Supplementary Data

Figure S1

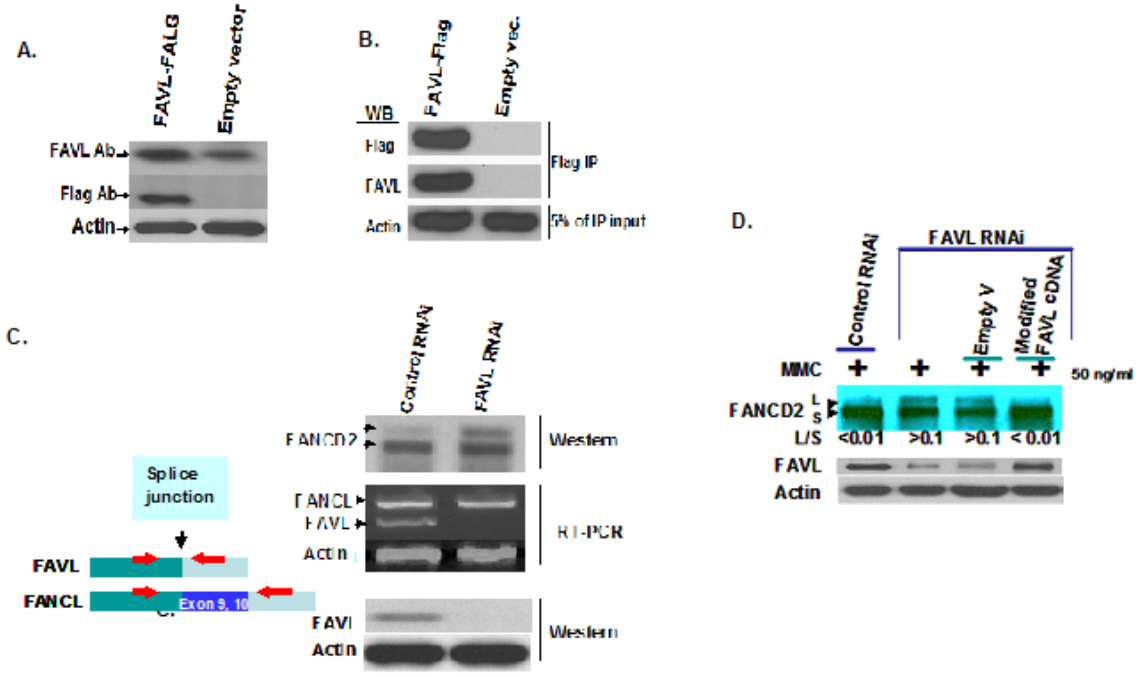


Figure S1. FAVL Antibody and FAVL RNAi oligos

Anti-FAVL Antibodies Recognize Both Endogenous and Exogenous FAVL. Analysis of the FAVL coding sequence revealed the presence of 13 amino acids (AAs) that differ between FANCL and FAVL owing to the fusion of FANCL exon 12 with exon 9 during splicing. We synthesized oligos encoding these 13 AAs coupled with EcoRI and SalI restriction enzyme sites at ends of the oligos, respectively, and ligated annealed oligos into the pGEX-4t-1 vector. Next, we followed standard procedures for expressing and purifying GST-fusion proteins and subsequently sent the fusion protein to COCALICO (Pennsylvania) for antibody production. FAVL antibody was affinity purified by MBP-FAVL-conjugated beads. MBP-FAVL fusion protein was similarly prepared as the GST-FAVL fusion protein.

(S1A) Lysates from Calu-6 cells expressing Flag-tagged FAVL, or with transfected-empty vector, were analyzed by Western blotting using the anti-FAVL antibody. A band of similar size is detected in cells expressing Flag-tagged FAVL as well as cells transfected with empty vector, indicating the anti-FAVL antibody detects exogenous and endogenous FAVL. In contrast, anti-Flag antibody only detects Flag-tagged FAVL.

(S1B) Flag-immunoprecipitation (IP) was performed by using the same lysates used above. FAVL only can be detected in the elutate of the IP pellet prepared from Flag-

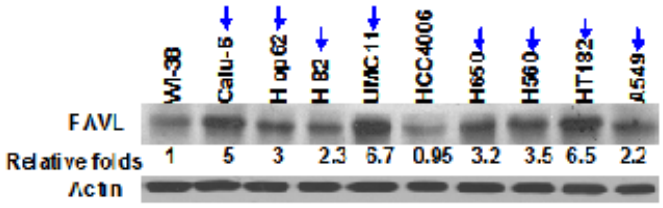
FAVL transfected cells but not from cells containing empty vector. The same blot was stripped and blotted with Flag antibody, and Flag was detected at the place where FAVL was detected. 5% of IP input was analyzed for β-actin expression to indicate the same amount of lysate used for IP.

(S1C). Specificity of FAVL antibody and FAVL RNAi oligos. Calu-6 cells were transfected with non-specific RNAi oligos, as a control, or FAVL RNAi oligos specifically targeting FAVL. Subsequently, the transfected cells were treated with 100 ng/ml MMC 24 hours after transfection for 16 hours. Lysate from half of these cells was analyzed by Western blot using antibodies against FANCD2, FAVL and β−actin, and the other half was used to isolate total RNA for performing RT-PCR (PCR primers as illustrated in the left). Monoubiquitinated FANCD2 is relatively increased in cells treated with RNAi oligos targeting FAVL as compared with cells treated with control RNAi oligos. In addition, FAVL expression levels, but not FANCL, are reduced in FAVL RNAi-transfected cells but not in cells treated with control RNAi oligos, and FAVL protein was also downregulated accordingly. These results suggest that FAVL RNAi oligos and FAVL antibody are specifically aimed at expected targets, and that silencing FAVL can partially restore activation of the FA pathway.

(S1D). FAVL RNAi oligos have no off-target effect. Calu-6 cells in one of 4 60 mm dishes were transfected with the combination of FAVL RNAi oligos and modified FAVL cDNA, which has the RNAi-targeting region containing modified nucleotide sequence encoding the same amino acids. The cells in another dish were transfected with the combination of FAVL RNAi oligos and empty vector control. The cells in the rest of 2 dishes were transfected with FAVL RNAi oligos or control RNAi oligos. 24 hr after transfection, cells in all 4 dishes were treated with 50 ng/ml MMC overnight. FANCD2, FAVL, and Actin were detected through Western blotting of lysates prepared from these cells. The compromised FANCD2 monoubiquitination detected in cells with control RNAi oligos as well as in cells transfected with the combination of FAVL RNAi oligos and modified FAVL cDNA indicates that FAVL RNAi oligos have no off-target effect. (L-monoubiquitinated FANCD2; S – unmonoubiquitinated FANCD2; L/S ratio was determined by the western band density generated through NIH image J program.)

Figure S2 FAVL Expression in Cancer Cell Lines and Cancer Tissues Sample

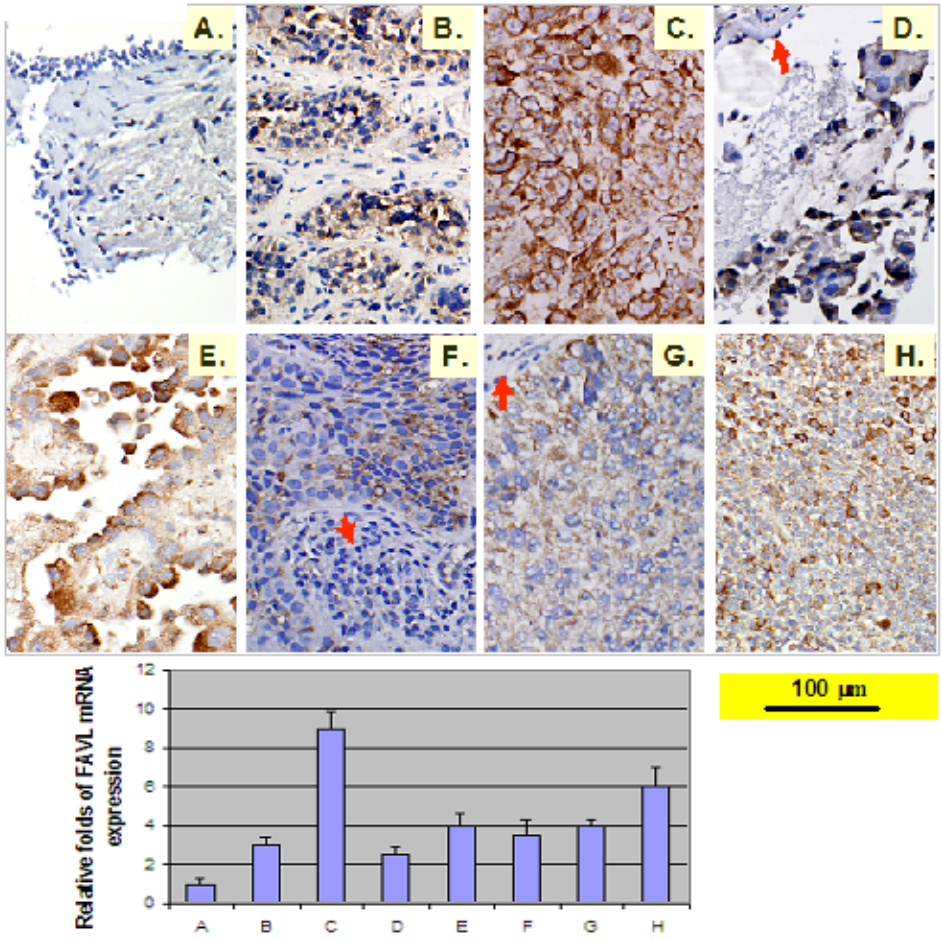
Figure S2A



(S2A). FAVL Is Highly Expressed in Lung Cancer Cell Lines Tested.

Total cell lysate of WI-38 cells (derived from fetus lung tissue) and cell lysates of 9 lung cancer cell lines were used to examine FAVL protein expression. Among these 9 lung cancer cell lines, eight cell lines express high levels of FAVL protein (marked with blue arrowheads) and the remain one expresses a similar level of FAVL protein compared with "normal" lung cells (Beas2B) found in the previous Western blotting analysis (Figure 2A). As compared with WI-38 cells, FAVL is also expressed at high levels in 8 of 9 lung cancer cell lines and a similar level to that in HCC4006 cells, consistent with the finding in previous study (Figure 2A) (The relative folds of FAVL expression were generated based on values of the gray density of Western bands standardized by levels of β-actin expression through the NIH image J program).

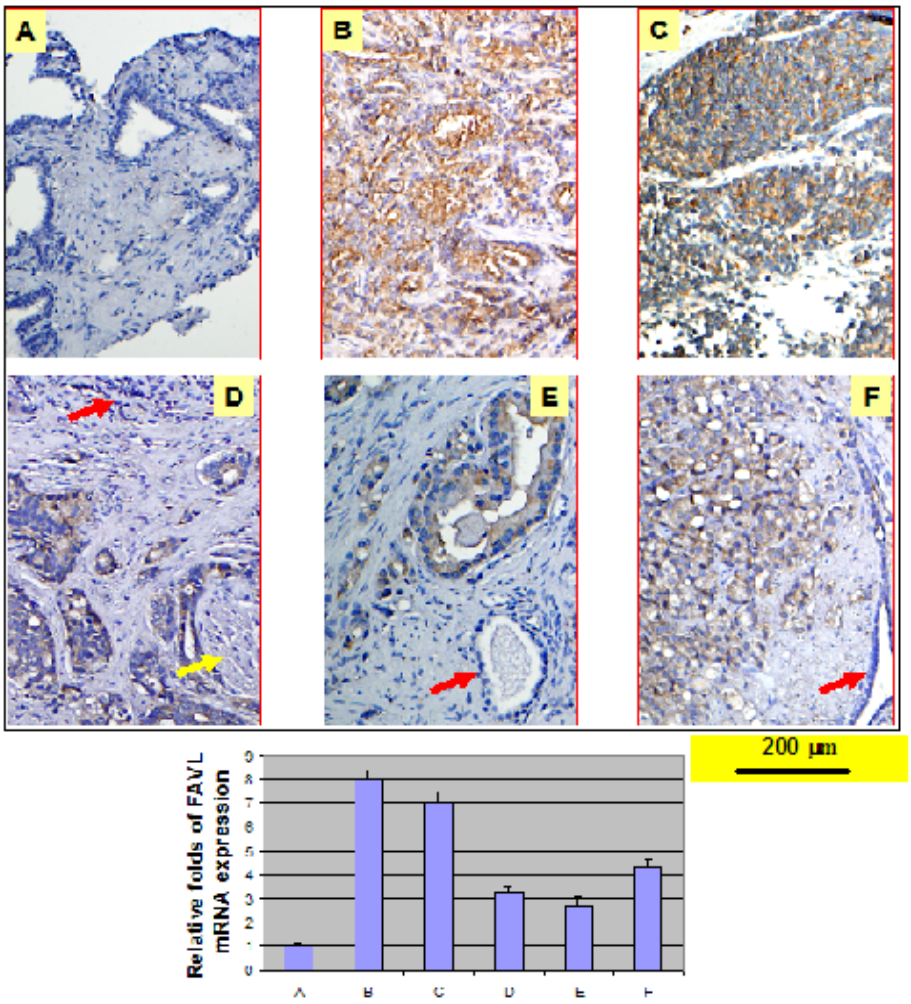
Figure S2B-1



(S2B-1). FAVL Staining Is Positive In Nearly Half of Lung Carcinomas Cases Tested.

Carcinoma cells were positive for FAVL staining, showing focal and/or diffuse cytoplasmic staining on 13 of 25 tested slides, which represented 25 lung carcinoma cases covering most types of lung cancer. All 5 tested non-malignant lung tissue samples were negative for FAVL staining, as indicated by the representative image A**.** Cancer types shown include: carcinoid in image B; adenocarcinoma in images C and D; bronchioloalveolar carcinoma in image E; squamous carcinoma in images F and G; and small cell lung cancer in image H. The selective expression of FAVL in carcinoma cells can be observed in the images D, F, and G with red arrows marking the normal gland in image D, and normal lymphocytes in image F and G. The bottom panel shows relative levels of FAVL mRNA in lung cancer tissue samples presented through using Quantitative RT-PCR (TaqMan assay) (Ratio average ± SEM with n=3).

Figure S2B-2



(S2B-2). FAVL Protein Elevation Was Found in More Than Half of Prostate Cancer Tissue Samples Tested.

IHC was conducted on 48 paraffin-embedded prostate tissue slides by using FAVL antibody. Among these slides, 5 slides are made from non-malignant tissue, and 43 slides were from prostate carcinomas. All non-malignant tissue samples are negative for FAVL staining (as shown in image A). Carcinoma cells are positive for FAVL showing focal or/and diffuse cytoplasmic staining on 30 of 43 tested slides, representing 43 carcinoma cases with Gleason score ranging from 6 to 10. Images B and C are representative ones of FAVL-staining in tumors with Gleason score 6 and 10, respectively. Images D, E, and F are images to indicate the staining of FAVL protein only in cancer cells. The yellow arrow marks typical perineural invasion (image D). Red arrows mark normal lymphocytes (image D) and normal glands (images E and F). TaqMan assay was also performed for FAVL mRNA expression, and results were consistent with those from IHC (Relative expression ratio average ± SEM with n=3).

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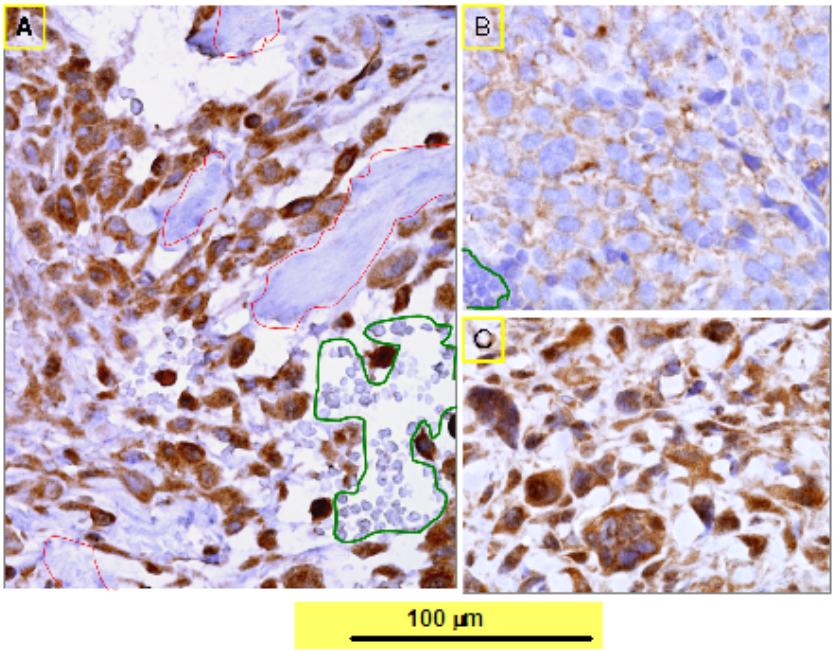
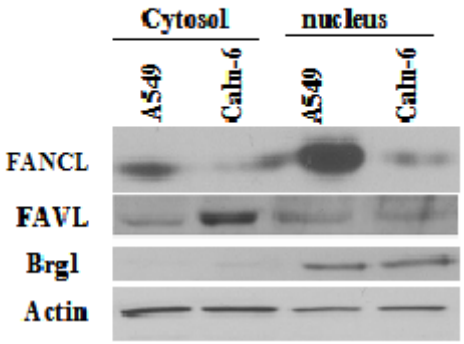
Figure S2B-3



(S2B-3). FAVL is Expressed in Osteosarcoma.

13 osteosarcoma tissue samples (paraffin embedded) were collected and detected for FAVL expression by IHC. Focal and/or diffused FAVL staining is positive in all 13 tissue samples tested, among which three samples display the intensity of staining similar to panel B, and the rest appears showing the staining density similar to panel A with osteoid or C without osteoid. [Negative FAVL stain is marked with the red line for osteoid-bone structure, and the green line for blood cells (panel A) and lymphocytes (panel B)]. Through TaqMan assay, levels of FAVL mRNA found in these osteosarcoma samples are consistent with FAVL protein levels detected by IHC (data not shown).

Figure S2C

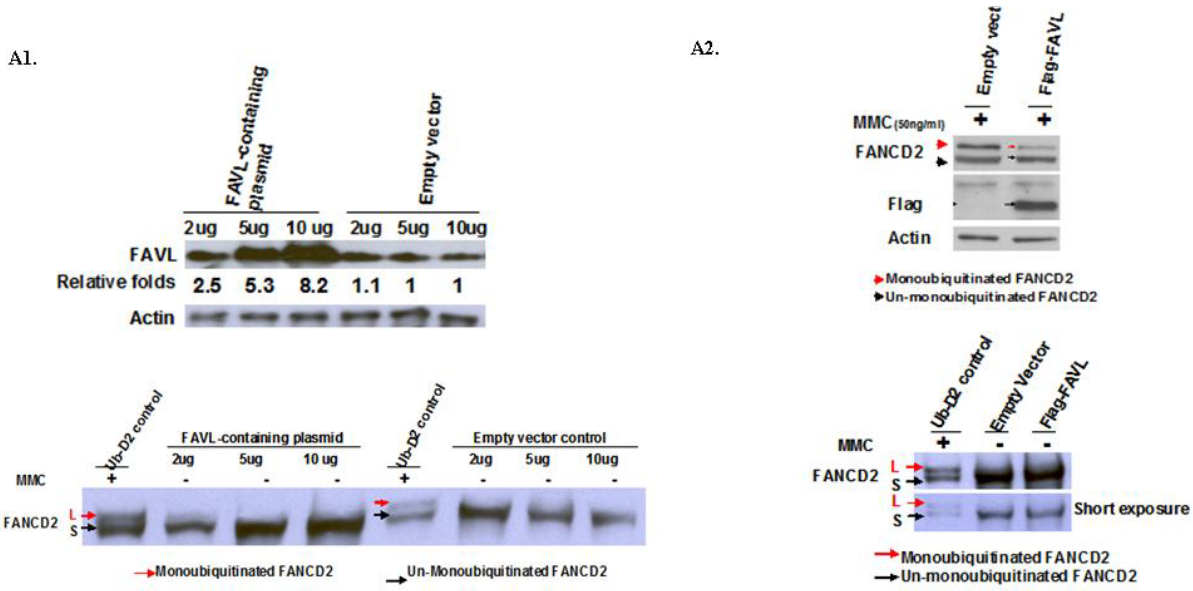


(S2C). FAVL Is Mainly Localized In the Cytoplasm.

Both cytoplasmic and nuclear fractions were prepared from A549 and Calu-6 cells known to harbor low and high levels of FAVL expression, respectively. A standard Western blot was performed by using these fractions. FAVL is mainly located in the cytoplasmic fraction prepared from Calu-6 cells; while FANCL is shown predominantly in nuclear fraction from A549 cells (Brg1 is a nuclear protein, and its signal shows only in the nuclear fraction, indicating that nuclear proteins mostly retained in the nuclear fractions we prepared).

Figure S3 Elevated FAVL Can Compromise the FA-BRCA Pathway

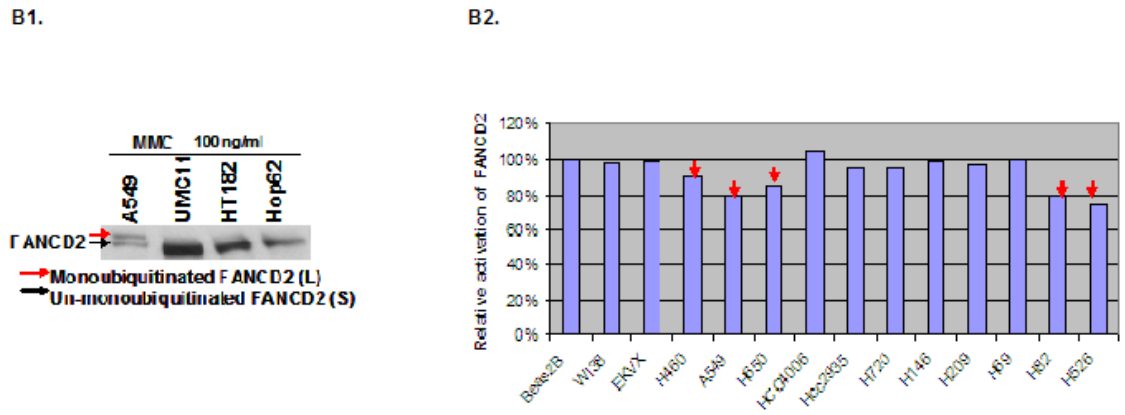
Figure S3A



(S3A-1). Overexpression of FAVL in U2OS cells. The different amount of FAVL-cDNA-containing plasmid and empty vector as controls were transfected into U2OS cells, respectively. Cells were split into two parts 24 hours after transfection. The part of cells was subjected to MMC treatment (Figure 3A), the part of cells transfected with 10 μg FAVL plasmid and empty vector control respectively was used for immunofluorenscence study (Figure 3A), and the remain part was used as non-treatment control for detecting FANCD2 monoubiquitination as well as levels of FAVL expression. Top panel: The levels of FAVL protein expression are positively proportioned to the amount of FAVL-cDNA plasmid transfected. In cells transfected with 10 μg of FAVL cDNA containing plasmid, the level of FAVL expression is the highest among all cells detected; whereas different amount of control plasmid did not affect the level of FAVL protein expression. Bottom panel: FANCD2 monoubiquitination is barely detectable in lysates prepared form MMC-untreated cells (Relative folds of FAVL expression were generated based on values of the gray density of Western bands normalized by levels of β-actin expression through the NIH image J).

(S3A-2). Activation of FANCD2 Is Compromised in PA1 Cells Expressing Flag-FAVL. The PA1 cells growing in 60mm dishes with 75% confluence were transfected with 5 μg of Flag-FAVL cDNA-containing plasmid and the same amount of empty vector as the control, respectively. The level of activated FANCD2 (marked with the red arrow) is relatively decreased in FAVL transfected PA cells following exposure to MMC. The level of monoubiquitinated FANCD2 is undetectable in corresponding untreated cells [bottom panel, FANCD2 monoubiquitination (D2-ub) was used as the control of Western band pattern].

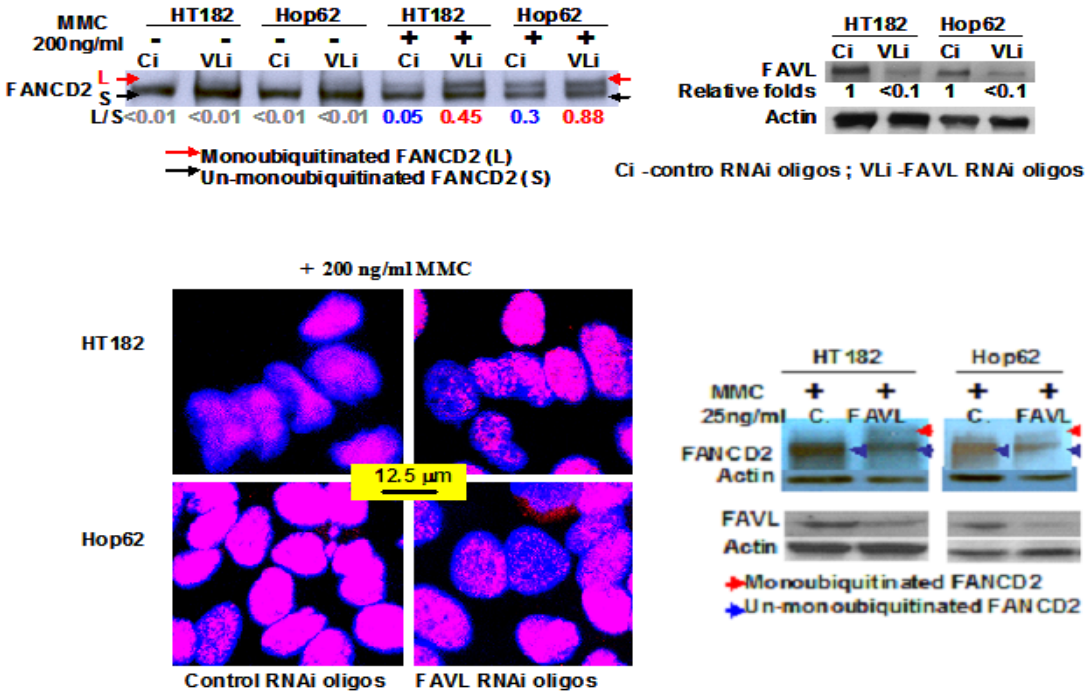
Figure S3A



(S3B-1). The monoubiquitinated FANCD2 could not be detected in UMC11, HT182, and Hop62 cells following MMC treatment compared with A549 cells

(S3B-2). FA-BRCA Pathway Is Slightly Impaired in Some of Lung Cancer Cell Lines. The ratio of monoubiquitinated FANCD2 over non-unmonoubiquitinated FANCD2 was generated based on the gray density of Western bands, derived from cells treated with 25 ng/ml MMC. Each ratio value was normalized with the one from Bras2B cells. Cells marked with (red arrowheads) show a little compromised FANCD2 monoubiquitination upon the treatment of 25 ng/ml MMC.
(The gray density was measured by the NIH image J.)

Figure 3C

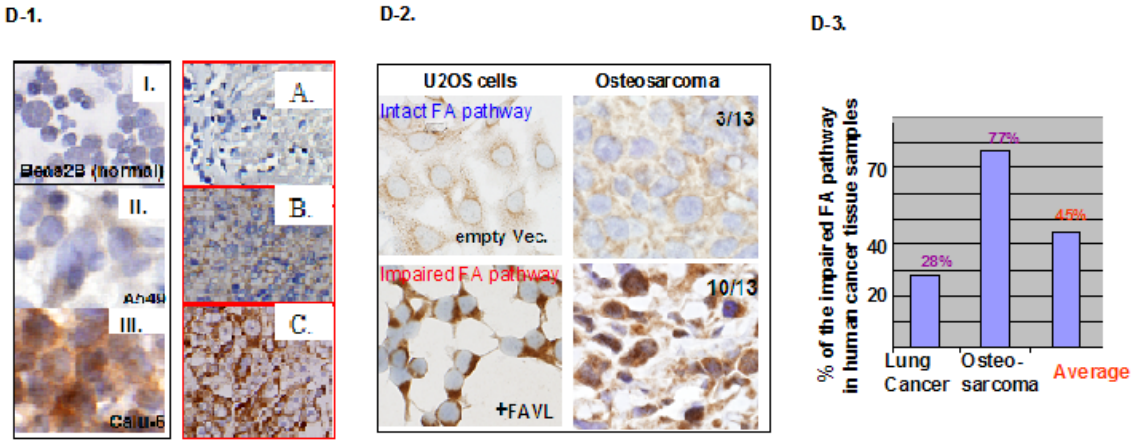


(Top panels) Reduction of FAVL Protein Levels in HT182, and Hop62 Cells Partially Restores the FA-BRCA Pathway. Total cell lysates prepared from HT182 and Hop62 cells transfected with either control (non-specific RNA oligos) or FAVL RNAi oligos (FAVL) and treated with 200 ng/ml MMC overnight, were analyzed by Western blotting using antibodies against FANCD2, FAVL, and β–actin as a loading control. The levels of MMC-induced FANCD2 monoubiquitination (the red arrowhead) were increased in HT182 and Hop62 cells with reduced FAVL protein level as compared with cells treated with control RNAi oligos. The low levels of FAVL protein detected in cells with FAVL specific RNAi oligos indicate an efficient FAVL knockdown. FANCD2 monoubiquitination was also examined in cells transfected with a half of amount of RNAi oligos used here, and FANCD2 activation can be restored to some extent (bottom panels).

Left of the bottom panels: The FANCD2 foci can be detected clearly in HT182 and Hop62 cells treated with 200 ng/ml MMC when FAVL protein was down regulated (top panels).

Right of the bottom panels: Monoubiquitinated FANCD2 was detectable in HT182 and Hop62 cells harboring downregulated FAVL when they were treated even with 25 ng/ml MMC (C. represents control RNAi oligos, FAVL labeled on the top of the images stands for RNAi oligos targeting FAVL).

Figure 3D



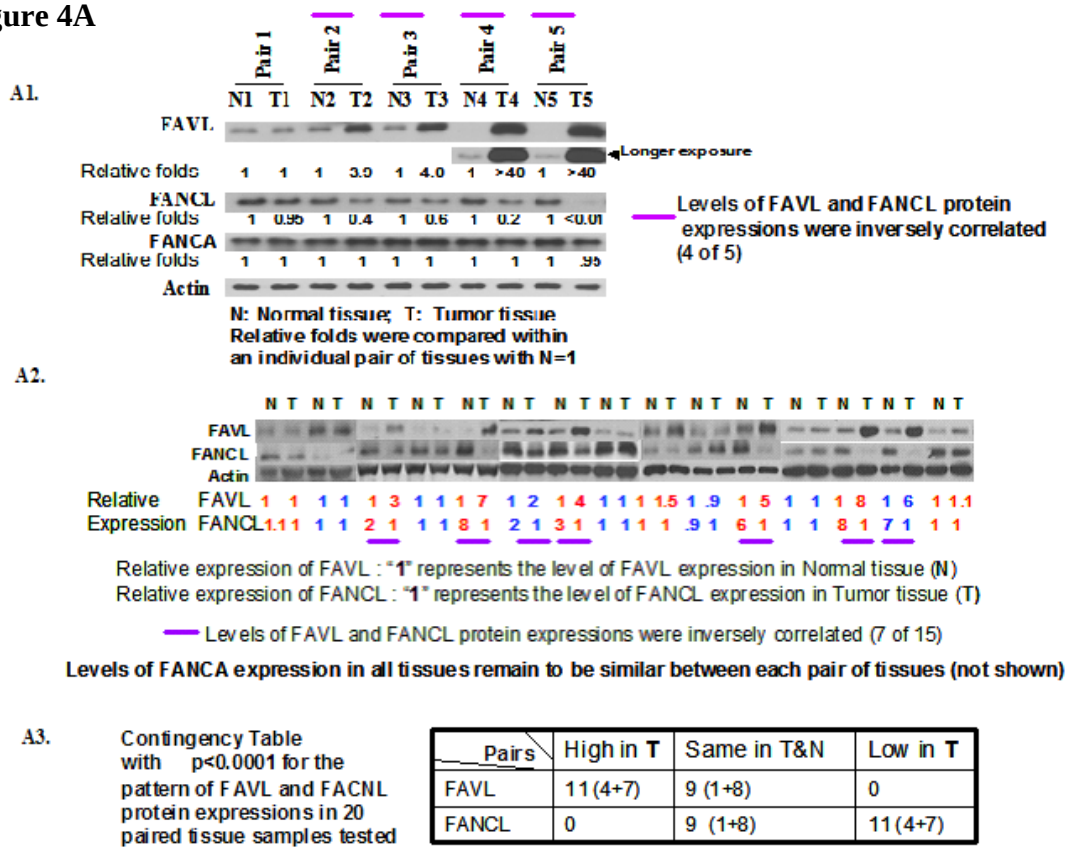
(S3D-1). Approximately One Quarter of Lung Carcinoma Cases Tested May Harbor a Defective FA-BRCA Pathway. The staining intensity observed for the benign lung tumor tissue in Figure 2B and S2B, image A here, is similar to that of normal Beas2B cells (**I**). In contrast, the staining intensity of the carcinoma samples shown in Figure S2B-1, image B here, is similar to that of A549 cells (**II**), in which FAVL is elevated but not high enough to impair the FA-BRCA pathway, while the staining intensity of the carcinoma samples shown in Figure 2B left panel, image C here, appears equivalent to that of Calu-6 cells (**III**), which harbor a defective FA-BRCA pathway. This degree of staining intensity shown for the tested slides was associated with 4 of 11 adenocarcinoma; 1 of 5 squamous carcinoma; 1 of 8 small cell lung cancer; and 1 of 1 carcinoid lung cancer for a total of approximately 7 of 25 carcinoma cases tested. The immunostaining conditions used for cells were exactly the same as those used for tissue staining. The basal level of FANCD2 monoubiquitination is usually too low to be detected; therefore, Western blot analysis of tissue samples was not performed to determine the levels of monoubiquitinated FANCD2, which would directly reflect the status of the FA-BRCA pathway.

(S3D-2). Most Osteosarcomas Tested May Harbor a Defective FA-BRCA Pathway. FAVL IHC was performed in U2OS stable cell pairs (Figure S5D-1) under the same condition used for detecting FAVL expression in osteosarcoma tissues. The brown color intensity in control cells, transfected with empty vector (empty vector), carrying an intact FA pathway, is comparable with the staining intensity found in 3 of 13 osteosarcoma cases tested. FAVL staining density in U2OS cells, overexpressing FAVL (+FAVL), harboring an impaired FA pathway, is similar to that intensity shown in the rest of osteosarcoma cases tested (10/13 cases). These results suggest that most of osteosarcomas might have harbored a compromised FA-BRCA pathway.

(S3D-3). Percentages of the impaired FA pathway are possibly present in human cancer tissues.

Figure S4. The Effect of Elevated FAVL May be partly Mediated through Downregulating FANCL Expression at the Protein Level.

Figure 4A



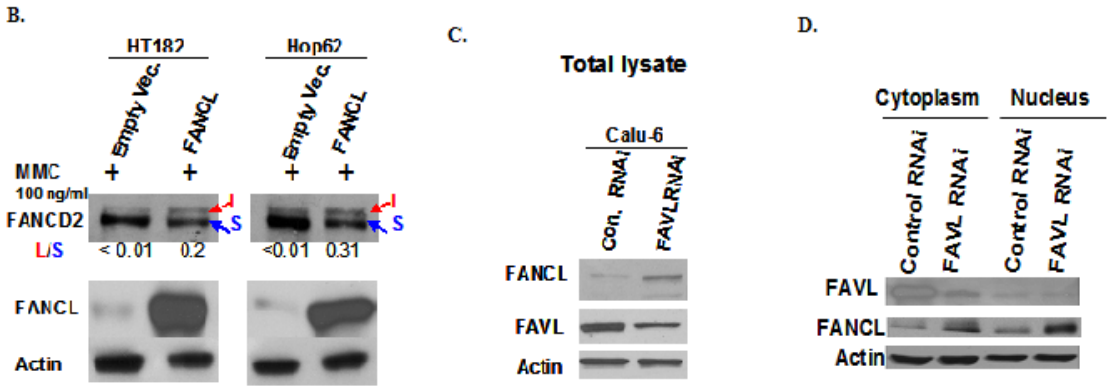
A3. Contingency Table with p<0.0001 for the pattern of FAVL and FACNL protein expressions in 20 paired tissue samples tested

Pairs	High in T	Same in T&N	Low in T
FAVL	11 (4+7)	9 (1+8)	0
FANCL	0	9 (1+8)	11 (4+7)

(S4A). The levels of FAVL and FANCL Proteins Are Inversely Related in Human Tissue Samples.

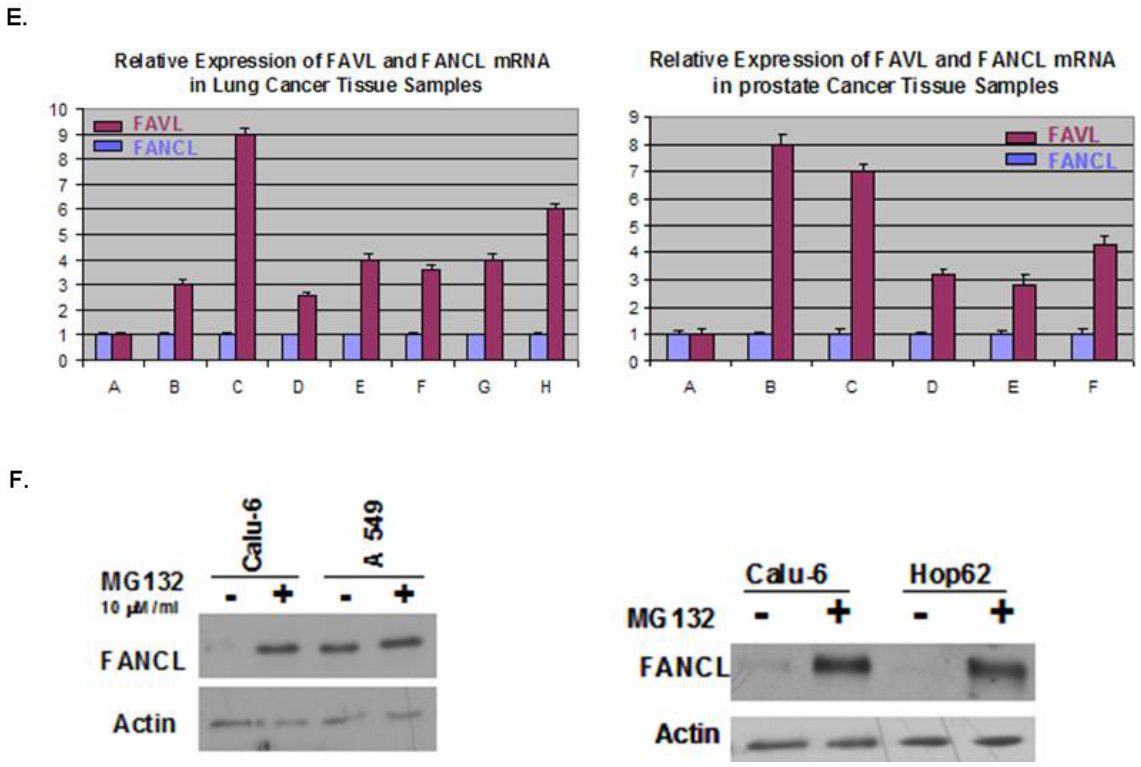
5 pairs of matched prostate tissue samples (A1) along with 15 pairs of matched lung tissue samples (A2) were used to detect the expression of FAVL and FA proteins. FAVL elevation in tumor tissues correlates with low levels of FANCL protein but not FANCM (data not shown) or FANCA. Relative folds of protein expression were determined with the gray density generated through using the NIH image J.) A total of 20 pairs of matched human tissue samples tested resulted in p value <0.0001 for the pattern of FAVL and FANCL protein expressions in human tissues (A3).

Figure 4B/C/D



(S4B). Ectopic Expression of FANCL Protein Can Partially Rescue FANCD2 Activation in Cells Expressing FAVL at a Higher Level.
HT182 and Hop62 cells were transfected with the plasmid containing FANCL cDNA or empty vectors as a control. 24 hours after transfection, these cells were treated with or without 100 ng/ml MMC. Subsequently, cell lysates were prepared following 18 hour treatment, and protein levels of FANCD2, FANCL, and β-actin were examined. The level of monoubiquitinated FANCD2 is relatively increased in cells expressing FANCL at high levels. The monoubiquitinated FANCD2 could not be detected in untreated cells (data not shown). (L/S ratio was determined with the gray density of western bands detected through using the NIH image J program. L: the long from of FANCD2 - the monoubiquitinated FANCD2; S: the short form of FANCD2 - unmonoubiquitinated FANCD2).
(S4C/D). **Downregulation of FAVL elevates FANCL protein mainly at nucleus.** Calu-6 cells were first transfected with RNAi oligos specifically targeting FAVL or control nonspecific oligos. 48 hours later, these transfected cells were collected and used for a standard western blot analysis with antibodies against FANCL, FAVL, and β–actin (as the loading control), respectively. The levels of FANCL protein were detected clearly in cells treated with RNAi oligos against FAVL compared with cells with control RNAi oligos (**C-total lysates; D-fraction**s).

Figure S4E/F

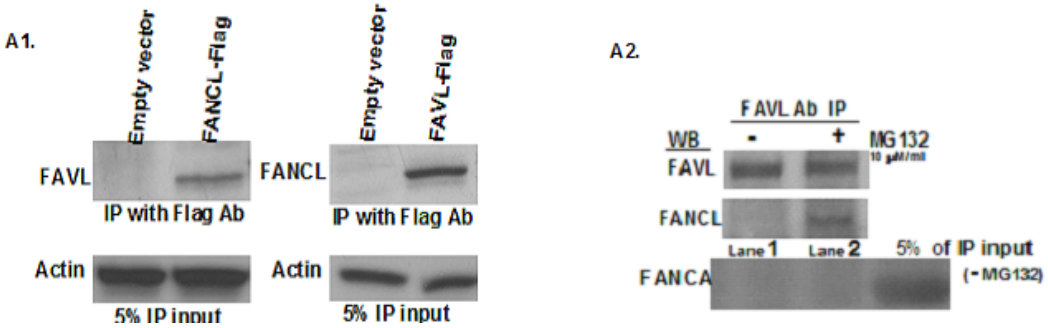


(S4E). The Levels of FANCL mRNA Expression in Tissue Samples Tested Appear to Be Similar. Tissue samples for verifying FAVL mRNA expression (Figure S2) were used for detecting both FANCL and FAVL mRNA expression with TaqMan assay. The levels of FAVL mRNA expression vary among tissue samples tested, but the levels of FANCL mRNA expression appear to be similar (Relative expression ratio average ±SEM with n=3).

(S4F). Inhibition of Proteosome Function Substantially Increases Levels of FANCL Protein in Calu-6 or Hop62 Cells But Not In A549 Cells. A549, Hop62, and Calu-6 lung cancer cells were treated with 10 μM MG132 over night, collected, and followed by a standard Western blotting for FANCL. The level of FANCL is increased substantially in MG132-treated Calu-6 or Hop62 cells but only slightly in treated A549 cells compared to untreated cells, respectively (β–actin signal is used as the loading control).

Figure S5. Overexpressed FAVL Can Promote FANCL Degradation.

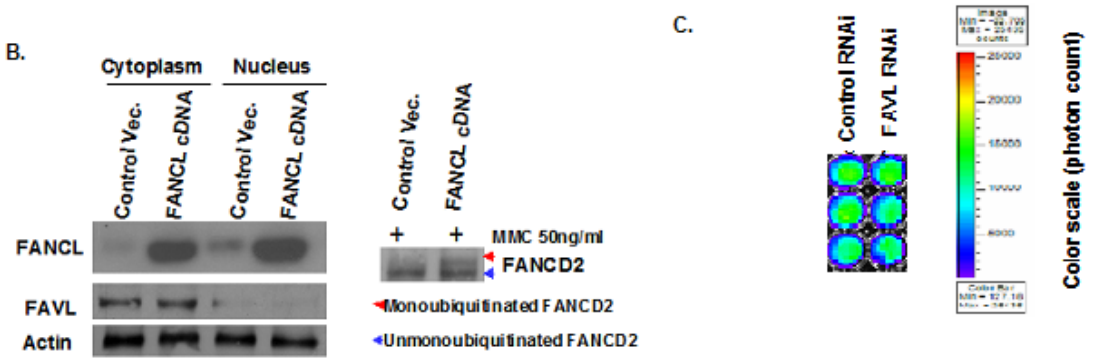
Figure S5A



(S5A-1). FAVL and FANCL Can Be Coimmunoprecipitated. Lysates prepared from cells transfected with empty vector or expressing Flag-tagged FANCL or FAVL were immunoprecipitated (IPed) using an anti-Flag antibody. The IPed samples were then analyzed by Western blot using anti-FAVL and anti-FANCL antibodies, respectively. FAVL is co-IPed with Flag-FANCL and FANCL is co-IPed with Flag-FAVL.

(S5A-2). The Interaction Between FAVL and FANCL Proteins Can Be Detected in MG132-Treated Calu-6 Cells. Calu-6 cancer cells were treated with or without MG132 over night, and collected. FAVL-IP was performed from lysates of these cells and followed by Western blotting for FANCL in the IP pellets. FANCL can be detected clearly in FAVL-IP pellet prepared from MG132-treated calu-6 cells (lane 2), but not in the pellet IPed with control antibodies, pooled rabbit IgG (data not shown). In addition, we could not detect FANCM (data not shown) or FANCA protein in the FAVL IP pellets prepared from calu-6 cells with or without MG132. [We can not perform reverse IP because FANCL antibody was generated from C-terminal part of FANCL, including a part of C-terminal of FAVL, but FAVL antibodies were raised from 13 unique amino acids of FAVL protein (Figure 1B).].

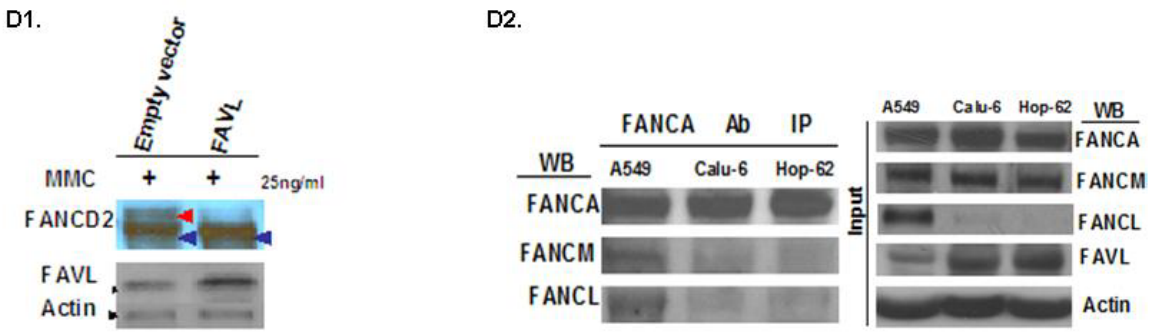
Figure S5B/C



(S5B). Overexpression of FANCL Can Complement FANCD2 Activation in Calu-6 Cells. Calu-6 cells were transfected with FANCL cDNA-containing plasmid and the empty vector (as the control). These transfected cells were collected with a portion of them for preparing total cell lysates, and cytoplasmic and nuclear fractions, respectively. Western blotting analysis of these lysates revealed that FANCL can be re-expressed in nucleus of Calu-6 cells and can, at least partly, restore FANCD2 monoubiquitination, consistent with the previous finding (24).

(S5C). Transfection Efficiency Was the Same Between Calu-6 Cells Treated with Control RNAi Oligos and Calu-6 Cells Treated with FAVL RNAi Oligos. Renilla luciferase reporter (1/10 of amount of pCEP-Flag-FANCL plasmid) was cotransfected into Calu-6 cells with control or FAVL RNAi oligos, respectively. A similar degree of luciferase activity displayed from both groups of cells indicates an equal amount of pCEP-Flag-FANCL plasmid was delivered into both groups of cells.

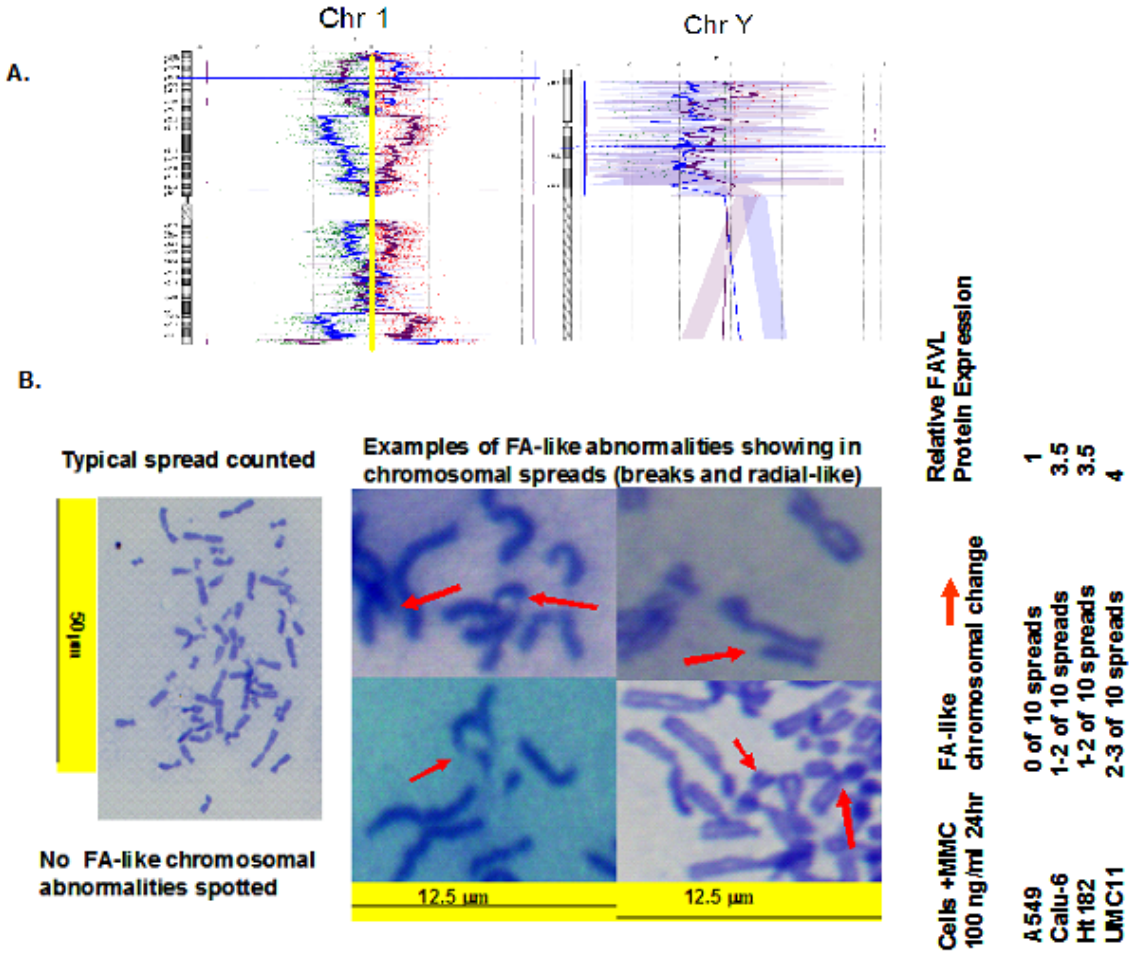
Figure S5D



(S5D-1). U2OS Stable Cells Overexpressing FAVL Carry an Impaired FA-BRCA Pathway. U2OS cells, transfected with plasmid containing FAVL cDNA or corresponding empty vector were pool-selected with 400 μg /ml neomycin 48 hours posttransfection. Three weeks after the selection, both types of cells were treated with 25 ng/ml of MMC for overnight, and subsequently were collected for analyzing protein levels of monoubiquitinated FANCD2, FAVL, and β–actin. The levels of monoubiquitinated FANCD2 is lower in cells overexpressing FAVL compared to control cells containing empty vector (The red arrow marks the monoubiquitinated FANCD2, and the blue one indicates un-monoubiquitinated FANCD2).

(S5D-2). Multi FA Protein Complex Is Instable in Lung Cancer Cells Expressing Lower Levels of FANCL Compared With A549 Lung Cancer Cells Harboring A Normal Level of FANCL. Nuclear extracts (NEs) were prepared from A549, Calu-6, and Hop62 lung cancer cells. FANCA immunoprecipitation from these NEs was performed. The pellets were analyzed for the presence of FANCA, FANCM, and FANCL. FANCM/L proteins were barely detectable in the pellets of FANCA-IP prepared from Calu-6 and Hop62 cells (Left panel). FANCM and FANCA input levels were similar among three types of cells; whereas FANCL is low in Calu-6 and Hop62 lung cancer cells (Right panel). Low levels of FANCL protein detected in the input appeared to be the cause of the insufficient FA complex formation. The levels of FAVL protein were found to be low in A549 cells and high in Calu-6 and Hop62 cells (Figure 4A) [FA proteins detected here were all negative in the pellets generated from the control antibody, the pooled rabbit IgG (data not shown)].

Figure S6. Overexpression of FAVL Promotes Chromosomal Instability.



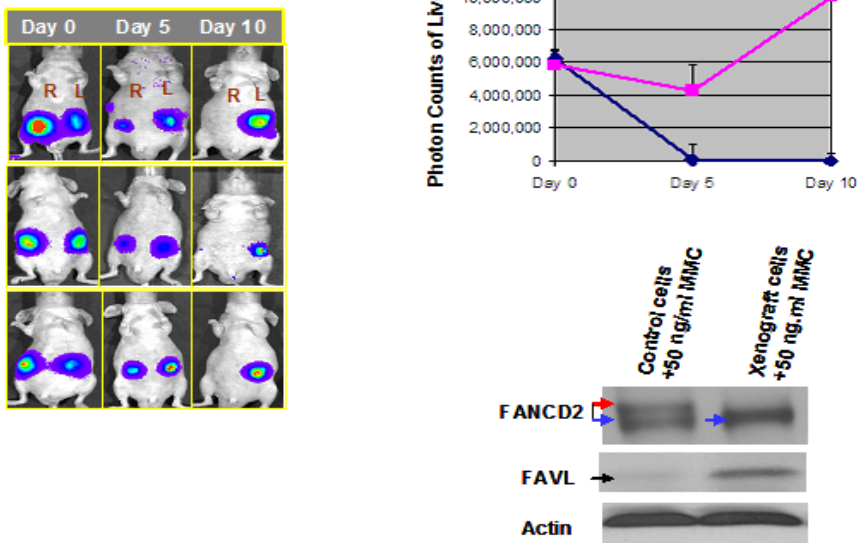
(S6A). Representation of typical output signal scanned from the CGH.
Blue line – the chromosome 1 profile of U2OS cells expressing a high level of FAVL, standardized by the chromosome 1 of control U2OS cells.
Purple line - the chromosome 1 profile of control U2OS cells normalized with chromosome 1 of FAVL-overexpressed U2OS cells.
Parts of blue / purple lines right or left of the center line (marked with the yellow color) stand for amplification or deletion, respectively.

Right panel (Y chromosome) shows the hybridization essentially negative on the basis of the output signal scanned. It was shown in the overall summary (Figure 6A) owing to the analysis software.

(**S6B**). Number of Chromosomal spreads carrying FA-like chromosomal abnormalities correlates with the relative level of FAVL protein expression in lung cancer cells (the relative expression of FAVL protein was calculated through image densities with the one generated in A549 cells as "1").

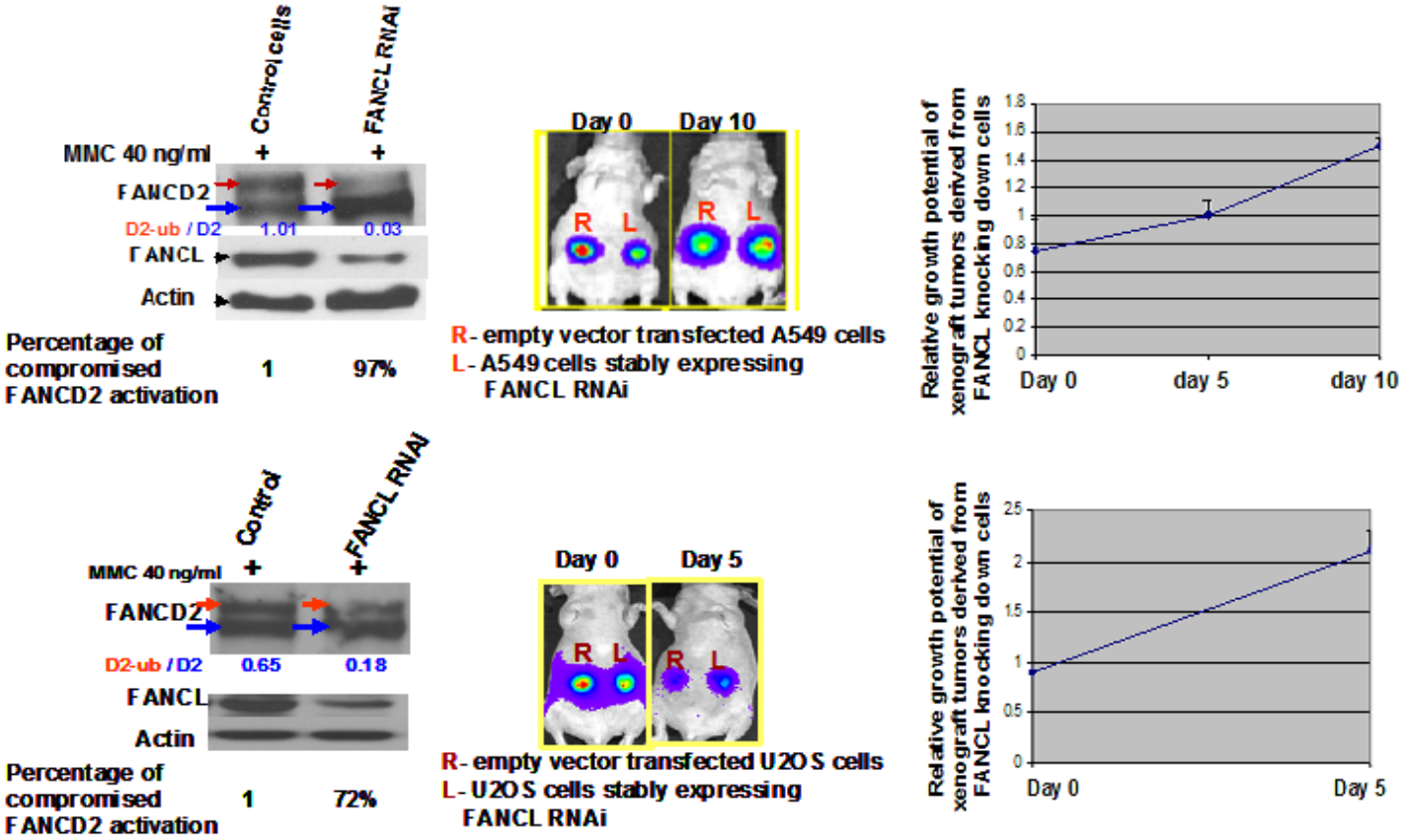
Figure S7. Overexpression of FAVL Promotes Tumor Formation and Tumor Progression.

Figure S7A



(S7A). U2OS Cells Expressing High Levels of FAVL Confer the Formation of U2OS Xenograft Tumors. 4 male nude mice with age of 5 weeks old were used to reveal in vivo growth advantages triggered by elevated FAVL. The left side of the mouse (L) was injected with U2OS cells overexpressing FAVL and the right side of the same mouse (R) was injected with control U2OS cells containing empty vectors that had been subjected to the same selection process. Live images taken from each mouse at the indicated time points show that U2OS cells overexpressing FAVL lived much longer and gained stronger growth potentials (1 of 4 mice in the group was shown in Figure 7A). The photon counts emitted from each image were plotted to indicate growth potentials of xenograft tumors (photon count average ±SEM with n=4) (the pink line represents the growth curve for tumors overexpressing FAVL (Figure S7A right). These mice were sacrificed at day 25, and portions of xenograft tumors were used to generate primary tumor cells. The xenograft tumor cells and original control cells were examined for FAVL expression and FANCD2 activation. FAVL expression persists at a high level and the FA-BRCA pathway remains defective in cells derived from xenograft tumors compared to control cells (bottom right).

Figure S7B



(S7B). Lower Expression of FANCL Protein Promotes the Growth of Xenograft Tumors.

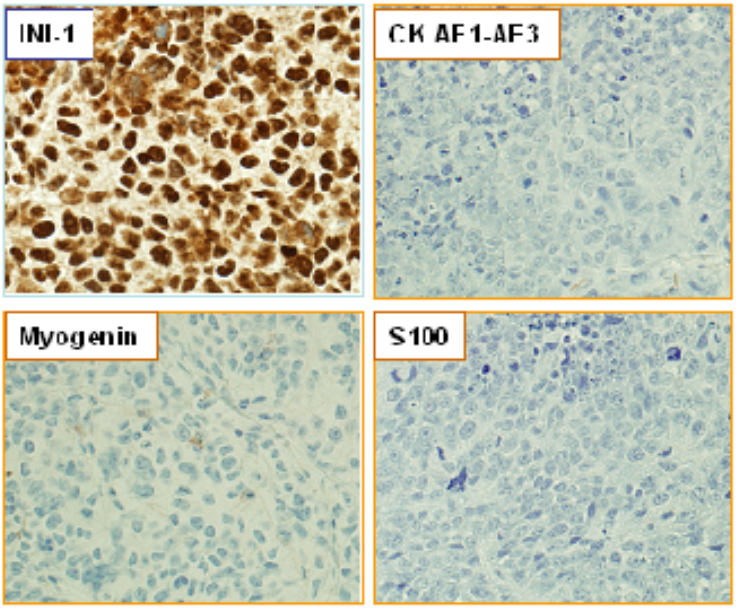
Left panels: FANCD2 monoubiquitination is compromised in A549 and U2OS stable cells. RNAi oligo specifically against human FANCL (18) was confirmed on our hands first capable of silencing FANCL expression (data not shown). And its sequence was then be used to generate a cDNA gene of FANCL RNAi oligo, which was made by synthesizing both two 64 nt long oligos (below) engineered with compatible cloning sites at the ends. These two oligos was subsequently annealed and ligated into siRNA expression pSuper-retro vector at sites of BamH1 and HindIII. Both A549 and U2OS cells carrying an intact FA pathway were then used as parental cells to generate stable cell pairs isogenic to the transgene expressing FANCL RNAi oligo performed as previously described (40). The FA pathway is impaired in A549 and U2OS cells expressing FANCL RNAi measured with a compromised FANCD2 monoubiquitination (marked with red arrowheads). The compromised FANCD2 activation was calculated by using band density ratio of FANCD2-ub over FANCD2 (D2-ub/D2); the formula is [D2-ub/D2 (control)-D2-ub/D2 (RNAi)] over D2-ub/D2 (control).

(F43: GATCCCCgacaagagctgtatgcactTTCAAGAGAagtgcatacagctcttgtcTTTTTGGAAA
R43: AGCTTTCCAAAAAgacaagagctgtatgcactTCTCTTGAAagtgcatacagctcttgtcGGGG).

Middle panels: Xenograft tumors expressing FANCL protein at a lower level gained growth potential. 3 male nude mice with age of 5 weeks old were used to reveal in vivo growth advantages triggered by the down-regulated FANCL. The left side of the mouse (L) was injected with A549 or U2OS cells constitutively expressing FANCL RNAi oligos and the right side of the same mouse (R) was injected with control A549 or U2OS cells containing empty vectors that had been subjected to the same selection process. These live images indicate that A549 or U2OS cells expressing FANCL RNAi oligos and thus a lower level of FANCL protein expression gain a stronger growth potential.

Right panels: Relative growth potential was plotted with photon counts of images at L side over R side (the curve represents photon count ratio average ±SEM, n=3).

Figure S7C



(S7C). Positive Immunostaining for INI-1 and Negative Immunostaining for CK AE1/AE3, Myogenin, and S100 in U2OS Xenograft Tumors.

Expressions of INI-1, CK AE1-AE3, Myogenin, and S100 in U2OS xenograft tumors were detected by IHC with polyclonal rabbit antibodies routinely used in the clinic pathology laboratory. A carcinoma marker CK AE1-AE3, a melanoma marker S100, and a rhabdomyosarcoma marker myogenin are undetectable in U2OS xenograft tumor samples; while, INI- 1, lost in more than 90% of epithelioid sarcomas, can be detected. [Positive or negative controls are always set up simultaneously by using other known samples to evaluate IHC in the clinical laboratory, and those controls for this experiment were turned out as expected (data not shown)]

Figure S8

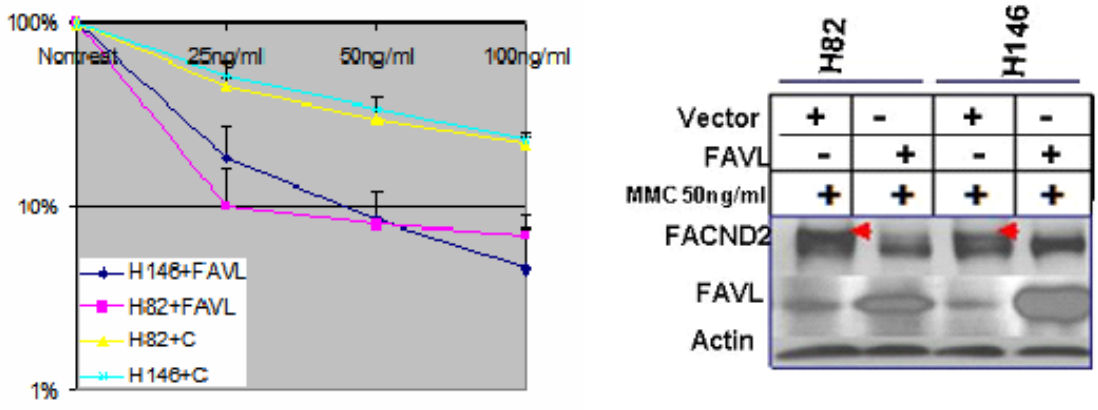


Figure S8. Cells with Overexpressed FAVL Are More Sensitive to MMC Treatment.

(Left panel) Cells from two SCLC cell lines, H82 and H146, were transfected with vector alone (+C) or a construct encoding for FAVL (+FAVL); treated with 25, 50, or 100 ng/ml MMC 12 hours post-transfection; and collected 72 hours following the treatment. The percentage of survived cells was then determined for each condition. The graph represents survive rate average ± SEM with n=3 (three independent experiments). SCLC cells expressing FAVL (pink and blue lines) had a lower survival rate following 72 hours of MMC treatment.

(Right panel) Total cell lysate was prepared from H82 and H146 cells transfected with vector alone or a construct encoding for FAVL and treated with 50 ng/ml MMC. A standard Western blot was then performed to examine the levels of FANCD2, FAVL, and β–actin (as a loading control). Indeed, overexpression of FAVL in H82 and H146 cells compromised FANCD2 activation, as indicated by reduced levels of monoubiquitinated FANCD2 (red arrows) (The similar result was also obtained by using PA1 cells, data not shown).