

## Supplementary

**Table S1. Open reading frame of *STE3.3* and *STE3.4* genes in *Melampsora* spp.**

Name	ORF length	Amino acid number	GenBank ID
<i>MlpSTE3.3-URE</i>	1158	385	MW363516
<i>MlpSTE3.3-TEL</i>	1158	385	MW927156
<i>MmedSTE3.3-URE</i>	1158	385	MW927144
<i>MmedSTE3.3-TEL</i>	1161	386	MW927159
<i>MpruSTE3.3-URE</i>	1152	383	MW927145
<i>MpruSTE3.3-TEL</i>	1152	383	MW927157
<i>MlpSTE3.4-URE</i>	1245	414	MW363515
<i>MlpSTE3.4-TEL</i>	1245	414	MW927160
<i>MmedSTE3.4-URE</i>	1245	414	MW927147
<i>MmedSTE3.4-TEL</i>	1245	414	MW927163
<i>MpruSTE3.4-URE</i>	1212	403	MW927149
<i>MpruSTE3.4-TEL</i>	1212	403	MW927161
<i>MmagSTE3.4-URE</i>	1212	403	MW927148
<i>MmagSTE3.4-TEL</i>	1212	403	MW927162

**Table S2. Predicted of physical and chemical properties of STE3.3 and STE3.4 protein in *Melampsora* spp.**

Name	Molecular weight	Theoretical pI	Formula	Instability index (II)	Average Hydropathy
<i>MlpSTE3.3-URE</i>	43128.73	8.97	C <sub>1981</sub> H <sub>3090</sub> N <sub>516</sub> O <sub>520</sub> S <sub>21</sub>	36.83	0.392
<i>MlpSTE3.3-TEL</i>	43046.67	8.97	C <sub>1974</sub> H <sub>3092</sub> N <sub>516</sub> O <sub>520</sub> S <sub>21</sub>	37.09	0.399
<i>MmedSTE3.3-URE</i>	43054.61	8.97	C <sub>1974</sub> H <sub>3084</sub> N <sub>516</sub> O <sub>521</sub> S <sub>21</sub>	36.05	0.384
<i>MmedSTE3.3-TEL</i>	43185.78	8.97	C <sub>1983</sub> H <sub>3093</sub> N <sub>517</sub> O <sub>521</sub> S <sub>21</sub>	36.76	0.397
<i>MpruSTE3.3-URE</i>	43135.58	8.78	C <sub>1974</sub> H <sub>3067</sub> N <sub>515</sub> O <sub>524</sub> S <sub>23</sub>	35.33	0.363
<i>MpruSTE3.3-TEL</i>	43135.58	8.78	C <sub>1974</sub> H <sub>3067</sub> N <sub>515</sub> O <sub>524</sub> S <sub>23</sub>	35.33	0.363
<i>MlpSTE3.4-URE</i>	45766.19	8.86	C <sub>2075</sub> H <sub>3223</sub> N <sub>549</sub> O <sub>571</sub> S <sub>24</sub>	30.60	0.282
<i>MlpSTE3.4-TEL</i>	45766.19	8.86	C <sub>2075</sub> H <sub>3223</sub> N <sub>549</sub> O <sub>571</sub> S <sub>24</sub>	30.60	0.282
<i>MmedSTE3.4-URE</i>	45766.19	8.86	C <sub>2075</sub> H <sub>3223</sub> N <sub>549</sub> O <sub>571</sub> S <sub>24</sub>	30.60	0.282
<i>MmedSTE3.4-TEL</i>	45814.28	8.93	C <sub>2080</sub> H <sub>3227</sub> N <sub>549</sub> O <sub>570</sub> S <sub>24</sub>	31.17	0.288
<i>MpruSTE3.4-URE</i>	44985.61	8.96	C <sub>2063</sub> H <sub>3199</sub> N <sub>535</sub> O <sub>551</sub> S <sub>21</sub>	37.62	0.325
<i>MpruSTE3.4-TEL</i>	44989.60	8.96	C <sub>2062</sub> H <sub>3199</sub> N <sub>535</sub> O <sub>552</sub> S <sub>21</sub>	36.39	0.326
<i>MmagSTE3.4-URE</i>	45085.68	8.96	C <sub>2070</sub> H <sub>3199</sub> N <sub>535</sub> O <sub>552</sub> S <sub>21</sub>	35.18	0.325
<i>MmagSTE3.4-TEL</i>	45051.71	9.03	C <sub>2068</sub> H <sub>3205</sub> N <sub>535</sub> O <sub>551</sub> S <sub>21</sub>	35.70	0.335

**Table S3. Hydrophilicity, hydrophobicity and transmembrane structure of STE3.3 and STE3.4 proteins in *Melampsora* spp.**

Sequences	Hydrophobicity peak	Hydrophilicity peak	Transmembrane helix	Number of helical amino acids in membrane	Probability of N-terminal on one side of plasma membrane
<i>MlpSTE3.3-URE</i>			7	153.85225	9.31%
<i>MlpSTE3.3-TEL</i>	Position:130 Score:2.800(max)	Position:252 Score:- 2.400(min)	7	153.85564	9.31%
<i>MmedSTE3.3-URE</i>			7	153.85235	9.31%
<i>MmedSTE3.3-TEL</i>	Position:131 Score:2.800(max)	Position:253 Score:- 2.400(min)	7	154.47249	7.65%
<i>MpruSTE3.3-URE</i>	Position:130	Position:252 Score:-	7	153.82699	11.18%
<i>MpruSTE3.3-TEL</i>	Score:2.800(max)	2.400(min)	7	153.82699	11.18%
<i>MlpSTE3.4-URE</i>			6	135.50797	84.80%
<i>MlpSTE3.4-TEL</i>	Position:281	Position:386 Score:-	6	135.50797	84.80%
<i>MmedSTE3.4-URE</i>	Score:2.989(max)	2.300(min)	6	135.50797	84.80%
<i>MmedSTE3.4-TEL</i>			6	134.85116	87.83%
<i>MpruSTE3.4-URE</i>			6	140.20469	70.39%
<i>MpruSTE3.4-TEL</i>	Position:281	Position:108 Score:-	6	141.6942	62.59%
<i>MmagSTE3.4-URE</i>	Score:3.333(max)	2.544(min)	6	141.71101	62.59%
<i>MmagSTE3.4-TEL</i>			6	141.68069	62.59%

**Table S4. Subcellular localization analysis of STE3.3 and STE3.4 protein in *Melampsora* spp.**

Sequences	Endoplasmic reticulum	Mitochondria	acuole	Cytoplasm	Nucleus	Vesicles	Golgi apparatus
<i>MlpSTE3.3-URE</i>	66.7%	33.3%	/	/	/	/	/
<i>MlpSTE3.3-TEL</i>	66.7%	33.3%	/	/	/	/	/
<i>MmedSTE3.3-URE</i>	66.7%	33.3%	/	/	/	/	/
<i>MmedSTE3.3-TEL</i>	66.7%	33.3%	/	/	/	/	/
<i>MpruSTE3.3-URE</i>	66.7%	33.3%	/	/	/	/	/
<i>MpruSTE3.3-TEL</i>	66.7%	33.3%	/	/	/	/	/
<i>MlpSTE3.4-URE</i>	33.3%	11.1%	11.1%	11.1%	11.1%	11.1%	11.1%
<i>MlpSTE3.4-TEL</i>	33.3%	11.1%	11.1%	11.1%	11.1%	11.1%	11.1%
<i>MmedSTE3.4-URE</i>	33.3%	11.1%	11.1%	11.1%	11.1%	11.1%	11.1%
<i>MmedSTE3.4-TEL</i>	33.3%	11.1%	11.1%	11.1%	11.1%	11.1%	11.1%
<i>MpruSTE3.4-URE</i>	77.8%	22.2%	/	/	/	/	/
<i>MpruSTE3.4-TEL</i>	66.7%	22.2%	/	/	11.1%	/	/
<i>MmagSTE3.4-URE</i>	66.7%	22.2%	/	/	11.1%	/	/
<i>MmagSTE3.4-TEL</i>	66.7%	22.2%	/	/	11.1%	/	/

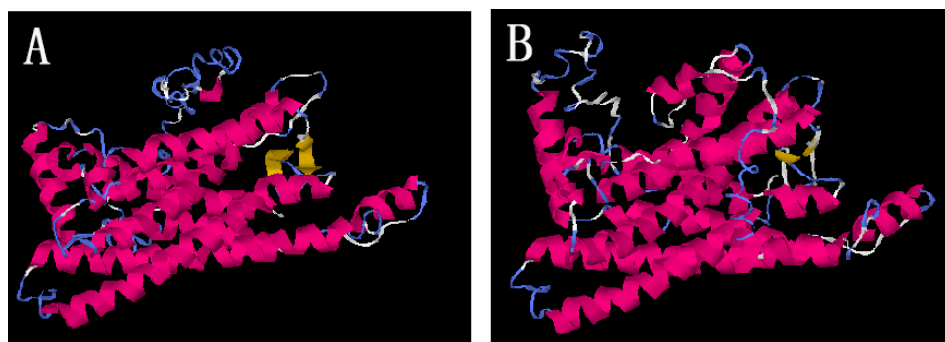
Note: “/” indicates that it does not exist.

**Table S5. Signal peptide of STE3.3 and STE3.4 protein in *Melampsora* spp.**

Sequences	Division value D	Signal peptide sequence
<i>MlpSTE3.3-URE</i>	0.578	1-26
<i>MlpSTE3.3-TEL</i>	0.578	1-26
<i>MmedSTE3.3-URE</i>	0.578	1-26
<i>MmedSTE3.3-TEL</i>	0.578	1-26
<i>MpruSTE3.3-URE</i>	0.584	1-18
<i>MpruSTE3.3-TEL</i>	0.584	1-18
<i>MlpSTE3.4-URE</i>	0.509	1-17
<i>MlpSTE3.4-TEL</i>	0.509	1-17
<i>MmedSTE3.4-URE</i>	0.509	1-17
<i>MmedSTE3.4-TEL</i>	0.509	1-17
<i>MpruSTE3.4-URE</i>	0.516	1-17
<i>MpruSTE3.4-TEL</i>	0.516	1-17
<i>MmagSTE3.4-URE</i>	0.516	1-17
<i>MmagSTE3.4-TEL</i>	0.516	1-17

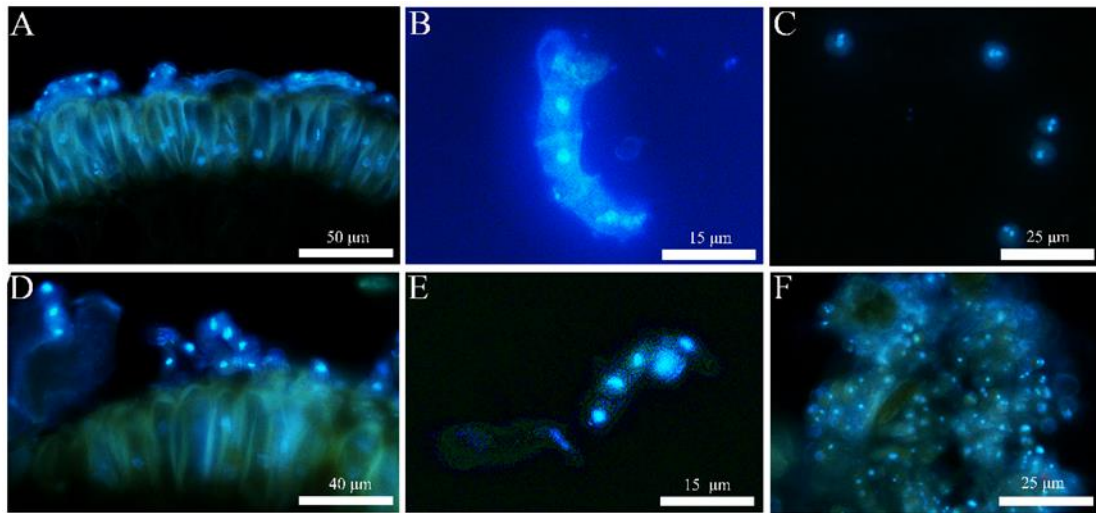
## Supplemental Figures

**Figure S1.** The tertiary structure of STE3.3 and STE3.4 protein based on Mlp



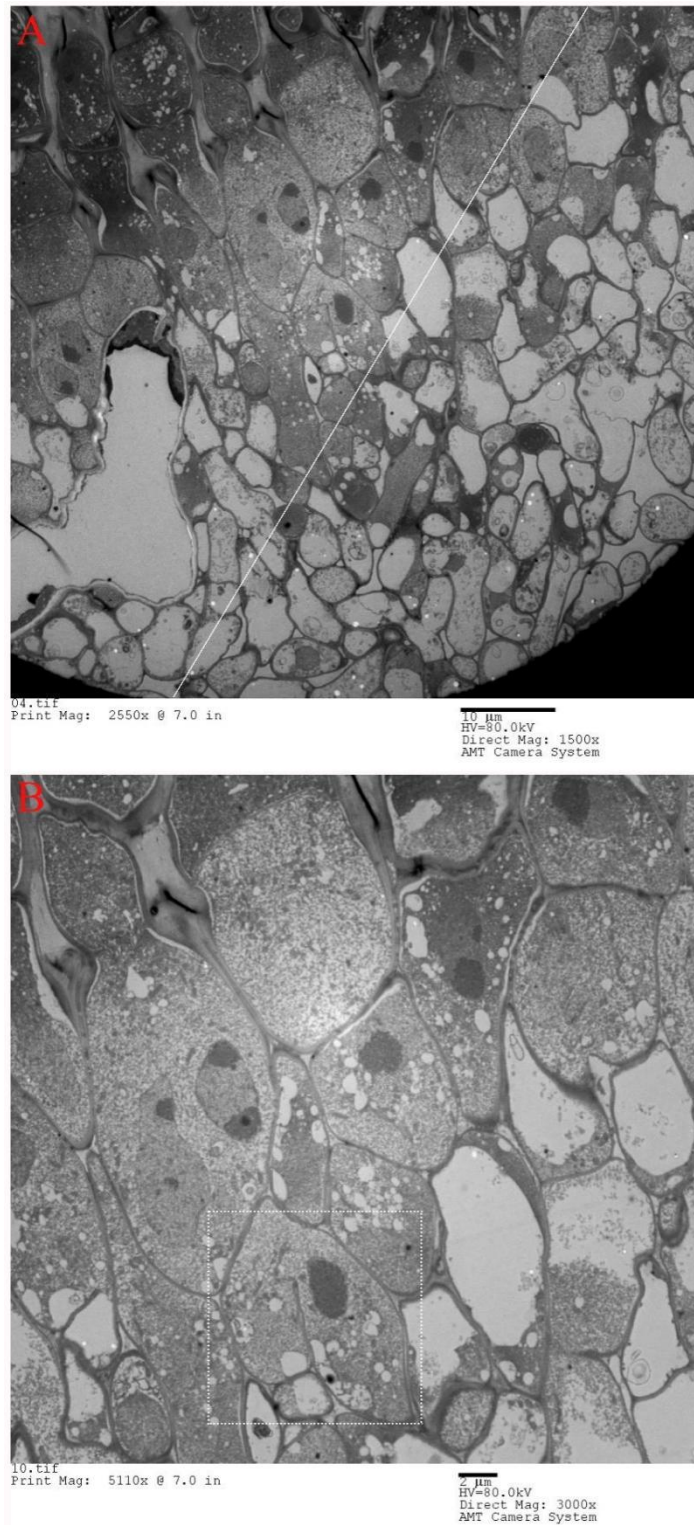
**Figure S2.** Nuclei of basidiospore under fluorescence microscopy

Note: A-C, Mlp; D-F, Mmed; A&D, Germinated teliospore; B&F, Probasidium; C&F, Basdiospora



**Figure S3.** Morphological characteristics of pycnia and aecia observed by transmission electron microscope (TEM)

Note: A, Ontogeny of aecia, the below under the line is monokaryotic, but the above is dikaryotic; B, Dicaryotization by cell membrane fusion





**Figure S4.** Buds of poplar collected in this study

