Online Supporting Information for

"A Targeted Quantitative Proteomic Approach for High-throughput Quantitative Profiling of Small GTPases in Brain Tissues of Alzheimer's Disease Patients"

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The Skyline files for the MRM spectral library containing the MS/MS and iRT information for all targeted small GTPase peptides and 10 standard BSA peptides are available at PeptideAtlas with the identifier number of PASS01380 (http://www.peptideatlas.org/PASS/PASS01380).

I. Supplementary Experimental Section

Heavy isotope-labeled synthetic peptides. Crude synthetic peptides labeled with $^{13}C/^{15}N$ on C-terminal lysine and arginine (~75% in peptide purity and >99% in isotopic purity) were synthesized and purified by New England Peptides (Cambridge, MA) in 96-well plate format. According to vendor's documentation, the average purity of a 15-mer peptide is ~75% with Tier Two Analysis (mass spectrum of every peptide) provided. The lyophilized form of peptides was reconstituted with 15% acetonitrile in 0.1% formic acid, aliquoted and stored at -80 °C until use to avoid freeze/thaw cycles. The crude heavy peptide standards of small GTPases were added to each sample at nominal concentrations of 0.5, 2, and 5 fmol/μL, respectively.

These peptide standards were used as received from the vendor without further purification, as, for relative quantification as employed in this study, all data points are normalized to a reference setting, thereby eliminating the peptide concentration as a variable.

Immunoblotting. After tissue homogenization, the protein concentration in the total brain tissue lysates was determined using Quick Start Bradford Protein Assay (Bio-Rad). Approximately 10 μg of lysates were mixed with 4× Laemmli SDS loading buffer, boiled at 95 °C for 5 min, and loaded onto 10% polyacrylamide gels. After electrophoresis, the proteins were transferred onto nitrocellulose membranes. After blocking with 5% non-fat milk in PBS with 0.1% Tween-20 (PBST) at 25 °C for 1 h, the membranes were incubated with primary antibodies against human RAB27B (Proteintech; rabbit polyclonal, 1:2,000) or β-actin (Thermo Fisher; rabbit polyclonal, 1:10,000). After overnight incubation with primary antibodies at 4°C with 5% bovine serum albumin (BSA) in PBST, the membranes were then incubated with peroxidase-labeled donkey

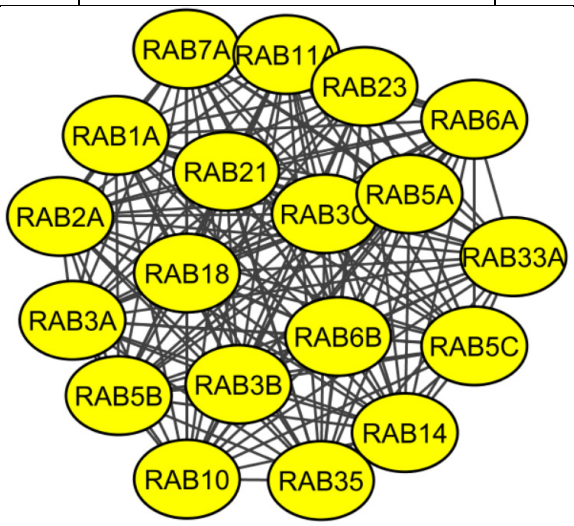
anti-rabbit secondary antibody (Thermo Fisher; 1:10,000) for 1 h at 25 °C. Amersham ECL Prime Western Blot Detecting Reagent (GE Healthcare) was used for visualization of protein bands.

II. Supplementary Tables

Table S1. A complete list of all small GTPases quantified in the scheduled LC-MRM analyses.

Table S2. A list of RAB GTPases involved with synaptic trafficking reported in literature.

Pavlos *et al*. (2010)[1]	Kokotos *et al.* (2018)[2]	Mignogna *et al*. (2018)[3]
RAB1A, RAB2A, RAB2B, RAB3A, RAB3B, RAB3C, RAB4A, RAB4B, RAB5A, RAB5B, RAB5C, RAB6A, RAB6B, RAB7A, RAB8B, RAB10, RAB11B, RAB12, RAB14, RAB15, RAB18, RAB21, RAB26, RAB27B, RAB33B, RAB34/39, RAB35, RAB43	RAB1A, RAB2A, RAB3A, RAB3B, RAB3C, RAB45, RAB5A, RAB5B, RAB5C, RAB6A, RAB6B, RAB7A, RAB10, RAB11A, RAB14, RAB18, RAB21, RAB23, RAB33A, RAB35	Cell body: RAB1, RAB2, RAB6, RAB7, RAB8, RAB33B, RAB39B Axon: RAB4, RAB5, RAB7, RAB8, RAB10, RAB11, RAB13, RAB14, RAB25, RAB26, RAB27B, RAB33A, RAB35 Dendrite: RAB8, RAB10, RAB11, RAB17, RAB21



Supplementary Figures

Figure S1. MRM analysis for the confirmation of the qualities of the crude stable isotope-labeled (SIL) peptides, i.e. lack of contamination of light version of the corresponding peptides.

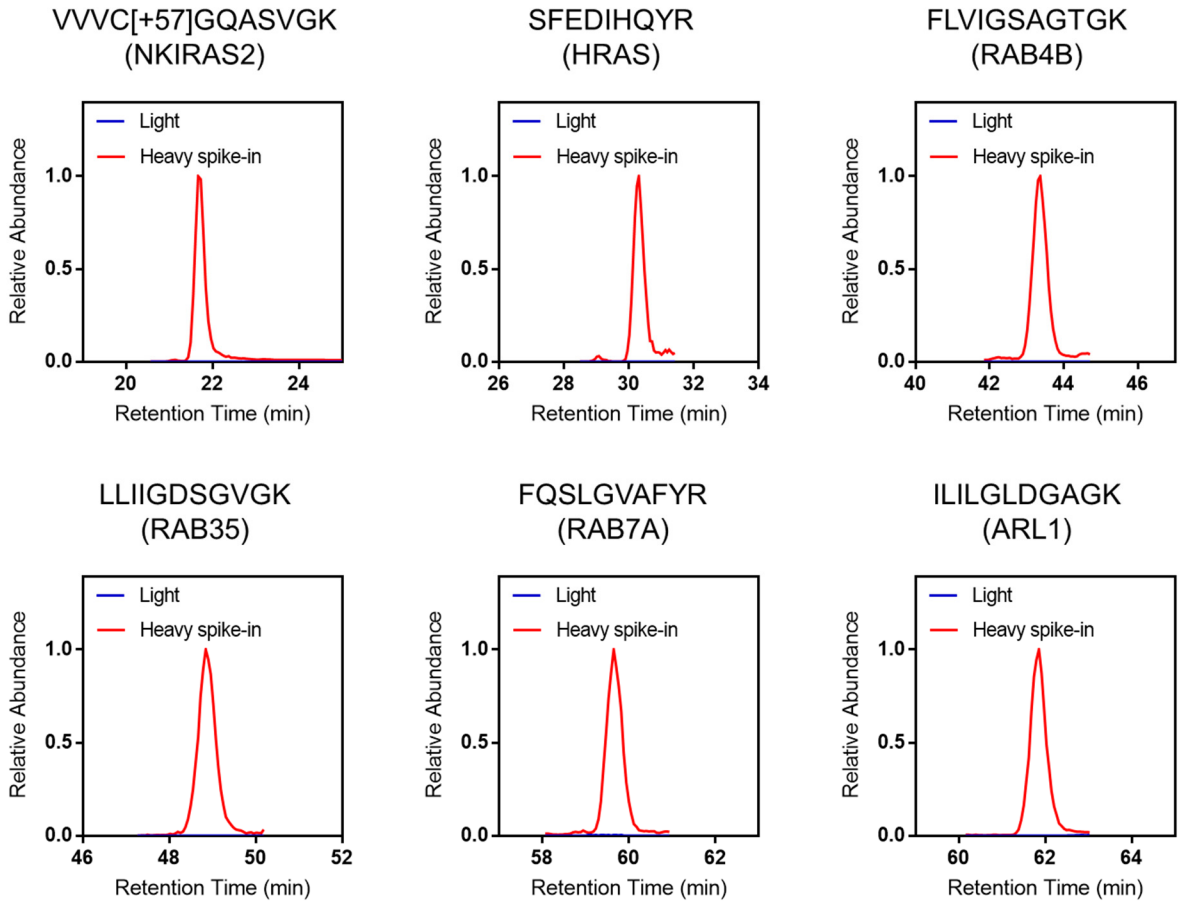
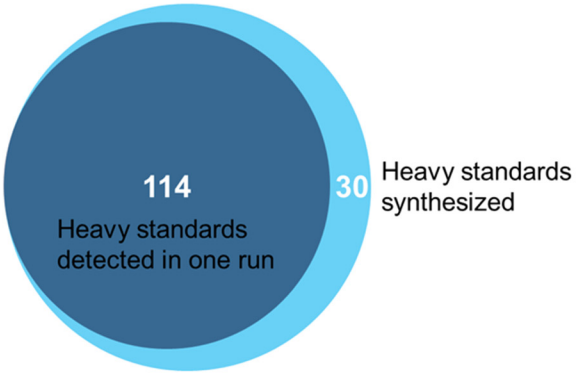
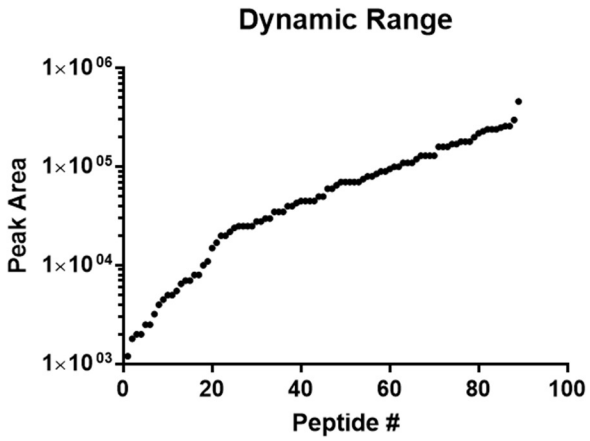


Figure S2. Coverage, dynamic range and optimization of spike-in concentration for the crude SIL peptides in the MRM analyses. (A) A Venn diagram showing the coverage of the SIL peptides detected in a single LC-MRM run; **(B)** A scatter plot depicting the distribution of peak areas resulting from LC-MRM analysis of 2 fmol each of crude SIL peptides; **(C)** A table describing the peak area ratios (light/heavy) obtained from three LC-MRM experiments.

A



B



C

	Light/Heavy Ratio			
Conc.	**Lowest**	**Median**	**Highest**	**Average**
0.5 fmol/μL	0.01	2.04	115	7.41
2.0 fmol/μL	0.005	0.34	23.4	1.71
5.0 fmol/μL	0.002	0.14	7.6	0.58

Figure S3. Extracted-ion chromatograms (XICs) showing selected MRM traces for the optimization of spiked-in concentrations of crude SIL peptides.

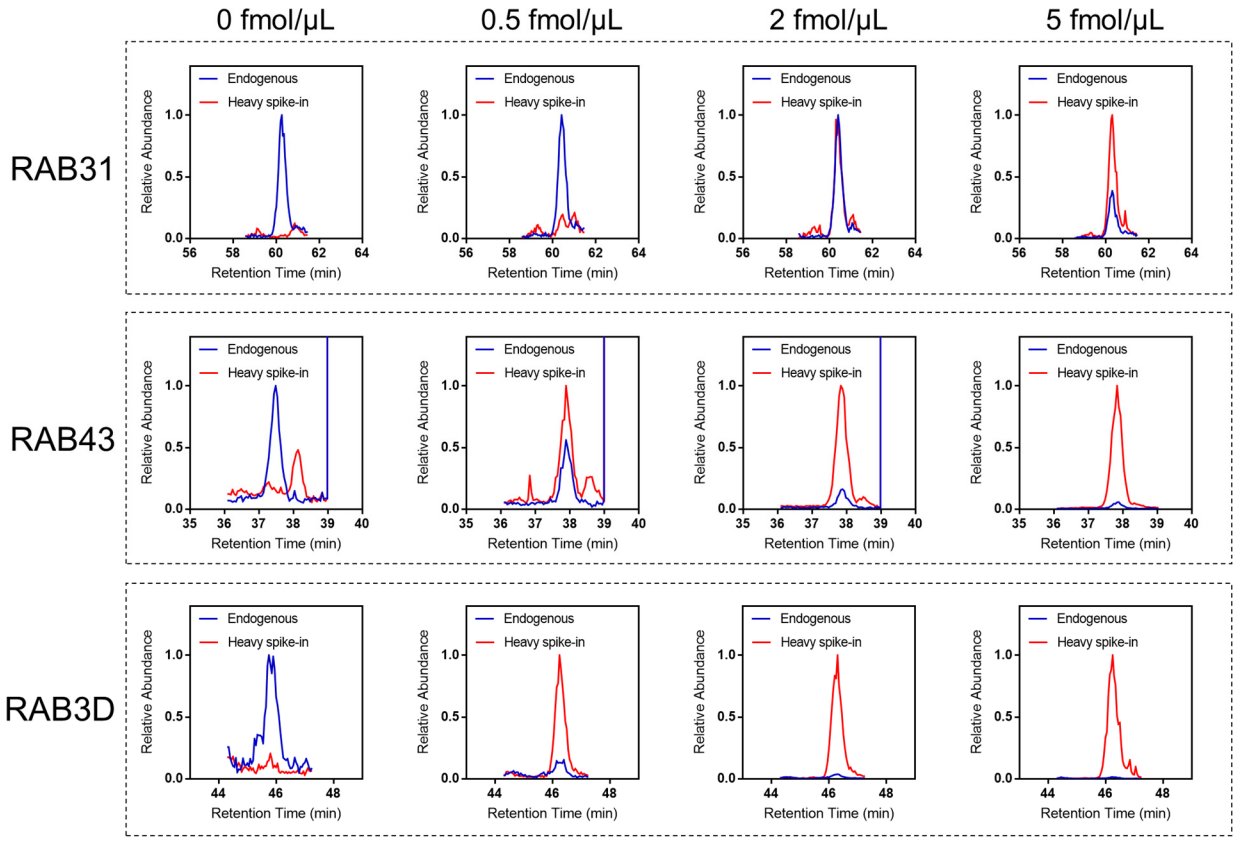


Figure S4. Selection for the twelve surrogate standard (SS) peptides across the designated chromatographic retention time windows.

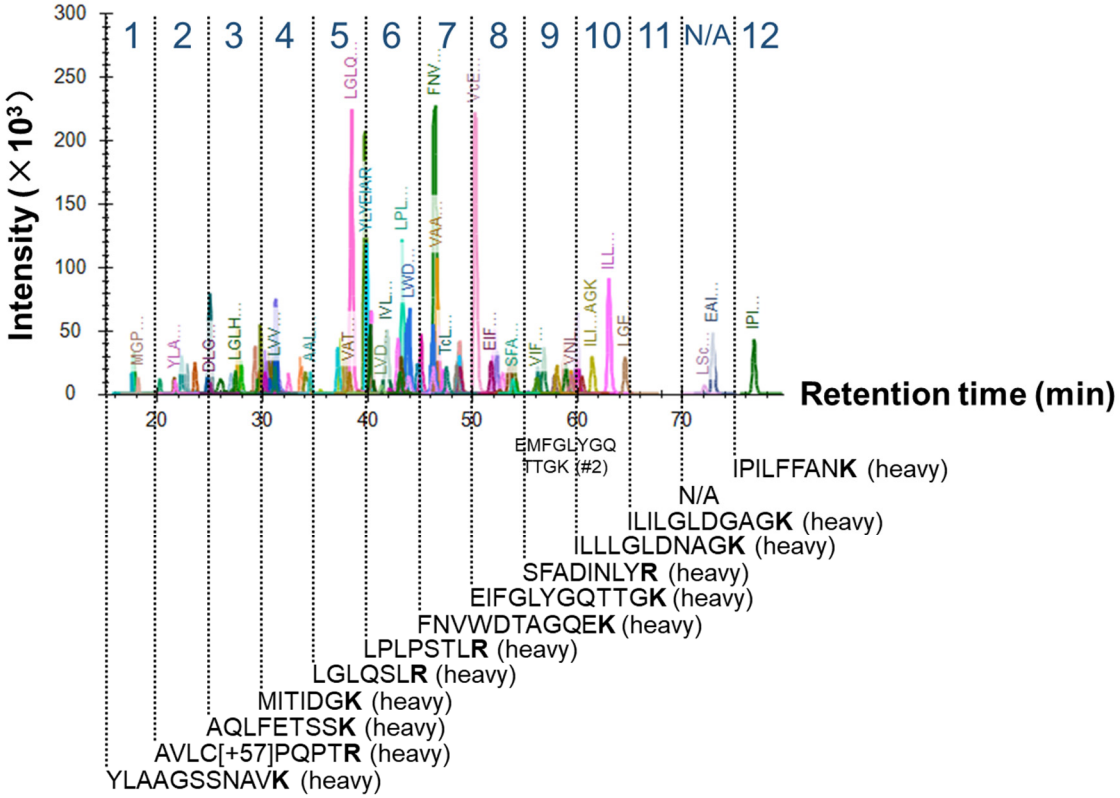


Figure S5. Comparison of the relative peak areas extracted for the selected SS peptides in the MRM analyses across similar or different retention time windows. (A) A connected scatter plot illustrating the relative peak area variations across three SS peptides (#1, #2, #3) eluted at similar retention time windows; **(B)** A connected scatter plot illustrating the relative peak area variations across three SS peptides (#1, #7, #12) eluted at different retention time windows.

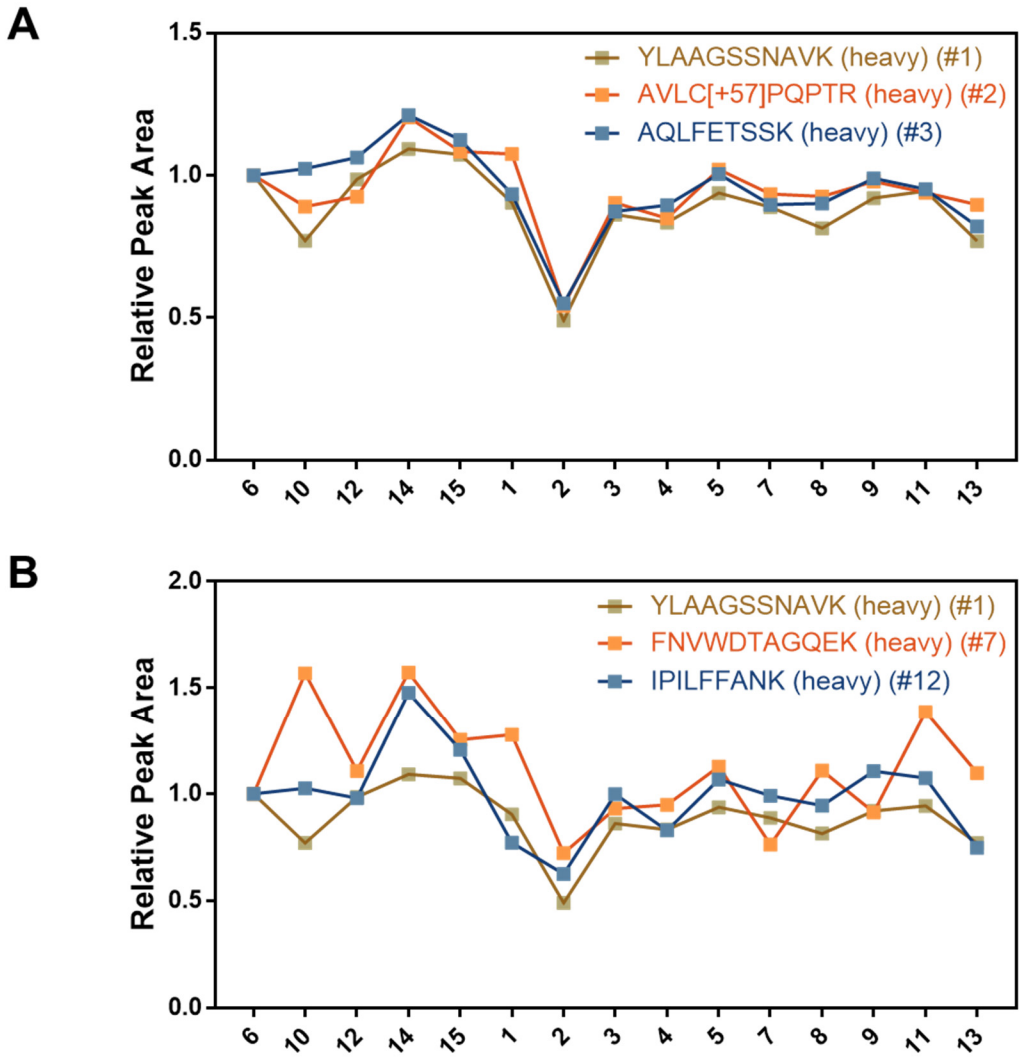


Figure S6. XICs showing selected MRM traces for quantification of various RAB3 isoforms (RAB3A, RAB3B, RAB3C and RAB3D)

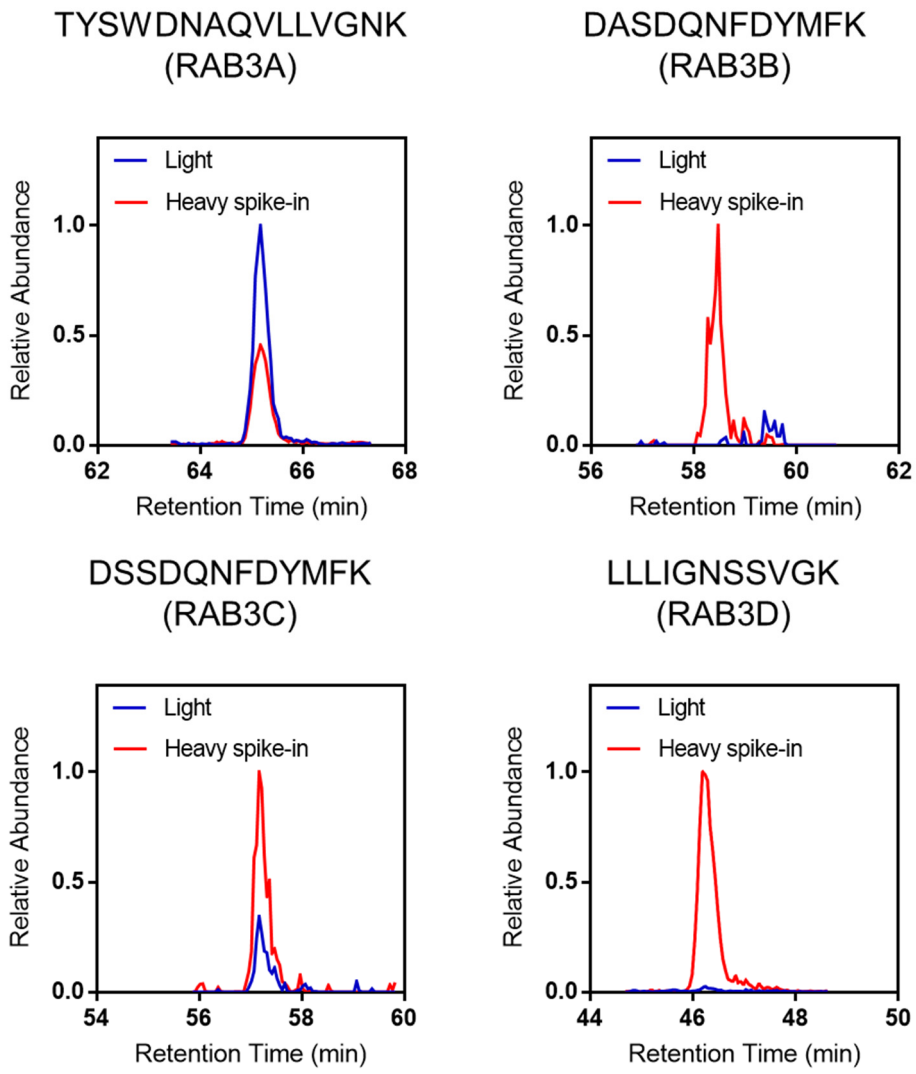
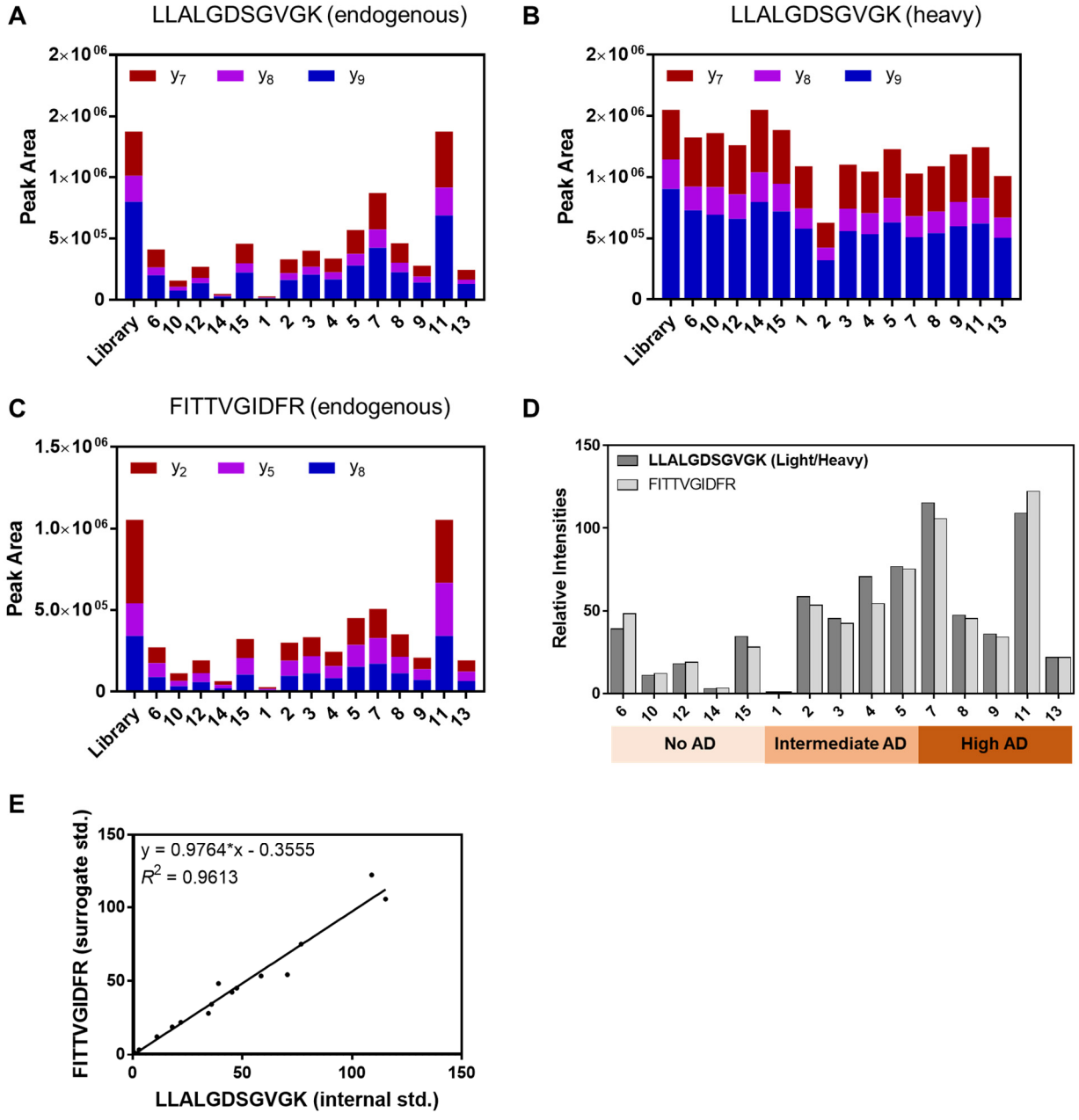


Figure S7. Quantification details for RAB27B by two normalization methods. (A) The relative contribution of each transition to targeted MRM analysis of the endogenous LLALGDSGVGK peptide derived from RAB27B; **(B)** The relative contribution of each transition to targeted MRM analysis of the isotope-labeled LLALGDSGVGK peptide; **(C)** The relative contribution of each transition to targeted MRM analysis of the endogenous FITTVGIDFR peptide; **(D)** Bar charts showing the quantification results normalized from IS peptide (LLALGDSGVGK) and SS peptide (FITTVGIDFR); **(E)** Linear regression of the quantification results shown in panel (D).



References:

1. Pavlos, N. J.; Gronborg, M.; Riedel, D.; Chua, J. J.; Boyken, J.; Kloepper, T. H.; Urlaub, H.; Rizzoli, S. O.; Jahn, R., Quantitative analysis of synaptic vesicle Rabs uncovers distinct yet overlapping roles for Rab3a and Rab27b in Ca2+-triggered exocytosis. *J. Neurosci.* **2010,** *30*, 13441-53.

2. Kokotos, A. C.; Peltier, J.; Davenport, E. C.; Trost, M.; Cousin, M. A., Activity-dependent bulk endocytosis proteome reveals a key presynaptic role for the monomeric GTPase Rab11. *Proc. Natl. Acad. Sci. U. S. A.* **2018,** *115*, E10177-E10186.

3. Mignogna, M. L.; D'Adamo, P., Critical importance of RAB proteins for synaptic function. *Small GTPases* **2018,** *9*, 145-157.