Supplementary Figure S1

Algorithm for genome-wide prediction of Nova’s action.

- Definition of YCAY clusters (SELEX, crystallography, biochemistry, CLIP).
- Define YCAY cluster scoring scheme (number, inter-motif distance, conservation).
- RNA map: relating cluster score to position (Nova splicing silencer vs. enhancer).

The RNA map relates to the mechanisms of Nova’s action \textit{in vitro}.

- Nova regulates assembly of the early spliceosomal complex.

The RNA map predicts the location of Nova’s action \textit{in vivo}.

- Genome-wide prediction of Nova-dependent splicing regulation.
- Validation (wt/ko brain): Nova action correctly predicted in 30/30 exons.
- Asymmetric Nova action on splicing intermediates in 19/30 tested pre-mRNA targets.
- Position of YCAY clusters predicts asymmetric Nova action in 19/19 pre-mRNAs.
Supplementary Figure S2

A

B

2.3 fold, p=0.002
2.9 fold, p<0.001
3.6 fold, p=0.005

average YCAY frequency

position

constitutive E

alternative E

constitutive E

alternative E

Nova +
Nova -
control
Nova + shuffle
Nova - shuffle
Supplementary Figure S3

a  Distribution of YCAY cluster widths.

b  Distribution of inter-YCAY distance within YCAY clusters.

c  Conservation of the YCAY clusters in human orthologous sequences.
Supplementary Figure S5

A

YCAV cluster score

St7, Dnajb5, Cacna1b, Agrn, APLP2, Lrp1b, Epb4.1

NISE2

NISE3

B

YCAV cluster score

St7, Dnajb5, Cacna1b, AGRN

NISE1

NISE2

NISE3

C

YCAV cluster score

Atp2b1, Gabrg2

NISE1

NISE2

NISE3

D

YCAV cluster score

NISS1, NESS

NISS2, NESS

NISS3

E

YCAV cluster score

NISS1, NESS

NISS2, NESS

NISS3

F

YCAV cluster score

NISS1, NESS

NISS2, NESS

NISS3
Supplementary Figure S7

a (NISS2 alignments)

**YCAV clusters in purple**

Putative silenced branch sites labeled by blue rectangles

Dab1 (NISS2 elements upstream of exons 7b and 7c, which are labeled in blue)

```
mouse
human
opossum
chick
cons.
```
Supplementary Figure S7

b (NISE1 alignments)

**YCAV clusters in purple**

Putative enhanced branch sites labeled by red rectangles

**Ank3 E3a/b (NISS2/NISE1)**

(the alternative part of the exon is in blue)

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**Brd9 (NISE1)**

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Supplementary Figure S7

c (NISE3 alignments)

Putative branch site labeled by black rectangles
Second putative branch site labeled by blue rectangles

Gabrg2 (NISE3)

mouse 1 TCATTTCGATCGATCTATCTATGATGATTTGGAAGTACTGTTATA 53
human 1 CCATTTCGATCGATCTACTGATGATTTGGAANACACGCTTAGA 53
opossum 1 TCATTTCGATCGATCTACTGATGATTTGGAANACACGCTTAGA 53

***** * ** ******* * *****   *   *** **   *
mouse 54 TAGCTCCAGTATAACTAACTTTGCTCTCTCCTCTACCTACCTT-GTCTCAG 104
human 54 TCTCTCGAGAACAACTAACT--GATCCCTCTCCTCTTACCCTCGTCGCCAG 103
opossum 53 ACTGCGTGGATCGATCAATTCTTT----CCTTCCCTTTTTATTTTATTCTTAG 100

Clstn1 (NISE3)

mouse 1 TGTCCATCCATCAAGCCACCACCACTGGTAACACGGCCATGAGACATAACCAG 54
human 1 TGTCCATCCACTGAGCTGCCAGCACCGGTAACACCGTGTTGAGTGAGAAGTGAG 54
opossum 1 TGCGACACATCTCAGAAACACCTATCACCTCATCCTCCATT--ACAGCTTATT 52

**  **  **        **  **     *****     *     * **    *
mouse 55 AG-CCTGCTGGGCCGCAAGCCCAACGCAACTCCTGCTTCTCTCTCCCT--CCCTAAG 105
human 55 TTGGGCGATGCTGGGTTGCACGCCTGACTTCGCCTTTTCTCCCCTGACCAAG 108
opossum 49 C-GACATAGTCAAGGATGTA---TTAACTTCTTATTT-CTTATTT--CTCAAAG 95

Skip (NISE3)

mouse 1 CATGTTTGTAGCTCAGAAACACCTATCACCTCAGTCTCCATCCTCCAT--ACAGCTTAT 52
human 1 GAAGCTTTTAGATCAGTAAGACCCATCGCCTCATCTTTCATTT-ACAGCTTATT 53
opossum 1 CAAAAAAGTAGAAGAGAA-ATTATATCATCTCAGTCTCCATCAGAATATCACAAT 52

***      ********* * **  *    *******      ** * ****
mouse 53 CTCTAAAACGATTTCAACATATATCT--GTCTTTTT-----CTCCCCAAAG 96
human 54 CTTCTAAAATGTACACAGATATATCT--GTCTTTTT-----CTCCCCAAAG 96
opossum 53 ATCTCAGGATTTACACACGACATGTCTTTATTATTATTTGCTGCTAAG 105

***      ********* * **  *    *******      ** * ****
Supplementary Figure S8

**Falz E4-intron-E5**

```
CAGCAACACAAGGAGCTAAGGAAGCAACGCCACAGAGTTTGAAGCTCACCTTCGCCAGA
TCATGCTGTCGCCATCCCTCATCTCCCTCTCACATGTTTCAGTCAGGTTGTCACATT
CTGTGTAGCCACTCTGTCAACCGGCACCACGTAGTCTCTCCTGCACAGCACACCACC
CACCTGCTTCTTTGGAAGGGGTGTTTCATGCTTCTCTTCATCCTTTCTAAAGCAGCTGT
```

**Plc13 E22a-intron-E23**

```
CTGTGTAGTACAGATCTGAATAGGAAGCAGCGCAAGGTAATCCCTCACCTTGAAAGCATA
GTAACATCATGACATTATCTGGGAAATATATGATAATATATATATATATATATATATACT
ATATTTATATAGATAGGTATATTTAGTATATTTATATATATATATATATATATATATATA
AAATGTCGATAAAACTTCTCAGACATAAGCATGATGAAATGAAATGAAATGAAATGAAATGAA
ATATTTTCTCTCTGCTGTAACAGTGTAAAGATACTGCATGAAAAAGAAAAACAAACT
AAACTGGAACACTGTAACAAAAAAATATTAAAATTGTTATGTTATGTTATGTTATGTTATG
```

**Lrp1b E79-intron-E80**

```
GCACCGCCCCTCTACACAATCTGATTTGGAAGAACACACTAATTTCTCGGGGTGAGAGA
ACACGCCATATCTTTTCTTTTCATCCATCCCATTTCCATACCCCTCCATACCAGACCCACT
CTGACTTGGACAGAGCTTGGCCTTATCATGTTTAAATATAACTGAGTTTGGCAAAATATAG
CATTAAAGTCTGACTTTTCTGCCACTTTTCTGCTATAGATGAAATGAAATGAAATGAAATGAA
ATATTTTCTCTCTGCTGTAACAGTGTAAAGATACTGCATGAAAAAGAAAAACAAACT
AAACTGGAACACTGTAACAAAAAAATATTAAAATTGTTATGTTATGTTATGTTATGTTATG
```

```
A  Analysis of the Nova-regulated exon sizes.

B  Analysis of the CACCA motif prevalence in NESS elements.

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Supplementary Figure S9
Supplementary Figure S10

(a) 

Maximum Entropy (MaxENT) score

- Constitutive exons
- Nova-regulated alternative exons

3' splice site 5' splice site

(b) 

RESCUE-ESE and FAS-ESS scores

- Constitutive exons
- Nova-regulated alternative exons

ES enhancers ES silencers

(c) 

Conserved alternative exon prediction by ACEScan

- Constitutive exons
- Nova-regulated alternative exons
Supplementary Figure S11

- Ube3a, E1a [YCAy] = -0.1
- Fuca2, E6a [YCAy] = 0.1
- Fubp1, E20 [YCAy] = -0.8
- Sdfr1, E1a [YCAy] = -0.5
- Ablim3, E15a [YCAy] = 0.8
- Ptbp1, E7 [YCAy] = 0.1
- Opl, E2a [YCAy] = -0.7
- Eif4g2, E15 [YCAy] = -0.8
- Abcc5, E10 [YCAy] = -1.0
- 2310030N02Rik, E4, [YCAy] = 0
Supplementary Figure S12

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Mock       ∆U2      ∆U1

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**j**

- rRNA 28S (4200nt)
- rRNA 18S (2300nt)
- U2 snRNA (189nt)
- U1 snRNA (165nt)
- U4 snRNA (146nt)
- rRNA 5S (121nt)
- U5 snRNA (117nt)
- U6 snRNA (107nt)
- tRNA (70-90nt)

Ethidium Bromide

Northern Blot
Supplementary Figure S13

(a) Diagram showing the relative sizes of L and S with Nova 0.06

(b) Graph showing the ratio L/S with Nova µM values 0 and 0.06

(c) Gel with bands labeled E-I-E-YCAY-E and Nova 0.06

(d) Table showing Nova µM 0 and 0.12 with TIME (min) 0, 5, 30, 60
Supplementary Figure S13

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### Diagram: E-YCAY-E
Supplementary Figure S14

- **WT dKO WT dKO**
- **NISE1**
- **Ddr1 E13a**
- **WT dKO**
- **6 fold**
- **wt ko**
- **wt ko**
- **4 fold**
- **wt ko**
- **6 fold**

Graphs showing the expression levels of NISE1, Ddr1 E13a, and Smarcc2 E16a in wild-type (wt) and knockout (ko) conditions.
Supplementary Figure S14 cont.

b

NISE2

WT dKO Aplp2 E14

WT dKO

10 fold

fraction

wt ko

NISE2

Aplp2 E12a

wt ko

NISE2

Bcas1 E9

wt ko

NISE2

9 fold

>50 fold

39 fold
Supplementary Figure S14 cont.

**b cont.**

![Graph showing expression levels of NISE2 and Plcl3 E22a genes in WT and dKO conditions.](image)

![Graph showing expression levels of Cacna1b E24a gene in WT and dKO conditions.](image)
Supplementary Figure S14 cont.

b cont.

**NISE2**

**Ptprf E6a**

**St7 E7**
Supplementary Figure S14 cont.

**c**

NISE2, NISE3

NISE2 & NISE3

Camk2g E13a

WT  dKO

WT  dKO

Agrn E31a

WT  dKO

WT  dKO

NISE2 & NISE3

Camk2g E13a

NISE2 & NISE3

Agrn E31a
Supplementary Figure S14 cont.

c cont.

NISE2, NISE3

Clsn1 E10

NISE2 & NISE3

NISE2 & NISE3

Cc64 C8

Epb4.1 E14

NISE2 & NISE3

>50 fold

>50 fold

>50 fold

>50 fold
Supplementary Figure S14 cont.

NISE2 & NISE3

NISE2, NISE3

Epb4.1l2 E12b

wt

ko

>50 fold

fraction

0.5

1

wt

ko

0

17 fold

>50 fold

fraction

0.5

1

wt

ko

0

0.5

1

wt

ko

>50 fold

Golga4 E22a

NISE2 & NISE3

wt

ko

11 fold

NISE2 & NISE3

wt

ko

5'

3'
Supplementary Figure S14 cont.

c cont.
Supplementary Figure S14 cont.

**c cont.**

![Diagram showing NISE2, NISE3 and their expression levels](image)

![Western blot images showing expression levels of Ptprf E19 and NISE2 & NISE3](image)

**Ptprf E19**

- 5' expression levels: wt > ko
- 3' expression levels: wt < ko

**NISE2 & NISE3**

- 5' expression levels: >50 fold greater in wt compared to ko
- 3' expression levels: wt = ko
Supplementary Figure S14 cont.

**d**  
![Supplementary Figure S14 d](image-url)
Supplementary Figure S14 cont.

**e**

NESS1

wt

ko

fraction

0

0.5

1

5 fold

wt

ko

2 fold

wt

ko

Neog E27

NESS1

3 fold

WT dKO

WT dKO

5 fold

wt

ko

0

0.5

1

3 fold

wt

ko

Stxbp2 E9
Supplementary Figure S14 cont.

**g**

![Diagram showing gene expression and fraction plots for WT dKO and NISS2 NESS2 combinations.](image)

- **NISS2** and **NESS2** gene expression levels are depicted with bars and fraction plots for WT and dKO conditions.
- The fraction plots show normalized expression levels for WT and dKO conditions, with 3 fold and 2 fold increases indicated.
- The 2x NISS2 bar chart indicates 7 fold and >50 fold increases.
- The 16 fold increase is highlighted in the 2x NISS2 condition.
Supplementary Figure S14 cont.

h

NISS1

NISE2/NISS1

WT  dKO  CP110 E14, E14a  WT  dKO

E14  E14a

NISS1 Itga3 E25

NISS1 Rap1ga1 E27

Itga3 E25

Rap1ga1 E27
Supplementary Figure S14 cont.

- **NISS1 (n=3)**
- **NISS2 or NESS1 (n=7)**
- **NISE1 (n=2)**
- **NISE2/3 (n=16)**
- **NESS2 (n=4)**