



**Figure 3 | Jaspalkinolide specifically halts actin dynamics of cells in which myosin II is inhibited.** **a**, Raw F-actin speckle flow measurements in the cell frame of reference (yellow arrows) superimposed on the corresponding FSM frames, for a cell in 50  $\mu\text{M}$  blebbistatin before (top) and approximately 2 min after (middle) addition of 1  $\mu\text{M}$  jaspalkinolide. Bottom, a separate cell in jaspalkinolide alone. **b**, F-actin flow magnitude maps corresponding to **a**. The combination of blebbistatin and jaspalkinolide immobilizes the actin network, an effect that is not achieved by either drug alone. **c–e**, Fixed, phalloidin-labelled keratocytes, untreated (**c**) or treated with 50  $\mu\text{M}$  blebbistatin (**d**) or 1  $\mu\text{M}$  jaspalkinolide (**e**). Consistent with impaired actin-network disassembly, blebbistatin-treated cells accumulate F-actin along the rear margin, jaspalkinolide-treated cells underneath the cell body. **f**, Treatment with either 5 nM latrunculin A, 50  $\mu\text{M}$  blebbistatin or

1  $\mu\text{M}$  jaspalkinolide can slow cells relative to the control population. The combination of blebbistatin and latrunculin A or jaspalkinolide and latrunculin A has no significant further effect on cell speed than either drug alone. The combination of blebbistatin and jaspalkinolide significantly ( $P < 0.05$  by Tukey's test) and synergistically slows cell locomotion. **g**, Blebbistatin causes significant ( $P < 0.05$  by Tukey's test) F-actin accumulation in the trailing 'tails' (but not in the cell body), whereas jaspalkinolide causes accumulation underneath the cell body (but not in the 'tails'), relative to untreated cells. a.u., Arbitrary units (see Methods). Compare **c–e**. Box-and-whisker plots (**f** and **g**) indicate the mean, 95% confidence interval (CI), extrema and quartiles for the indicated number of cells ( $n$ ) in each treatment group. Compare with Supplementary Movie 3.