

**Supplementary Information**  
**for**  
*A mutation in a splicing factor that causes retinitis pigmentosa has a transcriptome-wide effect on mRNA splicing*

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# 1 Quality Assessment

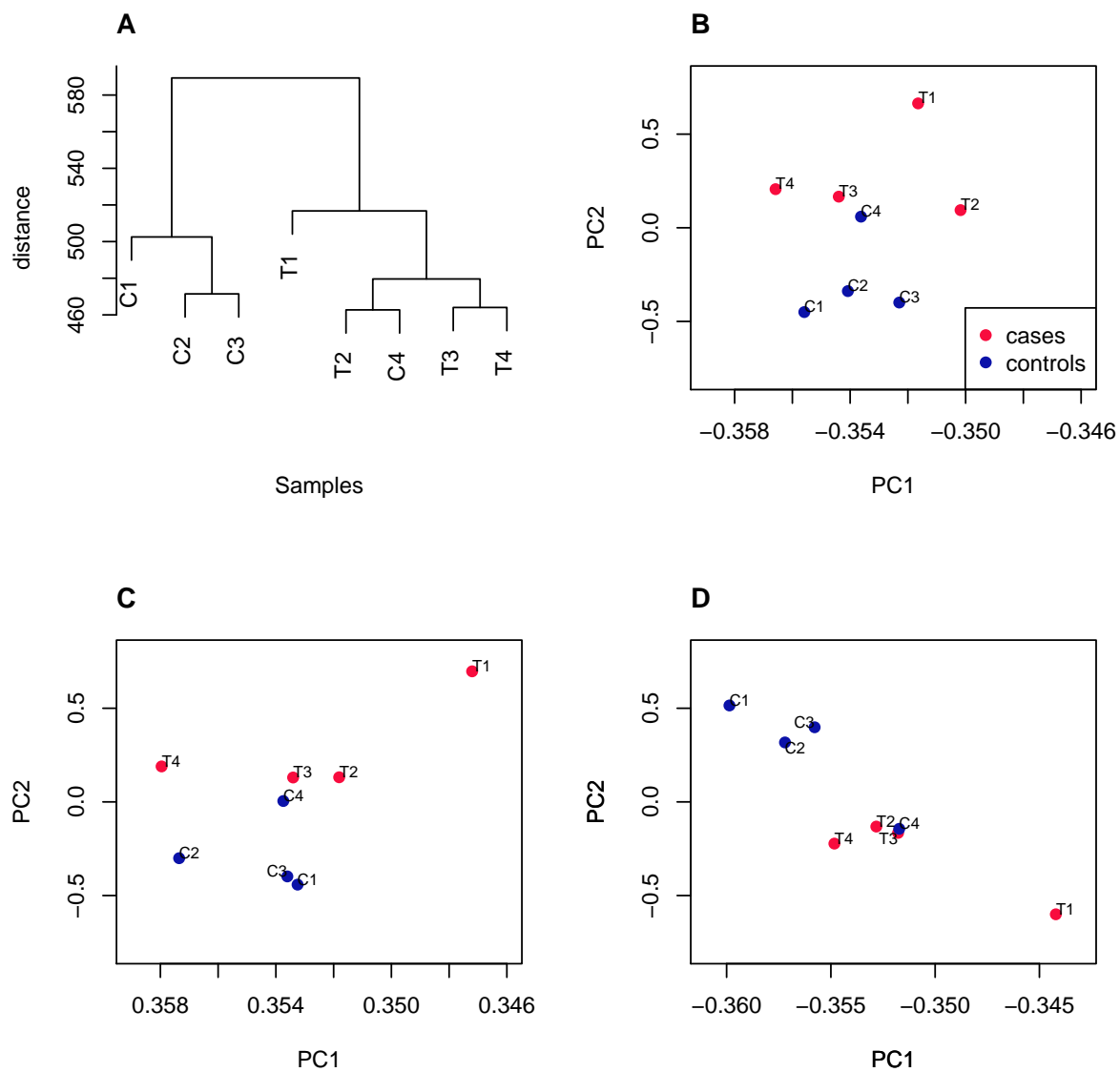


Figure S1: **Quality assessment of exon array data.** **A.** Hierarchical cluster of samples.  $T_i$  are cases,  $C_i$  are controls. **B.** Plot of first two principal components (PCs) for full probesets. **C.,D.** First two PCs for exonic and intronic probesets, respectively.

## 2 Sample Permutations

### 2.1 Core Probesets

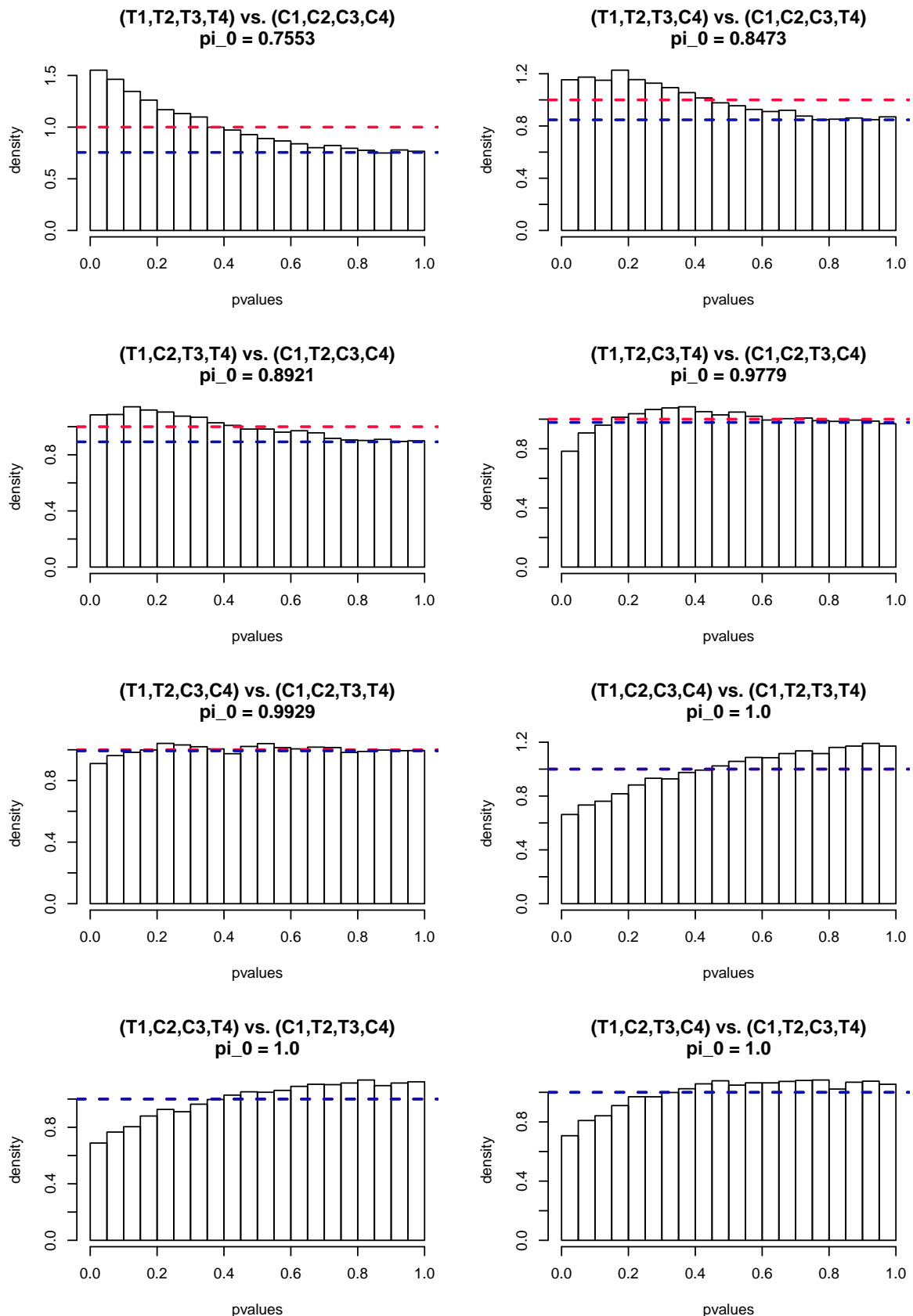


Figure S2: **Sample permutations for core probesets.** Plots representing the complete set (eight) of pairwise swaps between cases ( $T_i$ ) and controls ( $C_i$ ). Only core probesets are considered here. Plots are ordered from the top by the value of  $\pi_0$ . Dashed red line represents a uniform distribution; dashed blue lines represent the value of  $\pi_0$ .

## 2.2 Full Probesets

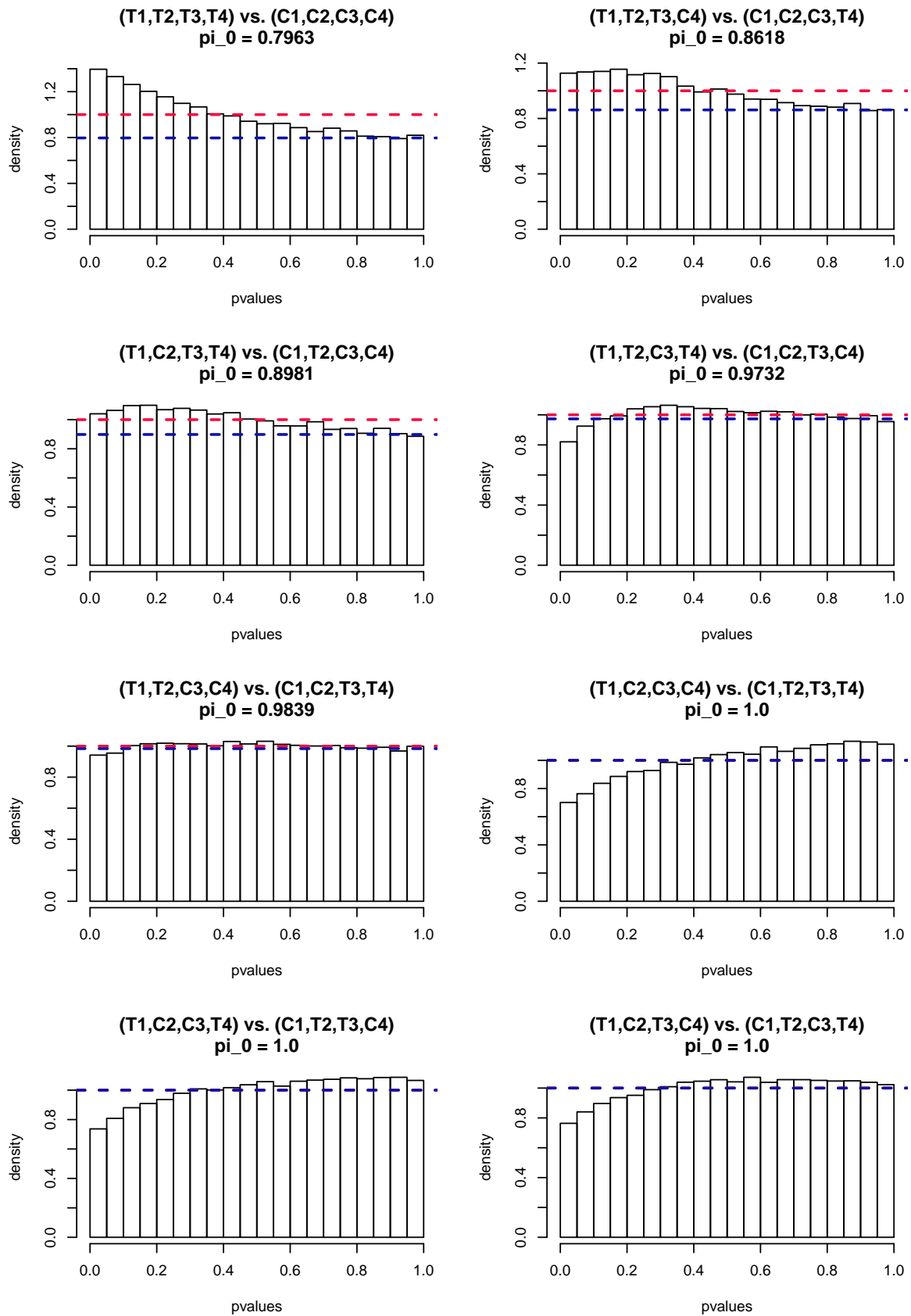


Figure S3: **Sample permutations for full probesets.** Plots representing the complete set (eight) of pairwise swaps between cases ( $T_i$ ) and controls ( $C_i$ ). Plots are ordered from the top by the value of  $\pi_0$ . Dashed red line represents a uniform distribution; dashed blue lines represent the value of  $\pi_0$ .

### 3 Additional Analyses

#### 3.1 Splice Site Scores

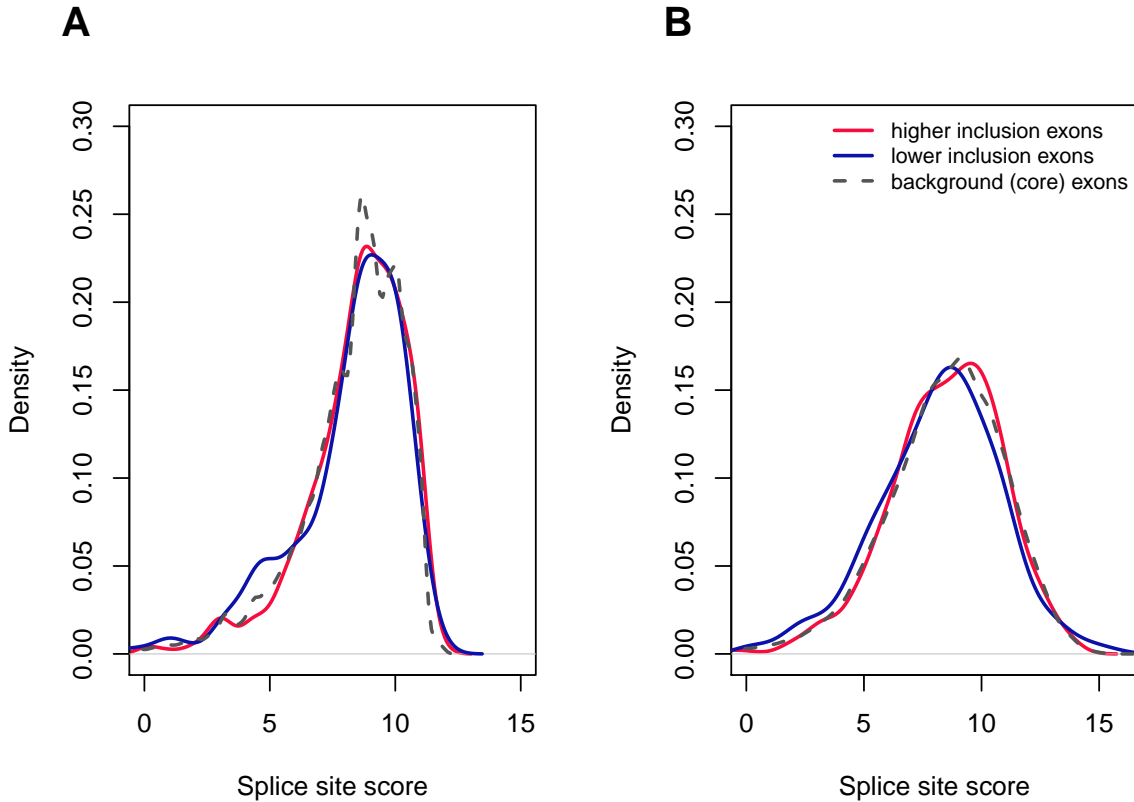


Figure S4: **Distribution of splice site scores.** Density plot of splice site scores of **A.** 5' splice sites and **B.** 3' splice sites. The dashed line indicates a similar plot for background exons (core exons).

#### 3.2 Constitutive versus Alternative Splicing Analyses

We reasoned that exons differentially included would also be enriched for alternatively spliced exons. However, we found no evidence of splicing bias for exons prone to alternative splicing. We tested this by constructing reference lists for constitutive and alternative exons in lymphoblastoid (similar to the whole blood samples we used) tissue [1] using the following procedure.

First, we aligned RNA-Seq data for 53 YRI samples to the hg19 build of the genome and isolated all junction spanning reads which numbered about 62 million reads. We then constructed a reference set of exons from the Ensembl gene model. Exons were defined at the transcript level by associating each transcript ID with the exon number (e.g. ENST\*:1 for the first exon in ENST\*). This method produced a redundant list because many exons overlap. Overlapping exons were eliminated using a randomisation procedure: from every set of exons that shared at least one terminal coordinate (start/stop) one exon was chosen at random.

For each internal exon we counted the number of reads that mapped to the upstream ( $C_1 : A$ ) and downstream ( $A : C_2$ ) junction as well as those that mapped only to flanking exons (skipping)

$(C_1 : C_2)$  [2]. We computed the percent-/proportion-spliced-in (PSI or  $\Psi$ ) using the expression

$$\Psi = \frac{\text{avg}(C_1 : A, A : C_2)}{\text{avg}(C_1 : A, A : C_2) + C_1 : C_2}.$$

An exon was considered constitutive if  $\Psi - 2 \cdot \text{SE}(\Psi) > 0.5$  and alternative if  $\Psi + 2 \cdot \text{SE}(\Psi) < 0.5$ . In this way we identified 19,685 constitutive and 3,078 alternative exons. From this list of constitutive and alternative exons we tallied those present in higher/lower inclusion in cases and compared them to similar counts in the background set of exons using Fisher's exact test. For exons included at higher levels in cases the odds ratio was 1.57 ( $p = 0.45$ ) while those included at lower levels had  $OR = 3.59$  ( $p = 0.27$ ) suggesting no splicing-specific influence.

### 3.3 U12-type Introns

We did not find any distinction in probeset intensities based on intron type. U2-type introns form the majority of introns and Fig. S6D adequately represents them. U12-type introns had a less even distribution (Supplementary Fig.S5) likely due to few probesets associated with them. No significant bias in inclusion levels was observed. We also compared U2-type and U12-type introns to each other based on their  $t$ -statistics. We found no significant difference in the  $t$ -statistics ( $p = 0.6268$ , 95% CI of difference of means:  $[-0.27, 0.17]$ ) suggesting similar distributions for both.

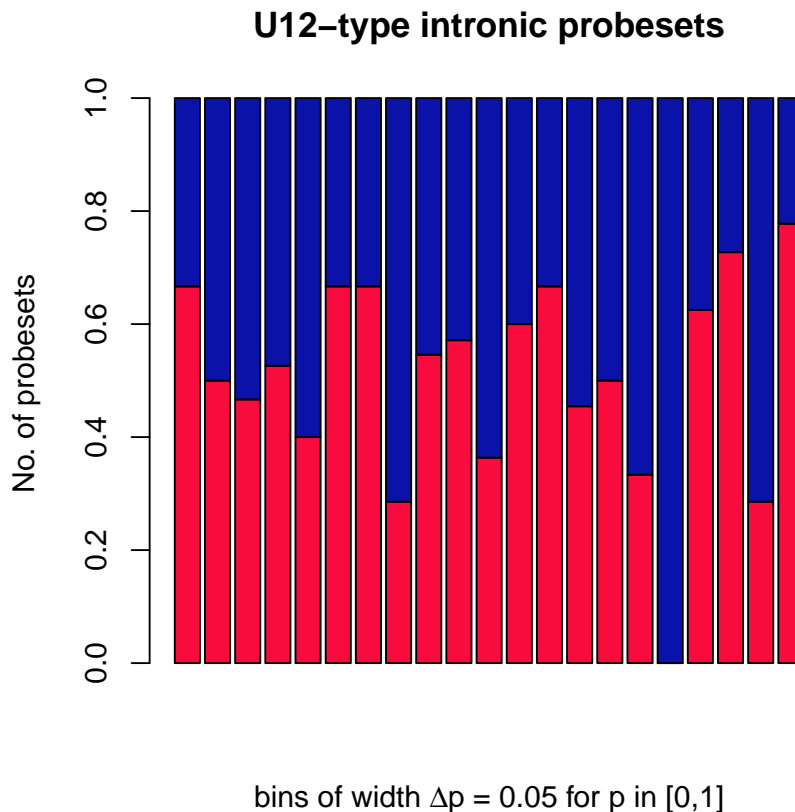


Figure S5: **Proportion of probesets indicating higher/lower inclusion in cases relative to controls for binned  $p$ -value thresholds for U12-type introns.** Each column represents all probesets (exons) for that bin. Red represent probesets included at higher levels in cases; blue are those included at lower levels in cases.

## 4 Number of Differentially Included Probesets

### 4.1 Differential Inclusion by Probeset Class

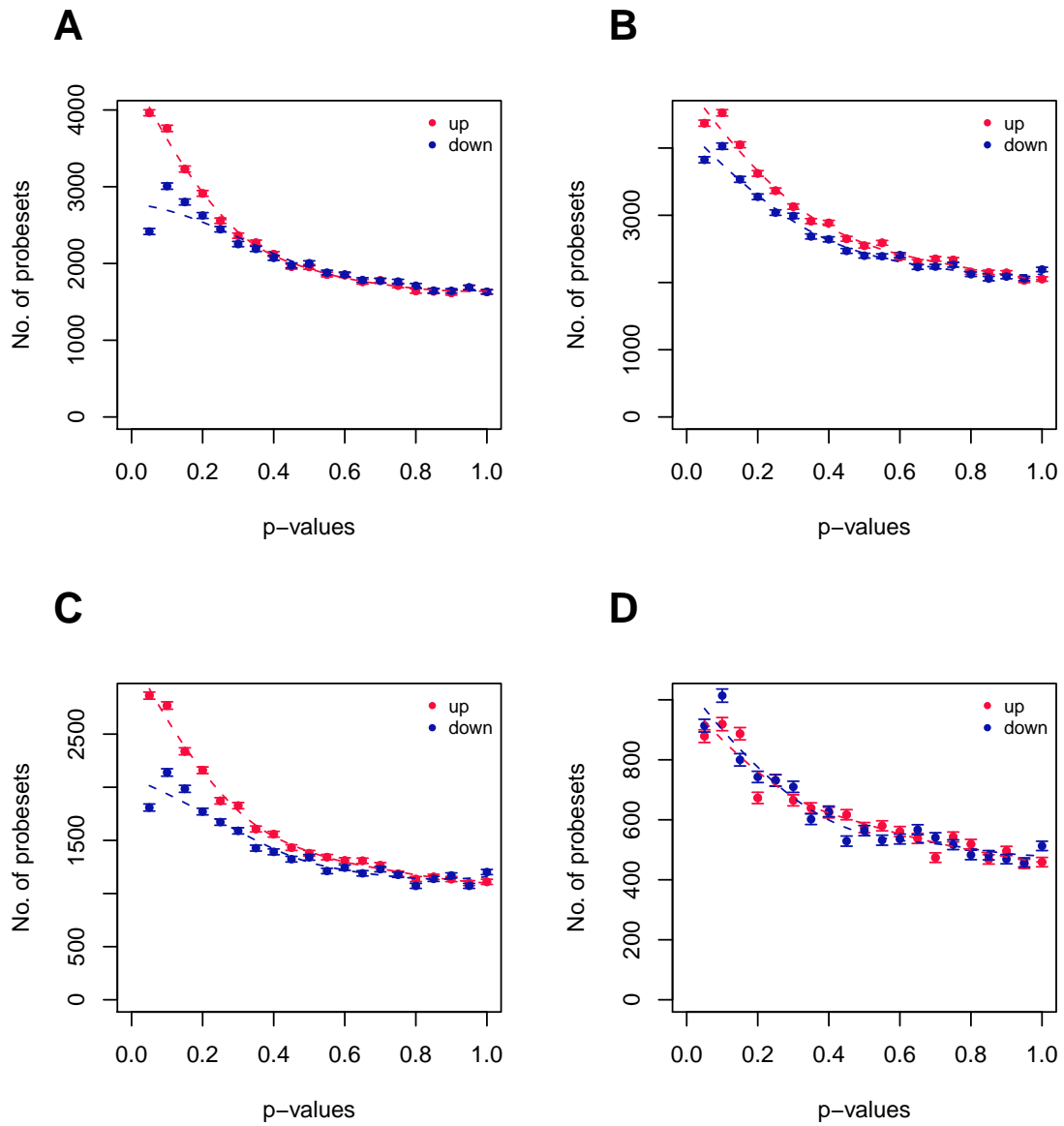


Figure S6: **Illustration of differential inclusion for different classes of probesets. A.** Core, **B.** full, **C.** exonic, and **D.** intronic probesets. Each point represents the number of probesets presenting higher ('up')/lower ('down') inclusion in cases relative to controls within  $\Delta p = 0.05$   $p$ -value bins.

## 4.2 Core Probesets

$p$ threshold	down	up	equal	$p$ -value	FDR
0.01	473	1287	0	$7.64 \times 10^{-87}$	$1.61 \times 10^{-85}$
0.05	2417	3963	0	$3.61 \times 10^{-84}$	$3.79 \times 10^{-83}$
0.1	3008	3760	0	$6.36 \times 10^{-20}$	$4.45 \times 10^{-19}$
0.15	2801	3230	0	$3.52 \times 10^{-8}$	$1.85 \times 10^{-7}$
0.2	2625	2913	0	$1.2 \times 10^{-4}$	$4.8 \times 10^{-4}$
0.25	2446	2557	0	0.12	0.36
0.3	2253	2362	0	0.11	0.36
0.35	2190	2271	0	0.23	0.58
0.4	2073	2122	0	0.46	0.81
0.45	1975	1959	0	0.81	0.99
0.5	2003	1956	0	0.46	0.81
0.55	1881	1856	0	0.69	0.99
0.6	1857	1848	0	0.90	0.99
0.65	1784	1761	0	0.71	0.99
0.7	1774	1779	0	0.95	0.99
0.75	1764	1713	0	0.40	0.81
0.8	1710	1642	0	0.25	0.58
0.85	1646	1637	0	0.89	0.99
0.9	1643	1617	0	0.66	0.99
0.95	1688	1685	0	0.97	0.99
1	1631	1629	207	0.99	0.99

Table S1: **Number of core probesets in each bin of width  $\Delta p = 0.05$ .** Data used to produce the plots in Fig. 2 and Fig. S6.



### 4.3 Full Probesets

$p$ threshold	down	up	equal	$p$ -value	FDR
0.01	1147	1400	0	$5.82 \times 10^{-7}$	$3.054 \times 10^{-6}$
0.05	3825	4370	0	$1.84 \times 10^{-9}$	$3.86 \times 10^{-8}$
0.1	4028	4524	0	$8.60 \times 10^{-8}$	$6.02 \times 10^{-7}$
0.15	3534	4048	0	$3.78 \times 10^{-9}$	$3.40 \times 10^{-8}$
0.2	3275	3622	0	$3.09 \times 10^{-5}$	0.00013
0.25	3040	3366	0	$4.88 \times 10^{-5}$	0.00017
0.3	2991	3129	0	0.080	0.1291
0.35	2685	2913	0	0.0024	0.0063
0.4	2642	2887	0	0.0010	0.0031
0.45	2470	2650	0	0.012	0.026
0.5	2399	2548	0	0.035	0.062
0.55	2391	2590	0	0.0050	0.012
0.6	2408	2378	0	0.68	0.68
0.65	2230	2306	0	0.27	0.35
0.7	2237	2352	0	0.093	0.14
0.75	2263	2337	0	0.28	0.35
0.8	2123	2155	0	0.64	0.68
0.85	2058	2150	0	0.16	0.23
0.9	2089	2143	0	0.42	0.48
0.95	2059	2032	0	0.68	0.68
1	2194	2052	25057	0.030	0.058

Table S2: Number of full probesets in each bin of width  $\Delta p = 0.05$

#### 4.4 Exonic Probesets

$p$ threshold	down	up	equal	$p$ -value	FDR
0.01	347	790	0	$2.95 \times 10^{-40}$	$4.59 \times 10^{-40}$
0.05	1809	2862	0	$6.61 \times 10^{-54}$	$2.059 \times 10^{-53}$
0.1	2139	2769	0	$2.46 \times 10^{-19}$	$2.55 \times 10^{-19}$
0.15	1985	2338	0	$8.48 \times 10^{-8}$	$5.29 \times 10^{-8}$
0.2	1771	2160	0	$5.89 \times 10^{-10}$	$4.59 \times 10^{-10}$
0.25	1672	1872	0	0.00083	0.00037
0.3	1590	1827	0	$5.37 \times 10^{-5}$	$2.79 \times 10^{-5}$
0.35	1427	1607	0	0.0012	0.00045
0.4	1392	1558	0	0.0024	0.00082
0.45	1323	1432	0	0.040	0.010
0.5	1340	1379	0	0.47	0.085
0.55	1212	1342	0	0.011	0.0033
0.6	1243	1311	0	0.19	0.038
0.65	1190	1308	0	0.019	0.0055
0.7	1229	1266	0	0.47	0.085
0.75	1176	1186	0	0.85	0.13
0.8	1072	1136	0	0.18	0.038
0.85	1138	1157	0	0.71	0.11
0.9	1170	1136	0	0.49	0.085
0.95	1072	1098	0	0.59	0.097
1	1202	1110	649	0.058	0.014

Table S3: Number of exonic probesets in each bin of width  $\Delta p = 0.05$

## 4.5 Intronic Probesets

<i>p</i> threshold	down	up	equal	<i>p</i> -value	FDR
0.01	280	304	0	0.34	0.52
0.05	914	879	0	0.42	0.52
0.1	1014	919	0	0.033	0.17
0.15	800	887	0	0.036	0.17
0.2	743	673	0	0.067	0.24
0.25	731	732	0	1	0.85
0.3	710	665	0	0.24	0.52
0.35	602	639	0	0.31	0.52
0.4	628	624	0	0.93	0.83
0.45	529	617	0	0.010	0.17
0.5	564	574	0	0.79	0.78
0.55	532	580	0	0.16	0.40
0.6	536	561	0	0.47	0.52
0.65	567	538	0	0.40	0.52
0.7	541	474	0	0.038	0.17
0.75	517	543	0	0.44	0.52
0.8	483	519	0	0.27	0.52
0.85	482	468	0	0.67	0.71
0.9	469	496	0	0.40	0.52
0.95	458	453	0	0.89	0.83
1	513	459	4235	0.089	0.26

Table S4: Number of intronic probesets in each bin of width  $\Delta p = 0.05$

## 4.6 U12-type Intronic Probesets

$p$ threshold	down	up	equal	$p$ -value	FDR
0.01	1	2	0	1	1
0.05	9	9	0	1	1
0.1	8	7	0	1	1
0.15	9	10	0	1	1
0.2	6	4	0	0.75	1
0.25	3	6	0	0.51	1
0.3	3	6	0	0.51	1
0.35	10	4	0	0.18	1
0.4	5	6	0	1	1
0.45	6	8	0	0.79	1
0.5	7	4	0	0.55	1
0.55	6	9	0	0.61	1
0.6	3	6	0	0.51	1
0.65	6	5	0	1	1
0.7	3	3	0	1	1
0.75	6	3	0	0.51	1
0.8	5	0	0	0.063	1
0.85	3	5	0	0.73	1
0.9	3	8	0	0.23	1
0.95	5	2	0	0.45	1
1	2	7	40	0.18	1

Table S5: Number of U12-type intronic probesets in each bin of width  $\Delta p = 0.05$

## 5 Transcript Cluster Identifiers (Metaprobesets) Associated with *PRPF8*

*PRPF8* is located on the reverse strand of chromosome 17 between base 1553923 and 1588154 (hg19 and Ensembl v.66). The following table shows corresponding metaprobesets.

ID	chr	strand	start	end
3705896	chr17	+	1577504	1577972
3705894	chr17	+	1574104	1574222
3705898	chr17	+	1581163	1581608
3740589	chr17	-	1574051	1574859
3740625	chr17	-	1583120	1583533
3740649	chr17	-	1587369	1587620

Table S6: Metaprobesets along *PRPF8*. The coordinates of transcript cluster IDs along the *PRPF8* gene.

Only 3740589 and 3740625 passed quality control filtering but were not differentially expressed between cases and controls.

## 6 Associated Genes

### 6.1 Higher Inclusion in Cases

ENSG00000000971 ENSG00000001036 ENSG00000001631 ENSG00000003147 ENSG00000003393  
ENSG00000003509 ENSG00000004660 ENSG00000004809 ENSG00000004975 ENSG00000005249  
ENSG00000005302 ENSG00000005471 ENSG00000005700 ENSG00000006432 ENSG00000006634  
ENSG00000006659 ENSG00000006831 ENSG00000007264 ENSG00000007350 ENSG00000007516  
ENSG00000007923 ENSG00000008083 ENSG00000008441 ENSG00000009307 ENSG00000009335  
ENSG00000009954 ENSG00000010404 ENSG00000010704 ENSG00000011007 ENSG00000011028  
ENSG00000011295 ENSG00000011566 ENSG00000012983 ENSG00000013306 ENSG00000013364  
ENSG00000013725 ENSG00000014138 ENSG00000014216 ENSG00000017260 ENSG00000018510  
ENSG00000023287 ENSG00000023318 ENSG00000023516 ENSG00000024048 ENSG00000028116  
ENSG00000029363 ENSG00000033122 ENSG00000034677 ENSG00000034693 ENSG00000039068  
ENSG00000040933 ENSG00000042832 ENSG00000042980 ENSG00000044115 ENSG00000047249  
ENSG00000047365 ENSG00000047410 ENSG00000047597 ENSG00000047617 ENSG00000048392  
ENSG00000048707 ENSG00000049246 ENSG00000049323 ENSG00000049541 ENSG00000049759  
ENSG00000049860 ENSG00000049883 ENSG00000052841 ENSG00000053524 ENSG00000054219  
ENSG00000054654 ENSG00000055917 ENSG00000055955 ENSG00000056586 ENSG00000058056  
ENSG00000058272 ENSG00000059378 ENSG00000059588 ENSG00000060140 ENSG00000060339  
ENSG00000061676 ENSG00000061987 ENSG00000062725 ENSG00000064042 ENSG00000064102  
ENSG00000064313 ENSG00000064393 ENSG00000064703 ENSG00000064763 ENSG00000064961  
ENSG00000065150 ENSG00000065243 ENSG00000065413 ENSG00000065485 ENSG00000065809  
ENSG00000065882 ENSG00000066855 ENSG00000066923 ENSG00000067066 ENSG00000067113  
ENSG00000067704 ENSG00000067798 ENSG00000067900 ENSG00000068878 ENSG00000068971  
ENSG00000069493 ENSG00000069696 ENSG00000069943 ENSG00000070159 ENSG00000070182  
ENSG00000070476 ENSG00000070961 ENSG00000071054 ENSG00000071537 ENSG00000072062  
ENSG00000072364 ENSG00000072415 ENSG00000072756 ENSG00000072849 ENSG00000072952  
ENSG00000073670 ENSG00000073803 ENSG00000074054 ENSG00000074276 ENSG00000074356  
ENSG00000074582 ENSG00000075275 ENSG00000075340 ENSG00000075651 ENSG00000075785  
ENSG00000075826 ENSG00000075884 ENSG00000075945 ENSG00000077097 ENSG00000077274  
ENSG00000077522 ENSG00000077713 ENSG00000077721 ENSG00000078328 ENSG00000078618  
ENSG00000078747 ENSG00000079263 ENSG00000079308 ENSG00000079805 ENSG00000079819  
ENSG00000079841 ENSG00000080298 ENSG00000080345 ENSG00000080546 ENSG00000080823  
ENSG00000081189 ENSG00000081923 ENSG00000082196 ENSG00000082258 ENSG00000082438  
ENSG00000082898 ENSG00000083093 ENSG00000083168 ENSG00000083642 ENSG00000084093  
ENSG00000084234 ENSG00000084754 ENSG00000084774 ENSG00000085491 ENSG00000085511  
ENSG00000085563 ENSG00000085719 ENSG00000085733 ENSG00000086232 ENSG00000086758  
ENSG00000086827 ENSG00000087245 ENSG00000087253 ENSG00000087274 ENSG00000087494  
ENSG00000087586 ENSG00000088205 ENSG00000088356 ENSG00000088827 ENSG00000088832  
ENSG00000088930 ENSG00000088970 ENSG00000089022 ENSG00000089048 ENSG00000089060  
ENSG00000089091 ENSG00000089094 ENSG00000089169 ENSG00000089234 ENSG00000089505  
ENSG00000089916 ENSG00000090238 ENSG00000090905 ENSG00000091039 ENSG00000091106  
ENSG00000091129 ENSG00000091157 ENSG00000091409 ENSG00000091436 ENSG00000092094  
ENSG00000092295 ENSG00000092330 ENSG00000092377 ENSG00000092758 ENSG00000092929  
ENSG00000093167 ENSG00000094880 ENSG00000095059 ENSG00000095066 ENSG00000095321  
ENSG00000096093 ENSG00000096401 ENSG00000099834 ENSG00000099910 ENSG00000099940  
ENSG00000099991 ENSG00000100077 ENSG00000100151 ENSG00000100225 ENSG00000100281  
ENSG00000100290 ENSG00000100294 ENSG00000100325 ENSG00000100330 ENSG00000100393  
ENSG00000100401 ENSG00000100416 ENSG00000100592 ENSG00000100604 ENSG00000100612  
ENSG00000100644 ENSG00000100650 ENSG00000100796 ENSG00000101017 ENSG00000101040  
ENSG00000101146 ENSG00000101294 ENSG00000101307 ENSG00000101442 ENSG00000101751

ENSG00000102043 ENSG00000102098 ENSG00000102125 ENSG00000102174 ENSG00000102189  
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ENSG00000104321 ENSG00000104341 ENSG00000104774 ENSG00000104812 ENSG00000104957  
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ENSG00000105609 ENSG00000105699 ENSG00000105810 ENSG00000105939 ENSG00000105963  
ENSG00000106012 ENSG00000106080 ENSG00000106327 ENSG00000106443 ENSG00000106546  
ENSG00000106605 ENSG00000106688 ENSG00000106804 ENSG00000107201 ENSG00000107242  
ENSG00000107262 ENSG00000107537 ENSG00000107581 ENSG00000107625 ENSG00000107672  
ENSG00000107758 ENSG00000107862 ENSG00000107897 ENSG00000108094 ENSG00000108100  
ENSG00000108278 ENSG00000108309 ENSG00000108344 ENSG00000108387 ENSG00000108389  
ENSG00000108576 ENSG00000108592 ENSG00000108799 ENSG00000108819 ENSG00000109458  
ENSG00000109685 ENSG00000110497 ENSG00000110628 ENSG00000110723 ENSG00000110799  
ENSG00000110911 ENSG00000111142 ENSG00000111231 ENSG00000111291 ENSG00000111371  
ENSG00000111554 ENSG00000111615 ENSG00000111644 ENSG00000111647 ENSG00000111666  
ENSG00000111696 ENSG00000111783 ENSG00000111799 ENSG00000111845 ENSG00000111886  
ENSG00000112038 ENSG00000112146 ENSG00000112186 ENSG00000112200 ENSG00000112249  
ENSG00000112294 ENSG00000112494 ENSG00000112511 ENSG00000112799 ENSG00000112893  
ENSG00000112983 ENSG00000112996 ENSG00000113108 ENSG00000113318 ENSG00000113327  
ENSG00000113369 ENSG00000113407 ENSG00000113441 ENSG00000113580 ENSG00000113595  
ENSG00000113645 ENSG00000113719 ENSG00000113946 ENSG00000113971 ENSG00000114030  
ENSG00000114098 ENSG00000114127 ENSG00000114166 ENSG00000114209 ENSG00000114248  
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ENSG00000114978 ENSG00000115009 ENSG00000115053 ENSG00000115091 ENSG00000115109  
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ENSG00000119820 ENSG00000119950 ENSG00000120029 ENSG00000120253 ENSG00000120693  
ENSG00000120756 ENSG00000120899 ENSG00000121486 ENSG00000121542 ENSG00000121848  
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## 6.2 Lower Inclusion in Cases

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## 7 Associated Exons

### 7.1 Higher Inclusion in Cases

The list of exons analysed represent a one-to-one mapping of probesets to exons (see *Methods*). The set of exons shown here represent a random choice of all possible exons that overlap with the exon array probeset. The number after the colon (':') represents the exon number with the transcript isoform.

ENST00000005340:4 ENST00000011700:2 ENST00000257931:20 ENST00000258243:8  
 ENST00000013125:29 ENST00000025301:2 ENST00000258526:12 ENST00000258538:9  
 ENST00000040877:23 ENST00000075503:8 ENST00000260408:5 ENST00000260508:12  
 ENST00000082259:9 ENST00000083182:7 ENST00000260818:42 ENST00000261207:17  
 ENST00000158149:10 ENST00000158771:2 ENST00000261405:39 ENST00000261483:15  
 ENST00000164133:13 ENST00000164133:5 ENST00000261483:17 ENST00000261483:19  
 ENST00000198765:9 ENST00000200135:14 ENST00000261483:4 ENST00000261517:56  
 ENST00000200135:5 ENST00000202017:4 ENST00000261530:7 ENST00000261584:10  
 ENST00000202816:5 ENST00000207549:16 ENST00000261667:10 ENST00000261681:5  
 ENST00000210060:8 ENST00000215862:19 ENST00000261755:9 ENST00000261867:6  
 ENST00000216115:4 ENST00000216492:6 ENST00000262134:5 ENST00000262262:2  
 ENST00000217800:7 ENST00000217909:4 ENST00000262352:8 ENST00000262455:2  
 ENST00000219070:4 ENST00000221973:2 ENST00000262464:61 ENST00000262539:17  
 ENST00000223642:40 ENST00000225308:3 ENST00000262738:27 ENST00000263119:31  
 ENST00000229214:5 ENST00000229729:17 ENST00000263253:20 ENST00000263354:6  
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## 7.2 Lower Inclusion in Cases

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