



Figure S2. CHCHD4-mediated tumour cell growth and mTORC1 signalling is coupled to CI activity. **a** Western blots show levels of AIF, and myc-tagged CHCHD4 in control U2OS cells, and cells overexpressing wild-type (WT.c11, WT.c13) or mutant (C66A/C68A) CHCHD4. β -Actin was used as a load control. **b** Chart shows mean fluorescence intensity of NADH in control U2OS cells, and U2OS cells expressing exogenous CHCHD4 (WT.c11). Cells treated at indicated time points with 1 mM cyanide (CN) and 1 μ M FCCP. 1 image per minute, 5 images per treatment, 3 fields of view per cell line. \pm SD. $n = 3$. Representative images of control U2OS cells at each condition also shown. **c** Western blots show levels of phosphorylated (P-) and total (T-) p70S6K, and puromycin labelled polypeptides in control U2OS cells treated with 0, 50 or 100 nM rotenone for 24 h, in the absence (NT) or presence of 10 mM aspartate (+D). β -Actin was used as a load control. **d** Agarose gel shows expression of *ND11* transcript in control U2OS cells and cells expressing CHCHD4 (WT.c11), stably transfected with empty vector (pWPI) or NDI1-containing vector (NDI1). *ACTB* transcript expression was used as a control.