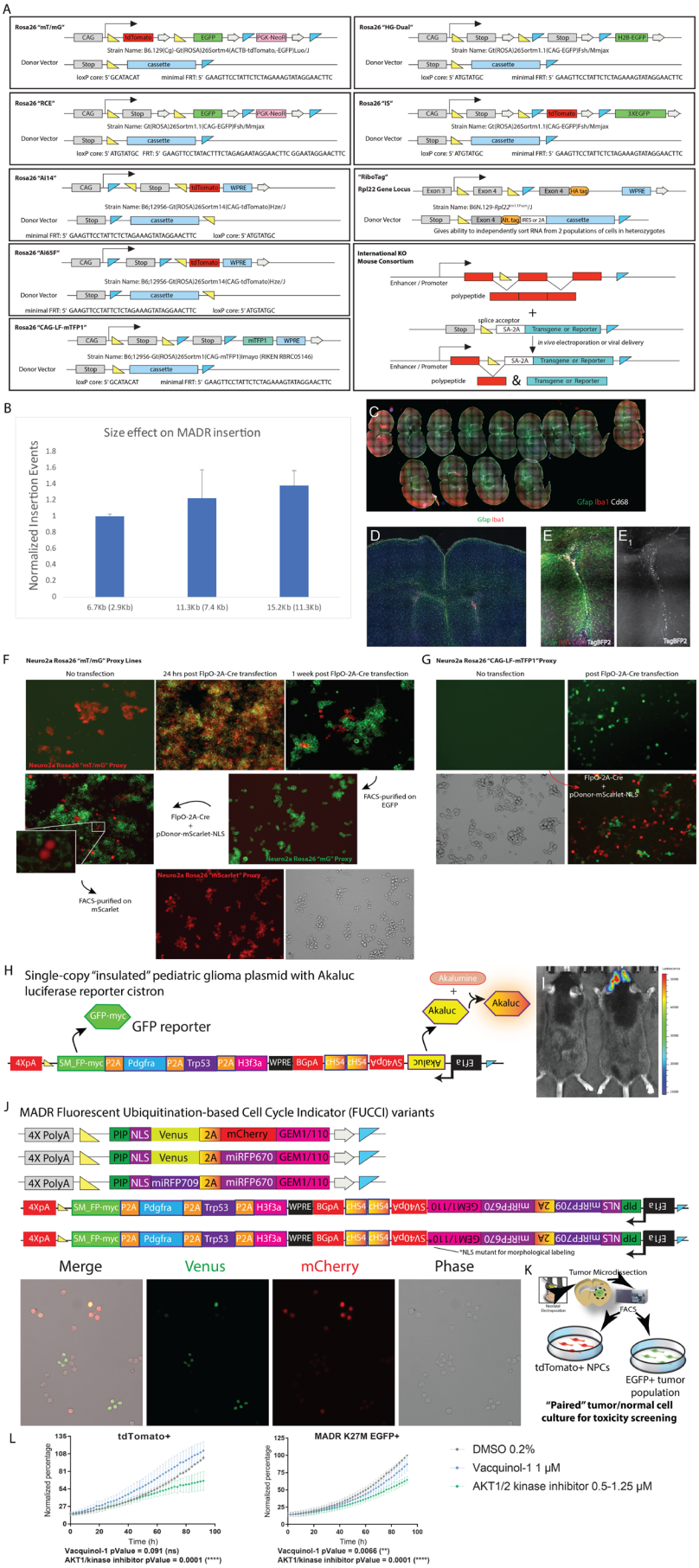

Data Supplement 1: Examples of MADR recipient lines, and miscellaneous data related to MADR usage, Related to STAR METHODS.

A. Mouse lines potentially compatible with *in vivo* MADR to allow lineage tracing studies or orthogonal RNA isolation using Ribotrap heterozygotes. Additionally, this method can extend to thousands of gene-trap mice that, as an example, flank loxP and FRT around important exons. *in vivo* MADR at such loci would enable 1) lineage tracing of heterozygous/homozygous null cells at the locus, as well as 2) swapping the locus with a transgene

B. Results from in vitro time-lapse imaging of integration using 3 different sized plasmids. No significant effect on MADR efficiency was observed when delivering 6.7Kb, 11.3Kb, and 15.2Kb plasmids at the same molar ratio (see below; Y axis is normalized MADR insertions [to 6.7Kb group]; no differences between groups were statistically significant; value in parentheses is LoxP-FRT insert size). This was done at 3 days post transfection. Note that these plasmid sizes span the typical range of plasmids used in most labs.

C. Sequential sections displaying the lack of an observable needle track or gross inflammation at EP site.

D. Lack of inflammation or gliosis at EP site

E. High magnification image of EPed cells from **D**

F. mT/mG-based "Proxy" cell lines for testing MADR constructs in vitro. Mouse N2a cells underwent CRISPR/Cas9-dependent homology dependent repair (HDR) with the same plasmids used for engineering ROSA26 mT/mG. Subsequent MADR transduction and sorting was used to clone alternate reporter lines.

G. Mouse N2a cells were created with a stable insertion of CAG-LF-mTFP1 in the ROSA26 locus. FlpO-2A-Cre and pDonor mScarlet is used to demonstrate dRMCE of this line.

H. Schematic of MADR plasmid for simultaneous generation of glioma and non-invasive imaging of tumor growth with Akaluc.

I. Control animal alongside littermate electroporated with plasmid from **T** and injected with akalumine.MADR

J. FUCCI variants, containing PIP degron fusions and hGEM1/110 fusions for descrimination of cell cycle events with different fluorescent proteins. Variants also have been generated for simultaneous generation of glioma and demarcation of cell cycle events with near infrared fluorescent proteins. Images show N2a proxy line with stable insertion of Venus/mCherry MADR FUCCI plasmid.

K. Schematic for derivation of tdTomato+ NPCs and EGFP+ tumor populations form the same microdissection for simultaneous "paired" toxicity screening.

L. Akt1/2 kinase inhibitor decreases proliferation in both NPCs and MADR K27M populations while Vacquinol-1 decreases proliferation preferentially in the K27M tumor population. Results are combined from 4 biological replicates and representative of two independent lines of each cell type.