

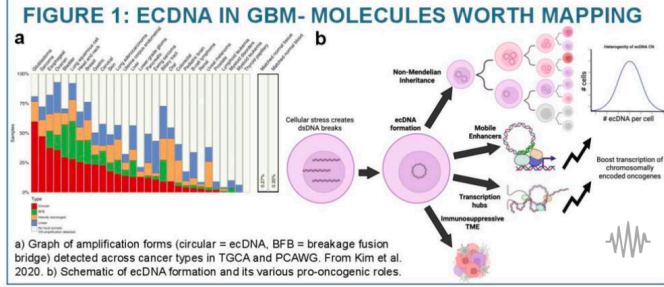
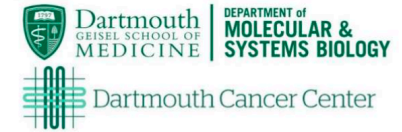


# Mapping Single-Cell ecDNA Dynamics in Glioblastoma with CRISPR Lineage Tracing

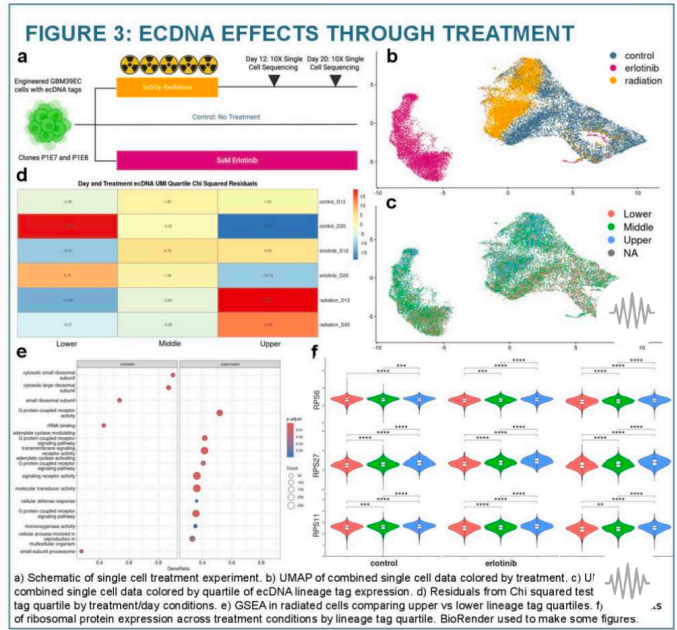
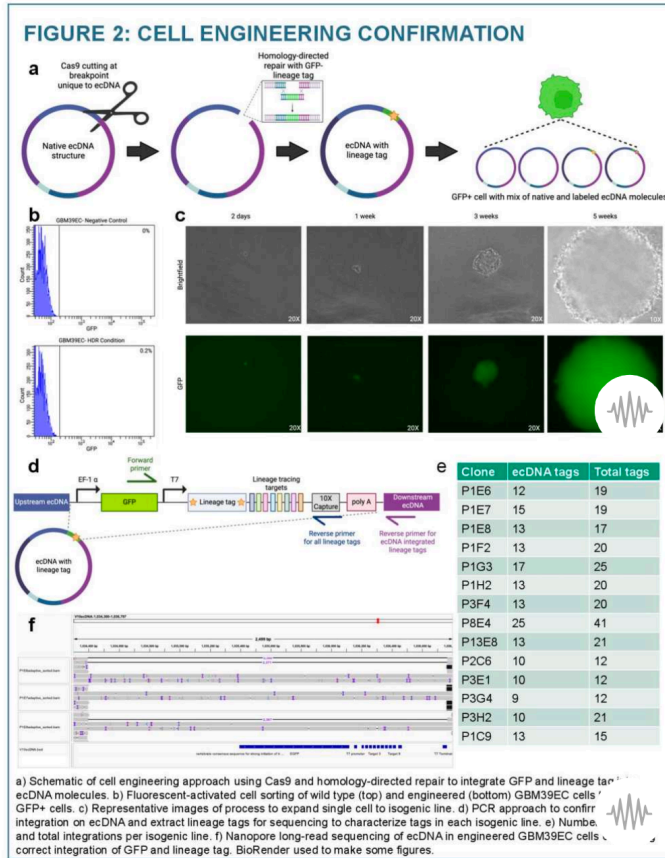
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**METHOD**  
The GBM39EC cell line (a gift from the Paul Mischel Lab at Stanford), established from a patient-derived xenograft of a primary GBM, was utilized as it has a well characterized ecDNA structure amplifying *EGFRvIII*. Via electroporation, an RNA guide against an ecDNA-unique sequence and Cas9 were delivered to cells to make double stranded breaks on ecDNA molecules. A construct encoding GFP and a lineage tag were also delivered to cells. Using homology-directed repair with sequence arms matching either side of the break, GFP and the lineage tag were integrated into ecDNA molecules (Fig 2a). After cells recovered, they were sorted by GFP (Fig 2b). Cells were then diluted to single cells per well to establish isogenic lines. Screening of isogenic lines to confirm correct integration of the lineage tag into ecDNA was conducted via PCR (Fig 2d). Overall, 14 isogenic lines were established with 9-25 confirmed ecDNA lineage tags per line (Fig 2e).



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