Phenotypic Screening

nature chemical biology

ARTICLE

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SMN2 splice modulators enhance U1–pre-mRNA association and rescue SMA mice

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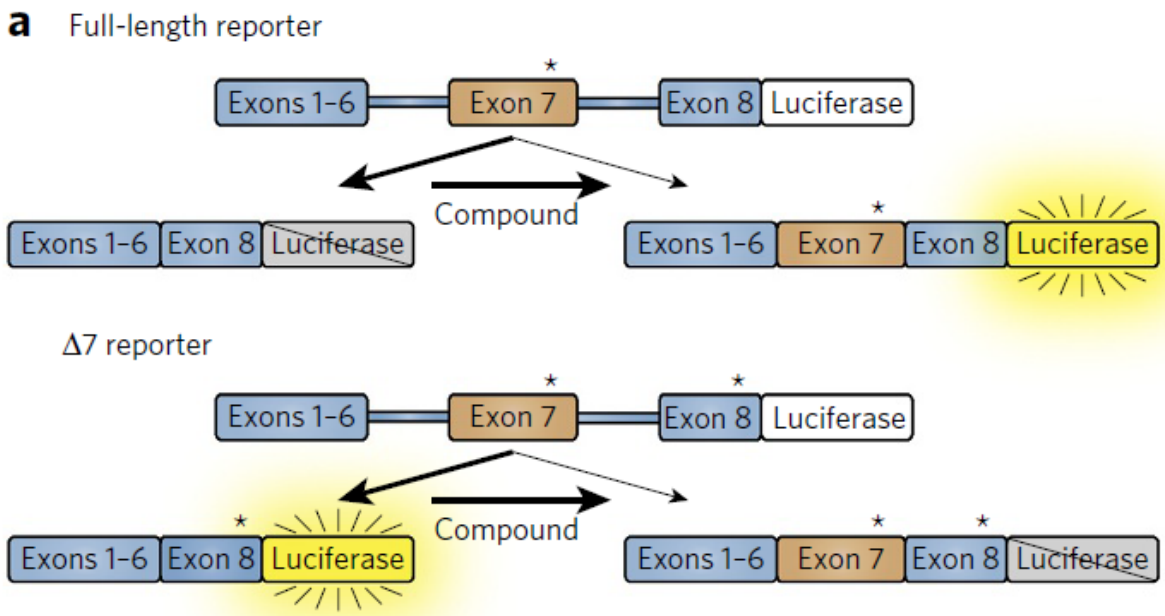
Spinal muscular atrophy (SMA), which results from the loss of expression of the survival of motor neuron-1 (*SMN1*) gene, represents the most common genetic cause of pediatric mortality. A duplicate copy (*SMN2*) is inefficiently spliced, producing a truncated and unstable protein. We describe herein a potent, orally active, small-molecule enhancer of *SMN2* splicing that elevates full-length SMN protein and extends survival in a severe SMA mouse model. We demonstrate that the molecular mechanism of action is via stabilization of the transient double-strand RNA structure formed by the *SMN2* pre-mRNA and U1 small nuclear ribonucleic protein (snRNP) complex. The binding affinity of U1 snRNP to the 5′ splice site is increased in a sequence-

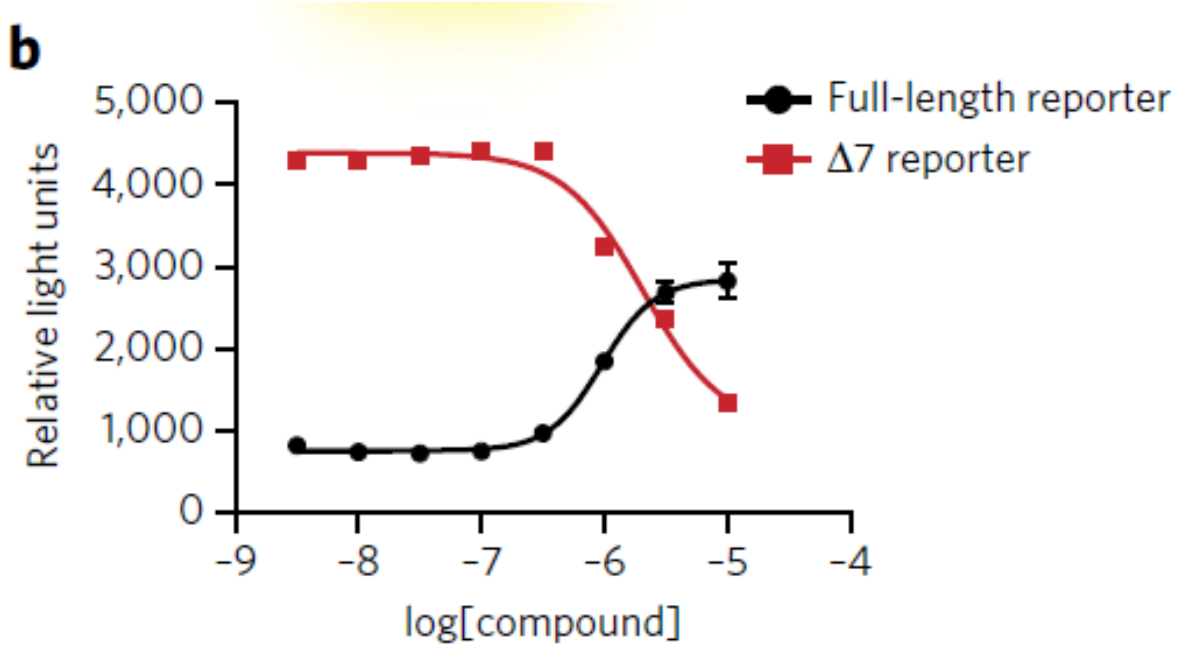
Relevant for exam: Figures 1 and 2

Figure 1a



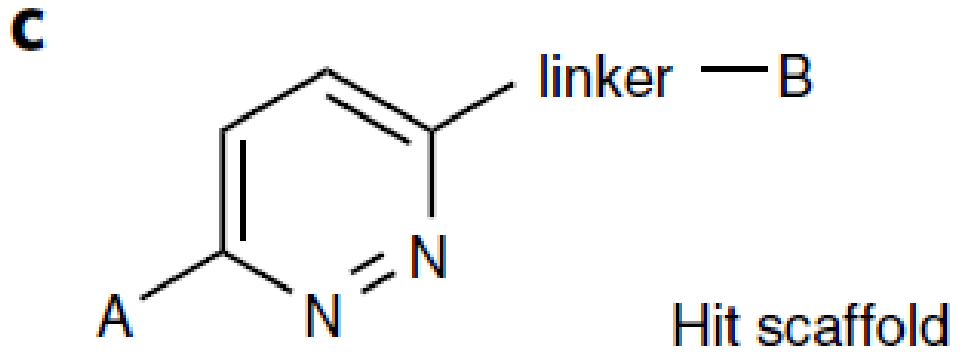
- What is an exon and an intron?
- Why is Exon 7 not efficiently included in SMN2?
- Why luciferase?
- Why two assays?

Figure 1b



- Unit on X-axis?
- Why is there luciferase activity at low compound concentration? (full-lenght reporter)

Figure 1c

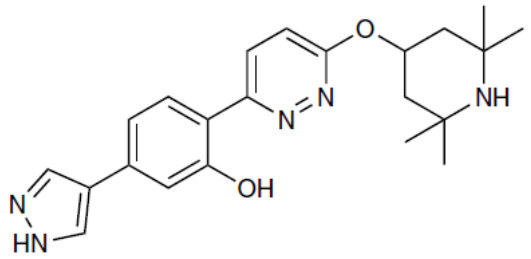


- What is «A» and «B»
- How many compounds were screened?
- At which concentration were compounds screened?

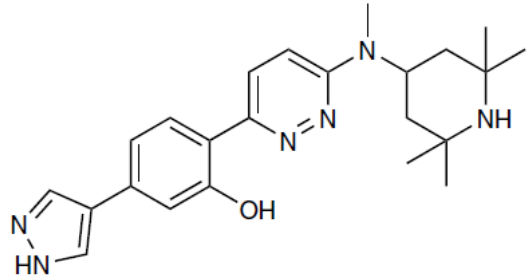
Figure 1d

d

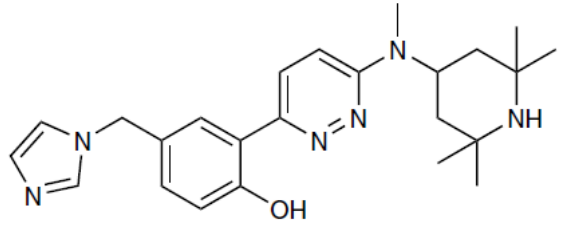
NVS-SM1
ELISA EC_{50}: 20 nM



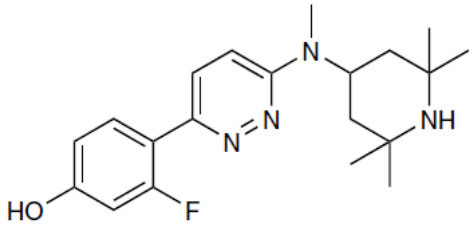
NVS-SM2
ELISA EC_{50}: 5 nM



NVS-SM3
ELISA EC_{50}: >10 µM

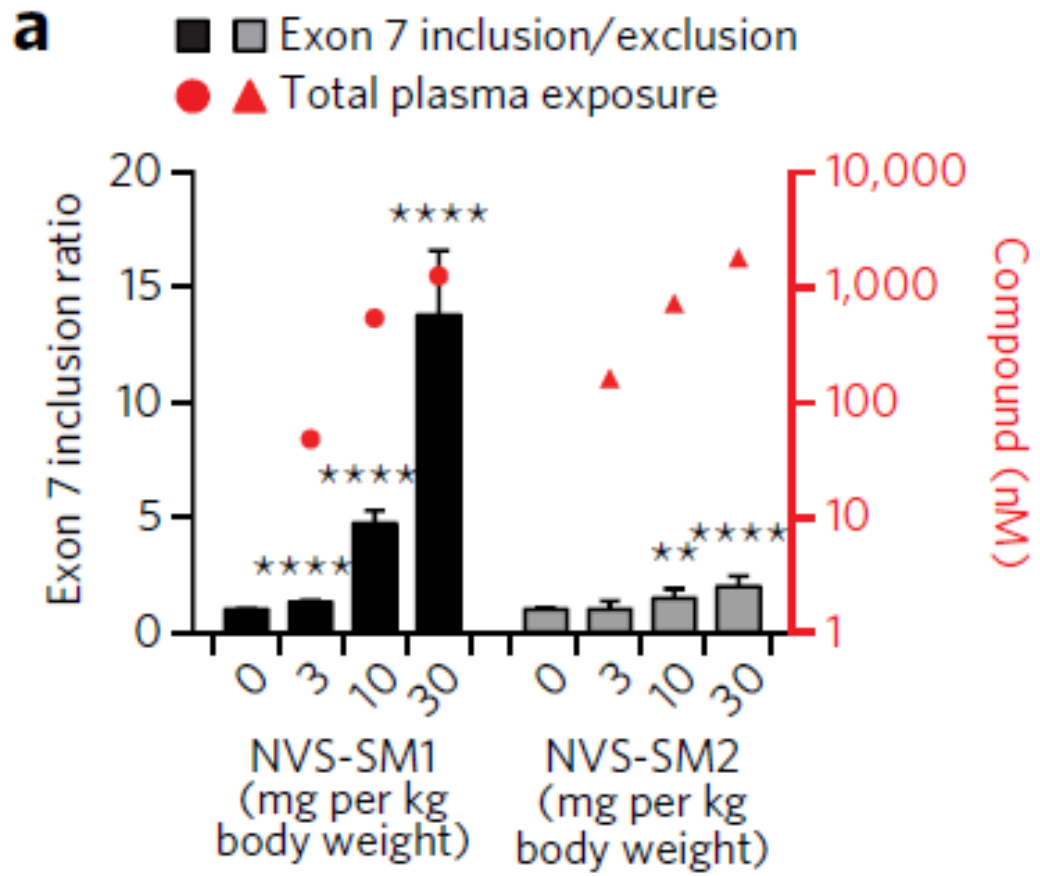


NVS-SM4
ELISA EC_{50}: >10 µM



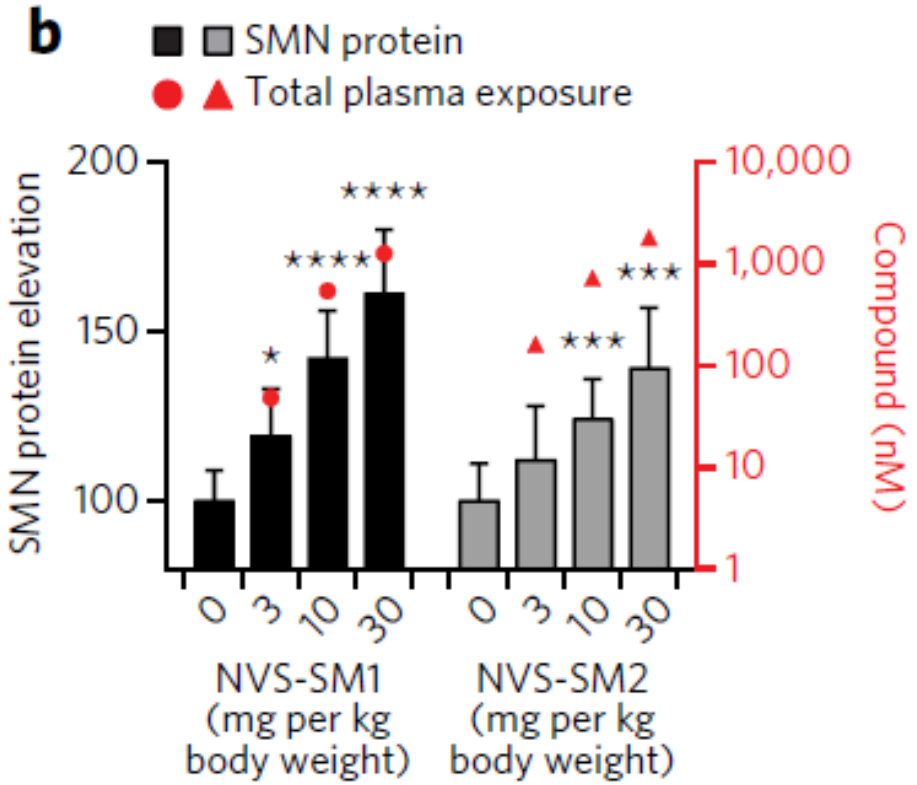
- What is «EC_{50} ELISA»
- Which compounds are active?
- Why were inactive compounds developed?

Figure 2a



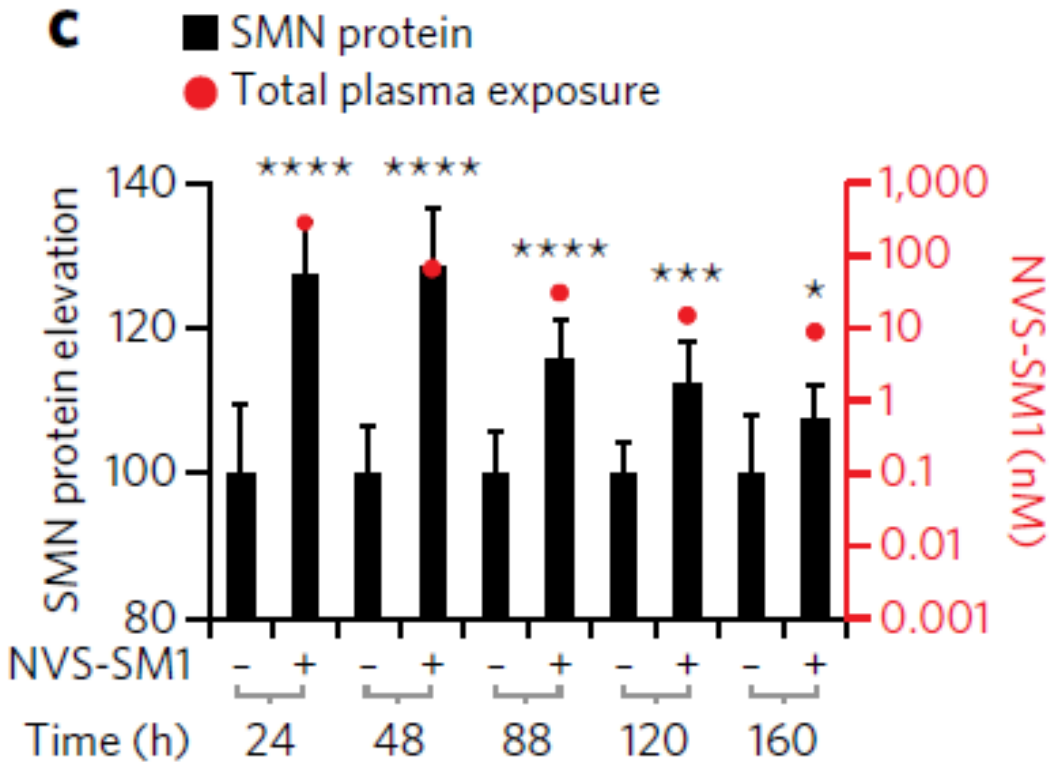
- Which type of animal experiment was performed here?
- What is the difference between «black» and «gray» bars?
- What is «plasma exposure»?
- How is «exon 7 inclusion ratio» measured experimentally?
- How much is this ratio without treatment?

Figure 2b



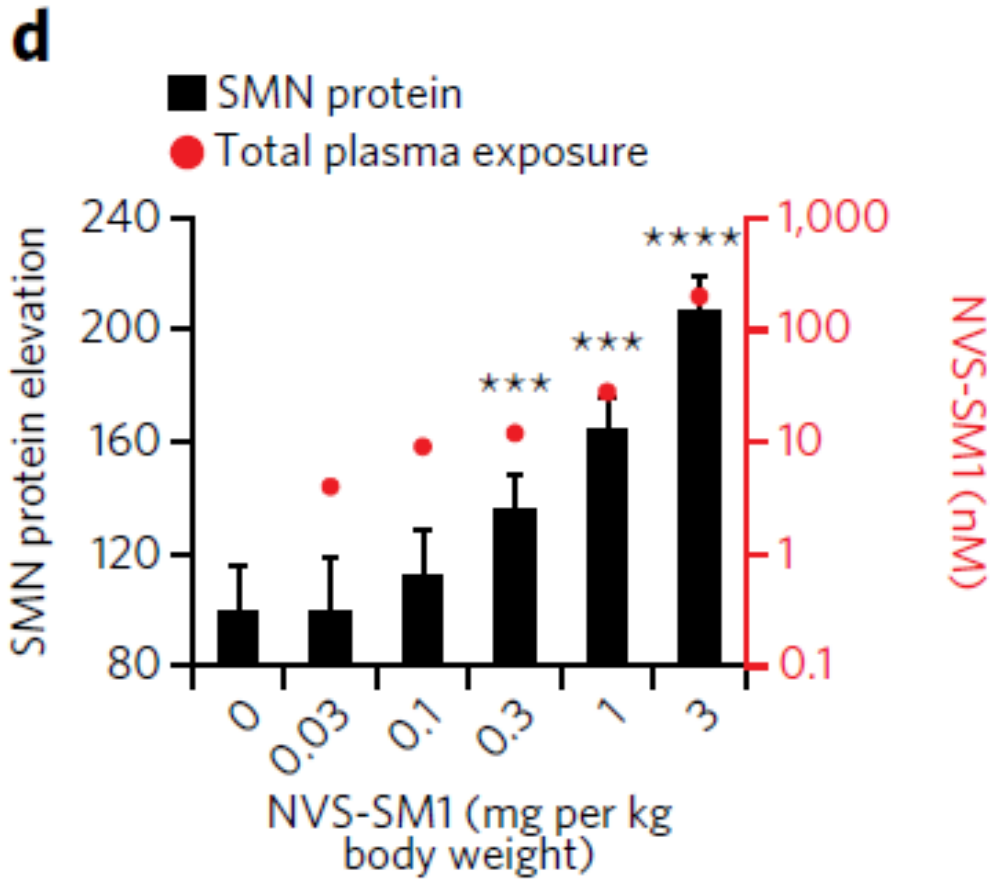
- What is different compared to Fig. 2a?
- How much does survival of motor neuron-1 conc. change maximally?

Figure 2c



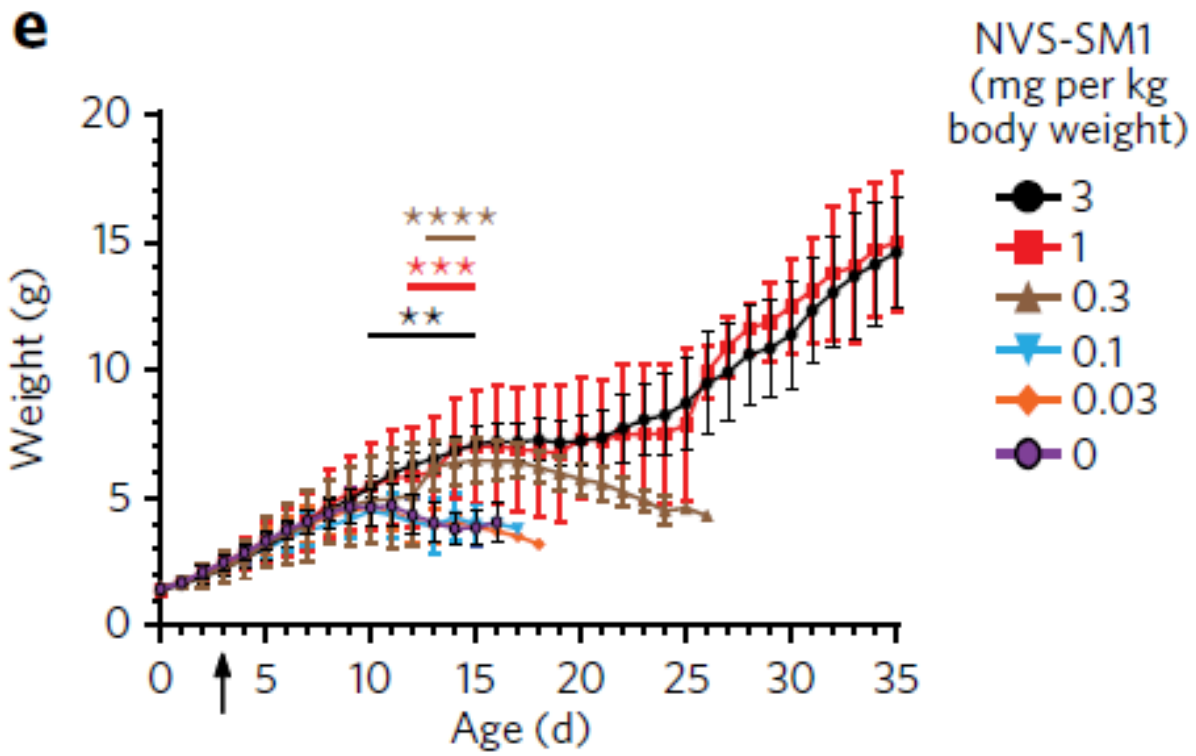
- Why was this experiment performed?
- Which concentration of NVS-SM1 is found after 6 days?

Figure 2d



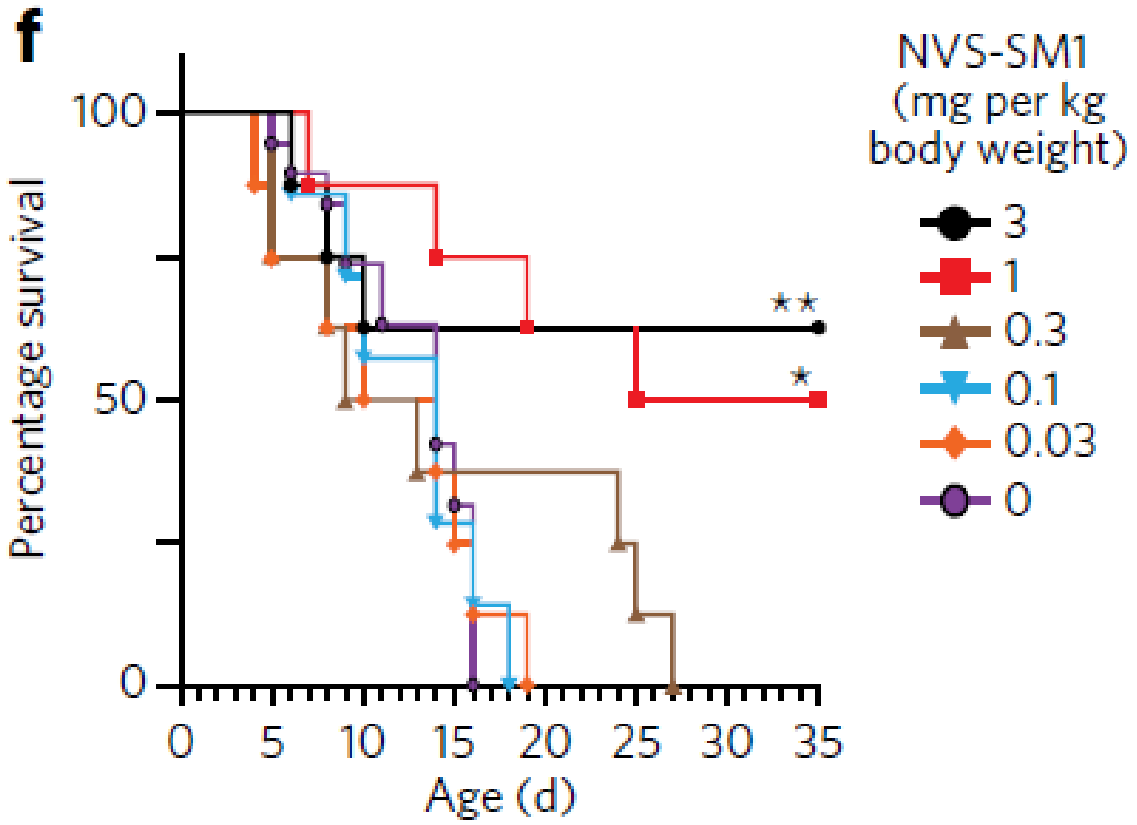
- Which animal model was used here?
- Are mice treated with more or less compound (dose) than in Fig. 2a?
- Is effect of NVS-SM1 better/worse than in Fig. 2a? Why?

Figure 2e



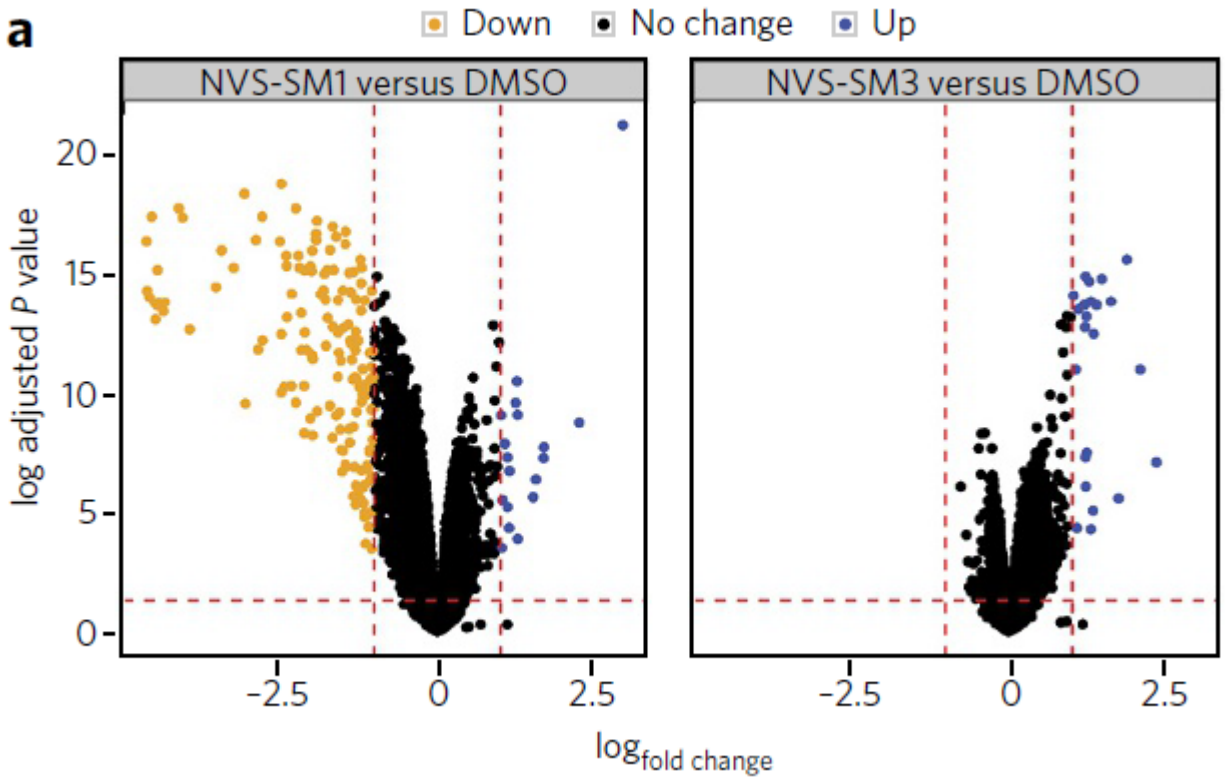
- Design of experiment?
- Which is the lowest conc. of compound needed to see a beneficial effect?
- How many mice are dying in treated groups (%)?
- Why are curves of 1 or 3 mg/kg groups getting flat before increasing again?

Figure 2f



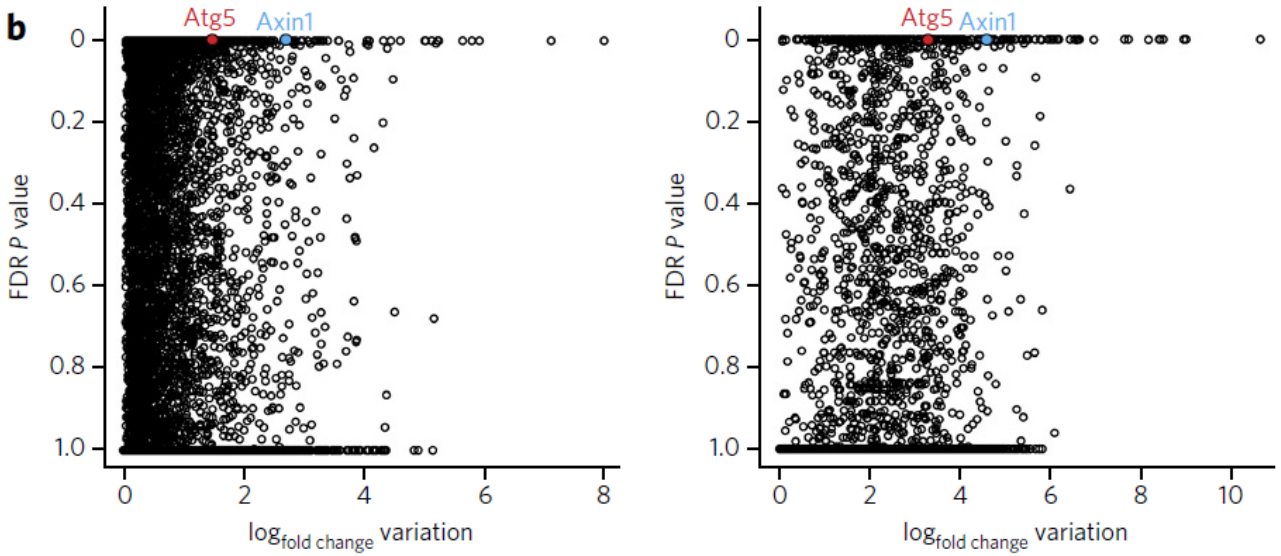
- How many mice are dying in treated groups (%)?

Figure 3a



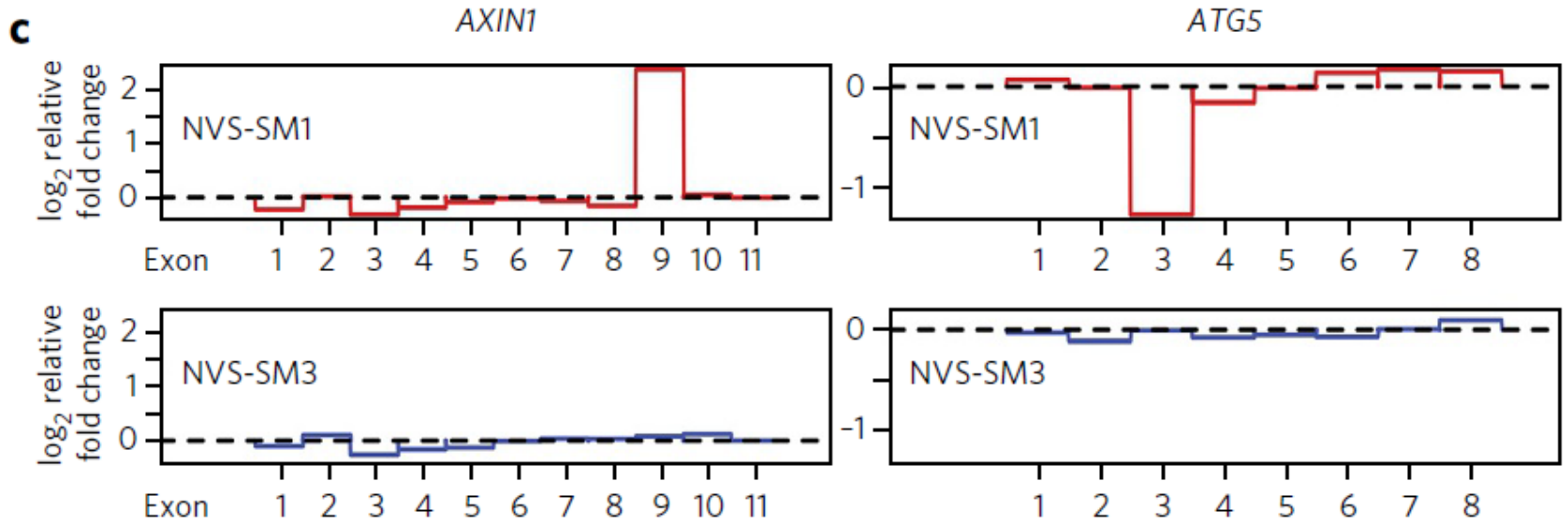
- Design of experiment?
- Vulcano plot: What is indicated on the X-axis? What on the Y-axis?
- Why are some points in «black»? «Blue»? «Yellow»?
- What does a $\log_{fold}$ change of 2.5 mean?
- Does NVS-SM1 change the transcription of many genes?

Figure 3b



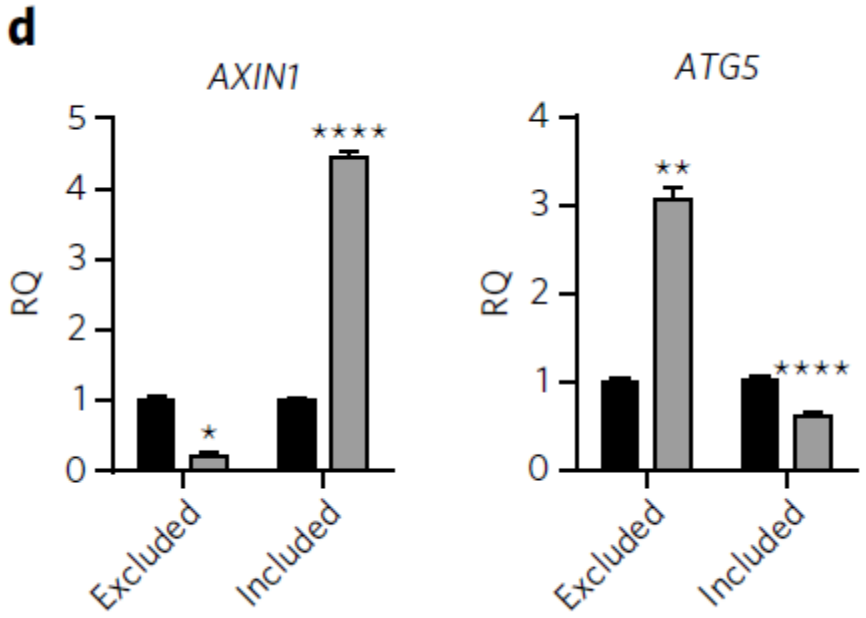
- Design of experiment?
- What is $\log_{fold}$ change variation in «left» and «right» graphs?
- What is «faulse discovery rate» FDR?
- What are «Atg5» and «Axin1»?
- Are many genes alternatively spliced due to compound (different exon levels; different exon-exon junction levels)?

Figure 3c



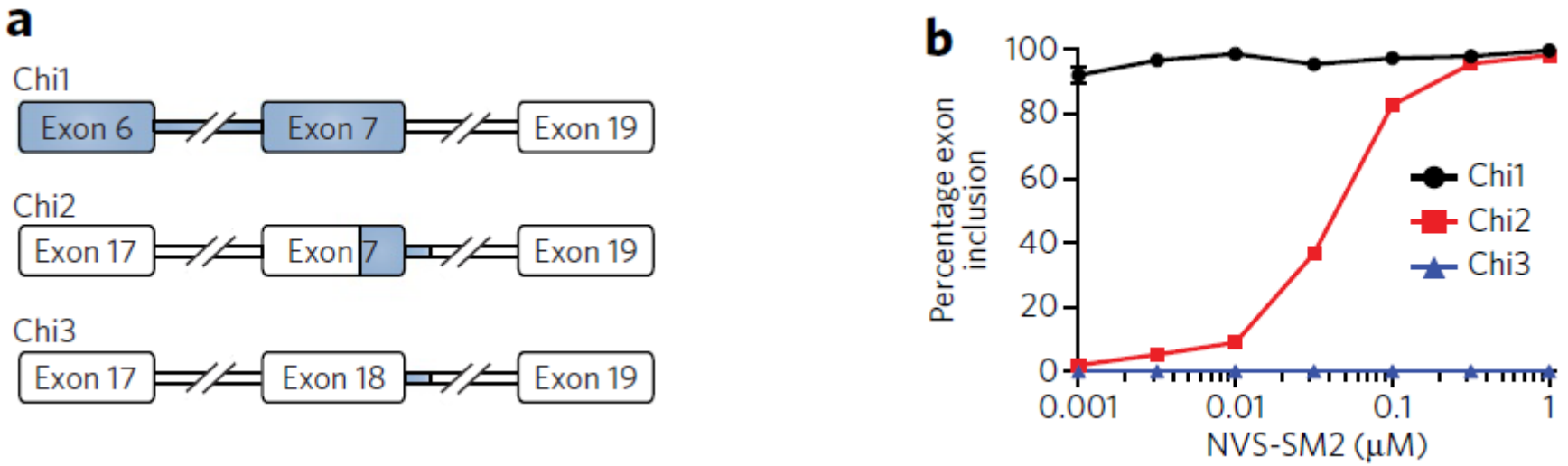
- What happens with AXIN1 upon treatment with NVS-SM1? What with ATG5?
- What is the «blue» curve?

Figure 3d



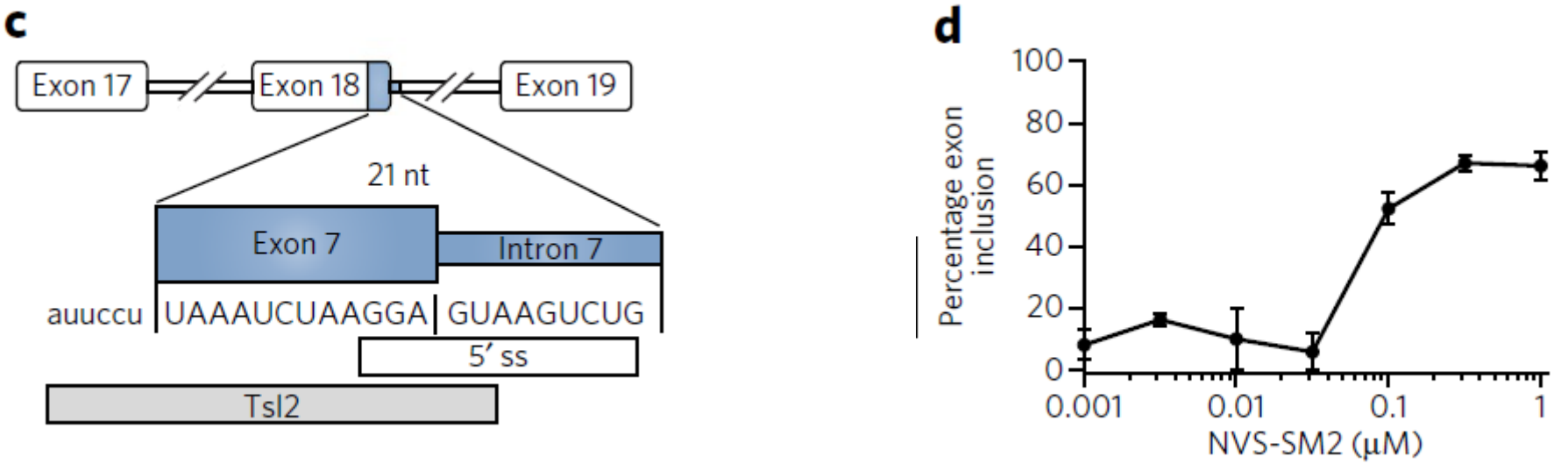
- Why was this experiment performed? Design?
- What is «RQ»? What are «black» bars? «Gray» bars?

Figure 4a and 4b



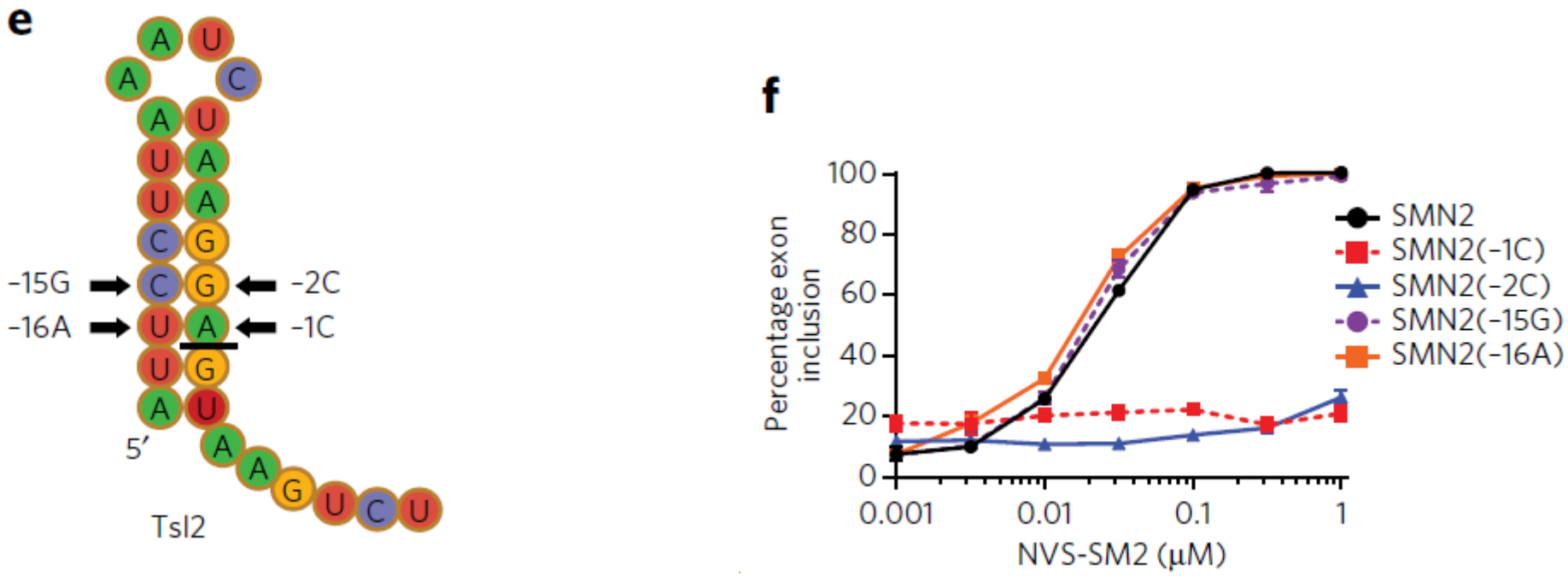
- Which question was addressed by this experiment?
- Why chimeric gene with BRCA1?

Figure 4c and 4d



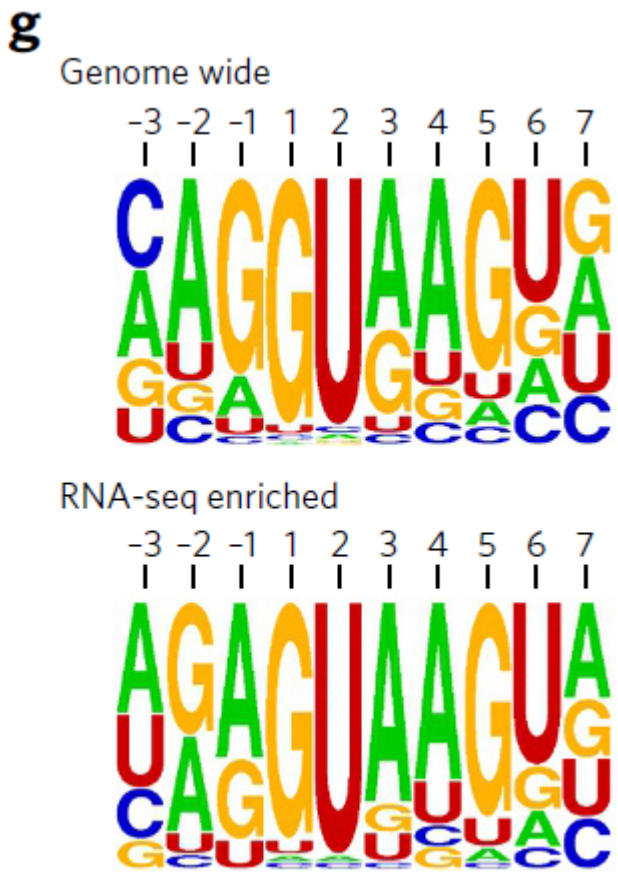
- Hypothesis based on the result of this experiment?

Figure 4e



- Why were indicated nucleotides mutated?
- Which effects did the nucleotide mutations have on splicing?
- Did the mutations alter the effect of NVS-SM2?
- To which region does NVS-SM2 most likely bind?

Figure 4g



- Question addresssed by this experiment?

Figure 4h

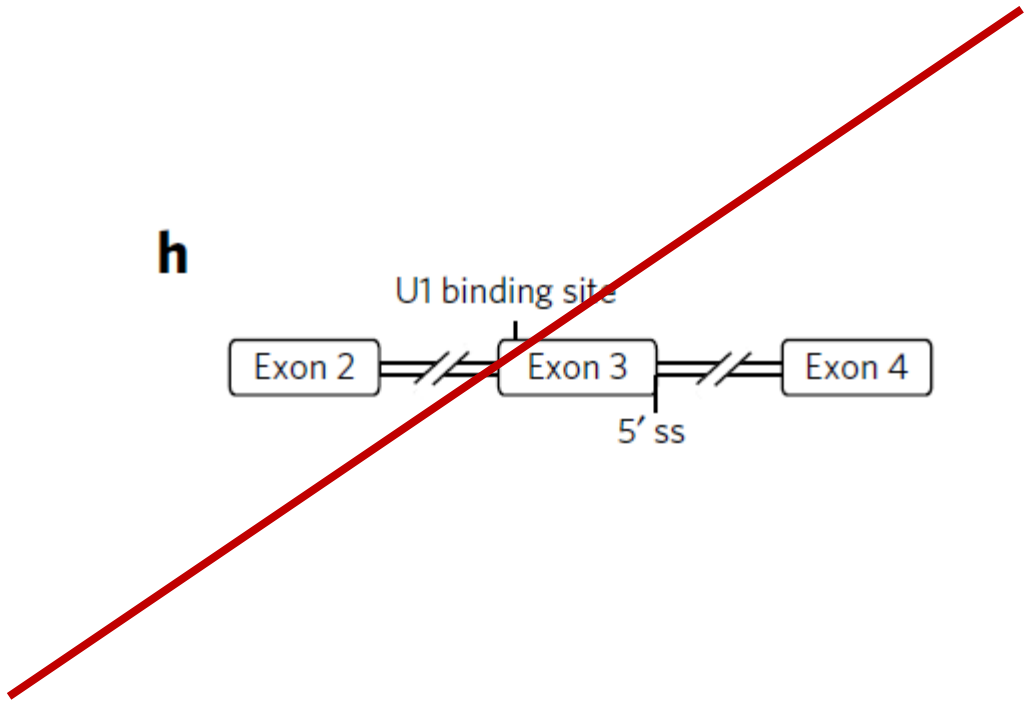


Figure 4i

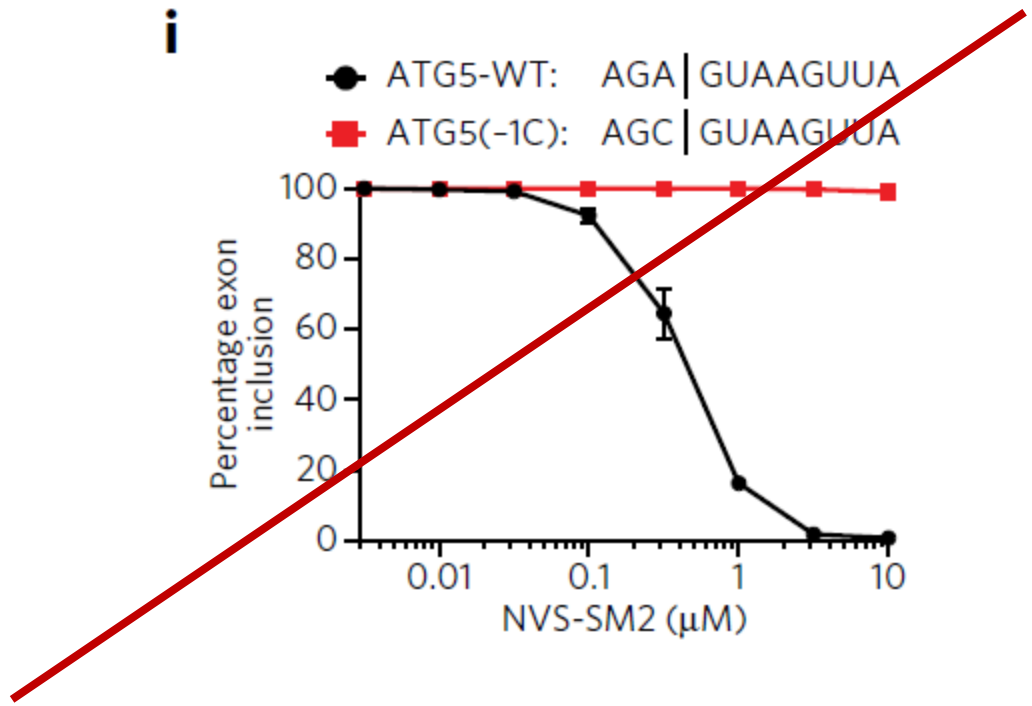
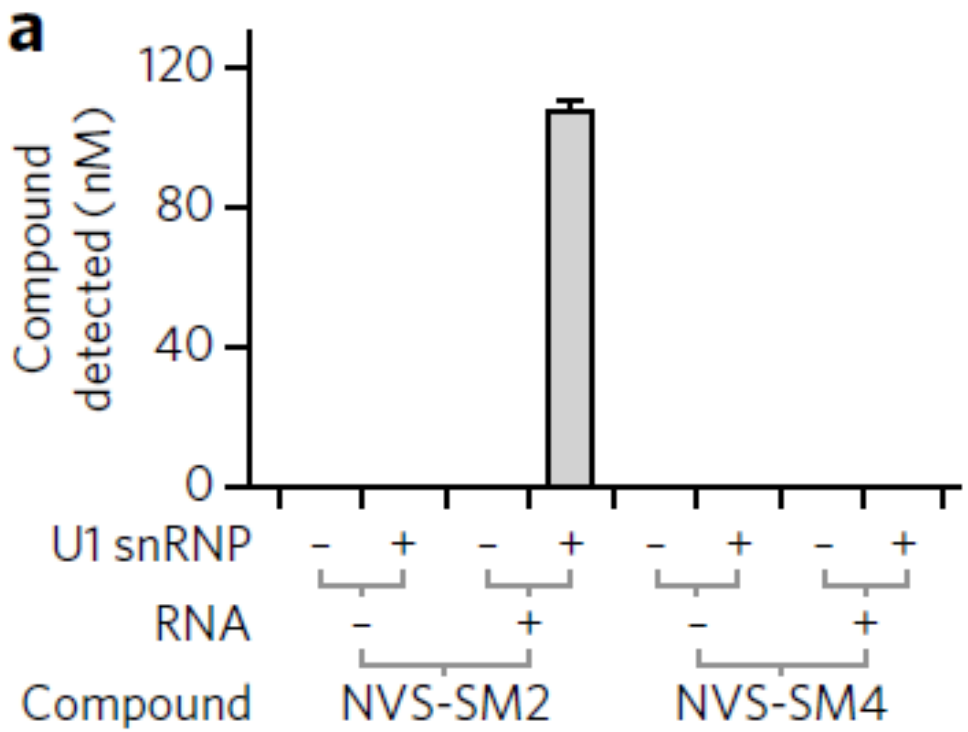
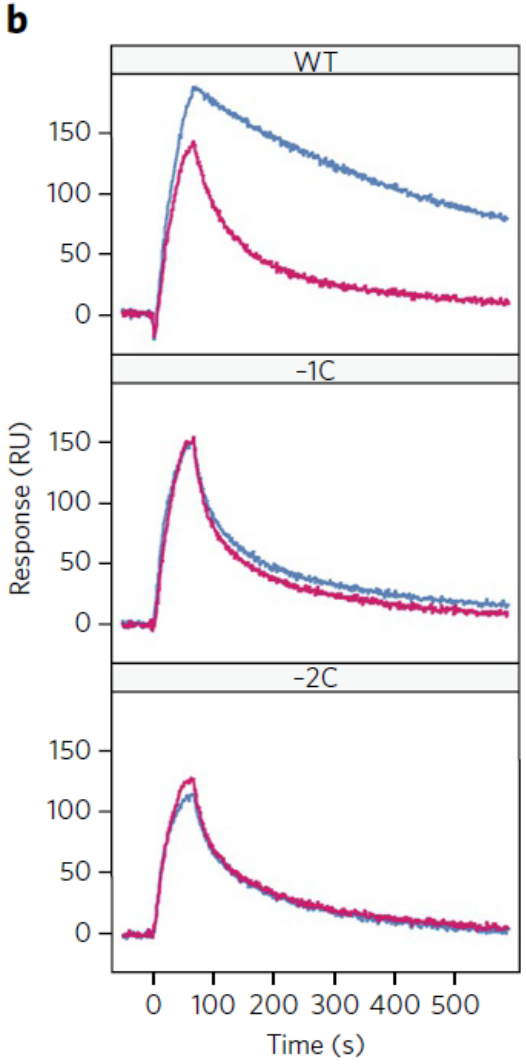

i
ATG5-WT: AGA | GUAAGUUA
ATG5(-1C): AGC | GUAAGUUA
Percentage exon inclusion
100
80
60
40
20
0
0.01
0.1
1
10
NVS-SM2 (μM)

Figure 5a



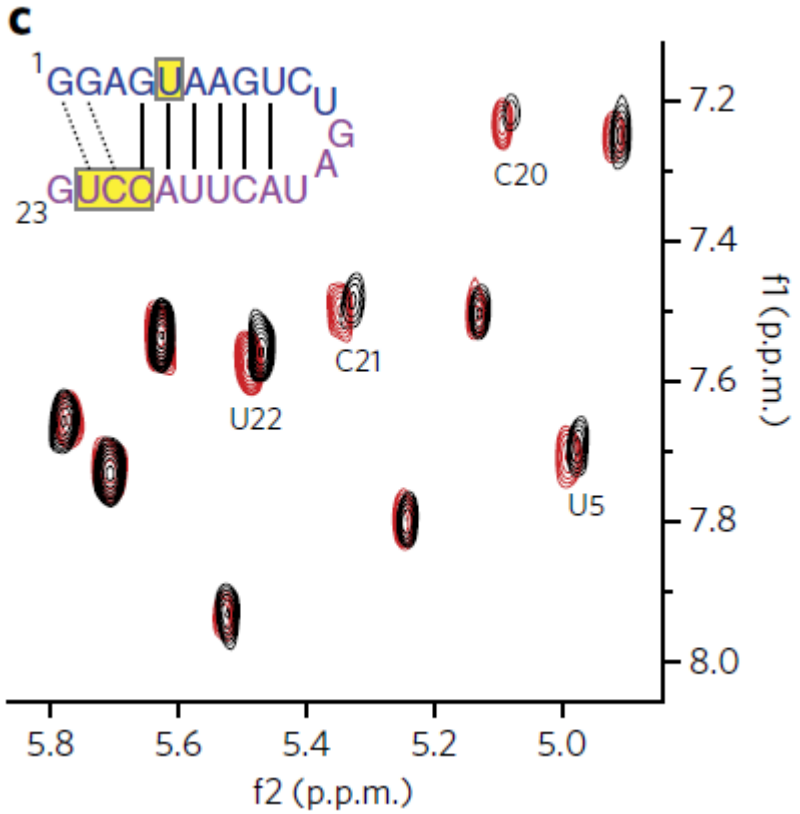
- Question addresssed by this experiment?
- How was this experiment performed?
- What is «U1 snRNP»?
- What is RNA?

Figure 5b



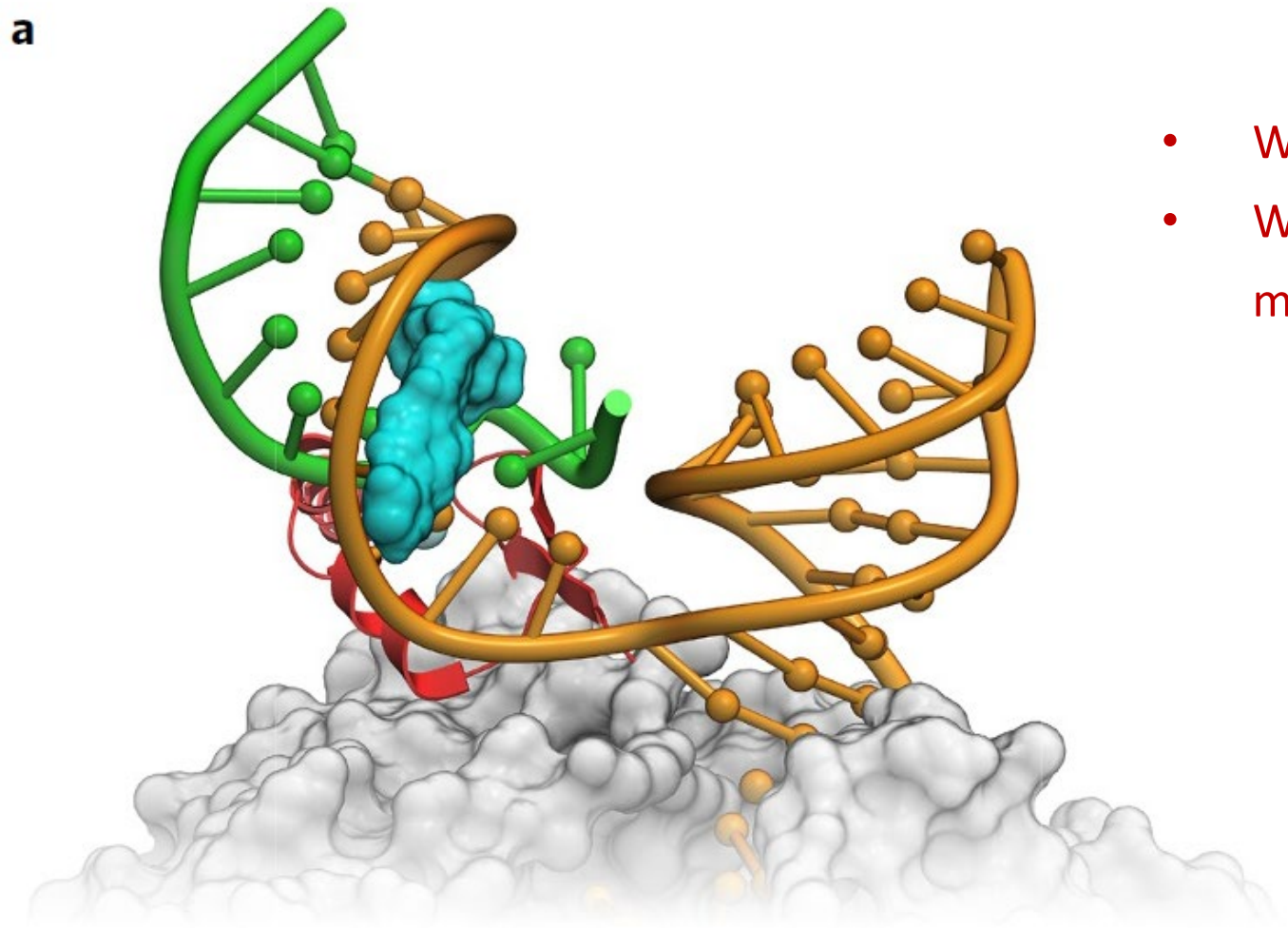
- Question addresssed by this experiment?
- How was this experiment performed? Which molecule was immobilized? Which molecule(s) were added?
- What is «-1C» and «-2C»?

Figure 5c



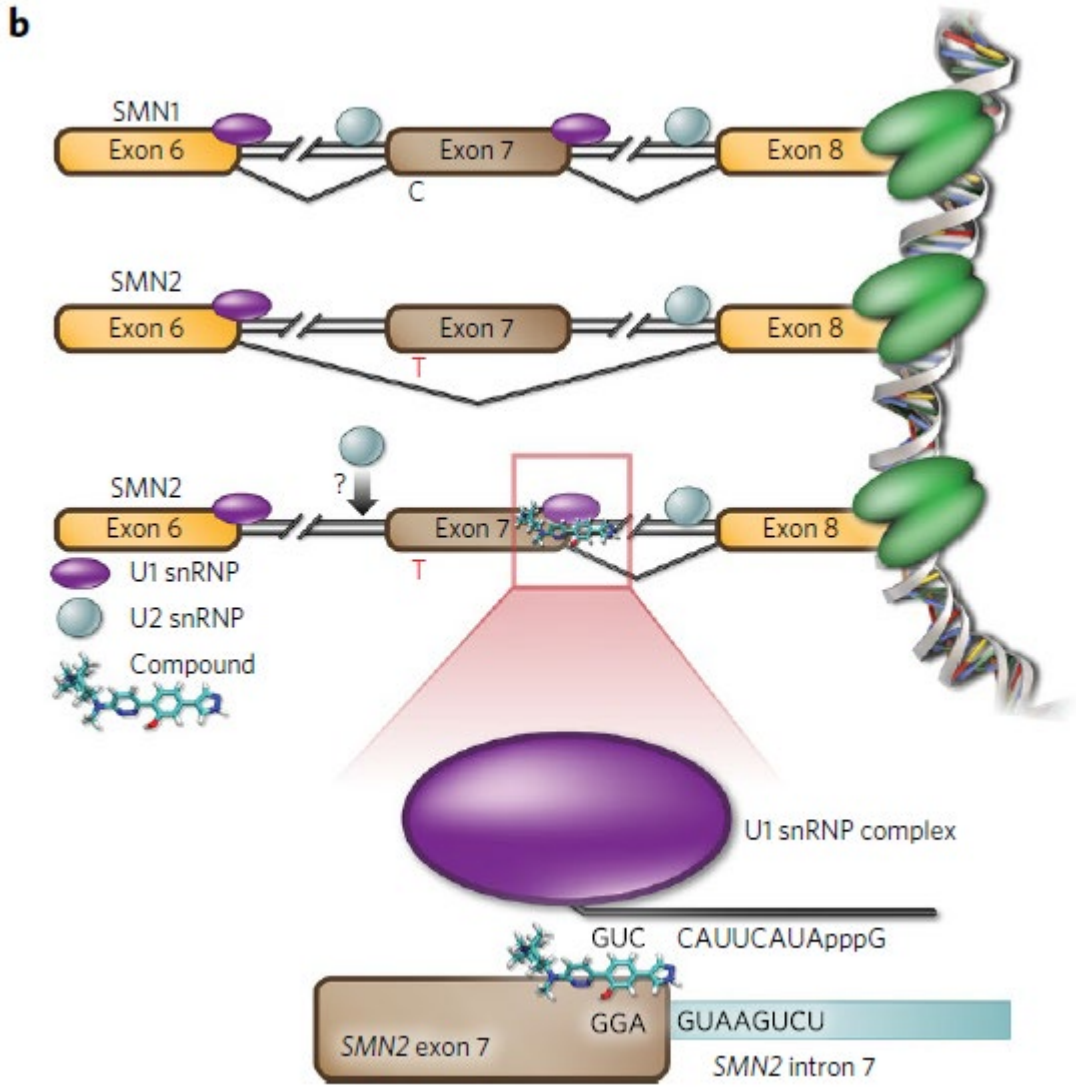
- Question addresssed by this experiment?
- How was this experiment performed?
- What is «blue» region in RNA? «Violet» region?
- What are nulcleotides highlighted in «yellow»?

Figure 6a



- What is this figure showing?
- Which color do the following molecules have?
  - SMN2 RNA
  - U1 snRNP (protein, RNA)
  - compound

Figure 6b


b
SMN1
Exon 6
Exon 7
Exon 8
C
SMN2
Exon 6
Exon 7
Exon 8
T
SMN2
Exon 6
?
Exon 7
Exon 8
T
U1 snRNP
U2 snRNP
Compound
U1 snRNP complex
GUC
CAUUCAUApppG
GGA
GUAAGUCU
SMN2 exon 7
SMN2 intron 7

Future development?

Jul 23, 2021

Novartis stops development of branaplam for SMA

Novartis has made the decision to discontinue development of branaplam, an investigational oral, once-weekly RNA splicing modulator, for the treatment of SMA.

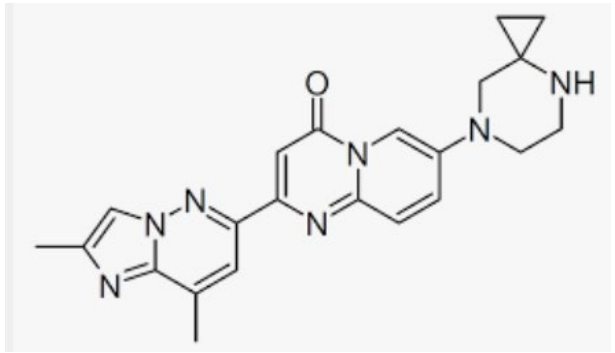
This was a difficult decision that was made as the result of rapid advancements in the SMA treatment landscape in recent years.

In October 2020, Novartis announced that branaplam lowers the level of huntingtin protein, which is one of the major therapeutic approaches in Huntington's disease. In 2021, U.S. Food and Drug Administration (FDA) granted an orphan drug status to branaplam for treatment of Huntington’s disease, and Novartis announced that they will set up clinical trials in 2021.[6] In December 2021, branaplam received a Fast Track designation from the FDA towards a phase IIb study in adult patients with early-stage HD manifestation.

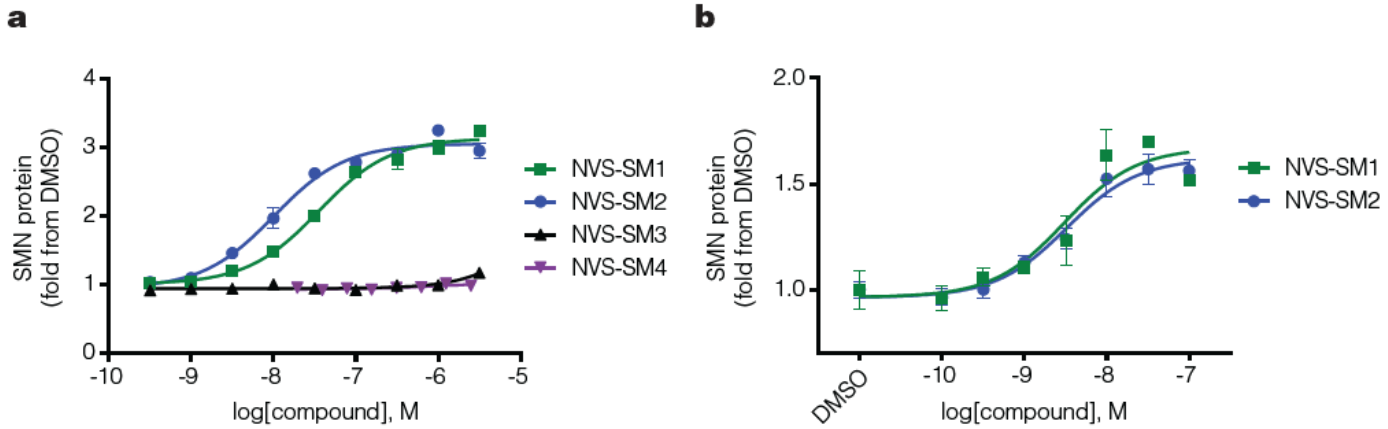
Spinal Muscular Atrophy (SMA) treatments

Zolgesma

Risdiplam



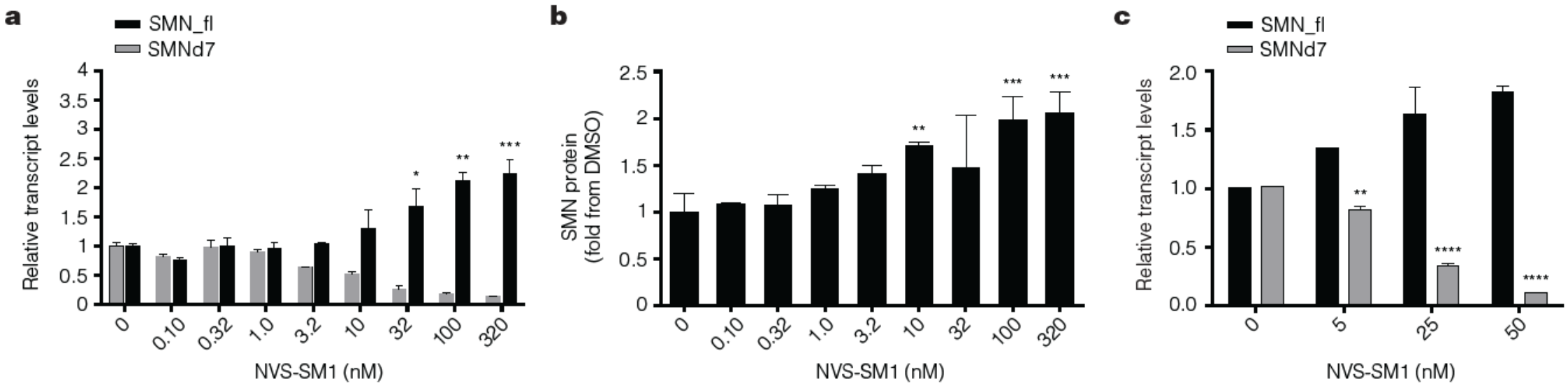
Supplementary Figures (no need to prepare)



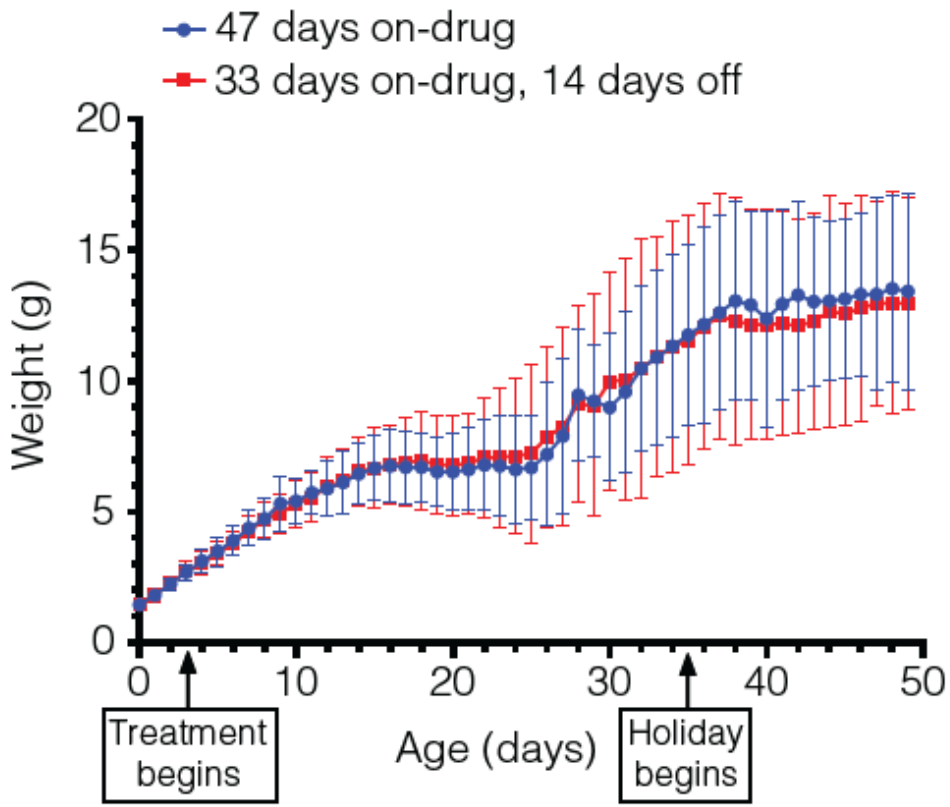
c

	NVS-SM1				NVS-SM2			
	mouse		rat		mouse		rat	
Parameter	IV 1 mg/kg	PO 3 mg/kg	IV 1 mg/kg	PO 3 mg/kg	IV 1 mg/kg	PO 3 mg/kg	IV 1 mg/kg	PO 3 mg/kg
Cl (mL/min/kg)	22.7		220		20.5		21.6	
$t_{1/2}$ (h)	8.5		9.8		10.5		12.2	
C_{max} (nM)		86		185		111		171
T_{max} (h)		4.3		7		3		4
V_{ss} (L/kg)	12.4		15.7		13.8		20.1	
AUC_{0-24h} (nM·h)	1788	1552	1615	3029	1704	1439	1477	2325
F (%)		29		63		29		53
Brain:Plasma @ 24h	1.5	1.4	1.8	1.3	1.1	0.7	1.8	1.4

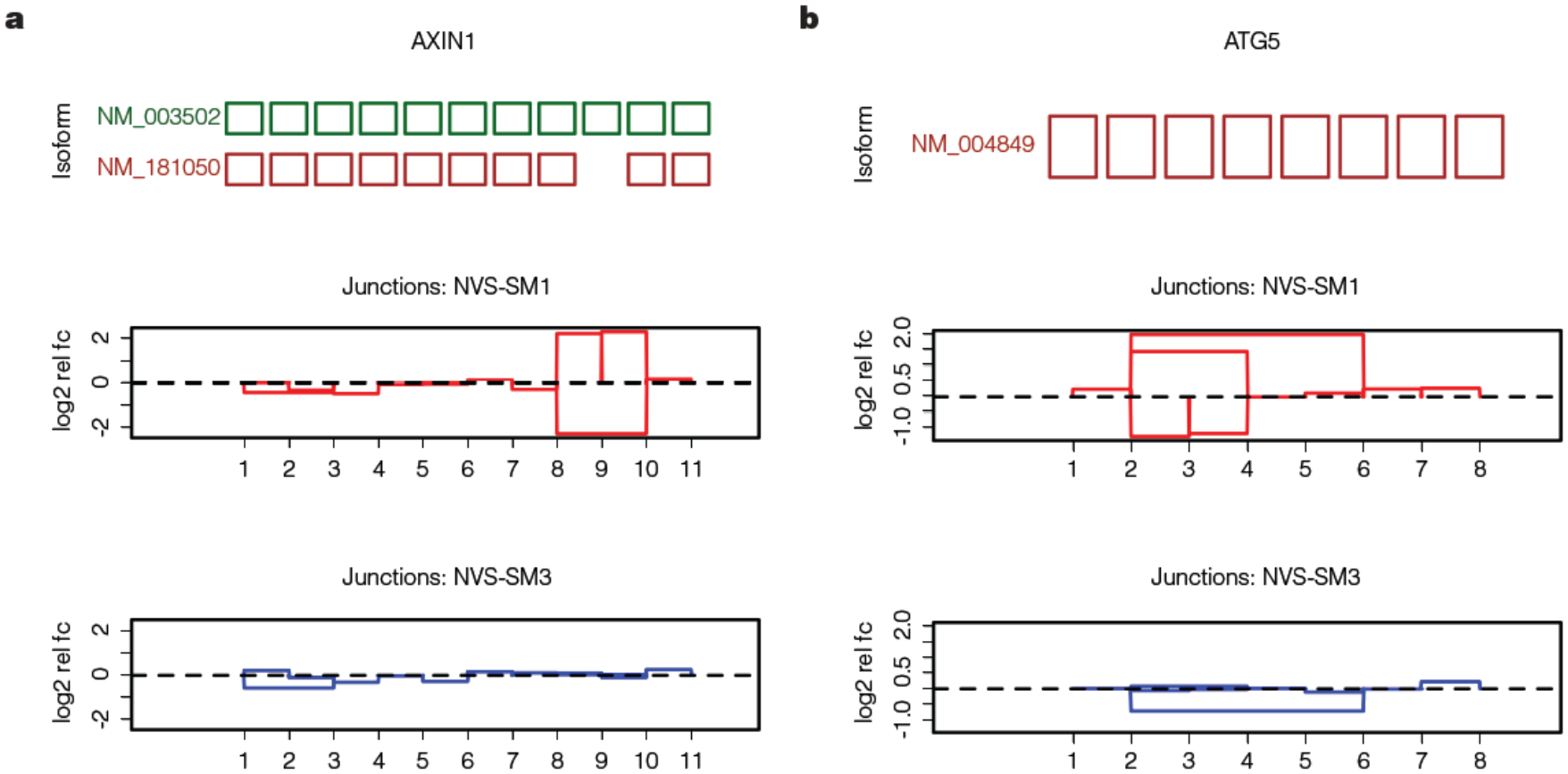
SI Figure 2


a
SMN_fl
SMNd7
Relative transcript levels
4
3.5
3
2.5
2
1.5
1
0.5
0
*
**
***
0
0.10
0.32
1.0
3.2
10
32
100
320
NVS-SM1 (nM)
b
SMN protein
(fold from DMSO)
2.5
2
1.5
1
0.5
0
**
***
***
0
0.10
0.32
1.0
3.2
10
32
100
320
NVS-SM1 (nM)
c
SMN_fl
SMNd7
Relative transcirpt levels
2.0
1.5
1.0
0.5
0.0
**
****
****
0
5
25
50
NVS-SM1 (nM)

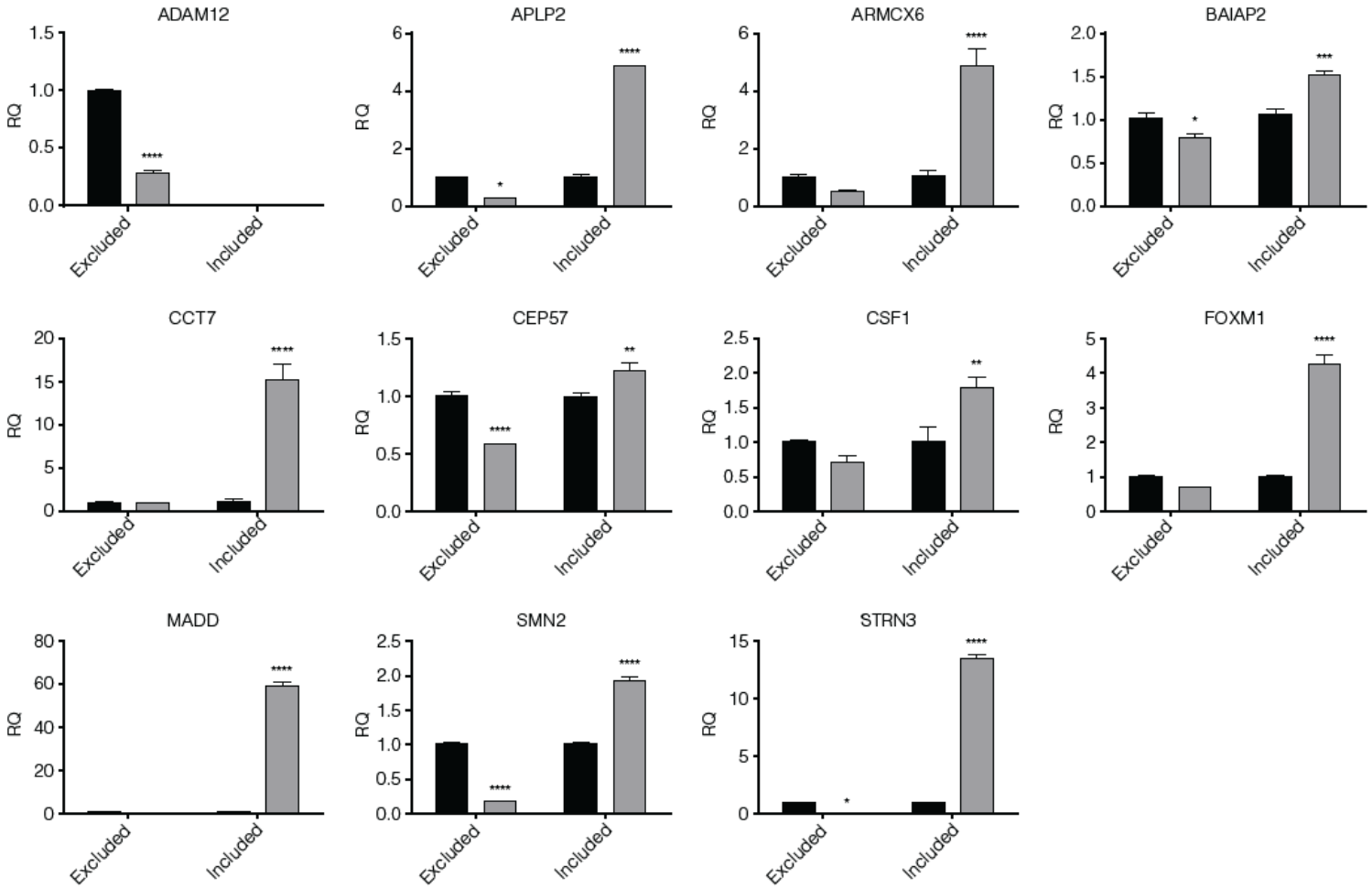
SI Figure 3


47 days on-drug
33 days on-drug, 14 days off
Weight (g)
20
15
10
5
0
0
10
20
30
40
50
Age (days)
Treatment begins
Holiday begins

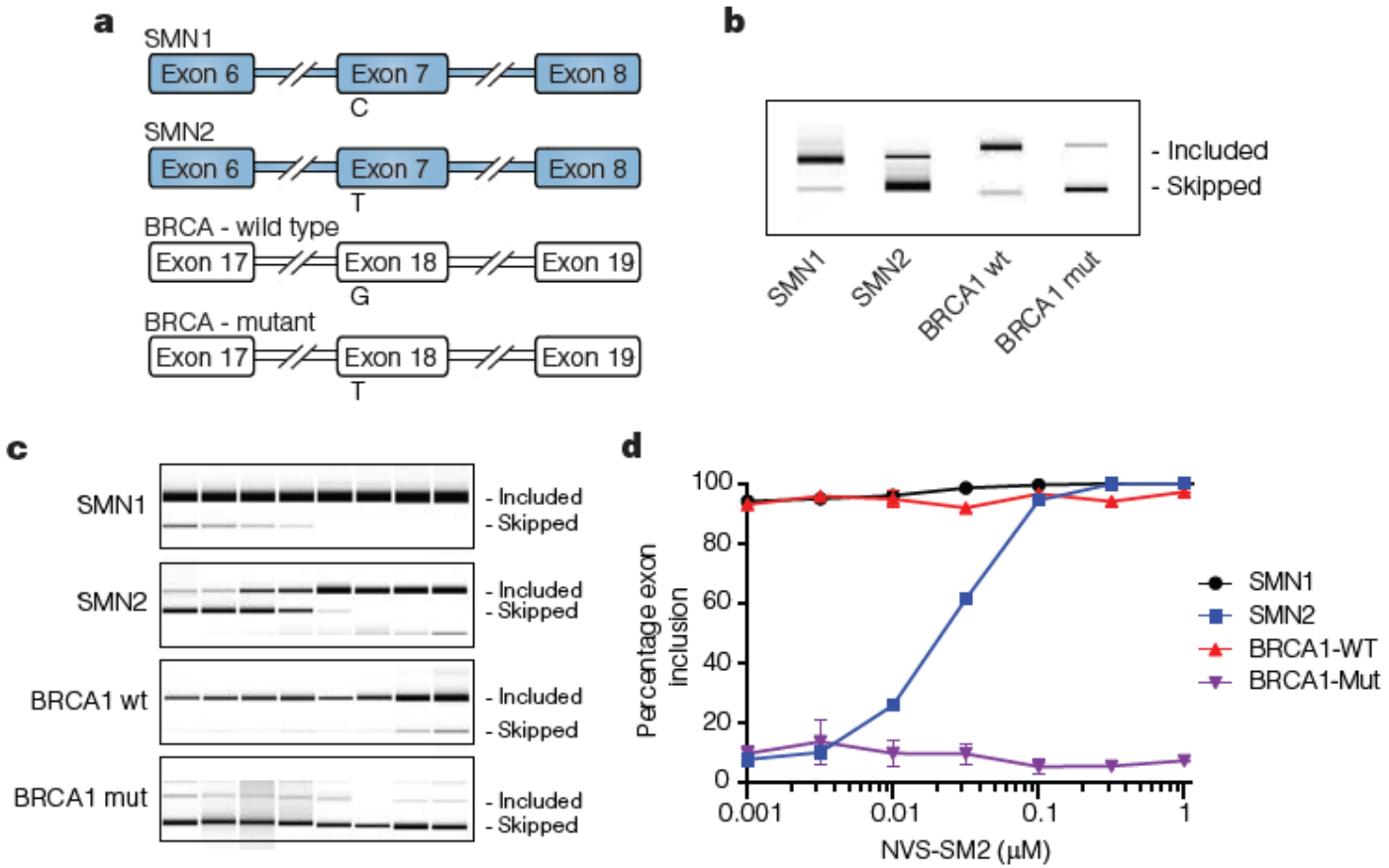
SI Figure 4


a
AXIN1
Isoform
NM_003502
NM_181050
Junctions: NVS-SM1
log2 rel fc
-2 0 2
1 2 3 4 5 6 7 8 9 10 11
Junctions: NVS-SM3
log2 rel fc
-2 0 2
1 2 3 4 5 6 7 8 9 10 11
b
ATG5
Isoform
NM_004849
Junctions: NVS-SM1
log2 rel fc
-1.0 0.5 2.0
1 2 3 4 5 6 7 8
Junctions: NVS-SM3
log2 rel fc
-1.0 0.5 2.0
1 2 3 4 5 6 7 8

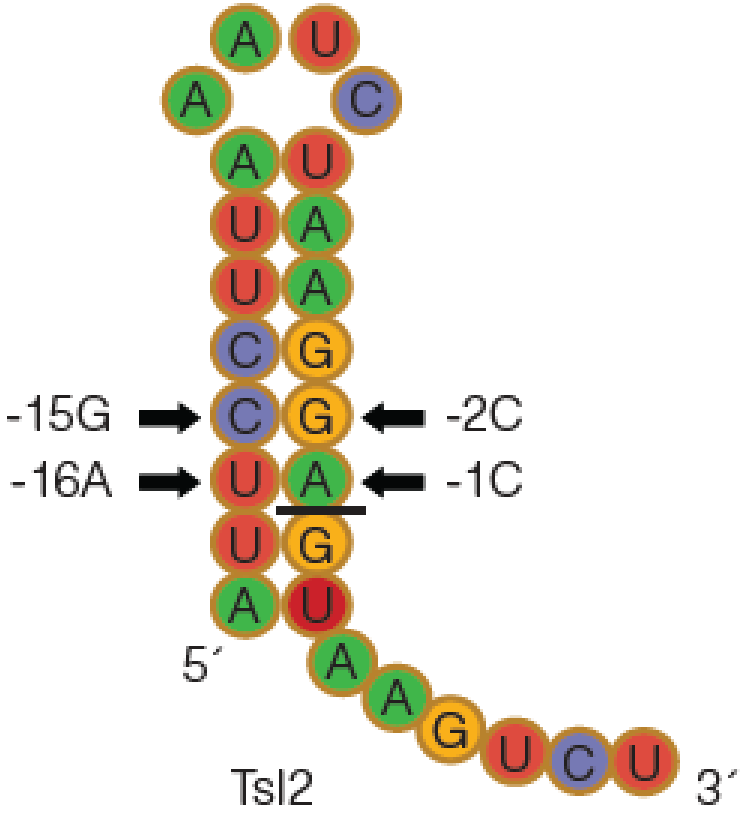
SI Figure 5


ADAM12
APLP2
ARMCX6
BAIAP2
CCT7
CEP57
CSF1
FOXM1
MADD
SMN2
STRN3
RQ
Excluded
Included

SI Figure 6

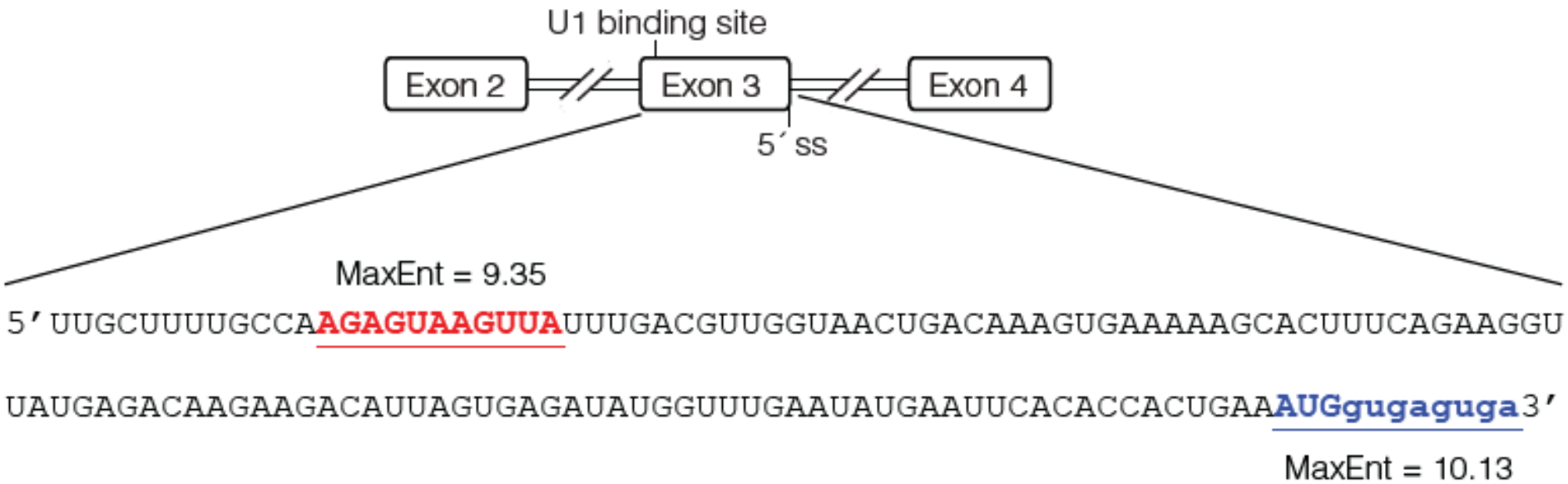

a
SMN1
Exon 6
Exon 7
Exon 8
C
SMN2
Exon 6
Exon 7
Exon 8
T
BRCA - wild type
Exon 17
Exon 18
Exon 19
G
BRCA - mutant
Exon 17
Exon 18
Exon 19
T
b
- Included
- Skipped
SMN1
SMN2
BRCA1 wt
BRCA1 mut
c
SMN1
SMN2
BRCA1 wt
BRCA1 mut
- Included
- Skipped
d
Percentage exon inclusion
100
80
60
40
20
0
0.001
0.01
0.1
1
NVS-SM2 (μM)
SMN1
SMN2
BRCA1-WT
BRCA1-Mut

SI Figure 7

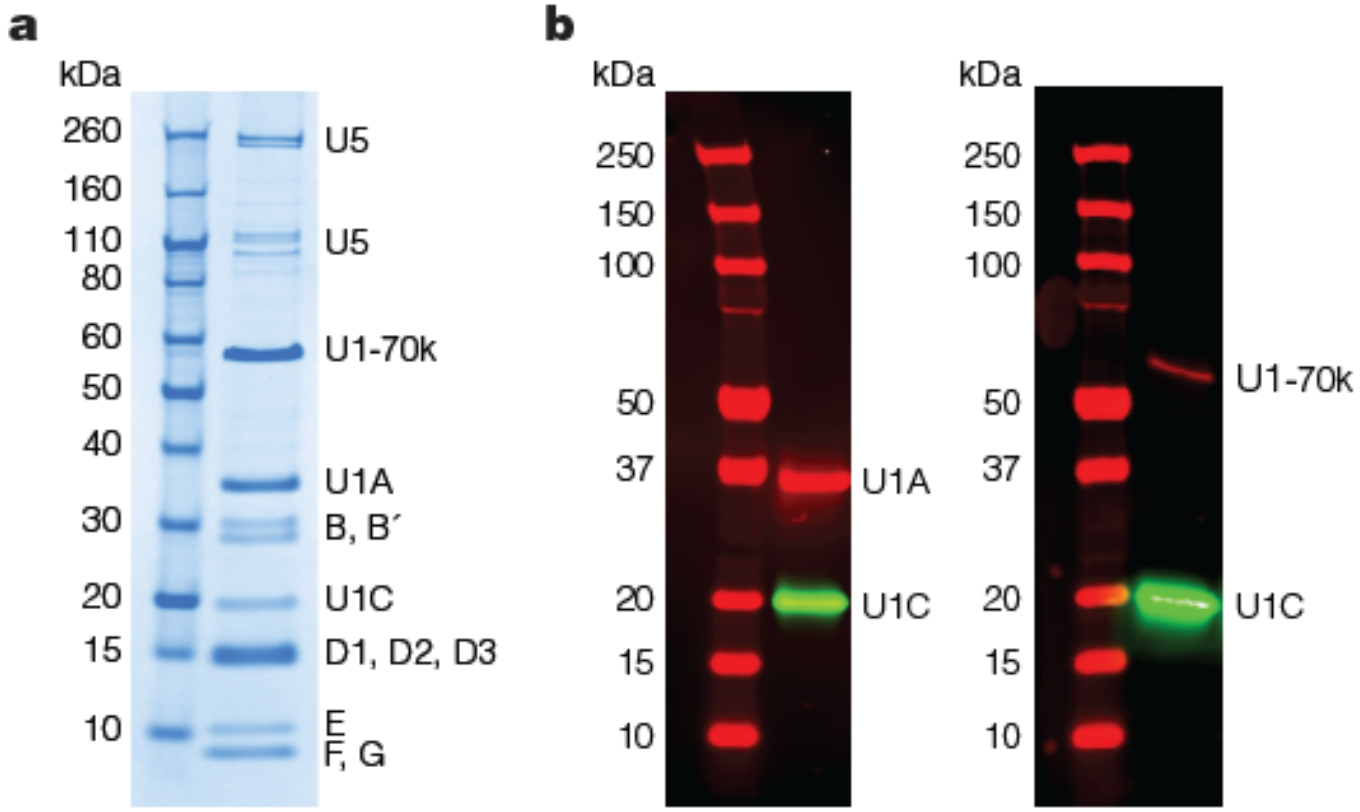


Tsl2	ΔG (kcal/mol)
Wild type	-5.66
-1C	-2.62
-2C	0.41
-15G	-1.28
-16A	-3.20

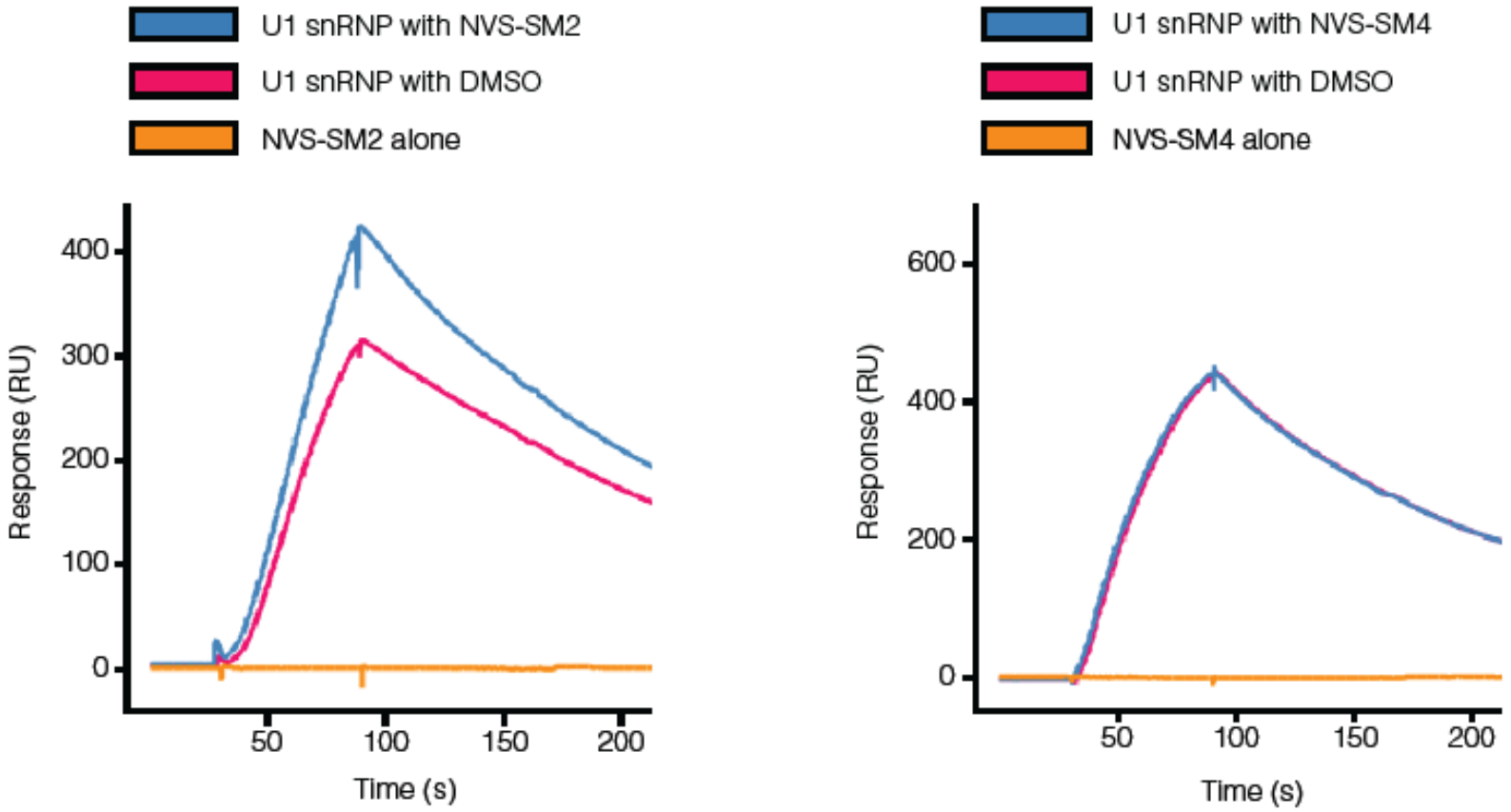
SI Figure 8


U1 binding site
Exon 2
Exon 3
Exon 4
5´ss
MaxEnt = 9.35
5′ UUGCUUUUGCCAAGAGUAAGUUAUUUGACGUUGGUAACUGACAAAGUGAAAAAGCACUUUCAGAAGGU
UAUGAGACAAGAAGACAUUAGUGAGAUAUGGUUUGAAUAUGAAUUCACACCACUGAAAUGgugaguga3′
MaxEnt = 10.13

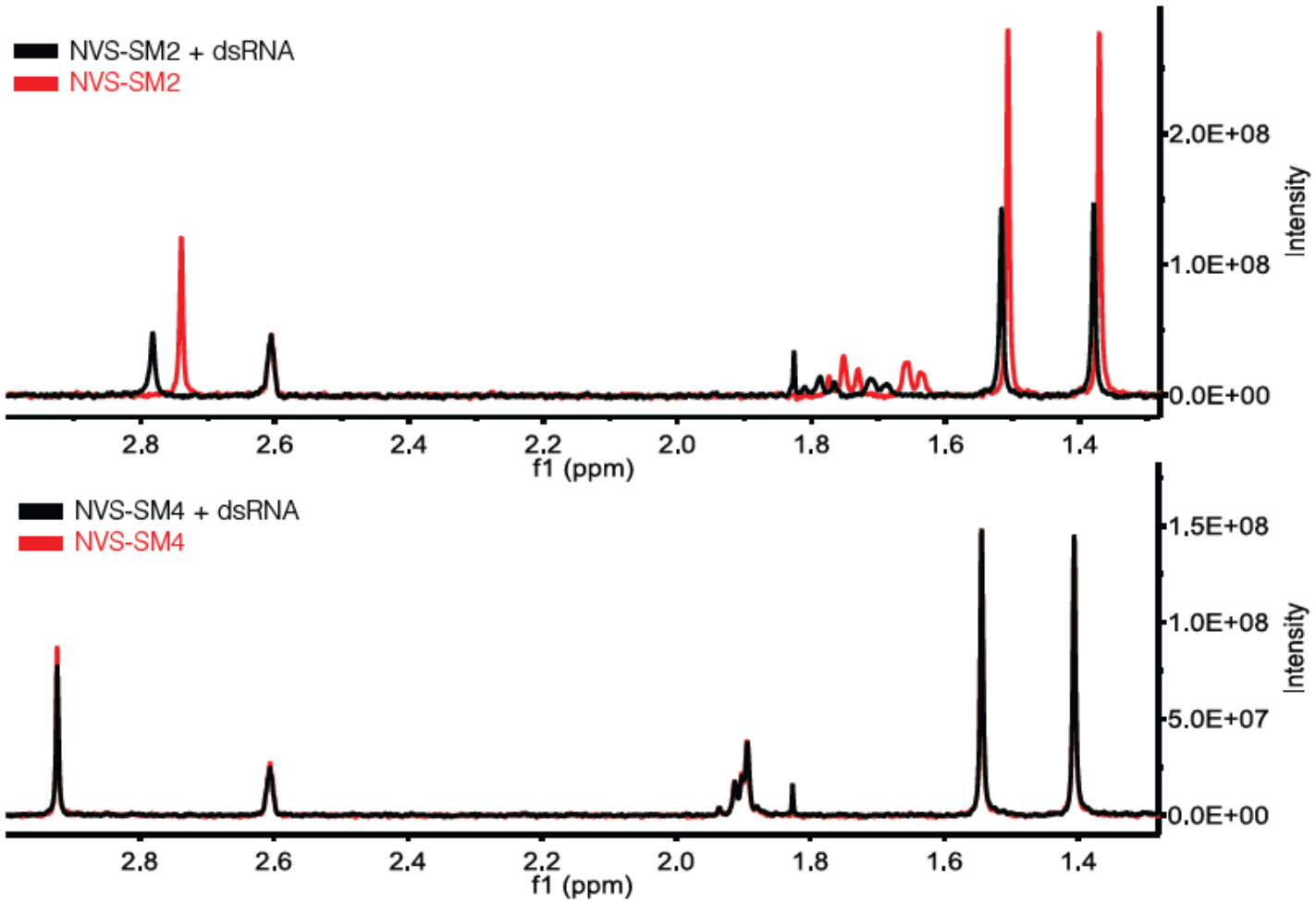
SI Figure 9


a
kDa
260
160
110
80
60
50
40
30
20
15
10
U5
U5
U1-70k
U1A
B, B´
U1C
D1, D2, D3
E
F, G
b
kDa
250
150
100
50
37
20
15
10
U1A
U1C
kDa
250
150
100
50
37
20
15
10
U1-70k
U1C

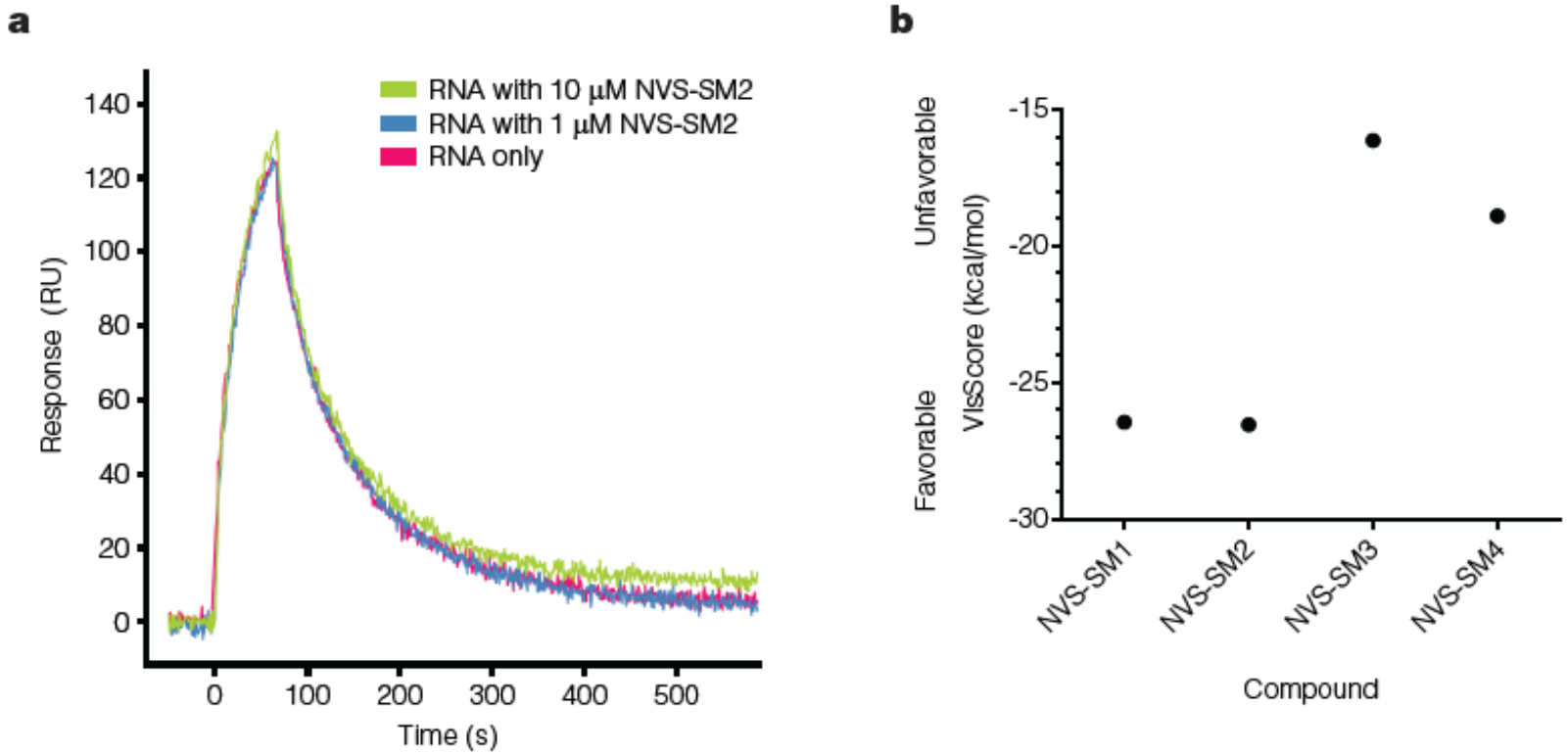
SI Figure 10


U1 snRNP with NVS-SM2
U1 snRNP with DMSO
NVS-SM2 alone
Response (RU)
400
300
200
100
0
50
100
150
200
Time (s)
U1 snRNP with NVS-SM4
U1 snRNP with DMSO
NVS-SM4 alone
Response (RU)
600
400
200
0
50
100
150
200
Time (s)

SI Figure 11


NVS-SM2 + dsRNA
NVS-SM2
2.0E+08
1.0E+08
0.0E+00
Intensity
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
f1 (ppm)
NVS-SM4 + dsRNA
NVS-SM4
1.5E+08
1.0E+08
5.0E+07
0.0E+00
Intensity
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
f1 (ppm)

SI Figure 12


a
RNA with 10 μM NVS-SM2
RNA with 1 μM NVS-SM2
RNA only
Response (RU)
140
120
100
80
60
40
20
0
0
100
200
300
400
500
Time (s)
b
Unfavorable
Favorable
VlsScore (kcal/mol)
-15
-20
-25
-30
NVS-SM1
NVS-SM2
NVS-SM3
NVS-SM4
Compound