Supplemental Information

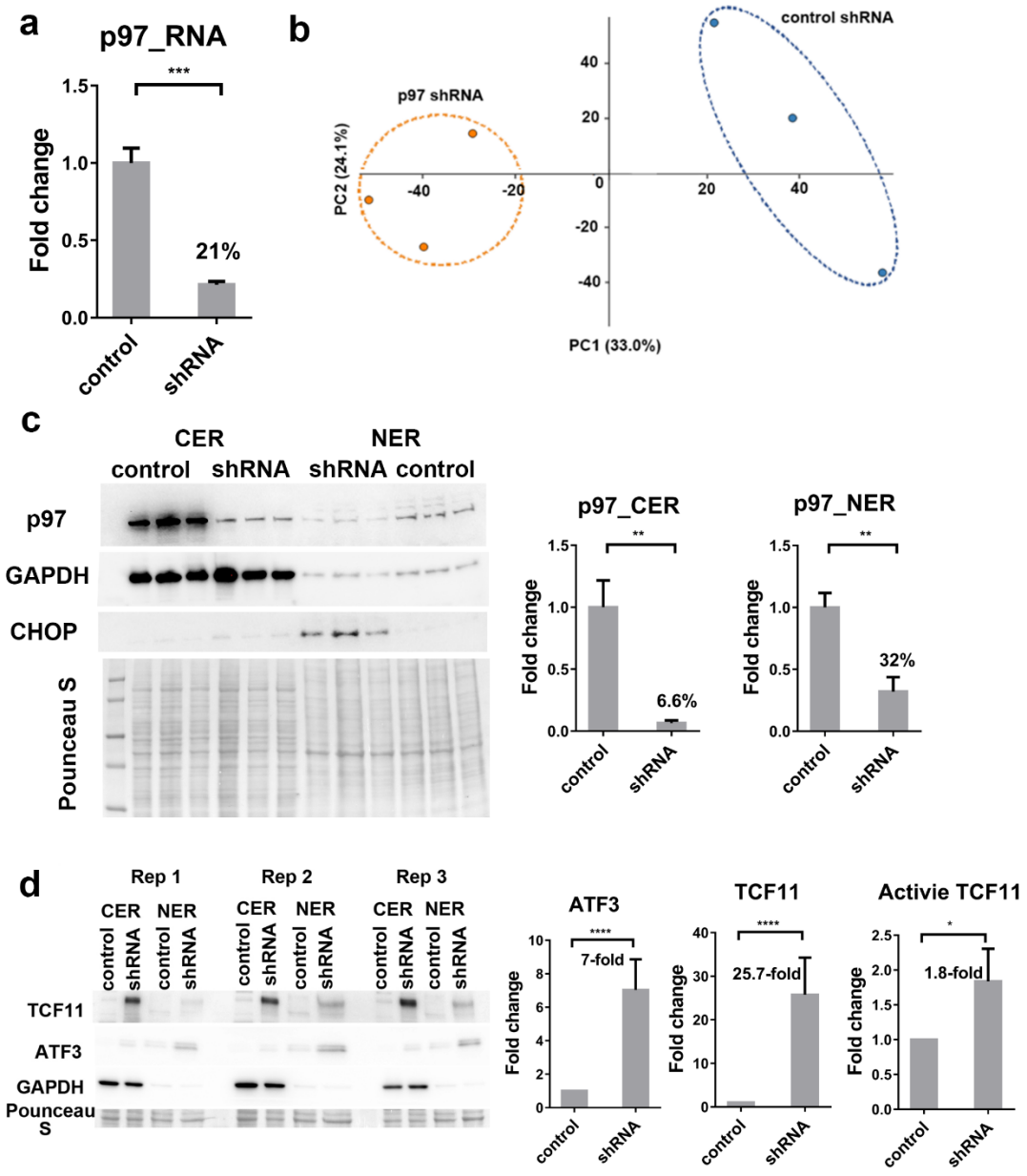

Figure S1. Cellular effects of p97 knockdown on HCT116 cells. Related to Fig. 1a-d. (a) qPCR analysis of the RNA level of p97 in HCT116 treated with p97 shRNA or control. (b) After 72h induction with Doxycycline, the mRNA level of p97 was reduced by 79%. Principal component analysis (PCA) on proteomic data from HCT116 cells treated with p97 shRNA (knockdown) or control shRNA showed that replicate samples had similar principal component (PC) scores. (c) Western blot showed that the p97 protein level was reduced by 93.4% in the cytosol and by 68% in the nucleus following p97 KD. (d) Western blot detected the ER stress markers ATF3 was upregulated 7 at the protein level by p97 KD. f) Quantification results of TCF11 detected by western blot. For all experiments, n=3, *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$. Data are represented as mean ± SD

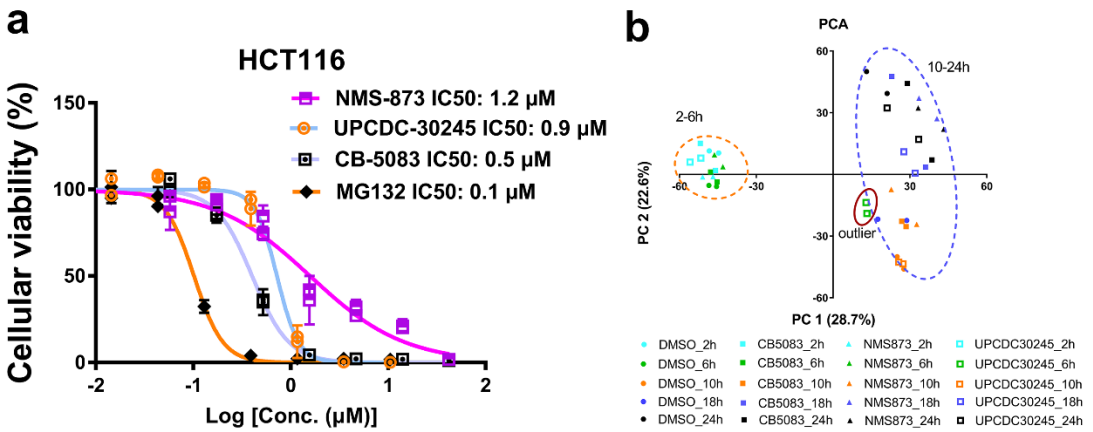

Figure S2. Cellular effects of p97 inhibitors and MG132 on HCT116 cells. Related to Fig. 1e-g. (a) Anti-proliferative effects CB-5083, NMS-873, UPCDC-30245 and MG132 on HCT116 cells. Cells were treated with the indicated inhibitor for 48h. Data are represented as mean ± SD (b) PCA analysis on temporal proteomics data showed that the samples clustered into two groups according to treatment time. With the exception of the 6h treatment with UPCDC-30245, the 2h and 6h treatments of CB-5083 and NMS-873 were grouped into one cluster while 8h, 18h and 24h were grouped into another cluster.

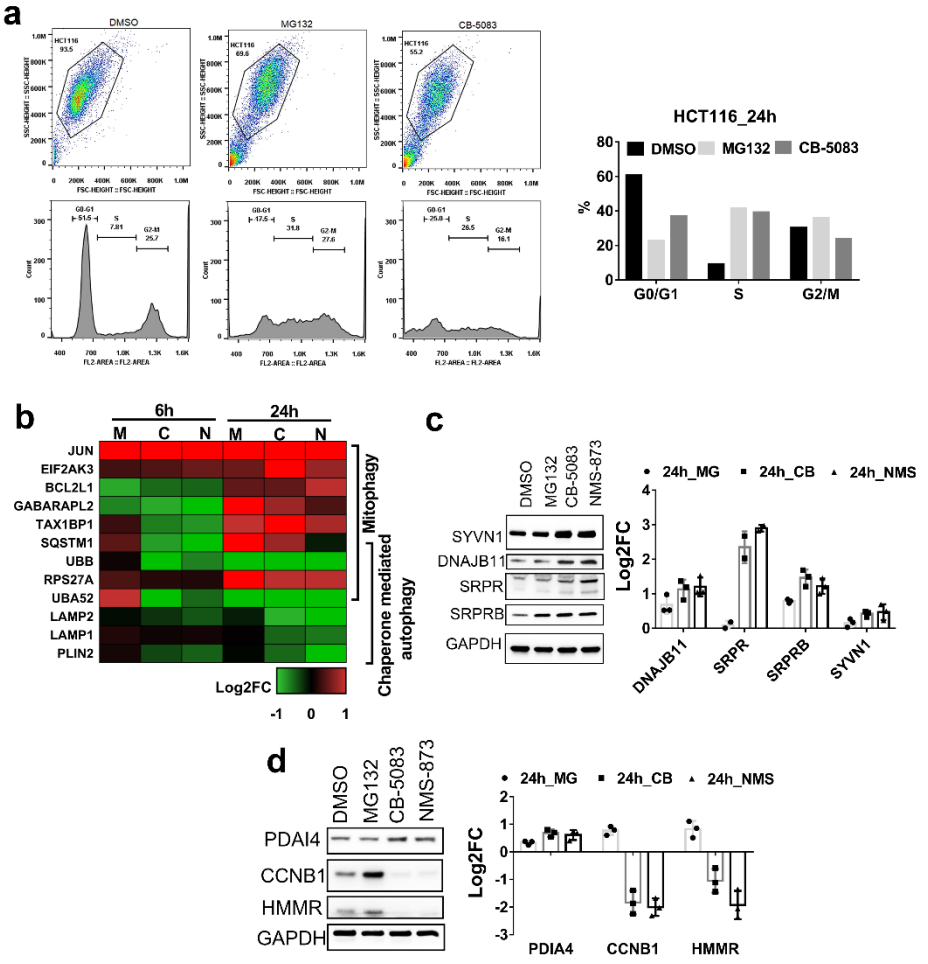

Figure S3. HCT116 cells respond differently to p97 inhibitors and MG132. Related to Fig.

2-3. (a), cell cycle analysis of HCT116 cells after 24h of treatments with DMSO, 1μM of MG132 (MG) or 2 μM of CB-5083. (b), heatmap of autophagy related proteins which were dysregulated by both CB-5083 and NMS-873 after 6h or 24h of treatment. Data were from TMT labeling proteomics. M, C and N indicates MG132, CB-5083 and NMS-873 treatment respectively. (c-d), western blotting validates the proteins identified from proteomic data. HCT116 cells were treated for 24h with DMSO, 1μM of MG132 (MG), 2 μM of CB-5083 (CB) or 4 μM of NMS-873 (NMS), n=3. Data are represented as mean ± SD

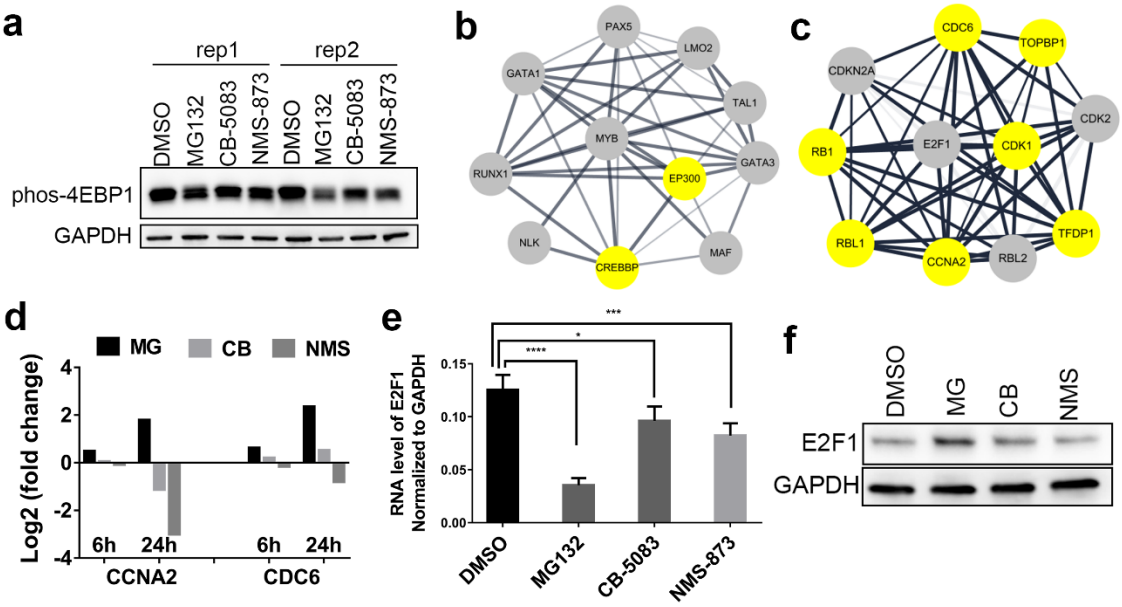

Figure S4. The effect of p97 inhibitors and MG132 on E2F1 pathway in HCT116 cells. Related to Fig. 4. (a) Western blotting indicates that both p97 inhibitors and MG132 reduced the levels of phosphorylated eIF4E-binding protein1 (phos-4EBP1). (b-c) Nnetwork of E2F1 (b) and cMyb (c). Proteins highlighted in yellow were identified in our TMT labeling proteomics. (d) CCNA2 and CDC6 which interact with E2F1 were dysregulated by CB-5083 (CB), NMS-873 (NMS) and MG132 (MG). (**e**) qPCR analysis revealed the RNA level of E2F1 was strongly downregulated by MG132 and slightly downregulated by p97 inhibitors. (**f**) Western blotting indicates that E2F1 protein levels were not significantly affected by p97 inhibitors but were increased by MG132. For both qPCR and western blot assays, HCT116 cells were treated for 6h with DMSO, 1μM of MG132 (MG), 2 μM of CB-5083 (CB) or 4 μM of NMS-873 (NMS), n=2, **** indicates p<0.0001, *** indicates p<0.001, * indicates p<0.05 according to unpaired t-test. Data are represented as mean ± SD.

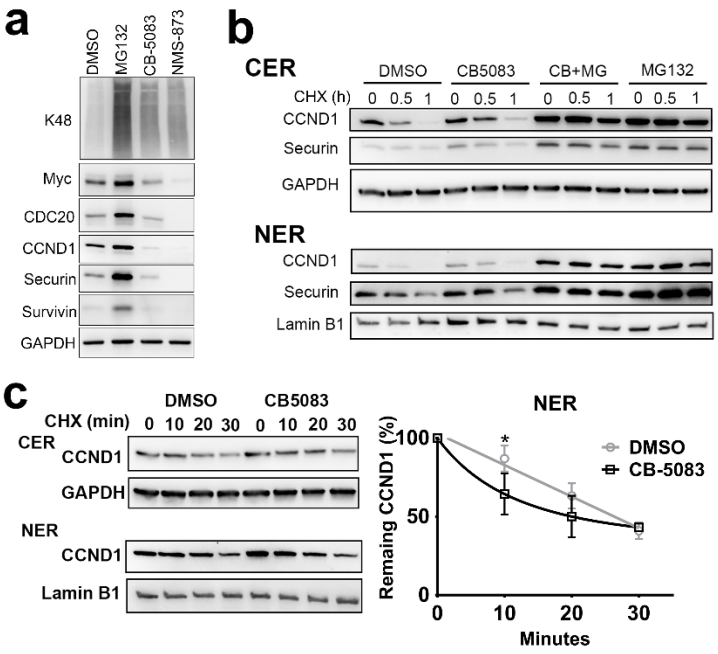

Figure S5. Western blot detecting the effects of p97 inhibitors and MG132 on cell cycle proteins in HT29 and HCT116 cells. Related to Fig. 5. (a) HT29 cells were treated for 6h with DMSO, 1μM of MG132, 2 μM of CB-5083 or 4 μM of NMS-873. (b) HCT116 cells were treated with DMSO, 1μM of MG132 (MG), 2 μM of CB-5083 (CB) or 1μM of MG132 plus 2 μM of CB-5083 (CB+MG) for 1h. Then, 50 μM of CHX was added and cells were collected at 0h, 0.5h, and 1h. (**c**) To determine the half-life of cyclin D1 in HCT116 cells. HCT116 cells were pre-treated with 5 μM of CB-5083 or DMSO for 30 minutes before adding 50 μM of CHX, and cells were collected at 0, 10, 20, 30 min (n=3), * indicates p<0.05 according to unpaired t-test.

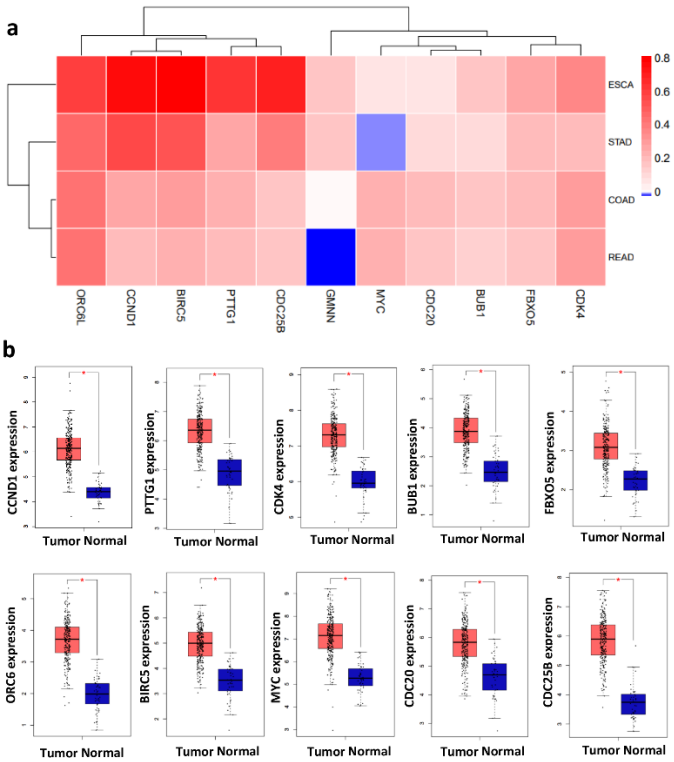

Figure S6. Expression analysis of p97 inhibitors specifically downregulated cell cycle proteins. Related to Table 1. (**a**) Expression of the eleven cell cycle genes that are specifically downregulated by p97/VCP inhibitors in different Gastrointestinal cancers. Batch adjusted normalized TCGA Pan-Cancer RNA-seq data was downloaded from the UCSC Xena Browser. Data represents log2 of the ratio of the mean expression of tumor samples to the mean expression of matched normal samples. (**b**) Boxplots depicting the mRNA expression of 10 out of the 11 proteins in colon cancer tumor tissue (N=275) and normal matched (N=41) using GEPIA web tool, * indicates $p<0.05$ and $|log2FC|>0.5$.

Table S5. Cyber green qPCR primer used in this study. Related to STAR Methods

Name	sequence
human GAPDH-F	5'- GAAGGTGAAGGTCGGAGTC -3'
human GAPDH-R	5'- GAAGATGGTGATGGGATTTC -3'
human DHFR F	5'- TGCACAAATG GGGACGA -3'
human DHFR R	5'- GGAAATATCTGAATTCATTCCTGAG -3'
human Cyclin D1 F	5'- AGCTGTGCATCTACACCGAC -3'
human Cyclin D1 R	5'- GAAATCGTGCGGGGTCATTG -3'
human CDC20 F	5'- GGCACCAGTGATCGACACATTCGCAT -3'
human CDC20 R	5'- GCCATAGCCTCAGGGTCTCATCTGCT -3'
human c-Myc F	5'- TCAGAGTCTGGATCACCTTCTGCT -3'
human c-Myc R	5'- TGCGTAGTTGTGCTGATGTGTGGA -3''
human PTTG1 F	5'- AAAGCTCTGTTCCTG CCTCA -3'
human PTTG1 R	5'- GAGAGGCACTCC ACTCAAGG -3'
human FBXO5 F	5'- CGCTGTAATTCACCTGCAAA -3'
human FBXO5 R	5'- GAGGAGCTTGCCATCTGAAC -3'