EXTENDED EXPERIMENTAL PROCEDURES

Plasmid Construction and *in vitro* Transcription

Entry clones and expression plasmids were generated by standard molecular biology procedures. The cytosolic Ca^{2+} sensor GCaMP6s (Chen et al., 2013) (gift from Emre Aksay), EGFP and the far-red fluorescent protein mKate2 (from Evrogen, henceforth mK2) were localized to the nucleus by C-terminally fusing them to the SV40 T antigen nuclear localization sequence in triplicate $(DPKKKRKV)_3$. mK2 was targeted to the plasma membrane (PM) by fusing it to the N-terminal palmitoylation/myristoylation signal of the human Lyn protein (MGCIKSKGKDSAGA). For *in vitro* transcription, the mMESSAGE mMACHINE® SP6 kit (Life Technologies) was used after NotI linearization of the pCS2+ vector encoding GCaMP6s-NLS and EGFP-NLS. To express GCaMP6s-NLS and mK2-NLS from a single ORF separately, at ratio of ~1:1, the highly efficient viral self-cleaving peptide, P2A was used as a linker between the two proteins (Kim et al., 2011).

To create plasmids for transgenesis using the Tol2kit system (Kwan et al., 2007), DNA fragments encoding GCaMP6s-NLS-P2A-mK2-NLS, PM-mK2 and $cPla_2$-mK2 (Enyedi et al., 2013a) were first subcloned into the pDONR221 backbone as entry clones, then recombined with the hsp70l or lysC promoter (Hall et al., 2007), and the SV40 polyadenylation sequence into the pDestTol2CG2 vector backbone with minimal tol2 elements.

For mammalian expression, $cPla_2$-EGFP, $cPla_2$-mK2, $cPla_2$-C2-mK2 (the C2-domain of zebrafish $cPla_2$ spanning amino acids 1-131), alone or fused to EGFP-laminB1 through a P2A peptide on the C-terminus, was subcloned into the pSB/CMV/MCS/Puro transposon plasmid (Enyedi et al., 2013b). The same backbone was used to express human and zebrafish 5-LOX (Ensembl: ENSG00000012779 and ENSDARG00000057273), that were cloned from cDNA, fused in frame with an N-terminal mK2 and linked on the 5' end by a P2A peptide to EGFP-laminB1. This backbone harbors a CMV-promoter driven expression site, along with another expression cassette that encodes a puromycin resistance gene, driven by an SV40-promoter. Both cassettes are between an inverted repeat domain of the transposon, allowing their mutual insertion into the genome when this plasmid is coexpressed with the hyperactive SB100X *Sleeping Beauty* transposase, as previously described (Izsvák et al., 2009). EGFP-NLS was expressed from the pEGFP-C1 backbone (Clontech). For bacterial expression, $cPla_2$-C2-EGFP-6HIS was cloned into a modified pGEX-4T backbone.

Single amino acid change mutants of $cPla_2$-mK2 (S498A and S498D) were created using the Stratagene QuikChange site-directed mutagenesis kit, following the manufacturer's instructions. To identify mutant clones, silent mutations were also introduced in the QuikChange primers that were automatically designed by the sequence-handling program "SeqHandler" (Czirják and Enyedi, 2006).

The plasmid pSpCas9(BB)-2A-GFP:$cPLA_2$-g1 was generated to encode a guide RNA sequence targeting human $cPLA_2$, using the primers: 5'-CACCGTTCCCACAAGTTTACGGTAG-3' and 5'-AAACCTACCGTAAACTTGTGGGAAC-3', following established guidelines (Ran et al., 2013).

Spinning Disk Confocal Imaging and Laser Wounding in Zebrafish

Experiments were performed at room temperature (~26°C) on a Nikon Eclipse FN1 microscope equipped with a 25x Apochromat LWD NA 1.1 water immersion objective lens, a Yokogawa CSU-X1 Spinning Disk unit, an Andor iXon3 897 EMCCD camera, 488 nm and 561 nm diode laser lines (Andor Revolution XD). For laser wounding experiments, anesthetized 2.5-3 dpf larvae were immobilized on a plastic dish by embedding in ~30 µl of 1% low-melting agarose dissolved in either standard hypotonic E3 or isotonic E3 (iso-E3 – E3 fish medium supplemented with 140 mM NaCl), supplemented with latrunculin B (LB) if indicated (2.5 µM, Sigma). The same respective medium was used for immersion. For experiments with LB, anesthetized larvae were preincubated in the drug for 30 minutes before imaging. Wounding was induced with quick successive laser pulses (3-4) at ~1 min using a microscope-mounted 435 nm UV MicroPoint laser (Andor). For ionomycin "shifting" experiments, anesthetized 2.5-3 dpf larvae were subjected to tail fin tip amputation using a needle knife (Fine Science Tools) in isotonic E3 and were immobilized on a plastic dish by embedding in isotonic agarose, as mentioned above. A bolus of 300 µL E3 (hypotonic or isotonic) "shifting" medium supplemented with 100 µM ionomycin was added to the imaging dish at 1 min.

For imaging nuclear Ca^{2+} signals, the TG(hsp70l:GCaMP6s-NLS-P2A-mK2-NLS) line was used for ratiometric measurements and GCaMP6s-NLS mRNA (~1ng) was injected into one-cell stage casper embryos to measure Ca^{2+} oscillations. $cPla_2$ localization was followed in the TG(hsp70l:$cPla_2$-mK2) line. 2.5-3 dpf embryos were heat shocked at 37 °C for 2 hours to induce expression of the reporters and imaged 8-12 hours later. GCaMP6s fluorescence was excited using the 488 laser line and emission was collected using a 535/20 bandpass filter (Chroma). mK2 fluorescence was excited using the 561 laser line and emission was collected using a 620/30 bandpass filter (Chroma). GCaMP6s and mK2 image acquisition was done simultaneously through triggered excitation and emission collection. Up to 30 Z-stack slices with a

resolution of 2-3 µm were acquired per field of view with the NIS-Elements software (Nikon). Images were acquired every 10 seconds for a period of 10-23 minutes.

Imaging Cells and Zebrafish Larvae by Widefield Fluorescence Microscopy

Experiments were performed at room temperature (~26°C) on a Nikon Eclipse Ti inverted microscope, equipped with a 20x Plan Apochromat NA 0.75 and a 10x Plan Apochromat NA 0.45 air objective lens (Nikon), an Andor Clara CCD camera and a motorized stage. Green and red fluorescence was excited with a LED light source (Lumencor) using the bandpass filters 475/28 and 549/15, respectively. For Fluo-4, SYTOX-green and EGFP measurements, the 470/40 excitation filter was used together with a multispectral dichroic (Chroma, 59022 bs) and the 525/50 emission filter set (Chroma). The far-red mK2 fluorophore was imaged with the 572/35 excitation filter combined with the previously mentioned dichroic and the 632/60 emission filter set (Chroma).

HeLa cells stably expressing fluorescent reporters were seeded on 25-mm diameter circular glass coverslips at a density of $2x10^5$ cells/35-mm wells 24h before imaging. Before the experiment, coverslips were placed into an imaging chamber and incubated in HEPES-buffered extracellular (EC) medium, containing 133 mM NaCl, 3.1 mM KCl, 1.2 mM $CaCl_2$, 0.5 mM KH_2PO_4, 0.5 mM $MgSO_4$, 2 mM $NaHCO_3$, 5 mM glucose, and 5 mM Na-HEPES, at pH 7.4 (~295 mOSM). When indicated, cells were preincubated in the following drugs: NVP-231 (100 µM, 6h, Cayman), staurosporine (100 nM, 30 min, Sigma), latrunculin A (100 nM, 30 min, Sigma).

For parallel cytosolic calcium [Ca^{2+}] and $cPla_2$-mK2 localization measurements, cells were loaded with Fluo-4 AM (2 µM, Life technologies) in EC medium containing probenecid (2.5 mM, Sigma) and Pluronic® F-127 (0.02%, Sigma) for 45 min at room temperature, followed by a 30 min equilibration step after washing in EC medium. A Z-stack of images was acquired every 10 s for a period of 9 min, with 1 µm slices spanning 5-6 µm to capture the full range of cell and nuclear swelling, predominately occuring along the Z-axis. During the experiments, stimuli (ATP, 100 µM) were added in 0.1 ml of buffer after removing 0.1 ml of medium from the cells. Hyposmotic shock was applied by diluting the incubating buffer with EC medium lacking NaCl (~28 mOSM). The osmolarity of all buffered solutions was measured on an osmometer. To measure F_{max} from the maximum Fluo-4 signal, 10 µM ionomycin (Sigma) was applied at the end of each run for 3 min.

For all permeabilized cell experiments, the following intracellular (IC) medium was used: 123 mM KCl, 12 mM NaCl, 1 mM KH_2PO_4, 1.94 mM $MgCl_2$, 10 mM MOPS, 0.28 mM $CaCl_2$, 1 mM NaEGTA at pH 7.2. Additional $CaCl_2$ was added to change the free Ca^{2+} concentration of the solution, which was measured on a spectrophotometer using Fura-2 (Life technologies) and a calcium calibration buffer kit (Life technologies). Cells were permeabilized in IC medium with 25 µg/ml digitonin (Cayman) in the presence of 2.5% PVP360 (Polyvinylpyrrolidone, Sigma). A Z-stack of images was acquired every 30 s for a period of 33 min, with 1 µm slices spanning 5-6 µm to capture the full range of nuclear swelling.

To image leukocyte recruitment in zebrafish larvae, 10 anaesthetized 2.5-3 dpf TG(lysC:PM-mK2) zebrafish larvae were simultaneously subjected to tail fin tip amputation in iso-E3 using a needle knife (Fine Science Tools), and were aligned and embedded in a small volume of 2% isotonic low melting agarose (~500 µl) in a glass-bottom dish (Matek Corporation). After solidification, a strip of agarose (~100 µl) was removed along the amputated tail fins, and the sample was mounted on the microscope. Meanwhile, a cell suspension was prepared from trypsinized wild type, $cPLA_2$ k.o. or $cPla_2$-mK2 expressing HeLa cells, and washed in EC or IC medium with low [Ca^{2+}]. The cells were then treated with ionomycin in EC medium (1 µM, 3 min) or permeabilized with digitonin in IC medium containing 2.5% PVP. After two additional washing steps in EC or IC medium, respectively, the cells were resuspended in 100 µl of normal EC (1.2 mM [Ca^{2+}]) or high [Ca^{2+}] IC medium (14 µM free [Ca^{2+}]). 4% PVP was used in the final suspending solution to prevent nuclear swelling of permeabilized cells as needed. The 100 µl cell suspension was then added to the agarose well, adjacent to the gaping tail fin wounds, right before acquisition was initiated (~10-15 min post-wounding). A Z-stack of light transmission and red fluorescence images (15 µm slices spanning 60 µm) was acquired every min of all larvae on the above described widefield microscope over the course of 1 h, using the 10x objective, to follow leukocyte migration toward the amputation site.

Confocal Measurement of Nuclear Volume in Cells and Zebrafish

Spinning disk confocal measurements on HeLa cells were carried out at room temperature (~26°C) on a Nikon Eclipse TiE inverted microscope, equipped with Nikon Plan Apo 100x/1.45 oil and Plan Apo 40x/0.95 air objectives, a Yokogawa CSU-X1 Spinning Disk unit, an ANDOR iXon ULTRA 897BV EMCCD camera, 488 nm and 561 nm solid-state laser lines (Andor Revolution). To measure nuclear volume changes in intact and latrunculin A pretreated HeLa cells (100 nM, 30 min), EGFP-NLS expressing cells were imaged every 10 sec before and after swelling in 150 mOSM EC-medium. To measure $cPla_2$ translocation in parallel to nuclear volume changes, $cPla_2$-mK2-P2A-EGFP-laminB1 stable cell lines were used, permeabilized with digitonin as described above, and imaged every 30 sec. Z-stack slices with a distance of 0.6-0.75 µm spanning 20 µm were acquired for every image using the 40x or 100x objective with the NIS-Elements software (Nikon).

To measure nuclear volume changes in the multi-layered tail fin tissue of zebrafish larvae, EGFP-NLS expression was restricted to a few cells by mosaic expression. This was achieved by 4-8-cell stage mRNA injection (~1 ng) (Gault et al., 2014), and allowed for the selection of embryos that showed expression restricted to a few cells in the suprabasal and basal epithelial layers of the tail fin. Measurements were carried out on a Leica TCS SP8 laser scanning confocal microscope, using a White Light Laser at 490 nm (15% output) and HyD3 as a detector with an emission bandwidth of 54 nm (495-549 nm). Images were acquired using a Leica HCX PL APO 40X water objective with the confocal pinhole set to 1 Airy units. Untreated and LB pretreated (2.5 mM, 30 min) anesthetized larvae were subjected to tail fin tip amputation using a needle knife (Fine Science Tools) in isotonic E3 and were immobilized in the 'vertical orientation' on a glass-bottom dish (Matek) by embedding in isotonic 1% agarose. A bolus of 500 µL hypotonic E3 "shifting" medium was added to the imaging dish at 1 min.

Nuclear Compression and Live Imaging

To compress permeabilized cells and their nuclei, a previously described protocol (Dumont and Mitchison, 2009) was modified as follows: A solution of 2% ultrapure agarose (Invitrogen 15510) in IC medium, containing 2.5% PVP was prepared and brought to boil, and 5 ml was put in a 35 mm Petri dish to solidify with 2 mm thickness. A 1 cm X 1 cm pad area was cut out and soaked in IC medium containing 2.5% PVP and ~550 nM free [Ca^{2+}] medium overnight at 4°C for equilibration. For compression, the agarose pad was deposited gently on the permeabilized cells, and a 2.75 g weight was carefully placed on the pad at the indicated time, using a micromanipulator. Imaging was carried out on the above mentioned spinning disk Nikon Eclipse TiE inverted microscope using the Plan Apo 40x/0.95 air objective. $cPla_2$ translocation was measured in $cPla_2$-mK2-P2A-EGFP-laminB1 stable cell lines, permeabilized with digitonin as described above, and imaged every 30 sec. Z-stack slices with a distance of 0.75 µm spanning 30 µm were acquired for every image using the NIS-Elements software (Nikon).

Fluorescence Recovery After Photobleaching (FRAP) experiments

FRAP experiments on HeLa cells stably expressing $cPla_2$-EGFP were carried out at 37°C, using a stage heater on a Leica TCS SP8 confocal microscope, running in "Fly mode," using PMT as a detector with the emission bandwidth of 65 nm (495-560 nm). 12-bit image data were acquired using Leica HC PL ApoCS2 63x/1.40 oil objective at 20× optical zoom. The confocal pinhole was set to 7.01 Airy units at 520 nm (i.e., 600 µm). The imaging window was set to 96 x 64 pixels (9.23 × 6.12 µm), and contained a 2.5 µm diameter circular bleach ROI in the middle. Samples were imaged and bleached with a 102 mW Argon laser, with a ~25 mW 488 nm line and a ~12 mW 458 nm line, limited to 20% of the total output (set to 0.2% of 488 nm laser line for the pre- and post-bleach and 100% of 458 and 488 nm laser lines each for the bleach step). Pre-bleach and post-bleach images were collected with line averaging of 2, and bleaching was performed in one scan. Time series images were collected every 80 ms, with no delay for measurements in low [Ca^{2+}], and every 150 ms in high [Ca^{2+}] IC buffer.

Quantifying Nuclear Volume Changes and FRAP Experiments

Spinning disk confocal Z-stack measurements of EGFP-NLS and EGFP-laminB1 were used to calculate nuclear volume changes in HeLa cells, using custom Python scripts. Briefly, individual nuclei were cropped from 3D stacks of EGFP-NLS expressing cells, and the background region was cropped by Otsu thresholding after Gaussian filtering to calculate the sum of the voxels in the remaining 3D mask, which is proportional to the nuclear volume. As EGFP-laminB1 only marks the outline of the nuclei, maximum intensity projection images were generated from the EGFP-laminB1 images from the time-lapse series of individual nuclei along all X-, Y- and Z-axis, which were converted to binary masks by Otsu thresholding after Gaussian filtering. Volumes were then calculated from these orthographic projections by summing up the overlapping voxels from the side projections.

Nuclear volume changes in zebrafish epithelial cells were measured from EGFP-NLS fluorescence, using the imaging software Imaris (Bitplane), which has a built-in function to calculate a rendered 3D surface and volume for objects such as nuclei.

FRAP experiments to measure diffusion coefficients (D_{Conf}) and mobile fractions (M_f) were quantified as previously described (Kang et al., 2012). Briefly, a custom Python script was developed to calculate the effective bleaching radius (r_e) from normalized postbleach profiles obtained from pre- and post-bleach images. FRAP curves were then background corrected and normalized for photobleaching, to calculate M_f and recovery half times ($\tau_{1/2}$). Diffusion coefficients were finally calculated from the determined values and the original bleaching spot radius (r_n = 1.25 µm), using the formula:

$$D_{Conf} = \frac{r_n^2 + r_e^2}{8\tau_{1/2}}$$

Image Processing and Data Analysis of Nuclear Ca^{2+} Signals in Zebrafish

All image processing tasks were performed with the open-source program Fiji (Schindelin et al., 2012) and the Anaconda distribution of the Python programming language, using custom scripts, based on the pandas, SciPy, NumPy and scikit-image libraries. To quantify nuclear Ca^{2+} signal intensity, background corrected and thresholded maximum intensity projection images of GCaMP6s and mK2 confocal time-lapse Z-stacks were generated and ratio images (GCaMP6s/mK2) were calculated using Fiji. The ratio value of every pixel was then remapped using a custom Python script, to express them as a function of binned distance (2 μm) from the moving wound margin. These spatiotemporal maps of the average Ca^{2+} signals were then averaged over multiple experiments (e.g., Figure S2A).

To quantify Ca^{2+} oscillation occurrence, a custom Python script was used. First, a "differential Z-stack image" is created by subtracting the subsequent time-lapse points of the GCaMP6s image stack, to highlight rapidly changing signal intensities, with positive values representing the onset of oscillatory events. The nuclei showing oscillation onsets are then identified in the individual frames by Otsu-thresholding across the Z-stacks, and their distance is determined from the wound margin. The spatiotemporal Ca^{2+} oscillation incidence is then represented by showing the average occurrence of Ca^{2+} oscillation events at a binned distance (10 μm bins) from the wound margin. Spatiotemporal maps of Ca^{2+} oscillation incidence were then averaged over multiple experiments (e.g., Figure 1C).

Quantification of Perinuclear Translocation and Ca^{2+} Signals in HeLa Cells

Two approaches were used to automatically quantify perinuclear translocation of mK2-labeled proteins in individual nuclei using custom Python scripts. The first approach (i) allows automatic classification of nuclei and differentiation between translocated and nontranslocated states of the mK2-labeled protein during the time course of an experiment. This can be assessed from single channel measurements, and ultimately compares the translocation state of the labeled protein to the average baseline (nucleoplasmic localization) and the average maximally translocated states (evoked with high [Ca^{2+}]), as described below. The other approach (ii) provides absolute quantification of the extent of translocation, as a normalized value of the average mK2 intensity, measured precisely along the nuclear envelope (NE) as detailed below. The latter requires parallel measurements of the mK2-labeled proteins and a reference EGFP-laminB1 fluorophore (precluding other measurements in the EGFP channel, e.g., parallel [Ca^{2+}] measurements with Fluo-4). Both methods rely on measuring the 'average radial profile plot' or 'radial distribution' of the mK2-labeled proteins in the nuclei. This plot is the average of the 'radial profile plots', measured between the centroid of the nucleus and each point of the 5 pixel dilated outline of the nucleus.

For both approaches, maximum intensity projection images are generated from the Z-stack time-lapse movies, followed by Gaussian filtering and auto local thresholding. The nuclei are then identified frame-by-frame, and separated in the masked images by segmentation to yield individual nuclear ROIs. An "average radial profile plot" is generated for the individual nuclei by averaging the raw profile plots, measured between the centroid of the nucleus and each point of the 5 pixel (~3.2 μm) dilated outline of the nucleus, after aligning the raw profile plots to the edge of the NE. Alignment of the raw profile plots to the outside edge of the NE is based on discriminating from the background by Otsu thresholding. The resulting "average radial profile plots" represent the radial fluorescence distribution in the nucleus, by showing the average fluorescence intensity along the axis perpendicular to the NE, which has a local maximum when the fluorophore is translocated and enriched on the NE.

To classify (i) if the protein is translocated or not in a given image, the "average radial profile plot" is compared to the average baseline (nucleoplasmic localization) and the average maximally translocated "average radial profile plot" curves, using the sum-of-squares method. Maximum translocation was achieved at the end of every measurement by applying ionomycin to intact cells or high [Ca^{2+}] to permeabilized cells. The Ca^{2+} signals from the same cells, measured in parallel using Fluo-4, are calculated from the maximum intensity projection images after background subtraction, using the same nuclear ROIs as described above. The values are then normalized to the average baseline F_{min} intensity and the ionomycin induced F_{max} value.

Quantitative translocation values (ii) are determined from the "average radial profile plots" after normalizing them to the intensity measured on the outside edge of the NE and the intensity measured 3 pixels (~2 μm) toward the center of the nucleus. To determine the location of the NE, the local emission intensity maximum of the EGFP-laminB1 fluorophore was used. The average intensity on the inner nuclear membrane was determined by measuring the value 1 pixel (~ 0.64 μm) inwards from the outside edge of the NE, and is in the range of ~0.4-0.5 in the non-translocated state, and ~1.5-2 when full translocation occurs (e.g., Figure 5A). The degree of translocation (%) is calculated by normalizing the measured absolute translocation values to the baseline (nontranslocated) and the maximally stimulated (fully translocated) states, measured at saturating, high [Ca^{2+}]. Swelling-induced accumulation rate of mK2-tagged proteins is calculated from the average change in

the degree of translocation (%), measured during the first 10 min following PVP washout. The apparent k_{off} of $cPla_2$-mK2, indicating debinding from the nuclear membrane, was calculated by exponential curve fitting using Python, assuming a first-order decay kinetics.

Quantifying Perinuclear $cPla_2$ Translocation in Zebrafish

To quantify $cPla_2$-mK2 translocation *in vivo*, the coordinates of the center of mass of the cells showing transient or persistent translocation were manually marked at the onset of translocation in every frame of the time-lapse movies using the Cell Counter plugin in Fiji. Translocation events were identified by visual inspection of the time-lapse movies. Spatiotemporal maps of permanent and transient $cPla_2$-mK2 translocation events were then generated by measuring the distance of the marked nuclei from the moving margin, using a custom Python script. To enhance image quality, 'blind deconvolution' was performed (using AutoQuant) on the original 3D image stacks, and partial maximum intensity projection images were created.

To quantify the extent of $cPla_2$–mK2 translocation in a selected group of cells (e.g., Fig. 3B, Fig. S1A and B, S2E and S4C), a binary mask of the cropped images containing individual nuclei was created after Gaussian filtering and Otsu thresholding, and a ratio was calculated from the intensity measured in a ring lining the outer ~0.5 µm perimeter of the nucleus (using the 3 pixel wide outer rim of the nuclear mask) and the intensity measured in the middle of the nucleus.

Quantifying Leukocyte Migration in Zebrafish

Leukocyte recruitment was determined by counting all migrating cells that arrived at the wound margin within 60 min, as judged from the 1 min / frame time-lapse movies of the TG(lysC:PM-mK2) larvae, labeling the granulocytes and a subset of macrophages (Hall et al., 2007). Cells that already resided at the wound margin at the beginning of the time-lapse sequence were not counted.

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