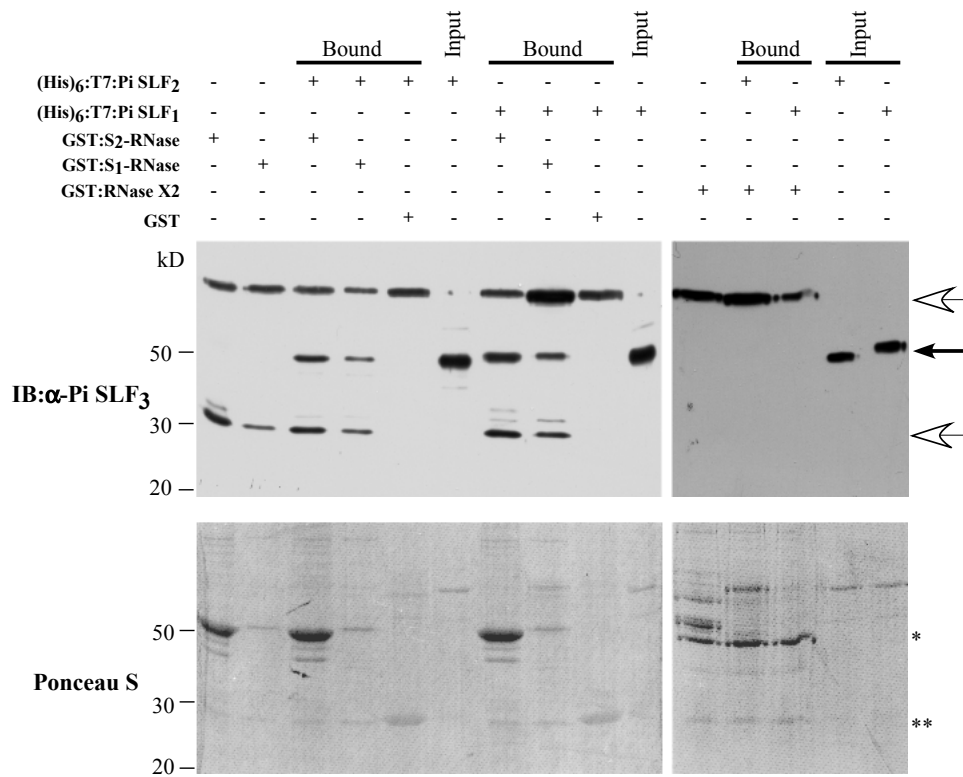


Hua and Kao, Supplemental Figure 3



Supplemental Figure 3. In vitro Binding Assay of Interactions of Pi SLFs with S₁-RNase, S₂-RNase, and RNase X2.

Purified (His)₆:T7:Pi SLF₂ and (His)₆:T7:Pi SLF₁ were separately incubated with GST:S₁-RNase-, GST:S₂-RNase-, GST:RNase X2-, and GST-bound Glutathione Sepharose 4 Fast Flow resin. The bound proteins were eluted and analyzed by immunoblotting using an anti-Pi SLF₃ antibody, which cross-reacts with Pi SLF₁ and Pi SLF₂, indicated as a darkened arrow in top panels. The PiSLF antibody was raised in rabbits against a synthetic peptide corresponding to the last 14 amino acids at the C-terminal end of Pi SLF₃. The open arrows indicate cross-reacting bacterial proteins that co-purified with the GST tagged proteins. The “input” lanes were used as positive controls for the (His)₆:T7 tagged proteins, and the lanes containing GST:S₁-RNase, GST:S₂-RNase and GST:RNase X2 alone were used as negative controls to show that there were no cross-reacting proteins of the sizes similar to those of the (His)₆:T7 tagged proteins. Bottom panels: Ponceau S staining of the immunoblots before immunoblotting. The single asterisk indicates the GST:S₁-RNase, GST:S₂-RNase and GST:RNase X2 bands, and the double asterisks indicate the GST band.