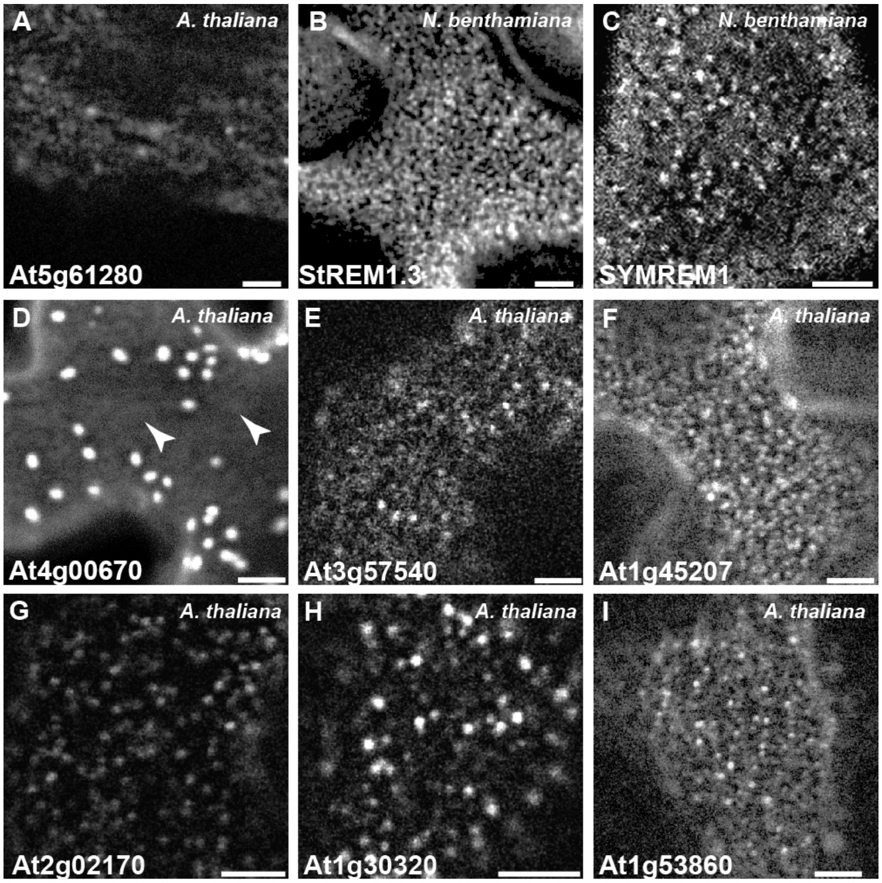


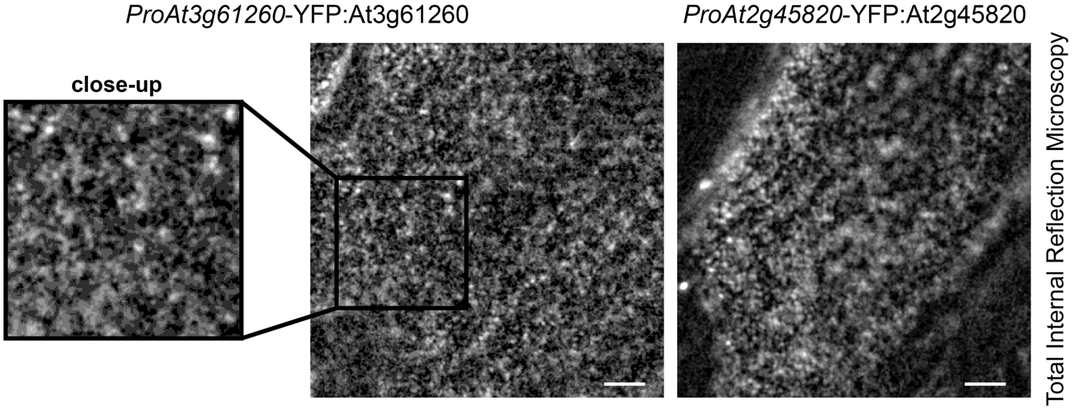
**Supplemental Figure 1 online.**

**Membrane domain localization of different Remorin proteins in *A. thaliana* and *N. benthamiana*.**

**(A)** Expression of At5g61280 under the control of its endogenous promoter in AvrPto DEX inducible *A. thaliana* resulted in labeling of distinct membrane domains. Scale bars indicate 5 µm.

**(B-C)** Heterologous and ectopic expression of the potato REM1.3 (B) and *M. truncatula* SYMREM1 (C) in *N. benthamiana* leaf epidermal cells. Scale bars indicate 5 µm.

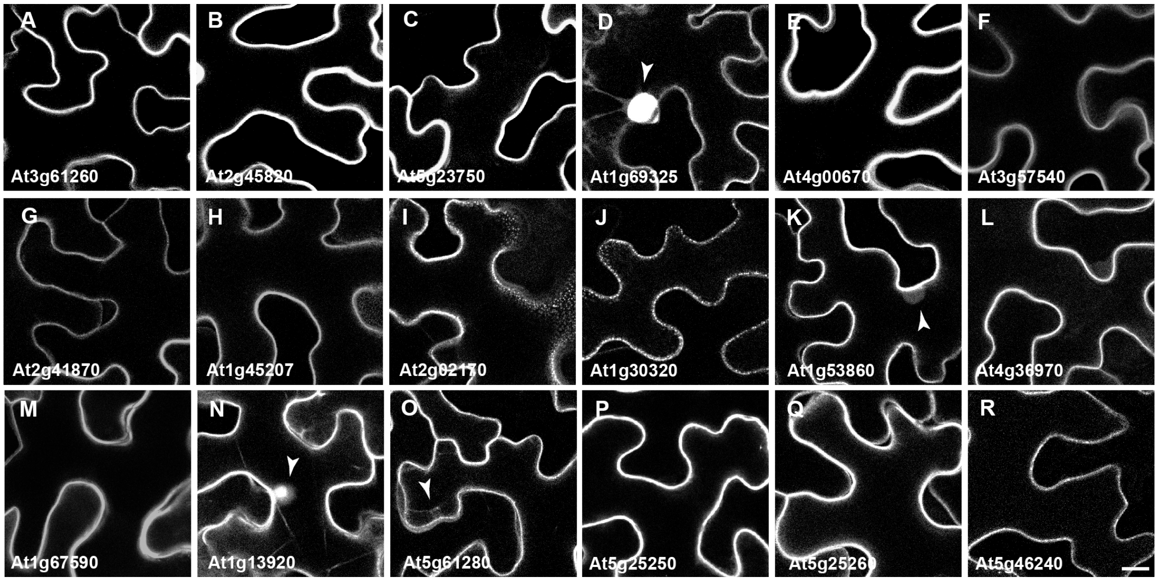
**(D-I)** Ectopic expression of different Remorins in leaf epidermal cells of transgenic *A. thaliana* plants transformed with a dexamethasone inducible AvrPto construct. Scale bars indicate 5 µm.



**Supplemental Figure 2 online.**

**Total Internal Reflection Microscopy (TIRFM) of upper plasma membrane planes.**

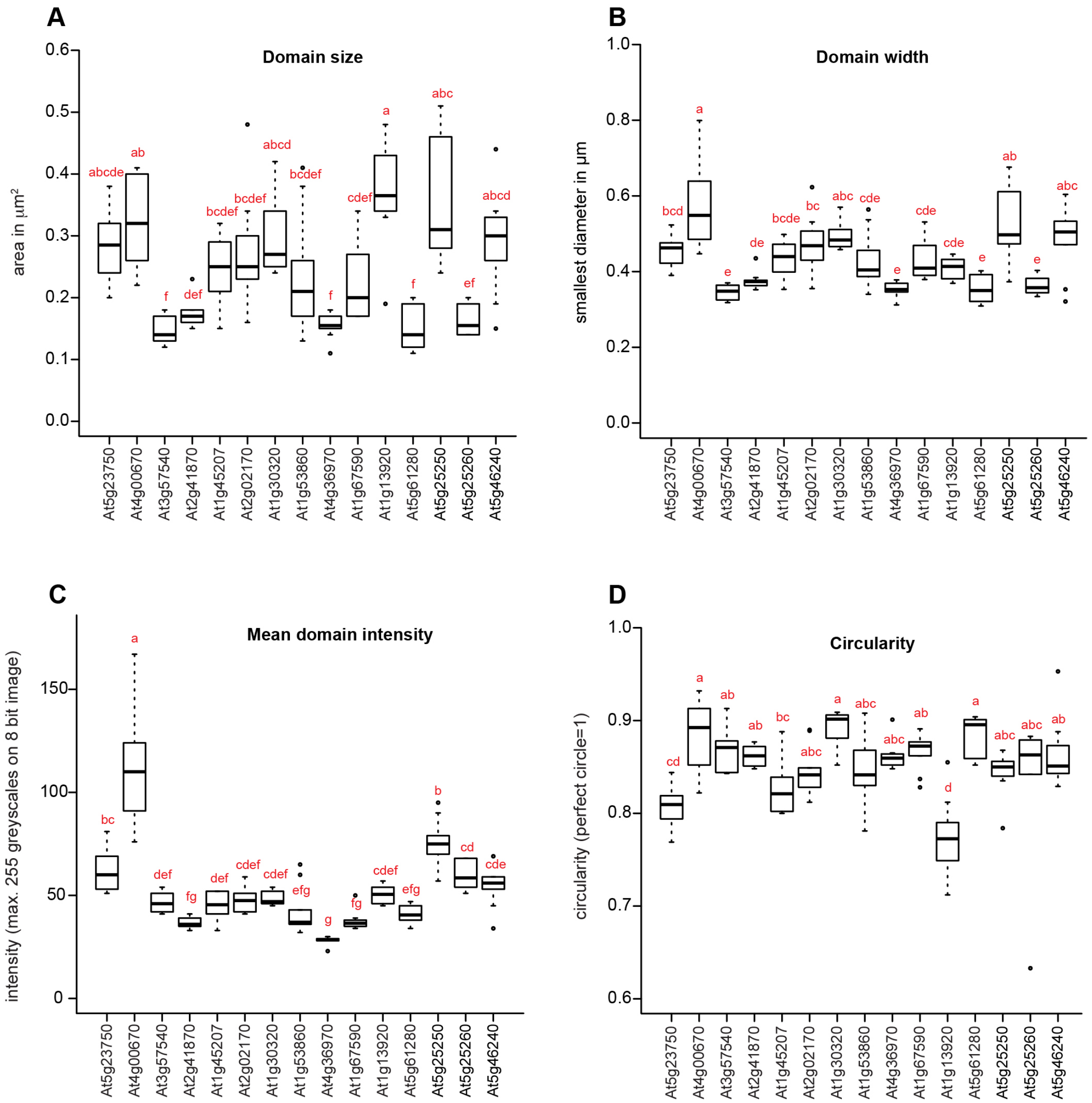
Null mutants expressing At3g61260 and At2g45820 as YFP fusion proteins were assessed by TIRFM. Both proteins localized to small yet highly mobile domains. Scale bars indicate 2 µm.



**Supplemental Figure 3 online.**

**Confocal images of secant planes illustrate plasma membrane localization of all marker proteins.**

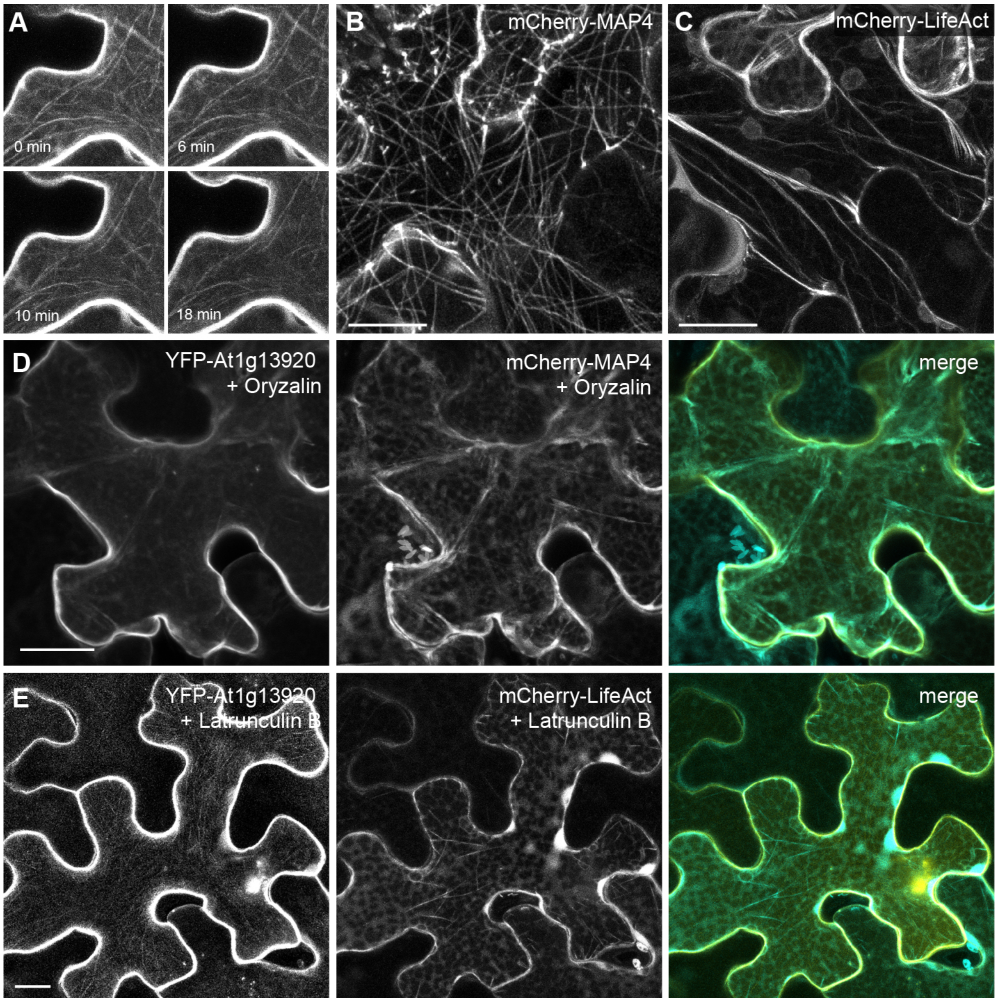
Eighteen different marker proteins were expressed in *N. benthamiana* leaf epidermal cells. Secant plant images are shown to demonstrate localization of the proteins to the plasma membrane. Arrowheads point towards partial nuclear and minor cytosolic localization of At1g69325, At1g53860, At1g13920 and At5g61280. Scale bar indicates 10 µm.



**Supplemental Figure 4 online.**

**Quantification of membrane domain parameters of all marker proteins.**

Boxplots representing ten independent images of single protein expressing *N. benthamiana* leaf epidermal cells were used to quantify domain parameters as described in the Methods section. Letters indicate results of a one-way analysis of variance (ANOVA) followed by a Tukey HSD test.



**Supplemental Figure 5 online.**

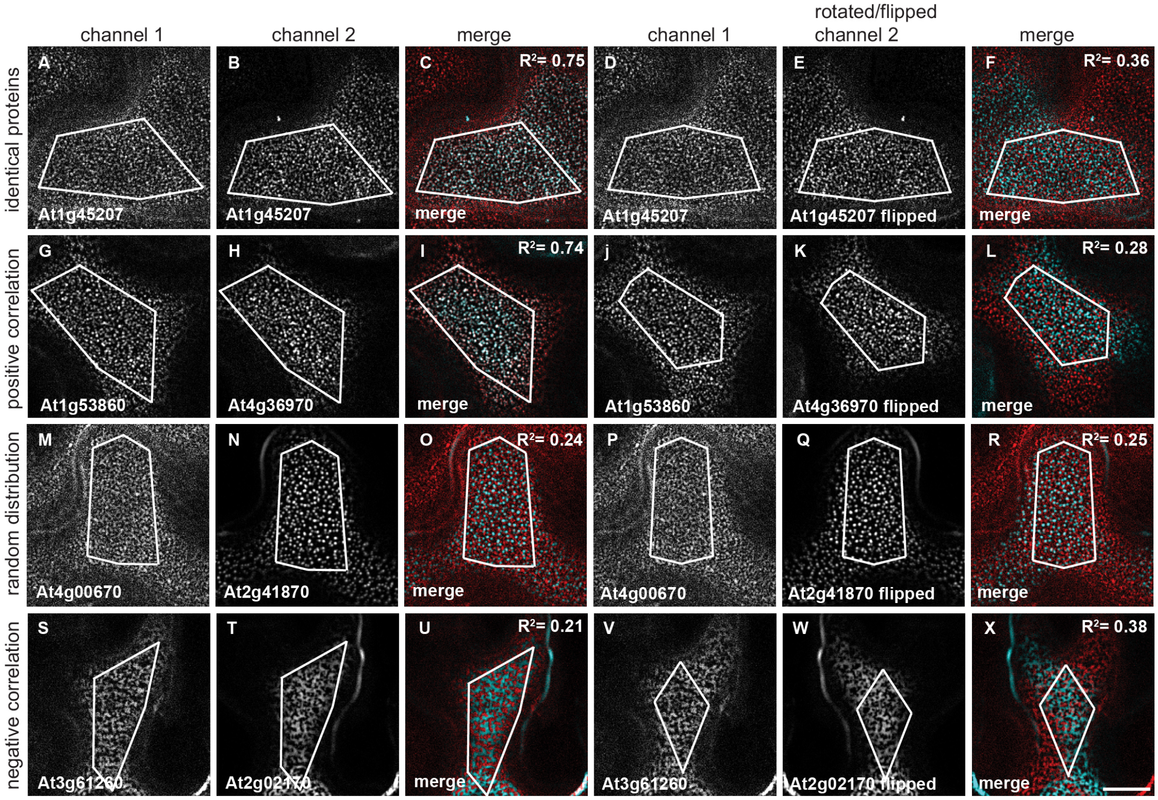
**Filament-like localization of At1g13920 is dependent on microtubules.**

**(A)** Time lapse experiments show that filament-like structures that are frequently labeled by At1g13920 are stable over an observation period of at least 20 minutes.

**(B-C)** Cells co-expressing the microtubule marker protein MAP4 (B) and the actin labeling peptide LifeAct (C) before treatment with oryzalin. Scale bars indicate 20 µm.

**(D)** Oryzalin treatment depolymerized microtubules and led to a loss of the At1g13920-labeled filaments and its predominant cytosolic localization. Scale bar indicates 20 µm.

**(E)** Depolymerization of the actin cytoskeleton by incubation in latrunculin B did not alter localization of At1g13920 but disrupted the majority of actin filaments. Scale bar indicates 20 µm.



**Supplemental Figure 6 online.**

**Image processing and randomization for quantitative analysis of co-localizations.**

To make any quantitative and robust statements on co-localization between two proteins, squared overlap coefficients ($R^2$) were calculated (C, I, O, U). For this, the channel 2 image was flipped or rotated (E, K, Q, W) to randomize the image. $R^2$ values were calculated for a new region of interest that contained image information of both channels (F, L, R, X). When mean $R^2$ values were significantly higher than the corresponding randomized values, pairs were ranked as positive correlations (co-localization). Pairs without any significant difference between these two values are randomly distributed and those where the randomized $R^2$ was significantly higher than the corresponding original $R^2$ value were ranked as negative correlation (mutual exclusion). Scale bars indicate 5 µm.

## Supplemental Table 1 online.

## Data from quantitative image analysis of co-localization experiments.

Protein pairs were grouped as ‚positive co-localization’ when $R^2$ was significantly higher ($p$<0.05) compared to $R^2_{random}$, as ‚random co-localization’ when $R^2$ was not significantly different compared to $R^2_{random}$ and ‚as ‚exclusion’ when $R^2$ was significantly lower ($p$<0.05) compared to $R^2_{random}$. Significant levels were calculated by Student ttest. rd= random; std.err= standard error; n= number of independent images that were analyzed.

| | sq. Manders | | Pearson | | | sq. Manders | | Pearson | | | sq. Manders | Pearson | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Protein pair** | **$R^2$** | **std. err.** | **Rr** | **std. err.** | **n** | **rd $R^2$** | **rd std. err** | **rd Rr** | **rd std. err.** | **n** | **ttest $R^2$** | **ttest Rr** | |
| **At1g45207xAt1g45207** | 0,752 | 0,020 | 0,661 | 0,026 | 11 | 0,364 | 0,021 | 0,016 | 0,012 | 10 | 3,47E-11 | 6,20E-15 | **identical proteins** |
| **At4g00670xAt4g00670** | 0,718 | 0,022 | 0,617 | 0,038 | 15 | 0,360 | 0,016 | 0,017 | 0,010 | 19 | 8,04E-14 | 1,30E-16 | |
| **At4g36970xAt4g36970** | 0,711 | 0,022 | 0,582 | 0,021 | 9 | 0,432 | 0,011 | -0,003 | 0,006 | 11 | 6,46E-10 | 8,82E-17 | |
| **At1g30320xAt1g30320** | 0,687 | 0,018 | 0,607 | 0,021 | 17 | 0,365 | 0,013 | 0,054 | 0,015 | 11 | 9,02E-13 | 5,67E-17 | |
| **At2g02170xAt2g02170** | 0,653 | 0,007 | 0,524 | 0,015 | 13 | 0,352 | 0,008 | 0,002 | 0,008 | 15 | 1,67E-20 | 2,08E-22 | |
| **At2g41870xAt2g41870** | 0,635 | 0,009 | 0,332 | 0,012 | 8 | 0,476 | 0,005 | -0,005 | 0,008 | 10 | 1,94E-11 | 2,93E-12 | |
| **At1g67590xAt1g67590** | 0,618 | 0,010 | 0,397 | 0,022 | 9 | 0,418 | 0,011 | -0,015 | 0,010 | 11 | 1,28E-10 | 4,98E-13 | |
| **At3g57540xAt3g57540** | 0,609 | 0,006 | 0,303 | 0,012 | 8 | 0,459 | 0,005 | -0,003 | 0,006 | 10 | 7,65E-13 | 5,32E-14 | |
| **At3g61260xAt3g61260** | 0,498 | 0,007 | 0,027 | 0,007 | 10 | 0,477 | 0,007 | -0,017 | 0,004 | 11 | 3,66E-02 | 3,15E-05 | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **At4g36970xAt1g53860** | 0,737 | 0,024 | 0,698 | 0,032 | 12 | 0,282 | 0,016 | 0,012 | 0,007 | 12 | 1,52E-13 | 4,70E-16 | |
| **At1g67590xAt2g02170** | 0,717 | 0,013 | 0,605 | 0,024 | 11 | 0,382 | 0,010 | 0,027 | 0,009 | 10 | 3,42E-14 | 5,11E-15 | **positive co-localization** |
| **At4g36970xAt1g45207** | 0,633 | 0,029 | 0,435 | 0,054 | 11 | 0,412 | 0,015 | 0,030 | 0,008 | 11 | 1,33E-06 | 3,51E-07 | |
| **At1g30320xAt2g02170** | 0,628 | 0,017 | 0,382 | 0,029 | 8 | 0,411 | 0,021 | 0,017 | 0,009 | 11 | 6,54E-07 | 1,37E-10 | |
| **At1g67590xAt1g30320** | 0,572 | 0,025 | 0,487 | 0,036 | 13 | 0,238 | 0,014 | -0,010 | 0,009 | 12 | 4,69E-11 | 6,11E-12 | |
| **At2g41870xAt3g57540** | 0,569 | 0,007 | 0,217 | 0,014 | 10 | 0,465 | 0,005 | -0,005 | 0,003 | 10 | 1,65E-10 | 1,21E-11 | |
| **At1g53860xAt1g45207** | 0,513 | 0,012 | 0,222 | 0,014 | 15 | 0,408 | 0,012 | -0,004 | 0,006 | 15 | 7,80E-07 | 7,68E-15 | |
| **At1g67590xAt1g53860** | 0,492 | 0,013 | 0,217 | 0,016 | 10 | 0,385 | 0,011 | -0,002 | 0,004 | 10 | 6,83E-06 | 8,75E-11 | |
| **At1g53860xAt2g02170** | 0,446 | 0,008 | 0,251 | 0,010 | 12 | 0,302 | 0,009 | -0,002 | 0,010 | 13 | 3,40E-11 | 9,97E-15 | |
| **At2g02170xAt1g45207** | 0,434 | 0,009 | 0,286 | 0,009 | 11 | 0,275 | 0,009 | 0,001 | 0,005 | 11 | 5,84E-11 | 8,13E-18 | |
| **At4g36970xAt3g57540** | 0,423 | 0,017 | 0,070 | 0,016 | 9 | 0,384 | 0,009 | -0,007 | 0,004 | 21 | 3,70E-02 | 7,92E-07 | |
| **At1g67590xAt3g57540** | 0,401 | 0,009 | 0,104 | 0,012 | 13 | 0,330 | 0,009 | -0,004 | 0,008 | 9 | 3,44E-05 | 1,71E-06 | |
| **At4g36970xAt1g30320** | 0,366 | 0,028 | 0,188 | 0,031 | 12 | 0,274 | 0,021 | 0,015 | 0,011 | 12 | 1,94E-02 | 5,43E-05 | |
| **At1g53860xAt1g30320** | 0,298 | 0,010 | 0,139 | 0,016 | 10 | 0,238 | 0,014 | -0,010 | 0,009 | 12 | 3,66E-03 | 3,36E-08 | |

*Supplemental Table 1 online (continued)*

| Protein pair | sq. Manders | | Pearson | | | sq. Manders | | Pearson | | | sq. Manders | Pearson | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | std. err. | Rr | std. err. | n | rd $R^2$ | rd std. err | rd Rr | rd std. err. | n | ttest $R^2$ | ttest Rr | |
| **At2g02170xAt3g57540** | 0,424 | 0,009 | -0,002 | 0,005 | 9 | 0,409 | 0,014 | -0,007 | 0,007 | 9 | 4,00E-01 | 5,60E-01 | **random co-localization** |
| **At1g45207xAt3g57540** | 0,417 | 0,009 | 0,039 | 0,006 | 19 | 0,396 | 0,008 | -0,006 | 0,003 | 19 | 8,24E-02 | 3,84E-08 | |
| **At4g36970xAt2g02170** | 0,381 | 0,013 | -0,002 | 0,010 | 10 | 0,366 | 0,011 | -0,005 | 0,004 | 13 | 3,84E-01 | 7,88E-01 | |
| **At1g53860xAt2g41870** | 0,374 | 0,009 | 0,003 | 0,007 | 10 | 0,366 | 0,012 | -0,005 | 0,006 | 12 | 5,87E-01 | 4,01E-01 | |
| **At1g67590xAt2g41870** | 0,361 | 0,019 | 0,087 | 0,010 | 13 | 0,312 | 0,022 | -0,005 | 0,006 | 10 | 1,10E-01 | 4,88E-08 | |
| **At1g67590xAt1g45207** | 0,354 | 0,029 | 0,058 | 0,011 | 10 | 0,322 | 0,024 | -0,011 | 0,003 | 10 | 3,90E-01 | 1,60E-05 | |
| **At2g41870xAt3g61260** | 0,353 | 0,007 | -0,027 | 0,005 | 12 | 0,356 | 0,009 | -0,018 | 0,007 | 12 | 8,37E-01 | 2,39E-01 | |
| **At1g45207xAt2g41870** | 0,353 | 0,010 | 0,045 | 0,021 | 12 | 0,333 | 0,010 | 0,012 | 0,008 | 12 | 3,33E-01 | 1,60E-01 | |
| **At1g45207xAt3g61260** | 0,318 | 0,011 | -0,019 | 0,003 | 11 | 0,319 | 0,012 | -0,020 | 0,004 | 11 | 9,54E-01 | 8,85E-01 | |
| **At2g41870xAt4g00670** | 0,239 | 0,021 | -0,031 | 0,013 | 11 | 0,246 | 0,017 | -0,007 | 0,005 | 11 | 7,88E-01 | 1,10E-01 | |
| **At1g30320xAt1g45207** | 0,133 | 0,008 | 0,050 | 0,007 | 18 | 0,116 | 0,009 | 0,002 | 0,005 | 17 | 1,53E-01 | 1,28E-06 | |
| **At1g30320xAt3g57540** | 0,132 | 0,005 | 0,014 | 0,007 | 12 | 0,122 | 0,005 | -0,011 | 0,004 | 12 | 1,03E-01 | 5,25E-03 | |
| **At2g02170xAt2g41870** | 0,410 | 0,005 | -0,042 | 0,009 | 10 | 0,436 | 0,006 | -0,003 | 0,006 | 10 | 2,89E-03 | 2,79E-03 | **exclusion** |
| **At1g53860xAt3g61260** | 0,373 | 0,011 | -0,117 | 0,011 | 15 | 0,434 | 0,004 | -0,005 | 0,006 | 14 | 2,52E-05 | 2,52E-09 | |
| **At4g36970xAt3g61260** | 0,296 | 0,014 | -0,128 | 0,026 | 11 | 0,353 | 0,006 | 0,010 | 0,008 | 12 | 9,82E-04 | 2,84E-05 | |
| **At1g67590xAt4g36970** | 0,286 | 0,012 | -0,009 | 0,012 | 13 | 0,385 | 0,011 | -0,002 | 0,004 | 10 | 6,41E-06 | 6,29E-01 | |
| **At1g67590xAt3g61260** | 0,208 | 0,013 | -0,368 | 0,027 | 10 | 0,357 | 0,008 | -0,019 | 0,009 | 11 | 5,12E-09 | 8,21E-11 | |
| **At2g02170xAt3g61260** | 0,207 | 0,016 | -0,432 | 0,047 | 15 | 0,383 | 0,012 | -0,012 | 0,010 | 14 | 2,29E-09 | 4,98E-09 | |
| **At4g00670xAt3g61260** | 0,193 | 0,014 | -0,136 | 0,017 | 11 | 0,247 | 0,016 | 0,004 | 0,009 | 11 | 2,00E-02 | 4,04E-07 | |
| **At1g30320xAt4g00670** | 0,191 | 0,023 | -0,153 | 0,021 | 12 | 0,265 | 0,018 | 0,003 | 0,009 | 12 | 1,70E-02 | 6,06E-07 | |
| **At1g30320xAt3g61260** | 0,153 | 0,006 | -0,106 | 0,032 | 10 | 0,192 | 0,011 | -0,017 | 0,010 | 10 | 5,61E-03 | 1,64E-02 | |
| **At1g67590xAt4g00670** | 0,102 | 0,011 | -0,291 | 0,019 | 13 | 0,217 | 0,015 | 0,004 | 0,008 | 13 | 1,60E-06 | 2,46E-13 | |

# Supplemental Table 2 online.

## List of primers used in this study.

**cloning of overexpression constructs**

| gene ID | forward primer | reverse primer | vector |
|---|---|---|---|
| At3g61260 | CACCATGGCGGAGGAACAGAAGA | TTAGAAACATCCACAAGTTGCCTT | 2 |
| At2g45820 | CACCATGGCGGAGGAGCAAAAGAC | TTAGAAACATCCACACGTTGCCTT | 2 |
| At5g23750 | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGCTGAAGAGGAACCG | GGGGACCACTTTGTACAAGAAAGCTGGGTTTTACATGCATCCGAAAAGC | 3 |
| At1g69325 | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAACGAATCCACAGTGC | GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGAGGCATGTAGAGGGTTTCC | 3 |
| At4g00670 | GGCGCGCCTACCATGGAGCCAAATATTCCGATCC | CGTTTAAACCTTAGAAGCAGCTCAAAGATGA | 1 |
| At3g57540 | GGCGCGCCTACCATGTTGACTTTGTACGGTCA | CGTTTAAACCTTAGGAAAGAGAGAAGAATGATC | 1 |
| At2g41870 | GGCGCGCCTACCATGCTGACTCTTTACCATCAAG | CGTTTAAACCTTAGGAGAAAGAGAAGAAGGAGC | 1 |
| At1g45207 | GGCGCGCCTACCATGCCGTCGGAGTCATCGTAC | CGTTTAAACCTTAGAATACATGGCAGGTGAAGC | 1 |
| At2g02170 | GGCGCGCCTACCATGGATTACGAACGAATCGG | GGCGCGCCTTAAGAACAAAAGCTAAAGC | 1 |
| At1g30320 | GGCGCGCCTACCATGGATTACGAGAGGATACAG | CGTTTAAACCTTATGAGAACCAACCACAACA | 1 |
| At1g53860 | GGCGCGCCTACCATGGACTTCACAAGAAACAG | CGTTTAAACCTTAATGACAAGTATTATTGC | 1 |
| At4g36970 | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAGAAAGACTTCTGTTTC | GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGAGAGCAGAAGAAGATTTTC | 3 |
| At1g67590 | CACCATGAGATCTAGTGTAGAAG | TTATTGACACCAACAACGAG | 2 |
| At1g13920 | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGATACCTTAATCAAGC | GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGAAACAGCATGCATTTC | 3 |
| At5g61280 | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGATAATTTGGTTAAGC | GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGTAACACCGAAAGCAGAAA | 3 |
| KAT1 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCGATCTCTTGGACTCG | GGGGACCACTTTGTACAAGAAAGCTGGGTTATTTGATGAAAAATACAAATGATCACC | 3 |
| FLOT1A | TTTGGTCTCTCACCATGTTCAAAGTTGCAAGAGC | AAAGGTCTCACCTTGCTGCGAGTCACTTGC | 4 |
| FLOT1B | TTTGGTCTCTCACCATGTTCAAGGTTGCAAGAGC | AAAGGTCTCACCTTCTTGCTTAGAGTACCGATCC | 4 |
| SYMREM1 | AGGCGCGCCTACCATGGAAGAATCGAAAAACAAAC | AGGCGCGCCCTAACTGAAAAACCTTAAACC | 1 |

**cloning of Pro:YFP:ORF constructs**

| | | | |
|---|---|---|---|
| ProAt3g61260 | TTTGGTCTCTCACCGTTGGCCGTCGTTG | AAAGGTCTCTTGTCAGTCGCCGCCTCTCAGCC | 4 |
| At3g61260 | TTTGGTCTCGAATATGGCGGAGGAACAG | AAAGGTCTCACCTTTTAGAAACATCCACAAGTTGC | 4 |
| ProAt2g45820 | TTTGGTCTCTCACCGGTATTCCTATGCTCAAATC | AAAGGTCTCTTGTCTGTCTCTCAGCCGAAGAAGAAG | 4 |
| At2g45820 | TTTGGTCTCAGAATATGGCGGAGGAGCAAAAG | AAAGGTCTCACCTTTTAGAAACATCCACACGTTGC | 4 |
| ProAt4g36970 | TTTGGTCTCTCACCTGCGTTGCATCGTTCGTGA | AAAGGTCTCTTGTCTGTTGGTTTCTCAAAGAACAAAATC | 4 |
| At4g36970 | TTTGGTCTCAGAATATGAGAAAGACTTCTGTTTC | AAAGGTCTCACCTTACTGAGAGCAGAAGAAGATTTTC | 4 |
| free YFP | TTTGGTCTCTGACAATGGTGAGCAAGGGCGAGG | AAAGGTCTCTATTCCTTGTACAGCTCGTCCATGC | 4 |

**qPCR primers**

| | | |
|---|---|---|
| At4g36970 | GAACATGAACAACAACAAGGG | CGAGGAGCAAAGCAAGTCATG |

*legend*

1= for cloning into pksi
2= for cloning into pENTR-D
3= for cloning into pDONR207
4= for cloning into pENTR-D BsaI
START and STOP codons are depicted in red; Pro=promoter