**Supplemental Figure Legends**

**Fig. S1. Relative expression of SLC2 family members compared to matched normal breast tissues.** Volcano plots show the statistical significance of differences of relative expression of GLUT mRNA family members; GLUT8 mRNA is circled. Dashed lines show commonly used thresholds of statistical significance and fold change.

**Fig. S2. Base composition of GLUT8.** Base composition analysis of GLUT8 (NM_014580.4) was performed using MacVector version 17.0.5. Mononucleotide base pairing shown in plot using a base window size of 50.

**Fig. S3. GLUT8 exon expression is similar across a large cancer panel.** The relative frequency of each exon (1-10) of GLUT8 is shown for 33 different types of tumor, scaled for total frequency of GLUT8 mRNA (partner to Fig. 4F).

**Fig. S4. Demonstration of specificity of new rabbit anti-GLUT8 antibody, BBA1.** Confocal microscopy of GLUT8 expression in MB231 cells. *Top Row:* parental MB231 cells (low endogenous expression of GLUT8) were transduced lentivirus expressing shRNA-GLUT8 (sh122 or 180; described in Fig. 4A) or scramble (scr) shRNA and stained with BBA1 antibody 48 hours later. *Middle, bottom rows:* MB231 cells stably expressing exogenous C-terminal FLAG GLUT8 were transduced with lentivirus expressing shRNA-GLUT8 or scramble shRNA and stained with BBA1. The sequence of CF-GLUT8 includes silent mutations that make it non-degradable by sh180. Scale bars show 10μM.

**Fig. S5. Lack of co-localization of GLUT8 with markers of early endosomes, peroxisomes and endoplasmic reticulum.** C-terminal FLAG GLUT8 protein was visualized in MB231 cells (using anti-FLAG), together with the early endosomal marker EEA1, peroxisomal marker PMP70 or endoplasmic reticulum maker PDI.

**Fig. S6. C-terminal FLAG v3 construct does not produce a FLAG tagged C-terminal peptide. A.** *Assay of mRNA expression*. qRT-PCR assay of RNA for MB231 cells expressing the constructs indicated. RT-qPCR assay was designed to be selective for v1 and v3 (as described for Fig.4C). **B**. *Assay of protein expression*. Western blot of lysates from the same cells showing that GLUT8-v3 constructs generate neither full-length nor detectable cleaved products. C. The protein and nucleotide sequences of the C-terminal and N-terminal FLAG sites of GLUT8.

**Fig. S7. Evaluation of fidelity of diffraction of red and green light (test of chromatic aberration).** C-terminal FLAG GLUT8 protein in MB231 cells was visualized after staining with primary anti-FLAG primary antibody and a mixture of anti-mouse secondary antibodies conjugated with $Alexa^{488}$ (green) or $Alexa^{546}$ (red).

**Fig. S8. Immunostaining of FLAG suggests masking of C-terminal FLAG epitope.** MB231 cells expressing either C-terminal or N-terminal FLAG tagged GLUT8 were stained with anti-FLAG, together with EEA1 (**A**) or PDI (**B**). Compare stain with the Western blotting data of Fig. 6A, which shows that N-FLAG and C-FLAG proteins are present in approximately equal amounts.

**Fig. S9. Increased GLUT8 does not protect TXNIP from degradation in starved cells re-fed amino acids.** MB231 cells expressing C-terminal-FLAG tagged GLUT8 were amino acid-starved overnight, and re-fed with single amino acids (I, isoleucine; L, leucine; V, valine) or all three (ILV) for 60 minutes (each added to final concentration of 0.8mM); cells were lysed, analyzed by Western blotting, and 15 μgs of lysate probed with the antibodies indicated.

**Table S1. Evaluation of specificity of commercial antibodies to GLUT8.** BBA1 (in house rabbit polyclonal anti-peptide antibody) passed the quality control assays, defined as loss of signal after knockdown of GLUT8 and gain of signal upon over-expression. The following commercial antibodies were assayed by the same criteria and did not pass:

| **Antibody**<br>**Cat#**<br>**Source** | **Species**<br>**Type** | **Epitope** | **Method tested**<br>**Western blotting (WB)**<br>**Immunofluorescent stain (IF)** |
|---|---|---|---|
| Anti-GLUT8 [EPR9477]<br>#Ab169779<br>**Abcam** | Rabbit<br>Monoclonal | Not disclosed | WB: 42kDa band is prominent.<br>Failed to show knockdown by shRNA<br>IF: ND |
| Anti-GLUT8 C-terminus<br>#07-1407<br>**Millipore** | Rabbit<br>Polyclonal | 11 amino acids near the C-terminus | WB: Not compatible<br>IF: Nuclear staining pattern, not decreased by shRNA expression |
| Anti-GLUT8<br>#bs-4241R<br>**Bioss** | Rabbit<br>Polyclonal | Immunogen:<br>AAs 278-292 | WB: 52, 45, 25kDa bands are prominent. Failed to show knockdown by shRNA.<br>Does not detect overexpression of FLAG tagged GLUT8.<br>IF: Nuclear staining pattern, not sensitive to shRNA expression.<br>No change in signal with overexpression |
| Anti-GLUT8<br>#Ab99132<br>**Abcam** | Rabbi<br>Polyclonal | Peptide derived from range AAs 323-372 | WB: 70kDa band is prominent. Not sensitive to KD by shRNA. Does not detect overexpression of FLAG tagged GLUT8<br>IF: ND |
| Anti-GLUT8<br>#Ab191269,<br>**Abcam** | Rabbit<br>Polyclonal | Synthetic peptide: Amino Acids 461-477 | WB: Does not detect overexpressed FLAG tagged GLUT8 or endogenous band<br>IF: ND |

**Supplemental Methods**

*Primer sequences for qPCR*

| | Primer sequence (5'-3') | | |
|---|---|---|---|
| Gene name | Forward | Reverse | NCBI Ref Seq Id |
| HPRT | CCTCATGGACTGATTATGGACAG | AATCCAGCAGGTCAGCAAAG | NM_000194 |
| YWHAZ | AAG ACA GCA CGC TAA TAA TGC | TTG GAA GGC CGG TTA ATT TTC | NM_003406 |
| GLUT1 | TTG CAG GCT TCT CCA ACT GGA C | ACG AAC CAG GAG CAC AGT GAA G | NM_006516 |
| GLUT8 all | ACG AAC CAG GAG CAC AGT GAA G | GAT CTC TGA CAT GAG GAG CCA G | NM_014580, NM_001271712 |
| GLUT8 variant 1 | GTT CGG GGC TGT CGT GAC | AGC ATC CAC ACG TCC TGG | NM_014580, NM_001271711 |
| GLUT8 variant 3 | TGG CGG CAG GTC TAC AT | TGC CGA CGA CGA CCA TTA | NM_001271712 |

Fig. S1

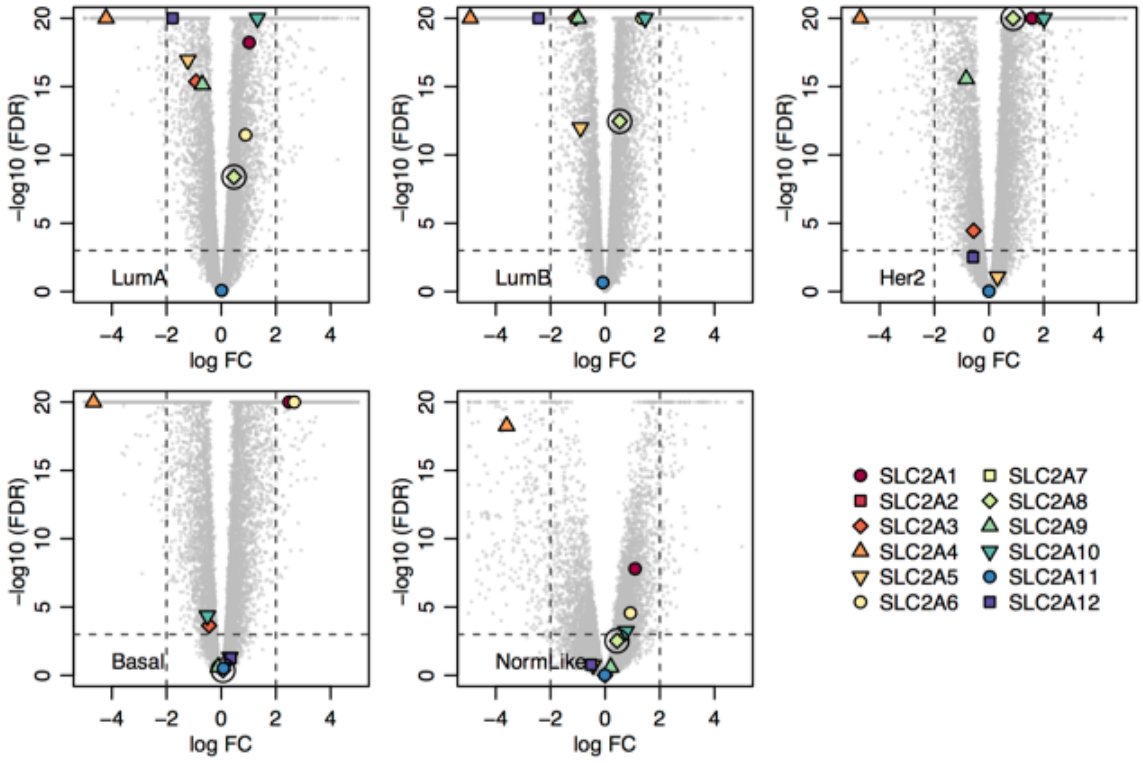

LumA
LumB
Her2
Basal
NormLike
−log10 (FDR)
log FC
SLC2A1
SLC2A2
SLC2A3
SLC2A4
SLC2A5
SLC2A6
SLC2A7
SLC2A8
SLC2A9
SLC2A10
SLC2A11
SLC2A12

Fig. S2

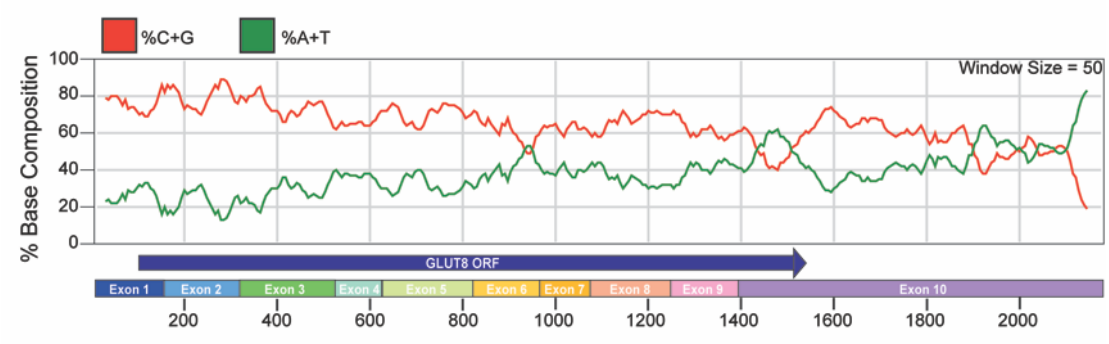

%C+G
%A+T
% Base Composition
100
80
60
40
20
0
Window Size = 50
GLUT8 ORF
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
200
400
600
800
1000
1200
1400
1600
1800
2000

Fig. S3

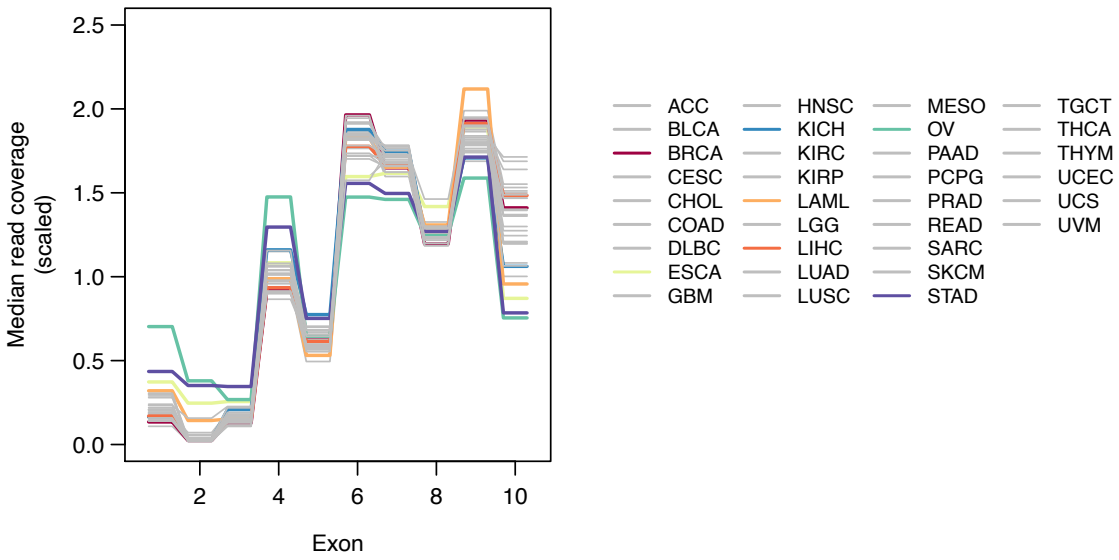

Median read coverage (scaled)
2.5
2.0
1.5
1.0
0.5
0.0
2
4
6
8
10
Exon
ACC
BLCA
BRCA
CESC
CHOL
COAD
DLBC
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LAML
LGG
LIHC
LUAD
LUSC
MESO
OV
PAAD
PCPG
PRAD
READ
SARC
SKCM
STAD
TGCT
THCA
THYM
UCEC
UCS
UVM

Fig. S4

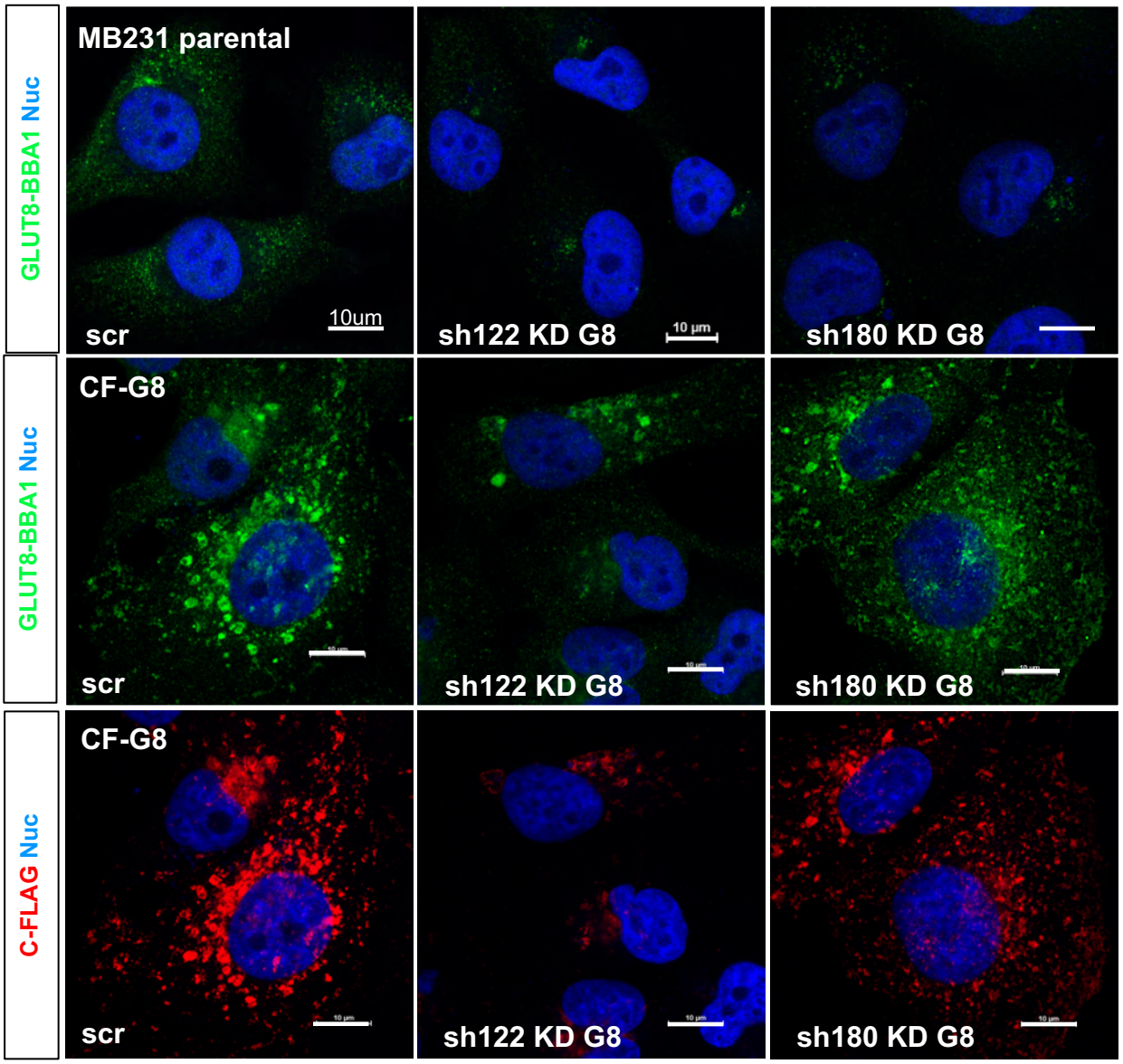

GLUT8-BBA1 Nuc
MB231 parental
scr
10um
sh122 KD G8
10 µm
sh180 KD G8
GLUT8-BBA1 Nuc
CF-G8
scr
sh122 KD G8
sh180 KD G8
C-FLAG Nuc
CF-G8
scr
sh122 KD G8
sh180 KD G8

Fig. S5

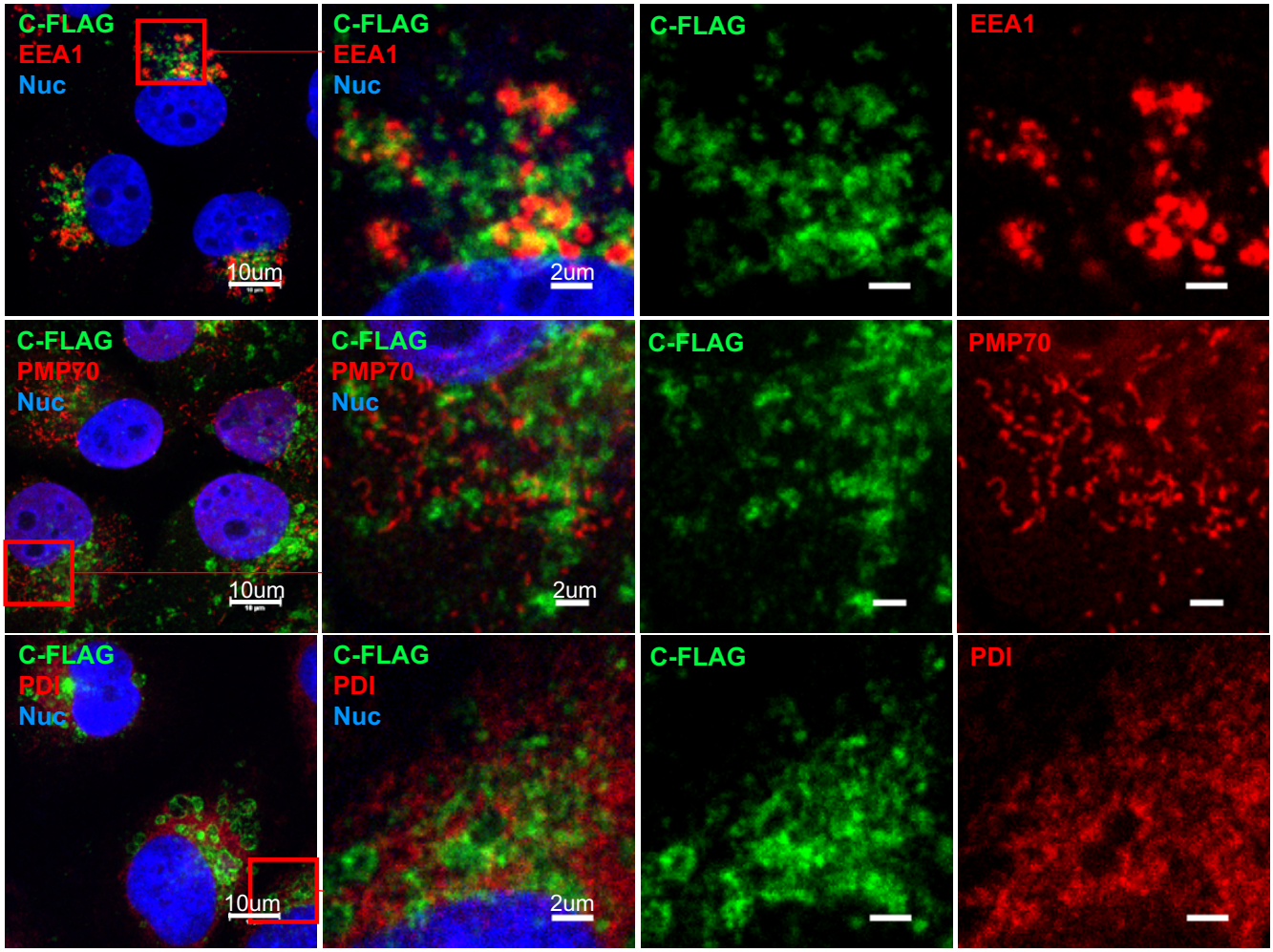
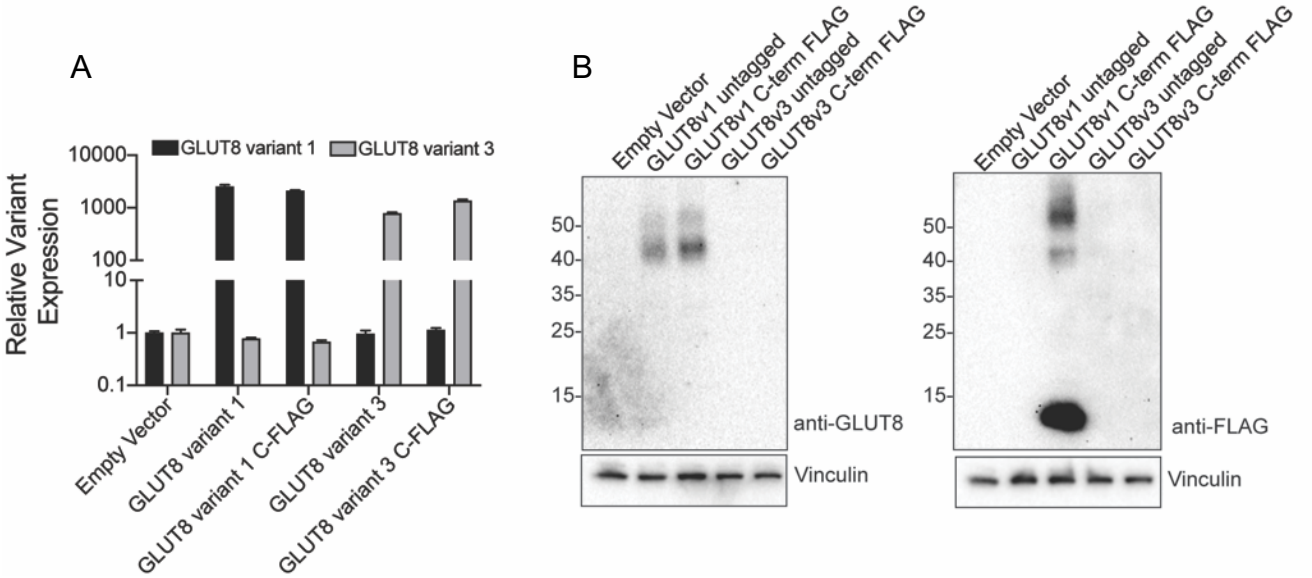

C-FLAG
EEA1
Nuc
10um
C-FLAG
EEA1
Nuc
2um
C-FLAG
EEA1
C-FLAG
PMP70
Nuc
10um
C-FLAG
PMP70
Nuc
2um
C-FLAG
PMP70
C-FLAG
PDI
Nuc
10um
C-FLAG
PDI
Nuc
2um
C-FLAG
PDI

Fig. S6




C

N-term FLAG Sequence

M D Y K D D D D K T P E

ATG GAT TAT AAA GAT GAT GAT GAT AAA ACG CCC GAC

FLAG epitope

C-term FLAG Sequence

E G R D Y K D D D D K * * *

GAG GGG CGA GAT TAT AAA GAT GAT GAT GAT AAA TGA

FLAG epitope

Fig. S7

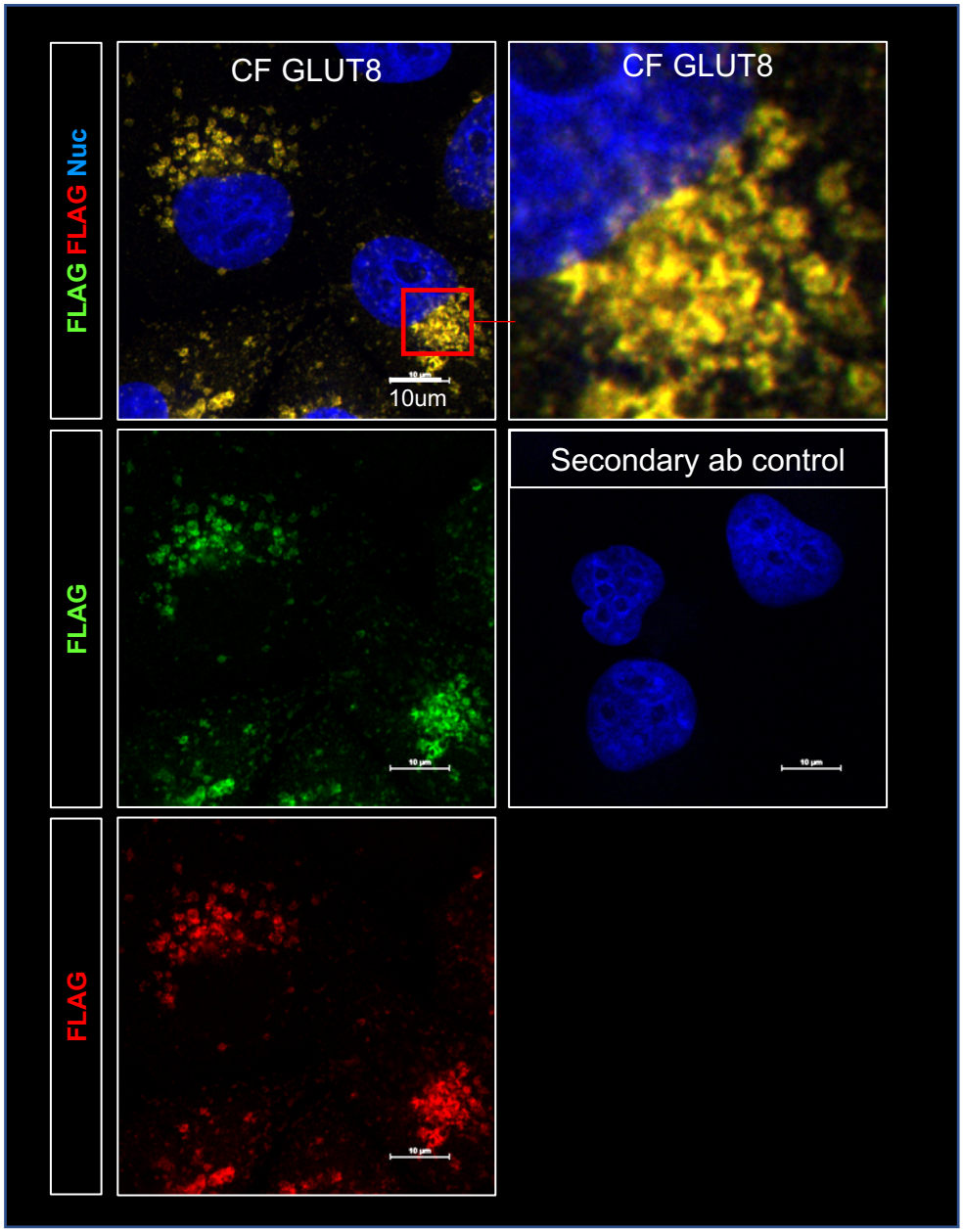

FLAG FLAG Nuc
CF GLUT8
10um
CF GLUT8
FLAG
Secondary ab control
FLAG

Fig. S8

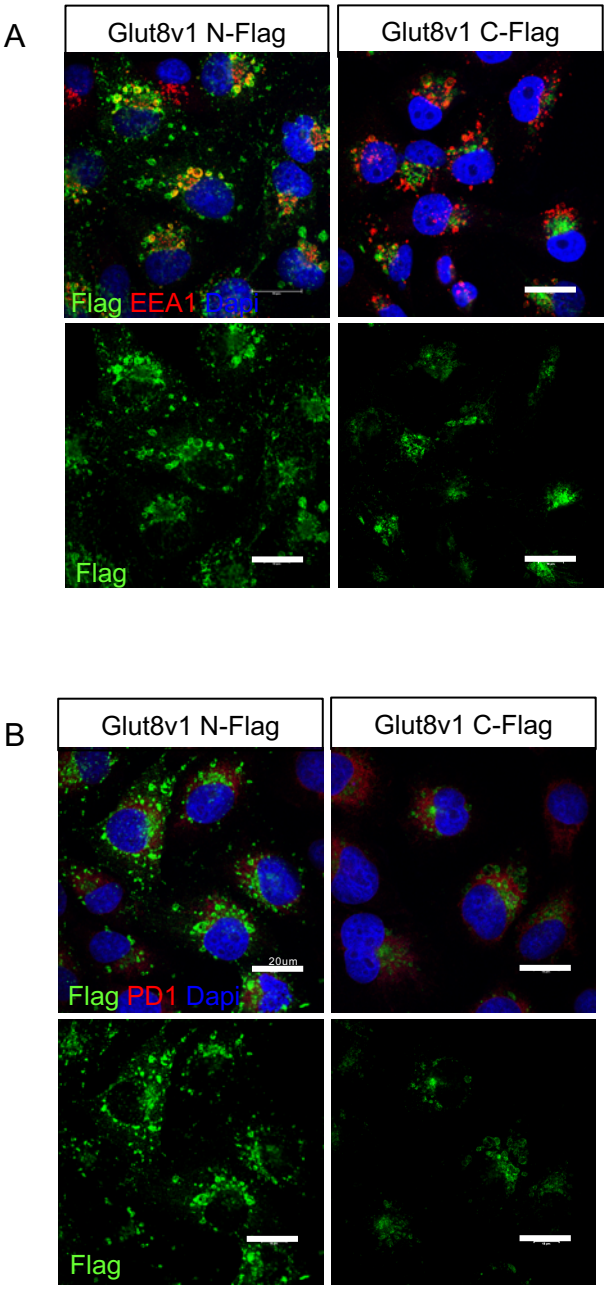
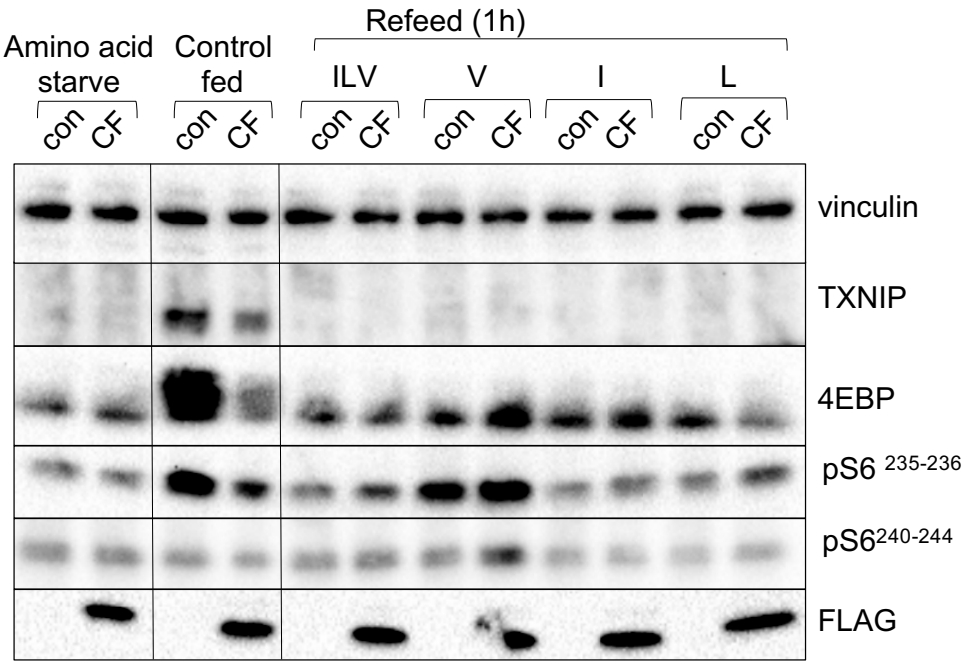

A
Glut8v1 N-Flag
Glut8v1 C-Flag
Flag EEA1 Dapi
Flag
B
Glut8v1 N-Flag
Glut8v1 C-Flag
20um
Flag PD1 Dapi
Flag

Fig. S9


Amino acid starve
Control fed
Refeed (1h)
ILV
V
I
L
con
CF
vinculin
TXNIP
4EBP
pS6 235-236
pS6240-244
FLAG