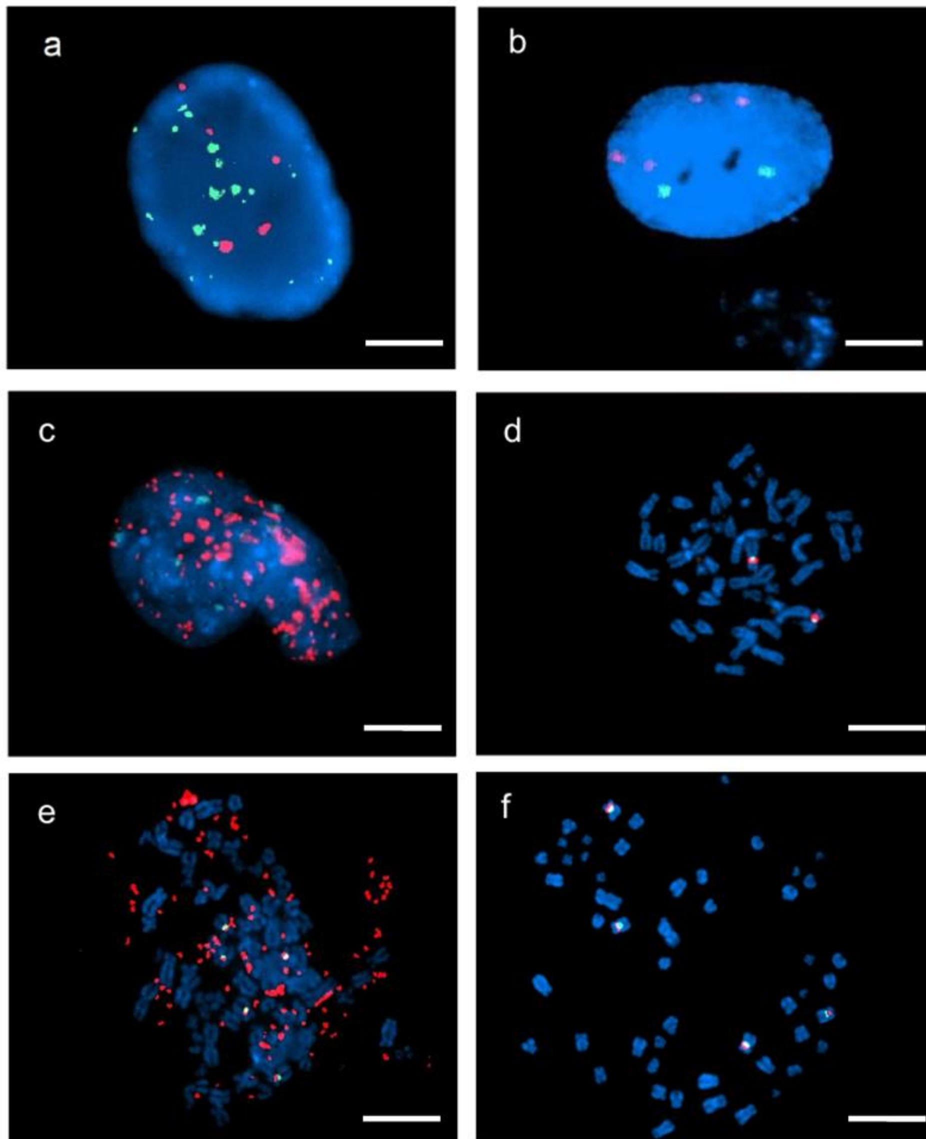
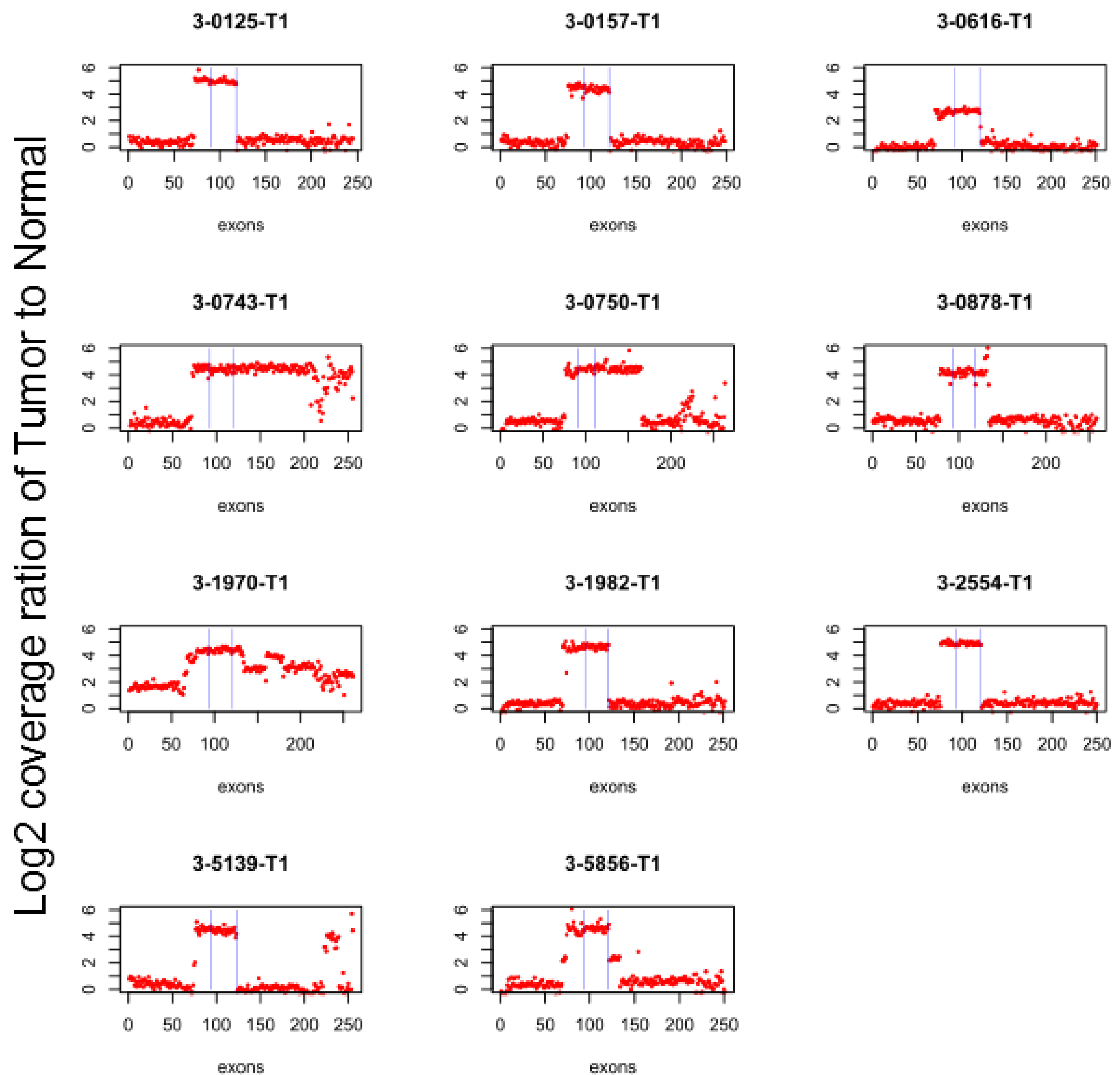


Supplementary Figure 1. FISH analysis for the detection of Double Minutes in GBM IV-34, GBM IV-39 and GBM6 tumors. Scale bars = 5µm.

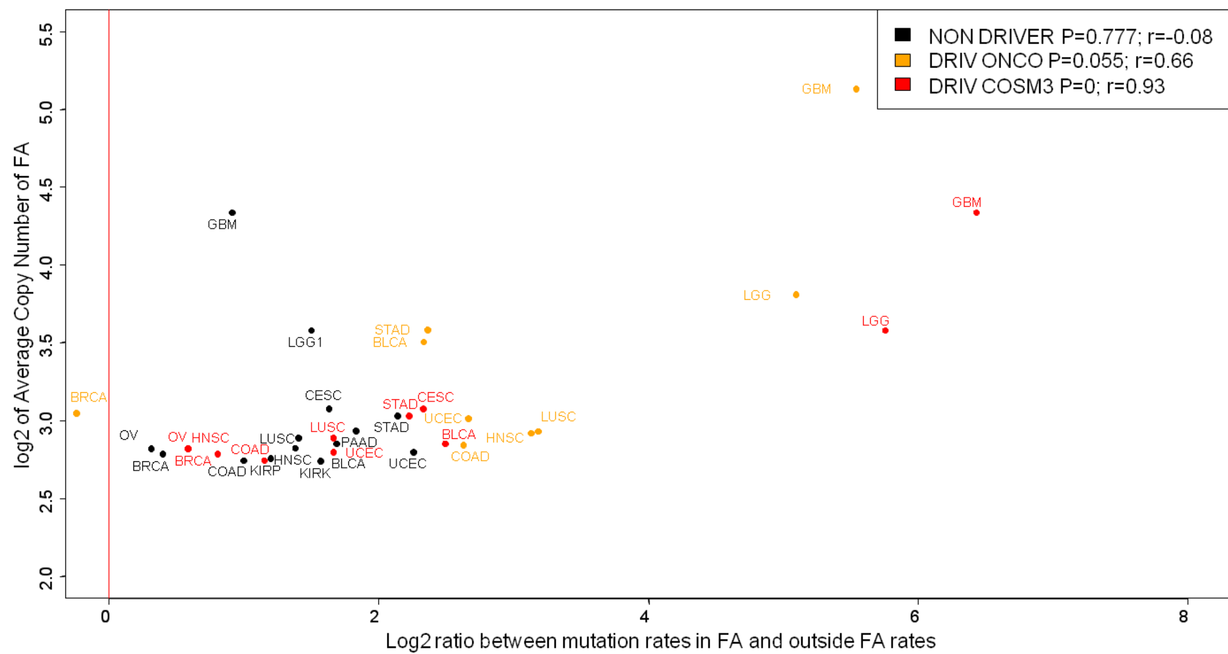
a and b. FISH analysis for the detection of PDGFRA amplification in GBM IV-34 cells**.** a. primary tumor cells shows multiple copies of the PDGFRA gene visualized as many green spots. b. Cultured tumor cells do not show PDGFRA amplification (green).

c and d. FISH analysis for the detection of EGFR amplification in GBM IV-39 cells**.** c. FISH in primary tumor cells demonstrates euploid chromosome 7 (green signal) and multiple copies of EGFR scattered all over the nucleus (red signal). d. Cultured tumor cells shows diploid chromosome 7 (green signal) and not amplified EGFR (red signal).

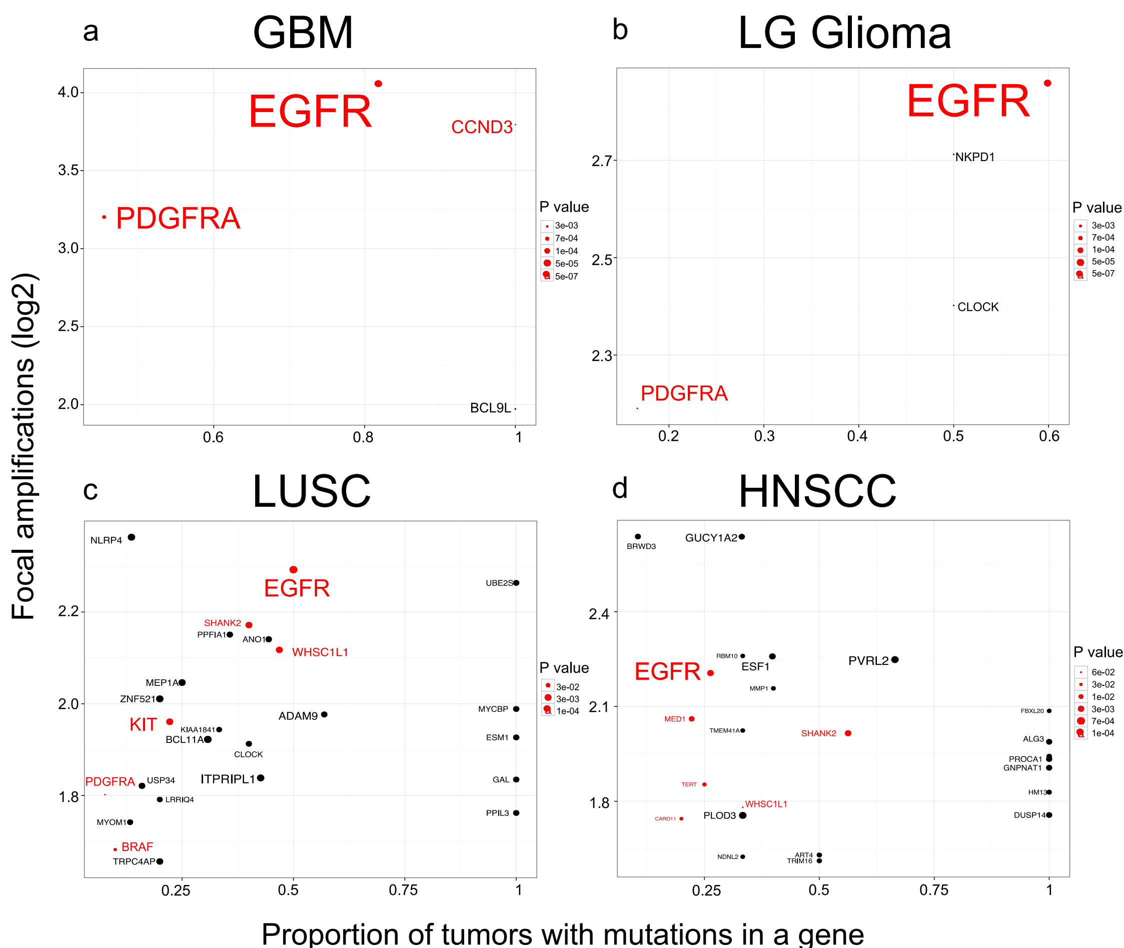
e and f. FISH analysis for the detection of EGFR amplification in GBM6 cells. e. FISH in primary tumor cells shows 5 copies of chromosome 7 (green signal) and multiple copies of EGFR scattered all over the nucleus in the form of Double Minutes (red signal). f. Cultured tumor cells shows chromosomal EGFR (red signal) in close proximity of the centromeres of chromosome 7 (green signal). No EGFR copies amplified in form of Double Minutes are detectable. [Methods].



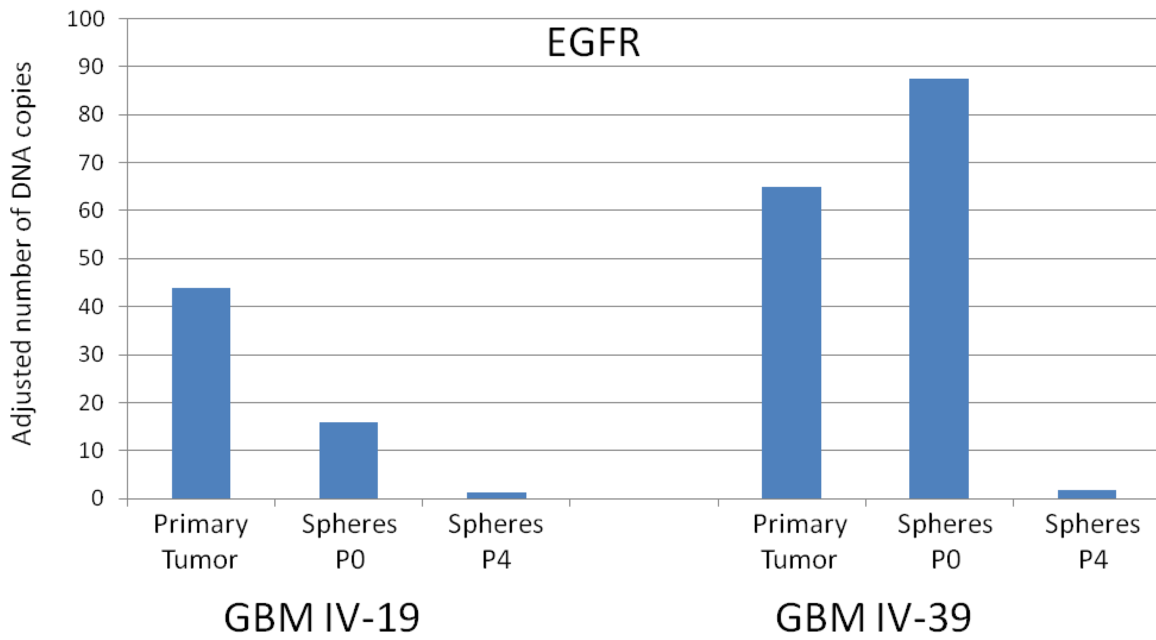
Supplementary Figure 2. Focal amplifications of EGFR locus by exome sequencing. Y axis – log2 of the ratios between the coverages of the tumor and the normal samples. X axis – equidistantly located exons. The blue vertical line depicts the first and last exons of EGFR. In Tumors 3-1970-T1 and 3-5856-T1 at least two overlapping FAs with different edges and copy numbers are present.

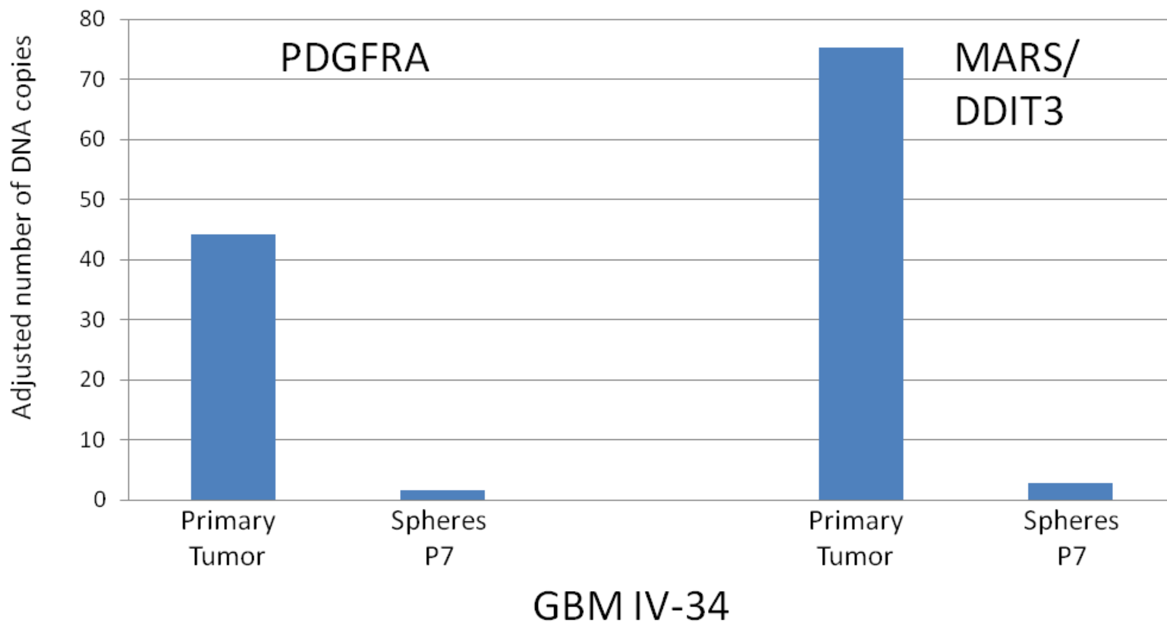


Supplementary Figure 3.Correlation of increase of mutation rates in FAs with the FA copy number. Statistical significance was assessed with ANOVA. X axis – log2 of the ratio between mutations rates inside FAs and outside. Y axis – log2 of the average copy number in FAs. Each data point represents the tumor type. Black – non-driver (passenger) mutations (N=14). Orange – mutations in oncogenes (N=9). Red – recurrent mutations (at least 3 times) in COSMIC v67 (N = 11). KICH was excluded from the analyses due to small amount of data. Red line represents equal mutations rates inside and outside of FAs.



Supplementary Figure 4. Co-localizations of mutations and amplifications on gene-by-gene basis. a. glioblastoma, b. low grade glioma, c. lung squamous cell carcinoma, d. head and neck squamous cell carcinoma. X-axis - proportion of mutations in Focal Amplifications, Y-axis - log2 of average copy number in FAs. The area in circle is inversely proportional to the log2 of the log2 of the Pvalue (Fisher test). All oncogenes are selected in red. All oncogenes are presented if they have at least one mutations in FAs and a Pvalue less than 0.15, all the other genes are presented if they have at least 2 mutations in FAs and Pvalue less than 0.01





Supplementary Figure 5. qPCR analysis of the Focal Amplifications (FA) in the tumors. Primary tumors and gliomaspheres of early passages (P0) confirm the focal amplifications with the number of copies of more than 40 per cell. FAs in the gliomaspheres at later passages (P4 and P7) are absent.

Supplementary Information

Supplementary Table 1. Tumor types from the TCGA collection used in this study

Tumor type	No. of samples	CNV analysis	Full tumor names
BLCA	26	SNP array	Bladder Urothelial Carcinoma
BRCA	763	SNP array	Breast invasive carcinoma
CESC	36	SNP array	Cervical squamous cell carcinoma and endocervical adenocarcinoma
COAD	269	SNP array	Colon adenocarcinoma
GBM1	174	aCGH	Glioblastoma multiforme
HNSC	302	SNP array	Head and Neck squamous cell carcinoma
KIRK	420	SNP array	Kidney renal clear cell carcinoma
KIRP	112	SNP array	Kidney renal papillary cell carcinoma
LGG1	218	SNP array	Brain Lower Grade Glioma
LUSC	178	SNP array	Lung squamous cell carcinoma
OV11	452	SNP array	Ovarian serous cystadenocarcinoma
PAAD	56	SNP array	Pancreatic adenocarcinoma
STAD	244	SNP array	Stomach Adenocarcinoma
UCEC	242	SNP array	Uterine Corpus Endometrioid Carcinoma

Supplementary Table 2. Average high throughput sequencing coverage per glioblastoma sample

TumID	Spheres	Tumor A	Tumor B	Normal
primary GBM IV-19	118	-	137	127
primary GBM IV-20	330	-	343	375
primary GBM IV-21	170	97	167	169
primary GBM IV-34	175	56	171	165
primary GBM IV-35	169	127	171	168
primary GBM IV-36	363	-	327	315
primary GBM IV-39	186	174	-	183
primary GBM IV-6	212	133	209	147

Supplementary Table 3. Primers for qPCR used in this study

target gene	qPCR primer name	sequence
EGFR	48462_EGFR_L	ATGACGATGAGCCTGTCTGAAG
EGFR	48463_EGFR_R	ACTCGGCAGGAATGCAGACT
PDGFRA	48470_PDGFRA_L	ACAGAGGAGGAGACTGCAAGAGA
PDGFRA	48471_PDGFRA_R	ATGTCCCACACATGGAGTAAAGAG
MARS/DDIT3	49418Mars_F	CCAGTGCCCTCCCTTACGTC
MARS/DDIT3	49419Mars_R	GCACTGAGCACACAACCAATG