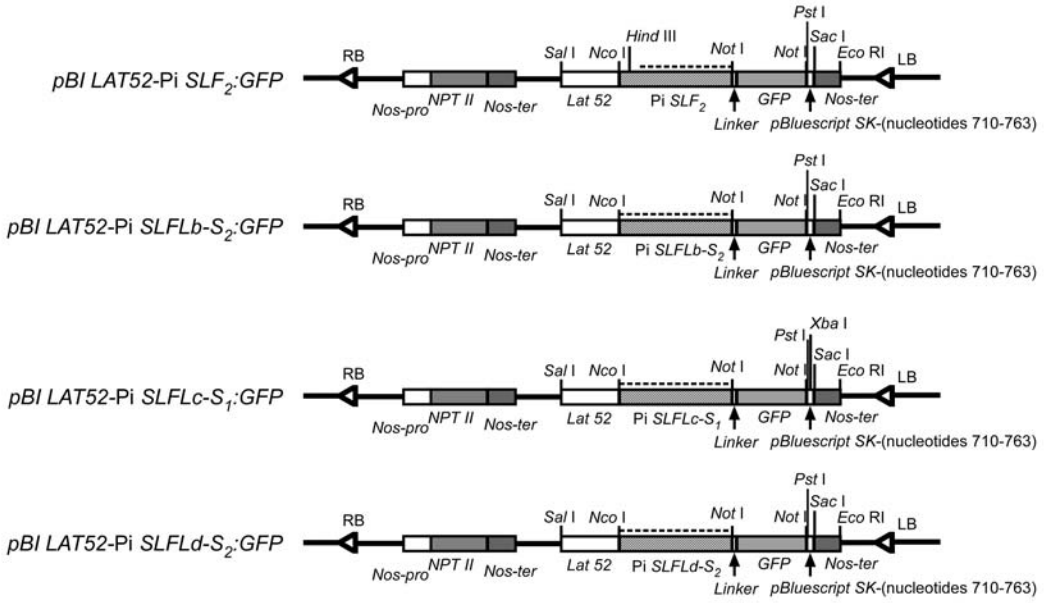
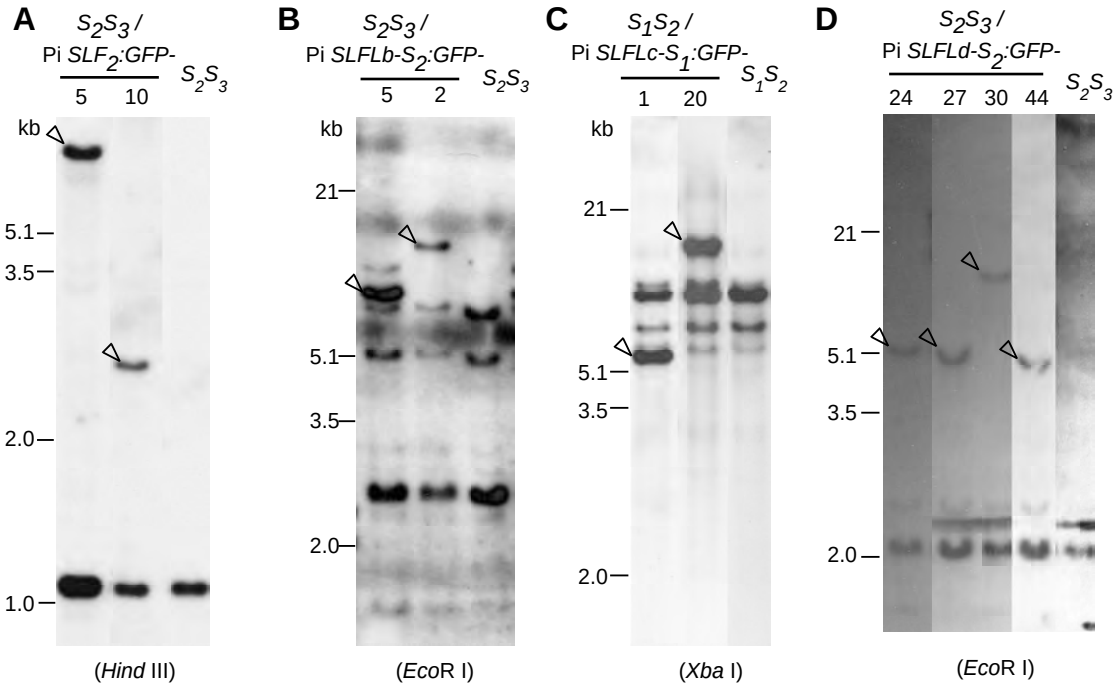
Supplemental Data. Hua et al. (2007). Comparison of *Petunia inflata S*-locus F-box protein (Pi SLF) with Pi SLF-like proteins reveals its unique function in S-RNase-based self-incompatibility.

**Hua et al., 2007; Supplemental Figure 1**



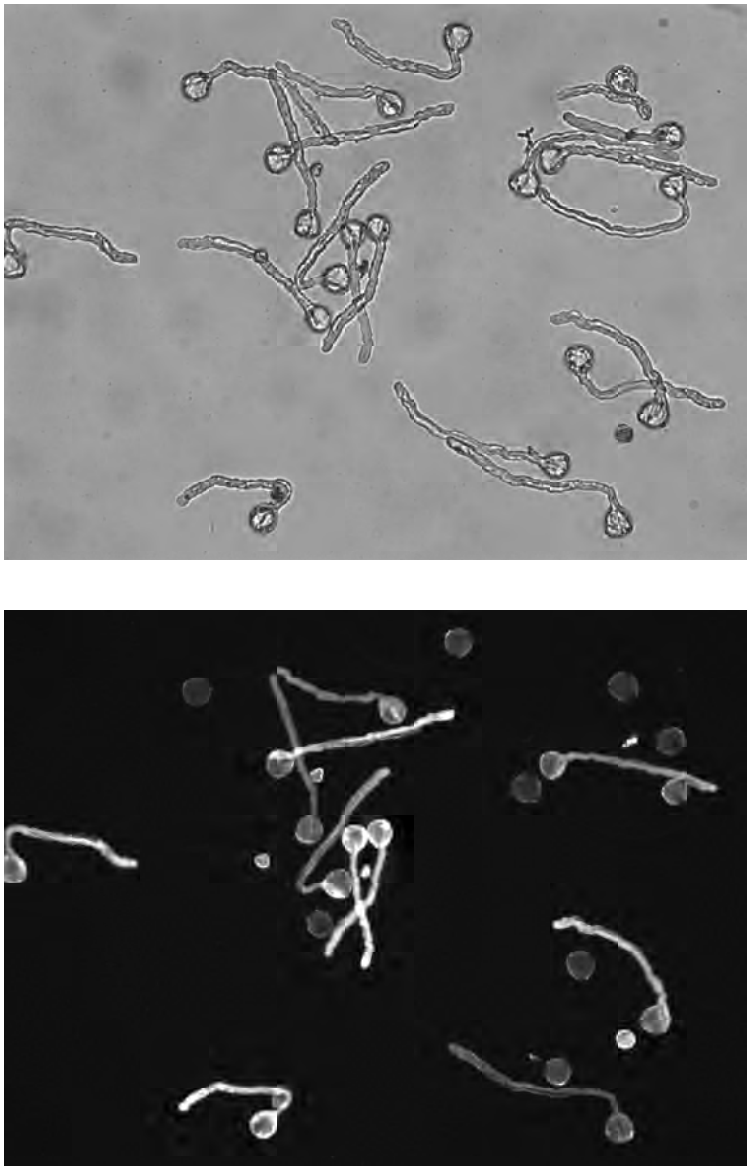

**Supplemental Figure 1. Schematics of the Ti-plasmid constructs used in plant transformation experiments.**

The name of each gene or sequence element is indicated under each construct and the restriction sites used for subcloning and digestion of genomic DNA for blotting analysis are indicated above each construct. The fragment used as probe in DNA-blotting analysis is marked with a broken line in each construct. The region between the right (RB) and the left border (LB) is integrated into transgenic plants. *NOS*: the gene encoding nopaline synthase; *pro*: promoter; *ter*: transcription terminator; *NPT II*: the gene encoding neomycin phosphotransferase II (conferring kanamycin resistance).



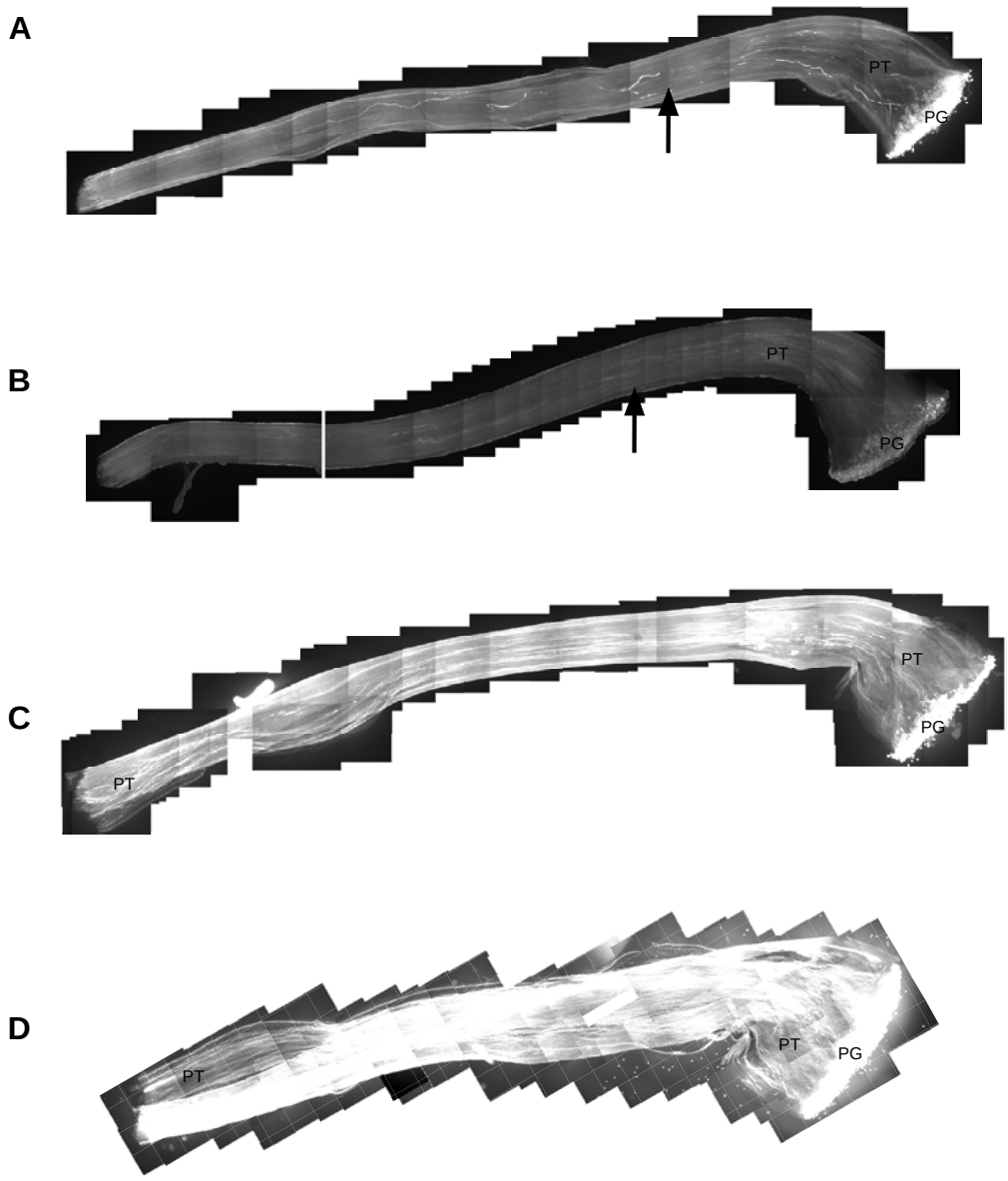

**Supplemental Figure 2. Genomic DNA gel blots showing independent transgenic lines that contain a single insert of (A) Pi *SLF$_2$:GFP*, (B) Pi *SLFLb-S$_2$:GFP*, (C) Pi *SLFLc-S$_1$:GFP*, or (D) Pi *SLFLd-S$_2$:GFP*.**

Each lane contains restriction digests of 15 μg genomic DNA isolated from the transgenic plant indicated, or from one of the two wild-type plants ($S_1S_2$ and $S_2S_3$). The restriction enzyme used for each blot is indicated at the bottom of the autoradiogram. cDNAs for Pi *SLF2(CTD)*, Pi *SLFLb-S$_2$*, Pi *SLFLc-S$_1$*, and Pi *SLFLd-S$_2$*, indicated in Supplemental Figure 1, were used as probes in (A), (B), (C), and (D), respectively. On each blot, the fragments that contain the transgene are indicated with small white triangles.



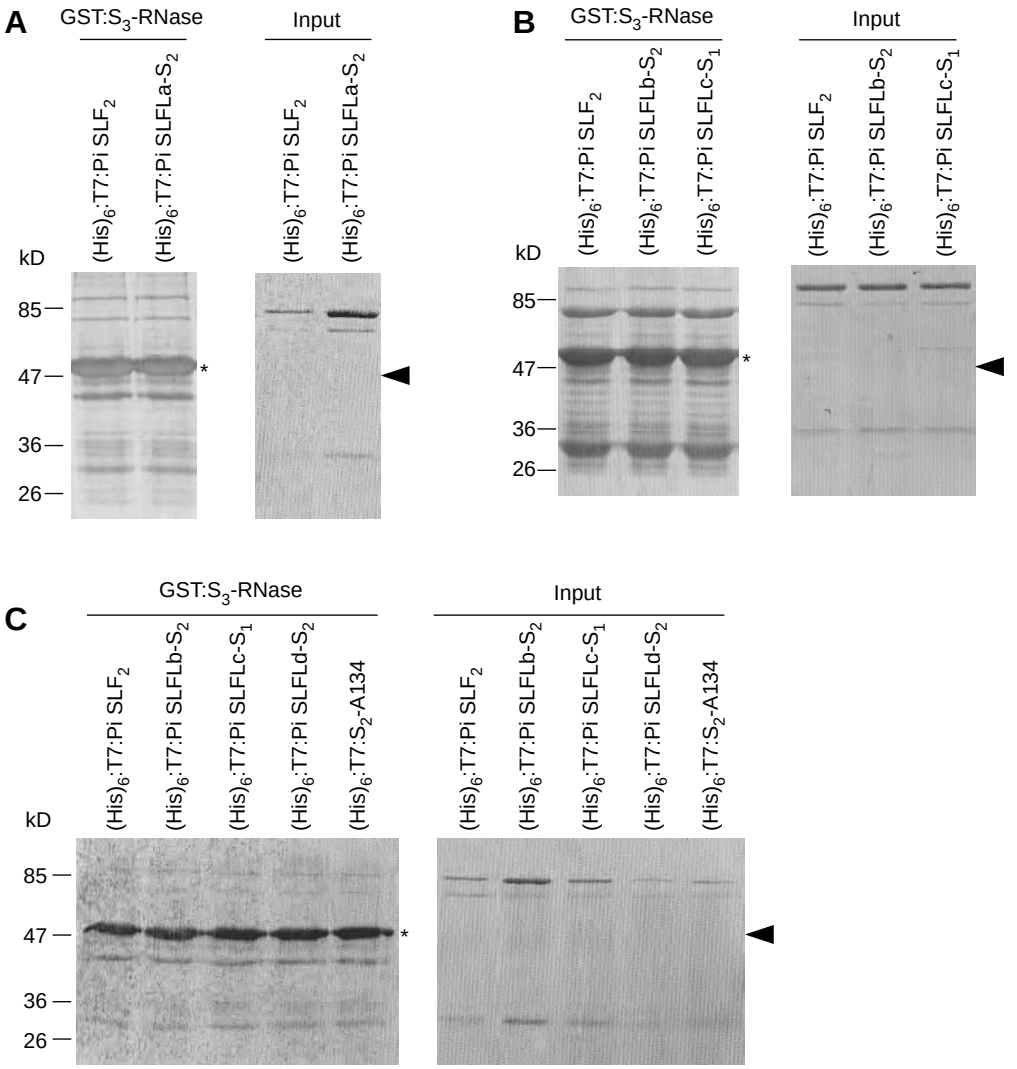
**Supplemental Figure 3. Bright field (top) and fluorescence (bottom) images of representative pollen tubes produced by a progeny plant from the cross between a wild-type $S_2S_3$ plant and the transgenic plant $S_2S_3$/Pi *SLF*$_2$*:GFP-5*.**

Pollen was germinated in a pollen germination medium, as described in Lee et al. (1996), for 2 hr with gentle shaking at 30 $^{o}$C, and the pollen tubes were observed under an epifluorescent microscope. Note that 11 of the 20 pollen tubes show GFP fluorescence.



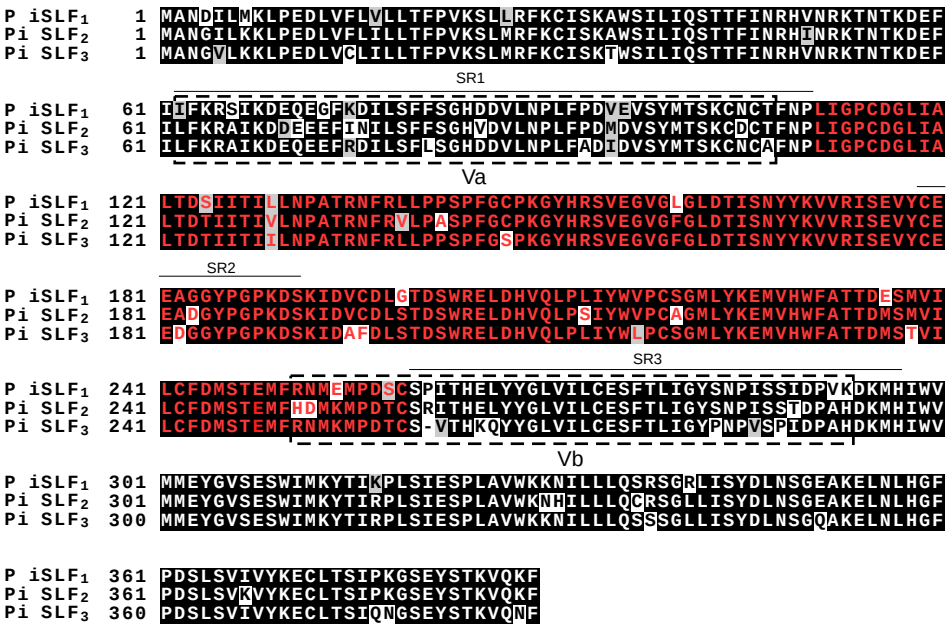

**Supplemental Figure 4. Fluorescence images of pollen tubes in pistils of a wild-type $S_2S_3$ plant 20 hr post-pollination with pollen from (A) transgenic plant $S_2S_3$/Pi *SLFLd-S$_2$:GFP*-30, (B) another wild-type $S_2S_3$ plant, (C) transgenic plant $S_2S_3$/Pi *SLF$_2$:GFP*-5, (D) a wild-type $S_1S_1$ plant.**

Pollen tubes (PT) were stained with aniline blue and visualized under an epifluorescent microscope. Note that most pollen tubes shown in (A) and (B) were stopped in the upper segment of the pistil (indicated with a black arrow), whereas most pollen tubes in (C) and (D) grew through the entire pistil to reach the ovary (not shown). PG: pollen grain.




**Supplemental Figure 5. Ponceau S staining of the immunoblots containing binding assays conducted at high concentrations to show that the amount of GST:$S_3$-RNase used in each binding reaction was in large excess over the $(His)_6$:T7 tagged protein.**

(A), (B), (C) show the part of immunoblots in Figures 4A, 4B, and 4C, respectively, that contains binding assays conducted at the high concentration (left panel). The same amount of GST:$S_3$-RNase shown was also used in all the binding assays conducted at low concentrations of each $(His)_6$:T7 tagged protein. For each $(His)_6$:T7 tagged protein, one tenth the input amount assayed at the high concentration is shown (right panel). The single asterisks indicate GST:$S_3$-RNase and the dark triangles indicate the predicted position where the $(His)_6$:T7 tagged proteins migrate on each gel.




**Supplemental Figure 6. Alignment of the deduced amino acid sequences of three allelic variants of Pi *SLF*.**

The alignment was performed as described in Figure 5. The three Pi SLF-specific regions, SR1, SR2, and SR3, are indicated by black lines above the aligned sequences. The two variable regions, Va and Vb, are boxed with dash lines. FD1 and FD3 are separated by FD2, which is highlighted in red.

**Hua et al., 2007; Supplemental Table 1. Percent pairwise nucleotide sequence identities between the coding regions of Pi *SLF* and Pi *SLFL* genes**

| | Pi *SLF*$_1$ | Pi *SLF*$_2$ | Pi *SLF*$_3$ | Pi *SLFLa-S*$_1$ | Pi *SLFLa-S*$_2$ | Pi *SLFLb-S*$_2$ | Pi *SLFLc-S*$_1$ | Pi *SLFLd-S*$_2$ | *S*$_1$*(S*$_2$*)-A113* | *S*$_3$*-A113* | *S*$_1$*-A134* | *S*$_2$*-A134* | *S*$_3$*-A134* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pi *SLF*$_1$ | ----- | 93.2 | 92.0 | 68.9 | 68.7 | 69.4 | 71.2 | 69.9 | 69.8 | 69.8 | 68.4 | 68.4 | 68.1 |
| Pi *SLF*$_2$ | | ----- | 93.4 | 69.1 | 69.1 | 69.8 | 71.5 | 69.2 | 70.2 | 69.8 | 68.2 | 68.4 | 67.8 |
| Pi *SLF*$_3$ | | | ----- | 69.6 | 69.8 | 69.5 | 70.6 | 69.1 | 68.8 | 68.8 | 67.2 | 67.0 | 66.7 |
| Pi *SLFLa-S*$_1$ | | | | ----- | 93.7 | 69.8 | 67.5 | 69.3 | 69.2 | 68.7 | 68.8 | 68.6 | 68.4 |
| Pi *SLFLa-S*$_2$ | | | | | ----- | 70.0 | 68.0 | 68.7 | 68.6 | 68.1 | 68.4 | 68.2 | 68.2 |
| Pi *SLFLb-S*$_2$ | | | | | | ----- | 67.3 | 66.4 | 67.9 | 67.2 | 67.2 | 67.1 | 67.1 |
| Pi *SLFLc-S*$_1$ | | | | | | | ----- | 69.1 | 70.4 | 69.7 | 66.4 | 65.7 | 66.0 |
| Pi *SLFLd-S*$_2$ | | | | | | | | ----- | 69.2 | 69.5 | 69.4 | 69.3 | 68.8 |
| *S*$_1$*(S*$_2$*)-A113* | | | | | | | | | ----- | 96.5 | 93.4 | 93.1 | 92.8 |
| *S*$_3$*-A113* | | | | | | | | | | ----- | 93.1 | 92.8 | 92.6 |
| *S*$_1$*-A134* | | | | | | | | | | | ----- | 99.2 | 99.3 |
| *S*$_2$*-A134* | | | | | | | | | | | | ----- | 98.7 |
| *S*$_3$*-A134* | | | | | | | | | | | | | ----- |

**Hua et al., 2007; Supplemental Table 2. Percent pairwise sequence identities between deduced amino acid sequences of Pi *SLF* and Pi *SLFL* genes**

| | Pi $SLF_1$ | Pi $SLF_2$ | Pi $SLF_3$ | Pi SLFLa-$S_1$ | Pi SLFLa-$S_2$ | Pi SLFLb-$S_2$ | Pi SLFLc-$S_1$ | Pi SLFLd-$S_2$ | $S_1(S_2)$-A113 | $S_3$-A113 | $S_1$-A134 | $S_2$-A134 | $S_3$-A134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pi $SLF_1$ | ----- | 89.5 | 88.2 | 54.3 | 54.6 | 51.3 | 53.8 | 51.3 | 51.2 | 51.2 | 48.4 | 47.6 | 47.4 |
| Pi $SLF_2$ | | ----- | 88.2 | 53.8 | 54.1 | 50.5 | 54.4 | 49.5 | 51.2 | 49.9 | 48.2 | 47.6 | 47.6 |
| Pi $SLF_3$ | | | ----- | 53.5 | 54.4 | 50.9 | 54.2 | 50.5 | 51.2 | 51.5 | 49.7 | 49.2 | 49.2 |
| Pi SLFLa-$S_1$ | | | | ----- | 88.6 | 50.9 | 53.0 | 52.5 | 50.4 | 51.2 | 50.0 | 49.7 | 49.5 |
| Pi SLFLa-$S_2$ | | | | | ----- | 50.3 | 52.6 | 52.1 | 49.3 | 50.9 | 50.3 | 50.0 | 49.5 |
| Pi SLFLb-$S_2$ | | | | | | ----- | 50.8 | 51.0 | 49.1 | 49.6 | 49.2 | 49.5 | 49.2 |
| Pi SLFLc-$S_1$ | | | | | | | ----- | 51.3 | 54.4 | 54.1 | 53.2 | 51.8 | 52.1 |
| Pi SLFLd-$S_2$ | | | | | | | | ----- | 51.2 | 51.7 | 50.5 | 50.3 | 48.9 |
| $S_1(S_2)$-A113 | | | | | | | | | ----- | 94.7 | 87.8 | 87.3 | 86.5 |
| $S_3$-A113 | | | | | | | | | | ----- | 88.6 | 88.1 | 87.3 |
| $S_1$-A134 | | | | | | | | | | | ----- | 97.6 | 97.9 |
| $S_2$-A134 | | | | | | | | | | | | ----- | 96.3 |
| $S_3$-A134 | | | | | | | | | | | | | ----- |

**Hua et al., 2007; Supplemental Table 3. List of recombinant proteins involved in this study**

| **$(His)_6$:T7 tagged proteins expressed produced (17 total)** |
|---|
| $(His)_6$:T7:Pi $SLF_1$ |
| $(His)_6$:T7:Pi $SLF_2$ |
| $(His)_6$:T7:Pi SLFLa-$S_2$ |
| $(His_)_6$:T7:PI SLFLb-$S_2$ |
| $(His)_6$:T7:Pi SLFLc-$S_1$ |
| $(His)_6$:T7:Pi SLFLd-$S_2$ |
| $(His)_6$:T7:$S_2$-A134 |
| $(His)_6$:T7:Pi $SLF_2$(FD2) |
| $(His)_6$:T7:Pi $SLF_2$(FD3) |
| $(His)_6$:T7:Pi $SLF_2$(FD1+FD2) |
| $(His)_6$:T7:Pi $SLF_2$(FD2+FD3) |
| $(His)_6$:T7:Pi $SLF_2$(FD1):Pi SLFLb-$S_2$(FD2):Pi $SLF_2$(FD3) |
| $(His)_6$:T7:Pi SLFLb-$S_2$(FD1):Pi $SLF_2$(FD2):$(His)_6$: Pi SLFLb-$S_2$(FD3) |
| $(His)_6$:T7:Pi $SLF_1$(FD1):Pi $SLF_2$(FD2+FD3) |
| $(His)_6$:T7:Pi $SLF_2$(FD1):Pi $SLF_1$(FD2+FD3) |
| $(His)_6$:T7:Pi $SLF_1$(FD1):Pi $SLF_2$(FD2):Pi $SLF_1$(FD3) |
| $(His)_6$:T7:Pi $SLF_2$(FD1):Pi $SLF_1$(FD2):Pi $SLF_2$(FD3) |
| **$(His)_6$:T7 tagged proteins produced for initial study but not mentioned in detail in the paper (11 total)** |
| $(His)_6$:T7:Pi $SLF_2$(81-160) |
| $(His)_6$:T7:Pi $SLF_2$(111-230) |
| $(His)_6$:T7:Pi $SLF_2$(244-389) |
| $(His)_6$:T7:Pi $SLF_2$(81-297) |
| $(His)_6$:T7:Pi $SLF_2$(81-389) |
| $(His)_6$:T7:Pi $SLF_2$(50-179) (FD1 without F-box domain + partial FD2) |
| $(His)_6$:T7:Pi $SLF_2$(50-191) (FD1 without F-box domain + partial FD2 with SR2) |
| $(His)_6$:T7:Pi $SLF_2$(50-260) (FD1 without F-box domain + FD2) |
| $(His)_6$:T7:Pi $SLF_2$(50-297) (FD1 without F-box domain + FD2 + SR3) |
| $(His)_6$:T7:Pi $SLF_2$(50-316) (FD1 without F-box domain + FD2 + partial FD3 with SR3) |
| $(His)_6$:T7:Pi $SLF_2$(50-389) (FD1 without F-box domain + FD2 + FD3) |
| **$(His)_6$:T7 tagged proteins unable to be expressed (4 total)** |
| $(His)_6$:T7:Pi $SLF_2$(50-110) (FD1 without F-box domain) |
| $(His)_6$:T7:Pi $SLF_1$(FD1) |
| $(His)_6$:T7:Pi $SLF_2$(FD1) |
| $(His)_6$:T7:Pi $SLF_3$(FD1) |
| **GST tagged proteins produced (2 total)** |
| GST:$S_2$-RNase |
| GST:$S_3$-RNase |