCT
Capzb	CCAGACAGATGGAGAAAGACG	CCTCCACCAGGTCGTTCTTA
Ddx58	GATAAAGGTTTCAAAAGGGCTGA	GATGGCTCCGTTGTTGAGAT
	CACAAAGCGTGCTCAGTGTT	CCATTTCTTCAGAGCATCCA
Gab1	CCTCTTCAGTCCGCGAATC	GGATCTTCTTCTGTGGGATTG
	CGTGGAAGAGAAGGTGGTTT	GGATCTTCTTCTGTGGGATTG
Itga6	GGCACTCAGGTTCGAGTGA	GGTATCGGGGAATGCTGTC
Myo10	GGACTTCGACTCCAGGTTTG	GCTTGTGTTGTCTATGATCTCCTT
	GGGCTCCATCATGTACAACC	GACTTCCATTGCCGTGATAA
Rabgap1l	GGAATTCAAAAGAATTACTCAGAAGG	CCGCATCGCCTGATATTC
	GCTCTTCTAAAGACCTCAAAGGA	CCGCATCGCCTGATATTC
Srpk2	GGAGCCGTTTCCCTTTATAGC	CCTTGCATATCCCAGCACA
Utrn	GGAGAATGCTCTTCAGGACAG	CCAAATGGGCCCTGATGCTA
	CTTAAAATGATGGCGGTGGT	CCAAATGGGCCCTGATGCTA