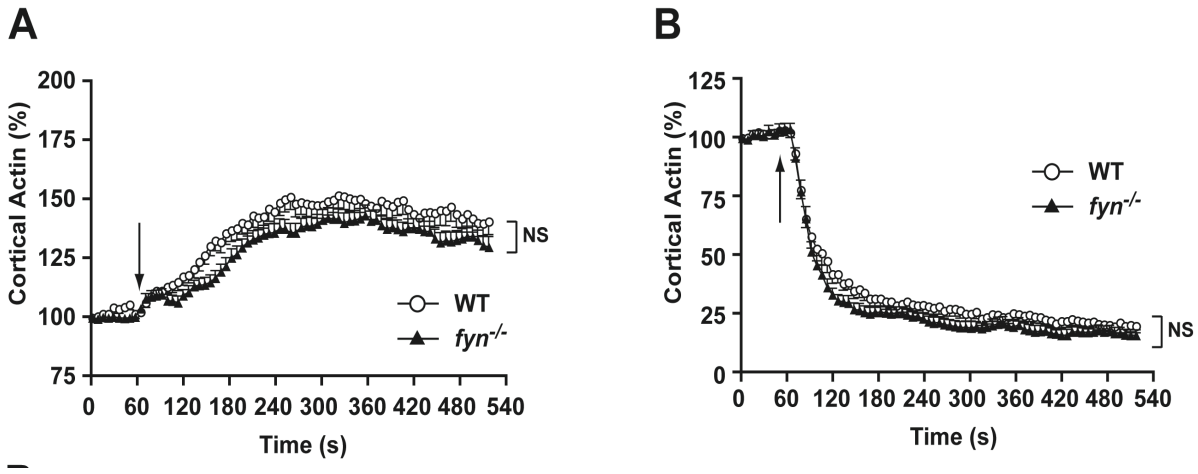
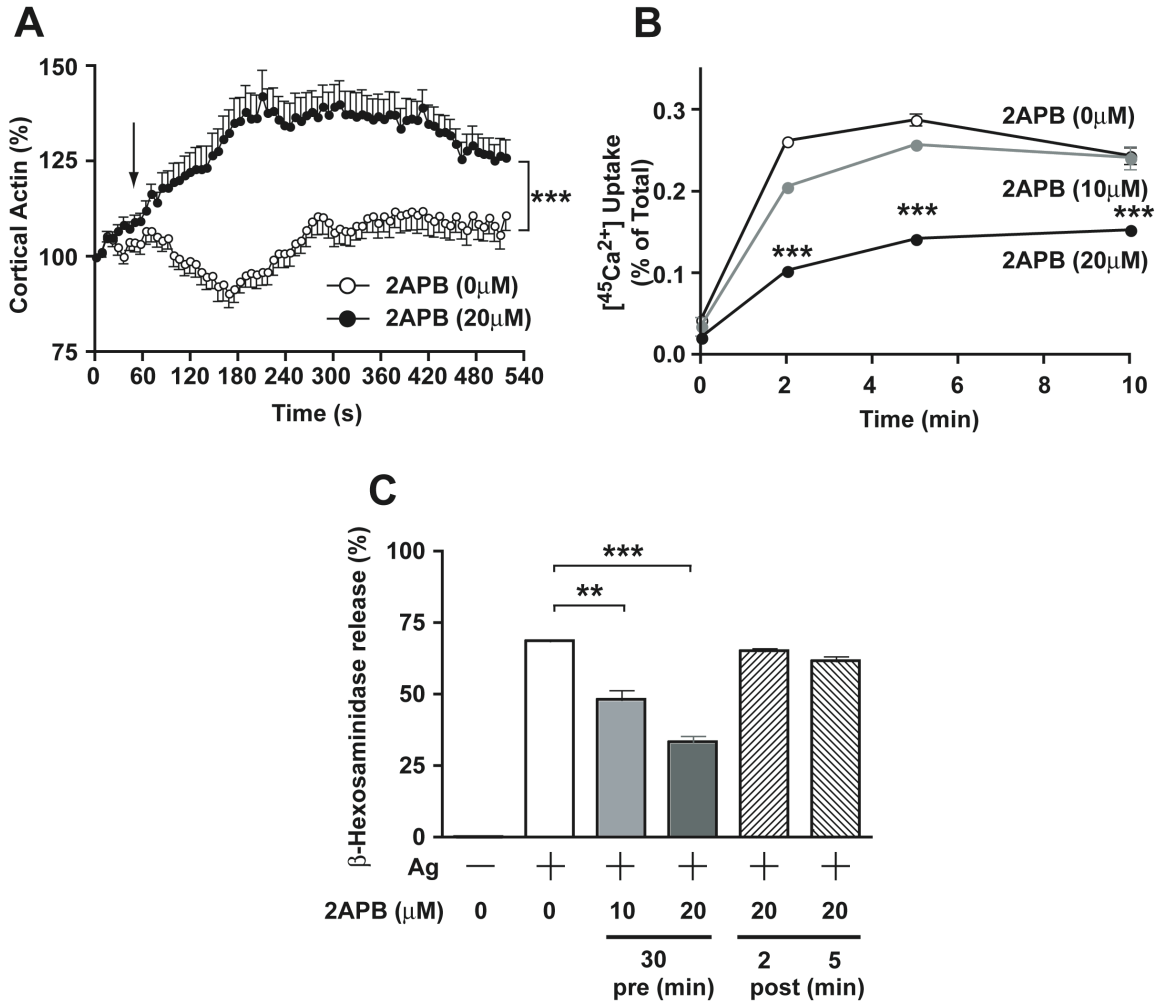
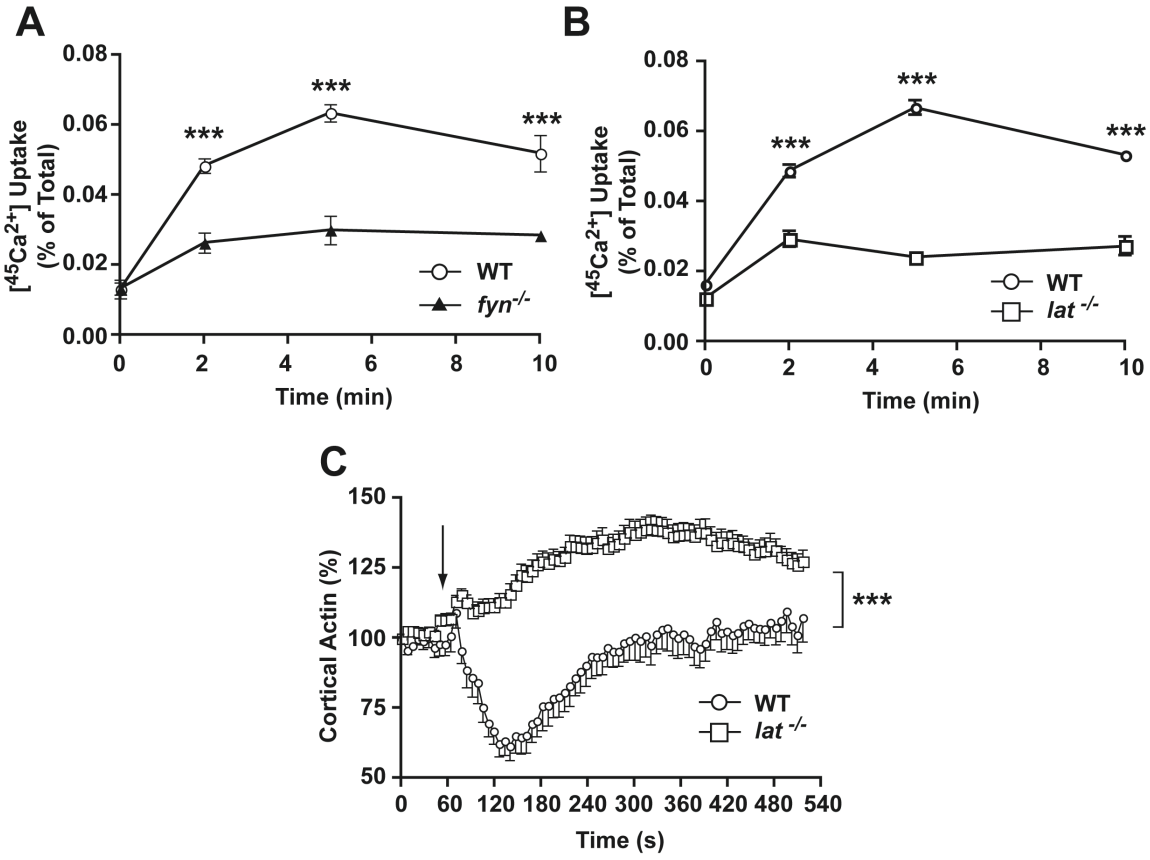


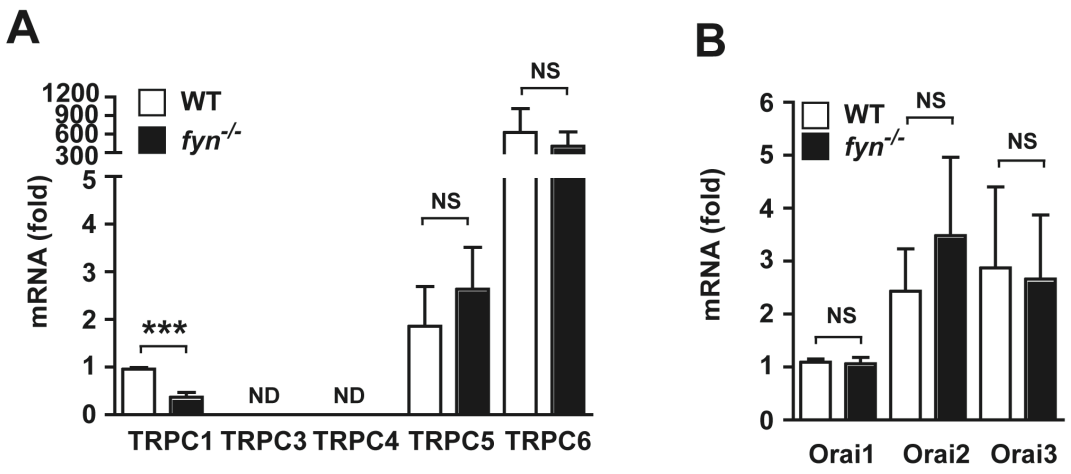
Supplemental Figure 1. Actin depolymerization is dependent on (extracellular) calcium. (A) WT or Fyn-null MCs were transfected with actin-YFP and sensitized with DNP-specific IgE for 3 h and activated in the absence of extracellular calcium with Ag (DNP-HSA). Changes in actin dynamics were measured as described in materials and methods. An averaged response is shown in the graph (35-38 cells were monitored in total). (B) WT or Fyn-null MCs were activated by exposure to the ionomycin (1 mM) in the presence of 1.8mM Ca^{2+} in the medium. An averaged response is shown in the graph (20-23 cells were monitored in total). For both A and B, an arrow indicates the time of Ag or ionomycin addition, respectively. Data is mean ± SE from at least 4 individual experiments.



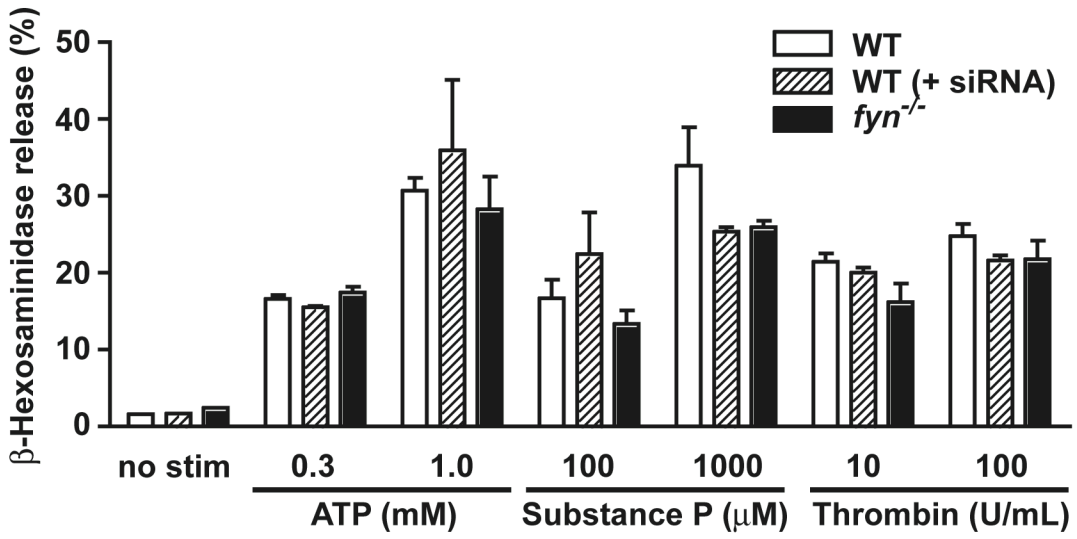
Supplemental Figure 2. 2-aminoethoxydiphenyl borate (2-APB), an inhibitor of store-operated calcium entry, prevents influx of calcium, cortical F-actin depolymerization, and MC degranulation. (A) Pretreatment of WT MCs with 2-APB (20 μM) caused a marked inhibition of F-actin depolymerization. The arrow indicates the time of Ag addition. The mean response (± SE) is shown in the graph (25-28 cells were monitored in total). Statistical significance relative to WT (without treatment) was $^{***}p < 0.001$. (B) Pretreatment of WT MCs with 2-APB (10 or 20 μM) impairs $^{45}Ca^{2+}$ uptake. One representative of 3 independent experiments is shown. Data is mean ± SE of quadruplicate samples. Statistical significance relative to WT MCs (without treatment) was $^{***}p < 0.001$. (C) Pretreatment of WT MCs with 2-APB (10 or 20 μM) inhibits mast cell degranulation in a dose dependent manner. No inhibition was seen when MCs were treated with 2-APB at 2 or 5 min post-stimulation (data not shown). Data is the mean ± SE and statistical significance relative to WT MCs (without treatment) was $^{**}p < 0.01$, $^{***}p < 0.001$. One representative of 3 experiments is shown.



Supplemental Figure 3. LAT-null MCs (which are known to have defective calcium responses) mimic the defective Ag-induced cortical F-actin depolymerization and degranulation of Fyn-null MCs. (A) Fyn-null MCs are defective in Ag-dependent $^{45}Ca^{2+}$ influx relative to WT MCs. Data is the mean ± SE of triplicate samples with a statistical significance relative to WT of $^{***}p < 0.001$. One representative of 3 experiments is shown. (B) LAT-null MCs are impaired in Ag-dependent $^{45}Ca^{2+}$ influx. Data is the mean ± SE of triplicate samples with a statistical significance relative to WT MCs of $^{***}p < 0.001$. (C) LAT-null MCs have an impairment in cortical F-actin depolymerization. The arrow indicates the time of Ag addition. The mean response (± SE) is shown in the graph (18-36 cells were monitored in total).



Supplemental Figure 4. Real-time quantitation of mRNA expression for TRPC and Orai isoforms reveals the reduced expression of TRPC1 in Fyn-null MCs. (A) mRNA levels of TRPC isoforms were determined by real-time PCR using specific probes for each isoform. Data was normalized to GAPDH mRNA levels and expressed as fold expression relative to the TRPC1 levels expressed in WT MCs. Statistical significance was $^{***}p < 0.001$. (B) Expression levels of Orai mRNA isoforms were determined by real-time PCR. Data was treated as in (A). Histograms are representative of at least 4 independent experiments.



Supplemental Figure 5. Stimulation of TRPC1 siRNA-silenced WT or Fyn-null MCs with ATP, substance P, or thrombin reveals normal degranulation. Degranulation responses observed in WT, TRPC1 siRNA-silenced WT (WT (+ siRNA)), and Fyn-null MCs. MCs were stimulated by ATP, substance P, and thrombin at the indicated concentrations. For some stimuli (substance P-1000 μM) a trend for reduced degranulation was observed. However, no statistical significant difference was achieved with any stimulus. One representative of three experiments is shown. Data is mean ± SE.