Mutations in Glucan, Water Dikinase Affect Starch Degradation and Gametophore Development in the Moss *Physcomitrella patens.*

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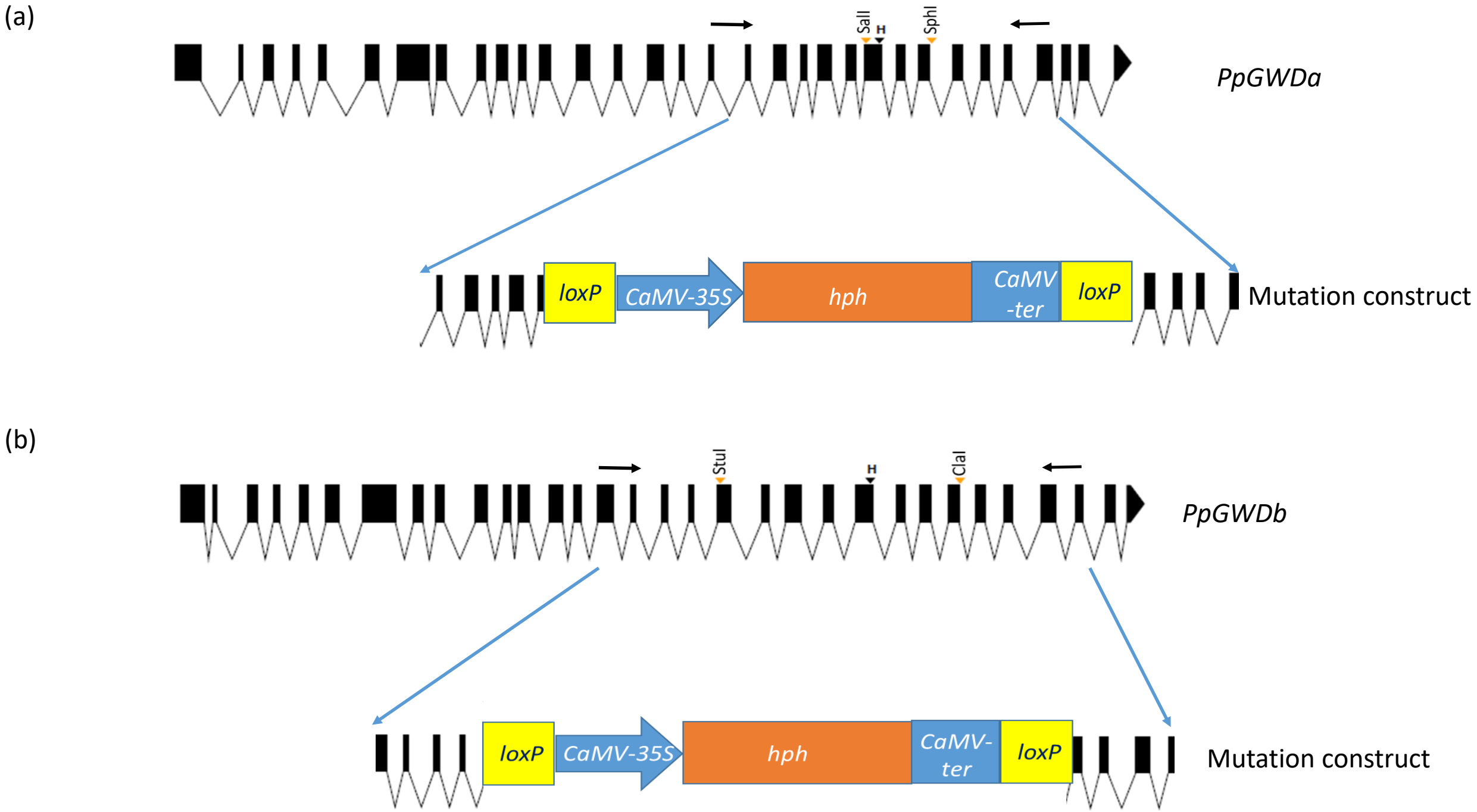
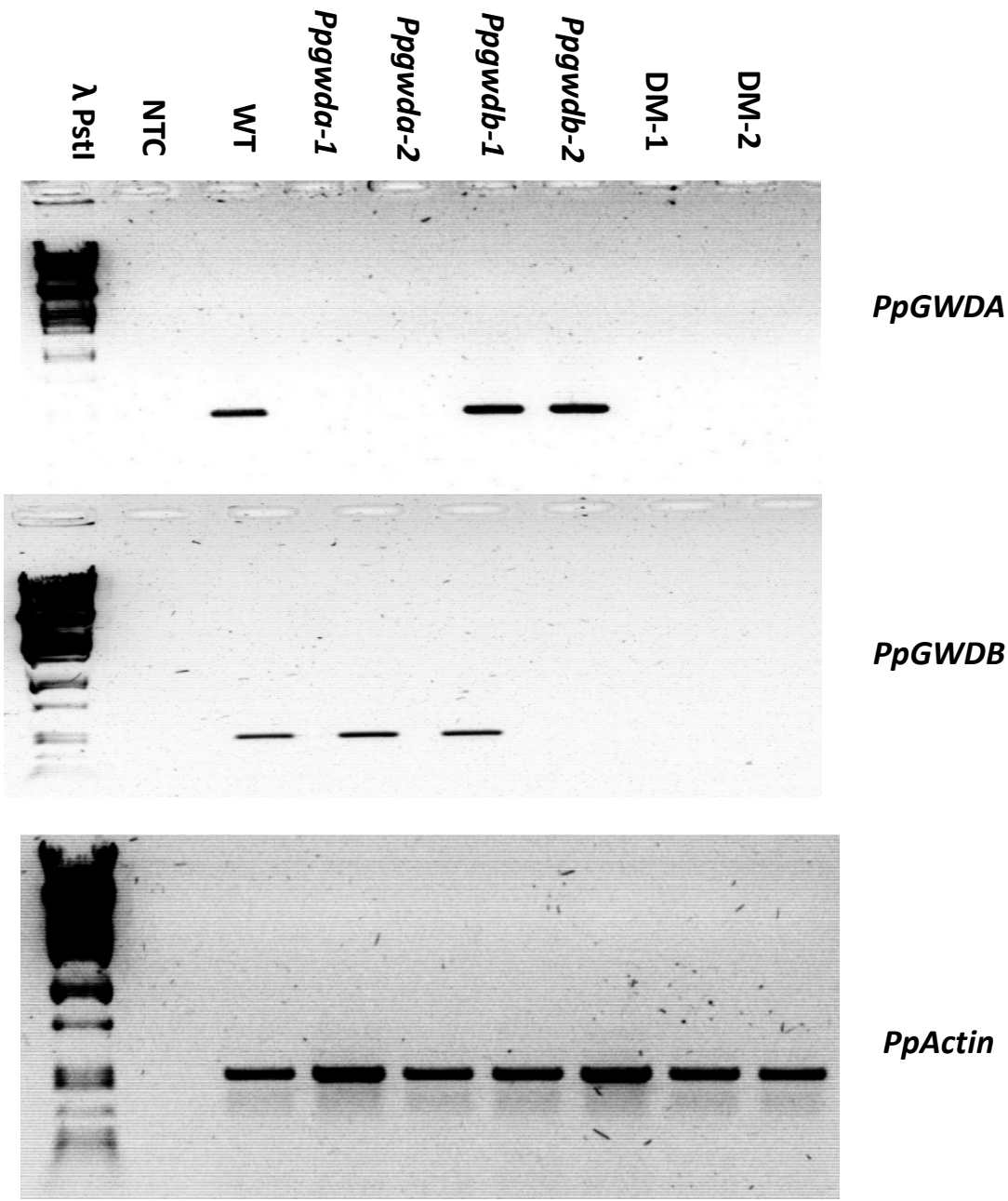
Supplementary Figure 1. Construct synthesis for mutant production. PCR was used to amplify parts of *PpGWDa* (a) or *PpGWDb* (b) genomic DNA. Arrows indicate approximate primer binding sites within the genes and the H represents the position of the codon encoding the catalytically essential histidine. The amplicons were ligated into pJET1.2/blunt and digested with the restriction sites shown in the gDNA. A resistance cassette containing the cauliflower mosaic virus 35S promoter (*CaMV-35S*), *Klebsiella* hygromycin B phosphotransferase (*hph*) and the cauliflower mosaic virus terminator (*CaMV-ter*) flanked with *loxP* sequences were ligated between those sites.

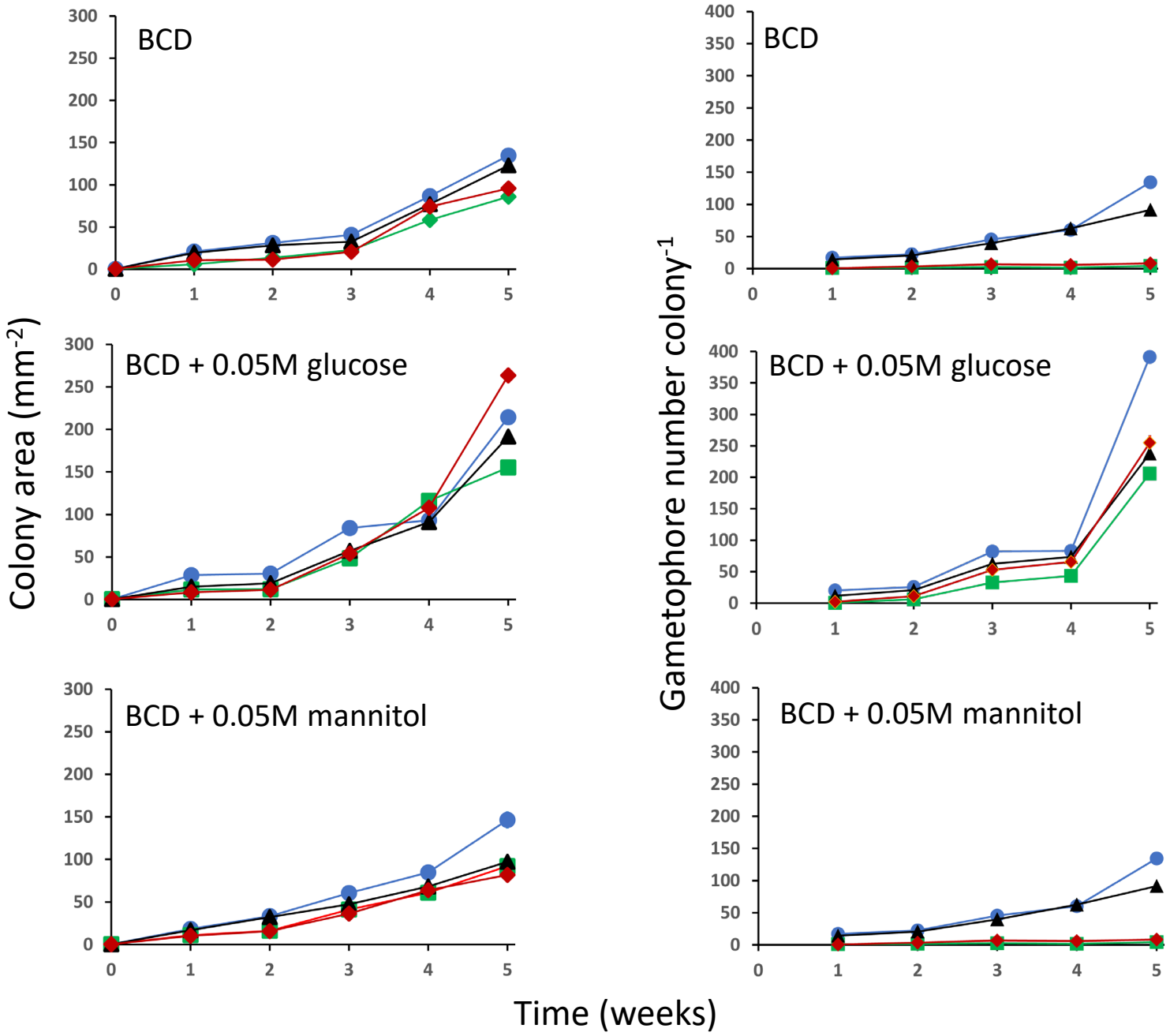
Supplementary Figure 2. Original DNA gels examining expression of *PpGWD1a*, *PpGWD1b* or *PpActin* via semi-quantitative RT-PCR in the wild-type (WT) or mutant lines. NTC denotes no template control and λPstI is DNA isolated form λ phage digested with PstI and used as a molecular weight marker.

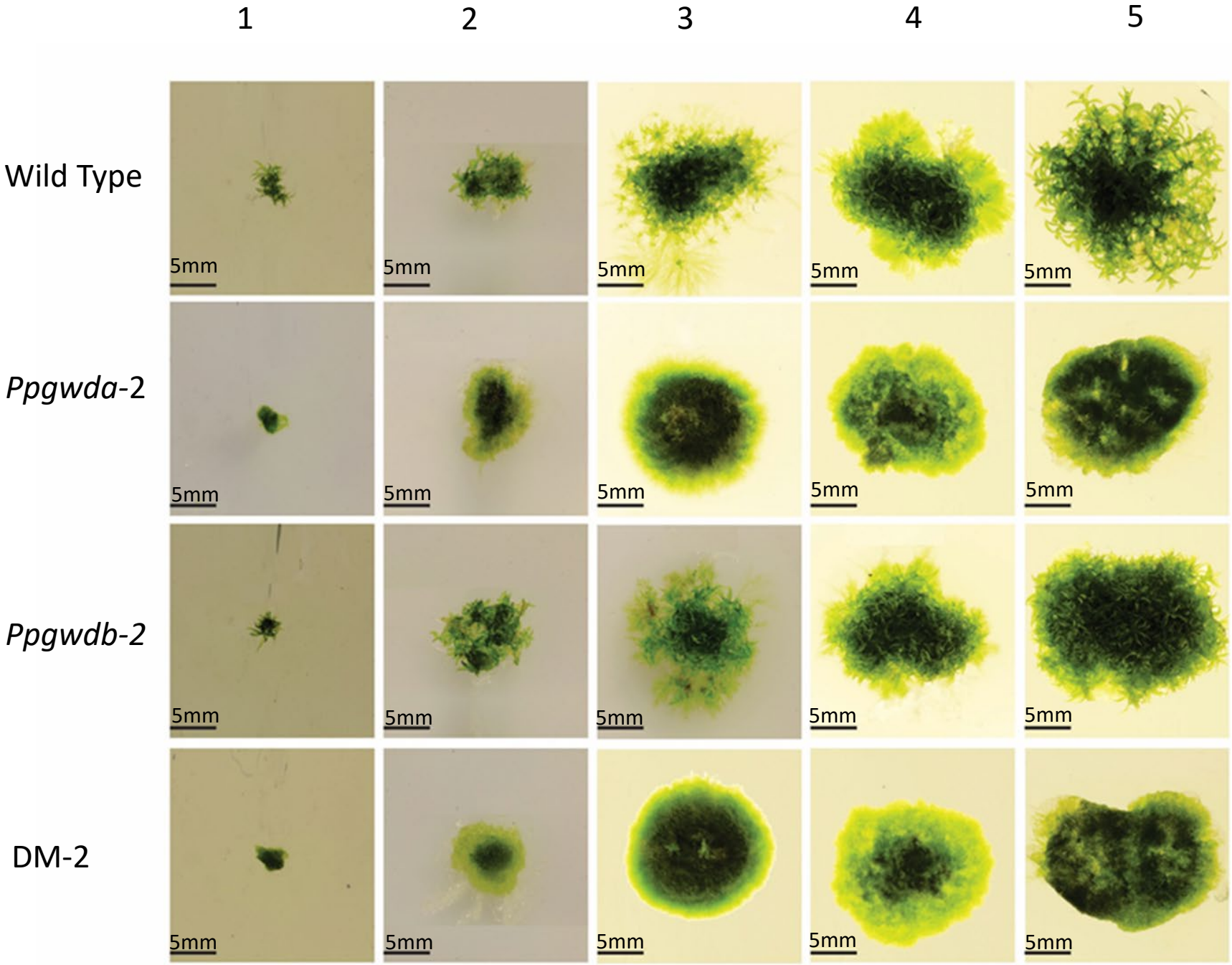
Supplementary Figure 3. Growth and gametophore number in the second set of mutant lines. Colonies were established on BCD media, BCD + 0.05M glucose and BCD + 0.05M mannitol and allowed to grow for 5 weeks. Data represents means of at least 3 colonies. Error bars are SEM and, if not visible, are within the symbol.

Supplementary Figure 4. Colony morphology of wild-type and second set of mutant lines grown on BCD medium for 1, 2, 3, 4 and 5 weeks.


(a)
SalI
H
SphI
PpGWDa
loxP
CaMV-35S
hph
CaMV -ter
loxP
Mutation construct
(b)
StuI
H
ClaI
PpGWDb
loxP
CaMV-35S
hph
CaMV- ter
loxP
Mutation construct


λ PstI
NTC
WT
Ppgwda-1
Ppgwda-2
Ppgwdb-1
Ppgwdb-2
DM-1
DM-2
PpGWDA
PpGWDB
PpActin


BCD
BCD + 0.05M glucose
BCD + 0.05M mannitol
Colony area (mm-2)
Gametophore number colony-1
Time (weeks)
Wild type
Ppgwda-2
Ppgwdb-2
DM-2


1
2
3
4
5
Wild Type
Ppgwda-2
Ppgwdb-2
DM-2
5mm