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Supplemental information

Lysosomal trafficking of the glucose transporter GLUT1 requires sequential regulation by TXNIP and ubiquitin

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Figure S1

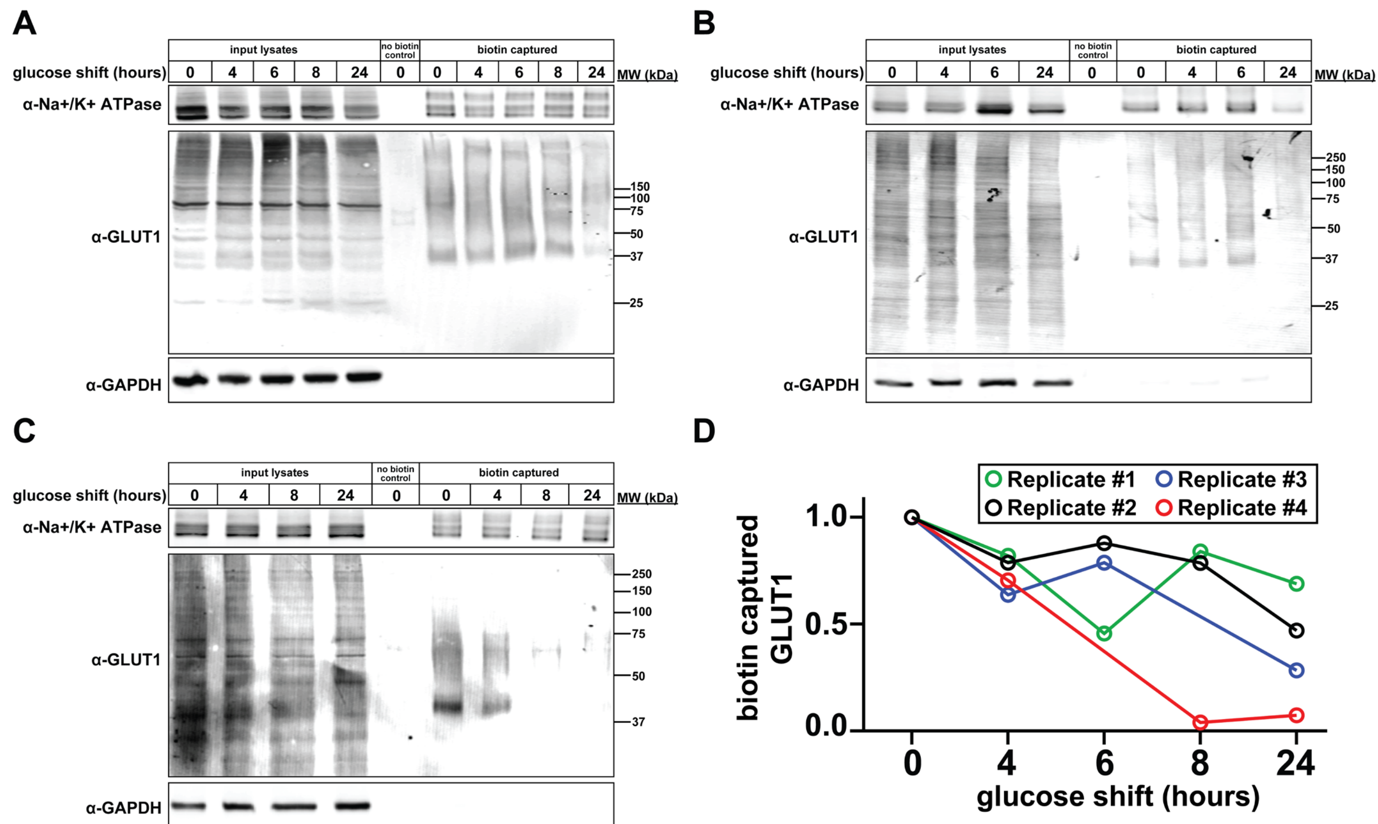


Figure S2

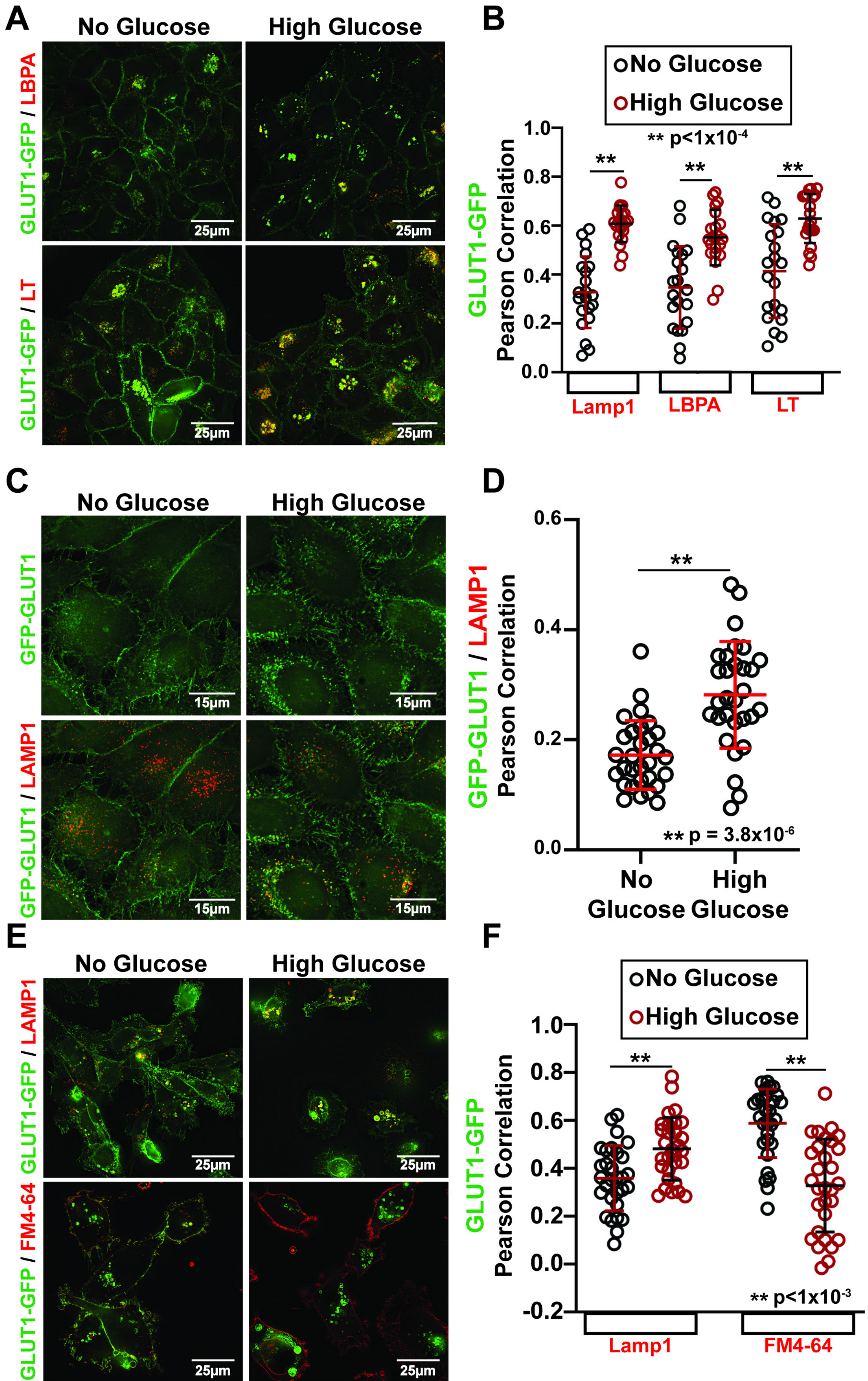


Figure S3

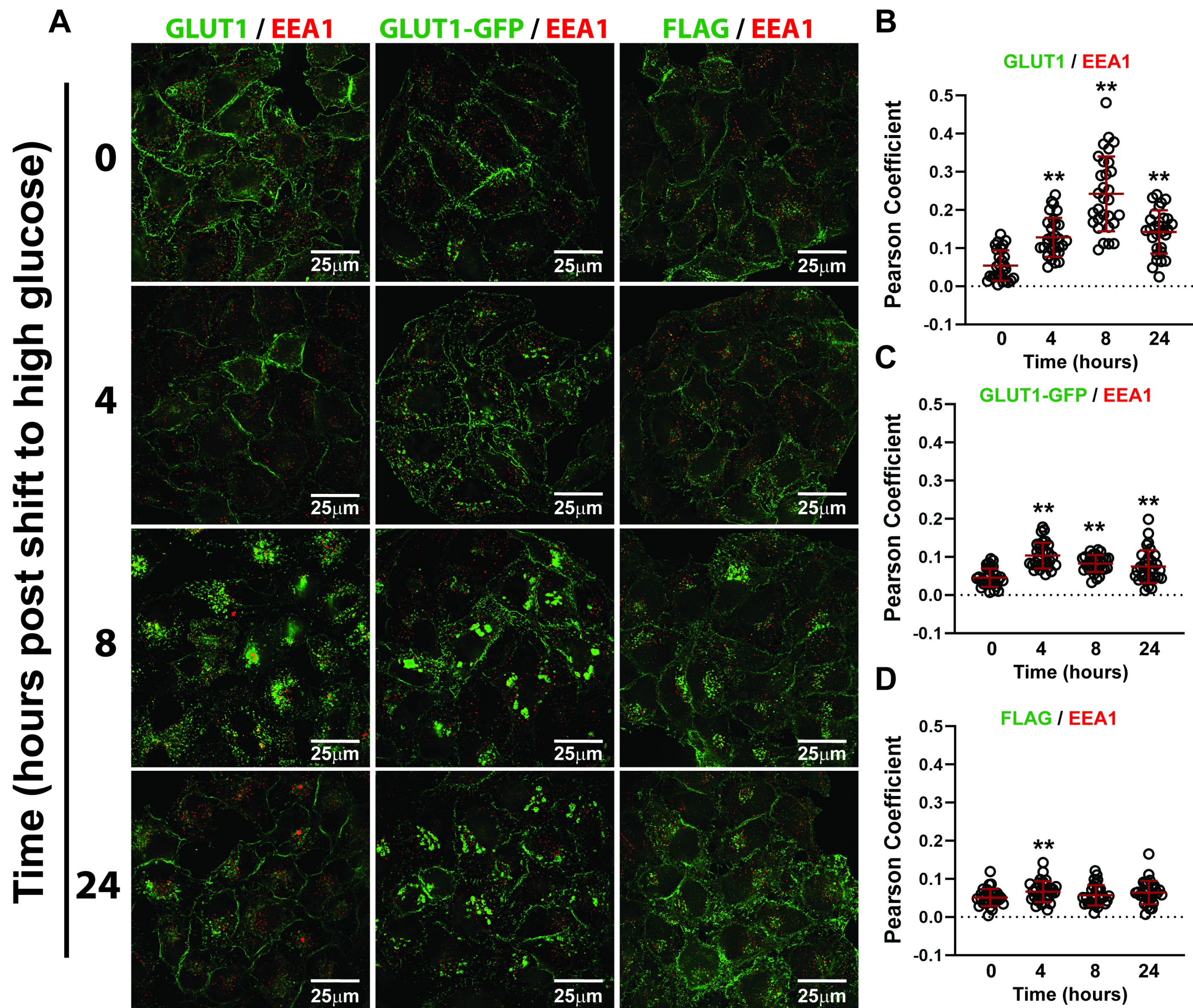


Figure S4

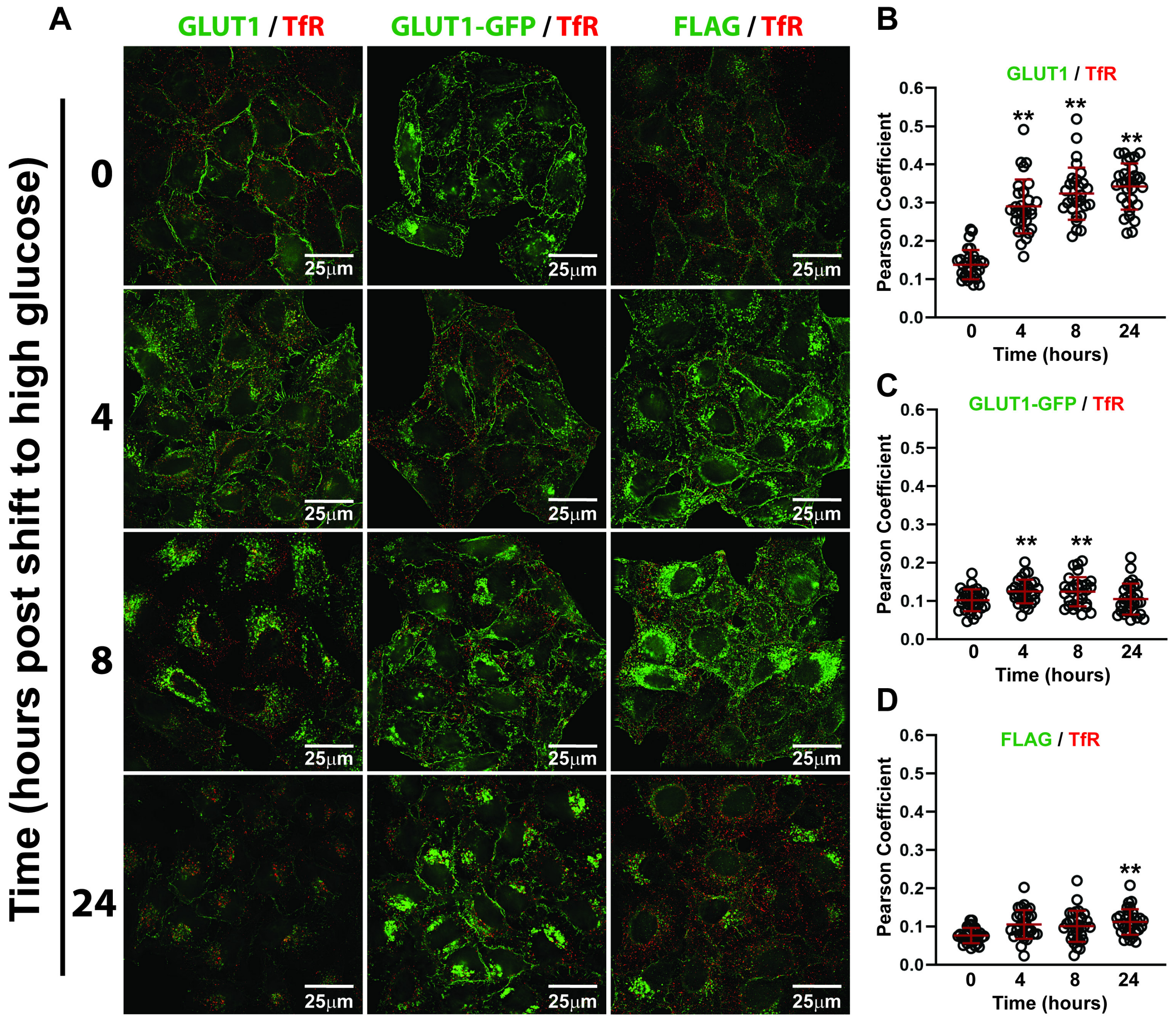
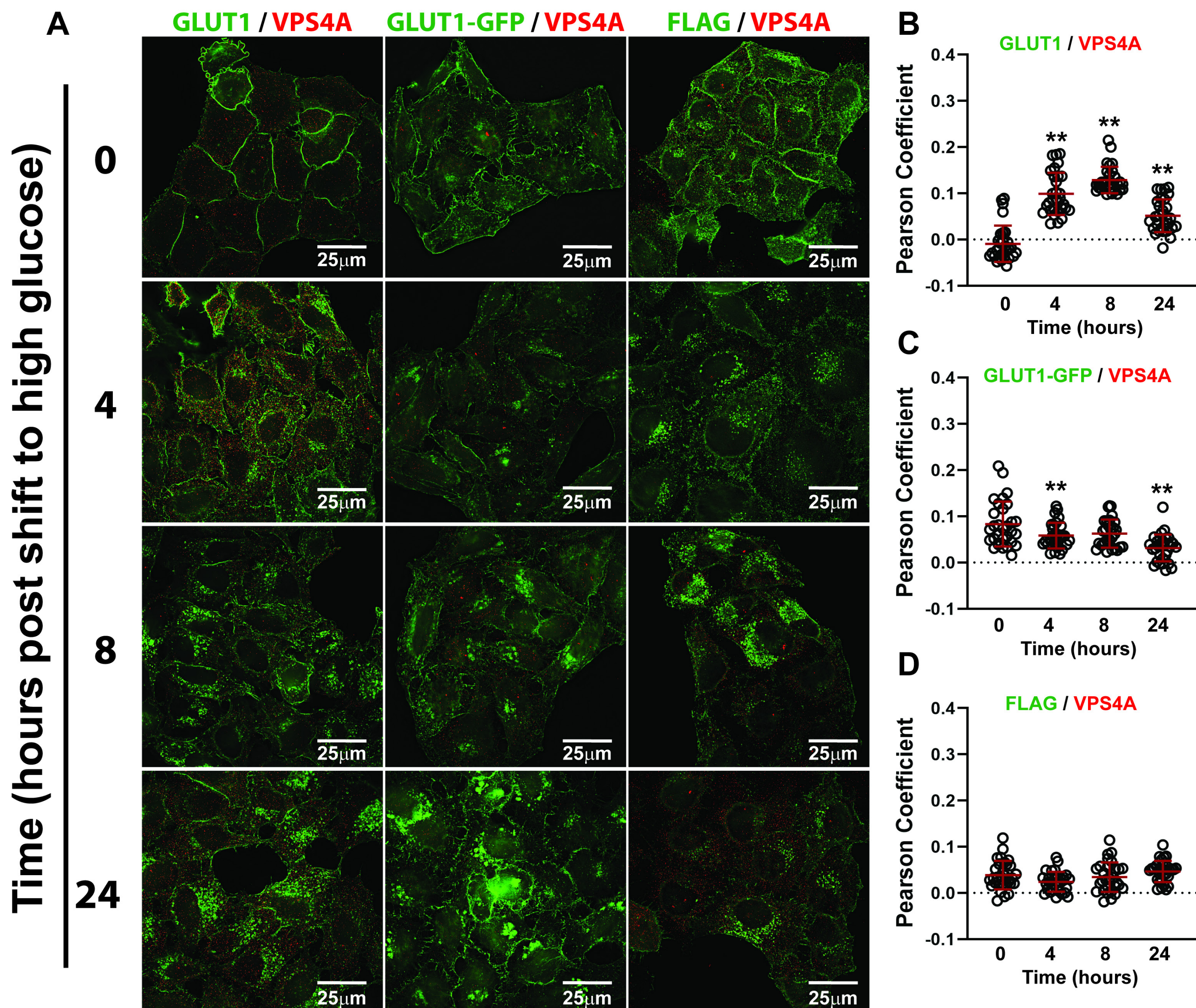


Figure S5



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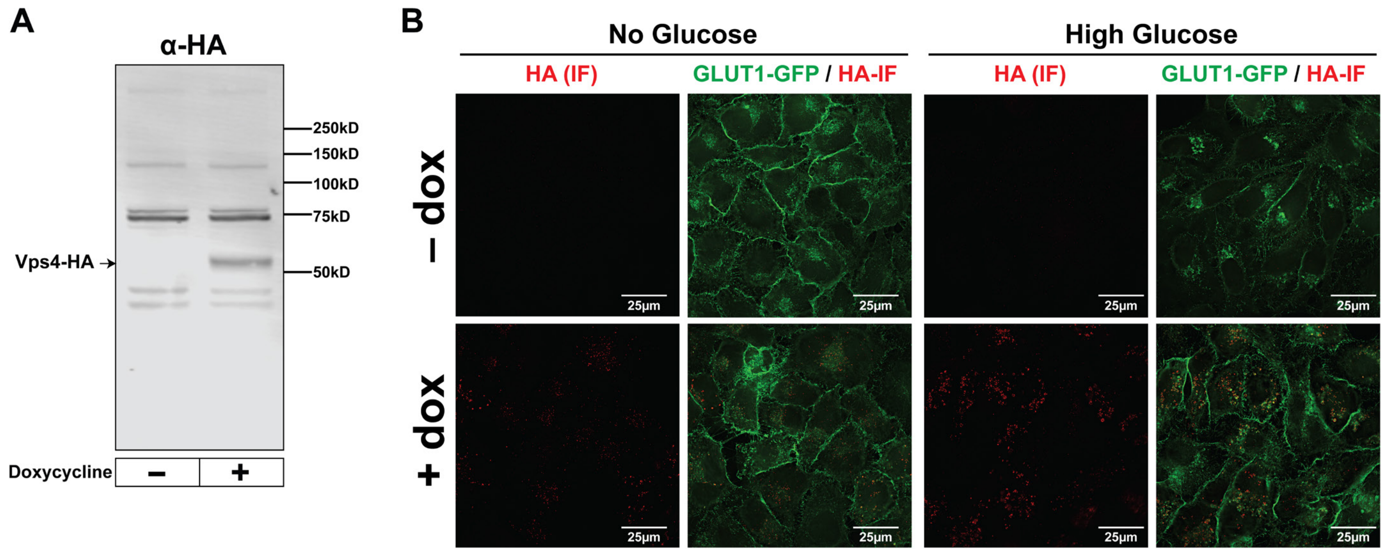
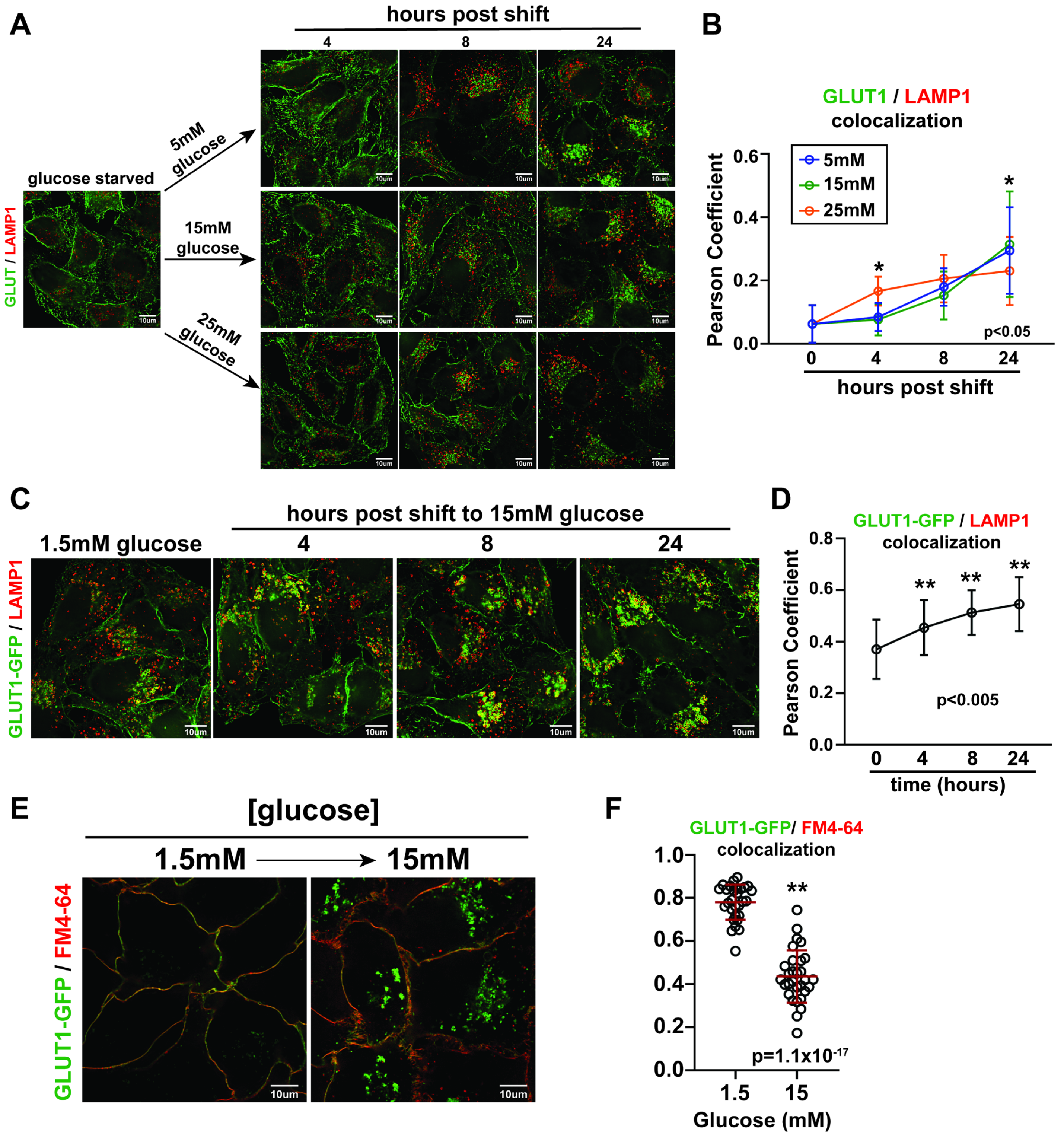


Figure S7



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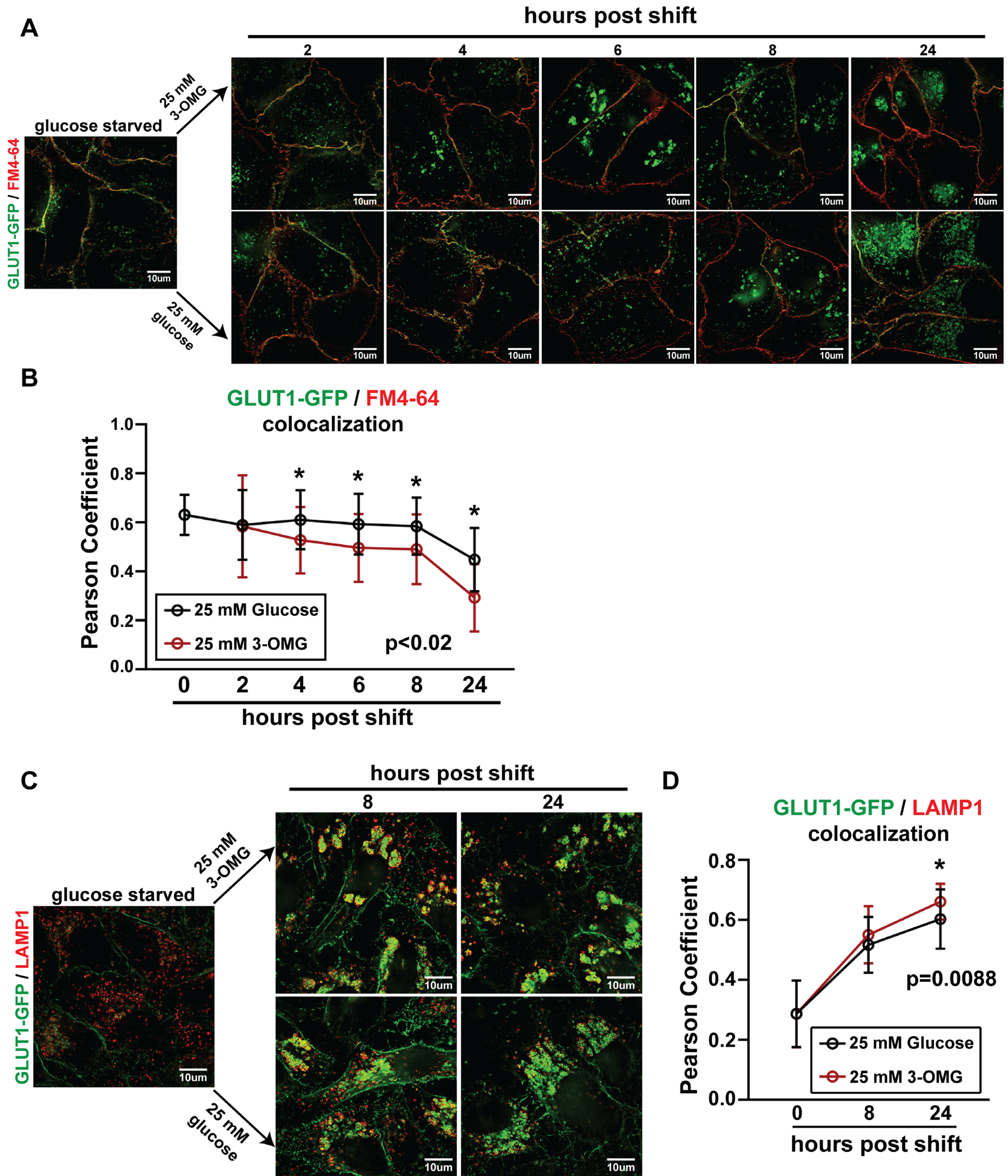

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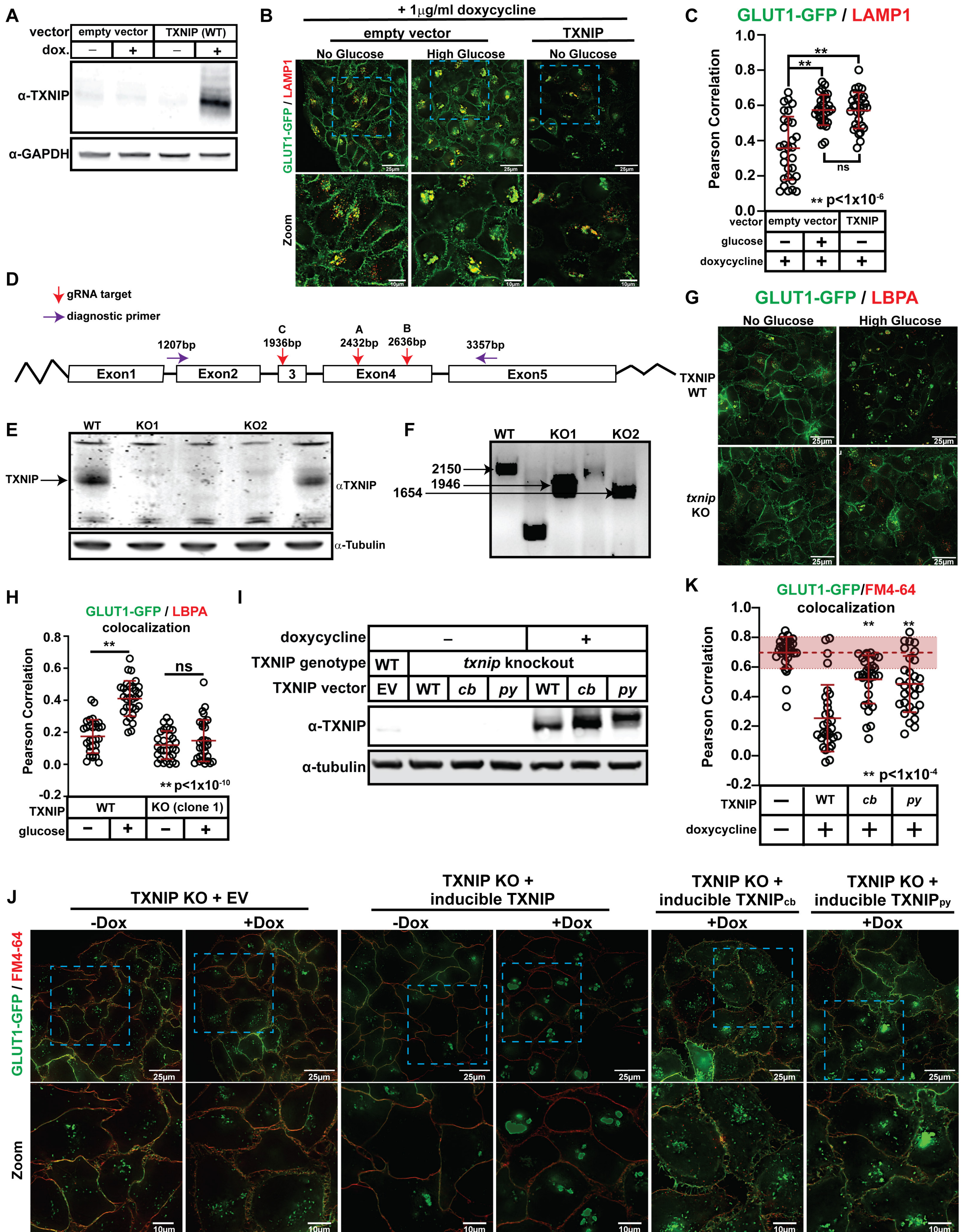

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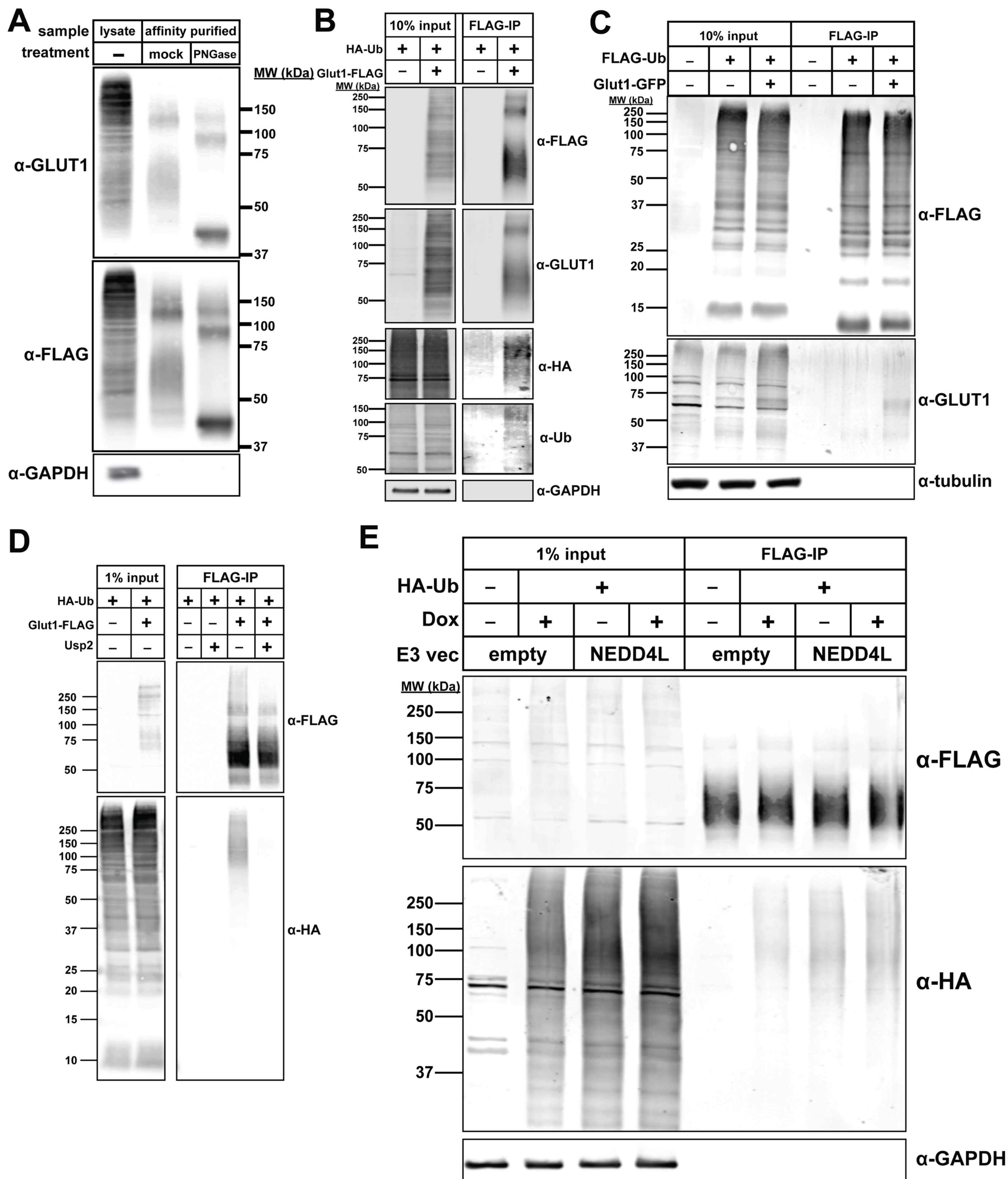

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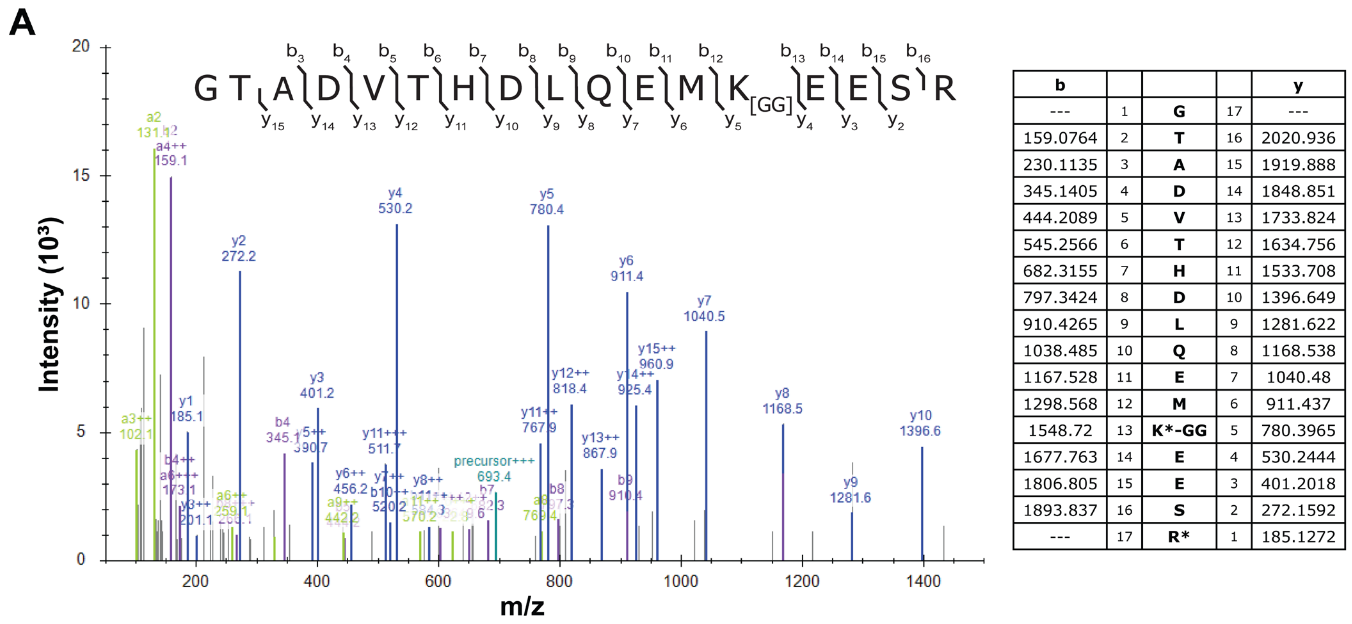

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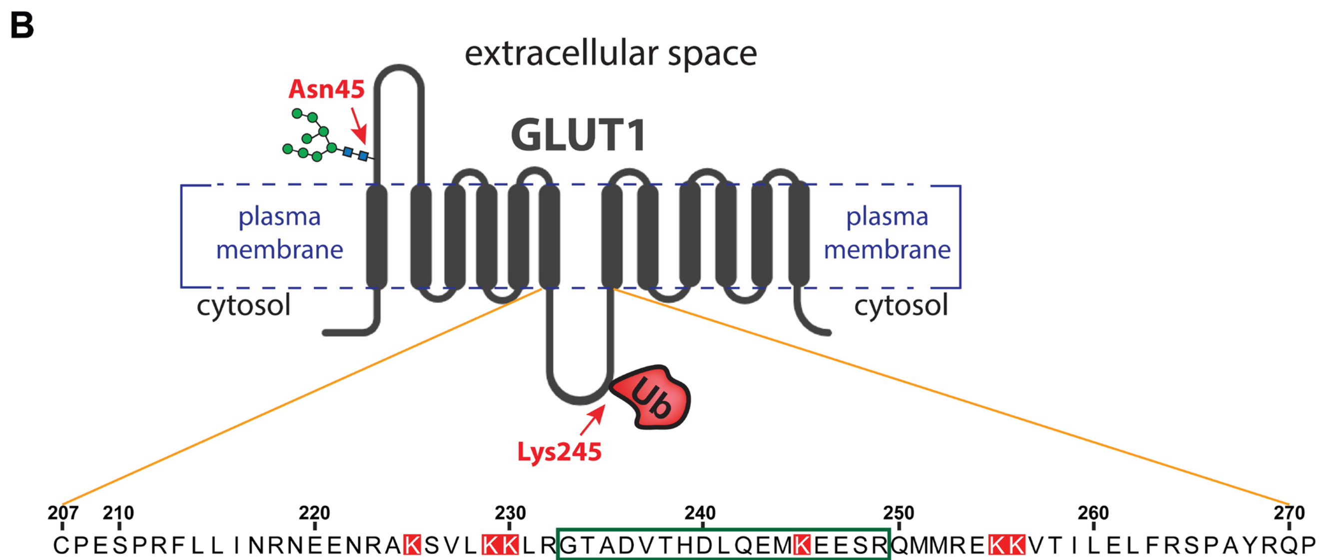


Figure S12

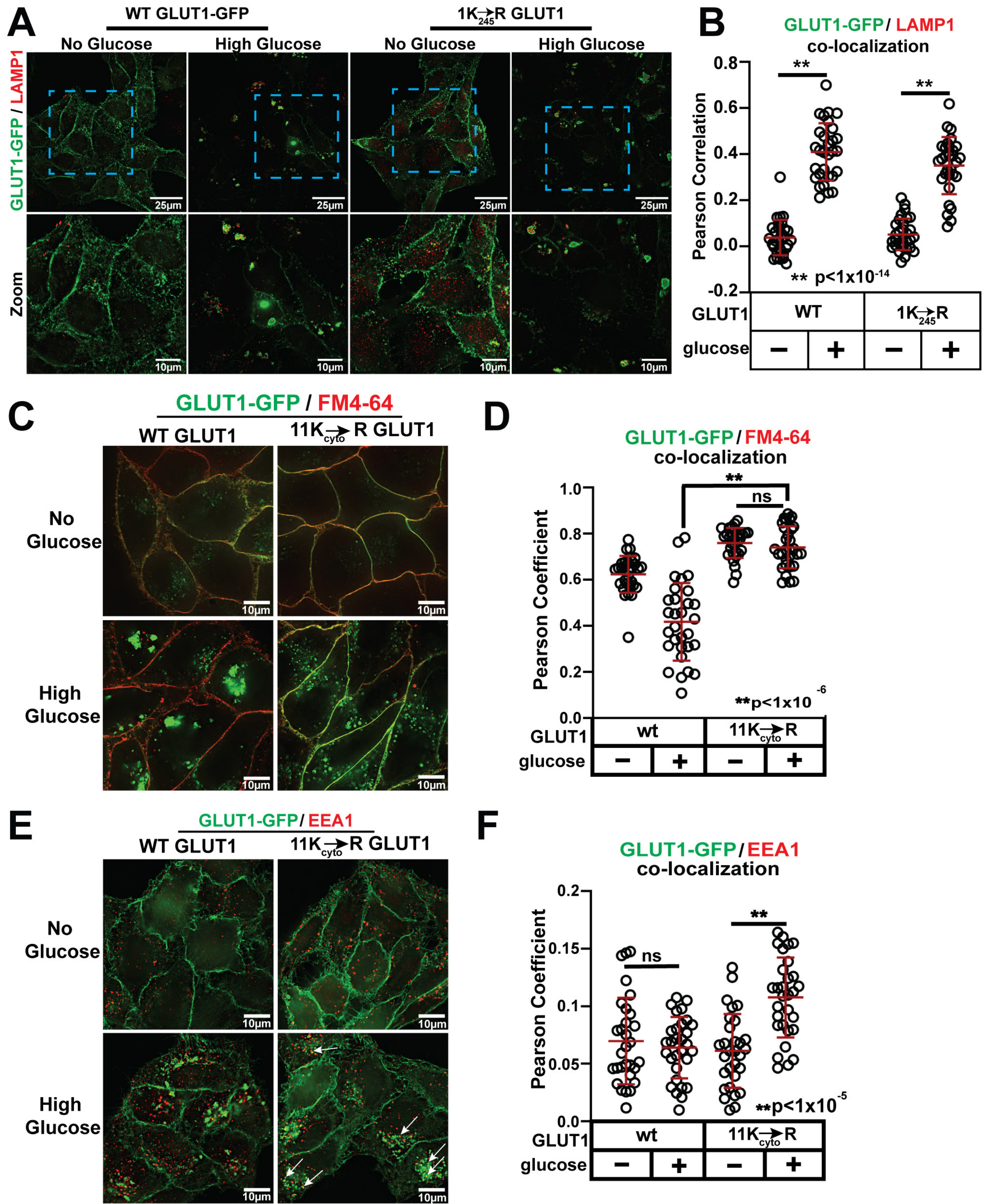
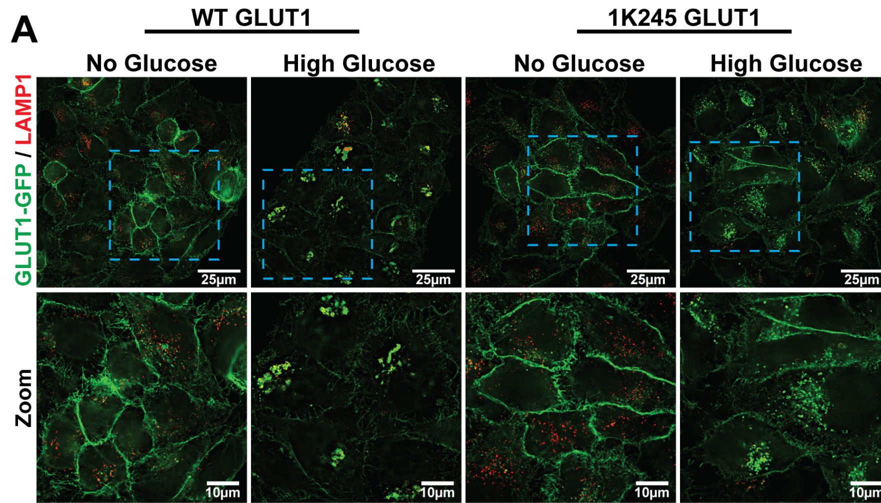
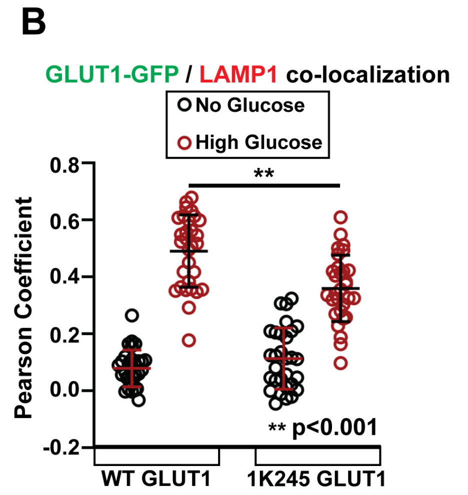
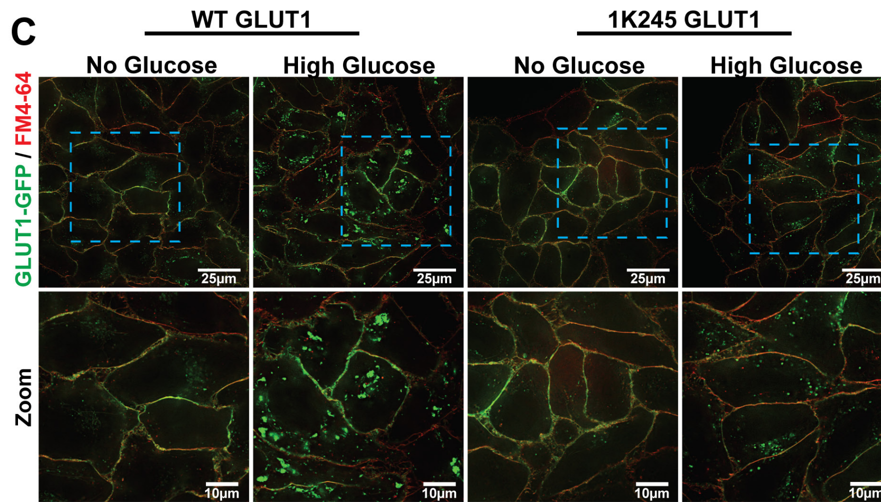


Figure S13







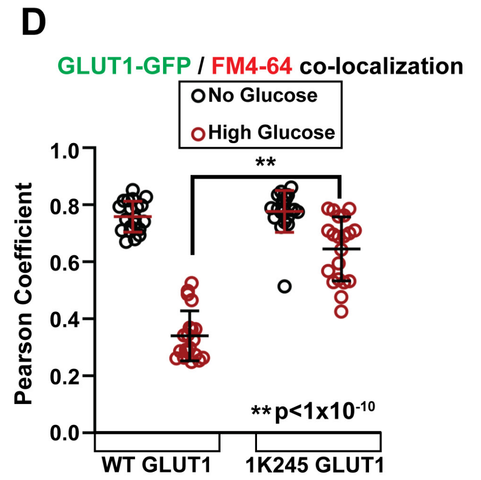
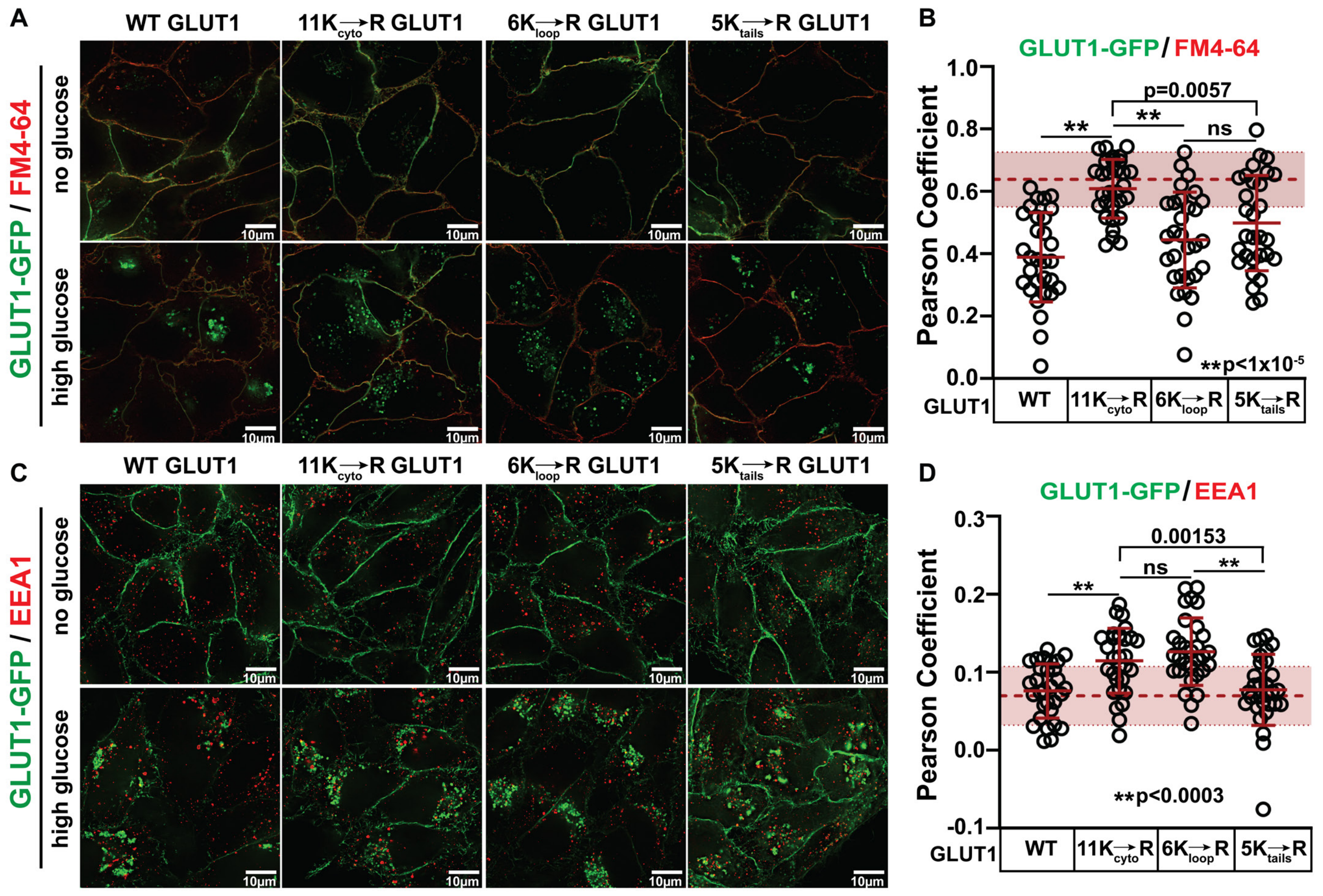


Figure S14



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Additional evidence for glucose-stimulated clearance of GLUT1 from the plasma membrane using surface biotinylation assays, Related to Figure 1. **(A-C)** Biological replicates of the experiment shown in FIG 1A. Quantification of these replicate experiments are depicted in **FIG 1B** and **FIG 1C**. **(D)** Line graph representation of the results summarized in **FIG 1B**, showing the time course quantification of each biological replicate. Measurements were taken for the whole lane using FIJI.

Figure S2. Additional evidence for glucose-stimulated clearance of GLUT1 from the plasma membrane using fluorescence microscopy assays, Related to Figure 2. **(A)** HeLa cells stably expressing GLUT1-GFP were cultured using the conditions indicated in **FIG 2** prior to labeling with LysoTracker (LT; bottom row, red) or fixation and imaging for immunofluorescence detection of LBPA (top row, red). **(B)** Quantification of the results shown in panel (A) was done by measuring the Pearson coefficient of correlation for at least 21 cells ($n \geq 21$) with each condition indicated. **(C)** HeLa cells stably expressing N-terminally tagged GFP-GLUT1 were cultured using conditions indicated in **FIG 2** prior to fixation and imaging for immunofluorescence detection of Lamp1 (red). **(D)** Quantification of the results shown in panel (C) was done by measuring the Pearson coefficient of correlation for 30 cells (n=30) for each condition indicated. **(E)** MDA-MB-231 cells stably expressing GLUT1-GFP were cultured using the conditions indicated in **FIG 2** prior to pulse-labeling with FM4-64 (red, bottom row) and live cell imaging, or fixation followed by immunofluorescence detection of LAMP1 (red, top row). **(F)** Quantification of the results shown in panel (E) was performed by measuring Pearson coefficient of correlation of 30 cells (n=30) for each condition indicated. All p-values measured using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by **. Data are represented as mean +/- SEM. All measurements of Pearson coefficient of correlation were performed using Softworx software.

Figure S3: Analysis of GLUT1 localization to EEA1-positive early endosomal compartments following glucose-stimulated endocytosis, Related to Figure 3. **(A)** GLUT1 co-localization with the early endosomal marker EEA1 (red) during a glucose stimulation time course was analyzed by fluorescence microscopy. In the left column, endogenous GLUT1 in HeLa cells was detected by immunofluorescence (green). In the middle column, HeLa cells stably expressing GLUT1-GFP were analyzed. In the right column, HeLa cells stably expressing GLUT1 harboring an exofacial FLAG tag (green) were analyzed. These different approaches for detecting and imaging GLUT1 are summarized in **FIG 2**. **(B,C,D)** Quantification of the Pearson coefficients of correlation for the experiments represented in (A). Pearson measurements were made using Softworx software for 30 cells (n=30) per condition shown. For all experiments p-values were computed using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by ** and refers to a significant difference from the T=0 condition. Data are represented as mean +/- SEM.

Figure S4: Analysis of GLUT1 localization to transferrin receptor (TfR)-positive endosomal compartments following glucose-stimulated endocytosis, Related to Figure 3. **(A)** GLUT1 co-localization with the endosomal marker TfR (red) during a glucose stimulation time course was analyzed by fluorescence microscopy. In the left column, endogenous GLUT1 in HeLa cells was detected by immunofluorescence (green). In the middle column, HeLa cells stably expressing GLUT1-GFP were analyzed. In the right column, HeLa cells stably expressing GLUT1 harboring an exofacial FLAG tag (green) were analyzed. These different approaches for detecting and imaging GLUT1 are summarized in **FIG 2**. **(B,C,D)** Quantification of the Pearson coefficients of correlation for the experiments represented in (A). Pearson measurements were made using Softworx software for 30 cells (n=30) per condition shown. For all experiments p-values were computed using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by ** and refers to a significant difference from the T=0 condition. Data are represented as mean +/- SEM.

Figure S5: Analysis of GLUT1 localization to VPS4A-positive late endosomal compartments following glucose-stimulated endocytosis, Related to Figure 3. **(A)** GLUT1 co-localization with the late endosomal marker VPS4A (red) during a glucose stimulation time course was analyzed by fluorescence microscopy. In the left column, endogenous GLUT1 in HeLa cells was detected by immunofluorescence (green). In the middle column, HeLa

cells stably expressing GLUT1-GFP were analyzed. In the right column, HeLa cells stably expressing GLUT1 harboring an exofacial FLAG tag (green) were analyzed. These different approaches for detecting and imaging GLUT1 are summarized in **FIG 2**. **(B,C,D)** Quantification of the Pearson coefficients of correlation for the experiments represented in (A). Pearson measurements were made using Softworx software for 30 cells (n=30) per condition shown. For all experiments p-values were computed using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by ** and refers to a significant difference from the T=0 condition. Data are represented as mean +/- SEM.

Figure S6: Validation of cell lines harboring a doxycycline-inducible dominant negative variant of Vps4, Related to Figure 3. **(A)** Immunoblot analysis of lysates prepared from HeLa cells stably transfected with an inducible expression vector for $Vps4^{E228Q}$-HA**. (B)** Fluorescence microscopy images of cells expressing GLUT1-GFP and harboring doxycycline-inducible $VPS4^{E228Q}$-HA. Cells with and without prior doxycycline induction were fixed and immunostained for HA.

Figure S7: GLUT1 trafficking in physiological glucose concentrations, Related to Figure 3. **A)** HeLa cells were cultured for 24 hours in media with no glucose, then switched to either 5mM, 15mM, or 25mM glucose media. Cells were fixed at the indicated time points for immunofluorescence detection with GLUT1 (green) and Lamp1 (red). **B)** Quantification of the experiments in (A). Measurements were done using the JACoP (BIOP version) FIJI plugin. Pearson correlation coefficient was measured for 30 cells under each condition (n=30). * indicates a statistically significant difference in the Pearson's coefficient between 5mM and 25mM glucose conditions. **C)** HeLa cells stably expressing GLUT1-GFP were cultured in 1.5mM glucose media, then switched to 15mM glucose media. The cells were fixed at the indicated times for immunofluorescence detection with Lamp1 (red). **D)** Quantification of the experiments in (C). Measurements were done using the JACoP (BIOP version) FIJI plugin. Pearson correlation coefficient was measured for 30 cells under each condition (n=30). ** indicates a statistically significant difference in Pearson's coefficient between the indicated time point and T=0. **E)** HeLa cells stably expressing GLUT1-GFP were cultured in 1.5mM glucose media then switched to 15mM glucose media for 24 hours. Prior to imaging, cells were placed on ice and switched to cold HBSS buffer containing 8μM FM4-64 in order to label the plasma membrane. Cells were imaged before FM4-64 internalized. **F)** Quantification of the experiments in (E). Measurements were done using the JACoP (BIOP version) FIJI plugin. Pearson correlation coefficient was measured for 30 cells under each condition (n=30). For all experiments, p-values were computed using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by ** unless otherwise indicated. Data are represented as mean +/- SEM.

Figure S8: GLUT1 is trafficked from the plasma membrane in response to substrate transport, Related to Figure 3. **A)** HeLa cells stably expressing GLUT1-GFP were cultured for 24 hours in media with no glucose, then switched to either 25mM glucose or 25mM 3-OMG. Prior to imaging, cells were placed on ice and switched to cold HBSS buffer containing 8μM FM4-64 in order to label the plasma membrane. **B)** Quantification of the experiments in (A). Measurements were done using the JACoP (BIOP version) FIJI plugin. Pearson coefficient of correlation was measured for 30 cells under each condition (n=30). * indicates a statistically significant difference in Pearson's coefficient between the glucose and 3-OMG condition at the indicated time point. **C)** HeLa cells stably expressing GLUT1-GFP were cultured in media without glucose for 24 hours then switched to either 25mM glucose or 25mM 3-OMG media. Cells were fixed at the indicated time points for immunofluorescence detection with Lamp1 (red). **D)** Quantification of the experiments in (C). Measurements were done using the JACoP (BIOP version) plugin. * indicates a statistically significant difference in Pearson's coefficient between the glucose and 3-OMG conditions at the 24 hour time point. Pearson correlation coefficient was measured for 30 cells under each condition (n=30). For all experiments, p-values were computed using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant. Data are represented as mean +/- SEM.

Figure S9: Characterization of the role of *TXNIP* in glucose-stimulated GLUT1 trafficking to lysosomes, Related to Figure 4. **(A)** HeLa cells were stably transfected with either empty vector (pINDUCER20) or vector expressing TXNIP under the control of Tet-on gene expression system. Doxycycline was added to induce expression of TXNIP for 24 hours prior to collection of cell lysate. Cell lysates were resolved by SDS-PAGE

and analyzed by immunoblot. Immunoblotting of GAPDH was performed as a loading control. **(B)** HeLa cells stably expressing GLUT1-GFP and a doxycycline-induced expression vector were cultured using the conditions described in FIG 1D and, for the induced samples, additionally treated with 1μg/ml doxycycline for the last 24 hours before fixation. Cells were fixed and imaged for detection of LAMP1 (red), a marker of lysosomal compartments. Zoomed images provided in the bottom row correspond to the blue dashed-line inset boxes of the top row. **(C)** Quantification of the experiments represented in panel (B) was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition shown. **(D)** A strategy to generate *txnip* knockout cell lines using CRISPR/Cas9 and gRNAs targeting exon 3 and 4 was developed. Cells were nucleofected twice with the TXNIP CRISPR/Cas9 KO plasmids (Santa Cruz Biotechnology), which target the *txnip* gene in 3 separate locations (red arrows) for a double strand break by the Cas9 nuclease. After recovering from electroporation, cells were sorted into 96 well plates for screening by immunoblot and PCR. **(E)** Clonal cell lines were isolated and screened for TXNIP expression by immunoblot. Two isolated clonal lines that lack detectable TXNIP expression are shown (KO1 and KO2). α-Tubulin was used as a loading control in immunoblots. **(F)** Clonal cell lines lacking TXNIP protein expression by immunoblot were further characterized for deletions at the chromosomal TXNIP locus. gDNA was collected from the two clones and the region of interest was amplified (purple arrows) and run on an agarose gel to compare with a wildtype sample. The size of the amplified DNA indicates at which gRNA target the *txnip* gene had been cut. KO1 is missing the region between gRNAs A and B while KO2 is missing the region between gRNAs A and C. **(G)** HeLa cells and *txnip* knockout equivalents (clone 1) stably expressing GLUT1-GFP were cultured as indicated in Fig1D then fixed for immunofluorescence detection of LBPA (red), a marker of late endosomes. **(H)** Quantification of the experiments represented in panel (G) was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition shown. All p-values were measured using a two sample Student's t-Test in Microsoft Excel. All measurements of Pearson coefficient of correlation were performed using Softworx software. **(I)** HeLa cells stably expressing GLUT1-GFP were stably transfected with either empty vector (pINDUCER20) or vector expressing wildtype, clathrin binding mutant, or py motif mutant TXNIP under the control of Tet-on gene expression system. Doxycycline was added to induce expression of TXNIP for 24 hours prior to collection of cell lysate. Cell lysates were resolved by SDS-PAGE and analyzed by immunoblot. Immunoblotting of α-tubulin was performed as a loading control. **(J)** Complementation analysis of HeLa cells stably expressing GLUT1-GFP with the *txnip* gene knocked out via CRISPR/Cas9. The knockout cells were complemented with either an empty vector, wildtype TXNIP, a clathrin binding mutant, or a py motif mutant expressed in a doxycycline-induced vector. Cells were cultured in media containing doxycycline to induce expression of the indicated protein. Prior to imaging, cells were placed on ice and switched to cold (4°C) buffer containing the lipophilic tracer dye FM4-64 (8μM) (red) in order to label the plasma membrane. Live cells were imaged in cold buffer immediately to ensure retention of FM4-64 at the plasma membrane. Zoomed images provided in the bottom row correspond to the blue dashed-line inset boxes of the top row. **(K)** Quantification of the results shown in panel (J) was performed by measuring the Pearson coefficient of correlation of 30 cells (n=30) for each condition indicated. All p-values were measured using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by **. Data are represented as mean +/- SEM. In panel K, the significant difference applies for comparison with conditions of no TXNIP expression and WT TXNIP expression. All measurements of Pearson coefficient of correlation were performed using Softworx software. Dotted red line on graphs indicated the average Pearson coefficient of correlation for the no TXNIP, no doxycycline condition and the shaded red area indicates the standard deviation.

Figure S10: Characterization of GLUT1 ubiquitin modification, Related to Figure 5. **(A)** GLUT1-FLAG stably expressed in HeLa cells was affinity purified from cell lysates and either mock-treated or treated with PNGase F at 37°C for an hour. **(B)** HeLa cells stably expressing an empty vector or GLUT1-FLAG were transiently transfected with an HA-Ub expression vector and cultured to 100% confluency before cell lysates were collected. Lysates were incubated with αFLAG magnetic beads followed by elution using FLAG peptide. Samples were resolved by SDS-PAGE and analyzed by immunoblot. GAPDH was used as a loading control. **(C)** HEK293T cells stably expressing FLAG-Ub were transiently transfected with a GLUT1-GFP expression plasmid. When cells reached 100% confluency, lysates were collected and incubated with αFLAG magnetic

beads. FLAG-Ub was eluted using FLAG peptide. Samples were resolved by SDS-PAGE and analyzed by immunoblot. α-tubulin was used as a loading control. **(D)** HeLa cells stably expressing an empty vector or GLUT1-FLAG were transiently transfected with an HA-Ub expression vector and cultured to 100% confluency before cell lysates were collected. Lysates were incubated with α-FLAG magnetic beads followed by elution using FLAG peptide. Half of each eluate was incubated with Usp2 at 37°C for an hour. Samples were resolved by SDS-PAGE and analyzed by immunoblot. **(E)** HeLa cells stably expressing constitutive GLUT1-FLAG and Dox-inducible TXNIP vectors were transiently transfected with either empty vector, HA-Ub, and/or Nedd4L, as indicated in the figures. 24 hours after inducing TXNIP with 1μg/ml doxycyxline, cells were collected in lysis buffer and incubated with α-FLAG magnetic beads for 1 hour at 4°C with rotation. GLUT1-FLAG was eluted with FLAG peptide and samples were resolved by SDS-PAGE then analyzed by immunoblot. GAPDH was used as a loading control. Quantification of results is provided in **FIG 5I**.

Figure S11: Evidence of GLUT1 ubiquitin modification on its major cytosolic loop, Related to Figure 5. **(A)** Enrichment of peptides containing ubiquitin remnant modifications (di-Gly) from tryptic digestion of MDA-MB-231 cell lysates revealed ubiquitin modification of GLUT1 at Lys245. The MS2 spectra of the peptide is shown at the top and the predicted fragmentation ions for the peptide is shown in the table below (generated using MS-Product (UCSF)). **(B)** Schematic of GLUT1 in the plasma membrane, illustrating the portions facing the cytosol. The detected ubiquitin modification on the major cytosolic loop of GLUT1 is shown. Below, the sequence corresponding to the major cytosolic loop of human GLUT1 is shown. All lysine residues in the major cytosolic loop, which represent potential sites of ubiquitin modification, are highlighted in red. The green box illustrates the ubiquitin-modified peptide that was resolved in (A).

Figure S12: Characterization of the role of cytosol-facing lysine residues in glucose-stimulated GLUT1 trafficking to lysosomes, Related to Figure 6. **(A)** HeLa cells stably expressing either wildtype GLUT1-GFP or GLUT1-GFP with lysine 245 mutated to arginine ($1K_{245}\rightarrow R$) were cultured as indicated prior to fixation and imaging for immunofluorescence detection of LAMP1 (red), a marker of lysosomal compartments. Zoomed images provided in the bottom row correspond to the blue dashed-line inset boxes of the top row. **(B)** Quantification of the results shown in panel A was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition indicated. **(C)** HeLa cells stably expressing either wildtype GLUT1-GFP or GLUT1-GFP with all cytosolic lysines mutated to arginine ($11K_{cyto}\rightarrow R$ GLUT1) were cultured as indicated. Prior to imaging, cells were placed on ice and switched to cold (4°C) buffer containing the lipophilic tracer dye FM4-64 (8μM) (red) in order to label the plasma membrane. Live cells were imaged in cold buffer immediately to ensure retention of FM4-64 at the plasma membrane. **(D)** Quantification of the results shown in panel C was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition indicated. **(E)** HeLa cells stably expressing either wildtype GLUT1-GFP or GLUT1-GFP with all cytosolic lysines mutated to arginine ($11K_{cyto}\rightarrow R$) were cultured as indicated prior to fixation and imaging for immunofluorescence detection of EEA1 (red), a marker of early endosomal compartments. **(F)** Quantification of the results shown in panel E was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition indicated. All p-values were measured using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by **. Data are represented as mean +/- SEM. All measurements of Pearson coefficient of correlation were performed using Softworx software.

Figure S13: A GLUT1 variant harboring a single cytosolic lysine (K245) only partially restores glucose-stimulated endocytic trafficking, Related to Figure 6. **(A)** HeLa cells stably expressing either wildtype GLUT1-GFP or GLUT1-GFP with all cytosolic lysines mutated to arginine except K245 (1K245) were cultured as indicated prior to fixation and imaging for immunofluorescence detection of LAMP1 (red), a marker of lysosomal compartments. **(B)** Quantification of the results shown in panel A was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition indicated. **(C)** HeLa cells stably expressing either wildtype GLUT1-GFP or GLUT1-GFP (green) with all cytosolic lysines mutated to arginine except K245 (1K245) were cultured as indicated. Prior to imaging, cells were placed on ice and switched to cold (4°C) buffer containing the lipophilic tracer dye FM4-64 (8μM) (red) in order to label the plasma membrane. Live cells were imaged in cold buffer immediately to ensure retention of FM4-64 at the plasma

membrane. **(D)** Quantification of the results shown in panel C was performed by measuring the Pearson coefficient of correlation for 20 cells (n=20) with each condition indicated. All p-values were measured using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by **. Data are represented as mean +/- SEM. All measurements of Pearson coefficient of correlation were performed using Softworx software. Zoomed images provided in the bottom row correspond to the blue dashed-line inset boxes of the top row.

Figure S14: Characterization of the role of cytosol-facing lysine residues in glucose-stimulated GLUT1 trafficking, Related to Figure 6. **(A)** HeLa cells stably expressing either wildtype GLUT1-GFP, GLUT1-GFP with all cytosolic lysines mutated to arginine ($11K_{cyto} \rightarrow R$), GLUT1-GFP with the 6 lysines on the major cytosolic loop mutated to arginine ($6K_{loop} \rightarrow R$), or GLUT1-GFP with the 5 cytosolic lysines outside of the major loop mutated to arginine ($5K_{tails} \rightarrow R$) were cultured as in Fig1D. Prior to imaging, cells were placed on ice and switched to cold (4°C) buffer containing the lipophilic tracer dye FM4-64 (8μM) (red) in order to label the plasma membrane. Live cells were imaged in cold buffer immediately to ensure retention of FM4-64 at the plasma membrane. **(B)** Quantification of the results shown in panel (C) was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition indicated. The red dashed line indicates the average Pearson's coefficient for WT GLUT1-GFP with FM4-64 in "no glucose" media. The shaded area represents the standard deviation. **(C)** HeLa cells stably expressing either wildtype GLUT1-GFP, GLUT1-GFP with all cytosolic lysines mutated to arginine ($11K_{cyto} \rightarrow R$), GLUT1-GFP with the 6 lysines on the major cytosolic loop mutated to arginine ($6K_{loop} \rightarrow R$), or GLUT1-GFP with the 5 cytosolic lysines outside of the major loop mutated to arginine ($5K_{tails} \rightarrow R$) were cultured as in Fig1D prior to fixation and imaging for immunofluorescence detection of EEA1 (red). **(D)** Quantification of the results shown in panel (C) was performed by measuring the Pearson coefficient of correlation for 30 cells (n=30) with each condition indicated. The red dashed line indicates the average Pearson's coefficient for WT GLUT1-GFP with EEA1 in "no glucose" media. All p-values were measured using a two sample Student's t-Test in Microsoft Excel. A P value < 0.05 was considered statistically significant and is indicated by **. Data are represented as mean +/- SEM. All measurements of Pearson coefficient of correlation were performed using Softworx software.

Table S1. Designations for mutant variants of GLUT1, TXNIP, and WWP1 used in this study, Related to STAR Methods.

Designation	Protein	Variant	Description
WT	GLUT1	Wildtype, isoform 1 (canonical)	wildtype
$1K_{245} \rightarrow R$	GLUT1	K245R	K245R mutation
$11K_{cyto} \rightarrow R$	GLUT1	K6R, K7R, K225R, K229R, K230R, K245R, K255R, K256R, K451R, K456R, K477R	All cytosolic-facing Lys residues mutated to Arg
$6K_{loop} \rightarrow R$	GLUT1	K225R, K229R, K230R, K245R, K255R, K256R	All cytosolic loop Lys residues mutated to Arg
$5K_{tails} \rightarrow R$	GLUT1	K6R, K7R, K451R, K456R, K477R	All N- and C-terminal tail Lys residues mutated to Arg
1K245	GLUT1	K6R, K7R, K225R, K229R, K230R, K255R, K256R, K451R, K456R, K477R	Single Lys variant: K245
WT	TXNIP	Wildtype, isoform 1 (canonical)	wildtype
cb	TXNIP	L351A, L352A	Clathrin binding domain mutated
py	TXNIP	$PPCY_{334} \rightarrow PACA_{334}$ and $PPTY_{378} \rightarrow PATA_{378}$	Two C-terminal PY motifs mutated
WT	WWP1	Wildtype, isoform 1 (canonical)	wildtype
4ww	WWP1	W377F, P380A, W409F, P412A, W484F, P487A, F524A, P527A	All four WW domains mutated

Table S2. Plasmids used in this study, Related to STAR Methods.

Designation	Backbone	Description	Source
pQCXIP	--	Retroviral vector for constitutive ORF expression in mammalian cells with puromycin resistance gene	ClonTech
pQCXIN	--	Retroviral vector for constitutive ORF expression in mammalian cells with neomycin resistance gene	ClonTech
pENTR1A	--	Gateway entry vector	Addgene
pInducer20	--	Gateway destination vector.Tet-inducible lentiviral vector for ORF expression in mammalian cells	Addgene
pRK5-HA	--	Mammalian expression plasmid	Addgene
WT TXNIP	pInducer20	Inducible WT TXNIP	This study
$TXNIP_{cb}$	pInducer20	Inducible TXNIP clathrin-binding mutant	This study
$TXNIP_{py}$	pInducer20	Inducible TXNIP py motif mutant	This study
WT TXNIP-FLAG	pQCXIN	WT TXNIP with C-terminal 3xFLAG tag	This study
$TXNIP_{py}$-FLAG	pQCXIN	TXNIP py motif mutant with C-terminal 3xFLAG tag	This study
WT GLUT1-GFP	pQCXIP	WT GLUT1 with a C-terminal GFP tag	This study
WT GLUT1-FLAG	pQCXIP	WT GLUT1 with a C-terminal 3xFLAG tag	This study
WT FLAG-GLUT1	pQCXIP	WT GLUT1 with a FLAG tag on the first exofacial loop	This study
$1K_{245}$→R GLUT1-GFP	pQCXIP	$1K_{245}$→R GLUT1 mutant with C-terminal GFP tag	This study
$11K_{cyto}$→R GLUT1-GFP	pQCXIP	$11K_{cyto}$→R GLUT1 mutant with C-terminal GFP tag	This study
1K245 GLUT1-GFP	pQCXIP	1K245 GLUT1 mutant with C-terminal GFP tag	This study
$6K_{loop}$→R GLUT1-GFP	pQCXIP	$6K_{loop}$→R GLUT1 mutant with C-terminal GFP tag	This study
$5K_{tails}$→R GLUT1-GFP	pQCXIP	$5K_{tails}$→R GLUT1 mutant with C-terminal GFP tag	This study
HA-Ub	pRK5-HA	Ubiquitin with an N-terminal HA tag	Addgene
WT FLAG-WWP1	pQCXIP	WT WWP1 with an N-terminal 3xFLAG tag	Nielsen, C.P. et. al.
4ww FLAG-WWP1	pQCXIP	WWP1 with all 4 WW domains mutated, with a N-terminal 3xFLAG tag	Nielsen, C.P. et. al.
WT WWP1	pQCXIN	WT untagged WWP1	This study
WT Nedd4L	pQCXIN	WT untagged Nedd4L	This study
$Vps4A^{E228Q}$-HA	pInducer20	3xHA-VPS4A with E228Q DN mutation in dox-inducible vector	This study