Title

Base-pairing requirements for small RNA-mediated gene silencing of recessive self-incompatibility alleles in *Arabidopsis halleri.*

Authors

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Abstract

Small RNAs (sRNA) are central regulators of gene expression, yet identifying the molecular alphabet of sRNA-target interactions remains challenging. Here, we take advantage of the dominance hierarchy among self-incompatibility alleles in *Arabidopsis halleri* to evaluate the base-pairing requirements for effective transcriptional silencing by a highly diversified set of sRNA-target interactions. We used RT-qPCR to follow temporal expression of the pollen (*SCR*) and pistil (*SRK*) determinants of self-

incompatibility in numerous heterozygous combinations. *SCR* and *SRK* had sharply distinct expression dynamics through flower development. Recessive *SCR* alleles were transcriptionally silenced in all heterozygote combinations examined, bringing levels of *SCR* transcripts below detection limits regardless of the position of the sRNA target along the *SCR* sequence. A simple threshold model of base-pairing for the sRNA-target interaction captures most of the variation in *SCR* transcript levels. In contrast, both *SRK* alleles were expressed at similar levels in all heterozygote genotypes. We show that the base-pairing requirements for effective transcriptional silencing by these sRNAs are broadly similar to those of canonical microRNAs, even though they are believed to function in sharply different ways. We discuss the implications for the evolutionary processes associated with the origin and maintenance of the dominance hierarchy among self-incompatibility alleles.

Key Words

Dominance/recessivity. Sporophytic self-incompatibility. RT-qPCR. heterochomatic siRNA. microRNA. *Arabidopsis halleri*.

47 # Introduction

48 Small non-coding RNAs are short RNA molecules (20-25nt) with
49 a range of regulatory functions whose central importance has
50 constituted a major discovery in the last 20 years (Vazquez *et al.*,
51 2010; Aalto & Pasquinelli, 2012). The best-known members of this
52 class of molecules are microRNAs, which are typically involved in
53 post-transcriptional gene silencing and regulate the activity of their
54 target gene in *trans* by either mRNA cleavage (quickly followed
55 by degradation) or by blocking translation (Li *et al.*, 2014). In
56 some cases, the action of microRNAs leads to the production of
57 secondary phased short interfering RNAs (pha-siRNAs) by their
58 target coding or non-coding sequence, which in turn can regulate
59 downstream targets (Fei *et al.*, 2013). Another major set of small
60 RNAs is heterochromatic short interfering RNAs (hc-siRNAs)
61 which are mediating transcriptional silencing of repeat sequences
62 in the genome through epigenetic modification by the RNA-
63 dependent DNA methylation pathway (RdDM, Matzke *et al.*,
64 2009).

65 Both microRNAs and siRNAs guide their effector molecules
66 (members of the ARGONAUTE gene family: AGO1 and AGO4,
67 respectively) to their target sites by sequence similarity through
68 base-pairing. For plant microRNAs, sequence similarity with the
69 target sequence is typically very high and appears to be a shared
70 feature of all functionally verified interactions (Wang *et al.*, 2015).
71 Total base-pairing complementarity, however, is not the sole
72 determinant of target specificity, and the position of the
73 mismatches along the microRNA:target duplex is also important.
74 Indeed, expression assays showed that while individual
75 mismatches typically have limited functional consequences, they
76 can also entirely inactivate the interaction when they hit specific

77 positions such as e.g. the 10th and 11th nucleotide, corresponding to
78 the site of cleavage (Jones-Rhoades *et al.*, 2006). Furthermore, the
79 position of mismatches along the microRNA:target duplex also
80 seems to be crucial, with a greater tolerance in the 3' than the 5'
81 region of the microRNA (up to four mismatches generally have
82 limited functional consequences in the 3' region, while only two
83 mismatches in the 5' region seem sufficient to abolish the target
84 recognition capability; Liu *et al.*, 2014, Mallory *et al.*, 2004;
85 Parizotto *et al.*, 2004; Schwab *et al.*, 2005). These observations
86 have led to the formulation of general "rules" for microRNA
87 targeting (Axtell & Meyers, 2018), but in the same time they also
88 revealed a large number of exceptions. As a result, *in silico*
89 prediction of microRNA target sites currently remains a difficult
90 challenge (Ding *et al.*, 2012; Axtell & Meyers, 2018). For other
91 types of small RNAs (pha-siRNAs and hc-siRNAs), even less is
92 known about the base-pairing requirements for targeting, mostly
93 because of the absence of experimentally confirmed examples of
94 discrete, single siRNA target sites either in *cis* or in *trans* (Wang *et*
95 *al.*, 2015).

96 In this context, the recent discovery by Tarutani *et al.* (2010),
97 Durand *et al.* (2014) and Yasuda *et al.*, (2016) of a highly
98 diversified set of small non-coding RNAs at the gene cluster
99 controlling self-incompatibility (SI) in Brassicaceae, provides an
100 experimentally tractable model to evaluate the base-pairing
101 requirements for silencing by a set of sRNAs that are regulating
102 expression of a single gene. Sporophytic SI is a genetic system that
103 evolved in several hermaphroditic plant lineages to enforce
104 outcrossing by preventing self-fertilization, hence avoiding
105 inbreeding depression (De Nettancourt, 2001). In the Brassicaceae
106 family, SI is controlled by a single genomic region called the "S-
107 locus", which contains two tightly linked genes, namely *SCR* and

108 *SRK,* that encode the pollen S-locus cysteine-rich and the stigma S-
109 locus receptor kinase recognition proteins, respectively. This
110 system involves a polymorphism in which multiple deeply
111 diverged allelic lines are maintained, and accordingly a large
112 number of S-alleles is typically found in natural populations of
113 self-incompatible species (Castric & Vekemans, 2004). With such
114 a large allelic diversity and the very process of self-rejection, most
115 individual plants are heterozygotes at the S-locus. Yet in most
116 cases, only one of the two S-alleles in a heterozygous genotype is
117 expressed at the phenotypic level in either pollen or pistil, as can
118 be revealed by controlled pollination assays on pollen or pistil
119 tester lines (Llaurens *et al.*, 2008; Durand *et al.*, 2014). Which of
120 the two alleles is expressed is determined by their relative position
121 along a dominance hierarchy, whose molecular basis for the pollen
122 phenotype has been initially studied in the genus Brassica. In this
123 genus, dominance is controlled at the transcriptional level in pollen
124 (Schopfer 1999, Kakizaki et al. 2003). Transcriptional silencing of
125 recessive alleles by dominant alleles is caused by 24nt-long trans-
126 acting small RNAs produced by dominant S-alleles and capable of
127 targeting a DNA sequence in the promoter sequence of the *SCR*
128 gene of recessive S-alleles, provoking DNA methylation (Shiba et
129 al. 2006). Details of how these sRNAs achieve their silencing
130 function remains incompletely understood (Finnegan *et al.*, 2011),
131 but it is clear that their biogenesis is similar to that of microRNAs
132 (*i.e.,* they are produced by a short hairpin structure), while their
133 mode of action is rather reminiscent of that of siRNAs (*i.e.,* the
134 transcriptional gene silencing functions through recruitment of the
135 methylation machinery). Strikingly, the full dominance hierarchy
136 in the Brassica genus seems to be controlled by just two small
137 RNAs called *Smi* and *Smi2* (Tarutani *et al.*, 2010, Yasuda *et al.*
138 2016). *Smi* and *Smi2* target distinct DNA sequences, but both are

139 located in the promoter region of *SCR*, and both seem to involve
140 DNA methylation and 24-nt active RNA molecules.

141 The dominance hierarchy in Brassica is however peculiar in that
142 only two ancestral allelic lineages segregate in that genus (the class
143 I and class II alleles referred to above, see *e.g.* Leducq *et al.*,
144 2014), whereas self-incompatible species in Brassicaceae typically
145 retain dozens of highly divergent ancestral allelic lineages (Castric
146 & Vekemans, 2004). A recent study showed that in *Arabidopsis*
147 *halleri*, a Brassicaceae species with multiple allelic lineages at the
148 S-locus, the dominance hierarchy among S-alleles in pollen is
149 controlled by not just two but as many as eight different sRNA
150 precursor families and their target sites, whose interactions
151 collectively determine the position of the alleles along the
152 hierarchy (Durand *et al.*, 2014). In that genus, much less is known
153 about the mechanisms by which the predicted sRNA-target
154 interactions translate into the dominance phenotypes. First, the
155 expression dynamics of the *SCR* gene across flower development
156 stages is poorly known. Indeed, Kusaba et al. (2002) measured
157 expression of *SCR* alleles in *A. lyrata,* but focused on only two S-
158 alleles (*SCRa* and *SCRb*, also known as *AlSCR13* and *AlSCR20*,
159 respectively, in Mable et al. 2003) and showed striking differences
160 in their expression dynamics in anthers. Hence, the developmental
161 stage at which the transcriptional control of dominance in pollen
162 should be tested is not clear. Second, while they did confirm
163 monoallelic expression, consistent with the observed dominance
164 relationship between the two alleles (*SCR*b > *SCR*a, Kusaba et al.
165 2002), the fact that only a single heterozygote combination was
166 measured among the myriad possible combinations given the large
167 number of S-alleles segregating in that species (at least 38 S-
168 alleles: Castric *et al.*, 2008) prevents generalization at this step.
169 Hence, a proper experimental validation of the transcriptional

170 control of dominance in Arabidopsis is still lacking. Third, Durand
171 *et al.*, (2014) observed rare sRNA-target interaction predictions
172 that did not agree with the observed dominance phenotype. In
173 particular, they identified cases where no sRNA observed as being
174 produced by a dominant allele was predicted to target the *SCR*
175 gene of a recessive allele, while the dominance phenotype had
176 been well established phenotypically by controlled crosses (*e.g.*
177 Ah04>Ah03) suggesting the possibility that mechanisms other than
178 transcriptional control may be acting. Conversely, in other rare
179 cases, sRNAs produced by a recessive S-allele were predicted to
180 target the *SCR* gene of a more dominant allele, suggesting
181 exceptions to the set of base-pairing rules used to predict target
182 sites. Fourth, although the target sites for the two sRNAs in
183 Brassica were both located in the promoter sequence, and can thus
184 be reasonably expected to prevent transcriptional initiation through
185 local modification of the chromatin structure associated with DNA
186 methylation, many of the predicted sRNA target sites in *A. halleri*
187 are rather mapped to the *SCR* intron or the intron-exon boundary
188 (beside some in the promoter as well), which suggests that distinct
189 silencing pathways might be acting (Cuerda-Gil & Slotkin, 2016).
190 It thus remains to be determined whether transcriptional control is
191 also valid when the targets are at other locations along the *SCR*
192 gene structure. Finally, the dominance hierarchy at the female
193 determinant *SRK* differs from that at *SCR*, co-dominance being
194 more frequent than on the pollen side both in Brassica
195 (Hatakeyama *et al.*, 2001) and in *A. halleri* (Llaurens *et al.*, 2008).
196 Limited transcriptional analysis in Brassica and Arabidopsis
197 suggests that dominance in pistils is not associated with *SRK*
198 expression differences, but again the number of interactions tested
199 has remained limited (Suzuki et al. 1999; Kusaba et al. 2002).

200 Here, we take advantage of the fact that dominance interactions in
201 Arabidopsis SI are controlled in pollen by a diversity of sRNAs
202 and the diversity of their target sites to determine the base-pairing
203 requirements for successful small-RNA mediated transcriptional
204 silencing of recessive *SCR* alleles in plants with controlled S-locus
205 genotypes. We first developed and validated a protocol for qPCR
206 expression analysis of a set of *SCR* and *SRK* alleles in *A. halleri*.
207 We then analysed the expression dynamics across four flower
208 developmental stages of nine *SCR* and five *SRK* alleles and tested
209 the transcriptional control of dominance for both genes in many
210 heterozygote combinations. We quantified the strength of silencing
211 of recessive *SCR* alleles and propose a quantitative threshold
212 model for how sequence identity between the small non-coding
213 RNAs and their target sites results in silencing. We discuss the
214 implications of this model on the evolutionary processes associated
215 with the origin and maintenance of the S-locus dominance
216 hierarchy in Brassicaceae.

217

218 # Material & Methods

219 ## Plant material

220 We used a collection of 88 *A. halleri* plants containing nine
221 different S-alleles (S1, S2, S3, S4, S10, S12, S13, S20, and S29) in
222 a total of 37 of all 45 possible homozygous and heterozygous
223 combinations. Each plant was genotyped at the S-locus using the
224 PCR-based protocol described in Llaurens et al. (2008). The plants
225 were obtained by controlled crosses (Llaurens *et al.*, 2008; Durand
226 *et al.*, 2014; Leducq *et al.*, 2014) and in a few instances were
227 cloned by cuttings. Hence, a given S-locus genotype can be either
228 represented in the collection by different clones (identical genetic

229 background) or by offspring from crosses of distinct parental
230 origins (different genetic backgrounds). Below we refer to these
231 two levels of experimental replicates as “clone replicates” and
232 “biological replicates”, respectively. On average, the collection
233 comprises *n*= 2.05 biological replicates per S-locus genotype and
234 clone replicates were available for three different S-locus
235 genotypes (Table S1 & S2). The pairwise dominance interactions
236 between these alleles as determined by pollen and pistil
237 compatibility phenotypes of heterozygote plants are reported in
238 Table S3.

239 RNA extraction and reverse transcription

240 On each plant, we collected flower buds at four developmental
241 stages: 1) five highly immature inflorescence extremities (more
242 than 2.5 days before opening, buds below 0.5mm, stages 1-10 in *A.*
243 *thaliana* according to Smyth *et al.*, 1990), 2) ten immature buds
244 (2.5 days before opening, between 0.5 and 1mm, approximately
245 stage 11), 3) ten mature buds (one day before opening, longer than
246 1mm, approximately stage 12), and 4) ten open flowers
247 (approximately stages 13-15). These stages were characterized by
248 establishing the size distribution within each stage and measuring
249 the time to flower opening based on ten buds. Samples collected
250 were flash-frozen in liquid nitrogen, then stored at - 80°C before
251 RNA extraction. Tissues were finely ground with a FastPrep-24 5G
252 Benchtop Homogenizer (MP Biomedicals, Model #6004-500)
253 equipped with Coolprep 24 x 2mL adapter (6002-528) and
254 FastPrep Lysis Beads & Matrix tube D. Total RNAs were
255 extracted with the Arcturus “Picopure RNA isolation” kit from
256 Life Science (PN: KIT0204) according to the manufacturer’s
257 protocol, including a step of incubation with DNAse to remove
258 gDNA contamination. We normalized samples by using 1 mg of
259 total RNA to perform reverse-transcription (RT) using the

260 RevertAid Fermentas enzyme following the manufacturer's
261 instructions.

262 Primer design

263 A major challenge to study expression of multiple S-alleles is the
264 very high levels of nucleotide sequence divergence among them,
265 precluding the possibility of designing qPCR primers that would
266 amplify all alleles of the allelic series (both for *SRK* and *SCR*).
267 Hence, we rather designed qPCR primers specifically targeted
268 towards each of the *SCR* and *SRK* alleles, and for each
269 heterozygote genotype we independently measured expression of
270 both alleles of each gene. Primers were designed based on genomic
271 sequences from BAC clones (Goubet et al 2012; Durand et al.
272 2014; Novikova et al. 2017), with a length of ~20 nucleotides, a
273 GC content around 50% and a target amplicon size of 200nt.
274 Whenever possible, we placed primers on either side of the *SCR*
275 intron to identify and discard amplification from residual gDNA.
276 However, because the coding sequence of the *SCR* gene is short,
277 the number of possible primers was limited and this was not
278 always possible. In two cases (*SCR01* and *SCR20*), both primers
279 were thus located in the same exon. For *SRK* alleles, the primers
280 were designed on either side of the first intron. To obtain relative
281 expression levels across samples, we used *actin 8* as a
282 housekeeping gene for standardization after we verified that the *A.*
283 *thaliana* and *A. halleri* sequences are identical at the primer
284 positions (An et al. 1996). Primer sequences are reported in Table
285 S4.

286 Quantitative real-time PCR

287 On each cDNA sample, at least three qPCR reactions (referred to
288 below as "technical" replicates) were performed for *actin 8* and for
289 each of the S-alleles contained in the genotype (one S-allele for

290 homozygotes, two S-alleles for heterozygotes). The runs were
291 made on a LightCycler480 (Roche) with iTaq Universal SYBR
292 Green Supermix (Bio-rad, ref 172-5121). Amplified cDNA was
293 quantified by the number of cycles at which the fluorescence signal
294 was greater than a defined threshold during the logarithmic phase
295 of amplification using the LightCycler 480 software release 1.5.0
296 SP3. The relative transcript levels are shown after normalisation
297 with actin amplification through the comparative $2^{-\Delta Ct}$ method
298 (Livak & Schmittgen, 2001). The Ct_{SCR} and Ct_{SRK} values of each
299 technical replicate were normalized relative to the average Ct_{actin}
300 measure across three replicates.

301 Validation of qPCR primers at the dilution limits

302 Given the very large nucleotide divergence between alleles of
303 either *SCR* or *SRK*, cross-amplification is unlikely. However, to
304 formally exclude that possibility, we first performed cross-
305 amplification experiments by using each pair of *SCR* primers on a
306 set of cDNA samples that did not contain that target *SCR* allele but
307 instead contained each of the other *SCR* alleles present in our
308 experiment.

309 In order to evaluate our ability to measure expression of *SCR*
310 alleles in biological situations where they are expected to be
311 transcriptionally silenced, we then used a series of limit dilutions
312 to explore the loss of linearity of the relationship between *Ct* and
313 the dilution factor. We used six to eight replicates per dilution level
314 to evaluate the linearity of the amplification curve. Then we
315 examined the shape of the melting curves to determine whether our
316 measures at this limit dilution reflected proper PCR amplification
317 or the formation of primer dimers. Finally, we used water in place
318 of cDNA to evaluate the formation of primer dimers in complete
319 absence of the target template DNA.

320 Expression dynamics and the effect of dominance

321 We used generalized linear mixed models (lme4 package in *R*;
322 Bates *et al.*, 2014) to decompose *Ct* values normalized by the *actin*
323 *8* control (as the dependent variable) into the effects of five
324 explanatory variables: biological and clone replicates -reflecting
325 the hierarchical structure of our dataset-, developmental stage,
326 dominance phenotype and allelic identity (Table S5). Because
327 expression of the different *SCR* (and *SRK*) alleles was quantified
328 by different primer pairs with inevitably different amplification
329 efficiencies, *Ct* values cannot be directly compared across alleles.
330 Most analyses were thus performed by comparing expression
331 levels of a given focal allele in different contexts (e.g. different
332 genotypic contexts, different developmental stages) and
333 accordingly we considered the identity of *SCR* or *SRK* alleles as
334 nuisance parameters in our statistical model by including them as
335 random effects. We visually examined normality of the residuals of
336 the model under different distributions of $2^{-\Delta Ct}$, including Gaussian,
337 Gamma and Gaussian with logarithmic transformations. We then
338 tested the effect of developmental stages and dominance on *SCR*
339 and *SRK* expression by considering them as fixed effects.
340 Phenotypic pairwise dominance relationships were obtained from
341 Llaurens *et al.*, (2008) and Durand *et al.*, (2014), and a set of
342 additional controlled crosses performed following the same
343 protocol (Table S3). Pollen and pistil dominance relationships
344 were used to assess the effect of dominance on *SCR* and *SRK*,
345 respectively. To test whether the different S-alleles have distinct
346 expression profiles across developmental stages, as suggested by
347 Kusaba *et al.* (2002) in *A. lyrata,* we used ANOVA to compare
348 nested models in which a random effect for the interaction between
349 the "allelic identity" and "stage" effects was introduced.

350 Target features and silencing effect.

351 We then sought to determine how the expression of *SCR* alleles
352 was affected by specific features of the small RNA-target
353 interactions between alleles within heterozygote genotypes. We
354 first used the small RNA sequencing data in Durand *et al.* (2014)
355 and Novikova *et al.* (2017) to identify the populations of 18-26nt
356 small RNA molecules produced by the small RNA precursors
357 carried at the S-locus by each of the nine S-alleles. For each
358 heterozygote combination, we then predicted the presence of
359 putative target sites of the small RNAs produced by one S-allele on
360 the genomic sequence of the *SCR* gene of the other S-allele
361 including 2kb of nucleotide sequence upstream and downstream of
362 *SCR* using a dedicated alignment algorithm and scoring matrix, as
363 described in Durand *et al.* (2014). The reciprocal analysis was also
364 performed regardless of the dominance relationship. Briefly,
365 alignment quality was assessed by a scoring system based on the
366 addition of positive or negative values for properly paired
367 nucleotides (+1), mismatches and gaps (-1), taking into account the
368 non-canonical G:U interaction (-0.5). For each pair of alleles
369 considered, only the sRNA/target combination with the highest
370 score was selected for further analysis (Table S6). We used Akaike
371 Information Criteria (AIC) to compare how well different base-
372 pairing scores for target site identification predicted the level of
373 *SCR* expression (and hence the silencing phenomenon), varying
374 the threshold from 14 to 22. Lower values of AIC are associated
375 with a best fit of the model. We then added a new fixed effect in
376 our model to test whether targets in the promoter or in the intron of
377 the *SCR* gene were associated with different strengths of silencing.
378 For this analysis, we included only targets above the threshold
379 identified (score >= 18).

380 To determine whether the base-pair requirement for silencing were
381 identical between Brassica and Arabidopsis, we calculated the
382 alignment score with our method between *Smi* & *Smi2* sRNAs and
383 their targets sites in the class II alleles in *Brassica rapa* (Tarutani
384 *et al.*, 2010, Yasuda *et al.*, 2016).

385 Finally, we used the phylogeny in Durand *et al.* (2014) to classify
386 sRNA/target interactions into "ancient" (mir867 and mirS4) and
387 "recent" (mirS1, mirS2 mirS3, mirS5, mir1887 and mir4239).
388 Based on this classification, we used a linear regression to compare
389 the alignment score for recent and ancient sRNAs in order to test
390 the hypothesis that interactions with base-pairing scores above the
391 threshold at which silencing was apparently already complete
392 correspond to recently emerged interactions that did not yet have
393 time to accumulate mismatches.

394 # Results

395 ## Validation of the qPCR protocol and the allele-
396 specific primers

397 Melting curves confirmed proper amplification and low primer
398 dimers formation unless template DNA concentration was very
399 low (data not shown). The specificity test confirmed the absence of
400 cross-amplification between alleles, as the *Ct* measures for water
401 control and cross amplification were comparably high (around
402 *Ct*=34) and both were higher than the positive controls (median
403 *Ct*=22, Figure S1). For each allele tested, we then evaluated the
404 linearity of *Ct* values through serial dilutions of the template
405 cDNA. Overall, the range of variation of *Ct* values spanned by a
406 given allele across the different developmental stages or
407 dominance status was generally well within the range over which
408 *Ct* varied mostly linearly with template cDNA concentration,

409 suggesting high power to detect these effects. For *SCR*, linearity
410 was good throughout most of the dilution range, but was lost as
411 expected at very low concentration (in particular for alleles *SCR01*,
412 *SCR02*, *SCR04*, *SCR13* and *SCR20*, Figure S2a). We note that
413 comparing levels of expression for a given allele between different
414 recessive contexts (*e.g.* when silenced by different sRNAs) should
415 therefore be challenging, especially for the above-mentioned
416 alleles. Linearity was good for most *SRK* alleles (Figure S2b)
417 except for *SRK12* (data not shown), so this allele was excluded
418 from further analyses.

419 *SCR* and *SRK* expression dynamics across flower 420 development stages

421 In total, we performed 344 RNA extractions and RT-PCR from the
422 37 different S-locus genotypes sampled at four developmental
423 stages and measured 1,838 $Ct_{SCR}/Ct_{\text{actin}}$ expression ratios, resulting
424 in an average of 26.9 measures of each S-allele for each diploid
425 genotype when combining clone, biological, and technical
426 replicates and 480 $Ct_{SRK}/Ct_{\text{actin}}$ (Table S1, Table S2). Distribution
427 of the residues of the generalized mixed linear model was closest
428 to normality after log-transformation of the ratios (Figure S3). As
429 expected, measured expression levels were more highly repeatable
430 across clones than across biological replicates for a given S-locus
431 genotype, but these sources of variation were minor as compared
432 to the technical error and the allele's expression dynamic in our
433 experiment (deviance estimates of 0.40, 1.07 and 6.08 and 4.56
434 respectively, Table S5a) after taking allele identity, developmental
435 stage and dominance status into account. To determine the
436 expression dynamics of the different *SCR* alleles, we focused on
437 genotypes in which a given focal allele was known to be dominant
438 at the phenotypic level (Figure 1a). Overall, we observed a

439 consistent pattern of variation among stages (F-value: 13.805, p-
440 value: 1.107e-05, Table S5c) with a very high expression in buds
441 at early developmental stages (<0.5 to 1mm), and low level of
442 expression in late buds right before opening and in open flowers,
443 consistent with degeneration in these stages of the anther tapetum
444 where *SCR* is expected to be expressed. Accordingly to Kusaba *et*
445 *al.*, (2002), we found evidence that the expression dynamics varied
446 across alleles (Chi^2: 217.32, p-value < 2.2e-16, Table S5b). The
447 *SRK* alleles had sharply distinct dynamics of expression, with
448 monotonously increasing expression in the course of flower
449 development (Chi^2: 6.9103, p-value 0.00857, Table S5g), with
450 lowest expression in immature buds (<0.5mm) and highest
451 expression in open flowers (Figure 1b).

452 ## Transcriptional control

453 Based on these results, we compared expression of *SCR* alleles
454 across genotypes by averaging $2^{-\Delta Ct}$ values across <0.5mm to 1mm
455 stages. Beside a few exceptions (see below), our expression data
456 were largely consistent with the hypothesis of transcriptional
457 control of the dominance hierarchy in pollen (31 of 37 genotypic
458 combinations, Figure 2). In the four S-alleles for which
459 homozygote genotypes were available (S1, S2, S3 and S20), *SCR*
460 alleles had substantial expression in homozygotes and this was the
461 only case where expression of the most recessive allele (*SCR*01)
462 could be detected. One of the two biological replicates for the
463 S1S1 homozygote genotype had consistently low expression across
464 two clone replicates (Figure S4), so we carefully confirmed
465 homozygosity of these two samples by analysing segregation after
466 crossing to plants that did not carry S1 (all of 58 tested progenies
467 indeed carried S1 as determined by PCR on gDNA). Climbing up
468 the dominance hierarchy from most recessive to most dominant,
469 the S-alleles measured were expressed in an increasing number of

470 heterozygous combinations. At the top of the dominance hierarchy,
471 the two most dominant alleles, *SCR*13 and *SCR*20, were expressed
472 in all heterozygous contexts, including when they formed a
473 heterozygote combination with one another (S13S20), as expected
474 given the codominance observed between them at the phenotypic
475 level (Durand *et al.*, 2014). This general rule had a few exceptions
476 however (Figure 2). For instance, we detected some expression for
477 both *SCR02* and *SCR*29 in heterozygote combination even though
478 phenotypic data indicate that S2>S29 in pollen (Table S3). We also
479 observed low expression for *SCR*10 and *SCR*12 when they were in
480 heterozygote combination with *SCR*01 and the absence of
481 expression for both *SCR*10 and *SCR*12 in the heterozygote
482 combination they formed together, which is not consistent with the
483 documented phenotypic dominance of these two alleles over
484 *SCR*01 and between them (*SCR*12>*SCR*10; see Table S3). We
485 confirmed proper phenotypic expression of S12 in pollen produced
486 by the S10S12 genotype, as five replicate pollinations on a S1S12
487 plant produced no silique.

488 Overall, in spite of these six exceptions, we observed a striking
489 contrast in transcript levels for a given allele according to its
490 relative phenotypic dominance status in the genotype, with at least
491 an overall 145-fold increase in transcript abundance in genotypes
492 where a given focal allele was phenotypically dominant as
493 compared to genotypes in which the same focal allele was
494 recessive at stages when *SCR* is expressed (F-value: 38.582; p-
495 value: < 2.2e-16, Table S5c). In most cases, the recessive allele
496 came close to or even below the detection limits of our method as
497 determined by the break of linearity of the dilution experiment
498 (Figure S1), so this fold-change value is probably under-estimated.
499 In strong contrast, we found no significant effect of dominance in
500 pistils on *SRK* expression (F-value: 6.8884 p-value: 0.068244;

501 Figure 3, Table S5h), confirming the absence of transcriptional
502 control of dominance for *SRK*.

503 Target features and silencing effect

504 Levels of *SCR* expression of any given focal allele varied sharply
505 with the alignment score of the "best" target available for the
506 repertoire of sRNAs produced by the other allele present in the
507 genotype (Figure 4a). Specifically, we observed high and variable
508 expression of *SCR* when the score of its best predicted target was
509 low, but consistently low *SCR* expression when the score of the
510 best target was high (Figure 4a, Table S5d). Strikingly, the
511 transition between high expression and low expression was very
512 abrupt (around an alignment score of 18), suggesting a threshold
513 effect rather than a quantitative model for transcriptional silencing.
514 In three cases, the presence of a target with a high score within the
515 *SCR* gene of the dominant allele was associated with high relative
516 *SCR* expression (in agreement with the dominant phenotype),
517 suggesting the absence of silencing in spite of the presence of a
518 target with high sequence similarity to the sRNA produced by the
519 recessive allele (sRNA from Ah03 on *SCR29*, score =18.5; sRNA
520 from Ah04 on *SCR20*, score=20; and sRNA from Ah10 on *SCR20*,
521 score =21; Figure 5a). Examining these targets in detail did not
522 reveal mismatches at the 10-11th nucleotide position, suggesting
523 that mismatches at other positions have rendered these sRNA-
524 target interactions inactive (Figure 5a). Another exception
525 concerns the observed low score (15.5) for the best match between
526 a sRNA from the dominant allele Ah04 and a target at *SCR* from
527 the recessive Ah03 allele (Figure 5b). Whether Ah04 silences *SCR*
528 from Ah03 through this unusual target or through another elusive
529 mechanism remains to be discovered. In spite of the generally very
530 low expression of all recessive alleles, we found some evidence
531 that the strength of silencing experienced by a given *SCR* allele

532 may vary across genotypic combinations for a given allele (F-
533 value=2.222, p-value = 0.0756, Table S5i). However, we found no
534 evidence that the position of the target site on the measured allele
535 (promoter; intron; intron-exon boundary *vs.* upstream/downstream)
536 could explain this variation (F-value=1.4432, n.s, TableS5e). The
537 alignment scores obtained in Brassica for *Smi* & *Smi2* on *SCR*
538 sequences show that dominant interactions are also strictly distinct
539 from recessive interactions, but at a threshold score of 16.5, hence
540 lower than that we observed in Arabidopsis (Table S6). Finally, we
541 found no effect of the inferred age of the miRNA on the mean
542 alignment score (mean= 20.41 and 20.22 for recent or ancient
543 miRNAs, respectively; F-value: 0.0362; ns, Table S5j).

544

545 # Discussion

546 Our main objective was to evaluate the base-pairing requirement of
547 the sRNA-target interactions controlling dominance/recessivity
548 interactions between alleles of the allelic series controlling SI in *A.*
549 *halleri*. Determining the base-pairing requirement for sRNA
550 silencing in plants has remained challenging because the "rules"
551 used for target prediction have typically been deduced from
552 observations that conflate distinct microRNA genes and their
553 distinct mRNA targets over different genes. Moreover, detailed
554 evaluations of the functional consequences of mismatches have
555 relied on heterologous reporter systems (typically GFP in transient
556 tobacco assays), hence limiting the scope of the phenotypic
557 consequences that can be studied. Here, we used a genetic system
558 (plant self-incompatibility) where multiple sRNAs regulate target
559 sites on a single gene (*SCR*), and in which we are able to make a
560 direct link between the sRNA-target interactions, the level of *SCR*

561 transcript and the encoded phenotype (dominance/recessivity
562 interaction).

563 The first step was to clarify several aspects of the expression
564 pattern of the genes controlling SI in *A. halleri,* as earlier accounts
565 had suggested that alleles of the allelic series may differ from one
566 another in their expression profile (Kusaba *et al.*, 2002). In line
567 with Kakizaki *et al.*, (2003), Suzuki *et al.*, (1999); Schopfer *et al.*,
568 (1999); Takayama *et al.*, (2000); Shiba *et al.*, (2002),we found
569 maximal expression of *SCR* in early buds but low or no expression
570 at the open flower stage. This expression pattern is consistent with
571 *in situ* hybridization experiments showing that *SCR* transcripts are
572 localized in the tapetum, a specialized layer of cells involved in
573 pollen grains coating (Iwano *et al.*, 2003), which undergoes
574 apoptosis and is quickly degraded as the development of pollen
575 grains inside the anther progresses (Murphy & Ross, 1998;
576 Takayama *et al.*, 2000). We confirmed that differences exist in the
577 temporal dynamics of expression among alleles, as suggested by
578 Kusaba *et al.* (2002) in *A. lyrata*, possibly as the result of strong
579 sequence divergence of the promotor sequences of the different
580 *SCR* alleles. Finally, we confirmed that *SCR* and *SRK* have sharply
581 distinct expression dynamics throughout flower development.
582 Indeed, transcript levels of *SRK* increased steadily along
583 development and were very low in early buds, consistent with the
584 observation that SI can be experimentally overcome to obtain
585 selfed progenies by "bud-pollination" (Llaurens et al. 2009).

586 Based on this clarified transcriptional dynamics, we confirmed the
587 generality of the transcriptional control of dominance for *SCR*. In
588 particular, we observed that even in the few heterozygote
589 genotypes where in our previous study (Durand *et al.*, 2014) no
590 sRNA produced by the phenotypically dominant allele was

591 predicted to target the sequence of the phenotypically recessive
592 *SCR* allele, transcripts from the recessive *SCR* allele were
593 undetected. This suggests either that some functional sRNAs or
594 targets have remained undetected by previous sequencing and/or
595 by our *in silico* prediction procedures, or that mechanisms other
596 than sRNAs may cause transcriptional silencing for some S-allele
597 combinations. In contrast, we confirmed the absence of
598 transcriptional control for *SRK*, for which both alleles were
599 consistently expressed at similar levels in all heterozygote
600 genotypes examined, irrespective of the (pistil) dominance
601 phenotype. For *SRK*, other dominance mechanisms must therefore
602 be acting, which are yet to be discovered (*e.g.* Naithani *et al.*,
603 2007).

604 An important feature of the silencing phenomenon is that the
605 decrease of transcript levels for recessive *SCR* alleles was very
606 strong in heterozygous genotypes, bringing down transcript levels
607 below the limits of detection in most cases. This is in line with the
608 intensity of transcriptional silencing by heterochromatic siRNAs
609 (typically very strong for transposable element sequences, see
610 Marí-Ordóñez *et al.*, 2013), while post-transcriptional gene
611 silencing by microRNAs is typically more quantitative (Liu *et al.*,
612 2014). As a result of this strong decrease of transcript levels, the
613 strength of silencing appeared independent from the position of the
614 sRNA target along the *SCR* gene (promoter *vs.* intron), although
615 we note that our power to distinguish among levels of transcripts
616 of recessive alleles, which were all extremely low, is itself fairly
617 low. It remains to be discovered whether the different positions of
618 the sRNA targets do indeed imply different transcriptional
619 silencing mechanisms (Durand *et al*., 2014).

620 Based on the many allelic combinations where we could compare
621 the agnostic prediction of putative target sites with the level of
622 transcriptional silencing, we find that a simple threshold model for
623 base-pairing between sRNAs and their target sites captures most of
624 the variation in *SCR* expression in heterozygotes. This result
625 provides a direct experimental validation of the *ad-hoc* criteria
626 used in Durand *et al.*, (2014). However, our results also indicate
627 that this quantitative threshold is not entirely sufficient to capture
628 the complexity of targeting interactions. Indeed, in two cases for
629 which the dominance relationship is known, this simple threshold
630 model would inappropriately predict that sRNAs from recessive
631 alleles should be able to target more dominant *SCR* alleles, yet the
632 dominant *SCR* alleles were expressed at normal levels with no sign
633 of silencing in these heterozygote genotypes (Figure 5a). The
634 position of the mismatches on these sRNAs (at position 15 and 18
635 of the sRNA for Ah03 on Ah29, and position 3 and 12 for the
636 others) therefore appear to be sufficient to abolish the function of
637 the targeting interaction. Similarly, a mismatch at position 10 in
638 the *Smi* interaction in Brassica (Tarutani *et al.*, 2010) and in other
639 microRNA-targets interactions (Franco-Zorrilla *et al.*, 2007) was
640 shown to result in loss of function of the interaction (Table S6).
641 Interestingly, quantitative differences may exist between
642 Arabidopsis and Brassica, as the experimentally validated targets
643 in Brassica (Tarutani *et al.*, 2010; Yasuda *et al.*, 2016) correspond
644 to base-pairing threshold below the one that we find in Arabidopsis
645 (*i.e.* a target score of 16.5 seems sufficient for silencing in Brassica
646 *vs.* 18 in Arabidopsis). For Brassica, both class I and class II alleles
647 have *Smi*, but a mismatch at the 10th position was proposed to
648 explain why the class II *Smi* is not functional. Here, we found that
649 this mismatch drives the alignment score under the 16.5 threshold
650 and could be sufficient to explain the loss of function, regardless of

651 its position. Overall, although these small RNAs achieve their
652 function in a way that is sharply different from classical
653 microRNAs (DNA methylation *vs.* mRNA cleavage), our results
654 suggest that the sRNA-target complementarity rules for silencing
655 in both cases are qualitatively consistent (Liu *et al.*, 2014). Better
656 understanding the molecular pathway by which these sRNAs
657 epigenetically silence their target gene (*SCR*) will now be key to
658 determine whether this threshold model can be generalized to more
659 classical siRNAs found across the genome, as evidence is still
660 missing for such classes of sRNAs.

661 The existence of a threshold model has important implications for
662 how the dominance hierarchy can evolve. In fact, our model
663 suggests that a single SNP can be sufficient to turn a codominance
664 interaction into a dominance interaction (and vice-versa), making
665 this a relatively trivial molecular event. This is actually what
666 Yasuda *et al.*, (2016) observed in *B. rapa*, where the combination
667 of single SNPs at the sRNA *Smi2* and its *SCR* target sequences
668 resulted in a linear dominance hierarchy among the four class II S-
669 alleles found in that species. Strikingly, in some cases, we
670 observed base pairing at sRNA-target interactions with very high
671 alignment scores (up to 22), *i.e.* above the threshold at which
672 transcriptional silencing was already complete (score =18). Under
673 our threshold model, such interactions are not expected since
674 complete silencing is already achieved at the threshold, and no
675 further fitness gain is therefore to be expected by acquiring a more
676 perfect target. A first possibility is that these interactions reflect the
677 recent emergence of these silencing interactions. In fact, one of the
678 models for the emergence of new microRNAs in plant genomes
679 involves a partial duplication of the target gene, hence entailing
680 perfect complementarity at the time of origin that becomes
681 degraded over time by the accumulation of mutations (Allen *et al.*,

682 2004). Under this scenario, the higher-than-expected levels of
683 sRNA-target complementarity could reflect the recent origin of
684 these sRNAs but we found no evidence of a difference in
685 alignment score for young *vs.* old microRNAs (Table S5j). A
686 second possibility is that selection for developmental robustness is
687 acting to prevent the phenotypic switch from mono- to bi-allelic
688 expression of *SCR* (especially during stress events, Boukhibar &
689 Barkoulas, 2016) that could be devastating for the plant
690 reproductive fitness. Indeed, we do observe strong variation in
691 overall *SCR* expression when the sRNA target score of the
692 companion allele is below the threshold, and it is possible that
693 under stress conditions the epigenetic machinery may be less
694 efficient, hence requiring stronger base-pairing to achieve proper
695 silencing than in the greenhouse conditions under which we
696 observed them in the present study. Finally, a third possibility is
697 that sRNA-target complementarity above the threshold reflects the
698 pleiotropic constraint of having a given sRNA from a dominant
699 allele control silencing of the complete set of target sequences
700 from the multiple recessive alleles segregating, and reciprocally of
701 having a given *SCR* target in a recessive allele maintaining
702 molecular match with a given sRNA distributed among a variety of
703 dominant alleles. Comparing the complementarity score of
704 sRNA/target interactions among sRNAs or targets that contribute
705 to high versus low numbers of dominance/recessive interactions
706 will now require a more complete depiction of the sRNA-target
707 regulatory network among the larger set of S-alleles segregating in
708 natural populations.

709

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Author Contribution

NB, SS, SB, ACH performed the molecular biology experiments. CP and ES obtained and took care of the plants. SS, IFL and XV provided advice on the experimental strategy and interpretations. NB performed the statistical analyses. VC supervised the work. NB and VC wrote the manuscript.

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912 small RNAs and their targets. *Nature Plants* **16206**: 1–6.

913

914 # Figure legends

915 **Figure 1**: Expression dynamics of **a.** *SCR* and **b.** *SRK* during
916 flower development, from early buds (<0.5mm) to open flowers.
917 For *SCR*, only genotypes in which a given allele was either
918 dominant or co-dominant were included (recessive *SCR* alleles
919 were strongly silenced at all stages and were therefore not
920 informative here). For each allele, $2^{-\Delta Ct}$ values were normalized
921 relative to the developmental stage with the highest expression. For
922 each stage, the thick horizontal line represents the median, the box
923 represents the 1st and 3rd quartiles. The upper whisker extends from
924 the hinge to the largest value no further than 1.5 * Inter Quartile
925 Range from the hinge (or distance between the first and third
926 quartiles). The lower whisker extends from the hinge to the
927 smallest value at most 1.5 * IQR of the hinge and the black dots
928 represents outlier values.

929 **Figure 2**: Expression of individual *SCR* alleles in different
930 genotypic contexts. Pollen dominance status of the S-allele whose
931 expression is measured relative to the other allele in the genotype
932 as determined by controlled crosses are represented by different
933 letters (**D**: dominant; **C**: codominant; **R**: recessive; **U**: unknown;
934 **H**: Homozygote, Table S3). In a few instances, relative dominance

935 status of the two alleles had not been resolved phenotypically and
936 were inferred from the phylogeny (marked by asterisks). Thick
937 horizontal bars represent the median of $2^{-\Delta Ct}$ values, 1st and 3rd
938 quartile are indicated by the upper and lower limits of the boxes.
939 The upper whisker extends from the hinge to the largest value no
940 further than 1.5 * Inter Quartile Range from the hinge (or distance
941 between the first and third quartiles). The lower whisker extends
942 from the hinge to the smallest value at most 1.5 * IQR of the hinge
943 and the black dots represents outlier values. We normalized values
944 relative to the highest median across heterozygous combinations
945 within each panel. Alleles are ordered from left to right and from
946 top to bottom according to their position along the dominance
947 hierarchy, with SCR01 the most recessive and SCR13 and SCR20
948 the most dominant alleles. Under a model of transcriptional control
949 of dominance, high expression is expected when a given allele is
950 either dominant or co-dominant and low expression when it is
951 recessive. Exceptions to this model are marked by black vertical
952 arrows and discussed in the text. "Na" marks homozygote or
953 heterozygote genotypes that were not available.

954 **Figure 3:** Expression of individual *SRK* alleles in different
955 genotypic contexts. Putative pistil dominance status of the S-allele
956 whose expression is measured relative to the other allele in the
957 genotype is represented by different letters (**D**: dominant; **R**:
958 recessive; **U**: unknown; **H**: Homozygote). Note that the pistil
959 dominance hierarchy of the S-allele have been less precisely
960 determined than the pollen hierarchy, and so many of the pairwise
961 dominance interactions were indirectly inferred from the
962 phylogenetic relationships (and marked by an asterisk) rather than
963 directly measured phenotypically. Thick horizontal bars represent
964 the median of $2^{-\Delta Ct}$ values, 1st and 3rd quartile are indicated by the
965 upper and lower limits of the boxes. The upper whisker extends

966 from the hinge to the largest value no further than 1.5 * Inter
967 Quartile Range from the hinge (or distance between the first and
968 third quartiles). The lower whisker extends from the hinge to the
969 smallest value at most 1.5 * IQR of the hinge and the black dots
970 represents outlier values.. We normalized the values for each allele
971 relative to the higher median across heterozygous combination. We
972 normalized values relative to the highest median across
973 heterozygous combinations within each panel. Alleles are ordered
974 from left to right and from top to bottom according to their position
975 in the pistil dominance hierarchy, with SRK01 the most recessive
976 and SRK04 the most dominant allele in our sample, based on the
977 phenotypic determination in Llaurens *et al.* (2008).

978 **Figure 4:** Base-pairing requirements for the transcriptional control
979 of *SCR* alleles by sRNAs suggest a threshold model. **a.** Relative
980 expression of *SCR* alleles as a function of the alignment score of
981 the "best" interaction between the focal allele (including 2kb of
982 sequence upstream and downstream of *SCR*) and the population of
983 sRNAs produced by sRNA precursors of the other allele in the
984 genotype. For each allele, expression was normalized relative to
985 the genotype in which the $2^{-\Delta Ct}$ value was highest. Dots are
986 coloured according to the dominance status of the focal *SCR* allele
987 in each genotypic context (black: dominant; white: recessive; grey:
988 undetermined). The black line corresponds to a local regression
989 obtained by a smooth function (loess function, span=0.5) in the
990 ggplot2 package (Wickham, 2009) and the grey area covers the
991 95% confidence interval. Vertical arrows point to observations that
992 do not fit the threshold model of transcriptional control and are
993 represented individually on Figure 5. **b.** Barplots of the Akaike
994 Information Criteria (AIC) quantifying the fit of the generalized
995 linear model for different target alignment scores used to define
996 functional targets. Lower AIC values indicate a better fit.

997 **Figure 5:** Predicted sRNA/target interactions that do not fit with
998 the documented dominance phenotype or the measured expression.
999 For each alignment, the sequence on top is the sRNA and the
1000 bottom sequence is the best predicted target site on the *SCR* gene
1001 sequence (including 2kb of sequence upstream and downstream of
1002 *SCR*). **a.** sRNA targets with a score above 18, while the S-allele
1003 producing the sRNA is phenotypically recessive over the S-allele
1004 containing the *SCR* sequence. **b.** sRNA target with a score below
1005 18, while the S-allele producing the sRNA (Ah04) is
1006 phenotypically dominant over the S-allele containing the *SCR*
1007 sequence and transcript levels of the *SCR03* allele is accordingly
1008 very low. This is the best target we could identify on SCR03 for
1009 sRNAs produced by Ah04.

1010

1011 **Supplementary figures**

1012 **Figure S1.** Validation of the *SCR* qPCR primers. "Positive
1013 control" corresponds to amplification with the Master Mix
1014 containing primers for *SCR* alleles that are present in the cDNA
1015 used. For the "Cross Amplification" assay, we used a Master Mix
1016 on cDNAs that do not contain alleles corresponding to the primer
1017 pair used. "Water": master mix with water instead of cDNA. Thick
1018 horizontal bars represent the median of $2^{-\Delta Ct}$ values, 1st and 3rd
1019 quartile are indicated by the upper and lower limits of the. The
1020 upper whisker extends from the hinge to the largest value no
1021 further than 1.5 * Inter Quartile Range from the hinge (or distance
1022 between the first and third quartiles). The lower whisker extends
1023 from the hinge to the smallest value at most 1.5 * IQR of the hinge
1024 and the black dots represents outlier values.

1025 **Figure S2:** qPCR amplification (non-transformed *Ct* values) in
1026 serial dilutions for each *SCR* (**a**) and *SRK* (**b**) allele. Solid lines are

1027 the linear regressions over all *Ct* values. Dashed lines are linear
1028 regressions excluding the highest dilution level.

1029 **Figure S3.** Generalized linear mixed model used to test the effect
1030 of developmental stage and dominance status on the expression of
1031 *SCR* alleles (*Ct* values). The distribution shows that the residues of
1032 the full model are approximately normally distributed when taking
1033 allele identity, developmental stage and dominance status into
1034 account and using a logarithmic transformation of the Ct_{SCR} /Ct_{actin}
1035 ratios.

1036 **Figure S4.** Expression of individual *SCR* alleles in different
1037 genotypic contexts, representing each biological and clone
1038 replicate separately. Symbols on top of the boxes indicate
1039 measures from identical clone replicates. See legend of Figure 2
1040 for a full description.

1041 **Table S1**. *SCR* samples analysed for each S-locus genotype,
1042 showing the number of biological and clone replicates over the
1043 four developmental stages sampled. "Allele 1" refers to the first
1044 allele noted in the genotype (for example in the S1S2 genotype,
1045 "allele 1" is S1 and "allele 2" is S2).

1046 **Table S2**: *SRK* samples analysed for each S-locus genotype,
1047 showing the number of biological and clone replicates over the
1048 four developmental stages sampled. The alleles are named
1049 accordingly to the Table S1.

1050 **Table S3**: Dominance relationships between alleles from the
1051 different genotypes included in this study as determined by
1052 controlled crosses.

1053 **Table S4:** qPCR primer sequences for each *SCR* and *SRK* alleles
1054 studied.

1055 **Table S5:** Detailed results from the generalized linear mixed
1056 models. **a**. Decomposition of the sources of variance across allele
1057 identity and the hierarchical levels biological, clones and technical
1058 replicates for *SCR*. **b**. Test of the variation of expression dynamic
1059 across *SCR* alleles. **c**. Test of the dominance and stage effects on
1060 *SCR* transcript levels, showing a significant interaction. **d**.
1061 Comparison of the fit of the model under different base-pairing
1062 score thresholds. **e**. Test of the effect of the position of the target
1063 on the strength of silencing. **f**. Decomposition of the source of
1064 variance across the technical replicates and the allele identity for
1065 *SRK*. **g**. Test of the variation of expression dynamic across *SRK*
1066 alleles. **h.** Test of the effect of stage and dominance on *SRK*
1067 transcript levels. **i**. Test of the effect of the identity of the
1068 companion allele on *SCR* transcript levels. **j**: Test of the effect of
1069 age on alignment score above the threshold of 18.

1070 **Table S6:** sRNA and target identified as the best match for every
1071 pair of alleles for *SCR*. Ct_{SCR}/Ct_{actin} ratios are given for the target
1072 allele in the interaction, calculated from the mean of Ct_{SCR}/Ct_{actin}
1073 ratios across the two earliest developmental stages (buds below
1074 1mm, see Figure 1). The positions of the targets are given relative
1075 to the beginning of the closest exon of *SCR* for targets upstream
1076 from the gene or in the intron), and relative to the stop codon for
1077 downstream targets. R: Recessive; D: dominant; H: homozygote.

Figure 1

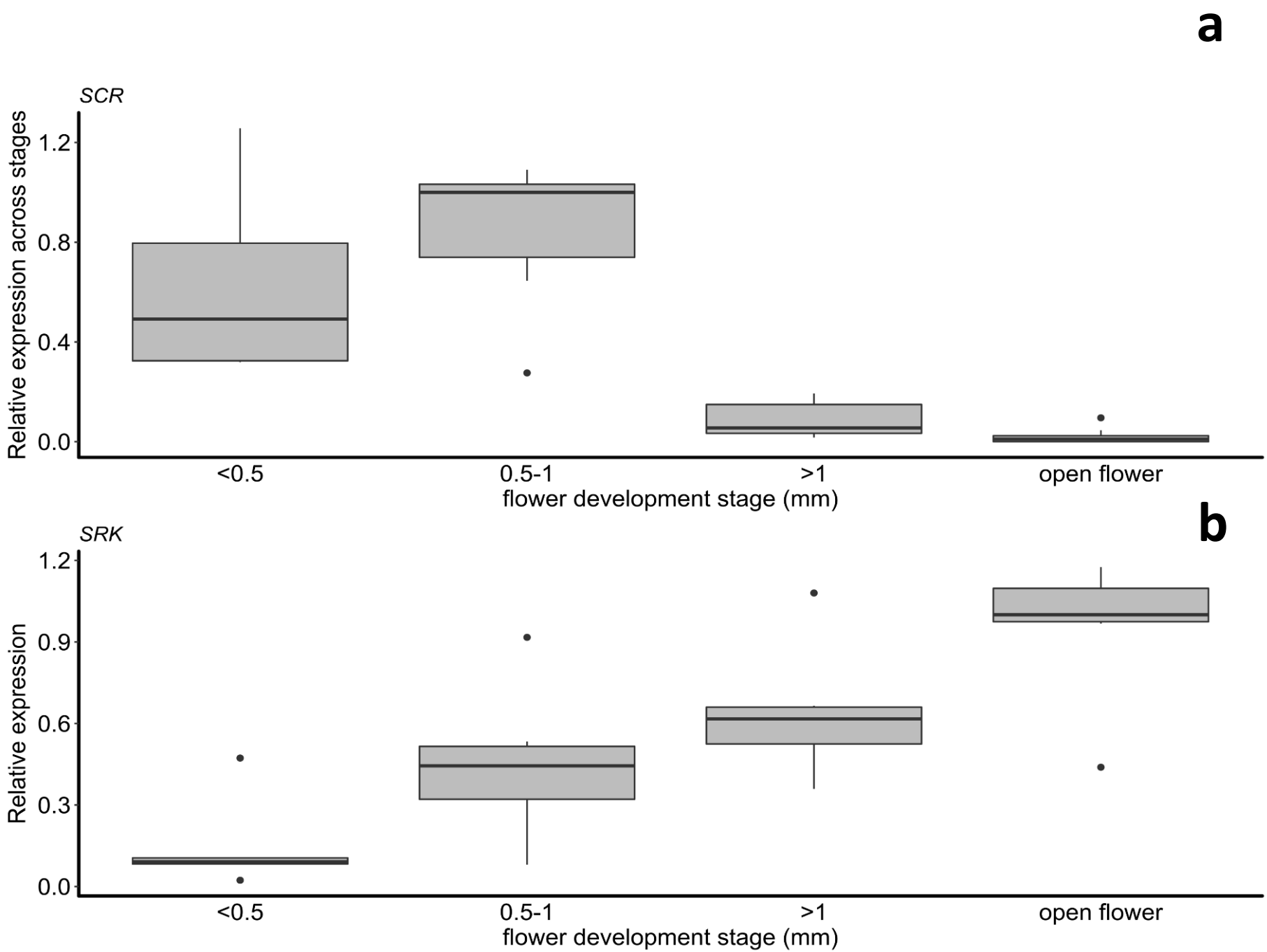
a
SCR
Relative expression across stages
1.2
0.8
0.4
0.0
<0.5
0.5-1
>1
open flower
flower development stage (mm)
b
SRK
Relative expression
1.2
0.9
0.6
0.3
0.0
<0.5
0.5-1
>1
open flower
flower development stage (mm)

Figure 2

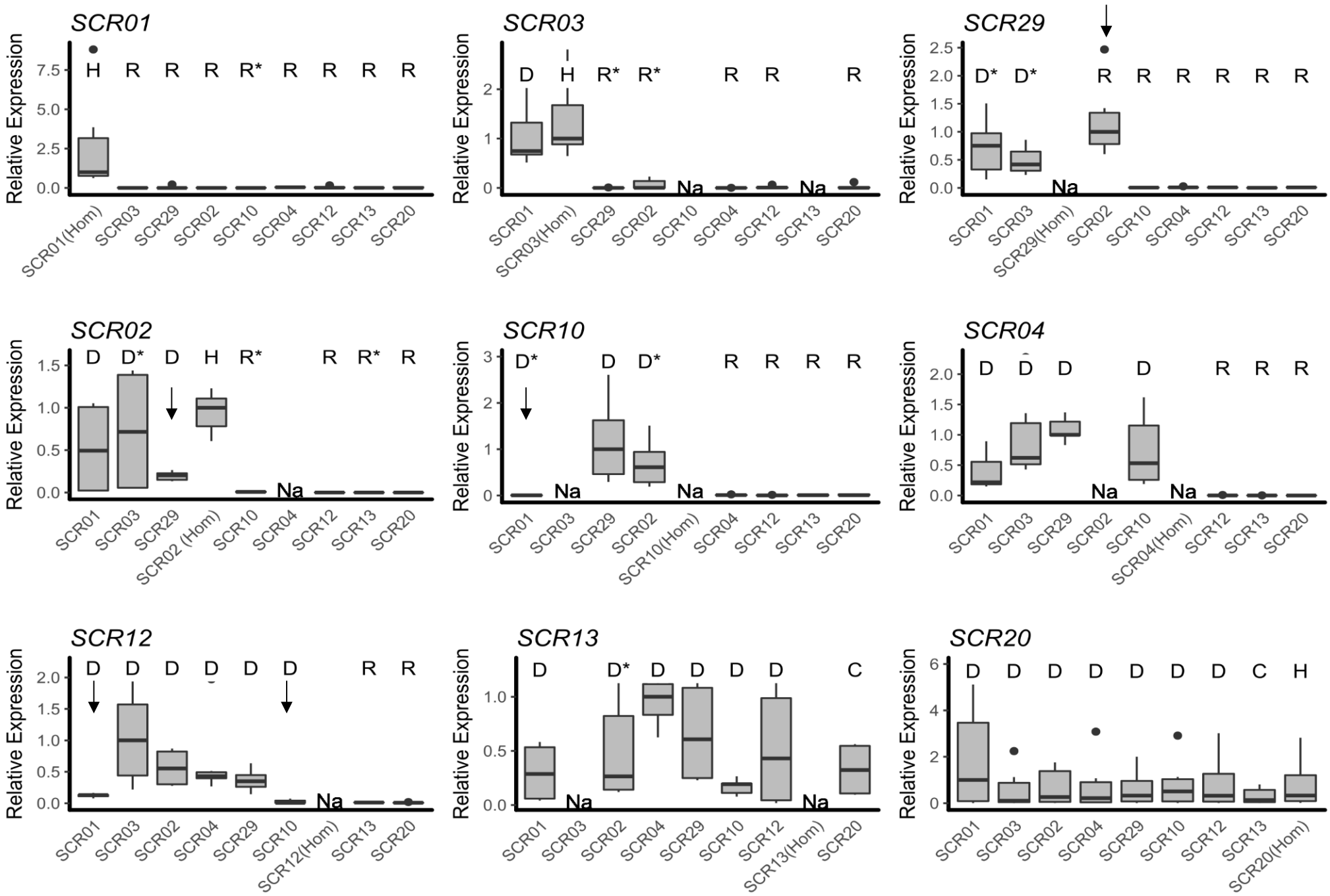
SCR01
Relative Expression
H R R R R* R R R R
SCR01(Hom) SCR03 SCR29 SCR02 SCR10 SCR04 SCR12 SCR13 SCR20
SCR03
D H R* R* R R R
Na Na
SCR01 SCR03(Hom) SCR29 SCR02 SCR10 SCR04 SCR12 SCR13 SCR20
SCR29
D* D* R R R R R R
Na
SCR01 SCR03 SCR29(Hom) SCR02 SCR10 SCR04 SCR12 SCR13 SCR20
SCR02
D D* D H R* R R* R
Na
SCR01 SCR03 SCR29 SCR02 (Hom) SCR10 SCR04 SCR12 SCR13 SCR20
SCR10
D* D D* R R R R
Na Na
SCR01 SCR03 SCR29 SCR02 SCR10(Hom) SCR04 SCR12 SCR13 SCR20
SCR04
D D D D R R R
Na Na
SCR01 SCR03 SCR29 SCR02 SCR10 SCR04(Hom) SCR12 SCR13 SCR20
SCR12
D D D D D D R R
Na
SCR01 SCR03 SCR02 SCR04 SCR29 SCR10 SCR12(Hom) SCR13 SCR20
SCR13
D D* D D D D C
Na Na
SCR01 SCR03 SCR02 SCR04 SCR29 SCR10 SCR12 SCR13(Hom) SCR20
SCR20
D D D D D D D C H
SCR01 SCR03 SCR02 SCR04 SCR29 SCR10 SCR12 SCR13 SCR20(Hom)

Figure 3

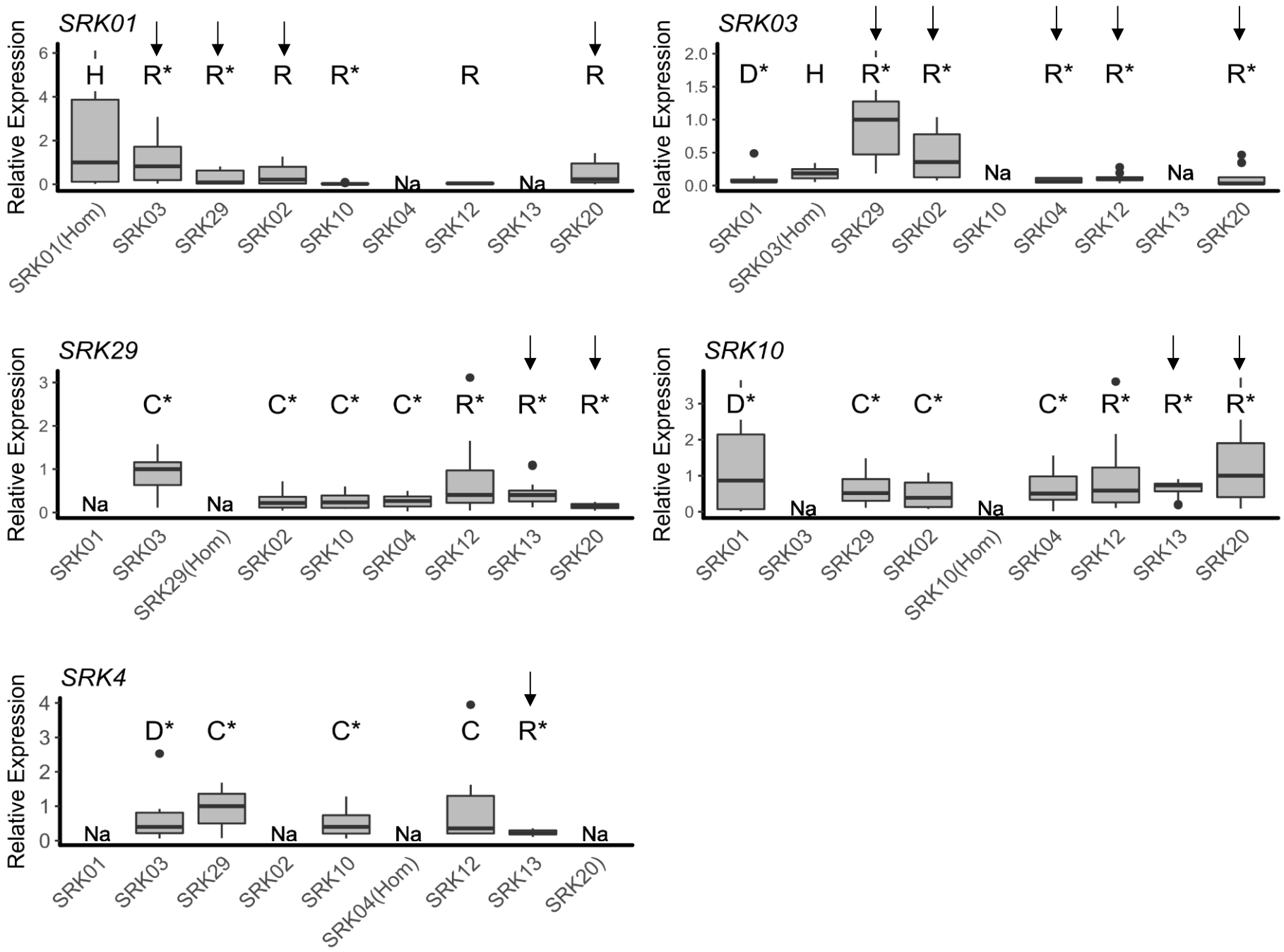
SRK01
Relative Expression
H
R*
R*
R
R*
R
R
Na
Na
SRK01(Hom)
SRK03
SRK29
SRK02
SRK10
SRK04
SRK12
SRK13
SRK20
SRK03
D*
H
R*
R*
R*
R*
R*
Na
Na
SRK01
SRK03(Hom)
SRK29
SRK02
SRK10
SRK04
SRK12
SRK13
SRK20
SRK29
C*
C*
C*
C*
R*
R*
R*
Na
Na
SRK01
SRK03
SRK29(Hom)
SRK02
SRK10
SRK04
SRK12
SRK13
SRK20
SRK10
D*
C*
C*
C*
R*
R*
R*
Na
Na
SRK01
SRK03
SRK29
SRK02
SRK10(Hom)
SRK04
SRK12
SRK13
SRK20
SRK4
D*
C*
C*
C
R*
Na
Na
Na
Na
SRK01
SRK03
SRK29
SRK02
SRK10
SRK04(Hom)
SRK12
SRK13
SRK20)

Figure 4

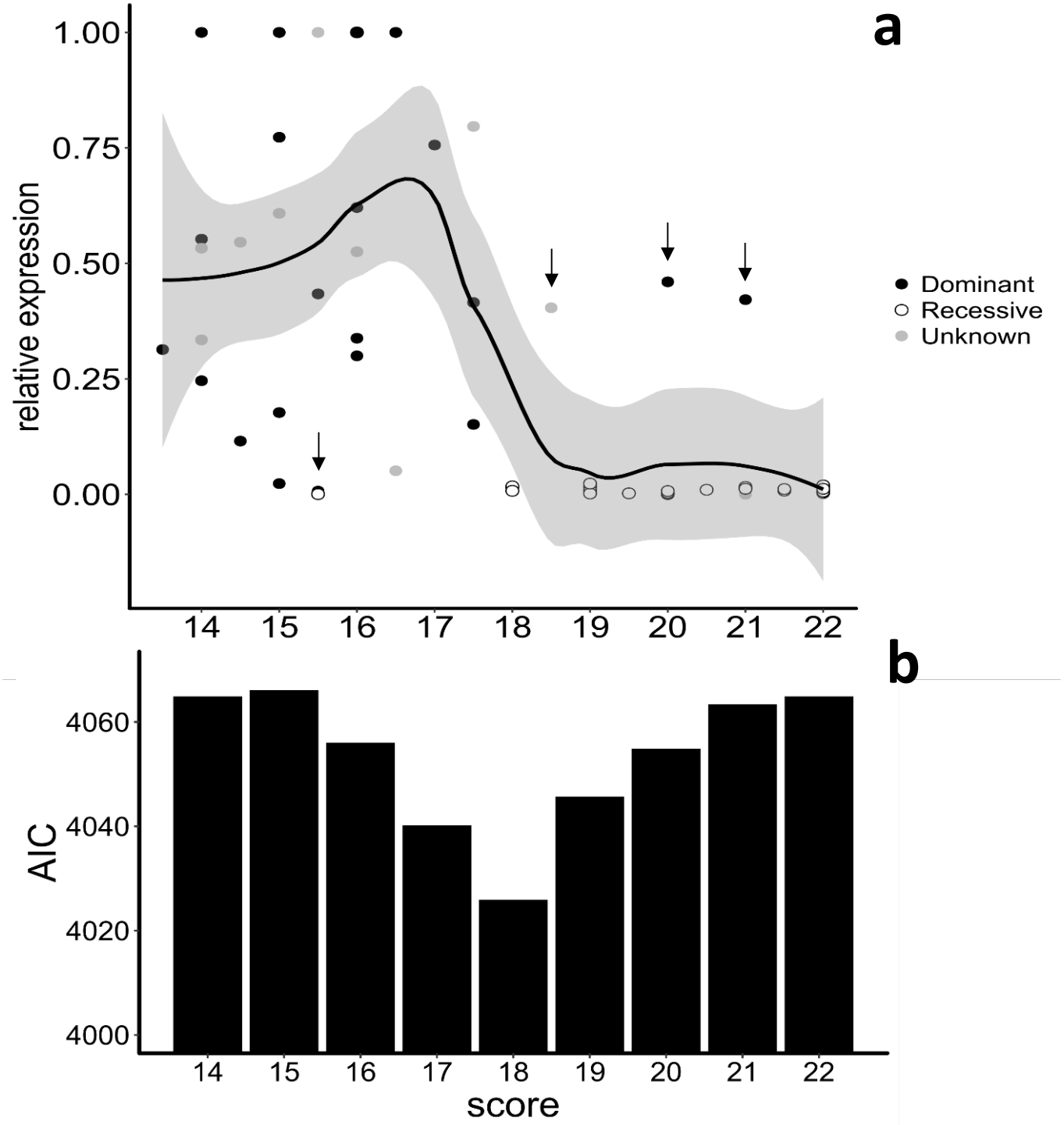
a
1.00
0.75
0.50
0.25
0.00
relative expression
14
15
16
17
18
19
20
21
22
Dominant
Recessive
Unknown
b
4060
4040
4020
4000
AIC
14
15
16
17
18
19
20
21
22
score

Figure 5

a

```
Ah03 MirS3    3' UACAAGUUCCAUUAUAUAAUCGAA 5'
                 ||||||||||||||x||x|||o||
Ah29 target   5' ATGTTCAAGGTAATTTACTAGTTT 3'
Score = 18.5


Ah04 Mir4239  3' CCUCGUACACCUUUAUUGCCUUUG 5'
                 |||x|||||||x||||||||||||
Ah20 target   5' GGAACATGTGGCAATAACGGAAAC 3'
Score = 20


Ah10 Mir4239  3' CCUCGUACACCUUUAUUGCCUUUGU 5'
                 |||x|||||||x||||||||||||||
Ah20 target   5' GGAACATGTGGCAATAACGGAAACA 3'
Score = 21
```

b

```
Ah04 MirS4    3' GUAUGAUUCUUGUUAGAUUCA 5'
                 ~~~||||||||o||||||||~
Ah03 target   5' ACTACTAAGAATAATCTAAGA 3'
Score = 15,5
```

Figure S1

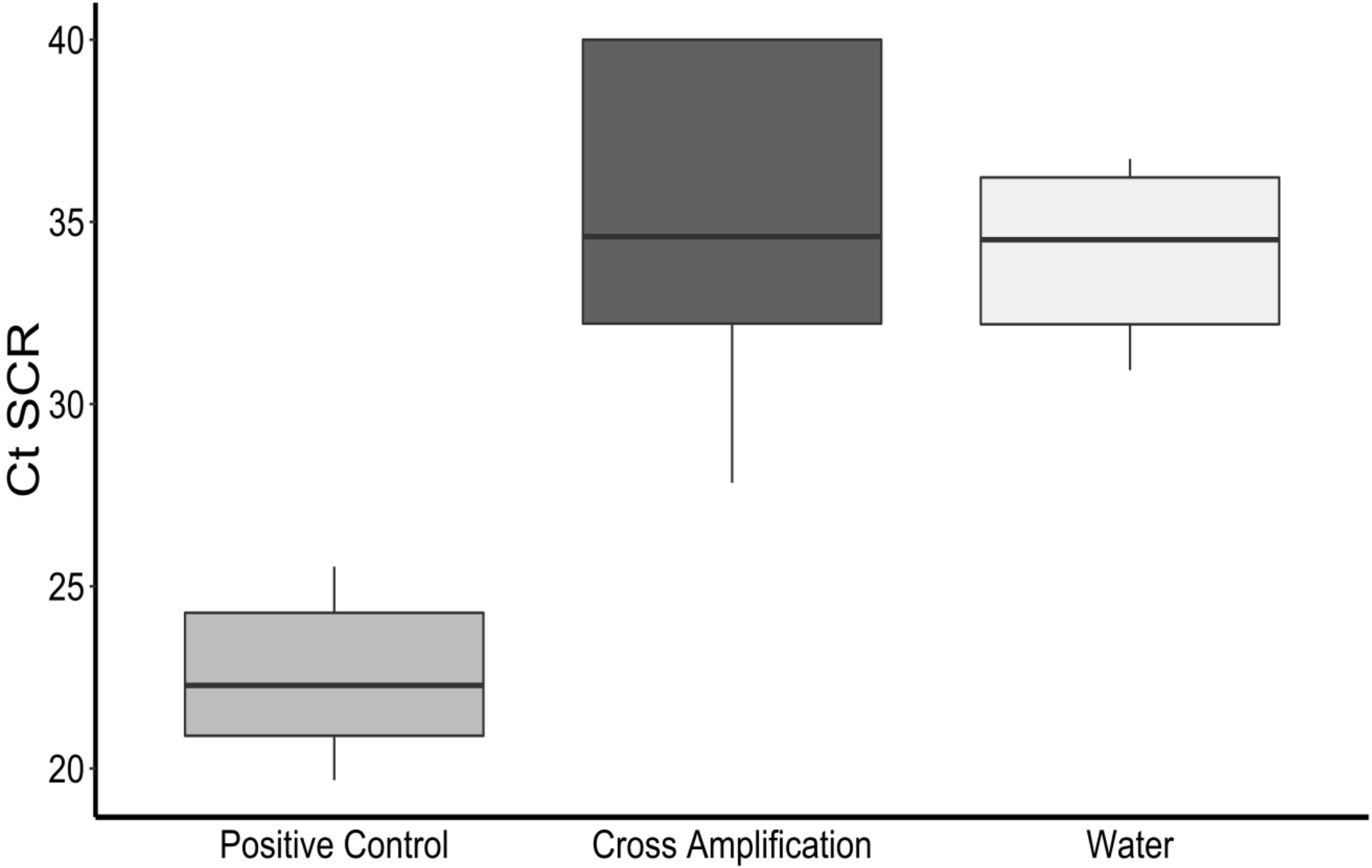
Ct SCR
40
35
30
25
20
Positive Control
Cross Amplification
Water

Figure S2

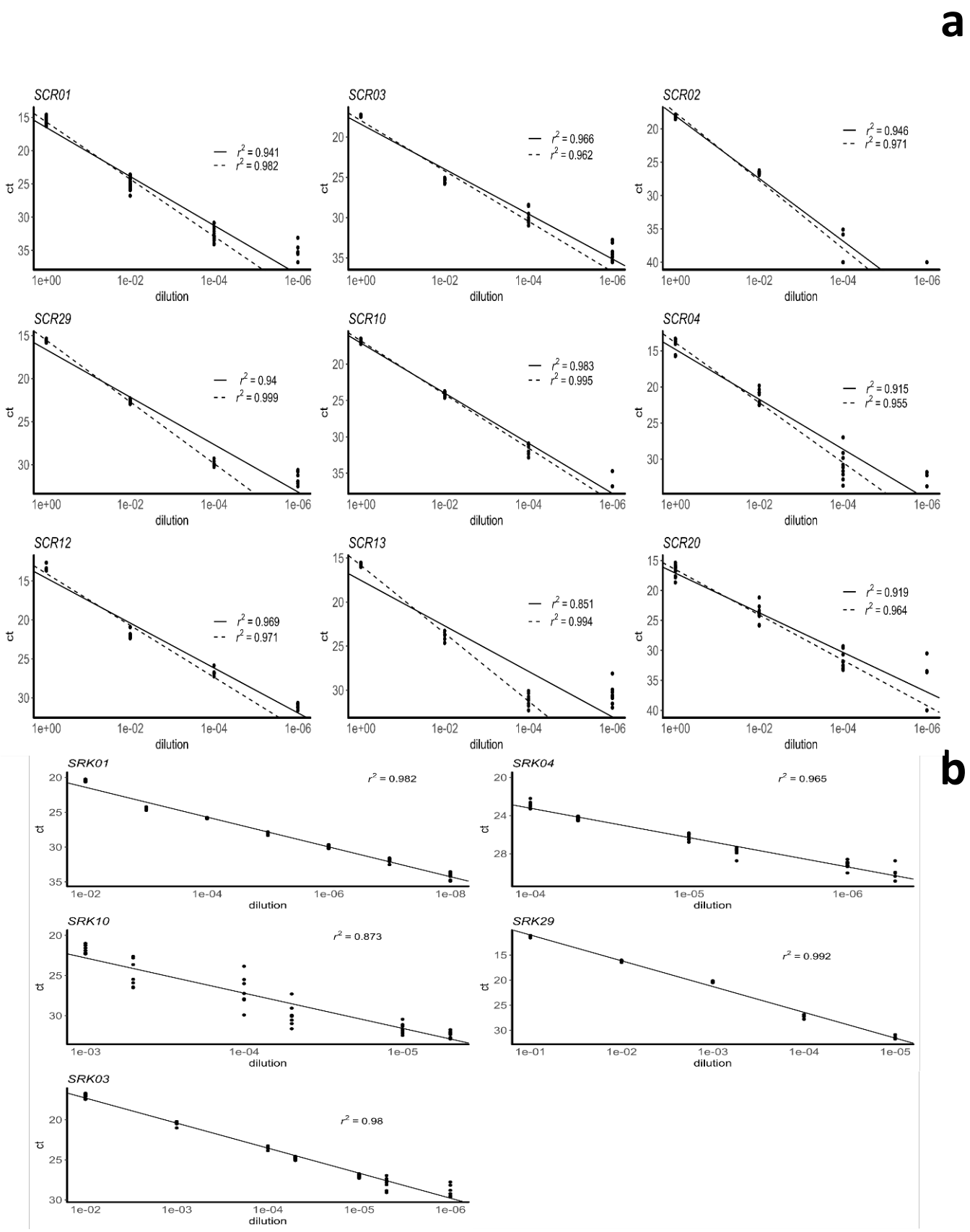
a
SCR01
r2 = 0.941
r2 = 0.982
SCR03
r2 = 0.966
r2 = 0.962
SCR02
r2 = 0.946
r2 = 0.971
SCR29
r2 = 0.94
r2 = 0.999
SCR10
r2 = 0.983
r2 = 0.995
SCR04
r2 = 0.915
r2 = 0.955
SCR12
r2 = 0.969
r2 = 0.971
SCR13
r2 = 0.851
r2 = 0.994
SCR20
r2 = 0.919
r2 = 0.964
ct
dilution
b
SRK01
r2 = 0.982
SRK04
r2 = 0.965
SRK10
r2 = 0.873
SRK29
r2 = 0.992
SRK03
r2 = 0.98

Figure S3

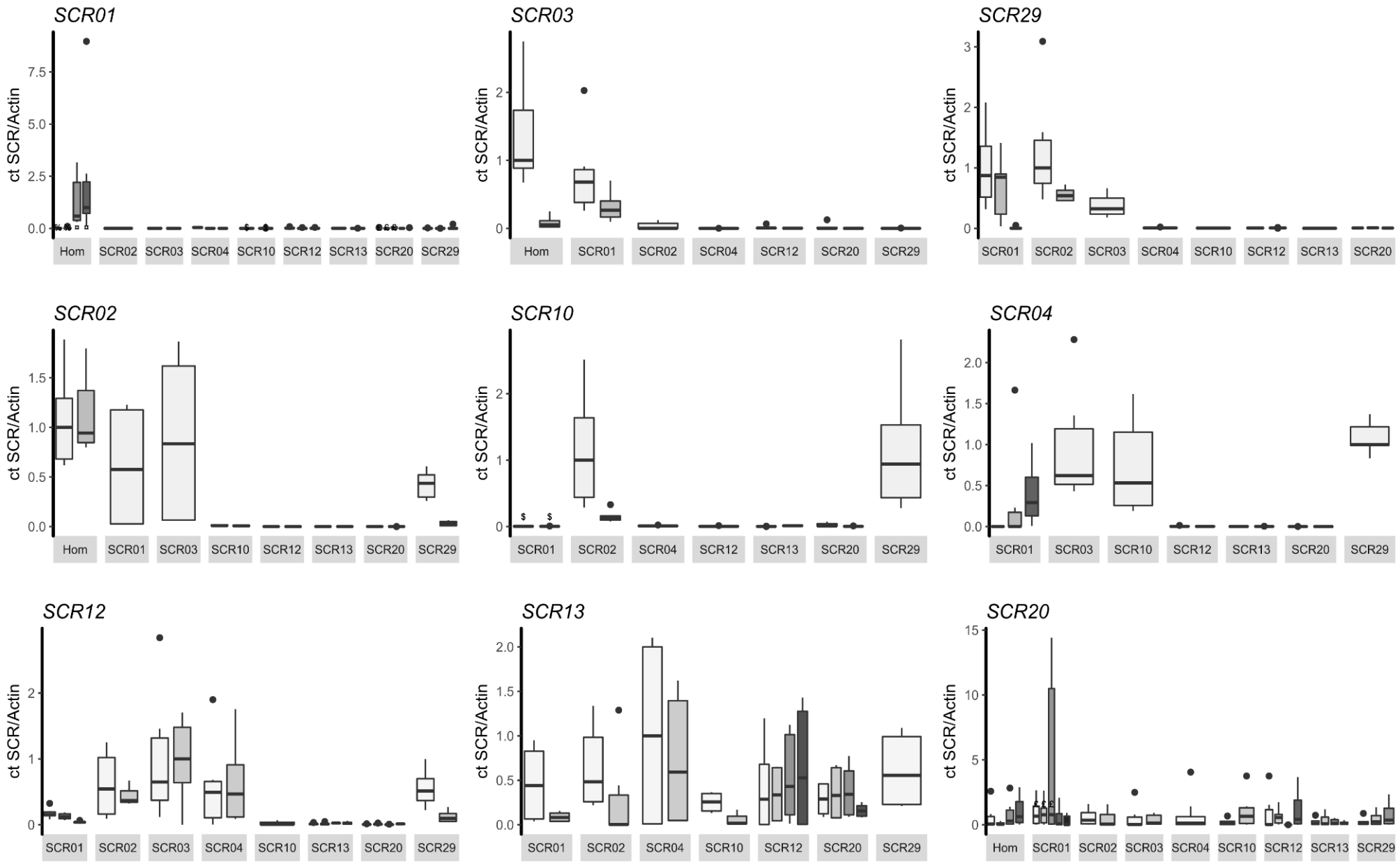
SCR01
ct SCR/Actin
Hom
SCR02
SCR03
SCR04
SCR10
SCR12
SCR13
SCR20
SCR29
SCR03
SCR29
SCR02
SCR10
SCR04
SCR12
SCR13
SCR20

Figure S4

```
lmer(log(s1$Ct_SCR.actine) ~ (1|allele_measured:stage)
+stage*dom_phenotype+
(1|replicatBiol_genotype/replicat_Techclone), na.action=na.omit)
```

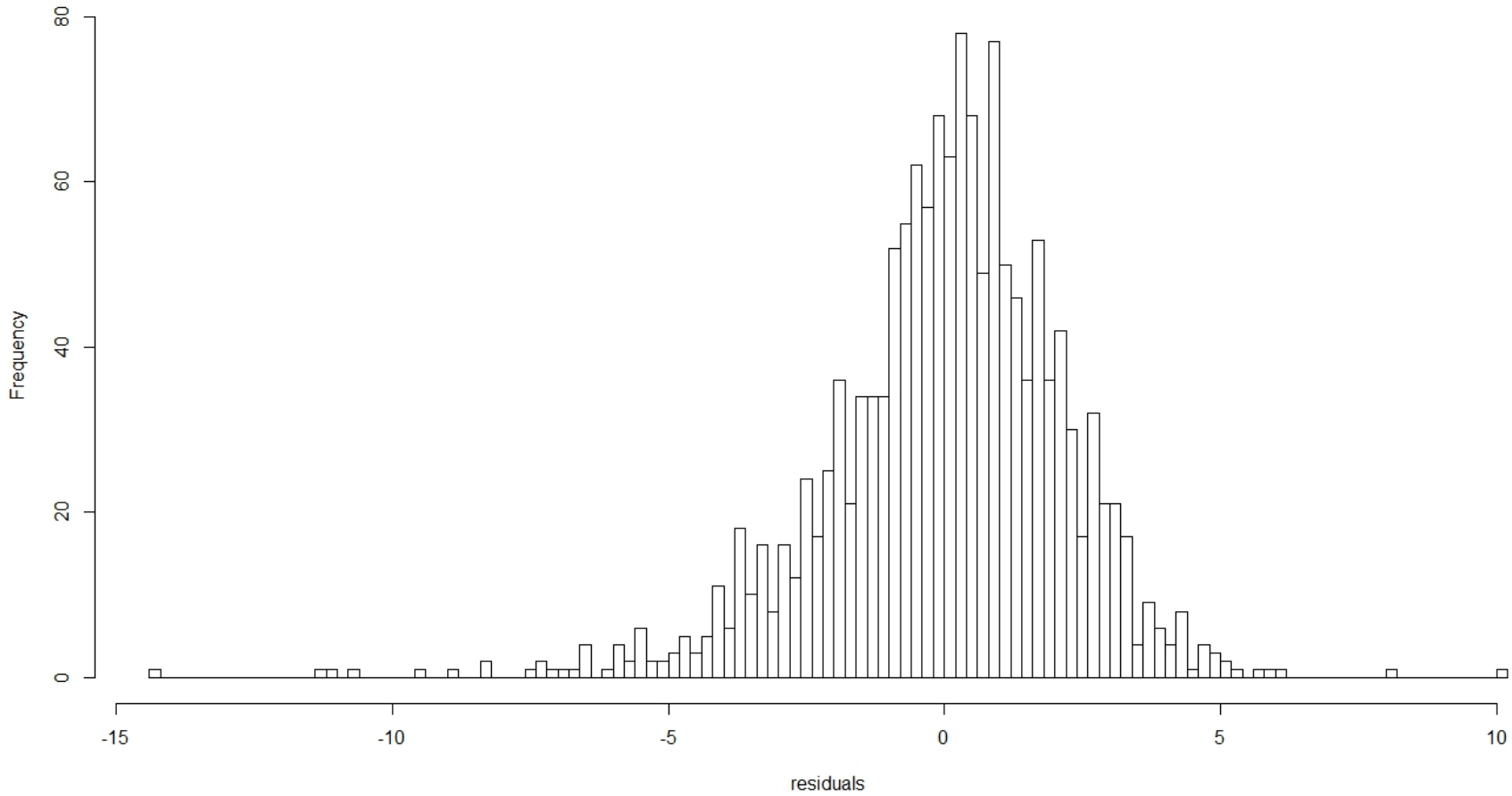

Table S1. SCR samples analysed for each S-locus genotype, showing the number of biological and clone replicates over the four developmental stages sampled. "Allele 1" refers to the first allele noted in the genotype (for example in the S1S2 genotype, "allele 1" is S1 and "allele 2" is S2).

	number of biological replicates	number of expression measure		
		allele 1	allele 2	actin
S1S1	2$	48	48	48
S1S2	1	12	12	12
S1S3	2	24	24	24
S1S4	3	36	36	36
S1S10	1$	24	24	24
S1S12	3	36	36	36
S1S13	2	24	24	24
S1S20	3£	60	60	60
S1S29	3	36	36	36
S2S2	2	24	24	24
S2S3*	2	18	18	18
S2S10	2	24	24	24
S2S12	2	24	24	24
S2S13	2	24	24	24
S2S20	2	24	24	24
S2S29	2	24	24	24
S3S3	2	24	24	24
S3S4	1	12	12	12
S3S12	2	24	24	24
S3S20*	3	30	30	30
S3S29	1	12	12	12
S4S10	1	12	12	12
S4S12	2	24	24	24
S4S13	2	24	24	24
S4S20	2	24	24	24
S4S29	1	12	12	12
S10S12	1	12	12	12
S10S13	2	24	24	24
S10S20	2	24	24	24
S10S29	1	12	12	12
S12S13	4	48	48	48
S12S20	4	48	48	48
S12S29	2	24	24	24
S13S20	4	48	48	48
S13S29	1	12	12	12
S20S20	4	48	48	48
S20S29	3	36	36	36

*: only the stages C and D were sampled for one of the biological replicates

$: two clone replicates per biological replicate

£: two of the three biological replicates are represented by two clone replicates

Table S2: SRK samples analysed for each S-locus genotype, showing the number of biological and clone replicates over the four developmental stages sampled. The alleles are named accordingly to the Table S1.

	number of expression measure		
	allele 1	allele 2	actin
S1S1	12	-	11
S1S2	10	-	12
S1S3	11	-	12
S1S10	9	12	11
S1S12	11	12	12
S1S13	11	-	12
S1S20	8	-	9
S1S29	10	-	9
S3S3	12	-	12
S3S4	8	-	12
S3S12	12	11	12
S3S20	12	-	12
S3S29	12	12	12
S4S10	12	12	12
S4S12	12	9	9
S4S13	8	-	12
S4S29	12	11	12
S10S2	12	-	12
S10S12	11	-	12
S10S13	12	-	12
S10S20	12	-	12
S10S29	12	12	12
S12S20	12	-	12
S12S29	11	12	12
S29S2	12	-	12

Table S3: Dominance relationships between alleles from the different genotypes included in this study as determined by controlled crosses.

pollen genotype	pistil phenotype	number of compatible crosses	dominance phenotype in pollen	reference	dominance phenotype in pistil	reference
S1S1	-	-	-	Durand et al. 2014	-	-
S1S2	[S1]	-	S2>S1	Llaurens et al. 2008	S2>S1	Llaurens et al. 2008
S1S2	[S2]	-		Llaurens et al. 2008		
S1S3	[S1]	-	S3>S1	Durand et al. 2014	-	-
S1S3	[S3]	-		Durand et al. 2014		-
S1S4	[S1]	-	S4>S1	Durand et al. 2014	S4>S1	Llaurens et al. 2008
S1S4	[S4]	-		Durand et al. 2014		
S1S10	[S1]	-	NA	-	-	-
S1S10	[S10]			-		-
S1S12	[S1]	-	S12>S1	Durand et al. 2014	S12>S1	Llaurens et al. 2008
S1S12	[S12]	-		Durand et al. 2014		
S1S13	[S1]	-	S13>S1	Durand et al. 2014	-	-
S1S13	[S13]	-		Durand et al. 2014		-
S1S20	[S1]	-	S20>S1	Durand et al. 2014	S20>S1	Llaurens et al. 2008
S1S20	[S20]	-		Durand et al. 2014		
S1S29	[S1]	-	NA	-	-	-
S1S29	[S29]			-		-
S2S3	[S2]	-	NA	-	-	-
S2S3	[S3]	-		-		-
S2S4	[S2]	-	S4>S2	Llaurens et al. 2008	S4>S2	Llaurens et al. 2008
S2S4	[S4]	-		Llaurens et al. 2008		
S2S10	[S2]	-	NA	-	-	-
S2S10	[S10]	-		-		-
S2S12	[S2]	-	S12>S2	Llaurens et al. 2008	S12>S2	Llaurens et al. 2008
S2S12	[S12]	-		Llaurens et al. 2008		
S2S13	[S2]	-	NA	-	-	-
S2S13	[S13]	-		-		-
S2S20	[S2]	-	NA	-	-	-
S2S20	[S20]	-		-		-
S2S29	[S2]	0/5	S2>S29	This study	-	-
S2S29	[S29]	4/7		This study		-
S3S4	[S3]	-	S4>S3	Durand et al. 2014	-	-
S3S4	[S4]	-		Durand et al. 2014		-
S3S10	[S3]	-	S10>S3	Durand et al. 2014	-	-
S3S10	[S10]	-		Durand et al. 2014		-
S3S12	[S3]	-	S12>S3	Durand et al. 2014	-	-
S3S12	[S12]	-		Durand et al. 2014		-
S3S13	[S3]	-	S13>S3	Durand et al. 2014	-	-
S3S13	[S13]	-		Durand et al. 2014		-
S3S20	[S3]	-	S20>S3	Durand et al. 2014	-	-
S3S20	[S20]	-		Durand et al. 2014		-
S3S29	[S3]	-	S29>S3	-	-	-
S3S29	[S29]	-		-		-
S4S10	[S4]	-	S4>S10	Durand et al. 2014	-	-
S4S10	[S10]	-		Durand et al. 2014		-
S4S12	[S4]	-	S12>S4	Durand et al. 2014	S12>S4	Llaurens et al. 2008
S4S12	[S12]	-		Durand et al. 2014		
S4S13	[S4]	-	S13>S4	Durand et al. 2014	-	-
S4S13	[S13]	-		Durand et al. 2014		-
S4S20	[S4]	-	S20>S4	Durand et al. 2014	S20>S4	Llaurens et al. 2008
S4S20	[S20]	-		Durand et al. 2014		

S4S29	[S4]	-	S4>S29	Durand et al. 2014	-	-
S4S29	[S29]	-		Durand et al. 2014		-
S10S12	[S10]	5/5	S12>S10	This study	-	-
S10S12	[S12]	0/5		This study		-
S10S13	[S10]	-	S13>S10	Durand et al. 2014	-	-
S10S13	[S13]	-		Durand et al. 2014		-
S10S20	[S10]	-	S20>S10	Durand et al. 2014	-	-
S10S20	[S20]	-		Durand et al. 2014		-
S10S29	[S10]	1/5	S10>S29	This study	-	-
S10S29	[S29]	3/3		This study		-
S12S13	[S12]	-	S13>S12	Durand et al. 2014	-	-
S12S13	[S13]	-		Durand et al. 2014		-
S12S20	[S12]	-	S20>S12	Durand et al. 2014	S20>S12	Llaurens et al. 2008
S12S20	[S20]	-		Durand et al. 2014		
S12S29	[S12]	-	S12>S29	Durand et al. 2014	-	-
S12S29	[S29]	-		Durand et al. 2014		-
S13S20	[S13]	-	S13=S20	Durand et al. 2014	-	-
S13S20	[S20]	-		Durand et al. 2014		-
S13S29	[S13]	-	S13>S29	Durand et al. 2014	-	-
S13S29	[S29]	-		Durand et al. 2014		-
S20S29	[S20]	-	S20>S29	Durand et al. 2014	-	-
S20S29	[S29]	-		Durand et al. 2014		-

Table S4: qPCR primer sequences for each *SCR* and *SRK* alleles studied.

		5' Primer Sequence 3'
SCR01	F	AAGTGGAAGCTTGGACGAGA
	R	AAAAGTCGTATCTGCGATTG
SCR02	F	GTCTTTCCTTATAAGCCATGG
	R	GGGGACCCAAGAGATTATGC
SCR03	F	CACATAAAAGAATCATGAAGTCTGC
	R	AATGACAGTGGCAAAGTCGC
SCR04	F	GACGTGTTGTTTTGTTCATGGG
	R	GGCGAGAGGGTCTGAAATTC
SCR10	F	CTCATTGTTTTCTTCACAAGCC
	R	GCGAATGTAAAGATGTTGATGGGG
SCR12	F	CGCTTGTTTTGTGTCACG
	R	GCTTTTAACAGAAACCAGGG
SCR13	F	AGACGTGCTACATTGTTCATAGT
	R	GAGACGGAAACTACAACTGCA
SCR20	F	GACATAGAAGTTCAGAAGGCGC
	R	TGCCGCTGTCAAGTTAATAGAG
SCR29	F	CATGTCTTTGCTTATAAGCC
	R	GCTGGTCGTCGATATTGCCG
SRK01	F	TCAGATTGGCGGCTTCTGAG
	R	TGGAAACAGAAGCAAGCAAGG
SRK03	F	AGGAATGTGAGGAGAGGTGC
	R	GGGCAACAACAACAGTAGGA
SRK04	F	CGGAGAGTTTCGAGATATCCG
	R	GGGTGGTAATGTCAAGTGGG
SRK10	F	ACTTGGGCTGGAAGAATGTG
	R	AGGAAACACAAGCGAGCAAG
SRK12	F	ATGGATGCGATTGTGGACAG
	R	CATTGGTTTGGTAGTTGGAATCA
SRK29	F	CCGAAATTATGCTGCCGATGG
	R	CTTGTGAGTTTCATCATGTACTGGT

Table S5: Detailed results from the generalized linear mixed models.

a. Decomposition of the sources of variance across allele identity and the hierarchical levels biological, clones and technical replicates for *SCR*.

```
lmer(log(Ct_SCR.actine) ~ stage*dom_sRNA_prediction+(1|allele_measured:stage)+(1|replicatBiol_genotype)+(1|replicat_Techclone), na.action=na.omit)
```

Random effects:	Groups	Name	Variance	Std.Dev.
	replicat_Techclone	(Intercept)	0.4091	0.6396
	replicatBiol_genotype	(Intercept)	1.0795	1.0390
	allele_measured:stage	(Intercept)	4.5674	2.1372
	Residual (Technical replicates)		6.0815	2.4461

---

b. Test of the variation of expression dynamic across *SCR* alleles.

```
model 1:  lmer(log(Ct_SCR.actine) ~ stage*dom_phenotype+(1|replicatBiol_genotype/replicat_Techclone))
model 2 : lmer(log(Ct_SCR.actine) ~ stage*dom_phenotype+(1|allele_measured:stage)+(1|replicatBiol_genotype/replicat_Techclone), na.action=na.omit)
```

	Df	AIC	BIC	logLik	deviance	Chisq Chi	Df	Pr(>Chisq)	
model 1:	11	6946.0	7004	-3462.0	6924.0				
model 2:	12	6730.6	6794	-3353.3	6706.6	217.32	1	< 2.2e-16	***

---

c. Test of the dominance and stage effects on *SCR* transcript levels, showing a significant interaction.

```
lmer(log(Ct_SCR.actine)~(1|allele_measured:stage)+stade*dom_phenotype+(1|replicatBiol_genotype/replicat_Techclone), na.action=na.omit
```

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
stade	213.51	71.17	3	27.53	13.805	1.107e-05	***
dom_phenotype	814.70	814.70	1	302.35	158.025	< 2.2e-16	***
stade:dom_phenotype	596.73	198.91	3	1358.37	38.582	< 2.2e-16	***

---

d. Comparison of the fit of the model under different base-pairing score thresholds.

```
lmer(log(Ct_SCR.actine) ~ (1|allele_measured:stage)+stage+Dom_score_based_xx+(1|replicatBiol_genotype/replicat_Techclone) , na.action=na.omit)
```

	Df	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)	
Dom_score_based_14	23	4064.9	4175.5	-2009.5	4018.9				
Dom_score_based_15	24	4066.1	4181.5	-2009.0	4018.1	0.8294	1	0.3624	
Dom_score_based_16	24	4056.0	4171.5	-2004.0	4008.0	10.0691	0	<2e-16	***
Dom_score_based_17	24	4040.2	4155.6	-1996.1	3992.2	15.8111	0	<2e-16	***
Dom_score_based_18	24	4025.9	4141.3	-1988.9	3977.9	14.3695	0	<2e-16	***
Dom_score_based_19	24	4045.7	4161.1	-1998.9	3997.7	0.0000	0	1.0000	
Dom_score_based_20	24	4054.9	4170.3	-2003.5	4006.9	0.0000	0	1.0000	
Dom_score_based_21	24	4063.4	4178.8	-2007.7	4015.4	0.0000	0	1.0000	
Dom_score_based_22	24	4064.9	4180.3	-2008.5	4016.9	0.0000	0	1.0000	

---

e. Test of the effect of the position of the target on the strength of silencing.

```
lmer(log(Ct_SCR.actine)~allele_measured:stage+stage+position_target+(1|allele_measured/replicatBiol_genotype/replicat_Techclone), na.action=na.omit)
```

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)
position_target	27.019	6.755	4	33.275	1.4432	0.241609

---

f. Decomposition of the source of variance across the technical replicates and the allele identity for *SRK*.

```
lmer(log(Ct_SRK.actine) ~ stade*dom_phenotype+(1|allele_measured:stade)+(1|replicat_Techclone), na.action=na.omit)
```

Random effects:	Groups	Name	Variance	Std.Dev.
	allele_measured:stade	(Intercept)	3.1451	1.7734
	replicat_Techclone	(Intercept)	0.6974	0.8351
	Residual		0.8089	0.8994

---

g. Test of the variation of expression dynamic across *SRK* alleles.

```
model 1 :lmer(log(Ct_SRK.actine) ~ stade*dom_phenotype+(1|replicatBiol_genotype/replicat_Techclone) , na.action=na.omit)
model 2 :lmer(log(Ct_SRK.actine) ~ (1|allele_measured:stade)+stade*dom_phenotype+(1|replicatBiol_genotype/replicat_Techclone), na.action=na.omit)
```

	Df	AIC	BIC	logLik	deviance	Chisq Chi	Df	Pr(>Chisq)	
model 1:	11	280.74	307.07	-129.37	258.74				
model 2:	12	275.82	304.56	-125.91	251.82	6.9103	1	0.00857	**

---

h. Test of the effect of stage and dominance on *SRK* transcript levels.

```
lmer(log(Ct_SRK.actine) ~ (1|allele_measured:stade)+stade*dom_phenotype+(1|replicatBiol_genotype/replicat_Techclone), na.action=na.omit)
```

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
stade	2.0520	0.6840	3	3.346	0.8456	0.546472	
dom_phenotype	5.5723	5.5723	1	3.442	6.8884	0.068244	.
stade:dom_phenotype	10.7038	3.5679	3	64.867	4.4107	0.006943	**

---

i. Test of the effect of the identity of the companion allele on *SCR* transcript levels.

```
lmer(log(Ct_SCR.actine) ~ (1|allele_measured:stade)+stage+Other_allele_inGenot+(1|replicatBiol_genotype/replicat_Techclone) , na.action=na.omit)
```

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
Other_allele_inGenot	52.061	10.412	5	32.843	2.2217	0.07558	.

---

j: Test of the effect of age on alignment score above the threshold of 18.

```
lm(score ~ age)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
age	1	0.066	0.06586	0.0362	0.8504
Residuals	29	52.708	1.81753		

Table S6: sRNA and target identified as the best match for every pair of alleles for SCR. CtSCR/Ctactin ratios are given for the target allele in the interaction, calculated from the mean of CtSCR/Ctactin ratios across the two earliest developmental stages (buds below 1mm, see Figure 1). The positions of the targets are given relative to the beginning of the closest exon of SCR for targets upstream from the gene or in the intron), and relative to the stop codon for downstream targets. R: Recessive; D: dominant; H: homozygote.

targeting allele	targeting sRNA precursor	targeted allele	dominance phenotype	Best predicted sRNA/target interaction	score	CtSCR /Ctactin	target location	relative target position	
								from	to
Ah01	Ah01_Mir4239			sRNA 3' AAAAUAAAAGCAAAAACGACGUA 5'					
				~~||||||--|||||x||||||	14			
		Ah01	H	target 5' TATTATTTT--TTTTTACTGCAT 3'		1.1342E-02	promotor	-598	-581
	Ah01_Mir4239			sRNA 3' AAAAU-AAAAGCAAAAACGAC-GUA 5'					
				~~||-|||||-||||||o||-||~	13.5				
		Ah02	D	target 5' UAUUAUUUUUC-UUUUUGUUGUCAA 3'		1.3053E-01	intron	-196	-177
	Ah01_Mir4239			sRNA 3' AAAAUA-AAAGCAAAAAC-GACGUA 5'					
				||||-|-||||||||||-||~~~~	15				
		Ah03	D	target 5' UUUU-UCUUUCGUUUUUGUCUAUUU 3'		2.8473E-02	promotor	-505	-486
	Ah01_Mir4239			sRNA 3' AAAUAAAAGCAAA-A-ACGACGUAC-UA 5'					
				||||x||||-|||-|-|-||-||||-||	14			
		Ah04	D	target 5' UUUACUUUC-UUUAUAU-CU-CAUGUAU 3'		1.2298E-01	promotor	-517	-493
	Ah01_Mir4239			sRNA 3' AGCAAAAACG-A-CGUAC-UAGGU--UCC 5'					
				||x|||||||-|-|o|||-|||||--|||	15.5			
		Ah10	D	target 5' UCAUUUUUGCAUCGUAUGUAUCCAUCAGG 3'		2.8487E-04	exon	103	131
	Ah01_Mir4239			sRNA 3' AAAAUAAA-AGCAAA-AA-CGACGUA 5'			2		
				||||||||-|x||||-||-|o|-|||	14.5			
		Ah12	D	target 5' UUUUAUUUGUAGUUUAUUAGUU-CAU 3'		2.1209E-02	downstream	530	554
	Ah01_Mir4239			sRNA 3' AG-CAAAAACGACGUACUAGGUUCC 5'					
				||-|||||||||x|o||---|||||	13.5			
		Ah13	D	target 5' UCUGUUUUUGCUACGUG---CAAGG 3'		2.0399E-02	downstream	1940	1961
	Ah01_Mir4239			sRNA 3' UCGUACGCCGUUUUUGCCUAUGUU 5'					
				~~||||o|||||x||||||x|||	16.5			
		Ah20	D	target 5' CACAUGUGGCAAUAACGGAAACAA 3'		2.0770E-01	exon	55	76
	Ah01_Mir4239			sRNA 3' AGCAAAAACGACGUACUA-GGUUCC 5'					
				~~|||||x||||||-||-|||||~	15			
		Ah29	D	target 5' UUUUUUUUUCUGCAU-AUGCCAAGA 3'		4.9834E-02	downstream	910	929
Ah02	Ah02_Mir1887			sRNA 3' GUGAAUCUAACAAGAAUCAUACUC 5'					
				~~|||||||||||||||x|||||~	19			
		Ah01	R	target 5' CUCUUAGAUUGUUCUUACUAUGAA 3'		5.4584E-05	promotor	-59	-39
	Ah02_MirS3			sRNA 3' AACUAACCAAUGAGCAUUUU-CA 5'					
				|||||x|x|||-|||||||-||	15			
		Ah02	H	target 5' UUGAUUUGCUAC-CGUAAAACGU 3'		2.3912E-01	upstream	-1545	-1524
	Ah02_Mir1887			sRNA 3' GUGAAUCUAACAAGAAUCAUACUC 5'					
				~~||||||||x||||||||o|x||	16.5			
		Ah03	R	target 5' CUCUUAGAUUAUUCUUAGUGUAAG 3'		1.8790E-03	promotor	-108	-87

```
        Ah02_MirS3                      sRNA  3'      AACUAACCAAUGAGCAUUUUCA      5'
                                                        |||||||||||||x|--||||~~          14
                    Ah04        D     target 5'      UUGAUUGGUUACAC--AAAAAA      3'          Na                 intron              -60    -43

        Ah02_MirS3                      sRNA  3'      AACUAACCAAUGAG-CAUUUUCA      5'
                                                        |||||||||||||o-|-||||~~         15.5
                    Ah10        D     target 5'      UUGAUUGGUUACUUAG-AAAACU      3'       4.1045E-02            intron              -56    -37

        Ah02_MirS3                      sRNA  3'     UUUAA-ACG-CUUAUU-GGUUAGUGU    5'
                                                     |||||-|x|-||||||-|||||||~~         16
                    Ah12        D     target 5'     AAAUUAUUCAGAAUAAGCCAAUCAGA    3'       9.6584E-02            intron             -601   -578

       Ah02_Mir1887                     sRNA  3'     GUGAAUCUAA-CA-AGAAUCAUACUC    5'
                                                     ~||||-||||-||-|||||||~~~~~         14
                    Ah13        D     target 5'     AACUU-GAUUAGUAUCUUAGUCACAA    3'       3.4676E-02          downstream            203    221

        Ah02_MirS3                      sRNA  3'    UUUAAA-CGCUUAU-U--G-GUUAGUGU   5'
                                                    ||||||-||x||||-|--|-||||||~~         14
                    Ah20        D     target 5'    AAAUUUAGCCAAUAUAUGCUCAAUCAAA   3'       6.9471E-02          downstream           1611   1636

       Ah02_Mir1887                     sRNA  3'      GUGAAUCUAACAAGAAUCAUACUC     5'
                                                      |o|||||||x|||||||||o|~~~         16
                    Ah29        R     target 5'      CGCUUAGAUAGUUCUUAGUGUAAU     3'       8.1926E-02           promotor             -53    -33

Ah03    Ah03_MirS3                      sRNA  3'      UACAAGUUCCAUUAUAUAAUCGAA     5'
                                                      |||||||||||||||||||x|||||         22
                    Ah01        R     target 5'      AUGUUCAAGGUAAUAUAUGAGCUU     3'       4.2927E-05             exon               59     82

        Ah03_MirS3                      sRNA  3'     AGUUCCAUUAUAUAAUC-GAAGAAA     5'
                                                     ||||||||||x||x|||-o||||||         17.5
                    Ah02        D     target 5'     UCAAGGUAAUUUACUAGUUUUCUUU     3'       1.9047E-01     intron-exon boundary      -19      5

        Ah03_MirS5                      sRNA  3'     AUACGAAAGUACAGA--AAAAAAAG     5'
                                                     ||||o||||||||-|--|||||||~         16.5
                    Ah03        H     target 5'     UAUGUUUUCAUGU-UGGUUUUUUUA     3'       3.6834E-02             exon               -21      1

        Ah03_MirS3                      sRNA  3'     ACA-AGUU-CCAUUAUAUAAUCGAAG    5'
                                                     |||-||||-||-||||||x||-|||~         15
                    Ah04        D     target 5'     UGUGUCAAUGG-AAUAUACUA-CUUU    3'       5.0001E-01            intron            -1221  -1199

        Ah03_MirS5                      sRNA  3'      AAAA-AAG-GUUCAGUACAAUUUC     5'
                                                      ||||-|||-||||x|||x||||||         16
                    Ah10        D     target 5'      UUUUCUUCACAAGCCAUAUUAAAG     3'          Na                 exon               84    107

        Ah03_MirS3                      sRNA  3'      ACAAGUUCCAUUAUAUAAUCGAAG     5'
                                                      |||||||||||||-|-x||-||||         16
                    Ah12        D     target 5'      UGUUCAAGGUAAU-U-CUA-CUUC     3'       1.8389E-01           promotor            -569   -549

        Ah03_Mirs3                      sRNA  3'     AGUUCCAUUAUAUAA-U-CGAAGAAA    5'
                                                     |||||||||||||||-|-x|||o|||         18.5
                    Ah13        D     target 5'     UCAAGGUAAUAUAUUAACACUUUUUU    3'          Na          intron-exon boundary       63     88

        Ah03_MirS5                      sRNA  3'     AUACGAAAGUACAGAA-AAA--AAAG    5'
                                                     |||-||||x|||||||-|||--||||         16
                    Ah20        D     target 5'     UAU-CUUUAAUGUCUUGUUUAAUUUC    3'       6.2263E-02          downstream           1478   1502
```

```
          Ah03_MirS3                          sRNA  3'     UACAAGUUCCAUUAUAUAAUCGAA      5'
                                                           ||||||||||||||x||x|||o||          18.5
                          Ah29        R      target 5'     AUGUUCAAGGUAAUUUACUAGUUU      3'        3.3086E-02       intron-exon boundary        -8     15

Ah04      Ah04_MirS4                          sRNA  3'     UAU-ACUUUUUUC-UUUUUUCU-UU      5'
                                                           |||-||-||||||-||||||||-||          17
                          Ah01        R      target 5'     AUAUUG-AAAAAGUAAAAAAGAGAA      3'        1.7497E-04             upstream             -1173  -1150

          Ah04_Mir867                         sRNA  3'    ACAGAAAGGAUUAUUUGGUACAAGUU      5'
                                                           ||||||||||x||||o|||||x||||          20.5
                          Ah02        R      target 5'    UGUCUUUCCUUAUAAGCCAUGGUCAA      3'            Na                   exon                 2     27

          Ah04_MirS4                          sRNA  3'       GUAUGAUUCUUGUUAGAUUCA        5'
                                                             ~~~||||||||o||||||||~          15.5
                          Ah03        R      target 5'       ACUACUAAGAAUAAUCUAAGA        3'        1.8947E-05       intron-exon boundary        -20     3

          Ah04_MirS4                          sRNA  3'   AA-AA-GAAAC-AGUAUUGUGAUAAAGA    5'
                                                          ||-||-|||||-||||||o|||||||~~       18.5
                          Ah04        H      target 5'   UUCUUACUUUGAUCAUAAUACUAUUUAU    3'            Na                   intron              -2057  -2032

          Ah04_MirS4                          sRNA  3'     AUAGGUCUUUGUCGCUAACAAUGA      5'
                                                           ||||||x|||o||o||||||||||          19
                          Ah10        R      target 5'     UAUCCAAAAAUAGUGAUUGUUACU      3'        6.6149E-04             intron               -872   -849

          Ah04_MirS4                          sRNA  3'     UAUAC-UUU-UUUC-UUUUUUCUUU      5'
                                                           ~||x|-|||-||||-||||||||||          16
                          Ah12        D      target 5'     UUAAGAAAAGAAAGAAAAAAAGAAA      3'        1.1418E-01             promotor             -273   -250

          Ah04_MirS4                          sRNA  3'    UAUACUUUUUU--C-UUUUU--UCUUU     5'
                                                          ~||||||||||--|-|||||--|||||         16
                          Ah13        D      target 5'    UUAUGAAAAAAGUGUAAAAAGUAGAAA     3'        6.5069E-02            downstream            1024   1049

        Ah04_Mir4239_copy1                    sRNA  3'     CCUCGUACACCUUUAUUGCCUUUG      5'
                                                           |||x|||||||x||||||||||||          20
                          Ah20        D      target 5'     GGAACAUGUGGCAAUAACGGAAAC      3'        9.5550E-02              exon                 51     74

          Ah04_Mir867                         sRNA  3'    ACAGAAAGGAUUAUUUGGUACAAGUU      5'
                                                          |||||||x||x||||o||||||||||         20.5
                          Ah29        R      target 5'    UGUCUUUGCUUAUAAGCCAUGUUCAA      3'        8.0761E-04              exon                 40     65

Ah10      Ah10_Mir1887                        sRNA  3'     GGAGUAUGAUUCUUGUUAGAUUCA      5'
                                                           |o|||||x|||||||||||||||~          19.5
                          Ah01        R      target 5'     CUUCAUAGUAAGAACAAUCUAAGA      3'        2.5148E-05             promotor             -61    -39

          Ah10_Mir867                         sRNA  3'     AGAAAGGAAUAUUCGGUACAAGUU      5'
                                                           |||||||||||||||||||x||||          22
                          Ah02        R      target 5'     UCUUUCCUUAUAAGCCAUGGUCAA      3'        2.0406E-03              exon                 2     25

          Ah10_Mir1887                        sRNA  3'     GGAGUAUGAUUCUUGUUAGAUUCA      5'
                                                           |||o|x||||||||o||||||||~          18
                          Ah03        R      target 5'     CCUUACACUAAGAAUAAUCUAAGA      3'            Na                   promotor             -109   -87

          Ah10_Mir867                         sRNA  3'     GCAACUUUUAACAAAUAUUCCU-UU      5'
                                                           ~~||x|||||x|||||||||||-||          17
                          Ah04        R      target 5'     UUUUCAAAAUGGUUUAUAAGGAGAA      3'        3.7813E-01              exon                 90    112

          Ah10_Mir4239                        sRNA  3'    UUGUUUC-UUACGUUUGUUCUG-GAA      5'
```

```
                                                  |||||||-||||--|||||-|o-|||          14.5
                    Ah10        H     target 5'   AACAAAGAAAUG--AACAA-AUGCUU    3'          Na                 dowstream          136    158

        Ah10_Mir867                     sRNA  3'    AGAAAGGAAUAUUCGGUACAAGUU     5'
                                                    ||||||o||-|||xox||||||||          15
                    Ah12        D     target 5'   UCUUUCUUU-UAAAUGAUGUUCAA    3'       4.2743E-03            promotor           -555   -533

        Ah10_Mir4239                    sRNA  3'    UUGUUUCUUAC-GUUUGUUCUGGAA    5'
                                                    |||-|||||||-|o||||x||o||~          15
                    Ah13        D     target 5'   AAC-AAGAAUGACGAACACGAUCUC    3'       1.1520E-02           downstream          714    736

        Ah10_Mir4239                    sRNA  3'    CCUCGUACACCUUUAUUGCCUUUGU    5'
                                                    |||x|||||||x|||||||||||||          21
                    Ah20        D     target 5'   GGAACAUGUGGCAAUAACGGAAACA    3'       8.7536E-02              exon              51     75

        Ah10_Mir867                     sRNA  3'    AGAAAGGAAUAUUCGGUACAAGUU     5'
                                                    |||||x|||||||||||||||||||          22
                    Ah29        R     target 5'   UCUUUGCUUAUAAGCCAUGUUCAA    3'       4.2462E-04              exon              42     65

Ah12    Ah12_Mirs3                      sRNA  3'    UUAGUUUUGGAUUUCCUCCAACUAU    5'
                                                    ||||||||||x||||||x|||||||          21
                    Ah01        R     target 5'   AAUCAAAACCAAAAGGAUGUUGAUA    3'       1.8177E-04             intron            -238   -215

        Ah12_MirS3                      sRNA  3'    UUUAGUUUUGGAUUUCCUCCAACUA    5'
                                                    |||||||||||x||||||x||||||          21
                    Ah02        R     target 5'   AAAUCAAAACCAAAAGGAUGUUGAU    3'       6.0314E-05             intron            -940   -916

        Ah12_MirS3                      sRNA  3'    UUAGUUUUGGAUUUCCUCCAACUAU    5'
                                                    |||||||||o|||||||x|||||||          21.5
                    Ah03        R     target 5'   AAUCAAAACUUAAAGGAUGUUGAUA    3'       4.1965E-04             intron            -211   -187

        Ah12_MirS3                      sRNA  3'    UUAGUUUUGGA-UUUCCUCCAACUAU    5'
                                                    ||||||||-||-||||||x|||||||          20
                    Ah04        R     target 5'   AAUCAAAA-CUAAAAGGACGUUGAUA    3'       1.6505E-03             intron             -79    -55

        Ah12_MirS3                      sRNA  3'    UUUAGUUUUGGA-UUUCCUCCAACUA    5'
                                                    ~||||||||-||-||||||x||||||          19
                    Ah10        R     target 5'   AAAUCAAAA-CUAAAAGGACGUUGAU    3'       3.2426E-04             intron             -74    -51

        Ah12_MirS3                      sRNA  3'    GACGAAAG-AAAAUCGAAACUAAGC    5'
                                                    ~~~~||||-||||o|||||||||~~          15.5
                    Ah12        H     target 5'   UUUCUUUCUUUUUGGCUUUGAUUGG    3'          Na                 downstream          123    123

        Ah12_MirS3                      sRNA  3'    UUUA-GUUUUGGAUUUCCUCCAACUA    5'
                                                    ~~||-|||||-x|||||||--|||||          14
                    Ah13        D     target 5'   AUAUCCAAAA-AUAAAGGA--UUGAU    3'       3.5946E-02            promotor           -179   -159

        Ah12_MirS3                      sRNA  3'   UUUAGUUUUGGA-UUUCC--UCCAACUA   5'
                                                   ~||||||||-||-|||||--|x||||o|         15.5
                    Ah20        D     target 5'   AAAUCAAAA-CUAAAAGGAUAUGUUGGU   3'       9.0139E-02             intron            -193   -168

        Ah12_MirS3                      sRNA  3'    UUAGUUUUGGA-UUUCCUCCAACUAU    5'
                                                    ||||||||-||-||||||x|||||||          20
                    Ah29        R     target 5'   AAUCAAAA-CUGAAAGGACGUUGAUA    3'       5.8038E-04             intron            -225   -201

Ah13    Ah13_MirS3                      sRNA  3'    CUCAUUGGUUAGUUUUGGAUUUCCU    5'
                                                    ~||||||||||||||||||x||||||          22
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		Ah01	R	target	5'	AAGUAACCAAUCAAAACCAAAAGGA	3'		6.6402E-05	intron	-231	-208
	Ah13_MirS3			sRNA	3'	UAGUUUUGGAUUUCCUUCAACUAU	5'					
						|||||||||x||||||x|||||||		20				
		Ah02	R	target	5'	AUCAAAACCAAAAGGAUGUUGAUA	3'		7.0608E-05	intron	-938	-915
	Ah13_MirS3			sRNA	3'	UAGUUUUGGAUUUCCUUCAACUAU	5'					
						||||||||o||||||x|||||||		20.5				
		Ah03	R	target	5'	AUCAAAACUUAAAGGAUGUUGAUA	3'		Na	intron	-211	-188
	Ah13_MirS3			sRNA	3'	CUCAUUGGUUAGUUUUGGA-UUUCCU	5'					
						~~||||||||||||||-||-||||||		20				
		Ah04	R	target	5'	GUGUAACCAAUCAAAA-CUAAAAGGA	3'		7.3639E-04	intron	-71	-49
	Ah13_MirS3			sRNA	3'	CU-AAAACGACUCAUUGGUUAGUUUUG	5'					
						||-||||-||x|||||||||||||||		21				
		Ah10	R	target	5'	GAGUUUU-CUAAGUAACCAAUCAAAAC	3'		4.9442E-04	intron	-59	-34
	Ah13_MirS2			sRNA	3'	AUAUAUACGCAUUACUGUAUUUGU	5'					
						||||||||||||||||||||~~		22				
		Ah12	R	target	5'	UAUAUAUGCGUAAUGACAUAAAAA	3'		3.5373E-03	promotor	-470	-449
	Ah13_MirS3			sRNA	3'	AAGAAAA-UCGAA-ACUAACCAAU	5'					
						||-||||-||-||-||||||||~		15				
		Ah13	H	target	5'	UU-UUUUGAG-UUCUGAUUGGUUG	3'		Na	intron	-95	-75
	Ah13_MirS3			sRNA	3'	UUAGUUUUGGA-UUUCCU-U-CAACUA	5'					
						||||||||-||-||||||-|-||||o|		17.5				
		Ah20	D	target	5'	AAUCAAAA-CUAAAAGGAUAUGUUGGU	3'		3.1419E-02	intron	-193	-168
	Ah13_MirS3		D	sRNA	3'	UUUGGA-UUUCCUUCAACUAUAUAU	5'					
						|||x||-||||||x|||||||||||		19				
		Ah29	R	target	5'	AAAACUGAAAGGACGUUGAUAUAUA	3'		1.4935E-04	intron	-229	-205
Ah20	Ah20_MirS3			sRNA	3'	AUAGUUGAAGGAAAACCAAAACUAG	5'					
						|||||||x||||||||||||||~		22				
		Ah01	R	target	5'	UAUCAACAUCCUUUUGGUUUUGAUU	3'		1.3491E-04	intron	-239	-216
	Ah20_MirS3			sRNA	3'	AUAGUUGAAGGAAAACCAAAACUAG	5'					
						|||||||x||||||||||||||~		22				
		Ah02	R	target	5'	UAUCAACAUCCUUUUGGUUUUGAUU	3'		1.9359E-05	intron	-938	-915
	Ah20_MirS3			sRNA	3'	AUAGUUGAAGGAAAACCAAAACUAG	5'					
						|||||||x||||||xx||||||||~		18				
		Ah03	R	target	5'	UAUCAACAUCCUUUAAGUUUUGAUU	3'		6.3463E-04	intron	-211	-188
	Ah20_MirS3			sRNA	3'	AUAGUUGAAGGAAAACCAAAACUAG	5'					
						|||||||x|||||||x||||||||~		20				
		Ah04	R	target	5'	UAUCAACGUCCUUUUAGUUUUGAUU	3'		3.0334E-04	intron	-79	-56
	Ah20_MirS3			sRNA	3'	UAGUUGAAGGAAAACCAAAACUAG	5'					
						||||||x|||||||x||||||||~		19				
		Ah10	R	target	5'	AUCAACGUCCUUUUAGUUUUGAUU	3'		9.5621E-04	intron	-74	-52
	Ah20_MirS2			sRNA	3'	CGUCGUAUUGUGCAUUUGUGUAU	5'					
						||||||||||||||||||o||||		21.5				
		Ah12	R	target	5'	GCAGCAUAACACGUAAACGCAUA	3'		1.4779E-03	promotor	-501	-479

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                Ah20_MirS2                          sRNA  3'    CACAAAUAC-ACAAAUUCACAAUAC    5'
                                                            ||-||||||-|-||||||x|||||~          16
                          Ah13        D      target 5'    GU-UUUAUGAU-UUUAAGCGUUAUU    3'        2.1991E-02          downstream          1322   1343

                Ah20_MirS3                          sRNA  3'    GUUG-A-AGGAAAACCAAAACUAGCC   5'
                                                            ||||-|-|||||||x||||||||o|~        17.5
                          Ah20        H      target 5'    CAACAUAUCCUUUUAGUUUUGAUUGU   3'        8.6244E-02           intron             -191   -167

                Ah20_MirS2                          sRNA  3'    CACAAAUACAGAAACACAUAUACA     5'
                                                            ||||||||||x|||-|||||||~~          18
                          Ah29        R      target 5'    GUGUUUAUGUGUUU-UGUAUAUUC     3'        6.2265E-04           promotor           -205   -185

Brassica S44    S44_Smi2                            sRNA  3'    UUCUGUAUGUGCUUAUUUCACACA     5'                    -
                                                            |o||x|x||||||||||o||||||          17
                          S29-pro     R      target 5'    AGGAAACACACGAAUAAGGUGUGU     3'        -           -

                S44_Smi2                            sRNA  3'    UUC-UGUAUGUGCUUAUUUCACACA    5'                    -
                                                            ~||-|||x||||x|||||o||||||        16.5
                          S40-pro     R      target 5'    AAGUACACACACAAAUAAGGUGUGU    3'        -           -

                S44_Smi2                            sRNA  3'    UUCUGUAUGUGCUUAUUUCACACA     5'                    -
                                                            ~~~|||x||||||||||o|||||~        16.5
                          S60-pro     R      target 5'    ACAACACACACGAAUAAGGUGUGA     3'        -           -

                S44_Smi2                            sRNA  3'    UUCUGUAUGUGCUUAUUUCACACA     5'                    -
                                                            |o||o|x||o||||-||||-||||        13.5
                          S44-pro     H      target 5'    AGGAUACACGCGAA-AAAG-GUGU     3'        -           -

Brassica S60    S60_Smi2                            sRNA  3'    UUCUAUAUGUGUUUAUUCCACACA     5'                    -
                                                            |o||x|x||||o||||||||||||
                          S29-pro     R      target 5'    AGGAAACACACGAAUAAGGUGUGU     3'   17   -           -

                S60_Smi2                            sRNA  3'    UUC-UAUAUGUGUUUAUUCCACACA    5'                    -
                                                            ~||-|x|x||||||||||||||||         18
                          S40-pro     R      target 5'    AAGUACACACACAAAUAAGGUGUGU    3'        -           -

                S60_Smi2                            sRNA  3'    UUCUAUAUGUGU--UUAUUCCACACA   5'                    -
                                                            ~~~~||x|||||--|||||||||||~        15
                          S60-pro     H      target 5'    UACAUACACACACGAAUAAGGUGUGA   3'        -           -

                S60_Smi2                            sRNA  3'    UUCUAUAUGUG-UUUAUUCCACACA    5'                    -
                                                            |o||||x||o|-|||-|||||||~~         14
                          S44-pro     D      target 5'    AGGAUACACGCGAAA-AAGGUGUAU    3'        -           -

Brassica S40    S40_Smi2                            sRNA  3'    UUCUAUAUGUGCUUAUUUCACACA     5'                    -
                                                            |o||x|x||||||||||o||||||          17
                          S29-pro     R      target 5'    AGGAAACACACGAAUAAGGUGUGU     3'        -           -

                S40_Smi2                            sRNA  3'    UUC-UAUAUGUGCUUAUUUCACACA    5'                    -
                                                            ~||-|x|x||||x|||||o||||||        14.5
                          S40-pro     H      target 5'    AAGUACACACACAAAUAAGGUGUGU    3'        -           -

                S40_Smi2                            sRNA  3'    UUCUAUAUGUGCUUAUUUCACACA     5'                    -
                                                            ~~~~~~~||||||||||o|||||~        14.5
                          S60-pro     D      target 5'    ACACGAAACACGAAUAAGGUGUGA     3'        -           -
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                S40_Smi2                          sRNA  3'      UUCUAUAUGUGCUUAUUUCACACA      5'                    -
                                                                |o||||x||o||||-||||-||||          15
                            S44-pro       D      target 5'      AGGAUACACGCGAA-AAAG-GUGU      3'        -           -

Brassica S29    S29_Smi2                          sRNA  3'      UUCUAUAUGUGCUUAUUGCACACA      5'                    -
                                                                |o||x|x||||||||||x||||||
                            S29-pro       H      target 5'      AGGAAACACACGAAUAAGGUGUGU      3'  16.5  -           -

                S29_Smi2                          sRNA  3'      UUC-UAUAUGUGCUUAUUGCACACA     5'                    -
                                                                ~||-|x|x||||x|||||x||||||
                            S40-pro       D      target 5'      AAGUACACACACAAAUAAGGUGUGU      3'   14   -           -

                S29_Smi2                          sRNA  3'      UUCUAUAUGUGCUUAUUGCACACA      5'                    -
                                                                ~~~~~~~||||||||||x|||||~
                            S60-pro       D      target 5'      ACACGAAACACGAAUAAGGUGUGA      3'   14   -           -

                S29_Smi2                          sRNA  3'      UUCUAUAUGUGCUUAUUGCACACA      5'                    -
                                                                |o||||x||o||||x||x||||~~
                            S44-pro       D      target 5'      AGGAUACACGCGAAAAAGGUGUAU      3'   13   -           -

Brassica S9     S9-Smi                            sRNA  3'      ACAUUGAUAAAAUGUGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||x||||||||||||          17
                            S29-SP11      R      target 5'      CUAUUCUAUUUCACACGUAAACAU      3'             -                    -

                S9-Smi                            sRNA  3'      ACAUUGAUAAAAUGUGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||x||||||||||||          17
                            S40-SP11      R      target 5'      CUAUUCUAUUUCACACGUAAACAU      3'             -                    -

                S9-Smi                            sRNA  3'      ACAUUGAUAAAAUGUGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||||||||||||||~          18
                            S60-SP11      R      target 5'      CUAUUCUAUUUUACACGUAAACAA      3'             -                    -

                S9-Smi                            sRNA  3'      ACAUUGAUAAAAUGUGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||x||||||||||||          17
                            S44-SP11      R      target 5'      CUAUUCUAUUUCACACGUAAACAU      3'             -                    -

Brassica S60    S60-smi                           sRNA          ACAUUGAUAAAAUGAGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||x||x|||||||||
                            S29-SP11      R      target         CUAUUCUAUUUCACACGUAAACAU      3'   15        -                    -

                S60-smi                           sRNA          ACAUUGAUAAAAUGAGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||x||x|||||||||
                            S40-SP11      R      target         CUAUUCUAUUUCACACGUAAACAU      3'   16        -                    -

                S60-smi                           sRNA          ACAUUGAUAAAAUGAGCAUUUGUA      5'                                  -
                                                                ~~~~~|||||||||x||||||||~
                            S60-SP11      H      target         CUAUUCUAUUUUACACGUAAACAA      3'   15        -                    -

                S60-smi                           sRNA          ACAUUGAUAAAAUGAGCAUUUGUA      5'                                  -
                                                                ~~~~~||||||x||x|||||||||
                            S44-SP11      D      target         CUAUUCUAUUUCACACGUAAACAU      3'   15        -                    -
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