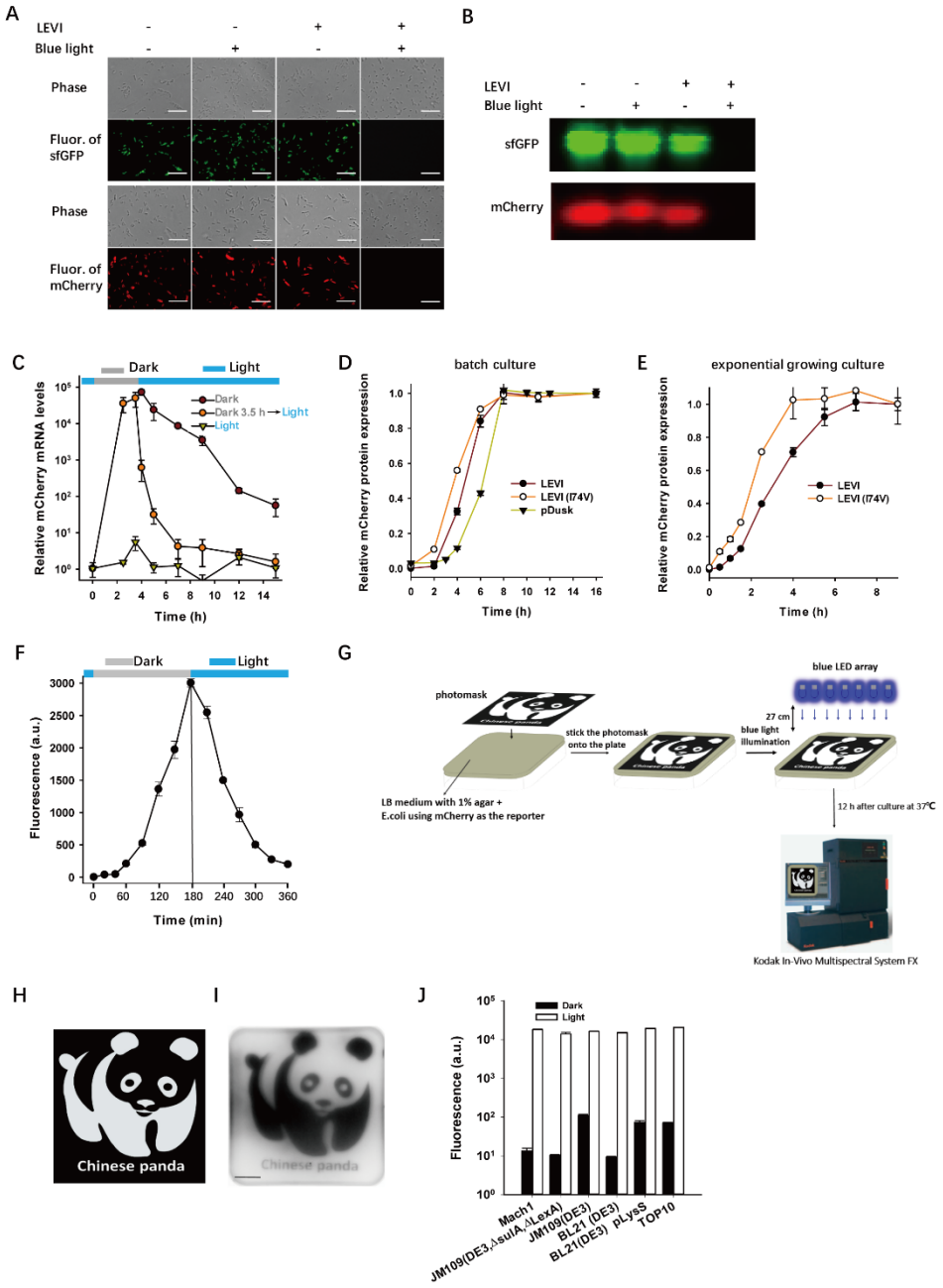
## Supplementary Figure 1




**Supplementary Figure 1 Characterization of LightOff system.**

(**A**)Phase contrast images and fluorescence images of *JM109(DE3, ΔsulA, ΔLexA)* cells transformed with pLEVI-sfGFP or pLEVI-mCherry and cultured in darkness or light. pColE-sfGFP and pColE-mCherry whose LEVI expression cassettes were removed based on pLEVI-sfGFP and pLEVI-mCherry were used as the control, respectively. Scale bars, 10 μm. (**B**) Native PAGE images of cell lysates. (**C**) Quantitative determination of mCherry mRNA levels. *JM109(DE3, ΔsulA, ΔLexA)*

cells transformed with pLEVI-mCherry were cultured in blue light illumination overnight. The overnight cultures were diluted 100-fold into fresh medium and firstly grown in darkness and then were transferred to blue light illumination. Cells always kept in darkness or blue light illumination were used as the controls. Cellular mCherry mRNA levels measured at the indicated time points. Error bars, mean ± SD (*n*=3 samples) from the same experiment. (**D**) and (**E**) Derepression kinetics of LightOff system in batch culture and exponential growing culture. **(D)** *JM109(DE3, ΔsulA, ΔLexA)* cells transformed with LightOff system with different VVD variants were cultured upon blue light exposure, the cells were transferred to darkness when $OD_{600}$ reached ~0.4. For comparison, light regulated mCherry expression from pDusk system in *BL21 (DE3)* cells was measured following the same experiment conditions. **(E)** *JM109(DE3, ΔsulA, ΔLexA)* cells transformed with LightOff system with different VVD variants were cultured upon blue light exposure overnight. The overnight cultures were diluted to $OD=10^{-6}$. The cells were preconditioned for 5 h in light illumination and then were transferred to darkness. mCherry fluorescence was measured at the indicated time points after transfer of cells to darkness. Data were normalized to the maximum mCherry expression in dark conditions, respectively. Error bars, mean ± s.e.m. from three independent experiments. (**F**) Rapid switching on and off gene expression in exponential growing culture. *JM109(DE3, ΔsulA, ΔLexA)* cells transformed with pLEVI-mCherry were cultured in blue light illumination overnight. The overnight cultures were diluted to $OD=10^{-6}$. The cells were preconditioned for 5 h in light illumination and then were transferred to darkness for 3 h, and were then transferred to blue light. Aliquots were taken at the given to for mCherry fluorescence measurements. Error bars, mean ± SD (*n*=3 samples) from the same experiment. a.u., arbitrary units. (**G**-I) Spatial control of gene expression by LightOff system using a plate-based assay. **(G)** Schematic diagram for the bacterial photography experiment demonstrating spatial control of gene expression by blue light. The overnight cultured cells were mixed with LB medium with 1% agar and immediately poured into a Petri dish with 12-cm internal diameter and allowed to harden at room temperature for 30 min. A photomask made of 0.2 mm thick

transparency was placed on the dish, and then the dish was incubated at 37 $^{o}$C and illuminated from top with blue light from a blue LED array lamp of 27 cm distance. Image of mCherry fluorescence was acquired using a Kodak In-Vivo Multispectral System FX (Carestream Health). **(H)** Panda image on the photomask. **(I)** Fluorescence image of mCherry reporter expression in bacteria film illuminated with blue light image. Scale bar, 2 cm. (**J**)Light-switched gene expression by LightOff system with $LexA_{408}$ mutant in different strains. mCherry expression by LightOff system containing $LexA_{408}$ mutant in LexA DNA binding motif and its cognate operator sequence was measured in different strains. Error bars, mean ± s.e.m. from three independent experiments. a.u., arbitrary units.