SUPPLEMENTAL MATERIALS

Supplemental Figure S1. Ca^{2+} mobilization monitored in RBL cells waves with genetically encoded GCaMP2 (n=80), as compared to indicator dyes Fluo-4 (n=40) and Fluo-5F (n=17). A) Images of cells sensitized with anti-DNP IgE, expressing GCaMP2 (top) or loaded with Fluo-4 (middle) or Fluo-5F (bottom), and stimulated with a puff of 1.7 μg/ml DNP-BSA. Right panels show time line analysis of the stimulated cells and changes in Ca^{2+} concentration occurring over time in the segments defined in the left panels. Changes in Ca^{2+} concentration are indicated by relative changes in brightness, where brighter colors represent higher Ca^{2+} concentrations. B) Percentage of indicator-labeled cells responding to stimulus with Ca^{2+} waves, averaged over multiple experiments ± SD; *$P < 0.05$ vs. cells expressing GCaMP2. C) Average velocity of Ca^{2+} waves measured with specified Ca^{2+} indicator. F) Average number of oscillations within 2 min after Ag stimulation. The first oscillation corresponds to the originating Ca^{2+} wave (dotted line). Error bars correspond to standard error of the mean (SEM).

Supplemental Figure S2. Sensitivity of Ca^{2+} responses to different doses of Ag: 1.7 ng/ml (n=25); 17 ng/ml (n=26); 170 ng/ml (n=26); 1.7 μg/ml (n=80). RBL cells expressing GCaMP2 were sensitized with anti-DNP IgE and stimulated with a puff from a pipette containing indicated concentrations of Ag. A) Percentage of cells responding with measurable Ca^{2+} elevation upon stimulation with Ag. B) Average velocity of Ca^{2+} wave. C) Bar graph showing percentage of cells responding to Ag stimulus with Ca^{2+} waves. Bar height shows total % waves; orange portion represents % waves originating in protrusions. D) Average lag time after Ag stimulation for initiation of Ca^{2+} wave. E) Average number of oscillations within 2 min after Ag stimulation. The first oscillation corresponds to the originating Ca^{2+} wave (dotted line). F) Percentage of

cells responding with Ca^{2+} puffs. Error bars correspond to SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplemental Figure S3. Quantification of TRPC1 knock-down by shRNA expression. Western blot analysis of whole cell lysates from of $1.5x10^6$ cells expressing shRNA targeting TRPC1 or a mock sequence. A) Expression levels of TRPC1 in knockdown cells or control cells expressing the mock shRNA sequence. B) Densitometry quantification of A, showing 50% reduction in TRPC1 expression (band a; blue bar) after shRNA treatment compared to control cells (band a; green bar). Protein expression levels as seen in nonspecific Ab labeling (band b) were unchanged by shRNA plasmids.

Supplemental Figure S4. Identification of rat BMMC by labeling with Alexa488-IgE and mAb AA4. Rat stem cells differentiated with IL3 and stem cell factor were plated overnight in MatTek wells and labeled as described in Materials and Methods. Representative field shows two cells, each labeled with Alexa488-IgE (green) and the mast cell-specific anti-ganglioside AA4 (red). Note polarized morphologies that are common for these cells after several hours on glass surfaces. Scale bar = 10 μm.

Supplementary movie 1. Ag stimulated Ca^{2+} waves in RBL-2H3 cells. Ca^{2+} wave in RBL cell expressing GCaMP2 sensitized with anti-DNP IgE and stimulated with a puff from a pipette containing 1.7μg/ml DNP-BSA (as depicted in Figure 1). Extracellular buffer is BSS with 2mM Ca^{2+} (as in Figure 2A, top panel). Note wave initiates from extended cellular protrusion. Image rate = 21 Hz (47.15 ms/frame). Movie =X3 real time.

Supplementary movie 2. Ca^{2+} waves in the absence of extracellular Ca^{2+}. Ca^{2+} wave in RBL cell expressing GCaMP2 sensitized with anti-DNP IgE and stimulated with a puff from a pipette containing 1.7μg/ml DNP-BSA. Extracellular buffer is BSS without Ca^{2+} (as in Figure 2A, bottom panel). Note wave initiates from cell body. Image rate = 17 Hz (59.6 ms/frame). Movie =X3 real time.

Fig S1

Ca^{2+} mobilization monitored in RBL cells waves with genetically encoded GCaMP2.

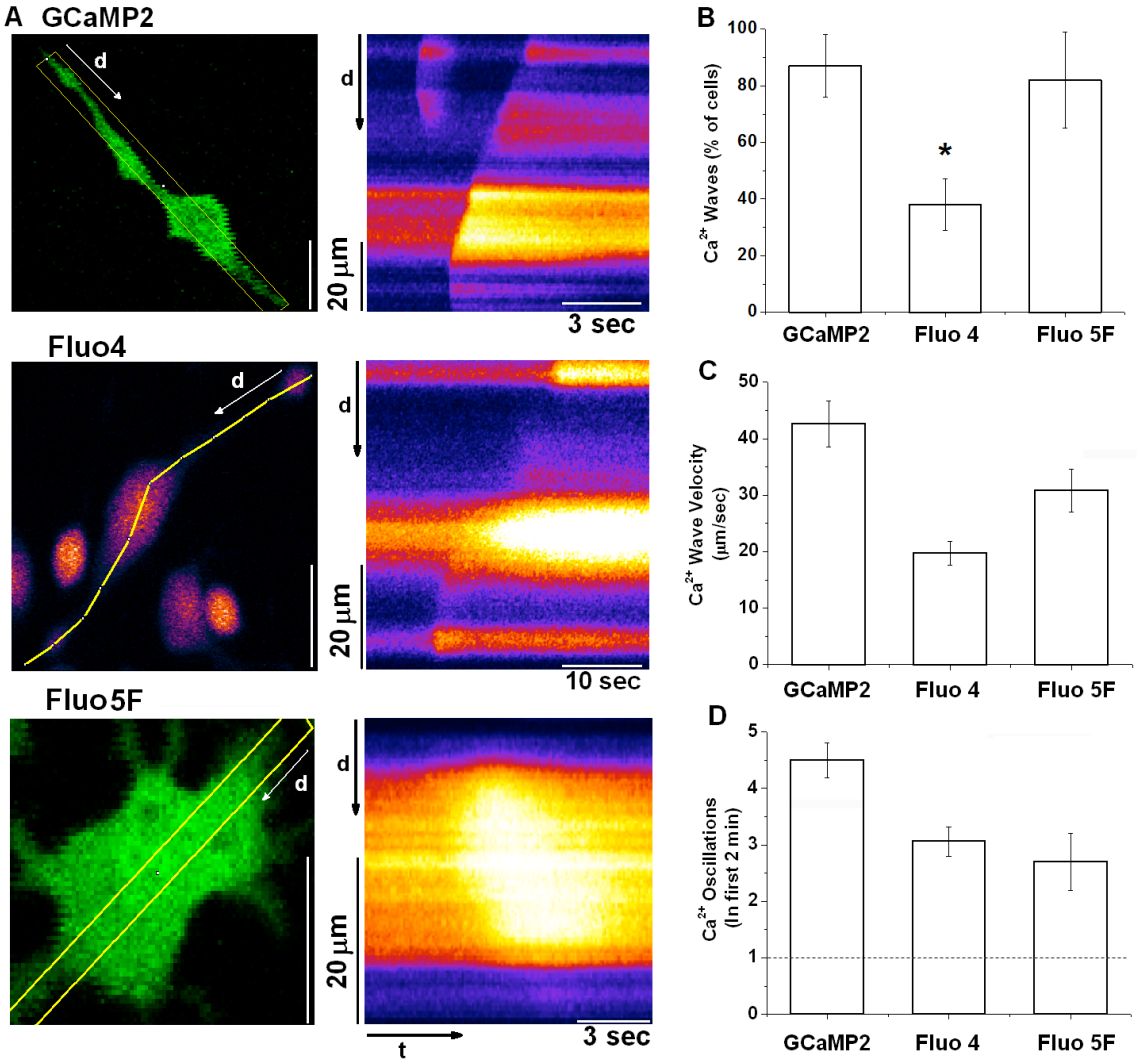

Fig S2

Sensitivity of Ca^{2+} responses to different doses of Ag.

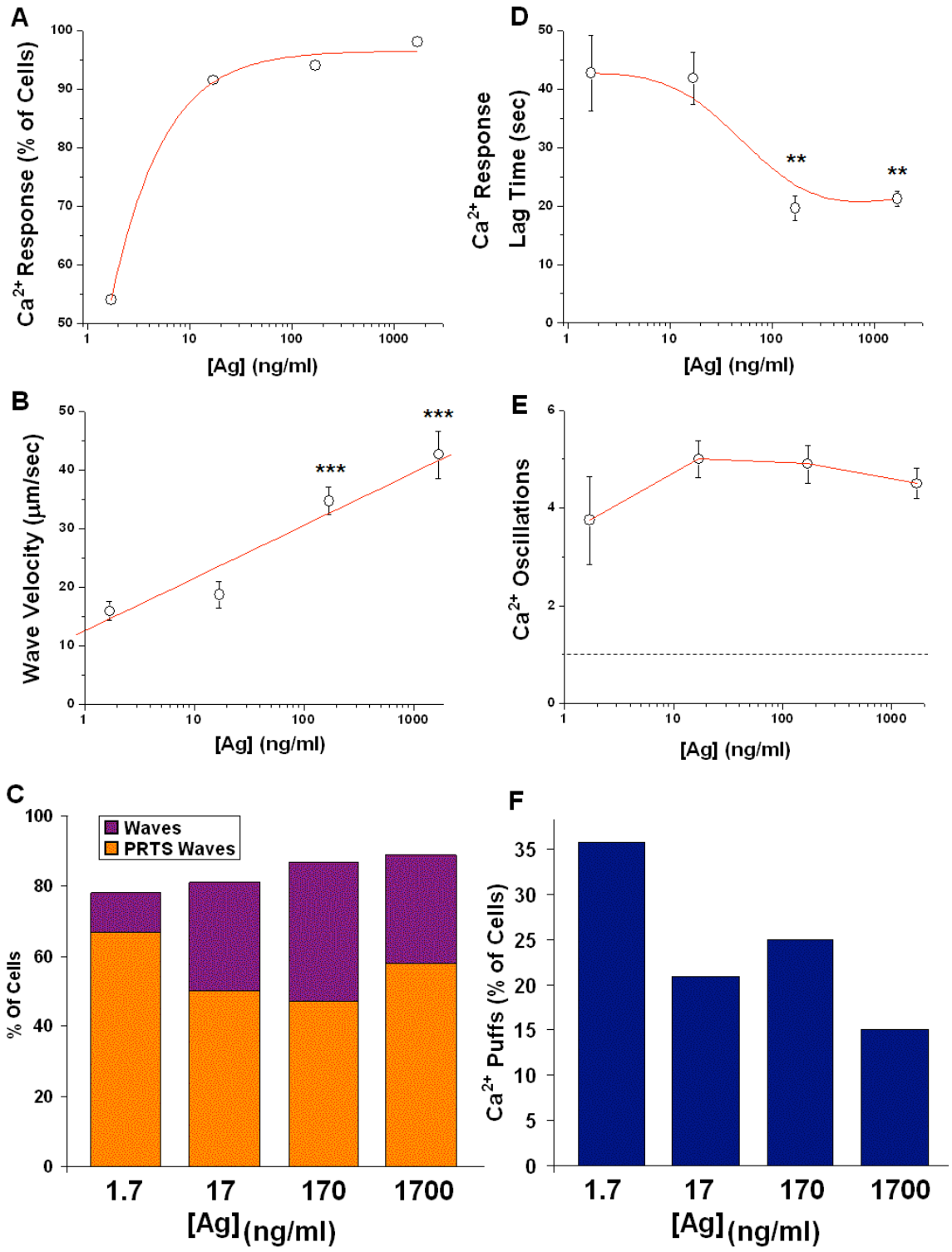

Fig S3

Quantification of TRPC1 knock-down by shRNA expression.

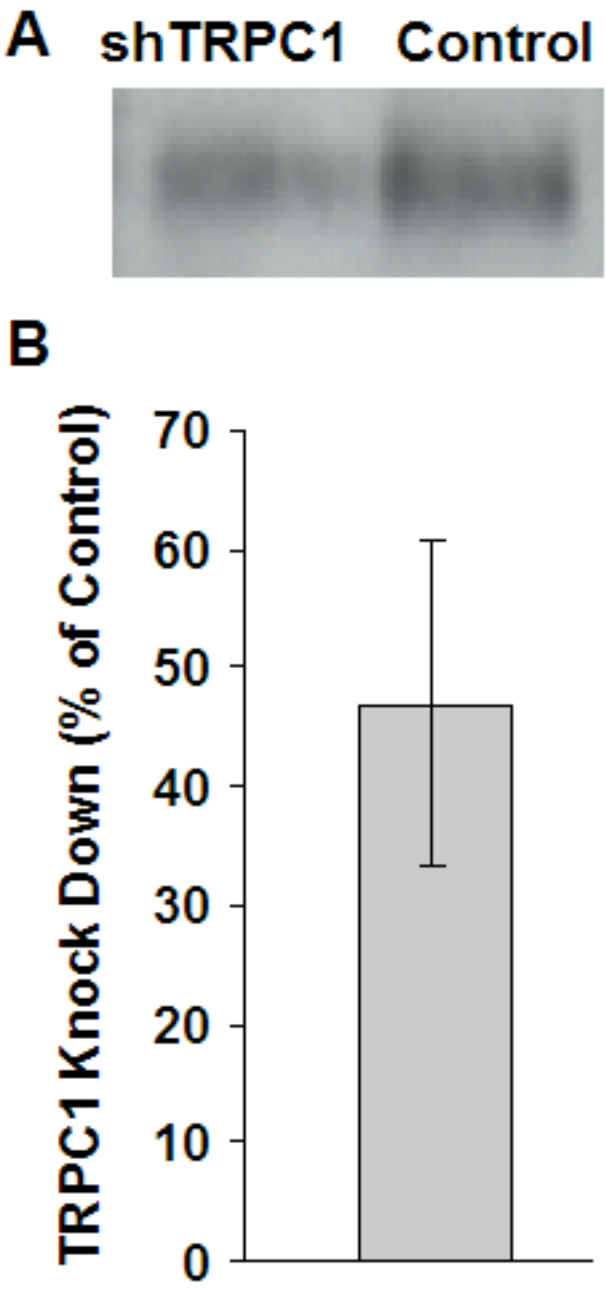

Fig S4

Identification of rat BMMC by labeling with Alexa488-IgE (green) and mAb AA4 (red)