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## Supplementary information

# Multiplexed single-cell analysis of organoid signaling networks

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# - Supplementary Information -

# Multiplexed Single-Cell Analysis of Organoid Signalling Networks

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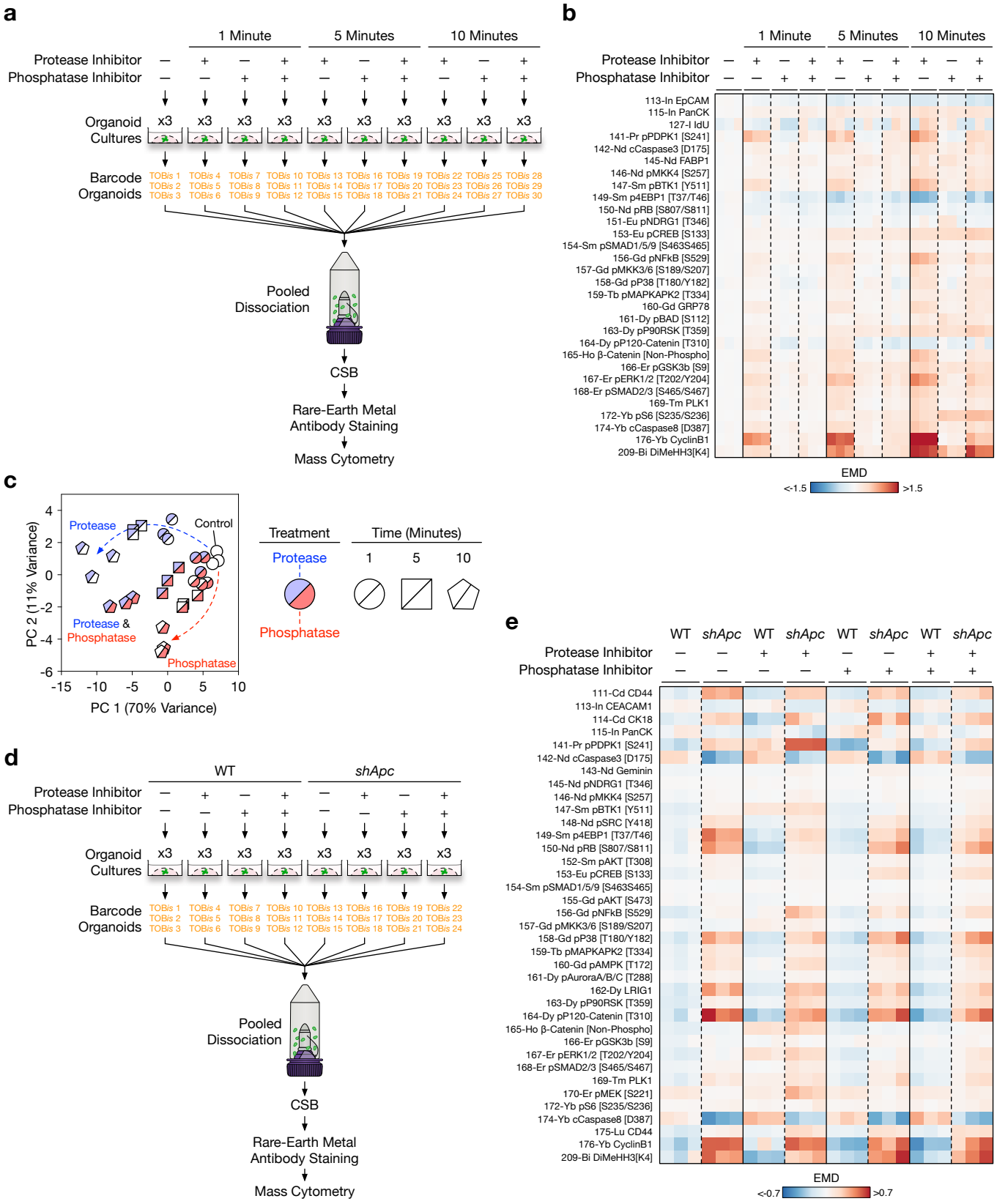
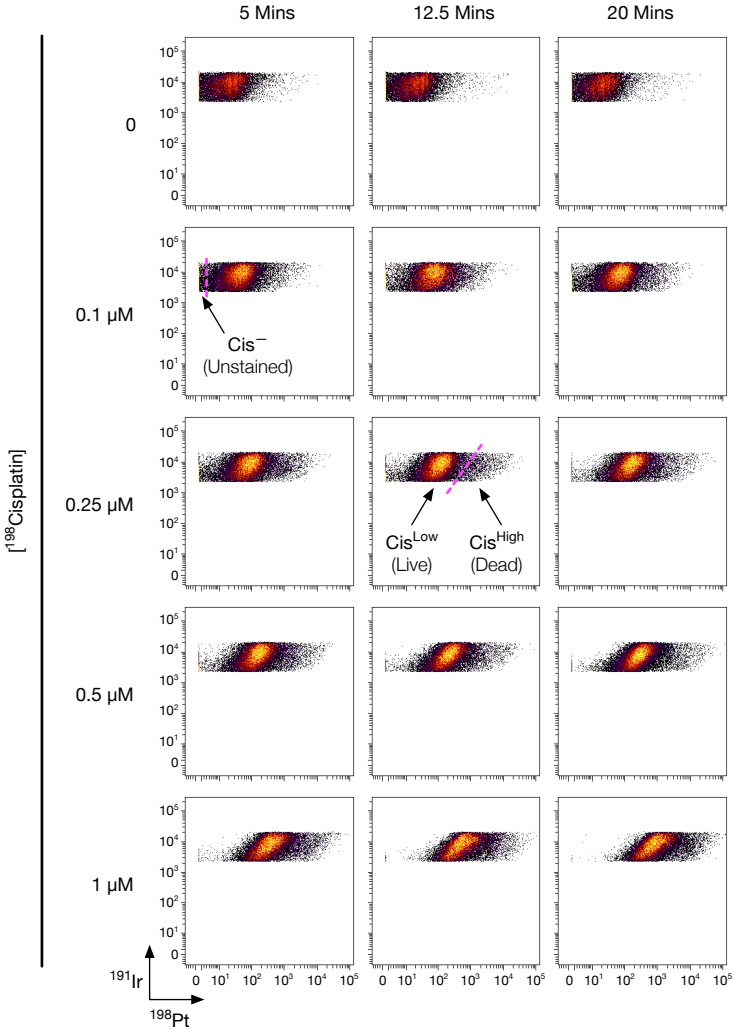
# Supplementary Figure 1



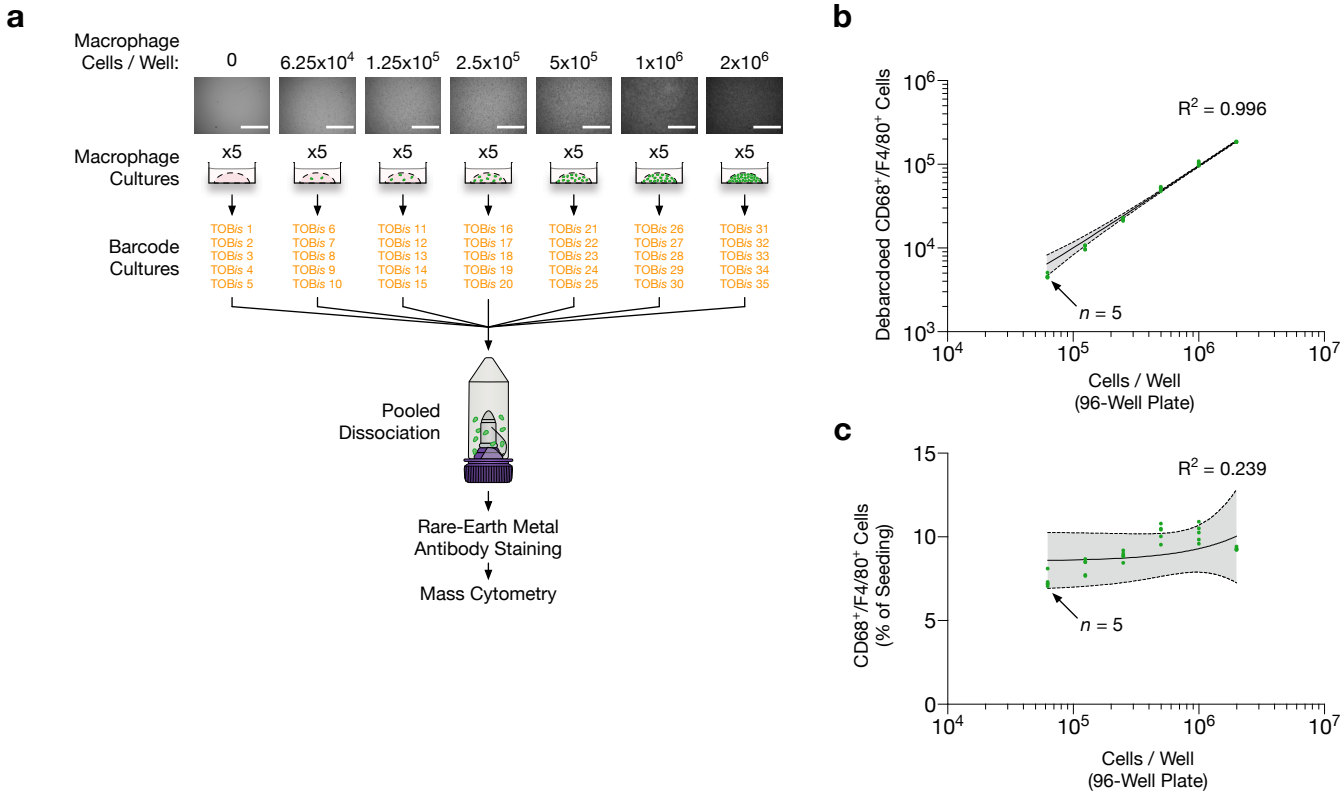
**Supplementary Figure 1 – Protease and Phosphatase Inhibitor Pre-Treatment.** a) Experimental overview. WT murine small intestinal organoids were treated with either protease inhibitors, phosphatase inhibitors, or both for 1, 5, or 10 minutes before fixation (n = 3). All conditions were TOB*is* barcoded, pooled for dissociation, stained with rare earth metal antibodies, and analysed by mass cytometry (MC). b) Earth Mover's Distance (EMD) heatmap of MC parameters following different organoid pre-treatments. Both protease and phosphatase inhibitor pre-treatments can alter heavy-metal antibody staining. c) Principal component analysis (PCA) of data in b). Protease and phosphatase inhibitor pre-treatment affects heavy-metal antibody staining in a time-dependent manner. Users are advised to empirically determine the duration of the treatment according to their experimental system and antibody panel. d) Experimental overview. Murine colonic organoids with either wild-type (WT) or Apc knockdown (*shApc*) were pre-treated for 5 minutes with either protease inhibitors, phosphatase inhibitors, or both, TOB*is* barcoded, dissociated, stained with rare-earth metal antibodies, and analysed by mass cytometry (MC). e) EMD heatmap of MC parameters following different inhibitor pre-treatments. Note how different inhibitor pre-treatments can alter antigen staining (e.g., pPDK1 [S241], pNFkB [S529]). CSB, cell staining buffer (see REAGENTS).

# Supplementary Figure 2



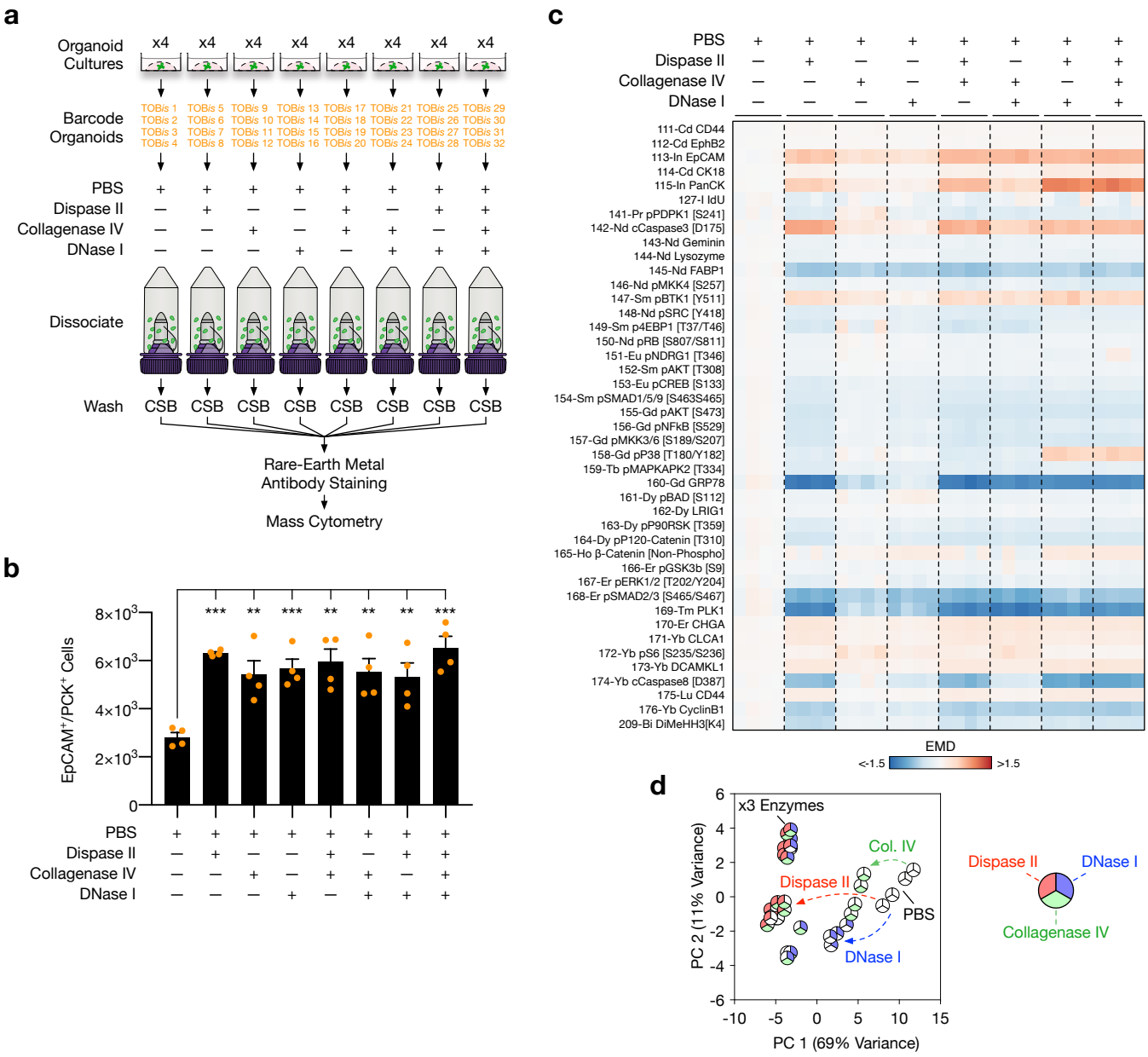
**Supplementary Figure 2 – Cisplatin Organoid Staining *in situ*.** 0 – 1 μM $^{198}$Cisplatin was added to murine small intestinal organoids over 5, 12.5, and 20 minutes and analysed by mass cytometry. Cisplatin$^{Low}$ cells can be retained as live cells and Cisplatin$^{High}$ cells can be gated out as dead cells. We advise users to select a combination of $^{198}$Cisplatin concentration and incubation time where all cells have had the opportunity to be stained with Cisplatin (i.e., no Cisplatin$^{-}$ cells). CSB, cell staining buffer (see REAGENTS).

# Supplementary Figure 3



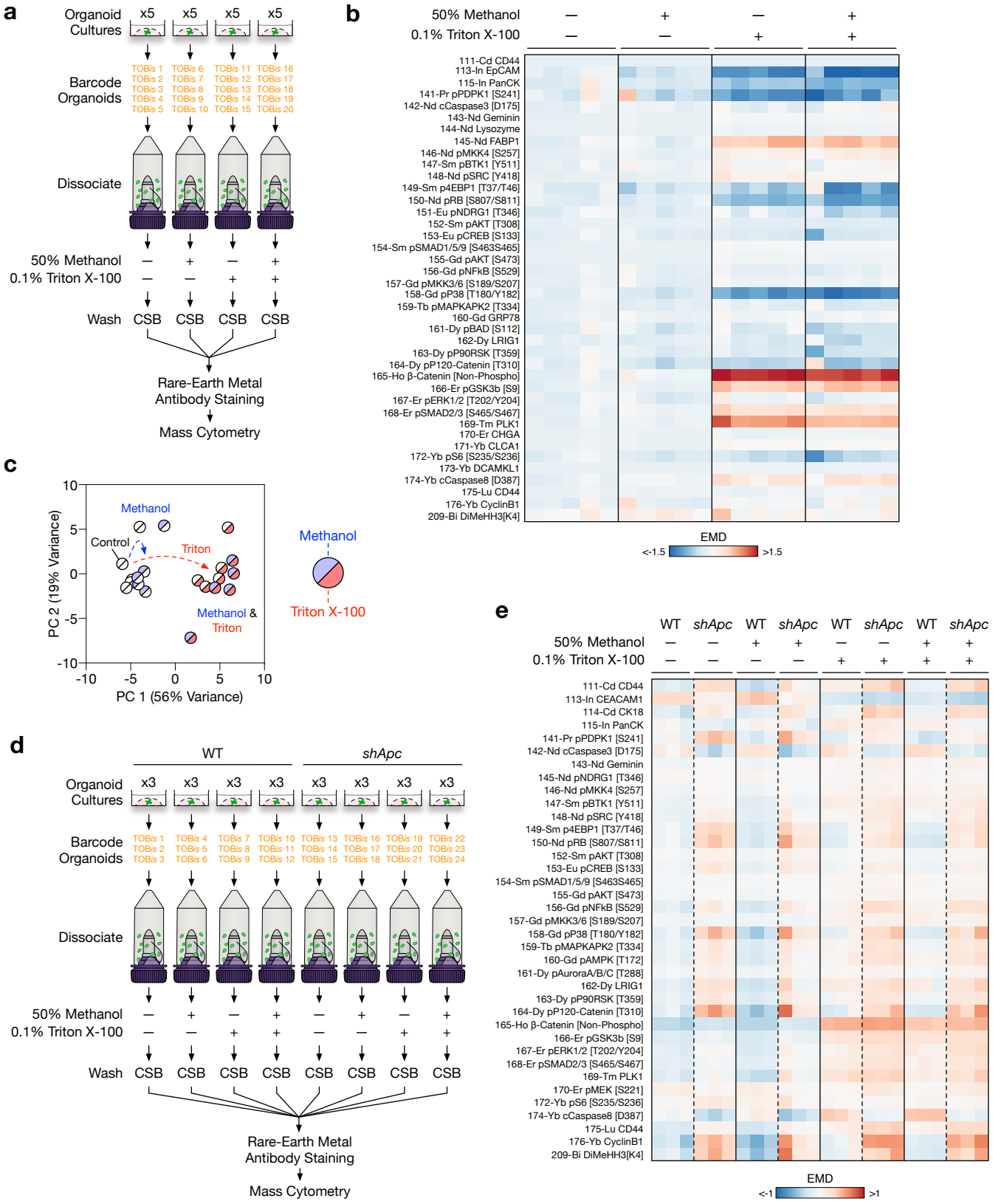
**Supplementary Figure 3 – TOB*is* Barcoding Capacity.** a) Experimental overview. Bone marrow derived macrophages (BMDMs) were plated in 96-well plates ranging from $6.25x10^4$ – $2x10^6$ cells per well in 3D Matrigel (n = 5). (Note: a typical seeding density for cells in 3D Matrigel in a 96-well plate is $<1x10^6$ per well). Cells were TOB*is* barcoded, dissociated, stained with rare-earth metal antibodies, and analysed by mass cytometry. Scale bar = 1 mm. b) and c) Debarcoded $CD68^+$ /$F4/80^+$ BMDMs display linear debarcoding recovery across all cell densities. Error represents 95% confidence interval (area shaded grey).

# Supplementary Figure 4



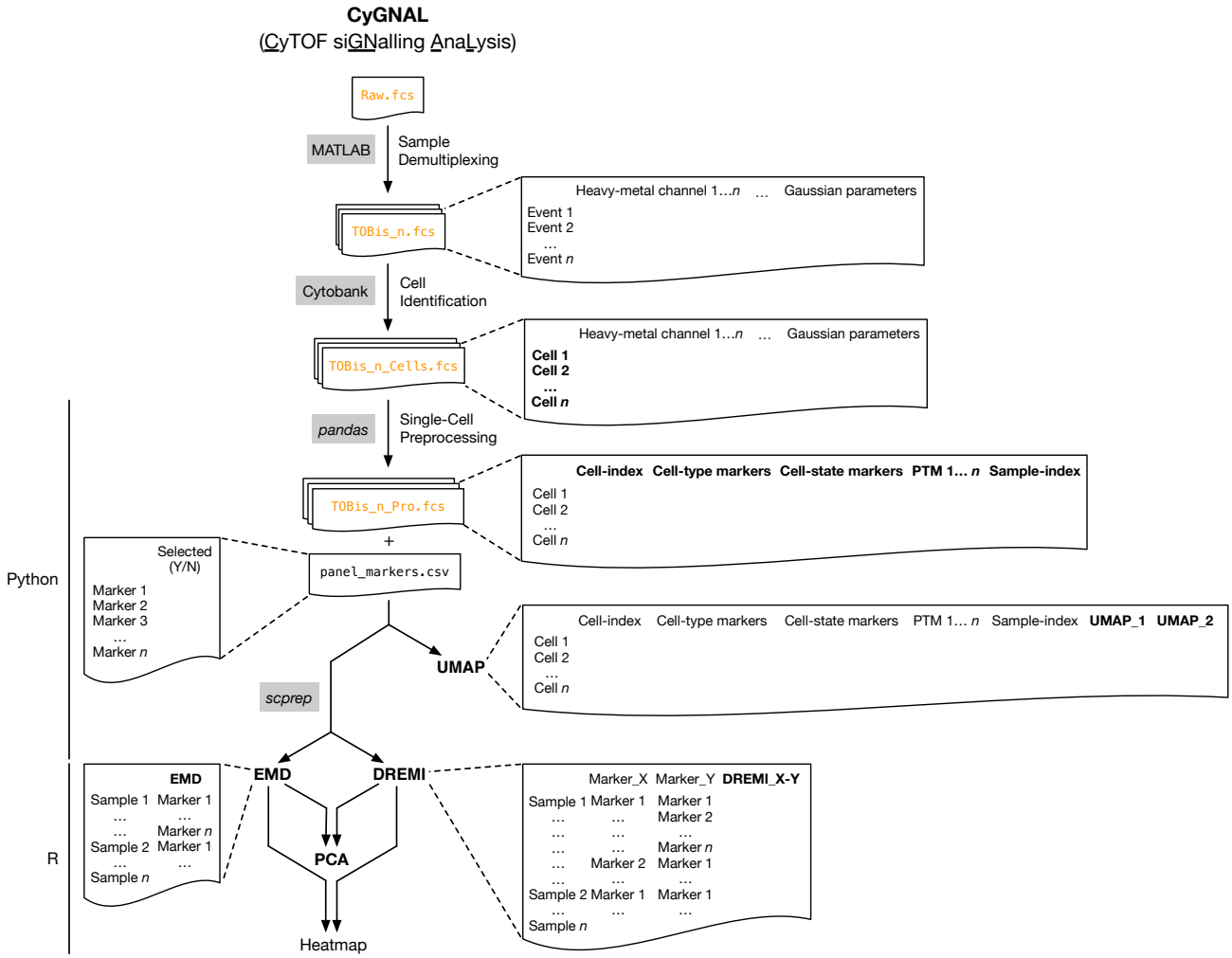
**Supplementary Figure 4 – Single-Cell Organoid Dissociation Enzymes.** a) Experimental overview. Murine small intestinal organoids were TOB*is* barcoded and dissociated in PBS supplemented with either Dispase II, Collagenase IV, and/or DNase I (n = 4) using the Miltenyi GentleMACS platform, stained with rare-earth metal antibodies, and analysed by mass cytometry (MC). b) Debarcoded $EpCAM^+$ /$PCK^+$ cells from each dissociation condition. All enzymes improve single-cell recovery relative to PBS alone (2-tailed unpaired *t*-test vs PBS, ** = $p<0.01$, *** = $p<0.001$). c) Earth Mover's Distance (EMD) heatmap of MC parameters following different dissociation conditions. Each dissociation enzyme can alter heavy-metal antibody staining and should be optimised for each biological system studied. d) Principal component analysis (PCA) of data in c). Heavy-metal antibody staining is affected by the combination of dissociation enzymes, and users are advised to test and titrate alternative dissociation enzymes for their experimental system. CSB, cell staining buffer (see REAGENTS).

# Supplementary Figure 5



**Supplementary Figure 5 – Cell Permeabilisation Buffers.** a) Experimental overview. Murine small intestinal organoids were TOB*is* barcoded (n = 5), dissociated, and permeabilised using 50% methanol, 0.1% Triton, or both, stained with rare-earth metal antibodies, and analysed by mass cytometry (MC). b) Earth Mover's Distance (EMD) heatmap of MC parameters following different permeabilisation conditions. In our experience, 0.1% Triton is a more effective permeabilisation buffer for small intestinal organoids, but this may vary with alternative models and should be optimised by for each biological system studied. c) Principal component analysis (PCA) of data in b). Permeabilisation conditions can substantially alter heavy-metal antibody staining, and users are advised to optimise the step based on their model system and antibody panel. d) Experimental overview. Wild-type (WT) or Apc knockdown (*shApc*) murine colonic organoids were TOB*is* barcoded, dissociated, and permeabilised using 50% methanol, 0.1% Triton, or both (n = 3), stained with rare-earth metal antibodies, and analysed by mass cytometry (MC). e) EMD heatmap of MC parameters following different permeabilisation conditions. In murine colonic organoids 50% methanol treatment yields similar antibody staining to untreated cells. Either 0.1% Triton alone or combined with 50% methanol yields strong staining across multiple parameters. CSB, cell staining buffer (see REAGENTS).

# Supplementary Figure 6



**Supplementary Figure 6 – CyGNAL Architecture.** File structure and processing architecture of CyTOF siGNalling AnaLysis (CyGNAL) pipeline for computing cell-type-specific signalling networks from TOB*is* MC data. Software environments and packages are indicated in grey boxes, computational processes are in bold text.

**Supplementary Table 1 – TOB*is* MC Costs.**

| | Item | Price / Well (£) | 35-plex TOB*is* | 126-plex TOB*is* |
|---|---|---|---|---|
| **Pre-Treatment** | $^{127}$IdU | 0.15 | 5.25 | 18.9 |
| | Protease Inhibitors | 0.035 | 1.23 | 4.41 |
| | Phosphatase Inhibitors | 0.078 | 2.73 | 9.83 |
| | PFA | 0.0084 | 0.29 | 1.1 |
| | $^{194}$Cisplatin | 0.088 | 3.08 | 11.09 |
| **TOB*is*** | TeMal * | 0.06 or 0.08 | 2.1 | 10.08 |
| | $^{196/8}$Cisplatin ** | 0.005 | - | 0.63 |
| | | **Price / C-Tube (£)** | **x5 C-Tubes** | **x15 C-Tubes** |
| **Dissociation** | C-Tubes | 3.9 | 19.5 | 58.5 |
| | Dispase II | 0.15 | 0.75 | 2.25 |
| | Collagenase IV | 0.16 | 0.8 | 2.4 |
| | DNase I | 0.18 | 0.9 | 2.7 |
| | | | **Price / 3M Cell Stain (£)** | **Price / 12M Cell Stain (£)** |
| **Staining** | 40-plex Metal-Antibody Panel *** | | 120 | 480 |
| | $^{191}$Ir + $^{193}$Ir | | 0.18 | 0.72 |
| | | **Total (£)** | 156.81 | 602.61 |

Costing guidelines for multiplexed TOB*is* MC experiments. Metal-antibody conjugates comprise 75-80% of the total cost of a TOB*is* MC experiment.

* Based on TeMal production in an academic lab (0.3 mmole = £2,000).

** Based on custom production of 196/8 Cisplatin from BuyIsotope (1 mL 10 mM = £1000).

*** Based on 100 ug Metal-antibody = £300 (£200 Ab, £100 conjugation), 1 ug / Metal-Ab / 3 million cell staining.

**Supplementary Table 2 – Murine Small Intestinal Organoid Mass Cytometry Antibody Panel.**

| Isotope-Metal | Antigen / Target | Antibody Clone | Supplier | Catalogue # |
|---|---|---|---|---|
| 89-Y | Phospho-Histone H3 [S28] | HTA28 | BioLegend | 641007 |
| 113-In | CD326 (EpCAM) | G8.8 | BioLegend | 118223 |
| 115-In | Pan-Cytokeratin (Pan-CK) | AE-1/AE-3 | BioLegend | 914204 |
| 141-Pr | Phospho-PDPK1 [S241] | J66-653.44.22 | BD Biosciences | 558395 |
| 142-Nd | Cleaved-Caspase 3 [D175] | D3E9 | CST | 9579 |
| 143-Nd | C-MYC | D84C12 | CST | 5605 |
| 144-Nd | Lysozyme | BGN/06/961 | Abcam | ab36362 |
| 145-Nd | FABP1 | 328605 | R&D Systems | MAB29641 |
| 146-Nd | Phospho-MKK4/SEK1 [S257] | C36C11 | CST | 4514 |
| 147-Sm | Phospho-BTK [Y551] | 24a/BTK | BD Biosciences | 558034 |
| 148-Nd | Phospho-SRC [Y418] | SC1T2M3 | Thermo | 14-9034-82 |
| 149-Sm | Phospho-4E-BP1 [T37/46] | 236B4 | CST | 2855 |
| 150-Nd | Phospho-RB [S807/811] | J112-906 | BD Biosciences | 558389 |
| 151-Eu | Phospho-PKCα [T497] | K14-984 | BD Biosciences | 610108 |
| 152-Sm | Phospho-AKT [T308] | J1-223.371 | BD Biosciences | 558316 |
| 153-Eu | Phospho-CREB [S133] | 87G3 | CST | 9198 |
| 154-Sm | Phospho-SMAD1 [S463/465]<br>Phospho-SMAD5 [S463/465]<br>Phospho-SMAD9 [S465/467] | D5B10 | CST | 13820 |
| 155-Gd | Phospho-AKT [S473] | D9E | CST | 4060 |
| 156-Gd | Phospho-NF-κB p65 [S529] | K10-895.12.50 | BD Biosciences | 558393 |
| 157-Gd | Phospho-MKK3 [S189] / MKK6 [S207] | D8E9 | CST | 12280 |
| 158-Gd | Phospho-p38 MAPK [T180/Y182] | D3F9 | CST | 4511 |
| 159-Tb | Phospho-MAPKAPK2 [T334] | 27B7 | CST | 3007 |
| 160-Gd | Phospho-AMPKα [T172] | 40H9 | CST | 2535 |
| 161-Dy | Phospho-BAD [S112] | 40A9 | CST | 5284 |
| 162-Dy | LRIG1 | Polyclonal | R&D Systems | AF3688 |
| 163-Dy | Phospho-p90RSK [T359] | D1E9 | CST | 8753 |
| 164-Dy | Phospho-p120-Catenin [T310] | 22/p120 (pT310) | BD Biosciences | 558203 |
| 165-Ho | β-Catenin [Active] | D13A1 | CST | 8814 |
| 166-Er | Phospho-GSK-3β [S9] | D85E12 | CST | 5558 |
| 167-Er | Phospho-ERK1/2 [T202/Y204] | 20A | BD Biosciences | 612359 |
| 168-Er | Phospho-SMAD2 [S465/467]<br>Phospho-SMAD3 [S423/425] | D27F4 | CST | 8828 |
| 169-Tm | GFP | 5F12.4 | Fluidigm | 3169009 |
| 170-Er | Phospho-MEK1/2 [S221] | 166F8 | CST | 2338 |
| 171-Yb | CLCA1 | EPR12254-88 | Abcam | ab180851 |
| 172-Yb | Phospho-S6 [S235/236] | D57.2.2E | CST | 4858 |
| 173-Yb | DCAMKL1 | 6F9 | Sigma | WH0009201M2 |
| 174-Yb | CHR-A | C-12 | Santa Cruz | sc-393941 |
| 175-Lu | CD44 | IM7 | BioLegend | 103051 |
| 176-Yb | Cyclin B1 | GNS-11 | BD Biosciences | 554179 |
| 209-Bi | Di-Methyl-Histone H3 [K4] | C64G9 | CST | 9725 |

Extracellular
Intracellular

## SUPPLEMENTARY METHOD

### Synthesis and Characterization of TeMal Isotopologues

## REAGENTS

(Triisopropylsilyl)acetylene (Sigma, Cat# 360031)

Acetone (Sigma, Cat# 179124)

*N*-bromosuccinimide (Sigma, Cat# B81255)

Silver(I) nitrate (Sigma, Cat# 209139)

Pentane (Sigma, Cat# 158941)

Sodium chloride (Sigma, Cat# S9888)

Sodium sulfate (Sigma, Cat# 238597)

*n*-Butylamine (Sigma, Cat# 471305)

Copper(I) chloride (Sigma, Cat# 224332)

Hydroxylamine hydrochloride (Sigma, Cat# 159417)

4-pentynoic acid (Sigma, Cat# 232211)

Ethyl acetate (Sigma, Cat# 319902)

Citric acid (Sigma, Cat# C0759)

Magnesium sulfate (Sigma, Cat# 746452)

Isotopically enriched tellurium (Trace Sciences International, custom order)

- $^{122}$Te – tellurium metal powder
- $^{123}$Te – tellurium oxide
- $^{124}$Te – tellurium metal powder
- $^{125}$Te – tellurium metal powder
- $^{126}$Te – tellurium metal powder

$^{128}$Te – tellurium metal powder

$^{130}$Te – tellurium metal powder

Hydrazine hydrate (Sigma, Cat# 225819)

Sodium borohydride (Sigma, Cat# 452882)

Tetrahydrofuran (THF) (Sigma, Cat# 401757)

Tetrabutylammonium fluoride, 1.0 M in THF (TBAF) (Sigma, Cat# 216143)

Ammonium chloride (Sigma, Cat# A9434)

Diethyl ether (Sigma, Cat# 673811)

Ethanol (Greenfield Global, Cat# P210EAAN)

Methanol (Sigma, Cat# 179337)

Glacial acetic acid (Caledon Laboratory Chemicals, Cat# 1000-1-29)

Dichloromethane (Sigma, Cat# D65100)

1-Hydroxybenzotriazole monohydrate (HOBt, TCI America, Cat# H0468)

*N,N*-diisopropylethylamine (DIPEA, Sigma, Cat# D125806)

HATU (Sigma, Cat# 445460)

*N*-(2-Aminoethyl)maleimide hydrochloride (TCI America, Cat# A2436)

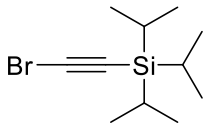
Sodium bicarbonate (Sigma, Cat# S6014)

Ammonium hydroxide (Caledon Laboratory Chemicals, Cat# 1525-1-29)

SiliaFlash silica gel P60 (Silicycle, Cat# R12030B)

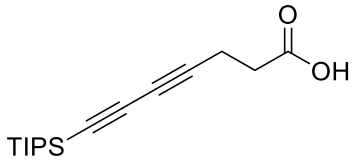
**Synthesis and Characterization of TeMal Isotopologues**

Reagents and solvents described below were purchased from Sigma-Aldrich, TCI America, Caledon laboratory Chemicals, or Greenfield Global (see reagents) and used as supplied unless otherwise indicated. Isotopically enriched tellurium metal was purchased from Trace Sciences. Solvents were degassed by sparging argon through vessels under sonication for at least one hour. Column chromatography was performed using SiliaFlash P60 (Silicycle); a combination of gravity elution and moderate air pressure was employed. NMR spectra were acquired using a 500 MHz Agilent DD2 spectrometer with an XSens C13 Cold Probe or a 400 MHz Bruker Avance III spectrometer with a dual resonance (BBFO) broad band probe. Mass spectra were obtained by positive mode electrospray (ESI+) on an Agilent 6538 Q-TOF mass spectrometer.

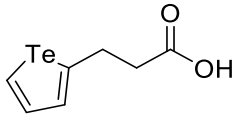



**(Bromoethynyl)triisopropylsilane (1).** This is a commercially available reagent but can easily be synthesized in-house. To a 250 mL round bottom flask was added acetone (65 mL), TIPS-acetylene (1.9 mL, 8.2 mmol, 1.0 eq), *N*-bromosuccinimide (1.47 g, 8.26 mmol, 1.0 eq), and catalytic silver nitrate (0.136 g, 0.80 mmol, 0.1 eq). The mixture was stirred at room temperature (observed formation of small amounts of white precipitate). After 3 hr, pentanes (~130 mL) was added and the mixture stirred for 30 min to precipitate succinimide and salts. The mixture was gravity filtered into a separatory funnel and the organic filtrate was washed with deionized water (4 x 50 mL) and brine (1 x 50 mL). The remaining organic layer was dried over sodium sulfate, gravity filtered, and concentrated by rotary evaporation until a clear oil remained. The oil was further dried on a high vacuum pump to remove pentanes. As the product is volatile, care was taken not to keep the product under high vacuum for prolonged periods of time or significant losses would occur. Final product was a clear, very pale-yellow oil (1.62 g, 76%). $^{1}$H NMR ($CDCl_3$, 500 MHz): δH 1.12-1.03 (21H, mult, Si-***CH***-

$(CH_3)_2$ and Si-CH-(***CH₃***)$_2$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δC 83.61 (s, Br-***C***≡C-Si-); 61.87 (s, Br-C≡***C***-Si-); 18.63 (s, Si-CH-(***CH₃***)$_2$); 11.40 (s, Si-***CH***-$(CH_3)_2$).




**7-(Triisopropylsilyl)hepta-4,6-diynoic acid (2)**. To a 50 mL RBF on ice was added degassed, deionized $H_2O$ (17.5 mL), *n*-butylamine (8.5 mL), and CuCl (0.039 g, 0.39 mmol, 0.05 eq) which generated a deep blue colour. $NH_2OH{\cdot}HCl$ was added until reaction changed from blue to colourless (~0.06 g). 4-pentynoic acid (0.870 g, 8.87 mmol, 1.1 eq) was added and the reaction stirred until solids were completely dissolved. The flask was purged with argon and (bromoethynyl)triisopropylsilane (2.04 g, 7.85 mmol, 1.0 eq) was delivered in neat form by syringe; an emulsion was formed which cleared to give a pale-yellow solution after vigorous stirring. After 1.5 hr, the reaction mixture was diluted with deionized water to ~50 mL and extracted with 2 x 50 mL EtOAc. The aqueous phase was acidified with 5% citric acid (~40 mL) and extracted with 2 x 50 mL EtOAc. The combined organic phase was washed with 3 x 40 mL 5% citric acid and 2 x 40 mL brine, dried over $MgSO_4$, and concentrated by rotary evaporation. This yielded a clear yellow oil which formed white crystals upon drying under high vacuum (1.90 g, 6.7 mmol, 91%). $^1H$ NMR ($CDCl_3$, 400 MHz): δH 2.62 (4H, s, COOH-***CH₂***-$CH_2$- and COOH-$CH_2$-***CH₂***-), 1.07 (21H, s, Si-***CH***-$(CH_3)_2$ and Si-CH-(***CH₃***)$_2$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δC 177.26 (s, COOH); 89.74, 81.41, 75.98, 66.85 (s, four *sp* carbons); 32.68 (s, COOH-***CH₂***-$CH_2$-), 18.68 (s, Si-***CH***-$(CH_3)_2$), 15.02 (s, (COOH-$CH_2$-***CH₂***-), 11.40 (s, Si-CH-(***CH₃***)$_2$).




**3-(Tellurophen-2-yl)propanoic acid (3)**. Where isotopically enriched tellurium samples were obtained in an oxidized state, the precursor was stirred overnight at room temperature in hydrazine hydrate (50-60% hydrazine in $H_2O$, 5mL per 100 mg tellurium). Contents were dried by careful rotary evaporation to yield a coating of

tellurium (0) which was then directly used in the cyclization reaction. The cyclization reaction and the TIPS-deprotection reaction were run concurrently. The deprotected diyne is somewhat unstable and thus no attempts were made to purify or characterize it before use in the cyclization reaction.

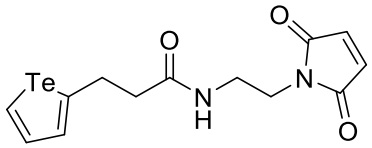
Cyclization Reaction: To a 10 mL RBF was added freshly powdered, isotopically enriched tellurium metal (0.100 g, 0.78 mmol, 1.0 eq) and sodium borohydride (0.237 g, 6.27 mmol, 8.0 eq). The flask was purged using argon for 15 min. Degassed water (3.0 mL) was injected into the flask and the reaction was stirred at room temperature for 1 hr, then heated to 40°C. The reduction of tellurium metal results in a series of colour changes from grey to light purple to very deep purple, then back to light purple, to pink/colourless. As soon as the cyclization reaction was set up, the deprotection reaction was begun.

Deprotection Reaction: To a dry 10 mL RBF under argon, on ice, was added **2** (0.284 g, 1.02 mmol, 1.3 eq. to Te metal) in THF (1.7 mL). TBAF (1.7 mL 1M solution in THF) was injected slowly. The mixture was allowed to stir on ice for 1 hour, after which the reaction mixture was partitioned over 5 mL saturated $NH_4Cl$ and 5 mL diethyl ether. The aqueous layer was extracted with 5mL ether, acidified with ~2.5 mL 5% citric acid (to reach pH ~4) and extract two more times with 5 mL diethyl ether. The combined organic phase as washed with 3 mL 5% citric acid, 3 mL brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to yield a pale yellow/orange oil. The flask containing TIPS-deprotected **2** was sealed and purged with argon. Contents were dissolved in degassed ethanol (3.0 mL) and transferred by syringe into the cyclization reaction.

Injection of diyne into the tellurium mixture resulted in darkening of reaction colour to purplish brown. After stirring for ~10-20 min, the reaction turned a peachy yellow colour. The mixture was stirred overnight at 40°C. After 15 hr, the cyclization reaction was opened to atmosphere and stirred for 1 hour to precipitate unreacted tellurium. Contents were filtered over a small celite column and the filter cake rinsed with minimal amounts of methanol. 5% citric acid was added to the filtrate until bubbling stopped and solution was acidic (pH~3-4). The mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic phase was washed with 10 mL 5% citric

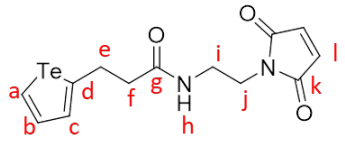
acid, 1mL brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Crude was purified by column chromatography using isocratic elution in 20% ethyl acetate in pentanes + 0.1% acetic acid. Due to "tail" of product co-eluting with an impurity, some samples were not completely purified (yellow oil containing traces of TIPS-based impurity; some colourless crystals formed upon cooling; NMR yield 45 to 70%).

Non-NMR active Te isotopologues ($^{122, 124, 126, 128, 130}$Te): $^{1}$H NMR ($CDCl_3$, 400 MHz): δH 8.72 (1H, dd, $^{3}J_{HH}$ = 6.9 Hz, $^{4}J_{HH}$ = 1.3 Hz, -Te-***CH***-CH-), 7.59 (1H, dd, $^{3}J_{HH}$ = 6.9, 3.9 Hz, -Te-CH-***CH***-), 7.45-7.33 (1H, m, -Te-CR-***CH***-), 3.24 (2H, t, $^{3}J_{HH}$ = 7.3 Hz, COOH-$CH_2$-***$CH_2$***-), 2.73 (2H, t, $^{3}J_{HH}$ = 7.3 Hz, COOH-***$CH_2$***-$CH_2$-). NMR active Te isotopologues: $^{123}$Te: $^{1}$H NMR ($CDCl_3$, 400 MHz): δH 8.72 (1H, ddd, $^{2}J_{HTe}$ = 82.8 Hz, $^{3}J_{HH}$ = 6.9 Hz, $^{4}J_{HH}$ = 1.2 Hz, -Te-***CH***-CH-), 7.59 (1H, ddd, $^{3}J_{HTe}$ = 15.1 Hz, $^{3}J_{HH}$ = 6.9, 3.9 Hz, -Te-CH-***CH***-), 7.45-7.33 (1H, m, -Te-CR-***CH***-), 3.30-3.17 (2H, m, COOH-$CH_2$-***$CH_2$***-), 2.73 (2H, t, $^{3}J_{HH}$ = 7.3 Hz, COOH-***$CH_2$***-$CH_2$-). $^{125}$Te: $^{1}$H NMR ($CDCl_3$, 400 MHz): δH 8.72 (1H, ddd, $^{2}J_{HTe}$ = 99.8 Hz, $^{3}J_{HH}$ = 6.9 Hz, $^{4}J_{HH}$ = 1.2 Hz, -Te-***CH***-CH-), 7.59 (1H, ddd, $^{3}J_{HTe}$ = 18.1 Hz, $^{3}J_{HH}$ = 6.9, 3.8 Hz, -Te-CH-***CH***-), 7.45-7.33 (1H, m, -Te-CR-***CH***-), 3.39-3.05 (2H, m, COOH-$CH_2$-***$CH_2$***-), 2.73 (2H, t, $^{3}J_{HH}$ = 7.3 Hz, COOH-***$CH_2$***-$CH_2$-).




**TeMal (4)**. To a 25 mL RBF was added DCM (2.1 mL), **2** (0.050 g, 0.20 mmol, 1.0 eq), HOBt (0.097 g, 0.72 mmol, 3.5 eq), DIPEA (0.22 mL, 1.3 mmol, 6.5 eq), and HATU (0.082 g, 0.22 mmol, 1.1 eq); contents were stirred for 10 minutes until completely dissolved. *N*-(2-aminoethyl)maleimide hydrochloride salt (0.037 g, 0.21 mmol, 1.0 eq) was added to begin the reaction. After stirring 3 hr at room temperature, the reaction mixture was diluted with DCM and concentrated to near dryness by rotary evaporation. The crude was redissolved in 7.5 mL EtOAc, washed with 1.5 mL 5% citric acid, 1 mL deionized $H_2O$, 1.5 mL saturated $NaHCO_3$, 1 mL deionized $H_2O$, and 1.5 mL brine. The organic phase was dried over MgSO4, concentrated by rotary

evaporation, and further purified by column chromatography using $NH_4OH$-pretreated silica (gradient elution of 50→60% EtOAc in pentanes). Isolated yields for isotopologues ranged between 0.040 to 0.063g; 53 to 81%.




Isotopologues with non-NMR active Te (**122, 124, 126, 128, 130-TeMal**): $^{1}$H NMR ($CDCl_3$, 500 MHz): δH 8.68 (1H, dd, $^{3}J_{HH}$ = 6.9 Hz, $^{4}J_{HH}$ = 1.2 Hz, $H^{a}$), 7.56 (1H, dd, $^{3}J_{HH}$ = 6.9, 3.9 Hz, $H^{b}$), 7.40-7.31 (1H, m, $H^{c}$), 6.70 (2H, s, $H^{l}$), 5.83 (1H, br s, $H^{h}$), 3.78-3.59 (2H, m, $H^{j}$), 3.46 (2H, q, $^{3}J_{HH}$ = 5.6 Hz, $H^{i}$), 3.20 (2H, td, $^{3}J_{HH}$ = 7.1 Hz, $^{4}J_{HH}$ = 1.1 Hz, $H^{e}$), 2.47 (2H, t, $^{3}J_{HH}$ = 7.1 Hz, $H^{f}$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δC 172.16 (s, $C^{g}$), 171.03 (s, $C^{k}$), 149.24 (s, $C^{d}$), 136.98 (s, $C^{b}$), 135.66 (s, $C^{c}$), 134.35 (s, $C^{l}$), 125.04 (s, $C^{a}$), 39.91 (s, $C^{f}$), 39.18 (s, $C^{i}$), 37.61 (s, $C^{j}$), 32.37 (s, $C^{e}$).

**123 TeMal**: $^{1}$H NMR ($CDCl_3$, 500 MHz): δH 8.67 (1H, ddd, $^{2}J_{HTe}$ = 81.1 Hz, $^{3}J_{HH}$ = 6.9 Hz, $^{4}J_{HH}$ = 1.2 Hz, $H^{a}$), 7.57 (1H, ddd, $^{3}J_{HTe}$ = 14.3 Hz, $^{3}J_{HH}$ = 6.9, 3.9 Hz, $H^{b}$), 7.42-7.29 (1H, m, $H^{c}$), 6.70 (2H, s, $H^{l}$), 5.80 (1H, br s, $H^{h}$), 3.78-3.58 (2H, m, $H^{j}$), 3.46 (2H, q, $^{3}J_{HH}$ = 5.6 Hz, $H^{i}$), 3.20 (2H, dt, $^{3}J_{HTe}$ = 14.2 Hz, $^{3}J_{HH}$ = 7.1 Hz, $H^{e}$), 2.47 (2H, t, $^{3}J_{HH}$ = 7.1 Hz, $H^{f}$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δC 172.16 (s, $C^{g}$), 171.03 (s, $C^{k}$), 149.24 (d, $^{1}J_{CTe}$ = 251.2 Hz, $C^{d}$), 136.98 (d, $^{2}J_{CTe}$ = 4.3 Hz, $C^{b}$), 135.66 (s, $C^{c}$), 134.35 (s, $C^{l}$), 125.04 (d, $^{1}J_{CTe}$ = 247.6 Hz, $C^{a}$), 39.91 (d, $^{3}J_{CTe}$ = 5.0 Hz, $C^{f}$), 39.18 (s, $C^{i}$), 37.61 (s, $C^{j}$), 32.37 (d, $^{2}J_{CTe}$ = 25.8 Hz, $C^{e}$).

**125 TeMal**: $^{1}$H NMR ($CDCl_3$, 500 MHz): δH 8.67 (1H, ddd, $^{2}J_{HTe}$ = 97.8 Hz, $^{2}J_{HH}$ = 6.9 Hz, $^{4}J_{HH}$ = 1.2 Hz, $H^{a}$), 7.57 (1H, ddd, $^{3}J_{HTe}$ = 17.3 Hz, $^{2}J_{HH}$ = 6.9, 3.9 Hz, $H^{b}$), 7.34 (1H, m, $H^{c}$), 6.70 (2H, s, $H^{l}$), 5.80 (1H, br s, $H^{h}$), 3.78-3.58 (2H, m, $H^{j}$), 3.51-3.42 (2H, m, $H^{i}$), 3.20 (2H, dt, $^{3}J_{HTe}$ = 16.0 Hz, $^{3}J_{HH}$ = 7.1 Hz, $H^{e}$), 2.47 (2H, t, $^{3}J_{HH}$ = 7.1 Hz, $H^{f}$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δC 172.16 (s, $C^{g}$), 171.03 (s, $C^{k}$), 149.24 (d, $^{1}J_{CTe}$ = 302.9 Hz, $C^{d}$), 136.97 (d, $^{2}J_{CTe}$ = 5.1 Hz, $C^{b}$), 135.66 (s, $C^{c}$), 134.35 (s, $C^{l}$), 125.04 (d, $^{1}J_{CTe}$ = 298.5 Hz, $C^{a}$), 39.90 (d, $^{3}J_{CTe}$ = 6.0 Hz, $C^{f}$), 39.18 (s, $C^{i}$), 37.61 (s, $C^{j}$), 32.37 (d, $^{2}J_{CTe}$ = 31.1 Hz, $C^{e}$).