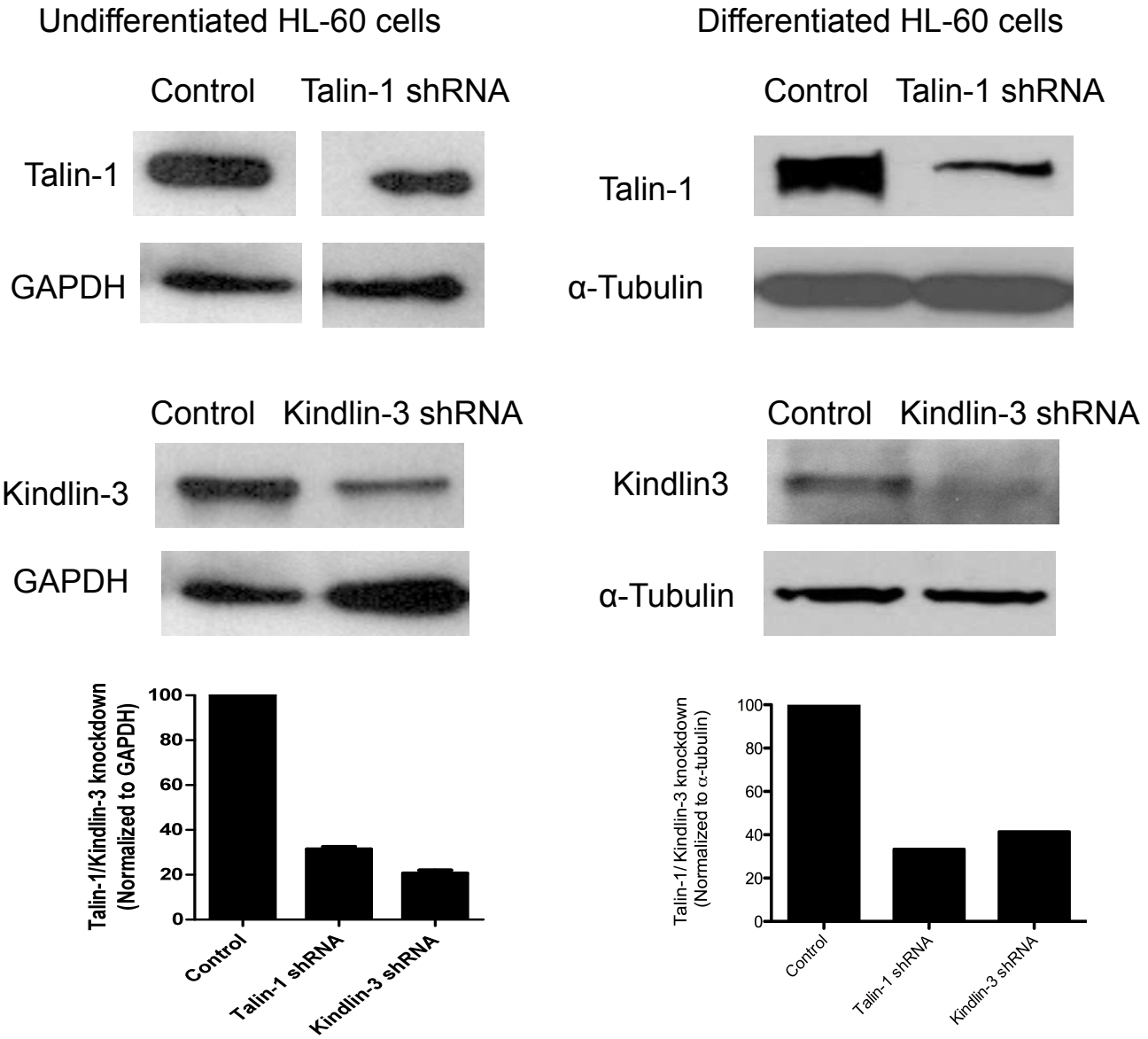
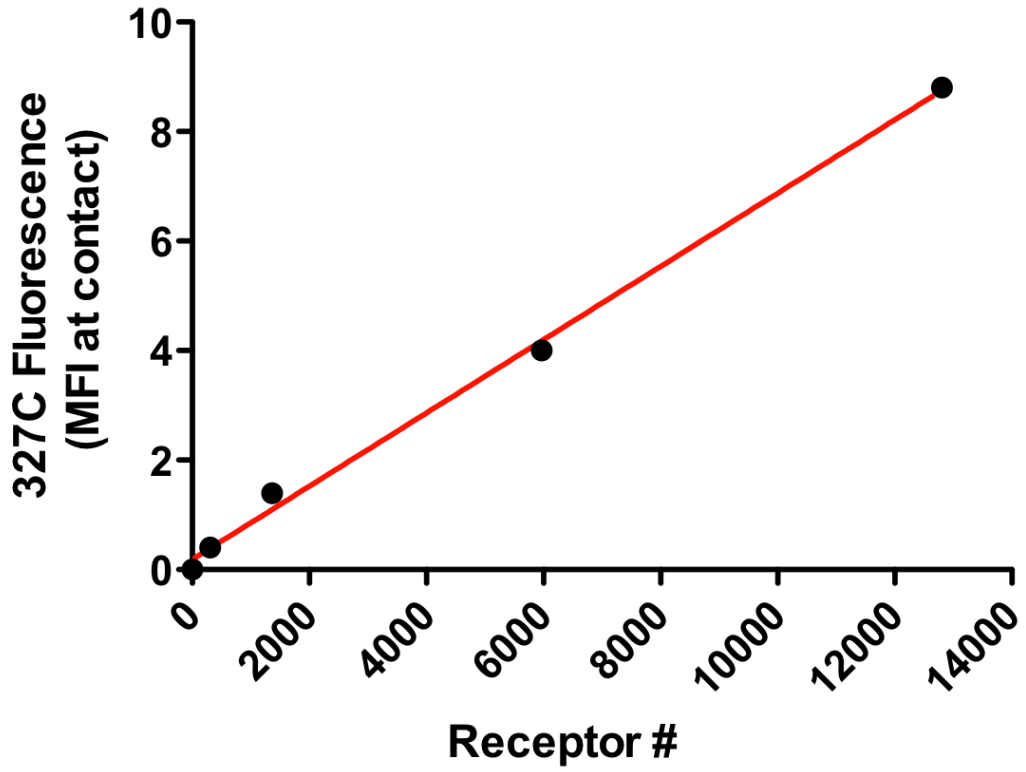
Supplementary Figure 1



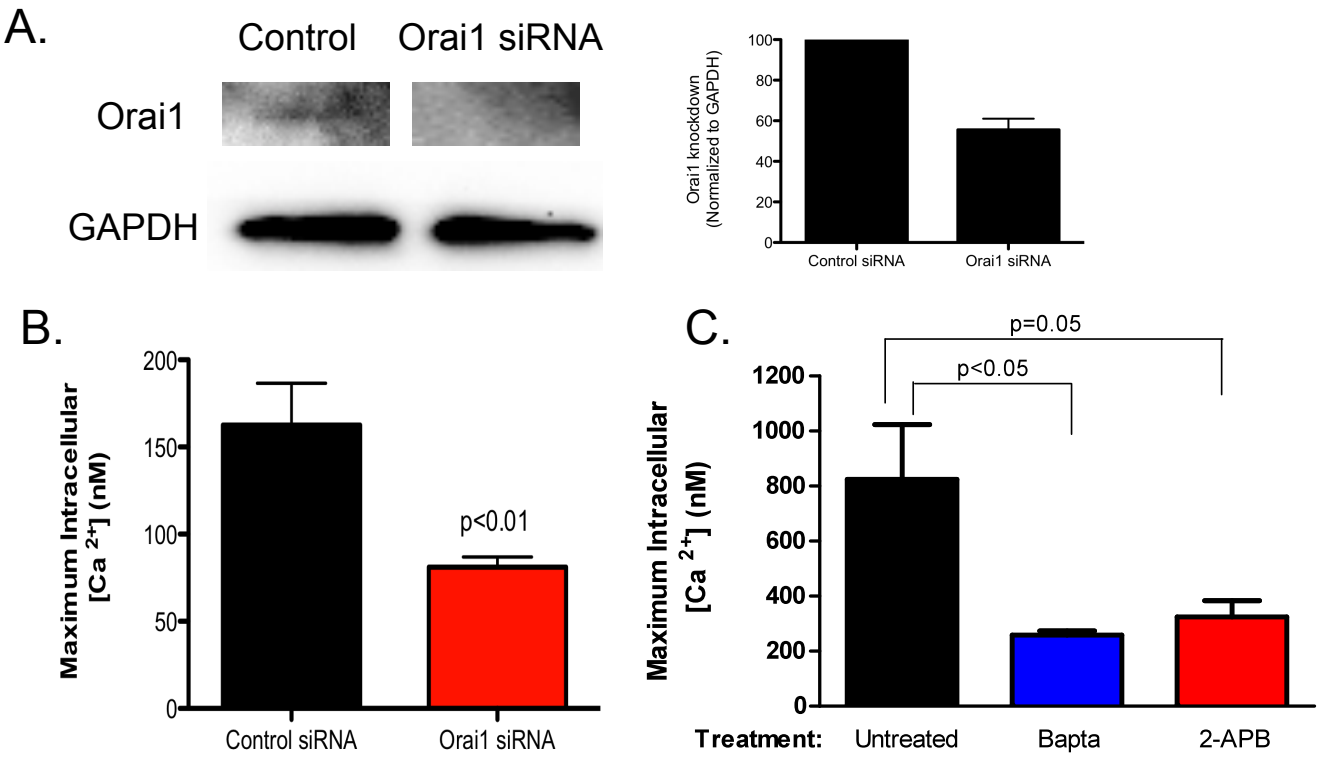
Supplementary figure 1: *Talin-1 and Kindlin-3 knockdown by lentiviral transfection of shRNA in HL-60 cells.* Control, Talin-1 and Kindlin-3 shRNA transfected cells were lysed before and after differentiation with 1.3% DMSO, 1mg protein lysate was run on SDS page and Talin-1 and Kindlin-3 protein was detected by western blot. Talin-1 and Kindlin-3 protein expression was normalized to GAPDH expression and revealed a 70% and 80% knockdown respectively. For blot images of Talin-1 knockdown in Talin-1 shRNA cells, the two blots are from the same lane.

Supplementary Figure 2



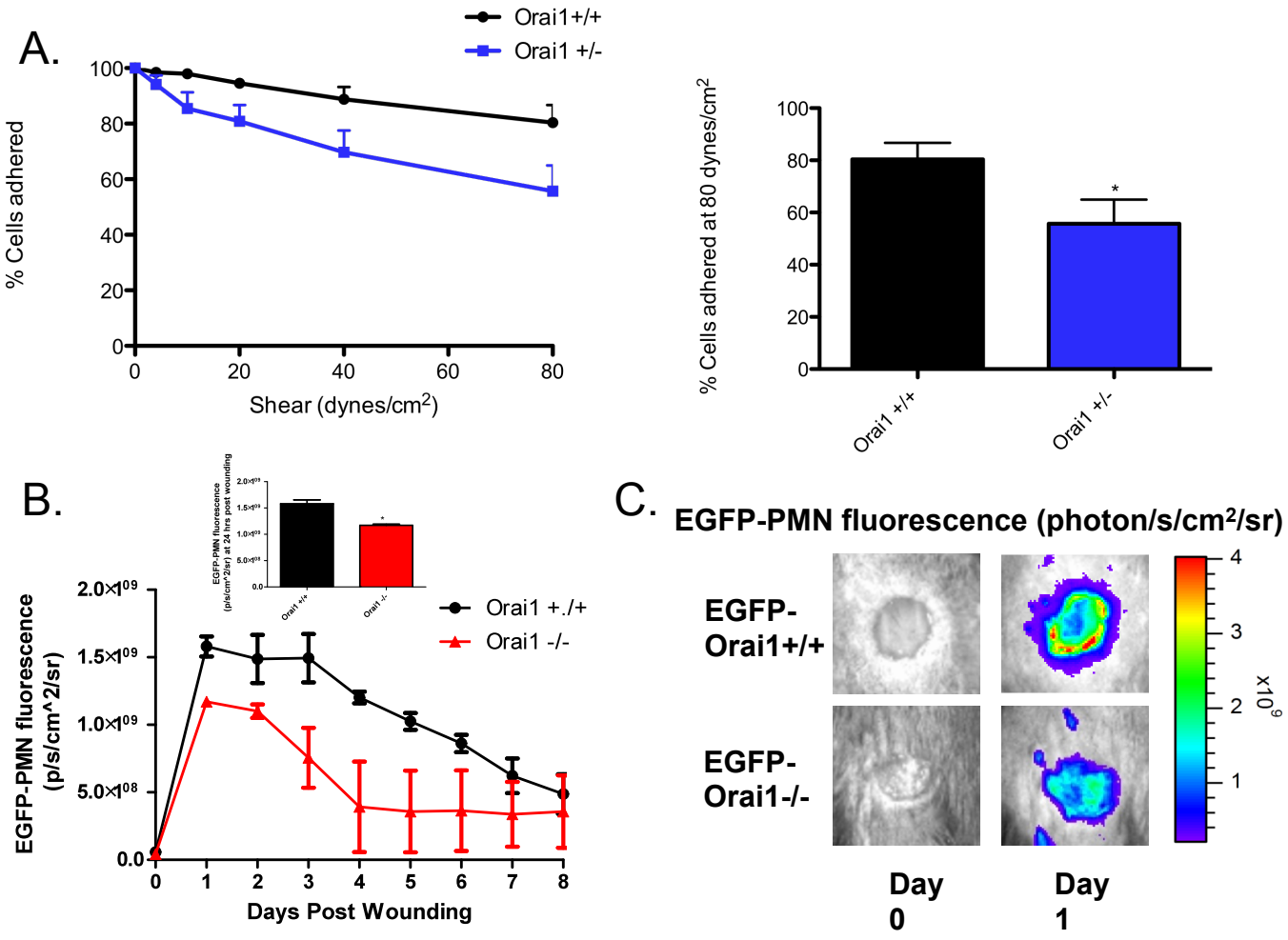
Supplementary Figure 2: *Correlating 327C MFI with antibody binding sites on beads at the contact site.* 7.65 μm beads with increasing antibody binding sites were incubated with 327C mAb-Alexa-488 for 30 minutes and allowed to settle on a glass substrate. 327C fluorescence was imaged by TIRF at settings equivalent to other experiments measuring 327C expression and mean fluorescence intensity was quantified for each bead condition. 327C MFI at contact was plotted against average receptor numbers at contact site to obtain a linear relationship.

Supplementary Figure 3



Supplementary Figure 3 *Orai1 knockdown, Bapta and 2-APB effectively inhibit intracellular calcium.* A. HL-60 cells transfected with control or Orai1 siRNA and differentiated over 3 days with 1.3% DMSO were lysed and 1mg protein lysate was run on SDS page and Orai1 protein was detected by western blot. Orai1 protein expression was normalized to GAPDH expression and revealed a 50% knockdown. For blot images of Orai1 knockdown the two blots are from the same lane. B and C. PMN were isolated from whole blood or HL-60 cells were transfected with control and Orai1 siRNA and differentiated to form neutrophil like cells. Cells were labeled with Fura-2AM, PMN were treated with 50uM Bapta and 100uM 2-APB and all cells were perfused over an ICAM-1+240Q substrate and intracellular calcium flux was measured over 2 minutes. To ensure LFA-1 dependent adhesion, cells were pretreated with anti-Mac-1 mAb ICRF44. Maximum calcium levels were plotted and data shown is mean+/- SEM from 3 independent experiments.

Supplementary Figure 4



Supplementary Figure 4: *Orai1 is required for PMN adhesion strengthening and migration to wound sites*. A. Bone marrow neutrophils were isolated from Orai1 $^{+/+}$ and Orai1 $^{+/-}$ mice, activated with Mn^{2+} and allowed to settle over an ICAM-1 coated substrate. Shear was ramped from 0,4, 10, 20, 40, 80 dynes/cm^2 at 30 second intervals and number of cells remaining bound were counted and plotted. To ensure LFA-1 dependent adhesion, cells were pretreated with anti-Mac-1 M1/70 for all experiments. B and C. Skin wounds were created on the backs of EGFP-Orai1 $^{+/+}$ and EGFP-Orai1 $^{-/-}$ mice and EGFP neutrophil fluorescence in the wound was tracked over 8 days. Representative images at Day 0 and 1 are shown. Data is plotted as mean+/- SEM from n=3 experiments.