

Let-7b, miR-29b and miR-125b as potential biomarkers for differentiating canine mammary carcinoma histological types

Supplementary Material

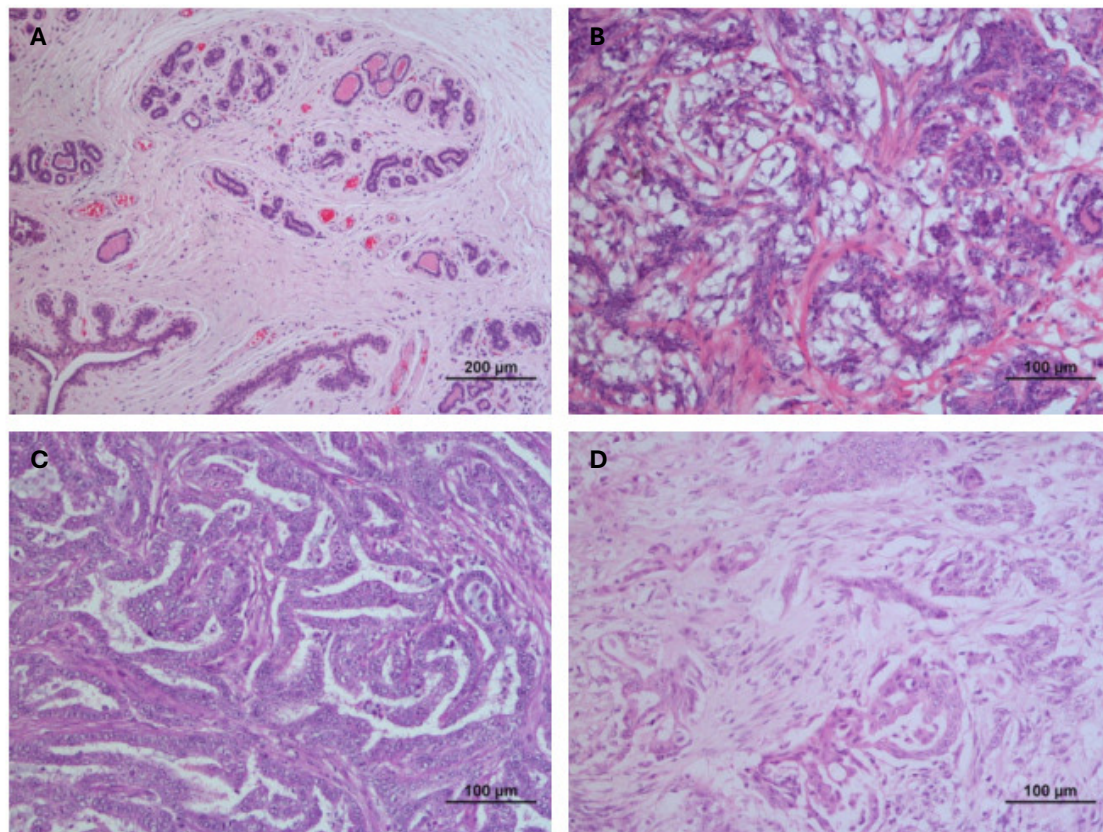


Figure S1. Histopathology microphotographs representative of the canine mammary gland tissues analyzed in the present study: controls (**A.** normal mammary gland); benign lesions (**B.** complex adenoma); malignant lesions (**C.** tubulopapillary carcinoma and **D.** complex carcinoma). Hematoxylin and eosin staining.

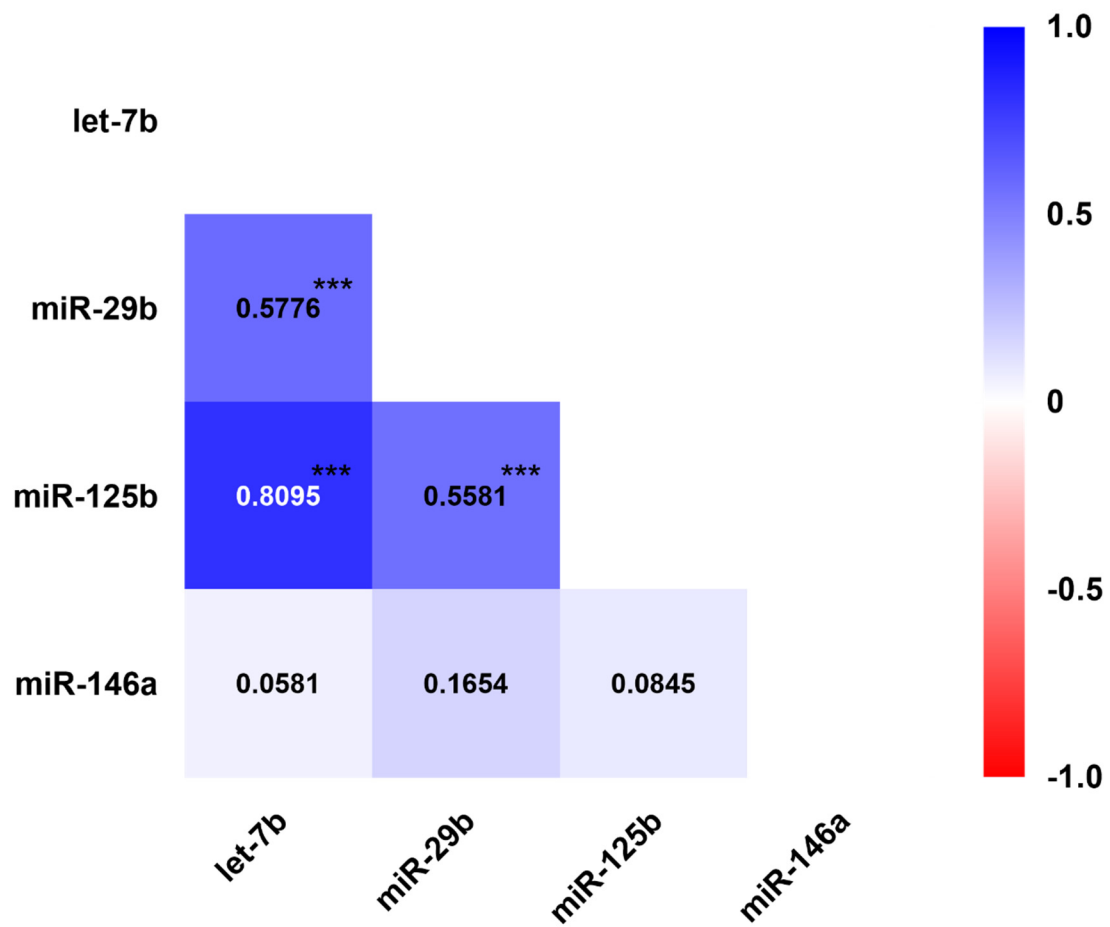


Figure S2. Heatmap of Pearson correlation coefficient matrix. The correlation matrix heatmap displays the Pearson correlation coefficients between microRNA expression data. In this matrix, 1 indicates a perfect positive correlation, 0 indicates no correlation and -1 indicates a perfect negative correlation between the studied microRNAs. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table S1. List of 1227 gene targets obtained from the mRNA/miRNA interaction, selected for STRINGapp Protein Query.

microRNA	Gene target
let-7b, miR-29b and miR-125b	RALGAPA2, ZNF641, NFASC, AGTPBP1, BCL9L, WDR59, PRRC2C, SYT16, CBX6, ZNF451, GTF3C6, FGD6, FBLN7, DUSP16, ISL1, OXR1, DBH, ATM, ZDHHC5, DEAF1, PLK4, MITF, DNAH1, CELSR3, OTOG, CLASP2, GORASP1, TTC21A, CIT, TACC2, PXDNL, VGLL3, LRP2, SYNE1, CIC, VPS13D, CHD1, RHOTB3, FUT11, NCOR1, WIPI2, PARP1, TOP2A, LCN10, LRRC8A, SMC1B, DNAH8, IRF5, AASS, NRG1, KIDINS220
let-7b and miR-29b	GABPB2, TCP11L1, SLC7A11, CCDC93, LOC119864502, EIF4E3, KBTBD8, RBM15B, ANKRD49, CLYBL, ACP3, FAM210B, BICD1, CCND2, RBM20, MAP1A, RFX7, BNIP2, LOC478384, FAM13A, DNAH5, MYNN, LOC100685048, ZC4H2, RLIM, ZNF516, SPIN1, CUL2, TRIM23, VWA5B1, PGD, NTRK3, MPZL3, SLC16A13, SLC35D1, SUN1, LOC119872207, DTL, FAM163A, ZBTB37, POU2F1, PLD5, CNST, SGPP1, CDC42BPB, NRXN1, FEM1C, HSPA9, SVEP1, KIAA1958, GJA9, PTPRQ, VANGL1, CSTF3, GJB6, NEXMIF, CD226, RFX3, PROM1, ARRDC3, ZDHHC4, RABGAP1L, PDE6B, ABCC1, COL1A1, GNB1, TRPV4, TES, ATP2B1, OPRM1, OR51L1, ATXN7L1, PCDH18, INPP4B, LRP1B, DNAH12, SYDE1, MYO7A, FAM160A2, E2F8, LPAR6, LOC119865171, ENTPD3, ATR, CPXM1, UGT1A6, PDS5B, DOCK10, CUX2, CNTN1, WNK1, FGFR2, GPR176, FAM214A, ACAP2, ATP13A3, ACTRT3, ZNF639, PRKAG3, IKBKE, EPRS1, CACNA1F, ERCC6L, NAA10, SPRY3, AHI1, LAMA2, TLE4, LOC476416, WAC, DHTKD1, FTO, UBR4, C2H10orf67, MAC1R, MCTP1, LYSMD4, USP54, RGR, FCSK, PHLPP2, FHOD1, BAZ1B, ATP5J2, AIMP2, DOC2A, WDR64, CEP76, ZFH2, NEK9, KCNK10, DHX8, DUSP14, PMPCA, RC3H2, PLAA, AARS2, MLIP, ASCC3, LRRC6, LNX1, AASDH, POLR2B, LOC482174, CALU, ANKIB1, MIOS, SP4, EPS15, ATPAF1, CNOT4, AGBL3, SH3BP5L, LOC119874670
miR-29b and miR-125b	EIF4E2, SLC1A2, PGRMC2, PGPEP1, YTHDF1, DCLK1, PATZ1, DENND5B, PARP11, BCL2L13, DAPK2, ARHGAP31, AMER1, SGK1, ASPN, SUV39H2, PURA, PI4K2B, RERE, IST1, SNX8, GGA2, IGF2BP3, ZNF862, HIPK1, HTD2, FBXO43, TRPV1, UGCG, EHBP1L1, PGLYRP2, EED, DLG2, KIAA1671, SLC38A2, INO80, HERC3, ANK2, DSP, ZKSCAN4, TLK1, ATP7A, PEG3, KLC3, PARP8, PATJ, TTC34, PSKH1, ELMO3, BRCA1, MYO1C, POLR3B, ECPAS, C5, SCIN, CRHR2, HIVEP3, TMEM154, DENND11, FAM98A, ARID5A, TENT5C
let-7b and miR-125b	CLCN1, C5AR1, NKX2-5, CGN, SLC23A2, TXNRD1, PIM1, COL9A3, MAPK3, CPT1A, PTAFR, NECTIN1, PDS5A, DSC1, POLR3GL, MINDY1, VSTM2A, HIPK3, CELF1, MADD, CREB3L1, TP53I11, SHANK2, CTSE, BBS1, NAA15, TPRA1, EFCC1, TMEM43, GRIP2, NR2C2, RFT1, PCBP4, ALS2CL, LOC484867, PTGER1, TNPO2, PDE4A, BSG, RNF141, PAK1, B3GNT6, SLC2B1, FAM168A, LOC106560171, TRIM66, UEVLD, GPALPP1, GRK1, CRTAP, LOC607986, MYRIP, XRN1, KCNAB1, GZF1, RBBP9, GPCPD1, TPX2, PLAGL2, PPP1R16B, HNF4A, RIMS4, ELMO2, SLC7A1, SLC25A15, FBXO16, DMTN, ZNF605, TMEM132B, HECTD4,



CFAP73, MED13L, FBXO21, KSR2, MLEC, OSBP2, CLTCL1, PPFIBP1, TESPA1, SPRYD3, DNAJC22, FGD4, C27H12orf60, EMP1, STYK1, GABARAPL1, C3AR1, PLCE1, TNKS2, LOXL4, HIF1AN, POLL, SLK, HABP2, ATE1, ASPH, FAM110B, NSMAF, PAG1, RBM12B, RAB27A, FMN1, ZNF106, MYEF2, CGNL1, ZNF609, NPTN, EDC3, HMG20A, ITSN1, B3GALT5, FRAS1, PDLIM5, CXXC4, PLA2G12A, NSUN3, ZPLD1, USF3, ECE2, LIPH, MASP1, KPNA4, PLD1, BTN1A1, ZKSCAN8, PDE1A, ABCB11, COBLL1, DCAF17, MAP3K20, SESTD1, HECW2, PPIL3, CDK15, NBEAL1, CREB1, PLEKHM3, ACADL, LMX1A, LOC609484, LOC478984, LOC612524, FANCB, SUV39H1, SMC1A, XKRX, TBC1D8B, LOC102157101, IRS4, TMEM164, LONRF3, LOC492092, NKAP, ZBTB33, ATP1B4, HMGB3, TBPL1, SCN1B, KLK13, ENPP1, ELAC1, UST, SOD2, PRKN, WDR27, PRUNE2, GCNT1, APBA1, TMEM252, S1PR3, BICD2, PRKCG, LOC484343, IGLON5, GYS1, ZNF529, LOC119863924, PLXDC2, KIF5B, FRMD4A, ECSCR, HBEGF, ZMAT2, KCTD16, STK32A, PLPP1, MAP3K1, CDK7, ZNF366, SERINC2, MED18, SLC30A2, WNT4, MTOR, TMC3, EFNA5, MTX3, LHFPL2, NIPA1, APBA2, SV2B, ABHD2, FANCI, ALPK3, HOMER2, RGS12, MSANTD1, EVC, TBC1D19, LGALS8, SPEF2, ZNF248, GNG4, EIF4EBP2, ERGIC1, KCNIP1, WWC1, LSM11, G3BP1, DAB2, NCAM1, RNF222, GLP2R, NLRX1, HYOU1, PAFAH1B2, GAS7, AKAP10, LOC489534, NATD1, SAMD11, ATAD3A, ANKRD65, MVD, ZNF469, LOC119863920, KLHL36, HSD17B2, RFWD3, ST3GAL2, KCNA2, BCL7B, SSC4D, KDELR2, LOC489893, MAD1L1, GPR146, STX1B, RNF40, ZNF48, ZKSCAN2, PRKCB, SYT17, RBFOX1, TBC1D24, PRKACB, TNFSF4, NAV1, DYRK3, C7H1orf74, TATDN3, ODR4, TNR, C7H1orf112, HCN3, UBE2Q1, ST8SIA5, C7H18orf25, GAREM1, COLEC12, CIDEA, TC2N, EFS, NOVA1, MAPK1IP1L, ESR2, AKAP5, GALNT16, SUS D6, MAP3K9, LIN52, CIPC, TBC1D16, C1QTNF1, AFMID, GPRC5C, CRHR1, CD300LG, KRT39, GSDMA, PSMD11, ADAP2, RAB11FIP4, NF1, NXN, INPP5K, METTL16, RAPGEF1, ABL1, EXOSC2, OLFML2A, NEMP1, SPRYD4, OS9, HMGA2, KIAA0930, TAB1, TCP11L2, TMEM182, CNM4, MAML1, ZNF454, SLC22A5, JADE2, NFIB, SLC24A2, UBE2R2, PIGO, SIT1, PAX5, PLPPR1, TAL2, ZNF483, RGS3, LOC100856137, SLC26A8, GLP1R, TTBK1, TRAM2, SIM1, QRSL1, LOC482176, TRHR, LIMCH1, TMEM33, SLC30A9, CLOCK, PLEKHA8, NEUROD6, PRSS38, PLXNA4, AGR2, ITGB8, RAPGEF5, HOXA13, TFEC, NT5C1A, GRIK3, KIAA0319L, MOB3C, RNF220, TIE1, SYT1, TCAF1, KDM7A, DGKI, GPAT4, UNC5D, CFAP97, STOX2, SPCS3, CENPO, DNAJC27, ASXL2, HADHA, CRIM1, GEMIN6, ATOH8, PAIP2B, CSDE1, WARS2, VNN1, MGAT4F, MTMR11, EHF, LRRC55, INTU, ANKRD24, CFAP97D2, C24H20orf96, SLA2, FLT3, PRSS56, PISD, MARCHF8, CALHM2, ACSL5, SMIM34A, TIGD2, ERAS, DRP2, AKT2, NHSL1, AGPAT4, CALHM5, ZSCAN22, SSC5D, ZFP14, GRXCR2, PDE4D, PIK3R1, ZNF683, FER, FAM193B, PLEKHN1, PRKCZ, HSDL1, OSGIN1, PCOLCE, ITGAM, ANKS4B, LOC610677, NVL, LOC490399, RIPK3, CCDC57, P2RX1, IL17REL, APOM, GRHL2, COL14A1, MYCL, TMEM139, LOC100688918, ANK1, UMOD, PGB1, KCNJ2, TJP1,



CUBN, PON2, CYP2E1, CD34, PDGFRB, CCL8, LOC448801, CCKAR, ATP7B, HGFAC, KDM5C, ARSL, AOX2, CD47, SSPO, LOC100049001, PMEL, ACAN, OAZ1, FMC1, ANPEP, SGK3, P2RY1, TPC3, PRF1, VDAC3, STK38L, GMPS, CASQ2, NOS2, CAPRIN1, LOC106557476, TAC3, OR6B1, OR8S9, OR10G9, OR2W11, OR3A1H, OR51H5, OR52E22, OR8S12, OR8D4, PDE4DIPP2, ANKRD35, OTUD7B, APH1A, ANP32E, C17H1orf54, ADAMTSL4, POGZ, OR10V10, HECW1, MAGI2, UPP1, LRRC4C, QSER1, RIN1, EFEMP2, AP5B1, LOC476047, LOC119864445, IWS1, MAP3K2, LYPD6, RIF1, LOC119864504, IQCF1, SLC41A3, MKRN2OS, IRAK2, RPUSD3, GRM7, CHL1, ADAMTS9, ATXN7, CFAP20DC, ITIH4, STAB1, C20H3orf18, MST1, USP4, SETD2, LRRC2, SLC6A20, PDE4C, TMEM38A, CHERP, CYP4F22, LOC484960, INSR, ZNF358, DUS3L, DPP9, REXO1, RNF126, MMP26, SLC6A5, MTMR2, AMOTL1, USP35, POLD3, LOC476816, LAMTOR1, DCHS1, CYB5R2, C21H11orf16, NRIP3, PLEKHA7, LGR4, DGKH, CHAMP1, STAC, MLH1, TGFB2, EFHB, PIK3R4, ZBTB38, PAQR9, EIF2A, IGSF10, BPIFB2, TMC2, KIF16B, JAG1, SLC52A3, ITCH, NCOA6, GGT7, NCOA5, ZNFX1, PRPF6, ACKR3, B3GLCT, PROSER1, NEK1, LOC486076, MTMR9, ESCO2, DPYSL2, CHMP7, XPO7, AGFG1, ATP6V0A2, SETD1B, TRAFD1, CABP1, SART3, MORC2, UFD1, CDKN1B, LOC486582, GPR84, HOXC6, PCBP2, DAZAP2, CSRNP2, TFCP2, ASIC1, KCNH3, DDN, LOC119866377, SOX5, GPRC5A, CLEC12A, SCNN1A, WASHC1, LBX1, TMEM273, RASGEF1A, BTA1F1, HPS1, PKD2L1, SEMA4G, PPRC1, ARL3, WBP1L, PDCD11, PDZD8, INPP5F, EDRF1, ADAM12, RB1CC1, YTHDF3, NCOA2, PRTG, DISP2, MGA, SPTBN5, GANC, STARD9, TMEM62, EPB42, ATP8B4, HERC1, DPP8, DIS3L, TLE3, C30H15orf39, USP25, TIAM1, IFNAR2, IFNGR2, SON, GK2, WDFY3, AFF1, CPOX, POLQ, SLC15A2, MYLK, CCDC14, ITGB5, WDR53, ADGRG7, LPCAT1, EHHADH, LOC478670, LOC102153616, GCNT2, MAK, NUP153, LOC100855640, PJVK, RBMS1, HOXD10, HOXD4, TRAK2, MAP2, UNC80, ABCB6, ACSL3, MPZ, KLHDC8A, CDK18, MARK1, MIA3, ATF6, ATP1A4, USH2A, LOC611589, FRMPD4, CASK, BRWD3, PWWP3B, RADX, AMOT, XPNPEP2, LOC100688437, SECISBP2, LOC612523, ATP9B, SALL3, CDH19, SERPINB13, POLI, STXBP5, SASH1, SCAF8, ARID1B, FNDC1, PDE10A, TBC1D32, PTPRK, CDC14B, RIC1, ZNF865, TMC4, LOC102152131, LOC119881386, LOC100686511, LOC484365, PRMT1, SPHK2, HIF3A, NOVA2, FOXA3, TOMM40, WDR87, LRFN3, ZNF599, SVIL, TAF3, SPATA24, HARS2, PCDHB1, TCERG1, RAB3C, SLC30A5, MRPS27, CNOT1, RSPRY1, ABCC11, ZSCAN20, TRIM62, SPOCD1, GPATCH3, UBXN11, MAN1C1, EIF4G3, ARHGEF10L, TRPM1, SLCO3A1, SH2D7, APC, VCAN, FAN1, VPS33B, EFL1, NWD2, CCDC149, ADO, NRBF2, UNC5B, SEC24C, ANXA11, CLINT1, CYFIP2, HMGCS1, NIPBL, LMBRD2, FLI1, ETS1, DCPS, RPUSD4, CDON, TMEM123, NLRP1, ENO3, ACAP1, MPDU1, DNAH9, SLC47A2, MFAP4, TOP3A, MXRA8, LOC489640, DPEP1, SPG7, PIEZO1, CNTNAP4, FA2H, TLE7, RANBP10, UPK3BL2, DNASE2B, CALN1, TRIM56, SLC12A9, AGFG2, LOC119875773, SMURF1, CARD11, TAOK2, MARF1,



LOC111096475, PDPK1, TEDC2, RAB26, TSC2, PIGQ, STXBP3, BCAR3, TGFB3, TNNT3, LRRC7, F13B, EMILIN2, GPR37L1, ZNF281, CRB1, UTP25, INTS7, CENPF, CEP350, SMG7, RASAL2, DNM3, MSTO1, CREB3L4, EPG5, FHOD3, TAF4B, TMEM241, SMCHD1, DLGAP1, RALGAP1, FBXO33, KLHL28, ZFYVE26, DCAF5, STON2, GTF2A1, FLRT2, EML5, GPR68, DICER1, SYNE3, DYNC1H1, GRIN1, FZD2, P4HB, TNRC6C, NT5C, TMEM104, TANC2, SMARCD2, GPATCH8, STAT5A, STAT5B, ODAD4, LOC490972, MSL1, CDK12, MED1, HOXB3, LRRC59, TRIM25, BCAS3, MYO19, MYO1D, SUZ12, NLK, GEMIN4, SGSM2, CACNA1B, PNPLA7, GTF3C4, NUP214, FIBCD1, CERCAM, GAPVD1, SSTR3, CACNA1I, TIMELESS, TSPAN31, LRIG3, SRGAP1, TBK1, TCF20, ZC3H7B, ELFN2, BTBD11, RANBP2, MRPS9, PPP4R3B, USP34, ZNF608, ALDH7A1, PRRC1, ACSL6, FSTL4, BNC2, DENND4C, FANCG, UNC13B, MELK, ZBTB5, TRIM32, RPP21, CCHCR1, MED23, VWA7, LOC474850, C2, LOC481722, DXO, PPT2, PHF1, PKHD1, SMPD2, HS3ST5, RECQL4, PKHD1L1, SLAIN2, ADGRL3, EREG, LOC482200, TRIM58, CPA2, KLHDC10, HDAC9, ADCYAP1R1, AVL9, ANLN, KCND2, ADPRS, 37104, OSBPL9, AGBL4, SPATA6, MUTYH, NAV3, CEP290, UHRF1BP1L, MAB21L2, C15H4orf45, RAPGEF2, TLL1, LOC482707, LOC482755, SLC37A3, UBN2, NUP205, KRBA1, GIMAP1, AGAP3, SFRP1, LOC607729, DUSP26, PDLIM3, CAPN14, TCHHL1, PRR9, BIRC6, GREB1, LPIN1, APOB, ADCY3, TMEM214, SLC35F6, TRIM54, IFT172, ALK, LCLAT1, LOC100685565, GPAT2, ACOXL, LOC111090401, DNAH6, LOXL3, ZNF638

Table S2. Protein-protein-interaction (PPI) network clusters according to Marckov Clusterring (MCL) analysis. Only clusters with five or more interactions were considered.

Cluster	Clustered proteins
Cluster 1	<i>WDR27, AKAP5, PRKACB, cOR10Q3, LOC489340, OR51L1, LOC610380, GNG4, GLP2R, ADGRG7, MYO1D, GNB1, GLP1R, OR8D4, CACNA1F</i>
Cluster 2	<i>KIF5B, INPP5F, PLCE1, C7H1orf74, PIK3R1, USP54, NF1, AGPAT4, FGFR2, DGKH, ALK, RBMS1</i>
Cluster 3	<i>FBXO43, TTC21A, DNAH12, DNAH1, SPEF2, SPATA6, DNAH6, SLC26A8, LOC611589, GIMAP1, LRRC6</i>
Cluster 4	<i>ACSL5, ACSL3, HSDL1, SLC30A2, ACSL6, HMGCS1, CPT1A, AASDH, ACADL</i>
Cluster 5	<i>RANBP10, NUP153, RANBP2, GEMIN4, NUP214, KPNA4, GEMIN6, NUP205, NEMP1</i>
Cluster 6	<i>SHANK2, DICER1, FBXO33, ZNFX1, LRP1B, DLGAP1, MRPS27</i>
Cluster 7	<i>DAB2, AGFG1, SYT1, STON2, APOB, EPS15, LRP2</i>
Cluster 8	<i>ADCY3, PDE1A, PDE4D, PDE10A, ENTPD3, PDE4A, PDE4C</i>
Cluster 9	<i>RHOBTB3, SLC16A13, DENND4C, RIC1, DENND11, AVL9</i>
Cluster 10	<i>WARS2, AIMP2, AARS2, HARS2, FAM193B, EPRS1</i>
Cluster 11	<i>DMTN, EPB42, TRIM58, OSBP2, ATP8B4, FAM210B</i>
Cluster 12	<i>RALGAPA1, PLD1, VANGL1, CELSR3, SLK, CIT</i>
Cluster 13	<i>ATPAF1, FHOD3, GPR68, FHOD1, PTAFR</i>
Cluster 14	<i>METTL16, VWA7, IGF2BP3, YTHDF3, YTHDF1, FTO</i>
Cluster 15	<i>ANKRD24, TLE3, APC, BCL9L, TLE4, RFWD3</i>
Cluster 16	<i>C5AR1, C5, OPRM1, C3AR1, SSTR3, GPR37L1</i>
Cluster 17	<i>NVL, DIS3L, EXOSC2, PDCD11, PMEL</i>
Cluster 18	<i>HNF4A, MED23, MED18, PLPPR1, MED1</i>
Cluster 19	<i>CAPRIN1, PCBP2, EIF2A, G3BP1, FAM98A</i>
Cluster 20	<i>HSPA9, C20H3orf18, HGFAC, DNAJC22, EFHB</i>
Cluster 21	<i>SMG7, ZBTB37, FER, PLXNA4, AACS</i>

Table S3. Gene Ontology (GO) Cellular Component enrichment analysis for terms with FDR p -value <0.05 .

Number of genes	Term name	Description	FDR value
56	GO:0005886	Plasma membrane	0.0236
13	GO:0036477	Somatodendritic compartment	0.0217
6	GO:0010494	Cytoplasmic stress granule	0.0095
32	GO:0042995	Cell projection	0.0052

FDR: False Discovery Rate

Table S4. Gene Ontology (GO) Molecular Function enrichment analysis for terms with FDR p -value <0.05 .

Number of genes	Term name	Description	FDR value
3	GO:0008569	Minus-end-directed microtubule motor activity	0.0481
10	GO:0140098	Catalytic activity, acting on RNA	0.0328
8	GO:0016874	Ligase activity	0.0188
9	GO:0042277	Peptide binding	0.0182
17	GO:0140657	ATP-dependent activity	0.0038
29	GO:0032559	Adenyl ribonucleotide binding	0.0038
7	GO:0008081	Phosphoric diester hydrolase activity	0.0038

FDR: False Discovery Rate

Table S5. KEGG enrichment analysis for terms with FDR p -value <0.05 .

Number of genes	Term name	Description	FDR value
4	cfa01522	Endocrine resistance	0.0457
8	cfa05014	Amyotrophic lateral sclerosis	0.045
6	cfa04020	Calcium signaling pathway	0.0405
4	cfa04911	Insulin secretion	0.0356
4	cfa05150	Staphylococcus aureus infection	0.0247
9	cfa04080	Neuroactive ligand-receptor interaction	0.0112
6	cfa04725	Cholinergic synapse	0.0056
5	cfa00071	Fatty acid degradation	0.0015
10	cfa04024	cAMP signaling pathway	2.00E-04
10	cfa05032	Morphine addiction	2.68E-07

FDR: False Discovery Rate

Table S6. Reactome pathway enrichment analysis for terms with FDR p -value <0.05 .

Number of genes	Term name	Description	FDR value
3	CFA-202040	G-protein activation	0.042
8	CFA-373076	Class A/1 (Rhodopsin-like receptors)	0.0238
3	CFA-180024	DARPP-32 events	0.0171
5	CFA-373080	Class B/2 (Secretin family receptors)	0.0088
7	CFA-8856825	Cargo recognition for clathrin-mediated endocytosis	0.0015
6	CFA-191859	snRNP Assembly	1.60E-04
21	CFA-372790	Signaling by GPCR	5.65E-07

FDR: False Discovery Rate