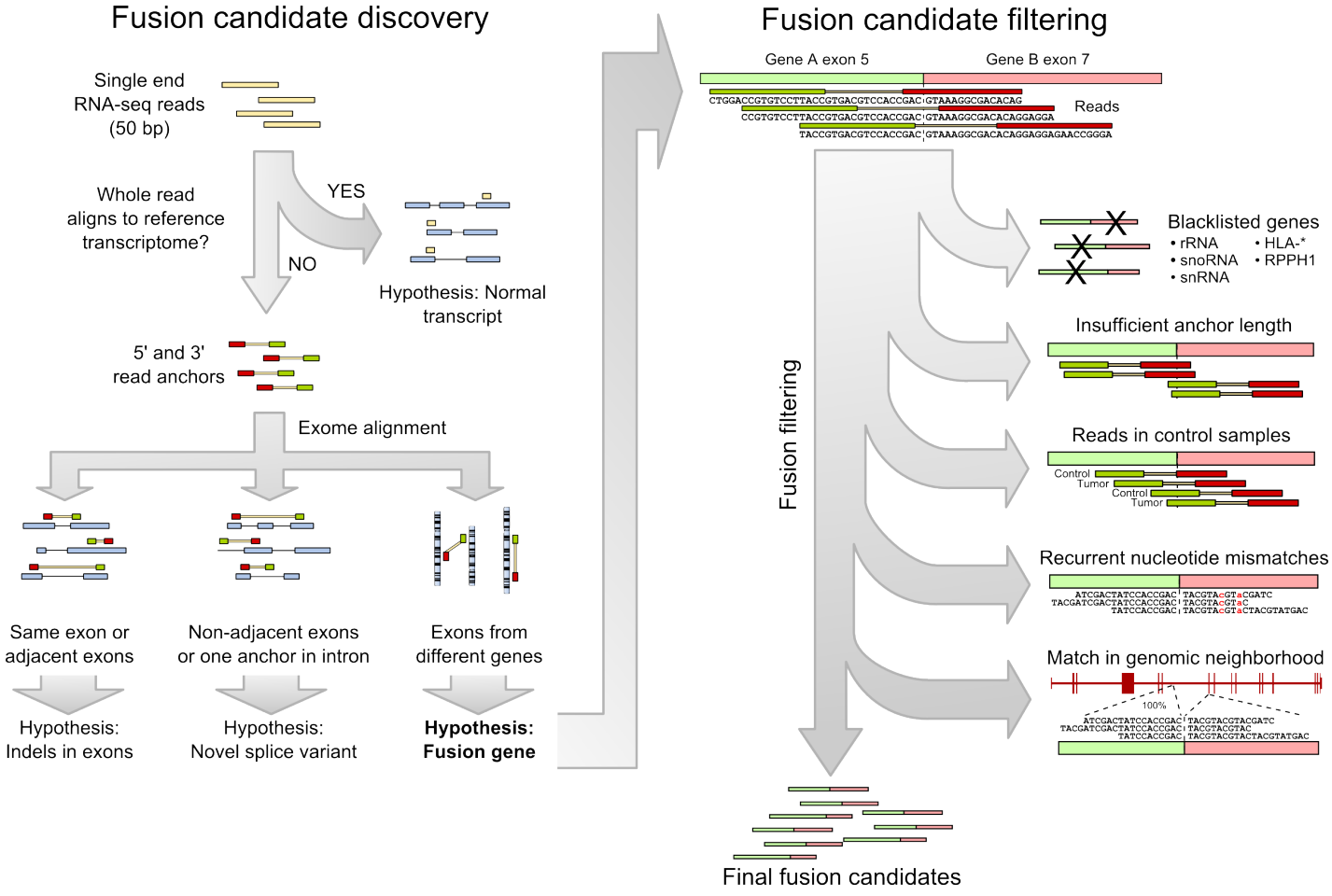
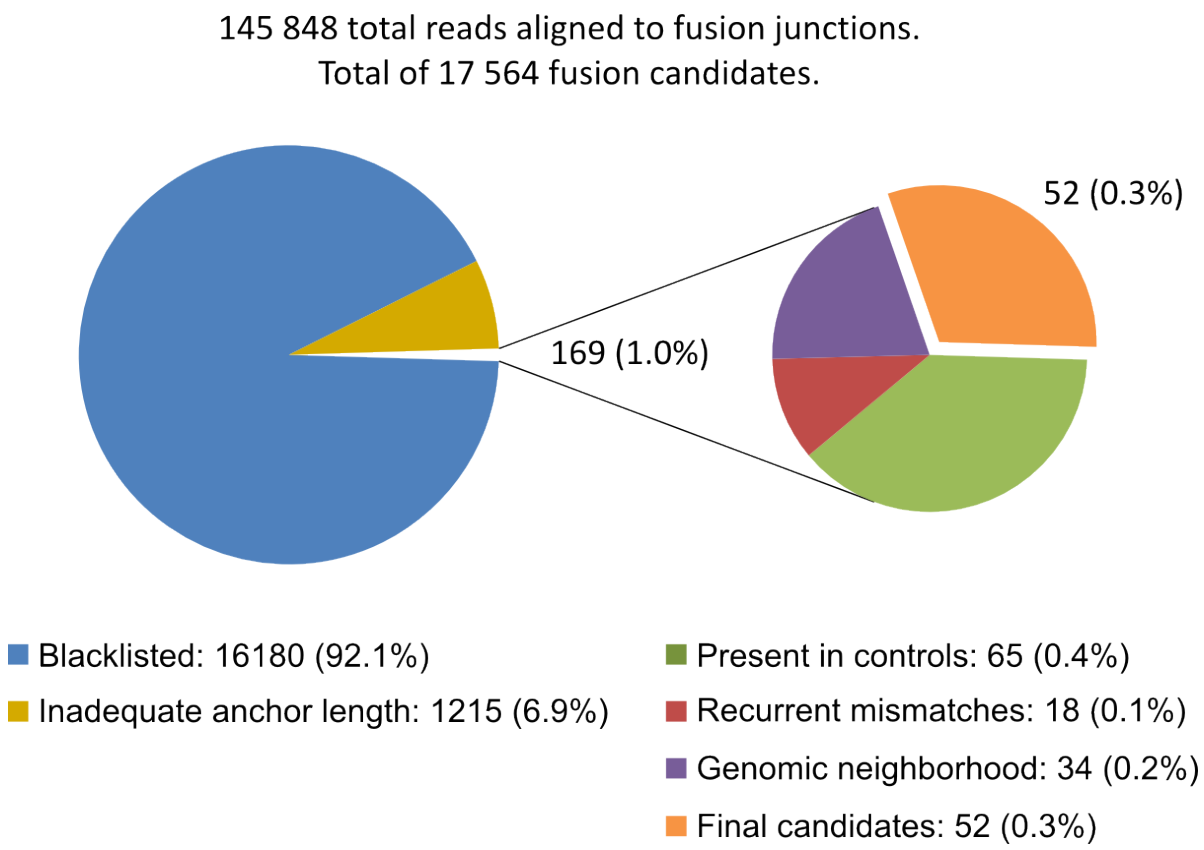
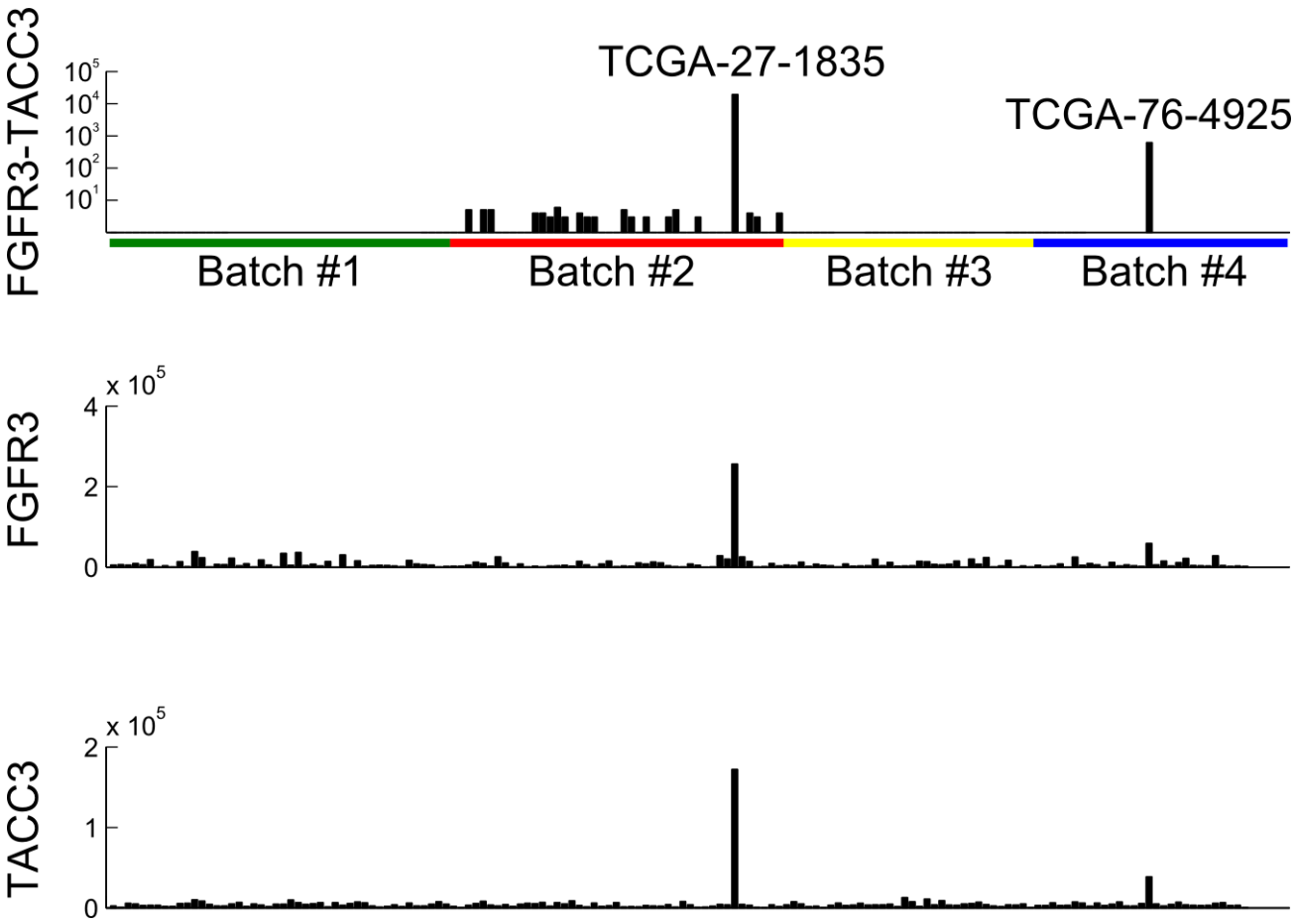


Supplementary Figure 1 | Schematic of the process used for fusion discovery and for filtering fusion candidates.



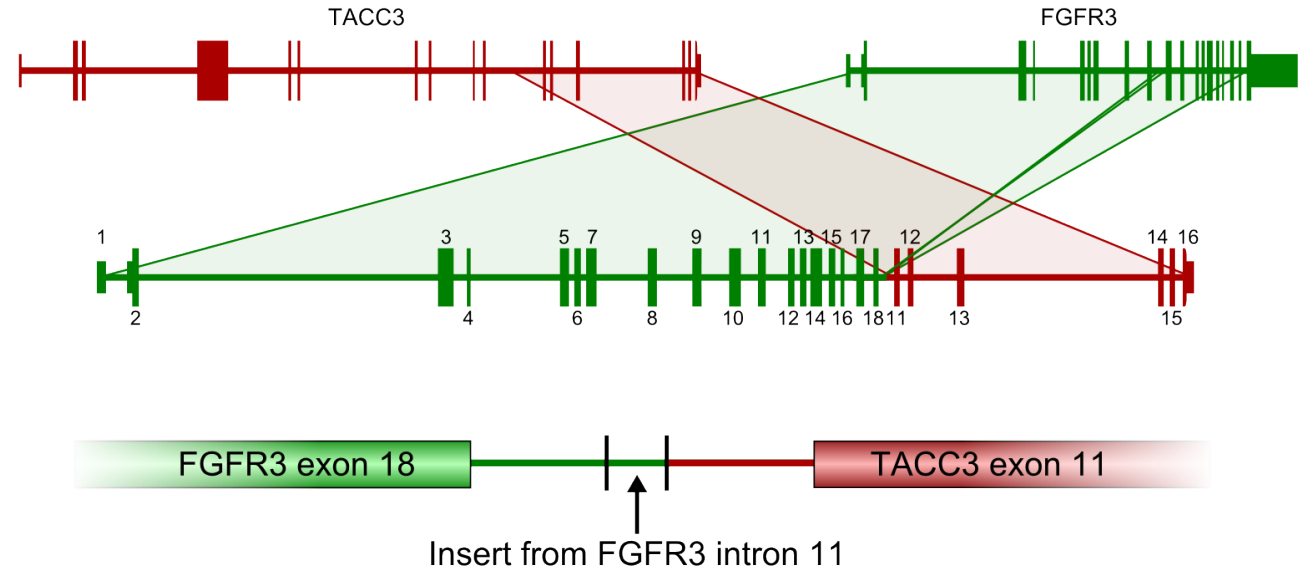
Supplementary Figure 2 | Total number of discovered fusion candidates in all 10 sample pools and after consecutive filtering based on different criteria. The filters were applied in the following order: blacklisted genes, inadequate anchors, presence in negative controls, recurrent mismatches in alignments.

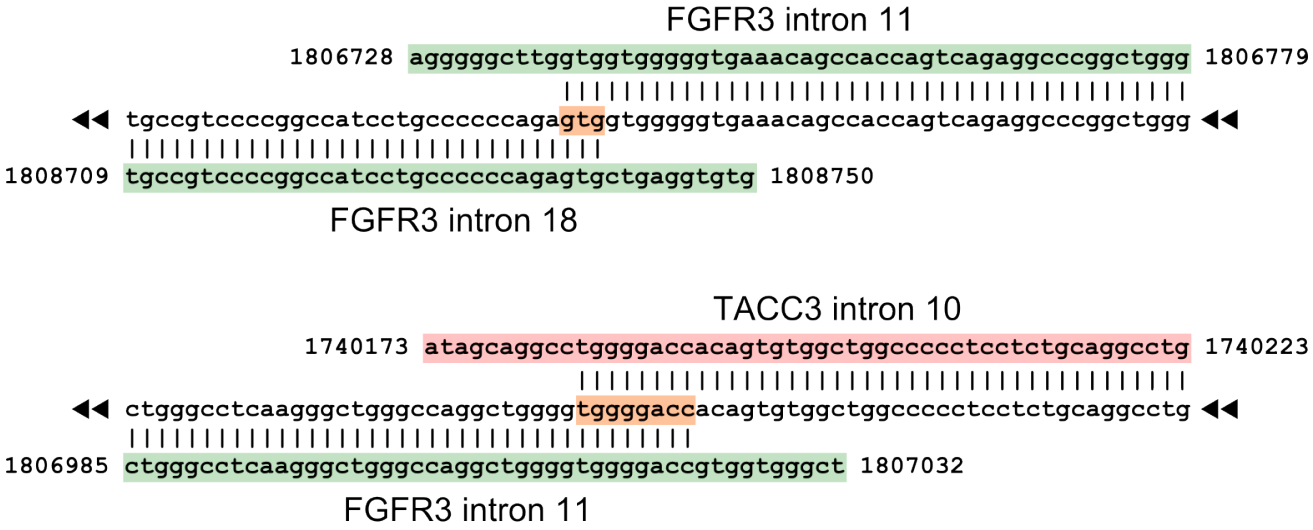
Supplementary Figure 3 | RNA-seq reads indicate a fusion between *FGFR3* exon 18 and *TACC3* exon 11 in a GBM sequencing pool that included patient GBM-13. The genomic reference is indicated in bold.



Supplementary Figure 4 | Transcriptome sequencing data from the Cancer Genome Atlas GBM project reveals two fusion positive tumors. We also observed that other samples in the same batch as the strongly fusion expressing sample TCGA-27-1835 have been contaminated with *FGFR3-TACC3* transcripts.

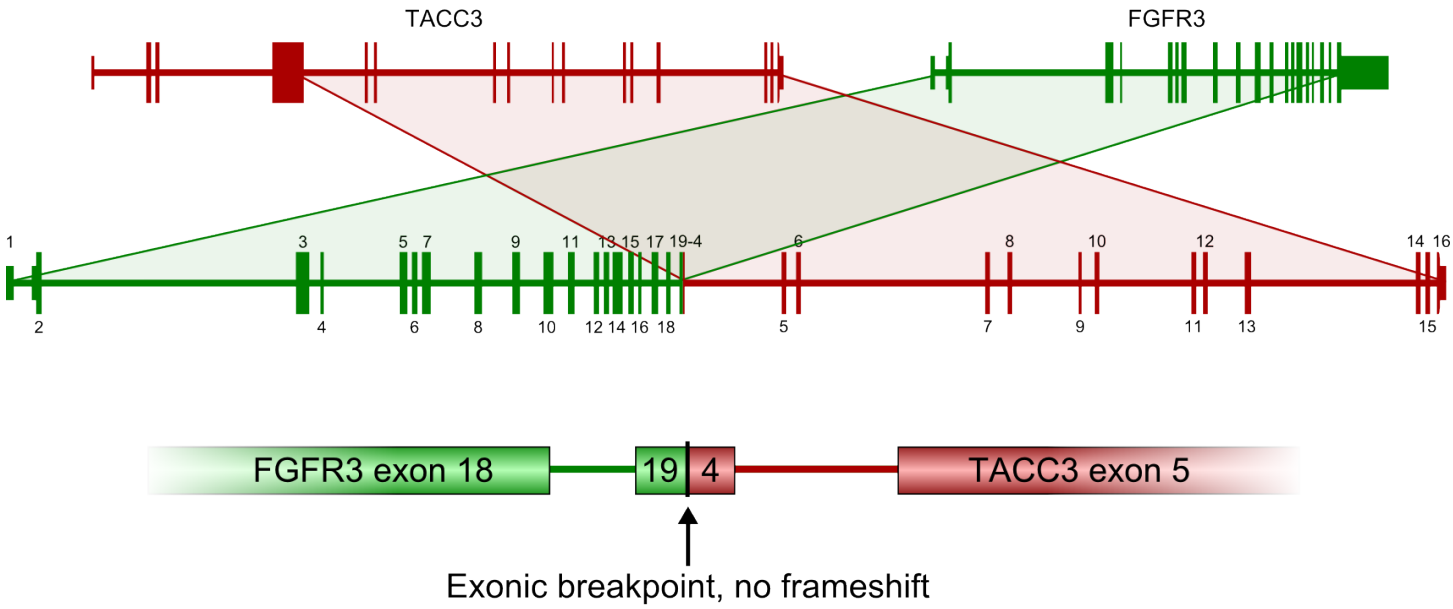
GBM-13 DNA sequencing

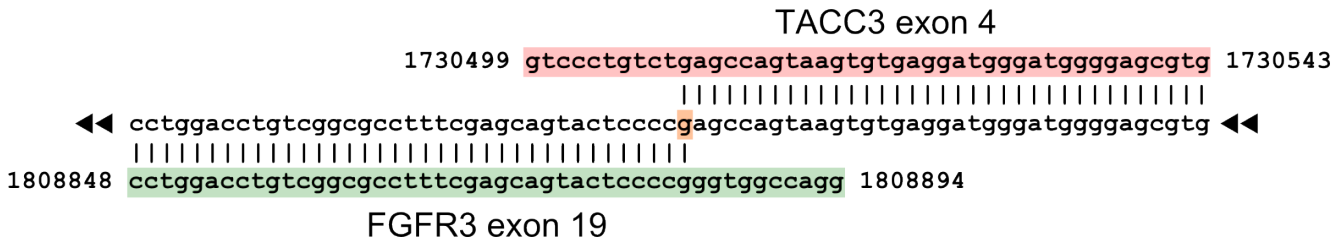




Supplementary Figure 5 | DNA-level schematic of *FGFR3-TACC3* in patient GBM-13 based on genomic DNA PCR and Sanger sequencing. A short fragment of *FGFR3* intron 11 is inserted at the breakpoint between *FGFR3* and *TACC3*. The shared nucleotide sequences are shown in peach.

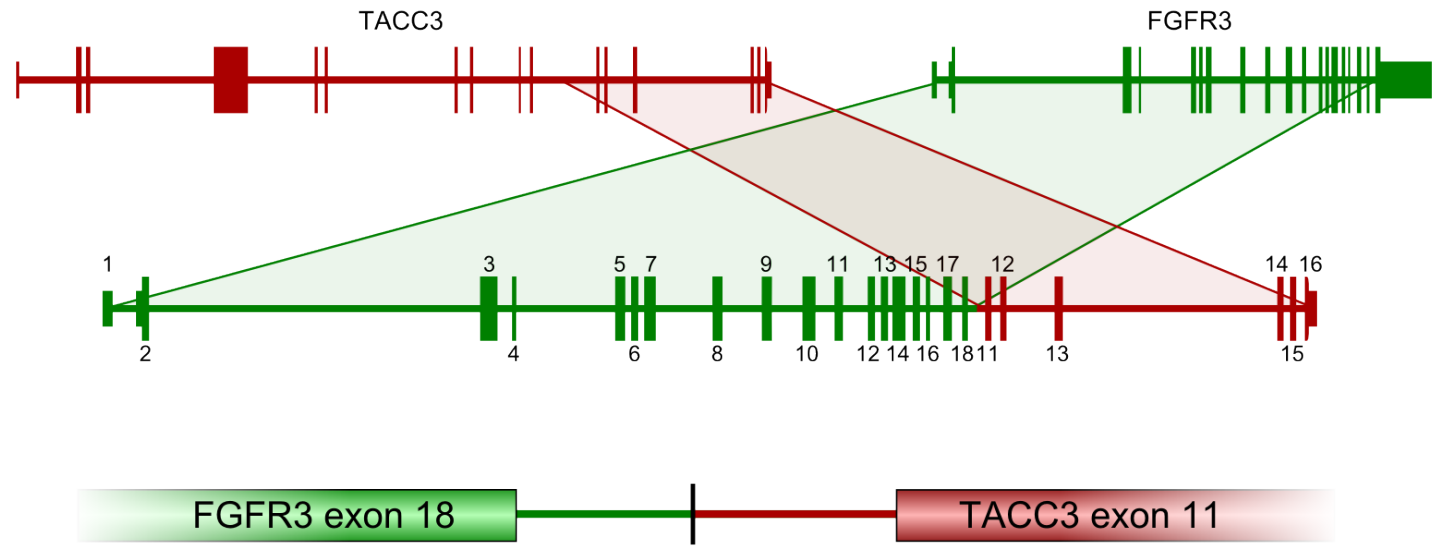
GBM-07 DNA sequencing

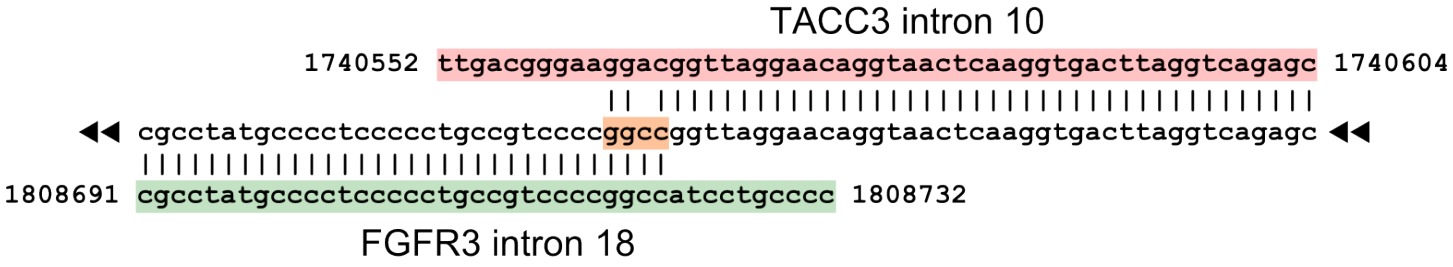




Supplementary Figure 6 | DNA-level schematic of *FGFR3-TACC3* in patient GBM-07 based on genomic DNA PCR and Sanger sequencing. The breakpoint interrupted exon 19 of *FGFR3* and exon 4 of *TACC3*, forming a chimeric exon. No frameshift was produced. The shared nucleotide is shown in peach.

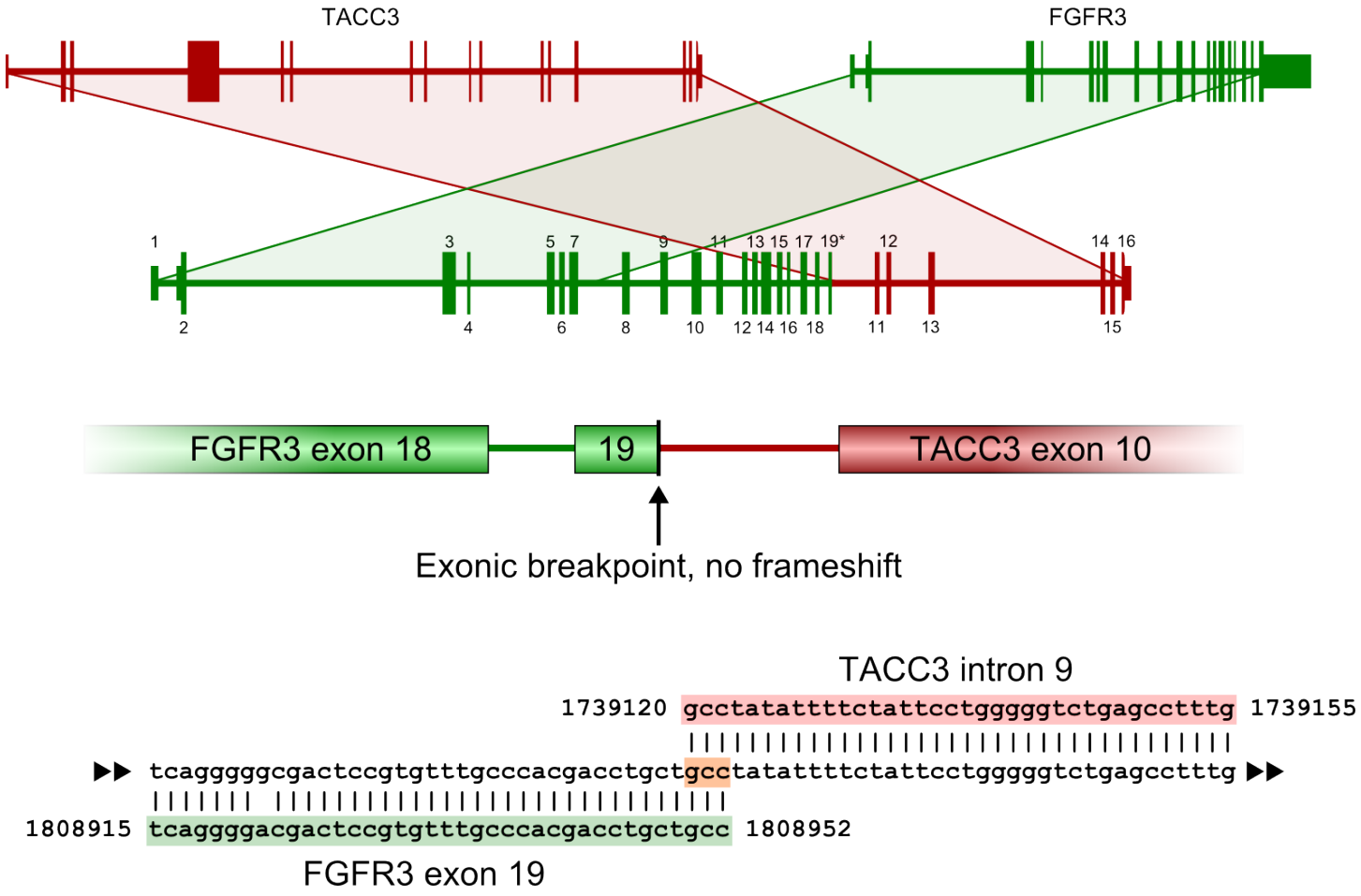
GBM-T01 DNA sequencing



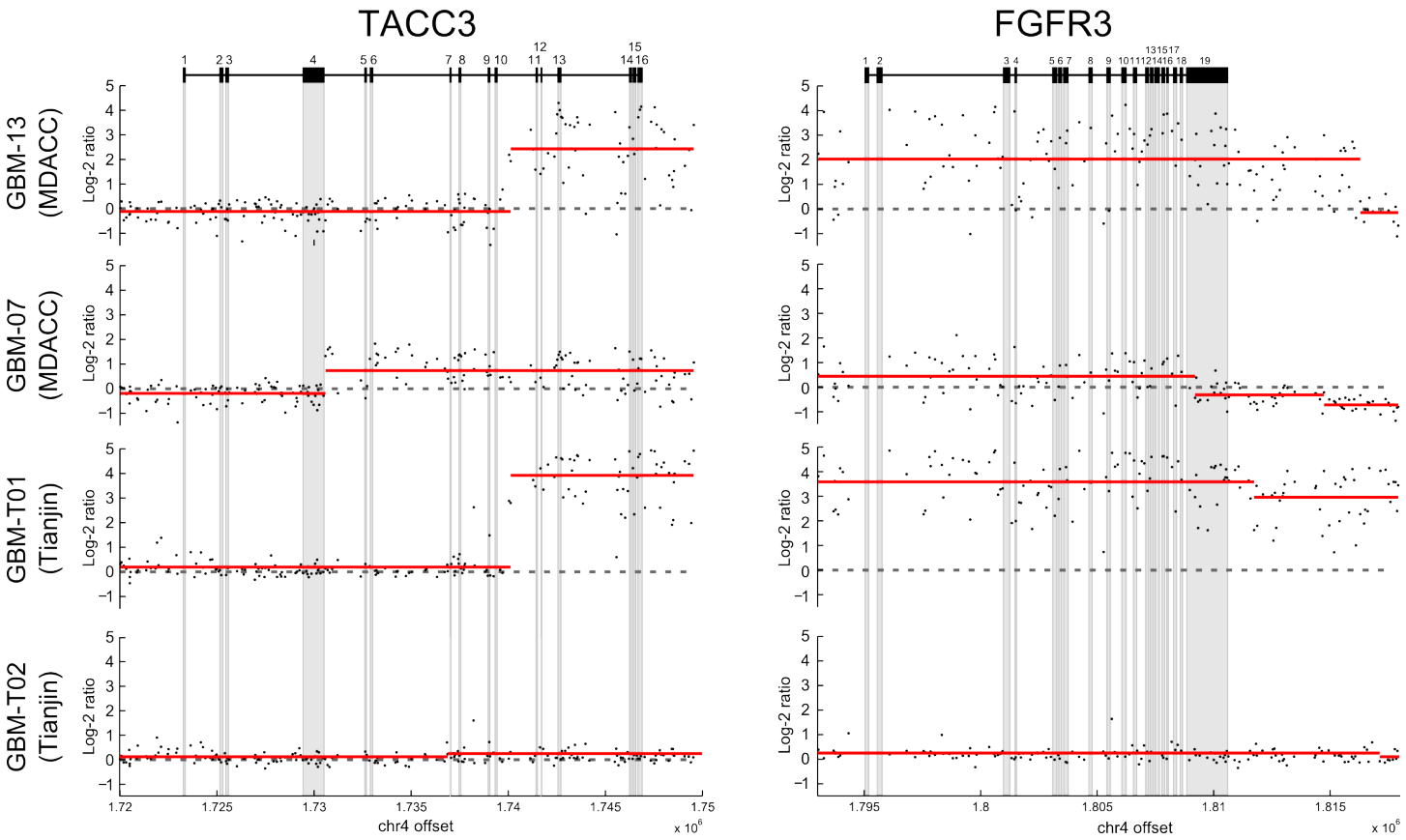


Supplementary Figure 7 | DNA-level schematic of *FGFR3-TACC3* in patient GBM-T01 based on genomic DNA PCR and Sanger sequencing. The shared nucleotide sequence is shown in peach.

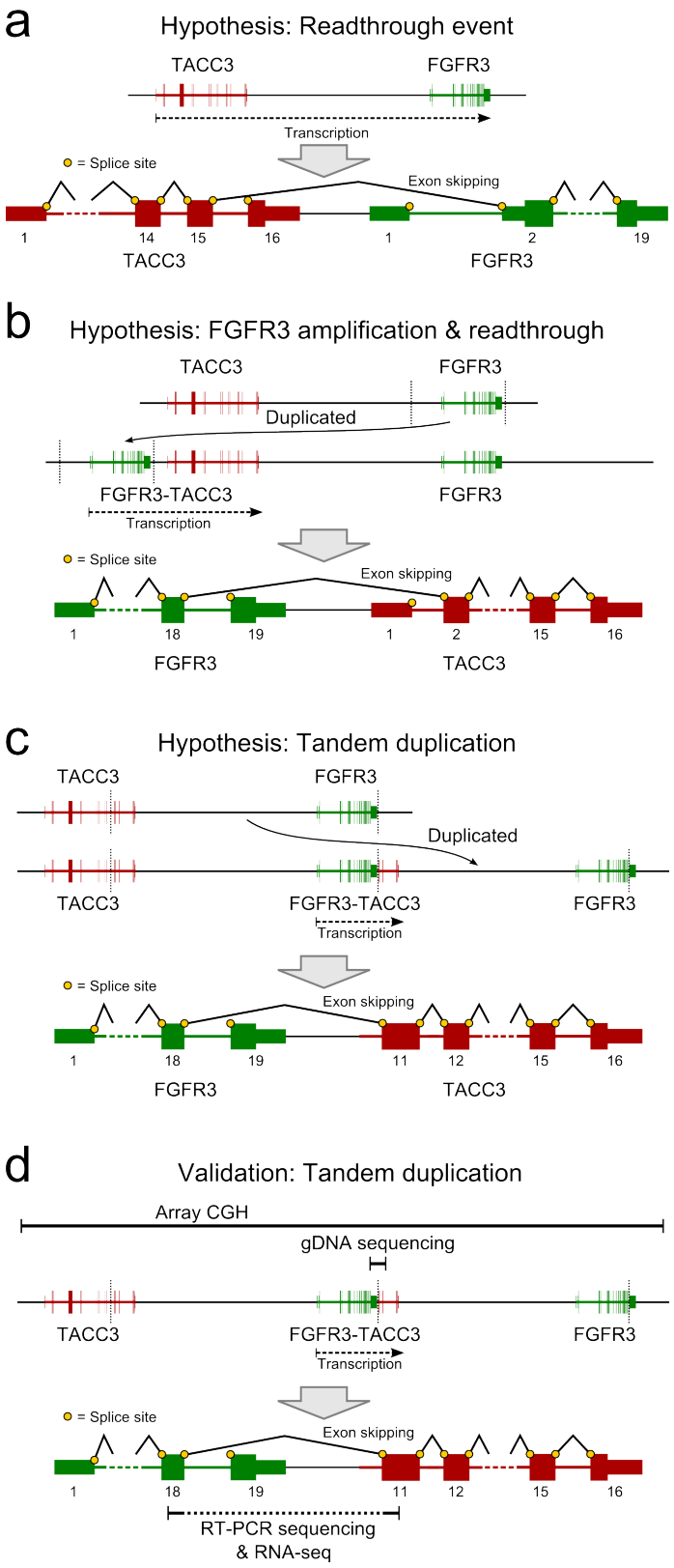
GBM-T02 DNA sequencing



Supplementary Figure 8 | DNA-level schematic of *FGFR3-TACC3* in patient GBM-T02 based on genomic DNA PCR and Sanger sequencing. The shared nucleotide sequence is shown in peach.

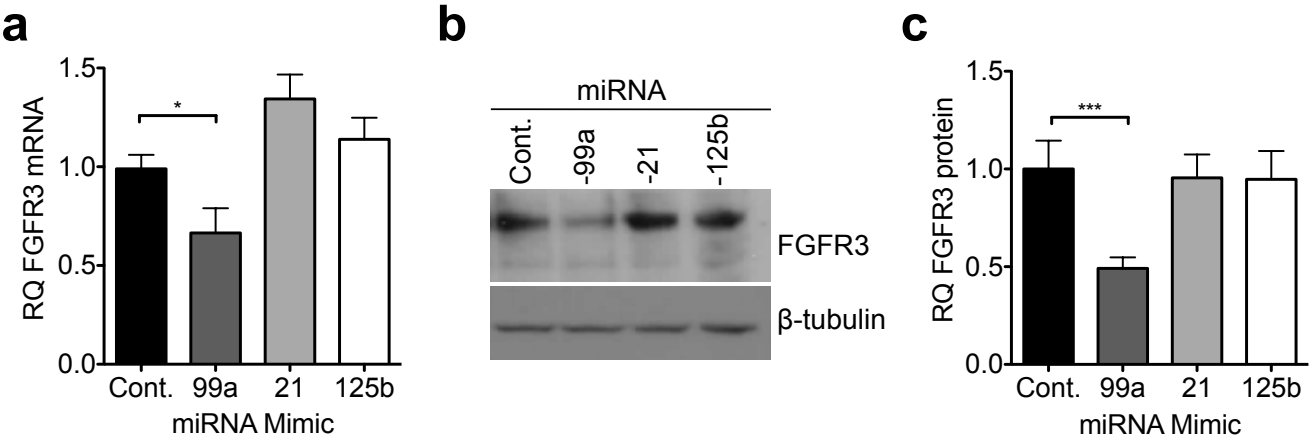


Supplementary Figure 9 | Detailed view of segmented probe logratios for the three fusion positive patients on the Agilent custom aCGH chip. Gene exon structures are shown at the top in black. In patients GBM-13 and GBM-T01, the TACC3 breakpoint was situated between exons 10 and 11; in GBM-07 it interrupted exon 4; in GBM-T02 it was between exons 9 and 10. There was considerable variation in the *FGFR3* breakpoint locations across samples.

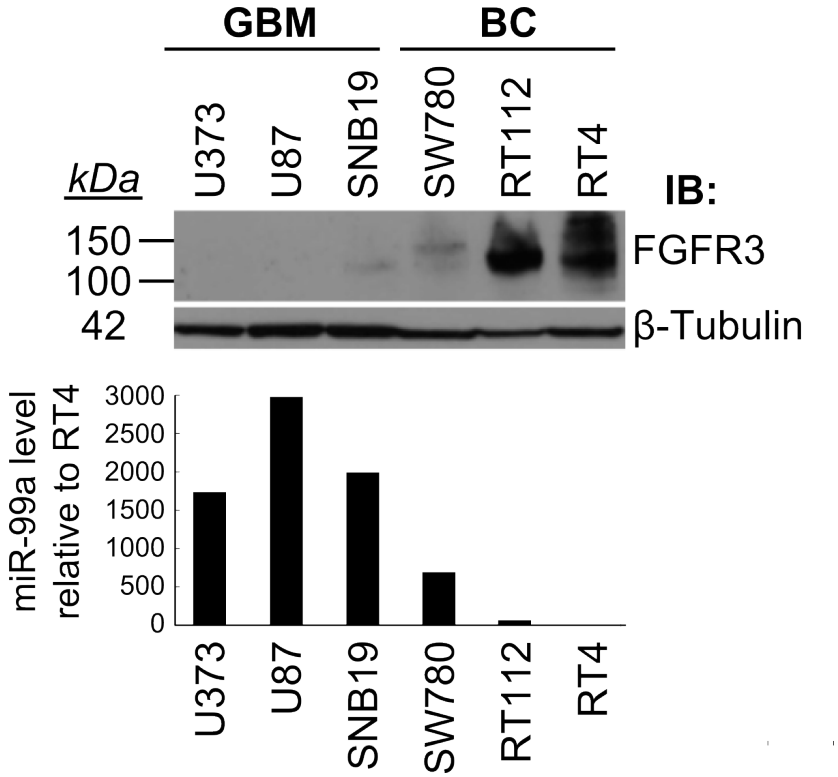


Supplementary Figure 10 | Alternative hypotheses for the mechanism generating the *FGFR3-TACC3* fusion. a) A read-through event is ruled out by *TACC3* being upstream of *FGFR3*. b) *FGFR3* amplification and translocation upstream of *TACC3* is ruled out by the fact that observed fusions only

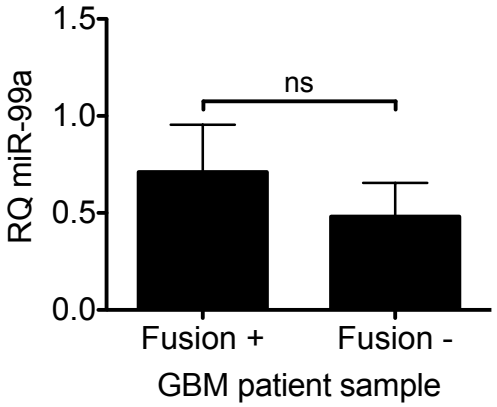
involve *TACC3* exons 5 and forward, and by the aCGH data which indicates a larger amplicon involving parts of *TACC3*. c) A tandem duplication can explain the observed fusion genes. d) Different experiments through which the presence of the tandem duplication was validated.



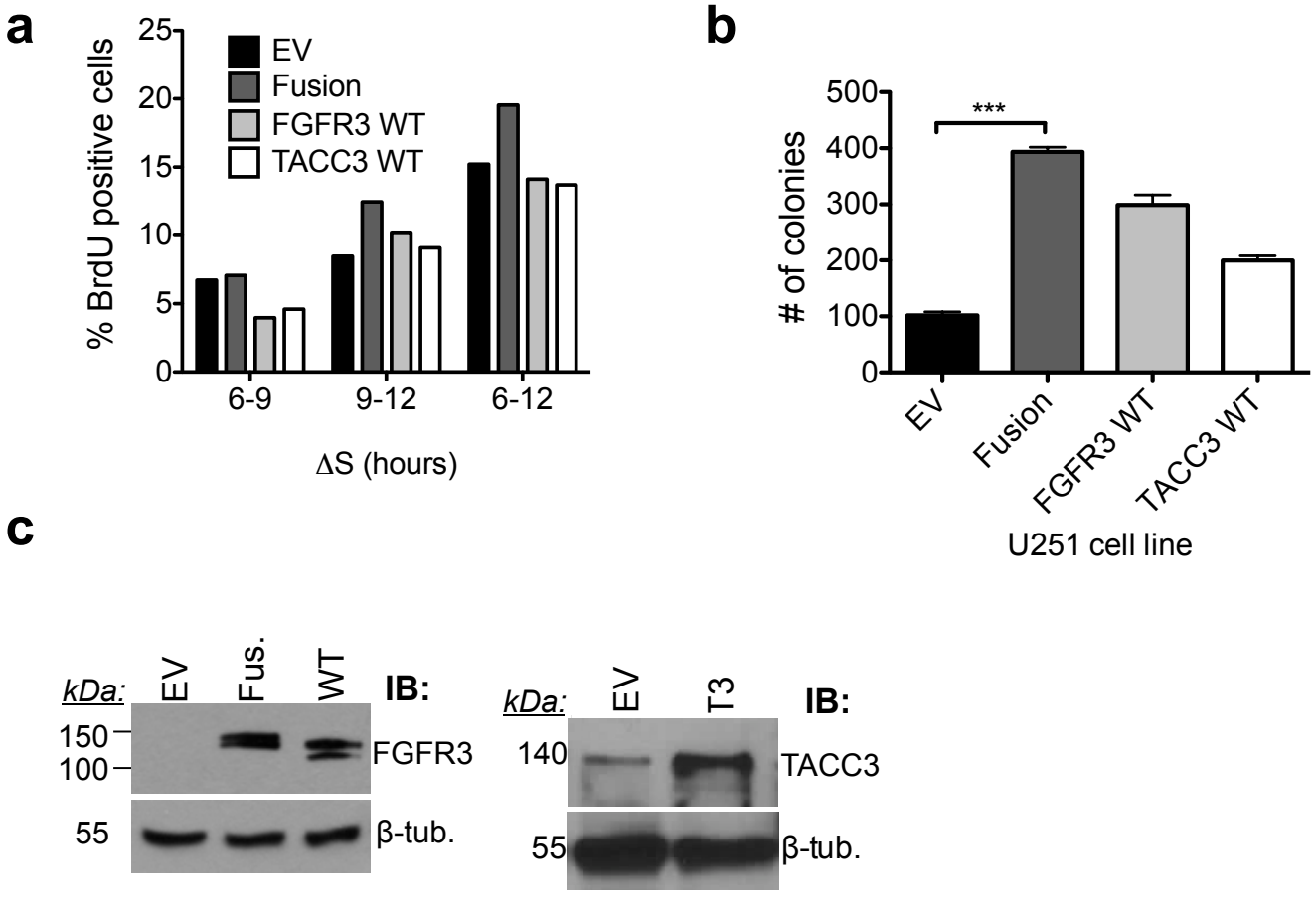
Supplementary Figure 11 | Of the highest expressed miRNA in GBM, only miR-99a suppresses *FGFR3* expression. Upon miR-99a, -21, or -125b transfection, relative *FGFR3* mRNA or protein levels were measured by qRT-PCR (a), immunoblotting (b), and densitometry (c), respectively. Error bars, s.e.m.; P-values calculated using Student's t-test. *P<0.05; ***P<0.001.



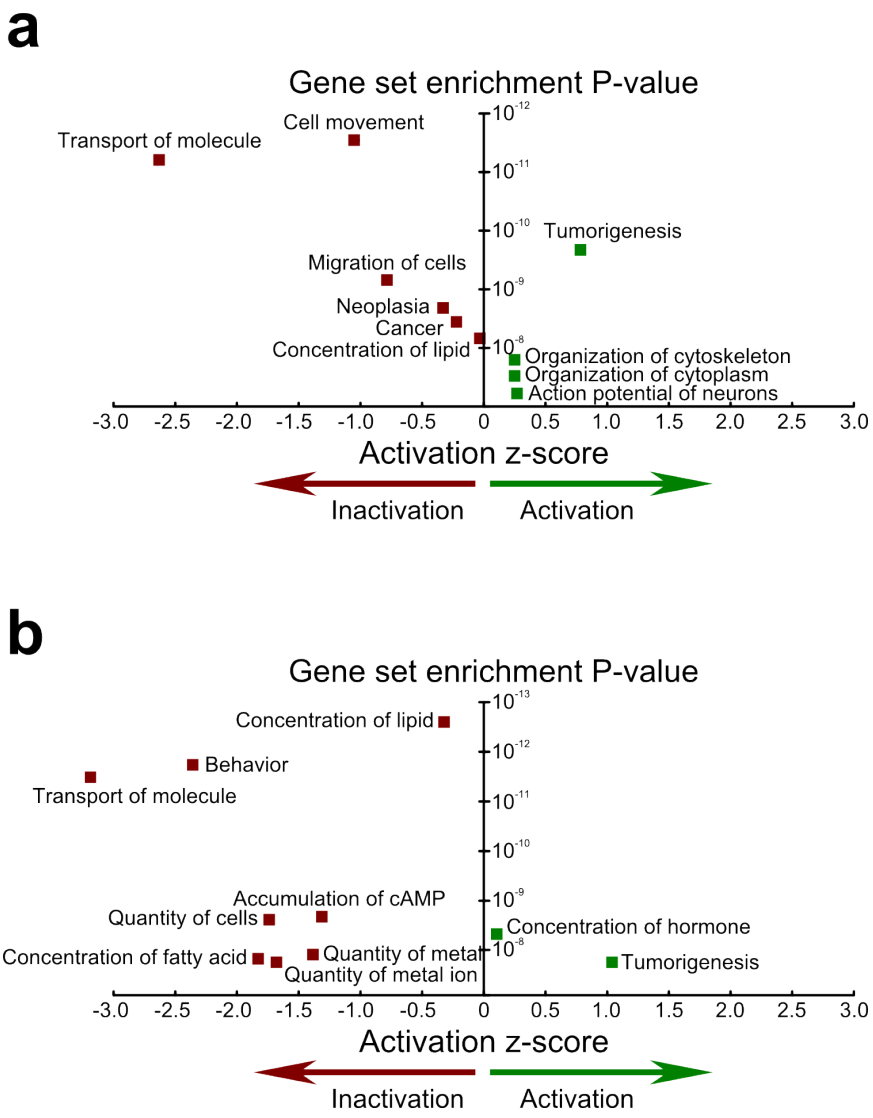
Supplementary Figure 12 | FGFR3 immunoblot and miR-99a qRT-PCR for 3 glioblastoma and 3 bladder cancer (BC) cell lines. FGFR3 protein level is inversely correlated with miR-99a.



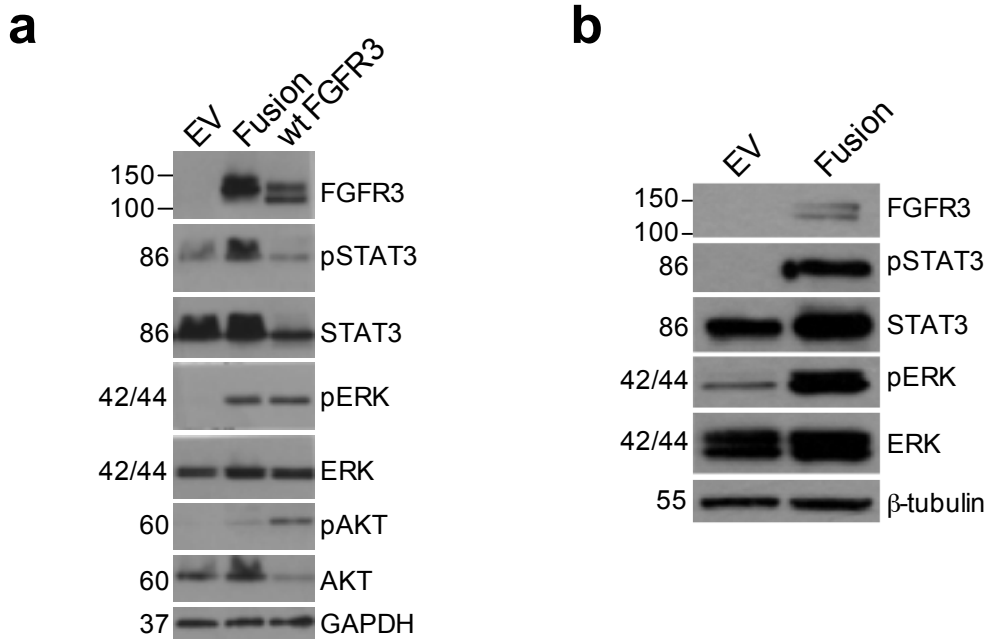
Supplementary Figure 13 | Relative levels of miR-99a were tested via qRT-PCR between fusion positive (n=4) and negative (n=8) patient samples. No significant difference between groups was observed (p = 0.46; two-tailed Student's t-test), suggesting the fusion does not regulate miR-99a expression. Relative miR-99a expression was normalized to endogenous U6 expression. Error bars, median absolute deviation.



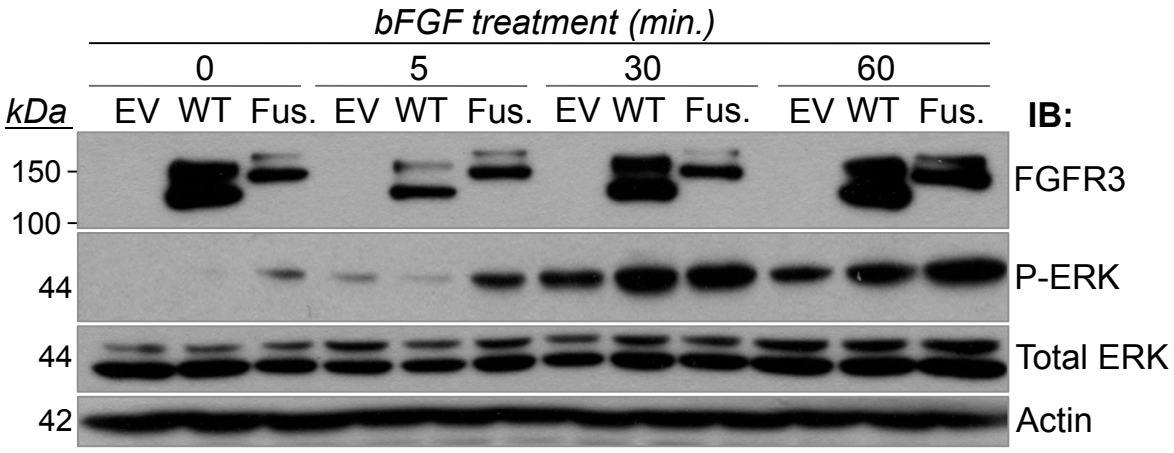
Supplementary Figure 14 | U251 cell line behaves similar to SNB19 when engineered to overexpress EV, fusion, wt *FGFR3*, or wt *TACC3* constructs. BrdU analysis (a) and soft-agar colony formation studies were performed on U251 stable cell lines. U251 fusion expressing cells grew significantly more colonies in soft agar (p=0.0001). Also, wt *FGFR3* cells also grew significantly more colonies (p= 0.0005). (d). Immunoblot of U251 stable cell lines, expressing EV, Fusion (Fus.), wt *FGFR3* (WT), or wt *TACC3* (T3) constructs (f). Error bars, s.e.m.; P-values calculated using two-tailed student's t-test. ***P<0.0001.



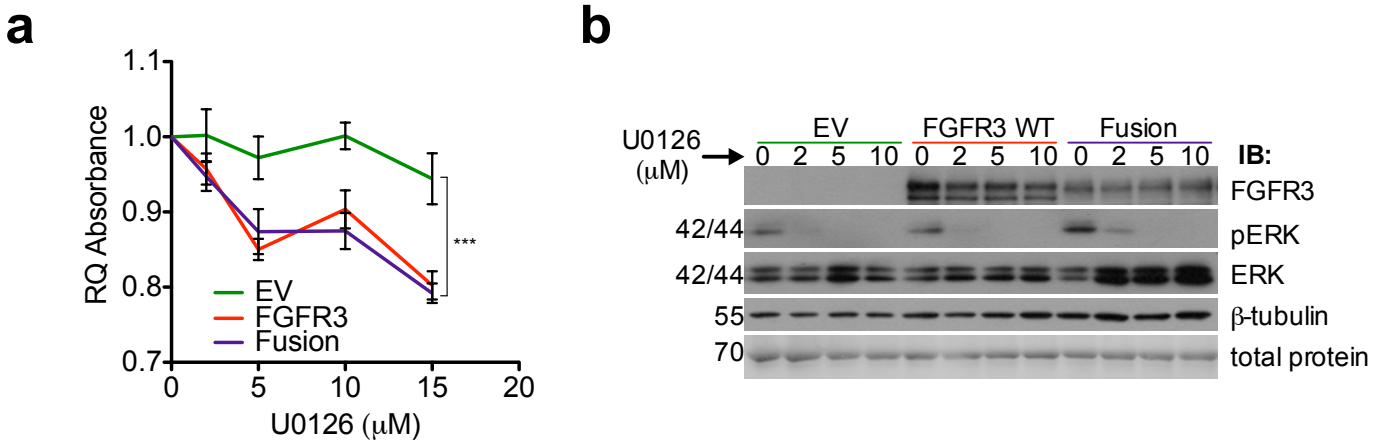
Supplementary Figure 15 | Top ten altered pathways identified by Ingenuity Pathway Analysis (IPA) for differential gene expression between SNB19 cell lines transfected with wt *FGFR3* and those transfected with empty vector (a), or wt *TACC3* and empty vector (b). Positive regulation z-scores indicate increased pathway activation. Only the top nine pathways are shown because IPA analysis did not allocate a regulation z-score for the next gene sets.



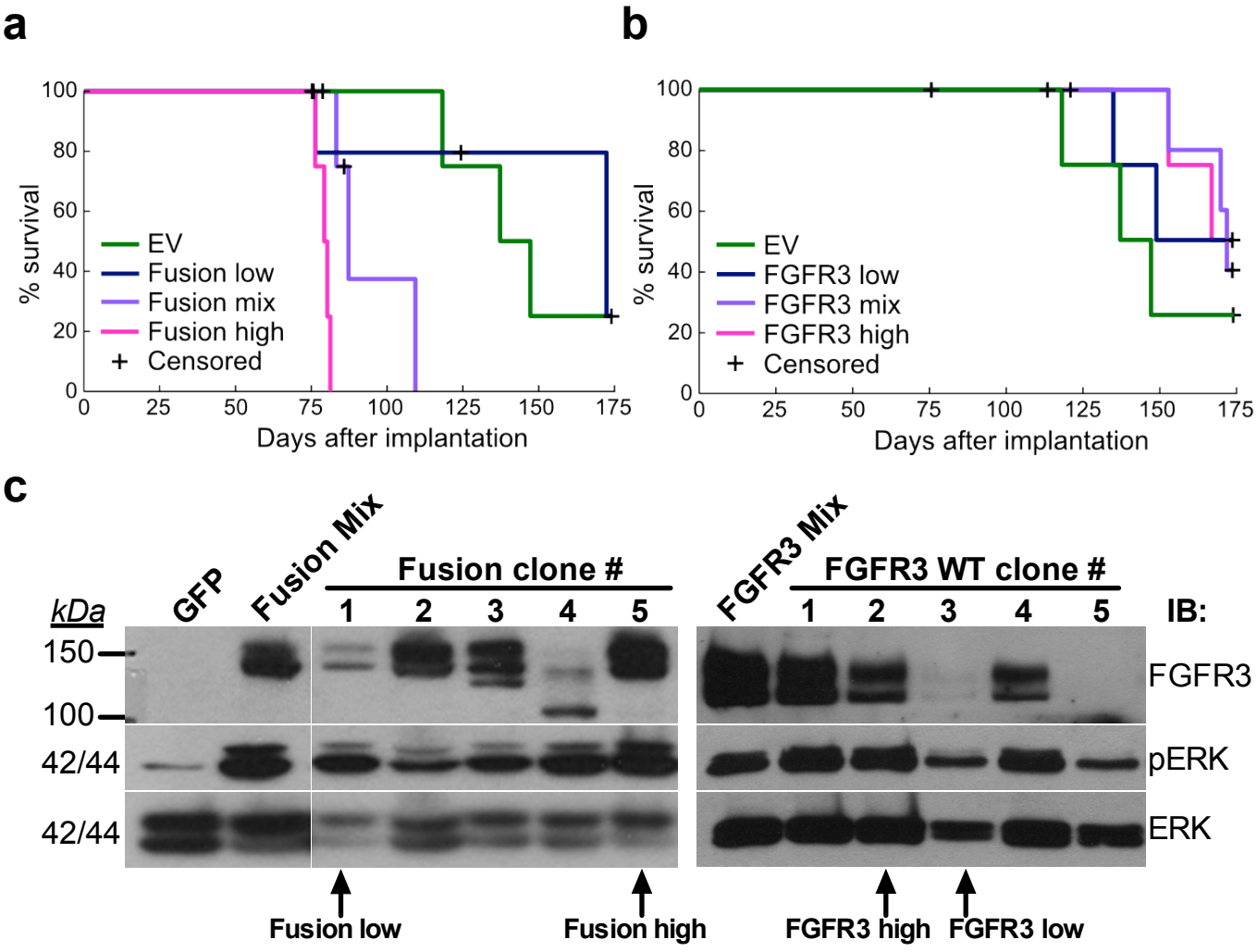
Supplementary Figure 16 | Downstream analysis of transient (a) and stable (b) fusion cell lines indicate the major downstream molecules activated are STAT3 and ERK. AKT is not significantly activated by the presence of the fusion.



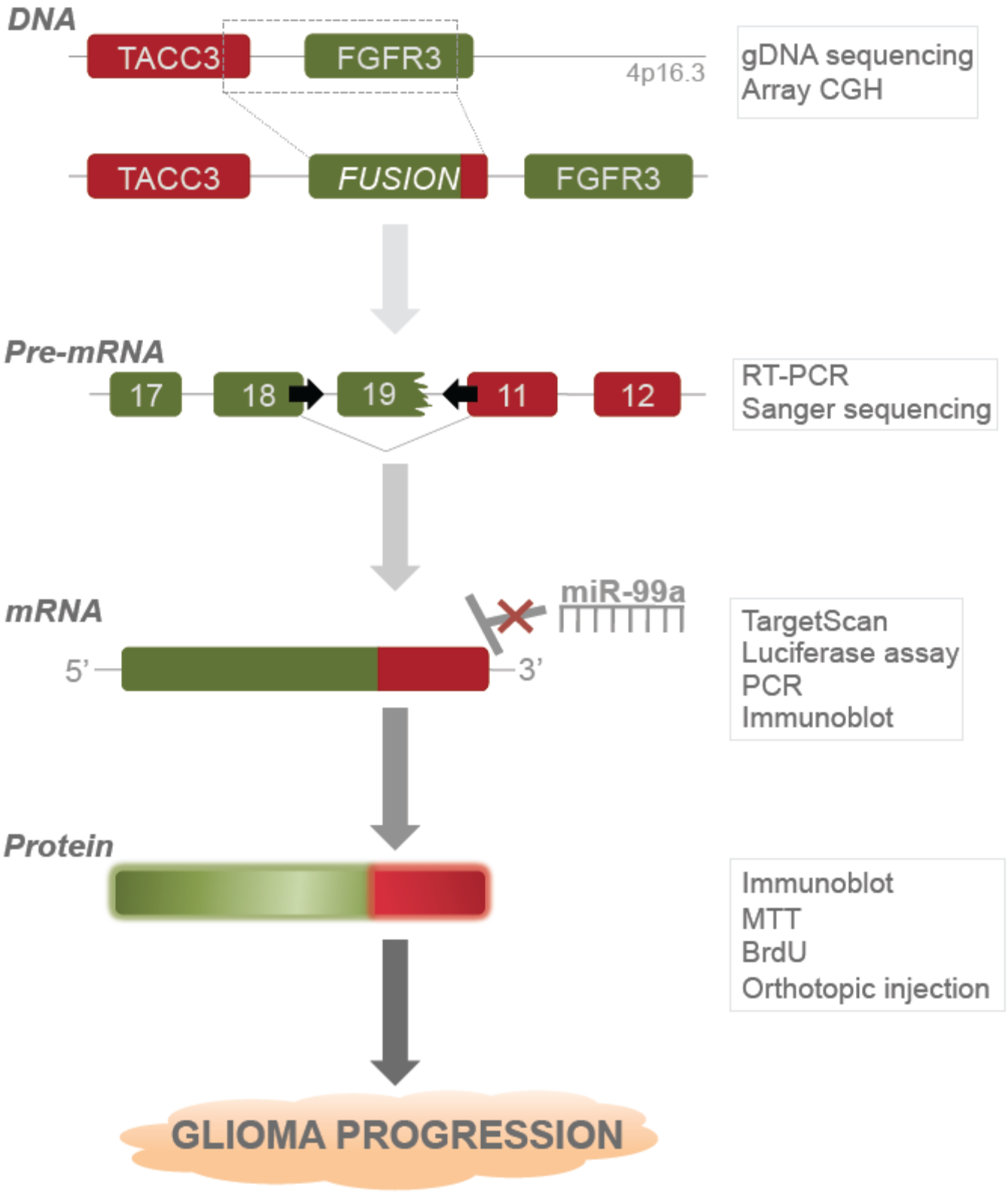
Supplementary Figure 17 | EV, wt *FGFR3* (WT) or Fusion (Fus.) overexpressing cell lines were serum starved and then incubated with bFGF ligand for the indicated times. All cell lines exhibited ligand-dependent ERK activation, although the fusion did show a slight increase in ERK activation in serum starved conditions. However, peak ERK activation was achieved upon incubation with ligand.



Supplementary Figure 18 | Cell viability assay of fusion, wt *FGFR3*, or EV cells treated with increasing concentration of ERK inhibitor U0126 (a). Immunoblot illustrating potency of U0126 (b)



Supplementary Figure 19 | Survival curves of fusion (a) or wt *FGFR3* (b) orthotopically implanted cell lines. SNB19 cell lines were transfected with either fusion or WT cDNA constructs and stable clones selected. Subsequent protein expression is can be visualized via immunoblot (c).



Supplementary Figure 20 | Schematic of the entire process of fusion discovery and further validation.

mRNA sequence for fusion variant 1 (NM_000142.4 bases 1-2530 + NM_006342.2 bases 2097-2837):

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Amino acid sequence of fusion variant 1:

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mRNA sequence for fusion variant 2 (NM_000142.4 bases 1-2571 + NM_006342.2 bases 1535-2847):

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CGATTTCTTGGGAGCACTGGACATTCCTGTGCCAGGCCCACCCCCAGGTGTTCCCGCGCCTGGGGGCCCACCCCTGTCCACCGGACCTATAGTGGACCTG
CTCCAGTACAGCCAGAAGGACCTGGATGCAGTGGTAAAGGCGACACAGGAGGAGAACCGGGAGCTGAGGAGCAGGTGTGAGGAGCTCCACGGGAAGAACC
TGGAACTGGGGAAGATCATGGACAGGTTCGAAGAGGTTGTGTACCAGGCCATGGAGGAAGTTCAGAAGCAGAAGGAACTTTCCAAAGCTGAAATCCAGAA
AGTTCTAAAAGAAAAAGACCAACTTACCACAGATCTGAACTCCATGGAGAAGTCCTTCTCCGACCTCTTCAAGCGTTTTGAGAAACAGAAAGAGGTGATC
GAGGGCTACCGCAAGAACGAAGAGTCACTGAAGAAGTGCGTGGAGGATTACCTGGCAAGGATCACCCAGGAGGGCCAGAGGTACCAAGCCCTGAAGGCCC
ACGCGGAGGAGAAGCTGCAGCTGGCAAACGAGGAGATCGCCCAGGTCCGGAGCAAGGCCCAGGCGGAAGCGTTGGCCCTCCAGGCCAGCCTGAGGAAGGA
GCAGATGCGCATCCAGTCGCTGGAGAAGACAGTGGAGCAGAAGACTAAAGAGAACGAGGAGCTGACCAGGATCTGCGACGACCTCATCTCCAAGATGGAG
AAGATCTGAcctccacggagccgctgtccccgccccctgctcccgtctgtctgtcctgtctgattctcttaggtgtcatgttctttttttctgtcttgtc
ttcaacttttttaaaaactagattgctttgaaaacatgactcaataaaagtttcctttcaatttaaacactgaaaaaaaaaaaa

Amino acid sequence of fusion variant 2:

MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGSGDAVELSCPPPGGGPMGPTVWVKDGTGLVPSERVLVGPQRL
QVLNASHEDSGAYSCRQRLTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGAPYWTRPERMDKKLLAVPAANTVRFRCPAAGNPTPSISW
LKNGREFRGEHRIGGIKLRHQQWSLVMESVVPSDRGNYTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSDAQ
PHIQWLKHVEVNGSKVGPDGTPYVTVLKTAGANTTDKELEVLSLHNVTFEDAGEYTCLAGNSIGFSHHSAWLVVLPAEEELVEADEAGSVYAGI
LSYGVGFFLFILVVAAVTLCRLRSPPKKGLGSPTVHKISRFPLKRQVSLESNASMSSNTPLVRIARLSSGEGPTLANVSELELPADPKWELSRA
RLTLGKPLGEGCFGQVVMAEAIGIDKDRAAKPVTVAVKMLKDDATDKDLSDLVSEMEMMKMIGKHKNIINLLGACTQGGPLYVLVEYAAKGNLR
EFLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCAYQVARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADFGLARDVHNLDYYKKTTNGRLPV
KWMAPEALFDRVYTHQSDVWSFGVLLWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWHAAPSQRPTFKQLVEDLDRVL
TVTSTDEYLDLSAPFEQYSPSQQLHSASAEDTPVVQLAAETPTAESKERALNSASTSLPTSCPGSEPVPTHQQGQPALELKEESFRDPAEVLGT
GAEVDYLEQFGTSSFKESALRKQSLYLKFDPLLRDSPGRPVPVATETSSMHGANETPSGRPREAKLVEFDFLGALDIPVPGPPPGVPAPGGPPL
STGPIVDLLQYSQKDLDAVVKATQEENRELRSRCEELHGKNLELGKIMDRFEEVVYQAMEEVQKQKELSKAEIQKVLKEKDQLTTDLNSMEKSF
SDLFKRFEKQKEVIEGYRKNEESLKKCVEDYLARITQEGQRYQALKAHAEEKLQLANEEIAQVRSKAQAEALALQASLRKEQMRIQSLEKTVEQ
KTKENEELTRICDDLISKMEKI-

mRNA sequence for fusion variant 3 (NM_000142.4 bases 1-2530 + NM_006342.2 bases 2097-2837):

gtcgcgggcagctggcgccgcgcggtcctgctctgccggtcgcacggacgcaccggcgggccgccggccggagggacggggcgggagctgggcccgcgga
cagcgagccggagcgggagccgcgcgtagcgagccgggctccggcgctcgccagtctcccgagcggcgcccgcctcccgccggtgcccgcgccgggccgt
gggggggcagcatgcccgcgcgcgctgcctgaggacgccgcggcccccgccccgccATGGGCGCCCCTGCCTGCGCCCTCGCGCTCTGCGTGGCCGTGGC
CATCGTGGCCGGCGCCTCCTCGGAGTCCTTGGGGACGGAGCAGCGCGTCGTGGGGCGAGCGGCAGAAGTCCCGGGCCCAGAGCCCGGCCAGCAGGAGCAG
TTGGTCTTCGGCAGCGGGGATGCTGTGGAGCTGAGCTGTCCCCCGCCCGGGGGTGGTCCCATGGGGCCCACTGTCTGGGTCAAGGATGGCACAGGGCTGG
TGCCCTCGGAGCGTGTCCTGGTGGGGCCCCAGCGGCTGCAGGTGCTGAATGCCTCCCACGAGGACTCCGGGGCCTACAGCTGCCGGCAGCGGCTCACGCA
GCGCGTACTGTGCCACTTCAGTGTGCGGGTGACAGACGCTCCATCCTCGGGAGATGACGAAGACGGGGAGGACGAGGCTGAGGACACAGGTGTGGACACA
GGGGCCCCTTACTGGACACGGCCCGAGCGGATGGACAAGAAGCTGCTGGCCGTGCCGGCCGCCAACACCGTCCGCTTCCGCTGCCCAGCCGCTGGCAACC
CCACTCCCTCCATCTCCTGGCTGAAGAACGGCAGGGAGTTCCGCGGCGAGCACCGCATTGGAGGCATCAAGCTGCGGCATCAGCAGTGGAGCCTGGTCAT
GGAAAGCGTGGTGCCCTCGGACCGCGGCAACTACACCTGCGTCGTGGAGAACAAGTTTGGCAGCATCCGGCAGACGTACACGCTGGACGTGCTGGAGCGC
TCCCCGCACCGGCCCATCCTGCAGGCGGGGCTGCCGGCCAACCAGACGGCGGTGCTGGGCAGCGACGTGGAGTTCCACTGCAAGGTGTACAGTGACGCAC
AGCCCCACATCCAGTGGCTCAAGCACGTGGAGGTGAATGGCAGCAAGGTGGGCCCGGACGGCACACCCTACGTTACCGTGCTCAAGACGGCGGGCGCTAA
CACCACCGACAAGGAGCTAGAGGTTCTCTCCTTGCACAACGTCACCTTTGAGGACGCCGGGGAGTACACCTGCCTGGCGGGCAATTCTATTGGGTTTTCT
CATCACTCTGCGTGGCTGGTGGTGCTGCCAGCCGAGGAGGAGCTGGTGGAGGCTGACGAGGCGGGCAGTGTGTATGCAGGCATCCTCAGCTACGGGGTGG
GCTTCTTCCTGTTCATCCTGGTGGTGGCGGCTGTGACGCTCTGCCGCCTGCGCAGCCCCCCCAAGAAAGGCCTGGGCTCCCCCACCGTGCACAAGATCTC
CCGCTTCCCGCTCAAGCGACAGGTGTCCCTGGAGTCCAACGCGTCCATGAGCTCCAACACACCACTGGTGCGCATCGCAAGGCTGTCCTCAGGGGAGGGC
CCCACGCTGGCCAATGTCTCCGAGCTCGAGCTGCCTGCCGACCCCAAATGGGAGCTGTCTCGGGCCCGGCTGACCCTGGGCAAGCCCCTTGGGGAGGGCT
GCTTCGGCCAGGTGGTCATGGCGGAGGCCATCGGCATTGACAAGGACCGGGCCGCCAAGCCTGTCACCGTAGCCGTGAAGATGCTGAAAGACGATGCCAC
TGACAAGGACCTGTCGGACCTGGTGTCTGAGATGGAGATGATGAAGATGATCGGGAAACACAAAAACATCATCAACCTGCTGGGCGCCTGCACGCAGGGC
GGGCCCCTGTACGTGCTGGTGGAGTACGCGGCCAAGGGTAACCTGCGGGAGTTTCTGCGGGCGCGGCGGCCCCCGGGCCTGGACTACTCCTTCGACACCT
GCAAGCCGCCCGAGGAGCAGCTCACCTTCAAGGACCTGGTGTCCTGTGCCTACCAGGTGGCCCGGGGCATGGAGTACTTGGCCTCCCAGAAGTGCATCCA
CAGGGACCTGGCTGCCCGCAATGTGCTGGTGACCGAGGACAACGTGATGAAGATCGCAGACTTCGGGCTGGCCCGGGACGTGCACAACCTCGACTACTAC
AAGAAGACAACCAACGGCCGGCTGCCCGTGAAGTGGATGGCGCCTGAGGCCTTGTTTGACCGAGTCTACACTCACCAGAGTGACGTCTGGTCCTTTGGGG
TCCTGCTCTGGGAGATCTTCACGCTGGGGGGCTCCCCGTACCCCGGCATCCCTGTGGAGGAGCTCTTCAAGCTGCTGAAGGAGGGCCACCGCATGGACAA
GCCCGCCAACTGCACACACGACCTGTACATGATCATGCGGGAGTGCTGGCATGCCGCGCCCTCCCAGAGGCCCACCTTCAAGCAGCTGGTGGAGGACCTG
GACCGTGTCCTTACCGTGACGTCCACCGAGGTGCCAGGCCCACCCCCAGGTGTTCCCGCGCCTGGGGGCCCACCCCTGTCCACCGGACCTATAGTGGACC
TGCTCCAGTACAGCCAGAAGGACCTGGATGCAGTGGTAAAGGCGACACAGGAGGAGAACCGGGAGCTGAGGAGCAGGTGTGAGGAGCTCCACGGGAAGAA
CCTGGAACTGGGGAAGATCATGGACAGGTTCGAAGAGGTTGTGTACCAGGCCATGGAGGAAGTTCAGAAGCAGAAGGAACTTTCCAAAGCTGAAATCCAG
AAAGTTCTAAAAGAAAAAGACCAACTTACCACAGATCTGAACTCCATGGAGAAGTCCTTCTCCGACCTCTTCAAGCGTTTTGAGAAACAGAAAGAGGTGA
TCGAGGGCTACCGCAAGAACGAAGAGTCACTGAAGAAGTGCGTGGAGGATTACCTGGCAAGGATCACCCAGGAGGGCCAGAGGTACCAAGCCCTGAAGGC
CCACGCGGAGGAGAAGCTGCAGCTGGCAAACGAGGAGATCGCCCAGGTCCGGAGCAAGGCCCAGGCGGAAGCGTTGGCCCTCCAGGCCAGCCTGAGGAAG
GAGCAGATGCGCATCCAGTCGCTGGAGAAGACAGTGGAGCAGAAGACTAAAGAGAACGAGGAGCTGACCAGGATCTGCGACGACCTCATCTCCAAGATGG
AGAAGATCTGAcctccacggagccgctgtccccgccccctgctcccgtctgtctgtcctgtctgattctcttaggtgtcatgttctttttttctgtcttg
tcttcAacttttttaaaaactagattgctttgaaaacatgactcaataaaagtttcctttcaatttaaacactgaa

Amino acid sequence of fusion variant 3:

MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGSGDAVELSCPPPGGGPMGPTVWVKDGTGLVPSERVLVGPQRLQVLNAS
HEDSGAYSCRQRLTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGAPYWTRPERMDKKLLAVPAANTVRFRCPAAGNPTPSISWLKNGREFRGEHR
IGGIKLRHQQWSLVMESVVPSDRGNYTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVGP
DGTPYVTVLKTAGANTTDKELEVLSLHNVTFEDAGEYTCLAGNSIGFSHHSAWLVVLPAEEELVEADEAGSVYAGILSYGVGFFLFILVVAAVTLCRLRS
PPKKGLGSPTVHKISRFPLKRQVSLESNASMSSNTPLVRIARLSSGEGPTLANVSELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAIGIDKDRAA
KPVTVAVKMLKDDATDKDLSDLVSEMEMMKMIGKHKNIINLLGACTQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCAYQ
VARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADFGLARDVHNLDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLLWEIFTLGGSPYPGIPV
EELFKLLKEGHRMDKPANCTHDLYMIMRECWHAAPSQRPTFKQLVEDLDRVLTVTSTDVPGPPPGVPAPGGPPLSTGPIVDLLQYSQKDLDAVVKATQEE
NRELRSRCEELHGKNLELGKIMDRFEEVVYQAMEEVQKQKELSKAEIQKVLKEKDQLTTDLNSMEKSFSDLFKRFEKQKEVIEGYRKNEESLKKCVEDYL
ARITQEGQRYQALKAHAEEKLQLANEEIAQVRSKAQAEALALQASLRKEQMRIQSLEKTVEQKTKENEELTRICDDLISKMEKI-

Sample	Fusion variant
MDA GBM-13	FGFR3-TACC3 e18-e11
MDA GBM-07	FGFR3-TACC3 e19*-e4*
Tianjin GBM-T01	FGFR3-TACC3 e18-e11
Tianjin GBM-T02	FGFR3-TACC3 e18-e10
TCGA-27-1835	FGFR3-TACC3 e18-e11
TCGA-12-0826	FGFR3-TACC3 e18-e8
TCGA-06-6390	FGFR3-TACC3 e18-e9
TCGA-19-5958	FGFR3-TACC3 e18-e8
TCGA-76-4925	FGFR3-TACC3 e18-e10
Singh GBM-22	FGFR3-TACC3 e18-e10
Singh GBM-1123	FGFR3-TACC3 e19*-e8
Singh GBM-51	FGFR1-TACC1

Supplementary Table 1 | Summary of all reported *FGFR-TACC* fusions. (*) Breakpoint disrupts an exon.