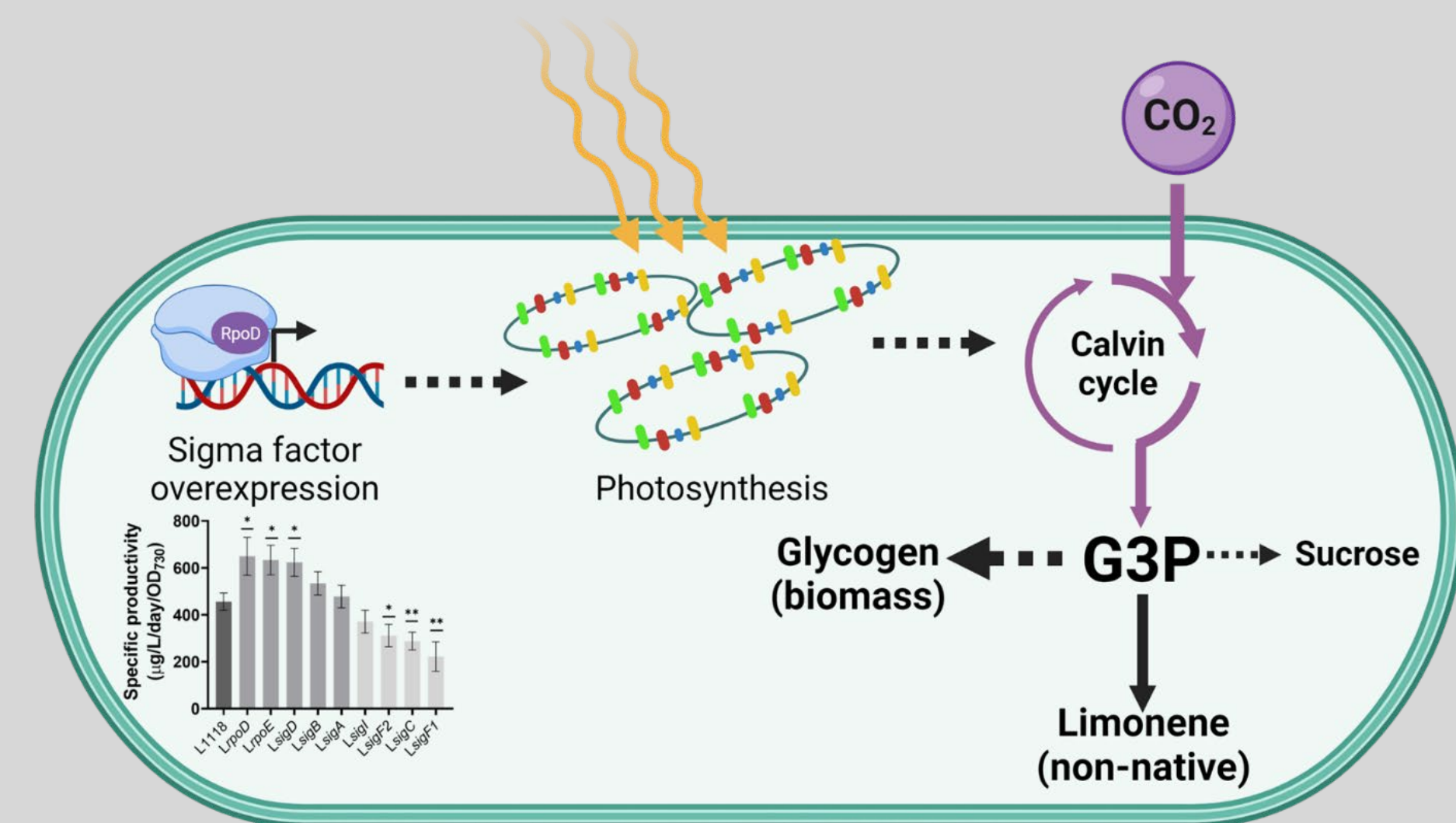


## Background

The terpene family is highly diverse with more than 50,000 unique chemical structures. Terpenes are condensed from two C5 precursor isomers, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Limonene, a C10 monoterpene, has been tested to use as a drop-in fuel additive for combustion engines and as the kerosene substitute for aviation fuels. Photosynthesis-driven CO<sub>2</sub> conversion to terpenes is particularly attractive for biofuel applications due to its low carbon footprint but is challenged with low productivities. We hypothesize that the low terpene yield in phototrophs is constrained by thermodynamics due to CO<sub>2</sub> delivery to cells.



## Methods

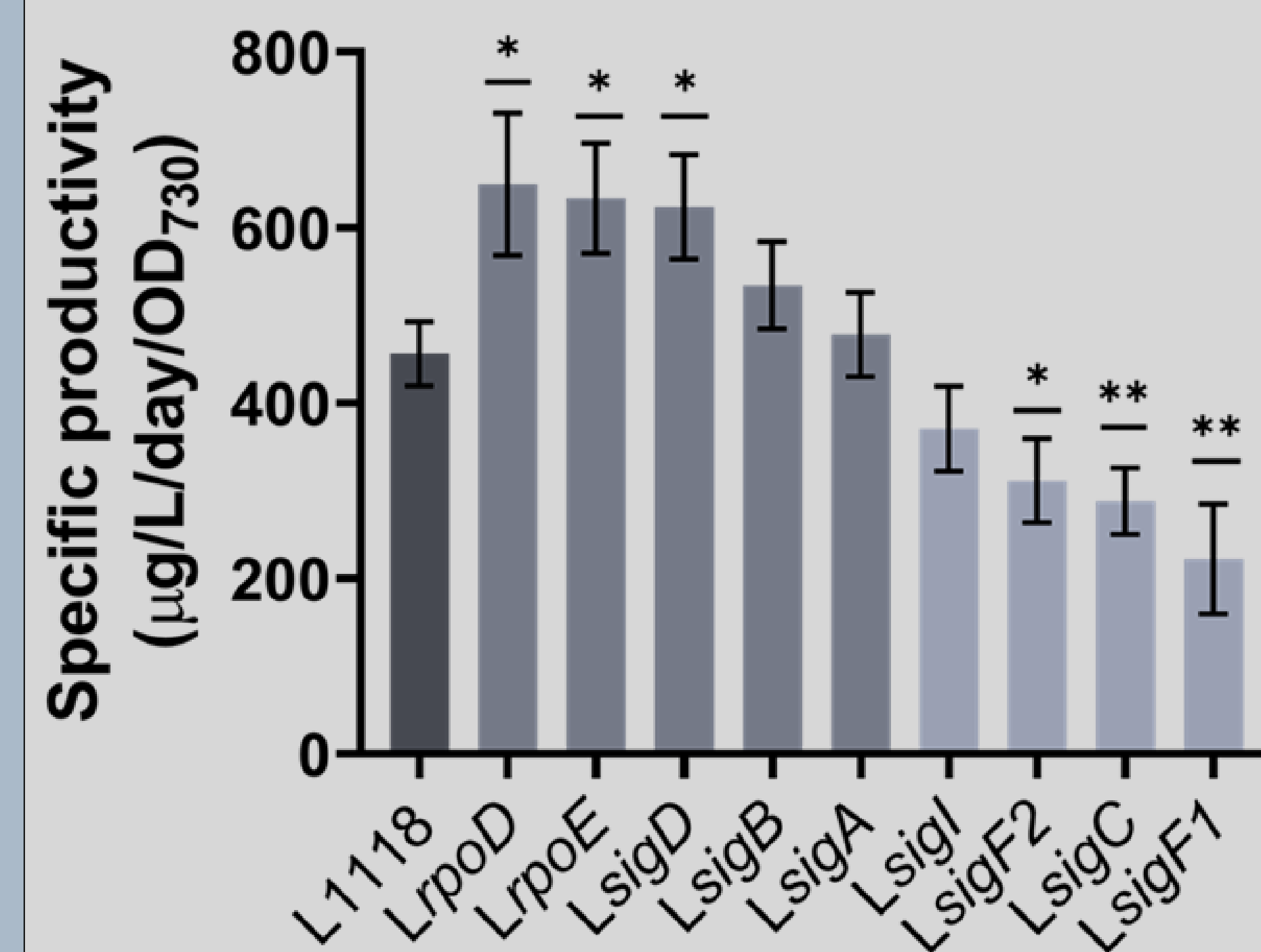
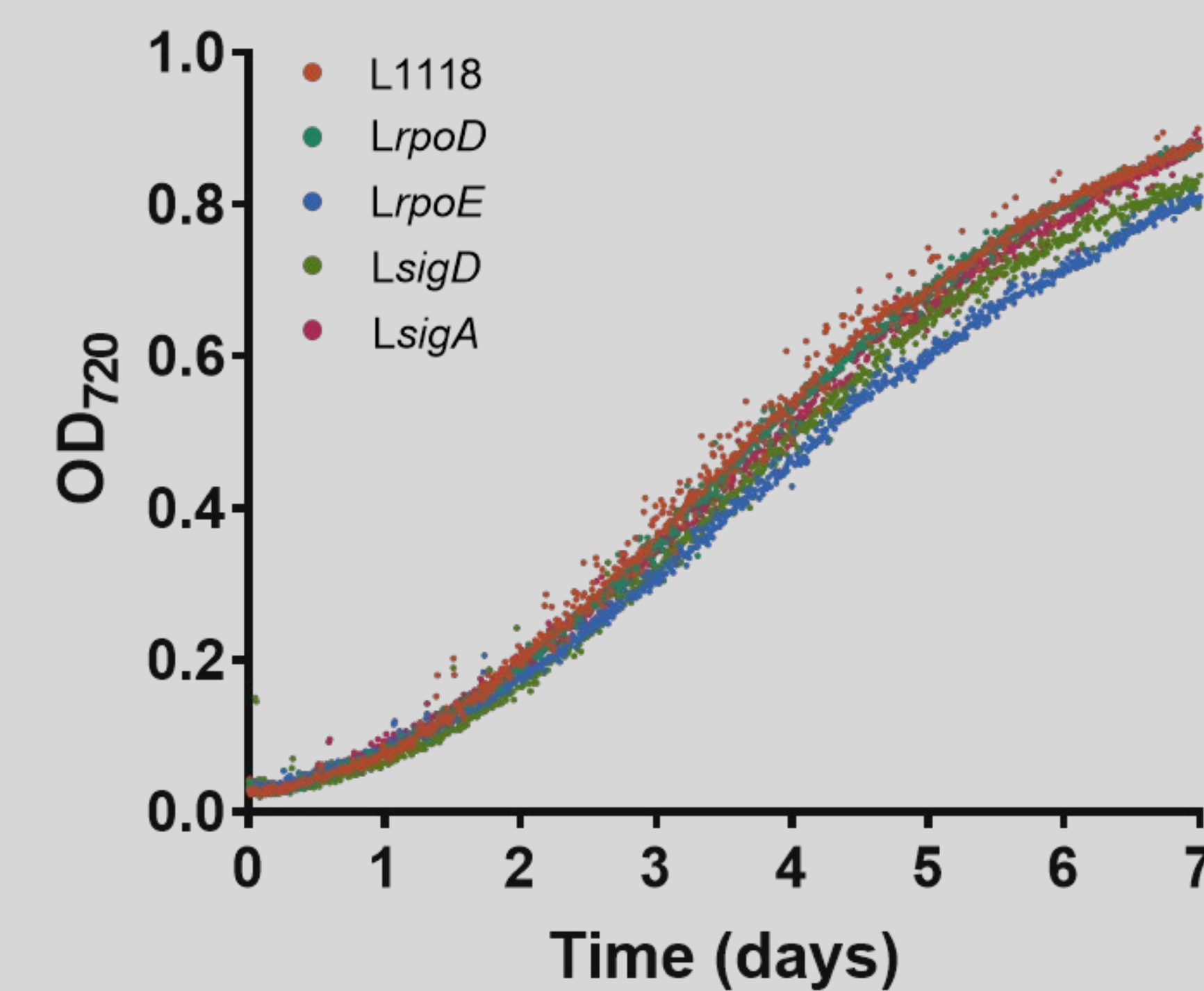
**Cyanobacteria growth.** Cyanobacteria were grown at 30 °C under 50 μmol/m<sup>2</sup>/s and varied intensity of cool white LED illumination in the Multi-Cultivator MC 1000-OD (Photon Systems Instruments, Czech Republic).

**Limonene measurement.** Limonene was collected using an absorbent trap containing HayeSep porous polymers (Sigma, USA) attached to the outlet of individual 1-L Roux bottles. Every day, limonene was eluted from the trap with 1 mL hexane supplemented with 10 μg/mL cedrene (Sigma, USA) as the internal standard. The eluted sample was analyzed by GC-MS in a Thermo Trace 1300 ISQ quadrupole (QD) (Thermo Fisher Scientific, USA) system.

**Oxygen evolution measurement.** Synthetic performance was measured using the Clark-type oxygen electrode (Qubit systems, Ontario, CN). The final oxygen evolution rate calculated was corrected with the dark respiration rate and normalized to chlorophyll content.

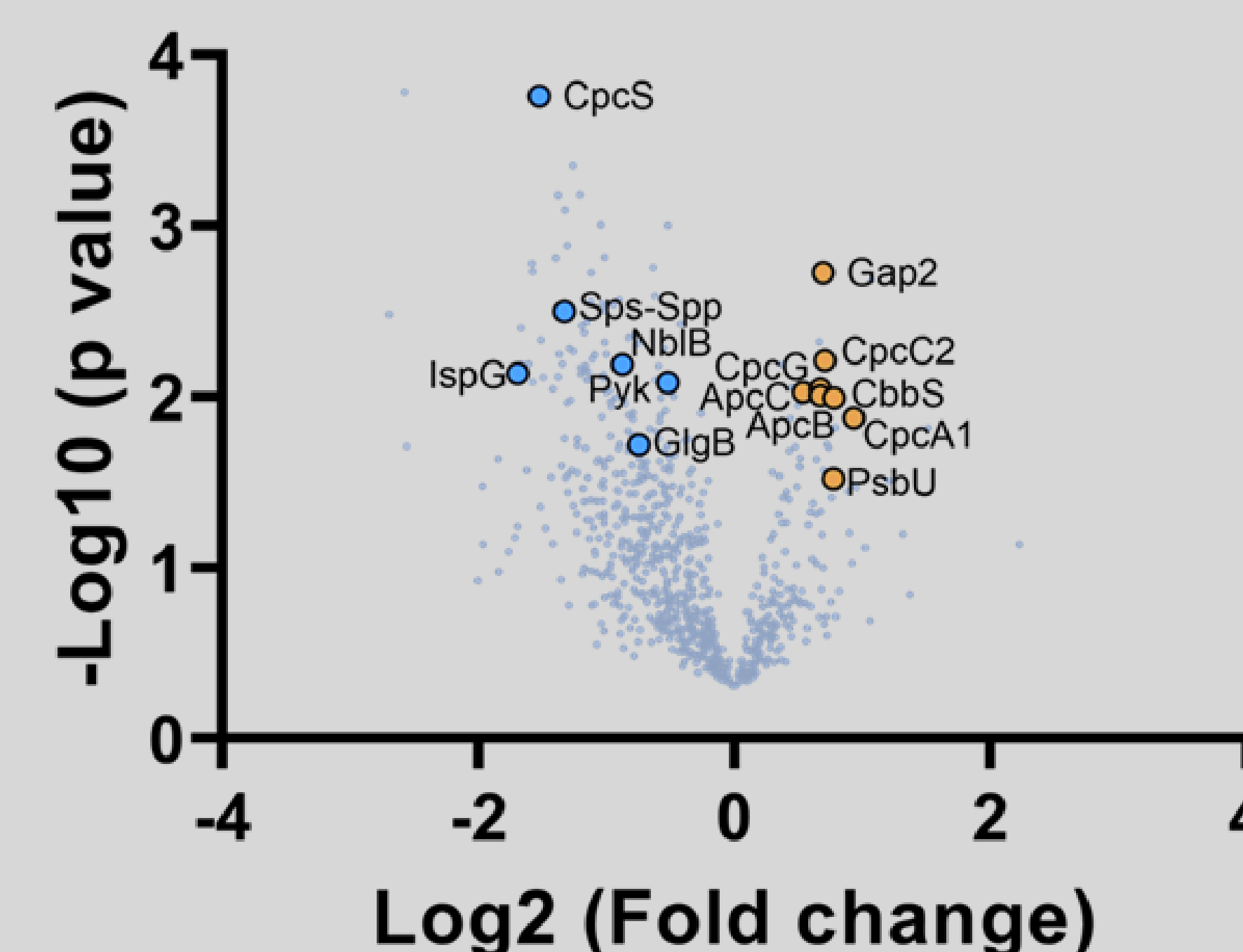
**Shotgun proteomics.** Comparative proteomics was conducted through LC-MS/MS on the Thermo LTQ Orbitrap XL by operating under the data-dependent mode. Normalized spectral abundance (NSAF) was used to compare the protein change between wild type and the engineered (*LrpD*) cells.

## Overexpression of sigma factors have varied effects on limonene productivity

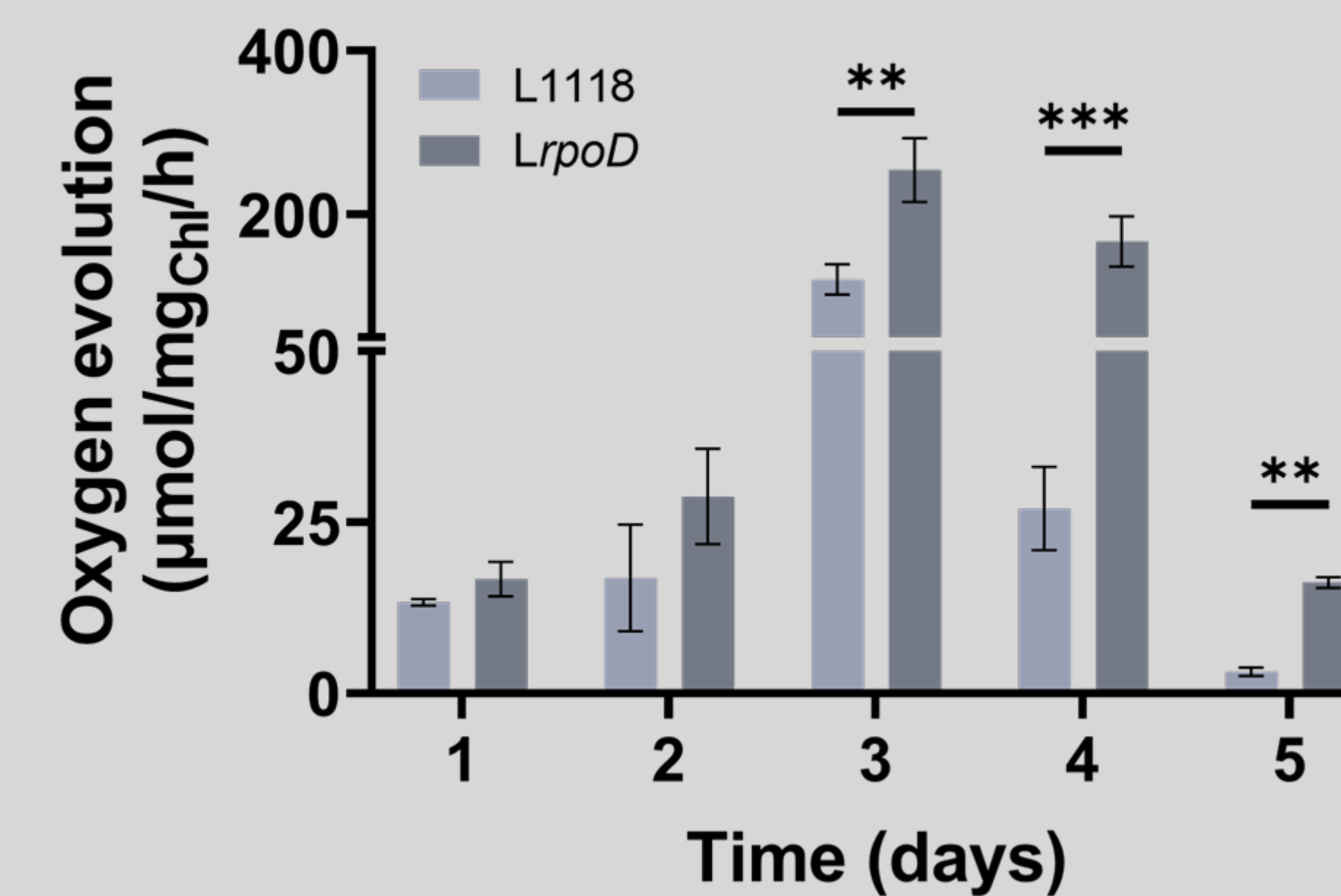


## Higher photosynthetic efficiency supports increased limonene production

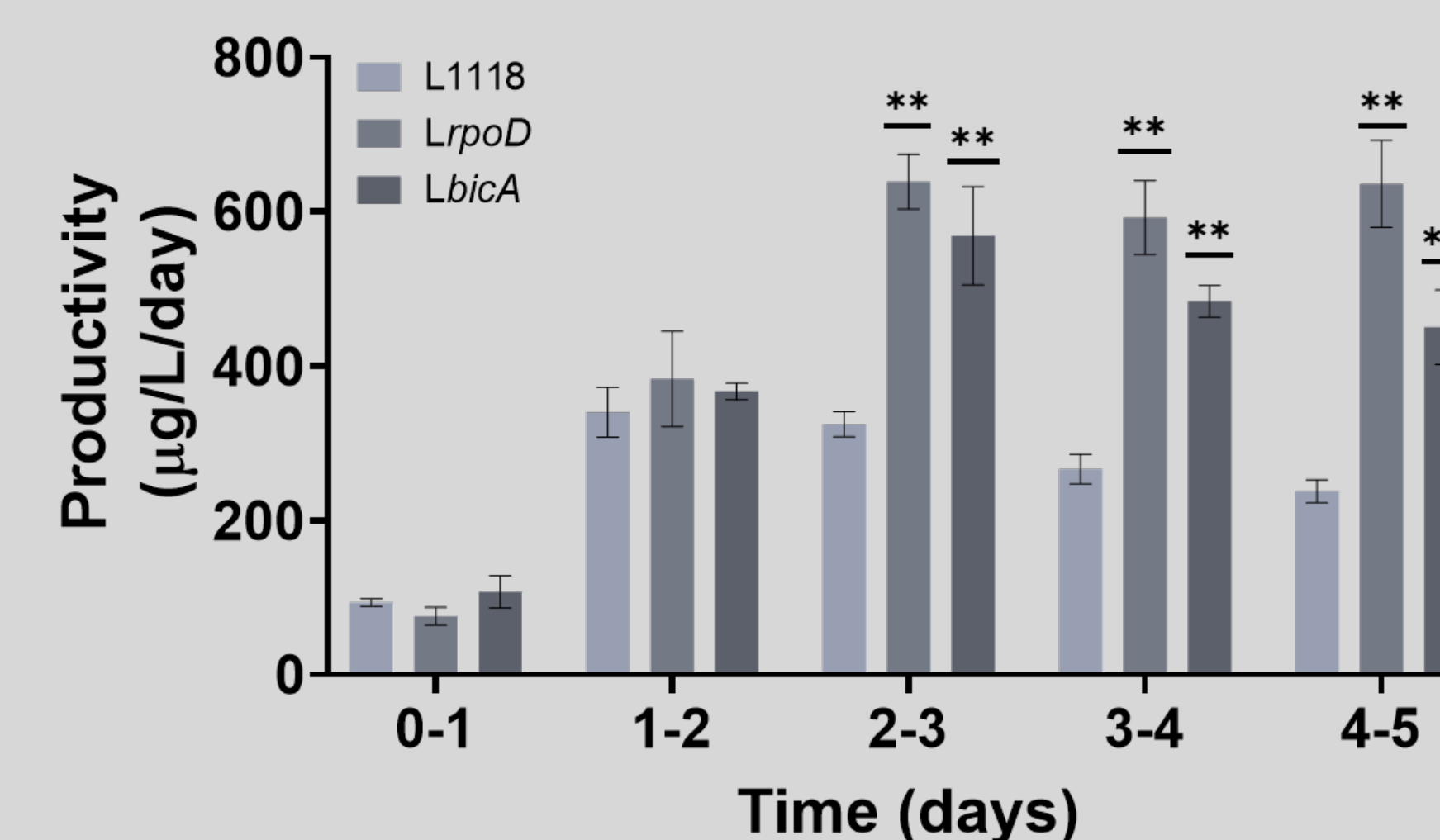
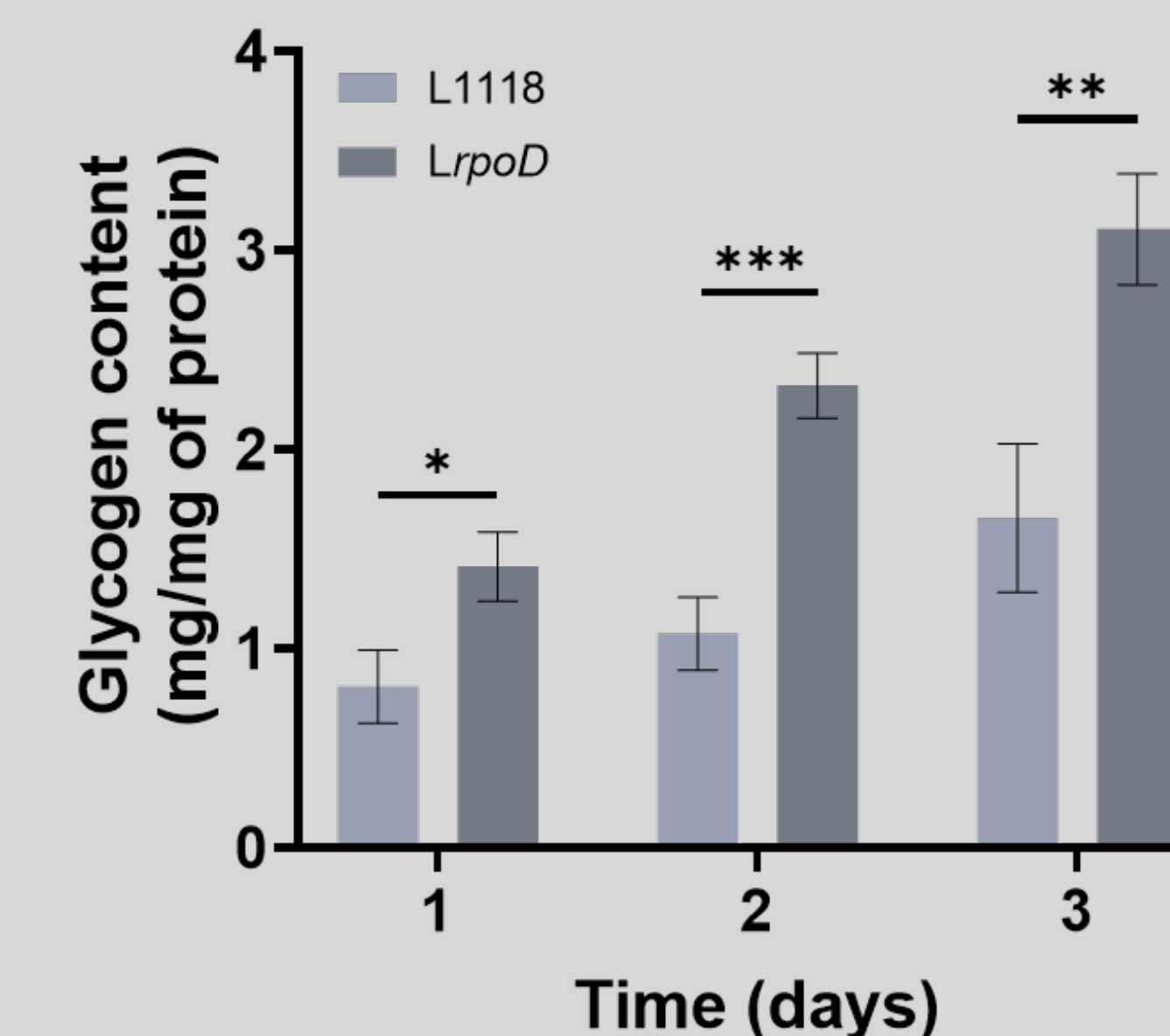
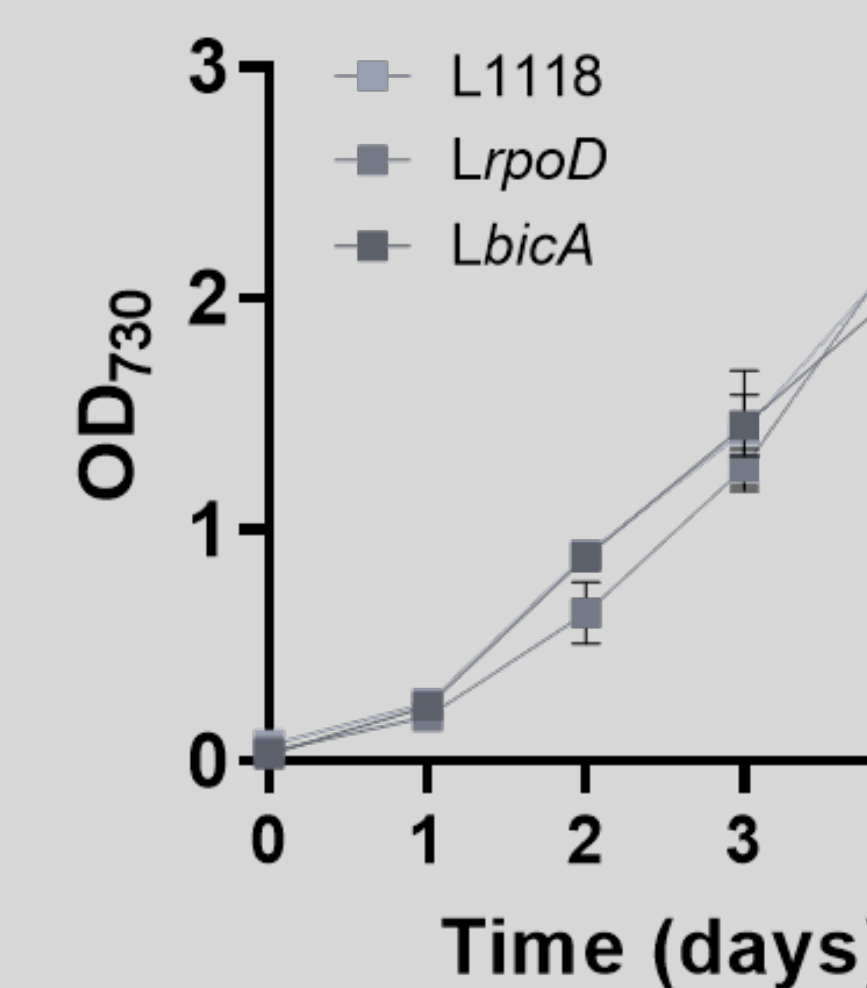
*Proteomics and O<sub>2</sub> evolution analyses indicate increased photosynthetic efficiency in LrpD cells*



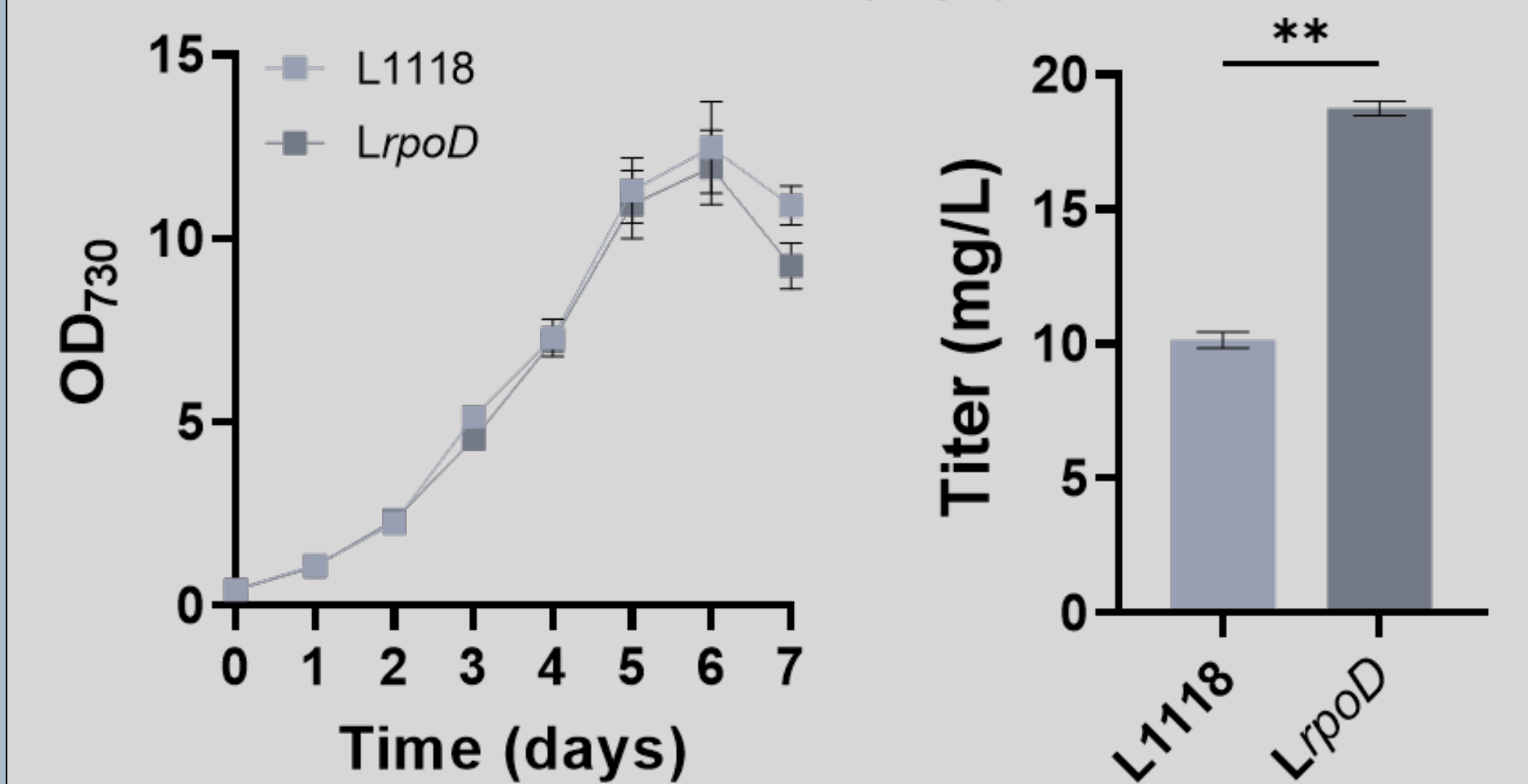
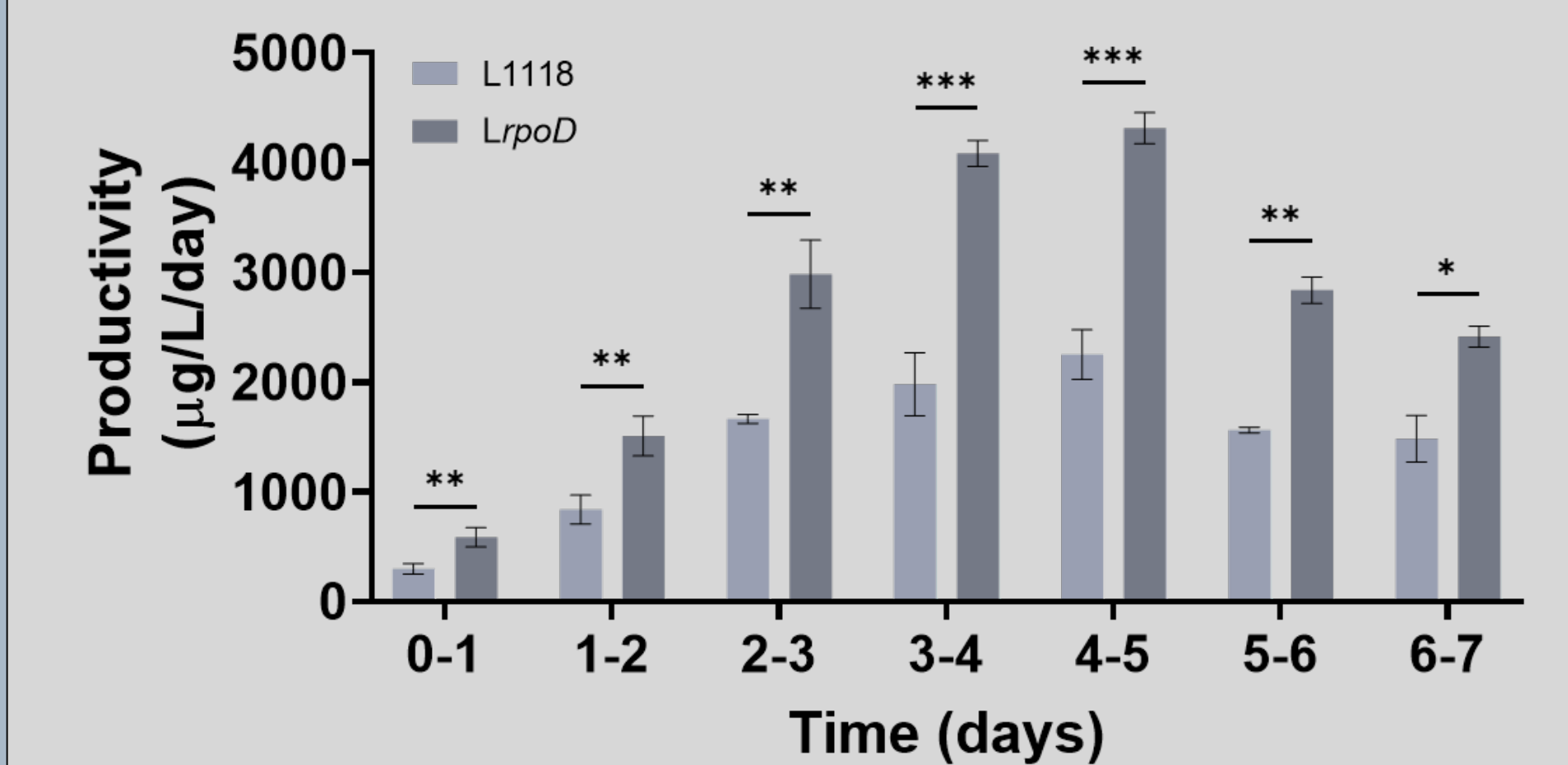
## Results



## Improving thermodynamics through bicarbonate transporter overexpression (*LbicA*) leads to increased glycogen and limonene production but similar growth among strains



## Improving thermodynamics through CO<sub>2</sub> delivery and high light leads to significantly improved limonene productivity



## Conclusion

- Enhancing photosynthesis through sigma factor engineering represents a key strategy to attain high productivity in secondary metabolic pathways such as terpenoids.
- Thermodynamics is revealed as the key determinant for high photosynthetic terpene productivity in cyanobacteria.

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## Acknowledgements

