Supplemental Table 1. Kinetics of G1 phase progression of NIH3T3 released from a nocodazole (G2/M) block. NIH3T3 cells expressing shRNAs against FBX4 or αB crystallin were released from a nocodazole (G2/M) block in media containing BrDU and percentage of cells in S phase was assessed by FACS.

Supplemental Fig. 1.

(A) *In vivo* association of FBX4 with cyclin D1. Western analysis of cyclin D1 immunoprecipitates from asynchronously growing NIH3T3 cells treated with DMSO or MG132. (B) FBX4 and αB crystallin form a functional $SCF^{FBX4\text{-}\alpha B\ crystallin}$ E3 ubiquitin ligase. Deleting the F-box of FBX4 abrogates SKP1 and CUL1, but not αB crystallin binding. SF9 cells were infected with viruses encoding Flag-FBX4 or Flag-(ΔF)FBX4, αB crystallin, CUL1, SKP1. Anti-FLAG immunoprecipitates were then probed with the indicated antibodies. (C) Expression of shRNAs directed against FBX4 induces cyclin D1 accumulation. U20S cells were transfected with shRNA vectors directed against firefly luciferase, β-TrCP2 or FBX4. Immunoblot analysis was performed with antibodies to the indicated proteins

(D) MCF7 cells were infected with empty virus (lane 1), or virus encoding αB crystallin (lane 3) or transfected with a vector encoding αB crystallin (lane 2). Cells lysates were subjected to western analysis to confirm αB crystallin levels.

Supplemental Fig. 2.

(A) Immunohistochemical analysis of an esophageal adenocarcinoma tissue microarray (TMA) for cyclin D1, αB crystallin and FBX4. Tissue sections were subjected to antigen retrieval using Vector antigen unmasking solution followed by blocking of endogenous peroxidase activity with 3% peroxide for 10 minutes. Sections were then incubated in Power Block (Biogenex, CA) for 10 minutes followed by

incubation with the primary antibody for 2hrs. Sections were washed with PBS and incubated with a secondary biotin conjugated antibody (Vector Laboratories), washed again and incubated with ABC reagent (Vector Laboratories) followed by detection with DAB substrate (Vector Laboratories). The following primary antibodies were used: cyclin D1 (AB3, Calbiochem, 1:20), αB crystallin (Stressgen, SPA-223, 1:250), and affinity purified FBX4 (6702, 12 μg/mL). Tumors were scored by a pathologist in a double blind mode. (B) Quantification of αB crystallin and FBX4 protein levels in esophageal TMA; (-) no detectable signal; (+) few cells weakly positive; (++) most cells positive; (+++) strongly positive.

Supplemental Fig. 3.

MDA-MB-231, MCF7 and MCF10A cells were cultured in media containing cycloheximide for the indicated intervals. Lysates were resolved on SDS-polyacrylamide gels, transferred to nitrocellulose membrane and subjected to immunoblot with the indicated antibodies.

Supplemental Table 1

Percentage of cells in S phase following nocodazole release

hrs	Mock	Experiment #1 alphaB shRNA1	Experiment #2 alphaB shRNA2
4	2.0	1.4	1.5
6	1.9	6.6	3.4
8	5.8	15.8	18.8
10	7.3	24.3	28.7
12	11.9	32.9	33.9
14	19.5	41.3	43.3

hrs	Mock	Experiment #3 alphaB shRNA1	Experiment #4 alphaB shRNA2
4	2.4	3.2	1.3
6	3.3	12.1	9.4
8	8.8	30.2	22.0
10	17.4	42.5	32.3
12	28.2	52.7	53.9
14	36.5	62.8	66.7

hrs	Mock	Experiment #1 FBX4 shRNA1	Experiment #2 FBX4 shRNA2
4	1.9	2.9	2.2
6	2.7	8.1	4.1
8	4.3	17.6	9.6
10	10.7	24.2	18.0
12	21.0	36.9	29.2
14	33.0	53.0	45.0

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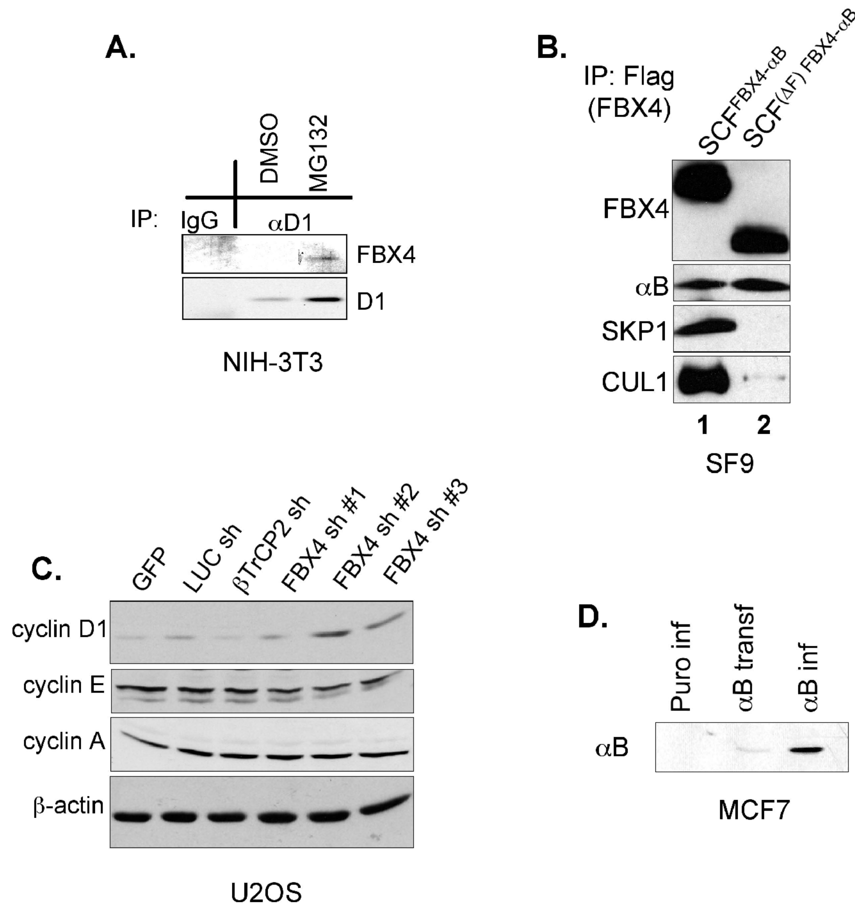
Supplemental Figure 1



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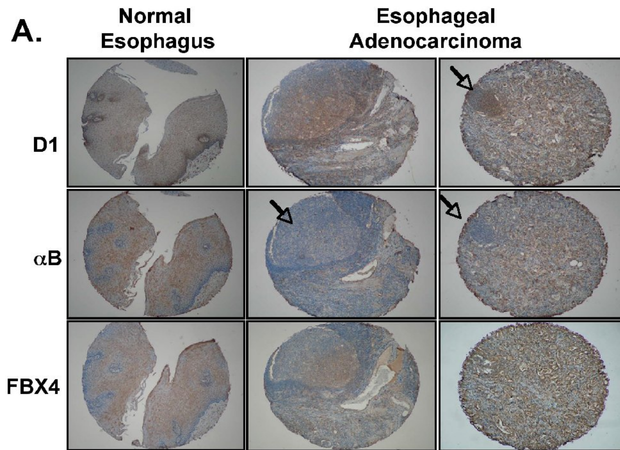
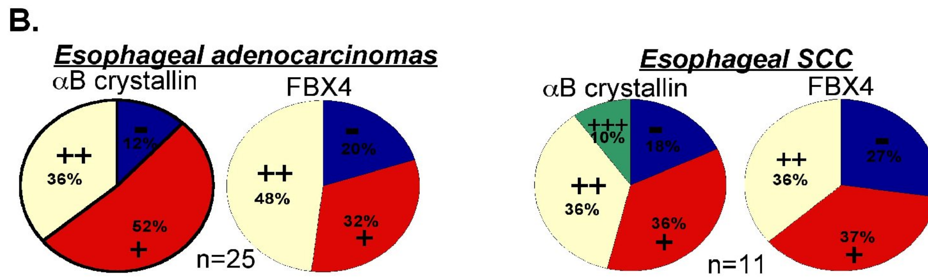
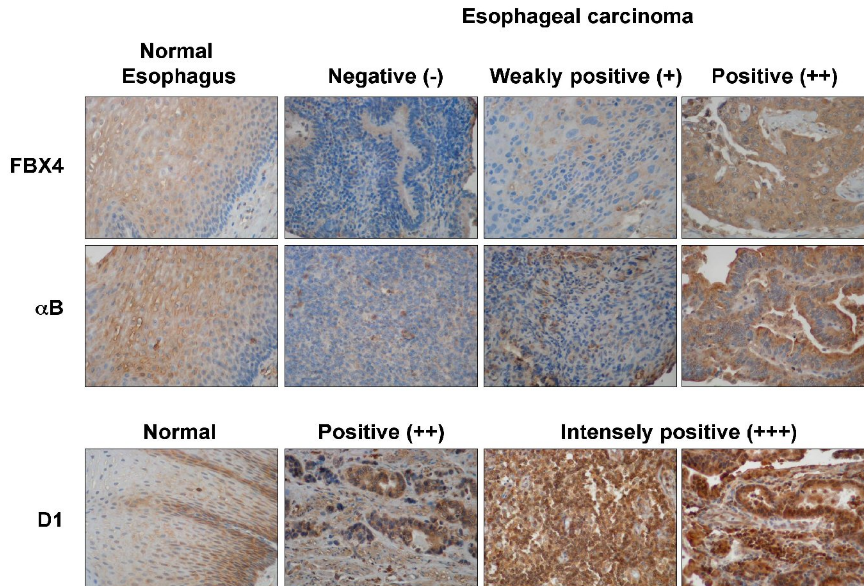
Supplemental Figure 2







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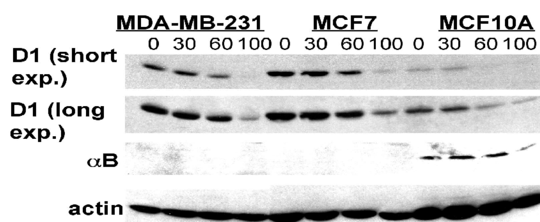

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