# Novel Gene Fusions in Glioblastoma Tumor Tissue and Matched Patient Plasma

**Lan Wang [1,†], Anudeep Yekula [1,†], Koushik Muralidharan [1], Julia L. Small [1], Zachary S. Rosh [1], Keiko M. Kang [1,2], Bob S. Carter [1,\*] and Leonora Balaj [1,\*]**

1   Department of Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02115, USA. lanwang.amber@gmail.com (L.W.); ayekula@mgh.harvard.edu (A.Y.); kmuralidharan@mgh.harvard.edu (K.M.); jsmall6@mgh.harvard.edu (J.S.); Zrosh@mgh.harvard.edu (Z.R.); kmkang@mgh.harvard.edu (K.M.K.)
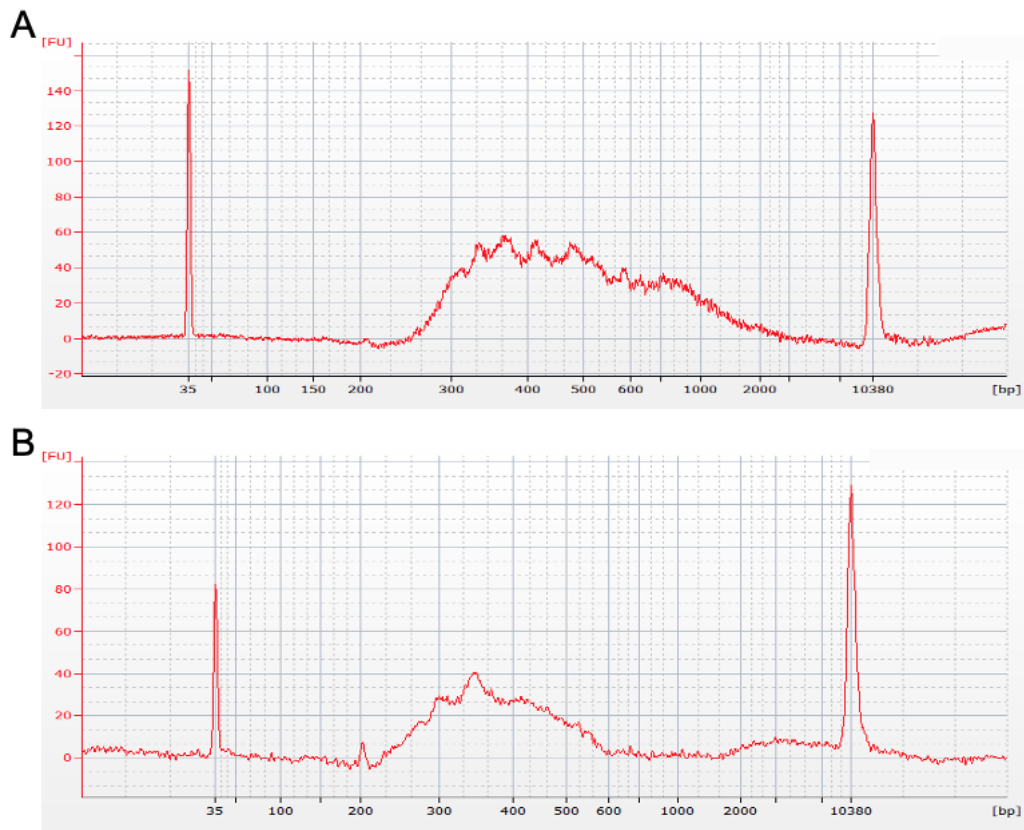2   School of Medicine, University of California San Diego, San Diego, CA 92092, USA
†   These authors contributed equally.
\*   Correspondence: bcarter@mgh.harvard.edu (B.S.C.); Balaj.Leonora@mgh.harvard.edu (L.B.)

## Supplementary
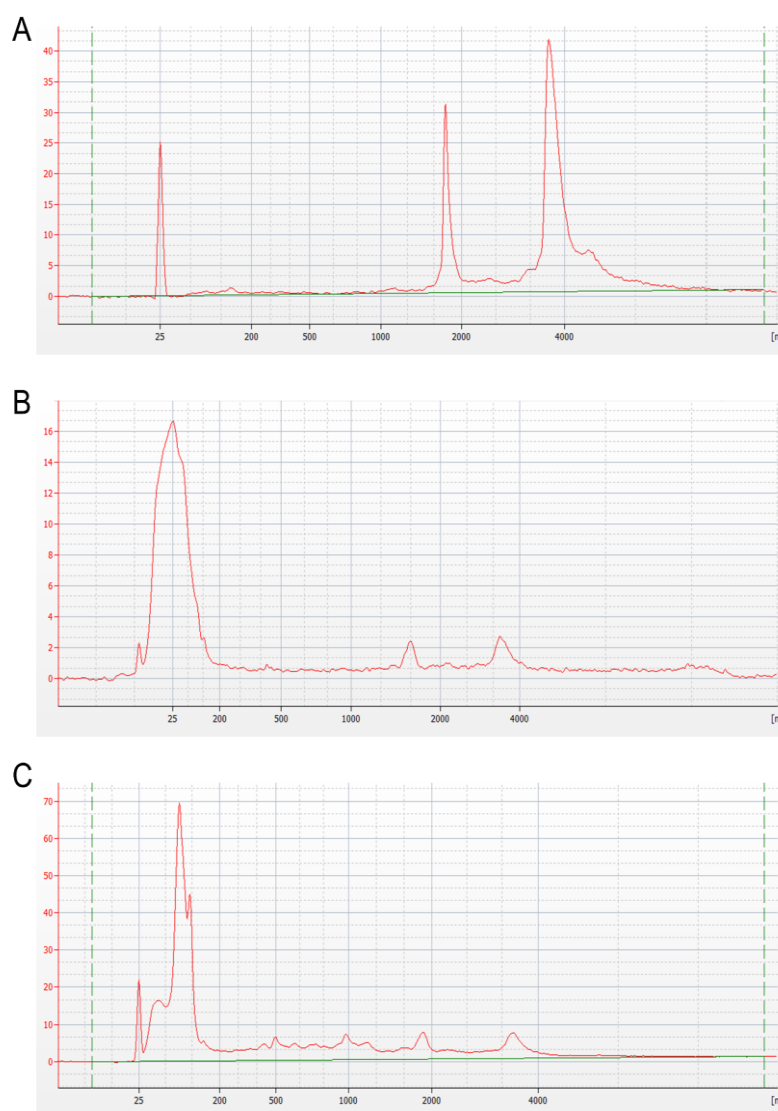


**Figure S1.** Representative Bioanalyzer profiles of RNA extracted from (A) GBM tumor, (B) patient plasma and (C) healthy control plasma.

**Figure S2.** Representative DNA Bioanalyzer profiles of libraries extracted from (A) GBM tumor, (B) patient plasma.
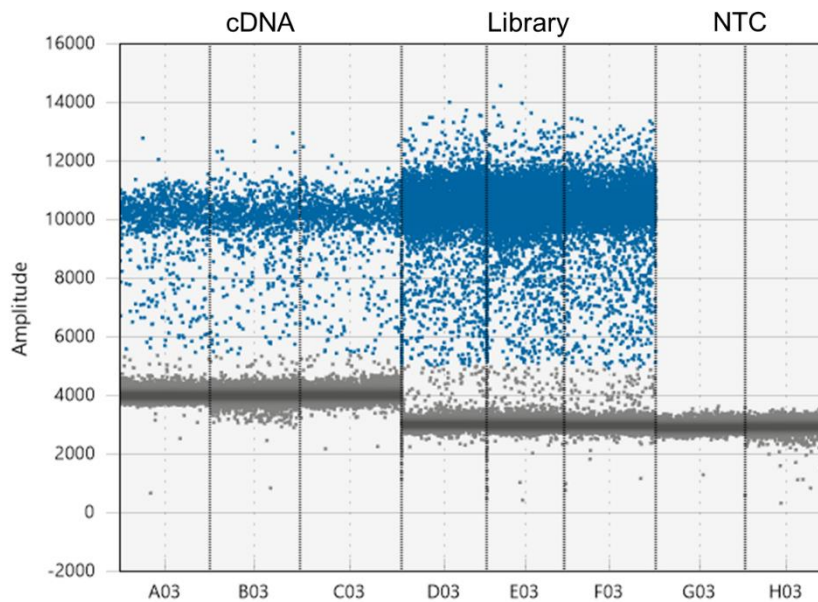


**Figure 3.** 1-D amplification plot of AGK-BRAF fusion droplet digital PCR validation assay of GBM tumor cDNA (left), Sample library (center) from patient P3 and No template control (right).

**Table 1.** MGH Solid Fusion Assay screens for fusions involving the above genes.

AKT3, ALK, AR, ARHGAP26, AXL, BRAF, BRD3, BRD4, CSF1, CSF1R, EGFR, ERG, ESR1, ESRRA
ETV1, ETV4, ETV5, ETV6, EWSR1, FGFR1, FGFR2, FGFR3, FGFR3, FGR, INSR, JAZF1, MAML2, MAST1,
MAST2, MET, MET, MSMB, MUSK, MYB, NOTCH1, NOTCH2, NRG1, NTRK1, NTRK2, NTRK3
NUMBL, NUTM1, PDGFRA, PDGFRB, PIK3CA, PKN1, PPARG, PRKCA, PRKCB, RAF1, RELA, RET,
ROS1, RSPO2, RSPO3, TERT, TFE3, TFEB, THADA, TMPRSS2

**Table 2.** QIAseq Targeted RNAscan Panel screens for fusions involving the above genes.

ABI1, ABL1, ABL2, ACTN4, AFF1, AFF3, AGK, AKAP9, ALK, ARHGAP26, ARHGEF12, ASPSCR1, ATF1,
ATIC, AUTS2, BAG4, BAIAP2L1, BCOR, BCR, BIRC, BRAF, CARS, CASC5, CBFA2T3, CBFB, CBL,
CCDC6, CCDC88C, CD74, CEP170B, CEP89, CHIC2, CIAO1, CLCN6, CLTC, CNTRL, COL1A1, COPA,
CREB1, CREBBP, CRTC1, CUX1, DAZL, DCTN1, DDIT3, DDX10, DEK, DHH, ELL, ELN, EML1, EML4,
EP300, EPS15, ERC1, ERG, ESRP1, ETV1, ETV4, ETV6, EWSR1, EZR, FAM131B, FCHSD1, FGFR1,
FGFR1OP, FGFR2, FGFR3, FIP1L1, FLI1, FLT3, FN1, FNBP1, FOXO1, FOXO4, FOXP1, FRYL, FRYL, FUS,
GAS7, GATA1, GATM, GLIS2, GNAI1, GOLGA5, GOPC, GOT1, GPHN, HACL1, HERPUD1, HIP1, HLF,
HOOK3, HOXA9, HOXD13, JAK2, KAT6A, KDM5A, KIF5B, KLC1, KMT2A, KRAS, LMNA, LNP1, LNP1,
LRIG3, LSM14A, MALT1, MAML2, MECOM, MKL1, MKRN1, MLF 1.00, MLLT1, MLLT1, MLLT10,
MLLT11, MLLT3, MLLT6, MLLT6, MN1, MNX1, MPRIP, MRPS14, MSN, MYB, MYH11, MYO1F, MYO5A,
NCOA2, NCOA4, NCOA4, NDE1, NPM1, NRG1, NSD1, NTRK1, NTRK3, NUMA1, NUP214, NUP98,
P2RY8, PAPSS1, PAX3, PAX5, PAX7, PAX8, PBX1, PCM1, PDGFB, PDGFRA, PDGFRB, PHF23, PICALM,
PLAG1, PML, PPARG, PPFIBP1, PRCC, PRDM16, PRKAR1A, PRKG2, PRRX1, PSIP1, PTPRR, PWWP2A,
RAF1, RANBP2, RAP1GDS1, RARA, RBM15, RCSD1, RET, RHEBL1, RNF130, ROS1, RPL22, RPN1,
RUNX1, RUNX1T1, SDC4, SEC31A, SEPT2, SEPT5, SEPT6, SEPT9, SET, SFPQ, SFPQ, SLC34A2, SLC45A3,
SND1, SQSTM1, SRGAP3, SS18, SSX1, SSX2, SSX2B, SSX2B, STAT5B, STIL, STRN, TACC1, TACC3, TAF15,
TAF15, TAL1, TCF3, TCF7L2, TET1, TFE3, TFG, TMPRSS2, TOP 1.00, TPM3, TPM4, TPR, TRIM24,
TRIM27, TRIM33, TRIP11, UBE2L3, UBE3C, USP42, VCL, VTI1A, WHSC1L1, WT1, ZBTB16, ZMIZ1,
ZMYM2, ZNF384, ZNF703, ZSCAN30

**Table 3.** RNA profiles indicated by Agilent Bioanalyzer 2100 Expert Software and/or Qubit 4 Fluorometer. Tumor tissue RNA concentration were determined by Qubit 4 Fluorometer. RIN, RNA fragments % > 200 bp of all RNA samples and plasma RNA concentration were calculated by 2100 Expert Software with baseline correction. RIN, RNA Integrity Number.

| Sample cohort | Patient/Healthy control ID | RIN | Concentration (ng/µL) | RNA fragment% >200bp |
|---|---|---|---|---|
| **Tumor tissue** | P1 | 8.0 | 45.4 | 92% |
| | P2 | 9.9 | 538.0 | 96% |
| | P3 | 7.7 | 53.9 | 80% |
| | P4 | 9.3 | 424.0 | 98% |
| | P5 | 9.1 | 209.0 | 88% |
| | P6 | 9.0 | 392.7 | 88% |
| | P7 | 8.7 | 70.4 | 94% |
| | P8 | 8.9 | 138.4 | 95% |
| | P9 | 9.6 | 81.2 | 96% |
| **Patient matched plasma** | P1 | 2.0 | 0.074 | 10% |
| | P2($t_1$) | 2.6 | 1.820 | 14% |
| | P2($t_2$) | 2.7 | 0.981 | 10% |
| | P2($t_3$) | 4.8 | 0.153 | 14% |
| | P2($t_4$) | 3.9 | 0.370 | 19% |
| | P3 | 6.8 | 0.062 | 6% |
| | P4 | 4.1 | 0.120 | 14% |
| | P5 | 1.4 | 0.082 | 21% |
| | P6 | 2.0 | 0.041 | 5% |
| | P7 | 5.5 | 0.181 | 17% |

| | | | | |
|---|---|---|---|---|
| | P8 | 5.3 | 0.135 | 24% |
| | P9 | 4.2 | 0.202 | 17% |
| | H1 | 2.9 | 1.206 | 11% |
| | H2 | 2.5 | 2.112 | 12% |
| | H3 | 2.6 | 1.869 | 13% |
| | H4 | 2.6 | 1.499 | 14% |
| **Healthy control plasma** | H5 | 2.7 | 4.274 | 14% |
| | H6 | 3.1 | 3.369 | 17% |
| | H7 | 2.8 | 3.187 | 16% |
| | H8 | 3.1 | 3.685 | 17% |
| | H9 | 2.3 | 2.610 | 14% |
| | H10 | 3.0 | 5.598 | 19% |

**Table S4.** Summary of fusion calling and droplet digital PCR (ddPCR) validation results. Fusions identified by MGH Solid Fusion Assay and RNA-seq are listed including the fusion categories (curated, high confidence or low confidence fusions) and number of supporting molecular tags (MTs). The corresponding ddPCR validation results for the curated and high confidence fusions. (NA, Not Available).

| Sample Cohort | Patient/ Healthy Control ID | MGH Solid Fusion Assay Fusions | RNA-seq Fusions | Fusion Category | Supporting MTs | ddPCR Positive Droplet Concentration (copies/μL) |
|---|---|---|---|---|---|---|
| GBM Tumor Tissue | P1 | FGFR3-TACC3 | FGFR3-TACC3 | Curated | 751 | 65550 |
| | | | RANBP2-RGPD8 | Low confidence | 150 | - |
| | | | PICALM-SYTL2 | Low confidence | 12 | - |
| | | | COL1A1-COL1A2 | Low confidence | 11 | - |
| | P2 | None | VTI1A-TCF7L2 | Curated | 1 | 151.5 |
| | | | COL1A1-COL1A2 | Low confidence | 136 | - |
| | | | COL1A1-COL2A1 | Low confidence | 27 | - |
| | P3 | AGK-BRAF | AGK-BRAF | Curated | 625 | 51650 |
| | | | SND1-TMEM178B | High confidence | 425 | 29250 |
| | | | CLU-TOP1 | High confidence | 7 | - |
| | | | PTPRZ1-ELN | High confidence | 8 | NA |
| | | | RANBP2-RGPD8 | Low confidence | 269 | - |
| | | | EWSR1-FUS | Low confidence | 10 | - |
| | | | PICALM-SYTL2 | Low confidence | 7 | FAIL |
| | P4 | None | RANBP2-RGPD8 | Low confidence | 212 | - |
| | | | MAP1B-TOP1 | Low confidence | 6 | - |
| | | | CD74-CLU | Low confidence | 5 | - |
| | | | CLU-TOP1 | Low confidence | 5 | - |
| | | | EWSR1-FUS | Low confidence | 6 | - |
| | P5 | None | VTI1A-TCF7L2 | Curated | 1 | 128.5 |
| | P6 | None | COL1A1-COL1A2 | Low confidence | 17 | - |
| | P7 | None | None | NA | 0 | - |
| | P8 | None | FGFR3-TACC3 | Curated | 2 | - |
| | | | RANBP2-RGPD8 | Low confidence | 171 | - |
| | | | SYN2-PPARG | Low confidence | 4 | - |
| | | | ATRNL1-MLLT10 | Low confidence | 3 | - |
| | P9 | None | RANBP2-RGPD8 | Low confidence | 248 | - |
| Patient Matched Plasma | P1 | Not tested | None | NA | 0 | - |
| | P2 (t$_1$) | Not tested | None | NA | 0 | - |
| | P2 (t$_2$) | Not tested | None | NA | 0 | - |
| | P2 (t$_3$) | Not tested | FGFR3-TACC3 | Curated | 2 | 507600 |
| | | | VTI1A-TCF7L2 | Curated | 2 | 16615 |
| | | | UBE2L3-VPS39 | High confidence | 55 | 18500 |
| | P2 (t$_4$) | Not tested | None | NA | 0 | - |
| | P3 | Not tested | CD74-GID8 | Filtered | 57 | - |
| | P4 | Not tested | TMEM91-TAL1 | High confidence | 12 | 33600 |
| | | | CRTC1-ABHD12 | High confidence | 49 | 8800 |
| | P5 | Not tested | None | NA | 0 | - |
| | P6 | Not tested | None | NA | 0 | - |
| | P7 | Not tested | RUNX1-RUNX3 | Low confidence | 4 | - |

| | | | | | |
|---|---|---|---|---|---|
| | P8 | Not tested | TMEM91-TAL1 | High confidence | 7 | 7725 |
| | | | FIP1L1-SCFD2 | Low confidence | 6 | - |
| | | | RAB7A-FOXP1 | High confidence | 8 | 8065 |
| | P9 | Not tested | RANBP2-RGPD8 | Filtered | 13 | - |
| | | | NPM1-FBXO38 | Filtered | 4 | - |
| | | | TMEM91-TAL1 | High confidence | 22 | 49400 |
| | H1 | Not tested | CDCA7L-MLLT3 | High confidence | 12 | 50400 |
| | | | UBA5-FOXP1 | High confidence | 14 | 17350 |
| | | | FIP1L1-SCFD2 | Low confidence | 6 | - |
| | H2 | Not tested | None | NA | 0 | - |
| **Healthy Control Plasma** | H3 | Not tested | FIP1L1-SCFD2 | High confidence | 8 | 34850 |
| | H4 | Not tested | ACTN1-ACTN4 | Low confidence | 8 | - |
| | H5 | Not tested | ELL-TAL1 | High confidence | 4 | NA |
| | H6 | Not tested | None | NA | 0 | - |
| | H7 | Not tested | FIP1L1-ATP8A1 | High confidence | 64 | NA |
| | H8 | Not tested | GLB1-TAL1 | High confidence | 6 | NA |
| | | | RASA3-TAL1 | High confidence | 6 | NA |
| | H9 | Not tested | None | NA | 0 | - |
| | H10 | Not tested | None | NA | 0 | - |

**Table S5.** Primers design for fusion transcript droplet digital PCR validation. F and R primers for fusion transcript sequences used for droplet digital PCR target amplification. F: forward primers; R: reverse primers.

| Fusion name | | Breakpoint Location | Primers | Sequences |
|---|---|---|---|---|
| **TMEM91-TAL1** | 5' | TMEM 91 exon 1, chr19:41,351,202 (+) | F | CGTGCGAGACGAGACACAT |
| | 3' | TAL1 exon 2, chr1:47,229,351 (-) | R | CTGAGAGGCCTGCAGTTACG |
| **CRTC1-ABHD12** | 5' | CRTC1 exon 1, chr19:18,683,828 (+) | F | ACAATCAGAAGCAGGCGGAG |
| | 3' | ABHD12 exon 3, chr20:25,323,430 (-) | R | TGATTCAAACCTTGATCCTGTGG |
| **FIP1L1-SCFD2** | 5' | FIP 1L1 exon 12, chr4:53,428,183 (+) | F | GCACTGCTCCACCTCTGATT |
| | 3' | SCFD2 exon 5, chr4:53,145,582 (-) | R | GCTGCTTTAACACAACGGACA |
| **UBE2L3-VPS39** | 5' | UBE2L3 exon 3, chr22:21,611,043 (+) | F | CCAAACATCGACGAAAAGGGG |
| | 3' | VPS39 exon 2, chr15:42,199,961 (-) | R | TGGTTCCCACAAGAAGCCATT |
| **RAB7A-FOXP1** | 5' | RAB7A exon 1, chr3:128,726,359 (+) | F | GTTTAGTCTCCTCCTCGGCG |
| | 3' | ROXP1 exon 12, chr3:71,015,653 (-) | R | TGGCCACTTGCATACACCAT |
| **CD74-GID8** | 5' | CD74 exon 9, chr5:150,402,152 (-) | F | CAGTCCCCATGTGAGAGCAG |
| | 3' | GID8 exon 5, chr20:62,944,739 (+) | R | TCCGTGGTAGACCGAATCCT |
| **CDCA7L-MLLT3** | 5' | CDCA7L exon 1, chr7:21,945,781 (-) | F | GCCCGGTTAGGAAGAATGGA |
| | 3' | MLLT3 exon 9, chr9:20,354,879 (-) | R | TGCTTATCTGATTTGCTTTGCTT |
| **UBA5-FOXP1** | 5' | UBA5 exon 1, chr3:132,660,698 (+) | F | GAGCTCAGAGGTGGTGGATTC |
| | 3' | FOXP1 exon 12, chr3:71,015,653 (-) | R | CAGCCTGGCCACTTGCATA |
| **FIP1L1-SCFD2** | 5' | FIP1L1 exon 9, chr4:53,399,839 (+) | F | TTGTTCAAGACTGGGCTTCCA |
| | 3' | SCFD2 exon 3, chr4:53,313,763 (-) | R | GCAGGTTTTCTCTGCTTGCC |
| **VTI1A--TCF7L2** | 5' | VTI1A exon 3, chr10:112,464,657 (+) | F | TCCGAGAGATACCACCCCAA |
| | 3' | TCF7L2 exon 5, chr10:113,141,184 (+) | R | GCACCACTGGCACTTTGTTAG |
| **FGFR3-TACC3** | 5' | FGFR3 exon 16, chr4:1,806,934 (+) | F | GACCTGGACCGTGTCCTTACC |
| | 3' | TACC3 exon 9, chr4:1,737,598 (+) | R | TGGAGCAGGTCCACTATAGGTC |
| **AGK-BRAF** | 5' | AGK exon 2, chr7:141,555,567 (+) | F | ACGCTTCGAAATCACTGGAAG |
| | 3' | BRAF exon 7, chr7:140,794,467 (-) | R | TGAAGGAGACGGACTGGTGA |
| **SND1-TMEM178B** | 5' | SND1 exon 10, chr7:127,721,400 (+) | F | ACGATTCACCTGTCCAGCAT |
| | 3' | TMEM178B exon 1, chr7:141,212,591 (+) | R | TAGGGCATGCCACTCATCCT |
| **PTPRZ1-ELN** | 5' | PTPRZ1 exon 21, chr7:122,040,979 (+) | F | GCCTGACATGGGAGTACCAG |
| | 3' | ELN exon 3, chr7:74,037,707 (+) | R | GGCTTAAGAGGTTTGCCTCCA |
| **PICALM-SYTL2** | 5' | PICALM exon 19, chr11:85,974,708 (-) | F | AAACCCCTTTGGCCCTGTATC |
| | 3' | SYTL2 exon 1, chr11:85,758,114 (-) | R | CAGCACACTCACTCTCTGGT |
| **ELL-TAL1** | 5' | ELL exon 1, chr19:18,521,921 (-) | F | GCGCTGAAGGAGGATAGGAG |
| | 3' | TAL1 exon 2, chr1:47,229,351 (-) | R | CTGAGAGGCCTGCAGTTACG |
| **FIP1L1-ATP8A1** | 5' | FIP1L1 exon 12, chr4:53,428,183 (+) | F | GCACTGCTCCACCTCTGATT |
| | 3' | ATP8A1 exon 5, chr4:42,616,078 (-) | R | GCGTTTGTTTCTTGTTCACTGC |
| **GLB1-TAL1** | 5' | GLB1 exon 1, chr3:33,097,011 (-) | F | CGGGGTTCCTGGTTCGCAT |
| | 3' | TAL1 exon 2, chr1:47,229,351 (-) | R | TTACGCTGCGGTGTGGTCC |
| **RASA3-TAL1** | 5' | RASA exon 1, chr13:114,132,435 (-) | F | GTCTTCCAGAGCGTGAAGATCA |
| | 3' | TAL1 exon 2, chr1:47,229,351 (-) | R | CTGAGAGGCCTGCAGTTACG |

**Table S6.** Overview of RNA-seq mapping metrics generated by GeneGlobe Data Analysis Center. RNA Control Primers refer to a set of RNA control primers targeting housekeeping genes that can be used as an indicator of RNA diversity (QIAseq™ Targeted RNAscan Data Analysis Handbook, Qiagen).

| Sample Cohort | Patient/ Healthy Control ID | Raw Reads/ Sample | Mapped Reads/ Sample | Average RNA Control Primers | Reads% > 200 bp |
|---|---|---|---|---|---|
| GBM Tumor Tissue | P1 | 3,254,559 | 3,084,254 | 4,712.00 | 31.04% |
| | P2 | 3,251,846 | 3,177,744 | 4,291.80 | 33.15% |
| | P3 | 3,739,661 | 3,488,775 | 2,857.80 | 23.65% |
| | P4 | 2,854,488 | 2,729,137 | 5,061.00 | 36.34% |
| | P5 | 2,120,682 | 2,025,337 | 4,000.80 | 35.87% |
| | P6 | 2,891,115 | 2,795,359 | 3,230.20 | 33.38% |
| | P7 | 2,563,322 | 2,432,636 | 5,364.20 | 35.42% |
| | P8 | 2,982,206 | 2,825,331 | 6,289.80 | 32.92% |
| | P9 | 3,109,352 | 2,970,338 | 6,059.50 | 43.20% |
| Matched Patient Plasma | P1 | 2,394,700 | 2,250,364 | 82.5 | 17.40% |
| | P2 (t1) | 2,741,135 | 2,600,817 | 116.2 | 20.31% |
| | P2 (t2) | 2,404,068 | 2,277,630 | 129.2 | 18.60% |
| | P2 (t3) | 3,227,719 | 3,060,547 | 184.8 | 9.90% |
| | P2 (t4) | 3,227,967 | 3,044,622 | 157.5 | 21.12% |
| | P3 | 2,742,402 | 2,536,385 | 174.5 | 21.15% |
| | P4 | 2,345,261 | 2,231,584 | 152.2 | 18.83% |
| | P5 | 2,745,203 | 2,608,936 | 153.5 | 21.52% |
| | P6 | 1,910,362 | 1,813,593 | 136.5 | 19.80% |
| | P7 | 1,429,178 | 1,352,839 | 115.5 | 19.30% |
| | P8 | 2,694,475 | 2,546,389 | 163.2 | 18.60% |
| | P9 | 3,157,529 | 3,005,320 | 202.2 | 20.50% |
| Healthy Control Plasma | H1 | 6,352,456 | 5,887,078 | 195.5 | 20.08% |
| | H2 | 5,955,852 | 5,607,881 | 290.8 | 19.90% |
| | H3 | 4,668,412 | 4,385,014 | 227.5 | 19.50% |
| | H4 | 5,681,289 | 5,211,746 | 232.8 | 17.67% |
| | H5 | 2,519,716 | 2,377,286 | 233 | 17.70% |
| | H6 | 2,016,223 | 1,906,711 | 127.2 | 18.30% |
| | H7 | 2,753,480 | 2,614,837 | 183.8 | 19.84% |
| | H8 | 2,809,201 | 2,663,076 | 255.8 | 20.53% |
| | H9 | 2,543,422 | 2,420,411 | 182.2 | 20.12% |
| | H10 | 2,617,655 | 2,487,716 | 180.2 | 19.80% |