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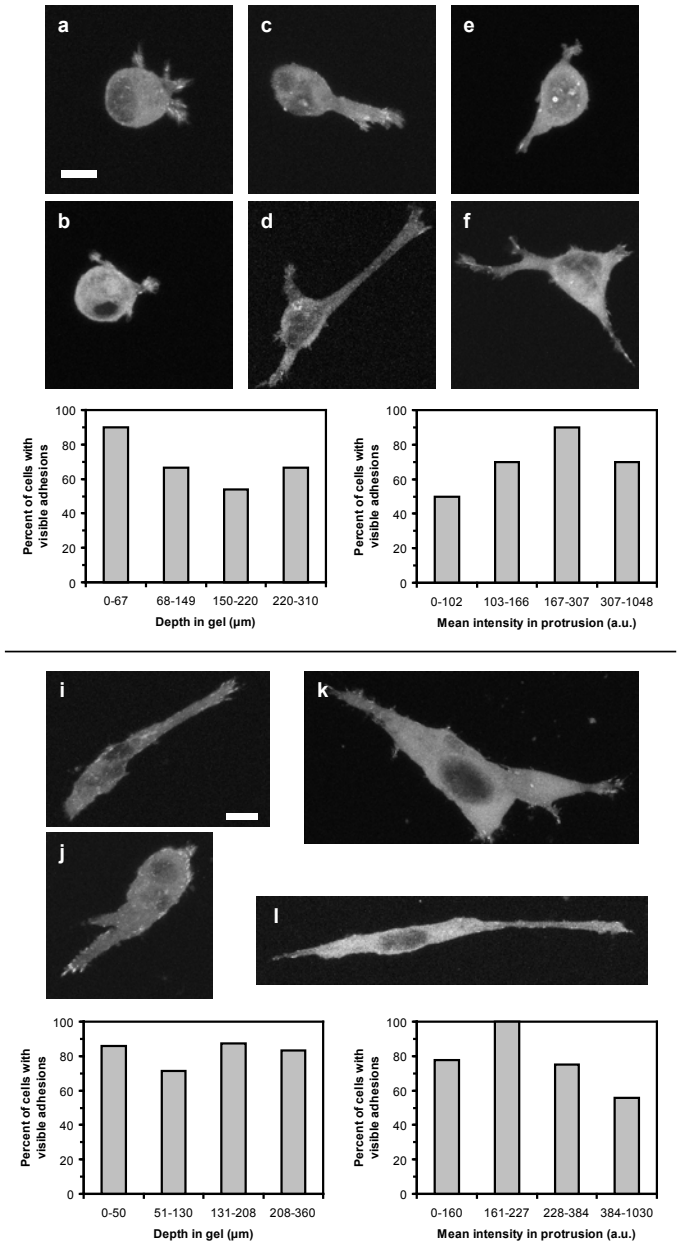


**Figure 1** U2OS (a-h) and HT-1080 (i-n) cells expressing promoter-truncated EGFP-paxillin in collagen gels. U2OS cells were cultured in bovine collagen gels and imaged 3-5 h after seeding. HT-1080 cells were cultured in rat-tail collagen and imaged at 18-24 h. All data were compiled from three independent experiments. All scale bars = 10 μm. **(a-f)** Representative fluorescence z-projections of U2OS cells with visible adhesions. Cell morphology was variable, as were protrusion dimensions, which ranged from fan-shaped (c-d, ~43% of cells) to tapered (e-f, ~32%). Approximately 25% of the cells (a-b) had multiple short protrusions. **(g)** Percent of fluorescent U2OS cells with at least one visible adhesion, binned according to depth in gel (i.e. distance from surface; see Methods). >50% of fluorescent cells at all assayed depths contained visible adhesions. A higher percentage of cells with visible adhesions was found near (0-67 μm) the glass surface—possibly because these areas were stiffer than areas distal to the glass. Left to right: n = 9, 13, 9, and 7 cells per bin. **(h)** Percent of fluorescent U2OS cells with at least one visible adhesion, binned according to the relative intensity of the protrusion background fluorescence (see Methods). A higher percentage of protrusions with intermediate background intensity had visible adhesions, possibly because high background eclipses adhesion signal whereas a very dim background indicates such low expression that adhesion intensity is also reduced. N = 10 cells for all bins. **(i-l)** Representative fluorescence z-projections of HT-1080 cells with visible adhesions. Cell morphology was variable, as were protrusion dimensions, which ranged from fan-shaped (i-j, ~57% of cells) to tapered (k-l, ~43%). **(m)** Percent of fluorescent HT-1080 cells with visible adhesions as a function of depth in the gel, presented as in (g), but showing little dependence of adhesion visibility on cell location. Left to right: n = 9, 7, 7, and 8 cells per bin. **(n)** Percent of fluorescent HT-1080 cells with visible adhesions as a function of the protrusion background intensity, presented as in (h) and showing a similar trend. Left to right: n = 9, 9, 8, and 9 cells per bin.

**Supplementary Movie Legends**

**Movie 1** A U2OS cell expressing promoter-truncated EGFP-paxillin in a 3D collagen gel about 3-5 h after seeding. The left panel shows paxillin signal; the right panel shows paxillin overlayed with the collagen reflectance image. The images are z-projections of nine z-slices. Frames were acquired every 10 s. Video plays at 10 frames/s. Corresponds to Figure 2.

**Movie 2** An HT-1080 cell expressing promoter-truncated EGFP-paxillin in a 3D collagen gel about 3-5 h after seeding. The left panel shows paxillin signal; the right panel shows paxillin overlayed with the collagen reflectance image. The images are z-projections of 20 z-slices. Frames were acquired every 10 s. Video plays at 10 frames/s.