Size of cell-surface Kv2.1 domains is governed by growth fluctuations

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Supporting Material

Kv2.1 channel counting

In order to obtain the density of Kv2.1 channels inside clusters, we measured both the cluster area and the number of channels. The intensity of an individual GFP was measured from the bleaching steps of single fluorophores (1). Individual channels located away from clusters were manually identified and they were then tracked with a custom-written center-of-mass algorithm. A 10x10 region of interest (ROI) was centered around the selected channels and the image was thresholded to reduce bias due to background fluorescence (2). The XY centroid of the thresholded image was obtained and the ROI was moved to the new location to account for channel motion. The center of mass in the next frame was then found and the algorithm was looped until the particle was lost. Particles are lost by the tracking algorithm either because they are photobleached or because they collide with a Kv2.1 cluster or with another individual channel. The tracking algorithm was implemented in LabView.

The photobleaching of an individual fluorophore occurs in a discrete manner and a step can be observed for each bleaching molecule. Because Kv2.1 is tetrameric, each channel is labeled with four GFP fluorophores and several steps are seen before the channel trajectory is lost. In order to characterize the intensity of a bleaching step the intensity of all the pixels in the threshold image is summed. Figure S1A shows characteristic bleaching steps. After a number of steps were collected from different trajectories in a cell the average step intensity is obtained. Fig. S1B shows a histogram of the measured intensity change during 39 steps in a single cell. The mean of the step intensity is 10418, measured in arbitrary units and the standard deviation 2374. Due to the tetrameric character of Kv2.1 channels, we obtain the channel number inside each cluster by dividing the sum of the intensities of the pixels in a cluster by 4 times the intensity of a fluorophore.

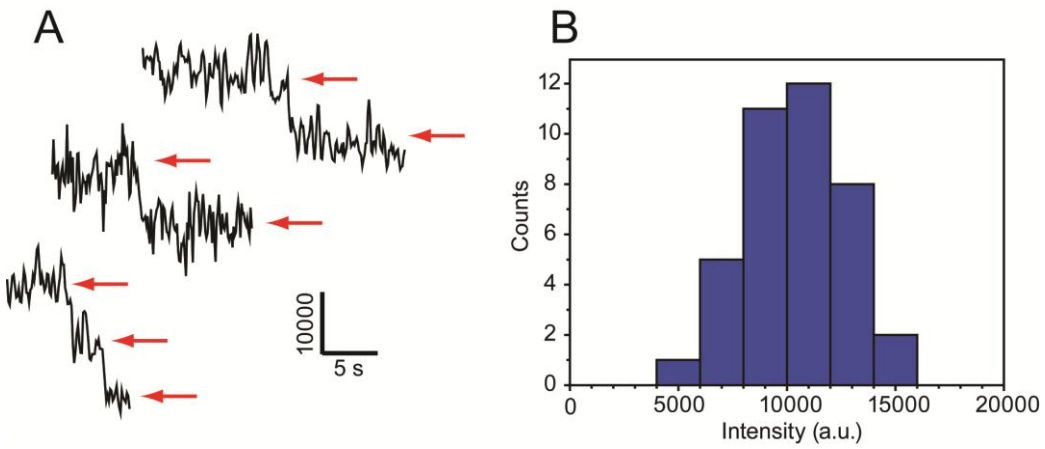

Fig. S1. Bleaching steps of individual GFP. (A) Time-course of the fluorescence emission of three characteristic channels obtained by thresholding and summing the intensities in a 10x10 pixel region of interest. The scale bars show an intensity of 10000 in arbitrary units and a time course equivalent to 5 s. (B) Distribution of the step intensities from the collection of 39 steps in a single cell.

Effect of latrunculin A on Kv2.1 cluster dynamics

We assessed the dynamics of individual clusters by tracking their center of mass. The trajectories from 31 clusters were obtained in control cells and 39 clusters in cells treated with LatA. The obtained trajectories were then characterized through their mean square displacement (MSD). The MSD provides a simple characterization of the mode of random motion. Unrestricted Brownian motion yields a MSD linear with time: in two dimensions, MSD ~ 4*Dt*, where *D* is the diffusion coefficient that depends on domain size. However, restricted diffusion results in a subdiffusive type of motion where the MSD becomes sublinear in time, $\mathrm{MSD} = K_\alpha t^\alpha$, with $0 < \alpha < 1$ (3). Figures S2A and S2B show log-log plots of the temporal MSD of trajectories in controls and LatA-treated cells, respectively. Dashed lines corresponding to MSD ~ t and MSD ~ $t^{0.7}$ are shown as a visual guide to the eye. Domains in control cells are seen to display anomalous subdiffusion while the domains in treated cells obey normal Brownian motion. Furthermore, the characteristic square displacement, or the diffusion coefficient, in treated cells is more than an order of magnitude higher than the values in control cells. Figure S2C shows the MSD in both cases after ensemble averaging the MSD of all the trajectories. Fitting to a power law yields an exponent $\alpha = 0.69$ for control cells. In LatA-treated cells, the diffusion becomes normal with an exponent of 0.99.

LatA experiments show that the actin cytoskeleton plays a crucial role in the anomalous subdiffusive motion of Kv2.1 domains. These experiments, as well as previous observations (4), also suggest that cortical actin hinders aggregation by forming physical barriers between domains.

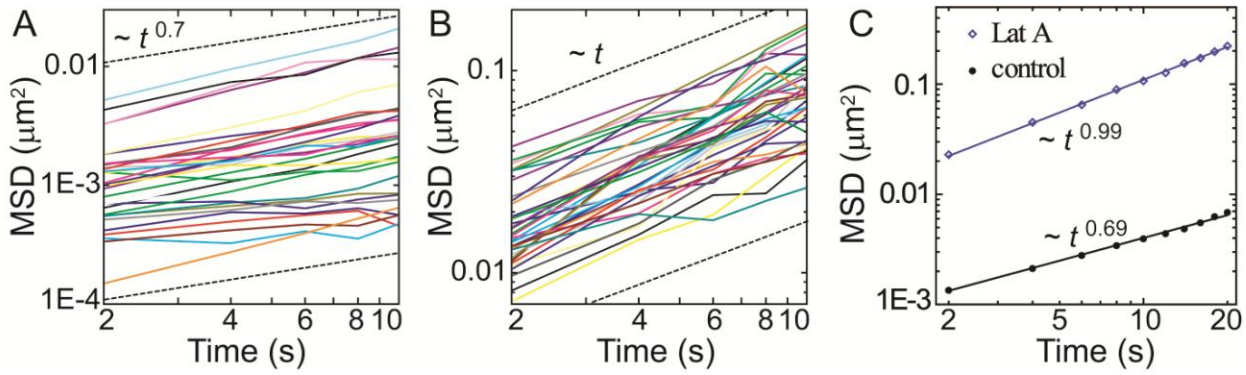

Fig. S2. Mean square displacement analysis of the motion of Kv2.1 domains on the cell surface. Log-log plot of the MSDs of domain trajectories from (A) control and (B) latrunculin A treated cells. In order to show sublinearity with control cells and normal diffusion in latrunculin A cells, dashed lines that scale as $t^{0.7}$ and t are shown (C) Ensemble averaged MSD of 31 control cell clusters (solid circles) and 39 clusters from cells treated with 5 μM latrunculin A (open diamonds). MSDs are fit to a power law, MSD ~ t^{α} to determine diffusion anomaly. Domains in control cells diffuse with an anomalous subdiffusive exponent of 0.69 ± 0.01. Domains in cells treated with latrunculin A are more mobile and display normal diffusion, ($\alpha = 0.99 \pm 0.01$).

SUPPORTING REFERENCES

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