CHAPTER 7

Synthetic lethal interactions with FA deficiency identified by genetic screens in head and neck cancer cell lines

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The Fanconi anemia (FA) pathway is an important DNA repair pathway to resolve DNA damage, in particular DNA interstrand crosslinks, during DNA replication. Despite the role of this repair pathway in counteracting replication stress to help cells to survive, FA-deficient cells can transform into tumor cells as demonstrated by the highly increased cancer risk seen in FA patients. This suggests the existence of compensatory mechanisms that are essential for FA-deficient cells to survive, and which are particularly required when cells transform and replication stress increases. By performing genome-wide high-throughput siRNA screening, we have investigated whether these compensatory mechanisms can be identified and might be exploited to develop new anti-cancer therapies for tumors in FA patients as well as FA-deficient tumors in non-FA patients. More lethal siRNAs were found in the FA-deficient tumor cell line compared to the corresponding FA-corrected tumor cell line (312 versus 253), indicating that FA-deficient cells may indeed rely on specific survival mechanisms. Our screen identified the proteasome, the Vacuolar ATPase, the nuclear pore complex and mitosis as promising targets to further investigate for development of novel treatment strategies that are specific for FA-deficient tumors.

**Introduction**

Fanconi anemia (FA) is a rare chromosomal instability syndrome characterized by a variety of congenital abnormalities, bone marrow failure and a high incidence of malignancies, in particular squamous cell carcinomas of the anogenital and head and neck region\(^1\). Since physical abnormalities can be subtle or absent, hematological problems are often the first indication for FA and form a main cause of disease complications, often requiring bone marrow transplantation\(^2,3\). Because bone marrow transplantation outcomes have been improved tremendously in recent years, the high cancer susceptibility is the next life-threatening problem that FA patients are now facing\(^4\). The risk to develop head and neck squamous cell carcinomas (HNSCC) is 500- to 700-fold higher than in the general population\(^5-7\). These tumors are difficult to treat in FA patients. More advanced stages of HNSCC are treated by either surgery with postoperative radiotherapy or by chemoradiation, the concomitant application of systemic cisplatin with locoregional radiotherapy. However, FA patients frequently develop treatment associated toxicities due to the high sensitivity to the commonly used chemotherapeutic drug cisplatin and radiotherapy\(^4\). Therefore, it is important to find new, preferably targeted, therapies to treat cancer in individuals with FA.

FA cells have a defect in an essential genome maintenance pathway that resolves problems during DNA replication\(^8,9\). Currently, bi-allelic mutations in one
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of 17 FA genes are known to be causative of FA\textsuperscript{8–10}. Together the FA proteins function in the FA pathway to repair DNA interstrand crosslinks. This repair pathway can be divided into an upstream part, in which 8 FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) together with several FA-associated proteins are responsible for mono-ubiquitination of FANCD2 and FANCI, and a downstream part (FANCD1/BRCA2, FANCI, FANCN/PALB2, FANCO/RAD51C, FANCQ/XPF and FANCS/BRCA1), which is not required for this posttranslational modification\textsuperscript{8,9}. Since tumor cells experience a lot of replication stress due to (epi)genetic alterations that deregulate cellular proliferation and apoptosis\textsuperscript{11,12}, it is surprising that cells with a deficient FA pathway can transform into a tumor. Moreover, FA pathway inactivation may even occur in sporadic tumors in non-FA patients\textsuperscript{13,14}. We therefore hypothesize that tumors with a defect in the FA pathway require compensatory mechanisms to survive. As a consequence, these mechanisms may represent an Achilles’ heel of the tumor and inactivation of such compensating mechanisms may result in reduced cellular fitness. These compensating processes will be synthetic lethal with the FA defect. By performing a high-throughput whole-genome RNA interference screen, we identified siRNAs targeting genes essential in FA-HNSCC but not the corrected cell line. Identification of these genes will aid in finding new treatment options for FA patients as well as for non-FA patients with FA-deficient tumors.

Results and discussion

**High-throughput siRNA screening in FA-deficient and FA-corrected HNSCC cell lines**

To identify genes that are essential for viability of FA head and neck tumor cells, we conducted high-throughput genome-wide siRNA screens in an FA-deficient HNSCC cell line with mutations in FANCC (VU-SCC-1131) and in the corresponding FANCC-corrected cell line (VU-SCC-1131+FANCC). The screening procedure was optimized for both cell lines to achieve uniform efficiency reflected by the sensitivity to transfection of a positive control siRNA SMARTpool (PLK1) and lack of sensitivity to transfection of a negative control siRNA SMARTpool (non-targeting siRNA (siCON)). Cells were reverse transfected in 384-wells format and after 5 days, cell viability was measured by adding CellTiter-Blue Reagent. Transfection of siRNAs targeting PLK1 (positive control) resulted in a reduction of at least 95% cell viability compared to cells transfected with the negative control siRNAs (data not shown). Toxicity of our transfection protocol was very modest since cell viability of negative control transfected cells was only slightly reduced (10-20%) compared to untransfected cells (data not shown). With these optimal transfection conditions, we conducted triplicate screens for each cell line. Raw viability values were normalized
by first log2-transformation and subsequently corrected for an overall plate effect across the cell lines by using a linear regression model. Subsequently, Z-scores were calculated and a cutoff of $Z=-2.75$, which was previously used by others\textsuperscript{15}, was chosen as a threshold to identify lethal siRNAs. Only 5 out of 3,264 negative controls (siCON) reached this threshold, whereas 4 out of 3,264 positive controls had Z-scores above -2.75 (Fig. 1A). The average Z-scores of untransfected, negative or positive control transfected VU-SCC-1131 cells was 0.30, 0.39 and -5.01, respectively (Fig. 1B). For VU-SCC-1131+FANCC cells, similar Z-scores (untransfected 0.19, negative control 0.33 and positive control -5.29) were obtained (Fig. 1B). Finally, Z' factors for each screen were calculated to determine the quality of the screens and varied between

Figure 1 Genome wide siRNA screening in FA and FA-corrected HNSCC cell lines
(A) Z-scores of negative (non-targeting siRNA\#2 (siCON)) and positive control (PLK1) used during high throughput siRNA screening in the FANCC-deficient FA-HNSCC cell line (VU-SCC-1131) and the corresponding genetically corrected cell line (VU-SCC-1131+FANCC). Symbols representing Z-scores above the cutoff of -2.75 for PLK1-transfected cells and below -2.75 for siCON-transfected cells are slightly enlarged. Note that there are some outliers (indicated with the arrow). These positive and negative controls were located on a plate with many lethal siRNAs and after normalization/re-scaling the data (see text), these Z-scores shifted upwards. (B) Average Z-score of untransfected (no siRNA (Buffer)), negative (siCON) and positive controls (PLK1). (C) Number of separate and overlapping lethal siRNAs (Z-score < -2.75) in VU-SCC-1131 and VU-SCC-1131+FANCC.
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Figure 2 Top twelve genes that contribute to survival of FA-deficient tumor cells

Top twelve proteins of which knockdown decreased cell viability more in FA-deficient VU-SCC-1131 cells than in the FA-corrected tumor cell line. Boxplots and triangles correspond to negative controls (siCON) and the indicated siRNAs, respectively.

0.55 and 0.75 (Table 1). Taken together, the transfection efficiency, discriminating power and reproducibility of the screening procedure were high and the toxicity was limited.

<table>
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Figure 3 Inhibition of the Vacuolar ATPase decreases cell viability in FA-HNSCC

(A) Knockdown of four subunits of the Vacuolar ATPase reduced cell viability of VU-SCC-1131 more than that of VU-SCC-1131+FANCC. (B) Average of normalized values of all subunits of the Vacuolar ATPase after knockdown in VU-SCC-1131 and VU-SCC-1131+FANCC. (C) Inhibition of the Vacuolar ATPase with Bafilomycin A1 in wild-type primary (BeBu and VU1131 fibroblasts) and SV40-immortalized fibroblasts (FEN5280SV), VU-SCC-1131 and the corrected cell line VU-SCC-1131+FANCC.
Top twelve FA-specific lethal siRNAs

By using tumor cells with either a defective or corrected FA pathway, two sets of essential genes might be found: one set of genes, of which knockdown is specifically lethal in tumor cells regardless of a functional FA pathway (tumor-specific), and a second set of genes, of which knockdown is only lethal in combination with the FA defect (FA-specific). Knockdown of some genes might turn out to be lethal in FA-deficient tumor cells but not in FA-deficient normal cells, making these genes excellent targets for novel therapies to treat tumors in FA patients.

Firstly, we addressed whether FA-specific hits could be identified by comparing lethal siRNAs in VU-SCC-1131 and VU-SCC-1131+FANCC. The cutoff of Z-score set at <-2.75 yielded 312 and 253 siRNAs that substantially reduced cell viability in VU-SCC-1131 cells or VU-SCC-1131+FANCC cells, respectively (Fig. 1C and Supplementary table 1). Interestingly, more lethal hits were found in VU-SCC-1131 compared to the corrected cell line (Fig. 1C).

Secondly, we used 3 linear regression models (see material and methods) to find siRNAs that displayed different lethality in the FA-deficient and FA-corrected cell line. The top twelve genes that showed the largest difference between the two cell lines are shown in Fig. 2. Although not much is known of most of these genes, some (RBBP9, RPL29 and PSMD11) have been implicated in carcinogenesis previously16–21 and might be promising targets in the development of anticancer drugs for FA-deficient tumors. In addition, the proteins encoded by 3 of the 12 top genes are part of the proteasome (PSMC1, PSMB2 and PSMD11), suggesting that proteasome inhibition by small molecule inhibitors such as bortezomib or carfilzomib might also be an effective strategy in the treatment of FA-deficient tumors.

Inhibition of the vacuolar-ATPase is a promising target in the treatment of FA-HNSCC

By comparing VU-SCC-1131 and VU-SCC-1131+FANCC we also noticed a difference in cell viability after knockdown of several subunits of the vacuolar-ATPase (V-ATPase). SiRNA-mediated depletion of ATP6V0A1, ATP6V1A, ATP6V1C1 and ATP6V1E1 resulted in decreased cell viability in VU-SCC-1131 compared to the corrected cell line (Fig. 3A and B). The multi-subunit V-ATPase functions as an ATP-dependent proton pump that is primarily involved in the acidification of intracellular compartments and extracellular environment, thereby regulating pH homeostasis22. Noteworthy, expression of the V-ATPase is upregulated in several cancers23–24. Since acidic pH alterations caused by high metabolic rates or altered metabolism of cancer cells favor cell proliferation, drug resistance and metastasis progression, interference with pH regulation has been suggested as an anti-cancer strategy25–31. Therefore, we tested whether FA-deficient tumor cells were more sensitive to the V-ATPase inhibitor Bafilomycin A1 than FA corrected cells. The difference in sensitivity in
terms of growth inhibition was small between the FA-deficient and FA-corrected cells. Whether this relates to drug specificity remains unclear, but the difference in sensitivity between tumor cells and normal fibroblasts derived from the same patient, was much larger (Fig. 3C). This suggests that inhibition of the V-ATPase might be beneficial in the treatment of HNSCC regardless of the FA defect.

The spindle assembly checkpoint is a potential therapeutic target for cancer therapy in FA patients

Based on Z-scores, many siRNAs (234) were lethal in both FA-deficient and corrected cells. Cluster analysis using the DAVID Functional annotation tool was performed on these hits (Z-score < -2.75) with the exception of the 15 genes that are now annotated as pseudogenes. This analysis revealed multiple clusters, of which the top ten with gene ontology enrichment scores above 2.67 are indicated in Fig. 4 and Supplementary table 2. The largest five clusters contained genes involved in 1) (m)RNA processing and splicing, 2) regulation of ubiquitination and proteasome proteolysis, 3) ribonucleoprotein and ribosome biogenesis, 4) macromolecular complex assembly and 5) mitosis. In particular the fifth cluster of genes involved in mitosis attracted our attention as regulators of mitosis have been proposed as candidate drug targets for antitumor therapies before. Antimitotic agents, such as taxol, have indeed been in clinical use for many years, including in neoadjuvant protocols for head and neck cancer, and therefore these hits were analyzed in more detail.

The fifth cluster harbors genes encoding proteins involved in the G2/M phase transition (e.g. WEE1 and CDK1) and (regulation of) mitotic spindle organization/assembly (e.g. NDC80, TPX2, KIF11 and CKAP5). In addition, the fifth cluster contained genes encoding spindle assembly checkpoint (SAC) proteins (e.g. BUB1B, BUB3 and MAD2) and chromosomal passenger complex (CPC) proteins (e.g. BIRC5, CDCA8 and INCENP), which are two complexes with an important role in the correct segregation of duplicated chromosomes during mitosis32,33 (Supplementary Fig. 1). In an independent experiment, we transfected VU-SCC-1131 and VU-SCC-1131+FANCC cells with siRNA SMARTpools targeting the six key components of the SAC: BUB1, BUB1B, BUB3, MAD1, MAD2 and TTK (Fig. 5A). Knockdown of all components except MAD1 resulted in over 50% reduction in cell viability in both cell lines. Inhibition of BUB3 and MAD2, which also had the lowest Z-scores, resulted in more than 80% cell death (Fig. 5B). Although BUB1 and TTK were not scored as hits in the primary genome-wide siRNA screens, knockdown of BUB1 or TTK markedly reduced cell viability in the validation experiment (Fig. 5B). Since TTK already has been suggested to be a candidate drug target for anticancer therapies and several TTK inhibitors have been synthesized, we analyzed this hit in more detail. TTK (also known as MPS1) is a dual specificity protein kinase and besides a key component of...
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the SAC, it is also involved in regulating the CPC by phosphorylating CDCA8 (ref 34). Overexpression of TTK has been reported in several tumor types\textsuperscript{35–38} and knockdown of this protein can lead to accelerated mitosis through checkpoint abrogation, followed by apoptosis specifically in cancer cells\textsuperscript{39}. Knockdown of TTK protein levels in TTK siRNA SMARTpool transfected cells was confirmed by western blotting (Fig. 5C). Deconvolution of the TTK siRNA smartpool, in which the four siRNAs that make up the pool were tested separately, resulted in > 40% cell death for all four siRNAs in both cell lines (Fig. 5D). This shows that TTK expression is essential in these tumor cell lines, regardless of FA status. To further explore TTK as a promising drug target, we tested two commercially available TTK inhibitors: Reversine and AZ3146. When treated with Reversine, primary fibroblasts (VU1131 fibroblasts) from the same FA patient from whom VU-SCC-1131 was derived, showed a very mild growth inhibition of approximately 10% compared to untreated cells (Fig. 5E). Similar results were obtained for other primary fibroblasts (BeBu) or for SV40-immortalized wild type fibroblasts (FEN5280SV). In contrast, tumor cell lines (VU-SCC-1131, VU-SCC-1365 and VU-SCC-1604) established from head and neck tumors from FA patients were more sensitive to Reversine treatment compared to wild type cells. Growth of these cell lines was reduced by approximately 60% to 90% (Fig. 5E). Previously, these three FA-HNSCC cell lines were functionally corrected for their FA defect\textsuperscript{40}. These corrected cell lines were also tested for Reversine sensitivity and only VU-SCC-1604 FANCL-corrected cells were less sensitive to Reversine compared to uncorrected cells. Primary wild type fibroblasts (BeBu) and FA-HNSCC cell lines were also tested

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**Figure 4** Cluster analysis of lethal hits in FA and FA-corrected HNSCC cell lines
Cluster analysis of lethal hits (Z-score below -2.75) in VU-SCC-1131 as well as VU-SCC-1131+FANCC. Using DAVID Functional annotation tool revealed multiple clusters, of which the top ten with gene ontology enrichment scores above 2.67 are shown.
Figure 5 Inhibition of the spindle assembly checkpoint decreases cell viability in HNSCC

(A) SiRNA SMARTpools targeting subunits of the spindle assembly checkpoint decreased cell viability in VU-SCC-1131 and VU-SCC-1131+FANCC. Cell viability was measured in triplicate and calculated relative to non-targeting transfected cells. (B) Z-score values of SAC proteins from the genome-wide siRNA screen.

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for another TTK-inhibitor: AZ3146. Again, FA-HNSCC cell lines were more sensitive to AZ3146 than wild type fibroblasts (Fig. 5F). It should be noted though that the FA-HNSCC cell lines divided faster than the wild-type and FA fibroblasts. Rather than their tumor background per se, this might explain their higher sensitivity to Reversine and AZ3146 (Fig. 5G). More research, preferably by using mouse models, is required to establish the potency of TTK inhibition in treatment of sporadic and FA-HNSCC.

The nuclear pore complex as candidate drug target in FA-HNSCC

To identify more genes that are essential for the survival of HNSCC tumors regardless of an FA defect, we also analyzed the sixth cluster, which contained genes involved in (m)RNA and protein transport (Fig. 4 and Supplementary Fig. 2). Many of these genes encode proteins that make up the nuclear pore complex (NPC). The NPC is the largest multiprotein complex in eukaryotic cells and its best-known and probably primary function is directing the transport of RNAs and proteins across the nuclear envelope. However, other functions, such as the regulation of genome organization, gene expression, mitosis and DNA repair, have been reported as well41–43. The NPC is built from a small number of proteins called nucleoporins (Nups), of which some (e.g. NUP88, NUP98, NUP214 and NUP358) have been implicated in cancer44–46. We selected 13 hits from the 6th cluster that are components of the NPC and 3 additional NPC genes with Z-scores above -2.75. For each gene, we tested the 4 separate siRNAs that composed the siRNA SMARTpools used in the screens, by transfecting VU-SCC-1131 and the corrected cells (Fig. 6A and B). Inhibition of 15 and 13 of the 16 selected genes in VU-SCC-1131 and VU-SCC-1131+FANCC, respectively, reduced cell viability by more than 50% with at least 2 of 4 siRNAs (Fig. 6A-C). Interestingly, knockdown of two genes (AHCTF1, also known as ELYS, and NUP35) with at least two separate siRNAs reduced cell viability in VU-SCC-1131 cells more than in the corrected cell line (VU-SCC-1131+FANCC). For 3 other genes (SEH1L, NUP107 and NUP155) the difference in viability between the two cell lines was even larger (Fig. 6D-F). Whereas our initial analysis did not reveal differential effects of knockdown of NCP genes, deconvolution of the SMARTpools targeting these genes showed that for at least 2 of 4 siRNAs VU-SCC-1131 cells were approximately 2-fold more sensitive to knockdown than VU-SCC-1131+FANCC cells (Fig. 6A-B).
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**Figure A**

Normalized values for genes AHCTF1, NUP35, NUP54, NUP62, NUP88, NUP93, NUP98, NUP107.

**Figure B**

Normalized values for genes NUP133, NUP153, NUP155, NUP160, NUP205, NUPL1, SEC13, SEH1L.

**Table C**

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**Figure D**

Normalized values for SEH1L.

**Figure E**

Normalized values for NUP107.

**Figure F**

Normalized values for NUP155.
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Although knockdown of these genes should be confirmed by RT-PCR and/or western blotting and more FA-HNSCC cell lines should be tested, it seems that the NPC is essential for the survival of FA-defective HNSCC tumor cells.

**Conclusion**

The larger number of lethal hits in the FA-deficient cell line VU-SSC-1131 compared to the corrected cell line suggests the existence of compensatory pathways that are essential for the survival of FA-defective tumor cells. Subsequent analyses revealed two classes of synthetic lethal interactions. The first class is tumor specific, but independent of FA status, and includes inhibition of the vacuolar ATPase and the spindle assembly checkpoint. This class may provide a cancer treatment strategy in both sporadic and FA patients. The second class is specific for the FA defect in HNSCC and includes inhibition of several nucleoporins and the proteasome. This class may be particularly interesting for the small subset of sporadic tumors with defects in the FA pathway.

**Materials and methods**

**Cell culture**

Primary (BeBu and VU1131 fibroblasts) and SV40-immortalized wild-type fibroblasts (FEN5280SV), VU-SSC-1131 (FANCC-deficient HNSCC cell line), VU-SSC-1365 (FANCA-deficient HNSCC cell line), VU-SSC-1604 (FANCL-deficient cell line) as well as the corresponding genetically corrected cell lines VU-SSC-1131+FANCC, VU-SSC-1365+FANCA and VU-SSC-1604+FANCL were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 1 mM sodium pyruvate (Gibco). FA-HNSCC cell lines were established as described previously.

**High throughput siRNA screens**

VU-SSC-1131 and VU-SSC-1131+FANCC cells were subjected to a genome-wide siRNA screen by reverse transfection in 384-well plates. Of in total 21,121 siRNA SMARTpools derived from the siARRAY Humane Genome library (Catalog items G-003500 (Sept05), G-003600 (Sept05), G-004600 (Sept05), and G-005000 (Oct05); Dharmacon, Thermo Fisher Scientific), 25 nmols were put in individual wells by using the Sciclone ALH 3000 workstation (Caliper LifeSciences) and a Twister II microplate handler (Caliper LifeSciences). The non-targeting siCONTROL #2 (siCON) and the PLK1 siGENOME SMARTpool were used as a negative and positive control, respectively, and manually added to 8 different wells on each plate. RNAiMAX (Invitrogen) at a final concentration of 0.01 μl/well was added to the siRNAs by using the Sciclone ALH 3000 workstation (Caliper LifeSciences) and a Twister II microplate handler (Caliper LifeSciences). The non-targeting siCONTROL #2 (siCON) and the PLK1 siGENOME SMARTpool were used as a negative and positive control, respectively, and manually added to 8 different wells on each plate. RNAiMAX (Invitrogen) at a final concentration of 0.01 μl/well was added to the siRNAs by using a Multidrop Combi (Thermo Fisher Scientific). Subsequently, cells were seeded using a μFill microplate dispenser (BioTek). Plates were incubated for 5 days at 37°C and 5% CO2. After 5 days, cell viability was determined by adding CellTiter-Blue reagent (Promega) using a Multidrop Combi (Thermo Fisher Scientific). Two hours later fluorescence was analyzed at 540 nm excitation and 590 nm emission wavelength using an Infinite F200 microplate reader (Tecan Group Ltd). Deconvolution of siRNA SMARTpools targeting TTK
and NUP genes was performed by using the same automated procedure as described above. The Z’ factor was used to assess screen quality and was calculated for each screen with the formula:

\[
Z' = 1 - \frac{(3\sigma_{\text{positive control}} + 3\sigma_{\text{negative control}})}{|\mu_{\text{positive control}} - \mu_{\text{negative control}}|}
\]

After measuring fluorescence using CellTiter Blue reagent (living cells convert resazurin (redox dye) into resorufin (fluorescent end product)), the obtained raw viability values (fluorescent signals) were re-scaled by first log2-transformation and subsequently corrected for an overall plate effect across the cell lines by using a linear regression model. These values were used to calculate Z-scores or were used together with 3 linear regression models to find siRNAs that caused a difference in cell viability between the FA-deficient and FA-corrected cells: We used a linear regression model to find siRNAs that differed significantly in their viability from the negative (siCON) controls, per cell line. Specifically, we explain normalized cell viability values by a factor indicating the siRNA or the siCON, using a linear regression that is fitted per siRNA and per cell line. P-values corresponding to the siRNA effect were corrected for multiple testing using Benjamini & Hochberg’s step-up FDR procedure. Subsequently, we used a linear regression model with both cell line and siRNA vs siCON effect, as well as the interaction of these, to find siRNAs that displayed different lethality in the two cell lines. Here we extracted p-values corresponding to the interaction effect, which were subsequently corrected for multiple testing as with the previous model.

**Western blot analysis of TTK/MPS1 protein expression**

Whole-cell extracts were prepared in lysis buffer (50 mM TRIS (pH 7.5), 150 mM NaCl and 1% Triton X-100 supplemented with protease (complete EDTA free tablets, Roche) and phosphatase (PhosSTOP, Roche) inhibitors). Proteins were separated on a 3-8% Tris-Acetate NUPAGE gradient gel (Invitrogen) and transferred to Immobilon-P membrane overnight. After blocking with 5% dry milk in TBST (10 mM TRIS-HCl (pH 7.5), 150 mM NaCl, 0.05% Tween-20), the membrane was incubated with TTK/MPS1 antibody (1:1,000, A300-296A, Bethyl Laboratories), followed by washing with TBST and incubation with horseradish peroxidase-conjugated secondary antibodies to visualize protein bands with ECL (GE Healthcare). Mouse monoclonal anti-α tubulin (1:5,000, B-5-1-2, SC23948, Santa Cruz Biotechnologies) was used as a control to ensure loading of equal amounts of protein in each western blot lane.

**Drug treatments**

Cells were seeded 6-well plates with increasing concentrations of drug (Reversine: R3904, Sigma-Aldrich, AZ3146: SC_361114, Santa Cruz, and Bafilomycin A1: B1793, Sigma-Aldrich). After two weeks of incubation with the indicated drugs or earlier after untreated cells had made 3 population doublings, the relative cell number compared to untreated cells for each drug concentration was determined using a Coulter counter.

**Acknowledgements**

We thank Irsan E. Kooi for help with the cluster analysis using the DAVID Functional annotation tool.
Supplementary figure 1 STRING protein analysis of the gene ontology term mitosis (GO:0007067) belonging to the fifth cluster.
Supplementary figure 2 STRING protein analysis of gene ontology terms RNA and protein transport.
Supplementary table 1. SiRNAs identified by genome wide screening that caused decreased cell viability (Z-score < -2.75) in VU-SCC-1131 and/or VU-SCC-1131+FANCC

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**Annotation cluster 6 (enrichment score 4.756)**

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References

Genetic screens in head and neck cancer cell lines