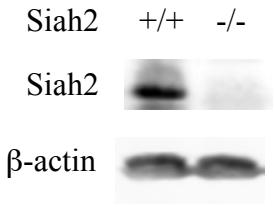
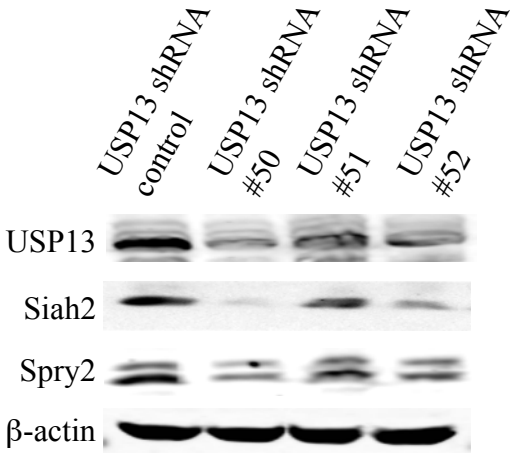
Sup. Fig. 1

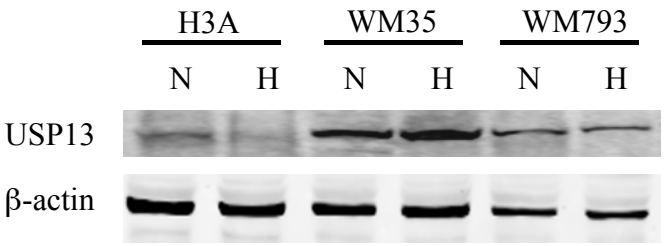


Sup. Fig. 2

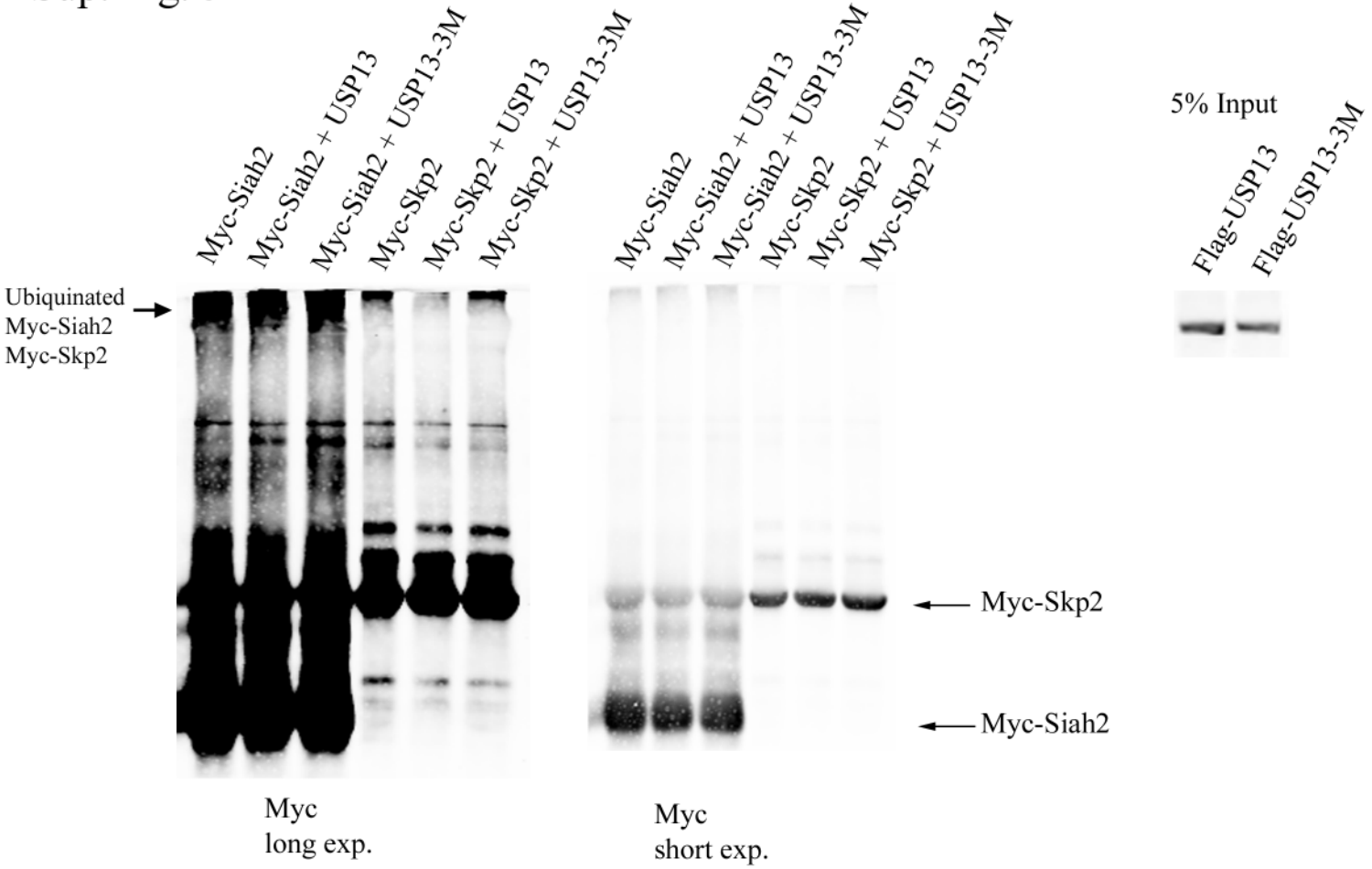


Sup. Fig. 3

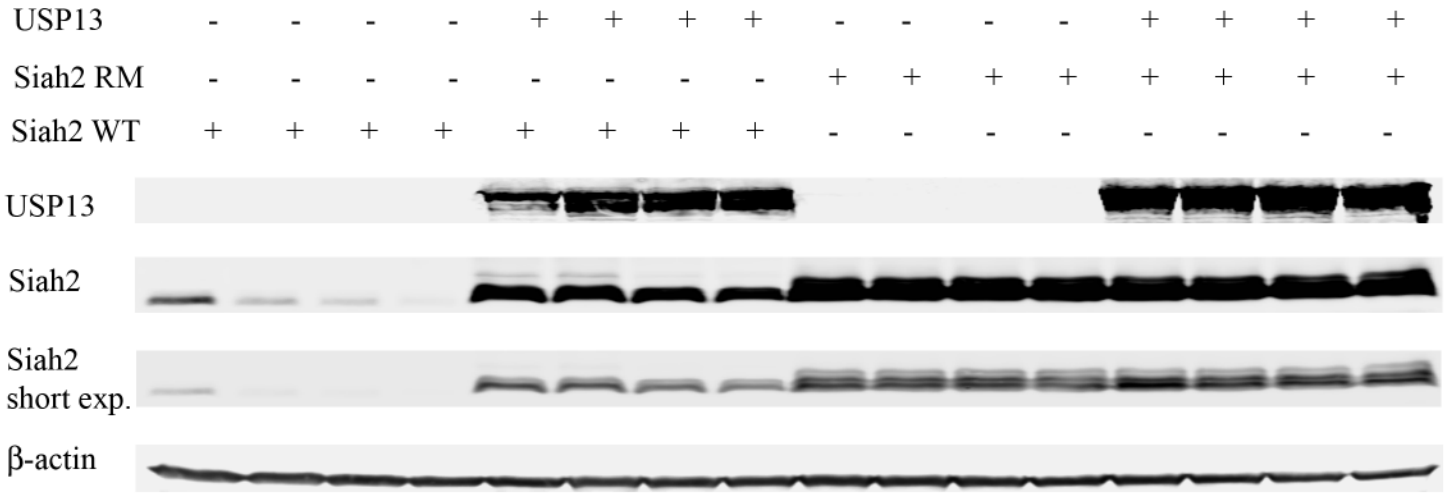
Sup. Fig. 4



Sup. Fig. 5



Sup. Fig. 6



Supplementary Figures

Supplementary Figure 1. Siah2 WT and Siah2 KO MEFs were harvested and cell lysates were subjected to western blot analysis to detect Siah2. Beta actin served as the loading control.

Supplementary Figure 2. Analysis of different USP13-KD by different USP13 shRNAs confirmed the major biochemical phenotypes. HeLa cells were transfected with different USP13 ShRNAs. Empty pLKO.1-Puro[3] vector served as control. Cells were harvested and cell lysates were subjected to western blot analysis to detect USP13, Siah2, and Spry2. Beta actin served as the loading control.

Supplementary Figure 3. Outline of USP13 functional domains. The positions of the single mutations are shown in this model.

Supplementary Figure 4. Melanoma cell lines WM35, WM793 and melanocytes were maintained in normoxia or subjected to hypoxia (2% O_2) conditions for 24 h. Cells were harvested and cell lysates were subjected to western blot analysis to detect USP13. Beta actin served as the loading control.

Supplementary Figure 5. 293T cells were transfected with ubiquitin along with Myc-Siah2 or Myc-Skp2 for 24 hours and incubated with MG132 (20 μM) for an additional 4 h. Siah2 and Skp2 were immunoprecipitated with anti-Myc antibody in the presence of protein A/G agarose. Immunoprecipitated Myc-Siah2 or Myc Skp2 bound to protein A/G

agarose beads were incubated with immunopurified Flag-USP13 for 1 h at 37^{o}C. The agarose beads-bound proteins were then washed and Myc-Siah2 and Myc Skp2 were eluted and separated on SDS-PAGE followed by immunoblotting using antibodies against myc, which allowed detection of ubiquitinated Siah2 and Skp2. Ubiquitination of Siah2 and Skp2 was confirmed using anti-ubiquitin antibody (data not shown).

Supplementary Figure 6. 293T cells were co-transfected with Siah2 or RING mutant Siah2 (Siah2RM) and myc-USP13. Following addition of cycloheximide for 1, 3, and 5 h, cells were harvested and the cell lysates were subjected to western blot to detect USP13 and Siah2.