

Text S2

Supplemental Results

The effects of calyculin A on cell speed and area are reduced when myosin contraction is inhibited with blebbistatin

Calyculin A is a phosphatase inhibitor that is thought to indirectly increase myosin II activity [1], and we have found that keratocytes treated with calyculin A migrate at higher speeds and are smaller than control cells (see Figure 8 in the main text). To determine whether these effects are primarily due to increased myosin II activity, we treated cells crawling on glass surfaces with either blebbistatin, a myosin-II inhibitor [2], or calyculin A alone, or both blebbistatin and calyculin A (Supplemental Figure 6). We found that cells treated with blebbistatin were significantly larger and slower than control cells (average area = $424 \mu\text{m}^2$ and average cell speed = $0.20 \mu\text{m/s}$ for control cells; average area = $503 \mu\text{m}^2$, $p = 0.005$, Student's T test, and average cell speed = $0.17 \mu\text{m/s}$, $p = 0.002$, for cells treated with blebbistatin). Cells treated with calyculin A were significantly smaller and faster than control cells (average area = $332 \mu\text{m}^2$, $p = 0.001$ and average cell speed = $0.28 \mu\text{m/s}$, $p = 10^{-5}$). The effects of calyculin A on cell area and speed were greatly reduced when the cells were treated with blebbistatin for 30 minutes prior to calyculin A treatment: cells treated with both blebbistatin and calyculin A were not significantly different from control cells (average area = $405 \mu\text{m}^2$, $p = 0.44$, and average cell speed = $0.19 \mu\text{m/s}$, $p = 0.38$). Although we cannot rule out the possibility that calyculin A affects the activity of signaling proteins that regulate adhesion and motility, these results suggest that the effects of calyculin A on cell shape and speed are primarily due to increased myosin II activity.

References

- [1] Ishihara H, Ozaki H, Sato K, Hori M, Karaki H, et al. (1989) Calcium-independent activation of contractile apparatus in smooth muscle by calyculin-a. *J Pharmacol Exp Ther* 250: 388–396.
- [2] Straight AF, Cheung A, Limouze J, Chen I, Westwood NJ, et al. (2003) Dissecting temporal and spatial control of cytokinesis with a myosin ii inhibitor. *Science* 299: 1743–1747.