Supplementary Information

Dual Chemical Probes Enable Quantitative System-Wide Analysis of Protein Prenylation and Prenylation Dynamics

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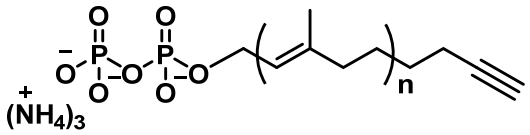
Supplementary Data Files (in separate Excel files)

Supplementary Figure 1. Structure of YnFPP and YnGGPP and peptide substrates used in biochemical enzyme assays. a) Structure of alkynyl-farnesyl pyrophosphate (YnFPP) and alkynyl-geranylgeranyl pyrophosphate (YnGGPP). b) Amino acid sequences of fluorescein amide (FAM)-labeled peptide substrates and prenylated product peptide used in FTase and GGTase-1 biochemical enzyme assays (Supplementary Figure 2). FAM-GerGer-RHOA(188-193) incorporates a geranylgeranyl cysteinyl thioether modification. FAM, 5/6-carboxyfluorescein; GerGer, geranylgeranyl.

a

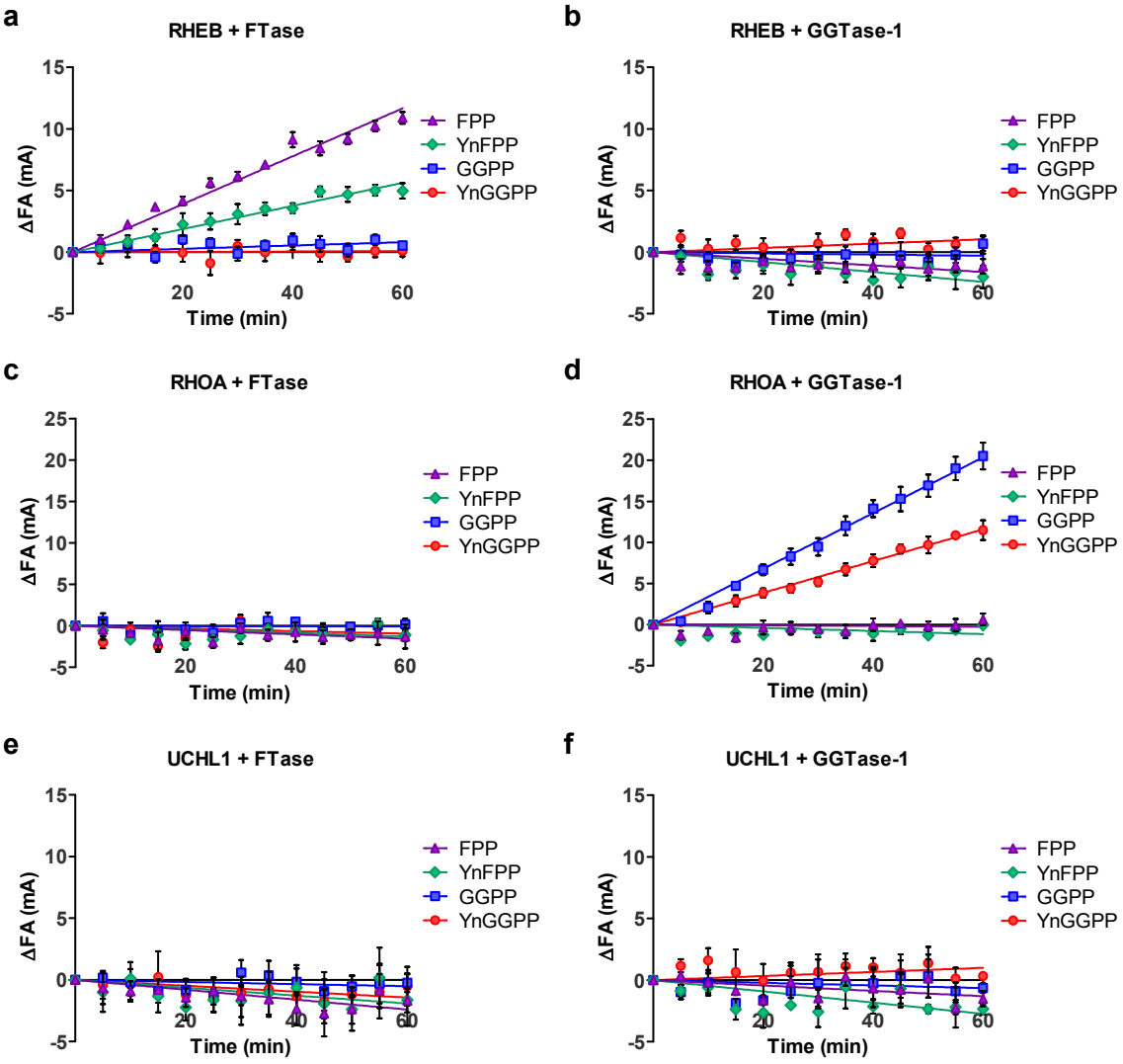


n=1 YnFPP
n=2 YnGGPP

b

Peptide	Sequence
FAM-UCHL1(218-223)	FAM-βAALCKAA-CO_2H
FAM-RHEB(179-184)	FAM-βASSCSVM-CO_2H
FAM-RHOA(188-193)	FAM-βASGCLVL-CO_2H
FAM-GerGer-RHOA(188-193)	FAM-βASGC(GerGer)LVL-CO_2H

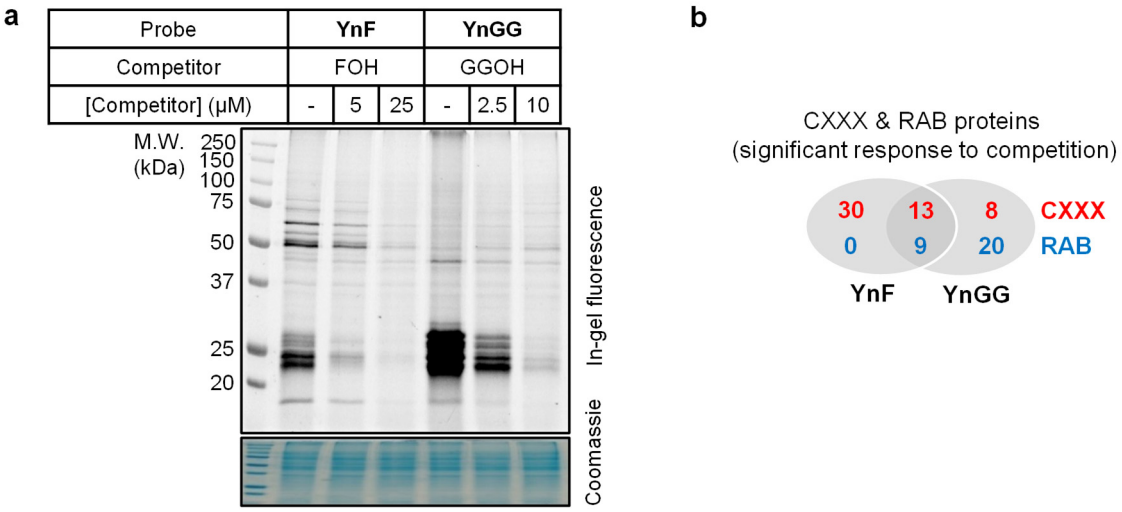
Supplementary Figure 2. FTase and GGTase-1 biochemical enzyme assays. Peptide prenylation was measured using fluorescence anisotropy (FA) as described in Biological Methods & Proteomics. Assays were performed with FAM-labeled peptides (1 μM), FTase or GGTase-1 (25 nM) and FPP, YnFPP, GGPP or YnGGPP (5 μM). Real-time analysis of *in vitro* prenylation of RHEB (a & b), RHOA (c & d) and UCHL1 (e & f) substrate peptides show that YnFPP and YnGGPP mimic the enzyme/peptide selectivity of the natural isoprenoid substrates. UCHL1 was found not to be a substrate for prenylation by FTase or GGTase-1 *in vitro*. g) V_{max} and K_m values for FPP and YnFPP or GGPP and YnGGPP prenylation reactions with FAM-RHEB(179-184) and FTase or FAM-RHOA(188-193) and GGTase-1, respectively (n = 2-3).



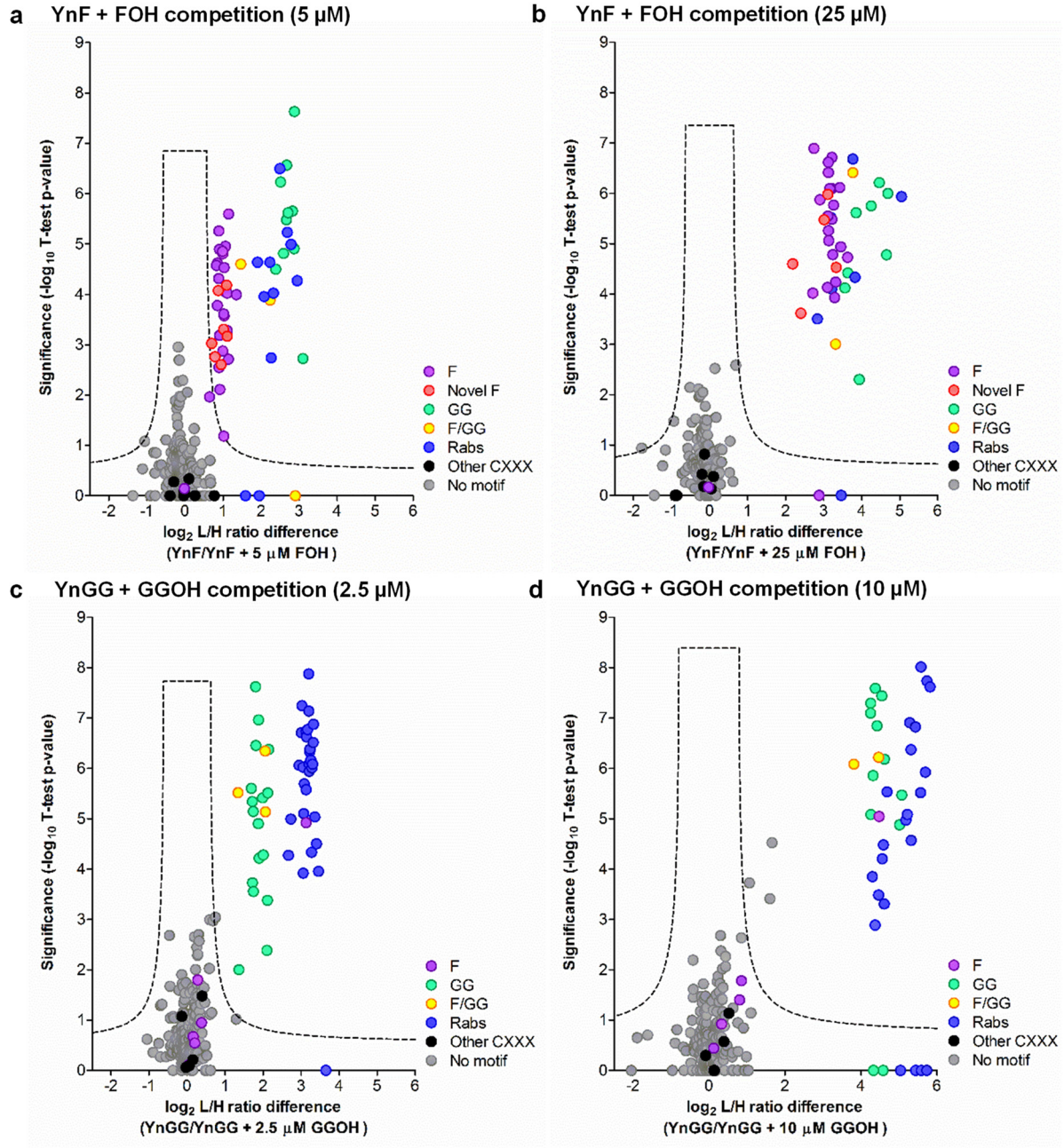
g

Peptide substrate	Isoprenoid	V_{max} (fmol/min)	K_m (μM)
FAM-RHEB(179-184)	FPP	6.9 (6.5-7.2)	0.042 (0.027 - 0.056)
	YnFPP	3.1 (2.8-3.5)	0.022 (0.00034 - 0.043)
FAM-RHOA(188-193)	GGPP	13.0 (12.0-14.0)	0.016 (0.0060 - 0.027)
	YnGGPP	8.4 (7.7-9.0)	0.026 (0.0094 - 0.042)

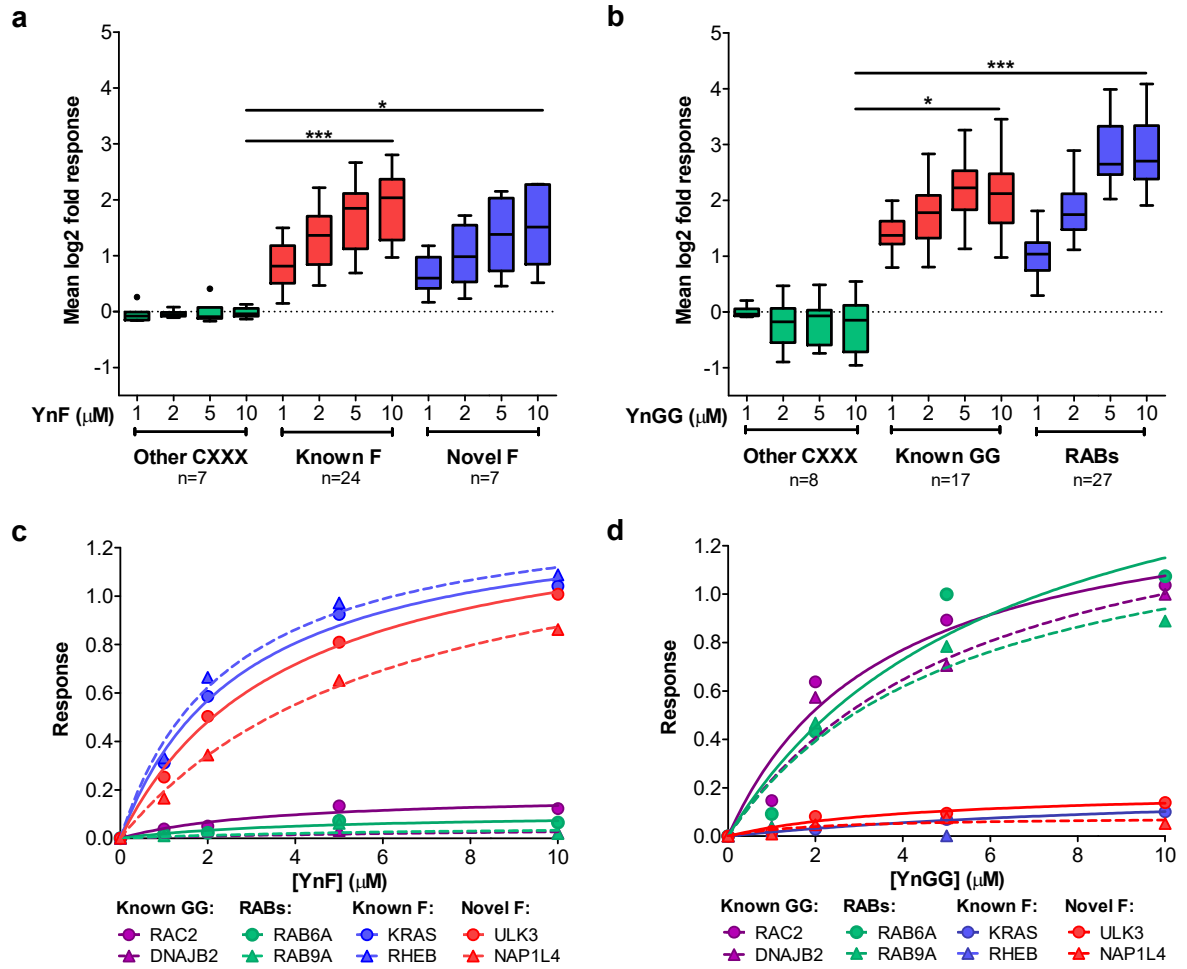
Supplementary Figure 3. Validation of YnF and YnGG labeling. a) EA.hy926 cells were cultured in medium supplemented with YnF (10 µM) +/- FOH or YnGG (10 µM) +/- GGOH for 24 hours. The lysate was subjected to CuAAC ligation with capture reagent AzTB, separated by SDS-PAGE and labeled proteins visualized by in-gel fluorescence (channel Cy3). Protein loading was assessed by Coomassie Blue staining. b) Overlap in protein identifications by YnF and YnGG.



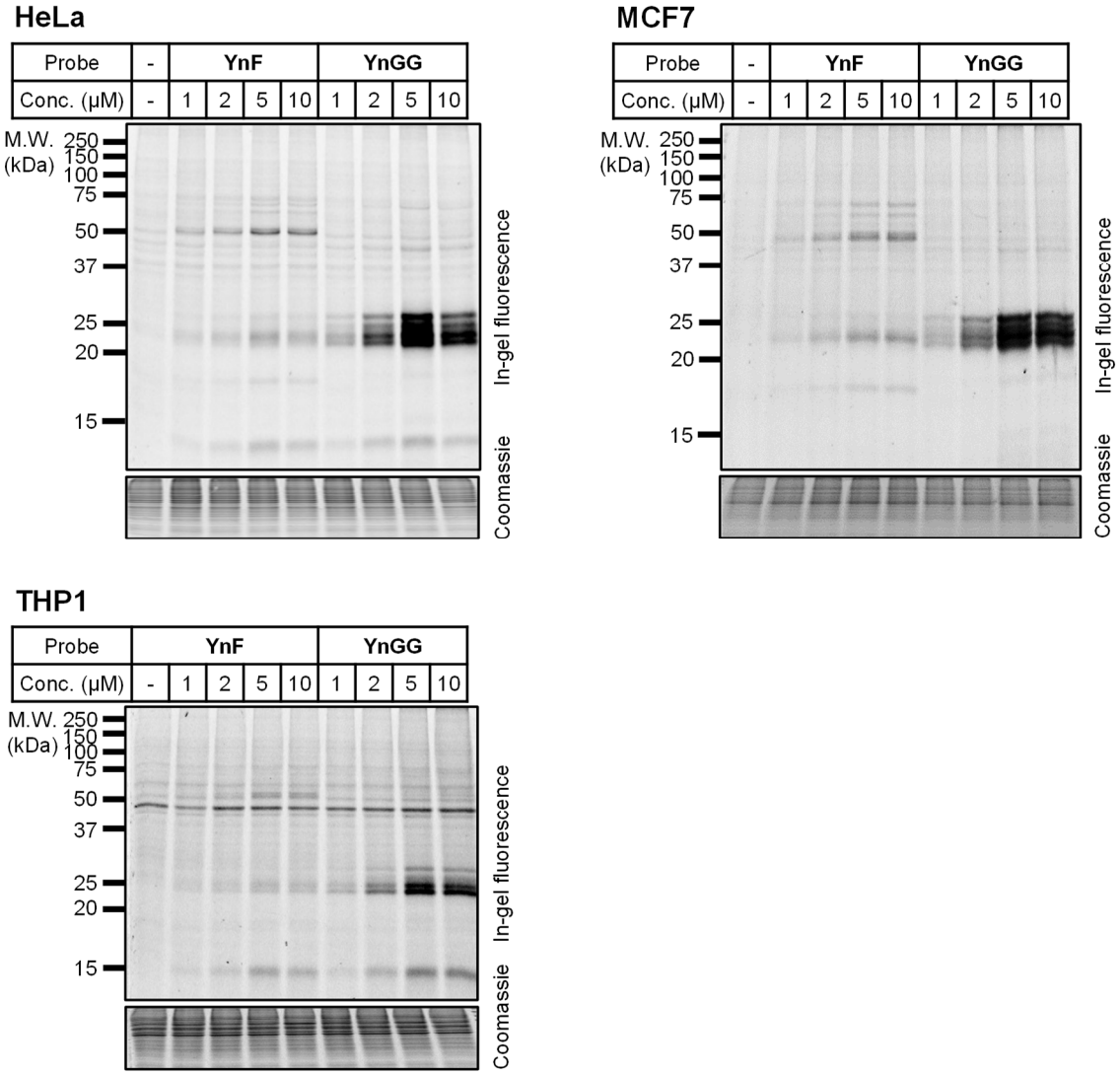
Supplementary Figure 4. YnF and YnGG labeling shows dose-dependent sensitivity to competition with natural isoprenoid substrate. EA.hy926 cell lysate labeled as above (Supplementary Figure 1a) was combined with a spike-in standard dually labeled with prenyl probe (YnF or YnGG) and R10K8 SILAC label. Following CuAAC to capture reagent AzRB-labeled proteins were enriched on NeutrAvidin agarose and subjected to on-bead trypsin digest. The resultant peptides were analyzed by nanoLC-MS/MS and the data processed in MaxQuant. The data was filtered to retain proteins identified with a minimum of 2 razor+unique peptides and 2 valid values in at least 2 replicates of the non-competition samples. A two-sample t-test was performed to compare the $\log_2$ L/H ratios of isoprenoid competition samples versus non-competition samples (n=3, Permutation-based FDR=0.02, S0=0.5).



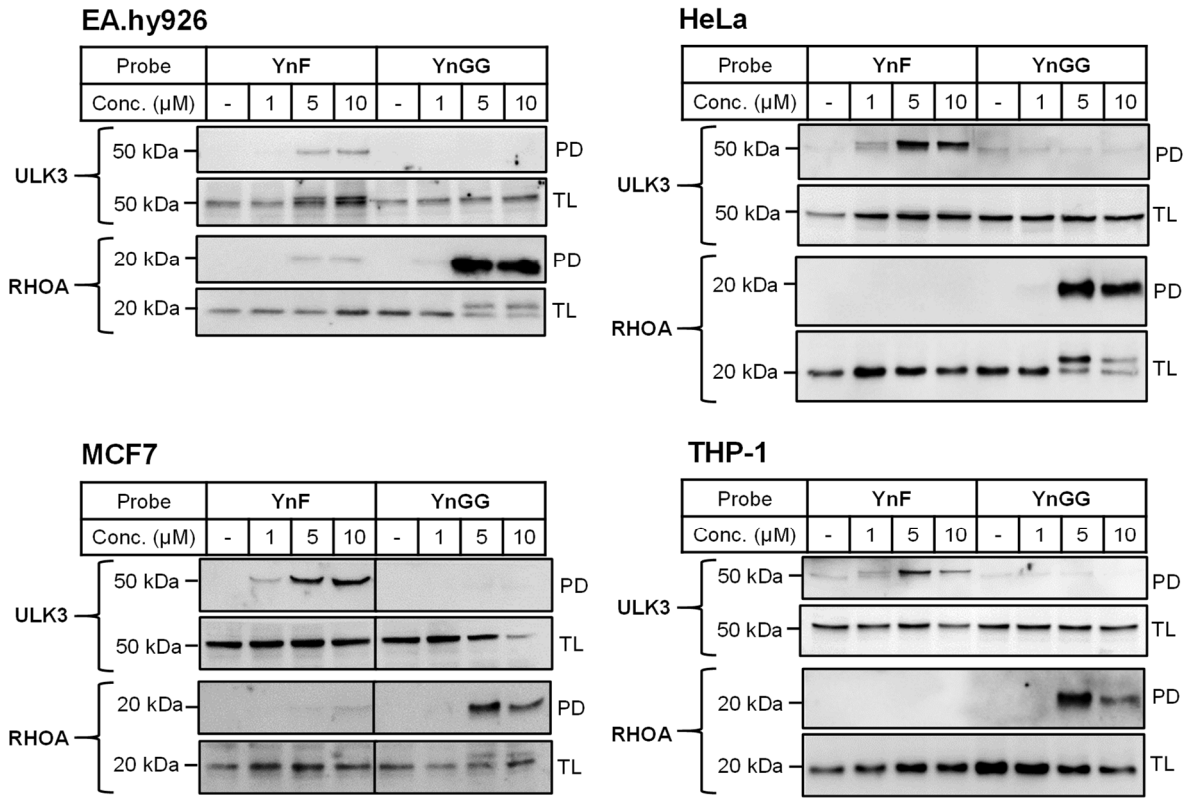
Supplementary Figure 5. YnF and YnGG show concentration- and substrate-dependent incorporation into prenylated proteins. EA.hy926 cells were treated with increasing concentrations of YnF or YnGG (1, 2, 5, 10 μM) or DMSO vehicle for 16 hours. Following CuAAC to capture reagent AzRB, labeled proteins were enriched on NeutrAvidin agarose and subjected to on-bead trypsin digest. The resultant peptides were labeled with 10-plex tandem mass tag (TMT) reagents and analyzed by nanoLC-MS/MS. The data was normalized as described in Biological Methods & Proteomics and proteins grouped according to their prenylation status as in Figure 2a and 2b. a) Known and novel farnesylated proteins show a dose-dependent increase in YnF labeling. b) Known geranylgeranylated and Rab proteins show a dose-dependent increase in YnGG labeling. Centre lines of box and whisker plots show the median and the box limits the 25th and 75th percentiles as calculated by GraphPad Prism, with Tukey whiskers. A Kruskal-Wallis test with a Dunn's post-test was performed to compare the $\log_2$ fold response at the highest probe concentration; * $p<0.05$, *** $p<0.001$. Dose-response curves of selected protein substrates show preferential incorporation of (c) YnF and (d) YnGG into farnesylated and geranylgeranylated substrates, respectively. Data points represent mean values (n=3).



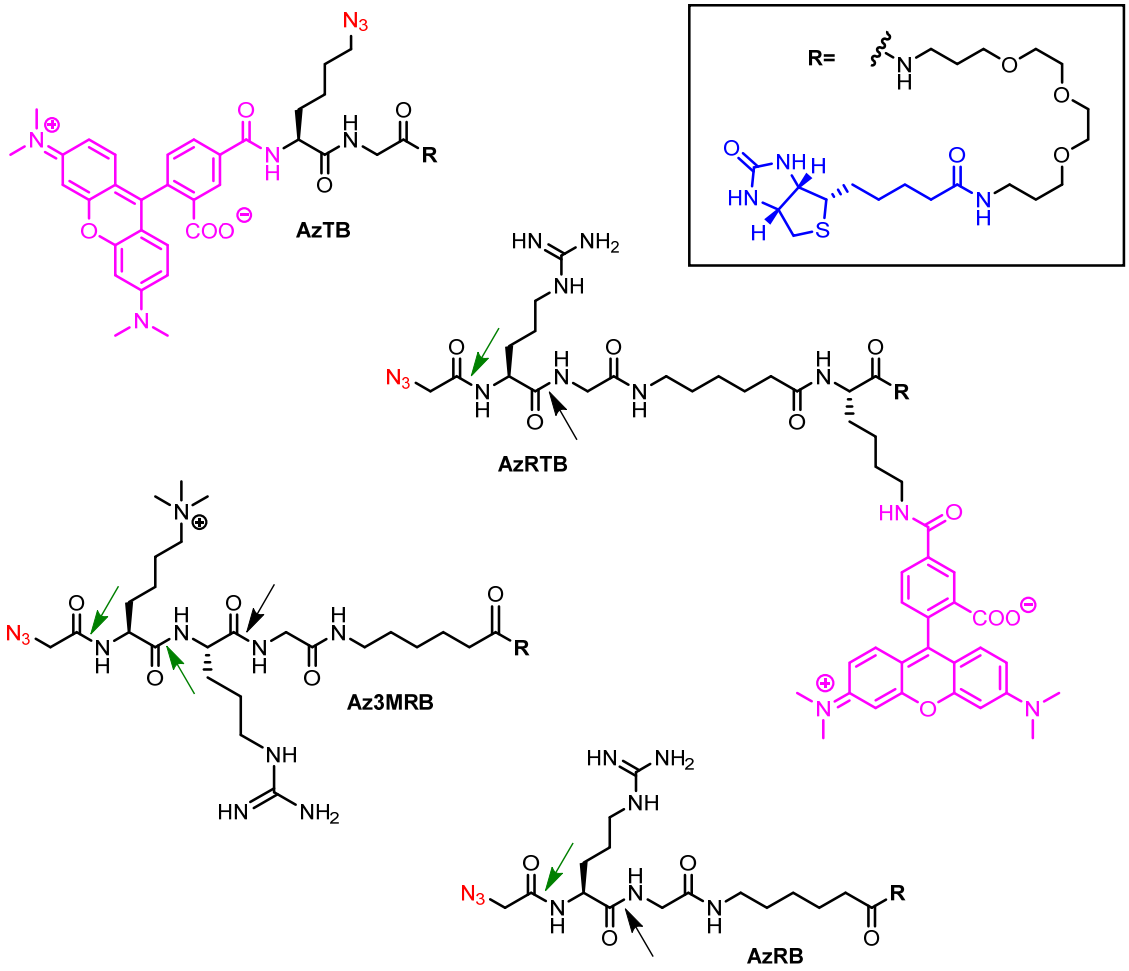
Supplementary Figure 6. In-gel fluorescence analysis of YnF and YnGG labeling in different cell types. HeLa, MCF7 and THP1 (monocytes) cells were cultured in medium supplemented with 1, 2, 5 or 10 µM YnF or YnGG for 16 hours. The lysate was subjected to CuAAC ligation with capture reagent AzTB, separated by SDS-PAGE and labeled proteins visualized by in-gel fluorescence (channel Cy3). Protein loading was assessed by Coomassie Blue staining.



Supplementary Figure 7. Immunoblot analysis of YnF and YnGG labeling in different cell types. EA.hy926, HeLa, MCF7 and THP1 (monocytes) cells were cultured in medium supplemented with 1, 2, 5 or 10 μM YnF or YnGG for 16 hours. The lysate was subjected to CuAAC ligation with capture reagent AzTB and labeled protein immobilized on NeutrAvidin resin. Protein pulldowns (PD) and total lysates before pulldown (TL) were analyzed by Western blot.

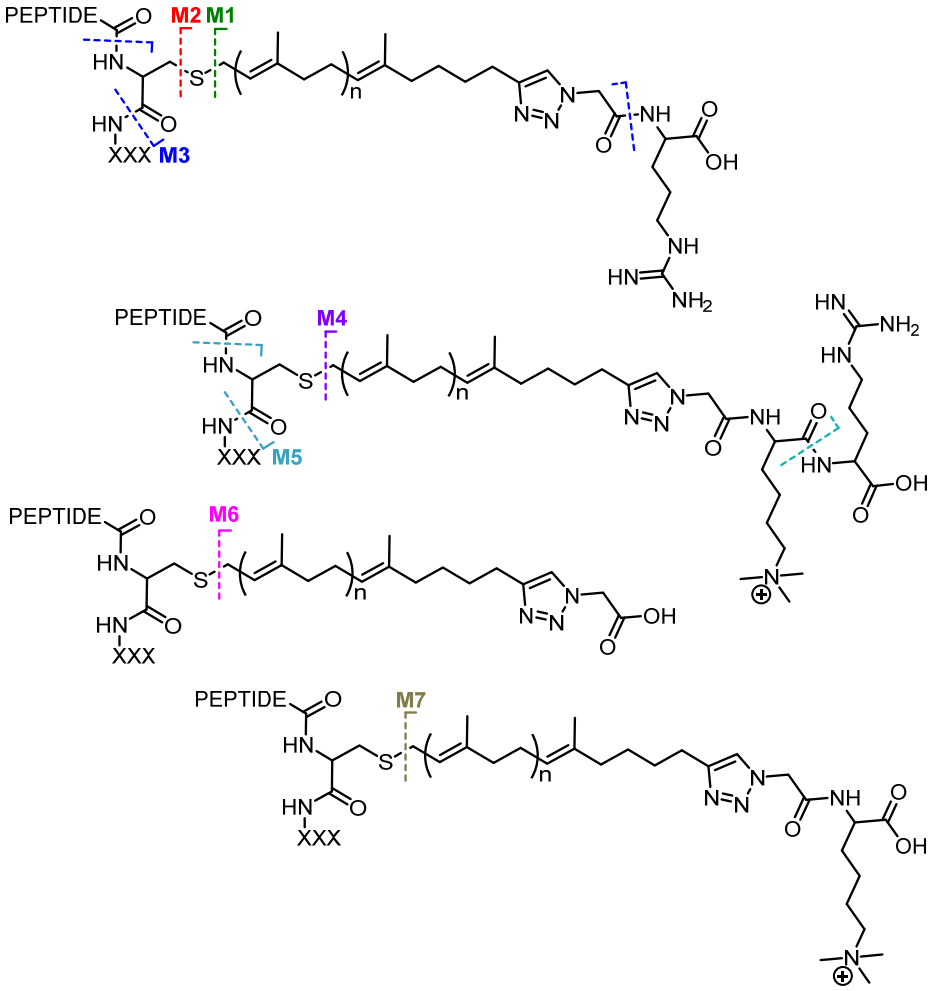


Supplementary Figure 8. Structures of capture reagents AzTB, AzRB, AzRTB and Az3MRB. Az = azide for CuAAC ligation, T = TAMRA fluorophore, B = biotin affinity handle, R = arginine for enzymatic cleavage, 3M = trimethyl-lysine. Trypsin (black arrow) and lysarginase (green arrow) cleavage sites are indicated.

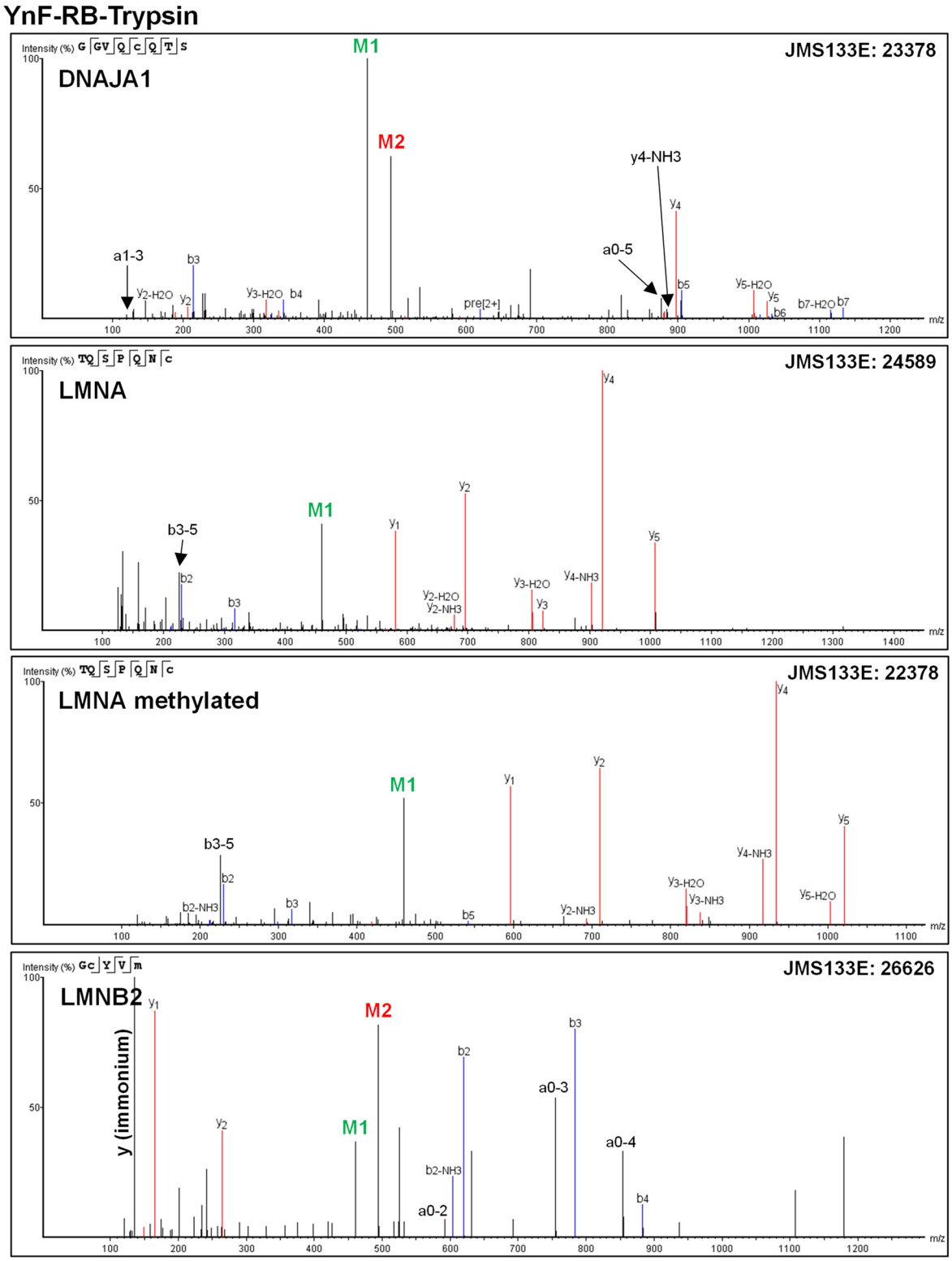


Supplementary Figure 9. Structures and *m/z* values of characteristic fragment ions of capture reagent-probe adduct in MS/MS spectra of modified peptides.

	CHARACTERISTIC FRAGMENT IONS (*m/z*)						
	AzRB/AzRTB-Trypsin			Az3MRB-Trypsin		AzRB/AzRTB-Lysarginase	Az3MRB-Lysarginase
	M1	M2	M3	M4	M5	M6	M7
YnF	460.3	494.3	392.2	630.4	-	-	-
YnGG	528.4	562.3	-	698.5	630.4	372.2	542.4

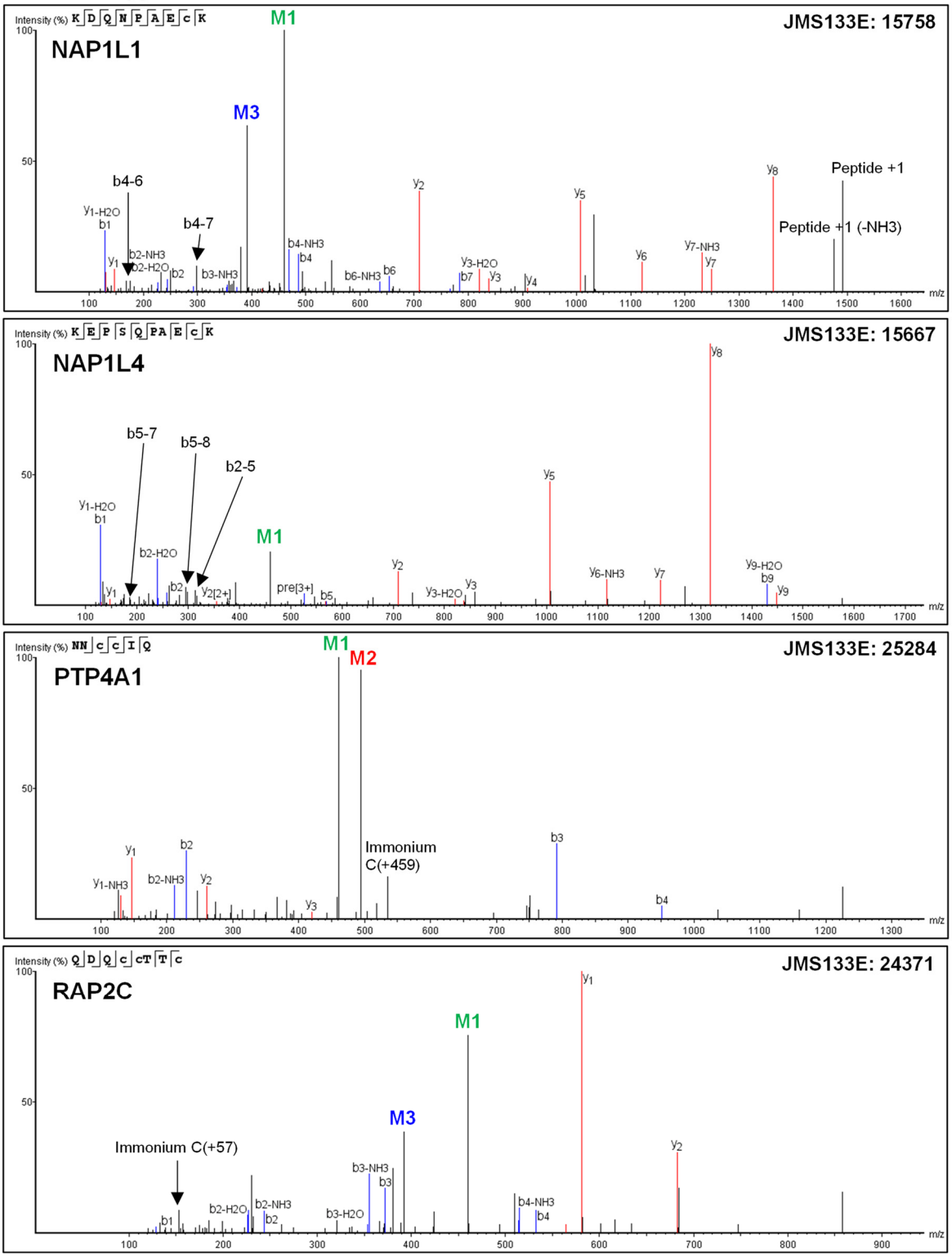


Supplementary Figure 10. MS/MS spectra of probe-modified peptides. Probe labeling (YnF/YnGG), capture reagent (AzRB/AzRTB/Az3MRB) and digest (trypsin/lysarginase) conditions are indicated in figure. Structures and *m/z* values of characteristic fragment ions (M1-M7) are detailed in Supplementary Figure 9.



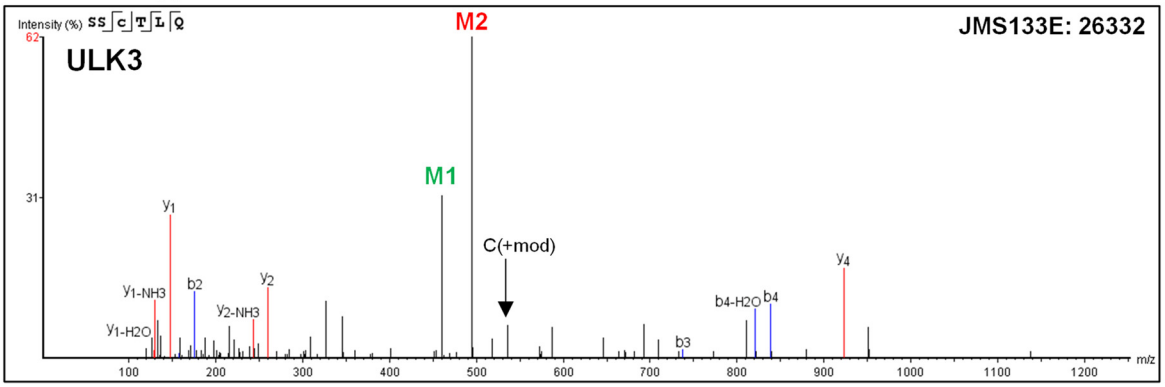
Supplementary Figure 10 cont.

YnF-RB-Trypsin



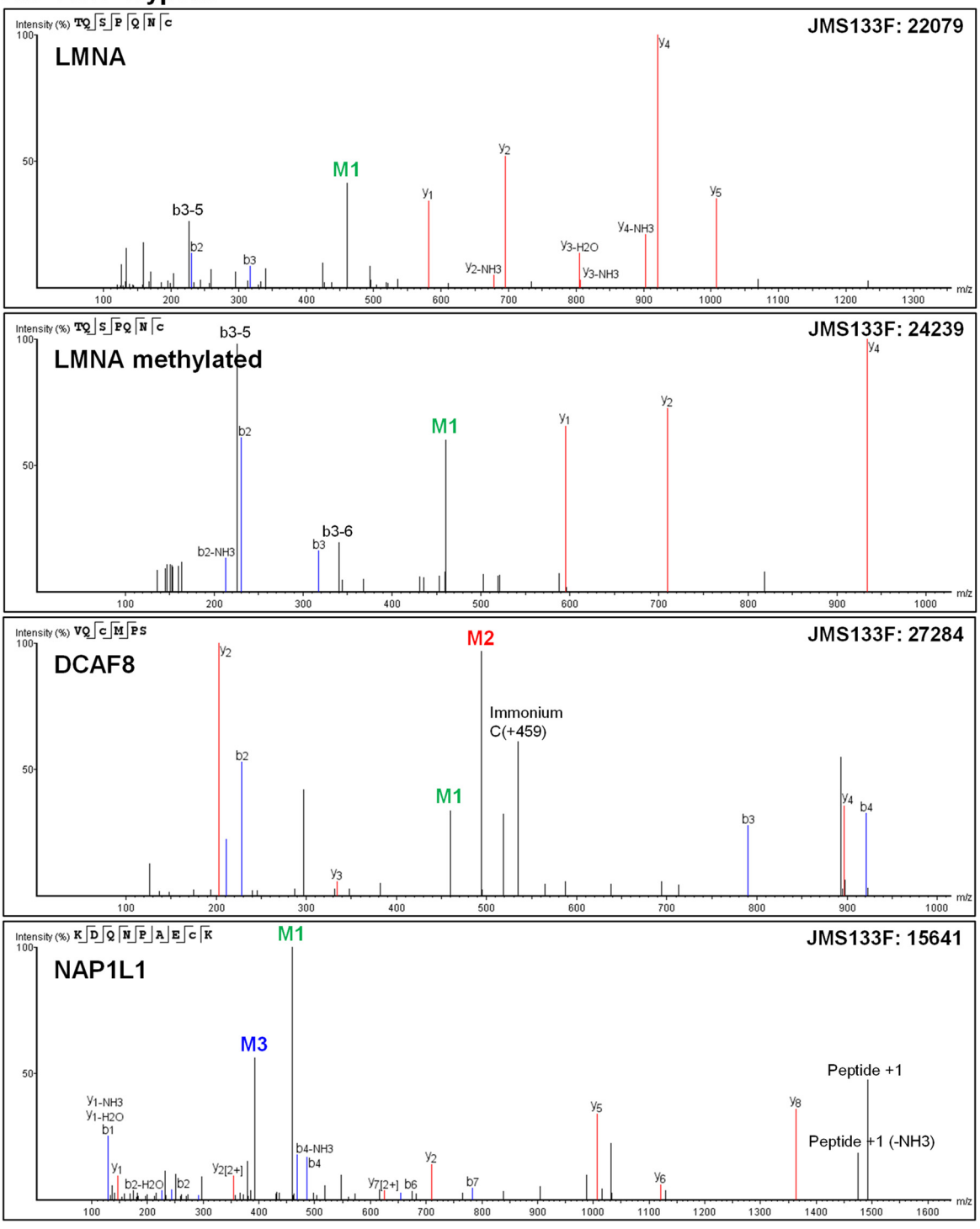
Supplementary Figure 10 cont.

YnF-RB-Trypsin



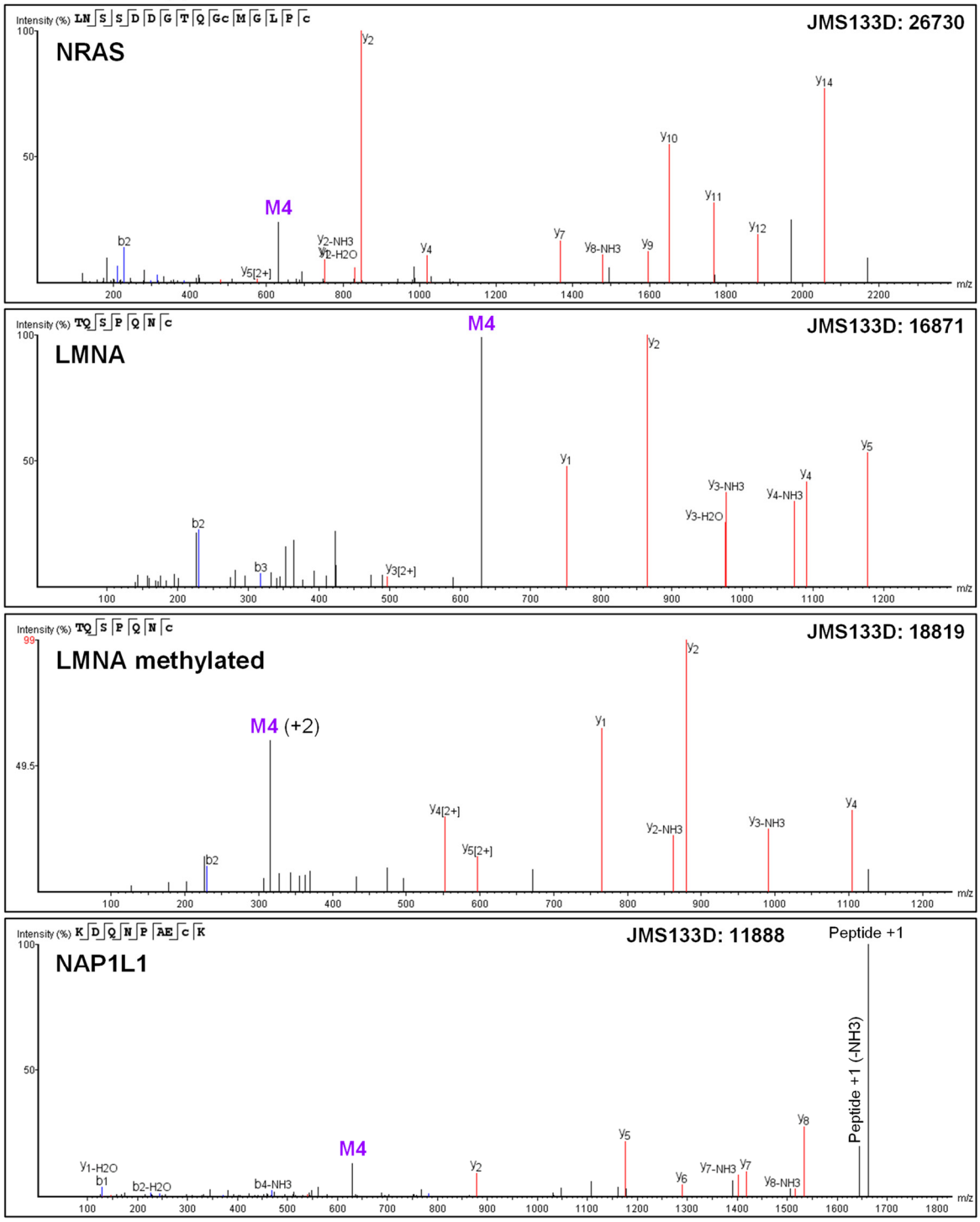
Supplementary Figure 10 cont.

YnF-RTB-Trypsin



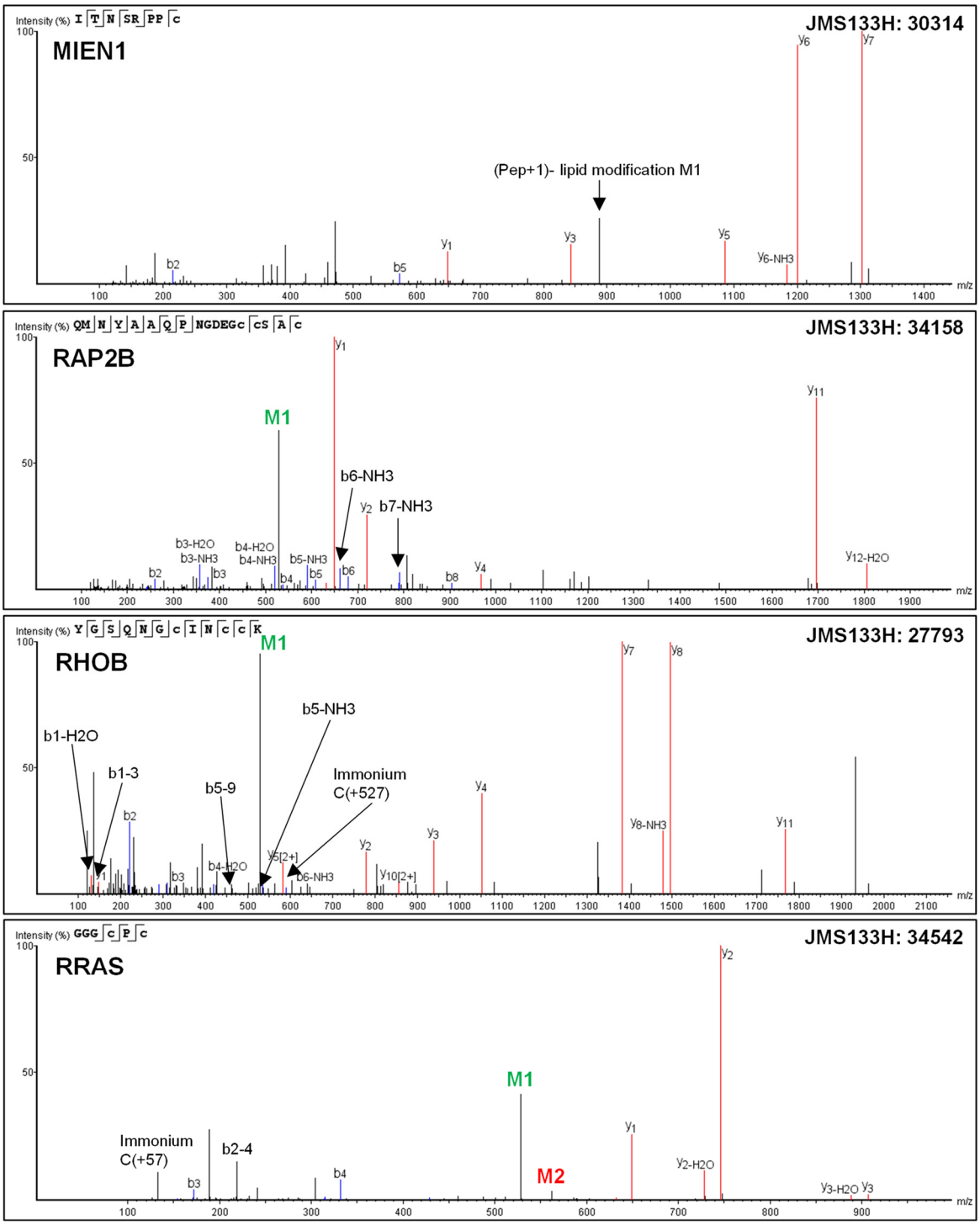
Supplementary Figure 10 cont.

YnF-3MRB-Trypsin



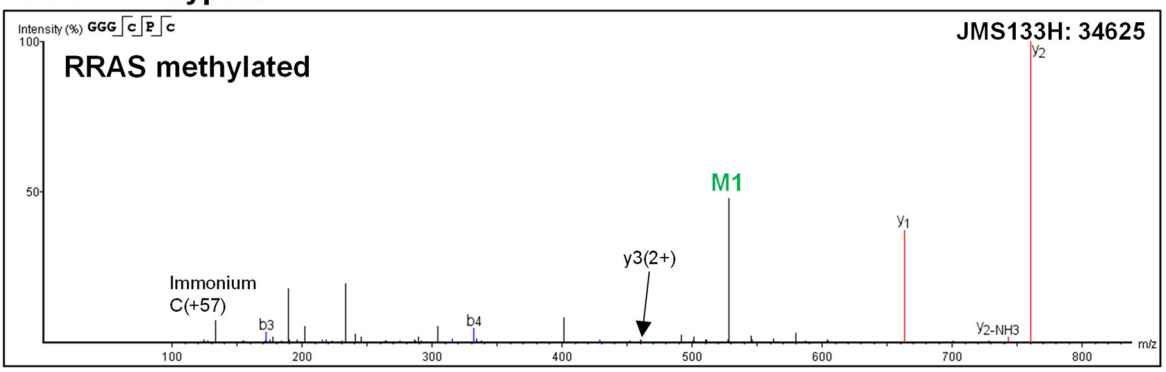
Supplementary Figure 10 cont.

YnGG-RB-Trypsin



Supplementary Figure 10 cont.

YnGG-RB-Trypsin



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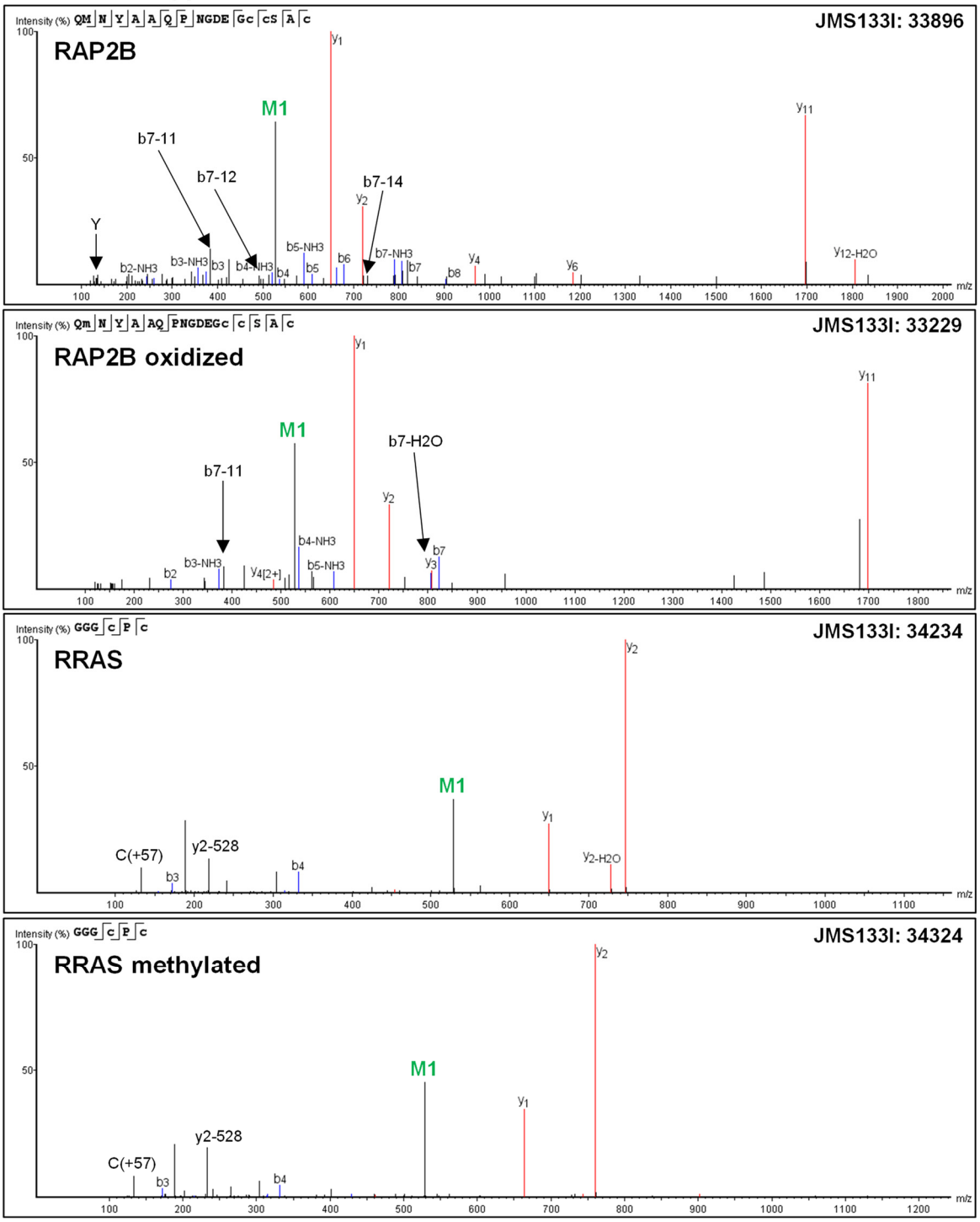
YnGG-RTB-Trypsin



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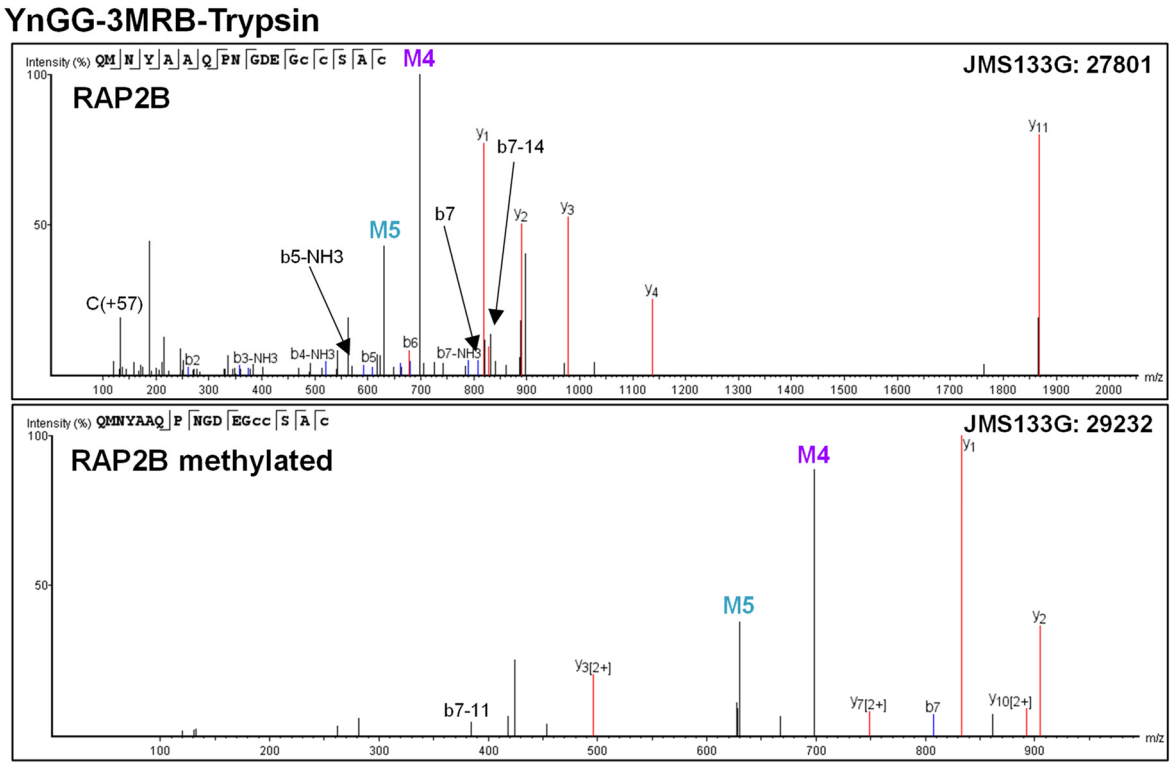
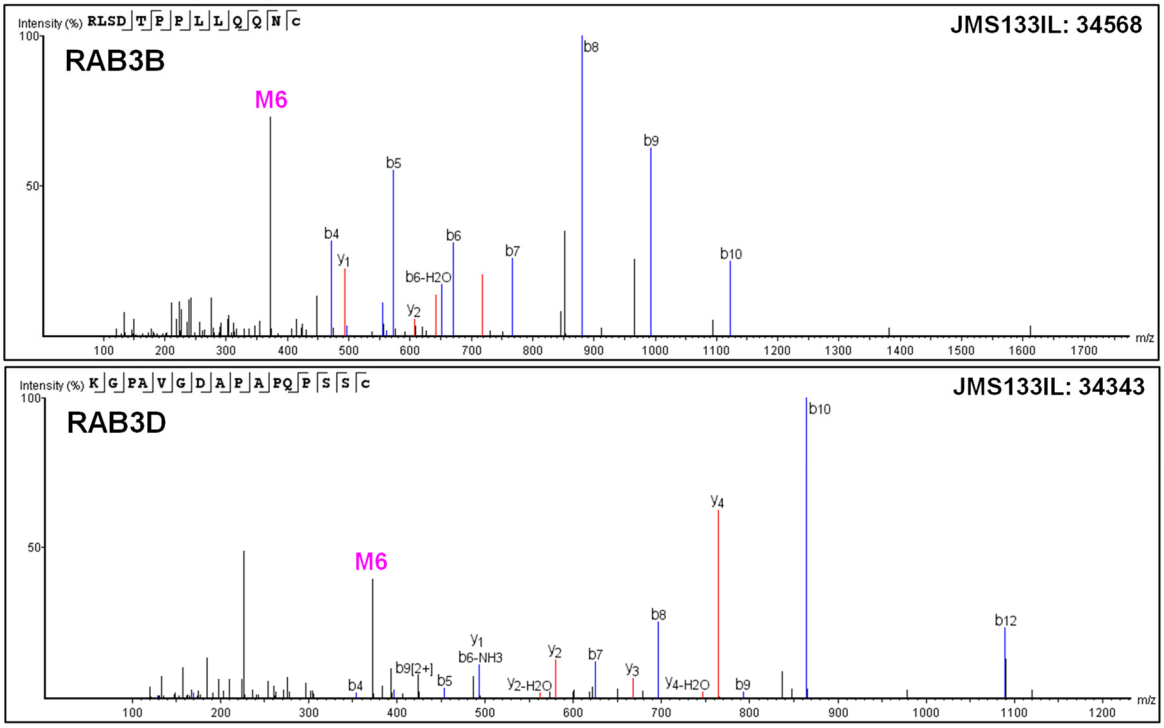
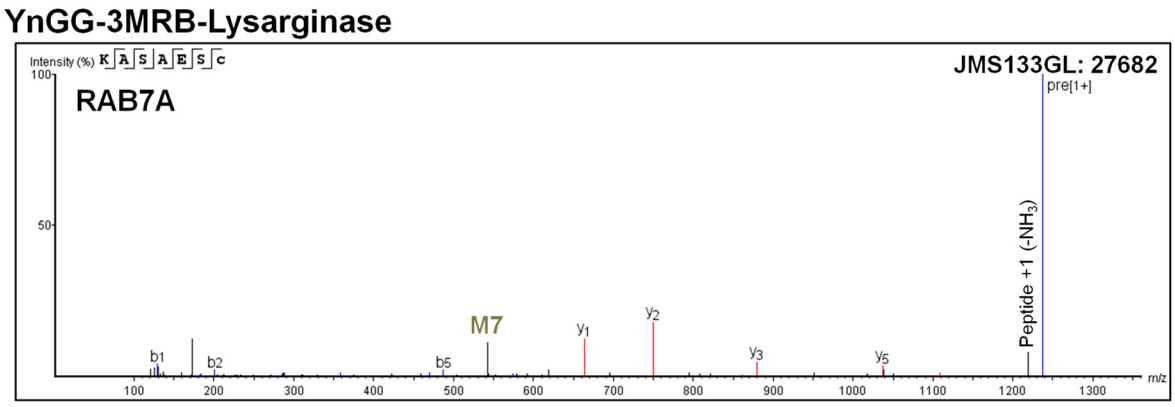


Supplementary Figure 10 cont.

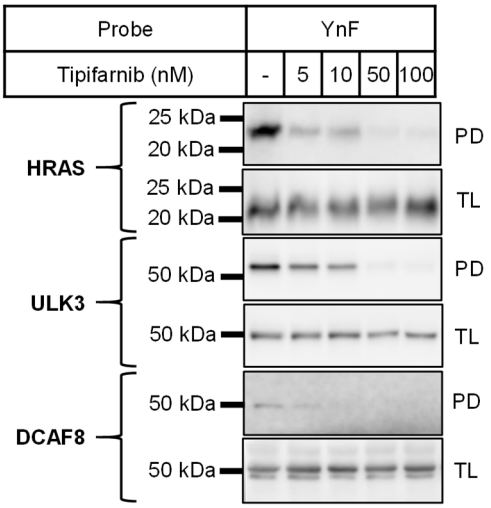
YnGG-RTB-Lysarginase



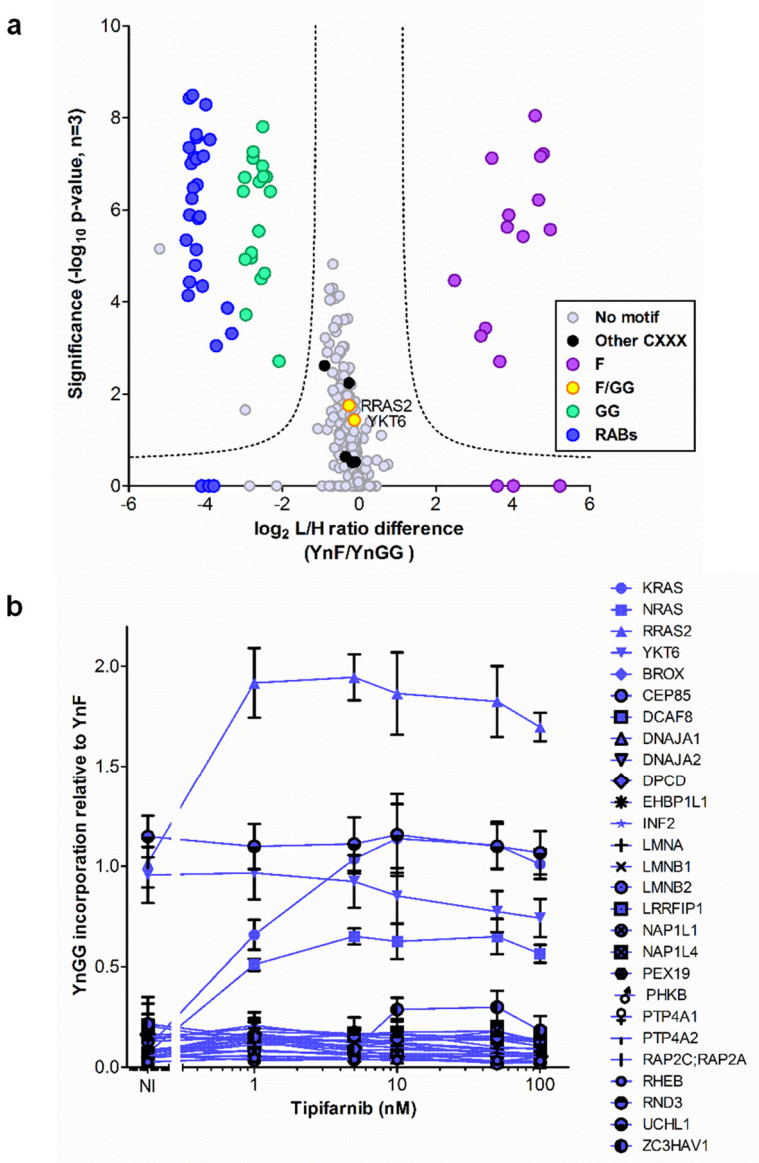
Supplementary Figure 10 cont.



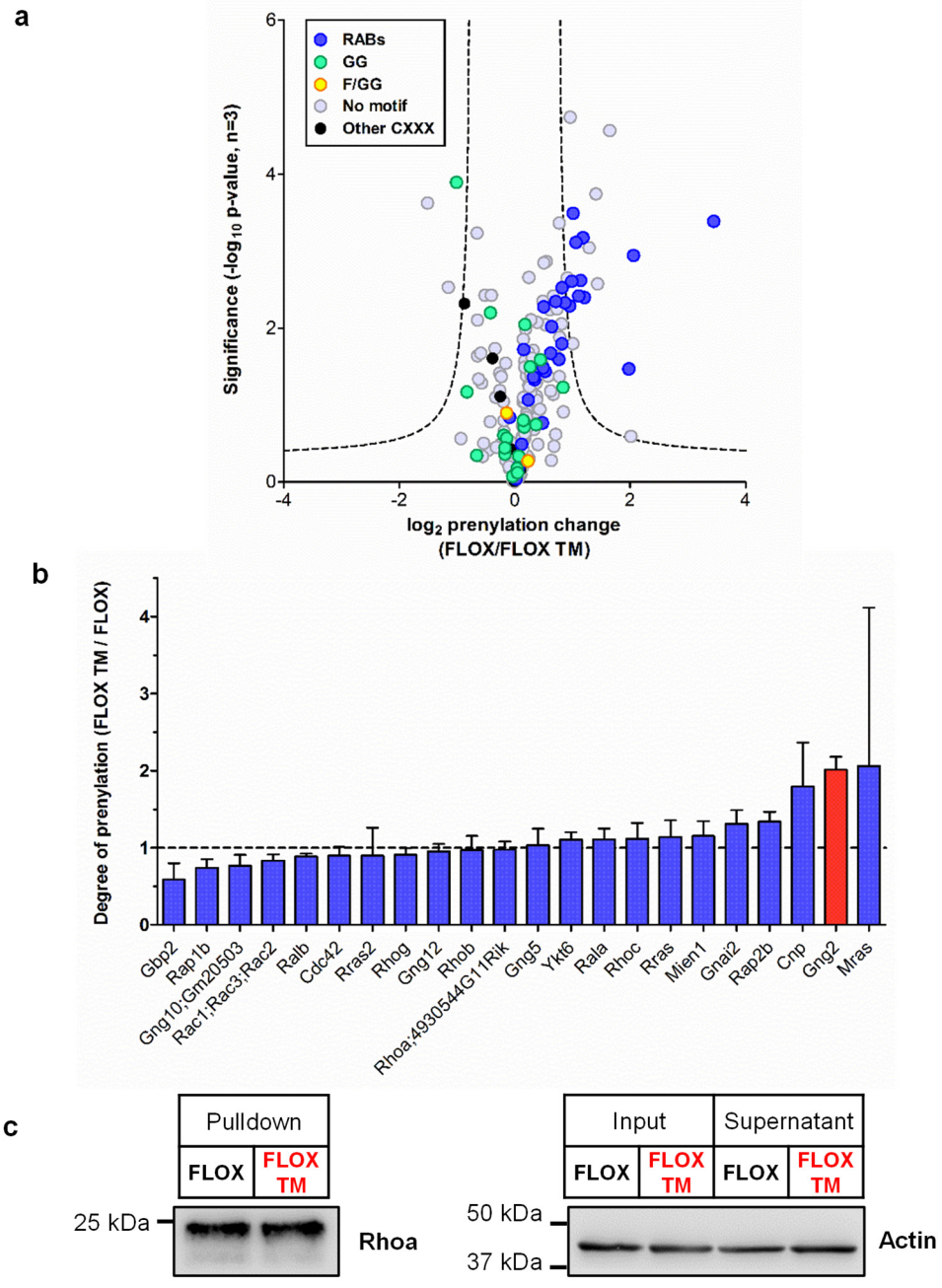
Supplementary Figure 11. Immunoblot analysis of YnF labeling in response to Tipifarnib treatment. EA.hy926 were pre-treated with increasing concentration of Tipifarnib for 1 hour. YnF (10 μM) was added to the medium and the cells were cultured for a further 8 hours. Protein lysates were subjected to CuAAC with capture reagent AzTB and labeled proteins immobilized on NeutrAvidin beads. Purified proteins were eluted and subjected to immunoblot analysis. PD = pulldown, TL = total lysate input.



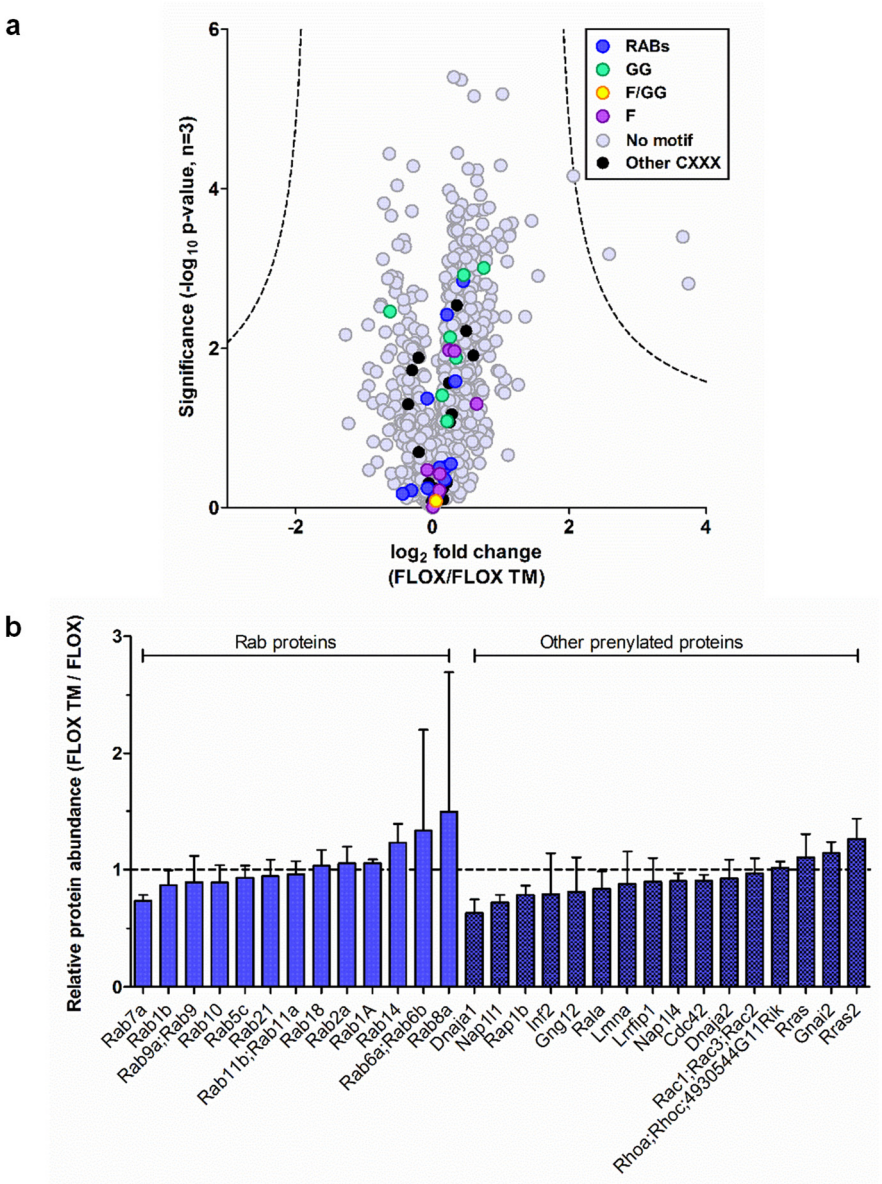
Supplementary Figure 12. YnF/YnGG probe preference analysis and prenylation switch in response to Tipifarnib treatment. EA.hy926 cells were cultured in the presence of YnF or YnGG (10 μM) for 24 hours. For Tipifarnib-treated samples cells were pre-treated with increasing concentrations of inhibitor for 1 hour before addition of YnGG. Cell lysates were combined with spike-in lysate triply labeled with YnF, YnGG and R10K8 SILAC label. Following CuAAC to capture reagent AzRB labeled proteins were enriched on NeutrAvidin agarose and subjected to on-bead trypsin digest. The resultant peptides were analyzed by nanoLC-MS/MS and the data processed in MaxQuant. a) Volcano plot summarizing the result of a two-sample t-test comparing the $\log_2$ L/H ratios of YnF versus YnGG labeled samples in the absence of inhibitor treatment (n=3, Permutation-based FDR=0.01, S0=1). b) Assessment of YnGG incorporation relative to YnF in response to Tipifarnib treatment for all farnesylated substrates quantified. Data points represent mean values (n=3) ± standard deviation.



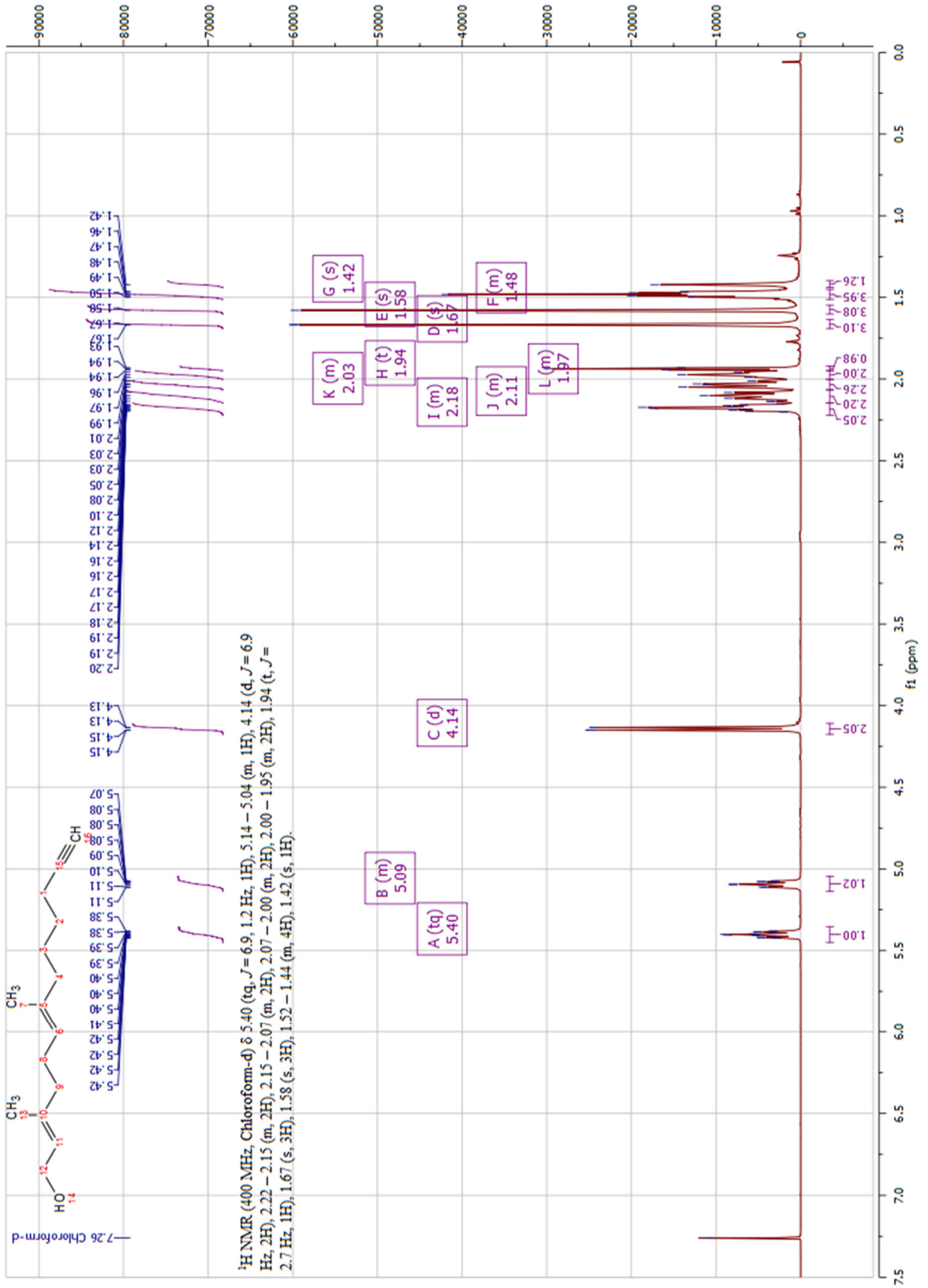
Supplementary Figure 13. Rep-1 knockout pulldown analysis. Rep-1 knock-out (FLOX TM) and control (FLOX) MEFs (mouse embryonic fibroblasts) were cultured in medium supplemented with YnGG (10 µM) for 24 hours. The protein lysates were combined with an aliquot of spike-in lysate prepared from NIH 3T3 cells dually labeled with YnGG and R10K8 SILAC label. The samples were subjected to CuAAC ligation, enrichment on NeutrAvidin beads and on-bead trypsin digest. The resultant peptides were analyzed by nanoLC-MS/MS and data processed in MaxQuant. a) Volcano plot summarizing the result of a two-sample t-test comparing the $\log_2$ L/H ratios of FLOX TM versus FLOX samples (n=3, Permutation-based FDR=0.01, S0=1). b) Degree of YnGG labeling of CXXX substrates in FLOX TM versus FLOX mouse fibroblasts. Protein with a statistically significant change in YnGG labeling (n=3, Permutation-based FDR=0.01, S0=1) is shown in red. Data presented as mean ± standard deviation. c) Immunoblot analysis of YnGG-labeled Rhoa in FLOX and FLOX TM mouse fibroblasts. Note that by convention protein names are in lowercase for mouse (e.g. Rep-1), and in uppercase for human (REP-1).



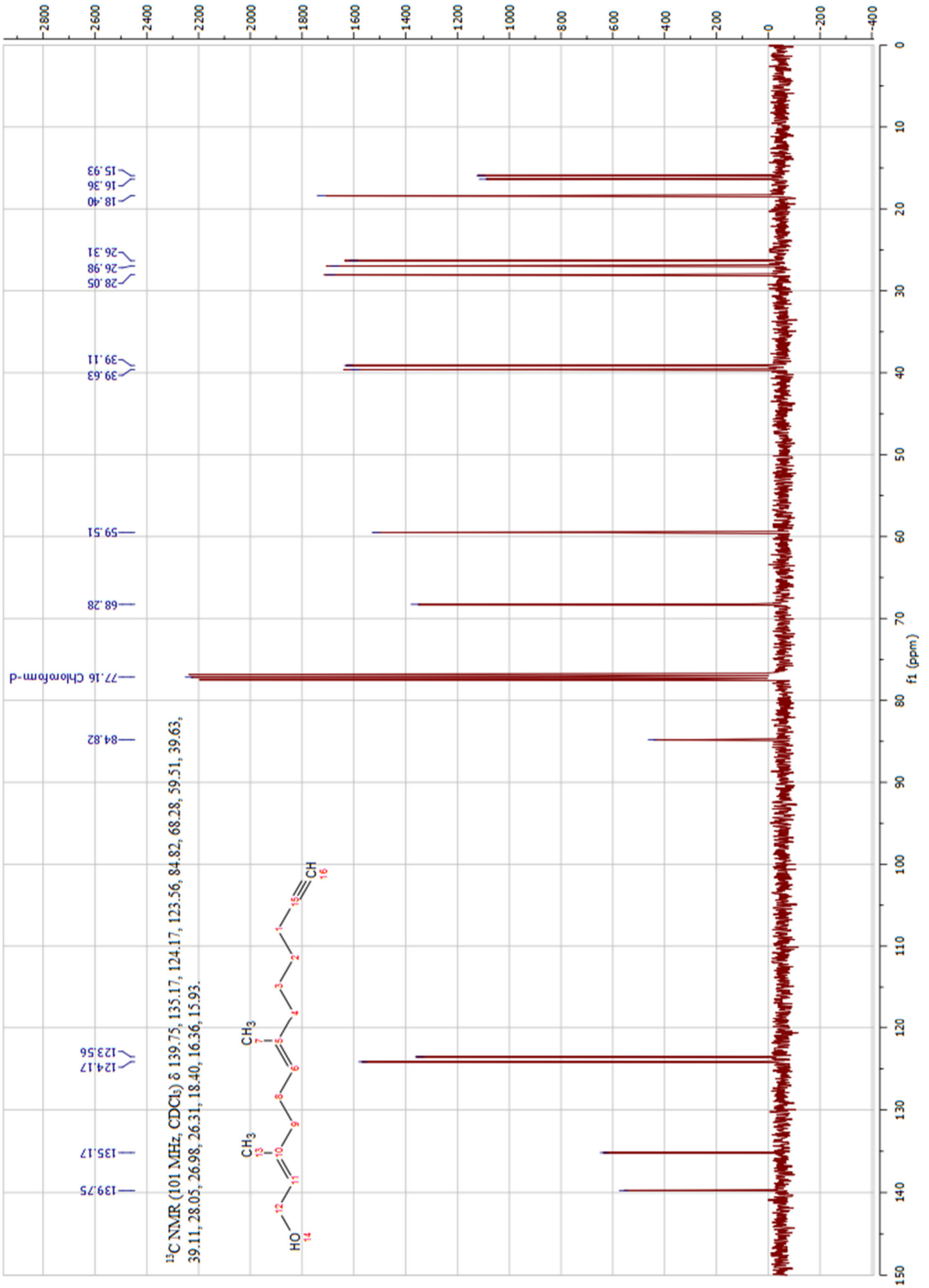
Supplementary Figure 14. Rep-1 knockout whole proteome analysis. FLOX TM and FLOX cell lysates were combined with an aliquot of NIH 3T3 spike-in lysate and subjected to trypsin digest. The resultant peptides were analyzed by nanoLC-MS/MS and data processed in MaxQuant. a) Volcano plot summarizing the result of a two-sample t-test comparing the $\log_2$ L/H ratios of FLOX TM versus FLOX samples (n=3, Permutation-based FDR=0.01, S0=1). b) Comparison of relative abundance of Rabs and other prenylated proteins in FLOX TM and FLOX cells. Data presented as mean (n=3) ± standard deviation. Note that by convention protein names are in lowercase for mouse (e.g. Rep-1), and in uppercase for human (REP-1).



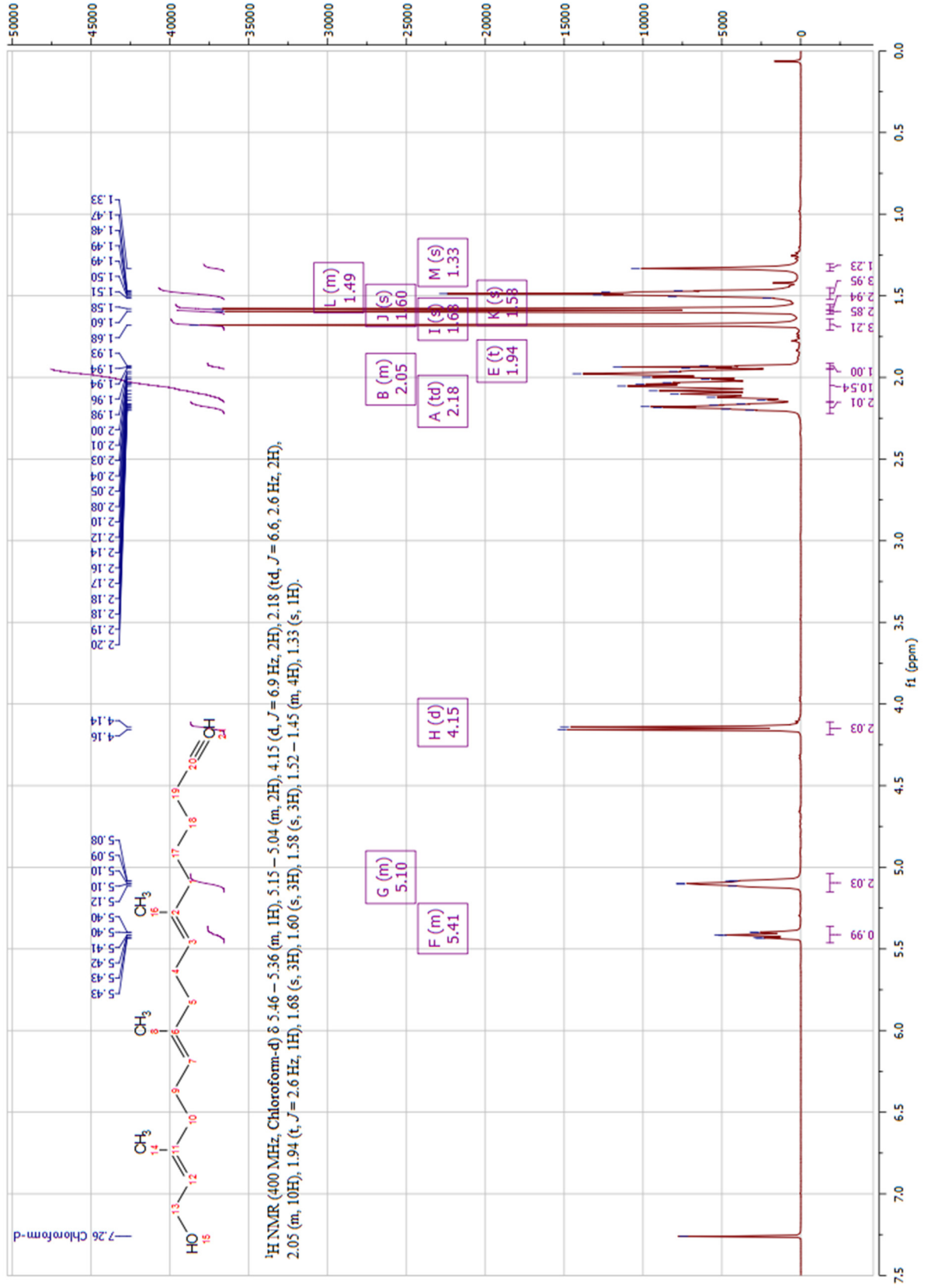
Supplementary Figure 15. 1H NMR Spectra of YnF



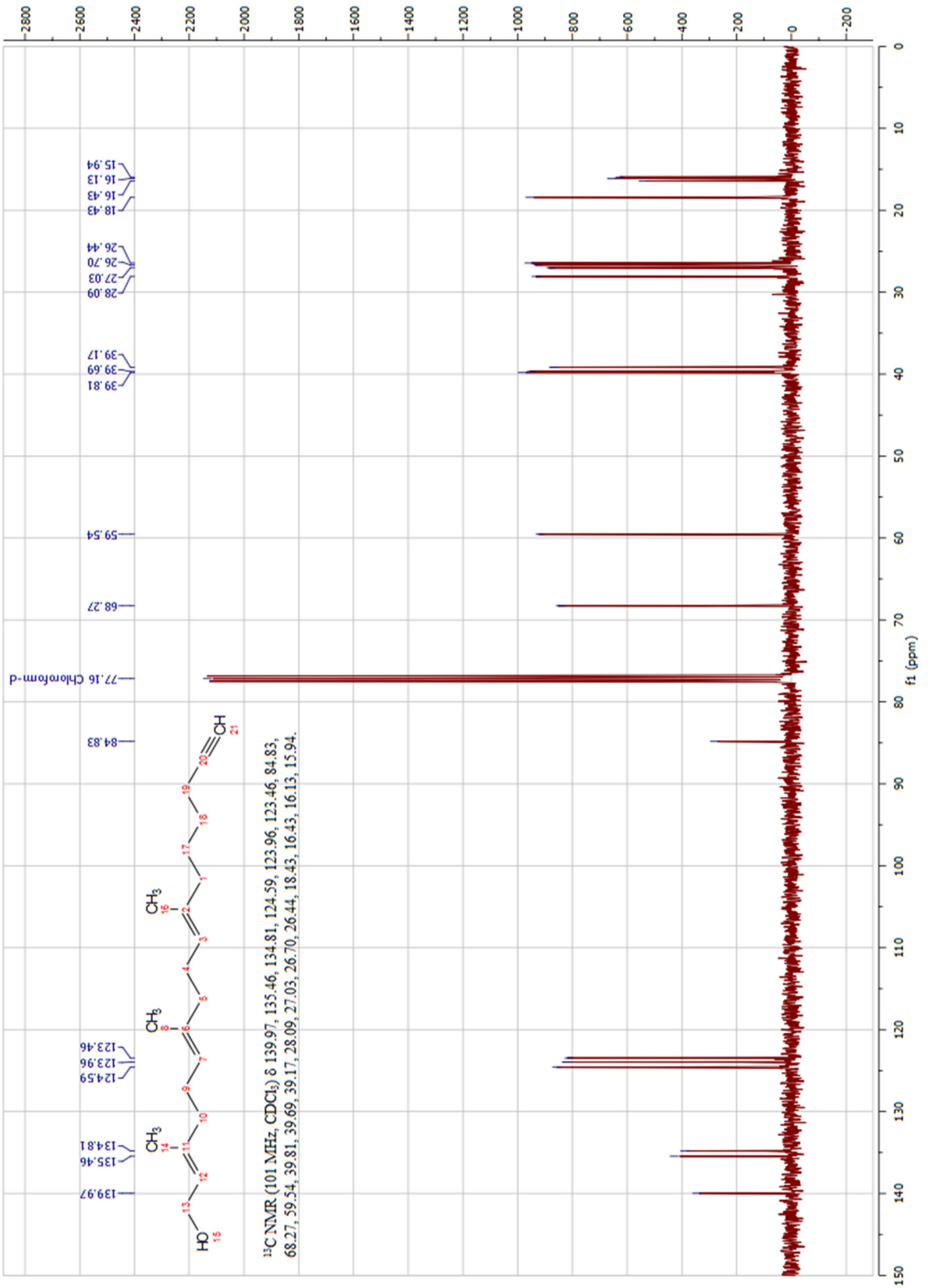
Supplementary Figure 16. 13C NMR Spectra of YnF



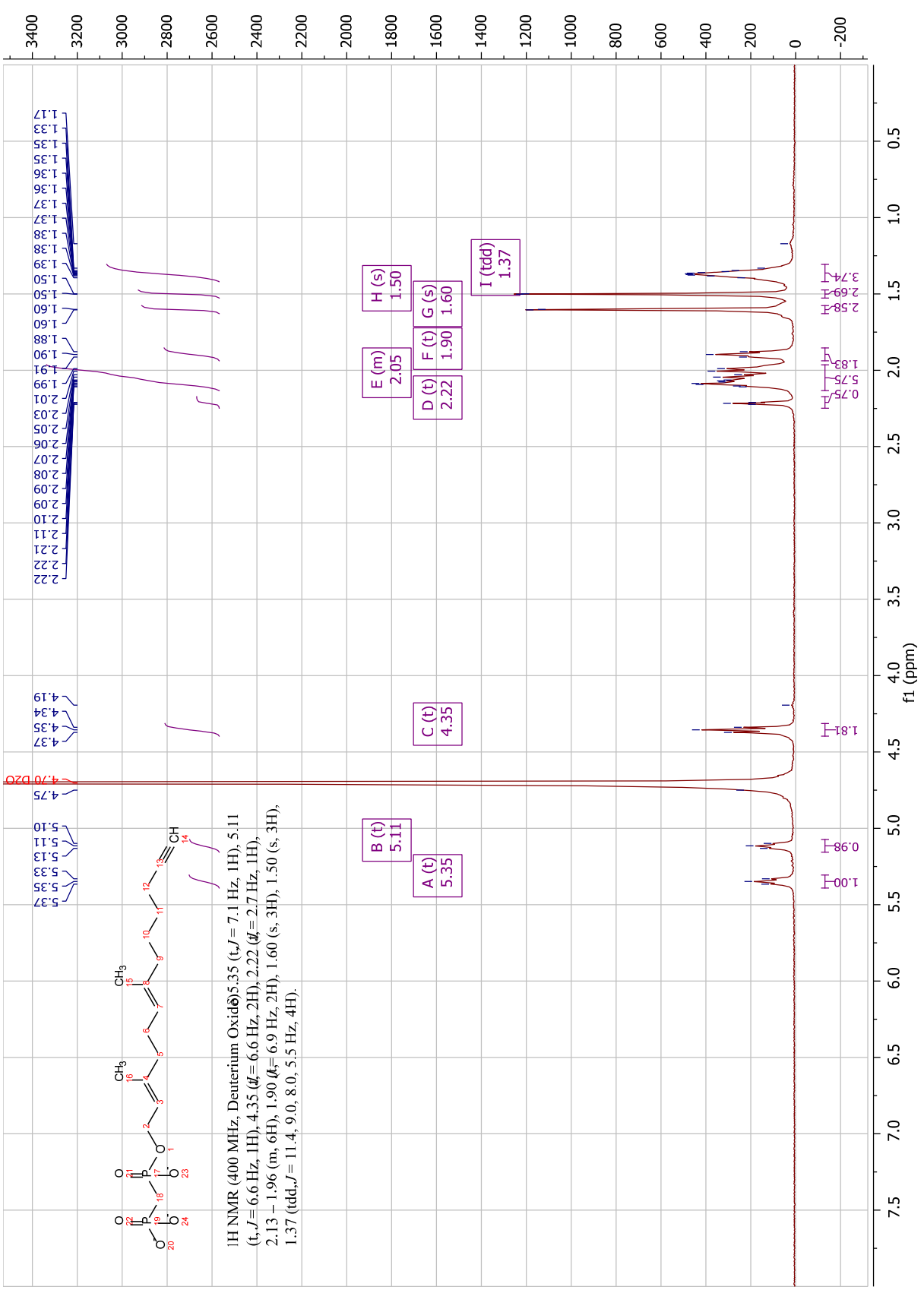
Supplementary Figure 17. 1H NMR Spectra of YnGG



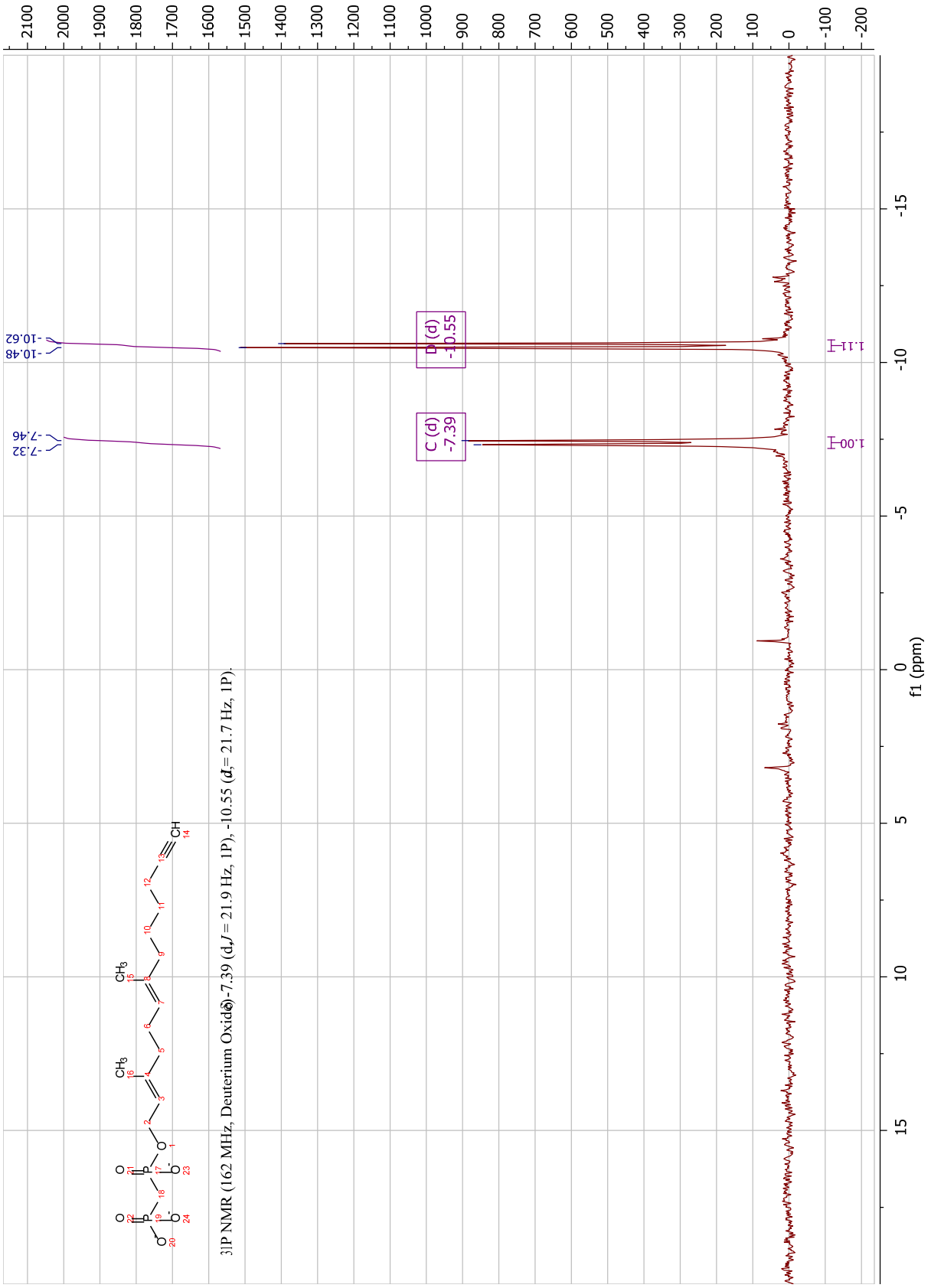
Supplementary Figure 18. 13C NMR Spectra of YnGG



Supplementary Figure 19. 1H NMR Spectra of YnFPP

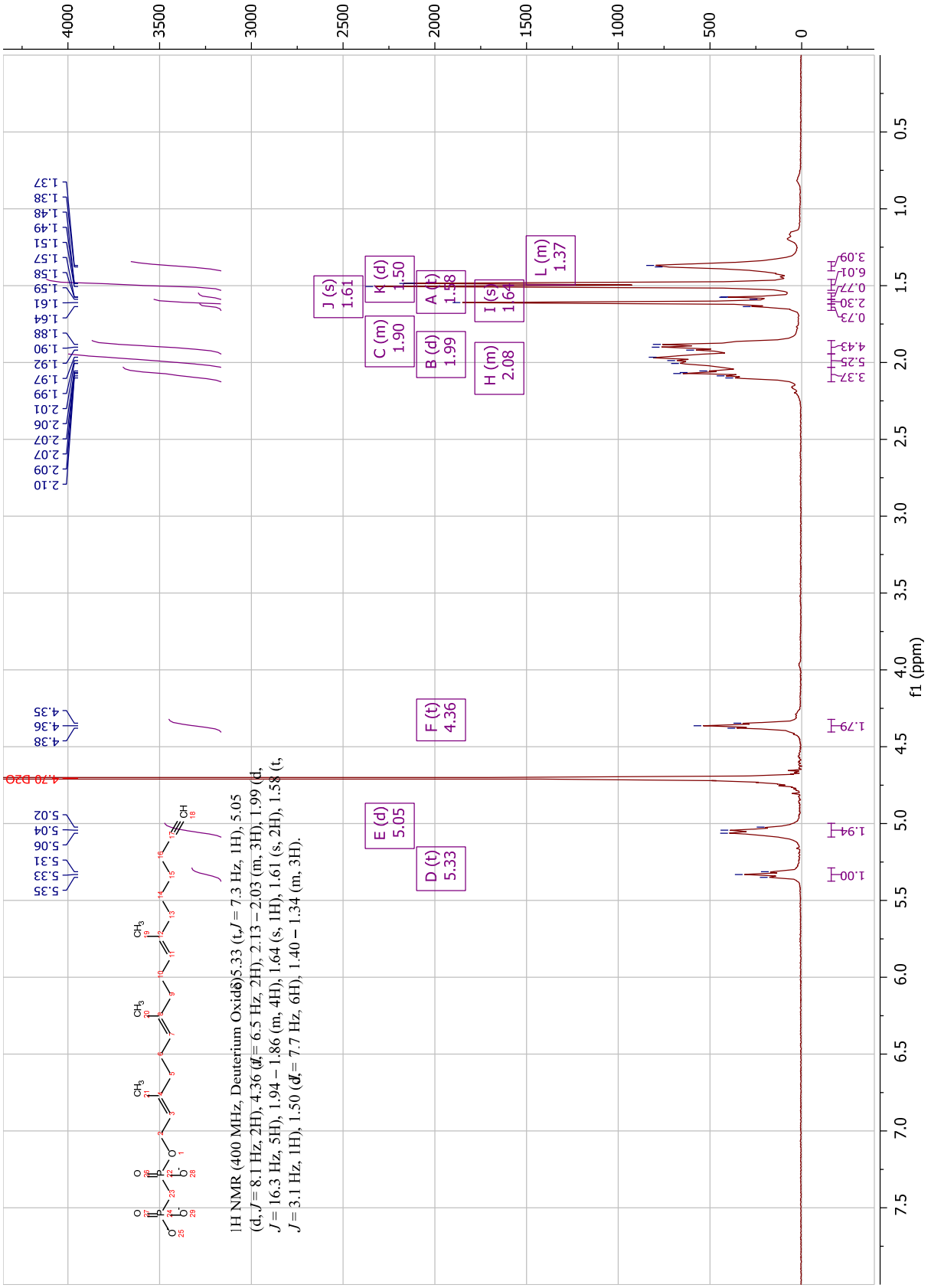


Supplementary Figure 20. 31P NMR Spectra of YnFPP

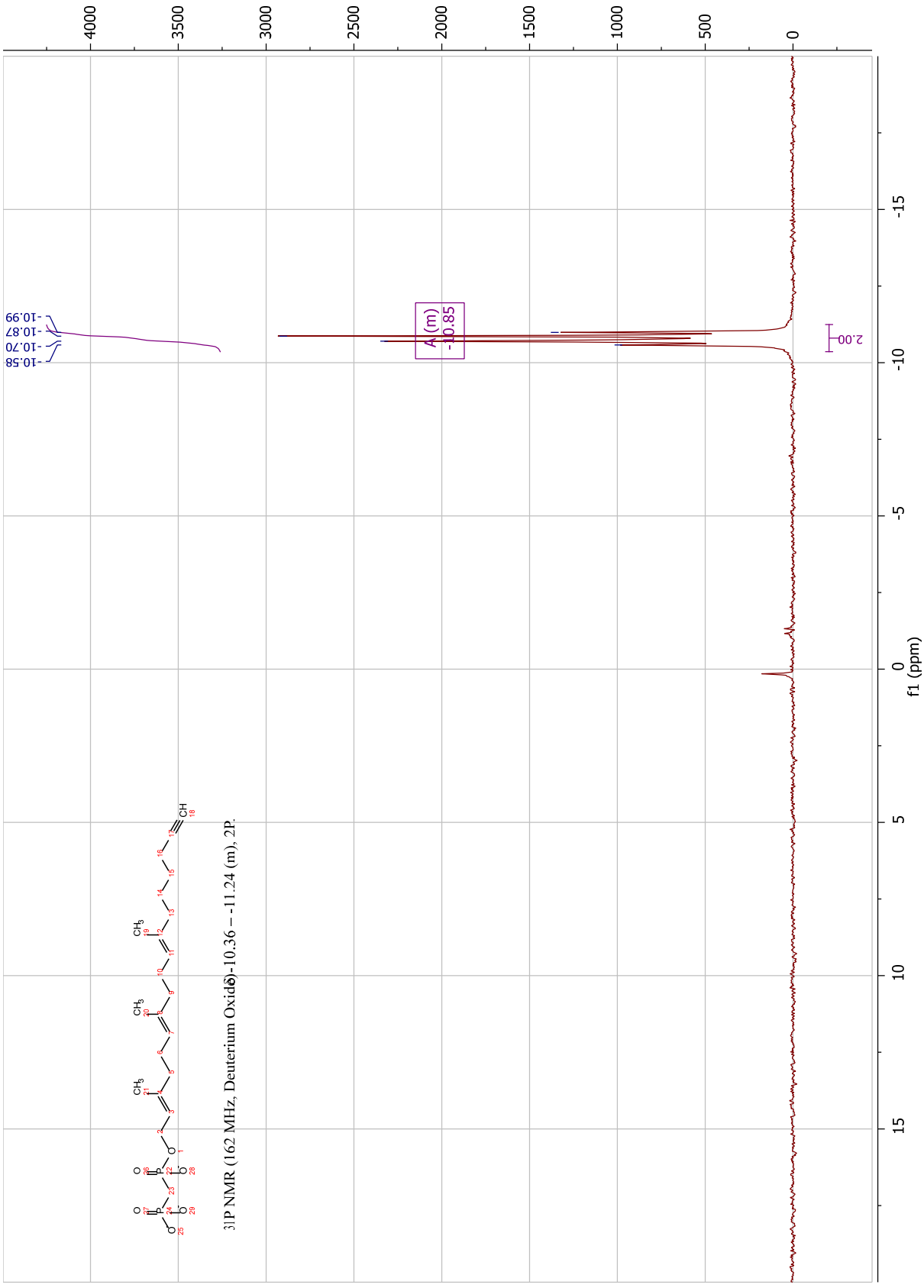


^{31}P NMR (162 MHz, Deuterium Oxide) δ -7.39 (d, J = 21.9 Hz, 1P), -10.55 (d, J = 21.7 Hz, 1P).

Supplementary Figure 21. 1H NMR Spectra of YnGGPP



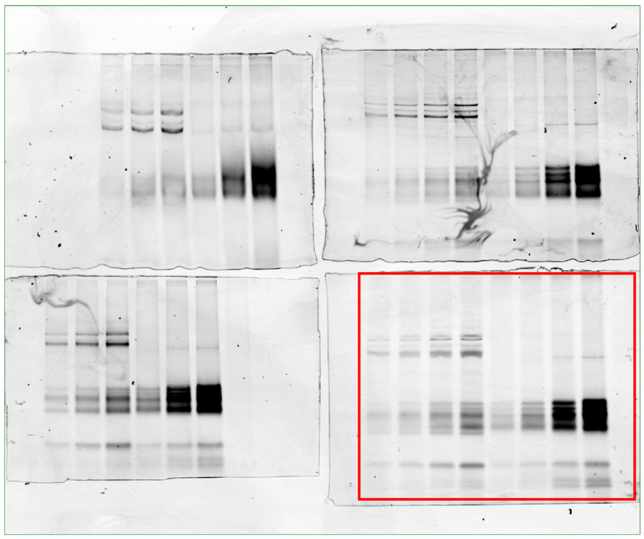
Supplementary Figure 22. 31P NMR Spectra of YnGGPP



Supplementary Figure 23. Uncropped gels from a) Figure 1c and b) Supplementary Figure 3a

a

In-gel fluorescence, channel Cy3



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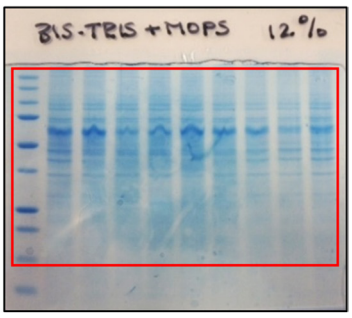
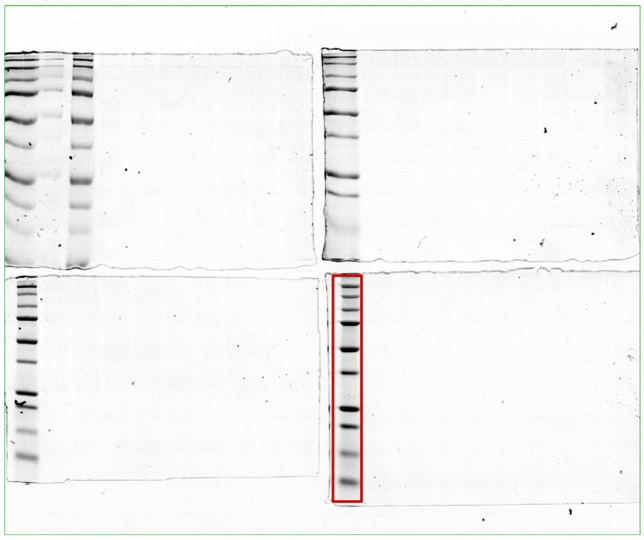
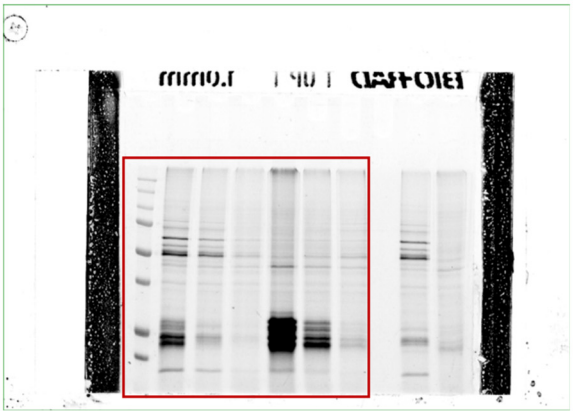


In-gel fluorescence, mw marker, channel Cy5

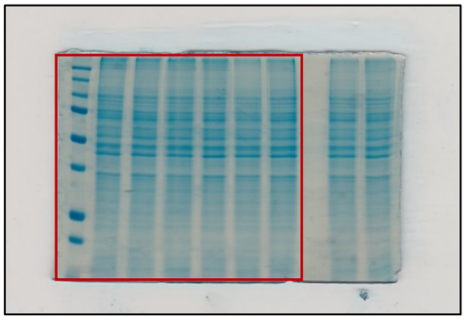


b

In-gel fluorescence, channel Cy3



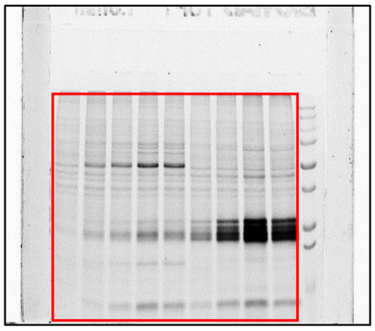
Coomassie



Supplementary Figure 24. Uncropped gels from Supplementary Figure 6

HeLa

In-gel fluorescence, channel Cy3



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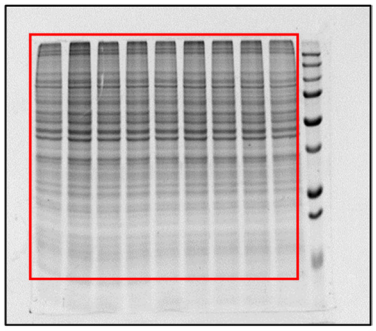
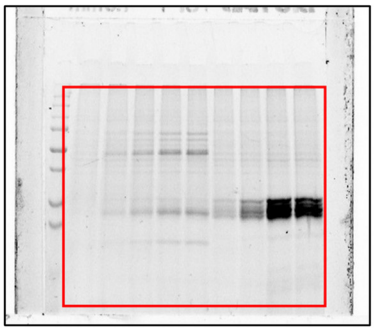


MCF7

In-gel fluorescence, channel Cy3



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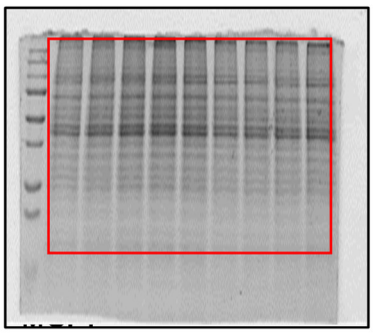
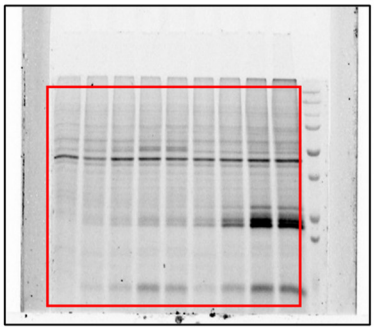


THP1

In-gel fluorescence, channel Cy3



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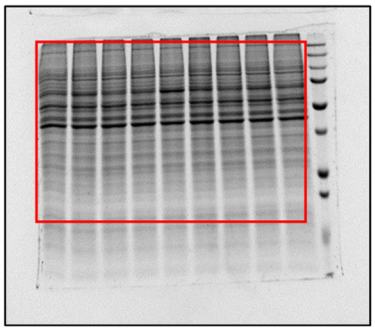
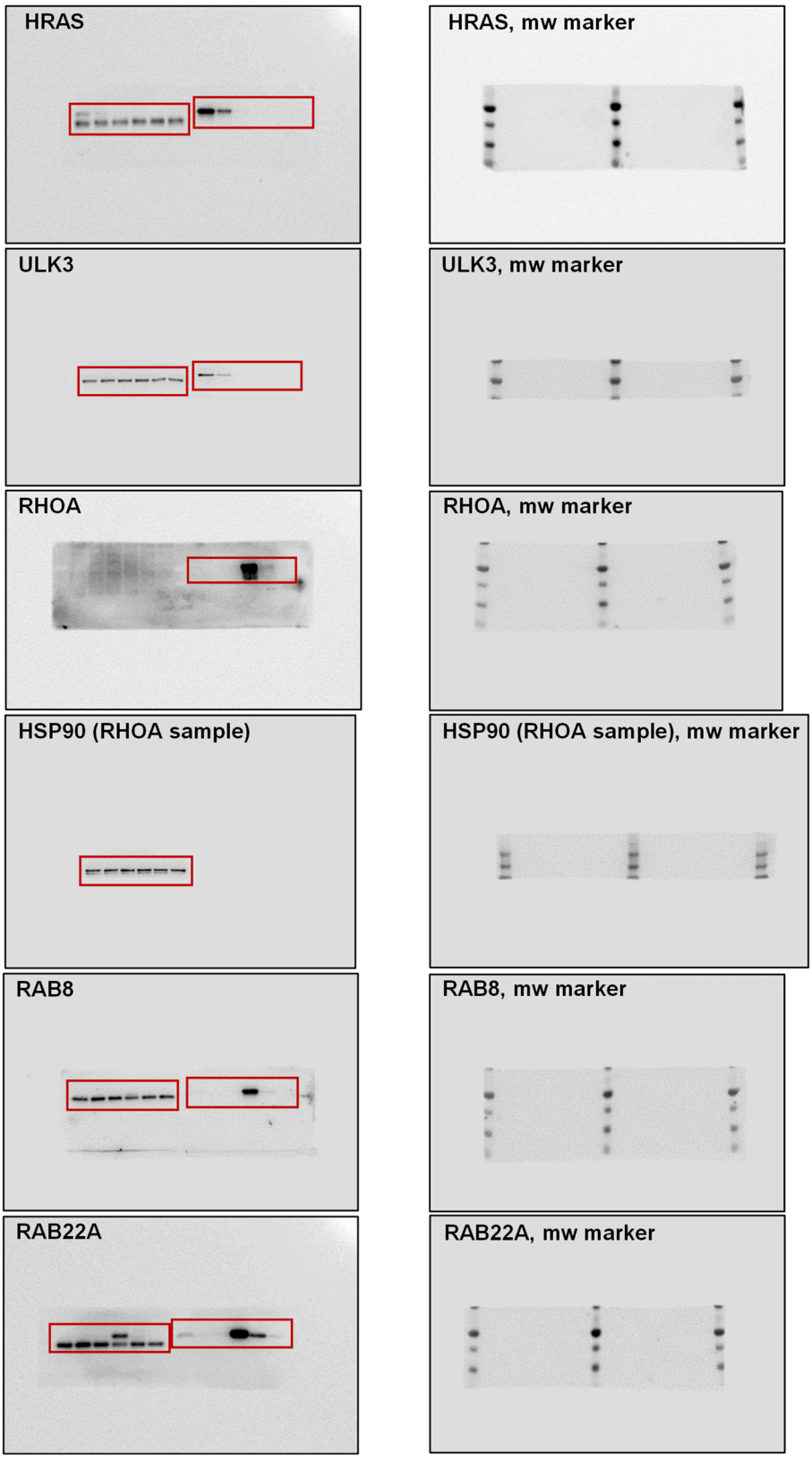
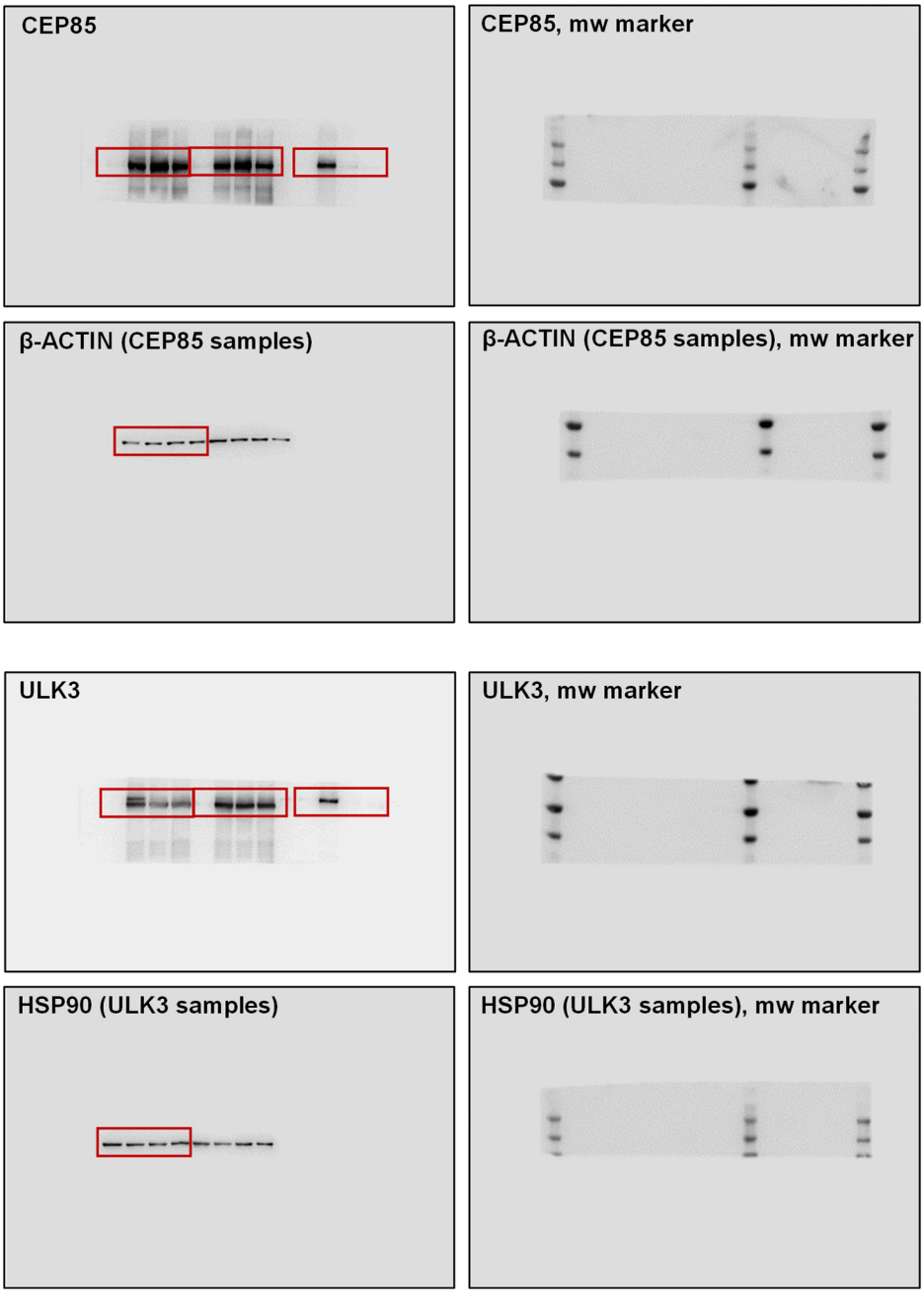


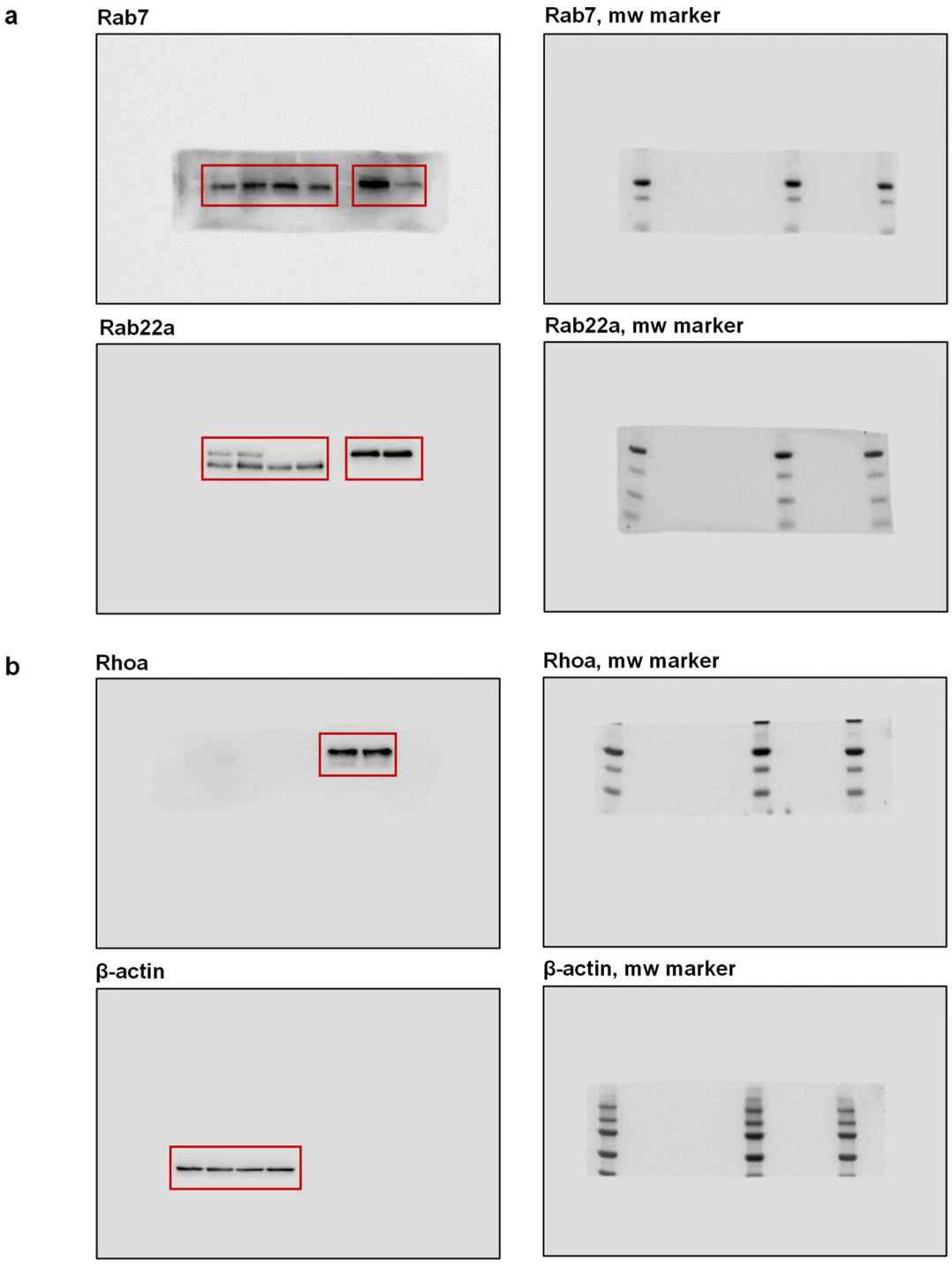
Supplementary Figure 25. Uncropped blots from Figure 2c



Supplementary Figure 26. Uncropped blots from Figure 2d



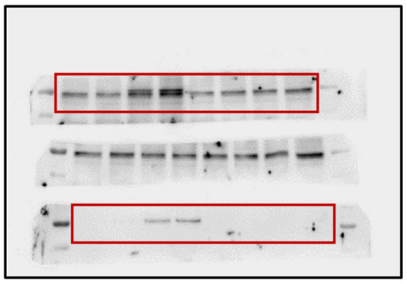
Supplementary Figure 27. Uncropped blots from a) Figure 6c and b) Supplementary Figure 13c



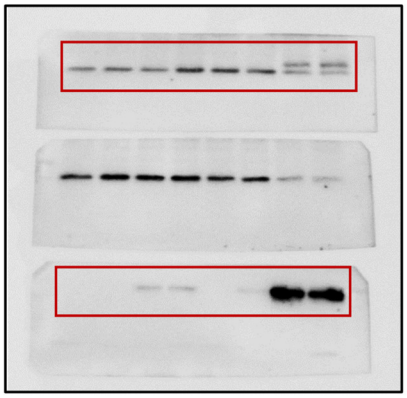
Supplementary Figure 28. Uncropped blots from Supplementary Figure 7

EA.hy926

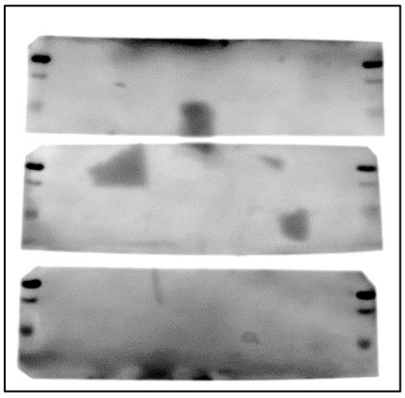
ULK3



RHOA

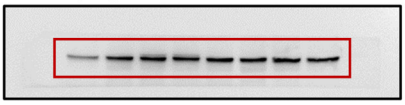


RHOA, mw marker

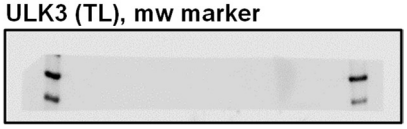


HeLa

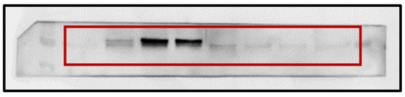
ULK3 (TL)



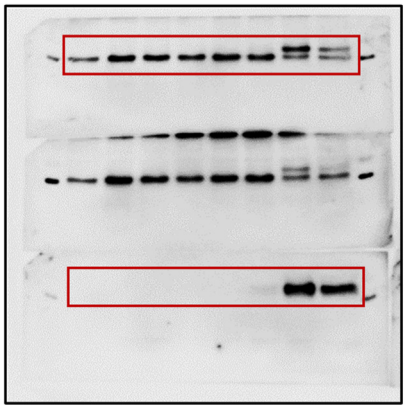
ULK3 (TL), mw marker



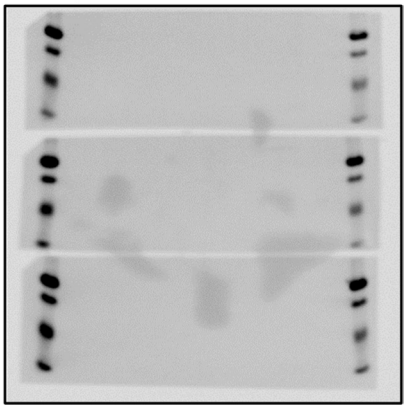
ULK3 (PD)



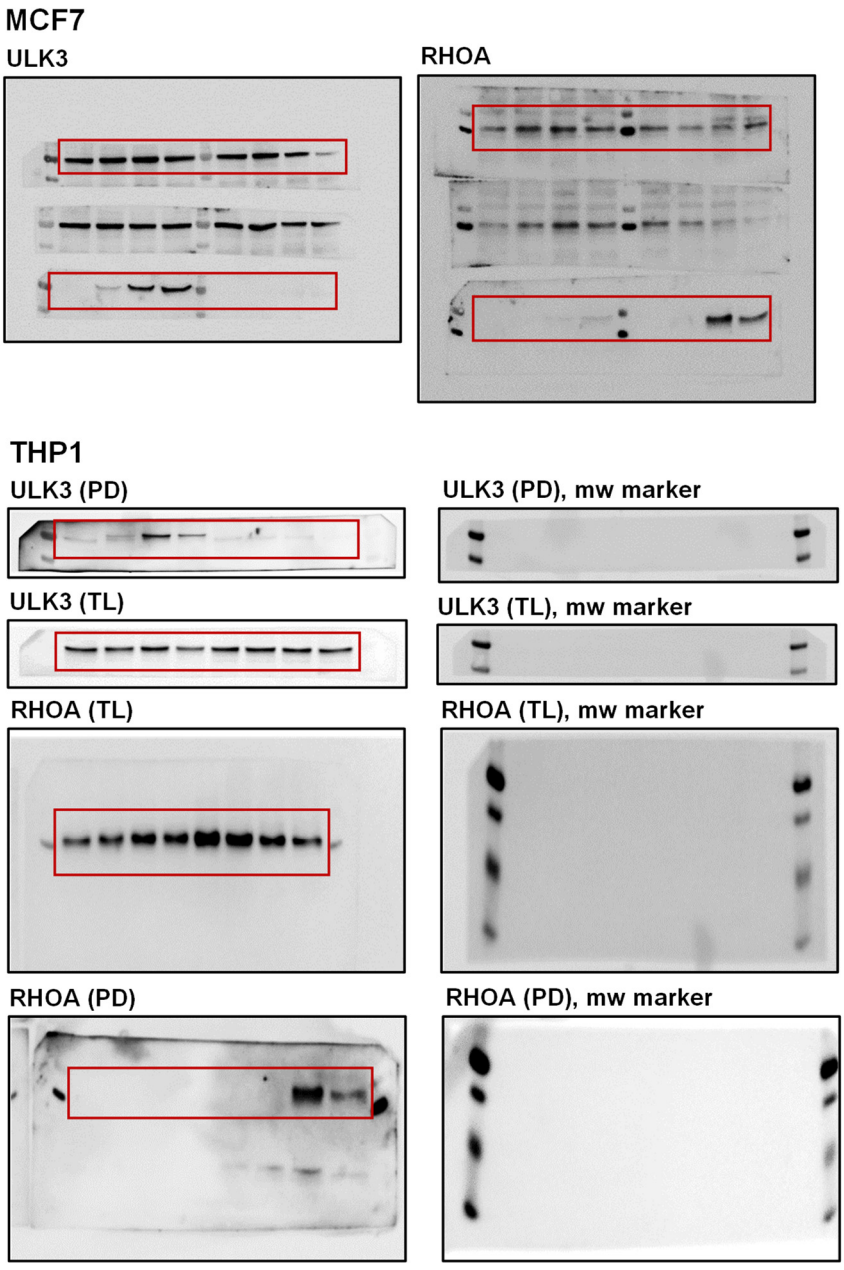
RHOA



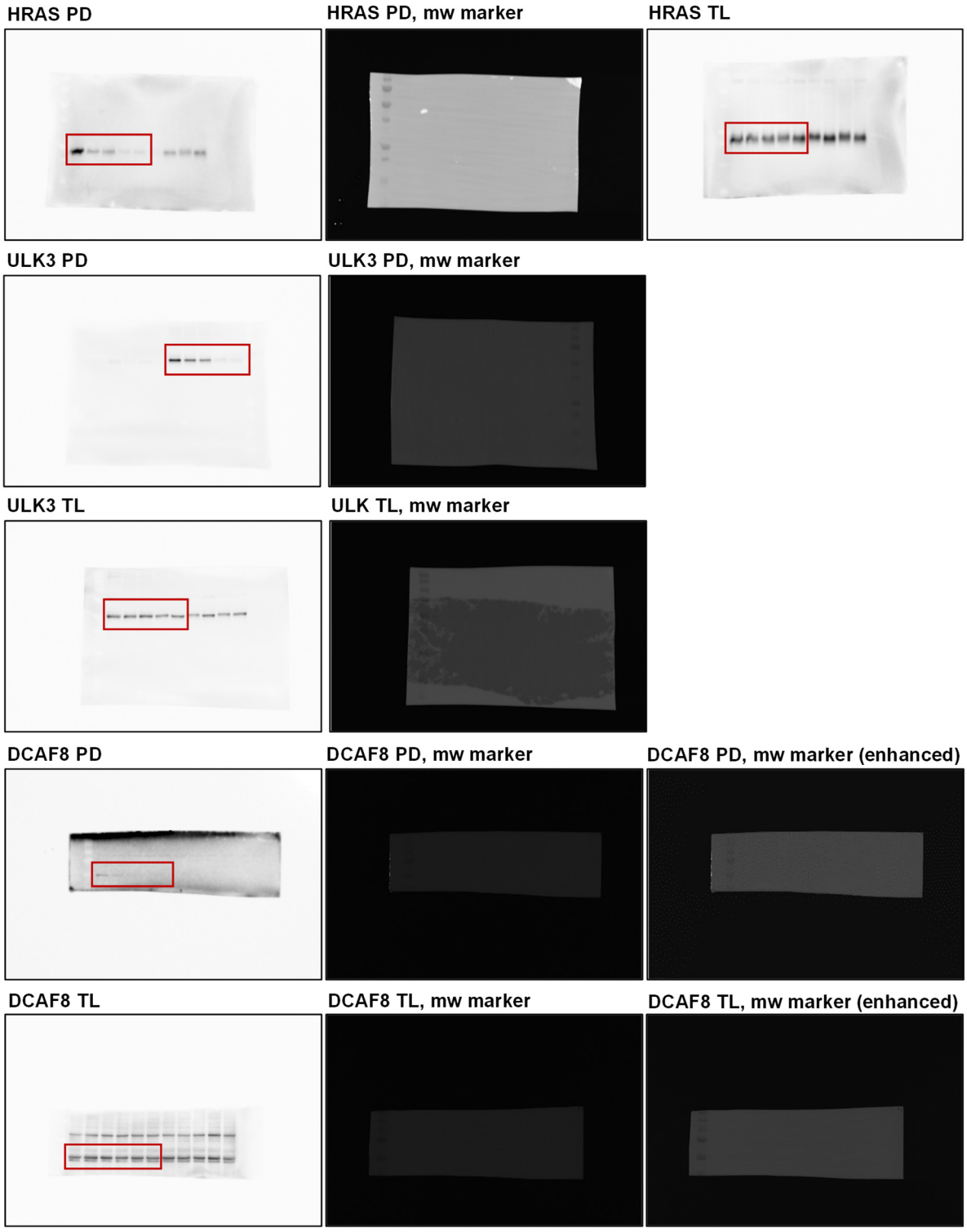
RHOA, mw marker



Supplementary Figure 28 cont.

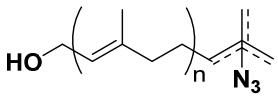


Supplementary Figure 29. Uncropped blots from Supplementary Figure 11

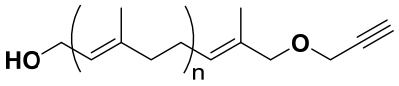


Supplementary Table 1. Comparative summary of probes used for whole-proteome metabolic labeling and mass spectrometry-based proteomic analysis of protein prenylation in mammalian cells. Probe structures are shown below the table. Notes: [a] allyl azides (such as AzF and AzGG) have been shown to be inherently unstable, undergoing spontaneous allylic rearrangement to yield a mixture of products[1,2]; [b] inhibition quantified for two spots identified by low-throughput 2D gel electrophoresis; [c] Structures of previously reported alkynyl probes Alk-FOH2/3 are included for comparative purposes, but are excluded from the table summary as they have not been tested in mass spectrometry-based applications[3]. N.D., not demonstrated at the whole proteome level.

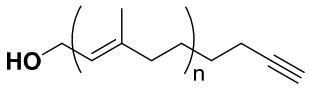
Probe system	Max # of prenylated proteins identified	Chemical stability	Proteome-level applications						Study
			Novel substrate identification	Statin-free substrate ID	F/GG selectivity	Quantification of inhibition	Probe site determination	Quantification of prenyl dynamics	
F-azide	18	x[a]	Yes	N.D.	x	x	x	x	Ref [4]
GG-azide	10	x[a]	x	N.D.	N.D.	x	x	x	Ref [5]
Alk-GOH	7	Yes	x	x	x	x	x	x	Ref [6]
Alk-FOH	22	Yes	Yes	x	x	x[b]	x	x	Ref [7]; Ref [8]
YnF/YnGG	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	This study



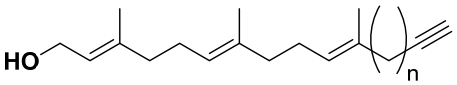
AzF (n=1); **AzGG** (n=2)
Mixtures of isomers



Alk-GOH (n=1); **Alk-FOH** (n=2)



YnF (n=2); **YnGG** (n=3)
This study



Alk-FOH-2[c] (n=1); **Alk-FOH-3**[c] (n=2)
Chemical stability unknown

Biological Methods & Proteomics

Reagents

FTI-277, GGTI-2133, Manumycin A, mevastatin, farnesol (FOH) and geranylgeraniol (GGOH) were obtained from Sigma-Aldrich. Due to low purity GGOH was re-purified by column chromatography prior to use. Tipifarnib was obtained from Biorbyt Ltd. Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Sigma-Aldrich. Fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Gibco®. R10K8 DMEM for SILAC cell culture was obtained from Dundee Cell products and dialyzed serum from Sigma-Aldrich. Sequence grade modified porcine trypsin was obtained from Promega. Lysarginase was obtained from Proteolysis Lab, IBMB-CSIC Barcelona Science Park. YnF, YnGG, YnFPP and YnGGPP were synthesized as described in chemical synthesis. FAM-labelled peptides were synthesized by solid phase peptide synthesis. Farnesyl pyrophosphate ammonium salt (FPP) and geranylgeranyl pyrophosphate ammonium salt (GGPP) were purchased from Sigma-Aldrich. FTase and GGTase-I enzymes were purchased from Jena Bioscience. Ultrapure water was obtained on a MilliQ water purification system (Millipore).

The syntheses of capture reagents AzTB, AzRB, AzRTB and Az3MRB have been described previously[9].

List of primary antibodies used:

Target	Species	Dilution	Catalogue no.	Supplier
β-ACTIN	Mouse	1:5000	ab6276	Abcam
DCAF8 (WDR42A)	Mouse	1:1000	ab54746	Abcam
HA-tag (C29F4)	Rabbit	1:1000	3724	Cell Signaling Technology
HRAS	Mouse	1:750	MAB3291	Millipore
HSP90	Mouse	1:1000	sc-69703	Santa Cruz Biotechnology
RAB22A	Rabbit	1:1000	ab137093	Abcam
RAB7	Rabbit	1:1000	9367	Cell Signaling Technology
RAB8A	Rabbit	1:1000	ab188574	Abcam
RHOA	Mouse	1:250	sc-418	Santa Cruz Biotechnology
ULK3	Rabbit	1:1000	ab124947	Abcam

FTase and GGTase-1 enzyme assay

Details of our biochemical enzyme assay will be reported fully elsewhere. Briefly, FAM-labeled peptides were solubilized in 1 mM DTT in H_2O and peptide concentrations were determined through A_{495} measurement in 0.1 M Tris-HCl (pH 8.0) on a Nanodrop ND-100 spectrophotometer (PerkinElmer, Waltham, MA) using a FAM molar extinction coefficient of 83,000 $M^{-1}cm^{-1}$.[10] Assays were performed with FAM-labelled peptides (1 μM), FTase or GGTase-1 (25 nM) and FPP, YnFPP, GGPP or YnGGPP (5 μM) in reaction buffer (50 mM TRIS, 20 mM KCl, 1 mM DTT, 50 μM $ZnCl_2$, 0.1% DDM (w/v), pH 7.5) at room temperature in black 384-well plates (Corning 3575, Corning, NY). Isoprenoid K_m values were determined using a two-fold serial dilution of FPP, YnFPP, GGPP or YnGGPP from 10 μM to 40 nM, and background corrected to reactions without prenyl pyrophosphates. V_{max} values were estimated using known ratios of FAM-RHOA(188-193) : FAM-GerGer-RHOA(188-193) peptide at a total concentration of 1 μM. Fluorescence measurements were recorded on an EnVision Xcite 2104 (PerkinElmer) for 1 hour using a FITC FP D505fp mirror module, FITC FP 480 nm excitation

filter (30 nm bandwidth), FITC FP P-pol 535 nm first emission filter (40 nm bandwidth), and FITC FP S-pol 535 nm second emission filter (40 nm bandwidth) using measurement height = 9.52 mm, 8 flashes/well, PMT gain = 319, and G-factor = 1.09.

Cell culture

Immortalized human umbilical vein endothelial cells (cell line EA.hy926) were cultured in DMEM supplemented with 10% FBS, 100 units/mL penicillin and 100 µg/mL streptomycin. SILAC-labelled EA.hy926 cells were grown in R10K8 DMEM supplemented with 10% dialyzed serum and penicillin/streptomycin as above. HEK-293, HeLa, and MCF7 cells were cultured in DMEM supplemented with 10% FBS. THP1 cells were cultured in RPMI supplemented with 10% FBS.

FLOX and FLOX TM mouse embryonic fibroblast cells (MEFs) were prepared from embryos of *CHM Flox, MerCreMer* mice[11]. *CHM Flox* allele is modified by insertion of 2 loxP-sites around exon 4 which on its own does not lead to changes in *Rep1* expression in comparison to wild type. *MerCreMer* is a tamoxifen (TM)-inducible Cre-recombinase transgene which causes recombination of the *CHM Flox* allele into *CHM* knock-out (KO) in the presence of TM. 'FLOX TM' are *CHM Flox, MerCreMer* cells treated with TM; 'FLOX' are control non-treated cells.

MEFs were cultured in DMEM F12 Nutrient mixture (Ham) [+] Glutamate (Invitrogen) supplemented with 15% FBS. SILAC-labelled NIH-3T3 cells were cultured in R10K8 DMEM supplemented with 10% dialyzed serum.

All cells were maintained in a humidified incubator (37°C, 5% CO_2). Cells were seeded on plates at least 24 hours before experiment. SILAC-labelled cells were maintained in SILAC medium for a minimum of 6 passages prior to use in experiments.

General procedure for preparation of cell lysate tagged with YnF or YnGG

Near-confluent (ca. 90%) plates of cells were cultured in medium (as above) supplemented with YnF or YnGG (1-10 µM, from 1000x stock in DMSO) or DMSO only for 8-24 hours. Cells were washed twice with cold PBS, aspirated and lysis buffer added (0.6 mL per 10 cm plate, 1% Triton X-100, 0.2% SDS in PBS supplemented with 1x Complete EDTA-free protease inhibitor cocktail (Roche)). Cells were scraped, transferred to a microcentrifuge tube and lysed on ice for 30 min. The lysate was clarified by centrifugation (14,000 x *g*, 4°C, 30 min) and the supernatant recovered. The protein concentration was determined using the DC™ protein assay (Bio-Rad) following the manufacturer's protocol.

Preparation of isoprenoid competition spike-in SILAC cell lysates

Spike-in standard cell lysate was prepared from EA.hy926 cells grown in R10K8 medium supplemented with YnF or YnGG (10 µM) for 24 hours. EA.hy926 cells were incubated with YnF (10 µM) + FOH (0, 5 or 25 µM) or YnGG (10 µM) + GGOH (0, 2 or 10 µM) for 8 hours. Biological triplicates were prepared for each isoprenoid concentration. After cell lysis each sample (600 µg) was mixed with spike-in lysate (200 µg). Protein concentration was normalized across all samples by addition of lysis buffer.

Preparation of samples for direct detection of prenylation site by LC-MS/MS

Cell lysates were prepared from EA.hy926 cells supplemented with YnF or YnGG (10 μM) for 24 hours. 1 mg of each protein lysate was subjected to click chemistry with each capture reagent (AzRB, AzRTB, Az3MRB) following the standard click chemistry protocol (see below). For the pull-down, 50 μL of NeutrAvidin® Agarose resin (Thermo Scientific) and after the reduction (see protocol described below for the preparation of samples for MS-based proteomics analysis) half of the samples was digested with trypsin (1 μg) and the other half with Lysarginase (1 μg in 50 μL Lysarginase buffer).

Preparation of spike-in SILAC cell lysates for inhibitor evaluation

Spike-in standard cell lysate was prepared from EA.hy926 cells grown in R10K8 medium supplemented with YnF or YnGG (10 μM) for 24 hours. Inhibitor treated EA.hy926 cells were pre-incubated in normal medium containing inhibitor (FTI-277, GGTI-2133, Manumycin A, Tipifarnib or DMSO control) for 1 hour. The medium was supplemented with 10 μM of YnF (FTI-277, Manumycin A, Tipifarnib or DMSO control samples) or YnGG (GGTI-2133 or DMSO control samples) and the cells incubated for a further 8 hours. Biological triplicates were prepared for each inhibitor concentration. After cell lysis each inhibitor treated sample (1 mg) was mixed with spike-in lysate (500 μg). Protein concentration was normalized across all samples by addition of lysis buffer.

Preparation of spike-in SILAC cell lysates for evaluation of YnF/YnGG probe preference

Spike-in standard cell lysate was prepared from EA.hy926 cells grown in R10K8 medium supplemented with YnF or YnGG (10 μM) for 24 hours. Inhibitor treated EA.hy926 cells were pre-incubated in normal medium containing Tipifarnib or DMSO control for 1 hour. The medium was supplemented YnF or YnGG (10 μM) and the cells incubated for a further 14 hours (overnight). Biological triplicates were prepared for each inhibitor concentration. After cell lysis each inhibitor treated sample (400 μg) was mixed with a YnF:YnGG spike-in lysate mixture 1:1 (400 μg). Protein concentration was normalized across all samples by addition of lysis buffer.

Preparation of spike-in SILAC cell lysates for quantification of Rab geranylgeranylation levels in a rodent model of Choroideremia

Spike-in standard cell lysate was prepared from NIH-3T3 cells grown in R10K8 medium supplemented with mevastatin (10 μM) and YnGG (10 μM) for 24 hours. FLOX and FLOX TM MEFs were incubated with YnGG (10 μM) for 24 hours. Biological triplicates were prepared for each cell line. After cell lysis each sample (450 μg) was mixed with spike-in lysate (150 μg). For whole proteome analysis, 75 μg of each sample was mixed with 75 μg of spike-in lysate. Protein concentration was normalized across all samples by addition of lysis buffer.

Click chemistry general protocol

Protein concentrations were normalized across all samples to 1-2 mg/mL by addition of lysis buffer. For each 100 μL of protein lysate click reagent mixture was prepared as follows: 1 μL capture reagent (10 mM in DMSO), 2 μL $CuSO_4$ (50 mM in H_2O) and 2 μL Tris(2-carboxyethyl)phosphine hydrochloride (50 mM in H_2O) were added sequentially to a microcentrifuge tube, mixed and left to stand for 2 min after which 1 μL Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (10 mM in DMSO) was added. The click reagent mixture was added to the protein lysate and the lysate incubated on a shaker at room temperature for 1 hour. The

click reaction was terminated by addition of 0.5M EDTA (5 µL). Methanol (400 µL), chloroform (100 µL) and ultrapure water (300 µL) were added sequentially, the mixture vortexed and centrifuged (14,000 x *g*, 5 min, RT). The top layer was removed, leaving the protein layer at the phase interface intact. Methanol (800 µL) was added, the sample vortexed and the protein pelleted as before. All solution was aspirated, and the protein pellet washed in methanol (800 µL) again, with vortexing and sonication to break the pellet. After a final centrifugation step and removal of the methanol the pellet was air-dried in an inverted tube for 5-10 min. Samples were re-suspended in 2% SDS, 10 mM EDTA in PBS (10 µL). Once dissolved, the samples were diluted to a final concentration of 0.2% SDS by addition of PBS.

Capture reagent AzTB was used for in-gel fluorescence and WB applications. Capture reagent AzRB was used for proteomics applications. AzRB, AzRTB and Az3MRB were used for the identification of modified peptides by proteomics. Reagent quantities were scaled accordingly for applications requiring larger quantities of protein lysate (>100 µL). For proteomics analysis the click reaction performed in 2 mL microcentrifuge tubes or 15 mL tubes and all centrifugation steps performed at room temperature to prevent precipitation of SDS.

SDS-PAGE and in-gel fluorescence imaging

Protein samples were combined with LDS loading buffer (4x stock) and incubated in a heat block at 95°C for 5 min. 10 µg of protein was separated on 12% Bis-Tris gels using MOPS running buffer at 100-160 V. Gels were fixed (40% methanol, 10% acetic acid, 50% ultrapure water) for 5 min and then washed in water for 5 min prior to imaging on a Ettan DIGE Imager or Typhoon FLA 9500 (GE Healthcare).

Protein enrichment for Western blot analysis

Protein samples (170 µg) were subjected to click chemistry with AzTB capture reagent, precipitated and re-suspended as described, except the proteins were re-suspended to a final concentration of 1-2 mg/mL. An aliquot (20 µg) of protein was kept aside for blots of total protein input before enrichment. The samples were incubated with Dynabead® MyOne™ Streptavidin C1 resin (10 µL per 100 µg protein, Invitrogen) for 1 hour at room temperature. The beads were washed with 0.2% SDS in PBS (3 x 0.5 mL) and eluted by boiling in 1x LDS sample loading buffer (95°C, 5 min). The proteins were separated by SDS-PAGE as described and transferred to a PVDF membrane (Immobilon-P^{SQ}, Millipore) by wet-tank transfer in a Tris-Glycine transfer buffer (Novex®) supplemented with 20% methanol for 2h at 100V or by iBlot transfer to nitrocellulose (P3 program, 8 min, Invitrogen). Membranes were blocked in 5% skimmed milk in Tris-buffered saline containing 0.01% Tween-20 (TBST). Subsequent incubation with primary antibody in blocking buffer was performed at 4°C overnight or for 1.5 hour at room temperature. After washing in TBST (3 x 15 min) the membranes were incubated with the appropriate HRP-conjugated secondary antibodies (α-mouse-HRP or α-rabbit-HRP, 1:5000, Advansta) in blocking buffer for 1 hour at room temperature. After washing as before, the membranes were incubated with HRP substrate (Luminata Crescendo, Millipore) and imaged on an ImageQuant LAS 4000 (GE Healthcare).

Preparation of ULK3 and CEP85 DNA constructs

WT or mutant forms of ULK3 were cloned as N-terminal HA fusions into the multiple cloning site of pCR3.1 plasmid using the EcoRI and XhoI restriction sites. WT ULK3 was subcloned

from the previously described pCAG-GST ULK3 plasmid[12]. ULK3 mutants C467A and C467S were generated by PCR using the following primers:

ULK3 forward: 5'-TATATAGAATTCATGGCGGGGCCCGGCTGGGGTCCCCCG-3'

ULK3 C467S reverse: 5'-TATATACTCGAGTCACTGAAGGGTTGCAGAGCTACG-3'

ULK3 C467S reverse: 5'-TATATACTCGAGTCACTGAAGGGTTGAAGAGCTACG-3'

WT or mutant forms of CEP85 were cloned as N-terminal HA fusions into the multiple cloning site of pCR3.1 plasmid using the NotI restriction site. The following primers were used to generate CEP85 constructs by PCR:

CEP85 forward: 5'-ATATATGCGGCCGCATGGCCATGCAGGAGAAATATCC-3'

CEP85 WT Reverse: 5'-ATATATGCGGCCGCTCACTGTGTGACACAGTTTTCTCC-3'

CEP85 C759A Reverse: 5'-TATATAGCGGCCGCTCACTGTGTGACTGCGTTTTCTCC-3'

CEP85 C759S Reverse: 5'-TATATAGCGGCCGCTCACTGTGTGACTGAGTTTTCTCC-3'

Overexpression and prenylation analysis of ULK3 and CEP85

HEK-293 cells were transfected with plasmid encoding for ULK3, ULK3 (C467S), ULK3 (C467A), CEP85, CEP85 (C759S) and CEP85 (C759A) utilizing Lipofectamine 2000 transfection reagent (7 h) according to the manufacturer's instructions. Next, the media was removed and replaced by fresh culture media supplemented with YnF (10 μM). After 16 hours incubation, the cells were washed with PBS (2x) and then lysed (PBS, 1% Triton X-100, 0.1% SDS, 1 x EDTA-free complete protease inhibitor). Protein samples (100 μg) were subjected to click chemistry with AzTB capture reagent, precipitated and re-suspended as described, except the proteins were re-suspended to a final concentration of 0.25 mg/mL. An aliquot (3 μg) of protein was kept aside for blots of total protein input before enrichment. Dynabeads® MyOne Streptavidin C1 (20 μL, Invitrogen) were washed with 0.2% SDS in PBS (3 x 300 μL). The sample (150 μL, 37.5 μg) was added to the beads and the beads were gently vortexed for 90 min. The supernatant was removed, the beads were washed with 0.2% SDS (3 x 300 μL) and eluted by boiling in 1x LDS sample loading buffer (95°C, 5 min). The proteins were separated by SDS-PAGE followed by iBlot transfer to a nitrocellulose membrane (P3 program, 8 min, Invitrogen). Membranes were blocked in 5% skimmed milk in TBST. Subsequent incubation with primary antibody in blocking buffer was performed overnight at 4 °C using HA-Tag (C29F4) antibody. After washing in TBST (3 x 15 min) the membranes were incubated with α-rabbit-HRP (1:5000, Advansta) in blocking buffer for 1 hour at room temperature. After washing as before, the membranes were incubated with HRP substrate (Luminata Crescendo, Millipore) and imaged on an ImageQuant LAS 4000 (GE Healthcare).

Preparation of samples for MS-based proteomic analysis of YnF and YnGG labelled proteins

Proteomics samples were prepared in a dust-free area using dedicated pipettes and pipette tips. Only low binding microcentrifuge tubes (Eppendorf® Protein LoBind) were used. All solutions were prepared fresh and filtered through a 0.22 μm syringe filter before use.

Protein lysates were subjected to click chemistry with AzRB capture reagent as described. After precipitation and re-suspension, the protein solution was centrifuged (4000 x *g*, 10 min, RT) to pellet any particulates. The clarified protein samples were incubated with NeutrAvidin® Agarose resin (50 µL per 1 mg protein, Thermo Scientific) for 2 hours at room temperature. The beads were pelleted (3,000 x *g*, 3 min) and the supernatant was removed. The beads were washed sequentially in 1% SDS in PBS (3 x 0.5 mL), 4M Urea in PBS (2 x 0.5 mL) and 50 mM ammonium bicarbonate (5 x 0.5 mL). For each wash step the beads were gently vortexed for 1 min followed by pelleting in a microcentrifuge (3,000 x *g*, 2-3 min).

After the final wash the beads were re-suspended in 50 mM ammonium bicarbonate (50 µL). DL-dithiothreitol (5 µL, 100 mM in 50 mM ammonium bicarbonate) was added and the beads incubated at 55°C for 30 minutes in a shaker. The beads were washed with 50 mM ammonium bicarbonate (2 x 0.5 mL) with vortexing and pelleting as before, leaving the beads covered in 50 µL solution after the second wash. Iodoacetamide (5 µL, 100 mM in 50 mM ammonium bicarbonate) was added and the beads incubated at room temperature for 30 minutes in the dark. The beads were washed as before. Sequence grade trypsin (5 µL, 0.2 µg/µL in 50 mM ammonium bicarbonate) was added and the beads incubated at 37°C overnight in a shaker. The beads were pelleted, and the supernatant collected. The beads were washed with 0.1% formic acid in ultrapure water (80 µL) with gentle shaking for 10 minutes. The beads were pelleted, and the supernatants pooled. The peptide solutions were purified on stage-tips according to a published protocol[13]. The peptides were eluted from the sorbent (Empore™ SDB-XC solid phase extraction discs, 3M) with 79% acetonitrile in water (60 µL), dried in a Savant SPD1010 SpeedVac® Concentrator (Thermo Scientific) and stored at -80°C until LC-MS/MS analysis. Peptides were reconstituted in 2% acetonitrile in water with 0.5% trifluoroacetic acid for LC-MS/MS analysis.

Preparation of samples for TMT MS-based proteomic analysis

Lysate modification with AzRB with click-chemistry and pull-down on Neutravidin Agarose resin was performed as described above. After the incubation with Neutravidin Agarose beads, the beads were washed sequentially in 1% SDS in PBS (3 x 0.5 mL), and 50 mM HEPES pH 8.0 (5 x 0.5 mL). For each wash step the beads were gently vortexed for 1 min followed by pelleting in a microcentrifuge (3,000 x *g*, 2-3 min). After the final wash the beads were re-suspended in 50 mM HEPES (50 µL). Tris(2-carboxyethyl)phosphine (5 µL, 50 mM in 50 mM HEPES) and chloroacetamide (5 µL, 150 mM in 50 mM HEPES) were added and the beads incubated at room temperature for 15 minutes in a shaker. Sequence grade trypsin (2 µL, 0.2 µg/µL in 50 mM HEPES) was added and the beads incubated at 37°C overnight in a shaker. The beads were pelleted, and the supernatant collected.

Isobaric labelling of the digested peptides was performed using the 10-plex tandem mass tag (TMT) reagents (Thermo fisher Scientific). Each TMT reagent (0.8 mg vial) was dissolved in 400 µL acetonitrile. The digested peptides were then mixed with 40 µL (1/10 vial) of the corresponding TMT reagent and incubated at room temperature for 2 hours with gentle shaking. The reaction was quenched with 5% hydroxylamine (1 µL) and the labeled peptides were then combined and dried in a Savant SPD1010 SpeedVac® Concentrator (Thermo Scientific).

The dried labeled peptide samples were re-suspended in 150 µL 1% TFA/H_2O (v/v), fractionated using SCX (Polystyrene-divinylbenzene copolymer modified with sulfonic acid)

solid phase extraction discs (3x discs) and separated into six fractions as previously reported[14]. Fractions 1-2, were combined, as were fractions 3-4 and fractions 5-6 and dried in a Savant SPD1010 SpeedVac® Concentrator (Thermo Scientific) and stored at -80°C until LC-MS/MS analysis. Peptides were reconstituted in 2% acetonitrile in water with 0.5% trifluoroacetic acid for LC-MS/MS analysis.

Preparation of samples for MS-based proteomic analysis of whole proteome

Protein lysates (30 µg) were precipitated using the chloroform-methanol method described above. The protein pellet was washed with 10% water in methanol (200 µL X 2) and pelleted by centrifugation (14,000 x *g*, 16 °C, 10 min). The pellet was dissolved in 5 mM DTT in 50 mM ammonium bicarbonate (48 µL) and the solution incubated at 55°C for 30 minutes. Cysteines were alkylated by the addition of iodoacetamide (2.4 µL, 100 mM in 50 mM ammonium bicarbonate) at room temperature for 30 minutes in the dark. Sequencing grade modified trypsin (Promega, 5 µL, 0.2 µg/µL in 50 mM ammonium bicarbonate) was added to the solutions and the samples were incubated at 37°C overnight in a shaker. Trifluoroacetic acid was added to a final concentration of 0.5% (100 µL 0.75% trifluoroacetic acid in ultrapure water). The peptide solutions were stage-tipped according to a published protocol[13]. The peptides were eluted from the sorbent (Empore™ SDB-XC solid phase extraction discs, 3M) with 79% acetonitrile in water, dried in a Savant SPD1010 SpeedVac® Concentrator (Thermo Scientific) and stored at -80°C until LC-MS/MS analysis. Peptides were reconstituted in 2% acetonitrile in water with 0.5% trifluoroacetic acid for LC-MS/MS analysis.

NanoLC-MS/MS analysis

Peptides were separated on an EASY-Spray™ Acclaim PepMap C18 column (50 cm × 75 µm inner diameter, Thermo Fisher Scientific) using a binary solvent system of 2% acetonitrile with 0.1% formic acid (Solvent A) and 80% acetonitrile with 0.1% formic acid (Solvent B) in an Easy nLC-1000 system (Thermo Fisher Scientific). 2 µL of peptide solution was loaded using Solvent A onto an Acclaim PepMap100 C18 trap column (2 cm x 75 µm inner diameter), followed by a linear gradient separation of 0-100% Solvent B over 2 hours at a flow rate of 250 nL/min.

Liquid chromatography was coupled to a QExactive mass spectrometer via an easy-spray source (Thermo Fisher Scientific). The QExactive was operated in data-dependent mode with survey scans acquired at a resolution of 75,000 at *m/z* 200 (transient time 256 ms). Up to 10 of the most abundant isotope patterns with charge +2 or higher from the survey scan were selected with an isolation window of 3.0 *m/z* and fragmented by HCD with normalized collision energies of 25. The maximum ion injection times for the survey scan and the MS/MS scans (acquired with a resolution of 17 500 at *m/z* 200) were 20 and 120 ms, respectively. The ion target value for MS was set to 10^6 and for MS/MS to 10^5, and the intensity threshold was set to 8.3×10^2.

Proteomics data analysis in MaxQuant

Processing of LC-MS/MS data was performed in MaxQuant version 1.5.0.25 using the built-in Andromeda search engine. Peptides were identified from the MS/MS spectra searched against the human reference proteome (Uniprot, accessed 16 July 2015). For the TMT experiment, TMT 10plex modifications at lysine side chains and peptide N-termini were selected as labels, cysteine carbamidomethylation was set as a fixed modification, and methionine oxidation was set as a variable modification. For spike-in SILAC experiments the multiplicity was set to 2 and

‘Arg10’ and ’Lys8’ chosen as heavy labels. Cysteine carbamidomethylation was set as a fixed modification. Methionine oxidation and N-terminal acetylation were set as variable modifications. ‘Trypsin/P’ was chosen as digestion mode enzyme. Minimum peptide length was set to 7 residues and maximum 2 missed cleavages were allowed. The ‘re-quantify’ and ‘match between run’ options were selected. ‘Unique and razor peptides’ were chosen for protein quantification. Other parameters were used as pre-set in the software. Processed data was analyzed using Perseus version 1.5.0.9, Microsoft Office Excel 2010 and GraphPad Prism version 5.03.

Proteomics data analysis with Peaks Suite

MS data were processed with PEAKS8.5[15], which as a default performs *de novo* peptide sequencing prior to database searches, in order to improve the accuracy of the results. The software also searches for common PTMs (PEAKS PTM) and point mutations (SPIDER). The data were searched against the same human reference proteome (with isoforms) that was used in MaxQuant analyses. A modified trypsin (cleave site: after K, R or C, none non-specific cleavage, up to five missed cleavages allowed) or Lysarginase (cleave site: after C and before K or R) selected for database searches, and no enzyme was chosen in *de novo* searches. The maximal mass error was set to 5 ppm for precursor ions and 0.01 Da for product ions. Cysteine carbamidomethylation, methionine oxidation, methylation (C-terminus) and the lipid-derived adducts (any cysteine residue) detailed in the table below were set as variable modifications. The maximal number of modifications per peptide was set as five. The false discovery rate was set to 0.01 for peptides and a minimum of 1 unique peptide per protein was required.

	YnF		**YnGG**	
	Trypsin	Lysarginase	Trypsin	Lysarginase
AzRB	459.30	303.19	527.36	371.26
AzRTB	459.30	303.19	527.36	371.26
Az3MRB	629.44	473.34	697.50	541.40

Isoprenoid competition data analysis

The “Ratio H/L” values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The “Ratio H/L” was inverted and divided by 2 to obtain the “ratio L/H”. The “ratio L/H” values were $\log_2$ transformed and replicates grouped. The data was filtered to require at least 2 valid values in the non-competition group. A two-sample t-test was performed to compare the $\log_2$ L/H ratios of isoprenoid competition samples versus non-competition samples (n=3, Permutation-based FDR=0.02, S0=0.5). Further analysis was performed in Excel. Proteins were filtered to retain those identified with a minimum of 2 razor+unique peptides in at least 2 replicates of the non-competition group. To determine a mean response to competition the mean $\log_2$ ratio L/H of competition samples were normalized to the mean $\log_2$ Ratio L/H of the non-competition samples by subtraction and the sign inverted. A mean response to competition for each isoprenoid concentration was only determined for proteins with at least 2 valid values in the competition group. The standard deviation was normalized according appropriate rules of error propagation.

The list of proteins was manually inspected and grouped as “F”, “Novel F”, “GG”, “RAB”, “No motif” or “Other CXXX” based on sequence analysis (presence of CXXX motif or member of RAB family) and literature annotation. CXXX proteins showing no significant response to

isoprenoid competition at either concentration tested were grouped as “Other CXXX”. Where significant responders were identified in both YnF and YnGG data-sets the protein was assigned to the “F” or “GG” group based on the probe preference analysis detailed below. High-quality data (normalized SD < 0.5) was imported into GraphPad Prism. Significance between the groups was determined by a Kruskal-Wallis test with Dunn’s post-test ($p<0.01$).

TMT data analysis

Protein groups and the corresponding corrected TMT intensity values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The values were grouped per condition (DMSO/YnF 1 μM/YnF 2 μM/YnF 5 μM/YnF 10 μM/YnGG 1 μM/YnGG 2 μM/YnGG 5 μM/YnGG 10 μM) and per sample (A/B/C). The values were $\log_2$ transformed, and protein groups with values present in less than 2 out of 3 of samples, removed. Mean values across TMT channels within samples were subtracted in rows, and then median values across all protein groups were subtracted in columns. Protein groups were matched and the categories (No motif/Known F/Novel F/Other CXXX) or (No motif/Known GG/RABs/Other CXXX) mapped from the isoprenoid competition experiments. The unmapped protein groups were removed. The data was imported into GraphPad Prism to construct plots presented in Supplementary Figure 5.

Prenyl transferase inhibitor evaluation data analysis

The “Ratio H/L” values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The “Ratio H/L” was inverted and divided by 3 to obtain the “ratio L/H”. The “ratio L/H” values were $\log_2$ transformed and the replicates grouped. The data was filtered to retain proteins with at least 2 valid values in the non-inhibition group. A multi-sample ANOVA test was performed (n=3, Permutation-based FDR=0.01, S0=1). Only proteins identified with a minimum of 2 razor+unique peptides were considered.

To establish a relative response to inhibition the mean L/H ratio of each inhibitor concentration was normalized to the mean L/H ratio of the non-inhibitor treated sample. The standard deviation was normalized according to appropriate rules for error propagation. Data was filtered to retain proteins with a prenylation motif (proteins bearing a C-terminal CXXX-motif and Rab proteins). ANOVA-significant proteins with a maximum relative response to inhibition of <0.5 were imported into GraphPad Prism. For the purpose of IC_{50} calculations the non-inhibitor treated samples were approximated at an inhibitor concentration of 3 log units below the lowest inhibitor concentration tested (1 nM for FTI-277, GGTI-2133 and Manumycin A samples, 1 pM for Tipifarnib samples). IC_{50} values were determined by non-linear regression using the “log(inhibitor) vs. response – variable slope (four parameters)” function. Top values were constrained to equal 1 and bottom values to be greater than 0. Proteins that returned an ambiguous IC_{50} were excluded from further analysis.

YnF/YnGG probe preference data analysis

The “Ratio H/L” values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The “Ratio H/L” was inverted and divided by 2 to obtain the “ratio L/H”. The “ratio L/H” values were $\log_2$ transformed and the replicates grouped. The data was filtered to retain

at least 2 valid values in at least one group. A two-sample t-test was performed to compare the log_2 L/H ratios of YnF samples versus YnGG samples (n=3, Permutation-based FDR=0.01, S0=1). Only proteins identified with a minimum of 2 razor+unique peptides were considered. For CXXX-motif proteins and RABs, probe preference was assigned as follows: Proteins quantified with a minimum of 2 valid values per probe were assigned as “YnF” or “YnGG” if significantly enriched by one probe, or “no preference” if no significant enrichment was found. Proteins quantified with a minimum of 2 valid values by one probe only was assigned as “YnF only” or “YnGG only” as appropriate.

YnF/YnGG prenylation switch in response to Tipifarnib data analysis

The “Ratio H/L” values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The “Ratio H/L” was inverted and divided by 2 to obtain the “ratio L/H”. The “ratio L/H” values were log_2 transformed and the replicates grouped. The data was filtered to retain at least 2 valid values in at least one group. A two-sample t-test was performed to compare the log_2 L/H ratios of YnF samples versus YnGG samples (n=3, Permutation-based FDR=0.01, S0=1). Only proteins identified with a minimum of 2 razor+unique peptides were considered. The mean L/H ratios were normalized to the mean L/H ratio of the YnF-labeled sample group to obtain a value for YnGG incorporation relative to YnF. ANOVA-significant proteins with a minimum YnGG incorporation of 25% relative to YnF upon Tipifarnib treatment were defined as prenylation switch substrates.

Rep-1 knockout data analysis (pull-down)

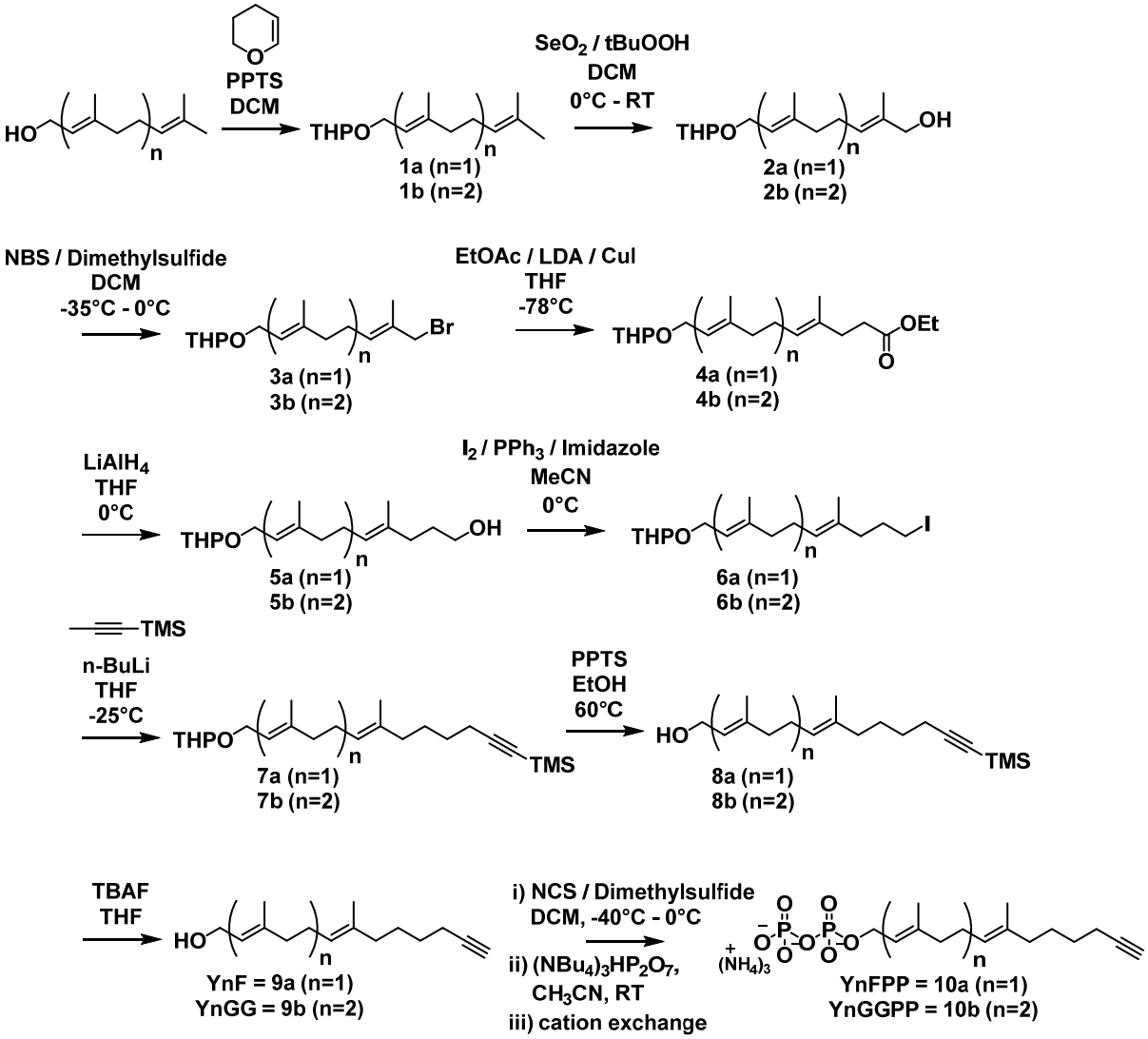
The “Ratio H/L” values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The “Ratio H/L” was inverted and divided by 2 to obtain the “ratio L/H”. The “ratio L/H” values were log_2 transformed and the replicates grouped. The data was filtered to retain proteins with at least 2 valid values in each group. A two-sample t-test was performed to compare the log_2 L/H ratios of FLOX TM versus FLOX samples (n=3, Permutation-based FDR=0.01, S0=1). Only proteins identified with a minimum of 2 razor+unique peptides were considered.

Rep-1 knockout data analysis (whole proteome)

The “Ratio H/L” values returned from MaxQuant processing were loaded in Perseus. The data was filtered to remove proteins categorized as “Only ID by site”, “Reverse” and “Potential contaminant”. The “Ratio H/L” was inverted to obtain the “ratio L/H”. The “ratio L/H” values were log_2 transformed and the replicates (n=3) grouped. The data was filtered to retain at least 2 valid values in each group. A two-sample t-test was performed to compare the log_2 L/H ratios of FLOX TM versus FLOX samples (n=3, Permutation-based FDR=0.01, S0=1). Only proteins identified with a minimum of 2 razor+unique peptides were considered.

Chemical Synthesis

Synthetic route to YnF, YnGG, YnFPP an YnGGPP:



General

All reagents were purchased from commercial sources (WVR or Sigma Aldrich) and used without further purification. Oven-dried glassware was used for all anhydrous reactions and flasks flushed with inert gas (nitrogen or argon) prior to use. Reaction progress was monitored by thin layer chromatography (TLC). TLC was performed on TLC Silica gel 60 F254 aluminum sheets (MERCK) and spots were visualized by UV (where possible) or vanillin stain.

Flash column chromatography was performed on a Biotage Isolera™ One fitted with a dual wavelength detector, with fraction collection at 254 nm for UV active compounds. For non-UV active compounds all fractions were collected, and presence of product confirmed by TLC.

Nuclear magnetic resonance spectra were recorded at room temperature on a 400 MHz Bruker instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C and 162 MHz for ^{31}P). Chemical shifts (δ) are reported in parts per million (ppm) relative to the $CDCl_3$ solvent peak (7.26 for ^{1}H and 77.16 for ^{13}C) or the D_2O solvent peak (4.70 for ^{1}H). Coupling constants are reported in Hertz (Hz). High resolution mass spectrometry (HRMS) was performed by the Mass Spectrometry Service (Department of Chemistry, Imperial College London).

(2*E*)-3,7-Dimethyl-1-(tetrahydro-2*H*-pyran-2-yloxy)octa-2,6-diene (1a)

To a solution of *trans*-geraniol (8.34 g, 54 mmol) and pyridinium *p*-toluene sulfonate (1.35g, 5.4 mmol) in anhydrous dichloromethane (10 mL) stirring under nitrogen was added 2,4-dihydropyran (19.7 mL, 216 mmol). The solution was stirred at room temperature overnight after which the solvent was removed *in vacuo* and the resulting residue taken up in diethyl ether. The organic layer was washed with saturated sodium bicarbonate (x2) and brine (x2), dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by flash column chromatography, eluting with 5% ethyl acetate in *n*-hexane. The isolated product was a clear liquid (6.73 g, 28 mmol, 52%).

R_f = 0.42 (Hexane – Ethyl acetate, 9:1); δ_H/ppm (400 MHz, $CDCl_3$) 5.40 – 5.29 (m, 1H), 5.12 – 5.02 (m, 1H), 4.64 – 4.57 (m, 1H), 4.22 (dd, *J* = 11.6, 6.2 Hz, 1H), 4.02 (dd, *J* = 11.9, 7.4 Hz, 1H), 3.88 (ddd, *J* = 11.2, 7.6, 3.5 Hz, 1H), 3.55 – 3.42 (m, 1H), 2.15 – 1.93 (m, 4H), 1.66 (s, 6H), 1.59 (s, 3H), 1.90-1.44 (m, 6H); δ_C/ppm (100 MHz, $CDCl_3$) 140.35, 131.70, 124.14, 120.68, 97.85, 63.73, 62.38, 39.74, 30.83, 26.47, 25.79, 25.62, 19.74, 17.78, 16.50; HRMS, found 256.2255 ($C_{15}H_{30}NO_2$, $[M + NH_4]^+$, requires 256.2277).

(2*E*,6*E*)-2,6-Dimethyl-8-(tetrahydro-2*H*-pyran-2-yloxy)octa-2,6-dien-1-ol (2a)

To a solution of **1a** (8.60 g, 36 mmol) in anhydrous dichloromethane (40 mL) stirring at 0°C under nitrogen was added selenium dioxide (400 mg, 3.6 mmol) followed by dropwise addition of *tert*-butyl hydroperoxide (5.5 M in decane, 7.25 mL, 40 mmol). The solution was allowed to warm to room temperature and stirred for 24 hours. The reaction was diluted with dichloromethane, and the organic layer washed with water (x2) and brine (x2), dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by flash column chromatography eluting in a gradient of 10-30% ethyl acetate in *n*-hexane. The isolated product was a pale-yellow oil (3.27 g, 12.9 mmol, 36%).

R_f = 0.23 (Hexane – Ethyl acetate, 7:3); δ_H/ppm (400 MHz, $CDCl_3$) 5.43 – 5.30 (m, 2H), 4.64 – 4.60 (m, 1H), 4.23 (dd, *J* = 11.9, 6.4 Hz, 1H), 4.01 (dd, *J* = 12.1, 7.6 Hz, 3H), 3.98 (s, 2H), 3.93 – 3.83 (m, 1H), 3.55 – 3.46 (m, 1H), 2.17 (td, *J* = 8.5, 7.7 Hz, 2H), 2.07 (t, *J* = 7.5 Hz, 2H), 1.90 – 1.45 (m, 6H), 1.67 (s, 3H), 1.66 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 139.82, 135.27, 125.81, 121.15, 97.99, 69.10, 63.82, 62.42, 39.28, 30.81, 25.86, 25.61, 19.72, 16.47, 13.83; HRMS, found 272.2230 ($C_{15}H_{30}NO_3$, $[M + NH_4]^+$, requires 272.2226).

(2*E*,6*E*)-1-Bromo-2,6-dimethyl-8-(tetrahydro-2*H*-pyran-2-yloxy)octa-2,6-diene (3a)

To a suspension of *N*-bromosuccinimide (2.48 g, 13.9 mmol) in anhydrous dichloromethane (20 mL) stirring at 0°C under nitrogen was added dimethylsulfide (1.23 mL, 16.7 mmol) dropwise. The bright orange suspension was stirred at 0°C for 15 minutes and then cooled to -40°C. **2a** (2.36 g, 9.28 mmol) in dichloromethane (1 mL) was added dropwise during which time the color of the reaction faded to a pale yellow. The reaction was allowed to warm to room temperature overnight. The reaction was poured into brine, and the organic layer was washed with water (x2) and brine (x1), dried over Na_2SO_4, filtered and evaporated *in vacuo* without heating (**IMPORTANT**: heating this compound above room temperature will result in decomposition). The crude product was purified by flash column chromatography eluting in a gradient of 1-20% ethyl acetate in *n*-hexane. The isolated product was a pale-yellow oil (498 mg, 1.57 mmol, 16.9%).

R_f = 0.47 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.58 (t, *J* = 6.8 Hz, 1H), 5.36 (t, *J* = 6.8 Hz, 1H), 4.65 – 4.59 (m, 1H), 4.24 (dd, *J* = 11.9, 6.4 Hz, 1H), 4.02 (dd, *J* = 11.9, 7.3 Hz, 1H), 3.96 (s, 2H), 3.93 – 3.84 (m, 1H), 3.56 – 3.46 (m, 1H), 2.21 – 2.11 (m, 2H), 2.11 – 2.02 (m, 2H), 1.90 – 1.37 (m, 6H), 1.75 (s, 3H), 1.67 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 139.45, 132.35, 130.95, 121.31, 98.02, 63.75, 62.45, 41.86, 38.79, 30.84, 26.64, 25.62, 19.76, 16.54, 14.81; HRMS, found 334.1376 ($C_{15}H_{29}NO_2Br$, $[M + NH_4]^+$, requires 334.1382).

Ethyl (4*E*,8*E*)-4,8-dimethyl-10-(tetrahydro-2*H*-pyran-2-yloxy)deca-4,8-dienoate (4a)

Preparation of lithium diisopropylamide (LDA): *n*-butyllithium (2.5M in hexanes, 1.25 mL, 3.1 mmol) was added dropwise to a stirring solution of diisopropylamine (440 µL, 3.1 mmol) in anhydrous tetrahydrofuran (3 mL) at -78°C under argon. The solution was stirred at 0°C for 15 minutes, and then cooled to -78°C.

The LDA was transferred dropwise to a suspension of ethyl acetate (307 µL, 3.1 mmol) and copper(I) iodide (1.2 g, 6.3 mmol) in anhydrous tetrahydrofuran (3 mL) stirring at -78°C under argon. Transfer was done in 1 mL aliquots to prevent warming of the LDA. The resulting suspension was stirred at -78°C for 30 minutes after which a solution of bromide **3a** (498 mg, 1.57 mmol) in tetrahydrofuran (2 mL) was cooled to -78°C and added dropwise. The resulting brown suspension was stirred at -78°C for 10 min, quenched by addition of saturated ammonium chloride and extracted with diethyl ether (x3). The combined organic layers were washed with water, brine and saturated ammonium bicarbonate, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by flash column chromatography eluting in a gradient of 3-10% ethyl acetate in *n*-hexane. The isolated product was a clear oil (474 mg, 1.46 mmol, 93%).

R_f = 0.39 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.39 – 5.30 (m, 1H), 5.17 – 5.10 (m, 1H), 4.62 (dd, *J* = 4.2, 3.0 Hz, 1H), 4.23 (dd, *J* = 11.9, 6.4 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.02 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.89 (ddd, *J* = 11.2, 7.6, 3.5 Hz, 1H), 3.55 – 3.46 (m, 1H), 2.43 – 2.33 (m, 2H), 2.33 – 2.24 (m, 2H), 2.16 – 2.06 (m, 2H), 2.06 – 1.99 (m, 2H), 1.90 – 1.45 (m, 6H), 1.67 (s, 3H), 1.60 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 173.64, 140.18, 133.76, 124.85, 120.84, 97.97, 63.78, 62.44, 60.37, 39.59, 34.80, 33.39, 30.85, 26.35, 25.63, 19.77, 16.55, 16.07, 14.40; HRMS, found 347.2193 ($C_{19}H_{32}O_4Na$, $[M + Na]^+$, requires 347.2198).

(4*E*,8*E*)-4,8-Dimethyl-10-(tetrahydro-2*H*-pyran-2-yloxy)deca-4,8-dien-1-ol (5a)

To a solution of **4a** (470 mg, 1.45 mmol) in anhydrous tetrahydrofuran (5 mL) stirring at 0°C under nitrogen was added lithium aluminum hydride (83 mg, 2.17 mmol). The reaction was stirred for 30 minutes and then quenched by addition of saturated ammonium chloride. The aqueous layer was extracted with diethyl ether (x3) and the combined organic layers washed with saturated sodium bicarbonate (x1) and brine (x2), dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by flash column chromatography eluting in a gradient of 1-30% acetone in *n*-hexane. The isolated product was a pale-yellow oil (349 mg, 1.23 mmol, 85%).

R_f = 0.58 (n-Hex – EtOAc, 1:1); δ_H/ppm (400 MHz, $CDCl_3$ 5.38 – 5.31 (m, 1H), 5.17 – 5.09 (m, 1H), 4.62 (dd, *J* = 4.3, 2.9 Hz, 1H), 4.28 – 4.20 (m, 1H), 4.02 (dd, *J* = 11.7, 7.3 Hz, 1H), 3.89 (ddd, *J* = 11.2, 7.5, 3.7 Hz, 1H), 3.61 (t, *J* = 6.5 Hz, 2H), 3.54 – 3.47 (m, 1H), 2.19 – 2.09 (m, 2H), 2.05 (t, *J* = 7.3 Hz, 4H), 1.89 – 1.46 (m, 8H), 1.66 (s, 3H), 1.60 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 139.91, 134.79, 124.54, 120.83, 97.99, 63.79, 62.47, 62.41, 39.51, 35.87, 30.71, 30.42, 26.02, 25.48, 19.67, 16.31, 15.84; HRMS, found 300.2542 ($C_{17}H_{34}NO_3$, $[M + NH_4]^+$, requires 300.2539).

(4*E*,8*E*)-1-Iodo-4,8-dimethyl-10-(tetrahydro-2*H*-pyran-2-yloxy)deca-4,8-diene (6a)

To a solution of **5a** (190 mg, 0.67 mmol), imidazole (64 mg, 0.94 mmol) and triphenylphosphine (265 mg, 1.01 mmol) in anhydrous acetonitrile stirring at 0°C under nitrogen was added iodine (239 mg, 0.94 mmol). The solution was stirred at 0°C for 2 hours, then at room temperature for 30 mins. The reaction was quenched by addition of saturated sodium thiosulfate and extracted with diethyl ether (3x). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo.* The crude product was purified by flash column chromatography in a gradient of 1-10% ethyl acetate in *n*-hexane. The isolated product was a pale-yellow oil (146 mg, 0.37 mmol, 56%).

R_f = 0.50 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.39 – 5.32 (m, 1H), 5.20 – 5.13 (m, 1H), 4.63 (dd, *J* = 4.1, 3.0 Hz, 1H), 4.27 – 4.20 (m, 1H), 4.02 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.89 (ddd, *J* = 11.2, 7.7, 3.6 Hz, 1H), 3.55 – 3.46 (m, 1H), 3.14 (t, *J* = 6.9 Hz, 2H), 2.08 (m, 6H), 1.90 (tt, *J* = 7.1, 7.1 Hz, 2H), 1.87 – 1.45 (m, 6H), 1.68 (s, 3H), 1.58 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) δ 139.93, 133.13, 125.46, 120.83, 97.84, 63.66, 62.29, 40.00, 39.50, 31.56, 30.74, 26.22, 25.52, 19.64, 16.39, 15.84, 6.63; HRMS, found 410.1533 ($C_{17}H_{33}NO_2I$, $[M + NH_4]^+$, requires 410.1556).

Trimethyl((7*E*,11*E*)-7,11-dimethyl-13-(tetrahydro-2*H*-pyran-2-yloxy)trideca-7,11-dien-1-yn-1-yl)silane (7a)

To a solution of 1-(trimethylsilyl)propyne (220 µL, 1.49 mmol) in anhydrous tetrahydrofuran (5 mL) stirring at -20°C under nitrogen was added *n*-butyllithium (2.5 M, 0.6 mL, 1.49 mmol) dropwise. The solution was stirred at -20°C for 45 minutes during which a pale-yellow color developed. **6a** (490 mg, 1.06 mmol) in tetrahydrofuran (1 mL) was added dropwise and the reaction stirred at -20°C for 1 hour. The reaction was diluted with H_2O and extracted with diethyl ether (3x). The combined organic layers were washed with saturated sodium bicarbonate and brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude was purified by flash column chromatography eluting in a gradient of 1-10% ethyl acetate in *n*-hexane. The isolated product was a clear oil (116 mg, 0.31 mmol, 83%).

R_f = 0.50 (Hexane – Ethyl acetate, 9:1); δ_H/ppm (400 MHz, $CDCl_3$) 5.40 – 5.29 (m, 1H), 5.10 (dt, *J* = 6.9, 3.4, 1H), 4.66 – 4.57 (m, 1H), 4.23 (dd, *J* = 11.9, 6.4, 1H), 4.02 (dd, *J* = 11.9, 7.4, 1H), 3.94 – 3.83 (m, 1H), 3.56 – 3.45 (m, 1H), 2.26 – 2.16 (m, 2H), 2.17 – 2.07 (m, 2H), 2.07 – 2.01 (m, 2H), 1.97 (t, *J* = 6.5, 2H), 1.90 – 1.38 (m, 10H), 1.67 (s, 3H), 1.58 (s, 3H), 0.14 (s, 9H); δ_C/ppm (100 MHz, $CDCl_3$) 140.35, 135.18, 124.25, 120.77, 107.77, 97.93, 84.50, 63.78, 62.42, 39.77, 39.15, 30.87, 28.18, 27.03, 26.41, 25.66, 19.85, 19.77, 16.56, 15.93, 0.32 (3C); HRMS, found 377.2883 ($C_{23}H_{41}O_2Si$, $[M + H]^+$, requires 377.2876).

(2*E*,6*E*)-3,7-Dimethyl-13-(trimethylsilyl)trideca-2,6-dien-12-yn-1-ol (8a)

A solution of **7a** (116 mg, 0.31 mmol) and pyridinium *p*-toluene sulfonate (7.7 mg, 0.03 mmol) in ethanol (5 mL) was stirred at 60°C for 4 hours. The solvent was evaporated *in vacuo* and the crude product purified by flash column chromatography eluting in a gradient of 5-30% acetone in *n*-hexane. The isolated product was a clear oil (77 mg, 0.26 mmol).

R_f = 0.25 (Hexane – Acetone, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.45 – 5.37 (m, 1H), 5.14 – 5.06 (m, 1H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.26 – 2.18 (m, 2H), 2.16 – 2.07 (m, 2H), 2.07 – 2.00 (m, 2H), 2.00 – 1.93 (m, 2H), 1.68 (s, 3H), 1.58 (s, 3H), 1.52 – 1.40 (m, 4H), 0.14 (s, 9H); δ_C/ppm (100 MHz, $CDCl_3$) 139.84, 135.27, 124.16, 123.56, 107.81, 84.53, 59.55, 39.66, 39.12, 28.14, 27.00, 26.36, 19.86, 16.40, 15.91, 0.31 (3C); HRMS, found 310.2579 ($C_{18}H_{36}NOSi$, $[M + NH_4]^+$, requires 310.2566).

(2*E*,6*E*)-3,7-Dimethyltrideca-2,6-dien-12-yn-1-ol (9a = YnF)

To a solution of **8a** (75 mg, 0.25 mmol) in anhydrous tetrahydrofuran (5 mL) stirring at room temperature under argon was added tetrabutylammonium fluoride (1 M in THF, 0.5 mL, 0.5 mmol). The reaction was stirred for 15 minutes after which the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography eluting in a gradient of 3-30% acetone in *n*-hexane. The isolated product was a clear oil (32 mg, 0.15 mmol, 59%).

R_f = 0.21 (Hexane – Acetone, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.40 (tq, *J* = 6.9, 1.2 Hz), 5.14 – 5.04 (m, 1H), 4.14 (d, *J* = 6.9 Hz, 2H), 2.22 – 2.15 (m, 2H), 2.15 – 2.07 (m, 2H), 2.07 – 2.00 (m, 2H), 2.00 – 1.95 (m, 2H), 1.94 (t, *J* = 2.7 Hz), 1.67 (s, 3H), 1.58 (s, 3H), 1.52 – 1.44 (m, 4H), 1.42 (s, 1H); δ_C/ppm (100 MHz, $CDCl_3$) 139.75, 135.17, 124.17, 123.56, 84.82, 68.28, 59.51, 39.63, 39.11, 28.05, 26.98, 26.31, 18.40, 16.36, 15.93; HRMS, found 238.2165 ($C_{15}H_{28}NO$, $[M + NH_4]^+$, requires 238.2171).

Trisammonium (2*E*,6*E*)-3,7-dimethyltrideca-2,6-dien-12-yn-1-yl pyrophosphate (10a = YnFPP)

The synthesis was adapted from a previously reported method[16]. To a solution of *N*-chlorosuccinimide (15 mg, 0.11 mmol) in anhydrous dichloromethane (1 mL) at -40°C under argon was added dimethyl sulfide (14 µL, 0.18 mmol) dropwise. The reaction was stirred at 0°C for 15 minutes and again cooled to -40°C. **9a** (20 mg, 0.09 mmol) in anhydrous dichloromethane (1 mL) was added dropwise and the reaction stirred at 0°C for 2 hours. The reaction was diluted with dichloromethane, washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The resulting isoprenyl chloride was used without further purification.

A solution of the isoprenyl chloride and tris(tetrabutylammonium) hydrogen pyrophosphate (163 mg, 0.18 mmol) in anhydrous acetonitrile (2 mL) was stirred at room temperature for 3 hours. The solvent was evaporated *in vacuo* and the product was dissolved in 25 mM ammonium bicarbonate buffer/isopropanol (39:1, 2 mL). The solution was passed through a 1x8 cm Dower 50X8 ion exchange column (NH_4^+ form). The eluent solution was lyophilized to yield a white solid. The solid was further purified by a tC18-Sep-Pak cartridge eluting in a gradient of 0-100% acetonitrile in 25 mM ammonium bicarbonate buffer. The isolated product was lyophilized to yield a white solid (7.2 mg, 0.019 mmol, 21%).

δ_H/ppm (400 MHz, D_2O/ND_4OD) δ 5.35 (t, *J* = 7.1 Hz, 1H), 5.11 (t, *J* = 6.6 Hz, 1H), 4.35 (t, *J* = 6.6 Hz, 2H), 2.22 (t, *J* = 2.7 Hz, 1H), 2.13 – 1.96 (m, 6H), 1.90 (t, *J* = 6.9 Hz, 2H), 1.60 (s, 3H), 1.50 (s, 3H), 1.37 (tdd, *J* = 11.4, 9.0, 8.0, 5.5 Hz, 4H); δ_P/ppm (162 MHz, D_2O/ND_4OD) δ -7.39 (d, *J* = 21.9 Hz, 1P), -10.55 (d, *J* = 21.7 Hz, 1P); HRMS, found 379.1081 ($C_{15}H_{25}O_7P_2$, $[M - H]^-$, requires 379.1081).

(2*E*,6*E*)-3,7,11-Trimethyl-1-(tetrahydro-2*H*-pyran-2-yloxy)dodeca-2,6,10-triene (1b)

As for **1a** except using *trans*-*trans*-farnesol (10 g, 45 mmol), pyridinium *p*-toluene sulfonate (1.13 g, 4.5 mmol) and 2,4-dihydropyran (16.4 mL, 180 mmol). The isolated product was a clear liquid (11.2 g, 36.4 mmol, 88%).

R_f = 0.25 (Hexane – Ethyl acetate, 95:5); δ_H/ppm (400 MHz, $CDCl_3$) 5.39 – 5.30 (m, 1H), 5.14 – 5.02 (m, 2H), 4.65 – 4.57 (m, 1H), 4.27 – 4.17 (m, 1H), 4.01 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.88 (ddd, *J* = 11.2, 7.7, 3.5 Hz, 1H), 3.54 – 3.43 (m, 1H), 2.16 – 1.90 (m, 8H), 1.90 – 1.40 (m, 6H), 1.66 (s, 6H), 1.58 (s, 6H); δ_C/ppm (100 MHz, $CDCl_3$) 140.30, 135.30, 131.34, 124.45, 124.00, 120.74, 97.84, 63.72, 62.33, 39.80, 39.73, 30.82, 26.83, 26.39, 25.77, 25.62, 19.71, 17.76, 16.50, 16.09; HRMS, found 324.2908 ($C_{20}H_{38}NO_2$, $[M + NH_4]^+$, requires 324.2903).

(2*E*,6*E*,10*E*)-2,6,10-Trimethyl-12-(tetrahydro-2*H*-pyran-2-yloxy)dodeca-2,6,10-trien-1-ol (2b)

As for **2a** with minor modifications as detailed below, using **1b** (12.6 g, 41 mmol), selenium dioxide (490 mg, 4.4 mmol) and *tert*-butyl hydroperoxide (8.22 mL, 45 mmol). After stirring overnight, a further volume of *tert*-butyl hydroperoxide (4.0 mL, 22 mmol) was added and the reaction stirred for a further 48 hours. The crude product was purified by flash column chromatography eluting in a gradient of 5-15% ethyl acetate in *n*-hexane. The isolated product was a clear oil (3.0 g, 9.3 mmol, 23%).

R_f = 0.18 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.44 – 5.30 (m, 2H), 5.15 – 5.06 (m, 1H), 4.65 – 4.58 (m, 1H), 4.27 – 4.18 (m, 1H), 4.07 – 4.00 (m, 1H), 3.98 (s, 2H), 3.88 (ddd, *J* = 11.2, 7.6, 3.4 Hz, 1H), 3.55 – 3.45 (m, 1H), 2.07 (m, 8H), 1.89 – 1.46 (m, 6H), 1.67 (s, 3H), 1.65 (s, 3H), 1.59 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 140.23, 134.95, 134.88, 125.97, 124.35, 120.81, 97.84, 69.03, 63.74, 62.40, 39.69, 39.38, 30.81, 26.31, 26.21, 25.62, 19.72, 16.53, 16.11, 13.82; HRMS, found 340.2861 ($C_{20}H_{38}NO_3$, $[M + NH_4]^+$, requires 340.2852).

(2*E*,6*E*,10*E*)-1-Bromo-2,6,10-trimethyl-12-(tetrahydro-2*H*-pyran-2-yloxy)dodeca-2,6,10-triene (3b)

As for **3a** using **2b** (1.41 g, 4.4 mmol), *N*-bromosuccinimide (1.17 g, 6.6 mmol) and dimethyl sulfide (0.58 mL, 7.9 mmol). The isolated product was a pale-yellow oil (1.22 g, 3.2 mmol, 73%)

R_f = 0.47 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.57 (t, *J* = 7.4 Hz, 1H), 5.40 – 5.32 (m, 1H), 5.15 – 5.07 (m, 1H), 4.66 – 4.59 (m, 1H), 4.24 (dd, *J* = 11.9, 6.4 Hz, 1H), 4.06 – 4.00 (m, 1H), 3.97 (s, 2H), 3.89 (ddd, *J* = 11.2, 7.6, 3.4 Hz, 1H), 3.55 – 3.46 (m, 1H), 2.17 – 1.94 (m, 8H), 1.91 – 1.46 (m, 6H), 1.75 (s, 3H), 1.68 (s, 3H), 1.59 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 140.28, 134.57, 132.06, 131.36, 124.67, 120.78, 97.97, 63.80, 62.45, 42.06, 39.69, 38.88, 30.86, 26.97, 26.38, 25.64, 19.78, 16.57, 16.11, 14.80; HRMS, found 402.2012 ($C_{20}H_{37}NO_2Br$, $[M + NH_4]^+$, requires 402.2008).

Ethyl (4*E*,8*E*,12E)-4,8,12-trimethyl-14-(tetrahydro-2*H*-pyran-2-yloxy)tetradeca-4,8,12-trienoate (4b)

As for **4a** using *n*-butyllithium (2.28 mL, 5.7 mmol), diisopropylamine (800 µL, 5.7 mmol), **3b** (1.10 g, 2.9 mmol), ethyl acetate (560 µL, 5.7 mmol) and copper(I) iodide (2.17 g, 11.4 mmol). The isolated product was a pale-yellow oil (753 mg, 1.92 mmol, 67%)

R_f = 0.50 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.43 – 5.35 (m, 1H), 5.19 – 5.08 (m, 2H), 4.68 – 4.62 (m, 1H), 4.26 (dd, *J* = 11.8, 6.4 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.05 (dd, *J* = 11.8, 7.5 Hz, 1H), 3.92 (ddd, *J* = 11.2, 7.6, 3.3 Hz, 1H), 3.59 – 3.49 (m, 1H), 2.45 – 2.37 (m, 2H), 2.37 – 2.27 (m, 2H), 2.19 – 1.94 (m, 8H), 1.94 – 1.50 (m, 6H), 1.71 (s, 3H), 1.63 (s, 3H), 1.62 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 173.69, 140.42, 135.24, 133.45, 125.19, 124.16, 120.70, 97.95, 63.79, 62.44, 60.38, 39.77, 39.68, 34.82, 33.43, 30.86, 26.74, 26.43, 25.64, 19.77, 16.58, 16.16, 16.06, 14.41; HRMS, found 415.2820 ($C_{24}H_{40}O_4Na$, $[M + Na]^+$, requires 415.2824).

(4*E*,8*E*,12*E*)-4,8,12-Trimethyl-14-(tetrahydro-2*H*-pyran-2-yloxy)tetradeca-4,8,12-trien-1-ol (5b)

As for **5a** using **4a** (750 mg, 1.9 mmol) and lithium aluminum hydride (108 mg, 2.9 mmol). The isolated product was a clear oil (459 mg, 1.3 mmol, 69%).

R_f = 0.33 (Hexane – Acetone, 7:3); δ_H/ppm (400 MHz, $CDCl_3$) 5.41 – 5.34 (m, 1H), 5.19 – 5.08 (m, 2H), 4.67 – 4.61 (m, 1H), 4.25 (dd, *J* = 11.6, 6.1, 1H), 4.09 – 4.01 (m, 1H), 3.95 – 3.87 (m, 1H), 3.64 (t, *J* = 6.5, 2H), 3.57 – 3.48 (m, 1H), 2.25 – 1.95 (m, 10H), 1.94 – 1.42 (m, 8H), 1.70 (s, 3H), 1.63 (s, 3H), 1.61 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 140.43, 135.28, 134.84, 124.93, 124.26, 120.83, 98.00, 63.87, 63.00, 62.50, 39.81, 36.21, 30.97, 30.93, 26.68, 26.45, 25.72, 19.87, 19.83, 16.62, 16.17, 16.08; HRMS, found 373.2731 ($C_{22}H_{38}O_3Na$, $[M + Na]^+$, requires 373.2719).

(4*E*,8*E*,12*E*)-1-Iodo-4,8,12-trimethyl-14-(tetrahydro-2*H*-pyran-2-yloxy)tetradeca-4,8,12-triene (6b)

As for **6a** using **5b** (450 mg, 1.3 mmol), imidazole (123 mg, 1.8 mmol), triphenylphosphine (506 mg, 1.9 mmol) and iodine (457 mg, 1.8 mmol). The isolated product was a pale-yellow oil (497 mg, 1.1 mmol, 84%).

R_f = 0.60 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.40 – 5.32 (m, 1H), 5.20 – 5.13 (m, 1H), 5.13 – 5.06 (m, 1H), 4.62 (dd, *J* = 4.2, 3.0 Hz, 1H), 4.23 (dd, *J* = 11.9, 6.4 Hz, 1H), 4.03 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.89 (ddd, *J* = 11.2, 7.6, 3.5 Hz, 1H), 3.55 – 3.45 (m, 1H), 3.14 (t, *J* = 7.0 Hz, 2H), 2.15 – 1.95 (m, 10H), 1.94 – 1.46 (m, 8H), 1.68 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H); δ_C/ppm (100 MHz, $CDCl_3$) 140.36, 135.16, 132.91, 125.94, 124.25, 120.75, 97.93,

63.79, 62.43, 40.13, 39.77, 39.71, 31.71, 30.87, 26.68, 26.44, 25.65, 19.77, 16.58, 16.10, 15.95, 6.79; HRMS, found 478.2196 ($C_{22}H_{41}NO_2I$, $[M + NH_4]^+$, requires 478.2182).

Trimethyl((7*E*,11*E,15E*)-7,11,15-trimethyl-17-(tetrahydro-2*H*-pyran-2-yloxy)heptadeca-7,11,15-trien-1-yn-1-yl)silane (7b)

As for **7a** using **6b** (490 mg, 1.1 mmol), 1-(trimethylsilyl)propyne (0.63 mL, 4.3 mmol), *n*-butyllithium (1.70 mL, 4.3 mmol). The isolated product was a clear oil (375 mg, 0.84 mmol, 80%).

R_f = 0.51 (Hexane – Ethyl acetate, 9:1); δ_H/ppm (400 MHz, $CDCl_3$) 5.41 – 5.30 (m, 1H), 5.15 – 5.05 (m, 2H), 4.62 (dd, *J* = 4.3, 2.9 Hz, 1H), 4.28 – 4.19 (m, 1H), 4.03 (dd, *J* = 11.9, 7.4 Hz, 1H), 3.89 (ddd, *J* = 11.3, 7.6, 3.7 Hz, 1H), 3.55 – 3.45 (m, 1H), 2.25 – 2.18 (m, 2H), 2.15 – 1.93 (m, 10H), 1.90 – 1.40 (m, 10H), 1.68 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H), 0.14 (s, 9H); δ_C/ppm (100 MHz, $CDCl_3$) 140.41, 135.38, 134.86, 124.59, 124.07, 120.73, 107.79, 97.93, 84.48, 63.79, 62.42, 39.85, 39.79, 39.16, 30.87, 28.18, 27.05, 26.77, 26.45, 25.66, 19.87, 19.77, 16.58, 16.16, 15.91, 0.32 (3C); HRMS, found 462.3776 ($C_{28}H_{52}NO_2Si$, $[M + NH_4]^+$, requires 462.3767).

(2*E*,6*E,10E*)-3,7,11-Trimethyl-17-(trimethylsilyl)heptadeca-2,6,10-trien-16-yn-1-ol (8b)

As for **8a** using **7b** (312 mg, 0.70 mmol) and pyridinium *p*-toluene sulfonate (17 mg, 0.07 mmol). The crude product was purified by flash column chromatography eluting in a gradient of 3-30% ethyl acetate in *n*-hexane. The isolated product was a clear oil (207 mg, 0.57 mmol, 82%).

R_f = 0.34 (Hexane – Ethyl acetate, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.46 – 5.37 (m, 1H), 5.15 – 5.04 (m, 2H), 4.15 (d, *J* = 7.0 Hz, 2H), 2.27 – 2.17 (m, 2H), 2.13 – 1.93 (m, 10H), 1.68 (s, 3H), 1.60 (s, 3H), 1.58 (s, 3H), 1.47 (m, 4H), 1.41 (br s, 1H), 0.14 (s, 9H); δ_C/ppm (100 MHz, $CDCl_3$) 140.00, 135.50, 134.89, 124.55, 123.93, 123.45, 107.80, 84.50, 59.56, 39.83, 39.69, 39.16, 28.17, 27.04, 26.74, 26.44, 19.87, 16.44, 16.16, 15.91, 0.32 (3C); HRMS, found 378.3182 ($C_{23}H_{44}NOSi$, $[M + NH_4]^+$, requires 378.3192).

(2*E*,6*E*,10*E*)-3,7,11-Trimethylheptadeca-2,6,10-trien-16-yn-1-ol (9b = YnGG)

As for **9a** using **8b** (205 mg, 0.57 mmol) and tetrabutylammonium fluoride (1.14 mL, 1.14 mmol). The crude product was purified by flash column chromatography eluting in a gradient of 3-20% acetone in *n*-hexane. The isolated product was a clear oil (58 mg, 0.19 mmol, 33%).

R_f = 0.29 (Hexane – Acetone, 8:2); δ_H/ppm (400 MHz, $CDCl_3$) 5.46 – 5.36 (m, 1H), 5.15 – 5.04 (m, 2H), 4.15 (d, *J* = 6.9 Hz, 2H), 2.18 (td, *J* = 6.6, 2.6 Hz, 2H), 2.05 (m, 10H), 1.94 (t, *J* = 2.6 Hz, 1H), 1.68 (s, 3H), 1.60 (s, 3H), 1.58 (s, 3H), 1.52 – 1.45 (m, 4H), 1.33 (br s, 1H); δ_C/ppm (100 MHz, $CDCl_3$) 139.97, 135.46, 134.81, 124.59, 123.96, 123.46, 84.83, 68.27, 59.54, 39.81, 39.69, 39.17, 28.09, 27.03, 26.70, 26.44, 18.43, 16.43, 16.13, 15.94; HRMS, found 306.2788 ($C_{20}H_{36}NO$, $[M + NH_4]^+$, requires 306.2797).

Trisammonium (2*E*,6*E*,10*E*)-3,7,11-trimethylheptadeca-2,6,10-trien-16-yn-1-yl pyrophosphate (10b = YnGGPP)

As for **10a** using **9b** (20 mg, 0.07 mmol), *N*-Chlorosuccinimide (13 mg, 0.08 mmol), dimethyl sulfide (11 µL, 0.14 mmol) and tris(tetrabutylammonium) hydrogen pyrophosphate (127 mg, 0.14 mmol). The isolated product was a white solid (10 mg, 0.02 mmol, 30%).

δ_H/ppm (400 MHz, D_2O/ND_4OD) δ 5.33 (t, *J* = 7.3 Hz, 1H), 5.05 (d, *J* = 8.1 Hz, 2H), 4.36 (t, *J* = 6.5 Hz, 2H), 2.13 – 2.03 (m, 3H), 1.99 (d, *J* = 16.3 Hz, 5H), 1.94 – 1.86 (m, 4H), 1.64 (s, 1H), 1.61 (s, 2H), 1.58 (t, *J* = 3.1 Hz, 1H), 1.50 (d, *J* = 7.7 Hz, 6H), 1.40 – 1.34 (m, 3H); δ_P/ppm

(162 MHz, D_2O/ND_4OD) δ -10.36 – -11.24 (m, 2P); HRMS, found 447.1704 ($C_{20}H_{33}O_7P_2$, $[M-H]^-$, requires 447.1707).

FAM-GerGer-RHOA(188-193)

FAM-RHOA(188-193) peptide (14 mg, 14 μmol) was dissolved in methanol (1.5 mL) and the pH adjusted to between 10 and 12 with 1 M sodium hydroxide solution (60 μL). The solution was cooled on ice for 5 min, then geranylgeranyl chloride (13 uL, 42 μmol, prepared according to published protocol[17]) was added. The reaction was stirred on ice for 30 min, quenched with acetic acid solution and purified on a C8 reverse phase HPLC column (Agilent Zorbax PrepHT 300SB-C8, 21.2x250 mm, 7 μm) using a linear solvent gradient of 28-90% acetonitrile/0.08% TFA in H_2O/0.08% TFA over 40 min at a flow rate of 8 mL/min.

Peptide Characterization

FAM-UCHL1(218-223):
HRMS (TOF MS ES+), m/z: calculated for $C_{48}H_{61}N_8O_{14}S$ $[M+H]^+$: 1005.4028, found: 1005.4012; Analytical RP-HPLC: Gradient: 5-98% acetonitrile/0.1% formic acid in H_2O/0.1% formic acid over 18 min, RT = 6.99 min.

FAM-RHEB(179-184):
HRMS (TOF MS ES+), m/z: calculated for $C_{46}H_{56}N_7O_{17}S_2$ $[M+H]^+$: 1042.3174, found: 1042.3218; Analytical RP-HPLC: Gradient: 5-98% acetonitrile/0.1% formic acid in H_2O/0.1% formic acid over 18 min, RT = 9.27 min.

FAM-RHOA(188-193)
HRMS (TOF MS ES+), m/z: calculated for $C_{49}H_{62}N_7O_{15}S$ $[M+H]^+$: 1020.4025, found: 1020.4039; Analytical RP-HPLC: Gradient: 5-98% acetonitrile/0.1% formic acid in H_2O/0.1% formic acid over 18 min, RT = 8.83 min.

FAM-GerGer-RHOA(188-193)
HRMS (TOF MS ES+), m/z: calculated for $C_{69}H_{94}N_7O_{15}S$ $[M+H]^+$: 1292.6529, found: 1292.6547; Analytical RP-HPLC: Gradient: 5-98% acetonitrile/0.1% formic acid in H_2O/0.1% formic acid over 18 min, RT = 14.37 min.

References

1 Berry, A. F. *et al.* Rapid multilabel detection of geranylgeranylated proteins by using bioorthogonal ligation chemistry. *Chembiochem* **11**, 771-773, doi:10.1002/cbic.201000087 (2010).

2 Gagneux, A., Winstein, S. & Young, W. G. Rearrangement of Allylic Azides. *J Am Chem Soc* **82**, 5956-5957 (1960).

3 Charron, G., Tsou, L. K., Maguire, W., Yount, J. S. & Hang, H. C. Alkynyl-farnesol reporters for detection of protein S-prenylation in cells. *Mol Biosyst* **7**, 67-73, doi:10.1039/c0mb00183j (2011).

4 Kho, Y. *et al.* A tagging-via-substrate technology for detection and proteomics of farnesylated proteins. *Proc Natl Acad Sci U S A* **101**, 12479-12484, doi:10.1073/pnas.0403413101 (2004).

5 Chan, L. N. *et al.* A novel approach to tag and identify geranylgeranylated proteins. *Electrophoresis* **30**, 3598-3606, doi:10.1002/elps.200900259 (2009).

6 DeGraw, A. J. *et al.* Evaluation of alkyne-modified isoprenoids as chemical reporters of protein prenylation. *Chem Biol Drug Des* **76**, 460-471, doi:10.1111/j.1747-0285.2010.01037.x (2010).

7 Charron, G., Li, M. M., MacDonald, M. R. & Hang, H. C. Prenylome profiling reveals S-farnesylation is crucial for membrane targeting and antiviral activity of ZAP long-isoform. *Proc Natl Acad Sci U S A* **110**, 11085-11090, doi:10.1073/pnas.1302564110 (2013).

8 Palsuledesai, C. C., Ochocki, J. D., Markowski, T. W. & Distefano, M. D. A combination of metabolic labeling and 2D-DIGE analysis in response to a farnesyltransferase inhibitor facilitates the discovery of new prenylated proteins. *Mol Biosyst* **10**, 1094-1103, doi:10.1039/c3mb70593e (2014).

9 Broncel, M. *et al.* Multifunctional reagents for quantitative proteome-wide analysis of protein modification in human cells and dynamic profiling of protein lipidation during vertebrate development. *Angew Chem Int Ed Engl* **54**, 5948-5951, doi:10.1002/anie.201500342 (2015).

10 Lanyon-Hogg, T. *et al.* Microfluidic Mobility Shift Assay for Real-Time Analysis of Peptide N-Palmitoylation. *SLAS Discov* **22**, 418-424, doi:10.1177/2472555216689529 (2017).

11 Tolmachova, T. *et al.* Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. *J Clin Invest* **116**, 386-394, doi:10.1172/JCI26617 (2006).

12 Caballe, A. *et al.* ULK3 regulates cytokinetic abscission by phosphorylating ESCRT-III proteins. *Elife* **4**, e06547, doi:10.7554/eLife.06547 (2015).

13 Rappsilber, J., Ishihama, Y. & Mann, M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal Chem* **75**, 663-670 (2003).

14 Goya Grocin, A., Serwa, R. A., Morales Sanfrutos, J., Ritzefeld, M. & Tate, E. W. Whole proteome profiling of N-myristoyltransferase activity and inhibition using Sortase-A. *Mol Cell Proteomics*, doi:10.1074/mcp.RA118.001043 (2018).

15 Zhang, J. *et al.* PEAKS DB: de novo sequencing assisted database search for sensitive and accurate peptide identification. *Mol Cell Proteomics* **11**, M111 010587, doi:10.1074/mcp.M111.010587 (2012).

16 Nguyen, U. T. *et al.* Exploiting the substrate tolerance of farnesyltransferase for site-selective protein derivatization. *Chembiochem* **8**, 408-423, doi:10.1002/cbic.200600440 (2007).

17 Corey, E. J., Kim, C. U. & Takeda, M. A method for selective conversion of allylic and benzylic alcohols to halides under neutral conditions. *Tetrahedron Letters* **13**, 4339-4342, doi:**https://doi.org/10.1016/S0040-4039(01)94310-2** (1972).