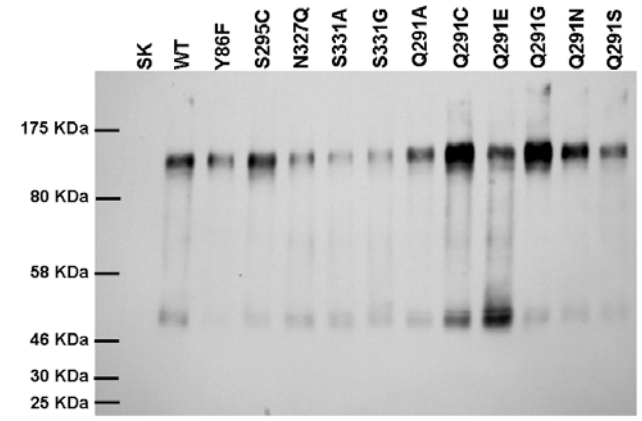
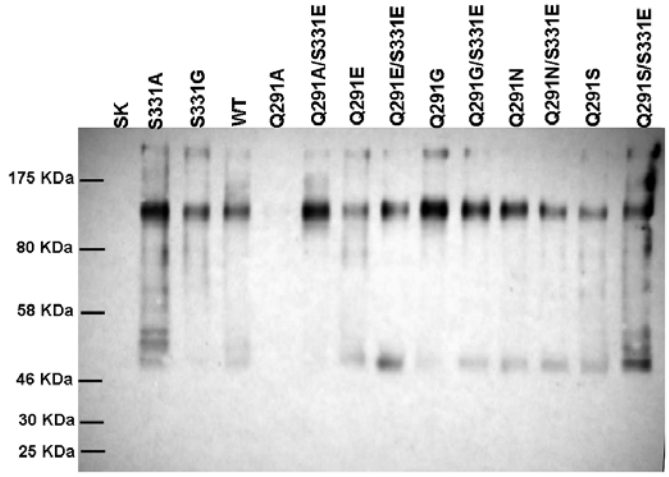
Supplementary Fig. 1

A

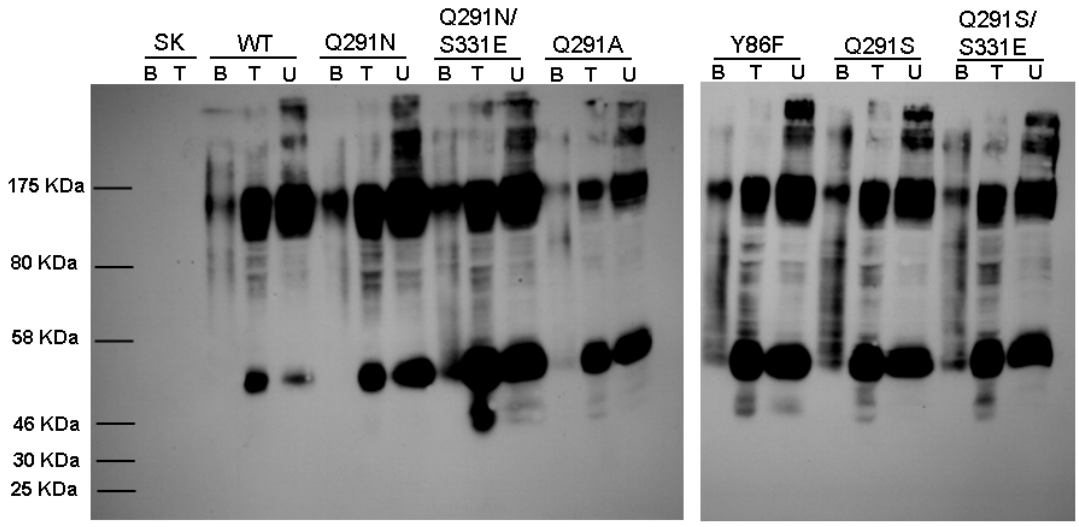


B



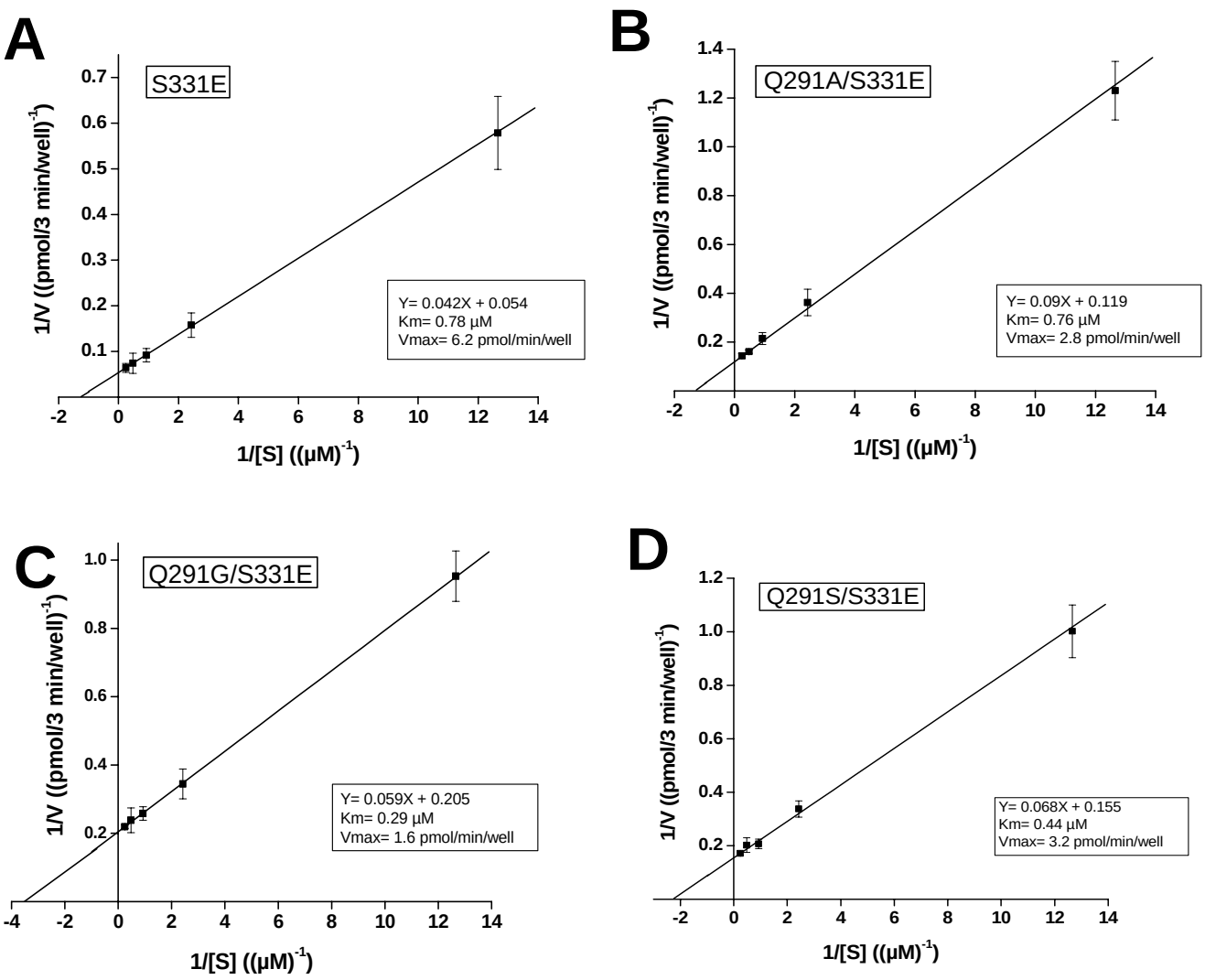
Cell surface biotinylation of GAT-1-WT and mutants. HeLa cells expressing GAT-1-WT (WT) and the indicated mutants, as well as HeLa cells transfected with the vector alone (SK), were biotinylated and processed as explained in Experimental Procedures. The markers shown were run in the lane to the left of SK and contain "Prestained Protein Marker, Broad Range", Cat. # P7708S from New England Biolabs. Two of the experiments are shown in panels A and B.

Supplementary Fig. 2



Total, unbound and biotinylated fractions from GAT-1-WT and mutants. HeLa cells expressing GAT-1-WT (WT) and the indicated mutants , as well as HeLa cells transfected with the vector alone (SK), were biotinylated and processed as explained in Experimental Procedures. The unbound fraction (U) represents the supernatant obtained after centrifugation of the mixture of the lysates of the cells, previously exposed to Sulfo-NHS-SS-Biotin, and the streptavidin-agarose beads. All of the biotinylated samples (B) but only 5% each of the total (T) and unbound fractions were loaded on the gel. The markers shown were run in the lane to the left of SK and contain "Prestained Protein Marker, Broad Range", Cat. # P7708S from New England Biolabs. Shown are the blots of two gels run in parallel. Because the biotinylated fraction is only a very small proportion of the total (and the unbound), the intensity of these bands is obviously not linear, in contrast to the intensity of the biotinylated fraction.

Supplementary Fig. 3



Kinetics of [^{3}H]-GABA transport of GAT-1-WT and mutants. Transport was done as described in Experimental Procedures for 3 min in quadruplicates, but the NaCl containing transport solution (200 µl/well) was supplemented with 6 µCi/ml of [^{3}H]-GABA (76.2 Ci/mMol) and unlabelled GABA at the following concentrations (µM): 0; 0.33; 1; 2 and 4. The results are shown as Lineweaver-Burk plots.