

Notch Pathway blockade affects the stem cell-like and the migratory capacity of brain tumor initiating cells



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Background

- Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults
- Brain tumor initiating cells (bTICs) are neural stem cell (NSC)-like cells found in GBM
- bTICs might be responsible for tumor-initiation, -progression, treatment resistance and relapse
- Notch signaling is important for maintaining an undifferentiated pool of normal NSC and in determination of cell fate
- Notch signaling is indicated to play a functional role in GBM and thereof derived bTICs

Materials and Methods

- Neurosphere cultures were established from human derived primary GBM xenografts and cultured in NB-media: Neurobasal™-A media supplemented with B-27, L-glutamine, EGF, bFGF (Invitrogen) and LIF (Chemicon). G1, G2 and G3 are three different primary GBM tumors and their corresponding neurosphere cultures
- Notch inhibition was accomplished using the γ -secretase inhibitor DAPT (Calbiochem) dissolved in DMSO (Sigma). 5 μ M DAPT was used, unless otherwise mentioned. Equal volumes of DMSO was used as a control
- Protein expression was determined by Western blot analysis (WB)
- mRNA expression was analyzed by Quantitative Real-Time Polymerase Chain Reaction (q-RT-PCR)

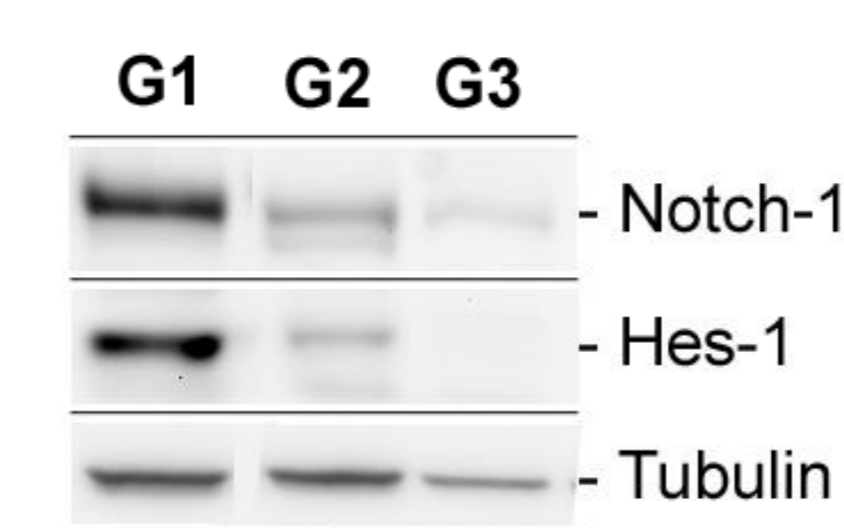
Aim

Investigate the significance of Notch expression and activation in GBM stem cell-like cultures

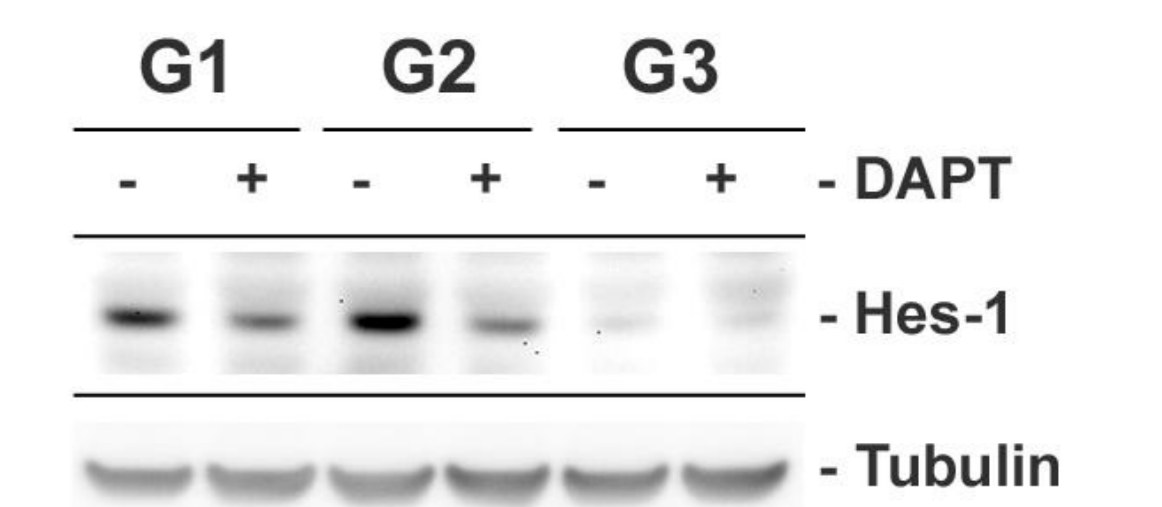
Results

The effect of Notch inhibition was more pronounced in cultures with high Notch expression and activation

A: Basal WB blot

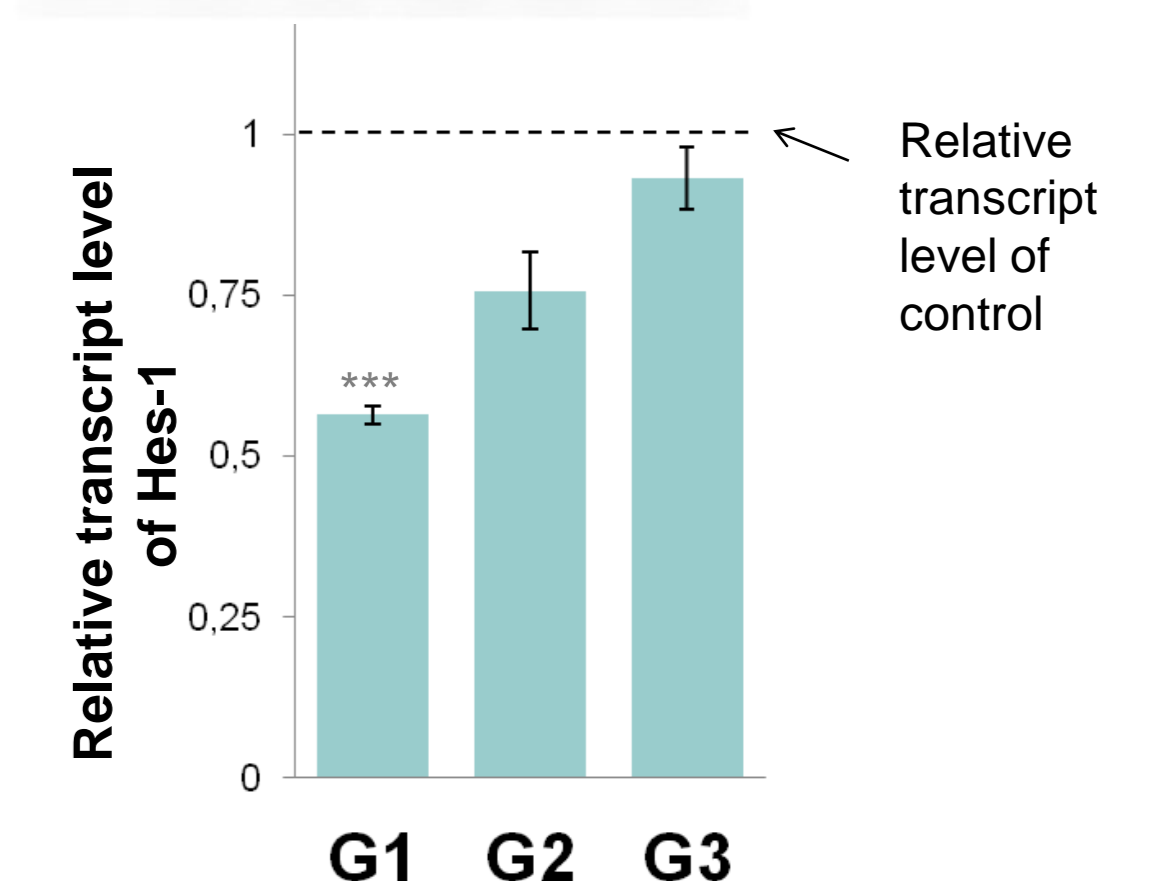


B: Effect of DAPT treatment on Hes-1 protein and mRNA level



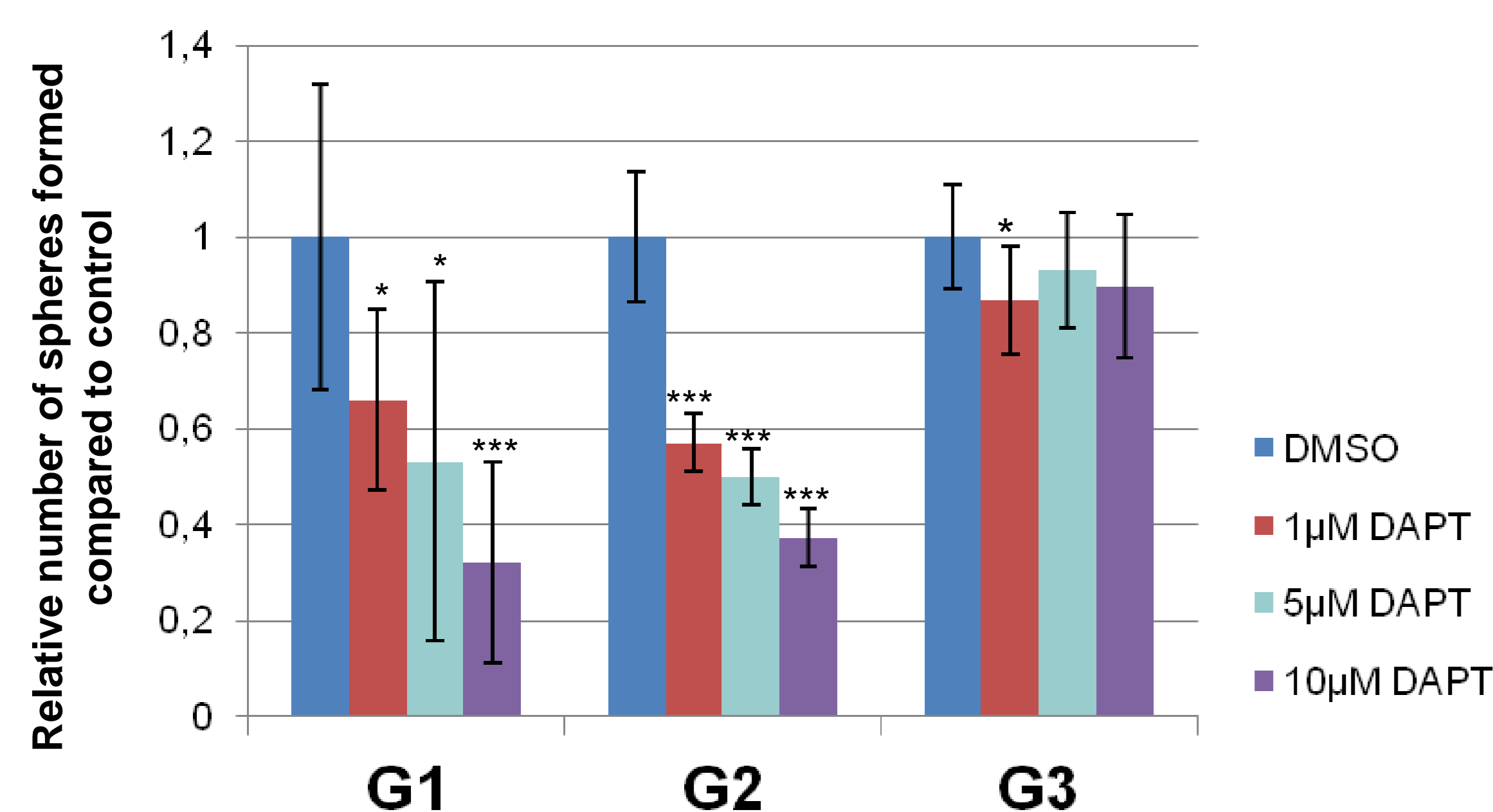
A: WB detection of the Notch-1 receptor and the Notch target Hes-1.

B: q-RT-PCR analysis and WB detection of Hes-1 in neurosphere cultures treated with DAPT compared to control.



Results

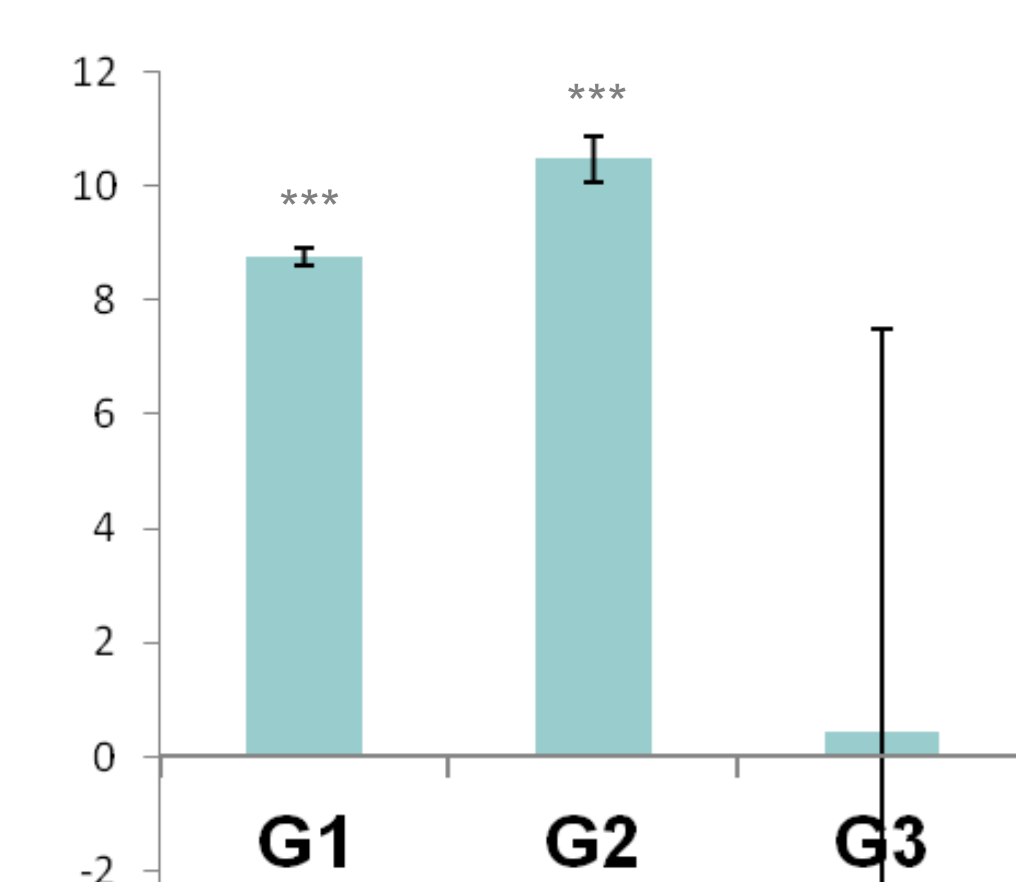
Primary sphere formation was reduced upon Notch pathway blockade



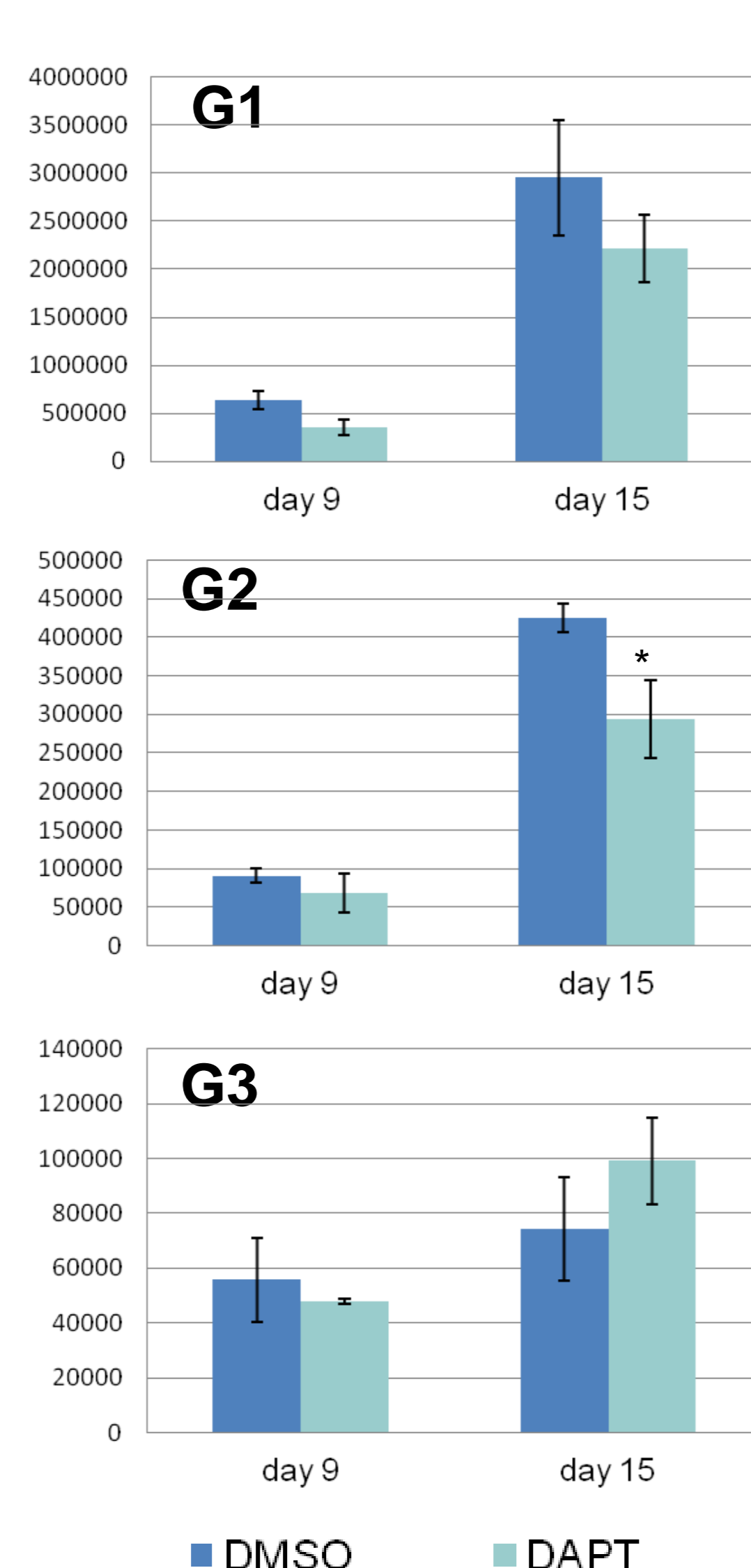
Primary sphere assay performed on cells from acutely dissociated xenograft GBM tissue indicates that GBM cells lose their NSC-like sphere forming capacity when Notch signaling is blocked

Notch inhibition induced cell cycle arrest

A: % change in G0/G1



B: Change in dead cells

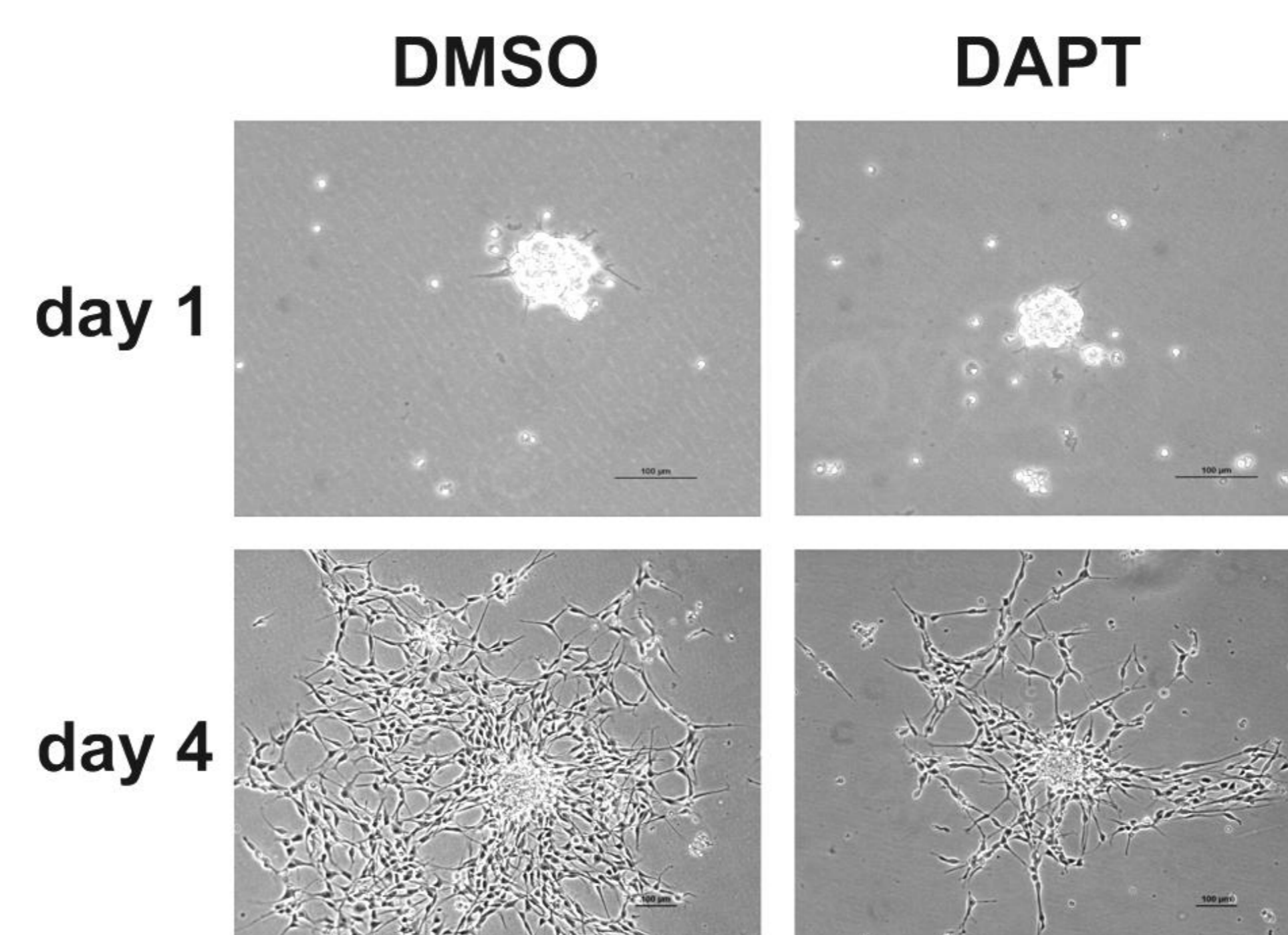


Cell cycle analyses showed an increase in the G0/G1 population upon DAPT treatment when compared to control (A), whereas no increase in Trypan blue positive cells was observed (B)

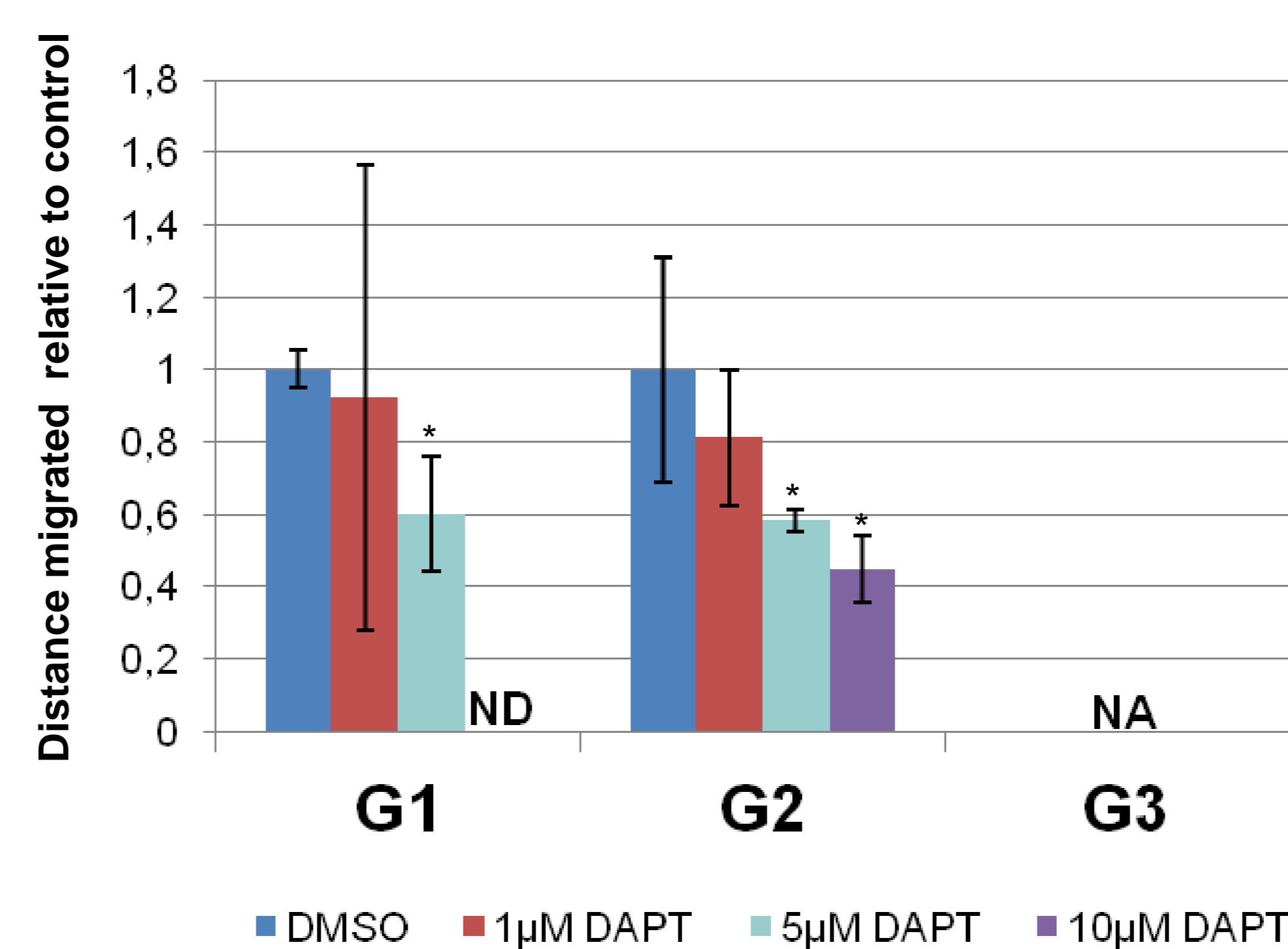
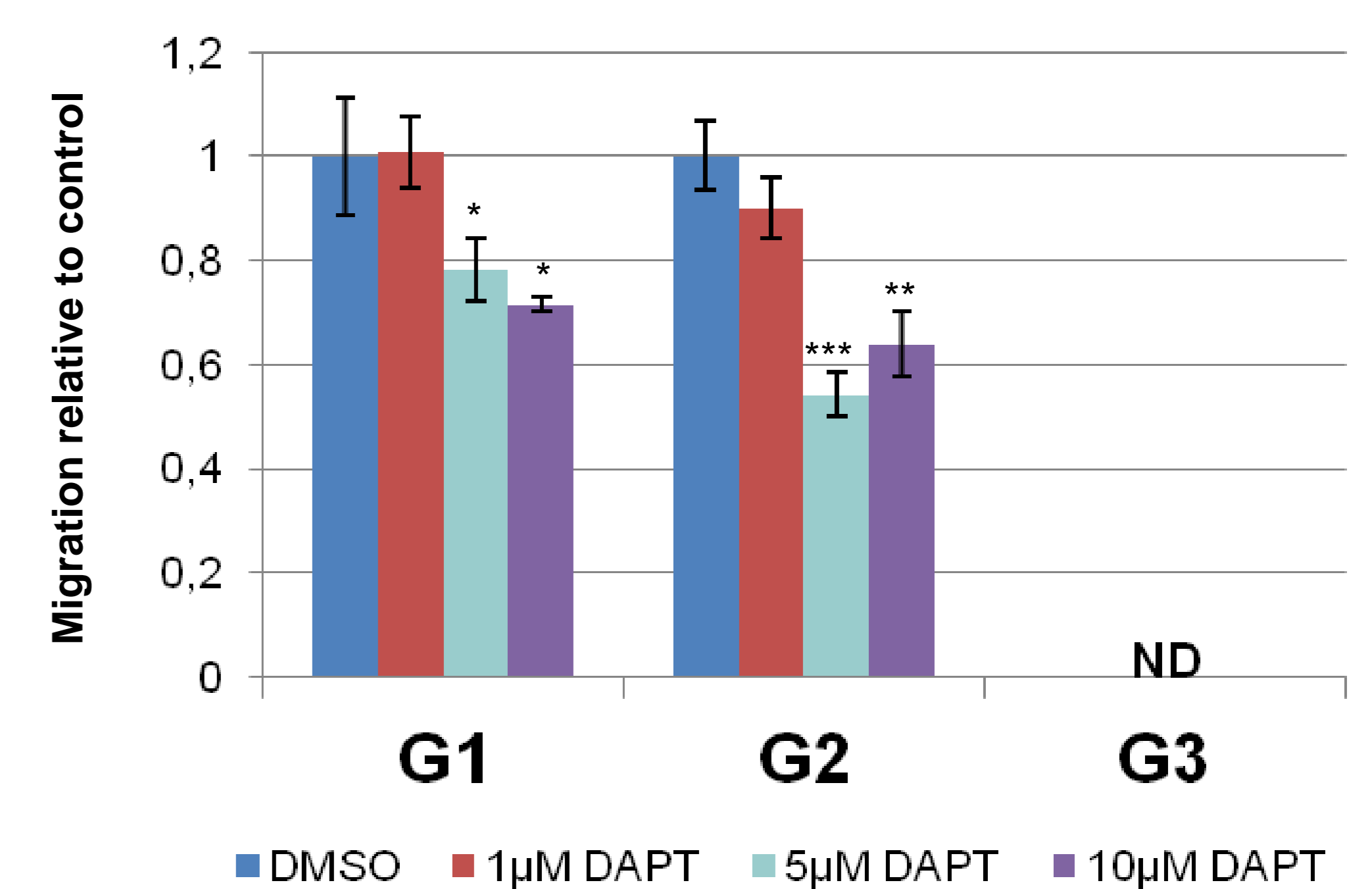
Together these data indicate that Notch pathway blockade leads to cell cycle arrest

The migratory potential of sphere cells was hampered upon Notch inhibition

A: Migration in a Hanging drop assay



B: Migration in a modified Boyden chamber



A top: Representative example of observations made in the hanging drop assay

A bottom: Distance migrated was calculated as the mean radius of migrated cell

B: Sphere cells were pretreated with different concentrations of DAPT and subsequently seeded in a modified Boyden chamber. The amount of migrated cells was quantified by MTT-staining

Together these results suggest that Notch blockade reduces the amount of migrating cells as well as the distance of migration

Conclusion

Notch signaling contributes to the NSC-like character and the malignant phenotype of bTICs, when these display dysregulated Notch pathway activation. It might be possible to target bTICs in human GBM through the Notch signaling pathway.