# Comprehensive map of age-associated splicing changes across human tissues and their contributions to age-associated diseases

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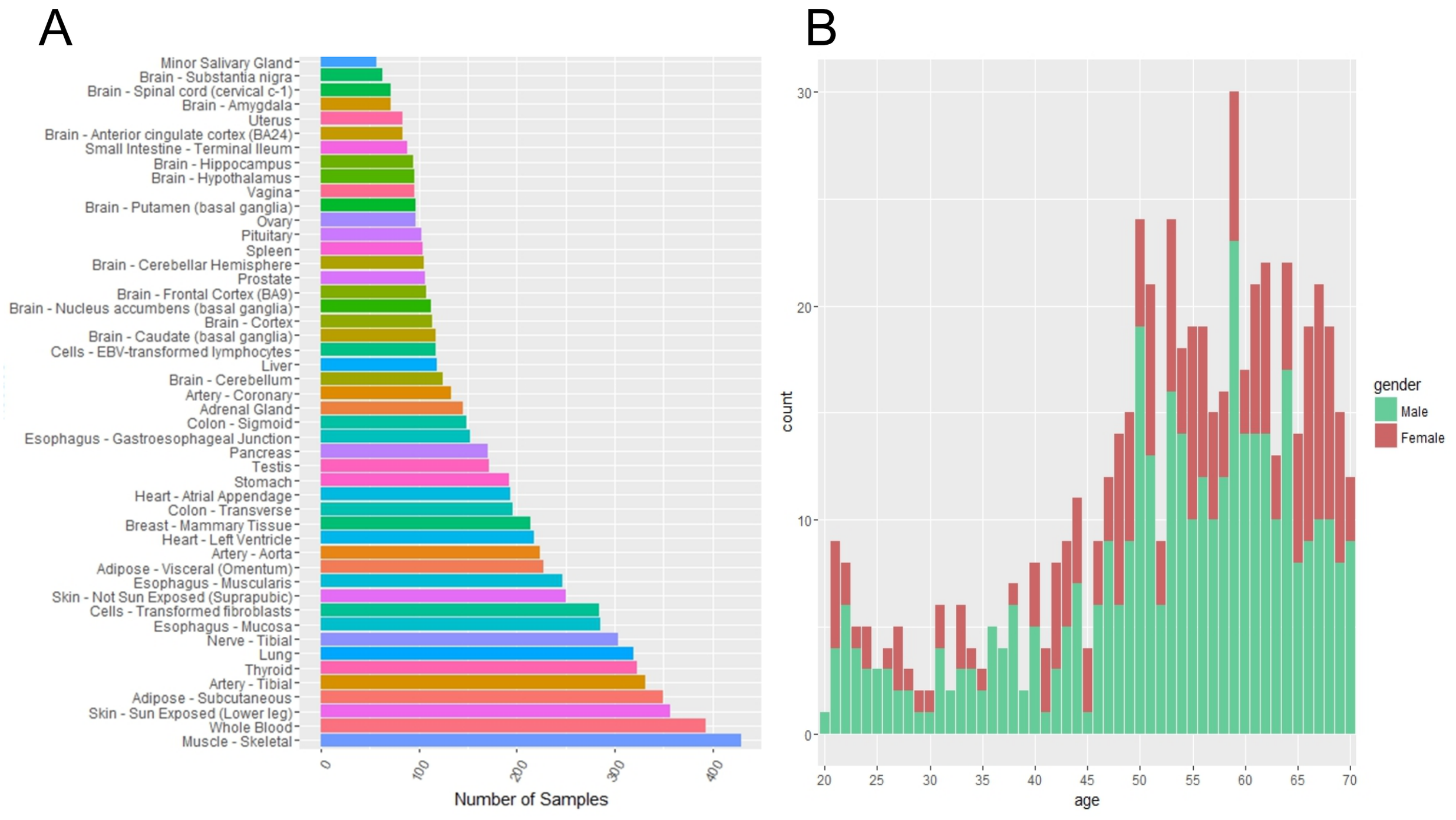



**Figure S1: Summary of GTEx data.** (A) Number of samples across 54 tissues. (B) Distribution of sample age and gender.

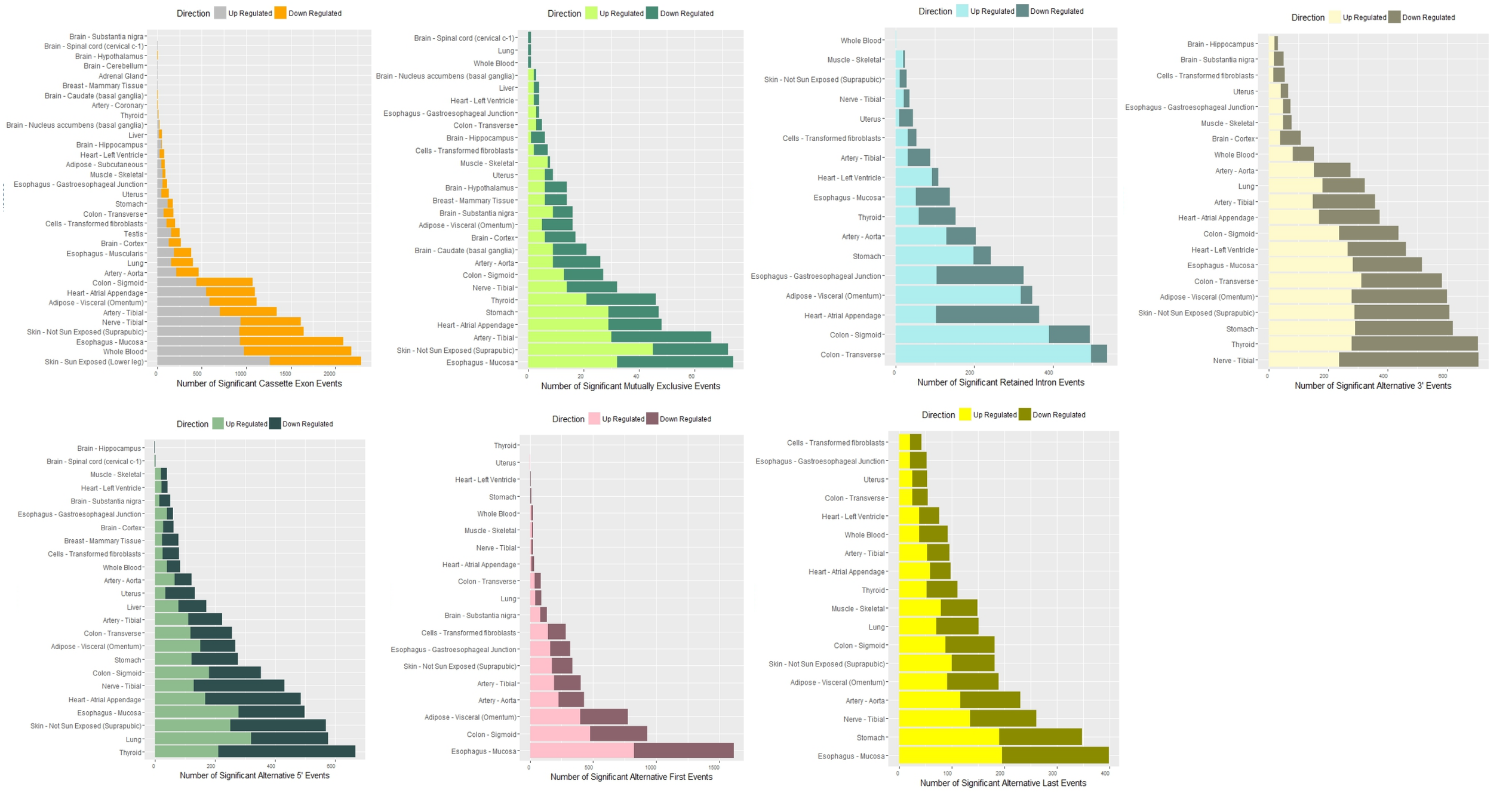
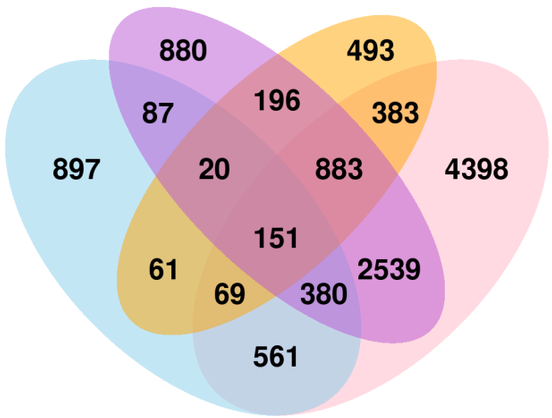



**Figure S2: Number of significant up and down-regulated splicing events across tissues for the 7 types of splicing events.**


**Figure S3: Functional enrichment among genes affected by age-associated splicing across tissues.** Columns correspond to tissues, rows to biological functions, colors indicate fold-change.

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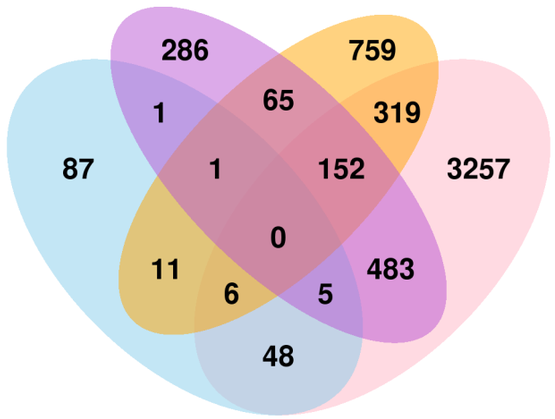


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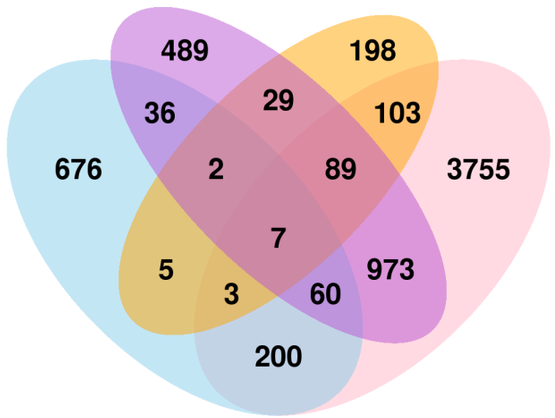


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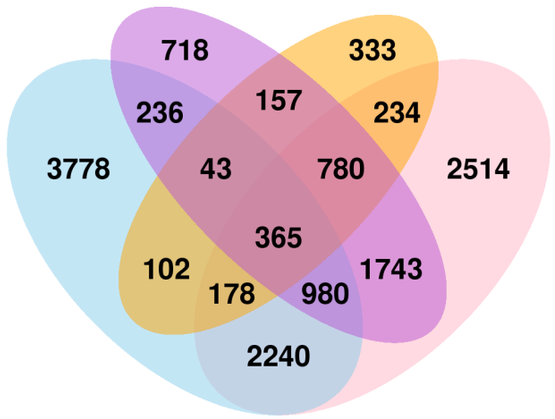


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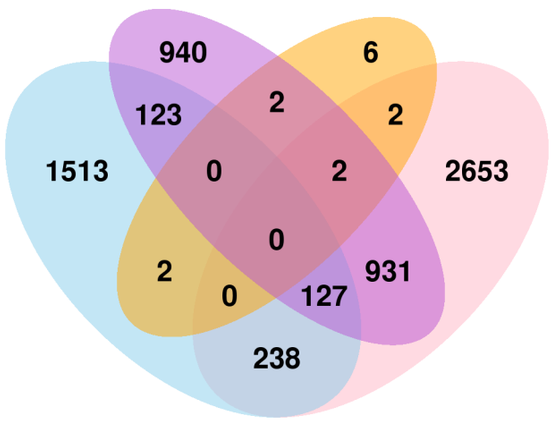


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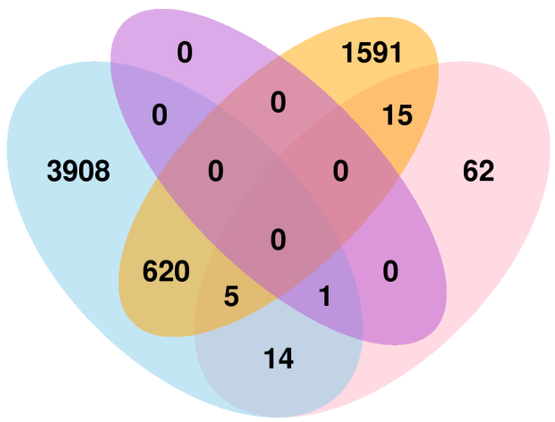


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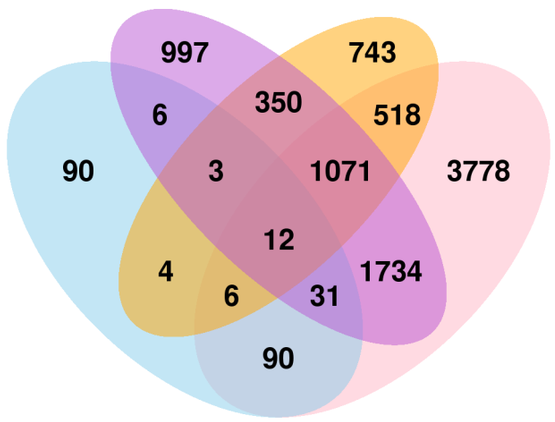


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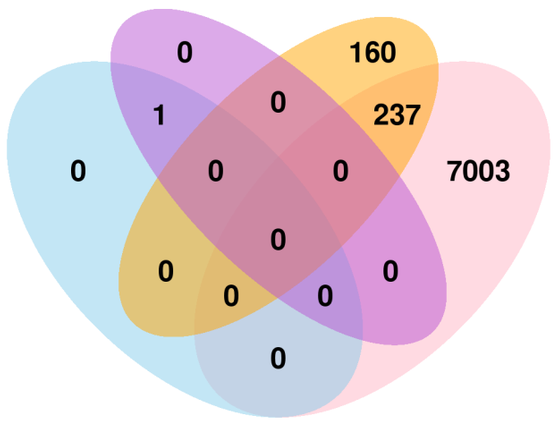


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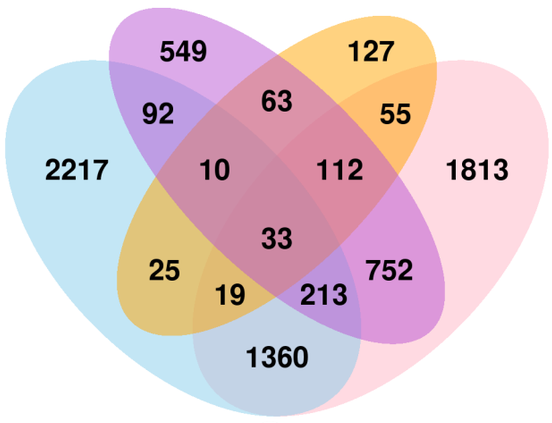


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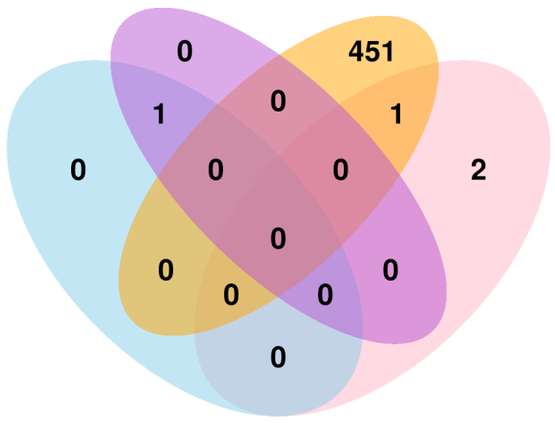


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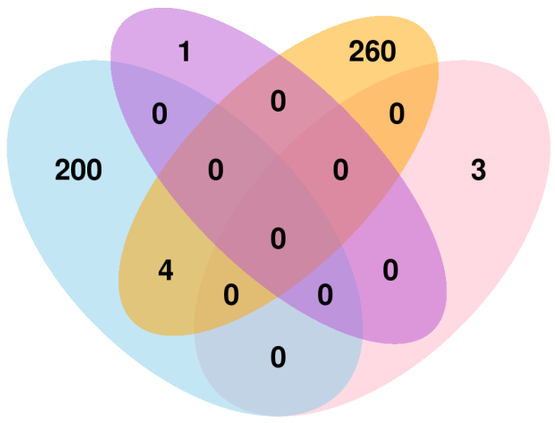


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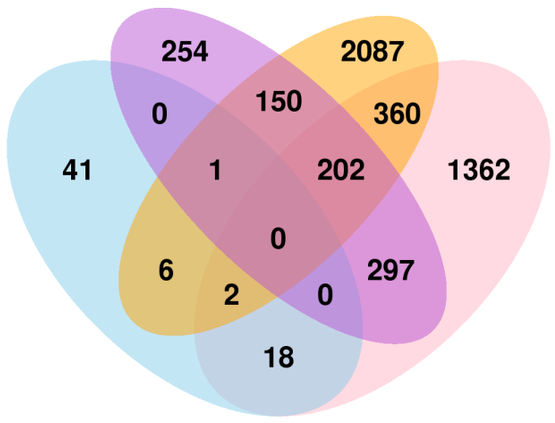


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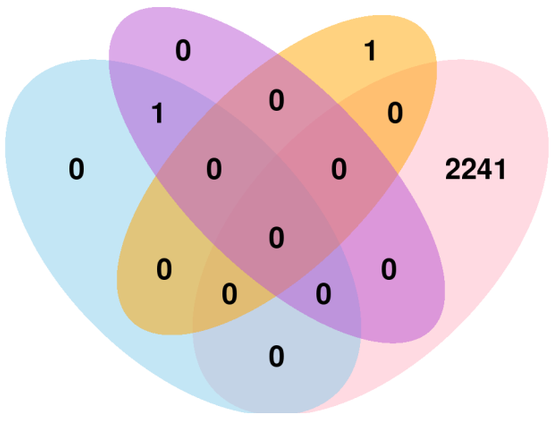


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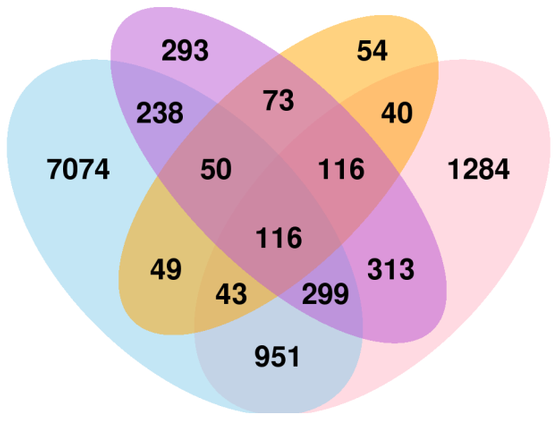


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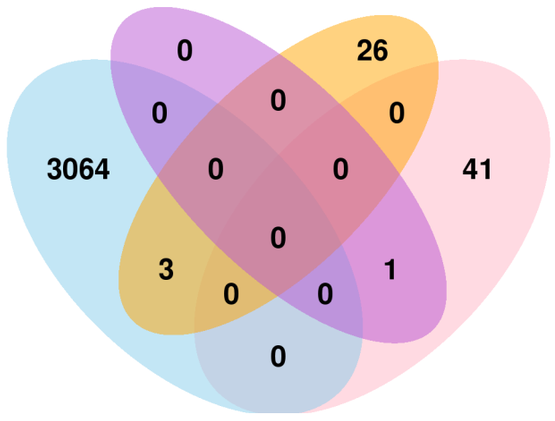


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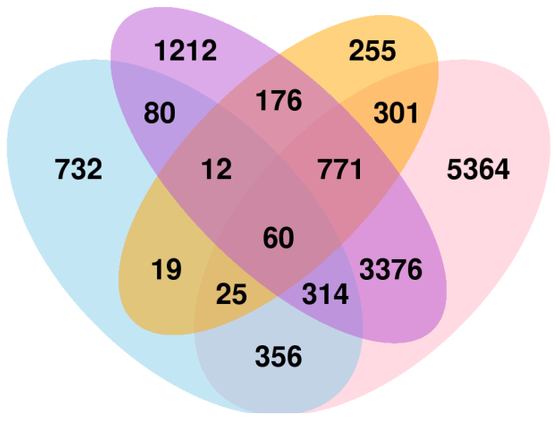


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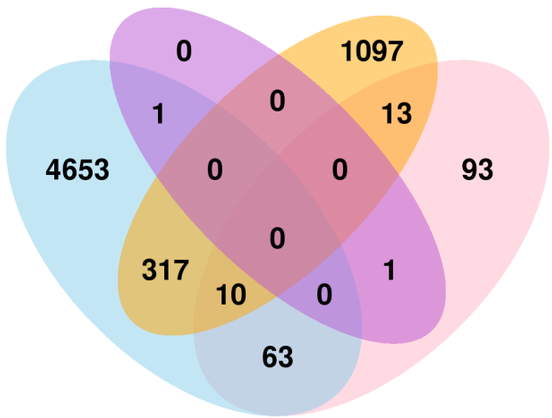


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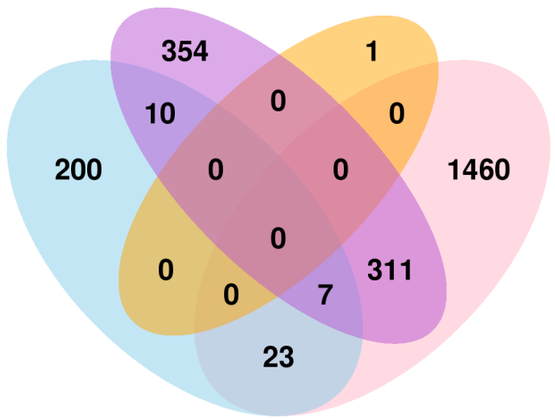


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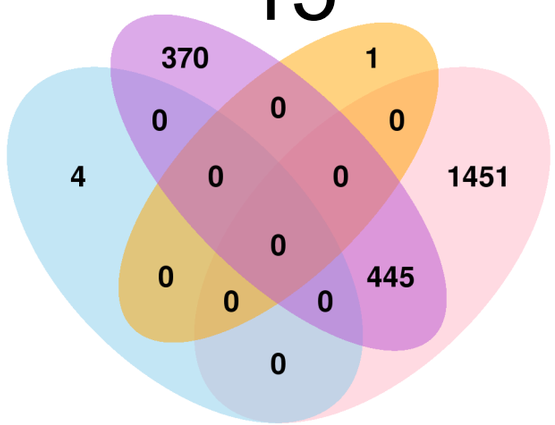


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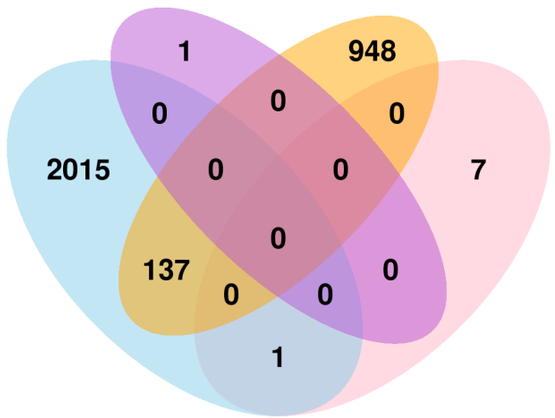


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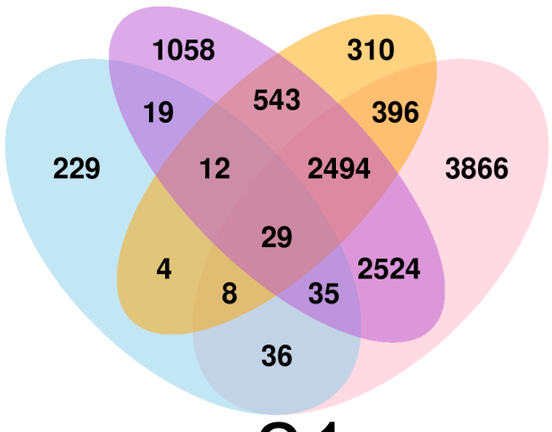


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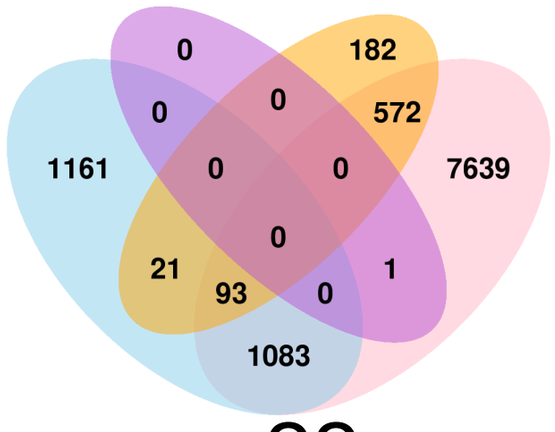


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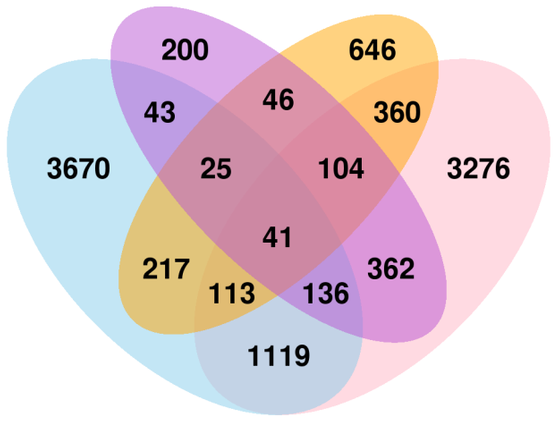


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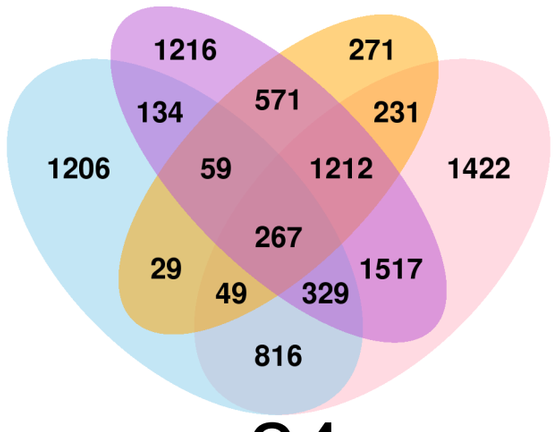


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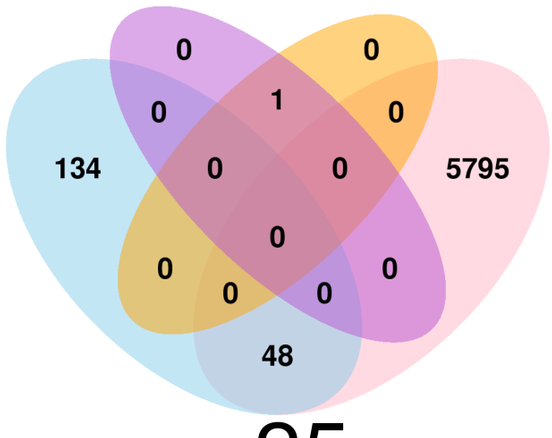


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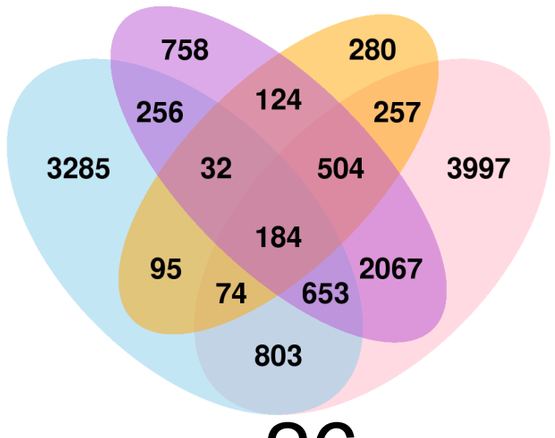


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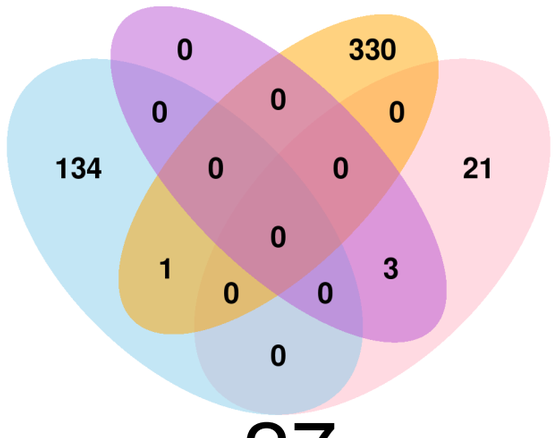


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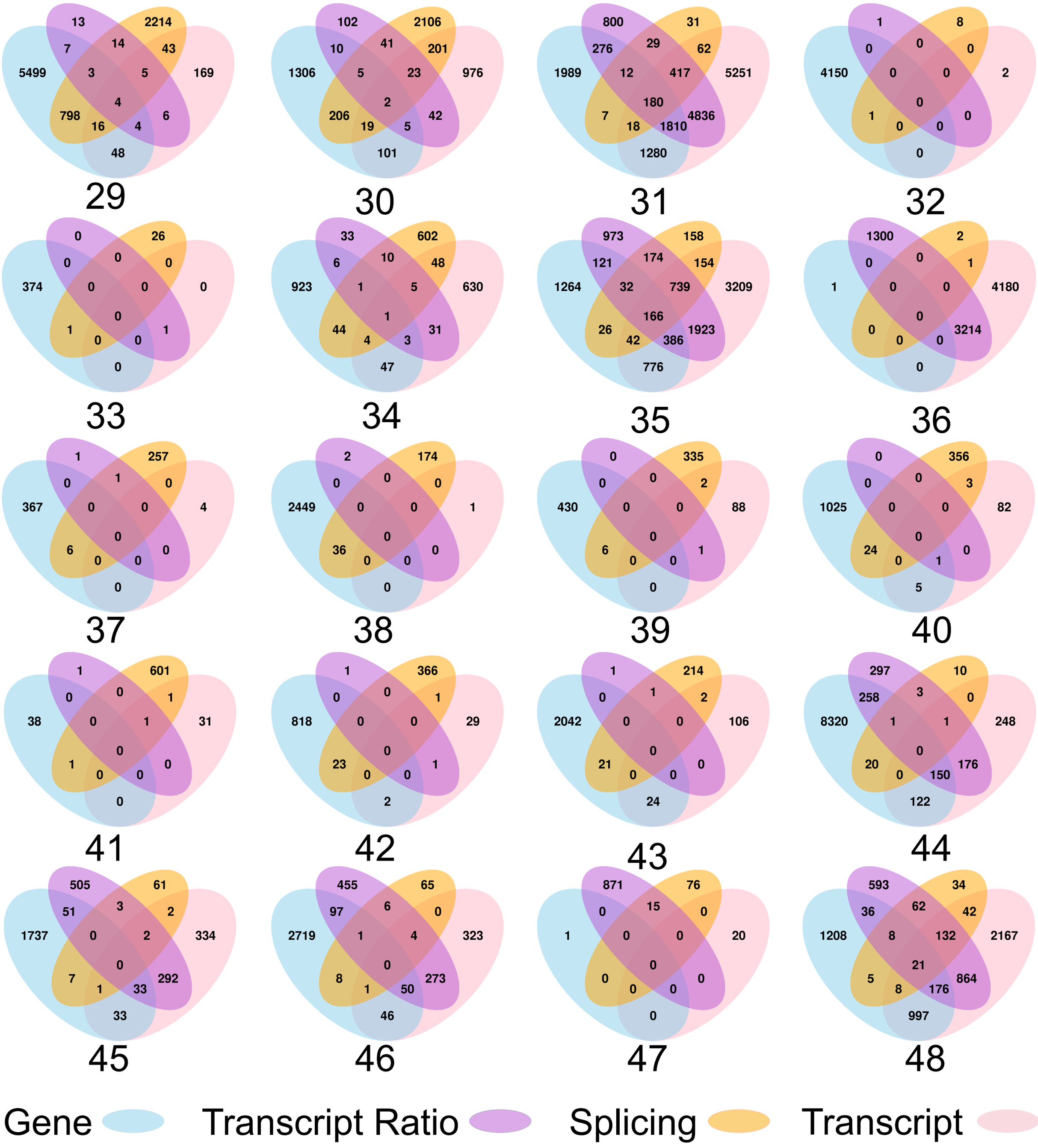


**Figure S4: Overlap of gene sets affected by age-associated changes detected at the level of gene expression (sky blue), transcript ratio (medium orchid), splicing events (orange) or transcript expression (pink).** (1) – (48) show the data for Whole Blood,Adipose - Subcutaneous,Muscle - Skeletal,Artery - Tibial,Artery - Coronary,Heart - Atrial Appendage,Adipose - Visceral (Omentum),Ovary,Uterus,Vagina,Breast - Mammary Tissue,Skin - Not Sun Exposed (Suprapubic), Minor Salivary Gland,Brain - Cortex,Adrenal Gland,Thyroid,Lung,Spleen,Pancreas,Esophagus - Muscularis,Esophagus - Mucosa,Esophagus - Gastroesophageal Junction,Stomach,Colon - Sigmoid,Small Intestine - Terminal Ileum,Colon - Transverse,Prostate,Testis,Skin - Sun Exposed (Lower leg),Nerve - Tibial,Heart - Left Ventricle,Pituitary,Brain - Cerebellum,Cells - Transformed fibroblasts,Artery - Aorta,Cells - EBV-transformed lymphocytes,Liver,Brain - Hippocampus,Brain - Substantia nigra,Brain - Anterior cingulate cortex (BA24),Brain - Frontal Cortex (BA9),Brain - Cerebellar Hemisphere,Brain - Caudate (basal ganglia),Brain - Nucleus accumbens (basal ganglia),Brain - Putamen (basal ganglia),Brain - Hypothalamus,Brain - Spinal cord (cervical c-1) and Brain – Amygdala respectively.

| Transcript Name | Number of Tissues |
|---|---|
| PFKL-001 | 12 |
| PFKL-002 | 1 |
| PFKL-003 | 4 |
| PFKL-004 | 12 |
| PFKL-005 | 9 |
| PFKL-006 | 11 |
| PFKL-007 | 5 |
| PFKL-008 | 1 |
| PFKL-009 | 2 |
| PFKL-010 | 0 |
| PFKL-011 | 4 |
| PFKL-201 | 3 |

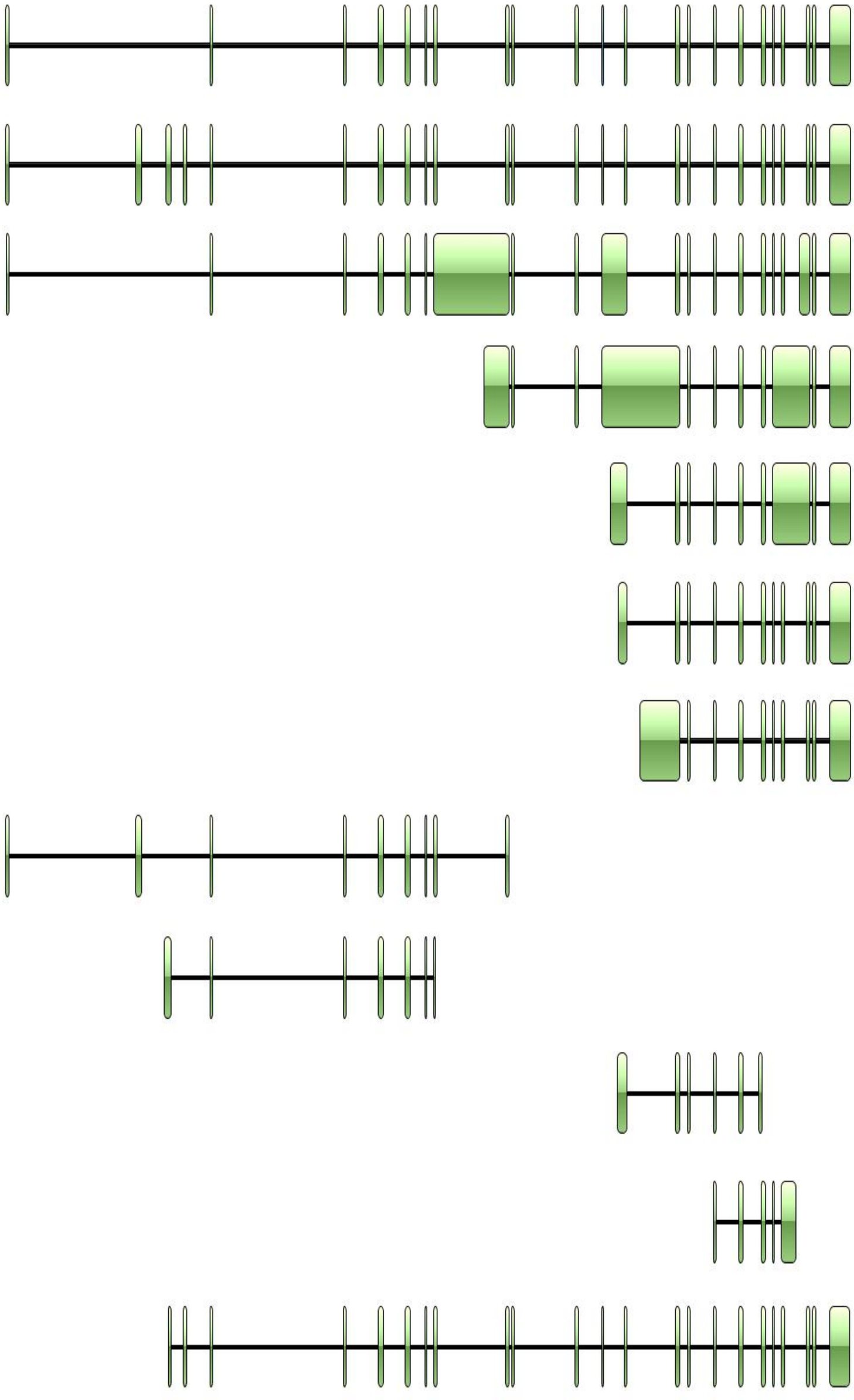

**Figure S5: The annotated 12 transcripts of PFKL gene and the number of tissues in which each transcript's expression/ratio changes significantly with age.**

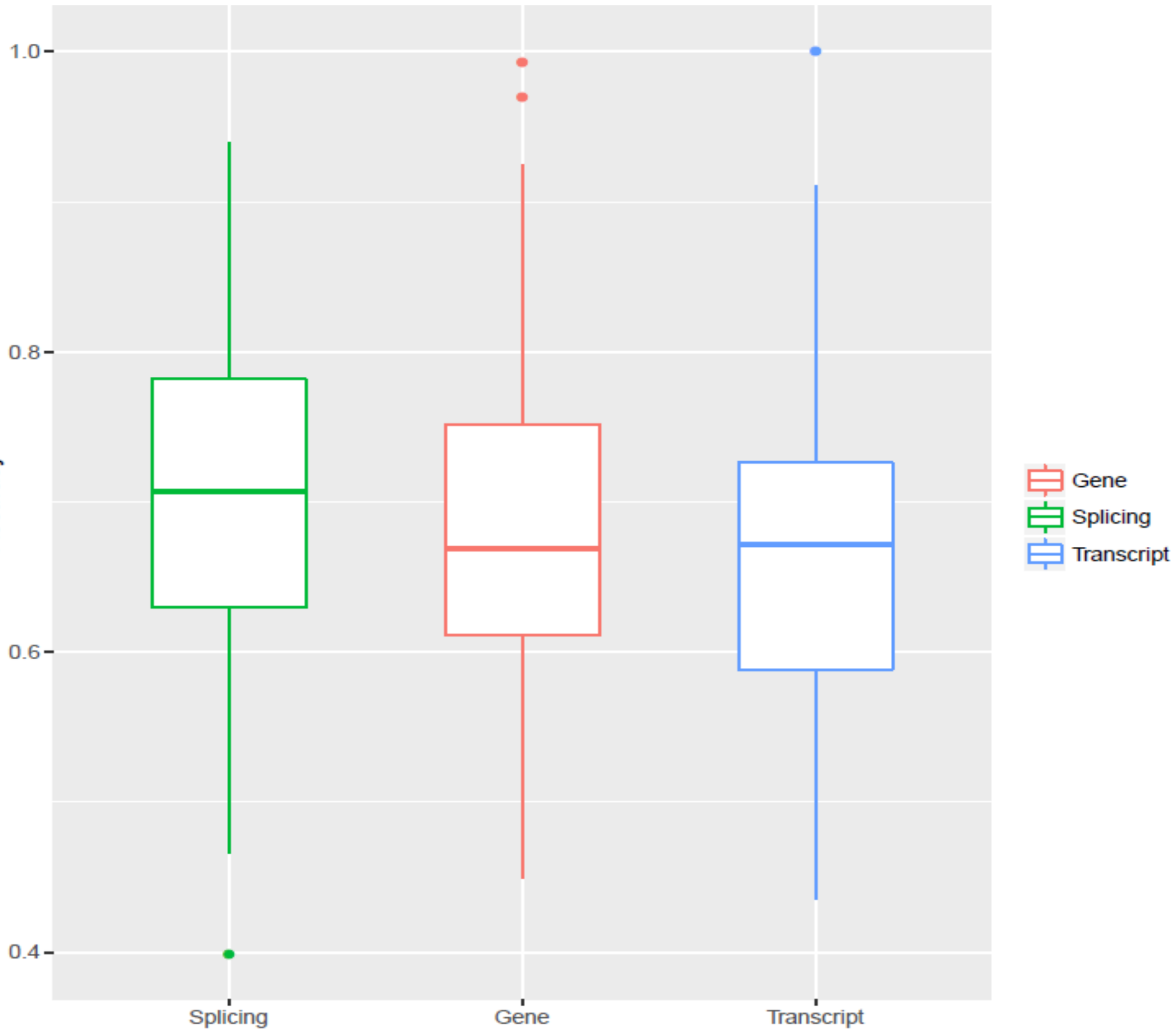


**Figure S6: Age classification accuracy for three models.** The green one is for splicing model, the red is for gene model, the blue is for transcript model.


**Figure S7: Accuracies of prediction of age using models based on 7 different types of splicing events across tissues.** The symbols represent the 7 types of splicing events (as defined in Fig. 5). The accuracy is measured by Pearson correlation between predicted and true ages based on cross-validation.

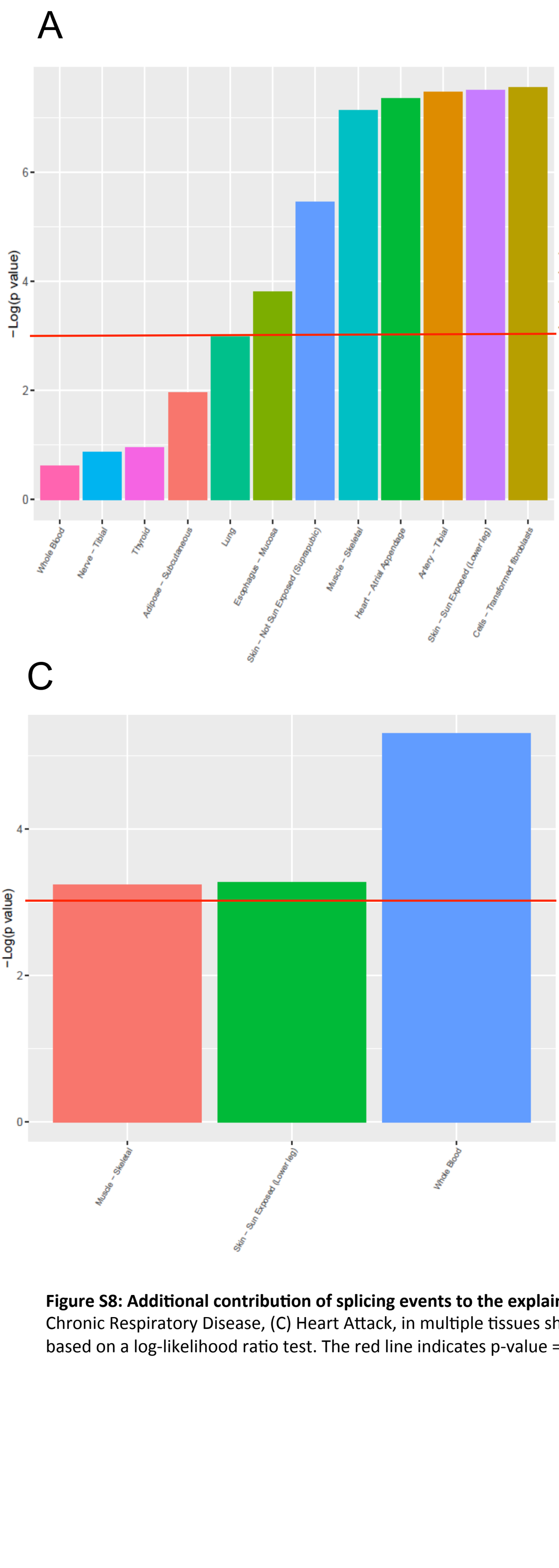


**Figure S8: Additional contribution of splicing events to the explained variance of complex diseases:** (A) Diabetes mellitus type II, (B) Chronic Respiratory Disease, (C) Heart Attack, in multiple tissues shown on x-axis. The y-axis denotes the significance (-log(p-value)) based on a log-likelihood ratio test. The red line indicates p-value = 0.05.

**Figure S9: TreeMap view of GO terms enriched in at least one tissue.** For each GO term, the number of tissues in which it is significant is used as the enrichment score for visualization.

**Table S1: Number of significant age associated splicing events across tissues.**

| Tissue Types | Number of samples | SE (up) | SE (down) | MX (up) | MX (down) | SS.A5 (up) | SS.A5 (down) | SS.A3 (up) | SS.A3 (down) | FL.AF (up) | FL.AF (down) | FL.AL (up) | FL.AL (down) | RI (up) | RI (down) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Whole Blood | 393 | 972 | 1207 | 0 | 1 | 41 | 44 | 81 | 71 | 11 | 12 | 39 | 54 | 2 | 0 |
| Adipose - Subcutaneous | 350 | 47 | 40 | 0 | 0 | 236 | 203 | 334 | 318 | 66 | 55 | 25 | 16 | 0 | 0 |
| Muscle - Skeletal | 430 | 56 | 34 | 7 | 1 | 20 | 21 | 49 | 29 | 14 | 11 | 80 | 69 | 20 | 4 |
| Artery - Tibial | 332 | 703 | 640 | 30 | 36 | 111 | 113 | 148 | 209 | 190 | 213 | 54 | 42 | 32 | 57 |
| Artery - Coronary | 133 | 3 | 6 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Heart - Atrial Appendage | 194 | 547 | 547 | 29 | 19 | 168 | 317 | 169 | 204 | 11 | 22 | 59 | 39 | 103 | 263 |
| Adipose - Visceral (Omentum) | 227 | 585 | 529 | 5 | 11 | 152 | 115 | 280 | 319 | 395 | 379 | 92 | 98 | 318 | 30 |
| Ovary | 97 | 0 | 0 | 23 | 13 | 29 | 35 | 0 | 4 | 3 | 2 | 160 | 149 | 0 | 0 |
| Uterus | 83 | 46 | 83 | 6 | 3 | 36 | 97 | 42 | 24 | 3 | 1 | 25 | 29 | 9 | 36 |
| Vagina | 96 | 0 | 0 | 30 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 81 | 303 |
| Breast - Mammary Tissue | 214 | 6 | 2 | 6 | 8 | 24 | 54 | 0 | 0 | 65 | 57 | 4 | 9 | 27 | 2 |
| Skin - Not Sun Exposed (Suprapubic) | 250 | 920 | 725 | 45 | 27 | 251 | 318 | 289 | 318 | 173 | 161 | 101 | 81 | 11 | 17 |
| Minor Salivary Gland | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brain - Cortex | 114 | 127 | 136 | 6 | 11 | 28 | 34 | 40 | 69 | 0 | 0 | 36 | 26 | 19 | 15 |
| Adrenal Gland | 145 | 5 | 3 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 7 | 4 | 0 | 0 |
| Thyroid | 323 | 10 | 6 | 21 | 25 | 211 | 455 | 280 | 424 | 1 | 0 | 53 | 58 | 60 | 93 |
| Lung | 320 | 154 | 244 | 0 | 1 | 320 | 255 | 181 | 142 | 43 | 45 | 71 | 80 | 0 | 0 |
| Spleen | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Pancreas | 171 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Esophagus - Muscularis | 247 | 188 | 190 | 0 | 0 | 136 | 317 | 92 | 139 | 3 | 0 | 0 | 0 | 30 | 32 |
| Esophagus - Mucosa | 286 | 928 | 1161 | 32 | 42 | 278 | 220 | 284 | 231 | 824 | 788 | 196 | 203 | 52 | 87 |
| Esophagus - Gastroesopha | 153 | 61 | 48 | 3 | 1 | 40 | 20 | 49 | 24 | 162 | 157 | 21 | 32 | 105 | 221 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| geal Junction | | | | | | | | | | | | | | | |
| Stomach | 193 | 115 | 62 | 29 | 18 | 123 | 153 | 291 | 328 | 2 | 8 | 191 | 157 | 198 | 45 |
| Colon - Sigmoid | 149 | 438 | 630 | 13 | 14 | 181 | 172 | 237 | 199 | 477 | 453 | 88 | 94 | 390 | 104 |
| Small Intestine - Terminal Ileum | 88 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Colon - Transverse | 196 | 69 | 111 | 3 | 2 | 119 | 137 | 312 | 271 | 39 | 46 | 26 | 29 | 497 | 41 |
| Prostate | 106 | 0 | 0 | 3 | 5 | 103 | 75 | 0 | 2 | 30 | 41 | 3 | 4 | 44 | 9 |
| Testis | 172 | 157 | 94 | 0 | 0 | 0 | 0 | 3 | 4 | 199 | 193 | 13 | 11 | 19 | 0 |
| Skin - Sun Exposed (Lower leg) | 357 | 1267 | 1025 | 0 | 0 | 365 | 216 | 291 | 216 | 4 | 5 | 153 | 150 | 41 | 60 |
| Nerve - Tibial | 304 | 935 | 675 | 14 | 18 | 130 | 300 | 237 | 468 | 12 | 14 | 135 | 126 | 21 | 15 |
| Heart - Left Ventricle | 218 | 26 | 50 | 2 | 2 | 22 | 20 | 266 | 195 | 2 | 4 | 39 | 38 | 93 | 16 |
| Pituitary | 103 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Brain - Cerebellum | 125 | 4 | 2 | 0 | 0 | 0 | 0 | 3 | 4 | 5 | 5 | 0 | 0 | 0 | 1 |
| Cells - Transformed fibroblasts | 284 | 106 | 97 | 2 | 5 | 26 | 55 | 17 | 38 | 142 | 142 | 21 | 22 | 32 | 21 |
| Artery - Aorta | 224 | 214 | 251 | 9 | 17 | 66 | 57 | 153 | 122 | 227 | 201 | 117 | 114 | 129 | 76 |
| Cells - EBV-transformed lymphocytes | 118 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Liver | 119 | 19 | 35 | 2 | 2 | 79 | 93 | 0 | 0 | 0 | 0 | 1 | 1 | 7 | 0 |
| Brain - Hippocampus | 94 | 43 | 12 | 1 | 5 | 0 | 1 | 20 | 12 | 0 | 0 | 2 | 5 | 83 | 6 |
| Brain - Substantia nigra | 63 | 0 | 3 | 9 | 7 | 16 | 36 | 18 | 33 | 79 | 56 | 0 | 0 | 6 | 13 |
| Brain - Anterior cingulate cortex (BA24) | 84 | 0 | 0 | 16 | 17 | 37 | 53 | 72 | 69 | 1 | 0 | 35 | 25 | 36 | 39 |
| Brain - Frontal Cortex (BA9) | 108 | 0 | 0 | 12 | 13 | 39 | 57 | 61 | 66 | 176 | 170 | 30 | 30 | 7 | 18 |
| Brain - | 105 | 0 | 0 | 1 | 6 | 43 | 58 | 44 | 57 | 25 | 20 | 34 | 24 | 44 | 31 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cerebellar Hemisphere | | | | | | | | | | | | | | | |
| Brain - Caudate (basal ganglia) | 117 | 3 | 6 | 9 | 12 | 0 | 0 | 82 | 82 | 18 | 9 | 13 | 8 | 0 | 0 |
| Brain - Nucleus accumbens (basal ganglia) | 113 | 19 | 8 | 2 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Brain - Putamen (basal ganglia) | 97 | 0 | 0 | 5 | 7 | 0 | 0 | 21 | 29 | 1 | 0 | 0 | 0 | 0 | 0 |
| Brain - Hypothalamus | 96 | 2 | 4 | 6 | 8 | 0 | 0 | 17 | 17 | 20 | 5 | 0 | 0 | 0 | 0 |
| Brain - Spinal cord (cervical c-1) | 71 | 4 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 29 | 40 | 0 | 0 | 0 | 0 |
| Brain - Amygdala | 72 | 0 | 0 | 11 | 9 | 31 | 39 | 44 | 56 | 0 | 0 | 0 | 0 | 9 | 8 |

**Table S2: Nineteen age-associated alternatively spliced genes shared by 9 tissues.**

| Gene name | Functions |
|---|---|
| PKD1 | GO annotations related to this gene include protein kinase binding and protein domain specific binding |
| RNF10 | GO annotations related to this gene include ubiquitin-protein transferase activity and transcription regulatory region DNA binding. |
| NFATC4 | GO annotations related to this gene include transcription factor activity, sequence-specific DNA binding and transcription coactivator activity. |
| CLK4 | GO annotations related to this gene include transferase activity, transferring phosphorus-containing groups and protein tyrosine kinase activity. |
| HDLBP | GO annotations related to this gene include nucleic acid binding and RNA binding. |
| ZMYM6 | GO annotations related to this gene include nucleic acid binding |
| LINC00963 | long non-coding RNA |
| TCOF1 | GO annotations related to this gene include poly(A) RNA binding and transporter activity. |
| ITGA7 | GO annotations related to this gene include protein heterodimerization activity and cell adhesion molecule binding. |
| CNTROB | GO annotations related to this gene include protein domain specific binding |
| WDR73 | May play a role in the regulation of microtubule organization and dynamics |
| VEZT | GO annotations related to this gene include myosin binding. |
| ARHGEF1 | GO annotations related to this gene include poly(A) RNA binding and Rho guanyl-nucleotide exchange factor activity. |
| ANAPC5 | GO annotations related to this gene include protein phosphatase binding. |
| LONP1 | GO annotations related to this gene include sequence-specific DNA binding and serine-type endopeptidase activity |
| STARD3 | GO annotations related to this gene include lipid binding and cholesterol transporter activity |
| TPM1 | GO annotations related to this gene include actin binding and cytoskeletal protein binding |
| UBA5 | GO annotations related to this gene include UFM1 activating enzyme activity |
| MFF | GO annotations related to this gene include protein homodimerization activity. |

**Table S3: Top 15 biological function terms based on number of tissues affected and enrichment (Fold Change).**

| Top Functional terms based on number of Tissues affected | Top Functional terms based on Fold Change |
|---|---|
| Ribonucleoprotein | large ribosomal subunit rRNA binding |
| cadherin binding involved in cell-cell adhesion | Peroxisome biogenesis |
| mRNA processing | protein import into peroxisome matrix |
| mRNA splicing, via spliceosome | RS domain binding |
| translational initiation | peptidyl-lysine monomethylation |
| cell-cell adhesion | execution phase of apoptosis |
| Transit peptide | mitochondrial proton-transporting ATP synthase complex, coupling factor F(o) |
| ATP-binding | activating transcription factor binding |
| Nucleotide-binding | histone H4 acetylation |
| Mitochondrion | hsa04970:Salivary secretion |
| translation | regulation of release of sequestered calcium ion into cytosol by sarcoplasmic reticulum |
| transit peptide: Mitochondrion | Viral nucleoprotein |
| nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | translation initiation factor activity |
| ribosome | error-prone translesion synthesis |
| Metal-binding | protein import into nucleus, docking |

**Table S4: Potential splicing factor drivers in skin fibroblast tissue.**

| Potential Driver | Location |
|---|---|
| SRSF7 | I1/I4 |
| SNRPA | I1 |
| U2AF2 | E2 |
| HNRNPC | E2 |
| PTBP1 | E2 |
| SRSF2 | E2 |
| TIA1 | E2 |
| TIAL1 | E2 |
| RALY | E2 |

**Table S5: Number of tested events across 48 tissues.**

| | SE | MX | SS.A5 | SS.A3 | FL.AF | FL.AL | RI |
|---|---|---|---|---|---|---|---|
| Total Number of Events | 35877 | 4626 | 13787 | 14387 | 68176 | 17552 | 5509 |
| Whole Blood | 21499 | 1814 | 8370 | 9199 | 29162 | 8091 | 4279 |
| Adipose - Subcutaneous | 23679 | 2049 | 9320 | 9843 | 32910 | 9208 | 4480 |
| Muscle - Skeletal | 23073 | 2007 | 9022 | 9553 | 32965 | 8971 | 4328 |
| Artery - Tibial | 23162 | 2011 | 9025 | 9560 | 32402 | 9044 | 4360 |
| Artery - Coronary | 21118 | 1664 | 8202 | 8877 | 26210 | 7518 | 4220 |
| Heart - Atrial Appendage | 22286 | 1824 | 8621 | 9325 | 29712 | 8375 | 4323 |
| Adipose - Visceral (Omentum) | 22923 | 1927 | 8927 | 9611 | 30636 | 8678 | 4450 |
| Ovary | 19880 | 1442 | 7867 | 8440 | 23610 | 6973 | 4113 |
| Uterus | 19601 | 1378 | 7694 | 8327 | 22607 | 6605 | 4078 |
| Vagina | 20461 | 1462 | 8083 | 8698 | 24301 | 6977 | 4223 |
| Breast - Mammary Tissue | 23020 | 1915 | 9029 | 9671 | 30945 | 8716 | 4475 |
| Skin - Not Sun Exposed (Suprapubic) | 22342 | 1830 | 8791 | 9495 | 29592 | 8394 | 4438 |
| Minor Salivary Gland | 18024 | 1062 | 6974 | 7693 | 18381 | 5268 | 3870 |
| Brain - Cortex | 19224 | 1419 | 7623 | 8288 | 23099 | 6743 | 4096 |
| Adrenal Gland | 21553 | 1699 | 8530 | 9154 | 27987 | 7919 | 4284 |
| Thyroid | 23333 | 2014 | 9207 | 9852 | 31971 | 9024 | 4547 |
| Lung | 24414 | 2125 | 9496 | 10253 | 33714 | 9386 | 4632 |
| Spleen | 18699 | 1358 | 7498 | 8210 | 21336 | 6475 | 4153 |
| Pancreas | 20358 | 1558 | 8073 | 8734 | 25505 | 7284 | 4226 |
| Esophagus - Muscularis | 22290 | 1868 | 8764 | 9393 | 30012 | 8470 | 4369 |
| Esophagus - Mucosa | 23495 | 2010 | 9297 | 9834 | 32795 | 8862 | 4512 |
| Esophagus - Gastroesophageal Junction | 20795 | 1597 | 8159 | 8838 | 26317 | 7575 | 4229 |
| Stomach | 22078 | 1760 | 8614 | 9392 | 28532 | 7993 | 4406 |
| Colon - Sigmoid | 21035 | 1609 | 8203 | 8949 | 26175 | 7555 | 4233 |
| Small Intestine - Terminal Ileum | 20009 | 1433 | 7865 | 8640 | 23292 | 6656 | 4264 |
| Colon - Transverse | 22556 | 1836 | 8832 | 9574 | 29592 | 8275 | 4476 |
| Prostate | 19788 | 1413 | 7809 | 8531 | 23197 | 6845 | 4238 |
| Testis | 26176 | 2326 | 10199 | 10756 | 35594 | 10418 | 4736 |
| Skin - Sun Exposed (Lower leg) | 23192 | 1974 | 9181 | 9854 | 31913 | 9008 | 4515 |
| Nerve - Tibial | 22831 | 1953 | 8987 | 9613 | 30718 | 8810 | 4455 |
| Heart - Left Ventricle | 21299 | 1727 | 8367 | 8984 | 27955 | 7965 | 4188 |
| Pituitary | 19805 | 1435 | 7833 | 8524 | 23339 | 6898 | 4212 |
| Brain - Cerebellum | 19422 | 1450 | 7745 | 8402 | 23815 | 6804 | 4135 |
| Cells - Transformed fibroblasts | 24123 | 2239 | 9443 | 9708 | 36058 | 9575 | 4196 |
| Artery - Aorta | 22125 | 1846 | 8695 | 9319 | 29445 | 8409 | 4345 |
| Cells - EBV-transformed lymphocytes | 21900 | 1898 | 8685 | 9021 | 29915 | 8065 | 4050 |
| Liver | 18280 | 1265 | 7186 | 7963 | 20563 | 6177 | 4018 |
| Brain - Hippocampus | 18070 | 1205 | 7089 | 7805 | 20016 | 5919 | 3896 |
| Brain - Substantia nigra | 16362 | 976 | 6298 | 7049 | 15912 | 4700 | 3614 |
| Brain - Anterior cingulate cortex (BA24) | 18160 | 1209 | 7112 | 7800 | 20241 | 5878 | 3893 |
| Brain - Frontal Cortex (BA9) | 19387 | 1411 | 7601 | 8290 | 23131 | 6669 | 4010 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Brain - Cerebellar Hemisphere** | 19362 | 1407 | 7626 | 8310 | 23118 | 6566 | 4045 |
| **Brain - Caudate (basal ganglia)** | 19488 | 1413 | 7639 | 8339 | 23329 | 6826 | 4071 |
| **Brain - Nucleus accumbens (basal ganglia)** | 19693 | 1433 | 7678 | 8378 | 23562 | 6760 | 4097 |
| **Brain - Putamen (basal ganglia)** | 18125 | 1228 | 7070 | 7786 | 20344 | 5991 | 3908 |
| **Brain - Hypothalamus** | 19086 | 1318 | 7449 | 8196 | 21790 | 6420 | 4021 |
| **Brain - Spinal cord (cervical c-1)** | 17546 | 1125 | 6795 | 7487 | 18105 | 5293 | 3756 |
| **Brain - Amygdala** | 16970 | 1062 | 6575 | 7261 | 17426 | 5125 | 3723 |

## Supplementary Note 1

We ascertained that the number of significant events detected was not correlated with the number of samples across tissues; Pearson correlation between sample size and fraction of events found to be 0.06 (p-value = 0.8). We also assessed, for each event type, the potential bias between up-regulated and down-regulated events using paired Wilcoxon test, and found most types of splicing events to be relatively balanced. There was a modest bias in alternative 5’ usage (toward down-regulated events; p-value = 0.047) and alternative first exons events (toward longer isoforms; p-value = 0.03).

**Supplementary Note 2**

PEER factors are expected to capture a variety of known and hidden confounders such as batch effects and global population variance. We explicitly remove the PEER factors that are correlated with age to minimize false negatives, because such PEER factors will explicitly occlude the associations between specific splicing events and age. Since some of the removed PEER factors are also correlated with race or ethnicity, we may falsely detect splicing events associated with race/ethnicity instead of age. Now we have tested whether our results are robust to human ancestry or not.

Recall that in our default approach we removed all PEER factors associated with age, with no consideration to ancestry. In Skin (sun exposed) tissue, for instance, we found that on average across all 23192 exon Skipping events, (1) Of the removed factors, almost none (8e-3%) are correlated with ethnicity and 2.4% are correlated with race. (2) even if we include PEER factors correlated with ethnicity (and age), all detected events were still deemed insignificant, and (3) if we include PEER factors correlated with race (and age), only 0.05% of detected events failed to qualify. We have done this assessment for all tissues and found that (1) Of the removed factors, on average 4.8% are correlated with ethnicity and on average 8.2% are correlated with race. (2) If we include PEER factors correlated with ethnicity, on average only 0.3% of detected events would be deemed insignificant, and (3) if we include PEER factors correlated with race, around 0.35% of detected events would be deemed insignificant in 45 tissues. However, we observed significant drop in detectability in three tissues (Blood, Lung and Colon – Sigmoid), prompting us to perform yet another robustness analysis.

As a potential cause for the drop in detectability in 3 tissues, consider the following. Since age and race can indeed be correlated due to population sampling bias, retaining race-associated PEER factor (thus indirectly retaining age-associated factors), we inadvertently control for age, which can lead to substantial false negatives. Thus instead of including PEER factors correlated with both age and race, we evaluated the model by directly including race as a confounding covariate (ethnicity information is too sparse to perform this analysis). We found that on average only 3% of detected events are deemed insignificant, and importantly, the three tissues showing substantial difference above do not show substantial differences any more (only ~5%).

Thus, overall, the possible false positive due to exclusion of PEER factors that might be associated with race/ethnicity is not substantial and our model should be robust to human ancestry.