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Deregulated expression of imprinted DLK1-DIO3 region in glioblastoma stem-like cells (GSCs): tumor suppressor role of lncRNA MEG3

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Disclosures

- M. Martini

- Consulting: Astra Zeneca, MSD, Merck & Co., Novartis, Pfizer, Roche
- Scientific Advisory Board: Diatech

Background

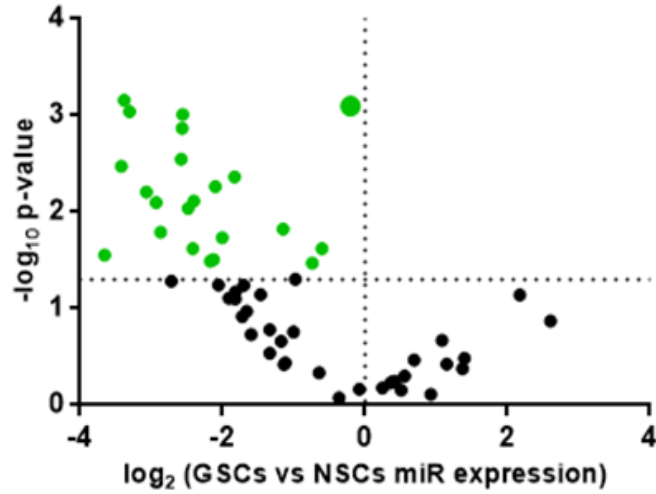
- The identification of GBM initiating stem-like cells (GSCs) has introduced a new paradigm in therapy, since these cells are likely to comprehend a population able to support tumor growth and should represent a primary therapeutic target¹
- During our recent study on GSCs profiles, where we identify two GSC clusters (proneural-like and mesenchymal-like phenotypes), we noticed that many of the miRNAs mapping to region q32.2 on Chr.14 were downregulated in both subtypes compared to normal neural stem cells²
- The region, contains the paternally expressed imprinted genes DLK1, DIO3 and RTL1, the maternally expressed imprinted lncRNAs (MEG3, MEG8, MEG9, LINC00524 and anti-sense RTL1), a large miRNA cluster (53 miRNAs), and two families of SNORDs (SNORD113 and SNORD114)
- The loss or the downregulation of MEG3 and MEG8 lncRNAs are recently described in an expanding list of human tumors, including neuroblastomas, meningiomas, and gliomas³

Material and Methods

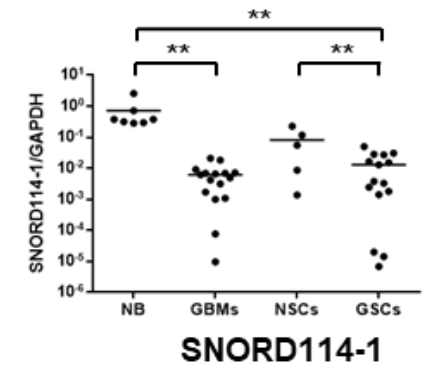
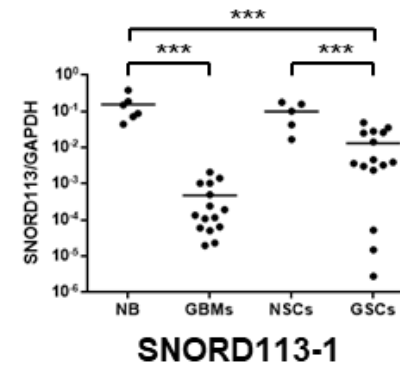
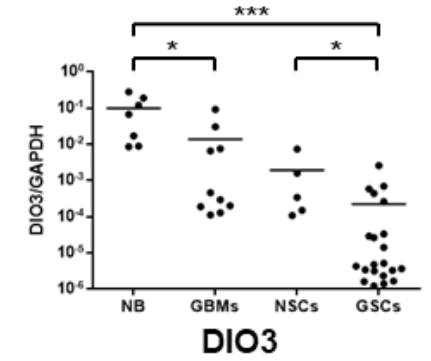
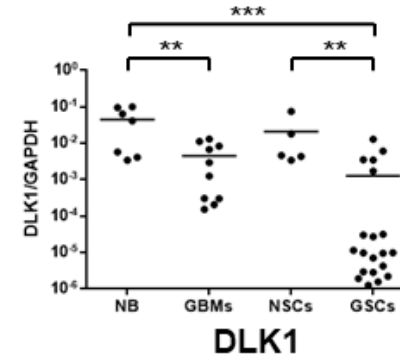
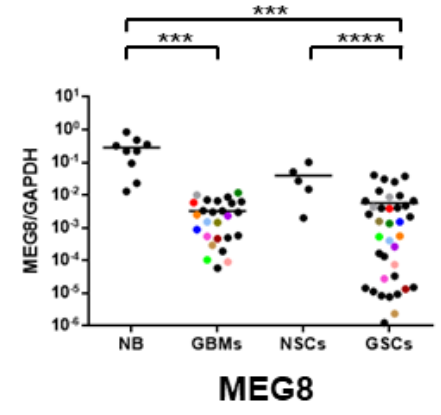
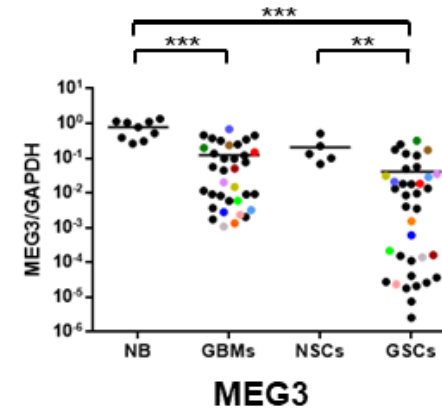
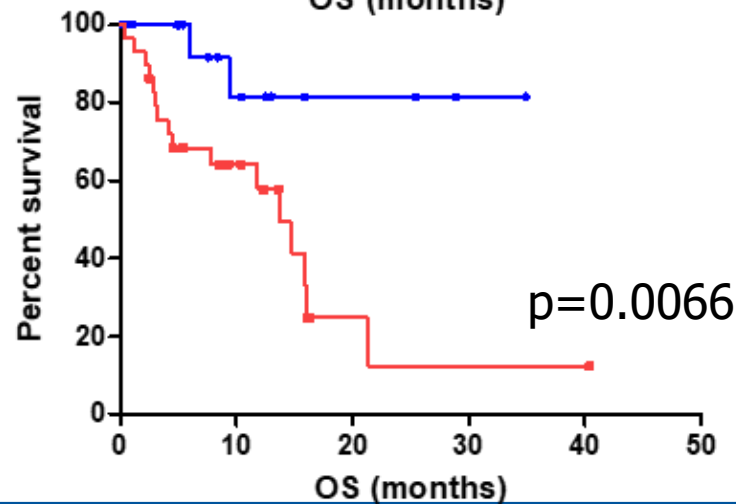
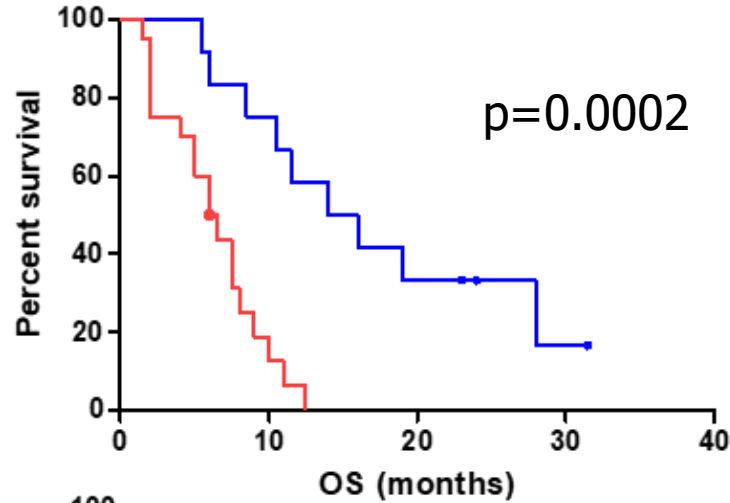
- 45 GSCs were isolated from surgical samples of adult patients with GBM. Human adult neural stem cell line, human neural progenitor cell lines were used as normal neural stem cells, while 293T, as Glioblastoma cell line.
- Real-time PCR was performed with SYBRTM Green Master Mix, MLPA analysis was performed by using ME032 UPD7-UPD14 A1 Kit, Methylation profile (Infinium Methylation 450K, Illumina) was performed by Genomix4life S.r.l
- Cell viability, cell proliferation, the motility assay and the colony formation ability was performed as previously described¹
- We used NOD-SCID mice for intracranial implantation and Nude athymic mice for subcutaneous implantation
- Gene array was performed using Agilent-019118 array for miRNAs and Affymetrix GeneChip1.0ST array while Reverse-Phase Protein Arrays was performed at the MD Anderson Cancer Center RPPA Core Facility on a per service basis
- Statistical analysis was performed using GraphPad-Prism 5 or MedCalc software

Results 1

- Dysregulation of DLK1-DIO3 transcripts in GBM and GSCs: expression of MEG3 and MEG8 lncRNAs is associated to GBM patient overall survival

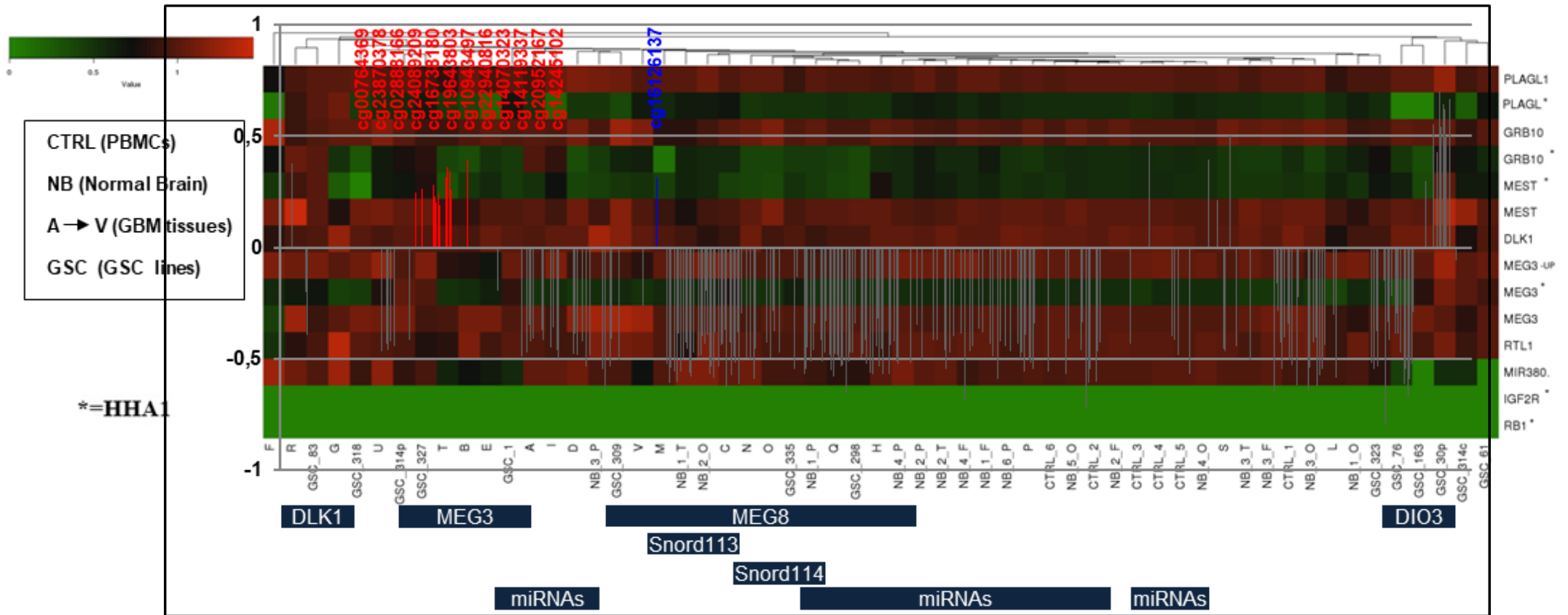


MEG3 and MEG8 expression levels
 — Above Median
 — Below Median



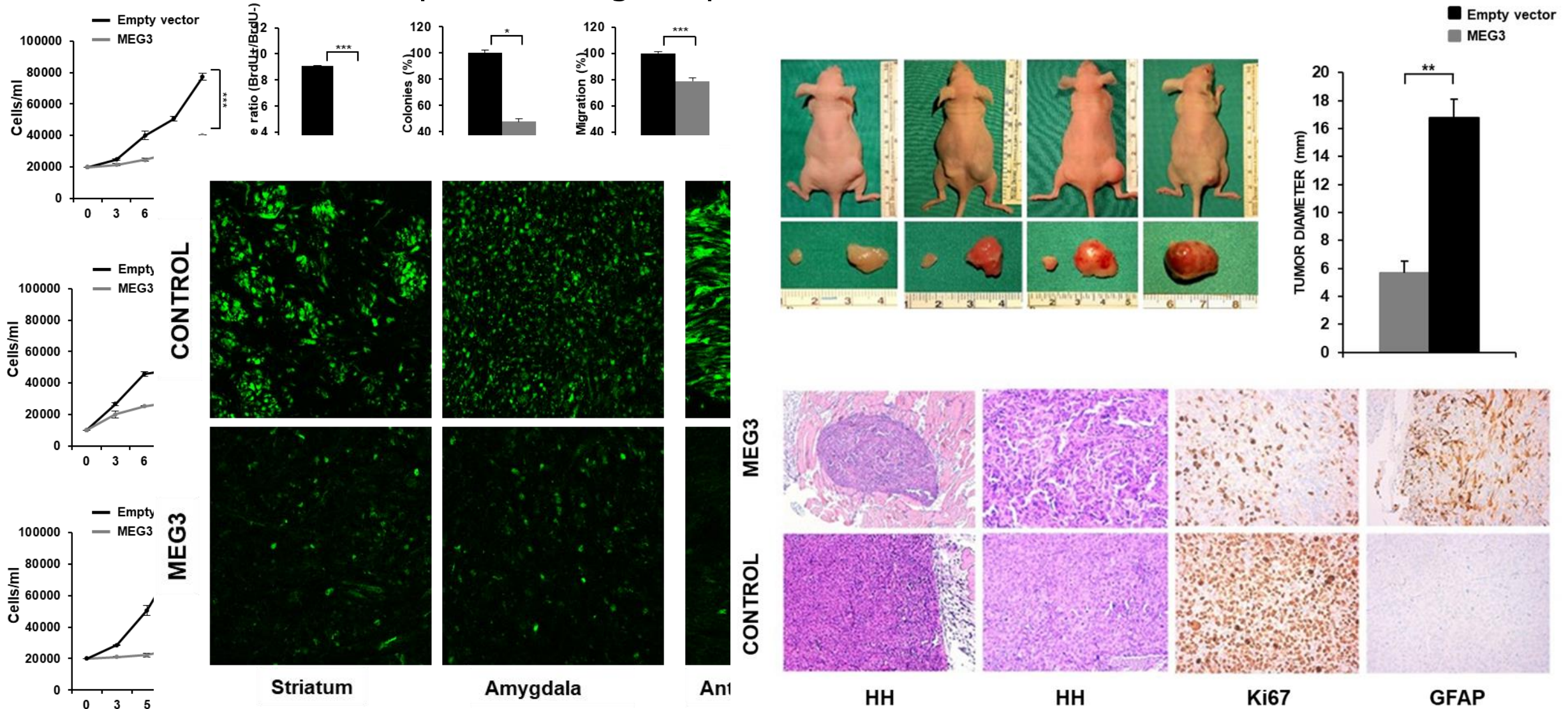
Results 2

- Epigenetic modifications are the main regulator of chromosome 14q32 gene expression in GSCs.



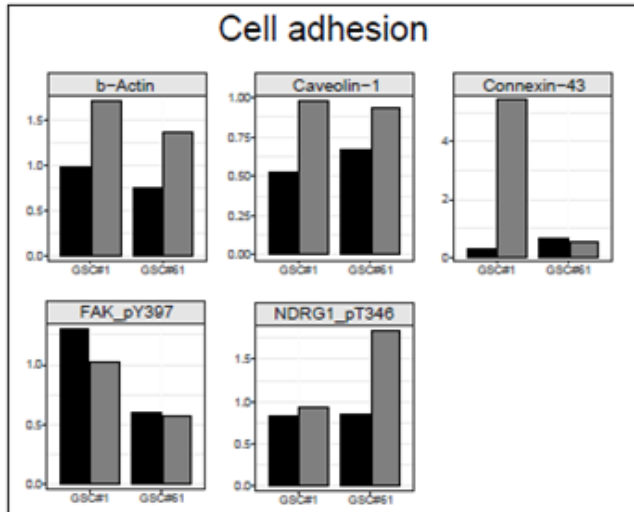
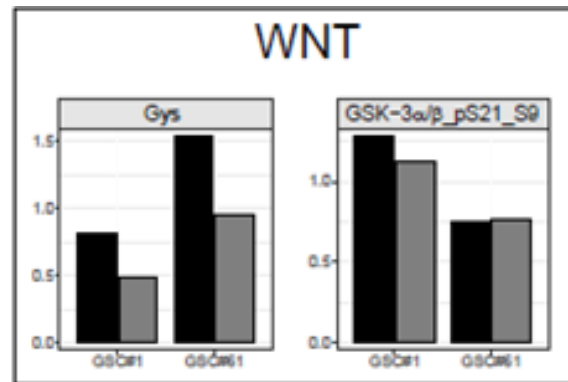
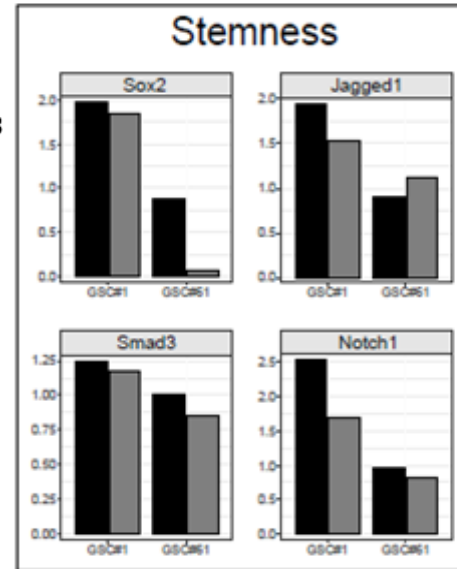
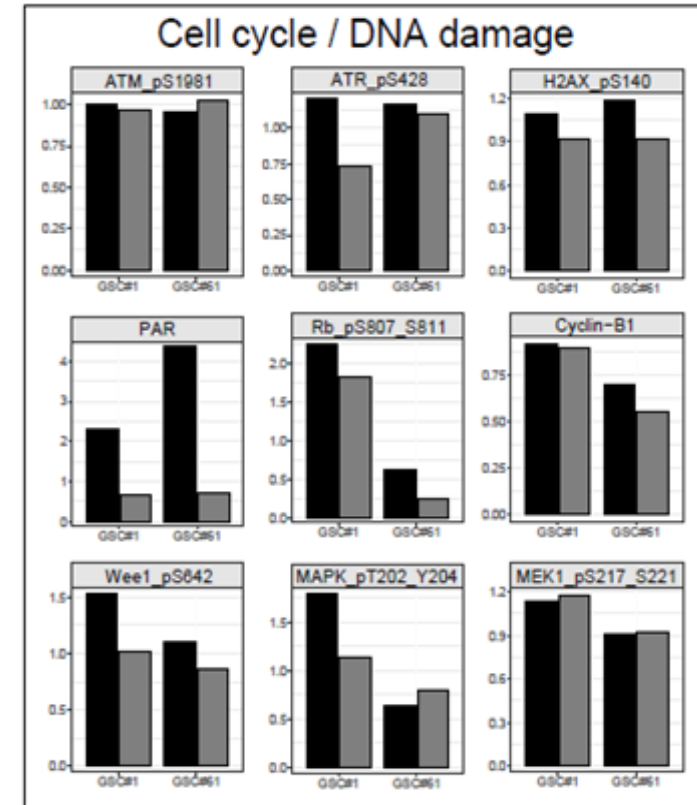
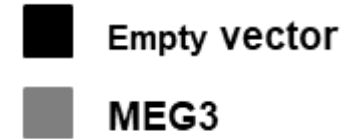
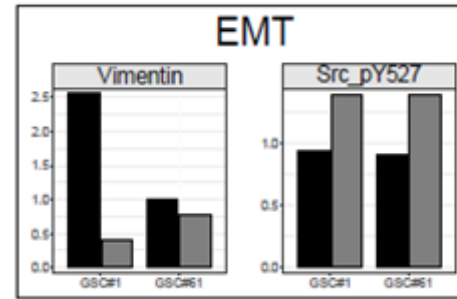
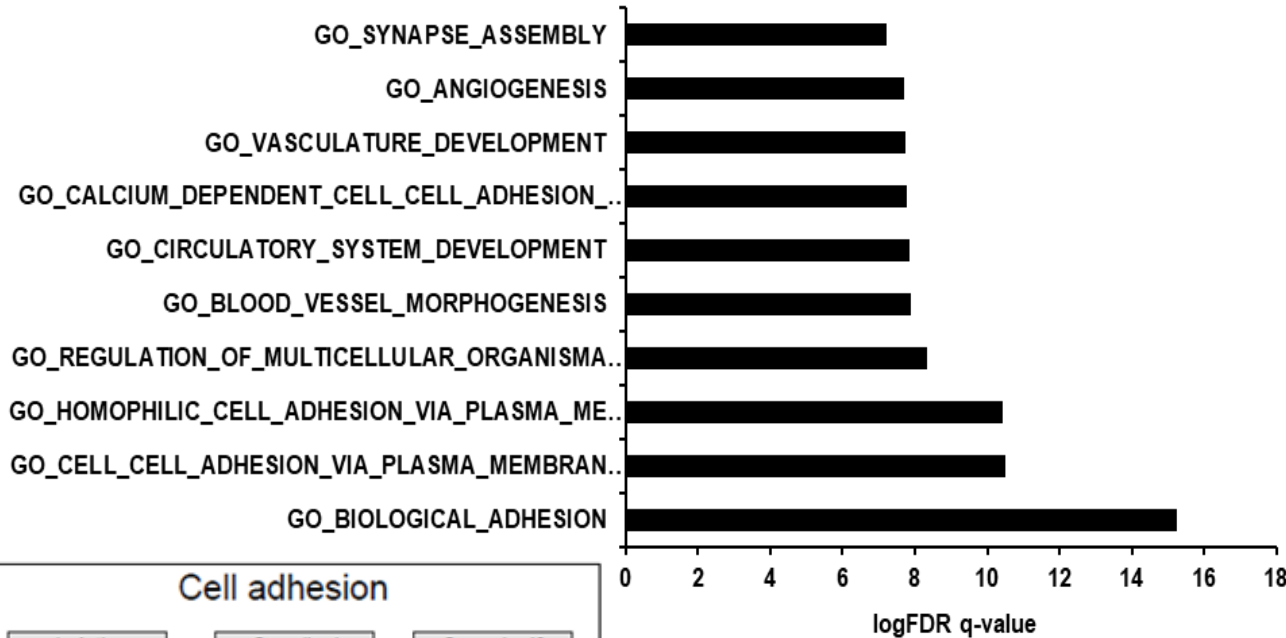
Results 3

• Restoration of MEG3 impairs tumorigenic properties of GSCs in vitro and in vivo



Results 4

- Tumor-suppressor function of MEG3 involved cell adhesion, EMT and stemness pathways



Conclusions

- Loss of expression of loci contained in the DLK1-DIO3 region on chromosome 14q32 is a frequent event in tumor samples and GSCs derived from GBM patients and is mainly mediated by epigenetic silencing
 - Lower MEG3 and MEG8 lncRNAs level was significantly associated to a poor outcome
 - In particular, MEG3 inhibited cell growth, migration and colony-forming ability of GSCs in vitro and significantly decreased the growth of GSC-derived tumors in vivo
- MEG3 negatively regulates the activity of several genes involved in EMT gene transcription (TGF β , c-Met), mainly acting as competing endogenous RNAs (ceRNAs).
- MEG3, through a direct action on protocadherin-beta, modulates the expression of Matrix Gla Protein (MGP), Periostin (POSTN) and Versican (VCAN) which play a key role in glioma invasion and migration
- MEG3 acts as a tumor-suppressor mainly regulating cell adhesion, EMT and cell proliferation, thus providing a potential candidate for novel GBM therapies.

Acknowledgments

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**WORKING
GROUP**

Thank you

