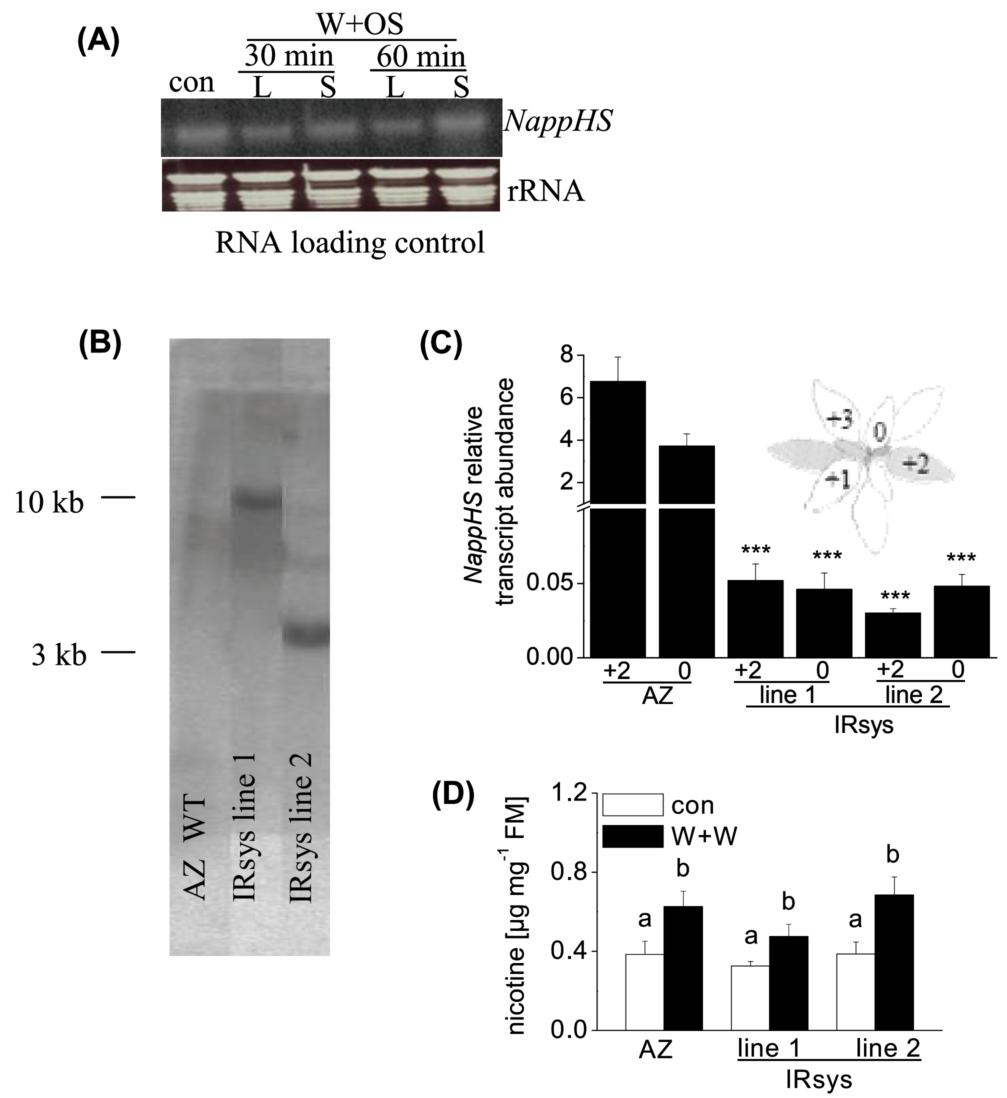
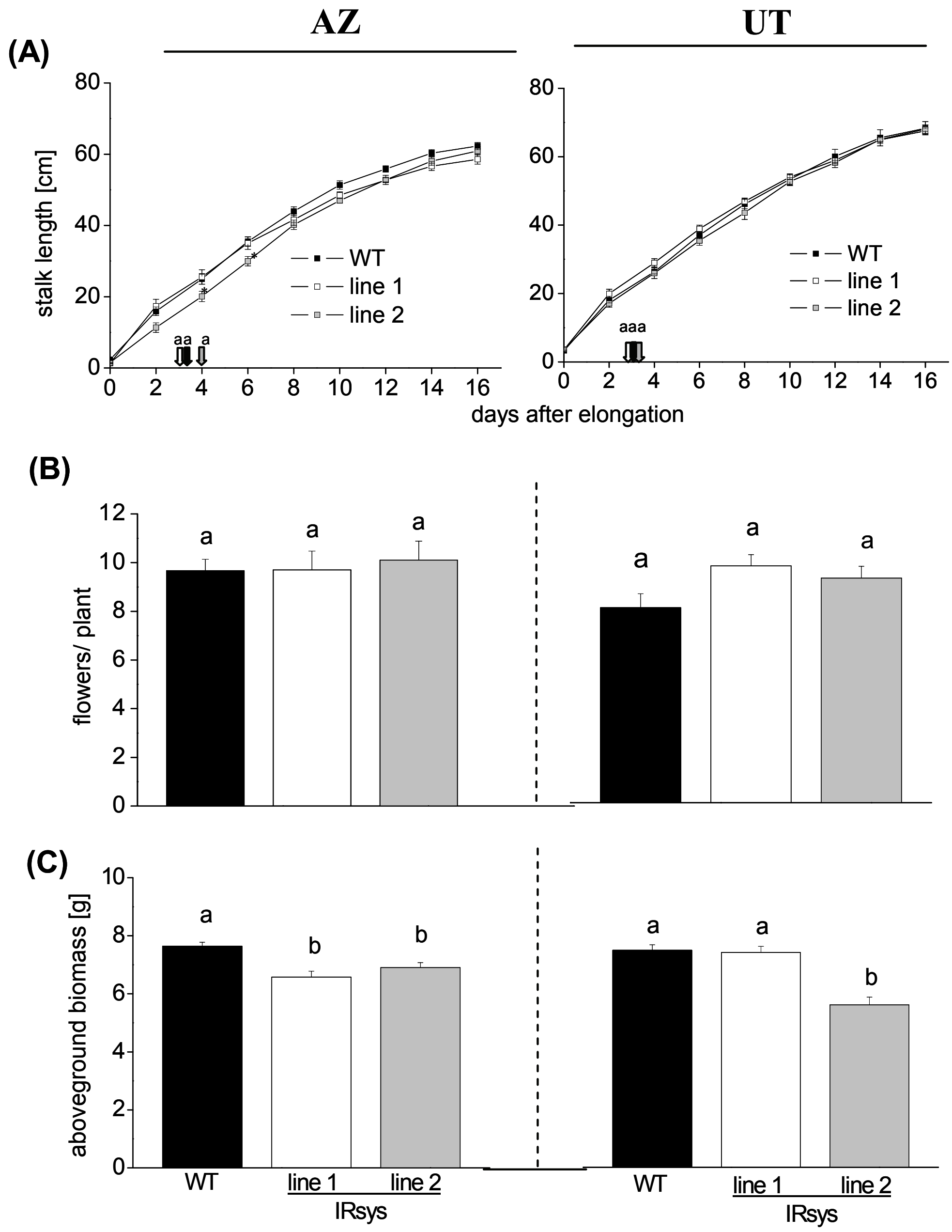
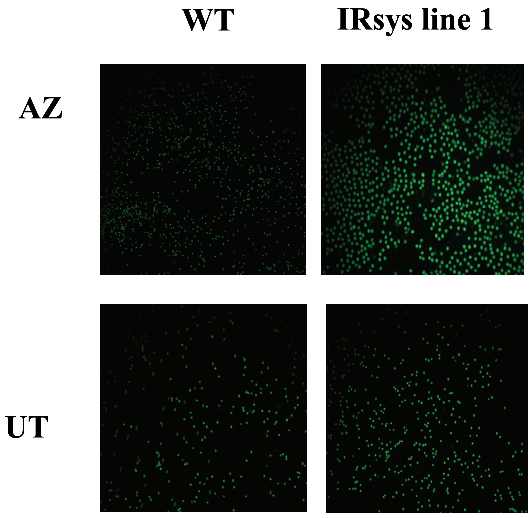


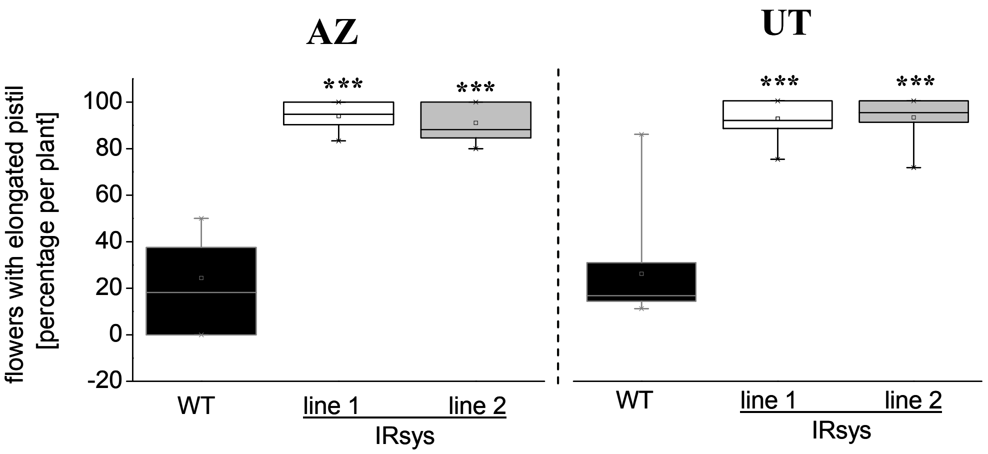
Supplemental Figure S1: **(A)** Northern blot analysis of *NappHS* transcripts in AZ wild-type (WT) plants. Rosette-stage leaves (position +2) were wounded with a pattern wheel (w), and 20 µL of 1:1 (v/v) diluted *M. sexta* oral secretions (OS) was applied to the resulting puncture wounds. Treated (L) and systemic (S) leaves from OS-elicited plants at position 0 were harvested after 30 min and 60 min, as well as unwounded (con) leaves at position +2 from five plants. **(B)** Southern blot analysis of AZ WT, AZ IRsys line 1 and AZ IRsys line 2 plants. Genomic DNA (5µg) was digested with *BamH1*, blotted onto a nylon membrane, and hybridized with a PCR fragment of the *hptII* gene, a selection marker on the transformation vector pRESC. **(C)** Relative *NappHS* transcript abundance in local (+2) and systemic (0) leaves of W+OS treated Arizona WT (AZ) and ppHS-silenced plants (IRsys line 1 and 2), (*** = $P < 0.001$, ANOVA). **(D)** Mean ± SE leaf (+2) nicotine content of 5 control (white bars) AZ WT, AZ IRsys line 1 and AZ IRsys line 2 plants. Nicotine accumulation was additionally determined in rosette-stage leaves that were wounded, followed by the application of 20 µL water (3 in a row, every 30 min), and harvested four days after the treatment (black bars).



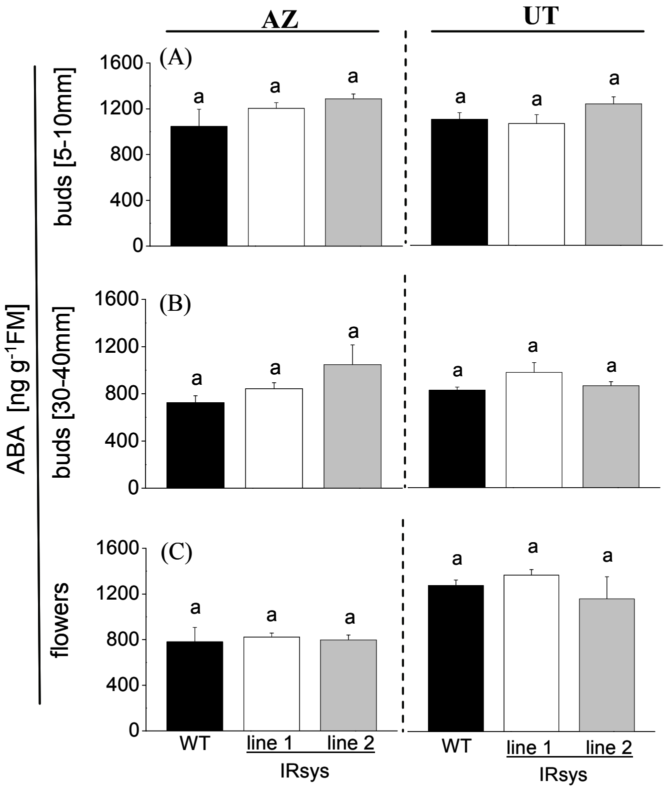
Supplemental Figure S2: *NappHS*-silenced plants do not differ from wild-type (WT) plants in flower production or stalk elongation. **(A)** Mean ± SE growth rates of 10 to 15 WT (black squares), and the two lines of ppHS-silenced plants in two accessions: IRsys line 1 (empty squares) and IRsys line 2 (gray squares) of AZ (left panel) and UT (right panel) accession. Asterisks represent significant differences from WT values of each accession (as determined by ANOVA followed by Bonferroni-corrected post-hoc test). First flowering is indicated by arrows in both panels (WT: black arrows, IRsys line 1: white arrows and IRsys line 2: gray arrows). Number of flowers **(B)** and above-ground biomass **(C)** in WT (black bars) and ppHS-silenced plants: IRsys line 1 (white bars); IRsys line 2 (gray bars) of the AZ (left panel) and the UT (right panel) accession. Bars represent means ± SE of 9 to 10 replicates. Different letters indicate significant differences among the genotypes.



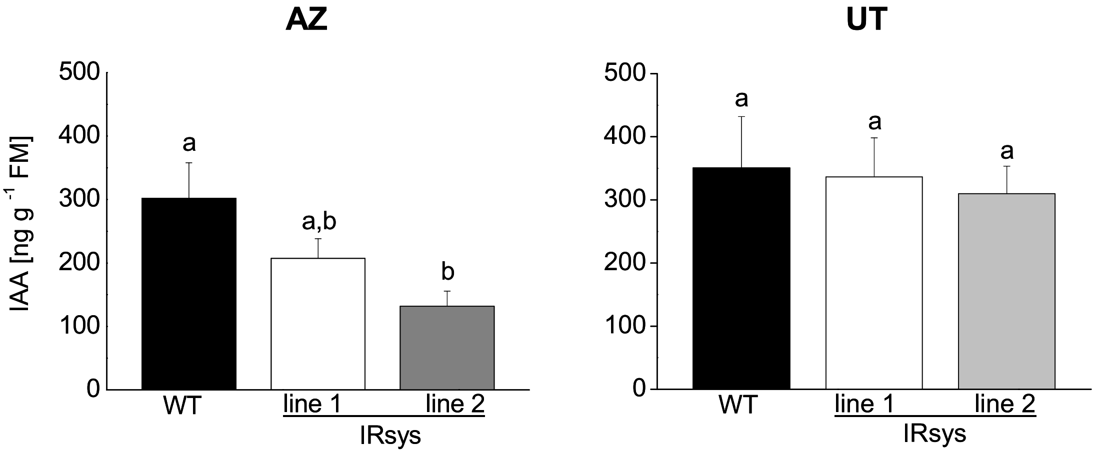
Supplemental Figure S3: Viability of pollen from WT and IRsys flowers. Pollen were stained with fluorescine diacetate and visualized using a fluorescence microscope.



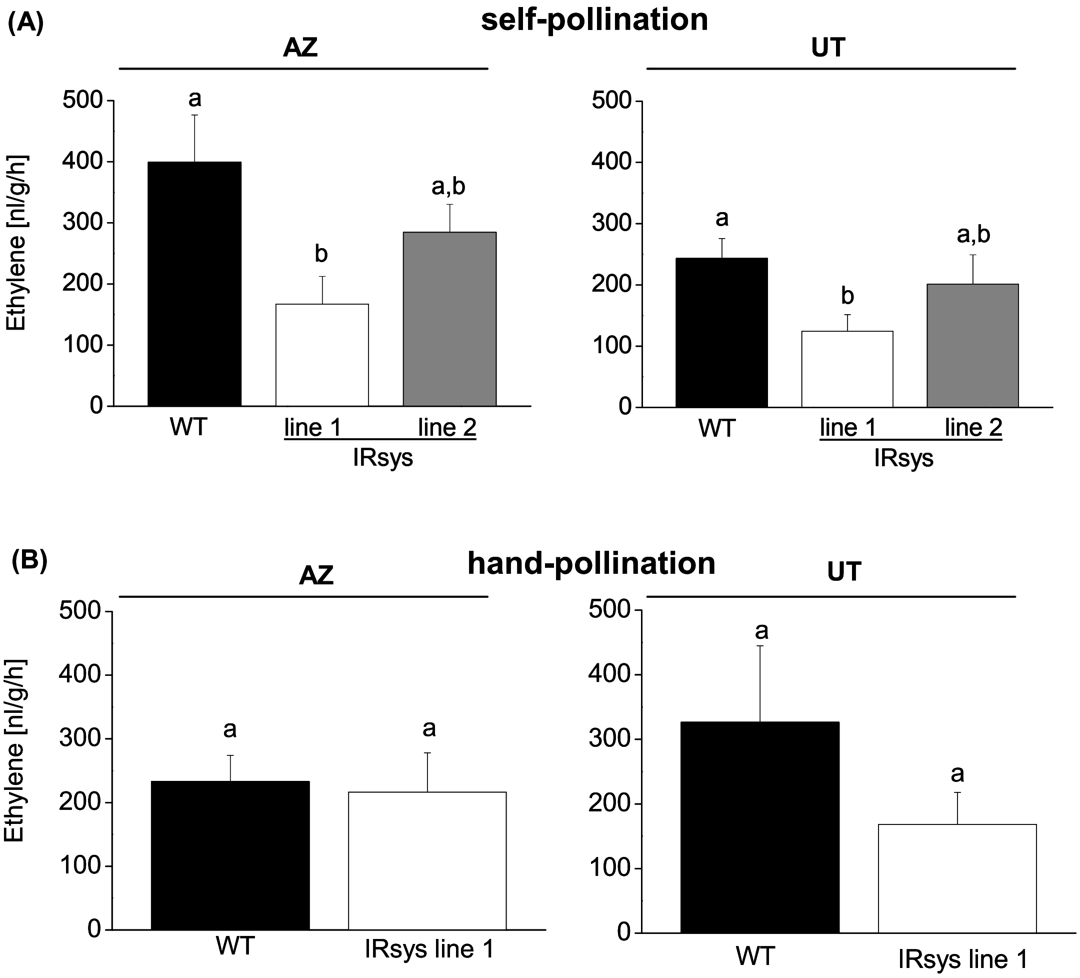
Supplemental Figure S4: Percentage of flowers with elongated pistils. Flowers with abnormal pistil growth in WT (black boxes), IRsys line 1 (white boxes) and IRsys line 2 (gray boxes) of AZ (left panel) and UT (right panel) were counted and divided by the total number of flowers per plant (n = 8 to10 plants/ per genotype).



Supplemental Figure S5: Mean ± SE abscisic acid (ABA) content of 4 to 5 small (A, 5 -10mm), elongated (B, 30 - 40mm) buds and flowers of WT (black bars) and IRsys line 1 (white bars) and IRsys line 2 (gray bars) AZ (left panel) and UT (right panel) accessions. Different letters indicate significant differences among WT plants and their respective IRsys-silenced lines (ANOVA followed by Bonferroni-corrected post-hoc test).

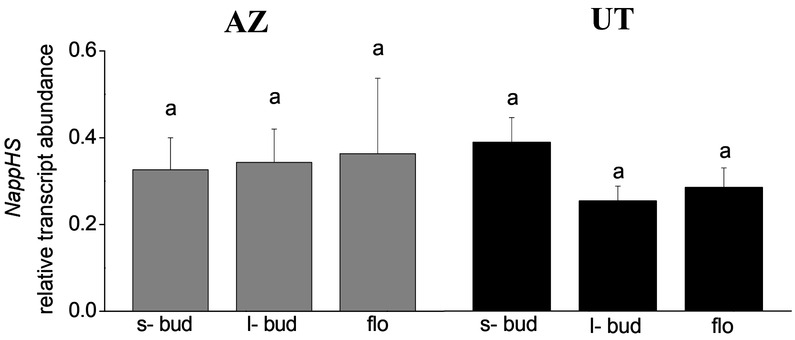


Supplemental Figure S6: Indole-3-acetic acid (IAA) concentration in WT and IRsys buds. Mean ± SE IAA content (n = 5) in buds of AZ (left panel) and UT (right panel) WT (black bars) and their respective IRsys lines 1(white bars) and IRsys lines 2 (gray bars). Different letters indicate significant differences as determined by ANOVA (Bonferroni-corrected post hoc tests, $P < 0.05$).

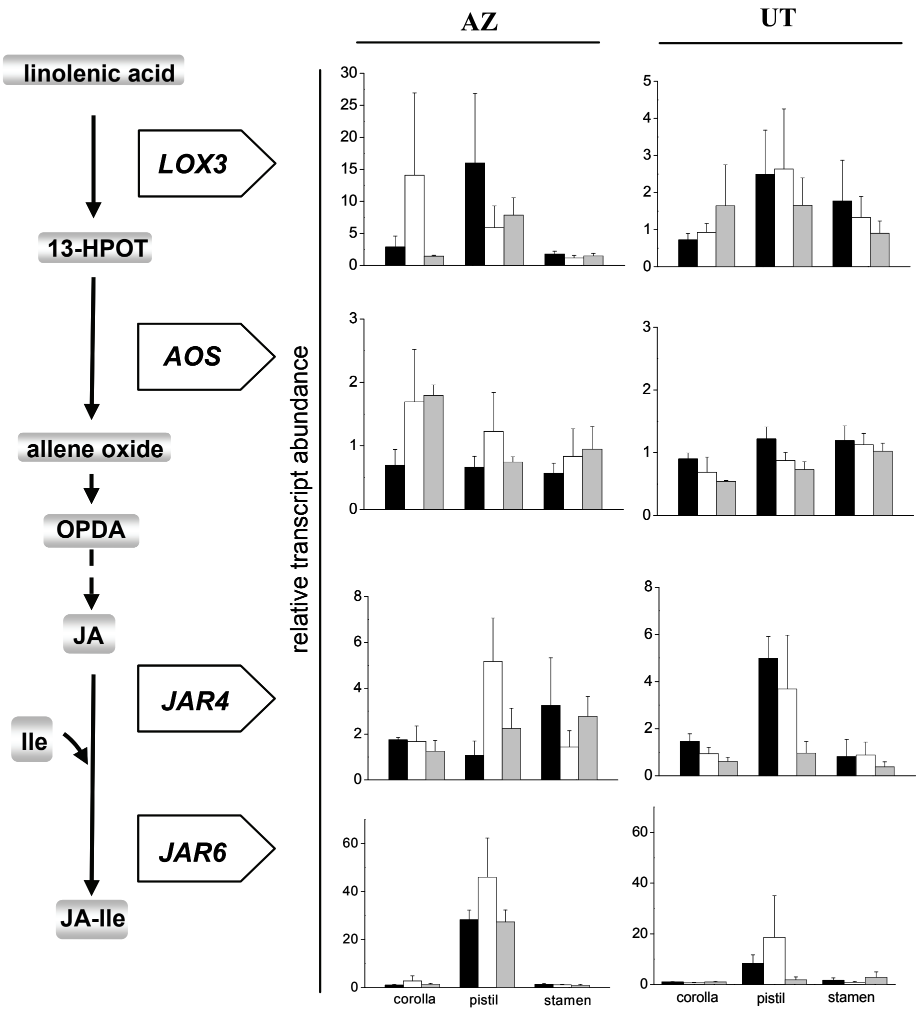


Supplemental Figure S7: Ethylene emissions are reduced in self-pollinated but not in hand-pollinated IRsys flowers.

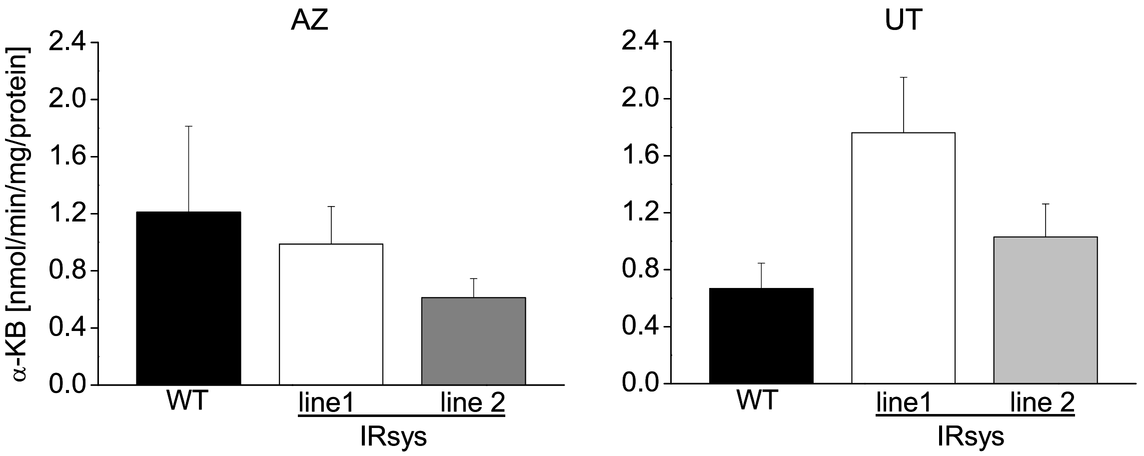
Ethylene emissions were measured by ’stop-flow’ measurements of single flowers. Mean values of AZ and UT WT (black bars), IRsys line 1 (white bars) and IRsys line 2 (gray bars) flowers are shown, error bars represent SE (n = 4 - 6), and different letters indicate significant differences as determined by ANOVA (Bonferroni-corrected post hoc tests, $P < 0.05$).



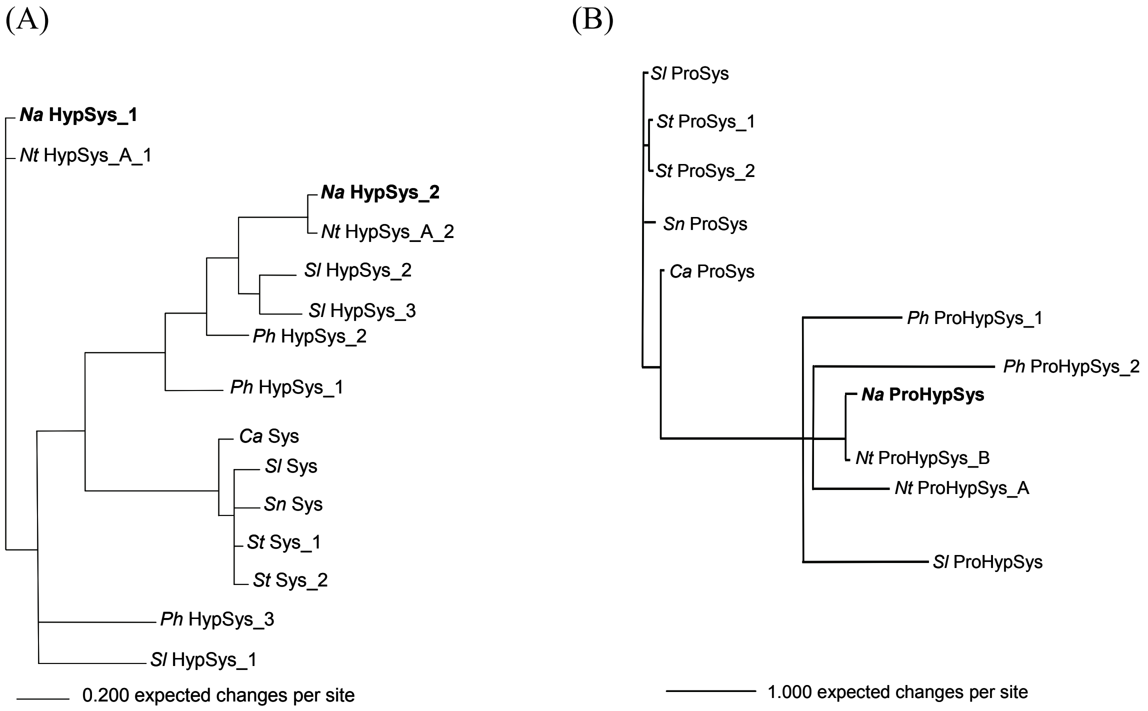
Supplemental Figure S8: Relative transcript abundance of *NappHS* in small buds (s-bud), buds (l-bud) and flowers (flo) of AZ (gray bars) and UT (black bars) WT plants. Different letters indicate significant differences (ANOVA followed by Bonferroni-corrected post-hoc test, n = 5).



Supplemental Figure S9: Transcript accumulation of genes involved in jasmonate synthesis and in the conjungation step of JA with Ile in the oxylipin pathway. Left panel shows a simplified model of the oxylipin pathway: A 13-lipoxygenase (*LOX3*) oxygenates linolenic acid to 13-HPOT (13-hydroperoxide linolenic acid) which is further converted to allene oxide and OPDA (12-oxo-phytodienoic acid) by *AOS* (allene oxide synthase). OPDA is converted to JA,and the conjugation of JA to Ile is mediated by *JAR4* (jasmonate resistant 4) and *JAR6* (jasmonate resistant 6). Relative transcript abundance of **(A)** *LOX3*, **(B)** *AOS*, **(C)** *JAR4* and **(D)** *JAR6* in corolla, pistil and stamen of 3 to 5 replicated wild-type (black bars) and IRsys line 1 (white bars) and IRsys line 2 (gray bars) plants of AZ (left panel) and UT (right panel) plants. Asterisks represent significant differences from WT values of each accession (as determined by ANOVA followed by Bonferroni-corrected post-hoc test).



Supplemental Figure S10: α-ketobutyrate (α-KB) concentration in buds of 4 - 5 replicates from AZ and UT WT (black bars) and their respective inverted repeat (IRsys) lines 1 (white bars) and lines 2 (gray bars).



Supplemental Figure S11: Phylogenetic relationship of **(A)** *Na* HypSys and HypSys as well as Sys from other plant species and of **(B)** *Na* ProHypSys, ProHypSys and ProSys of other plant species (based on deduced amino-acids sequences).
GenBank accession numbers: *Capsicum annuum* (*Ca* ProSys: AF000375), *Nicotiana attenuata* (*Na* ProHypSys: AY456270), *Nicotiana tabacum* (*Nt* ProHypSys-A: AY033148, *Nt* ProHypSys-B: AY033149), *Petunia hybrida* (*Ph* ProHypSys: EF552428), *Solanum lycopersicon* (*Sl* ProHypSys: AY292201, *Sl* ProSys: M84801), *Solanum nigrum* (*Sn* ProSys: AF000375) *Solanum tuberosum* (*St* ProSys_1: AF000373, *St* ProSys_2: AF000374).

Supplemental Figure S12: AZ WT and IRsys flowers at different developmental stages. Small buds **(A),** buds before anther dehiscence **(B)**, buds after anther dehiscence **(C)** and fully opened flowers **(D)**.