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**Appendix Figure S1. Aster-C is an ER protein.** Confocal image analysis depicting the colocalization of Aster-C with ER in COS-7 cells transiently expressing GFP-Aster-C and DsRed-ER under starvation and in response to AA stimulation. Scale bar, 5 µm.



**Appendix Figure S2. Network of Aster-C interacting proteins.** Co-immunoprecipitation and protein mass spectrometry analysis demonstrate that Aster-C mainly interacts with three clusters of protein families, including coatomer protein complex I (COP I), myosin and cytoskeleton related proteins, and the nuclear pore complex (NPC), which resembles the SEA-complex in budding yeast which contains TORC1 and its upstream regulators of GATOR1 and GATOR2. The protein interaction network was generated using the STRING online tool (<u>https://string-db.org/</u>).



**Appendix Figure S3. Arf1 deficiency inhibited mTORC1 activation and lysosomal association.** Confocal imaging analysis depicting the co-localization of mTOR with lysosomes in C2C12-Vector and Arf1 KO cells transiently expressing YFP-mTOR and stained with Lysotracker Red under starvation and in response to AA stimulation. Scale bar, 10 μm.



Expression cutoff	1.4 FPKM	2.2 FPKM
5-year survival high 5-year survival for patients with higher expression than the expression cutoff.	76%	78%
5-year survival low 5-year survival for patients with lower expression than the expression cutoff.	48%	59%
Log-rank P value for Kaplan-Meier plot showing results from analysis of correlation between mRNA expression level and patient survival.	4.22e-15 (Prognostic , favorable)	7.58e-10

Appendix Figure S4. The expression level of Aster-C is negatively correlated with the

## prognosis of human renal cancer

Data obtained from:

https://www.proteinatlas.org/ENSG00000178075-GRAMD1C/pathology