**Figure S7: Supplemental Note 2 - Statistical analysis of** **features of introns affected by differential alternative splicing in the *sac3a* mutant**

The 5’ splice site strength, 3’ splice site strength, guanine-cytosine (GC) content and length were compared for “non-changing-events” and the “up and down” regulated events. The “const. introns” group represents all the constitutive introns in the Arabidopsis AtRTD2 transcriptome, for which no SUPPA events have been annotated. The “No change” category always describes the intron(s), the features of intron(s), of the particular alternative splicing event type which is not changing in the *prp4ka* mutant, relative to wild-type. The significance of the differences between groups were tested using t-tests. Significant findings (p-value < 0.01) are marked in red.

**Intron retention**

Included less, the intron is included less relative to wild-type

Included more, the intron is included more (more retained) relative to wild-type

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **5’ splice site strength** | | | | | **3’ splice site strength** | | | | |
|  | **CSI** | **No change** | **Included less** | **Included more** |  | **CSI** | **No change** | **Included less** | **Included more** |
| **mean** | 75.002 | 72.805 | 71.683 | 69.967 | **mean** | 73.275 | 71.586 | 73.649 | 70.444 |
| **median** | 75.808 | 73.696 | 72.671 | 71.294 | **median** | 73.654 | 72.187 | 74.060 | 70.450 |
| **CSI x no change** | | | 2.275e-200 | | **CSI x no change** | | | 2.485e-149 | |
| **CSI x included less** | | | 6.557e-05 | | **CSI x included less** | | | 0.598 | |
| **CSI x included more** | | | 9.325e-19 | | **CSI x included more** | | | 2.541e-07 | |
| **No change x included less** | | | 0.170 | | **No change x included less** | | | 0.00407 | |
| **No change x included more** | | | 2.322e-07 | | **No change x included more** | | | 0.035 | |
| **Included less x included more** | | | 0.078 | | **Included less x included more** | | | 0.00034 | |
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| **GC content** | | | | | **Intron size** | | | | |
|  | **CSI** | **No change** | **Included less** | **Included more** |  | **CSI** | **No change** | **Included less** | **Included more** |
| **min** | 0.070 | 0.120 | 0.210 | 0.140 | **min** | 4 | 8 | 42 | 13 |
| **5%** | 0.240 | 0.260 | 0.270 | 0.260 | **5%** | 75 | 73 | 77 | 74 |
| **25%** | 0.300 | 0.310 | 0.310 | 0.310 | **25%** | 86 | 88 | 99 | 97 |
| **median** | 0.330 | 0.330 | 0.340 | 0.340 | **median** | 99 | 117 | 145 | 139 |
| **75%** | 0.350 | 0.360 | 0.360 | 0.370 | **75%** | 162 | 243 | 231 | 281 |
| **95%** | 0.400 | 0.410 | 0.400 | 0.410 | **95%** | 474 | 552 | 513 | 575 |
| **max** | 0.820 | 0.750 | 0.670 | 0.620 | **max** | 57631 | 5480 | 909 | 1090 |
| **CSI x no change** | | | 8.327e-175 | | **CSI x no change** | | | 1.770e-76 | |
| **CSI x included less** | | | 0.00011 | | **CSI x included less** | | | 0.021 | |
| **CSI x included more** | | | 1.997e-10 | | **CSI x included more** | | | 3.608e-06 | |
| **No change x included less** | | | 0.331 | | **No change x included less** | | | 0.754 | |
| **No change x included more** | | | 0.00591 | | **No change x included more** | | | 0.070 | |
| **Included less x included more** | | | 0.343 | | **Included less x included more** | | | 0.142 | |
|  | | | | |  | | | | |

**Exitron**

Included less, the exitron is included less relative to wild-type

Included more, the exitron is included more (more retained) relative to wild-type

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **5’ splice site strength** | | | | | **3’ splice site strength** | | | | |
|  | **CSI** | **No change** | **Included less** | **Included more** |  | **CSI** | **No change** | **Included less** | **Included more** |
| **mean** | 75.002 | 65.268 | 58.410 | 63.903 | **mean** | 73.275 | 63.095 | 59.640 | 63.243 |
| **median** | 75.808 | 67.950 | 62.515 | 67.981 | **median** | 73.654 | 64.342 | 62.444 | 64.843 |
| **CSI x no change** | | | 1.934e-204 | | **CSI x no change** | | | 2.196e-305 | |
| **CSI x included less** | | | 0.016 | | **CSI x included less** | | | 0.00298 | |
| **CSI x included more** | | | 7.291e-05 | | **CSI x included more** | | | 7.326e-06 | |
| **No change x included less** | | | 0.259 | | **No change x included less** | | | 0.347 | |
| **No change x included more** | | | 0.579 | | **No change x included more** | | | 0.938 | |
| **Included less x included more** | | | 0.392 | | **Included less x included more** | | | 0.376 | |
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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **GC content** | | | | | **Intron size** | | | | |
|  | **CSI** | **No change** | **Included less** | **Included more** |  | **CSI** | **No change** | **Included less** | **Included more** |
| **min** | 0.070 | 0.180 | 0.350 | 0.320 | **min** | 4 | 16 | 69 | 21 |
| **5%** | 0.240 | 0.320 | 0.355 | 0.330 | **5%** | 75 | 63 | 72 | 42 |
| **25%** | 0.300 | 0.370 | 0.390 | 0.355 | **25%** | 86 | 81 | 79 | 78 |
| **median** | 0.330 | 0.410 | 0.440 | 0.420 | **median** | 99 | 98 | 89 | 90 |
| **75%** | 0.350 | 0.460 | 0.645 | 0.495 | **75%** | 162 | 114 | 183 | 122 |
| **95%** | 0.400 | 0.640 | 0.660 | 0.660 | **95%** | 474 | 462 | 331 | 247 |
| **max** | 0.820 | 0.740 | 0.660 | 0.690 | **max** | 57631 | 2847 | 432 | 289 |
| **CSI x no change** | | | 0.0 | | **CSI x no change** | | | 0.00793 | |
| **CSI x included less** | | | 0.00136 | | **CSI x included less** | | | 0.576 | |
| **CSI x included more** | | | 8.514e-07 | | **CSI x included more** | | | 1.222e-05 | |
| **No change x included less** | | | 0.116 | | **No change x included less** | | | 0.807 | |
| **No change x included more** | | | 0.432 | | **No change x included more** | | | 0.00026 | |
| **Included less x included more** | | | 0.252 | | **Included less x included more** | | | 0.277 | |
|  | | | | |  | | | | |

**Alternative 5’ splice sites**

Used more, the 5’ splice site used more compared to the other 5’ splice site defining the event

Used less, the 5’ splice site used less compared to the other 3’ splice site defining the event

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| --- | --- | --- | --- | --- |
| **5’ splice site strength** | | | | |
|  | **CSI** | **No change** | **Used less** | **Used more** |
| **mean** | 75.002 | 69.265 | 67.085 | 69.235 |
| **median** | 75.808 | 70.037 | 67.995 | 70.449 |
| **CSI x no change** | | | 0.0 | |
| **CSI x used less** | | | 1.407e-13 | |
| **CSI x used more** | | | 9.860e08 | |
| **No change x used less** | | | 0.022 | |
| **No change x used more** | | | 0.976 | |
| **Used less x used more** | | | 0.120 | |
|  | | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **GC content** | | | | | **Intron size** | | | | |
|  | **CSI** | **No change** | **Used less** | **Used more** |  | **CSI** | **No change** | **Used less** | **Used more** |
| **min** | 0.070 | 0.140 | 0.250 | 0.220 | **min** | 4 | 14 | 73 | 67 |
| **5%** | 0.240 | 0.270 | 0.290 | 0.290 | **5%** | 75 | 77 | 94 | 86 |
| **25%** | 0.300 | 0.310 | 0.330 | 0.320 | **25%** | 86 | 96 | 162 | 113 |
| **median** | 0.330 | 0.330 | 0.350 | 0.330 | **median** | 99 | 144 | 285 | 173 |
| **75%** | 0.350 | 0.360 | 0.370 | 0.360 | **75%** | 162 | 304 | 526 | 336 |
| **95%** | 0.400 | 0.400 | 0.416 | 0.400 | **95%** | 474 | 666 | 865 | 742 |
| **max** | 0.820 | 0.710 | 0.660 | 0.670 | **max** | 57631 | 5016 | 1403 | 1064 |
| **CSI x no change** | | | 1.602e-127 | | **CSI x no change** | | | 1.649e-192 | |
| **CSI x used less** | | | 1.066e-06 | | **CSI x used less** | | | 5.628e-12 | |
| **CSI x used more** | | | 0.00049 | | **CSI x used more** | | | 1.834e-05 | |
| **No change x used less** | | | 0.00115 | | **No change x used less** | | | 2.940e-06 | |
| **No change x used more** | | | 0.084 | | **No change x used more** | | | 0.471 | |
| **Used less x used more** | | | 0.251 | | **Used less x used more** | | | 0.00052 | |
|  | | | | |  | | | | |

**Alternative 3’ splice sites**

Used more, the 3’ splice site used more compared to the other 3’ splice site defining the event

Used less, the 3’ splice site used less compared to the other 3’ splice site defining the event

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| --- | --- | --- | --- | --- |
| **3’ splice site strength** | | | | |
|  | **CSI** | **No change** | **Used less** | **Used more** |
| **mean** | 73.275 | 69.011 | 68.146 | 68.416 |
| **median** | 73.654 | 69.434 | 68.454 | 68.613 |
| **CSI x no change** | | | 0.0 | |
| **CSI x used less** | | | 1.068e-07 | |
| **CSI x used more** | | | 7.385e-07 | |
| **No change x used less** | | | 0.342 | |
| **No change x used more** | | | 0.522 | |
| **Used less x used more** | | | 0.834 | |
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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **GC content** | | | | | **Intron size** | | | | |
|  | **CSI** | **No change** | **Used less** | **Used more** |  | **CSI** | **No change** | **Used less** | **Used more** |
| **min** | 0.070 | 0.100 | 0.220 | 0.240 | **min** | 4 | 21 | 51 | 69 |
| **5%** | 0.240 | 0.260 | 0.270 | 0.273 | **5%** | 75 | 74 | 83 | 78 |
| **25%** | 0.300 | 0.310 | 0.310 | 0.310 | **25%** | 86 | 90 | 110 | 101 |
| **median** | 0.330 | 0.330 | 0.340 | 0.330 | **median** | 99 | 119 | 198 | 159 |
| **75%** | 0.350 | 0.360 | 0.370 | 0.355 | **75%** | 162 | 244 | 334 | 317 |
| **95%** | 0.400 | 0.400 | 0.420 | 0.407 | **95%** | 474 | 558 | 684 | 700 |
| **max** | 0.820 | 0.710 | 0.690 | 0.680 | **max** | 57631 | 5673 | 1500 | 1515 |
| **CSI x no change** | | | 1.744e-114 | | **CSI x no change** | | | 2.311e-108 | |
| **CSI x used less** | | | 0.00083 | | **CSI x used less** | | | 5.883e-06 | |
| **CSI x used more** | | | 0.00878 | | **CSI x used more** | | | 0.00037 | |
| **No change x used less** | | | 0.023 | | **No change x used less** | | | 0.00427 | |
| **No change x used more** | | | 0.114 | | **No change x used more** | | | 0.054 | |
| **Used less x used more** | | | 0.674 | | **Used less x used more** | | | 0.559 | |
|  | | | | |  | | | | |

**Exon skipping**

Skipped more, the exon is less often included.

Skipped less, the exon is more often included.

upstream

5’ss

downstream

3’ss

3’ss

5’ss

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Upstream 5’ss strength** | | | | **Downstream 3’ss strength** | | | |
|  | **No change** | **Skipped more** | **Skipped less** |  | **No change** | **Skipped more** | **Skipped less** |
| **mean** | 74.262 | 76.244 | 69.305 | **mean** | 72.752 | 77.244 | 71.626 |
| **median** | 74.818 | 73.350 | 68.553 | **median** | 72.886 | 77.264 | 70.211 |
| **No change x skipped more** | | | 0.495 | **No change x skipped more** | | | 0.033 |
| **No change x skipped less** | | | 0.041 | **No change x skipped less** | | | 0.503 |
| **Skipped more x skipped less** | | | 0.067 | **Skipped more x skipped less** | | | 0.029 |
|  | | | |  | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **3’ss strength** | | | | **5’ss strength** | | | |
|  | **No change** | **Skipped more** | **Skipped less** |  | **No change** | **Skipped more** | **Skipped less** |
| **mean** | 72.521 | 74.272 | 73.614 | **mean** | 73.626 | 74.421 | 73.414 |
| **median** | 73.153 | 75.969 | 75.208 | **median** | 74.271 | 74.453 | 72.815 |
| **No change x skipped more** | | | 0.418 | **No change x skipped more** | | | 0.755 |
| **No change x skipped less** | | | 0.467 | **No change x skipped less** | | | 0.890 |
| **Skipped more x skipped less** | | | 0.797 | **Skipped more x skipped less** | | | 0.738 |
|  | | | |  | | | |

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| --- | --- | --- | --- |
| **5’ss strength** | | **3’ss strength** | |
| **No change - Upstream 5’ss x 5’ss** | 0.00872 | **No change - Upstream 3’ss x 3’ss** | 0.291 |
| **Skipped less - Upstream 5’ss x 5’ss** | 0.149 | **Skipped less - Upstream 3’ss x 3’ss** | 0.371 |
| **Skipped more - Upstream 5’ss x 5’ss** | 0.629 | **Skipped more - Upstream 3’ss x 3’ss** | 0.285 |

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Upstream intron GC content** | | | | **Downstream intron GC content** | | | |
|  | **No change** | **Skipped more** | **Skipped less** |  | **No change** | **Skipped more** | **Skipped less** |
| **min** | 0.14 | 0.3 | 0.29 | **min** | 0.15 | 0.23 | 0.3 |
| **5%** | 0.26 | 0.312 | 0.29 | **5%** | 0.25 | 0.262 | 0.316 |
| **25%** | 0.3 | 0.33 | 0.3 | **25%** | 0.3 | 0.33 | 0.33 |
| **median** | 0.33 | 0.34 | 0.35 | **median** | 0.33 | 0.34 | 0.34 |
| **75%** | 0.35 | 0.36 | 0.36 | **75%** | 0.35 | 0.34 | 0.35 |
| **95%** | 0.39 | 0.36 | 0.382 | **95%** | 0.39 | 0.376 | 0.384 |
| **max** | 0.67 | 0.36 | 0.39 | **max** | 0.69 | 0.4 | 0.4 |
| **No change x skipped more** | | | 0.079 | **No change x skipped more** | | | 0.923 |
| **No change x skipped less** | | | 0.177 | **No change x skipped less** | | | 0.00898 |
| **Skipped more x skipped less** | | | 0.823 | **Skipped more x skipped less** | | | 0.311 |
|  | | | |  | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Upstream intron size** | | | | **Downstream intron size** | | | |
|  | **No change** | **Skipped more** | **Skipped less** |  | **No change** | **Skipped more** | **Skipped less** |
| **min** | 36 | 85 | 79 | **min** | 40 | 73 | 83 |
| **5%** | 74 | 87 | 88 | **5%** | 76 | 79 | 91 |
| **25%** | 95 | 98 | 142 | **25%** | 94 | 90 | 117 |
| **median** | 150 | 163 | 186 | **median** | 143 | 92 | 147 |
| **75%** | 297 | 183 | 200 | **75%** | 290 | 168 | 160 |
| **95%** | 654 | 351 | 415 | **95%** | 636 | 1997 | 319 |
| **max** | 3929 | 422 | 442 | **max** | 2713 | 3158 | 388 |
| **No change x skipped more** | | | 0.093 | **No change x skipped more** | | | 0.525 |
| **No change x skipped less** | | | 0.093 | **No change x skipped less** | | | 0.00275 |
| **Skipped more x skipped less** | | | 0.582 | **Skipped more x skipped less** | | | 0.412 |
|  | | | |  | | | |