GATA-4, a potential novel therapeutic target for high-grade meningioma, regulates miR-497, a potential novel circulating biomarker for high-grade meningioma

Caterina Negroni[1], PhD, David A Hilton[2], Dr, Emanuela Ercolano[1], PhD, Claire L Adams[1], PhD, Kathreena M Kurian[3], Dr, Daniele Baiz[1, §], PhD, and C Oliver Hanemann[1, §*], MD PhD, FRCP.

[1]University of Plymouth, Faculty of Medicine and Dentistry, The Institute of Translational and Stratified Medicine, The John Bull Building, Plymouth Science Park, Research Way, Plymouth UK, PL6 8BU

[2]Cellular and Anatomical Pathology, University Hospitals Plymouth NHS Trust, Derriford Road, Plymouth UK, PL6 8DH

[3]Institute of Clinical Neuroscience, University of Bristol and Southmead Hospital – North Bristol Trust, Bristol UK, BS8 1QU

[§]Equal contribution

[*]**Corresponding author**: Prof Clemens Oliver Hanemann MD, FRCP, University of Plymouth, Faculty of Health: Medicine, Dentistry and Human Sciences, Plymouth Science Park, Research Way, Plymouth UK, PL6 8BU. Phone: +44 1752437418, Fax: +441752517846, E-mail: oliver.hanemann@plymouth.ac.uk

Supplemental Figures

Supplemental Figure 1

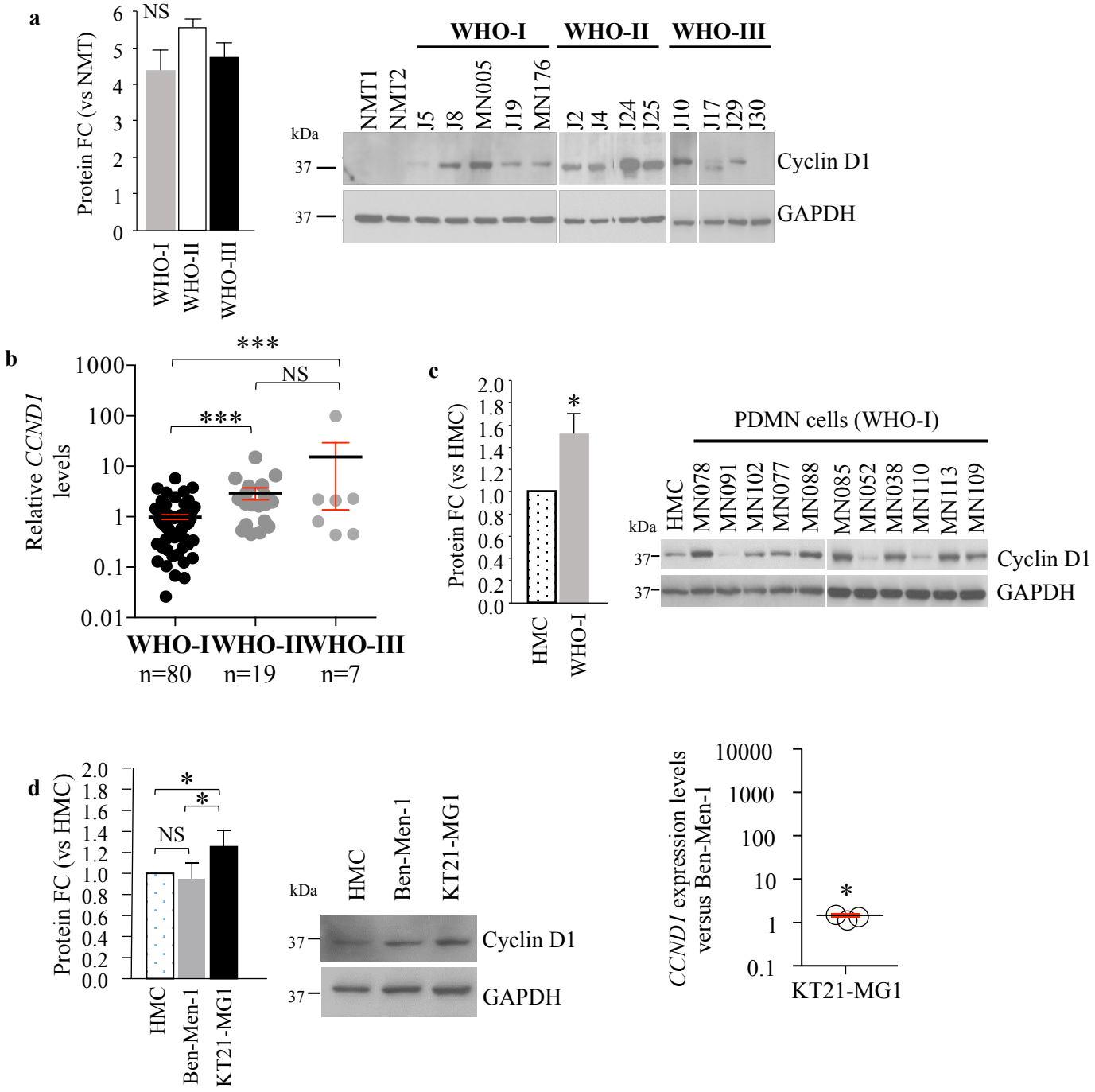

Figure S1 Protein analysis of Cyclin D1 demonstrated its overexpression in meningioma tissues and cells. a Densitometry analysis and representative Western blot of Cyclin D1 in tumour specimens (WHO I n=5, WHO II n=4, WHO III n=~~5~~4). **b** qPCR analysis of Cyclin D1 in tumour specimens. Data have been normalised to the mean of WHO I meninigomas (n=8~~2~~0). **C** Densitometry analysis and representative Western blot of Cyclin D1 in PDMN cells (WHO-I, n = 11) when compared to HMC cells, showing higher levels in WHO I PDMN cells (1·5 folds, when compared to HMC). **d** On the left, densitometry analysis and representative Western blot of Cyclin D1 in meningioma cell lines Ben-Men-1 and KT21-MG1. On the right, relative gene expression analysis (conducted by RT-qPCR) of Cyclin D1 (0·16 Log_{10} folds increase), in KT21-MG1 cells when compared to Ben-Men-1 cells. All cell lines were profiled at three different passages to ensure data consistency. (One-way ANOVA, Student's t-Test; NS=not significant; *=p<0·05, ***=p<0·005).

Supplemental Figure 2

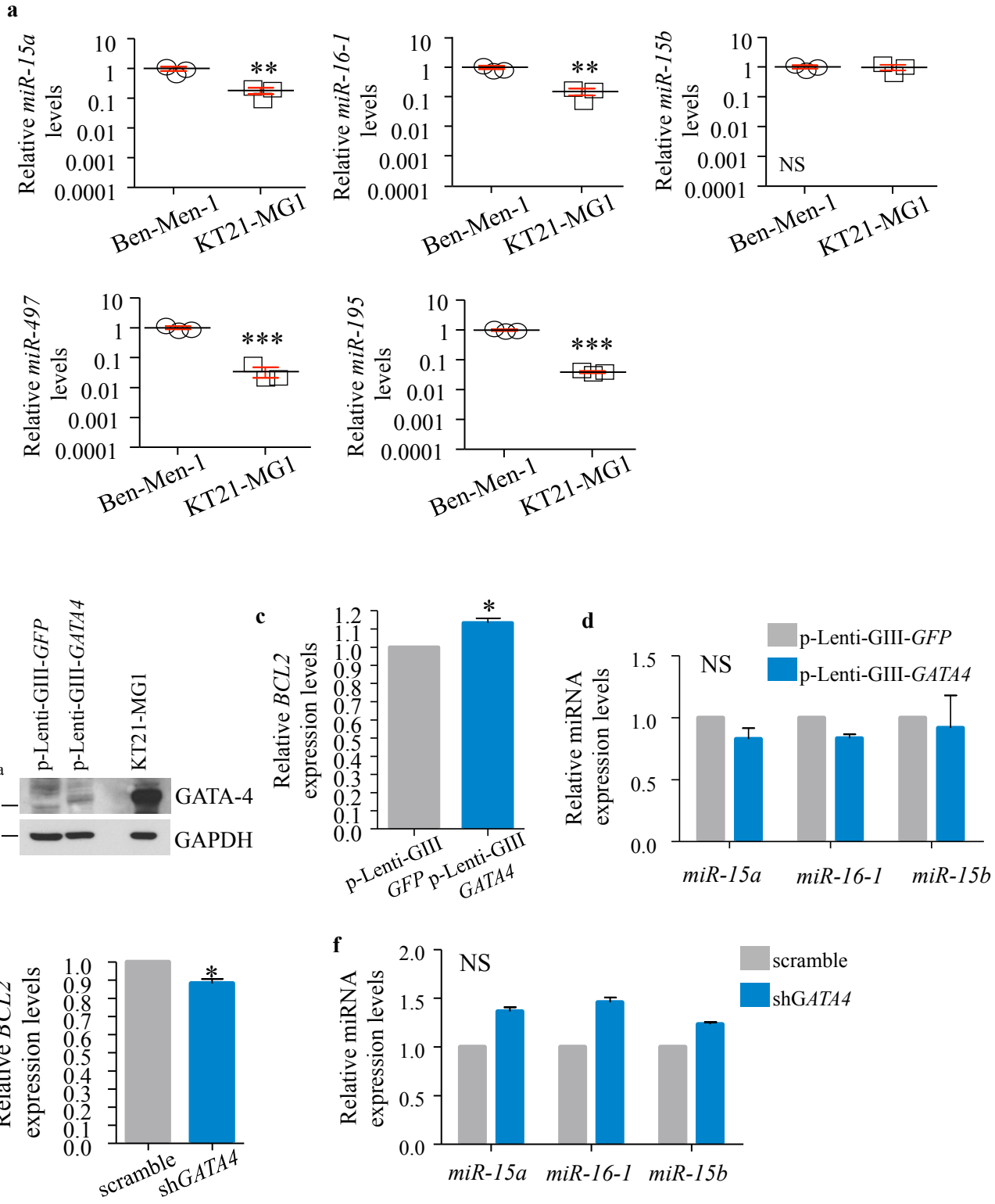

Figure S2 Lentiviral-mediated modulation of GATA-4 in a model of meningioma, *in vitro*. **a** RT-qPCR analysis showing downregulation of the miR-15 family in KT21-MG1 cells, compared to Ben-Men-1 cells. All cell lines have been analysed at three different passages to ensure consistency of the results. **b** Representative Western blot showing specificity of GATA-4 antibody, based on high expression in KT21-MG1 cells. **c** RT-qPCR of *BCL2* after *GATA4* overexpression, compared to GFP. **d** Relative expression levels of the miR-15a, -16-1, and -15b in Ben-Men-1 cells after ectopic expression of *GATA4*, compared to GFP. **e** RT-qPCR revealed a significant decrease of *BCL2* levels when compared to scramble. **f** Relative expression levels of the miR-15a, -16-1, and -15b following *GATA4* knockdown in KT21-MG1 cells, when compared to scramble. Lentiviral-mediated transduction has been performed in three independent experiments, with one replica per experiment. (Student's t-Test; NS=not significant; *=p<0·05, **=p<0·01, *** = p<0·005).

Supplemental Figure 3

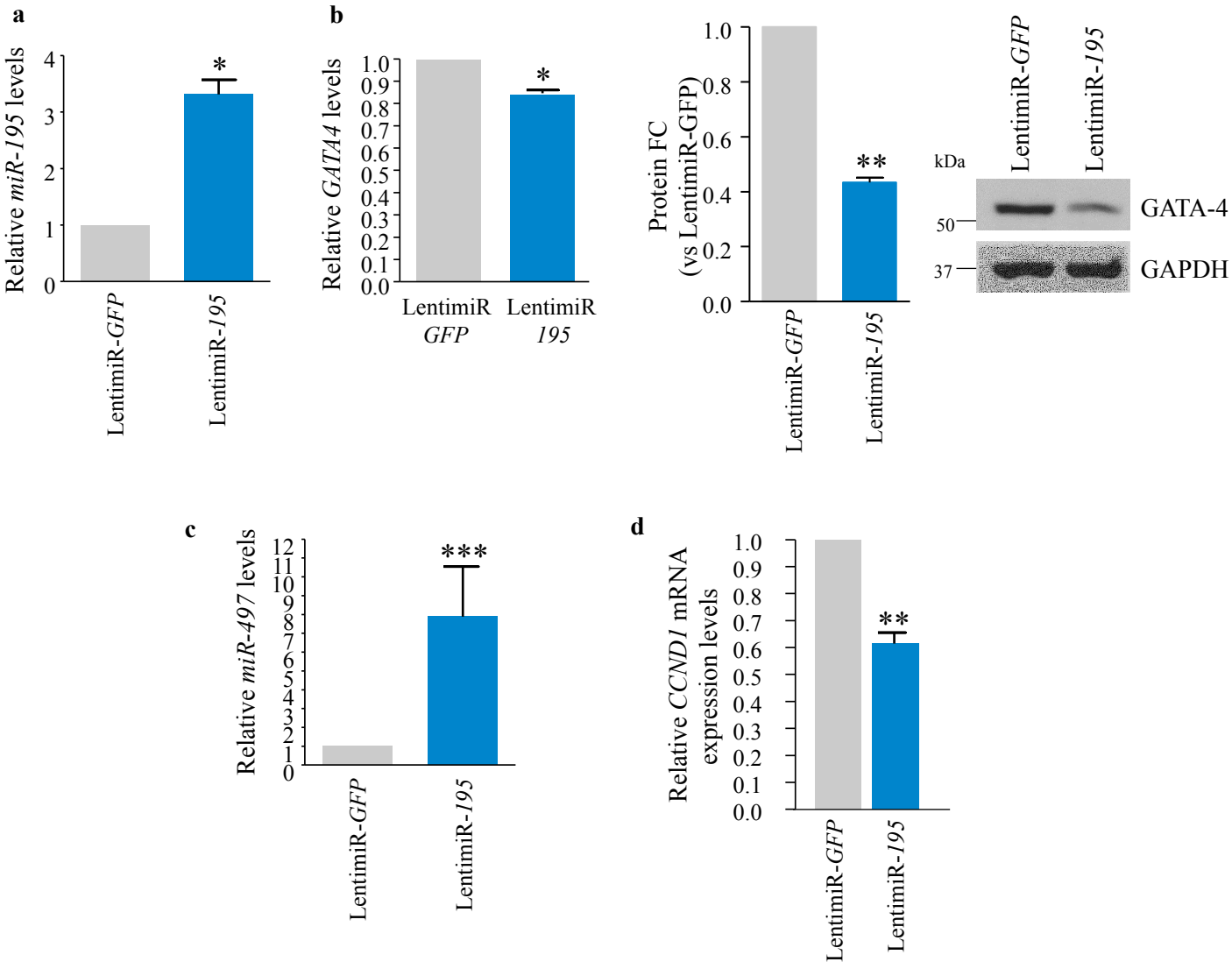

Figure S3 Lentiviral-mediated overexpression of miR-195 in KT21-MG1 cells. a RT-qPCR analysis of KT21-MG1 cells infected with the LentimiRa-GFP-hsa-miR-195-5p (LentimiR-*195*), detected a 3·31 fold increase of miR-195, when compared to LentimiR-GFP-infected cells. **B** Left, relative expression levels of *GATA4* in KT21-MG1 cells after ectopic expression of miR-195-5p, when compared to LentimiR-GFP. Right, densitometry analysis and representative Western blot showing decreased GATA-4 protein levels after overexpression of miR-195-5p. **c** RT-qPCR analysis of miR-497 after overexpression of miR-195-5p. **d** RT-qPCR analysis showing a significant decrease of the expression levels of *CCND1* following miR-195-5p overexpression, when compared to LentimiR-GFP-infected cells. miR-195 overexpression has been performed in three biological replicates to ensure consistency. (Student's t-Test; *=p<0·05, **=p<0·01, ***=p<0·005).

Supplemental Figure 4

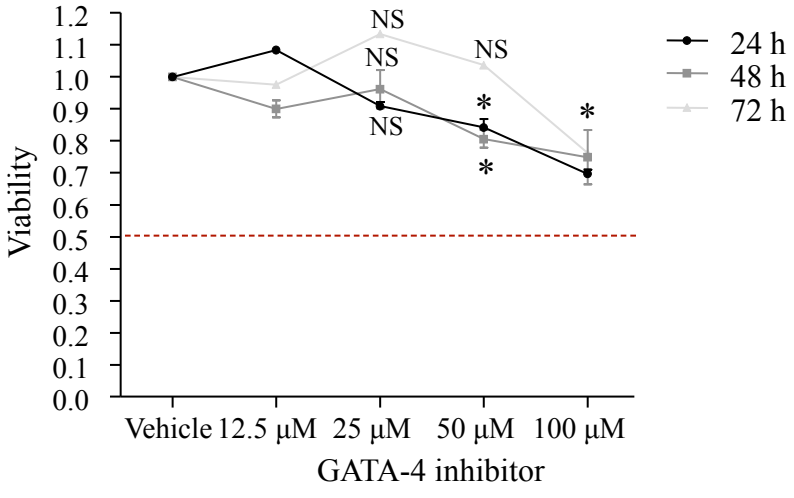

Figure S4 Cell viability measured by ATP assay following treatment of KT21-MG1 cells with the GATA-4 small-molecule inhibitor NSC 140905 for 24, 48, and 72 hours. Vehicle treated cells were administered 0·1% DMSO, which corresponds to the highest concentration of DMSO used. The experiment has been performed in three biological and technical replicas to ensure data consistency. (Two-way ANOVA; NS=not significant, *=p<0·05).

Supplemental Figure 5

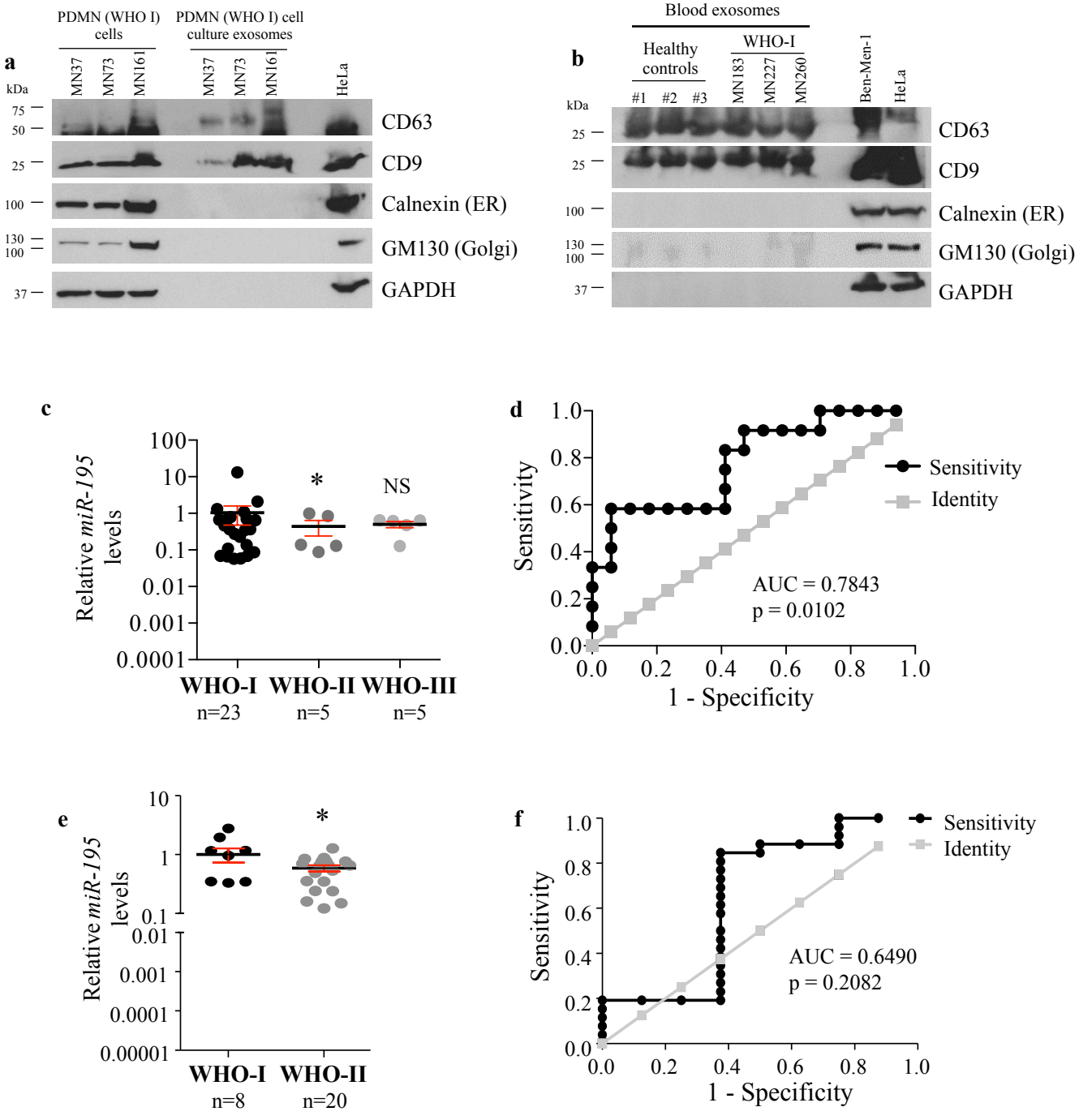

Figure S5 Analysis of miRNA cargo in meningioma exosomes. a Representative Western blot showing assessment of cell culture media-derived exosomes enrichments. **b** Representative Western blot showing assessment of circulating exosomes enrichments. **c, d** Discovery cohort for the investigation of miR-195 expression levels and diagnostic value in circulating exosomes. Data have been normalised to the mean of WHO I serum exosomes (n=234). ROC analysis of the miRNA-195 comparing low- *vs.* higher-grade patients (AUC and p-value reported in figure, 95% confidence interval=0·6127 to 0·9559 ~~0·5446-0·9048~~). **e, f** miR-195 expression levels in an independent validation cohort. Data have been normalised to the mean of WHO I serum exosomes (n=8). ROC analysis of miR-195 comparing WHO-I and -II meningioma patients (AUC and p-value reported in figure, 95% confidence interval=0·5134-0·7796). N numbers for WHO I, II, and III serum-derived exosomes are reported in figure. (One-way ANOVA, Student's t-Test; NS=not significant, *=p<0·05).