Supplemental Material

A Novel Transcription Complex that Selectively Modulates Apoptosis of Breast Cancer Cells through Regulation of FASTKD2.

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1) Supplemental Legends to Tables and Figures

Supplemental Table 1. Components of the DIF-1 complex(es) identified by mass spectrometry and their Mascot confidence scores. A Mascot score of 50 was applied as a cut-off for significantly confident ($p<0.05$) protein identification by mass spectrometry. All ribosomal, keratin-related, and heat shock related proteins were not listed, as they are usually considered as contaminates in protein complex purification and mass spectrometry. The protein Mascot scores are listed in parenthesis and listed with the highest Mascot scores at the top.

Supplemental Table 2. siRNA sequences.

Supplemental Table 3. Sequences of primers used in qRT-PCR for FASTKD2 expression studies or ChIP analyses.

Supplemental Figure 1. Gel electrophoresis of purified DIF-1 protein complexes from HeLa and T-47D cells. This is a representative silver-stained gel comparing the pooled HA peptide purified eluates from HeLa or T-47D cells stably expressing FLAG-HA-DIF-1 or control (FLAG-HA tag only) cells. The molecular weight markers are as shown on the left.

Supplemental Figure 2. Localization of DIF-1, BP1, and EAP1 in the cell nucleus and in regions of the brain. Nuclear localization of DIF-1, BP1, and EAP1. DIF-1 was previously shown to be a nuclear protein {Tinnikov, 2009 #463}. **(A)** The expression of GFP-DIF-1, GFP-BP1, and GFP-EAP1 (in green) in HeLa cells indicates that all three proteins localize in the nucleus (in blue) with a distinct nucleoli exclusion pattern. **(B)** Colocalization of EAP1 mRNA with the mRNAs encoding NRIF3, BP1 and DIF-1 in neuronal populations of the rat brain. The transcripts were detected using a double *in situ* hybridization procedure in which an EAP1 cRNA probe was labeled with digoxygenin and NRIF3, BP1 and DIF-1 cRNAs were labeled with ^{35}S-UTP. ***Upper panels***, Neurons of the piriform cortex (PIR CTX) containing EAP1 mRNA transcripts (purple color) that colocalize with NRIF3, BP1 and DIF-1 mRNAs (white grains). ***Middle panels***, Neurons of the arcuate (ARC) and ventromedial nucleus (VMH) of the hypothalamus showing a similar colocalization as in the PIR CTX. ***Lower panel***, Higher magnification image showing colocalization of EAP1 mRNA (purple color) with BP1 mRNA (white grains) in the VMH of the hypothalamus. Bars in upper panels = 50μm; in middle panels = 100 μm; in lower panel = 20 μm. 3V = third ventricle.

Supplemental Figure 3. Knockdown of BP1 or EAP1 leads to disruption of the DIF-1 complex(es). (**A**) The affinity purified DIF-1 assoicated proteins prior to chromatography using (**I**) HeLa FLAG-HA-DIF1 or (**II**) T-47D FLAG-HA-DIF1 stable cells. In addition, HeLa cells stably expressing FLAG-HA-DIF1 were transfected with 40 nM of either (**B**) control siRNA, (**C**) BP1 siRNA, (**D**) EAP1 siRNA, or (**E**) a combination of BP1 and EAP1 siRNA. As described in **Figure 2B**, cells also received zVAD-fmk and zVDVAD-fmk to prevent apoptosis that might result from siRNA transfection. Panel A shows the affinity purified DIF-1 associated proteins prior to chromatography. The purified DIF-1 complex(es) was size-fractionated in a Superdex 200 gel filtration column as described in Figure 2B. The collected fractions were analyzed by SDS-PAGE followed by silver staining. The siRNA used in each panel is indicated in brackets [ ]. Position of protein standards for the size fractionation are indicated at the top. The molecular weight markers for the SDS-PAGE are as shown on the left.

Supplemental Figure 4. HeLa DD1-ERT2 and T-47D DD1(S28A)-ERT2 stable cell lines do not undergo apoptosis after 24 h of 4OHT treatment. HeLa DD1-ERT2 or T-47D DD1(S28A)-ERT2 stable cells were seeded on coverslips in 48 spot multiwells 24 h before treatment with 1

uM 4OHT for 24 h. Cells were fixed and permeabilized for immunofluorescence for FLAG (green) and for TUNEL assay (red). Nuclei were stained with DAPI (blue).

Supplemental Figure 5. siRNA knockdown of FASTKD2 protects SKBR3 cells from apoptosis resulting from GFP-DD1 expression or DIF-1 knockdown. SKBR3 cells were transfected with FASTKD2 siRNA (40 nM) or control siRNA (40 nM). Twenty-four h later, cells were transfected with GFP-DD1, GFP-DD1(S28A), or DIF-1 siRNA (40 nM). Twenty-four h later, cells were fixed and processed for TUNEL (red) and GFP expression (green). Nuclei were stained with DAPI (blue).

Supplemental Figure 6. ChIP studies of the FASTKD2 gene with primer sets A, C, and D. ChIP studies indicated that DIF-1 associates with a region downstream of the FASTKD2 promoter as determined with Primer set B. This figure shows the results using Primer sets A, C, D. Short fragments (150-300 bp) of chromatin from the stable cell lines expressing either FLAG-HA tag (T-47D or HeLa) or FLAG-HA-DIF-1 (T-47D DIF-1 and HeLa DIF-1) were used for ChIP. ChIP was performed using FLAG-M2 agarose beads, and was followed by qRT-PCR using primer set A, C, or D to amplify the regions of the FASTKD2 gene (see **Figure 7A** for the Primer set positions). The ChIP-qRT-PCR results shown represent the mean +/- SEM of three independent experiments.

Supplemental Figure 7. BP1 or EAP1 mediated transcriptional repression is not dependent on DIF-1. HeLa cells were transfected with either DIF-1 siRNA (40 nM) or control siRNA (40 nM). Cells were incubated throughout the experiment with the caspase inhibitors zVAD-fmk and zVDVAD-fmk to prevent apoptosis resulting from decreased DIF-1 expression. Forty h later cells were transfected with 100 ng of pG5-SV-BCAT and 200 ng of vectors expressing Gal4-BP1, Gal4-EAP1, Gal4-DIF-1 or 150 ng (equal molar amount of plasmid) of vector expressing only the Gal4-DBD. Twenty-four h later the cells were harvested for CAT activity. The results represent the mean +/- SEM of three independent experiments.