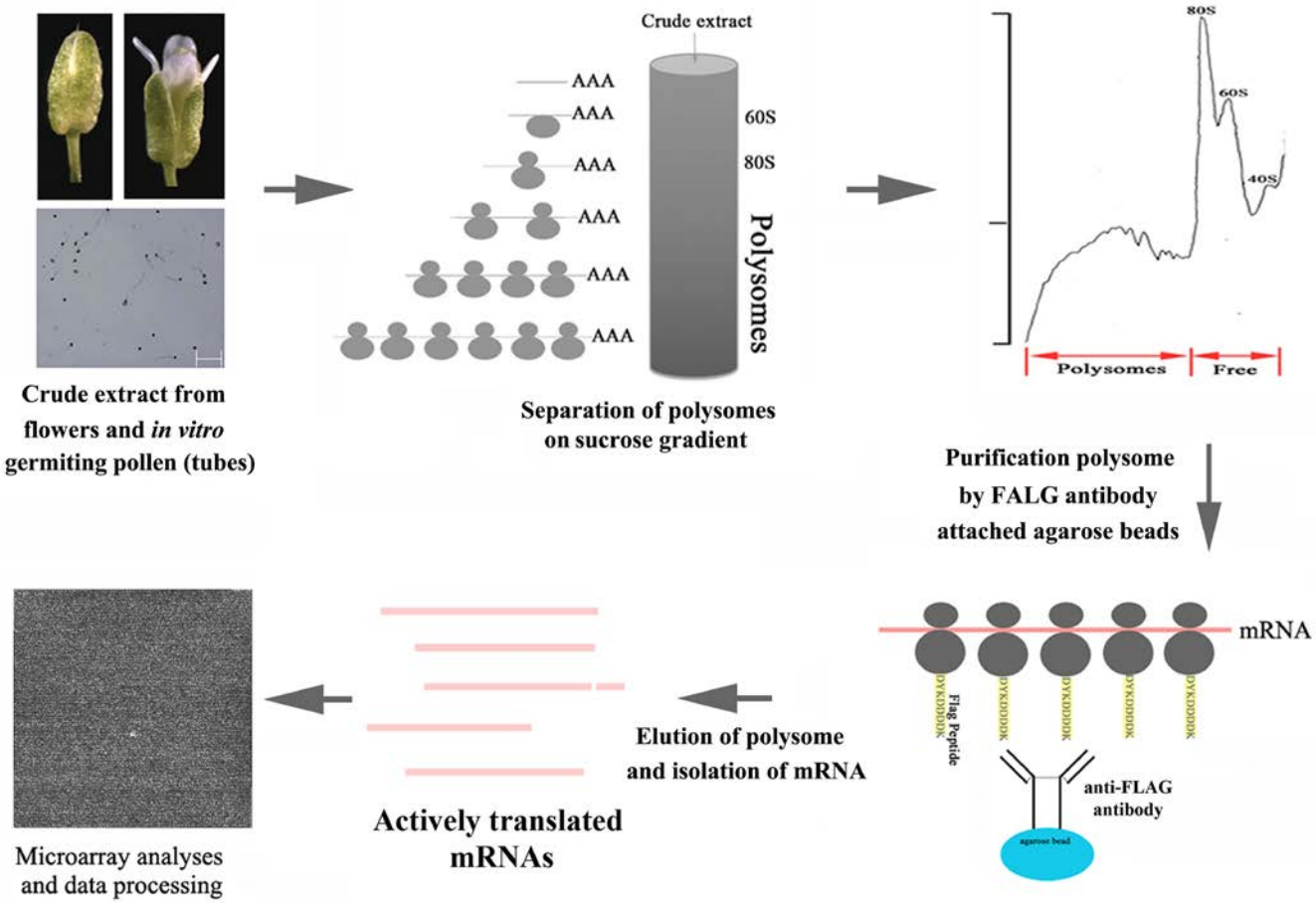
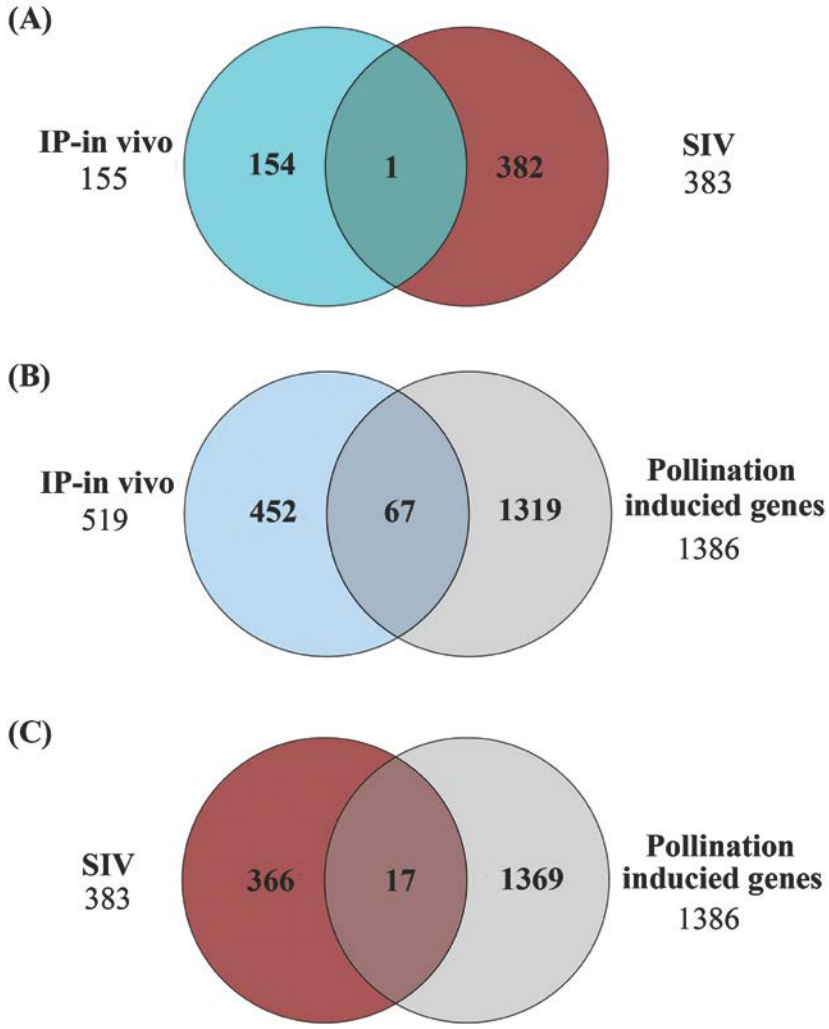


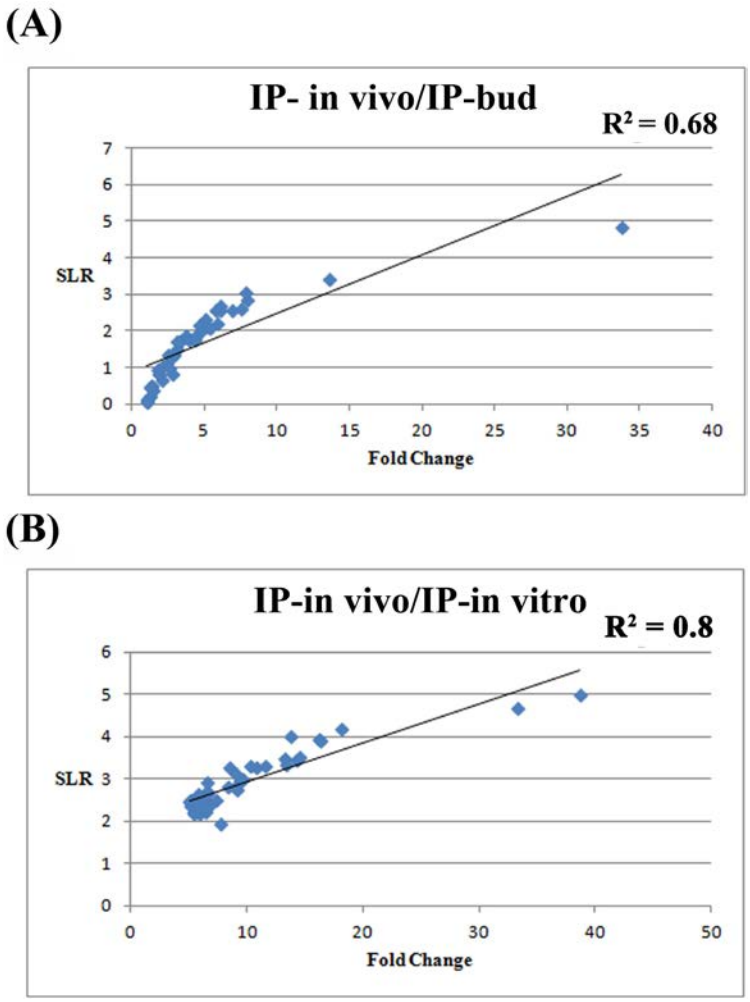

**Supplemental Figure 1. Scheme for extraction of polysome-mRNA from *in vitro* germinated pollen (*in vitro*), pollinated (*in vivo* stage, ST14 and ST15a) and non-pollinated (Bud stage, ST10-12) floral buds.**

The crude extracts were fractionated by 20% to 60% (w/v) sucrose density gradients and the UV absorbance (260 nm) was recorded. The polysomal fractions were passed through FLAG agarose beads to isolate the FLAG-RPL18 containing polysome-mRNA complex. The actively translated mRNAs isolated from affinity-purified polysomes were used for microarray analyses.



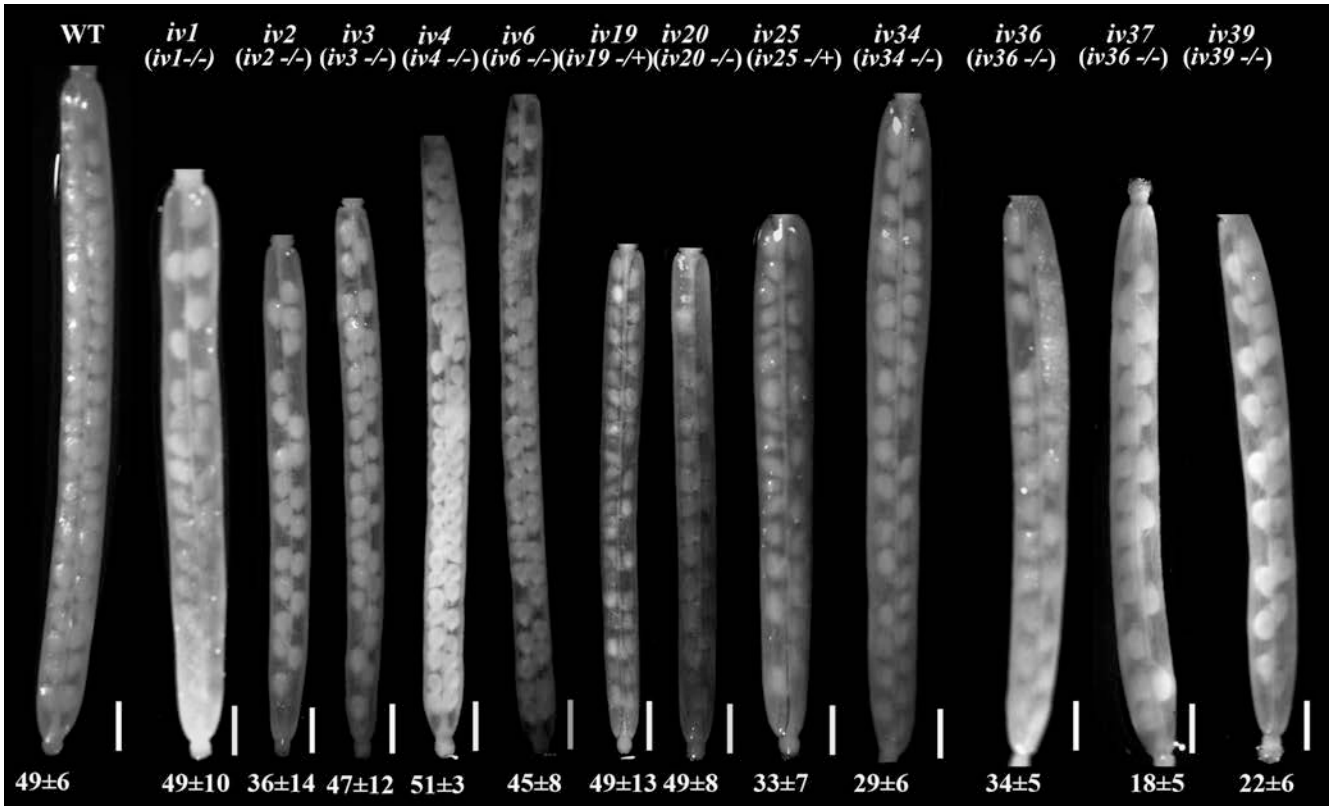

**Supplemental Figure 2**. **Three Venn diagram comparisons.**

(**A**) Comparison of highly-enriched *in vivo* pollen tube polysomal mRNAs (probe pair sets) to semi-*in vivo* (SIV) enriched mRNAs (Qin et al., 2009). (**B**) Comparison between highly-enriched *in vivo* pollen tube polysomal mRNA (probe pair sets) pollen-pistil interaction upregulated mRNAs (Boavida et al., 2011). (**C**) Comparison of semi-*in vivo* (SIV) enriched mRNAs to pollen-pistil interaction upregulated mRNAs.



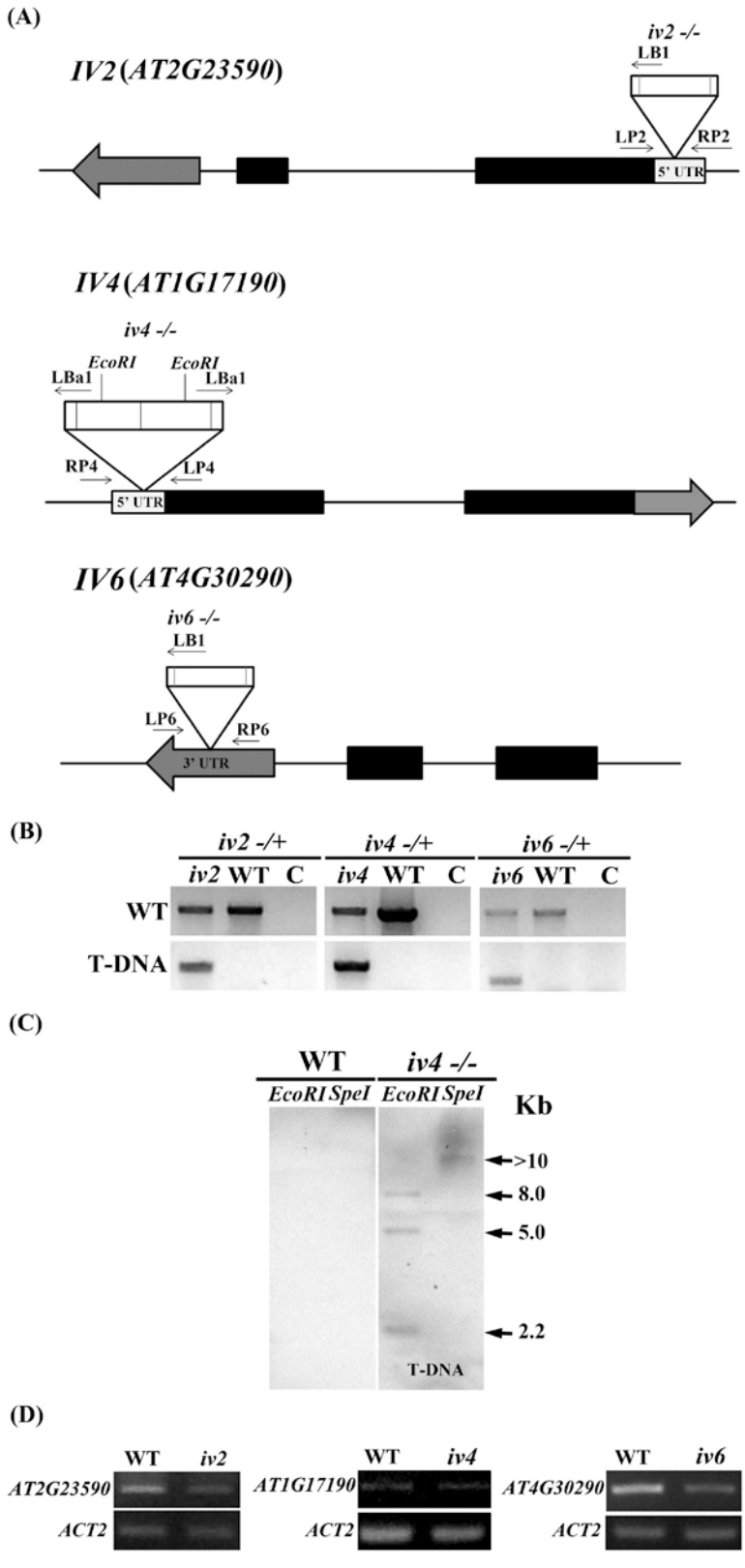

**Supplemental Figure 3. Comparison of fold change data generated with GeneSpring and SLR data generated with R program.**

Fold change data and SLR data indicate consistent up-regulation of the 41 transcripts in IPIVV/IPBUD (**A**) and IPIVV/IPIVT (**B**) comparisons.




**Supplemental Figure 4. The silique phenotypes and seed number per silique of several self-pollinated *iv* mutants.**

*iv1*, *iv2*, *iv3*, *iv4*, *iv6*, *iv20*, *iv34*, *iv36*, *iv37* and *iv39* are homozygous mutants; *iv19* and *iv25* are heterozygous mutants. The number under each silique represents the average seed number per silique and more over 10 siliques were used for the statistic analysis. WT, wild type silique.




**Supplemental Figure 5**. **T-DNA insertion patterns and investigation of *iv2*, *iv4*, and *iv6* specific mRNAs detected in flowers of corresponding homozygous mutants.**

(**A**) T-DNA inserted mutation (triangles) of *iv2*, *iv4*, and *iv6* mutants. LB1, the left border primer of SAIL line mutant. LP2 and RP2, the primers for *IV2* gene. LBa1, the left border primer of SALK line mutant. LP4 and RP4, the primers for *IV4* gene. LP6 and RP6, the primers for *IV6* gene. (**B**) PCR analysis of *iv2* (*iv2-/+*), *iv4* (*iv4-/+*), and *iv6* (*iv6-/+*) heterozygous plants. *iv2*, *iv2* heterozygous genomic DNA. *iv4*, *iv4* heterozygous genomic DNA. *iv6*, *iv6* heterozygous genomic DNA. WT, wild type genomic DNA. C, the water used as template for PCR control. (**C**) Southern blot analysis of *iv4* (*iv4 -/-*) homozygous plant. Genomic DNA was digested with *EcoRI* and *SpeI* two restriction enzymes, respectively; *EcoRI* is a restricted site on the T-DNA of *iv4* mutant, but *SpeI* is not. WT, wild type genomic DNA. (**D**) Investigation of *iv2*, *iv4*, and *iv6* specific mRNA. mRNAs were detected in flowers of corresponding homozygous mutants. WT, mRNA was from wild type. *ACT2*, the transcripts used as total RNA loading control.

**Supplemental Table 1. Number of the flowers used for the polysome-mRNA extraction of the Bud stage, *in vivo* stage, and *in vitro* germinated pollen in each of three biological replicate samples.**

| Sample ID | Flowers NO. | RNA Conc. (ng/μl) | aRNA Conc. (ng/μl) |
|---|---|---|---|
| Bud | 800-900 | 8.761 | 198.1 |
| *in vivo* | 500-700 | 0.455 | 1159.1 |
| *in vitro* | 7200 | 2.897 | 1036.4 |

aRNA: antisense RNA synthesized

**Supplemental Table 2. Investigation of pollen viability and seeds abortion pattern of selected *IV* mutants**

| Mutant | AGI number | Genotype | *in vitro* pollen germination | Seeds abortion | Publicly mutant name | Predicted gene function |
|---|---|---|---|---|---|---|
| Col-0 | | | Normal | No | | |
| *IV 1* | AT4G18425 | Homozygous | Normal | Yes | SALK_063946C | Hypothetical protein |
| *IV 2* | AT2G23590 | Homozygous | Normal | Yes | SAIL_270_A07 | Methyl esterase 8 |
| *IV 3* | AT1G64405 | Homozygous | Normal | Yes | SAIL_198_G04.V1 | Expressed protein |
| *IV 4* | AT1G17190 | Homozygous | Normal | Yes | SALK_047724C | Glutathione S-transferase TAU26 |
| *IV 6* | AT4G30290 | Homozygous | Normal | Yes | SAIL_62_A10 | Xyloglucan endotransglucosylase/hydrolase |
| *IV 19* | AT1G70090 | Heterozygous | Abnormal | Yes | SAIL_510_E07 | Galacturonosyl transferase-like 9 |
| *IV 20* | AT1G70090 | Homozygous | Abnormal | Yes | SAIL_510_E07 | Galacturonosyl transferase-like 9 |
| *IV25* | AT1G29430 | Heterozygous | Abnormal | Yes | CS804061 | Putative auxin-induced protein |
| *IV 34* | AT2G27500 | Homozygous | Abnormal | Yes | SALK_068499C | Glycosyl hydrolase superfamily protein |
| *IV 36* | AT5G48540 | Homozygous | Abnormal | Yes | CS809476 | Receptor-like protein kinase-related family protein |
| *IV 37* | AT2G01080 | Homozygous | Normal | Yes | CS806210 | LEA hydroxyproline-rich glycoprotein family |
| *IV 39* | AT4G38950 | Homozygous | Abnormal | Yes | SALK_080688 | ATP binding microtubule motor family protein |

**Supplemental Table 3. Primer pairs used in this study.**

| Primers | Sequence 5'→ 3' |
|---|---|
| **Genotyping** | |
| **RP2** | TCCATAGCTATGACCCACGAG |
| **LP2** | TGTAGTTGTGAACTATTAATGTGCAGTC |
| **RP4** | TTTTTGTCGATGAATTCAGCC |
| **LP4** | AACCGATGCATCTTGATTAGC |
| **RP6** | TCAAAAACTCGATTCGAATGG |
| **LP6** | GAATCTTGGACCAGTACGTG |
| **LB1** | GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC |
| ***Lba1*** | TGGTTCACGTAGTGGGCCATCG |
| **RT-PCR** | |
| ***AT1G17190*** | CCCCCCAAAACCGCAACCTCTA |
| | AGCCAATGCCTTGACCACACTCT |
| ***AT4G30290*** | CTCTTTGCAGCACAATCTATCAGCG |
| | CGGTGAAAGGAGCTTTTGACCAATC |
| ***AT2G23590*** | CGCGTGGTGCTGGTACAAGGTGAA |
| | ACGGTGCTTCGTGAACTCAGCG |
| ***ACTIN2*** | CATCAGGAAGGACTTGTACGG |
| | GATGGACCTGACTCGTCATAC |
| **Q-PCR** | |
| ***AT1G17190*** | TGGCGAACGACCAAGTGATT |
| | CATCTTCGTCCTCATCCCGAA |
| ***AT2G02650*** | AACGAAATCACTCGCCTCACTG |
| | AGTGGCAATGACAGAAAATGCC |
| ***AT5G55590*** | GCAAAGAGATATGGCGCGATC |
| | ACCCGGTATCTTCAGTCTCCGA |
| ***AT2G32690*** | AGTCGCTGAATTTTCGTTCGG |
| | TGTAAATGCCATGCTTGTGGAG |
| ***AT4G02050*** | CAGCGAACATGGTTAATTACGG |
| | AAGAAATACCCGCCAAGCGT |
| ***AT2G23590*** | CTTTCATGCCCGACACCAAA |
| | GGTGTCATGGTGCTTGCAAAC |
| ***AT2G27500*** | ACCGGTTGGCCTTCT AAAGGA |
| | CCCATTATACAAAGCCGCGTT |
| ***AT3G60290*** | CTCACGTGCCTACCCGTTACAT |
| | CGAGCATTGGTCGTTGTGAAG |
| ***AT4G18425*** | CTTTCCATCACCGTCAGCAGA |

| | | |
|---|---|---|
| | | AGAAAACACCCCTACGCCTACC |
| ***AT4G30290*** | | TATGAATGCCGAGTCACGTGG |
| | | TCCCCTTGTAGCCCAATGCTCT |
| ***AT5G13580*** | | TAGAAACGCGGCGAATACG |
| | | GAGATTCCACGGTGTCCTTCA |
| ***AT2G17845*** | | TGGAGTCGCATACGCTTGTT |
| | | CCTGGTGCGATCGAATTCA |
| ***AT1G66120*** | | ACCTGCCATGTACGAGATGCA |
| | | GGTTAAGTACGGCTCCGGTCAT |
| ***AT1G70090*** | | TCTGCCGTTTTAGCGTTTGC |
| | | AAAGCCATTTCCGACGGTG |
| ***AT1G64405*** | | AAGAAGAGCGTGTCCTGGTTCC |
| | | ACTCAAGCTCACTAGCCGTCGA |
| ***AT1G66540*** | | ATGCGGCTGATCATTTACCG |
| | | CTTTACCCGCCTCTCGAAATC |
| ***AT3G53300*** | | TTCCGCGAGTGCGATTACA |
| | | TCTGTCTCTTGCACCAGCTCTT |
| ***AT4G18425*** | | TACCAACAGGAACCGTTCTCG |
| | | ATCGCATTGACCGCCATT |
| ***AT2G20595*** | | ATGTTCTCAGCCTTGTCGCAGT |
| | | GGACCACCGCATTCGATTT |
| ***AT3G55870*** | | GATGAAGTTCGCGGAAGGTTCT |
| | | ATGGTGTGACGAAGCGGAA |
| ***AT5G22970*** | | GGATGTGGTTCGTTTTGTACGA |
| | | GGCAAAAATGGAACACGAGTCT |
| ***GAPDH*** | | TTACAGTTCCCGTGTGGTCGA |
| | | GCTTAGGCCTTTGACATGTGGA |
| ***Actin2*** | | GGCTCCTCTTCTTAACCCAAAGGC |
| | | CACACCATCACCAGAATCCAGC |
| ***FLAG-RPL18*** | ***QF*** | TATGCGAGACGCCTATGATCG |
| | ***QR*** | CGTGCACAACAGAATTGAAAGC |
| ***AP1*** | ***QF*** | TACTCTTACGCCGAAAGACAGC |
| | ***QR*** | TGTATTGACGTCGGACTCAGGT |
| ***CRP1*** | ***QF*** | AGGTGCGAACAAGAGGTGTCAC |
| | ***QR*** | GCAAGCCTTGTTCCCATCATT |
| ***SHP1*** | ***QF*** | GTACCTGCGAGCAAAGATAGCC |
| | ***QR*** | TCACACTCGATTCCTGCTGGTC |
| ***SR1*** | ***QF*** | TGATATCTGGTGTGGCGCTTG |
| | ***QR*** | TGGTTCAGCGGCTATAATCCG |
| ***VGD1*** | ***QF*** | GTTAAACTCAGCCCTGGAACCA |
| | ***QR*** | AACCTGGACTGTGCCACTAAGG |
| ***PLIM2 (CRP1)*** | ***QF*** | GACTTGCTTCAGGTGCACACAC |
| | ***QR*** | AGGACGCCATTAAGAGAAGCG |

## SUPPLEMENTAL METHODS:

### Analysis for T-DNA Mutants

SALK and SAIL lines were obtained from the ABRC and they were analyzed the genotype according to the primers offered from T-DNA express website (http://signal.salk.edu/tdnaprimers.2.html).

### Genomic DNA Extraction and Southern Blot Analysis

Genomic DNA was extracted from the wild type and *iv4* homozygous plants. About 1g plant sample was grinded with liquid nitrogen briefly and then grinded with 5 mL extraction buffer (0.1 M Tris HCl pH8.5, 50 mM EDTA, 0.1 M NaCl, 2% Sodium dodecyl sulfate). The crude extract was poured into a 50 mL centrifuge tube and added proteinase K (0.1 mg/ mL) for degrading protein in 1hour at room temperature. The 5 mL of phenol/chloroform/isoamyl alcohol (12:12:1) was added into crud extract and mixed gently, and then separated the phases by centrifuge at 4000g, 5 min at room temperature. Supernatant was transferred into new tube and repeated the phenol/chloroform/isoamyl alcohol purification. Genomic DNA was precipitated with 3M sodium acetate (1mL, pH 5.5) and 100% ethanol (10 mL) from the supernatant. After centrifugation (12000rpm, 20 min, 4°C), the pellet was suspended in 0.2-0.3 mL Tris-EDTA (TE) buffer (pH 7.0). Genomic DNA for Southern blot was digested with *EcoR1* and *Spe1* restriction enzymes, respectively. The probe designed and detail steps for Southern blot were followed the protocol of DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche, Mannheim, Germany). The primers for DNA probe preparation are Southern SALKF (5'ATCGGGGAAATTCGAGCTCGGTACC 3') and Southern SALKR (5'CCCACTGAATCAAAGGCCATGG 3').

**RT-PCR analysis of RNA**

Total RNA was extracted by use of the RNeasy mini kit (Qiagen). For RT-PCR, 92 ng of total RNA was treated with DNaseI (Invitrogen Life Technologies, Carlsbad, CA, USA) for 30 min and first-strand cDNA was synthesized with 10 μM oligo dT primer and MMLV reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA). The PCR mix contained 1.5 mM MgCl2 0.2 mM dNTPs, 0.5 μM each of sense and antisense primers, 2.5 U of Taq polymerase (MDBio, Taipei, Taiwan) and 1 × PCR buffer supplied with the Taq polymerase. The PCR program was conducted at 94°C for 5 min, 25–35 cycles at 94°C for 1 min, annealing at 55–59°C for 1 min, 72°C for 1–3 min, final elongation at 72°C for 7 min with use of a Biometra® T3 Thermocycler (Whatman Biometra, Gottingen, Germany). PCR products were analyzed on a 1% (w/v) agarose gel containing 0.01% (w/v) ethidium bromide. *ACTIN 2* (*ACT2*; AT3G18780) was used as a positive control for monitor cDNA synthesized in PCR amplification, and a 761 bp fragment was obtained by use of the primer sets listed in Supplemental Table 3 online.

**Boavida, L.C., Borges, F., Becker, J.D., and Feijó, J.A.** (2011). Whole genome analysis of gene expression reveals coordinated activation of signaling and metabolic pathways during pollen-pistil interactions in *Arabidopsis*. Plant Physiol. **155,** 2066-2080.

**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.