Mapping mechanosensitive channel number to survival probability

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**Abstract**

**Importance**

**Introduction**

Current state of the field, disparity between channel counts, and why cells need so many. “We chose to examine the contribution of a single mechanosensitive channel (MscL) responsible for sensing and responding to large membrane tension.”

**Materials and Methods**

**Strains, media, and growth conditions.** HJ will provide information.

**Flow cell.** HJ will provide information.

**Imaging conditions.** HJ will provide information.

**Data analysis and image processing.**

Cells were identified manually as “survivors” or “fatalities”. Fatalities were defined as cells which did not perform two complete division events in during the acquisition time.

Phase contrast and fluorescence images were acquired before the shock. Individual cells were segmented using the phase-contrast image and total cellular fluorescence intensities were computed. The segmented cells were then matched with the manually curated identification of survival or death. Each day’s data set was processed individually. See the Supplemental Information for a more detailed discussion of segmentation.

**Calibration of channel number.**

Using previously known fluorescence calibration

Calculation of “per cell” measurement using a reference area.

**Logistic regression.**

Common problem in machine learning. Assume that there is a smooth, linear increase in the log-odds of survival with channel number.

The parameters were estimated through Markov Chain Monte Carlo using the Generalized Linear Models utility in the open-source software PyMC3.

**Results**

To our knowledge, there has been no single-cell measurement of survival with a known number of channels. We have engineered a system in which the expression of the MscL protein is modulated across two orders of magnitude in copy number using RBS modification. By pooling this data together, we can directly map a cells’ MscL copy number and measure its probability of survival. Previous work (Booth et al.,) have measured this quantity using a combination of super-resolution microscopy and bulk survival essays. Understanding the precise number of channels needed to have appreciable survival is critical to our understanding of the biological and physical implications of mechanosensation.

The need for a standard candle.

Agreement/disagreement between dilution method, quantitative western, and photobleaching assays.

Distribution of channel number (per cell or per area – need to choose one and specify the issues. Should show cells in some figure). Distribution among survivors and fatalities across all shock rates.

Compare channel distribution of survivors and fatalities between fast ( >= 1.0 Hz ) and slow (< 1.0 Hz) shock rates. This is particularly interesting for the low expressing strains (SD4, SD6).

Inferring survival probability using Logistic regression on pooled data set, low shock rate, and fast shock rate.

Comment on the apparent number of channels needed for appreciable survival.

**Discussion**

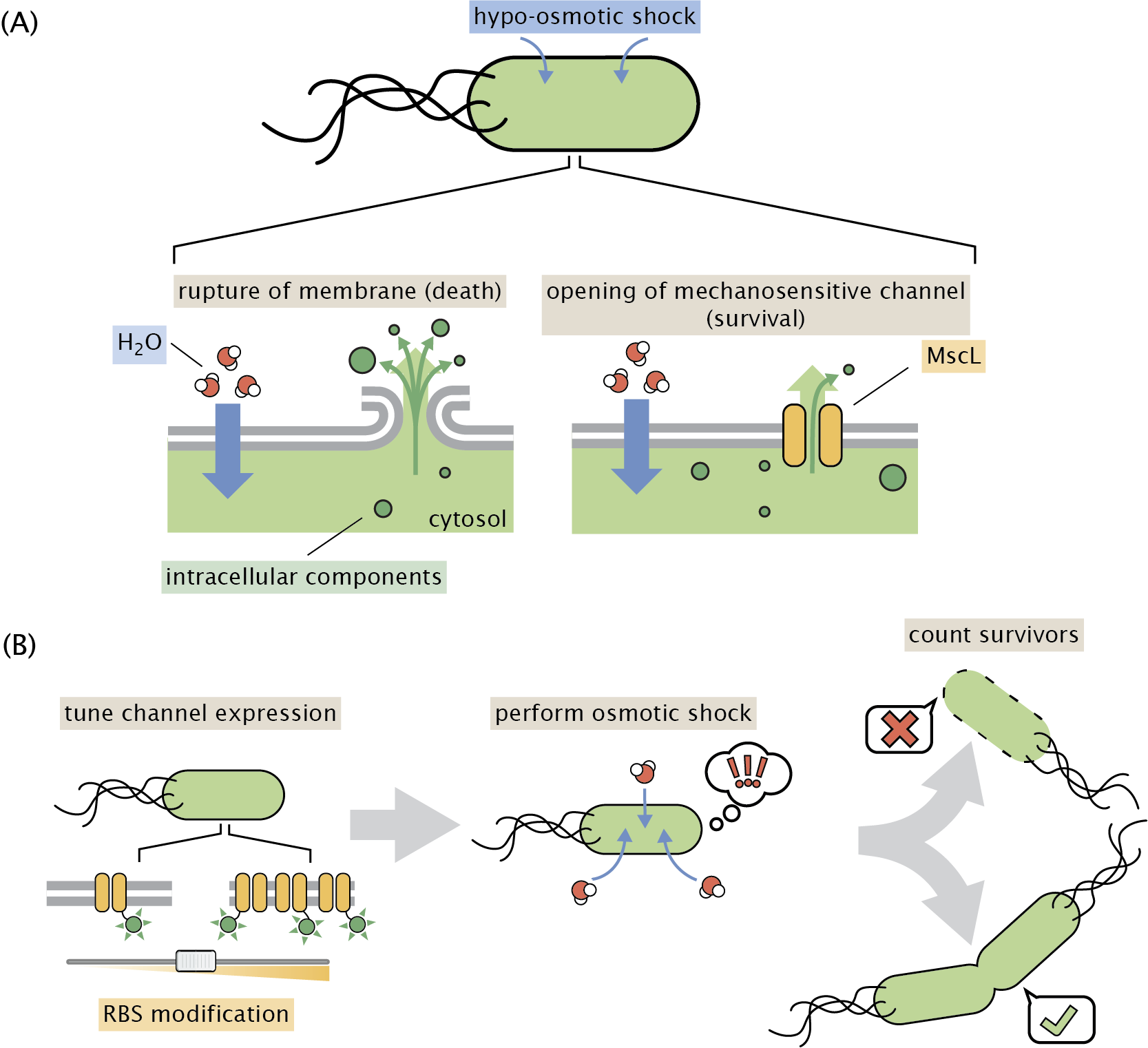
Emphasize highly quantitative measurement of a single channel number and survival probability using the same methodology. Allows for the estimation of single-cell survival probability.

Touch with theory work in some manner, point out that this is (as reported in Booth paper) well above what one would expect given the theory.

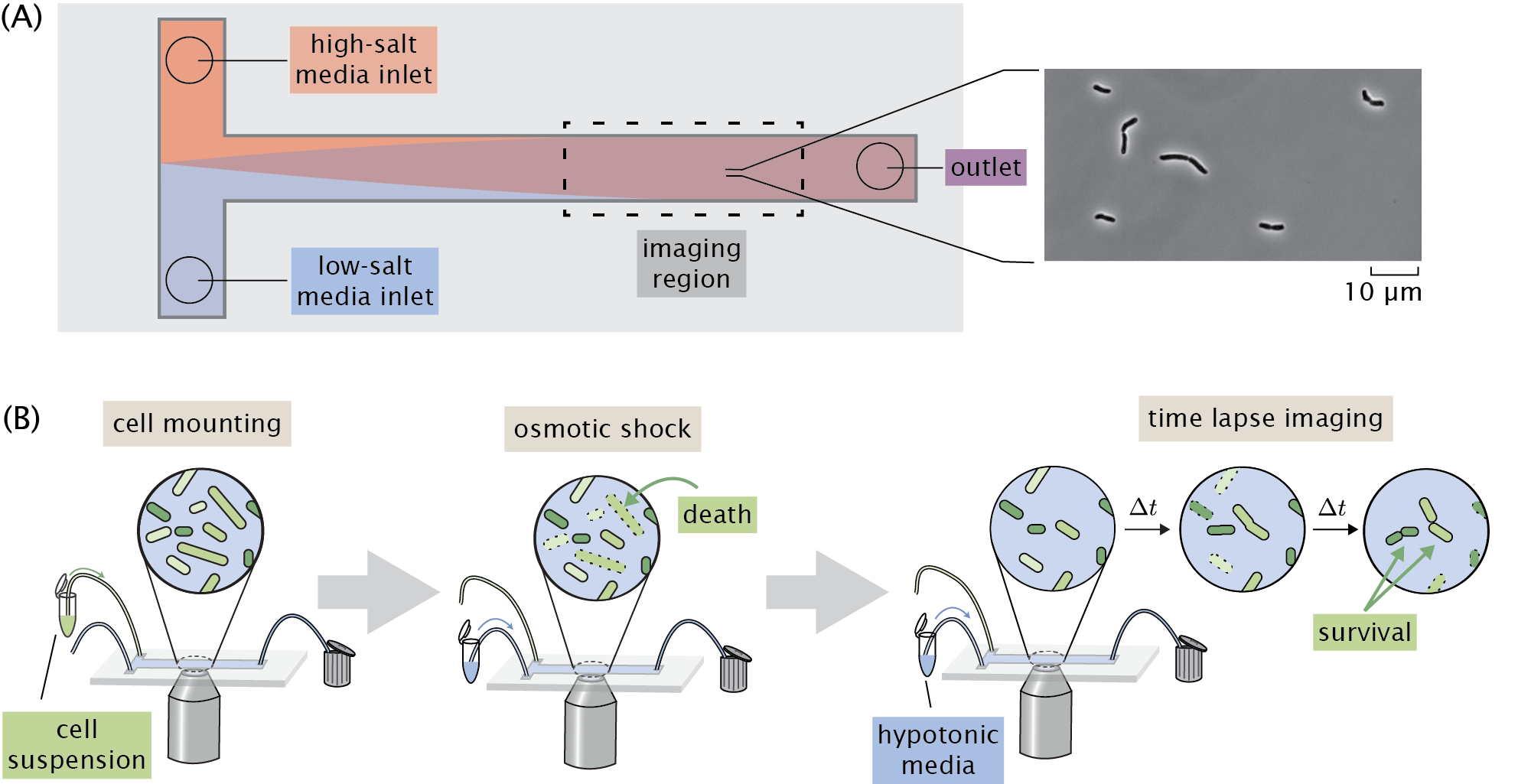
Theoretical predictions require only a few MscL channels to be present to relieve even large osmotic downshocks. However, our data suggests that between 400 and 600 copies of MscL to survive slow osmotic shocks to nearly 100%. The estimates for channel copy numbers in *E. coli* growing in standard LB Miller medium is around this number, indicating

**Acknowledgements**

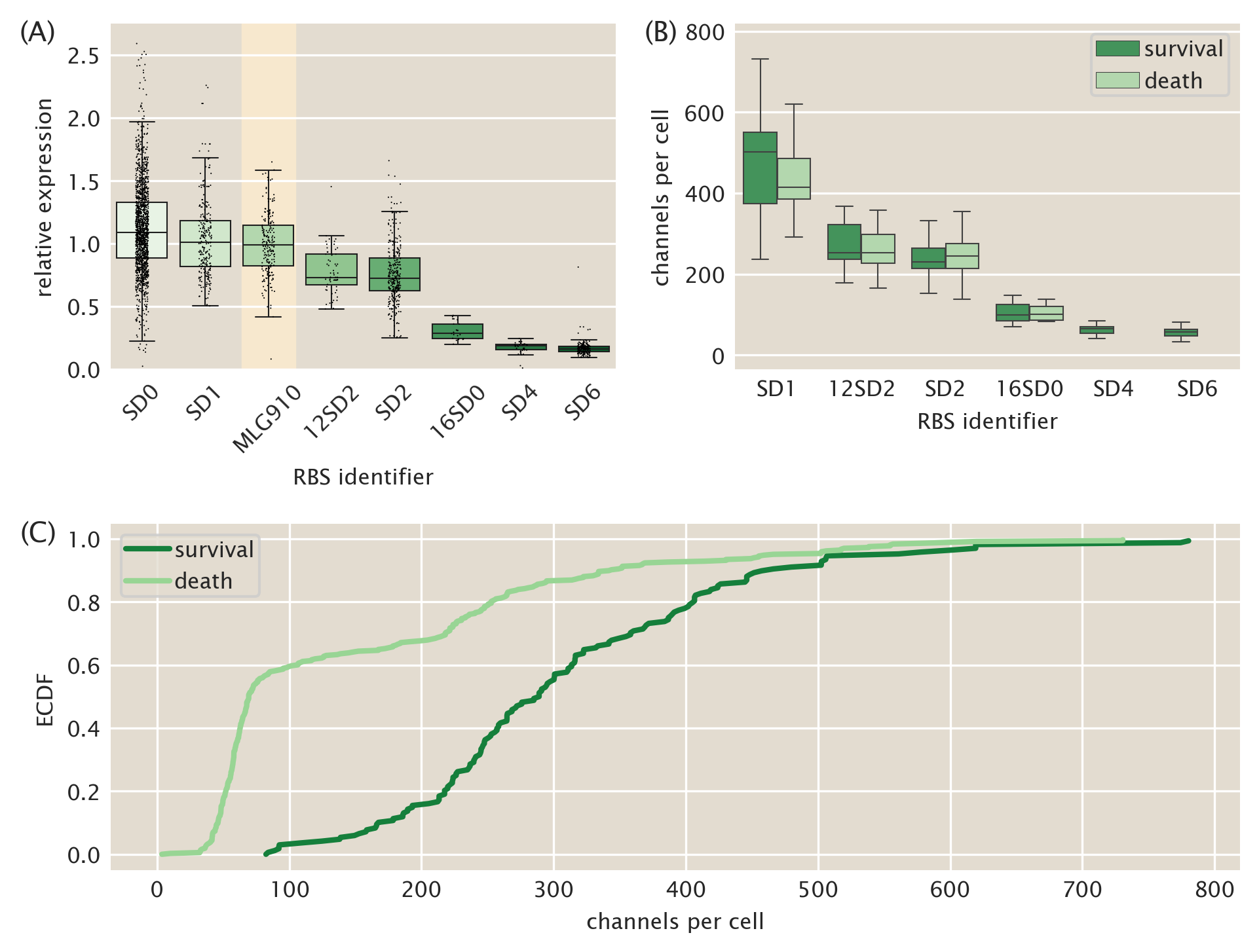
**References**



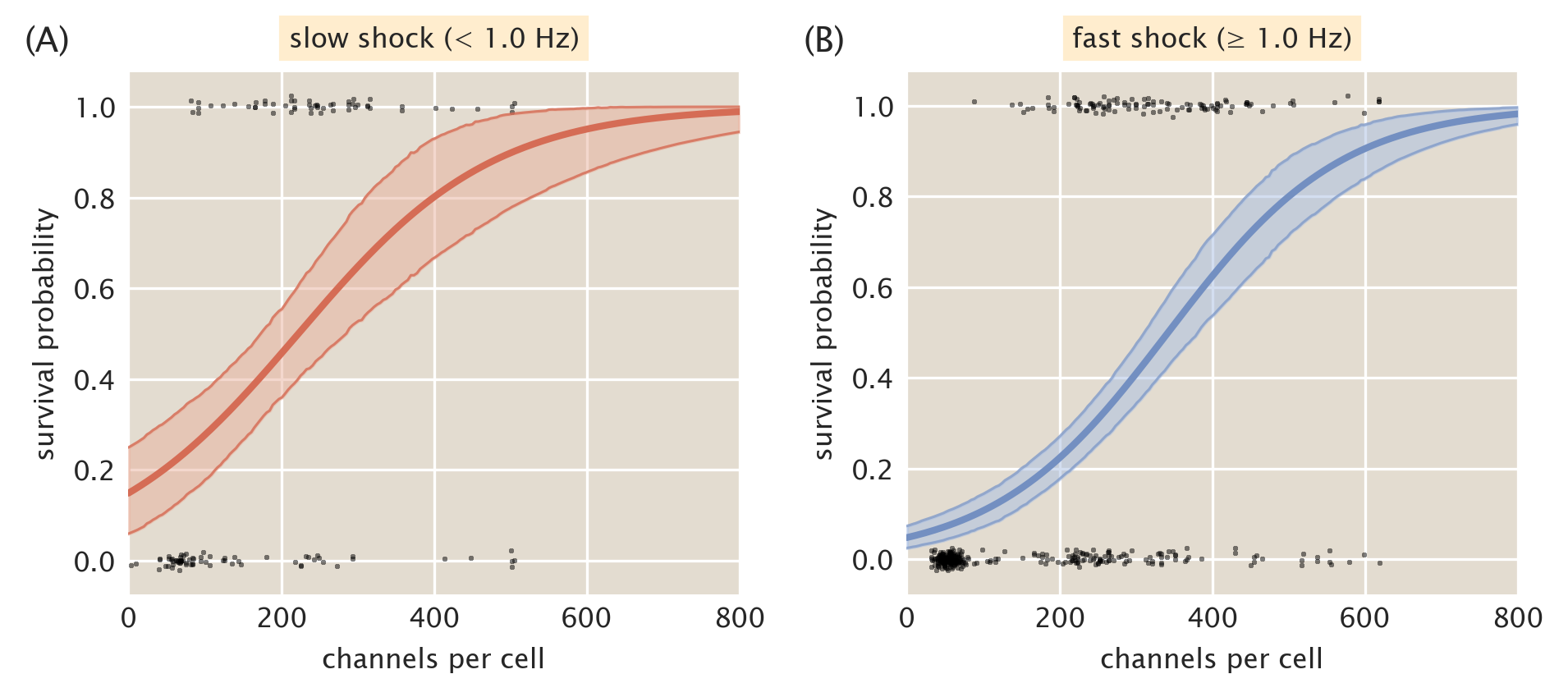
**Figure 1**: **Role of mechanosensitive channels during hypo-osmotic shock.** (A) Water rushes into a cell during hypo-osmotic shock resulting in increased turgor pressure and tension in the cell membrane. If no mechanosensitive channels are present and membrane tension is high (left panel), the membrane ruptures and cell death occurs. If mechanosensitive channels are present (right panel) and membrane tension is beyond the gating tension, the mechanosensitive channel MscL opens, releasing cytoplasm and small intracellular molecules into the environment, relieving pressure and membrane tension. (B) The experimental approach in this work. The number of mechanosensitive channels tagged with a fluorescent reporter is tuned through RBS modification of the mscL gene. The cells are then subjected to a hypo-osmotic shock and the number of surviving cells is computed.



**Figure 2**: **Experimental approach to measuring survival probability.** (A) Layout of a home-made flow cell for subjecting cells to osmotic shock.(A) Cells attached to a glass coverslip within the flow chamber are shocked by addition of a low salt medium into the flow chamber. (B) The typical experimental procedure. Cells are loaded into a flow chamber as shown in (A) and mounted to the glass coverslip surface. Cells are subject to a hypo-osmotic shock by flowing hypotonic medium into the flow cell. After shock, the cells are monitored for several hours and surviving cells are identified.



**Figure 3**: **Control of MscL expression and distribution of survival under osmotic shock.** (A) Variability in expression across designed RBS mutants. The boxes represent the interquartiles of the distribution, the center line displays the median, and the whiskers represent XXX. Individual cells are denoted as black points. The strain used for calibration of channel copy number (MLG910) is highlighted. (B) XXX. (C) The Empirical Cumulative Distribution Function (ECDF) of the data shown in (B). The cells were separated by survival or death and the CDFs were computed individually.



**Figure 4**: **Probability of survival as a function of MscL copy number.** Predicted survival probabilities from a one-dimensional logistic regression for samples exposed to a slow shock (< 1.0 Hz, A) and fast shock ( ≥ 1.0 Hz, B). Shaded regions represent the 95th percent credible regions of the