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At3g10350.2 STEFVIVTIPTVMAVSESSRLSASLKKESVPVKRLIVNQLLPPS---SSDCKFC SIKRKD
At5g60730.1 TTEFVIVTIPTVMAINESSRLHASLRKENVPVHRLIVNQLLPQS---ESDCKFC SIRRKE
M_jannaschii RTAFRLVVIPEEMSILESERAMKALQKYGIPIDAVIVNQLIPE----DVQCDFC RARREL
S_cervisiae LTTTFVCVCIPEFSLYETERLIQELISYDMDVNSIIIVNQLLFAENDQEHNCKRC QARWKM
At1g01910.1 MTTFVCVCIPEFSLYETERLVQELAKFEIDTHNIIINQVLYDD--EDVESKLLRARMRM
H_sapiens QTTFICVCIAEFSLYETERLIQELAKCKIDTHNIIIVNQLVFPD--PEKPKMCEARHKI
* * * * * : : * : * * * . : . : * : * : : . . . : .

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At3g10350.2 QMRALDMIREDSSELSALTLM EAPLVDMEIRGVPALRFLGDIWK-----
At5g60730.1 QTRVLGLIQNDEL SGLKLIQSPLLD AEIRGVPALKFMGDLIWK-----
M_jannaschii QLKRLEMIKEKF--GDKVIAYVPLLRTEAKGIETLTKQIAKILYEEEEKEE-----QKIEQ
S_cervisiae QKKYLDQIDELY--EDFHVVKMPLCAGEIRGLNLT KFSQFLNKEYNPITDGKVIYELED
At1g01910.1 QQKYLDQFYMLY--DDFNITKLP LLPEEVTGVEALKAF SHKFLTPYHPTTSRSNVEELER
H_sapiens QAKYLDQMEDLY--EDFHIVKLP LLPEEVRGADKVNTFSALLLEPYKPPSAQ-----
* : * : : : * * * * : : :

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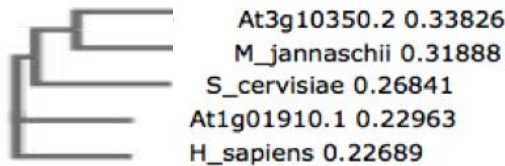
At3g10350.2 -----
At5g60730.1 -----
M_jannaschii KVGQ-----
S_cervisiae KE-----
At1g01910.1 KVHTLRLQLKTAEEELERVKSG
H_sapiens -----

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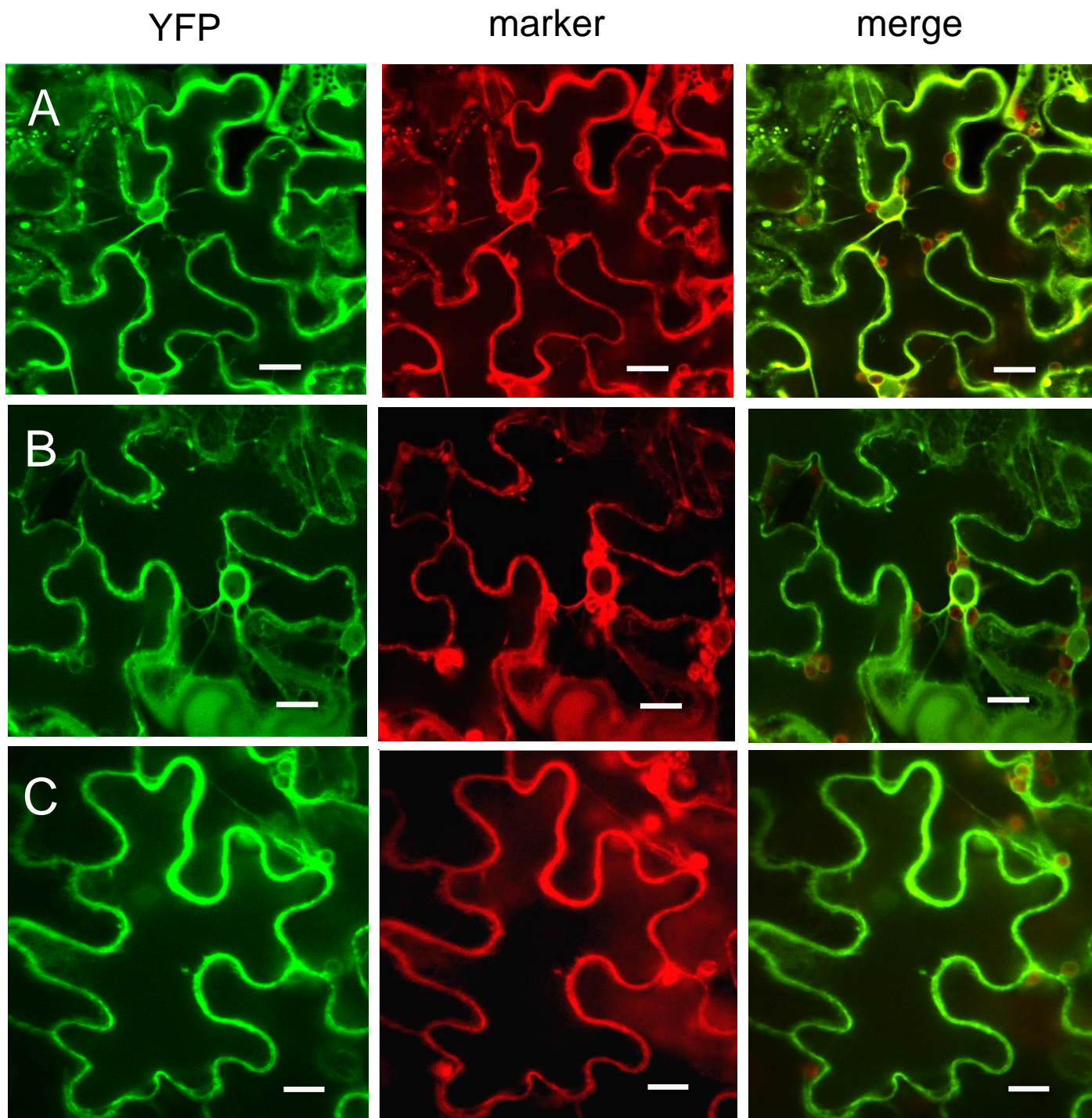
>Get3_Sacch = NCBI DAA11760
>Get3_M_jannaschii = AAB99142
>H_sapiens = NP_004308

```



Supplemental Figure S1. Alignment by Clustal omega (EMBL-EBI) of protein sequences for yeast (*Saccharomyces cerevisiae*) GET3 and homologs in other organisms. The Arabidopsis homolog (At1g01910.1) is the principal focus of this study. The conserved CXXC motif found in other homologs except At1g01910.1 is highlighted in red. *M. jannaschii* is *Methanocaldococcus jannaschii*, a thermophilic methanogenic archaean. Shown below is a cladogram constructed from the sequence data.

Fig. S1 (cont.)

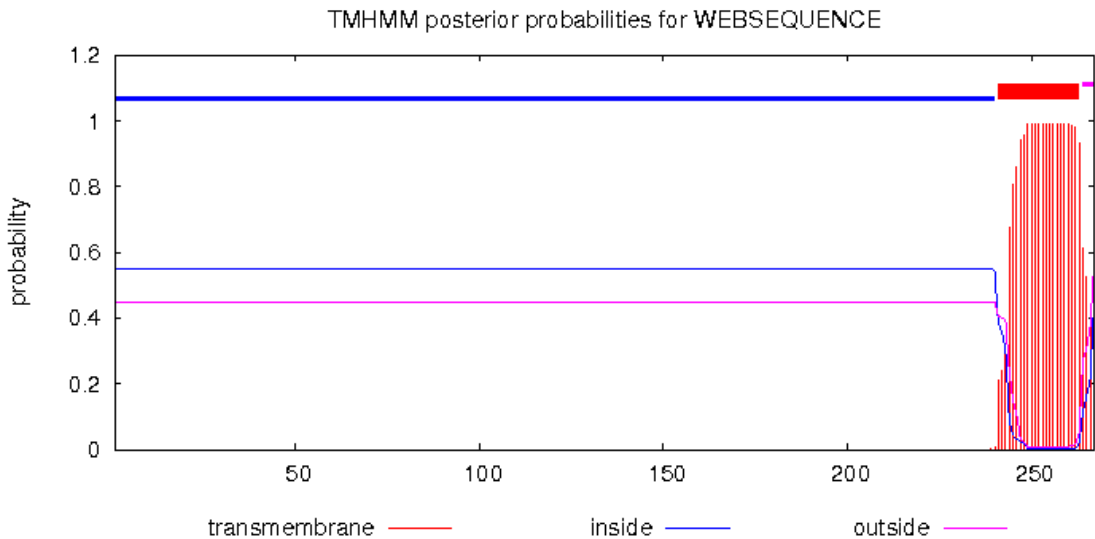


Supplemental Figure S2: Cellular localization of components of the GET pathway. A) GET1-YFP colocalization with the ER marker CDC-960; B) GET3-YFP colocalization with the ER marker CDC-960; C) GET4-YFP colocalization with cytoplasmic marker mCherry. Bar=20 μ M

Fig. S2

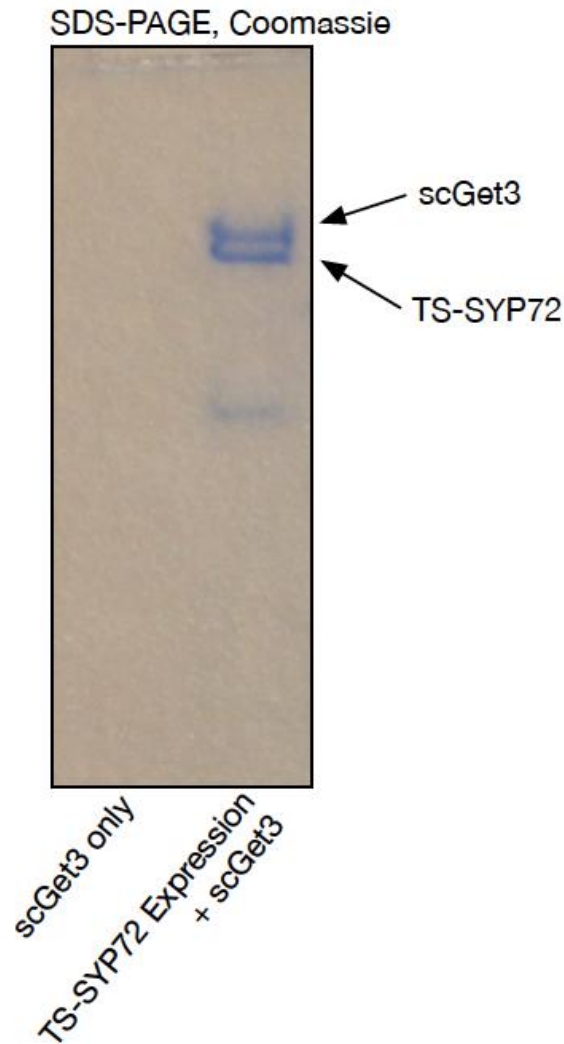
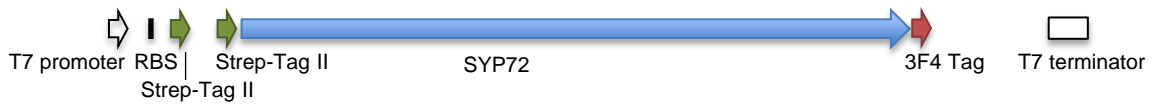
At3g45280

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1  MPVIDIIFRV  DEICKKYDKY  DIDKHREIGA  SGDDAFSRLF  TSIDSDIEAV
51  LRKAELASTE  KNRAAAVAMN  AEVRRTKARL  AEDVVKLQKL  AVKKIKGLTR
101 EERESRCDLV  IALADRLQAI  PDGNEHGAKQ  ANSDWGGASA  PNKNIKFDMS
151 EEDMDDGFFQ  QSEESSQFRQ  EYEMRRKKQD  EGLDIISEGL  DALKNLARDM
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251 LLCVILGIVS YIYNALN
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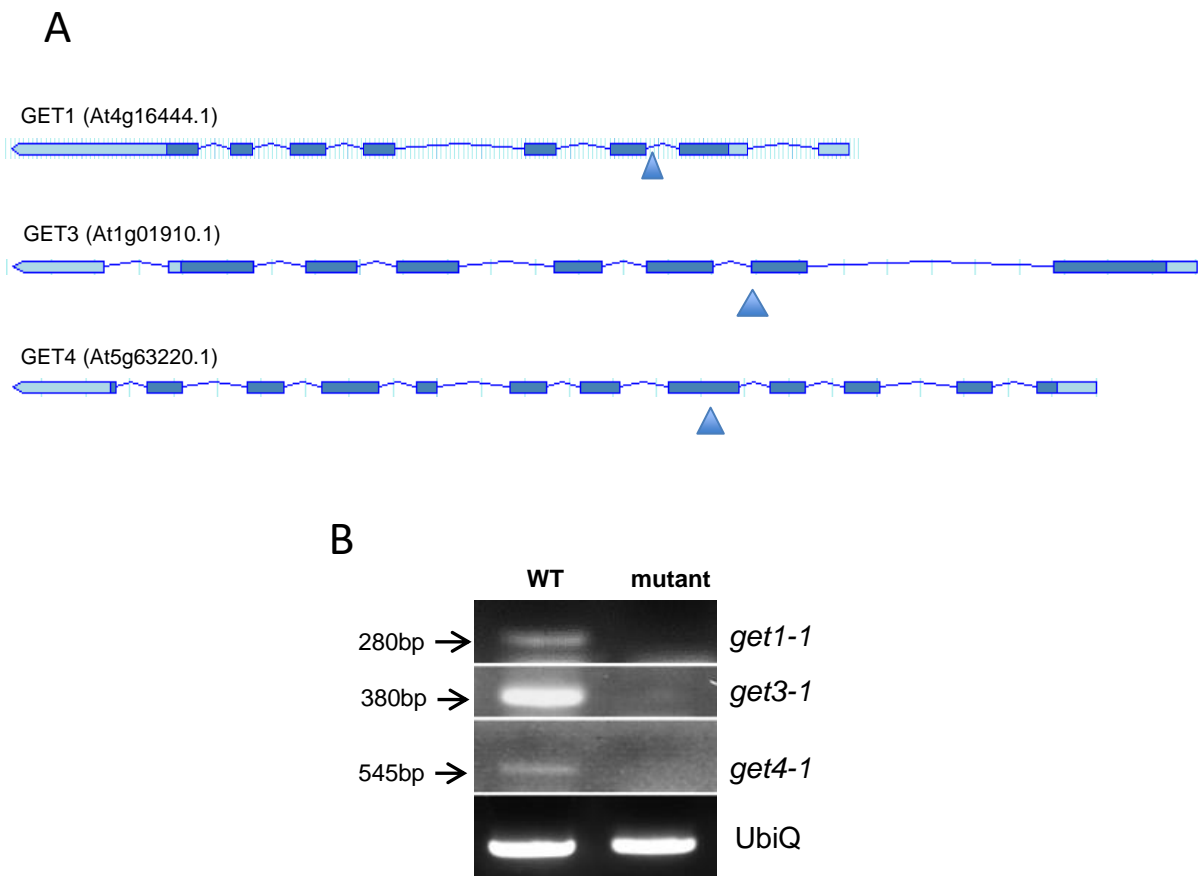
Supplemental Figure S3. Analysis of syntaxin 72 (At3g45280) protein sequence. Plot below shows the posterior probabilities for transmembrane helices according to the TMHMM Server v.2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). Region with high transmembrane helix probabilities is underlined in the sequence.

Fig. S3

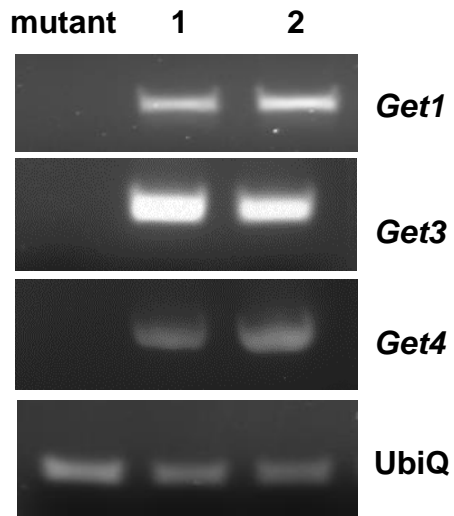


Supplemental Figure S4. Synthesis of the components used in the proteinase K protection assays. *In vitro* expression and Streptactin purification: Twin-Strep (TS) tagged SYP72-3F4 was expressed in a 250 mL PURExpress reaction in the presence of [³⁵S]-methionine and scGet3. This mixture was subjected to purification via Streptactin resin in parallel with a scGet3-only control to show that scGet3 was only pulled down by forming a complex with TS-SYP72-3F4.

Fig. S4

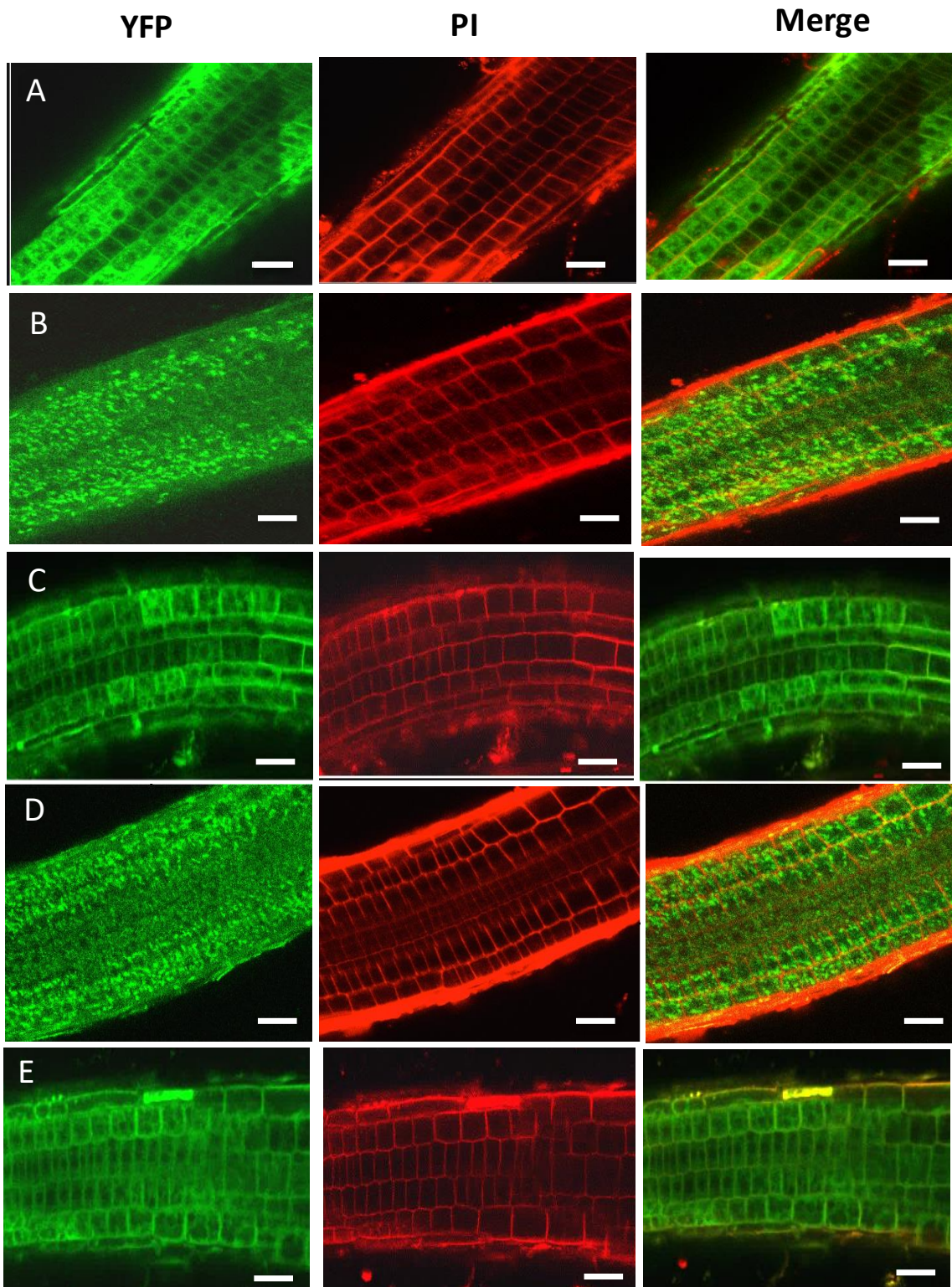


Supplemental Figure S5. A) Gene models for Arabidopsis GET gene homologs as indicated. Triangles indicate approximate positions of T-DNA insertions in *get1-1* (SAIL line CS861559), *get3-1* (SALK_033189) and *get4-1* (Salk_121195). B) RT-PCR of the cognate RNA transcripts in *get* mutant seedlings. Ubiquitin was used as a loading control.

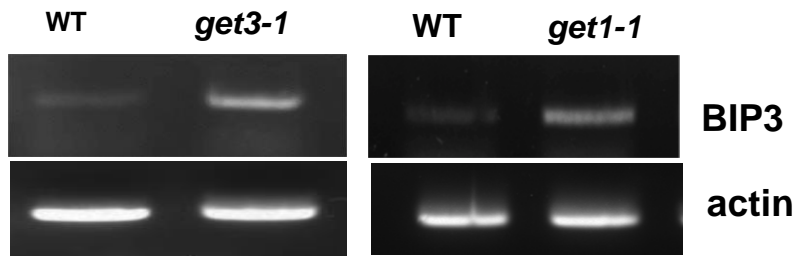


Supplemental Figure S6. Expression of the GET transgenes in complemented mutant lines. RT-PCR of GET 1, 3 and 4 expression in *get* mutant lines complemented with their cognate 35S:*GET* construct. Ubiquitin was used as a loading control.

Fig. S6



Supplemental Figure S7. Localization of YFP-tagged SYP72 in various *get* mutant lines. Confocal microscopy of roots from seedlings expressing YFP-SYP72 in WT and various mutant backgrounds as indicated. YFP-SYP72 in A) WT; B) *get1-1*; C) *get1-1* complemented with 35S:*GET1*; D) *get4-1*; E) *get4-1* complemented with 35S:*GET4*. All counterstained with propidium iodide (PI). Bar = 50 μ m.



RT-PCR for BIP3 Induction in *get3* and *get1* lines under unstressed/normal conditions

Supplemental Figure S8. Expression of an ER stress indicator in *get* mutant lines. Expression of BIP3 (At1g09080) in *get* mutants which have not been subjected experimentally to stress. RT-PCR analysis of *get3-1* and *get1-1* 7-day old seedlings.