SUPPLEMENTAL INFORMATION

FIGURE LEGENDS

Fig. S1. **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.

Fig. S2. **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.

Fig. S3. **ARD1 and AGB1 expression.** *A.* ARD1 is localized in the cytoplasm of both wildtype and *agb1-2 Arabidopsis* protoplasts. Bar = 10 μm. 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 Gβ 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).

Fig. S5. **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.

Fig. S6. **AGB1 and ARDs interact physically.** *A.* Growth of yeast strain AH109 containing the genes indicated (AtGβ1γ1 alone or AtGβ1γ1 + 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 AtGβ1γ1 alone and positive growth for the strains containing both AtGβ1γ1 and one of the three ARDs. *B.* 6x-His-tagged Gβγ 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 μM.

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 nostpfusrv	ARD1 reverse without stop codon	AgCCgAggCATTgATCATgAAgTTATC 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
5g43850 rv2	ARD4 reverse full-length	TTAATgTgCTTTAACggTTTCTCCAAA CTT
5g43850 rvnostp	ARD4 reverse without stop codon	ATgTgCTTTAACggTTTCTCCAAACTTg TAggT

Fig S1

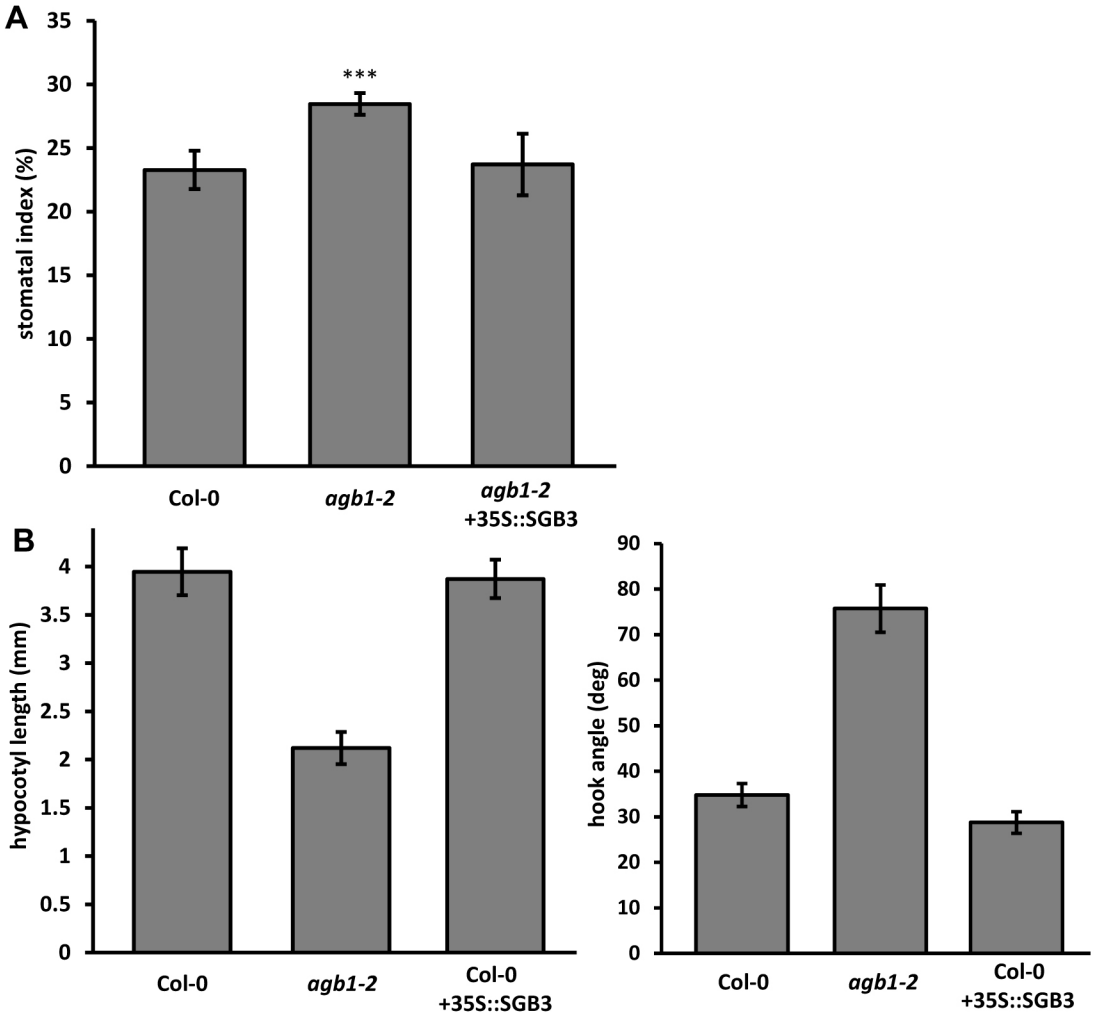
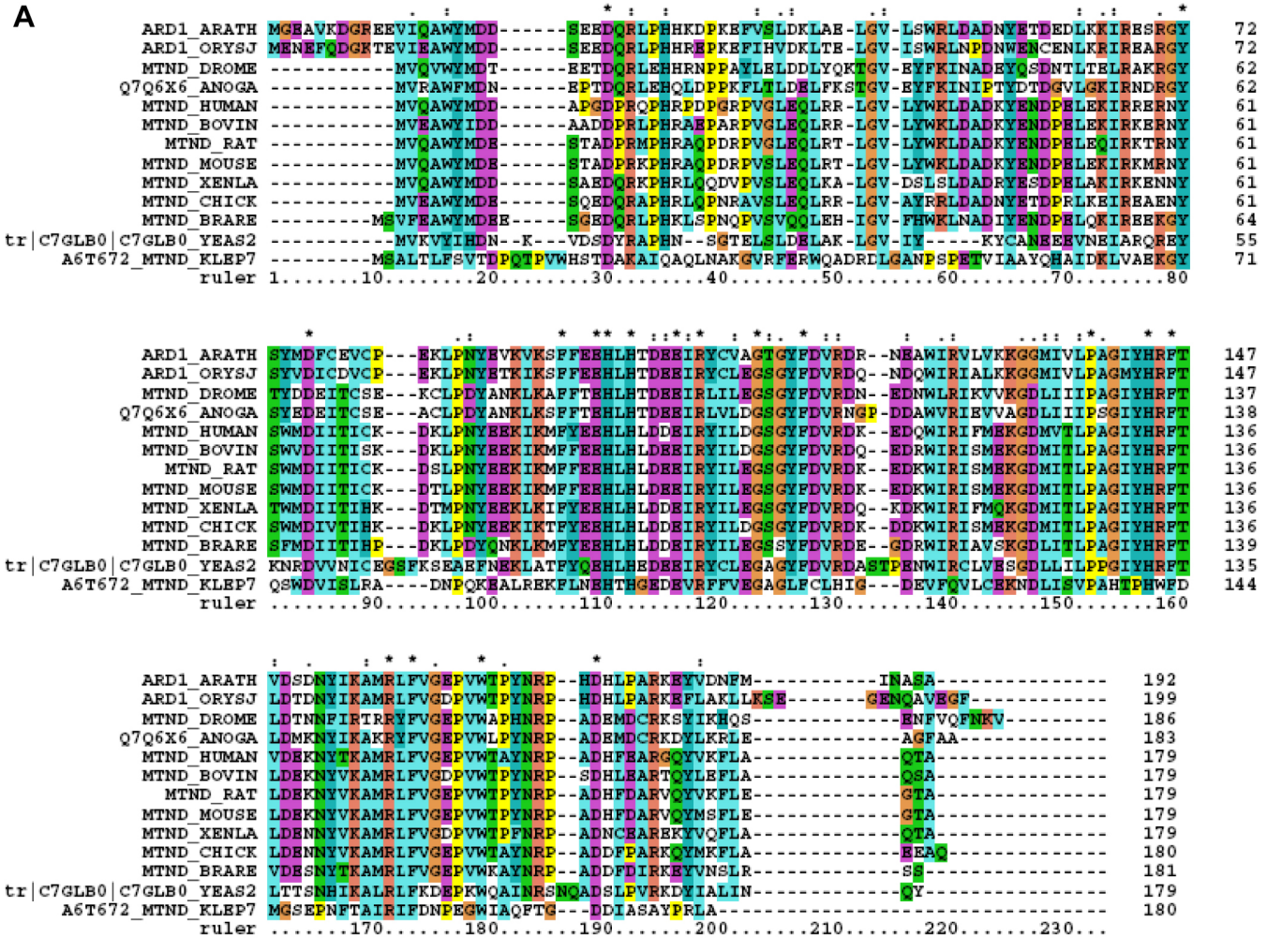

A
stomatal index (%)
35
30
25
20
15
10
5
0
***
Col-0
agb1-2
agb1-2
+35S::SGB3
B
hypocotyl length (mm)
4
3.5
3
2.5
2
1.5
1
0.5
0
Col-0
agb1-2
Col-0
+35S::SGB3
hook angle (deg)
90
80
70
60
50
40
30
20
10
0
Col-0
agb1-2
Col-0
+35S::SGB3

Fig S2

A


ARD1_ARATH
ARD1_ORYSJ
MTND_DROME
Q7Q6X6_ANOGA
MTND_HUMAN
MTND_BOVIN
MTND_RAT
MTND_MOUSE
MTND_XENLA
MTND_CHICK
MTND_BRARE
tr|C7GLB0|C7GLB0_YEAS2
A6T672_MTND_KLEP7
ruler

B

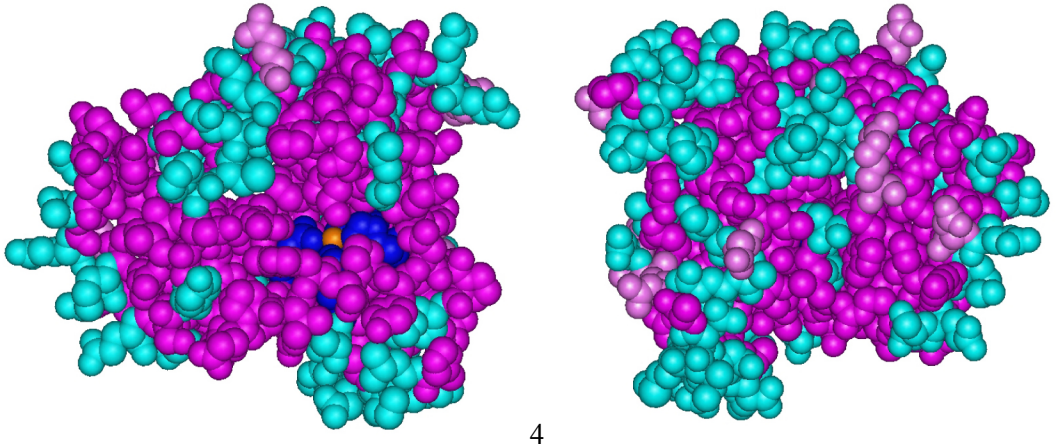
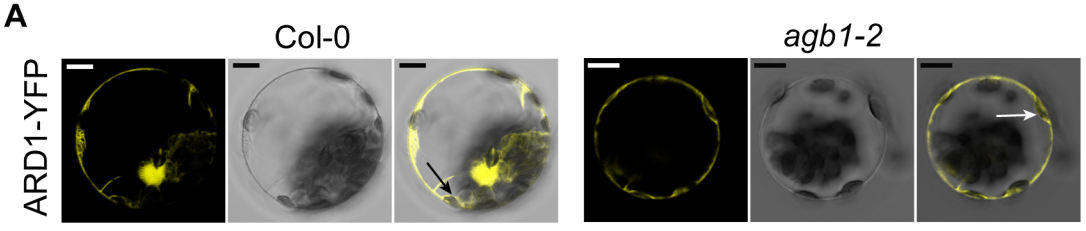


Fig S3


A
Col-0
agb1-2
ARD1-YFP

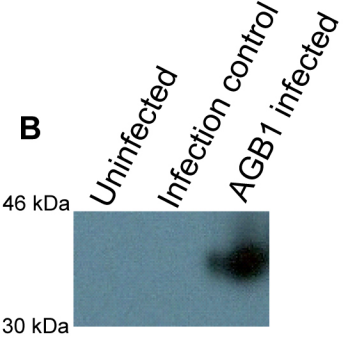
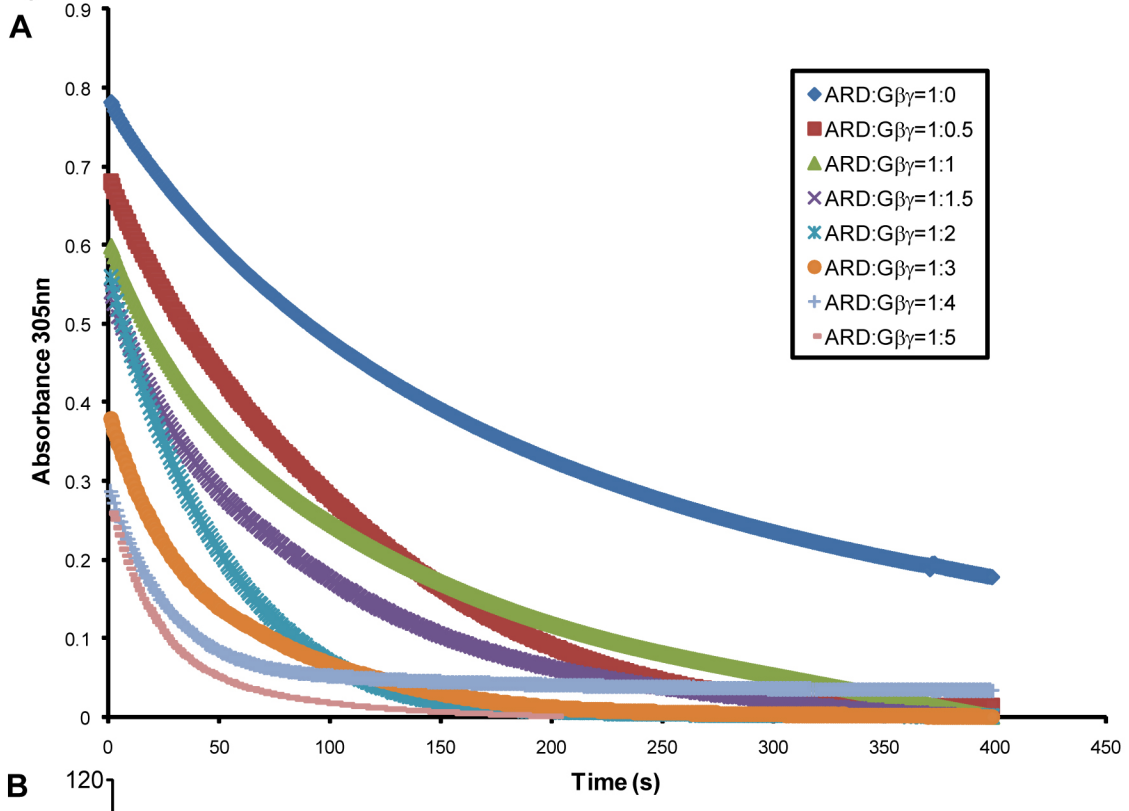

B
Uninfected
Infection control
AGB1 infected
46 kDa
30 kDa

Fig S4


A
Absorbance 305nn
Time (s)
ARD:Gβγ=1:0
ARD:Gβγ=1:0.5
ARD:Gβγ=1:1
ARD:Gβγ=1:1.5
ARD:Gβγ=1:2
ARD:Gβγ=1:3
ARD:Gβγ=1:4
ARD:Gβγ=1:5

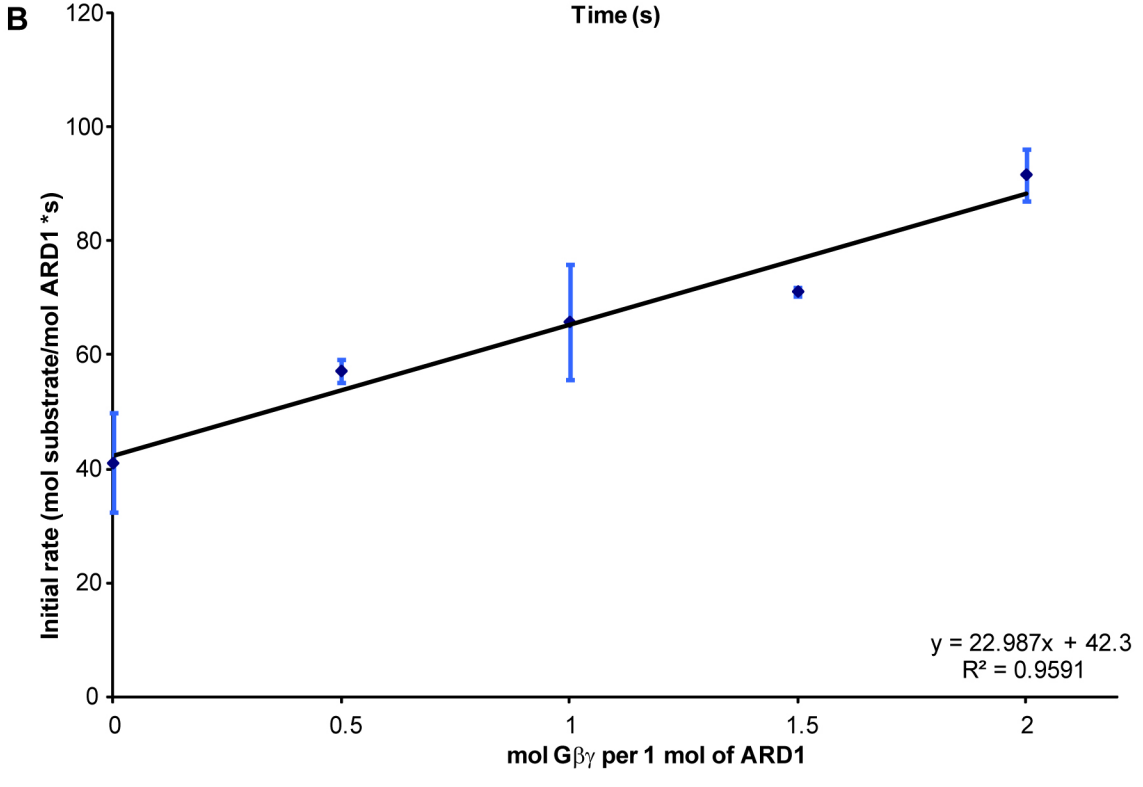

B
Initial rate (mol substrate/mol ARD1 *s)
mol Gβγ per 1 mol of ARD1
y = 22.987x + 42.3
R² = 0.9591

Fig S5

A

```
ARD1      MGEAVKDGREEVIQAWYMDDSEEDQRLPHHKDPKEFVSLDKLAELGVLSWRLDADNYETD  60
ARD3      MGEAAKDQTEEVIQAWYLDNKEEDQKLPHHKDPKEFVSLDKLAELGVLCWRLDADNYETD  60
ARD2      MGEVVKDGREEVIQAWYMDDSEEDQRLPHHKDPKEFLSLDKLAELGVLSWRLDADNYETD  60
ARD4      ----------MALEAWFMDDSNEDQRLPHHRNPKELVSLDYLAELGVLYWKLNPENYEND  50
                    .::**::*:.:***:****::***::*** ******* *:*:.:***.*

ARD1      EDLKKIRESRGYSYMDFCEVCPEKLPNYEVKVKSFFEEHLHTDEEIRYCVAGTGYFDVRD 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
           *:. :
```

B

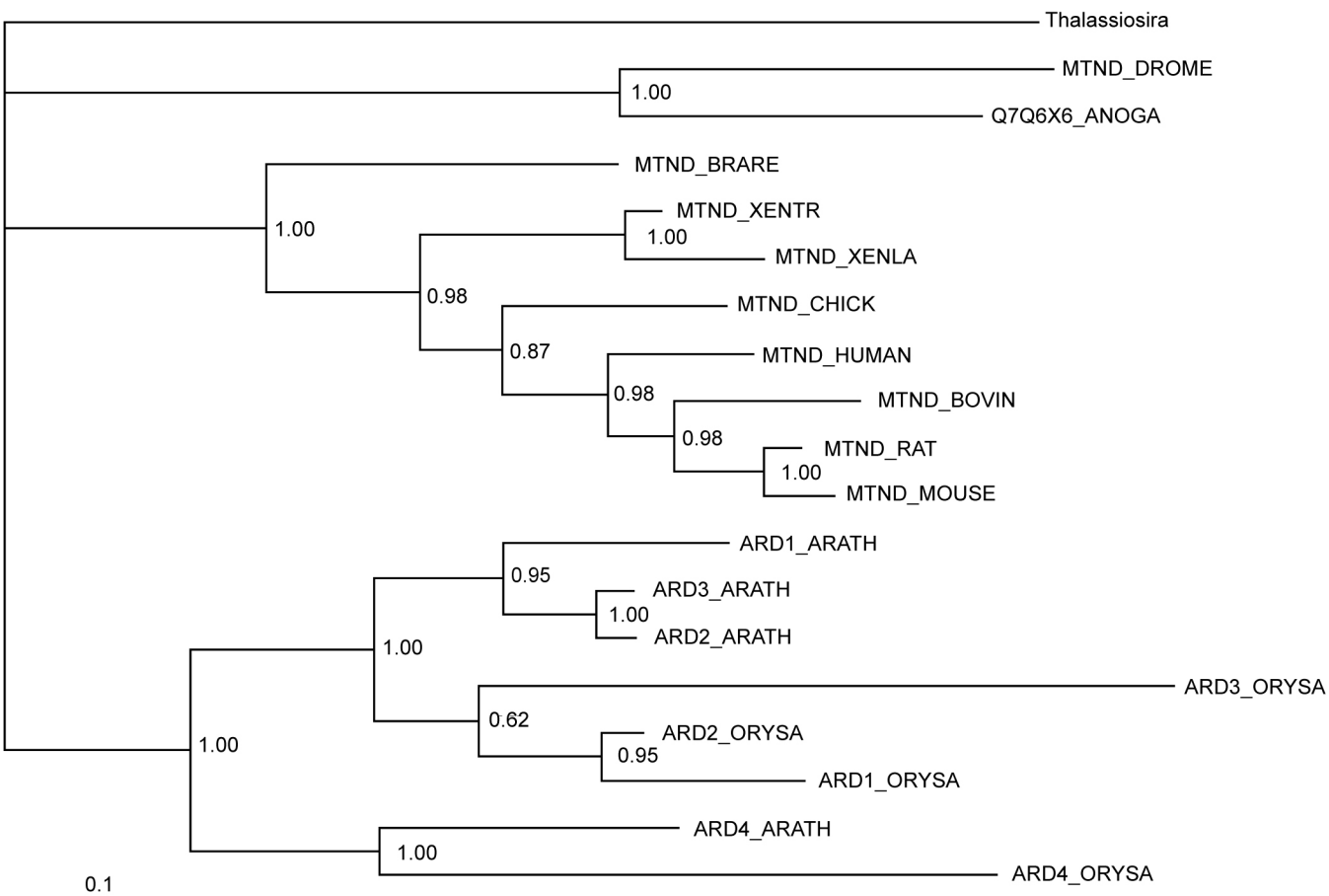
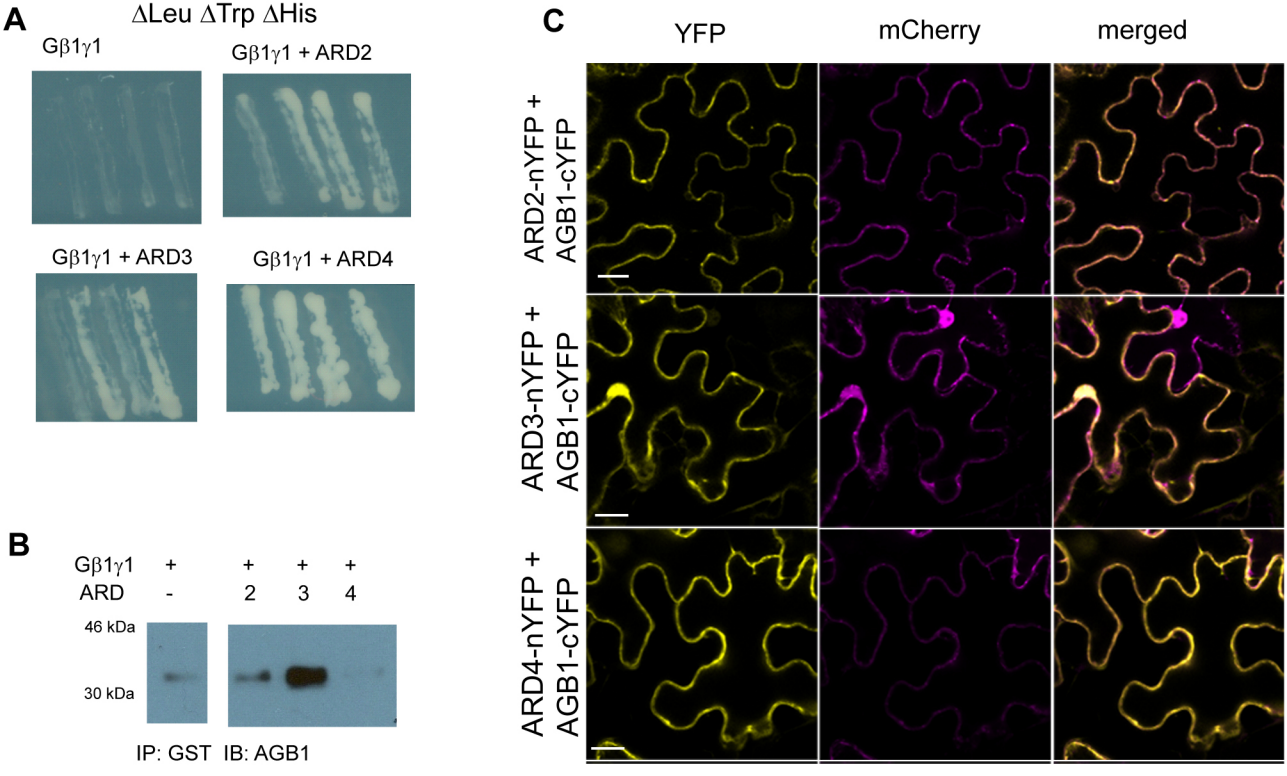


Figure S6


A
ΔLeu ΔTrp ΔHis
Gβ1γ1
Gβ1γ1 + ARD2
Gβ1γ1 + ARD3
Gβ1γ1 + ARD4
B
Gβ1γ1 + + + +
ARD - 2 3 4
46 kDa
30 kDa
IP: GST IB: AGB1
C
YFP
mCherry
merged
ARD2-nYFP + AGB1-cYFP
ARD3-nYFP + AGB1-cYFP
ARD4-nYFP + AGB1-cYFP