**Table S1 Mouse Tumor Samples - Related to Figure 6**

| Type of Sample | Protocol | Area | Time from EP | Cell Line Created* | ChIP-Seq | pDonor variant |
|---|---|---|---|---|---|---|
| **K27M-1** | 10X 3' scRNA-seq | Disseminated | 150 days | **X** | **X** | pDonor-H3F3A-K27M-EGFP pTV1 Pdgfra D842V COTv1 Trp53-V5 WPRE |
| **K27M-2** | 10X 3' scRNA-seq | Striatal | 106 days | **X** | **X** | pDonor-smFP-mycBRIGHT- pTV1 Pdgfra D842V COTv1 Trp53 270h-P2ACO3-H3F3A K27M WPRE |
| **K27M-3** | 10X 3' scRNA-seq | Striatal | 149 days | **X** | **X** | pDonor-H3F3A-K27M-EGFP pTV1 Pdgfra D842V COTv1 Trp53-V5 WPRE |
| **K27M-4** | 10X snATACseq | Disseminated | 222 days† | | | pDonor-smFP-mycBRIGHT- pTV1 Pdgfra D842V COTv1 Trp53 270h-P2ACO3-H3F3A K27M WPRE |
| **K27M-5** | 10X snATACseq | Striatal | 251 days† | | | pDonor-smFP-mycBRIGHT- pTV1 Pdgfra D842V COTv1 Trp53 270h-P2ACO3-H3F3A K27M WPRE |

*-Cell lines created from parallel processing of additional GFP+ cells. All 10X scRNA- or snATAC-sequencing was done acutely from the dissociated brain tissue.

†-Initial EPed population size was decreased compared with typical results in this group leading to increased tumor formation span

**Table S3. Comparison of approaches for in vivo genetic manipulation - Related to STAR METHODS**

| Method | GEMM | Standard EP | Transposition-mediated EP | Virus | CRISPR Cas9/Cpf1 | HITI | SLENDR | Base writing | MADR |
|---|---|---|---|---|---|---|---|---|---|
| Time for engineering and generation | Months | ~2 weeks per plasmid | ~2 weeks per plasmid | >4-6 weeks | ~2 weeks per plasmid | ~2 weeks (plasmid); months (virus) | ~2 weeks (plasmid); months (virus) | ~2 weeks per plasmid | ~2 weeks per plasmid |
| Copy number | 1-2 per knock-in | Highly Variable | Highly Variable (up to hundreds) | Variable but likely less than EP | 1-2 but not readily controllable | 1-2 but not readily controllable | 1-2 but not readily controllable | 1-2 but not readily controllable | 1-2 depending on zygosity of recipient |
| Breeding | More complex for conditional alleles | Not necessary | Not necessary | Only necessary for RCAS/Tva | Not necessary | Not necessary | Not necessary | Not necessary | 1 line per targeted stain |
| Stability of Expression | Generally stable depending on locus silencing | Prone to dilution and/or silencing | Prone to silencing and insertional effects | Prone to silencing and insertional effects | Expression dependent on mutation site | Expression dependent on insertion site or fusion partner | Expression dependent on insertion site or fusion partner | Expression dependent on mutation site | Generally stable depending on locus silencing |
| Payload | Limited by targeting construct* | Typically governed by plasmid limits* | Typically governed by plasmid limits* | Limited to viral payloads | Typically governed by plasmid limits but viral variant is subject to viral payloads* | Typically governed by plasmid limits but viral variant is subject to viral payloads* | Typically governed by plasmid limits but viral variant is subject to viral payloads* | Typically governed by plasmid limits but viral variant is subject to viral payloads* | Typically governed by plasmid limits* |
| Focality | Depends on cis regulatory elements | Focality depends on electrode orientation | Focality depends on electrode orientation | Diffusion pattern unidirectional from injection site | Focality depends on electrode orientation (plasmid version) or viral spread (AAV/LV) | Focality depends on electrode orientation (plasmid version) or viral spread (AAV) | Focality depends on electrode orientation (plasmid version) or viral spread (AAV) | Focality depends on electrode orientation (plasmid version) or viral spread (AAV/LV) | Focality depends on electrode orientation |
| Efficiency | Typically 100% | 100% | 100% | 100% | approaching 100% but off-targets and heterogeneity unclear; largely LOF | Typically <20% but requires minicircle DNA production to reach this | Typically <5% | up to 80% but off-targets and heterogeneity unclear especially when multiplexing | Can be titered to approach 100% insertion** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Other notes | Least amenable to mixing and matching mutations | Plasmids rarely integrate or integrate unpredictably | Random insertions, supraphysiological expression, can be silenced, in and out hopping of transgenes | Random insertions, potential supraphysiological expression, can be silenced, can incite cellular immunity, RCAS/Tva models often use injection of >50,000 avian virus producing cells--causing potential immune interactions and trauma | immunogenic, hard to definitively lineage trace, low HDR efficiency | Multiplexing mutant alleles challenging | Multiplexing mutant alleles challenging | immunogenicity unclear, challenging to definitively lineage trace mutant cells | Transgenes can potentially hop in and out before Flp/Cre dilution; potentially compatible/complementary with virtually all methods (Orthogonal to CRISPR/Cas variants; HITI; Slendr; Base writers) |

*-BAC DNA can be utilized

**-this decreases total cell yields

See text for further details