Figure 1. Experimental overview and timing of flg22-activated responses.

1. Overview of the sampling strategy. Four-week-old Col-0 *Arabidopsis thaliana* plants grown in soil were sprayed with 10uM flg22 peptide + 0.025% Silwett L-77 solution or H2O + 0.025% Silwett L-77 as a control. Rosette leaf tissue was harvested for total PM protein enrichment and RNA-seq at the indicated time points. The line graph represents expression of the FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (FRK1) marker gene after flg22 treatment as determined by real-time RT-PCR. Expression values were normalized to Elongation factor-1ɑ. Plotted values represent the mean ratio ± SD of FRK1 expression in flg22-treated samples relative to the H2O control treatment at each time point.
2. Robust mitogen-activated protein kinase (MPK) activation is observed 10-15 minutes post-flg22 treatment. Plants were treated as in (A). Total leaf protein was extracted from tissue harvested at the indicated time points and 10ug was subjected to SDS-PAGE and immunoblotting using an antibody recognizing phospho-p44/p42 MPK. Signals corresponding to MPK3 and MPK6 are indicated with arrows.
3. Reproducible callose deposition is observed 720 minutes (12h) post-flg22 treatment as determined by aniline blue staining. Plants were treated as in (A) and tissue was processed according to (Adam and Somerville, 1996).

Supplemental Figure 1: RNA/protein isolations and data analysis/filtering pipelines

Supplemental Table 1: Summary of Xtandem parameters and Scaffold identification modeling

Supplemental Table 2: MS/MS isoform count data, protein ID probability, exclusive spectrum counts

Supplemental Table 3: MS/MS locus count data and QProt analysis

Supplemental Table 4: GeneCounter summary of total read mapping

Supplemental Table 5: pre-filtered gene counts

Supplemental Table 6: 10ct filtered - 10min - reads mapped to locus and NBP-seq analysis

Supplemental Table 7: 10ct filtered - 60min - reads mapped to locus and NBP-seq analysis

Supplemental Table 8: 10ct filtered - 180min - reads mapped to locus and NBP-seq analysis

Supplemental Table 9: 10ct filtered - 360min - reads mapped to locus and NBP-seq analysis

Supplemental Table 10: 10ct filtered - 720min - reads mapped to locus and NBP-seq analysis

Supplemental Figure 2: Rank-order scatter plot of mRNA (FPKM) and PM protein (NSAF) relative abundance values

Supplemental Table 11: List of zero and low mRNA proteins highlighted in Supplemental Figure 02

Figure 2. Identification overlap across datasets and Gene Ontology (GO) classification of identified genes

1. Left, Venn diagram of overlap in total gene identifications in the RNA-seq and PM protein datasets. Right, Venn diagram of the overlap in filtered gene identifications in RNA-seq and PM protein datasets. The filtered datasets were used for differential expression analysis. The filtered RNA-seq data includes only genes with ten or more counts in all three replicates of either treatment (flg22) or control (H2O) at a given time point. The filtered MS/MS data includes proteins identified in at least two replicates of either treatment or control at a given time point.
2. Comparison of plant GO Slim Biological Process term annotations in filtered RNA-seq and PM-enriched protein identifications with the *A. thaliana* genome. Annotations were retrieved from the BioMart ‘plantsmart21’ database. Data are presented as the percentage of total annotations in each respective dataset.Complete results can be found in Supplemental Table 12.
3. Comparison of plant GO Slim Molecular Function term annotations in filtered RNA-seq and PM-enriched protein identifications with the *A. thaliana* genome. Annotations were retrieved and presented as in (B). Complete results can be found in Supplemental Table 12.

Supplemental Table 12: Complete results from comparison of mRNA/protein identifications with *A. thaliana* genome

Figure 3. Predicted sub-cellular localization of proteins identified by MS/MS and genes identified by RNA-seq **I will modify B and C to include PM association evidence as well**

1. Comparison of plant GO Slim Molecular Function term annotations in filtered RNA-seq and PM-enriched protein identifications with the *A. thaliana* genome. Annotations were retrieved and presented as in (Figure 2B). Asterisks (\*) indicate the top 3 most significant terms (hypergeometric test with Benjamini-Hochberg correction, p<0.001) using the *A. thaliana* genome as the background set. Complete results can be found in Supplemental Table 12.
2. Predicted transmembrane domains and lipid modifications in filtered genes identified by RNA-seq. Transmembrane domains were predicted using TMHMM2.0 (retrieved from TAIR); myristoylation was predicted using PlantsP database myristoylation predictions; palmitoylation evidence was obtained from experimental data (Helmsley, 2012); GPI anchor predictions were retrieved from the Aramemnon webserver**;** prenylation was predicted using. Complete results can be found in Supplemental Table 13.
3. Predicted transmembrane domains and lipid modifications found in filtered proteins identified by MS/MS. The same resources were used as described in (B).

Supplemental Table 13: Summary of membrane association evidence from all prediction programs

Supplemental Figure 3: Venn of overlap in PM association evidence from GOslim, SUBA, and AtSubP

Figure 4. PM-associated proteins exhibit transcription dependent and independent regulation.

1. An overview of the percentage of genes that are differentially expressed (DE) in the RNA-seq and MS/MS data at the indicated time points. The largest percentage of RNA-DE genes was detected at 60 min post-treatment while the largest percentage of protein-DE genes was detected at 720 min post-treatment.
2. An overall weak correlation is observed between RNA and protein regulation. Scatter plot of RNA and protein log2 fold changes for all mRNA and protein pairs identified. The red dotted line is a linear regression of the plotted data. Pearson correlation coefficients are reported*.*
3. A stronger correlation in fold change was observed for genes that were differentially expressed at both the RNA and protein level.Scatter plot of RNA and protein log2 fold changes for all genes identified as differentially expressed in mRNA and protein datasets. The red dotted line is a linear regression of the plotted data. Pearson correlation coefficients are reported*.*
4. Hierarchical clustering of expression patterns for all genes detected as differentially expressed in the protein data (DE proteins and their associated RNA-DE expression). Asterisks on the dendrogram indicate the clades (lowercase letters a-j) queried for term enrichment analysis. ClueGO was used for Gene Ontology (GO) term enrichment with all identified proteins used as the background set. Selected GO terms significantly over-represented (p < 0.1 after Benjamini-Hochberg adjustment) in specific clusters are indicated on the right. Red boxes highlight clusters where differential protein expression was detected without corresponding changes detected at the transcript level. See Supplemental Table 13for full GO term enrichment results.

Supplemental Table 14: GO term enrichment in hierarchical clustering clades in Figure 4D

Figure 5. Overview of cellular responses to FLS2 activation.

Stacked bar charts depicting the number of differentially expressed genes and proteins with the indicated GOslim Biological Process annotations at each time point. Astericks (\*) indicate time points where the corresponding term is significantly enriched (hypergeometric test with Benjamini-Hochberg multiple test correction, p<0.1) relative to the background sets of filtered mRNA and protein identifications, respectively.

1. Immune system, protein targeting, stress and transmembrane transport processes are up-regulated at the mRNA and PM protein level during FLS2-PTI.
2. Kinase and transmembrane transporters are highly up-regulated at the PM in response to flg22 perception.
3. Major transcriptome changes are associated with the cell wall, extracellular region, and plasma membrane.

Supplemental Table 15: Summary of GOSlim term enrichment at each RNA-seq and MS/MS timepoint

Supplemental Table 16: Identified transporters exhibiting differential protein regulation during FLS2-PTI

Figure 6. Major temporal expression patterns during FLS2-PTI

**I am still working on this figure. We should know by next week whether it will be worth it to include the TF/cis element analysis of RNA-seq clusters. If not, we will not include Fig. 6A or Suppl. Fig.4 in the manuscript.**

1. RNA expression clusters identified using Splinecluster in Supplemental Figure4 were combined into thirteen non-overlapping groups according the first major time point of differential expression and expression patterns over the course of the experiment. Transcription factors and over-represented cis-element that are present in each cluster are indicated on the graphs. Complete transcription factor and cis-element analysis are located in Supplemental Tables 16 and 17.
2. Protein expression clusters identified from Splinecluster in Supplemental Figure5 were partitioned into seven non-overlapping groups according the first timepoint of differential expression and expression patterns over the course of the experiment. Enriched domains (based on the hypergeometric test with Benjamini-Hochberg adjustment, p < 0.1) present in at least two proteins within each cluster group are indicated on the plots. Complete protein domain enrichment results are located in Supplemental Table 18. **I will rework the protein group names to be in order**

Supplemental Figure 4: Splinecluster analysis RNA-seq data - individual clusters

Supplemental Table 17: Transcription factors differentially expressed during FLS2-PTI

Supplemental Table 18: cis-element over-representation in temporal RNA-seq clusters

Supplemental Figure 5: Splinecluster analysis MS/MS data - Individual clusters

Supplemental Table 18: Protein domain over-representation in PM protein temporal clusters

Supplemental Figure 6: LRR-RLK full phylogeny, expression patterns, and PM relative abundance

Supplemental Figure 7: Non-LRR RLK full phylogeny, expression patterns, and PM relative abundance

Figure 7. Network expression clustering highlights known regulators of plant immunity and development

1. BAK1-interacting proteins represent a major expression module identified through Autosome clustering (Morris, et al. 2011) of differentially expressed mRNA and protein fold changes using physical interactions within the RLK + first neighbor protein-protein interaction network. Nodes are colored according to their expression index in the RNA-seq data. The expression index is the sum of the number of time points a gene is identified as up-regulated minus the number of time points a gene was identified as down-regulated. Nodes that were significantly up- and down- regulated at different time points are represented by a triangle.
2. Nodes within the BAK1 sub-network exhibit different patterns of RNA and protein expression over time. Line charts representing mRNA and protein fold change values for the indicated genes over time. Asterisks indicate time points with significant differential expression for either mRNA (red line) or protein (blue line).
3. Same as in (A) but nodes are colored according their protein expression index.

Supplemental Figure 8: Complete results of Autosome network expression clustering

Figure 8. Calmodulin-like protein TCH3/CML12 interacts with Receptor-like kinases and contributes to plant immunity

1. TCH3/CML12 and RLK expression sub-network identified through Autosome clustering (see Figure 7A for details).
2. Nodes within the TCH3/RLK sub-network exhibit different patterns of RNA and protein expression over time. Line charts representing mRNA and protein fold change values for the indicated genes over time. Asterisks indicate time points with significant differential expression for either mRNA (red line) or protein (blue line).
3. Same as in (A) but nodes are colored according their protein expression index.
4. Enhanced pathogen growth is observed in *tch3-1* knockout plants (Student’s t-test, p<0.05). Wild-type Col-0 and *tch3-1* plants were sprayed with *Pseudomonas syringae* pv. tomato DC3000 (*Pto*) at a concentration of 1x108 cfu/mL. Leaf tissue was harvested at four days post-inoculation, homogenized, and dilution plated to assay *Pto* growth.
5. TCH3 associates with the PM during plant immune responses **Should have this data early next week**

Supplemental Figure 9: T-DNA insertion lines (tch3-1 and crk14-1) and RT-PCR knockout validation

Figure 9. Cysteine-rich receptor-like kinases (CRKs) are coordinately expressed after FLS2 activation and CRK14 contributes to plant immunity

1. Most of the CRK family is up-regulated at the mRNA level. CRK PM protein changes Left: Unrooted phylogeny of the annotated CRK family in Arabidopsis generated from full-length protein sequences. Middle: Heatmap of RNA and protein differential expression. Right: Bar chart of RLK relative protein expression in PM-enriched fractions. #, kinase domain only; \*, no kinase domain present.
2. Enhanced pathogen growth is observed in *crk14-1* knockout plants (Student’s t-test, p<0.001).
3. Several CRK phosphopeptides identified by MS/MS are conserved across multiple CRK proteins. Conserved phosphorylated residues are represented on the CRK13 domain architecture.
4. Functional analysis of the role of CRK13 phosphorylation in inducing cell death. Alanine mutants of CRK13T392 and CRK13S438 do not elicit cell death when transiently expressed in *Nicotiana tabacum.*

Supplemental Figure 10: Amino acid alignment of identified phosphopeptides

Supplemental Table 19: Complete list of phosphopeptide identifications