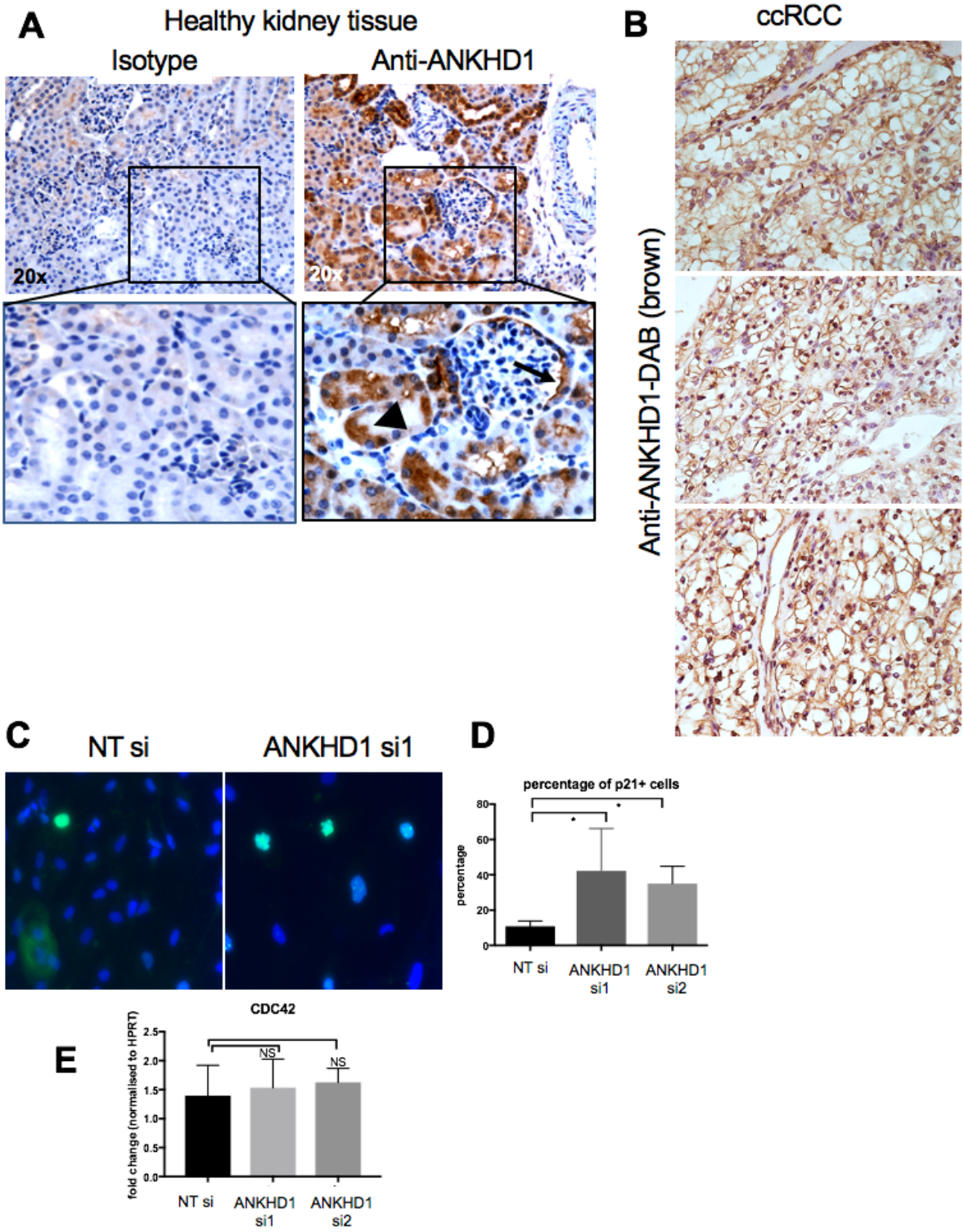
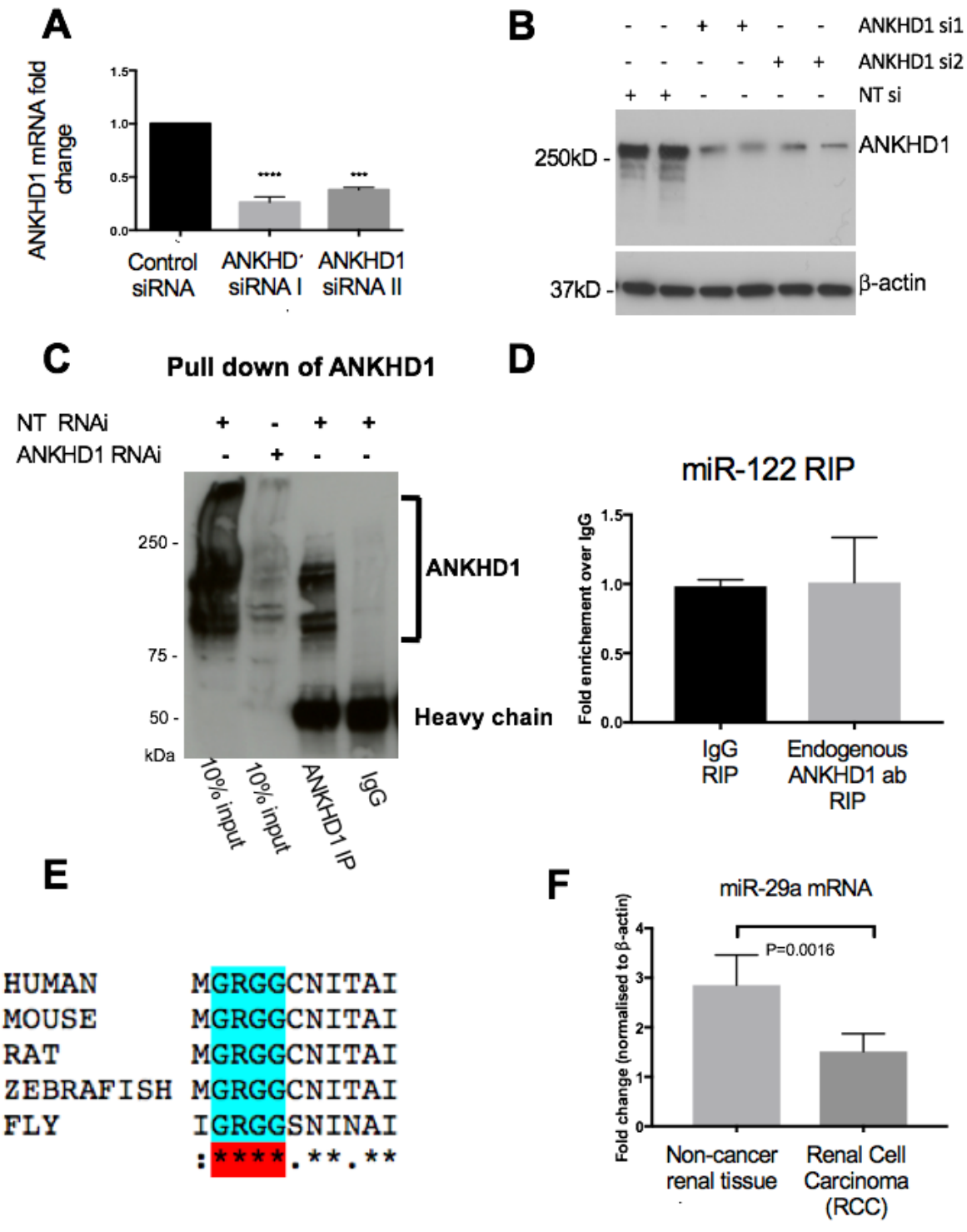
Supplementary material 1



SI Figure 1: A. IHC of normal healthy kidney tissue was carried out and isotype control (left panel) was examined as well as anti-ANKHD1 (right panel). **B.** More examples of anti-ANKHD1 staining in RCC human tissues are shown, each vertical panel

represents a different RCC patient. **C.** Cells were stained by IF with anti-p21 antibody to study whether ANKHD1 silencing results in alterations of p21 as previously suggested in literature, p21 is in green and nuclei are counterstained in blue (dapi). **D.** Three independent experiments were performed and number of p21 positive cells were counted and shown as a percentage. Student t-test was carried out and p value of less than 0.05 was considered as significant. **E.** qPCR of one of the bioinformatics-identified target of miR-29a, CDC42, did not change significantly following knockdown of ANKHD1.

Supplementary material 2



SI Figure 2: A. qPCR validation of the level of ANKHD1 knockdown following transfection with two independent siRNAs. **B.** Confirmation of the level of knockdown using Western blotting. **C.** The level of pull down used in RIP assays is assessed using Western blotting. **D.** RIP-qPCR of miR-122, one of the identified altered miRNAs, was

performed showing that it does not physically associate with ANKHD1. **E.** Actual amino acid homology of the GxxG box of ANKHD1 in human, mouse, rat, zebrafish and Drosophila is shown. F. qPCR analysis of miR-29a in RCC and healthy control tissues was carried out.

Table 1

miRNA name	GO function	Validated genes	P value With Benjamini correction *	Validated by qPCR in this study
miR-29a-3p	Cell cycle control	CDC42, CCND1, CCNT2, CDK2, CDK6,BCL2, VEGFA, KLF4, NASP	**4.70E-04** ***1.40E-02**	VEGFA, KLF4, CCND1 (not validated CDC42)
miR-196a	Cell cycle not-significant	None identified		N/A
miR-205	Cell proliferation	BCL2, E2F1, AR, VEGFA	4.50E-03 *0.3	VEGFA

Table 1: Bioinfomatics analysis was performed to analyse the known (Experimentally validated) targets of miRNAs followed by classification into functional categories with emphasis on cell division/proliferation using David gene ontology database.