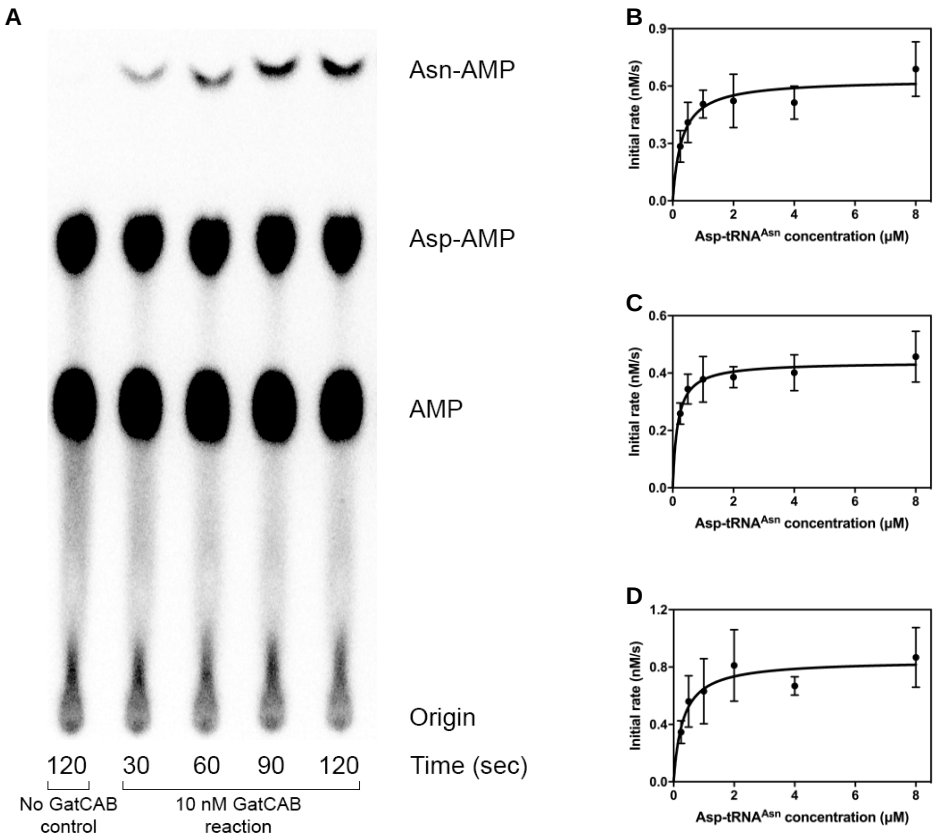

Supplementary Figure 2. GatCAB amidotransferase assay with ^{32}P-labeled tRNA.
(A) Representative phosphorimage of the separation of Asn-[α-^{32}P]AMP, Asp-[α-^{32}P]AMP, [α-^{32}P]AMP, by PEI cellulose chromatography. Aliquots (5 μL) of the amidotransferase reaction at 37 °C (10 nM GatCAB from *M. tuberculosis* , 0.5 μM Asp-tRNA^{Asn}, 1 mM Gln, 4 mM ATP, 25 mM KCl, 8 mM $MgCl_2$, 40 mM HEPES-KOH pH 7.5) were taken at the time points indicated and quenched / digested at 37 °C with 5 μL of 100 mM sodium citrate pH 4.7, and 0.66 mg/mL of nuclease P1. Digested samples (3 μL) were then spotted onto a 20 × 20 cm PEI-cellulose glass plate, which was developed in 10 mM ammonium chloride, 5% acetic acid for ~2 hours. Aliquot from no GatCAB reaction at 120 seconds was taken as the background control. Michaelis-Menten curves of WT (n = 4) **(B)**, G444S (n = 4) **(C)** and K61N (n = 3) **(D)** GatCAB are shown for their amidotransferase activity. GraphPad Prism was used to calculate kinetic parameters by nonlinear regression. Error bars represent the standard deviation of independent biological replicates.