

Human spliceosomal snRNA sequence variants generate variant spliceosomes

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Supplemental Materials:

Figure S1. Related to Figure 1. Characterization of snRNA variant genes.

Figure S2. Mapping RNA-sequencing reads to snRNA genes.

Figure S3. Related to Figure 2. Sanger-sequencing RTqPCR amplicons.

Figure S4. Related to Figure 2C. Differential cell line expression of human snRNA variants.

Figure S5. Genomic alignments of snRNA gene variants.

Figure S6. Genomic alignments of snRNA gene promoters.

Figure S7. Related to Figure 5. Variant snRNAs generate variant containing spliceosomes.

Table S1. Overview of human snRNA gene families.

Table S2. List of DNA oligos for RTqPCR.

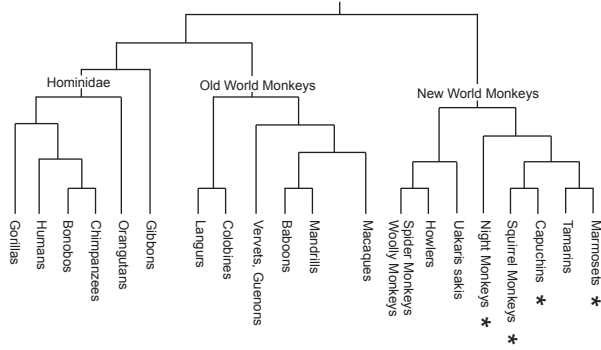
Table S3. Characteristics of human snRNA variants.

Table S4. Related to Figure 4. List of snRNA variant half-lives.

Table S5. List of DNA oligos for Northern blotting.

Supplemental Figure 1

A



B



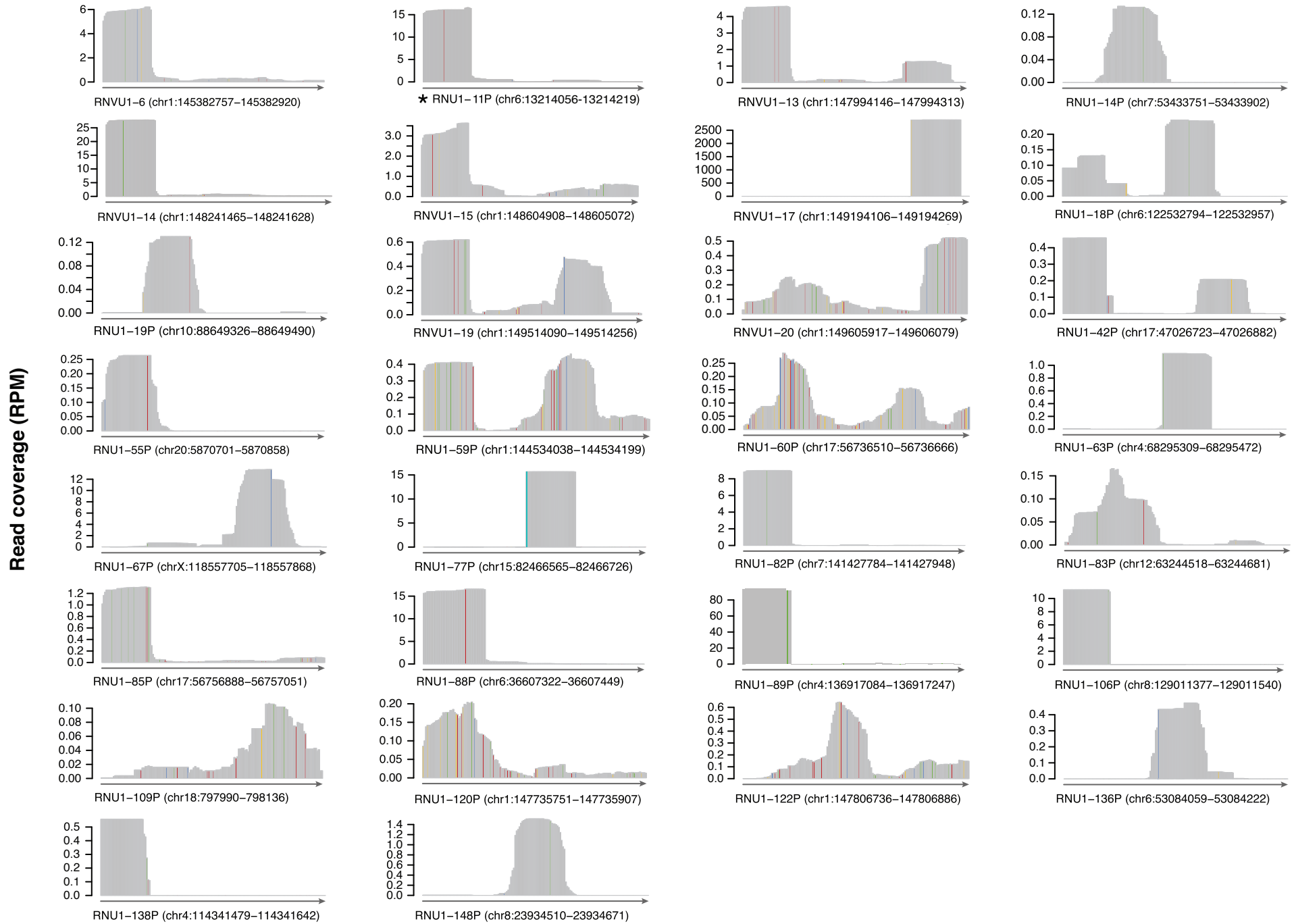
Human snRNA sequence variant genomic loci

Supplemental Figure 2

A

Sequence variation
compared to RNU1-1

■ ■ ■ ■
A C G T



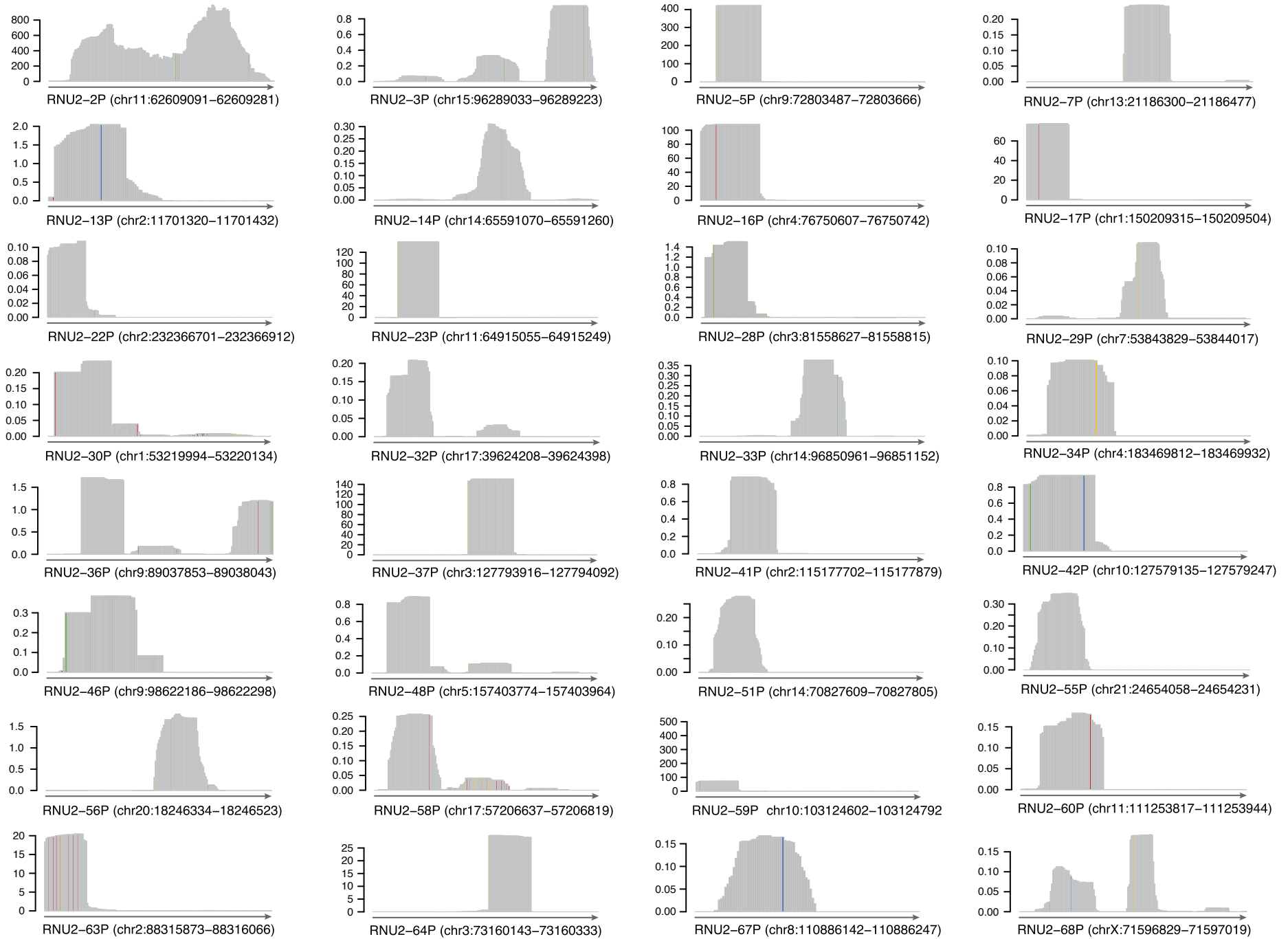
Supplemental Figure 2

B

Sequence variation
compared to RNU2-1

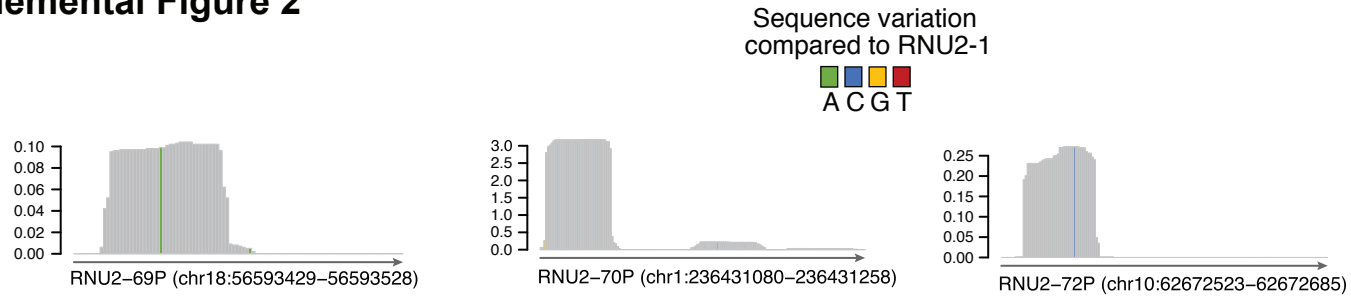
■ ■ ■ ■
A C G T

Read coverage (RPM)

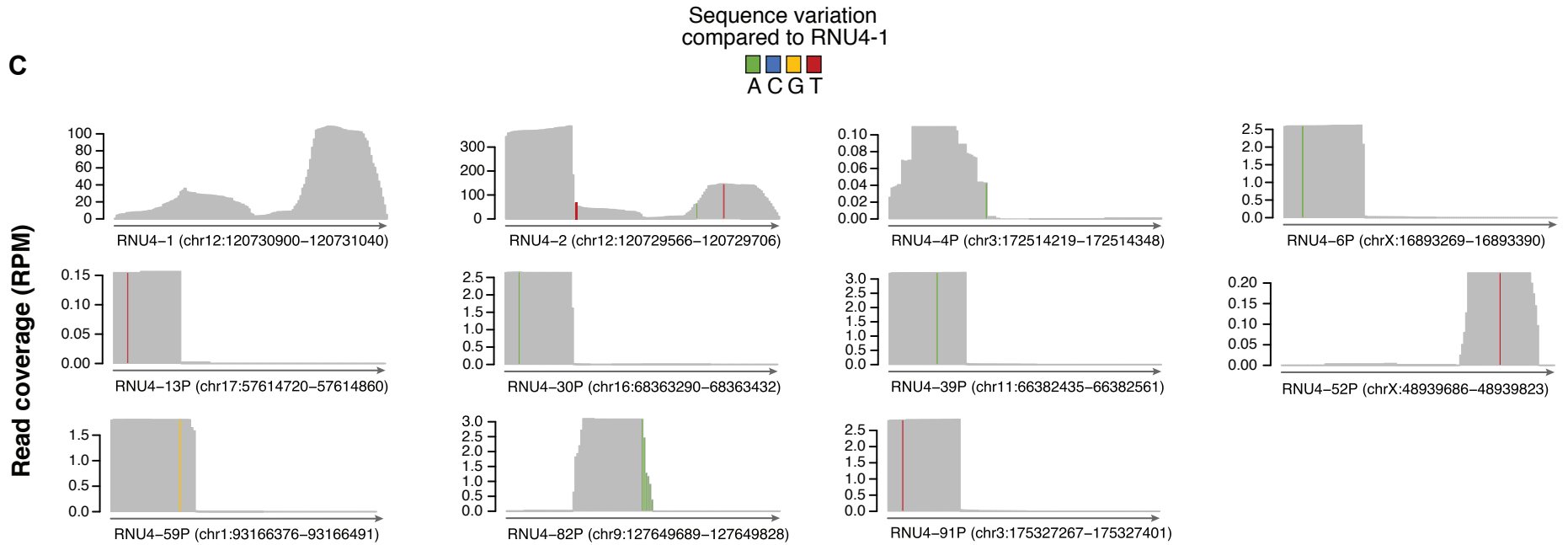


Supplemental Figure 2

B



C

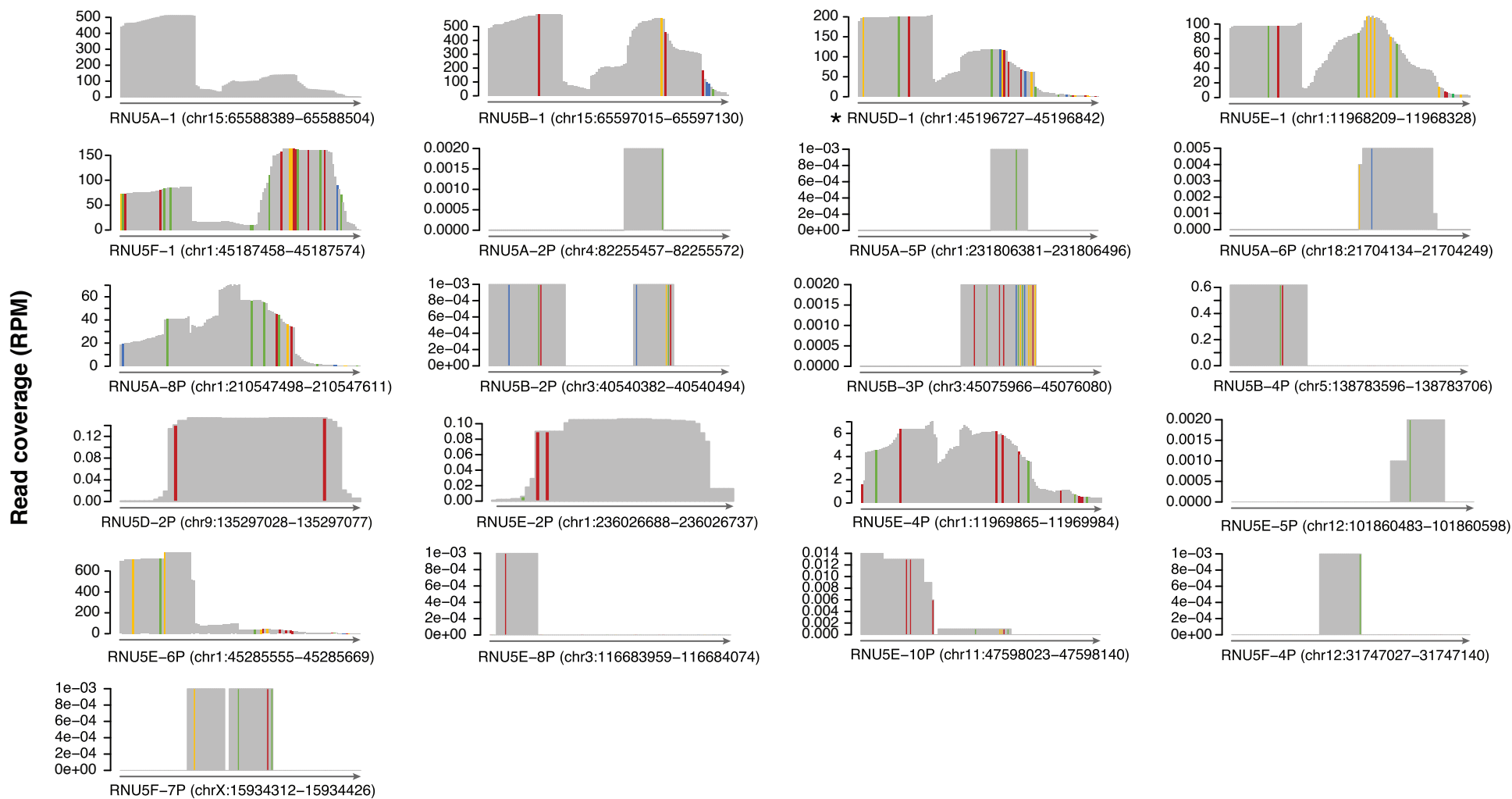


Supplemental Figure 2

D

Sequence variation
compared to RNU5A-1

■ ■ ■ ■
A C G T



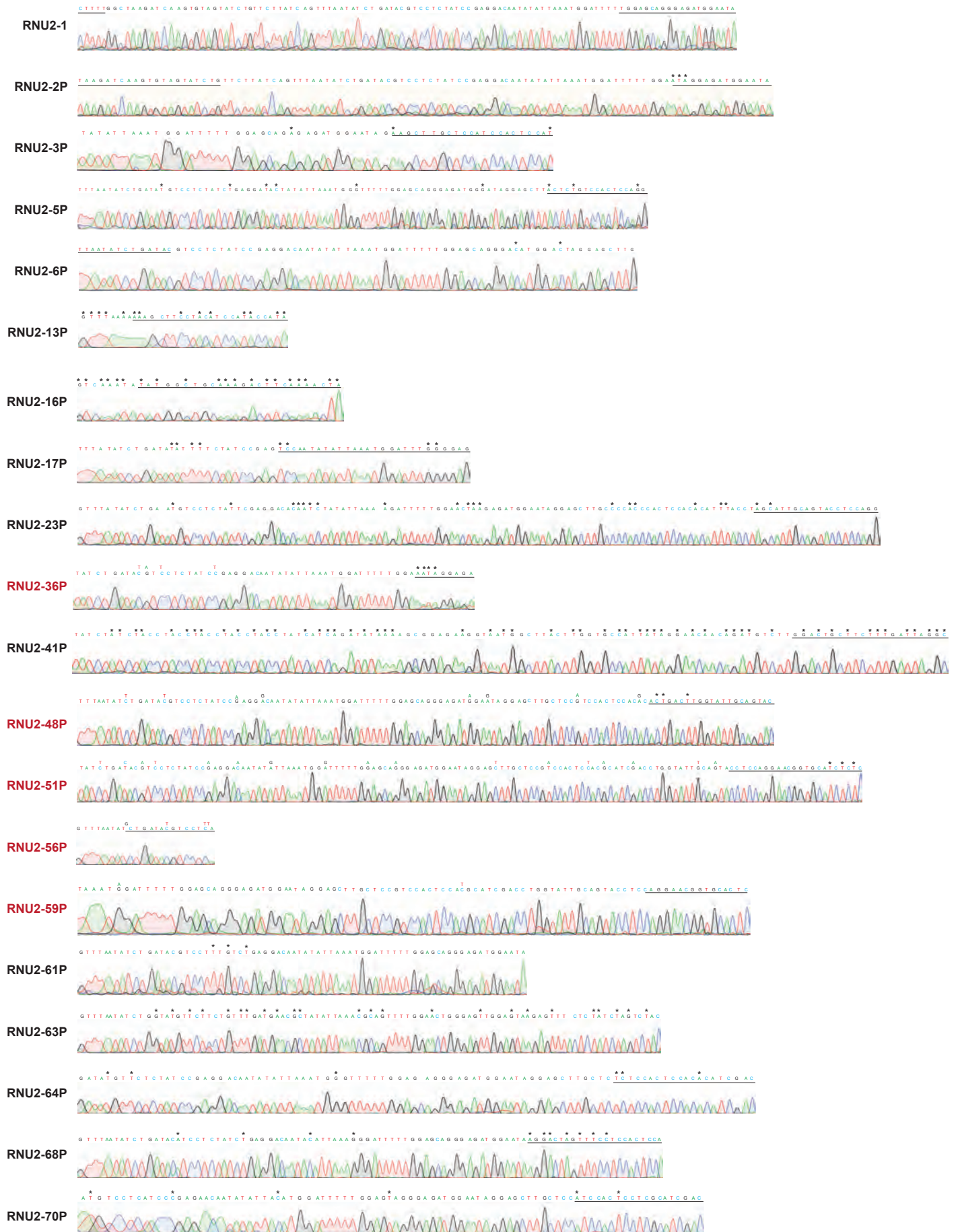
Supplemental Figure 3

A



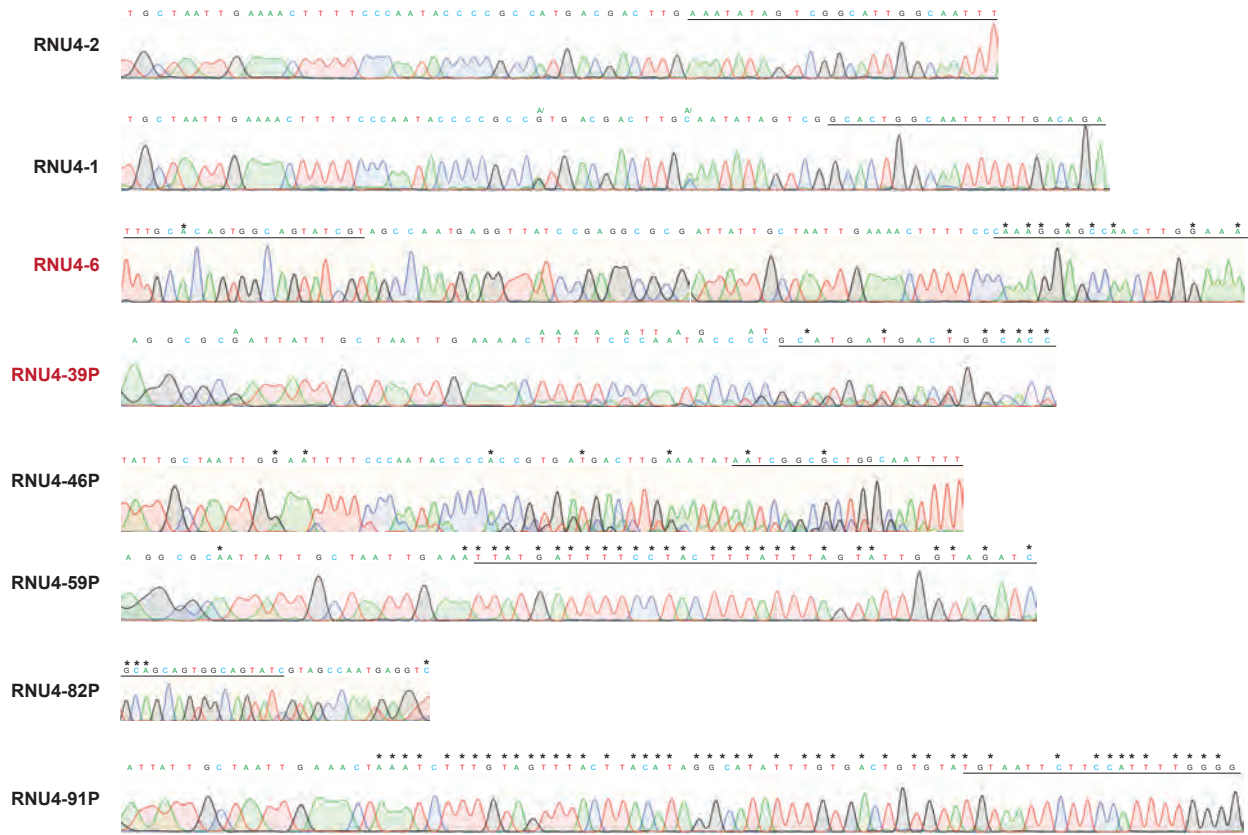
Supplemental Figure 3

B



Supplemental Figure 3

C



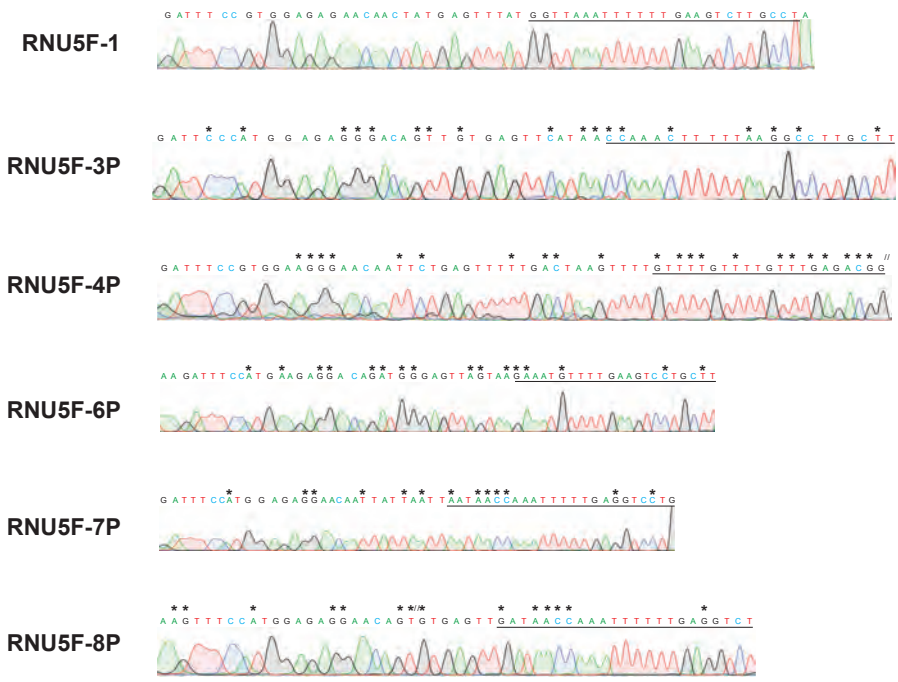
Supplemental Figure 3

D



Supplemental Figure 3

D

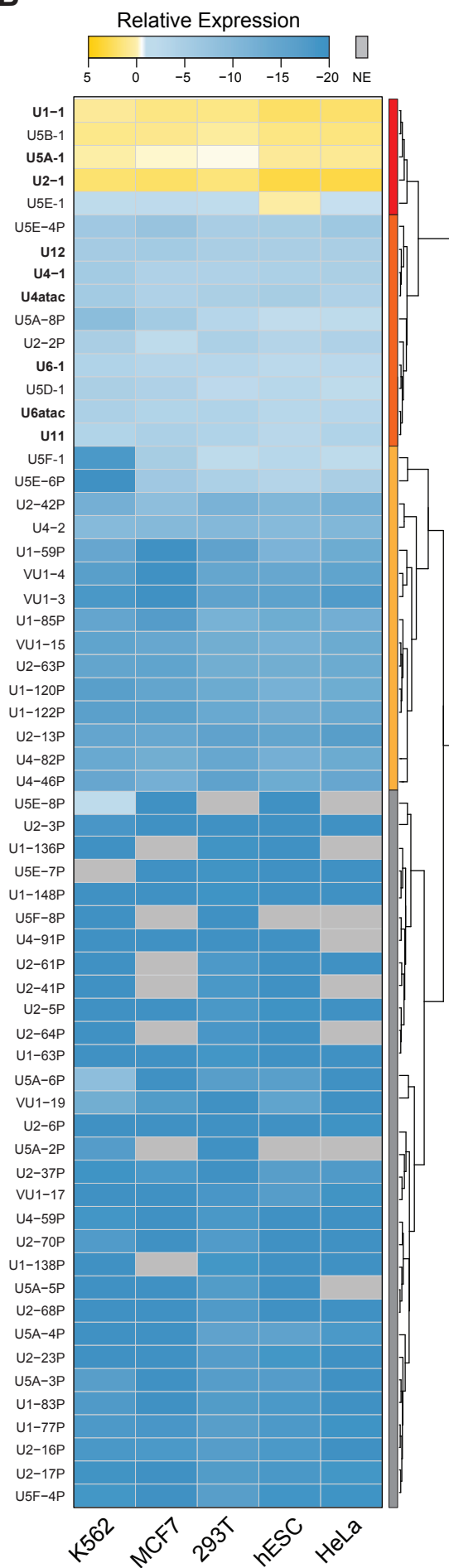


Supplemental Figure 4

A

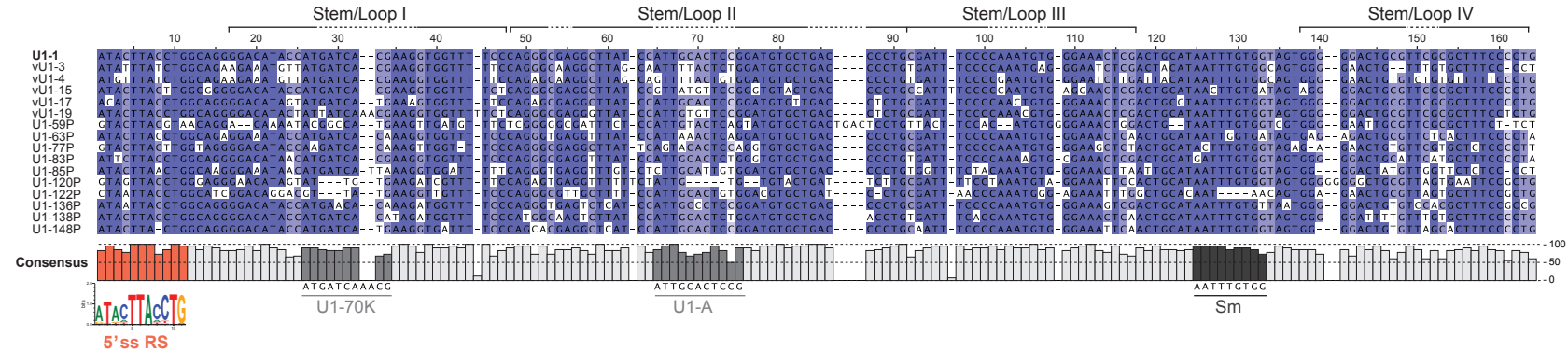
| snRNA | Gene Dosage | % Rel. Expr. |
|----------|-------------|---------------|
| U1-1 | 7 | 100.0 ± 0.75% |
| U2-1 | 5-20 | 76.8 ± 0.30% |
| U4-1 | 1 | 1.6 ± 0.11% |
| U5A-1 | 1 | 13.0 ± 0.22% |
| U5B-1 | 1 | 18.5 ± 0.53% |
| U5D-1 | 1 | 3.4 ± 0.10% |
| U5E-1 | 1 | 8.4 ± 0.25% |
| U5F-1 | 1 | 6.1 ± 0.20% |
| U5A-8P | 1 | 1.8 ± 0.10% |
| U5E-4P | 1 | 0.6 ± 0.01% |
| U5E-6P | 1 | 0.8 ± 0.01% |
| Total U5 | 8 | 52.0 ± 0.20% |
| U6-1 | 1 | 4.2 ± 0.41% |
| U11 | 1 | 1.6 ± 0.11% |
| U12 | 1 | 0.6 ± 0.06% |
| U4atac | 1 | 0.7 ± 0.21% |
| U6atac | 1 | 2.3 ± 0.20% |

B

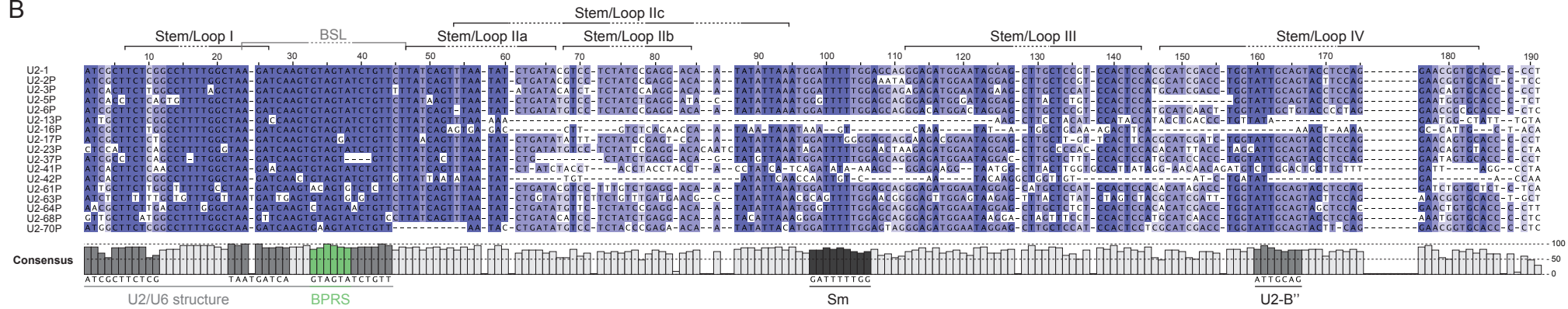


Supplemental Figure 5

A

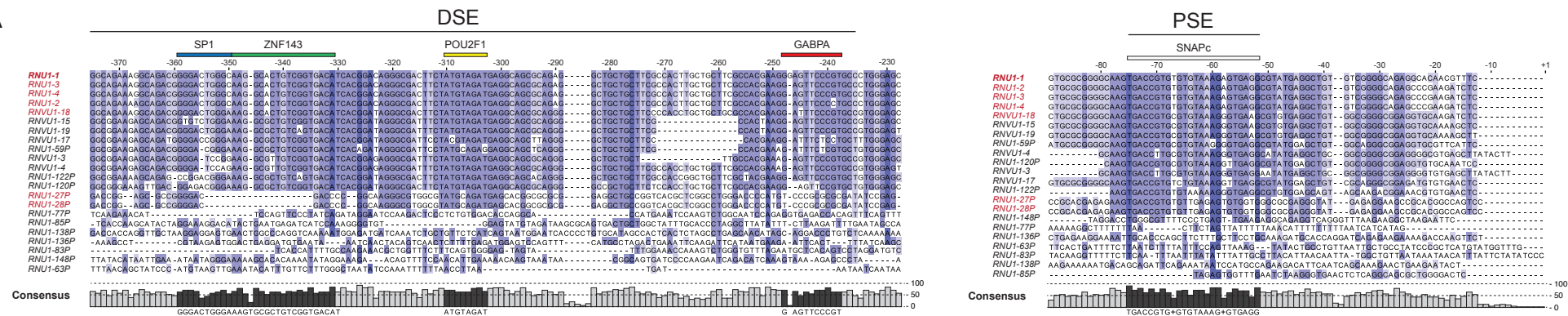


B

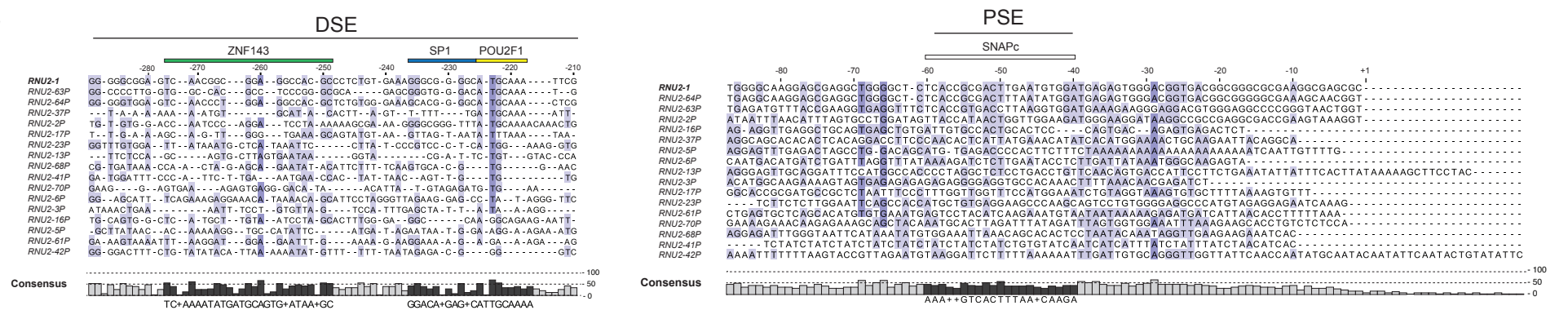


Supplemental Figure 6

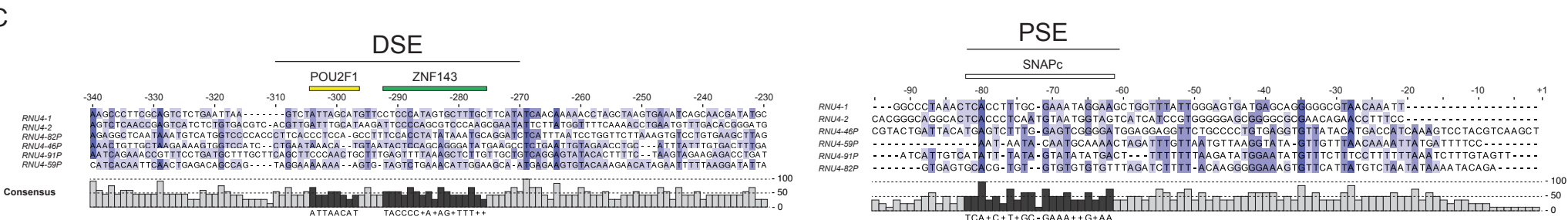
A



B

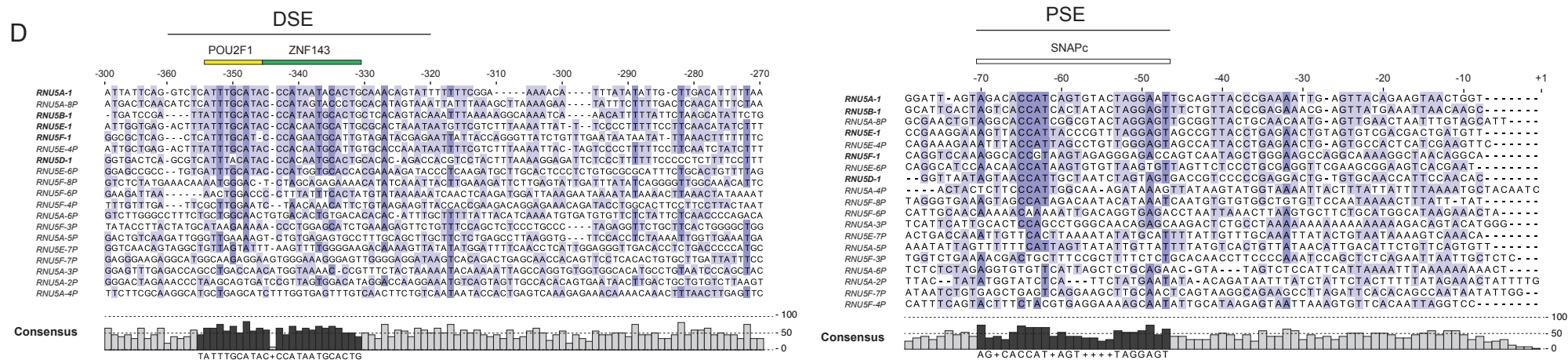


C

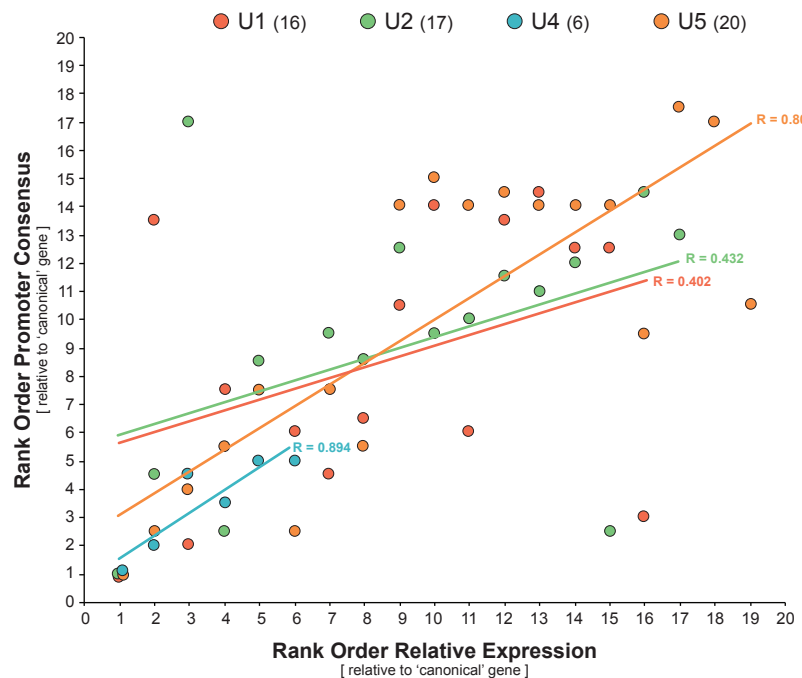


Supplemental Figure 6

D



E



Supplemental Figure 7

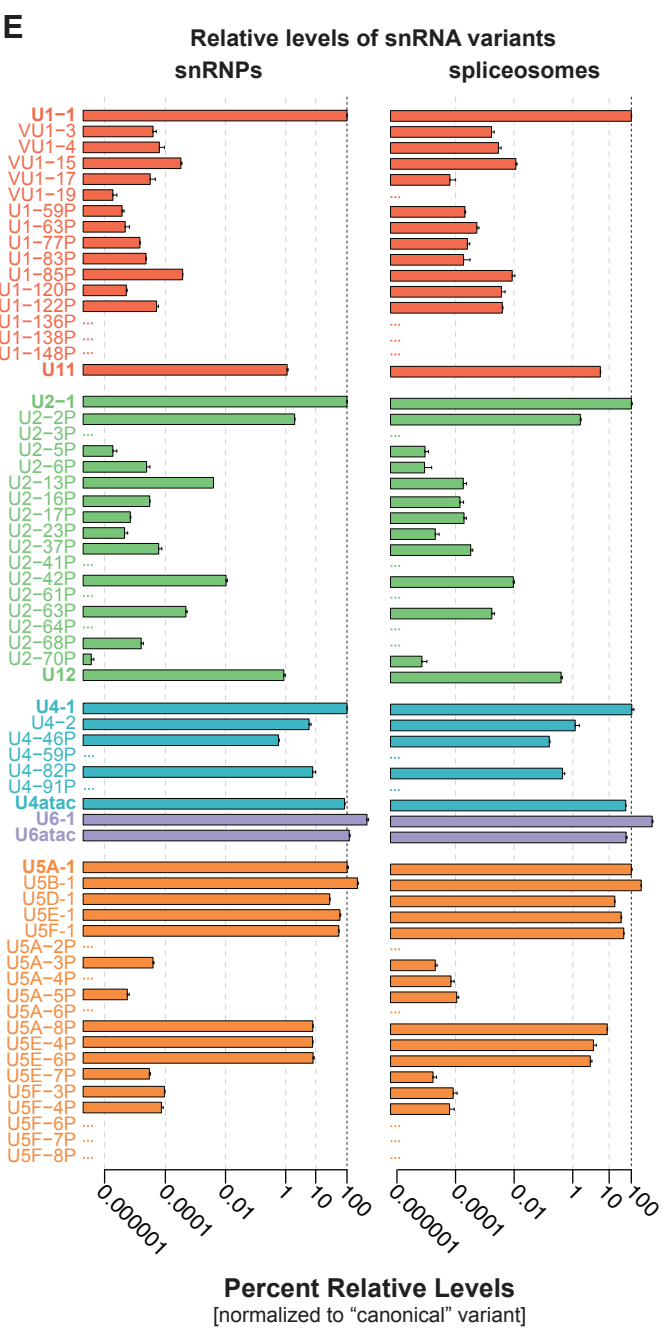
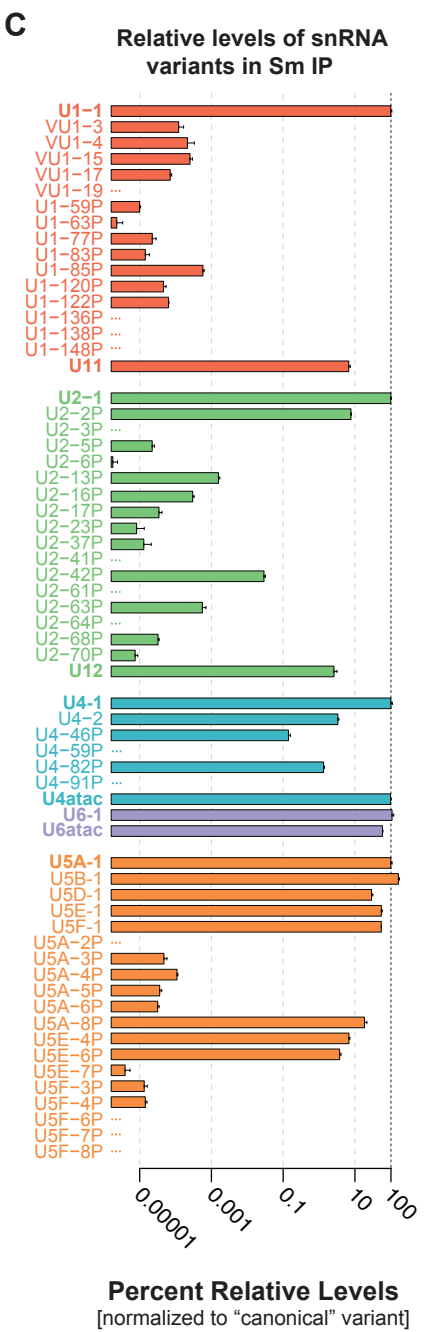
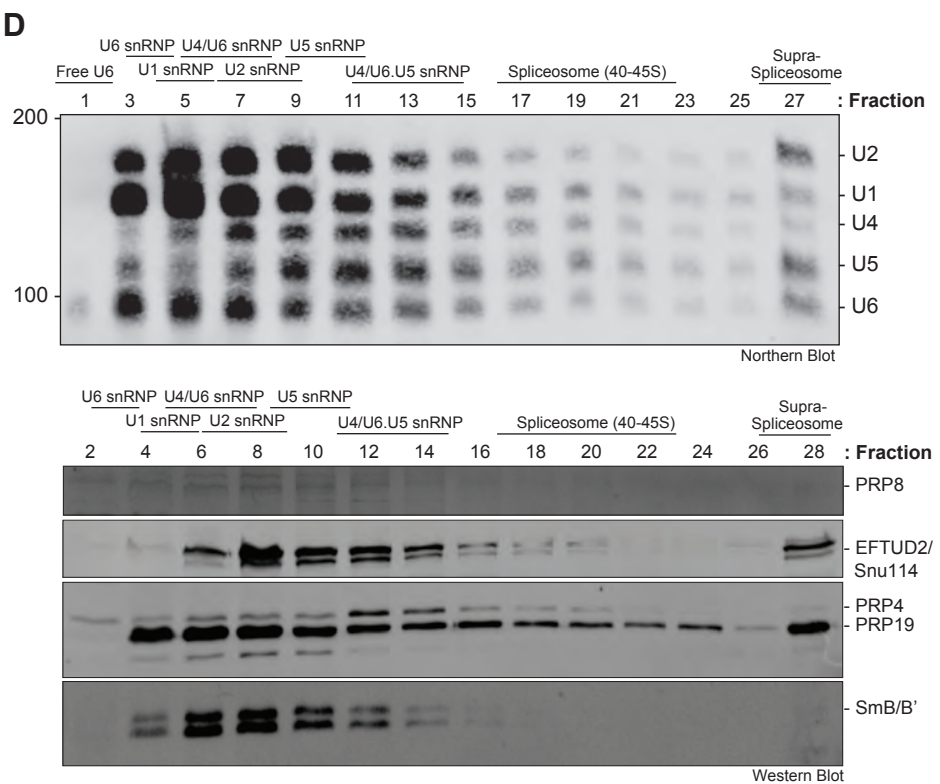
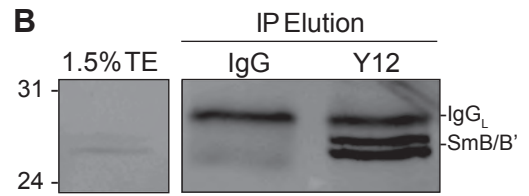
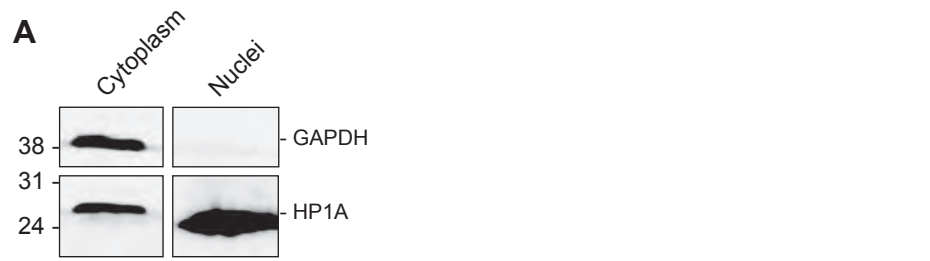


Table S1: Overview of human snRNA gene families, based on annotation in Ensembl database v. 91.**snRNA:** snRNA gene family**Length:** mature length of the canonical snRNA**Polymerase:** gene transcribed by RNA polymerase II or III**snRNP assembly:** cellular location where snRNP biogenesis occur(s)**# variants:** number of total annotated snRNA variant genes in Ensembl database v. 91**Variant length mean (sd):** mean length of all snRNA variants (standard deviation)**% identity mean (sd):** mean percent nucleotide identity as compared to the canonical snRNA (standard deviation)

| snRNA | Length | Polymerase | snRNP assembly | # genes | Variant length mean (sd) | % identity mean (sd) |
|-------|--------|------------|-----------------------|---------|--------------------------|----------------------|
| U1 | 164 | RNAP II | Nucleus and cytoplasm | 178 | 159 (11) | 77 (12) |
| U2 | 191 | RNAP II | Nucleus and cytoplasm | 93 | 170 (34) | 75 (17) |
| U4 | 141 | RNAP II | Nucleus and cytoplasm | 99 | 135 (23) | 72 (13) |
| U5 | 116 | RNAP II | Nucleus and cytoplasm | 33 | 109 (17) | 78 (9) |
| U6 | 107 | RNAP III | Nucleus only? | 1437 | 104 (7) | 72 (13) |

Table S2: List of DNA oligos for RTqPCR.**Gene:** snRNA name**Forward oligo:** Forward oligo sequence (5'-3')**Reverse oligo:** Reverse oligo sequence (5'-3')**Amplicon:** PCR amplicon length in base pairs**Sequencing:** positive or negative sequencing result by Sanger sequencing PCR amplicons, or no amplification (NA)

| snRNA | Forward oligo | Reverse oligo | Amplicon | Sequencing |
|---------|--------------------------------|-------------------------------|----------|------------|
| U1-1 | ATACTTACCTGGCAGGGGAGA | GCAGTCGAGTTTCCCACATT | 122 | + |
| VU1-3 | ATATTATCTGGCAGAAGAAATGTTATG | AGATTCCTCATTTGGGGAAATCA | 115 | + |
| VU1-4 | ATGTTTATCTGGCAGAAGAAATGTTA | ATTATGTAATCAAGATTCCCACATTC | 127 | + |
| VU1-6 | ACCTGGCAGGAGAGATACCCTGG | AAGTTTCCCACATTTGGGGAAACT | 107 | NA |
| VU1-14 | CCTGGAAGGGGAGATACCAT | ATGCACTCGAGTTTCCCACAC | 118 | - |
| VU1-15 | ATACTTACTTGGCGGGGAGA | TTCCTCACATTTGGGGAAATG | 114 | + |
| VU1-17 | CAGGGGAGATAGTATGATCATGAAA | CGCAGTCGAGTTTCCCACAG | 111 | + |
| VU1-19 | GCAGGGGAGATACTATTATCAAA | TTATGCAGTCGAGTTTCCCACG | 119 | + |
| U1-42P | CAGGGGAGATACCATGATCCT | CCACATTTGGGGAAATCGCG | 95 | - |
| U1-59P | TTACGTAACAGGAGAAAATACGGC | ACATGTGGAAGTAACAGGAGTCA | 103 | + |
| U1-63P | AGCTGGCAGAGGAAATACCA | GGAAAGTGAGAACGCAGTCTCT | 154 | + |
| U1-67P | ATGCTTAACTGGCAGGGGAGATA | CACAGTCCCCACTACCACAAATG | 142 | - |
| U1-77P | TGGTAGGGGAGATACCAAGATCA | GGAGAGCACGAACACAGTCTCT | 149 | + |
| U1-82P | GGTGGTTTTTCCAGCGTGG | GTCGAGTTTTCCACATTTGGGAAT | 82 | - |
| U1-83P | TCTTACCTGGCAGGGGAGATAA | AGTTTCGCACTTTTGGGGAAATCA | 113 | + |
| U1-85P | GGCAAGGGAATAACATGATCATT | GGGGAGAGAACCAACATAGTCC | 153 | + |
| U1-88P | CCTGGCAGGGGAGATACTATG | TTATTTTCTTTGAATTTTTTTTCATTTGG | 121 | - |
| U1-120P | CCTGGGAGGGAAGATAGTATTG | TCCTACATTTAGGAAATCGCAAGAA | 95 | + |
| U1-122P | TGGCATCGGAGAGGACGTTAT | GTTATTGTGCAGCCAAATTTCTCC | 117 | + |
| U1-136P | GGGGAGATACCATGAACACAAAGA | GTGGACACAGTCCCCATTAA | 139 | + |
| U1-138P | GGGAGATACCATGATCACATAGA | CAGGGGAAAGCACAAACAAAATC | 149 | + |
| U1-148P | ATCATGAAGGTGATTTTCCAGCA | CCACTACCACAAATTATGCAGTTGAA | 111 | + |
| U2-1 | GGCCTTTTGCTAAGATCAA | TATTCATCTCCCTGCTCCA | 123 | + |
| U2-2P | GGCTAAGATCAAGTGATGATCTG | CAAGCTCTATTCCATCTCTATT | 113 | + |
| U2-3P | TGTTTTTATCAGTTTAATATGATACATCTT | ATGGAGTGGATGGAGCAAGCTT | 105 | NA |
| U2-5P | ATCACCTCTCAGTGTTTGGCTAA | CAATACCTGGAGTGGACAGAGT | 152 | + |
| U2-6P | TATCTGTTCTATCAGTTAATATCTGATAT | GTACAGCAATACCAAGTTGATGCA | 132 | + |

| | | | | |
|--------|---------------------------------|--------------------------------------|-----|----|
| U2-13P | TTCTCGGCTTTTGGCTAAG | GTATGGTATGGATGTAGGAAGCTTTTT | 80 | + |
| U2-14P | TCACTTCTTGGTCTTTTGGCTAAG | GGTCAATGTGTGGAGTGGATGT | 155 | NA |
| U2-16P | TGGCCTTTTGGCTAAGATCAA | AGTTTTGAAGTCTTTGCGCCATA | 109 | + |
| U2-17P | CTTTTGGCTAAGATCAAGTGTAGG | CTCCCCAAATCCATTTAATATATTGGA | 95 | + |
| U2-23P | TCCATTCTCAGCCTTTTGGG | TCCTGGAGGTACTGCAATGCT | 180 | + |
| U2-36P | TCAGCCTTTTGGCTAAGATCGG | GTAAGCTCCTATTCCATCTCCTATT | 124 | NA |
| U2-37P | TATCTGCTATCTGAGGACAGTATG | CCTTGCTCTTCCACTCCATG | 81 | + |
| U2-41P | TCTCAACCTTTTGGCTAAGAACAA | GGCCTAATCAAAGAAGCAGTCC | 170 | + |
| U2-42P | TTCTCGGCTTTTGGCTAAG | TTCATATCAGATACAACCAGCCTTG | 100 | + |
| U2-48P | GCTTCTAGATATTTTGGCTAAGATCAAG | GAGGTACTGCAATACCAAGTCAAGTGT | 169 | - |
| U2-51P | AGTGAACCTTATCCTTTTGGCTAAGA | GAGAGATGCACCGTTCCTGG | 197 | - |
| U2-55P | AATTCTCGGCTTTTGGCTG | ATTCATGCGGGGCTTAAAC | 158 | NA |
| U2-56P | GCTTCTTGGCCTTTTGGTTAAGTT | GCTGATATGCTTTTATCTGAGGA | 81 | - |
| U2-59P | GTTTAGTATCTGATATGCTCTATCT | AGCGAGTGCACCGTTCCTG | 140 | - |
| U2-61P | GCCTAAGATCAAGTACAGTGTCTC | GGTACTGCAATACCAGGTCTATGT | 152 | + |
| U2-63P | ATCTCTTTTTGCTGTTTGGTTAATGATT | GTAGACTAGATAGAGTAAACTCTTAC | 149 | + |
| U2-64P | TTTGGGCTAAGATCAAGTCTAGTAA | GGTCGATGTGTGGAGTGGAGA | 141 | + |
| U2-68P | GCTTCATGGCCTTTTGGCTAAGT | TGGAGTGGAGGAACTAGTCTCCT | 142 | + |
| U2-70P | TAAGATCAAGTGAAGTATCTGTTAATAC | GGTCGATGCGAGGAGTGGAT | 123 | + |
| U4-1 | TGGCAGTATCGTAGCCAATG | CTGTCAAAAATTGCCAGTGC | 108 | + |
| U4-2 | GCAGTATCGTAGCCAATGAGGTT | AAATTGCCAATGCCACTATATTT | 108 | + |
| U4-6P | TTGCACAGTGGCAGTATCGT | TTTCCAAGTTGGCTCCTTTG | 106 | - |
| U4-39P | CTTTGCGCAGTGGCAGTATCA | ACAGGTGCCAGTCATCATGC | 103 | - |
| U4-46P | TGCAGTGGCAGTACCGTAGC | AAAAATTGCCAGCGCTGATT | 116 | + |
| U4-59P | GCAGTGGCAGTATCGTAGCCAG | GATCTACCAATACTAAATAAAGTAGGAAAATCATAA | 95 | + |
| U4-82P | TTTGCAGCAGTGGCAGTATC | ACATGGGTCTGCTTTCTGTT | 137 | + |
| U4-91P | TGCAGTGGCAGTATCGTAGC | CCCCAAAATGGAAGAATTACA | 124 | + |
| U6 | CGCTTCGGCAGCACATATAC | ATGGAACGCTTACGAATTT | 96 | + |
| U11 | CTTCTGTCGTGAGTGGCACA | CAGCTGCCCAAATACCAAAA | 102 | + |
| U4atac | CCATCCTTTTCTTGGGGTTG | TAGTTGATGCGGGTGTGTTG | 90 | + |
| U6atac | AGGTTAGCACTCCCCTTGACA | ACGATGGTTAGATGCCACGA | 101 | + |
| U12 | AACCTTATGAGTAAGGAAAATAACGATTCTG | CGACCTTTACCCGCTCAAAA | 88 | + |
| U5A-1 | CTCTGGTTTCTTTCAGATCGC | CAAGGCCTCAAAAATTGGG | 100 | + |
| U5B-1 | ATACTCTGGTTTCTTTCAGATCGT | GAACAAGGCCTCAAAAATTAGC | 106 | + |
| U5D-1 | GCTCTGGTTTCTTTCAAATCGT | GGGCTTCAAAAATTGCTTG | 99 | + |
| U5E-1 | ATACTCTGGTTTCTTTCAAATCGT | AGGCCTCAAAAATTGGTTTC | 101 | + |
| U5F-1 | GATCTCTGGTTTCTTTCATAACGA | AGGCAAGACTTCAAAAATTTAACC | 107 | + |
| U5A-2P | ATTCTCCAGTTTCTTTCAGATCA | TGCCAAAATAAGGCCTCAAAA | 110 | + |
| U5A-3P | ATACTCTGGTTTCTTTCAGATCATACA | GATGCAGGGCCTCAAAAAT | 106 | + |
| U5A-4P | ATACTCCAGTTTCTTTCAGATCAT | CCAACGCAAGGTCTCACAATA | 105 | + |
| U5A-5P | TCCTGGTTTCTTTCAGATCATA | TTATACAAAGCACCAAGGCTGAA | 111 | + |
| U5A-6P | ATACTCCAGTTACCCTTCAGATCA | GCAGATACAGGGCCTCAAAA | 110 | + |
| U5A-7P | TCTGGCTTGTTCAGGTCAC | AAAGGAAGACCTCAAAAATTAGCTA | 103 | - |
| U5A-8P | ACACTGGTTTCTTTCAGATCGA | GGCAAGGCTTACAAAATTAGC | 102 | + |
| U5B-2P | TGGCTTCTTTCAGATCATATAAATC | GCCATTCTAACTGGCATGAGA | 105 | - |
| U5B-3P | GATTTCTCCTGAGATCGTATAAATCG | CAGCAAGAGGACAAATGCAG | 103 | - |
| U5B-4P | ATACTCTGGTTTCTTTCAGATCATATAAA | AGCAAAGGCTCAAAAATTAGCTA | 100 | - |
| U5E-4P | TACTCTAGTTTCTTTCATATCGCAT | CAAGACCTTCAAAAATTGGTTAAA | 104 | + |
| U5E-5P | ATACTCTGGTATCTTTCAGATTATATCAATT | AGGCCTCAAAAATTGGTTAAG | 101 | - |
| U5E-6P | ATACTCGGGTTTCTTTCAAAGC | AATAAGAGGCCTCAAAAATTGAATA | 105 | + |

| | | | | |
|---------|--------------------------------|--------------------------|-----|----|
| U5E-7P | TCAGATCATATAAATCTTTTGCCTTTT | AGCCCTGCTAAAGCGAGAC | 101 | + |
| U5E-8P | TAACTCTGTTTCTCTTCAGATCATGTA | GAGGCCTCAGAAATTGGATAAAG | 101 | + |
| U5E-9P | TGGTTTCTCTTCAGATCATATAAATCTTTT | TTTGAGGCCTCTGTTTAAGGAT | 101 | NA |
| U5E-10P | ATACTCTGGTTTCTCTTCAGATTGATA | TGAGCCCTCAAAAAGTTGGG | 103 | - |
| U5F-2P | AAGCTCTGGTTTCTCTTCAAAA | AACAACCTGTTCTCTCCACGA | 75 | NA |
| U5F-3P | AAGCTCTGGTTTCTTGTGAGAAC | AAGCAAGGCCTTAAAAAGTTGG | 107 | + |
| U5F-4P | ATACTCTGGTTTCTCTTCAGATCGT | CCGTCTCAAACAAAACAAAAC | 114 | + |
| U5F-6P | TCATTATGGTTTCTCTTCAGAATGA | CAAGCAGGACTTCAAAACATTTTC | 107 | + |
| U5F-7P | TCTGGTTTCTCTTCAGAACAAAT | CAGGACCTCAAAAATTTGGTTATT | 99 | + |
| U5F-8P | ATGCTCATTCTTTAGAATGAATAAGTTT | CAAGACCTCAAAAATTTGGTTATC | 98 | + |

Table S3: Characteristics of human snRNA variants, based on annotation in Ensembl database v. 91.

Gene name (aliases): snRNA gene name (associated aliases)

Ensembl ID: Ensembl gene IDs

Chr: chromosome on which the gene is located

Location: position of first base pair of gene

Strand: orientation of gene, left to right (+) or right to left (-)

Length (nts): predicted mature length of snRNA in nucleotides

Nucleotide differences: number of nucleotide differences between canonical and variant snRNA (indels/mismatches)

| Gene name (aliases) | Ensembl ID | Chr | Location | Strand | Length (nts) | Nucleotide differences |
|---------------------|-----------------|-----|-------------|--------|--------------|------------------------|
| RNU1-1 | ENSG00000206652 | 1 | 16,514,122 | - | 164 | 0 |
| RNU1-2 | ENSG00000207005 | 1 | 16,895,980 | + | 164 | 0 |
| RNU1-3 | ENSG00000207513 | 1 | 16,666,785 | - | 164 | 0 |
| RNU1-4 | ENSG00000207389 | 1 | 16,740,516 | + | 164 | 0 |
| RNVU1-18 | ENSG00000206737 | 1 | 143,729,407 | - | 164 | 0 |
| RNU1-27P | ENSG00000206596 | 14 | 34,546,714 | + | 164 | 0 |
| RNU1-28P | ENSG00000206588 | 14 | 34,556,226 | - | 164 | 0 |
| RNVU1-3 | ENSG00000201183 | 1 | 148,402,715 | + | 161 | 27 |
| RNVU1-4 | ENSG00000277610 | 1 | 120,942,600 | + | 164 | 33 |
| RNVU1-6 | ENSG00000201558 | 1 | 146,052,081 | - | 164 | 15 |
| RNVU1-14 | ENSG00000207501 | 1 | 145,281,116 | + | 164 | 15 |
| RNVU1-15 | ENSG00000207205 | 1 | 144,412,576 | + | 165 | 18 |
| RNVU1-17 | ENSG00000207349 | 1 | 143,699,456 | - | 164 | 11 |
| RNVU1-19 | ENSG00000275538 | 1 | 120,850,819 | - | 167 | 12 |
| RNU1-42P | ENSG00000200903 | 17 | 48,949,361 | - | 160 | 18 |
| RNU1-59P (VU1-24) | ENSG00000201699 | 1 | 149,162,783 | - | 162 | 51 |
| RNU1-63P | ENSG00000206629 | 4 | 67,429,591 | - | 164 | 17 |
| RNU1-67P | ENSG00000207175 | X | 119,423,742 | + | 164 | 6 |
| RNU1-77P | ENSG00000212170 | 15 | 82,174,224 | - | 162 | 23 |
| RNU1-82P | ENSG00000212153 | 7 | 141,727,984 | + | 165 | 13 |
| RNU1-83P | ENSG00000200296 | 12 | 62,850,738 | - | 164 | 13 |
| RNU1-85P (VU1-34) | ENSG00000200997 | 17 | 58,679,527 | - | 164 | 36 |
| RNU1-88P | ENSG00000238554 | 6 | 36,639,545 | - | 128 | 52 |
| RNU1-120P (VU1-22) | ENSG00000199879 | 1 | 148,263,476 | + | 157 | 47 |
| RNU1-122P (VU1-21) | ENSG00000202408 | 1 | 148,334,612 | - | 156 | 41 |
| RNU1-136P | ENSG00000206908 | 6 | 53,219,261 | + | 164 | 16 |
| RNU1-138P | ENSG00000206820 | 4 | 113,420,323 | + | 164 | 15 |

| | | | | | | |
|-----------|-----------------|----|-------------|---|-----|-----|
| RNU1-148P | ENSG00000207201 | 8 | 24,076,997 | - | 162 | 11 |
| RNU11 | ENSG00000274978 | 1 | 28,648,600 | + | 134 | 94 |
| RNU2-1 | ENSG00000274585 | 17 | 43,233,787 | - | 191 | 0 |
| RNU2-2P | ENSG00000222328 | 11 | 62,841,619 | - | 191 | 8 |
| RNU2-3P | ENSG00000222076 | 15 | 95,745,804 | + | 191 | 13 |
| RNU2-5P | ENSG00000222465 | 9 | 70,188,571 | - | 180 | 29 |
| RNU2-6P | ENSG00000223336 | 13 | 46,374,401 | - | 190 | 13 |
| RNU2-13P | ENSG00000251718 | 2 | 11,561,194 | + | 113 | 114 |
| RNU2-14P | ENSG00000222985 | 14 | 65,124,352 | - | 191 | 21 |
| RNU2-16P | ENSG00000222644 | 4 | 75,829,454 | - | 136 | 113 |
| RNU2-17P | ENSG00000222222 | 1 | 150,236,967 | + | 190 | 21 |
| RNU2-23P | ENSG00000222477 | 11 | 65,147,584 | + | 195 | 28 |
| RNU2-36P | ENSG00000222293 | 9 | 86,422,938 | - | 191 | 20 |
| RNU2-37P | ENSG00000222627 | 3 | 128,075,073 | - | 177 | 28 |
| RNU2-41P | ENSG00000222923 | 2 | 114,420,125 | + | 178 | 84 |
| RNU2-42P | ENSG00000222629 | 10 | 125,890,566 | - | 113 | 116 |
| RNU2-48P | ENSG00000222626 | 5 | 157,976,766 | - | 191 | 21 |
| RNU2-51P | ENSG00000222640 | 14 | 70,360,892 | + | 197 | 39 |
| RNU2-55P | ENSG00000223078 | 21 | 23,281,736 | - | 174 | 81 |
| RNU2-56P | ENSG00000252635 | 20 | 18,265,690 | - | 190 | 20 |
| RNU2-59P | ENSG00000222414 | 10 | 101,364,845 | - | 191 | 9 |
| RNU2-61P | ENSG00000223001 | 6 | 89,063,500 | - | 191 | 18 |
| RNU2-63P | ENSG00000222724 | 2 | 88,016,354 | - | 194 | 46 |
| RNU2-64P | ENSG00000223247 | 3 | 73,110,992 | + | 191 | 17 |
| RNU2-68P | ENSG00000222810 | X | 72,376,979 | - | 191 | 23 |
| RNU2-70P | ENSG00000222650 | 1 | 236,267,780 | + | 179 | 24 |
| RNU12 | ENSG00000276027 | 22 | 42,615,244 | + | 150 | 114 |
| RNU4-1 | ENSG00000200795 | 12 | 120,293,097 | - | 141 | 0 |
| RNU4-2 | ENSG00000202538 | 12 | 120,291,763 | - | 141 | 6 |
| RNU4-7P | ENSG00000201628 | 6 | 150,326,623 | - | 141 | 9 |
| RNU4-39P | ENSG00000199325 | 11 | 66,614,964 | - | 127 | 48 |
| RNU4-46P | ENSG00000222750 | 16 | 19,498,610 | + | 138 | 13 |
| RNU4-59P | ENSG00000201317 | 1 | 92,700,819 | - | 116 | 58 |
| RNU4-82P | ENSG00000199313 | 9 | 124,887,410 | - | 140 | 50 |
| RNU4-91P | ENSG00000201648 | 3 | 175,609,479 | + | 135 | 47 |
| RNU4atac | ENSG00000264229 | 2 | 121,530,881 | + | 127 | 81 |
| RNU6-1 | ENSG00000206625 | 15 | 67,839,939 | - | 107 | 0 |
| RNU6atac | ENSG00000221676 | 9 | 134,164,439 | - | 126 | 72 |
| RNU5A-1 | ENSG00000199568 | 15 | 65,296,051 | + | 116 | 0 |
| RNU5B-1 | ENSG00000200156 | 15 | 65,304,677 | + | 116 | 7 |
| RNU5D-1 | ENSG00000200169 | 1 | 44,731,055 | - | 116 | 18 |
| RNU5E-1 | ENSG00000199347 | 1 | 11,908,152 | + | 120 | 13 |
| RNU5F-1 | ENSG00000199377 | 1 | 44,721,786 | - | 117 | 19 |
| RNU5A-2P | ENSG00000207065 | 4 | 81,334,303 | + | 116 | 20 |
| RNU5A-3P | ENSG00000254172 | 8 | 30,643,689 | + | 114 | 17 |
| RNU5A-4P | ENSG00000252397 | 13 | 33,829,539 | - | 111 | 19 |
| RNU5A-5P | ENSG00000222986 | 1 | 231,670,635 | + | 116 | 32 |

| | | | | | | |
|-----------|-----------------|----|-------------|---|-----|----|
| RNU5A-6P | ENSG00000206863 | 18 | 24,124,170 | - | 116 | 19 |
| RNU5A-7P | ENSG00000251788 | 12 | 64,228,560 | - | 116 | 20 |
| RNU5A-8P | ENSG00000200972 | 1 | 210,374,154 | - | 114 | 22 |
| RNU5B-2P | ENSG00000199906 | 3 | 40,498,891 | - | 113 | 28 |
| RNU5B-3P | ENSG00000252410 | 5 | 139,447,907 | + | 111 | 42 |
| RNU5E-4P | ENSG00000201801 | 1 | 11,909,808 | - | 120 | 17 |
| RNU5E-5P | ENSG00000202249 | 12 | 101,466,705 | + | 116 | 22 |
| RNU5E-6P | ENSG00000202444 | 1 | 44,819,883 | - | 115 | 22 |
| RNU5E-7P | ENSG00000202160 | 2 | 15,864,935 | + | 117 | 18 |
| RNU5E-8P | ENSG00000200372 | 3 | 116,965,112 | - | 116 | 17 |
| RNU5E-9P | ENSG00000223096 | 2 | 178,142,548 | + | 104 | 22 |
| RNU5E-10P | ENSG00000200376 | 11 | 47,576,471 | - | 118 | 24 |
| RNU5F-2P | ENSG00000251875 | 1 | 179,576,268 | + | 82 | 48 |
| RNU5F-3P | ENSG00000200637 | 18 | 6,017,723 | - | 117 | 21 |
| RNU5F-4P | ENSG00000252390 | 12 | 31,594,093 | - | 114 | 26 |
| RNU5F-6P | ENSG00000199849 | 1 | 164,351,273 | + | 116 | 27 |
| RNU5F-7P | ENSG00000200566 | X | 15,916,189 | - | 115 | 17 |
| RNU5F-8P | ENSG00000251750 | 1 | 240,653,367 | + | 111 | 27 |

Table S4: snRNA variant half-lives.

snRNA: snRNA variant name

 $t_{1/2} \pm \text{SEM}$: Estimation of snRNA variant half-life after actinomycin D-mediated transcription inhibition \pm SEM from three technical replicates, N.D. indicates snRNAs with half-lives that were undeterminable within the assayed time points

| snRNA | $t_{1/2} \pm \text{SEM}$ |
|-------------|--------------------------|
| U1-1 | N.D. |
| VU1-3 | 31.51 \pm 0.10 |
| VU1-4 | 20.39 \pm 0.06 |
| VU1-15 | 7.96 \pm 0.04 |
| VU1-17 | 86.64 \pm 0.06 |
| VU1-19 | 43.32 \pm 0.11 |
| U1-59P | 15.40 \pm 0.04 |
| U1-63P | 26.66 \pm 0.11 |
| U1-77P | 23.11 \pm 0.04 |
| U1-83P | 53.32 \pm 0.03 |
| U1-85P | 8.16 \pm 0.02 |
| U1-120P | 9.24 \pm 0.03 |
| U1-122P | 10.35 \pm 0.06 |
| U1-136P | 19.25 \pm 0.08 |
| U1-138P | 13.10 \pm 0.08 |
| U1-148P | 38.51 \pm 0.10 |
| U11 | N.D. |
| U2-1 | N.D. |
| U2-2P | 49.51 \pm 0.05 |
| U2-3P | 21.66 \pm 0.14 |
| U2-5P | 13.59 \pm 0.05 |
| U2-6P | 11.02 \pm 0.05 |
| U2-13P | 49.51 \pm 0.04 |
| U2-16P | 23.90 \pm 0.08 |
| U2-17P | 23.90 \pm 0.17 |
| U2-23P | 43.32 \pm 0.14 |
| U2-37P | 43.32 \pm 0.09 |
| U2-41P | 16.91 \pm 0.10 |
| U2-42P | 20.39 \pm 0.07 |
| U2-61P | 28.88 \pm 0.09 |
| U2-63P | 8.56 \pm 0.06 |
| U2-64P | 15.10 \pm 0.07 |
| U2-68P | 34.66 \pm 0.08 |
| U2-70P | 28.88 \pm 0.13 |
| U12 | N.D. |

| snRNA | $t_{1/2} \pm \text{SEM}$ |
|--------------|--------------------------|
| U4-1 | N.D. |
| U4-2 | N.D. |
| U4-46P | 77.02 \pm 0.04 |
| U4-59P | 21.00 \pm 0.04 |
| U4-82P | 17.33 \pm 0.05 |
| U4-91P | 16.91 \pm 0.12 |
| U4atac | N.D. |
| U5A-1 | N.D. |
| U5A-2P | 13.59 \pm 0.04 |
| U5A-3P | 19.80 \pm 0.05 |
| U5A-4P | 10.50 \pm 0.03 |
| U5A-5P | 17.33 \pm 0.03 |
| U5A-6P | 18.73 \pm 0.06 |
| U5A-8P | N.D. |
| U5B-1 | N.D. |
| U5D-1 | N.D. |
| U5E-1 | N.D. |
| U5E-4P | N.D. |
| U5E-6P | N.D. |
| U5E-7P | 28.88 \pm 0.06 |
| U5F-1 | N.D. |
| U5F-2P | 26.66 \pm 0.04 |
| U5F-3P | 23.11 \pm 0.03 |
| U5F-4P | 13.33 \pm 0.05 |
| U5F-6P | 17.33 \pm 0.05 |
| U5F-7P | 27.73 \pm 0.10 |
| U5F-8P | 10.66 \pm 0.10 |
| U6 | N.D. |
| U6atac | N.D. |

Table S5: List of DNA oligos for Northern blotting.

Gene: snRNA gene name

DNA oligo: modified antisense DNA oligo as ordered from IDT with a conjugated 5' IR 700nm fluorophore

| Gene | DNA oligo |
|------|---|
| U1 | /5IRD700/CAGAGGACAGCGCGAACGCAGTCCCCACTACCACAAATTATGCAGTCGAGTTCCACATTTGGGGAACGGCAGGGGTCAGCACATCC |
| U2 | /5IRD700/CGGAGCAAGCTCCTATTCCATCTCCTGCTCAGAAAATCCATTTAATATATTGCCTCGGATAGAGGACGTATCAGATATTAAGTATA |
| U4 | /5IRD700/TCACGGCGGGGTATTGGGAAAAGTTTTCAATTAGCAATAATCGCGCCTCGGATAGACCTCATTGGCTACGATACTGCCACTGCGCAAAGC |
| U5A | /5IRD700/AGCCTTGCCAAAGCAAGGCCTCAAAAAATTGGGTTAAGACTCAGAGTTGTTCTCTCCACGGAAATCTTTAGTAAAAGGCGAAAGATTTA |
| U6 | /5IRD700/CACGAATTTGCGTGTATCCTTGCGCAGGGGCCATGCTAATCTTCTGTATCGTTCCAATTTTAGTATATGTGCTGCCAAGCGAGCAC |