

**Supplemental Figure 1. Exchange of DAD on full-length FRL2 and FRL3 proteins confers the expected autoregulatory effect.** Epitope-tagged derivatives of full-length FRL2 and full-length FRL3 were expressed by transient transfection in NIH 3T3 cells (0.3  $\mu$ g DNA). Protein expression was detected with 9E10 anti-myc monoclonal (red); F-actin was visualized with fluorescein phalloidin (green). **(A)** Full-length FRL3 is distributed diffusely throughout the cytoplasm of transfected cells and does not induce stress fiber formation (stress fibers (SF) in 24% of cells, N=161). **(B)** Full-length FRL3+2 is distributed diffusely throughout the cytoplasm and weakly localized to the periphery of transfected cells and induces thick stress fiber formation (SF in 70%, N=50). **(C)** Full-length FRL3 $\Delta$ DAD is distributed diffusely throughout the cytoplasm and concentrated at the periphery of transfected cells and induces robust stress fiber formation (SF in 80%; N=50). **(D)** Full-length FRL2 is distributed diffusely throughout the cytoplasm as well as notably concentrated at the periphery of transfected cells and strongly induces stress fiber formation (SF in 92%, N=50). **(E)** Full-length FRL2+3 is distributed diffusely throughout the cytoplasm of transfected cells and does not strongly induce stress fiber formation (SF in 37%, N=51). **(F)** Expression of full-length FRL3+2 or FRL3 $\Delta$ DAD (0.1, 0.3, 1  $\mu$ g DNA transfected) induces robust activation of an SRF reporter gene while full-length FRL3 has minimal effect on activation. Reporter gene activity was standardized to activation induced by expression of an SRF-VP16 control fusion protein. Error bars show S.E.M, N=3. **(G)** Expression of full-length FRL2 (0.1, 0.3, 1  $\mu$ g DNA transfected) induces robust activation of an SRF reporter gene. Full-length FRL2+3 had minimal effect on activation of the reporter gene. Reporter gene activity was standardized as in (F). Error bars show S.E.M, N=3.

