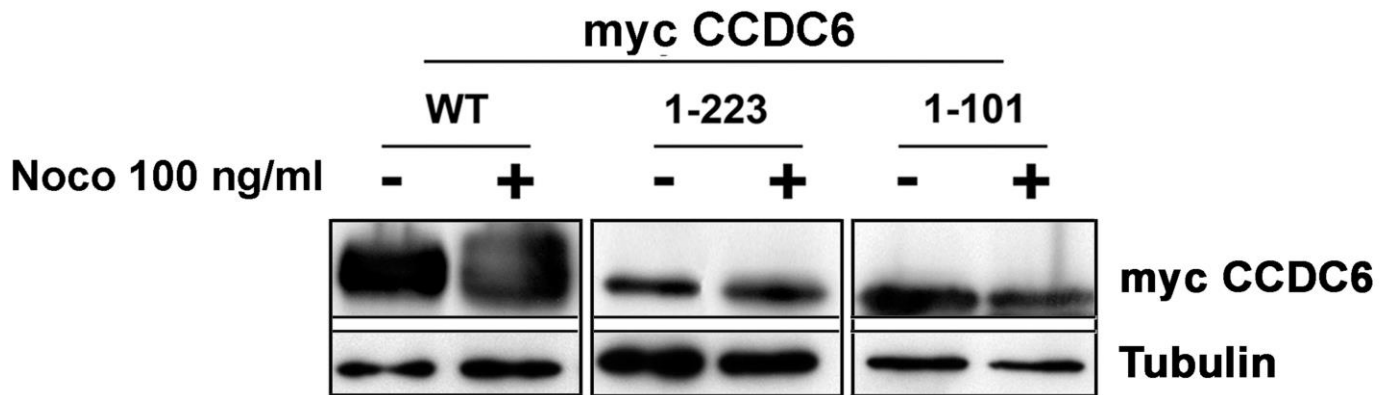


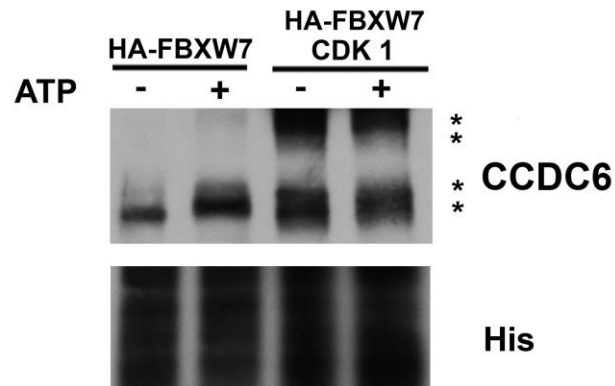
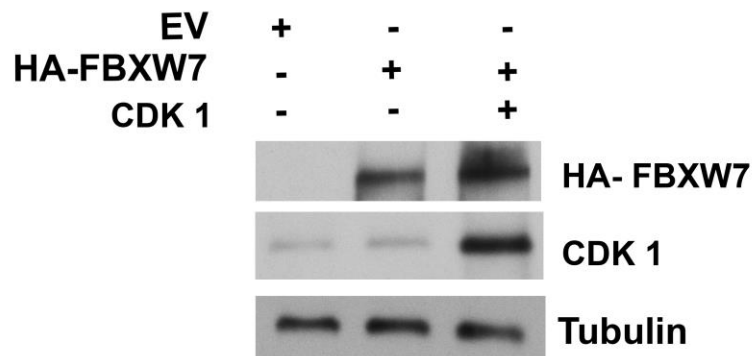
FBXW7 and USP7 regulate CCDC6 turnover during the cell cycle and affect cancer drugs susceptibility in NSCLC

Supplementary Material

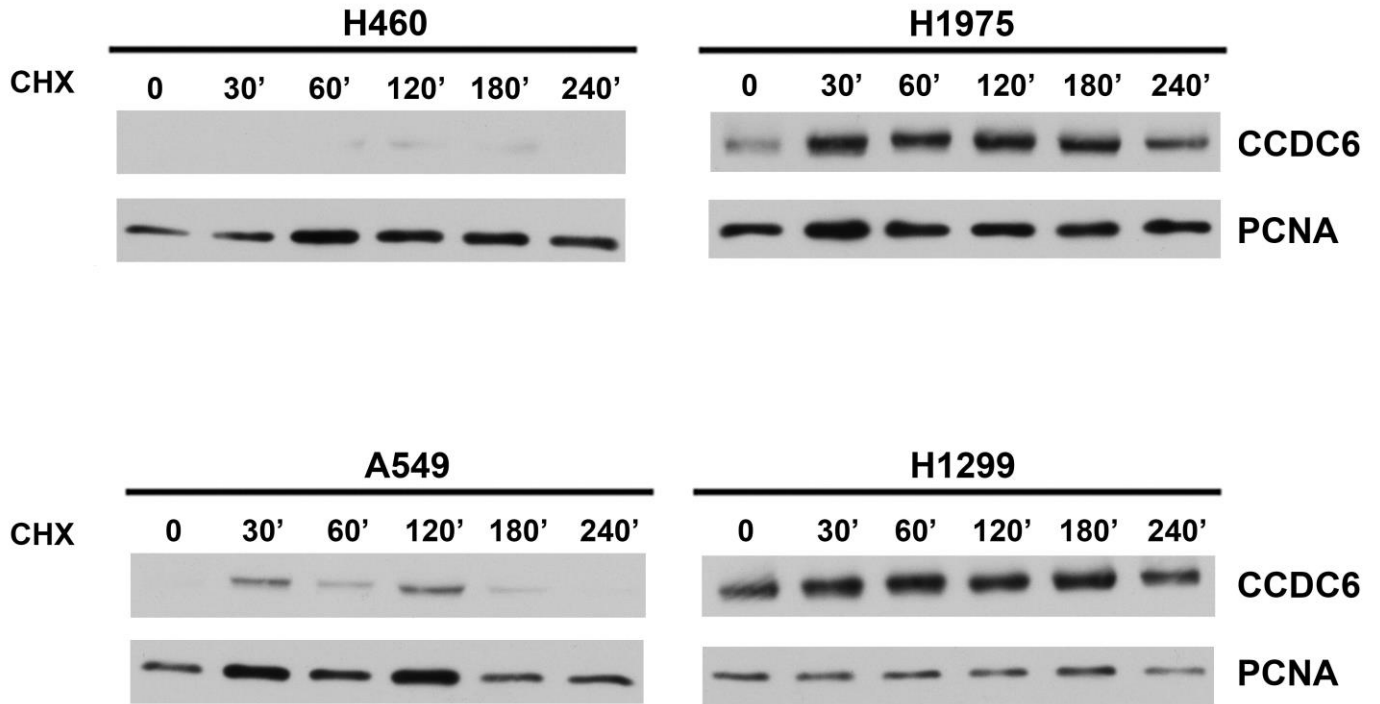


Supplementary Figure 1: Restriction of the CCDC6 mitotic phosphorylated target region

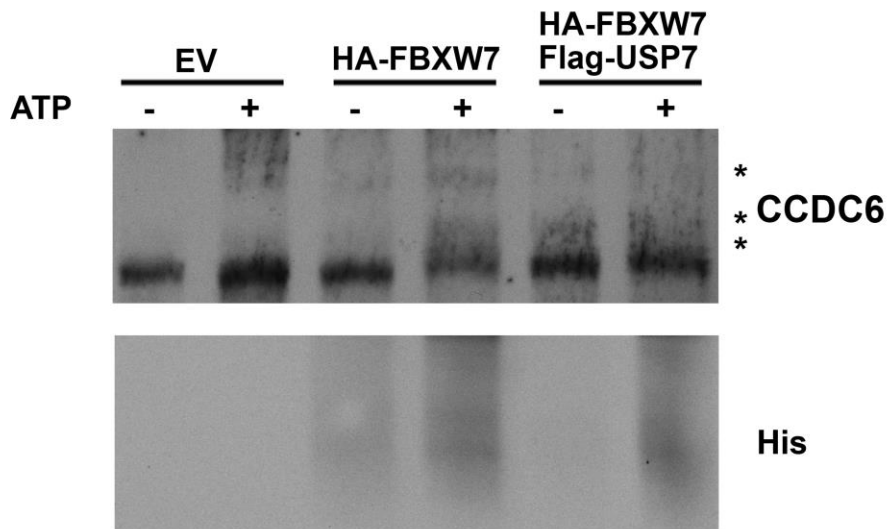
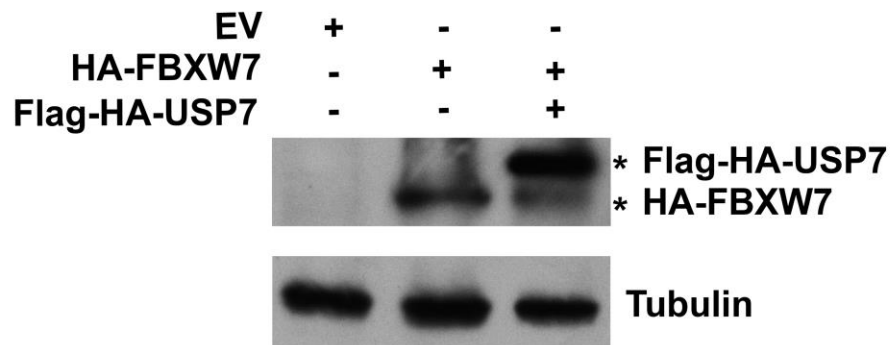
Human HeLa cells, transiently transfected with CCDC6 wild type or the CCDC6 (1-223) and (1-101) deleted mutant constructs, were treated with 100 ng/ml nocodazole for 16 hours; samples were collected and analysed by immunoblotting using the indicated antibodies.

A**B**

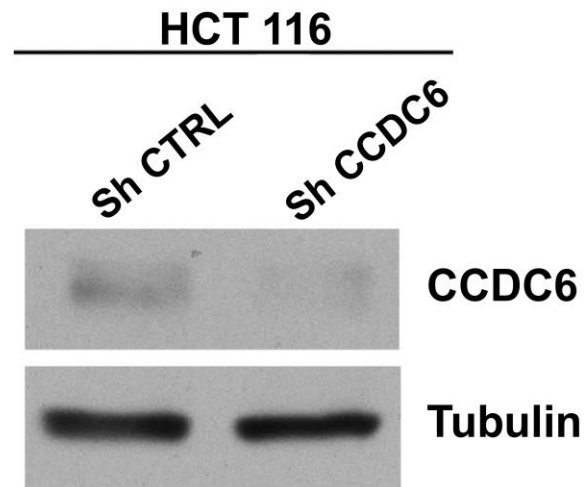
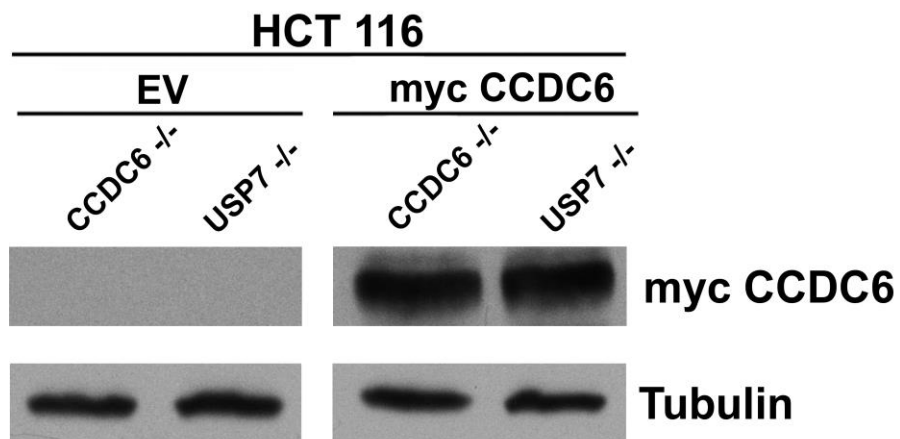
Supplementary Figure 2: CDK1 elevated FBXW7 mediated ubiquitination of recombinant GST-CCDC6 *in vitro* **A)** Affinity-purified GST-CCDC6 recombinant protein (Abnova H00008030-P01) was incubated with purified E1, E2, His-ubiquitin and 293T cell lysate, as source of E3 ligase FBXW7, from cells transiently transfected with HA-FBXW7, in presence or absence of CDK1 construct, at 37°C for 60 min (according to manufacturer instructions; see M&M). The ubiquitination reaction products were resolved by SDS-PAGE and probed with anti-Histidine antibody and anti-CCDC6 antibody. **B)** The transfection efficiency of the above mentioned plasmids is shown in the immunoblot hybridized with the indicated antibodies.



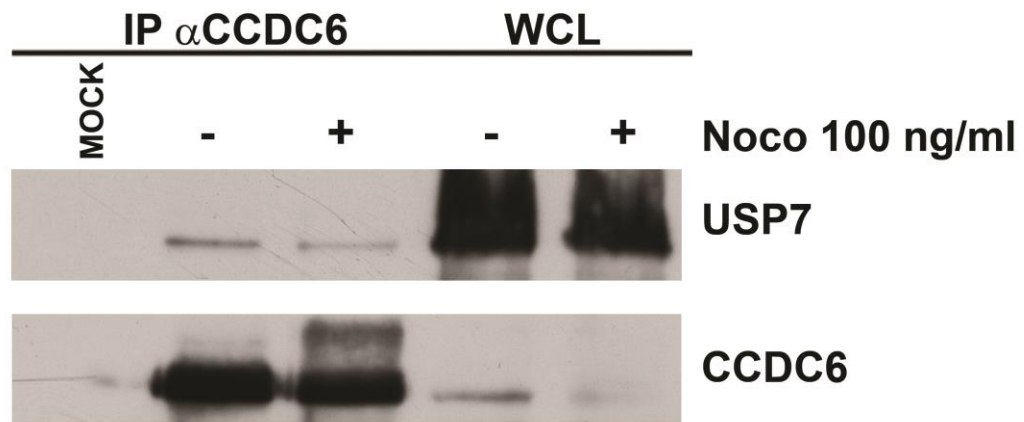
Supplementary Figure 3: In NSCLC cell lines the CCDC6 half life differences account for the observed variations in the CCDC6 abundance. H460, H1975, A549 and H1299 cells were treated with cycloheximide (CHX) (100 ng/ml) for the indicated times. Cell lysates were immunoblotted with the indicated antibodies.

A**B**

Supplementary Figure 4: USP7 impairs the CCDC6 ubiquitination mediated by FBXW7. **A)** Affinity-purified GST-CCDC6 recombinant protein (Abnova H00008030-P01) was incubated with purified E1, E2, His-ubiquitin and 293T cell lysate, as source of E3 ligase FBXW7, from cells transiently transfected with HA-FBXW7, in presence or absence of FLAG-HA-USP7, at 37°C for 60 min (according to manufacturer instructions; see M&M). The ubiquitination reaction products were resolved by SDS-PAGE and probed with anti-Histidine antibody and anti-CCDC6 antibody. **B)** The transfection efficiency of the above mentioned plasmids is shown in the immunoblot hybridized with the indicated antibodies.

A**B**

Supplementary Figure 5: **A)** HCT116 cells were transfected with control shRNAs (shCTRL) or sh-CCDC6. The CCDC6 protein depletion was assessed by Western blot. **B)** Myc-empty vector (EV) and myc-CCDC6 (CCDC6+) expression in HCT116 CCDC6^{-/-} and in HCT116 USP7^{-/-} are shown. Anti-myc hybridization is shown. Antitubulin immunoblots are shown at bottom of panels A and B, respectively.



Supplementary Figure 7: USP7-CCDC6 interaction is reduced in mitosis.

Endogenous CCDC6 from asynchronous or mitotic HeLa cell extracts were analysed by SDS-PAGE and immunoblotted with the anti-USP7 and anti-CCDC6 antibodies, as shown. The immunoblots of the whole cell lysates (WCL) are shown at the right of the panel.

Supplementary Table 1:

OLIGO NAME	OLIGO SEQUENCE
CCDC6 _{S359A} Fwd	gatcccttacacacctgctccgagttcaagcag
CCDC6 _{S359A} Rev	ctgcttgaactcggagcaggtgtgtaagggatc
CCDC6 _{S413A} Fwd	ggatcacaaggcctgcaccacggagaagca
CCDC6 _{S413A} Rev	tgcttctccgtgggtgcaggccttgatacc
CCDC6 _{T427A} Fwd	gatggaggcggcgcgggcccgttgaat
CCDC6 _{T427A} Rev	attcaaacggcccgcgccctccatc