# Supporting Information

## Shtutman et al. 10.1073/pnas.1103842108

### SI Methods

**Cell Lines.** Tumor cell lines were obtained from American Type Culture Collection. BJ normal foreskin fibroblasts immortalized with human telomerase reverse (line BJ1-hTERT) were obtained from Clontech Laboratories, BJ-ELN, BJ-ELB, and BJ-ELR cells (1) were kindly provided by W. Hahn (Massachusetts General Hospital, Boston, MA). Human prostate epithelial cells were obtained from LifeLine Cell Technologies. MCF7 cells were maintained in DMEM supplemented with 0.1 mmol/L of non-essential amino acids, 1 mmol/L of sodium pyruvate, 0.01 mg/mL of bovine insulin (Sigma), and 10% FBS (HyClone) or 10% FC2 (Invitrogen). MDA-MB231, HeLa, SW480, HCT116, HT1080, ACHN, K562, SK-MEL-28, SK-MEL-103, SK-MEL-19, SK-MEL-29, SK-ML-147, T24, and U251 cell lines were grown in DMEM with 10% FC2. HL60 cells were grown in DMEM with 20% FBS, and CCRF-CEM cells were grown in RPMI with 10% FBS. LNCaP, PC3, NCI-H69, and A2780 cells were grown in RPMI with 10% FC2. A549 and Malme-3M cells were grown in Ham's F-12 and L-15 medium, respectively, with 10% FC2. Saos-2 cells were grown in McCoy's 5A medium with 20% FBS. BJ fibroblasts were maintained in BJ medium (4:1 DMEM/ M199) supplemented with 1 mmol/L of sodium pyruvate and 10% FBS. Human prostate epithelial cells were maintained using the ProstaLife Media kit (LifeLine Cell Technologies). Primary human melanocytes were grown in 254CF medium supplemented with HMGS-2 supplement (Invitrogen). Cell lines expressing tTR-KRAB (2) for regulated genetic suppressor element (GSE) expression were prepared as described (3). Full-length coatomer protein complex ζ1 (COPZ1) and coatomer protein complex ζ2 (COPZ2) cDNAs (Open Biosystems) were cloned into a lentiviral vector, pLenti6-bsd-FLAG, which adds a FLAG tag at the C terminus, constructed in our laboratory. Retroviral vector expressing GFP-LC3 (4) was obtained from Addgene. Lentiviral vector expressing miR-152 precursor was from SBI Bioscience. The recipient cell populations transduced with these and the corresponding insert-free control vectors were selected with blasticidin or puromycin before analysis.

**Retroviral and Lentiviral Infections.** Retroviral infections were performed as described previously (5). Lentiviral transduction was carried out as described (6) using pCMV-Δ8.9 and pVSV-G packaging constructs. The vector plasmid, pCMV-Δ8.9, and pVSV-G DNA were mixed at a 5:4:1 ratio and cotransfected using the polyethylenimine protocol (7) into 293FT cells. Lentivirus-containing supernatants were harvested three times, at 24, 48, and 72 h after transfection. Lentiviral library transduction and BrdU selection of growth-inhibitory sequences was carried out as previously described (3). Twenty-five percent of the transduced cells were used for DNA extraction immediately, and the rest were subjected to selection for doxycycline-dependent resistance to BrdU suicide (3), followed by DNA extraction. Library-derived cDNA inserts were amplified by PCR from genomic DNA using vector-specific primers and were subjected to 454 massive parallel sequencing. BLAST analysis (3) was used to identify genes giving rise to cDNA fragments enriched by GSE selection.

**Construction of the GSE Library.** Total RNA preparations of the following 18 tumor and leukemia cell lines were used for library construction: A549 and H69, lung cancer; HCT116 and SW480, colon cancer; MCF-7 and MDA-MB-231, breast cancer; LNCaP and PC3, prostate cancer; HeLa, cervical cancer; A2780, ovarian cancer; ACHN, renal carcinoma; HT1080, fibrosarcoma; Saos-2, osteosarcoma; MALME-3M, melanoma; U251, glioblastoma; K562, chronic myelogenous leukemia; and CCRF-CEM, acute lymphoblastic leukemia. cDNA was prepared with the SMART cDNA synthesis kit (Clontech Laboratories). Normalization of the cDNA mixtures was carried out by Evrogen using duplex-specific nuclease (8, 9). Normalized cDNA mixture was fragmented as described (10), ligated with adapters generated by annealing one long and one short strand containing AgeI or SphI restriction sites, and then was PCR-amplified using the following adapter-specific PCR primers:

AgeI long adapter: AAACGTGGAGAATAAGCCGAATC-CACCGGTACAATGGATGGATGG
AgeI short adapter: CCATCCATCCATTGTACCGGTG
SphI long adapter: AAATCCTAAACCGACCGAGTATC-CACGAGCATGCCTAACTAACTA
SphI short adapter: TAGTTAGTTAGGCATGCTCGTG
AgeI amplified primer: CGTGGAGAATAAGCCGAATC
SphI amplified primer: TCCTAAACCGACCGAGTATC

Amplified cDNA fragments were digested with AgeI and Sph I and cloned into the corresponding restriction sites of the pLLCEm lentiviral vector. The latter vector was prepared from LLCEP-U6× (11) by removing the hU6 promoter and replacing EGFP-puro with a multiple cloning site containing AgeI and SphI restriction sites. The resulting GSE library contained ~$2.6 \times 10^8$ clones, with an average insert length of 135 bp.

**Quantitative RT-PCR Analysis of Gene Expression.** Total cellular RNA was purified either with RNAeasy kit (Qiagen) or with TRIzol (Invitrogen) to include small RNAs, according to manufacturers' protocols. First-strand cDNA was prepared using Maxima First-Strand cDNA Synthesis (MBI Fermentas). Coatomer protein complex 1 (COPI) gene expression was analyzed by quantitative RT-PCR (qRT-PCR) with corresponding primers (Table S4) using $RT^2$ SYBR Green qRT-PCR Master Mixes (Qiagen). Expression of microRNA 152 (miR-152) was analyzed as described previously (12) with universal and miR-152–specific primers (Table S4). L32 ribosomal protein and GAPDH were used for normalization.

**siRNAs and miRNAs.** siRNAs were obtained from Qiagen and Thermo Scientific/Dharmacon. The siRNA target sequences are listed in Table S3. Control siRNA (negative control; All-Star) and the cytotoxic siRNA mixture (positive control) were obtained from Qiagen. Mimetics of human miR-152 (hsa-miR-152), miRIDIAN Mimic (catalog no. C-300614–05-0005), and microRNA Mimic Negative Control #1 were obtained from Thermo Scientific. Synthetic hsa-miR-152 was obtained from Applied Biosystems/Ambion. siRNAs and miRNAs were transfected using the reverse format (96-well plates) or fast-forward format (other plates) siRNA transfection procedures, in antibiotic-free medium with Silentfect siRNA transfection reagent (Bio-Rad Life Science). Cell numbers were determined 4–8 d after transfection by flow cytometry or by staining cellular DNA with Hoechst 33342.

**Protein Expression Assays.** Protein levels were analyzed by immunoblotting using the enhanced chemiluminescence method (Thermo Scientific), with the following primary antibodies: goat anti-COPZ1 D-20 (Santa Cruz Biotechnology), guinea pig anti-COPZ2 (13), mouse anti-FLAG M2 (Sigma), and mouse anti-GFP (Roche). Brightness, contrast, and final size of the images were adjusted using Adobe Photoshop CS software.

**Immunofluorescence Assays.** Cells cultured on glass coverslips (Bellco Glass) were fixed with 3.7% paraformaldehyde in PBS for

20 min at room temperature and permeabilized with 0.5% Triton X-100 in PBS for 3 min. The coverslips were incubated with mouse anti-GM130 antibodies (BD Bioscience PharMingen) and DAPI (Invitrogen). The secondary antibody was Alexa Fluor 594-conjugated goat anti-mouse IgG (Invitrogen). Fluorescence images were acquired using a Leica DM IRE2 microscope equipped with a Leica cooled CCD camera, Leica FW4000 software, and an HCX PL Apo 63/1.4 NA objective. Brightness, contrast, color balance, and final size of the images were adjusted using Adobe Photoshop CS software.

**Time-Lapse Video Microscopy.** Phase-contrast time-lapse video microscopy was done using Leica DM IRE2 inverted microscopes in a climate-controlled 37 °C room using GIBCO $CO_2$-independent culture medium (18045; Invitrogen). The microscopes interfaced with personal computers running Leica's FW4000 program, which controlled the camera (Leica DC350FX) as well as focus position, filter cube, light source, and transmitted light intensity. The FW4000 program was used to run acquisition protocols over at least 5 d after siRNA transfection, and images were taken every 6 min.


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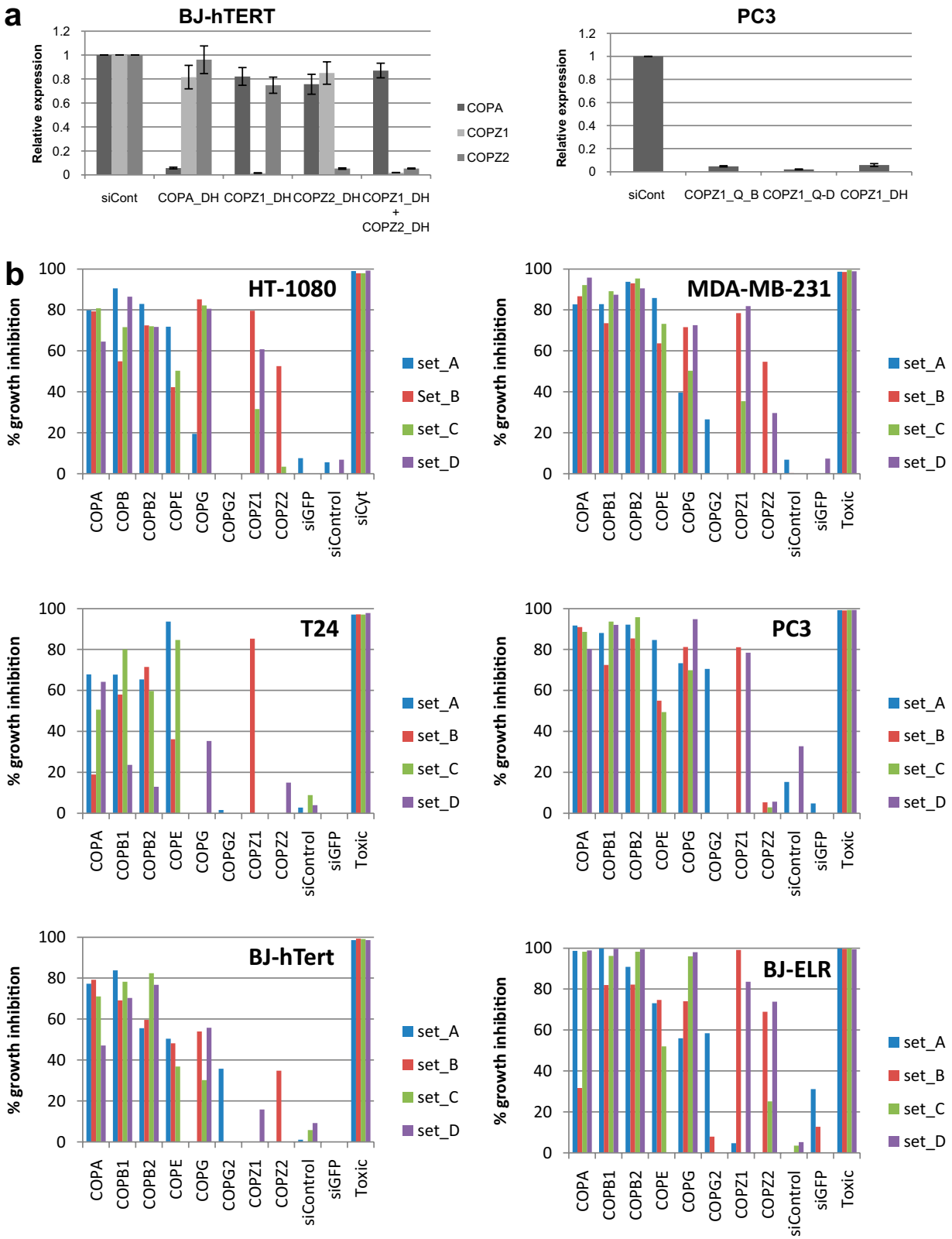



**Fig. S1.** Effects of COPI subunit knockdown in different cell lines. (*A*) qRT-PCR analysis of knockdown efficacy of COPA, COPZ1, and COPZ2 mRNAs in BJ-hTERT cells (*Left*) and PC3 cells (*Right*). mRNA levels in cells transfected with the indicated siRNAs are shown relative to cells transfected with control siRNA. Data shown are mean ± SD for duplicate measurements. (*B*) Effects of siRNAs targeting different COPI subunit proteins on the growth of different tumor cell lines and BJ fibroblast derivatives. The indicated cell lines were transfected with four siRNAs per gene [sets A–D (Qiagen)], at 5-nM concentrations targeting the indicated COPI subunits, with negative controls targeting no known genes (siControl), with GFP (siGFP), or with a toxic positive control comprising a mixture of cytotoxic siRNAs (siCyt) (Qiagen). Cell numbers were determined 4 d posttransfection by flow cytometry (in triplicate) and are plotted as mean percent growth inhibition (percent decrease in cell numbers relative to cells transfected with control siRNA).

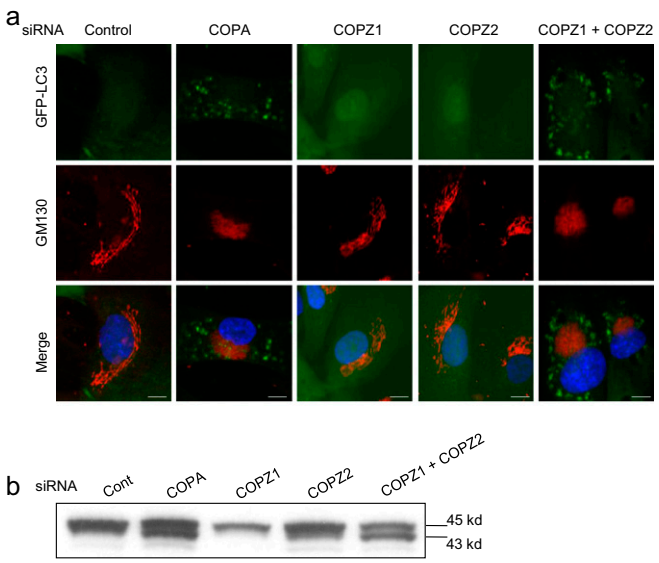



**Fig. S2.** Effects of COPZ1, COPZ2, and COPA knockdown in BJ-hTERT cells on Golgi apparatus, autophagosomes, and cell death. (*A*) BJ-hTERT cells expressing the autophagosome marker GFP-LC3 were transfected with control siRNA or siRNAs targeting COPA, COPZ1, COPZ2, or COPZ1 + COPZ2 (at 10-nM concentrations) and were analyzed by fluorescence microscopy for GFP fluorescence (green), indirect immunofluorescence staining for Golgi marker GM130 (red), and nuclear DNA staining with DAPI 72 h posttransfection with the indicated siRNAs. (Scale bars: 10 μM.) (*B*) GFP-LC3 electrophoretic mobility of the cells in *A* analyzed by immunoblotting with anti-GFP antibody.

**Table S1. mRNA expression of COPI subunit genes in normal human tissues (qRT-PCR)**

| | COPA | | COPB1 | | COPB2 | | COPZ1 | | COPZ2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD |
| Adipose | 2.86 | 1.8E-02 | 0.93 | 2.1E-03 | 1.26 | 1.2E-02 | 2.14 | 1.8E-02 | 3.20 | 1.1E-02 |
| Bladder | 0.91 | 2.3E-03 | 0.44 | 1.0E-05 | 0.53 | 1.4E-03 | 0.84 | 3.5E-03 | 1.23 | 3.8E-03 |
| Brain | 1.29 | 9.6E-03 | 0.32 | 5.3E-03 | 0.69 | 2.8E-03 | 1.45 | 5.1E-03 | 0.38 | 2.6E-03 |
| Cervix | 1.37 | 2.7E-02 | 0.35 | 4.9E-03 | 0.80 | 5.8E-03 | 1.40 | 1.1E-02 | 1.18 | 8.9E-03 |
| Colon | 1.31 | 2.3E-02 | 0.22 | 1.4E-04 | 0.67 | 4.5E-03 | 1.93 | 1.1E-02 | 0.90 | 4.0E-03 |
| Esophagus | 0.71 | 2.1E-02 | 0.51 | 5.8E-03 | 0.53 | 4.2E-04 | 1.53 | 2.1E-03 | 1.05 | 6.4E-03 |
| Heart | 0.35 | 4.4E-03 | 0.12 | 2.3E-03 | 0.20 | 7.5E-04 | 0.56 | 6.9E-03 | 0.35 | 6.0E-04 |
| Kidney | 1.26 | 8.2E-03 | 0.67 | 3.4E-03 | 0.68 | 2.3E-03 | 1.49 | 6.6E-05 | 0.49 | 2.3E-03 |
| Liver | 0.92 | 4.1E-03 | 0.38 | 1.6E-03 | 0.57 | 6.5E-03 | 1.06 | 1.2E-03 | 0.50 | 1.7E-03 |
| Lung | 1.24 | 4.4E-03 | 0.41 | 1.9E-03 | 0.80 | 2.6E-03 | 1.71 | 5.5E-04 | 0.58 | 3.3E-03 |
| Ovary | 1.90 | 3.3E-03 | 0.50 | 1.3E-03 | 1.25 | 1.2E-02 | 2.23 | 4.6E-02 | 0.61 | 7.4E-03 |
| Placenta | 1.32 | 6.3E-04 | 0.74 | 9.9E-03 | 1.25 | 6.4E-03 | 1.82 | 2.1E-02 | 0.55 | 4.7E-04 |
| Prostate | 1.22 | 3.5E-04 | 0.27 | 1.9E-04 | 0.90 | 7.4E-03 | 1.07 | 2.7E-03 | 1.39 | 2.4E-03 |
| Skeletal muscle | 0.10 | 4.9E-04 | 0.03 | 1.7E-04 | 0.04 | 9.9E-05 | 0.13 | 2.9E-03 | 0.21 | 7.3E-05 |
| Small Intestine | 1.27 | 4.4E-03 | 0.61 | 3.5E-03 | 0.82 | 5.9E-03 | 0.90 | 1.3E-02 | 0.29 | 1.2E-03 |
| Spleen | 1.05 | 3.0E-04 | 0.53 | 2.1E-05 | 0.79 | 2.1E-03 | 0.68 | 3.6E-04 | 0.28 | 2.3E-03 |
| Testes | 1.07 | 2.2E-03 | 0.69 | 5.5E-03 | 0.82 | 6.4E-03 | 0.93 | 7.8E-03 | 0.87 | 2.8E-04 |
| Thymus | 0.55 | 1.9E-03 | 0.21 | 6.9E-03 | 0.40 | 1.3E-03 | 0.69 | 9.3E-03 | 0.02 | 5.6E-05 |
| Thyroid | 1.87 | 1.3E-02 | 1.00 | 8.4E-05 | 1.27 | 3.1E-02 | 4.08 | 4.7E-03 | 0.82 | 3.2E-03 |
| Trachea | 0.71 | 4.6E-03 | 0.40 | 4.2E-04 | 0.66 | 3.8E-03 | 0.96 | 6.9E-03 | 0.41 | 7.4E-04 |
| BJ-hTERT | 1.00 | 8.5E-04 | 1.00 | 5.7E-03 | 1.00 | 3.4E-03 | 1.00 | 1.2E-03 | 1.00 | 2.1E-02 |

Colors mark different gene expression levels, from the lowest (dark blue) to the highest (orange).

**Table S2. Expression of COPZ1, COPZ2, and miR-152 in human tumor cell lines (qRT-PCR)**

| | | COPZ1 | | COPZ2 | | miR-152 | |
|---|---|---|---|---|---|---|---|
| Tumor cell line | Tumor type | Mean | ± SD | Mean | ± SD | Mean | ± SD |
| BJ-hTERT | Immortalized fibroblasts | 1.000 | 5.20E-02 | 1.000 | 6.89E-02 | 1.00 | 1.69E-02 |
| A549 | Lung cancer | 0.774 | 3.23E-02 | 0.084 | 1.28E-02 | 0.30 | 1.05E-02 |
| HT1080 | Fibrosarcoma | 0.347 | 2.19E-02 | 0.088 | 2.86E-03 | 0.34 | 5.80E-02 |
| WM 793 | Melanoma | 0.526 | 7.54E-02 | 0.310 | 7.76E-03 | 1.78 | 1.12E-01 |
| MCF7 | Breast cancer | 0.456 | 1.49E-02 | 0.010 | 4.28E-04 | 0.14 | 2.27E-03 |
| MDA-MB157 | Breast cancer | 0.988 | 2.35E-01 | 0.004 | 1.24E-03 | 0.05 | 3.30E-03 |
| MDA-MB231 | Breast cancer | 0.962 | 3.78E-03 | 0.019 | 5.98E-04 | 0.05 | 1.10E-02 |
| HeLa | Cervical cancer | 0.724 | 2.10E-02 | 0.002 | 4.32E-04 | ND | |
| PC3 | Prostate cancer | 0.636 | 8.01E-02 | 0.001 | 2.13E-05 | 0.01 | 7.91E-04 |
| Raji | Burkitt lymphoma | 0.464 | 6.75E-02 | 0.004 | 5.79E-04 | 0.11 | 5.47E-03 |
| Ramos | Burkitt lymphoma | 0.581 | 6.10E-03 | 0.010 | 6.06E-05 | 0.10 | 1.10E-03 |
| Jurkat | T-cell leukemia | 0.822 | 3.32E-01 | 0.008 | 7.09E-03 | 0.10 | 2.86E-04 |

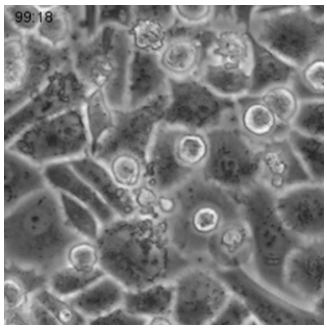
Colors mark different gene expression levels, from the lowest (dark blue) to the highest (orange).

**Table S3. Target sequences of siRNAs used for the knockdown of the indicated genes**

| Gene | siRNA | Target sequence |
|---|---|---|
| *COPZ1* | Qiagen A | AGCGATTTAAATTGTATTGAA |
| *COPZ1* | Qiagen B | TTGGCTGTGGATGAAATTGTA |
| *COPZ1* | Qiagen C | TTGGGAATAGTTCATAGGGAA |
| *COPZ1* | Qiagen D | TCCCAGCATATTTAGATAATA |
| *COPZ1* | Thermo Scientific (pool of four siRNAs) | GGACAAUGAUGGAGAUCGA |
| | | CAACAAGACCCAUCGGACU |
| | | GGGAAUAGUUCAUAGGGAA |
| | | AUUGGAGCUCCUAUGAAA |
| *COPA* | Qiagen A | TCCCACTGAGTTCAAATTCAA |
| *COPA* | Qiagen B | CTGGATTTCAACAGCTCCAAA |
| *COPA* | Qiagen C | CTGGCGCATGAATGAATCAAA |
| *COPA* | Qiagen D | AAGCTTAATGACCTCATCCAA |
| *COPA* | Qiagen 5 | CACACGGGTGAAGGGCAACAA |
| *COPA* | Thermo Scientific (pool of four siRNAs) | ACUCAGAUGGUGUGUAAUA |
| | | GCAAUAUGCUACACUAUGA |
| | | GAACAUUCGUGUCAAGAGU |
| | | GCGGAGUGGUUCCAAGUUUU |
| *COPB1* | Qiagen A | CAGGATCACACTATCAAGAAA |
| *COPB1* | Qiagen B | CAAGGATTGGTTATAATATAA |
| *COPB1* | Qiagen C | CAGAATTGCTAGAACCTTTAA |
| *COPB1* | Qiagen D | CACCAACATGGTTGATTTAAA |
| *COPB2* | Qiagen A | ACGATTCTTCAGAGTATGCAA |
| *COPB2* | Qiagen B | CAGGTTTCAAGGGTAGTGAAA |
| *COPB2* | Qiagen C | CAGTACGTATTTGGCATTCAA |
| *COPB2* | Qiagen D | CTGCTAGATCTGATCGAGTTA |
| *COPE* | Qiagen A | CCGGAAGGAGCTGAAGAGAAT |
| *COPE* | Qiagen B | CAGAGCTGTCAGGACCATGAA |
| *COPE* | Qiagen C | CCCGGAAGGAGCTGAAGAGAA |
| *COPE* | Qiagen D | ATCTGTTAATAAATATCTCAA |
| *COPG* | Qiagen A | AGGCCCGTGTATTTAATGAAA |
| *COPG* | Qiagen B | CCGAGCCACCTTCTACCTAAA |
| *COPG* | Qiagen C | CACCGACTCCACTATGTTGAA |
| *COPG* | Qiagen D | TCCGTCGGATGTGCTACTTGA |
| *COPG2* | Qiagen A | CAGGTGACTGTCAGAAGTAAA |
| *COPG2* | Qiagen B | CTGCATCAAGTGATAATATTA |
| *COPG2* | Qiagen C | GACGCGATTGTTTCAATCTAA |
| *COPG2* | Qiagen D | AGGCTCGTATATTCAATGAAA |
| *COPS8* | Qiagen D | CTGCATTTGTTCAATAAATAT |
| *COPZ2* | Qiagen A | CTGGCCTTAACTCATATCTTA |
| *COPZ2* | Qiagen B | CAGCATTGACCTCTTCCTATA |
| *COPZ2* | Qiagen C | AACAAATTAAATGGTCGTTAT |
| *COPZ2* | Qiagen D | CCGGCTGCTGGCCAAGTATTA |
| *COPZ2* | Thermo Scientific (pool of four siRNAs) | GGGCUCAUCCUACGAGAAU |
| | | UCUUGGUGCUGGACGAGAU |
| | | CAACAAGACCAGCCGGACU |
| | | GAACAAAUUAAAUGGUCGU |

**Table S4. Primer sequences for qRT-PCR of the indicated genes**

| Gene | Sense | Antisense |
|---|---|---|
| *GAPDH* | AGGTGAAGGTCGGAGTCA | GGTCATTGATGGCAACAA |
| *RPL13A* | AGATGGCGGAGGTGCAG | GGCCCAGCAGTACCTGTTTA |
| *COPZ1* | ACACTGGGGTAGGTGTCGTC | AAGATGGAGGCGCTGATTTT |
| *COPZ2* | CCTTCTGGATCACTTGCTGG | GGTTGCTGGAGAACATGGAC |
| *COPA* | TATCAACCTCCCATGCCTTT | ACCCCACTATGCCCCTTATT |
| *COPB1* | TCTGAAACTTGTGGAAAAGC | ACACAATTTCTGTCACTTGC |
| *COPB2* | GCTCTGTAGGATGCAGATCCA | GTAGCCGGTAACAAACGAGG |
| Universal miRNA | AACGAGACGACGACAGACTTT | |
| miR-152 | | TCAGTGCATGACAGAACTTG |




**Movie S1.** Time-lapse phase-contrast video microscopy of PC3 cells transfected with 10 nM COPZ1 siRNA. Numbers in the upper left show the time (hours and minutes) after transfection. Note multiple apoptotic events (cell shrinking and blebbing) and the formation of rounded cells, some of which (i.e., the cell in the center) undergo apoptosis. Such events were rare among cells transfected with control siRNA.

Movie S1