Supplemental Materials

Molecular Biology of the Cell

Hussain et al.

Supplementary Figures

Figure S1. Western blot using the affinity-purified Rabbit anti-ABCG1 antibody on samples of INS1 cells without and with siRNA-mediated depletion of ABCG1.

Figure S2.(A) Distribution of carboxypeptidase E (CPE), mitochondrial succinate-ubiquinone oxidoreductase (SUO), and the ER chaperone calnexin (CalNx). Quantification is from two separate fractionation experiments. For CPE, the data relate to the western blot shown in Figure 1D; quantification of SUO and CalNx documents that comparable amounts of cell were fractionated and that levels of ER and mitochondria are not affected by ABCG1 knockdown. Data are presented as mean $\pm$ SEM. (B) Western blots of fractions from continuous sucrose gradients following centrifugation of postnuclear supernatants to equilibrium. Individual blots were run for Control and ABCG1 knockdown samples and were normalized during quantification using identical samples included on both gels. The blots document the nearly parallel loss of carboxypeptidase E, secretogranin III, and phogrin in the main fractions (9-11) containing CpepSfGFP and also show the negligible loss of VAMP4 in the samples from ABCG1 knockdown.

Figure S3. Fluorescent labeling in GRINCH cells showing extensive co-localization of SfGFP (mainly marking Cpep-SfGFP), transiently expressed NPY-mCherry, and immunostained endogenous insulin (blue).

Figure S4. (A) Relative levels of free cholesterol in GRINCH cells incubated without or with 20 μM or 50 μM exogenous cholesterol for 21-24 h before processing parallel samples for cholesterol determination and protein assay. Results are from 4 and 5 independent experiments for 20 μM and 50 μM cholesterol, respectively. (B) Levels of free cholesterol in ABCG1-, OSBP-, ABCA1- and ABCG1/A1 knockdown cells are comparable to those in the control. Results are from 5 independent experiments. Data are presented as mean $\pm$ SEM.

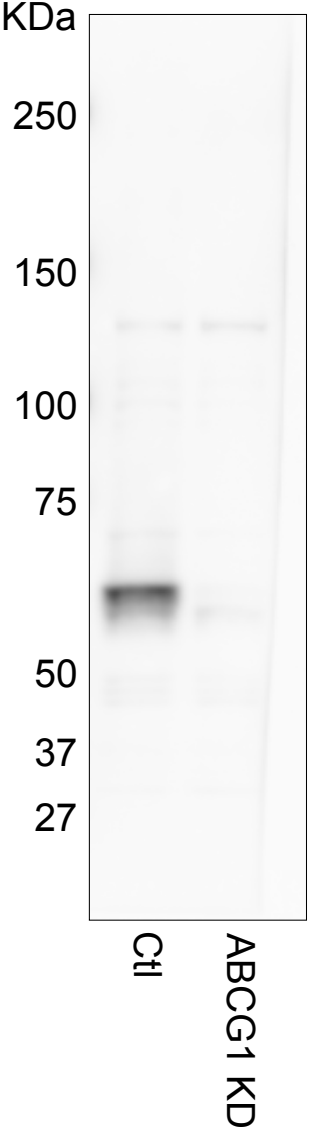
Figure S5. (A) siRNA-mediated knockdown of ABCA1 quantified from western blots; n=16. Western blot documenting the specificity of ABCA1 antibody. (B) Distributions of ABCA1 and ABCG1 in GRINCH cells examined by centrifugation on continuous sucrose gradients (0.6-1.6M). Fractions were processed for western blotting. The two transporters are broadly distributed but quite similar, although relative amounts in the two peaks differ.

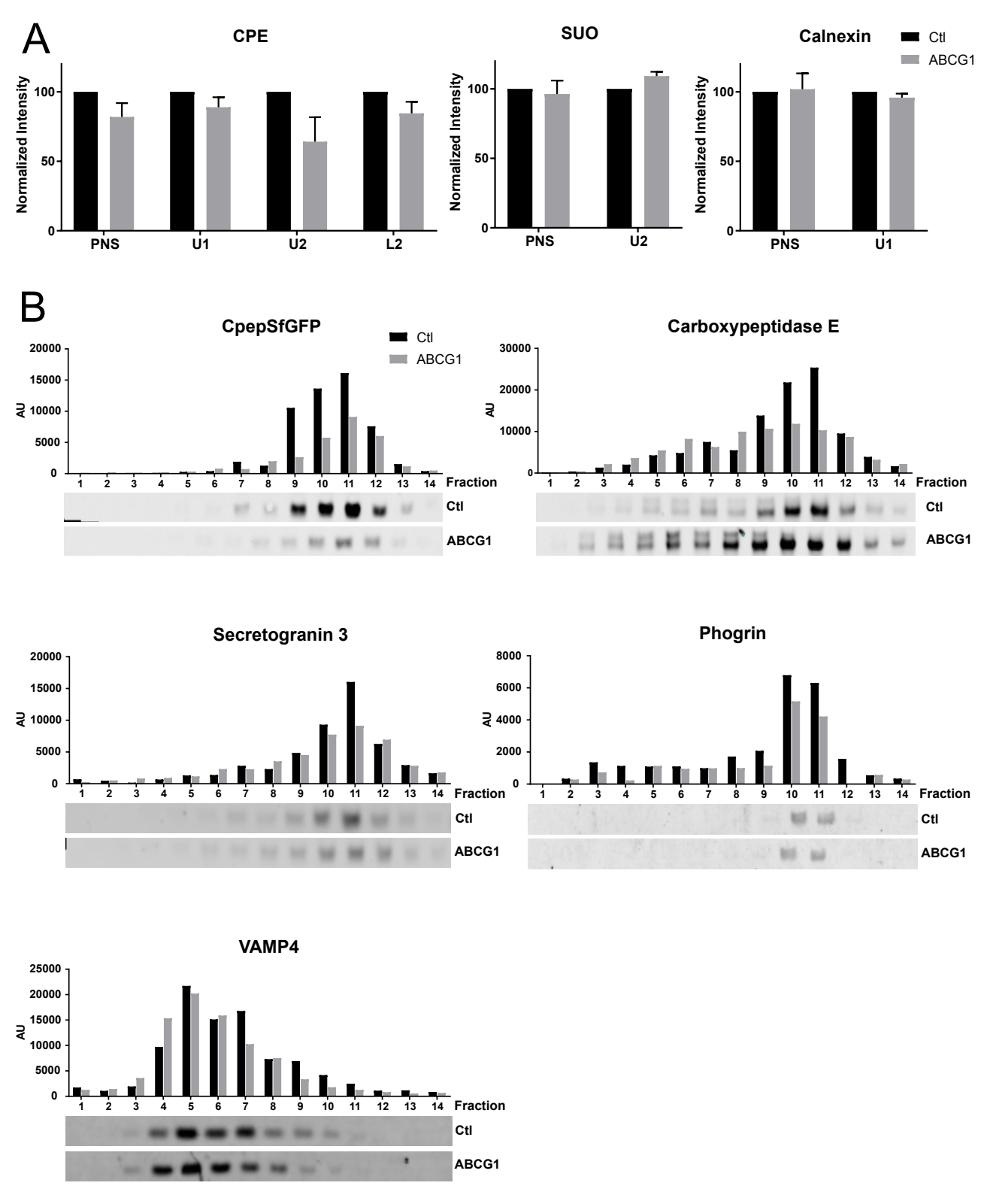
Figure S6. (A) RNAi-mediated knockdown targeted to the 3'-UTR of OSBP shows comparable loss of OSBP, hPro-CpepSfGFP, and CpepSfGFP as observed with siRNA Smart Pool induced knockdown (used in most experiments). Quantification from western blots; n = 2. Data are presented as mean $\pm$ SEM. Accompanying fluorescence images document knockdown and antibody specificity. (B) Loss of hPro-CpepSfGFP and CpepSfGFP caused by RNAi-mediated knockdown targeted to the 3'-UTR of OSBP can be partially reversed by co-expressing recombinant OSBP-mCherry, arguing that the effects of OSBP knockdown are on-target.

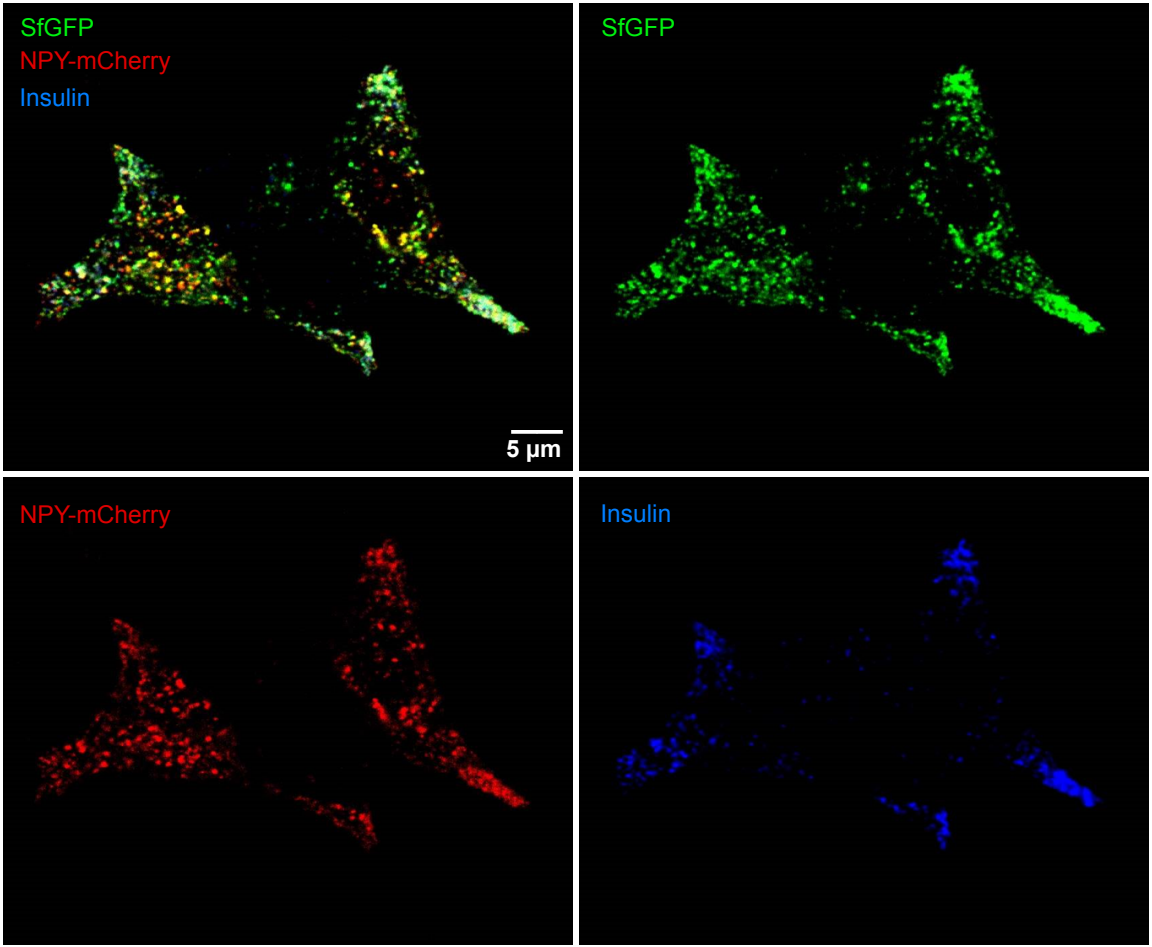
Figure S7. Replots of data shown in Figure 7A after normalization to equalize the initial level of hPro-CpepSfGFP between Control and OSBP-depleted cells. This confirms similar processing kinetics of hPro-CpepSfGFP and shows that CpepSfGFP levels remain depressed in OSBP knockdown as compared to the control.

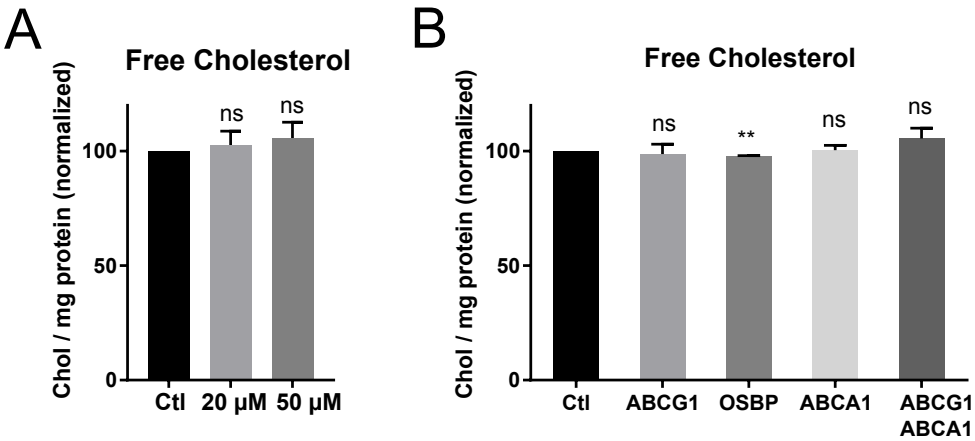
Figure S8. Fluorescence emission scans measuring GFP levels in GRINCH cell lysate and media following basal (3 mM glucose) and stimulated (20 mM glucose) secretion. Intensity at

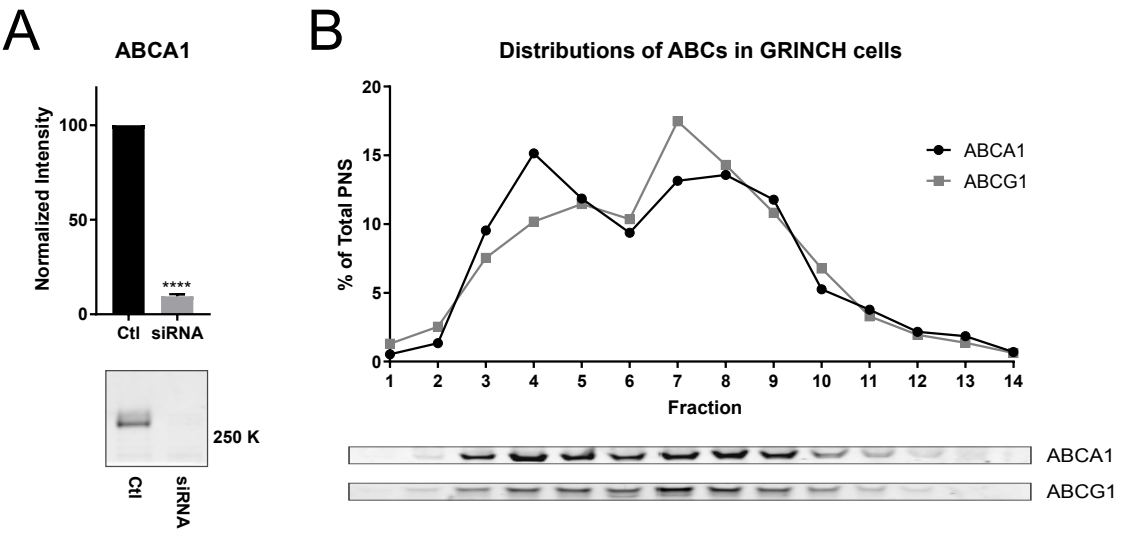
the emission maximum (510 nm) was used to quantify percent secretion. Accompanying western blots show the bands detected in cell lysates and stimulated secretion.

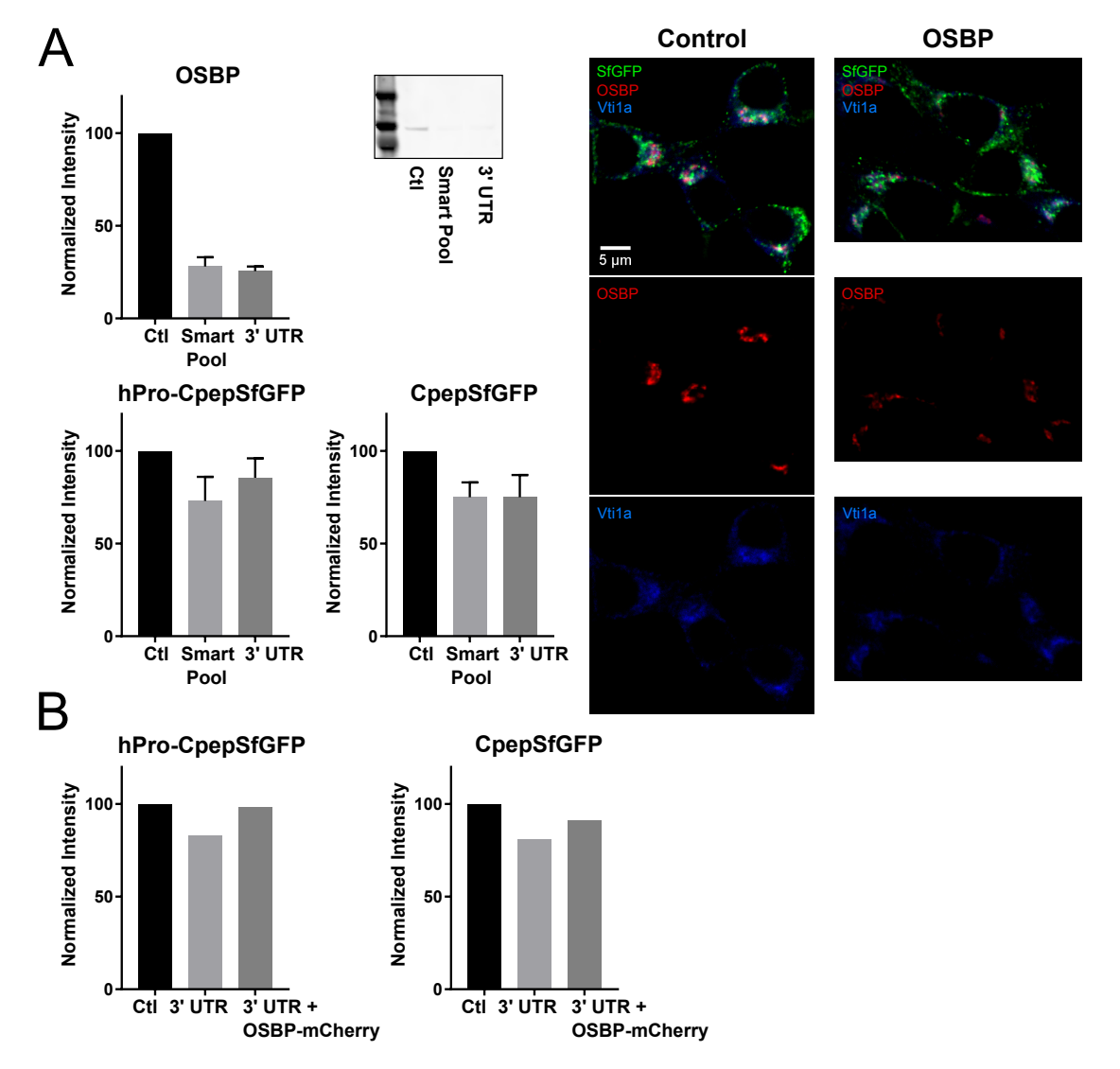

KDa
250
150
100
75
50
37
27
Ctl
ABCG1 KD

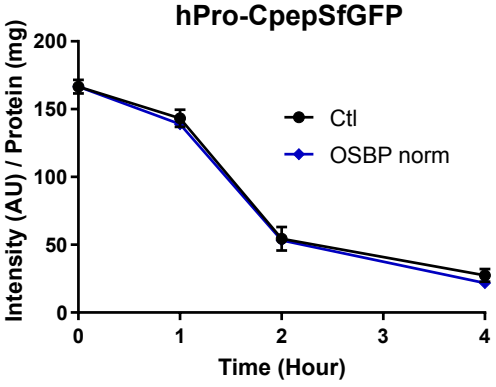

A
CPE
SUO
Calnexin
Ctl
ABCG1
Normalized Intensity
100
50
0
PNS
U1
U2
L2
B
CpepSfGFP
Carboxypeptidase E
Secretogranin 3
Phogrin
VAMP4
AU
Fraction
Ctl
ABCG1

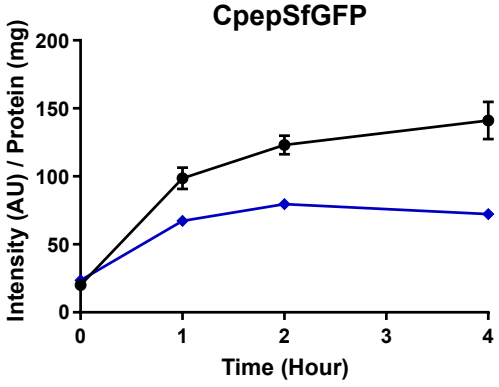

SfGFP
NPY-mCherry
Insulin
5 µm
SfGFP
NPY-mCherry
Insulin

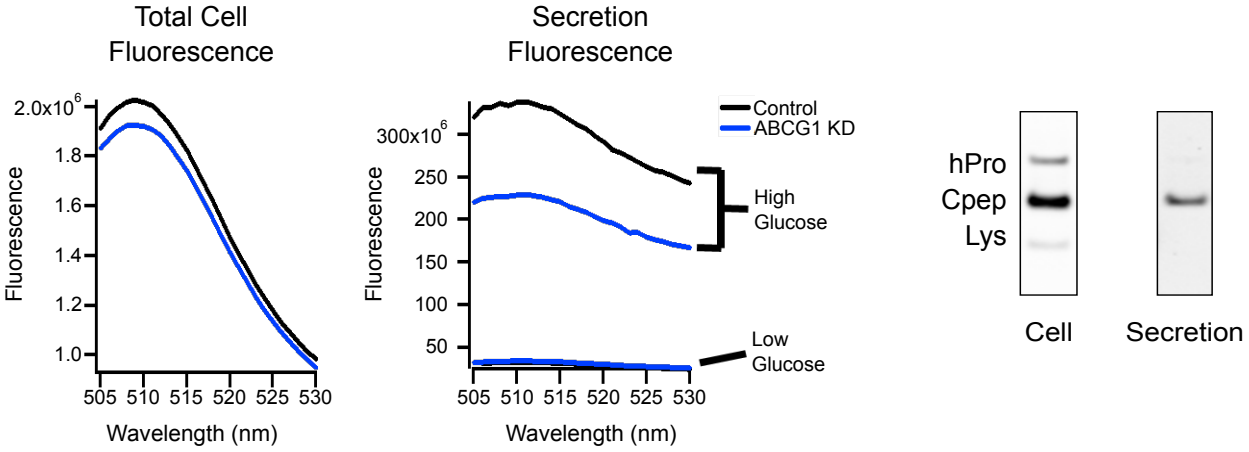

A
Free Cholesterol
Chol / mg protein (normalized)
100
50
0
ns
ns
Ctl
20 μM
50 μM
B
Free Cholesterol
Chol / mg protein (normalized)
100
50
0
ns
**
ns
ns
Ctl
ABCG1
OSBP
ABCA1
ABCG1
ABCA1


A
ABCA1
Normalized Intensity
100
50
0
****
Ctl
siRNA
250 K
Ctl
siRNA
B
Distributions of ABCs in GRINCH cells
% of Total PNS
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Fraction
ABCA1
ABCG1
ABCA1
ABCG1


A
OSBP
Normalized Intensity
100
50
0
Ctl
Smart Pool
3' UTR
Ctl
Smart Pool
3' UTR
hPro-CpepSfGFP
CpepSfGFP
Control
OSBP
SfGFP
OSBP
Vti1a
5 µm
OSBP
Vti1a
B
hPro-CpepSfGFP
CpepSfGFP
Ctl
3' UTR
3' UTR + OSBP-mCherry


hPro-CpepSfGFP
Intensity (AU) / Protein (mg)
200
150
100
50
0
0
1
2
3
4
Time (Hour)
Ctl
OSBP norm


CpepSfGFP
Intensity (AU) / Protein (mg)
200
150
100
50
0
0
1
2
3
4
Time (Hour)


Total Cell
Fluorescence
2.0x10^6
1.8
1.6
1.4
1.2
1.0
Fluorescence
505 510 515 520 525 530
Wavelength (nm)
Secretion
Fluorescence
300x10^6
250
200
150
100
50
Fluorescence
Control
ABCG1 KD
High
Glucose
Low
Glucose
505 510 515 520 525 530
Wavelength (nm)
hPro
Cpep
Lys
Cell
Secretion