

Supporting Information

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

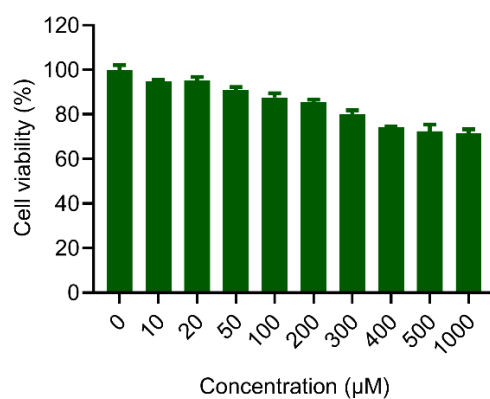
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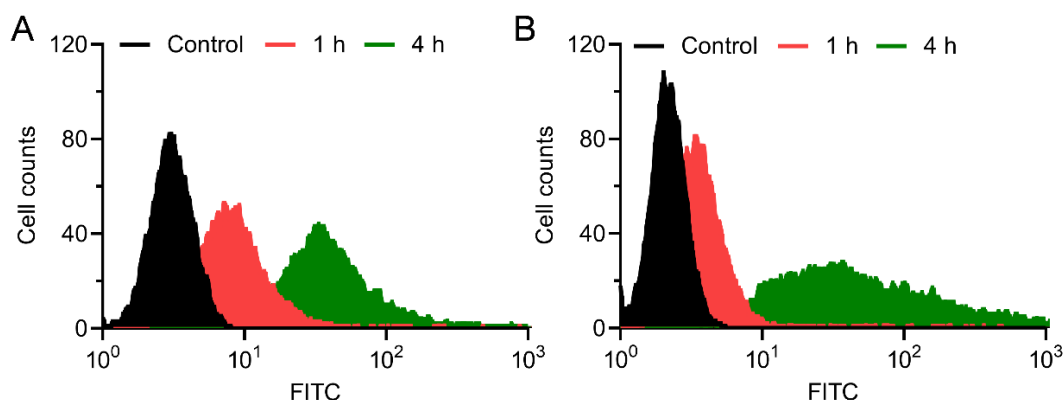
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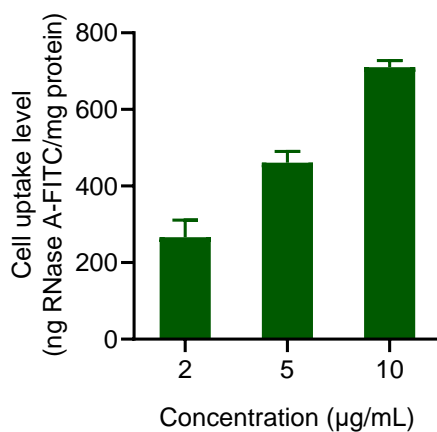
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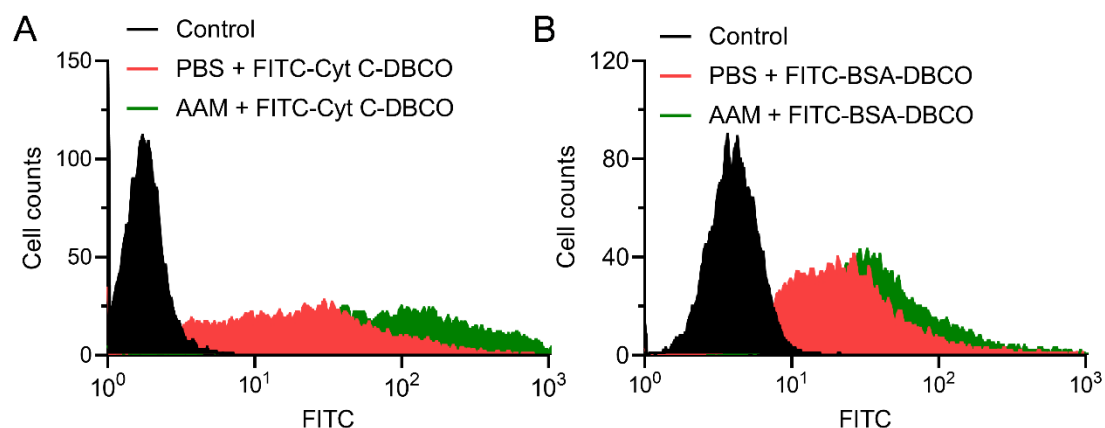
**Fig. S1.** Viability of HeLa cells after 48-h incubation with AAM at different concentrations (n = 3).



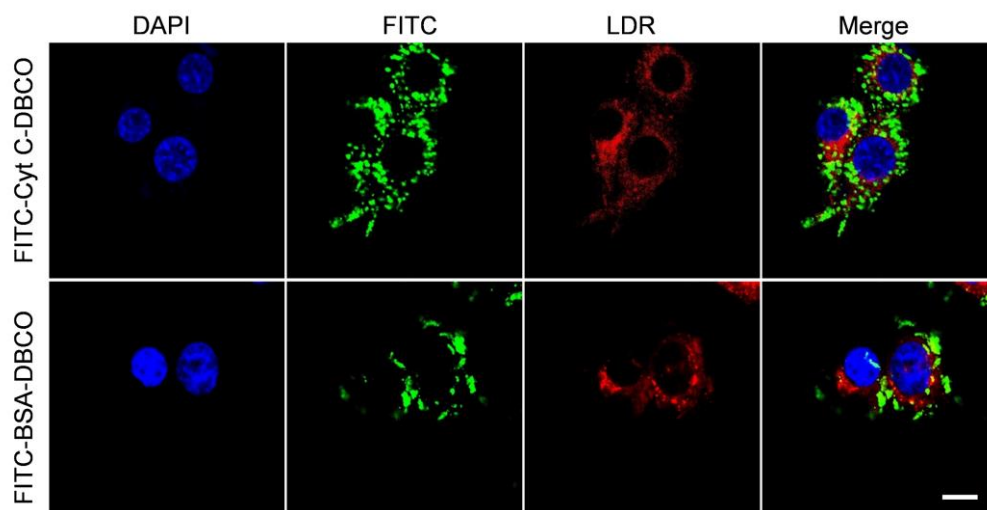
**Fig. S2.** Flow cytometric profiles of HeLa (A) and B16F10 (B) cells after treatment with AAM (50 μM) for 48 h followed by incubation with FITC-RNase A-DBCO (2 μg/mL) for different time.



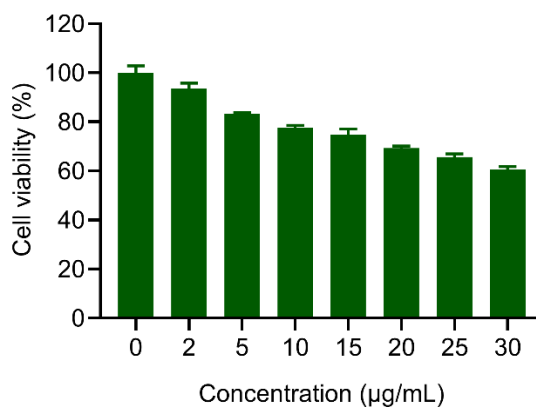
**Fig. S3.** Cell uptake level of FITC-RNase A-DBCO at different concentrations in AAM-pretreated (50 μM, 48 h) HeLa cells after 4-h incubation (n = 3).



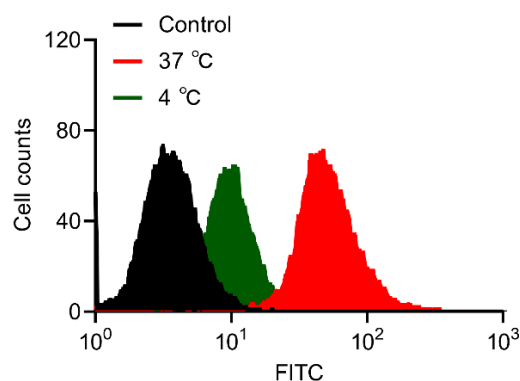
**Fig. S4.** Flow cytometric profiles of HeLa cells after treatment with AAM (50  $\mu$ M) or PBS for 48 h followed by incubation with FITC-Cyt C-DBCO (2  $\mu$ g/mL, A) and FITC-BSA-DBCO (2  $\mu$ g/mL, B) for 4 h.



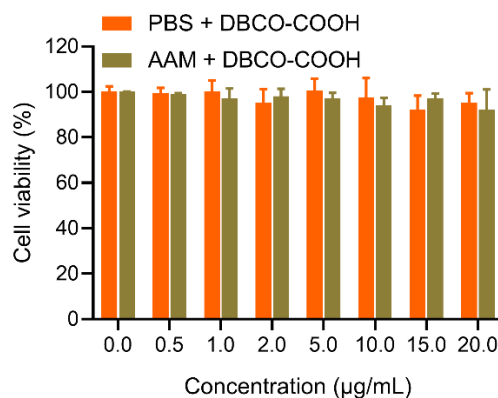
**Fig. S5.** CLSM images of HeLa cells after treatment with AAM (50  $\mu$ M) for 48 h followed by incubation with FITC-Cyt C-DBCO or FITC-BSA-DBCO (2  $\mu$ g/mL) for 4 h (scar bar = 10  $\mu$ m). Cell nuclei were stained with DAPI, and endolysosomes were stained with LDR.



**Fig. S6.** Viability of HeLa cells pre-treated with AAM (50  $\mu$ 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  $\mu$ M) for 48 h followed by incubation with FITC-RNase A-DBCO (2  $\mu$ g/mL) at 37 or 4  $^{\circ}$ C for 4 h ( $n = 3$ ).



**Fig. S8.** Viability of HeLa cells after treatment with PBS (control) or AAM (50  $\mu$ 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                     |