Large-scale identification of ubiquitination sites on membrane-associated proteins in *Arabidopsis thaliana* seedlings

Lauren E. Grubb\(^{1,2,+}\), Paul Derbyshire\(^2\), Katherine E. Dunning\(^1\), Cyril Zipfel\(^{2,3}\), Frank L.H. Menke\(^{2,*}\), and Jacqueline Monaghan\(^{1,2,*}\)

\(^1\)Department of Biology, Queen’s University, Kingston, Canada
\(^2\)The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, United Kingdom
\(^3\)Department of Plant and Microbial Biology, Zurich-Basel Plant Science Center, University of Zurich, Zurich, Switzerland

*Current address: John Innes Centre, Norwich Research Park, Norwich, United Kingdom

*Senior Authors authors:  
frank.menke@tsl.ac.uk
jacqueline.monaghan@queensu.ca

Author for Contact: jacqueline.monaghan@queensu.ca

Short title: Site-specific ubiquitination of membrane proteins

One-sentence summary: An analysis of the identification of ubiquitination sites on proteins found at the cell periphery, including over 100 protein kinases.
Dear Editor,

Protein phosphorylation and ubiquitination are two of the most frequently observed post-translational modifications in eukaryotes, regulated by thousands of protein kinases, phosphatases, E3 ubiquitin ligases, and ubiquitin proteases. Although previous studies have catalogued several ubiquitinated proteins in plants (Walton et al. 2016), few ubiquitinated membrane-localized proteins have been identified. Receptor kinases (RKs) initiate phosphorylation signal relays that regulate plant growth, development, and stress responses. While the regulatory role of phosphorylation on protein kinase function is well-documented (Couto and Zipfel 2016), considerably less is known about the significance of ubiquitination on protein kinases, even though their turnover is critical to signaling competence and cellular homeostasis. Here we describe the large-scale identification of ubiquitination sites on Arabidopsis (Arabidopsis thaliana) proteins associated with or integral to the plasma membrane, including over 100 protein kinases.

Proteins can be mono-, poly-, and/or multi-mono-ubiquitinated, each affecting protein function in different ways (Vierstra 2012; Swatek and Komander 2016). Dynamic interplay between phosphorylation and ubiquitination has been observed in several proteins involved in immune signaling (Mithoe and Menke 2018), including layered post-translational regulation of the receptor-like cytoplasmic kinase (RLCK) BOTRYTIS-INDUCED KINASE1 (BIK1). BIK1 is directly phosphorylated and activated by several ligand-bound RKs (Couto and Zipfel 2016), and can be dephosphorylated by the phosphatase PP2C38 (Couto et al. 2016). Precise control of BIK1 abundance is regulated by poly-ubiquitination by the E3 ligases PLANT U-BOX25 (PUB25) and PUB26 (Wang et al. 2018), as well as phosphorylation by CALCIUM-DEPENDENT PROTEIN KINASE28 (CPK28; Monaghan et al. 2014;
Wang et al. (2018) and the mitogen-activated protein kinase kinase kinase kinase kinase (MAP4K) SERINE/THREONINE KINASE1 (SIK1)/MAP4K4 (Zhang et al. 2018; Jiang et al. 2019). Most recently, it was shown that BIK1 is also mono-ubiquitinated by the E3 ligases RING-H2 FINGER A3A (RHA3A) and RHA3AB to regulate its activation and endocytosis (Ma et al. 2020).

Proteomics and mutagenesis approaches have resulted in the discovery of several phosphorylated residues on BIK1 (Liang and Zhou 2018). To help us understand the role of ubiquitination on BIK1 function, we set out to identify in vivo ubiquitination sites on BIK1. We enriched for plasma membrane-localized BIK1 by isolating microsomal protein fractions from Col-0/pBIK1:BIK1-HA, cpk28-1/pBIK1:BIK1-HA and CPK28-OE1/pBIK1:BIK1-HA genotypes, which express 100-fold higher levels of BIK1 and differentially accumulate BIK1 protein compared to wild-type (Monaghan et al. 2014). To increase protein abundance of non-integral proteins and allow us to potentially capture immune-induced ubiquitination, proteasomal machinery was inhibited with 50 μM MG-132 an hour before treatment with water or 1 μM elf18 (an immunogenic peptide derived from bacterial EF-Tu (Zipfel et al. 2006)). Microsomal protein fractions were digested with trypsin, and anti-K-ε-GG agarose beads (Udeshi et al. 2013) were used to enrich ubiquitinated peptides by affinity binding. Ubiquitinated lysines were identified based on a shift of ~114 Da—the mass of two glycine remnants that remain covalently bound to lysines following trypsin digestion—using liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) (Supplementary Methods). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al. 2019) partner repository with the dataset identifier PXD021992 and 10.6019/PXD021992.
We filtered our data for peptides with the diGly ubiquitin remnant, setting a threshold Mascot ion score of >20 and required multiple spectra for each peptide. This resulted in the identification of a total of 916 ubiquitinated peptides on 450 proteins across several biological replicates with a peptide false discovery rate of 0.025 (Supplemental Table S1), and an additional 526 peptides on 398 proteins observed in single experiments (Supplemental Table S2). Included in these data were seven ubiquitinated lysines on BIK1 (Table 1, Figure 1, Supplemental Tables S1 and S2). Given our particular interest in BIK1, we manually inspected all spectra mapping to BIK1 and found an additional three sites (Figure 1 and Supplemental Figure S1), altogether corroborating five of the ubiquitinated residues reported by (Ma et al. 2020) and revealing five novel ones (Figure 1). Thus, BIK1 is ubiquitinated on multiple surface-exposed lysines in vivo: three in the N-terminal variable domain (K31, K41, K61), seven in the canonical kinase domain (K95, K106, K155, K170, K186, K286, K337), and five in the C-terminal region (K358, K366, K369, K374, K388) (Figure 1). Whether RHA3A/B and PUB25/26 compete for these sites or ubiquitinate distinct lysines remains to be tested experimentally, as does clarifying which E2 conjugating enzymes work with respective E3 ligases to catalyze these events (Turek et al. 2018). Furthermore, as the phospho-status of BIK1 has been shown to affect its regulation by both RHA3A/B and PUB25/26 (Wang et al. 2018; Ma et al. 2020), another challenge will be resolving the biochemical mechanisms underlying this interplay.

Analysis of gene ontology (GO) terms associated with proteins identified in the high-confidence dataset (Supplemental Table S1) indicated an enrichment of proteins localized to the ‘plasma membrane’ \((p=1.53 \times 10^{-114})\) (Supplemental Table S3). Because we analysed the samples in the mass spectrometer in data-dependent
mode, without quantification, we are unable to comment on differences between genotypes or immune treatments. Therefore, any immune-triggered events must be corroborated experimentally. Multiple sequence alignments of peptides spanning -10 to +10 amino- and carboxyl-terminal to the modified lysines indicated very little consensus and no significant motifs (Supplemental Figure S2). Unlike other post-translational modifications, the ubiquitination reaction requires coordination between E1 activating, E2 conjugating, and E3 ligase enzymes (Vierstra 2012). While it may be possible for individual E2-E3 pairs to exhibit residue-level specificity on their target proteins, data from multiple species suggest that surface-availability may be the only unifying feature of ubiquitinated residues (Danielsen et al. 2011).

We identified ubiquitinated peptides mapping to proteins from diverse families, including aquaporins, H\(^{+}\) and Ca\(^{2+}\) ATPases, remorins, several classes of transporters, cellulose synthases, and others (Supplemental Tables S1 and S2). Comparison between our dataset and 8 published Arabidopsis ubiquitome datasets, as well as manual inspection of the literature, revealed 265 novel ubiquitin targets (Supplemental Table S4). We noted that molecular function GO terms ‘protein modification’ \(p=1.79 \times 10^{-12}\), ‘phosphorylation’ \(p=2.15 \times 10^{-26}\), and ‘response to stimulus’ \(p=6.44 \times 10^{-21}\) were particularly enriched in our dataset (Supplemental Table S3). Interestingly, we identified multiple ubiquitinated lysines on over 70 RKs representing diverse subgroups, including FLS2, EFR, CERK1, LORE, RLK7, SOBIR1/EVR, LIK1, RKL1, WAK1, WAK2, FER, ER, BAM1, BAM2, and others (Table 1). We also identified ubiquitination sites on more than 20 plasma membrane-associated cytoplasmic protein kinases from several subgroups (Table 1). Because analysis of tryptic peptides with ubiquitinated lysine residues enriched by anti-K\(\epsilon\)-GG does not allow for discrimination between mono- or poly-ubiquitination, it is likely
that we have captured both degradative and non-degradative ubiquitination on these protein kinases. Given the broad interest in phosphorylation-based signal transduction and protein homeostasis, we expect this information will be valuable to the plant research community and look forward to future studies that explore the function of these ubiquitination events.

Supplemental Data

Supplemental Methods.

Supplemental Figure S1. Ubiquitinated residues identified on BIK1.

Supplemental Figure S2. Consensus motif analysis of ubiquitinated lysines.

Supplemental References.

Supplemental Table S1. High-confidence peptides identified in multiple experiments.

Supplemental Table S2. Peptides identified in single experiments.

Supplemental Table S3. Gene ontology terms associated with proteins identified in this study.

Supplemental Table S4. Comparative analysis reveals 265 unique ubiquitin targets identified in this study.

Footnotes

Acknowledgements

We thank Jan Sklenar for helpful suggestions and technical assistance and are grateful to Melissa Bredow for help using Phyre2 and PyMol. We thank Libo Shan and Ping He for sharing data prior to publication.
Funding

This research was funded through a Biotechnology and Biological Sciences Research Council (BBSRC) Anniversary Future Leader Fellowship (J.M.), a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant (J.M.), a John R. Evans Leader’s Fund grant from the Canadian Foundation for Innovation and the Ontario Ministry of Research and Innovation (J.M.), Queen’s University start-up funds (J.M.), a grant from the European Research Council under the Grant Agreement 309858 (grant "PHOSPHinnATE", C.Z.), and through generous support of the Gatsby Charitable Foundation (C.Z. and F.L.H.M). L.E.G. and K.D. were supported by NSERC Canada Graduate Scholarships for Masters students (CGS-M), NSERC Michael Smith Foreign Study Supplements, and Ontario Graduate Scholarships (OGS).

Author Contributions

F.L.H.M. and J.M. designed the research; L.E.G. and F.L.H.M. performed the experiments; L.E.G., P.D., K.D., F.L.H.M., and J.M. analyzed the data; C.Z., F.L.H.M., and J.M. supervised the work; J.M. wrote the letter with input from all authors.

Tables and Figures

Table 1: Ubiquitinated protein kinases identified in this study.

Proteins matching the gene ontology term “kinase activity” were filtered from Supplementary Tables S1 and S2 and classified based on phylogenies presented by (S.-H. Shiu and Bleecker 2001; S. H. Shiu and Bleecker 2003). *Residues that are
only supported by a single observation (Table S2) are indicated by an asterisk and should be interpreted with caution. **Residues that were observed only after manual inspection of mass spectra matching BIK1 are indicated with two asterisks and shown in Supplementary Figure S1.

**Receptor-like protein kinases**

| Protein family | Accession | Protein name | K-GG     |
|----------------|-----------|--------------|----------|
| SD-1           | AT1G11300  | EGM1         | K514, K527 |
|                | AT4G27300  | SD1-1        | K518*, K550*, K648 |
|                | AT4G21380  | ARK3/RK3/SD1-8 | K661    |
|                | AT1G11350  | CBRLK1/RKS2  | K528     |
|                | AT1G61550  | S-locus lectin protein kinase family protein | K507     |
|                | AT1G11330  | RDA2         | K529, K542 |
|                | AT1G61380  | LORE/SD1-29  | K493, K506* |
| SD-2           | AT2G19130  | S-locus lectin protein kinase family protein | K498*, K591 |
|                | AT1G34300  | Lectin protein kinase family protein | K489*, K710 |
|                | AT4G32300  | SD2-5        | K641*, K727 |
| L-LEC          | AT4G28350  | LecRK-VII.2  | K339      |
|                | AT3G53380  | LecRK-VIII.1 | K374      |
|                | AT2G37710  | RLK/LecRK-IV.1 | K350*, K370 |
| C-LEC          | AT1G52310  | C-type lectin receptor kinase | K265*, K278*, K292 |
|                | AT1G70520  | CRK2         | K379*     |
|                | AT4G23180  | CRK10/RLK4   | K438*, K449 |
| CRK/DUF26      | AT4G23190  | CRK11/RLK3   | K349, K366, K368, K400*, K451 |
|                | AT4G23300  | CRK22        | K352, K369, K371, K381 |
| Gene ID    | Protein Name          | K Values         |
|-----------|-----------------------|------------------|
| AT4G05200 | CRK25                 | K448, K507       |
| AT4G11530 | CRK34                 | K363, K399       |
| AT4G04570 | CRK40                 | K376*, K402      |
| AT5G20050 | URK-II family protein | K199*, K156      |
| AT5G54380 | THE1                  | K480*, K526, K534, K560*, K657*, K753* |
| AT3G51550 | FERONIA               | K530, K534, K549, K561, K672, K759, K771, K773, K781*, K843* |
| AT3G46290 | HERK1                 | K479, K498, K501 |
| AT1G30570 | HERK2                 | K518             |
| AT2G23200 | CrRLK1L-1 family protein | K710            |
| AT5G38990 | MDS1                  | K541, K554*, K646* |
| AT1G51800 | IOS1                  | K721             |
| AT1G51890 | LRR-la family protein | K543*            |
| AT2G37050 | BSR050                | K740             |
| AT4G33430 | SERK3/BAK1            | K339*            |
| AT2G13800 | SERK5/BAK8            | K303             |
| AT5G10290 | LRR-II family protein | K276, K314, K469 |
| AT5G16000 | NIK1                  | K320*            |
| AT2G23950 | CLERK                 | K317             |
| AT3G17840 | RLK902                | K315, K336, K347 |
| AT1G48480 | RKL1                  | K353, K506       |
| AT5G58300 | LRR-III family protein | K326*           |
| AT2G26730 | LRR-III family protein | K293, K315*, K416 |
| AT2G36570 | PXC1                  | K319             |
| ATG       | Description            | K-Value |
|-----------|------------------------|---------|
| AT3G08680 | LRR-III family protein | K407    |
| AT5G16590 | LRR1                   | K317    |
| AT1G53730 | SRF6                   | K392*   |
| AT3G14350 | SRF7                   | K322    |
| AT4G22130 | SRF8                   | K344*, K353 |
| AT5G63410 | LRR-VI family protein  | K397, K427 |
| AT2G02780 | LRR-VI family protein  | K403*   |
| AT3G28040 | LRR-VIIa family protein| K742, K728 |
| AT1G80870 | LRR-VIIa family protein| K89     |
| AT5G49760 | HPCA                   | K600*, K605, K625, K685* |
| AT3G14840 | LIK1                   | K666, K677, K688*, K700, K774, K793, K808, K821, K963* |
| AT1G66150 | TMK1                   | K640, K746 |
| AT2G01820 | TMK3                   | K601, K637*, K743, K812 |
| AT3G23750 | BARK1                  | K736*   |
| AT5G48380 | BIR1                   | K354, K562 |
| AT3G28450 | BIR2                   | K290, K514 |
| AT5G42440 | LRR-Xb family protein  | K109    |
| AT2G01820 | PSKR1                  | K757*   |
| AT5G65700 | BAM1                   | K785    |
| AT3G49670 | BAM2                   | K781, K914 |
| AT1G28440 | HSL1                   | K845, K957* |
| AT1G09970 | RLK7/LRR XI-23         | K689, K703*, K818, K904, K966* |
| AT2G33170 | LRR XI family protein  | K835*   |
| Accession | Protein name | KGGs |
|-----------|--------------|------|
| AT5G25930 | LRR XI family protein | K701*, K941* |
| AT1G72180 | LRR XI family protein | K704* |
| AT5G20480 | EFR | K999, K1004 |
| AT5G46330 | FLS2 | K924, K940 |
| AT1G27190 | BIR3 | K339 |
| AT1G31420 | FEI1 | K358 |
| AT4G08850 | MIK2/BSR850 | K770, K788, K793, K803, K818 |
| AT2G26330 | ERECTA | K668 |
| AT3G02130 | RPK2/TOAD2/CLI1 | K1144 |
| AT2G31880 | SOBI1/EVR | K640* |
| AT3G21630 | CERK1 | K452 |
| AT1G25390 | LRK10L4 | K309 |
| AT4G60800 | Protein kinase superfamily protein | K293 |
| AT3G55950 | CCR3 | K514 |
| AT1G11050 | Protein kinase superfamily protein | K449 |
| AT1G21250 | WAK1/PRO25 | K403, K425, K437 |
| AT1G21270 | WAK2 | K420, K432, K668* |
| AT2G23450 | WAKL family protein | K653* |
| AT3G45780 | PHOT1/NPH1/RPT1 | K526, K899 |

**Cytoplasmic protein kinases**

| Protein family | Accession | Protein name | KGGs |
|----------------|-----------|--------------|------|
| RLCK-V         | AT3G59110 | RLCK-V family protein | K206 |
| RLCK-VII       | AT2G39660 | BIK1 | K31*, K41, K61, K155**, K337*, K358*, K366**, K369**, K374*, K388 |
| Gene ID       | Description                  | Reference Numbers |
|--------------|------------------------------|-------------------|
| AT2G17220    | PBL32/KIN3                   | K99, K242, K347   |
| AT5G13160    | PBS1                         | K204              |
| AT5G18610    | PBL27                        | K201              |
| AT5G03320    | PBL40                        | K115*             |
| AT1G06700    | PTI1-1                       | K71               |
| AT2G30740    | PTI1-2                       | K38, K74          |
| AT3G59350    | PTI1-3                       | K116, K133        |
| AT2G47060    | PTI1-4                       | K46, K303*        |
| AT3G17410    | PTI1-7/CARK1                 | K89, K190, K299   |
| AT4G35230    | BSK1                         | K85               |
| AT4G00710    | BSK3                         | K67, K481*        |
| AT5G59010    | BSK5                         | K64*              |
| AT3G54030    | BSK6                         | K65               |
| AT1G63500    | BSK7                         | K68*, K105, K304* |
| AT1G52540    | RLCK-XV family protein       | K249*             |
| AT3G24550    | PERK1                        | K303              |
| AT4G32710    | PERK14                       | K18*              |
| AT3G20410    | CPK9                         | K71, K115, K427   |
| AT4G04720    | CPK21                        | K84, K526*        |
| AT5G66210    | CPK28                        | K25, K34, K48*, K97, K108, K206*, K217, K351, K401*, K785 |
| AT3G63260    | MRK1/RAF48                   | K342              |
| AT1G65950    | Protein kinase superfamily   | K421              |
| AT4G00300    | Fringe-related protein       | K775              |
| AT1G56145    | LRR transmembrane protein    | K725              |
| Gene ID       | Description                                           | Kinase |
|--------------|-------------------------------------------------------|--------|
| AT3G27560    | ATN1                                                  | K44*   |
| AT1G03740    | Protein kinase superfamily protein                    | K56*   |
| AT3G25840    | PRP4KA                                                | K462*  |
| AT4G35500    | Protein kinase superfamily protein                    | K247*  |
| AT5G05200    | Protein kinase superfamily protein                    | K31*   |
| AT5G40540    | Protein kinase superfamily protein                    | K44*   |
| AT5G38480    | GRF3/RCI1                                             | K52    |
| AT1G12000    | Phosphofructokinase family protein                    | K23    |
| AT4G21534    | SPHK2                                                 | K49, K59|
| AT4G09320    | NDPK1                                                 | K106   |
| AT5G50780    | AtMORC4                                               | K736*, K766|
| AT1G12330    | Cyclin-dependent kinase-like protein                  | K184   |
| AT4G36080    | Inositol or phosphatidylinositol kinase                | K3581* |
| AT1G20930    | CDKB2;2                                               | K88*   |
| AT5G26667    | PYR6                                                  | K54*   |
| AT4G29130    | GIN2/HXK1                                             | K117*  |
| AT1G10900    | Phosphatidylinositol-4-phosphate 5-kinase family protein | K28*   |
**Figure 1: BIK1 is ubiquitinated on multiple lysines *in vivo*.**

A comparison between this study and (Ma et al. 2020) indicates that BIK1 is ubiquitinated on three lysines at its amino (N) terminus, seven in its kinase domain, and five at its carboxyl (C) terminus. Ubiquitinated lysines identified in (Ma et al. 2020) are shown in green, those identified in this study are shown in blue, and residues identified in both studies are in magenta. The ATP-binding site (ABS), catalytic loop (CL), activation loop (AL) and P +1 loop (PL) are indicated; the ABS is not surface-exposed, but the CL is shown in dark gray, the AL in white, and the PL in black. Although the structure of the BIK1 canonical kinase domain was recently solved (Lal et al. 2018), we modelled BIK1 in Phyre2 intensive mode (Kelley et al. 2015) in order to include the disordered N- and C-terminal ends in this surface representation in PyMol (The PyMol Molecular Graphics System, Version 2.0 Schrodinger, LLC). Phyre2 intensive modeling maximises sequence coverage and confidence to model regions for which there is no template information by an *ab initio* simplified-folding physics simulation; while 354/395 (90%) of the residues were modelled at >90% accuracy, it is likely that the model does not completely reflect the protein structure.
Couto, Daniel, Roda Niebergall, Xiangxiu Liang, Christoph A. Bücherl, Jan Sklenar, Alberto P. Macho, Vardis Ntoukakis, et al. 2016. “The Arabidopsis Protein Phosphatase PP2C38 Negatively Regulates the Central Immune Kinase BIK1.” *PLoS Pathogens* 12 (8): e1005811.

Couto, Daniel, and Cyril Zipfel. 2016. “Regulation of Pattern Recognition Receptor Signalling in Plants.” *Nature Reviews. Immunology* 16 (9): 537–52.

Danielsen, Jannie M. R., Kathrine B. Sylvestersen, Simon Bekker-Jensen, Damian Szklarczyk, Jon W. Poulsen, Heiko Horn, Lars J. Jensen, Niels Mailand, and Michael L. Nielsen. 2011. “Mass Spectrometric Analysis of Lysine Ubiquitylation Reveals Promiscuity at Site Level.” *Molecular & Cellular Proteomics: MCP* 10 (3): M110.003590.

Jiang, Yunhe, Baoda Han, Huoming Zhang, Kiruthiga G. Mariappan, Jean Bigeard, Jean Colcombet, and Heribert Hirt. 2019. “MAP4K4 Associates with BIK1 to Regulate Plant Innate Immunity.” *EMBO Reports* 20 (11).

Jiang, Xiangxiu, and Jian-Min Zhou. 2018. “Receptor-Like Cytoplasmic Kinases: Central Players in Plant Receptor Kinase-Mediated Signaling.” *Annual Review of Plant Biology* 69 (April): 267–99.

Ma, Xiyu, Lucas A. N. Claus, Michelle E. Leslie, Kai Tao, Zhiping Wu, Jun Liu, Xiao Yu, et al. 2020. “Ligand-Induced Monoubiquitination of BIK1 Regulates Plant Immunity.” *Nature* 581 (7807): 199–203.

Mithoe, Sharon C., and Frank Lh Menke. 2018. “Regulation of Pattern Recognition Receptor Signalling by Phosphorylation and Ubiquitination.” *Current Opinion in Plant Biology* 45 (Pt A): 162–70.

Monaghan, Jacqueline, Susanne Matschi, Oluwaseyi Shorinola, Hanna Rovenich, Alexandra Matei, Cécile Segonzac, Frederikke Gro Malinovsky, et al. 2014. “The Calcium-Dependent Protein Kinase CPK28 Buffers Plant Immunity and Regulates BIK1 Turnover.” *Cell Host & Microbe* 16 (5): 605–15.

Perez-Riverol, Yasset, Atilia Csordas, Jingwen Bai, Manuel Bernal-Llinares, Suresh Hewapathirana, Deepti J. Kundi, Avinash Inuganti, et al. 2019. “The PRIDE Database and Related Tools and Resources in 2019: Improving Support for Quantification Data.” *Nucleic Acids Research* 47 (D1): D442–50.

Shiu, S-H, and A. B. Bleecker. 2001. “Plant Receptor-Like Kinase Gene Family: Diversity, Function, and Signaling.” *Science Signaling.* https://doi.org/10.1126/stke.2001.113.re22.

Shiu, Shin Han, and Anthony B. Bleecker. 2003. “Expansion of the Receptor-like kinase/Pelle Gene Family and Receptor-like Proteins in Arabidopsis.” *Plant Physiology* 132 (2): 530–43.

Swatek, Kirby N., and David Komander. 2016. “Ubiquitin Modifications.” *Cell*
Research 26 (4): 399–422.
Turek, I., N. Tischer, R. Lassig, and M. Trujillo. 2018. “Multi-Tiered Pairing Selectivity between E2 Ubiquitin–conjugating Enzymes and E3 Ligases.” The Journal of Biological Chemistry. http://www.jbc.org/content/293/42/16324.short.
Udeshi, Namrata D., Philipp Mertins, Tanya Svinkina, and Steven A. Carr. 2013. “Large-Scale Identification of Ubiquitination Sites by Mass Spectrometry.” Nature Protocols 8 (10): 1950–60.
Vierstra, Richard D. 2012. “The Expanding Universe of Ubiquitin and Ubiquitin-like Modifiers.” Plant Physiology 160 (1): 2–14.
Walton, A., E. Stes, N. Cybulski, M. Van Bel, and S. Inigo. 2016. “It’s Time for Some ‘site’-Seeing: Novel Tools to Monitor the Ubiquitin Landscape in Arabidopsis Thaliana.” The Plant. http://www.plantcell.org/content/28/1/6.short.
Wang, Jinlong, Lauren E. Grubb, Jiayu Wang, Xiangxiu Liang, Lin Li, Chulei Gao, Miaomiao Ma, et al. 2018. “A Regulatory Module Controlling Homeostasis of a Plant Immune Kinase.” Molecular Cell 69 (3): 493–504.e6.
Zhang, Meixiang, Yi-Hsuan Chiang, Tania Y. Toruño, Donghyuk Lee, Miaomiao Ma, Xiangxiu Liang, Neeraj K. Lal, et al. 2018. “The MAP4 Kinase SIK1 Ensures Robust Extracellular ROS Burst and Antibacterial Immunity in Plants.” Cell Host & Microbe 24 (3): 379–91.e5.
Zipfel, Cyril, Gernot Kunze, Delphine Chinchilla, Anne Caniard, Jonathan D. G. Jones, Thomas Boller, and Georg Felix. 2006. “Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation.” Cell 125 (4): 749–60.
Couto, Daniel, Roda Niebergall, Xiangxiu Liang, Christoph A. Bücherl, Jan Sklenar, Alberto P. Macho, Vardis Ntoukakis, et al. 2016. "The Arabidopsis Protein Phosphatase PP2C38 Negatively Regulates the Central Immune Kinase BIK1." PLoS Pathogens 12 (8): e1005811.

Google Scholar: Author Only Title Only Author and Title

Couto, Daniel, and Cyril Zipfel. 2016. "Regulation of Pattern Recognition Receptor Signalling in Plants." Nature Reviews. Immunology 16 (9): 537–52.

Google Scholar: Author Only Title Only Author and Title

Danielsen, Jannie M. R., Kathrine B. Sylvestersen, Simon Bekker-Jensen, Damian Szklarczyk, Jon W. Poulsen, Heiko Horn, Lars J. Jensen, Niels Maland, and Michael L. Nielsen. 2011. "Mass Spectrometric Analysis of Lysine Ubiquitylation Reveals Promiscuity at Site Level." Molecular & Cellular Proteomics: MCP 10 (3): M110.003590.

Google Scholar: Author Only Title Only Author and Title

Jiang, Yunhe, Baoda Han, Huoming Zhang, Kiruthiga G. Mariappan, Jean Bigeard, Jean Colcombet, and Heribert Hirt. 2019. "MAP4K4 Associates with BIK1 to Regulate Plant Innate Immunity." EMBO Reports 20 (11).

https://www.embopress.org/doi/abs/10.15252/embr.201947965.

Google Scholar: Author Only Title Only Author and Title

Kelley, Lawrence A., Stefans Mezulis, Christopher M. Yates, Mark N. Wass, and Michael J. E. Sternberg. 2015. "The Phyre2 Web Portal for Protein Modeling, Prediction and Analysis." Nature Protocols 10 (6): 845–58.

Google Scholar: Author Only Title Only Author and Title

Liang, Xiangxiu, and Jian-Min Zhou. 2018. "Receptor-Like Cytoplasmic Kinases: Central Players in Plant Receptor Kinase-Mediated Signaling." Annual Review of Plant Biology 69 (April): 267–99.

Google Scholar: Author Only Title Only Author and Title

Ma, Xiyu, Lucas A N. Claus, Michelle E. Leslie, Kai Tao, Zhiping Wu, Jun Liu, Xiao Yu, et al. 2020. "Ligand-Induced Monoubiquitination of BIK1 Regulates Plant Immunity." Nature 581 (7807): 199–203.

Google Scholar: Author Only Title Only Author and Title

Monaghan, Jacqueline, Susanne Matschi, Oluwaseyi Shorinola, Hanna Rovenich, Alexandra Matei, Cécile Segonzac, Frederikke Gro Malinovsky, et al. 2014. "The Calcium-Dependent Protein Kinase CPK28 Buffers Plant Immunity and Regulates BIK1 Turnover." Cell Host & Microbe 16 (5): 605–15.

Google Scholar: Author Only Title Only Author and Title

Shiu, Shin Han, and Anthony B. Bleecker. 2003. "Expansion of the Receptor-like kinase/Pelle Gene Family and Receptor-like Proteins in Arabidopsis." Plant Physiology 132 (2): 530–43.

Google Scholar: Author Only Title Only Author and Title

Swatek, Kirby N., and David Komander. 2016. "Ubiquitin Modifications." Cell Research 26 (4): 399–422.

Google Scholar: Author Only Title Only Author and Title

Turek, I., N. Tischer, R. Lassig, and M. Trujillo. 2018. "Multi-Tiered Pairing Selectivity between E2 Ubiquitin–conjugating Enzymes and E3 Ligases." The Journal of Biological Chemistry. http://www.jbc.org/content/293/42/16324.short.

Google Scholar: Author Only Title Only Author and Title

Udeshi, Namrata D., Philipp Mertins, Tanya Svinkina, and Steven A. Carr. 2013. "Large-Scale Identification of Ubiquitination Sites by Mass Spectrometry." Nature Protocols 8 (10): 1950–60.

Google Scholar: Author Only Title Only Author and Title

Vierstra, Richard D. 2012. "The Expanding Universe of Ubiquitin and Ubiquitin-like Modifiers." Plant Physiology 160 (1): 2–14.
Walton, A., E. Stes, N. Cybulski, M. Van Bel, and S. Inigo. 2016. "It's Time for Some 'site'-Seeing: Novel Tools to Monitor the Ubiquitin Landscape in Arabidopsis Thaliana." The Plant. http://www.plantcell.org/content/28/1/6.short.

Wang, Jinlong, Lauren E. Grubb, Jiayu Wang, Xiangxiu Liang, Lin Li, Chulei Gao, Miaomiao Ma, et al. 2018. "A Regulatory Module Controlling Homeostasis of a Plant Immune Kinase." Molecular Cell 69 (3): 493–504.e6.

Zhang, Meixiang, Yi-Hsuan Chiang, Tania Y. Toruño, Donghyuk Lee, Miaomiao Ma, Xiangxiu Liang, Neeraj K. Lal, et al. 2018. "The MAP4 Kinase SIK1 Ensures Robust Extracellular ROS Burst and Antibacterial Immunity in Plants." Cell Host & Microbe 24 (3): 379–91.e5.

Zipfel, Cyril, Gernot Kunze, Delphine Chinchilla, Anne Caniard, Jonathan D. G. Jones, Thomas Boller, and Georg Felix. 2006. "Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation." Cell 125 (4): 749–60.