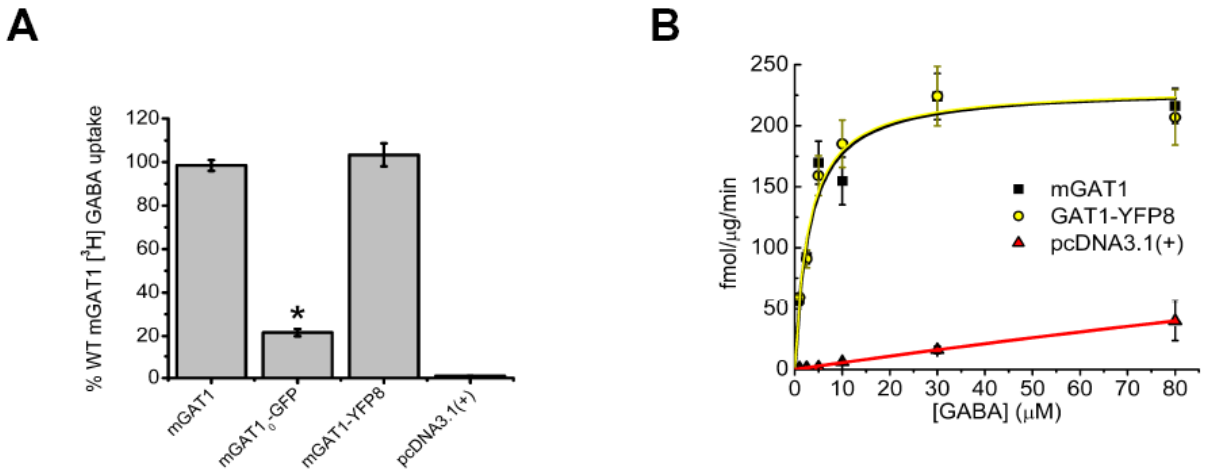
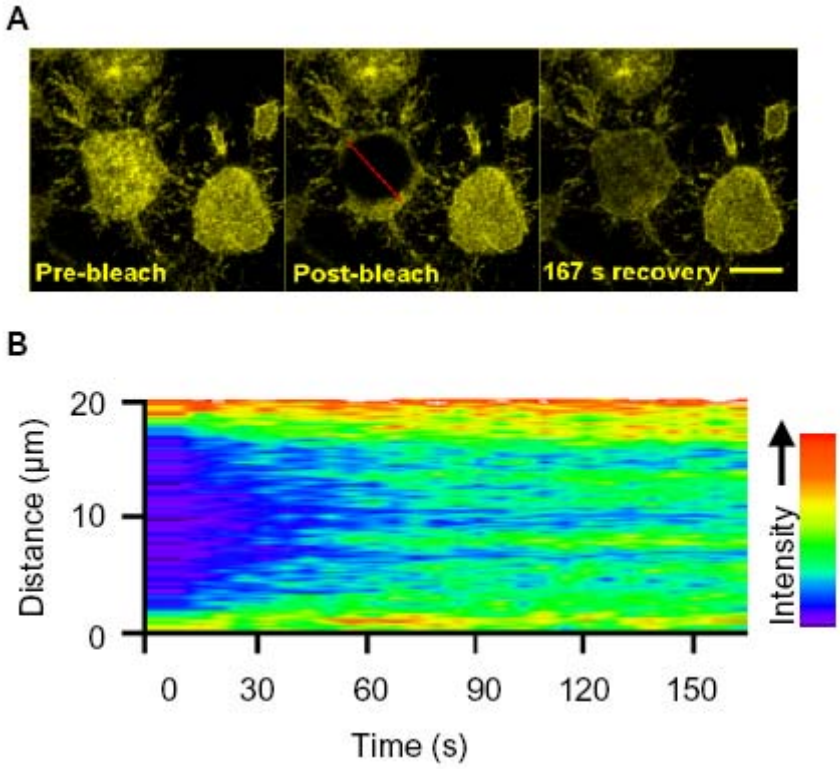
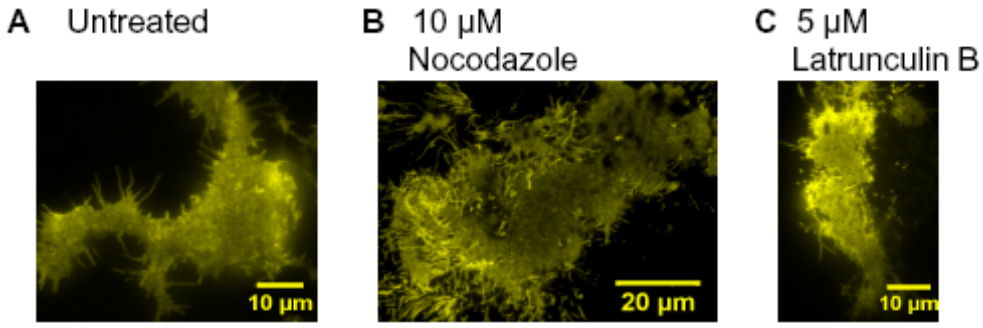


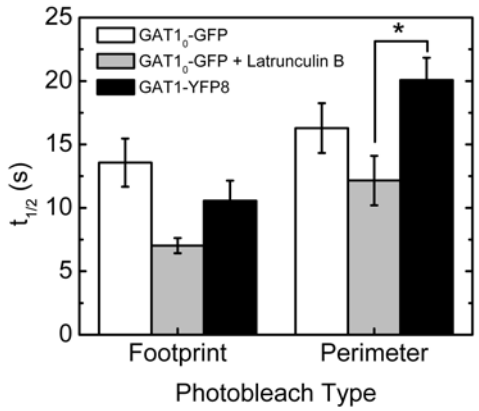
Supplemental figure 1: [^{3}H]-GABA uptake is like wild-type mGAT1 for mGAT1YFP8 but not for mGAT1$_0$-GFP. A) 20 min [^{3}H]-GABA uptake from N2a cells transfected with 100 ng/well of GAT1 wild-type plasmid, GAT1-YFP8, or blank pcDNA3.1(+) vector. Results represent the mean ± SEM of 6 - 18 transfections for each construct. * Denotes significant difference compared to wild-type, $p \leq 0.05$ (one-way analysis of variance (ANOVA) with Tukey's post hoc test). B) GABA concentration versus [^{3}H]-GABA uptake for N2a cells transfected with 100 ng/well of GAT1 wild-type plasmid (K_m= 3.0 ± 0.8 mM and V_{max}= 231 ± 15 fmol/µg/min), GAT1-YFP8 (K_m= 2.9 ± 0.6 mM and V_{max}=231 ± 11 fmol/µg/min), or blank pcDNA3.1(+) vector. Each data point represents the mean of 6 transfections ± SEM.



Supplemental figure 2: Whole footprint photobleach of latrunculin B treated cells. A) Confocal images of GAT1-YFP8 localized at the cell footprint are shown of a pre-, and post-photobleached region of interest representing > 90% of the footprint surface area. Scale bar, 10 µm. B) The kymograph is obtained by measuring the intensity along the red line, which is a section of the photobleached region; the line profile is plotted over time. The intensity key shows that photobleached regions are represented by "cooler" colors, and increased fluorescence is represented by "hotter" colors.



Supplemental figure 3: Total internal reflection fluorescence images of N2a cells transfected with GAT1-YFP8 A) GAT1-YFP8 localizes on the cell membrane and in filopodia. Neither overnight treatment with (B) 10 µM nocodazole nor (C) 1 hr treatment with 5 µM latrunculin B affect cell attachment to the coverslip. C) Filopodial contacts are visible following latrunculin B treatment.



Supplemental figure 4: Comparison of GAT1 mobile fractions. Perimeter photobleach indicates that disrupting actin filaments via latrunculin B treatment results in a significantly faster recovery time constant for $GAT1_0$-GFP compared to GAT1-YFP8 ($p < 0.05$, Mann-Whitney). Therefore, a GAT1 that can interact with PDZ proteins has a higher effective molecular weight than a GAT1 that cannot interact with PDZ proteins (recovery time constant increases with the molecular weight of the species). [$GAT1_0$-GFP footprint: n = 9, $GAT1_0$-GFP + latrunculin B footprint: n = 9 GAT1-YFP footprint: n=18, $GAT1_0$-GFP perimeter: n = 12, $GAT1_0$-GFP + latrunculin B perimeter: n = 10, GAT1-YFP8 perimeter: n=12].