

**Inducible *Exoc7/Exo70* knockout reveals a critical role of the exocyst
in insulin-regulated GLUT4 exocytosis**

Shifeng Wang^{1,2#}, Lauren Crisman^{1#}, Jessica Miller¹, Ishara Datta¹, Daniel R. Gulbranson¹, Yuan Tian³,
Qian Yin³, Haijia Yu^{1,4}, and Jingshi Shen^{1*}

From the ¹Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309, USA; ²Department of Chinese Medicine Information Science, Beijing University of Chinese Medicine, Beijing, 102488, China; ³Department of Biological Sciences and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA. ⁴Jiangsu Key Laboratory for Molecular and Medical Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing, 210023, China.

* To whom correspondence should be addressed: Jingshi Shen, Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO 80309, USA. Phone: 303-492-6166; Fax: 303-492-7744; E-mail: jingshi.shen@colorado.edu

#These authors contributed equally to this work.

This SI file contains: Supplemental Figures 1 and 2.

Supplemental files not included in this SI file: Supplemental Tables 1 and 2.

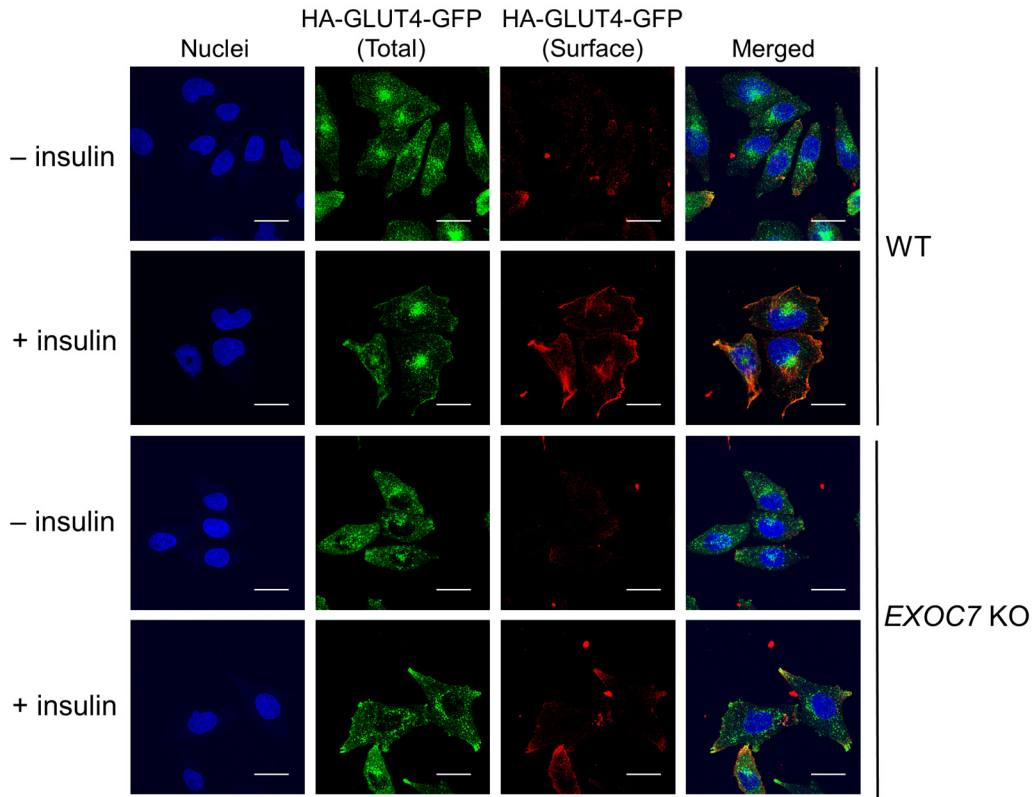


Figure S1. *EXOC7* knockout (KO) inhibits insulin-stimulated GLUT4 translocation in HeLa cells. Wild-type (WT) and *EXOC7* KO HeLa cells expressing an HA-GLUT4-GFP reporter were either untreated or treated with 100 nM insulin for 30 minutes. GLUT4 reporters on the surfaces of non-permeabilized cells were labeled using anti-HA antibodies and Alexa Fluor 568-conjugated secondary antibodies. Nuclei were stained with Hoechst 33342. The images were captured using a 100× oil immersion objective on a Nikon A1 Laser Scanning confocal microscope. Representative images are shown. Scale bars: 25 μm.

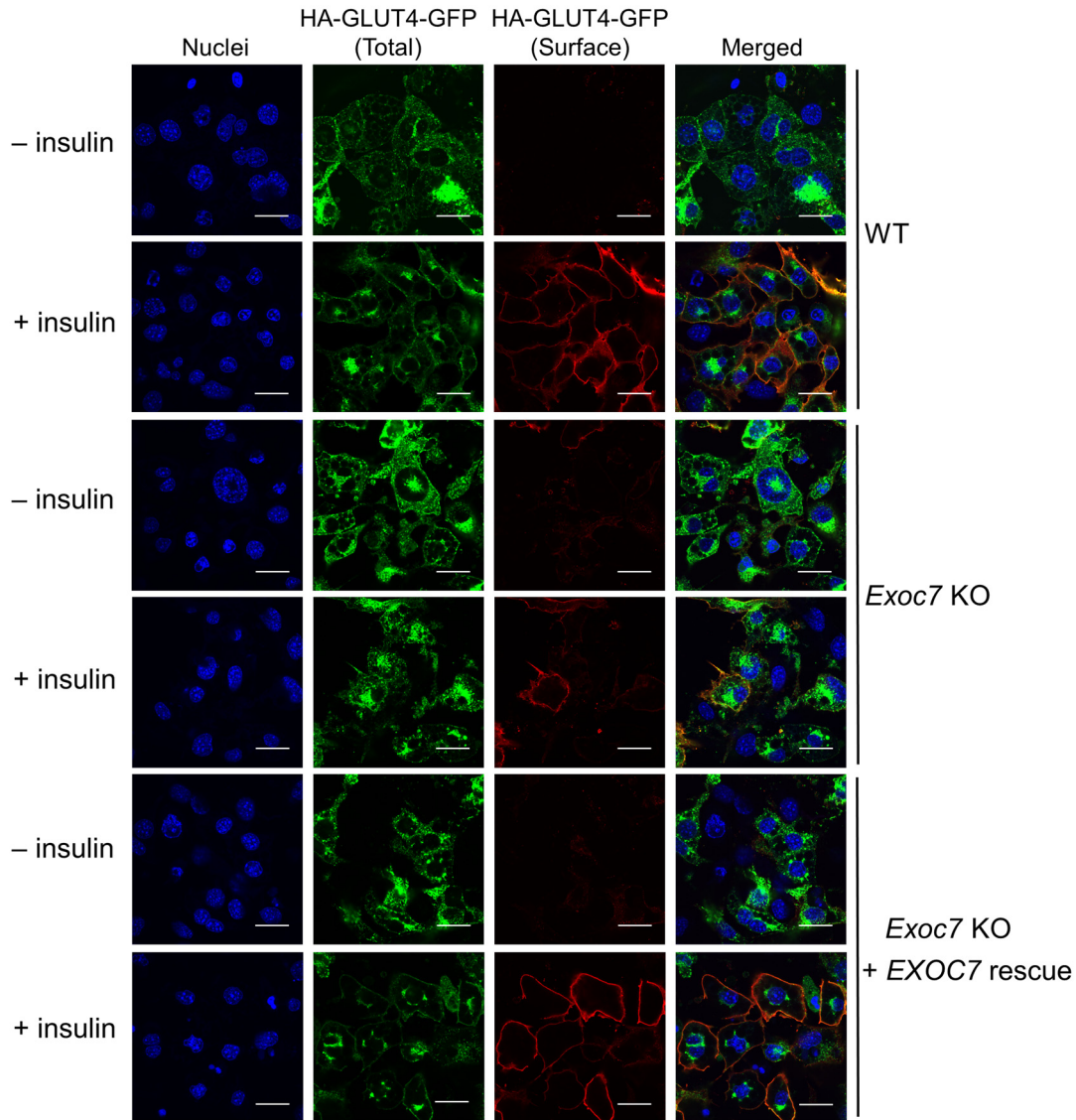


Figure S2. *Exoc7* KO inhibits insulin-stimulated GLUT4 translocation in adipocytes. Preadipocytes expressing the HA-GLUT4-GFP reporter were cultured and differentiated as described in Figure 6C. The cells were either untreated or treated with 100 nM insulin for 30 minutes. GLUT4 reporters on the surfaces of non-permeabilized cells were labeled using anti-HA antibodies and Alexa Fluor 568-conjugated secondary antibodies. Nuclei were stained with Hoechst 33342. The images were captured using a 100 \times oil immersion objective on a Nikon A1 Laser Scanning confocal microscope. Representative images are shown. Scale bars: 25 μ m.