

# Notch Pathway blockade affects the differentiating and 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

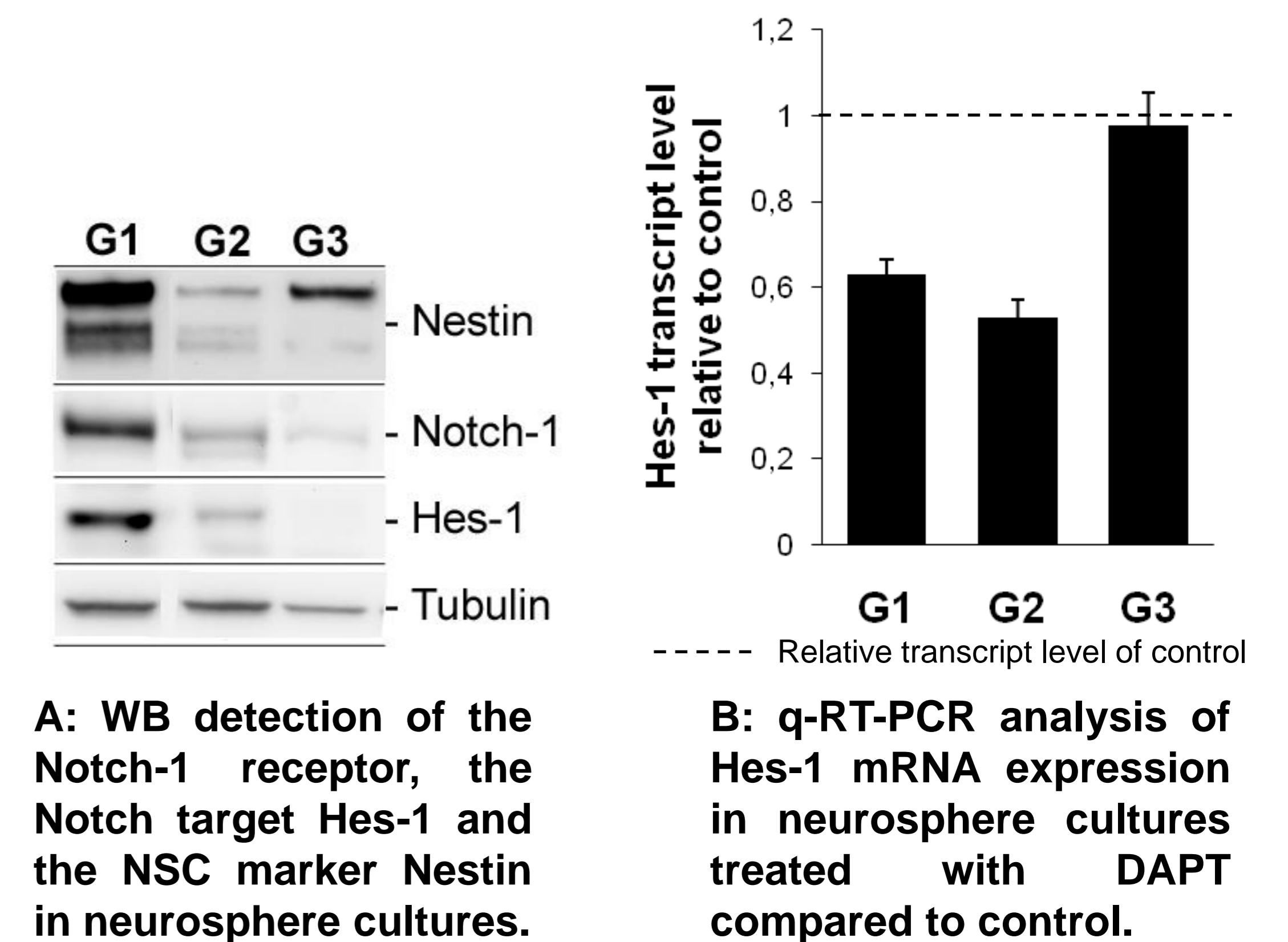
- Neurosphere cultures were established from human derived primary GBM xenografts and cultured in NB-media: Neurobasal™-A media supplemented with B-27, L-glutamine, EGF, bFGF (Invitrogen) and LIF (Chemicon). G1, G2 and G3 are three different primary GBM tumors and their corresponding neurosphere cultures
- Notch inhibition was accomplished using the  $\gamma$ -secretase inhibitor DAPT (Calbiochem) dissolved in DMSO (Sigma). 5 $\mu$ 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)

## Objective

Investigate the significance of Notch expression and activation in GBM stem cell-like cultures originating from human primary GBM

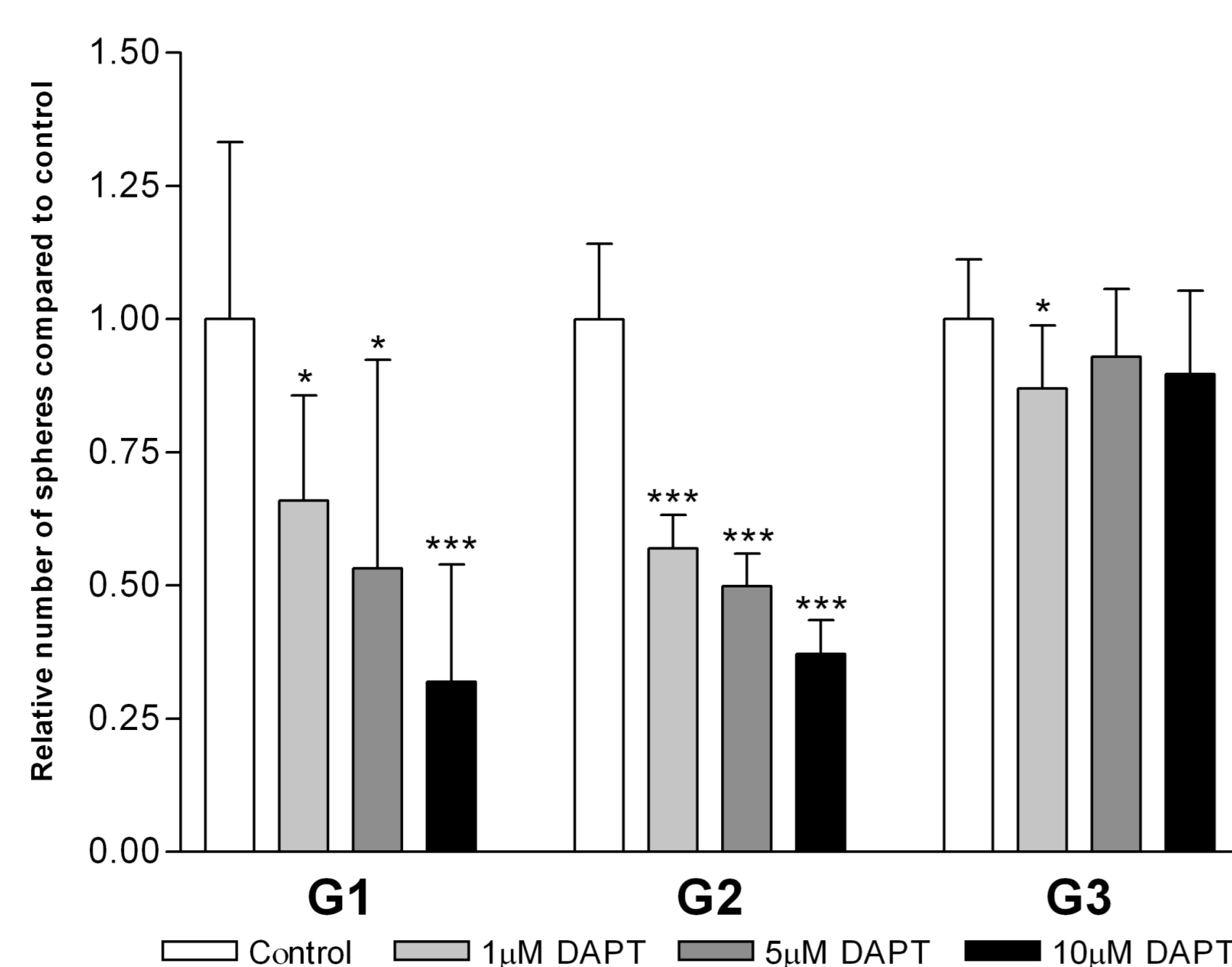
## Results

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



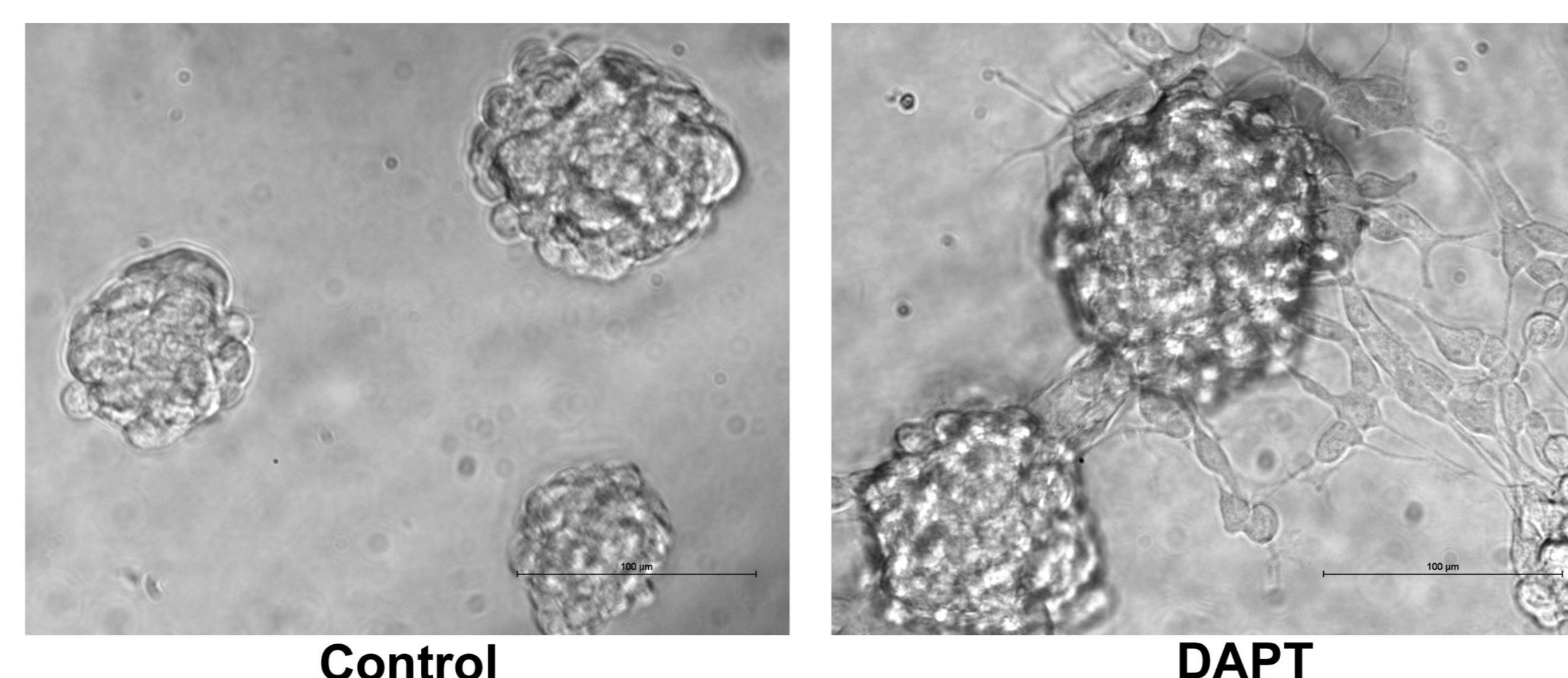
## Results

**Primary sphere formation was reduced upon Notch pathway blockade**

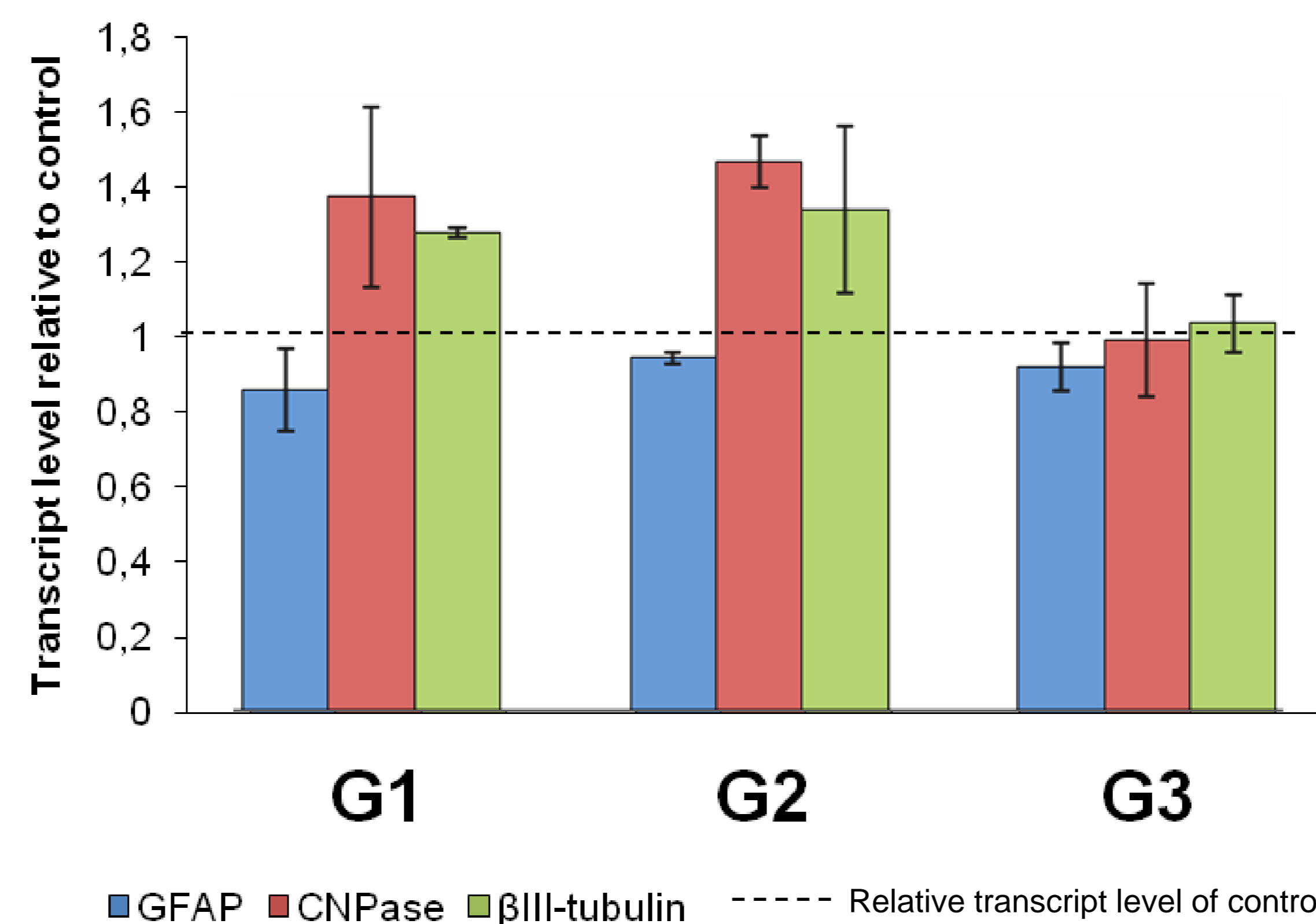


Primary sphere assay performed on cells from acutely dissociated xenograft GBM tissue.

**Notch blockade initiates differentiation and alters the differentiation pattern**

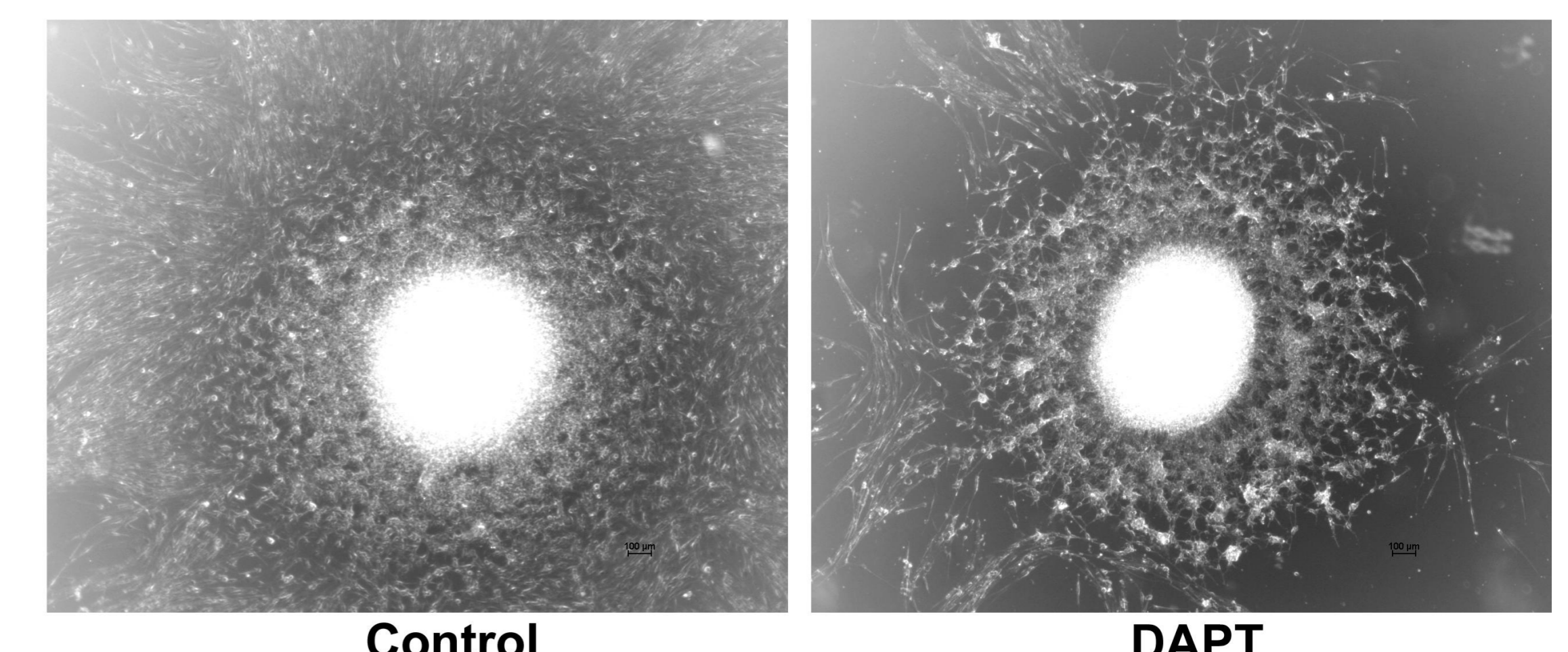


**A:** Spheres formed in NB-media during DAPT treatment had a tendency to adhere to the culture plate and cells with a differentiated morphology migrated away from the spheres

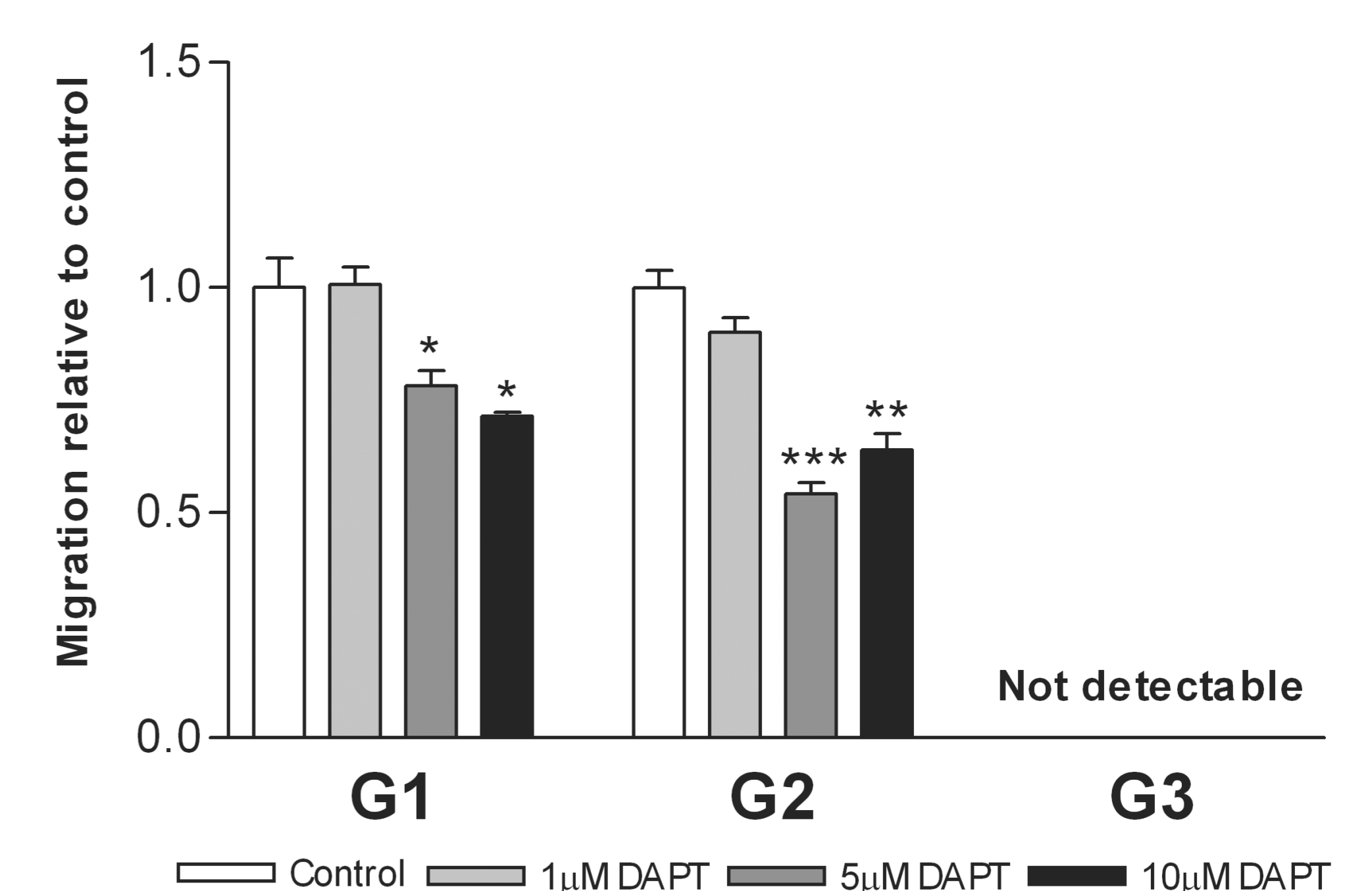


**B:** Sphere cells were induced to differentiate during concurrent DAPT treatment. q-RT-PCR analysis of markers for the three neural lineages was performed

**The migratory potential of sphere cells was reduced upon Notch inhibition**

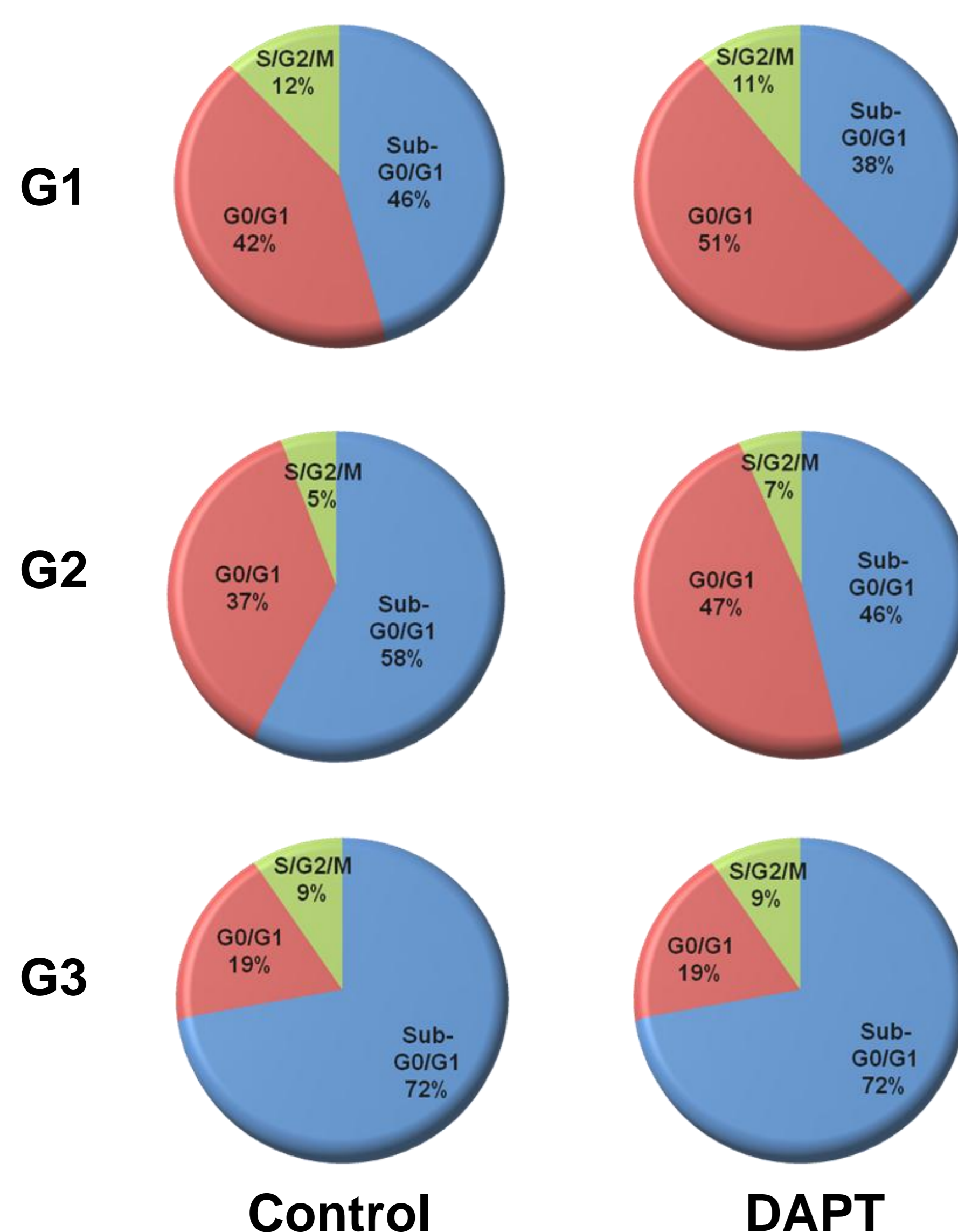


**A:** When whole spheres were transferred to serum-containing media, they adhered to the culture plate and cells began to migrate away from the sphere.



**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

**Notch inhibition induces cell cycle arrest**



Cell cycle analyses showed an increase in the G0/G1 population upon DAPT treatment

## 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.