

Supplementary Material

Table S1. Primer sequences.

| Primer | Sequence 5'-3' | Purpose |
|-------------------|--------------------------|---|
| MdDAM1-Fq | TCAAGCGTGGGTACGTTGCTTC | qRT-PCR |
| MdDAM1-Rq | GATGACCTGAGCGATAAAGTTGGC | qRT-PCR and genotyping of transgenic plants |
| <i>MdDAM4</i> -Fq | CATACTGGTGGGGAAAAATCG | qRT-PCR |
| MdDAM4-Rq | CTCAGCTTGCGGGTCTTATC | qRT-PCR |
| MdEF1alpha_F | TCAAGCGTGGGTACGTTGCTTC | qRT-PCR |
| MdEF1alpha_R | GATGACCTGAGCGATAAAGTTGGC | qRT-PCR |
| nptII_F | ACAAGATGGATTGCACGCAGG | Genotyping of transgenic trees |
| nptII_R | AACTCGTCAAGAAGGCGATAG | Genotyping of transgenic trees |
| tOCS_R | ATCATGCGATCTAGGCGTC | Genotyping of transgenic trees |
| virG_F | GCCGGGGCGAGACCATAG | Genotyping of transgenic trees |
| _virG_R | CGCACGCGCAAGGCAACC | Genotyping of transgenic trees |

Table S2. Fold Changes of the 53 differentially expressed genes in line P35S:MdDAM1#1 and their corresponding level of expression in 'Golden delicious' during a bud dormancy time course

| | | | log2F | C in 'Golden de | licious' normalize | ed to "October" v | alues | | | | | | | |
|----------------|--|-------------------------------------|--------------|-----------------|--------------------|-------------------|--------------|-------------------------------------|--------------|--------------------|----------|----------|----------|---------------------|
| Gene ID | log2FC in P35S: <i>MdDAM1</i> #1 compared to NT control | D=Down-regulated; U=Up-regulated | November | December | January | February | March | Gene description | Base Mean | log2Fold Change | lfcSE | stat | p-value | p-adjusted value |
| MDP0000013331 | 3.102008678 | U | 5.323777361 | 5.219265464 | 5.132224509 | 2.382071306 | 5.122451642 | MADS-box TF (MdAP1) | 37.93058 | 3.102009 | 0.422284 | 7.345787 | 2.05E-13 | 1.20936E-10 |
| MDP0000306273 | -1.765324523 | D | -4.039082471 | -8.411557551 | -5.081813402 | -4.077032721 | -3.914831834 | Cytochrome P450 | 127.8228 | -1.76532 | 0.435069 | -4.05758 | 4.96E-05 | 0.002414223 |
| MDP0000232313 | -2.028923988 | D | 0.007427254 | -0.991325717 | -3.104317329 | -7.041121229 | -4.74071452 | MADS-box TF (MdDAM4) | 102.4041 | -2.02892 | 0.234514 | -8.65163 | 5.08E-18 | 6.75369E-15 |
| MDP0000154764 | -3.574346057 | D | 0.442054783 | 1.07493231 | 0.283823052 | -3.628397812 | -5.866732565 | ERF TF | 76.98169 | -3.57435 | 0.423111 | -8.44777 | 2.97E-17 | 3.35142E-14 |
| MDP0000517262 | -2.174799841 | D | 2.725017536 | 2.89907733 | 1.590991199 | -0.477453471 | -0.450648799 | AP2 TF | 107.3594 | -1.09719 | 0.278619 | -3.93794 | 8.22E-05 | 0.003514481 |
| MDP0000142134 | -1.371959521 | D | 0.885393104 | 1.878934016 | 1.387006718 | 0.730400012 | -0.945293083 | Beta-amylase | 418.1697 | -1.37196 | 0.166457 | -8.24214 | 1.69E-16 | 1.70286E-13 |
| MDP0000197219 | -1.320494194 | D | 1.646206286 | 2.865225196 | 1.896792402 | 0.554264209 | -0.118793867 | bZip TF | 243.4806 | -1.32049 | 0.249784 | -5.28655 | 1.25E-07 | 1.77202E-05 |
| MDP0000165880 | -1.359287916 | D | 1.090650763 | 2.645518221 | 2.745179792 | 0.581787466 | -1.192573437 | ERF TF | 181.0693 | -1.35929 | 0.201182 | -6.75651 | 1.41E-11 | 5.48431E-09 |
| MDP0000149950 | -2.329176978 | D | 2.035986436 | 3.152300132 | 3.080024204 | 2.036100625 | -1.194778901 | Beta-amylase | 171.1249 | -2.56044 | 0.279437 | -9.16287 | 5.05E-20 | 8.55607E-17 |
| MDP0000218882 | -2.076243815 | D | 2.608407537 | 2.408170486 | 2.486722227 | 1.873075336 | -0.256698457 | Cellulose synthase-like | 106.0868 | -2.07624 | 0.498984 | -4.16094 | 3.17E-05 | 0.00167449 |
| MDP0000130030 | -2.305458588 | D | 2.231309487 | 2.916064311 | 2.362873672 | 1.159869709 | -0.656154546 | Isoflavone 2'-hydroxylase-like | 35.18403 | -2.30546 | 0.467564 | -4.93079 | 8.19E-07 | 8.84208E-05 |
| MDP0000547069 | -1.055181541 | D | 1.777410171 | 2.057836188 | 1.49467537 | -0.858322594 | -2.193400201 | Glycoside hydrolase 17 | 314.2396 | -1.05518 | 0.209225 | -5.04329 | 4.58E-07 | 5.30961E-05 |
| MDP0000252855 | -1.043430114 | D | 1.13305786 | 1.072721136 | 0.736229376 | -0.637396175 | -1.037033923 | Endo-beta-mannanase | 216.0798 | -1.04343 | 0.289367 | -3.6059 | 0.000311 | 0.009264854 |
| MDP0000867709 | -1.247196782 | D | 0.694345511 | -0.322923738 | 0.291576483 | 0.185218238 | -0.776991646 | Cytochrome P450 | 434.9515 | -1.2472 | 0.155632 | -8.01377 | 1.11E-15 | 9.8655E-13 |
| MDP0000271354 | 1.936128804 | U | 0.347576809 | 0.303761663 | -0.265744914 | 1.895238107 | -0.89192327 | Oxygen oxidoreductase | 68.95418 | 1.936129 | 0.39853 | 4.858182 | 1.18E-06 | 0.000117669 |
| MDP0000147201 | -1.345055444 | D | 0.587634219 | 0.811897925 | 1.338263964 | 1.404522944 | -0.051528816 | Glycoside Hydrolase 17 | 44.9735 | -1.32307 | 0.35982 | -3.67702 | 0.000236 | 0.007590104 |
| MDP0000707539 | -1.628383451 | D | -0.338049421 | 0.148465037 | 0.367685763 | 0.770688765 | 0.658012873 | WRKY TF | 44.64627 | -1.62838 | 0.448463 | -3.63103 | 0.000282 | 0.008634265 |
| MDP0000324398 | 2.127427854 | U | 0.616135408 | 0.034562742 | 0.048585632 | 1.398324732 | 1.614345852 | MdIAA6 | 37.33274 | 2.127428 | 0.444985 | 4,780899 | 1.75E-06 | 0.00016373 |
| MDP0000655623 | 1.335121018 | U | -0.094125008 | -0.577521039 | 0.061809461 | 0.762454064 | 1.492695989 | No apical meristem (NAM) TF | 41.93428 | 1.335121 | 0.368629 | 3.621857 | 0.000292 | 0.008900814 |
| MDP0000259294 | -2.422074935 | P | -0.724518138 | -1.334792067 | -2.172188419 | -3.466670797 | -4.941138902 | MADS-box TF (MdDAM2) | 112.6455 | -2.42207 | 0.234916 | -10.3104 | 6.33E-25 | 2.35599E-21 |
| MDP0000241650 | -1.052397594 | D | -0.841600326 | -1.226951883 | -3.044196769 | -1.923599425 | -3.115033118 | ERFTF | 197.6707 | -1.0524 | 0.19117 | -5.50503 | 3.69E-08 | 5.82943E-06 |
| MDP0000690168 | -4.903849779 | D | -1.807653632 | -3.574704992 | -2.541159049 | -3.165751151 | -4.018894702 | No apical meristem (NAM) TF | 110.5939 | -4.90385 | 1.137845 | -4.30977 | 1.63E-05 | 0.001019609 |
| MDP0000270602 | -2.156593915 | D | -3.388673248 | -3.283569314 | -1.875339737 | -3.324889771 | -3.750827026 | 3'.5'-hvdroxvlase | 101.5539 | -2.15659 | 0.346884 | -6.21704 | 5.07E-10 | 1.33832E-07 |
| MDP0000389795 | -1.131347002 | D | -2.443717436 | -2.582504459 | -2.242040503 | -2.931616208 | -4.003158918 | Cytochrome P450 | 888.8723 | -1.13135 | 0.199803 | -5.66233 | 1.49E-08 | 2.67418E-06 |
| MDP0000910006 | -2.34829143 | D | -3.34883594 | -4.93302134 | -3.34693843 | -2.719046885 | -2.226485811 | Cytochrome P450 | 57.51566 | -2.34829 | 0.384325 | -6.11017 | 9.95E-10 | 2.43878E-07 |
| MDP0000578301 | -1.459017174 | D | -1.310229829 | -2.280640454 | -2.851255867 | -2.891971868 | -1.402629005 | WRKY Family Protein | 88,48401 | -1.45902 | 0.329133 | -4.43292 | 9.3E-06 | 0.000652115 |
| MDP0000141889 | -1.380175295 | D | -1.947680916 | -2.408882289 | -2.630593672 | -3.002086054 | -1.289968716 | Methionine-tRNA ligase | 61.92002 | -1.38018 | 0.379629 | -3.63559 | 0.000277 | 0.008530013 |
| MDP0000753736 | -1.512239083 | D | -3.007757943 | -3.943915646 | -2.279345682 | -1.938831751 | -1.201866181 | MdIAA122 | 65.38263 | -1.51224 | 0.342544 | -4.41473 | 1.01E-05 | 0.000691185 |
| MDP0000292868 | -1.811121443 | D | -2.953326503 | -3.110207197 | -2.726705207 | -1.108773467 | -1.78001353 | Cvtochrome P450 | 44.07092 | -1.57627 | 0.387996 | -4.06258 | 4.85E-05 | 0.002378614 |
| MDP0000183534 | -1.302731934 | D | -2.243759948 | -2.249209535 | -2.342569496 | -1.579905161 | -1.897664167 | Zinc finger TF | 99.92978 | -1.30273 | 0.352931 | -3.69118 | 0.000223 | 0.007293178 |
| MDP0000266003 | 1.50319907 | U | -0.844407507 | -5.408951138 | -1.231631753 | -0.656397599 | 0.283498996 | Fructose-bisphosphate aldolase | 47.49853 | 1.503199 | 0.411233 | 3.65535 | 0.000257 | 0.008106958 |
| MDP0000191851 | 1.008989601 | U | 0.572189116 | -2.865071024 | -1.505574819 | -2.954217557 | -0.207871749 | Chorismate mutase | 88.67534 | 1.00899 | 0.275978 | 3.656056 | 0.000256 | 0.008091534 |
| MDP0000827821 | -1.180624206 | D | -1.931172766 | -1.260113384 | -1.017789606 | -1.1115674 | -0.851993582 | NF-YA TF | 132.8057 | -1.18062 | 0.27845 | -4.23999 | 2.24E-05 | 0.001284873 |
| MDP0000231993 | -2.665595949 | D | -1.717597147 | -2.295361437 | -1.244964068 | -0.004026756 | -0.723762313 | WRKY TF | 58.11666 | -2.6656 | 0.498378 | -5.34854 | 8.87E-08 | 1.30021E-05 |
| MDP0000464704 | -1.646477394 | D | -1.157628935 | -1.247657156 | -0.596736464 | -2.373154482 | -2.088648847 | ERF TF | 72.46021 | -1.64648 | 0.332563 | -4.95088 | 7.39E-07 | 8.16553E-05 |
| MDP0000834642 | -1.165073996 | D | -1.214282665 | -1.414913186 | -0.281169537 | -1.167032022 | -1.702733755 | bZip TF | 103.3053 | -1.16507 | 0.304648 | -3.82432 | 0.000131 | 0.005004377 |
| MDP0000426372 | -1.414807605 | D | -1.633041024 | -0.864100318 | -1.139067074 | -2.108664718 | -2.144396104 | Dof TF | 189.3233 | -1.41481 | 0.212346 | -6.66276 | 2.69E-11 | 9.62466E-09 |
| MDP0000143173 | -1.154786705 | D | -2.235910208 | -0.595539268 | -1.101857781 | -1.27128824 | -1.828455658 | TALE family protein | 506.8575 | -1.15479 | 0.307094 | -3.76037 | 0.00017 | 0.006043064 |
| MDP0000560179 | 1,349486767 | U | 0.349763136 | -0.684554066 | -0.44055133 | -1.579331271 | 0.669154972 | Chorismate mutase | 101,7293 | 1.349487 | 0.247617 | 5,449891 | 5.04E-08 | 7.72543E-06 |
| MDP0000162509 | -2 567049596 | D | 0.064475533 | -0.531875798 | -1 297254999 | -0 756306464 | -1 199042086 | Glycoside hydrolase 3 | 64 17803 | -2 56705 | 0 422768 | -6 072 | 1 26F-09 | 3 01624E-07 |
| MDP0000120881 | -1.898132971 | | -0 375173922 | -0 245653905 | -0.093083013 | -0 354164847 | -1 425216763 | No apical meristem (NAM) TE | 83 05418 | -1 89813 | 0.500578 | -3 79188 | 0.00015 | 0.005530176 |
| MDP0000321920 | 1 171056965 | U | -0.659820129 | -0.055235414 | -0 140245638 | -0 782139467 | -0 175959053 | ABC transporter | 137 251 | 1 171057 | 0.20213 | 4 008689 | 6 11E-05 | 0.00282507 |
| MDP0000151003 | 1.080454306 | 5 | 0.414526276 | 0.1/3300658 | 0.5106733 | 0.778776715 | 1 267013010 | Cutochrome p450 | 164 4502 | 1 02045 | 0.23213 | 4.000003 | 2 205 00 | 6 095205 07 |
| MDP0000171003 | -1.909404090 | | -0.414320270 | 1 022251609 | 1 020647127 | 1 160471166 | 2 02127420 | | 104.4392 | 1.30343 | 0.330230 | -3.91085 | 3.200-03 | 0.98339E=07 |
| MDP0000176320 | 1.004000091 | U | -3.140700393 | -1.932331008 | -1.920047137 | 1.156471155 | 0.742206245 | DZIF IF | 40.30070 | 1.534555 | 0.38/2/9 | 3.902349 | 7.42E-05 | 0.003237134 |
| MDD00000006440 | 1.096243896 | | -1.20001/248 | -2.409/01998 | -1./3/106963 | -0.056776161 | 0.7 13390245 | | 82.623 | 1.098244 | 0.30428 | 3.609314 | 0.000307 | 0.003363310 |
| MDP0000286448 | 1.124304427 | <u> </u> | -1.492120471 | -2.112/142/7 | -2.515688929 | -0.770997792 | 1.028942584 | ORC6-like protein | 86.75472 | 1.124304 | 0.304471 | 3.692644 | 0.000222 | 0.007267316 |
| WDP0000237499 | 1.250000187 | U | -2.894779743 | -4.331110302 | -4.725014272 | -5.704418021 | 0.071164547 | MIAA1U7 | 109.5031 | 1.2566 | 0.31972 | 3.93031 | 8.48E-05 | 0.003603083 |
| MDP0000311765 | -2.0108648 | D | -6.10911244 | -6.85/269148 | -4.671908917 | -1.346/2/795 | -0.361391551 | Aylogiucan:xylogiucosyl transferase | 114.3166 | -2.01086 | 0.413523 | -4.86276 | 1.16E-06 | 0.000115596 |
| MDP0000199273 | -1.204240417 | D | -4.161101355 | -4.716180565 | -4.134476381 | -2.080775132 | -0.089402194 | Glycoside hydrolase 9 | 298.7596 | -1.20424 | 0.193216 | -6.23261 | 4.59E-10 | 1.22047E-07 |
| MDP0000321215 | 1.072584438 | U | -2.75037237 | -3.678874159 | -2.912148022 | -0.597493537 | 1.86017383 | Replication factor-a protein 1 | 188.0246 | 1.072584 | 0.242664 | 4.420047 | 9.87E-06 | 0.000680744 |
| MDP0000248100 | 1.713224259 | U | -2.005826279 | -4.815899039 | -2.746572609 | -0.777840906 | 1.256983429 | DNA repair protein | 35.17381 | 1.713224 | 0.468556 | 3.656391 | 0.000256 | 0.008088056 |
| MDP0000131617 | 1.013233752 | U | -3.04172421 | -3.956660686 | -3.805042828 | -1.047892226 | 0.944045674 | Pol II | 183.4405 | 1.013234 | 0.251191 | 4.033715 | 5.49E-05 | 0.002614998 |
| MDP0000613174 | 1.130680656 | U | -2.665347219 | -4.138882759 | -4.399481132 | -1.720746076 | 0.815811048 | SINE-1 like | 355.2206 | 1.130681 | 0.283825 | 3.983728 | 6.78E-05 | 0.003045075 |



Figure S1. Environmental parameters during the apple bud dormancy time course experiment.

Temperatures in °C and day lengths in hours (h), indicated by a red and a blue line, respectively, were recorded each month from 2011 to 2019 at the orchard and the mean value plotted. Months are indicated by abbreviations. Buds from the cultivars 'Golden Delicious', 'Anna', and 'Dorsett Golden' were harvested at six time points from October to March as indicated by vertical arrows. The date of full bloom in 2019 is also indicated for each cultivar.



MdDAM1_'Golden_delicious' MdDAM1_'Dorsett_Golden' MdDAM1=`Anna'

MdDAM1_'Golden_delicious' MdDAM1_'Dorsett_Golden' MdDAM1-'Anna'

MdDAM1_'Golden_delicious' MdDAM1_'Dorsett_Golden' MdDAM1-'Anna'

MdDAM1_'Golden_delicious' MdDAM1_'Dorsett_Golden' MdDAM1-'Anna'

MdDAM1_'Golden_delicious' MdDAM1_'Dorsett_Golden' MdDAM1-`Anna'

MdDAM1_'Golden_delicious' MdDAM1_'Dorsett_Golden' MdDAM1-'Anna'

CTCTTTGATTTCTCAAGCTCCAGGACCAAGGATGTGATTGCAAGGTACAA CTCTTTGATTTCTCAAGCTCCAGGACCAAGGATGTGATTGCAAGGTACAA TTCACATATCGGTGGGGAAAAATCGGATCAACCCACGATTCATCAGCTAC TTCACATATCGGTGGGGAGAAATCGGATCAACCCACGATTCATCAGCTAC TTCACATATCGGTGGGAGAAATCGGATCAACCCACGATTCATCAGCTAC

AGTTGGAGAAAGAAAACAATATCAGGCTGAGGAAGGAACTTGAGGATAAG AGTTGGAGAAAGAAAACAATATCAGGCTGAGGAAGGAACTTGAGGATAAG AGTTGGAGAAAGAAAACAATATCAGGCTGAGGAAGGAACTTGAGGATAAG

AGTTGCAAGTTGAGGCAGATGAAGGGTGTGGACCTTGAAGACTTGGATCT AGTTGCAAGTTGAGGCAGATGAAGGGTGTGGACCTTGAAGACTTGGATCT AGTTGCAAGTTGAGGCAGATGAAGGGTGTGGACCTTGAAGACTTGGATCT

TGATTCAAACTAAGGAAGAAAAGATTATGAGTGAGGTTATGGCACTTGAG TGATTCAAACTAAGGAAGAAAAGATTATGAGTGAGGTTATGGCACTTGAG TGATTCAAACTAAGGAAGAAAAGATTATGAGTGAGGTTATGGCACTTGAG

GGTGATGTATCCCAGAGGAGATATCGGACCGGAGGCCATCCTGGAGTTGG

AATGTCACCACCTGCTCCAACAGCTCTCTTTCCCTTGAAGATGATTGCTC AATGTCACCACCTGCTCCAACAGCTCTCTTTCCCTTGAAGATGATTGCTC AATGTCACCACCTGCTCCAACAGCTCTCTTTCCCTTGAAGATGATTGCTC

.icious' CGACATCTTGTCTCTCAAACTGGGGTGA >lden' CGACATCTTGTCTCTCAAACTGGGGTGA CGACATCTTGTCTCTCAAACTGGGGTGA Β

| GCAAGGCAGGTGAC | MdDAM1 'Dorsett Golden' | MKIKIKKIDYLPAROVTFSKRRRGIFKKAGELSILCESEVAVIIFSOTGK |
|----------------|----------------------------|---|
| GCAAGGCAGGTGAC | MdDAM1-`Anna' | MKIKIKKIDYLPAROVTFSKRRRGIFKKAGELSILCESEVAVIIFSOTGK |
| GCAAGGCAGGTGAC | MdDAM1 'Golden delicious' | MKTKTKKTDYLPAROVTFSKRRRGTFKKAGELSTLCESEVAVITFSOTGK |
| ***** | habitii_ coracii_actrerous | *************************************** |
| TGGAGAGCTGTCGA | | |
| TGGAGAGCTGTCGA | | |
| TGGAGAGCTGTCGA | MdDAM1_'Dorsett_Golden' | LFDFSSSSTKDVIARYNSHIGGEKSDQPT IHQLQLEKENNIRLRKELEDK |
| ***** | MdDAM1-`Anna' | LFDFSSSSTKDVIARYNSHIGGEKS <mark>DQPT<i>IHQLQLEKENNIRLRKELEDK</i></mark> |
| CTCAAACTGGCAAG | MdDAM1 'Golden delicious' | LFDFSSSSTKDVIARYNSHIGGEKS <mark>DQPT<i>IHQLQLEKENNIRLRKELEDK</i></mark> |
| CTCAAACTGGCAAG | | ********************* |
| CTCAAACTGGCAAG | | |
| | MdDAM1 'Dorsett Golden' | SCKI.ROMKGVDI.EDI.DI.DEI.OKI.EKI.VEASI.GRVIOTKEEKIMSEVMAI.E |
| ATTGCAAGGIACAA | MdDAM1 - `Anna' | SCKIROMKGVDLEDIDIDELOKLEKIVEASIGRVIOTKEEKIMSEVMALE |
| ATTCCALCCTACAA | MdDM1 (Colden deligious) | CCKI DOMICUDI EDI DI DEI OKI EKI VER CI CDVIOWKERKIMCEVMAI E |
| **** | MaDAMI_ GOIden_delicious | SCRERQMAGVDEEDEDEDEEQREEREVEASEGRVIQIREERIMSEVMALE |
| GATTCATCAGCTAC | | * |
| GATTCATCAGCTAC | | |
| GATTCATCAGCTAC | MdDAM1_'Dorsett_Golden' | KKGAELIEA NNQLSHRMVMYPRGDIGPEAILELENLNNIGEES <mark>V</mark> TSESTT |
| ***** | MdDAM1-`Anna' | KKGAELIEA NNQLSHRMVMYPRGDIGPEAILELENLNNIGEES <mark>V</mark> TSESTT |
| | MdDAM1 'Golden delicious' | <i>KKGAELIEA</i> NNQLSHRMVMYPRGDIGPEAILELENLNNIGEES <mark>M</mark> TSESTT |
| AACTTGAGGATAAG | | *************************************** |
| AACTTGAGGATAAG | | |
| AACTTGAGGATAAG | MdDAM1 /Dorsett Golden/ | NUTTOSNESI SI FODOSDI I SI KI C- |
| ***** | MdDAM1_ Dorsect_Gorden | NUTICONSCIENCEDITICINIC |
| | MODAMI Anna | NVIICSNSSLSLEDDCSDILSLALG= |
| GAAGACTTGGATCT | MaDAMI Golden_delicious, | NALLC2N22F2FFDDC2D1F2FVFG- |
| GAAGACTTGGATCT | | ************** |
| GAAGACTTGGATCT | | |
| ******** | | |
| | | |
| | | |

Figure S2. Single nucleotide polymorphisms detected in MdDAM1 sequence of low-chill cultivars.

The nucleotide (A) and amino acid (B) sequences of *MdDAM1* from 'Golden Delicious' was aligned to those of the low-chilling cultivars 'Dorsett Golden' and 'Anna'. The single nucleotide polymorphisms (SNPs) in (A) are highlighted in blue. The M (green) to V (blue) amino acid mutation at position 194 is indicated. The MADS domain (Interpro domain IPR002100) is highlighted in gray and the K-box domain indicated in bold characters.



Figure S3. Molecular characterization of the 35S: MdDAM1 transgenic lines.

Schematic representation of the pP35S:*MdDAM1* construct. The sequences of the primers shown are indicated in Table S2. The BamHI restriction site used to digest the genomic DNA for the Southern blot analysis is indicated (A). End-point PCR amplification of different regions of pP35S:*MdDAM1* construct in the transgenic lines 35S:MdDAM1#1-4 and the non-transformed control (NT control). The plasmid was used as a control for PCR amplification (B). Southern blot analysis using a *nptll* probe. The base pair (bp) ladder is indicated on the left (C).

| | 1 | | | | | | | | | | | | 130 |
|--|---|--|--|--|---|---|--|--|--|--|--|--|--|
| MdDAM1 | | AT | GAAGATCAAG | ATAAAAAAGA | TCGACTACTT | GCCGGCAAGG | CAGGTGACCT | TCTCAAAGAG | GAGAAGGGGG | ATTTTCAAGA | AAGCTGGAGA | GCTGTCGATT | CTGTGTGAAT |
| MdDAM4 | ATGGTGAAAA | GGATGAATGA | GAAGAT T AAG | ATCAGGAGGA | TCGACTACTT | GCCGGCAAGG | CAGGTGACCT | TCTCAAAGAG | GAGAAGAGGG | ATTTTCAAGA | AAGCTGAAGA | GCTGTCGATT | CTGTGTGAAT |
| Consensus | | aa | GAAGATCAAG | ATaAaaAaGA | TCGACTACTT | GCCGGCAAGG | CAGGTGACCT | TCTCAAAGAG | GAGAAGaGGG | ATTTTCAAGA | AAGCTGaAGA | GCTGTCGATT | CTGTGTGAAT |
| | | | | | | | | | | | | | |
| | 131 | | | | | | | | | | | | 260 |
| MdDAM1 | CTGAAGTTGC | TGTTATCATC | TTTTCTCAAA | CTGGCAAGCT | CTTTGATTTC | TCAAGCTCCA | GCACCAAGGA | TGTGATTGCA | AGGTACAATT | CACATATCGG | TGGGGAAAAA | TCGGATCAAC | CCACGATTCA |
| MdDAM4 | CTGAAGTTGC | TGTTATCATC | TTTTCTCAAA | CTGGCAAGCT | CTTTGATTAC | TCAAGCACCA | GTACCAAGGA | TGTGATTGCA | AGGTACAAAT | CACATACTGG | TGGGGAAAAA | TCGGATCAAA | TCACGCTTCA |
| Consensus | CTGAAGTTGC | TGTTATCATC | TTTTCTCAAA | CTGGCAAGCT | CTTTGATTaC | TCAAGCaCCA | GCACCAAGGA | TGTGATTGCA | AGGTACAAaT | CACATAccGG | TGGGGAAAAA | TCGGATCAAa | CCACGaTTCA |
| | | | | | | | | | | | | | |
| | 261 | | | | | | | | | | | | 390 |
| MdDAM1 | TCAGCTACAG | TTGGAGAAAG | AAAACAATAT | CAGGCTGAGG | AAGGAACTTG | AGGATAAGAG | TTGCAAGTTG | AGGCAGATGA | AGGGTGTGGA | CCTTGAAGAC | TTGGATCTGG | ATGAACTACA | GAAGTTAGAA |
| MdDAM4 | CCAACTGCAG | TCGGAGAAAG | AAAACACGAT | CAGGCTGAGT | AAGGAACTTG | AGGATAAGAC | CCGCAAGCTG | AGGCATATGA | AGGGTGAGGA | CCTTCAAGAC | TTGGATCTGG | ATCAACTGAA | CAAGTTAGAA |
| Consensus | CCAaCTaCAG | TCGGAGAAAG | AAAACAagAT | CAGGCTGAGg | AAGGAACTTG | AGGATAAGAC | CCGCAAGCTG | AGGCAGATGA | AGGGTGaGGA | CCTTCAAGAC | TTGGATCTGG | ATCAACTaaA | CAAGTTAGAA |
| | | | | | | | | | | | | | |
| | 201 | | | | | | | | | | | | 500 |
| MADAMI | 391 | AACCAACCOM | maccacement | 20002222000 | | C300030C3C0 | CACCERTATICC | CACHINGACAA | | CACCHCARAC | | CCA CCMA A CC | 520 |
| MdDAM1 | 391 AAATTGGTGG | AAGCAAGCCT | TGGCCGTGTG | ATTCAAACTA | AGGAAGAAAA | GATTATGAGT | GAGGTTATGG | CACTTGAGAA | AAAGGGAGCT | GAGCTGATAG | AAGCTAACAA | CCAGCTAAGC | 520 CACAGGATGG |
| MdDAM1 MdDAM4 | 391 AAATTGGTGG AAATTGGTGG | AAGCAAGCCT AAGTAAGCAT | TGGCCGTGTG TGGCCGTGTA | АТТСАААСТА АТАААААСТА | AGGAA G AAAA AGGAAAAAAA | GATTATGAGT GATAATGAGT | GAGGTTATGG GAGATTATGG | CACTTGAGAA CACTTACGAA | AAAGGGAGCT CAAGGGAGCT | GAGCTGATAG GAGCTTATAG | AAGCTAACAA AAGCTAACAA | CCAGCTAAGC CCAACTAAAG | 520 CACAGGATGG CAGAGGTTGG |
| MdDAM1 MdDAM4 Consensus | 391 AAATTGGTGG AAATTGGTGG AAATTGGTGG | AAGCAAGCCT AAGTAAGCAT AAGCAAGCaT | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTa | АТТСАААСТА АТАААААСТА АТааАААСТА | AGGAA G AAAA AGGAAAAAAA AGGAAaAAAA | GATTATGAGT GATAATGAGT GATAATGAGT | GAGGTTATGG GAGATTATGG GAGATTATGG | CACTTGAGAA CACTTACGAA CACTTaaGAA | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT | GAGCTGATAG GAGCTTATAG GAGCTGATAG | ААССТААСАА ААССТААСАА ААССТААСАА | CCAGCTAAGC CCAACTAAAG CCAACTAAac | 520 CACAGGATGG CAGAGGTTGG CACAGGaTGG |
| MdDAM1 MdDAM4 Consensus | 391 AAATTGGTGG AAATTGGTGG AAATTGGTGG | AAGCAAGCCT AAGTAAGCAT AAGcAAGCaT | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTa | АТТСАААСТА АТАААААСТА АТааАААСТА | AGGAA G AAAA AGGAA A AAAA AGGAA a AAAA | GATTATGAGT GATAATGAGT GATaATGAGT | GAGGTTATGG GAGATTATGG GAGaTTATGG | CACTTGAGAA CACTTACGAA CACTTaaGAA | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT | GAGCTGATAG GAGCTTATAG GAGCTGATAG | ААGСТААСАА ААGСТААСАА ААGСТААСАА | CCAGCTAAGC CCAACTAAAG CCAaCTAAac | 520 CACAGGATGG CAGAGGTTGG CAcAGGaTGG 650 |
| MdDAM1 MdDAM4 Consensus MdDAM1 | 391 AAATTGGTGG AAATTGGTGG AAATTGGTGG 521 TGATGTATCC | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTa | АТТСАААСТА АТАААААСТА АТааАААСТА | AGGAAGAAAA AGGAAAAAAA AGGAAaAAAA | GATTATGAGT GATAATGAGT GATAATGAGT | GAGGTTATGG GAGATTATGG GAGaTTATGG | CACTTGAGAA CACTTACGAA CACTTaaGAA | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT | GAGCTGATAG GAGCTTATAG GAGCTGATAG | AAGCTAACAA AAGCTAACAA AAGCTAACAA | CCAGCTAAGC CCAACTAAAG CCAaCTAAac | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 |
| MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 | 391 AAATTGGTGG AAATTGGTGG 521 TGATGTATCC TGATGTATCC | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT CAGAGGA | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTA GATATCGGAC | ATTCAAACTA ATAAAAACTA ATaaAAACTA CGGAGGCCAT | AGGAAGAAAA AGGAAAAAAA AGGAAaAAAA CCTGGAGTTG | GATTATGAGT GATAATGAGT GATAATGAGT GAAAACCTGA | GAGGTTATGG GAGATTATGG GAGATTATGG ATAATATTGG | CACTTGAGAA CACTTACGAA CACTTAAGAA AGAAGAAAAGC | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT ATGACATCTG | GAGCTGATAG GAGCTTATAG GAGCTGATAG AATCAACCAC | AAGCTAACAA AAGCTAACAA AAGCTAACAA AAATGTCACC | CCAGCTAAGC CCAACTAAAG CCAaCTAAac ACCTGCTCCA | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 ACAGCTCTCT GCAGTGCTCT |
| MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 Consensus | 391 AAATTGGTGG AAATTGGTGG AAATTGGTGG 521 TGATGTATCC TGATGTTATC TGATGTAACC | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT CAGAGGA CGCTGGAGGA | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTA GATATCGGAC GATATCGAAC | ATTCAAACTA ATAAAAACTA ATaaAAACTA CGGAGGCCAT CGGCGGCGAT | AGGAAGAAAA AGGAAAAAAA AGGAAAAAAA CCTGGAGTTG CATGGAGTTG CATGGAGTTG | GATTATGAGT GATAATGAGT GATAATGAGT GAAAACCTGA GAAAACCTGA | GAGGTTATGG GAGATTATGG GAGATTATGG ATAATATTGG ATAATGTTGG ATAATGTTGG | CACTTGAGAA CACTTACGAA CACTTAAGAA AGAAGAAAGC AGAAGAAAGC AGAAGAAAGC | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT ATGACATCTG ATGACATCTG ATGACATCTG | GAGCTGATAG GAGCTTATAG GAGCTGATAG AATCAACCAC AATCAGCCAC | AAGCTAACAA AAGCTAACAA AAGCTAACAA AAATGTCACC AAATGTCACC | CCAGCTAAGC CCAACTAAAG CCAACTAAAC ACCTGCTCCA ACCTGCTCCA | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 ACAGCTCTT GCAGTGCTCT aCAGCGCTCT |
| MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 Consensus | 391 AAATTGGTGG AAATTGGTGG AAATTGGTGG 521 TGATGTATCC TGATGTATCC TGATGTAACC | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT CAGAGGA CGCTGGAGGA CaGAGGA | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTA GATATCGGAC GATATCGAAC GATATCGAAC | ATTCAAACTA ATAAAAACTA ATaaAAACTA CGGAGGCCAT CGGCGGCGAT CGGaGGCCAT | AGGAAGAAAA AGGAAAAAAA AGGAAAAAAA CCTGGAGTTG CATGGAGTTG CATGGAGTTG | GATTATGAGT GATAATGAGT GATAATGAGT GAAAACCTGA GAAAACCTGA GAAAACCTGA | GAGGTTATGG GAGATTATGG GAGATTATGG ATAATATTGG ATAATGTTGG ATAATATTGG | CACTTGAGAA CACTTACGAA CACTTAAGAA AGAAGAAAGC AGAAGAAAGC AGAAGAAAGC | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT ATGACATCTG ATGACATCTG ATGACATCTG | GAGCTGATAG GAGCTTATAG GAGCTGATAG AATCAACCAC AATCAACCAC AATCAACCAC | AAGCTAACAA AAGCTAACAA AAGCTAACAA AAATGTCACC AAATGTCACC AAATGTCACC | CCAGCTAAGC CCAACTAAAG CCAACTAAAC ACCTGCTCCA GCCTGCTCCA aCCTGCTCCA | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 ACAGCTCTCT GCAGTGCTCT aCAGCgCTCT |
| MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 Consensus | 391 AAATTGGTGG AAATTGGTGG AAATTGGTGG 521 TGATGTATCC TGATGTATCC TGATGTAACC 651 | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT CAGAGGA CGCTGGAGGA CaGAGGA | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTA GATATCGGAC GATATCGAAC GATATCGAAC | ATTCAAACTA ATAAAAACTA ATaaAAACTA CGGAGGCCAT CGGCGGCGAT CGGaGGCCAT | AGGAAGAAAA AGGAAAAAAA AGGAAAAAAA CCTGGAGTTG CATGGAGTTG CATGGAGTTG | GATTATGAGT GATAATGAGT GATAATGAGT GAAAACCTGA GAAAACCTGA GAAAACCTGA 705 | GAGGTTATGG GAGATTATGG GAGATTATGG ATAATATTGG ATAATGTTGG ATAATATTGG | CACTTGAGAA CACTTACGAA CACTTAAGAA AGAAGAAAGC AGAAGAAAGC AGAAGAAAGC | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT ATGACATCTG ATGACATCTG ATGACATCTG | GAGCTGATAG GAGCTTATAG GAGCTGATAG AATCAACCAC AATCAGCCAC AATCAACCAC | AAGCTAACAA AAGCTAACAA AAGCTAACAA AAATGTCACC AAATGTCACC AAATGTCACC | CCAGCTAAGC CCAACTAAAG CCAACTAAAG ACCTGCTCCA GCCTGCTCCA aCCTGCTCCA | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 ACAGCTCTCT GCAGTGCTCT aCAGCgCTCT |
| MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 Consensus MdDAM1 | 391 AAATTGGTGG AAATTGGTGG 521 TGATGTATCC TGATGTATCC TGATGTAACC 651 TTCCCTTGAA | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT CAGAGGA CGCTGGAGGA CaGAGGA GATGATTGCT | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTA GATATCGGAC GATATCGAAC GATATCGAAC CCGACATCTT | ATTCAAACTA ATAAAAACTA ATaaAAACTA CGGAGGCCAT CGGCGGCGAT CGGGGGCCAT GTCTCTCAAA | AGGAAGAAAA AGGAAAAAAA AGGAAAAAAA CCTGGAGTTG CATGGAGTTG CATGGAGTTG CTGGGGTGA | GATTATGAGT GATAATGAGT GATAATGAGT GAAAACCTGA GAAAACCTGA GAAAACCTGA 705 | GAGGTTATGG GAGATTATGG GAGATTATGG ATAATATTGG ATAATGTTGG ATAATATTGG | CACTTGAGAA CACTTACGAA CACTTAAGAA AGAAGAAAGC AGAAGAAAGC AGAAGAAAGC | AAAGGGAGCT CAAGGGAGCT aAAGGGAGCT ATGACATCTG ATGACATCTG ATGACATCTG | GAGCTGATAG GAGCTTATAG GAGCTGATAG AATCAACCAC AATCAACCAC AATCAACCAC | AAGCTAACAA AAGCTAACAA AAGCTAACAA AAATGTCACC AAATGTCACC AAATGTCACC | CCAGCTAAGC CCAACTAAAG CCAACTAAAC ACCTGCTCCA GCCTGCTCCA aCCTGCTCCA | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 ACAGCTCTT GCAGTGCTCT aCAGcgCTCT |
| MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 Consensus MdDAM1 MdDAM4 | 391 AAATTGGTGG AAATTGGTGG 521 TGATGTATCC TGATGTATCC 651 TTCCCTGAA | AAGCAAGCCT AAGTAAGCAT AAGCAAGCAT CAGAGGA CGCTGGAGGA CaGAGGA GATGATTGCT GATGACTGCT | TGGCCGTGTG TGGCCGTGTA TGGCCGTGTA GATATCGGAC GATATCGAAC GATATCGAAC CCGACATCTT CCGACATCTT | ATTCAAACTA ATAAAACTA ATaaAAACTA CGGAGGCCAT CGGGGGGCGAT CGGGGGCCAT GTCTCTCAAA GTCTCTCAAA | AGGAAGAAAA AGGAAAAAAA AGGAAAAAAA CCTGGAGTTG CATGGAGTTG CATGGAGTTG CTGGGGTGA CTGGGGGTAC | GATTATGAGT GATAATGAGT GAAAACCTGA GAAAACCTGA GAAAACCTGA 705 CTTAG | GAGGTTATGG GAGATTATGG ATAATATTGG ATAATATTGG ATAATGTGG ATAATATTGG | CACTTGAGAA CACTTACGAA CACTTAAGAA AGAAGAAAGC AGAAGAAAGC AGAAGAAAGC | AAAGGAAGCT CAAGGGAGCT aAAGGGAGCT ATGACATCTG ATGACATCTG ATGACATCTG | GAGCTGATAG GAGCTTATAG GAGCTGATAG AATCAACCAC AATCAACCAC AATCAACCAC | AAGCTAACAA AAGCTAACAA AAGCTAACAA AAATGTCACC AAATGTCACC AAATGTCACC | CCAGCTAAGC CCAACTAAAG CCAACTAAAC ACCTGCTCCA GCCTGCTCCA ACCTGCTCCA | 520 CACAGGATGG CAGAGGTTGG CACAGGATGG 650 ACAGCTCTTT GCAGTGCTCT aCAGCgCTCT |

Β



Figure S4. Similarity between MdDAM1 and MdDAM4 and sRNA abundance mapping on MdDAM4

Alignment of *MdDAM1* and *MdDAM4* shows a high similarity at the nucleotide level. Similar nucleotides are indicated in red (A). The abundance of small RNAs (sRNA) generated in 35S:*MdDAM1* transgenic lines and mapping on *MdDAM4* with a perfect match is indicated (B). The number of small RNA (sRNA) ranging from 19 nt to 24 nt mapping in sense (+ strand) or in antisense (- strand) are indicated by the black and blue lines, respectively. The flat lines shown in the NT control and in line P35S:*MdDAM1*#3 indicate that there were no sRNAs mapping on *MdDAM4*.