Supplementary Information

A New Tandem Enrichment Strategy for Simultaneous Profiling of O-GlcNAcylation and Phosphorylation in RNA-binding Proteome

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Materials and reagents

Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were purchased from Corning (NY, USA). Fetal bovine serum (FBS) and penicillin-streptomycin was purchased from Gibco (NY, USA). Trifluoroacetic acid (TFA), trypsin (from bovine pancreas, TPCK treated), ammonium bicarbonate (NH_4HCO_3), dithiothreitol (DTT), iodoacetamide (IAA), and formic acid (FA) were all purchased from Sigma. ZIC-HILIC (5μm, 200Å) was purchased from Merck KGaA (Darmstadt, Germany). Formaldehyde (37%), tris-hydroxypropyltriazolylmethylamine (THPTA), sodium L-ascorbate, Cy5-azide, myoglobin, NHS-alkyne, biotin-azide, acetonitrile (ACN) and uridine were all purchased from Sigma-Aldrich (Germany). Ethynyluridine (EU), actinomycin D and hydroxyurea were obtained from J&K Scientific Co. Ltd. (Beijing, China). Complete protease inhibitor cocktail tablets were received from Roche (Mannheim, Germany). Ribonucleoside Vanadyl Complex RNase inhibitor, PNGase F and galactose oxidase were purchased from New England Biolabs (USA). RNase A (100 mg mL^{-1}) was purchased from Kangwei Century Biotechnology Co., Ltd. (Beijing, China). Albumin from bovine serum V (BSA) was purchased from Beijing BioDee B iotechnology Co. Ltd (Beijing, China). NE-PER Nuclear and Cytoplasmic Extraction Reagents, Dynabeads® M-280 Streptavidin-coated magnetic beads and TRIzol reagent were all purchased from ThermoFisher (USA). DEPC-treated water was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The deionized water used in all experiments (Resistance > 18.2 MΩ cm^{-1}) was prepared using a Millipore purification system (Billerica, MA). All the buffers used in the RNA related experiments were prepared using DEPC-treated water containing RNase inhibitor and all the experimental supplies were RNase-free grade, unless otherwise mentioned.

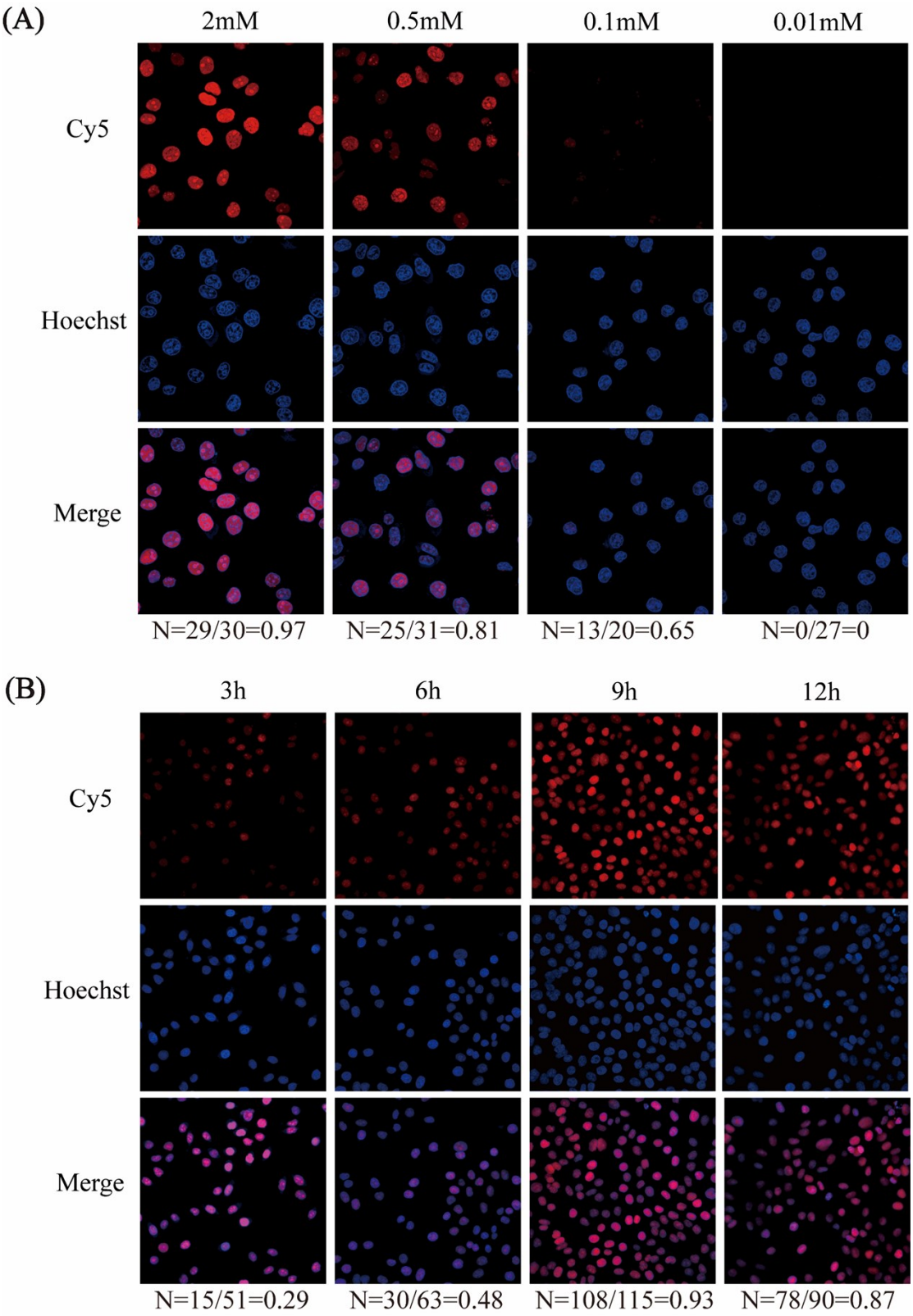

Fig.S1 (A) Confocal laser scanning microscopy (CLSM) images and the corresponding labeling efficiency N of HeLa cells treated with 2mM EU, 0.5mM EU, 0.1mM EU and 0.01mM EU, respectively. (B) CLSM images and the corresponding labeling efficiency N of HeLa cells treated with 0.5mM EU for 3h, 6h, 9h and 12h, respectively.

Table 1. Number of O-GlcNAc-peptides and phospho-peptides enriched by HILIC under different conditions[a].

	Number of O-GlcNAc-peptides	Number of phospho-peptides
NC-5%FA	10	72
	19	164
	20	123
NC-0.1%TFA	13	79
	74	120
	86	117
NC-1%TFA	234	476
	415	590
	462	550

[a] The table highlights results extracted from Orbitrap Fusion MS data obtained from HILIC enrichments from nucleocytoplasmic proteins. The experiments were performed with three technical replicates. NC, nucleocytoplasmic proteins.