

- A total of  $1 \times 10^5$  to  $1 \times 10^6$  32D cells were plated into 12-well plates with 32D cell media (DMEM containing 10% fetal bovine serum and 1x penicillin/streptomycin in the presence mouse IL-3).
- 32D cells were then spin-infected with following lentiviruses at MOI of 1:1 at 250 *g* for 2 h.
  - FUW-M2rtTA (#20342)
  - TETO-dCas9-D3A (#78254)
  - TETO-dCas9-mD3A (#78255)
  - pL-sgRNA1 and/or pL-sgRNA2
- 24 h post-transduction, transduced cells were washed and re-plated in fresh media with IL3 for their expansion in the presence of 0.25  $\mu\text{g/ml}$  doxycycline (Sigma, D9891-100G).
- Four days post transduction, GFP+ and/or mCherry+ 50,000 – 100,000 cells were sorted (directly into fresh media in the presence of 0.25  $\mu\text{g/ml}$  doxycycline) to establish stable cell lines.
- Every other day, a half-media change with (0.5  $\mu\text{g/ml}$ ) doxycycline was performed.
- Cells were then harvested at different time points and were subjected to bisulfite sequencing and qRT-PCR analysis (see the manuscript for detailed time point for cell harvest).