

Supplementary Materials:

LNX1 Modulates Notch1 Signaling to Promote Expansion of the Glioma Stem Cell Population During Temozolomide Therapy in Glioblastoma

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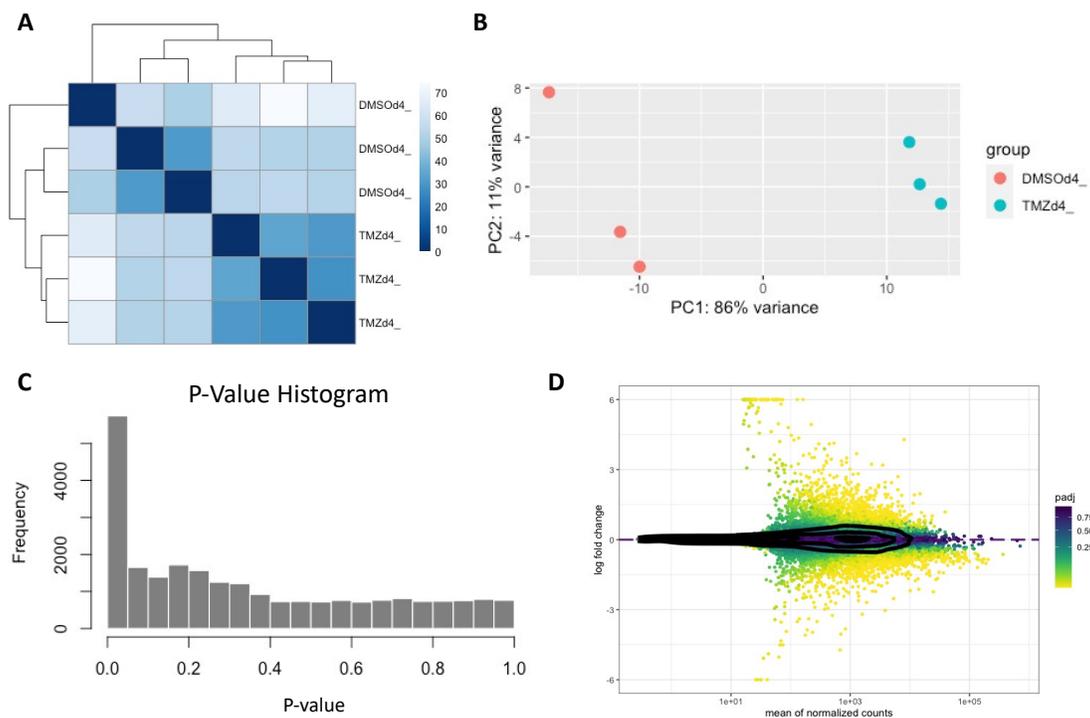


Figure S1. RNA-Seq Quality Metrics. **(A)** Sample distance clustering shows that replicates are more similar to each other and that there are differences between our control and treatment groups. **(B)** PCA shows that the greatest element of difference between our samples comes from the treatment performed. **(C)** Waterfall plot of p-values shows that we have a number of genes that were significantly altered **(D)** MA-plot represents appropriate shape for trustworthy RNA-Seq data.

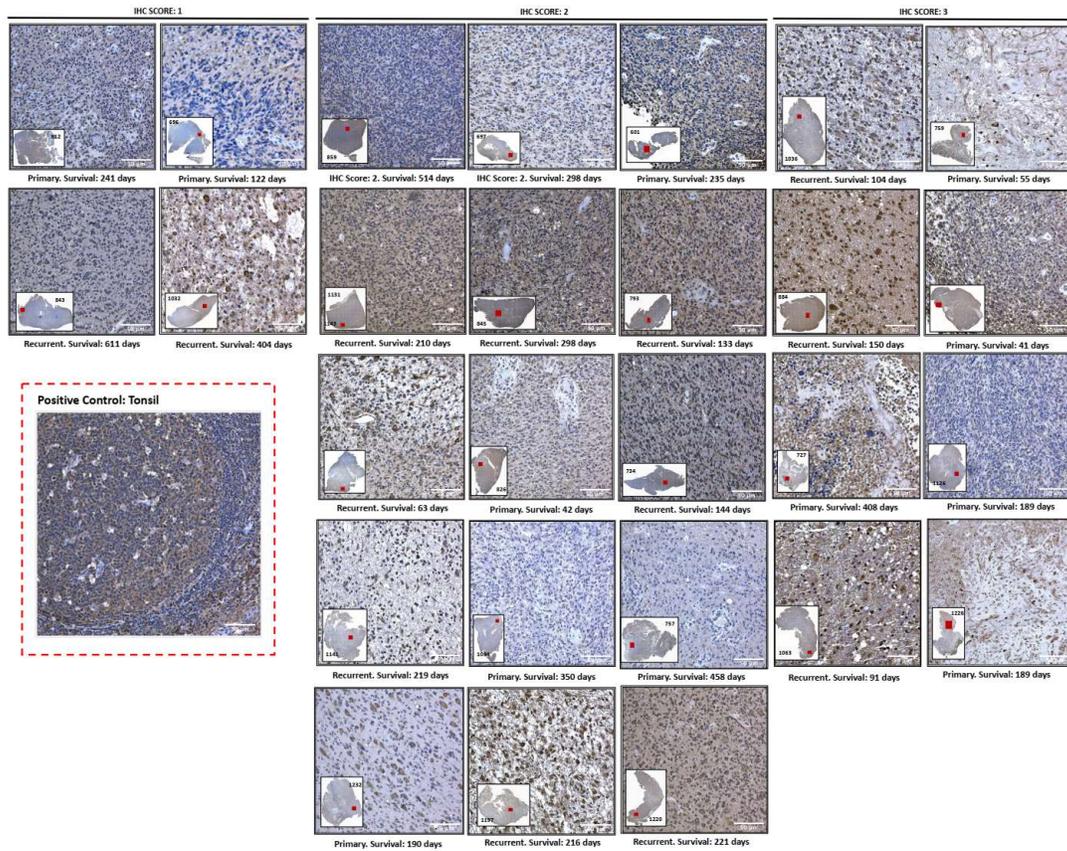


Figure S2. LNX1 Expression in Patient Samples. Images represent immunohistochemistry against LNX1 in GBM patient samples isolated from consenting patients at our institution. Samples were scored by a board-certified neuropathologist and de-identified survival data was correlated with the scores. Positive control for staining was tonsillar tissue, which is known to express LNX1 at high levels.

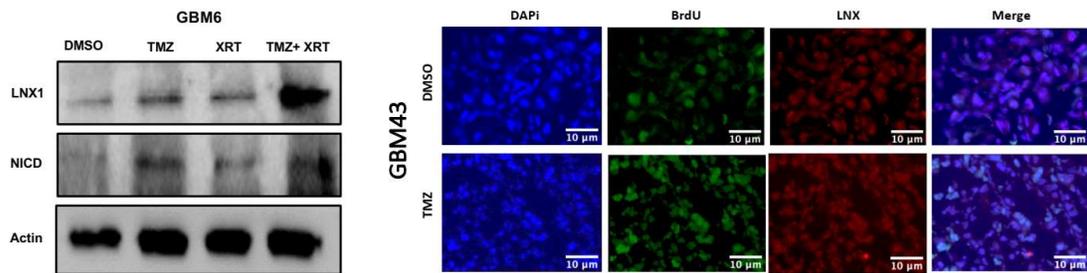


Figure S3. LNX1 Expression Elevates After Exposure to Therapeutic Stress. (A) LNX1 and NICD levels were assessed by western blot after exposure to TMZ, radiation, and combination therapy. Results showed that both were elevated upon exposure to therapeutic stress. (B) GBM43 cells were implanted intracranially to generate an animal tumor model. Tissues were then sectioned and stained for LNX1 and the proliferation marker BrdU. Increased expression of both is noted in tumor areas after TMZ therapy as compared to control DMSO.

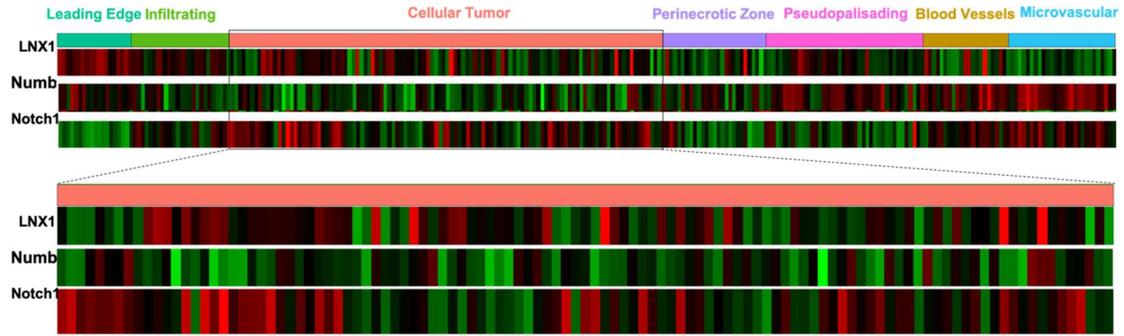


Figure S4. IvyGap provides in situ RNA hybridization data showing expression of genes within various regions of patient tumors. The cellular tumor was assessed for LNX1, Numb, and Notch1 expression and reflected that LNX1 was elevated, corresponding with Numb loss, and Notch1 elevations.

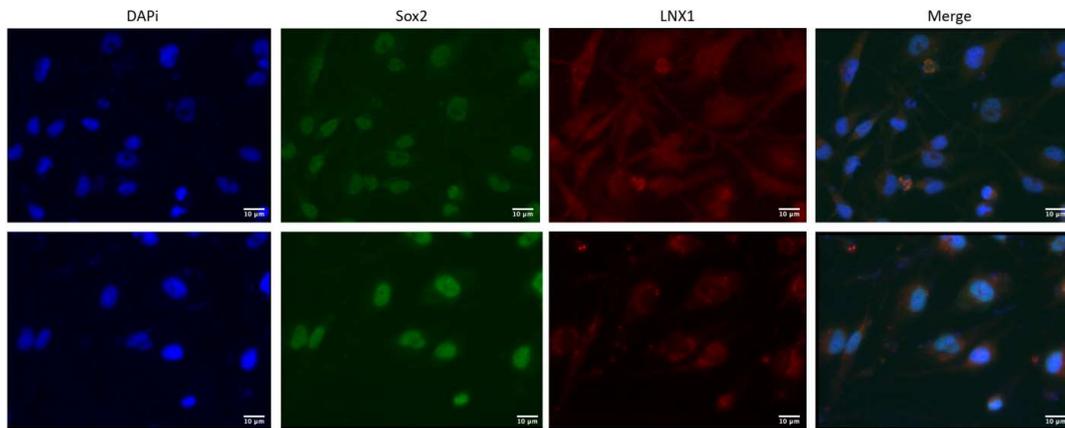


Figure S5. Cell identity confirmation was performed by immunostaining for LNX1 and Sox2, a well-known stem cell marker. Results showed that cells expressing LNX1 consistently expressed Sox2, thus confirming their identity as GSCs.

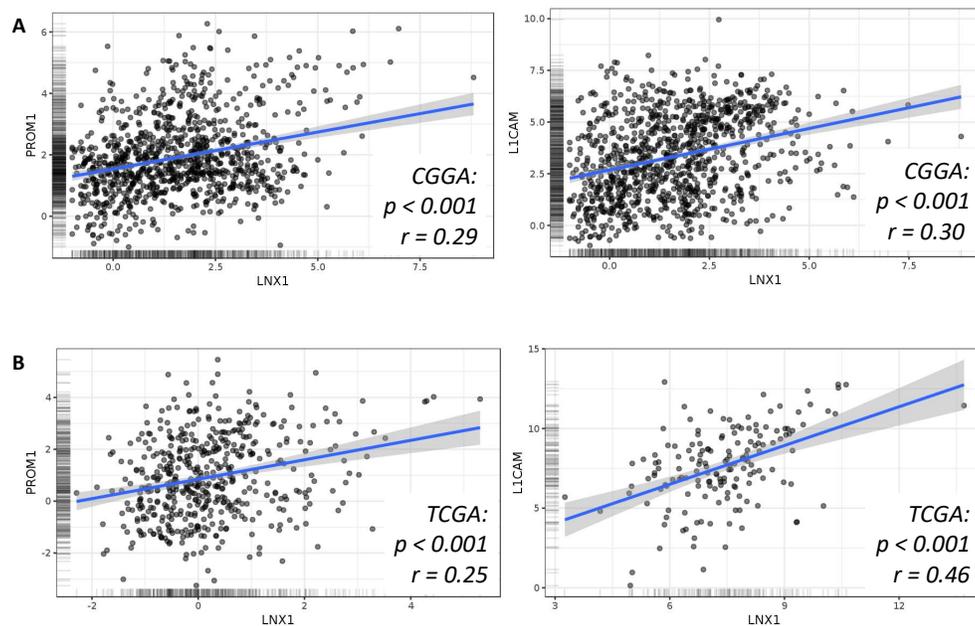
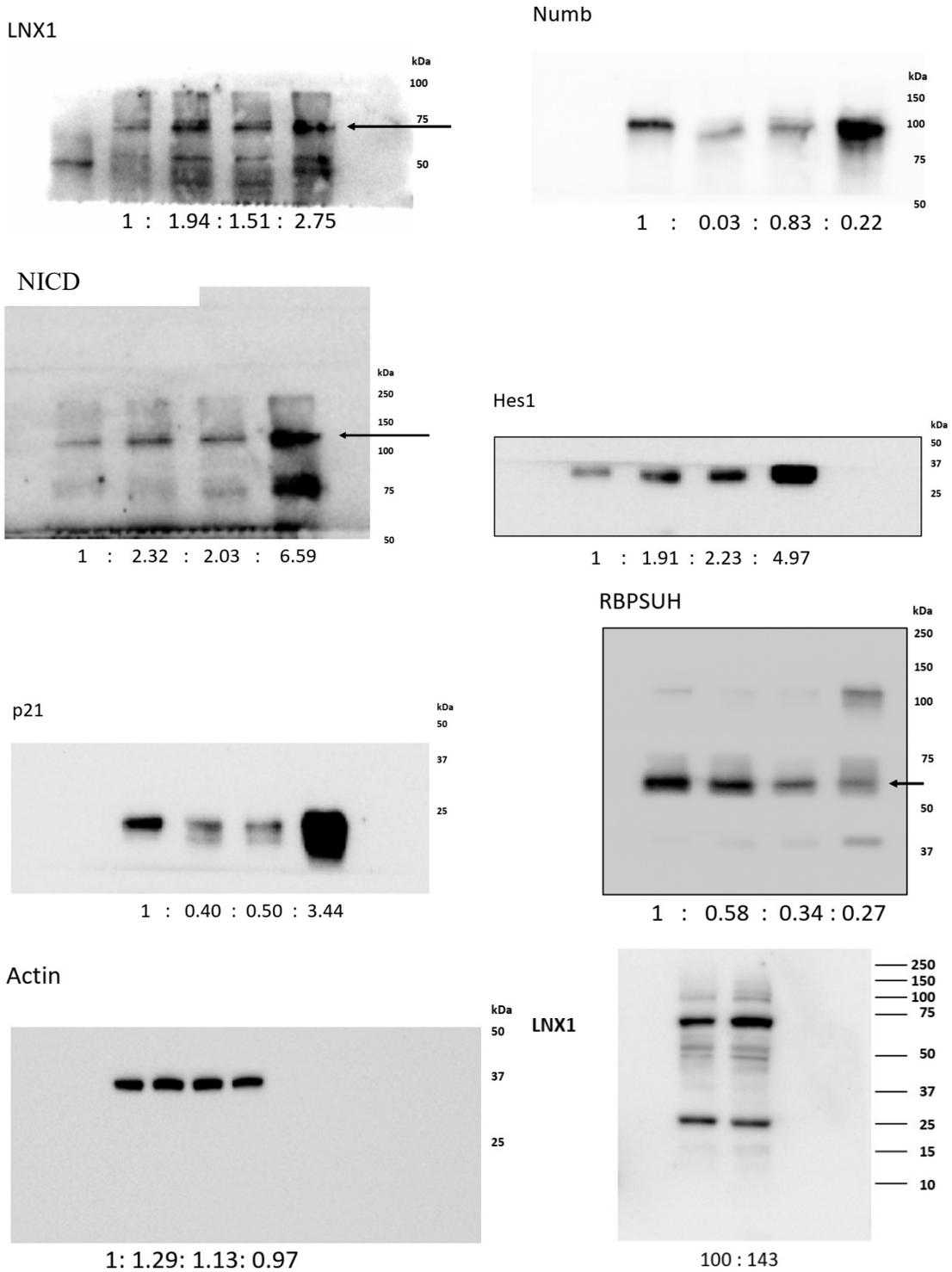
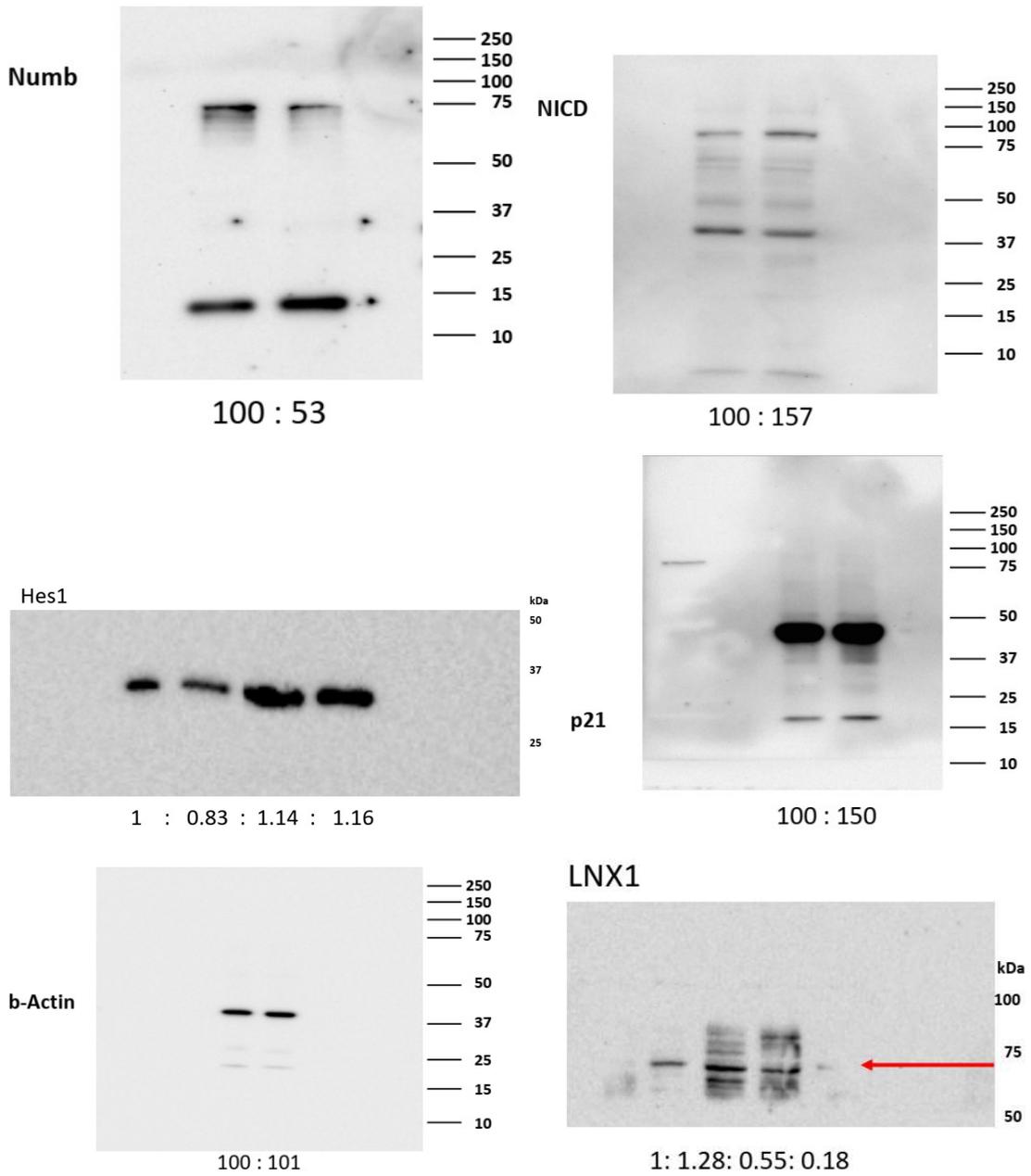
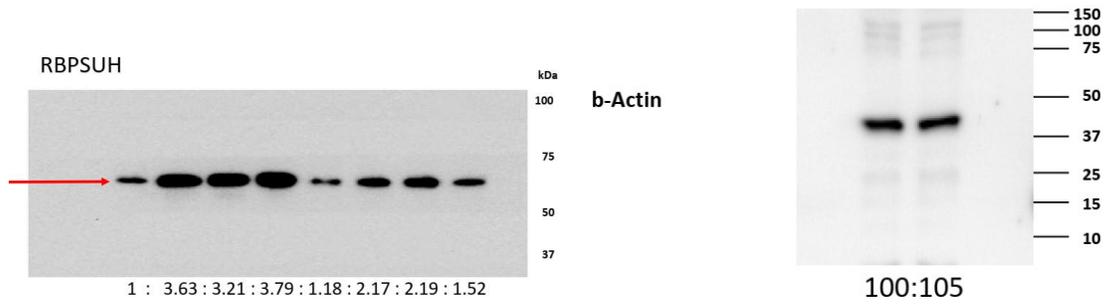
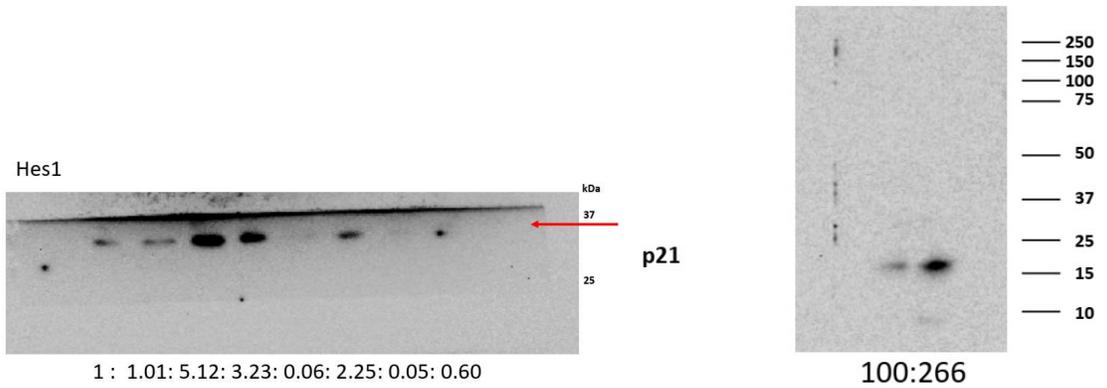
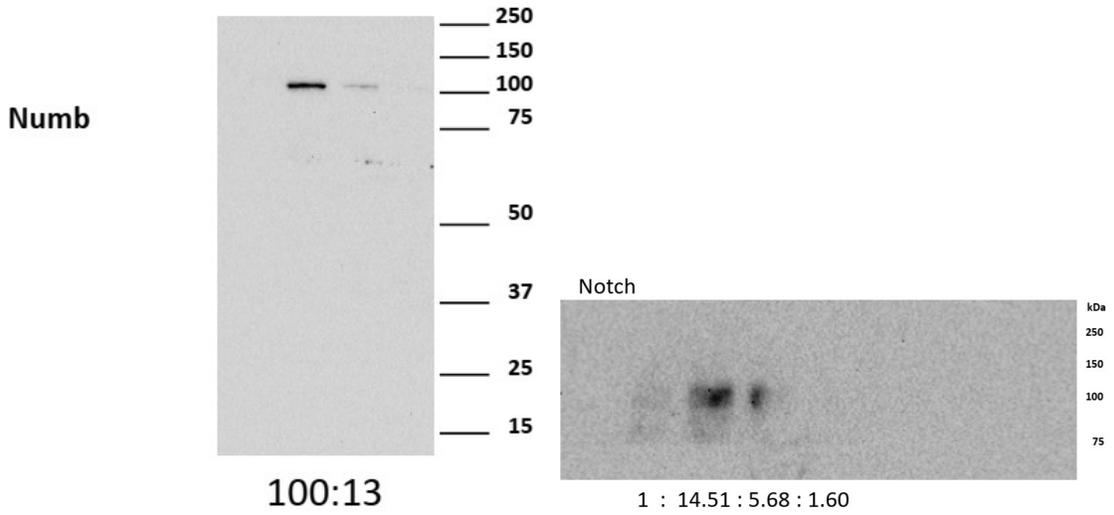


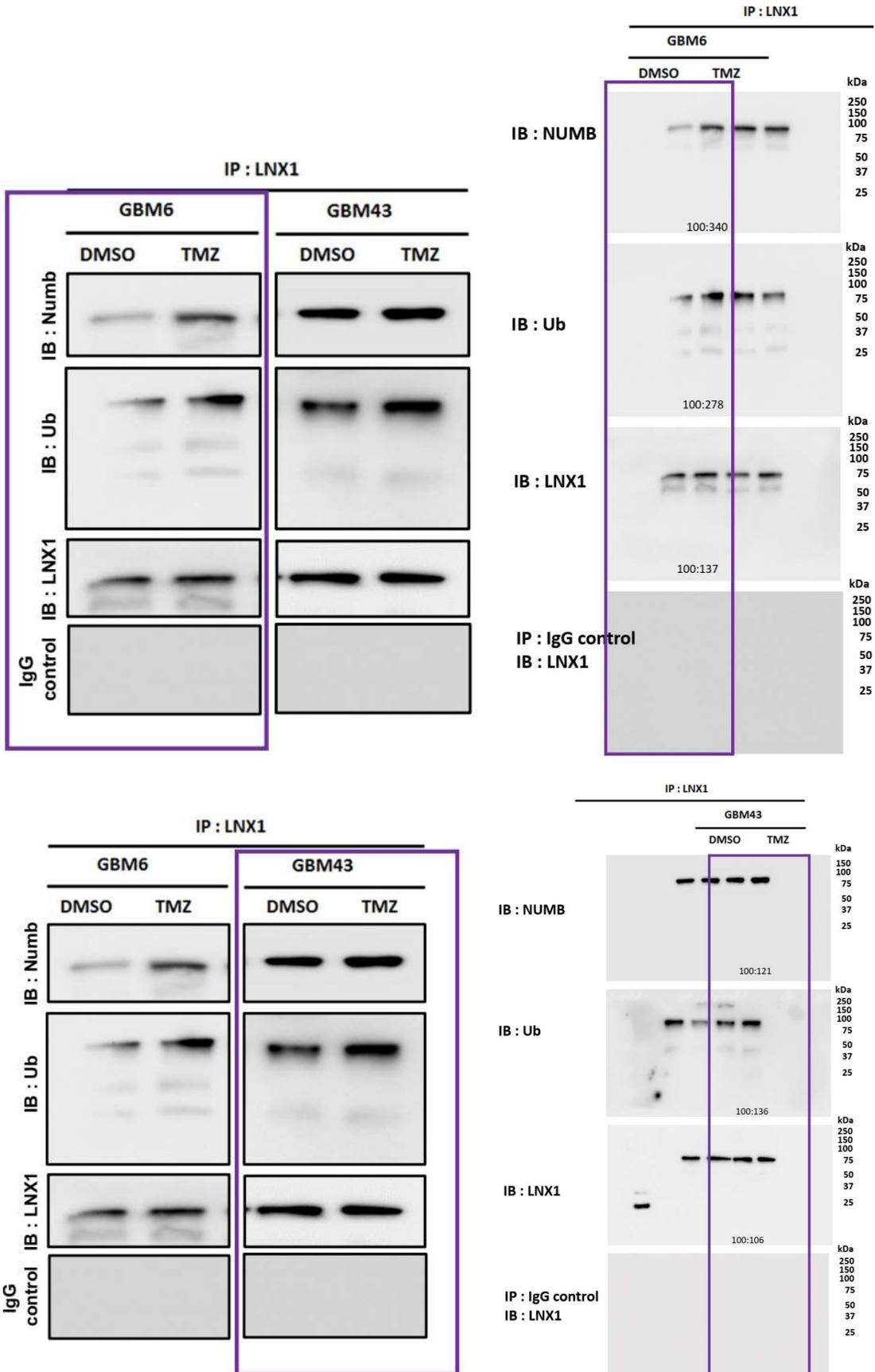
Figure S6: GlioVis datasets CGGA and TCGA were examined for correlations between LNX1 and GSC markers CD133 (PROM1) and L1CAM. **(A)** In the CGGA dataset, we noted positive correlations between LNX1 and PROM1/L1CAM. **(B)** In the TCGA_GBM dataset, we noted positive correlations between LNX1 and PROM1/L1CAM

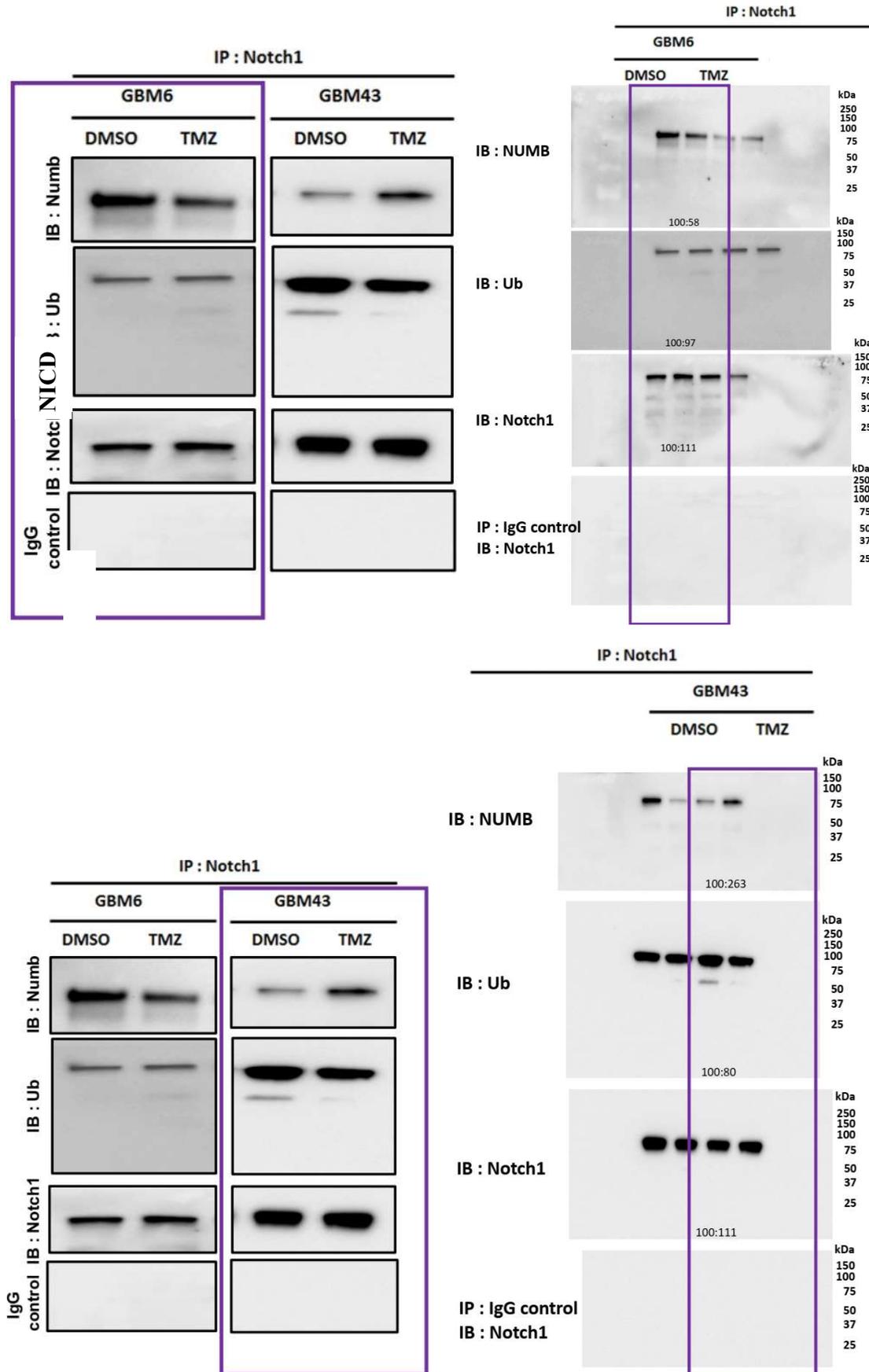
Original Western Blot Figures

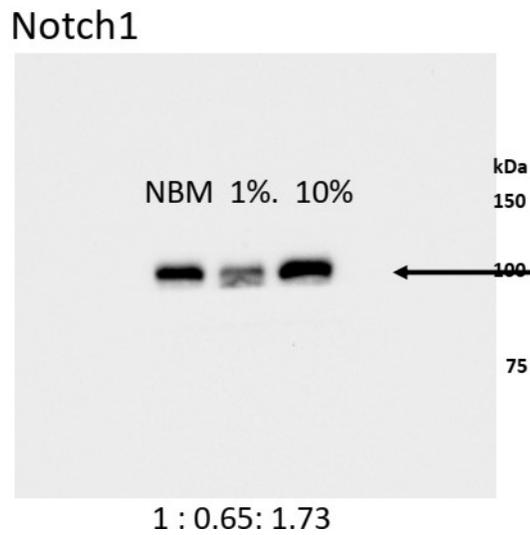
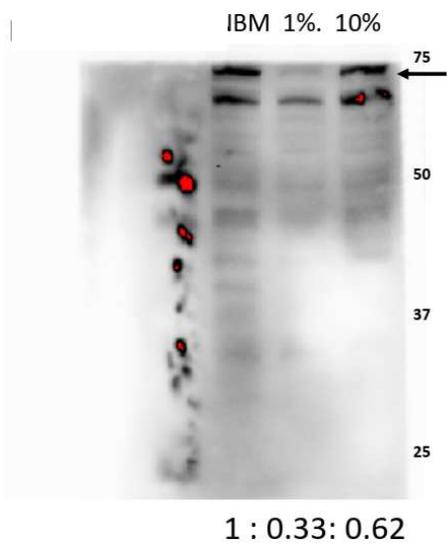
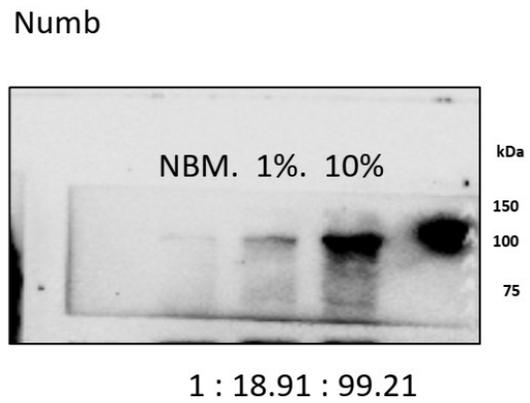
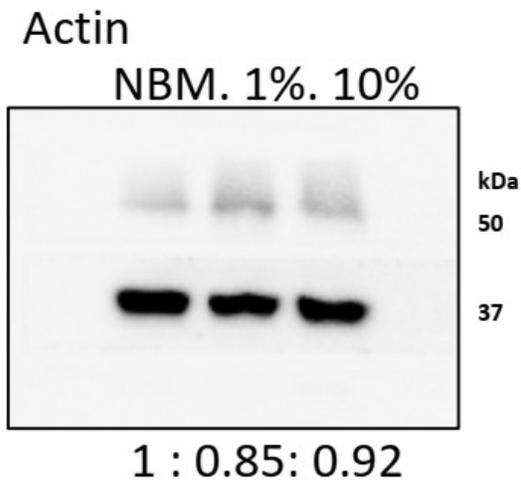
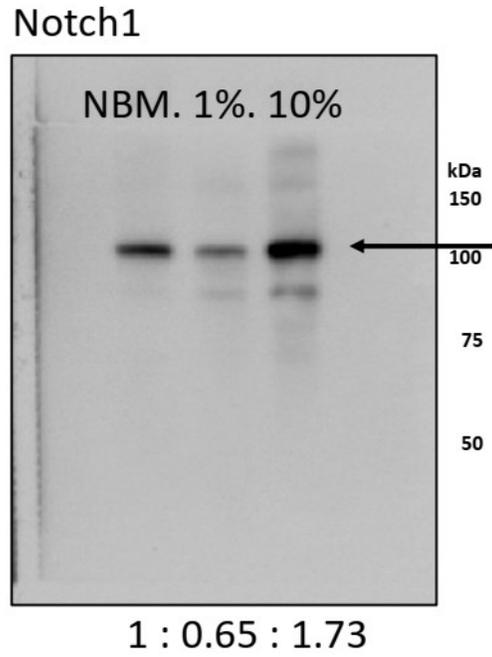
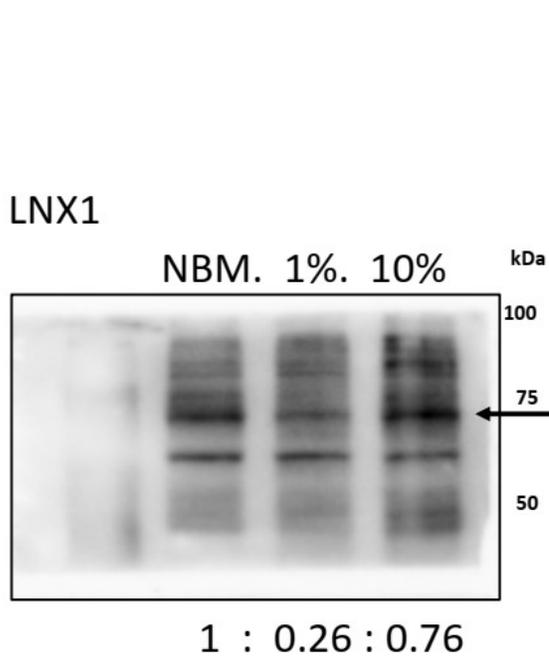






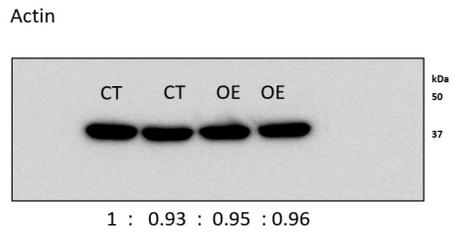
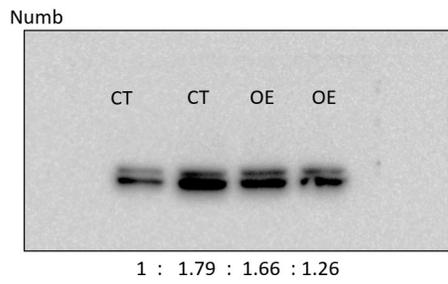
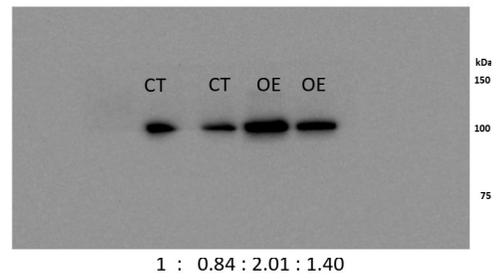
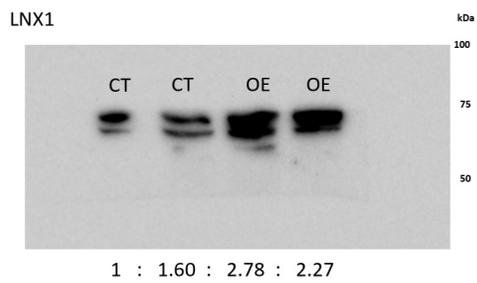
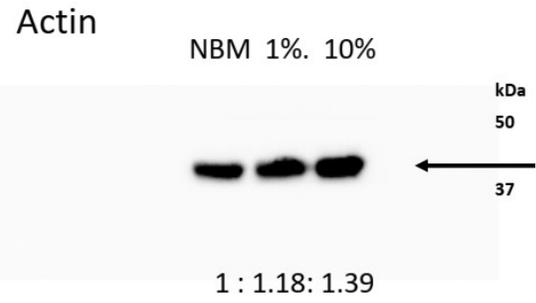
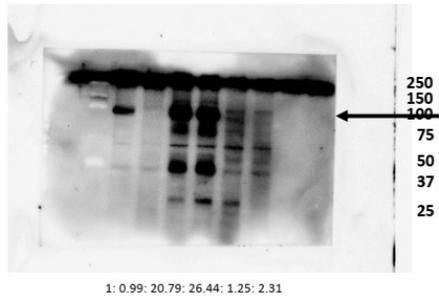


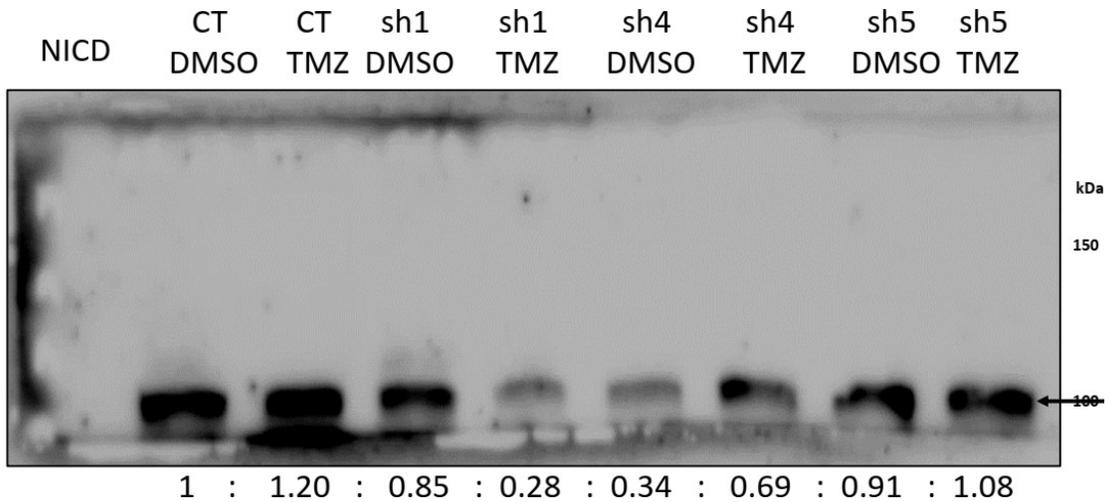
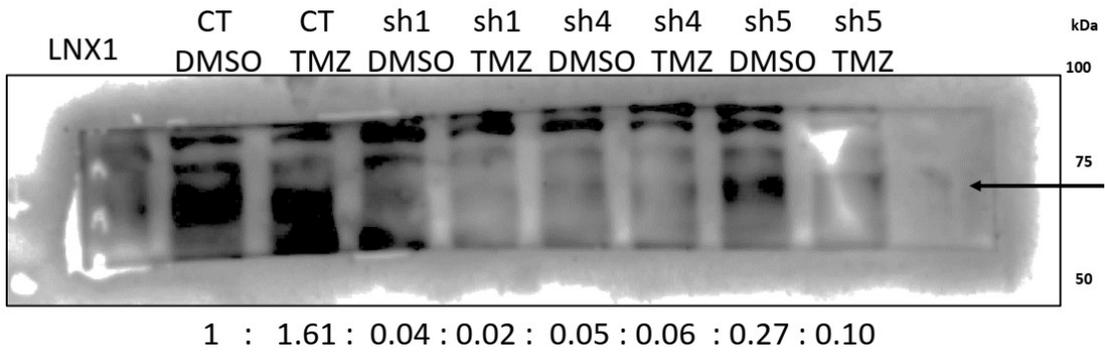
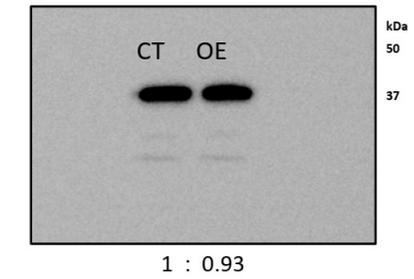
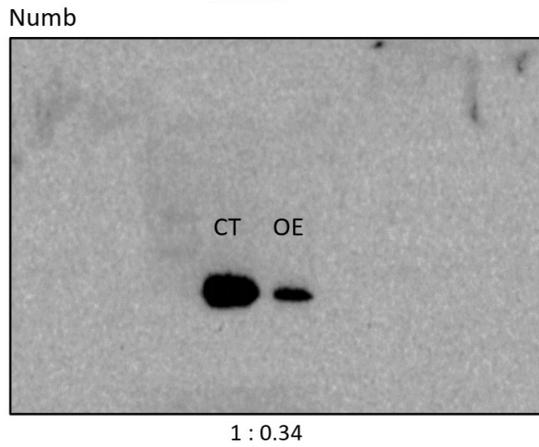
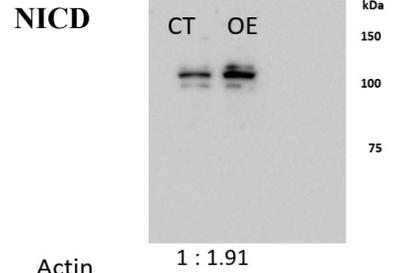
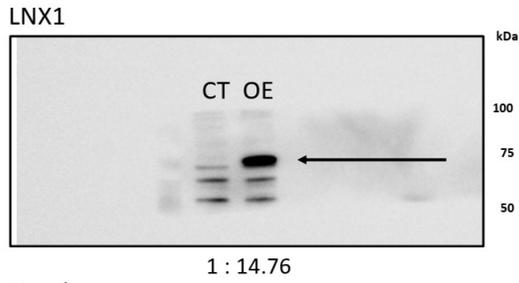




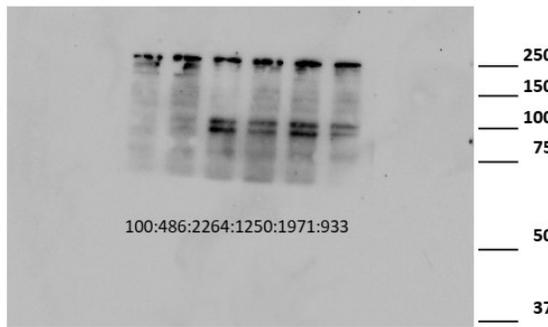
Numb

GBM5 - 6 - 43 (NBM - 1% for each) -

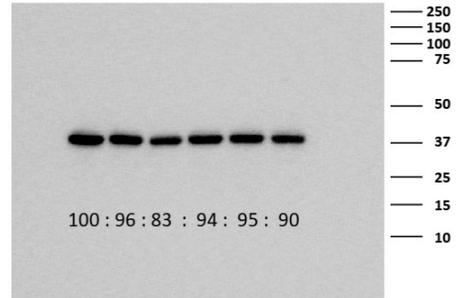




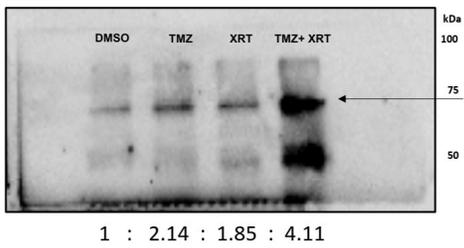
Numb (lanes are as above)



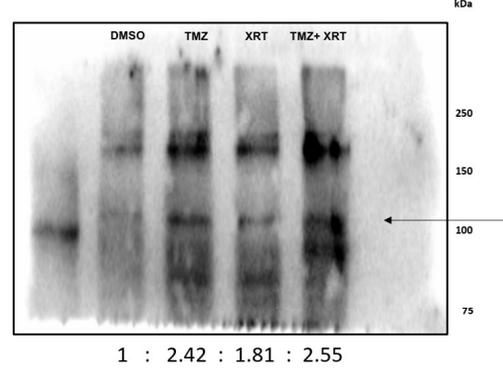
Actin



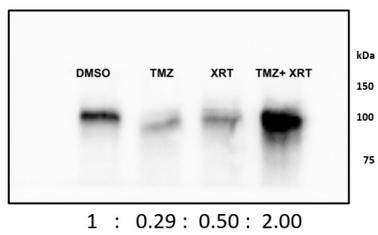
LNK1



NICD



Numb



Actin

