Supplementary Materials

Supplementary Materials and Methods:

Synthetic scheme for the preparation of metarrestin

Metarrestin synthesis

2-Amino-1-benzyl-4,5-diphenyl-1H-pyrrole-3-carbonitrile (fig. S12). A modified Voigt reaction/ Knoevenagel condensation sequence was carried out using the procedure of Roth and Eger (*74*). Thus, benzoin (21.22 g, 100.0 mmol), benzylamine (16.07 g, 150.0 mmol, 1.5 equiv.), and trifluoroacetic acid (0.57 g, 5.0 mmol, 0.05 equiv.) were heated at reflux for 1 h connected to a Dean-Stark trap, and the mixture was removed from the oil bath. Malononitrile (9.91 g, 150.0 mmol, 1.5 equiv.) was added to the mixture, and the reaction heated at reflux for 1 h connected to a Dean-Stark trap. The reaction mixture was allowed to cool to room temperature and stirred for 16 h, affording the crude pyrrole as a dark brown solid. The product was further precipitated with ethyl ether and the solid washed with additional ethyl ether until the filtrate was colorless, affording the previously reported pyrrole product (*75*) as a light orange solid (24.50 g, 70.12 mmol, 70% yield), which was used without further purification. R_f = 0.22 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 (s, 2 H), 7.06–7.37 (complex, 15 H); ^{13}C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d (CH, CH_3): 125.8, 126.3, 127.9, 128.1, 128.2, 128.6, 128.7, 129.2, 131.0; u (C, CH_2): 46.9, 117.5, 120.9, 125.6, 130.8, 133.1, 136.0, 146.0; IR 2195, 1663, 1556 cm^{-1}; HRMS calculated for $C_{24}H_{20}N_3$ $[M + H]^+$ 350.1657, found 350.1648.

(E)-Ethyl N-(1-benzyl-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)formimidate (fig. S13). 2-Amino-1-benzyl-4,5-diphenyl-1H-pyrrole-3-carbonitrile (6.18 g, 17.7 mmol) and triethylorthoformate (26.20 g, 176.9 mmol, 10.0 equiv.) were heated at 75 °C for 14 h, and the excess triethylorthoformate was removed *in vacuo*. The residue was dissolved in a minimum of CH_2Cl_2, adsorbed onto celite, and chromatographed on silica to afford the previously reported formimidate product (*75*) as a tan solid (6.70 g, 16.5 mmol, 93% yield). R_f = 0.47 (20% EtOAc in hexanes); mp = 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (t, *J* = 7.2 Hz, 3 H), 4.27 (dq, *J* = 0.8, 8.2 Hz, 2 H), 5.05 (s, 2 H), 6.86 (dd, *J* = 2.0, 8.0 Hz, 2 H), 7.06 (dd, *J* = 1.6, 8.0 Hz, 2 H), 7.14–7.29 (complex, 11 H), 8.51 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d (CH, CH_3): 14.0, 126.5, 126.6, 127.3, 128.2, 128.3, 128.5, 129.0, 131.2, 158.4; u (C, CH_2): 47.0, 63.3, 117.9, 123.2, 128.6, 130.9, 132.9, 137.7, 143.9; IR 2208, 1627, 1605 cm^{-1}; HRMS calculated for $C_{27}H_{24}N_3O$ $[M + H]^+$ 406.1919, found 406.1915.

***trans*-4-(7-Benzyl-4-imino-5,6-diphenyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)cyclohexanol (metarrestin)** (fig. S14). A solution of (E)-ethyl N-(1-benzyl-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)formimidate (2.61 g, 6.44 mmol), *trans*-4-aminocyclohexanol hydrochloride (1.95 g, 12.87 mmol, 2.0 equiv), and potassium *tert*-butoxide (2.17 g, 19.32 mmol, 3.0 equiv.) in MeOH (75 mL) was heated at 50 ºC for 19 h, and then cooled to room temperature and filtered. The precipitate was washed with MeOH (2 x 20 mL) and water (2 x 20 mL). The solid was recrystallized from hot MeOH (200 mL) to afford the pyrrolopyrimidine product, metarrestin, as a white solid (1.70 g, 3.58 mmol, 56% yield). R_f = 0.39 (1:1 acetone:CH_2Cl_2 with 1% Et_3N); mp = 222–224 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55–1.64 (m, 4 H), 2.01–2.12 (m, 4 H), 3.68 (t, *J* = 9.4 Hz, 1 H), 5.05 (t, *J* = 11.2 Hz, 1 H), 5.27 (s, 2 H),

6.47 (s, 1 H), 6.96 (d, J = 6.4 Hz, 2 H), 7.04 (d, J = 6.6 Hz, 2 H), 7.18–7.24 (complex, 11 H), 7.74 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$, APT pulse sequence)δ d (CH, CH_3): 51.5, 70.0, 126.79, 126.83, 127.2, 128.0, 128.1, 128.3, 128.5, 130.6, 131.1, 142.5; u (C, CH_2): 30.5, 34.9, 46.0, 103.3, 118.3, 130.7, 132.7, 133.9, 138.0, 142.3, 155.5; IR 1625, 1604 cm^{-1}; HRMS (ESI) m/z calculated for $C_{31}H_{31}N_4O$ $[M + H]^+$ 475.2498, found 475.2492.

Preparation of biotin-metarrestin (P1)

2-Amino-1-(3-bromobenzyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile (fig. S15). A modified Voigt reaction/Knoevenagel condensation sequence was carried out using the procedure of Roth and Eger (*74*) Thus, benzoin (5.26 g, 24.8 mmol), 3-bromobenzylamine (4.61 g, 24.8 mmol), and trifluoroacetic acid (0.14 g, 1.24 mmol, 0.05 equiv.) were heated at reflux for 1 h connected to a Dean-Stark trap, and then the mixture was removed from the oil bath. Malononitrile (4.91 g, 74.3 mmol, 3.0 equiv.) was added to the mixture, and the reaction again heated at reflux for 1 h connected to a Dean-Stark trap. The reaction mixture was allowed to cool to room temperature and stirred for 19 h, affording the crude pyrrole as a dark red solid. The product was further precipitated with ethyl ether and the solid washed with additional ethyl ether until the filtrate was colorless, affording the pyrrole product as a light purple solid (6.80 g, 15.87 mmol, 64% yield), which was used without further purification. R_f = 0.53 (50% EtOAc/hexanes); mp = 184–190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.96 (s, 2 H), 6.80 (d, J = 7.8 Hz, 1 H), 7.03–7.28 (complex, 12 H), 7.43 (d, J = 9.0 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6, APT pulse sequence) δ d (CH, CH_3): 125.6, 126.6, 128.3, 128.5, 128.9, 129.0, 129.6, 130.4, 131.1, 131.5; u: (C, CH_2): 45.4, 118.4, 120.7, 122.0, 124.0, 131.3, 133.9, 140.4,

149.1; IR 2203, 1632 cm^{-1}; HRMS (ESI) m/z calculated for $C_{24}H_{19}BrN_3$ $[M + H]^+$ 428.0757, found 428.0749.

(E)-Ethyl N-(1-(3-bromobenzyl)-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)formimidate (fig. S16). 2-Amino-1-(3-bromobenzyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile (2.71 g, 6.33 mmol) and triethylorthoformate (7.50 g, 50.60 mmol, 8 equiv.) were heated at 65 °C for 18 h, and the excess triethylorthoformate was removed *in vacuo*. The residue was dissolved in a minimum of CH_2Cl_2, adsorbed onto celite, and chromatographed on silica to afford the formimidate product as a light orange solid (2.60 g, 5.37 mmol, 85% yield). R_f = 0.81 (50% EtOAc/hexanes); mp = 130–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, *J* = 7.1 Hz, 3 H), 4.29 (q, *J* = 7.1 Hz, 2 H), 5.01 (s, 2 H), 6.79 (d, *J* = 7.7 Hz, 1 H), 7.03–7.35 (complex, 13 H), 8.55 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d (CH, CH_3): 14.0, 125.2, 126.7, 128.2, 128.5, 128.7, 129.0, 130.0, 130.1, 130.5, 131.2, 158.5; u (C, CH_2): 46.3, 63.4, 117.8, 122.5, 123.3, 128.4, 130.7, 132.7, 139.8, 143.7; IR 2209, 1627 cm^{-1}; HRMS (ESI) m/z calculated for $C_{27}H_{22}BrN_3O$ $[M + H]^+$ 484.1019, found 484.1004.

***trans*-4-(7-(3-Bromobenzyl)-4-imino-5,6-diphenyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)cyclohexanol** (fig. S17). A solution of (E)-ethyl N-(1-(3-bromobenzyl)-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)formimidate (440 mg, 0.91 mmol), *trans*-4-aminocyclohexanol hydrochloride (275 mg, 1.82 mmol, 2.0 equiv), and potassium *tert*-butoxide (306 mg, 2.73 mmol, 3.0 equiv.) in MeOH (20 mL) was heated at 50 ºC for 19 h, and then cooled to room temperature and filtered. The reaction was diluted with CH_2Cl_2, (30 mL),

adsorbed onto celite, and purified by silica chromatography to afford the pyrrolopyrimidine product as a tan solid (465 mg, 0.84 mmol, 92% yield). R_f = 0.28 (10% MeOH in CH_2Cl_2); mp = 197–201 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52–1.73 (m, 4 H), 2.11 (t, *J* = 13.3 Hz, 4 H), 3.61–3.70 (m, 1 H), 4.89 (t, *J* = 11.2 Hz, 1 H), 5.22 (s, 2 H), 6.36 (br s, 1 H), 6.86 (d, *J* = 7.4 Hz, 1 H), 7.02–7.11 (m, *4* H), 7.18-7.23–7.34 (complex, 9 H), 7.78 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d (CH, CH_3): 52.3, 69.5, 125.6, 127.1, 128.35, 128.37, 128.4, 130.09, 130.14, 130.5, 130.6, 131.1, 142.6; u (C, CH_2): 30.7, 34.5, 45.1, 103.2, 118.3, 122.6, 130.3, 133.1, 133.5, 140.0, 142.3, 155.8; IR 1624 cm^{-1}; HRMS (ESI) m/z calculated for $C_{31}H_{30}BrN_4O$ $[M + H]^+$ 553.1598, found 553.1574.

***N*-(6-(3-((3-(*trans*-4-hydroxycyclohexyl)-4-imino-5,6-diphenyl-3,4-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl)phenyl)hex-5-yn-1-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (biotin-metarrestin, P1)** (fig. S18). A microwave vial was charged with *trans*-4-(7-(3-Bromobenzyl)-4-imino-5,6-diphenyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)cyclohexanol (55 mg, 0.10 mmol), *N*-(hex-5-yn-1-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (96 mg, 0.30 mmol), copper (I) iodide (11 mg, 0.06 mmol), and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium (II) (27 mg, 0.03 mmol). The vial was capped and the atmosphere evacuated and backfilled with argon twice (the yield of the cross-coupling reaction was much lower if oxygen was not removed). Diisopropylethylamine (1 mL) and DMF (2 mL) were added via syringe and the reaction heated at 100 ºC for 2 h under microwave irradiation. After cooling to room temperature (RT), the reaction was adsorbed onto Celite and immediately purified by silica gel chromatography (0 to 30% MeOH + 0 to 2%

$NH_4OH_{(aq)}$ in CH_2Cl_2) to afford the biotin-metarrestin analog P1 (23 mg, 0.029 mmol, 29% yield) as an orange solid. R_f = 0.26 (10% MeOH + 2% NH_4OH in CH_2Cl_2); mp = 138–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36–1.44 (m, 2 H), 1.51–1.72 (complex, 12 H), 2.04–2.12 (m, 4 H), 2.17 (t, *J* = 7.4 Hz, 2 H), 2.39 (t, *J* = 6.4 Hz, 2 H), 2.64 (d, *J* = 13.2 Hz, 1 H), 2.81 (dd, *J* = 4.8, 12.8 Hz, 1 H), 3.09 (q, *J* = 4.7 Hz, 1 H), 3.24 (q, *J* = 6.0 Hz, 2 H), 3.63–3.70 (m, 1 H), 4.23–4.26 (m, 1 H), 4.38–4.42 (m, 1 H), 5.03 (t, *J* = 11.8 Hz, 1 H), 5.20 (s, 2 H), 5.66 (br s, 1 H), 6.28 (t, *J* = 5.2 Hz, 1 H), 6.43 (br s, 1 H), 6.78 (d, *J* = 8.0 Hz, 1 H), 7.00–7.03 (m, 2 H), 7.11–7.26 (complex, 10 H), 7.78 (s, 1 H);^{13}C NMR (101 MHz, $CDCl_3$, APT pulse sequence) δ d (CH, CH_3): 51.9, 55.6, 30.1, 61.8, 69.6, 126.0, 127.0, 128.1, 128.2, 128.3, 128.4, 129.8, 130.51, 130.54, 131.0, 142.6; u (C, CH_2): 19.1, 25.7, 26.0, 28.1, 28.2, 28.8, 30.5, 34.7, 36.0, 38.9, 40.5, 45.7, 80.8, 90.2, 103.1, 118.2, 124.1, 130.4, 132.9, 133.7, 138.0, 142.4, 155.3, 163.9, 173.2; IR 3283, 2934, 1696, 1624 cm^{-1}; HRMS (ESI) m/z calculated for $C_{47}H_{54}N_4O$ $[M + H]^+$ 796.4003, found 796.3997; $[\alpha]_{589}$ = + 0.30 (c = 0.84 g/100 mL in CH_2Cl_2, 22 °C).

Quantitative metarrestin analysis – UPLC-MS/MS for pharmacokinetics

An ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed to determine metarrestin concentrations in plasma and tissue samples. A Waters Xevo TQ-S triple quadrupole instrument with electrospray ionization in the selected reaction monitoring mode was used. The separation was performed on an Acquity BEH C18 column (50 × 2.1 mm, 1.7 microns) using a Waters Acquity UPLC system with 0.6 mL/min flow rate and gradient elution. Tissue samples were homogenized with 3 or 6 volumes (v/w) of water on the day of analysis. 10 µL of plasma or tissue homogenate sample was mixed with 200

μL acetonitrile containing internal standard to precipitate proteins in a 96-well plate. 1.0 μL supernatant was injected for the UPLC-MS/MS analysis. Data were analyzed using MassLynx V4.1.

Pharmacokinetics

BALB/c mice, 17–19 g, female, n = 66, were purchased from BK Laboratory Animal Co. LTD and had free access to food and water. They were dosed with metarrestin at 5 or 25 mg/kg (10 ml/kg) in 5% NMP [NMP (Sigma-Aldrich cat#270458), 20% PEG400 (Sigma-Aldrich cat# 202398), and 75% 10% HPBCD (Sigma-Aldrich cat# H107)] via lower left abdominal quadrant injection (n = 33 each dose). The animals were anesthetized with isoflurane and restrained manually at the designated time points. Approximately 120 μL of blood were taken from the animals into K_2EDTA tubes via retro-orbital puncture. Blood samples were placed on ice and centrifuged (2,000 g, 5 min under 4 ℃) to obtain plasma samples within 15 minutes. An aliquot of 20 μL plasma sample was protein-precipitated with 200 μL acetonitrile that contained 50 ng/mL IS (dexamethasone). The mixture was vortexed for 2 min, centrifuged at 14,000 rpm for 5 min, and an aliquot of 30 μL supernatant was diluted with 70 μL MeOH:H_2O (1:1,v/v), then vortexed for 2 min. A 2 μL aliquot of supernatant was injected into UPLC-MS/MS. Analysis of samples was done with an LC-MS/MS-02 (API4000), Waters-BEH-C18 (2.1 × 50 mm, 1.7 μm) column, flow rate 0.6 mL/min, with a mobile phase consisting of solvent A (H_2O- 0.025%FA-1 mM NH_4OAc) and solvent B (MeOH- 0.025%FA-1 mM NH_4OAc). The gradient was 10, 90, 90, 10% B at 0.30, 0.60, 1.20, 1.21, 1.80 (stop) min. Retention times for metarrestin and dexamethasone were 1.09 and 1.08 min, respectively. Calibration curves were established with known serially diluted concentrations of metarrestin before analysis. SCID Beige mice were purchased from

Charles River and dosed with metarrestin at 5 and 25 mg/kg, once daily at 24 hour intervals for 4 weeks as above. 24 h after the final dose, concentrations of metarrestin in plasma were analyzed as above.

Cell culture and drug treatments: PC-3M cells were maintained in RPMI 1640 medium (Invitrogen). Other cell lines including HeLa, Panc1, PACADD159, PACADD183, PA-TU-8988T, KP-3, PK-8, NOR-P1, L3.6sl, Colo357, Suit-2, HPAC were maintained in DMEM (Invitrogen) with 10% FBS (GEMINI Bio Products) and 100 units/mL of penicillin and streptomycin (Invitrogen). Cells were generally plated ~24 h before treatment with metarrestin for 5 h or 24 h at 1 μM.

***Immunofluorescence*:** The experiments were performed as previously described (*3*). Primary antibodies: 1:300 dilution for SH54 (anti-PTB antibody) (*6*); 1: 300 for Nopp140 (anti-human Nopp140 rabbit polyclonal serum RS8) (*76*); 1: 50 for UBF (F-9) (Santa Cruz Biotechnology); 1:100 for RPA194 (Santa Cruz Biotechnology); 1:100 for CUG-BP1 (3B1) (Santa Cruz Biotechnology); 1:10 for fibrillarin (Sigma Cat #: ANA-N); 1:1000 for SC35 (*38*); 1:300 for paxillin (ab32084); 1:600 for vinculin (Sigma V9131). The fluorescein-conjugated secondary antibodies (Jackson ImmunoResearch) and Alexa Fluor antibodies (Thermofisher) were used at a dilution of 1:200. Samples were visualized on a Nikon Eclipse Ti-E inverted fluorescence microscope using NIS-Elements AR 3.2 software (Nikon).

ChIP-qPCR: 2-5×10^7 cells were used for each ChIP assay according to previously described protocols (*77*). Briefly, HeLa cells with or without metarrestin treatment were crosslinked with 1% formaldehyde for 10 min at room temperature with rotation, and then crosslinking was quenched by the addition of glycine. Fixed chromatin was sonicated by Covaris and used for immunoprecipitation with the indicated antibody. Isolated DNA was analyzed by qPCR using SYBR green on CFX Connect Real-Time PCR Detection System (BioRad). The comparative cycle threshold method was applied to evaluate occupancy from replicate PCR reactions relative to the level of input. The primer sets used in the study included: rDNA promoter (F: 5'-GCT GCG ATG GTG GCG TTT TTG GGG and R: 5'-ATA TAA CCC GGC GGC CCA AAA TTG CC), 5'ETS (F: 5'-CGTGCCTGAGGTTTCTCC and R: 5'-CCACCAACGGACGTGAAG), 5.8S (F: 5'- GCA GGA CAC ATT GAT CAT CGA CAC and R: 5'- GCG CGG CGG CAA GAG GAG), 28S (F: 5'-GGAGGAAAAGAAACTAACCAGGAT and R: 5'-GCCTCGATCAGAAGGACTTG), and *U12* (F: 5'GATCTGCCCGACCTTATTCA and R 5'AAACGCATTCACCACCTACC).

In vivo Br-UTP incorporation: Cells were rinsed once in glycerol buffer (20 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 25% glycerol, 0.5 mM PMSF, 0.5 mM EGTA) and followed by Br-U incorporation assay protocol (*8*). The cells were co-immunolabeled with C23 (Santa Cruz Biotechnology) at a 1:100 dilution and anti-BrdU that also recognizes Br-U (Sigma) at a 1:50 dilution.

Western blot: Western blots were performed according to the manufacturer's protocols (Li-COR). Transfer membrane was from Millipore (cat. no. IPFL00010). The primary antibodies used were phospho-γH2AX (Upstate 07-164, rabbit, 1:1000); p53 (Santa Cruz Biotechnology sc-6243 rabbit 1:500); UBF (F-9) (Santa Cruz Biotechnology, mouse, 1:500); RPA194 (Santa Cruz Biotechnology, sc-48385, mouse, 1:500); p53BP1 (Novus, NB100-304, rabbit 1:10,000); EF1a (Millipore 05-235, mouse, 1:1000), HA-Tag (C29F4, Cell Signaling, rabbit 1:1000) and actin (Sigma A5060, rabbit, 1:3000, A4700, mouse, 1:1000) as a loading control. LI-COR IRDye secondary antibodies (1:10,000), goat anti-mouse-680RD (926-68070), goat anti-mouse-800CW (926-32210), goat-anti-rabbit-680RD (926-68071), goat-anti-rabbit-800CW (926-32211) were used for visualization. Protein bands were detected using LI-COR Odyssey Image Studio (LI-COR Biosciences).

***RT- PCR*:** Total RNA was isolated from cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized with Random Hexamer (IDT DNA Technologies) or gene-specific primers using M-MLV Reverse Transcriptase (Invitrogen 28025-013), The DNA was PCR-amplified with gene-specific primers. 5'ETS-F: CCTCCAGTGGTTGTCGACTT; 5'ETS-R: GAACGACACACCACCGTTC; eEF1A1-F: AACATTGTCGTCATTGGACA; eEF1A1-R: TTGATCTTTCCCTTTCTGGT; eEF1A2-F: 5' CCATGTGTGTGGAGAGCTTCTC; eEF1A2-R: 5' TCTCCACGTTCTTGATGACGCC; GAPDH-F: 5'ACCACAGTCCATGCCATCAC; GAPDH-R: 5'TCCACCACCCTGTTGCTGTA. The PCR products were resolved on a 1–2% agarose gel.

qPCR: Real time PCR was performed with ABI PRISM 7900HT instrument and Power SYBR Green PCR Master Mix (Life Technologies Cat# 4367659) at the Northwestern University Core Facility.

RNA interference: 75 nM Pol I1 Stealth siRNA (Invitrogen Cat#: 10620318) or Stealth negative control siRNA (Invitrogen Cat#: 12935-300) were transfected into cells with Lipofectamine RNAiMAX Transfection Reagent (Thermofisher Scientific) according to the manufacturer's instructions. The experiments were performed after 72 hours transfection. For eEF1A2 knockdown, 50 nM eEF1A2 DsiRNA (IDT Duplex pool HSC.RNAI.N001958.12/ HSC.RNAI.N001958.12.2) or DS Scramble Negative Control siRNA (IDT) were transfected with Lipofectamine RNAiMAX as described above for 3 days. pcDNA3.1-HA-eEF1A2 plasmid (*78*) was transfected after DsiRNA RNAi for 1 day with Lipofectamine 2000. The cells were then fixed for immunofluorescent labeling.

Flow cytometry: DNA content/cell cycle measurement with three metarrestin-treated cell lines was carried out with DAPI staining protocol in Northwestern University Core Facility.

Electron microscopy (EM): Cells were treated with metarrestin and DMSO, then fixed in 2% paraformaldehyde-2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3 and EM. For analysis of nucleoli of treated and untreated cells, the largest nucleolus in each cell was chosen. Nucleolar area (mm^2) was estimated with the formula $((\text{largest} + \text{shortest diameter})/2)^2 \times \pi$. Comparisons were also made using the largest diameter (L) and the modified ellipsoid formula for estimated

nucleolar volume ½ (length (L) × width $(W)^2$). The data were analyzed using Mann Whitney U test.

24-well Transwell Permeable Matrigel Invasion assay: The effect of metarrestin on invasion activity of PANC1 and PC3M cells was measured using 24-well Transwell Permeable Matrigel Invasion Chambers with 8 μm pores (Corning, Cat #354480). Membranes were rehydrated with 500 μL of serum-free DMEM (Thermo Fisher, Cat #11965084) both inside the chamber and inside the well and incubated for two hours at 37 °C, 5% CO_2. Cells were detached, washed, resuspended in serum-free DMEM, counted, and diluted to a final concentration of 1×10^5 cells/mL in six separate 15 mL conical tubes. Metarrestin or vehicle was added to each tube in decreasing concentrations, as indicated in Fig. 1D, and 500 μL of resuspended cells in metarrestin or vehicle-containing medium were placed in the upper chambers in triplicates. 750 μL of DMEM plus 10% FBS acting as a chemoattractant were placed in the basal wells. Plates were incubated at 37 °C, 5% CO_2 for 24 hours. To count the number of invading cells, medium from the top insert was removed and a cotton swab was used to wipe out non-invading cells from the top side of the membrane on the insert. Invading cells on the bottom of the membrane on the insert were fixed and stained using the three-step Diff Quik Rapid Stain Set (Siemens) followed by three water washes and air drying. Cell invasion for each compound treatment was performed in triplicate. Representative photographs were taken of each insert.

Incucyte cell proliferation assay: PC3M and PANC1 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin and dissociated at 70% confluence. 40 μL of PC3M cells at a density of 1.25×10^4 cells/ml or PANC1 cells at a density of 2.5×10^4

cells/mL were seeded in tissue culture-treated 384-well plates (Corning, cat#3712) and incubated overnight at 37 °C, 5% CO_2. 40 µL of DMEM containing 2 × final concentration of metarrestin or DMSO were added to the wells with cells, creating three replicates per cell line. Plates were placed in the Incucyte Zoom (Essen), and brightfield photographs were taken every three hours for approximately five days. Incucyte Zoom analysis software was used to measure percent confluence.

***Small molecule pull-down*:** PC3M cells were harvested and then lysed with homogenization buffer, and the affinity precipitation experiment was processed as previously described (*79*). A biotinylated version of metarrestin (P1) was immobilized to streptavidin resin as previously described (*79*). Pulled down proteins were separated on a 10% Bis-Tris gel (Life Technologies) and visualized by silver staining using a Pierce Silver Stain Kit for Mass Spectrometry (Pierce Chemical). In addition, competition with soluble ligand was assessed by adding metarrestin (63 µM) to the PC3M cell lysates during binding to the matrix. Pull-down using recombinant eEF1A was done in a similar way.

Proteomics: The excised gel bands were destained and digested with sequencing-grade trypsin overnight at 37 ºC (*80*). The digested peptides were extracted with 1% formic acid and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis by Agilent 1100 nanoflow LC system coupled on-line with hybrid linear ion trap-FT-ICR instrument (LTQ-FT, Thermo Electron) (*81*). Tandem MS data were used to search the entire NCBInr human database

using SEQUEST. Positive hits were re-evaluated by Scaffold (Proteome Softwares, Inc.), and hits that showed 95% probability in Scaffold were considered as significant hits.

Cellular thermal shift assay: Cell lysates were used for the cellular thermal shift assays as previously described (*82*). Cultured PC3M cells were harvested and washed with PBS. The cell lysates was prepared and divided into two aliquots. One aliquot was treated with metarrestin, and the other aliquot was treated with DMSO. After incubation for 30 min at room temperature, the lysates were further divided into smaller aliquots (50 μL) and heated individually for 3 min from 62 ºC to 89 ºC. The heated lysates were centrifuged at 16,000 × g for 20 minutes at 4 °C, and the supernatants were separated from precipitates. The supernatants were analyzed by SDS-PAGE followed by Western blot using anti-eEF1A antibody.

Psoralen crosslinking: Psoralen crosslinking was performed as described (*31*) with the following adjustments: Trioxsalen solution was 200 μg/mL in EtOH (SIGMA T6137). Cells were lysed in 0.25 mL lysis buffer. Proteinase K was added to a final concentration of 2 mg/ml. One phenol/chloroform extraction was performed. After staining with EtBr, psoralen crosslinks were reversed by irradiation at 254 nm for 5 minutes per side. The gel was soaked in 1 L depurination solution (0.25 M HCl) for 30 minutes before neutralization for 2 × 20 minutes with 1.5 M NaCl/0.5 M NaOH. The gel was then soaked in 1.5 M NaCl/0.5 M Tris pH 7.0 for 2 × 20 minutes. The gel was transferred to a Hybond XL membrane in 20 × SSC transfer solution. The membrane was washed with 2 × SSC for 10 minutes before crosslinking twice at 254 nm on Optimal Crosslink (Fisher, FB-UVXL-1000). Hybridization was performed overnight at 65 °C

using random-primed labeled DNA in hybridization solution (7.5 × Denhardt's solution, 5X SSPE, 0.1% SDS, 1 mL 10 mg/mL E coli tRNA). Membranes were washed for 20 minutes at 65 °C in 5 × SSPE, 0.1% SDS. Two additional washes of 0.5 × SSPE, 0.1% SDS were performed at 65 °C (2 × 20 mins) before membranes were analyzed using a phosphorimager or exposed to film.

Pancreatic cancer model: 60,000 luciferase-tagged PANC1 spheres were injected into the pancreas of Nod/IL2gamma (null) mice (obtained from NCI Mouse Repository, Frederick, MD; on ACUC-approved animal protocol SB-211-2). At 4 weeks after inoculation, mice were treated once daily with metarrestin (5 mg/kg or 25 mg/kg) or vehicle via IP injections, which extended out for 6 more weeks. Mice were injected with luciferin 5 minutes before being sacrificed by CO_2 euthanasia. Organs were then individually dissected, subjected to quantitative xenogene imaging, and fixed with 4% non-buffered formaldehyde. Impact of metastatic burden was calculated and graphed as liver and lung/primary tumor ratios based on a five-minute photon count for the liver normalized to liver weight (g), which was then compared to the average photon count per weight (g) of the primary pancreatic tumor of the same animal. This calculation corrects for variations in luciferin injection, mouse heart rate, and perfusion (*17*). For overexpression of eEF1A2, 6×10^4 PANC1 3D spheres transduced with eEF1A2 (eEF1A2 OE) were injected into the tail of the pancreas of NSG mice. Mice of both groups were harvested at 6 weeks after implantation and subjected to necropsy. Liver metastasis/mm^2 was calculated as the sum of the volumes of the individual metastases. Tumor volume (mm^3) was calculated as ($L \times W^2$), where L = length (mm) and W = width (mm). Animal survival was measured from the first day of treatment until death. Animals in control and metarrestin-treatment cohorts were allowed to progress under continuous treatment conditions until they

reached a predetermined study end-point (15% weight loss, recognizable signs of morbidity, general lack of reflexes, abnormal posture, loss of ability to ambulate, labored respiration, inability to drink or feed) where, in order to prevent animal suffering, animals were euthanized in accordance with local animal care guidelines. Only 'warm' necropsy specimens (blood, primary tumor, liver, and lungs) were used for PK analysis. All applicable institutional guidelines for the care and use of animals were followed. All animal procedures were approved by the National Cancer Institute Animal Care and Use Committee (ACUC) of the National Institutes of Health (NIH).

Breast cancer model: The model derived from 0.2 liter of pleural effusion from a breast cancer patient (model #373342). These cancer cells had metastasized to the lung pleura from the initial tumor detected in the breast. The effusion was centrifuged at 5000 RPM for 10 minutes. The supernatant was removed, and the cells were inoculated with Matrigel into a mouse mammary fat pad. After the size of the tumor reached 1 cm, a 2 x 2 mm fragment of mammary fat pad was re-transplanted into a mammary fat pad of another mouse. The tumors used in this experiment went through 4 passages. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac strain of mice from Taconic was used in the experiment. The animals were acclimated up to 7 days before tumor inoculation. The 2 x 2 mm tumor was inoculated into the mammary fat pad. The study started after the tumor reached 150-200 mm^3. Doses of 25 mg/kg metarrestin in 5% NMP, 20% PEG400, and 75% of 10% 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) in water were injected IP 5 days a week for 4 weeks. Tumor size and total body weight were measured twice a week.

Prostate cancer model: Mice were implanted in the flank with 3 x 10^6 human PC-3M-luc-C6 pancreatic tumor cells (Caliper Life Sciences) and split into 4 groups of 10 mice. Dosing with 5 or 25 mg/kg drug or vehicle alone (negative control) was initiated after 2 weeks and continued until the experimental endpoint, 6 weeks after implantation. Primary tumor size (measured with calipers), live tumor cell content (measured by biophotonic imaging), and total body weight were determined each week. At the endpoint, animals were euthanized, metastatic organs removed, and metastases quantitated by biophotonic imaging of the organs.

Supplemental Figures and Tables:

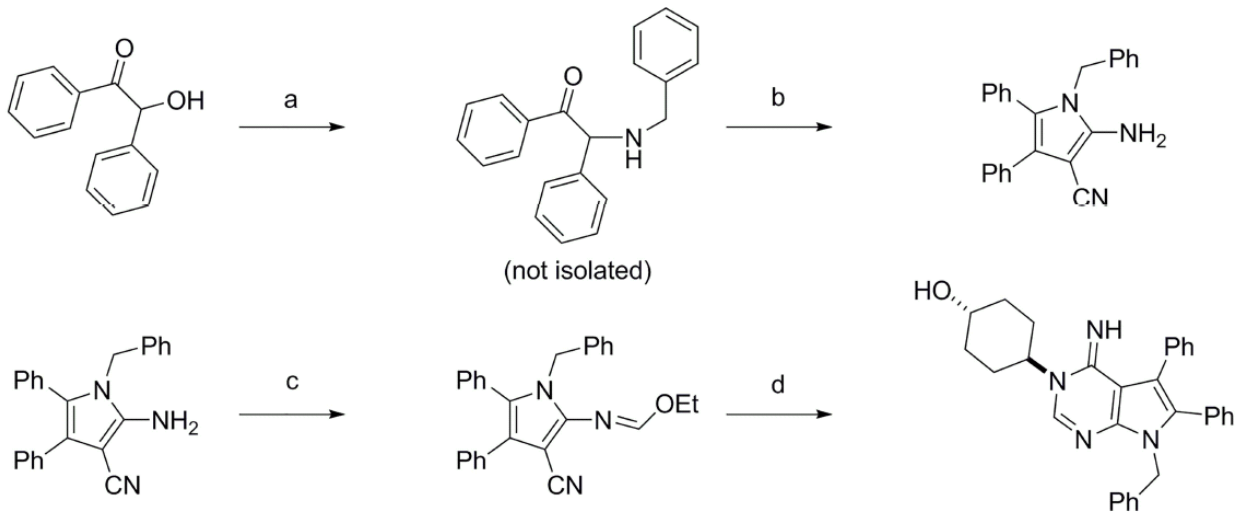


Fig. S1. **Synthetic scheme for metarrestin** Reagents and conditions: (a) benzyl amine, trifluoroacetic acid (5 mol %), toluene; (b) malononitrile, toluene (24.5 g, 70% yield, two steps, product isolated by precipitation and used without further purification); (c) triethylorthoformate, neat (6.7 g, 93% yield, product purified by silica gel chromatography); (d) trans-4aminocyclohexanol hydrochloride, KOtBu MeOH (1.7 g, 56% yield, product purified by recrystallization from MeOH).

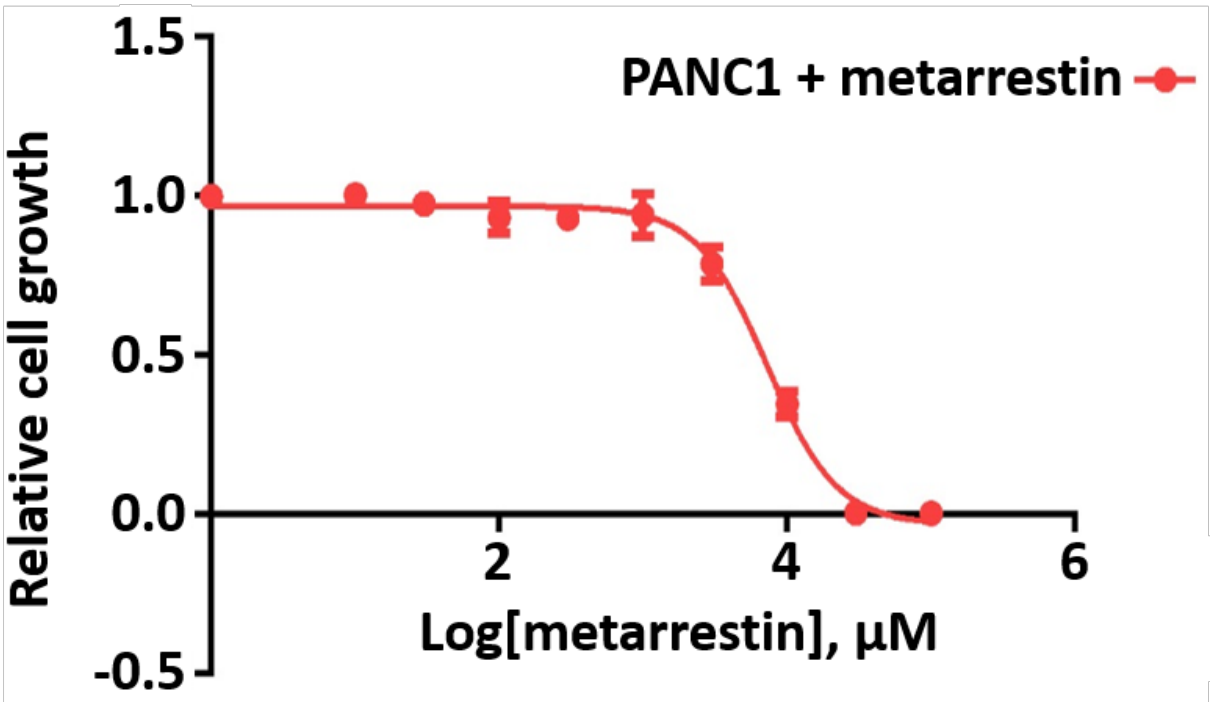


Fig. S2. Drug response of PANC1 cells treated with metarrestin Relative cell growth was measured by CellTiterGlo normalized to DMSO control samples (set to 1.0) after 72 hours. Mean cell viability values are plotted with standard error of the mean (SEM) from at least 2 independent experiments done in triplicate.

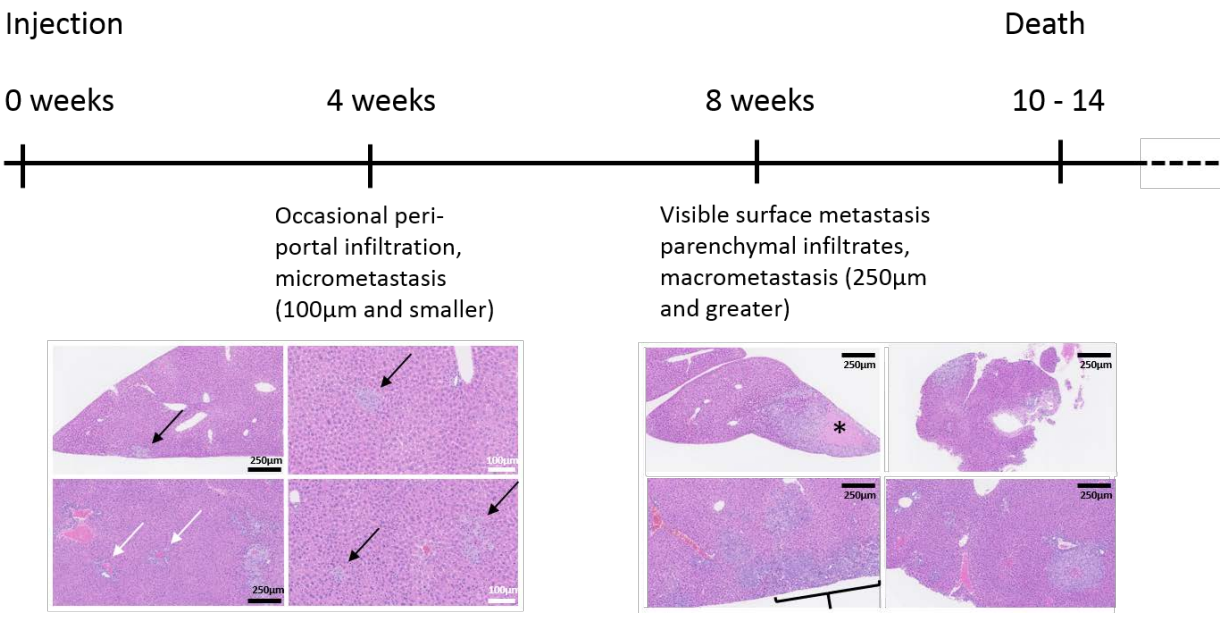
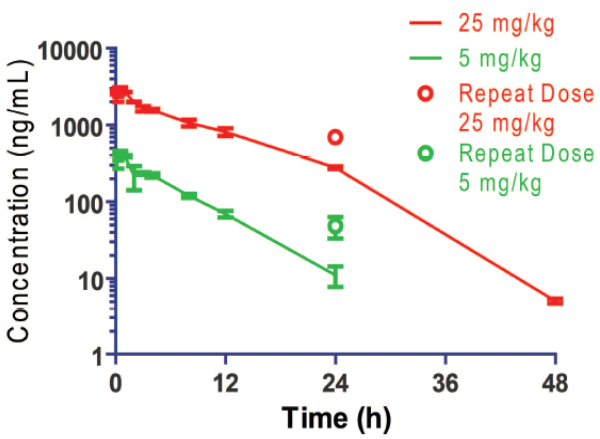


Fig. S3. Metastatic cancer progression in PANC1 NSG mice After intrapancreatic injection of sixty thousand luciferase-expressing 3D PANC1-luc cells derived from PANC-luc cells grown as spheroids in NSG mice, micrometastasis (black arrows) with periportal infiltration (white arrows) developed within ~4 weeks (representative H&E staining, — scale bar=250 µm, white scale bar=100 µm). Macrometastasis with visible surface metastases (indicated with —) developed after 8 weeks. * indicates necrosis. Timeline depicted at the top.

A



Single dose pharmacokinetics in Plasma				
	5 mg/kg IP		25 mg/kg IP	
Sampling time (h)	ng/mL	mM	ng/mL	mM
0.083	308	0.649	2180	4.593
0.25	430	0.906	2660	5.605
0.5	429	0.904	2930	6.174
1	391	0.824	2680	5.647
2	217	0.457	2000	4.214
3	235	0.495	1630	3.434
4	221	0.466	1563	3.293
8	119	0.251	1065	2.244
12	69.3	0.146	818	1.724
24	11.1	0.023	279	0.588
48	B Q L	BQL	5.09	0.011

Repeat dose pharmacokinetics in Plasma				
	5 mg/kg IP		25 mg/kg IP	
Sampling time (h)	ng/mL	mM	ng/mL	mM
24	48.4	0.102	692	1.458

B

Animal	Pancreas primary tumor (µmol/kg)	Liver metastasis (µmol/kg)
1	49.5	87.2
2	55.2	79
3	20.6	117.2
4	20.4	43.2
5	34.1	64.6
6	63	149.6
7	23.6	59.8

Fig. S4. Metarrestin plasma and tumor pharmacokinetics (**A**) Single dose, intraperitoneal injection pharmacokinetics in female BALB/c mice, n=3, and repeat dose pharmacokinetics in SCID-beige mice, n=3 per time point. Repeat dose study mice were given daily intraperitoneal injections for 4 weeks, with concentration measured 24 h after the last dose. Metarrestin was formulated in 5% NMP + 20% PEG400 + 75% (10% HP-β-CD in water). (**B**) Metarrestin tumor concentrations after daily 10 mg/kg intraperitoneal injections for 7 days.

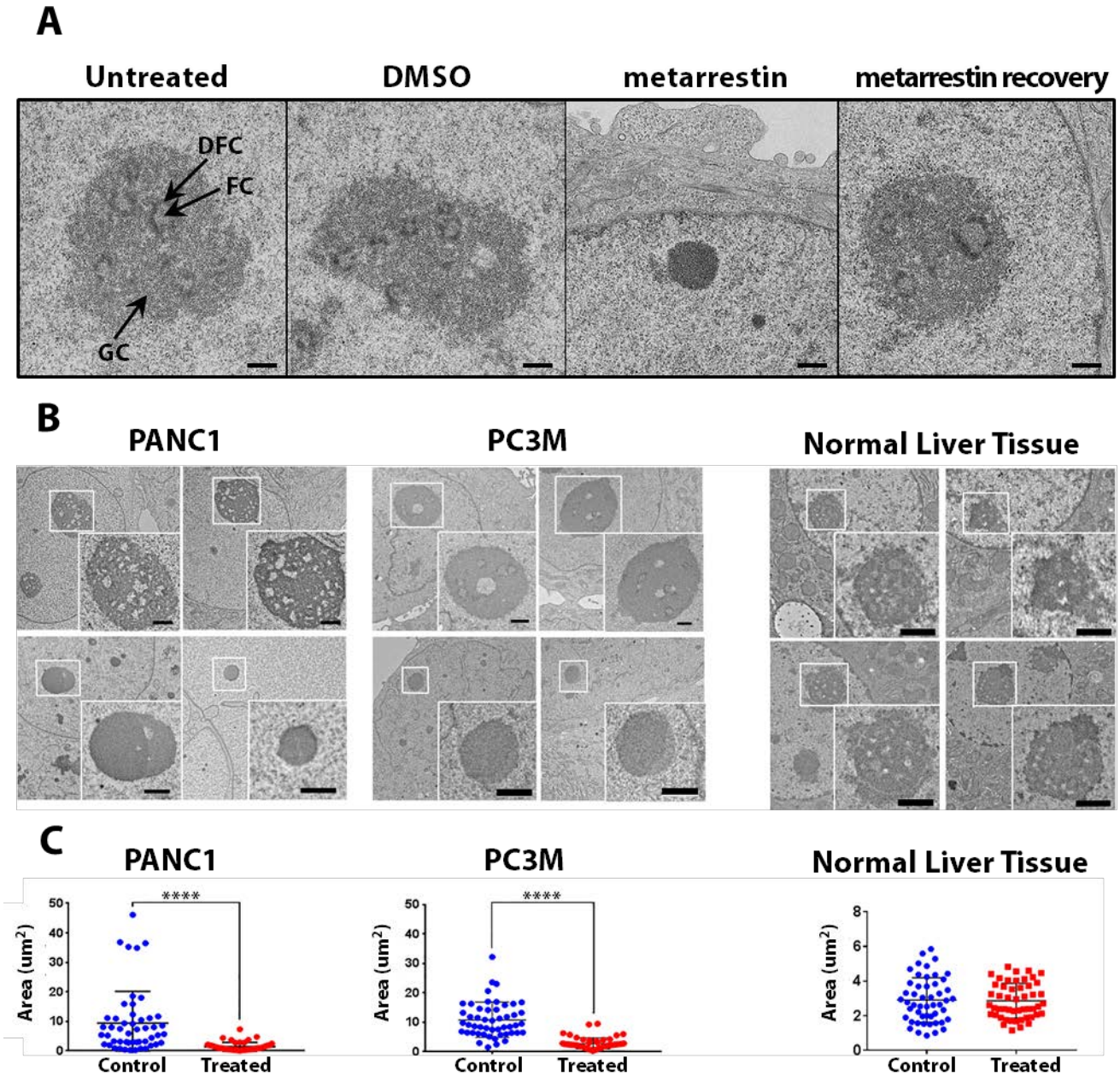


Fig. S5. Metarrestin-induced nucleolar structure changes as observed by electron microscopy (**A**) The structure of the nucleolus loses the typical three substructures seen in untreated or DMSO-treated cells (DFC, dense fibrillary components, FC, fibrillar components, and GC, granular components, are specified by arrows and labels), and becomes highly

segregated (third panel) upon treatment with metarrestin at 1 μM for 24 hours in HeLa cells. However, withdrawing the treatment allows for the recovery of the nucleolus (fourth panel). Scale bar = 500 nm. (**B**) Similar changes were observed in PANC1 and PC3M cells (left two panels), but not observed in treated normal liver tissues (right panels). Scale bar = 1 μm. (**C**) Quantitative evaluation of the EM images demonstrated that nucleolar volume was reduced in the treated cancer cell lines (left two graphs), but not in treated normal liver tissues (right graph). **** $P<0.0001$.

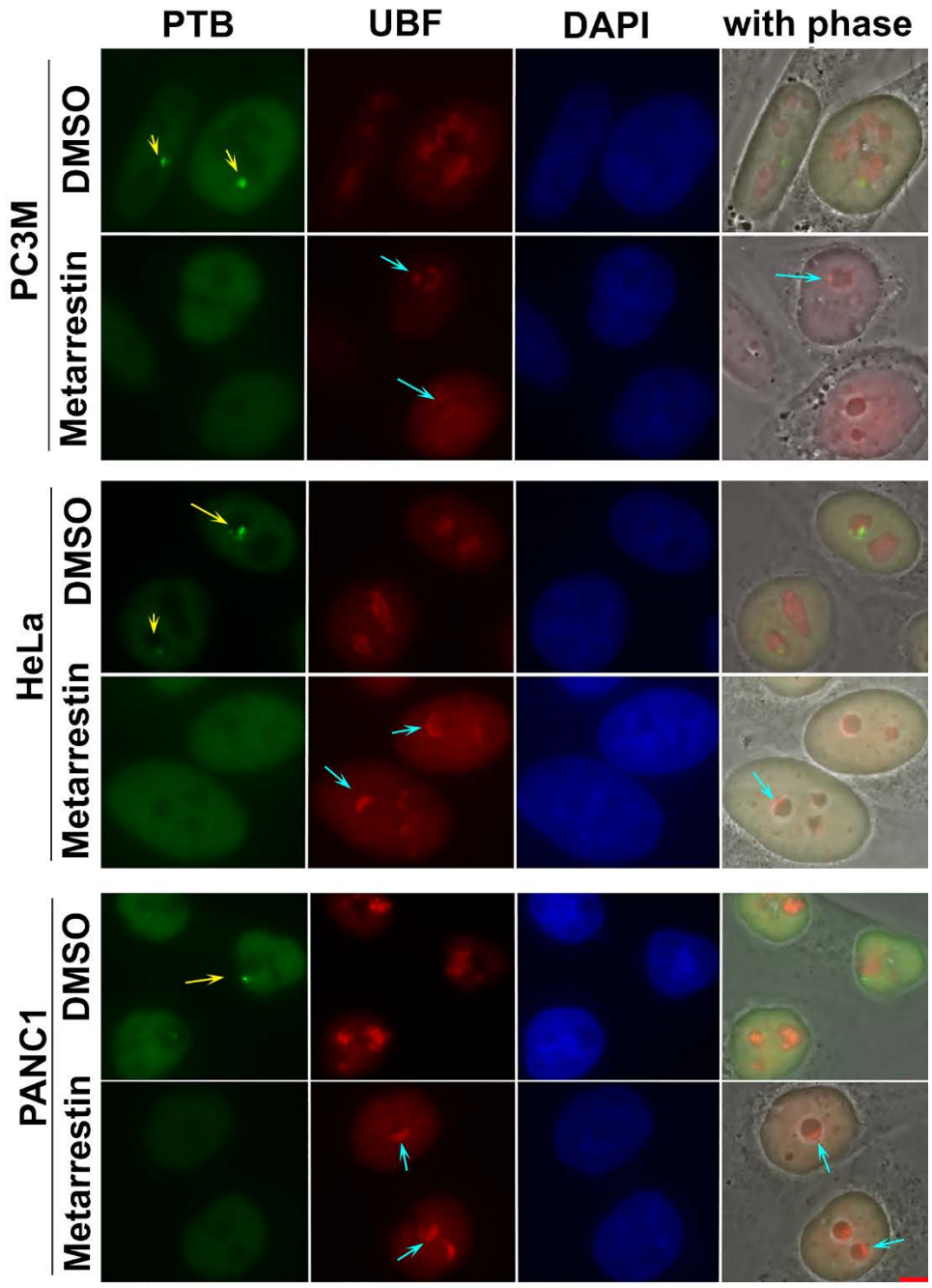


Fig. S6. Changes of nucleolar structure in metarrestin-treated cells The nucleolar structure changes (UBF labeling in red, blue arrows) are closely associated with the loss of PNC (PTB labeling in green, yellow arrows)**.** Metarrestin treatment (1 μM for 18 hours) was tested in three cell lines, PANC1, PC3M, and HeLa. The capping of the Pol I transcription factor UBF in

metarrestin-treated cells is evident as shown in merged images (indicated by arrows). Scale bar = 10 μm.

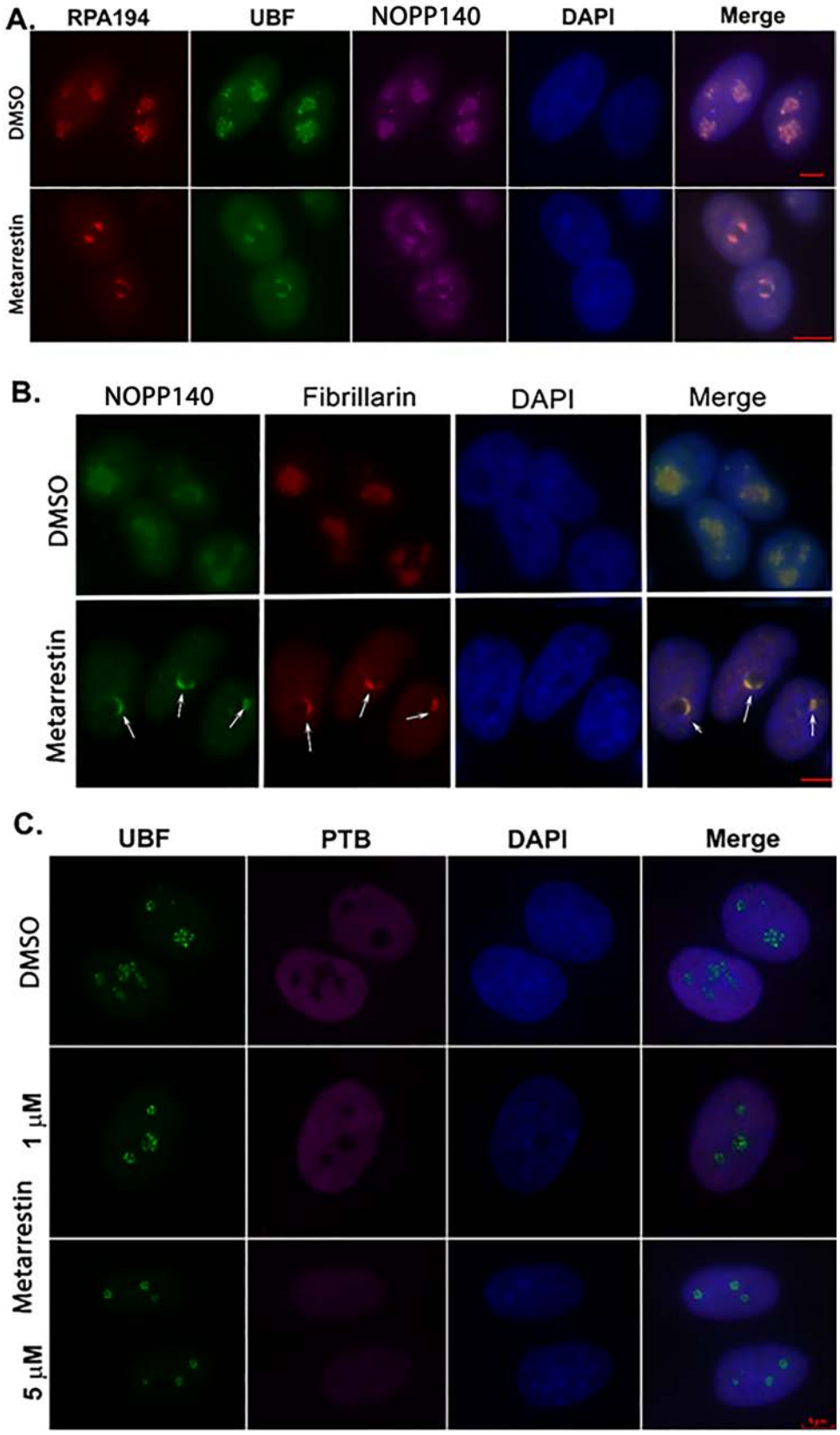
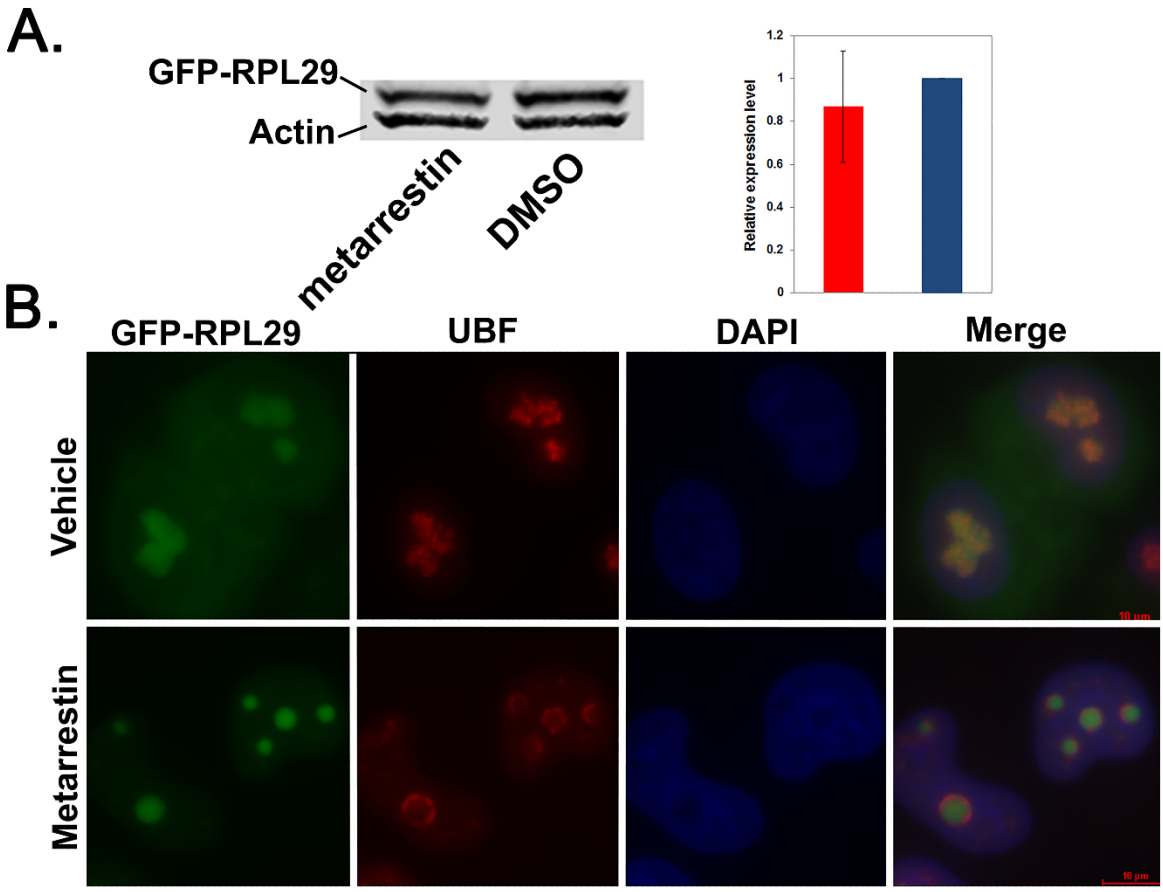

A.
RPA194
UBF
NOPP140
DAPI
Merge
DMSO
Metarrestin
B.
NOPP140
Fibrillarin
DAPI
Merge
DMSO
Metarrestin
C.
UBF
PTB
DAPI
Merge
DMSO
1 μM
Metarrestin
5 μM

Fig. S7. Capping structure of Pol I transcription factors in metarrestin-treated cells (A) Pol I transcription factors, UBF (green), RPA194 (red), and pre-ribosomal RNA processing factor, NOPP140 (pink) showed similar capping structure in metarrestin-treated cells**.** Scale bar = 10 μm. (**B**) Immunolabeling of two pre-ribosomal RNA processing factors, fibrillarin (red) and NOPP140 (green) demonstrated the cap-like structure in metarrestin-treated cells (arrows). Scale bar = 5 μm. (**C**) The capping of Pol I transcription factors was not evident in normal fibroblast cell line, GM02153, under metarrestin treatment with the same protocol (1 μM for 18 hours). These cells do not have PNCs as PTB labeling (red) did not show any perinucleolar enriched labeling. UBF did not become completely capped even when treated with 5 μM in these cells. Scale bar = 5 μm.



Suppl. Fig. 8. No impact of metarrestin treatment on Pol II transcription, translation, or cytoplasmic-nuclear trafficking in metarrestin-treated cells. (**A**) Stably transfected HeLa cells were induced to express the GFP-RPL29 fusion protein after treatment with metarrestin (1 μM for 5 hours). GFP-RPL29 expression was not significantly changed. (**B**) In metarrestin-treated cells, nucleoli altered with nucleolar capping can be detected by immunolabeling of UBF (red). The newly synthesized GFP-RPL29 entered nuclei and localized to the distorted nucleoli, but was not assembled into mature ribosomes in metarrestin-treated cells to be transported into the cytoplasm (green). Scale bar = 10 μm.

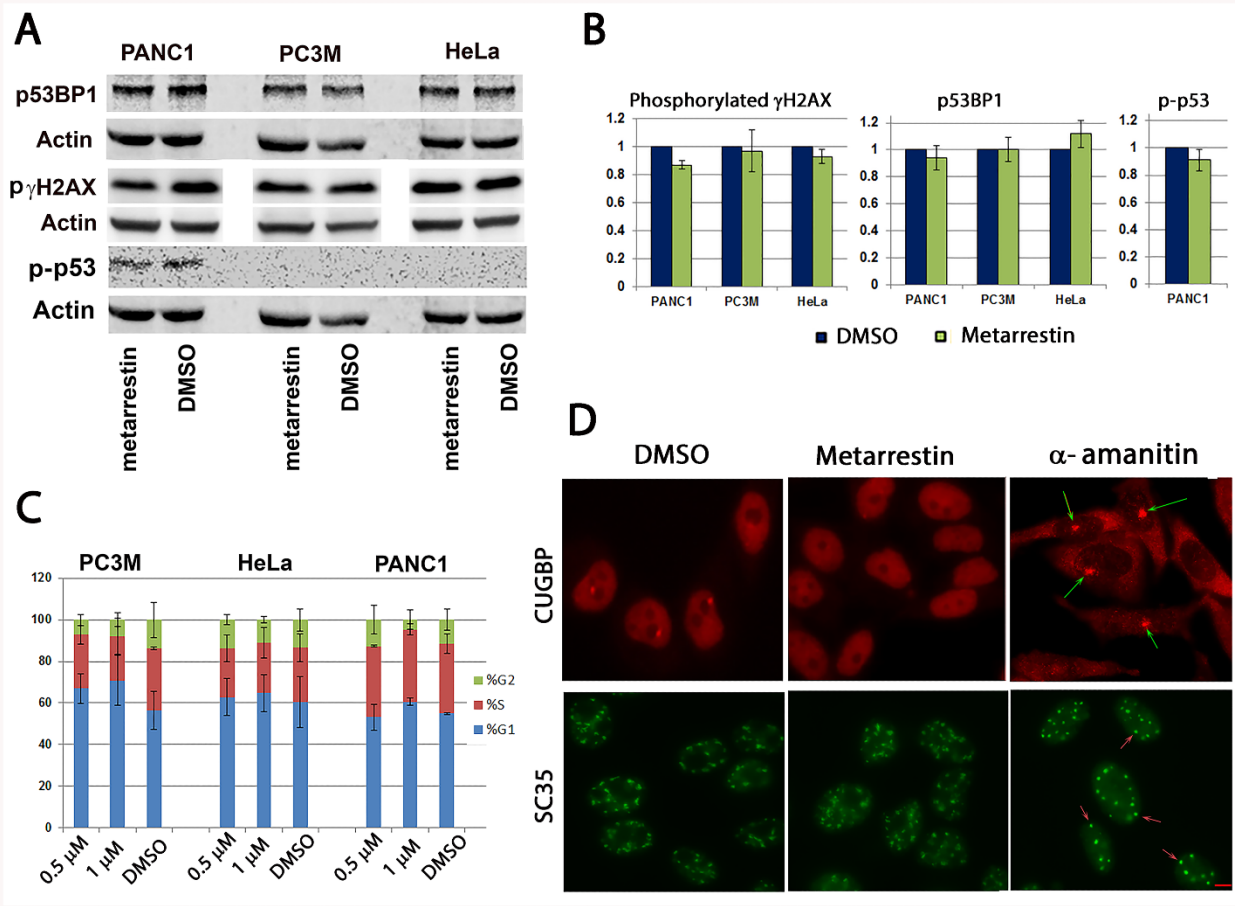


Fig. S9. No impact on DNA damage response, cell cycle, or pol II transcription in metarrestin-treated cells. (**A**) Western blot analyses of DNA damage response signature phosphorylated proteins or p53 (**B**) did not show significant change when cells were treated with metarrestin at 1 μM for 24 hours. (**C**) Cell cycle analyses of DNA content by flow cytometry did not show significant cell cycle block within 24 hours of metarrestin treatment at two different concentrations. (**D**) Immunolabeling of CUGBP and SC35 in metarrestin-treated cells did not show the signature changes of their labeling patterns that are seen during pol II transcription inhibition by α-amanitin. Metarrestin treatment disassembles PNCs without causing cytoplasmic relocation of CUGBP (top middle panel), as compared to treatment by α-amanitin, which induces cytoplasmic relocation of the protein, but does not affect proteins localized to the PNCs

(top right panel, arrows). Similarly, metarrestin treatment did not significantly impact the speckled distribution pattern (bottom middle panel), unlike α-amanitin that induced round aggregates (bottom right panel, arrow). Scale bar = 5 μm.

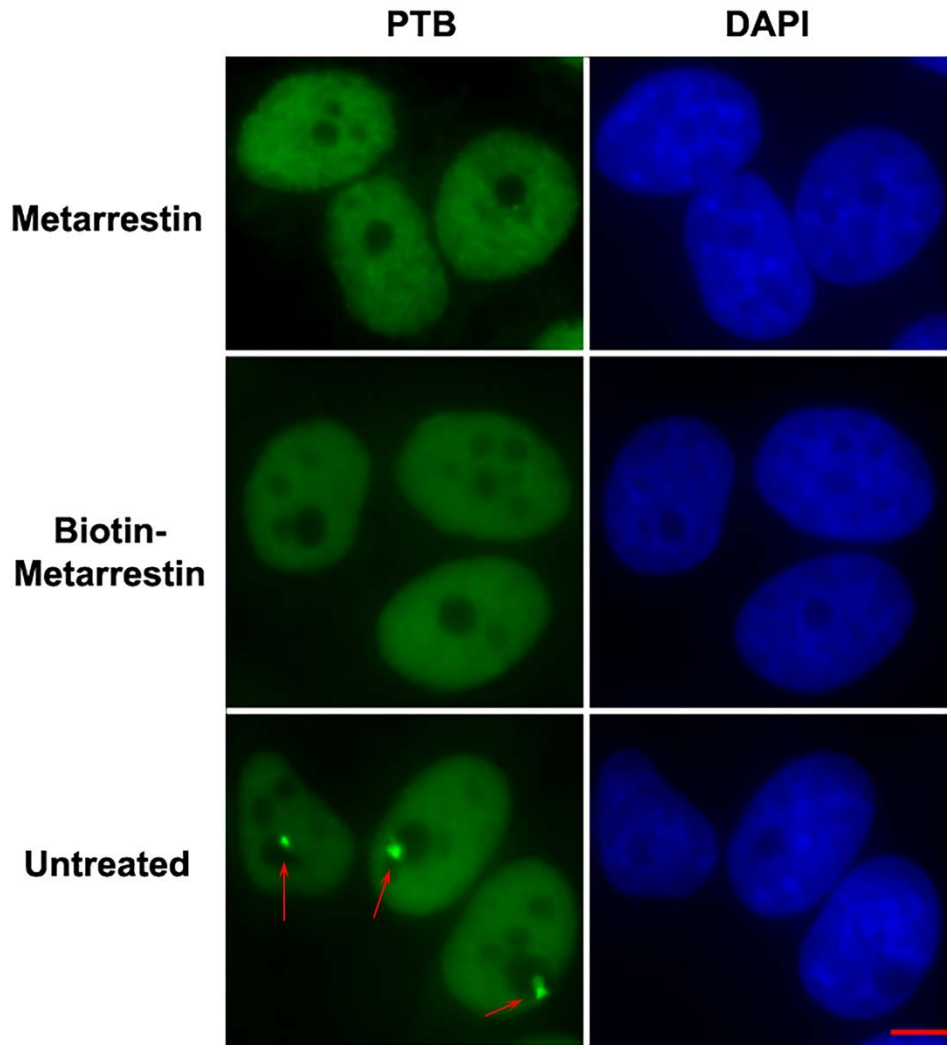


Fig. S10. Effectiveness of biotin-metarrestin in disassembling PNCs The effects of metarrestin and biotin-metarrestin were compared in PC3M cells treated at 1 μM or untreated for 24 hours. Arrows indicate PNCs. Scale bar= 5 μm.

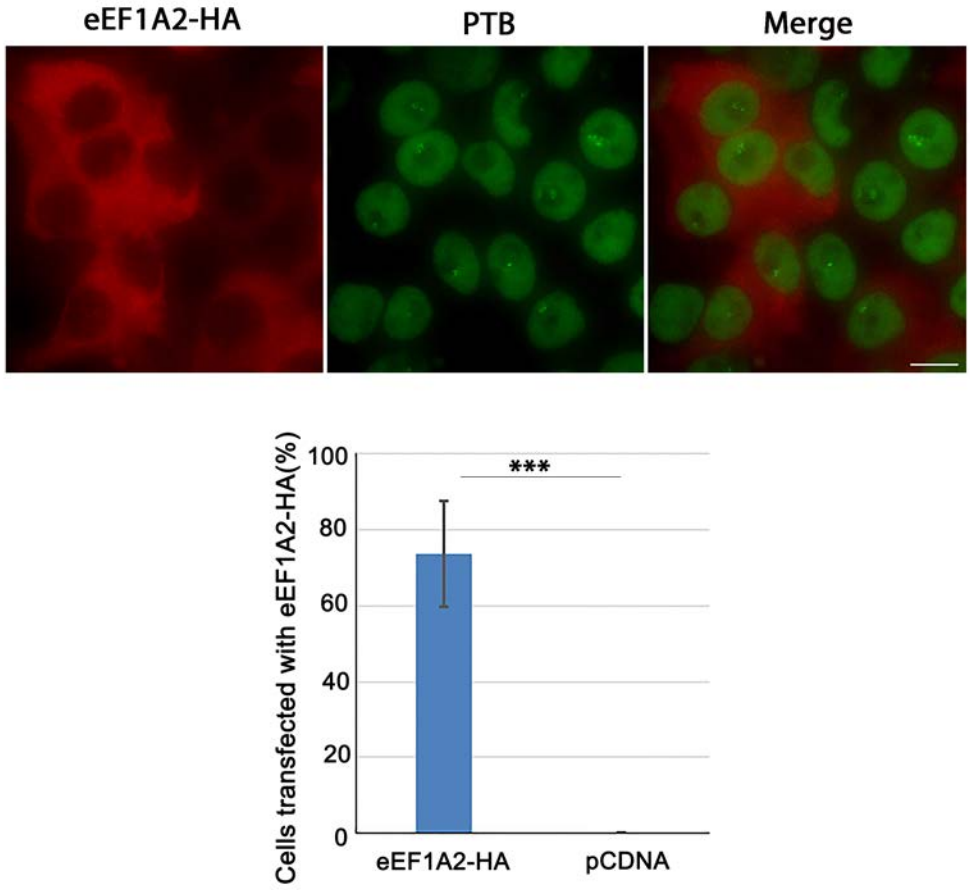


Fig. S11. Expression of eEF1A2-HA in transfected cells The protein (red) is predominantly in the cytoplasm. Immunolabeling using SH54 demonstrates the nucleoplasmic and PNC distribution of PTB. The transfection efficiency was around 70%. *** $P < 0.001$. Scale bar = 20 μm.

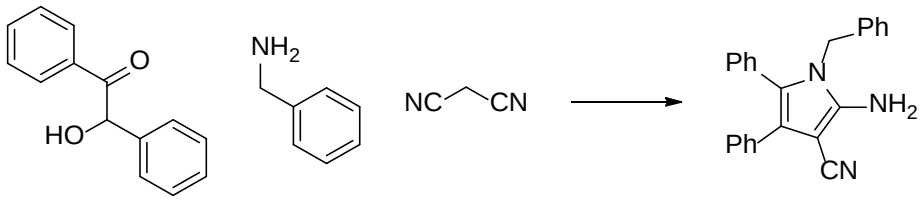


Fig. S12. Synthesis of 2-amino-1-benzyl-4,5-diphenyl-1H-pyrrole-3-carbonitrile.

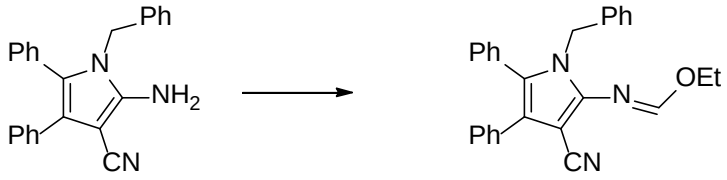


Fig. S13. Synthesis of (E)-ethyl N-(1-benzyl-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)formimidate.

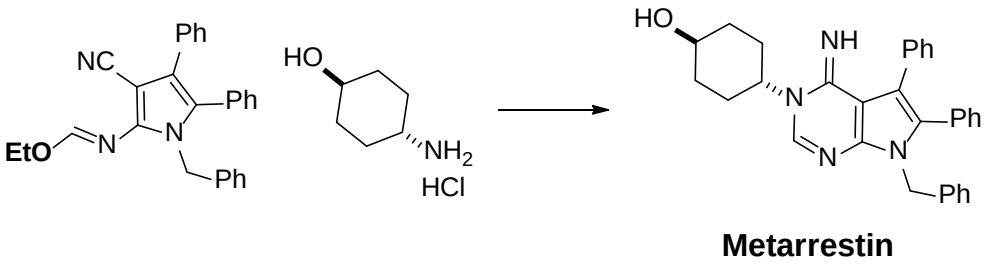


Fig. S14. Synthesis of *trans*-4-(7-benzyl-4-imino-5,6-diphenyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)cyclohexanol (metarrestin).

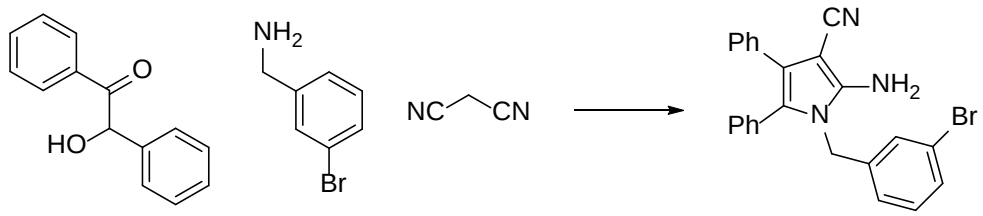


Fig. S15. Synthesis of 2-amino-1-(3-bromobenzyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile.

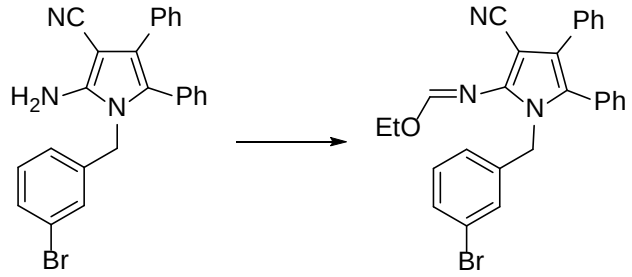


Fig. S16. Synthesis of (E)-ethyl N-(1-(3-bromobenzyl)-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)formimidate.

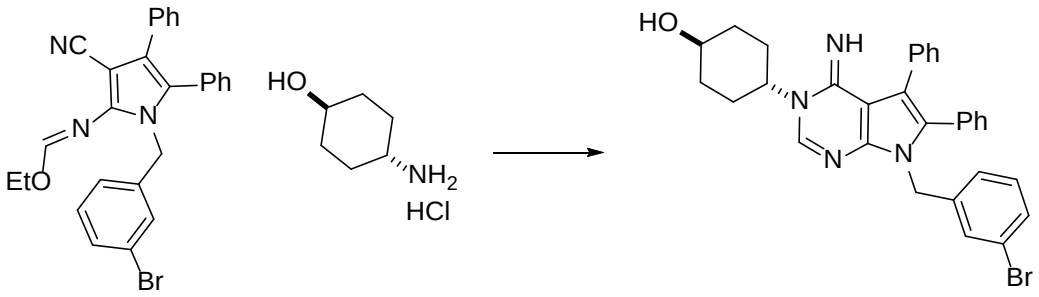


Fig. S17. Synthesis of *trans*-4-(7-(3-bromobenzyl)-4-imino-5,6-diphenyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)cyclohexanol.

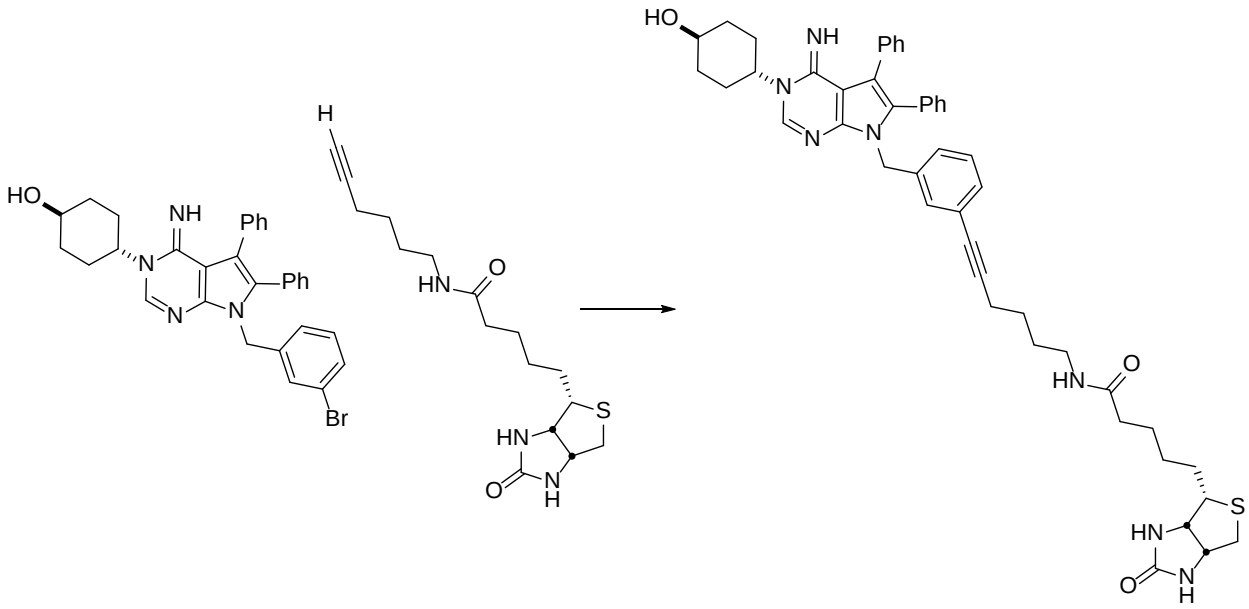


Fig. S18. Synthesis of *N*-(6-(3-((3-(*trans*-4-hydroxycyclohexyl)-4-imino-5,6-diphenyl-3,4-dihydro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl)phenyl)hex-5-yn-1-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (biotin-metarrestin, P1).

Supplementary Tables:

Cell line	Origin
T47D	breast cancer
CG	neuroblastoma
TKF-1	cholangiocellular carcinoma
Panc1	pancreatic ductal carcinoma
83 Mer	neuroblastoma cancer stem cells
PACADD159	pancreatic carcinoma
KP-3	adenosquamous carcinoma of the human pancreatic duct
SKOV3	ovarian adenocarcinoma
PACADD183	pancreas carcinoma from ascites
PA-TU-8988T	liver metastasis of a primary pancreatic adenocarcinoma
HEK293T	293 cells expressing a mutant version of the SV40 large T antigen
NOR-P1	metastatic subcutaneous tumor of a patient with pancreatic cancer
colo205	colorectal carcinoma
L3.6sl	liver metastasis from injecting COLO-357 cells into mouse spleen
PC-3M	human prostate carcinomas
HeLa	cervix adenocarcinoma
Colo357	liver metastasis of pancreatic adenocarcinoma
Suit-2	liver metastasis of pancreatic carcinoma.
HPAC	pancreatic adenocarcinoma cell line from a nude mouse xenograft of a primary tumor

Table S1. Cancer cell lines examined for PNC prevalence with or without metarrestin treatment (from Fig. 1C).

Cell Lines	(1) Primary (2) Met	PNC prevalence (%)	Detailed origin
Panc 8.13	1	3	Primary tissue culture line - E. Jaffe JHU
SB06	1	7	LN met
SB.07	1	13	primary - distal pancreatectomy
Panc3.27	1	17	Primary tissue culture line - E. Jaffe JHU
Panc10.05	1	30	Primary tissue culture line - E. Jaffe JHU
PSN1	1	3	patient-derived xenograft - primary
KLM1	1	22	primary
KP-3	1	50	patient-derived xenograft - primary
SU86	1	8	ppancreas (not SU86.86, not obtained from ATCC) primary explant
L3.3	2	50	COLO357 propagated through pancreas in nude mice
YAPC	2	10	explant from ascites
L3.6pl	2	55	COLO357 propagated through liver in nude mice
L3.6sl	2	80	COLO357 propagated through spleen in nude mice
Colo357	2	93	liver met
PK-8	2	55	liver met
PATU-8988T	2	58	liver met
NOR-P1	2	70	subcutaneous met
SUIT-2	2	97	liver met

Table S2. Pancreatic cancer cell lines evaluated for PNC prevalence in Fig. 2A.

	KPC-Metarrestin Female	KPC-Metarrestin Male	KPC-Vehicle Female	KPC-Vehicle Male	C57B/L6 Metarrestin Female	C57B/L6 Metarrestin Male	C57B/L6 Vehicle Female	C57B/L6 Vehicle Male
Diagnosis	F01	M01	F02	M02	F03	M03	F04	M04
Adrenal hyperplasia, subcapsular cell	2/3		4/5		3/4		2/4	1/4
Adrenal lipogenic pigment		3/4		4/4		4/4		4/4
Brain hemorrhage						1/4		
Cecum hyperplasia, crypt				1/4				
Cecum proliferative typhlitis		1/4				1/4		
Duodenum dilatation	3/3	1/4	2/5	1/4				
Duodenum hyperplasia, crypt		1/4						
Epididymis aspermia						1/4		
Femur marrow hyperplasia, granulocytic	1/3	4/4	4/5	3/4		1/4		
Gall Bladder hyalinosis							1/4	
Gall Bladder hyperplasia, papillary	3/3	4/4	5/5	4/4				
Gall Bladder inflammation, acute					3/4	3/4	2/4	3/4
Heart dilation, atrium		1/4						
Heart mineralization		1/4		1/4		1/4		

Ileum segmented filamentous bacteria	3/3	4/4	4/5	4/4	3/4	3/4	4/4	3/4
Kidney dilatation, tubule			1/5		3/4	2/4		1/4
Kidney hydronephrosis	1/3							
Kidney infarct					1/4			
Kidney lymphocytic infiltrate				1/4				
Kidney mineralization	1/3	1/4	4/5	3/4	2/4	2/4	1/4	
Kidney regeneration, tubule			1/5	1/4	1/4	3/4		2/4
Kidney vacuolation, proximal tubule		1/4		1/4		1/4		3/4
Liver deformity	1/3							
Liver extramedullary hematopoiesis, mixed			1/5					
Liver inflammation, subacute	3/3	2/4	5/5	3/4	4/4	3/4	4/4	4/4
Liver lymphocytic infiltrate		2/4						
LN, mandibular hyperplasia, lymphoid		1/4						
LN, mandibular plasmacytosis		1/4						
LN, mesenteric dilatation, sinusoids			1/5					
Lung lymphocytic infiltrate	3/3	3/4	3/5	2/4	4/4	3/4	4/4	2/4
Lung pneumonia,				1/4				

acidophilic macrophage								
Lymph node plasmacytosis			1/5					
Pancreas duct, dilation	3/3	4/4	4/5	3/4				
Pancreas necrosis			2/5	1/4				
Pancreas neoplasm, graded	3/3	4/4	5/5	4/4				
Salivary gland lymphocytic infiltrate	2/3		1/5					
Skin/ subcutis inflammation, chronic active		1/4						
Spleen congestion			1/5					
Spleen extramedullary hematopoiesis, increased mixed erythropoiesis			1/5	1/4				
Spleen extramedullary hematopoiesis, erythrocytic, increased erythropoiesis	1/3	1/4	2/5		2/4			
Spleen fibrosis	1/3	2/4	1/5	3/4				
Spleen hyperplasia, lymphoid							1/4	
Spleen melanin	1/3					1/4	2/4	1/4
Stomach, glandular, hyperplasia, foveolar cells	3/3	2/4	4/5	2/4				
Stomach, nonglandular, erosion		1/4	2/5					
Testis degeneration, seminiferous tubule						1/4		

Thymus cyst						1/4		
Thyroid ectopic thymus	1/3							
Thyroid hyperplasia, papillary		1/4	1/5	1/4				
Vertebra hyperplasia, granulocytic, marrow	1/3	4/4	4/5	3/4		1/4		

Table S3. Veterinary pathology read of the organs in KPC mice treated by metarrestin.

Both tumor-bearing autochthonous KPC mice (Pdx-Cre; $KRAS^{G12D/+}$;$TP53^{R172H/+}$ genotype; female and male) and non-tumor bearing C57BL/6 mice (male and female) were exposed to daily treatment with metarrestin for three months. 6-week-old KPC and C57BL/6 mice (male and female) were assigned to groups (n=8) that were fed with control vehicle (NIH Haslan 31 chow) or metarrestin-infused 10 mg/kg (70 ppm equivalent) chow for 12 weeks. Animals were clinically monitored on a daily basis under ACUC guided protocols, and harvested at signs of distress as directed by the study veterinarian, or after 12 weeks on vehicle or metarrestin chow. Veterinary pathology review of 12 organ systems (15 slides per organ) revealed few, low-frequency incidental lesions due to the $KRAS^{G12D/+}$;$TP53^{R172H/+}$ genotype but no histopathological changes attributable to extended exposure to 10 mg/kg metarrestin in the treated animals compared to vehicle control animals.

CBC summary	KPC/metarrestin			KPC/control		
	total number of mice	number with low values	number with high values	total number of mice	number with low values	number with high values
WBC (K/µL)	9	1	2	9		2
NE (K/µL)	9		4	9		6
LY (K/µL)	9			9		
MO (K/µL)	9		4	9		6
EO (K/µL)	9		2	9		5
BA (K/µL)	9		1	9		2
NRBC (K/µL)	9			9		
NE (%)	9		3	9		3
LY (%)	9		3	9	6	
MO (%)	9		2	9		1
EO (%)	9		1	9		2
BA (%)	9		1	9		2
NRBC (%)	9			9		
Erythrocytes						
RBC (M/µL)	9		1	9		5
Hb (g/dL)	9			9	1	
HCT (%)	9			9	1	2
MCV (fL)	9	7		9	7	
MCH (pg)	9	8		9	6	
MCHC (g/dL)	9	2		9	1	
RDW (%)	9			9		
Retics (M/µL)	9			9		
Retics (%)	9			9		
Thrombocytes						
PLT (K/µL)	9	1		9	2	
MPV (fL)	9	1		9		
PDW (%)	9			9		
PCT (%)	9			9		

Blood chemistry summary	KPC/metarrestin			KPC/control		
	total number of mice	number with low values	number with high values	total number of mice	number with low values	number with high values
ALB	9		7	8		6
ALP	9	5		8	6	
ALT	9	8	1	8	7	
AMY	9	6		8	6	
TBIL	9			8		
BUN	9			8		
Ca	9		1	8		
PHOS	9	1		8	1	
CRE	9	3	1	8	2	
GLU	9	2		8	1	
Na+	9			8		
K+	9	1		8		
TP	9			8		1
GLOB	9		9	8		8

	C57B/L6 / metarrestin			C57B/L6 / vehicle		
	total number of mice	number with low values	number with high values	total number of mice	number with low values	number with high values
WBC (K/µL)	7			8		
NE (K/µL)	7			8		
LY (K/µL)	7			8		
MO (K/µL)	7			8		2
EO (K/µL)	7		3	8		5
BA (K/µL)	7			8		1
NRBC (K/µL)	7			8		
NE (%)	7			8		
LY (%)	7	1		8	1	
MO (%)	7		2	8		5
EO (%)	7		4	8		6
BA (%)	7		4	8		5
NRBC (%)	7			8		

Erythrocytes								
RBC (M/µL)	7			5	8			7
Hb (g/dL)	7	1			8	1		
HCT (%)	7	1		1	8			
MCV (fL)	7	7			8	8		
MCH (pg)	7	7			8	7		
MCHC (g/dL)	7	2			8			
RDW (%)	7				8			
Retics (M/µL)	7				8			
Retics (%)	7				8			
Thrombocytes								
PLT (K/µL)	7	3			8	3		
MPV (fL)	7				8	2		
PDW (%)	7				8			
PCT (%)	7				8			

	C57B/L6 / metarrestin			C57B/L6 / vehicle		
	total number of mice	number with low values	number with high values	total number of mice	number with low values	number with high values
ALB	8		8	8		8
ALP	8	3		8	4	
ALT	8	5		8	4	
AMY	8	8		8	8	
TBIL	8			8		
BUN	8			8		
Ca	8		1	8		
PHOS	8			8	1	
CRE	8	2		8	4	1
GLU	8			8		
Na+	8			8		
K+	8	1	1	8		
TP	8		7	8		8
GLOB	8		8	8		8

Table S4. Hematology and blood biochemistry assessment of metarrestin toxicity in tumor-bearing KPC and wild type mice. WBC, white blood cells (K=1,000 per microliter

blood); NE, neutrophils; LY, leukocytes; MO, monocytes; EO, eosinophils; BA, basophils; NRBC, non-red blood cells; RBC, red blood cells (M=millions per microliter); Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume (fL=femtoliter); MCH, mean corpuscular hemoglobin (pg=picogram); MCHC, mean corpuscular hemoglobin concentration (dL=deciliter); RDW, red cell distribution width; Retics, reticulocytes; PLT, platelets; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; TBIL, total bilirubin; BUN, blood urea nitrogen; Ca, calcium; PHOS, phosphorus; CRE, creatinine; GLU, glucose; Na+, sodium; K+, potassium; TP, total protein; GLOB, globulin.