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Supporting Information Appendix for

A synthetic agent ameliorates polycystic kidney disease by promoting apoptosis of cystic cells through increased oxidative stress

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This PDF file includes:

Supporting text (Supplemental Methods)
Figures S1 to S17

Supporting Information Text

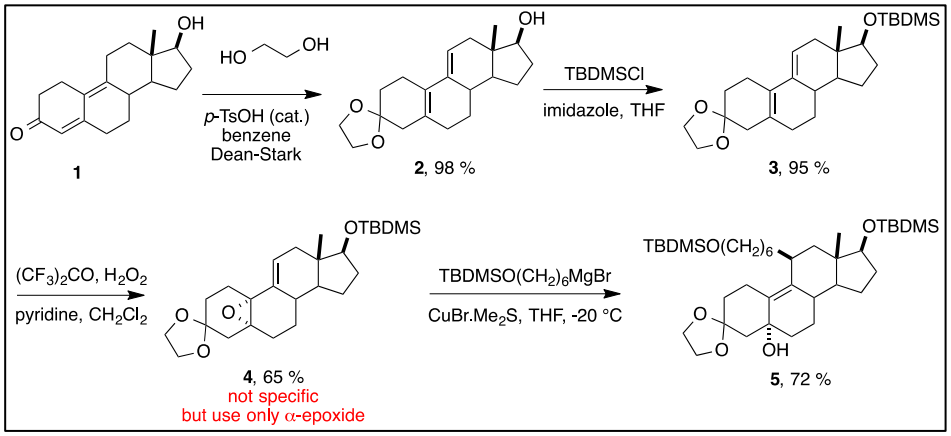
Supplemental Methods

Synthesis of 11beta-dipropyl

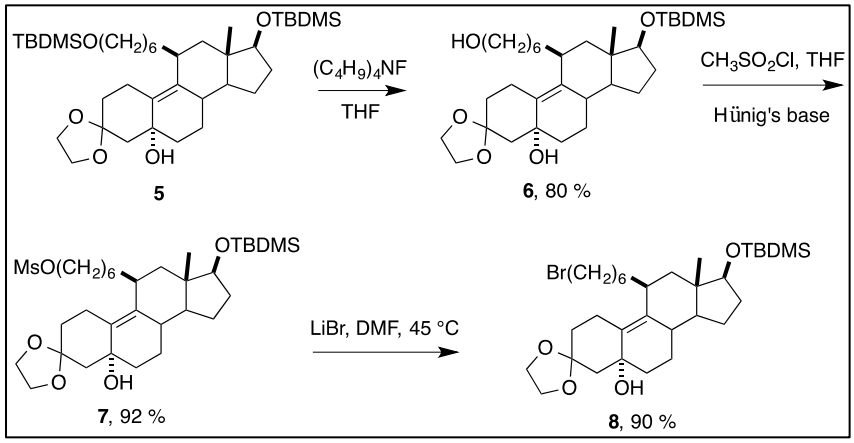
General Procedures: All reactions were carried out under an inert atmosphere (nitrogen or argon where stated) with dry solvents under anhydrous conditions. Glassware for anhydrous reactions was dried in an oven at 100 °C for minimum 6 h prior to use. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at a high commercial quality (typically 97 % or higher) and used without further purification, unless otherwise stated. High field NMR spectra were recorded at 300, 400 or 500 MHz for ^{1}H, and 75, 100 or 125 MHz for ^{13}C, as indicated. Chemical shifts of ^{1}H and ^{13}C spectra were referenced to the NMR solvents. Flash chromatography was performed using silica gel (230–600 mesh) or aluminum oxide 90 standardized. Thin layer chromatography was performed using TLC-PET foil coated with silica gel 60 F254 or aluminum oxide as stated. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, dq = double quartet m = multiplet, br = broad. The yield of each step is specified as a percentage below each intermediate in the reaction scheme.

Synthetic schemes

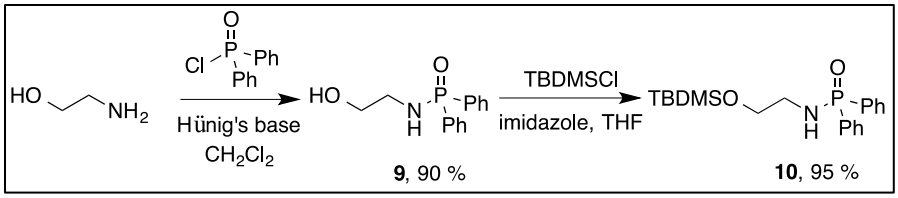
Scheme 1:



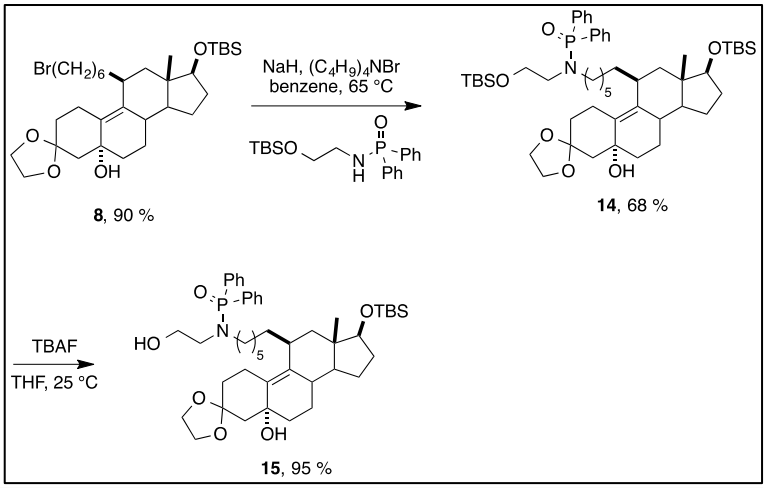
Scheme 2:



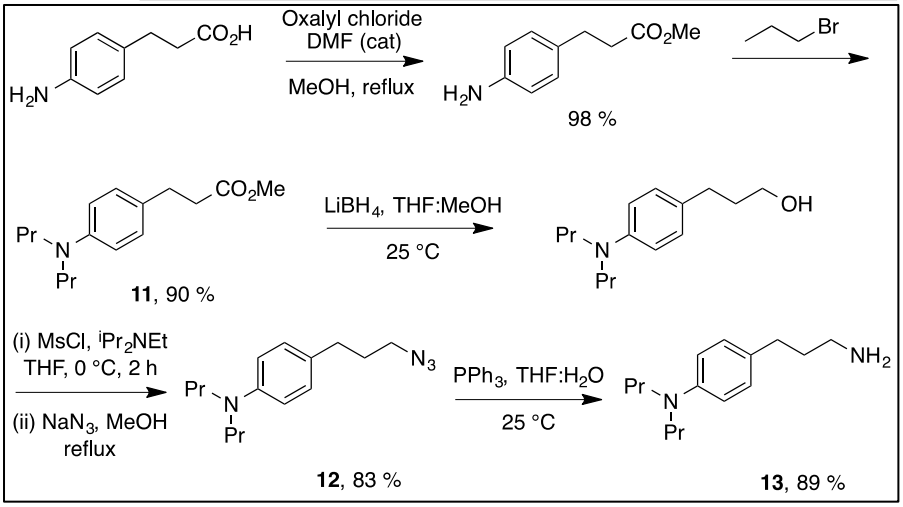
Scheme 3:



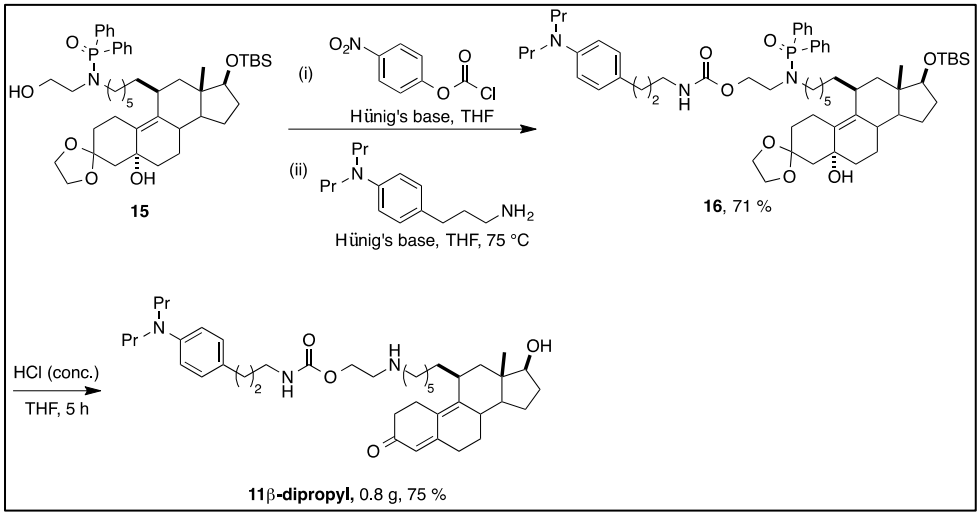
Scheme 4:



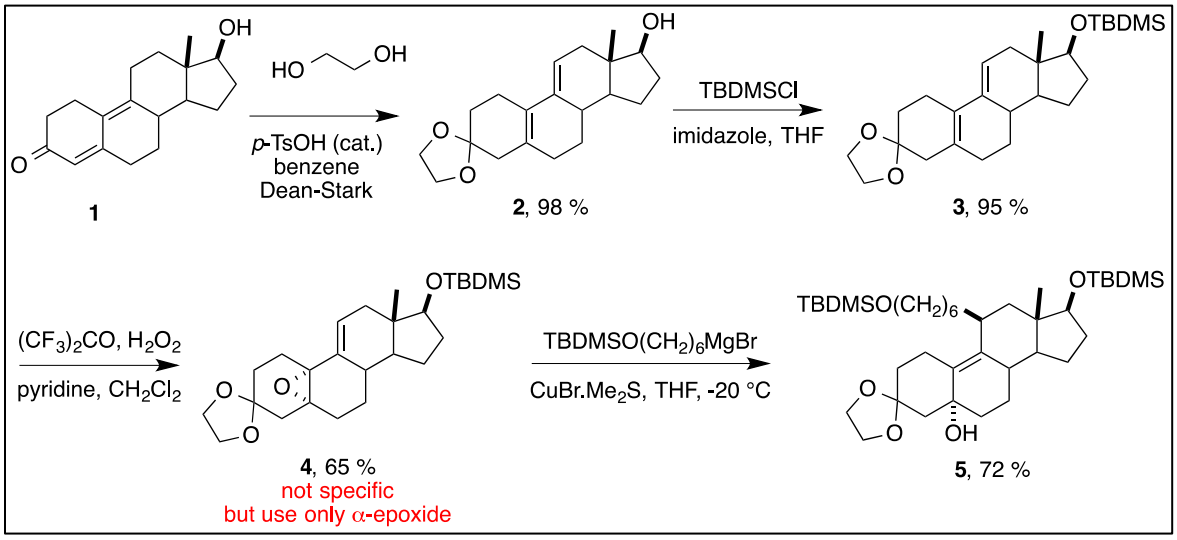
Scheme 5:



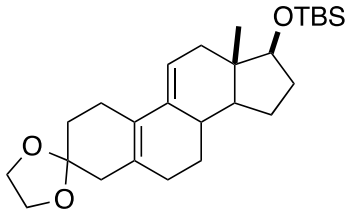
Scheme 6:



Scheme 1: Synthesis of intermediate *5*



Synthesis of *tert*-butyldimethyl(((13*S*,17*S*)-13-methyl-1,2,4,6,7,8,12,13,14,15,16,17-dodeca-hydrospiro[cyclopenta[*a*]phenanthrene-3,2'-[1,3]dioxolan]-17-yl)oxy)silane (intermediate *3*)



(8ξ,14ξ)-17-Hydroxyestra-4,9-dien-3-one and ethylene glycol were dissolved in benzene. TsOH was added and the mixture was refluxed using a Dean-Stark trap until no water was being distilled from the reaction mixture. The solvent was removed under reduced pressure and the crude oil was redissolved in ethyl acetate. The organic layer was washed with $NaHCO_3$ (sat.), water, brine and dried over Na_2SO_4 and solvent was removed under reduced pressure to yield compound **2** [**(13*S*,17*S*)-13-methyl-1,2,4,6,7,8,12,13,14,15,16,17-dodecahydrospiro[cyclopenta[*a*] phenanthrene-3,2'-[1, 3]dioxolan]-17-ol]** which was used without further purification.

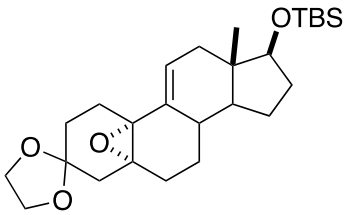
Compound **2** and imidazole were dissolved in DMF and *tert*-butyldimethyl-silylchloride was added to solution. The mixture was stirred at room temperature overnight. $NaHCO_3$ (sat.) was added and the mixture was extracted with CH_2Cl_2 (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography using EtOAc:hexane (9:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.59 – 5.51 (m, 1H), 4.18 – 3.91 (m, 5H, CHOTBS and OCH_2CH_2O), 3.76 – 3.61 (m, 1H), 2.51 (d, *J* = 16.2 Hz, 1H), 2.25 – 2.06 (m, 3H), 2.04 (s, 1H), 1.97 – 1.92 (m, 2H), 1.88 (t, *J* = 4.1 Hz, 2H), 1.85 – 1.69 (m, 4H), 1.66 (s, 1H), 1.58 – 1.32 (m, 2H), 1.32 – 1.08 (m, 3H), 0.89 (d, *J* = 9.0 Hz, 12H, TBS+Me), 0.70 (s, 3H, TBS), 0.09 (s, 3H, TBS).

^{13}C NMR (75 MHz, $CDCl_3$) δ 136.9, 130.0, 126.5, 118.4, 108.4[$C(OCH_2)_2$], 82.1, 64.7, 64.6, 58.8, 58.5, 47.0, 42.2, 41.5, 39.5, 38.5, 31.5, 31.4, 31.3, 27.7, 26.1, 25.9, 24.9, 24.2, 18.3, 11.8, -4.2, -4.6.

HRMS (ESI) calculated for [$(C_{26}H_{42}O_3Si)^+$]: 430.2898; found: 430.2916

Synthesis of *tert*-butyldimethyl(((5'*R*,10'*R*,13'*S*,17'*S*)-13'-methyl-1',2',7',8',12',13', 14',15',16',17'-decahydro-4'*H*,6'*H*-spiro[[1,3]dioxolane-2,3', [5,10]epoxycyclopenta-[*a*]phenanthren] -17'-yl)oxy)silane (intermediate *4*)



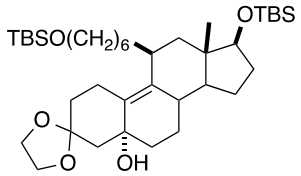
Compound **3** was dissolved in CH_2Cl_2. Hexafluoroacetone and pyridine were added to the solution and the mixture was cooled to 0 °C. H_2O_2 (30%) was added dropwise and the mixture was stirred at ambient temperature overnight. Water was added and the organic layer was isolated in a sep funnel, washed with another portion of water, then brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by alumina column chromatography using EtOAc:hexane (9:1). Compound **4** (epoxide) was isolated as a white foamy solid.

^{1}H NMR (300 MHz, C_6D_6) δ 5.96-5.94 (m, 1H), 3.57-3.42 (m, 5H, CHOTBS and OCH_2CH_2O), 2.52-2.46 (m, 1H), 2.38 (d, J = 25 Hz, 1H), 2.17-1.92 (m, 5H), 1.81-1.20 (m, 10H), 1.10-0.97 (m,1H), 1.00 (s, 9H, TBS), 0.76 (s, 3H, Me), 0.056 (s, 3H, TBS), 0.046 (s, 3H, TBS).

^{13}C NMR (75 MHz, C_6D_6) δ 137.6, 126.3, 107.7 [$C(OCH_2)_2$], 82.4, 64.6, 64.3, 61.5, 60.4, 46.4, 42.7, 41.5, 39.6, 38.7, 32.8, 31.5, 29.1, 26.4 (3C), 26.0, 24.3, 23.2, 18.7, 12.1,-3.9, -4.2.

HRMS (ESI) calculated for [$(C_{26}H_{42}O_4Si)^+$]: 446.2847; found: 446.2853.

Synthesis of (5*R*,11*S*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-11-(6-((*tert*-butyldimethyl-silyl)oxy)hexyl)-13-methyl-1,2,6,7,8,11,12,13,14,15,16,17-dodecahydrospiro[cyclopenta[*a*]-phenanthrene-3,2'-[1,3]dioxolan]-5(4*H*)-ol (intermediate *5*)



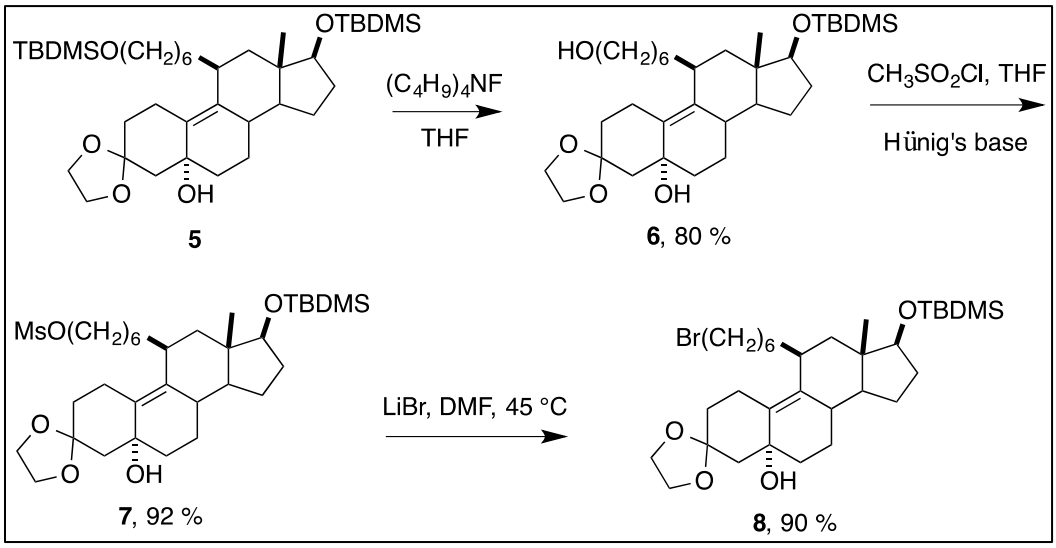
Magnesium was dried under vacuum, kept under argon and anhydrous THF (8 mL) was added. Bromoalkane compound was added to the solution and the mixture was heated using heat gun until some bubbles of gas form in the mixture. Heating of the mixture continued using oil bath at 70 ˚C for 4 h. After cooling to room temperature, THF (12 mL) was added and the mixture was further cooled to -20 °C. $CuBr.SMe_2$ was added to the mixture and stirred for 5 min. Compound **4** (steroid) dissolved in 10 mL THF was added dropwise to the mixture and stirred for 1.5-2 h. The mixture was added to a mixture of NH_4Cl (sat.) (100 mL) and ethyl acetate (100 mL) and stirred for 10 min. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the product was purified by alumina column chromatography (hexane then 2-3 % EtOAc:hexane), yielding a colorless oil.

^{1}H NMR (300 MHz, C_6D_6) δ 4.51 (s, 1H), 3.55 (t, J = 10.5 Hz, 2H, CH_2OTBS), 3.47-3.3.31 (m, 5H, CHOTBS and OCH_2CH_2O), 2.91-2.84 (m, 1H), 2.76-2.60 (m, 2H), 2.08-1.67 (m, 8H), 1.64-1.16 (m, 17H), 1.03-1.01 (m, 21H, Me, TBS), 0.95-0.80 (m, 1H), 0.11-0.076 (m, 12H, TBS).

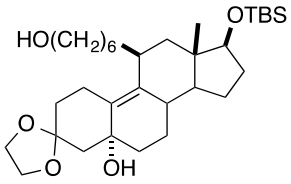
^{13}C NMR (75 MHz, C_6D_6) δ 139.0, 131.9, 109.7 [$C(OCH_2)_2$], 83.5, 70.3, 64.9, 64.3, 63.6, 51.5, 48.5, 43.4, 41.9, 39.7, 37.3, 36.6, 36.5, 33.6, 31.6, 30.2, 29.4, 26.6, 26.55 (3C), 26.53 (3C), 24.4, 24.3, 23.9, 18.9, 18.7, 14.4, -3.8, -4.1, -4.7 (2C).

HRMS (ESI) calculated for [($C_{38}H_{70}O_5Si_2$)$^+$] [M + Na]: 685.4654; found: 685.4649

Scheme 2: Synthesis of intermediate *8*

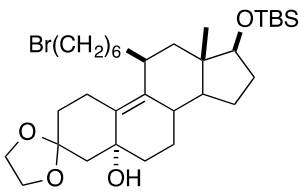


Synthesis of (5*R*,11*S*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-11-(6-hydroxyhexyl)-13-methyl-1,2,6,7,8,11,12,13,14,15,16,17-dodecahydrospiro[cyclopenta[*a*]phenanthrene-3,2'-[1,3]dioxolan]-5(4*H*)-ol (intermediate *6*)



Intermediate **5** was dissolved in THF and TBAF (1 M in THF) was added. The mixture was stirred for 3-5 h (monitored by TLC) at room temperature. Upon completion of the reaction, the mixture was poured into water and extracted with ethyl acetate (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and product was purified by alumina column chromatography (50 % EtOAc:hexane) producing a viscous syrup.

Synthesis of (5*R*,11*S*,13*S*,17*S*)-11-(6-bromohexyl)-17-((*tert*-butyldimethylsilyl)oxy)-13-methyl-1,2,6,7,8,11,12,13,14,15,16,17-dodecahydrospiro[cyclopenta[*a*]phenan-threne--3,2'-[1,3]dioxolan]-5(4*H*)-ol (intermediate *8*)



Steroid **6** was dissolved in THF and diisopropyl-ethylamine was added. The mixture was cooled to 0 °C and a solution of MsCl in THF was added dropwise. The mixture was stirred for 1-4 h (monitored by TLC) and then poured into a saturated $NaHCO_3$ solution. The resulting solution was extracted with ethyl acetate (3 times). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to yield the methanesulfonate product (intermediate **7**), a yellowish oil which was used for the next reaction without further purification.

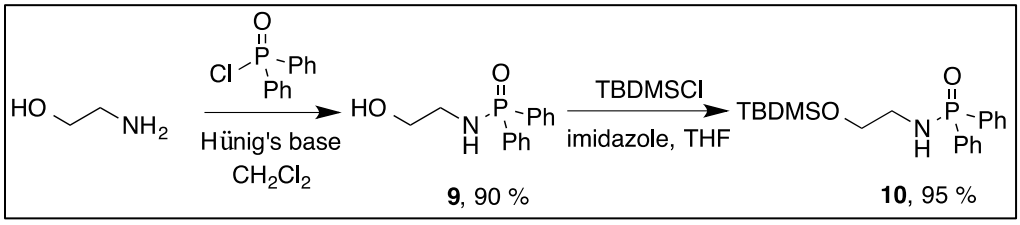
Compound **7** was dissolved in DMF and LiBr was added. The mixture was stirred at 45 °C for 4 h (TLC). $NaHCO_3$ (sat.) was added and the mixture was extracted with ethyl acetate (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude was purified by alumina column chromatography using (30 % EtOAc:hexane) to yield a colorless oil that became a white solid on standing.

^{1}H NMR (500 MHz, C_6D_6) δ 4.47 (s, 1H), 3.47-3.3.37 (m, 4H, OCH_2CH_2O), 3.35-3.32 (m, 1H, CHOTBS), 2.95 (t, J = 7 Hz, 2H, CH_2Br), 2.85-2.81 (m, 1H), 2.72-2.61 (m, 2H), 2.05-2.00 (m, 3H), 1.93-1.88 (m, 2H), 1.81-1.68 (m, 4H), 1.61-1.58 (m, 1H), 1.53-1.43 (m, 5H), 1.39-1.32 (m, 2H), 1.27-10.3 (m, 9H), 1.02 (s, 9H, TBS), 0.99 (s, 3H, Me), 0.92-0.86 (m, 1H), 0.10 (s, 3H, TBS), 0.07 (s, 3H, TBS).

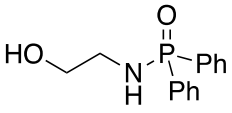
^{13}C NMR (125 MHz, C_6D_6) δ 138.9, 131.9, 109.6, 83.5, 70.3, 64.9, 64.3, 51.5, 48.5, 43.4, 41.9, 38.7, 38.5, 37.2, 36.6, 36.5, 33.9, 33.4, 31.6, 29.4, 29.1, 28.6, 26.5 (3C), 24.4, 24.3, 23.9, 18.7, 14.4, -3.8, -4.1.

HRMS (ESI) calculated for $[(C_{32}H_{56}O_4SiBr)^+]$+Na: 633.2945; found: 633.2944

Scheme 3: Synthesis of intermediate *10*

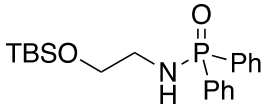


Synthesis of *N*-(2-hydroxyethyl)-*P*,*P*-diphenylphosphinic amide (intermediate *9*)



Ethanolamine was dissolved in CH_2Cl_2 and Et_3N was added to the solution under argon. Diphenyl-phosphinic chloride was added dropwise and the mixture was stirred at room temperature for 3 h (TLC). Water was added and the mixture was extracted with CH_2Cl_2 (3 times). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure. The crude was purified by column chromatography using CH_2Cl_2:MeOH (9:1) to yield a thick syrup.

Synthesis of *N*-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-*P*,*P*-diphenylphosphinic amide (intermediate *10*)

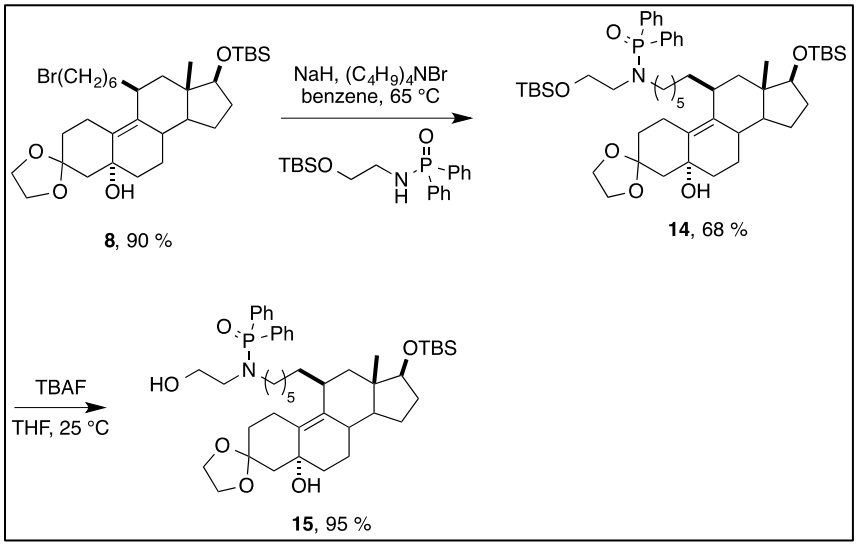


N-(2-Hydroxyethyl)-*P*,*P*-diphenylphosphinic amide and imidazole were dissolved in DMF and *tert*-butyldimethylsilylchloride was added to solution. The mixture was stirred at room temperature overnight. $NaHCO_3$ (sat.) was added and the mixture was extracted with ethyl acetate (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude was purified by column chromatography using CH_2Cl_2:MeOH (95:5).

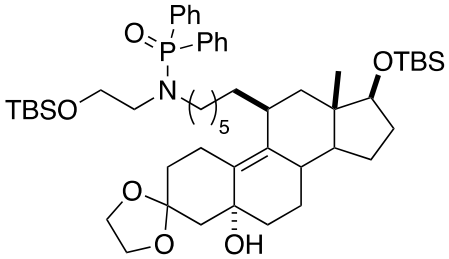
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 – 7.77 (m, 5H), 7.58 – 7.36 (m, 5H), 3.73 (t, *J* = 5.3 Hz, 2H), 3.34 (d, *J* = 6.9 Hz, 1H), 3.06 (ddt, *J* = 8.9, 6.8, 5.2 Hz, 2H), 0.88 (s, 9H), 0.17 – -0.09 (m, 6H).

^{31}P NMR (121 MHz, CDCl3) δ 25.01.

Scheme 4: Synthesis of intermediate *15*



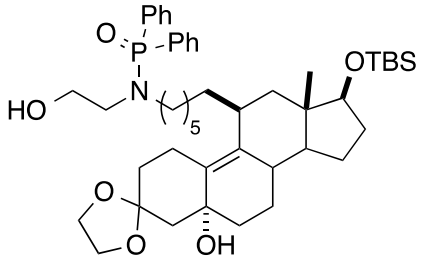
Synthesis of *N*-(6-((5*R*,11*S*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-13-methyl-1,2,4,5,6,7,8,11,12,13,14,15,16,17-tetradecahydrospiro[cyclopenta[*a*] phenanthrene-3,2'-[1,3]dioxolan]-11-yl)hexyl)-*N*-(2-((*tert*-butyldimethylsilyl)oxy) ethyl)-*P*,*P*-diphenylphosphinic amide (intermediate *14*)



Compound **10** and tetrabutyl-ammonium bromide were dissolved in benzene under argon atmosphere. Sodium hydride was added and the mixture was stirred for 30 min. Steroid **8** dissolved in benzene was added dropwise and then the mixture was heated to 65 °C for 4 h. $NaHCO_3$ (sat.)

was added and the mixture was extracted with ethyl acetate (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude was purified by alumina column chromatography using (50 % EtOAc:hexane).

Synthesis of *N*-(6-((5*R*,11*S*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-13-methyl-1,2,4,5,6,7,8,11,12,13,14,15,16,17-tetradecahydrospiro[cyclopenta[*a*] phenanthrene-3,2'-[1,3]dioxolan]-11-yl)hexyl)-*N*-(2-hydroxyethyl)-*P*,*P*-diphenyl phosphinic amide (intermediate 15)

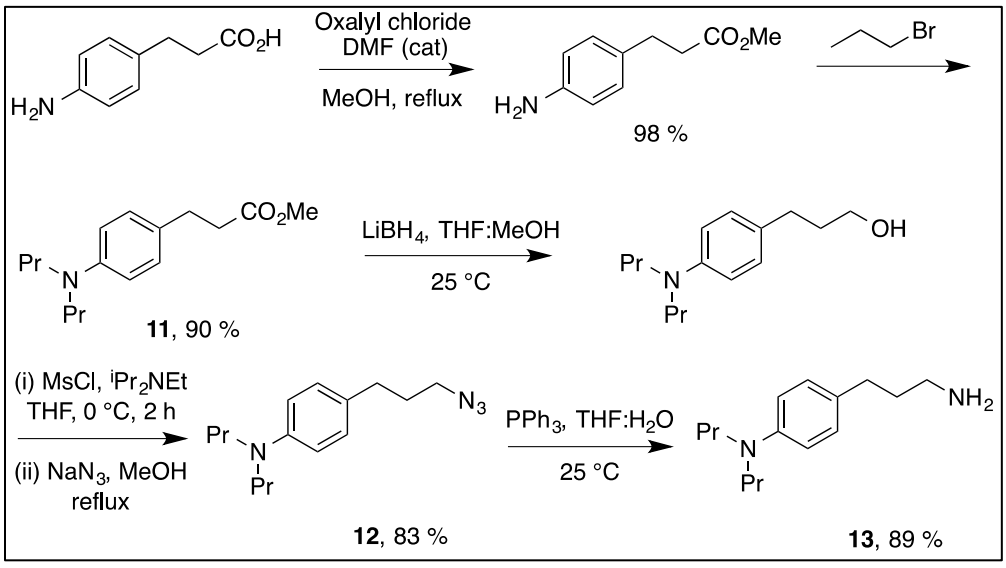


Steroid **14** was dissolved in THF and TBAF (1M in THF) was added. The mixture was stirred for 3-5 h (TLC) at room temperature. Upon the completion, the mixture was poured into water and extracted with ethyl acetate (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and product was purified by alumina column chromatography using ethyl acetate as eluent, yielding a white solid.

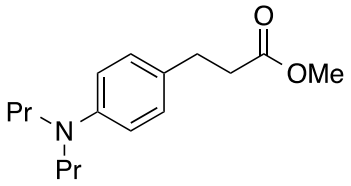
^{1}H NMR (300 MHz, CDCl3) δ 7.88 (ddt, J = 11.9, 6.5, 1.6 Hz, 4H), 7.59 – 7.41 (m, 6H), 5.30 (d, J = 1.2 Hz, 7H), 4.21 – 3.89 (m, 5H), 3.66 (t, J = 4.5 Hz, 2H), 3.49 (dd, J = 8.8, 7.3 Hz, 1H), 3.29 – 3.15 (m, 2H), 2.99 – 2.75 (m, 3H), 2.62 (d, J = 14.7 Hz, 1H), 2.33 (t, J = 14.4 Hz, 1H), 1.99 – 1.75 (m, 5H), 1.67 – 1.43 (m, 7H), 1.42 – 1.18 (m, 8H), 1.17 – 1.06 (m, 4H), 1.06 – 0.79 (m, 13H).

^{13}C NMR (75 MHz, CDCl3) δ 139.64, 132.77, 132.65, 132.27, 132.23, 131.99, 130.59, 130.27, 128.99, 128.82, 109.11, 83.02, 70.25, 64.90, 64.31, 59.01, 53.68, 51.36, 49.18, 47.46, 45.95, 42.96, 41.13, 37.99, 37.74, 36.72, 36.07, 35.92, 31.10, 29.52, 28.77, 28.68, 26.86, 26.09, 23.97, 23.70, 23.20, 18.33, 13.87, -4.25, -4.55.

Scheme 5: Synthesis of intermediate *13*



Synthesis of methyl 3-(4-(dipropylamino)phenyl)propanoate (intermediate *11*)



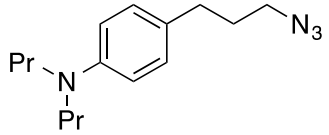
Methyl 3-(4-aminophenyl)propanoate and K_2CO_3 were dissolved in DMSO. Propylbromide was added and the mixture was stirred at room temperature for 3 days. Water was added to the solution and the mixture was extracted with ethyl acetate (3 times). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude was purified by column chromatography using EtOAc:hexane (9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, *J* = 8.8 *Hz*, 2H), 6.59 (d, *J* = 8.8 *Hz*, 2H), 3.65 (s, 3H), 3.21 (t, *J* = 8.0 *Hz*, 4H), 2.83 (t, *J* = 8.0 *Hz*, 2H), 2.56 (t, *J* = 8.0 *Hz*, 2H), 1.66-1.54 (m, 4H), 0.92 (t, *J* = 8.0 *Hz*, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 146.8, 129.3, 128.4, 112.2, 62.8, 53.3, 34.8, 31.2, 20.7, 11.7;

HRMS (ESI) calculated for $[(C_{16}H_{25}O_2N)^+]$ 263.1885, found 263.1883

Synthesis of 4-(3-azidopropyl)-*N*,*N*-dipropylaniline (intermediate *12*)



Compound **11** was dissolved in THF:MeOH (10:1) and $LiBH_4$ (2 eq) was added at 0 °C. The reaction mixture was then brought to ambient temperature and stirred for 12 h. The mixture was poured into 1N HCl and extracted with ethyl acetate (3 times). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to yield the primary alcohol product, which was used for the next reaction without further purification.

3-(4-(Dipropylamino)phenyl)propan-1-ol was dissolved in THF and diisopropyl-ethylamine was added. The mixture was cooled to 0 °C and a solution of MsCl in THF was added dropwise. The mixture was stirred for 1-4 h (TLC) and then poured into $NaHCO_3$ (sat.). The solution was extracted with ethyl acetate (3 times). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to yield the 3-(4-(dipropylamino)phenyl)propyl methanesulfonate which was used for the next reaction without further purification.

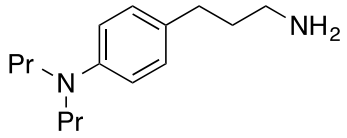
The 3-(4-(dipropylamino)phenyl)propyl methanesulfonate was dissolved again in MeOH and NaN_3 was added. The mixture was stirred and refluxed for 4 h (TLC) and the solvent was removed under reduced pressure and the crude was redissolved in CH_2Cl_2. The solid was filtered and washed with CH_2Cl_2. The solvent was removed under reduced pressure and the product **12** was purified by column chromatography using EtOAc:hexane (9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, *J* = 16.0 *Hz*, 2H), 6.58 (d, *J* = 16.0 *Hz*, 2H), 3.28 (t, *J* = 9.2 *Hz*, 2H), 3.21 (t, *J* = 8.0 *Hz*, 4H), 2.59 (t, *J* = 10.0 *Hz*, 2H), 1.92-1.82 (m, 2H), 1.66-1.53 (m, 4H), 0.92 (t, *J* = 8.0 *Hz*, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 146.9, 129.9, 127.4, 112.1, 53.2, 51.0, 31.8, 30.9, 20.7, 11.7;

HRMS (ESI) calculated for $[(C_{15}H_{24}N_4)^+]$ 260.2001, found 260.1996

Synthesis of 4-(3-aminopropyl)-*N*,*N*-dipropylaniline (intermediate *13*)



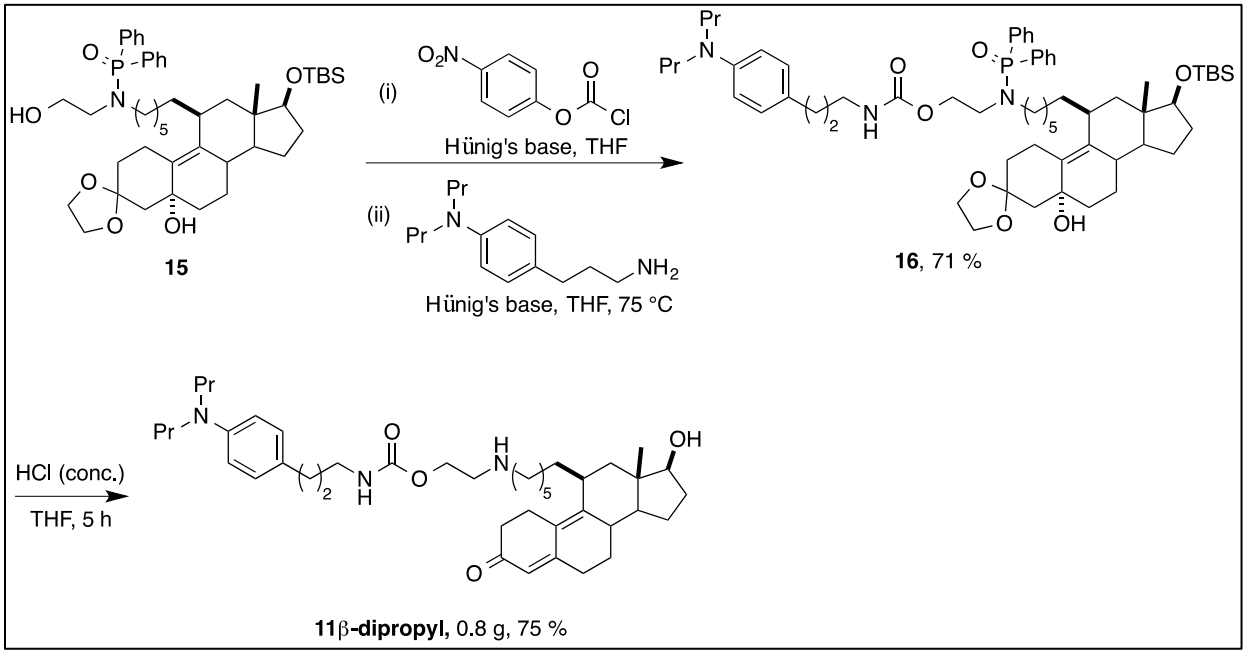
Compound **12** was dissolved in THF:H_2O (10:1). Triphenyl-phosphine was added and the mixture was stirred at room temperature for overnight. Water was added followed by ethyl acetate. The organic layer was separated and extracted with 1 N HCl (3 times). The combined aqueous layers were adjusted the pH to 11 (using NaOH) then extracted with ethyl acetate (3 times). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to yield product which was used without further purification.

^{1}H NMR (400 MHz, CDCl3) δ 7.02 (d, J = 11.7 Hz, 1H), 6.58 (d, J = 11.7 Hz, 1H), 3.20 (t, J = 10.4 Hz, 4H), 2.74 (t, J = 9.4 Hz, 2H), 2.67 (br, 1H), 2.57-2.46 (m, 2H), 1.76 (p, J = 9.4 Hz, 2H), 1.58 (p, J = 9.4 Hz, 4H), 0.92 (t, J = 9.6 Hz, 6H);

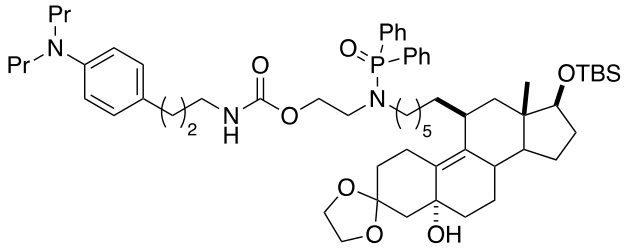
^{13}C NMR (100 MHz, CDCl3) δ 146.7, 129.2, 128.8, 112.1, 53.2, 42.1, 36.0, 32.3, 20.6, 11.7;

HRMS (ESI) calculated for [($C_{15}H_{26}N_2$)$^+$] 234.2096, found 234.2095

Scheme 6: Synthesis of 11beta-dipropyl



Synthesis of 2-((6-((5*R*,11*S*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-13-methyl-1,2,4,5,6,7,8,11,12,13,14,15,16,17-tetradecahydrospiro[cyclopenta[*a*] phenanthrene-3,2'-[1,3] dioxolan]-11-yl)hexyl)(diphenylphosphoryl)amino)ethyl (3-(4-(dipropylamino)phenyl)- propyl) carbamate (intermediate *16*)



Steroid **15** and diisopropylethylamine were dissolved in THF. *p*-Nitrophenyl chloroformate was added dropwise and the mixture was stirred at room temperature for 3-5 h. Na_2CO_3 (sat.) was added to the mixture and the aqueous layer was extracted with ethyl acetate (3 times). The combined organic layers were washed with Na_2CO_3 (sat.) (3 times), water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude was used without further purification.

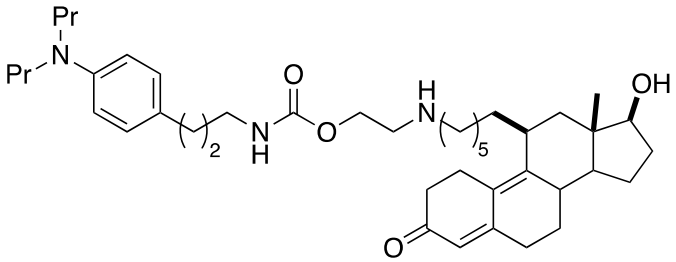
The product from last step and diisopropylethylamine were dissolved in THF. Amine **13** dissolved in THF was added dropwise and the mixture was stirred at 75 °C for 5 h. Na_2CO_3 (sat.) was added to the mixture and the aqueous layer was extracted with CH_2Cl_2 (3 times). The combined organic layers were washed with Na_2CO_3 (sat.) (3 times), water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude was purified by column chromatography using CH_2Cl_2 followed by CH_2Cl_2:MeOH (95:5) as eluent.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85-7.78 (m, 3H), 7.69-7.62 (m, 1H), 7.56-7.39 (m, 6H), 6.98 (d, *J* = 11.7 *Hz*, 2H), 6.56 (d, *J* = 11.7 *Hz*, 2H), 4.85 (s, 1H), 4.23 (s, 1H), 4.03-3.92 (m, 4H), 3.47 (t, *J* = 12.0 *Hz*, 2H), 3.27 (t, *J* = 8 *Hz*, 2H), 3.21-3.11 (m, 8H), 3.00-2.92 (m, 2H), 2.76 (q, *J* = 8.0 Hz, 2H), 2.63-2.55 (m, 2H), 2.50 (t, *J* = 12 *Hz*, 4H), 2.35-2.25 (m, 2H), 2.10-2.06 (m, 1H), 1.93 (s, 2H), 1.91 (s, 1H), 1.88-1.72 (m, 6H), 1.62-1.51 (m, 9H), 1.45-1.39 (m, 2H),1.34 (dd, *J* = 13.9, 7.3 *Hz*, 3H), 1.04-0.99 (m, 5H), 0.92-0.82 (m, 15H), 0.01 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 139.7, 133.2, 132.7, 132.6, 132.5, 132 (2 peaks), 132.2, 132.1,132.0 (2 peaks), 131.5, 130.5, 129.3, 128.8 (2 peaks), 128.6 (2 peaks), 112.1, 109.2, 83.0 (2 peaks), 70.3, 64.9 (2 peaks), 64.3, 63.2, 53.2 (2 peaks), 51.4, 47.4, 43.0, 41.1, 38.0, 37.7, 36.7, 36.1, 35.9 (2 peaks), 35.8, 32.1, 31.1, 29.6, 28.8, 27.1, 26.1, 24.0 (3 peaks), 23.7, 23.5, 23.2 (2 peaks), 21.3 (2 peaks), 20.7, 18.3, 13.9, 11.7, -4.2, -4.5

HRMS (ESI) calculated for [($C_{62}H_{94}N_3O_7PSi)^+$] 1051.6599, found 1051.6602

Synthesis of 2-((diphenylphosphoryl)(6-((11*S*,13*S*,17*S*)-17-hydroxy-13-methyl-3-oxo-2,3,6,7,8,11,12,13,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-11-yl)hexyl)amino)ethyl (3-(4-(dipropylamino)phenyl)propyl)carbamate (11beta-dipropyl)



The steroid **16** was dissolved in THF and conc. HCl (37%) was added dropwise to the solution. The mixture was stirred at room temperature for 6 h and then $NaHCO_3$ (solid) was added

until pH 7. The mixture was filtered through celite and washed with CH_2Cl_2. The solvent was removed under reduced pressure and the crude was purified by column chromatography using CH_2Cl_2 followed by CH_2Cl_2:MeOH (95:5) as eluent.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 11.6 *Hz*, 2H), 6.55 (d, *J* = 11.6 *Hz*, 2H), 5.66 (s, 1H), 4.91 (br, 2H), 4.18-4.13 (m, 3H), 3.72-3.70 (m, 1H), 3.70-3.52 (m, 2H), 3.38 (t, *J* = 8.0 *Hz*, 1H), 3.23-3.13 (m, 5H), 3.00-2.90 (m, 2H), 2.88-2.78 (m, 3H), 2.62 (t, *J* = 11.6 *Hz*, 2H), 2.55-2.46 (m, 2H), 2.46-2.29 (m, 5H), 2.26 (s, 1H), 2.19 – 1.97 (m, 3H), 1.96-1.83 (m, 2H), 1.79-1.69 (m, 2H), 1.63 – 1.51 (m, 6H), 1.42 (s, 2H), 1.35-1.25 (m, 5H), 1.19-1.07 (m, 2H), 1.01 (t, *J* = 12.0 *Hz*, 1H), 0.97-0.87(m, 8H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0, 171.3, 158.2, 151.3, 146.8, 129.3, 128.0, 126.2, 125.7, 122.3, 112.2, 112.1, 82.9, 64.1, 53.2, 51.6, 49.8, 48.9, 48.8, 43.0, 40.9, 39.9, 37.6, 37.5, 36.8, 36.6, 36.5, 34.3, 32.1 (2 peaks), 32.0, 30.9, 30.6, 30.0, 29.6, 28.6, 27.4, 27.2, 26.5, 23.6, 13.7, 11.7.

HRMS (ESI) calculated for [$(C_{42}H_{65}N_3O_4)^+$] 675.4795, found 675.4791

Methods for supplemental figures

Immunofluorescence

The immunofluorescence studies were performed on the renal cryosections (5–7 µm) as per the standardized protocols. The antibodies used in the study are as follow: mouse anti-malondialdehyde (MDA) (1:200, Invitrogen, catalog no. MA5-27560) and rabbit anti-gamma-H2AX (1:200, Bethyl Laboratories, catalog no. A700-053). Additionally, the lectins used as tubular markers were rhodamine (RL-1032)-conjugated *Dolichos biflorus* agglutinin (DBA) (1:50, Vectors Laboratories) and FITC-*Lotus tetragonolobus* agglutinin (LTA) (1:200, Vectors Laboratories, catalog no. FL-1321). For detection of primary signals, Alexa-647-conjugated secondary antibodies (Molecular Probes) were used. Hoechst 33342 (DAPI, Molecular Probes, catalog no. H-3570) was used for nuclear staining.

Gamma-H2AX quantification

To quantify the extent of DNA Damage, the immunofluorescence images were evaluated for the nuclei showing positive signal of gamma-H2AX protein (white). The values of the DNA Damage were expressed as positive nuclei/100 nuclei counted in a random field; >400 nuclei/genotype counted.

4-HNE quantification

For the quantification of extent of 4-HNE (byproduct of lipid peroxidation), immunofluorescence images were processed in ImageJ software (NIH, version-1.47) using the IHC profiler application. Briefly, images were split into respective channels, converted to binary image and areas exhibiting protein expression (green) were expressed as percent area against the background. Images from at least 3 independent mice/genotype were used.

Immunoblotting

Immunoblotting was performed in the renal tissues to deduce the extent of ferroptosis and inflammation using antibodies as follows: rabbit anti- FTH1 (1:1000, Cell Signal Technology, catalog no. 4393), rabbit anti- Slc7a11 (1:1000, Cell Signal Technology, catalog no. 12691), rabbit anti-Nrf2 (1:1000, Cell Signal Technology, catalog no. 12721), rabbit-keap1 (1:1000, Cell Signal Technology, catalog no. 8047), rabbit anti-Slc3a2 (1:1000, Cell Signal Technology, catalog no.

47213), and rabbit anti-IL1β (1:1000, Cell Signal Technology, catalog no. 31202). Goat anti-rabbit secondary antibody was used against the primary antibodies at a dilution of 1:10000.

4NBP assay

The model nucleophile 4-(*p*-nitrobenzyl)-pyridine (4NBP) was used to estimate reactivity of 11beta compounds towards DNA, as previously described. A large excess of the 4NBP nucleophile was used to allow for reactions with a pseudo-first order kinetics in the test compounds. Chlorambucil, a simpler aniline mustard, was used as a positive control. Briefly, a 200 mM 4NBP solution in acetone was mixed 1:1 with a buffered (0.1 M sodium acetate, pH 4.6) aqueous solution of 100 µM test compound. Test compounds stocks (2.5 mM) were prepared in DMSO. Final reaction volume was 200 µl. Reactions were incubated on a water bath at 66 C for various amounts of time, and then quenched by immediately placing on ice for 1 min. The reactions were subsequently diluted with 200 µl acetone, 500 µl ethyl acetate and 200 µl sodium hydroxide (0.25 M). After vortexing and centrifugation, the purple-blue 4NBP alkylation product separates in the organic phase and can be quantified spectrophotometrically using absorbance at 550 nm. DMSO only reactions were used as blanks for each time point. Data were plotted as Absorbance vs time, and pseudo-first order rate constants were calculated.

Renal epithelial Pkd1 knockout cell line generation from renal primary cells

Primary cells from kidney were isolated using enzymatic digestion procedures maintaining sterile conditions. Genotype of the mice was $Pkd1^{flox/flox}$;$Dtr^{flox/flox}$;$Pax8^{rtTA}$;TetO-Cre. Briefly, minced tissue pieces were transferred to GentleMACS C-tube (Miltenyi Biotech, Cat. no. 130093237) containing 5 ml of freshly prepared dissociation buffer (DMEM containing D, P, A, Y enzymes). The C-tube was inserted into GentleMACS Octo-Dissociator (Miltenyi Biotech, Cat. no. 130096427) and run with pre-set program '37 multi E 01' (run time 30 min). After cell dissociation, 5 ml of DMEM with 10% FBS was added to stop digestion, and the mixture was passed through 70 µm strainer followed by centrifugation at 400 x*g* for 10 minutes. Cells were resuspended in REGM medium (Lonza, REGM Renal Epithelial Cell Growth Medium BulletKit, Cat. no. cc-3190) and let attach for 48 hours and then treated with doxycycline (Sigma, Cat. no. D9891-100G) 1 µg/ml for 72hrs, followed by serum starvation in 0.1% FBS containing media for 24 hours prior to preparation of protein or RNA. For quality control purposes, cells were seeded in parallel, treated similarly to experimental conditions and tested to assess Cre dependent deletion by genotyping after doxycycline treatment. Cell line generation from primary cells was performed using transduction with lentivirus of SV40 large T antigen (Gentarget, Cat. no. LVP016-Hygro) as per manufacturer's instructions. Hygromycin antibiotic was used for selection of the transduced and transformed cells. Cell lines were validated by genotyping and by protein analysis for target alleles.

Primers

Gene	Forward Primer	Reverse Primer
$Pax8^{rtTA}$	5'-CCATGTCTAGACTGGACAAGA	5'-CTCCAGGCCACATATGATTAG
$TetO^{Cre}$	5'-GCAGAGCTCGTTTAGTGAAC	5'-TCGACCAGTTTAGTTACCC
$Pkd1^{fl}$	5'-CACAACCACTTCCTGCTTGGTG	5'-CCAGCATTCTCGACCCACAAG
Dtr^{fl}	5'-ACCATGAAGCTGCTGCCGTC	5'-TCAGTGGGAATTAGTCATGC

Cell culture, cell toxicity assay and flow cytometry

The immortalized renal epithelial cell lines (WT and *Pkd1*$^{-/-}$) were maintained and passaged in high glucose DMEM media containing GlutaMAX (Gibco), and supplemented with 1 mM pyruvate (Gibco), 100 IU penicillin (Gibco), 100 µg/ml streptomycin (Gibco) and 10% FBS (VWR International), in a humidified incubator at 37 °C, in an atmosphere containing 5% CO_2. Dose-response viability assays were carried out using the MultiTox-Glo® assay (Promega) according to the manufacturer instructions. Briefly, cells were seeded in black-clear bottom 96-well plates (Corning) at a density of 5x10^3 cells per well, and incubated for 24 h. Media was then removed and replaced with media containing 11beta-dichloro at concentrations ranging from 0 to 6 µM. All solutions, including the vehicle-only solution (0 µM) contained 0.1% DMSO. Cells were incubated in drug solutions for 24 h. The MultiTox-Glo® assay was carried out as follows: after drug incubation, 50 µl of the live-cell reagent (GF-AFC Reagent) was added to each sample, control and background wells. The plate was incubated for 30 min at 37 °C and then fluorescence (ex/em 400/505 nm) was measured in a SpectraMax® M2e (Molecular Devices) plate reader. Subsequently, 50 µl of dead-cell reagent (AAF-Glo™ Reagent) was added in each well, and the plate was incubated at room temperature for 15 min. Luminescence was then recorded in each well using the SpectraMax M2e plate reader. Fractional viability was calculated as the ratio between the live-cell signal and dead-cell signal, and then normalized to the untreated control. For flow cytometry experiments, the cells were seeded at 150x10^3 cells per well in 6-well plates and grown for 24 h. The media was then removed and replaced with media containing 11beta-dichloro at concentrations ranging from 0 to 10 µM. All treatment solutions contained 0.1% DMSO. After a 2 h incubation, the cells were washed twice with HBSS 1X (Gibco) and then stained with HBSS containing 1.5 µM MitoSOX™ (Invitrogen) for 1 h. Subsequently, the cells were collected by trypsinization and analyzed on a Beckman-Coulter CytoFLEX flow cytometer. A minimum of 10,000 gated cells were used to estimate the median MitoSOX fluorescence (FL2 channel) for each sample using CytExpert 2.4 software (Beckman-Coulter).

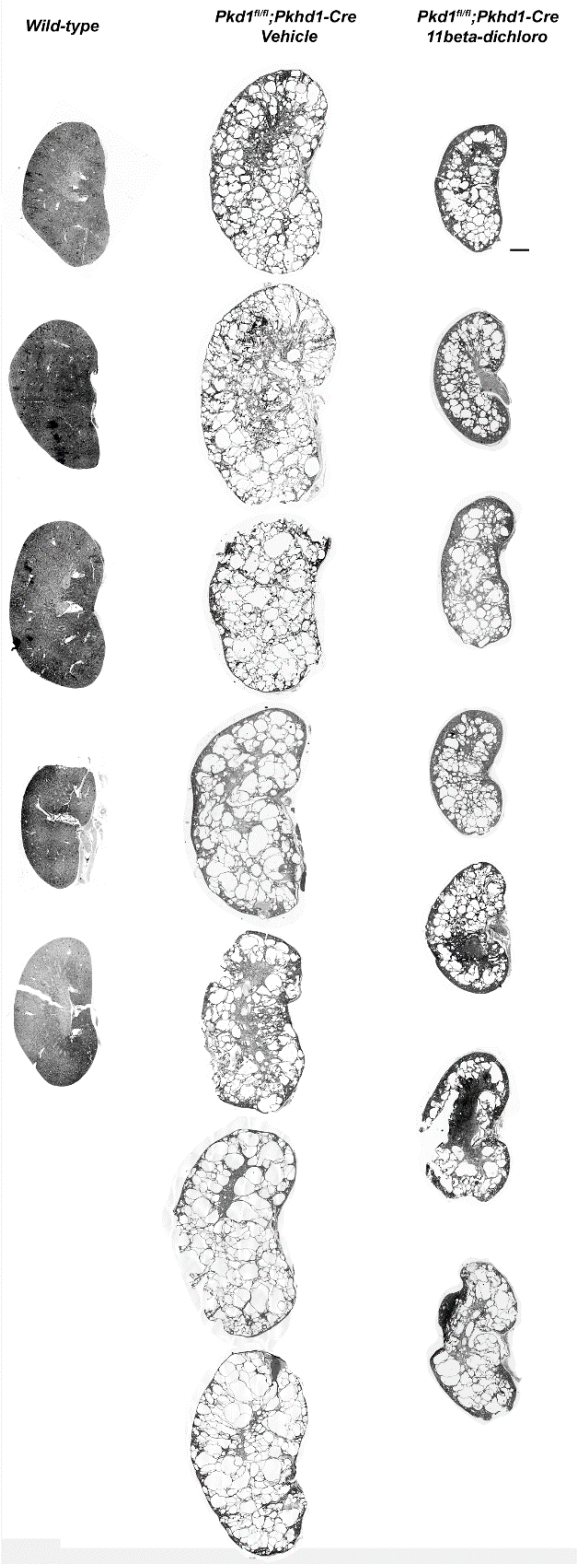


Fig. S1. Kidney panel for data shown in Fig. 1b-f. Size marker denotes 1 mm. One kidney per animal is shown; only one kidney was used to determine the cystic index for each animal.

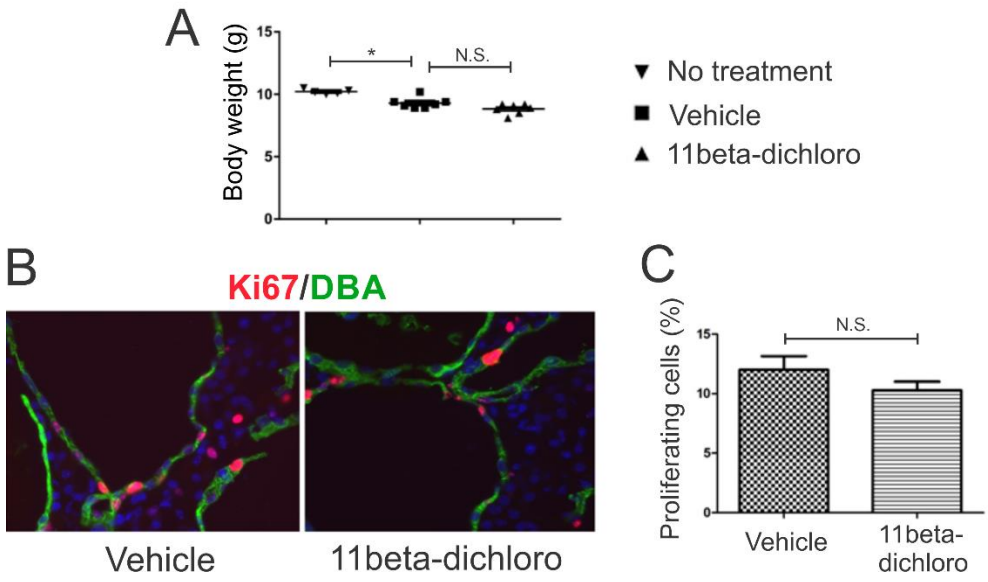


Fig. S2. Body weight and proliferation for the neonate ADPKD (Pkhd1-Cre) model. (*A*) Body weight variation of Pkhd1-Cre mice during treatment with 11beta-dichloro or vehicle. Left column shows untreated non-cystic mice; middle column denotes vehicle treated; right column denotes 11beta-dichloro treated animals. * denotes P<0.05. N.S denotes P>0.05 (not significant) using two-way ANOVA; n=7. (*B*) Ki67 staining on *Pkd1*$^{-/-}$ collecting ducts cyst cells treated with vehicle or 11beta-dichloro. (*C*) Quantification of the data from b, as percentage of Ki67 positive cells. Three kidneys from 3 different mice per experimental condition were analyzed and >1000 DBA-positive cells were counted for each sample. N.S. denotes P>0.05 not significant by *t* test; data are shown as mean +/- s.e.m.

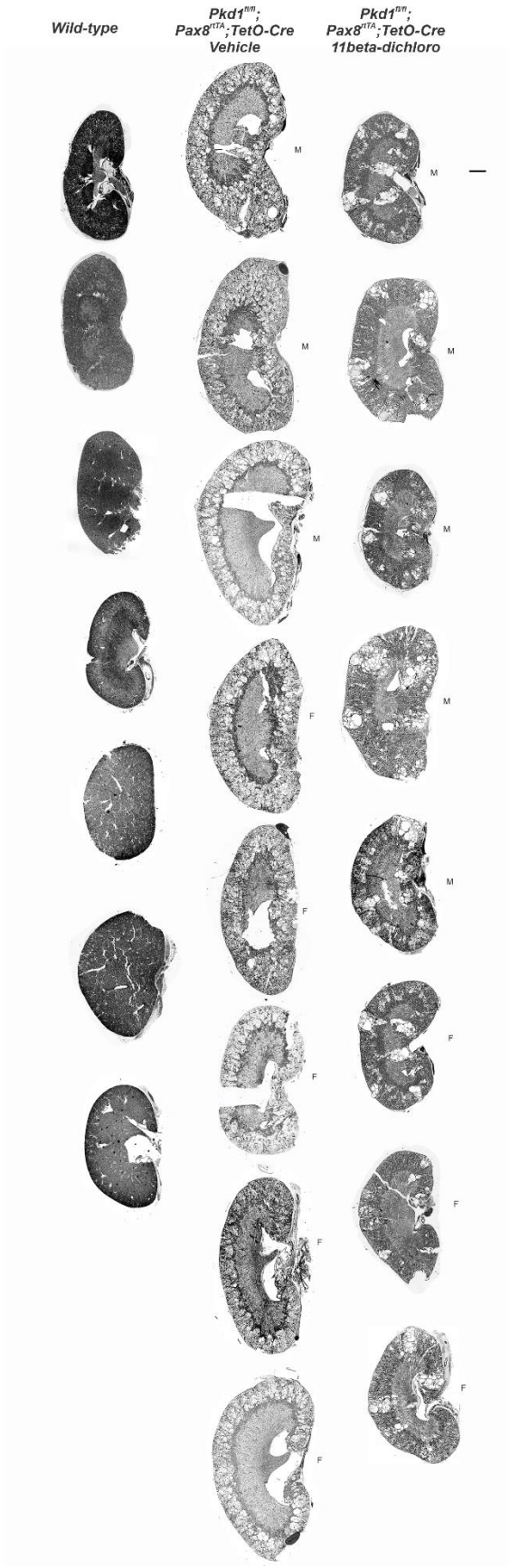


Fig. S3. Kidney panel for data shown in Fig. 2. Size marker denotes 1 mm. One kidney per animal is shown; only one kidney was used to determine the cystic index for each animal. For vehicle or 11beta-dichloro-treated animals, the sex is indicated next to each kidney, M (male) or F (female).

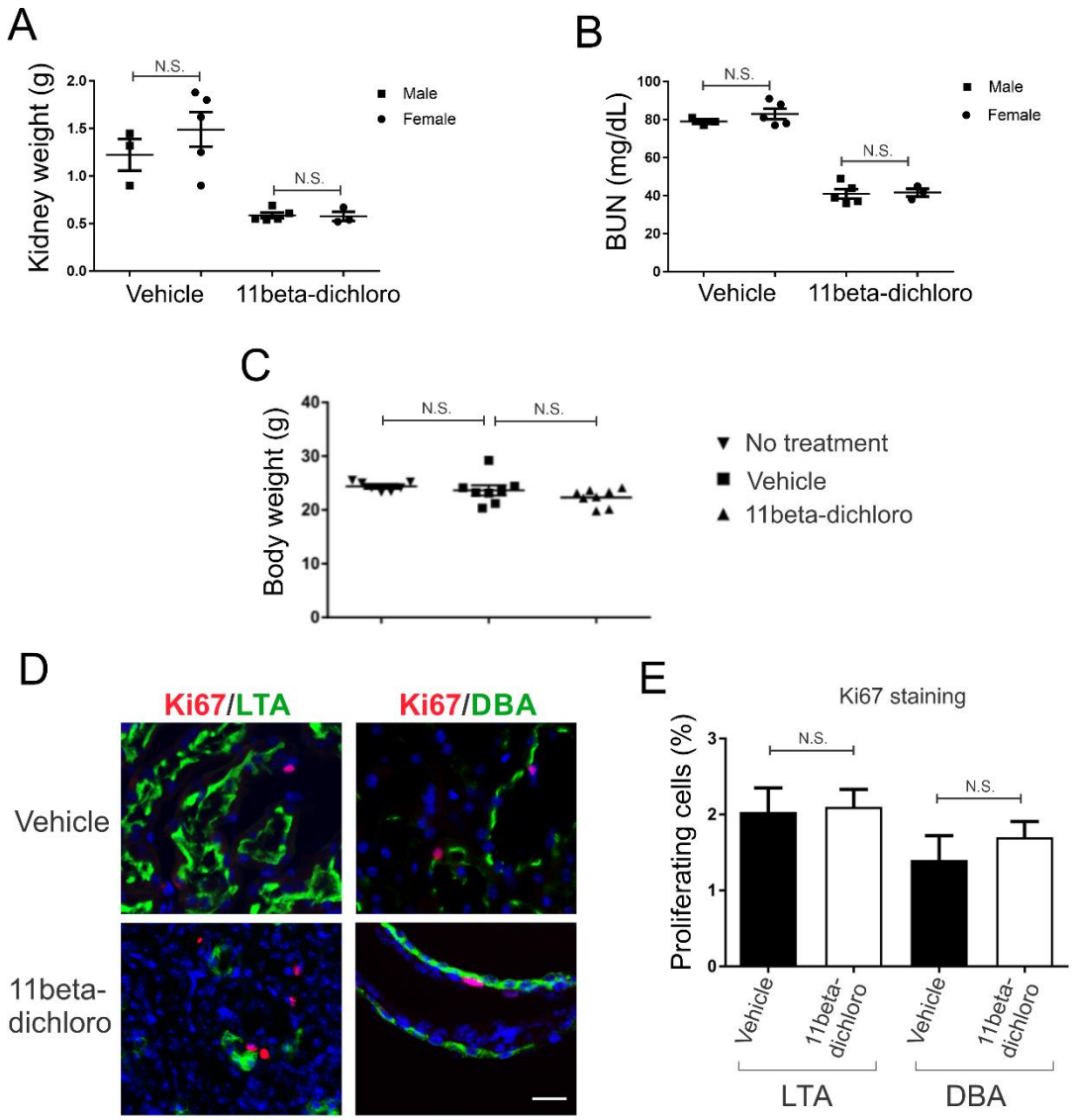


Fig. S4. Kidney weight, body weight and proliferation following 11beta-dichloro treatment in the adult Pax8 ADPKD model. (*A*) Total kidney weight (two kidneys) in vehicle-treated and 11beta-dichloro-treated animals, grouped by sex**.** No sex-dependent variation was observed. N.S. denotes p>0.05, not significant by ANOVA. Error bars denote s.e.m. (*B*) BUN, a measure of kidney function, in vehicle-treated and 11beta-dichloro-treated animals, grouped by sex. No sex-dependent difference was observed. N.S. denotes p>0.05, not significant by ANOVA. Error bars denote s.e.m. (*C*) Body weight variation of the Pax8 mice. Left column shows untreated mice; middle column denotes vehicle treated; right column denotes 11beta-treated animals. Two-way ANOVA shows P>0.05 (not significant) for any two groups; n=8. (*D*) Ki67 staining on the $Pkd1^{-/-}$ tissues in collecting ducts (DBA positive) or proximal tubules (LTA positive). Scale bar is 20 µm. (*E*) Quantification of the data from d, as percentage of Ki67 positive cells. Three kidneys from 3 different mice per experimental condition were analyzed and >1000 DBA-positive or LTA-positive cells were counted for each sample. N.S. denotes P>0.05 not significant by *t* test; data is shown as mean +/- s.e.m.

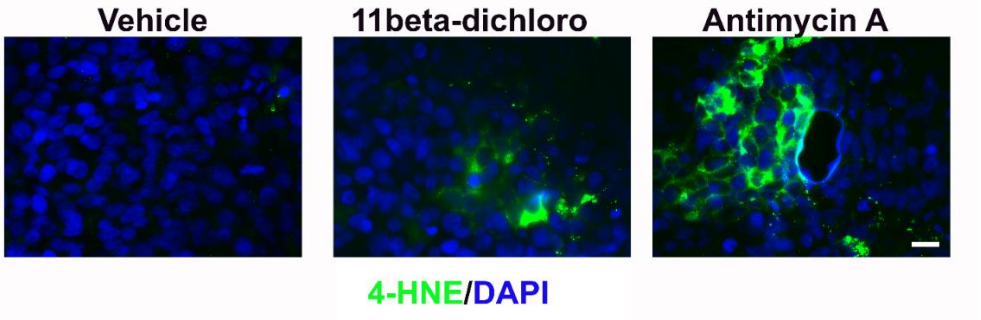


Fig. S5. Validation of 4-HNE antibody for immunofluorescence. IMCD3 cells (a commercially-available established murine kidney cell line) were treated with antimycin A (1 h, 100 µM) or 11beta-dichloro (4 h, 5 µM) and the status of ROS induction was examined via 4-HNE staining. As seen in the middle (11beta-dichloro) and right (antimycin A) panels, 4-HNE detects a positive signal compared with DMSO treated cells. Size bar, 10 µm.

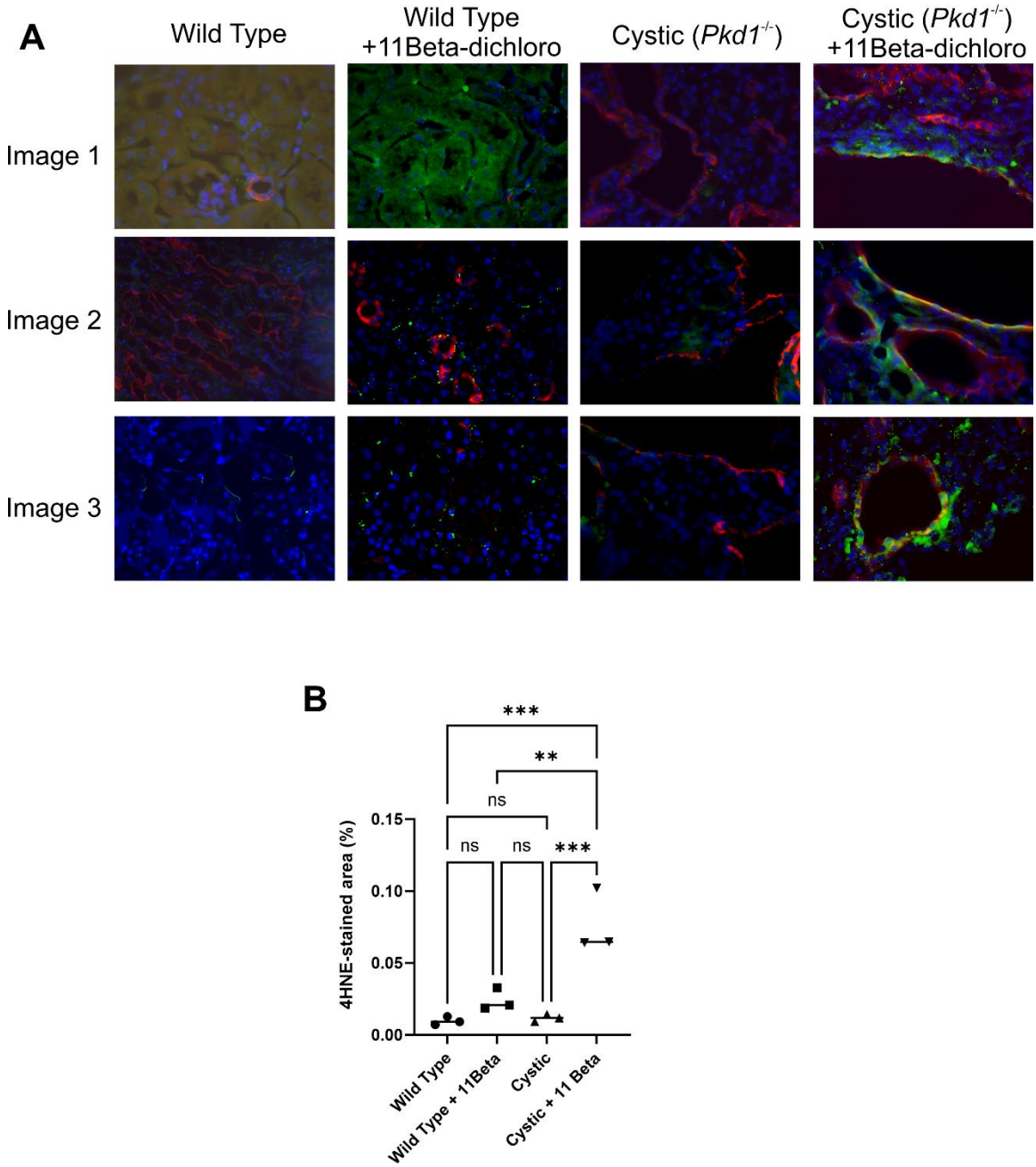


Fig. S6 Quantification of 4-HNE signal in the Pkhd1-Cre (neonate) model of ADPKD. (*A*)Three independent images are shown for each of the experimental conditions indicated (WT, WT+11beta-dichloro, Cystic and Cystic+11beta-dichloro). The 4-HNE signal is in bright green. Nuclei were stained with DAPI. DBA-positive (collecting duct cells) are stained in red. (*B*) Quantification of the 4-HNE signal from images in (A) as the area percentage of green stain normalized to the number of nuclei present in each image. Statistics: "ns" denotes "not significant" ($p>0.05$); **$p<0.01$, ***$p<0.001$ by t test.

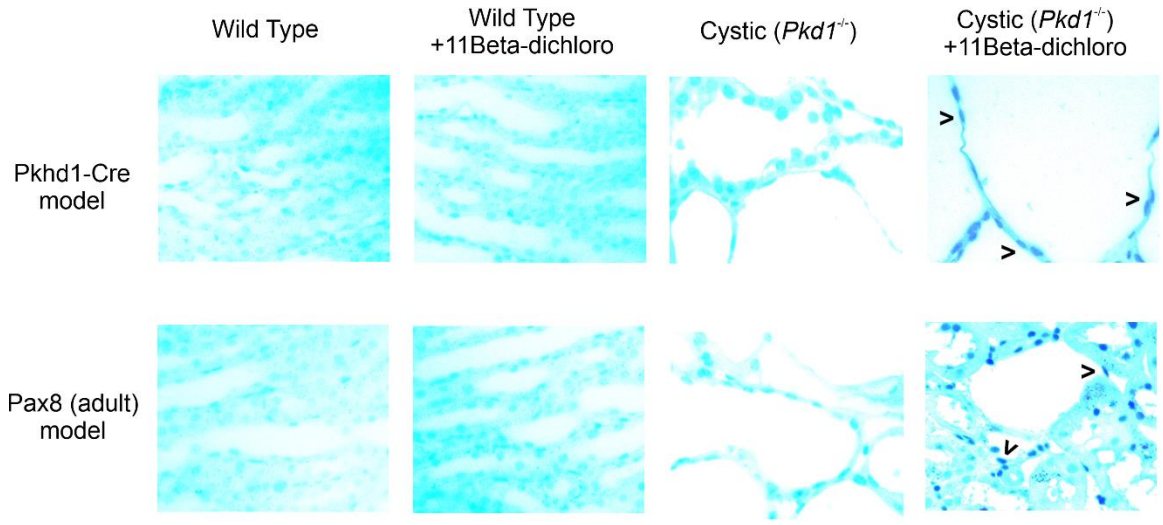


Fig. S7 Staining for oxidative biomarker 8-oxoguanine (8-oxoG) in kidney sections after treatment with 11beta-dichloro. 8-oxoG staining was performed in kidney sections from both the neonate (top row) or adult (bottom row) models. The images here are in pseudo-color, with the nuclei shown in cyan, whereas the 8-oxoG staining is in dark blue. As discussed in the main text, the 8-oxoG signal appears only in cystic (Pkd1$^{-/-}$) kidneys treated with 11beta-dichloro, in either model.

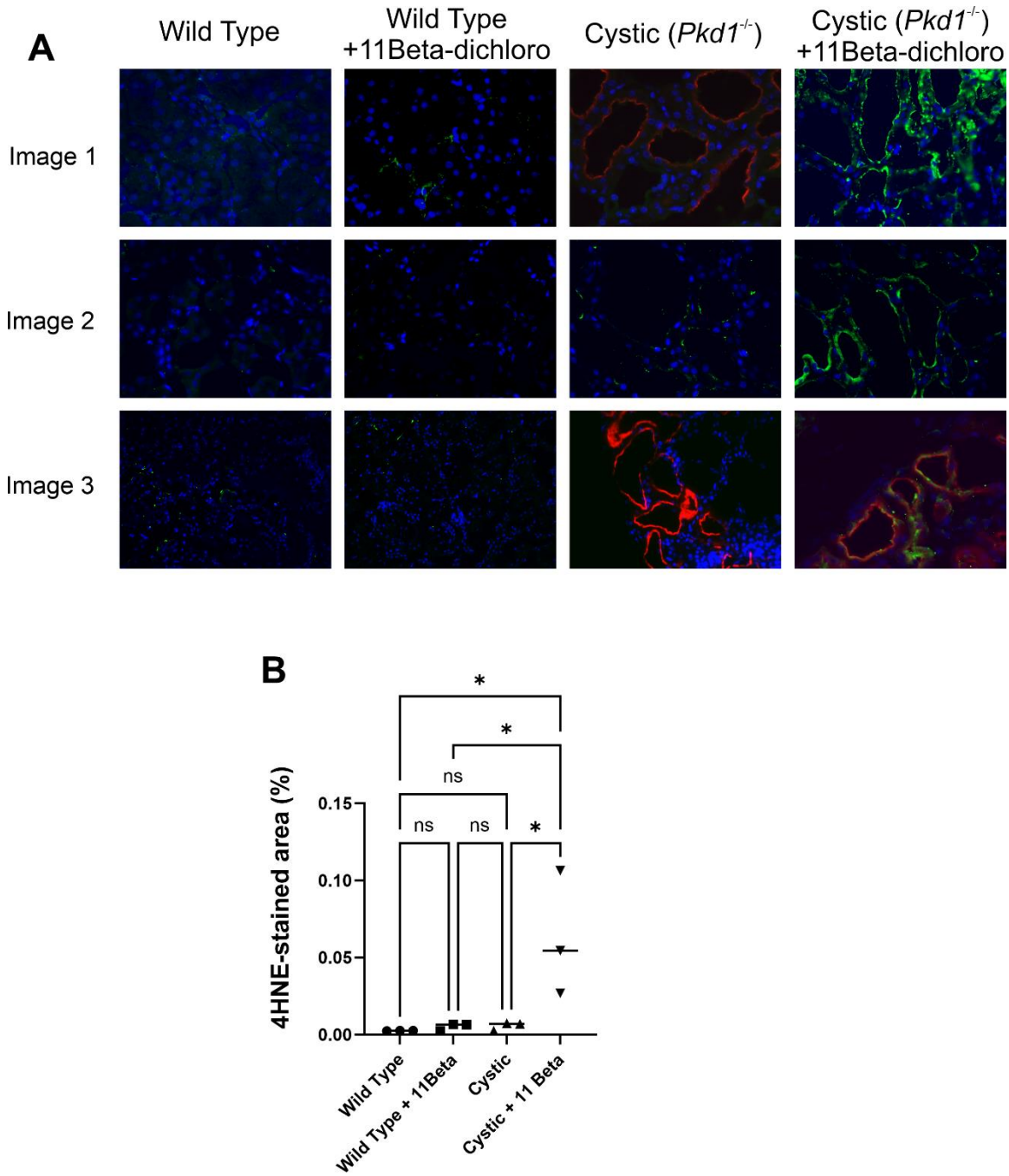


Fig. S8 Quantification of 4-HNE signal in the Pax8 (adult) model of ADPKD. (*A*) Three independent images are shown for each of the experimental conditions indicated (WT, WT+11beta-dichloro, Cystic and Cystic+11beta-dichloro). The 4-HNE signal is in bright green. Nuclei were stained with DAPI. DBA-positive (collecting duct) cells are stained in red. (*B*) Quantification of the 4-HNE signal from images in (A) as the area percentage of green stain normalized to the number of nuclei present in each image. Statistics: "ns" denotes "not significant" ($p>0.05$); *$p<0.05$ by t test.

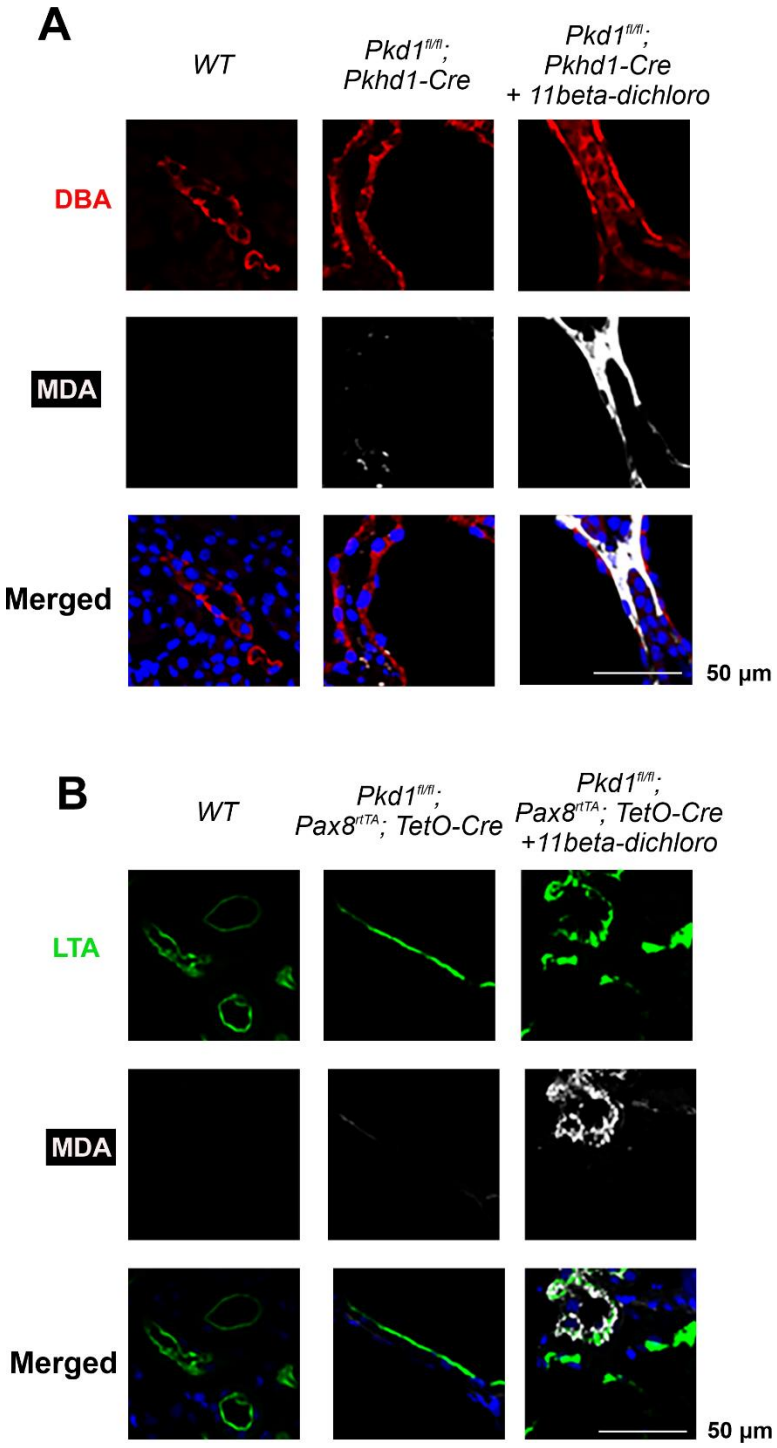


Fig. S9 Evaluation of MDA (malonyl-dialdehyde) production in kidney tissues after 11beta-dichloro treatment. MDA signal is in white, nuclei are stained blue (DAPI), DBA-positive cells (collecting ducts) are stained red, LTA-positive cells are shown in green. Top images (A) correspond to the Pkhd1-Cre (neonate) model; bottom images (B) show the Pax8 (adult) model.

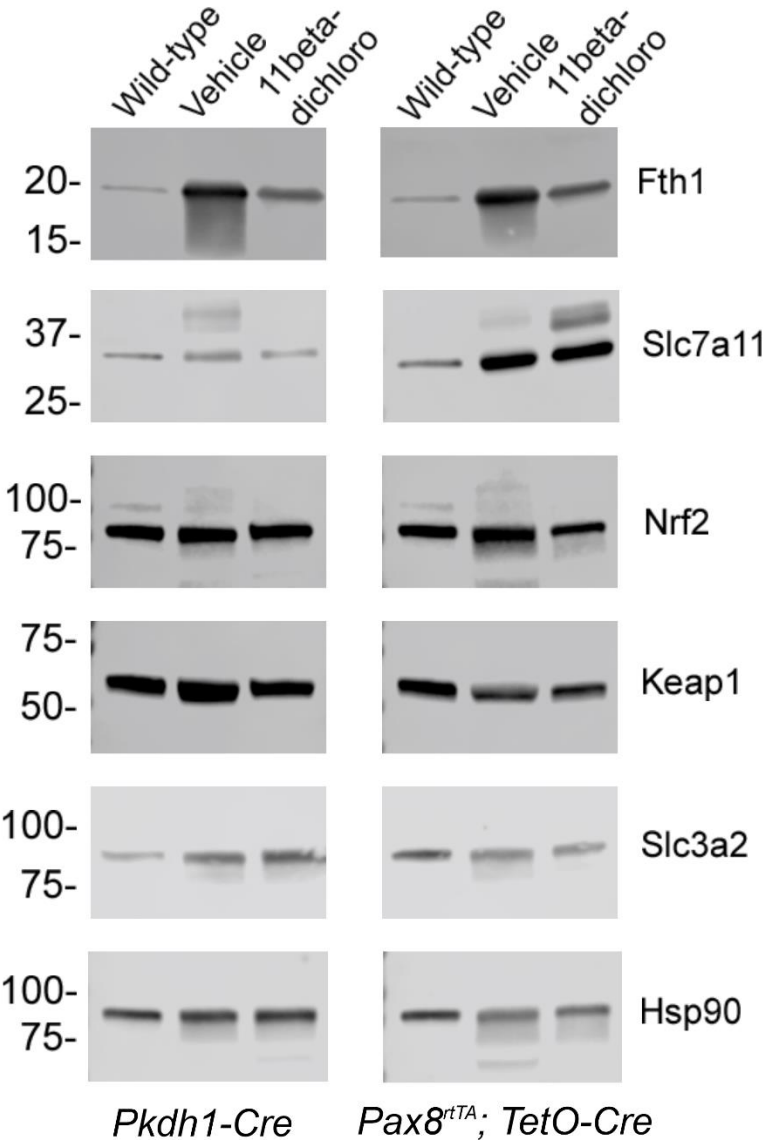


Fig. S10 Ferroptosis biomarkers analysis in whole kidney lysates. The protein levels of key biomarkers in ferroptosis (FTH1, SLC3A2, SLC7A11, NRF2, KEAP1) were assessed by western blot in kidney lysates from WT, vehicle-treated cystic (*Pkd1*$^{-/-}$) kidneys and cystic kidneys treated with 11beta-dichloro, in both the neonatal (Pkhd1-Cre) and adult (Pax8) models of ADPKD. Hsp90 was used as a loading control. Ferritin heavy chain 1 (FTH1) levels were increased in the *Pkd1*$^{-/-}$ kidney lysates (compared with wild-type) in both models; FTH1 levels were down following treatment with 11beta-dichloro. All other proteins (SLC3A2, SLC7A11, NRF2, KEAP1) were unchanged between cystic samples and cystic samples treated with 11beta-dichloro. Taken together, these results indicate that ferroptosis is not a major contributor to the cell death induced by 11beta-dichloro in cystic kidneys.

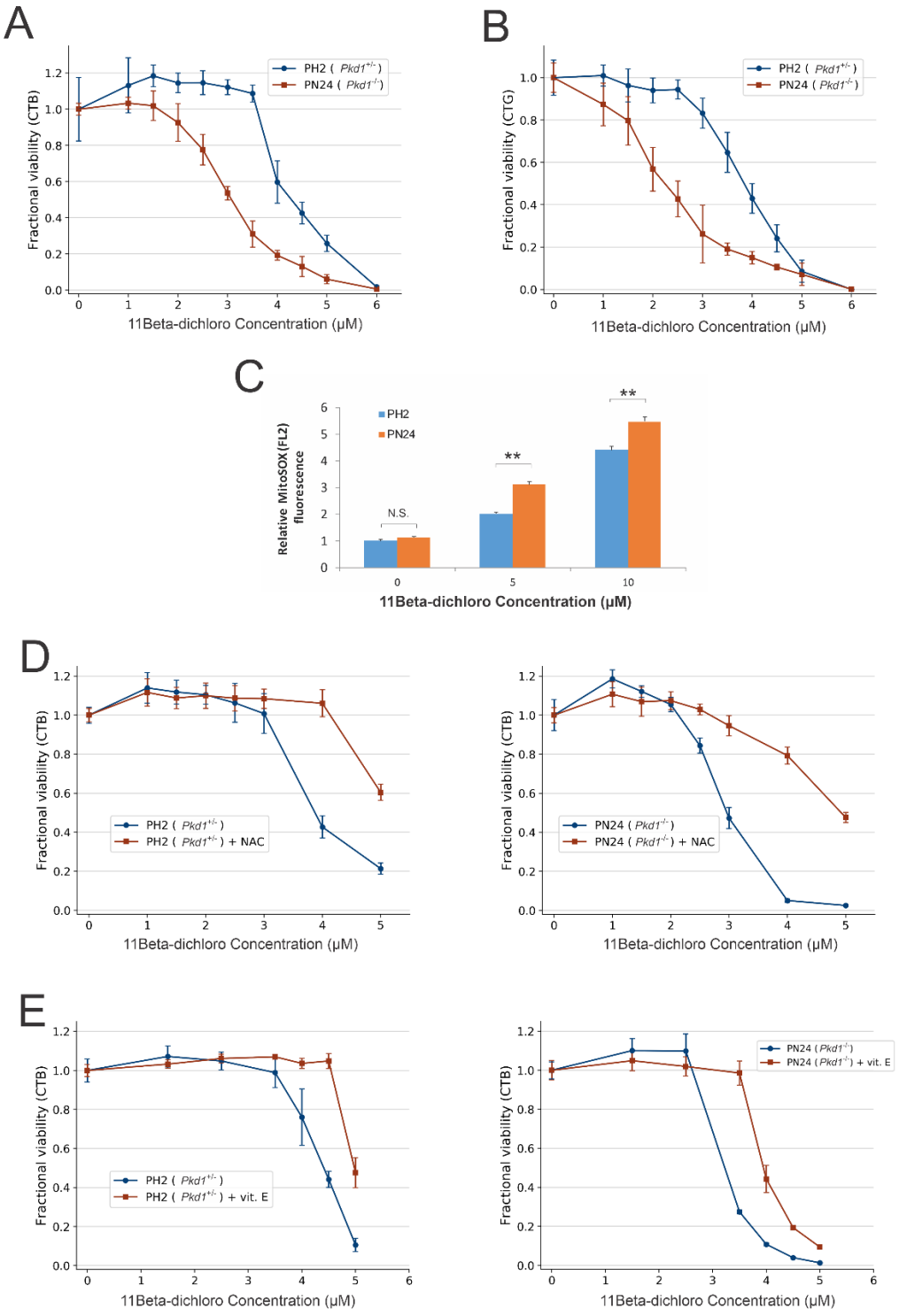


Fig. S11 Dose-response of PH2 (*Pkd1*$^{+/-}$) and PN24 (*Pkd1*$^{-/-}$) murine kidney cell lines to 11beta-dichloro treatment for 24 h. (*A*) Viability was evaluated with the CellTiter-Blue® assay (CTB, Promega), which relies on the detection of the metabolic reduction of a fluorescent dye. (*B*) Viability was evaluated with the CellTiter-Glo ® (CTG) assay, which measures total ATP content of the cells. (*C*) Induction of oxidative stress in PH2 and PN24 kidney cell lines by 11beta-dichloro after 2 h exposure. MitoSOX, an oxidation sensitive probe that detects primarily mitochondrial

superoxide, was used to stain the cells, and its fluorescence was quantified by flow cytometry in the FL2 channel. (*D*)(*E*) Antioxidants N-acetylcysteine (NAC, 10 mM) and vitamin E (vit. E, DL α-tocopherol acetate, 100 μM) alleviate 11beta-dichloro toxicity in PH2 and PN24 kidney cell lines. Viability of PH2 and PN24 cells was analyzed using the CTB assay following 24 h exposure to 11beta or 11beta+antioxidant co-treatment. Statistics: error bars denote standard deviations, with n=4; N.S., not significant; **p<0.01 by t test (panel C).

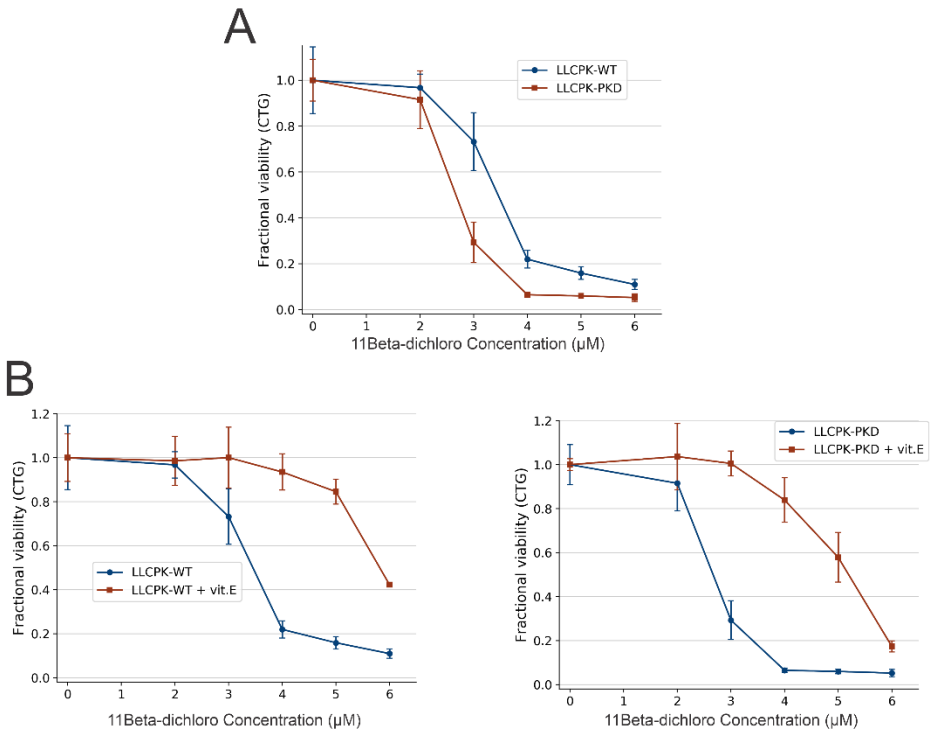


Fig. S12. Dose-response of LLCPK WT and *Pkd1*$^{-/-}$ porcine kidney cell lines to 11beta-dichloro treatment for 24 h. (*A*) Viability was assessed with CellTiter-Glo ® (CTG) assay (Promega), an assay that quantifies total cellular ATP amount with a luminescent probe. (*B*) Antioxidant vitamin E (vit.E, DL α-tocopherol acetate, 100 μM) alleviates 11beta-dichloro toxicity in LLCPK WT (left panel) and *Pkd1*$^{-/-}$ (right panel) kidney cell lines. Error bars denote standard deviations; n=4.

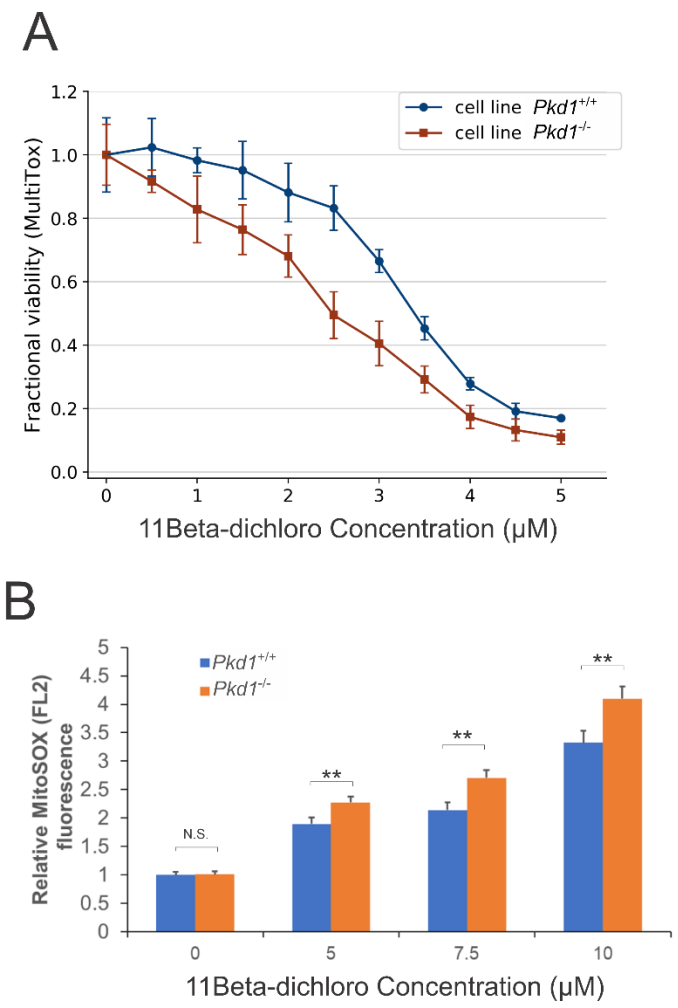


Fig. S13 Dose-response of an independent set of isogenic mouse kidney cell lines (WT and *Pkd1*$^{-/-}$) to 11beta-dichloro treatment for 24 h. (*A*) Viability was assessed with MultiTox-Glo® assay (Promega), an assay that uses fluorescent and luminescent probes to quantify both the number of live and number of dead cells in each well. This allows for a viability estimate that is independent of the cell number. (*B*) Induction of oxidative stress in the WT and *Pkd1*$^{-/-}$ kidney cell lines by 11beta-dichloro after 2 h exposure. MitoSOX, an oxidation sensitive probe that detects primarily mitochondrial superoxide, was used to stain the cells, and its fluorescence was quantified by flow cytometry in the FL2 channel. Statistics: error bars denote standard deviations, with n = 4 (panel A); n = 3 (panel B); N.S., not significant ($p>0.05$); **$p<0.01$ by t test (panel B).

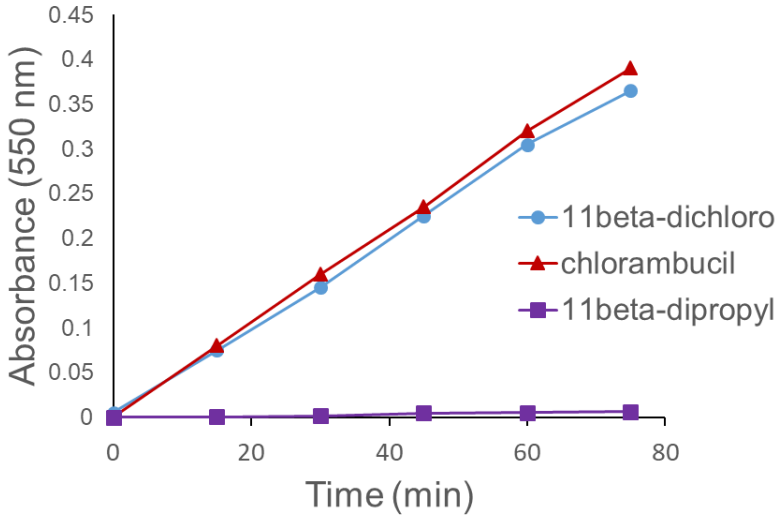


Fig. S14 Relative reactivity of 11beta compounds towards model nucleophile 4NBP. Excess 4NBP (4-(p-nitrobenzyl)-pyridine) was reacted at 66 °C with 11beta-dichloro, 11beta-dipropyl and chlorambucil (positive control), and reaction rates were estimated from the amount of alkylated product measured by absorbance at 550 nm. Each point is an average of 2 measurements. The data show that the aniline mustards 11beta-dichloro and chlorambucil have comparable reaction rates towards 4NBP, whereas, under the same conditions 11beta-dipropyl is unreactive towards 4NBP. This suggests that while aniline mustards can readily alkylate nucleophilic sites in the cell (such as DNA nucleobases), 11beta-dipropyl is unable to do so.

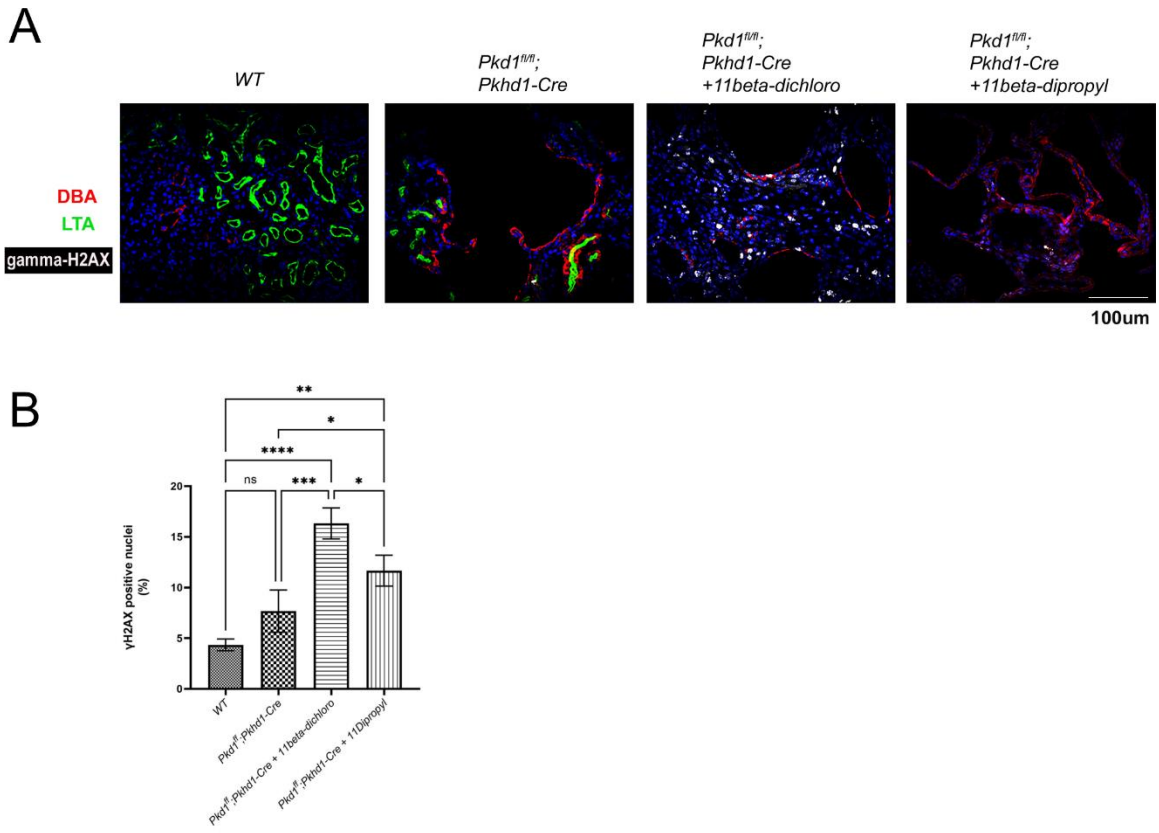


Fig. S15 Relative DNA damage ability of 11beta compounds as measured by the levels of phosphorylated histone gamma-H2A.X. (A) Kidney sections from the Pkhd1-Cre (neonate) mouse model of ADPKD were stained for nuclei (DAPI-blue), DBA (red), LTA (green) and gamma-H2AX (white). The panels on the right show cystic kidneys treated with 11beta-dichloro or 11beta-dipropyl as indicated. (B) Quantification of the gamma-H2AX signal in the images from (A) as a percentage of nuclei positive for the gH2AX stain. Statistics: “ns” denotes not significant, $p>0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$, by t test.

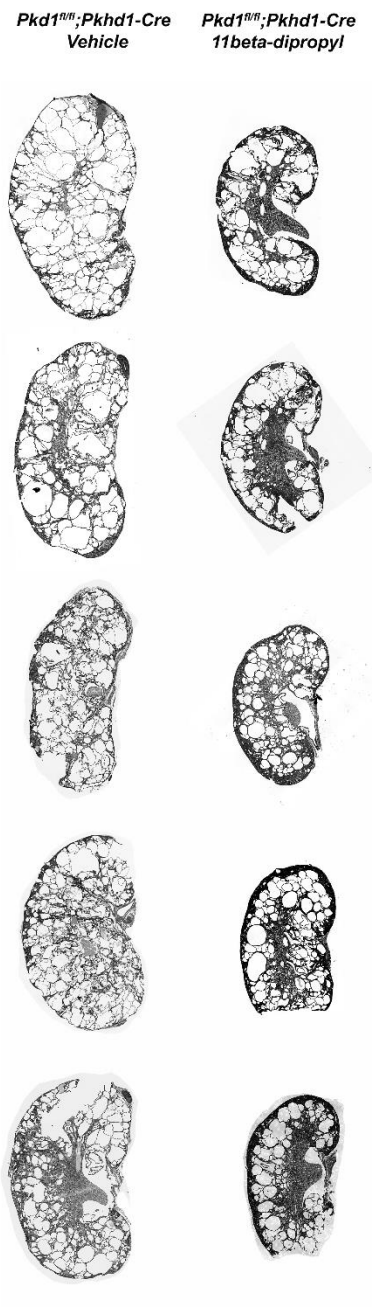


Fig. S16. Kidney panel for data shown in Fig. 5. Size marker denotes 1 mm. One kidney per animal is shown; only one kidney was used to determine the cystic index for each animal.

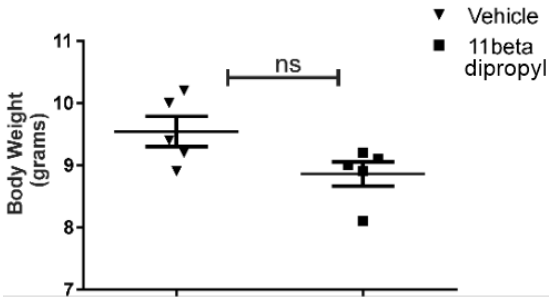


Fig. S17. Body weight for the neonate ADPKD (Pkhd1-Cre) model treated with 11beta-dipropyl. Left column shows vehicle treated mice; right column denotes 11beta-dipropyl treated animals. N.S denotes P>0.05 (not significant) using ANOVA; n=5. Data are shown as mean +/- s.e.m.