Supporting Information

A functional cancer genomics screen identifies a druggable synthetic lethal interaction between *MSH3* and *PRKDC*

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SUPPLEMENTARY METHODS

Assessment of Apoptosis

Cells were seeded in 6cm dishes at 30% confluence. After 24 hr, medium was refreshed and cells were treated with KU60648 (0, 0.1, 0.5 or 1µM) for 24 - 96 hr. After treatment, cells were harvested by trypsinization, washed with ice-cold PBS, incubated with accutase solution (Sigma Aldrich) and finally re-suspended in antibody-binding buffer.

We used the annexin-V/FITC Apoptosis Detection Kit I (BD Biosciences) in order to stain cells for annexin-V, as well as propidium iodide (PI). After 20 minutes of incubation in the dark, samples were analyzed on a FACS Gallios Flow Cytometer (Beckman Coulter). We used FACS Kaluza software (Beckman Coulter), in order to quantify populations. Apoptosis was calculated as the fractional difference of annexin-V/PI double-positive population in treated and untreated samples. At least 100,000 events were assessed per measurement.

Cell cycle analysis

Cells were seeded at 30% confluence and exposed to KU60648 (1µM) for 0, 6, 12, 24, 48, 72 or 96 hr. Subsequently, cells were detached by incubation (37°C) with trypsin (Gibco Life Technologies) and washed with ice-cold PBS. A 70% ethanol / 15% accusase (Sigma Aldrich) mixture was used for cell fixation over night. The next day, samples were washed twice and resuspended in PBS. After incubation (20min, room temperature) with RNAse

A (1mg/ml, Qiagene), cells were stained with proidium iodide (0.05mg/ml, Sigma Aldrich) and analyzed on a FACS Gallios Flow Cytometer (Beckman Coulter). We used FACS Kaluza Analysis Software (Beckman Coulter) in order to quantify the distribution of the relative cellular DNA content, thereby inferring the cell cycle profile of each sample. Cell doublets were excluded from analysis by plotting the FL-3 signal integral against the signal peak. At least 40,000 events were measured for each sample.

Immunofluorescence

Cells were seeded on 18mm² coverslips and either left untreated or exposed to 1µM of etoposide for 1 hr. Subsequently, etoposide was washed out and cells were incubated for 4, 48 or 72 hr with or without 0.5µM of KU60648.

After treatment, cells were fixed in 3% PFA/ 2% succrose, incubated with cytoskeleton buffer (10mM PIPES pH 6.8, 100mM NaCl, 300mM sucrose, 3mM $MgCl_2$, 1mM EDTA, 0.5% Triton X-100) for 10 minutes on ice, followed by 10-minute incubation with cytoskeleton stripping buffer (10mM Tris-Hcl pH 7.4, 10mM NaCl, 3mM MgCl2, 1% Tween-40, 0.5% sodium deoxycholate) on ice. Cells were washed with ice-cold PBS and blocked in PBS+5%BSA+ 2% goat serum + 0,1% Triton X for 60 minutes at room temperature.

Cells were stained with RAD51 (1:500, ab63801, Abcam), RPA (1:200, #2267S, Cell Signaling Technology) and γ-H2AX (Ser139) (1:500, clone JBW301, 05-636, Millipore) antibodies at 4°C over night, and incubated with Alexa Fluor® 488 conjugated anti-rabbit and anti-mouse (both 1:600, Molecular Probes, Life Technologies) antibodies for 2 hr at room temperature. Cell nuclei were counterstained with 4',6'-diamidino-2-phenylindole (DAPI) and cells were mounted on slides in ProLong Gold Antifade Reagent (Invitrogen).

Finally, cells were imaged on an Axiovert 200M inverted fluorescence-imaging microscope equipped with a CCD camera (Zeiss). Zen 2012 Lite (Zeiss) software was used for image analysis. For quantification, at least 80 cells were counted in at least eight independent high power fields, equally distributed over the slide.

Retroviral gene delivery

Production of retrovirus was performed as described previously (50). In brief, amphotropic retrovirues were packaged in HEK293T cells by co-transfection with pMDg, pMDg/p as well as pMLP (control), V2HS 233593 (*PRKDC*-targeting shRNA), pBabe-HRAS^{G12V} or pBabe-MYC plasmids using Trans-IT LT1 transfection reagent (Mirus, USA). After 12 hr of transfection, cell medium was replaced and virus soup was harvested after 24 and 48 hrs.

Cells were transduced with virus-containing supernatants in the presence of 10µg/ml polybrene (Santa Cruz Biotechnology) for 24 hr and transfected cells were selected with puromycin (1-6µg/ml, Sigma Aldrich) for at least 48 hr.

Immunoblotting assays

For lysate preparation, cells were washed twice with ice-cold PBS and resuspended in lysis buffer (Cell Signaling) supplemented with protease (Roche) and phosphatase inhibitor (Calbiochem) cocktails. After 20 minutes of incubation, we centrifuged lysates at 14,000 rpm at 4°C for 20 minutes. We quantified their protein concentration using bicinchoninic acid for colorimetric protein detection (Pierce BCA Protein Assay Kit, Thermo Scientific), in order to resuspend equal amounts of protein (30µg) in Laemmli sample buffer. We separated samples on 4-12% SDS-PAGE gels (Novex, Life Technologies) and blotted them on nitrocellulose membranes (Amersham Hybond-C Extra). We used caspase-3 (#9662S), cyclin A2 (clone BF683, #4656) (both Cell Signaling Technology), γ-H2AX (Ser139) (clone JBW301, 05-636, Millipore), DNA-PKcs (18-2, ab1832, Abcam) and β-actin (clone C4, sc-47778, Santa Cruz Biotechnology) antibodies for protein detection by the ECL Western Blotting Detection Kit (GE Healthcare) on chemiluminescence films (Amersham Hyperfilm ECL, GE Healthcare).

LEGENDS TO SUPPLEMENTARY FIGURES AND TABLES

Figure S1. A. Potency (y-axis) of KU60648 across 94 cancer cell lines. Cell lines were classified into sensitive (green) and resistant (red) groups according to their half maximal growth inhibitory KU60648 concentration (GI_{50} threshold: 400nM). Cell lines without mutation annotation (CCLE database) are depicted in transparent colors. Histology representation in the screening cell line panel is shown as bar plot (inset). **B.** Calculation of the KU60648 sensitivity threshold. Compound concentrations (25 nM steps, x-axis) are plotted against the distance to their nearest neighbor in the KU60648 activity screening profile (y-axis). Significance values (Student's t-tests) of 10^{-k} are indicated by *k* blue concentric circles. **C.** Concentration-growth curves (Cell Titer Glo© assay) are shown for a validation panel comprising 7 $MSH3^{mut}$ (blue), 1 $BRCA1^{mut}$ (green), 1 Brd4Nut fused (red) as well as 18 control lines. Significance values (concentric circles) were derived from concentration-wise t-testing of the relative viability in $MSH3^{mut}$ vs. $MSH3^{wt}$ control groups.

Figure S2. Scatter blots illustrate comparison of KU60648-GI_{50} values in mutant (left) vs. wildtype (right) cell lines for 9 exemplary damage repair associated genes (*BRCA1*, *BRCA2*, *RAD50*, *CHEK2*, *FANCD2*, *ATM*, *PAXIP1*, *MLL3*, *MSH3*); vertical bars represent group averages. Frameshift (fs), nonsense (ns) and nucleotide repeat (rep) mutations in *ATM*, *MLL3* and *MSH3* are highlighted. Significance values were calculated by Fisher's exact test.

Figure S3. A. Schematic representation of the MSH3 protein (*MSH3* wt) as well as the two mutant variants (*MSH3* R1, R2) recurrently detected in cancer cell lines. Boxes indicate MLH1 interaction (orange) and ATP binding (red) domains; dots represent the poly- (brown) and mononucleotide (green) repeats in the *MSH3* gene. The *MSH3* variant R2 translates the K381fs mutation, frequently reported in colon cancer. **B, C.** Exemplary chromatograms derived from Sanger sequencing of *BRCA1* exons in H2347 (**B**) and H1563 (**C**) cell lines. Arrows indicate direction (5'->3') of the normal *BRCA1* cDNA sequence (blue) as well as confirmed sequence deviances

(magenta) detected in cell lines. **D.** Tile representation of Sanger sequencing results of 23 *BRCA1* exons in H1563, H2347, H1838 and H1568 lines.

Figure S4. Cell cycle analysis of nine cell lines (HCC2429 [Brd4Nut], HCC44, H1838, H1703, H2030 [$MSH3^{mut}$], H1563 [$BRCA1^{mut}$], H2347, HCC1359, H1915 [controls]) under 96-hour exposure to KU60648 (1μM). Cells were examined at 7 time points (0, 6, 12, 24, 48, 72, 96 hours); normalized S- (x-axis) and G_2/M- (y-axis) phases were determined by flow cytometry and compared (Δ_{rel}) to the initial cell cycle constitution (0 hrs).

Figure S5. A. Exemplary viability heat maps for H1838 (upper panel) and HCC1359 (lower panel) are shown after 48 hr exposure to combinations of KU60648 (x-axis) and doxorubicin (y-axis). Colors encode relative viability of 120 concentration combinations of both compounds. The observed viability (left panel), determined by CTG assays, was compared to the expected viability, assuming independent effects of both compounds (middle panel), in order to determine the synergistic effect (right panel) of each concentration combination (right panel). **B.** Apoptosis levels after 48 hr combination treatment were quantified for 3 *MSH3*-mutant lines (HCT116, HCC44, H1838, upper panel) and 3 KU60648-resistant controls (H1915, H1568, HCC1359, lower panel). Cells were exposed to five concentrations of doxorubicin (0, 30, 100, 250, 1000nM) with (orange) or without (blue) addition of KU60648 (1μM). Strongest synergistic combinations are highlighted by arrows.

Figure S6. HCC44 (left), HCC2429 (middle) and H1568 (right) cells after 120-hour transduction with either control or shDNA-PKc virus were analyzed for protein expression of DNA-PKc, cleaved Caspase 3 and β-actin by immunoblotting.

Figure S7. A. HCC44 [$MSH3^{mut}$] (left), and H1568 [cntrl] (right) cells after 120 hr transduction with either control or five shXRCC5 viruses were analyzed for protein expression of Ku80 and β-actin by immunoblotting. **B.** Representative morphology (100x magnification) of HCC44 [$MSH3^{mut}$], HCT116 [$MSH3^{mut}$], HCC1359 [cntrl] and H1568 [cntrl] cells two weeks after transduction with

viruses encoding either control or XRCC5 targeting shRNA. **C.** Quantification of relative viability two weeks after viral transduction.

Figure S8. A. Genotyping of knockout ($MSH3^{-/-}$) and control ($MSH3^{wt/wt}$) murine embryonic fibroblasts (MEFs). Primer pair AB amplifies the MSH3 null allele, whereas pair AC is specific for the wildtype allele. **B.** Concentration-growth curves (Cell Titer Glo© assay) are shown for knockout ($MSH3^{-/-}$) and control ($MSH3^{wt/wt}$) MEFs. Significance was derived from t-testing of the relative viability in the $MSH3^{-/-}$ (red) against the $MSH3^{wt/wt}$ (blue) control group. **C.** $MSH3^{-/-}$ (left) and $MSH3^{wt/wt}$ (red) MEFs were analyzed for protein expression of cleaved Caspase 3 and β-actin by immunoblotting after 48-hour exposure to KU60648 (0, 0.1 and 1μM).

Figure S9. A. Double strand break repair signaling was monitored (0hrs, 4hrs, 72hrs) after short (1hr) expose to high-dose (1μM) pulses of etoposide. Representative immunofluorescence images (green: γ-H2AX and Rad51 foci, blue: DAPI) are shown for HCC2429 [Brd4Nut], H1703 [$MSH3^{mut}$] and HCC1359 [ctrl] cancer cell lines. **B.** Double strand break repair signaling was monitored (4hrs, 72hrs) after short (1hr) expose to high-dose (1μM) pulses of etoposide and permanent DNA-PKC inhibition (1μM KU60648). Representative immunofluorescence images (green: γ-H2AX, Rad51 and RPA1 foci, blue: DAPI) are shown for HCC2429 [Brd4Nut], H1703 [$MSH3^{mut}$] and HCC1359 [ctrl] cancer cell lines.

Figure S10. A, B, C, D Waterfall plots display relative changes of individual tumor volumes after 11-day treatment with KU60648 (red, 40 mg/kg b.i.d.) and vehicle solution (blue control), respectively, in nude mice engrafted with HCT116 (**A**), A549 (**B**), MEF (MYC/RAS transduced) $Msh3^{-/-}$ (**C**) and control MEF (MYC/RAS transduced) $Msh3^{wt/wt}$ (**D**) tumors. Constant levels (100%) are marked (green dashed line). **E, F** Representative images of nude mice engrafted with HCT116 [$MSH3^{mut}$] and A549 [$KRAS^{mut}$] tumors after 11-day treatment with KU60648 (40 mg/kg b.i.d.) and vehicle solution (control), respectively. Subcutaneous tumors are encircled (yellow dashed lines).

Table S1. List of all cell lines included in this study. Cell line name (green), GI_{50} of KU60648 from initial cell line screen (red), sensitivity classification (magenta) as well as mutation annotation source (blue) are recorded.