

SUPPLEMENTARY TABLES S1-10

RNF13 dileucine motif variants L311S and L312P interfere with endosomal localization and AP-3 complex association

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Table S1. Statistical values - Manders overlap coefficient, Figure 1A-E

Marker		RNF13 WT	RNF13 L311S	RNF13 L312P
EEA1 (panel A)	Number of cells analyzed (after removal of outliers)	29	30	30
	Mean \pm SEM	0.0825 \pm 0.0075	0.0849 \pm 0.0076	0.0973 \pm 0.0070
	Exact p value (WT)		>0.9999	0.3167
	Exact p value (L311S)	>0.9999		0.6795
	Exact p value (L312P)	0.3167	0.6795	
Lamp 1 (panel B)	Number of cells analyzed (after removal of outliers)	29	30	30
	Mean \pm SEM	0.2778 \pm 0.0152	0.1435 \pm 0.0151	0.1726 \pm 0.0160
	Exact p value (WT)		<0.0001	0.0004
	Exact p value (L311S)	<0.0001		0.6402
	Exact p value (L312P)	0.0004	0.6402	
Rab5 (panel C)	Number of cells analyzed (after removal of outliers)	30	29	30
	Mean \pm SEM	0.2141 \pm 0.0186	0.2210 \pm 0.0198	0.2068 \pm 0.0169
	Exact p value (WT)		>0.9999	>0.9999
	Exact p value (L311S)	>0.9999		>0.9999
	Exact p value (L312P)	>0.9999	>0.9999	
Rab7 (panel D)	Number of cells analyzed (after removal of outliers)	30	30	30
	Mean \pm SEM	0.3669 \pm 0.0188	0.3224 \pm 0.0228	0.3145 \pm 0.0195
	Exact p value (WT)		0.2209	0.2197
	Exact p value (L311S)	0.2209		>0.9999
	Exact p value (L312P)	0.2197	>0.9999	
Rab9 (panel E)	Number of cells analyzed (after removal of outliers)	30	30	30
	Mean \pm SEM	0.3606 \pm 0.0275	0.3419 \pm 0.0196	0.3731 \pm 0.0205
	Exact p value (WT)		>0.9999	>0.9999
	Exact p value (L311S)	>0.9999		0.9260
	Exact p value (L312P)	>0.9999	0.9260	

Table S2. Statistical values - Vesicle diameter, Figure 1F-J

Marker		NT	RNF13 WT	RNF13 L311S	RNF13 L312P
EEA1 (panel F)	Number of vesicles analyzed (after removal of outliers)	75	71	75	74
	Mean \pm SEM (μ m)	0.4048 \pm 0.0089	0.3877 \pm 0.0093	0.5119 \pm 0.0126	0.5151 \pm 0.0100
	Exact p value (NT)		>0.9999	<0.0001	<0.0001
	Exact p value (WT)	>0.9999		<0.0001	<0.0001
	Exact p value (L311S)	<0.0001	<0.0001		>0.9999
	Exact p value (L312P)	<0.0001	<0.0001	>0.9999	
Lamp 1 (panel G)	Number of vesicles analyzed (after removal of outliers)	75	75	74	73
	Mean \pm SEM (μ m)	0.6415 \pm 0.0124	0.6365 \pm 0.0138	0.8104 \pm 0.0238	0.7541 \pm 0.0183
	Exact p value (NT)		>0.9999	<0.0001	<0.0001
	Exact p value (WT)	>0.9999		<0.0001	<0.0001
	Exact p value (L311S)	<0.0001	<0.0001		>0.9999
	Exact p value (L312P)	<0.0001	<0.0001	>0.9999	
Rab5 (panel H)	Number of vesicles analyzed (after removal of outliers)	75	75	75	75
	Mean \pm SEM (μ m)	0.5175 \pm 0.0106	0.5161 \pm 0.0083	0.6325 \pm 0.0088	0.6520 \pm 0.0089
	Exact p value (NT)		>0.9999	<0.0001	<0.0001
	Exact p value (WT)	>0.9999		<0.0001	<0.0001
	Exact p value (L311S)	<0.0001	<0.0001		>0.9999
	Exact p value (L312P)	<0.0001	<0.0001	>0.9999	
Rab7 (panel I)	Number of vesicles analyzed (after removal of outliers)	75	75	75	75
	Mean \pm SEM (μ m)	0.7512 \pm 0.0128	0.7679 \pm 0.0122	1.061 \pm 0.0170	1.012 \pm 0.0144
	Exact p value (NT)		>0.9999	<0.0001	<0.0001
	Exact p value (WT)	>0.9999		<0.0001	<0.0001
	Exact p value (L311S)	<0.0001	<0.0001		>0.9999
	Exact p value (L312P)	<0.0001	<0.0001	>0.9999	
Rab9 (panel J)	Number of vesicles analyzed (after removal of outliers)	75	75	75	75
	Mean \pm SEM (μ m)	0.7947 \pm 0.0115	0.8017 \pm 0.0108	0.6149 \pm 0.0090	0.6089 \pm 0.0101
	Exact p value (NT)		>0.9999	<0.0001	<0.0001
	Exact p value (WT)	>0.9999		<0.0001	<0.0001
	Exact p value (L311S)	<0.0001	<0.0001		>0.9999
	Exact p value (L312P)	<0.0001	<0.0001	>0.9999	

Table S3. Statistical values - Number of punctae (EGF), Figure 2B

Time		NT	RNF13 WT	RNF13 L311S	RNF13 L312P
0 min	Number of cells analyzed (after removal of outliers)	44	41	45	43
	Mean \pm SEM	264 \pm 19	221 \pm 20	220 \pm 41	228 \pm 38
	Exact p value (NT)		0.3787	0.3478	0.9996
	Exact p value (WT)	0.3787		>0.9999	0.4402
	Exact p value (L311S)	0.3478	>0.9999		0.4071
	Exact p value (L312P)	0.9996	0.4402	0.4071	
15 mins	Number of cells analyzed (after removal of outliers)	44	46	48	43
	Mean \pm SEM	157 \pm 14	154 \pm 10	152 \pm 14	156 \pm 16
	Exact p value (NT)		0.9994	0.9978	>0.9999
	Exact p value (WT)	0.9994		>0.9999	0.9998
	Exact p value (L311S)	0.9978	>0.9999		0.9988
	Exact p value (L312P)	>0.9999	0.9998	0.9988	
30 mins	Number of cells analyzed (after removal of outliers)	46	46	45	42
	Mean \pm SEM	108 \pm 7	115 \pm 9	98 \pm 7	103 \pm 8
	Exact p value (NT)		0.9939	0.9788	0.9976
	Exact p value (WT)	0.9939		0.9138	0.9701
	Exact p value (L311S)	0.9788	0.9138		0.9968
	Exact p value (L312P)	0.9976	0.9701	0.9968	
60 mins	Number of cells analyzed (after removal of outliers)	46	47	50	45
	Mean \pm SEM	55 \pm 5	76 \pm 9	84 \pm 7	83 \pm 6
	Exact p value (NT)		0.8489	0.6785	0.7015
	Exact p value (WT)	0.8489		0.9898	0.9930
	Exact p value (L311S)	0.6785	0.9898		>0.9999
	Exact p value (L312P)	0.7015	0.9930	>0.9999	

Table S4. Statistical values - Pearson's coefficient (EGF), Figure 2C

Time		NT	RNF13 WT	RNF13 L311S	RNF13 L312P
0 min	Number of cells analyzed (after removal of outliers)	45	40	38	42
	Mean \pm SEM	0.363 \pm 0.008	0.355 \pm 0.012	0.307 \pm 0.005	0.282 \pm 0.010
	Exact p value (NT)		0.9558	0.0041	<0.0001
	Exact p value (WT)	0.9558		0.0155	0.0002
	Exact p value (L311S)	0.0041	0.0155		0.3568
	Exact p value (L312P)	<0.0001	0.0002	0.3568	
15 mins	Number of cells analyzed (after removal of outliers)	45	45	44	42
	Mean \pm SEM	0.222 \pm 0.005	0.259 \pm 0.013	0.275 \pm 0.003	0.275 \pm 0.022
	Exact p value (NT)		0.0927	0.0070	0.0066
	Exact p value (WT)	0.0927		0.6983	0.6850
	Exact p value (L311S)	0.0070	0.6983		>0.9999
	Exact p value (L312P)	0.0066	0.6850	>0.9999	
30 mins	Number of cells analyzed (after removal of outliers)	45	37	44	42
	Mean \pm SEM	0.155 \pm 0.014	0.239 \pm 0.030	0.214 \pm 0.020	0.202 \pm 0.006
	Exact p value (NT)		0.0093	0.0020	0.0173
	Exact p value (WT)	0.0093		0.9379	0.9948
	Exact p value (L311S)	0.0020	0.9379		0.8432
	Exact p value (L312P)	0.0173	0.9948	0.8432	
60 mins	Number of cells analyzed (after removal of outliers)	45	45	41	43
	Mean \pm SEM	0.073 \pm 0.004	0.077 \pm 0.005	0.093 \pm 0.008	0.082 \pm 0.004
	Exact p value (NT)		0.9932	0.5486	0.9312
	Exact p value (WT)	0.9932		0.7115	0.9870
	Exact p value (L311S)	0.5486	0.7115		0.8827
	Exact p value (L312P)	0.9312	0.9870	0.8827	

Table S5. Statistical values - Number of punctae (Tf) Figure S2B

Time		NT	RNF13 WT	RNF13 L311S	RNF13 L312P
0 min	Number of cells analyzed (after removal of outliers)	40	43	39	44
	Mean \pm SEM	473 \pm 38	368 \pm 33	434 \pm 46	406 \pm 38
	Exact p value (NT)		0.2133	0.8888	0.6000
	Exact p value (WT)	0.2133		0.6058	0.8925
	Exact p value (L311S)	0.8888	0.6058		0.9527
	Exact p value (L312P)	0.6000	0.8925	0.9527	
15 mins	Number of cells analyzed (after removal of outliers)	42	44	36	45
	Mean \pm SEM	392 \pm 64	317 \pm 56	327 \pm 69	295 \pm 54
	Exact p value (NT)		0.5126	0.6643	0.2830
	Exact p value (WT)	0.5126		0.9980	0.9764
	Exact p value (L311S)	0.6643	0.9980		0.9409
	Exact p value (L312P)	0.2830	0.9764	0.9409	
30 mins	Number of cells analyzed (after removal of outliers)	40	42	42	44
	Mean \pm SEM	261 \pm 28	233 \pm 30	172 \pm 41	158 \pm 14
	Exact p value (NT)		0.9557	0.3634	0.2347
	Exact p value (WT)	0.9557		0.6752	0.5088
	Exact p value (L311S)	0.3634	0.6752		0.9934
	Exact p value (L312P)	0.2347	0.5088	0.9934	
60 mins	Number of cells analyzed (after removal of outliers)	31	36	34	33
	Mean \pm SEM	5 \pm 1	16 \pm 3	4 \pm 1	8 \pm 2
	Exact p value (NT)		0.9968	>0.9999	>0.9999
	Exact p value (WT)	0.9968		0.9957	0.9985
	Exact p value (L311S)	>0.9999	0.9957		0.9999
	Exact p value (L312P)	>0.9999	0.9985	0.9999	

Table S6. Statistical values - Pearson's coefficient (Tf), Figure S2C

Time		NT	RNF13 WT	RNF13 L311S	RNF13 L312P
0 min	Number of cells analyzed (after removal of outliers)	42	38	39	42
	Mean \pm SEM	0.398 \pm 0.012	0.397 \pm 0.028	0.404 \pm 0.009	0.405 \pm 0.006
	Exact p value (NT)		>0.9999	0.9987	0.9979
	Exact p value (WT)	>0.9999		0.9982	0.9972
	Exact p value (L311S)	0.9987	0.9982		>0.9999
	Exact p value (L312P)	0.9979	0.9972	>0.9999	
15 mins	Number of cells analyzed (after removal of outliers)	40	41	36	38
	Mean \pm SEM	0.340 \pm 0.008	0.371 \pm 0.030	0.391 \pm 0.028	0.403 \pm 0.048
	Exact p value (NT)		0.8595	0.5741	0.3878
	Exact p value (WT)	0.8595		0.9573	0.8437
	Exact p value (L311S)	0.5741	0.9573		0.9888
	Exact p value (L312P)	0.3878	0.8437	0.9888	
30 mins	Number of cells analyzed (after removal of outliers)	43	39	41	40
	Mean \pm SEM	0.328 \pm 0.027	0.353 \pm 0.013	0.348 \pm 0.033	0.385 \pm 0.041
	Exact p value (NT)		0.9115	0.9552	0.4654
	Exact p value (WT)	0.9115		0.9989	0.8477
	Exact p value (L311S)	0.9552	0.9989		0.7734
	Exact p value (L312P)	0.4654	0.8477	0.7734	
60 mins	Number of cells analyzed (after removal of outliers)	41	40	35	42
	Mean \pm SEM	0.322 \pm 0.009	0.292 \pm 0.019	0.321 \pm 0.015	0.358 \pm 0.052
	Exact p value (NT)		0.8671	>0.9999	0.7965
	Exact p value (WT)	0.8671		0.8818	0.3472
	Exact p value (L311S)	>0.9999	0.8818		0.7781
	Exact p value (L312P)	0.7965	0.3472	0.7781	

Table S7. Statistical values – Manders overlap coefficient, Figure 3A-C

Marker		RNF13 WT	RNF13 LL / AA
EEA1 (panel A)	Number of cells analyzed (after removal of outliers)	30	28
	Mean \pm SEM	0.07313 \pm 0.00614	0.05979 \pm 0.00673
	Exact p value		0.0631
Lamp 1 (panel B)	Number of cells analyzed (after removal of outliers)	30	29
	Mean \pm SEM	0.3095 \pm 0.0209	0.1688 \pm 0.0195
	Exact p value		<0.0001
Rab7 (panel C)	Number of cells analyzed (after removal of outliers)	30	30
	Mean \pm SEM	0.2866 \pm 0.0154	0.2794 \pm 0.0154
	Exact p value		0.5945

Table S8. Statistical values – Vesicle diameter, Figure 3D-F

Marker		NT	RNF13 WT	RNF13 LL / AA
EEA1 (panel D)	Number of vesicles analyzed (after removal of outliers)	75	75	75
	Mean \pm SEM (μm)	0.4747 \pm 0.0079	0.4701 \pm 0.0082	0.5828 \pm 0.0112
	Exact p value (NT)		>0.9999	<0.0001
	Exact p value (WT)	>0.9999		<0.0001
	Exact p value (LL/AA)	<0.0001	<0.0001	
Lamp 1 (panel E)	Number of vesicles analyzed (after removal of outliers)	73	75	75
	Mean \pm SEM (μm)	0.6129 \pm 0.0110	0.6307 \pm 0.0118	0.7957 \pm 0.0131
	Exact p value (NT)		0.9636	<0.0001
	Exact p value (WT)	0.9636		<0.0001
	Exact p value (LL/AA)	<0.0001	<0.0001	
Rab7 (panel F)	Number of vesicles analyzed (after removal of outliers)	75	75	75
	Mean \pm SEM (μm)	0.6941 \pm 0.0108	0.7197 \pm 0.0132	0.8659 \pm 0.0118
	Exact p value (NT)		0.5839	<0.0001
	Exact p value (WT)	0.5839		<0.0001
	Exact p value (LL/AA)	<0.0001	<0.0001	

Table S9. Statistical values – Manders overlap coefficient, Figure 6C and 6F

Marker		CTL	si#1	si#2
Lamp1 (panel C)	Number of cells analyzed (after removal of outliers)	30	30	30
	Mean \pm SEM	0.3161 \pm 0.0207	0.1869 \pm 0.0195	0.1635 \pm 0.0165
	Exact p value (CTL)		0.0001	<0.0001
	Exact p value (si#1)	0.0001		>0.9999
	Exact p value (si#2)	<0.0001	>0.9999	
Rab7 (panel F)	Number of cells analyzed (after removal of outliers)	29	30	30
	Mean \pm SEM	0.3722 \pm 0.0324	0.3221 \pm 0.0265	0.3389 \pm 0.0277
	Exact p value (CTL)		>0.9999	>0.9999
	Exact p value (si#1)	>0.9999		>0.9999
	Exact p value (si#2)	>0.9999	>0.9999	

Table S10. Statistical values – Vesicle diameter, Figure 6D and G

Marker		CTL+ vector	CTL+ WT	si#1+ vector	si#1+ WT	si#2+ vector	si#2+ WT
Lamp1 (panel D)	Number of vesicles analyzed (after removal of outliers)	75	72	75	75	75	73
	Mean \pm SEM (μ m)	0.6621 \pm 0.0118	0.6244 \pm 0.0107	0.8384 \pm 0.0150	0.8488 \pm 0.0151	0.8296 \pm 0.0126	0.8338 \pm 0.0136
	Exact p value (CTL+vector)		>0.9999	<0.0001	<0.0001	<0.0001	<0.0001
	Exact p value (CTL+WT)	>0.9999		<0.0001	<0.0001	<0.0001	<0.0001
	Exact p value (si#1+vector)	<0.0001	<0.0001		>0.9999	>0.9999	>0.9999
	Exact p value (si#1+WT)	<0.0001	<0.0001	>0.9999		>0.9999	>0.9999
	Exact p value (si#2+vector)	<0.0001	<0.0001	>0.9999	>0.9999		>0.9999
	Exact p value (si#2+WT)	<0.0001	<0.0001	>0.9999	>0.9999	>0.9999	
Rab7 (panel G)	Number of vesicles analyzed (after removal of outliers)	75	75	75	75	75	75
	Mean \pm SEM (μ m)	0.7037 \pm 0.0116	0.7183 \pm 0.0102	0.8689 \pm 0.0115	0.8692 \pm 0.0133	0.8577 \pm 0.0116	0.8637 \pm 0.0133
	Exact p value (CTL+vector)		>0.9999	<0.0001	<0.0001	<0.0001	<0.0001
	Exact p value (CTL+WT)	>0.9999		<0.0001	<0.0001	<0.0001	<0.0001
	Exact p value (si#1+vector)	<0.0001	<0.0001		>0.9999	>0.9999	>0.9999
	Exact p value (si#1+WT)	<0.0001	<0.0001	>0.9999		>0.9999	>0.9999
	Exact p value (si#2+vector)	<0.0001	<0.0001	>0.9999	>0.9999		>0.9999
	Exact p value (si#2+WT)	<0.0001	<0.0001	>0.9999	>0.9999	>0.9999	