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Figure 3 displays a series of Western blots (Fig. 2d, 2e, 3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h, 3i, 3j, 3k, 3l, 3m, 3n, 3o, 3p, 3q, 3r, 3s, 3t, 3u, 3v, 3w, 3x, 3y, 3z, 3aa, 3ab, 3ac, 3ad, 3ae, 3af, 3ag, 3ah, 3ai, 3aj, 3ak, 3al, 3am, 3an, 3ao, 3ap, 3aq, 3ar, 3as, 3at, 3au, 3av, 3aw, 3ax, 3ay, 3az, 3ba, 3bb, 3bc, 3bd, 3be, 3bf, 3bg, 3bh, 3bi, 3bj, 3bk, 3bl, 3bm, 3bn, 3bo, 3bp, 3bq, 3br, 3bs, 3bt, 3bu, 3bv, 3bw, 3bx, 3by, 3bz, 3ca, 3cb, 3cc, 3cd, 3ce, 3cf, 3cg, 3ch, 3ci, 3cj, 3ck, 3cl, 3cm, 3cn, 3co, 3cp, 3cq, 3cr, 3cs, 3ct, 3cu, 3cv, 3cw, 3cx, 3cy, 3cz, 3da, 3db, 3dc, 3dd, 3de, 3df, 3dg, 3dh, 3di, 3dj, 3dk, 3dl, 3dm, 3dn, 3do, 3dp, 3dq, 3dr, 3ds, 3dt, 3du, 3dv, 3dw, 3dx, 3dy, 3dz, 3ea, 3eb, 3ec, 3ed, 3ee, 3ef, 3eg, 3eh, 3ei, 3ej, 3ek, 3el, 3em, 3en, 3eo, 3ep, 3eq, 3er, 3es, 3et, 3eu, 3ev, 3ew, 3ex, 3ey, 3ez, 3fa, 3fb, 3fc, 3fd, 3fe, 3ff, 3fg, 3fh, 3fi, 3fj, 3fk, 3fl, 3fm, 3fn, 3fo, 3fp, 3fq, 3fr, 3fs, 3ft, 3fu, 3fv, 3fw, 3fx, 3fy, 3fz, 3ga, 3gb, 3gc, 3gd, 3ge, 3gf, 3gg, 3gh, 3gi, 3gj, 3gk, 3gl, 3gm, 3gn, 3go, 3gp, 3gq, 3gr, 3gs, 3gt, 3gu, 3gv, 3gw, 3gx, 3gy, 3gz, 3ha, 3hb, 3hc, 3hd, 3he, 3hf, 3hg, 3hi, 3hj, 3hk, 3hl, 3hm, 3hn, 3ho, 3hp, 3hq, 3hr, 3hs, 3ht, 3hu, 3hv, 3hw, 3hx, 3hy, 3hz, 3ia, 3ib, 3ic, 3id, 3ie, 3if, 3ig, 3ih, 3ii, 3ij, 3ik, 3il, 3im, 3in, 3io, 3ip, 3iq, 3ir, 3is, 3it, 3iu, 3iv, 3iw, 3ix, 3iy, 3iz, 3ja, 3jb, 3jc, 3jd, 3je, 3jf, 3jg, 3jh, 3ji, 3jj, 3jk, 3jl, 3jm, 3jn, 3jo, 3jp, 3jq, 3jr, 3js, 3jt, 3ju, 3jv, 3jw, 3jx, 3jy, 3jz, 3ka, 3kb, 3kc, 3kd, 3ke, 3kf, 3kg, 3kh, 3ki, 3kj, 3kl, 3km, 3kn, 3ko, 3kp, 3kq, 3kr, 3ks, 3kt, 3ku, 3kv, 3kw, 3kx, 3ky, 3kz, 3la, 3lb, 3lc, 3ld, 3le, 3lf, 3lg, 3lh, 3li, 3lj, 3lk, 3ll, 3lm, 3ln, 3lo, 3lp, 3lq, 3lr, 3ls, 3lt, 3lu, 3lv, 3lw, 3lx, 3ly, 3lz, 3ma, 3mb, 3mc, 3md, 3me, 3mf, 3mg, 3mh, 3mi, 3mj, 3mk, 3ml, 3mm, 3mn, 3mo, 3mp, 3mq, 3mr, 3ms, 3mt, 3mu, 3mv, 3mw, 3mx, 3my, 3mz, 3na, 3nb, 3nc, 3nd, 3ne, 3nf, 3ng, 3nh, 3ni, 3nj, 3nk, 3nl, 3nm, 3nn, 3no, 3np, 3nq, 3nr, 3ns, 3nt, 3nu, 3nv, 3nw, 3nx, 3ny, 3nz, 3oa, 3ob, 3oc, 3od, 3oe, 3of, 3og, 3oh, 3oi, 3oj, 3ok, 3ol, 3om, 3on, 3oo, 3op, 3oq, 3or, 3os, 3ot, 3ou, 3ov, 3ow, 3ox, 3oy, 3oz, 3pa, 3pb, 3pc, 3pd, 3pe, 3pf, 3pg, 3ph, 3pi, 3pj, 3pk, 3pl, 3pm, 3pn, 3po, 3pp, 3pq, 3pr, 3ps, 3pt, 3pu, 3pv, 3pw, 3px, 3py, 3pz, 3qa, 3qb, 3qc, 3qd, 3qe, 3qf, 3qg, 3qh, 3qi, 3qj, 3qk, 3ql, 3qm, 3qn, 3qo, 3qp, 3qq, 3qr, 3qs, 3qt, 3qu, 3qv, 3qw, 3qx, 3qy, 3qz, 3ra, 3rb, 3rc, 3rd, 3re, 3rf, 3rg, 3rh, 3ri, 3rj, 3rk, 3rl, 3rm, 3rn, 3ro, 3rp, 3rq, 3rr, 3rs, 3rt, 3ru, 3rv, 3rw, 3rx, 3ry, 3rz, 3sa, 3sb, 3sc, 3sd, 3se, 3sf, 3sg, 3sh, 3si, 3sj, 3sk, 3sl, 3sm, 3sn, 3so, 3sp, 3sq, 3sr, 3ss, 3st, 3su, 3sv, 3sw, 3sx, 3sy, 3sz, 3ta, 3tb, 3tc, 3td, 3te, 3tf, 3tg, 3th, 3ti, 3tj, 3tk, 3tl, 3tm, 3tn, 3to, 3tp, 3tq, 3tr, 3ts, 3tt, 3tu, 3tv, 3tw, 3tx, 3ty, 3tz, 3ua, 3ub, 3uc, 3ud, 3ue, 3uf, 3ug, 3uh, 3ui, 3uj, 3uk, 3ul, 3um, 3un, 3uo, 3up, 3uq, 3ur, 3us, 3ut, 3uu, 3uv, 3uw, 3ux, 3uy, 3uz, 3va, 3vb, 3vc, 3vd, 3ve, 3vf, 3vg, 3vh, 3vi, 3vj, 3vk, 3vl, 3vm, 3vn, 3vo, 3vp, 3vq, 3vr, 3vs, 3vt, 3vu, 3vv, 3vw, 3vx, 3vy, 3vz, 3wa, 3wb, 3wc, 3wd, 3we, 3wf, 3wg, 3wh, 3wi, 3wj, 3wk, 3wl, 3wm, 3wn, 3wo, 3wp, 3wq, 3wr, 3ws, 3wt, 3wu, 3wv, 3wv, 3wx, 3wy, 3wz, 3xa, 3xb, 3xc, 3xd, 3xe, 3xf, 3xg, 3xh, 3xi, 3xj, 3xk, 3xl, 3xm, 3xn, 3xo, 3xp, 3xq, 3xr, 3xs, 3xt, 3xu, 3xv, 3xw, 3xx, 3xy, 3xz, 3ya, 3yb, 3yc, 3yd, 3ye, 3yf, 3yg, 3yh, 3yi, 3yj, 3yk, 3yl, 3ym, 3yn, 3yo, 3yp, 3yq, 3yr, 3ys, 3yt, 3yu, 3yv, 3yw, 3yx, 3yy, 3yz, 3za, 3zb, 3zc, 3zd, 3ze, 3zf, 3zg, 3zh, 3zi, 3zj, 3zk, 3zl, 3zm, 3zn, 3zo, 3zp, 3zq, 3zr, 3zs, 3zt, 3zu, 3zv, 3zw, 3zx, 3zy, 3zz, 4a, 4b, 4c, 4d, 4e, 4f, 4g, 4h, 4i, 4j, 4k, 4l, 4m, 4n, 4o, 4p, 4q, 4r, 4s, 4t, 4u, 4v, 4w, 4x, 4y, 4z, 5a, 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 5j, 5k, 5l, 5m, 5n, 5o, 5p, 5q, 5r, 5s, 5t, 5u, 5v, 5w, 5x, 5y, 5z, 6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h, 6i, 6j, 6k, 6l, 6m, 6n, 6o, 6p, 6q, 6r, 6s, 6t, 6u, 6v, 6w, 6x, 6y, 6z, 7a, 7b, 7c, 7d, 7e, 7f, 7g, 7h, 7i, 7j, 7k, 7l, 7m, 7n, 7o, 7p, 7q, 7r, 7s, 7t, 7u, 7v, 7w, 7x, 7y, 7z, 8a, 8b, 8c, 8d, 8e, 8f, 8g, 8h, 8i, 8j, 8k, 8l, 8m, 8n, 8o, 8p, 8q, 8r, 8s, 8t, 8u, 8v, 8w, 8x, 8y, 8z, 9a, 9b, 9c, 9d, 9e, 9f, 9g, 9h, 9i, 9j, 9k, 9l, 9m, 9n, 9o, 9p, 9q, 9r, 9s, 9t, 9u, 9v, 9w, 9x, 9y, 9z, 10a, 10b, 10c, 10d, 10e, 10f, 10g, 10h, 10i, 10j, 10k, 10l, 10m, 10n, 10o, 10p, 10q, 10r, 10s, 10t, 10u, 10v, 10w, 10x, 10y, 10z, 11a, 11b, 11c, 11d, 11e, 11f, 11g, 11h, 11i, 11j, 11k, 11l, 11m, 11n, 11o, 11p, 11q, 11r, 11s, 11t, 11u, 11v, 11w, 11x, 11y, 11z, 12a, 12b, 12c, 12d, 12e, 12f, 12g, 12h, 12i, 12j, 12k, 12l, 12m, 12n, 12o, 12p, 12q, 12r, 12s, 12t, 12u, 12v, 12w, 12x, 12y, 12z, 13a, 13b, 13c, 13d, 13e, 13f, 13g, 13h, 13i, 13j, 13k, 13l, 13m, 13n, 13o, 13p, 13q, 13r, 13s, 13t, 13u, 13v, 13w, 13x, 13y, 13z, 14a, 14b, 14c, 14d, 14e, 14f, 14g, 14h, 14i, 14j,

**Supplementary Note 1: Extended Discussion.**

Here, we demonstrate an unexpected and conserved dependency of HGG growth on microenvironmental neuroligin-3 across multiple classes of pediatric and adult HGG. The magnitude of this effect both underscores its potential as a therapeutic target and suggests that the mechanisms by which NLGN3 promotes HGG growth are incompletely understood. We have furthered our understanding of the mechanisms by which NLGN3 stimulates glioma proliferation and now show that NLGN3 activates FAK upstream of the PI3K-mTOR pathway, as well as stimulating numerous additional oncogenic signaling pathways and upregulating genes associated with known mechanisms of glioma malignancy. However, loss of a single robust mitogen is unlikely to account entirely for the complete stagnation of xenograft growth observed in the Nlgn3-deficient brain. Future work will need to elucidate further mechanisms by which NLGN3 regulates glioma progression as well as by which the cancer may circumvent this dependency.

NLGN3 is cleaved and secreted from both neurons and OPCs. The physiological function of NLGN3 cleavage remains to be determined. Whether activity-regulated cleavage of NLGN3 in OPCs, likely occurring at axo-glial synapses, plays a role in the adaptive responses of myelin-forming precursor cells to neuronal activity<sup>1</sup> remains to be seen. However, these findings define a previously unrecognized place for OPCs as a microenvironmental cell type contributing to glioma growth.

Targeting NLGN3 holds great promise for glioma therapy as an adjuvant to traditional cytotoxic treatment modalities such as radiation and chemotherapy. Possible strategies to prevent the glioma growth-promoting effects of NLGN3 include blocking NLGN3 cleavage and release, sequestering soluble NLGN3 in the tumor

microenvironment or blocking the as-of-yet unidentified NLGN3 binding partner/receptor on glioma cells. Here, we focus on the former strategy, identify ADAM10 as the protease mediating NLGN3 cleavage and demonstrate robust stagnation of tumor growth with ADAM10 inhibition in preclinical models of HGG. The specific ADAM10 inhibitor GI254023X is presently in the preclinical phase of development, but the ADAM10 inhibitor INCB7839 has been through phase II clinical testing for other indications and could be re-purposed now for HGG therapy. ADAM10 mediates cleavage of numerous cell surface proteins, prominently targeting synapse-associated proteins<sup>2</sup>, and also plays an important role in amyloid protein processing<sup>3</sup>. While ADAM10 inhibition appears well-tolerated in clinical trials<sup>4,5</sup>, long-term effects on synaptic plasticity, amyloid deposition and neurological function should be carefully evaluated as part of any effort towards clinical translation of ADAM10 inhibition for this group of lethal cancers.

The present study highlights a means to target the growth-promoting effects of neuronal activity on high-grade gliomas that span a range of clinically and molecularly distinct glioma types. Further elucidation of neural influences on other classes of brain cancers may provide additional therapeutic insights. Moreover, a critical role is emerging for nervous system regulation of a variety of cancers, including prostate<sup>6</sup>, gastric<sup>7</sup>, pancreatic<sup>8</sup> and skin<sup>9</sup> cancers. Deeper understanding of the neural mechanisms driving malignancy may broadly highlight avenues towards more effective cancer control.

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