



Role of adenosine receptor A_{2B} in the maintenance of GSCs stemness under hypoxia.

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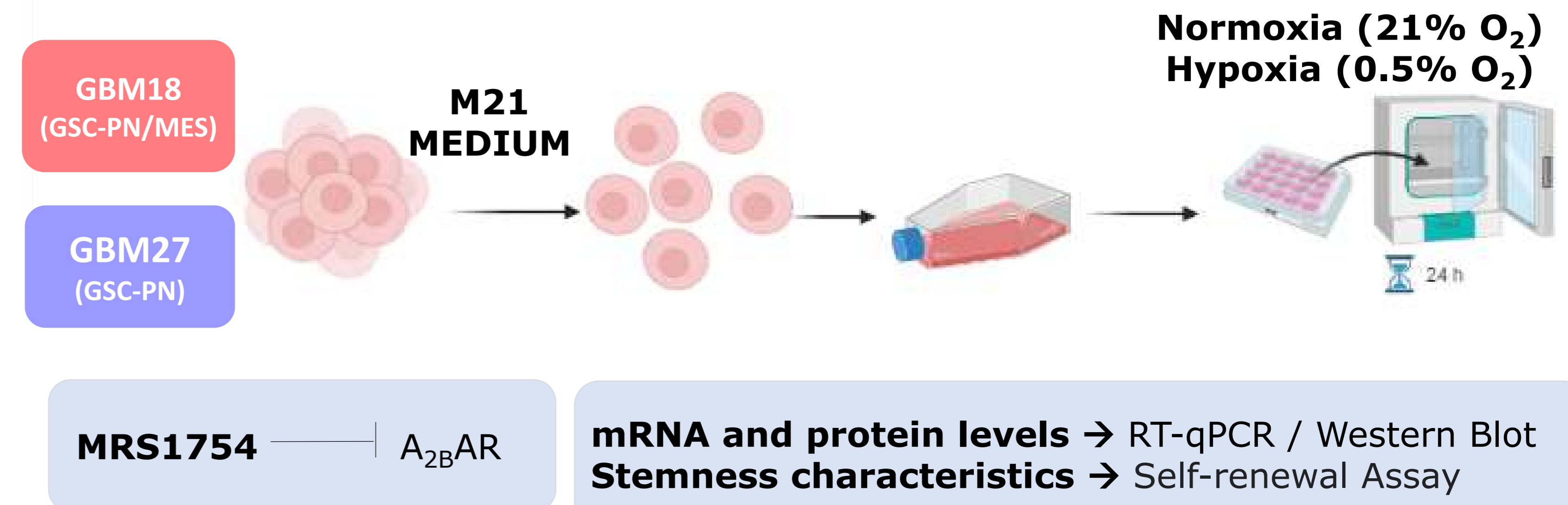
INTRODUCTION

Glioblastoma (GB) is the primary brain tumor with the worst prognosis. This is mainly due to a cellular subpopulation with stemness characteristics called Glioblastoma Stem Like Cells (GSCs). Two GSC subtypes has been described: proneural (GSC-PN) and mesenchymal (GSC-MES). These cells secrete large amounts of adenosine, which increases even more under hypoxia conditions. High adenosine levels signal through it lowest affinity receptor, A_{2B} (A_{2B}AR), thereby modulating signaling pathways involved in stemness maintenance.

OBJECTIVE

The aim of this study is to determine the role of A_{2B}AR in the maintenance of GSCs stemness under hypoxia using primary GBM cultures with different subtypes.

METHODOLOGY



RESULTS

A

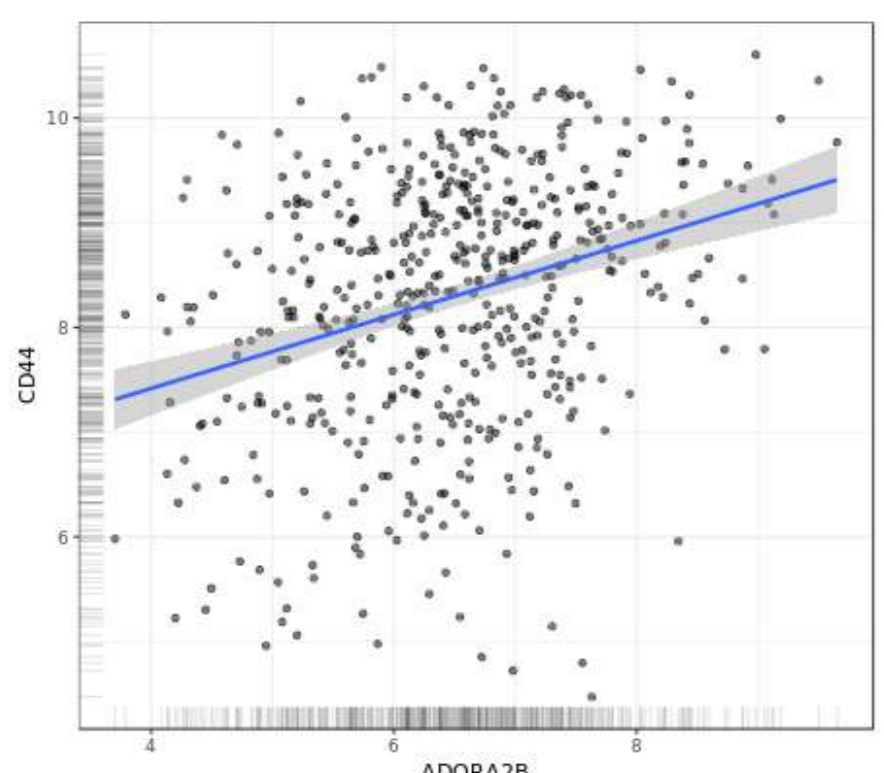
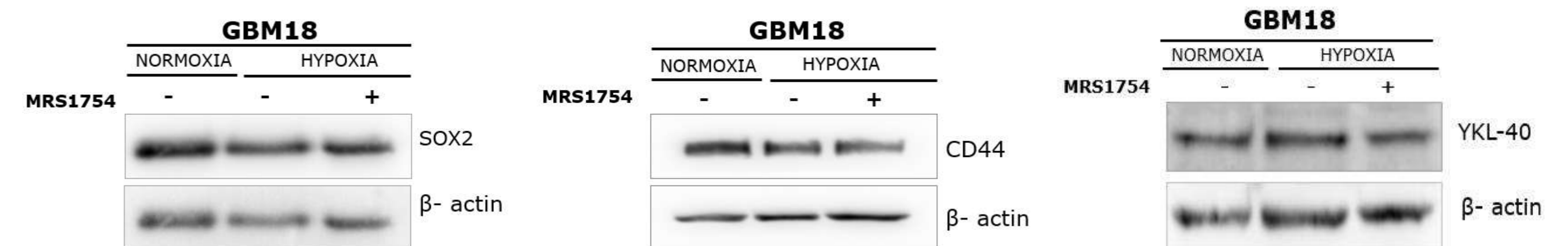


Figure 1. Expression correlation analysis. Receptor A_{2B}AR gene expression versus proteins expression related to stemness characteristics. **A)** CD44 by Gliosis platform.

A



B

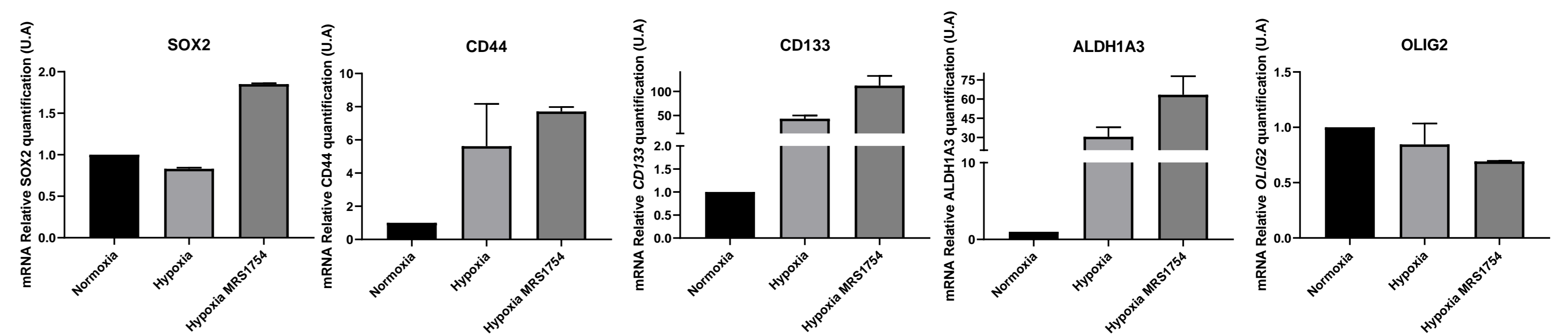
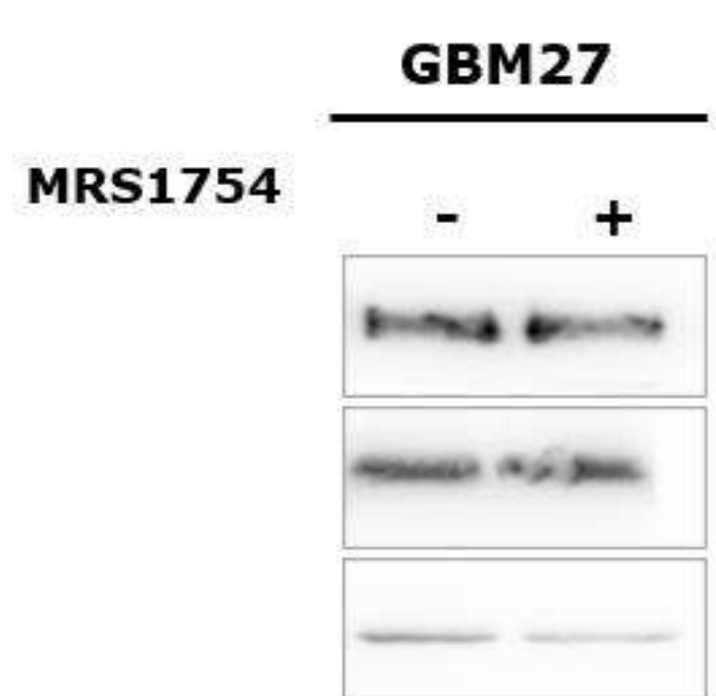


Figure 4. Effect of A_{2B}AR blockage with MRS1754 on the expression of stem and differentiation genes on mRNA and protein levels in hypoxia in GBM18.

A) SOX2, CD44 and YKL-40 protein levels of GBM18 under normoxia or hypoxia treated with 50uM MRS1754 for 24h by western blot. **B)** SOX2, CD44, CD133 and ALDH1A3 transcript levels of GBM18 under normoxia or hypoxia treated with 50uM MRS1754 for 24h by RT-qPCR.

A



B

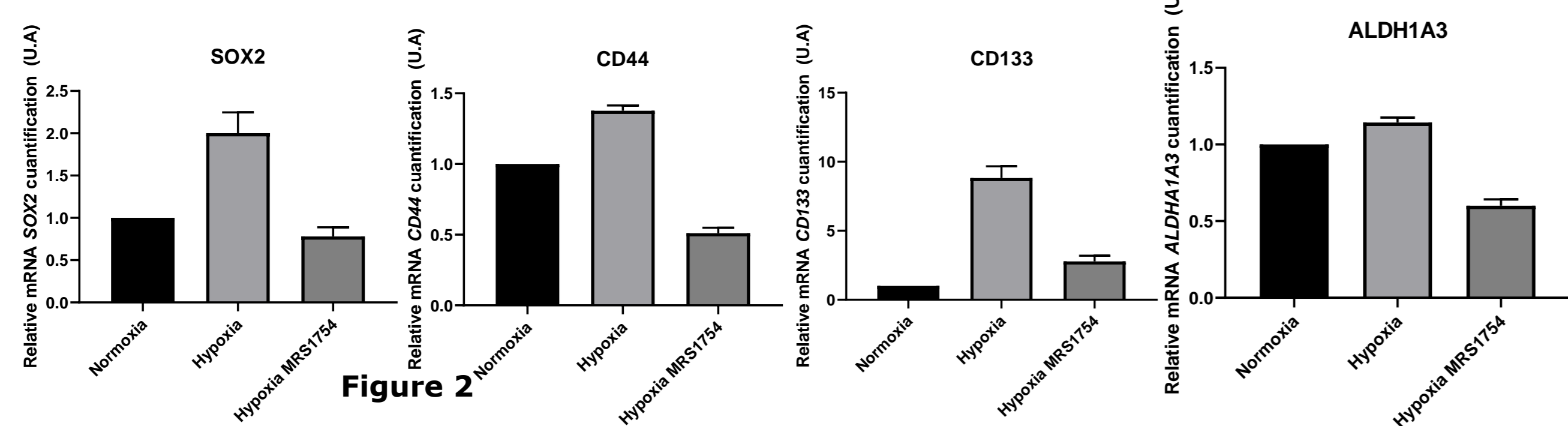
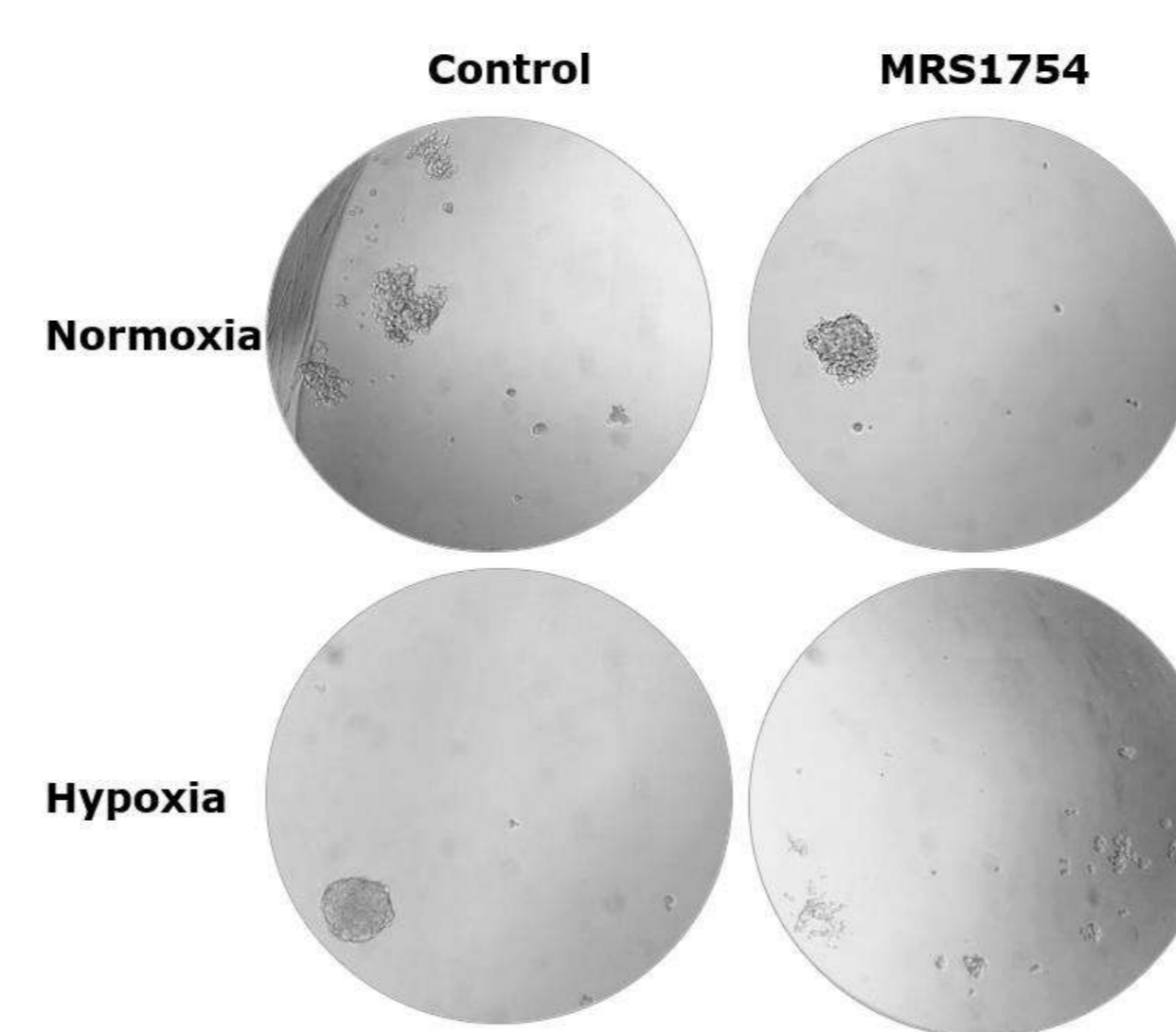


Figure 2. Effect of A_{2B}AR blockage with MRS1754 on the expression of stem and differentiation genes on mRNA and protein levels in hypoxia in GBM27.

A) SOX2 and CD44 protein levels of GBM27 under normoxia or hypoxia treated with 50uM MRS1754 for 24h by western blot. **B)** CD133, ALDH1A3, SOX2 and CD44 transcript levels of GBM27 under normoxia or hypoxia treated with 50uM MRS1754 for 24h by RT-qPCR.

A



B

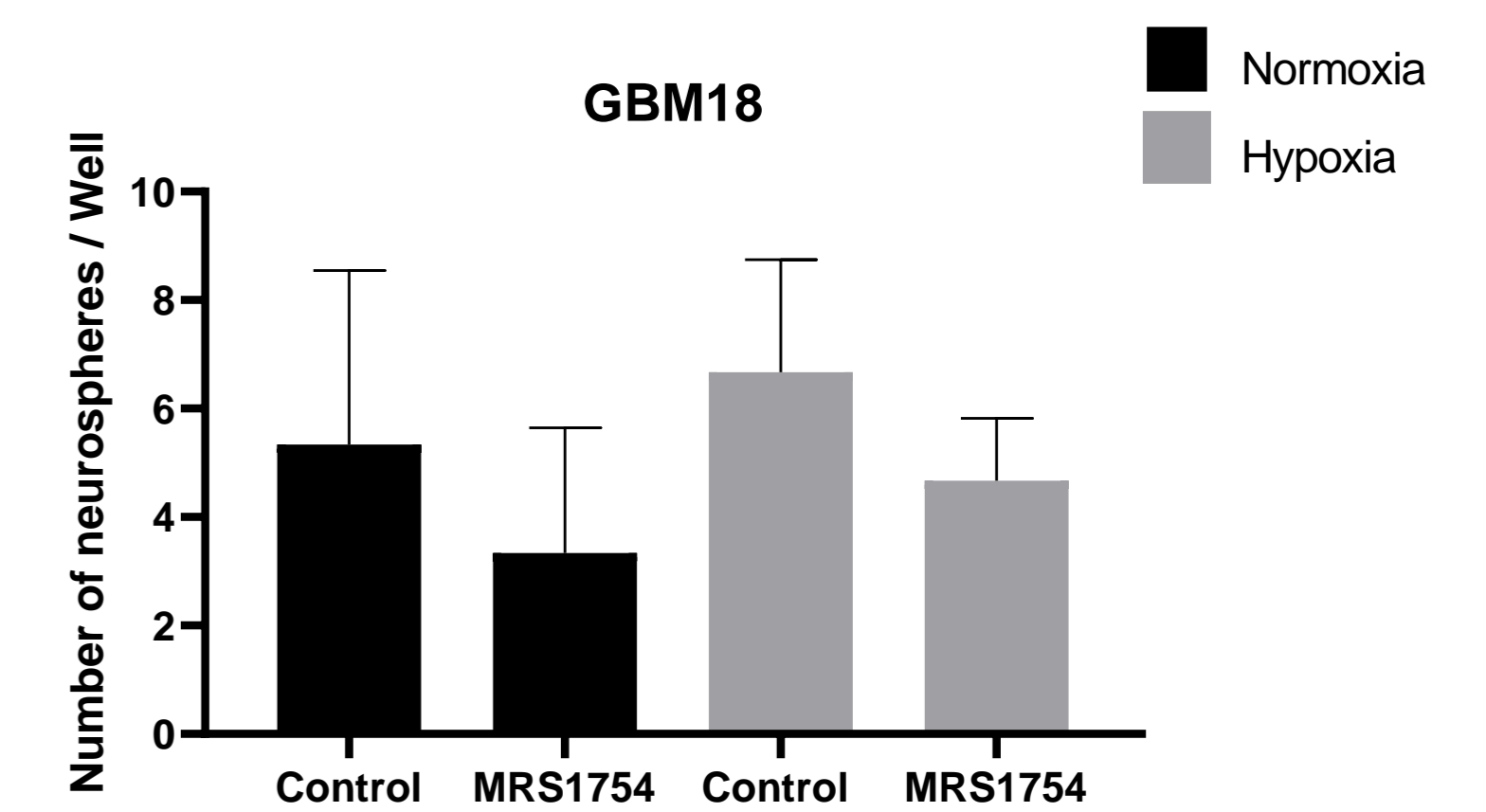
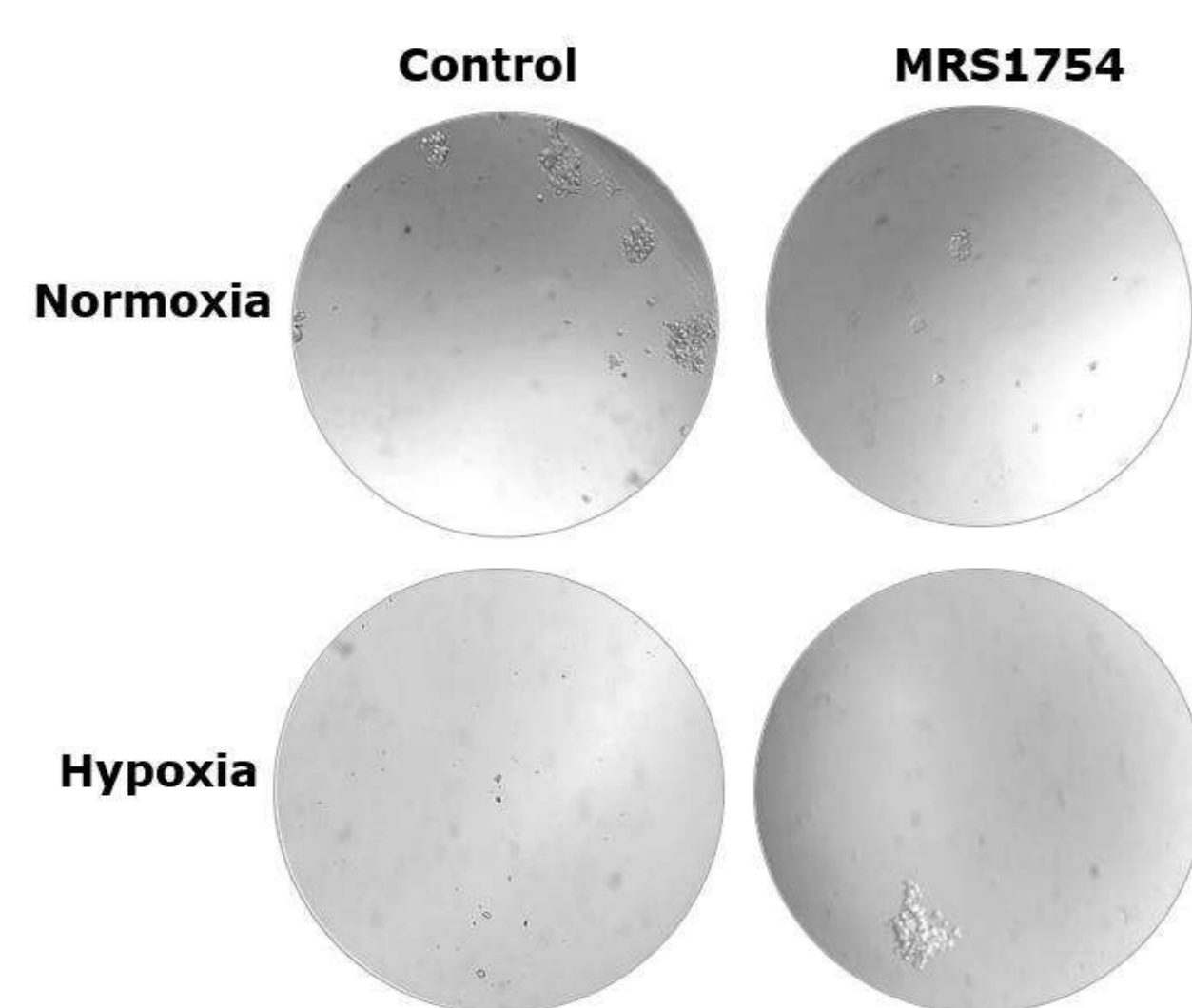


Figure 5. Effect of A_{2B}AR blockage with MRS1754 on GBM18 self-renewal capacity under normoxia and hypoxia.

A) Neurospheres formed for 14 days after being treated with MRS1754 50uM for 14 days under Normoxia (21% O₂) or Hypoxia (0.5%O₂) and **B)** and the results were plotted as a number of Neurospheres /Well.

A



B

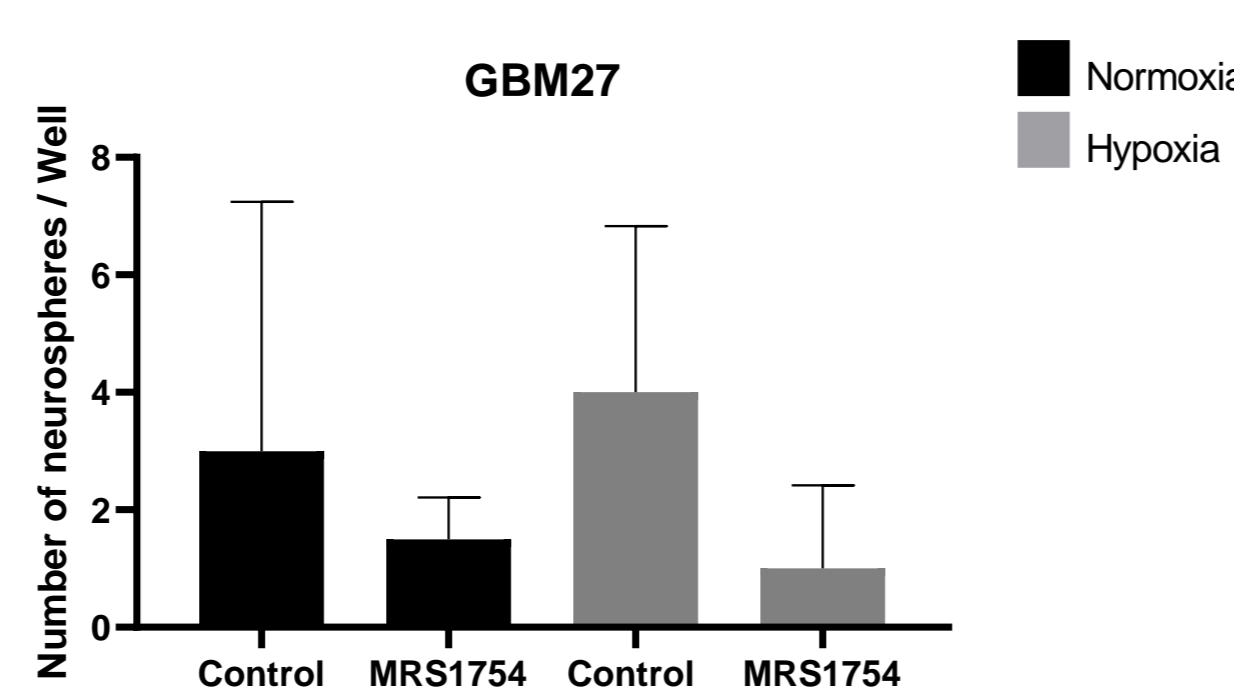
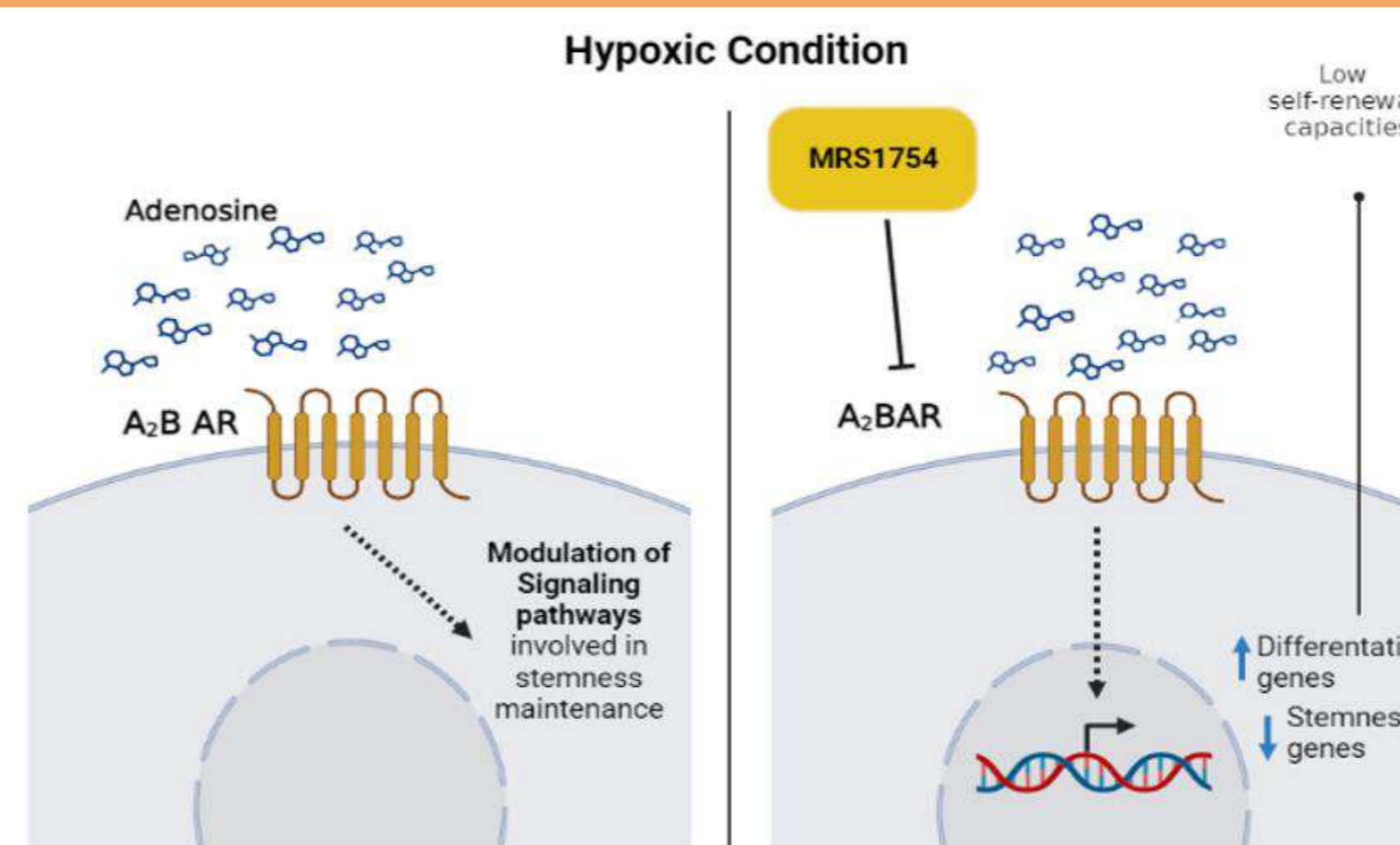


Figure 3. Effect of A_{2B}AR blockage with MRS1754 Block on GBM27 self-renewal capacity under normoxia and hypoxia.

A) Neurospheres formed for 14 days after being treated with MRS1754 50uM for 14 days under Normoxia (21% O₂) or Hypoxia (0.5%O₂) and **B)** and the results were plotted as a number of Neurospheres/Well.

CONCLUSION



The best effect of blockade of A_{2B}AR on the maintenance of GSC stemness was visualized in culture GBM27. This GSC subtype is probably more susceptible to this treatment

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