An ARF GTPase module promoting invasion and metastasis through regulating phosphoinositide metabolism

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The signalling pathways underpinning cell growth and invasion use overlapping components, yet how mutually exclusive cellular responses occur is unclear. Here, we report development of 3-Dimensional culture analyses to separately quantify growth and invasion. We identify that alternate variants of IQSEC1, an ARF GTPase Exchange Factor, act as switches to promote invasion over growth by controlling phosphoinositide metabolism. All IQSEC1 variants activate ARF5- and ARF6-dependent PIP5-kinase to promote PI(3,4,5)P3-AKT signalling and growth. In contrast, select pro-invasive IQSEC1 variants promote PI(3,4,5)P3 production to form invasion-driving protrusions. Inhibition of IQSEC1 attenuates invasion in vitro and metastasis in vivo. Induction of pro-invasive IQSEC1 variants and elevated IQSEC1 expression occurs in a number of tumour types and is associated with higher-grade metastatic cancer, activation of PI(3,4,5)P3 signalling, and predicts long-term poor outcome across multiple cancers. IQSEC1-regulated phosphoinositide metabolism therefore is a switch to induce invasion over growth in response to the same external signal. Targeting IQSEC1 as the central regulator of this switch may represent a therapeutic vulnerability to stop metastasis.
central conundrum in biology is that highly overlapping signalling pathways regulate distinct biological outputs. For instance, activation of receptor tyrosine kinases (RTKs), and their downstream effectors such as the MAPK-ERK and PI3K-Akt pathway, can induce either growth or invasion. Since the development of invasive features and the progressive loss of normal tissue organisation are hallmarks of tumour progression understanding how cells decode their response to external signals has significant clinical implications.

Metastasis is the major cause of cancer-related death, increasingly recognised as a collective migration event. For a collection of epithelial cells to invade they must undergo rearrangement of normal apical–basal cellular polarisation to clusters or chains of cells lead by an invasive front. The use of 3-dimensional (3D) culture systems, whereby epithelial cells are embedded in gels of extracellular matrix (ECM) to undergo collective morphogenesis, has illuminated the molecular mechanisms of collective cell polarisation. Such polarity rearrangements can be achieved via altered membrane trafficking of morphogenesis-regulating proteins. For collective invasion to occur, signalling receptors, such as RTKs, must be directed to domains where a pro-invasive ligand is exposed. Some RTKs, such as the HGF receptor Met, require internalisation and endosomal localisation for full oncocogenic signalling. Additional membrane trafficking steps, such as recycling back to the cortex, can provide sustained signalling to effectors. However, what controls whether such signals result in tumour cell growth versus the induction of collective invasion and metastasis remains unclear.

The ARF family of small GTPases (ARF1–6) are implicated in the membrane trafficking and signalling mechanism underpinning single cell invasion. ARF GTPases have fundamental roles in vesicular transport by regulating assembly of coat complexes, lipid-modifying enzymes, and recruiting regulators of other GTPases onto membranes undergoing scission. ARF6 in particular controls internalisation and recycling of RTKs by acting in concert with a number of GTP exchange factors (GEFs) and GTPase-activating proteins (GAPs). Accordingly, small molecule ARF GEF or GAP inhibitors have been used to control invasion in vitro and metastasis in vivo.

Despite the core requirement for ARF GTPases in membrane trafficking steps controlling RTK signalling, it remains unclear how they could promote an invasion response, rather than growth, from an RTK. Are particular ARF-regulated trafficking pathways induced in invasive cells, such as to enhance endocytic recycling and sustained RTK activation, to promote invasion over growth? Here, we describe that specific pro-invasive transcript variants of the ARF GEF IQSEC1 are upregulated in invasive tumours. These alternate variant proteins act as scaffolds to direct phosphoinositide metabolism to induce invasive protrusions. We identify that IQSEC1 can be targeted to inhibit collective invasion in vitro and metastasis in vivo.

Results

Expression of the ARF GEF IQSEC1 is associated with poor clinical outcome. We examined whether ARF GTPase expression was associated with the acquisition of invasive behaviours. We used 3D culture of prostate cancer cell lines to represent the transition from non-tumorigenic to highly metastatic (Fig. 1a). Non-tumorigenic RWPE-1 cells formed acini with a central lumen (Fig. 1a, b). RWPE-2, an oncogenic KRAS-expressing RWPE-1 variant formed lumen-lacking aggregates, some of which developed invasive cell chains (Fig. 1b, white arrowheads). Bone metastasis-derived PC3 cells grew as heterogeneous acini, variably forming round, locally spread, or spindle-shaped invasive cell chains (Fig. 1c). Multiday live imaging revealed that invasive

IQSEC1 is a regulator of collective cell invasion. We examined the contribution of IQSEC1 to cell growth and movement. Publicly available IQSEC1 transcript information revealed multiple variants occurring through combinatorial use of alternate translational initiation sites and alternate splicing (Fig. 2a; Supplementary Table 1). Western blotting suggested simultaneous expression of multiple variants in PC3 cells, with three IQSEC1 bands depleted by IQSEC1-specific shRNAs (Supplementary Fig. 1a). IQSEC1 depletion reduced proliferation proportional to knockdown efficiency (Supplementary Fig. 1a, b). As PC3 cells grow as a mixed morphology 3D culture (Fig. 1c), we developed a machine learning approach to determine whether this heterogeneity also occurred in 2D (Supplementary Fig. 1c). Mirroring 3D collective phenotypes (Fig. 1c), single PC3 cells in 2D culture could be classified into round (54%), spread (21%) and spindle phenotypes (17%) (Supplementary Fig. 1d, e). IQSEC1 depletion selectively abolished spindle characteristics, causing increased spread behaviours (Supplementary Fig. 1f).

We examined whether IQSEC1-dependent spindle shape was required for cell movement. Live imaging of wounded 2D monolayers revealed cells of various shapes rapidly move into the wound (Supplementary Fig. 1g). IQSEC1-depleted cells displayed a modest defect in migration. In contrast, in 3D invasion assays wounded monolayers embedded in ECM (Matrigel) invade by forming spindle-shaped protrusions that develop into multicellular chains (Supplementary Fig. 1h, white arrowheads). In IQSEC1-depleted cells, although some protrusions formed, multicellular chain formation and invasive activity was strongly compromised (Supplementary Fig. 1h, i). Thus, IQSEC1 is required for growth and multicellular invasive chain formation, resulting in defects in morphogenesis where cell elongation is required (Supplementary Fig. 1j).

IQSEC1 is a regulator of collective 3D invasion. We dissected IQSEC1 contribution to growth and/or invasion in 3D PC3 acini, which can form without (round) or with (spindle) invasive characteristics (Fig. 1c). In order to quantify this growth versus invasive behaviour we developed an automated method of quantitation from hourly imaging of hundreds to thousands of 3D acini over multiple days (see the “Methods” section and Supplementary Fig. 2a).

Elevated IQSEC1 expression in PC3 cells, compared to RWPE-1 cells, was maintained upon plating of both cell types into 3D culture (Fig. 2b). Growth of acini from single cells could be measured by increased area over time (Supplementary Fig. 2b, c). The progressive development of protrusive invasion could be determined using a ‘Compactness’ measurement
Similar to 2D, IQSEC1 depletion modestly decreased 3D acinar growth (Supplementary Fig. 2c), proportional to depletion levels (Supplementary Fig. 1a). Mirroring 3D wound invasion defects (Supplementary Fig. 1h), the most prominent effect of IQSEC1 depletion was abolished protrusive invasion (Supplementary Fig. 2b, c).

We examined whether protrusion-forming activity was a common feature of all IQSEC1 isoforms. We focused on the four annotated variants (v1–4; Fig. 2a). IQSEC1 v1–4 possess the IQSEC family-defining features of a calmodulin-binding IQ domain, a catalytic SEC7 ARF GEF domain, and a lipid-binding PH domain. Alternate initiation sites provide v1 or v2 with unique N-termini, followed by a common N-terminal extension.
Three alternate C-termini can occur, with the v4-type tail truncating a potential nuclear localisation sequence. We stably, individually restored each RNAi-resistant GFP-tagged IQSEC1 variant in the background of depletion of all endogenous IQSEC1 transcripts. All IQSEC1 variants could restore growth and/or invasion to levels matching or above control cells (Fig. 2c–f). Variants containing the N-terminal extension (v1, v2) conferred the strongest effect on growth. In contrast, protrusive activity occurred in an isoform-selective manner: only v2 (also known as BRAG2b) restored spindle behaviours in both 2D and 3D to levels surpassing controls (Fig. 2e, f; S2D). To corroborate the live imaging approach we examined fixed acini through 3D confocal imaging. Restoration of v2 to IQSEC1 KD cells increased the total number of nuclei, the level of a proliferation marker (Ki67) and suppressed apoptosis without changing total acinus volume (Supplementary Fig. 2e–h). This represented formation of protrusions and disruption to lumen formation. In contrast, v4 failed to rescue IQSEC1 KD-induced growth defects, but instead increased area by increasing acinus volume without changing cell number, due to the presence of a lumen. Thus, IQSEC1 v2 is a major regulator of 3D growth and invasion. We explored the IQSEC1 v2 properties that promote invasion. IQSEC1 can function in ARF GTPase-dependent endocytosis at the cell cortex, and in the nucleus to control nucleolar architecture. V3 showed predominantly nuclear localisation, while v4 was cortical (Figs. 2g, S2i). V1 showed mixed cortical and cytoplasmic localisation. In contrast, v2 displayed a mix of cytoplasmic, cortical and nuclear labelling (Fig. 2g, green arrowheads, S2i). We reasoned that in overexpressed GFP-tagged variants may mask vesicular pools. Accordingly, anti-IQSEC1 antibodies directed to either all isoforms or to v2-specifically (Fig. 2h) labelled both the cytoplasm and tubulovesicular compartments behind the tips of invasive acinar protrusions in 3D (Fig. 2i–k, arrowheads, S2j, k). Thus, one locale of IQSEC1 v2 function is focally at endosomes in protrusion tips (Fig. 2i–k).

We mapped the domains of v2 responsible for its localisation and potent invasion-inducing activity. We expected that this would be conferred by the unique v2 N-terminus. However, using mutants and chimeras of IQSEC1 isoforms revealed a nuanced and combinatorial effect of alternate N- and C-termini on IQSEC1 localisation and protrusion induction (Supplementary Fig. 3a–g). Surprisingly, the unique v2 N-terminus (2N) was not required for invasion. Any variant containing the v2-tail and any N-terminal extension promoted invasion. Accordingly, replacing the unique v2 N-terminus (2N) with that from v1 (1N) even enhanced invasion over and above that of v2 (Supplementary Fig. 3e–g). This was concomitant with a shift away from nuclear or cortical localisation, towards the cytoplasm (Supplementary Fig. 3c, 1N/2C chimera). This is in contrast to expression of v1 which did not induce invasion (Fig. 2f). This is due to the presence of a v1-type C-terminus (1C) which in all experiments inhibits invasion, concomitant with a more general cortical recruitment. A v2 tail without N-terminal extension (i.e. v3) similarly does not promote invasion. This maps the invasion-inducing activity of IQSEC1 to the N-terminal extension that is common to only v1 and v2. However, the type of C-terminus influences whether this N-terminus induces invasion (Supplementary Fig. 3a–g).Mutual inactivation of GEF activity (IQSEC1 v2GEF inactive) confirmed that IQSEC1 v2-induced spindle shape in 2D (Supplementary Fig. 3b) and protrusive invasion in 3D (Supplementary Fig. 3d–g) are due to its ability to activate ARF GTPases. Thus, the N-terminal common extension in v2 confers enhanced invasive activity in a GEF-dependent manner.

IQSEC1 activates ARF5/6 in distinct locations within protrusions. IQSEC1 functions with both ARF5 and ARF6 to control integrin endocytosis and focal adhesion disassembly. Given IQSEC1 GEF activity-dependency for invasion (Supplementary Fig. 3d–g), we examined whether ARF5 and ARF6 are required for this process. While individual ARF depletion caused some changes that mimicked IQSEC1 loss, only combined ARF5/6 depletion reduced all aspects of 2D spindle shape and 3D growth and invasion (Fig. 3a–c, S4a). We examined whether individual or co-overexpression (OX) of ARF5 or ARF6, wild-type (WT) or fast-cycling mutants (Supplementary Fig. 4b, c) induced invasive behaviours. Only ARF5/6 WT co-overexpression increased 2D spindle shape, 3D growth and 3D invasion (Fig. 3d–f, S4d–g). While fast-cycling mutants induced some 2D spindle behaviours, they failed to induce 3D effects, either alone or in combination, indicating that normal GTPase cycling was required. Crucially, IQSEC1 depletion abolished all effects of ARF5/6 co-overexpression (Fig. 3d–f, 54h). This identifies IQSEC1 as the major GEF-regulating growth and invasion with ARF5/6 as its major targets. Despite identifying IQSEC1 GEF activity as essential for growth and invasion (Supplementary Fig. 3e–g) we did not observe global defects in GTP-loading of ARF5 or ARF6 upon IQSEC1 depletion (Supplementary Fig. 4i). As IQSEC1 v2 localised to a discrete pool at tips of protrusions (Fig. 2j), we reasoned that IQSEC1 may regulate a small but crucial ARF5/6 pool. We developed an imaging-based approach to determine the localisation of the IQSEC1–ARF complex. Endogenous IQSEC1 v2 localised with ARF5-mNeonGreen and ARF6-TagRFP in tubulovesicular structures near the leading edge lamellipodium of spindle 2D cells (Fig. 3g, white arrowheads). In 3D, ARF5 displayed a prominent vesicular localisation in the acinus body, which overlapped with IQSEC1 in some regions, while ARF6 was prominent at cell–cell contacts lacking IQSEC1 (Fig. 3h, yellow arrowheads and white arrows, respectively). Both ARF5 and ARF6 could be found with IQSEC1 v2 in protrusion tips (Fig. 3h, white arrowheads).
To identify the location of active ARF, we expressed a sensor that detects GTP-loaded ARF proteins (GGA1 NGAT domain) (Fig. 3i). GGA1 NGAT-TagRFP-T colocalized with ARF6 most notably in the tips of protrusions (Fig. 3j, green arrowheads), and with ARF5 in puncta in the body of the protrusion (white arrowheads) and the acinus body itself (white arrows).

To confirm that IQSEC1 was controlling this localised GTP loading of ARF5/6 we developed an automated method to analyse the percentage of overlap of the GGA1-NGAT probe with ARF5- or ARF6-positive puncta. We validated the sensitivity of this approach by first examining whether chemical inactivation of ARF GEF or GAP activity could be detected (Fig. 3i).
identified that a reported ARF6 inhibitor (NAV-2729)29, but not the Cytohesin family ARF GEF inhibitor (Secin-H3)37,38, also reduces IQSEC1-catalysed nucleotide exchange on ARF5 (Supplementary Fig. 4j, k). In cells, NAV-2729 significantly decreased GGA1-NATG recruitment to both ARF5 and ARF6-positive puncta (Fig. 3k, l). In contrast, the ARF GAP inhibitor Q511 significantly increased GGA1-NATG recruitment to ARF6, while decreasing recruitment to ARF5. Thus, our approach sensitively detects GTP-loading of specific ARF subpopulations (Fig. 3j–l). Applying this approach in IQSEC1-depleted cells we observed a robust decrease, but not complete loss, of recruitment of GGA1-NATG to both ARF5 and ARF6 (Fig. 3j–l). These data confirmed that IQSEC1 controls GTP loading of a pool of ARF5/6 that is crucial for invasive activity (Fig. 3m).

We determined the morphogenetic effect of modulating ARF activity. Secin-H3 treatment, which minimally acts on IQSEC1-mediated ARF activation (Supplementary Fig. 4k) modestly decreased 3D growth, without affecting invasion (Supplementary Fig. 4l–o). ARFGAP inhibition (Q511)27,38 increased 3D invasion, without affecting growth. However, in line with being a dual IQSEC1-ARF6/5 inhibitor, NAV-2729 treatment abolished 3D growth and invasion. Thus, ARF5 and ARF6 are the major targets of IQSEC1 GEF activity. Chemical or genetic inhibition of IQSEC1-directed ARF activation strongly attenuates 3D growth and invasion.

**IQSEC1 is a scaffold for Met and Akt signalling.** All IQSEC1 variants possess the SEC7-PH domains required to activate ARF GTP loading (Fig. 4a). The enhanced invasion-promoting capabilities of IQSEC1 v2 are conferred by the shared N-terminal extension (encoded by Exon 3; Fig. 4a). We used mass spectrometry–protein–protein interaction analysis of IQSEC1 chimeras to identify domain-specific IQSEC1 binding partners. We classified interactors as unique to v1 or v2 N-termini (1N or 2N), shared between v1 or v2 N-terminal extension (Exon 3), to the core IQSEC7-PH region (Core), or specific to the v2 C-terminus (2C) (Fig. 4a). We prioritised interactors where multiple components of a complex could be identified. We ordered these by mRNA expression change from two pairs of PC3-derived sublines which possess epithelioid (PC3E, Epi) compared to mesenchymal expression change from two pairs of PC3-derived sublines which possess epithelioid (PC3E, Epi) compared to mesenchymal expression change from two pairs of PC3-derived sublines which possess epithelioid (PC3E, Epi) compared to mesenchymal expression change from two pairs of PC3-derived sublines which possess epithelioid (PC3E, Epi) compared to mesenchymal

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**Fig. 2 IQSEC1 isoforms differentially regulate collective invasion. a Schema, domain structure of IQSEC1 variants (v). Common domains, grey. Unique domains, colour: blue, v1; pink, v2 and v3; green, v4. b Western blot in 2D and 3D using anti-IQSEC1 or GAPDH antibodies. Relative expression of all IQSEC1 bands normalised to 2D RWPE1 is shown. Mean ± s.d., n = 6 independent experiments. p-values (Student’s 2-tailed t-test), *p ≤ 0.05; n.s., not significant. c Schema, pipeline for identifying isoform-specific IQSEC1 functions. d Western blot of PC3 cells expressing GFP or GFP-IQSEC1 v1–v4, and either Scr or IQSEC1 KD4 shRNA using anti-IQSEC1, GFP and GAPDH antibodies (all used on same membrane). Different exposures demonstrate expression of all variants. Upper and lower parts of same GFP blot demonstrate expression of GFP-IQSEC1 variants and GFP control. N = 3 independent experiments. e and f Phase images of acini (GFP-positive, yellow) from cells described in (d). Scale bars, 100 μm. Heatmap, area and compactness measurements Z-score-normalised to control values, p-values, one-way ANOVA, greyscale values as indicated. n = 3 independent experiments, 3 replicates/condition, 300–650 acini/condition in total. g PC3 acini described in (d) were stained for F-actin (black) and nuclei (magenta). Localisation of GFP-IQSEC1 can be appreciated from FIRE pseudo-coloured Look Up Table (FIRE LUT) (green arrowheads, cytoplasmic localisation). Magnified images are inset. n = 3 independent experiments. Scale bars, 20 μm. Cartoon, localisation of GFP-IQSEC1 variants in PC3 acini. h Western blot of PC3 cells expressing GFP or GFP-IQSEC1 v1–v4 using anti-IQSEC1 (v2-specific) or GAPDH (loading control) antibodies. Different exposures allow detection of all variants. Upper and lower parts of same GFP blot are shown to demonstrate expression of GFP-IQSEC1 variants and GFP control. I Schema, summary of the effect of IQSEC1 KD and GFP-IQSEC1 v2 expression on growth and proliferative ability of PC3 acini. j Endogenous IQSEC1 v2 (green) co-stained with F-actin (red). Magnified images are shown. Arrowhead indicates localisation at protrusion tip. n = 3 independent experiments. Scale bars, 10 μm. k IQSEC1-positive protrusion tips were quantified (% positive/acinus) using antibodies which detect all variants (49 acini) or are specific for v2 (55 acini). Violin plots show data distribution, n = 2 independent experiments. p-values, Student’s 2-tailed t-test. n.s. not significant.
Notably, while SIN1 depletion decreased spindle behaviours in 2D, it robustly increased invasion in 3D, suggesting 3D-specific differences in this pathway (Fig. 4h, i, S5g, m). Finally, we examined ARFGAP1 as a potential antagonist of IQSEC1-ARF function. Unexpectedly, ARFGAP1 depletion phenocopied IQSEC1 depletion: decreasing pAkt levels, spindle shape in 2D, modestly decreasing 3D growth, and profoundly inhibiting 3D invasion (Supplementary Fig. 5g, n–p). This suggests ARFGAP1 as an effector of ARF5/6 in regulating invasion and signalling to the mTORC2-Akt pathway (Supplementary Fig. 5q), similar to observation of dual effector-terminator functions of other ARFGAPs46–48. Thus, an IQSEC1-ARF5/6-ARFGAP1 complex
may control LRP1-Met signalling to mTORC2 during formation of invasive 3D protrusions.

An IQSEC1–LRP1 complex modestly regulates Met endocytic trafficking. Oncogenic Met signalling requires internalisation9. We investigated whether IQSEC1 controls HGF-Met signalling to Akt by controlling Met endocytic trafficking. HGF stimulation increased 3D growth and robustly induced invasion, which was substantially blunted by IQSEC1 depletion (Supplementary Fig. 6a–d). ARF5/6 co-overexpression resulted in a modest but consistent increase in pAkt levels, which was nonetheless attenuated to levels similar to parental cells by IQSEC1 depletion (Figs. 3d, 5a, b, 6a, b). This suggests that IQSEC1-ARF5/6 control signalling to the Akt pathway.

Paradoxically, IQSEC1-depleted cells displayed decreased total Met levels (Supplementary Fig. 6a, b), but an increased half-life of Met (Supplementary Fig. 6e), suggesting altered trafficking routes. We examined whether this was due to altered endocytosis and/or recycling of Met (Fig. 5c). We developed an image-based analysis of Met trafficking using a fluorescently conjugated anti-Met antibody (Fig. 5c, d). This allowed dual quantitative analysis of internalisation levels and localisation to cellular sub-regions (membrane, cytoplasm, juxtanuclear). We detected some impact of IQSEC1 depletion on Met trafficking, though in all instances the magnitude of effect was modest. In control cells Met was efficiently labelled at the cell surface, appeared in peripheral endosomes after 10 min internalisation, then clustered in the juxtanuclear region by 30 min (Fig. 5d–f). IQSEC1-depleted cells showed a modest but significant delay in the internalisation and transit of Met from the periphery to the juxtanuclear region (Fig. 5d–f), representing a decrease in total Met internalisation levels. We observed no significant difference in recycling of internalised Met (Fig. 5e, 10 min), but defects in internalisation were re-apparent in extended time points of recycling that allowed for re-internalisation (30 min). Thus, IQSEC1 is required for efficient internalisation, but not recycling, of Met (Fig. 5f).

We examined whether the positive (LRP1) and negative (SORL1) interactors of IQSEC1-regulated invasion we identified may also function by controlling activated Met trafficking. LRP1/SORL1 are regulators of endocytic sorting of a number of transmembrane proteins49,50. In control cells, puncta containing activated Met (pY1234/5-Met, pMet) were distributed throughout the cytoplasm. Treatment with HGF induced a reduction in peripheral pMet puncta, concomitant with puncta clustering in the juxtanuclear region (Fig. 5g, h), which was abolished by

IQSEC1 depletion (Fig. 5h–j). Consistent with a positive role for LRP1 in invasion LRP1, but not SORL1, depletion also blocked redistribution of pMet away from the cell periphery in response to HGF (Fig. 5h–j). These data reveal that IQSEC1 and LRP1 control Met-induced invasion by regulating Met transport to the juxtanuclear region (Fig. 5k), an essential function for signalling from Met9. The magnitude of effects of IQSEC1 depletion on trafficking, however, were modest. We therefore turned to potential effects on IQSEC1 in regulating signalling from Met.
combination of level and asymmetry of PIP₃ promotes invasive function (Supplementary Fig. 7d).

To align total levels of PIPs with analysis of bulk peripheral distribution we validated that anti-PIP antibodies detected total cellular PIP levels, observing they are responsive to PI3K inhibition (LY294002) or activation (using myristoylated PI3K) (Supplementary Fig. 7g–i). In contrast to PI3K inhibition, which decreases total and bulk peripheral levels of PIPs (Supplementary Fig. 7h, j), IQSEC1-depleted cells displayed an overall increase in total PI(4,5)P₂ and PIP₃ levels (Supplementary Fig. 7k), concomitant with a decrease in bulk peripheral PIPs (Figs. 6a, S7c) and the appearance of PIPs in intracellular compartments.
(Supplementary Fig. 7a). This reduction of bulk peripheral PIP3, in the absence of IQSEC1 was observed equivalently whether the cell cortex was defined using either F-actin or a cytoplasmic stain (CellMask) (Supplementary Fig. 7l), and irrespective of cell shape (Supplementary Fig. 7l), representing that bulk differences in PIP3 levels were not simply a consequence of altered shape. Reduced bulk peripheral PIP3 levels in IQSEC1-depleted was comparable to the magnitude of P13K-inhibited cells (Supplementary Fig. 7j). These data suggest that IQSEC1-ARF-PIP5K is not essential for general PI(4,5)P2-PIP3 generation, but rather that a LRPI–Met–IQSEC1 v2 complex promotes bulk peripheral PIP3 production that leads to invasive protrusion formation (S7d, g).

IQSEC1 v2 promotes single cell spindle shape (Supplementary Fig. 2d) and 3D invasion (Fig. 2e, f), while IQSEC1 v4 promotes round cell shape. No difference in bulk peripheral PI(4,5)P2 levels between cell shape classes (spindle, spread, round) was observed (Supplementary Fig. 7a, b). Accordingly, rescue of IQSEC1 depletion with either IQSEC1 v2 or v4 partially restored total PI(4,5)P2 to levels to those observed in control cells (Supplementary Fig. 7m). In contrast, the invasion-inducing IQSEC1 v2 supported more robust activation of PIP3 generation than could IQSEC1 v4 (Supplementary Fig. 7m). Given that IQSEC1 v2 induces a switch to spindle shape, this suggests that IQSEC1 v2-directed bulk peripheral production of PIP3 drives invasion.

We next examined the components of PIP3 generation that drive invasion. Iso-type-selective Class I PI3K inhibitors revealed that either P13Kβ or P13Kδ inhibition decreased pAkt levels, while P13Kα or P13Kγ inhibition paradoxically increased pAkt levels (Supplementary Fig. 8a). However, only P13Kβ or Akt inhibition significantly attenuated 3D growth and invasion (Supplementary Fig. 8b–d). Depletion of PIP5K1A-C revealed that PIP5K1β depletion showed the most robust decrease in growth, invasion, and pAkt levels (Supplementary Fig. 8e–g). These data suggest a PIP5K1β → P13Kβ → Akt pathway for 3D growth and invasion.

Our data suggest that IQSEC1-ARF5/6 controls the PIP production required for PIP5K1β → P13Kβ → Akt signalling during invasion (Supplementary Figure 7e). We tested whether generalised cortical targeting of this PIP-Akt pathway could overcome the need for IQSEC1 (Fig. 6b). We targeted each of PIP5K1β, P13Kβ, Akt1 to the cortex through addition of a myristoylation sequence, and inhibited IQSEC1 by shRNA or chemical means (NAV-2729) (Fig. 6b, c).

Myristoylation of P13Kβ (P13Kβm) or PIP5K1β (PIP5K1βm), but not PIP5K1α (PIP5K1αm), increased Akt phosphorylation, 3D growth and invasion, with the most robust increase occurring upon P13Kβm expression (Fig. 6c–f). As expected in control cells, IQSEC1 depletion reduced pAkt levels and attenuated 3D growth and invasion (Fig. 6c–f). Myristoylation of P13Kβ (P13Kβm) or PIP5K1β (PIP5K1βm), but not PIP5K1α (PIP5K1αm), increased 3D growth and invasion with the most robust increase occurring upon P13Kβm expression (Fig. 6c, e, f). Strikingly, IQSEC1 perturbation (shRNA, NAV-2729) had a disproportionate effect on invasion versus growth. While growth was blunted, in IQSEC1-perturbed conditions invasion was abolished (Fig. 6c, e, f). NAV-2729 treatment blocked the ability of all cells in 3D to become acini. Single cells initially increased slightly in area and adopted irregular shapes, manifested in increased compactness, but not in invasion. Thus, generalised cortical recruitment of PKP signalling is sufficient to drive growth, but not invasion. Invasion requires IQSEC1-dependent ARF activity at invasive protrusions (Fig. 6i).

IQSEC1-ARF regulates localisation and activation of Akt. Akt signalling showed a different influence on 3D behaviours than that observed for P13Kβ or PIP5K1β. Our data suggest that IQSEC1 may contribute to Akt S473 levels by at least two mechanisms: influencing PIP5K1β-directed PI(4,5)P2 production, and interaction with RICTOR-SIN1 (Supplementary Fig. 7e). While IQSEC1 depletion lowered endogenous pAkt levels, expression of myristoylated Akt1 (Akt1m) overcame the requirement of IQSEC1 for pS473 phosphorylation (Fig. 6d, g, h). As predicted, general cortical targeting of Akt1m robustly increased 3D area compared to control cells, but not invasion (Fig. 6g, h). Strikingly, although Akt1m no longer required IQSEC1 for phosphorylation events normally indicating ‘active-ness’ (i.e. pS473-Akt, Fig. 6d), IQSEC1 depletion still reversed the Akt1m -induced 3D growth increase to levels below control cells, and abolished invasion; NAV-2729 was similarly an inhibitor of these processes (Fig. 6g, h). Thus an effector of PIP3 signalling, Akt, requires IQSEC1 for full signalling output.

As experimentally ‘active’ Akt (asymmetrically cortically targeted Akt1m, as defined by pS473 levels) was unable to induce oncogenic signalling in IQSEC1-depleted cells we examined whether this was due to altered Akt localisation. In 2D, pAkt localised to cortical regions and to puncta distributed through the cytoplasm (Supplementary Fig. 8h). In 3D, a pool of pAkt was enriched in protrusion tips (Supplementary Fig. 7f, white arrowheads), as well as to puncta throughout the protrusion and acinus body. In contrast, IQSEC1-depleted cells displayed an increased cortical pAkt aggregate size, but with strongly reduced intensity (Supplementary Fig. 8h–k). These data
reveal that IQSEC1 is required for the signalling from, and asymmetry of, the PIP3 effector Akt.

**IQSEC1 regulates growth and invasion in vitro and in vivo.**

Our data indicate that LRP1-Met-IQSEC1-promoted enrichment of ARF5/6-dependent PIP3 signalling to induce invasion rather than growth. We tested the generalisability of IQSEC1 inhibition to inhibit growth and invasion mechanisms in commonly used prostate cancer models. mRNA expression and western blotting indicated that, with the exception of LRP1 in 22Rv1, all components of the LRP1-Met-IQSEC1-ARF5/6 pathway are expressed in examined prostate cancer cell lines (Supplementary Fig. 9a, b).
Chemical inhibition (NAV-2729, Supplementary Fig. 9c–h) or genetic depletion (Supplementary Fig. 10a–f) of IQSEC1 attenuated growth and/or invasion in a range of 3D cancer cell models. This included upon ectopic OX of the LRPI–IQSEC complex in cells with low endogenous levels (LRP1<sup>TM</sup>-GFP in 22Rv1, GFP-IQSEC1 v2 in DU145), HGF treatment of the mixed morphology DU145 cultures to resemble the spindle-type invasion of PC3 (Supplementary Fig. 9, S10), highly invasive human breast cancer cells (MDA-MB-231), murine pancreatic ductal adenocarcinoma cells (PDAC, KC-PTEN, K-rasG12D/PTEN-null<sup>90</sup>) and patient-derived PDAC cells (TKCC-07) (Supplementary Fig. 10a, c–f). Thus, IQSEC1 is required for growth and invasion across a number of 3D cell models from different cancer types.

We examined the in vivo role of IQSEC1 by intraprostatic xenograft of IQSEC1-depleted PC3 cells (Fig. 7a). While IQSEC1 depletion did not significantly attenuate tumour incidence, tumour area and volume were significantly reduced (Fig. 7b–e). As predicted from our 3D in vitro studies, metastatic activity was strongly decreased in IQSEC1-depleted cells. Both the incidence and number of macrometastases were significantly decreased in IQSEC1-depleted cells (Fig. 7f, g). Wide-spread dissemination of macrometastases was observed in controls. In the few mice presenting macrometastases in IQSEC1-depleted cells, these were limited to prostatic proximal lymph nodes (with the exception of a singular diaphragm-located tumour) (Fig. 7h), and showed no difference in proliferation or apoptosis to controls, confirming a bona fide effect on movement (Fig. 7i, j). These data indicate an essential requirement for IQSEC1 in metastasis in vivo.

Elevation in IQSEC1, ARF5 and ARF6 levels was associated with clinical outcome in prostate cancer across 12 studies, representing 2910 patients (Fig. 8a). Increased Copy Number (CN) of IQSEC1 occurred most frequently in advanced prostate cancers (Fig. 8b, CR, castrate-resistant; NE, neuroendocrine), associated with a gain in 3p Status (Fig. 8c; IQSEC1, 3p25.2–25.1). Primary prostate tumours with elevated IQSEC1 were of significantly higher grade (Fig. 8d), were associated with tumour-bearing lymph node positivity (Fig. 8e) and metastases (Fig. 8h). Mirroring the ablation of wide-spread metastasis of IQSEC1-depleted PC3 xenografts (Fig. 7b), IQSEC1 increase occurred most frequently in samples from diverse metastatic sites, but particularly bone, liver and lymph node (Fig. 8g). IQSEC1 increase occurred exclusively with androgen deprivation therapy, retained presence of tumour after therapy, and increased levels of the androgen receptor V7 variant, a major mechanism for escape from androgen deprivation (Fig. 8h–j). A clinical indicator of disease, serum PSA levels, was elevated in patients displaying combined IQSEC1 and ARF5/6 elevation (Fig. 8k). This suggests a clinical association of IQSEC1–ARF5/6 gain with therapy resistance and metastasis.

We examined the association of IQSEC1, ARF5, and ARF6 elevation with frequent genomic alterations in prostate cancer. IQSEC1 increase was associated with amplifications particularly in AR and MYC and to a lesser extent with loss of PTEN, and prominently with TP53 mutation, but not common gene fusion events (Fig. 8l–n, S11a). ARF6 increase followed a similar profile, while ARF5 increase was associated with a broader range of mutational events. IQSEC1 allowed robust stratification of prostate cancer patients, stratifying a 24-month decrease in median overall survival in IQSEC1-elevated patients (Fig. 8o, 665 patients). Inclusion of ARF5/6 increase did not change the median overall survival of this group, but rather extended the median survival of the control arm, resulting in a 35-month survival difference compared to the IQSEC1-ARF5-ARF6 elevated group. IQSEC1 increase was similarly associated with TP53 mutation and MYC amplification across the pan-cancer Cancer Genome Atlas (TCGA) dataset, representing 10,449 patients (Supplementary Fig. 11a–d). Strikingly, IQSEC1, ARF5 and ARF6 increase similarly stratified patient survival when all tumour types were considered together, providing an exceptional >9-year (111 months) median survival increase for non-IQSEC1-ARF5/6-amplified patients (across 9307 patients) (Fig. 8p).

Comparison of matched normal–tumour tissue from the TCGA prostate cohort (n = 52) showed a significant increase in overall IQSEC1 mRNA in tumours (Fig. 8q). This was accompanied by a switch from the non-invasive IQSEC1 v1 isoform, to the pro-invasive IQSEC1 v2 in tumours (Fig. 8r). Analysis of the pan-cancer TCGA dataset for tumour types with profiled normal tissue revealed that four tumour types (kidney, KICH; liver, LIHC; prostate; sarcoma, SARC) displayed IQSEC1 mRNA higher than normal tissue (Fig. 8s). However, seven tumour types showed that rather than an increase in overall IQSEC1, instead tumours underwent an isoform switch from v1-to-v2 (Fig. 8s). This demonstrates that isoform switching to IQSEC1 v2 is a major event associated with tumourigenesis in patients.

Finally, we examined the association of IQSEC1 mRNA levels across the pan-cancer TCGA dataset with common tumour-associated signalling pathways from reverse phase protein array
(RPPA) data. Within each cancer type IQSEC1 expression was divided into high and low using a median split of mRNA levels, and protein differences between each group that consistently and significantly trended in the same direction across a quarter of all cancer types was calculated (Supplementary Fig. 11e). Mirroring IQSEC1 regulation of phosphoinositide signalling in PC3 cells, a clear PI3K-AKT signature was associated with high IQSEC1 levels across ten tumour types (Fig. 8t). Together, these data indicate a role for tumour-associated isoform switching to IQSEC1 v2 to promote PI3K-AKT signalling and metastasis across cancer types, resulting in treatment resistance and a robust decrease in patient survival.
Fig. 6 IQSEC1-ARF signalling controls phosphoinositide generation during invasion. a Quantitation of PI(4,5)P$_2$ or PIP$_3$ in the presence or absence of IQSEC1 is shown in box-and-whiskers plot; 10–90 percentile; + mean; dots outliers; midline median; boundaries quartiles. Values, peripheral/total intensity/cell, n = 3 independent experiments, 4 replicates/condition, 1674/1959 (Scr and IQSEC1 in upper panel) and 2684/2083 (Scr and IQSEC1 in lower panel) cells quantified; p-values; one-way ANOVA. ****p ≤ 0.0001. b Cartoon, PI3K targeting in presence or absence of IQSEC1. c Western blot of PC3 cells expressing Myr-FLAG-Cre (Control), Myr-FLAG-PIP5K$_{α}$, Myr-FLAG-PIP5K$_{β}$ or Myr-FLAG-P3K and either Scr or IQSEC1 shRNA (KD4). Anti-IQSEC1, phospho-S473 Akt, total Akt and GAPDH (loading control for IQSEC1) antibodies were used. n = 2 independent experiments. d Western blot of PC3 cells expressing Myr-FLAG-Cre (Control) or Myr-Akt1 and either Scr or IQSEC1 KD1 shRNA using anti-IQSEC1, phospho-S473 Akt, total Akt and GAPDH (loading control for IQSEC1) antibodies. n = 2 independent experiments. e and f Phase images of PC3 acini described in (c) are shown. Scr acini were also treated with NAV-2729 (IQSEC1 inhibitor). Scale bars, 100 μm. Heatmap, area and compactness measurements Z-score-normalised to control. p-values; one-way ANOVA, greyscale values as indicated. n = 3 independent experiments, 4 replicates/condition, 1693–2435 acini/condition in total. Cartoon, acini phenotype representative of each condition. g–h Phase images of PC3 acini described in (d) are shown at 96 h. Scr acini were treated with IQSEC1-inhibiting compound NAV-2729. Scale bars, 100 μm. Heatmap, area and compactness measurements Z-score-normalised to control. p-values; one-way ANOVA, greyscale values as indicated. n = 2 independent experiments, 4 replicates/condition, 1287–2363 acini/condition in total. Cartoon, acini phenotype representative of each condition. i Schema, summarises the relationship between location and level of peripheral PIP$_3$ and 2D and 3D PC3 phenotype.

Discussion

Our results clarify a long-held conundrum of how the same signalling pathways lead to distinct biological outputs: alternate isoforms of the same RTK effector, IQSEC1, differentially localise to control ARF GTase-dependent phosphoinositide metabolism at these distinct locales.

We describe that IQSEC1 is a key regulator of bulk peripheral PIP$_3$ signalling to promote invasion. Our data support a model whereby pro-invasive IQSEC1 isoforms form a complex with the HGF receptor Met and the endocytic receptor LRPI at membranes that will develop into invasive protrusions (Fig. 7k). IQSEC1 activates GTP loading on ARF6 to (1) stimulate PI5K-mediated PI(4,5)P$_2$ production, which is (2) a precursor to PI3K-mediated PIP$_3$ production to (3) promote invasive protrusion formation. Elevation of bulk PIP$_3$ levels also triggers cell growth, comcomitant with internalisation of Met and (4) activation of pAkt downstream of the mTORC2 complex. Met and pAkt are retrograde transported in the body of protrusions on ARF5 endosomes to a junctional signalling compartment, essential for growth and invasion. Inhibiting this IQSEC1-mediated ARF5/6 activation abolishes the collective growth and invasion/metastasis in vitro and in vivo.

Multiple isoforms of IQSEC1 exist through combination of alternate start sites and alternate splicing, though the regulators of these events are unknown. Such N-terminal and C-terminal extensions operate in a hierarchy; N-terminal extensions provide association with endocytic co-receptors LRP1 and SORL1, while alternate C-termini can either positively or negatively enhance invasive activity. SORL1 and LRPI are members of the LDLR family, co-receptors involved in endocytic control of a milieu of processes including ARFGEF1, which in our hands functioned as an effector rather than an antagonist of ARF signalling, similar to the dual effector terminator function described for other ARFGEF,46–48. That this GAP associates with the GEF supports the notion of tight control over GTP cycling. Whether ARF5/6 both, or separately, interact with the two identified effectors (ARFGAP1, PIP5K1β), is unclear.

The ARF GTase-dependent recycling of Met promotes sustained ERK signalling required for migration in other cells.57,70 Internalisation of Met is key to full oncogenic output.8,9. IQSEC1 has modest effects on internalisation and junctional trafficking of Met, suggesting the major role of IQSEC1 is to couple Met to its downstream component Akt. Met recycling was not perturbed in IQSEC1-depleted cells, suggesting another ARFGEF may promote this function. The PI(4,5)P$_2$/PI$_3$-dependent Cytohesin-1 ARFGEF is one candidate for this, as we note upregulation in PC3, and it is required downstream of Met for invasive activity in other cells.71 Cytohesin-1 alternate splicing controls its recruitment to membranes by controlling differential affinity for
Such a GEF would likely act subsequently to IQSEC1-directed control of peripheral PI(4,5)P₂ levels. Thus, IQSEC1 may sit at the top of a cascade of ARF GEFs controlling Met endocytosis, recycling, and signal output. This may explain why IQSEC1 inhibition alone can reverse ARF5/6 OX, despite co-expression of numerous ARFGEFs in PC3 cells.

A striking result is that although IQSEC1-ARF5/6 would classically be considered ‘upstream’ of PI(4,5)P₂, PIP₃, and Akt, inhibition of IQSEC1 nonetheless counteracts the experimental activation of this pathway at multiple levels. Our data suggests that this is due to alternate isoforms of IQSEC1 possessing different locations, thereby likely influencing where downstream
**Methods**

**Cell culture.** PC3 (ATCC), PC3 E-Cad+, TEM4–18, TEM2–5, GS689.Li, GS694. Ld, GS883.LALN, JD1203.Lu, GS672.Ug (M. Henry, University of Iowa), PC3-Epi

**Macrometastasis incidence was counted and is presented as (f) count per mouse, values, mean ± s.e.m. 12 and 10 mice were transplanted with PC3 cells expressing Scr or IQSEC1 KD4 shRNA, respectively. p-values (Chi-squared and Mann–Whitney test (2-tailed)) respectively:** p ≤ 0.05.

**Heatmap shows the frequency at which metastasis occurred at locations with different proximity to the prostate. Cartoon, summarises the locations metastasis was observed. i and j The (f) tumour and (j) lymph node (LN) sections were stained with anti Ki67 and Cleaved Caspase 3 (CC3) antibodies and the percentage of positive cells quantified. Each mouse is represented by a differently coloured point on the bar graphs.**

**Live 3D culture and analysis.** Culture of cell lines as 3D acini was adapted from previous protocols. Briefly, single cell suspensions were made (1.5 × 104 cells per ml) in the appropriate medium supplemented with 2% growth factor reduced matrigel (GFRM; BD Biosciences), 150 μl of this mix was plated per well in a well-96 ImageLock plate (Essen Biosciences) pre-coated with 10 μg/ml blasticidin (InvivoGen) or 200 μg/ml hygromycin (Merck). Stable knockdown of proteins was achieved using plKO-1-purozymic, plKO-1-hygrozymic or plKO1-membrane tagged Venus (substituted for pcDNA) lentiviral shRNA vectors. ShRNA sequences are listed in Supplementary Table 3. GFP-IQSEC1 v2 and GST-GGA3-GAT were kindly gifted from J. Casanova (University of Virginia). All RNAi-resistant variants and chimeras were made by mutagenesis or sub-cloning using fragment synthesis (GeneArt). GFP/FluoroDPIs were kindly gift from P. Melancon (University of Alberta) and alternate fluorescent tags and mutations generated by sub-cloning. EGF-PH-GRP1 and EGF-PH-PLC6 were described previously and sub-cloned into mNeonGreen. Myr-FLAG-Cre, Myr-FLAG-PIP-SKs, Myr-FLAG-PIKs, Myr-Akt1 and RFP-GGA1-NGAT were purchased from Addgene. LRP1-EGF was a kind gift from S. Kins (Technical University Kaiserslautern).

**IQSEC1 variant information.** The nomenclature for IQSEC1 variants has been complicated by alternate names for IQSEC1 in literature and changing isoform designations at NCBI. Unification of nomenclature is presented in Supplementary Table 4.

**2D and 3D acini immunofluorescence and imaging.** 3D acini were set up as described above in either eight-well Costar slides (Nunc, LabTek-II) or in 96-well plates (Greiner) pre-coated with 60 or 10 μg/ml GFRM for 15 min at 37 °C. Plates were incubated at 37 °C for 4 h, then imaged using an Incucyte® ZOOM (Essen Bioscience) with Incucyte ZOOM Live Cell Analysis System Software 2018A. Images were taken every hour for 4 days at 2 positions per well using a x10 objective lens. Sample size (n) and replicate number are stated for each experiment in figure legends. Outlines of phase and GFP-positive (where appropriate) acini were generated using a custom pipeline in CellProfiler (Version 3.1.8). A custom macro in Fiji software (2.0.0) was then used to colour code images from each time point, progressively coloured along a blue-red rainbow time-scale, and concatenate them into one image per 12-h block to reduce data dimensionality of multiday imaging. Using CellProfiler, measurements such as area and compactness, which could reliably measure size and protrusiveness of 3D PC3 objects, respectively, were generated for each 12-h block. Custom pipelines designed in KNIME Data Analytics Platform (Version 3.3.1) were then used to collate data from multiple experiments, normalise to controls, calculate Z-score and perform statistical analysis using one-way ANOVA. Normalised data and p-values are presented as heatmaps generated in PRISM 7 (GraphPad). The number of independent experiments (n), technical replicates, and the number of acini quantified per condition are stated in each figure legend.
microscope were processed using the Zeiss proprietary ZEN 3.2 software, exported as TIFF files and processed in Fiji. Cells or acini on 96-well plates were imaged using an Opera Phenix high content analysis system and where appropriate Harmony High-Content Imaging and Analysis Software (PerkinElmer, Version 4.6) was used to perform machine learning.

Antibodies used: Alexa Fluor 488 (A12379), 568 (A12380) or 647 (A22287) phallodin (Thermo Fisher Scientific), anti-IQSEC1 (Sigma, G4798), anti-IQSEC1 (Calbio-Carex, PS0-8009), anti-LRP1 (Sigma, L2295), anti-Met (CST, 3127), anti-Met phospho 1234/1235 (CST, 3077), anti-Akt phospho S473 (CST, 3787), DYKDDDDK Tag (CST, 14793, 1800), anti-Ki67 (Thermo Fisher Scientific, 18-}

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Fig. 8 IQSEC1 is associated with metastasis, treatment resistance, and poor clinical outcome. a and b CN increase in IQSEC1, ARFS, ARFs across indicated cohorts. AD, prostate adenocarcinoma; NE neuroendocrine; CR Castrate-resistant. c-j Clinical metrics (% samples) between IQSEC1 CN-amplified (±) or non-amplified group for c 3p Status (−, n = 420; +, n = 32), d Gleason Score (−, n = 779; +, n = 53), e Tumour Lymph Node Status. N0, no positivity. NI, positivity. (−, n = 381; +, n = 36), f Sample Type (−, n = 1,797; +, n = 272), g Tumour Anatomic Site (−, n = 525; +, n = 78), h androgen deprivation therapy. Luteinizing hormone-releasing hormone, LHRH (−, n = 484; +, n = 30), i person neoplasms status (−, n = 299; +, n = 77), and j androgen receptor (AR) isoform v7 mRNA levels (Log2) (−, n = 227; +, n = 69). p values, c-i Chi-squared Test or J Kruskal–Wallis Test; Q values, Benjamini–Hochberg adjustment. Box-and-whiskers plots: 10–90 percentile; +, mean; dots, outliers; midline, median; boundaries, quartiles. k Prostate-specific antigen (PSA) serum levels (Log2) in IQSEC1, ARFS, ARFs CN increase (+, n = 2,308) patients. Q value, Kruskal–Wallis Test. Box-and-whiskers plots: 10–90 percentile; +, mean; dots, outliers; midline, median; boundaries, quartiles. l-m Copy Number Alteration (l, m) or (n) mutation frequencies between IQSEC1 CN-amplified (n = 849) or unaltered (n = 2,308) patients. Group association: Red, IQSEC1 CN increase; blue, unaltered. Q-values, one-sided Fisher exact test with Benjamini–Hochberg adjustment. a and p Overall survival (% patients, months), unaltered compared to (i) IQSEC1 CN increase or (ii) combined IQSEC1, ARFS, ARFs CN increase for a cohorts in A (n = 665) or p pan-cancer TCGA cohorts (n = 9,307). p-values, Log-rank t-test. q and r Matched Normal (blue) and prostate tumour (Tumour, red) Log2-normalised IQSEC1 mRNA for q total isoforms or v2-v1 ratio. values. Line: unbroken, tumour-increased; dotted, tumour-decreased. n = 52. p-values, Independent Groups t-test. s IQSEC1 mRNA log2-normalised normal–tumour ratio across pan-cancer TCGA cohorts for total (green-magenta), individual blue, red, or green-violet ratio (grey-orange). Circle size, p-value, Independent Groups t-test. Normal tissue (n = 643), tumour tissue (n = 6,716). Supplementary Table 2 contains tumour-type data. t PK3-AKT pathway (RPPA of pan-cancer TCGA cohorts) from median-split of Log2-transformed IQSEC1 mRNA. Red, high IQSEC1 co-occuring; blue, low IQSEC1 co-occuring. Only cancer types presenting increased AKT_p5473 included. Row sorting from dendrogram. Circle size, p-value, Independent Groups t-test.

Technical replicates, number of cells/ACI imaged per experiment, statistical test performed and significance is stated in the appropriate figure legend.

Harmony High-Content Imaging and Analysis Software (PerkinElmer, Version 4.6) was also used to quantify the colocalisation between mNeonGreen-tagged ARF5 or ARF6 and RFP-GGA1-NGAT. PC3 cells stably expressing ARF/GGA1 were FACS sorted and a population that was positive for both mNeonGreen and RFP selected. These cells were plated for 24 h then treated with either NAV-2729 or QS11 overnight. Cells were then fixed, stained with CellMask (1:500) and Hoechst (1:1000) and imaged using an Opera Phenix. A custom pipeline was used to identify red (GGA1+) and green (ARF5+) cells sub-cellular regions i.e. nuclear, cytoskeletal and cytoplasmic reagents described above and used to collect and combine these populations for each condition. Each cell was imaged in three consecutive planes (2 μm step size) and analysis was processed on each individual plane. Cells were detected based on nucleus localisation and defined by either F-actin or HCS CellMask staining and machine learning then used to classify cells as either spindle, spread or round phenotypes. Each cell was imaged in three consecutive planes (2 μm step size) and analysis was processed on each individual plane. Cells were detected based on nucleus localisation and defined by either F-actin or HCS CellMask staining. Cells in contact to the image border or without green channel positivity were discarded. The morphological properties of each object were then calculated to classify them into three different categories (‘round’, ‘spindle’ and ‘spread’) using machine learning following manual training. Different cell regions were defined as a ‘peripheral’, using Method reside region [μm/xμm, region type ‘membrane region’, Outer border: – 2 μ (1.194 μm), Inner border: 5 μ (2.95 μm), (b) ‘nuclear’ using method ‘greymask’, (c) ‘cytoplasm’, using method ‘restrict by mask’; population ‘green cells’, mask regions ‘Peripheral’ and ‘Nucleus’. Finally, intensity properties of PIP probe channel (Alexa 488) were calculated by standard method in the different cell regions within each object. As an alternate approach, images were also stack processed using maximum projection all slices, followed by a similar pipeline as described above. No significant difference was obtained between methods. A custom pipeline was generated using KNIME Data Analytics Platform (Version 3.3.1) to collate data, calculate the log2 fold change of each phenotype over control and to calculate statistical significance using one way ANOVA. Data obtained from three different replicate wells was collected and combined with experiment keys providing information about replicates and samples. For image analysis using single plane stack processing, objects that were in more than one plane were detected as a similar object using a similarity search of objects in subsequent planes based on euclidean distance search of nearest neighbour and distance from the imaging object X and Y positions. Upper bound (use range checking) used was 5.0. Automated selection of one single plane per object based on area (or alternatively, average of the three planes of the same object) was used for subsequent analysis. Selection of samples to be analysed, followed by selection of control sample, and log2 transformation of intensity data and normalisation to control sample is used. In some cases this analysis was carried out in sub-populations of cells that either expressed a fluorescently tagged plasmid or were stained with a specific antibody.

The use of the nuclear, cytoskeletal and cytoplasmic reagents described above also allowed each cell to be segmented into specific sub-cellular regions i.e. nuclear, cytoplasmic and peripheral (defined as 5 pixels from outer edge of cell). When used in combination with fluorescently tagged proteins (such as reporters for phosphoepitopes) or antibodies the mean intensity of a specific protein per cell (intensity/cell) or per sub-cellular region per cell (i.e. mean peripheral intensity/mean total intensity/cell) could be measured. Where appropriate ‘spots’ of positive staining were detected, using method ‘Standardisations’ to more accurately calculate the mean intensity/cell or mean spot area/cell.

Custom KNIME Data Analytics Platform (Version 3.3.1) pipelines described previously were then used to collate data, to calculate the ratio over control and to determine statistical significance. In addition, these pipelines were adapted to measure the total intensity of specific antibodies in 3D acini. Data are presented as box and whiskers plots as z-score normalised or as log2 fold change over control where each point represent one cell. The number of independent experiments (n),
Metuptake assay. Metuptake assays were performed using a fluorescently labelled Met-647 antibody (BD Biosciences) to assess the cells’ ability to internalise and trap 5 × 10^5–6.7 × 10^5 6-Carboxyfluorescein diacetate succinimidyl ester (CFSE) in antibody binding medium (RPMI + 0.5% BSA + 1 M CaCl₂, pH 7.4). Cells were washed twice with ice-cold PBS, incubated with Met-647-conjugated antibody for 1 h at 4 °C (1:100) then washed again with PBS. One plate was fixed as described above. Two plates were treated with HGF (50 ng/μl) and cholera toxin (100 μM) diluted in pre-warmed medium for 10 or 30 min at 37 °C. They were then acid stripped (washed three times at 3 °C with 0.5 M acetic acid, 0.5 M NaCl in PBS) and fixed as previously described. The remaining two plates were incubated at 17 °C with pre-warmed medium supplemented with HGF and cholera toxin for 1 h. Acid stripping was performed and plates maintained at 37 °C without HGF or cholera toxin for 10 min or 30 min prior to fixation. Cells were stained with Hoechst and phalloidin and imaged using the Opera Phenix® High Content analysis system.

Data are presented as line graphs that show normalised (Z-score) relative region intensities compared to the intensity of the whole cell. Box and whiskers plots show total normalised (Z-score) intensity throughout the cells. N = 2 independent experiments, 4 replicates/condition with the following total number of cells examined per experiment: For internalisation at 0 min (Scramble 421, shIQSEC1 546), 10 min (Scramble 122, shIQSEC1 126), or 30 min (Scramble 93, shIQSEC1 120), and for recycling at 10 min (Scramble 150, shIQSEC1 136) or 30 min (Scramble 219, shIQSEC1 282). Cells examined per experiment, p-values were calculated using Welch’s 2-tailed t-test and are shown on each graph as follows: n.s. = not significant, *p < 0.01, **p < 0.001 and ***p < 0.0001.

Invasion and migration assays. ImageLock plates were coated with 10% GFRM diluted in medium overnight at 37 °C. Cells were re-suspended in 100 μl medium and plated for 4 h at 37 °C. The plates were then separated by SDS-PAGE, transferred to a PVDF membrane and immunoblotted. Lysates from cells expressing GFRM-tagged proteins were immunoprecipitated using a GFRM-Trap Kit (Chromotek) as per manufacturer’s instructions and immunoblotting performed as above. n = 3 and quantitation is shown as mean ± s.d.

Fluorescent polarization assay. Nucleotide exchange of ARF5 protein was examined by observing changes in fluorescent polarization. 100 μM recombinant ARF protein was incubated with 200 μM mGDP and 30 μM EDTA in gel filtration buffer (GF) (20 mM Tris, 150 mM NaCl, 1 mM EDTA) overnight at 18 °C. 100 μM MgCl₂ was added to stop the reaction. A PD10 (GE Healthcare) desalting column was then washed with GF buffer containing 5 mM MgCl₂, 500 μl of sample and then eluted with GF buffer. Protein concentration was determined using a Bradford assay as per manufacturer’s instructions. 20 μM of GDP nucleotide and 2 μM of GEP was sequentially added to 1 μM ARF and polarization changes were monitored with a Photon Multiplier Detection System (Photon Technology International). Excitation was set to 366 nm and emission to 450 nm. Inhibitors were added to the reaction prior to addition of GEF protein.

Proliferation assays. PC3 cells were plated in a 96-well ImageLock plate, in triplicate for 24 h. Imaging was carried out on IncuCyte® ZOOM every 4 h for 48 h. Cells were then harvested and measured using the XCelligence software. n = 3 independent experiments with three replicates per condition. p-values (Student’s t-test): **p < 0.01 and ***p < 0.0001.

Immunoblotting. Cells were plated for 24 h prior to treatment with growth factors or inhibitors for a further 24 h. Plates were washed twice with ice cold PBS then lysis buffer added for 15 min (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.5 mM MgCl₂, 0.2 mM EGTA, and 1% Triton X-100 with complete protease inhibitor cocktail and PhosSTOP tablets (Roche)). Cells were scraped and lysates clarified by centrifugation at 2160×g at 4 °C for 15 min. BCA Protein Assay kit (Pierce) was used to determine protein concentration. SDS-PAGE was then performed and proteins transferred to PVDF membranes using the iBlot 2 transfer system (Thermo Fisher Scientific). Membranes were incubated for an hour in Rockland blocking buffer (Rockland) and primary antibodies added overnight at 4 °C (1:1000 unless stated otherwise). Secondary antibodies used were: anti-GAPDH (Novus Biologicals NB-110-85530), anti-ARF1 (Novus Biologicals N0000038-1M01), anti-ARF5 (Sigma A5230), ARF5 (Novus Biologicals H00000381-M01), anti-ARF-GAPSP (Sigma HPA051019), anti-SORL1 (BD 611860), anti-S1 (CST 1286), anti-AKT phosphorylated (CST 3787), anti-AKT (CST 2920), anti-AKT phospho S473 (CST 3787), anti-ARF (Novus Biologicals NB-110-85530), anti-ARF-GAPSP (Sigma A5230), ARF5 (Novus Biologicals H00000381-M01), anti-ARF-GAPSP (Sigma HPA051019), anti-SORL1 (BD 611860), anti-RICTOR (CST 2114), anti-PP2C (Sigma HPA050940 1250), anti-14-3-3 (CST 7413), anti-PI3Kα (CST 9693) and PI3Kβ (Sigma K0767). After addition of appropriate secondary antibodies for 45 min, membranes were washed three times in TBSI and imaged using the ChemiDoc Imaging System (BioRad) or Odyssey Imaging System (LI-COR Biosciences). Bands were quantified using Image Lab 6.1 (BioRad) or Image Studio Software 6.0 (LI-COR Biosciences). The number of independent experiments (n) is stated in the appropriate figure legend and quantitation is shown as mean ± s.d., p-values were calculated using a Student’s 2-tailed t-test, unless otherwise stated, and are shown on each graph as follows: n.s. = not significant, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. GAPDH was used as a loading control for each immunoblot and a representative image for each sample set is shown where appropriate.

GFP Trap and Immunoprecipitation. Immune complexes were collected when 1 mg of cell lysate was immunoprecipitated with anti-Met antibody (CST 3127, 1:300) overnight at 4 °C with rotation. Anti-mouse agarose or mouse agarose (both Sigma-Aldrich) was added for 1 h at 4 °C with rotation added to three wells. The complexes were then separated by SDS-PAGE, transferred to a PVDF membrane and immunoblotted. Lysates from cells expressing GFP-tagged proteins were immunoprecipitated using a GFP-Trap Kit (Chromotek) as per manufacturer’s instructions and immunoblotting performed as above. n = 3 and quantitation is shown as mean ± s.d.

Mass spectrometry. For mass spectrometry analysis agarose beads were resuspended in a 2 M urea and 100 mM ammonium bicarbonate buffer and stored at −20 °C. On bead digestion was performed from the supernatants. Triplicate biological replicates were digested with 25 μl 2 M urea in 50 mM Tris, pH 7.5, 1 mM EDTA, and 150 ng EndoLysC (Alpha Laboratories) and 150 ng trypsin (Promega) on beads Lys-C (Alpha Laboratories) and trypsin (Promega) on beads according to manufacturer’s instructions. Trypsin digests were separated using reverse phase Reprosil Pur Basic 1.9 μm (Dr. Maisch GmbH) using an EASY-nLC 1200 (Thermo Fisher Scientific) coupled online to an Orbitrap Q-Exactive HF mass spectrometer (Thermo Fisher Scientific) via nanoelectrospray ion source (Thermo Fisher Scientific). For the full scan a resolution of 60,000 at 250Th was used. The top ten most intense ions in the full MS were isolated for fragmentation with a target of 50,000 ions at a resolution of 15,000 at 250Th. MS data acquisition were performed using the XCalibur software (Thermo Fisher Scientific). The MaxQuant software version 1.5.5.172 was used to process MS Raw files and searched with Andromeda search engine®. Database was defined as Uniprot and allowing maximum two missed cleavages. Methionine oxidation and N-terminal acetylation were specified as variable modifications, and Cysteine carboxylmethylolation as fixed modification. The peptide and protein false discovery rate (FDR) was set to 0.1% and 0.01. The common reverse and contaminant hits (as defined in MaxQuant) were removed. Only protein groups identified with at least one uniquely assigned peptide were used for quantification. For label-free quantitation, proteins in all three replicates in at least one group, were measured.
according to the label-free quantitation algorithm available in MaxQuant. The RNAseq data from PC3 sublines have been deposited to the Gene Expression Omnibus (GEO) (accession no. GSE54820). The proteomic data generated in this work have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository. The RNAseq data from PC3 sublines in this study are available in the Short Read Archive database for PC3, SRS354082, or the Gene Expression Omnibus (GEO) database for PC3-Epi and PC3-EM14, SRS534082. These datasets can be accessed, analysed and downloaded using in-platform cBioportal.org tools. Analysis of patient cohorts was performed using the TCGA Splicing Variants Database (www.TSVdb.com). RPPA dataset using the TCGA Splicing Variants Database (www.TSVdb.com) (2012). RPPA and IQSEC1 variant data were analysed using custom KNIME Data Analytics Platform (Version 3.3.1) pipelines.

**Data availability**

The proteomic data generated in this work have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository. The RNAseq data from PC3 sublines in this study are available in the Short Read Archive database for PC3, SRS354082, or the Gene Expression Omnibus (GEO) database for PC3-Epi and PC3-EM14, SRS534082. These datasets can be accessed, analysed and downloaded using in-platform cBioportal.org tools. Analysis of patient cohorts was performed using the TCGA Splicing Variants Database (www.TSVdb.com) (2012). RPPA and IQSEC1 variant data were analysed using custom KNIME Data Analytics Platform (Version 3.3.1) pipelines.

**Reporting summary**

Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

**References**

1. Halouin, R. & McCaffrey, L. Rewiring cell polarity signaling in cancer. Oncogene 34, 939–950 (2015).
2. Pampaloni, F., Reynaud, E. G. & Stelzer, E. H. The third dimension bridges the gap between cell culture and live tissue. Nat. Rev. Mol. Cell Biol. 8, 839–845 (2007).
3. Burgh, M. D. & Mostov, K. E. From cells to organs: building polarized tissue. Nat. Rev. Mol. Cell Biol. 9, 887–901 (2008).
4. Zajac, O. et al. Tumour spheres with inverted polarity drive the formation of peritoneal metastases in patients with hypermetastatic colorectal carcinomas. Nat. Cell Biol. 20, 296–306 (2018).
5. Bryant, D. M. et al. A molecular switch for the orientation of epithelial cell polarization. Dev. Cell 31, 171–187 (2014).
6. Shami, E. R. & Ewald, A. J. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. Nat. Rev. Mol. Cell Biol. 15, 647–664 (2014).
7. Parachoniak, C. A. & Park, M. Dynamics of receptor trafficking in tumorigenesis. Trends Cell Biol. 22, 231–240 (2012).
8. Toft, C. et al. A dire role for Met endocytosis in tumorigenesis. Nat. Cell Biol. 13, 827–837 (2011).
9. Menard, L., Parker, P. J. & Kermorgant, S. Receptor tyrosine kinase c-Met controls the cytokine skeleton from different endosomes via different pathways. Nat. Commun. 5, 3907 (2014).
10. Parachoniak, C. A., Luo, Z., Abella, J. V., Keen, J. H. & Park, M. GGA3 functions as a switch to promote Met receptor recycling, essential for sustained ERK and cell migration. Dev. Cell 20, 75–763 (2011).
11. Humphreys, D., Davidson, A. C., Hume, P. J., Makin, L. E. & Koronakis, V. ARF6 coordinates actin assembly through the WAVE complex, a mechanism usurped by Salmonella to invade host cells. Proc. Natl. Acad. Sci. USA 110, 16860–16865 (2013).
12. Montagnac, G. et al. ARF6 Interacts with JIP4 to control a motor switch mechanism regulating endosome traffic in cytokinesis. Curr. Biol. 19, 184–195 (2009).
13. Santy, L., Rivachandran, K. S. & Casanova, J. E. The DOK180/Elmo complex couples ARNO-mediated ARF activation to the downstream activation of Rac1. Curr. Biol. 15, 1749–1754 (2005).
14. Stammes, M. A. & Rothman, J. E. The binding of AP-1 clathrin adaptor particles to Golgi membranes requires ARF-ribosylation factor, a small GTP-binding protein. Cell 73, 999–1005 (1993).
15. Donaldson, J. G. & Jackson, C. L. ARF family G proteins and their regulators: roles in cell migration, transport, development and disease. Nat. Rev. Mol. Cell Biol. 12, 362–375 (2011).
16. Hashimoto, A. et al. ARF6-AMAP1-AMAP1-cortactin pathway frequently used in cancer invasion is activated by VEGFR2 to promote angiogenesis. PLoS ONE 6, e23539 (2011).
17. Hashimoto, S. et al. Lyso phosphatidic acid activates ARF6 to promote the mesenchymal malignancy of renal cancer. Nat. Commun. 7, 10656 (2016).
18. Kinoshita, R. et al. Overexpression of ARF6 and AMAP1 proteins correlates with rapid local recurrence after breast conservative therapy. PLoS ONE 8, e67913 (2013).
19. Morishige, M. et al. ARF6 links epidermal growth factor receptor signalling to ARF activation to induce breast cancer invasion. Nat. Cell Biol. 10, 85–92 (2008).
20. Hashimoto, S. et al. Targeting AMAP1 and cortactin binding bearing an atypical src homology 3/proline interface for prevention of breast cancer invasion and metastasis. Proc. Natl. Acad. Sci. USA 103, 7036–7041 (2006).
21. Grossmann, A. H. et al. The small GTPase Arf6 stimulates beta-catenin transcriptional activity during WNT/β-catenin-mediated melanoma invasion and metastasis. Sci. Signal. 6, ra14 (2013).
22. Marchesin, V. et al. ARF6-JIP3/4 regulate endosomal tubules for MT1-MMP recycling in cancer invasion. J. Cell Biol. 211, 339–358 (2015).
23. Marchesin, V., Montagnac, G. & Chavrier, P. ARF6 promotes the formation of Rac1 and WAVE-dependent ventral F-actin rosettes in breast cancer cells in response to epidermal growth factor. PLoS ONE 10, e0121747 (2015).
24. Muradilhan-Chari, V. et al. ADP-ribo-sylation factor 6 regulates tumorigenic and invasive properties in vivo. Cancer Res. 69, 2201–2209 (2009).
25. Zhang, Q. et al. Small-molecule synergist of the Wnt/beta-catenin signaling pathway. Proc. Natl. Acad. Sci. USA 104, 7444–7448 (2007).
26. Singh, M. K. et al. Structure-activity relationship studies of QS11, a small molecule Wnt synergistic agonist. Bioorg. Med. Chem. Lett. 25, 4838–4842 (2015).
27. Yoo, J. H. et al. ARF6 is an actionable node that orchestrates oncogenic GNAQ signaling in uveal melanoma. Cancer Cell 29, 889–904 (2016).
28. Bello, D., Webber, M. M., Kleiman, H. K., Wartinger, D. G. & Rhim, J. S. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. Carcinogenesis 18, 1215–1223 (1997).
29. Kaihn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F. & Jones, L. W. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest. Urol. 17, 16–25 (1979).
30. Somney, A. et al. ARF-GEP100, a guanine nucleotide-exchange protein for ADP-ribosylation factor 6. Proc. Natl. Acad. Sci. USA 98, 2413–2418 (2001).
31. Dunphy, J. L., Ye, K. & Casanova, J. E. Nuclear functions of the Arf guanine nucleotide exchange factor BRA2G. Traffic 8, 661–672 (2007).
32. Manavska, Y. et al. BRA2G differentially regulates beta1- and beta3-integrin-dependent adhesion in endothelial cells and is involved in developmental and pathological angiogenesis. J. Exp. Med. 199, 493 (2004).
33. Santy, J. C. Characterization of a fast cycling ADP-ribosylation factor 6 mutant. J. Biol. Chem. 277, 40185–40188 (2002).
Hafner, M. et al. Inhibition of cytokines by SecinH3 leads to hepatic insulin resistance. *Nature 444*, 941–944 (2006).

Benabdi, S. et al. Family-wide analysis of the inhibition of Arf guanine nucleotide exchange factors with small molecules: evidence of unique inhibitory profiles. *Science 365*, 4119–4127 (2019).

Lu, Z. X. et al. Transcriptome-wide landscape of pre-mRNA alternative splicing associated with metastatic colonization. *Mol. cancer Res. 13*, 305–318 (2015).

Roca, H. et al. Transcripton factors OVOL1 and OVOL2 induce the mesenchymal to epithelial transition in human cancer. *PloS ONE 8*, e76773 (2013).

Lillis, A. P., Mikhailenko, L. I. & Strickland, D. K. Beyond endocytosis: LRK function in cell migration, proliferation and vascular permeability. *J. Thromb. Haemost. 3*, 1884–1893 (2005).

Herz, J. et al. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J. 7*, 4119–4127 (1988).

Li, H. et al. Targeting of mTORC2 prevents cell migration and promotes apoptosis in breast cancer. *Breast Cancer Res. Treat. 134*, 1057–1066 (2012).

Zhang, F. et al. mTORC complex component Rictor interacts with PKCeta and regulates cancer cell metastasis. *Cancer Res. 70*, 9360–9370 (2010).

Liu, P. et al. Simultaneous phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorgenesis. *Nat. Cell Biol. 15*, 1340–1350 (2013).

Jian, X. et al. Autoinhibition of Arf GTPase-activating protein activity by the BAR domain in ASAP1. *J. Biol. Chem. 284*, 1652–1663 (2009).

Lewis, S. M., Poon, P. P., Singer, R. A., Johnston, G. C. & Säng, A. The ArfGAP Glo3 is required for the generation of COPI vesicles. *Mol. Cell 15*, 4064–4072 (2004).

Zhang, C. J., Cavenagh, M. M. & Kahn, R. A. A family of Arf effectors determines as suppressors of the loss of Arf function in the yeast Saccharomyces cerevisiae. *J. Biol. Chem. 273*, 19792–19796 (1998).

Theret, L. et al. Identification of LRK-1 as an endocytosis and recycling receptor for betai-integrin in thyroid cancer cells. *Oncotarget 8*, 78614–78623 (2017).

Kang, H. S. et al. LR P1-dependent pepsin clearance induced by 2'-hydroxycoinnamaldehyde attenuates breast cancer cell invasion. *J. Biochem. Cell Biol. 53*, 15–23 (2014).

Stephens, L. et al. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science 279*, 710–714 (1998).

Sarbasov, D. G., Guertin, D. A., Ali, S. M. & Sabatini, D. M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science 307*, 1098–1101 (2005).

Brown, H. A., Gutowski, S., Moonaw, C. R., Slaughter, C. & Sternweiss, P. C. ADP-ribosylation factors, a small GTP-dependent regulatory family, stimulate phospholipase D activity. *Cell 75*, 1137–1144 (1993).

Cockcroft, S. et al. Phospholipase D: a downstream effector of ARF in granulocytes. *Science 263*, 523–526 (1994).

Honda, A. et al. Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation. *Cell 99*, 321–332 (1999).

Yoo, J. H. et al. The small GTPase ARF6 activates PI3K in melanoma to induce a pro-metastatic state. *Cancer Res. 79*, 2892–2908 (2019).

Tsai, M. T. et al. Regulation of HGF-induced hepatocyte proliferation by the orthotopic treatment model of prostate cancer and metastasis in the immunocompetent mouse: efficacy of Bin3 ligand immunotherapy. *Int. J. Cancer 107*, 773–780 (2003).

Hübner, N. C. et al. Quantitative proteomics combined with BAC Transgeneomics reveals in vivo protein interactions. *J. Cell Biol. 189*, 739–754 (2010).

Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol. 26*, 1367–1372 (2008).

Cox, J. et al. Andromeda: a peptide search engine integrated into the MaxQuant environment. *J. Proteomics 10*, 1794–1805 (2011).

Uniprot, C. The Universal Protein Resource (UniProt) in 2010. *Nucleic Acids Res. 38*, D142–D148 (2010).

Cox, J. et al. Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Mol. Cell. Proteom. 13*, 2513–2526 (2014).

Cerami, E. et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov. 2*, 401–404 (2012).

Gao, J. et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal. 6*, pl1 (2013).

Sun, W. et al. TSVdb: a web-tool for TGCA splicing variants analysis. *BMC Genomics 19*, 405 (2018).

Perez Riverol, Y. et al. The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic acids Res. 47*, D442–D450 (2019).

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Author contributions

M.N., D.M.B., K.N., E.S. designed experiments and analysed data. M.N., D.M.B., K.N., E.S. developed high-throughput imaging and bioinformatics. E.S., A.R.-F, L.M., S.Z., and S.L. generated and/or provided cell lines. All other authors have no competing interests.

Competing interests

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