Figure Legends

Figure 1. Skp2 is acetylated by p300 at K68 and K71.

A. Immunoblot (IB) analysis of 293T whole cell lysates (WCL) and anti-p300 immunoprecipitates (IP). Rabbit IgG was used as a negative control for the immunoprecipitation procedure.

B-C. Immunoblot (IB) analysis of whole cell lysates derived from HeLa cells that were serum-starved for 24 hours and then collected after 1 hour following addition of insulin to activate the PI3K/Akt signaling pathway. Cells were pretreated with TSA (2 μM) and NAM (10 mM) for 1 hour before the addition of insulin.

D. Schematic representation of the Skp2 deletion mutants used in **E-F**.

E-F. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Flag immunoprecipitates (IP) derived from 293T cells transfected with HA-p300 and various Flag-human Skp2 constructs.

G. Sequence alignment of the putative acetylation sites K68 and K71 in Skp2 from different species.

H. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Flag immunoprecipitates (IP) derived from 293T cells transfected with HA-p300 and the indicated Flag-mouse Skp2 constructs.

(see also Figure S1)

Figure 2. SIRT3 interacts with, and deacetylates Skp2.

A. Autoradiography of ^{35}S-labelled Sirtuins bound to HA-Skp2 immunoprecipitated from the transfected 293T cells, empty vector (EV) transfection was used as a negative control.

B. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Skp2 immunoprecipitates (IP) derived from HeLa cells that were infected with the indicated lentiviral vector. Anti-HA IgG was used as a negative control for the immunoprecipitation procedure.

C. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Flag immunoprecipitates (IP) derived from 293T cells transfected with HA-p300, Flag-Skp2 and the various Flag-SIRT3 constructs.

D. Schematic representation of the various biotinylated peptides used in **E**. Where indicated, the K68 and/or K71 residue is acetylated.

E. 2 μg of indicated peptides were incubated in the presence or absence of recombinant SIRT3 at 37°C for 2 hours. Where indicated, 1.5 mM NAD or 20 mM NAM was added into the reaction. The indicated amount of peptides after reaction were spotted on nitrocellulose membrane and immunoblotted with the Ac-K68K71-Skp2 antibody.

F. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Skp2 immunoprecipitates (IP) derived from HeLa cells that were infected with the indicated lentiviral vector. Before Skp2 immunoprecipitation, the indicated cells were serum starved for 24 hours, and then collected at the indicated time periods following the addition of insulin. Cells were pretreated with TSA (2 μM) for 1 hour before addition of insulin.

G-I. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Skp2 immunoprecipitates (IP) derived from WT or *SIRT3*$^{-/-}$ (**G-H**), *SIRT4*$^{-/-}$ (**I**) MEFs. Before Skp2 immunoprecipitation, the indicated cells were serum starved for 24 hours, and then collected 2 hours following addition of insulin. Cells were pretreated with TSA (2 μM) for 1 hour before the addition of insulin. Where indicated, 10 mM ROS inhibitor NAC was added for 16 hours before harvesting for IP (**H**).

J. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Skp2 immunoprecipitates (IP) derived from HeLa cells that were infected with the indicated lentiviral vector. Before Skp2 immunoprecipitation, the indicated cells were serum starved for 24 hours, and then collected at the indicated time periods following the addition of insulin. Cells were pretreated with TSA (2 μM) for 1 hour before addition of insulin.

K. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from WT or *SIRT3*$^{-/-}$ MEFs.

L-M. Representative images of SIRT3 and Skp2 expression in breast tumor cells as assessed by immunohistochemistry (**L**). Both Skp2 and SIRT3 levels were classified as Low, Medium or High based on the intensities of the IHC staining, and the percentages of patients classified in each category are depicted in histogram in **M**.

N. Growth curves for the xenograft experiments with the indicated tumor cells that were inoculated subcutaneously. 8 $x10^6$ cells were injected into each flank of 9 nude mice. The visible tumors were measured at the indicated days. Error bars, $\pm$SEM and * indicates $p < 0.05$ (Student's t-test).

(see also Figure S2)

Figure 3. p300-dependent acetylation of Skp2 impairs Cdh1-mediated Skp2 proteolysis pathway.

A. Immunoblot analysis of HeLa cells transfected with the indicated siRNA oligos, after synchronization with nocodazole and release at the indicated time periods.

B. Immunoblot analysis of HeLa cells transfected with limiting amount of the indicated Flag-Skp2 constructs, along with a green fluorescent protein (GFP) as a transfection control. HeLa cells were synchronized in the M phase with nocodazole and then released into G1 for the indicated time periods.

C. Autoradiography of ^{35}S-labelled Cdh1 bound to the indicated GST fusion proteins.

D. Schematic representation of the various biotinylated peptides used in Figures **3E-F** and **5D-E**, which are derived from the Cdh1-interaction motif of Skp2. Where indicated, the K68 and/or K71 residue is acetylated.

E. Autoradiography of ^{35}S-labelled Cdh1 bound to the indicated biotinylated peptides.

F. Autoradiography of ^{35}S-labelled Cdh1 bound to the indicated biotinylated peptides that have been subject to SIRT3 *in vitro* deacetylation assays as described in **2E**.

(see also Figure S3)

Figure 4. Acetylation of Skp2 positively regulates Skp2 oncogenic functions.

A. Immunoblot analysis of LNCaP cell lines stably transfected with the indicated HA-Skp2 constructs.

B. Cell growth curves of the various LNCaP cell lines stably expressing the indicated HA-Skp2 constructs. Results were presented as means $\pm$ SD from three independent experiments.

C. Various LNCaP cell lines stably expressing the indicated HA-Skp2 constructs were pulsed with BrdU for 30 minutes and the BrdU incorporation rate was measured. Results were presented as means $\pm$ SD from three independent experiments.

D-E. LNCaP cells stably transfected with the indicated HA-Skp2 constructs (with empty vector as a negative control) were injected subcutaneously into nude mice (n=5 for each group) and examined over time for *in vivo* tumorigenesis. Pictures in **E** were taken 6 weeks after injection. Results were presented as means $\pm$ SD and * indicates $p < 0.05$ (Student's t-test).

(see also Figure S4)

Figure 5. Acetylation of Skp2 by p300 promotes Skp2 cytoplasmic localization.

A. Sequence alignment of the Skp2 NLS with p21, p27 and FOXO1 NLS.

B. Immunofluorescence and DAPI staining of 293T cells transfected with the indicated Flag-Skp2 constructs.

C. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Flag immunoprecipitates (IP) derived from 293T cells transfected with the indicated Flag-Skp2 constructs.

D. Autoradiography of ^{35}S-labelled importin α1 or importin α5 bound to the indicated biotinylated peptides.

E. Autoradiography of ^{35}S-labelled importin α5 or importin α7 bound to the indicated biotinylated peptides which have been subject to SIRT3 *in vitro* deacetylation assays as described in **2E**.

(see also Figure S5)

Figure 6. Cytosolic Skp2 plays a critical role in cellular migration.

A-B. Skp2 is required for cell migration. WT and *Skp2*$^{-/-}$ MEFs were plated for *in vitro* wound healing assays (**A**). Results in **A** were quantified in **B** and presented as means ± SD from three independent experiments.

C. WT and *Skp2*$^{-/-}$ MEFs were infected with the indicated viral constructs before plating for transwell assay. The results were quantified and presented as means ± SD from three independent set of experiments.

D. Immunoblot analysis of WT and *Skp2*$^{-/-}$ MEFs, synchronized by serum starvation for 72 hours and then released by re-addition of serum for the indicated periods.

E-F. Immunoblot analysis of normal human fibroblasts (**E**) or SKOV3 epithelial cancer cell line (**F**) infected with the indicated lentiviral shRNA vectors.

G. Immunoblot analysis of WT and *Skp2*$^{-/-}$ MEFs infected with the indicated viral constructs.

H. Immunoblot analysis of DU145 cells infected with the indicated viral constructs.

I-J. Immunoblot analysis of MCF7 (**I**) or ZR75 epithelial cancer cell line (**J**) infected with the indicated lentiviral shRNA vectors.

K. Immunoblot (IB) analysis of MCF7 whole cell lysates (WCL) and anti-E-cadherin immunoprecipitates (IP). Anti-HA IgG was used as a negative control for the immunoprecipitation procedure.

L. Immunoblot (IB) analysis of whole cell lysates (WCL) of HeLa cells transfected with the indicated plasmids. Twenty hours post-transfection, cells were treated with the proteasome inhibitor MG132 overnight before harvesting.

M-N. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from DU145 (M) or SKOV3 (N) cells treated with 10 μM MG132 for 12 hours before harvesting.

O-P. Representative images of E-cadherin and Skp2 expression in breast tumor cells as assessed by immunohistochemistry (**O**). Both Skp2 and E-cadherin levels were classified as Low, Medium or High based on the intensities of the IHC staining, and the percentages of patients classified in each category are depicted in histogram in **P**.

(see also Figure S6)

Figure 7. Skp2 promotes the ubiquitination and destruction of E-cadherin in a CKI-dependent manner.

A. Sequence alignment of the putative CKI phosphorylation sites in Skp2 from various species.

B. Indicated GST-fusion proteins were incubated with CKIδ and [γ-^{32}P]ATP. The kinase reaction products were resolved by SDS-PAGE and phosphorylation was detected by autoradiography.

C-D. Autoradiography of ^{35}S-labelled Skp2 bound to the indicated GST-fusion proteins. Where indicated, GST-fusion proteins were pretreated with CKIδ before pull-down assays were performed.

E. Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Myc immunoprecipitates (IP) derived from HeLa cells transfected with the indicated Myc-E-cadherin constructs.

F. Immunoblot (IB) analysis of whole cell lysates (WCL) from HeLa cells transfected with the indicated plasmids.

G. SCF^{Skp2} E3 ligase complex promotes E-cadherin ubiquitination *in vitro*. Where indicated, GST-E-cadherin proteins were pretreated with CKI before the *in vitro* ubiquitination assays.

H. Proposed model for how acetylation of Skp2 is governed by both p300 and SIRT3, which subsequently regulates Skp2 stability and cellular localization to influence its oncogenic functions.

(see also Figure S7)

Figure 1

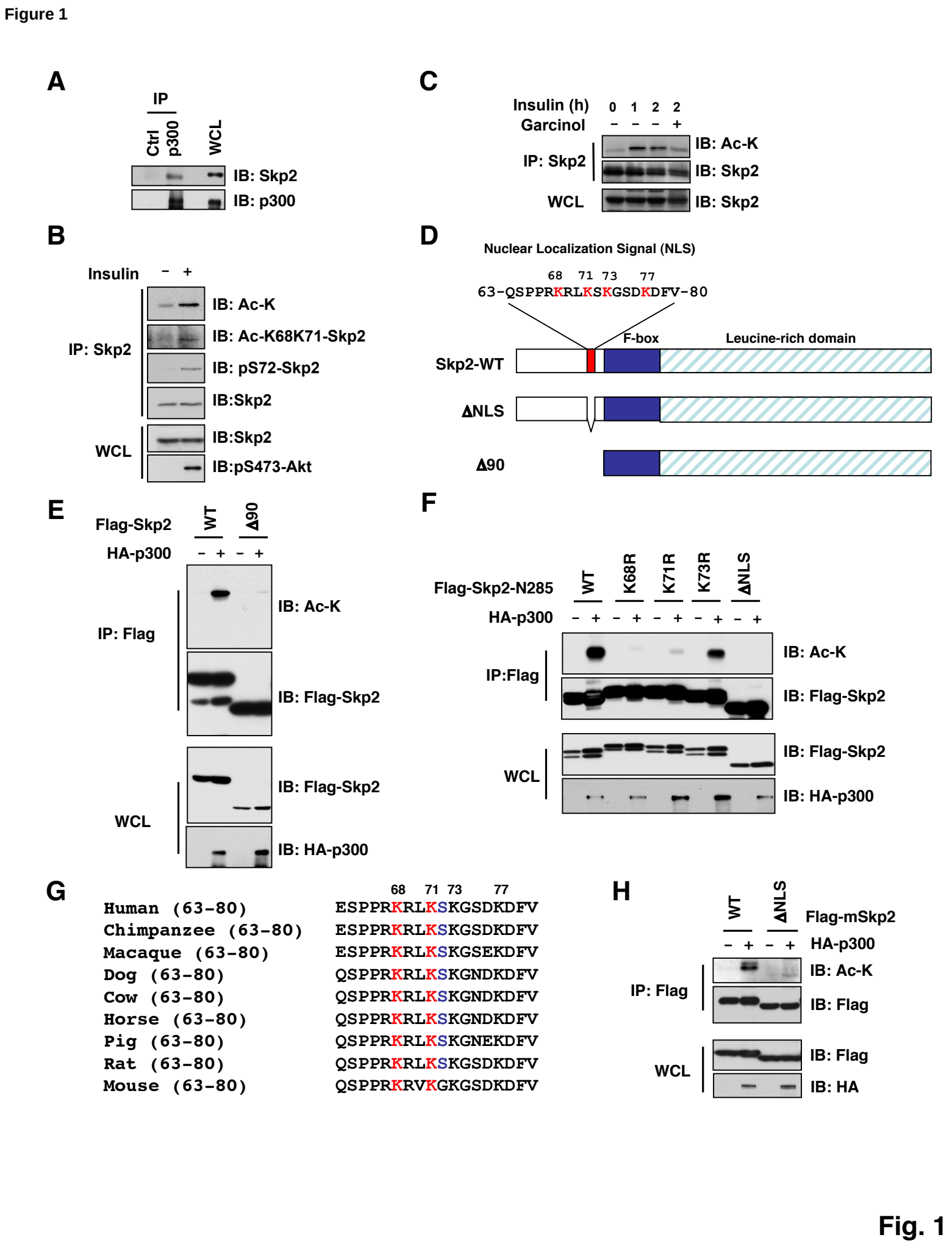

Fig. 1

Figure 2

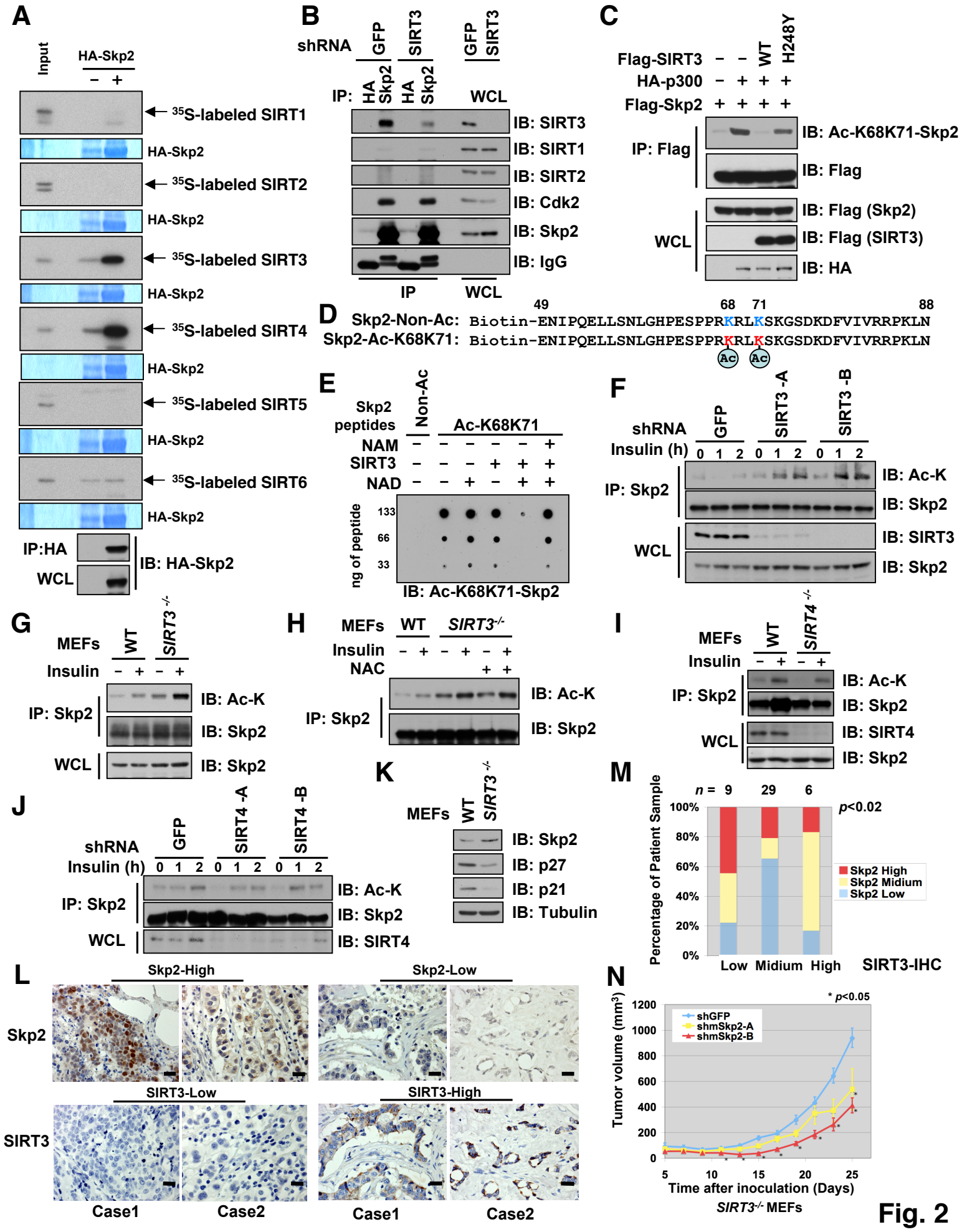

Fig. 2

Figure 3

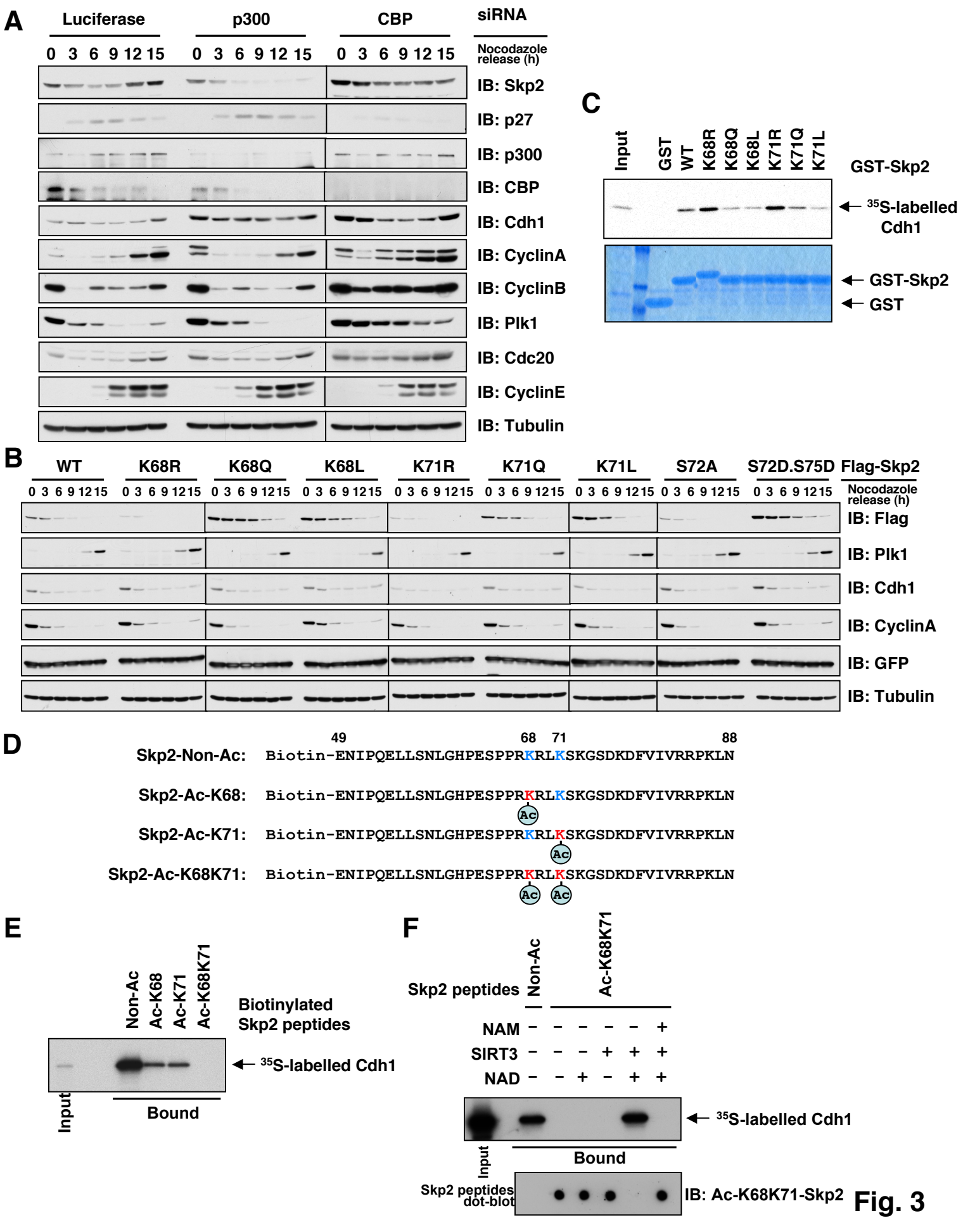

Fig. 3

Figure 4

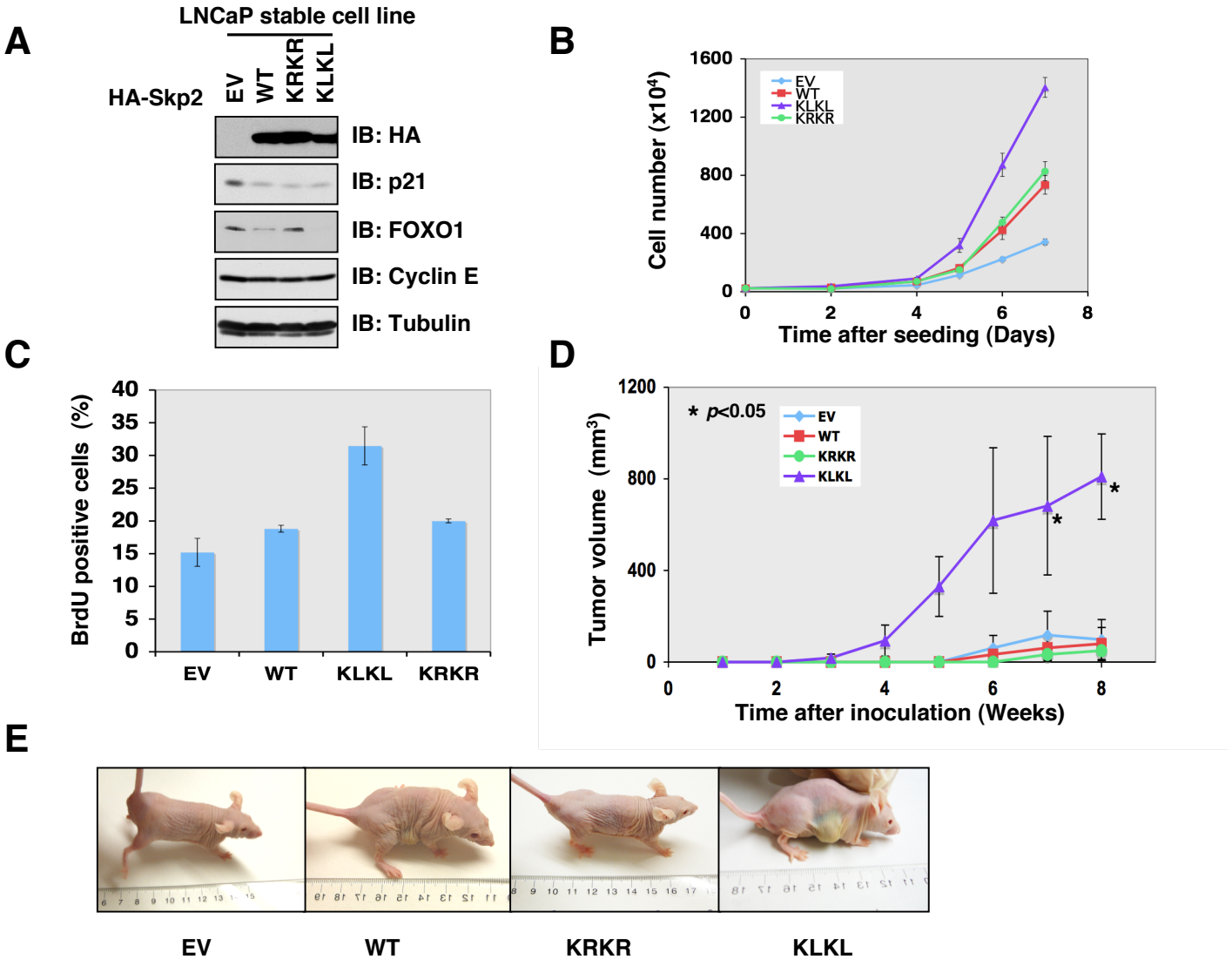

Fig. 4

Figure 5

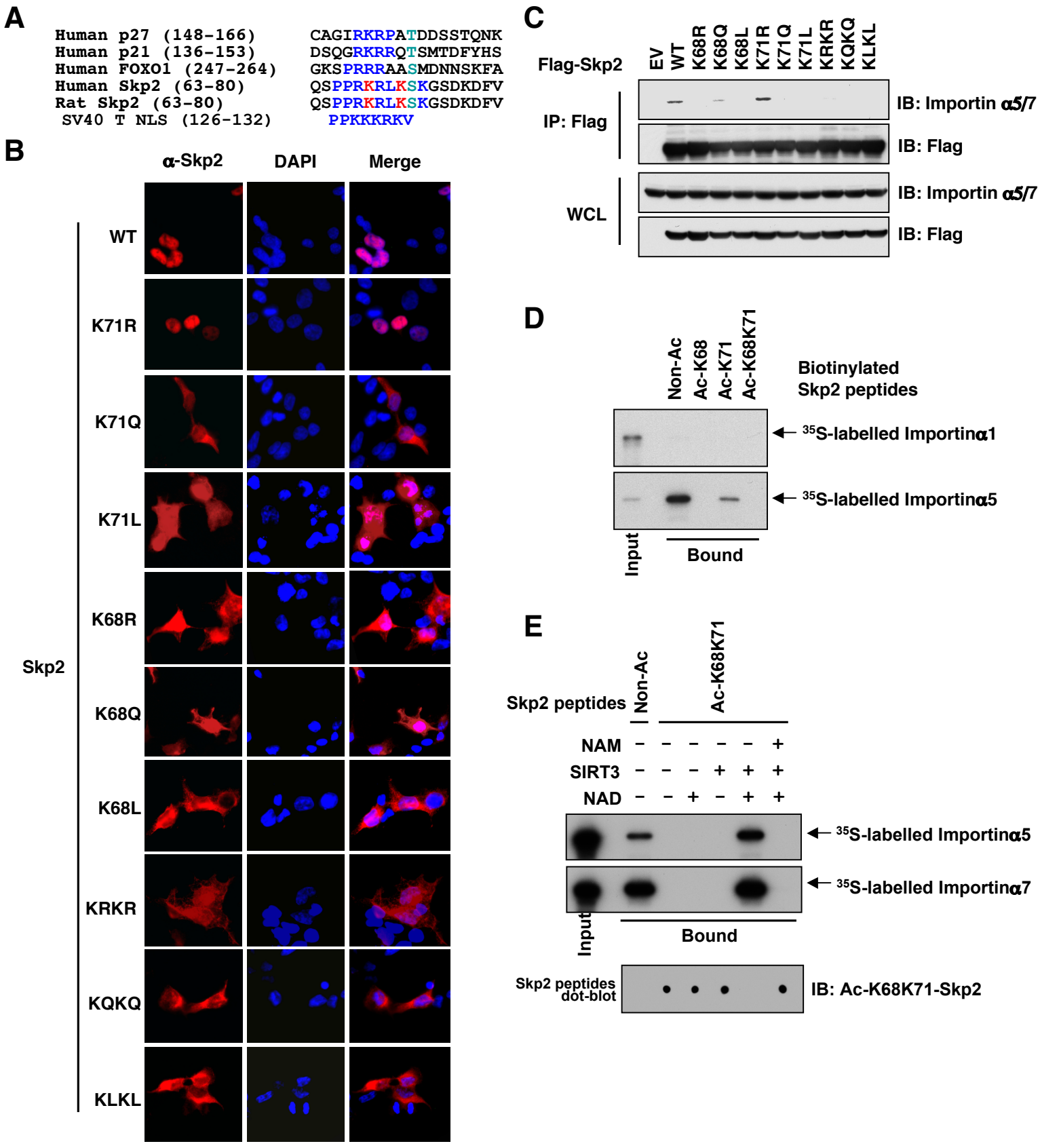

Fig. 5

Figure 6

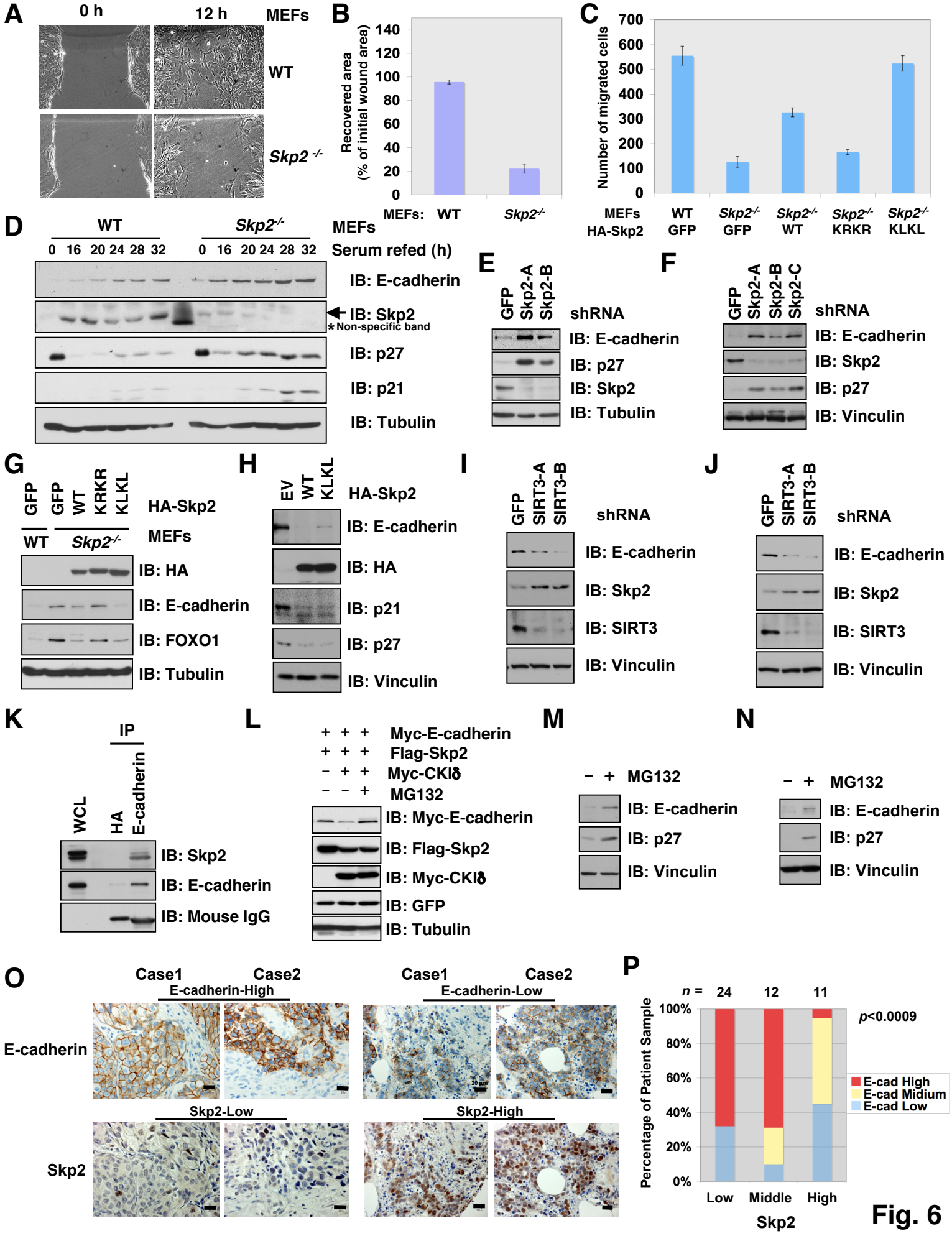

Fig. 6

Figure 7

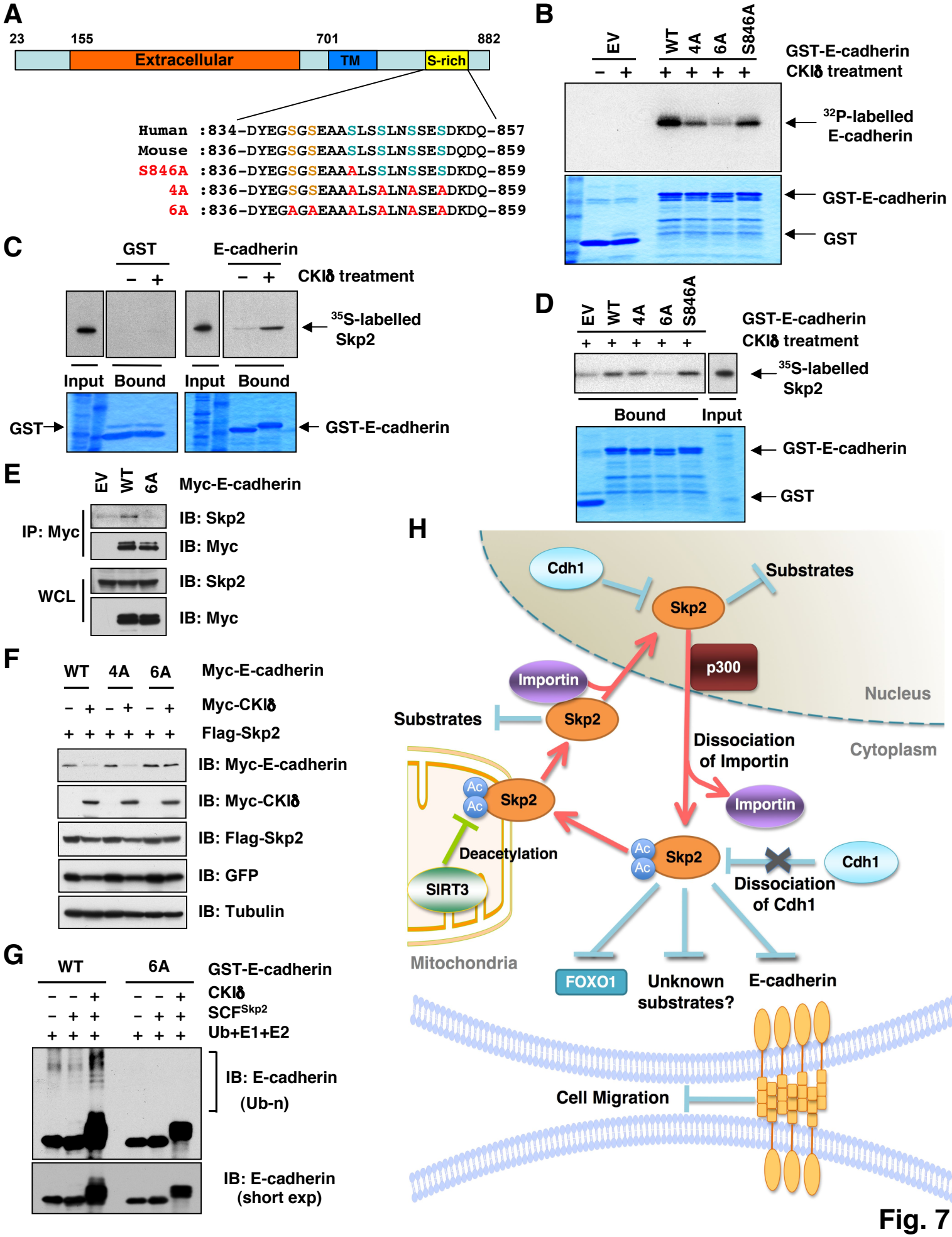

Fig. 7