Supplemental Figures.

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Movie S1: Glut1 Trafficking In Response to Acute Glucose Starvation In Autophagy Competent Cells.

Movie S2: Glut1 Trafficking In Response to Acute Glucose Starvation In Autophagy Deficient Cells.

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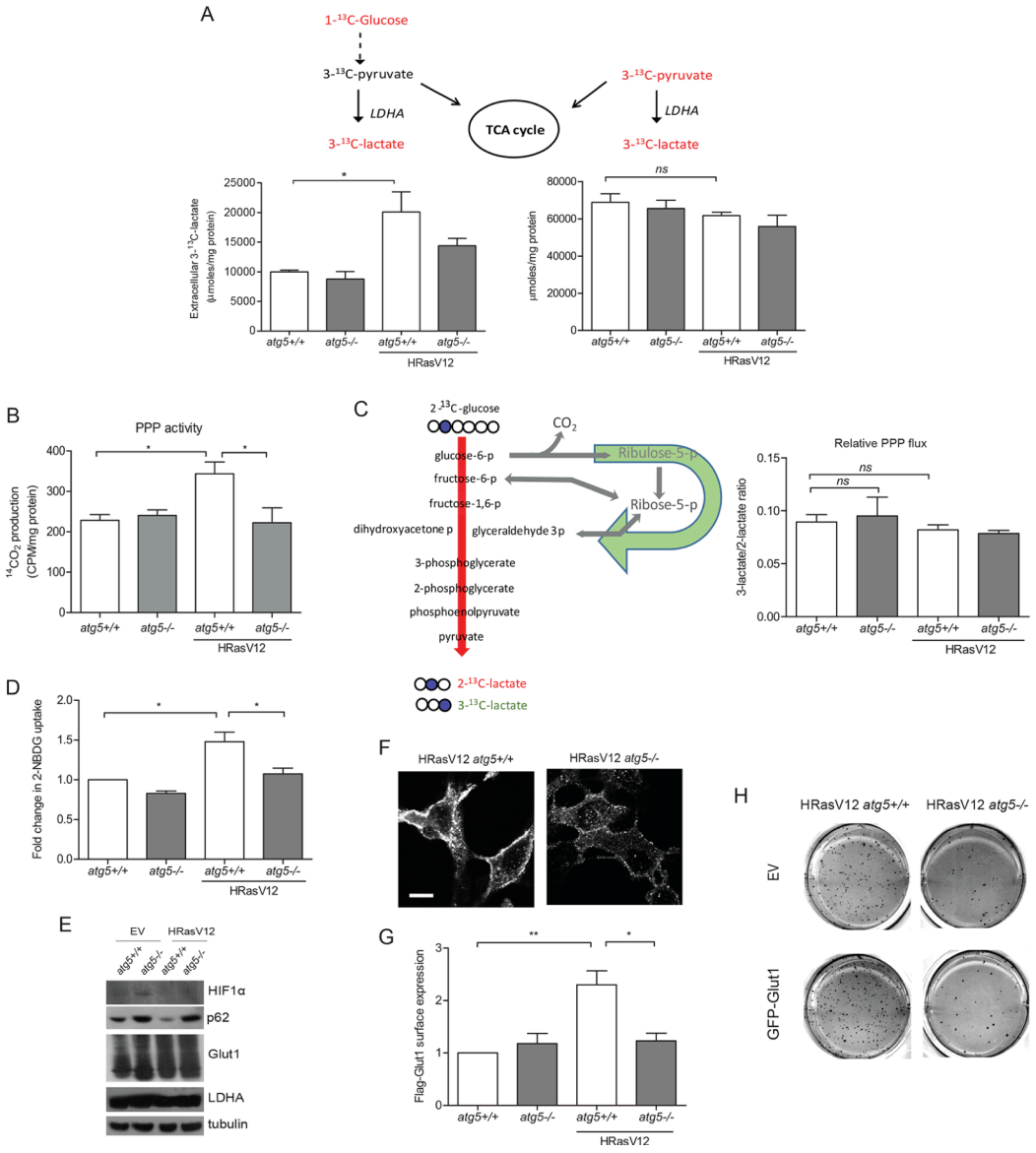

Figure S1: Autophagy facilitates glucose uptake and lactate production in HRasV12 transformed cells by promoting Glut1 cell surface expression (Related to Figure 1). (A) Indicated cell types cultured in media containing 1-^{13}C-glucose (left panel) or 3-^{13}C-pyruvate (right panel) for 24h; extracellular 3-^{13}C-lactate was measured by NMR spectroscopy (mean ± SEM, n=3 independent experiments). Similar results for 1-^{13}C-glucose derived extracellular 3-^{13}C-lactate (left panel) were also reported in Lock et al. 2011. (B) Oxidative pentose phosphate pathway (PPP) activity was assayed by measuring $^{14}CO_2$ formation from 1-^{14}C-glucose (mean ± SEM, n=4 independent experiments). (C) Left panel: Schematic of the flux of 2-^{13}C-glucose through the glycolytic and oxidative PPP, resulting in the generation of 2-^{13}C-lactate and 3-^{13}C-lactate respectively. Right panel: 2-^{13}C-lactate and 3-^{13}C-lactate levels were measured by NMR spectroscopy and the ratio of these metabolites was calculated to determine relative PPP flux (mean ± SEM, n=3 independent experiments). (D) MEFs labeled with 2-NBDG (100 µM) for 3h to measure glucose uptake (mean ± SEM, n=3 independent experiments). (E) The indicated cell types were immunoblotted for HIF1alpha, p62, Glut1 and LDHA. (F) HRasv12 transformed *atg5+/+* and *atg5-/-* cells immunostained with α-Glut1. Scale bar, 10µm. (G) Cells transfected with Flag-Glut1 and the surface expression of Flag-Glut1 was FACS quantified (mean ± SEM, n=3 independent experiments). (H) Soft agar colony formation of the indicated cell types. P-values calculated using ANOVA followed by Tukey's HSD test. ***P*<0.01, **P*<0.05; *ns*, not significant.

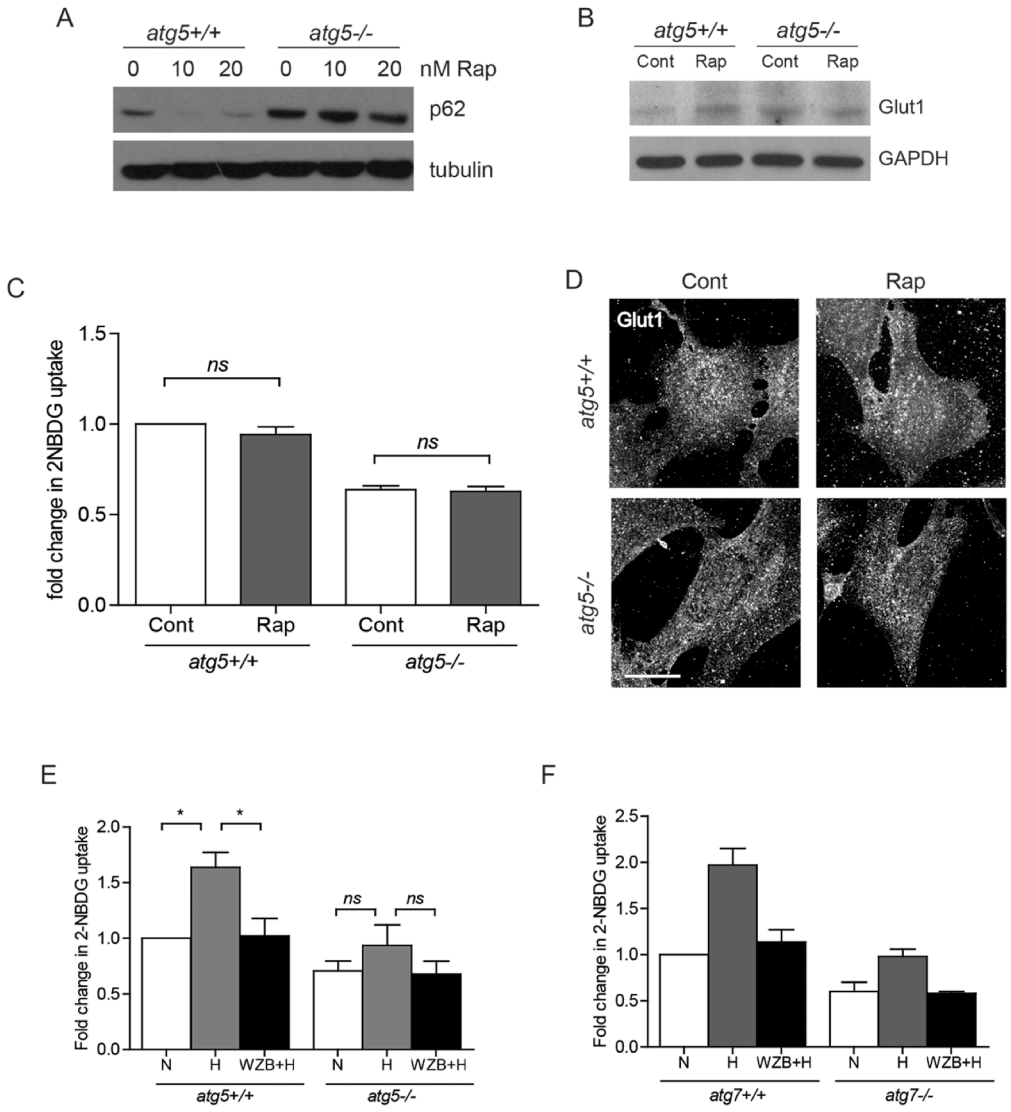

Figure S2: Rapamycin treatment does not impact glucose uptake and Glut1 localization (Related to Figure 1). (A-B) Cells were treated with indicated doses of Rap for 24h and lysates were immunoblotted for (A) p62 or (B) Glut1. (C) MEFs were treated with 20nM dose of Rapamycin (Rap) for 24h and then labeled with 2-NBDG (100 μM) for 3h to measure glucose uptake (mean ± SEM, n=3). (D) Cells were treated with Rap (20nM) for 24h, fixed in acetone at 4°C for 15 mins and immunostained with α-Glut1. (E and F) Indicated cell types were exposed to hypoxia (H, 1% O2) for 24h in the presence of WZB117 (WZB, 10μM) and FACS analyzed for glucose uptake (mean ± SEM, n=3 for Figure S2F, n=2 for Figure S2G). **P*<0.05; *ns*, not significant.

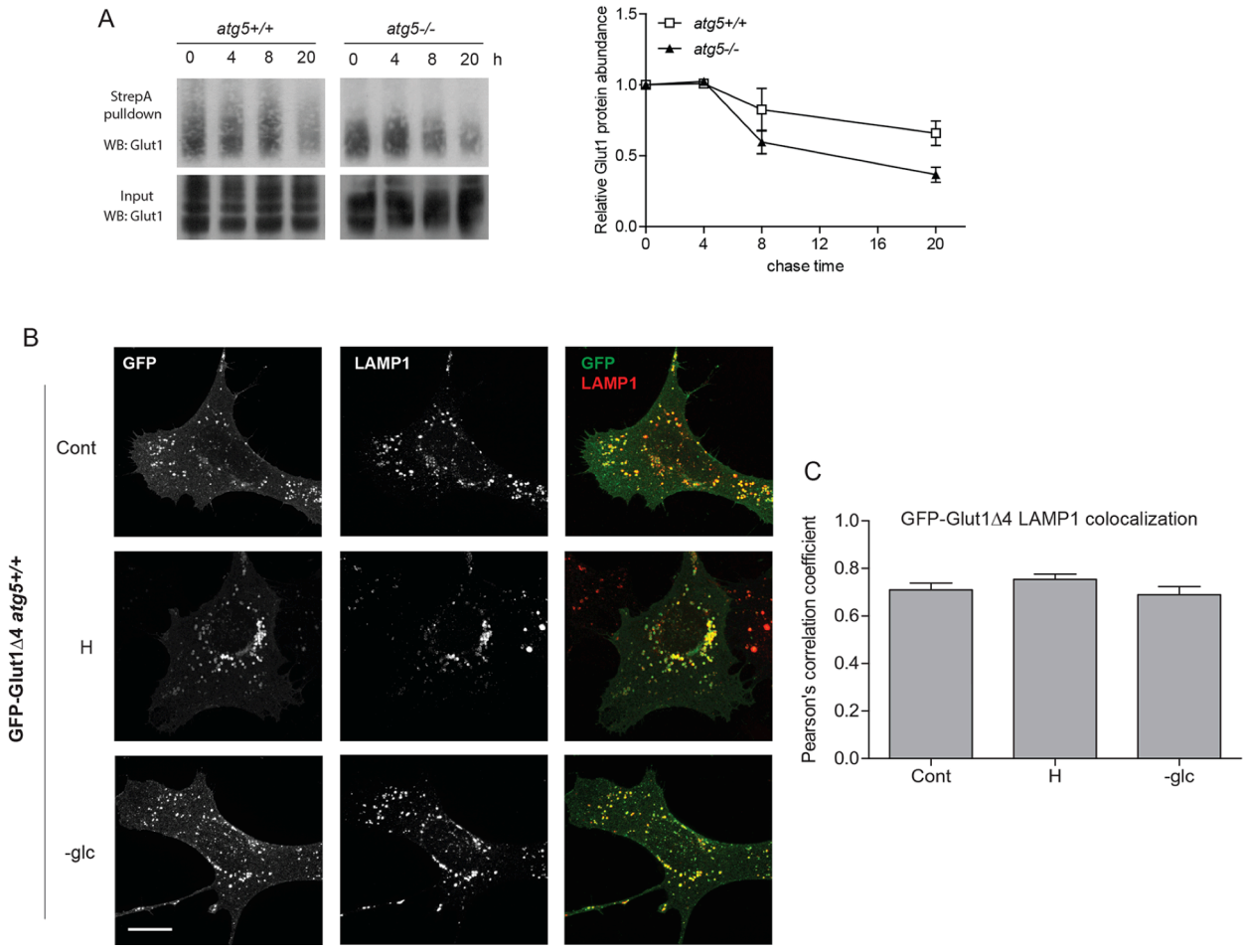

Figure S3: Autophagy mediated Glut1 recycling is C-terminal PDZ domain dependent (Related to Figure 3). (A) Left: Following pulse labeling of cell surface proteins with biotin at 4C, the turnover of biotinylated Glut1 was measured via streptavidin (Strep A) pulldown followed by Glut1 immunoblotting from lysates at the indicated times upon return to 37C. Input: Immunoblotting of total protein lysates used to confirm endogenous Glut1 levels prior to Strep A pulldown. Right: Densitometric quantification of biotinylated Glut1 from Strep A pulldowns shown on left. Relative Glut1 protein abundance was quantified as the fraction of signal intensity remaining compared to the 0h time point. Mean ± SEM of 2 independent experiments. (B) Wild type MEFs stably expressing GFP-Glut1Δ4 were exposed to hypoxia (H, 1% O_2) for 24h or acute glucose starvation (-glc) for 18h and immunostained with α-GFP and α-LAMP1. Scale bar, 20μm. (C) Pearson's correlation coefficient for GFP-LAMP1 colocalization (mean ±SEM, n=30 cells from 3 independent experiments).

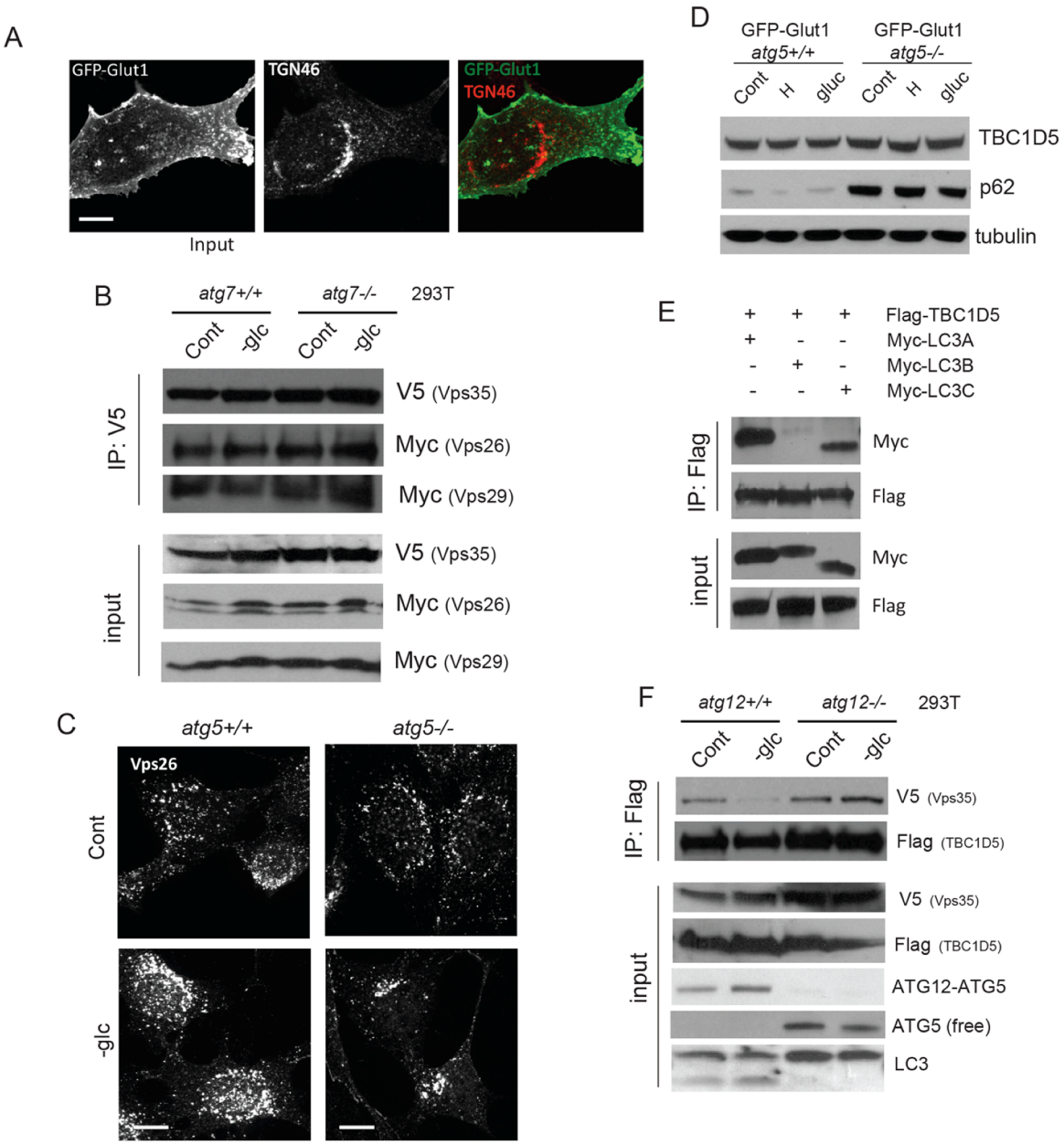

Figure S4: Autophagy inhibits endosomal membrane recruitment of retromer but does not impact retromer assembly (Related to Figures 4 and 5). (A) Wild type MEFs stably expressing GFP-Glut1 were glucose starved (-glc) for 18h and immunostained for GFP and TGN46. Scale bar, 20µm. (B) HEK293T cells with or without ATG7 deletion were transfected with V5-Vps35, Myc-Vps26 and Myc-Vps29. Cells were either kept in full media conditions or glucose starved for 8h. Lysates were prepared, immunoprecipitated with α-V5, resolved on SDS-PAGE and immunoblotted as indicated. (C) *atg5+/+* and *atg5-/-* MEFs were glucose starved for 18h and immunostained for Vps26. Scale bar, 25µm. (D) MEFs were exposed to H (1% O_2) or subject to glucose starvation (-glc) for 18h and lysates were immunoblotted for indicated proteins. (E) HEK293T cells were transiently transfected with Flag-TBC1D5 and Myc-LC3A, Myc-LC3B or Myc-LC3C. Lysates were immunoprecipitated with α-Flag, resolved on SDS-PAGE and immunoblotted with α-Flag and α-Myc. (F) HEK293T cells with or without CRISPR-Cas9 mediated ATG12 deletion were transiently transfected with Flag-TBC1D5 and V5-Vps35; cells were glucose starved and lysates were immunoprecipitated with α-Flag and immunoblotted as indicated.

Supplemental Movie Legends

Movie S1: Glut1 Trafficking In Response to Acute Glucose Starvation In Autophagy Competent Cells (Related to Figure 2). Spinning disk confocal imaging of *atg5*+/+ MEFs stably expressing GFP-Glut1 following 2h of glucose starvation. Images were acquired every 20s. The video plays at 4 frames per second.

Movie S2: Glut1 Trafficking In Response to Acute Glucose Starvation In Autophagy Deficient Cells (Related to Figure 2). Spinning disk confocal imaging of *atg5-/-* MEFs stably expressing GFP-Glut1 following 2h of glucose starvation. Images were acquired every 20s. The video plays at 4 frames per second.