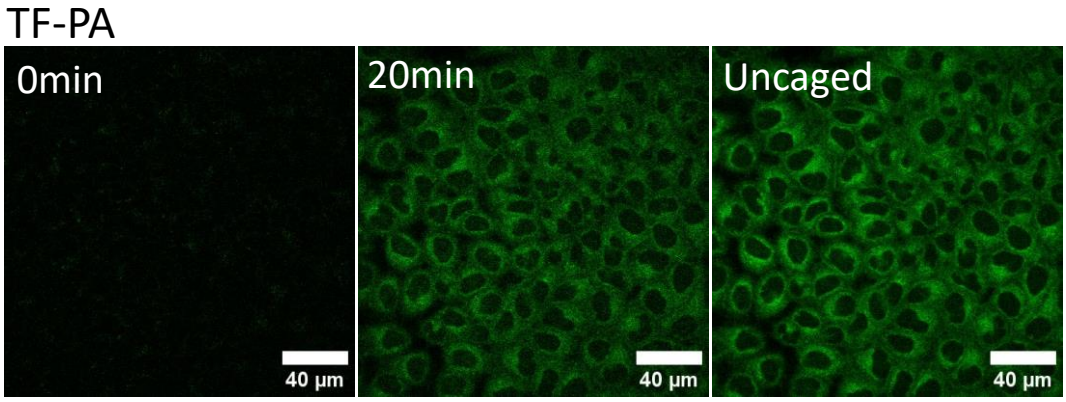
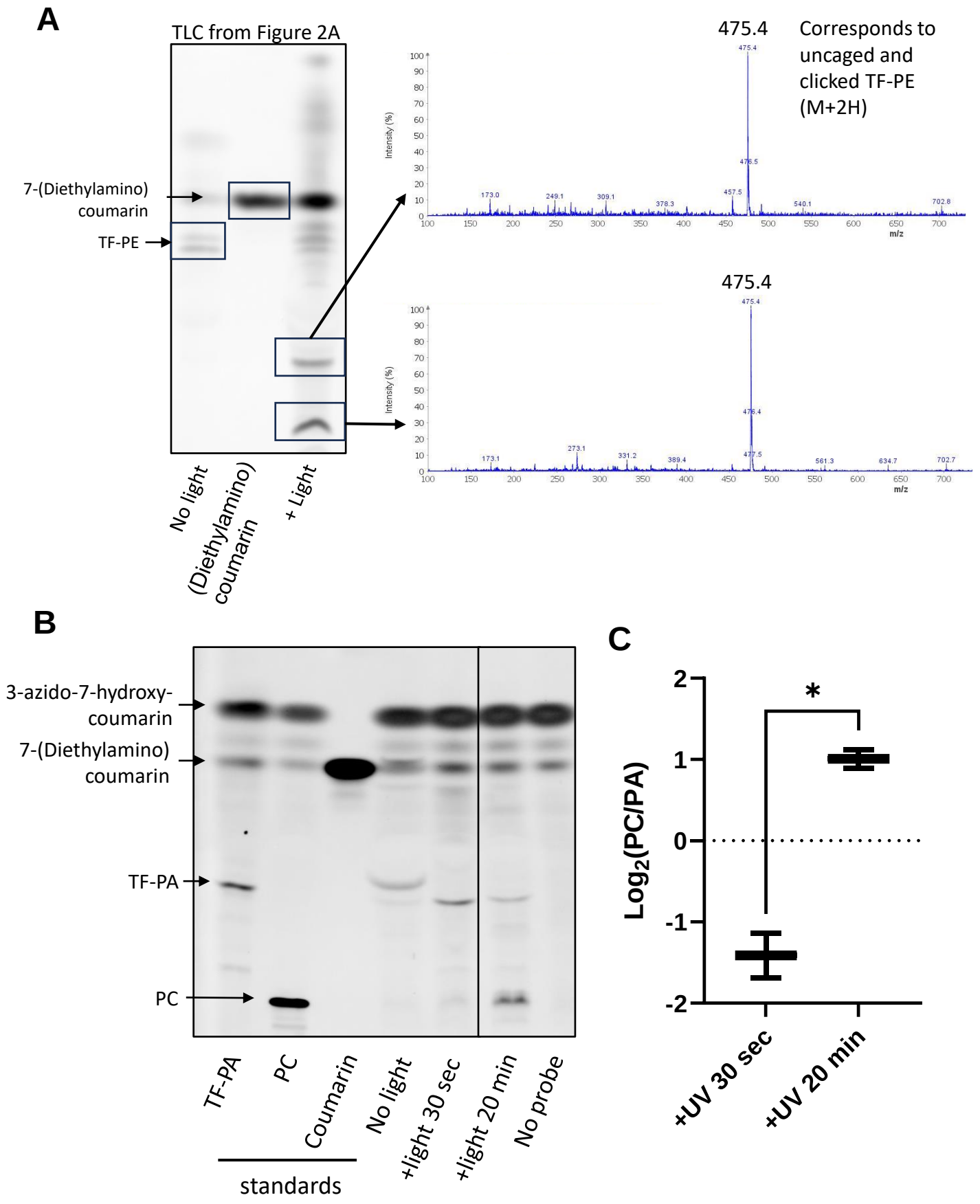
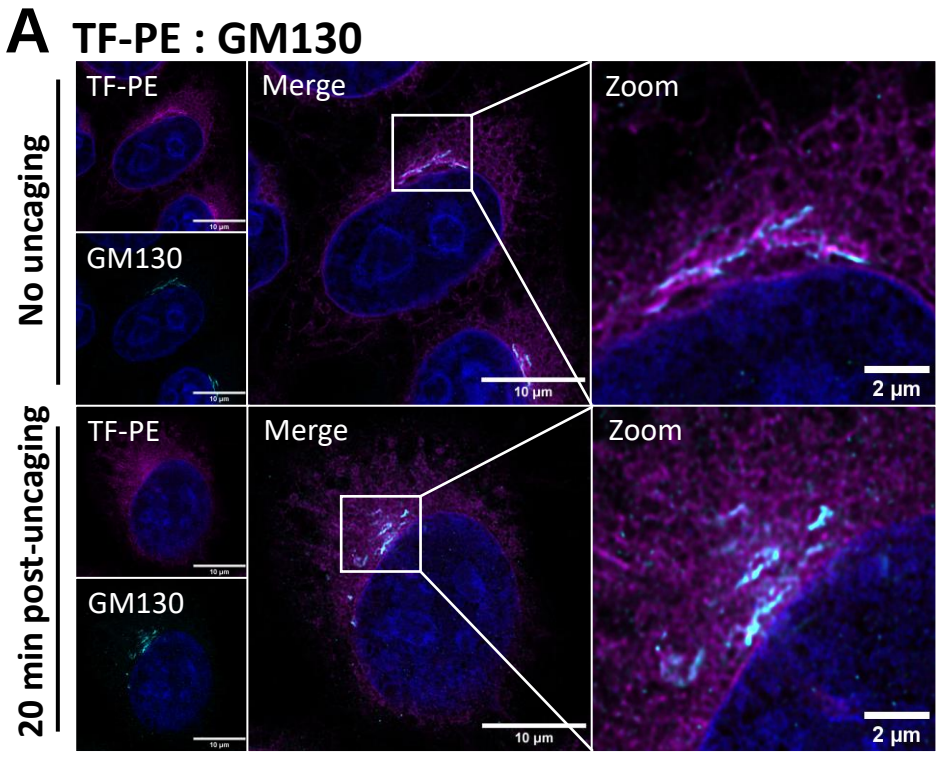
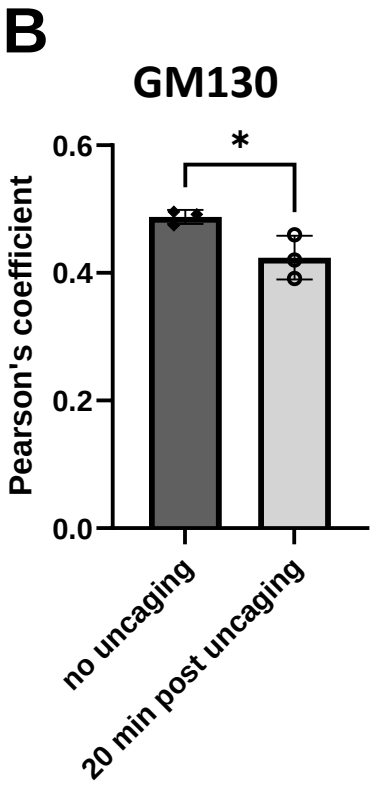


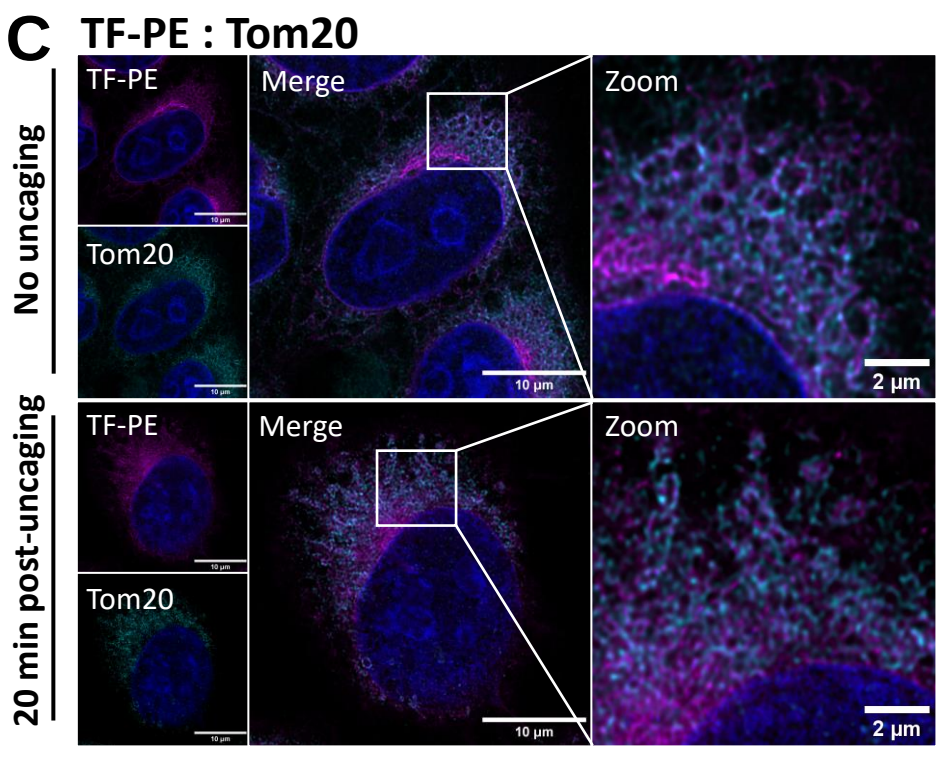
Supplementary Figure 1. Confocal micrograph showing loading of trifunctional PA (5 µM) derivatives into HeLa cells. Illumination of cells with 405 nm laser light (uncaging) led to a rapid increase of emission intensity due to the higher quantum yield of the released 7-diethylamino-4-hydroxymethyl-coumarin.

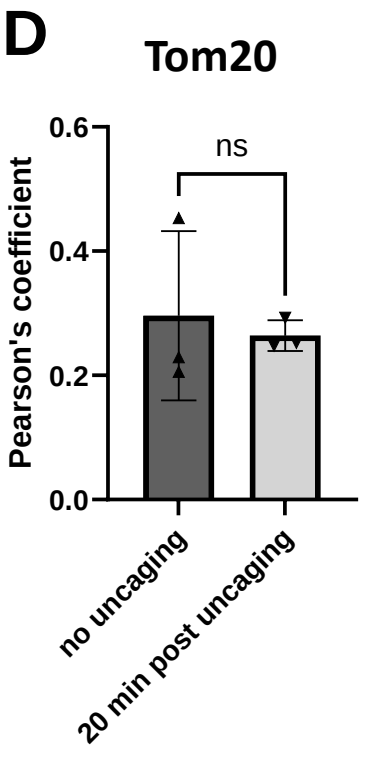


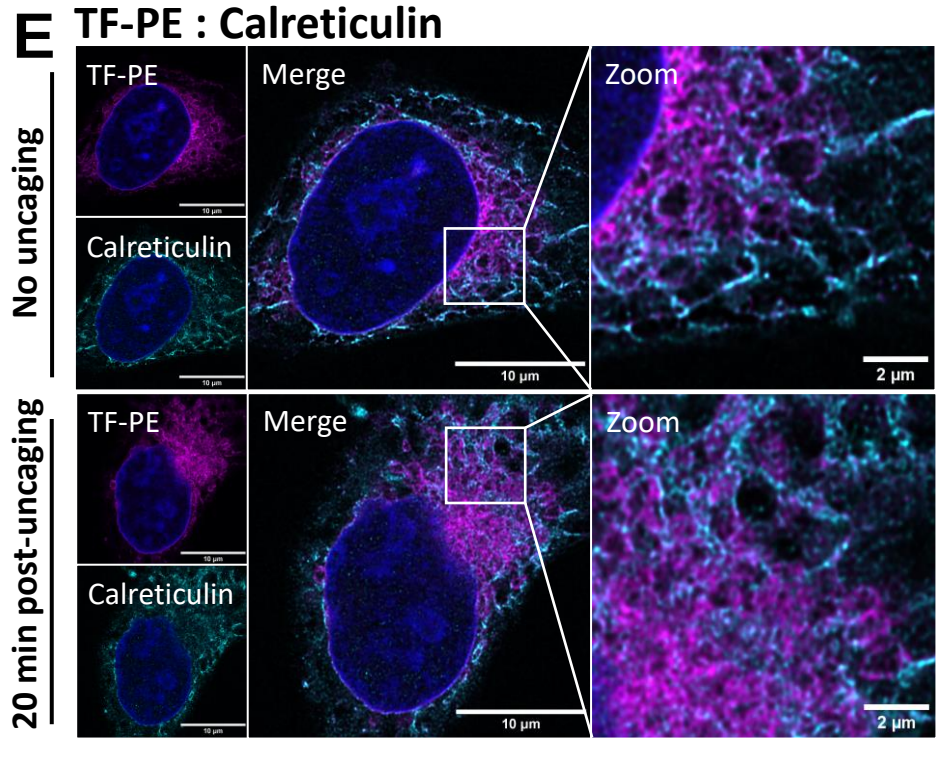
Supplementary Figure 2. A. Annotation of figure 2A is represented with ESI positive spectra of indicated TLC bands using direct TLC plate mass analysis with Plate Express from Advion. The peak at 475.4 corresponds to the predicted M+2H mass of uncaged TF-PE clicked with a 3-azido-7-hydroxy-coumarin. **B.** TLC (HPTLC silica 60) of the standards TF-PA (uncaged), alkyne PC and 7-(Diethylamino)coumarin in the first three lanes followed by lipid extracts from HeLa cells initially treated with 10 μM of TF-PA derivative **2** with and without subsequent UV light irradiation and metabolization for the time indicated. Lipids were labeled with 3-azido-7-hydroxy-coumarin prior to TLC. 65:25:4:1 chloroform : methanol : water, acetic acid for 6 cm, followed by 1:1 hexanes : ethyl acetate for 9 cm. **C.** Integrated densities following background subtraction of lipid spots corresponding to PA and PC 30 sec or 20 min after light irradiation (from A). Two biological replicates.

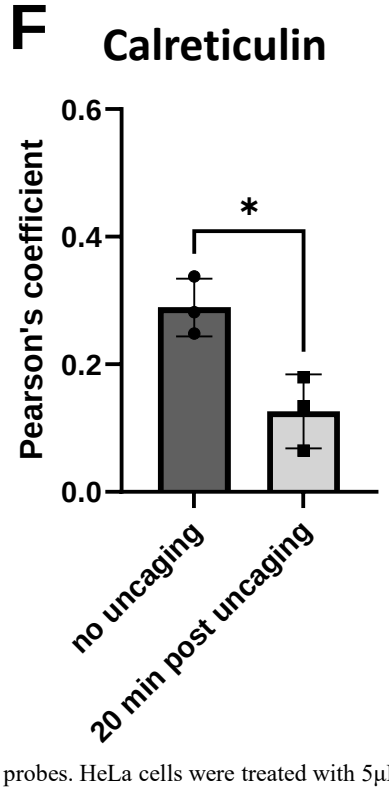




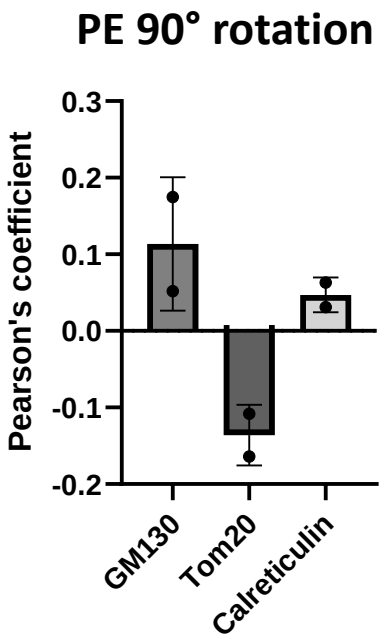




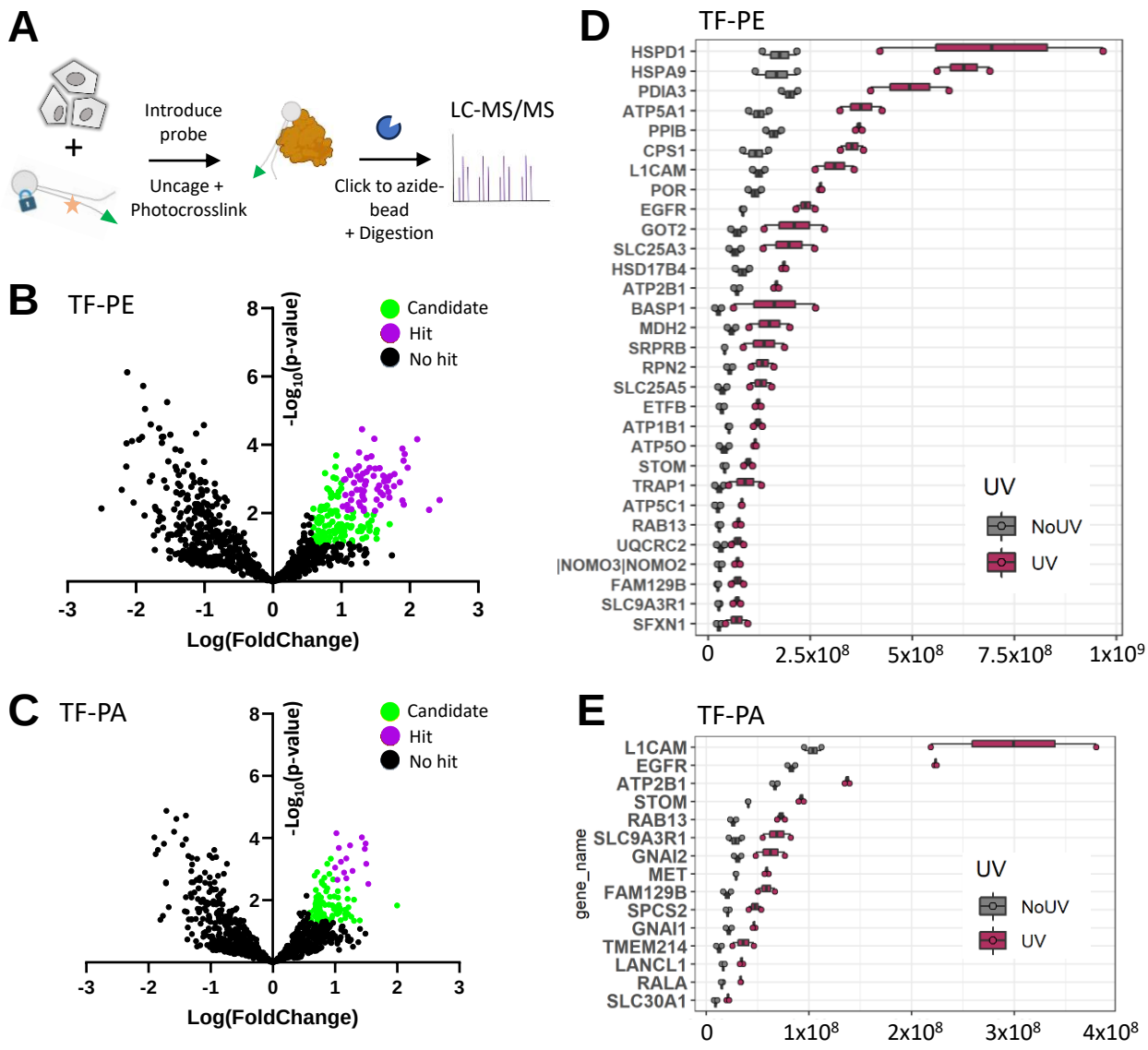




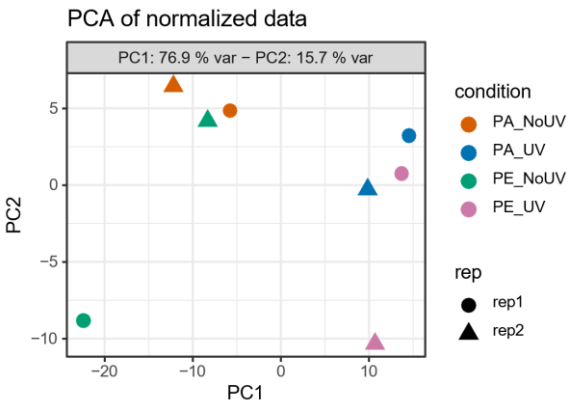
Supplementary Figure 3. A,C,E Confocal micrograph showing subcellular localization of lipid probes. HeLa cells were treated with 5µM of TF-PE and either incubated for 20 min and photo-crosslinked with 350 nm light or uncaged with 400 nm light and allowed to metabolized for 20 min prior to photo-crosslinking. The cells were then fixed, subjected to click reactions with a fluorescent azide (in magenta), and immuno-stained with the organelle markers (A) GM130 (Golgi), (B) Tom20 (Mitochondria), and (C) Calreticulin (ER)(in cyan). Images are representative of three independent experiments. The overlay of the two signals will appear white. **B.D,F.** Colocalization measurement using Pearson's coefficient between TF-PE and (B) GM130 (Golgi), (D) Tom20 (Mitochondria), and (F) Calreticulin (ER). Pearson's correlation coefficients were calculated using an ImageJ pipeline. Each experiment was performed in biological triplicates and 7 cells from different field of views were analyzed with Pearson per replicates. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 (Unpaired t-test)..



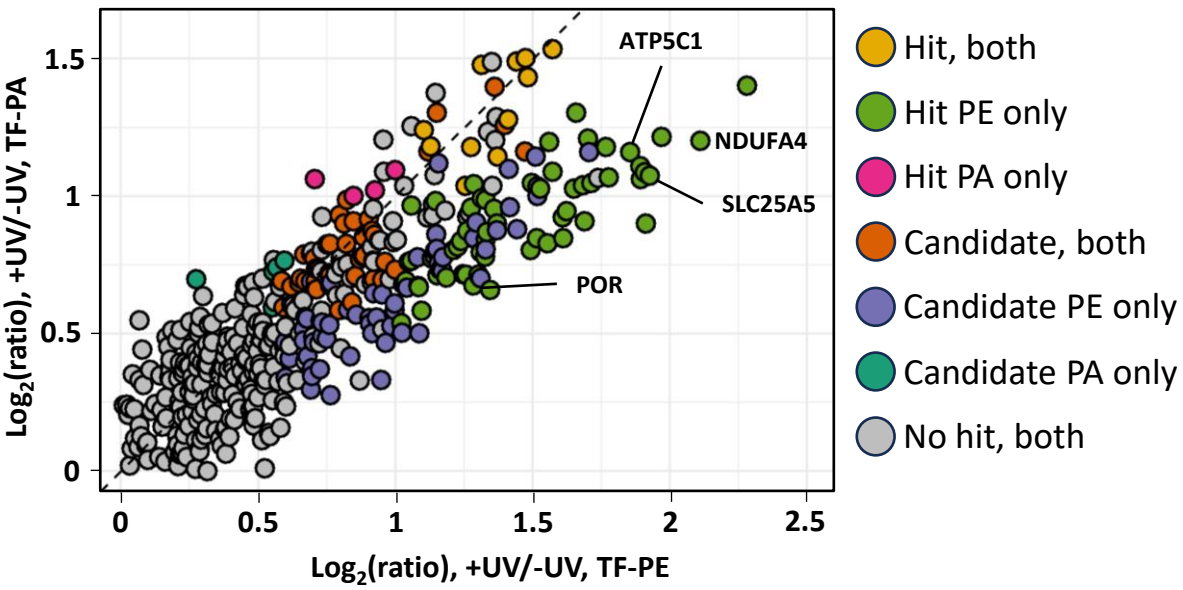
Supplementary Figure 4. Colocalization measurement using Pearson's coefficient between TF-PE after 90° rotation of the filed of view and Calreticulin (ER), Tom20 (Mitochondria) or GM130 (Golgi)



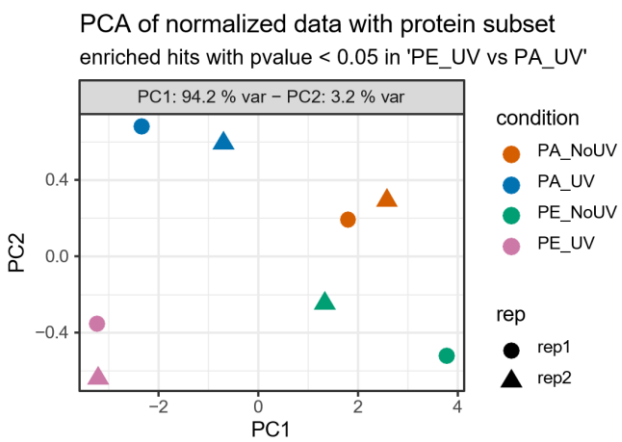
Supplementary Figure 5. A. Experimental design pipeline. **B-C.** Volcano plots of identified hit proteins for each probe, by Limma analysis. “Hits” are defined as proteins with a false discovery rate less than 0.05 and a fold change of at least 2-fold in the +UV over the -UV. “Candidates” are defined as proteins with a false discovery rate less than 0.2 and a fold change of at least 1.5-fold. **D-E.** Normalized intensities of proteins identified as hits by the Limma analysis for (E) TF-PE; (F) TF-PA.



Supplementary Figure 6. PCA for quality control of lipid-protein interactome replicates. PCA was performed on the normalized protein abundance values. Each point represents a single replicate. The first two principal components account for 76.9% (PC1) and 15.7% (PC2) of the total variance.



Supplementary Figure 7. Proteins pull down by TF-PA and TF-PE, colored by the degree of enrichment in the (+) UV condition over the (-) UV condition. Log_2(ratio) values bellow 0 for both TF-PA and TF-PE were not included in the graph for clarity.



Supplementary Figure 8. PCA for quality control of lipid-protein interactome replicates. PCA was performed on a subset of proteins (ETFB, ATP5A1, ACAT1, TMEM214, SHMT2, GNB2, ATP5O, CS, OXCT1, PDIA3, AIFM1) corresponding to enriched hits with differences between the photo-crosslinked PE (PE_UV) and the photo-crosslinked PA (PA_UV). Each point represents a single replicate. The first two principal components account for 94.2% (PC1) and 3.2% (PC2) of the total variance.

TF-PE : Mitochondrial hits

GO terms	Gene	logFC	pvalue
respiratory chain	NDUFA4	2.1094	0.0001
	ETFB	1.8922	0.0001
	ATP5C1	1.8541	0.0013
	SDHA	1.6885	0.0024
	ATP5O	1.6234	0.0012
	ATP5A1	1.6104	0.0008
	COX4I1	1.3285	0.0022
	UQCRC2	1.2745	0.0061
	ATP5F1	1.2247	0.0021
	UQCRC1	1.1517	0.0027
metabolic pathways	CS	2.2800	0.0080
	FH	1.9067	0.0003
	SHMT2	1.8917	0.0043
	PDHB	1.7764	0.0007
	SDHA	1.6885	0.0024
	ACAT1	1.6812	0.0011
	CPS1	1.6496	0.0009
	ECH1	1.5722	0.0058
	GOT2	1.5142	0.0040
	OXCT1	1.3439	0.0015
	MDH2	1.3315	0.0040
protein transport	SLC25A5	1.9266	0.0002
	SLC25A6	1.7670	0.0016
	SLC25A3	1.5530	0.0016
	SFXN1	1.3278	0.0085
gene regulation	HSPD1	1.9122	0.0056
	TRAP1	1.7001	0.0035
	PPIF	1.6091	0.0030
	SLIRP	1.4909	0.0086
	TRMT10C	1.3265	0.0073
redox and apoptosis	AIFM1	1.7120	0.0008
	SCCPDH	1.0578	0.0029
Biosynthesis of Fe/S	HSPA9	1.9696	0.0005
	ACO2	1.0844	0.0058

Supplementary Figure 9. Table summarizing mitochondrial proteins identified as enriched hits for TF-PE. Proteins are ordered by logFC values within each of the color-coded GO mitochondrial biological process categories.