

SUPPLEMENTARY ONLINE DATA Serine/threonine kinase 16 and MAL2 regulate constitutive secretion of soluble cargo in hepatic cells

Julie G. IN*1, Anneliese C. STRIZ*, Antonio BERNAD† and Pamela L. TUMA*2

*Department of Biology, The Catholic University of America, Washington, DC 20064, U.S.A.

+Department of Regenerative Cardiology, Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III, Melchor Fernández Almagro 3, E-28029 Madrid, Spain

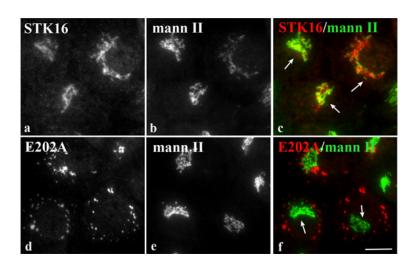


Figure S1 In non-polarized hepatic Clone 9 cells, exogenous STK16 is present at the Golgi whereas E202A is in peripheral puncta

Clone 9 cells were infected with recombinant adenovirus expressing STK16 (a–c) or E202A (d–f). Cells were double-labelled for mannosidase II (mann II) and the V5 tag. Merged images are shown in c and f. Arrows indicate the Golgi in infected cells. Scale bar, 10 μ m.

¹ Present address: Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, U.S.A.

² To whom correspondence should be addressed (email tuma@cua.edu).

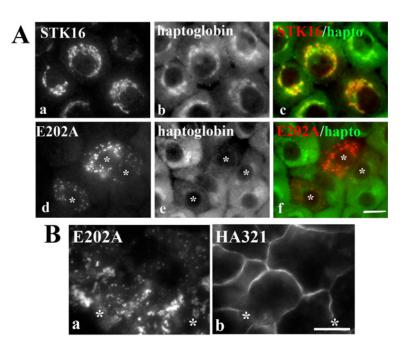


Figure S2 Haptoglobin, but not HA321, expression is decreased in E202A-expressing cells

(A) WIF-B cells were infected with recombinant adenovirus expressing STK16 (a–c) or E202A (d–f) and double-labelled for haptoglobin and the V5 tag. Merged images are shown in c and f. Asterisks indicate E202A-infected cells with decreased haptoglobin staining. (B) E202A-infected cells were double-labelled for HA321 and the V5 tag. Asterisks mark selected bile canaliculi. Scale bar, 10 μ m.

Table S1 Y2H screens identify STK16 and 18 other putative MAL2 interactors

Four Y2H screens were performed as described in the Results section of the main paper of which only three yielded usable clones. Out of the 54 clones, 19 putative MAL2 interactors were identified.

Bait	Library	Putative Interactors
MAL2-Cub	x-NubG library (Type I)	STK16
MAL2-Cub	NubG-x library (Type II)	31 clones, four false positives
Cub-MAL2	x-NubG library (Type I)	Seven clones, all false positives
Cub-MAL2	NubG-x library (Type II)	15 clones, eight false positives

Table S2 The secretion rates are unchanged in infected cells

The secretion rates for albumin (% of total secreted/min) were calculated for control, knockdown or overexpressing cells as indicated. Values are expressed as the means \pm S.E.M. Measurements were done on at least three independent experiments.

Condition	Rate (% of total secreted/min)
Control	2.2 + 0.2
STK16	2.9 + 0.3
E202A	2.2 ± 0.5
AS MAL2	1.9 + 1.0

Received 9 April 2014/18 July 2014; accepted 1 August 2014

Published as BJ Immediate Publication 1 August 2014, doi:10.1042/BJ20140468