

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: Pulsatile activation of the *sinI* promoter in an *expR*⁻ microcolony. Full time-lapse microscopy movie of the *expR*⁻ microcolony used to demonstrate pulsatile expression from the *sinI* promoter in Fig. 1c. Expression rate over the whole experiment runtime is shown in Supplementary Fig. 3a (first column). The cells in the colony carry the *PsinI-mVenus* fusion at the chromosomal locus and the integrated suicide plasmid *pK18mob3-PwgeA-mCerulean* that also includes a *Ptrp-mCherry* fusion for image segmentation. (Left) mVenus channel false-coloured according to the NIS-elements 'red fire' lookup table displayed in Fig. 1c, (right) phase contrast channel of the same colony.

File Name: Supplementary Movie 2

Description: *sinI* expression pulse frequency is elevated in the wild type. Full time-lapse microscopy movie of a wild-type microcolony likewise carrying the chromosomal *PsinImVenus* fusion and the integrated *pK18mob3-PwgeA-mCerulean* with the *Ptrp-mCherry* fusion. Expression rate over the whole experiment runtime is shown in Supplementary Fig. 7e (right). Pulse frequency is clearly elevated in the wild type compared to the *expR*⁻ microcolony shown in Movie 1, while pulse amplitudes greatly vary from cell to cell both in the wild-type and in the *expR*⁻ background. (Left) mVenus channel false-coloured according to the NIS-elements 'red fire' lookup table displayed in Fig. 1c, (right) phase contrast channel of the same colony.

File Name: Supplementary Data_Custom code

Description: This folder contains a ReadMe Word file, 5 Matlab scripts with the custom code, a subfolder 'data' with subfolders containing data of all colonies analysed with respect to pulsing in the manuscript, and a Word file with tables linking the respective colonies/data to different genotypes/growth conditions.