

Supplementary File for

Homeostatic Response of Phospholipid Pathways to PCYT2 Deficiency and Impaired *de novo* Synthesis of Phosphatidylethanolamine

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Supplementary Figure Legend

Supplementary Figure 1. A. The fatty acid composition in total lipids: Total lipids were extracted with chloroform : methanol (2:1) in the presence of C17:0 fatty acid as an internal standard and the lower lipid layer was saponified and methylated with BF₃-MeOH. The methylated fatty acids were separated and their amounts determined by GC-FID as described in Experimental procedures. The shown are results from four independent experiments. Differences between WT and KD cells were determined using the pair wise comparisons with Student t- test at p<0.05. **B, C: The lipid separation and detection by TLC.** The TLC plate B shows the separation and the amount of the main lipid classes in WT and KD cells. None of the lipids including the phospholipids were different in WT and KD cells. The TLC plate in C. shows the separation of phospholipids that were collected and their fatty acid composition individually determined by GC-FID. As it can be seen in the TLC plate (C), no significant differences in individual phospholipid species (SM, PC, PS, PI, PE) between WT and KD cells was detected in 3 individual experiments. D. The actual amounts of phospholipids determined by GC-FID confirmed the TLC results. PL are determined in 20 ug of the cell pellet.

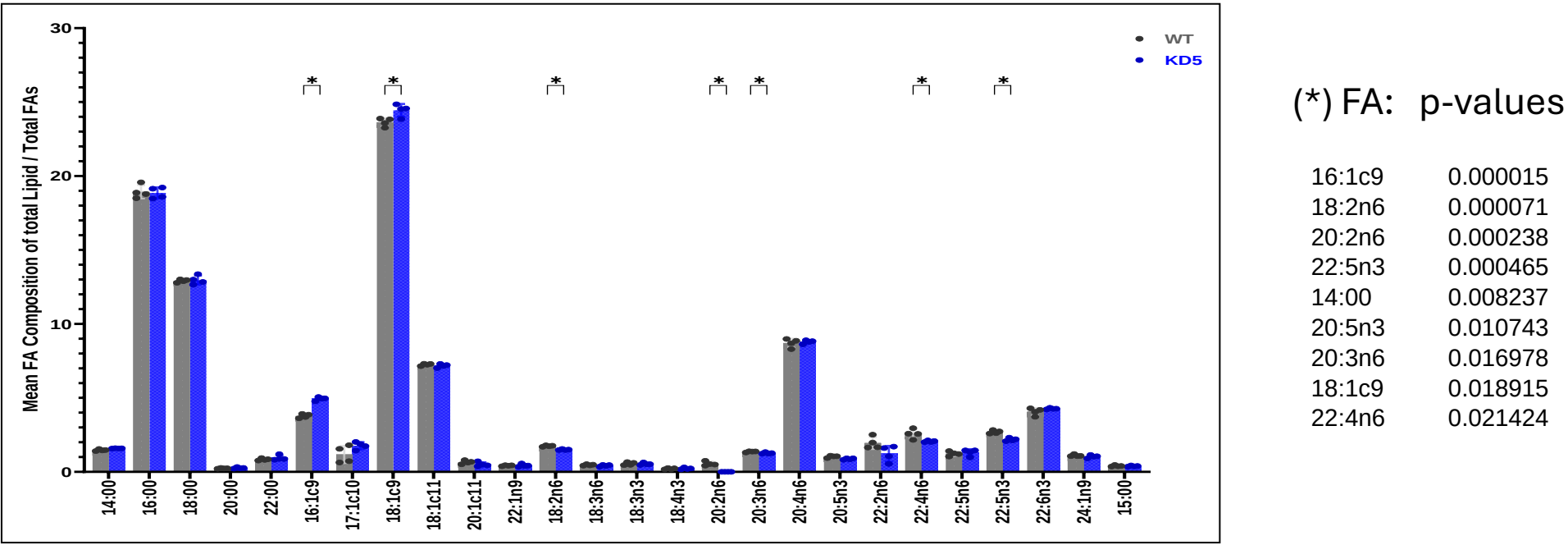
Supplementary Figure 2. The unsupervised hierarchical clustering of the entire PL datasets for PC, PE, PS, PI and SM identifies the two major clusters that differentiate KD and WT PLs based on their FA composition. The two clusters negatively correlated with each other, demonstrating that a significant portion of FAs was distributed differently in KD and WT PLs.

The 1st cluster (red) identifies that n-6 are overrepresented in KD cells in all PLs except SM (Bold). All KD cell PLs, except PC, also have higher presence of specific MUFA (Italics). Only PI and SM accumulate specific SFA, while PS, PI and PE accumulate specific n-3.

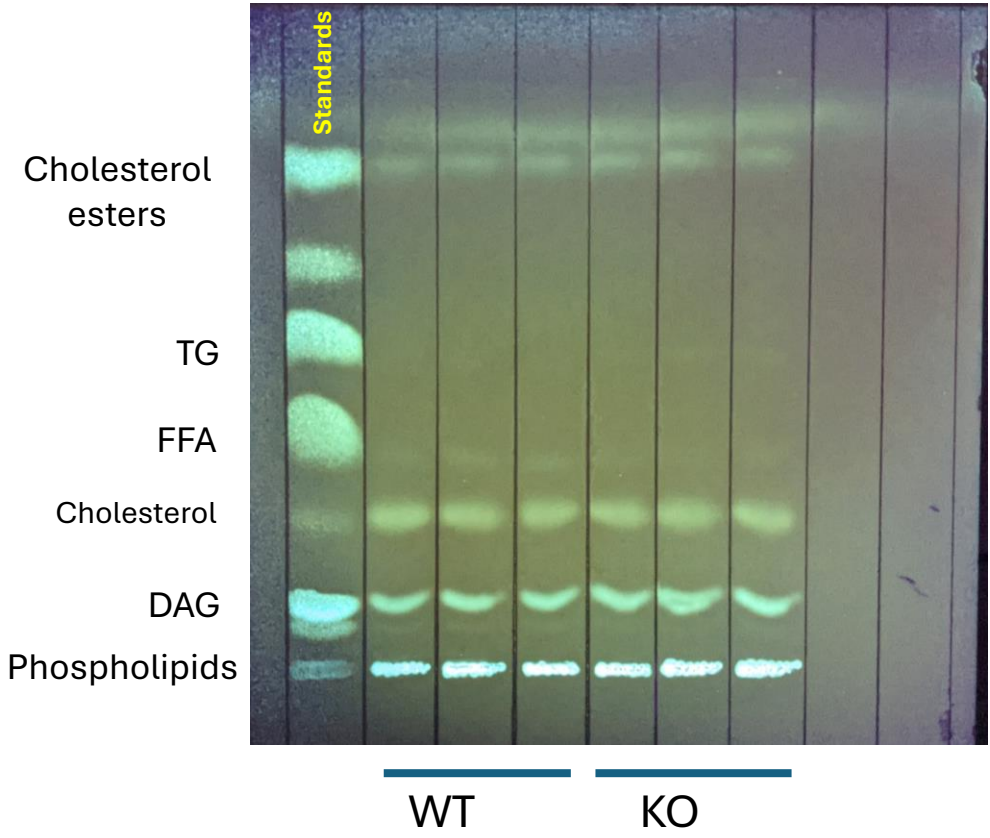
The 2nd cluster (blue) identifies that KD cells have reduced contribution of SAF (Italics) in most PLs: PC, PE, PS and SM, but not in PI. KD cells also have lower n-3 in PC and PE and SM (Bold). There is also underrepresentation of n-9 and n-11 MUFA in PC, PE, PI and PS but not in SM (Underlined). Some n-6 FAs, including the essential 18:2n6 FA in PE are less in KD cells.

Supplementary Figure 3. The cell viability test : Cell viability was determined using trypan blue exclusion assay after media starvation for 0, 1, 2, and 3 h. No significant differences in cell viability were observed between WT and KD cells. All measurements were performed in triplicate and expressed as means $\pm$ SEM from two separate experiments.

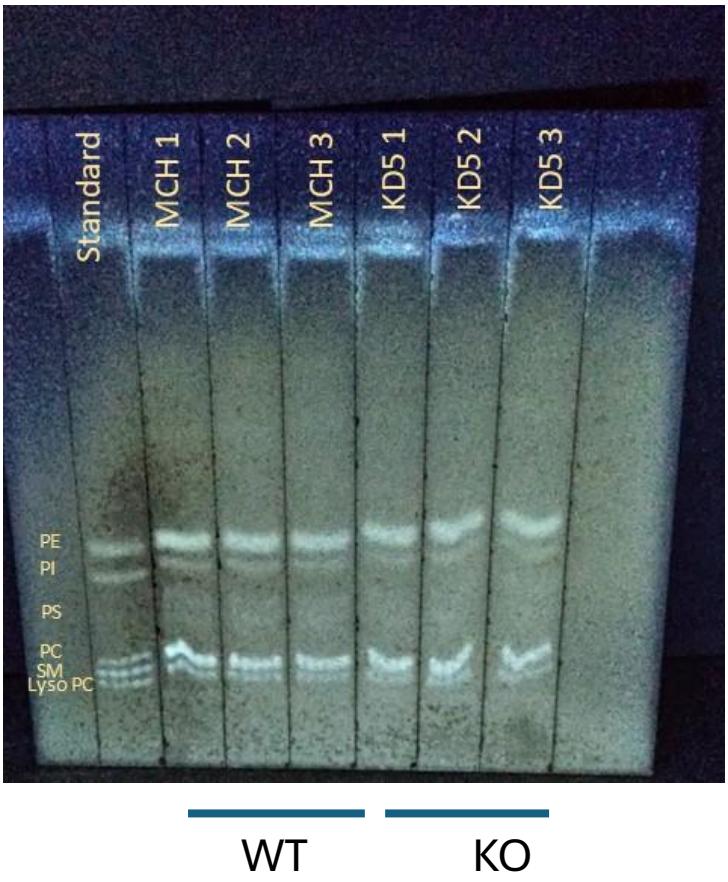
A.



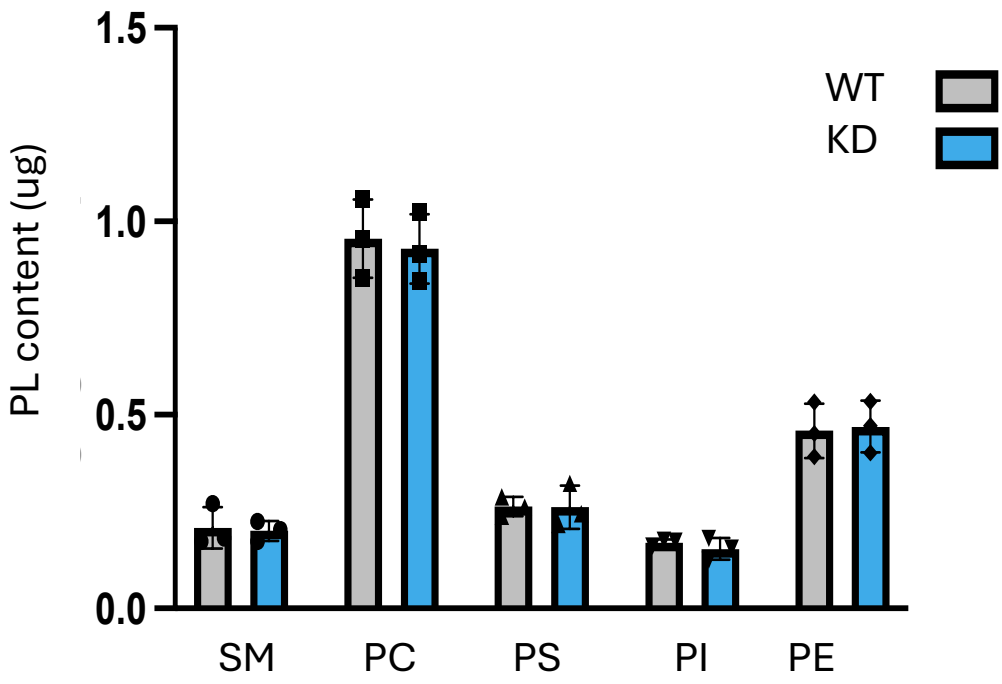
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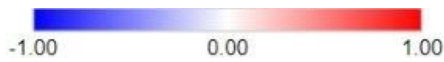
C



D.



Suppl. Figure 1



Cluster 1

PC20:4n6 PC22:2n6 PC22:4n6

PE16:1c9 PE17:1c10
PE20:4n6 PE22:4n6 PE20:3n6
PE22:5n3 PE20:3n9

PS18:1c11 PS20:1c11 PS24:1n9
PS20:3n6 PS20:4n6
PS18:3n3 PS22:6n3

PI18:00 PI19:00
PI18:1c11 PI16:1c9
PI18:2n6 PI22:4n6 PI20:4n6
PI18:3n3

SM15:00 SM16:00 SM24:00
SM18:1c9 SM18:1c11 SM16:1c9

Cluster 2

PC18:00 PC19:00 PC23:00
PC16:1c9 PC18:1c9
PC18:3n6
PC18:4n3 PC20:5n3

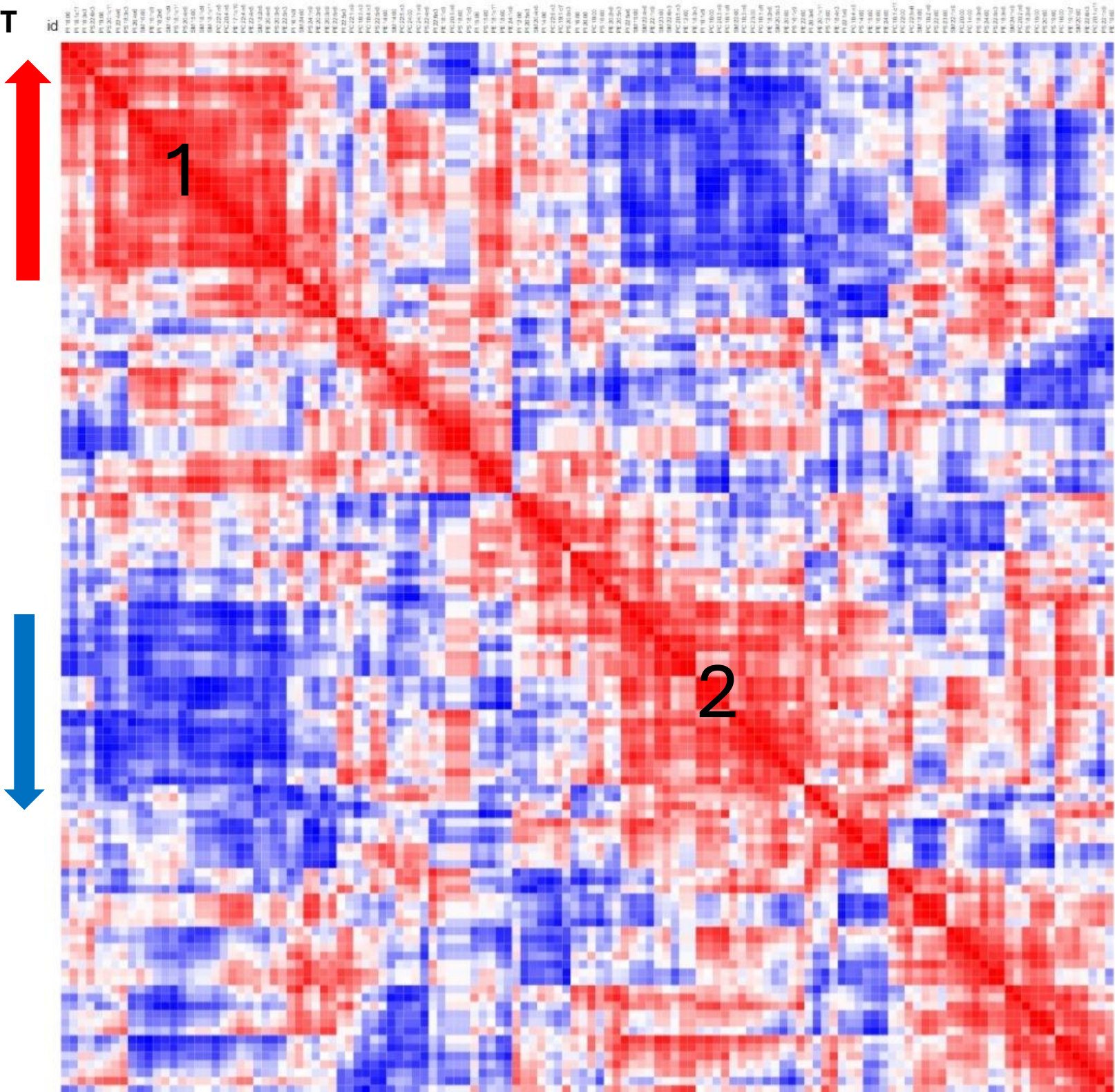
PE12:00 PE15:00 PE16:00 PE20:00 PE22:00
PE20:1c11 PE22:1n9
PE18:2n6 PE20:2n6
PE18:4n3 PE18:3n3 PE20:5n3

PS16:00 PS14:00 PS12:00
PS16:1c9

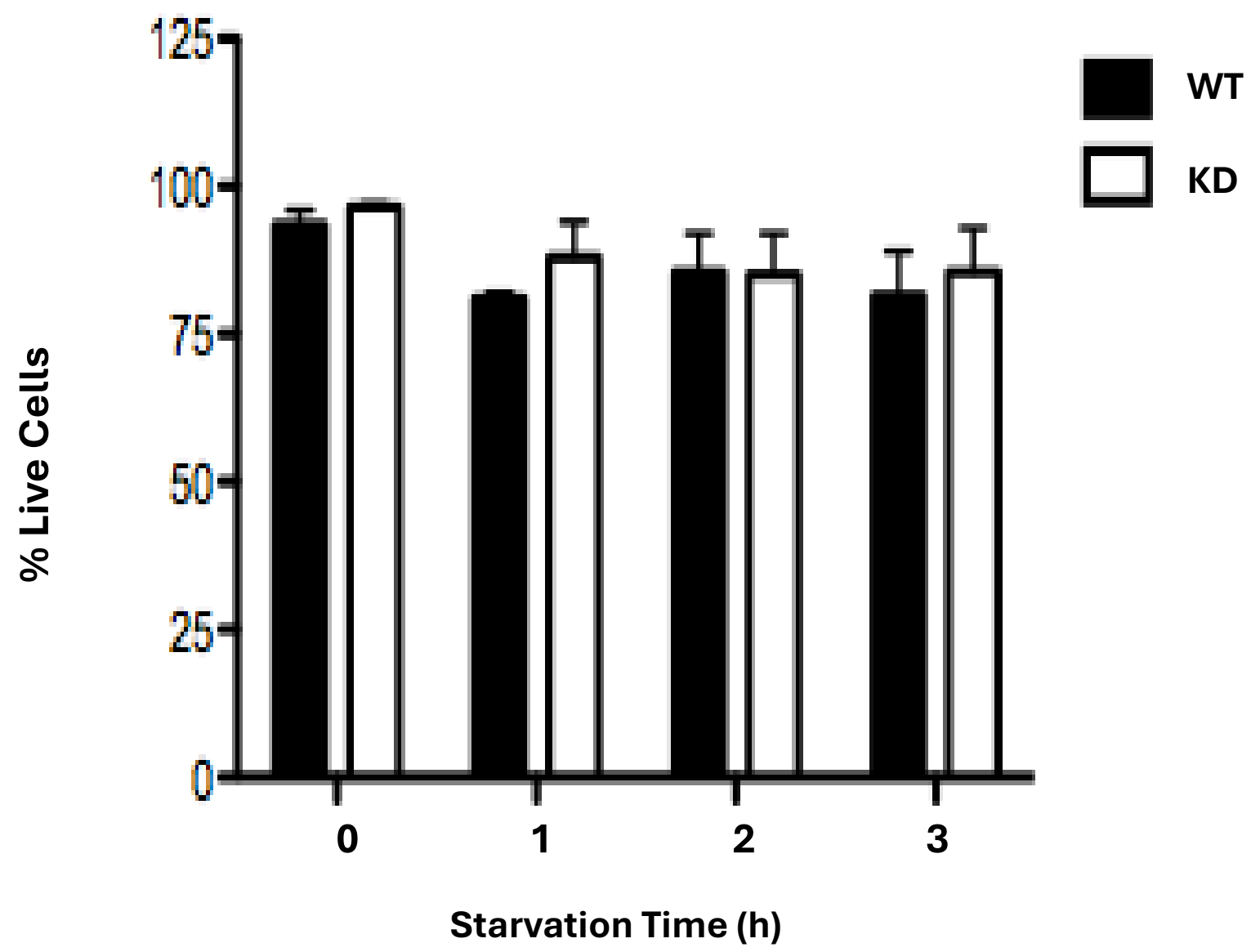
PI18:1c9 PI22:1n9
PI20:3n6 PI22:5n6

SM19:00 SM22:00 SM23:00
SM22:4n6
SM22:6n3 SM20:5n3

KD/WT



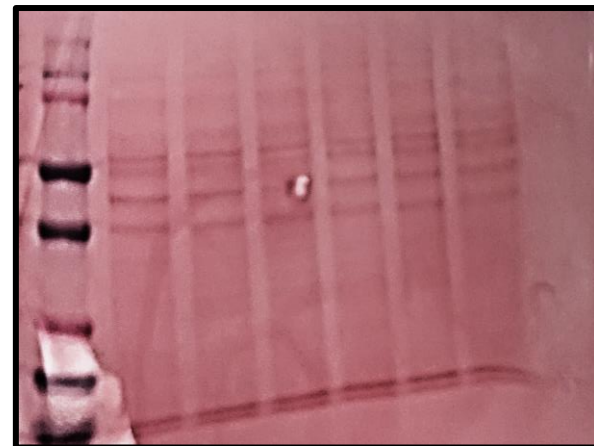
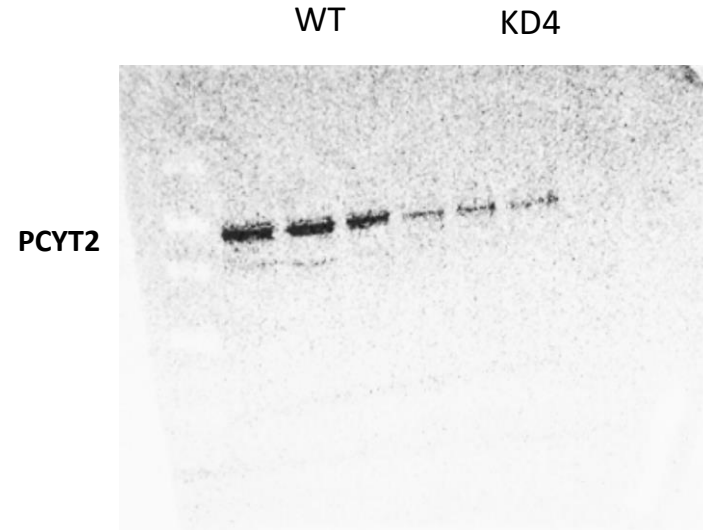
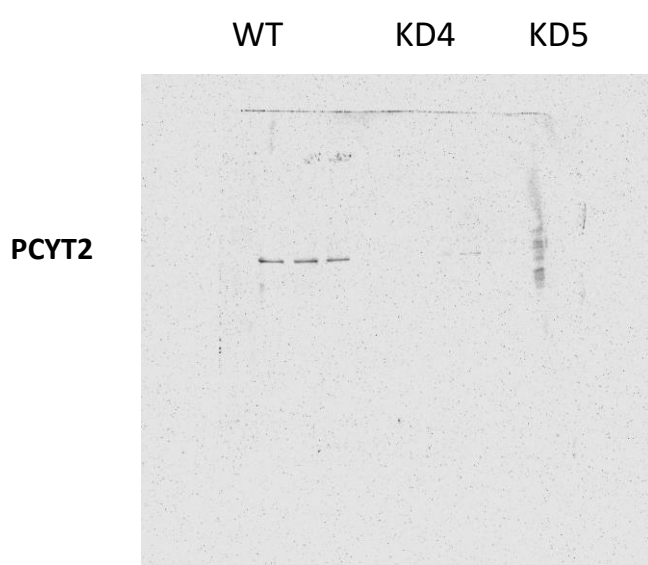
Suppl. Figure 2



Suppl. Figure 3

Fig. 1

Fig 2A Original western blots: PCYT2 protein amount was drastically reduced in KD cells, as shown; this was determined in two separate experiments performed in triplicates. Ponceau S was used to demonstrate the equal protein loading in control (WT) and Pcyt2 knock-down (KD4 and KD5) cells.



Original western blots for PCYT1 in Fig 4. The results are from three separate cell cultures. Cells were grown under conditions without (0) and in the presence of 2.5 mg/L choline in the media. The cells were also treated with 5 mg/L choline but these blots were not used. The membrane was cut from that area and then re-probed with control tubulin

Fig. 2F

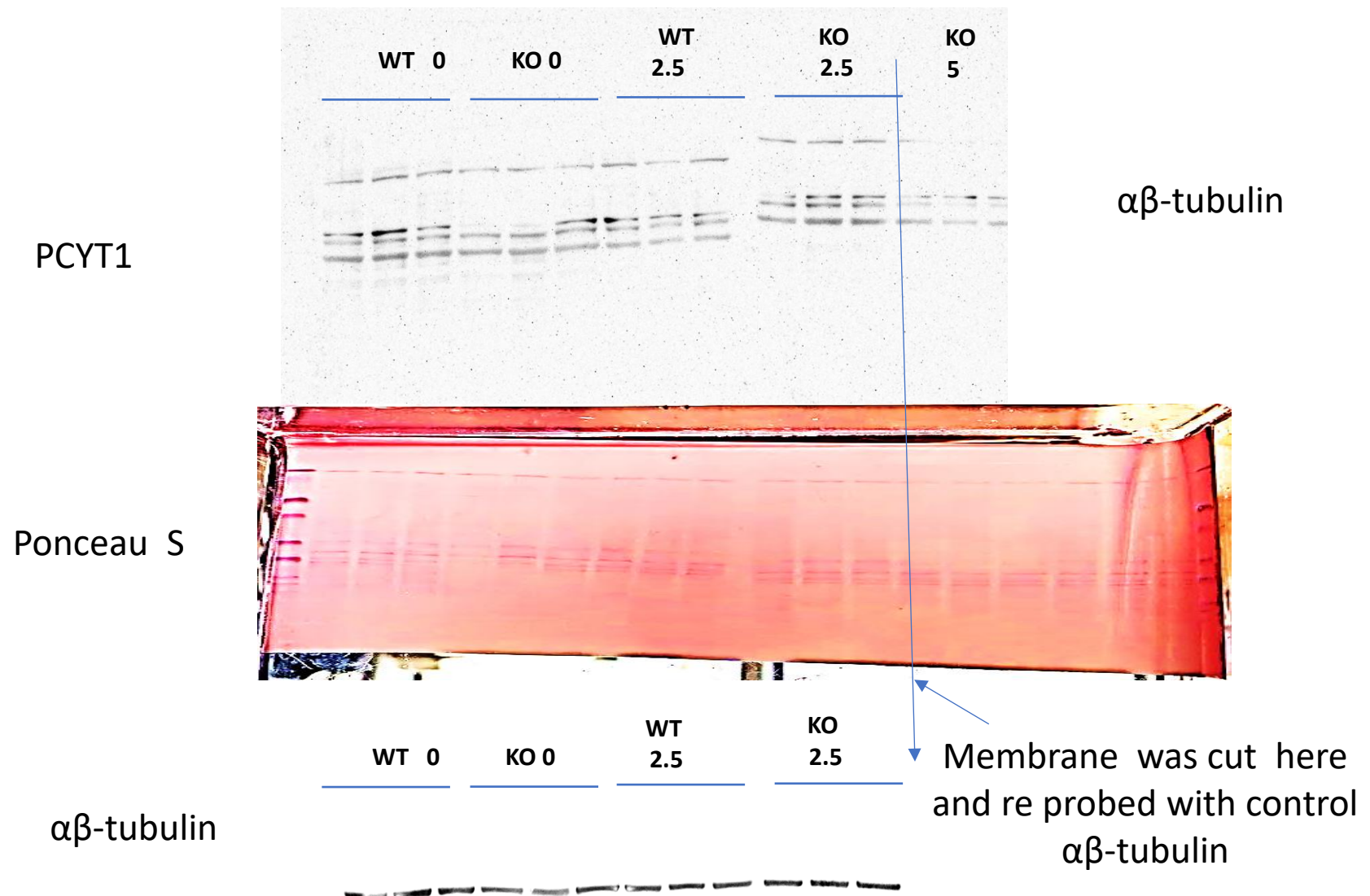
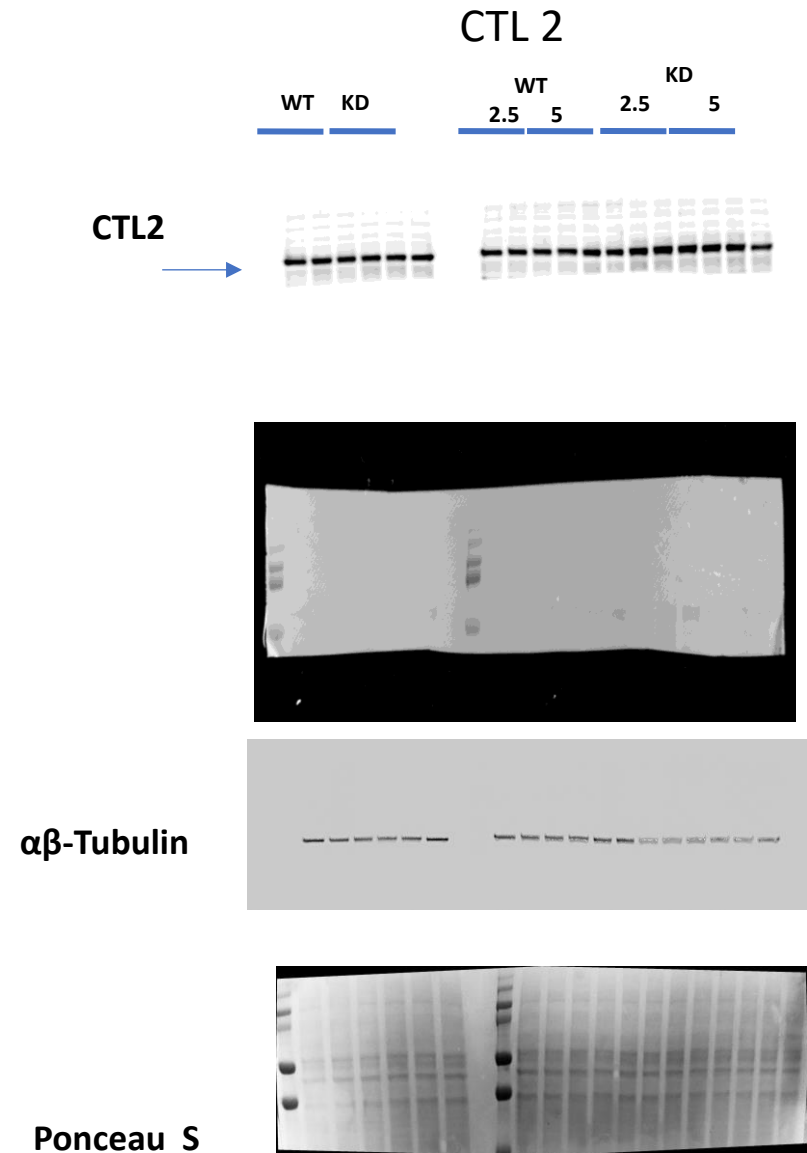
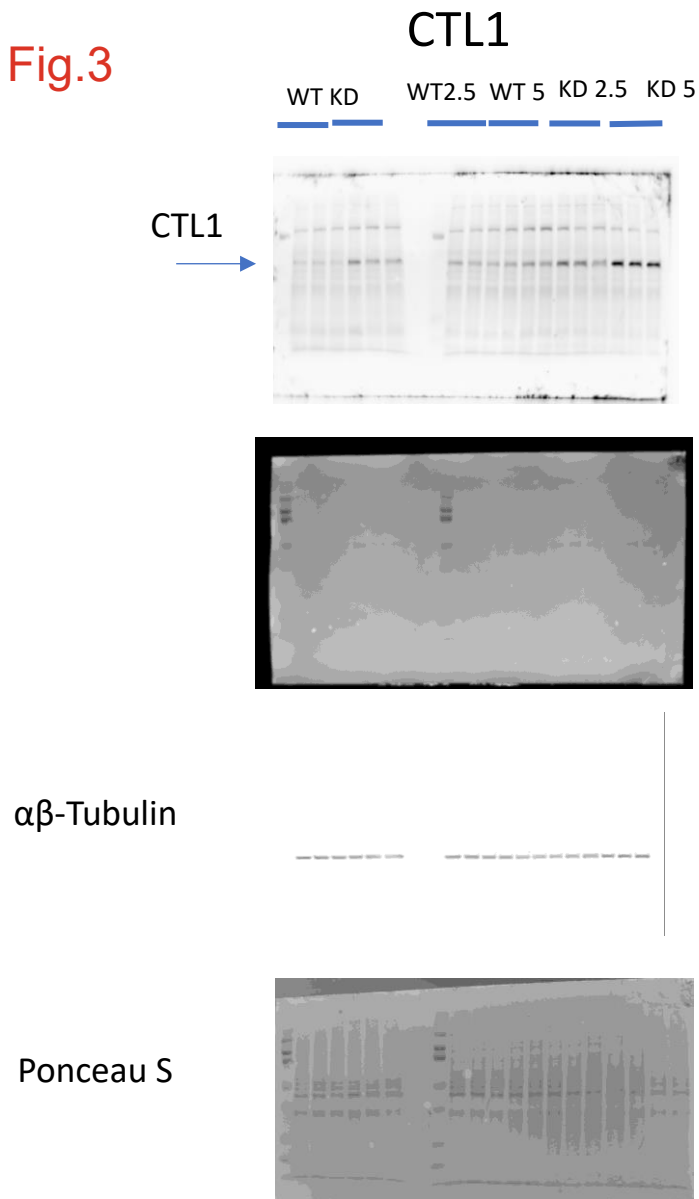
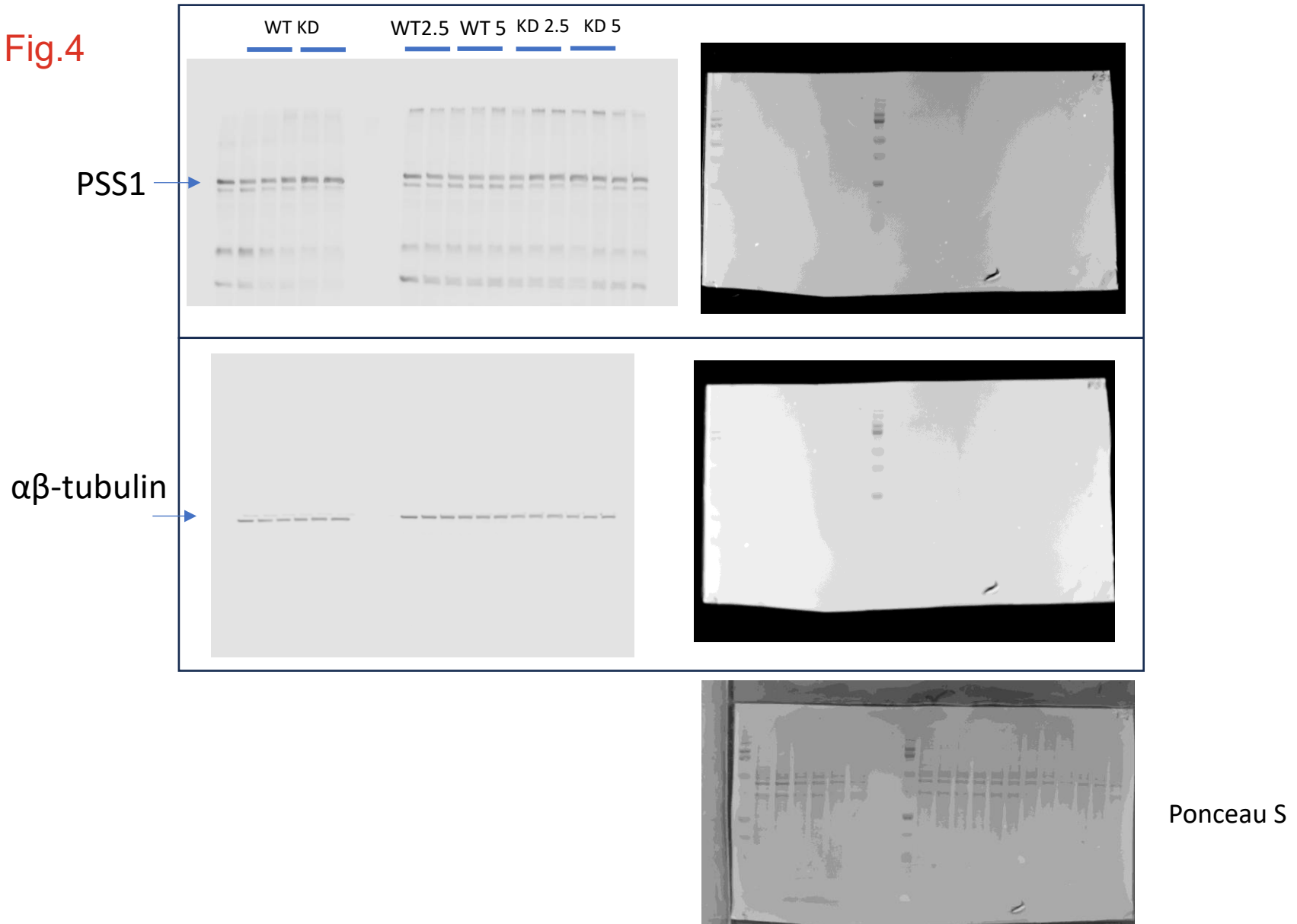


Fig.3



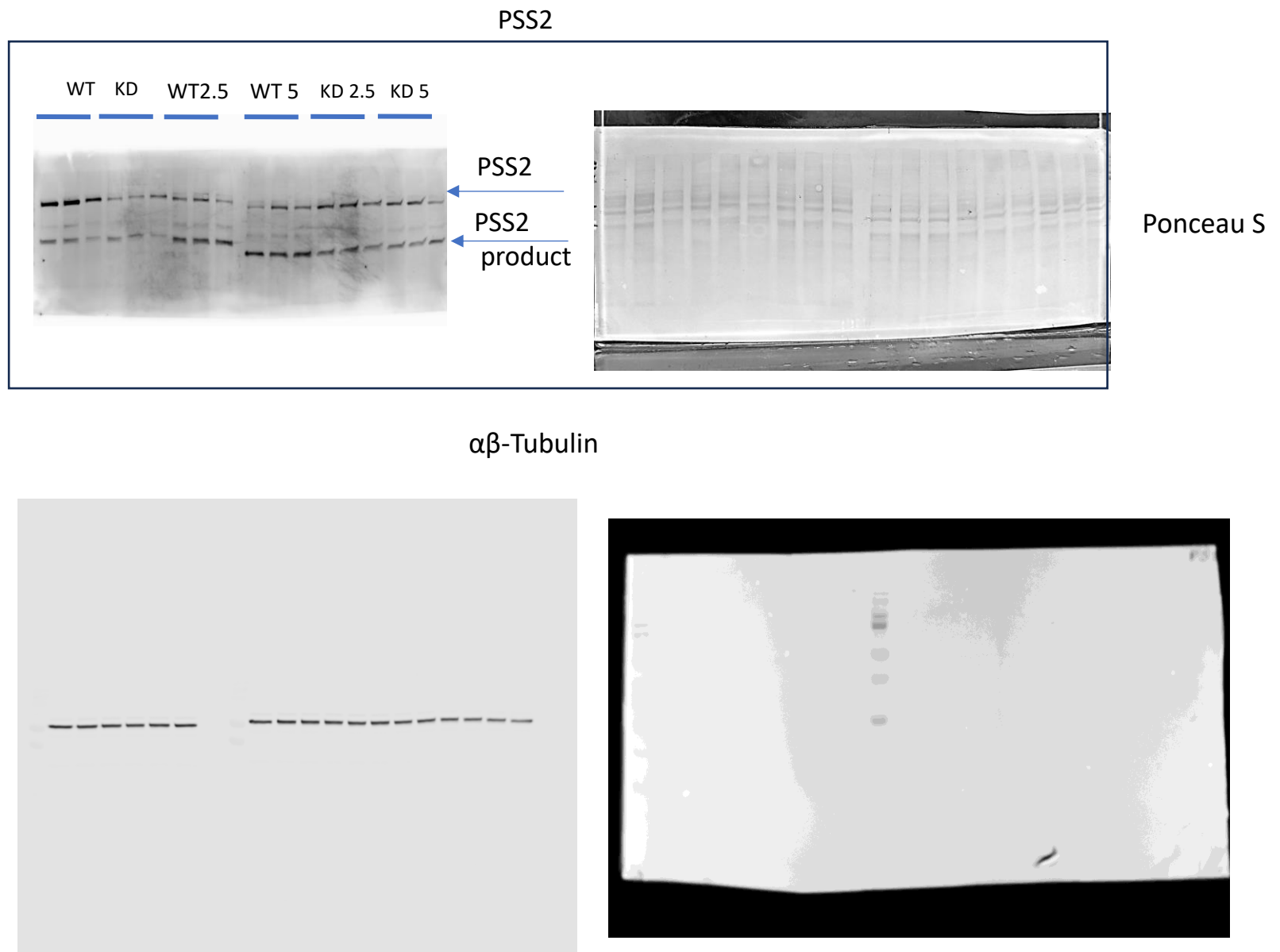
Western blots for CTL2 and CTL1 used in Fig 3 Also shown are the membranes stained with Ponceau S . Tubulin controls were determined after the same membrane stripping

Fig.4



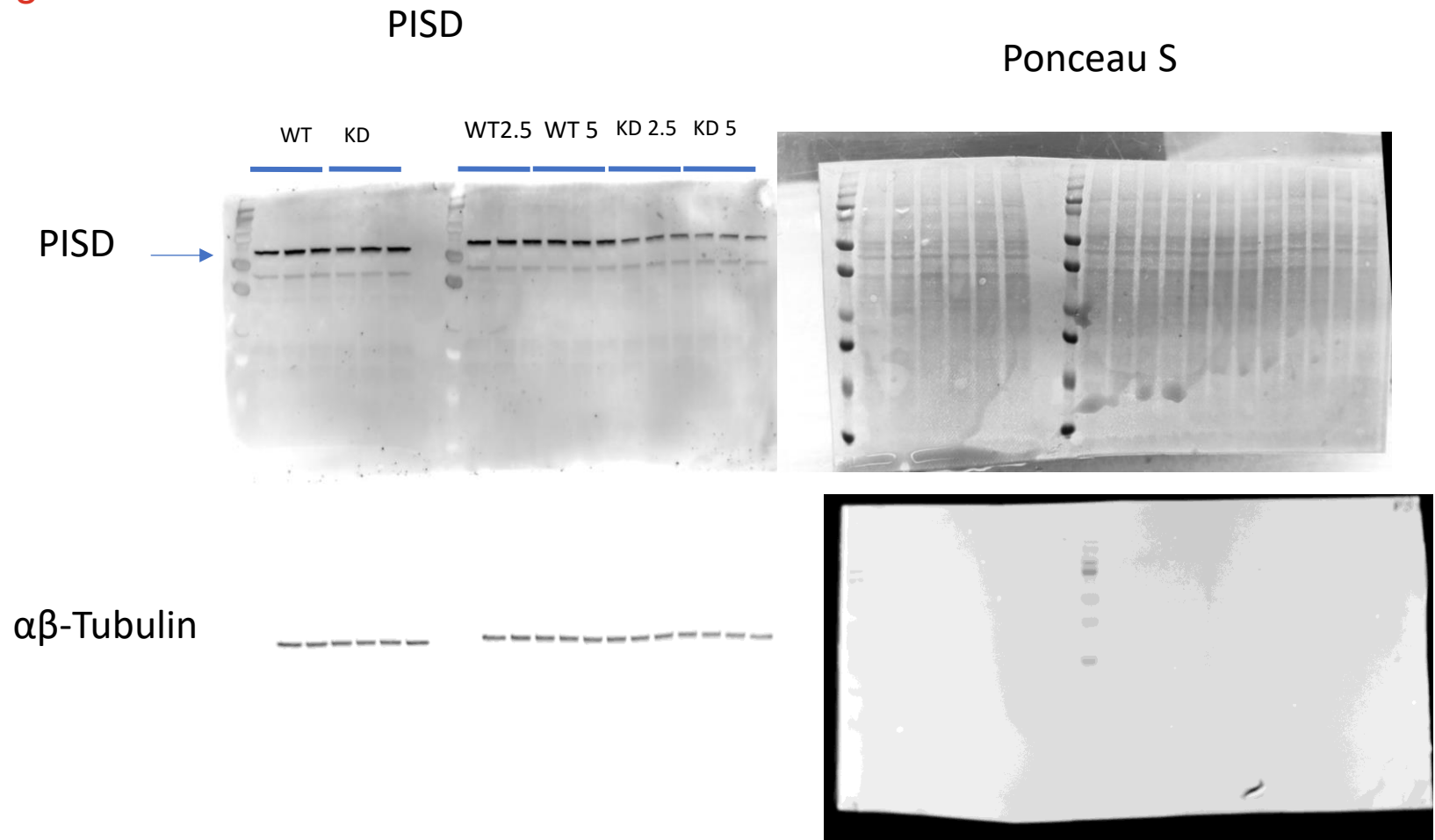
The original western blots for PSS1 and control tubulin used in Fig. 5. The conditions are described in the previous page for PCYT1.

Fig.5



The original western blots for PSS2 and control tubulin used in Fig. 5. Tubulin was determined on the same membrane by stripping and re probing. The conditions are described in the previous figure for PCYT1.

Fig.6



The original western blots for PSS1 and control tubulin used in Fig. 6. Tubulin was determined on the same membrane by stripping and re probing. The conditions are described in the figure for PCYT1.