Literature Report

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Chemical Profiling of the Endoplasmic Reticulum Proteome Using Designer Labeling Reagents

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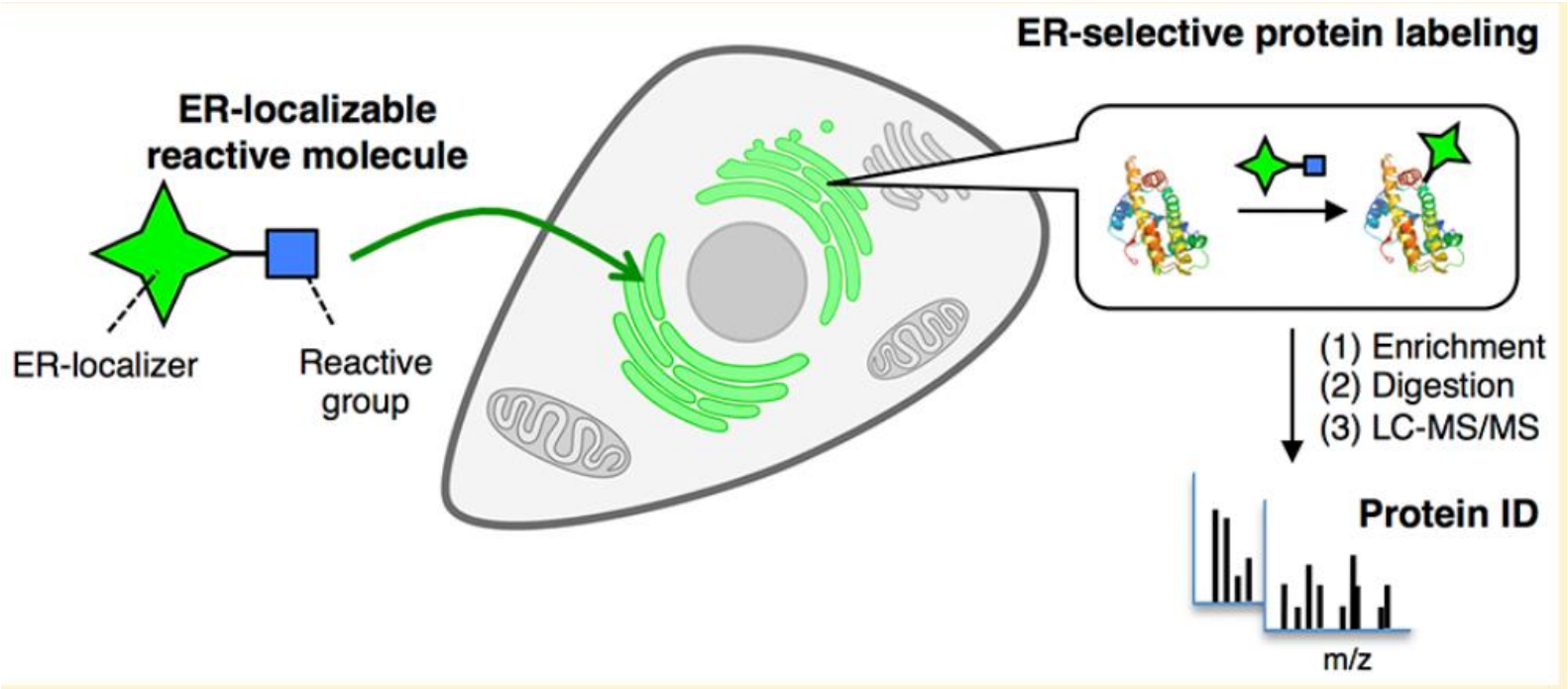

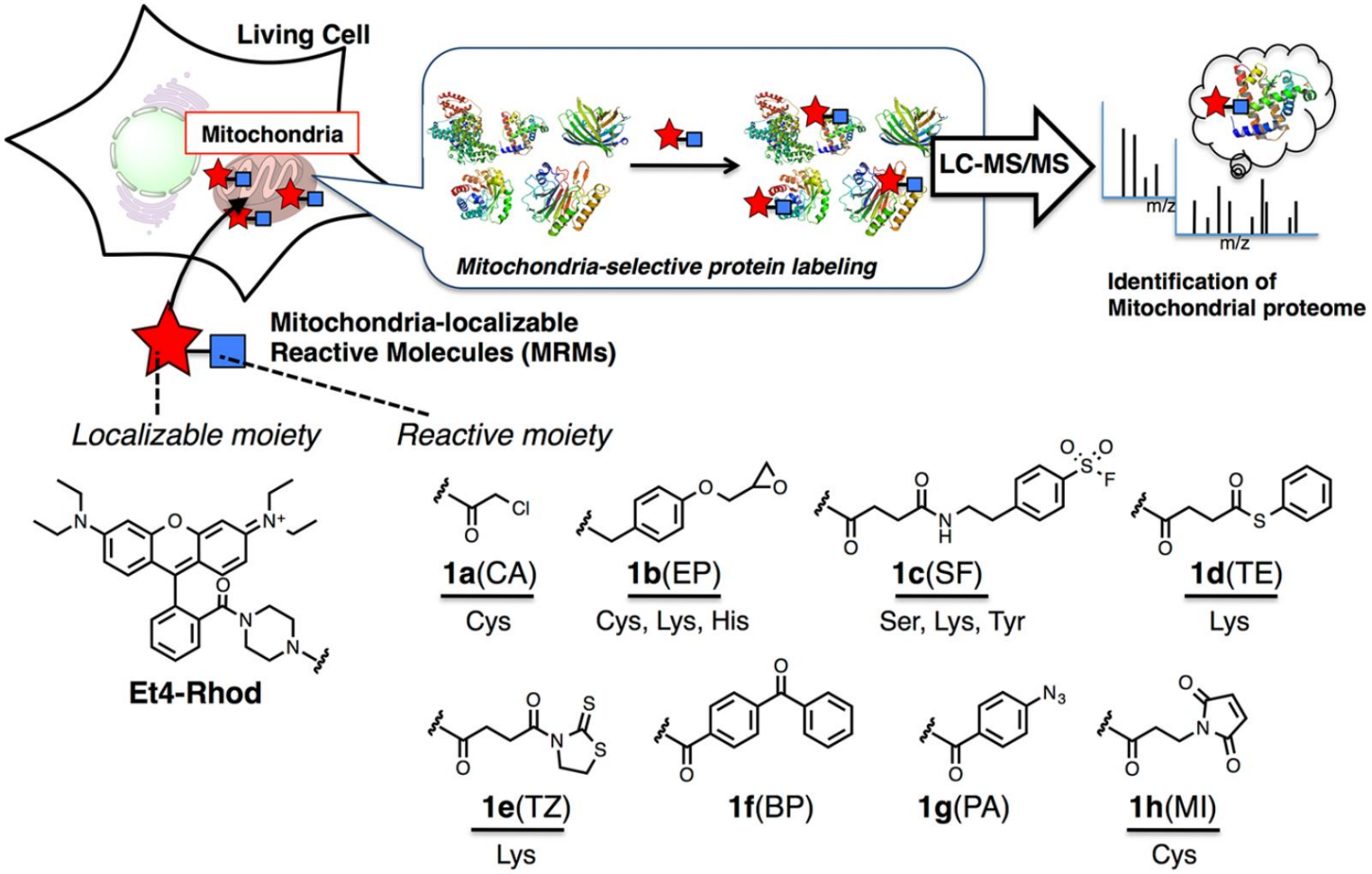

Figure 1. Schematic illustration of mitochondria selective protein labeling and profiling by a set of mitochondria-localizable reactive molecules (MRMs). Et4-Rhod, tetraethyl-rhodamine; CA, chloroacetyl; EP, epoxide; SF, sulfonyl fluoride; TE, thiophenyl ester; TZ, thiazolidinethione; BP, benzophenone; PA, phenylazide; MI, maleimide

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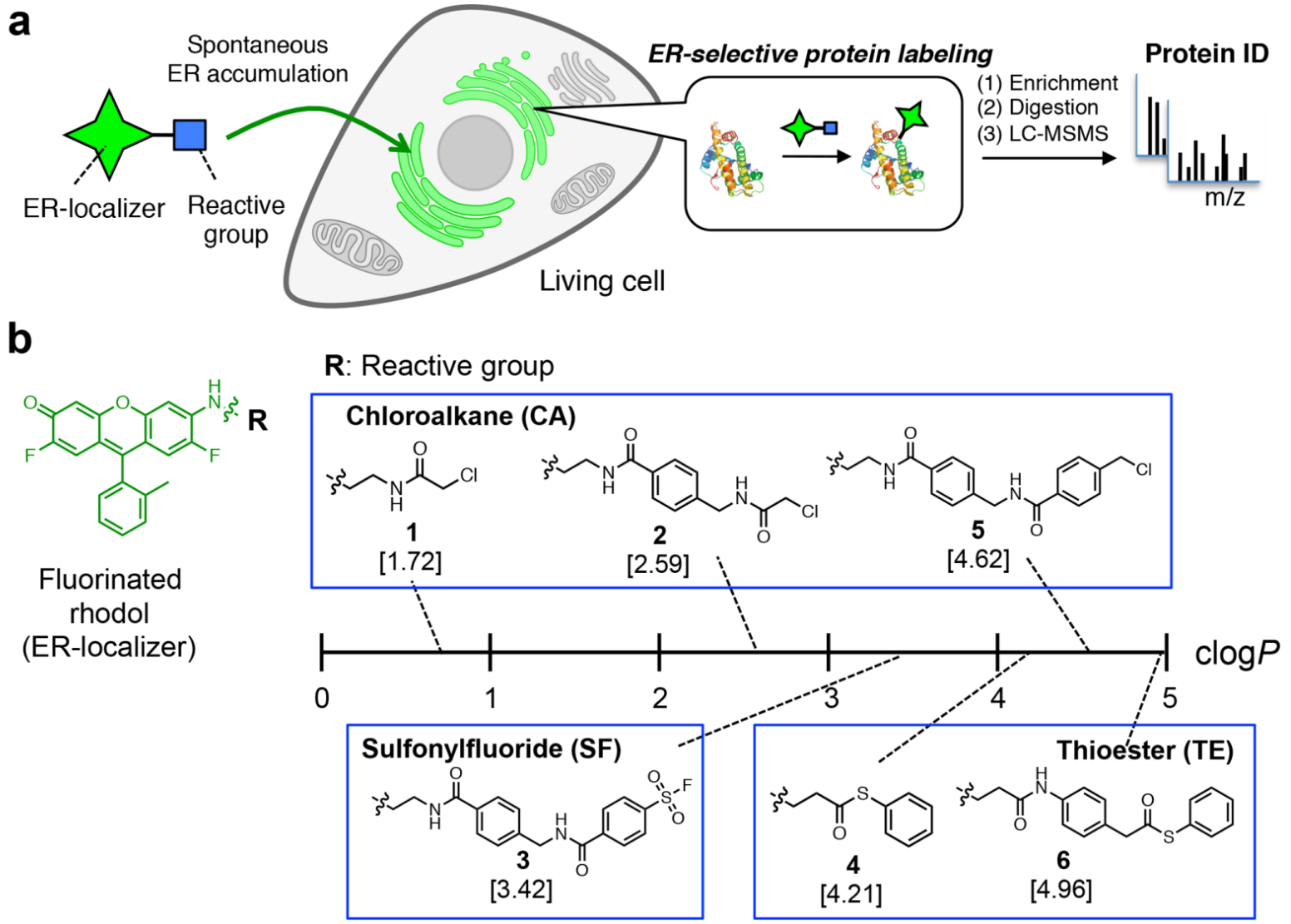


Figure 1. ER-focused chemical proteomics with a set of ER-localizable reactive molecules (ERMs). (a) Schematic of ERM-mediated selective labeling and profiling of ER-associated proteins. (b) Molecular design of ERM 1-6 with the different reactive groups. The calculated logP (clogP) value for each of these compounds is shown in brackets.

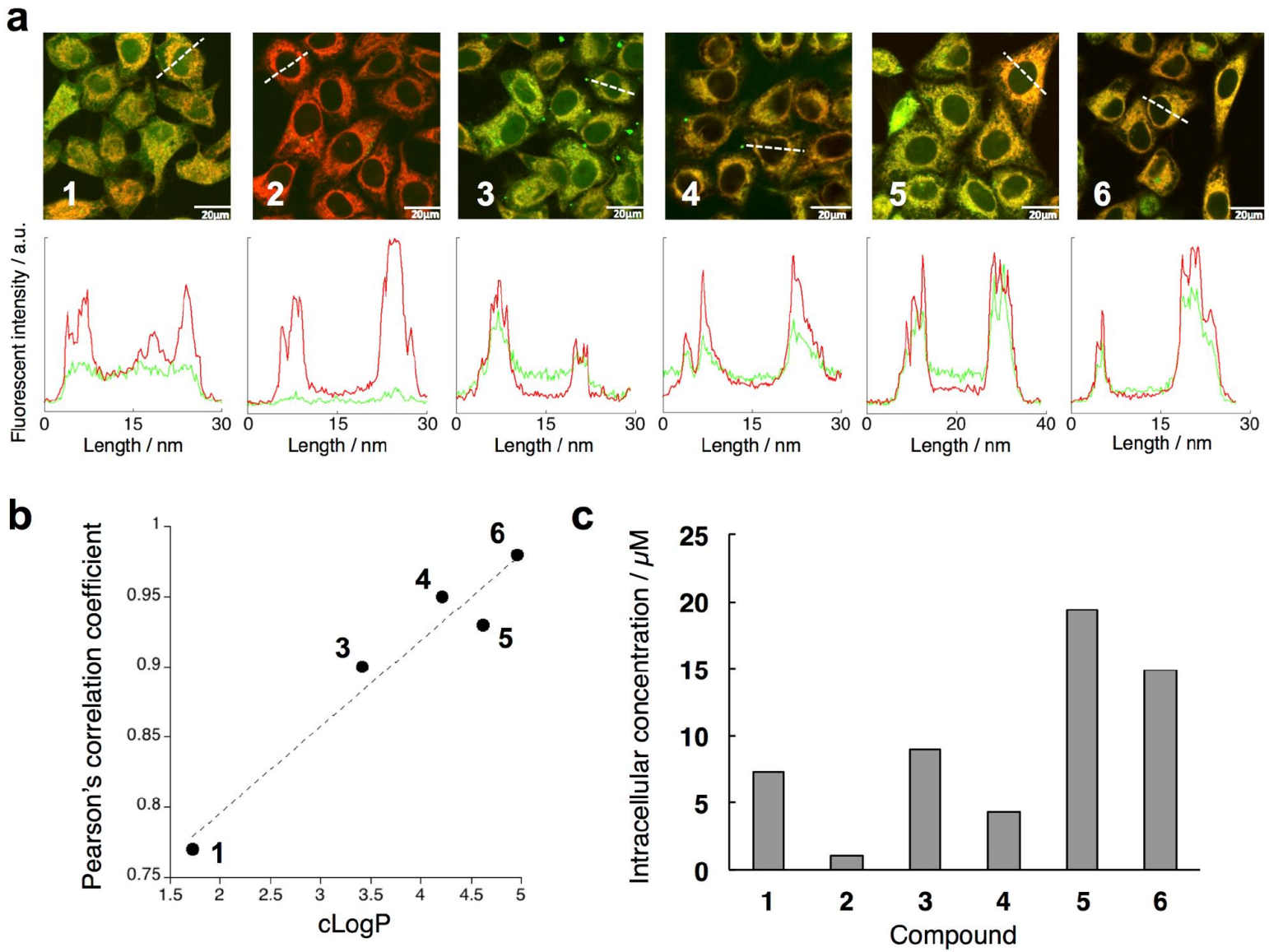


Figure 3. Cell permeability and spontaneous ER localizability of the ERMs 1-6. (a) Representative CLSM merged images of HeLa cells treated with 100 nM of each ERM (green) and ER-Tracker (red) for 15 min. Colocalization areas are in yellow. Line-plot graphs indicate the fluorescence intensity profiles of the ERM (green) and ER-Tracker (red) along the white dotted lines indicated in the CLSM images. Scale bar: 20 μm. (b) Correlation of Pearson's correlation coefficients of the ERM/ER-Tracker signal overlap observed in the CLSM images against the clogP values of the cell-permeable ERMs (1, 3-6). (c) Intracellular concentration of each ERM in HeLa cells determined by spectroscopic measurement of rhodol fluorescence

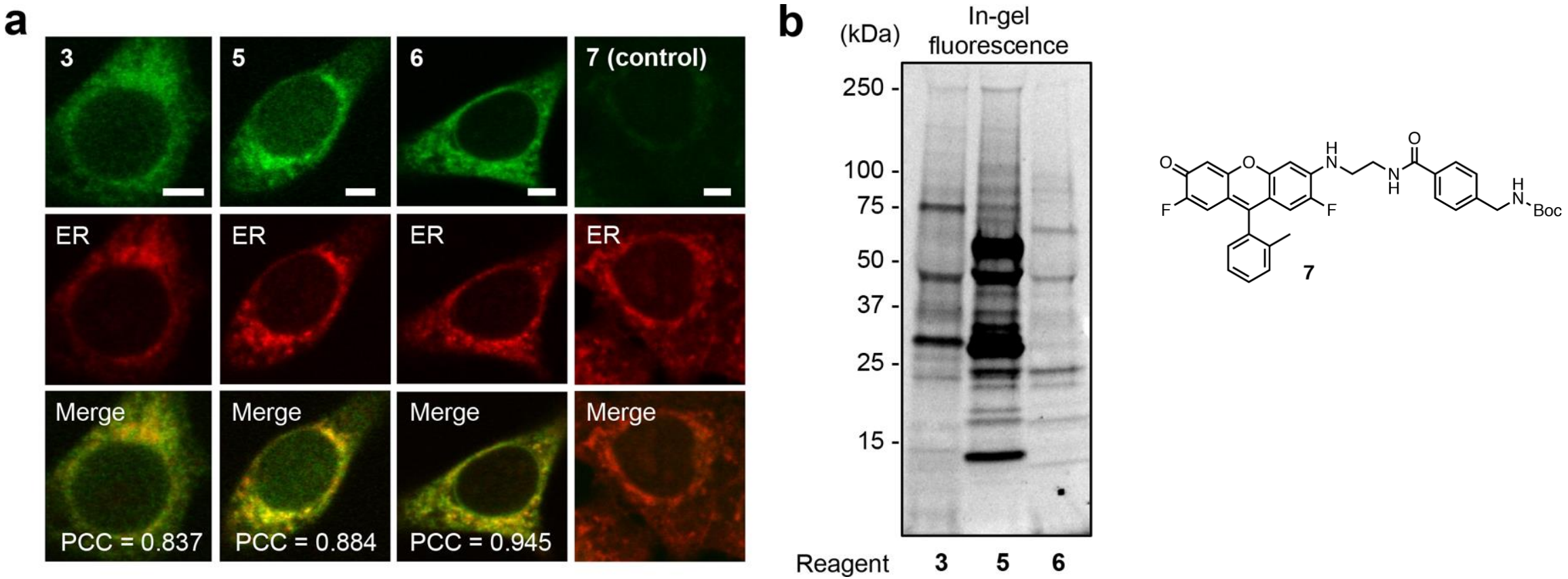


Figure 4. Covalent protein modification in the ER with ERMs. (a) CLSM images of HeLa cells treated with ERMs 3, 5, 6, and 7 (green) and ERTracker (red), followed by methanol fixation. Colocalization was quantified using Pearson's correlation coefficient (PCC). Scale bar: 5 μm. (b) SDS-PAGE analysis with in-gel fluorescence imaging. Reaction conditions: HeLa cells were incubated with each ERM (100 nM) in DMEM at 37 °C for 1 h. After cell lysis, the labeled proteins were enriched by immunoprecipitation with anti-rhodol antibody to enhance the fluorescent signal, followed by SDS-PAGE (a 4-12% gradient gel).

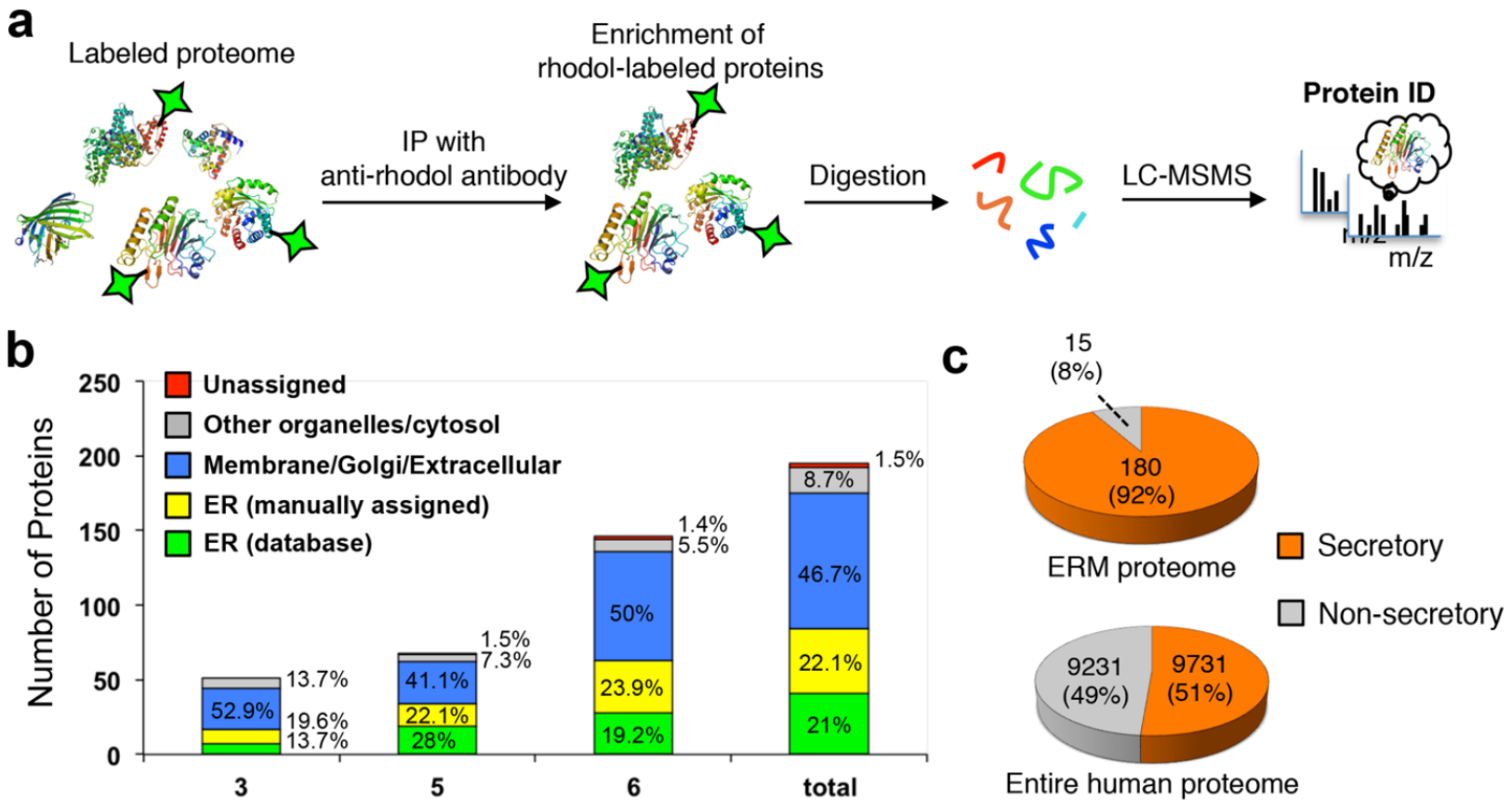


Figure 5. ERM-based identification and profiling of rhodol-labeled proteins in the ER. (a) Schematic of the approach used in this study for protein identification. The rhodol-labeled proteins were enriched by IP with anti-rhodol antibody and then identified by LC-MS/MS analysis. (b) Number of proteins identified in HeLa cells using the ERM-based approach and the ratio of proteins with ER annotations to proteins with other annotations. The identified proteins were classified as “ER”, “membrane/Golgi/extracellular”, “other organelles/cytosol”, or “unassigned” according to the UniProtKB database, the Gene Ontology (GO) cell component annotation terms, and manual literature surveys. (c) Pie charts of the percentage of identified proteins with secretory pathway annotation according to the GO database or the Phobius predictive tool (ref 43) among proteins identified with our method (top) and reported for the entire human proteome (bottom).

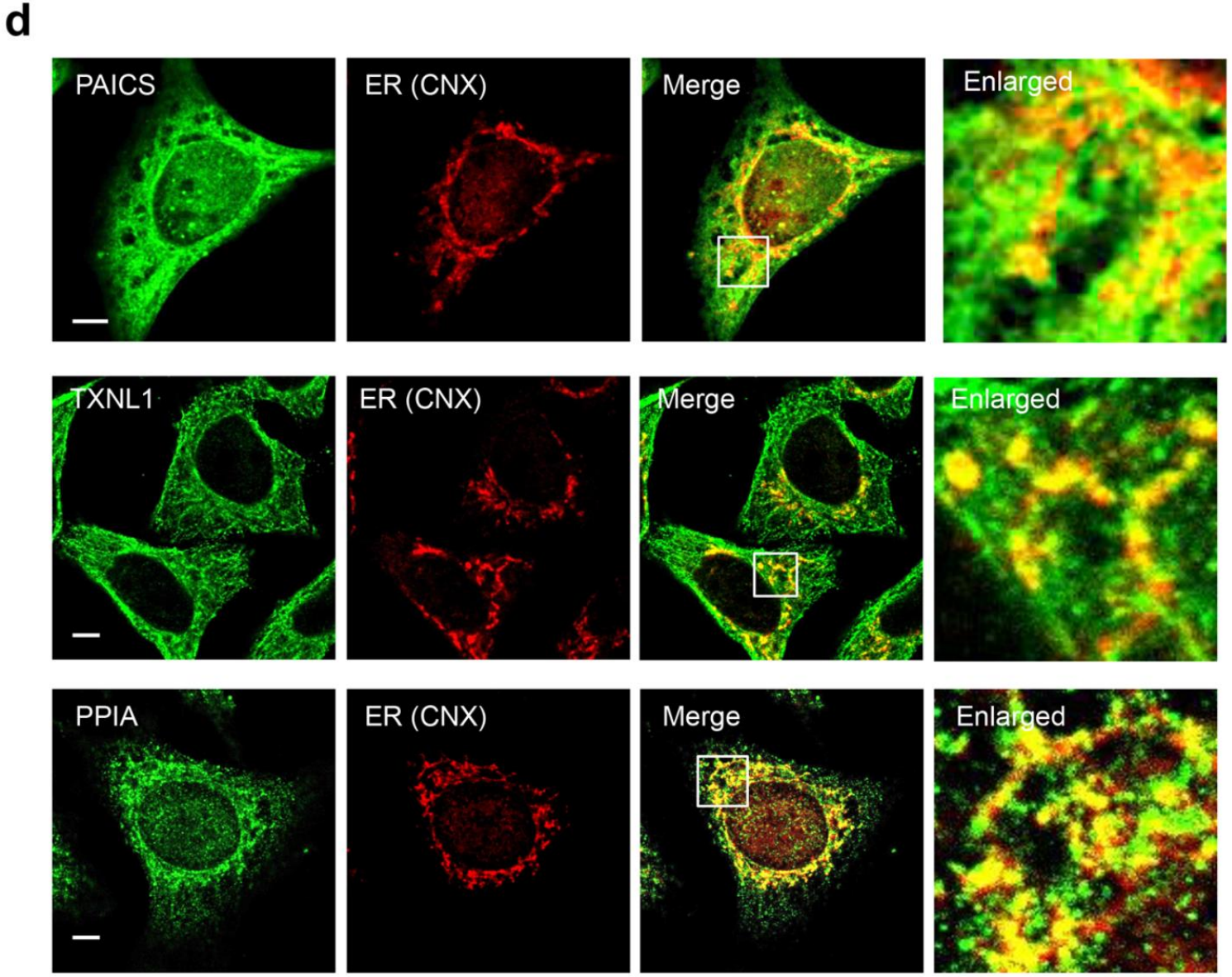
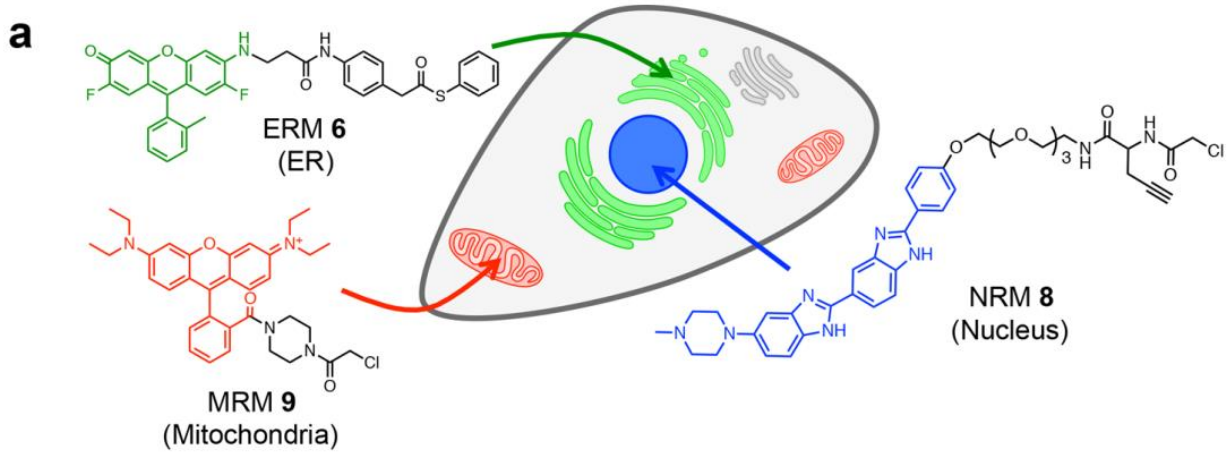
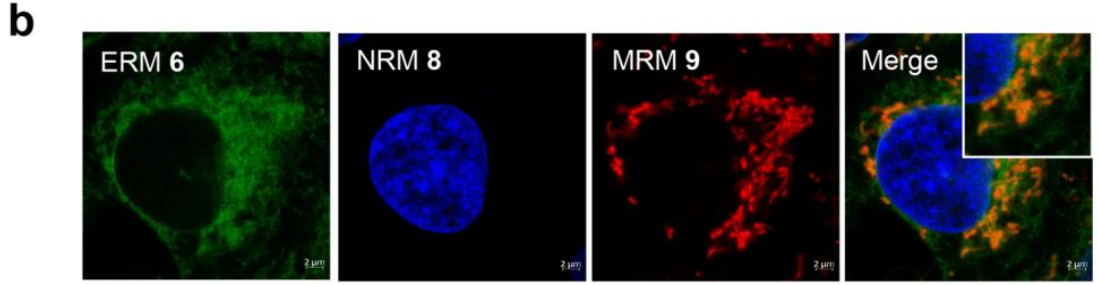
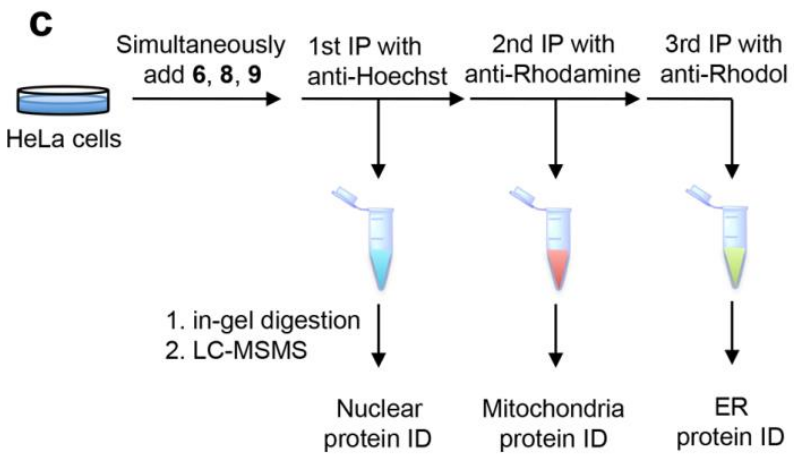


Figure 5. (d) Subcellular localization of the three proteins PAICS, TXNL1, and PPIA, newly assigned to the ER in this study.HeLa cells were fixed and stained with anti-PAICS, anti-TXNL1, and anti-PPIA (green) and anti-calnexin (CNX) (red) to visualize the ER. Colocalization regions are shown in yellow in the merged images. Scale bar: 5 μm.







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	NRM	MRM	ERM
Target organelle	44	223	ER: 15 Secretory: 32
Whole	56	282	36
%	79	79	ER: 42 Secretory: 89

Figure 6. Simultaneous use of NRM, MRM, and ERM for targeted protein enrichment in HeLa cells. (a) Molecular structures of ERM 6, NRM 8, and MRM 9. The reaction properties of 8 and 9 are reported in refs 19 and 18, respectively. (b) CLSM images of a live HeLa cell concurrently treated with ERM 6 (100 nM, green), NRM 8 (5 µM, blue), and MRM 9 (0.5 µM, red). (c) Workflow of the sequential IP protocol for the enrichment of proteins labeled with each reagent. (d) The cellular locations of the identified proteins were analyzed according to the protein ontology information provided in the UniProt database and from manual literature surveys.

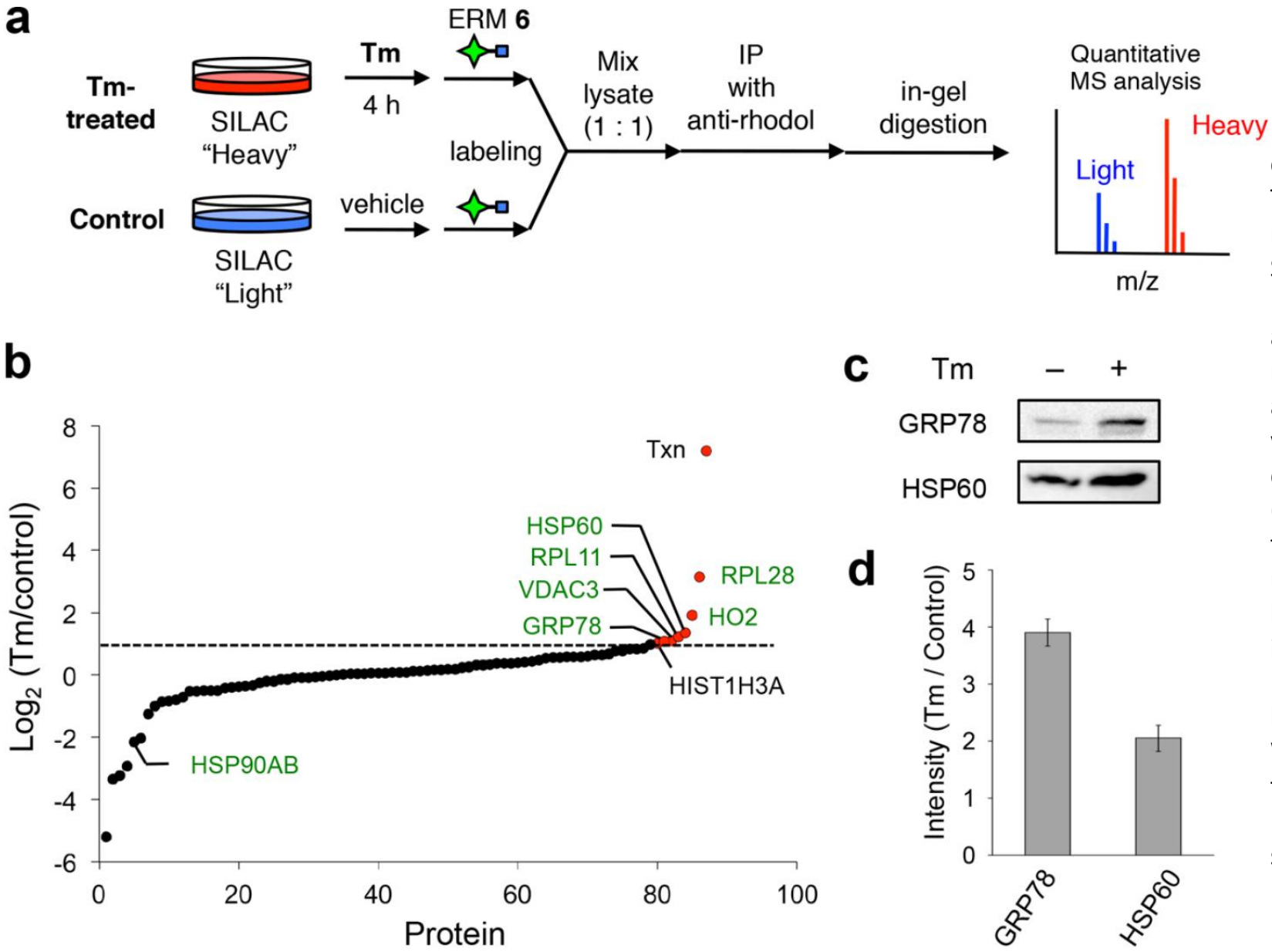

Figure 7. SILAC-based quantitative MS analysis of ERM proteome change induced by ER stress. (a) Design of SILAC experiments coupled with ERM-mediated protein labeling to quantify ER proteome changes in tunicamycin (Tm)-treated HeLa cells. Tm-treated cells were grown in heavy SILAC medium, and nontreated cells were grown in light SILAC medium (control), followed by labeling with ERM 6. After cell lysis, the lysates from the light and heavy cells were mixed in a 1:1 ratio and the rhodol-labeled proteins were enriched by IP using an anti-rhodol antibody. The enriched proteins were subjected to in-gel tryptic digestion and quantitative LC-MS/MS analysis. (b) Log2 ratio plot (Tm-treated cells (heavy)/ control cells (light)) of the identified proteins. Proteins more than 2-fold upregulated, and the others are shown in red and black in the plot, respectively. Gene names written in green or black letters are proteins assigned as ER-associated proteins (assigned by database and literature) or non-ER proteins, respectively. (c) Western blotting analysis of Tm-induced changes in the expression levels of GRP78 and HSP60. (d) Relative intensities of the Western blotting analysis shown in (c). Data represent the mean ± SD, n = 3.

Thanks !