

Supporting Information

Figure S1

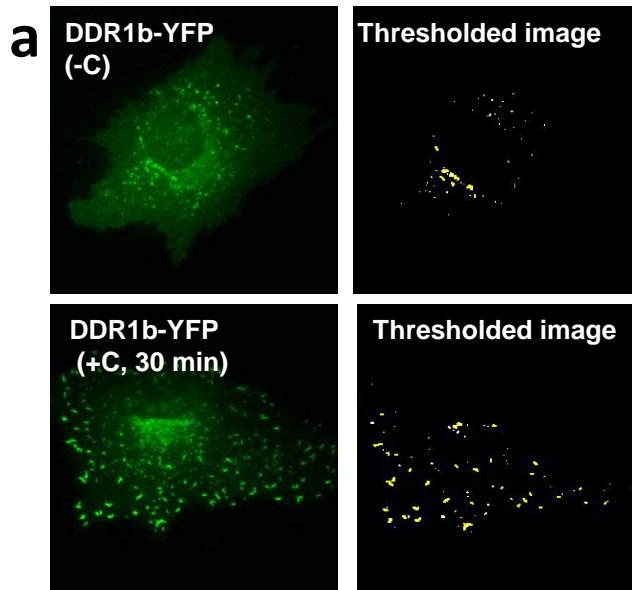


Figure S1a: Methodology for quantitative analysis of the number of punctate structures in DDR1b-YFP cells. Images were thresholded and particles with circularity < 0.99 and area $< 10 \mu\text{m}^2$ were selected for analysis. As shown above these settings selected the majority of DDR1b-YFP clusters while excluding very smaller globular particles or nuclear signal present in cells. The selected structures are marked by yellow overlay on the thresholded particles (white).

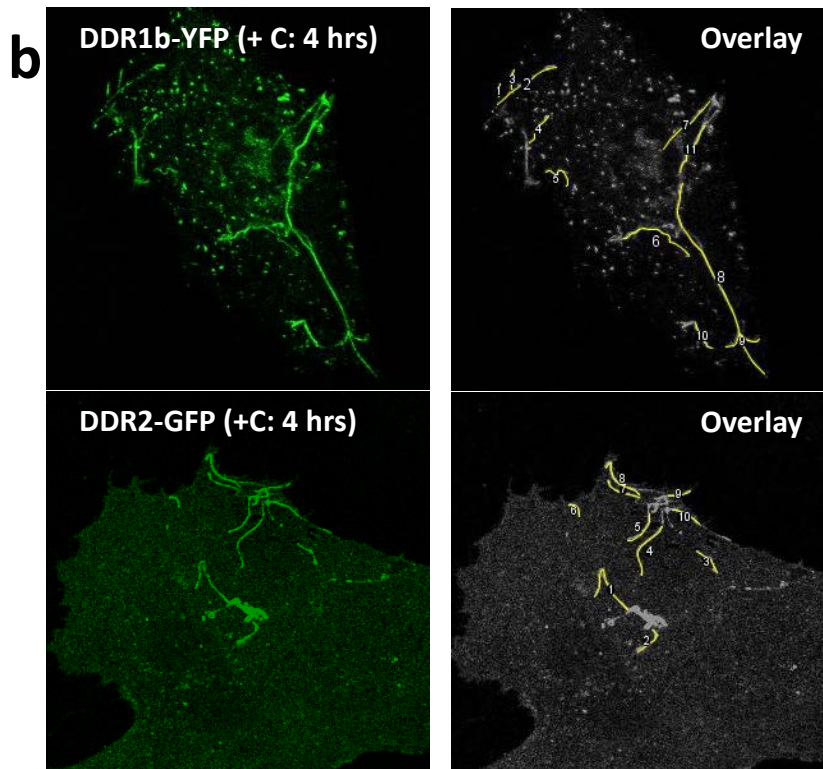


Figure S1b: Methodology for quantitative analysis of the length of filamentous structures in DDR1b-YFP- or DDR2-GFP- expressing cells. The maximum uninterrupted contour length of filaments was manually traced using a freehand line, until an overlap, joint or tangle was encountered. Tracked filaments are shown in yellow in the overlay images.

Figure S2

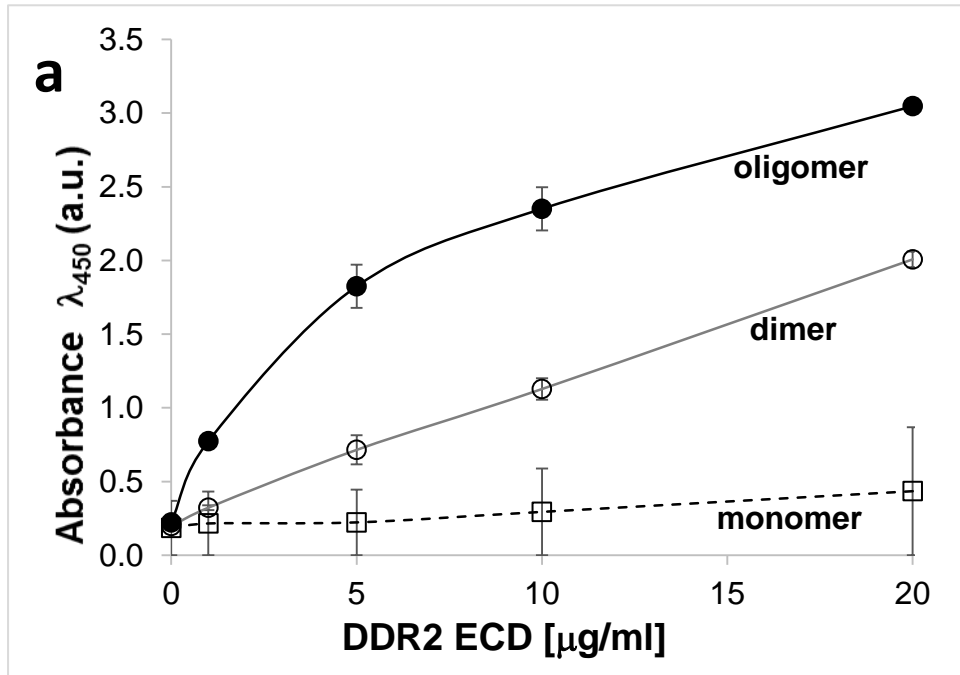


Figure S2a: Binding of DDR2 ECD to rat-tail collagen. Solid phase binding assay for binding of various oligomeric forms of recombinant DDR2 ECD to immobilized rat collagen. Experimental details were similar to that used for bovine collagen (Figure 2a). EC_{50} for oligomeric DDR2 (3.6 ± 0.37) was ~ 4 times lower than that for dimeric DDR2 ($14.15 \pm 0.84 \mu\text{g/ml}$), $p < 0.0001$.

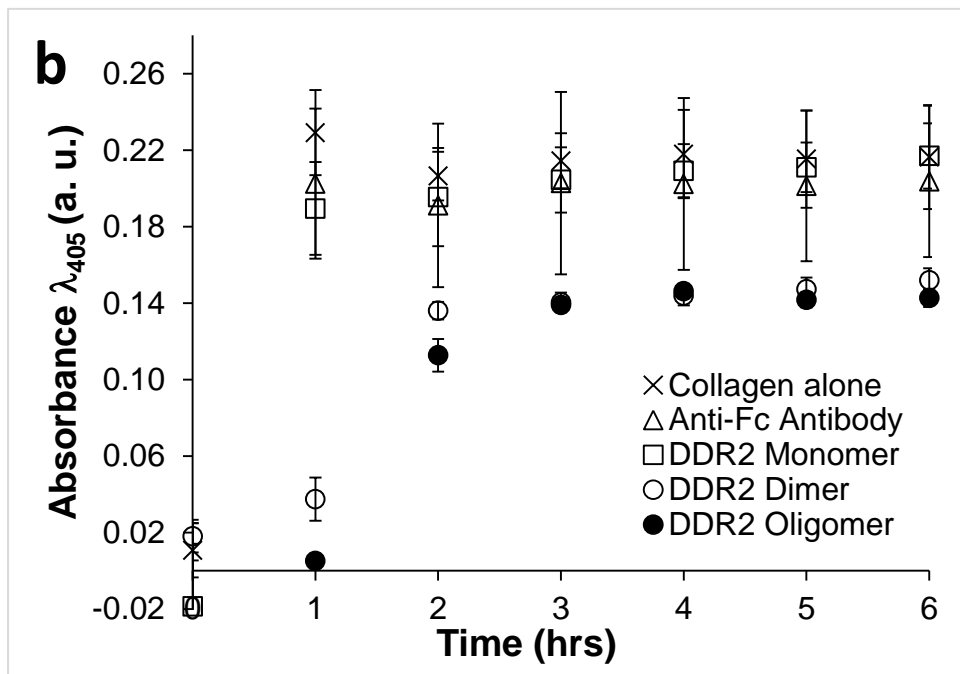


Figure S2b: Effect of the oligomeric state of DDR2 ECD on fibrillogenesis of rat-tail collagen. Turbidity of collagen solutions with or without DDR2 ECD (as indicated) was monitored every hour for 6 hours. Experimental details are similar to that used for bovine collagen as in Figure 2b.

Figure S3

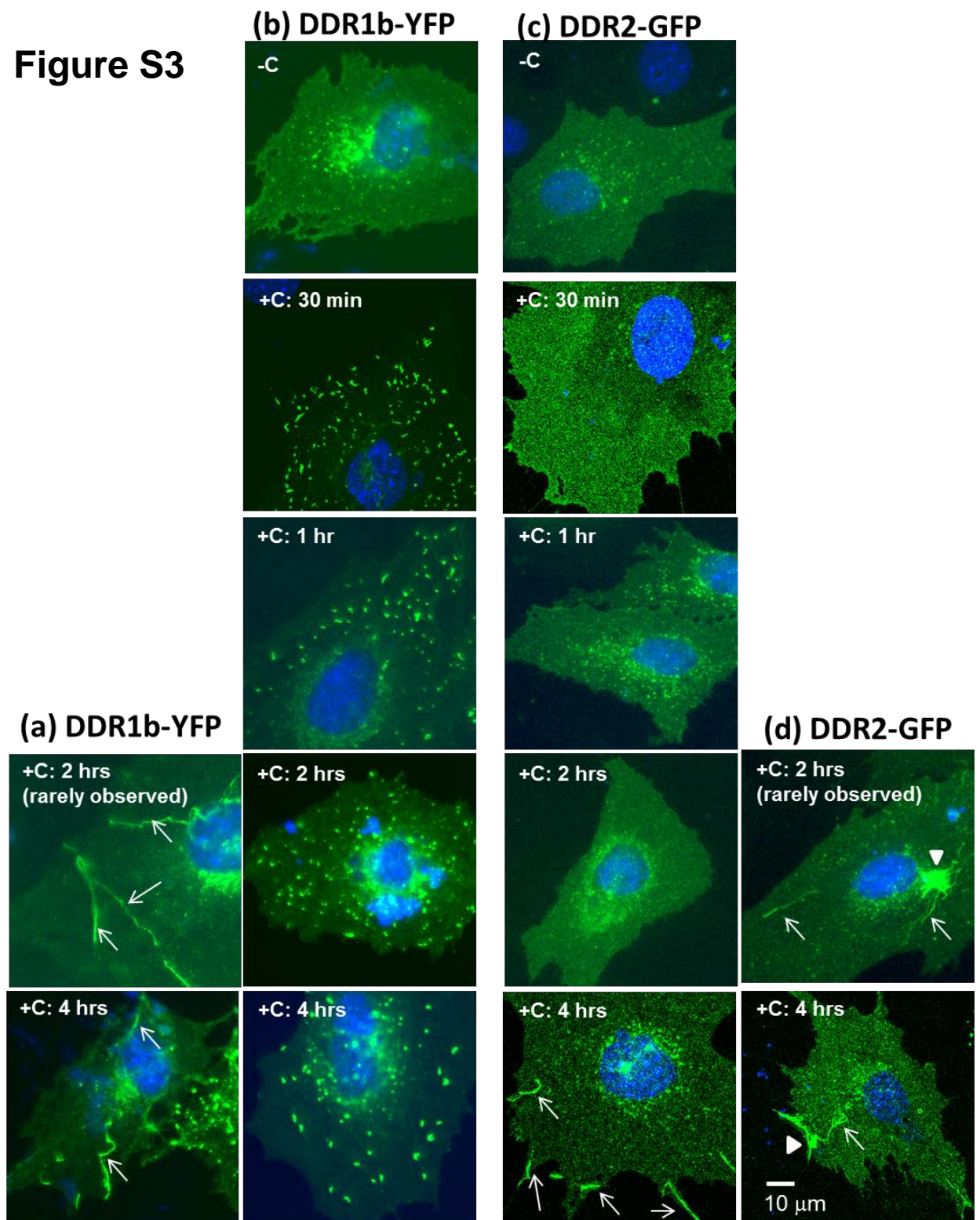


Figure S3: Effect of bovine-dermal collagen on spatio-temporal distribution of DDR1b-YFP and DDR2-GFP in fixed MC3T3 cells. The central columns (b) and (c) show the commonly observed spatial distribution of DDRs (green) in cells. Upon stimulation with soluble collagen (+C), DDR1b-YFP forms clusters which persist in several cells even up to 4 hrs. DDR2-GFP on the other hand does not show evidence of cluster formation. At 4 hrs of collagen stimulation, a sub-population of both DDR1b-YFP and DDR2-GFP expressing cells (columns, a and d), show spatial distribution of DDRs into filamentous structures (arrows) and tangles (arrowheads). Such filamentous structures were rarely observed at the 2hr (or earlier) time-points. The morphology of filamentous structures when observed at 2hrs resembled those that were formed at 4 hrs. Scale bar is same for all panels. Blue indicates nuclear staining.

Figure S4

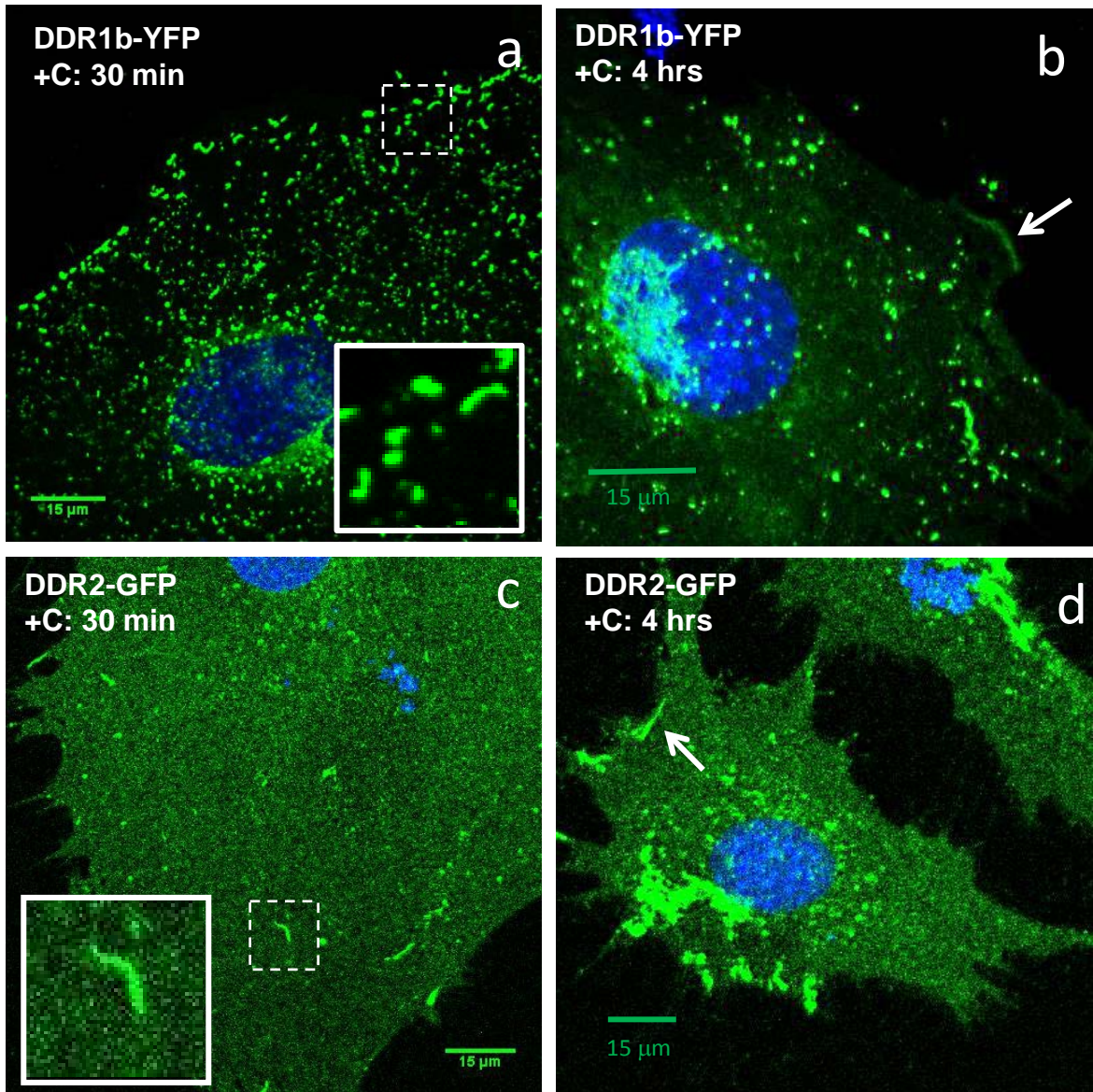


Figure S4: Effect of rat-tail collagen on spatial distribution of DDR1b-YFP and DDR2-GFP in MC3T3 cells. Similar to results with bovine collagen (Fig. 4), DDR1b-YFP (in a) shows evidence of small, punctate structures or ‘cluster formation’ upon stimulation with rat tail collagen, which persisted in several cells even after 4 hr of collagen stimulation (in b). Such a cluster formation was not observed for DDR2-GFP (in c) but DDR2-GFP was observed to assemble into small filamentous structures as early as 30 min post collagen stimulation. After 4 hrs of stimulation (b, d) YFP/GFP positive filamentous structures (arrows) and/or tangles of filaments were visible on both DDR1b-YFP- and DDR2-GFP- expressing cells.

Figure S5

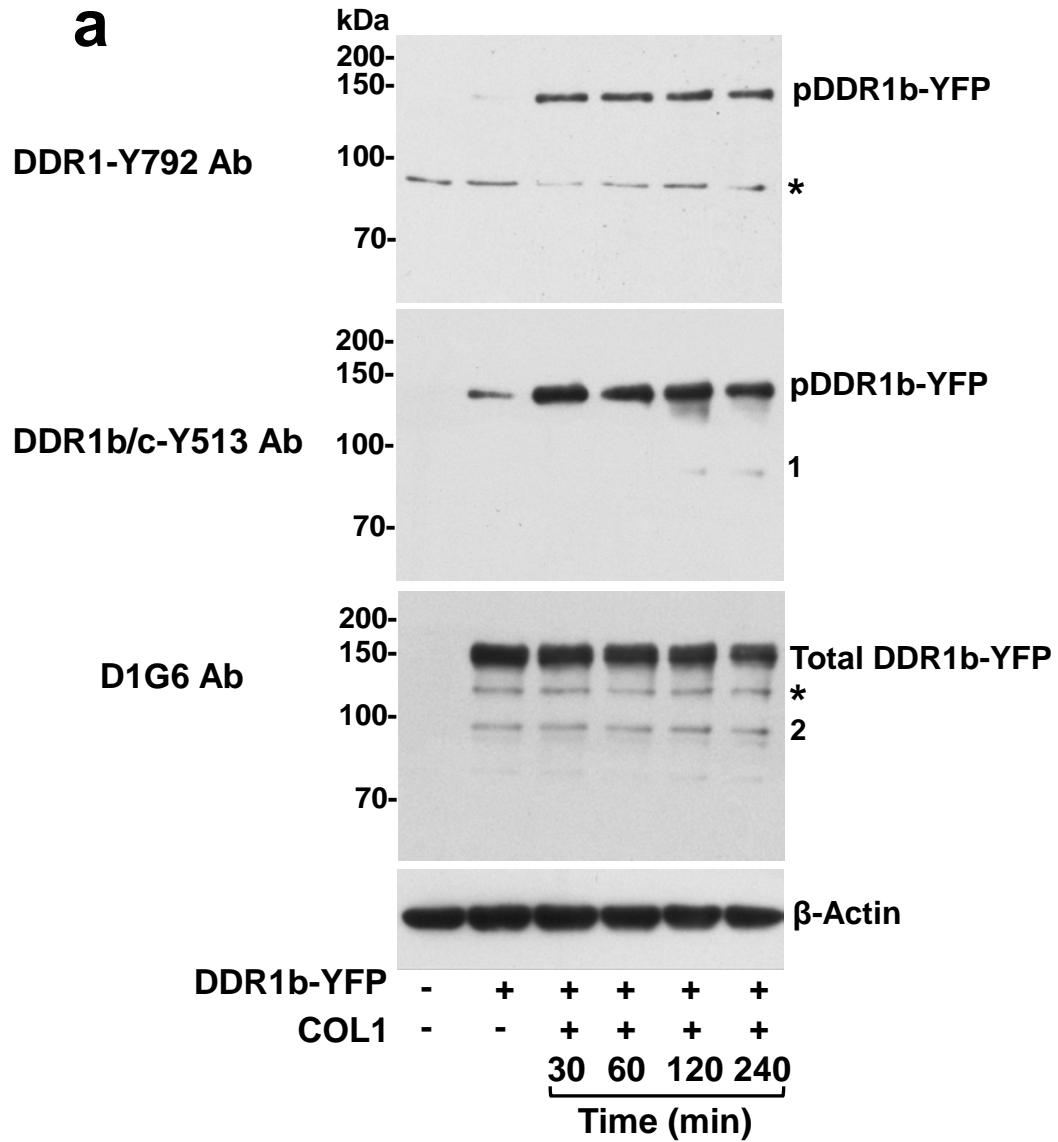


Figure S5, cont'd

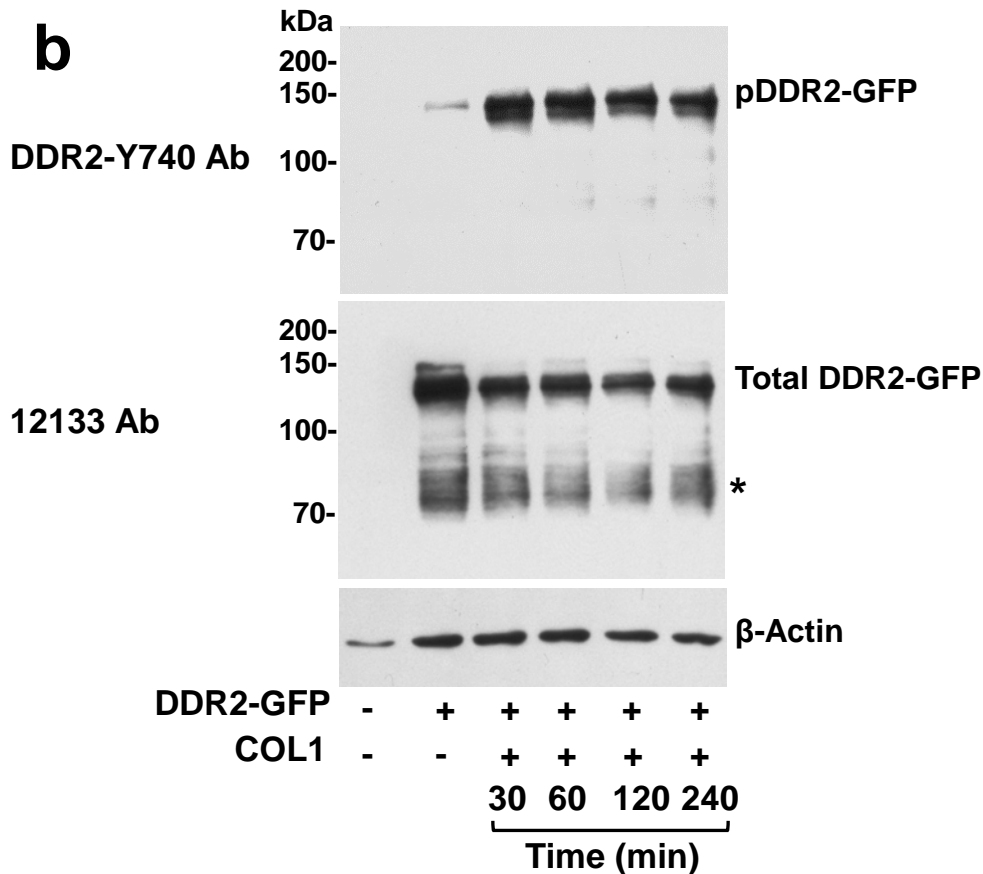


Figure S5. Expression and phosphorylation of DDR1b-YFP and DDR2-GFP. Cos1 cells were either untransfected (-) or transfected (-) with expression vectors encoding either DDR1b-YFP (a) or DDR2-GFP (b), as described in Materials and Methods. Twenty-six hrs after transfection, the cells were washed with DPBS and serum starved O/N. Subsequently, the cells were treated with vehicle (20 ml of 0.02N acetic acid) (-) or 20 μ g/ml (final concentration) of rat tail collagen I (+) for various times, as indicated. The cells were then lysed in RIPA buffer and equal protein concentrations (25 μ g/lane) were resolved by reducing 7.5% SDS-PAGE followed by immunoblotting. In the case of DDR1b-YFP (Panel a), the membrane was first probed with antibodies to DDR1-Y792, followed by stripping, and re-probing with DDR1b/c-Y513 antibodies. The membrane was stripped again and then re-probed with D1G6 antibodies against total DDR1. In the case of DDR2-GFP (Panel b), the membrane was first probed with DDR2-Y740 antibodies followed by stripping and re-probing with 12133 antibodies against total DDR2. Numbers 1 and 2, in the DDR1b/c-Y513 and total DDR1 blots of pDDR1b-YFP, respectively, likely represent the cleaved C-terminal fragment of DDR1³³. Asterisks (*): non-specific bands.

Figure S6

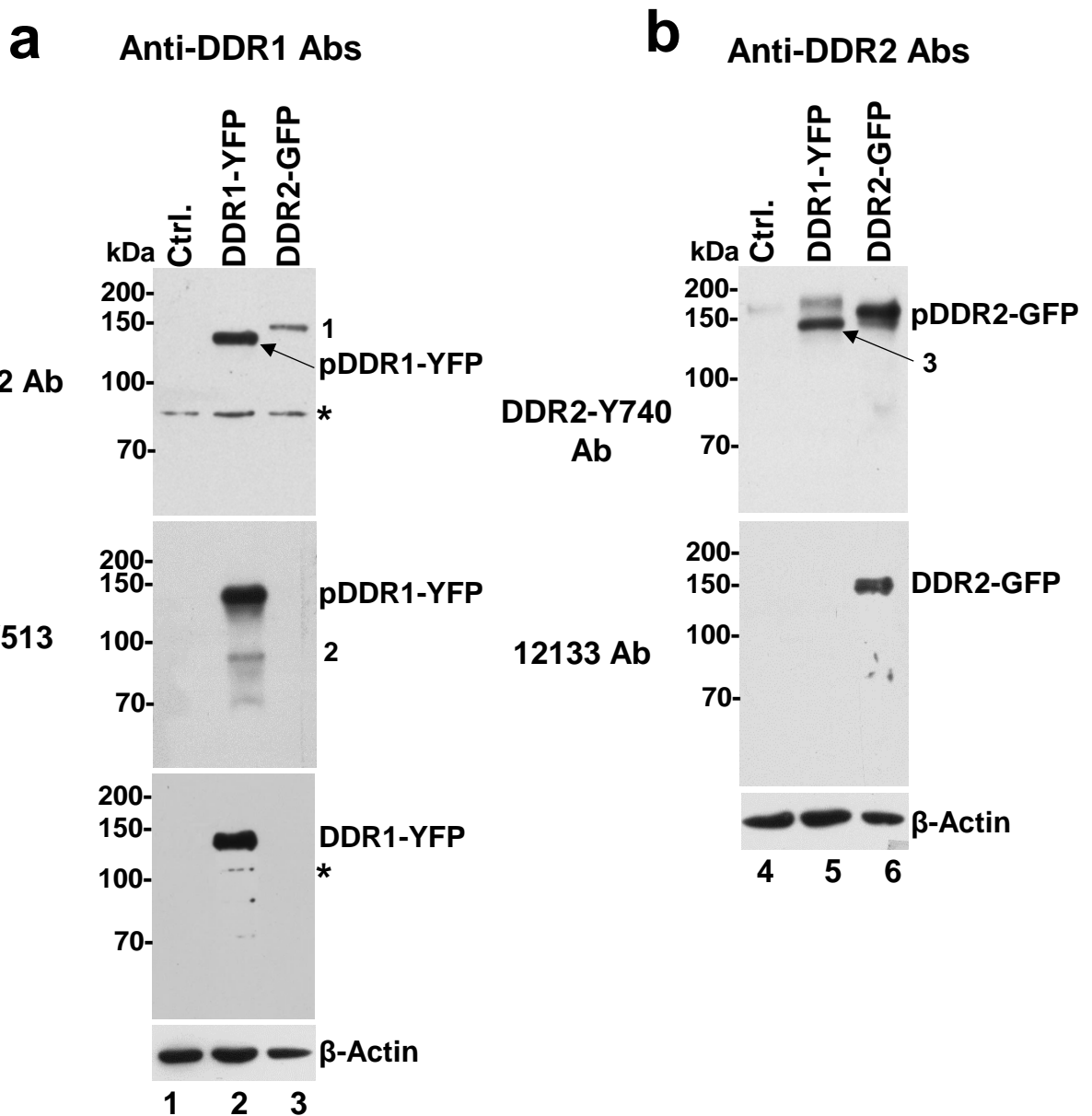


Figure S6: Specificity and cross-reactivity of DDR antibodies. Cos1 cells were untransfected (lanes 1 and 4, Ctrl.) or transfected with plasmid vectors encoding human DDR1b-YFP (lanes 2 and 5) or DDR2-GFP (lanes 3 and 6). After 26 hrs, the cells were washed with DPBS and serum starved O/N. Subsequently, the cells were treated with 20 $\mu\text{g/ml}$ (final concentration) of rat tail collagen I for 2 hrs. The cells were then lysed in RIPA buffer and equal protein concentrations (25 $\mu\text{g/lane}$) were resolved by reducing 7.5% SDS-PAGE followed by immunoblot analyses using various antibodies to total or phosphorylated DDRs, as indicated. β -actin was used as loading control. Number 1 in the DDR1-Y792 blot (lane 3) may represent cross reactivity of this pDDR1 antibody with pDDR2-GFP. Number 2 in the DDR1b/c-Y513 blot (lane 2) likely represents the C-terminal fragment of pDDR1b-YFP. Number 3 in the DDR2-Y740 blot (lane 5) may represent a cross reactivity of this pDDR2 antibody with pDDR1b-YFP. Asterisk (*): non-specific band.

Figure S7

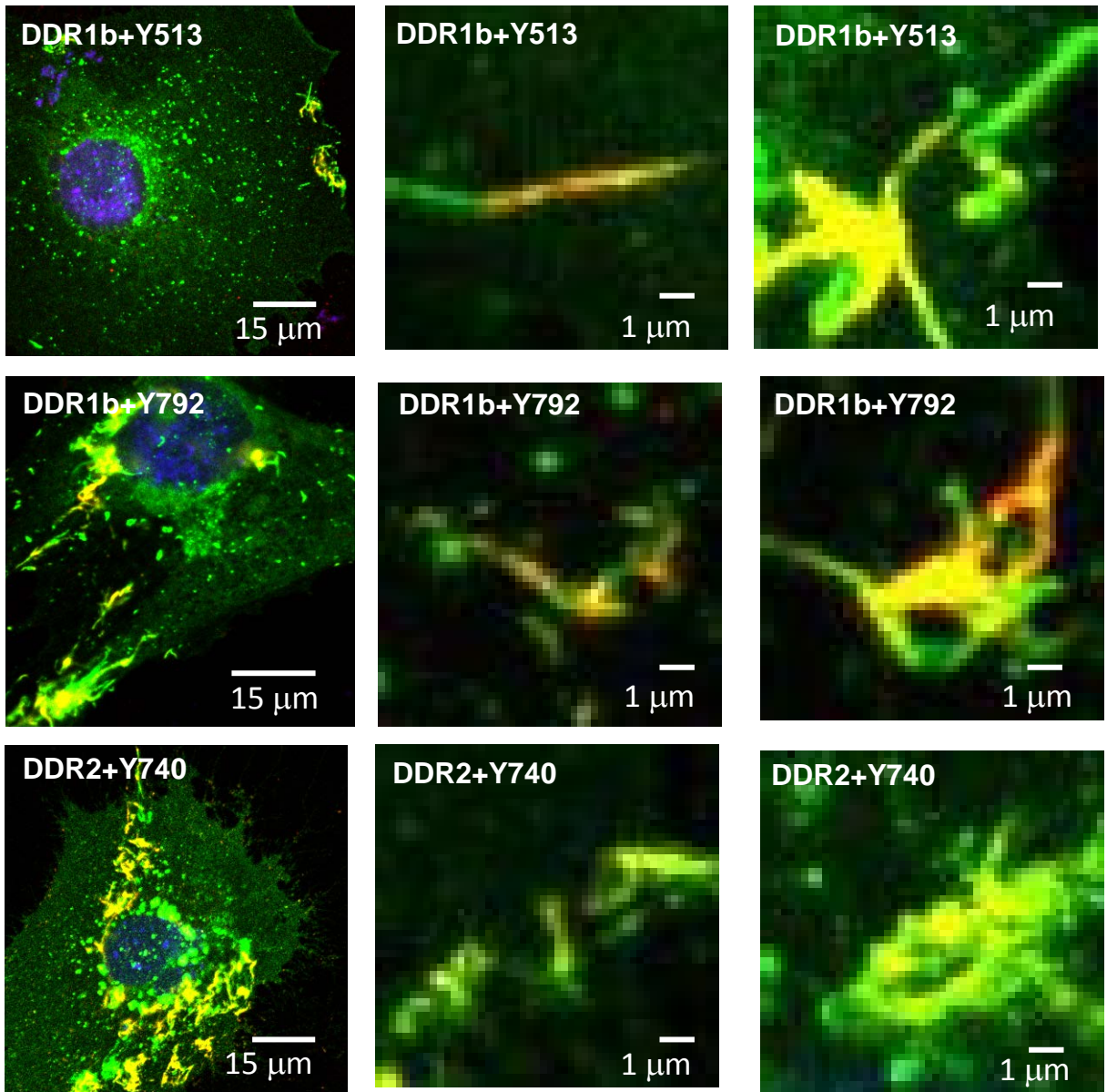


Figure S7: Immunocytochemistry (ICC) for spatial distribution of DDR1b-YFP and DDR2-GFP phosphorylation upon stimulation with rat tail collagen. After 4 hrs of stimulation with rat tail collagen the cells were stained for pDDR antibodies as indicated. Column 1 shows a whole cell with YFP/GFP signal (green) and its co-localization (yellow) with pDDR antibodies as indicated. Columns 2 and 3 are regions selected from two different cells showing that phosphorylation (yellow) was pre-dominantly present on filamentous structures and tangles of filaments as observed with bovine collagen in Figures 9 and 10b.

Figure S8

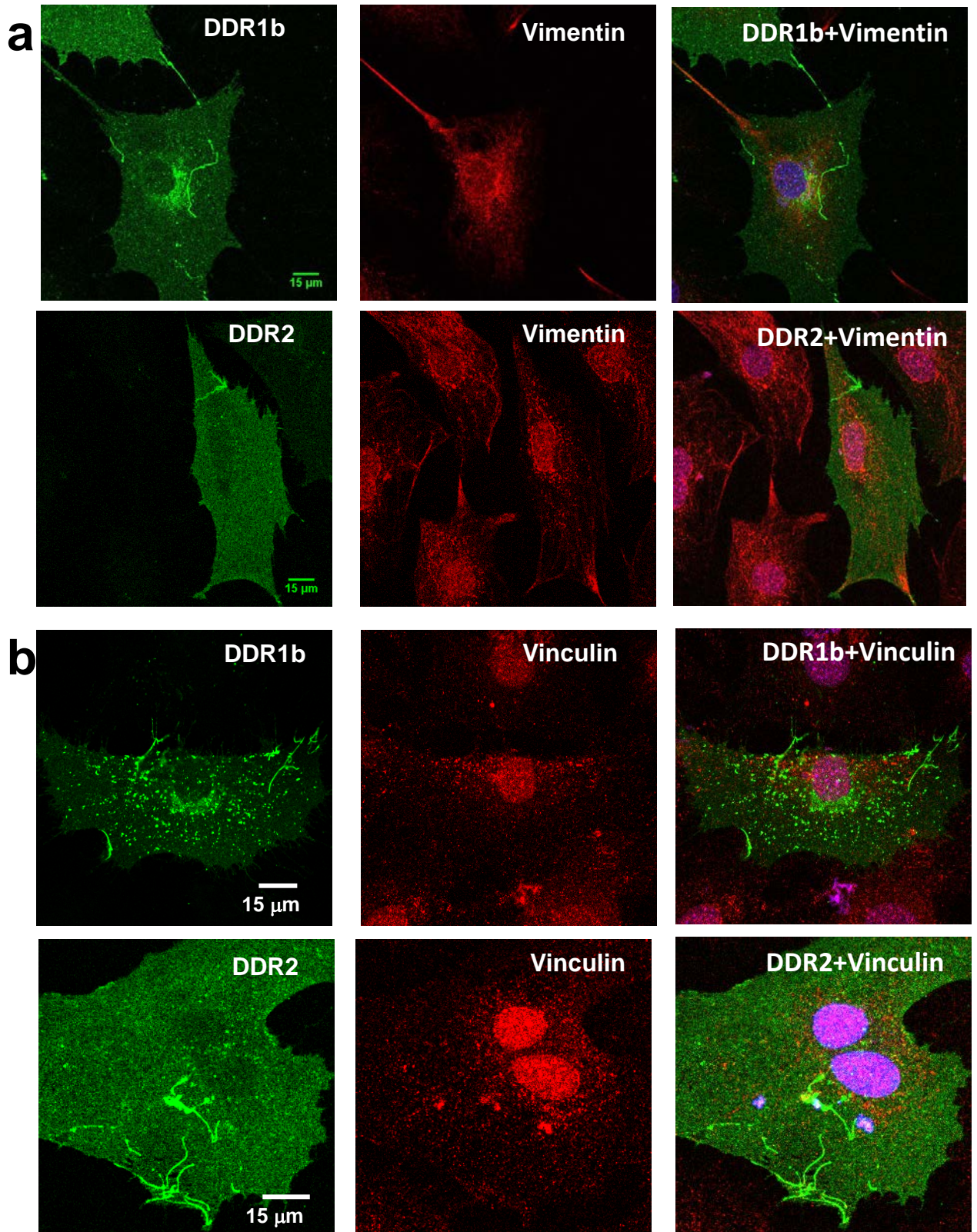


Figure S8: ICC for spatial distribution of DDRs and vimentin/vinculin. DDR1b-YFP- or DDR2-GFP-expressing cells stimulated with bovine-dermal collagen for 4 hrs revealed the formation of filamentous structures (green). However immunostaining for vimentin (red) (a) or vinculin (red) (b) revealed lack of co-localization (yellow) with the filamentous structures formed by DDR1b-YFP or DDR2-GFP.

Figure S9

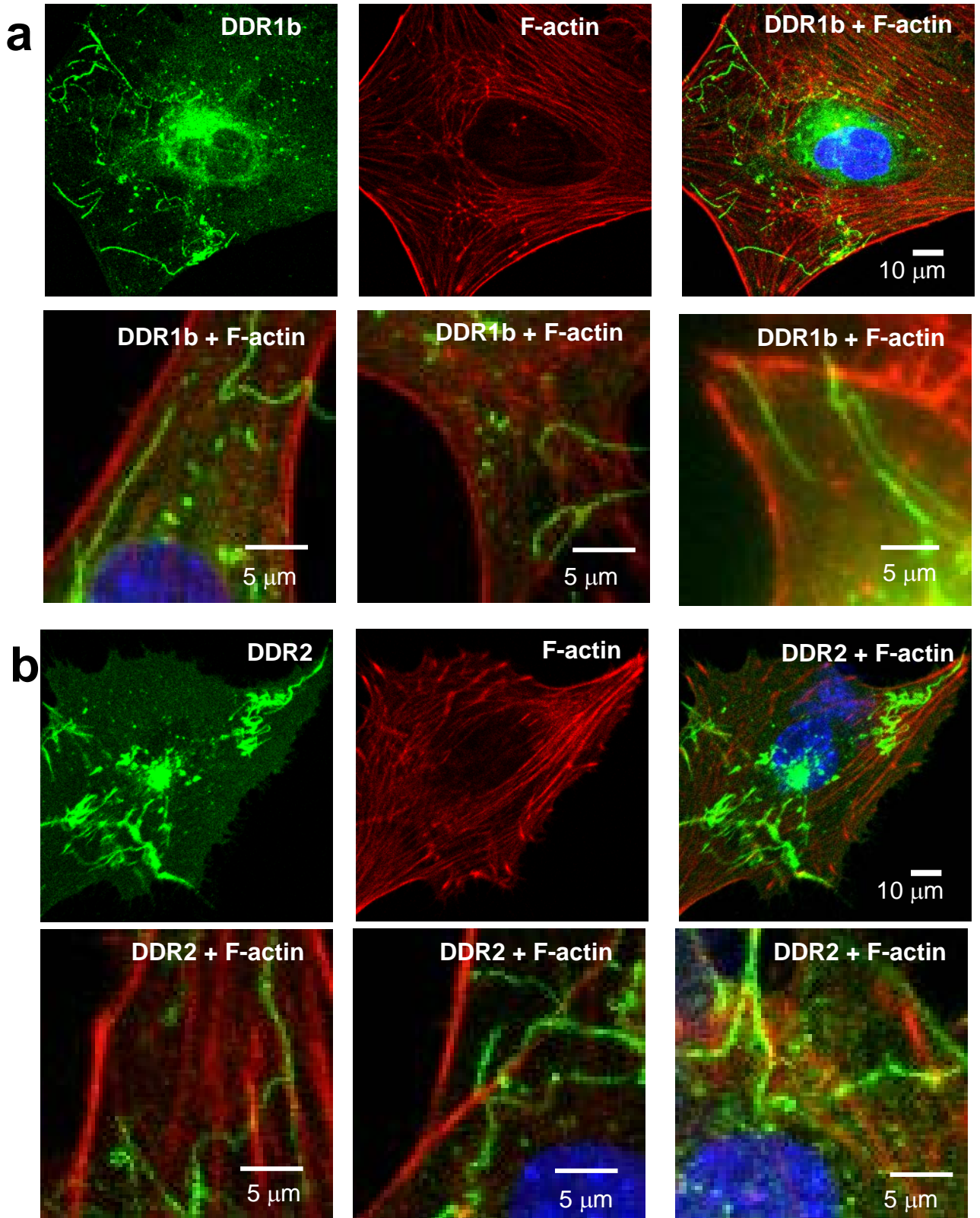


Figure S9: ICC for spatial distribution of DDRs and F-actin. The filamentous structures (green) formed in (a) DDR1b-YFP- or (b) DDR2-GFP- expressing cells after 4 hrs of administration of bovine-dermal collagen did not co-localize with f-actin (red). Bottom rows in panels a and b show selected regions from three different cells showing how the trajectories of DDRs and f-actin filaments were distinct with little to no co-localization (yellow).

Figure S10

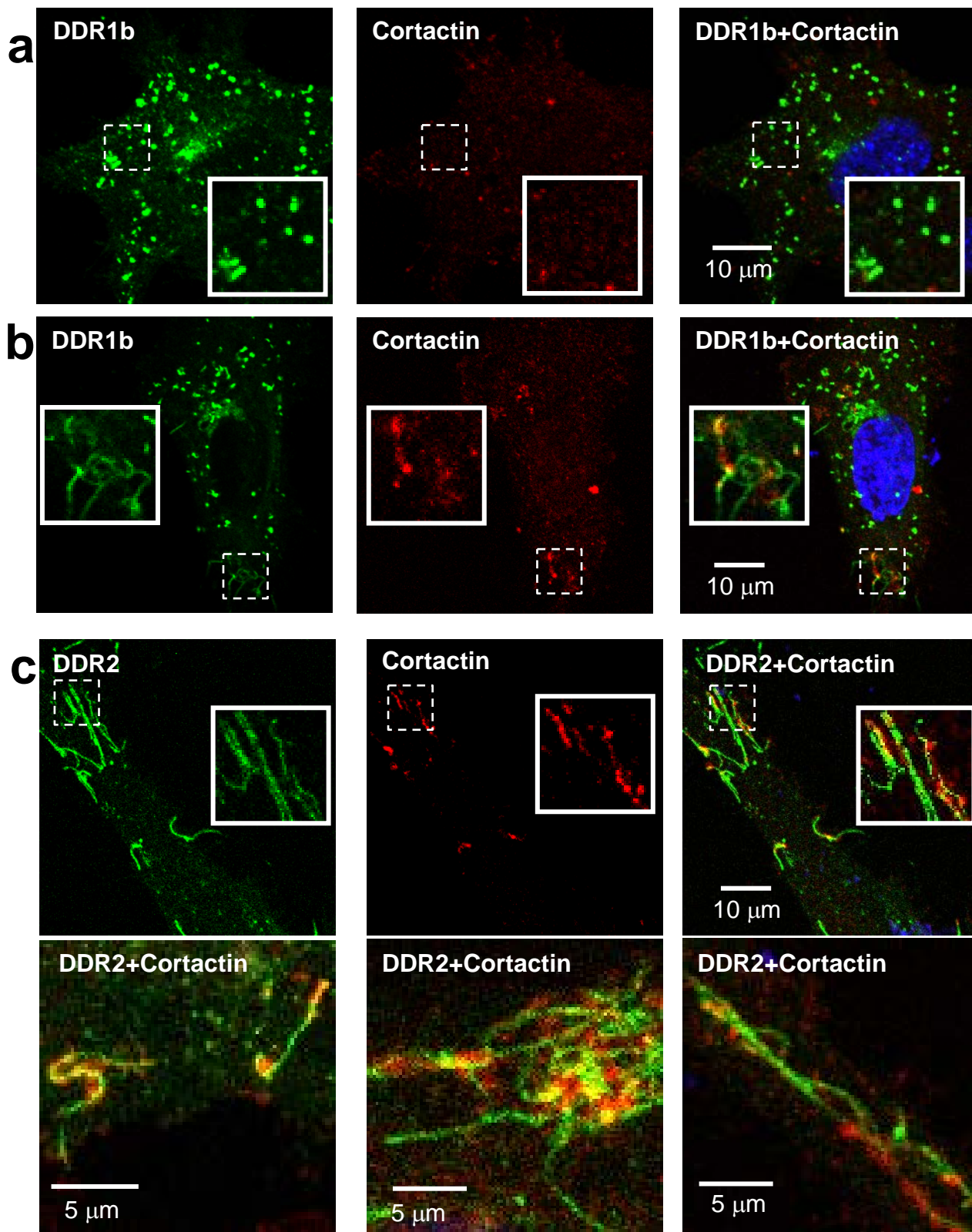


Figure S10: ICC for spatial distribution of DDRs and cortactin. After 4 hrs of administration of bovine dermal collagen, DDR1b-YFP clusters (green) (in a) show no co-localization with cortactin (red). However the regions of filamentous structures formed in DDR1b-YFP- (in b) and DDR2-GFP- (in c) expressing cells were enriched with cortactin with few segments showing partial co-localization ($R=0.65 \pm 0.22$). Selected regions from three different cells are included in panel c.