Figure S1. Western blot analysis of c-Myc and GS expression, and global protein synthesis in MDA-MB-231 cells starved of glutamine and fed with asparagine. (A) MDA-MB-231 cells were cultured in the indicated medium for 24 or 72 h. γ-tubulin was used as the loading control. (B) Western blot analysis of global protein synthesis in MDA-MB-231 cells cultured for 24 h in the indicated medium. Puromycin (2.5 μM) was added during the last 10 min before sample collection. Puromycin incorporation was revealed with an anti-puromycin antibody. Vinculin was used as the loading control. (C) GS expression was analyzed in cells grown for 24 h in the medium indicated with or without the c-Myc inhibitor 10074-G5 (50 μM; indicated in the figure as G5). γ-tubulin was used as the loading control. Gln, glutamine; Asn, asparagine; GS, glutamine synthetase.

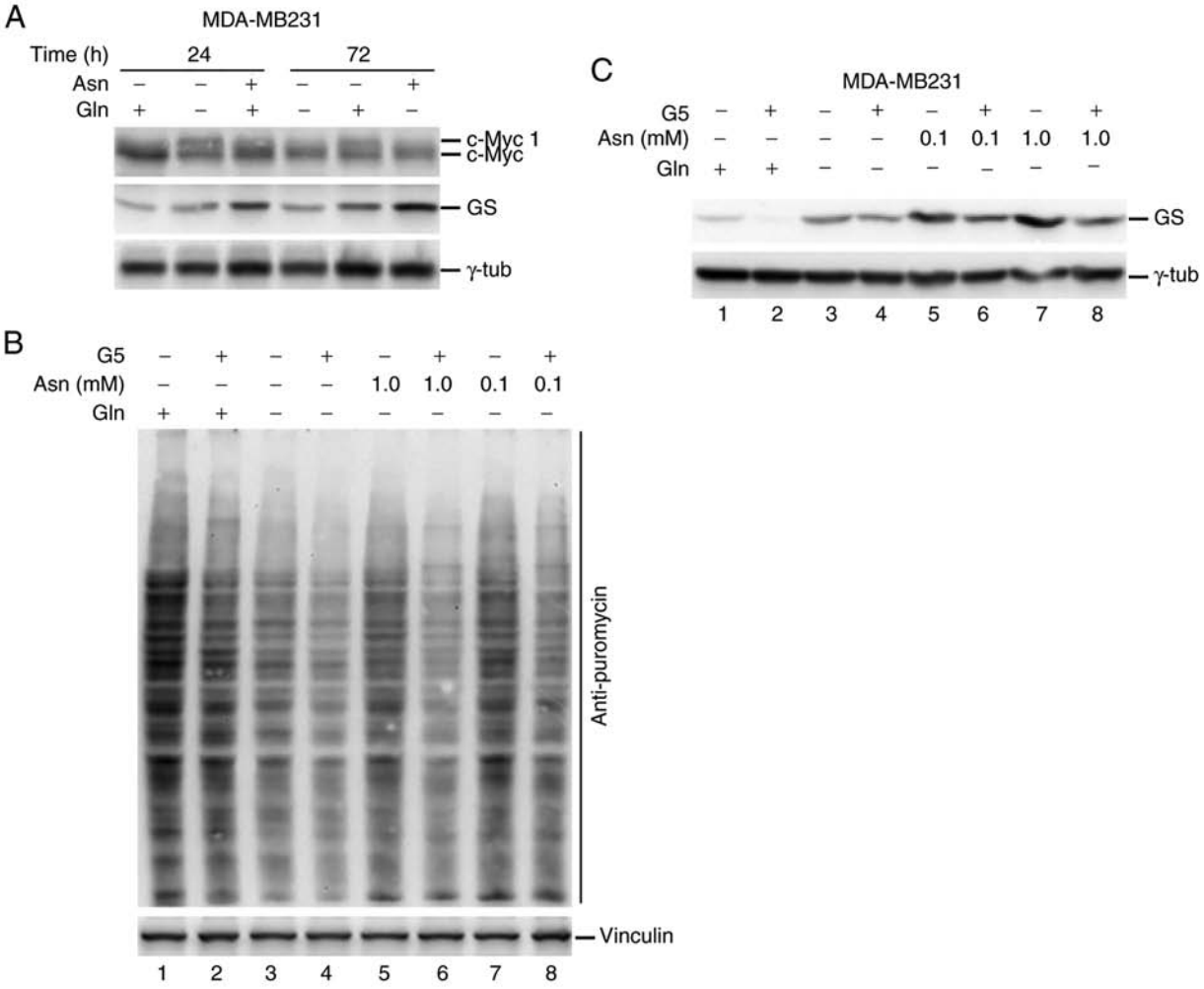

Figure S2. Short-term growth of glutamine-starved cells in the presence of single or pooled NEAAs. Cen3tel cells were cultured in the indicated medium for 24 h (A) or 24 and 72 h (B) NEAAs, a pool of alanine, asparagine, aspartic acid, glutamic acid and proline at a concentration of 0.1 mM each; single NEAAs were used at a concentration of 1 mM. Time 0 is the time point at which cells were incubated in the media with different compositions. NEAAs, non-essential amino acids; Ala, alanine; Asn, asparagine; Asp, aspartic acid; Glu, glutamic acid; Pro, proline; Gln, glutamine.