We thank both reviewers for their constructive reviews. We hope that our manuscript will be found suitable by the editor for publication in Plant Direct considering the additional experiments we have performed.

Reviewer #1:

An excellent careful study.

We thank the reviewer for his comment.

On page 7, the authors list a set of genes co-expressed wth GFY3-5; many of these genes show the same pattern with acetate boost (Goodenough et al 2014), which could be cited as confirmation. They also note the dfferences between patterns of GF1/2 vs GF3-5 expression.

We have added the reference p7.

There are two Lauersen et al 2016 references. Please cite as 2016a or 2016b.

We have corrected the references.

It should be MaterialS and Methods

Corrected (p15)

The Hayashi paper is 2015, not 4

We thank the reviewer for his careful review. The references Hayashi et al. 2014 has been deleted, considering that the microbodies where the GFY1_5 proteins localize are not glyoxylate cycle-containing peroxisomes (see our response to reviewer 2 below).

Reviewer #2:

This manuscript describes the characterisation of 5 members of the GFY family of proteins from Chlamydomonas. The five members are located on chromosome 17 in 2 distinct loci comprising GFY1-3 and GFY 4 and 5 respectively. All display a high degree of sequence identity and contain the typical signatures of the GFY superfamily. Gene expression analysis by qRT PCR showed strong upregulation of GFY3, 4 and especially 5 in the presence of acetate and co-expression analysis provide strong circumstantial evidence for a role in acetate metabolism. GFY3-5 were co-expressed with genes involved in acetate metabolism such as the glyoxylate cycle, many of which are peroxisomal, and with peroxisome membrane proteins. Unfortunately individual KO lines of GFY 1 2 and 3 did not have phenotypes suggesting redundancy. The close linkage of the isoforms prevented isolation of double mutant combinations. Molecular modelling suggest the chlamydomonas proteins have a similar structure to the functionally and structurally characterised Sat P acetate channels from bacteria. Crucial residues that make interactions with acetate are conserved. Tagging the proteins with venus revealed a punctate localisation suggestive of peroxisomes. Sequences similar to the recognition motifs for the peroxisome membrane protein receptor PEX19 are found in all 5 GFY isoforms. The manuscript is well written and provides interesting data concerning

the chlamydomonas GFY isoforms.

Major comment
GFY proteins have been reported in the plasma membrane and mitochondria in other organisms. The suggestion of a peroxisome localisation is novel. There is a strong circumstantial argument for such a location but it is not directly proven. Punctate staining could mean the protein is in other subcellular organelles and the function of the putative PEX19 binding sites is not tested. Ideally one would like to see co expression of the labelled protein with a validated peroxisome marker or imunofluorescence with an antibody recognising a bona fidae peroxisome protein. Alternatively co-localisation on a density gradient. Such data would greatly strengthen the story. If that is not possible the conclusion should be more nuanced that, on the balance of evidence it is likely that the proteins localise to peroxisome membranes.

Additional transformations using a citrate synthase 2 (CIS2) fluorescent-PST2 recipient strain have been performed. Fluorescent signals of CrGFY fusion constructs were observed as small spots spread throughout the cell for all CrGFY variants (Figure 5). However, none of them colocalizes with the glyoxylate cycle-containing peroxisomes, regardless of CrGFY isoform. Therefore, we have nuanced our discussion and conclusion, considering this last result and as suggested by reviewer 2. The mention to PEX19 binding sites has been deleted, since as the reviewer pointed out, we have no experimental evidence for them.

Minor points
1) Intro line 123-124 this paper does not provide 'evidence' for being channel proteins its inferred form conservation of sequence and likely structure plus co-expression data

We agree with the reviewer. We have now used the verb 'suggest' (p4).

2) It would be nice to see the coexpression data as a plot and a p-value showing the tightness of the coexpression

We have indicated in Supplemental Table S2 the PCC (Pearson's correlation coefficient) and MR (Mutual Rank) values from Phytozome and ALCOdb respectively. In addition, and in order to minimize the noisy presentation of data in this work, we have indicated that the data can be easily called upon in respective databases by the references numbers in the manuscript: "An expanded and detailed co-expression gene list can be consulted by using the accession numbers in the corresponding databases."

3) Please explain more clearly how the putative PEX19 binding sites were identified

We have deleted that part since the GFY proteins do not colocalize with the peroxisomes.

4) Fig2 legend Δ symbol is missing

We are unclear as to what the reviewer means by this request, the figure and the caption are complete.

5) Ribas seems to be cited twice in the ref list.

We thank the reviewer for his careful review. We have corrected the mistake.