Supplementary Figures

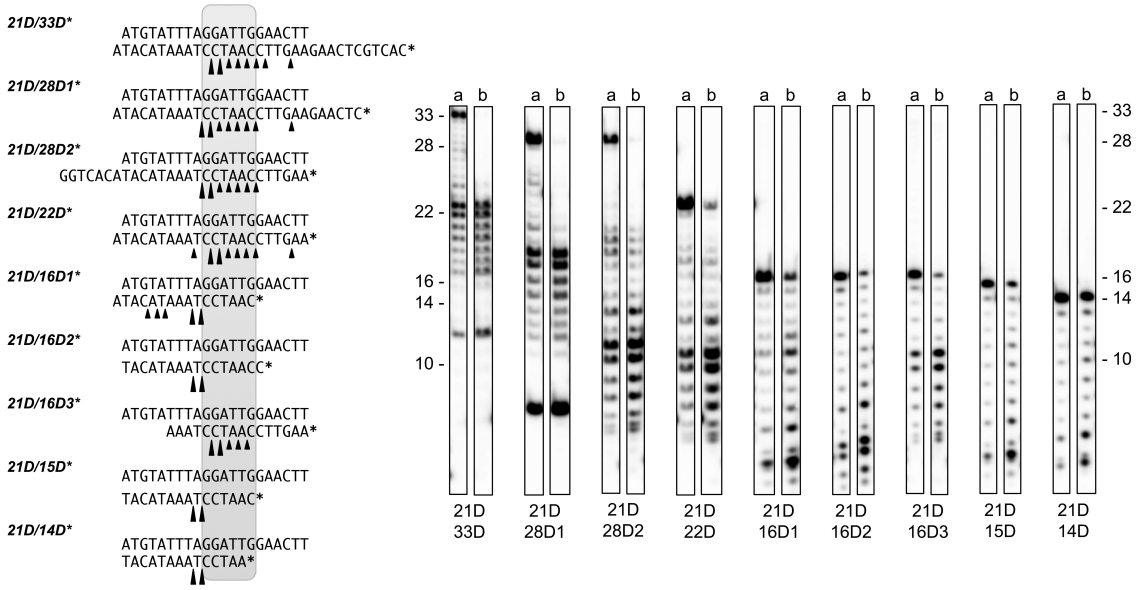

Figure S1. pUL15C cleavage of a 21nt/33nt DNA duplex and variants thereof, labeling the lower strand at the 5' terminus with ^{32}P. *Left,* Sequence of duplex DNA substrates. Asterisk denotes the position of the ^{32}P label. *Right*, Cleavage patterns of specificity, following incubation with pUL15C for 5 and 30 min (Lanes a and b, respectively). A summary of the primary cleavage sites (trianges) is illustrated below each duplex sequence.

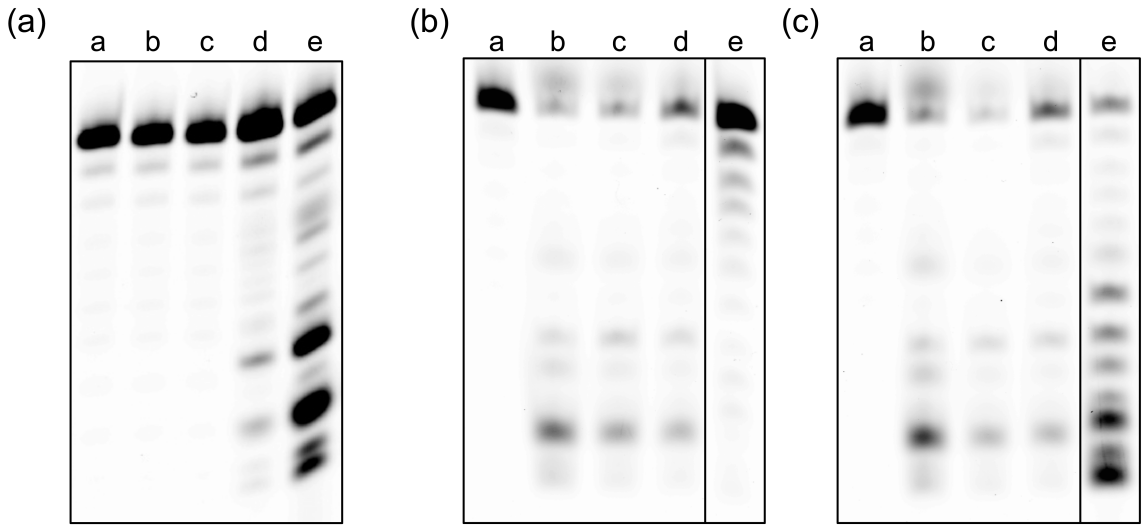

Figure S2. Gel-based analysis of DNase I activity in the presence of Mg^{++} and Mn^{++}. (a) Lanes a - c, incubation of the model duplex *21D/33D with 1.2 μM purified pUL15C for 0, 15 and 30 mins, respectively, in 10 mM $MgCl_2$; Lane d and e, 5-sec and 15-sec DNase I digestion, respectively, of the model duplex in 10 mM $MgCl_2$. (b), (c) Dnase I activity in the presence or absence, respectively, of 20 μM β-thujaplicinol. Lanes a – d, incubation of the model duplex with DNase I concentration of 0, 0.01, 0.002, and 0.0004 U/μl, respectively, for 15 min in 1 mM $MnCl_2$; Lanes e, incubation of the model duplex with 1.2 μM purified pUL15C for 15 min in 1 mM $MnCl_2$.

Supplementary Methods

Analysis of pUL15 nuclease activity on lower strand of short DNA duplexes - To prepare ^{32}P-labeled duplex DNA substrates, the oligonucleotides (33D, 28D1, 28D2, 22D, 16D1, 16D2, 16D3, 15D, and 14D) were labeled at their 5' termini using [γ-^{32}P]ATP (6000 Ci/mmol; PerkinElmer Life Sciences) and T4 polynucleotide kinase (Fermentas) according to the manufacturer's instructions, and subsequently purified using Sephadex G-50 spin columns (GE Healthcare). 5'-labeled oligonucleotides were annealed to complementary 21-nt DNA (21D: 5′-ATGTATTTAGGATTGGGACTT-3′), yielding the duplexes 21D/33D*, 21D/28D1*, 21D/28D2*, 21D/22D*, 21D/16D1*, 21D/16D2*, 21D/16D3*, 21D/15D*, and 21D/14D*. Annealing was performed by heating a mixture containing 10 μM Cy5-21D, 10 μM each complementary DNA, in 10 mM Tris-HCl pH 7.6, and 25 mM NaCl to 80°C and slow cooling to 4°C. Nuclease digestion was initiated by incubating 0.1 μM of duplex DNA carrying ^{32}P-labeled strand in a reaction containing 20 mM Tris-HCl pH 7.0, 1 mM $MnCl_2$, 10 mM NaCl, and 0.3 μM pUL15C at 37°C and terminated after 5 or 30 min by adding an equal volume of 8 M urea. Hydrolysis products were fractionated by 15% denaturing urea polyacrylamide gel electrophoresis (19:1 acrylamide:bisacrylamide) and visualized by using autoradiography (Typhoon Trio+, GE Healthcare).

Synthesis and characterization of compounds 11 and 21 - *(a) 4-([1,1'-biphenyl]-4-carbonyl)-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trien-1-one* (compound **11**). To a suspension of 5-hydroxy-4-methoxy-2-methylpyrylium trifluoromethanesulfonate (100 mg, 0.345 mmol) and 1-([1,1'-biphenyl]- 4-yl)prop-2-yn-1-one (712 mg, 3.45 mmol, 10 eq) in CH_2Cl_2 (5 mL) was added N,N-diisopropylaniline (81 μL, 0.414 mmol, 1.2 eq). After microwave irradiation at 100 °C for 1 hr, the reaction mixture was concentrated and purified by chromatography (Silica [10 g], 0% EtOAc/hexane to 35% EtOAc/hexane gradient over 20 column volumes), yielding bicycle 6-([1,1'-biphenyl]-4-carbonyl)-3-methoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one as an orange solid (92.8 mg, 77% yield). MP= 156-159 °C. Rf= 0.22 in 20% EtOAc in hexanes. **IR (thin film, KBr)**

3063 (w), 2979 (w), 2935 (w), 2837 (w), 1711 (s), 1641 (m), 1603 (s), 1449 (w), 1323 (m), 1127 (m), 1043 (w), 989 (w), 844 (m), 744 (s), 698 (m) cm-1. **^{1}H NMR (400 MHz, CDCl3)** δ 7.93 (d, *J* = 8.3 Hz, 2H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.65 – 7.61 (m, 2H), 7.51 – 7.38 (m, 3H), 6.83 (d, *J* = 2.4 Hz, 1H), 6.30 (s, 1H), 5.20 (d, *J* = 2.5 Hz, 1H), 3.60 (s, 3H), 1.77 (s, 3H). **^{13}C NMR (100 MHz, CDCl3)** δ 190.37, 188.72, 155.32, 146.66, 145.31, 139.84, 138.77, 135.73, 129.91, 129.29, 128.71, 127.63, 127.54, 120.62, 87.37, 87.01, 54.98, 21.07. **HRMS (ESI+)** *m/z* calc'd for $C_{22}H_{19}O_4$+: 347.1278. Found: 347.1280.

To the above bicycle 6-([1,1'-biphenyl]-4-carbonyl)-3-methoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (46.4 mg, 0.134 mmol) in CH_2Cl_2 (1.5 mL) was added trifluoromethanesulfonic acid (47.3 µL, 0.536 mmol, 4 eq). The reaction was allowed to stir for 30 minutes, at which time it was quenched with sodium acetate (110 mg, 1.34 mmol, 10 eq), stirred for 20 min, and concentrated under reduced pressure to generate the methoxytropolone. The methoxytropolone was then dissolved in 25% HBr in acetic acid (2 mL), and heated to 90 ºC for 4 hr. The reaction was cooled to room temperature, quenched with pH 7 phosphate buffer (10 mL), and diluted with CH_2Cl_2 (5 mL). The organic layer was washed with phosphate buffer (3 X 10 mL), dried over Na_2SO_4, filtered, and concentrated to yield 11 as a brown oil (26.2 mg, 59% yield). **IR (thin film, KBr)** 3262 (br), 3060 (w), 2961 (w), 1669 (s), 1601 (s), 1534 (s), 1398 (m), 1284 (s), 1232 (s), 1191 (s), 1083 (s), 906 (m), 859 (m), 750 (s), 696 (m) cm-1. **^{1}H NMR (400 MHz, CDCl3)** δ 7.88 (d, *J* = 8.2 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.54 (s, 1H), 7.52-7.39 (m, 3H), 7.36 (s, 1H), 2.36 (s, 3H). **^{13}C NMR (100 MHz, CDCl3)** ä 197.02, 168.70, 159.03, 157.33, 147.49, 140.15, 139.85, 138.42, 134.57, 131.05, 129.41, 128.96, 128.01, 127.70, 124.67, 119.18, 24.86. **HRMS (ESI+)** *m/z* calc'd for $C_{21}H_{17}O_4$+: 333.1121. Found: 333.1124.

(b) Methyl 7-bromo-4,6-dihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (compound **21**). To a suspension of 5-hydroxy-4-methoxy-2-methylpyrylium trifluoromethanesulfonate (122.4 mg, 0.422 mmol) and previously-made methyl 3-bromopropiolate[(25)] (687.4 mg, 4.21 mmol) in CH_2Cl_2 (800 µL) was added N,N-diisopropylaniline (98 µL, 0.505 mmol). After microwave irradiation at 120 °C for 20 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column,

solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (8 CV); 10-15% EtOAc in hexanes (10 CV); 15-20% EtOAc in hexanes (10 CV); 20-25% EtOAc in hexanes (10 CV); 25-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield bicycle methyl 7-bromo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate as a red/orange oil (65.8 mg, 54% yield). Rf= 0.36 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 3446 (w), 2945 (w), 1715 (s), 1615 (w), 1317 (m), 1380 (w), 1168 (w), 1124 (w), 862 (w), 700 (w) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 6.09 (s, 1H), 4.95 (s, 1H), 3.86 (s, 3H), 3.57 (s, 3H), 1.77 (s, 3H). **^{13}C NMR (100 MHz, CDCl3)** δ 186.93 (s), 162.86 (s), 145.20 (s), 144.02 (s), 132.10 (s), 119.34 (s), 90.73 (s), 88.08 (s), 55.11 (s), 52.54 (s), 22.09 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{11}H_{12}BrO_5$+: 302.9863. Found: 302.9868.

To a solution of above referenced bicycle, methyl 7-bromo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate, (53.80 mg, 0.1775 mmol) in CH_2Cl_2 (1.77 mL) was added triflic acid (66.3 μL, 0.7496 mmol). The reaction was allowed to stir at room temperature for 30 minutes before being quenched with pH 7 phosphate buffer (10 mL). The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield the methoxytropolone as a red oil (36.4 mg, 68% yield). The methoxytropolone (19.9 mg, 0.066 mmol) was then dissolved in 33% HBr/AcOH (875 μL) and heated to reflux at 120 °C for 6 hours before being quenched with pH 7 phosphate buffer (10 mL). The organic layer was isolated and the aqueous later was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield Methyl 7-bromo-4,6-dihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (21) as a brown oil (18.2 mg, 96%). **IR (thin film, KBr)** 3426 (br), 2952 (w), 1732 (s), 1533 (m), 1342 (w), 1287 (m), 1218 (s), 1061 (w), 954 (w), 668 (w) cm-1. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.41 (s, 1H), 3.97 (s, 3H), 2.45 (s, 3H). **^{13}C NMR (100 MHz, CDCl3)** δ 168.25 (s), 166.08 (s), 157.72 (s), 156.15 (s), 138.00 (s), 135.36 (s), 123.44 (s), 118.80 (s), 53.37 (s), 25.89 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{10}H_8BrO_4$+: 270.9601. Found: 270.9608.