

Additional file 2

CRISPR-directed evolution of the spliceosome for resistance to splicing inhibitors

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Additional File 2:

Table S1: Sequences of sgRNAs with PAM targeting SF3B1

Table S2: List of primers annealed to synthesis sgRNAs with *BsaI* compatible overhangs

Table S3: List of primers used for genotyping

Table S4: CDE-platform for SF3B1 resistance to splicing inhibitor

Table S5: List of primers used for Intron retention analysis

Table S1: Sequences of sgRNAs with PAM targeting SF3B1

sgRNA	5'-sgRNA sequence-3' (PAM is underlined)
1	GGAGCTCGCGCGGGCGCAGG <u>AGG</u>
2	GGAGCGCAAGAAGATGGAGG <u>AGG</u>
3	<u>CCG</u> CCGGCGCGCCCATGGCCGTC
4	<u>CCT</u> TCGACACCGATCTCTACGGC
5	<u>CCG</u> ACCCCAACCGCTTCGCCGGC
6	<u>CCT</u> CCATCCCCGCCTCCGAGGAC
7	CTCCGAGGACGACGCGCCCG <u>AGG</u>
8	<u>CCG</u> AGGCGGCCGTCAACCCCGCC
9	<u>CCG</u> GCCGCCTCGCCTCCTACACC
10	<u>CCG</u> GCCACGCCGTCGCCGCCGCC
11	CGACATCCCCCGCGCCGCCG <u>AGG</u>
12	<u>CCA</u> AGAAGTCGCAGCGCATCATC
13	<u>CCG</u> CGAGGATGACTATCGCCGCC
14	GCTGGCCCGCATCATCTCGCCG <u>G</u>
15	<u>CCG</u> GAGCGCCATGATCCCTTCGC
16	CCACCCCGGACCCTTCCGTG <u>GCGG</u>
17	<u>CCG</u> ATGCCATGCGCGAGAATGAC
18	<u>CCT</u> GCAGAAGCAGAAGGAGCAGC
19	TGACATAGCTCAGAAGAAGA <u>AGG</u>
20	GGAGAAGGCCAAGGAGAAGA <u>AGG</u>
21	GCCGAAGCGCCGCAATAGATG <u>GGG</u>
22	CAATAGATGGGATCAGTCG <u>CAGG</u>
23	GGTGATGCTTCTGCTGCTGCT <u>G</u>
24	GGCGAAGACCTCCTCCGATT <u>GGG</u>
25	GATGCTCCTGATGCAACTCCT <u>G</u>
26	GGGCGCTGGGATGCCACCCCT <u>G</u>
27	<u>CCC</u> CTGGTCGTGTCGGGGATGCG
28	GTCGGTGAGAAGGAATCGTT <u>GGG</u>
29	<u>CCG</u> TCCGGTGAGAAGGAATCGTTG
30	<u>CCG</u> ATGCGGATGCAACACCTGCA
31	GCAGCTGGTGGCATTACTCC <u>AGG</u>
32	<u>CCT</u> TCTGGAGCTTGGGATGCTAC
33	<u>CCT</u> GGTGGGCTTGTCACGCCAAC
34	<u>CCA</u> ACACCCAAGAAGCAGAGATC
35	<u>CCT</u> GCAAGTATGGGTAGTGCTAC
36	<u>CCT</u> GGAGGCACTGGTGCTGCGAC
37	<u>CCT</u> GCAGGTTACACTCCTGGTCC
38	<u>CCA</u> TTTGGTGGTGACAACCTTGC
39	<u>CCA</u> ACACCCGCCAGATTGCTTC
40	CAGAGCAGTACCAGCTCTTG <u>C</u> GG
41	GAACAGGCCTCTTACGGATG <u>AGG</u>
42	GCTTGATACTATGTTCCCGC <u>AGG</u>
43	<u>CCG</u> CAGGAAGGGTACAAGATTCT
44	<u>CC</u> AGCTTCATACCAACCCATACG
45	<u>CC</u> AGCAAGAAAGCTGCTCGCTAC
46	ACCATTGTATGCTATTCTG <u>AGG</u>
47	GCTATTCTGAGGAGAATCGT <u>G</u>
48	GTGCCAAGGAGTTGCCTGGT <u>G</u>
49	GTTGCCCTCATGAAGCCAG <u>AGG</u>
50	<u>CCA</u> GTA CTTTGAACATTGCTGA
51	GCTATCTCCAGAAGAGCAGA <u>AGG</u>
52	GATCATGAAGCTCCTGCTCA <u>AGG</u>
53	<u>CC</u> ACAGCGGAAGACAGCACTTCG

54	<u>G</u> CTCATGCAGCCAACACTT <u>GAGG</u>
55	TGACAGGGTACTGTATAAGCT <u>G</u> G
56	<u>CCG</u> TCCGTTTGTGCATAAGATTC
57	GACTACTATGCTCGTGT <u>TGAGGG</u>
58	<u>CCGGG</u> AGATTATCTCAAATCTTA
59	TCAAATCTTAGCAAAGCTGCT <u>G</u> G
60	<u>CCGCC</u> ATGAGACCAGATATTGAT
61	CACTGCTAGAGCATT <u>TAGCGTGG</u>
62	TAGCGTGGTGGCTTCTGCTT <u>TGG</u>
63	TCCTGGCAAGCTCGGCACACT <u>G</u> G
64	<u>CCTGG</u> CAAGCTCGGCACACTGGT
65	CAGCAGATTGCTATTCTTAT <u>GGG</u>
66	<u>CCTAG</u> TTGAGATCATTGAGCATG
67	GTGACGAGAACCAGAAAGT <u>GCGG</u>
68	<u>CCAG</u> AAAGTGC GGACAATCACTG
69	AAGGGTATCAGATCTCACCGT <u>G</u> G
70	<u>CCGTG</u> GAAAGGTTCTTGCTGCCT
71	GCTGCCTTCTTGAAAGCTAT <u>TGG</u>
72	<u>CCTCT</u> TATGGATGCGCTGTATGC
73	<u>CCTG</u> ATTAGGGAGTTCAGTCAC
74	GATGAAGAAGATTGCCTGA <u>AGG</u>
75	GTGTAGAGGCTGATTATAT <u>CCGG</u>
76	GGAGAATGGCTCTGGACCGC <u>AGG</u>
77	GTAGAGATGGCAAATAAGGT <u>TGG</u>
78	TATTGTTGGGAGGATTGTTG <u>AGG</u>
79	GCCTTACAGGAGAATGGTGAT <u>G</u> G
80	GGTGATGGAAACAATTGAGA <u>AGG</u>
81	TTGGAGGAGCTGCTTATTGAT <u>TGG</u>
82	<u>CCTT</u> ACCTTCCTCAGATTTGTGG
83	AAGCTGCTGATCTGATCTCG <u>AGG</u>
84	GTGTCAGGAGGAGCAGCTTAT <u>G</u> G
85	GAAGAGTACCCTGAGGTGTT <u>GGG</u>
86	<u>CCGCC</u> AATCAAGGATCTTCTTCC
87	GAATAGGCATGAGAAGGTCC <u>AGG</u>
88	GAGAACTGCATTGATCTAGT <u>TGG</u>
89	GTTGGTAGGATTGCTGATCGT <u>G</u> G
90	TGCCTGCTAGGGAGTGGATG <u>AGG</u>
91	AATGTTGAAGGCTCACAAGA <u>AGG</u>
92 (HR-target)	ATGTTGAAGGCTCACAAGA <u>AGG</u>
93	AGGCTCACAAGAAGGGTATT <u>AGA</u>
94	CTCACAAGAAGGGTATTAGA <u>AGA</u>
95	GGTTATATTGCGAAGGCTATT <u>G</u> G
96	GTTGAATAACTTGAAGGTGC <u>AGG</u>
97	<u>CCGTG</u> TCTGCACAAGTTCGCAA
98	<u>CCTG</u> ATGAATGAGTACCGAGTCC
99	GTATATTGGTGAGATGGGCA <u>AGG</u>
100	<u>CCTG</u> CTTGAAGATGCTCTAATG
101	GCTGTGAAGCACATGGCTCT <u>TGG</u>
102	TGTTGCTGGCTTGGGCTGTG <u>AGG</u>
103	GCTGTCATGGAGGCGATTG <u>AGGG</u>
104	GAGGGGATGAGAGTTGCTCT <u>TGG</u>
105	<u>CCAG</u> CTGTGATCTTGAATTACTG
106	<u>CCAT</u> CCAGCAAGGAAAGTACGTG
107	TCCTGCACTGGATGACGAT <u>GGGG</u>

108	<u>TATCTACAGCCGTCCAGAGCTGG</u>
109	GATGAGACTCCTGCAAGTAT <u>GGG</u>
110	<u>CCAGAAGAGCAGAAGGAGAGGAA</u>
111	AAAGCAAGGGAATTTGGTGCT <u>GG</u>
112	<u>CCATTGTTCAACAAGATTCTACC</u>
113	<u>CCAGATATTGATAACATTGATGA</u>
114	ATAACATTGATGAGTATGTG <u>GAGA</u>
115	<u>CCACTGCTAGAGCATTTAGCGTG</u>
116	<u>CCAGTCACCAGATGAAGAGATGA</u>
117	<u>CCAGATGAAGAGATGAAGAAGAT</u>
118	<u>CCGGCAAACCTGCTGCCTCTGCTG</u>
119	CACATTGGTTATATTGCGA <u>AAGG</u>

Table S2: List of primers annealed to synthesis sgRNAs with *BsaI* compatible overhangs

Name of the primer	Sequence (5' - 3')
F1	<u>GGCAGGAGCTCGCGCGGGCGCAGG</u>
F2	<u>GGCAGGAGCGCAAGAAGATGGAGG</u>
F3	<u>GGCAGACGGCCATGGGCGCGCCGG</u>

F4	<u>GGC</u> AGCCGTAGAGATCGGTGTCGA
F5	<u>GGC</u> AGCCGGCGAAGCGGTTGGGGT
F6	<u>GGC</u> AGTCCTCGGAGGCGGGGATGG
F7	<u>GGC</u> ACTCCGAGGACGACGCGCCCG
F8	<u>GGC</u> AGGCGGGGTTGACGGCCGCCT
F9	<u>GGC</u> AGGTGTAGGAGGCGAGGCGGC
F10	<u>GGC</u> AGGCGGCGGCGACGGCGTGGC
F11	<u>GGC</u> ACGACATCCCCCGCGCCGCCG
F12	<u>GGC</u> AGATGATGCGCTGCGACTTCT
F13	<u>GGC</u> AGGCGGCGATAGTCATCCTCG
F14	<u>GGC</u> AGCTGGCCCGCATCATCTCGC
F15	<u>GGC</u> AGCGAAGGGATCATGGCGCTC
F16	<u>GGC</u> ACCACCCCGACCCTTCCGTG
F17	<u>GGC</u> AGTCATTCTCGCGCATGGCAT
F18	<u>GGC</u> AGCTGCTCCTTCTGCTTCTGC
F19	<u>GGC</u> ATGACATAGCTCAGAAGAAGA
F20	<u>GGC</u> AGGAGAAGGCCAAGGAGAAGA
F21	<u>GGC</u> AGCCGAAGCGCCGCAATAGAT
F22	<u>GGC</u> ACAATAGATGGGATCAGTCGC
F23	<u>GGC</u> AGGTGATGCTTCTGCTGCTGC
F24	<u>GGC</u> AGGCGAAGACCTCCTCCGATT
F25	<u>GGC</u> AGATGCTCCTGATGCAACTCC
F26	<u>GGC</u> AGGGCGCTGGGATGCCACCCC
F27	<u>GGC</u> ACGCATCCCCGACACGACCAG
F28	<u>GGC</u> AGTCGGTGAGAAGGAATCGTT
F29	<u>GGC</u> ACAACGATTCTTCTCACCGA
F30	<u>GGC</u> ATGCAGGTGTTGCATCCGCAT
F31	<u>GGC</u> AGCAGCTGGTGGCATTACTCC
F32	<u>GGC</u> AGTAGCATCCCAAGCTCCAGA
F33	<u>GGC</u> AGTTGGCGTGACAAGCCCACC
F34	<u>GGC</u> AGATCTCTGCTTCTTGGGTGT
F35	<u>GGC</u> AGTAGCACTACCCATACTTGC
F36	<u>GGC</u> AGTCGCAGCACCAGTGCCTCC
F37	<u>GGC</u> AGGACCAGGAGTGTAACCTGC
F38	<u>GGC</u> AGCAAGGTTGTCACCACCAAA
F39	<u>GGC</u> AGAAGCAATCTGGCCGGGTGT
F40	<u>GGC</u> ACAGAGCAGTACCAGCTCTTG
F41	<u>GGC</u> AGAACAGGCCTCTTACGGATG
F42	<u>GGC</u> AGCTTGATACTATGTTCCCGC
F43	<u>GGC</u> AGAATCTTGTACCCTTCTG
F44	<u>GGC</u> ACGTATGGGTTGGTATGAAGC
F45	<u>GGC</u> AGTAGCGAGCAGCTTTCTTGC
F46	<u>GGC</u> AACCATTGTATGCTATTCCTG
F47	<u>GGC</u> AGCTATTCTGAGGAGAATCG

F48	<u>GGCAGT</u> GCCCAAGGAGTTGCCTGG
F49	<u>GGCAGTTG</u> CCCCCATGAAGCCAG
F50	<u>GGC</u> ATCAGCAATGTTCCAAAGTAC
F51	<u>GGCAG</u> CTATCTCCAGAAGAGCAGA
F52	<u>GGCAGAT</u> CATGAAGCTCCTGCTCA
F53	<u>GGC</u> ACGAAGTGCTGTCTTCCGCTG
F54	<u>GGCAGCT</u> CATGCAGCCAACACTTG
F55	<u>GGC</u> ATGACAGGGTACTGTATAAGC
F56	<u>GGC</u> AGAATCTTATGCACAAACGGA
F57	<u>GGCAGACT</u> ACTATGCTCGTGTTGA
F58	<u>GGC</u> ATAAGATTTGAGATAATCTCC
F59	<u>GGC</u> ATCAAATCTTAGCAAAGCTGC
F60	<u>GGC</u> AATCAATATCTGGTCTCATGG
F61	<u>GGC</u> ACACTGCTAGAGCATTTAGCG
F62	<u>GGC</u> ATAGCGTGGTGGCTTCTGCTT
F63	<u>GGC</u> ATCCTGGCAAGCTCGGCACAC
F64	<u>GGC</u> AACCAGTGTGCCGAGCTTGCC
F65	<u>GGC</u> ACAGCAGATTGCTATTCTTAT
F66	<u>GGC</u> ACATGCTCAATGATCTCAACT
F67	<u>GGCAGT</u> GACGAGAACCAGAAAGTG
F68	<u>GGC</u> ACAGTGATTGTCCGCACTTTC
F69	<u>GGC</u> AAAGGGTATCAGATCTCACCG
F70	<u>GGC</u> AAGGCAGCAAGAACCTTTCCA
F71	<u>GGCAGCT</u> GCCTTCTTGAAAGCTAT
F72	<u>GGCAGC</u> ATACAGCGCATCCATAAG
F73	<u>GGCAGT</u> GACTGGAACCTCCATAATC
F74	<u>GGCAGAT</u> GAAGAAGATTGTCCTGA
F75	<u>GGCAGT</u> GTAGAGGCTGATTATATC
F76	<u>GGCAGG</u> GAGAATGGCTCTGGACCGC
F77	<u>GGCAGT</u> AGAGATGGCAAATAAGGT
F78	<u>GGC</u> ATATTGTTGGGAGGATTGTTG
F79	<u>GGCAGC</u> CTTACAGGAGAATGGTGA
F80	<u>GGCAGG</u> TGATGGAAACAATTGAGA
F81	<u>GGC</u> ATTGGAGGAGCTGCTTATTGA
F82	<u>GGC</u> ACCACAAATCTGAGGAAGGTA
F83	<u>GGC</u> AAAGCTGCTGATCTGATCTCG
F84	<u>GGCAGT</u> GTCAGGAGGAGCAGCTTA
F85	<u>GGC</u> AGAAGAGTACCCTGAGGTGTT
F86	<u>GGCAGG</u> AAGAAGATCCTTGATTGG
F87	<u>GGC</u> AGAATAGGCATGAGAAGGTCC
F88	<u>GGCAG</u> GAGAACTGCATTGATCTAGT
F89	<u>GGCAGTT</u> GGTAGGATTGCTGATCG
F90	<u>GGC</u> ATGCCTGCTAGGGAGTGGATG
F91	<u>GGC</u> AAATGTTGAAGGCTCACAAGA

F92 (HR-target)	<u>GGCA</u> ATGTTGAAGGCTCACAAGAA
F93	<u>GGCA</u> AGGCTCACAAGAAGGGTATT
F94	<u>GGCA</u> CTCACAAGAAGGGTATTAGA
F95	<u>GGCA</u> GGTTATATTGCGAAGGCTAT
F96	<u>GGCA</u> GTTGAATAACTTGAAGGTGC
F97	<u>GGCA</u> TTGCGACAGTTGTGCAGACA
F98	<u>GGCA</u> GGACTCGGTAATTCATC
F99	<u>GGCA</u> GTATATTGGTGAGATGGGCA
F100	<u>GGCA</u> CATTAGAGCATCTTCAAGCA
F101	<u>GGCA</u> GCTGTGAAGCACATGGCTCT
F102	<u>GGCA</u> TGTTGCTGGCTTGGGCTGTG
F103	<u>GGCA</u> GCTGTCATGGAGGCGATTGA
F104	<u>GGCA</u> GAGGGGATGAGAGTTGCTCT
F105	<u>GGCA</u> CAGTAATTCAGATCACAGC
F106	<u>GGCA</u> CACGTAATTCCTTGTGGA
F107	<u>GGCA</u> TCCTGCACTGGATGACGATG
F108	<u>GGCA</u> TATCTACAGCCGTCAGAGC
F109	<u>GGCA</u> GATGAGACTCCTGCAAGTAT
F110	<u>GGCA</u> TTCTCTCCTTCTGCTCTC
F111	<u>GGCA</u> AAAAGCAAGGGAATTTGGTGC
F112	<u>GGCA</u> GGTAGAATCTTGTGAACAA
F113	<u>GGCA</u> TCATCAATGTTATCAATATC
F114	<u>GGCA</u> ATAACATTGATGAGTATGTG
F115	<u>GGCA</u> CACGCTAAATGCTCTAGCAG
F116	<u>GGCA</u> TCATCTCTTCATCTGGTGAC
F117	<u>GGCA</u> ATCTTCTTCATCTCTTCATC
F118	<u>GGCA</u> CAGCAGAGGCAGCAGTTTGC
F119	<u>GGCA</u> CACATTTGGTTATATTGCGA
R1	<u>AAAC</u> CCTGCGCCCGCGGAGCTCC
R2	<u>AAAC</u> CCTCCATCTTCTTGCCTCC
R3	<u>AAAC</u> CCGGCGCGCCCATGGCCGTC
R4	<u>AAAC</u> TCGACACCGATCTCTACGGC
R5	<u>AAAC</u> ACCCCAACCGCTTCGCCGGC
R6	<u>AAAC</u> CCATCCCCGCCTCCGAGGAC
R7	<u>AAAC</u> CGGGCGCGTCGTCCTCGGAG
R8	<u>AAAC</u> AGGCGGCCGTCACCCCGCC
R9	<u>AAAC</u> GCCGCCTCGCCTCCTACACC
R10	<u>AAAC</u> GCCACGCCGTCGCCGCCGCC
R11	<u>AAAC</u> CGGCGGCGGGGGATGTGC
R12	<u>AAAC</u> AGAAGTCGACGCGATCATC
R13	<u>AAAC</u> CGAGGATGACTATCGCCGCC
R14	<u>AAAC</u> GCGAGATGATGCGGGCCAGC
R15	<u>AAAC</u> GAGCGCCATGATCCCTTCGC
R16	<u>AAAC</u> CACGGAAGGGTCCGGGGTGG

R17	<u>AAACATGCCATGCGCGAGAATGAC</u>
R18	<u>AAACGCAGAAGCAGAAGGAGCAGC</u>
R19	<u>AAACTCTTCTTCTGAGCTATGTCA</u>
R20	<u>AAACTCTTCTCCTTGGCCTTCTCC</u>
R21	<u>AAACATCTATTGCGGCGCTTCGGC</u>
R22	<u>AAACGCGACTGATCCCATCTATTG</u>
R23	<u>AAACGCAGCAGCAGAAGCATCACC</u>
R24	<u>AAACAATCGGAGGAGGTCTTCGCC</u>
R25	<u>AAACGGAGTTGCATCAGGAGCATC</u>
R26	<u>AAACGGGGTGGCATCCCAGCGCCC</u>
R27	<u>AAACCTGGTCGTGTCGGGGATGCG</u>
R28	<u>AAACAACGATTCCCTTCTCACCGAC</u>
R29	<u>AAACTCGGTGAGAAGGAATCGTTG</u>
R30	<u>AAACATGCGGATGCAACACCTGCA</u>
R31	<u>AAACGGAGTAATGCCACCAGCTGC</u>
R32	<u>AAACTCTGGAGCTTGGGATGCTAC</u>
R33	<u>AAACGGTGGGCTTGTCACGCCAAC</u>
R34	<u>AAACACACCCAAGAAGCAGAGATC</u>
R35	<u>AAACGCAAGTATGGGTAGTGCTAC</u>
R36	<u>AAACGGAGGCACTGGTGCTGCGAC</u>
R37	<u>AAACGCAGTTACACTCCTGGTCC</u>
R38	<u>AAACTTTGGTGGTGACAACCTTGC</u>
R39	<u>AAACACACCCGCCAGATTGCTTC</u>
R40	<u>AAACCAAGAGCTGGTACTGCTCTG</u>
R41	<u>AAACCATCCGTAAGAGGCCTGTTC</u>
R42	<u>AAACGCGGGAACATAGTATCAAGC</u>
R43	<u>AAACCAGGAAGGGTACAAGATTCT</u>
R44	<u>AAACGCTTCATACCAACCCATACG</u>
R45	<u>AAACGCAAGAAAGCTGCTCGCTAC</u>
R46	<u>AAACCAGGAATAGCATACAATGGT</u>
R47	<u>AAACCGATTCTCCTCAGGAATAGC</u>
R48	<u>AAACCCAGGCAACTCCTTGGGCAC</u>
R49	<u>AAACCTGGCTTCATGAGGGGCAAC</u>
R50	<u>AAACGTACTTTGGAACATTGCTGA</u>
R51	<u>AAACTCTGCTCTTCTGGAGATAGC</u>
R52	<u>AAACTGAGCAGGAGCTTCATGATC</u>
R53	<u>AAACCAGCGGAAGACAGCACTTCG</u>
R54	<u>AAACCAAGTGTGGCTGCATGAGC</u>
R55	<u>AAACGCTTATACAGTACCCTGTCA</u>
R56	<u>AAACTCCGTTTGTGCATAAGATTC</u>
R57	<u>AAACTCAACACGAGCATAGTAGTC</u>
R58	<u>AAACGGAGATTATCTCAAATCTTA</u>
R59	<u>AAACGCAGCTTTGCTAAGATTTGA</u>
R60	<u>AAACCCATGAGACCAGATATTGAT</u>

R61	<u>AAAC</u> CGCTAAATGCTCTAGCAGTG
R62	<u>AAACA</u> AGCAGAAGCCACCACGCTA
R63	<u>AAAC</u> GTGTGCCGAGCTTGCCAGGA
R64	<u>AAAC</u> GGCAAGCTCGGCACACTGGT
R65	<u>AAAC</u> ATAAGAATAGCAATCTGCTG
R66	<u>AAAC</u> AGTTGAGATCATTGAGCATG
R67	<u>AAAC</u> CACTTTCTGGTTCTCGTCAC
R68	<u>AAAC</u> GAAAGTGGGACAATCACTG
R69	<u>AAAC</u> CGGTGAGATCTGATACCCTT
R70	<u>AAAC</u> TGGAAAGGTTCTTGCTGCCT
R71	<u>AAAC</u> ATAGCTTCAAGAAGGCAGC
R72	<u>AAAC</u> CTTATGGATGCGCTGTATGC
R73	<u>AAAC</u> GATTAGGGAGTTCCAGTCAC
R74	<u>AAAC</u> TCAGGACAATCTTCTTCATC
R75	<u>AAAC</u> GATATAATCAGCCTCTACAC
R76	<u>AAAC</u> GCGGTCCAGAGCCATTCTCC
R77	<u>AAAC</u> ACCTTATTTGCCATCTCTAC
R78	<u>AAAC</u> CAACAATCCTCCCAACAATA
R79	<u>AAAC</u> TCACCATTCTCCTGTAAGGC
R80	<u>AAAC</u> TCTCAATTGTTTCCATCACC
R81	<u>AAAC</u> TCAATAAGCAGCTCCTCCAA
R82	<u>AAAC</u> TACCTTCTCAGATTTGTGG
R83	<u>AAAC</u> CGAGATCAGATCAGCAGCTT
R84	<u>AAAC</u> TAAGCTGCTCCTCCTGACAC
R85	<u>AAAC</u> AACACCTCAGGGTACTCTTC
R86	<u>AAAC</u> CCAATCAAGGATCTTCTCC
R87	<u>AAAC</u> GGACCTTCTCATGCCTATTC
R88	<u>AAAC</u> ACTAGATCAATGCAGTTCTC
R89	<u>AAAC</u> CGATCAGCAATCCTACCAAC
R90	<u>AAAC</u> CATCCACTCCCTAGCAGGCA
R91	<u>AAAC</u> TCTTGTGAGCCTTCAACATT
R92 (HR-target)	<u>AAAC</u> TTCTTGTGAGCCTTCAACAT
R93	<u>AAACA</u> ATACCCTTCTTGTGAGCCT
R94	<u>AAAC</u> TCTAATACCCTTCTTGTGAG
R95	<u>AAAC</u> ATAGCCTTCGCAATATAACC
R96	<u>AAAC</u> GCACCTTCAAGTTATTCAAC
R97	<u>AAAC</u> TGTCTGCACAACTGTCGCAA
R98	<u>AAAC</u> GATGAATGAGTACCGAGTCC
R99	<u>AAAC</u> TGCCCATCTCACCAATATAC
R100	<u>AAAC</u> TGCTTGAAGATGCTCTAATG
R101	<u>AAAC</u> AGAGCCATGTGCTTACAGC
R102	<u>AAAC</u> CACAGCCAAGCCAGCAACA
R103	<u>AAAC</u> TCAATCGCCTCCATGACAGC
R104	<u>AAAC</u> AGAGCAACTCTCATCCCCTC

R105	<u>AAAC</u> GCTGTGATCTTGAATTACTG
R106	<u>AAACT</u> CCAGCAAGGAAAGTACGTG
R107	<u>AAAC</u> CATCGTCATCCAGTGCAGGA
R108	<u>AAAC</u> GCTCTGGACGGCTGTAGATA
R109	<u>AAAC</u> CATACTTGCAGGAGTCTCATC
R110	<u>AAAC</u> GGAAGAGCAGAAGGAGAGGAA
R111	<u>AAAC</u> GCACCAAATTCCCTTGCTTT
R112	<u>AAACT</u> TGTTCAACAAGATTCTACC
R113	<u>AAAC</u> GATATTGATAACATTGATGA
R114	<u>AAAC</u> CACATACTCATCAATGTTAT
R115	<u>AAAC</u> CTGCTAGAGCATTAGCGTG
R116	<u>AAAC</u> GTCACCAGATGAAGAGATGA
R117	<u>AAAC</u> GATGAAGAGATGAAGAAGAT
R118	<u>AAAC</u> GCAAACCTGCTGCCTCTGCTG
R119	<u>AAACT</u> CGCAATATAACCAAATGTG

Table S3: List of primers used for genotyping

Name of the primer	Sequence (5' - 3')
F1	CTAACCGCCTCTCCCCCGCC
F2	AAGAAGATGGAGGAGGCCCTT
F3	CTTCCGTGCGGACCTATGCC
F4	AAGGAGAAGAAGGCCGTGCC
F5	ACCGTCGGTGAGAAGGAATCGT

F6	AGAAAGCTGCTCGCTACACCA
F7	GCGGAAGACAGCACTTCGACAA
F8	CAAAGCTGCTGGTCTTGCTACC
F9	CCACATCTCAAGTCCCTAGTT
F10	GATGGAAACAATTGAGAAGGTT
F11	ACAAGCTGCTGATCTGATCTCG
F12	GCAAAAACCGTGTCTGCACA
F13	TTGCTTAACTATGTCTGGCCA
R1	CAACGGCAACAAAGTAAACATT
R2	CCACCGAGTTCAAGCTTCACAG
R3	CATAGAATTCTAACATATCAATGTA
R4	CAATAATATATGCCACAAAATTTAAGC
R5	TCATCAGGGCAGGCAGAACC
R6	ATAAGCTGCTCCTCCTGACAC
R7	TGAAGCACCCAAATTAGCCACA
R8	GTCTCATGGCGGCAATCATG
R9	GGTAATCCTCTGGCTTCATGAG
R10	ACGATTCTTCTCACCGACGGT
R11	CTGCCACTGGCTGCTGCTCG
R12	CTTCCTTCTTCTTCTGAGCTATG
T-DNA-F	CACAGGAAACAGCTATGAC
T-DNA-R	CGCGCTAAAAACGGACTAG

Table S4: CDE-platform for SF3B1 resistance to splicing inhibitor

	CRISPR-directed evolution of SF3B1 via targeted sgRNA library			Directed evolution of SF3B1 via domain-specific sgRNAs				
No. of transformation	2			1 (One for each sgRNA)				
No. of sgRNAs	119 (in an sgRNA library transformed to Agrobacterium)			3				
				sgRNA-92		sgRNA-119	PTG	
No. of calli transformed	~15,000			1000		1000	1000	
No. of shoots/plants recovered on GEX1A supplemented media	21 seedlings from 0.4 μ M			8 seedlings from 0.4 μ M		4 seedlings from 0.6 μ M	No seedlings	5 seedlings from 0.4 μ M
No. of plants showing SF3B1 editing	<i>SGR1</i>	<i>SGR2</i>	<i>SGR3</i>	<i>SGR3</i>	<i>SGR5</i>	<i>SGR4</i>	—	<i>SGR6</i>
	2	2	3	3	2	2		2
Heritability of GEX1A resistance in seed progeny (T1)	<i>SGR1</i>	<i>SGR2</i>	<i>SGR3</i>	<i>SGR3</i>	<i>SGR5</i>	<i>SGR4</i>	—	<i>SGR6</i>
	2	2	3	3	2	2		2

*Please note that all false positives exhibit no SF3B1 editing and their seed progeny is sensitive to GEX1A

**Please note that one set of transformation was conducted without GEX1A selection for targeted mutagenesis of SF3B1 and no functional knock out mutant could be recovered from these (except one in-frame mutant *SGS1*).

Table S5: List of primers used for Intron retention analysis

Name of the primer	Sequence (5' - 3')
03g55590_F	GCTACTCCAAAGCAAATCAGGGAG
03g55590_R	CGGACATAGCCTTCCGACTTG
04g37790_F	GTCTTCTAATTCGGTTCCTGACACG
04g37790_R	GGGGACGGCTTGAATCCTTG
04g57440_F	GCTAAGGTTGTGAGCATCGATGG
04g57440_R	CCAAGAGTTGCGGACAATCCAG
07g08460_F	GGGAGGAGGGCTGTATGTGAAG
07g08460_R	CTGGCTTCAGAGCCCTTCATG
07g35350_F	CGGACTGCAGTGCTATCCAACAG
07g35350_R	CATTGAATGCATAGGTCGCATGCG
07g48510_F	GGAGGTGCTGTTCTTGCAAGTG
07g48510_R	TTGGGACAAGGTCTTGGTTTGG
08g36910_F	GAGATCGATCAGTAGTGGTTAGCAG
08g36910_R	GCGTCCAGGTCGTAGAGACG
08g44390_F	GAGAAGCTCTACGCAGTCTTGG
08g44390_R	GGACAATGAAGGAATGACCATTG
10g31850_F	GCATGGGGAAATACTTCTGCG
10g31850_R	TCGAAGCAGACAGGACAGTTG