Figure S1. **Microdensitometer traces of ribonuclease S1 and V1 lanes**. A) Scan of wild type S1 pattern (lane 7 of Fig. 6A); B) S1 pattern of G4309A (lane 7 of Fig 6B); C) V1 pattern of wild type (lane 9 of Fig 6A); D) V1 pattern of G4309A (lane 9 of Fig 6B). Numbers below (A) and (C) signify nucleotide numbers. * below nt 50 in (B) indicates increased S1 sensitivity at this position in G4309A. Vertical arrow at nt 49 in (D) shows reduced V1 sensitivity of G4309A at this position. Underlining in (C), (D) indicates relatively little change on the 3' side of the acceptor stem and at the top of the D stem.
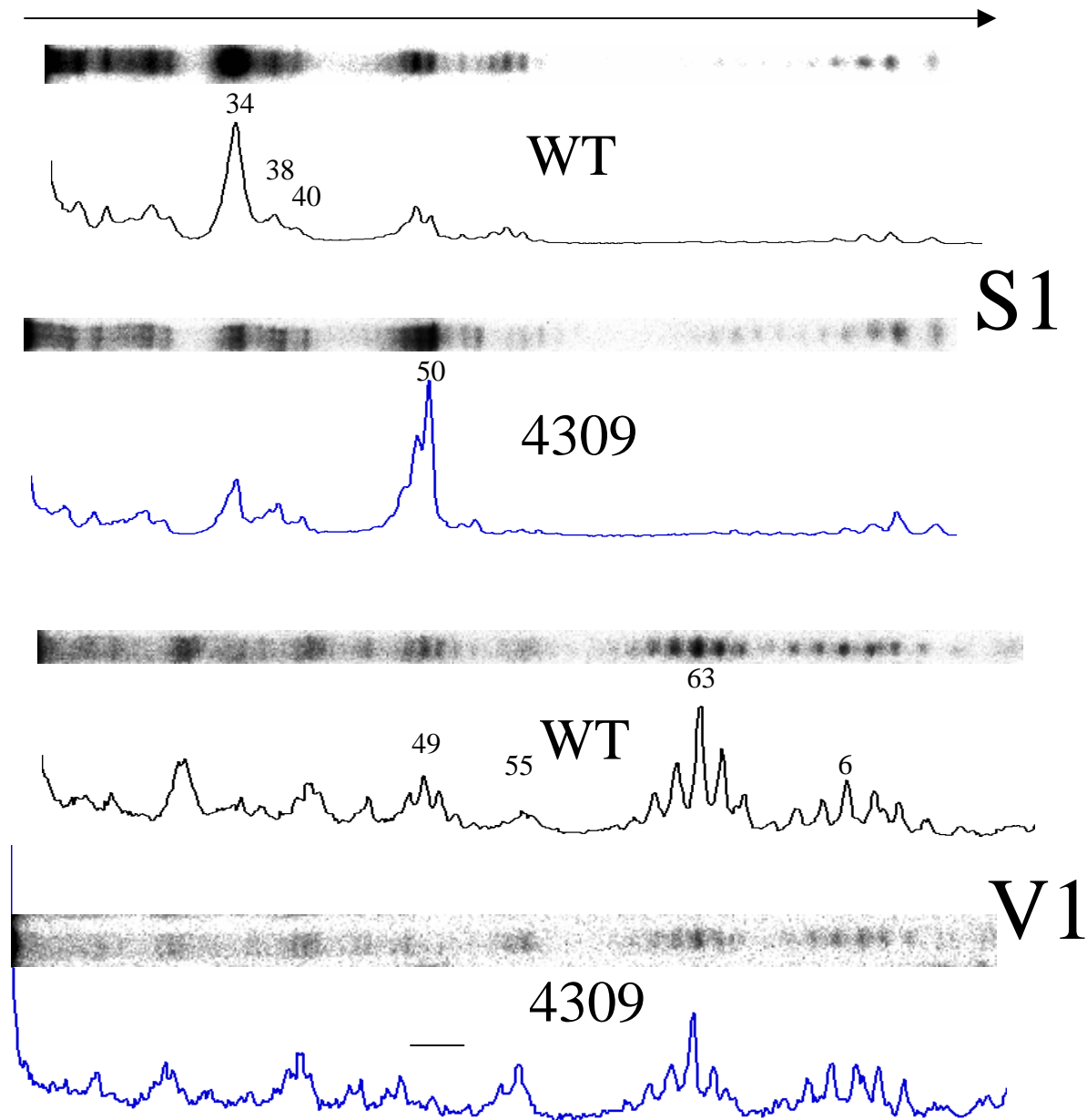
Figure S2. **Structure probing with 3' end labeled tRNA$^{\text{Ile}}$ precursors**. tRNA$^{\text{Ile}}$ precursors were labeled at their 3' ends with T4 RNA ligase and ^{32}P-Cp. RNAs were cleaved with structure probing nucleases and electrophoresed on an 8% denaturing polyacrylamide gel. Arrow at top indicates direction of electrophoresis from left to right. Gel lanes and traces are (from top to bottom) S1 WT; S1 G4309A; V1 WT; V1 G4309A. Small numbers above traces designate nt numbers in the tRNAs. Horizontal bar over trace B2 indicates the bottom of the T stem (nt 49-53) which displays altered sensitivities in the G4309A (G51A) mutant tRNA$^{\text{Ile}}$. Although the counts of labeled RNAs loaded were not precisely matched in this experiment, the highlighted structural differences are reproducibly observed in the normalized microdensitometer traces.