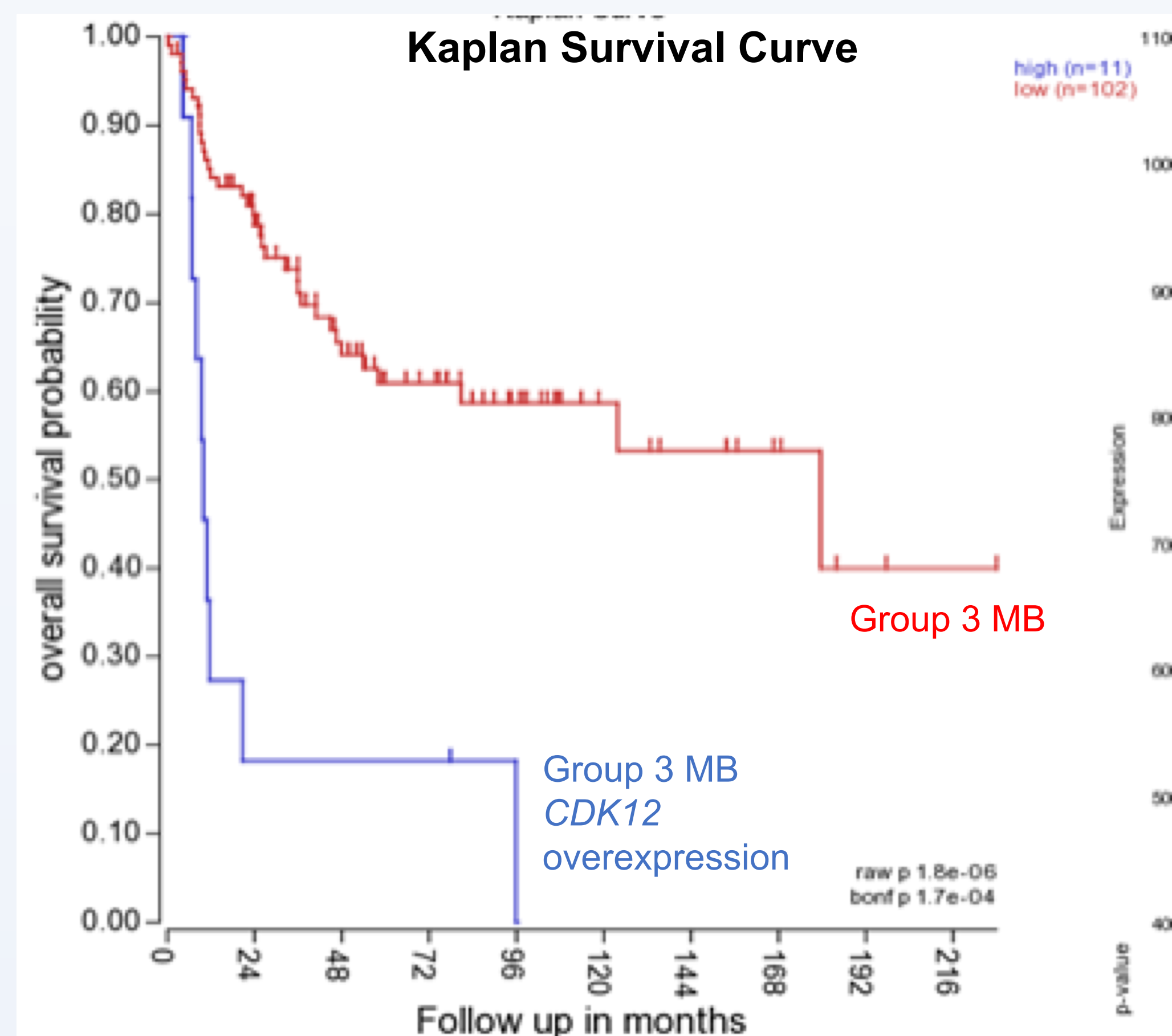


# The Role of *CDK12* in Pediatric MYC-Amplified Medulloblastoma

## BACKGROUND

- Medulloblastoma is the most common type of malignant brain cancer in children, originating in the cerebellum
- MB is subdivided into four subtypes
  - WNT is the most common with the best prognosis
  - Group 3 has the worst prognosis
  - This group is MYC-amplified
- Vibhakar lab performed a functional genomic screen using CRISPR-Cas9 technology.
  - Cyclin-dependent kinase 12, *CDK12*, was identified as a top essential gene for Group 3 Myc-MB viability
- Using microarray data, *CDK12* overexpression was identified in Group 3 MB
- Additional data also suggests that higher *CDK12* expression confers worse survival rate in Group 3 MB

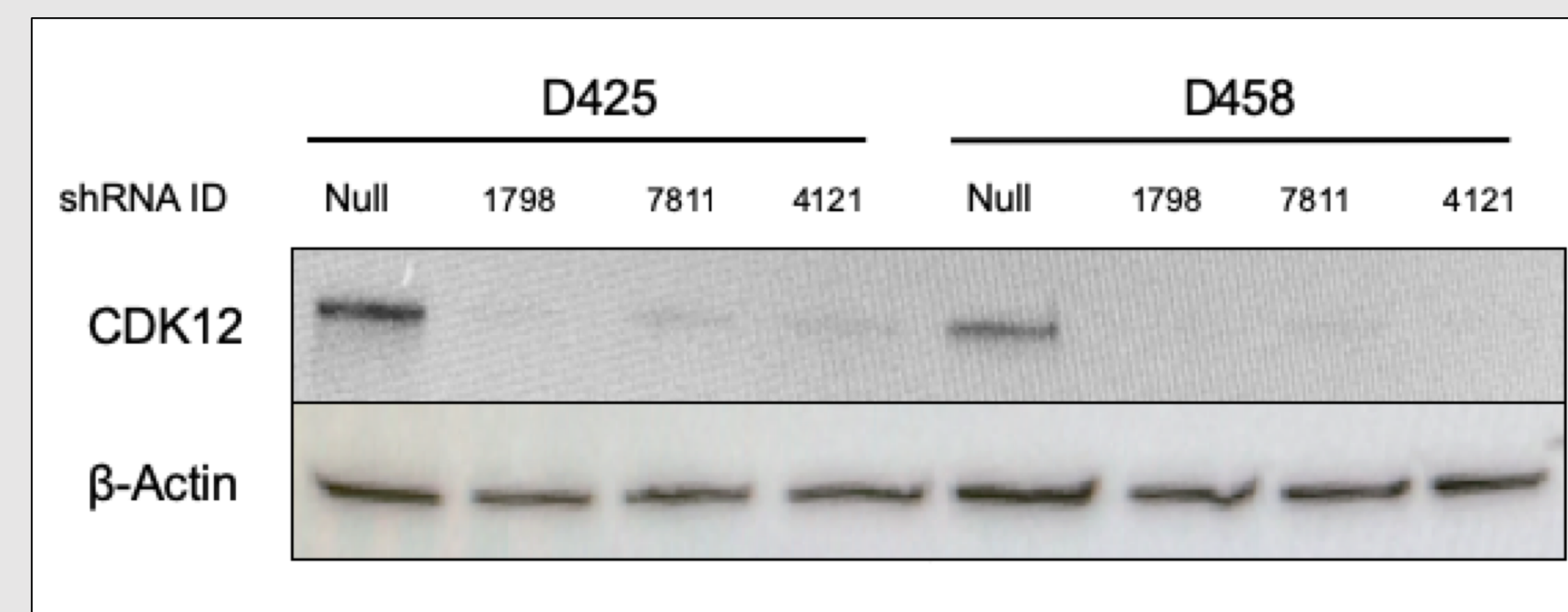
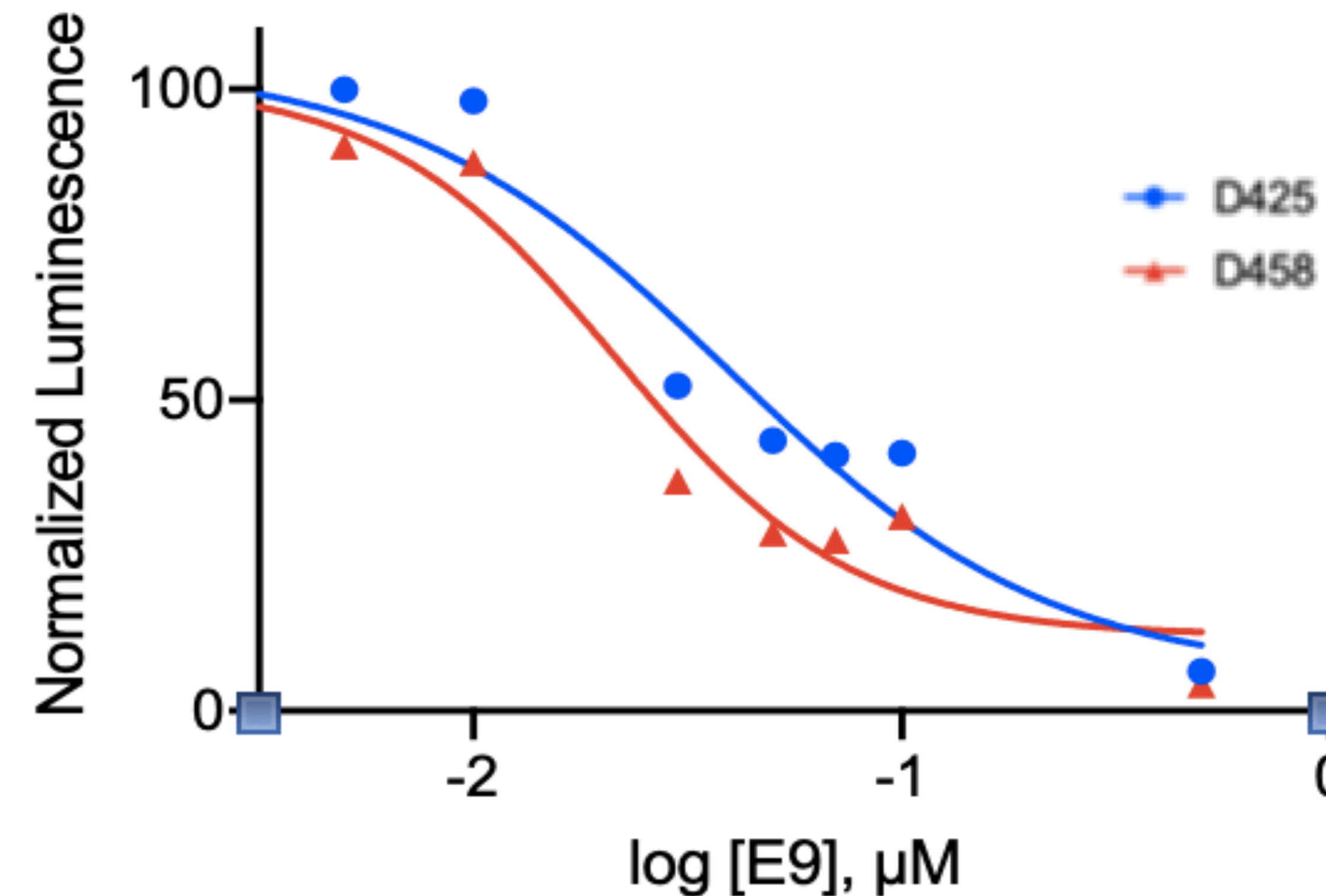


- CDK12* phosphorylates the C-terminal domain (CTD) of RNA pol II
- CDK12* regulates the expression of various genes involved in DNA repair

## OBJECTIVES

- What is the role of *CDK12* in Group 3 MB tumors?
- What effect do *CDK12* inhibitors have on Group 3 MB cell lines?
- Does *CDK12* in Group 3 MB regulate radiation sensitivity?

## E9 Dose Response Curve

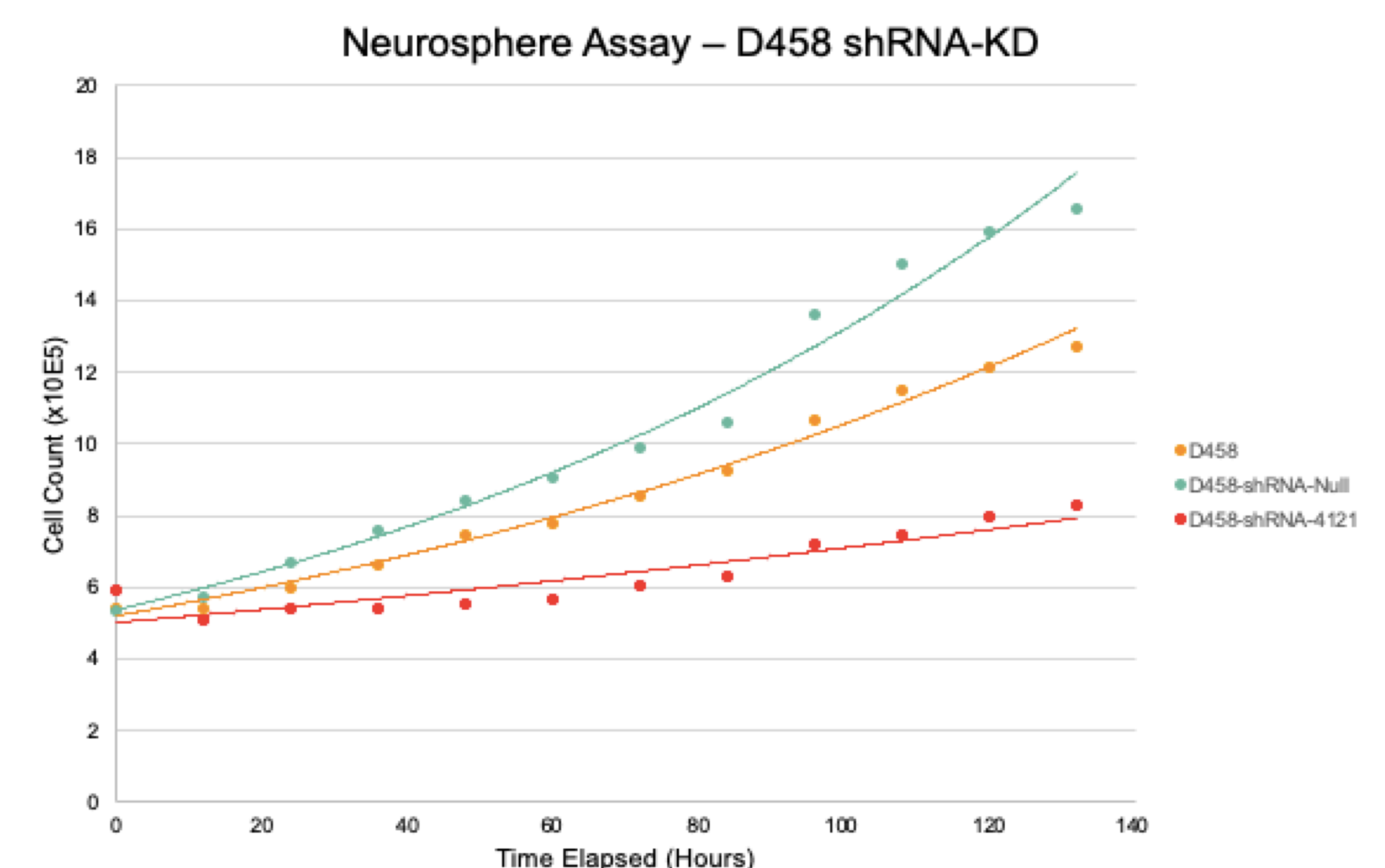
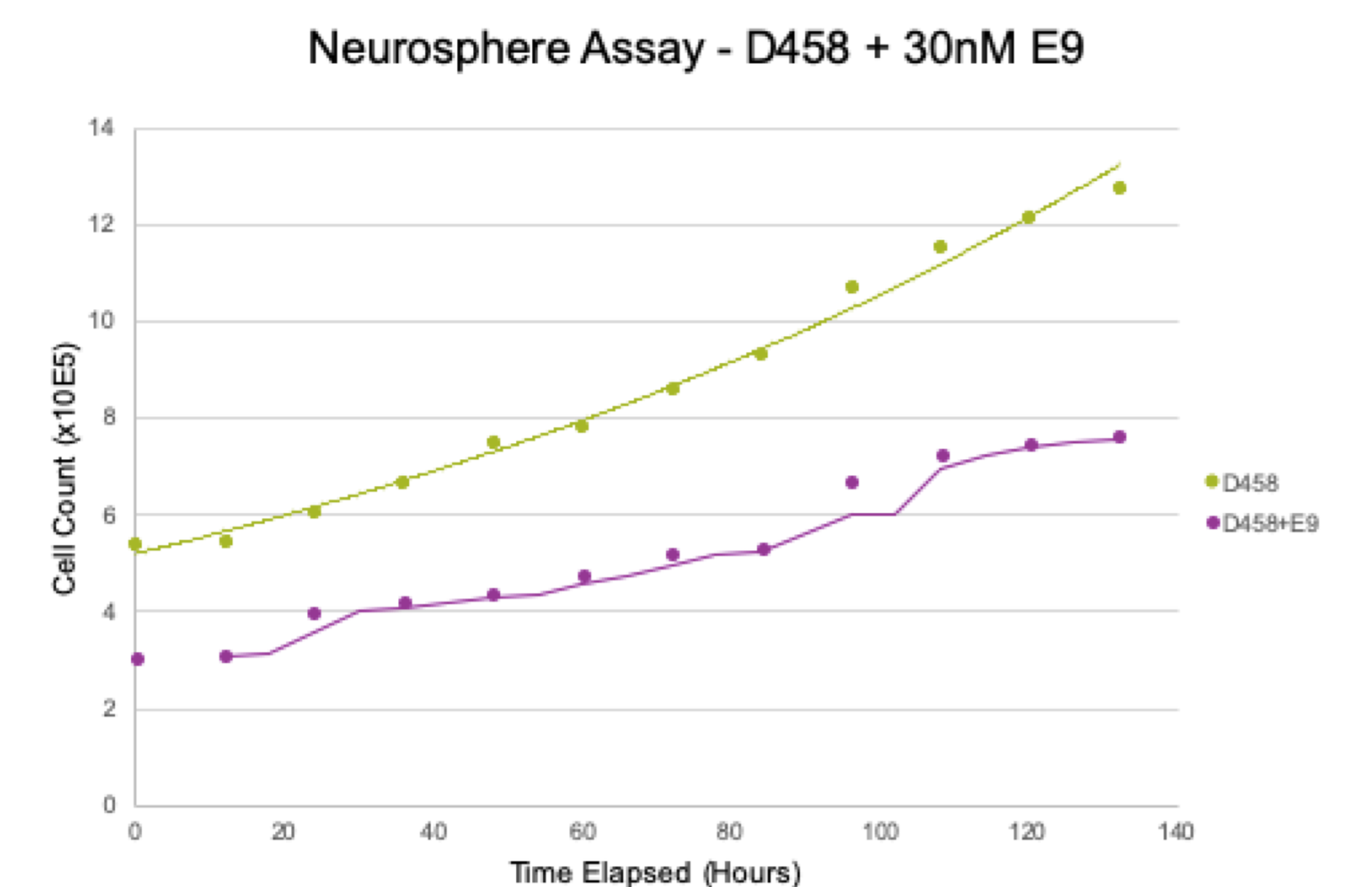


## MATERIALS AND METHODS

- Verified group 3 MB cell lines used:
  - D458 (Med (RRID:CVCL\_1161)) – MYC-amplification with *CDK12* overexpression
  - D425 (Med (RRID:CVCL\_1275)) – has additional *TP53* mutation
- CDK12* knock down cell lines were developed using three different Sigma Aldrich *CDK12*-shRNAs
  - HEK293T transfection was followed by lentiviral transduction of both D458s and D425s
- Knock down was confirmed using western blot
- CDK12* inhibitor E9 (MedChemExpress) IC<sub>50</sub> was performed using D425s and D458s to find an IC<sub>50</sub> of 30nm
- Neurosphere growth assay performed for KD cell lines and control cell lines treated with E9
- Western blotting using shRNA KD cell lines as well as controls
  - Probed for total RNA Pol II, c-Caspase 3/7, c-MYC, P-Rpb1-Ser2 and beta-actin

## RESULTS

- Successful shRNA knock down confirmed by western blot
- Neurosphere growth assays are good indicators that group 3 MB relies heavily on *CDK12*



## FUTURE WORK

- Further exploration of E9 mechanism via western blotting
- RT-PCR on shRNA-KD cell lines
- In vivo* mouse experiments with knock downs and E9
- RNAseq data analysis for other potential therapeutic/synergistic targets