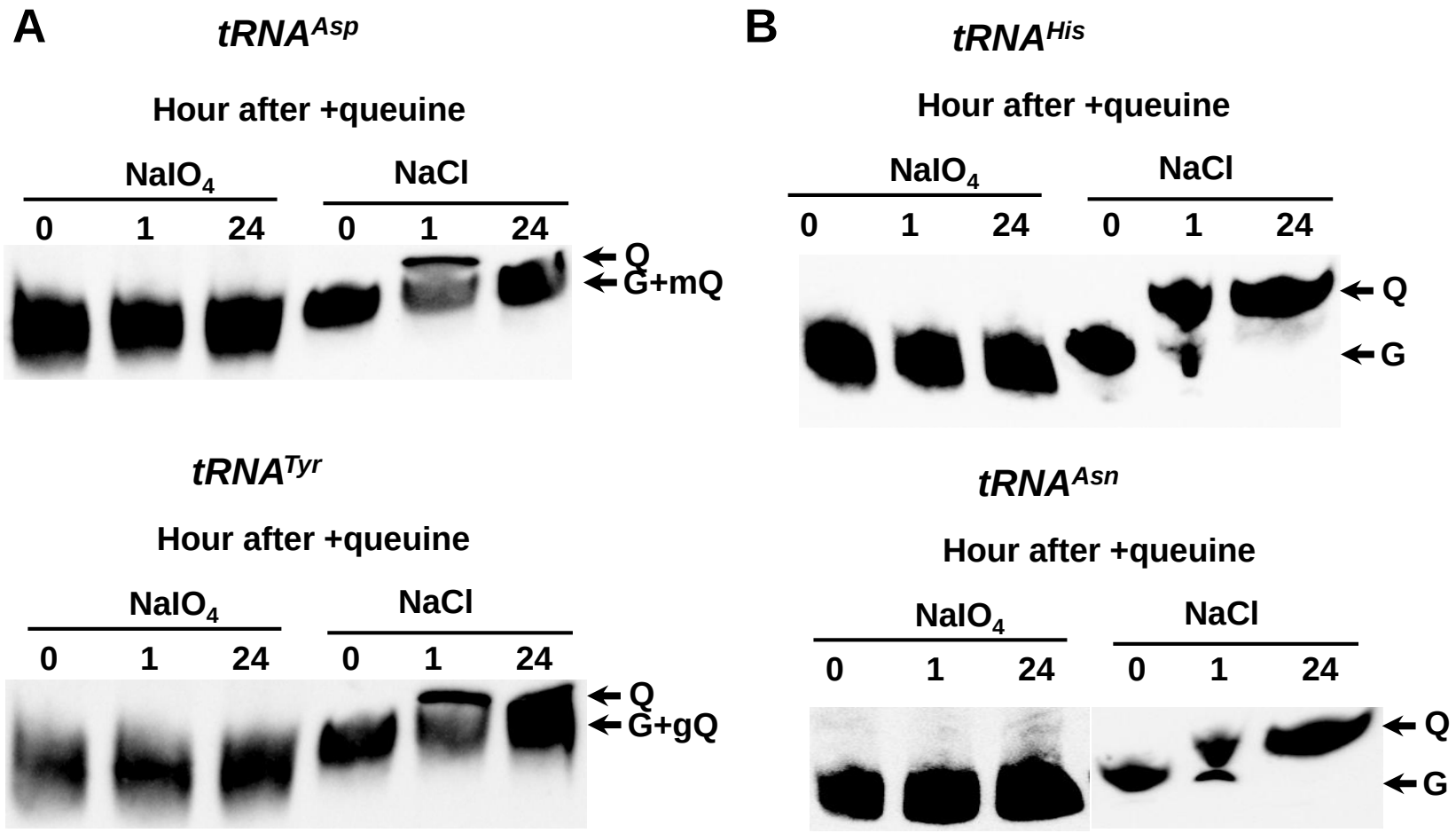

Figure S1: Analysis of Q and glycosylated Q-modified tRNAs using APB gels. Individual tRNA with ($NaIO_4$) and without (NaCl) periodate treatment. 0: total RNA from 0Q cells; 1, 24: total RNA from cells 1 and 24 hours after the addition of queuine to 0Q cells. Total RNA was from MCF7 cells.

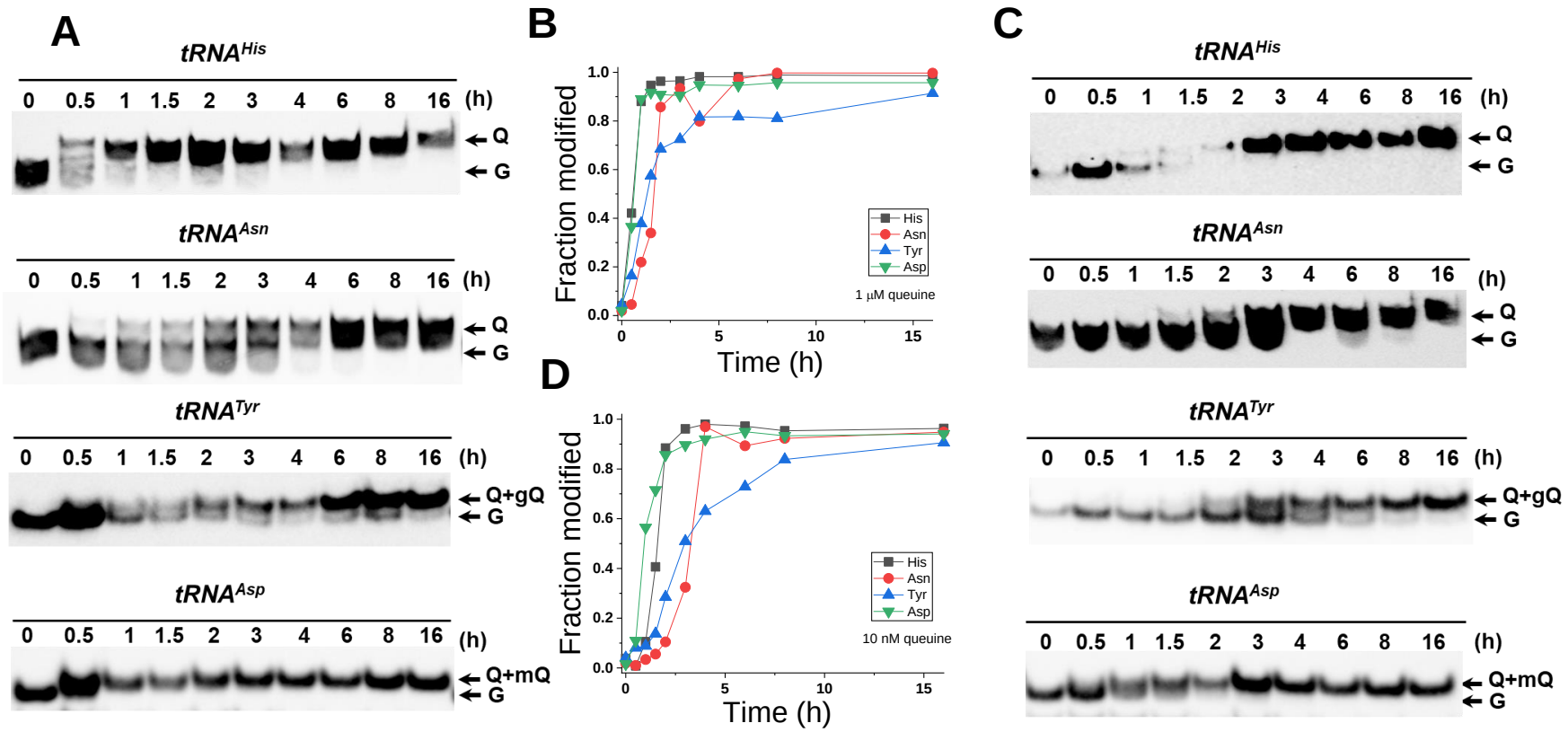

Figure S2: Q and glycosylated Q-modification kinetics of HeLa cells by APB ($tRNA^{His}$ and $tRNA^{Asn}$) and acid denaturing ($tRNA^{Tyr}$ and $tRNA^{Asp}$) gels. (A) At t = 0, 1 μM queuine was added to the medium of 0Q cells, total RNA was extracted after designated time points in hours. G: unmodified tRNA, Q: Q-modified tRNA, mQ: mannosyl-Q, gQ: galactosyl-Q. (B) Quantitative modification fraction over time for individual tRNAs, from 1 μM queuine data. For $tRNA^{His}$ and $tRNA^{Asn}$, the modification fraction represents Q; for $tRNA^{Asp}$ and $tRNA^{Tyr}$, the modification fraction represents the sum of the Q and glycosylated Q modifications. (C), (D) Same as (A), (B) except at t = 0, 10 nM queuine was added to the medium of 0Q cells.

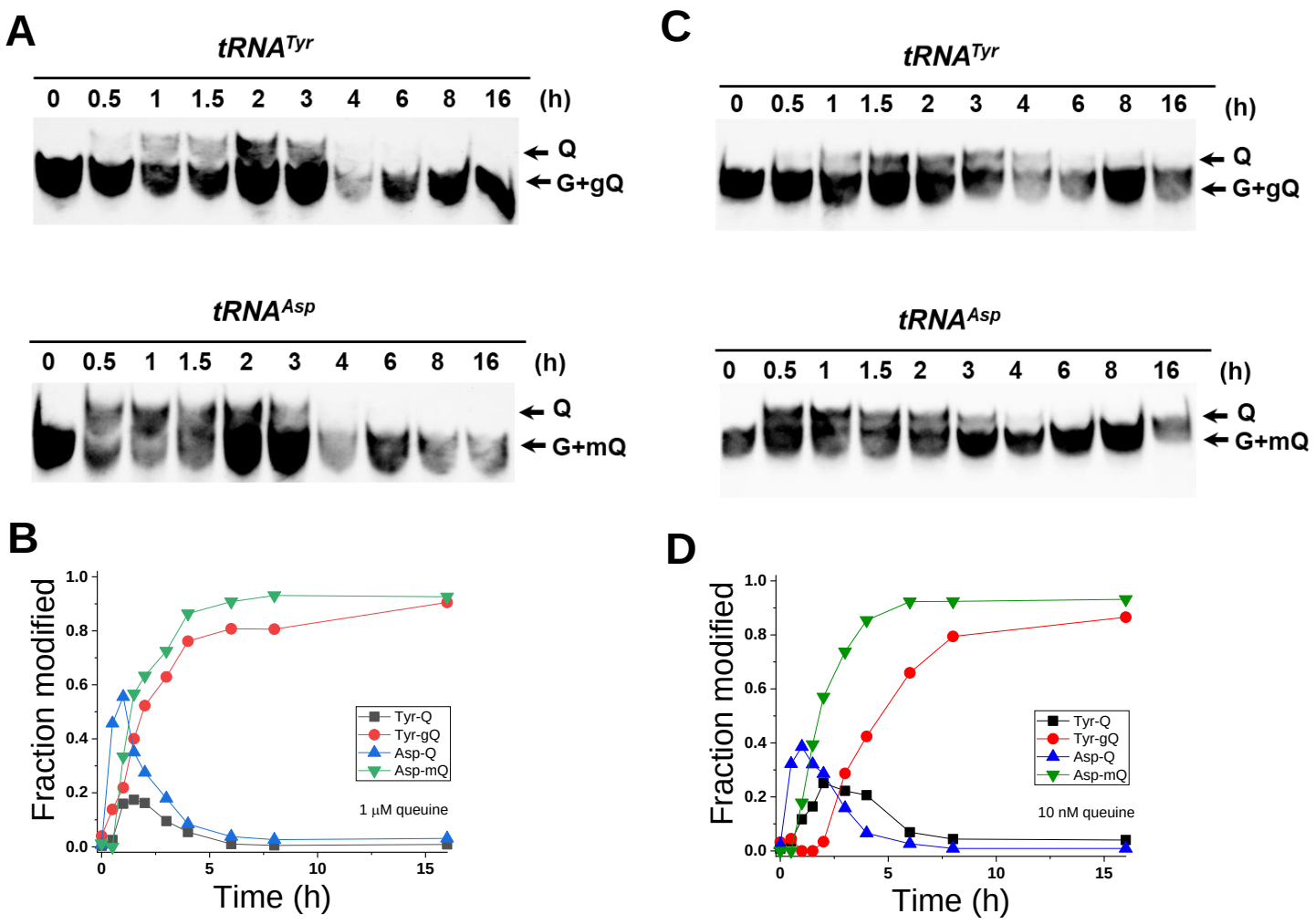

Figure S3: Q-modification kinetics of tRNA^{Asp} and tRNA^{Tyr} in HeLa cells by APB gels. (A) At t = 0, 1 μM queuine was added to the medium of 0Q cells, total RNA was extracted after designated time points in hours. G: unmodified tRNA, Q: Q-modified tRNA, mQ: mannosyl-Q, gQ: galactosyl-Q. The reversal to fast migration in later time points corresponds to gQ or mQ products as indicated by the acid denaturing gel results from Figure S2. (B) Quantitative modification fraction over time for individual tRNAs, from 1 μM queuine data. For comparison the total modification fraction from acid denaturing gel results in Figure S2 are also shown. (C), (D) Same as (A), (B) except at t = 0, 10 nM queuine was added to the medium of 0Q cells.

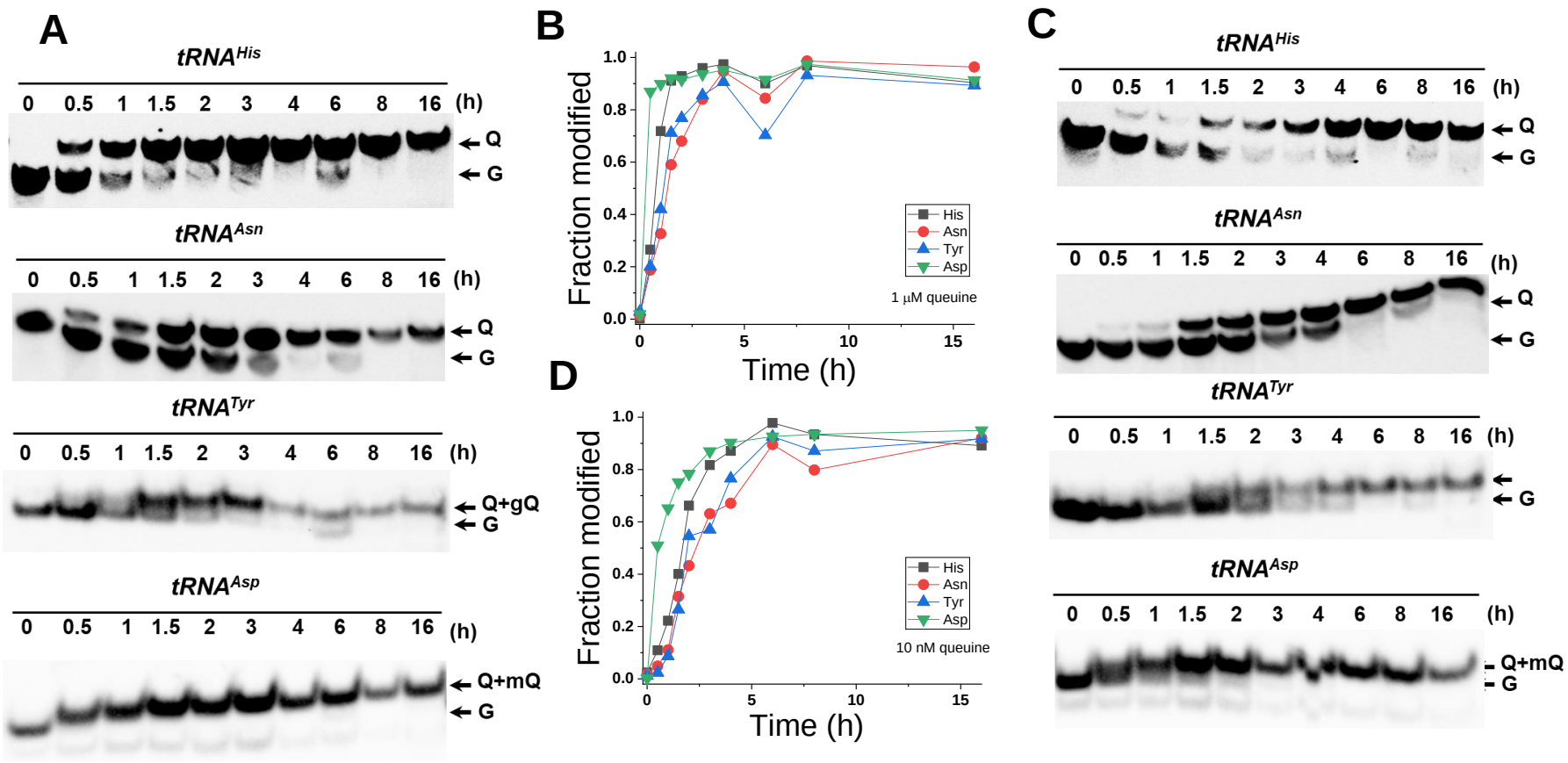

Figure S4: Q and glycosylated Q-modification kinetics of MCF7 cells by APB ($tRNA^{His}$ and $tRNA^{Asn}$) and acid denaturing ($tRNA^{Tyr}$ and $tRNA^{Asp}$) gels. (A) At t = 0, 1 μM queuine was added to the medium of 0Q cells, total RNA was extracted after designated time points in hours. G: unmodified tRNA, Q: Q-modified tRNA, mQ: mannosyl-Q, gQ: galactosyl-Q. (B) Quantitative modification fraction over time for individual tRNAs, from 1 μM queuine data. For $tRNA^{His}$ and $tRNA^{Asn}$, the modification fraction represents Q; for $tRNA^{Asp}$ and $tRNA^{Tyr}$, the modification fraction represents the sum of the Q and glycosylated Q modifications. (C), (D) Same as (A), (B) except at t = 0, 10 nM queuine was added to the medium of 0Q cells.

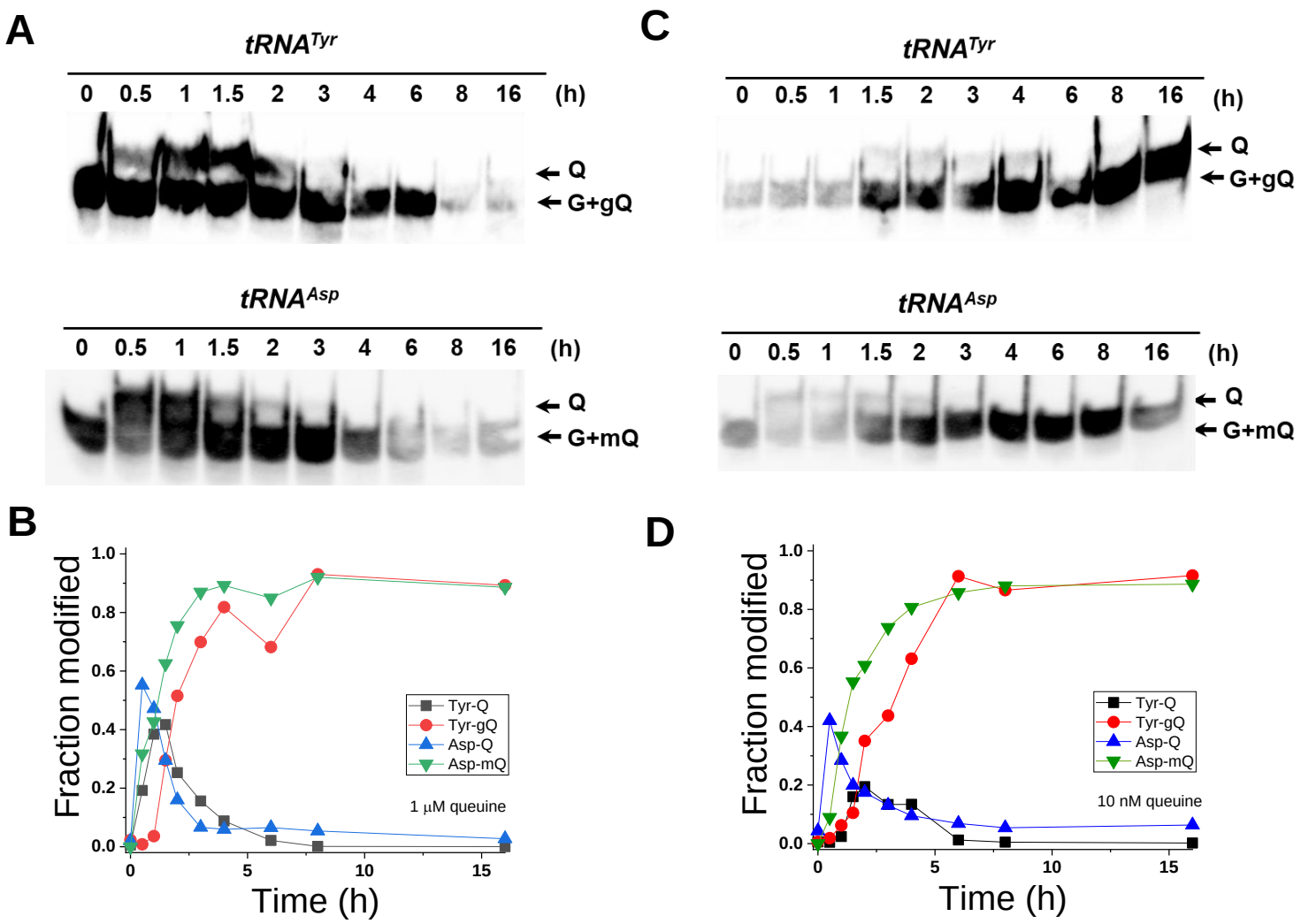

Figure S5: Q-modification kinetics of tRNA^{Asp} and tRNA^{Tyr} in MCF7 cells by APB gels. (A) At t = 0, 1 μM queuine was added to the medium of 0Q cells, total RNA was extracted after designated time points in hours. G: unmodified tRNA, Q: Q-modified tRNA, mQ: mannosyl-Q, gQ: galactosyl-Q. The reversal to fast migration in later time points corresponds to gQ or mQ products as indicated by the acid denaturing gel results from Figure S4. (B) Quantitative modification fraction over time for individual tRNAs, from 1 μM queuine data. For comparison the total modification fraction from acid denaturing gel results in Figure S4 are also shown. (C), (D) Same as (A), (B) except at t = 0, 10 nM queuine was added to the medium of 0Q cells.