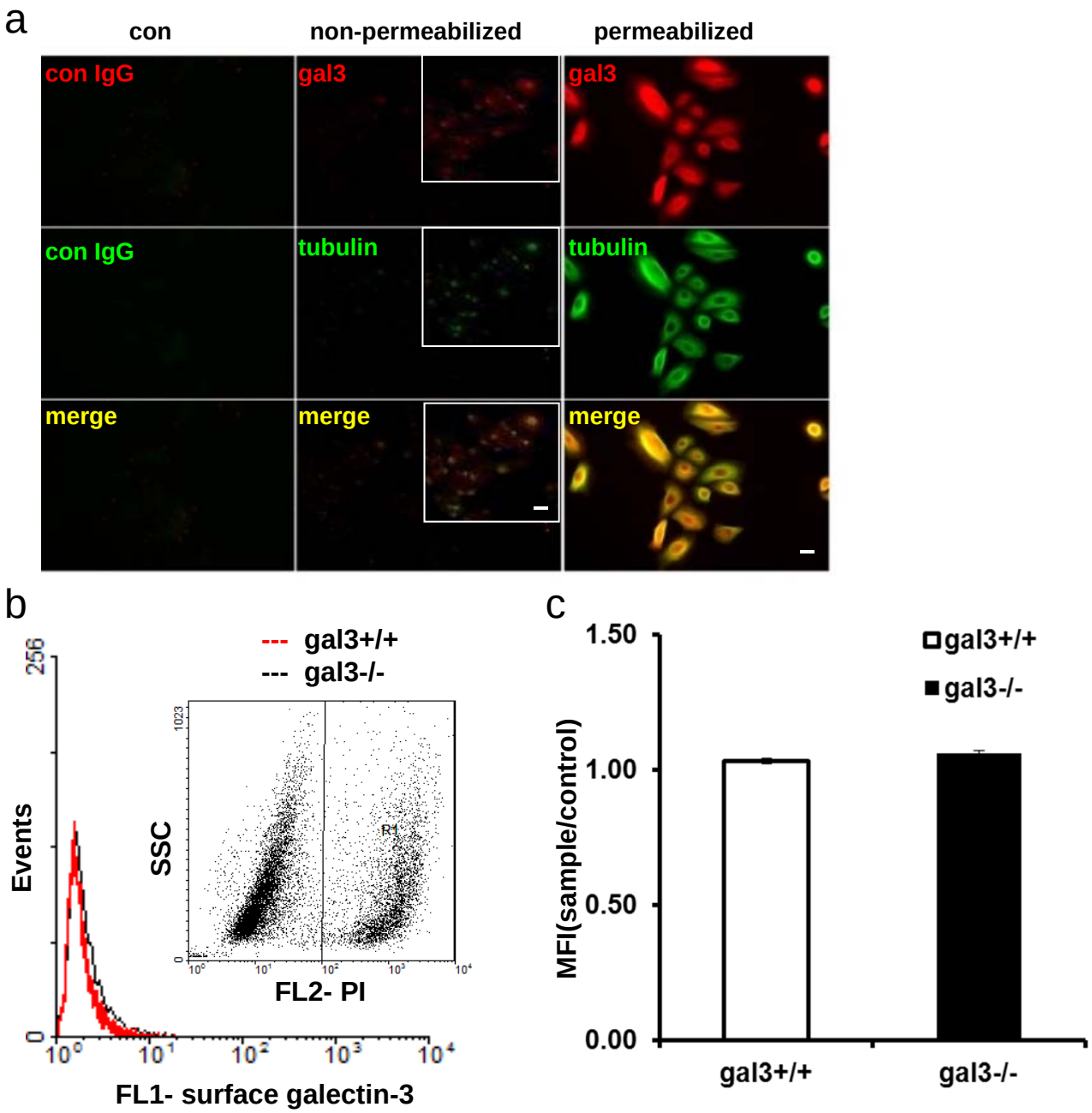
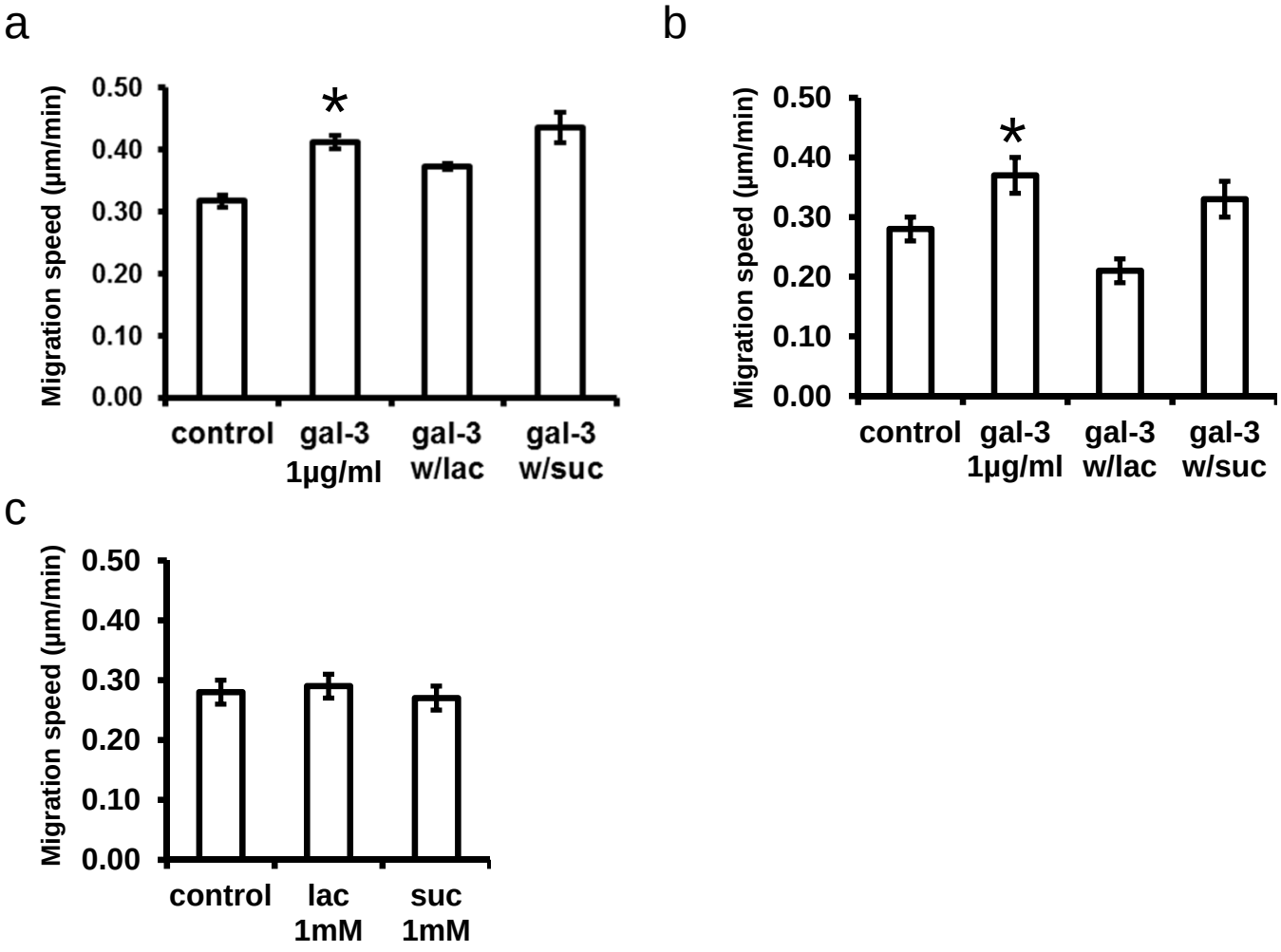
Supplementary Figure 1



Supplementary Figure 1. Detection of galectin-3 on the cell surface and in the cytoplasm of keratinocytes. (a) Human keratinocytes (NHK) were cultured on collagen I-coated glass coverslips. Cells were either untreated or permeabilized with 0.1% Triton X-100 for 1 min, and stained for galectin-3 (red) and a cytoplasmic protein, tubulin (green). The image intensity has been artificially increased by 40% in the insets to show absence of staining in non-permeabilized cells. Scale bar = 20 µm. (b) Gal3$^{+/+}$ and gal3$^{-/-}$ keratinocytes were cultured till 80% confluence. Cells were dissociated with Cellstripper for 30 min. Surface galectin-3 was detected by flow cytometry. Propidium Iodide was used to exclude dead cells (R1). (c) Comparisons of MFI ratio (specific antibody/control) of surface galectin-3 show no difference between gal3$^{+/+}$ and gal3$^{-/-}$.

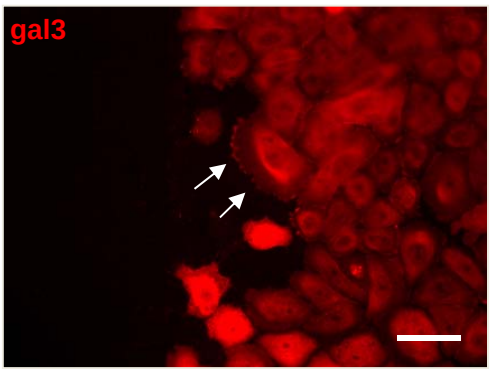
Supplementary Figure 2



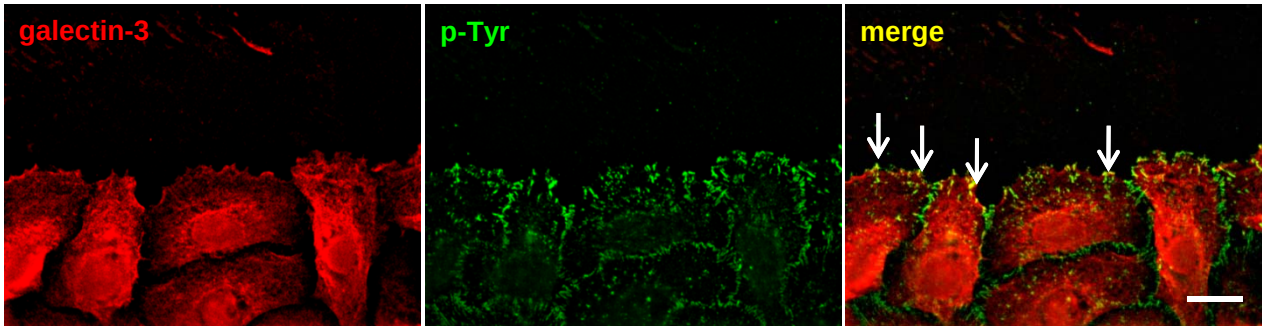
Supplementary Figure 2. (a) Wild type mouse keratinocytes were incubated in PBS alone, or supplemented with recombinant galectin-3 (1µg/ml), galectin3 with 25mM lactose, or galectin-3 with 25mM sucrose in growth factor-deficient medium. Single cell migration speeds are represented as µm/min. *, P =0.003. (b) NHK cells were serum starved overnight and the cells were then exposed to PBS (control), recombinant human galectin-3 (1 µg/ml), galectin-3 with 1 mM lactose, or galectin-3 with 1 mM sucrose in growth factor-deficient media. Cells were monitored microscopically for 1 h, and single cell migration speeds were calculated as µm/min. * $P < 0.05$. (c) NHK cells as described in (b) were exposed to PBS (control), 1 mM lactose, or 1 mM sucrose in growth factor-deficient media, and single cell migration speeds (µm/min) were calculated.

Supplementary Figure 3

a



b



Supplementary Figure 3. Galectin-3 translocates to the leading edge of keratinocytes prior to active migration. NHK cells were grown on collagen I-coated glass coverslips till confluent in human keratinocyte medium. Scratches were made using a sterile pipette tip. Cells were fed with medium containing growth factors for 30 min and then fixed for immunofluorescence microscopy. (a) NHK cells were stained for galectin-3 (red) 30 min after scratching. Arrows show galectin-3 localization at the leading edge of keratinocytes. Scale bar = 20 µm. (b) NHK cells were stained for galectin-3 (red) and phosphotyrosine (green) 30 min after scratching. Arrow shows colocalization of galectin-3 and phosphotyrosine (yellow). Scale bar = 10 µm.