Engineering a synthesis-friendly constitutive promoter for mammalian cell expression

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Supporting Information

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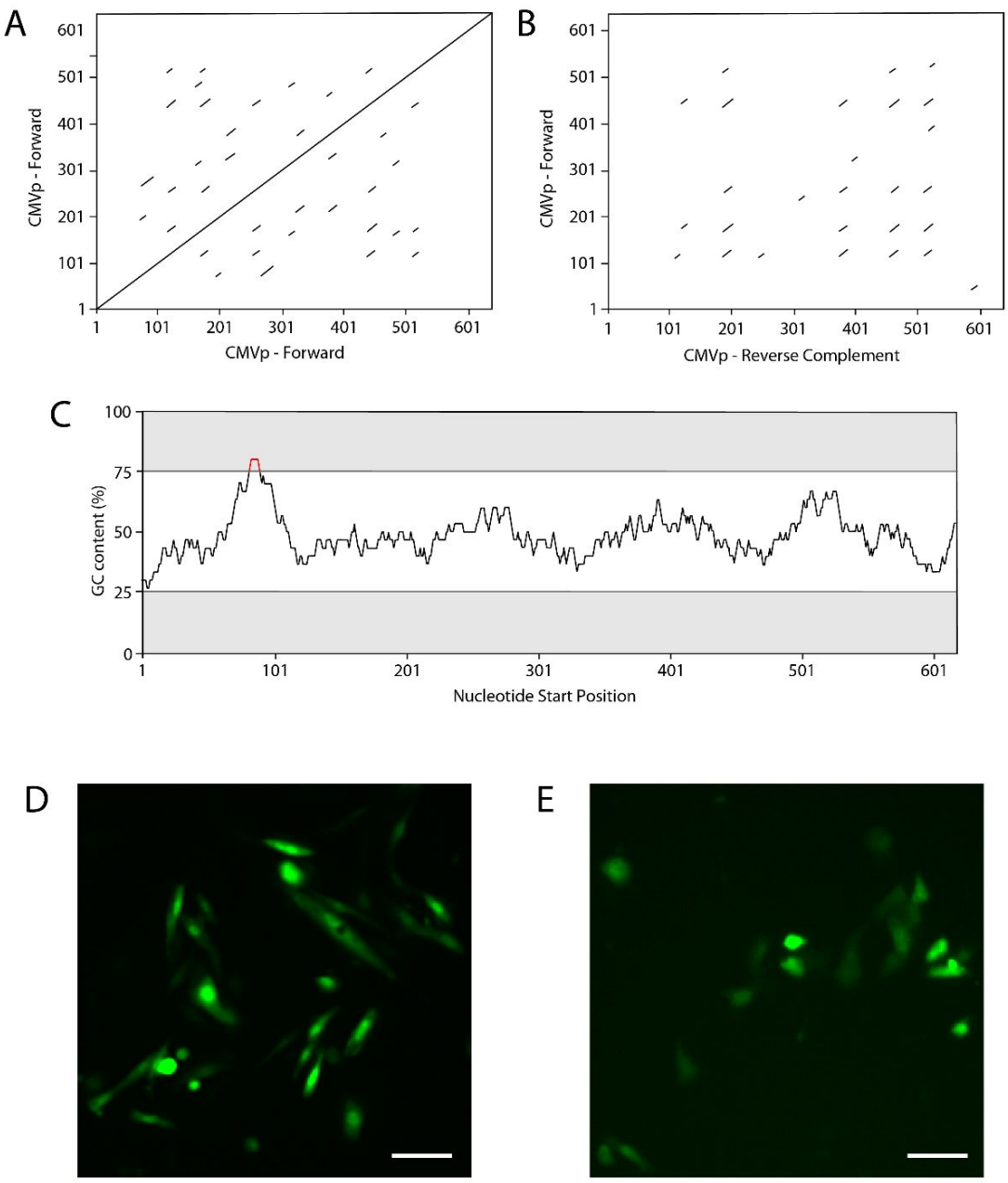

Figure S1. Synthesis-related CMVp features and stable cell lines. (A, B) Dot plots for visualization of repeating segments found in two sequences placed along the axes. Minimum window size used is 9bp. (A) Forward direction repeats present in full-length CMV promoter displayed as a dot plot with the full sequence along each axes. (B) Reverse, indirect repeats present in full-length CMV promoter displayed as a dot plot with the full sequence along the Y-axis and reverse complement along the X-axis. (C) GC-content plot for CMVp calculated with window size of 30bp. Red GC contents show sequences outside of the synthesis-friendly GC content limits of 25% and 75%. Pseudo-coloured fluorescence microscopy images of stable CHO cells (D) and MDCK cells (E) expressing Venus regulated by SFC promotor. Scale bar represents 100µm.

Supplementary Video 1. Fluorescence microscopy timelapse of HEK293 cells stably transfected with SFCp-driven CaRQ and Venus fusion construct. Images are taken at 5 second intervals following observation period of cells to ensure absence of blebbing. Bolus 10uM ATP stimulus is added at 10 seconds. Rapid blebbing begins at 140 seconds.

Supplementary Video 2. Fluorescence microscopy timelapse of HEK293 cells stably transfected with CMVp-driven red fluorescence Ca^{2+} indicator, RCaMP, in tandem with SFCp-driven Ceru-NanoLuc. Images are taken at 5 second intervals. Bolus 10uM ATP stimulus is added at 20 seconds.