Supporting Information

Lee *et al.* 10.1073/pnas.0807668105

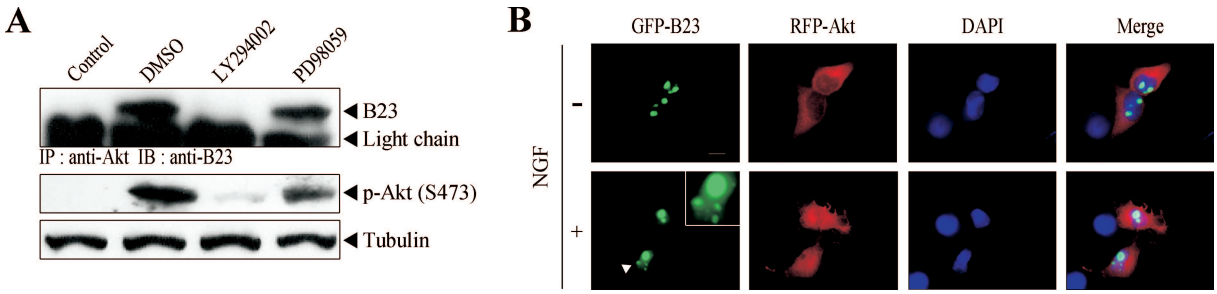


Fig. S1. B23 colocalized with active Akt in the nucleus. (*A*) Active Akt binds B23. B23 stably transfected PC12 cells were pretreated with or without LY294002 (20 μM) or PD98059 (20 μM) for 30 min, followed by 100 ng/ml NGF for 30 min. The endogenous B23 was immunoprecipitated with anti-B23 antibody. LY294002, but not PD98059, blocks the interaction between Akt and B23. NGF stimulated Akt phosphorylation (*Top*), PI3K inhibitor, LY294002 disturbs Akt phosphorylation, but the MEK inhibitor PD98059 does not affect Akt phosphorylation (*Middle*). (*B*) Naïve PC12 cells were cotransfected with GFP-B23 and RFP-Akt. After NGF stimulation for 30 min, cytoplasmic Akt translocated into the nucleus. However, B23 resides mostly in the nucleolus and is partly dispersed in the nucleoplasm. Nuclei counterstained with DAPI. (Scale bar, 10 μm.)

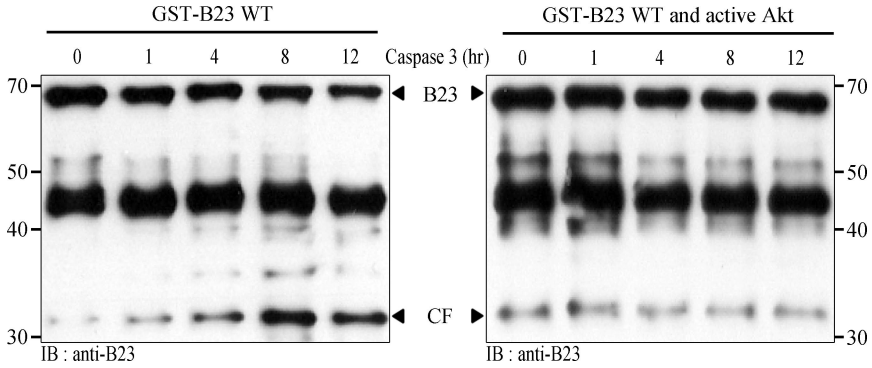


Fig. S2. Active Ak protect caspase-3 mediated cleavage of B23. Purified GST-B23 WT protein was preincubated in the absence or presence of active purified Akt for 15 min, followed by 25 ng/ml of active caspase-3 protein applied at the indicated times. The reaction mixture was then analyzed with anti-B23 antibody. Caspase-3 induces B23 cleavage in a time dependent manner (*Left*), whereas active Akt protects B23 cleavage by caspase-3 (*Right*).

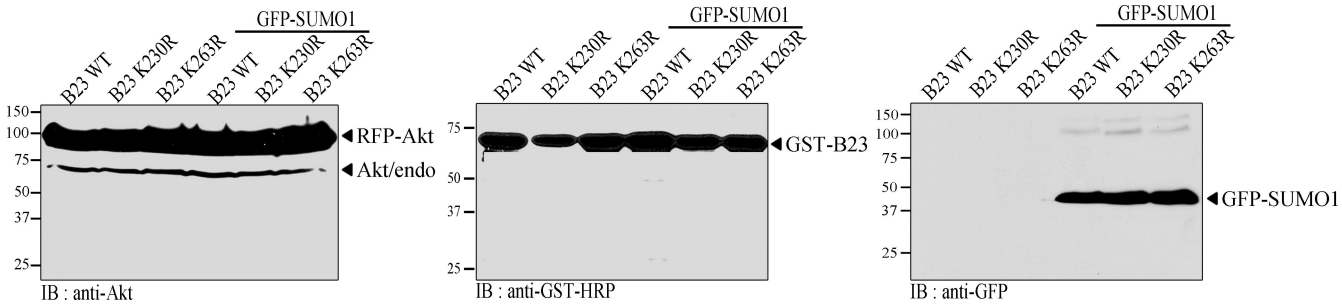


Fig. S3. The transfected protein expression level for Fig. 4*A*. Various GST-B23 constructs were cotransfected into HEK293 cells with GFP-Sumo1 and RFP-Akt. Immunoblotting was performed with antibodies against GST, GFP, and RFP.

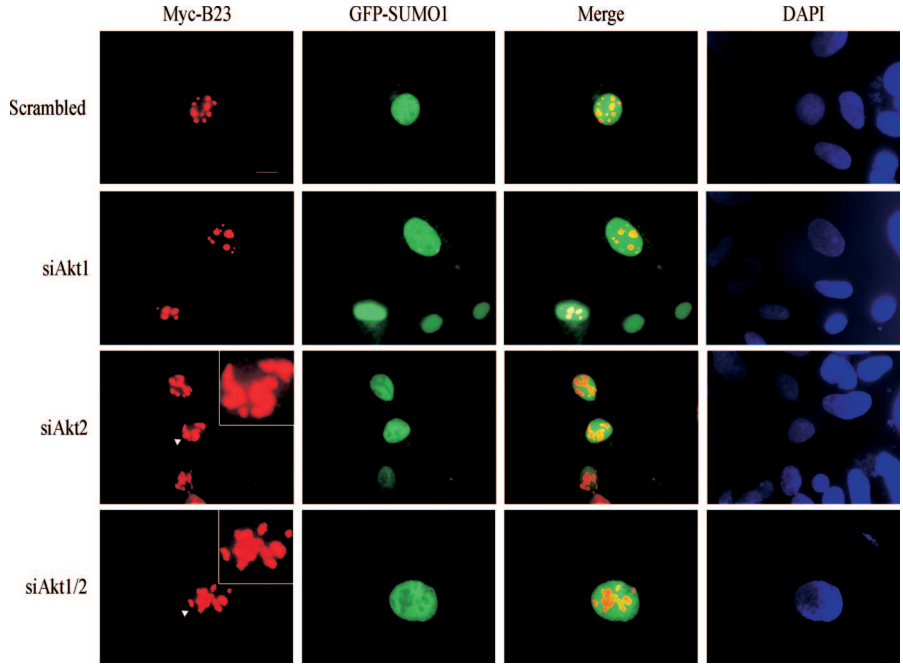
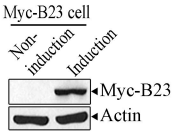


Fig. S4. Knocking down of Akt2 disrupts the colocalization of B23 and GFP-Sumo1 in the nucleolus. Myc-B23 and siRNAs were cotransfected into HEK293 cells with GFP-Sumo1. The transfected cells were stained with anti-myc antibody (red) and DAPI (blue). GFP-smo1 distributed in the nucleus and colocalized with B23 in scrambled siRNA or Si-RNA-Akt1 transfected cells. B23 coagulated in the nucleus of siRNA-Akt2 or siRNA-Akt1/2 transfected cells, resulting in expulsion GFP-Sumo1 from the nucleolus. An arrowhead indicates the magnified position in the *Insets*.(Scale bar, 10 μm.)

A

Growth phase (%)	Non-induction	Induction
G1	67.6 ± 1.91	55.4 ± 1.75
S	8 ± 0.59	17.6 ± 1.20
G2/M	24.4 ± 1.37	27 ± 0.56



B

MEF WT

Growth phase (%)	Control	pGE	shB23	pEGFP	GFP-B23
SubG1	3.8 ± 0.70	3.6 ± 0.36	23.3 ± 1.76	6.2 ± 1.18	7.5 ± 0.60
G1	66.6 ± 1.27	67.6 ± 2.78	54.1 ± 1.55	65.1 ± 1.20	55.9 ± 0.42
S	13.3 ± 0.66	12.3 ± 1.17	10.5 ± 0.80	12.6 ± 0.93	20.6 ± 0.67
G2/M	16.3 ± 1.18	16.5 ± 2.01	12.1 ± 3.12	15.9 ± 1.63	16 ± 0.89

MEF 1KO

Growth phase (%)	Control	pGE	shB23	pEGFP	GFP-B23
SubG1	3.9 ± 0.35	4.1 ± 0.38	19 ± 0.60	3.7 ± 0.25	4.8 ± 0.31
G1	34.1 ± 0.81	34.1 ± 1.25	29.9 ± 0.70	35.1 ± 0.59	32.8 ± 0.30
S	7.1 ± 0.15	5.5 ± 0.29	4.8 ± 0.15	6.2 ± 0.32	15.4 ± 0.15
G2/M	54.9 ± 0.41	56.3 ± 0.64	45.7 ± 0.27	54 ± 0.17	46 ± 0.15

MEF 2KO

Growth phase (%)	Control	pGE	shB23	pEGFP	GFP-B23
SubG1	1 ± 0.31	1 ± 0.26	15 ± 0.61	3.5 ± 0.21	2.7 ± 0.26
G1	70 ± 1.10	72.2 ± 1.00	63.5 ± 0.76	68.8 ± 0.46	64.1 ± 0.23
S	8.2 ± 0.60	7.2 ± 0.74	5.2 ± 0.36	8.4 ± 0.42	11.5 ± 0.25
G2/M	19.8 ± 1.01	19.6 ± 0.58	16.3 ± 0.79	19.2 ± 1.04	21.7 ± 0.57

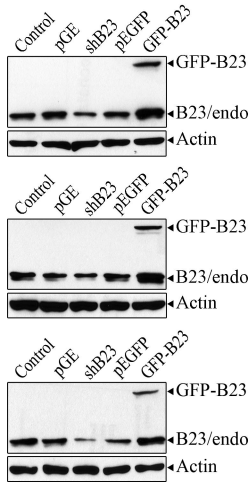


Fig. S5. Overexpression of B23 increases S phase. (*A*) Myc-B23 stably transfected PC12 cells were cultured in induced or noninduced conditions for 30 h before FACS analysis. Induction of B23 enhanced the cell numbers in the S phase. The distribution of cell phase is shown in the table. Verification of induction condition and loading control is shown by using anti-myc and anti-β actin antibodies (*Right*). (*B*) Akt regulates cell cycle progression an isoform specific manner, cooperating with B23. MEF WT, MEF 1KO, MEF 2KO, and MEF DKO cell lines were transfected with pGE-1(control vector for shRNA), shRNA-B23, pEGFP-C2, or pEGFP-B23 constructs for 30 h. Then the cells were stained with PI before FACS analysis.

A Hela cell

Growth phase (%)	Control	Scrambled	siAkt1	siAkt2	siAkt1/2
G1	59.5	59.3	61.1	62	62.9
S	10.9	14.8	15.8	12	16.5
G2/M	29.6	25.9	23.1	26	20.6

HEK293

Growth phase (%)	Control	Scrambled	siAkt1	siAkt2	siAkt1/2
G1	64.4	66.3	64.2	63.3	63.3
S	15.7	12.8	14.8	14.7	14.9
G2/M	19.9	20.9	21	22	21.8

B

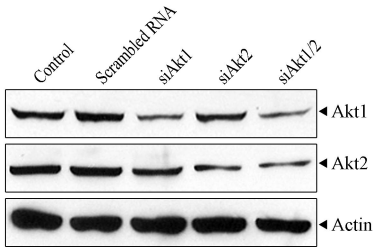


Fig. S6. Transient transfection of si-RNA-Akt does not alter cell cycle progression. (*A*) HEK 293 cells or HeLa cells were transfected with Si-Akts for 30h. Then the cells were stained with PI before FACS analysis. (*B*) Knocking down of Akt was analyzed by Western blotting by using HeLa cell extract.