



## Engineering the spatial organization of metabolic pathways

Albertsen, Line; Maury, Jerome; Bach, Lars Stougaard; Nielsen, Jens; Mortensen, Uffe Hasbro

*Publication date:*  
2009

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Albertsen, L., Maury, J., Bach, L. S., Nielsen, J., & Mortensen, U. H. (2009). *Engineering the spatial organization of metabolic pathways*. Poster session presented at Biochemical Engineering XVI, Burlington, Vermont, USA.

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

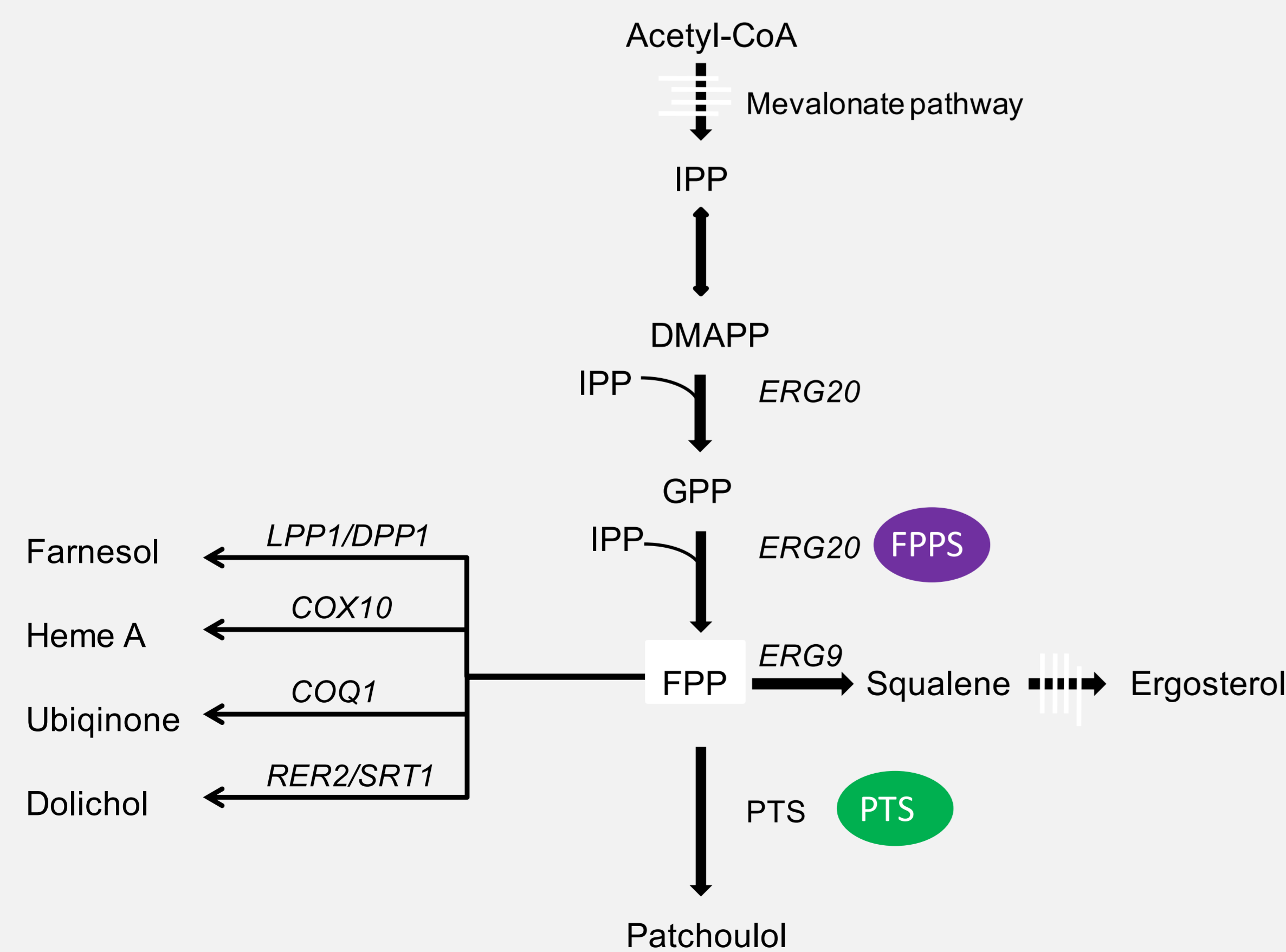
- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## Abstract

Several natural systems for ensuring optimal spatial arrangement of biosynthetic enzymes exist. Sequentially acting enzymes can for example be positioned in close proximity by attachment to cellular structures, up-concentration in membrane enclosed organelles or assembly into large complexes. The vision is that by positioning sequentially acting enzymes in close proximity, the cell can accelerate reaction rates and thereby prevent loss of intermediates through diffusion, degradation or competing pathways. The production of valuable metabolites in cell factories often depends on both heterologous and host enzymes. In this case, no spatial coordination of the biosynthetic enzymes can be expected to be in place. Presumably this contributes to the low productivity regularly observed for heterologous pathways. In one test case, we investigated whether a heterologous pathway could be optimized by positioning two sequentially acting enzymes in close proximity. More specifically, we fused patchouli synthase originating from Patchouli (*Pogostemon Cablin*) to the natural yeast enzyme, farnesyl diphosphate synthase (FPPS) and expressed it in the well-characterised cell factory *Saccharomyces cerevisiae*. Successfully, the sesquiterpene production was increased two-fold when the enzymes were fused compared to when they were expressed from the same promoters as free enzymes. Moreover, the strategy could be used in combination with other traditional metabolic engineering strategies to increase the production of a desired product, as enzyme fusion combined with down-regulation of a competing pathway and up-regulation of a selected pathway enzyme resulted in a five-fold higher sesquiterpene production. This simple test case demonstrates that engineering of the spatial organization of pathways has great potential for diverting flux towards a desired product.

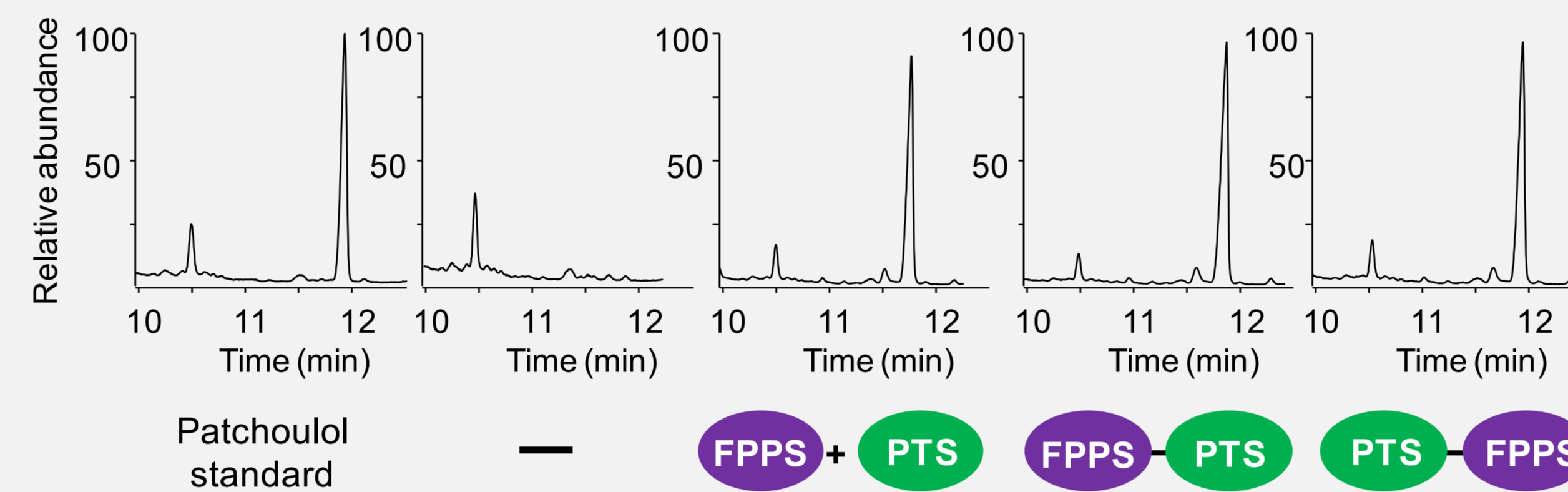
## Pathway



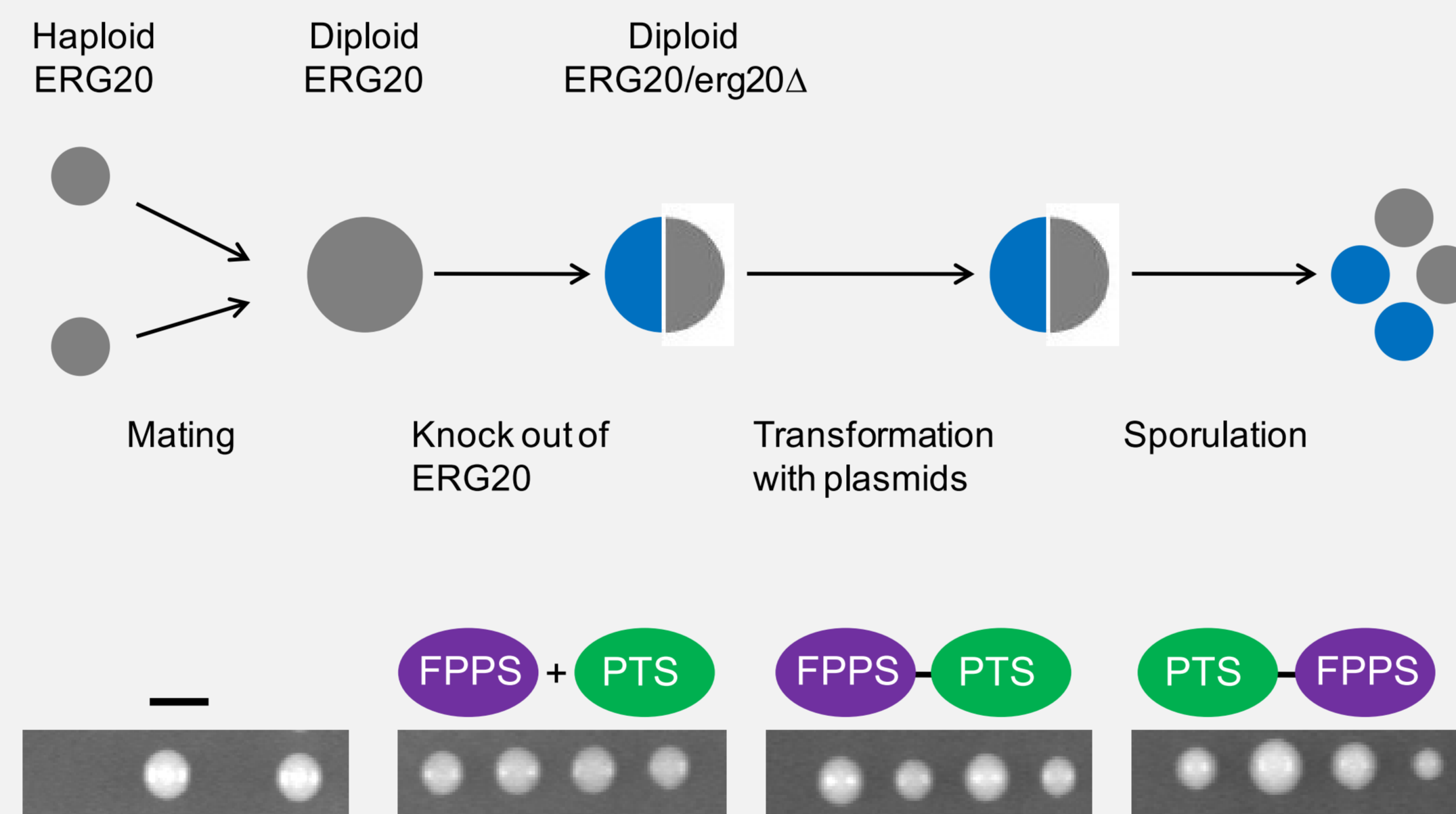
## Functionality

### Is PTS functional when fused to FPPS?

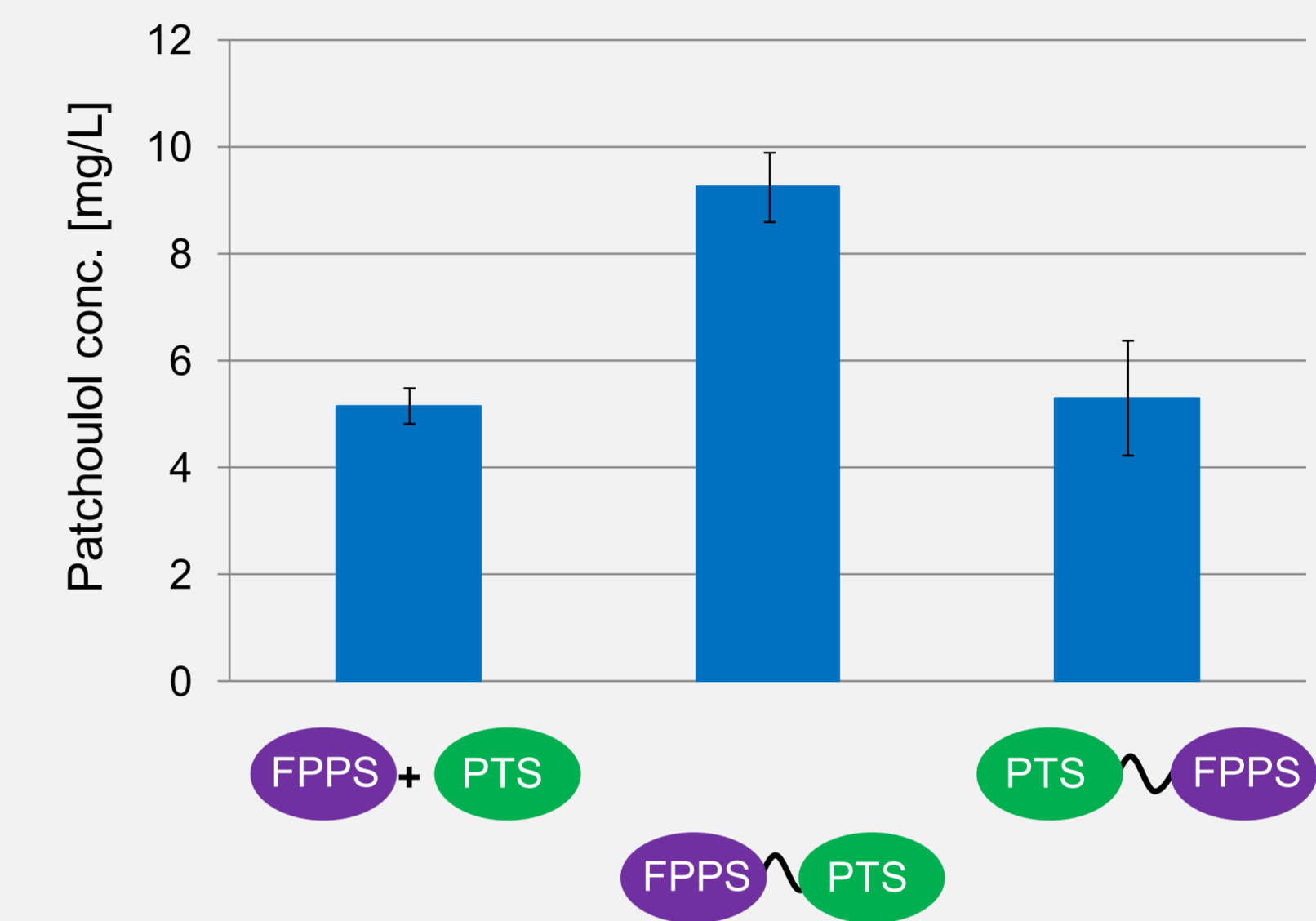
Results of GC-MS analysis:



### Is FPPS functional when fused to PTS?

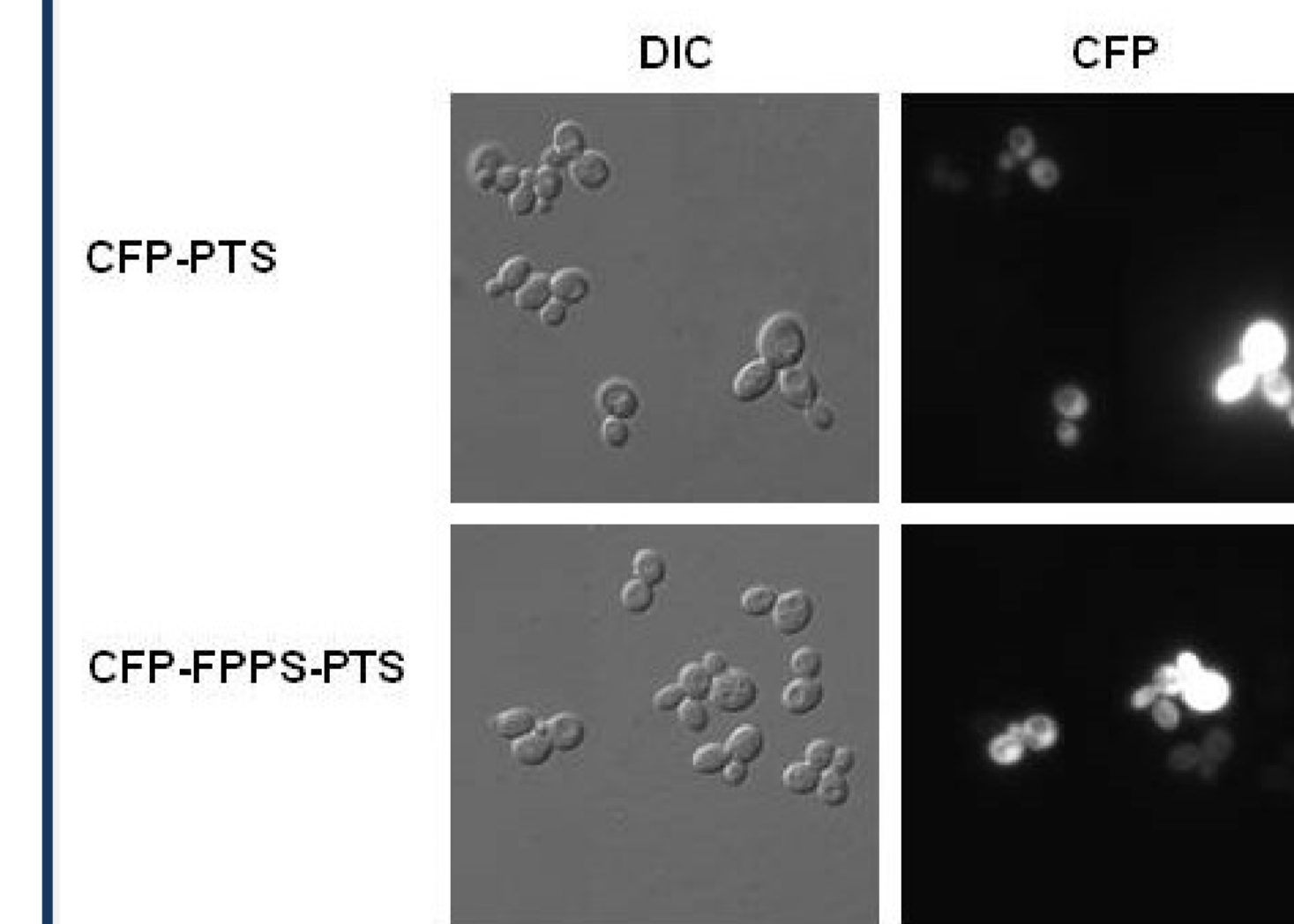


## Effect of enzyme fusion

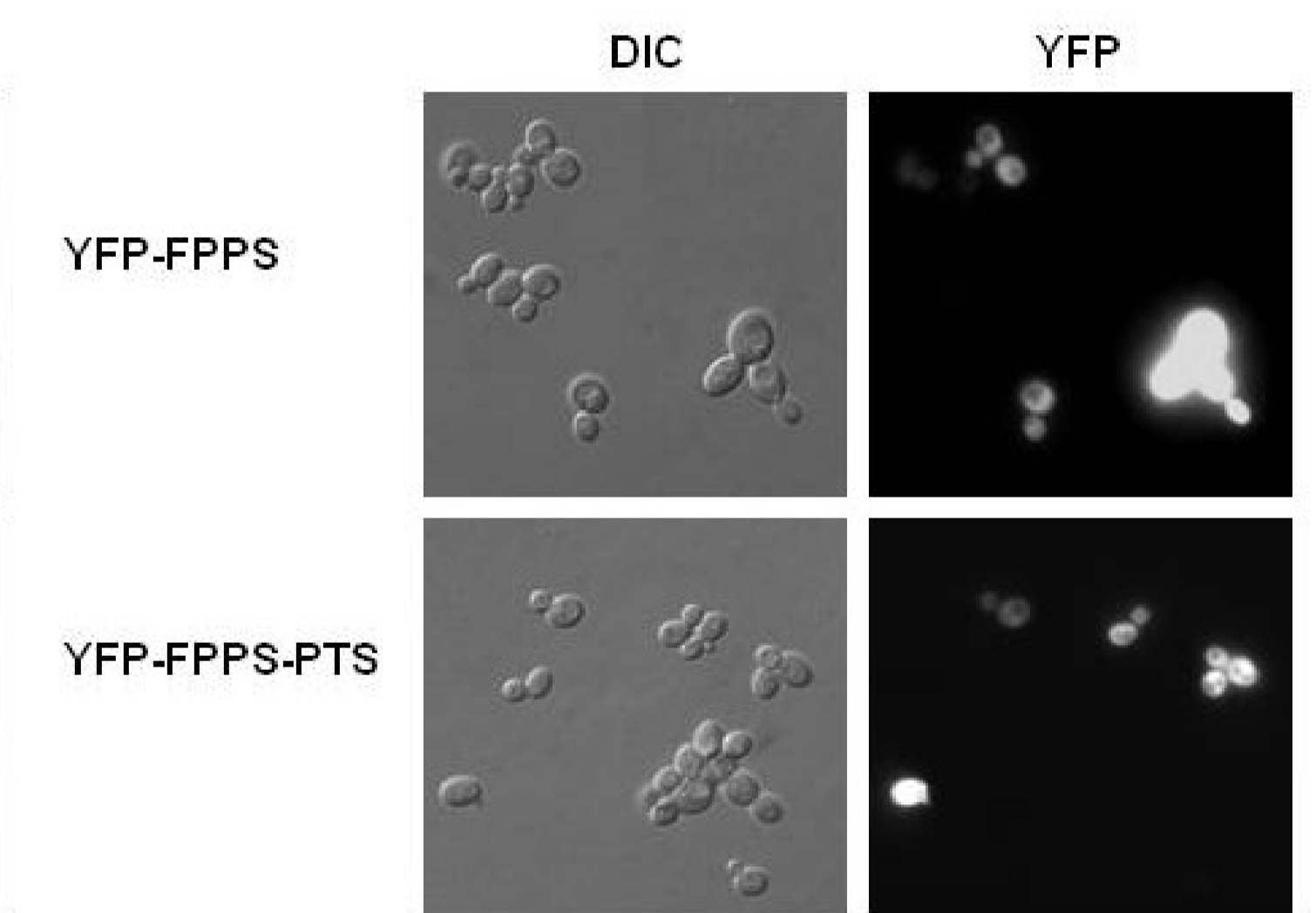


## Expression levels

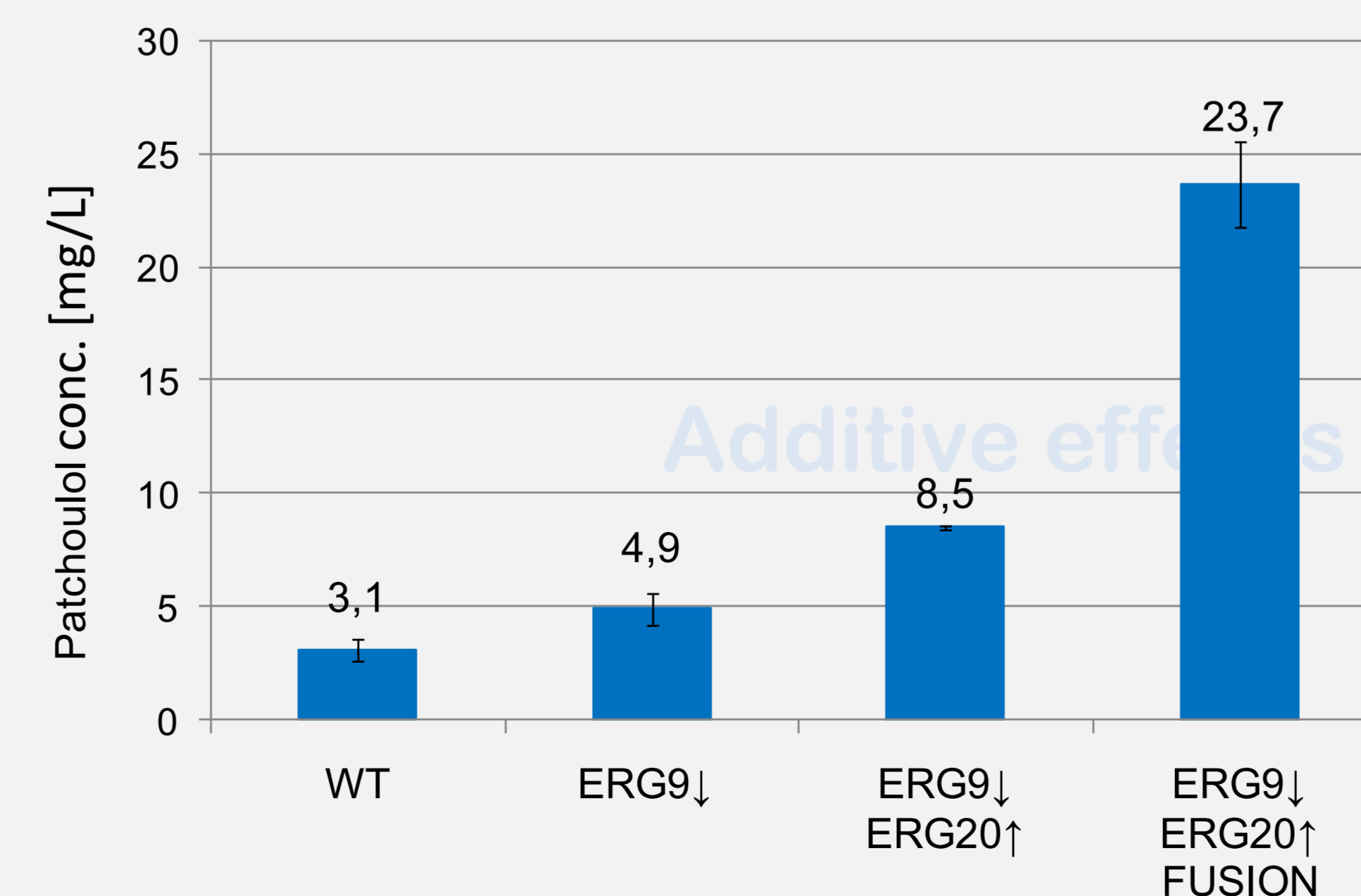
### Expression level PTS



### Expression level FPPS



## Additive effects



## Effect of linker

