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Title: A new insect cell glycoengineering approach provides baculovirus-inducible glycogene expression and increases human-type glycosylation efficiency



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## ACCEPTED MANUSCRIPT

1 A new insect cell glycoengineering approach provides baculovirus-2 inducible glycogene expression and increases human-type 3 glycosylation efficiency 4 5 Ann M. Toth<sup>2</sup>, Chu-Wei Kuo<sup>3</sup>, Kay-Hooi Khoo<sup>3</sup>, Donald L. Jarvis<sup>1,2</sup> 6 7 <sup>1</sup>To whom correspondence should be addressed: Tel: 1-307-766-4284; Fax: 1-307-766-5098; email: dljarvis@uwyo.edu 8 <sup>2</sup>Department of Molecular Biology, University of Wyoming, Laramie WY 82071, USA 9 10 <sup>3</sup>Institute of Biological Chemistry, Academia Sinica, Nankang, Taipei 115, Taiwan 11 12 Keywords: baculovirus expression vector system; glycoengineering; baculovirus promoters; insect cell 13 glycosylation 14 15 Abbreviations: 16 17 39K, baculovirus delayed early gene encoding phosphoprotein of 39 kDa in apparent 18 molecular weight; AmBic, ammonium bicarbonate buffer; BCA, bicinchoninic acid; 19 B4GALT1, β1,4-galactosyltransferase I; BEVS, baculovirus expression vector system; CMAS, CMP-sialic acid synthetase; CSAT, CMP-sialic acid transporter; E1-ecto, 8X-20 histidine tagged form of the ectodomain of Western equine encephalitis virus E1 21 glycoprotein; GNPE, N-acetyl-D-glucosamine-6-phosphate 2'-epimerase; hEPO, human 22 erythropoietin; hEPO-His, 6X-histidine tagged form of human erythropoietin; hpi, hours 23 post-infection; ie1, immediate early gene 1; MAL, Maackia amurensis lectin; MALDI-24 25 TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; MGAT1, *β*1,2-glucosaminyltransferase I; MGAT2, *β*1,2-glucosaminyltransferase II; 26 RCA, *Ricinus communis* agglutinin; RT-PCR, reverse transcriptase-polymerase chain 27 reaction; SAS, sialic acid-9-phosphate synthase; SEAP, secreted alkaline phosphatase; 28 SNA, Sambucus nigra agglutinin; ST3GAL3, α2,3-sialyltransferase III; ST6GAL1, 29  $\alpha$ 2,6-sialyltransferase I; PNGase F, peptide-*N*-glycosidase F; TBA, thiobarbituric acid; 30

- 31 TBS, Tris-buffered saline.
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