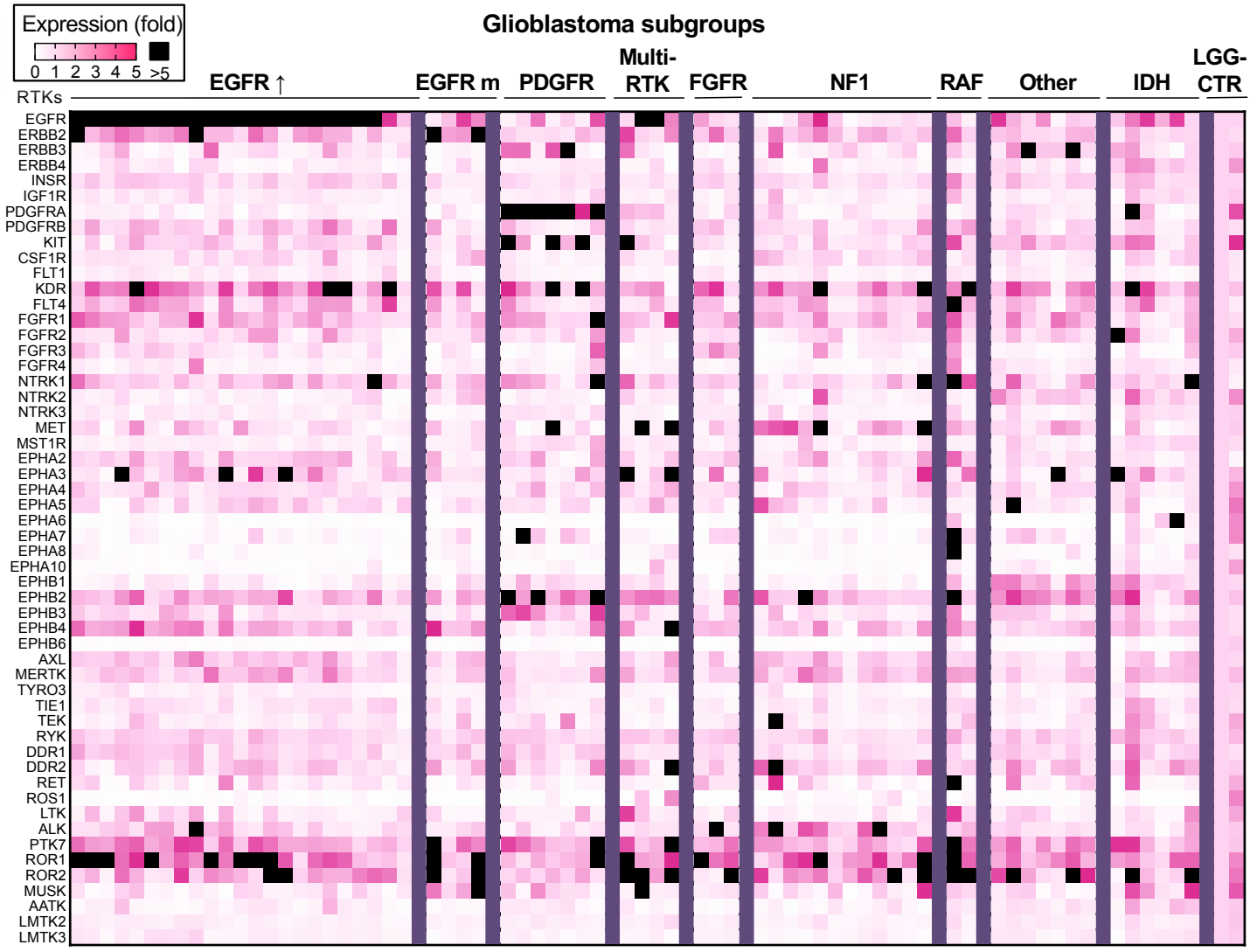
## SUPPLEMENTAL FIGURES S1-S6

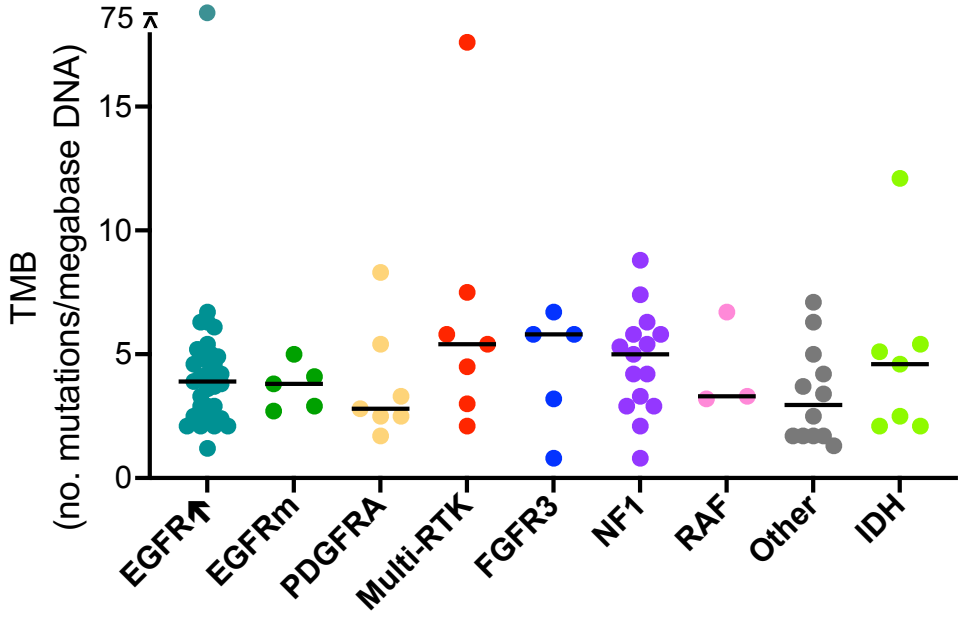
S1: Glioblastoma molecular subgroups: landscape of RTK expression
S2: Glioblastoma molecular subgroups: tumor mutation burden
S3: Glioblastoma molecular subgroups: proliferation
S4: Glioblastoma molecular subgroups: correlation matrix
S5: Glioblastoma IDH-wild-type: characteristic nuclear morphology of the histologic patterns
S6: Glioblastoma IDH-wild-type: correspondence between histologic patterns and molecular subgroups

## SUPPLEMENTAL TABLES S1-S2

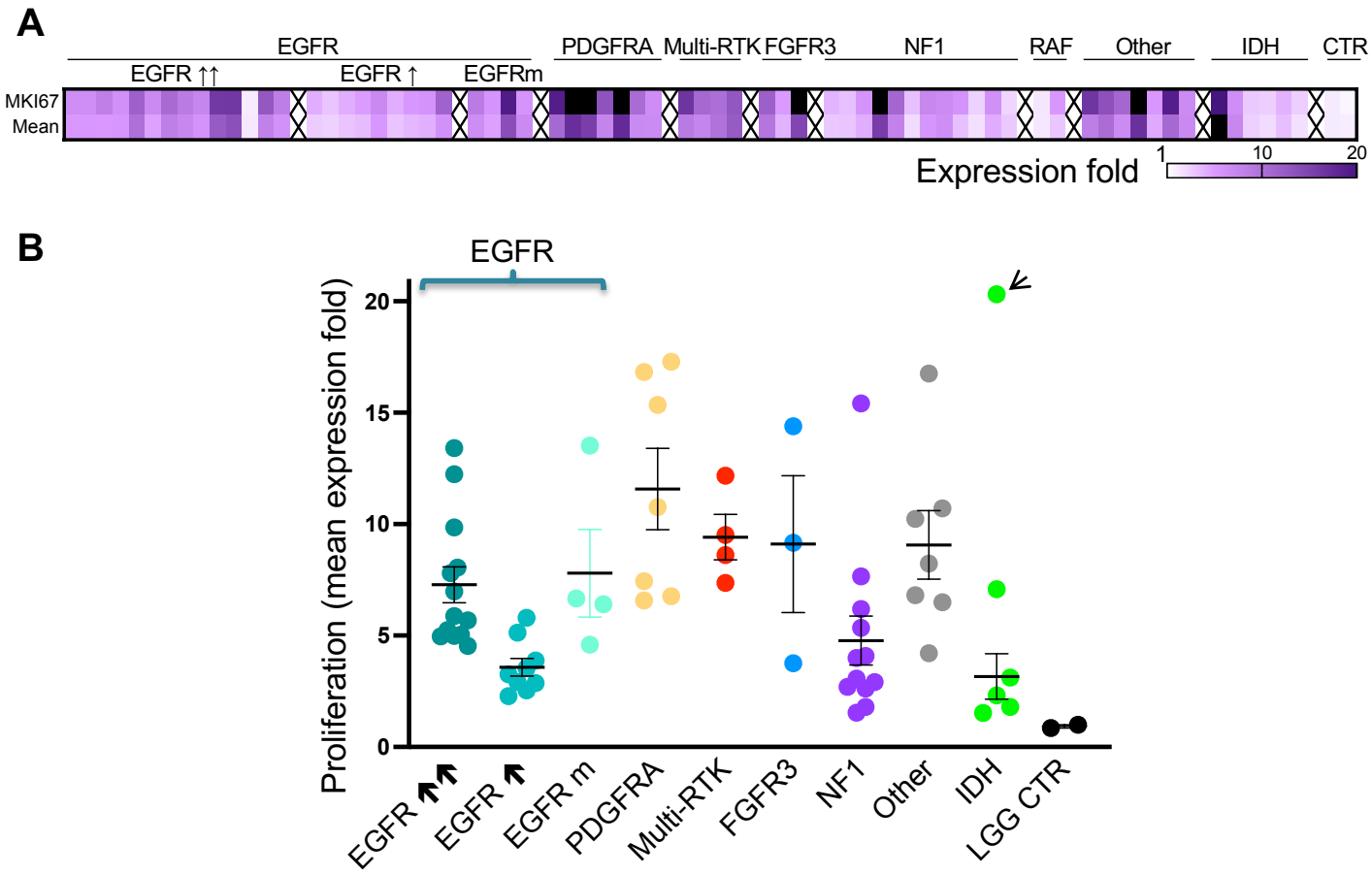
S1: Glioblastoma genomic alterations
S2: RTK classes and genes



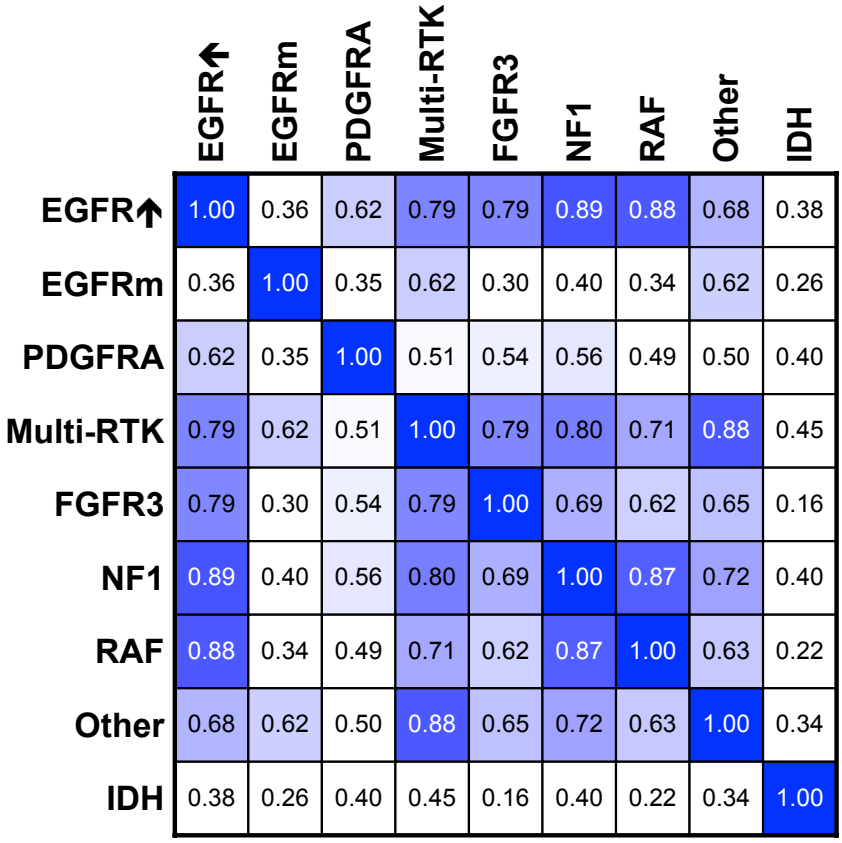

**Supplemental Figure S1.** **Landscape of RTK expression in glioblastoma subgroups.** RTK fold-expression relative to precursor low-grade glioma control (LGG-CTR) showing only the RTKs with raw values >500 in at least one sample (see Materials and Methods for RNA expression analysis). EGFR↑, *EGFR* amplification; EGFRm, *EGFR* point mutation. Note strong upregulation of *EGFR* expression in almost all cases with *EGFR* amplification. Note also increased expression of various RTKs in the absence of genetic alteration in some of the samples.




**Supplemental Figure S2.** **Tumor mutation burden (TMB) in glioblastoma subgroups.** Plot of individual TMB values medians indicated by horizontal bars. In the EGFRm subgroup, the samples from multifocal cases with EGFR mutations without amplification were added to the analysis. All values are from tumors in the absence of any treatment. Note only three tumors with TBM>10, and only three subgroups with median values >5.

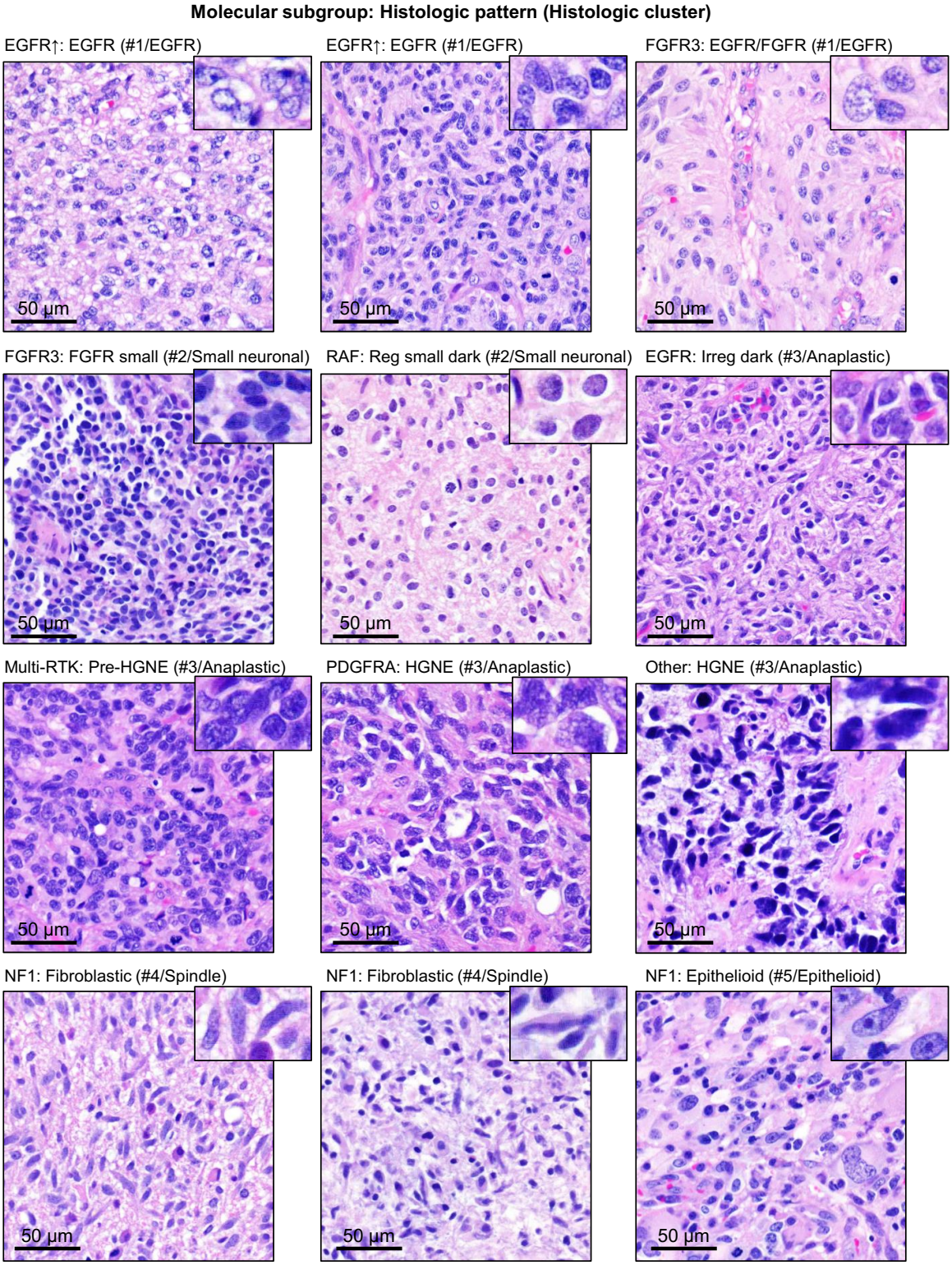



**Supplemental Figure S3.** **Glioblastoma subgroups proliferation.** **A.** Expression analysis heatmap of *MIK67* (shown), *TYMS*, *CCNB2*, *CDK1*, *CHEK1* and *EZH2* yielding a mean expression index, as fold overexpression relatively to precursor low-grade glioma controls (LGG). The sample order is the same as in Figure 1. Note separation of the EGFR subgroup in three proliferation clusters: with amplification and high overexpression (↑↑), with amplification and medium overexpression (↑), and with mutation only. The EGFR#12 tumor shows very low values due to recent prior radiation, and was excluded from further analysis. **B.** Mean±SEM plot of individual proliferation values from (A). Note different proliferation indices in the three EGFR subgroups. The outlier value from the IDH subgroup indicated by arrow corresponds to IDH#1 and was excluded from the mean calculation.

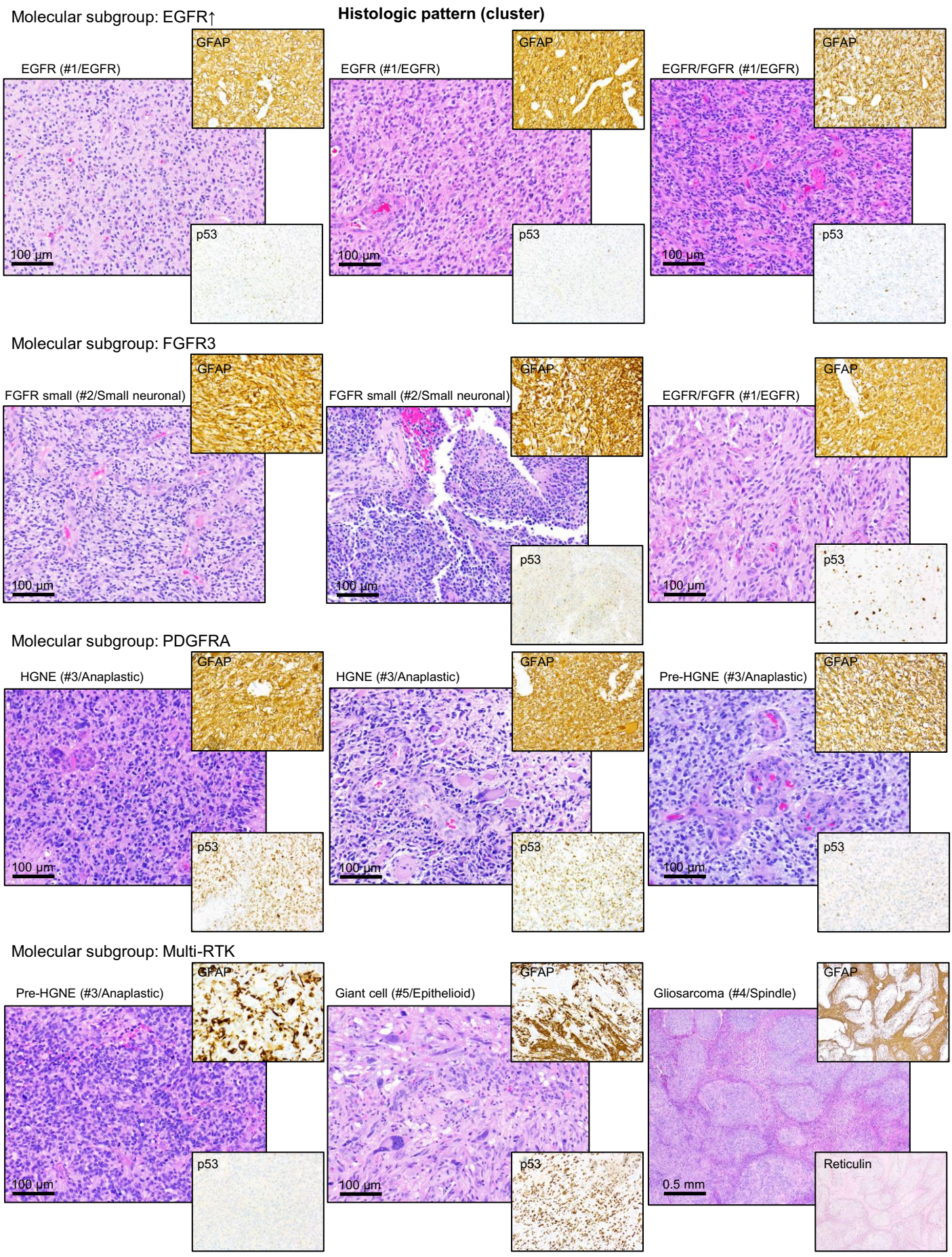


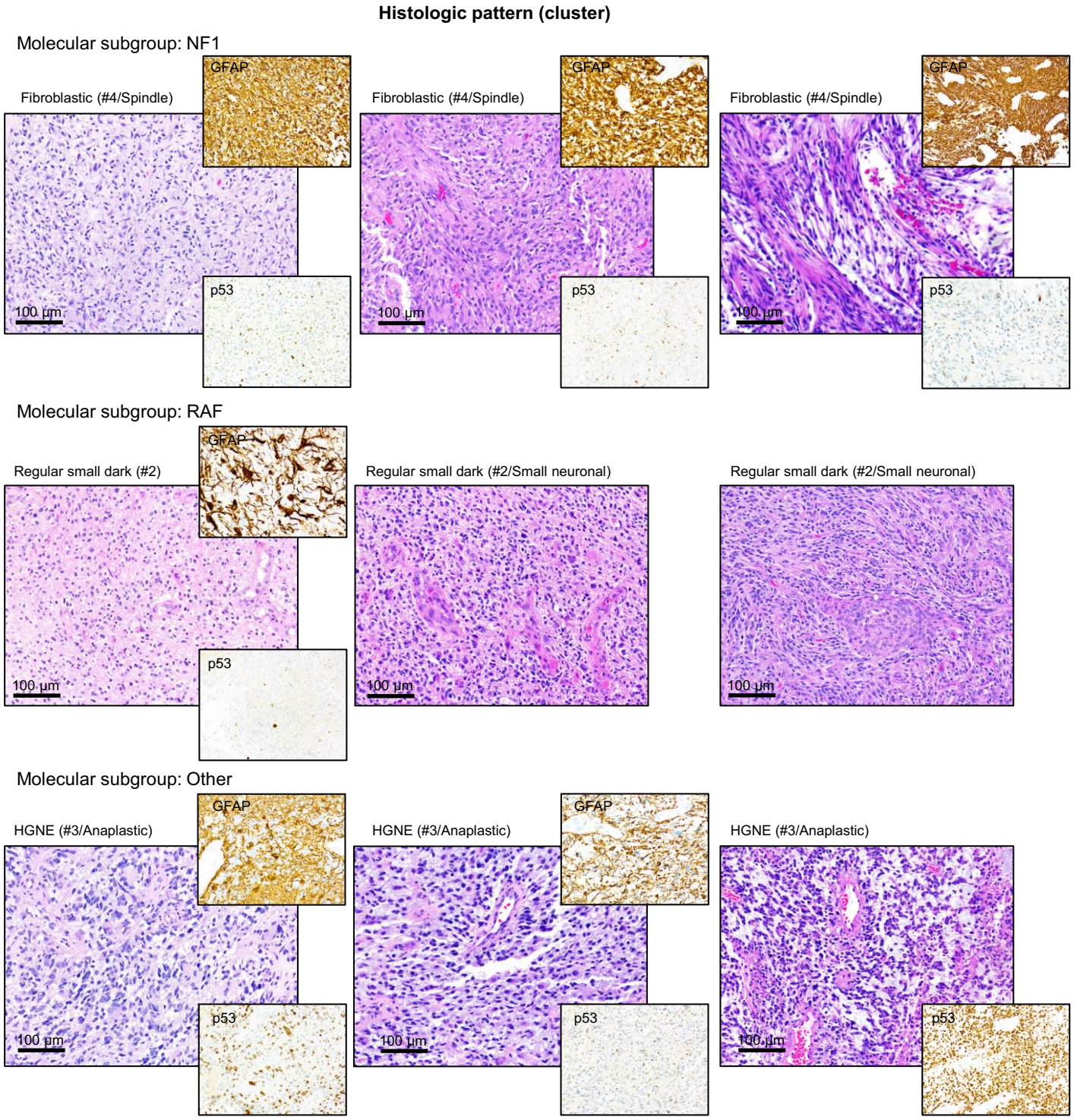
| | EGFR↑ | EGFRm | PDGFRA | Multi-RTK | FGFR3 | NF1 | RAF | Other | IDH |
|---|---|---|---|---|---|---|---|---|---|
| **EGFR↑** | 1.00 | 0.36 | 0.62 | 0.79 | 0.79 | 0.89 | 0.88 | 0.68 | 0.38 |
| **EGFRm** | 0.36 | 1.00 | 0.35 | 0.62 | 0.30 | 0.40 | 0.34 | 0.62 | 0.26 |
| **PDGFRA** | 0.62 | 0.35 | 1.00 | 0.51 | 0.54 | 0.56 | 0.49 | 0.50 | 0.40 |
| **Multi-RTK** | 0.79 | 0.62 | 0.51 | 1.00 | 0.79 | 0.80 | 0.71 | 0.88 | 0.45 |
| **FGFR3** | 0.79 | 0.30 | 0.54 | 0.79 | 1.00 | 0.69 | 0.62 | 0.65 | 0.16 |
| **NF1** | 0.89 | 0.40 | 0.56 | 0.80 | 0.69 | 1.00 | 0.87 | 0.72 | 0.40 |
| **RAF** | 0.88 | 0.34 | 0.49 | 0.71 | 0.62 | 0.87 | 1.00 | 0.63 | 0.22 |
| **Other** | 0.68 | 0.62 | 0.50 | 0.88 | 0.65 | 0.72 | 0.63 | 1.00 | 0.34 |
| **IDH** | 0.38 | 0.26 | 0.40 | 0.45 | 0.16 | 0.40 | 0.22 | 0.34 | 1.00 |

**Supplemental Figure S4. Glioblastoma subgroups correlation matrix.** Pearson correlation coefficient between each pair of subgroups is shown. Closest correlations are indicated with white labeling on blue background. Note unique profiles for the IDH, EGFRm and PDGFRA subgroups.




**Supplemental Figure S5. <u>Characteristic nuclear morphology of the glioblastoma histologic patterns</u>.** The nuclear morphology is detailed for the representative histologic patterns seen in the IDH-wild-type glioblastoma molecular subgroups, as labeled on top of the figure. H&E images were acquired at 40x magnification, and all panels and all insets are shown at the same magnification, respectively. Note characteristic small nuclei with open vesicular chromatin in EGFR-like cluster#1 tumors, small, regular nuclei with finely stippled or “salt-and-pepper” chromatin in Small neuronal-like cluster#2 tumors, hyperchromatic, irregular nuclei in Anaplastic cluster#3 tumors, spindle nuclei in Spindle cluster#4 tumors, and centrally-placed nuclei with conspicuous nucleolus in the epithelioid pattern of Epithelioid cluster#5 tumors.


Molecular subgroup: EGFR↑
Histologic pattern (cluster)
EGFR (#1/EGFR)
GFAP
p53
100 µm
EGFR (#1/EGFR)
GFAP
p53
100 µm
EGFR/FGFR (#1/EGFR)
GFAP
p53
100 µm
Molecular subgroup: FGFR3
FGFR small (#2/Small neuronal)
GFAP
100 µm
FGFR small (#2/Small neuronal)
GFAP
p53
100 µm
EGFR/FGFR (#1/EGFR)
GFAP
p53
100 µm
Molecular subgroup: PDGFRA
HGNE (#3/Anaplastic)
GFAP
p53
100 µm
HGNE (#3/Anaplastic)
GFAP
p53
100 µm
Pre-HGNE (#3/Anaplastic)
GFAP
p53
100 µm
Molecular subgroup: Multi-RTK
Pre-HGNE (#3/Anaplastic)
GFAP
p53
100 µm
Giant cell (#5/Epithelioid)
GFAP
p53
100 µm
Gliosarcoma (#4/Spindle)
GFAP
Reticulin
0.5 mm




**Supplemental Figure S6. <u>Correspondence between histologic patterns and molecular subgroups in IDH-wild-type glioblastoma</u>.** This figure is displayed on two pages and shows three examples of representative histologic patterns for each of the IDH-wild-type glioblastoma molecular subgroups. H&E images were acquired at 20x magnification, as described in Materials and Methods. Corresponding IHCs for GFAP and p53 are also shown in insets. The images show the characteristic morphology of tumors from 21 different patients.

**Supplemental Table S1. RTK-MAPK pathway genetic alterations**

Colored font: recurrent mutations
Bold font: di-sulfide bond mutations
Fold overexpression from whole transcriptomics
* Unless otherwise mentioned, the mutations are clonal

| Case | Gene | Amplif | Fold | Mutation/fusion | Amino aci | Effect | Associations* | NM |
|---|---|---|---|---|---|---|---|---|
| EGFR#1 | EGFR | Yes | 72 | | | | | _005228 |
| EGFR#2 | EGFR | Yes | 56 | Exons 2-7 | | Splice | vIII | _005228 |
| EGFR#3 | EGFR | Yes | 46 | c.1951G>A | V651M | Missense | w/ amplificatior | _005228 |
| EGFR#4 | EGFR | Yes | 43 | **c.1907G>T** | **C636F** | Missense | w/ amplificatior | _005228 |
| | EGFR | Yes | | EGFR-SEPT14 | | In-frame | w/ amplificatior | _005228 |
| EGFR#5 | EGFR | Yes | 42 | | | | | _005228 |
| EGFR#6 | EGFR | Yes | 37 | c.866C>T | A289V | Missense | | _005228 |
| | EGFR | Yes | | Exons 25-27 | | Splice | vIVa | _005228 |
| EGFR#7 | EGFR | Yes | 34 | c.323G>A | R108K | Missense | w/ amplificatior | _005228 |
| EGFR#8 | EGFR | Yes | 30 | EGFR-SEPT14 | | In-frame | w/ amplificatior | _005228 |
| | EPHB1 | No | | c.2138A>G | D713G | Missense | | _004441 |
| EGFR#9 | EGFR | Yes | 29 | Exons 2-7 | | Splice | vIII | _005228 |
| EGFR#10 | EGFR | Yes | 26 | | | | | _005228 |
| EGFR#11 | EGFR | Yes | 26 | | | | | _005228 |
| EGFR#12 | EGFR | Yes | 25 | | | | | _005228 |
| EGFR#13 | EGFR | Yes | 25 | EGFR-SEPT14 | | In-frame | w/ amplificatior | _005228 |
| EGFR#14 | EGFR | Yes | 24 | Exons 2-7 | | Splice | vIII | _005228 |
| | EGFR | Yes | | EGFR-VOPP1 | | In-frame | w/ amplificatior | _005228 |
| EGFR#15 | EGFR | Yes | 18 | Exons 25-27 | | Splice | vIVa | _005228 |
| | ALK | No | | c.3402G>C | Q1134H | Missense | | _004304 |
| EGFR#16 | EGFR | Yes | 13 | Exons 2-7 | | Splice | vIII | _005228 |
| | ERRFI1 | | | CN loss | | | Homozygous | |
| | ALK | No | | c.502T>C | F168L | Missense | Likely germline | _004304 |
| EGFR#17 | EGFR | Yes | 12 | c.866C>T | A289V | Missense | | _005228 |
| | EGFR | Yes | | Exons 25-28 | | Deletion | C-terminal | _005228 |
| | ALK | No | | c.3587T>A | L1196Q | Missense | | _004304 |
| EGFR#18 | EGFR | Yes | 10 | **c.884G>A** | **C295Y** | Missense | | _005228 |
| EGFR#19 | EGFR | Yes | 9.4 | Exons 2-7 | | Splice | vIII | _005228 |
| EGFR#20 | EGFR | Yes | 7.8 | Exons 2-7 | | Splice | vIII | _005228 |
| | EGFR | Yes | | CTDSP2-EGFR | | In-frame | Fusion | _005228 |
| EGFR#21 | EGFR | Yes | 5.8 | SEC61G-EGFR | | In-frame | Fusion | _005228 |
| EGFR#22 | EGFR | Yes | 4.5 | EGFR-SEPT14 | | In-frame | w/ amplificatior | _005228 |
| EGFR#23 | EGFR | Yes | 1.4 | | | | | _005228 |
| EGFR#24 | EGFR | Yes | | c.1793G>T | G598V | Missense | w/ amplificatior | _005228 |
| EGFR#25 | EGFR | Yes | | | | | | _005228 |
| EGFR#26 | EGFR | Yes | | c.866C>T | A289V | Missense | | _005228 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| EGFR#27 | EGFR | Yes | | c.323G>A | R108K | Missense | w/ amplificatio | _005228 |
| EGFR#28 | EGFR | Yes | | **c.976T>C** | **C326R** | Missense | w/ amplificatio | _005228 |
| EGFR#29 | EGFR | Yes | | c.787A>C | T263P | Missense | w/ amplificatio | _005228 |
| | EGFR | Yes | | c.1793G>T | G598V | Missense | | _005228 |
| EGFR#30 | EGFR | Yes | | **c.932G>A** | **C311Y** | Missense | w/ amplificatio | _005228 |
| EGFR#31 | EGFR | Yes | | | | | | _005228 |
| EGFR#32 | EGFR | Yes | | Exon 20 | | Insertion | | _005228 |
| EGFR#33 | EGFR | Yes | | c.1088C>T | T363I | Missense | w/ amplificatio | _005228 |
| EGFR#34 | EGFR | No | 1.5 | c.874G>T | V292L | Missense | | _005228 |
| | PDGFRA | No | | c.2930T>G | V977G | Missense | Germline | _006206 |
| EGFR#35 | EGFR | No | 4.3 | **c.1773C>G** | **C591W** | Missense | | _005228 |
| EGFR#36 | EGFR | No | 2.8 | | P596L | Missense | | _005228 |
| | EGFR | No | | c.1280G>A | R427H | Missense | | _005228 |
| | EPHA8 | No | | c.181G>A | D61N | Missense | | _020526 |
| | | | | | | | | |
| PDGFRA#1 | PDGFRA | Yes | 25 | **c.704G>A** | **C235Y** | Missense | | _006206 |
| | KIT | Yes | 5.4 | | | | | |
| | KDR | Yes | 4.9 | | | | | _002253 |
| PDGFRA#2 | PDGFRA | Yes | 22 | c.1607T>A | V536E | Missense | | _006206 |
| | EPHB2 | No | 14 | c.2375G>A | W792* | Nonsense | Germline | _001309193 |
| PDGFRA#3 | PDGFRA | Yes | 15 | **c.704G>T** | **C235F** | Missense | w/ amplificatio | _006206 |
| PDGFRA#4 | PDGFRA | Yes | 15 | | | | | _006206 |
| | KIT | Yes | 21 | | | | | |
| | KDR | Yes | 10 | | | | | _002253 |
| | MET | No | 27 | PTPRZ1-MET | | In-frame | Fusion | _000245 |
| PDGFRA#5 | PDGFRA | No | 9.8 | c.925G>C | V309L | Missense | w/ overexpres | _006206 |
| PDGFRA#6 | PDGFRA | Yes | 4.9 | | | | | _006206 |
| | KIT | Yes | 7 | | | | | |
| | KDR | Yes | 10 | | | | | _002253 |
| | LZTR1 | | | c.321-8_324del | R107fs | Frameshift | | _006767 |
| | ARAF | No | | c.83A>C | K28T | Missense | | _001654 |
| PDGFRA#7 | PDGFRA | Yes | 26 | c.932-1_934delGAGA | K312del | Splice | w/ overexpres | _006206 |
| | H3F3A | No | | | G34R | Missense | | _002107 |
| | IDH2 | No | | | G190S | Missense | Likely germline | _02168 |
| | | | | | | | | |
| Multi-RTK#1 | PDGFRA | Yes | 1.8 | | | | | _006206 |
| | KIT | No | 13 | | | | | |
| | FGFR2 | No | | c.1501G>A | A501T | Missense | Subclonal | _022970 |
| | NRAS | No | | c.181C>A | Q61K | Missense | Subclonal | _002524 |
| Multi-RTK#2 | PDGFRA | Yes | 1.5 | | | | | _006206 |
| | KIT | Yes | 1.4 | | | | | |
| | MET | Yes | 39 | | | | | _000245 |
| | EGFR | No | 9.6 | | | | | _005228 |
| Multi-RTK#3 | PDGFRA | Yes | 1.6 | | | | | _006206 |
| | EGFR | No | 6.2 | | | | | _005228 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Multi-RTK#4 | EPHA8 | No | | c.593G>A | R198H | Missense | | _020526 |
| | EPHB1 | No | | c.1817C>T | A606V | Missense | | _004441 |
| | MET | No | 28 | | | | | _000245 |
| | MSH6 | No | | c.2615_2616dup | G873fs | Frameshift | Germline | _000179 |
| Multi-RTK#5 | MET | Yes | very high | | | | | _000245 |
| Multi-RTK#6 | ERRFI1 | | | CN loss | | | Homozygous | |
| | FGFR2 | No | | c.252G>T | R84S | Missense | Likely germline | _022970 |
| | PTPN11 | | | c.181G>A | D61N | Missense | Subclonal | _002834 |
| | LZTR1 | | | CN loss | | | Homozygous | _006767 |
| Multi-RTK#7 | NTRK1 | No | | LMNA-NTRK1 | | | | |
| | FGFR3 | No | | C.505G>A | A169T | Missense | Subclonal | _000142 |
| | | | | | | | | |
| FGFR#1 | FGFR3 | No | | FGFR3-TACC3 | | In-frame | Fusion | _000142 |
| | FRS2 | Yes | 44 | | | | | |
| FGFR#2 | FGFR3 | No | | FGFR3-TACC3 | | In-frame | Fusion | _000142 |
| FGFR#3 | FGFR3 | No | | FGFR3-TLN1 | | In-frame | Fusion | _000142 |
| FGFR#4 | FGFR3 | No | | c.2274+26_2346dup | S782ins(7 | Insertion | C-terminal | _000142 |
| | FRS2 | Yes | | | | | | |
| FGFR#5 | FGFR3 | No | | c.1672G>A | A558T | Missense | | _000142 |
| | | | | | | | | |
| NF1#1 | NF1 | | | c.5977C>T | Q1993* | Nonsense | LOH | _001042492 |
| | PDGFRA | No | | c.853A>G | S285G | Missense | Germline | _006206 |
| NF1#2 | NF1 | | | c.4790G>T | G1597V | Missense | Germline; LOH | _001042492 |
| NF1#3 | NF1 | | | c.8033T>G | L2678* | Nonsense | LOH | _001042492 |
| NF1#4 | NF1 | | | c.499_502delTGTT | C167fs | Frameshift | LOH | _001042492 |
| NF1#5 | NF1 | | | c.7549C>T | R2517* | Nonsense | LOH | _001042492 |
| | PTPN11 | | | c.215C>T | A72V | Missense | Subclonal | _002834 |
| | PTPN11 | | | c.226G>C | E76Q | Missense | Subclonal | _002834 |
| NF1#6 | NF1 | | | c.5797G>T | G1933* | Nonsense | | _001042492 |
| | NF1 | | | c.6390dup | H2131fs | Frameshift | | _001042492 |
| NF1#7 | NF1 | | | c.6852_6855del | Y2285fs | Frameshift | LOH | _001042492 |
| | PDGFRA | No | | c.3149T>C | I1050T | Missense | Likely germline | _006206 |
| NF1#8 | NF1 | | | c.7897G>T | E2633* | Nonsense | | _001042492 |
| | NF1 | | | NF1-NUDT12 | S1935fs | Frameshift | Fusion | _001042492 |
| | PTPN11 | | | c.1520C>A | T507K | Missense | | _002834 |
| NF1#9 | NF1 | | | c.5032_5034dupTAT | Y1678du | Insertion | Germline | _001042492 |
| | NF1 | | | c.1020dupT | V341fs | Frameshift | | _001042492 |
| | PTPN11 | | | c.205G>A | E69K | Missense | Subclonal | _002834 |
| | LZTR1 | | | c.321-8_324del | R107fs | Frameshift | LOH | _006767 |
| | FRS2 | Yes | 3.7 | | | | | |
| NF1#10 | NF1 | | | c.6007-1G>A | | Splice | | _001042492 |
| | NF1 | | | c.1381C>T | R461* | Nonsense | | _001042492 |
| | ERBB4 | No | | c.2539C>T | R847C | Missense | Likely germline | _005235 |
| NF1#11 | NF1 | | | c.4600C>T | R1534* | Nonsense | | _001042492 |
| | NF1 | | | c.4625T>C | L1542P | Missense | | _001042492 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| NF1#12 | NF1 | | NF1-chr2p15 | G1597fs | Frameshift | Fusion | _001042492 |
| NF1#13 | NF1 | | c.4267G>T | E1423* | Nonsense | | _001042492 |
| NF1#14 | NF1 | | c.479G>A | R160K | Splice | LOH | _001042492 |
| NF1#15 | NF1 | | c.6772C>T | R2258* | Nonsense | LOH | _001042492 |
| | PTPN11 | | c.417G>C | E139D | Missense | | _002834 |
| | | | | | | | |
| RAF#1 | BRAF | No | c.1799T>A | V600E | Missense | | _004333 |
| RAF#2 | BRAF | No | c.1780G>A | D594N | Missense | | _004333 |
| RAF#3 | BRAF | No | c.1447A>G | K483E | Missense | | _004333 |
| RAF#4 | RAF1 | Yes | c.770C>T | S257L | Missense | w/ overexpress | _002880 |
| | | | | | | | |
| IDH#1 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| | FGFR2 | Yes | 68 | | | | _022970 |
| IDH#2 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| IDH#3 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| IDH#4 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| IDH#5 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| IDH#6 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| IDH#7 | IDH1 | | c.395G>A | R132H | Missense | | _001282387 |
| IDH#8 | IDH1 | | | R132H | Missense | | _001282387 |

**Supplemental Table S2.** Human RTK classes and genes.

| Class | Family name | Members (proteins) | Members (genes) |
|---|---|---|---|
| I | EGFR | EGFR, ERBB2-4 | Same |
| II | Insulin receptor | INSR, IGFR | INSR, IGF1R |
| III | PDGFR | PDGFRα, PDGFRβ, KIT, M-CSFR, FLT3 | PDGFRA, PDGFRB, KIT, CSF1R, FLT3 |
| IV | VEGFR | VEGFR1-3 | FLT1, KDR, FLT4 |
| V | FGFR | FGFR1-4 | Same |
| VI | CCK | CCK4 | PTK7 (inactive; WNT pathway) |
| VII | NGFR | TRKA-C | NTRK1-3 |
| VIII | HGFR | MET, RON | MET, MST1R |
| IX | EPHR | EPHA1-8, EPHA10, EPHB1-4, EPHB6 | Same |
| X | AXL | AXL, MER, TYRO3 | AXL, MERTK, TYRO3 |
| XI | TIE | TIE, TEK | TIE1, TEK |
| XII | RYK | RYK | Same |
| XIII | DDR | DDR1-2 | Same |
| XIV | RET | RET | Same |
| XV | ROS | ROS | ROS1 |
| XVI | LTK | LTK, ALK | Same |
| XVII | ROR | ROR1-2 | Same; (pseudokinases; WNT pathway) |
| XVIII | MUSK | MUSK | Same |
| XIX | LMR | AATYK1-3 | AATK, LMTK2, LMTK3 |

EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; CCK, colon carcinoma kinase; NGFR, nerve growth factor receptor; HGFR, hepatocyte growth factor receptor; EPHR, ephrin receptor; AXL, from the Greek word anex-elekto, or uncontrolled; TIE, tyrosine kinase receptor in endothelial cells; RYK, receptor related to tyrosine kinases; DDR, discoidin domain receptor; RET, rearranged during transfection; ROS, RTK expressed in some epithelial cell types; LTK, leukocyte tyrosine kinase; ROR, receptor orphan; MUSK, muscle-specific kinase; LMR, Lemur.