Additional File 1: CICR follows a diffusion path integrating all channels and pores

We investigated the impact of pores on the propagation of Ca^{2+} signals on either side of the NE, considering calcium release channels on both sides of the NE, Fig 1. We studied the release of Ca^{2+} from a channel, N_1, and the activation of another channel, N_2, on the nucleosolic side of the NE. We incorporated channels, C_1 and C_2, on the cytosolic side of the NE. The propagation of a Ca^{2+} burst across both sides of the NE for different pore distributions and a given channel arrangement is shown in Fig 1. As in the main text, the inner nuclear membrane is characterised by a lower diffusion constant D. Pores clustered around the channels C_1 and C_2 are located far from the diffusion range of the channel N_1, which results in the firing of N_1 being unable to trigger further release events, Fig 1A (crossed beige arrow). However, when pores were clustered around channels N_1 and N_2 the situation changed. In Fig 1B the arrows follow Ca^{2+} propagation from its release event in N_1 as it crosses the NE through the nearby pore to activate channel C_1 where it spreads to activate C_2, reenters the nucleoplasm and finally triggers the firing of channel N_2.

It is the presence of Ca^{2+} release units on both sides of the NE that enables alternative paths, from channel to channel through nuclear pores, to be exploited. Diffusion across the NE would be expected to equilibrate Ca^{2+} concentration on both sides of the NE, thus driving Ca^{2+} transmission to a halt. Moreover, by doubling the space that Ca^{2+} can potentially diffuse in, a permeable nuclear envelope could reduce the local Ca^{2+} concentration, preventing CICR activation. Channels C_1 and C_2, however, act as Ca^{2+} amplifying units and allow for concentrations differences to be maintained. The arising concentration differences drive the reentry of Ca^{2+} back into the nucleus and trigger the firing of the second nuclear channel N_2, as shown in

Fig 1B.

Thus, pores may contribute to the robustness of Ca^{2+} signalling and allow for firing to proceed in conditions where CICR would otherwise fail. This also suggests a reason for the existence of signalling machinery to be on both sides of the NE, such as during plant symbiosis [1, 2] where all the Ca^{2+} decoding elements seem to be located inside the nucleus and the existence of a Ca^{2+} signalling machinery on the outer side of the membrane may seem superfluous. This coupled signalling machinery might offer an advantage to cells that need to maintain oscillations in a fluctuating environment for long durations. For instance, during legume symbiosis a minimum number of sustained nuclear Ca^{2+} spikes is required to elicit a response [3]. The role of a Ca^{2+} signalling machinery on the outer nuclear membrane may be to sustain nucleosolic Ca^{2+} oscillations, facilitated by pores, elements of which are essential for nuclear Ca^{2+} signalling during legume symbiosis [4, 5].

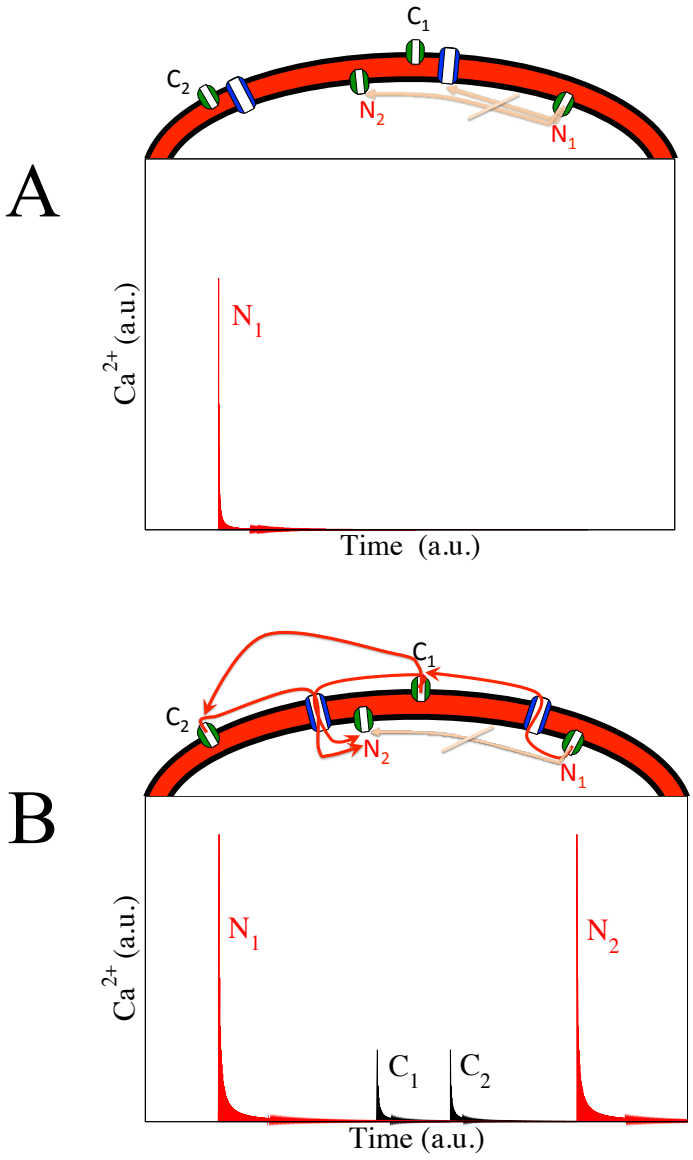

Figure 1: **Ca^{2+} diffusion through pores may create additional activation paths, permitting the exploitation of more favourable propagation conditions on the other side.** We cluster 4 pores around either the outer nuclear membrane (ONM) channels C_1 and C_2 (upper panel) or the inner nuclear membrane (INM) channels N_1 and N_2 (lower panel). The simplified scheme illustrates how Ca^{2+} spreads. The crossed beige arrows represent the disruption of Ca^{2+} spread caused by a low diffusion constant, while the red arrows show the spreading of Ca^{2+} as it activates channels leading them to fire in sequence as seen in the main plots that show Ca^{2+} levels surrounding channels (arbitrary units). Diffusion on the nuclear surface is D_N=5 $\mu m^2/s$, and on the cytosolic side is D_C=20 $\mu m^2/s$

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