Supporting information

Light-controlled cell factories – Employing photocaged IPTG for light-mediated optimization of *lac*-based gene expression and (+)-valencene biosynthesis in *Corynebacterium glutamicum*

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DNA-Sequence of the synthetic codon-usage optimized CnVS gene (oCnVS):

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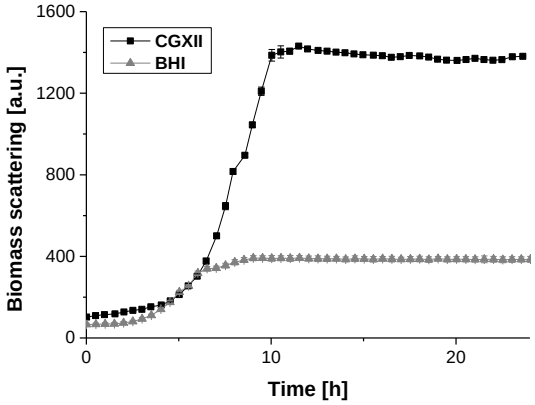

FIG S1 Growth curves of *C. glutamicum* cultures in triplicates in BHI complex (grey triangles) and CGXII minimal medium (black squares).

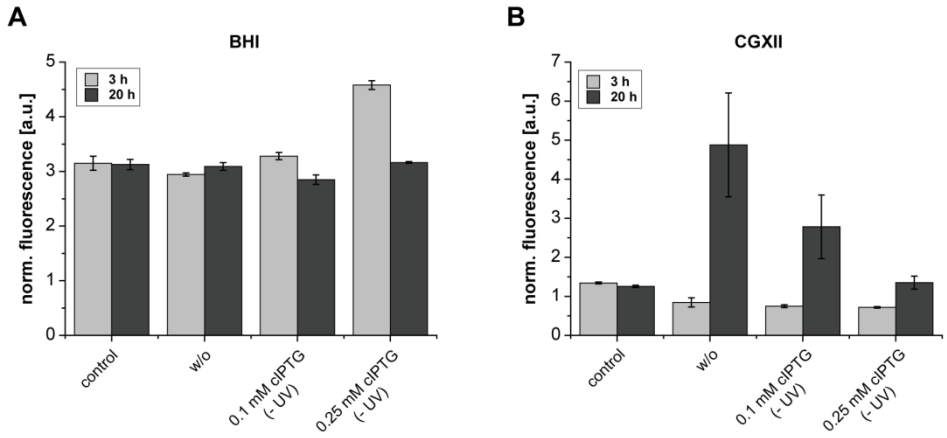

FIG S2 Basal expression background of cIPTG supplemented cultures in BHI (**A**) and CGXII medium (**B**) in the dark. Normalized fluorescence values originate from biomass-normalized triplicates analog to values for induced gene expression depicted in Fig. 2B,D. Control: Wildtype control strain without the pEKEx-2-EYFP plasmid.

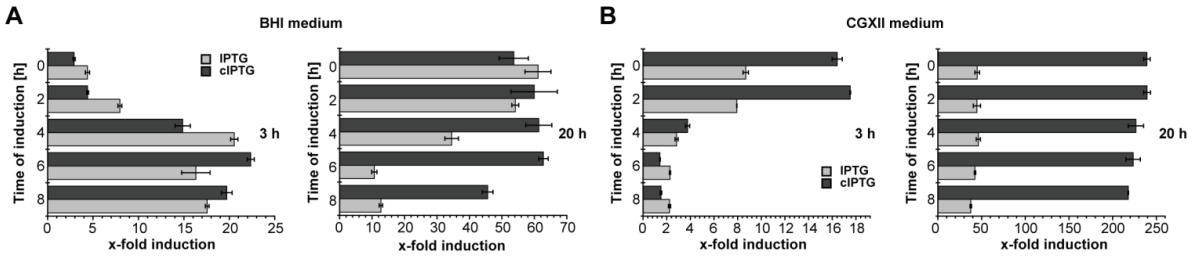

FIG S3 Dynamic range of induction for IPTG (light grey) and cIPTG-based (dark grey) induction after 3 (left) and 20 h (right) of expression in BHI (**A**) and CGXII medium (**B**) using *C. glutamicum* ATCC13032 (pEKEx-2-EYFP). Calculations originate from data depicted in Fig.2 B,C (biomass-normalized fluorescence) and Fig.S2 (basal fluorescence levels).

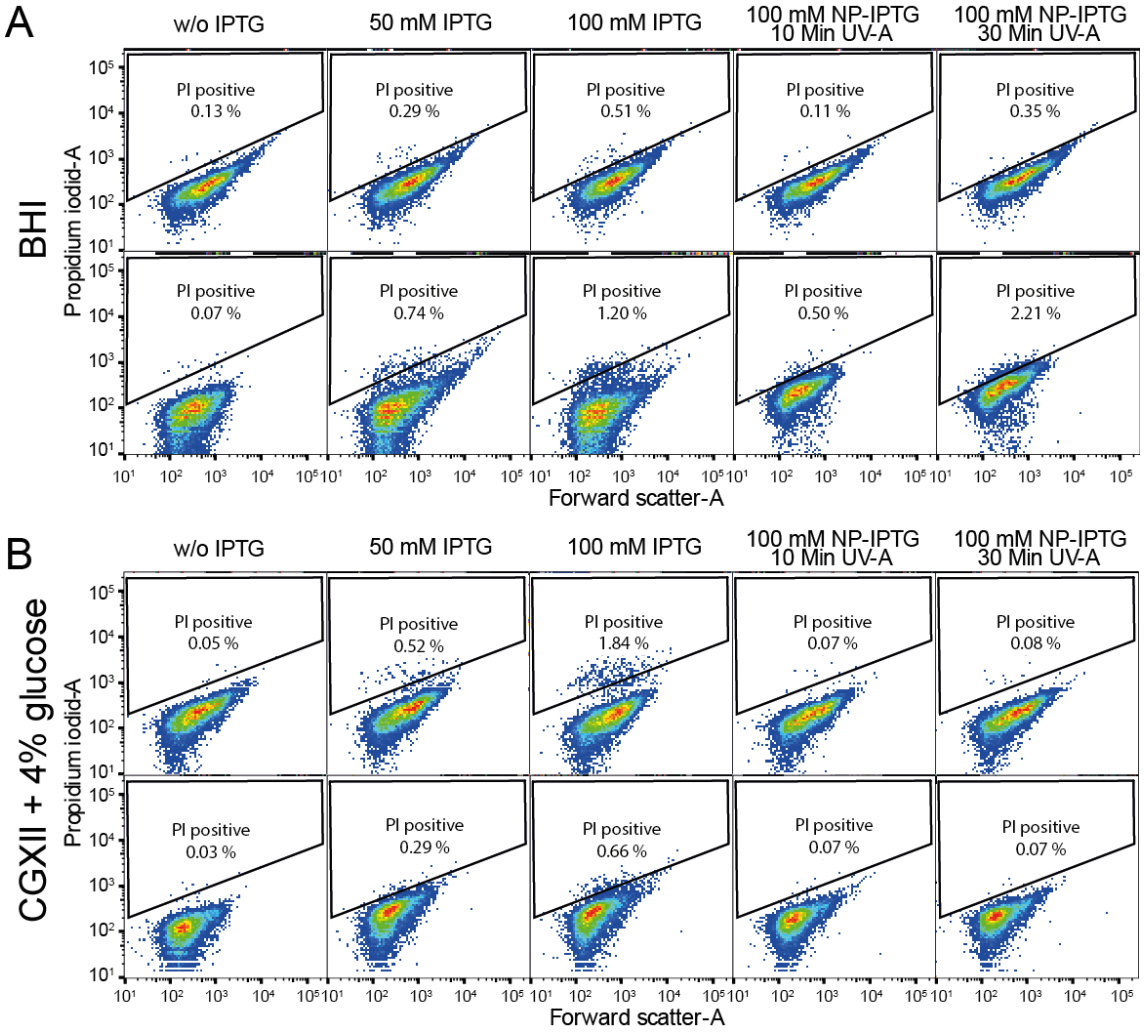

FIG S4 Propidium iodide-based live-dead-staining using flow cytometric single-cell analysis to evaluate cell viability.

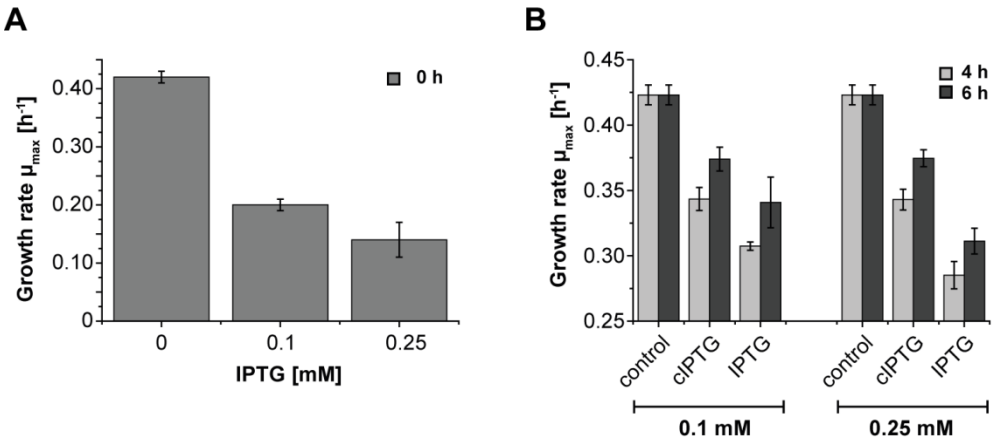

FIG S5 Reduction of growth impairment during (+)-valencene production in VLC6 *via* delayed induction and application of cIPTG-based light induction. **A**) Growth rates of VLC6 cultures for direct induction (0 h) are depicted for different IPTG inducer concentrations. **B**) Growth rates of VLC6 cultures are shown for delayed IPTG and cIPTG-induction after 4 (light grey) and 6 h (dark grey) together with un-induced cultures (control). All means and standard deviation derive from biological triplicates.

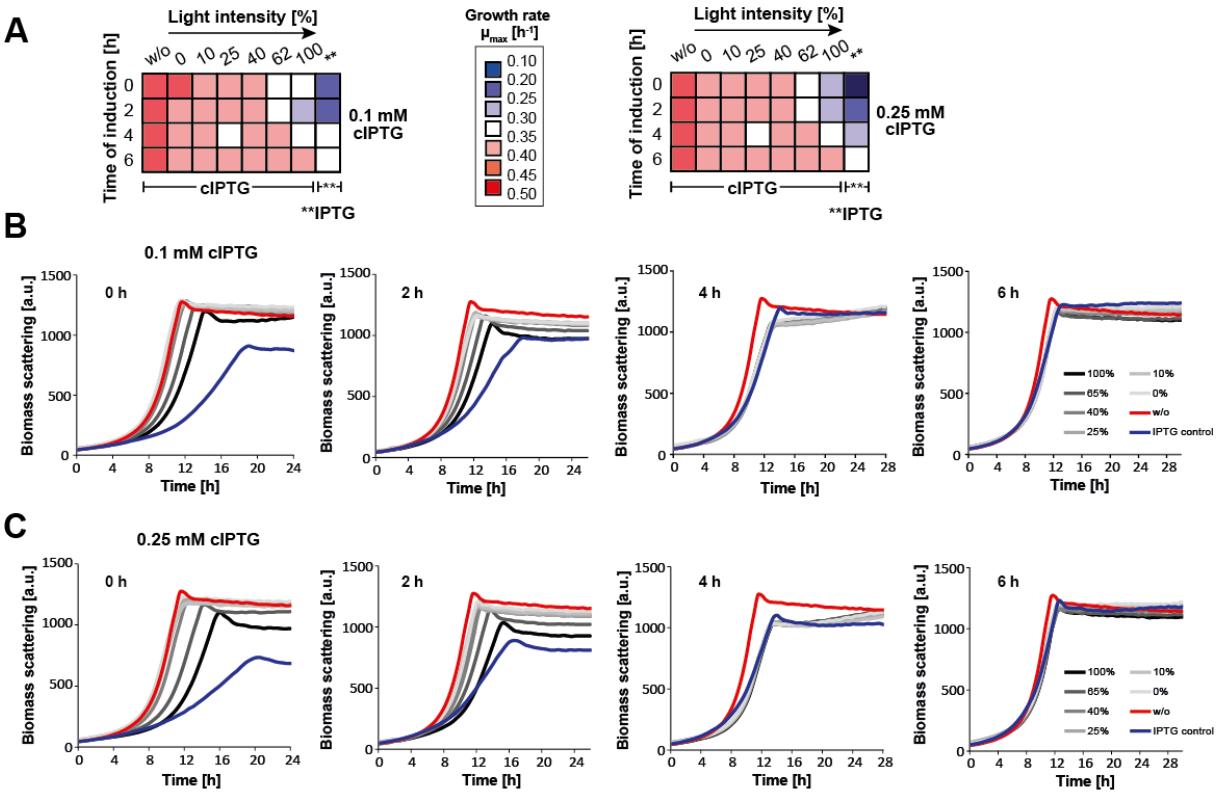

FIG S6 Growth analysis of *C. glutamicum* VLC6 during IPTG- and cIPTG-induced (+)-valencene production for different induction time points. (**A**) Maximum growth rate μ_{max} intervals from low (blue) to high (red) in h^{-1} after 24 h of production using different times of induction as well as different (c)IPTG concentrations (0.1 and 0.25 mM). (**B**) Growth curves of (+)-valencene production cultures supplemented with 0.1 mM (c)IPTG. Light induction (grey to black curves) is compared to IPTG induction (blue curve) after 0, 2, 4 and 6 h of cultivation and plotted against uninduced control cultures (w/o, red curve). (**C**) Growth curves of (+)-valencene production cultures analogous to Fig. S6 B supplemented with 0.25 mM (c)IPTG. All averaged data originate from at least three independent biological triplicates.

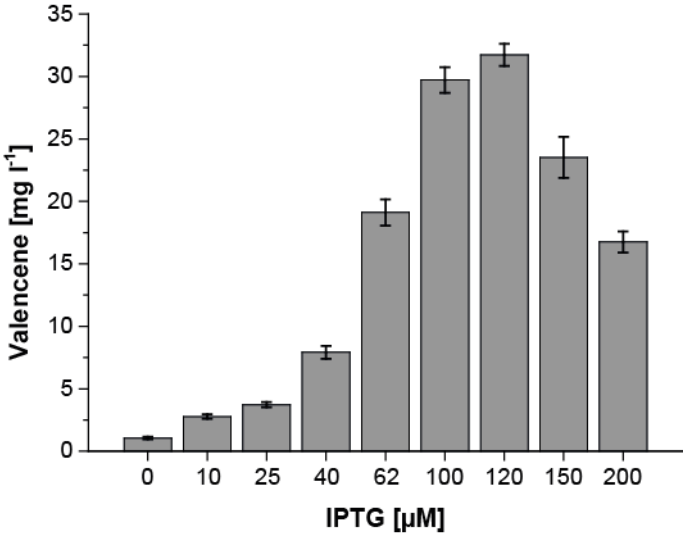

FIG S7 Optimization of IPTG concentrations for *C. glutamicum* VLC6 production cultures induced after 4 h of cultivation. All averaged data originate from at least three independent biological triplicates. Error bars indicate the respective standard deviations.

TAB S1 Summary of titers as well as volumetric and biomass-specific productivities for the conducted (+)-valencene productions in different *C. glutamicum* strains using CGXII minimal medium. Values for volumetric productivity were calculated using the overall cultivation times (28 h for induction after 4 h and 30 h for induction after 6 h).

Strain	Condition	Titer [mg l^{-1}]	Final OD_{600}	Volumetric productivity [µg l^{-1} h^{-1}]	Biomass-specific productivity [µg g CDW^{-1} h^{-1}]
VLC3	0.1 mM IPTG, 6h, Flask	7.2±0.6	33.5±2.1	240 ± 20	29 ± 2
VLC4	0.1 mM IPTG, 6h, Flask	10.8±1.1	34.1±1.8	360 ± 37	42 ± 4
VLC5	0.1 mM IPTG, 6h, Flask	10.5±3.5	33.9±2.3	350 ± 117	41 ± 14
VLC6	0.1 mM IPTG, 6h, Flask	27.1±0.6	35.2±1.1	903 ± 20	103 ± 2
VLC6	0.1 mM IPTG, 6h, Flowerplate	29.0±0.1	59.7±3.4	967 ± 3	65 ± 0
VLC6	0.1 mM IPTG, 4h, Flask	14.8±1.1	30.3±1.2	529 ± 39	70 ± 5
VLC6	0.1 mM IPTG, 4h, Flowerplate	28.4±1.7	57.8±2.9	1014 ± 61	70 ± 4
VLC6	0.1 mM cIPTG, full light induction after 4h, Flowerplate	41.0±0.7	60.8±1.9	1464 ± 25	96 ± 2