Background: Small Extracellular Vesicles (sEVs) are major intercellular communicators, with growing evidence of a role in establishing favourable metastatic niches during cancer progression. We have shown that, relative to their paired primary counterpart, Group 4 (Grp4) metastatic Medulloblastoma (MB) cell-lines release significantly more sEVs with increased functionally active MMP-2 on their surface, resulting in enhanced extracellular matrix degradation and subsequently increased cell invasiveness. Methods: Transcriptomic analysis of sEV microRNA cargoes derived from metastatic/recurrent and primary cell-line and patient CSF samples, followed by microRNA target prediction of differentially abundant microRNAs using TargetScan and miRDB was conducted. Patient dataset analysis was used to confirm microRNA target mRNA expression levels. Finally, microRNA inhibition followed by RT-qPCR or invasion
assays were used to quantify changes at the gene and functional level. RESULTS: Transcriptomic analysis demonstrated >75% miR-106b-5p depletion in Grp4 MB metastatic sEVs compared to their primary sEV counterparts derived from cell-line and patient CSF samples. This microRNA is predicted to target TIMP-2, which plays a crucial role in MMP-2 activation. We confirmed TIMP-2 to be upregulated in paired metastatic Grp4 tumours relative to their primary counterpart using a publicly available patient dataset. Following miR-106b-5p inhibition in the primary cell-line, RT-qPCR analysis and invasion assays determined the existence of a complex functional response, with increasing TIMP-2 gene expression leading to decreasing invasiveness. This suggests a delicately balanced process where TIMP-2 overabundance can ultimately impede MMP-2 activation. In support of this, analysis of patient datasets showed intermediate TIMP-2 gene expression amongst Grp3/4 patients compared to less frequently metastatic subgroups. CONCLUSIONS: Our data support the hypothesis that sEV-derived miR-106b-5p regulates enhanced ECM degradation through fine-tuning MMP-2 activation via differential TIMP-2 abundance. Future work will quantify changes in cell/sEV surface-bound TIMP-2 and MMP-2 alongside MMP-2 activation via flow cytometry and gelatin zymography respectively.