

## **Juxta-membrane S-acylation of plant receptor-like kinases is likely fortuitous and does not necessarily impact upon function.**

Charlotte H. Hurst<sup>1,2</sup>, Kathryn M. Wright<sup>2</sup>, Dionne Turnbull<sup>1</sup>, Kerry Leslie<sup>1,2,3</sup>, Susan Jones<sup>4</sup>, and Piers A. Hemsley<sup>1,2,\*</sup>

1. Division of Plant Science, School of Life Science, University of Dundee (at JHI), Invergowrie, Dundee DD2 5DA, UK.

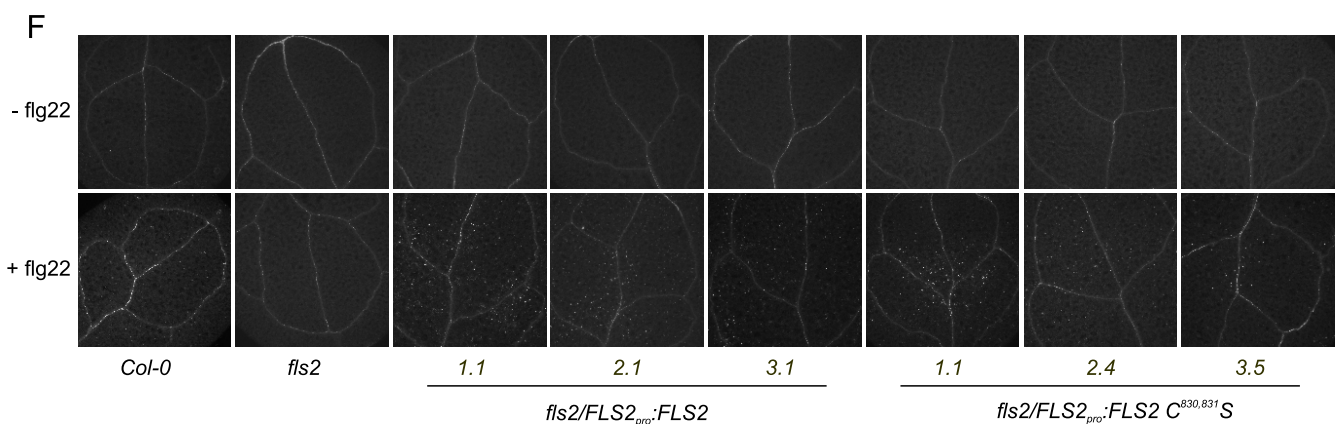
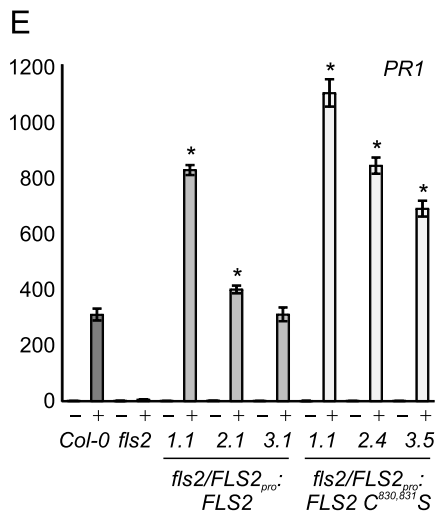
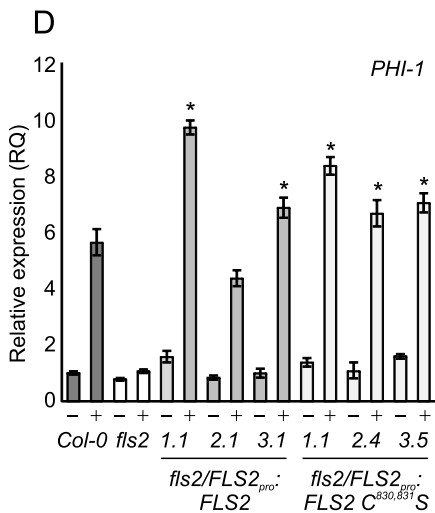
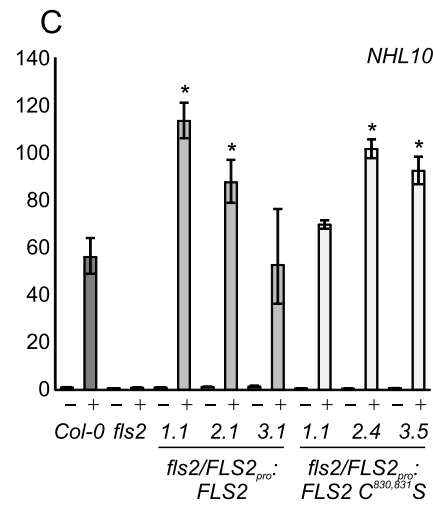
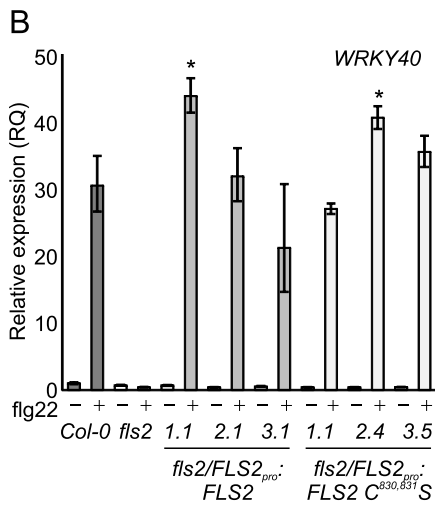
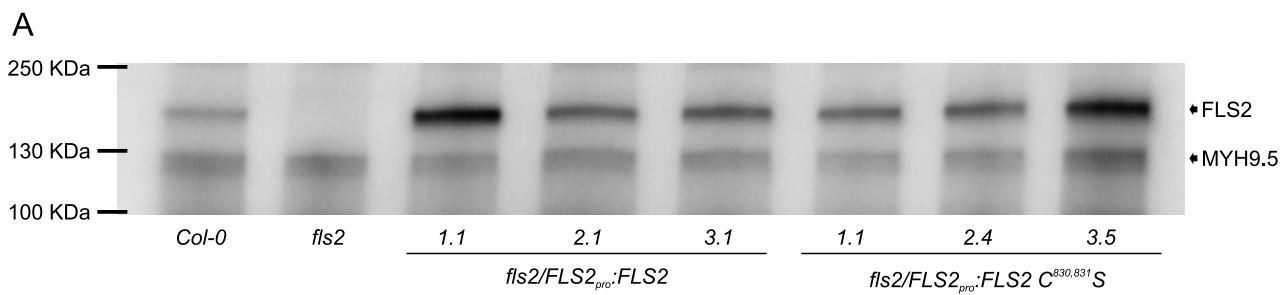
2. Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

3. Present address: Biomedical Sciences Research Complex, University of St Andrews, St Andrews, KY16 9ST, UK.

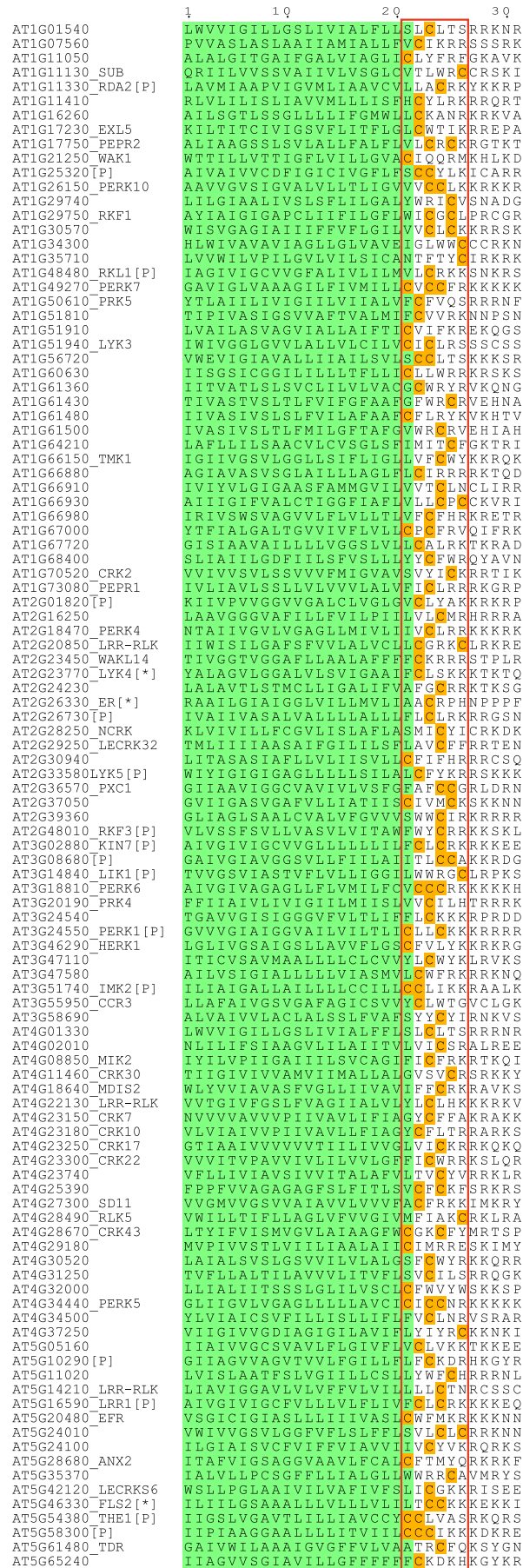
4. Information and Computer Sciences, James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

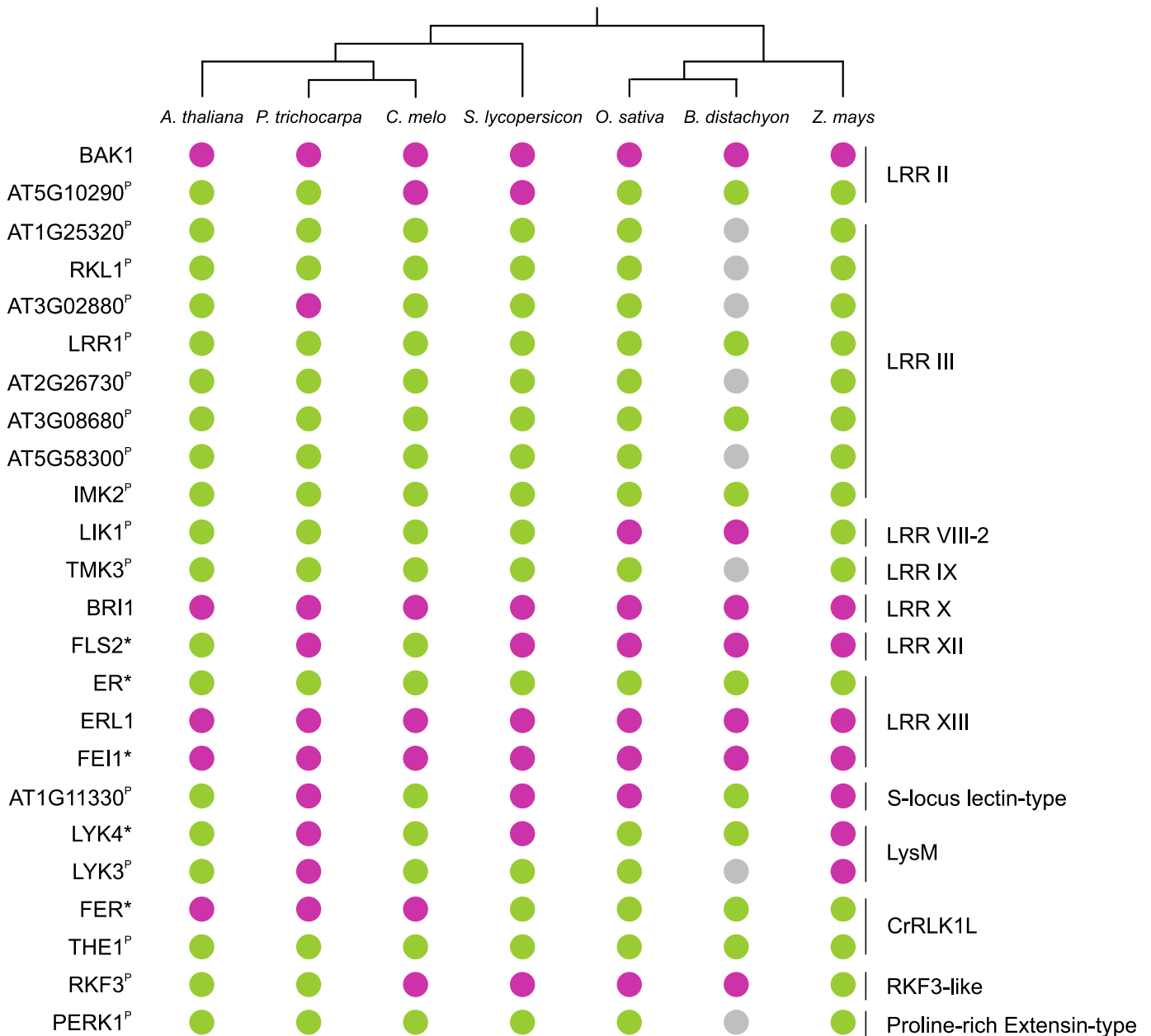
\* Author for correspondence: [p.a.hemsley@dundee.ac.uk](mailto:p.a.hemsley@dundee.ac.uk)

## Supplemental Data



**Supplemental figure 1. FLS2 C<sup>830,831</sup>S expressing plant responses to flg22 stimulation are indistinguishable from wild type (A).** FLS2 protein expression in *fls2/FLS2<sub>pro</sub>:FLS2* and *fls2/FLS2<sub>pro</sub>:FLS2 C<sup>830,831</sup>S* seedlings in transgenic lines used in this study. Lower band labelled MYH9.5 is also recognised by the antibody used and serves as a loading control. **(B).** Induction of *WRKY40*, **(C)** *NHL10* and **(D)** *PHI-1* gene expression after 1 hour treatment with 1 $\mu$ M flg22 in *fls2/FLS2<sub>pro</sub>:FLS2* and *fls2/FLS2<sub>pro</sub>:FLS2 C<sup>830,831</sup>S* seedlings as determined by qRT-PCR. **(E)** Induction of *PR1* gene expression after 24 hours treatment with 1 $\mu$ M flg22 in *fls2/FLS2<sub>pro</sub>:FLS2* and *fls2/FLS2<sub>pro</sub>:FLS2 C<sup>830,831</sup>S* seedlings as determined by qRT-PCR. Values were calculated using the  $\Delta\Delta C_T$  method from 3 technical replicates, error bars represent RQMIN and RQMAX and constitute the acceptable error level for a 95% confidence interval according to Student's t-test. Asterisks denote statistically significant differences in flg22 treated samples compared with that of the Col-0 flg22 treated sample (\*,  $P < 0.05$ ). Similar data were obtained over 2 biological repeats (additional data shown in figure 1). **(F)** Representative images of callose deposition following 12 hours treatment with water or 1 $\mu$ M flg22 used to generate graph in figure 1F.





**Supplemental figure 3.** Juxta-transmembrane cysteines are not necessarily conserved between orthologues. Top hit orthologues of Arabidopsis proteins identified as S-acylated through proteomics (denoted by superscript P) or tested in small scale assay (denoted by \*) were identified for species contained in the Aramemnon membrane protein database. Arabidopsis proteins that lack juxta-TM cysteines were also included for reference. The presence (green) or absence (magenta) of juxta-TM cysteines for any given receptor-like kinase in any given species is indicated. Grey indicates that no orthologous receptor-like kinase was found in the database. Phylogenetic relationships between species are indicated by the tree above the species names. Accepted receptor-like kinase family groupings are indicated on the right.

**Supplemental Table 1 - RLKs identified through S-acyl-proteomics (data from Hemsley et al 2013 New Phytologist 197(3):805-14**

<b>Locus Identifier</b>	<b>Gene name and (symbol) where known</b>
AT1G11330	S DOMAIN1 13 (SD1-13); RESISTANT TO DFPM INHIBITION OF ABA SIGNALING 2 (RDA2)
AT1G25320	
AT1G48480	RECEPTOR-LIKE KINASE 1 (RKL1)
AT2G01820	
AT2G26730	
AT2G33580	LYSM-CONTAINING RECEPTOR-LIKE KINASE 5 (LYK5)
AT2G48010	RECEPTOR-LIKE KINASE IN IN FLOWERS 3 (RKF3)
AT3G02880	KINASE 7 (KIN7)
AT3G08680	
AT3G14840	LYSM RLK1-INTERACTING KINASE 1 (LIK1)
AT3G24550	PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASE 1 (PERK1)
AT3G51740	INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2 (IMK2)
AT5G10290	
AT5G16590	LEUCINE RICH REPEAT PROTEIN 1 (LRR1)
AT5G54380	THESEUS1 (THE1)
AT5G58300	

Minutes 0 5 15 0 5 15 0 5 15 0 5 15 0 5 15

Active

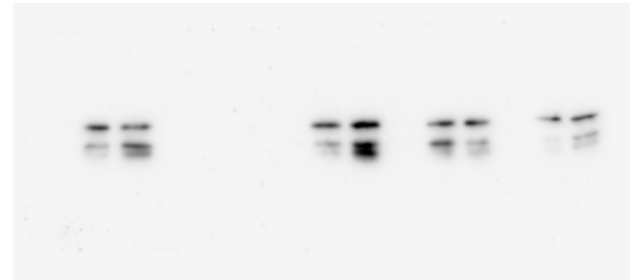
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 37 kDa →

Total

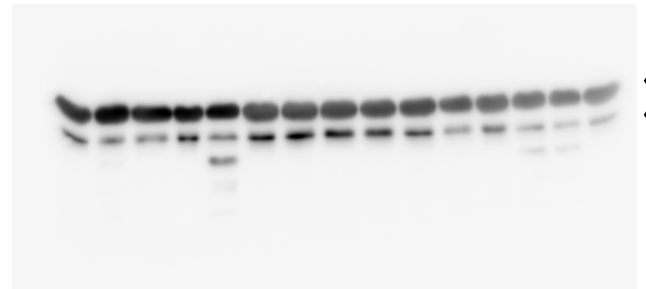
55 kDa →  
 37 kDa →

Col-0    *fls2*    1.1    2.1    3.1  
*fls2/FLS2<sub>pro</sub>:FLS2*

0 5 15 0 5 15 0 5 15 0 5 15 0 5 15



♦ pMAPK6  
 ♦ pMAPK3



♦ Rubisco  
 ♦ MAPK6

Col-0    *fls2*    1.1    2.4    3.5  
*fls2/FLS2<sub>pro</sub>:FLS2<sup>G30,831S</sup>*

A

