Bait ID	Hit Gene Name	Pep #	Unique Pep #	Peptide
1724	LRP10	2	1	SQVTPSAAPLEALDGGTGPAR
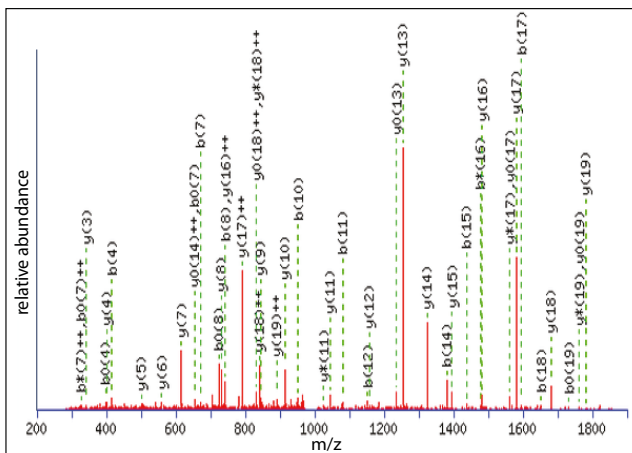
Monoisotopic mass of neutral peptide Mr(calc): 1994.01
Ions Score: 120
Matches (Bold Red): 47/230 fragment ions using 68 most intense peaks

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	88.04	44.52			70.03	35.52	S							21
2	216.10	108.55	199.07	100.04	198.09	99.55	Q	1907.98	954.49	1890.96	945.98	1889.97	945.49	20
3	315.17	158.09	298.14	149.57	297.16	149.08	V	1779.92	890.47	1762.90	881.95	1761.91	881.46	19
4	416.21	208.61	399.19	200.10	398.20	199.61	T	1680.86	840.93	1663.83	832.42	1662.84	831.93	18
5	513.27	257.14	496.24	248.62	495.26	248.13	P	1579.81	790.41	1562.78	781.89	1561.80	781.40	17
6	600.30	300.65	583.27	292.14	582.29	291.65	S	1482.75	741.88	1465.73	733.37	1464.74	732.88	16
7	671.34	336.17	654.31	327.66	653.33	327.17	A	1395.72	698.36	1378.70	689.85	1377.71	689.36	15
8	742.37	371.69	725.35	363.18	724.36	362.68	A	1324.69	662.85	1307.66	654.33	1306.67	653.84	14
9	839.43	420.22	822.40	411.70	821.42	411.21	P	1253.65	627.33	1236.62	618.81	1235.64	618.32	13
10	952.51	476.76	935.48	468.25	934.50	467.75	L	1156.60	578.80	1139.57	570.29	1138.59	569.80	12
11	1081.55	541.28	1064.53	532.77	1063.54	532.27	E	1043.51	522.26	1026.49	513.75	1025.50	513.25	11
12	1152.59	576.80	1135.56	568.29	1134.58	567.79	A	914.47	457.74	897.44	449.22	896.46	448.73	10
13	1265.67	633.34	1248.65	624.83	1247.66	624.34	L	843.43	422.22	826.41	413.71	825.42	413.21	9
14	1380.70	690.85	1363.67	682.34	1362.69	681.85	D	730.35	365.68	713.32	357.16	712.34	356.67	8
15	1437.72	719.36	1420.70	710.85	1419.71	710.36	G	615.32	308.16	598.29	299.65	597.31	299.16	7
16	1494.74	747.88	1477.72	739.36	1476.73	738.87	G	558.30	279.65	541.27	271.14	540.29	270.65	6
17	1595.79	798.40	1578.76	789.89	1577.78	789.39	T	501.28	251.14	484.25	242.63	483.27	242.14	5
18	1652.81	826.91	1635.79	818.40	1634.80	817.90	G	400.23	200.62	383.20	192.11			4
19	1749.87	875.44	1732.84	866.92	1731.85	866.43	P	343.21	172.11	326.18	163.59			3
20	1820.90	910.95	1803.88	902.44	1802.89	901.95	A	246.16	123.58	229.13	115.07			2
21							R	175.12	88.06	158.09	79.55			1

Bait ID	Hit Gene Name	Pep #	Unique Pep #	Peptide
1724	SDCBP	3	1	LYPELSQYMGLSLNEEEIR
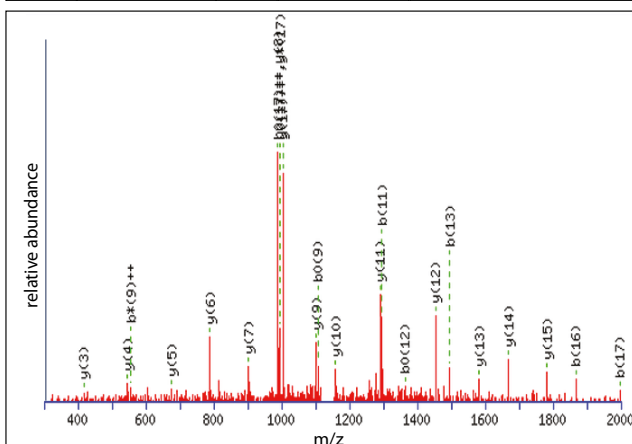
Monoisotopic mass of neutral peptide Mr(calc): 2283.11
Ions Score: 129
Matches (Bold Red): 24/194 fragment ions using 29 most intense peaks

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	114.09	57.55					L							19
2	277.15	139.08					Y	2171.03	1086.02	2154.01	1077.51	2153.02	1077.01	18
3	374.21	187.61					P	2007.97	1004.49	1990.94	995.97	1989.96	995.48	17
4	503.25	252.13			485.24	243.12	E	1910.92	955.96	1893.89	947.45	1892.91	946.96	16
5	616.33	308.67			598.32	299.67	L	1781.87	891.44	1764.85	882.93	1763.86	882.44	15
6	703.37	352.19			685.36	343.18	S	1668.79	834.90	1651.76	826.39	1650.78	825.89	14
7	831.42	416.22	814.40	407.70	813.41	407.21	Q	1581.76	791.38	1564.73	782.87	1563.75	782.38	13
8	994.49	497.75	977.46	489.23	976.48	488.74	Y	1453.70	727.35	1436.67	718.84	1435.69	718.35	12
9	1125.53	563.27	1108.50	554.75	1107.52	554.26	M	1290.64	645.82	1273.61	637.31	1272.63	636.82	11
10	1182.55	591.78	1165.52	583.27	1164.54	582.77	G	1159.60	580.30	1142.57	571.79	1141.58	571.30	10
11	1295.63	648.32	1278.61	639.81	1277.62	639.32	L	1102.57	551.79	1085.55	543.28	1084.56	542.79	9
12	1382.67	691.84	1365.64	683.32	1364.66	682.83	S	989.49	495.25	972.46	486.74	971.48	486.24	8
13	1495.75	748.38	1478.72	739.87	1477.74	739.37	L	902.46	451.73	885.43	443.22	884.45	442.73	7
14	1609.79	805.40	1592.77	796.89	1591.78	796.39	N	789.37	395.19	772.35	386.68	771.36	386.19	6
15	1738.84	869.92	1721.81	861.41	1720.83	860.92	E	675.33	338.17	658.30	329.66	657.32	329.16	5
16	1867.88	934.44	1850.85	925.93	1849.87	925.44	E	546.29	273.65	529.26	265.13	528.28	264.64	4
17	1996.92	998.96	1979.89	990.45	1978.91	989.96	E	417.25	209.13	400.22	200.61	399.24	200.12	3
18	2110.00	1055.51	2092.98	1046.99	2091.99	1046.50	I	288.20	144.61	271.18	136.09			2
19							R	175.12	88.06	158.09	79.55			1

| Bait ID	Hit Gene Name	Pep #	Unique Pep #	Peptide
1724	VAMP3	1	1	ADALQAGASQFETSAAK
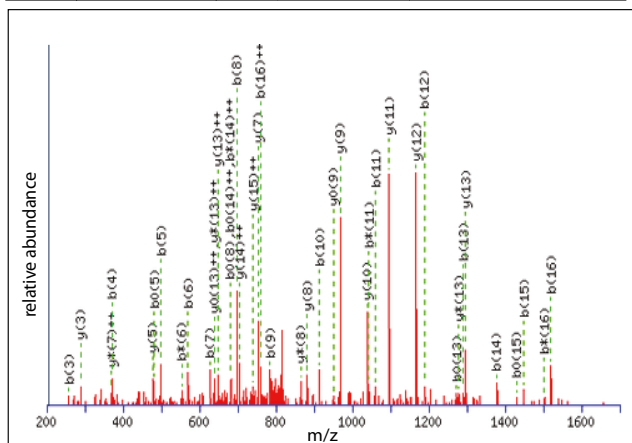
Monoisotopic mass of neutral peptide Mr(calc): 1664.80
Ions Score: 89
Matches (Bold Red): 42/176 fragment ions using 82 most intense peaks

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	72.04	36.53					A							17
2	187.07	94.04			169.06	85.03	D	1594.77	797.89	1577.74	789.38	1576.76	788.88	16
3	258.11	129.56			240.10	120.55	A	1479.74	740.38	1462.72	731.86	1461.73	731.37	15
4	371.19	186.10			353.18	177.09	L	1408.71	704.86	1391.68	696.34	1390.70	695.85	14
5	499.25	250.13	482.22	241.62	481.24	241.12	Q	1295.62	648.31	1278.60	639.80	1277.61	639.31	13
6	570.29	285.65	553.26	277.13	552.28	276.64	A	1167.56	584.29	1150.54	575.77	1149.55	575.28	12
7	627.31	314.16	610.28	305.65	609.30	305.15	G	1096.53	548.77	1079.50	540.25	1078.52	539.76	11
8	698.35	349.68	681.32	341.16	680.34	340.67	A	1039.51	520.26	1022.48	511.74	1021.49	511.25	10
9	785.38	393.19	768.35	384.68	767.37	384.19	S	968.47	484.74	951.44	476.22	950.46	475.73	9
10	913.44	457.22	896.41	448.71	895.43	448.22	Q	881.44	441.22	864.41	432.71	863.43	432.22	8
11	1060.51	530.76	1043.48	522.24	1042.50	521.75	F	753.38	377.19	736.35	368.68	735.37	368.19	7
12	1189.55	595.28	1172.52	586.76	1171.54	586.27	E	606.31	303.66	589.28	295.15	588.30	294.65	6
13	1290.60	645.80	1273.57	637.29	1272.59	636.80	T	477.27	239.14	460.24	230.62	459.26	230.13	5
14	1377.63	689.32	1360.60	680.80	1359.62	680.31	S	376.22	188.61	359.19	180.10	358.21	179.61	4
15	1448.67	724.84	1431.64	716.32	1430.65	715.83	A	289.19	145.10	272.16	136.58			3
16	1519.70	760.35	1502.68	751.84	1501.69	751.35	A	218.15	109.58	201.12	101.07			2
17							K	147.11	74.06	130.09	65.55			1

Supplementary Figure 1. MS/MS spectra for proteins identified on the basis of a single peptide in two biological replicates. "Bait ID" is the unique identifier for the sample in ProHIts, our internal interaction database (Liu et al., 2010). "Hit Gene Name" is the gene name of the interactor. "Pep #" is the total number of peptides. "Unique Pep #" is the number of unique peptides (as defined by the Mascot search engine). VAMP3, SDCBP, LRP10 were identified with at least one unique peptide in both PI4K2A biological replicates and thus were identified as high-confidence interactors.
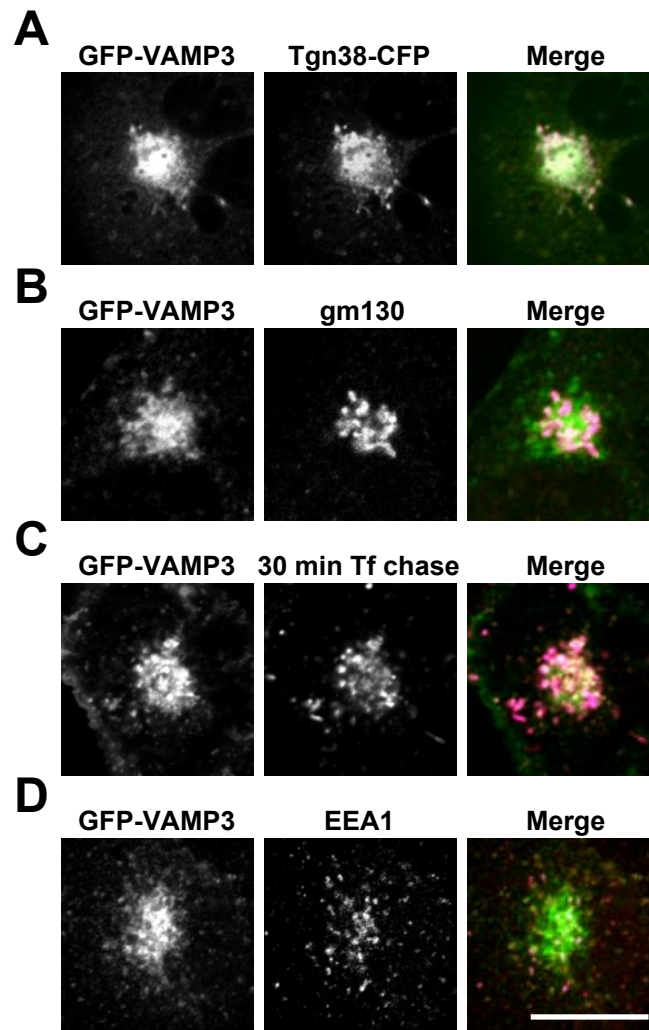
Supplementary Figure 2. Characterization of GFP-VAMP3 distribution. COS-7 cells were transfected with GFP-VAMP3 (B-D) or together with Tgn38-CFP (A). Cell were either fixed and stained (B,D) or incubated with Alexa Fluor 555-conjugated Tf for 10 min followed by 30min chase in complete medium prior to fixation (C). Scale bars: 10 μm.

A
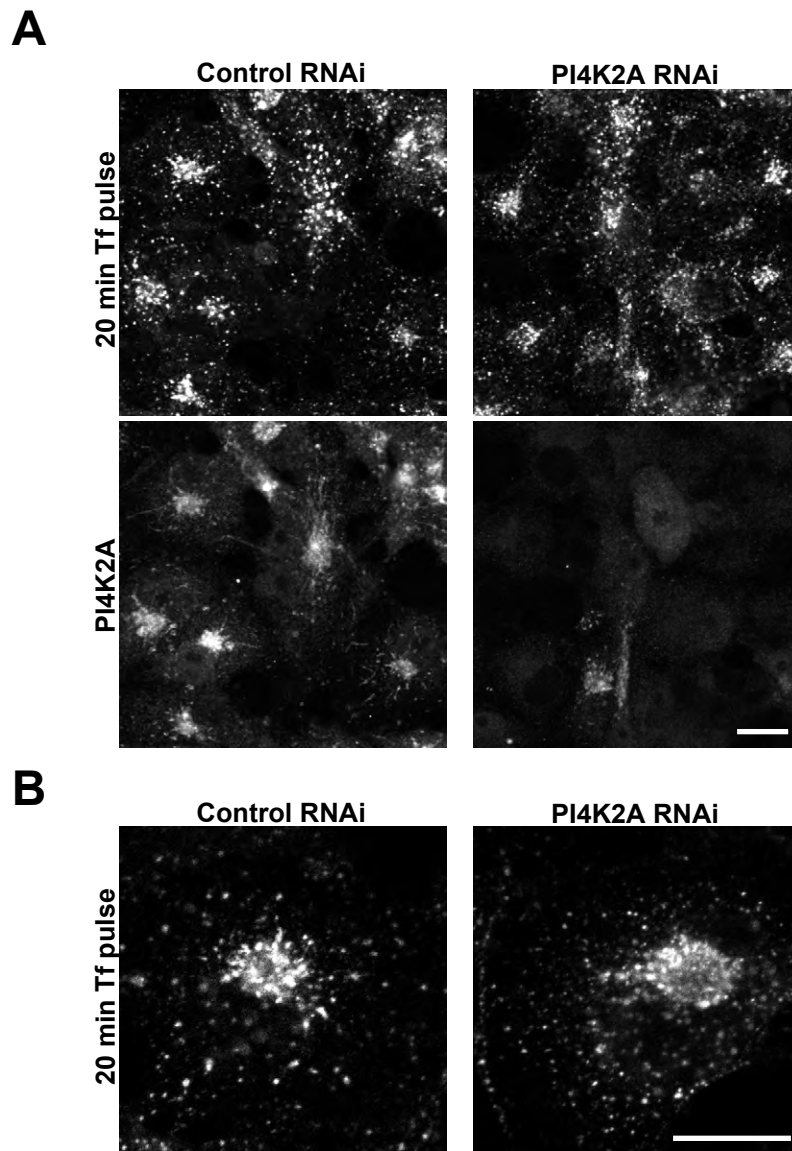
Supplementary Figure 3. PI4K2A knockdown does not disrupt ERC morphology. (A,B) COS-7 cells pre-treated with control siRNA or siRNA directed against PI4K2A were serum-starved for 30 min, incubated with Alexa Fluor 488-conjugated Tf for 20 min and fixed. Cells were then stained with antibody directed against PI4K2A (A, bottom panel). Prolonged pulse, shown here, results in appearance of Tf in the ERC even in PI4K2A-depleted cells (A, upper panel). (B) Tf in the ERC of control and PI4K2A-depleted cell after 20 min pulse.

Bait ID	Hit Gene Name	Pep #	Unique Pep #	Peptide
1724	VAMP7	1	1	AQTALPYAMNSEFSSVLAAQLK
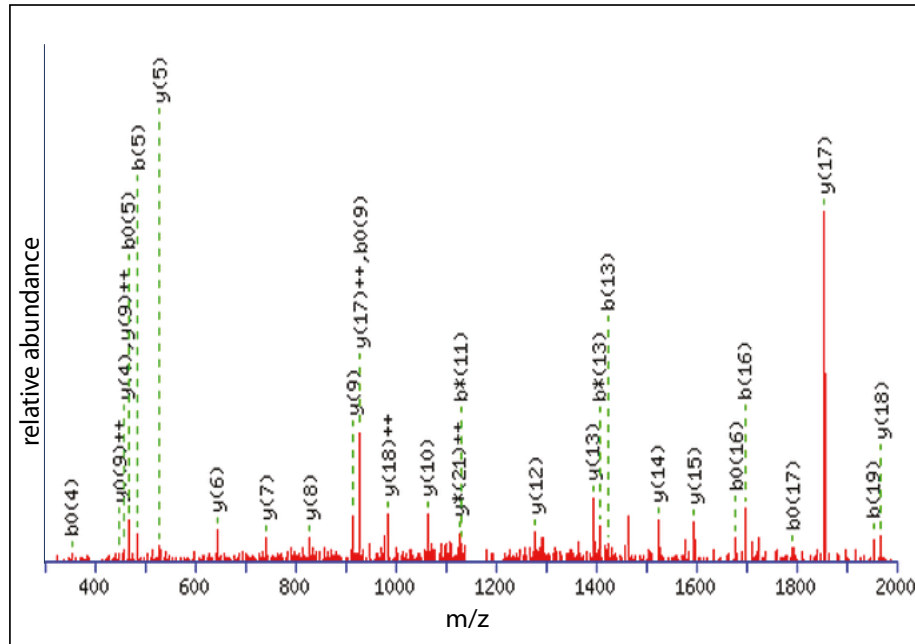
Monoisotopic mass of neutral peptide Mr(calc): 2339.18

Ions Score: 92

Matches (Bold Red): 29/232 fragment ions using 40 most intense peaks

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	72.04	36.53					A							22
2	200.10	100.56	183.08	92.04			Q	2269.15	1135.08	2252.13	**1126.57**	2251.14	1126.08	21
3	301.15	151.08	284.12	142.57	283.14	142.07	T	2141.09	1071.05	2124.07	1062.54	2123.08	1062.05	20
4	372.19	186.60	355.16	178.08	**354.18**	177.59	A	2040.05	1020.53	2023.02	1012.01	2022.04	1011.52	19
5	**485.27**	243.14	468.25	234.63	**467.26**	234.13	L	**1969.01**	**985.01**	1951.98	976.50	1951.00	976.00	18
6	582.32	291.67	565.30	283.15	564.31	282.66	P	**1855.93**	**928.47**	1838.90	919.95	1837.92	919.46	17
7	745.39	373.20	728.36	364.68	727.38	364.19	Y	1758.87	879.94	1741.85	871.43	1740.86	870.93	16
8	816.43	408.72	799.40	400.20	798.41	399.71	A	**1595.81**	798.41	1578.78	789.90	1577.80	789.40	15
9	947.47	474.24	930.44	465.72	**929.45**	465.23	M	**1524.77**	762.89	1507.75	754.38	1506.76	753.88	14
10	1061.51	531.26	1044.48	522.74	1043.50	522.25	N	**1393.73**	697.37	1376.71	688.86	1375.72	688.36	13
11	1148.54	574.77	**1131.51**	566.26	1130.53	565.77	S	**1279.69**	640.35	1262.66	631.83	1261.68	631.34	12
12	1277.58	639.30	1260.56	630.78	1259.57	630.29	E	1192.66	596.83	1175.63	588.32	1174.65	587.83	11
13	**1424.65**	712.83	**1407.62**	704.32	1406.64	703.82	F	**1063.61**	532.31	1046.59	523.80	1045.60	523.31	10
14	1511.68	756.35	1494.66	747.83	1493.67	747.34	S	**916.55**	**458.78**	899.52	450.26	898.54	**449.77**	9
15	1598.72	799.86	1581.69	791.35	1580.70	790.86	S	**829.51**	415.26	812.49	406.75	811.50	406.26	8
16	**1697.78**	849.40	1680.76	840.88	**1679.77**	840.39	V	**742.48**	371.74	725.46	363.23			7
17	1810.87	905.94	1793.84	897.42	**1792.86**	896.93	L	**643.41**	322.21	626.39	313.70			6
18	1881.91	941.46	1864.88	932.94	1863.89	932.45	A	**530.33**	265.67	513.30	257.16			5
19	**1952.94**	976.97	1935.92	968.46	1934.93	967.97	A	**459.29**	230.15	442.27	221.64			4
20	2081.00	1041.00	2063.97	1032.49	2062.99	1032.00	Q	388.26	194.63	371.23	186.12			3
21	2194.08	1097.55	2177.06	1089.03	2176.07	1088.54	L	260.20	130.60	243.17	122.09			2
22							K	147.11	74.06	130.09	65.55			1

Supplementary Figure 4. MS/MS spectra for VAMP7. "Bait ID" is the unique identifier for the sample in ProHIts our internal interaction database (Liu et al., 2010). "Hit Gene Name" is the gene name of the interactor. "Pep #" is the total number of peptides. "Unique Pep #" is the number of unique peptides (as defined by the Mascot search engine). VAMP7 was identified with only one peptide in one PI4K2A biological replicate (1724) and thus did not meet our cutoffs to be identified as a high-confidence interactor.

Bait ID	Bait Gene Name	Hit Gene Name	Hit Gene ID	Hit Protein ID	Hit Score	Pep #	Unique Pep #	Coverage	Freq
1st rep									
1724	PI4K2A	PI4K2A	55361	13559514	2160	775	31	61.4	0.45
1724	PI4K2A	GABARAPL2	11345	6005768	96	5	3	28.2	0.31
1724	PI4K2A	GNA11	2767	115511049	116	4	3	6.7	1.48
1724	PI4K2A	SLC25A4	291	55749577	169	9	3	10.1	4.04
1724	PI4K2A	TNFRSF10B	8795	22547116	134	7	3	7.7	0.31
1724	PI4K2A	ABCB1	5243	42741659	171	2	2	3.7	0.54
1724	PI4K2A	DSG2	1829	116534898	94	5	2	2.9	4.8
1724	PI4K2A	IFNGR1	3459	4557880	90	3	2	7.2	0.22
1724	PI4K2A	TMEM59	9528	20070191	109	6	2	7.7	0.36
1724	PI4K2A	WDR6	11180	11072093	87	3	2	3.9	2.2
1724	PI4K2A	LRP10	26020	32490559	120	2	1	2.9	0.27
1724	PI4K2A	SDCBP	6386	55749490	129	3	1	6.4	0.4
1724	PI4K2A	VAMP3	9341	4759300	89	1	1	17	1.75
2nd rep									
1748	PI4K2A	PI4K2A	55361	13559514	2118	674	33	66.6	0.45
1748	PI4K2A	DSG2	1829	116534898	406	14	7	10.2	4.8
1748	PI4K2A	IFNGR1	3459	4557880	229	5	4	14.1	0.22
1748	PI4K2A	SDCBP	6386	55749490	228	4	3	17.8	0.4
1748	PI4K2A	TNFRSF10B	8795	22547116	157	5	3	9.5	0.31
1748	PI4K2A	VAMP3	9341	4759300	224	5	3	40	1.75
1748	PI4K2A	WDR6	11180	11072093	123	3	3	2.9	2.2
1748	PI4K2A	ABCB1	5243	42741659	196	6	2	3.3	0.54
1748	PI4K2A	GABARAPL2	11345	6005768	104	3	2	22.2	0.31
1748	PI4K2A	GNA11	2767	115511049	137	3	2	10.3	1.48
1748	PI4K2A	LRP10	26020	32490559	134	4	2	4.6	0.27
1748	PI4K2A	SLC25A4	291	55749577	118	5	2	7.4	4.04
1748	PI4K2A	TMEM59	9528	20070191	94	5	2	7.7	0.36

Supplementary Table 1. Detailed summary of AP-MS data for PI4K2A interaction partners. "Bait ID" is the unique identifier for the sample in ProHIts, our internal interaction database (Liu et al., 2010). "Bait Gene Name" is the gene name of the bait (referred throughout the paper as PI4K2A). "Hit Gene Name" is the HUGO gene name of each filtered interactor. "Hit Gene ID" is the NCBI Gene ID for each hit. "Hit Protein ID" is the NCBI protein ID which can be used to refer to the protein sequence. "Hit Score" is the score from the Mascot search engine. "Pep #" is the total number of peptides, or spectral counts. "Unique Pep #" is the number of unique peptides (as defined by the Mascot search engine). "Coverage" is the percent of the protein sequence that has been detected by mass spectrometry. "Freq" is the frequency with which the protein is identified in our internal SLRI data base.