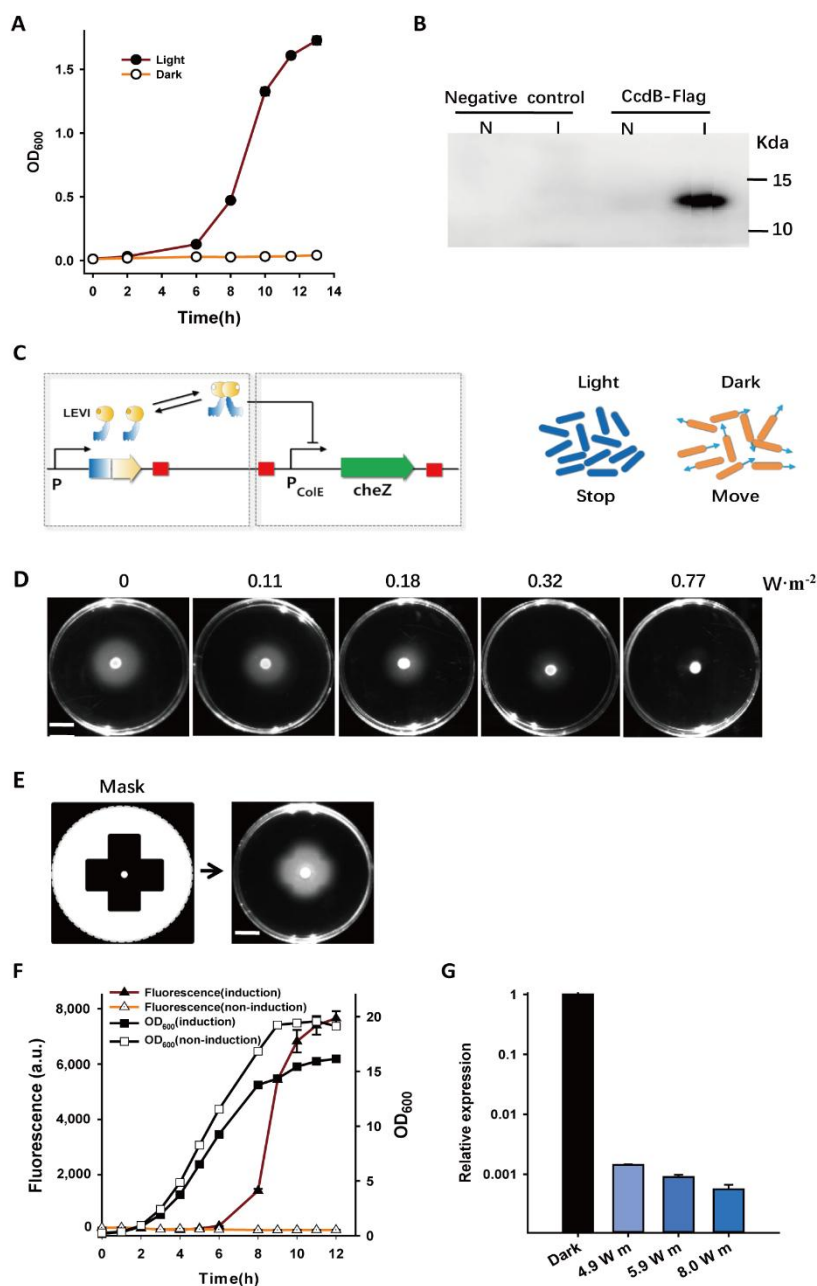


Supplementary Figure 2



Supplementary Figure 2 Application of LightOff system in many quantitative and synthetic biology studies.

(A) and (B) Expression of toxic protein by LightOff system. (A) The toxicity of CcdB-flag to *E.coli* cells. Error bars, mean \pm s.e.m. from three independent experiments. (B) Detection of CcdB-flag expression from LightOff system by western blot. Lysates were derived from non-induced (N) or induced (I) cells. Cells transformed with pCDFDuet 1 empty vector were used as the negative control. (C-E)

Controlling bacteria mobility using LightOff system. **(C)** Expression of cheZ protein, which plays an important role in bacteria mobility, was under the control of LightOff system. **(D)** The engineered bacteria were spotted onto the semisolid media and kept in different light irradiance for 18 h before imaging. **(E)** The engineered bacteria were cultured using a printed mask with a specific image as the “light guide”. Scale bars in (b) and (c), 1 cm. **(F)** Large-scale production of recombinant protein using fermenter by LightOff system. *JM109(DE3, ΔsuaA, ΔLexA)* cells transformed with pLEVI-mCherry vector, 5% (v/v) of the seed culture was inoculated to 5-L fermenter. The cells were illuminated with blue light illumination or transferred to dark condition to induce mCherry expression when OD₆₀₀ reached ~0.2. Fluorescence and OD₆₀₀ were measured at indicated time points. Error bars, mean ± SD (*n*=3 samples) from the same experiment. a.u., arbitrary units. **(G)** More stringent control of gene expression with higher light intensity by LEVI (I74V). *E.coli* cells were cultured in darkness or upon 4.9 W m⁻², 5.9 W m⁻² or 8.0 W m⁻² blue light exposure. Data were normalized to the mCherry expression in dark conditions. Error bars, mean ± s.e.m. from three independent experiments.