## SUPPLEMENTAL FIGURE LEGENDS

**Fig S1:** Cell morphodynamics of hMSCs does not depend on substrate rigidity or vinculin head-tail interaction.

- (A) Typical surface mask (in black) of protrusions and retractions used for morphodynamics analysis.
- (B) Normalized area of protrusions and retractions for hMSCs expressing either WT or T12 vinculin as a function of substrate rigidity.

Mean+/-SEM. ANOVA and Tukey's HSD test, n=4-5 cells per condition. No significant difference between conditions.

**Fig. S2:** FA protein steady-state recruitment to the cell-ECM interface is rigidity-dependent. Vinculin conformation change is required for the rigidity-dependence of its recruitment and that of Talin, but not that of Paxillin and FAK.

- (A) GFP-tagged FA protein recruitment patches in hMSCs spread on substrates of varied rigidities.
- (B) Recruitment patches of GFP-tagged proteins co-expressed with T12-mCherry in hMSCs spread on substrates of varied rigidities.

Bar=10 $\mu$ m.

**Fig S3:** FA protein mobilities are rigidity-dependent, and that of vinculin and talin depend on vinculin head-tail interaction.

(A) Typical auto-correlation curve of vinculin-mCherry (red) fitted with a 2-species 3D diffusion model.

(B-D) Fast and slow apparent diffusion coefficients of (B) vinculin and T12, (C) talin and talin coexpressed with T12, and (D) Paxillin as a function of subtrate rigidity. Mean+/-SEM. ANOVA

and Tukey's HSD test or Student t-test, n=8 per condition. Shown p-values are between extreme rigidities and WT vs. mutant conditions at lowest rigidity.

(E) Typical cross-correlation curve of the GFP-mCherry tandem (red) as a positive control for cross-correlation, fitted with a 1-species 3D diffusion model.

(F) Typical cross-correlation curve of co-transfected GFP and mCherry as a negative control for cross-correlation.

(G) Typical cross-correlation curve of co-transfected Talin-GFP and Vinculin-mCherry.

**Fig.S4**: Fluorescence lifetime differences between conditions are not due to variable expression ratios of Talin-GFP and vinculin- or T12-mCherry.

GFP fluorescence lifetime of Talin-GFP as a function of Talin-GFP/Vinculin-mCherry (WT or mutant) expression ratio as measured from the GFP/mCherry intensity ratio.