SUPPLEMENTAL INFORMATION

FIGURE LEGENDS

<u>Fig. S1.</u> **ARD1 genetically interacts with AGB1.** *A*. Stomatal index (SI) in Col-0, *agb1-2*, and 35S:SGB3 in *agb1-2* plants. *agb1-2* plants display an SI significantly higher than Col-0. SI is rescued by the overexpression of SGB3 in *agb1-2* plants. *** p<0.0001 *B*. Quantification of hypocotyl length and hook angle in plants overexpressing SGB3 in Col-0 as shown in Fig. 1D.

<u>Fig. S2.</u> **ARD1 is an aci-reductone dioxygenase protein.** *A*. Multiple sequence alignment of ARD proteins (ARATH, Arabidopsis; ORYSJ, rice; DROME, drosophila; ANOGA, mosquito; HUMAN, human; BOVIN, bovine; RAT, rat; MOUSE, mouse; XENLA, xenopus; CHICK, chicken; BRARE, fish; YEAS2, yeast; KLEP7, bacteria). Circled star indicates four conserved metal-binding residues. *B*. Homology model of ARD1 threaded into the crystal structure of MmADI1 (PDB ID 1VR3, homology model score = 0.74). Magenta and pink residues are identical and similar between the two, respectively, while cyan residues are plant-specific. The four metal-binding residues are navy, and the bound metal ion is red.

<u>Fig. S3.</u> **ARD1 and AGB1 expression.** *A*. ARD1 is localized in the cytoplasm of both wildtype and *agb1-2 Arabidopsis* protoplasts. Bar = 10 μ m. Arrows indicate fluorescent regions surrounding the chloroplasts, confirming that ARD1 is localized in the cytoplasm. *B*. The anti-AGB1 antibody detects AGB1 in AGB1-infected Sf9 cells, but does not detect Sf9 G β protein in uninfected Sf9 cells or those infected with an unrelated protein (infection control).

Fig. S4. **ARD1 enzymatic activity in the presence and absence of purified wildtype Gβγ.** *A*. Enzymatic activity (measured by the absorbance of the substrate at 305 nm) of ARD1 alone (ARD:Gβγ =1:0) and in the presence of an increasing concentration of purified Gβγ protein (molar ratio indicated). *B*. The initial rate of the ARD1 enzyme from the graph shown in (A) +/- standard deviation in the presence of five different concentrations of Gβγ. The rates are expressed in moles of substrate per mole of enzyme per second +/- standard deviation and were recorded as initial rates. These rates account for the average oxygen-induced decay rate (baseline, see Experimental Procedures).

<u>Fig. S5.</u> **Plants contain four ARD proteins.** *A*. Multiple sequence alignment of the four *Arabidopsis* ARD proteins (red=ARD domain, underline = non-canonical NES, gray=putative metal binding residues). *B*. Phylogeny of metazoan ARD proteins generated in MrBayes. Diatom (Thalassiosira) served as an outgroup. Species indicated are as follows: ARATH, Arabidopsis; ORYSA, rice; DROME, drosophila; ANOGA, mosquito; HUMAN, human; BOVIN, bovine; RAT, rat; MOUSE, mouse; XENLA, xenopus; CHICK, chicken; BRARE, fish.

<u>Fig. S6.</u> **AGB1 and ARDs interact physically.** *A*. Growth of yeast strain AH109 containing the geness indicated (AtG β 1 γ 1 alone or AtG β 1 γ 1 + ARD2, ARD3, or ARD4) on yeast dropout media missing leucine, tryptophan, and histidine. This selects for a positive interaction between each of the two genes, resulting in no growth for the strain containing AtG β 1 γ 1 alone and positive growth for the strains containing both AtG β 1 γ 1 and one of the three ARDs. *B*. 6x-His-tagged G $\beta\gamma$ was pulled down with ARD-GST (ARD2, ARD3, or ARD4) on a glutathione resin and detected by immunoblotting with anti-AGB1. *C*. Bifluorescence molecular complementation in tobacco leaf epidermal cells. Bar = 20 μ M.

| Table S1. Primers used for PCR reactions. | Names of primers used for RT-PCR (Fig. 2) are denoted in |
|---|--|
| parenthesis. | |

| Name | Purpose | Sequence 5'-3' |
|-----------------|---------------------------------------|------------------------------|
| 4g14716 gtwfd1 | | |
| (D) | ARD1 cDNA Gateway cloning forward | CACCATgggTgAAgCggTCAAggATg |
| 4g14716 rv2 | ARD1 reverse full-length | CTAAgCCgAggCATTgATCATgAAg |
| 4g14716 | | AgCCgAggCATTgATCATgAAgTTATC |
| nostpfusrv | ARD1 reverse without stop codon | gACATA |
| ARD2 LP (C) | ARD1 Internal reverse primer (exon 3) | TAACCACgAgATTCACggATC |
| ARD2+3 rt-F (B) | ARD1 Internal forward primer (exon 4) | TgCCCggAAAAgCTTCCAAACTA |
| ARD2 rt-R (A) | ARD1 RT-PCR reverse primer | AgCCgAggCATTgATCATgAAgT |
| 4g14710 gtwfd | ARD2 cDNA Gateway cloning forward | CACCATgggTgAAgTggTTAAggATg |
| 4g14710 rv2 | ARD2 reverse full-length | CTAggCTgACgCgTCTATgACACCA |
| 4g14710 | | |
| rvnostp | ARD2 reverse without stop codon | ggCTgACgCgTCTATgACACCACCT |
| 2g26400 gtwfd | ARD3 cDNA Gateway cloning forward | CACCATgggTgAAgCCgCTAAggATC |
| 2g26400 rv2 | ARD3 reverse full-length | TTACgCTgAAgCgTCTATgTTACgg |
| 2g26400 | | |
| rvnostp | ARD3 reverse without stop codon | CgCTgAAgCgTCTATgTTACgggTCC |
| 5g43850 gtwfd | ARD4 cDNA Gateway cloning forward | CACCATggCTCTCgAggCATggTTTA |
| | | TTAATgTgCTTTAACggTTTCTCCAAA |
| 5g43850 rv2 | ARD4 reverse full-length | СТТ |
| 5g43850 | | ATgTgCTTTAACggTTTCTCCAAACTTg |
| rvnostp | ARD4 reverse without stop codon | TAggT |





| Fig S2 | | |
|--------------------------|--|-----|
| Δ | * | |
| A ARD1_ARATH | MGEAVKDGREEVIQAWYMDDBEEDQRLPHHKDPKEFVELDKLAE-LGV-LSWRLDADNYETDEDLKKIRESRGY | 72 |
| ARD1_ORYSJ | MENEFODGETEVIEAWYMDD <mark>S</mark> EEDORLPHHR <mark>E</mark> PKEFIHVDKLTE-LGV-ISWRLNPDNWENCENLKRIREARGY | 72 |
| MTND_DROME | MVQVWYMDTBETDQRLEHHRNPPAYLELDDLYQKTGV-BYFKINADEYQSDNTLTELRAKRGY | 62 |
| Q7Q6X6_ANOGA | MVRAWFMDNBPTDQRLEHQLDPPKFLTLDELFKSTGV-EYFKINIPTYDTDGVLGKIRNDRGY | 62 |
| MIND_HUMAN | MVQAWYMDDAPGDPROPHRPDPGREVGLEOLRR-LGV-LYWKLDADKYENDPBLEKIRKERNY | 61 |
| MTND BOVIN | | 61 |
| MTND MOUSE | | 61 |
| MTND XENLA | MYOAWYMDDSAEDORKPHRLOODYPYSLEOLKA-LGY-DSLSLDADRYESDPBLAKIRKENNY | 61 |
| MTND CHICK | MVEAWYMDBSQEDQRAPHRLOPNRAVSLEQLRR-LGV-AYRRLDADNYETDPRLKEIREAENY | 61 |
| MTND_BRARE | MEVFEAWYMDEESGEDORLPHKLSPNOPVSVOOLEH-IGV-FHWKLNADIYENDPELOKIREEKGY | 64 |
| tr C7GLB0 C7GLB0 YEAS2 | MVKVYIHDNKVDSDYRAPHNSGTELSLDELAK-LGV-IYKYCANEEEVNEIAROREY | 55 |
| A6T672_MTND_KLEP7 | MEALTLFSVTDPOTPVWHSTDAKAIQAQLNAKGVRFERWQADRDLGANPSPETVIAAYQHAIDKLVABKGY | 71 |
| ruler | 11020304050607080 | |
| | | |
| | • | |
| | | 147 |
| ARD1_ARAIN ARD1_ORYSJ | SYUDICUCC | 147 |
| MIND DROME | TYDDETTCSE KCLPDY ANKLKAFFTEHLHTDEETELTLEGS GYPDYEDN EDNULET KVVKGDLT I TPAGTYHEPT | 137 |
| Q7Q6X6 ANOGA | SYEDEITCSEACLPDYANKLKSFFTEHLHTDEEIRLVLDGSGYPDVRNGP-DDAWVRIEVVAGDLIIIPSGIYHRFT | 138 |
| ~ MTND HUMAN | SWMDIITICKDKLPNYEEKIKMFYEEHLHLDDEIRYILDCSGYPDVRDKEDQWIRIFMEKGDMVTLPAGIYHRFT | 136 |
| MTND_BOVIN | SWVDIITISKDKLPNYBEKIKMFFEEHLHLDEEIRYILDGSGYFDVRDQEDRWIRISMEKGDMITLPAGIYHRFT | 136 |
| MTND_RAT | SWMDIITICKDSLPNYEEKIKMFFEEHLHLDEEIRYILEGSGYFDVRDKEDKWIRISMEKGDMITLPAGIYHRFT | 136 |
| MTND_MOUSE | SWMDIITICKDTLPNYBEKIKMFFEBHLHLDBBIRYILEGSGYPDVRDKEDKWIRISMBKGDMITLPAGIYHRFT | 136 |
| MTND_XENLA | TWMDIITIHKDTMPNYBEKLKIFYEBHLHLDDBIRVILLEGSGYPDVRDQKDKWIRIFMQKGDMITLPAGIYHRPT | 136 |
| MIND_CHICK | SUMDIVTIHKDKLPNYESKIKTFYESHLHLDDBINYILDGSGYPDVRKDDKWIRISMEKGDMITLPAGIYHRFT | 120 |
| tr C7CLPO C7CLPO VEAC | BERNIILING PURLPHINKLAMFIBERLEUDBIKILDESSIFVER - SDAWIKIAVSAGDLI BAGIIRFI | 125 |
| A6T672 MTND KLED7 | ANALYVNIC BEGER SEASTABLAIFIYBALDA DABIAL DABAAL PURAA IYAMA IYAMA KU SU SUBLAIFIYAL AAF | 144 |
| ruler | 90100110120130140150160 | |
| | | |
| | | |
| | | |
| ARD1_ARATH | VDSDNYIKAMRLFVGEPVWTPYNRPHDHLPARKEYVDNFMINASA | |
| ARD1_ORYSJ | LDTDNYIKAMRIFVGDPVWTPYNRPHDHLPARKEFLAKLLKSEGENQAVEGF199 | |
| MTND_DROME | LDTNNFIFTRFYFVGEPVWAPHNRPADEMDCRKSYIKHQSBNFVQFNKV186 | |
| Q7Q6X6_ANOGA | LUMKNYIKAKRYFYGEFYWLFYNRP - ADKMDCRKDYLKRLE - AGFAA - 183 | |
| MIND HOMAN | | |
| MTND PAT | | |
| MTND MOUSE | LDERNYVEAMELFVGEDVWTDVNED - ADHFDAEVOYMSFLE | |
| MTND XENLA | LDENNYVKAMRLFVGDPVWTPFNRP-ADNCBAREKVVOPLA-0TA-0TA-179 | |
| MTND CHICK | LDENNYVKAMRLFVGEPVWTAYNRP-ADDFPARKOYMKFLA | |
| MTND BRARE | VDESNYTKAMRLFVGEPVWKAYNRPADDFDIRKBYVNSLR | |
| tr C7GLB0 C7GLB0 YEAS2 | LTTSNHIKALRLFKDEPKWQAINRSNQADSLPVRKDYIALINQY | |
| A6T672_MTND_KLEP7 | MGSEPNFTAIRIFDNPEGWIAQFTGDDIASAYPRLA | |
| - ruler | | |





В

Fig S3

Α

A Col-0 agb1-2

⁴ Uninfected Infection AGB₁ infected 30 kDa



| Fig S5 A | | |
|--------------------|---|-----|
| ARD1 | MGEAVKDGREEVIQAWYMDDSEEDQRLPHHKDPKEFVSLDKLAELGVLSWRLDADNYETD | 60 |
| ARD3 | MGEAAKDQTEEVIQAWYLDNKEEDQKLPHHKDPKEFVSLDKLAELGVLCWRLDADNYETD | 60 |
| ARD2 | ${\tt MGEVVKDGREEVIQAWYMDDSEEDQRLPHHKDPKEFLSLDKLAELGVLSWRLDADNYETD$ | 60 |
| ARD4 | MALEAWFMDDSNEDQRLPHHRNPKELVSLDYLAELGVLYWKLNPENYEND | 50 |
| | ···**··**·**************************** | |
| ARD1 | ED <u>LKKIRESRGY</u> SYMDFCEVCPEKLPNYEVKVKSFFEEHLHTDEEIRYCVAGTGYFDVRD | 120 |
| ARD3 | EELKRIRESRGYSYMDLCEVCPEKLPNYEEKVKMFFEEHLHIDEEIRYCLAGSGYFDVRD | 120 |
| ARD2 | EDLKKIRESRGYSYMDFCEVCPEKLPNYEVKVKSFFEEHLHTDEEIRYCVAGSGYFDVRD | 120 |
| ARD4 | SELSKIREDRGYDYMDLLDLCPEKVSNYEEKLKNFFTEHIHKDEEIRYCLAGSGYFDVRD | 110 |
| | ·:*·:*** *** *** · ::**** * *** *** *** | |
| ARD1 | RNEAWIRVLVKKGGMIVLPAGIYHRFTVDSDNYIKAMRLFVGEPVWTPYNRPHDHLPARK | 180 |
| ARD3 | LNDIWIRVWVKKGGLIVFPAGIYHRFTVDSDNYMKAMRLFVGGPVWTAYNRPHDHLPARK | 180 |
| ARD2 | RNEAWIRVWVKKGGMIVLPAGIYHRFTVDSDNYIKAMRLFVGEPVWTPYNRPHDHLPARK | 180 |
| ARD4 | KDDRWIRIWMQPGDLIVLPAGIYHRFTLDASNYIKLMRLFVGEPVWTPYNRPQEEHPVRK | 170 |
| | :: ***: :: *.:**:*******:*:*:********** | |
| ARD1 | EYVDNFMINASA 192 | |
| ARD3 | AYMKKFLKVIGDRNIDASA 199 | |
| ARD2 | EYIDNFVKVNEGGVIDASA 199 | |
| ARD4 | KYIHGLTYKFGETVKAH 187 | |
| _ | *:. : | |
| R | | |



