



**Figure S1.** Queuine addition to serum-free medium results in full Q-modification of histidyl and asparaginyl tRNA in *HeLa* cells. Cells were seeded at  $3 \times 10^4$  per  $\text{cm}^2$  in serum free medium (queuine deficient) for 5 days. They were then either supplemented with  $1 \mu\text{M}$  queuine base and incubated for 24 or 48 hours, or left untreated (U). RNA was isolated and the tRNA modification status determined by electrophoretic separation on APB gels, followed by Northern blotting. The tRNA signals were detected using  $^{32}\text{P}$ -labelled oligonucleotide probes specific for  $\text{tRNA}^{\text{His}}$  or  $\text{tRNA}^{\text{Asn}}$ .