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=10µm.

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 substrate 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.