Supporting Information

Wu et al. 10.1073/pnas.1221538110

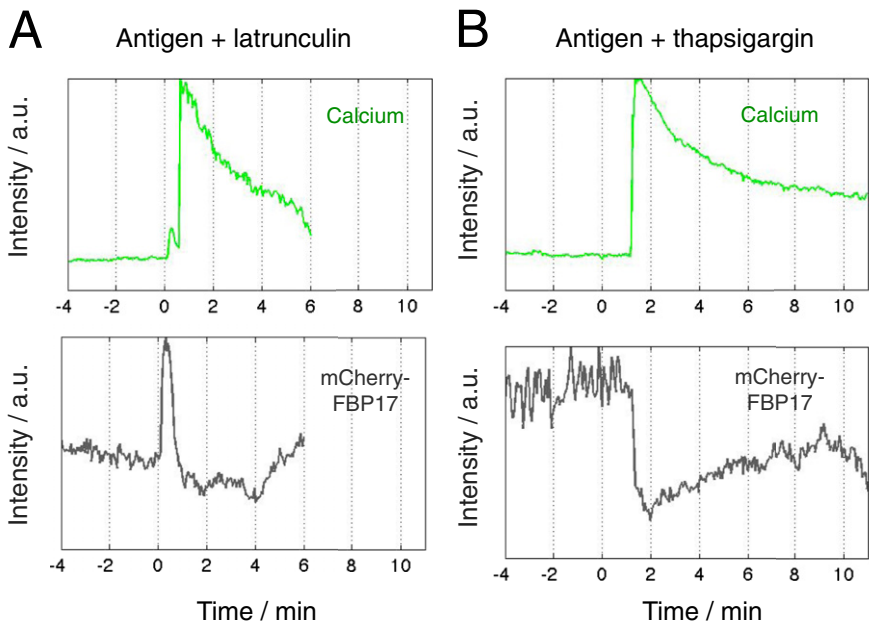


Fig. S1. Standing oscillations of FBP17 require a normal calcium homeostasis and actin dynamics. (*A*) Addition of 12.5 μM latrunculin B together with antigen induced one transient pulse of FBP17 recruitment to the membrane without oscillations. (*B*) Addition of 2 μM thapsigargin together with antigen stimulation abolished both calcium oscillations and FBP17 oscillations. For both *A* and *B*, time 0 is the time of antigen addition.

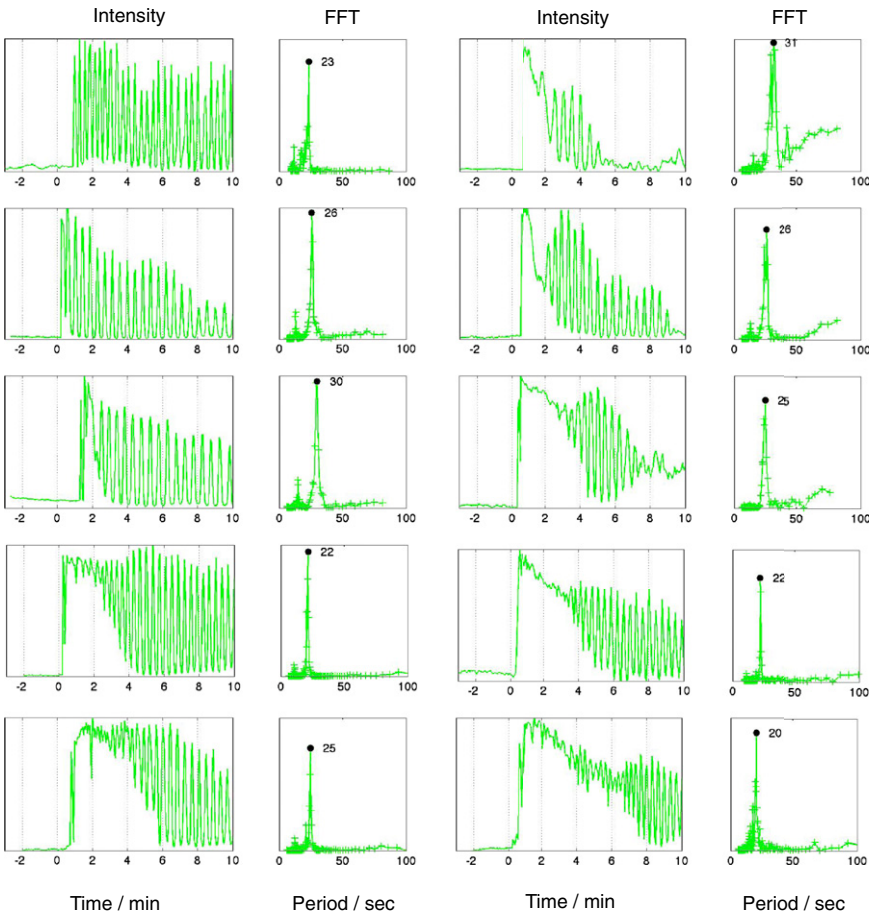


Fig. S2. Additional examples of antigen stimulation-induced cytosolic calcium oscillations. Note that the time of onset of calcium oscillations relative to the stimulus, and the duration of calcium oscillations, are heterogeneous. Time 0 is the time of antigen addition.

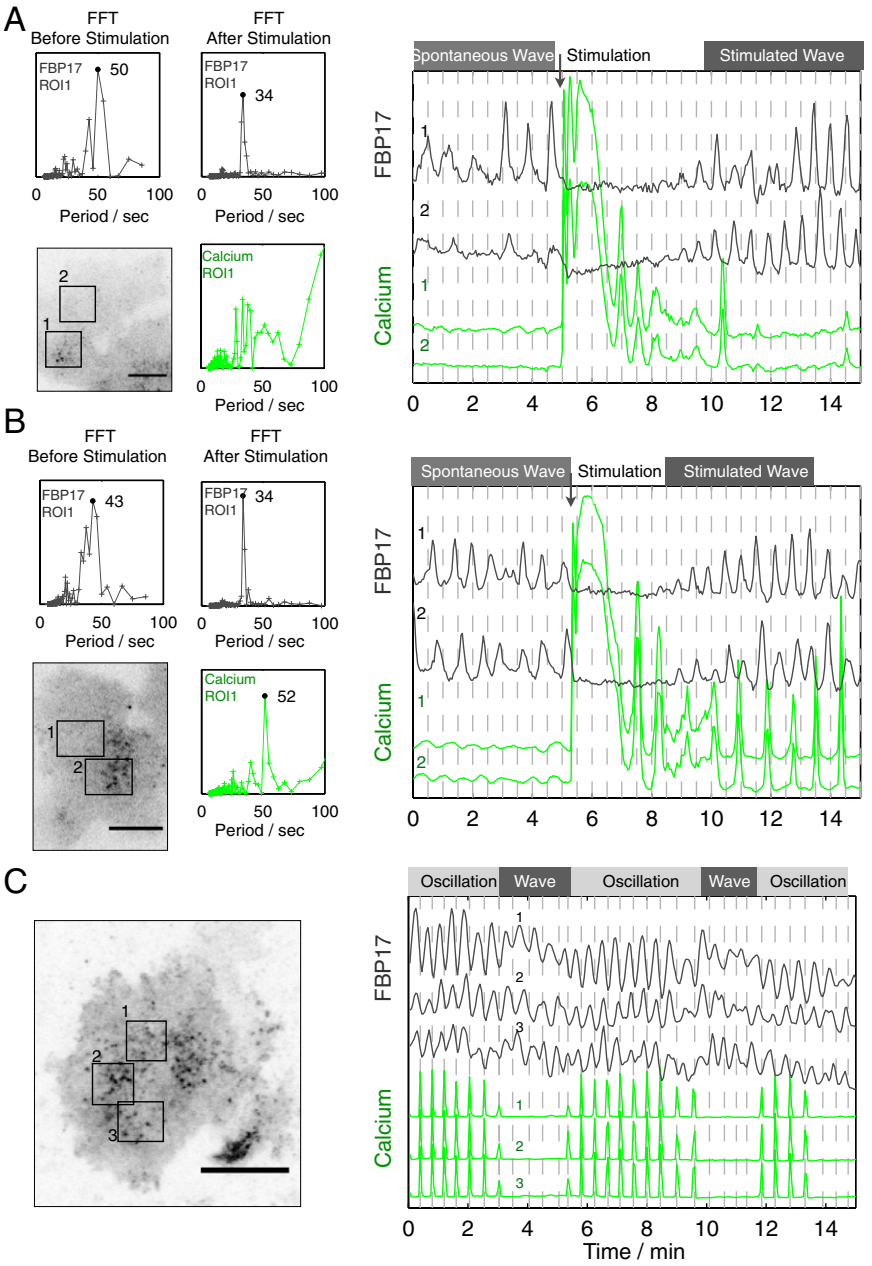
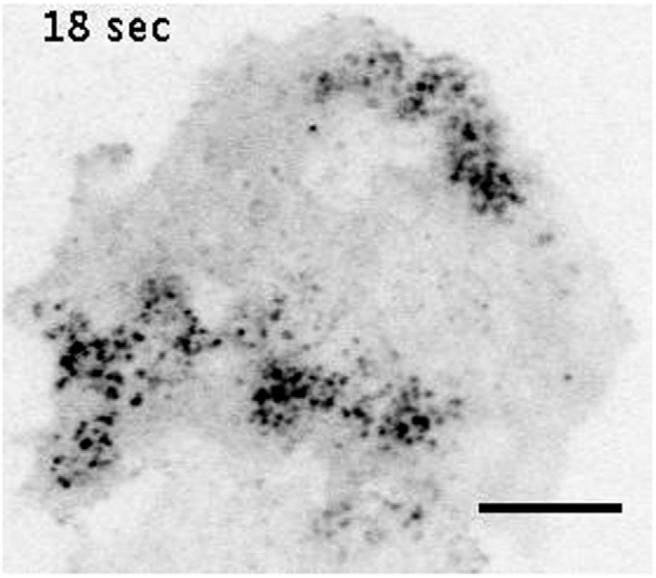
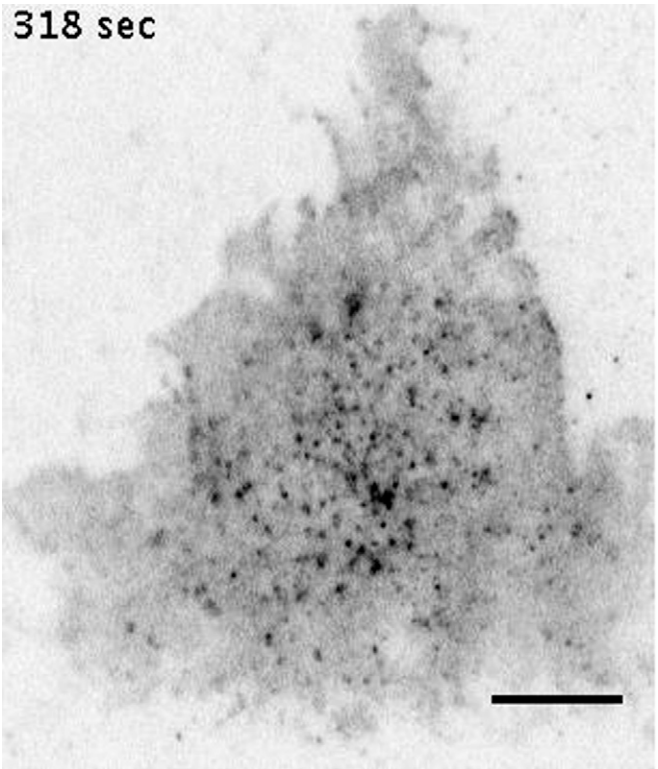


Fig. S3. FBP17 waves (spontaneous or stimulated) are dissociated from calcium fluctuations. (*A* and *B*) Cells displaying spontaneous mCherry-FBP17 waves in the absence of calcium oscillations were stimulated. FBP17 waves (mismatching phases for the two different ROIs outlined in the image on the *Right*) stopped transiently during the initial elevation of calcium level and then resumed without calcium oscillations (*A*) or with weak calcium oscillations (relative to the initial elevation) whose frequency did not match that of FBP17 waves (*B*). (*Left*) Frequency analysis of FBP17 (before and after stimulation) and calcium (after stimulation) are shown. (*C*) Switches from traveling waves to standing oscillations and vice versa during the poststimulus period were coupled with the onset and disappearance of calcium oscillations, respectively, as shown by the analysis of the three ROIs outlined in the image (Movie S7). Vertical dashed lines are evenly spaced grid lines in the graphs of *A* and *B*, but indicate peaks of calcium spikes, which are also valleys of FBP17 signal during calcium oscillation in *C*. (Scale bar: 10 μm.)



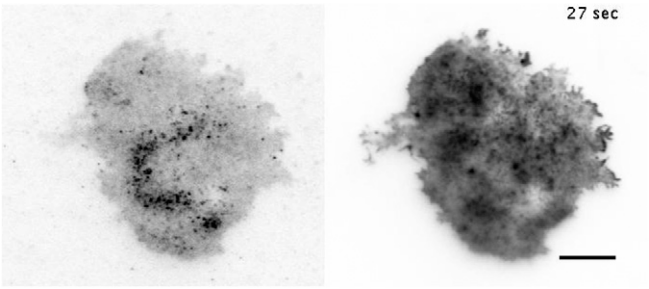
Movie S1. Total internal reflection fluorescence microscopy (TIRFM) movie of mast cell showing dramatic recurring traveling waves of mCherry-FBP17. The movie was acquired at 3-s intervals starting 100 min after antigen stimulation and is played at 20 frames per second (60× real time). (Scale bar: 10 μm.)

Movie S1



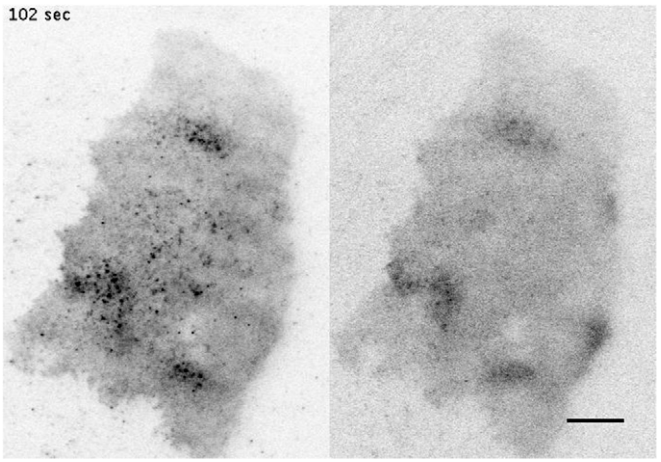
Movie S2. TIRFM movie of mast cell showing oscillatory recruitment of mCherry-FBP17. The movie was acquired at 3-s intervals starting 40 min after antigen stimulation and is played at 20 frames per second (60× real time). (Scale bar: 10 μm.)

Movie S2



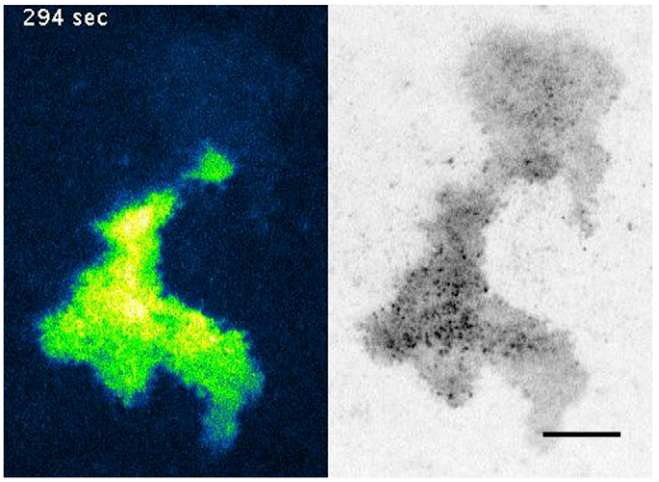
Movie S3. Recurring traveling waves of mCherry-FBP17 (*Left*) and LifeAct-EGFP (*Right*) induced by antigen stimulation were abolished by the addition of latrunculin B at frame 223. The movie was acquired at 3-s intervals and is played at 30 frames per second (90× real time). (Scale bar: 10 μm.)

Movie S3



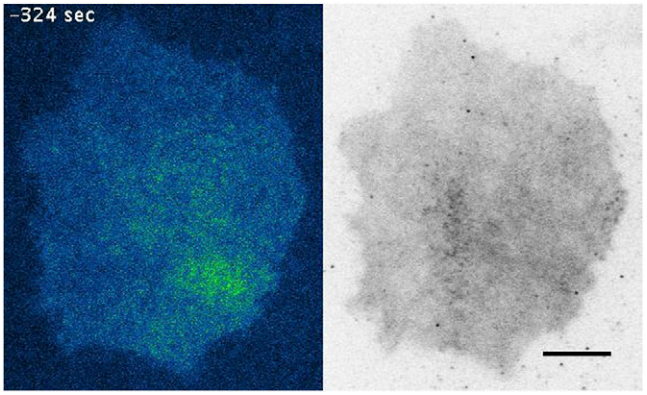
Movie S4. Two-color TIRFM movie of mast cell showing traveling waves of coexpressed mCherry-FBP17 (*Left*) and Cdc42 activity probe CBD-EGFP (*Right*). Note that waves precisely overlap both spatially and temporally. The movie was acquired at 2-s intervals starting 100 min after antigen stimulation and is played at 20 frames per second (40× real time). (Scale bar: 10 μm.)

Movie S4



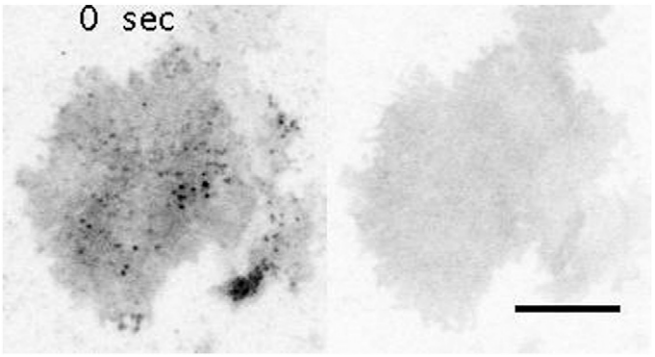
Movie S5. Two-color TIRFM movie of mast cell expressing the calcium sensor GCAMP3 (*Left*) and mCherry-FBP17 (*Right*). Antigen stimulation elicited dramatic calcium and FBP17 oscillations. Antigen was added at frame 121. The sample was imaged for 400 frames at 3-s intervals, and the movie is played at 40 frames per second (120× real time). An inverted grayscale lookup table was used in the case of FBP17, and fluorescence is shown in black. (Scale bar: 10 μm.)

Movie S5



Movie S6. Two-color TIRFM movie of mast cell expressing the calcium sensor GCAMP3 (*Left*) and mCherry-FBP17 (*Right*). Stimulation induced a conversion of mCherry-FBP17 fluorescence fluctuations from spontaneously occurring waves to standing oscillations that have the same frequency as robust calcium oscillations. Antigen was added at frame 108. The sample was imaged at 3-s intervals for 300 frames, and the movie is played at 20 frames per second (60× real time). (Scale bar: 10 μm.)

Movie S6



Movie S7. Two-color TIRFM movie of mast cell expressing the calcium sensor GCAMP3 (*Right*) and mCherry-FBP17 (*Left*). Note spontaneous conversion of mCherry-FBP17 fluctuations from oscillations to waves during the poststimulation period. The transition from oscillations to waves correlates with the interruption of calcium oscillations. The movie was acquired at 3-s intervals 35 min after stimulation and is played at 20 frames per second (60× real time). (Scale bar: 10 μm.)

Movie S7