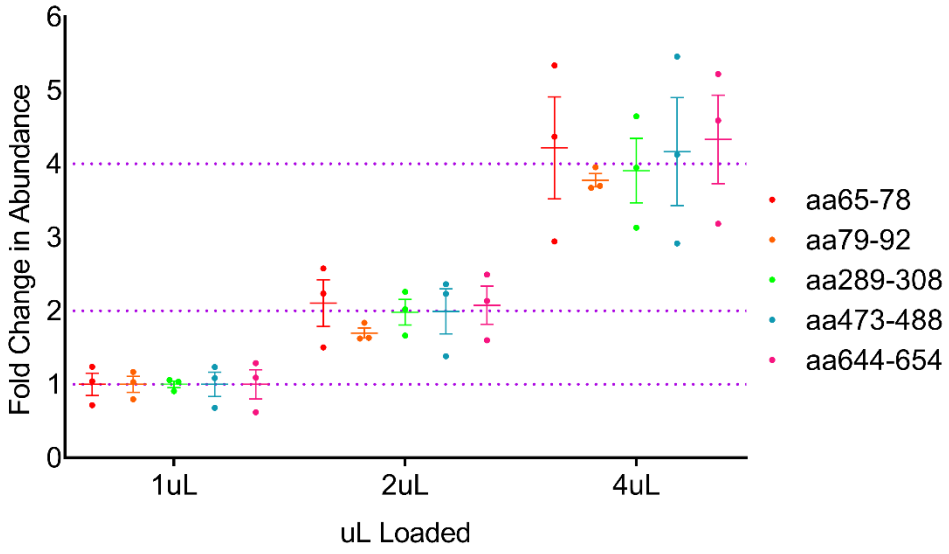
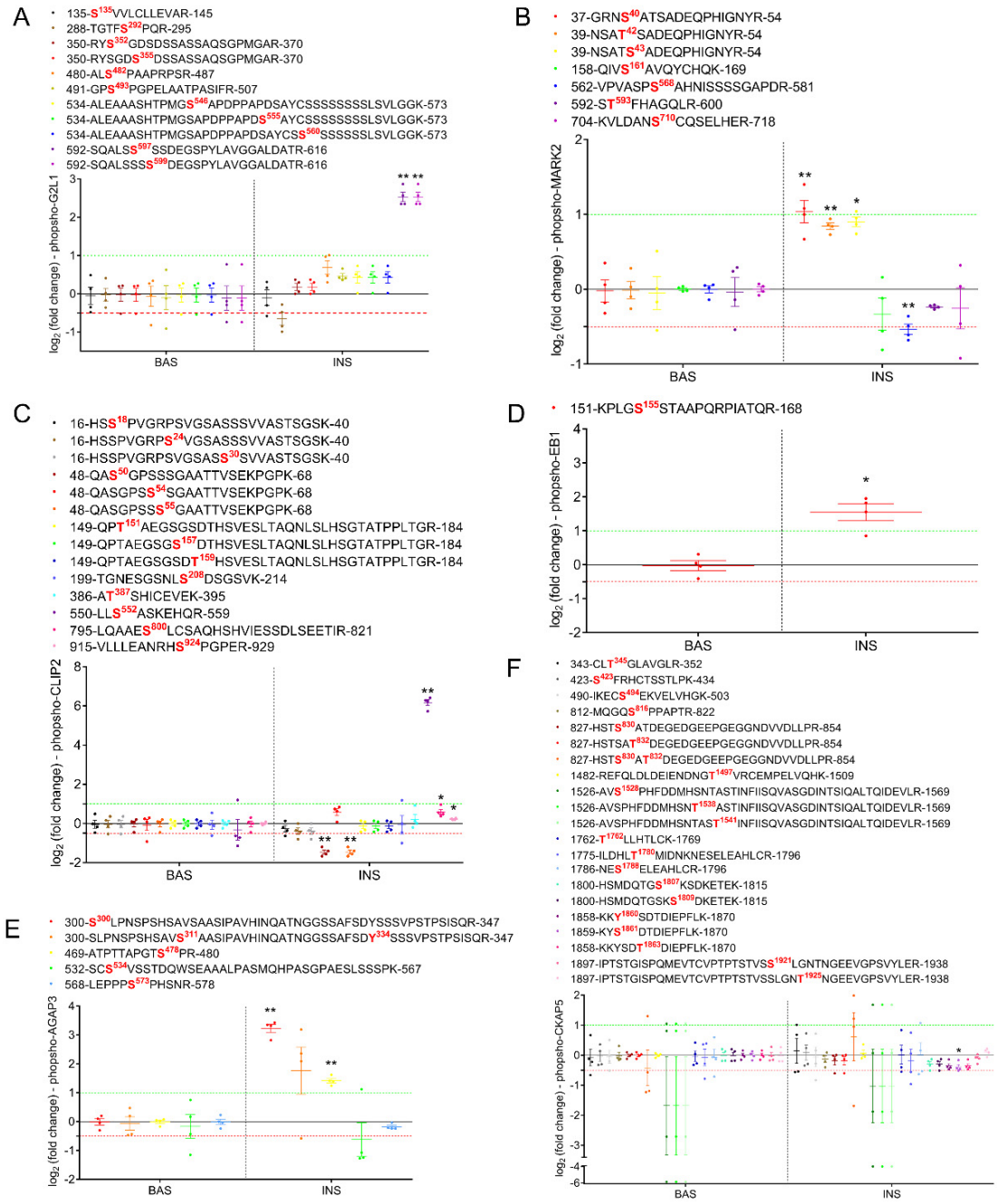
Supplemental Figures



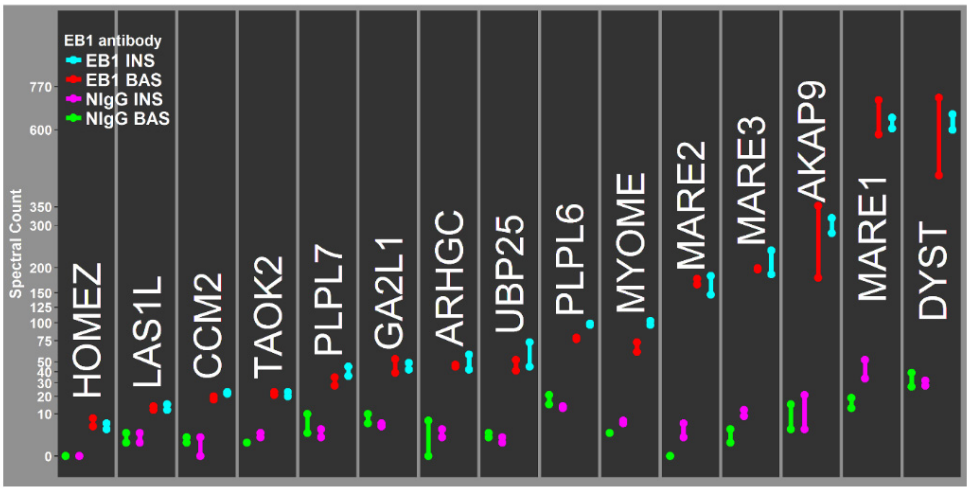
Supplemental Figure 1

Supplemental Figure 1. Extracted ion abundance dose curve. Immunoprecipitated CLASP2 was prepared for mass spectrometry analysis as described in Experimental Procedures (n=3). Five CLASP2 peptide ions (denoted above in the graph with the different colored circles) were chosen according to the previously established rules described in Experimental Procedures. The nomenclature in the Legend denotes the start and stop amino acids of each individual rat CLASP2 peptide chosen. Either 1uL, 2uL, or 4uL of the final digest was injected for mass spectrometry analysis. Each peptide ion's normalized abundance value was normalized by the mean value of the 1uL sample and then expressed as a fold change over the 1uL samples ±SEM.



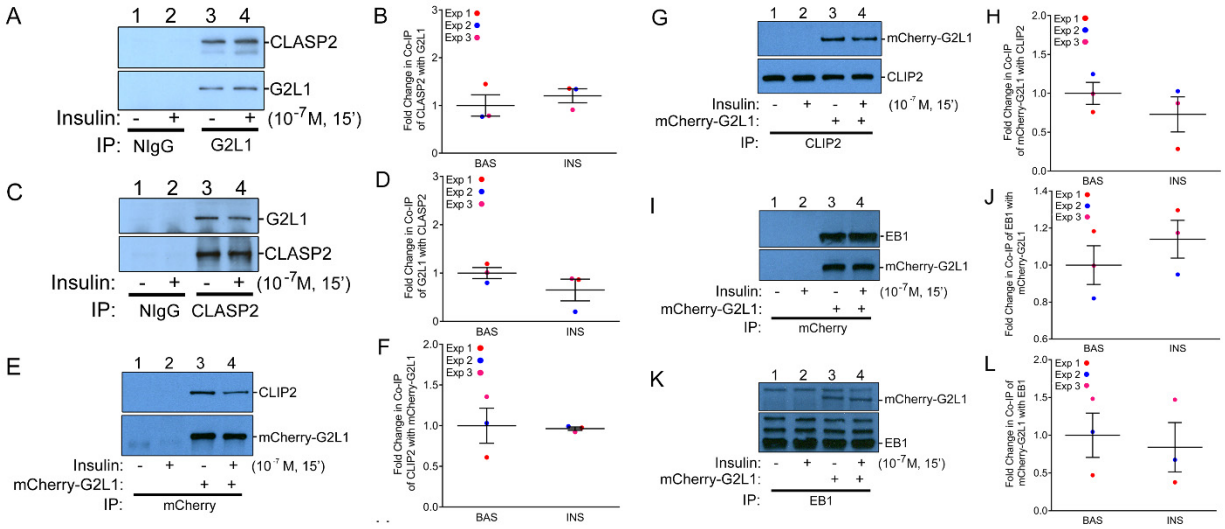
Supplemental Figure 2

Supplemental Figure 2. All quantitative phosphoproteomics findings for G2L1, MARK2, CLIP2, EB1, AGAP3, and CKAP5. Phosphorylation was analyzed as described in Experimental Procedures (n=4 per protein). All data points are included and plotted on a log2 scale, each unique phosphopeptide has a unique color. The basal versus insulin data is separated by the vertical dashed black line. The horizontal red dashed line represents a 50% decrease in phosphorylation while the horizontal green dashed line represents a 2-fold increase in phosphorylation (non-log2 scale). *p≤0.05; **p≤0.01 insulin compared to basal; T-test. BAS, basal. INS, insulin.



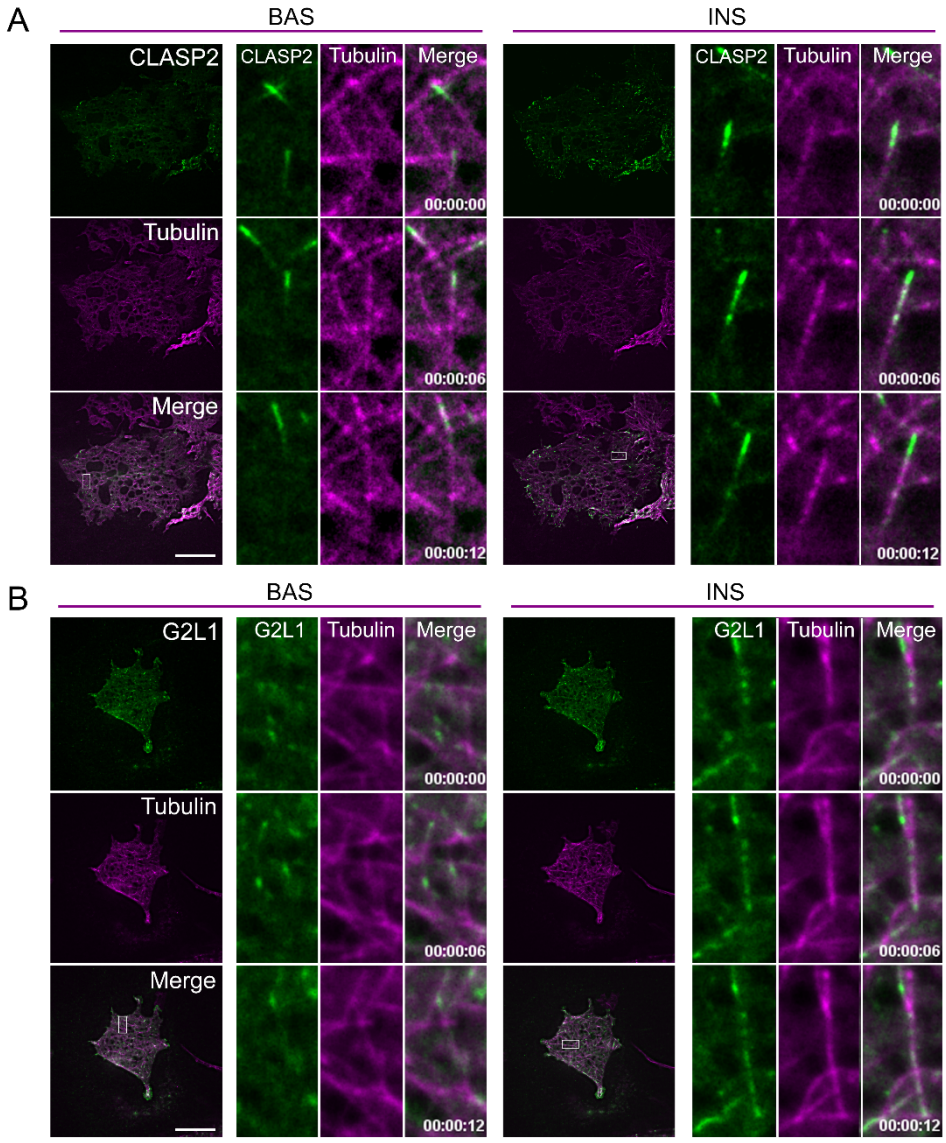
Supplemental Figure 3

Supplemental Figure 3. SAINT scoring and Spectrum Count Profile analysis of the EB1 interactome. IPs and tandem mass spectrometry was performed as described in Experimental Procedures. The 15 "SAINT-qualified" proteins were ordered in a hierarchical manner, from lowest spectrum counts identified (HOMEZ) to highest (DYST), and results from two experiments were individually plotted in a Spectrum Count Profile ("SCP"). Basal NIgG IPs (green), insulin NIgG IPs (magenta), basal EB1 IPs (red), and insulin EB1 IPs (turquoise). The Uniprot ID for EB1 is MARE1.



Supplemental Figure 4

Supplemental Figure 4. G2L1 IP and reciprocal CLASP2, CLIP2 EB1 IPs. Adenoviral infection, cell treatment and lysis, and IPs were performed as described in Experimental Procedures. The IPs were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. The membranes containing the immunoprecipitated proteins were subjected to western blot with the antibodies indicated. ***A***, A representative blot of a G2L1 IP and CLASP2 co-IP ***B***, Densitometry-based quantification of three G2L1 IPs and CLASP2 co-IPs. ***C***. A representative blot of a CLASP2 IP and G2L1 co-IP ***D***, Densitometry-based quantification of three CLASP2 IPs and G2L1 co-IPs. ***E***, A representative blot of a mCherry-G2L1-myc anti-mCherry IP and CLIP2 co-IP ***F***, Densitometry-based quantification of three mCherry-G2L1-myc anti-mCherry IPs and CLIP2 co-IPs. ***G***, A representative blot of a CLIP2 IP and mCherry-G2L1-myc co-IP ***H***, Densitometry-based quantification of three CLIP2 IPs and mCherry-G2L1-myc co-IPs. ***I***, A representative blot of a mCherry-G2L1-myc anti-mCherry IP and EB1 co-IP ***J***, Densitometry-based quantification of three mCherry-G2L1-myc anti-mCherry IPs and EB1 co-IPs. ***K***, A representative blot of an EB1 IP and mCherry-G2L1-myc co-IP ***L***, Densitometry-based quantification of three EB1 IPs and mCherry-G2L1-myc co-IPs.



Supplemental Figure 5

Supplemental Figure 5. Insulin stimulates CLASP2 and G2L1 plus-end trailing along microtubules. Live-cell imaging of the effect of insulin (10min) on adipocytes cultured in serum-starved conditions co-overexpressing ***A***, GFP-CLASP2-HA (green) and mRuby2-Tubulin (magenta) or ***B***, mCherry-G2L1-myc (green) and iRFP670-Tubulin (magenta). Live cells were imaged using TIRFM on a 2-second acquisition interval. The time series images display the +TIP dynamics in the magnified ROI. Scale bar = 20μm.