



**Figure S6. Aminoacylation of tRNA<sup>Ile</sup> with purified isoleucine.**

A,  $k_{\text{obs}}$  in tRNA<sup>Ile</sup> aminoacylation by IleRS was obtained with 30 mM purified (●) or unpurified (○) isoleucine to test if the purification procedure introduced contaminants that may inhibit enzymatic activity. The *inset* shows the steady state portion of the progress curve.

B,  $k_{\text{obs}}$  in tRNA<sup>Ile</sup> aminoacylation was also obtained with a low and limiting isoleucine concentration – 0.01 mM purified (●) or unpurified (○), to test the concentration of the purified isoleucine sample. WT IleRS was present at 20 nM concentration and tRNA<sup>Ile</sup> was present at 10 μM concentration.