

Supporting Information

**Cytosolic protein delivery *via* metabolic glycoengineering and
bioorthogonal click reaction**

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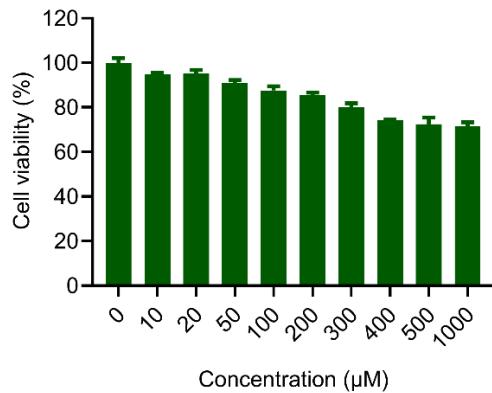


Fig. S1. Viability of HeLa cells after 48-h incubation with AAM at different concentrations ($n = 3$).

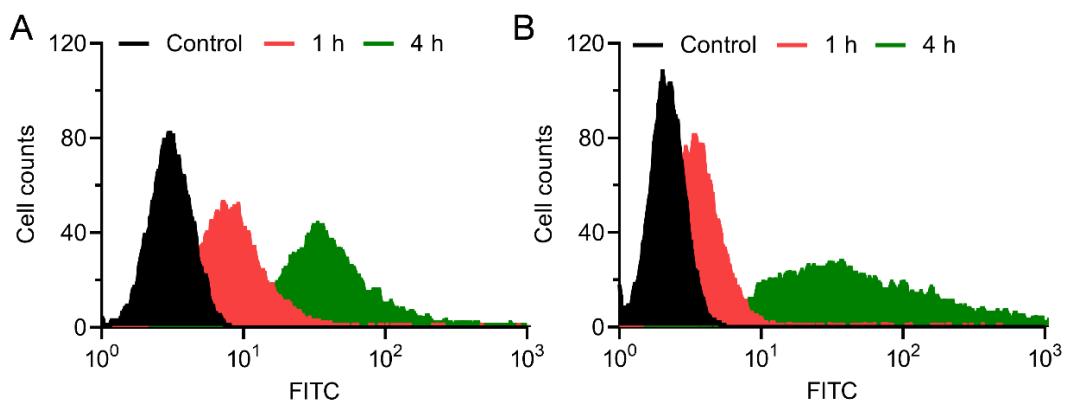


Fig. S2. Flow cytometric profiles of HeLa (A) and B16F10 (B) cells after treatment with AAM (50 μM) for 48 h followed by incubation with FITC-RNase A-DBCO (2 $\mu\text{g}/\text{mL}$) for different time.

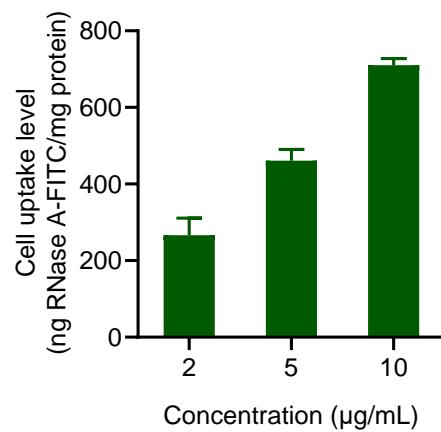


Fig. S3. Cell uptake level of FITC-RNase A-DBCO at different concentrations in AAM-pretreated (50 μM , 48 h) HeLa cells after 4-h incubation ($n = 3$).

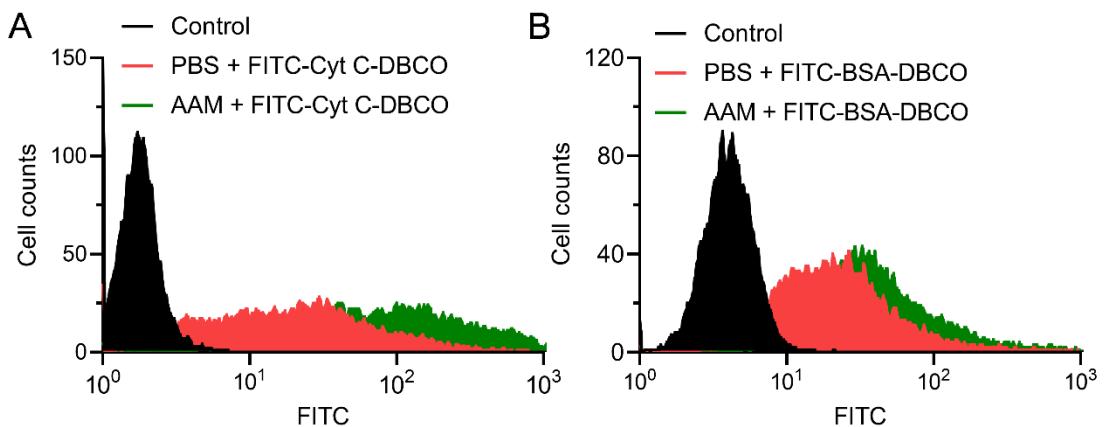


Fig. S4. Flow cytometric profiles of HeLa cells after treatment with AAM (50 μ M) or PBS for 48 h followed by incubation with FITC-Cyt C-DBCO (2 μ g/mL, A) and FITC-BSA-DBCO (2 μ g/mL, B) for 4 h.

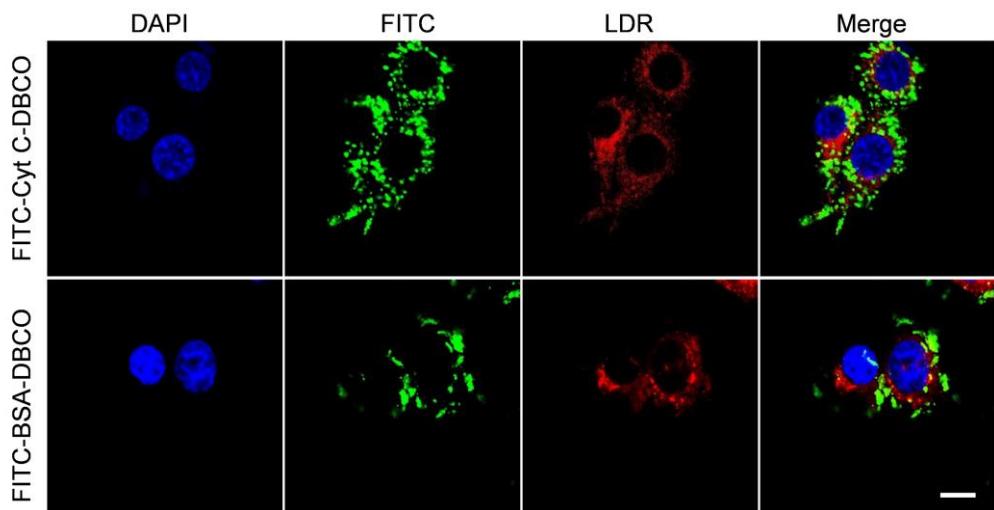


Fig. S5. CLSM images of HeLa cells after treatment with AAM (50 μ M) for 48 h followed by incubation with FITC-Cyt C-DBCO or FITC-BSA-DBCO (2 μ g/mL) for 4 h (scale bar = 10 μ m). Cell nuclei were stained with DAPI, and endolysosomes were stained with LDR.

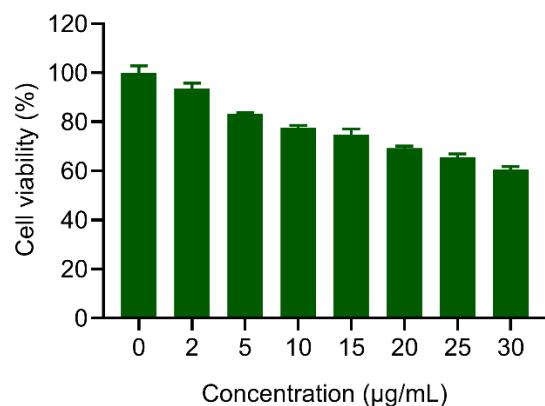


Fig. S6. Viability of HeLa cells pre-treated with AAM (50 μ M, 48 h) after incubation with FITC-RNase A-DBCO for 48 h at different concentrations (n = 3).

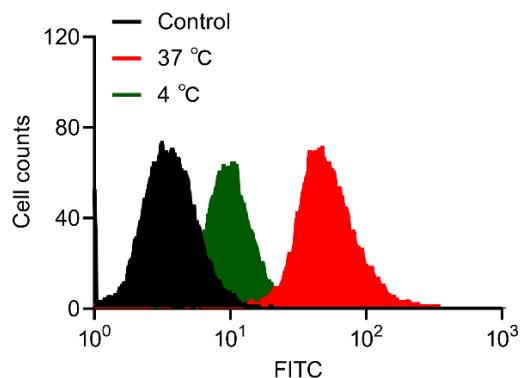


Fig. S7. Flow cytometric profiles of HeLa cells after treatment with AAM (50 μ M) for 48 h followed by incubation with FITC-RNase A-DBCO (2 μ g/mL) at 37 or 4 °C for 4 h (n = 3).

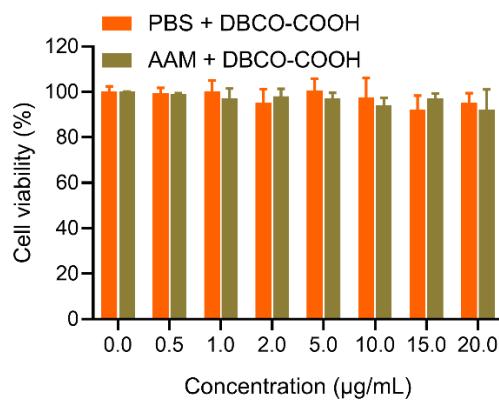


Fig. S8. Viability of HeLa cells after treatment with PBS (control) or AAM (50 μ M) for 48 h, followed by incubation with DBCO-COOH at various concentrations for 48 h (n = 3).

Table S1. The molar ratios of DBCO/protein for the modification of different proteins.

Protein	MWs (kDa)	pIs	DBCO/Protein (mol/mol)
Cyt C	12.4	10.3	3
RNase A	14.7	8.6	3
BSA	67.0	4.7	20