Identification of potential sialic acid binding proteins on cell membrane by proximity chemical labeling

Supporting Information

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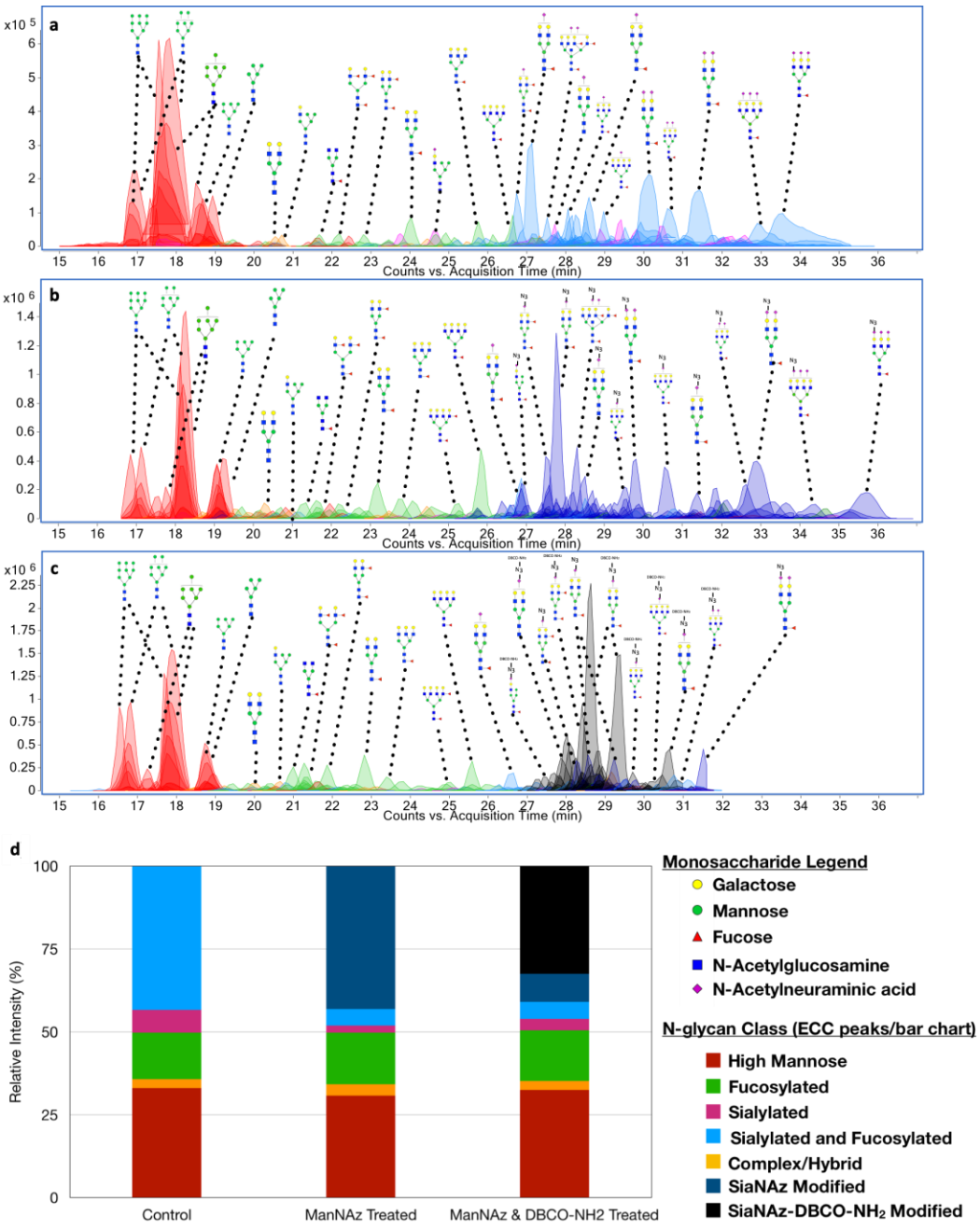

Fig. S1. (**a**) LC-MS profile of N-Glycans released from PNT2 cells. Annotated structures are putative based on mass and compositions. LC-MS peaks are color coded to assign glycan subtype. Over 400 structures are generally observed in a chromatogram. (**b**) N-Glycan profile from cells after treatment with ManNAz. SiaNAz containing compounds are labeled accordingly. (**c**) N-Glycan profile after treatment with ManNAz followed by DBCO-NH_2. (**d**) Relative abundances of glycan subtypes.

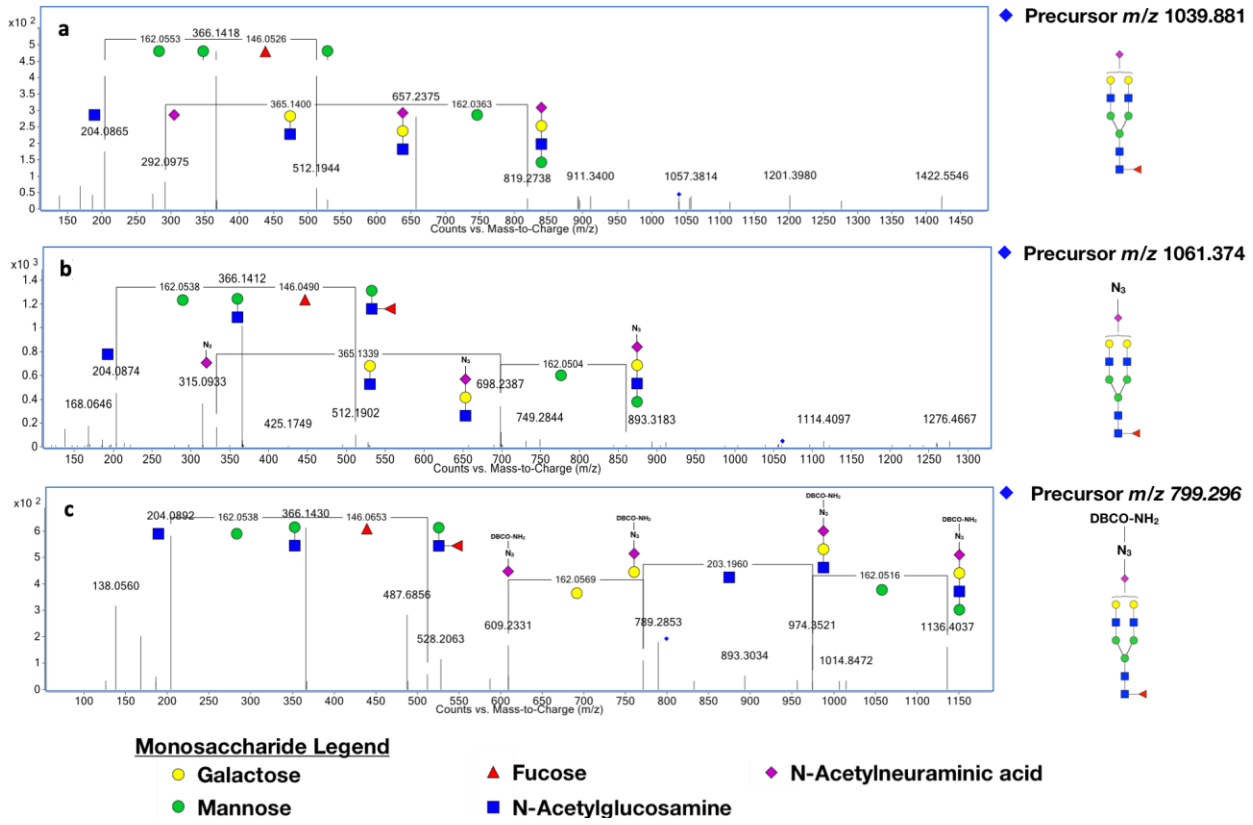

Fig. S2. Tandem mass spectra of selected compounds (**a**) $Hex_5HexNAc_4Fuc_1Sia_1$ (**b**) $Hex_5HexNAc_4Fuc_1SiaNAz_1$, and (**c**) $Hex_5HexNAc_5Fuc_1(SiaNAz\text{-}DBCO\text{-}NH_2)_1$. The spectra are representative of SiaNAz containing species. The assigned fragment structures are proposed.

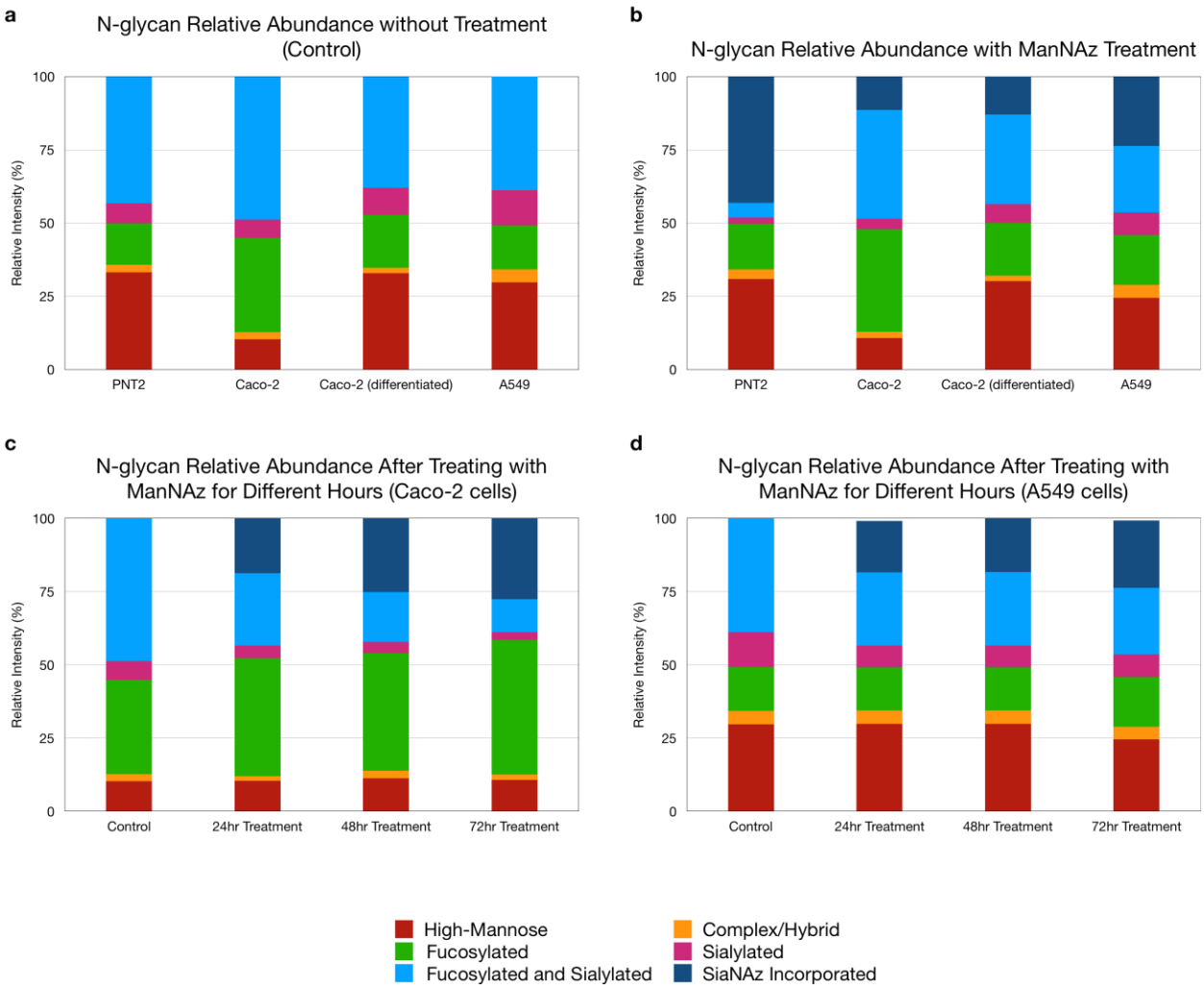

Fig. S3. Relative abundances of cell surface N-glycans color coded for glycan types. (**a**) Control, no ManNAz treatment of cell lines PNT2, undifferentiated Caco-2, differentiated Caco-2, and A549. (**b**) Cell lines treated by adding ManNAz to supernatant. (**c**) Caco-2 treated with ManNAz while allowing cells to grow with variable time after addition of ManNAz. (**d**) A549 treated with ManNAz and variable growing times.

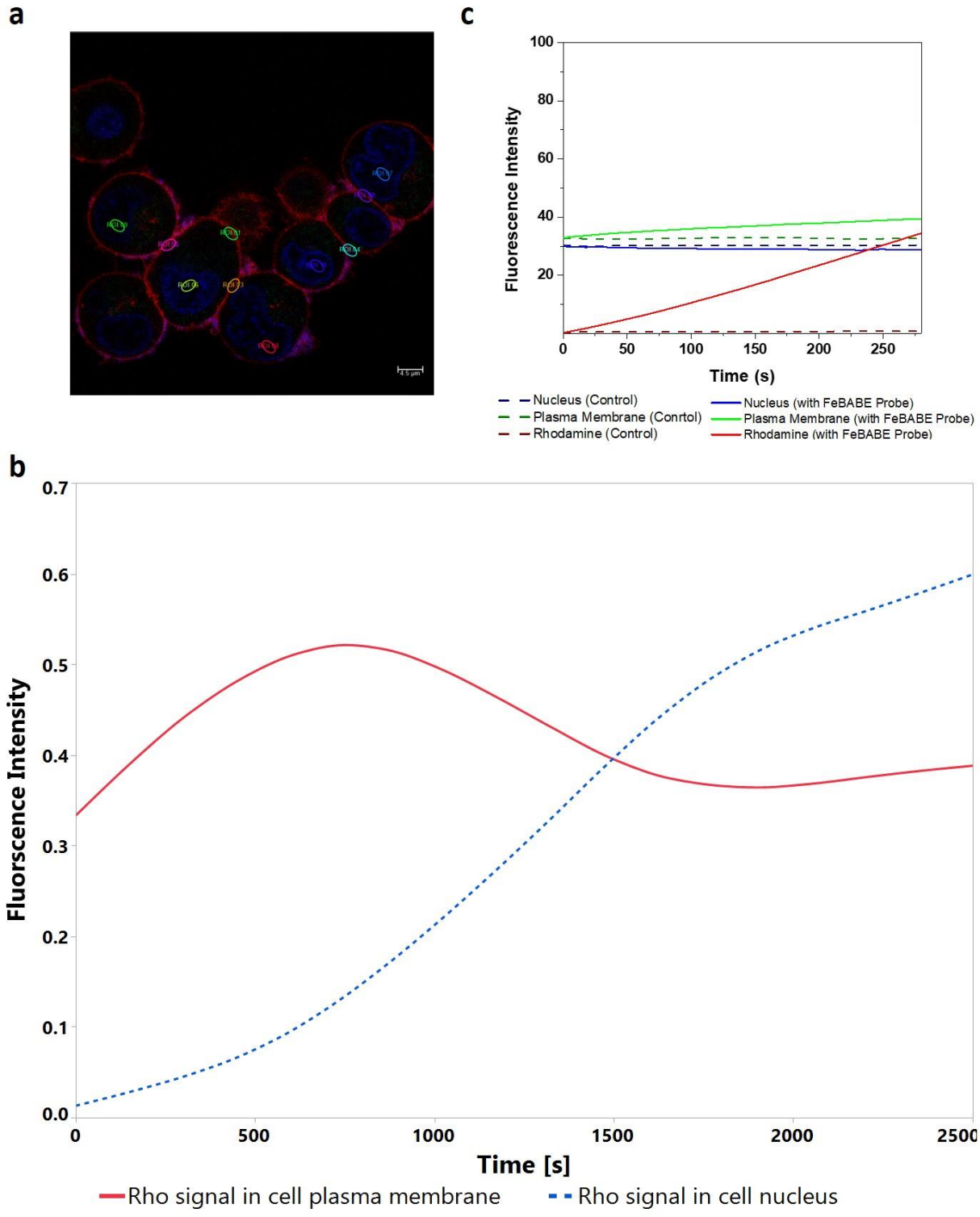

Fig. S4. (a) To monitor the rhodamine signals at different sublocations of the cells, regions of equal sizes were selected for quantitation. They include five regions on cell plasma membrane (ROI 01-05) and five from cell nuclei (ROI 06-10). **(b)** Averaged rhodamine fluorescence intensity changes over the time on the cell plasma membrane (red, solid line) and the cell nucleus (blue, dash line) **(c)** Variation of the

overall fluorescence intensities with various stains versus the time. The intensities of the nucleus (dark blue, dash line), plasma membrane (dark green, dash line), rhodamine (without FeBABE - dark red, dash line), and rhodamine (with FeBABE – red, solid line). DMSO and hydrogen peroxide were added in the experiments. The intensities change slightly with time except for the production of FeBABE probe and additional with hydrogen peroxide (red, solid line) indicating that the nascent radicals are increasing significantly relative to all other intensities.

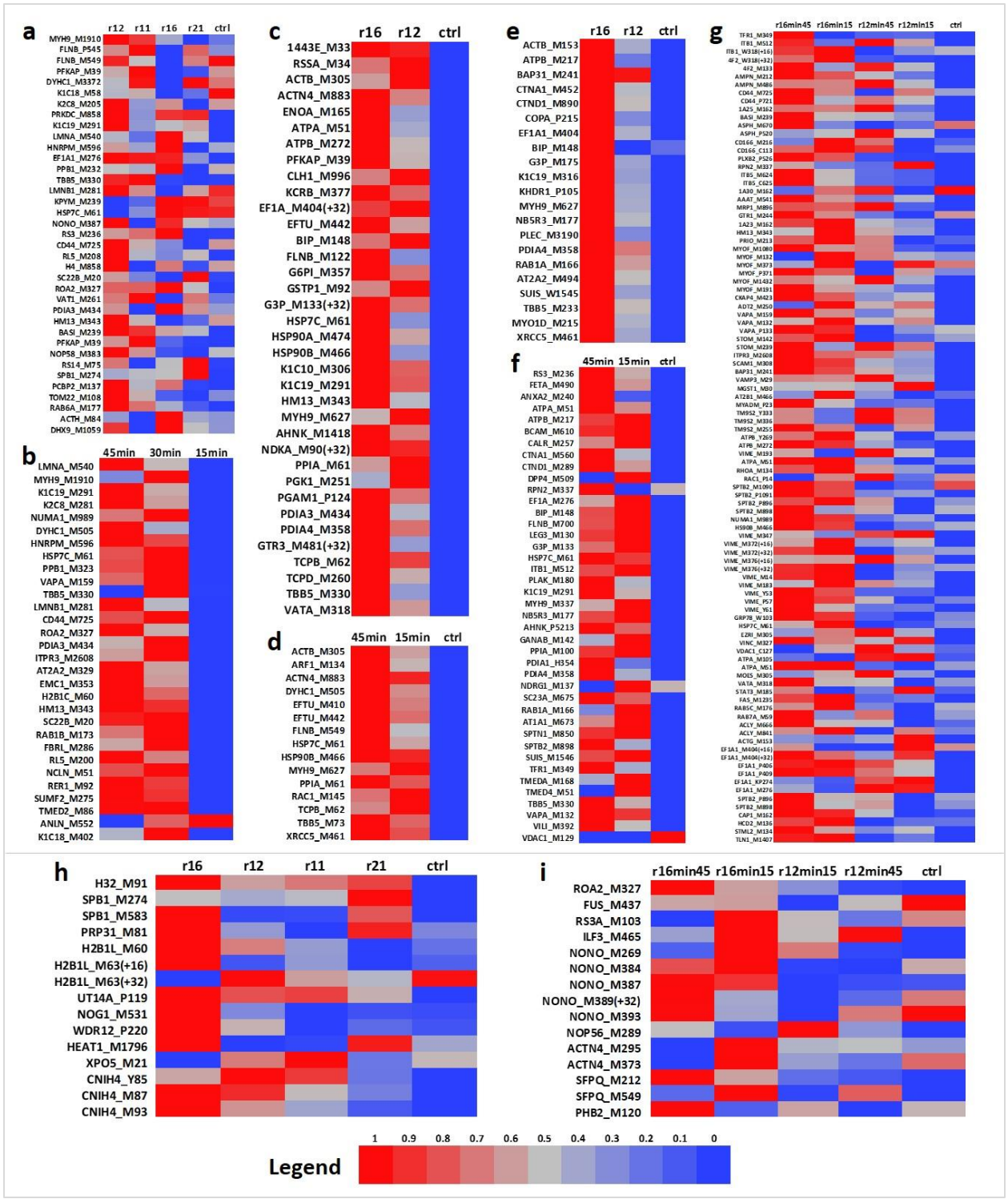

Fig. S5. The extent of oxidation quantified using Skyline for cell membrane proteins. Each column represents one treatment condition and each row represents one oxidation site of a selected protein. The label 'r12' means that the ratio of the concentrations of FeBABE and H_2O_2 is 1:2. (**a**) Oxidation of selected proteins in PNT2 with 50 μM FeBABE and various concentrations of H_2O_2. With the increasing

concentration of H_2O_2, oxidation of peptides also increases. (**b**) Protein oxidation in PNT2 cell line with r12 and various reaction times including 15, 30, and 45 minutes. With 45-minute reaction time, oxidation of peptides reaches highest level. (**c**) Protein oxidation in undifferentiated Caco-2 treated with 50 μM FeBABE and various concentrations of H_2O_2. (**d**) Protein oxidation in undifferentiated Caco-2 treated with various reaction times and the ratio of FeBABE to H_2O_2 being 1:6. (**e**) Protein oxidation in differentiated Caco-2 with 50 μM FeBABE and various concentrations of H_2O_2. (**f**) Protein oxidation in differentiated Caco-2 with various reaction times and the ratio of FeBABE to H_2O_2 being 1:6. (**g**) Protein oxidation in A549 with various concentration ratios of FeBABE and H_2O_2 with variable times. (**h**) Oxidation of selected nucleus proteins in PNT2 with 50 μM FeBABE and various concentrations of H_2O_2. Except with the condition r16, oxidation of nucleus proteins under other conditions are comparable to the control group. Considering both the background oxidation and nucleus oxidation, the condition r12 was used for PNT2 cell line. (**i**) Oxidation of selected nucleus proteins in A549 with various concentration ratios of FeBABE and H_2O_2 with variable times. Similar as cell line PNT2, the condition r16 gave higher oxidation of nucleus proteins compared to others so that the ratio r12 was used as the optimal condition.

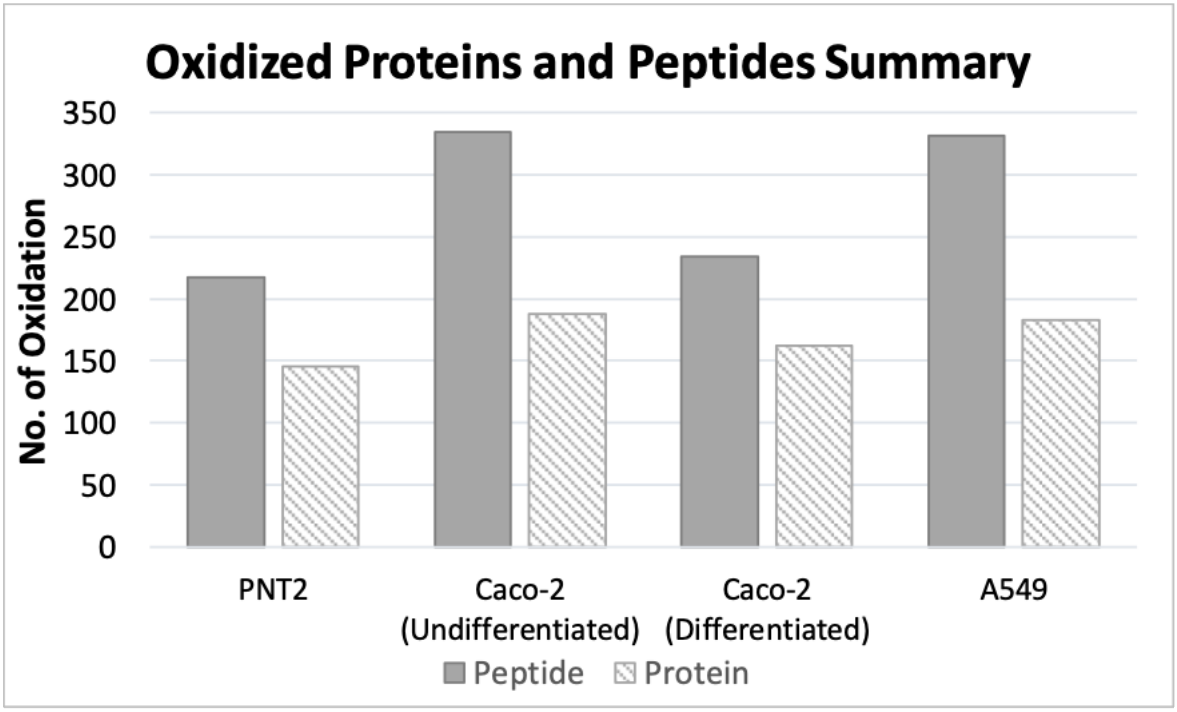

Fig. S6. The number of oxidized proteins and peptides of each cell line generated under optimal POSE conditions.

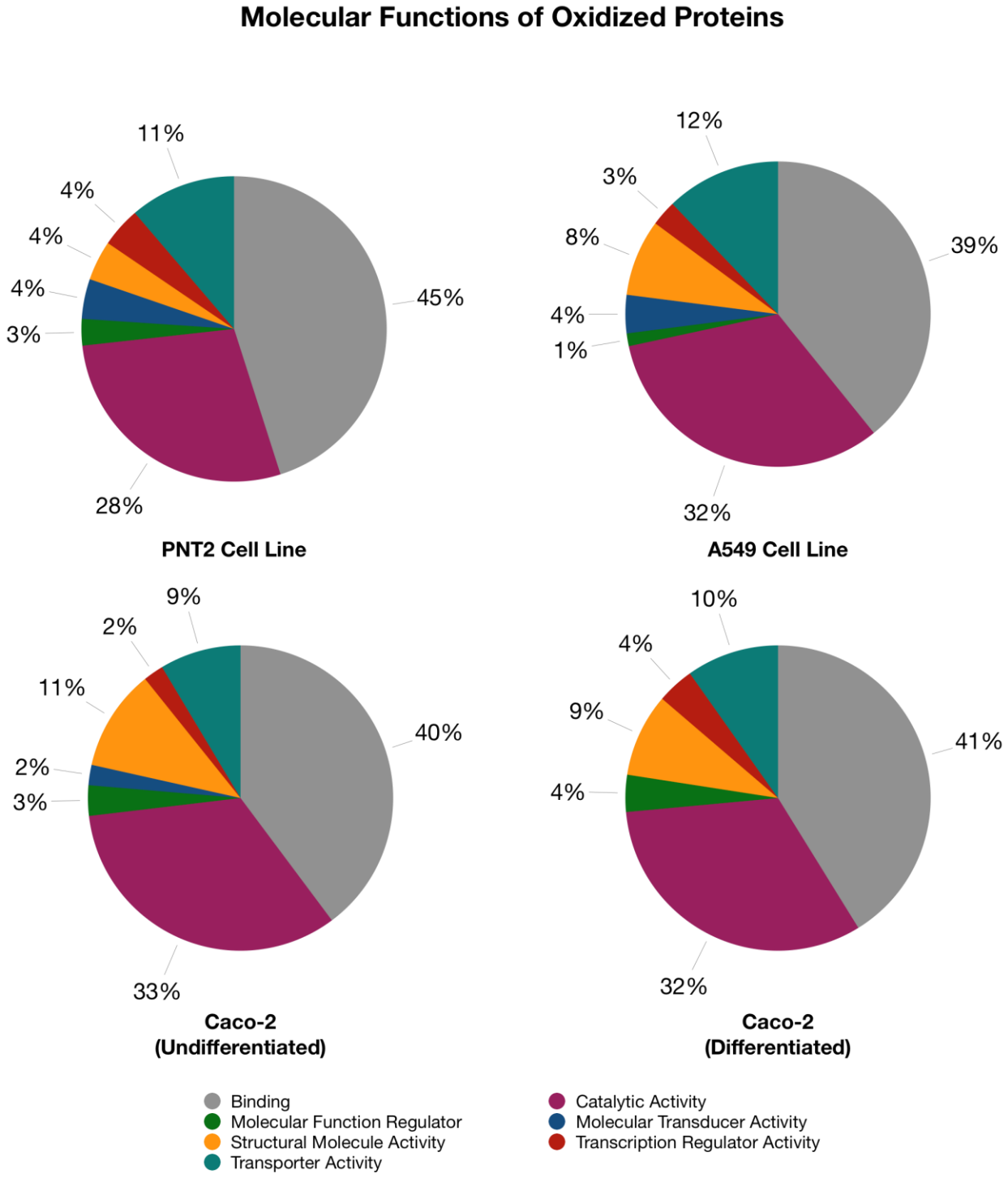

Fig. S7. Molecular functions of oxidized proteins in each cell line. The functions were assigned based on gene ontology using PANTHER.

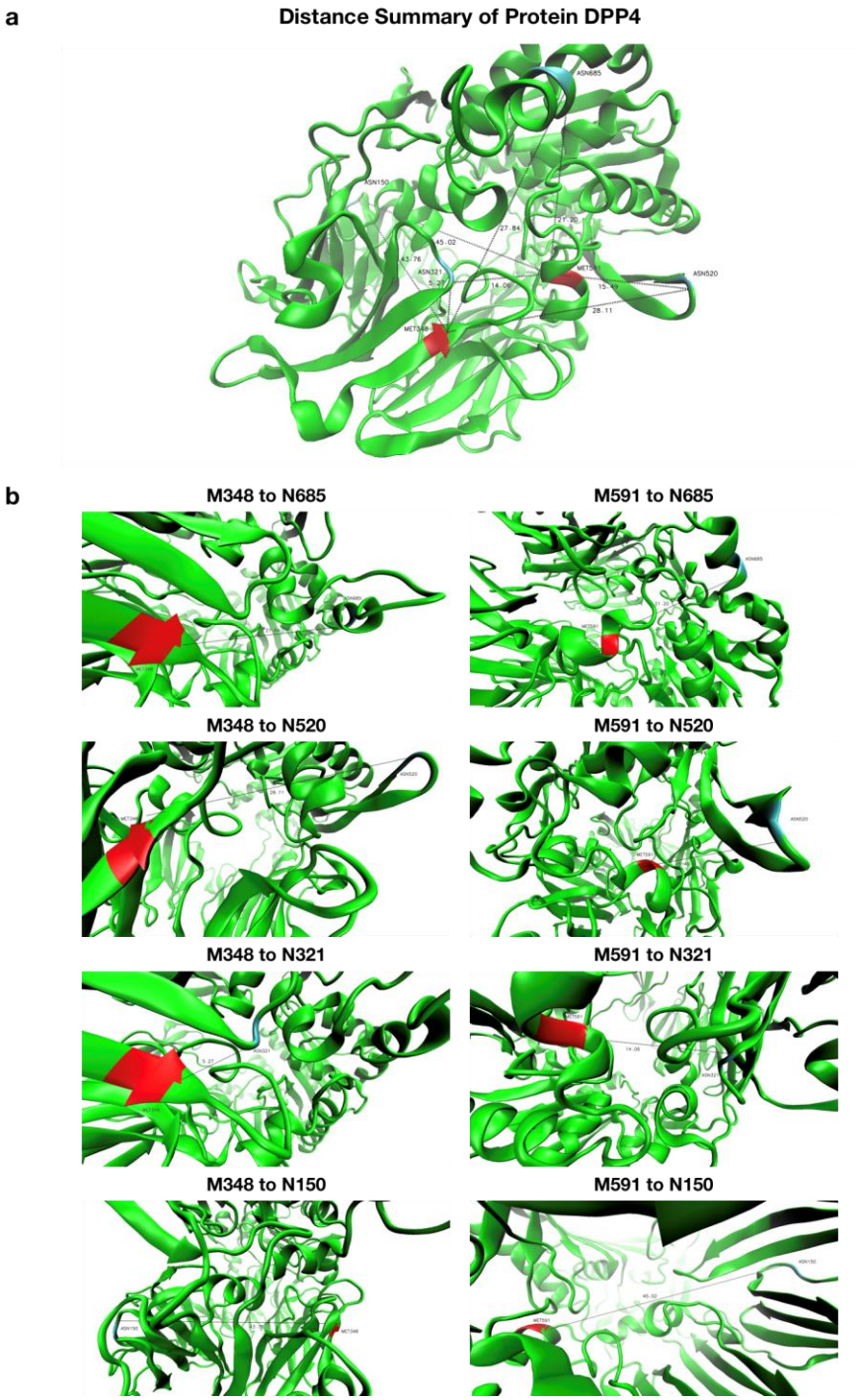

Fig. S8. (**a**) The summary of distances between oxidation sites (red) and sialylation sites (cyan) of protein Dipeptidyl peptidase 4 (DPP4). (**b**) Distance analysis of oxidation sites M348 and M591 to each sialylation sites N685, N520, N321, and N150. The crystal structure of DPP4 (ID: 1J2E) was obtained from Protein Data Bank (PDB).

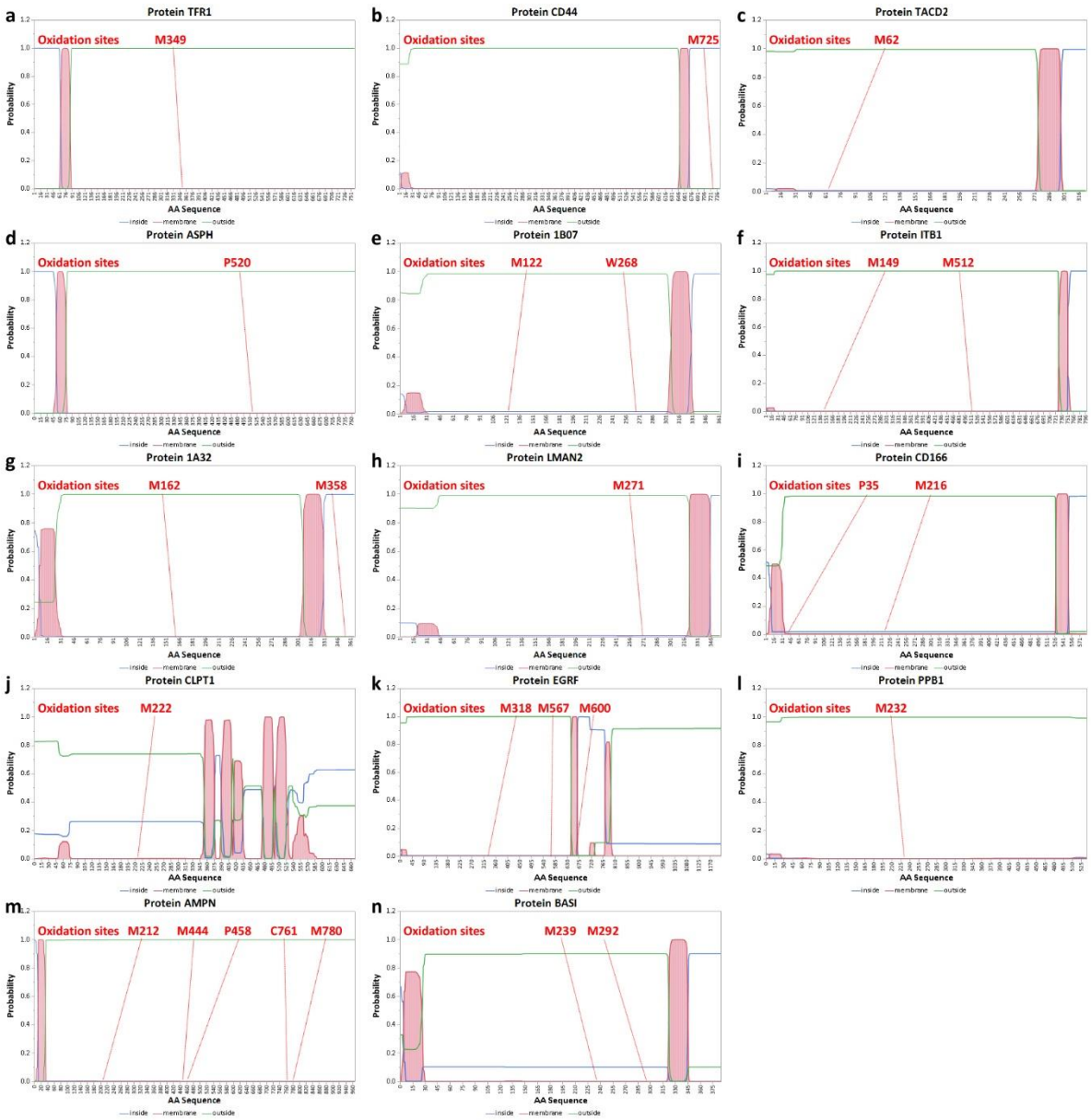

Fig. S9. Transmembrane location predicted by TMHMM and corresponding oxidation sites for PNT2 cell line. (**a**) TFR1. (**b**) CD44. (**c**) TACD2. (**d**) ASPH. (**e**) 1B07. (**f**) ITB1. (**g**) 1A32. (**h**) LMAN2. (**i**) CD166. (**j**) CLPT1. (**k**) EGRF. (**l**) PPB1. (**m**) AMPN. (**n**) BASI.

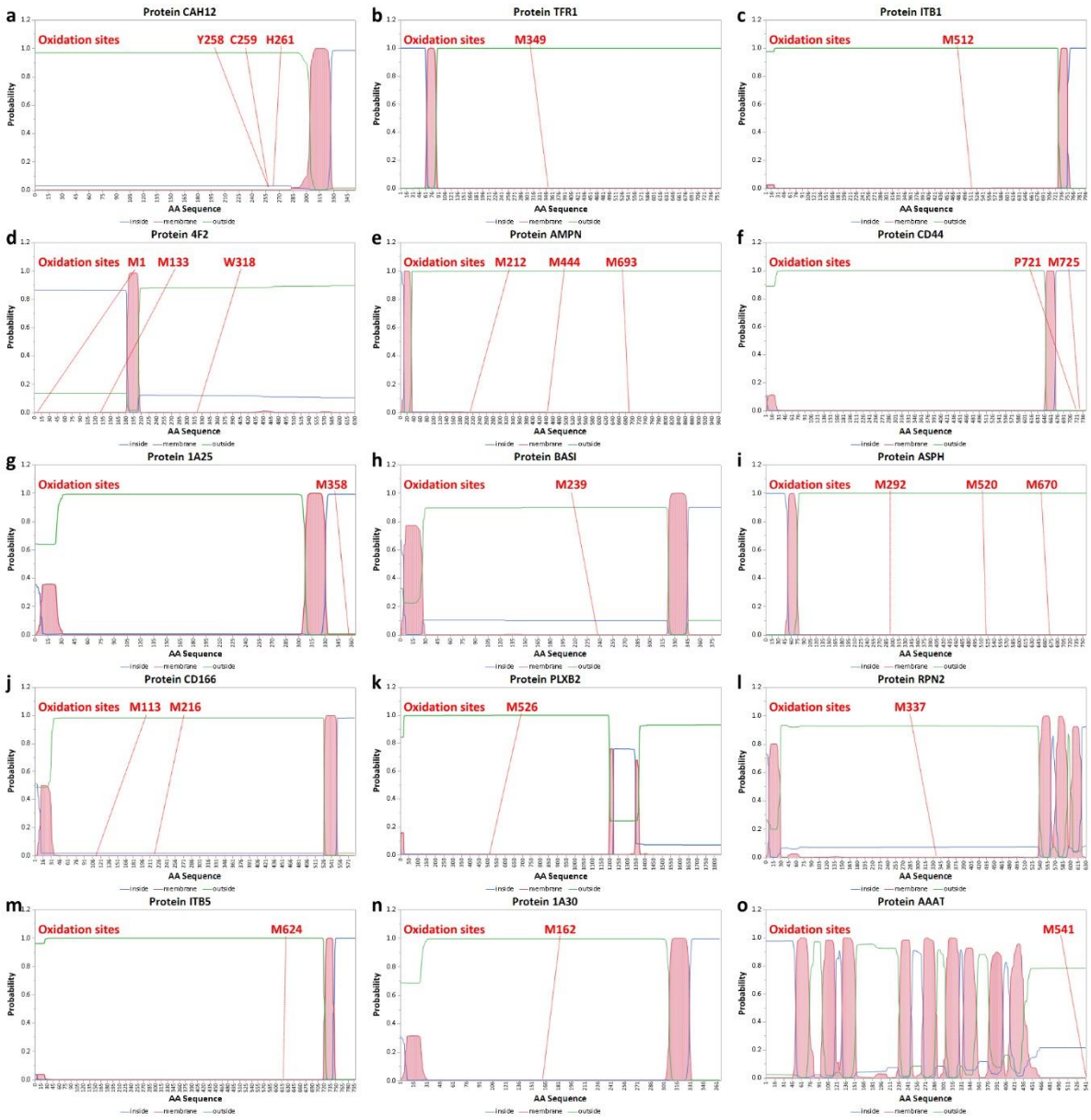

Fig. S10. Transmembrane location predicted by TMHMM and corresponding oxidation sites for A549 cell line. (**a**) CAH12 (**b**) TFR1. (**c**) ITB1. (**d**) 4F2. (**e**) AMPN. (**f**) CD44. (**g**) 1A25. (**h**) BASI. (**i**) ASPH. (**j**) CD166. (**k**) PLXB2. (**l**) RPN2. (**m**) ITB5. (**n**) 1A30. (**o**) AAAT.

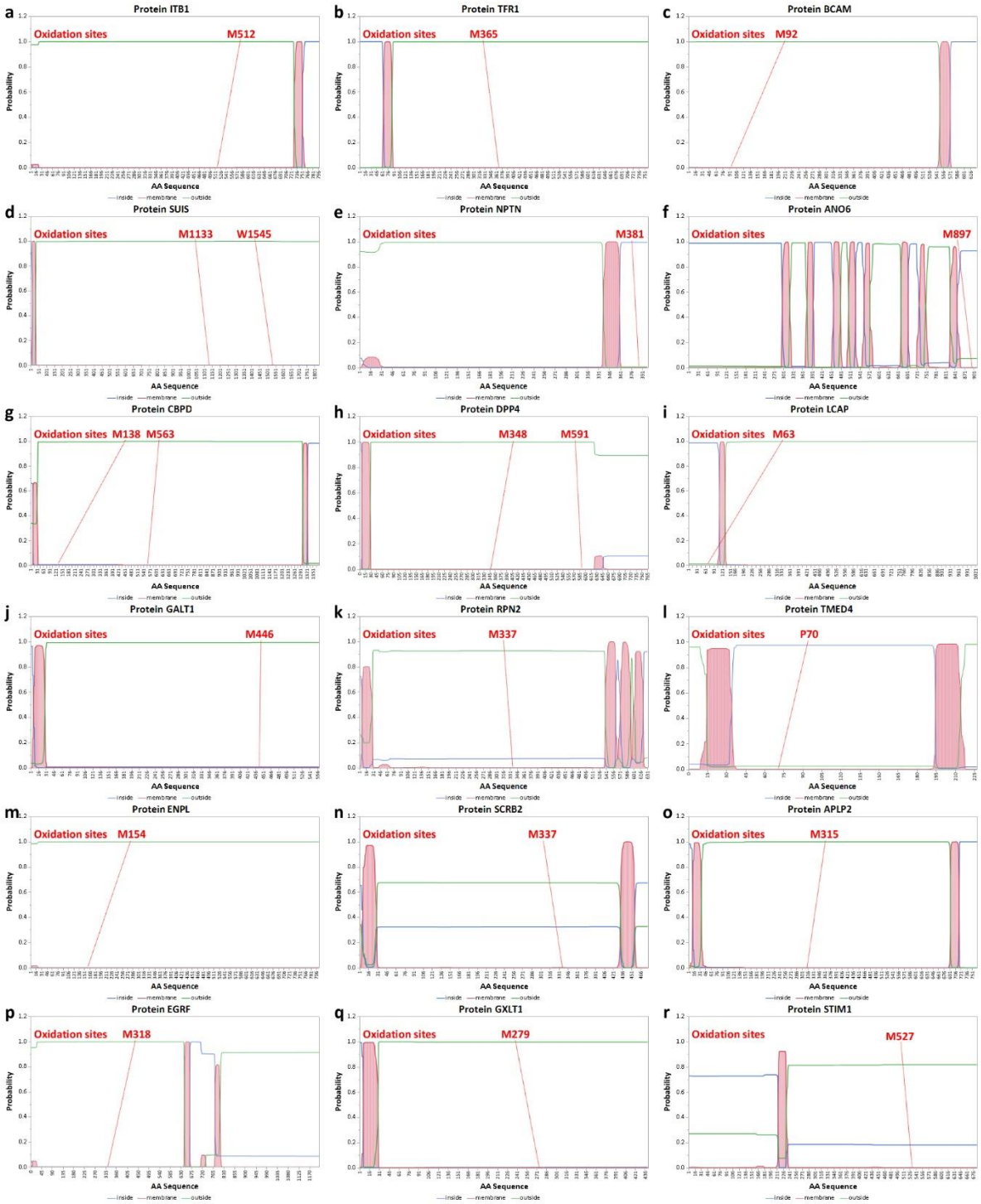

Fig. S11. Transmembrane location predicted by TMHMM and corresponding oxidation sites for Caco-2 cell line. (**a**) ITB1. (**b**) TFR1. (**c**) BCAM. (**d**) SUIS. (**e**) NPTN. (**f**) ANO6. (**g**) CBPD. (**h**) DPP4. (**i**) LCAP. (**j**) GALT1. (**k**) RPN2. (**l**) TMED4. (**m**) ENPL. (**n**) SCRB2. (**o**) APLP2. (**p**) EGRF. (**q**) GXLT1. (**r**) STIM1.