SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Discordant pairs from whole-genome sequencing indicating the presence of multiple distinct *EGFR* rearrangements co-existing in individual TCGA samples

(A) Sample TCGA-06-0214 exhibited at least four distinct rearrangements at the *EGFR* locus, three of which would result in the vIII variant (ex. 2-7 deletion) and one would lead to the C-terminal truncation (ex. 25-28 deletion). (B) Sample TCGA-06-5415 exhibited two distinct deletions both resulting in EGFRvIII. (C) Sample TCGA-14-2554 harbored at least three distinct deletions that would result in EGFRvIII and one that would result in an ex. 13-15 deletion. (D) Sample TCGA-26-5132 contained at least three distinct deletions that would result in EGFRvIII. Both vIII and C-terminal truncations were also validated and detected in RNA-Seq of each sample; the ex. 13-15 deletion was not detected by RNA-Seq.

Supplementary Figure 2. Evaluation of the quality of whole-genome amplifications in SNS libraries

(A) Cumulative K-S metric (a lower number represents a more uniform genome coverage) for 50 single MDA libraries derived from BT340. (Two nuclei were removed from analysis due to detected contamination). (B) Comparison between the pileup metric and the cumulative metric. (C) Cumulative K-S metric for 60 single MDA libraries derived from BT325.

Supplementary Figure 3. *EGFR* amplification and rearrangements in BT325 and BT340

Integrated genome viewer (IGV) screenshot showing the 5' and 3' ends of the *EGFR* amplicon from the bulk tumor sequencing of BT325 (A) and BT340 (B). (C) IGV screenshot showing two distinct deletions observed in bulk BT340 tumor. (D) Fluorescence *in situ* hybridization (FISH) for total *EGFR* and the chromosome 7 centromere (CEP7) confirming varying total *EGFR* copy number levels in BT340.

Supplementary Figure 4. Clonality analysis of BT325

(A) Bulk average somatic copy-number profile derived from whole-genome sequencing of GBM BT325. (B) Clonality analysis suggested subclonal deletions in chromosomes 1, 4, and 6.

Supplementary Figure 5. Detection of loss-of-heterozygosity in single nuclei by haplotype clustering

(A) Heterozygous variants in the germline serve as markers for segregating two haplotypes in a diploid individual. (B) Single-cell genotypes derived from sequencing of whole-genome amplified libraries are incomplete representations of the true genotype. (C) Tumor nuclei with loss-of-heterozygosity share the same retained haplotype and can be distinguished from nuclei with heterozygous

genotypes. (D) Hierarchical clustering of 60 SNS libraries from BT325 based on the genotypes in chromosome 10. (E) Inference of the retained "R" haplotype and the deleted "D" haplotype from combined sequencing of all LOH cells. (F) Haplotype compositions in the two clusters derived in (D) at germline heterozygous sites in a region containing *PTEN* (Chr10:87-90Mb). (G) Ratio between the number of sites showing the deleted "D" haplotype and those of the retained "R" haplotype within each cell. Two SNS in cluster 2 with ratio ~1 are diploid (complete heterozygosity); two libraries with ratio ~0.5 are probably derived from a mixture of DNA from a tumor nucleus and a normal nucleus. See Supplementary Fig. 7 for more details. (H) Estimates of single-base errors introduced by MDA based on the discordant sites in LOH nuclei in comparison to the "R" haplotype.

Supplementary Figure 6. Constructing the clonal hierarchy of 48 single nuclei derived from BT340

(A) The final clonal hierarchy was constructed from interrogation of both clonal and subclonal SCNA events in each of 48 single-nuclei. (B) and (C) Clonal events including hemizygous deletion of chromosome 10 and amplification of chromosome 7 confirmed the identity of 44 nuclei as tumor derived. (D) Subclonal populations were segregated by deletions in 6q and 9p arms that only existed in the major subclone (35 out of 44 nuclei). The minor subclone are heterozygous in the regions of deletion in chromosome 6 and homozygous in the regions of deletion in chromosome 9; the major subclone are homozygous in the regions of deletion in chromosome 6 and homozygously deleted in the regions of subclonal deletion in chromosome 9. The haplotype ratio between the deleted and the retained haplotype in regions of subclonal deletion in chromosome 6 showed that the minor population had both alleles. Due to the relatively small number of germline SNVs in these regions, the ratio was not always close to one. (E) Loss-of-heterozygosity in Chr. 16 occurred in a sub-population of nuclei as inferred from their shared genotype (bottom 7 nuclei). Single-nuclei sequencing clearly indicates that these 7 nuclei were derived from the 35 nuclei that had both deletions in Chr. 6q and 9p.

Supplementary Figure 7. Clonal alterations in BT325

(A) Haplotype clustering of LOH in Chr. 9p. The homozygous cluster (56/60) contained tumor nuclei with LOH in this region (9-35Mb). The heterozygous cluster contained 4 samples with heterozygous haplotypes. (B) The haplotype ratio (deleted vs. retained) in chromosome 10 confirmed the LOH status of the tumor cluster and suggested two samples as 1:1 mixture of tumor and normal nuclei (gray filled circles). (C) The chromosome 7 copy ratio confirmed that most tumor nuclei had trisomy 7 but the normal nuclei were disomic. Discordant read pairs spanning the EGFR amplicon (F) and the translocation between chromosomes 9 and 3 (E) were detected in both tumor/normal mixture samples despite their heterozygosity in Chr. 9 and Chr. 10. (F) (Upper panel) Schematic drawing of the complex rearrangement involving chromosomes 1, 3, 8 and 9 producing loss of *CDKN2A/CDKN2B*. (Lower panel) Sequence coverage plots

near the *CDKN2A* locus from the bulk library, from a representative single-nucleus library, and from the pool of all tumor nuclei. Despite amplification bias, single-nuclei libraries showed no background in the deleted regions.

Supplementary Figure 8. Complex rearrangements in BT340
(A) Complex rearrangement involving chromosomes 2 and 8 near the telomeric regions. (B) Complex rearrangement event within chromosome 12. (C) Complex rearrangement event within chromosome 14. Two boundaries could not be determined due to frequent templated insertions near the breakpoints. (D) Two deletion events hitting the *CDKN2A/CDKN2B* locus. The chromothripsis event between chromosome 6 and chromosome 9 not only resulted in the deletion of *CDKN2A* but also generated a circular extrachromosomal amplicon. IGV screenshots showed adjacent breakpoints in Chr. 6 and Chr. 9 that were separately translocated to the amplicon and to the derivative chromosome. A subsequent deletion removed some rearranged segments in the derivative chromosome and created the major subclone in the tumor. (E) Fluorescence *in situ* hybridization using red probes for Chr. 6 and green for Chr. 9 on FFPE tissue highlighted the highly heterogeneous amplifications of the extrachromosomal amplicon where bulk average suggested ~22 copies per cell. Sites of FISH probes are indicated in (D). The boundaries of rearranged segments and genes within the amplicon are listed in Supplementary Table S2.

Supplementary Figure 9. Interrogation of clonal alterations in single nuclei derived from BT325
(A) Sub-clonal deletions (as reflected by incomplete SNV allelic frequency segregation) and copy numbers in chromosomes 1, 4, and 6. (B) Hierarchical clustering of 56 tumor nuclei from BT325 based on the haplotypes in regions with sub-clonal LOH showed three clusters: Samples in red have complete LOH in all the segments; samples in blue are heterozygous in these regions. (C) Samples in blue have a D:R ratio ~1, suggesting that they are diploid; samples in purple are heterozygous but their D:R ratio is well below 1, suggesting that they are likely a mixture of two cells from both two populations. (D) The allele frequency plot for the putative sub-clonal population showed complete SNV segregation. (E) Rearrangement signatures confirmed that the sub-clonal deletions resulted from a chromothripsis event. (F) Hierarchical clustering of 60 nuclei from BT325 generated from haplotyping analysis of clonal and subclonal deletions. (See Supplementary Fig. 7 for the determination of clonal events.) Open circles are nuclei that are heterozygous and contain diploid chromosome 10. Gray circles are nuclei that are heterozygous in chromosome 10 but exhibit ~1:2 ratio between the deleted and the retained haplotype, consistent with a 1:1 mixture of tumor (LOH) and normal (diploid) nuclei. Fifty-six nuclei with complete LOH in chromosome 10 are further separated into two distinct clusters based on the haplotype in subclonally deleted regions of Chrs. 1, 4, and 6. Red circles are nuclei that have LOH in all these regions and blue circles are heterozygous in these regions with diploid genotype; purple circles are likely to be a mixture of

two cells from both populations based on the ratio between the deleted and the retained haplotype.

Supplementary Figure 10. EGFRvII deletion mutant expressing Ba/F3 cells are sensitive to EGFR inhibitors.
(A-D) Drug sensitivity analysis to assess cell survival after 72-hour treatment with EGFR small molecule inhibitors erlotinib (A), lapatinib (B), afatinib (C), and neratinib (G). The relative IC50 values are listed below.

Supplementary Table Legends:

Table S1: *EGFR* alterations in TCGA samples with matching RNA and DNA sequencing

Table S2: Genes identified in the chromosome 6-9 amplicon in BT340

Table S3: BT325 Segmented copy number data

Table S4: BT340 Segmented copy number data

Table S5: BT340 copy number aberrations

Table S6: BT340 chromosomal rearrangements

Table S7: BT325 copy number aberrations and chromosomal rearrangements

Table S8: Somatic SNVs and indels in BT325 detected from bulk whole-exome sequencing

Table S9: Somatic SNVs and indels in BT340 detected from bulk whole-exome sequencing