Manumycin Polyketides Act as Molecular Glues Between UBR7 and P53

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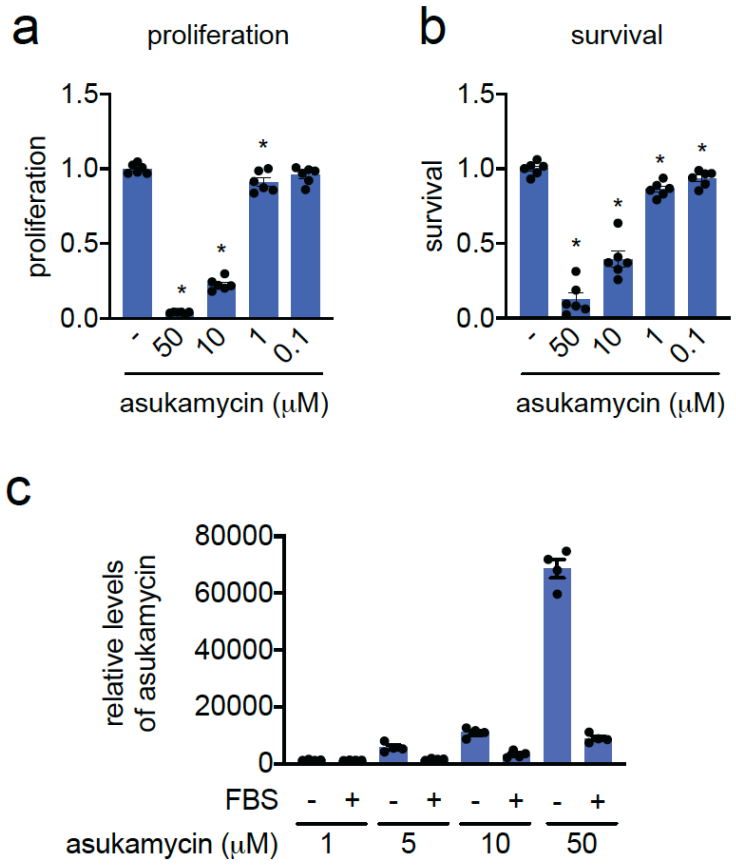
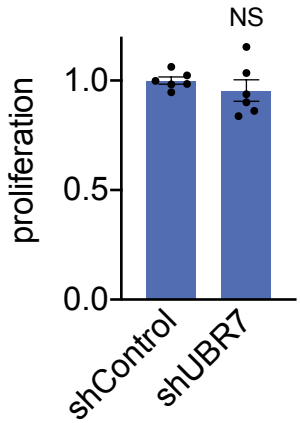
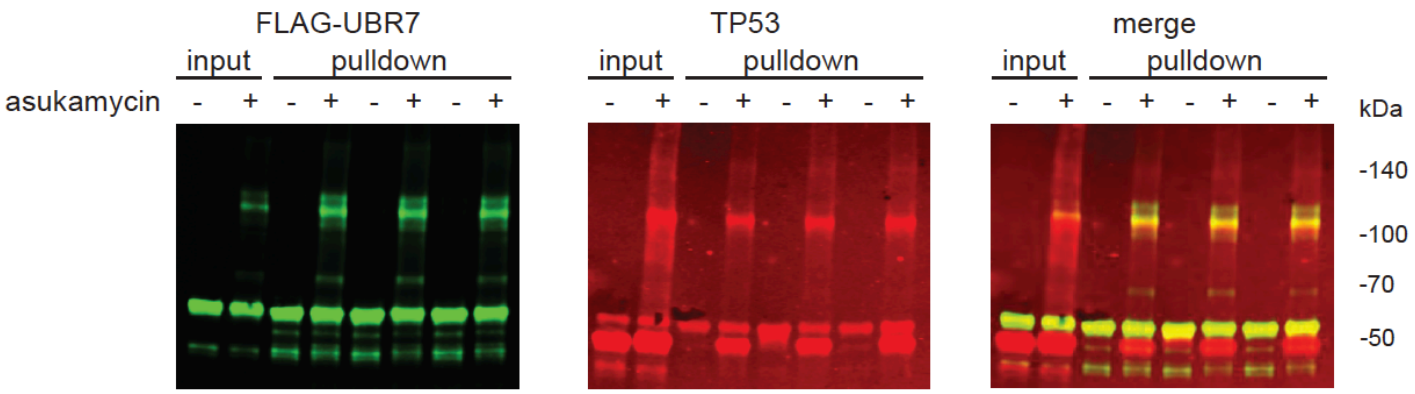


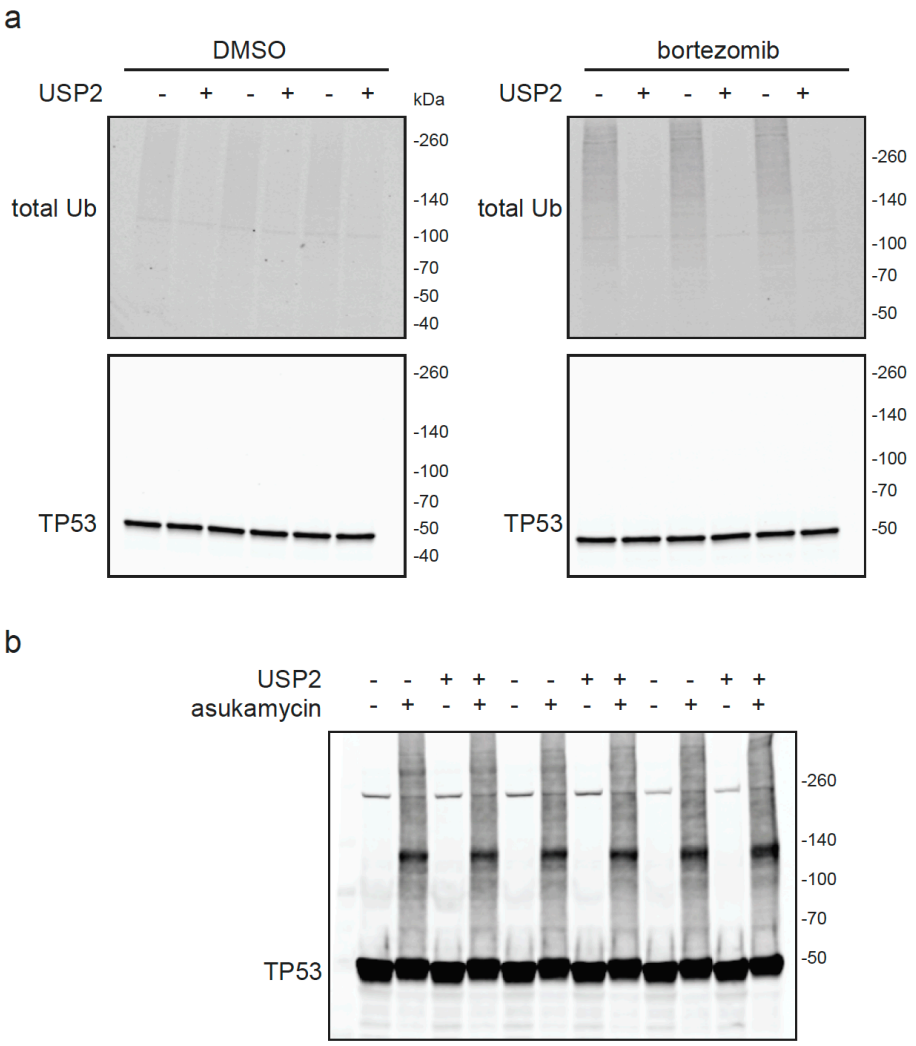
Supplementary Figure 1. Asukamycin effects in HCC38 cells. (a, b) Proliferation and serum-free cell survival in HCC38 breast cancer cells treated with DMSO vehicle or asukamycin for 48 h, assessed by Hoechst stain. **(c)** Intracellular levels of asukamycin in fetal bovine serum (FBS)-containing media or serum-free media in 231MFP cells treated with DMSO vehicle or asukamycin for 1.5 h, measured by LC-MS/MS. Data shown as individual replicate values and average ± sem and are n=6 for **(a, b)** and n=4 for **(c)** biologically independent samples/group. Statistical significance was calculated in **(a, b)** with two-tailed unpaired Student's t-tests and are shown as *p<0.05 compared to vehicle-treated controls within each group. Source data can be found in **Supplementary Data 1.**



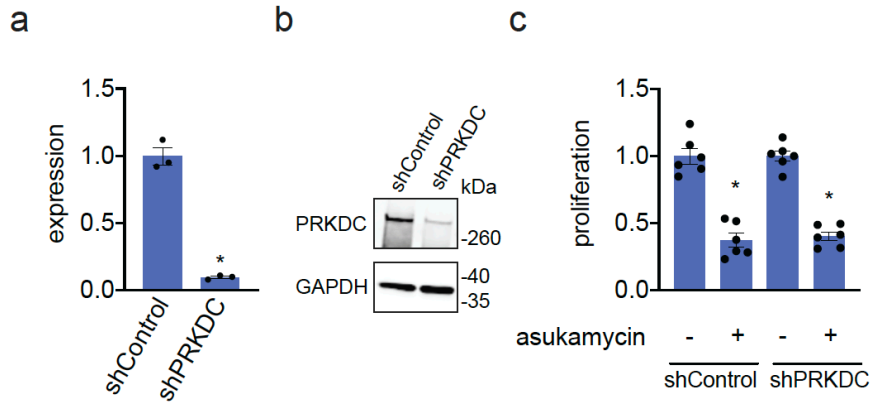
Supplementary Figure 2. Proliferation in 231MFP cells. Proliferation in 231MFP shControl and shUBR7 breast cancer cells for 48 h, assessed by Hoechst stain. Data shown as individual replicate values and average ± sem and are n=6 biologically independent samples/group. NS denotes that shUBR7 proliferation is not significantly changed ($p>0.05$) compared to the shControl group by a Student's two-tailed t-test. Source data can be found in **Supplementary Data 2.**



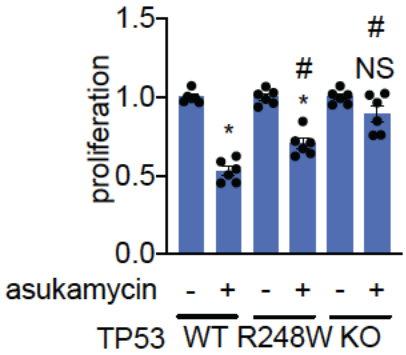
Supplementary Figure 3. Dual color Western blots of FLAG-UBR7. 231MFP cells stably expressing FLAG-UBR7 were treated with DMSO vehicle or asukamycin (50 μM) for 3 h. FLAG-UBR7 interacting proteins were subsequently enriched and then subjected to dual color Western blotting analysis of FLAG-UBR7 (in green) and TP53 (in red). Blot shown on the right is a merged blot of FLAG-UBR7 and TP53 with the yellow color indicating overlap. These blots show n=3 biologically independent samples/group. Uncropped blots can be found in **Supplementary Figure 8.**



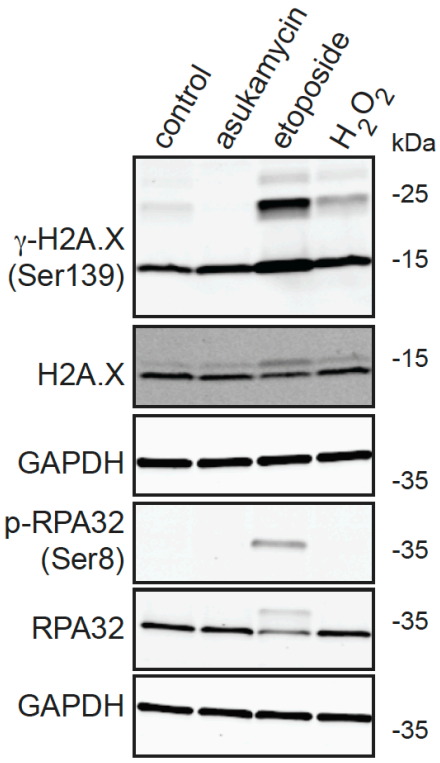
Supplementary Figure 4. Asukamycin-mediated higher molecular weight TP53 species are not polyubiquitinated protein. (a) Total proteome ubiquitin Western blot from 231MFP cell lysate treated with deubiquitinase USP2 (0.3 micrograms, 1 h). 231MFP cells were treated either with DMSO vehicle or proteasome inhibitor bortezomib (1 μM, 3 h) to accumulate ubiquitinated proteins. This experiment is a positive control experiment to show that our conditions for USP2 incubation can show removal of ubiquitin from polyubiquitinated proteins. **(b)** 231MFP cells treated *in situ* with DMSO vehicle or asukamycin (50 μM) for 4 h. Cell lysates were then treated with USP2 (0.3 micrograms, 1 h). The 260 kDa band is likely a non-specific band not corresponding to TP53. Blots show n=3 biologically independent samples/group.



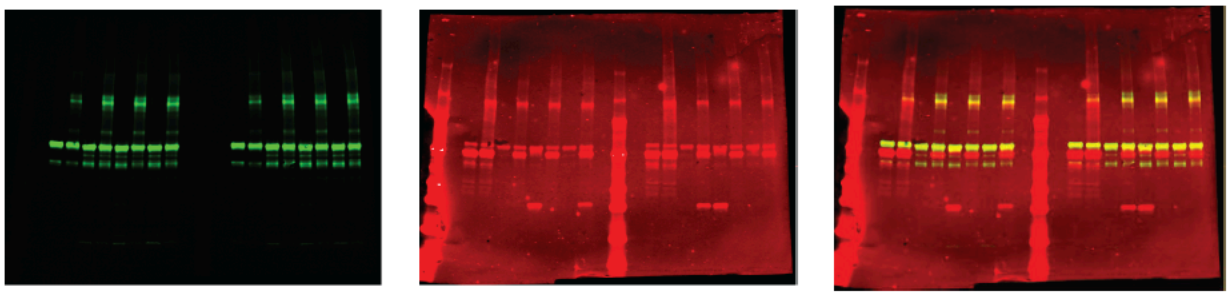
Supplementary Figure 5. PRKDC knockdown in 231MFP breast cancer cells. **(a, b)** PRKDC was stably knocked down using shRNAs and PRKDC knockdown was confirmed by qPCR **(a)** and by Western blotting **(b)** compared to shControl cells**. (c)** Proliferation in 231MFP breast cancer cells treated with DMSO vehicle or asukamycin (50 μM, 48 h), and assessed by Hoechst staining. Data shown as individual replicate values and average ± sem and are n=3 biologically independent samples/group for **(a)** and n=6 biologically independent samples/group for **(c)**. Western blot shown in **(b)** is a representative blot from n=3 biologically independent samples/group. Statistical significance was calculated with two-tailed unpaired Student's t-tests and are shown as *p<0.05 compared to shControl groups in **(a, c)** and compared to respective shControl or shPRKDC vehicle-treated controls in **(c)**. Uncropped blots can be found in **Supplementary Figure 9.** Raw source data can be found in **Supplementary Data 3.**



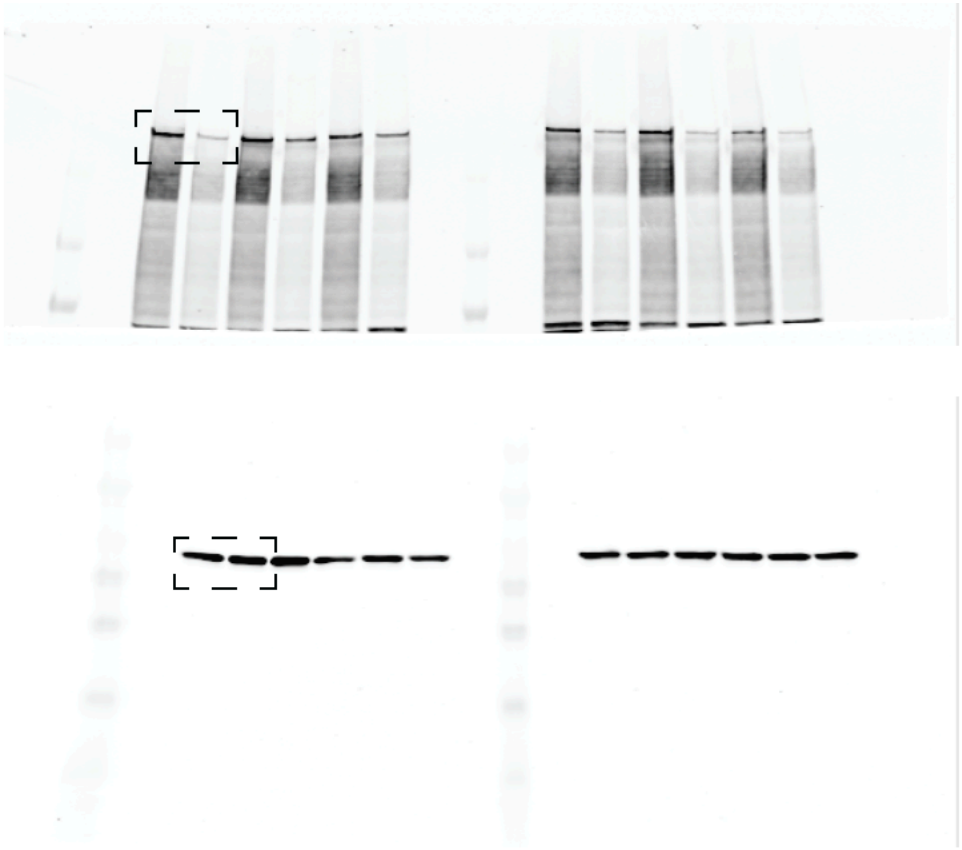
Supplementary Figure 6. Cell proliferation in HCT116 isogenic lines with different TP53 status. Cell proliferation in HCT116 cell lines expressing a wild-type, R248A mutant, or knockout TP53 treated with DMSO vehicle or asukamycin (30 μM) for 24 h, assessed by Hoechst stain. Data shown are individual replicate values and average ± sem from n=6 biologically independent samples/group. Statistical significance is calculated with two-tailed unpaired Student's t-tests and are shown as *p<0.05 compared to corresponding vehicle-treatment group for each genotype, and #p<0.05 compared to TP53 wild-type expressing asukamycin-treated HCT116 groups. Source data can be found in **Supplementary Data 4.**



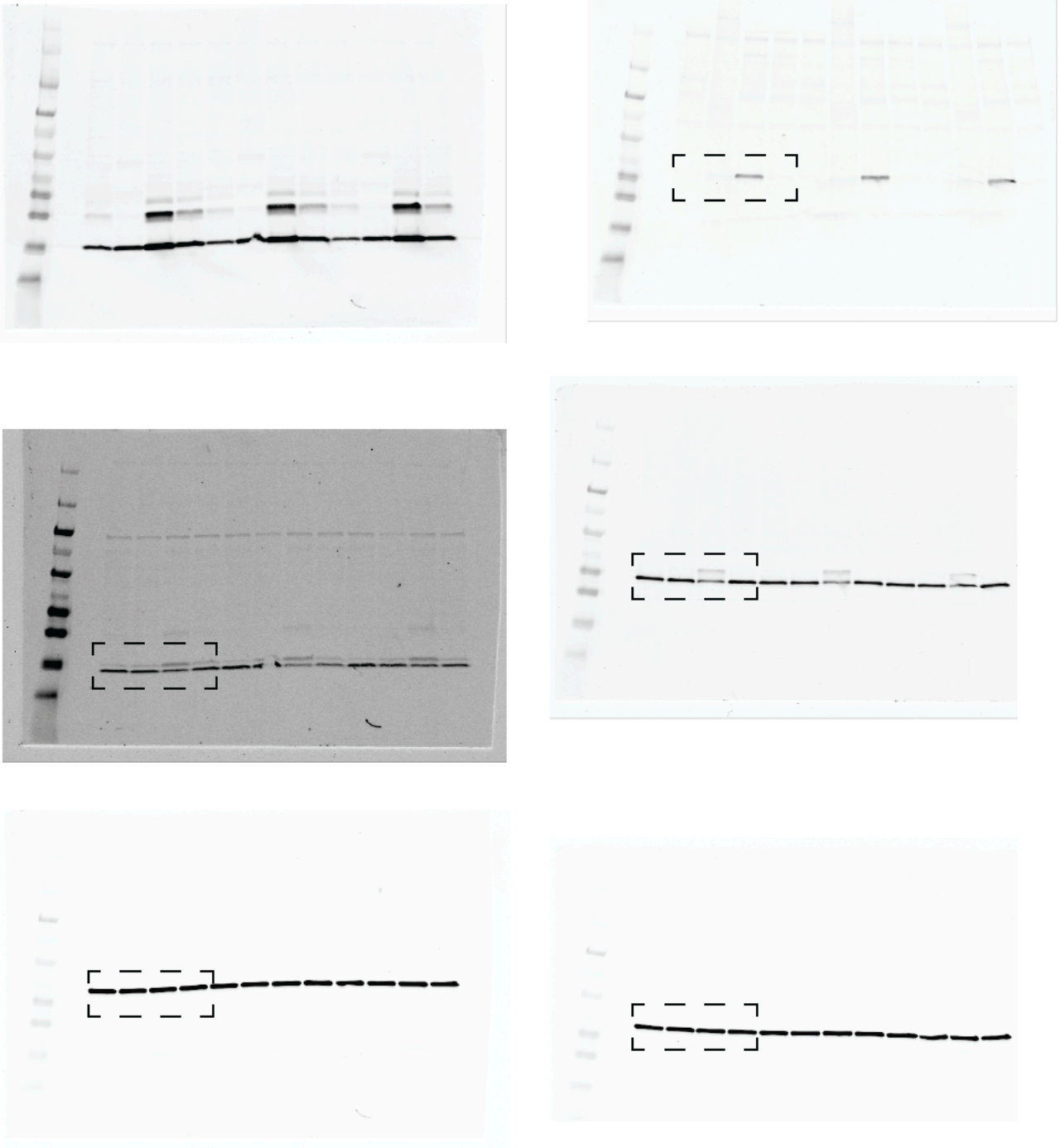
Supplementary Figure 7. Assessment of DNA damage markers with asukamycin, etoposide and hydrogen peroxide treatment. 231MFP cells were treated with DMSO vehicle, asukamycin (50 μM) for 24 h or etoposide (50 μM) for 12 h, or with hydrogen peroxide (1 mM) for 2 h. Cells were assessed for DNA damage using DNA damage markers looking at H2A.X Ser 139 phosphorylation, total H2A.X levels, loading control GAPDH levels, phosphorylated Ser 8 RPA32 levels, total RPA32 levels, and corresponding GAPDH loading control levels. The gels are representative of n=3 biologically independent samples/group. Uncropped blots can be found in **Supplementary Figure 10.**



Supplementary Figure 8. Source Data for Supplementary Figure 3.



Supplementary Figure 9. Source data for Supplementary Figure 5



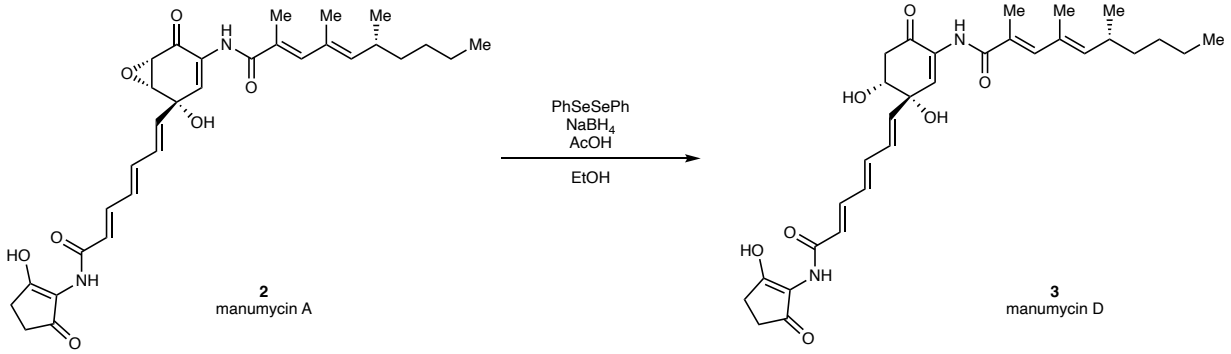
Supplementary Figure 10. Source Data for Supplementary Figure 7.

Synthetic Procedures

General Procedures

Unless otherwise stated, all reactions were performed in oven-dried or flame-dried Fisherbrand® borosilicate glass tubes (Fisher Scientific, 1495925A, 13 × 100 mm) with a black phenolic screw cap (13-425) under an atmosphere of dry nitrogen. Manumycin A was purchased from Cayman Chemicals, and used directly without further purification. Reactions were monitored by thin layer chromatography (TLC) on TLC silica gel 60 F_{254} glass plates (EMD Millipore) and visualized by UV irradiation and staining with *p*-anisaldehyde, phosphomolybdic acid, or potassium permanganate. Volatile solvents were removed under reduced pressure using a rotary evaporator. Flash column chromatography was performed using Silicycle F60 silica gel (60Å, 230-400 mesh, 40-63 µm). Ethyl acetate and hexanes were purchased from Fisher Chemical and used for chromatography without further purification. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AV-600 spectrometer operating at 600 MHz for ^{1}H, and 150 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm) with respect to the residual solvent signal $CDCl_3$ (^{1}H NMR: δ = 7.26; ^{13}C NMR: δ = 77.16). Peak multiplicities are reported as follows: *s* = singlet, *d* = doublet, *t* = triplet, *dd* = doublet of doublets, *tt* = triplet of triplets, *m* = multiplet, *br* = broad signal, *app* = apparent. High-resolution mass spectra (HRMS) were obtained by the QB3/chemistry mass spectrometry facility at the University of California, Berkeley.

Synthesis of manumycin D (3)



Synthesis of manumycin D (**3**)**:** The following procedure was conducted based on a slight modification to known procedures [1,2]. To a stirring solution of diphenyl diselenide (4.2 mg, 0.015 mmol, 1.5 eq.) in ethanol (0.15 mL) under a nitrogen atmosphere was added sodium borohydride (1.0 mg, 0.027 mmol, 3.0 eq.) at room temperature. After evolution of hydrogen ceased, the light-yellow solution was cooled to 0 ºC, followed by the addition of acetic acid (0.52 µL, 0.015 mmol, 1.5 eq.), and the mixture was further stirred at 0 ºC for 5 min. The resulting light-yellow solution was then added to a solution of manumycin A (**2**) (5.0 mg, 0.0091 mmol, 1.0 eq.) in ethanol (0.1 mL) at room temperature, and upon complete consumption of manumycin A (monitored by TLC, 5 min) the reaction mixture was diluted with ethyl acetate (1.0 mL), and oxygen was passed through the solution for 5 minutes. Volatiles were removed under reduced pressure, and dichloromethane (1.0 mL) was added. Insoluble materials were removed by passing the mixture through a plug of celite, and the crude material was purified by preparative TLC ($CHCl_3$:MeOH = 9:1) to give manumycin D (**3**) (2.9 mg, 58% yield) as a dark-orange oil whose data was in agreement with that previously reported: [3,4] R_f = 0.33 (SiO_2, $CHCl_3$:MeOH = 9:1, UV, $KMnO_4$); ^{1}H NMR: (600 MHz, $CDCl_3$) δ 13.54 (br, 1H), 8.29 (br, 1H), 7.56 (m, 2H), 7.32 (dd, *J* = 14.8, 11.3 Hz, 1H), 6.80 (s, 1H), 6.66 – 6.51 (m, 2H), 6.46 – 6.36 (m, 1H), 6.09 – 5.97 (m, 2H), 5.34 (d, *J* = 9.6 Hz, 1H), 4.17 – 4.03 (m, 1H), 3.23 (br, 1H), 2.90 (dd, *J* = 17.1, 6.1 Hz, 1H), 2.84 (br, 1H), 2.76 (dd, *J* = 17.1, 3.4 Hz, 1H), 2.65 – 2.58 (m, 2H), 2.55 – 2.50 (m, 2H), 2.50 – 2.39 (m, 1H), 2.06 (s, 3H), 1.82 (s, 3H), 1.39 – 1.22 (m, 6H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.4, 191.9, 174.0, 169.0, 165.5, 143.7, 142.9, 140.3, 139.9, 138.7, 132.4, 131.6, 131.5, 130.1, 128.5, 126.1, 121.5, 115.1, 73.7, 71.9, 40.7, 37.2, 33.0, 32.3, 29.9, 25.8, 23.0, 20.9, 16.7, 14.23, 14.18; HRMS: (ESI-TOF, m/z) *calcd*. For $C_{31}H_{39}N_2O_7$ $[M–H]^–$ calc.: 551.2763; found: 551.2765.

References for Synthetic Methods

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