Supplemental Materials and Methods

Yeast Two hybrid screen

AH109 yeast strain was co-transformed with both Gal4-DBD-SAM68 vector and the LNCaP cDNA library and plated on synthetic selective media (SD without leucine, tryptophan and histidine) for the low stringency screen. The ~ 1500 yeast positive colonies obtained were subsequently tested in high stringency condition by plating in SD lacking leucine, tryptophan, histidine and adenine. Plasmids from positive clones were recovered and sequenced to identify the SAM68 interacting protein(s).

Plasmid constructs

pcDNA3.1-GFP-SAM68, pcDNA3.1-Myc-SAM68, pEGFP-C1-SAM68$_{V229F}$, pEGFP-C1-SAM68$_{G187E}$, pGEX4-T1-SAM68, pGEX4-T1-SAM68$_{321-443}$, pGEX4-T1-SAM68$_{351-443}$, and BCL-X minigene have been previously described [1,2]. BCL-X$_{\Delta 1-500}$ minigene was amplified using primers #(21-30) from BCL-X minigene [2] and cloned into XhoI-NotI restriction sites of the original BCL-X minigene to introduce the deletion.

pGBKT7-Gal4-DBD-SAM68 was subcloned from pGEX-4T1-GST-SAM68 using the EcoRI–SalI restriction sites. The human FBI-1 cDNA was amplified from PC3 cells by the Phusion Hot Start High-Fidelity DNA polymerase (Finnzymes) using primers #(1-2) and cloned into SalI-XbaI restriction sites of p3XFLAG (Sigma-Aldrich). FBI-1$_{274-584}$ was amplified using primers #(3-2) from p3XFLAG-FBI-1 and cloned into SalI-XbaI restriction sites of pcDNA3.1-Myc vector. All the GST fusion protein of SAM68 and FBI-1 were cloned in pGEX4-T1 plasmid. The SAM68$_{365-443}$, SAM68$_{351-400}$ and SAM68$_{351-434}$ were amplified from pGEX-4Ti-SAM68 using primers #(4-5), #(6-7), #(6-8), respectively. The mut1-BCL-X$_{\Delta 1-500}$, mut2-BCL-X$_{\Delta 1-500}$ and SAM68$_{\Delta 400-420}$ deletion mutants were constructed by mega primers strategy [3] using primers #(21-30-22) #(21-30-23) #(5-9-10), respectively. The pGEX4-T1-FBI-1, pGEX4-T1-FBI-1$_{1-276}$, and pGEX4-T1- FBI-1$_{274-584}$ were amplified from p3XFLAG-FBI-1 using primers #(11-12), #(13-12), #(11-14). All these

constructs were cloned into EcoRI–SalI restriction site of pGEX-4T1. pBIND-Gal4-SAM68 and pBIND-Gal4-SAM68$_{\Delta 400\text{-}420}$ were amplified using primers #(15-16) from pGEX-4T1-SAM68 and pGEX-4T1-SAM68$_{\Delta 400\text{-}420}$, respectively, and cloned in frame with Gal-4 domain using the SalI-NotI restriction sites of pBIND. Full-length FBI-1 was subcloned from p3XFLAG-FBI into the SalI-XbaI restriction sites of pACT. FBI-1$_{274\text{-}584}$ was amplified from p3XFLAG-FBI using primers #(17-2) and cloned into the SalI-XbaI restriction sites of pACT. pEGFP-C1-SAM68$_{\Delta 400\text{-}420}$ and pCDNA3.1-Myc-SAM68$_{\Delta 400\text{-}420}$ were amplified from pGEX-4T1-GST-SAM68 using mega primer strategy and primers #(5-10-17), and cloned in the EcoRI–SalI restriction sites of pEGFP-C1 or the EcoRI–XhoI restriction sites of pCDNA3.1-Myc. p14ARF promoter has been amplified from PC3 cells using #(19-20) primers and cloned into XhoI-HindIII restriction site of pGL3 luciferase reporter vector. All oligonucleotides used as primers in this study are listed in the Supplemental Table 1.

Cell cultures, transfections and cell extract preparation

HEK293T, BPH1, LNCaP or PC3 cells, plated in 35-mm dishes, were transfected with various combination of vectors (750ng total plasmid DNA) as indicated using Lipofectamine 2000 (Invitrogen). For RNAi, cells were transfected with siRNAs (Sigma-Aldrich) using Lipofectamine RNAi Max (Invitrogen) and Opti-MEM medium (Invitrogen). Sequences for SAM68 siRNA and pLKO-shSAM68 were previously described [4,5]. The FBI-1 siRNA sequence is listed in the Supplemental Table 1. Twenty four hours after transfection, whole extract preparation was performed by diluting cells in lysis buffer [50 mM Hepes, pH 7.4, 150 mM NaCl, 15 mM $MgCl_2$, 10% glycerol, 1 mM dithiothreitol, 20 mM β-glycerophosphate, 0.5 mM $NaVO_4$, protease inhibitor cocktail (Sigma-Aldrich), 0.5% Triton X-100]. After 10 min incubation in ice, cell suspension was centrifuged for 10 min at 12,000x*g* at 4°C, and the supernatant fractions were collected and used for further analyses. Cytosol/nuclear fractionation was performed as previously reported [1].

Subcellular fractionation experiments

Cytosol/nuclear fractionation was performed by resuspending the cells in RSB10 buffer (10 mM Tris/HCl 7.4, 2.5 mM $MgCl_2$, 10mM NaCl) supplemented with 20 mM β-glycerophosphate, 0.5 mM $NaVO_4$, 1 mM dithiothreitol and protease inhibitor cocktail. Cells suspension was incubated 15 min in ice and then centrifuged at 700 *xg* for 8 min. The supernatant was collected (cytosol fraction) and the pellet was then resuspended in RSB100 buffer (10 mM Tris/HCl 7.4, 2.5 mM $MgCl_2$, 100mM NaCl, 0.5% Triton X-100) supplemented with 20 mM β-glycerophosphate, 0.5 mM $NaVO_4$, 1 mM dithiothreitol and protease inhibitor cocktail. The suspension was homogenized and incubated in ice for 5min. Then, nuclear extracts were layered onto 30 % (wt/vol) sucrose cushion and centrifuged for 15 min at 7,000 *xg*. The supernatant (nuclear fraction) was collected and used for further experimental manipulations. Sucrose gradient fractionation of the nuclear compartment was performed as previously described [6].

GST and Poly -A/-U pull-down assays and Protein cross-link

GST pull down assays were performed as previously described [1,7]. In some experiments, GST-fusion proteins were cross-linked to the GSH-Sepharose beads by 1h incubation in 200 mM Triethanolamine, pH 8.9, supplemented with 50mM dimethylpimelimidate dihydrochloride, blocked with 100mM ethanolamine, pH 8.9. Beads were washed in phosphate buffered solution and used for the experiments. Poly-A/-U pulldown assays were performed as described [1].

Western-blot analysis

Western-blot analysis were performed as previously described [1] using 1:1000 dilution of the following primary antibodies: rabbit anti-ERK2, mouse anti-Myc, rabbit anti-SAM68 and goat anti-lamin B (Santa Cruz Biotechnology); rabbit anti-FBI-1, rabbit anti HDAC1 and mouse anti-Tubulin, anti-Flag and anti-hnRNPA1 (Sigma-Aldrich); and rabbit anti-GFP (Molecular Probes).

Splicing assays and RT-PCR analyses

BCL-X minigene splicing assays and RT-PCR analyses were performed as previously described [1]. Quantitative real-time PCRs (qPCR) were performed by using LightCycler 480 SYBR Green I Master using the LightCycler 480 System (Roche), following manufacture's instructions. The

oligonucleotide primers used are listed in the Supplemental Table 1. The qRT-PCR analysis of *BCL-X* variant in Figure 3B was calculated by delta-delta Ct (cycle threshold) method. Briefly, ΔCt was calculated as the difference between $Ct_{BCL\text{-}Xs}$ and $Ct_{BCL\text{-}XL}$ ($\Delta Ct = Ct_{BCL\text{-}Xs} - Ct_{BCL\text{-}XL}$). ΔΔCt was then calculated as the difference between ΔCt of si-FBI-1 samples and ΔCt of control samples (si-CTRL) ($\Delta\Delta Ct = \Delta Ct_{si\text{-}FBI} - \Delta Ct_{si\text{-}CTRL}$). Fold variation (F) of each samples compare to the control was then estimated as follow: $F = 2^{(-\Delta\Delta Ct)}$.

Immunofluorescence analysis and image acquisition and manipulation

Immunofluorescence analysis was performed as described [1,5]. Confocal images were taken using a Leica confocal microscope equipped with a Plan-Neofluar HCX 40.0x/1.25 oil UV objective and aquired using the IAS AF Lite software (Leica Microsystems). Images were acquired as TIFF files, and Photoshop (Adobe) was used for composing the panels.

UV crosslink RNA/protein immunoprecipitation (CLIP)

Briefly, cells were washed with PBS, UV-irradiated (400mJ/cm^2) and incubated 10min on ice in lysis buffer: 50mM Tris–HCl, pH 7.4; 100mM NaCl; 1mM MgCl2; 0.1mMCaCl2; 1% NP-40; 0.5% sodium deoxycholate; 0.1% SDS, protease inhibitor cocktail, RNase inhibitor (Promega). Samples were then sonicated and incubated with Turbo DNase (Ambion) for 3min at 37°C, and centrifuged at 15000 x *g* for 3 min at 4°C. Extracts (50μg) were treated with Proteinase K for 30min at 37°C, RNA purified by standard methods (Input), pre-cleared for 1h on protein G-Dynabeads and incubated on protein G-Dynabeads with anti-c-Myc or rabbit anti-SAM68 (Santa Cruz Biotechnology) antibodies for 2 hrs at 4°C under rotation. Immunoglobulins from rabbit or mouse were used as control. After three washes with High-salt wash buffer (50mM Tris–HCl, pH 7.4; 1M NaCl;1mM EDTA; 1% NP-40; 0.5% sodium deoxycholate; 0.1% SDS) and two washes in PK buffer (100mM Tris–HCl, pH 7.4; 50 mM NaCl; 10mM EDTA) an aliquot (10%) was kept as control of immunoprecipitation and the rest incubated with 50μg Proteinase K at 37°C for 20min and RNA was isolated by standard procedures. Each sample was normalized with respect to its

input. *BCL-X* RNA associated with SAM68 is represented as fold enrichment relative to control samples.

References

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