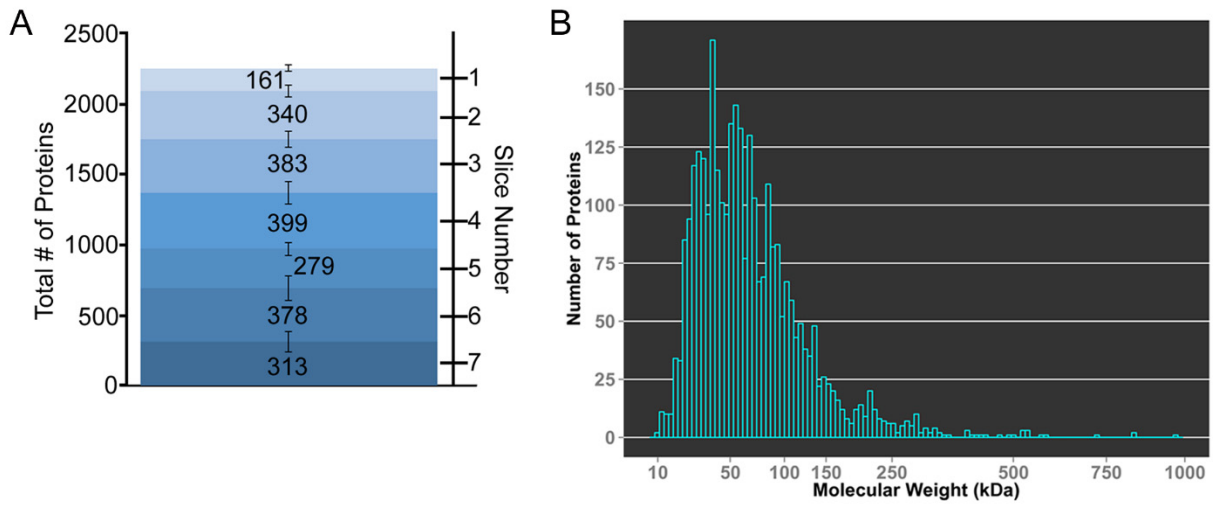
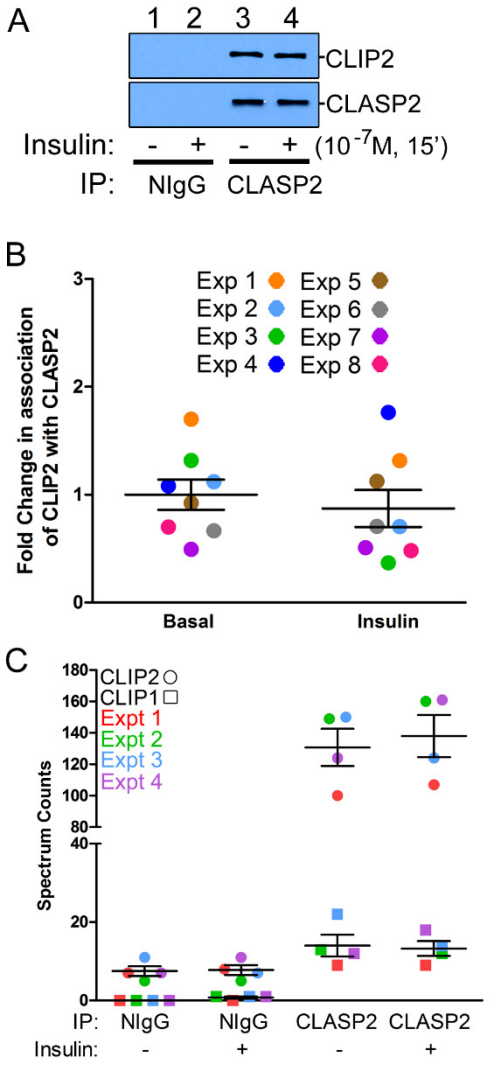


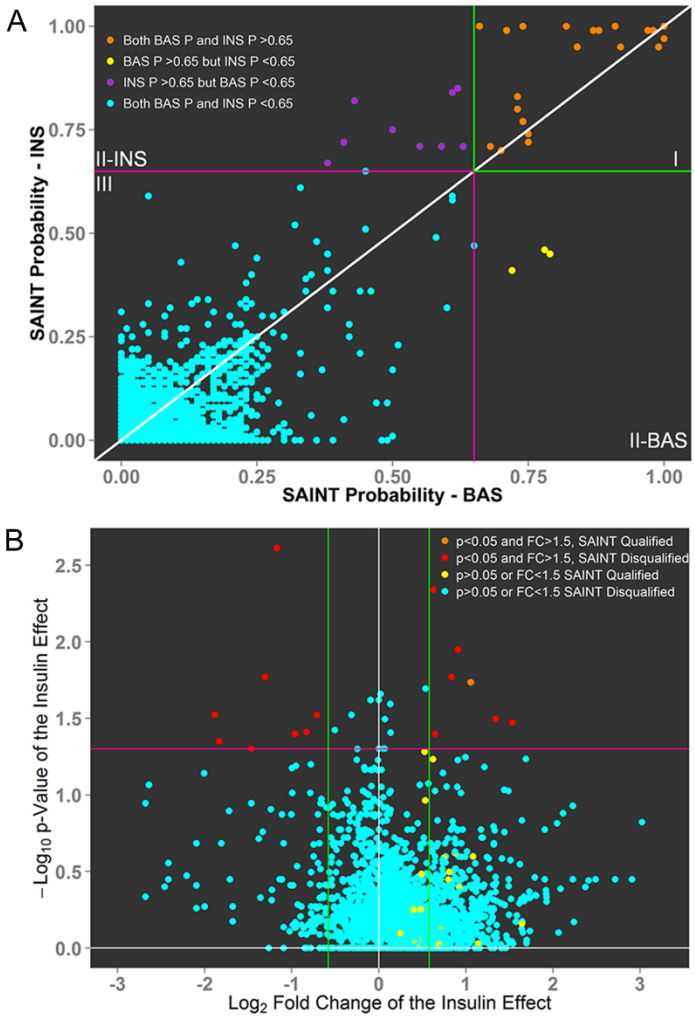
Supplemental Figure 1

Supplemental Figure 1. Detailed breakdown of the proteomic numbers associated with the CLASP2 Antibody #1 interactome. 150mm plates of serum-starved, differentiated 3T3-L1 adipocytes were either left untreated or treated with 100 nM insulin for 15 mins. The cells were lysed in CHAPS lysis buffer and the NIgG and CLASP2 Antibody #1 interactome experiments were performed as described in Experimental Procedures (n=4). *A*, This graph depicts the average number of proteins per slice in the SDS-PAGE gel, +/- SD for the CLASP2 Antibody #1 experiment (the NIgG and CLASP2 Antibody #1 IPs, both basal and insulin, were all grouped together). Slice 1: ~180-250kDa, Slice 2: ~100-180kDa, Slice 3: ~75-100kDa, Slice 4: ~55-75kDa, Slice 5: ~40-55kDa, Slice 6: ~30-40kDa, Slice 7: ~20-30kDa. Using this CLASP2 Antibody #1 experiment numbers as an example for the type of data acquired using this particular proteomic method, each lane of the basal and insulin NIgG IPs had an average of 1659 and 1624 proteins identified, respectively, whereas the basal and insulin CLASP2 Antibody #1 IPs had an average of 1632 and 1537 proteins. These protein numbers were derived from combining the Mascot data files from each lane's slices into one sample using the MuDPIT function in the program Scaffold. The total number of proteins identified in all sixteen samples of the CLASP2 Antibody #1 experiment was 3034. *B*, Using CLASP2 Antibody #1 as an example, this histogram represents the average distribution of all the proteins identified across all four experiments for CLASP2 Antibody #1, relative to their molecular weight. Myosin-10 and Myosin-9 were the top two most abundant proteins for the CLASP2 Antibody #1 experiment, followed closely behind by unconventional myosin-Ic, which when combined represent only 0.1% of the number of proteins identified, yet an average of 19.4% of the identified spectra per sample. CLASP2 averaged 12 and 13 assigned spectra in the four NIgG basal and insulin IP samples, respectively, versus 330 and 387 in the CLASP2 Antibody #1 basal and insulin IP samples, respectively. This represents a 28 and 30-fold enrichment, resulting from CLASP2 immunoprecipitation, with an average of 60% sequence coverage of CLASP2 across all eight CLASP2 Antibody #1 IPs.



Supplemental Figure 2

Supplemental Figure 2. CLASP2 Antibody #1 co-IP of CLIP1/CLIP-115 and CLIP2/CLIP-170. 150mm plates of serum-starved, differentiated 3T3-L1 adipocytes were either left untreated or treated with 100 nM insulin for 15 mins. The cells were lysed in CHAPS lysis buffer and the NIgG and CLASP2 Antibody #1 IPs were performed as described in Experimental Procedures. The IPs were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. *A*, The membranes containing the immunoprecipitated proteins were subjected to Western blot for CLIP2 (top panel) and CLASP2 (bottom panel). *B*, Western blot densitometry was used to quantify the amount of CLIP2 in the CLASP2 IPs. CLIP2 was normalized to the amount of CLASP2 and expressed as the fold change over basal (n=8). NIgG IPs were omitted due to a lack of the Western blot to detect a CLIP2 signal. *C*, Raw spectrum counts are plotted for CLIP1/CLIP-170 and CLIP2/CLIP-115 from either the NIgG or CLASP2 Antibody #1 IPs (basal and insulin-treated) for the four interactome experiments performed.



Supplemental Figure 3

Supplemental Figure 3. Detailed breakdown of the SAINT data associated with the CLASP2 Antibody #1 interactome. 150mm plates of serum-starved, differentiated 3T3-L1 adipocytes were either left untreated or treated with 100 nM insulin for 15 mins. The cells were lysed in CHAPS lysis buffer and the NIgG and CLASP2 Antibody #1 interactome experiments were performed as described in Experimental Procedures (n=4). *A*, Each protein's SAINT P-scores for basal and insulin were plotted against each other, again using the CLASP2 Antibody #1 experiment as a representative example for this experimental approach. When viewing the 3034 proteins from the CLASP2 Antibody #1 experiment, 2981 proteins possessed a P-Score < 0.65 in both the BAS and INS samples ("Quadrant III"), 3 proteins had a P-Score > 0.65 in the BAS samples only ("Quadrant II-BAS"), 11 proteins had a P-Score > 0.65 in the INS samples only ("Quadrant II-INS"), and 39 proteins (1.3% of the interactome) had a P-Score > 0.65 in both the BAS and INS samples ("Quadrant I"). *B*, Volcano plot of the CLASP2 Antibody #1 interactome data (n=4) analyzing the Log_2 fold change of the insulin effect versus the $–Log_{10}$ p-value of the insulin effect. For calculating the effect of insulin in the CLASP2 Antibody #1 experiment, the spectral counts of each protein were normalized to those of CLASP2 within the respective sample. A paired 2-tailed T-test was performed to obtain a p-value for the significance of the effect of insulin. The green

vertical lines demarcate the 1.5 fold increase and decrease cut-offs, and the magenta horizontal line indicates the p-value=0.05 cut-off for the insulin effect. SAINT-disqualified proteins fell in two categories: the turquoise circles represent proteins that were either non-significantly affected by insulin or possessed less than a 1.5-fold effect of insulin and the red circles are proteins that were both significantly affected by insulin and possessed greater than a 1.5-fold effect of insulin. SAINT-qualified proteins also fell in 2 groups: non-significantly affected by insulin or possessed less than a 1.5-fold effect of insulin (yellow circles), or the lone orange circle which was significantly affected by insulin and possessed greater than a 1.5-fold effect of insulin (FAM13A).