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Supplementary Materials for

Cell-specific cross-talk proteomics reveals cathepsin B signaling as a driver of glioblastoma malignancy near the subventricular zone

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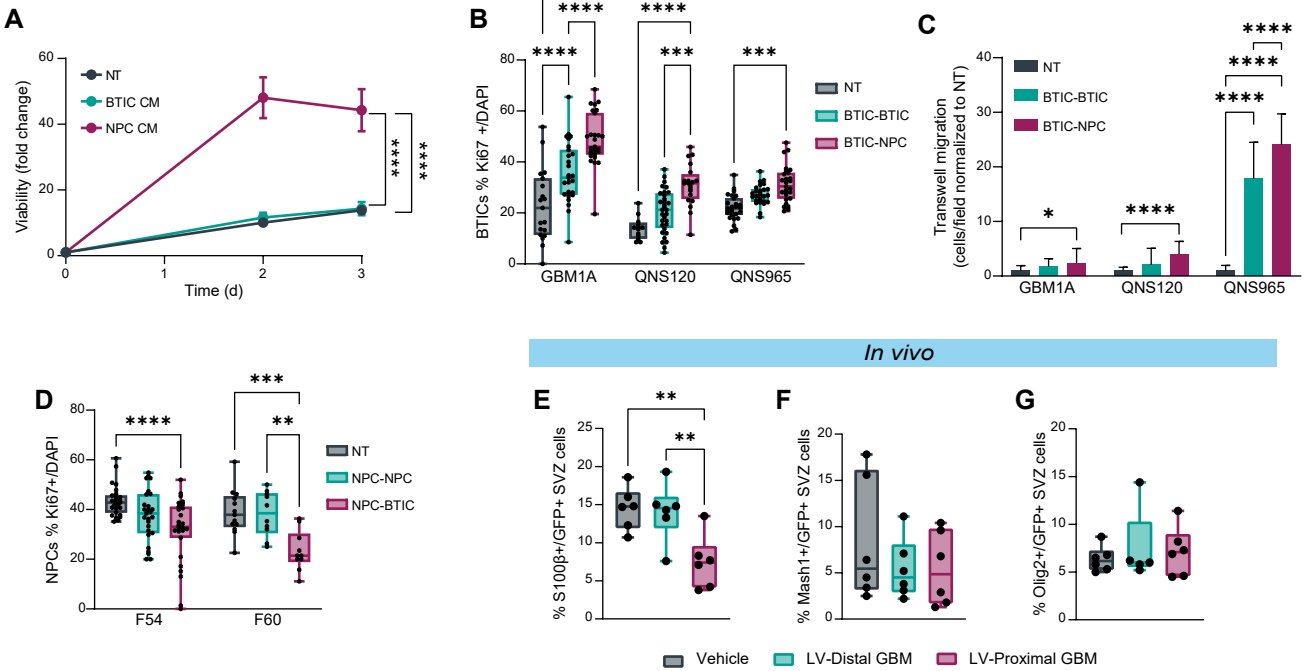
The PDF file includes:

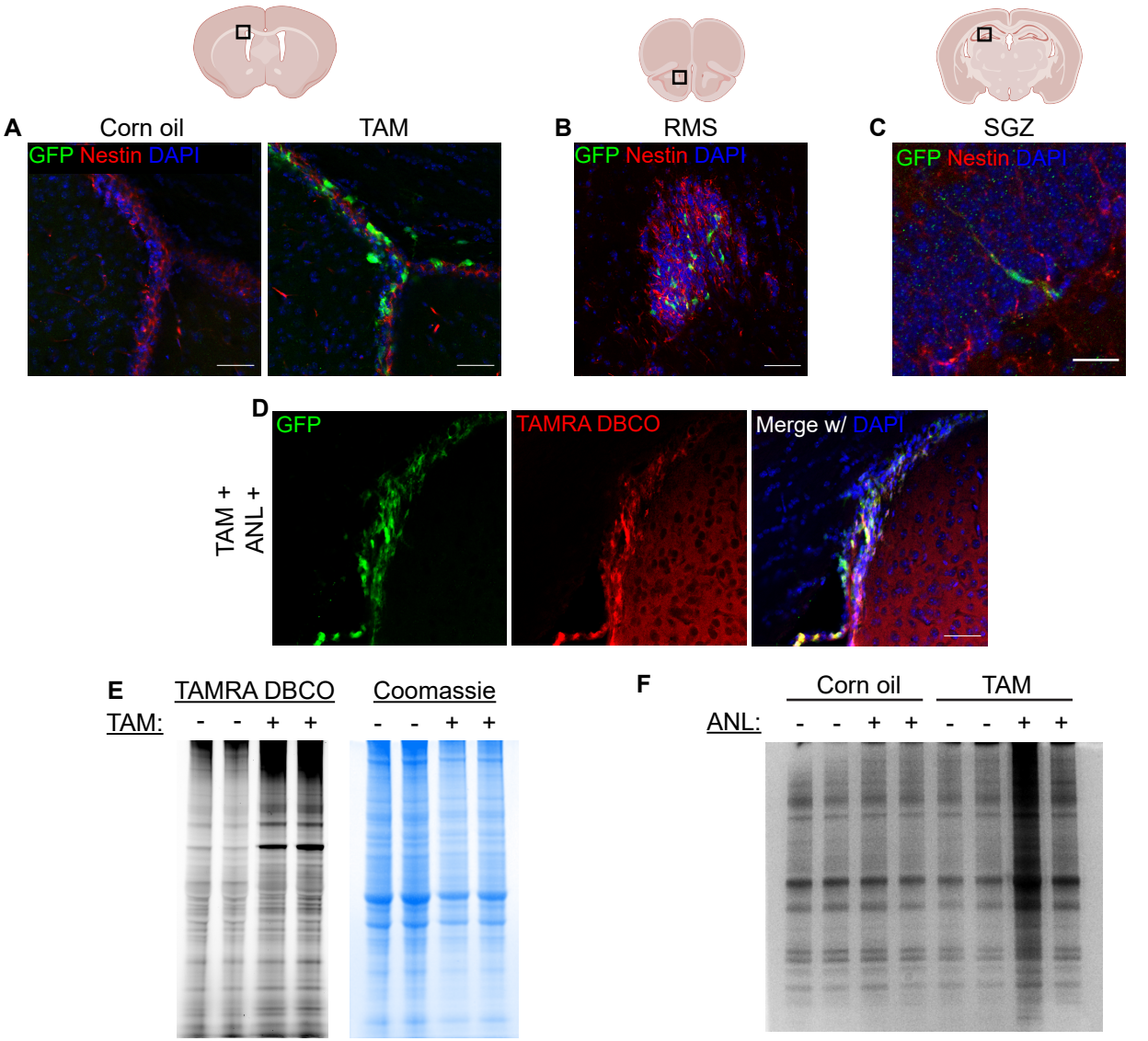
Other Supplementary Material for this manuscript includes the following:

Supplementary Figure 1

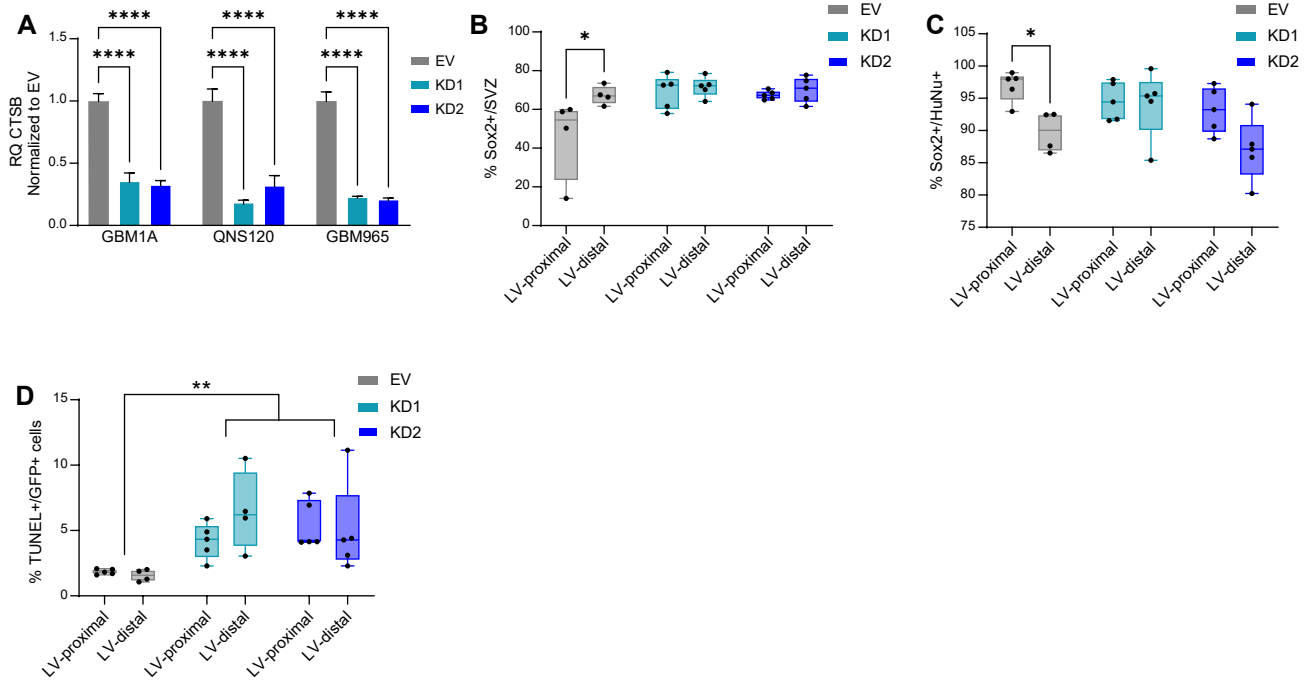


Supplementary Figure 1: Related to main Figure 1.
(A) Viability assay over time of BTICs treated with conditioned medium from themselves or hfNPCs. Data tested with two-way ANOVA with Tukey multiple comparisons. (B) Proliferation assay indicating percentage of Ki67+ BTICs when co-cultured with themselves or hfNPCs for 48h. Data tested with two-way ANOVA with Šídák's multiple comparisons test. (C) Transwell migration assay of BTICs co-cultured with themselves or hfNPCs for 24h. Compared to NT conditions. Data tested with two-way ANOVA with Šídák's multiple comparisons test. (D) Proliferation assay indicating percentage of Ki67+ hfNPCs when co- cultured with themselves or BTICs for 48h. Compared to NT conditions. Data tested with two-way ANOVA with Tukey multiple comparisons. (E) Percentage quantifications of GFP+ cells in the SVZ that are S100β+ (n = 5-6 biological replicates). Data tested with ordinary one-way ANOVA with Holm-Šídák's multiple comparisons test. (F) Percentage quantifications of GFP+ cells in the SVZ that are Mash1+ (n = 5-6 biological replicates). (G) Percentage quantifications of GFP+ cells in the SVZ that are Olig2+ (n = 5-6 biological replicates). Data represented as median ± maximum/ minimum (box and whisker) or mean ± standard deviation (line graphs, bar charts), $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****.

Supplementary Figure 2

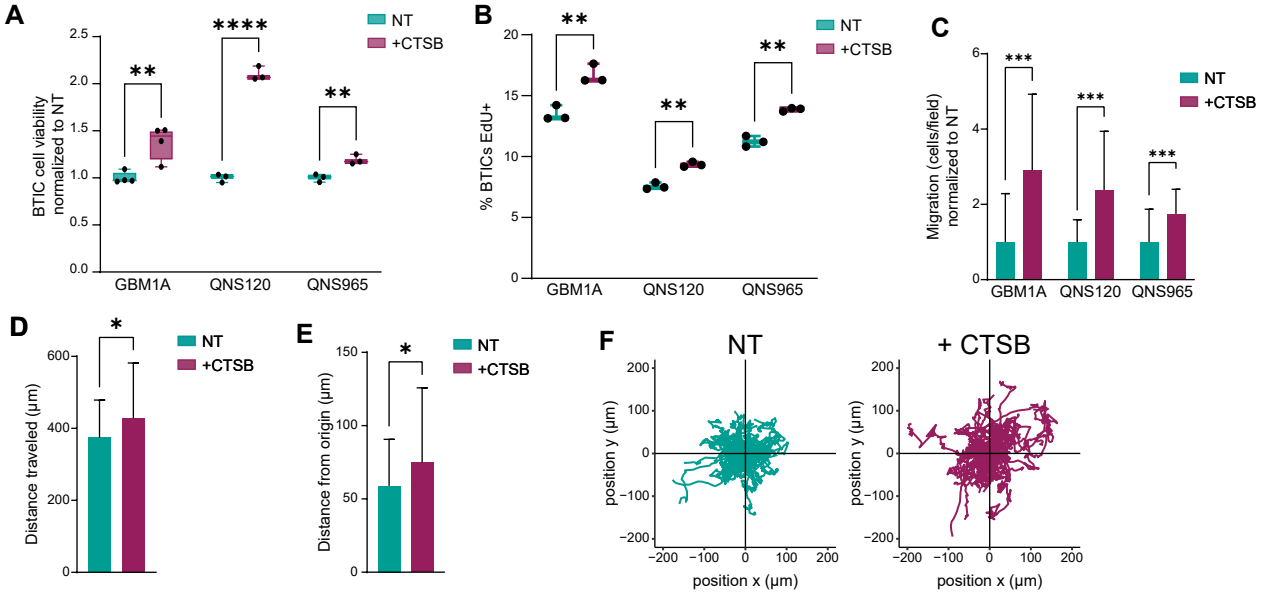


Supplementary Figure 2: Nes-MetRS* mice effecttively express GFP and MetRS* in NSCs when activated.
(A) IHC showing TAM-inducible GFP expression in Nestin+ SVZ cells. Scale bar = 50 μm. Schematic created with Biorender.com. (B) IHC showing GFP expression in rostral migratory stream (RMS). Scale bar = 50 μm. Schematic created with Biorender.com. (C) IHC showing GFP expression in subgranular zone (SGZ) of hippocampus. Scale bar = 25 μm. Schematic created with Biorender.com. (D) IHC showing TAMRA DBCO co-localization to GFP+ cells in the SVZ. Scale bar = 50 μm. (E) Gel indicating increased TAMRA DBCO incorporation into SVZ lysate following TAM induction of NSCs. (F) Silver stain of lysate immunoprecipitation showing pulldown enrichment in animals receiving both TAM and ANL.



Supplementary Figure 3

Supplementary Figure 3: Related to main Figure 5.
(A) RT-qPCR of CTSB expression in BTIC lines transduced with EV or CTSB KD hairpins (n = 3 technical replicates). Data was tested with one-way ANOVA with Tukey multiple comparisons. (B) Percentage of Sox2+ cells in the SVZ in the presence of EV or KD shRNA GBM1A tumors proximal or distal to LV (n = 4-5 biological replicates). Data was tested with two-way ANOVA with Šídák's multiple comparisons test. (C) Percentage of Sox2+ cells in EV or KD shRNA GBM1A tumors proximal or distal to LV (n = 4-5 biological replicates). Data was tested with two-way ANOVA with Šídák's multiple comparisons test. (D) Percentage of TUNEL+ cells in EV or KD shRNA GBM1A tumors proximal or distal to LV (n = 4-5 biological replicates). Data was tested wth Data was tested with two-way ANOVA with Šídák's multiple comparisons test. Data represented as median ± maximum/minimum (box and whisker) or mean ± standard deviation (bar chart), $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****.



Supplementary Figure 4

Supplementary Figure 4: Soluble CTSB contributes to malignancy-associated phenotypes in BTICs

(A) Cell viability measurements for NT and + recombinant CTSB at 48h. Data was tested with multiple unpaired t-tests with Benjamini FDR. (B) Proliferation assay indicating percentage of EdU+ cells in NT and + recombinant CTSB. Data was tested with multiple unpaired t-tests with Benjamini FDR. (C) Transwell migration of NT and + recombinant CTSB. Data was tested with multiple unpaired t-tests with Benjamini FDR. (D) Total distance traveled by NT and + recombinant CTSB in timelapse migration. Data tested with two-tailed unpaired t-test. (E) Distance from origin traveled by NT and + recombinant CTSB in timelapse migration. Data tested with two-tailed unpaired t-test. (F) Representative plots of migration from origin for NT and + recombinant CTSB in BTIC line QNS120. Data represented as median ± maximum/minimum (box and whisker) or mean ± standard deviation (bar chart), $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****.

Supplemental Table 1: gProfiler SVZ proteomics GO and KEGG. Table indicating the dataset source, the comparison to LV-proximal GBM group, the term name, the term id, the adjusted p-value, the negative log of the adjusted p-value, the term size, the query size, the number of intersecting proteins with the term, and the identities of the intersecting proteins from the SVZ proteomics analysis.

Supplemental Table 2: gProfiler BTIC proteomics GO and KEGG. Table indicating the dataset source, the term name, the term id, the adjusted p-value, the negative log of the adjusted p-value, the term size, the query size, the number of intersecting proteins with the term, the percent of significant proteins in the term, the effective domain size, and the identities of the intersecting proteins from the co-culture proteomics analysis.

Supplementary Table 3: Patient information for intraoperative specimen collection

Patient Sample ID	Primary or recurrent	Tumor diagnosis	Extent of Resection	Age at Surgery	Sex	MGMT Promoter Methylation	Other mutations
QNS484b	Recurrent	GBM	Gross Total	68	Male	Positive	P53 negative ATRX retained
QNS669	Primary	GBM	Sub-total	81	Male	Negative	P53 positive ATRX Retained
QNS679	Primary	GBM	Sub-total	56	Female	Positive	ATRX retained PTEN mutation positive TERTp mutation positive CDKN2A/B loss
QNS690	Primary	GBM	Sub-total	70	Male	Negative	ATRX retained P53 positive
QNS783	Primary	GBM	Sub-total	67	Male	Negative	TERTp mutation positive PTEN mutation positive
QNS794	Primary	GBM	Sub-total	52	Female	Positive	ATRX retained P53 positive
QNS891	Primary	GBM	Sub-total	58	Male	Negative	ATRX retained P53 positive
QNS893	Primary	GBM	Sub-total	71	Male	Negative	ATRX retained P53 positive

Supplemental Table 3: Patient information for intraoperative sample collection. Table indicating the sample ID, whether the tumor was primary or recurrent, the tumor diagnosis from pathology, the extent of resection, the age at surgery, the biological sex, the MGMT promoter methylation status, and other mutations detected in the tumor.