

Evidence of Cancer Promoting Roles for AMPK and Related Kinases

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Abstract:

The discovery that the 5'AMP-activated protein kinase, AMPK, serves to link the tumour suppressors LKB1 and the Tuberous Sclerosis Complex (TSC), and functions to slow macromolecular synthesis through attenuation of the mechanistic Target of Rapamycin Complex 1 (mTORC1), revealed a role for AMPK in tumour suppression. On the other hand, the well-recognized role of AMPK in maintaining ATP homeostasis, through suppression of anabolism and promotion of catabolism, as well as the role of AMPK in neutralising reactive oxygen species (ROS), via maintenance of NADPH-dependent reductive capacity, point to tumour-protective roles in the context of metabolic stress, which is a key feature of many solid tumours. A growing number of studies thus suggest a duality of functions for AMPK that are either pro- or anti-cancer, depending upon context. Importantly, AMPK is comprised of 3 subunits and multiple isoforms exist for all three, allowing for different permutations to assemble and the potential for specific AMPK complexes to regulate distinct cellular processes. Moreover, certain subunits of the AMPK complex are frequently overexpressed in a spectrum of human cancer types, suggesting an outright oncogenic function for specific AMPK complexes. Adding complexity to this picture, the catalytic AMPK alpha subunits belong to a family of 14 kinases that can all be activated by LKB1 and studies are beginning to reveal a similar duality of roles in cancer for other members of the AMPK-related kinase family.

Cancer cells divert enormous resources into fuelling the growth required to sustain their unscheduled proliferation. Commonly arising oncogenic mutations resulting in RAS and PI3K pathway activation, p53 inactivation or MYC overexpression, directly impinge upon core cellular metabolism, at once driving proliferation and at the same time signalling to cells to redirect the breakdown products of nutrients into the synthesis of macromolecules required for cell growth [1, 2]. This diversion of nutrients comes at a cost however and cancer cells must continuously rebalance their rate of macromolecular synthesis and cell growth with the energetic cost of supporting that growth, measured in ATP. The fragility of this balancing act is underscored by the observation that cancer cells often exhibit exquisite sensitivity to nutrient deprivation, rapidly undergoing cell death where non-transformed counterparts respond by downregulating proliferative signalling and undergoing arrest [3-6]. In the context of a growing solid tumour, cancer cells are continuously exposed to a range of pathophysiological metabolic strains, including nutrient limitation, hypoxia and microenvironment acidification, owing to the inefficient and disorganised nature of the tumour vasculature. Indeed, poorly vascularised tumour regions typically show high levels of necrotic cell death [7]. Strategies to exploit the intrinsic metabolic vulnerabilities of tumour cells are thus now gaining in credibility and may have broad utility in the treatment of a spectrum of cancers [8-11].

AMPK Maintains ATP Homeostasis

The 5'AMP-activated protein kinase, AMPK, is a key regulator of the balance between cell growth and bioenergetic homeostasis. In general, AMPK promotes processes that generate or preserve cellular ATP, including glycolysis, oxidative phosphorylation, β -oxidation of fatty acids and autophagy, and inhibits processes that consume ATP, such as protein translation, ribosome assembly and lipid synthesis [12, 13]. As its name suggests, AMPK activity increases with rising [AMP], or more precisely, upon an increase in the cellular [AMP]:[ATP] ratio. AMPK is a trimeric complex comprised of a catalytic alpha subunit and a regulatory gamma subunit held together by a scaffolding beta subunit. The gamma subunit can bind up to 3 molecules of AMP, at least 2 of which can exchange for ATP which reduces activity, thereby allowing AMPK to directly detect changes in the [AMP]:[ATP] ratio [14]. This ability to simultaneously bind activating and inhibitory adenosine phosphate residues ensures a graded rather than binary response to the cellular metabolic state, enabling cells to continuously "fine-tune" their rate of macromolecular synthesis in line with energetic fluctuations. AMPK is thus activated indirectly by a wide variety of compounds that increase the cellular

[AMP]:[ATP] ratio, such as biguanides Metformin and Phenformin, mitochondrial toxins and modulators, 2-deoxyglucose, and indeed by nutrient deprivation [15]. The importance of AMPK's role in ATP homeostasis is underlined by the fact that many such compounds are profoundly toxic in cells that lack functional AMPK, yet are well tolerated by AMPK-expressing counterparts [16].

AMPK in Protection from Reactive Oxygen Species

Reactive oxygen species (ROS) are produced primarily as a natural by-product of mitochondrial respiratory chain activity [17]. While moderate levels of ROS, in particular H₂O₂, participate in signal transduction, high levels of ROS can result in macromolecular damage and cytotoxicity. ROS levels are elevated by impaired mitochondrial function, driven by mitochondrial mutations, oncogenic signalling and, importantly, by hypoxia [18]. Notably, AMPK is activated by hypoxia in a ROS-dependent manner [19] and is implicated in hypoxia-driven angiogenesis [20]. Treatment of cells with exogenous H₂O₂ likewise activates AMPK [21]. AMPK was shown to play a key role in cellular antioxidant defence by preserving NADPH levels, via inhibition of ACC1/2-mediated fatty acid synthesis and activation of fatty acid oxidation [22]. NADPH is a major antioxidant required for maintaining the reductive capacity of glutathione and lowering ROS levels, thereby protecting cells from oxidative stress-induced death [23]. Consistent with a tumour-promoting role for AMPK-mediated ROS defence, the tumour suppressor Folliculin was recently identified as a negative regulator of AMPK, and loss of Folliculin was shown to protect cells from death induced by H₂O₂, amongst other stresses, through an evolutionarily conserved mechanism involving AMPK-dependent activation of autophagy [24, 25].

The Paradox of Tumour Suppression by AMPK

The picture that emerges is that AMPK plays a central role in the adaptive responses to cellular metabolic stress. This ability to respond dynamically to a spectrum of metabolic insults is of obvious benefit to tumour cells in a hostile microenvironment, where nutrients, growth factors and oxygen are limiting, while metabolic waste accumulates, as tumours outgrow their vascular supply. Thus AMPK may be critical for maintaining cancer cell viability in established tumours, making it an attractive target for pharmacological inhibition. [Somewhat paradoxically however](#), one mechanism by which AMPK can preserve ATP is through the inhibition of mTORC1-driven protein translation [26, 27], [and](#) this very activity has fuelled the notion that AMPK can function as a tumour suppressor, given that mTORC1

activity is increased in cancer via activation of upstream oncogenic signalling through PI3K and AKT and/or loss of upstream tumour suppressors PTEN, TSC and LKB1. The discovery that LKB1 directly activates AMPK, thereby linking LKB1 to suppression of mTORC1, and that AMPK in turn activates the Tuberous Sclerosis Complex (TSC), another negative regulator of mTORC1, seemed to place AMPK squarely in a tumour suppressive role [28-31]. More recently, the discovery that MAGE A3/6 targets AMPK α 1 for degradation appears to buttress this interpretation: expression of MAGE A3/6 proteins is normally restricted to the testes but is reportedly widespread in human cancer [32]. Expression of MAGE A3/6 is sufficient to increase focus formation and anchorage-independent growth of immortalised cell lines. Expression of MAGE A3/6 moreover increases mTORC1 signalling and suppresses autophagy in a manner that requires AMPK α 1 degradation, whereas depletion of MAGE A3/6 has the opposite effects. These observations collectively indicate that reducing AMPK activity can have tumour-promoting consequences. However, reducing AMPK activity is not the same as completely suppressing it, and a number of [observations](#) confound the simple designation of AMPK as a *bona-fide* tumour suppressor.

Firstly, although the predominant kinase upstream of AMPK in many cells, [LKB1](#) is not the only kinase capable of activating AMPK and we, amongst several other groups [22, 33-35], observe robust activation of AMPK in LKB1-deficient tumour cells, such as A549 and HeLa, upon treatment with both direct (A769662, Salicylate) and indirect (2DG, Phenformin, Ca⁺⁺ Ionophore) AMPK stimuli (Figure 1). Indeed, the TGF β -activated kinase TAK1 and the Ca⁺⁺/Calmodulin-dependent kinase CaMKK β have both been shown to directly phosphorylate the AMPK α subunit on the same T172 residue that is targeted by LKB1 [33, 36]. Activation of AMPK by CaMKK β may be of particular relevance in the context of tumour hypoxia, as hypoxia-induced ROS has been linked to Ca⁺⁺ release from the endoplasmic reticulum, leading to activation of CaMKK β and AMPK [37]. Moreover the Androgen Receptor/CaMKK β /AMPK axis has been proposed to play a prominent role in the etiology of prostate cancer [38]. [Thus, loss of LKB1 does not necessarily equate with loss of AMPK activity.](#)

Secondly, there is little evidence of AMPK deletion or inactivating mutation in human cancer. In mammals there are 2 genes [each](#) encoding alpha (*PRKAA1*, *PRKAA2*) and beta (*PRKAB1*, *PRKAB2*) subunits and three encoding gamma subunits (*PRKAG1*, *PRKAG2*, *PRKAG3*), and this genetic redundancy is often cited as a plausible explanation for the retention of wild type alleles [39]. On the contrary however, there is now clear evidence that specific AMPK subunits, notably *PRKAA1* (AMPK α 1) and *PRKAB2* (AMPK β 2), are frequently amplified across a broad spectrum of human cancers (Figure 2)[40, 41]. This selective amplification is also

observed in established human tumour cell lines and correlates closely with elevated mRNA expression (Figure 3A)[42]. Amplification of *PRKAA1* and *PRKAB2* coincides significantly with activation and/or amplification of dominant oncogenes such as *KRAS*, *BRAF* and *AKT*, while *PRKAB2* amplification in particular coincides significantly with *MYC* amplification across several cancer types including Melanoma, Breast and Bladder cancers (Figure 3B & C and data available via cBioPortal). Although [these](#) data [do not provide](#) definitive evidence of an outright oncogenic role for these subunits, [they](#) clearly [necessitate](#) a rethink of the possible roles of AMPK in human cancer.

A third and related point is that “AMPK” refers not to one complex but potentially to many complexes: Not accounting for splice variants, the 7 genetically encoded AMPK subunits in principle allow for assembly of up to 12 distinct AMPK complexes. It is tempting to speculate that different AMPK complexes might selectively regulate specific cellular processes via distinct downstream effectors, [or indeed respond differentially to specific upstream stimuli](#). There is already some evidence to suggest that this is the case: Deletion of [STK11](#), [\(encoding LKB1\)](#) in cardiac myocytes suppresses activation of AMPK α 2 and downstream phosphorylation of [Acetyl-CoA Carboxylase 2 \(ACC2\)](#) in response to ischemia, however, activation of AMPK α 1 is unaffected – by inference AMPK α 1 plays a minor role in the regulation of ACC2, at least in this context [43]. Additionally, FRET biosensors of AMPK activity directed to specific subcellular compartments reveal that plasma membrane-, lysosome- and golgi-associated AMPK complexes preferentially contain α 1 over α 2, suggesting a physical segregation of function for such complexes [44]. It is thus possible that certain AMPK complexes might promote tumour suppression while others favour tumour survival.

Finally, with the striking exception of [Pulmonary Adenocarcinoma](#), where [STK11](#) is mutated in up to 20% of cases, genetic loss of LKB1 is relatively infrequent in sporadic human cancer (Figure 2). Although promoter methylation at the *STK11* locus has been reported in sporadic colorectal cancer, it appears to be a relatively rare event [45]. Thus, it would seem that the vast majority of human cancers retain the capacity to call upon a functional LKB1/AMPK pathway for protection in the face of metabolic stress. Consistent with this perspective, although deletion of [Stk11](#) profoundly accelerates KRas-driven tumours in a mouse model of lung cancer, such tumours are exquisitely sensitive to the mitochondrial inhibitor Phenformin [46]. Similarly, deletion of [Stk11](#) in an ErbB2-driven mouse model of breast cancer, and deletion of [Prkaa1](#) in E μ -MYC-driven lymphoma, both accelerate tumourigenesis but in both instances render the tumour cells profoundly sensitive to

metabolic stress [47, 48]. Thus, even under circumstances where loss of the LKB1/AMPK pathway promotes tumour development, loss of this pathway simultaneously elicits a metabolic vulnerability that can potentially be exploited for therapy [49, 50].

Tumour promoting roles of AMPK-related Kinases (ARKs)

The alpha subunits of AMPK belong to an extended family [along with another](#) 12 related kinases: BRSK1, BRSK2, MARK1 (PAR-1c), MARK2 (PAR-1b), MARK3 (PAR-1a), MARK4 (PAR-1d), MELK, NUA1 (ARK5), NUA2 (SNARK), SIK1, SIK2 (QIK) and SIK3 (QSK). All of the ARKs bar MELK can be phosphorylated by LKB1 [29], although for some ARKs additional kinases are implicated as upstream regulators [51-54]. Several of the family members are broadly conserved across evolution, even as far as the plant kingdom [55], indicative of the ancient origin of these proteins and their crucial importance for most life forms on Earth. While our understanding of the ARKs lags some distance behind that of AMPK itself, to generalise, the physiological roles of these kinases fall into three categories: regulation of cell polarity; regulation of cell migration and regulation of metabolism at both cellular and organismal levels.

NUAK1

NUAK1 was initially isolated as the 5th AMPK-related mammalian kinase and hence is also termed ARK5. Early reports linked NUA1 to AKT signalling and specifically to IGF-induced cell migration and invasion [56, 57]. NUA1 was mechanistically linked to cell detachment via direct phosphorylation of the myosin phosphatase complex subunit MYPT1, supporting a role for NUA1 in facilitating cell motility [58]. Reduced expression of miRNAs targeting NUA1 is associated with invasion in Melanoma and metastasis in Head and Neck Squamous Cell Carcinoma [59, 60]. Additionally, NUA1 has been identified as a risk factor in Ovarian cancer [61] and is mutated in a small percentage of Oesophageal cancers [62].

We [recently](#) identified NUA1 in a synthetic lethal RNAi screen for kinases that are selectively required to support tumour cell viability when MYC is overexpressed [63], a result that was [independently reproduced by the Goga lab](#) [64]. The synthetic lethal interaction was observed using multiple distinct small interfering and short hairpin RNA sequences and could be rescued by genetic complementation using a non-targeted NUA1 cDNA. Confirming these results, a recently described highly-selective small molecule inhibitor of NUA1, HTH-01-015 [65], selectively kills MEFs when MYC is acutely deregulated (Figure 4). Acute MYC deregulation in cells depleted of NUA1 results in ATP collapse, revealing an unexpected role

for NUAK1 in ATP homeostasis, at least in the context of MYC deregulation. The energetic stress induced upon suppression of NUAK1 isn't limited to the *in vitro* setting: We showed that depletion of NUAK1 suppressed tumour formation and extended survival in an orthotopic mouse model of MYC-driven Hepatocellular Carcinoma, suggesting that NUAK1 is a potential target for cancer therapy [63].

Functionally, we linked NUAK1 to maintenance of mitochondrial fitness, or rather to mitochondrial plasticity: MYC activation increases expression of specific respiratory chain components, thereby enhancing respiratory capacity [66], and this adaptive effect was abrogated upon depletion of NUAK1. Moreover, we also exposed an unexpected role for NUAK1 in MYC-dependent activation of AMPK and [found that](#) depletion of NUAK1 resulted in enhanced activity of mTORC1. Importantly, AMPK α 1 was also identified in our original synthetic lethal with MYC screen (while AMPK β 1 was identified in an independent synthetic lethal with MYC screen [67]) and the synthetic lethal effect of depleting either AMPK α 1 or NUAK1 was rescued by slowing ATP consumption, via inhibition of mTORC1 with Rapamycin. NUAK1 depleted tumour cells thus have reduced ATP-generating capacity and elicit an impaired AMPK response to MYC. Our data therefore also implicate AMPK as having a tumour-protective role in MYC-overexpressing cells, which [at first glance](#) appears to conflict with evidence that loss of AMPK accelerates E μ -MYC-driven lymphomagenesis [48]. A plausible explanation is that blood-borne cancers [are unlikely to](#) be subject to the same metabolic stress encountered by solid tumours. Consistent with this possibility, the Jones group showed that AMPK-deficient E μ -MYC lymphoma cells are extremely sensitive to metabolic stress whereas AMPK-replete counterparts are much more resistant [48]. Thus, a mutation that offers a selective advantage at the time of tumour initiation may become a liability later in malignancy, particularly in the context of solid tumours.

NUAK2

Closely related to NUAK1, NUAK2 (aka SNARK) is frequently amplified across a spectrum of human cancers (Figure 5), forming part of the 1q32 amplicon common in Melanoma, Glioblastoma and Mammary cancers [68-70] [and a](#) specific role for NUAK2 in Acral Melanoma has been proposed [69]. Co-amplification of the potent p53 suppressor MDMX (encoded by *MDM4*) along with the RAS-pathway effector ELK4 [71] complicates interpretation of the significance of NUAK2 amplification. However, numerous expression analyses accessible through Oncomine do indicate frequent overexpression of NUAK2 mRNA in human cancers, suggesting a potential role in disease maintenance. Similar to AMPK, NUAK2 activity was

shown to increase upon nutrient deprivation or H₂O₂ treatment, suggesting a role for NUA2 in protection from nutrient and oxidative stress [72]. On the other hand, mice constitutively haplo-insufficient for NUA2 are sensitized to azoxymethane-induced colonic tumour formation, although it is unclear if this reflects an enterocyte-autonomous phenotype rather than a consequence of whole-body haploinsufficiency, and loss of NUA2 heterozygosity in the tumours was not reported [73].

Microtubule Affinity Regulating Kinases (MARKs/PAR-1 proteins)

The MARK sub-family of kinases are implicated in cell motility and the physiological regulation of energy metabolism [52, 74]. Constitutive deletion of *MARK2*, *3* or *4* all result in hypermetabolic phenotypes of varying severity, increased Insulin sensitivity, and resistance to high-fat diet-induced obesity, suggesting that these proteins contribute systemically to diabetes [75-77], which is a well-recognised risk factor in many cancers [78]. *MARK1* is amplified in roughly 12% of Breast and Liver cancers and is co-amplified with NUA2 across multiple cancers, likely reflecting a broader amplification of the Q arm of chromosome 1 (Figure 5). *MARK1* and *4* were recently shown to co-ordinately mediate LKB1's ability to suppress epithelial to mesenchymal transition (EMT) via DIXDC-dependent inhibition of SNAIL expression [79]. As such, these kinases may play an important role in suppression of metastasis of certain cancers, however, their function in primary tumours was not addressed in this study.

MARK2 was recently found to be overexpressed in [23](#) of 77 primary NSCLC tumour samples relative to paired non-malignant tissue, [and overexpression correlated with copy number gains and/or locus hypomethylation](#) [80]. Analysis of the TCGA cohort [81] revealed overexpression of *MARK2* in over 50% of NSCLC, irrespective of histological subtype, and overexpression correlated more with hypomethylation than with copy number gains, especially amongst the Squamous subtype. In NSCLC cell lines with high levels of *MARK2* protein expression, depletion of *MARK2* by RNAi suppressed proliferation and was associated with decreased WNT, HIF1 α and MYC pathway activity, while high expression of *MARK2* correlated with resistance to Cisplatin, as had been previously reported [82]. Intriguingly, the *Xenopus* homologues of *MARK2* and *MARK3* are important for both canonical and non-canonical WNT signalling [83] and the WNT pathway is widely activated in human cancer [84, 85].

Regulation of the Hippo Pathway

Several ARKs have recently been implicated as important regulators of the Hippo pathway, which controls organ size and is deregulated in many cancers (for excellent reviews see refs. [86, 87]). The Hippo pathway comprises a transcription module, made up of YAP1 and TAZ, which are negatively regulated by a kinase module, including the effector kinases LATS1 & 2 and their upstream activators MST1 & 2. NUAK1 and 2 were shown to be able to directly phosphorylate LATS1 leading to LATS1 degradation [88], which is predicted to result in increased YAP1/TAZ activity. On the other hand, MARK1 and 4 were shown to promote LATS1 activation by driving membrane re-localization of another Hippo factor, SCRIB, required for MST1/2-dependent phosphorylation of LATS1/2 and consequent inactivation of YAP1 [89]. Adding complexity to this picture, AMPK was recently shown by three independent groups to suppress YAP1 activity in response to energetic stress [90-92]. An earlier study showed LKB1-dependent regulation of YAP1, independent of either AMPK or LATS, suggesting that additional ARKs participate in regulation of this pathway (Nguyen). Clearly, the LKB1 pathway intersects the Hippo pathway at multiple levels (see Figure 6) and it will be fascinating to determine how this regulation is coordinated and indeed if it is bi-directional. As is the case for LKB1 signalling, the Hippo pathway appears to have both tumour promoting and tumour suppressive functions [87] and the role of both pathways in human cancer is likely to be highly context dependent.

Concluding Remarks

The duality of roles for AMPK in the adaptive response to metabolic stress, versus the attenuation of biosynthetic processes and cell growth, point to a complex and dynamic relationship with Cancer that defies restrictive designation as either “Oncogene” or “Tumour-Suppressor”. Moreover, the term “AMPK” is itself deceptive in that it captures an as yet undetermined number of potential trimeric complexes. Hints are emerging that multiple distinct AMPK complexes co-exist in cells and a number of intriguing questions are on the cusp of investigation: How do different AMPK complexes respond to different signalling and metabolic cues? Do they regulate distinct biological processes via select downstream targets? Is their activity coordinated and does cross-talk between complexes occur? Is there similar coordination with and amongst the 12 AMPK α -related ARKs? A growing list of small molecules that directly bind to AMPK suggests that these complexes can be targeted pharmacologically, although the bias to-date has been for compounds with AMPK-activating potential [93]. Selective small molecule inhibitors of AMPK would be useful, at the very least as tool compounds, and in the right context may have therapeutic potential in light of AMPK’s

tumour protective roles. There is moreover a clear need to develop reagents that can distinguish between complexes of different subunit composition, while conditional allelic mice promise to shed much light on the roles of individual AMPK subunits and ARKs in normal physiology and indeed in the context of Cancer. Disentangling the specific roles of this family of kinases is likely to yield many more surprises and intriguing insights relevant to Cancer, Metabolism, Physiology and beyond in the years to come.

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Conflict of Interest Statement

The authors declare no conflict of interest in the publication of this work.

Figure Legends

Figure 1

LKB1-independent activation of AMPK. Equal numbers of U2OS (LKB1 w/t), A549 (LKB1 deficient) and HeLa (LKB1 deficient) cells were treated with Salicylate (**10mM**), Phenformin (**3mM**), Calcium Ionophore (**3μM**) or DMSO vehicle control (vc) for 1hr, then lysed in RIPA buffer, fractionated by SDS-PAGE and blotted for the canonical AMPK target, phosphor-ACC1-Ser79. Note that A769662 (100μM) was used in lieu of Salicylate in HeLa cells. Consistent with published reports, acute activation of AMPK is unimpeded by the absence of LKB1, except in the instance of Phenformin treatment of HeLa cells.

Figure 2

Amplification of AMPK subunits in human cancer. Graphs accessible through cBioPortal (<http://www.cbioportal.org>) show the cumulative frequency of genetic alterations at the *STK11* (top), *PRKAA1* (middle) and *PRKAB2* loci, across a spectrum of human cancers. Green bars reflect the frequency of mutation (inclusive of activating and inactivating); red bars, the frequency of gene amplification; blue bars show the frequency of gene deletion and grey bars reflect multiple alterations at the same locus. Data are sourced from the TCGA, except where noted, and published TCGA cohorts are indicated with an asterisk. Other cohorts are 1) SU2C; 2) Broad; 3) Yale; 4) MSKCC; 5) AMC; 6) Mich; 7) BCCRC; 8) Genetech; 9) ICGC; 10) UHK; 11) JHU; 12) Pfizer; 13) BGI; 14) Cornell; 15) DFCRC; 16) TSP; 17) Sanger.

Figure 3

Specific Overexpression of *PRKAA1* and *PRKAB2* in established human cancer cell lines.

A) The Cancer Cell Line Encyclopedia (<http://www.broadinstitute.org/ccle/home>) dataset reveals selective amplification of *PRKAA1* ($\alpha 1$) and *PRKAB2* ($\beta 2$) that correlates with mRNA overexpression across a broad spectrum of cancer derived cell lines. *PRKAA2* ($\alpha 2$) and *PRKAB1* ($\beta 2$) by comparison show little evidence of consistent copy number alterations. Gene copy numbers are graphed along the X-axis while mRNA expression levels are graphed along the Y-axis. B) Analysis of the CCLE dataset through cBioPortal shows the mutation spectra of individual cell lines, arrayed from left to right, analysed for the indicated genes, revealing coincident amplification/mutation of *PRKAA1* and *PRKAB2*, both with each other and with selected dominant oncogenes, including *KRAS* and *MYC*. Note that the graphic is truncated from the right for visualization purposes. C) Statistical analysis of data from the graphic in (B) shows significant co-occurrence of *PRKAA1* and *PRKAB2* genetic alterations with those in

dominant oncogenes including KRAS and MYC. Similar results can be retrieved through analysis of primary tumour datasets for Breast, Ovarian and Pancreatic cancers amongst others, through cBioPortal.

Figure 4

Synthetic Lethality of MYC deregulation with pharmacological inhibition of NUA1.

Primary mouse embryo fibroblasts expressing MYC fused to the Estrogen Receptor ligand-binding domain (MycER^{T2}), rendering MYC activity dependent upon the synthetic ligand 4-hydroxy-Tamoxifen (4-OHT), were treated with 0, 25nM or 100nM 4-OHT and/or the selective NUA1 inhibitor HTH-01-015 (10 μ M), and the surviving cultures were stained with crystal violet after 48hrs. V.C. = vehicle control.

Figure 5

NUAK2 is frequently amplified in human cancer. Mutation spectra of *NUAK2* (SNARK) and *MARK1* (PAR-1C) across human cancer types, as per Figure 2. Data are adapted from cBioPortal, sourced from the TCGA, except where noted, and published TCGA cohorts are indicated with an asterisk. Other cohorts are 1) SU2C; 2) Broad; 3) Yale; 4) MSKCC; 5) AMC; 6) Mich; 7) BCCRC; 8) Genetech; 9) ICGC; 10) UHK; 11) JHU; 12) Pfizer; 13) BGI; 14) Cornell; 15) DFCRC; 16) TSP; 17) Sanger.

Figure 6

Regulation of Hippo Signalling by AMPK & ARKs. Phosphorylation of the transcriptional co-activators YAP1 and TAZ by LATS1 or LATS2 leads to cytosolic sequestration, thereby suppressing TEAD-driven transcription. LATS1/2 are negatively regulated by NUA1/2 but activated directly by AMPK and indirectly by MARK1/4 kinases, downstream of LKB1. AMPK has additionally been shown to directly phosphorylate YAP1, preventing its interaction with TEAD transcription factors.

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Figure 1

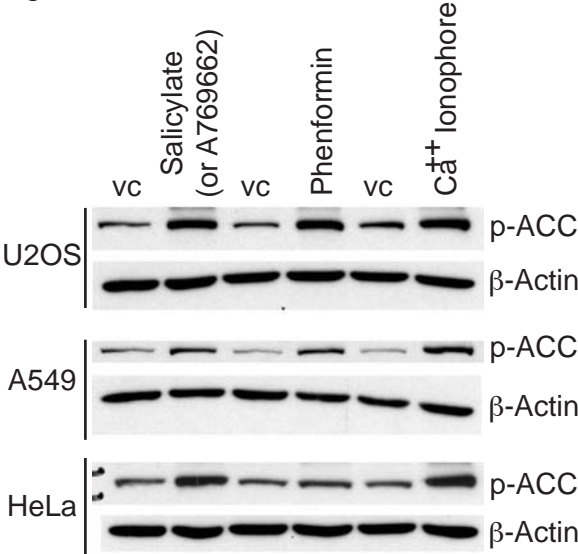


Figure 2

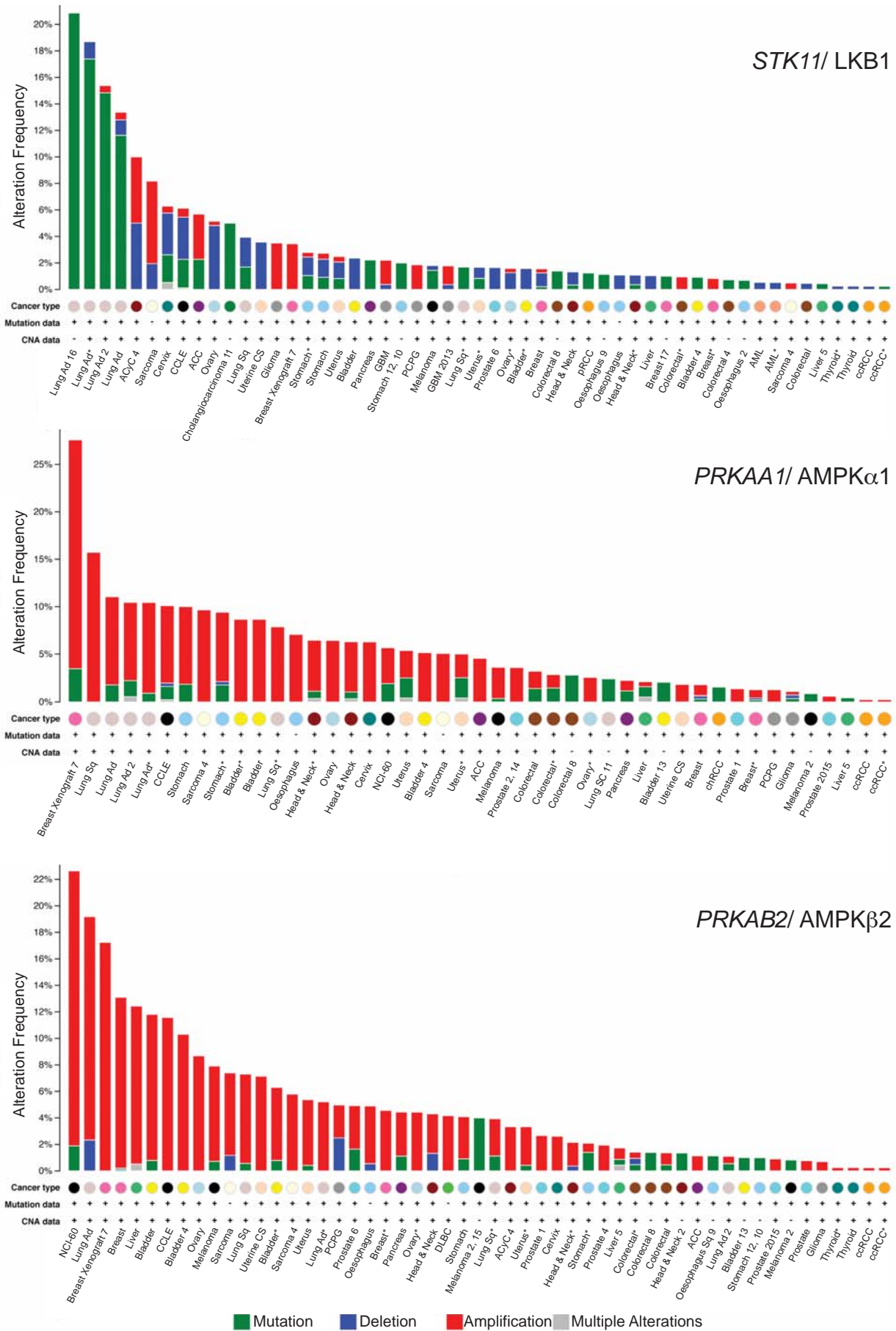
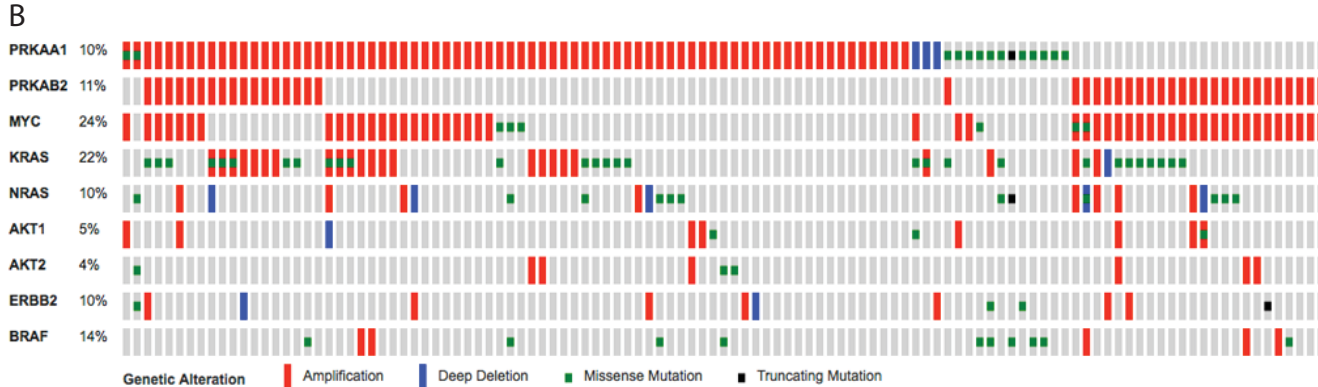
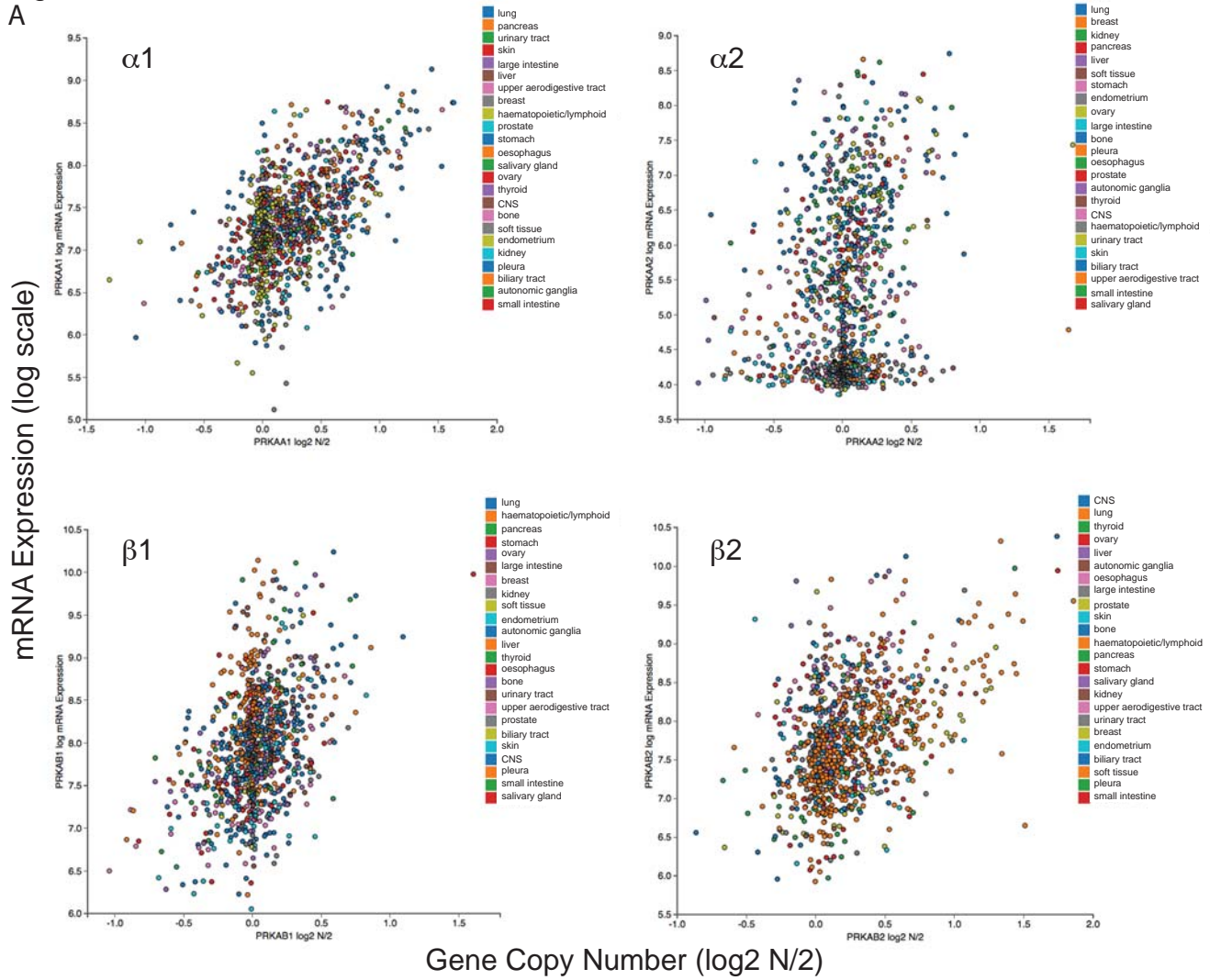


Figure 3



C

Gene A	Gene B	p-Value	Log Odds Ratio	Association
PRKAA1	KRAS	<0.001	0.886	Tendency towards co-occurrence Significant
PRKAB2	MYC	<0.001	0.960	Tendency towards co-occurrence Significant
PRKAB2	KRAS	<0.001	0.800	Tendency towards co-occurrence Significant
MYC	KRAS	<0.001	0.625	Tendency towards co-occurrence Significant
NRAS	AKT1	<0.001	1.297	Tendency towards co-occurrence Significant
PRKAB2	NRAS	0.004	0.814	Tendency towards co-occurrence Significant
PRKAA1	PRKAB2	0.009	0.759	Tendency towards co-occurrence Significant

Figure 4

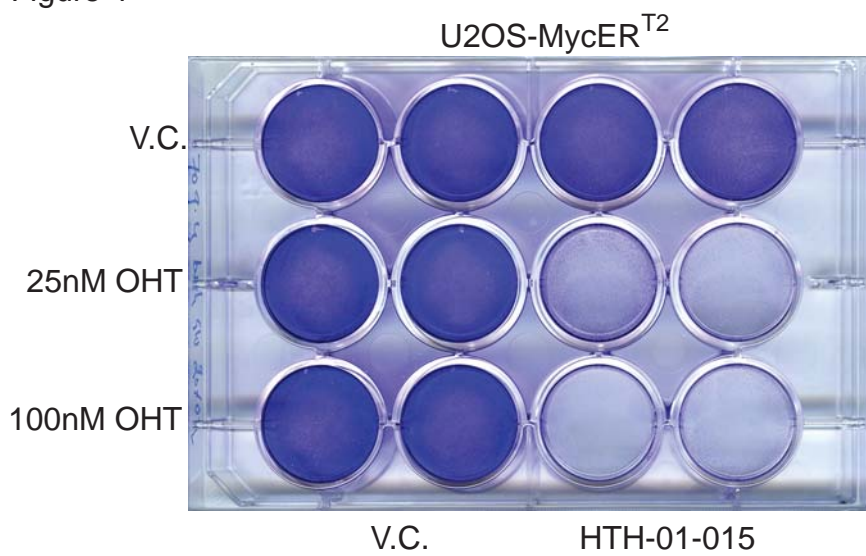
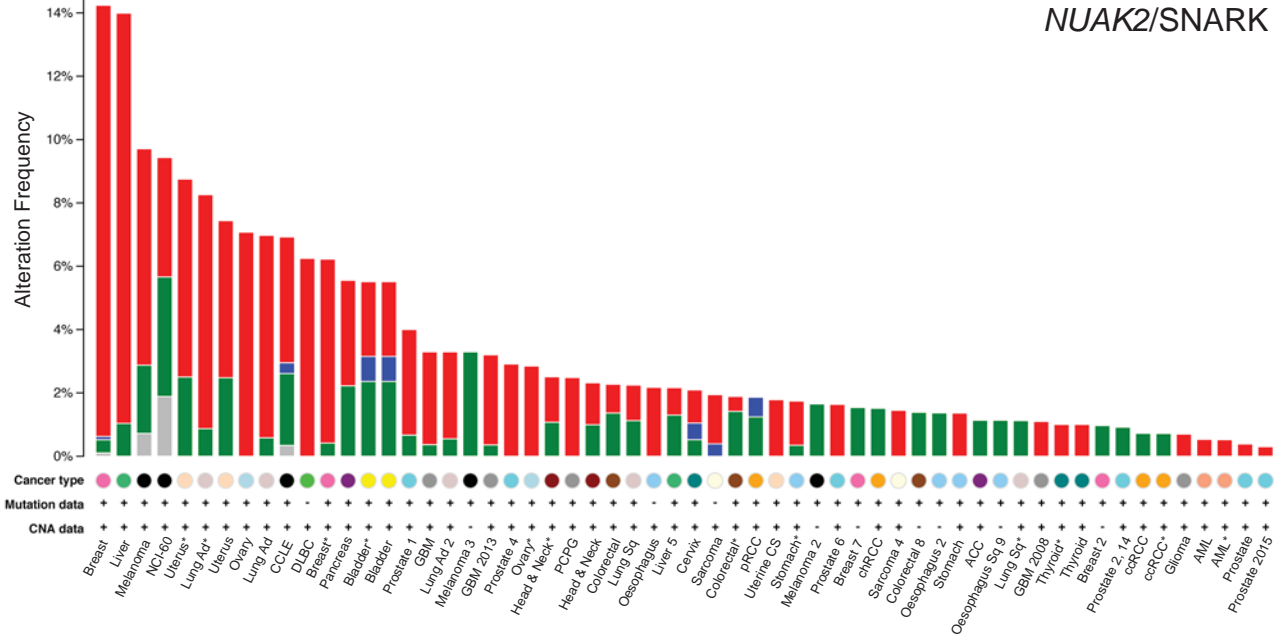


Figure 5

NUAK2/SNARK



MARK1/Par-1c

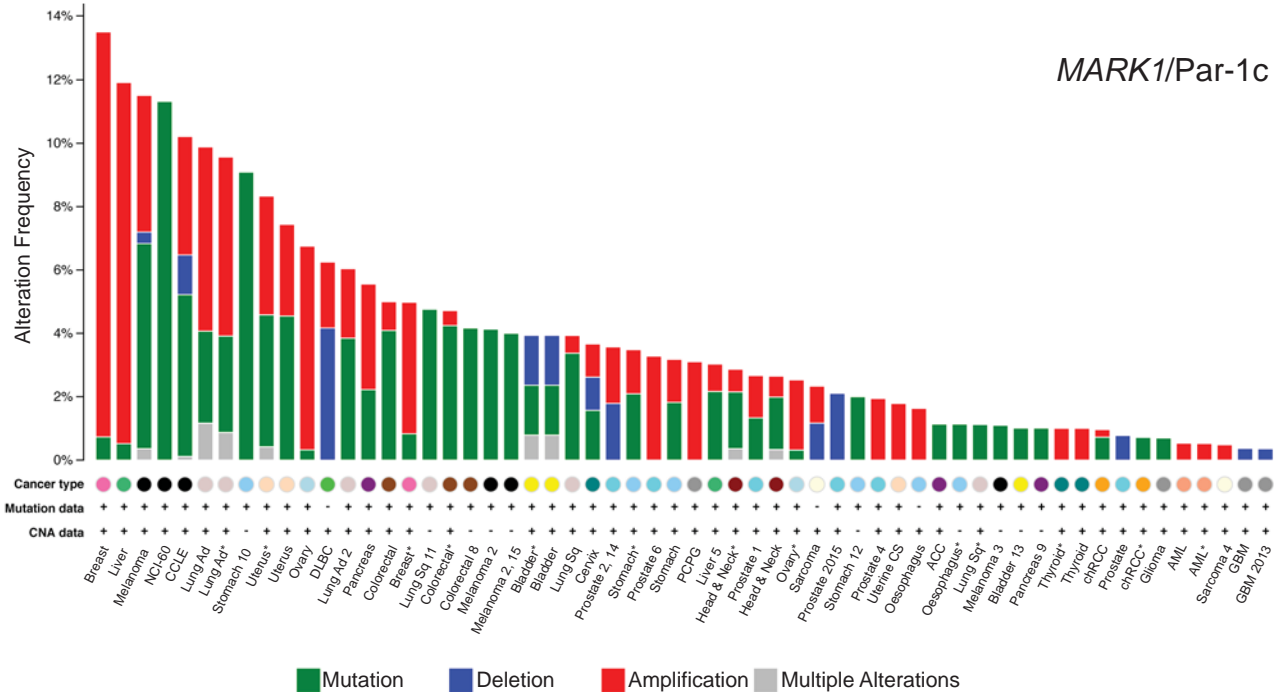


Figure 6

