Supplemental Data

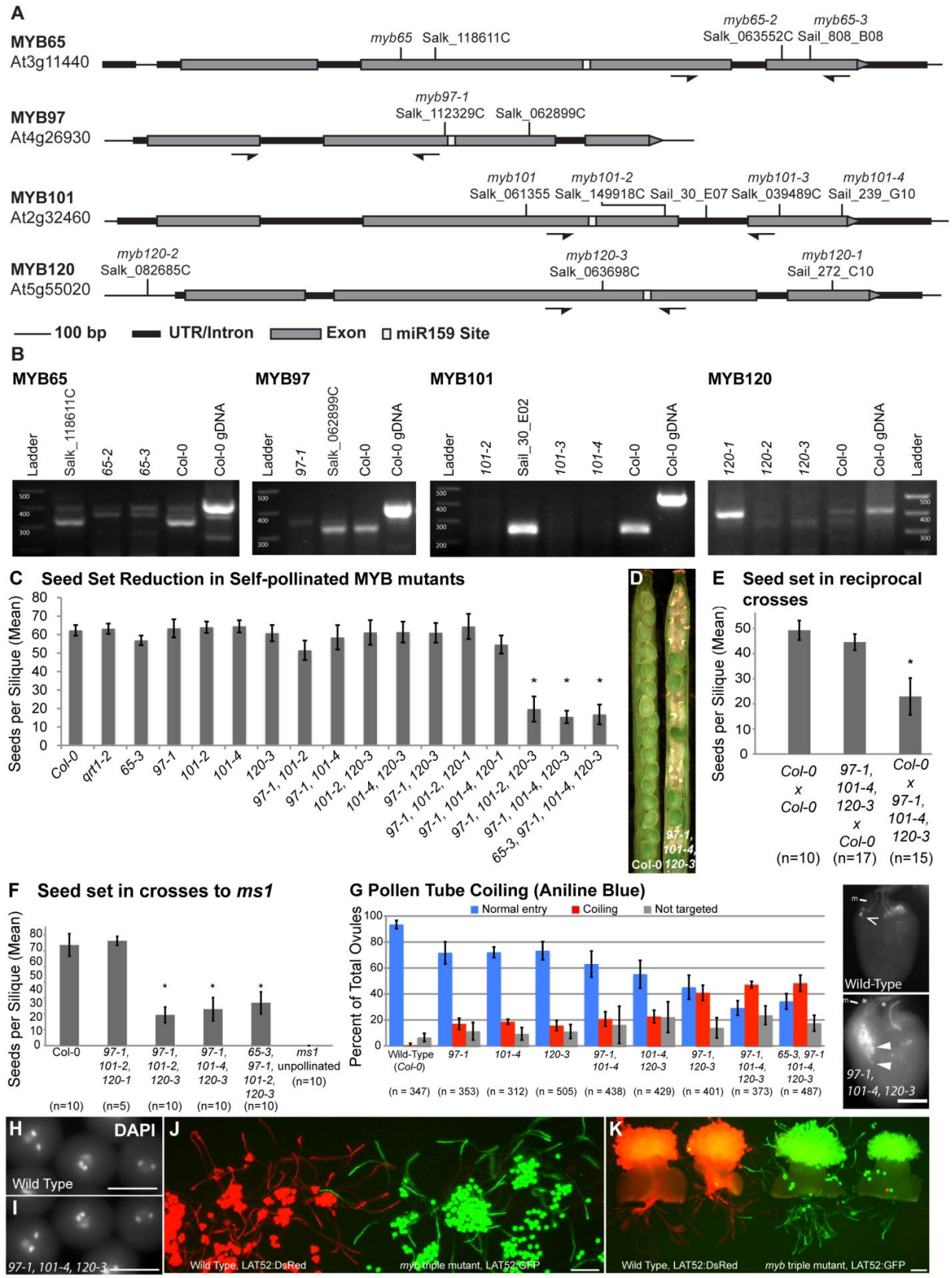
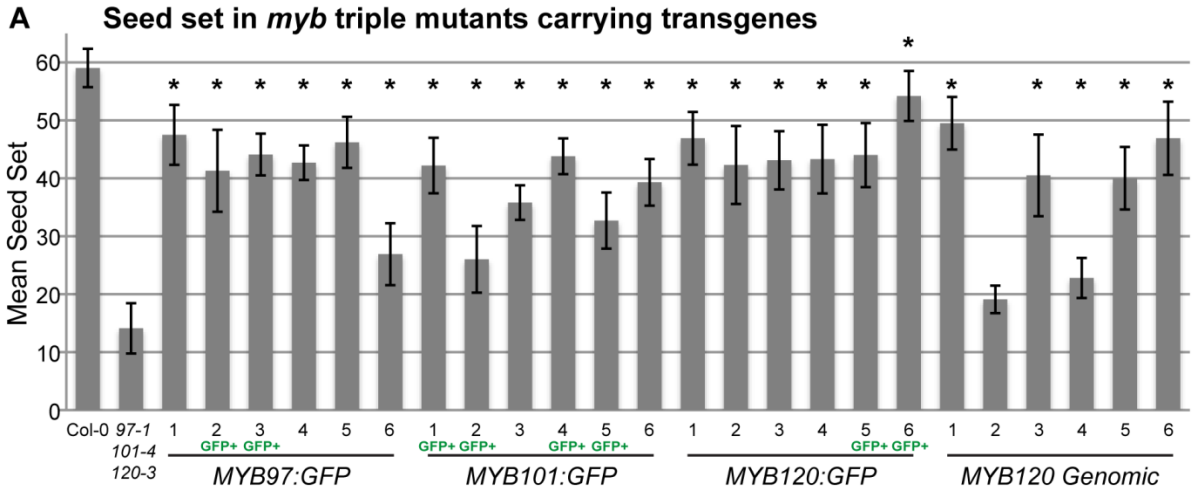


Figure S1. A summary of T-DNA insertion lines used in this study and their impact on RNA abundance, seed set, and pollen tube function, related to Figure 2. (A) Gene maps utilized in this study. Allele designations were given only to insertion lines for which a PCR product could confirm the insertion location in the gene of interest. Arrows denote the location of primers used for RT-PCR analysis in B. Un-translated regions (black bars) are based on previously published full length cDNAs or our experimental results (MYB120). Previously published MYB alleles: *myb65* [1], *myb101* [2]. (B) RT-PCR analysis for floral cDNA. The gene being analyzed is in bold type. Genotypes being analyzed are directly above each lane. (C) Seed set from wild-type (Col-0, *qrt1-2*) and *myb* single, double, triple and quadruple mutants. Seed set analysis is reported as the average seed number in ten consecutive siliques on the primary bolt, excluding the first five flowers (+/- standard deviation). Asterisks indicate seed set reductions significantly different than wild-type controls (p-value < 0.001, students t-test). (E) Reciprocal crosses using wild-type (Col-0 accession) and *myb* triple mutant flowers identify male-specific defects in seed production. All flowers were emasculated a minimum of 12 hours before being manually pollinated and allowed to set seed. (F) *myb* triple mutants display drastic reductions in seed production when used as a pollen donor. *ms1* pistils were pollinated with wild-type or mutant pollen and allowed to set seeds before seeds were counted in each silique. Asterisks (*) indicate significantly different quantities of seeds produced per silique by a students t-test (P-value<0.001). n, number of crosses performed. (G) Quantitative analysis of coiling events and targeting measured by aniline blue staining. *ms1* pistils were pollinated with wild-type or *myb* triple mutant pollen 24 hours prior to dissection and staining with

aniline blue for fluorescence microscopy. Insets: Wild-type pollen tubes grow into the micropyle (m) and arrest growth (arrow) at the entrance to the female gametophyte. Multiple *myb* triple mutant pollen tubes (askterisk) grow into the micropyle (m) and overgrow (coil) in the embryo sac (solid arrowhead). (H) Wild-type (Col-0) pollen grains and (I) *myb* triple mutant pollen grains each develop two compact sperm nuclei and a diffuse vegetative nucleus. (J,K) *myb* triple mutants express *LAT52:GFP* and grow normally in vitro (2 hours) and in the semi in vivo growth system (4 hours). *LAT52:GFP* was introgressed into the *myb* triple mutant and was grown next to wild-type (*qrt1-2*, *LAT52:DsRed*) pollen in vitro (J), and in the semi in vivo condition (K).



B The *myb* triple mutant genotype is transmitted through the male germline at reduced frequency

	Genotype	% Transmission	N
Single Mutants	*myb65-3/MYB65*	48.78	123 (B)
	myb97-1/MYB97	51.85	189 (P)
	myb101-2/MYB101	57.29	192 (P)
	myb101-4/MYB101	48.09	445 (B)
	myb120-1/MYB120	46.83	568 (B)
	myb120-3/MYB120	54.42	190 (P)
	Control Heterozygote	50.74	1078 (B)
Double Mutants	*myb97-1, myb101-4/MYB101*	49.56	428 (B)
	myb120-3, myb101-4/MYB101	43.49	347 (B)
	myb120-3, myb97-1/MYB97	45.50	189 (P)
	myb97-1, myb120-1/MYB120	46.09	473 (B)
	myb120-3, myb65-3/MYB65	46.45	394 (B)
Triple Mutants	*myb120-3, myb97-1, myb101-4/MYB101*	15.45*	261 (B)
	myb120-3, myb97-1, myb101-2/MYB101	17.18*	192 (P)
	myb97-1, myb101-2, myb120-1/MYB120	43.60	270 (B)
Quadruple Mutant	*myb65-3, myb101-4, myb120-3, myb97-1/MYB97*	17.86*	84 (P)
Rescue Lines	*myb97-1, myb120-3, myb101-4/MYB101, MYB101:GFP/+* (1-1)	45.17	292 (B)
	myb97-1, myb120-3, myb101-4/MYB101, MYB101:GFP/+ (1-2)†	38.36	753 (B)
	myb97-1, myb120-3, myb101-4/MYB101, MYB97:GFP/+ (1-4)	37.61	435 (B)
	myb97-1, myb120-3, myb101-4/MYB101, MYB97:GFP/+ (3-1)	36.29	566 (B)
	myb97-1, myb120-3, myb101-4/MYB101, MYB120:GFP/+ (5-2)	39.27	950 (B)
	myb97-1, myb120-3, myb101-4/MYB101, MYB120:GFP/+ (1-1)	40.05	565 (B)

Figure S2. MYB:GFP fusion constructs are capable of rescuing the *myb* triple mutant phenotype, related to Figure 1. (A) Seed set was analyzed in self-pollinated primary transformants (carrying indicated constructs) and compared to the parental *myb* triple mutant and wild type (Col-0). All values are the averages (+/- sd) of at least 10 consecutive siliques on the primary inflorescence. Data from six representative primary

transformants (of 23-24 analyzed) are shown for each construct. *MYB101:GFP* lines were assayed for GFP accumulation in the vegetative nucleus of mature pollen grains. *MYB97:GFP* and *MYB120:GFP* were assayed for GFP accumulation in the vegetative nucleus of pollen tubes grown in the semi in vivo assay. Lines that were GFP+ are indicated. Asterisks (*) indicate seed set increases that are statistically significantly different from the *myb* triple mutant parent by a students t-test (P-value <0.001). (B) *MYB:GFP* constructs rescue transmission of the *myb* triple mutant genotype to progeny. % Mutant transmission is the percentage of heterozygous F_1 progeny following crosses of the indicated genotype with *ms1* females; determined either by selection on Basta plates ('B') or by PCR ('P') genotyping (Methods). Transmission through the female gametophyte is not affected (1:1, *myb120-3, myb97-1, myb101-4* : *myb120-3, myb97-1, MYB101,* 50.93% triple mutant, n = 540). Asterisks (*) indicate transmission ratios that differ significantly from 50% transmission expected for alleles that do not affect male gametophyte function (students t-test, P-value <0.001). †, indicates constructs that were first transformed into wild type and then crossed into the *myb* triple mutant background.

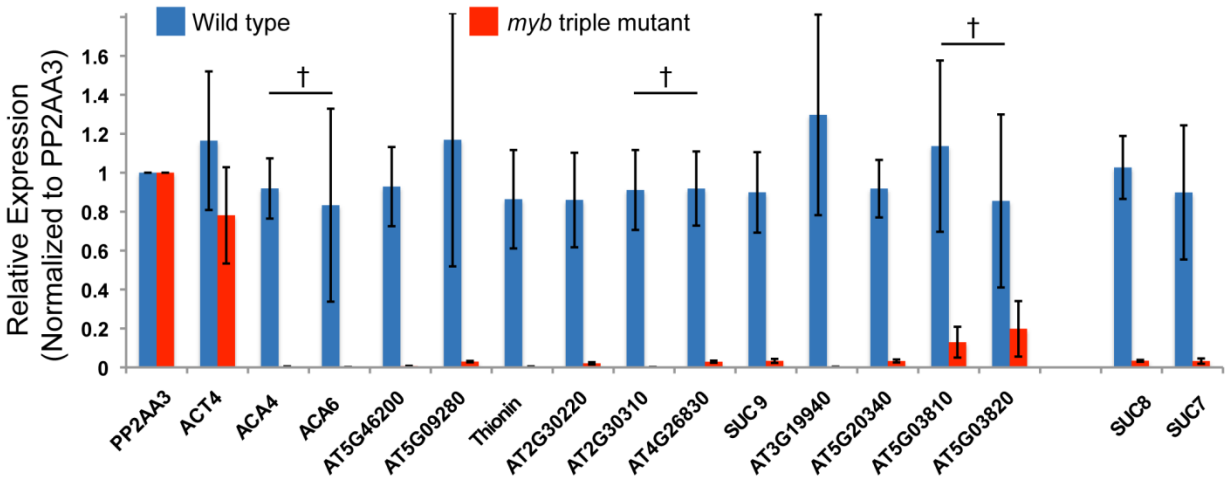


Figure S3. RT-qPCR analysis of MYB-regulated genes, related to Figure 3.

RT-qPCR analysis of the 14 genes found to have the lowest mRNA abundance in myb triple mutants compared to wild type by microarray analysis. SUC8 and SUC9 were also analyzed. Three biological replicates, normalized to *PP2AA3* (AT1G13320); standard deviation is shown for normalized expression values. ACT4 (AT5G59370) is a pollen specific actin, which was not differentially expressed between *myb* triple mutant pollinations and wild type pollinations. For analysis of related gene pairs gene specific primers were designed where possible; this is denoted by a bar and † symbol above expression data.

Table S1. Differentially regulated genes and small peptides identified by Microarray Analysis, related to Figure 3 (Table S1.xlsx). Average normalized expression data are given for *ms1* unpollinated pistils, *ms1* x wild type (Col-0) 8 hours, *ms1* x *myb* triple mutant 8 hours, and selected experiments from publically available sources. Only genes with a p-value less than 0.01 are shown (Column H). Genes that are down regulated in the *myb* triple mutant are shown in rows 3-40, while genes upregulated in *myb* triple mutant are shown in rows 42-43. The table has been sorted by fold change (Wild type / *myb* triple mutant) (Column I, descending). Affymetrix probe sets associated with a single nuclear gene do not contain '_s_' in their Array Element name (Column A). Array Elements that identify multiple genes have multiple Locus identifiers (Columns C and D). RefSeq Transcript IDs are given in Column G, multiple genes are separated by ///. Publically available microarray data were renormalized and incorporated for comparison. Uninucleate microspores, Bicellular pollen, and Tricellular pollen[3]. Dry Pollen, 0.5 Hour Pollen Tubes, 4 Hour Pollen Tubes, Semi in vivo [4], Sperm cels[5] Max sporophyte (Column W) represents the maximum value from the tissue types in columns Y through AF. Ovules and Stigma[6], 7 Day Root, 17 Day Root, 8 Day Seedling, 21 Day Seedling, Rosette 17 Day[7]. Regulatory sequence analysis of differentially regulated genes was performed on the RSAT server (http://rsat.ulb.ac.be/) using the HvGAMYB position weight matrix (Gubler F, Raventos D, Keys M, Watts R, Mundy J, Jacobsen JV, Plant J. 1999 Jan;17(1):1-9). The presence of a predicted site within -3000 upstream of the differentially regulated gene is indicated (HvGAMYB core site identified (Y/N)). For each predicted site the locus is shown (Locus Identifier),

followed by the strand (Strand) and position relative to the start site (start, and end). Sequences were analyzed in two rounds. The first round used the default settings where the regulatory sequence was searched from -3000 to -1 bp of the start site or to the closest upstream ORF, the second searched from -3000 to -1 regardless of the inclusion of upstream ORFs. The location of the predicted HvGAMYB site with respect to the upstream ORF is indicated (before upstream ORF (Y/N)). The sequences identified are listed (sequence), followed by the weight, and measures of significance (Pval, ln_Pval, sig). Sheet 3. S18MYB-regulated small peptides. The number of amino acids encoded by the gene model is listed (#aa, Column E), followed by the predicted localization listed on TAIR 10 (Column F). The amino acid sequence including any putative signal peptides (aa seq, Column H), was used to independently predict subcellular localization using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/). If the protein was predicted to contain a signal peptide this is noted (Signal peptide?, Column H, where T = true, and F = false). The position of the signal peptide cleavage site is also noted (Cleavage site after aa#, column I). Any known Gene Ontology information from TAIR 10 have been included (GO Molecular Function, Columns J and K).

Supplemental Experimental Procedures

Seedling selection and plant growth

Seeds were grown on solid Murashige and Skoog (MS) medium (MP Biomedicals, LLC, Solon, OH, USA). SAIL (Syngenta Arabidopsis Insertion Library, [8] lines were grown on solid media supplemented with 25µg/ml glufosinate ammonium (Basta; Chem service, INC, West Chester, PA, USA). *MYB:GFP* constructs were selected on solid media supplemented with 25µg/ml Hygromycin B (Research Products International, Mount Prospect, IL USA). *Arabidopsis* plants were grown at 21°C and received 100 µmol/m^2/sec illumination for 16 hours each day (Environmental growth chambers, Chagrin Falls, OH, USA). Seedlings were transplanted to sterile 2MIX potting media (www.fafard.com) between 7 and 10 days after plating. In vitro pollen tube growth was performed as previously described [9]. SIV pollinations were performed as previously described [4, 10].

MYB120 cDNA cloning

We obtained multiple identical cDNAs following amplification with RT-PCR primers (see Oligonucleotides used in this study, below) complementary to predicted 3' and 5' untranslated regions. These clones (Genbank Accession #: KC544014) contained an open reading frame (Supplemental Fig. S1) encoding a different C-terminus from that predicted (TAIR 10, http://www.arabidopsis.org/).

Oligonucleotides used in this study

	Gene	Primer Name	Sequence
T-DNA Identification	MYB101	SAIL_30_E07 LP	TCTCAACCATCAAAAAGGCTG
	MYB101	SAIL_30_E07 RP	ATGGGCAGGTTCCAAACTAAC
	MYB101	SALK_039489C LP	TCATCCTCGTCTAACGAGGTG
	MYB101	SALK_039489C RP	TGCAAAATTAGGTTTTGTCCC
	MYB120	SALK_063698C LP	AACTCCATCCACATCCACATC
	MYB120	SALK_063698C RP	TGTTGTGGTTCGGAAGAGAAG
	MYB120	SALK_082685C LP	GACAAAAGAGCATTTTGCCAC
	MYB120	SALK_082685C RP	ACGTGTAACGCAGGTAACACC
	MYB97	SALK_112329C LP	TGGCAGGTATATTCCACTTGC
	MYB97	SALK_112329C RP	GTATCCTCAACAGCCCTCCTC
	MYB65	SAIL_808_B08 LP	AAAACGATGAACCAATGCAAG
	MYB65	SAIL_808_B08 RP	ACTCAGAATGTACCACGTGGC
	MYB65	SALK_063552C LP	CCAATGCAAGAAGAAGTTTGC
	MYB65	SALK_063552C RP	CATCGCAGGTAGAGGAGTCAG
	SALK LB	LBb1.3	ATTTTGCCGATTTCGGAAC
	SAIL LB	LB3	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC
	SAIL LB	LB2	GCTTCCTATTATATCTTCCCAAATTACCAATACA
	SAIL LB	LB1	TAGCATCTGAATTTCATAACCAATCTCGATACAC
MYB120 cDNA	MYB120	120 5pRTF2	CTCTTCTTGAATCGGCAAGG
	MYB120	1203pUTRR5	AGTTAACAGATGATTTGCAACAAT
RT-PCR analysis of mutants	MYB101	101 3pRTF	AGGGTTTCCTCCAGTGACCT
	MYB101	101 3pRTR	TGCATTTCTGTCACCCGATA
	MYB120	120 3pRTF	CCGCTCAAACCGCTACTTAC
	MYB120	SALK_063698C RP	AACTCCATCCACATCCACATC
	MYB97	97 ex1F	CCACTCTCAGCTAGGCAACA
	MYB97	97 ex2R1	GTCGGAGACGAGACAAGGAA
	MYB65	65 3pRTF2	CGCTCCGAGATTACAAGTCC
	MYB65	65 cDNAR	CAGCGACCAAACAGGAGGCAT
Genetic Constructs	MYB101:	BamHI-MYB101F1	***GGATCC***AGGTCCGACTCACAGTCACA
	MYB101:GFP	BamHI-MYB101F2	***GGATCC***GTCAACACAATGGGCTGAAG
	MYB101:GFP	SalI-Myb101R	***GTCGAC***TGCACAGATGCTAGGCATGTTGCTCCAAT
	MYB97:GFP	BamHI-MYB97F	***GGATCC***AGCGCTAGCGGTTTGACTAA
	MYB97:GFP	SalI-MYB97R	***GTCGAC***TGCGCAGATCCCTGGCAAGTTGTTGGTG
	MYB120:GFP	Myb120gatewayGenF	***CACC***TTTGGCCGAGTCTTCAAGTT
	MYB120:GFP	Myb120forGFPR	ATGTTTATGATCAAAACGCAGATT
	MYB120 Genomic	Myb120gatewayGenR	GCAGTTCTCCACCGTTGATT
	THIONIN:GFP	AT5G36720GenomicF	***CACC***CGGTCAAAATTGTTTTTGGAA
	THIONIN:GFP	AT5G36720NoStopR	GGTATCACATAGAAACCAGAATAATCC
RT-qPCR Primers	PP2AA3	PP2AA3qPCRF	GAGTTTGGTCCTGAATGGGCAATG
	PP2AA3	PP2AA3qPCRR	ACTGGAGCGAGAAGCGATACTG
	ACTIN4	ACT4F1	GATTGCGAAAGCAGAGTACG
	ACTIN4	ACT4R1	ACTTCCGTCTTCGTTTGGTG
	AT4G20990	AT4G20990_F2	CTCTTAGGCAAGCCGTTGAC
	AT4G20990	AT4G20990_R2	TCTTCCCTTTGAGTCTTGAACC
	AT4G21000	AT4G21000_F2	ATCGTGGAAAGGAGATGGTG
	AT4G21000	AT4G21000_R2	TCGTGTGAACCATGTGAAGC

AT5G46200	AT5G46200_F2	ATTGGCCACGTCAAAGTCAG
AT5G46200	AT5G46200_R2	AGACGATGCAGTGTTTCCTG
AT5G09280	AT5G09280_F4	CAGTCGCAAACGCTAAACAG
AT5G09280	AT5G09280_R4	AGACGGAGTTTCTGGTGCAG
AT5G36720	AT5G36720_F1	GCAGAGTTTTGTGCAAGCAG
AT5G36720	AT5G36720_R1	ATACGCGAGTACGGTGGAAG
AT2G30310	AT2G30310_F3	TTGCGGAACAGGATACTTGG
AT2G30310	AT2G30310_R1	ATCGGAGTGATTCGGACAAG
AT2G30220	AT2G30220_F1	GATGGACATGATCCGAAACC
AT2G30220	AT2G30220_R1	TATCCTGTTCCGCAACATCC
AT4G26830	AT4G26830_F1	TGGTACGTGCTTCTTTGGTG
AT4G26830	AT4G26830_R1	AATGTCCGGTGGGAAACTC
AT5G06170	AT5G06170_F2	ATTCCATTTGCATTAGCTTCC
AT5G06170	AT5G06170_R2	CCACCTCCAAATAACGCATC
AT3G19940	AT3G19940_F4	TGCGATCATGACGGTTTTC
AT3G19940	AT3G19940_R4	GCTTCCAAACTCTTCCCATC
AT5G20340	AT5G20340_F1	ACCCATCTAGCATCCGTTTG
AT5G20340	AT5G20340_R1	TAGCCCAGTGATCCATCTTG
AT5G03810	AT5G03810_F4	TCAAGAAGGGCTTGTTGTGG
AT5G03810	AT5G03810_R3	TAGCATTCGAGCATGTACCC
AT5G03820	AT5G03820_F3	GTGGGTACATGTTCGAATGC
AT5G03820	AT5G03820_R3	GAGATGAGGGGTATTCCTTGG
SUC7	SUC7_F2	CGTTTGGAGTTGGTCCTATTG
SUC7	SUC7_R2	TGCCACAATACTGCTGACTG
SUC8	SUC8_F2	CGCTTTTTGGAGGTGGAAAC
SUC8	SUC8_R2	CACGGTAAATGCCACAACAC

GFP Fusion constructs and Genomic Clones

To generate *MYB101:GFP* and *MYB97GFP*, genomic fragments containing the promoter (*MYB101*, 2.3kb upstream of the start codon; *MYB97*, 1.2kb), introns and exons (excluding the stop codon) were cloned into pCR2.1 (Invitrogen). These were subcloned into a pCAMBIA1300 (www.cambia.org) variant containing *GFP* and a *NOS* terminator. The *MYB120:GFP* genomic fragment was based on the incorrect TAIR 10 annotation and consequently did not include the experimentally determined third exon. *MYB120:GFP* contains the promoter (1 kb upstream of start codon) and the experimentally determined first two introns and exons and was cloned into the pENTR-D vector (Invitrogen). The *MYB120:GFP* vector was created by recombination into

pMDC107 [11] using LR Clonase II (Invitrogen). *qrt1-2*; Col-0, *HTR10:HTR10:RFP*; or *myb97-1, myb101-4, myb120-3* (Col-0) plants were transformed as previously described [12]. For each construct at least 20 primary transformants were recovered by selecting for hygromycin resistance. GFP was detectable by epifluorescence microscopy in pollen grains from *MYB101:GFP* primary transformants (26/27), but not for *MYB97:GFP* (0/12) or *MYB120:GFP* (0/20).

To generate *THIONIN:GFP,* a genomic fragment containing the promoter (1 kb upstream of the start codon of *AT5G36720*) and a single exon were cloned into the pENTR-D vector (Invitrogen) and transferred to pMDC107 [11] using LR Clonase II (Invitrogen). The current Arabidopsis genome annotation, (TAIR10, http://www.arabidopsis.org/) states that *AT5G36720* is perfectly duplicated as *AT5G36805*. We suspect this to be an annotation error based on analysis of the genome sequences of other Arabidopsis accessions [13].

PCR to identify T-DNA insertion sites

T-DNA insertion sites were identified using left border (SAIL - LB3, LB2, and/or LB1, SALK - LBb1.3) and gene-specific flanking primers (see Oligonucleotides used in this study, above), designed at http://signal.salk.edu/tdnaprimers.2.html) in PCR reactions. The PCR program used for this reaction was: 94°C for 5 min followed by 36 cycles of 94°C for 15 sec, 60°C for 30 sec, 72°C for 2 min, and a final elongation step of 72°C for 4 min.

Imaging of pollen tube growth

Aniline blue analysis was performed on crosses dissected 24 hours after pollination as reported previously [14]. We made the following adjustments; all fixative and exchange steps were extended to 12 hours, and aniline blue incubation was extended from overnight to a period of 4-12 days before analysis by epifluorescence microscopy on a Zeiss Axiovert 200M Fluorescence Microscope with an UV filter. For live imaging, crosses were performed under a dissecting microscope (Zeiss Stemi 2000C) by pollinating *ms1* or emasculated *ACT11:MSI1:GFP* stage 14 stigmas [15] with pollen from male donors. Ovary walls were removed 12 hours post-pollination as previously described [16]. Dissected pistil tissues were mounted in 80mM sorbitol for analysis by confocal laser scanning microscopy using a 40x objective with DIC capability (Zeiss LSM510 Meta inverted microscope). Standard emission and detection wavelength settings were used to image GFP expression (argon laser 458/477/488/514mn at 30mV) and DsRed/mRFP expression (helium neon laser 633nm at 3mV). Signal intensities were optimized for each individual fluorophore and then combined in overlay. Final images represent a merge of single planes at varying depths (z stacks). Semi in vivo (SIV) imaging was performed on a Zeiss Lumar V12 Fluorescence Stereomicroscope.

RNA extraction, RT-PCR and RT-qPCR

RNA was purified using the Qiagen RNeasy kit (http://www.qiagen. com). The yield and RNA purity were determined by Nano-Drop (Thermo Scientific, Wilmington, DE, USA). Gene-specific first strand synthesis and PCR was performed using the Access RT-PCR

System (Promega). For RT-PCR, RNA was DNAse treated with RNAse-Free DNAse (Qiagen). cDNA libraries were constructed from 2.5ug of total RNA using Super Script VILO Master Mix (random hexamer and oligo dT, Super Script III), (Invitrogen). For Real-time RT-qPCR, RNA was DNAse treated using the Ambion TURBO DNA-free kit from Invitrogen (AM1907). Primers were designed at Primer3Plus (primer3plus.com/), using qPCR-optimized settings. All primers were tested to assure that they have near 100% efficiency and linearity prior to use with an ABI 7000 real time PCR machine. RT-qPCR was performed in triplicate. The comparative Ct method (User Bulletin #2, Applied Biosystems) was used for quantification. Expression was normalized to the ubiquitous PP2AA3 gene[17]. RT-qPCR was performed with cDNA libraries using primers of equivalent efficiency in triplicate reactions using SYBR Green qPCR Master Mix (Invitrogen) on an ABI 7000 PRISM Sequence Detection System (Applied Biosystems).

Microarray Analysis

Total RNA was extracted from unpollinated *ms1* pistils and *ms1* pistils pollinated with wild-type pollen or *myb* triple mutant pollen and grown for 8 hours. Total RNA was extracted using the RNeasy kit (Qiagen). The yield and RNA purity were determined by Nano-Drop (Thermo Scientific). RNA integrity was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies). Hybridization and post hybridization processing was performed as per the manufacturer's instructions (Affymetrix, ATH1) by the Brown University Core Facility (www.brown.edu/Research/CGP/core/). Analysis of differential expression was performed on the Partek Genomics Suite 6.6 beta (www.partek.com).

Renormalization of publicly available microarray data was performed with RMAExpress (rmaexpress.bmbolstad.com/), and compared using Microsoft Excel.

Bioinformatic Analysis

MYB97, MYB101, MYB120-regulated small peptides were analyzed for predicted signal peptides using the SignalP 4.1 server [70]. Analysis of microarray differential expression was performed on the Partek Genomics Suite 6.6 beta (www.partek.com). Renormalization of publicly available microarray data (Figure 3, Table S1) was performed with RMAExpress (rmaexpress.bmbolstad.com/).

References

1. Millar, A.A., and Gubler, F. (2005). The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. Plant Cell *17*, 705-721.
2. Alonso-Peral, M.M., Li, J., Li, Y., Allen, R.S., Schnippenkoetter, W., Ohms, S., White, R.G., and Millar, A.A. (2010). The microRNA159-regulated GAMYB-like genes inhibit growth and promote programmed cell death in Arabidopsis. Plant Physiol *154*, 757-771.
3. Honys, D., and Twell, D. (2004). Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol *5*, R85.
4. Qin, Y., Leydon, A.R., Manziello, A., Pandey, R., Mount, D., Denic, S., Vasic, B., Johnson, M.A., and Palanivelu, R. (2009). Penetration of the stigma and style elicits a novel transcriptome in pollen tubes, pointing to genes critical for growth in a pistil. PLoS Genet *5*, e1000621.
5. Borges, F., Gomes, G., Gardner, R., Moreno, N., McCormick, S., Feijo, J.A., and Becker, J.D. (2008). Comparative transcriptomics of Arabidopsis sperm cells. Plant Physiol *148*, 1168-1181.
6. Swanson, R., Clark, T., and Preuss, D. (2005). Expression profiling of Arabidopsis stigma tissue identifies stigma-specific genes. Sexual Plant Reproduction *18*, 163-171.
7. Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Scholkopf, B., Weigel, D., and Lohmann, J.U. (2005). A gene expression map of Arabidopsis thaliana development. Nat Genet *37*, 501-506.
8. Sessions, A., Burke, E., Presting, G., Aux, G., McElver, J., Patton, D., Dietrich, B., Ho, P., Bacwaden, J., Ko, C., et al. (2002). A high-throughput Arabidopsis reverse genetics system. Plant Cell *14*, 2985-2994.

9. Boavida, L.C., and McCormick, S. (2007). Temperature as a determinant factor for increased and reproducible in vitro pollen germination in Arabidopsis thaliana. Plant J *52*, 570-582.
10. Palanivelu, R., and Preuss, D. (2006). Distinct short-range ovule signals attract or repel Arabidopsis thaliana pollen tubes in vitro. BMC Plant Biol *6*, 7.
11. Curtis, M.D., and Grossniklaus, U. (2003). A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol *133*, 462-469.
12. Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J *16*, 735-743.
13. Cao, J., Schneeberger, K., Ossowski, S., Gunther, T., Bender, S., Fitz, J., Koenig, D., Lanz, C., Stegle, O., Lippert, C., et al. (2011). Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat Genet *43*, 956-963.
14. Mori, T., Kuroiwa, H., Higashiyama, T., and Kuroiwa, T. (2006). GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization. Nat Cell Biol *8*, 64-71.
15. Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in Arabidopsis. Plant Cell *2*, 755-767.
16. Johnson, M.A., and Kost, B. (2010). Pollen tube development. Methods Mol Biol *655*, 155-176.
17. Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., and Scheible, W.R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiol *139*, 5-17.