Amino acid metabolism is an essential part of cancer metabolism regulation. Here, we discovered that micropeptide hSPAR is downregulated in breast cancer and acts as a tumor suppressor through its cytoplasmic C-terminus (hSPAR-C). Mechanistically, we found that hSPAR (or hSPAR-C) is an inhibitor of cellular glutamine uptake in cancer cells and triggers lysosomal localization of P27KIP1, where P27KIP1 disrupts Ragulator complex and inactivates mTORC1 signaling. In addition, hSPAR (or hSPAR-C) can stabilize P27KIP1 through blocking P27KIP1’s interaction to its E3 ligase TRIM21. Interestingly, the accumulated P27KIP1 can only be translocated to lysosomes when hSPAR is overexpressed or simultaneously knocking down both TRIM21 and SLC38A2, the glutamine transporter that is downregulated by hSPAR. Moreover, we demonstrate that such effects of hSPAR do not occur in non-cancerous cells. Mouse tail vein injection of hSPAR-C dramatically prevents the growth of triple-negative breast cancer. Collectively, we define hSPAR as a cancer-specific glutamine metabolism inhibitor and uncover a previously unidentified hSPAR-P27KIP1-mTORC1 regulatory pathway.