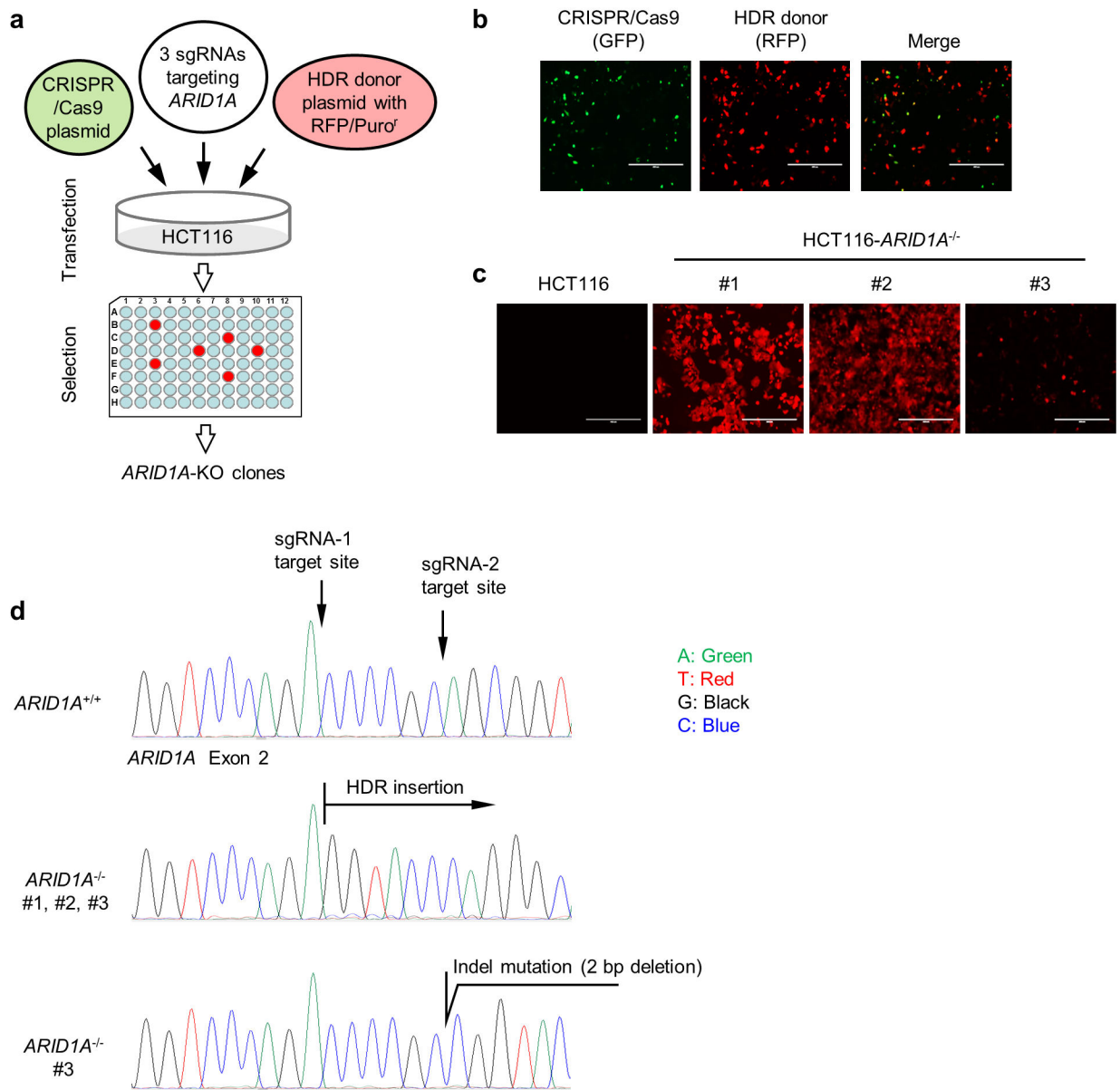


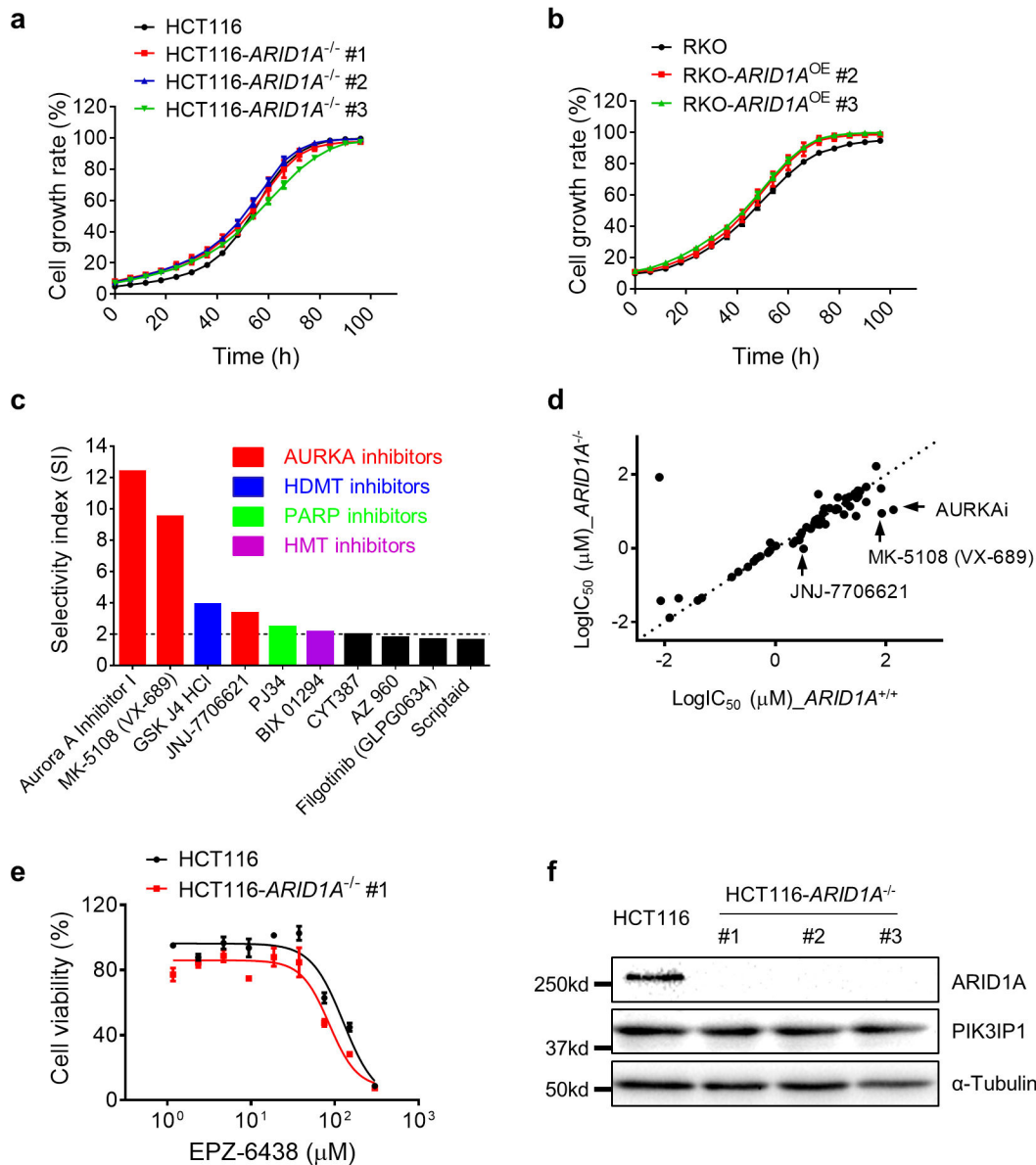
## Supplementary Information

**Targeting AURKA-CDC25C axis to induce synthetic lethality in ARID1A-deficient colorectal cancer cells**

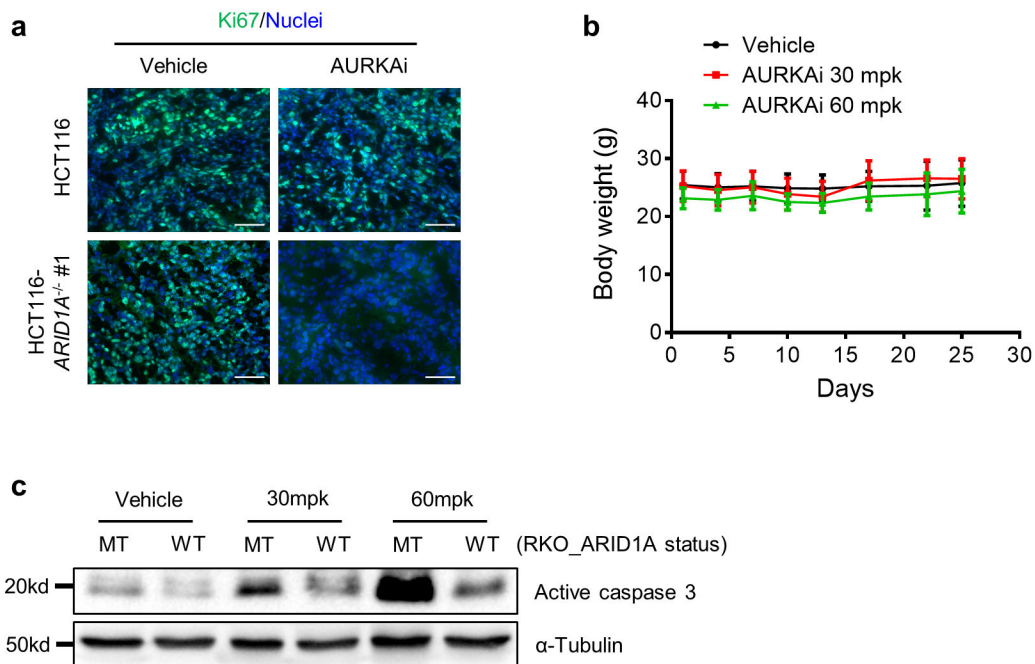
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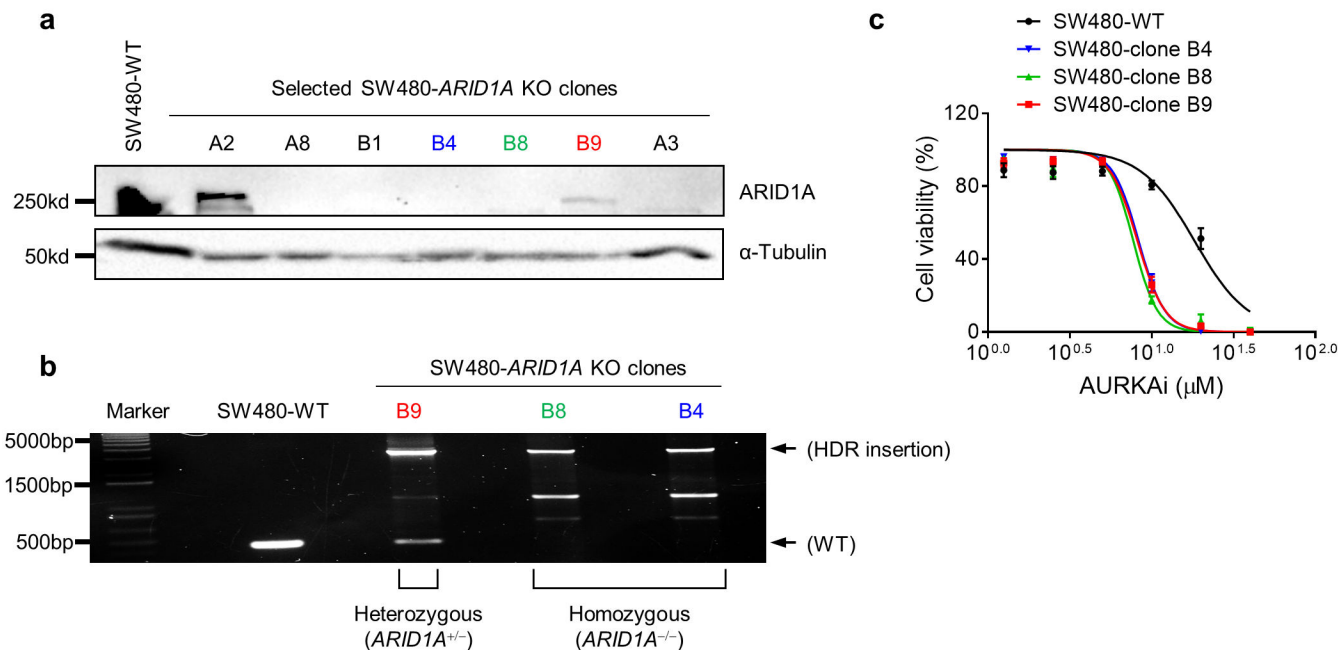
**Supplementary Figure 1. Generation of *ARID1A* knockout (KO) HCT116 cells.** **a**, HCT116 cells were transfected with CRISPR/Cas9 plasmid containing green fluorescence protein (GFP), 3 sgRNAs targeting *ARID1A* gene and HDR donor plasmid containing red fluorescence protein (RFP) and puromycin resistant gene (Puro<sup>r</sup>). After 48 h of transfection, puromycin was added. After 72 h, the cells were trypsinized and plated in 96-well plates at approximately 0.5-1 cell per well for clone selection. Single clones were picked up after they form colonies. **b**, Transfection efficiency was assessed with GFP (CRISPR/Cas9) and RFP (HDR donor plasmid). Co-transfectants are shown as yellow in the merged image. Scale bars, 400  $\mu$ m. **c**, After 2 weeks of selection and clone isolation, *ARID1A*<sup>-/-</sup> clones have red fluorescence only. Scale bars, 400  $\mu$ m. **d**, Sequencing analysis of the sgRNA target sites on *ARID1A* exon 2 in HCT116-*ARID1A*<sup>+/+</sup> and HCT116-*ARID1A*<sup>-/-</sup> clones. *ARID1A*<sup>-/-</sup> #1 and #2 have the HDR insertion at the sgRNA-1 target site. *ARID1A*<sup>-/-</sup> #3 is a heterozygote mutant that has the HDR insertion at the sgRNA-1 target site and an indel mutation (2 bp deletion) at the sgRNA-2 target site. No mutation was observed in sgRNA-3 target site on *ARID1A* exon 4 in the three *ARID1A*<sup>-/-</sup> clones.



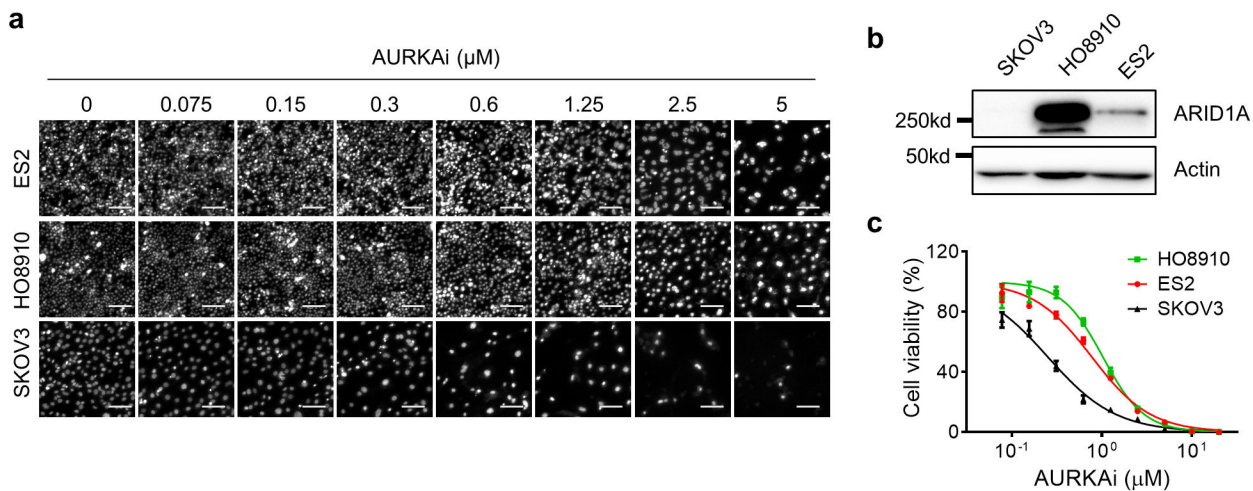
**Supplementary Figure 2. The growth rates of ARID1A-isogenic colorectal cancer cells and synthetic lethality screening.** ARID1A-isogenic HCT116 (a) and RKO (b) cell lines were grown in a 96-well ImageLock Microplate until confluent and assessed for real-time growth rate with IncuCyte-ZOOM. Error bars represent s.d. No significant difference was observed in in vitro growth rate between ARID1A<sup>+/+</sup> and ARID1A<sup>-/-</sup> cells. c, Selectivity index (SI) of the synthetic lethality candidates for ARID1A.  $SI = IC_{50}^{ARID1A(+)} / IC_{50}^{ARID1A(-)}$ . Among all the epigenetics compounds tested, top 10 candidates are shown in the graph and drugs with SI>2 are indicated. d, AURKA inhibitors identified from the screening. e, Dose response curves of ARID1A-isogenic HCT116 cell lines with EPZ-6438 (EZH2 inhibitor) treatment for 96 h. Error bars represent s.d. f, Immunoblot analysis of ARID1A and PIK3IP1 expression in ARID1A-isogenic HCT116 cell lines.



**Supplementary Figure 3. ARID1A-isogenic CRC mouse xenograft model.** **a**, Immunofluorescence staining of tumor tissues isolated from mice with a cell proliferation marker, Ki67 (green) and a nuclear counter staining with Hoechst33342 (blue). *ARID1A*<sup>-/-</sup> tumor show significant lower level of Ki67 staining upon AURKai treatment. Scale bars, 50  $\mu$ m. **b**, Mouse body weight measurement of mice treated with AURKai. Error bars represent s.d. A daily i.p. injection of AURKai (30 and 60 mg kg<sup>-1</sup> (mpk)) did not significantly affect mice body weights. **c**, Induction of apoptosis in ARID1A-deficient RKO tumor by AURKai treatment. Tumor tissues were lysed and subjected to immunoblot analysis of active casepase-3.

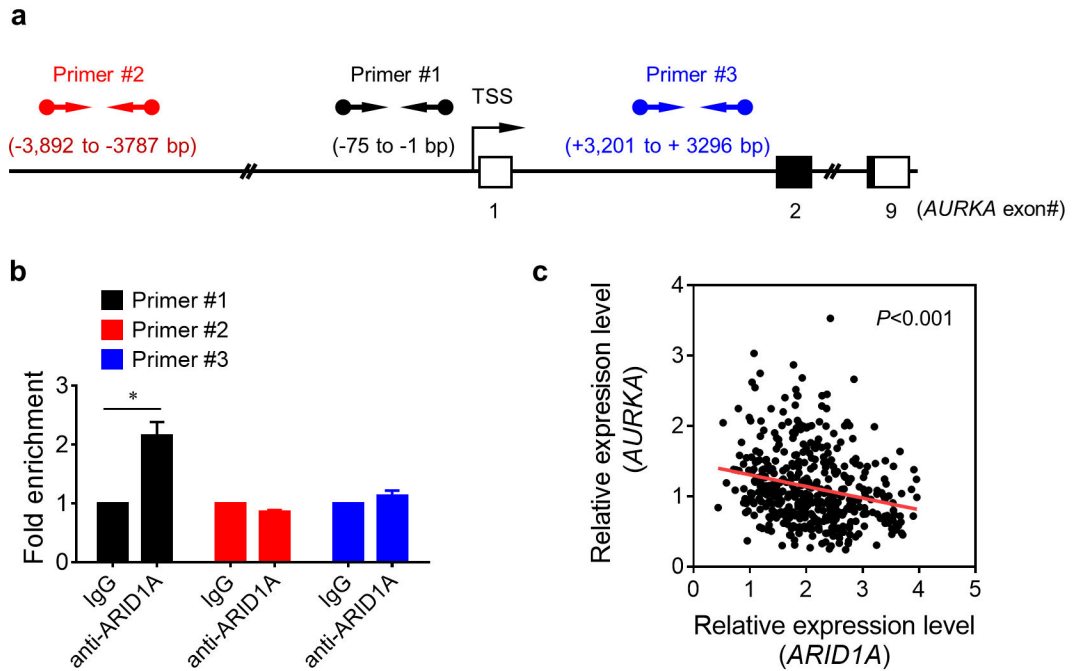


**Supplementary Figure 4. Generation of SW480 *ARID1A*-KO cells and validation of *ARID1A*-AURKA synthetic lethality.** SW480 colorectal cancer cells harboring wildtype *ARID1A* was transfected with CRISPR/Cas9 plasmid, sgRNAs targeting *ARID1A* genomic locus, and homologous-directed repair (HDR) donor plasmid containing puromycin-resistance and red fluorescence protein (RFP) genes to generate *ARID1A* KO cell lines. The RFP fluorescent cells were picked up and the KO clones were further selected with puromycin. **a**, Immunoblot analysis of *ARID1A* expression in SW480 parental and puromycin-selected *ARID1A* KO clones. Clones B4 and B8 have no detectable *ARID1A* expression, while clone B9 has barely detectable *ARID1A* expression. **b**, The three clones (B4, B8, and B9) were selected for genomic PCR analysis with primer pairs specific for sgRNA target site within *ARID1A* locus. Clone B9 has both *ARID1A* wildtype amplicon (WT) and an amplicon with HDR sequences (HDR insertion), suggesting an *ARID1A*-heterozygous KO clone. B8 and B4 clones only have HDR insertion amplicon, suggesting *ARID1A*-homozygous KO clones. **c**, Dose response curves of SW480 *ARID1A*-isogenic cell lines with AURKAi treatment. Error bars represent s.d. Both heterozygous and homozygous *ARID1A* KO clones show significantly increased sensitivity to AURKAi treatment.

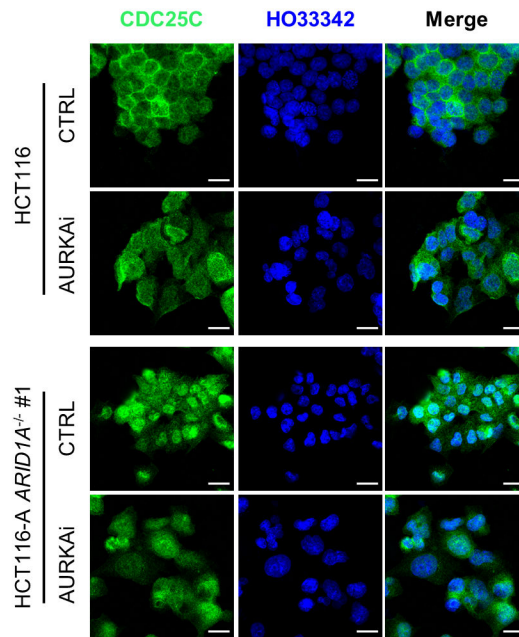


**Supplementary Figure 5. Validation of ARID1A-AURKA synthetic lethality in ovarian cancer cell lines.** **a**, Three ovarian cancer cell lines with different ARID1A status, ES2 (*ARID1A*-WT), HO8910 (*ARID1A*-WT), and SKOV3 (*ARID1A*-MT) were treated with AURKai for three days and nuclei were stained with Hoechst 33342. Scale bars, 100  $\mu\text{m}$ . **b**, Western blot analysis of ARID1A status in three ovarian cancer cell lines. Actin was used as an internal control. **c**, The cell viability was measured by counting the number of cell nuclei with Image J software. Error bars represent s.d.





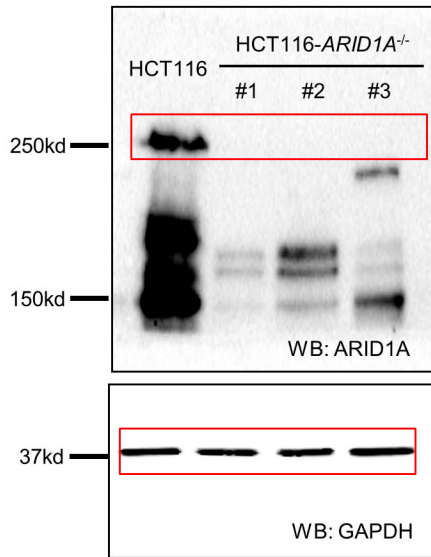
**Supplementary Figure 6. Negative regulation of *AURKA* expression by *ARID1A*.** **a**, Target regions of primer pairs used for chromatin immunoprecipitation (ChIP) of *AURKA* promoter with an anti-*ARID1A* antibody. TSS denotes the transcription start site. **b**, *AURKA* promoter ChIP was done with an anti-*ARID1A* antibody and three indicated primer pairs in HCT116 cells. Error bars represent s.d. \* $P < 0.05$ , One sample *t*-test. **c**, The transcriptome profiling data of 440 colorectal cancer patients' samples were obtained from The Cancer Genome Atlas (TCGA). The relative mRNA expression levels of *ARID1A* and *AURKA* in each sample were analyzed. Spearman correlation analysis was used to assess statistical significance.



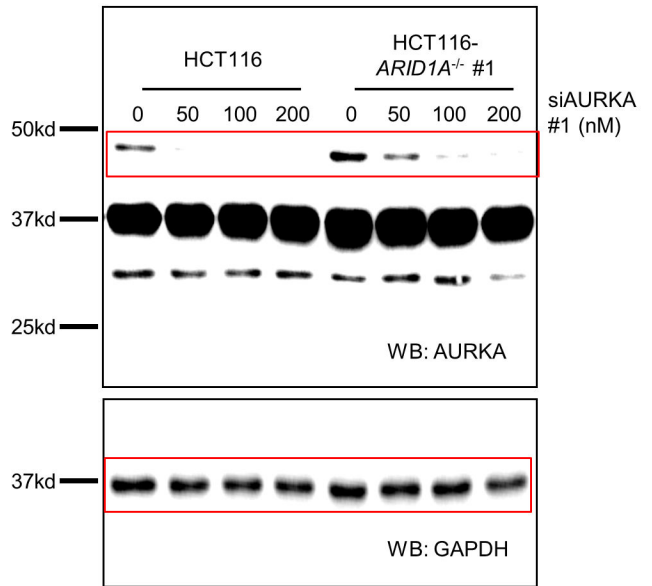
**Supplementary Figure 7. Inhibition of CDC25C nuclear localization by AURKAI.** *ARID1A*-isogenic HCT116 cells were treated with or without AURKAI and analyzed for immunofluorescence of CDC25C (green). Nuclei were stained with Hoechst33342 (HO33342). Note the significant nuclear localization of CDC25C in *ARID1A*-KO cells and its reversal by AURKAI treatment. Scale bars, 20  $\mu$ m.



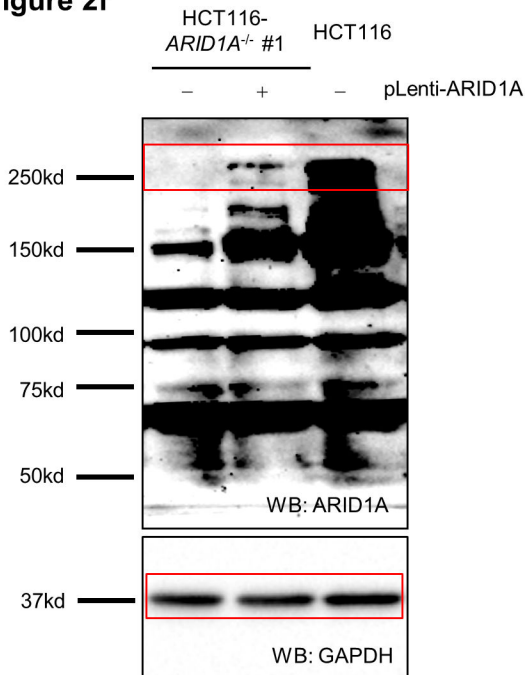
**Figure 1c**



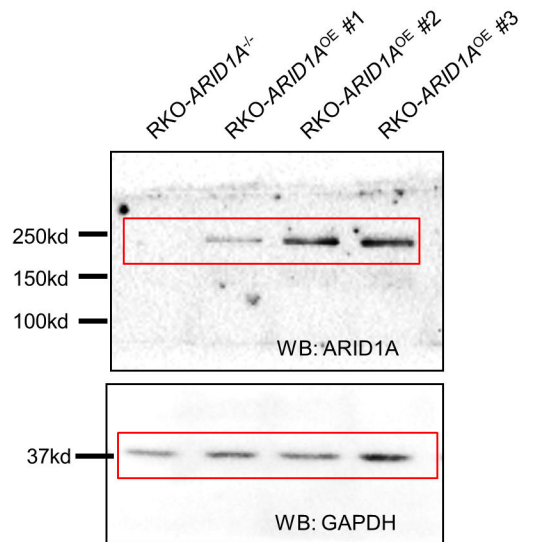
**Figure 2c**



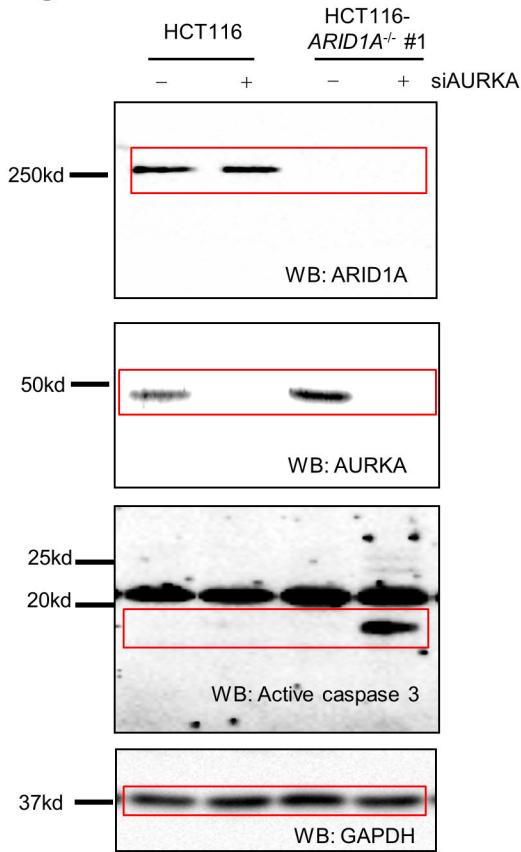
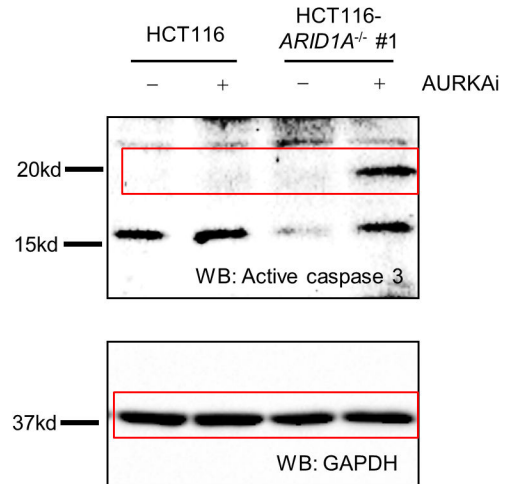
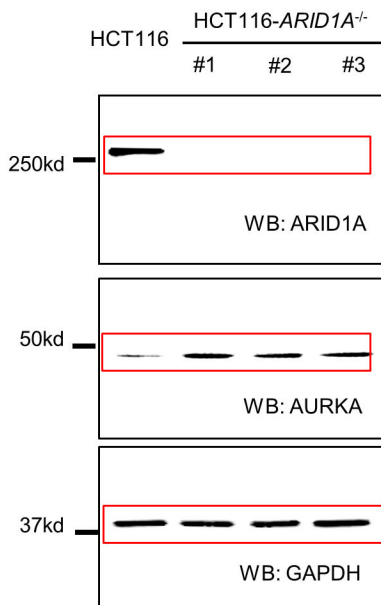
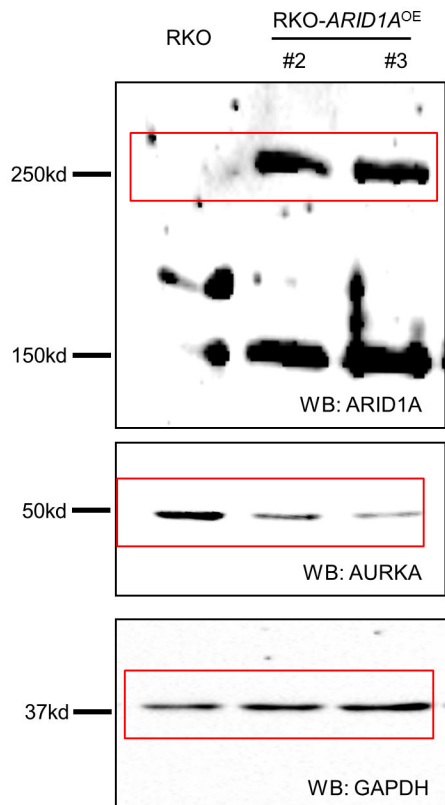
**Figure 2f**



**Figure 3a**

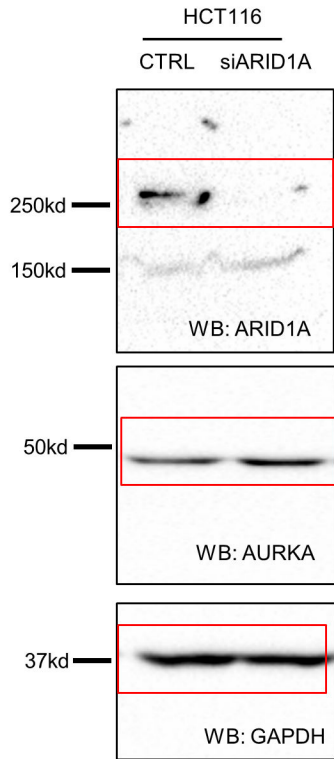


**Supplementary Figure 8. Original Western blots shown in Figures 1-3. Each figure corresponds to the Western blots in the indicated Figure number.**

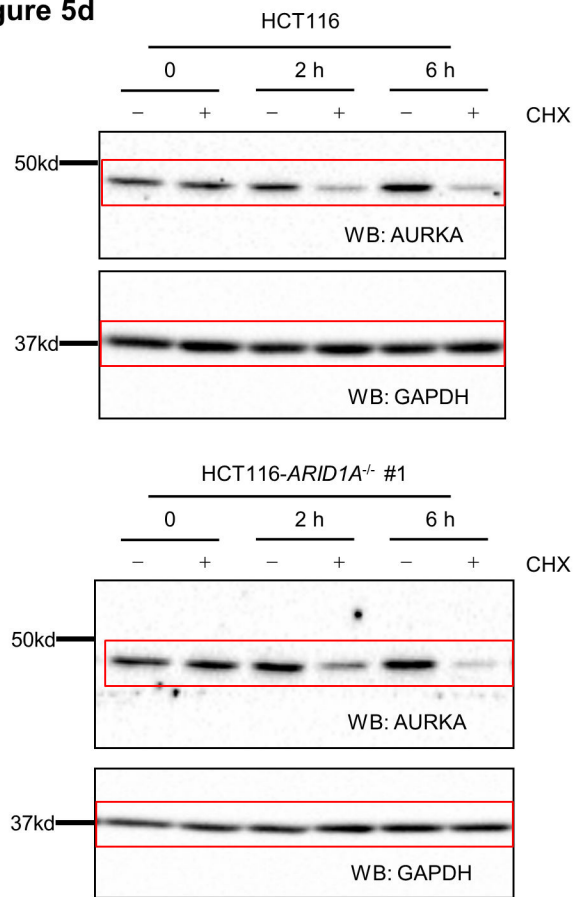
**Figure 4h****Figure 4i****Figure 5a****Figure 5b**

**Supplementary Figure 9. Original Western blots shown in Figures 4-5. Each figure corresponds to the Western blots in the indicated Figure number.**

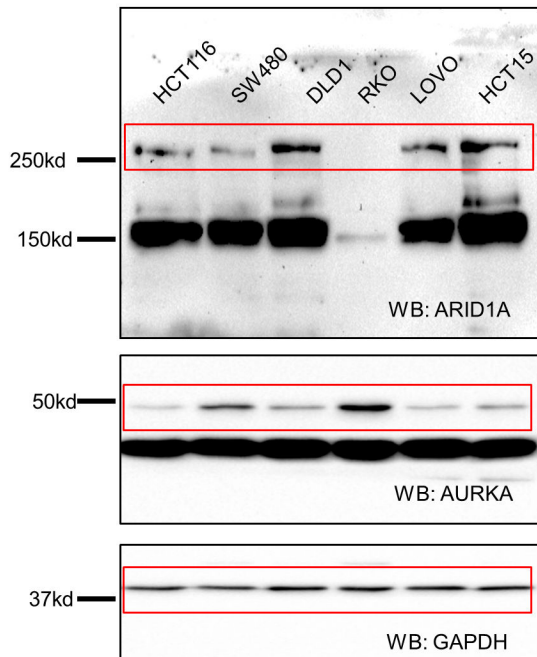
**Figure 5c**



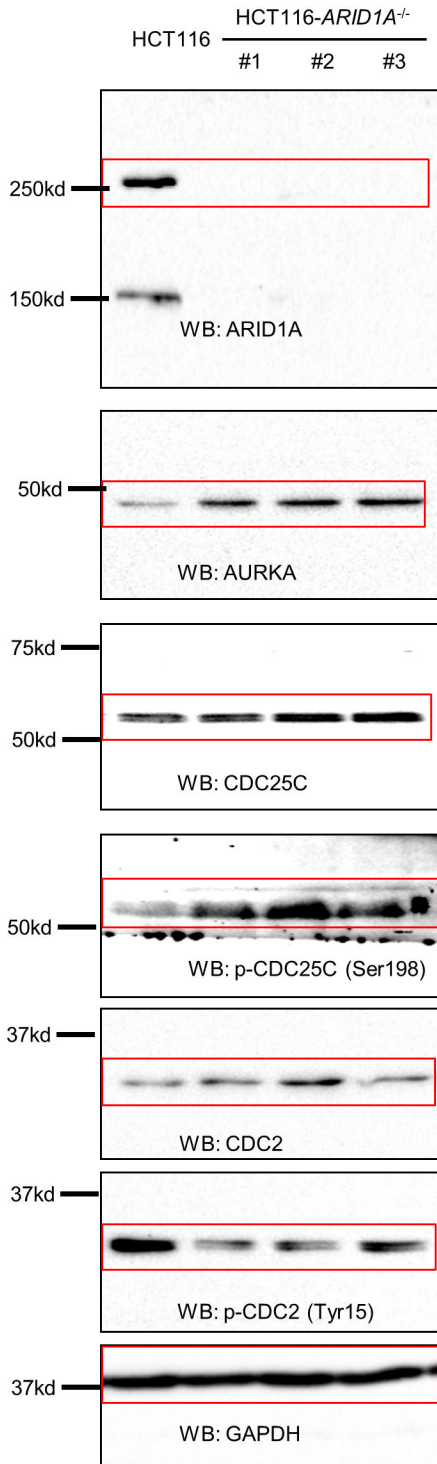
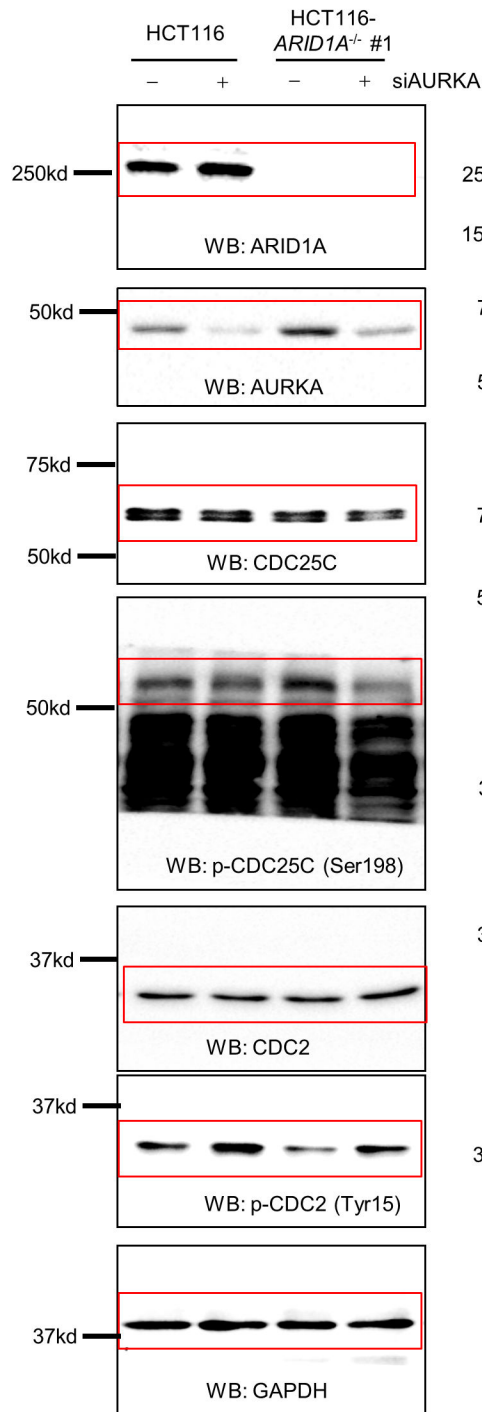
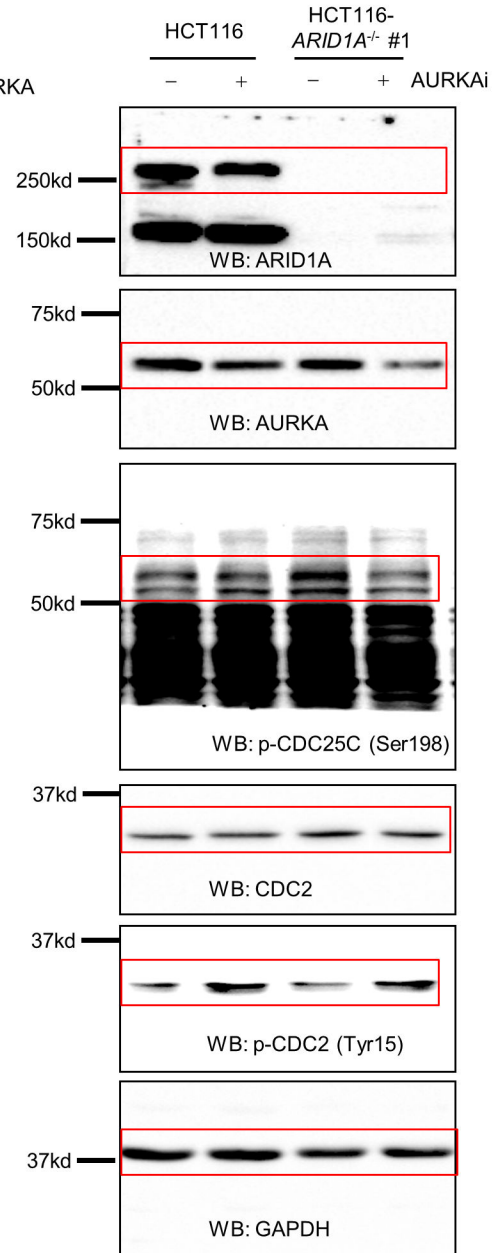
**Figure 5d**



**Figure 5m**

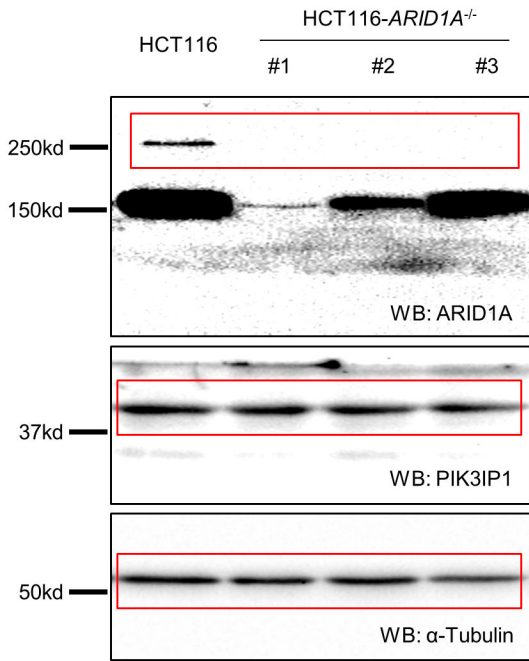


**Supplementary Figure 10. Original Western blots shown in Figure 5. Each figure corresponds to the Western blots in the indicated Figure number.**

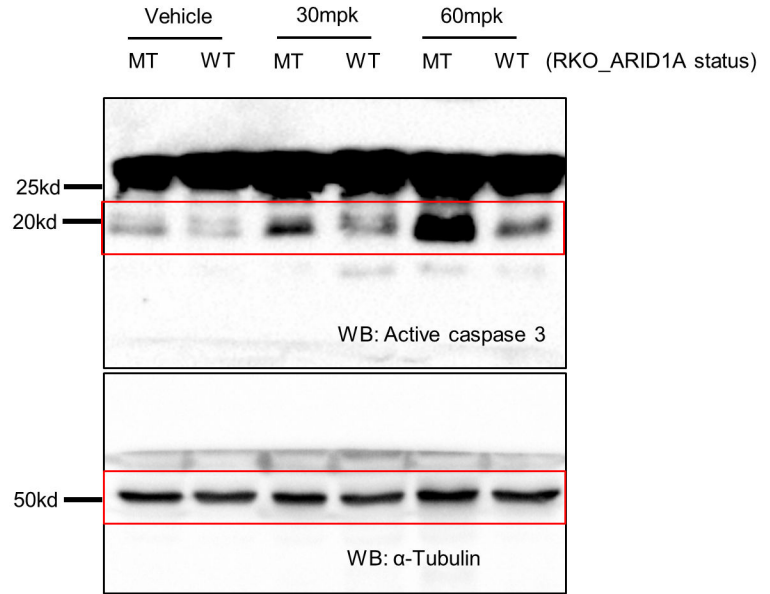
**Figure 6a****Figure 6b****Figure 6c**

**Supplementary Figure 11. Original Western blots shown in Figure 6. Each figure corresponds to the Western blots in the indicated Figure number.**

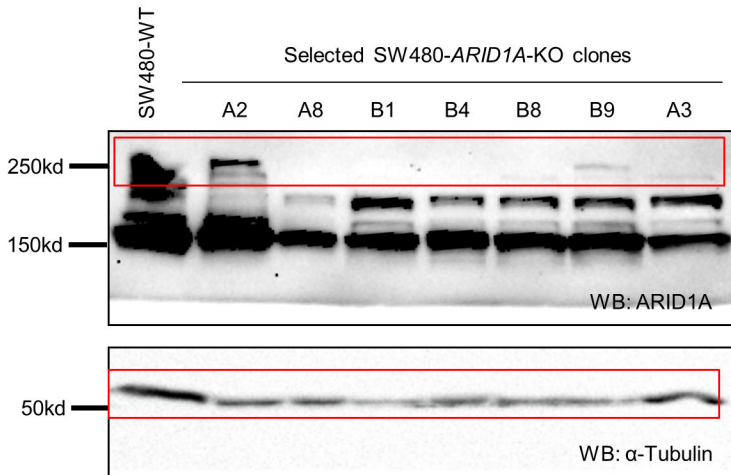
### Supplementary Figure 2f



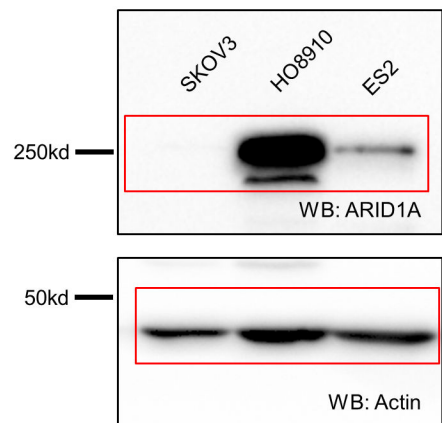
### Supplementary Figure 3c



### Supplementary Figure 4a



### Supplementary Figure 5b



**Supplementary Figure 12.** Original Western blots shown in Supplementary Figures 2-5. Each figure corresponds to the Western blots in the indicated Figure number.