## Supplementary Information

# Sex-Specific Isolation and Propagation of Human Premeiotic Fetal Germ Cells and Germ Cell-Like Cells

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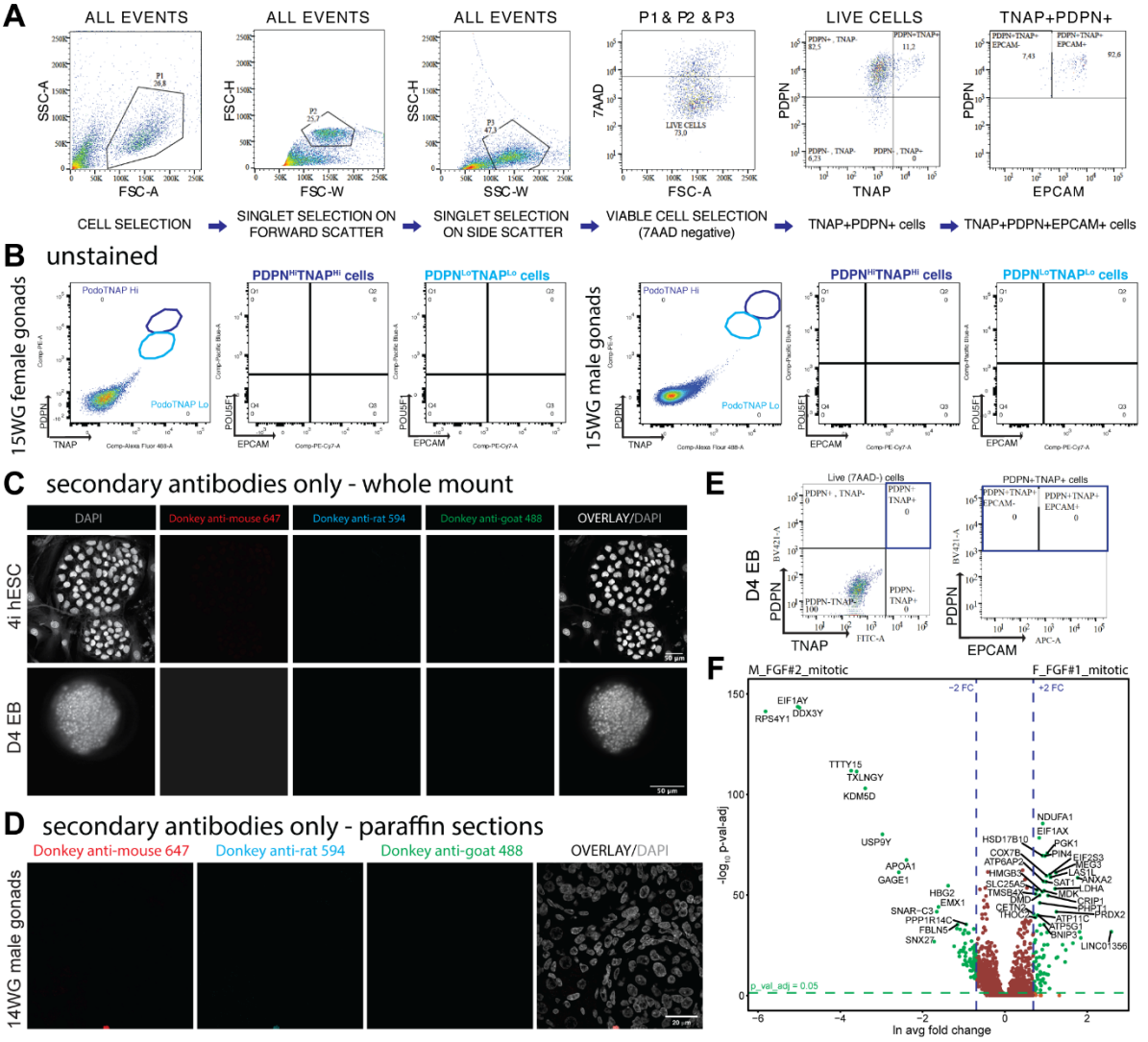


**Figure S1: Gating strategies, negative controls and volcano plot. A)** FACS plots showing the gating strategy used for the analysis of the different cell populations in D4 EBs. **B)** FACS plots showing the gating of the unstained controls on 15WG female gonads (left panels) and 15WG male gonads (right panels). **C)** Negative control for whole mounts (DAPI and secondary antibodies only) for 4i-hESCs (top panel) and D4 EBs (bottom panel). Scale bars are 50µm. **D)** Negative Control for paraffin sections (DAPI and secondary antibodies only) from 14WG male gonads. Scale bars is 20µm. **E)** FACS plots showing the gating of the unstained controls on live cells from D4-EBs. **F)** Volcano plot showing differentially expressed genes between male (M) mitotic FGCs (#2) and female (F) mitotic FGCs (#1).

**Table S1. List of antibodies used for immunofluorescence and FACS.**

| Antibody | Host Species | Stock Concentration | Dilution used | Company / Product No. |
|---|---|---|---|---|
| **Primary antibodies for immunofluorescence** | | | | |
| POU5F1 (OCT3/4) | mouse anti-human | 0.2mg/ml | 1:100 | Santa Cruz / SC-5279 |
| SOX17 | goat anti-human | 0.2mg/ml | WM: 1:175<br>IHC: 1:200 | R&D Systems / AF1924 |
| PDPN | rat anti-human | 0.5mg/ml | WM: 1:200<br>IHC: 1:500 | eBiosciences / 14-9381-80 |
| TNAP (ALPL) | goat anti-mouse | 0.2mg/ml | 1:200 | R&D system / AF2910 |
| DDX4 | rabbit anti-human | 1mg/ml | WM: 1:200<br>IHC: 1:500 | Abcam / Ab13840 |
| **Secondary antibodies for immunofluorescence** | | | | |
| Alexa 488 | donkey anti-goat | 2mg/ml | 1:500 | Abcam / ab150129 |
| Alexa 594 | donkey anti-rat | 2mg/ml | 1:500 | Invitrogen / A21209 |
| Alexa 647 | donkey anti-mouse | 2mg/ml | 1:500 | Invitrogen / A31571 |
| Alexa 647 | donkey anti-rabbit | 2mg/ml | 1:500 | Invitrogen / A31573 |
| **Conjugated antibodies for FACS** | | | | |
| BV421 conj. PDPN | mouse anti-human | 0.2mg/ml | 1:100 | BD Biosciences / 566456 |
| PE conj. PDPN | rat anti-human | 0.05mg/ml | 1:3000 | eBioscience / 12-9381-80 |
| Alexa 488 conj. TNAP | mouse anti-human | 1ml in 100ml of staining volume (for 250K cells) | 1:100 | BD Biosciences / 561495 |
| BV421 conj. ITGA6 | mouse anti-human | 1ml in 100ml of staining volume (for 250K cells) | 1:100 | BioLegend / 313624 |
| APC conj. EPCAM | mouse anti-human | 1ml in 100ml of staining volume (for 250K cells) | 1:100 | BioLegend / 324207 |
| PE/Cyanine7 conj. EPCAM | mouse anti-human | 1ml in 100ml of staining volume (for 250K cells) | 1:100 | BioLegend / 324221 |
| BV421 conc. POU5F1 | mouse anti-human | 0.2mg/ml | 1:100 | BD Biosciences / 565644 |

**Table S2. List of differentially expressed genes between male mitotic and female mitotic FGCs.**

| | p_val | ln avg fold change | pct.1 | pct.2 | p_val_adj |
|---|---|---|---|---|---|
| RPS4Y1 | 2.245E−146 | −5.815331595 | 0.033 | 0.997 | 4.524E−142 |
| EIF1AY | 9.779E−149 | −5.031866128 | 0.007 | 0.997 | 1.97E−144 |
| DDX3Y | 2.888E−148 | −4.992315905 | 0.005 | 0.994 | 5.819E−144 |
| TTTY15 | 6.79E−117 | −3.73437114 | 0.005 | 0.843 | 1.368E−112 |
| TXLNGY | 1.716E−116 | −3.601192326 | 0.007 | 0.843 | 3.458E−112 |
| KDM5D | 4.374E−108 | −3.3932949 | 0.002 | 0.795 | 8.812E−104 |
| USP9Y | 3.0382E−85 | −2.975198277 | 0 | 0.663 | 6.1211E−81 |
| GAGE1 | 2.486E−66 | −2.577180458 | 0.042 | 0.639 | 5.0084E−62 |
| APOA1 | 1.962E−72 | −2.390622397 | 0.044 | 0.663 | 3.9528E−68 |
| SNAR−C4 | 7.7607E−32 | −1.718893356 | 0.314 | 0.678 | 1.5635E−27 |
| SNAR−C3 | 1.049E−46 | −1.655379224 | 0.759 | 0.979 | 2.1134E−42 |
| EMX1 | 4.4218E−49 | −1.612674343 | 0.096 | 0.617 | 8.9085E−45 |
| HBG2 | 1.2471E−59 | −1.377466958 | 0.094 | 0.645 | 2.5125E−55 |
| PRTG | 1.5742E−29 | −1.232335501 | 0.208 | 0.602 | 3.1715E−25 |
| ZADH2 | 2.4357E−38 | −1.167510598 | 0.183 | 0.672 | 4.9071E−34 |
| FBLN5 | 2.6929E−40 | −1.149191149 | 0.494 | 0.934 | 5.4254E−36 |
| S100A11 | 2.1599E−26 | −1.111847619 | 0.344 | 0.744 | 4.3516E−22 |
| LOC100996255 | 1.7205E−24 | −1.103450201 | 0.478 | 0.78 | 3.4664E−20 |
| KRCC1 | 6.5745E−38 | −1.086252179 | 0.19 | 0.684 | 1.3246E−33 |
| SLC35D2 | 1.9228E−27 | −1.074560572 | 0.398 | 0.786 | 3.874E−23 |
| SPRY4 | 5.1802E−27 | −1.027175727 | 0.419 | 0.792 | 1.0437E−22 |
| ENPP4 | 6.4306E−36 | −1.012812873 | 0.347 | 0.837 | 1.2956E−31 |
| KRT17P5 | 1.9894E−20 | −1.01015447 | 0.368 | 0.714 | 4.008E−16 |
| PRSS23 | 1.0446E−35 | −1.004520785 | 0.564 | 0.961 | 2.1045E−31 |
| B3GALNT1 | 5.0882E−28 | −1.001859857 | 0.237 | 0.651 | 1.0251E−23 |
| SYCE1 | 6.0571E−29 | −0.993202541 | 0.344 | 0.765 | 1.2203E−24 |
| PIP5K1B | 1.6858E−37 | −0.974347924 | 0.478 | 0.937 | 3.3964E−33 |
| STOX1 | 1.6455E−22 | −0.968211627 | 0.255 | 0.617 | 3.3152E−18 |
| CD53 | 6.171E−31 | −0.9625812 | 0.279 | 0.738 | 1.2433E−26 |
| IDS | 1.5236E−24 | −0.958967284 | 0.422 | 0.783 | 3.0696E−20 |
| ZNF560 | 2.7358E−26 | −0.950568717 | 0.225 | 0.627 | 5.5118E−22 |
| PPP1R14C | 2.1546E−40 | −0.939439473 | 0.649 | 0.991 | 4.3409E−36 |
| FBLN2 | 2.1454E−20 | −0.937438214 | 0.363 | 0.705 | 4.3222E−16 |
| MSL1 | 2.1522E−21 | −0.934180643 | 0.333 | 0.69 | 4.336E−17 |
| ENTPD1 | 7.4728E−20 | −0.915827694 | 0.384 | 0.711 | 1.5055E−15 |
| PIGT | 3.9939E−36 | −0.901035468 | 0.639 | 0.97 | 8.0464E−32 |
| SLC35B2 | 4.256E−36 | −0.875229251 | 0.698 | 0.955 | 8.5745E−32 |
| RNASE1 | 2.716E−22 | −0.860773915 | 0.494 | 0.837 | 5.472E−18 |
| LBH | 1.0418E−21 | −0.846731505 | 0.513 | 0.834 | 2.0989E−17 |
| MROH8 | 3.2199E−23 | −0.84450453 | 0.237 | 0.62 | 6.4871E−19 |
| TEKT4P2 | 7.0669E−28 | −0.844208534 | 0.232 | 0.648 | 1.4238E−23 |
| LOC653786 | 2.0543E−17 | −0.840282822 | 0.302 | 0.611 | 4.1387E−13 |
| ZNF264 | 4.0733E−14 | −0.825770152 | 0.347 | 0.614 | 8.2064E−10 |
| H2AFY | 2.0712E−20 | −0.814941568 | 0.461 | 0.798 | 4.1729E−16 |

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|---|---|---|---|---|---|
| PEAK1 | 1.018E−19 | −0.812122919 | 0.295 | 0.645 | 2.051E−15 |
| DDX4 | 4.3716E−32 | −0.809024217 | 0.684 | 0.97 | 8.8076E−28 |
| C9orf156 | 2.7889E−17 | −0.804487435 | 0.382 | 0.696 | 5.6188E−13 |
| HACD4 | 1.7688E−19 | −0.794728412 | 0.281 | 0.636 | 3.5635E−15 |
| SNX27 | 6.5307E−29 | −0.778815375 | 0.836 | 0.973 | 1.3157E−24 |
| DCAF4L1 | 1.2182E−21 | −0.776590454 | 0.689 | 0.952 | 2.4543E−17 |
| MLLT3 | 4.0909E−21 | −0.775763195 | 0.253 | 0.608 | 8.2419E−17 |
| CTSH | 6.8461E−22 | −0.771850354 | 0.438 | 0.786 | 1.3793E−17 |
| SGSM2 | 8.0206E−18 | −0.766648405 | 0.375 | 0.723 | 1.6159E−13 |
| ANKRD20A12P | 1.6652E−14 | −0.75809797 | 0.44 | 0.699 | 3.3548E−10 |
| LINC00467 | 2.3077E−28 | −0.75584772 | 0.651 | 0.955 | 4.6492E−24 |
| DAZL | 3.6891E−38 | −0.755059147 | 0.81 | 0.997 | 7.4325E−34 |
| MARCH8* | 5.4784E−26 | −0.749775179 | 0.649 | 0.925 | 1.1037E−21 |
| SERPING1 | 1.4649E−28 | −0.749279779 | 0.918 | 0.994 | 2.9514E−24 |
| PER3 | 1.5315E−23 | −0.747973104 | 0.283 | 0.666 | 3.0856E−19 |
| TJP3 | 6.9908E−23 | −0.744473926 | 0.637 | 0.892 | 1.4084E−18 |
| STK39 | 1.1814E−18 | −0.743047536 | 0.532 | 0.892 | 2.3801E−14 |
| RAB6B | 1.1106E−19 | −0.74270123 | 0.333 | 0.69 | 2.2375E−15 |
| ZNF473 | 2.4975E−21 | −0.742492915 | 0.532 | 0.855 | 5.0318E−17 |
| HDAC9 | 2.657E−24 | −0.741574136 | 0.548 | 0.898 | 5.3531E−20 |
| PTCH1 | 2.1952E−22 | −0.740849297 | 0.267 | 0.657 | 4.4227E−18 |
| ICA1 | 3.4635E−27 | −0.739654736 | 0.33 | 0.783 | 6.978E−23 |
| LMBR1L | 1.8688E−23 | −0.734934697 | 0.398 | 0.819 | 3.765E−19 |
| LINC00339 | 5.3781E−19 | −0.733037619 | 0.279 | 0.627 | 1.0835E−14 |
| COL22A1 | 1.5608E−13 | −0.732414689 | 0.525 | 0.765 | 3.1445E−09 |
| AGGF1 | 5.6422E−30 | −0.731540303 | 0.693 | 0.964 | 1.1367E−25 |
| STK31 | 5.2834E−15 | −0.728976875 | 0.436 | 0.723 | 1.0645E−10 |
| TDRD9 | 1.0273E−23 | −0.716849627 | 0.393 | 0.771 | 2.0697E−19 |
| SKAP2 | 6.3066E−27 | −0.715284023 | 0.621 | 0.922 | 1.2706E−22 |
| NR1H2 | 6.8461E−24 | −0.707402344 | 0.719 | 0.952 | 1.3793E−19 |
| RAB11B | 3.0624E−20 | −0.705575752 | 0.604 | 0.877 | 6.1699E−16 |
| CDH2 | 1.4899E−26 | −0.70375383 | 0.775 | 0.979 | 3.0017E−22 |
| RHOG | 1.5037E−21 | −0.700048529 | 0.4 | 0.786 | 3.0296E−17 |
| C2orf74 | 8.9902E−25 | −0.699532446 | 0.646 | 0.925 | 1.8113E−20 |
| PRR15 | 7.7557E−14 | −0.69907575 | 0.344 | 0.614 | 1.5625E−09 |
| RPRM | 2.4825E−22 | −0.698793285 | 0.527 | 0.873 | 5.0016E−18 |
| ZNF16 | 1.6854E−16 | −0.696337515 | 0.335 | 0.645 | 3.3957E−12 |
| ZNF879 | 7.6354E−17 | −0.695976082 | 0.326 | 0.648 | 1.5383E−12 |
| SETD9 | 5.9144E−18 | −0.694565159 | 0.319 | 0.66 | 1.1916E−13 |
| SYAP1 | 1.0356E−25 | 0.696128406 | 0.934 | 0.916 | 2.0864E−21 |
| NKAP | 1.1532E−19 | 0.698613682 | 0.888 | 0.822 | 2.3233E−15 |
| CETN2 | 1.2207E−45 | 0.698858231 | 0.995 | 0.991 | 2.4593E−41 |
| LGALS1 | 1.357E−21 | 0.708947692 | 0.991 | 0.985 | 2.734E−17 |
| FHL2 | 5.686E−10 | 0.715162506 | 0.724 | 0.605 | 1.1455E−05 |
| UPF3B | 2.5064E−31 | 0.719652309 | 0.948 | 0.94 | 5.0497E−27 |
| DPPA5 | 5.8083E−12 | 0.719977419 | 0.925 | 0.937 | 1.1702E−07 |

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|---|---|---|---|---|---|
| CLDN7 | 1.3322E−08 | 0.726268983 | 0.649 | 0.524 | 0.0002684 |
| THOC2 | 6.8972E−44 | 0.740649866 | 1 | 0.958 | 1.3896E−39 |
| TSR2 | 1.1666E−32 | 0.741558609 | 0.979 | 0.976 | 2.3503E−28 |
| CYB5A | 9.2881E−19 | 0.746803755 | 0.916 | 0.861 | 1.8713E−14 |
| TMSB4X | 1.4802E−55 | 0.747375538 | 1 | 1 | 2.9822E−51 |
| PPP1R15A | 5.7929E−22 | 0.750559833 | 0.911 | 0.825 | 1.1671E−17 |
| PYGL | 8.0558E−09 | 0.756942769 | 0.726 | 0.675 | 0.0001623 |
| OGT | 1.0798E−26 | 0.757069373 | 0.965 | 0.931 | 2.1754E−22 |
| GSPT2 | 3.7759E−08 | 0.7613267 | 0.637 | 0.539 | 0.00076073 |
| PLIN2 | 1.1036E−23 | 0.76935916 | 0.925 | 0.825 | 2.2235E−19 |
| PCSK1N | 2.5619E−18 | 0.772128697 | 0.984 | 0.994 | 5.1614E−14 |
| ATRX | 4.2127E−37 | 0.776073115 | 0.991 | 0.967 | 8.4874E−33 |
| SLC25A5 | 1.7795E−57 | 0.777908651 | 1 | 1 | 3.5851E−53 |
| RNF113A | 3.3715E−14 | 0.790091395 | 0.735 | 0.578 | 6.7926E−10 |
| QTRT1 | 3.0617E−15 | 0.791055784 | 0.803 | 0.741 | 6.1684E−11 |
| ATP11C | 5.8543E−45 | 0.79455939 | 0.986 | 0.985 | 1.1795E−40 |
| NMT2 | 3.5803E−13 | 0.7985535 | 0.785 | 0.645 | 7.2132E−09 |
| ATP5G1 | 6.4042E−45 | 0.805022199 | 0.993 | 0.982 | 1.2903E−40 |
| PDHA1 | 5.6829E−20 | 0.818387702 | 0.862 | 0.69 | 1.1449E−15 |
| TCEAL4 | 2.9675E−20 | 0.829049098 | 0.885 | 0.795 | 5.9787E−16 |
| EIF1AX | 1.7764E−83 | 0.83421497 | 0.998 | 1 | 3.5789E−79 |
| DBI | 4.1487E−24 | 0.84088565 | 0.937 | 0.904 | 8.3583E−20 |
| DMD | 6.5097E−55 | 0.844933547 | 0.988 | 0.973 | 1.3115E−50 |
| PHPT1 | 4.1446E−51 | 0.848538631 | 0.995 | 0.988 | 8.3502E−47 |
| ALPPL2 | 3.904E−11 | 0.8531822 | 0.71 | 0.566 | 7.8654E−07 |
| FAM60A | 4.9018E−40 | 0.857126872 | 0.967 | 0.955 | 9.8757E−36 |
| PRPS1 | 8.0598E−17 | 0.860180539 | 0.827 | 0.726 | 1.6238E−12 |
| NUSAP1 | 1.5623E−15 | 0.862297448 | 0.965 | 0.919 | 3.1476E−11 |
| LPCAT4 | 1.0852E−07 | 0.863853915 | 0.618 | 0.5 | 0.00218628 |
| SLC2A1 | 1.5067E−12 | 0.879870181 | 0.721 | 0.566 | 3.0356E−08 |
| CRYM | 5.7288E−10 | 0.880329163 | 0.625 | 0.458 | 1.1542E−05 |
| IFITM1 | 1.2316E−35 | 0.884027673 | 0.993 | 0.994 | 2.4812E−31 |
| DIAPH2 | 7.7274E−28 | 0.908010669 | 0.904 | 0.774 | 1.5568E−23 |
| HMGB3 | 1.1329E−56 | 0.909929067 | 0.995 | 1 | 2.2824E−52 |
| HSD17B10 | 1.3383E−74 | 0.915124575 | 0.993 | 1 | 2.6962E−70 |
| PI4KAP2 | 2.3698E−12 | 0.915855296 | 0.71 | 0.566 | 4.7744E−08 |
| REC8 | 9.3001E−24 | 0.916568833 | 0.855 | 0.672 | 1.8737E−19 |
| NDUFA1 | 1.3151E−90 | 0.920445929 | 1 | 1 | 2.6495E−86 |
| APOC1P1 | 2.9874E−13 | 0.932229235 | 0.611 | 0.364 | 6.0188E−09 |
| IDH3G | 5.0253E−24 | 0.935794377 | 0.845 | 0.714 | 1.0124E−19 |
| ATP6AP2 | 7.8391E−62 | 0.938017704 | 0.998 | 0.991 | 1.5794E−57 |
| PRKX | 7.7501E−16 | 0.940454002 | 0.609 | 0.343 | 1.5614E−11 |
| ACTG2 | 6.2774E−25 | 0.948798301 | 0.951 | 0.864 | 1.2647E−20 |
| BNIP3 | 2.5315E−40 | 0.950246556 | 0.998 | 0.997 | 5.1001E−36 |
| STAG2 | 3.4495E−30 | 0.952531627 | 0.92 | 0.816 | 6.9498E−26 |
| FKBP11 | 3.8911E−16 | 0.953011951 | 0.749 | 0.645 | 7.8394E−12 |

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|---|---|---|---|---|---|
| MDK | 3.6426E−57 | 0.953998575 | 1 | 0.997 | 7.3388E−53 |
| PIN4 | 1.7194E−74 | 0.97341047 | 0.991 | 0.994 | 3.4642E−70 |
| RHOBTB3 | 2.9358E−11 | 0.977806015 | 0.616 | 0.431 | 5.9148E−07 |
| SAT1 | 1.1857E−61 | 1.000273302 | 1 | 1 | 2.3887E−57 |
| COL1A2 | 1.3021E−15 | 1.011314823 | 0.639 | 0.467 | 2.6234E−11 |
| COX7B | 1.3563E−64 | 1.012192301 | 0.993 | 0.988 | 2.7325E−60 |
| BRDT | 2.0261E−36 | 1.02425211 | 0.92 | 0.744 | 4.0819E−32 |
| PGK1 | 8.3864E−76 | 1.030532987 | 0.998 | 1 | 1.6896E−71 |
| NLRP7 | 2.6231E−30 | 1.039416876 | 0.93 | 0.828 | 5.2848E−26 |
| CRIP1 | 8.8147E−55 | 1.048008315 | 0.988 | 0.946 | 1.7759E−50 |
| DEPTOR | 1.996E−15 | 1.05139215 | 0.637 | 0.434 | 4.0214E−11 |
| ELF3 | 1.3192E−19 | 1.075156533 | 0.674 | 0.395 | 2.6578E−15 |
| HSPB1 | 1.7833E−08 | 1.085057722 | 0.979 | 0.973 | 0.00035929 |
| EIF2S3 | 3.4857E−65 | 1.095885869 | 0.998 | 0.979 | 7.0226E−61 |
| LAS1L | 8.0952E−64 | 1.111708673 | 0.977 | 0.922 | 1.6309E−59 |
| LDHA | 4.0217E−58 | 1.219874914 | 1 | 0.997 | 8.1026E−54 |
| MEG3 | 3.4804E−66 | 1.239552588 | 0.998 | 0.997 | 7.0121E−62 |
| SLC35F2 | 1.1048E−23 | 1.249868091 | 0.689 | 0.395 | 2.2259E−19 |
| PRDX2 | 1.143E−46 | 1.25055862 | 0.934 | 0.798 | 2.3028E−42 |
| GDPD2 | 3.9745E−24 | 1.281205743 | 0.785 | 0.56 | 8.0074E−20 |
| ID1 | 2.8218E−30 | 1.403248743 | 0.878 | 0.846 | 5.6851E−26 |
| ARRDC3 | 1.1065E−21 | 1.463934874 | 0.719 | 0.527 | 2.2292E−17 |
| NGFRAP1 | 1.1167E−27 | 1.481777737 | 0.834 | 0.714 | 2.2498E−23 |
| TUBB2B | 4.731E−31 | 1.65301173 | 0.707 | 0.377 | 9.5316E−27 |
| ANXA2 | 1.1838E−63 | 1.768904759 | 0.934 | 0.617 | 2.385E−59 |
| PTN | 1.159E−36 | 1.8157341 | 0.66 | 0.253 | 2.335E−32 |
| CA2 | 1.13E−33 | 1.845621888 | 0.696 | 0.349 | 2.2766E−29 |
| LINC01356 | 9.7734E−37 | 2.587518332 | 0.696 | 0.331 | 1.969E−32 |