SUPPLEMENTARY DATA

Supplementary Figure 1. Identification and validation of the genes supporting drug resistance by shRNA-mediated phenotype screen. (A) Diagram explaining how the phenotype screen was performed. (B) Diagram explaining how the validation phase of the screen was performed. (C) HCT116p53KO cells singularly infected with viral stocks of each shRNA plasmid recovered in the screen and puromycin selected were treated 72hs with 200 μM 5FU before counting. Each batch of infected cells is labeled as KO53pRS followed by the name of the silenced gene. In parallel, as a positive control, also empty vector-infected HCT116 (WTPRS) have been treated. A representative experiment out of 3 is shown. (D) An aliquot of each batch on infected cells from (C) was used for colony assay performed as described in Materials and Methods. A representative experiment out of 5 is shown.

Supplementary Figure 2. GSK3B silencing by siRNA abolishes drug resistance of p53-null colon carcinoma cell lines. siRNA transfection was performed using 100nM commercial oligos for the indicated genes; oligos targeting luciferase (luc) were used as a control. 24 hs after transfection medium was replaced with complete medium containing 200μM 5FU and cells were harvested 48hs later for analyzing GSK3B levels by western blot and counting dead cells after Trypan blue staining.

Supplementary Figure 3. GSK3B depletion do not alter the response of HCT116p53KO resistant cells to targeted drugs. Cell death 72 hrs after treatment of HCT116p53KO-pRS and –pRSGSK3B with 10 μg/ml cetuximab, 75 μg/ml panitumumab, 25 μg/ml bevacizumab was evaluated by Trypan blue staining.

Supplementary Figure 4. Caspases are not activated after 5FU treatment in GSK3B-silenced cells. HCT116p53KO-pRS and HCT116p53KO-pRSGSK3B were treated for the indicated times with 200 M 5FU and total cell lysates blotted with antibodies recognizing: (A) total and cleaved form of caspase-9 and -8; (B) total and cleaved form of caspase-2; Notably no (or barely visible) processing of the caspase enzymes is evident. Actin and vinculin levels served as loading controls. Solid arrows indicate active caspase
cleaved fragment; white arrows indicate pro-caspase; asterisks indicate intermediate fragments derived from proteolytic processing of the caspases

Supplementary Figure 5. tAIF is released from mitochondria upon 5FU treatment in GSK3B-silenced HCT116p53KO cells. (A) mitochondrial and cytoplasmic fractions from untreated and 5FU-treated HCT116p53KO-pRS and –pRSGSK3B cells were probed with anti-AIF and anti-cytochrome C antibodies to assess the release of mitochondrial proteins in the cytoplasm; uncleaved (AIF) and truncated (tAIF) derived from proteolytic processing are indicated; anti-porin and anti-actin were used to assess the loading and purity of the mitochondrial and cytoplasmic fractions, respectively. (B) siRNA transfection in HCT116p53KO-pRSGSK3B was performed using custom-made oligos for AIF. 24hs after transfection medium was replaced with complete medium containing 200μM 5FU and cells were harvested 72hs later for analyzing AIF levels by western blot and counting dead cells after Trypan blue staining.

Supplementary Figure 6. GSK3B is activated in colon carcinoma samples. (A) Representative sections of colon carcinoma (upper) and non-neoplastic pathology i.e. diverticulosis (lower) stained with anti-pTyr216-GSK3B antibodies. Representative sections of 10 sample pairs from colon carcinoma patients (upper: colon cancer; lower: peri-tumoral colon) stained with anti-pTyr216-GSK3B antibodies

Supplementary Table 1. Known genetic alterations characterizing the different colon carcinoma cell lines used in the paper. Information about genetic defects were retrieved from the database of the Wellcome Trust Sanger Institute Catalogue Of Somatic Mutations In Cancer (COSMIC, http://www.sanger.ac.uk/genetics/CGP/cosmic).

Supplementary Table 2. Cohort of patients characterization
Expression of MHL1, p53, MDM2, p21, pTyr216-GSK3B were studied by IHC staining on TMA. In the table are also shown: age, sex, overall survival, disease-free survival, tumor grade, and clinical outcome (relapse). Sample triplicates arrayed in tissue microarray were stained with specific antibodies and graded accordingly to an increasing intensity by blind reading by two experienced operators.
Supplementary Table 3. Correlation between activated GSK3B and patients outcome. The cohort of 50 patient was analyzed for the expression of active GSK3B, expressed as immuno-histochemically pTyr216 positive, in correlation with the relapse of the tumor disease. pTyr216-GSK3B was significantly higher in samples from patients that relapsed after 5FU treatment than in patient that responded to therapy

* 48.7% vs 63.6% P(two-sided) = 0.002599 McNemar’s test
OR = 0.2105; 95% CI (0.05209, 0.6335)

Supplementary Materials and Methods

Materials. Cetuximab (Erbitux, Ely Lilly), panitumumab (Vectibx, Amgen), bevacizumab (Avastin, Genentech) were from San Gerardo Hospital, Monza . SB216763, SB415286, were from Sigma-Aldrich. anti-p53 (DO-1, sc-126), anti-caspase-9 sc-56073, anti-caspase-8 sc-70503 were from SantaCruz Biotechnology; anti-p21 (clone EA10) and anti-MDM2 (clone IF2) were from Merck Chemicals; anti-caspase-2 was a kind gift of Prof. Claudio Brancolini (Dept Medical and Biological Sciences, University of Udine, Italy)

Reporter assay. 5 x 10^4 cells/well in a 96-well plate were seeded in triplicate the day before transfection. 0.2 μg TopFlash (containing two sets of three copies of the TCF binding site upstream of the Thymidine Kinase minimal promoter and Luciferase open reading frame) + 0.2 μg pGL4.75 (encoding for Renilla luciferase, used as an internal control for transfection efficiency) reporters were transfected in each well using Lipofectamine2000 following the manufacturer’s protocol. 48 hrs later cells were washed, lysed and assayed for Luciferase signals directly in the well by using the Dual- Glo Luciferase Assay System (Promega), according manufacturer’s instructions. Firefly luciferase intensity was normalized over Renilla luciferase signal.

siRNA transfection. Transient siRNA transfection were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Commercial siRNAs targeting GSK3B sequence GCTAGATCACTGTAACATA (#4390824 Ambion Applied Biosystems covering nt 1292-1310); GSK3A (#S100288554 Qiagen); luc (Luciferase GL2 Eurofins MWG Operon); siRNA AIF: AUGUCACAAAAGACACUGCA were used.

Fractionation. Cells were washed once with cold PBS buffer before lysis and pellet resuspended in 900 I fractionation buffer (10 mM Hepes, 250 mM sucrose, 1mM EDTA, 1mM EGTA, 1mM DTT,1% PIC) and left on ice 1h. The suspension was then passed
3x10 times through a 25g needle and then 3x10 times through a 27g needle and centrifuged using a microcentrifuge at 4000 rpm 4°C, 10 min. The pellets (formed by unbroken cells, nuclei, high molecular weight membranes) were discarded and the supernatants centrifuged using a microcentrifuge at maximum speed, 4°C, 20 min. Supernatants (cytoplasms) were saved and freeze as such. Pellets (mitochondria) were resuspended in 65 μl RIPA buffer + inhibitors and freeze until further processing.