

New *Phytologist* Supporting Information Figs S1 & S2 and Tables S1–S3

Article title: *Oakleaf*: an S locus-linked mutation of *Primula vulgaris* that affects leaf and flower development

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AtKNAT1

MEEYQHDNSTTPQRVSFLYSPISSSNKNNDNTSDTNNNNNNNNNSNYGPGYNNTNNNNHHHQMHLFPHMSSLLPQTTENCFRSDHD
QPNNNNNPSVKSEASSRINHYSMLMRAIHNTQEANNNDNDVSDVEAMKAKIIAHPHYSTLLQAYLDCQKIGAPPDVVDRITAA
RQDFEARQQRSTPSVSASSRDPQLDQFMEAYCDMLVKYREELTRPIQEAMEFIRRIEQLSMLCQSPIHILNNDGKSDNMGSSD
EEQENNSGGETELPEIDPRAEDRELKHNLLKKYSGYLSSLKQELSKKKKKGKLPKEARQKLLTWELHYKWPYPSESEKVALAES
TGLDQKQINNWFINQRKRHWKPSSEDMQFMVMDGLQHPHHAALYMDGHYMGDGPYRLGP

PvKNL1

MEDYNENTSSQSGNFLYGGSLLATNNNTTSTNTTSSLYGRNSSHQSHMMNMMNMMNMSMNINSFHLGGEYPIVKSEAGGSGTSQN
FIFPNSVIRGIQTGLSNESCSNNDVESIKAKIISHPQYSNLLLEAYMDCQKVGAPPEVAAQLTAVREEFEGQRASTIGKNATKDP
ELDQFMEAYYNMLVKYREELTRPIQEAMDFMRRVEAQLNILLNNNNNSTAPVRIFNSEEKCEGVVSSEEDQENSGGETELAEIDPR
AEDQELKNYLLRKYSGYLGLSKQELSKKKKKGKLPKDARQKLLSWWELHYKWPYPSESEKVALAESTGLDQKQINNWFINQRKRH
WKPSSEDMQFMVMDGIHPQSASLYMDGHYMGEGPYRLGP

PvKNL2

MDDIYRQVYMSPDNLMPVDYQALYSSSSASVASGGDHQSRFPFYGSDELISISDQALIRRSTNIHEDDDVLKRKIIASHPWYPKL
LDAYIGCQKVGAPPEISCLLEEIQRENDVLNHAASSCLGEDPELDEFMNTFCEILLKYKSDLKPLEEATTFLNKIELQFGNLC
KDEGPVSSDDDLGGGEVEGLDNRITQSEEDLKDRLLRKFGSHISNLKLEFSKKKKKGKLPKEGRQTLLEWNNLHYKWPYPTEGDK
ISLAETTGLDQKQINNWFINQRKRHWKPSSEDMQFMVMDGLQHPHHAALYMDGHYMGEGPYRLGP

PvKNL6

MDELYRLHSSISSDDLTINRLNGNIQFDTIIVREMSDLKQAIIASHPLYPNLVSAYIECRKVGAPPEMALLLDEISKENIPIST
CGEIGVDPEDDFMGTYCAVLQRYKEDLSKPFNEATTFLLTSIESQLHDLCKDTIITPTTTSLASDDMAGSSEEMSCGEVEAVES
QESSVPRKGDKELKEMLLRKYSGYLSSLRDKDFLKKRKKGKLPNDARTALLDWWNTHYRWYPTEEEKVKLSEVTGLDQKQINNWF
INQRKRHWKPSSEDMRFALMEGVSGNSNTGSSMYVDHGDGTGSNLVKGQLPRFI

PvSTL1

MSLMNFGENLNSGGANEMMILPNSSSSNNNTLFFQQNHNNFNTSNQNNITPFLLENYNNNQLNVYSTDHQGASSLRAKIIAHPHY
HRLLAAYVNCQKIGAPPEVVAGLEEAAYSVAVRGRVDSRVGEDPGLDQFMEAYCEMLTKYEQLSKPFKEAMLFLSKIESQFKA
LALPSSSDSGFDGYGSSEEEAEFIHNTVDPQAEENELKQQLLRKYSGYLGLSKQEFMKKRKKGKLPKEARNQLEWWSRNYKWPYP
SESQKMALAESTGLDSKQINNWFINQRKRHWKPSSEDMQFVMDSNSHPHNSAAAHYMDHVLANPFPMDTSPSFI

PvSTL2

MYANNSSCSMAFGEHLNSGGIYPMMTMPNSSPSNNNTLFFENNHTSENQTSFFMENNQNINSNDDHGGSTSSASSISLRAKIMAH
HYHRLLAAYVNCQKIGAPQEVVARLEEAQVMIGRVPVGGVGGDDPGLDQFMEAYCEMLNKYEQLSKPFKEAMLFLSKFESQFRA
LTLSSSTGHSFERNSSSEEEADTDYSIVDRQEDKDLKGRLLRKYSGYLGLSKQEFMKKKKKGKLPKEARENLLEWWSRNNHWPY
PSESQKMELEAESTGLDPKQINNWFINQRKRHWKPTDEMDFVMDPNPNAAAHYMDHVMVNPFPMDI

PvKNL3

MDMQEMAGLHQHYSDQQLSDTSSVLRISITVDGSTDDQQNPHHQQQGGPTWLNSAILQTNFLNLQTNSESPTRPHHHQGHNNNSN
AGQWTPRSMDTNNNNNNNNNNNNNNNNNNNEVVEDPGGMSWQTARHKAELIAHPLYEQLLSAHVACLRIATPVDQLPRIDAQL
AQSQQVAKYSGMAHGGISMGDDKELDQFMTHYVLLLCFSFKEQLQQHVRVHAMEAVMACWELEQSLQSLTGVSPGEGTGATMSDD
EEDQLESDNNLFDGSLDGHSTGFGPLVPTEESERSLMERVRQELKHELKQGYKEKIVDIREEILRKRKAGKLPDGTTSVLKAWWQ
SHSKWPYPTEEDKARLVQEPGLELQKQINNWFINQRKRHWKPSSEDMQFVMDPNPNAAAHYMDHVMVNPFPMDI

PvKNL4

MAFHHTQELPLHHFSDSSGGPPPTWLNSEILRQNHYGGTGGRSFLQTIINNNTNNNNSENDEFSSRSESDGSGEEREWQTAKCK
SDILSHPLYEQLLSAHVQCLRIATPVDQLPRIDAQLAQSQQVAKYVGLGHGNPPLDDKDLQFMTHYVLLLSFKEQLQQHVRV
HAMEAVMACWELEQSLQSLTGVAPGEGTGATMSDDDEDQADSDTNIYDGLDGTDMGMFPTESERSLMARVRQELKLELQGY
KEKIVDIREEILRKRKAGKLPDGTTSHLKAWWQSHSKWPYPTEEDKARLVQETGLQKQINNWFINQRKRHWKPSSEDMQFVMDPNPNA
AAHYMDHVMVNPFPMDI

PvKNL7

MQEQQGNMGMGGGDHFGSDVGGGEMKAEIAAHPLCEQLLAHVACLRVATPIDQLPMIEAQLAQAQGIIVRAYAASVQGNRPLSP
HERQELDNFLGQYIMVLSFKDQLQQHVRVHAMEAVMACREIEHNLQALTGVTLGEGSGATMSDDEADDTNNIMQQMDFSMHQ
SAEGHDMGMGFPPLPTEESERSLMERVRQELKIELKQGFRRSRIEDVREEILRKRKAGKLPDGTTSVLKNWWQLHAKWPYPTEEDKA
KLVEETGLQKQINNWFINQRKRHWKPSSEDMQFVMDPNPNAAAHYMDHVMVNPFPMDI

Fig. S1 Predicted amino acid sequences of PvKNOX proteins. Amino acid sequences of wild type PvKNOX proteins are shown together with the *Arabidopsis thaliana* KNAT1 (AtKNAT1) protein sequence. Names of *Primula* proteins reflect similarity to *A. thaliana* KNAT and STM proteins. The standard single letter amino acid code is used. The sequences were used to generate the phylogenetic tree (Fig. 6b) and protein sequence alignments (Fig. S2).

AtKNAT1	-----MEEYQHDN-----S-----TTPQRVSFYLYSP	21
PvKNL1	-----MEDYNNEN-----TSQSGNFLYGG	18
PvKNL2	-----	0
PvKNL6	-----	0
PvSTL1	-----	0
PvSTL2	-----	0
PvKNL3	MDMQEMAGLHQHYSDQQLSDTSSVLRISITVDGSTDDQQNPH----HQQQQGGPTWLNISA	55
PvKNL4	-----MAFH---HTQELPLHHFSDSSGGPPPTWLNSE	30
PvKNL7	-----	0

AtKNAT1	ISSSNKNDNTSDTNNNNNNSSNYGPGYNNTNNNNHHQHMLFPHMSSLLPQTTECNFR	81
PvKNL1	SLLATATNNNTTST----NTTSSLYGRN-----SSHQSHMMNNMNMNMMS-NINSFH	65
PvKNL2	-----MDDIYRQVYMSPDNLMMPVQYQALYS	26
PvKNL6	-----MDELYR	6
PvSTL1	-----MSLMNFGENLNSGG---ANEMMILPNSSS--SNNNTLFLQ	35
PvSTL2	-----MYANNSSSCSMAFGEHLNSGG---IYPMTMPNSSP--SNN-NTLFE	41
PvKNL3	ILQTNFLNLQT-----NSESPSTRPHHQGHNN-----SNAGQWTPR----SMD	95
PvKNL4	ILRQNHYYGGT-----GG---R---SFL	46
PvKNL7	-----MQ	2

AtKNAT1	SDHDQPNNNNPNVSKSEASSSRIN-HYSMLMRAIH--NTQEANNNDNVSDVEAMKAK	137
PvKNL1	Q-----LGEYPIVKSEAGSGTSQNFIFPNSVIR---GIQTGLSNESCSNNDVESIKAK	116
PvKNL2	SSS-----ASVASGGDQSRFPFYGSDELISISDQALIRRSTNIHEDDDVLKRR	75
PvKNL6	L-----HSSISSDDLIT-INRLNGN-----IQF---DTIIVREMSDLKMAQ	43
PvSTL1	QNHNNFN-----TSNQNNITPFLLENYN--NNQLNV--YSTDHQGASSLRK	78
PvSTL2	NNHTSE-----NQTSFFMENNQ---NINSNDDHGGSTASSISLRK	80
PvKNL3	TNNNNNNNNN-----NNNNNNN-----NESVVEDPGGMSWQTARHKAE	136
PvKNL4	QTINNNNNNN-----SENDEFS-----RRSESDGSGEEREWQTAKCKSD	87
PvKNL7	-----EQGGNM-----GMMGGDHFSGDVGGMKAE	29

Helix Loop Helix

AtKNAT1	IIAHPHYSTLLQAYLDCQKIGAPPDVRITAAARQDFE ARQQR-STP---SVSASSRDP	192
PvKNL1	IIISHPQYSNLLLEAYMDCQKVGAPPEVAAQLTAVREEFE GRQRA-STI---G-KNATKDP	170
PvKNL2	IASHPWPYKLLDAYIGCQKVGAPPEISCLLEETQREND VLNH---KA---ASSCLGEDP	128
PvKNL6	IASHPLYPNLVSAYIECRKVGAPPEMALLLEISKENI PIS-----TCGEIGVDP	93
PvSTL1	IIAHPHYHRLLAAYVNCQKIGAPPEVVAGLEEAAYSVA VRG---RV---DSSRVGEDP	130
PvSTL2	IMAHPHYRLLAAYVNCQKIGAPQEVVARLEEAYQVM- IG---RV---GPGCVGDDP	130
PvKNL3	ILAHPLYEQLLSAHVAQLRIATPVDQLPRIDAQLAQSQ QVVAKYSGMAHGGISMG--DDK	194
PvKNL4	ILSHPLYEQLLSAHVQCLRATPVDQLPRIDAQLAQSQ QVVAKYSVLGHGNPPL---DDK	144
PvKNL7	IAAHPLCEQLLAHVAQLRVATPIDQLPMTIEAQLAQSQ GIVRAYAASVQGNRPLSPHERQ	89

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Amphipathic Helix H3

AtKNAT1	ELDQFMAYCDMLVKYREELTRPIQ ---EAMEFIRRIESQLSMLCQ---SPIHILNN	243
PvKNL1	ELDQFMAYYNMLVKYREELTRPIQ ---EAMDFMRRVEAQLNILLNNNNSTAPVRFNS	226
PvKNL2	ELDEFMNTFCEILLKYKSDLSKPLE ---EATTFLNKIELQFGNLCCKDEGPVSS-----	178
PvKNL6	ELDDFMGTYCAVLQRKYKEDLTKFPFN ---EATTFLTLSIESQLHDLCKDTIITPTTSLAS	149
PvSTL1	GLDQFMAYCEMLTKYEQELSKPFFK ---EAMLFLSKIESQFKALALPS-SDS-----	178
PvSTL2	GLDQFMAYCEMLNKYEQELSKPFFK ---EAMLFLSKFESQFRALTLSSSTGHS-----	179
PvKNL3	ELDQFMTHYVLLLSCFKQQLQHVVRVHAMEAVMACWELEQSLQSL TGVSPEGETGATMSD	254
PvKNL4	DLQFMTHYVLLLSFKQQLQHVVRVHAMEAVMACWELEQSLQSL TGVAPGEGTGATMSD	204
PvKNL7	ELDNFLGQYIMVLSFFKDLQHVVRVHAMEAVMACREIHNQLAL TGVTLGEGSGATMSD	149

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ELK Domain

AtKNAT1	PDGK-----S---DNMGSSDEEQENNSGGETEELP---EIDPRAED RELKNHLLRKY	288
PvKNL1	EE-K-----C---EGVV-SSEEDQENSGETELA---EIDPRAED QELKNYLLRKY	269
PvKNL2	-----DDDLGGGEVEGL---DNRTQSE KDLKDRLLRKF	209
PvKNL6	-----DDMAGSSEE---EMSCGEVEAVESQESSVPRKGD KELKEMLLRKY	191
PvSTL1	---G-----F---DGYGSSEEE---AEFIHN---TVDPQAE ENELKGQLLRKY	214
PvSTL2	---G-----F---ERNGSSEE---ADTDYS---IVDRQED KDLKGRLLRKY	215
PvKNL3	DEEDQLESMDNLF-----DGSLDGHDSTGFGLVPTES-ERSLMERVR QELKHELKQGY	307
PvKNL4	DEEDQADSDTNIY-----DGSLDGTDNMGFG--MPTES-ERSLMARVR QELKLELKQGY	255
PvKNL7	DEADDTNNIMQMDFSMDHQASAEGHDMMGFGPLPTES-ERTLMERVR QELKTELKQGF	208

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ELK Domain

Homeodomain

AtKNAT1	SGYLSLKLQELS KKKKKGKLPKEARQKLLTWELHYKWPYPSESEKVALAESTGLDQKQI	348
PvKNL1	SGYLGLKQELS KKKKKGKLPKIDARQKLLSWELHYKWPYPSESEKVALAESTGLDQKQI	329
PvKNL2	GSHISNLKLEFS KKKKKGKLPKEGRQTLLEWNLHYKWPYPTEGDKI SLAETTGLDQKQI	269
PvKNL6	SGYLSLRLKDFL KKKKKGKLPNDARTALLDWNTHYRWYPTEEEKVKLSEVTGLDQKQI	251
PvSTL1	SGYLGLKQEFM KKKKKGKLPKEARNQLLEWWSRNYKWPYPSESQKMALAESTGLDQKQI	274
PvSTL2	SGYLGLKQEFM KKKKKGKLPKEARENLEWWSRNYKWPYPSESQKMELEAESTGLDPKQI	275
PvKNL3	KEKIVDIREIIL RKRRAGKLPDGTTSVLKAWWQSHSKWPYPTEEDKARLVQEPGLKQI	367
PvKNL4	KEKIVDIREIIL RKRRAGKLPDGTTSVLKAWWQSHSKWPYPTEEDKARLVQETGLQKQI	315
PvKNL7	RSRIEDVREIIL RKRRAGKLPDGTTSVLKNWQLHAKWPYPTEEDKAKLVEETGLQKQI	268

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Homeodomain

AtKNAT1	NNWFINQRKRHWKPS EDMQFMVMDGLQHPHAA---LYMDGHYMGDGPYRLGP-----	398
PvKNL1	NNWFINQRKRHWKPS EDMQFMVMDGI-HPQSAS---LYMDGHYMGEGPYRLGP-----	378
PvKNL2	NNWFINQRKRHWKPS ENMQLAVMDNLTGQFYAEDD-----	304
PvKNL6	NNWFINQRKRHWKPS EDMRFALMEGVSGNSNTGSS-MYVDHGD-GTGSNLVKGQLPRFI	308
PvSTL1	NNWFINQRKRHWKPS EDMQFVVMDSNSHPHNSAAAHHYMDHVLANPFPMDTSPSFI---	330
PvSTL2	NNWFINQRKRHWKPT DEMDFVMDPNSPN-AAAHHYMDHVMVNPFPMDI-----	325
PvKNL3	NNWFINQRKRHWHSNP STSTILKSKRSSSGDKNRDQFA-----	406
PvKNL4	NNWFINQRKRHWHSN PSSSTVVKSKRKSAGEPSNERFM-----	354
PvKNL7	NNWFINQRKRHWHSN SQSVTALKSKRKR-----	296

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Fig. S2 Multiple sequence alignment of PvKNOX proteins. Clustal Omega multiple sequence alignment of the eight predicted wild type PvKNOX proteins, and *Arabidopsis thaliana* KNAT1 (AtKNAT1) is shown. The standard single letter amino acid code is used. Amino acid sequence numbers for each line are indicated at right. The final number in brackets indicates the full length amino acid sequence of each protein. The Helix Loop Helix domain is indicated in red, the Amphipathic Helix H3 in blue, the ELK domain in green, and the Homeodomain in orange. (-), alignment gaps. (*), fully conserved residues; (:), strongly similar property residues; (.), weakly similar property residues.

Table S1 RNA-Seq read data from six paired-end read libraries

Sample	Paired-end reads	Total no. of bases	Transcript assemblies
<i>Oakleaf</i> open flowers	24,995,179	5.0×10^9	33,197
wild type open flowers	33,400,153	6.7×10^9	35,809
<i>Oakleaf</i> young leaves	14,310,589	2.9×10^9	27,457
wild type young leaves	45,723,021	9.1×10^9	33,711
Mixed stage pin flowers	100,768,798	2.0×10^{10}	39,688
Mixed stage thrum flowers	81,045,610	1.6×10^{10}	41,488

The number of paired-end sequence reads, the total number of bases and the number of transcript assemblies obtained from each of the six tissue samples are shown, sequences <200 bp have not been included in the transcript assembly numbers.

Table S2 Differential gene expression of *PvKNOX* genes

<i>Primula vulgaris</i> gene	<i>Arabidopsis thaliana</i> gene	<i>Knox gene</i> Class	Normalised read counts				Log ₂ fold change	
			Leaf		Flower		leaf	flower
			<i>Oakleaf</i>	wild type	<i>Oakleaf</i>	wild type		
<i>PvKNL1</i>	<i>KNAT1</i>	I	0	1	307	194	–	0.66
<i>PvKNL2</i>	<i>KNAT2</i>	I	27	73	232	135	-1.45	0.78
<i>PvKNL6</i>	<i>KNAT6</i>	I	15	7	1502	1077	0.96	0.48
<i>PvSTL1</i>	<i>STM1</i>	I	2	0	135	79	–	0.77
<i>PvSTL2</i>	<i>STM1</i>	I	0	0	558	255	–	1.13
<i>PvKNL3</i>	<i>KNAT3</i>	II	1971	1591	1704	1424	0.31	0.26
<i>PvKNL4</i>	<i>KNAT4</i>	II	1716	3368	1232	2595	-0.97	-1.07
<i>PvKNL7</i>	<i>KNAT5</i>	II	2690	672	1690	3056	2.00	-0.85

The eight *P. vulgaris* *KNOX* genes, identified from gene models based on assembled *P. vulgaris* genome sequence data and RNA-Seq transcript assemblies, were named according to the similarity of their encoded proteins to *Arabidopsis* *KNOX* proteins. *Primula vulgaris* genes are shown alongside the corresponding *A. thaliana* gene and its unigene identifier (ID). RNA-Seq normalized read counts from leaf and flower tissues of *Oakleaf* and wild type plants are shown; the log₂ fold change in expression between the leaf samples and flower samples is shown. The wild type was a pin plant.

Table S3 Analysis of single nucleotide polymorphisms in *PvKNL* genes

Gene	Position in genomic DNA	Base Change	Amino acid change	Reads for <i>Oak</i> SNP in flower	Total reads at this position	Reads for <i>Oak</i> SNP in leaf	Total reads at this position	Genotype and Impact of SNP on encoded protein
PvKNL1	671	TCT - ACT	Ser35 - Thr	7	7	0	0	Homozygous
PvKNL1	1882	ACG - ATG	Thr161 - Met	3	3	0	0	Homozygous
PvKNL2	3534	GCT - GGT	Ala120 - Gly	3	3	No SNP	No SNP	Homozygous
PvKNL2	25367	GGG - GCG	Gly233 - Ala	9	10	No SNP	No SNP	Also found in pin
PvKNL2	30146	CAG - TAG	Gln298 - Stop	2	15	No SNP	No SNP	Truncated protein
PvSTL1	8202	Frameshift -2 bp	aa12: stop16	3	8	No SNP	No SNP	Truncated protein
PvSTL1	8188	GAA - GCA	Glu17 - Ala	8	15	2	2	Substitution tolerated
PvSTL1	8068	Frameshift +2 bp	aa57: stop63	4	11	No SNP	No SNP	Truncated protein
PvSTL1	2413	TCG - CCG	Ser271 - Pro	5	10	No SNP	No SNP	Substitution tolerated
PvSTL1	2278	GTC - ATC	Val316 - Ile	8	13	No SNP	No SNP	Substitution tolerated
PvSTL2	7432	GAA - CAA	Glu47 - Gln	37	37	0	0	Homozygous and in Pin
PvSTL2	7336	GCG - ACG	Ala79 - Thr	29	29	0	0	Homozygous and in Pin
PvSTL2	3225	ATT - ATG	Ile197 - Met	31	31	0	0	Homozygous and in Pin
PvKNL3	7545	CAG - CAT	Gln65 - His	67	98	50	63	Substitution tolerated
PvKNL3	2860	TTG - TCG	Leu335 - Ser	No SNP	No SNP	2	15	Substitution not tolerated
PvKNL3	2034	AGT - AGA	Ser395 - Arg	No SNP	No SNP	2	11	Also found in pin
PvKNL4	2083	AAC - AGC	Asn28 - Ser	17	69	23	66	Substitution tolerated
PvKNL7	8564	GGG - GAG	Gly6 - Glu	0	0	66	132	Substitution not tolerated

Single nucleotide polymorphisms (SNPs) detected in *PvKNL* genes, as compared with the wild type draft genome sequence. The position of each polymorphism in the genomic DNA is indicated. The resulting codon and amino acid (aa) changes are also shown. For frameshifts, the aa at which the peptide sequence diverges from wild type is shown, as is the next stop codon in the shifted reading frame. The Illumina read counts with the *Oakleaf* SNP variant, are shown for flower and leaf transcriptomes. The total number of reads at this position (*Oakleaf* and wild type) are also shown. Where no SNP was detected in either flower or leaf RNA-Seq data this is indicated as 'No SNP'. The final column indicates whether the *Oakleaf* SNP as compared with the wild type draft genome is homozygous or heterozygous in *Oakleaf*. Where the same *Oakleaf* SNP was also found in the wild type pin plant transcriptome data this is also indicated. Where the SNP is heterozygous, the impact of the SNP on the encoded protein as determined by SIFT (Ng & Henikoff, 2003) (<http://sift.bii.a-star.edu.sg/>) is shown.

Reference

Ng PC, Henikoff S. 2003. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Research* **31**: 2812–3814.