Genome wide functional screen for calcium transients in *E. coli* identifies decreased membrane potential adaptation to persistent DNA damage

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Running Head: High-throughput calcium screen in *E. coli*

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1. Abstract

Importance:

All eukaryotic cells use calcium as a critical signaling molecule. There is tantalizing evidence that bacteria also use calcium for cellular signaling, but much less is known about the molecular actors and physiological roles. To identify genes regulating cytoplasmic calcium in *Escherichia coli*, we created a single cell screen for modulators of calcium dynamics. The genes uncovered in this screen helped refine a model for voltage mediated bacterial mechanosensation. Additionally, we were able to more carefully dissect the mechanisms of adaptation to long term DNA damage, which has implications for both bacteria and mitochondria in the face of unrepaired DNA.

Abstract:

Calcium plays numerous critical roles in signaling and homeostasis in eukaryotic cells. Unlike eukaryotic cells, far less is known about calcium signaling in bacteria, and few genes controlling influx and efflux have been identified. Previous work in *Escherichia coli* showed calcium influx is induced by voltage depolarization, which were enhanced by mechanical stimulation, suggesting a role in bacterial mechanosensation. To identify proteins and pathways affecting calcium handling in bacteria, we designed a live cell screen to monitor calcium dynamics in single cells across a genome wide knockout panel in *E. coli*. The screen measured cells from the Keio collection of knockouts and quantified calcium transients across the population. Overall, we found 143 gene knockouts that decreased calcium transients, and 32 genes knockouts that increased transients. Knockouts involved in energy production and regulation appeared, as expected, as well as knockouts of the voltage sink, the F1Fo-ATPase. Knockouts in exopolysaccharide and outer membrane synthesis showed reduced transients and refined our model of electrophysiology mediated mechanosensation in *E. coli*. Additionally, knockouts annotated in DNA repair had reduced calcium transients and voltage. However, acute DNA damage did not affect voltage, and suggested that only long term adaptation to DNA damage decreased membrane potential and calcium transients. Our work showed a distinct separation between the acute and long term DNA damage responses in bacteria, which has implications for mitochondrial DNA damage in eukaryotes.

2. Introduction

Calcium is an important and ubiquitous signaling molecule in eukaryotic cells¹, but much less is known about its role in bacteria^{2,3}. High resolution tools to measure cellular calcium,

including fluorescent biosensors, have enabled detailed knowledge of calcium pathways and dynamics at the sub-cellular level in eukaryotic cells^{4,5}, and have recently been applied in bacteria⁶. In bacteria, intracellular calcium concentration changes have been associated with infection⁷, cell division⁸, motility^{9,10}, along with other important cellular processes³. Bacteria are known to tightly regulate the cytoplasmic calcium levels^{11,12}, while changes in the chemical or mechanical environment have been shown to induce calcium transients^{6,13–15}. Given the overwhelming importance of calcium in eukaryotes, as well as the many potential bacterial signals, it is critical to understand exactly how and why bacteria regulate calcium levels, as well as the proteins that bind calcium and exert calcium-responsive cellular functions.

One main challenge in uncovering the spectrum of signals encoded by calcium dynamics in bacteria has been a lack of tools to monitor calcium concentration in bacteria with high resolution. Luminescent tools have been used to monitor populations of cells with low time resolution¹¹, but they lack the sensitivity to investigate individual cells. The advent of ultrasensitive genetically encoded calcium sensors⁴ was recently applied to *E. coli* and revealed these bacteria act as electrically excitable cells where a voltage depolarization leads to a transient calcium influx, which was tied to mechanical stimulation⁶. These new fluorescent sensors can bridge the single cell gap and potentially reveal new facets of calcium signaling in bacterial cells.

Recently, our group used genetically encoded fluorescent sensors to establish a link between mechanosensation, voltage depolarization, and calcium influx⁶. Similar to vertebrate sensory neurons, mechanically stimulated *E. coli* induced a voltage depolarization, which in turn caused an increase in calcium influx into the cytoplasm. Thus, there is a direct link between mechanosensation and cytoplasmic calcium concentration. Furthermore, mechano- and surface-sensing by bacteria is a critical step in processes associated with human health such as host infection¹⁶ and biofilm formation^{17,18}.

Given the importance of calcium in eukaryotic cells along with the implications in human health, we sought to understand more about the proteins involved in regulating bacterial cytoplasmic calcium concentration. In this paper, we created a genome wide screen of mechanically induced calcium transients in a library of knockouts in *E. coli*. The screen could detect knockouts that decreased or increased the calcium transients. Many hits corresponded with proteins known to influence calcium concentration including genes associated with voltage generation through the electron transport chain and ATP generation through the F₁F₀-ATPase. However, we also discovered several pathways that were strongly enriched including outer membrane lipid synthesis and DNA repair. The outer-membrane synthesis knockouts had voltage levels higher than wildtype which suggested a model of mechanosensation in which the chemical structure of the outer-membrane plays a critical role in relaying mechanical signals. Knockouts in the DNA damage repair pathway showed increased DNA damage, but also lower voltage. However, acute DNA damage induced chemically did not change the voltage or calcium transients, which showed that long term adaptation to survive DNA damage included lowering the membrane voltage and growth rates. Though we did not definitely identify a single mechanosensor or voltage gated calcium channel, several hits could potentially provide these elusive functions. Overall, our screening strategy revealed several pathways important in voltage and calcium homeostasis and provided several intriguing hits to follow in future work.

3. Results

High-throughput screen for calcium effectors in *E. coli*

Our model of *E. coli* calcium transients suggested that mechanical stimulation induced voltage depolarization, which led to a transient calcium influx⁶ (Fig 1A). Therefore, a screen for modulators of calcium transients would report conditions that altered mechanosensation, membrane voltage, or calcium handling proteins. To identify genes that modulate mechanically induced calcium transients in *E. coli*, we developed a live cell screen (Fig 1B) using automated 96 well plate imaging of cells expressing a fusion of GCaMP6f (calcium sensor) and mScarlet (insensitive control). The Keio collection of knockouts was transformed with the GCaMP6-mScarlet plasmid and grown on dual selection plates to maintain the gene knockout (kanamycin) and the sensor plasmid (carbenicillin). After transformation, ~97% of knockouts grew on double selection plates which were moved forward for imaging. Cells were spotted onto agarose pads and pressed into glass bottom well plates (Supp fig 1). Thus, within each well were many cells, each of which had a genotype defined by the Keio collection.

From each well, we acquired a 90 second video of the mechanically induced calcium activity to identify genetic knockouts with altered calcium handling. From each field of view (FOV), individual cells were segmented and the mean GCaMP and mScarlet fluorescence was extracted from each frame (see methods, Supp fig 1). From each cell's fluorescence, we defined transients and calculated the total calcium area under the curve (AUC). Combining every cell from a given genetic identity we assembled a population level AUC as the median of the sum from all cells within a given field of view. Comparing the median population AUC from a given genotype, we were able to identify knockouts that reduced or enhanced the number of Ca⁺⁺ transients (Fig 1B).

To test the resolving power of the screen, CCCP and apramycin were used as positive controls, respectively. These chemical treatments had Z' factors of 0.32 for CCCP (low transients⁶), and a Z' factor of 0.28 for apramycin, an aminoglycoside that induces increased transients¹⁹ (Fig 2A, Supp fig 2). Though the Z' score was < 0.5, we considered it sufficient to move forward into a genome wide screen by accepting potential false positives during the primary data collection.

We then proceeded to use our assay to screen the Keio collection, a genome wide knockout library in *E. coli*²⁰. The Keio collection consisted of 45x 96 well plates. We screened all 45 plates as a primary screen, which consisted of raw video data of 7.9 TB. After removing unconfirmed strains, wells too dim to image, and any other artifacts we could determine from the fluorescence images, we extracted time traces from a total of 4.04M individual cells from 3504 genetic knockouts. The top hits from the primary screen were re-imaged in biological triplicate to confirm true positive hits. Across the entire screen, we generated criteria to call knockouts with high and low population wide calcium AUC. Overall, we found 143 knockouts that decreased calcium AUC, and 32 genes that increased calcium AUC (Tables S1,S2). A random sample of traces from Δ recG (low transients) and Δ btuR (high traces) qualitatively matched the expectations from the median AUC screen measurements (Fig 2C) when compared to wildtype (WT).

Expected knockouts in the electron transport chain affect calcium transients

Given our previous work linking membrane voltage with calcium transients, we expected that knockouts known to reduce membrane potential^{21,22} would have a corresponding reduction of observed calcium transients. Indeed, many knockouts of genes in the electron transport chain showed reduced calcium AUC as compared to WT (Table S1). Gene ontology (GO) enrichment analysis^{23,24} of knockouts with reduced calcium transients also showed high enrichment in molecular functions associated with energy production including cytochromes and the TCA

cycle. This data provided confirmation that our screen could identify known pathways affecting voltage regulation, which in turn affected voltage transients^{6,25}.

In addition to genes involved with voltage generation, we observed that gene knockouts of several components of the F1Fo-ATPase had higher calcium AUC as compared to WT. Considering our current model, these data are also in agreement with the F1Fo-ATPase as a sink of membrane voltage. We expected that inhibiting or eliminating that pathway would result in a higher overall membrane potential and increased calcium transients¹⁵. Measurements with TMRM confirmed the higher membrane potential as well (Supp Fig 3). Though it has long been known that the electron transport chain generates a voltage that is used by the F1Fo-ATPase²⁶, these hits gave confidence that our screen could detect genetic knockouts that affected both voltage generation and mechanically induced calcium transients.

Outermembrane integrity and exopolysaccharides are critical for mechanosensation

In addition to components regulating voltage, we also expected to find knockouts that affect bacterial mechanosensation. Several inner-membrane (IM) proteins with domains of unknown function were hits with reduced calcium transients, which may be the channels or sensors involved in relaying the mechanical signal across the plasma membrane (Table S1). However, no single IM protein eliminated all calcium transients, which could be due to functional redundancy or a non-proteinaceous calcium channel²⁷. However, we did see an enrichment in genes associated with outer-membrane and polysaccharide synthesis that had reduced calcium AUC (Fig 3A). We hypothesized that a fully intact outer-membrane and the presence of polysaccharides are critical components of the mechanical relay. Earlier work showed these knockouts had no reduction in growth rate as compared to WT cells²⁸. Furthermore, these knockouts showed no reduction in basal voltage as measured by TMRM when compared to WT cells (Fig 3B), which suggested these knockouts were a part of the mechanosensitive relay.

Based on this data and the previous work showing mechanically induced calcium transients, we proposed a model of mechanosensation in which *E. coli* use the outer-membrane and polysaccharide adhesion to stick to the surface of the glass. Upon mechanical deformation with the agarose pad, the outer-membrane undergoes a shear stress which induces the activation of the mechanical sensor, followed by voltage and calcium transients. To test this model, we measured a knockout of 4 components of exopolysaccharide synthesis²⁹ (Δ 4pol) which showed very low calcium transients in our assay (Fig 3C). A second experiment measured calcium transients of bacteria either sandwiched between the glass which is standard in this assay, or encased in agarose which solidified around the cells and exerts no shear force. Cells encased in agarose showed significantly lower calcium transients relative to the glass sandwiched cells (Fig 3D), but continued to grow. Combined, these data are consistent with mechanosensation arising from a shear force relayed through the outer-membrane to induce voltage and calcium transients (Fig 3E).

Knockouts involved in DNA repair reduce voltage and calcium transients

One unexpected pathway that showed dramatically decreased calcium AUC was genes associated with recombination and DNA repair (Fig 4A). These knockouts had extremely low calcium transients compared to WT cells. This striking data suggested a link between persistent DNA damage and membrane voltage in *E. coli*. DNA damage is known to reduce respiration in bacteria via the SOS response, but respiration is restored upon repair of the damage³⁰. Furthermore, DNA damage is associated with depolarized membrane potential in eukaryotic

mitochondria^{31–33}. Changes to the metabolic state could affect membrane voltage, and previous work established the voltage transients require oxygen²⁵.

To first determine if defects in recombination could lead to increased DNA damage, we assayed damage by measuring terminal deoxynucleotidyl transferase dUTP nick labeling (TUNEL). As expected, the knockouts tested showed significantly more DNA damage as compared to WT cells (Fig 4B). Proteins associated with DNA repair are reported to reduce expression of components of the electron transport chain in and mitochondria, so we hypothesized these cells with unrepaired DNA would have lower membrane potential. TMRM measurements showed that recombination knockouts indeed had lower voltage (Fig 4C) as compared to WT cells. The exception was Δ recG which had a significantly higher TMRM fluorescence than WT cells. We suspected this could be due to cytoplasmic pH differences. For all gene knockouts of DNA repair, there was a corresponding reduction in the growth rate of these cells as compared to WT (Fig 4D). Despite these signatures, the knockout cells observed were not elongated which suggested the SOS response and *sulA* expression had been reduced³⁴. Together, we observed that cells with incomplete repair machinery and persistent DNA damage have reduced voltage and calcium transients, and a reduced growth rate, as compared to wildtype cells.

Persistent, not acute, adaptation to DNA damage lowers membrane voltage

DNA damage in eukaryotic mitochondria is known to be associated with depolarized membrane potential, and the depolarization has been attributed to ROS buildup and damage to the membrane³⁵. In bacteria, it has been suggested the recA filaments interact with the membrane, which could provide a mechanism for reduced voltage^{36,37}. The high temporal and spatial resolution of the fluorescent measurements offered the opportunity to investigate the dynamics between DNA damage and depolarization. Genetic knockouts from the Keio collection have many generations of growth and adaptation before imaging is possible, so we instead turned to genotoxic chemicals to induce damage and monitor the acute voltage response of cells.

To control the onset of DNA damage, we used mitomycin C (MMC), a DNA crosslinker purified from *Streptomyces caespitosus*^{38,39}. Addition of 5 μ M MMC showed bactericidal activity within two hours when measured by CFUs (Fig 5A), confirming high DNA damage within a short time frame. The SOS response was upregulated as measured by filamenting cells 2 hours after treatment (Supp Fig 4). Unexpectedly, calcium transients in cells treated with 5 μ M MMC were identical to untreated cells (Fig 5B), and even after 8 hours of treatment, cells showed no difference in calcium AUC as compared to untreated cells (Fig 5C), suggesting the voltage was unchanged. *E. coli* were then grown over 36 hours in liquid culture in the presence of 5 μ M MMC, and the cells reached stationary phase which indicated some fraction of the population were adapted to the new environment. These cells, which were continuously treated with the drug MMC, showed severely reduced transients, similar to knockouts of the DNA repair machinery (Fig 5D). Together, our data support a model whereby cells undergo a long-term adaptation to persistent DNA damage by lowering their membrane potential followed by a reduction in the SOS pathway. The adaptation could be through mutation, toxin/antitoxin systems, or others, but they occur at long timescales compared to ROS generation (seconds to minutes).

4. Discussion

In this paper, we created a single cell, genome wide screen for modulators of cytoplasmic calcium in *E. coli*. Using this screen, we identified both known and novel pathways that modulate the level of cytoplasmic calcium. The imaging assay had a throughput of 384 genotypes per day, each consisting of dozens to hundreds of single cells, with 450 time points for each measurement. The high signal to noise ratio from the fluorescent signal combined with the automated analytical scripts were able to distinguish positive and negative chemical controls with high confidence. This setup could be easily adapted to other conditions including a chemical screen on calcium AUC, additional bacterial species, or other fluorescent sensors. We believe that single cell, physiological dynamics have the potential to uncover new information and biological processes not available from static imaging.

One of the most interesting and significant hits in our screen was the identification of genes associated with DNA repair, which had reduced voltage and calcium transients as compared to the WT cells. Our data showed the DNA damage clearly results in an impaired voltage, but that the decrease in voltage occurs only over the course of adaption over ~48 hours. Acute treatment had no impact on the calcium transients, even at concentrations high enough to prevent cells from future cell division. This data suggests a model whereby DNA damage does not immediately lower voltage through ROS production³⁰ or interaction with the plasma membrane^{36,37}, but rather occurs only as a mutation or transcriptional adaptation that allows a fraction of the cells to continue to divide in spite of persistent DNA damage. Furthermore, the cells turn off the SOS response despite this persistent DNA damage. Understanding the exact mechanisms by which cells accommodate DNA damage via voltage suppression is an important path forward that we plan to study.

DNA damage using MMC also revealed an interesting relationship between cell division as measured by CFUs, and cell metabolism as measured by cytoplasmic calcium maintenance. Addition of 5 µg/mL MMC was enough to prevent > 99% of cells from forming colonies within two hours. Yet, the cells were metabolically active for 8 hours after treatment. This intermediate time period, between treatment and cell death, is similar to previous results with aminoglycosides¹⁹, and suggests this could be an important consideration during antibiotic treatment if those dying cells can still signal to neighbors, and influence the population level activity.

The mechanisms that tie DNA damage to decreased membrane potential could have impact in the mitochondria of eukaryotic cells as well. Depolarized mitochondria are marked for mitophagy via the PINK-1 pathway, and one trigger is via mitochondrial DNA damage^{40,41}. An existing model suggests that DNA damage directly reduces membrane potential through increased ROS^{40,42}, but would be the opposite of what we observed in bacteria. One interesting avenue will be to perform similar experiments on eukaryotic cells monitoring the mitochondrial potential upon mitochondrial DNA damage.

5. Methods and materials

Strains and growth

E. coli BW25113 was acquired from the Yale Coli Genetic Stock Center and was used as the wildtype strain for all experiments except for MG1655 which was used for comparisons to the Δ4pol strain. The Keio collection was purchased from Dharmacon (OEC4988). Keio strains were grown in 10 µg/mL kanamycin to ensure maintenance of the genetic insertion. Cells were grown overnight in LB at 37 °C while shaking at 190 RPM. All strains that carried the GCmS

calcium indicator plasmid were grown in 100 µg/mL carbenicillin in addition to other antibiotics necessary to maintain those strains. Δ₄pol strain was maintained in media with 10 µg/mL chloramphenicol, 30 µg/mL kanamycin, and 25 µg/mL zeocin.

Plasmids and transformation

The constitutive GCaMP-mScarlet plasmid was generated in a previous publication¹⁹ and is available on Addgene (#158979). The plasmid was transfected into *E. coli* knockouts by using the Transformation and Storage Solution (TSS) buffer in a 96 well plate. Briefly, cells were grown overnight in a deep well 96 plate in LB and kan. Cells were then spun down at 1900x g for 7 min, and the supernatant was removed by pouring. TSS buffer was added, and incubated with 1 µL per well from a miniprep of the plasmid. Upon incubation for 1 hour, 5 µL of the cells were placed onto a double selection plate (carb – 100 µg/mL, kan – 50 µg/mL) using a multichannel pipet, and the plates were incubated overnight at 37 °C.

Colonies of the transformed cells were then picked using a pin transfer tool, and grown up overnight in LB (carb – 100 µg/mL, kan – 50 µg/mL), and glycerol stocks were made from this suspension. To grow cells for imaging, the glycerol stock in 96 well plates was placed into a 96 well plate with LB (carb – 100 µg/mL, kan – 10 µg/mL) using a pin transfer and cells were grown overnight at 37 °C while shaking.

TMRM assays

A 1 mL Falcon polystyrene round-bottom tube with 940 µL of M9 minimal media with 0.4% glucose and 1X NEB amino acids was seeded with 50 µL of overnight growth suspension of the strain to be tested (in biological triplicate), and grown at 28°C with 200 rpm shaking. When the cells reached ~0.4 OD, 10 µL of TMRM was added to the suspension at a final concentration of 0.2 mM TMRM. Thirty minutes later, cells were quantified for their TMRM incorporation by counting 100,000 events per condition using a BDFACSCellesta Flow Cytometer with the following Voltage settings: FSC at 700, SSC at 350, with 561 nm laser D585/15 at 500, C610/20 at 500 and B670/30 at 481. Emission for each event was collected at the 585/15 nm wavelengths.

Tunel Assay

DNA damage was measured using the TUNEL APO-DIRECT kit (BD 556381) using the manufacturer's suggested protocol. Cells were fixed with ethanol followed by washing and staining.

Keio imaging

Imaging took place on an inverted microscope setup using 96 well glass bottom plates (Brooks, MGB096-1-2-LG-L), similar to earlier work¹⁹. Cells grown in overnight cultures in 96 wells at 37 °C in LB with carbenicillin to maintain the plasmid. Agarose pads in a 96 well format were created using a custom 3D printed mold (<https://www.shapeways.com/shops/kraljilab>). The mold was designed so that each well held a 200 µL agarose pad. Agarose was dissolved at a final concentration of 2% in partial minimal medium (PMM, 1x M9 salts, 0.2 % glucose, 200 µM MgSO₄, 10 µM CaCl₂, 1x MEM amino acids) and added into the mold which was covered by a piece of glass (McMaster Carr, 8476K43). A second piece of glass was added to the to create flat surfaces on both sides. After the agarose gel solidified (typically > 30 minutes), 2 µL of the cell suspension was added onto

each pad and the solution was allowed to fully diffuse into the gel. The pads were then pressed into the glass bottom plate using a second 3D printed piece. There is an inversion of the plate (A1 from cell suspension -> A12 in the glass bottom plate) that was accounted for in the analysis software.

Imaging took place on an automated inverted microscope (Nikon Ti2) using a 40x NA 0.95 objective to image onto two sCMOS cameras (Hamamatsu, Flash 4 v2) using a custom dichroic splitter in the emission path. Emission filters were a 525/50 (GCaMP) and 568 LP (mScarlet) and we used a 561 LP dichroic to separate the two colors. Cells were illuminated using an LED source (Lumencor, Spectra X), using simultaneous excitation with 470 nm and 550 nm light. Images were captured continuously with an exposure time of 200 ms (5 Hz) for a total of 90 seconds.

To enable automated imaging of all 96 wells without additional user input, a single point was selected in each well by the user such that there were cells, but not too dense. Selecting all 96 points took on average ~20 minutes. Enabling the Perfect Focus on the Ti2 kept the plate in focus across the entire well plate. Capture of the entire movie took ~2.5 hours per plate, so we were typically able to image 2-3 plates per day. Image data was stored with the metadata in the .nd2 format for downstream processing.

Image processing

Image processing from the movie files was similar to our previous reports¹⁹, with a few changes due to the nature of the movie collection. Image analysis occurred in Matlab using custom .m scripts. Image processing followed the general scheme of (1) estimating the illumination profile for all experiments on a given day, (2) correcting the uneven illumination for each movie, (3) registering drift and jitter in XY, (4) subtracting an estimated background, (5) segmenting cells using a Hessian algorithm, (6) extracting time traces for individual cells, (7) processing each time trace to calculate calcium area under the curve (AUC).

1. Estimating the illumination profile: For a given day, every movie was averaged across time, and opened using a morphological operator and blurred using a 2D Gaussian filter. Each of these experimental images were then averaged together to give an estimate of the uneven illumination. These images were smooth across the entire field of view, and varied by ~50% across the entire image.
2. Correcting uneven illumination. Each individual movie was then loaded into memory sequentially. Each frame of the movie was converted to a double, and then divided by the uneven illumination. This image was then multiplied by the average value of the movie and converted back into a uint16 to maintain consistent intensity values. Each frame was then reassembled into an illumination corrected movie.
3. Registering drift and jitter in XY: Each frame was aligned to the previous frame using a convolution of the 2D Fourier transform. Each sequential image was first estimated by applying the XY warping from the previous frame. Then, the 2DFT was taken for each image, and multiplied to the previous frame. The optimal updated XY position was then calculated and applied. Due to the short time of these movies (90 sec), little drift was observed for most measurements.
4. Subtracting the estimated background: The background was estimated for each frame individually using a morphological operator. A disk structured element with radius 9 μm was blurred with a Gaussian filter. This background estimation was then subtracted

from the original image. To protect against potential negative values, the minimum of the entire movie was set to 50 counts.

5. Segmenting cells using a Hessian algorithm: To segment cells, first the foreground was estimated using Otsu's method from the background subtracted image. The Hessian was then calculated on the background subtracted image, and then elementwise multiplied to a logical image of the foreground. Otsu's method was again used on this modified Hessian image to identify individual cells. Hard limits were set to remove potential noise that did not fit given criteria for size or minimum intensity. We found that first increasing the size of the image using a spline interpolation gave superior segmentation results. Using this method, not all cells were identified within a microcolony, though we estimate that it can identify ~96% of the cells accurately.
6. Extracting time traces for individual cells: From a given identified cell, for each time point in the movie, we extracted the mean intensity using the Matlab command, `regionprops`. The mean intensity for both the GCaMP6f and the mScarlet were extracted using this method, or any other fluorophore the cells expressed.
7. Processing each time trace to calculate the calcium AUC. To identify calcium transients in a given cell, we first removed any low frequency photobleaching using a moving median filter, followed by a wavelet denoising (`wdencmp.m`). To identify the transient starts, any point of the differentiated that rose above zero (rising slope) and enforced that a peak have an area under the curve $> 3\sigma$. For any given cell, all the transients that met these criteria were summed to give a cellular AUC over the duration of the movie. To calculate the genotype AUC, the median AUC of the population was calculated to minimize any outliers from non-stationary cells.

CFUs

CFUs were measured by plating treated cells onto LB-agarose without antibiotic and counting growing colonies. CFU measurements were conducted trying to mimic the experiments performed via microscopy. Briefly, cells were grown overnight in LB and diluted 1:20 in 5 mL PMM. These cultures were grown at room temperature and shaking for 2 hours ($t = 0$) followed by the addition of antibiotic. At each time point, the culture was removed from the shaker, and 100 μ L was removed. A 10x series dilution was then conducted by removing 20 μ L and adding to 180 μ L LB alone in a 96 well plate. The 10-fold dilution was performed 7 times, leading to the original concentration to a dilution of 10^7 . From each of the 10x dilution series, 3 μ L was plated onto an LB agar pad and left to dry (1 colony = 333 cells/mL, lower end of our dynamic range). After an entire experiment (typically 5 hours), the agar was placed into an incubator and grown overnight. Colonies were then manually counted the next morning.

Growth curves

Growth curves were collected in a 96 well plate using a Tecan Spark plate reader. The plate reader was set to 37 °C and data was collected from each well every 5 minutes over 10 hours.

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Figures and figure legends:

Figure 1:

Design for a genome wide screen for calcium effectors in bacteria. (A) Current model underlying the observed calcium transients in *E. coli*. Mechanical stimulation induces voltage depolarizations, which induce calcium influx into the cell. Voltage is generated by respiration via the electron transport chain, and is consumed by the F1Fo-ATPase. (B) Outline of the live cell screen. The Keio collection is transformed with a constitutive GCaMP-mScarlet expressing plasmid and imaged on an automated microscope. Custom analyses segment out individual cells and calculate the calcium AUC. All the cells from a given genotype are then combined, giving rise to the AUC score, which is then screened for knockouts that increase or decrease calcium transients.

Figure 2:

The live cell screen can identify knockouts with altered calcium flux. (A) Box plot of the calcium AUC from cells treated with DMSO (negative control), CCCP (eliminates transients) or apramycin (increases transients). The box plots each have 16 biological replicates. * $p < 0.001$. (B) Calcium AUC from the primary screen. Each gene in the Keio is plotted on the x-axis, and the log of the calcium AUC is plotted on the y axis. The genes from 4300-5400 are not present in the Keio collection. Right: a histogram of the calcium AUC from the entire screen. (C) Random sample of individual cells from wildtype cells (left), ΔrecG (middle, reduced calcium AUC), or ΔbtuR (right, increased calcium AUC). For all box plots: red line = median, blue box = 25/75 limits, black line = 10/90 limits, red x are outliers.

Figure 3:

Knockouts of outer-membrane synthesis and EPS secretion refine model for mechanosensation. (A) Box plot of knockouts involved in OM synthesis and EPS secretion had reduced calcium AUC as compared to WT cells. Each boxplot is comprised of 5 biological replicates. (B) Knockouts of the OM synthesis and EPS secretion did not have a reduced membrane potential compared to WT cells. Each genotype was measured in biological triplicate. (C) Box plot of calcium AUC of wildtype cells adhered with poly-l-lysine or an agarose pad compared to a Δ4pol knockout under the same conditions. Each box plot is comprised of 4 biological replicates. *represents $p < 0.01$ (D) Box plot of calcium AUC for *E. coli* adhered under agarose similar to other measurements, or encased in agarose by adding them before solidification of the gel. Each boxplot is comprised of 5 biological replicates. *represents $p < 0.01$ (E) Proposed model for *E. coli* mechanosensation. A force is applied by the agarose pad, which is opposed by an equal and opposite force from the coverslip. The coverslip force arises from adherence with secreted EPS which then interacts with LPS on the outer membrane of the cell. This force is relayed to a mechanosensor in the inner membrane which remains to be identified. For all box plots: red line = median, blue box = 25/75 limits, black line = 10/90 limits, red x are outliers.

Figure 4:

Knockouts of genes associated with DNA repair have reduced calcium AUC. (A) Box plot of knockouts involved in DNA repair compared to WT cells. Each boxplot is comprised of 5 biological replicates. (B) TUNEL fluorescence measured by a flow cytometer for WT, ΔrecA , and ΔrecG . *represents $p < 0.05$. (C) TMRM fluorescence for comparing WT to rec knockouts. Each marker indicates one biological replicate. Addition of 50 μM CCCP is used to show fluorescence from zero voltage. (D) Growth curves measured by OD600 from WT compared to rec knockout cells. The dark line shows the mean, and the shaded line shows the standard deviation of 3 biological replicates. For all box plots: red line = median, blue box = 25/75 limits, black line = 10/90 limits, red x are outliers.

Figure 5:

Persistent DNA damage induces an adaption via voltage reduction. (A) CFU assay of cells treated with 5 μM MMC compared to untreated cells. Gentamicin treated cells are a positive control for a bactericidal compound. (B) Time course of calcium AUC taken from 90 sec movies comparing untreated vs 5 μM MMC treated cells. Each point was taken from a unique well, alternating between treated and untreated conditions. (C) Time course of calcium AUC from a titration of MMC concentrations over 8 hours. AUC was calculated from 90 second movies and is normalized to untreated cells. (D) Calcium AUC comparing persistent DNA damage (ΔrecA , 36 hour of 5 μM MMC) to acute DNA damage. * indicates $p < 0.01$. For all box plots: red line = median, blue box = 25/75 limits, black line = 10/90 limits, red x are outliers.

Supplementary figures and tables:

Supplementary Figure 1:

Loading and imaging cells in a 96 well plate. (A) Pictures of the 3D printed agarose mold to immobilize *E. coli* in glass bottom plates. The green mold is filled with agarose between 2 pieces of glass until solidification. Cells are added to the bottom of the pad in 2 μ L drops. (B) The red press is used to push each agarose pad into (C) the bottom of the glass bottom dish. (D) Top: 160 x 160 μ m² FOV of cells expressing GCaMP mScarlet immobilized under an agarose pad. Bottom: Overlay of the image with the Hessian calculated cell mask shown in purple. The mask calculated 1460 individual cells in this particular FOV. Right: Zoom in on one section of cells and mask.

Supplementary Figure 2:

Control plates to test high and low calcium AUC relative to WT cells. (A) A plate map showing cells treated with DMSO (negative control), 50 μ M CCCP (positive control, low AUC), or 100 μ g/mL apramycin (positive control, high blinks). (B) Measured Z-score from the test plate with the Z-score for each well color coded.

Supplementary Figure 3:

Knockouts of the F1Fo-ATPase had altered membrane voltage that correlated with calcium transients. Each mark is the median of 100k events measured on the cytometer, and each genotype was measured in biological triplicate. Δ atpA had reduced calcium transients and voltage, while Δ atpD and Δ atpF had increased voltage and calcium transients compared to WT.

Supplementary figure 4:

Cell morphology before and after treatment with 5 μ M MMC. (A) GCaMP fluorescence (green) and the segmented mask (purple) before addition of drug and (B) 2 hours after drug. (C) Minor axis (orange) and major axis (blue) distributions at $t = 0$ hours before drug addition calculated by the masks shown in (A). (D) Minor axis (orange) and major axis (blue) distributions at $t = 2$ hours before drug addition calculated by the masks shown in (B).

Table S1: List of genes knockouts that reduced calcium AUC.

Gene ID	Mappe d IDs	Gene function	Panther Family/Subfamily
ECOLI EcoGene=EG12913 U niProtKB=P45544	frlR	Probable fructoselysine utilization operon transcriptional repressor;frlR;ortholog	MANNOSYL-D- GLYCERATE TRANSPORT/METAB OLISM SYSTEM REPRESSOR MNGR- RELATED (PTHR44846:SF1)
ECOLI EcoGene=EG10459 U niProtKB=P08956	hsdR	Type I restriction enzyme EcoKI R protein;hsdR;ortholog	TYPE I RESTRICTION ENZYME ECOKI R PROTEIN (PTHR47396:SF1)
ECOLI EcoGene=EG13274 U niProtKB=P77136	yqeK	Uncharacterized protein YqeK;yqeK;ortholog	
ECOLI EcoGene=EG10218 U niProtKB=P09549	dedD	Cell division protein DedD;dedD;ortholog	CELL DIVISION PROTEIN DEDD (PTHR38687:SF1)
ECOLI EcoGene=EG13487 U niProtKB=P76230	ydjK	Putative metabolite transport protein YdjK;ydjK;ortholog	SYNAPTIC VESICLE 2-RELATED PROTEIN (PTHR24064:SF445)
ECOLI EcoGene=EG10836 U niProtKB=P0C079	relB	Antitoxin RelB;relB;ortholog	ANTITOXIN DINJ- RELATED (PTHR38781:SF1)
ECOLI EcoGene=EG14353 U niProtKB=P18393	ybdZ	Enterobactin biosynthesis protein YbdZ;ybdZ;ortholog	ENTEROBACTIN BIOSYNTHESIS PROTEIN YBDZ (PTHR38444:SF1)
ECOLI EcoGene=EG12908 U niProtKB=P45539	frlA	Probable fructoselysine/psicosely sine transporter FrlA;frlA;ortholog	FRUCTOSELYSINE/P SICOSELYSINE TRANSPORTER FRLA-RELATED (PTHR11785:SF512)
ECOLI EcoGene=EG10579 U niProtKB=P17109	menD	2-succinyl-5- enolpyruvyl-6-hydroxy- 3-cyclohexene-1- carboxylate synthase;menD;ortholog	PROTEIN PHYLLO, CHLOROPLASTIC (PTHR42916:SF1)
ECOLI EcoGene=EG11225 U niProtKB=P0ABN5	dcuA	Anaerobic C4- dicarboxylate transporter DcuA;dcuA;ortholog	ANAEROBIC C4- DICARBOXYLATE TRANSPORTER DCUA (PTHR36106:SF2)

ECOLI EcoGene=EG12590 UniProtKB=P39400	yjjN	L-galactonate-5-dehydrogenase;lgoD;ortholog	L-GALACTONATE-5-DEHYDROGENASE (PTHR43401:SF3)
ECOLI EcoGene=EG11821 UniProtKB=P69681	amtB	Ammonia channel;amtB;ortholog	AMMONIUM TRANSPORTER MEP2 (PTHR43029:SF10)
ECOLI EcoGene=EG12992 UniProtKB=Q46836	pppA	Prepilin peptidase PppA;pppA;ortholog	PREPILIN PEPTIDASE PPPA-RELATED (PTHR30487:SF0)
ECOLI EcoGene=EG10830 UniProtKB=P21893	recJ	Single-stranded-DNA-specific exonuclease RecJ;recJ;ortholog	SINGLE-STRANDED-DNA-SPECIFIC EXONUCLEASE RECJ (PTHR30255:SF2)
ECOLI EcoGene=EG13871 UniProtKB=P75915	ycdY	Chaperone protein YcdY;ycdY;ortholog	CHAPERONE PROTEIN YCDY (PTHR34227:SF1)
ECOLI EcoGene=EG11238 UniProtKB=P12994	ybhB	UPF0098 protein YbhB;ybhB;ortholog	UPF0098 PROTEIN YBHB (PTHR30289:SF12)
ECOLI EcoGene=EG12261 UniProtKB=P37655	yhjQ	Putative cellulose biosynthesis protein BcsQ;bcsQ;ortholog	CELLULOSE BIOSYNTHESIS PROTEIN BCSQ-RELATED (PTHR43384:SF12)
ECOLI EcoGene=EG10098 UniProtKB=P0ABB0	atpA	ATP synthase subunit alpha;atpA;ortholog	ATP SYNTHASE SUBUNIT ALPHA, MITOCHONDRIAL (PTHR48082:SF2)
ECOLI EcoGene=EG11729 UniProtKB=P31471	yieL	Uncharacterized protein YieL;yieL;ortholog	ENTEROCHELIN ESTERASE (PTHR48098:SF3)
ECOLI EcoGene=EG13560 UniProtKB=P0AAA1	yagU	Inner membrane protein YagU;yagU;ortholog	
ECOLI EcoGene=EG11899 UniProtKB=P33228	recT	Protein RecT;recT;ortholog	
ECOLI EcoGene=EG10827 UniProtKB=P15032	recE	Exodeoxyribonuclease 8;recE;ortholog	
ECOLI EcoGene=EG11223 UniProtKB=P11447	argH	Argininosuccinate lyase;argH;ortholog	ARGININOSUCCINATE LYASE (PTHR43814:SF1)
ECOLI EcoGene=EG12825 UniProtKB=P67662	aaeR	HTH-type transcriptional activator AaeR;aaeR;ortholog	HTH-TYPE TRANSCRIPTIONAL ACTIVATOR AAER (PTHR30126:SF42)

ECOLI EcoGene=EG12318 UniProtKB=P36649	cueO	Blue copper oxidase CueO;cueO;ortholog	MULTICOPPER OXIDASE LPR2 (PTHR11709:SF2)
ECOLI EcoGene=EG13687 UniProtKB=P75820	ybjR	N-acetylmuramoyl-L-alanine amidase AmiD;amiD;ortholog	N- ACETYLMURAMOYL -L-ALANINE AMIDASE AMID (PTHR30417:SF10)
ECOLI EcoGene=EG20050 UniProtKB=Q6BF17	dgoD	D-galactonate dehydratase;dgoD;ortholog	D-GALACTONATE DEHYDRATASE (PTHR48080:SF2)
ECOLI EcoGene=EG13472 UniProtKB=P75796	yliA	Glutathione import ATP-binding protein GsiA;gsiA;ortholog	GLUTATHIONE IMPORT ATP- BINDING PROTEIN GSIA (PTHR43776:SF7)
ECOLI EcoGene=EG10408 UniProtKB=P0A825	glyA	Serine hydroxymethyltransferase;glyA;ortholog	SERINE HYDROXYMETHYLTRANSFERASE (PTHR11680:SF50)
ECOLI EcoGene=EG12287 UniProtKB=P37680	sgbE	L-ribulose-5-phosphate 4-epimerase SgbE;sgbE;ortholog	L-RIBULOSE-5- PHOSPHATE 4- EPIMERASE SGBE (PTHR22789:SF8)
ECOLI EcoGene=EG11997 UniProtKB=P33347	yehK	Uncharacterized protein YehK;yehK;ortholog	
ECOLI EcoGene=EG13420 UniProtKB=P76187	ydhF	Oxidoreductase YdhF;ydhF;ortholog	OXIDOREDUCTASE YDHF (PTHR43364:SF1)
ECOLI EcoGene=EG13236 UniProtKB=P54746	mngB	Mannosylglycerate hydrolase;mngB;ortholog	MANNOSYLGlycerate HYDROLASE (PTHR46017:SF2)
ECOLI EcoGene=EG12920 UniProtKB=P45551	yhfY	Uncharacterized protein YhfY;yhfY;ortholog	
ECOLI EcoGene=EG12341 UniProtKB=P0AFX7	yfiJ	Anti-sigma-E factor RseA;rseA;ortholog	ANTI-SIGMA-E FACTOR RSEA (PTHR38104:SF1)
ECOLI EcoGene=EG12912 UniProtKB=P45543	frlD	Fructoselysine 6- kinase;frlD;ortholog	FRUCTOSELYSINE 6- KINASE (PTHR43085:SF41)
ECOLI EcoGene=EG13446 UniProtKB=P0AEI6	ymfB	Phosphatase NudJ;nudJ;ortholog	NUDIX HYDROLASE 23, CHLOROPLASTIC (PTHR43222:SF2)
ECOLI EcoGene=EG12892 UniProtKB=P45760	gspI	Putative type II secretion system protein I;gspI;ortholog	TYPE II SECRETION SYSTEM PROTEIN I-

			RELATED (PTHR38779:SF2)
ECOLI EcoGene=EG14159 UniProtKB=P0ACR7	yfeR	Uncharacterized HTH-type transcriptional regulator YfeR;yfeR;ortholog	LYSR FAMILY TRANSCRIPTIONAL REGULATOR- RELATED (PTHR30419:SF14)
ECOLI EcoGene=EG13692 UniProtKB=P75825	hcp	Hydroxylamine reductase;hcp;ortholog	HYDROXYLAMINE REDUCTASE (PTHR30109:SF0)
ECOLI EcoGene=EG12197 UniProtKB=P0AFY8	seqA	Negative modulator of initiation of replication;seqA;ortholog	
ECOLI EcoGene=EG11400 UniProtKB=P0AFV2	yhiD	Putative magnesium transporter YhiD;yhiD;ortholog	MAGNESIUM TRANSPORTER YHID-RELATED (PTHR33778:SF1)
ECOLI EcoGene=EG10599 UniProtKB=P0A6F5	groL	60 kDa chaperonin;groL;ortholog	60 KDA HEAT SHOCK PROTEIN, MITOCHONDRIAL (PTHR45633:SF3)
ECOLI EcoGene=EG12909 UniProtKB=P0AC00	frlB	Fructoselysine 6-phosphate deglycase;frlB;ortholog	FRUCTOSELYSINE 6- PHOSPHATE DEGLYCASE (PTHR10937:SF15)
ECOLI EcoGene=EG11051 UniProtKB=P0AGA6	uhpA	Transcriptional regulatory protein UhpA;uhpA;ortholog	TRANSCRIPTIONAL REGULATORY PROTEIN UHPA (PTHR43214:SF22)
ECOLI EcoGene=EG11014 UniProtKB=P14294	topB	DNA topoisomerase 3;topB;ortholog	DNA TOPOISOMERASE 3- ALPHA (PTHR11390:SF21)
ECOLI EcoGene=EG11332 UniProtKB=P0A8F0	upp	Uracil phosphoribosyltransferase;upp;ortholog	URACIL PHOSPHORIBOSYL TRANSFERASE 2 (PTHR10285:SF135)
ECOLI EcoGene=EG12475 UniProtKB=P39282	yjeM	Inner membrane transporter YjeM;yjeM;ortholog	INNER MEMBRANE TRANSPORTER YGJI- RELATED (PTHR43243:SF70)
ECOLI EcoGene=EG11690 UniProtKB=P0ADL3	yicN	Uncharacterized protein YicN;yicN;ortholog	

ECOLI EcoGene=EG10834 UniProtKB=P0A7H6	recR	Recombination protein RecR;recR;ortholog	RECOMBINATION PROTEIN RECR (PTHR30446:SF0)
ECOLI EcoGene=EG12544 UniProtKB=P39352	yjhB	Putative metabolite transport protein YjhB;yjhB;ortholog	METABOLITE TRANSPORT PROTEIN YJHB- RELATED (PTHR23508:SF2)
ECOLI EcoGene=EG13862 UniProtKB=P69432	pgaD	Biofilm PGA synthesis protein PgaD;pgaD;ortholog	
ECOLI EcoGene=EG13693 UniProtKB=P75829	ybjX	Uncharacterized protein YbjX;ybjX;ortholog	HOMOLOG OF VIRK (PTHR38785:SF1)
ECOLI EcoGene=EG11919 UniProtKB=P0AF43	yjbB	Uncharacterized protein YjbB;yjbB;ortholog	SUBFAMILY NOT NAMED (PTHR10010:SF39)
ECOLI EcoGene=EG10831 UniProtKB=P05824	recN	DNA repair protein RecN;recN;ortholog	DNA REPAIR PROTEIN REC N (PTHR11059:SF0)
ECOLI EcoGene=EG13556 UniProtKB=P77183	yagQ	Molybdenum cofactor insertion chaperone PaoD;paoD;ortholog	MOLYBDENUM COFACTOR INSERTION CHAPERONE PAOD (PTHR30388:SF4)
ECOLI EcoGene=EG12083 UniProtKB=P0AFC7	nuoB	NADH-quinone oxidoreductase subunit B;nuoB;ortholog	NADH DEHYDROGENASE [UBIQUINONE] IRON- SULFUR PROTEIN 7, MITOCHONDRIAL (PTHR11995:SF14)
ECOLI EcoGene=EG12333 UniProtKB=P0AFP0	yadS	UPF0126 inner membrane protein YadS;yadS;ortholog	UPF0126 INNER MEMBRANE PROTEIN YADS (PTHR30506:SF3)
ECOLI EcoGene=EG11253 UniProtKB=P22525	ycbB	Probable L,D- transpeptidase YcbB;ycbB;ortholog	L,D- TRANSEPTIDASE YCBB-RELATED (PTHR30582:SF28)
ECOLI EcoGene=EG10833 UniProtKB=P15043	recQ	ATP-dependent DNA helicase RecQ;recQ;ortholog	BLOOM SYNDROME PROTEIN (PTHR13710:SF105)
ECOLI EcoGene=EG11414 UniProtKB=P28632	holD	DNA polymerase III subunit psi;holD;ortholog	
ECOLI EcoGene=EG10776 UniProtKB=P0AFM6	pspA	Phage shock protein A;pspA;ortholog	MEMBRANE- ASSOCIATED

			PROTEIN VIPP1, CHLOROPLASTIC (PTHR31088:SF6)
ECOLI EcoGene=EG10829 UniProtKB=P24230	recG	ATP-dependent DNA helicase RecG;recG;ortholog	ATP-DEPENDENT DNA HELICASE HOMOLOG RECG, CHLOROPLASTIC (PTHR47964:SF1)
ECOLI EcoGene=EG13188 UniProtKB=P76168	intQ	Putative defective protein IntQ;intQ;ortholog	PROPHAGE INTEGRASE INTR-RELATED (PTHR30349:SF36)
ECOLI EcoGene=EG14308 UniProtKB=P76356	yoeA	Protein YoeA;yoeA;ortholog	COLICIN I RECEPTOR-RELATED (PTHR30069:SF10)
ECOLI EcoGene=EG10394 UniProtKB=P13035	glpD	Aerobic glycerol-3-phosphate dehydrogenase;glpD;ortholog	GLYCEROL-3-PHOSPHATE DEHYDROGENASE, MITOCHONDRIAL (PTHR11985:SF15)
ECOLI EcoGene=EG11206 UniProtKB=P09162	yjaA	Uncharacterized protein YjaA;yjaA;ortholog	
ECOLI EcoGene=EG11351 UniProtKB=P27127	rfaB	Lipopolysaccharide 1,6-galactosyltransferase;rfaB;ortholog	LIPOPOLYSACCHARIDE 1,6-GALACTOSYLTRANSFERASE (PTHR12526:SF593)
ECOLI EcoGene=EG10259 UniProtKB=P15047	entA	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase;entA;ortholog	2,3-DIHYDRO-2,3-DIHYDROXYBENZOATE DEHYDROGENASE (PTHR43231:SF1)
ECOLI EcoGene=EG11842 UniProtKB=P32137	yihP	Putative 2,3-dihydroxypropane-1-sulfonate exporter;yihP;ortholog	2,3-DIHYDROXYPROPANE-1-SULFONATE EXPORTER-RELATED (PTHR11328:SF39)
ECOLI EcoGene=EG10209 UniProtKB=P0A6K1	dapF	Diaminopimelate epimerase;dapF;ortholog	DIAMINOPIMELATE EPIMERASE, CHLOROPLASTIC (PTHR31689:SF0)
ECOLI EcoGene=EG10932 UniProtKB=P07014	sdhB	Succinate dehydrogenase iron-	SUCCINATE DEHYDROGENASE [UBIQUINONE] IRON-

		sulfur subunit;sdhB;ortholog	SULFUR SUBUNIT, MITOCHONDRIAL (PTHR11921:SF29)
ECOLI EcoGene=EG14287 UniProtKB=P0AAZ7	ycaR	UPF0434 protein YcaR;ycaR;ortholog	ZGC:162634 (PTHR33505:SF4)
ECOLI EcoGene=EG13176 UniProtKB=P0A8D3	yaiI	UPF0178 protein YaiI;yaiI;ortholog	UPF0178 PROTEIN YAI (PTHR35146:SF1)
ECOLI EcoGene=EG10982 UniProtKB=P0AGE9	sucD	Succinate--CoA ligase [ADP-forming] subunit alpha;sucD;ortholog	SUCCINATE--COA LIGASE [ADP/GDP-FORMING] SUBUNIT ALPHA, MITOCHONDRIAL (PTHR11117:SF2)
ECOLI EcoGene=EG12098 UniProtKB=P33643	rldD	Ribosomal large subunit pseudouridine synthase D;rldD;ortholog	RIBOSOMAL LARGE SUBUNIT PSEUDOURIDINE SYNTHASE D (PTHR21600:SF57)
ECOLI EcoGene=EG11017 UniProtKB=P13482	treA	Periplasmic trehalase;treA;ortholog	TREHALASE (PTHR23403:SF1)
ECOLI EcoGene=EG14048 UniProtKB=P76343	msrQ	Protein-methionine-sulfoxide reductase heme-binding subunit MsrQ;msrQ;ortholog	PROTEIN-METHIONINE-SULFOXIDE REDUCTASE HEME-BINDING SUBUNIT MSRQ (PTHR36964:SF1)
ECOLI EcoGene=EG12575 UniProtKB=P39385	yjiN	Uncharacterized protein YjiN;yjiN;ortholog	INNER MEMBRANE PROTEIN-RELATED (PTHR38442:SF1)
ECOLI EcoGene=EG12528 UniProtKB=P39333	yjiI	Cyclic-di-GMP-binding biofilm dispersal mediator protein;bdcA;ortholog	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 4 (PTHR43943:SF2)
ECOLI EcoGene=EG12209 UniProtKB=P37002	crcB	Putative fluoride ion transporter CrcB;crcB;ortholog	FLUORIDE ION TRANSPORTER CRCB-RELATED (PTHR28259:SF14)
ECOLI EcoGene=EG12102 UniProtKB=P33650	feoB	Fe(2+) transporter FeoB;feoB;ortholog	FE(2+) TRANSPORTER FEOB (PTHR43185:SF1)
ECOLI EcoGene=EG10712 UniProtKB=P16681	yjdN	Protein YjdN;yjdN;ortholog	PROTEIN YJDN (PTHR33990:SF1)
ECOLI EcoGene=EG12034 UniProtKB=P33026	setB	Sugar efflux transporter B;setB;ortholog	SUGAR EFFLUX TRANSPORTER A-

			RELATED (PTHR23535:SF2)
ECOLI EcoGene=EG11544 UniProtKB=P63204	gadE	Transcriptional regulator GadE;gadE;ortholog	
ECOLI EcoGene=EG11774 UniProtKB=P31979	nuoF	NADH-quinone oxidoreductase subunit F;nuoF;ortholog	NADH DEHYDROGENASE [UBIQUINONE] FLAVOPROTEIN 1, MITOCHONDRIAL (PTHR11780:SF10)
ECOLI EcoGene=EG11736 UniProtKB=P63228	gmhB	D-glycero-beta-D-manno-heptose-1,7-bisphosphate 7-phosphatase;gmhB;ortholog	D-GLYCERO-BETA-D-MANNO-HEPTOSE-1,7-BISPHOSPHATE 7-PHOSPHATASE (PTHR42891:SF1)
ECOLI EcoGene=EG13557 UniProtKB=P77489	yagR	Aldehyde oxidoreductase molybdenum-binding subunit PaoC;paoC;ortholog	ALDEHYDE OXIDOREDUCTASE MOLYBDENUM-BINDING SUBUNIT PAOC (PTHR11908:SF123)
ECOLI EcoGene=EG12349 UniProtKB=P75717	ybcC	Putative uncharacterized protein ExoD;exoD;ortholog	
ECOLI EcoGene=EG11287 UniProtKB=P25728	ygbA	Uncharacterized protein YgbA;ygbA;ortholog	
ECOLI EcoGene=EG10404 UniProtKB=P09832	gltD	Glutamate synthase [NADPH] small chain;gltD;ortholog	GLUTAMATE SYNTHASE [NADPH] SMALL CHAIN (PTHR42783:SF3)
ECOLI EcoGene=EG13570 UniProtKB=P0ACC9	wcaB	Putative colanic acid biosynthesis acetyltransferase WcaB;wcaB;ortholog	SERINE ACETYLTRANSFERASE-RELATED (PTHR42811:SF5)
ECOLI EcoGene=EG10502 UniProtKB=P0ADF8	ilvN	Acetolactate synthase isozyme 1 small subunit;ilvN;ortholog	
ECOLI EcoGene=EG13791 UniProtKB=P77790	ddpX	D-alanyl-D-alanine dipeptidase;ddpX;ortholog	D-ALANYL-D-ALANINE DIPEPTIDASE (PTHR43126:SF1)
ECOLI EcoGene=EG13568 UniProtKB=P76387	wzc	Tyrosine-protein kinase wzc;wzc;ortholog	TYROSINE-PROTEIN KINASE ETK-RELATED (PTHR32309:SF25)

ECOLI EcoGene=EG12242 UniProtKB=P63201	gadW	HTH-type transcriptional regulator GadW;gadW;ortholog	HTH-TYPE TRANSCRIPTIONAL REGULATOR ADIY-RELATED (PTHR43280:SF20)
ECOLI EcoGene=EG12264 UniProtKB=P0ADJ5	bcsF	Cellulose biosynthesis protein BcsF;bcsF;ortholog	
ECOLI EcoGene=EG11479 UniProtKB=P69423	tatC	Sec-independent protein translocase protein TatC;tatC;ortholog	SEC-INDEPENDENT PROTEIN TRANSLOCASE PROTEIN TATC, CHLOROPLASTIC-RELATED (PTHR30371:SF0)
ECOLI EcoGene=EG10813 UniProtKB=P15033	racC	Protein RacC;racC;ortholog	
ECOLI EcoGene=EG20283 UniProtKB=P0AG74	rusA	Crossover junction endodeoxyribonuclease RusA;rusA;ortholog	
ECOLI EcoGene=EG11890 UniProtKB=P37194	slp	Outer membrane protein Slp;slp;ortholog	OUTER MEMBRANE PROTEIN SLP (PTHR37530:SF1)
ECOLI EcoGene=EG13389 UniProtKB=P64524	cbtA	Cytoskeleton-binding toxin CbtA;cbtA;ortholog	
ECOLI EcoGene=EG12677 UniProtKB=P0A6B7	iscS	Cysteine desulfurase IscS;iscS;ortholog	CYSTEINE DESULFURASE, MITOCHONDRIAL (PTHR11601:SF34)
ECOLI EcoGene=EG10042 UniProtKB=P0AE14	ampE	Protein AmpE;ampE;ortholog	PROTEIN AMPE (PTHR38684:SF1)
ECOLI EcoGene=EG10923 UniProtKB=P0A809	ruvA	Holliday junction ATP-dependent DNA helicase RuvA;ruvA;ortholog	HOLLIDAY JUNCTION ATP-DEPENDENT DNA HELICASE RUVA (PTHR33796:SF1)
ECOLI EcoGene=EG11352 UniProtKB=P27128	rfaI	Lipopolysaccharide 1,3-galactosyltransferase;rfaI;ortholog	LIPOPOLYSACCHARIDE 1,3-GALACTOSYLTRANSFERASE (PTHR13778:SF10)
ECOLI EcoGene=EG11052 UniProtKB=P09835	uhpB	Signal transduction histidine-protein kinase/phosphatase UhpB;uhpB;ortholog	SIGNAL TRANSDUCTION HISTIDINE-PROTEIN KINASE/PHOSPHATA

			SE UHPB (PTHR24421:SF49)
ECOLI EcoGene=EG13720 UniProtKB=P75869	yccR	Protein Sxy;sxy;ortholog	PROTEIN SXY (PTHR36121:SF1)
ECOLI EcoGene=EG13460 UniProtKB=P77650	hcaD	3-phenylpropionate/cinnamic acid dioxygenase ferredoxin--NAD(+) reductase component;hcaD;ortholog	SD06560P (PTHR43557:SF2)
ECOLI EcoGene=EG11158 UniProtKB=P0AC28	ygfA	5-formyltetrahydrofolate cyclo-ligase;ygfA;ortholog	5-FORMYLTETRAHYDROFOLATE CYCLO-LIGASE (PTHR23407:SF1)
ECOLI EcoGene=EG10832 UniProtKB=P0A7H3	recO	DNA repair protein RecO;recO;ortholog	DNA REPAIR PROTEIN RECO (PTHR33991:SF1)
ECOLI EcoGene=EG12766 UniProtKB=P42906	agaA	Putative N-acetyl galactosamine-6-phosphate deacetylase;agaA;ortholog	
ECOLI EcoGene=EG11194 UniProtKB=P0AGM9	yicE	Xanthine permease XanP;xanP;ortholog	PURINE PERMEASE C1399.01C-RELATED (PTHR42810:SF2)
ECOLI EcoGene=EG20265 UniProtKB=P0A6S3	flgI	Flagellar P-ring protein;flgI;ortholog	FLAGELLAR P-RING PROTEIN (PTHR30381:SF0)
ECOLI EcoGene=EG11728 UniProtKB=P31470	yieK	Uncharacterized protein YieK;yieK;ortholog	GLUCOSAMINE-6-PHOSPHATE ISOMERASE (PTHR11280:SF5)
ECOLI EcoGene=EG12669 UniProtKB=P40874	solA	N-methyl-L-tryptophan oxidase;solA;ortholog	PEROXISOMAL SARCOSINE OXIDASE (PTHR10961:SF7)
ECOLI EcoGene=EG10361 UniProtKB=P22256	gabT	4-aminobutyrate aminotransferase GabT;gabT;ortholog	4-AMINO BUTYRATE AMINOTRANSFERASE GABT (PTHR43206:SF2)
ECOLI EcoGene=EG13567 UniProtKB=P0AAB2	wzb	Low molecular weight protein-tyrosine-phosphatase Wzb;wzb;ortholog	LOW MOLECULAR WEIGHT PHOSPHOTYROSINE PROTEIN

			PHOSPHATASE (PTHR11717:SF7)
ECOLI EcoGene=EG10676 UniProtKB=P0AFH6	oppC	Oligopeptide transport system permease protein OppC;oppC;ortholog	OLIGOPEPTIDE TRANSPORT SYSTEM PERMEASE PROTEIN OPPC (PTHR43386:SF2)
ECOLI EcoGene=EG11426 UniProtKB=P27241	rfaZ	Lipopolysaccharide core biosynthesis protein RfaZ;rfaZ;ortholog	
ECOLI EcoGene=EG12789 UniProtKB=P64599	yhbT	SCP2 domain-containing protein YhbT;yhbT;ortholog	EUCALYPTUS, ISOFORM B-RELATED (PTHR10094:SF25)
ECOLI EcoGene=EG11387 UniProtKB=P26612	amyA	Cytoplasmic alpha-amylase;amyA;ortholog	ALPHA-AMYLASE (PTHR43447:SF7)
ECOLI EcoGene=EG50010 UniProtKB=P25437	fdh	S-(hydroxymethyl)glutathione dehydrogenase;frmA;ortholog	ALCOHOL DEHYDROGENASE CLASS-3 (PTHR43880:SF12)
ECOLI EcoGene=EG12364 UniProtKB=P39347	intB	Putative protein IntB;intB;ortholog	PROTEIN INTB-RELATED (PTHR30629:SF9)
ECOLI EcoGene=EG13154 UniProtKB=Q47149	yafQ	mRNA interferase toxin YafQ;yafQ;ortholog	MRNA INTERFERASE TOXIN YAFQ (PTHR40588:SF1)
ECOLI EcoGene=EG10838 UniProtKB=P67910	rfaD	ADP-L-glycero-D-manno-heptose-6-epimerase;hldD;ortholog	ADP-L-GLYCERO-D-MANNO-HEPTOSE-6-EPIMERASE (PTHR43103:SF3)
ECOLI EcoGene=EG12559 UniProtKB=P39368	yjhQ	Uncharacterized N-acetyltransferase YjhQ;yjhQ;ortholog	SLL6060 PROTEIN (PTHR43617:SF2)
ECOLI EcoGene=EG12651 UniProtKB=P71244	wcaM	Colanic acid biosynthesis protein WcaM;wcaM;ortholog	
ECOLI EcoGene=EG10823 UniProtKB=P0A7G6	recA	Protein RecA;recA;ortholog	MITOCHONDRIAL DNA REPAIR PROTEIN RECA HOMOLOG (PTHR45900:SF1)
ECOLI EcoGene=EG14320 UniProtKB=P56258	wecF	TDP-N-acetylfucosamine:lipid II N-acetylfucosaminyltransferase;wecF;ortholog	

ECOLI EcoGene=EG12172 UniProtKB=P0ACE7	hinT	Purine nucleoside phosphoramidase;hinT;ortholog	HISTIDINE TRIAD NUCLEOTIDE-BINDING PROTEIN 1 (PTHR23089:SF33)
ECOLI EcoGene=EG13262 UniProtKB=P0AFP4	ybbO	Uncharacterized oxidoreductase YbbO;ybbO;ortholog	ALCOHOL DEHYDROGENASE-RELATED 31 KDA PROTEIN (PTHR43313:SF1)
ECOLI EcoGene=EG13011 UniProtKB=P67244	yqhA	UPF0114 protein YqhA;yqhA;ortholog	UPF0114 PROTEIN YQHA (PTHR38596:SF1)
ECOLI EcoGene=EG10981 UniProtKB=P0A836	sucC	Succinate--CoA ligase [ADP-forming] subunit beta;sucC;ortholog	SUCCINATE--COA LIGASE [ADP-FORMING] SUBUNIT BETA (PTHR11815:SF1)
ECOLI EcoGene=EG11153 UniProtKB=P0A7X6	rimM	Ribosome maturation factor RimM;rimM;ortholog	RIBOSOME MATURATION FACTOR RIMM (PTHR33692:SF1)
ECOLI EcoGene=EG11320 UniProtKB=P0A8D0	ybaD	Transcriptional repressor NrdR;nrdR;ortholog	TRANSCRIPTIONAL REPRESSOR NRDR (PTHR30455:SF2)
ECOLI EcoGene=EG10824 UniProtKB=P08394	recB	RecBCD enzyme subunit RecB;recB;ortholog	RECBCD ENZYME SUBUNIT RECB (PTHR11070:SF23)
ECOLI EcoGene=EG13800 UniProtKB=P77294	ydeR	Uncharacterized fimbrial-like protein YdeR;ydeR;ortholog	PROTEIN FIMG (PTHR33420:SF27)
ECOLI EcoGene=EG12362 UniProtKB=P38051	menF	Isochorismate synthase MenF;menF;ortholog	ISOCHORISMATE SYNTHASE 2, CHLOROPLASTIC (PTHR47253:SF4)
ECOLI EcoGene=EG10243 UniProtKB=P03007	dnaQ	DNA polymerase III subunit epsilon;dnaQ;ortholog	DNA POLYMERASE III SUBUNIT EPSILON (PTHR30231:SF32)
Unmapped ID	eceH		
Unmapped ID	nfnB		
Unmapped ID	rcfR		

Table S2: List of gene knockouts that increased calcium AUC.

Gene ID	Mappe	Gene function	Panther Family/Subfamily
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	d IDs			
ECOLI EcoGene=EG11748 UniProtKB=P31665	yad D	Recombination-promoting nuclease RpnC;rpnC;ortholog	INACTIVE RECOMBINATION-PROMOTING NUCLEASE-LIKE PROTEIN RPNE-RELATED (PTHR34611:SF2)	
ECOLI EcoGene=EG14144 UniProtKB=P0AA49	yfd V	Uncharacterized transporter YfdV;yfdV;ortholog	AUXIN EFFLUX CARRIER FAMILY PROTEIN (PTHR36838:SF1)	
ECOLI EcoGene=EG12251 UniProtKB=P37645	yhj G	AsmA family protein YhjG;yhjG;ortholog	ASMA FAMILY PROTEIN YHJG (PTHR30441:SF2)	
ECOLI EcoGene=EG10233 UniProtKB=P18776	dms B	Anaerobic dimethyl sulfoxide reductase chain B;dmsB;ortholog	ANAEROBIC DIMETHYL SULFOXIDE REDUCTASE CHAIN B-RELATED (PTHR43177:SF5)	oxidoreductase(PC00176)
ECOLI EcoGene=EG10963 UniProtKB=P09158	spe E	Polyamine aminopropyltransferase;speE;ortholog	SPERMIDINE SYNTHASE (PTHR11558:SF11)	transferase(PC00220)
ECOLI EcoGene=EG13397 UniProtKB=P0AGK8	isc R	HTH-type transcriptional regulator IscR;iscR;ortholog	HTH-TYPE TRANSCRIPTIONAL REGULATOR ISCR (PTHR33221:SF10)	winged helix/forkhead transcription factor(PC00246)
ECOLI EcoGene=EG10302 UniProtKB=P06971	fhu A	Ferrichrome outer membrane transporter/phage receptor;fhuA;ortholog	FERRICHROME OUTER MEMBRANE TRANSPORTER/PHAGE RECEPTOR (PTHR32552:SF64)	
ECOLI EcoGene=EG11368 UniProtKB=P0ABU0	menB	1,4-dihydroxy-2-naphthoyl-CoA synthase;menB;ortholog	1,4-DIHYDROXY-2-NAPHTHOYL-COA SYNTHASE, PEROXISOMAL	epimerase/racemase (PC00096)

			(PTHR43113:S F1)	
ECOLI EcoGene=EG13060 UniProtKB=Q46810	ygf J	Molybdenum cofactor cytidyltransferase; mocA; ortholog	MOLYBDENUM COFACTOR CYTIDYLYLTRANSFERASE (PTHR43777:SF1)	
ECOLI EcoGene=EG14162 UniProtKB=P76535	yfe U	N-acetylmuramic acid 6-phosphate etherase; murQ; ortholog	GLUCOKINASE REGULATOR Y PROTEIN (PTHR10088:SF4)	protein-binding activity modulator(PC00095)
ECOLI EcoGene=EG12608 UniProtKB=P39206	cai E	Carnitine operon protein CaiE; caiE; ortholog	CARNITINE OPERON PROTEIN CAIE-RELATED (PTHR13061:SF14)	microtubule binding motor protein(PC00156)
ECOLI EcoGene=EG11402 UniProtKB=P28904	tre C	Trehalose-6-phosphate hydrolase; treC; ortholog	NEUTRAL AND BASIC AMINO ACID TRANSPORT PROTEIN RBAT (PTHR10357:SF179)	amylase(PC00048)
ECOLI EcoGene=EG13380 UniProtKB=P76349	yee L	Putative uncharacterized protein YeeL; yeeL; ortholog	ADP-HEPTOSE--LPS HEPTOSYLT RANSFERASE 2 (PTHR30160:SF7)	carbohydrate kinase(PC00065)
ECOLI EcoGene=EG12167 UniProtKB=P36566	cm oM	tRNA 5-carboxymethoxyuridine methyltransferase; cmoM; ortholog	UBIQUINONE BIOSYNTHESIS O-METHYLTRANSFERASE, MITOCHONDRIAL (PTHR43464:SF19)	methyltransferase(PC00155)

ECOLI EcoGene=EG10843 UniProtKB=P09377	rhaS	HTH-type transcriptional activator RhaS;rhaS;ortholog	HTH-TYPE TRANSCRIPTIONAL ACTIVATOR RHAS (PTHR43280:SF6)	helix-turn-helix transcription factor(PC00116)
ECOLI EcoGene=EG14075 UniProtKB=P76445	yeiU	Lipid A 1-diphosphate synthase;lpxT;ortholog		
ECOLI EcoGene=EG12301 UniProtKB=P0ACU2	ycdC	HTH-type transcriptional regulator RutR;rutR;ortholog	HTH-TYPE TRANSCRIPTIONAL REGULATOR RUTR (PTHR30055:SF196)	
ECOLI EcoGene=EG10721 UniProtKB=P16679	phnL	Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase subunit PhnL;phnL;ortholog	ALPHA-D-RIBOSE 1-METHYLPHOSPHONATE 5-TRIPHOSPHATE SYNTHASE SUBUNIT PHNL (PTHR42798:SF1)	ATP-binding cassette (ABC) transporter(PC00003)
ECOLI EcoGene=EG12135 UniProtKB=P0A917	ompX	Outer membrane protein X;ompX;ortholog	OUTER MEMBRANE PROTEIN X (PTHR35892:SF3)	
ECOLI EcoGene=EG10103 UniProtKB=P0ABA0	atpF	ATP synthase subunit b;atpF;ortholog	ATP SYNTHASE SUBUNIT B (PTHR33445:SF1)	
ECOLI EcoGene=EG10101 UniProtKB=P0ABB4	atpD	ATP synthase subunit beta;atpD;ortholog	ATP SYNTHASE SUBUNIT BETA, MITOCHONDRIAL (PTHR15184:SF58)	ATP synthase(PC00002)
ECOLI EcoGene=EG10798 UniProtKB=P08178	purM	Phosphoribosylformylglycinamide cyclo-ligase;purM;ortholog	TRIFUNCTIONAL PURINE BIOSYNTHETIC PROTEIN ADENOSINE-	ligase(PC00142);transferase(PC00220)

			3 (PTHR10520:S F12)	
ECOLI EcoGene=EG137 73 UniProtKB=P76114	ync C	HTH-type transcriptional regulator McbR;mcbR;ortholog	HTH-TYPE TRANSCRIPT IONAL REGULATOR MCBR (PTHR43537:S F15)	winged helix/forkhead transcription factor(PC00246)
ECOLI EcoGene=EG121 13 UniProtKB=P34749	hof Q	DNA utilization protein HofQ;hofQ;ortholog	DNA UTILIZATION PROTEIN HOFQ (PTHR30604:SF1)	
ECOLI EcoGene=EG101 05 UniProtKB=P0ABA4	atp D	ATP synthase subunit delta;atpH;ortholog	ATP SYNTHASE SUBUNIT O, MITOCHOND RIAL (PTHR11910:S F1)	ATP synthase(PC00002)
ECOLI EcoGene=EG101 74 UniProtKB=P0ABK2	cyd B	Cytochrome bd-I ubiquinol oxidase subunit 2;cydB;ortholog	CYTOCHRO ME BD-I UBIQUINOL OXIDASE SUBUNIT 2 (PTHR43141:S F5)	oxidoreductase(PC0 0176)
ECOLI EcoGene=EG100 08 UniProtKB=P14377	zra S	Sensor protein ZraS;zraS;ortholog	SENSOR PROTEIN ZRAS (PTHR43065:S F38)	histidine kinase receptor of two- component system(PC00265)
ECOLI EcoGene=EG135 39 UniProtKB=P77231	cit G	2-(5''- triphosphoribosyl)- 3'- dephosphocoenzy me-A synthase;citG;ortho log	2-(5''- TRIPHOSPHO RIBOSYL)-3'- DEPHOSPHO COENZYME- A SYNTHASE (PTHR30201:S F2)	transferase(PC0022 0)
ECOLI EcoGene=EG125 71 UniProtKB=P39381	yjiJ	Uncharacterized protein YjiJ;yjiJ;ortholog	SUGAR TRANSPORTER- RELATED (PTHR23537:SF1)	

ECOLI EcoGene=EG20274 UniProtKB=P0ABR9	mh pB	2,3-dihydroxyphenylpropionate/2,3-dihydroxicinnamic acid 1,2-dioxygenase;mhpB;ortholog	
ECOLI EcoGene=EG10061 UniProtKB=P0A9Q1	arc A	Aerobic respiration control protein ArcA;arcA;ortholog	AEROBIC RESPIRATION CONTROL PROTEIN ARCA (PTHR48111:SF55)
ECOLI EcoGene=EG13977 UniProtKB=P77378	ydi R	Putative electron transfer flavoprotein subunit YdiR;ydiR;ortholog	ELECTRON TRANSFER FLAVOPROTEIN SUBUNIT YDIR-RELATED (PTHR43153:SF10) oxidoreductase(PC00176)
ECOLI EcoGene=EG10163 UniProtKB=P0AE82	cpx A	Sensor histidine kinase CpxA;cpxA;ortholog	SENSOR HISTIDINE KINASE CPXA (PTHR45528:SF1)

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