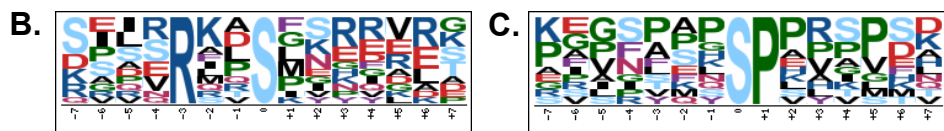


Supplemental Figure S1. Phosphosite distribution of differentially phosphorylated peptides in *mlk* mutants

The of threonine (T) phosphorylation, serine (S) phosphorylation, and tyrosine (Y) phosphorylation sites identified as having altered abundance in *mlk1/2/3* or *mlk1/3/4* mutants compared to wildtype samples at ZT12 (A) or ZT14 (B).

	#	Motif	Motif Score	Foreground Matches	Foreground Size	Background Matches	Background Size	Fold Increase
Decreased	1R.S.....	5.08	12	48	55686	1013104	4.55
Increased	1SP.....	5.71	11	37	53111	1013104	5.67



Supplemental Figure S2. Motif-X analysis of phosphopeptides with decreased abundance in *mlk1/2/3* mutants.

Phosphopeptides with altered abundance in *mlk1/2/3* (ZT12) mutants were extended and aligned using PEPTIDEXTENDER (<http://schwartzlab.uconn.edu/pepextend>). Motif-X analysis was then performed with the probability threshold was set to p-value $\leq 10^{-6}$, the occurrence threshold was set to 10. The default IPI Arabidopsis Proteome data set was used as the background data set.

Supplemental Table S1. Summary of Pairwise Comparisons

Pairwise Comparison	Global Proteome			Phosphoproteome		
	Total Peptides with Altered Abundance	Proteins with Increase Abundance	Proteins with Decrease Abundance	Total Peptides with Altered Abundance	Proteins with Increase Abundance	Proteins with Decrease Abundance
<i>mlk123_ZT12/WT_ZT12</i>	15	0	13	113	44	50
<i>mlk134_ZT12/WT_ZT12</i>	232	28	95	429	103	190
<i>mlk123_ZT14/WT_ZT14</i>	20	6	7	170	87	63
<i>mlk134_ZT14/WT_ZT14</i>	228	76	36	306	108	115

Supplemental Table S2. REVIGO Cellular Component GO-term Enrichment Analysis

<i>mlk1/2/3</i> ZT12					
GO-term ID	Description	Frequency	Log ₁₀ p-value	Uniqueness	Dispensability
GO:0005654	nucleoplasm	1.37%	-3.0182	0.15	0
GO:0044451	<i>nucleoplasm part</i>	0.90%	-2.0482	0.17	0.82
GO:0043226	organelle	20.79%	-2.5969	0.75	0
GO:0005634	nucleus	8.97%	-4.4283	0.13	0.51
GO:0043231	<i>intracellular membrane-bounded organelle</i>	13.76%	-2.6364	0.12	0.75
GO:0043227	membrane-bounded organelle	14.45%	-3.382	0.17	0.57
GO:0043229	<i>intracellular organelle</i>	19.92%	-2.6144	0.16	0.7

<i>mlk1/3/4</i> ZT12					
GO-term ID	Description	Frequency	Log ₁₀ p-value	Uniqueness	Dispensability
GO:0005575	cellular_component	100.00%	-1.7986	1	0
GO:0005623	cell	53.55%	-12.1624	0.97	0
GO:0005654	nucleoplasm	1.37%	-13.8069	0.31	0
GO:0044451	<i>nucleoplasm part</i>	0.90%	-10.1113	0.33	0.82
GO:0070013	<i>intracellular organelle lumen</i>	2.74%	-8.9747	0.3	0.95
GO:0031981	<i>nuclear lumen</i>	2.29%	-10.58	0.29	0.87
GO:0043233	<i>organelle lumen</i>	2.74%	-8.9747	0.33	0.97
GO:0031974	membrane-enclosed lumen	2.74%	-8.9747	0.94	0
GO:0032991	macromolecular complex	14.01%	-3.6517	0.95	0
GO:0043226	organelle	20.79%	-11.9788	0.95	0
GO:0043234	protein complex	6.42%	-4.0467	0.88	0
GO:0005829	cytosol	2.55%	-6.6478	0.72	0.17
GO:0044424	intracellular part	35.65%	-11.7077	0.68	0.22
GO:0005622	intracellular	41.18%	-11.4711	0.77	0.32
GO:0044444	cytoplasmic part	12.66%	-2.011	0.66	0.35
GO:0044464	cell part	52.39%	-10.7959	0.77	0.38
GO:0043227	membrane-bounded organelle	14.45%	-12.9172	0.47	0.4

GO:0043229	<i>intracellular organelle</i>	19.92%	-11.5935	0.39	0.7
GO:0000785	chromatin	0.47%	-9.0726	0.41	0.46
GO:0044427	<i>chromosomal part</i>	1.12%	-7.4962	0.39	0.84
GO:0000790	<i>nuclear chromatin</i>	0.23%	-1.6216	0.34	0.78
GO:0005694	chromosome	1.51%	-6.6556	0.5	0.49
GO:0005652	nuclear lamina	0.00%	-2.2358	0.53	0.51
GO:0043228	non-membrane-bounded organelle	8.41%	-2.4597	0.5	0.56
GO:0044428	nuclear part	3.12%	-10.7447	0.37	0.57
GO:0044446	<i>intracellular organelle part</i>	8.94%	-7.9355	0.32	0.73
GO:0044422	organelle part	9.43%	-7.8861	0.5	0.58
GO:0005634	nucleus	8.97%	-19.4547	0.39	0.58
GO:0043231	<i>intracellular membrane-bounded organelle</i>	13.76%	-12.6216	0.37	0.75
GO:0031965	nuclear membrane	0.10%	-1.7721	0.46	0.6
GO:0005849	mRNA cleavage factor complex	0.05%	-1.71	0.42	0.62
GO:0016607	nuclear speck	0.09%	-3.6498	0.42	0.65
GO:0016604	<i>nuclear body</i>	0.19%	-2.4895	0.39	0.7
GO:0043232	intracellular non-membrane-bounded organelle	7.95%	-2.4597	0.41	0.67

mlk1/2/3 ZT14

GO-term ID	Description	Frequency	Log ₁₀ p-value	Uniqueness	Dispensability
GO:0005623	cell	53.55%	-1.3298	0.93	0
GO:0031974	membrane-enclosed lumen	2.74%	-1.5258	0.87	0
GO:0044427	chromosomal part	1.12%	-3.9547	0.26	0
GO:0000785	<i>chromatin</i>	0.47%	-1.9872	0.27	0.84
GO:1902494	<i>catalytic complex</i>	3.73%	-3.6925	0.82	0
GO:0044422	organelle part	9.43%	-1.5686	0.35	0.36
GO:0043228	non-membrane-bounded organelle	8.41%	-1.3565	0.36	0.51
GO:0031981	nuclear lumen	2.29%	-2.757	0.23	0.53
GO:0043233	<i>organelle lumen</i>	2.74%	-1.5258	0.24	0.97
GO:0070013	<i>intracellular organelle lumen</i>	2.74%	-1.5258	0.23	0.95

GO:0005694	chromosome	1.51%	-2.9914	0.37	0.54
GO:0005634	nucleus	8.97%	-2.8239	0.32	0.55
GO:0044428	nuclear part	3.12%	-2.063	0.27	0.61
GO:0044446	<i>intracellular organelle part</i>	8.94%	-1.5918	0.22	0.73
GO:0043232	intracellular non-membrane-bounded organelle	7.95%	-1.3565	0.28	0.67

mlk1/3/4 ZT14

GO-term ID	Description	Frequency	Log₁₀ p-value	Uniqueness	Dispensability
GO:0005623	cell	53.55%	-5.2218	0.95	0
GO:0005654	nucleoplasm	1.37%	-6.6778	0.29	0
GO:0043233	<i>organelle lumen</i>	2.74%	-2.4559	0.31	0.97
GO:0044451	<i>nucleoplasm part</i>	0.90%	-4.9706	0.31	0.82
GO:0070013	<i>intracellular organelle lumen</i>	2.74%	-2.4559	0.27	0.95
GO:0031981	<i>nuclear lumen</i>	2.29%	-2.9547	0.26	0.87
GO:0031974	membrane-enclosed lumen	2.74%	-2.4559	0.91	0
GO:0043226	organelle	20.79%	-4.0615	0.92	0
GO:0044464	cell part	52.39%	-4.4935	0.72	0.12
GO:0005829	cytosol	2.55%	-3.8268	0.67	0.17
GO:0044424	intracellular part	35.65%	-3.8386	0.62	0.35
GO:0005622	intracellular	41.18%	-3.52	0.71	0.38
GO:0044427	chromosomal part	1.12%	-3.1024	0.36	0.5
GO:0000785	<i>chromatin</i>	0.47%	-1.8861	0.4	0.84
GO:0070449	elongin complex	0.01%	-1.719	0.48	0.53
GO:0005694	chromosome	1.51%	-2.1232	0.47	0.54
GO:0044428	nuclear part	3.12%	-1.857	0.33	0.57
GO:0005634	nucleus	8.97%	-8.9136	0.35	0.58
GO:0043231	<i>intracellular membrane-bounded organelle</i>	13.76%	-3.3686	0.33	0.75
GO:0043229	intracellular organelle	19.92%	-4.091	0.35	0.62
GO:0043227	<i>membrane-bounded organelle</i>	14.45%	-3.6861	0.44	0.7

Supplemental Table S3. REVIGO Biological Process GO-term Enrichment Analysis

<i>mlk1/2/3</i> ZT12					
GO-term ID	Description	Frequency	Log ₁₀ p-value	Uniqueness	Dispensability
GO:0040029	regulation of gene expression, epigenetic	0.13%	-1.4572	1	0
<i>mlk1/3/4</i> ZT12					
GO-term ID	Description	Frequency	Log ₁₀ p-value	Uniqueness	Dispensability
GO:0006325	chromatin organization	0.67%	-10.4078	0.88	0
GO:0016043	<i>cellular component organization</i>	7.24%	-2.5575	0.86	0.75
GO:0007064	<i>mitotic sister chromatid cohesion</i>	0.05%	-2.1267	0.89	0.54
GO:0051276	<i>chromosome organization</i>	1.48%	-10.1433	0.87	0.53
GO:0016570	<i>histone modification</i>	0.37%	-2.0696	0.77	0.9
GO:0016569	<i>covalent chromatin modification</i>	0.42%	-4.2168	0.8	0.7
GO:0006996	<i>organelle organization</i>	3.60%	-4.5768	0.86	0.65
GO:0007623	circadian rhythm	0.06%	-1.6968	0.98	0
GO:0009987	cellular process	63.78%	-1.6536	0.99	0
GO:0010033	response to organic substance	0.90%	-4.1475	0.76	0
GO:0001101	<i>response to acid chemical</i>	0.12%	-1.5017	0.79	0.59
GO:1901701	<i>cellular response to oxygen-containing compound</i>	0.35%	-2.6778	0.71	0.8
GO:1901700	<i>response to oxygen-containing compound</i>	0.50%	-1.9101	0.77	0.68
GO:0097305	<i>response to alcohol</i>	0.06%	-3.15	0.76	0.55
GO:0009737	<i>response to abscisic acid</i>	0.03%	-3.224	0.77	0.67
GO:0009725	<i>response to hormone</i>	0.34%	-2.3585	0.74	0.79
GO:0071359	<i>cellular response to dsRNA</i>	0.02%	-1.8794	0.73	0.81
GO:0033993	<i>response to lipid</i>	0.23%	-2.6289	0.74	0.77
GO:0070887	<i>cellular response to chemical stimulus</i>	1.01%	-2.4437	0.72	0.73
GO:0043331	<i>response to dsRNA</i>	0.03%	-1.8794	0.76	0.83
GO:0071407	<i>cellular response to organic cyclic compound</i>	0.17%	-2.6615	0.7	0.67
GO:0071310	<i>cellular response to organic substance</i>	0.65%	-1.9393	0.69	0.87

GO:0014070	<i>response to organic cyclic compound</i>	0.23%	-2.821	0.74	0.75
GO:0010605	negative regulation of macromolecule metabolic process	1.17%	-5.5421	0.51	0
GO:0031050	<i>dsRNA fragmentation</i>	0.02%	-1.9355	0.59	0.98
GO:0031047	<i>gene silencing by RNA</i>	0.09%	-4.1379	0.56	0.75
GO:0048523	<i>negative regulation of cellular process</i>	1.83%	-2.0635	0.57	0.92
GO:0031324	<i>negative regulation of cellular metabolic process</i>	1.17%	-3.3862	0.52	0.97
GO:0031327	<i>negative regulation of cellular biosynthetic process</i>	0.77%	-4.1463	0.51	0.99
GO:0035194	<i>posttranscriptional gene silencing by RNA</i>	0.03%	-3.3893	0.59	0.84
GO:0035196	<i>production of miRNAs involved in gene silencing by miRNA</i>	0.01%	-2.5287	0.41	0.94
GO:0035195	<i>gene silencing by miRNA</i>	0.02%	-2.8097	0.56	0.97
GO:0051253	<i>negative regulation of RNA metabolic process</i>	0.63%	-1.5129	0.47	0.96
GO:0070918	<i>production of small RNA involved in gene silencing by RNA</i>	0.02%	-1.9355	0.41	0.98
GO:2000113	<i>negative regulation of cellular macromolecule biosynthetic process</i>	0.72%	-3.4461	0.49	0.98
GO:0045934	<i>negative regulation of nucleobase-containing compound metabolic process</i>	0.70%	-1.8539	0.49	0.92
GO:0010629	<i>negative regulation of gene expression</i>	0.78%	-5.1073	0.51	0.93
GO:0009892	<i>negative regulation of metabolic process</i>	1.26%	-4.8962	0.57	0.87
GO:0009890	<i>negative regulation of biosynthetic process</i>	0.77%	-4.0535	0.54	0.93
GO:0010558	<i>negative regulation of macromolecule biosynthetic process</i>	0.74%	-4.4935	0.5	0.92
GO:0016458	<i>gene silencing</i>	0.17%	-4.8125	0.55	0.8
GO:0016441	<i>posttranscriptional gene silencing</i>	0.03%	-2.7447	0.59	0.84
GO:0051172	<i>negative regulation of nitrogen compound metabolic process</i>	0.79%	-3.8477	0.53	0.93
GO:0032502	developmental process	2.81%	-2.6946	0.98	0

GO:0048511	rhythmic process	0.08%	-1.4271	0.98	0
GO:0048856	anatomical structure development	2.54%	-2.4547	0.97	0
GO:0048731	<i>system development</i>	1.26%	-1.342	0.95	0.86
GO:0050896	response to stimulus	12.21%	-4.4584	0.98	0
GO:0065007	biological regulation	20.50%	-8.3458	0.99	0
GO:0071840	cellular component organization or biogenesis	8.57%	-1.5986	0.98	0
GO:0046483	heterocycle metabolic process	29.66%	-5.9066	0.85	0.05
GO:0006807	nitrogen compound metabolic process	38.74%	-2.7423	0.94	0.06
GO:1901360	organic cyclic compound metabolic process	30.32%	-4.8928	0.89	0.08
GO:0006396	RNA processing	3.21%	-4.2725	0.68	0.14
GO:0018193	peptidyl-amino acid modification	1.50%	-2.2958	0.8	0.18
GO:0043170	macromolecule metabolic process	39.49%	-2.4622	0.88	0.21
GO:0010467	gene expression	19.67%	-5.3325	0.81	0.22
GO:0006725	cellular aromatic compound metabolic process	29.63%	-5.3325	0.85	0.24
GO:0097298	regulation of nucleus size	0.00%	-2.8327	0.82	0.28
GO:0048519	negative regulation of biological process	1.98%	-3.7352	0.74	0.32
GO:0048518	positive regulation of biological process	1.74%	-2.1864	0.74	0.33
GO:0040029	regulation of gene expression, epigenetic	0.13%	-4.4157	0.68	0.34
GO:0006397	mRNA processing	0.56%	-4.266	0.7	0.36
GO:0008380	<i>RNA splicing</i>	0.41%	-2.5214	0.71	0.62
GO:0000377	<i>RNA splicing, via transesterification reactions with bulged adenosine as nucleophile</i>	0.32%	-1.8729	0.71	0.99
GO:0000375	<i>RNA splicing, via transesterification reactions</i>	0.32%	-1.7721	0.71	0.95
GO:0000398	<i>mRNA splicing, via spliceosome</i>	0.32%	-2.266	0.7	0.88
GO:0009719	response to endogenous stimulus	0.53%	-2.1871	0.83	0.37
GO:0016071	mRNA metabolic process	0.80%	-3.0283	0.75	0.37

GO:0090304	nucleic acid metabolic process	21.45%	-5.9706	0.66	0.39
GO:0032774	<i>RNA biosynthetic process</i>	10.93%	-3.317	0.61	0.6
GO:0018130	<i>heterocycle biosynthetic process</i>	17.39%	-2.9957	0.71	0.52
GO:0016070	<i>RNA metabolic process</i>	15.95%	-5.0969	0.65	0.58
GO:0006351	<i>transcription, DNA-templated</i>	10.66%	-2.7986	0.58	0.74
GO:0097659	<i>nucleic acid-templated transcription</i>	10.72%	-3.0814	0.6	0.88
GO:0006139	<i>nucleobase-containing compound metabolic process</i>	26.55%	-5.9747	0.7	0.6
GO:0019438	<i>aromatic compound biosynthetic process</i>	16.95%	-2.7258	0.72	0.52
GO:1901362	<i>organic cyclic compound biosynthetic process</i>	17.87%	-2.5017	0.73	0.51
GO:0034654	<i>nucleobase-containing compound biosynthetic process</i>	14.53%	-2.7167	0.66	0.71
GO:0048583	regulation of response to stimulus	1.12%	-2.6925	0.63	0.4
GO:0044260	cellular macromolecule metabolic process	34.28%	-3.4685	0.74	0.43
GO:0034641	cellular nitrogen compound metabolic process	34.14%	-4.1543	0.77	0.44
GO:0050789	regulation of biological process	19.37%	-7.8794	0.66	0.45
GO:0080090	<i>regulation of primary metabolic process</i>	11.68%	-6.5952	0.55	0.87
GO:0010468	<i>regulation of gene expression</i>	10.82%	-5.2541	0.49	0.86
GO:0031323	<i>regulation of cellular metabolic process</i>	11.66%	-4.2907	0.52	0.87
GO:0031326	<i>regulation of cellular biosynthetic process</i>	10.82%	-4.4377	0.49	0.89
GO:0051252	<i>regulation of RNA metabolic process</i>	10.03%	-3.3936	0.42	0.86
GO:2001141	<i>regulation of RNA biosynthetic process</i>	9.97%	-2.9469	0.41	0.9
GO:0019219	<i>regulation of nucleobase-containing compound metabolic process</i>	10.26%	-3.7799	0.46	0.85
GO:2000112	<i>regulation of cellular macromolecule biosynthetic process</i>	10.68%	-4.1681	0.45	0.85
GO:0006355	<i>regulation of transcription, DNA-templated</i>	9.92%	-2.6576	0.41	0.9
GO:0060255	<i>regulation of macromolecule metabolic process</i>	11.72%	-7.0004	0.51	0.67

GO:0019222	<i>regulation of metabolic process</i>	11.94%	-5.8539	0.65	0.67
GO:0050794	<i>regulation of cellular process</i>	18.84%	-5.0937	0.6	0.76
GO:1903506	<i>regulation of nucleic acid-templated transcription</i>	9.97%	-2.9469	0.41	0.89
GO:0009889	<i>regulation of biosynthetic process</i>	10.83%	-4.3382	0.53	0.86
GO:0010556	<i>regulation of macromolecule biosynthetic process</i>	10.75%	-4.4401	0.48	0.89
GO:0051171	<i>regulation of nitrogen compound metabolic process</i>	10.93%	-6.4908	0.52	0.86
GO:0042221	response to chemical	3.07%	-2.6162	0.81	0.46
GO:0051716	<i>cellular response to stimulus</i>	9.56%	-3.384	0.74	0.63
GO:0006281	<i>DNA repair</i>	2.23%	-1.4724	0.59	0.9
GO:0033554	<i>cellular response to stress</i>	2.97%	-1.4737	0.75	0.81
GO:0006974	<i>cellular response to DNA damage stimulus</i>	2.36%	-1.7122	0.75	0.51
GO:0080134	<i>regulation of response to stress</i>	0.34%	-1.684	0.65	0.62
GO:0044271	cellular nitrogen compound biosynthetic process	22.50%	-1.7328	0.71	0.47
GO:0018205	peptidyl-lysine modification	0.36%	-1.3134	0.83	0.48

mlk1/2/3 ZT14

GO-term ID	Description	Frequency	Log₁₀ p-value	Uniqueness	Dispensability
GO:0051276	chromosome organization	1.48%	-2.7852	0	0
GO:0007064	<i>mitotic sister chromatid cohesion</i>	0.05%	-1.7645	0	0.54
GO:0006325	<i>chromatin organization</i>	0.67%	-1.3072	0	0.53

mlk1/3/4 ZT14

GO-term ID	Description	Frequency	Log₁₀ p-value	Uniqueness	Dispensability
GO:0009628	response to abiotic stimulus	0.57%	-1.857	0.91	0
GO:0009987	cellular process	63.78%	-2.1512	0.97	0
GO:0010629	negative regulation of gene expression	0.78%	-1.644	0.62	0
GO:0050896	response to stimulus	12.21%	-1.8962	0.94	0

GO:0051276	chromosome organization	1.48%	-2.1175	0.86	0
GO:0006325	<i>chromatin organization</i>	0.67%	-1.308	0.86	0.53
GO:0065007	biological regulation	20.50%	-2.4425	0.94	0
GO:0009058	biosynthetic process	31.61%	-1.6655	0.88	0.02
GO:0044249	cellular biosynthetic process	30.05%	-2.762	0.65	0.06
GO:1901576	<i>organic substance biosynthetic process</i>	30.37%	-2.2013	0.68	0.66
GO:0018130	<i>heterocycle biosynthetic process</i>	17.39%	-1.7144	0.68	0.53
GO:0019438	<i>aromatic compound biosynthetic process</i>	16.95%	-1.5114	0.68	0.53
GO:1901362	<i>organic cyclic compound biosynthetic process</i>	17.87%	-1.752	0.7	0.54
GO:1901360	organic cyclic compound metabolic process	30.32%	-1.301	0.86	0.07
GO:0071704	organic substance metabolic process	58.36%	-1.5157	0.89	0.08
GO:0006807	nitrogen compound metabolic process	38.74%	-1.4377	0.88	0.09
GO:0044237	cellular metabolic process	53.06%	-2.2358	0.83	0.18
GO:0046483	heterocycle metabolic process	29.66%	-1.3439	0.81	0.25
GO:0097305	response to alcohol	0.06%	-1.5686	0.91	0.29
GO:0019222	regulation of metabolic process	11.94%	-3.5229	0.56	0.38
GO:0050794	<i>regulation of cellular process</i>	18.84%	-1.5935	0.52	0.76
GO:0050789	<i>regulation of biological process</i>	19.37%	-3.0804	0.57	0.67
GO:0080090	<i>regulation of primary metabolic process</i>	11.68%	-2.6003	0.49	0.87
GO:0009889	<i>regulation of biosynthetic process</i>	10.83%	-1.7986	0.4	0.86
GO:0031323	<i>regulation of cellular metabolic process</i>	11.66%	-1.9957	0.45	0.87
GO:0031326	<i>regulation of cellular biosynthetic process</i>	10.82%	-1.8665	0.38	0.86
GO:0060255	<i>regulation of macromolecule metabolic process</i>	11.72%	-2.8069	0.47	0.62
GO:0051171	<i>regulation of nitrogen compound metabolic process</i>	10.93%	-2.7959	0.49	0.86