Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma

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High-grade gliomas (HGG) are a devastating group of cancers, and represent the leading cause of brain tumour-related death in both children and adults. Therapies aimed at mechanisms intrinsic to glioma cells have translated to only limited success; effective therapeutic strategies will need also to target elements of the tumour microenvironment that promote glioma progression. Neuronal activity promotes the growth of a range of molecularly and clinically distinct HGG types, including adult and paediatric glioblastoma (GBM), anaplastic oligodendroglioma, and diffuse intrinsic pontine glioma (DIPG).1 An important mechanism that mediates this neural regulation of brain cancer is activity-dependent cleavage and secretion of the synaptic adhesion molecule neuroligin-3 (NLGN3), which promotes glioma proliferation through the PI3K–mTOR pathway.1 However, the necessity of NLGN3 for glioma growth, the proteolytic mechanism of NLGN3 secretion, and the further molecular consequences of NLGN3 secretion in glioma cells remain unknown. Here we show that HGG growth depends on microenvironmental NLGN3, identify signalling cascades downstream of NLGN3 binding in glioma, and determine a therapeutically targetable mechanism of secretion. Patient-derived orthotopic xenografts of paediatric GBM, DIPG and adult GBM fail to grow in Nlgn3 knockout mice. NLGN3 stimulates several oncogenic pathways, such as early focal adhesion kinase activation upstream of PI3K–mTOR, and induces transcriptional changes that include upregulation of several synapse-related genes in glioma cells. NLGN3 is cleaved from both neurons and oligodendrocyte precursor cells via the ADAM10 sheddase. ADAM10 inhibitors prevent the release of NLGN3 into the tumour microenvironment and robustly block HGG xenograft growth. This work defines a promising strategy for targeting NLGN3 secretion, which could provide transformative promise for HGG therapy. To determine the necessity of microenvironmental NLGN3 to glioma growth, we xenografted patient-derived HGG cells expressing green fluorescent protein (GFP) and luciferase into Nlgn3 knockout mice2 (Nlgn3−/−;NSG). Patient-derived paediatric glioblastoma xenografts (SU-pcGBM2) to the frontal cortex were monitored using bioluminescence in vivo imaging systems (IVIS) over the course of 6 months (Fig. 1a) and evaluated histologically (Fig. 1b). Initial engraftment was equivalent in knock out and wild-type Nlgn3 mice (Extended Data Fig. 1a, b). A notable inhibition of glioma growth was evident in Nlgn3 knockout animals for up to 6 months (Fig. 1a–f and Extended Data Fig. 1c, d). By 4.5 months, a subset of tumours circumvented this apparent NLGN3 dependency and began to exhibit growth (Fig. 1e, f, Extended Data Fig. 1c, d). The observed degree of growth inhibition was unexpected, as our previous work indicated that brain-derived neurotrophic factor (BDNF) also contributes to activity-regulated glioma proliferation.1 Conditioned medium from optogenetically stimulated acute cortical slices from Nlgn3 wild-type or transgenic knockout Thy1::ChR2 mice demonstrated that the increase in glioma cell proliferation induced by active conditioned medium is incompletely abrogated in the context of the Nlgn3 knockout (Extended Data Fig. 2a), replicating the degree of differential proliferation previously accounted for by activity-regulated BDNF.1 Taken together, these findings indicate that glioma growth is more dependent on NLGN3 in situ than would have been predicted from these in situ /in vitro experiments. The nearly normal neurological function of Nlgn3 knockout mice3–5 is attributed to the compensatory expression of other neuroligins3,6. We found no effect of Nlg1, Nlg4X, Nlg4Y (Extended Data Fig. 2b, c) or Nlg2 on glioma proliferation. Thus, compensatory expression of other neuroligins would not be expected to influence glioma growth, supporting a unique role for NLGN3 in glioma pathobiology. To determine the role of NLGN3 in the growth of additional HGG types, patient-derived xenografts of DIPG (SU-DIPG-VI and SU-DIPG-XIII-FL) and adult glioblastoma (SU-GBM035) were tested in the Nlgn3-deficient mouse brain. We found that DIPG, xenografted to pons or to frontal cortex, and adult glioblastoma each exhibited marked growth stagnation in Nlgn3 knockout mice (Fig. 1g–k). By contrast, patient-derived HER2+ breast cancer brain metastasis xenografts (DF-BM354)7 did not exhibit differential growth in wild-type or knockout Nlgn3 mouse brains (Fig. 1l). These results indicate a conserved dependency on NLGN3 across molecularly and clinically distinct types of HGG. The observed growth inhibition is more profound than can be explained by known effects of NLGN3 on glioma PI3K–mTOR signalling3. To delineate the signalling consequences of NLGN3 exposure in glioma better, we used phosphoproteomics (Fig. 2a, Extended Data Table 1). Phospho-antibody array analyses at 5 and 30 minutes after NLGN3 exposure revealed focal adhesion kinase (FAK) phosphorylation and numerous phosphorylation events classically downstream of FAK, including activation of the SRC kinase cascade, PI3K–mTOR cascade, and SHC-RAS-RAF-MEK-ERK cascade (Fig. 2a). Additional oncogenic proteins exhibiting increased phosphorylation include integrin β3, growth factor receptors EGF, FGFR and VEGFR, and others (Extended Data Table 1). FAK activity is central to many of these signalling events. Phospho-tyrosine pull-down analysis at 5–10 min after NLGN3 exposure demonstrated FAK phosphorylation. FAK inhibition blocked the effects of NLGN3 on glioma proliferation (Fig. 2b, c). FAK phosphorylation peaked at 5–10 min after NLGN3 exposure, placing FAK early in the signalling cascade (Fig. 2d). FAK activity was necessary for PI3K stimulation by NLGN3 as demonstrated by AKT (Ser473) phosphorylation (Fig. 2e).

We performed RNA sequencing (RNA-seq) after glioma exposure to NLGN3. Genes involved in cell proliferation were upregulated, as were several genes known to promote malignancy in glioma, including...
PDGFA^{8-10}, TTYH1^{11} and several potassium channel genes^{12} (Extended Data Fig. 3). In addition to NLGN3\(^3\), numerous genes involved in synapse function were upregulated after exposure to NLGN3 (Extended Data Fig. 3). While the meaning of this intriguing observation remains to be clarified, it suggests the biology of NLGN3 may extend beyond the role of mitogen.

The notable inhibition of glioma growth observed in the NLGN3-deficient mouse brain highlights its therapeutic potential. Blocking release into the tumour microenvironment is one therapeutic strategy, so we sought to determine the proteolytic and cellular details of NLGN3 shedding. Full-length NLGN3 is cleaved and secreted in an activity-regulated fashion with shedding of the N-terminal ectodomain\(^1\) (Fig. 3a–c). Optogenetic stimulation of acute cortical slices increased NLGN3 shedding, whereas the addition of tetrodotoxin (TTX) inhibited NLGN3 shedding (Fig. 3c).

Neuroligin-3 is highly expressed in both neurons and oligodendrocyte precursor cells\(^13\) (OPCs; Extended Data Fig. 4a), known to form bona fide synapses with pre-synaptic neurons and serve as a post-synaptic cell\(^14-18\). To test the relative contribution of neurons and OPCs to secretion of NLGN3, mice expressing cell type-specific, inducible Cre drivers were bred to Nlgn3\(^{Tg}\) mice. Tamoxifen was administered for 5 days starting at P28, and resulted in recombination in approximately 40% of cortical neurons in the CamKII\(\alpha\)-CreER driver mouse (in which expression of tamoxifen-activated CreER recombinase is driven by a Camk2a promoter), and in around 80% of OPCs in the PDGFR\(\alpha\)-CreER driver mouse (Extended Data Fig. 4b, c). Using the acute slice model together with inducible, conditional deletion of Nlgn3 from either neurons or OPCs, we find that both cell types contribute to activity-regulated NLGN3 secretion, and notably OPCs are a major source of secreted NLGN3 (Fig. 3d, e), defining a role for OPCs as a microenvironmental cell type contributing to glioma growth.

NLGN3 exposure results in feed-forward glioma expression of NLGN3\(^3\), and we thus asked whether glioma cells contribute to the pool of secreted NLGN3. Increased levels of NLGN3 were found in conditioned medium from glioma xenograft-bearing, NLGN3 wild-type brain slices compared to non-xenograft-bearing slices (Extended Data Fig. 5a, b). Conditioned medium from NLGN3-primed glioma cultures also exhibit upregulated secretion (Extended Data Fig. 5c). By contrast, xenograft-bearing brain slices from Nlgn3 knockout mice secrete no detectable NLGN3 (Extended Data Fig. 5b). These data indicate that glioma cells contribute to NLGN3 in the tumour microenvironment in a manner regulated by neuroligin-3 exposure from normal stromal cells.

To determine the enzyme responsible for the cleavage of NLGN3, we analysed the C-terminal transmembrane domain for putative cleavage...
Figure 2 | Signalling consequences of NLGN3 in glioma. a. Schematic illustration of signalling pathways activated after NLGN3 exposure; red circles represent proteins exhibiting increased phosphorylation. b. Proliferation index of SU-pcGBM2 cells exposed to plain medium (aCSF), aCSF plus 5 nM FAK inhibitor (FAKi), NLGN3 (50 nM), or NLGN3 (50 nM) plus 5 nM FAK inhibitor (NLGN3 + FAKi), (n = 3 wells per condition). **P < 0.01, one-way ANOVA with Tukey’s post hoc test for multiple comparisons. NS indicates P > 0.05. Data are mean ± s.e.m. c. Representative confocal images of SU-pcGBM2 cells as in b exposed to NLGN3 in the absence (top) or presence (bottom) of FAK inhibitor. Vimentin, green; phospho-FAK, white; DAPI, blue; EdU, red. Scale bar, 50 μm. d. Representative western blots demonstrating increased phosphorylation of FAK (Tyr861) after exposure to NLGN3 for 0, 5, 10 or 15 min. e. Representative western blots demonstrating AKT (Ser473) phosphorylation in SU-pcGBM2 cells exposed to plain medium (aCSF), aCSF plus 5 nM FAK inhibitor (FAKi), NLGN3 (50 nM), or soluble NLGN3 (50 nM) plus 5 nM FAK inhibitor (NLGN3 + FAKi). Experiments in d and e, performed in biological duplicate and technical triplicate (6 replicate western blots). The 95% CI in b are: aCSF versus aCSF + FAKi [−0.10, 0.08]; aCSF versus NLGN3 [−0.24, −0.05]; aCSF versus NLGN3 + FAKi [−0.08, 0.11]; aCSF + FAKi versus NLGN3 [−0.23, −0.04]; aCSF + FAKi versus NLGN3 + FAKi [−0.07, 0.12]; NLGN3 versus NLGN3 + FAKi [0.07, 0.25].

Figure 3 | ADAM10 mediates activity-regulated neuroligin-3 shedding from both neurons and OPCs. a. Schematic depicting NLGN3 cleavage. FL, full-length; sNLGN3, soluble NLGN3. b. NLGN3 western blot of slice lysate and conditioned medium (CM). c. NLGN3 western blot of conditioned medium from optogenetically stimulated Thy1::ChR2 (ChR2) slices or wild-type slices at baseline neuronal activity with or without tetrodotoxin (TTX). d. NLGN3 western blot and quantification of conditioned medium from CamKIIα−Cre;NLGN3fl/fl (no Cre) slices (n = 3 animals). e. As in d, but from the PDGFRα−Cre;NLGN3fl/fl model (n = 3 animals). f. NLGN3 western blots of conditioned medium from optogenetically stimulated Thy1::ChR2 slices incubated without (left lanes of each blot) or with (right lanes of each blot) inhibitors as indicated. g. NLGN3 western blot of optogenetically stimulated Thy1::ChR2 slice homogenates with or without ADAM10 or MMP2/9 inhibitors. h. NLGN3 western blot of conditioned medium from optogenetically stimulated Thy1::ChR2 slices with or without indicated concentration of ADAM10 inhibitor. i. NLGN3 western blot and quantification of wild-type and Mmp9−/− (Mmp9 KO) slice conditioned medium (n = 3 animals). j, k. As in i, but in CamKIIα−Cre;Adam10fl/fl (n = 3 animals) (j), or PDGFRα−Cre;Adam10fl/fl (n = 3 animals) (k) models. l. ADAM10 western blot and quantification of slice conditioned medium with or without TTX. Quantifications expressed as ratio of experiment to control. P values determined by two-tailed Student’s t-test. NS indicates P > 0.05. Data are mean ± s.e.m. All western blots performed with n = 3 biologically independent samples. The 95% CI are: [−0.48, −0.03] (d); [−0.93, −0.46] (e); [−0.49, 0.55] (j); [−0.56, −0.30] (j); [−0.18, 0.31] (k); [−0.70, −0.45] (l).
ADAM10 inhibitors have been developed for clinical use, and the ADAM10 inhibitor GI254023X was assessed and found to be sufficient (Extended Data Table 2). Given the effects of ADAM10 inhibition on glioma self-renewal and therefore tumour initiation, drug treatment started well after engraftment. Mice bearing paediatric glioblastoma (SU-pcGBM2) or DIPG (SU-DIPG-VI or SU-DIPG-XIX) orthotopic xenografts were treated with GI254023X or vehicle control. Paediatric GBM and both DIPG xenografts in mice treated with the ADAM10 inhibitor exhibited pronounced growth reduction compared to vehicle-treated controls (Fig. 4a–c). Histological analyses revealed reduced glioma proliferation in ADAM10 inhibitor-treated animals (Fig. 4d, e and Extended Data Fig. 7a). Furthermore, ADAM10 inhibition abrogated glioma cell secretion of NLGN3 from both xenograft-bearing brain slices and NLGN3–primed glioma cells (Extended Data Fig. 5b, c), suggesting that this therapeutic strategy addresses all cellular sources of NLGN3.

We next investigated the therapeutic potential of ADAM10 inhibition. ADAM10 is expressed in gliomas as reported in the literature and demonstrated in gene expression datasets from paediatric and adult HGG samples (Extended Data Fig. 5d). Cell-intrinsic effects of ADAM10 inhibition have been reported in adult HGG, so we assessed possible direct effects of ADAM10 inhibition on the paediatric HGG cells used here. ADAM10 inhibition did not change proliferation, cell viability or invasion of paediatric HGG cells (Extended Data Fig. 6e–g). ADAM10 inhibition mildly affected paediatric HGG self-renewal (Extended Data Fig. 6h, i).

We next tested the influence of ADAM10 inhibition on HGG growth in vivo. Brain penetration of the specific ADAM10 inhibitor GI254023X was assessed and found to be sufficient (Extended Data Table 2). Given the effects of ADAM10 inhibition on glioma self-renewal and therefore tumour initiation, drug treatment started well after engraftment. Mice bearing paediatric glioblastoma (SU-pcGBM2) or DIPG (SU-DIPG-VI or SU-DIPG-XIX) orthotopic xenografts were treated with GI254023X or vehicle control. Paediatric GBM and both DIPG xenografts in mice treated with the ADAM10 inhibitor exhibited pronounced growth reduction compared to vehicle-treated controls (Fig. 4a–c). Histological analyses revealed reduced glioma proliferation in ADAM10 inhibitor-treated animals (Fig. 4d, e and Extended Data Fig. 7a). Furthermore, ADAM10 inhibition abrogated glioma cell secretion of NLGN3 from both xenograft-bearing brain slices and NLGN3–primed glioma cells (Extended Data Fig. 5b, c), suggesting that this therapeutic strategy addresses all cellular sources of NLGN3.

ADAM10 inhibitors have been developed for clinical use, and the ADAM10/17 inhibitors INCB7839 or vehicle control; (n = 5 control, n = 4 treated mice) h. Schematic summary. NRXN, neurexin; sNLGN3, soluble neuroligin-3. P values determined by Student's two-tailed t-test (a, b, g) or two-sided Mann–Whitney test (c, d). Each dot represents one mouse. Data are mean ± s.e.m except in f, where data are mean ± s.d. The 95% CI are: [−4.53, −1.22] (a); [−4.32, −0.05] (b); [−2.91, −1.04] (g). The 97% CI are: [−8.63, −1.66] (c); [−19.39, −2.32] (d).

Figure 4 | ADAM10 inhibition blocks glioma growth. a–c, Orthotopic xenograft growth (fold change in photon flux) after systemic administration of GI254023X or vehicle control for SU-pcGBM2 (n = 7 control, n = 8 treated mice) (a), SU-DIPG-VI (n = 5 control, n = 4 treated mice) (b), SU-DIPG-XIX (n = 4 control, n = 4 treated mice) (c) xenografts. d, In vivo proliferation index of SU-pcGBM2 cells in vehicle control and ADAM10i treated mice (n = 4 mice per group). e, Representative confocal images (Ki67+, green; human nuclear antigen (HNA)+, red) of SU-pcGBM2 xenografts in vehicle-treated or ADAM10i-treated mice (n = 4 mice per group) as in d. Scale bar, 50 μm. f, Pharmacokinetics of INCB7839; (n = 3 mice per data point). g, Growth (fold change in photon flux) of SU-pcGBM2 xenografts after systemic administration of INCB7839 or vehicle control; (n = 5 control, n = 4 treated mice) h. Schematic summary. NRXN, neurexin; sNLGN3, soluble neuroligin-3. P values determined by Student's two-tailed t-test (a, b, g) or two-sided Mann–Whitney test (c, d). Each dot represents one mouse. Data are mean ± s.e.m except in f, where data are mean ± s.d. The 95% CI are: [−4.53, −1.22] (a); [−4.32, −0.05] (b); [−2.91, −1.04] (g). The 97% CI are: [−8.63, −1.66] (c); [−19.39, −2.32] (d).

The possibility of cross-inhibition with pharmacological protease inhibition, genetic models were used to definitively determine the proteolytic mechanism. Acute cortical slices from Mmp9 knockout mice showed no change in NLGN3 secretion (Fig. 3i), suggesting that MMP9 is not responsible for NLGN3 cleavage. Conditional knockout of Adam10 from CamKIIα neurons resulted in an approximately 50% decrease in levels of secreted NLGN3 (Fig. 3j), whereas conditional deletion of Adam10 from OPCs did not influence NLGN3 secretion (Fig. 3k). Incubation with TTX substantially abrogates ADAM10 release into active slice conditioned medium (Fig. 3l), demonstrating activity-regulated release of ADAM10, a finding consistent with reports of ADAM10 localized to synaptic vesicles. Taken together, these data indicate that ADAM10, released in activity-dependent fashion from neurons, is the chief enzyme responsible for NLGN3 shedding.

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ADAM10 inhibitors have been developed for clinical use, and the ADAM10/17 inhibitors INCB7839 and XL-784 have been through phase II clinical trials for other diseases. Brain penetration of INCB7839 was superior to XL-784 (Fig. 4f and Extended Data Fig. 7b) and sufficient to inhibit ADAM10 enzymatic function (Fig. 4f). INCB7839 robustly inhibited the growth of paediatric GBM orthotopic xenografts (Fig. 4g). ADAM10 mediates the cleavage of several cell
surface proteins, prominently targeting synapse-associated proteins\textsuperscript{19}, and also has an important role in amyloid protein processing\textsuperscript{25,26}. While ADAM10 inhibition appears well-tolerated in clinical trials\textsuperscript{25,26}, and caused no overt neurotoxicity here (Extended Data Fig. 8), long-term effects on neurological function should be carefully evaluated.

The dependency of HGG growth on microenvironmental NLGN3 is conserved across several classes of paediatric and adult HGG (Supplementary Note 1). The magnitude of this effect both underscores its potential as a therapeutic target and suggests that the mechanisms by which NLGN3 promotes HGG growth (Fig. 4h) are incompletely understood. Future work will need to determine further mechanisms by which NLGN3 regulates glioma progression and clarify how the cancer may circumvent this dependency. Targeting the ADAM10 sheddase represents a potentially transformative strategy to modulate NLGN3 levels in the tumour microenvironment for HGG therapy.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to Methods, along with any additional Extended Data display items and levels in the tumour microenvironment for HGG therapy.

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Methods

Mice and housing conditions. All in vivo experiments were conducted in accordance with protocols approved by the Stanford University Institutional Animal Care and Use Committee (IACUC) and performed in accordance with institutional guidelines. Animals were housed according to standard guidelines with free access to food and water in a 12 h light/12 h dark cycle. For brain tumour xenograft experiments, the IACUC does not set a limit on maximal tumour volume but rather on indications of morbidity; mice were euthanized if they exhibited signs of neurological defects or if they lost 15% or more of their body weight.

For constitutive Nlgn3 knockout studies, Nlgn3 knockout mice (The Jackson Laboratory) were intercrossed with Nsg mice (non-obese diabetic (NOD)-severe combined immunodeficiency (SCID) N2g2+/− (NSG); The Jackson Laboratory) to produce the Nlgn3 knockout;NSG genotype. All Nlgn3 mouse model experiments were performed with male mice, either hemizygous wild-type Nlgn3 (Nlgn33+/−;NSG) or hemizygous null Nlgn3 littermates (Nlgn3−/−;NSG).

For conditional knockout experiments, Nlgn33/− mice (a gift from T. Sudhof) or Adam10−/− mice (The Jackson Laboratory) were crossed to CamKII−CreER (The Jackson Laboratory) or PDGFβR−CreER (The Jackson Laboratory). Cre+ or control Cre−/− mice were tested with 100 mg kg−1 tamoxifen intraperitoneally for 5 days and experiments were performed 7 days after the end of treatment. Tamoxifen was given from postnatal day 28 (P28) to P33 and brain slice experiments performed at P40. Mmp9−/− mice (The Jackson Laboratory) were used in brain slice experiments at P40. Thy1−:ChR2 (Jackson Labs, line 18) were used in brain slice experiments at 4–7 weeks of age.

Bioluminescence imaging. For in vivo monitoring of tumour growth, bioluminescence imaging was performed using an IVIS imaging system (Xenogen). Mice orthotopically xenografted with luciferase-expressing glioma cells were placed under isofluorane anaesthesia and injected with luciferin substrate. Animals were imaged at baseline and randomized based on tumour size by a blinded investigator so that experimental groups contained an equivalent range of tumour sizes. All total flux values were then normalized to baseline values to determine fold change of tumour growth. Statistical analysis between tumour burden in each group was assessed using Student’s two-tailed t-test (parametric data) or Mann–Whitney test (non-parametric data). On the basis of the variance of xenograft growth in control mice, we used at least 3 mice per genotype to give 80% power to detect an effect size of 20% with a significance level of 0.05.

Orthotopic xenografting. Patient-derived DIPC cells (SU-DIPG-V1) from a pontine DIPG tumour or SU-DIPG-XIII-FL, from a frontal lobe DIPG metastasis, paediatric glioblastoma cells (SU-PGMB2 cells), adult glioblastoma cells, (SU-GMBM35) or HER2 breast cancer brain metastasis cells (DF-MBM35) expressing firefly luciferase were xenografted into the orthotopic location of derivation in Nlgn3−/−;NSG and Nlgn3−/−;NSG mice. A single-cell suspension from cultured neural spheres was prepared in sterile PBS immediately before the xenograft procedure. Animals at P34–P36 were anaesthetized with 1–4% isoflurane and placed in a stereotactic apparatus. The cranium was exposed via midline incision under isofluorane anaesthesia and injected with luciferin substrate. Animals were then moved into a stereotactic apparatus. The cranium was exposed via midline incision under isofluorane anaesthesia and injected with luciferin substrate. Animals were then placed in a stereotactic apparatus. The cranium was exposed via midline incision under isofluorane anaesthesia and injected with luciferin substrate. Animals were then placed in a stereotactic apparatus. The cranium was exposed via midline incision under isofluorane anaesthesia and injected with luciferin substrate.

Perfusion and immunohistochemistry. Animals were anaesthetized with intra-peritoneal Avertin (tribromoethanol), then transcardially perfused with 20 ml of PBS. Brains were fixed in 4% paraformaldehyde overnight at 4°C, then transferred to 30% sucrose for cryoprotection. Brains were embedded in Tissue-Tek OCT (Sakura) and sectioned in the coronal plane at 40 μm using a sliding microtome (Microm HM450; Thermo Scientific). For immunohistochemistry, coronal sections were incubated in blocking solution (3% normal donkey serum, 0.3% Triton X-100 in TBS) at room temperature for 30 min. Chicken anti-GFP (1:500, Abcam), rat anti-MBP (1:300, Abcam), mouse anti-human nuclei clone 235-1 (1:100; Millipore), rabbit anti-cleaved caspase 3 (1:200, Cell Signalling), or mouse anti-NeuN (Millipore, 1:2,000) were diluted in 1% blocking solution (1% normal donkey serum in 0.3% Triton X-100 in TBS) and incubated overnight at 4°C. Sections were then rinsed three times in 1× TBS and incubated in secondary antibody solution (Alexa 488 donkey anti-chicken IgG, 1:500 (Jackson Immuno Research); Alexa 594 donkey anti-mouse IgG, 1:500 (Life Technologies)); Alexa 647 donkey anti-rabbit IgG, 1:500 (Life Technologies); Alexa 594 donkey anti-rat IgG, 1:1,000 (Life Technologies)) in 1% blocking solution at 4°C overnight. Sections were rinsed three times in TBS and mounted with ProLong Gold Mounting medium (Life Technologies).

Cell culture. For all human tissue studies, informed consent was obtained and tissue was used in accordance with protocols approved by the Stanford University or Dana Farber Cancer Institute Institutional Review Board (IRB). For all patient-derived cultures, short tandem repeat (STR) DNA fingerprinting was performed at least three months after cryopreservation to verify authenticity. The STR fingerprints and clinical characteristics for the patient-derived cultures have been previously reported1 with the exception of SU-DIPG-XIX which is a H3.3K27M mutant tumour that was derived from the brainstem at the time of autopsy from a male who was 2 years of age at diagnosis, treated with radiotherapy and cabazitaxel, and survived 18 months. STR fingerprint for SU-DIPG-XIX is: X/Y (AMEL), 10/11 (CSF1P01), 13/14 (D13S317), 9/13 (D16S539), 30/30 (D21S11), 11/12 (DSS818), 10/10 (D7S820), 9/3.9 (TH01), 8/11 (TPOX), 17/18 (WGA). All cell cultures were routinely tested for mycoplasma.

All high-grade glioma cultures were generated as previously described1. In brief, tissue was obtained from high-grade glioma (WHO grade III or IV) tumours at the time of biopsy or from early post-mortem donations. Tissue was dissociated both mechanically and enzymatically and grown in a defined, serum-free medium designated ‘tumour stem media’ (TSM), consisting of Neurobasal-A (Invitrogen), B27-(A) (Invitrogen), human-bFGF (20 ng ml−1) (Shenandoah Biotech), human-EGF (20 ng ml−1) (Shenandoah), human PDGF-AA (10 ng ml−1) and PDGF-BB (10 ng ml−1) (Shenandoah) and heparin (2 ng ml−1) (Stem Cell Technologies). Breast cancer brain metastasis line, PDX-DF-BM354, was provided by the Zhao laboratory and developed as previously described1. Generation of conditioned medium from acute cortical slices. Conditioned medium was generated as previously described1. Mice (genotype varied based on experiment) were anesthetized with 1–4% isoflurane and immediately cervical disconnected and decapitated. Extracted brains were placed in oxygenated high-sucrose solution and sliced in 350-μm sections. Slices were then placed in buffering solution (aCSF) and allowed to recover for at least 1 h. After recovery, slices were then moved into fresh aCSF in a 24-well plate and slices optogenetically stimulated using a blue-light LED to observe the effects of elevated neuronal activity (in the case of Thy1−:ChR2 brain slices) or unstimulated to observe the effects of baseline neuronal activity. After recovery, medium was conditioned for 30 min. For various experiments, conditioned medium was prepared in the presence of various protease inhibitors (described below) or tetrodotoxin at 1 μM (Tocris). Surrounding medium was then collected for immediate use or frozen at −80°C for future experiments. All slice experiments were performed in three biological replicates unless otherwise indicated.

EdU incorporation assay. 8-well chamber slides were coated with poly-1-lysine. Cells were then seeded at 40,000 cells per well and exposed to various conditions based off the assay (aCSF, aCSF plus the relevant inhibitor (see below), active conditioned medium (conditioning methods vary by assay), recombinant NLGN3 (Origene Technologies), NLGN1 (R&D Systems), NLGN4 (R&D Systems), or NLGN4Y (R&D Systems) 10 μM EdU was added to each well, fixed 24 h after 4 μM parafomaldehyde in PBS and stained with the Click-IT EdU kit and protocol (Invitrogen). Proliferation index was then determined by quantifying percentage of EdU labelled cells using confocal microscopy at 200× magnification. Group mean differences were otherwise assessed using one-way analysis of variance (one-way ANOVA) with Tukey’s post hoc tests to further examine pairwise differences. All experiments were performed in three biological replicates. CellTiter-Glo assay of cell viability. To assess overall cell number, 5,000 glioma cells were seeded in minimal growth medium in a 96-well plate with varying concentrations of ADAM10 inhibitor. After 24, 48 or 72 h, CellTiter-Glo reagent (Promega) was added at 1:1 ratio. Luminescence was measured after a 10-min incubation at room temperature to stabilize signal. All experiments were performed in three biological replicates.

Inhibitors used. Batimastat at 20 nM (Pan MMP inhibitor; BB-94; Selleck Chemicals); MMP2/MMP9 Inhibitor II at 50 nM (sc-311430; Santa Cruz Biotechnology); ARP 100 at 20 nM (MMP2 inhibitor) (R&D Systems); MMP13 Inhibitor at 10 nM (sc-205756; Santa Cruz Biotechnology); MMP9 Inhibitor I at 100 nM (sc-311437; Santa Cruz Biotechnology); MMP9/MMP13 Inhibitor II at 10 nM (sc-311439; Santa Cruz Biotechnology); MMP2/MMP3 Inhibitor I at 20 nM (sc-295483; Santa Cruz Biotechnology); TAPI-1 at 20 μM (ADAM17 inhibitor; Selleck Chemicals); Batimastat at 20 μM (ADAM10 inhibitor; Sigma Aldrich); PF-00526271 at 5 nM (FAK inhibitor; S2672; Selleck Chemicals).

Analysis of NLGN3 secretion from glioma. For in vivo studies demonstrating NLGN3 secretion from xenograft-bearing slices, mice were xenografted as above with SU-DIPG-XIII or SU-GMBM35 cells in premotor cortex. Brains were extracted...
and used for conditioned medium experiments in comparison to non-epidermal
littermate controls at 5 months (SU-DIPG-XIII) or 6 weeks (SU-GBM035). These experiments were performed in duplicate for SU-DIPG-XIII xenograft-bearing cortical slices and in triplicate for SU-GBM035 xenograft-bearing cortical slices (five biological replicates in total). For in vitro studies demonstrating that neurilin secretion from xenograft-bearing slices can be blocked by ADAM10 inhibition, xeno were xenografted as above with SU-GBM035 cells in premotor cortex and brains were extracted at 6 weeks post xenograft. Cortical slices were made and incubated in the presence or absence of 200 nM ADAM10 inhibitor, GI254023X.
Conditioned medium was then analysed using western blot analyses, comparing non-xenograft bearing slices to xenograft-bearing slices from wild-type or Ngn3 knockout mice in the presence of absence of an ADAM inhibitor. These experiments were performed in triplicate.

For in vitro studies of Nlgn3 secretion from glioma cells, SU-pcGBM2 cells were seeded at 5 million cells per well in the presence of either vehicle, 10 nM recombinant Nlgn3, 200 nM GI254023X, or 100 nM Nlgn3 + 200 nM GI254023X for 48 h. After thorough rinsing of the cells, cells were left in either fresh medium or 200 nM GI254023X for another 48 h. After 48 h, medium was collected and analysed for presence of cleaved Nlgn3 using western blot analyses as described below. Experiments were performed in three biological replicates.

The spherical invasion assay was performed as previously described12.

Neurosphere formation assay. Extreme limiting dilution analysis (ELDA) was performed to evaluate self-renewal capacity12. SU-pcGBM2 cells were dissociated in TrypLE (+DNase and HEPES) for 5 min at 37°C. Cells were triturated into a single-cell solution. The solution was incubated with Hoechst (Thermo, 33342) for 30 min at 37°C. Live cells were identified using a LIVE/DEAD staining kit (Thermo, L10119). Live cells were sorted into 96-well plates. Spheres were counted at 14 days. Cell density per well ranged from 1, 10, 25, 50, 100, 260, 500 to 1,000. Each condition was tested in 10 independent wells. Volume of medium per well was 200 μl with growth factors spike-ins every 3–4 days. The ADAM10 inhibitor was reconstituted in DMSO. Each well was adjusted to have 0.1% DMSO, except for the no-DMSO control wells. Neurosphere-forming capability was determined using the ELDA web-based tool (http://bioinfo.wehi.edu.au/software/elda/).

Western blot analyses. Western blot analyses were used to probe for protein levels present in either brain slice homogenate or secreted into slice conditioned medium. For slice homogenates, brain slices were lysed using RIPA buffer and protease inhibitors. Lysates were incubated on ice for 10 min and then centrifuged at 10,000g for 10 min at 4°C. All samples were normalized to protein concentration, mixed with Laemmli loading buffer (1:4), boiled for 5 min, and loaded onto BioRad Mini-Protein TGE precast gels. Protein was transferred to PVDF membranes and blocked with 5% bovine serum albumin (BSA) in TBST for 1 h. Anti-neurologin-3 (NovusBio; 1:250), anti-phospho FAK pTyr861 (Thermo Fisher Scientific; 1:500), anti-neuroligin-3 (Nlgn3) (4.6 mm UFLC system. LC separation was carried out on a ZORBAX SB-Phenyl column (5 μm, 4.6 mm × 50 mm, 3.5 μm) (Agilent) at room temperature. The analysis time was 3 min. The injection volume was 5–10 μl. Isocratic elution was carried out with a mobile phase composed of 55% water and 45% acetonitrile with 0.1% of formic acid and a flow rate of 0.5 ml min−1. The mass spectrometer was operated in the positive mode with multiple-reaction monitoring (MRM) with the transition m/z 392.2 to 361.2. Data acquisition and analysis were performed using the Analyst 1.6.1 software (AB SCIEX).

LC–MS/MS analysis of GI254023X concentrations in tissues and serum. A single 100 mg kg−1 dose was delivered intraperitoneally in NSG mice, and tissue samples collected 30 min later for analysis using LC–MS/MS. Tissues samples were weighed and 1 volume of buffer solution (NextSeq 500 by Stanford Functional Genomics Facility. Reads were mapped to hg19 annotation using TopHat228 (version 2.0.13) and transcript expression was quantified against RefSeq gene annotations using featureCounts37. Differential testing and log2 fold change calculation was performed using DESeq238 with default multiple hypothesis adjustment to control false positives (Benjamini–Hochberg, FDR = 0.1). Gene Ontology analyses were performed using DAVID39,40.

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Model Animal Research Center of Nanjing University. Study was conducted by Crown Biosciences, in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of CrownBio and in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Compounds were formulated in 2% DMSO, 2% Tween 80, 48% PEG300, 48% water. Compounds were administered intraperitoneally, with a dosing volume of 10μl g⁻¹ and concentration of 5 mg ml⁻¹. Compounds were dosed at 50 mg kg⁻¹. A cohort of 24 male, NSG mice, age 6–8 weeks, body weight 18–22 g, were used for each study. Animals were housed at room temperature, under 40–70% humidity, with a 12 h light/12 h dark schedule. Mice were fed with Co²⁺ dry granule food, with free access to reverse osmosis water. Eight time points were collected for each compound (0.25, 0.5, 1, 2, 4, 6, 8 and 24 h), with an n = 3 for each time point. Blood was collected via cardiac puncture and collected into potassium–EDTA Eppendorf tubes. Samples were centrifuged within 30 min to afford plasma samples. Brains were collected at each time point, PBS (4 ×) was added, and the material homogenized with a Tissue Lyser II to give a fine homogenate. Brain homogenate (50 μl) was treated with 250 μl acetonitrile (containing 200 ng ml⁻¹ tolbutamide), which was then vortexed and then centrifuged at 4,000 r.p.m. (3220g) for 20 min. The supernatant was collected and mixed with a 0.1% aqueous formic acid solution. Samples were analysed on a Waters UPLC or Agilent 1200 Liquid chromatography system, with an API 4000 mass spectrometer, and a 10μl injection volume, with tolbutamide as an internal standard. Pharmacokinetics were analysed using WinNonlin6.3 (non-compartmental model).

Mouse drug treatment studies. For all drug studies, NSG mice were xenografted as above with either SU-pcGBM2, SU-DIPG-VI, or SU-DIPG-XIX cells. Four weeks post-xenograft, animals were treated with either systemic administration of ADAM10 inhibitor, GI254023X (Sigma-Aldrich; formulated in 10% DMSO in aqueous formic acid solution. Samples were analysed on a Waters UPLC or Agilent 1200 Liquid chromatography system, with an API 4000 mass spectrometer, and a 10μl injection volume, with tolbutamide as an internal standard. Pharmacokinetics were analysed using WinNonlin6.3 (non-compartmental model).

Confocal imaging and quantification of cell proliferation. Cell quantification was performed by a blinded investigator using live counting at 400 × magnification using a Zeiss LSM700 scanning confocal microscope and Zen 2011 imaging software (Carl Zeiss). The area for quantification was selected as follows: of a 1 in 6 series of 40μm coronal sections, 3 consecutive sections were selected at approximately 1.1–0.86 mm anterior to bregma (figures 22, 23 and 24 of Franklin and Paxinos3); using our stereotactic coordinates for tumour xenograft, these sections are expected to include the tissue most proximal to the site of tumour cell implantation in the coronal plane. For each of the three consecutive sections, the cingulum bundle was first identified as an anatomic landmark, and six 160 × 160-μm field area for quantification were selected centred around this landmark within cortical layer 6b of M2. Within each field, all human nuclear antigen (HNA)-positive tumour cells were quantified to determine tumour burden within the areas quantified. HNA-positive tumour cells were then assessed for double-labelling with Ki67. To calculate the proliferation index (the percentage of proliferating tumour cells for each animal), the total number of HNA-positive cells co-labelled with Ki67 across all areas quantified was divided by the total number of human nuclei-positive cells counted across all areas quantified. Differences in proliferation indices were calculated using unpaired, two-tailed Student’s t-tests (parametric data) or Mann–Whitney test (non-parametric data).

Statistical analyses. Statistical tests were conducted using Prism (GraphPad) software unless otherwise indicated. Gaussian distribution was confirmed by the Shapiro–Wilk normality test. For parametric data, unpaired, two-tailed Student's t-tests and one-way ANOVA with Tukey's post hoc tests to further examine pairwise differences were used. For non-parametric data, two-sided Mann–Whitney test was used. The limiting dilution assay to test for neurosphere forming capacity was analysed with a chi-squared test using the ELDA web-based tool (http://bioinf.wehi.edu.au/software/elda/). A level of P < 0.05 was used to designate significant differences. On the basis of the variance of xenograft growth in control mice, we used at least 3 mice per genotype to give 80% power to detect an effect size of 20% with a significance level of 0.05. For all animal experiments, the number of independent mice used is listed in figure legend. For all western blots, analyses were done in biological triplicate, except in the case of Fig. 2d, e and Extended Data Fig. 6a, in which analyses were done in biological duplicates and technical triplicates.

Statistical analyses for proteomic and RNA-seq data are described above in the respective sections.

Materials availability. All glioma cultures are freely available and can be obtained by contacting the corresponding author and with a standard materials transfer agreement.

Data availability. RNA-seq data are deposited in the Gene Expression Omnibus (GEO) under accession number GSE99045. The source data for the main figures and all western blots are included in the supplementary information for this paper.

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Extended Data Figure 1 | Orthotopic xenografts of paediatric glioblastoma fail to grow in the NLGN3-deficient brain.

a, Engraftment is equivalent in Nlgn3 knockout and wild-type mice. In vivo bioluminescence imaging of SU-pcGM2 xenografts 2 weeks after xenograft in WT;NSG (WT; left) or Nlgn3 KO;NSG mice (KO; right).

The heat map superimposed over the mouse heads represents the degree of photon emission by cells expressing firefly luciferase. b, Absolute flux of pHGG cells in identically manipulated WT;NSG (n = 11) and Nlgn3 KO;NSG (n = 14) mice, measured by IVIS imaging 2 weeks after xenograft illustrates no significant difference (P > 0.05) in tumour engraftment (two-sided Mann–Whitney test). Data are mean ± s.e.m.

c, d, Data from Fig. 1 shown on the same axis (c) and with each independent cohort colour-coded for comparison of littermates (d). Data illustrate growth of pHGG (SU-pcGBM2) xenografts in identically manipulated WT;NSG (black dots, n = 11) and Nlgn3 KO;NSG (grey dots, n = 14) mice, measured by IVIS imaging (fold change in total photon flux) and shown at 6, 12, 18 and 24 weeks post-xenograft. Data were replicated in five independent cohorts (litters) of mice xenografted with different cell preparations on different days and the data from these five biological replicates are shown combined with each cohort colour-coded (that is, littermates are shown in the same colour). **P < 0.01, ****P < 0.0001, two-sided Mann–Whitney test. Data are mean ± s.e.m.
Extended Data Figure 2  |  Neuroligin-3 is the only neuroligin family member that promotes glioma proliferation.  
a. Schematic representation of active conditioned medium generation (left). Proliferation index (EdU+ and DAPI co-positive nuclei/total DAPI+ nuclei) of pHGG cells (SU-pcGBM2) exposed to plain medium (aCSF), optogenetically stimulated Nlgn3 wild-type cortical slice conditioned medium, or optogenetically stimulated Ngn3 knockout cortical slice conditioned medium ($F = 30.8, P < 0.001$).

b, c. Proliferation index of patient-derived paediatric cortical glioblastoma (SU-pcGBM2) cells as measured by EdU incorporation 24 h after in vitro exposure to recombinant human NLGN1 at concentrations ranging from 0 to 100 nM (b) or recombinant human NLGN4X or NLGN4Y at 100 nM (c). $n = 3$ wells per condition. All data are mean ± s.e.m. *$P < 0.05$, **$P < 0.001$, one-way ANOVA with Tukey’s post hoc test for multiple comparisons. n.s. indicates $P > 0.05$. 

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Extended Data Figure 3 | Gene expression changes induced by neuroligin-3 in glioma. a, Scatterplot showing SU-pcGBM2 (n = 2) gene expression changes after 16h of treatment with vehicle (~1% DMSO) or NLGN3 (100 nM). The x axis shows mean fragments per kilobase of transcript per million mapped reads (FPKM) value in vehicle-treated cells, and the y axis shows log2(fold change) of NLGN3 compared to vehicle. Points shown in red represent genes showing statistically significant change (adjusted P < 0.1, Benjaminin–Hochberg for multiple comparison testing). b, Gene Ontology (GO) biological processes enriched in significantly upregulated genes with NLGN3 treatment, as identified by DAVID with P values shown with Benjamini–Hochberg adjustment for multiple comparison testing. c, Genes associated with each GO biological processes terms shown in b.
Extended Data Figure 4 | Neuroglgin-3 expression data and efficiency of Cre driver mice. a, Nlgn3 RNA expression (FPKM values) in various cell types (data from brain RNA-seq dataset of Barres and colleagues\textsuperscript{13}). b, c, Recombination rate of inducible Cre driver models 7 days after treatment with tamoxifen (100 mg kg\textsuperscript{-1} for 5 days) in Rosa26::tdTomato\textsuperscript{lox-stop-lox} reporter mice. b, To assess the neuron-specific CamKII\textalpha–CreER Cre driver, recombination efficiency was quantified as a percentage of NeuN\textsuperscript{+} neurons that co-express tdTomato\textsuperscript{+} in the cortex of either CamKII\textalpha–CreER\textsuperscript{−} or CamKII\textalpha–CreER\textsuperscript{+} mice 7 days after completion of tamoxifen administration. c, To assess the OPC-specific Cre driver PDGFR\textalpha–CreER, recombination efficiency was quantified as the number of PDGFR\textalpha\textsuperscript{+} OPCs that co-express tdTomato in the cortex of either PDGFR\textalpha–CreER\textsuperscript{−} or PDGFR\textalpha–CreER\textsuperscript{+} mice. n = 3 mice per group.
Extended Data Figure 5  |  NLGN3 shedding from glioma cells is regulated by NLGN3 exposure and is mediated by ADAM10. a, NLGN3 western blot illustrating NLGN3 secreted into conditioned medium from optogenetically stimulated Thy1::ChR2; NSG cortical slices (ChR2 stim slice) or SU-DIPG-XIII xenograft-bearing Thy1::ChR2; NSG cortical slices (ChR2 stim slice with xenograft). Performed in biological duplicate. b, NLGN3 western blot illustrating NLGN3 secreted into conditioned medium from wild-type brain slices, wild-type brain slices bearing xenografts of adult GBM SU-GBM035 (WT + xeno), or from Nlgn3 knockout brains bearing SU-GBM035 xenografts (Nlgn3 KO + xeno) in the absence (left three lanes) or presence (right three lanes) of 200 nM ADAM10 inhibitor GI254023X (+ADAM10i). Performed in biological triplicate. c, NLGN3 western blot illustrating glioma cell secretion of NLGN3 in vitro at baseline medium conditions (aCSF), following exposure to recombinant NLGN3 with subsequent washing (NLGN3), at baseline medium conditions in the presence of ADAM10 inhibitor GI254023X (aCSF + ADAM10i) or after NLGN3 exposure in the presence of ADAM10 inhibitor (NLGN3 + ADAM10i). Performed in biological triplicate. d, mRNA expression levels of ADAM10 in primary tumour and cultures of DIPG by RNA-seq with values reported as FPKM12,42 (left; n = 8 primary samples, n = 7 culture samples) and in 493 individual adult glioblastoma samples from TCGA43 (right). Values are reported as robust multi-array averages (RMA; right). Boxes show the median, 25th and 75th percentiles, error bars show the minima and maxima.
Extended Data Figure 6 | Functional consequences for glioma of protease inhibition in situ and in vitro. a, SU-pcGBM2 cells (EdU, red; DAPI, blue) exposed to conditioned medium generated in the presence or absence of ADAM10 inhibitor. Scale bar, 50 μm. b–d, Proliferation indices of SU-pcGBM2 cells exposed to plain medium (aCSF) or active conditioned medium generated in the presence of pan-MMP inhibitor (BAT) (b), ADAM10 inhibitor (c) or ADAM10 inhibitor with or without NLGN3 rescue (d), n = 3 wells per condition. e, Cell viability of SU-pcGBM2 cells exposed to ADAM10 inhibitor (GI254023X, 10 nM–2 μM) at 24, 48 and 72 h (n = 3 wells per condition). f, Proliferation index of SU-pcGBM2 cells exposed to GI254023X (0–2 μM) (n = 3 wells per condition). g, Spheroid invasion index of SU-DIPG-VI cells exposed to ADAM10i (0–5 μM) at 24, 48 and 72 h expressed as the diameter of the sphere of glioma cells relative to the initial diameter at time 0 h. h, Neurosphere formation assay in SU-pcGBM2 cells in the presence of GI254023X (0–2 μM; n = 10 wells per condition. i, Extreme limiting dilution assay (ELDA) data presented in h re-plotted here as a log fraction plot with the slope of the solid line representing the log-active cell fraction and confidence intervals shown as dotted lines. SU-pcGBM2 cells treated with ADAM10 inhibitor GI254023X at 0.5 μM (black), 1 μM (red) or 2 μM (green), with vehicle (DMSO) control (royal blue) or no DMSO (cyan) and analysed for neurosphere formation at 2 weeks. In b–d and f, P values as indicated; one-way ANOVA with Tukey's post hoc test for multiple comparisons. Data are mean ± s.e.m. In h, χ² test; data are mean ± confidence intervals. n.s. indicates P > 0.05.
Extended Data Figure 7 | Glioma xenograft proliferation after ADAM10 inhibition and pharmacokinetics of XL-784.  

**a.** Representative confocal images (Ki67, green; human nuclear antigen, red; MBP, white) of vehicle-treated or ADAM10i-treated mice bearing frontal cortex SU-pcGBM2 xenografts; images similar to but lower magnification than those shown in Fig. 4e; n = 4 vehicle, n = 4 ADAM10i-treated mice. Scale bar, 100 μm.  

**b.** Brain tissue and plasma levels of XL-784 at various time points following a single 50 mg kg⁻¹ intraperitoneal dose in NSG mice as assessed by LC–MS/MS. n = 3 mice at each data point. Data are mean ± s.d.
Extended Data Figure 8 | Lack of detectable neurotoxicity after treatment with INCB7839. Histological assessment of neuronal integrity was performed in mice treated with INCB7839 or vehicle control. The cortex, CA1 region of the hippocampus and dentate gyrus of the hippocampus were examined immunohistologically in the hemisphere contralateral to glioma xenografts in mice treated with INCB7839 or vehicle control. Brain sections were immunostained with NeuN (green) to mark neuronal nuclei and cleaved caspase-3 (red) to mark apoptotic cells and counterstained with DAPI (blue). Representative sections from $n = 4$ INCB7839-treated mice and $n = 4$ vehicle control mice were examined. Neuronal nuclei appeared morphologically normal and non-pyknotic. Extremely few cleaved caspase-3$^+$ cells were identified in either group; a total of one cleaved caspase-3$^+$ cell was found in each group across all mice examined (white arrows). Scale bar, 200 μm.
Extended Data Table 1 | Phospho-antibody array analysis in glioma cells following NLGN3 exposure

| 5 minute phosphorylation | ratio of fold change | 30 minute phosphorylation | ratio of fold change |
|--------------------------|----------------------|---------------------------|----------------------|
| LYN                      | 2.72                 | NFKB1                     | 4.13                 |
| PLCG2                    | 2.21                 | CCND1                     | 3.67                 |
| FGFR1                    | 1.97                 | FLT3                      | 3.37                 |
| SHC1                     | 1.88                 | FAK2                      | 2.13                 |
| SYK                      | 1.88                 | MEK1                      | 2.06                 |
| NF-KB1                   | 1.86                 | JUN                       | 1.91                 |
| TP53                     | 1.79                 | EFN1/B2/B3                | 1.87                 |
| DOK1                     | 1.79                 | CDK1                      | 1.80                 |
| AURKA                    | 1.78                 | PLD2                      | 1.65                 |
| EGFR                     | 1.76                 | PTPN11                    | 1.62                 |
| RAF1                     | 1.67                 | CDH5                      | 1.58                 |
| RPS6KB1                  | 1.56                 | ELK1                      | 1.52                 |
| PAK2                     | 1.55                 | GSK3A                     | 1.49                 |
| PTK2B                    | 1.53                 | PAK1                      | 1.45                 |
| MET                      | 1.52                 | ETK                       | 1.44                 |
| JUN                      | 1.52                 | ITGB3                     | 1.36                 |
| VEGFR2                   | 1.51                 | CRK                       | 1.30                 |
| PLD1                     | 1.47                 | TP53                      | 1.30                 |
| GAB2                     | 1.47                 | PXN                       | 1.29                 |
| TNK2                     | 1.47                 | MTOR                      | 1.28                 |
| CCNE1                    | 1.41                 |                           |                      |
| ITGB3                    | 1.39                 |                           |                      |
| LCK                      | 1.37                 |                           |                      |
| BCAR1                    | 1.35                 |                           |                      |
| PXN                      | 1.31                 |                           |                      |

Phosphorylation ratio of NLGN3-exposed versus control SU-pcGBM2 cell lysates after 5-min or 30-min exposure to NLGN3, as assessed by phospho-antibody array.
A single 100 mg kg⁻¹ dose was delivered intraperitoneally in NSG mice (n = 3), and tissue samples collected 30 min later for analysis using LC-MS/MS. Brain tissue concentrations show reasonable penetration across the blood brain barrier, achieving 2–4 μM concentration of drug at this time point.

**Extended Data Table 2 | Brain penetration of ADAM10 inhibitor GI254023X**

| Tissue            | ADAM10 inhibitor(GI254023X) levels |
|-------------------|-----------------------------------|
| Serum             | 38 μg/ml (97 μM)                  |
| Kidney            | 106 μg/ml (270 μM)                |
| Cerebral cortex   | 1.6 μg/ml (4 μM)                  |
| Pons              | 887 ng/ml (2.2 μM)                |
Experimental design

1. Sample size
   Describe how sample size was determined.
   Based on the variance of xenograft growth in control mice, power calculations indicated use of at least 3 mice per genotype to give 80% power to detect an effect size of 20% with a significance level of 0.05

2. Data exclusions
   Describe any data exclusions.
   No data were excluded from the analyses.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All attempts at replication were successful.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   For patient-derived xenograft studies, animals were IVIS imaged at baseline and randomized based on tumor size by a blinded investigator so that experimental groups contained an equivalent range of tumor sizes.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   The experimenter performing IVIS imaging and histological quantifications was blinded to group allocation.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a | Confirmed
   --- | ---
   □ | □ The exact sample size \( (n) \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   □ | □ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   □ | □ A statement indicating how many times each experiment was replicated
   □ | □ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   □ | □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   □ | □ The test results (e.g. \( P \) values) given as exact values whenever possible and with confidence intervals noted
   □ | □ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   □ | □ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
Software

7. Software

Describe the software used to analyze the data in this study.

Statistical tests were conducted using Prism (GraphPad) software for most analyses. The limiting dilution assay to test for neurosphere forming capacity was analyzed with a chi-squared test using the Extreme Limiting Dilution Analysis (ELDA) web-based tool (http://bioinf.wehi.edu.au/software/elda/). For RNA-seq analyses, reads were mapped to hg19 annotation using TopHat2 (version 2.0.13) and transcript expression was quantified against RefSeq gene annotations using featureCounts. Differential testing and log2 fold change calculation was performed using DESeq2. Gene Ontology analyses were performed using DAVID. For mass spectrometry, data acquisition and analysis were performed using the Analyst 1.6.1 software (AB SCIEX). Western blots were quantified and analyzed using ImageJ 1.48v. Pharmacokinetics were analyzed using WinNonlin 6.3

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials such as patient-derived cell cultures are freely available and can be obtained by contacting the corresponding author and with a standard MTA with Stanford University.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies used in Western blots: Anti-Neuroligin-3 (NovusBio; 1:250; #NB1-90090 Lot A00876), Anti-phospho FAK pY861 (Thermo Fisher Scientific; 1:500; #44-626G Lot QJ221024), and anti-FAK (Cell Signaling Technologies; 1:500; #3285S Lot 9), anti-ADAM10 (Abcam; 1:500; #Ab1997 Lot GR282958-1), anti-rabbit IgG HRP-linked Antibody (Cell Signaling Technologies; 1:2000; #70745 Lot 26)

Antibodies used in immunohistochemistry: Chicken anti-GFP (Abcam; 1:500; #Ab13970 Lot GR236651-11), Rat anti-MBP (Abcam; 1:300; #Ab7349 Lot GR267330-2), Mouse anti-human nuclei clone 235-1 (Millipore; 1:100; #MAB1281 Lot 2886689), rabbit anti-Ki67 (Abcam; 1:500; #Ab15580 Lot GR292681-1), rabbit anti-cleaved caspase 3 (Cell Signaling; 1:200; #9661S Lot 43), mouse anti-NeuN (Millipore; 1:2000; #MAB377), Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) (Jackson Immuno Research; 1:500; #703-54S-155 Lot 129193), Goat anti-Mouse IgG Secondary Antibody Alexa Fluor Fluor 594 (Life Technologies; 1:500; #R37121 Lot 1572551), Goat anti-Rat IgG Secondary Antibody Alexa Fluor 594 (Life Technologies; 1:1000; #A21209 Lot 1547508), Goat anti-Rabbit IgG Secondary Antibody Alexa Fluor 647 (Life Technologies; 1:500; #A31573 Lot 1693297)

Antibody used in Phospho-tyrosine pull down assay: Anti-phosphotyrosine pY-1000 antibody (Cell Signaling Technologies; #8803)

All antibodies have been validated in the literature and/or in Antibodypedia for use in mouse immunohistochemistry or human cell Western blot analyses. To further validate the antibodies on our hands, we confirmed that each antibody stained in the expected cellular patterns and brain-wide distributions (for immunohistochemistry) and at the correct mobility (for Westerns). For the case of cleaved caspase-3 staining, we confirmed antibody staining in mouse brain tissue of a disease model as a positive control. For NLGN3 Westerns, we confirmed staining of recombinant NLGN3 protein.
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used.
      The eukaryotic cell cultures used are patient-derived cultures of high-grade gliomas generated in the Monje lab from biopsy (SU-pcGBM2, SU-GBM035) or autopsy tissue (SU-DIPG-VI, SU-DIPG-XIII-FL, SU-DIPG-XIX).
   b. Describe the method of cell line authentication used.
      Sort Tandem Repeat (STR) fingerprinting is performed every 3 months on all cell cultures to ensure authenticity.
   c. Report whether the cell lines were tested for mycoplasma contamination.
      All cell cultures are routinely tested for mycoplasma contamination and all cultures used tested negative.
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
      N/A

11. Description of research animals
   Provide details on animals and/or animal-derived materials used in the study.
   Mice of the following genotypes were used: NOD-SCID-IL2R gamma chain-deficient (NSG); monitored over 6 months
   Nlgn3y-/-;NSG; monitored over 6 months
   Nlgn3fl/fl;Pdgfra::CreER; used at p40
   Nlgn3fl/fl;CAMKII::CreER; used at p40
   Thy1::ChR2; used between 4-6 weeks of age
   MMP9-/-; used between 4-6 weeks of age
   ADAM10fl/fl;CamKII::CreER; used at p40
   ADAM10fl/fl;Pdgfra::CreER; used at p40
   Because Nlgn3 is a gene on the X-chromosome, all Nlgn3 mouse model experiments were performed with male mice. For all other experiments mice of both sexes were used equally.

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants.
   Patient-derived cell cultures and xenograft models were used and the clinical characteristics of the subject described in the methods or in previous reports. No living human research participants were involved in this study.