### **Supplementary Materials**

### **Supplementary Figure S1. T24 is required for optimal OSC19 invadopodia function.**

**A.** Representative epifluorescent images of OSC19 cells with stable shRNA scramble control (Ctl) or anti-cortactin shRNA combined with siRNA knockdown (KD) transduced with murine FLAG-WT, -ΔDDW, -T24A and -T24D cortactin lentiviruses. Cells were plated on Oregon Green (OG)-488 gelatin coated coverslips for 12 hours, fixed, and labeled with anti-FLAG and rhodamine phalloidin (Actin). Gelatin panels are pseudo-colored white; degradation is evident as black areas indicating loss of fluorescence. Scale bar represents 20 μm. **B.** Total cell lysates from (**A**) evaluated for endogenous and FLAG-cortactin expression by immunoblotting with antibodies against cortactin (top) and β-actin (bottom). **C.** Quantification of gelatin matrix degradation for cortactin KD and FLAG-cortactin expressing OSC19 cells. Data represents the mean + S.E.M. of n ≥ 100 cells for each line analyzed from at least three independent experiments. All conditions were normalized to control (Ctl) OSC19 cells. n.s., not significant; \*, P ˂ 0.05, Welch’s *t* test vs. Ctl. **D.** Representative epifluorescent images of UMSCC1 cells stably expressing shRNA scramble control (Ctl) or murine FLAG-WT, -ΔDDW, -T24A and -T24D cortactin lentiviruses plated on Oregon Green (OG)-488 gelatin coated coverslips for 12 hours, fixed, and labeled with anti-cortactin or anti-FLAG and rhodamine phalloidin (Actin), as indicated. Gelatin panels are pseudo-colored white; degradation is evident as black areas indicating loss of fluorescence. Scale bar represents 20 μm. Insets denote invadopodia precursors (yellow; top right corners) and active invadopodia (magenta; bottom left corners). Invadopodia precursors comprise cortactin and actin puncta lacking associated gelatin degradation (yellow arrowheads). Active invadopodia contain cortactin and actin with associated gelatin degradation (magenta arrowheads). Inset scale bar represents 2 μm.

**Supplementary Figure S2. Effects of stable and transient cortactin knockdown on HNSCC invadopodia function.**

**A.** Representative epifluorescent images of OSC19 cells with stable scramble shRNA control (Ctl), anti-cortactin siRNA knockdown (siCTTN), anti-cortactin shRNA knockdown (shCTTN), or both shRNA and siRNA cortactin treatment (KD). Cells were plated on OG-488 gelatin coverslips for 12 hours and labeled with an anti-cortactin antibody and rhodamine phalloidin (Actin). Gelatin is pseudo-colored white. Scale bar represents 20 μm. Effects of each knockdown condition on cortactin expression are found in the indicated RNAi lanes of the Western blot in Supplementary Figure S1B. **B.** Quantification of matrix degradation area per cell area for the indicated OSC19 cell lines. All conditions were normalized to control OSC19 gelatin degradation (Ctl). Data represents the mean + S.E.M. of n ≥ 100 cells per condition analyzed from at least three independent experiments. \*, P ˂ 0.05, Welch’s *t* test vs. Ctl. Ctl and KD conditions are identical to those displayed in Supplemental Figure S1. **C.** Representative epifluorescent images of UMSCC1 cells with stable scramble control (Ctl), cortactin siRNA knockdown (siCTTN), cortactin shRNA knockdown (shCTTN), or both RNAi conditions (KD). Cells plated on OG-488 gelatin for 12 hours were labeled with an anti-cortactin antibody and rhodamine phalloidin (Actin). Gelatin is pseudo-colored white. Scale bar represents 20 μm. Cortactin expression for the indicated RNAi lanes of the Western blot are shown in Figure 2D. **D.** Quantification of matrix degradation area per cell area for the indicated UMSCC1 cell lines. Conditions were normalized to control (Ctl). Data represents the mean + S.E.M. of n ≥ 100 cells per condition analyzed from at least three independent experiments. \*, P ˂ 0.05, Welch’s *t* test vs. Ctl. Ctl and KD conditions are identical to those displayed in Figure 2.

**Supplementary Figure 3. Silmitasertib inhibits CK2-dependent phosphorylation.**

HNSCC cells incubated with 0, 1 or 10 μM Silmitasertib for 24 hours (MDA1586) or 12 hours (OSC19 and UMSCC1) were lysed and evaluated for CK2α inhibition by Western blotting with antibodies against phospho-serine 473 AKT (pS473; top), pan AKT (middle), and β-actin (bottom).

**Supplementary Figure 4. Silmitasertib treatment does not impact collective invasive groups at the tumor invasive front.**

**A.** Quantitation of invasive distance and **B**. area of collective invasive group in tumors from mice treated with Silmitasertib or vehicle (DMSO). Data represent the mean + S.E.M. of two serial sections from N = 4 tumors, n ≥ 54 collective groups per condition. n.s., not significant; Welch’s *t* test vs DMSO.

**Supplementary Materials and Methods**

**Plasmid constructs**

FLAG-tagged murine cortactin expression constructs were generated as described (1). Briefly, murine cortactin cDNAs were PCR amplified as XbaI-SalIfragments and subcloned into XbaI-SalI digested pLenti CMV GFP Hygro (#17446, Addgene). GST-tagged full length and NTA human cortactin and N-WASp VCA expression constructs were generated as before (2). Full length human cortactin cDNAs were PCR amplified as ClaI-BglII fragments and subcloned into ClaI-BglII digested pGST-parallel2. Purification of recombinant proteins were performed as before (3), with GST tags removed by AcTEV protease (#12575015, Invitrogen). The pLU-Luc2 lentiviral vector was obtained from Elena Pugacheva (West Virginia University, Morgantown, WV).

**Tissue sectioning, staining and microscopy**

HNSCC patient and PDX tissue samples were fixed in 10% neutral-buffered formalin and paraffin embedded (FFPE). Extracted orthotopic tongue tumors were frozen in O.C.T. media (4583, Scigen) using a HM 525 cryostat (Thermo Scientific). For patient tissue blocks, five micron sections were cut and dried onto charged glass slides at 60-65oC for 30 minutes. Slides were washed three times in xylene (#8400-1, Statlabs) for two minutes each, rinsed in 100%, 95%, and 80% ethanol (#7100-1, Statlabs) sequentially for 20 seconds each, rinsed with distilled water twice for 10 seconds each, then incubated in hematoxylin (#7211, Richard Allan Scientific) for 90 seconds. Slides were washed twice in distilled water for 20 seconds each, then incubated with bluing solution (0.3% ammonium hydroxide, A669-212, Fisher Scientific) for 10 seconds and rinsed twice in distilled water for 20 seconds. Slides were washed with acid alcohol solution (0.1% hydrochloric acid in 70% ethanol, A144-212, Fisher Scientific), twice with distilled water for 1 minute, then with 80% ethanol for 10 seconds. Slides were incubated in eosin (#3801600, Lieca Biosystems) for 15 seconds, washed twice with 95% ethanol, three times with 100% ethanol, then three times with xylene for 20 seconds. Slides were mounted using a Tissue-Tek SCA coverslipper (Model 4764, Sakura).

#### For PDX tumors, FFPE sections were incubated three times in microwave-heated xylene for 3 minutes each. PDX tumor and orthotopic tongue tumor sections were washed thrice in 100% ethanol, then once in 96% ethanol for 1 minute. Samples were washed with distilled water, incubated with hematoxylin for 30 seconds, rinsed with water for 1 minute, then with 96% ethanol. Slides were subsequently incubated with eosin for 2 minutes, washed with water for 10 seconds, then rinsed in 96% ethanol. Slides were washed with 100% ethanol, followed by three 1 minute xylene washes. Slides were dried and mounted with glass coverslips using Permount (SP15-500, Fisher). Histological images were acquired with an Olympus VS120 Virtual Slide microscope with an UPlanSApo 20X/0.75 objective using VS-ASW-S6 software (Olympus Corporation).

**Predictive analysis of human cortactin T24 phosphorylating kinases**

The 84 amino acid sequence containing the entire human cortactin NTA domain was evaluated for potential phosphorylating kinases targeting the T24 site using the publicly available web-based programs ScanSite 2.0 (4), Minimotif Miner (5), PhosphoMotif finder (6), NetPhosK 1.0 (7), GPS 2.0 (8), PPSP (9) and KinasePhos (10). Potential kinases were ranked by frequency based on the number of different programs identifying the same kinase and are displayed in Supplementary Table S1.

**Tumor invasive group characterization and quantitation**

Collective groups were defined as independent groups of tumor cells that were discontinuous with the main tumor mass and progressed towards the base of tongue. Invasive distance represents the difference between the farthest edge of the collective group and the nearest border of the primary tumor mass. Quantitation of these groups was carried out as described for tumor invasive protrusions in the Materials and Methods.

***References***

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