

The atypical cyclin P regulates cancer cell stemness through the activation of the WNT pathway

Cellular Oncology

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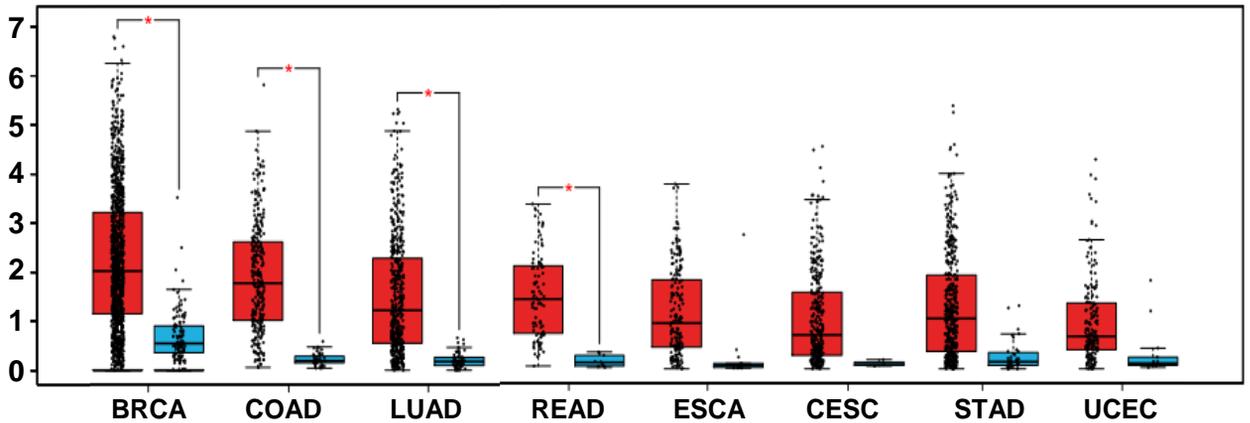
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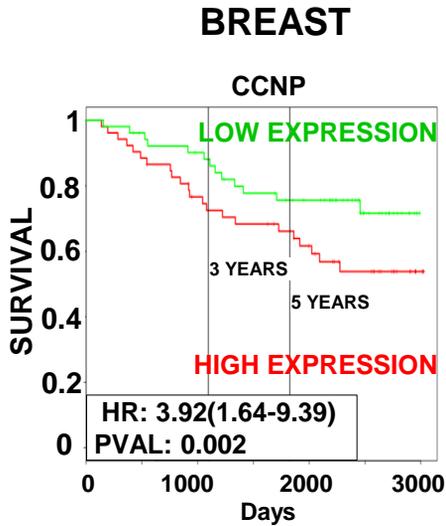
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a

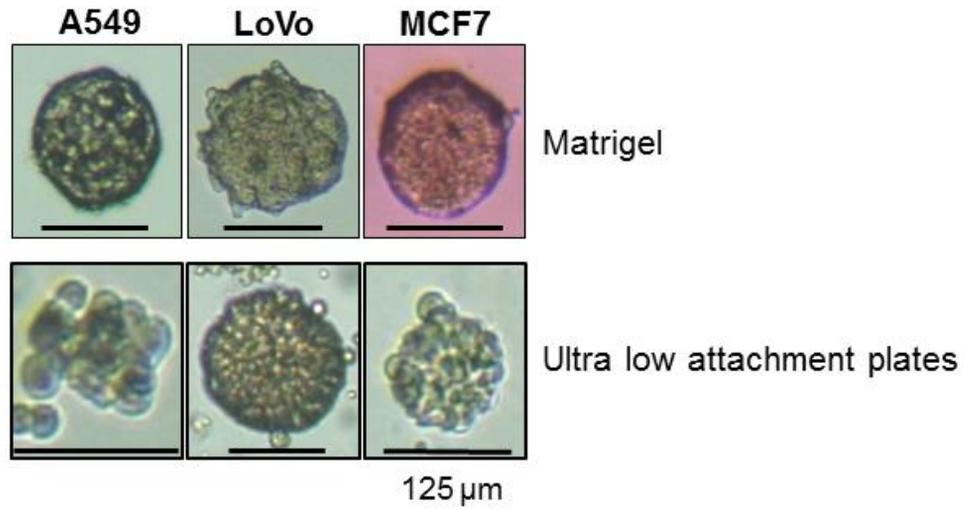


| | |
|---------------------------------|---|
| BRCA: Breast invasive carcinoma | ESCA: Esophageal carcinoma |
| COAD: Colon adenocarcinoma | CESC: Cervical squamous cell carcinoma and endocervical carcinoma |
| LUAD: Lung adenocarcinoma | STAD: Stomach adenocarcinoma |
| READ: Rectum adenocarcinoma | UCEC: Uterine Corpus Endometrial Carcinoma |

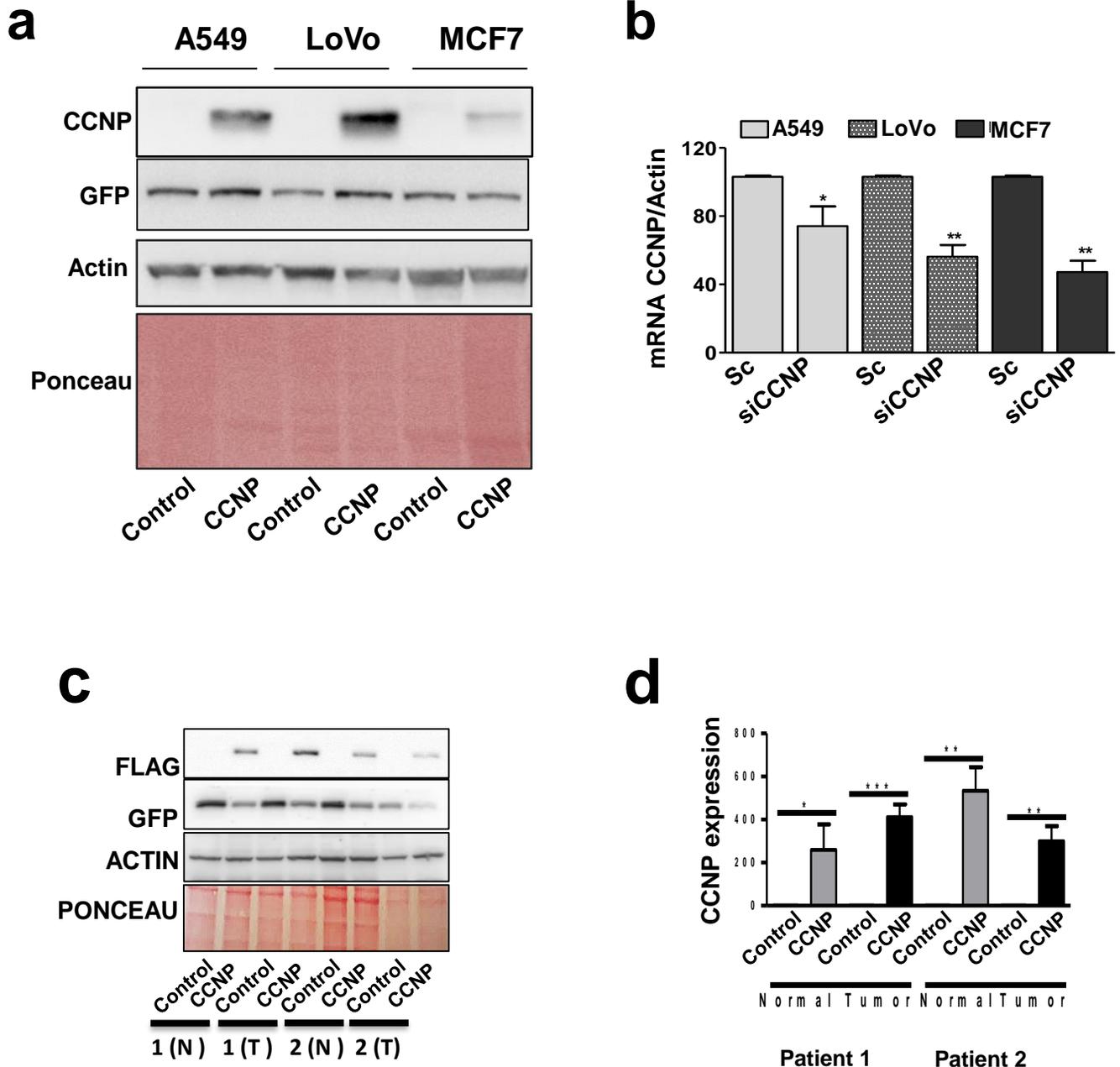
b



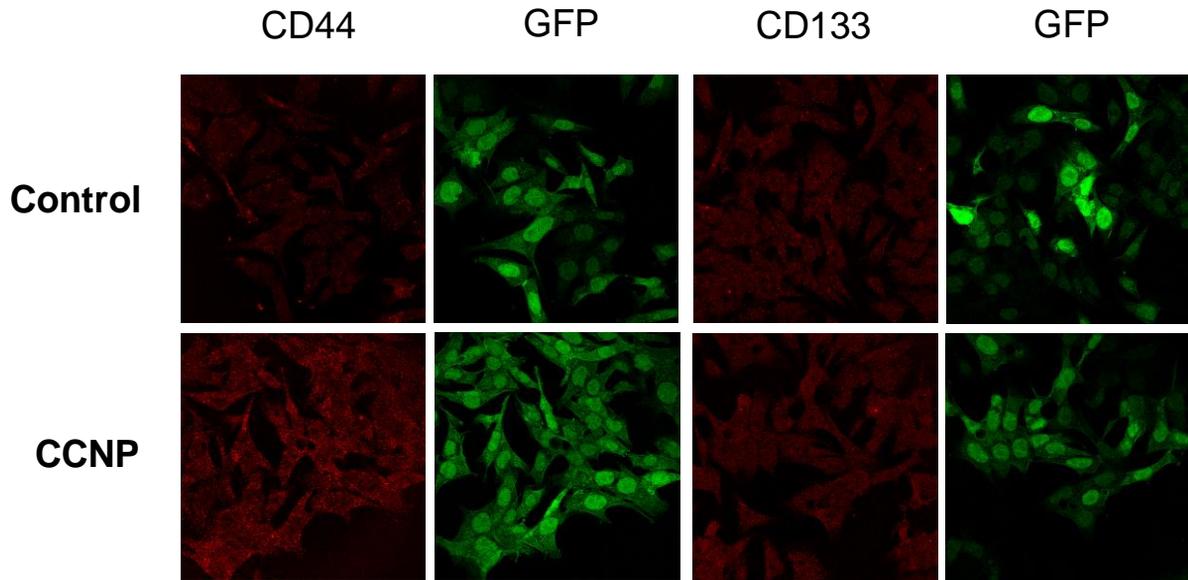
Supplementary Fig. S1 CCNP is upregulated in tumor tissues. (A) mRNA expression in tumor (red boxes) and adjacent healthy (blue boxes) tissues in various types of cancers according to the GEPIA database (<http://gepia.cancer-pku.cn/>). Data accessed in August 2018. (B) Survival curves in breast cancer patients presenting high and low levels of CCNP expression according to the PROGgeneV2 tool (GSE42568 database, n = 104). Data accessed in July 2018.



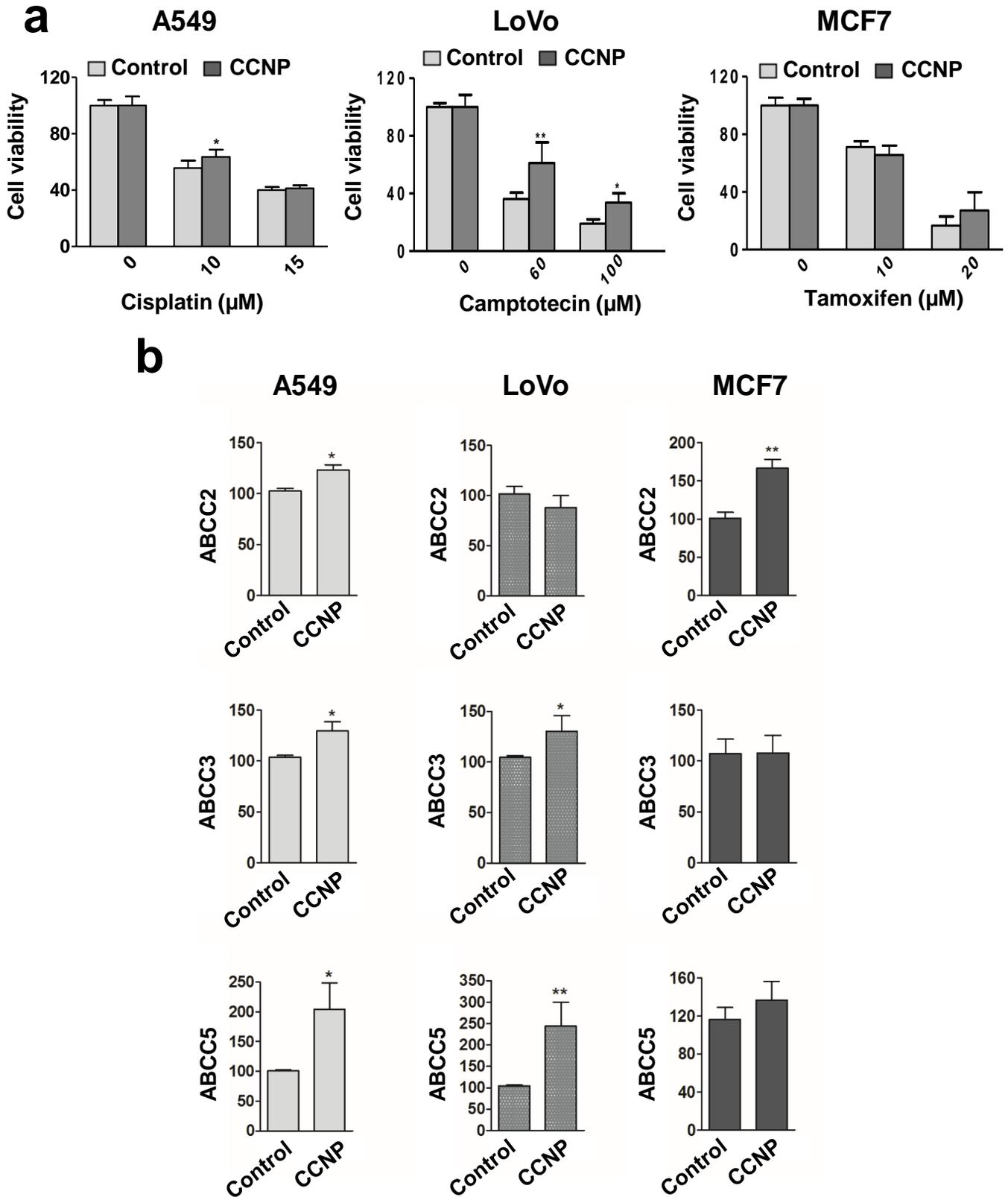
Supplementary Fig. S2 Comparison of spheroid morphology. The image shows representative images of spheroids obtained by seeding in matrigel or ultra-low attachment plates.



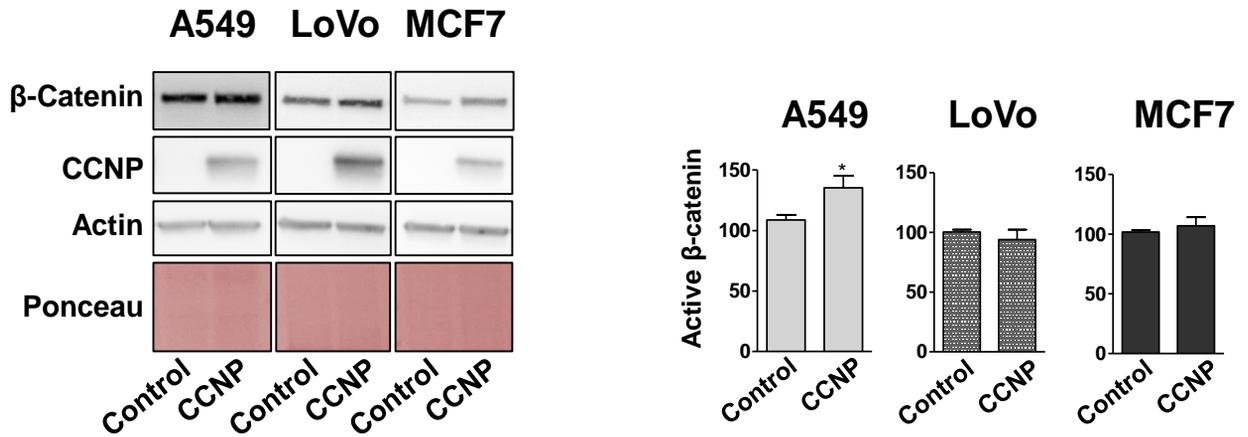
Supplementary Fig. S3 CCNP overexpression and downregulation in cancer cell lines and patient-derived organoids. (A) Cells were transduced with an empty lentiviral vector (control) or with CCNP expression constructs. Protein overexpression was confirmed by Western blot analysis. (B) Cells were transfected with scrambled control (Sc) or siRNA targeting siRNA (siCCNP) at 50 nM. After 72 h, CCNP expression was monitored by RT-qPCR. The columns represent the mean \pm SEM of five independent, ** $P < 0.01$, * $P < 0.05$ vs. scrambled control (Sc), Mann-Whitney test. (C-D) Normal and tumor-derived organoids of patients 1 and 2 were transduced empty vector (control) or CCNP-expression construct. CCNP-Flag expression was followed by western blot (C) and RT-qPCR (D)



Supplementary Fig. S4 Immunofluorescence of stemness markers CD44 and CD133 in LoVo cells overexpressing CCNP. Cells were transduced with an empty vector or CCNP expression construct and seeded on poly-lysine coated coverslips. Forty-eight hours later, cells were fixed in cold paraformaldehyde, blocked for 1 h, and incubated for 1 h with CD44 or CD133 antibodies. Coverslips were then washed several times with PBS and incubated with anti-rabbit fluorescent secondary antibody.

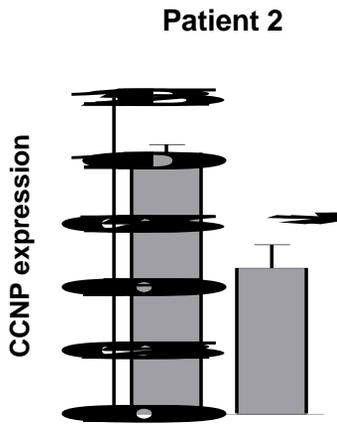


Supplementary Fig. S5 Overexpression of CCNP decreases cancer cell sensitivity to drug treatment. Cells were transduced with an empty vector (control) or with a CCNP-expressing construct and treated with the indicated concentrations of drugs. Cell viability was measured by the MTT assay (A), and the expression of ABC transporters was monitored by RT-qPCR (B). The columns represent the mean \pm SEM of six independent experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$ vs control. The results are represented as a percentage of control

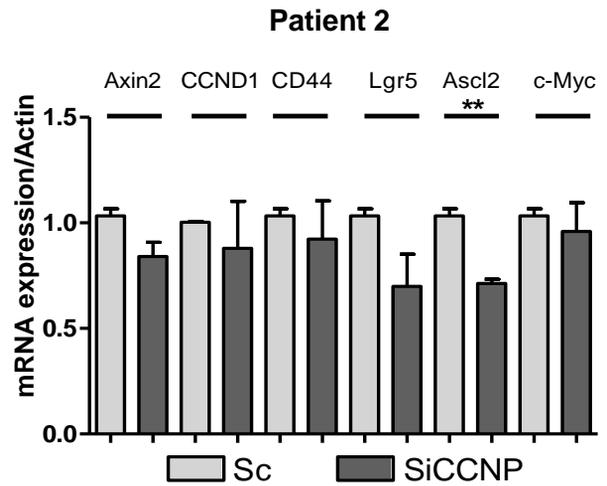


Supplementary Fig. S6 Overexpression of CCNP decreases cancer cell sensitivity to drug treatment. Cells were transduced with an empty vector (control) or with a CCNP-expressing construct, and the levels of active β -catenin were monitored by western blot. Representative images are shown in the left panel, whereas the right panel shows activated β -catenin quantification. The columns represent the mean \pm SEM of nine independent experiments performed in triplicate. * $P < 0.05$ vs. control, Mann-Whitney test. The results are represented as a percentage of control.

a



b



Supplementary Fig. S7 Knockdown of CCNP reduces the expression of Ascl2 in Patient 2 tumor-derived organoids. Tumor organoids of patient 2 were transfected with scrambled control (Sc) or with siRNA targeting CCNP (siCCNP). Knockdown efficiency (A) and the expression of stem cell and WNT targets (B) were evaluated by RT-qPCR. The columns represent the mean \pm SEM of three independent experiments, ** P <0.01 vs. control, T-student test. The results are represented as a percentage of control

| Cell line | Source | Catalog nº | STR |
|------------|---|------------|---|
| A549 | European Collection of Authenticated Cell Cultures | 86012804 | Amelogenin: X,Y; CSF1PO: 10,12; D13S317: 11; D16S539: 11,12; D5S818: 11; D7S820: 8,11; THO1: 8,9,3; TPOX: 8,11; vWA: 14 |
| HEK293-T | Authenticated by the American Type Culture Collection | | CSF1PO: 11,12; D13S317: 12,14; D16S539: 9,13; D5S818: 8,9; D7S820: 11; THO1: 7, 9,3; TPOX: 11; vWA: 16,19; Amelogenin: X |
| HT-29 | European Collection of Authenticated Cell Cultures | 91072201 | Amelogenin: X; CSF1PO: 11,12; D13S317: 11,12; D16S539: 11,12; D5S818: 11,12; D7S820: 10; THO1: 6,9; TPOX: 8,9; vWA: 17,19 |
| LoVo | Authenticated by the American Type Culture Collection | | Amelogenin: XY; CSF1PO: 11,13,14; D13S317: 8, 11; D16S539: 9, 12; D5S818: 11, 13; D7S820: 9,3,10, 11; THO1: 9,3; TPOX: 8,9; vWA: 17,18 |
| MCF7 | Authenticated by the American Type Culture Collection | | Amelogenin: X; CSF1PO: 10; D13S317: 11; D16S539: 11,12; D5S818: 11,12; D7S820: 8,9; THO1: 6; TPOX: 9,12; vWA: 14,15 |
| MDA-MB-231 | Authenticated by the American Type Culture Collection | | Amelogenin: X; CSF1PO: 12,13; D13S317: 13; D16S539: 12; D5S818: 12; D7S820: 8,9; THO1: 7,9,3; TPOX: 8,9; vWA: 15,18 |
| NCI-H1395 | American Type Culture Collection | CRL-5868 | Amelogenin: X; CSF1PO: 12; D13S317: 10,14; D16S539: 11,13; D5S818: 12; D7S820: 8; THO1: 6,9,3; TPOX: 8; vWA: 14,17 |

Supplementary Table S1 List of cell lines used.

| Gene | Sequence |
|---------------|----------------------------|
| 18S Forward | CTACCACATCCAAGGAAGGCA |
| 18S Reverse | TTTTTCGTCACTACCTCCCCG |
| CCNP Forward | CTGGTGGTAGACTGGCTGGT |
| CCNP Reverse | AGCACGCACTCTTCCATTTT |
| OCT4 Forward | GTGGAGAGCAACTCCGATG |
| OCT4 Reverse | TGCAGAGCTTTGATGTCCTG |
| Sox2 Forward | TGG CGA ACC ATC TCT GTG GT |
| Sox2 Reverse | CCA ACG GTG TCA ACC TGC AT |
| Nanog Forward | CAG AAG GCC TCA GCA CCT AC |
| Nanog Reverse | ATT GTT CCA GGT CTG GTT GC |
| ABCC1 Forward | TGCCCTACCTGACCCTCGGC |
| ABCC1 Reverse | ACACCCAGTCAGGCTCCGCA |
| ABCC2 Forward | TCTCTCGATACTCTGTGGCAC |
| ABCC2 Reverse | CTGGAATCCGTAGGAGATGAAGA |
| ABCC3 Forward | GCTGGGCTGGGAAACCGGAC |
| ABCC3 Reverse | TGGGTGCAGCCTGGAACAGC |
| ABCC5 Forward | CCCGCCCCAGGCAGGGAATG |
| ABCC5 Reverse | TGTGTCCTGACGGCGTCTCCTT |

Supplementary Table S2 List of RT-qPCR primers

| Cat. No | Antibody | Species | Company |
|-----------------|---------------------------------------|---------|----------------|
| 2956 | GFP | Rabbit | Cell Signaling |
| HPA045615 | CCNP | Rabbit | Sigma |
| GTX627419 | OCT-4 | Mouse | GeneTex |
| Sc-7297 | CD44 | Mouse | Santa Cruz |
| E-AB-33462 | CD133 | Rabbit | Elabscience |
| Sc-374429 | INTEGRIN β | Mouse | Santa Cruz |
| 8814 | ACTIVATED β -CATENIN | Rabbit | Cell Signaling |
| Sc-58628 | Na ⁺ K ⁺ ATPase | Mouse | Santa Cruz |
| CSB-PA00025A0Rb | GAPDH | Rabbit | CusAb |
| Sc-374015 | LAMIN B | Mouse | Santa Cruz |
| Sc-17824 | SP1 | Mouse | Santa Cruz |
| F3165 | FLAG | Mouse | Sigma-Aldrich |
| A5316 | β -ACTIN | Mouse | Sigma-Aldrich |

Supplementary Table S3 List of antibodies used