

Manuscript number: DMD-AR-2021-000597R1

Title: Factors Influencing Luciferase-Based Bioluminescent Imaging in Preclinical Models of Brain Tumor

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Supplemental Figures

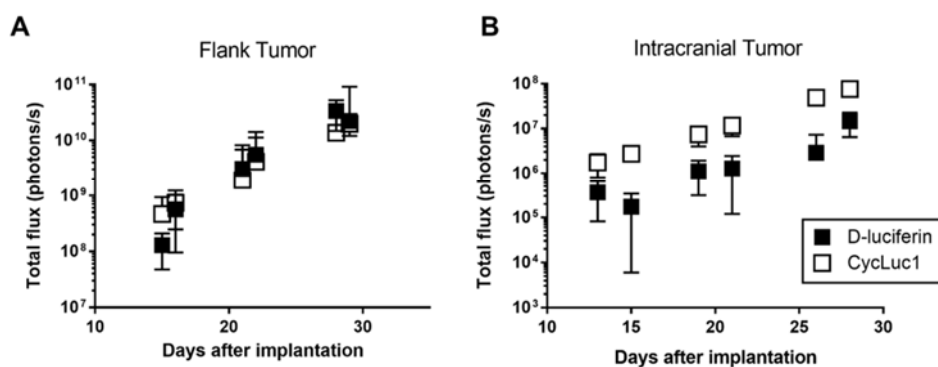


Figure S1. The intensity of BLI light signal from D-luciferin and CycLuc1 with different orders of cross-over imaging with GBM6 implanted on the different locations, flank and brain.

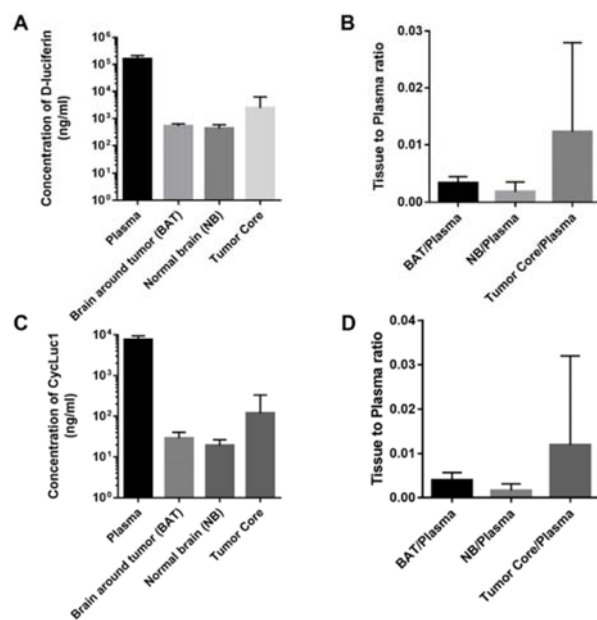
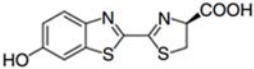
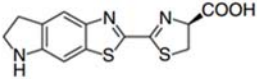


Figure S2. Concentration of D-luciferin (A and B) and CycLuc1 (C and D) measured by LC-MS/MS in and around the brain tumor following a single intraperitoneal dose of 150 mg/kg D-luciferin and 5 mg/kg CycLuc1. Tissues were harvested at 10 min post dosing.

Table S1

Summary of physicochemical properties of D-luciferin and CycLuc1

	D-luciferin	CycLuc1
Structure		
Molecular Weight ¹	280.32	305.37
XLogP ¹	0.9	2.6
Hydrogen bond donor count ¹	2	2
Hydrogen bond acceptor count ¹	7	7
Polar surface area (Å ²) ¹	129	128
Emission wavelength (nm) ²	553	603
K _m (μM) ²	6.76±0.3	0.1±0.01

¹Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>)²Harwood, Katryn R., et al. "Identification of mutant firefly luciferases that efficiently utilize animoluciferins." Chemistry & biology 18.12 (2011): 1649-1657.