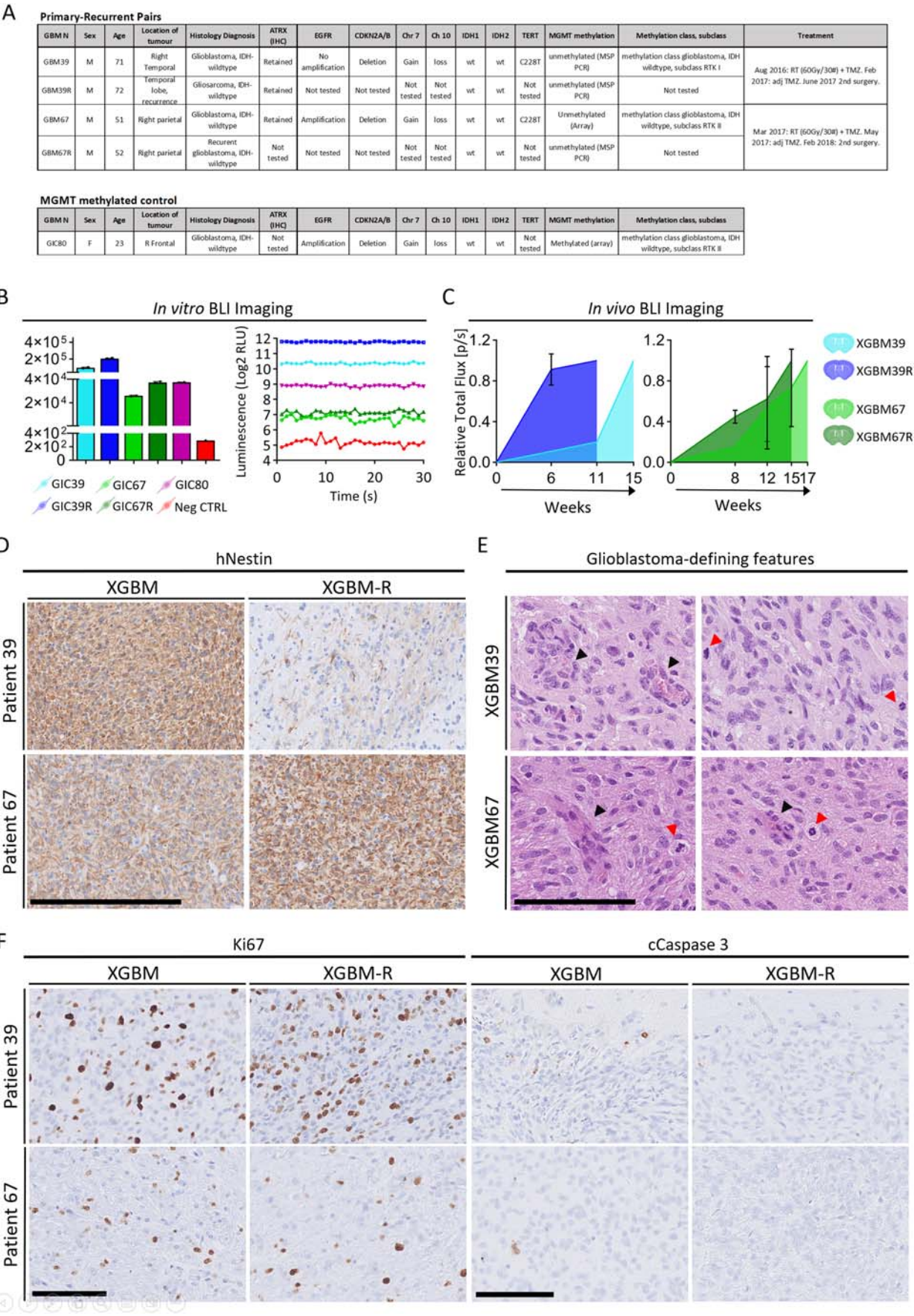
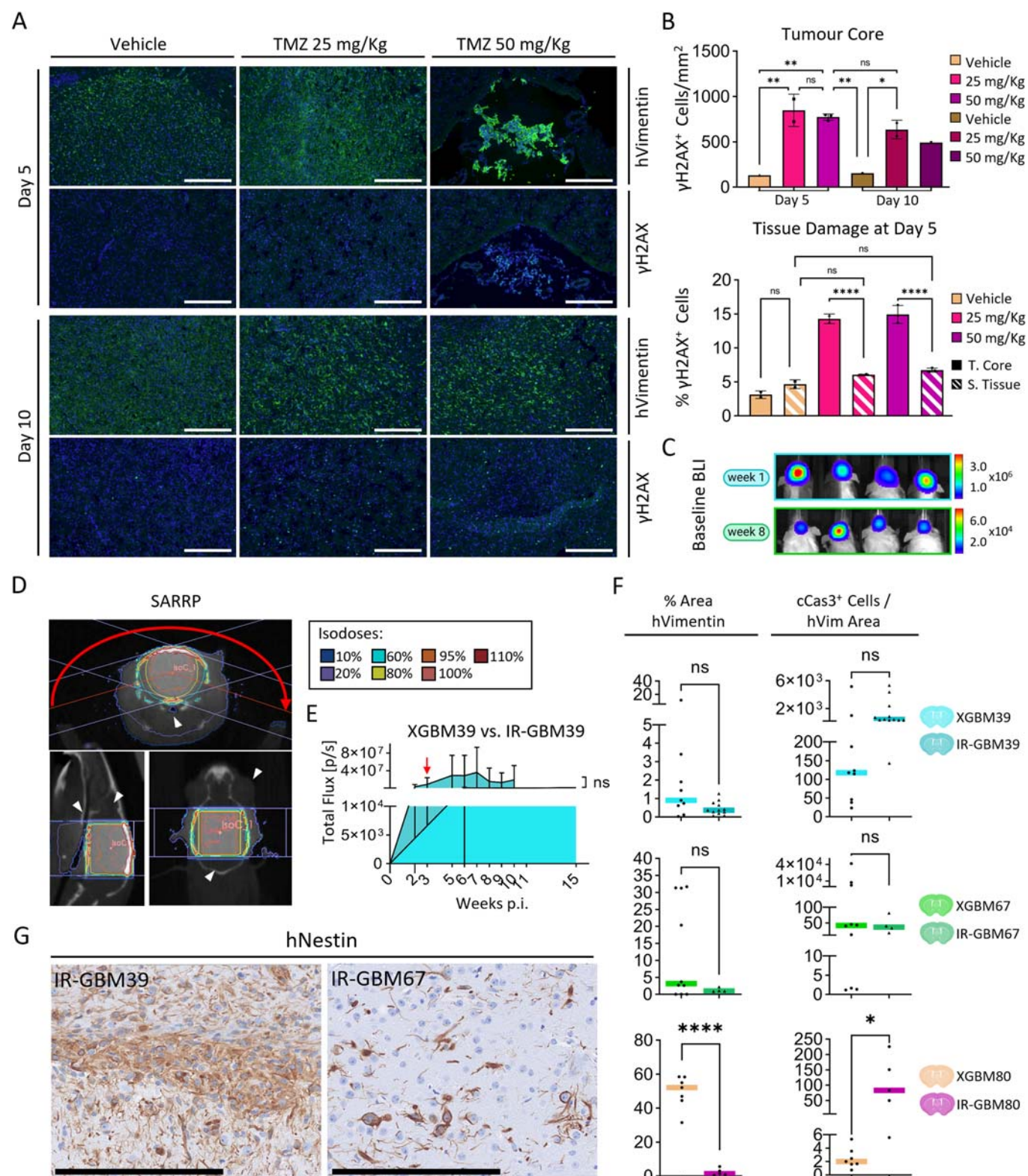


Expanded View Figures

Figure EV1. Histological characterization of PDX models.

(A) Summary table of clinical and histo-molecular features of glioblastoma patients. (B) In vitro assessment of successful integration of F-Luciferase reporter genes in each GIC line used for this study. Left: quantification of Luminescence counts after imaging the cells with IVIS and measuring the ROIs ($n = 2$ for all lines; $n = 1$ for negative control). Error bars represent Mean $\pm$ SD. Right: 30 s kinetic measurement of firefly luminescence signal with plate reader ($n = 1$, with each line being measured with and without luciferin addition. Neg CTRL is represented by the average of all GIC without luciferin addition). (C) Curves showing the average tumour growth, measured via BLI, for all PDX derived from the injection of longitudinal primary and recurrent GICs: on the left both GBM39 and GBM39R ($n = 2$ and $n = 3$, respectively), while on the right GBM67 and GBM67R ($n = 3$). Error bars represent Mean $\pm$ SD. Each mouse's signal was normalized to the highest signal measured within the same animal over time. (D) Representative ICH staining for hNestin of each untreated PDX model generated in this study. Patient 39 both primary and recurrent tumours at the top and patient 67 at the bottom. Scale bars are 250 μm . (E) Representative H&E of both primary untreated PDX models generated in this study highlighting typical malignant features of glioblastoma (XGBM39 at the top and XGBM67 at the bottom). Black arrows indicate vascular proliferation; red arrows indicate mitosis. All images show spread nuclear atypia and high cellularity. Scale bars are 100 μm . (F) Representative ICH staining for KI67 (left) and cCaspase3 (right) of each untreated PDX model generated in this study. Patient 39 both primary and recurrent tumours at the top and patient 67 at the bottom. Scale bars are 100 μm .





◀ **Figure EV2. Optimization of PDX model of induced recurrence.**

(A) TMZ tolerability assay: hVimentin and γ H2AX IF stainings of brain sections for each time point and each administered dose. Scale bars are 250 μ m. (B) Quantification of DNA damage at timepoints and doses of TMZ, tumour core and surrounding tissue are shown. Top: DNA damage quantification within the tumour core for each treatment group after 5 and 10 days of continuous administration. $n = 1$ for both vehicles, and Day10 50 mg/kg; $n = 2$ for both Day5 and Day10 25 mg/kg; $n = 3$ for Day5 50 mg/kg. Bottom: comparison of DNA damage between the tumour core and the surrounding tissue as an indication of the toxicity to non-neoplastic cells. $n = 2$ for both vehicles and both 25 mg/kg; $n = 3$ for both 50 mg/kg. One-way ANOVA. $*p = 0.0202$; [25 mg/kg vs. D5 Vehicle]: $**p = 0.0052$; [50 mg/kg vs. D5 Vehicle]: $**p = 0.0062$; [50 mg/kg vs. D10 Vehicle]: $**p = 0.0070$. $****p < 0.0001$. Error bars represent Mean $\pm$ SD. (C) IVIS BLI of mice at the chosen timepoint of start of treatment. Each mouse showed a signal at 8 or 1 weeks post injection, respectively, for patients 39 (top) and 67 (bottom). (D) Radiation tolerability assay: CT image showing the isodoses of radiation received by the targeted tissue in one representative mouse. White arrows show successful avoidance of radiation-sensitive tissues. (E) Comparison of tumour growth curves, measured with BLI, between untreated primary PDXs and treated IR-PDXs derived by the injection of primary GIC39. The red arrow indicates a first scan performed after the sham injection and before radiation and TMZ administration. Error bars represent Mean $\pm$ SD. (F) Column scatter plots showing the quantification of immunohistochemical staining for hVimentin (%area of brain section surface which is positive for hVim; left) and cCaspase3 (right) in XGBM compared to IR-GBM. $n = 9$ for XGBM39, $n = 12$ for IR-GBM39, $n = 10$ for XGBM67, $n = 4$ for IR-GBM67, $n = 7$ for XGBM80, $n = 5$ for IR-GBM80. Unpaired t-test. $****p < 0.0001$; $*p = 0.0106$. (G) Representative ICH staining for hNestin of both IR-PDX models generated in this study. Patient 39 on the left, patient 67 on the right. Scale bars are 250 μ m. Source data are available online for this figure.

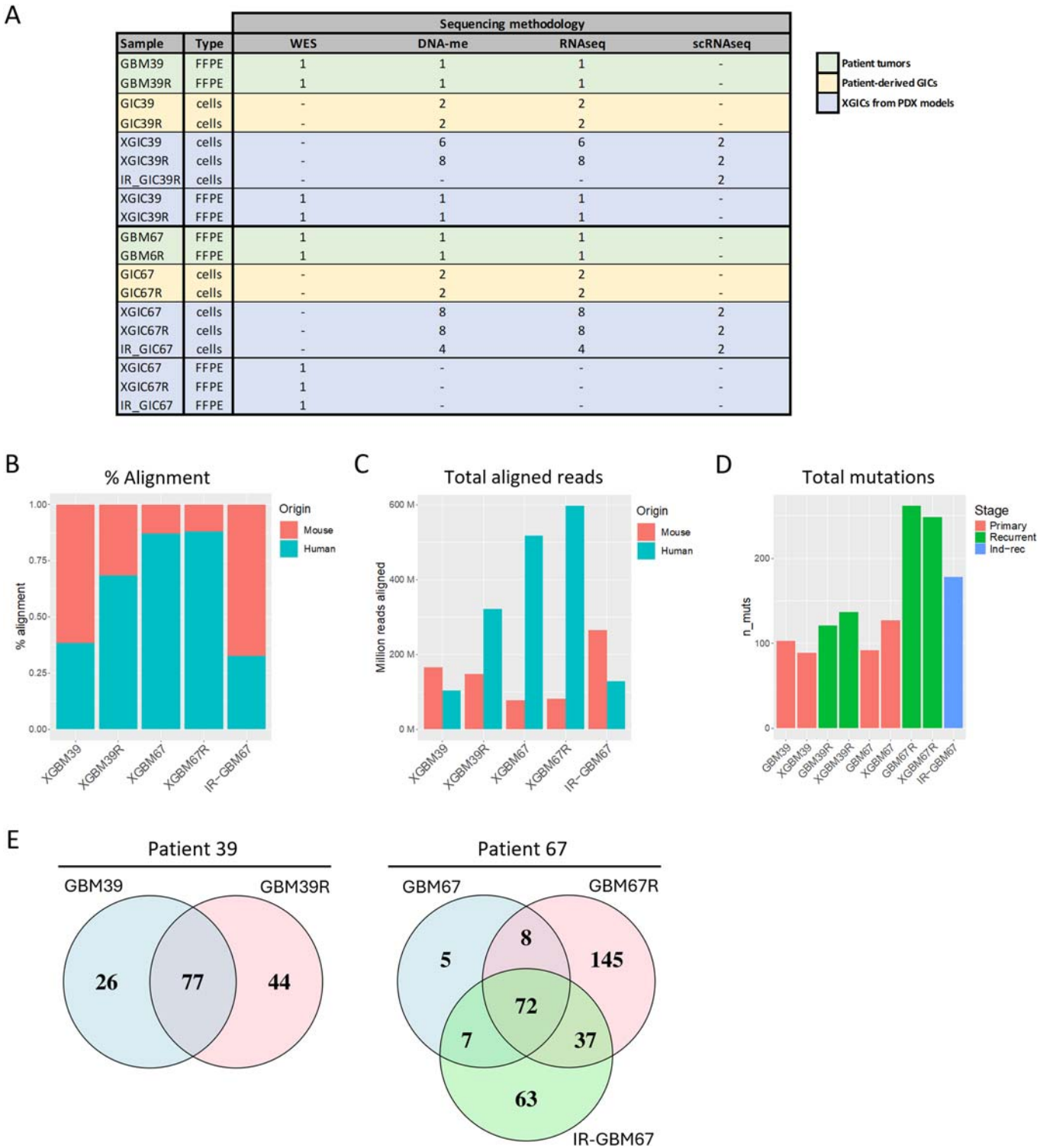


Figure EV3. Whole exome sequencing of patient tumours and PDX models.

(A) Summary of GBM samples used for multi-omic characterization of PDX models. (B) % of WES reads aligning to human vs mouse genome. (C) Total number of reads aligning to human vs mouse proportion of concatenated genome. (D) Total number mutations detected in WES samples. (E) Venn diagrams showing the number over overlapping mutations between primary, recurrent and modelled recurrence samples in patient 39 (left) and patient 67 (right).

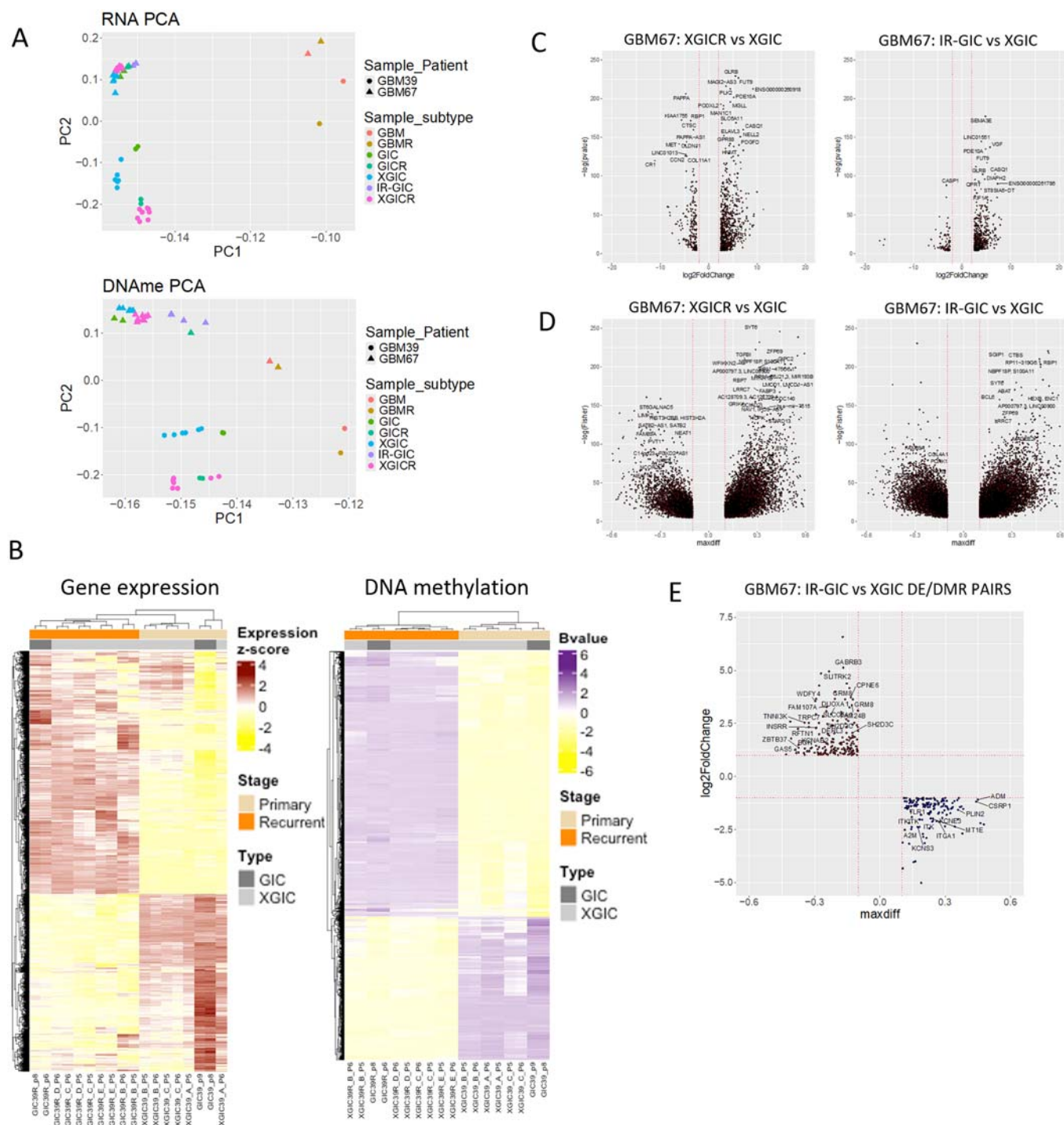


Figure EV4. Transcriptional and epigenetic analysis of PDX models.

(A) Principal component analysis of all RNA (top) and DNAm (bottom) GBM and patient- and xenograft-derived GIC samples. Samples are coloured according to their subtype (Primary GBM, recurrent GBM, patient-derived primary GICs or recurrent GICRs, xenograft-derived primary XGIC, induced-recurrence IR-GIC or true recurrence XGICR) and shaped according to their patient of origin with patient 39 shown as circles and patient 67 shown as triangles. (B) Semi-supervised hierarchical clustering of RNA (left) and DNAm (right) patient- and xenograft-derived GIC samples from patient 39. (C) Volcano plot of differential gene expression between XGIC67 and XGIC67R (left) or between XGIC67 and IR-GIC67 (right) samples. Y-axis shows $-\log(P\text{-value})$ calculated in DEseq2 using a Wald's test. (D) Volcano plot of differential DNA methylation between XGIC67 and XGIC67R (left) or between XGIC67 and IR-GIC67 (right) samples. Y-axis shows $-\log(\text{Fisher})$ calculated in DMRcate using a Fisher's exact test. (E) Scatter plot of the concordantly regulated genes identified between GIC67 vs IR-GIC67 samples. Hypomethylated and overexpressed genes are red, hypermethylated and downregulated genes are blue. Labelled genes were also found in the XGIC vs XGICR comparison.

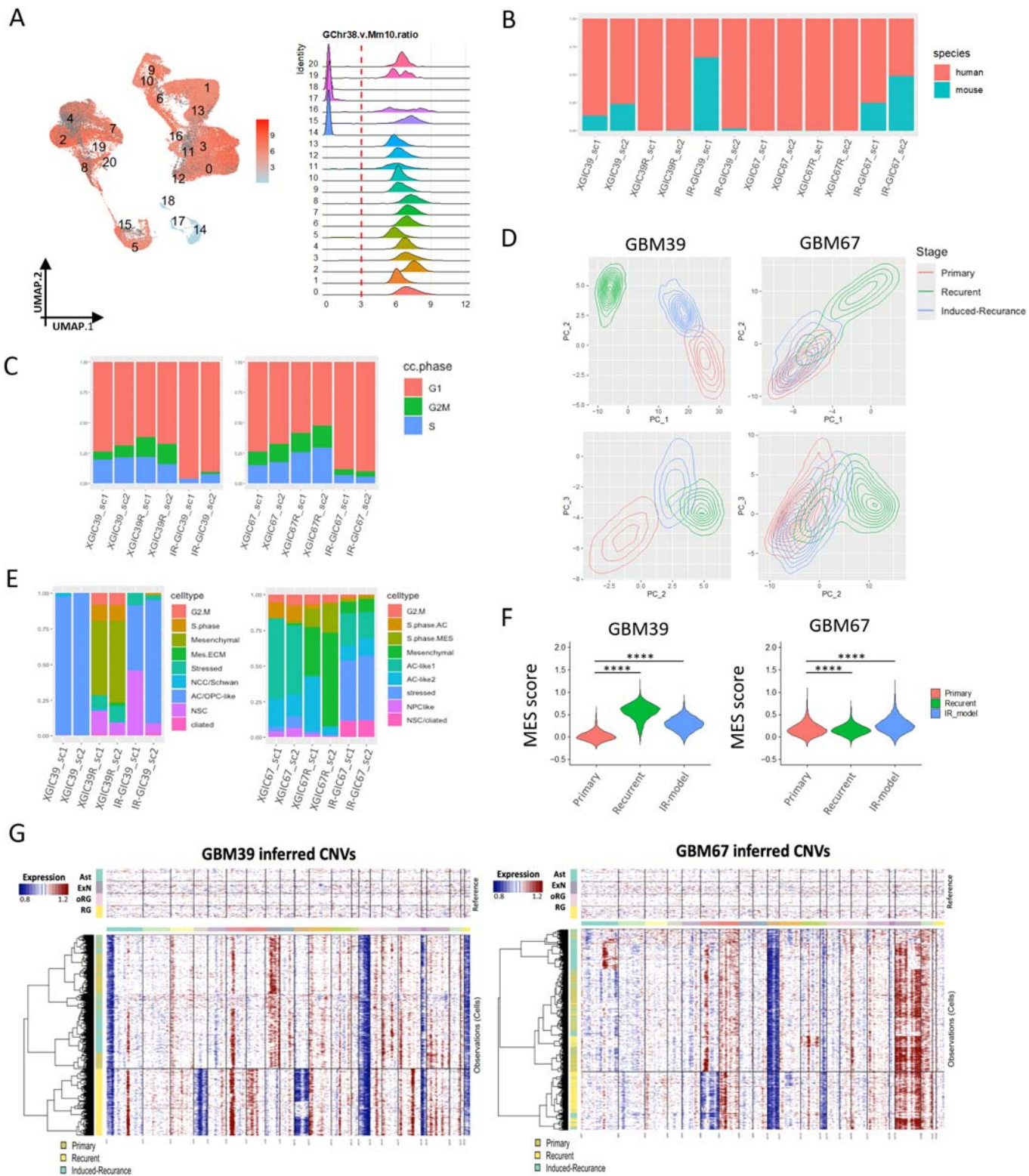


Figure EV5. scRNAseq of PDX models.

(A) UMAP of PDX samples with cells coloured by ratio of reads aligning to human (GChr39) vs Mouse (Mm10) reference genome (left), ridge plot showing GChr39vsMm10 ratio cutoff used to remove clusters of Mouse cells (right). (B). Bar plot showing the proportion of human and mouse cells in each sample. (C) Bar plots showing the cell cycle phase proportion of cells in each sample of GBM39 (left) and GBM67 (right). (D) PCA plots of PDX cells. (E) Bar plots showing the cell type proportion in each sample of GBM39 (left) and GBM67 (right). (F) Violin plot showing Neftel et al MES-like signature scores in PDX samples. Wilcoxon test with Bonferroni correction (for GBM9 n = Primary, 3727; Recurrent, 20638 IR-model, 979. For GBM67 n = Primary, 20984; Recurrent 19099, IR-model, 2197). **** p < 0.0001. (G) Heatmap showing inferred Copy Number Variants in down samples PDX cells.