Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information for:

A combination of metabolic labeling and 2D-DIGE analysis in response to a farnesyltransferase inhibitor facilitates the discovery of new prenylated proteins

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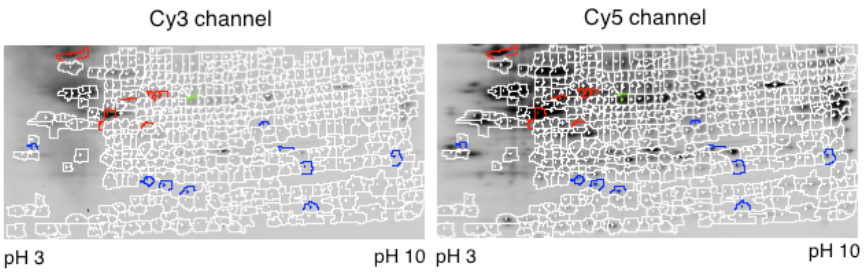


Figure S1. 2D-DIGE analysis shows that when identical samples (50 µM C15Alk-treated HeLa cells) are reacted with Cy3-N3 and Cy5-N3 and run together on a 2D gel, the corresponding Cy3- and Cy5-labeled spots co-migrate on the gel. >96% spots were found to have 1:1 ratio.

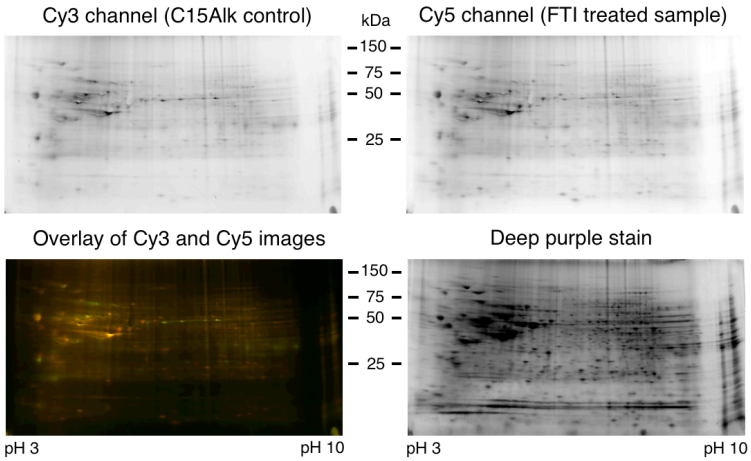


Figure S2. Representative gel showing 2D DIGE analysis of samples from FTI-treated HeLa cells. HeLa cells were treated with 50 µM **1** in presence or absence of 10 µM FTI. The lysate of FTI-treated cells was reacted with Cy5-azide while the C15-alkyne treated (control) was reacted with Cy3-azide. After mixing the samples, the proteins were first resolved using a pH 3-10 NL IPG strip and then with a 10-20% polyacrylamide gradient gel.

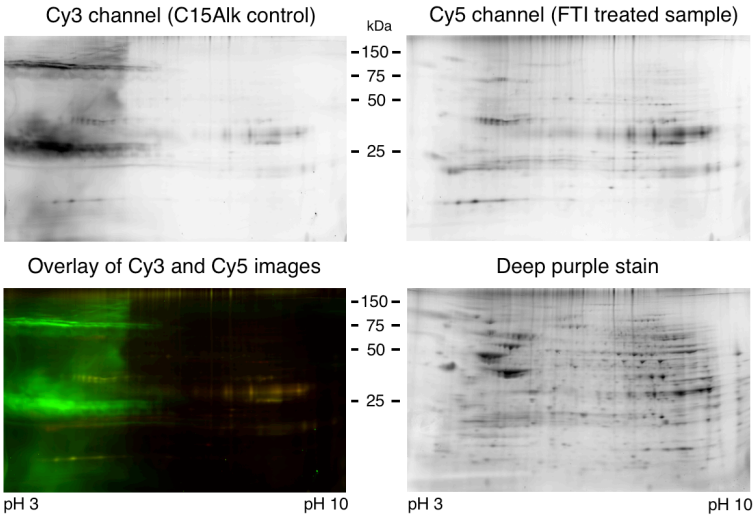


Figure S3. DIGE of Triton X-114 fractionated FTI-treated HeLa cells. HeLa cells were treated with 50 µM **1** in presence or absence of 10 µM FTI. Cell lysates were fractionated into aqueous and Triton X-114 phases. Triton

X-114 phase of FTI treated cells was reacted with Cy3-N_3 and C15-alkyne treated Triton X-114 phase was reacted with Cy5-N_3. Proteins were first resolved on pH 3-10 NL IPG strip and then on 10-20% polyacrylamide gel.

Table S1. List of protein spots used for protein identification from the 2D-DIGE gel comparing C15Alk labeling of HeLa cells in presence or absence of an FTI (Analysis without Triton X-114 fractionation).

Spot number[a]	C15Alk+FTI/C15Alk Ratio in gel used for protein ID[b]	C15Alk+FTI/C15Alk Avg ratio from three gels[c]	T-test score[c]
91	-2.37	-1.97	0.0027
216	-1.53	-	-
265	1.97	1.27	0.33
362	-1.79	-1.71	0.014
414	2.39	2.57	0.0071
473	1.63	1.44	0.074
207	-2.09	-2	0.064
213	-2.77	-2.24	0.023
357	-1.57	-1.49	0.029
418	2.2	1.73	0.12
519	2.47	2.44	0.0000094
408	-1.78	-1.36	0.52

[a] Spot numbers are derived from the DIA analysis performed on the gel used for spot excision. [b] Ratios from DeCyder DIA analysis performed on the Cy3 and Cy5 images of the gel. [c] Values from DeCyder BVA analysis on three biological replicates of 2D-DIGE comparing C15-alkyne labeling in presence and absence of an FTI. A value is not provided for the spot that could not be detected in gels other than the one used for spot excision.

Table S2. Migration pattern and 3D spot view (from DIA analysis) of the spots used for protein identification from the 2D-DIGE gel comparing C15Alk labeling of HeLa cells in presence or absence of an FTI (Analysis without Triton X-114 fractionation).

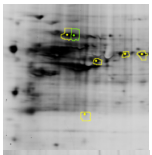
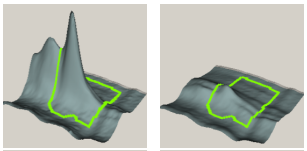
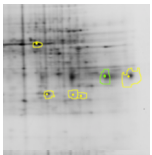
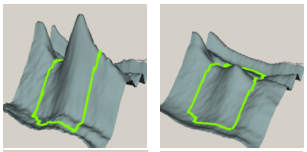
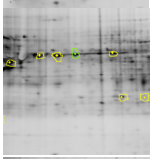
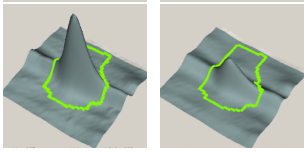
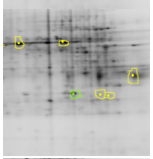
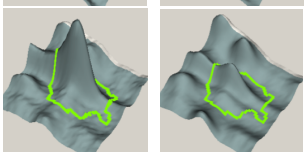
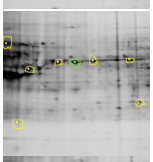
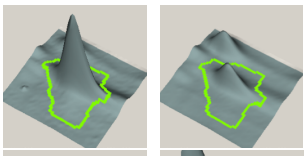
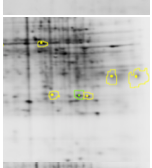
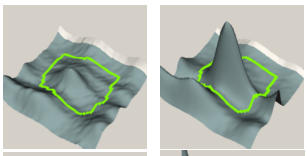
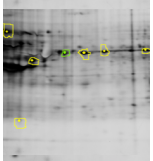
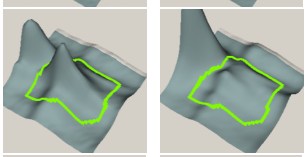
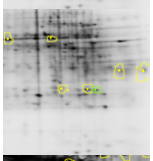
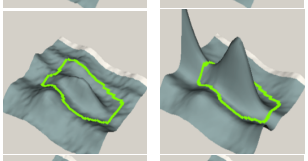
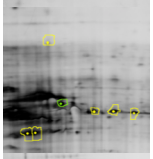
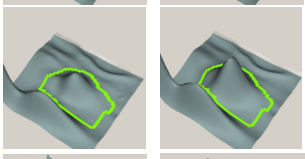
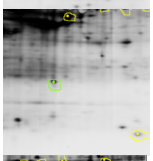
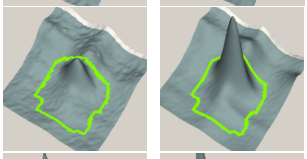
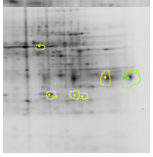
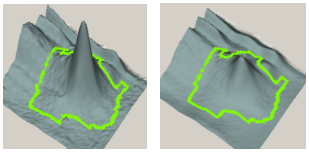
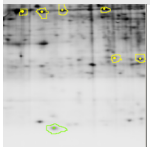
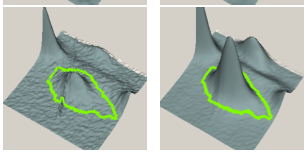
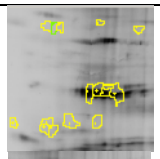
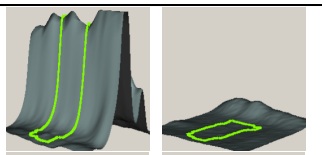
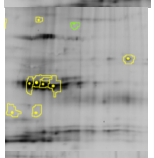
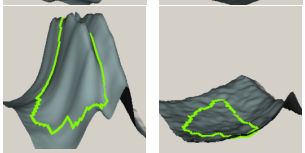
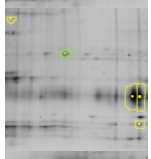
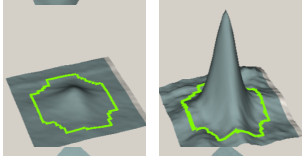
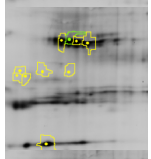
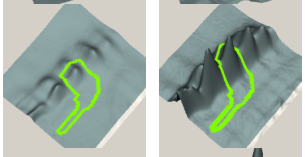
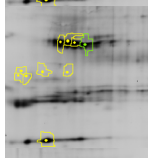
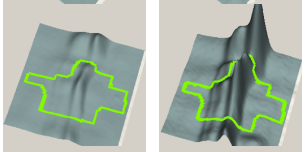
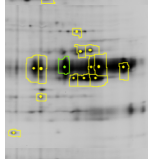
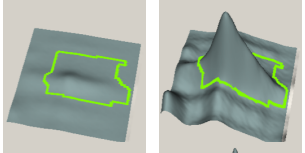
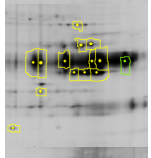
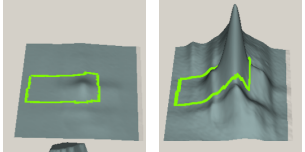
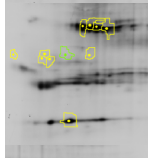
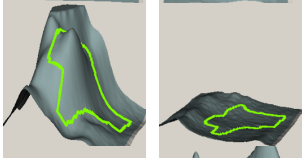
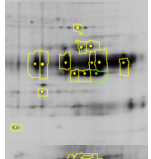
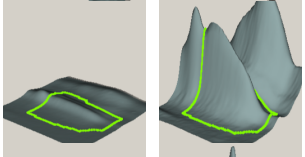
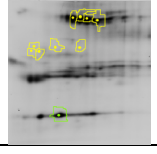
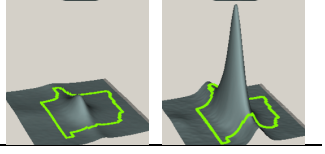
Spot number	Migration pattern	DIA 3D view	Spot number	Migration pattern	DIA 3D view
91			362		
207			408		
213			414		
216			418		
265			473		
357			519		

Table S3. List of protein spots used for protein identification from the 2D-DIGE gel comparing C15Alk labeling of HeLa cells in presence or absence of an FTI (Analysis with Triton X-114 fractionation).

Spot number	Ratio	Migration pattern	DIA 3D view
2	-93.06		
5	-13.18		
6	18.78		
9	7.96		
11	10.50		
19	23.97		
20	25.82		
22	-21.84		
24	16.36		
32	9.12		

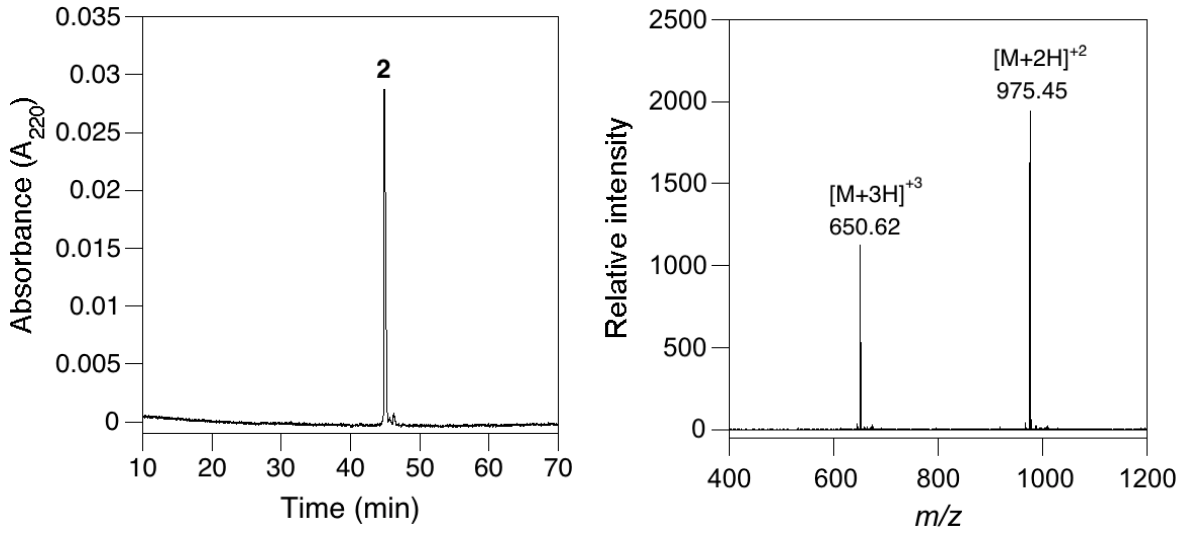


Figure S4. Left: HPLC analysis of purified peptide **2** showed 94.5% purity. Right: ESI-MS analysis showed presence of expected peptide. Calculated $[M+2H]^{2+}$ = 975.45, observed $[M+2H]^{2+}$ = 975.43, calculated $[M+3H]^{3+}$ = 650.63, observed $[M+3H]^{3+}$ = 650.62.

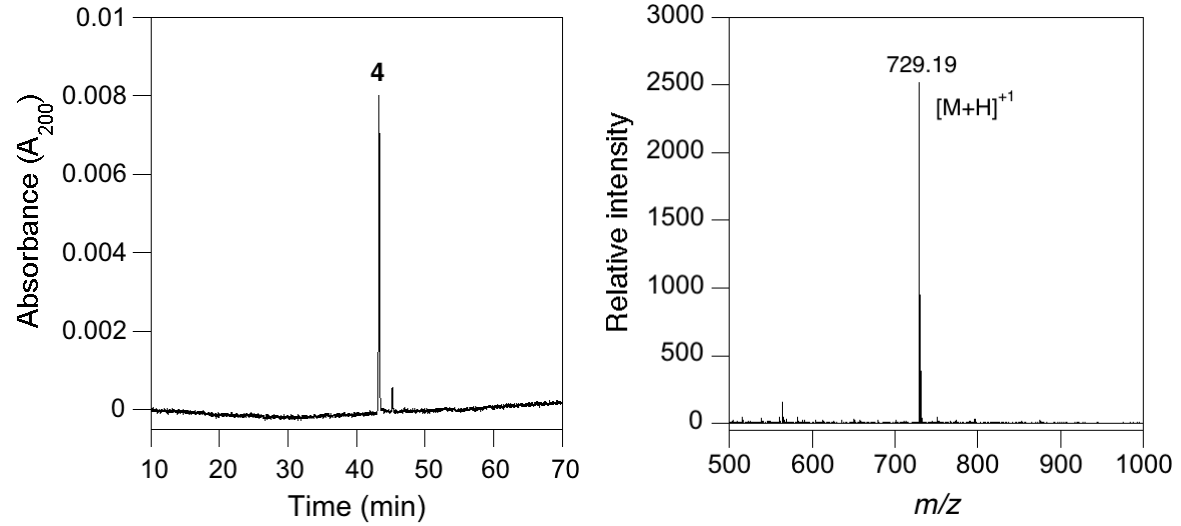
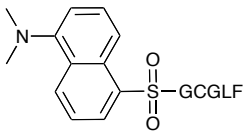


Figure S5. Left: HPLC analysis of purified peptide **4** showed 98.5% purity. Right: ESI-MS analysis showed presence of expected peptide. Calculated $[M+H]^{+}$ = 729.27, observed $[M+H]^{+}$ = 729.19.



Dansyl-GCGLF
4

Figure S6. Structure of dansyl-GCGLF, **4**.

y = m1*x/(m2+x)		
	Value	**Error**
m1	0.010653	9.9177e-4
m2	7.9377	1.7691
Chisq	1.3938e-6	NA
R	0.99085	NA

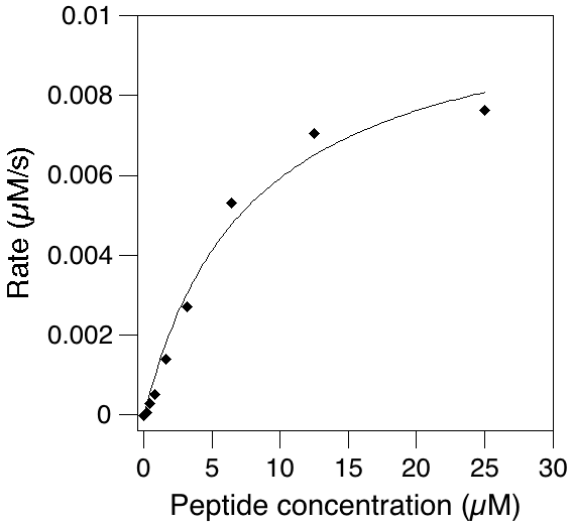


Figure S7. Continuous fluorescence assay carried out using a peptide concentration range of 0.2-25 μM, 10 μM FPP and 100 nM rPFTase.