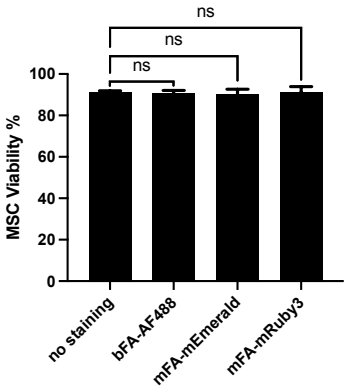


Figure S1. Fluorescent Fetuin-A proteins are non-toxic in mesenchymal stromal cells (MSC). Viability was tested by Trypan Blue dye exclusion 72 h after a 2 h incubation with the indicated fluorescent fetuin-A derivatives. Cells were treated in triplicated wells of a 24-well plate. A minimum of 400 cells was counted in each well. Values represent mean ± SE, n=3.

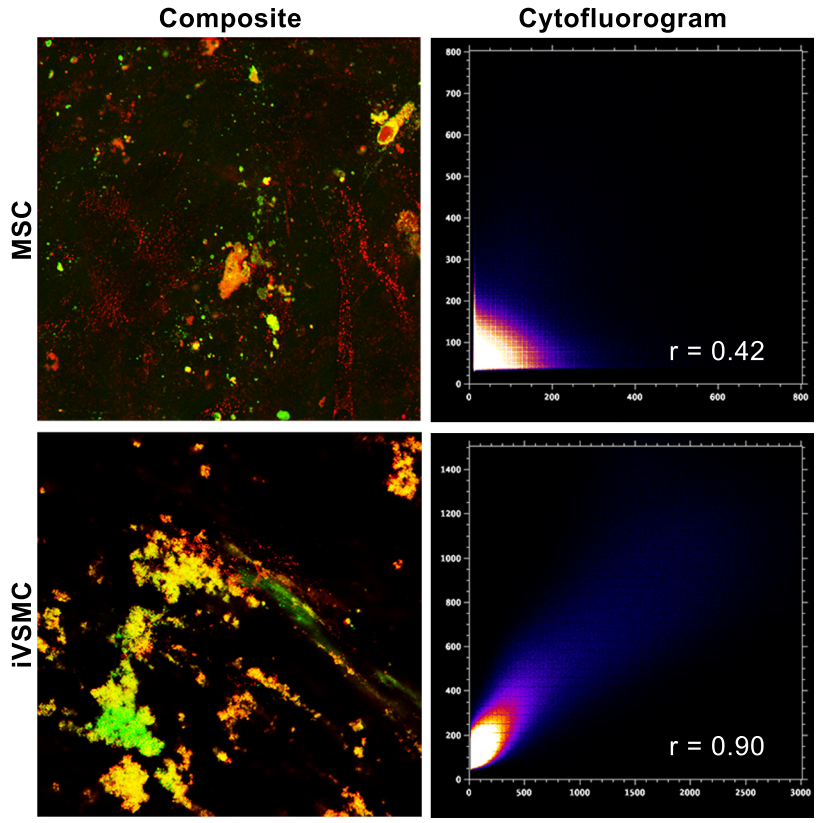


Figure S2. Composite images and co-localization analysis of mineral deposits in mFA-mRuby3- (left panel, red) and calcein-stained cells (left panel, green). Right panel: corresponding cytofluorograms. The intensity of pixel fluorescence increase is shown using a heatmap. r-value: the Pearson correlation coefficient.

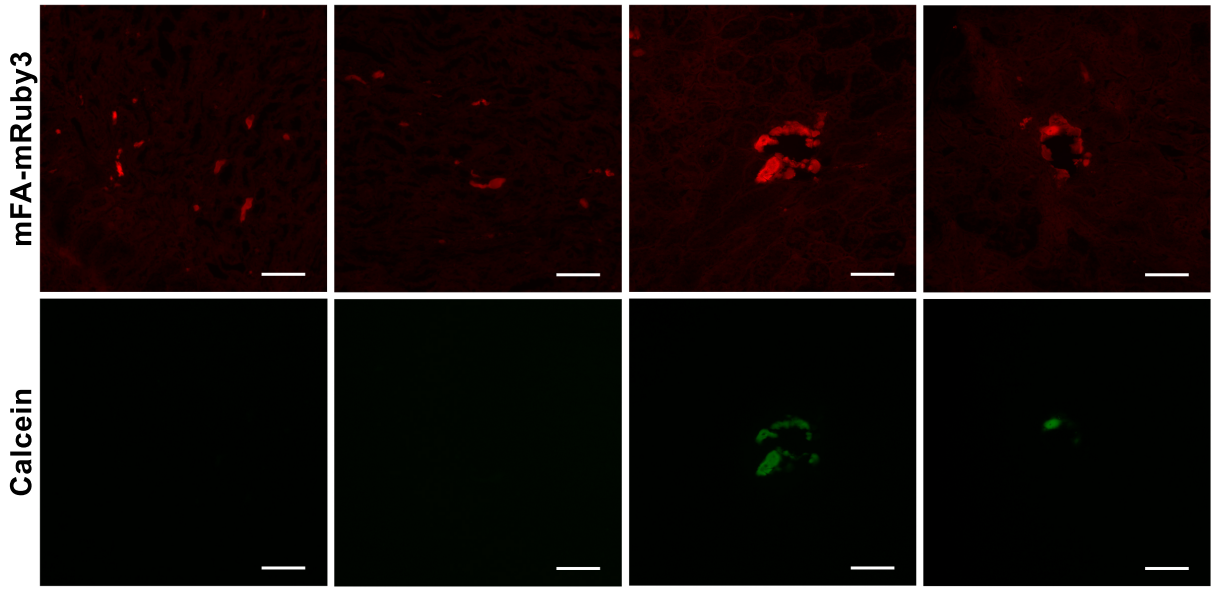
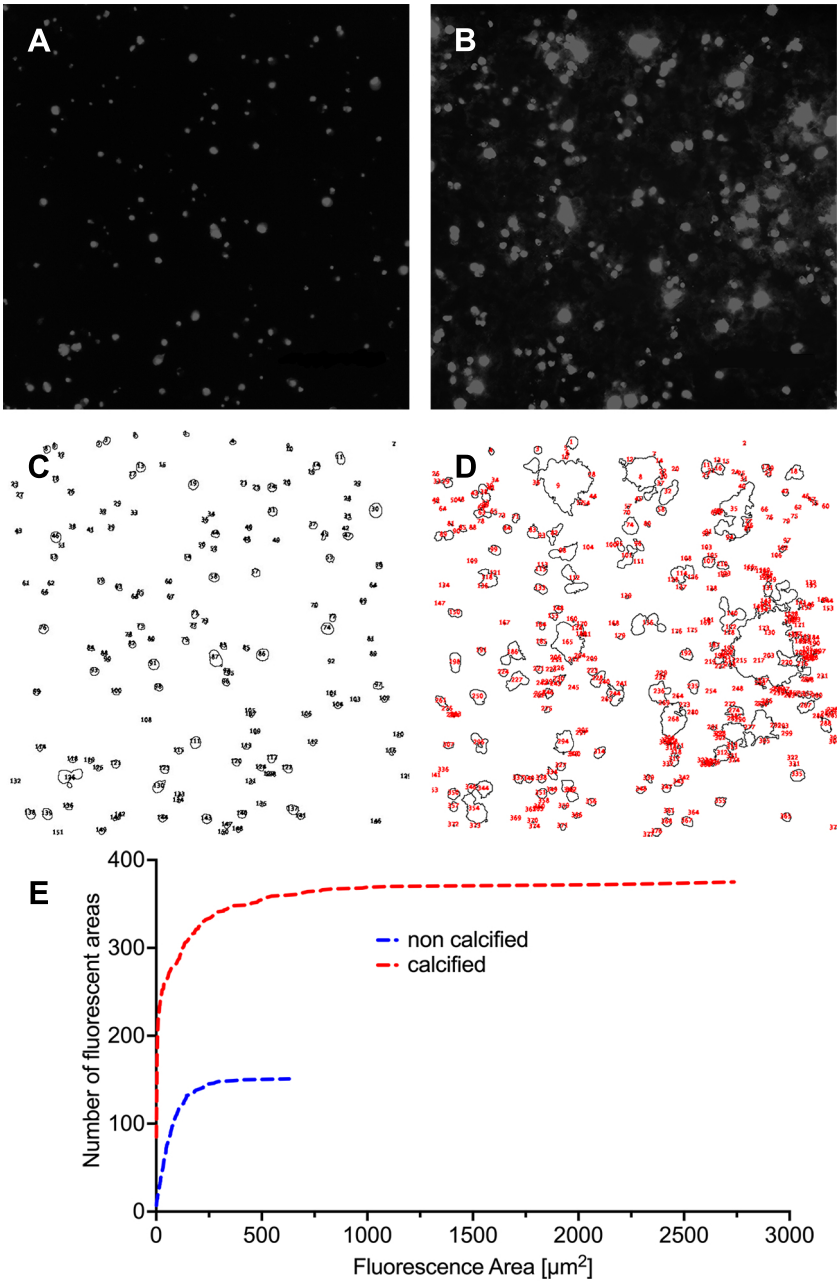


Figure S3. Simultaneous staining of kidney calcifications with mFA-mRuby3 and calcein. Upper panel: mFA-mRuby3-derived signal (red). Lower panel: calcein-derived fluorescence (green). Scale bars 100 µm.



779

780 **Figure S4.** Analysis of calcified areas depicted in Figures 7C, D. **(A, B)** Typical micrographs were
781 converted to 8-bit grayscale images. **(C, D)** Particle analysis was performed with a threshold set manually
782 to match the contours of fluorescent areas. (**E)** Accumulate frequency distribution of areas identified in C
783 and D showing that both the number and the size of areas increased in calcified HEK293 cells expressing
784 mFA-mRuby3.

785

786 Table S1. Comparison of stains used for histopathological assessment of calcification.

Stain	**Chemical composition**	**Sensitivity**	**Specificity**	**Suitability for histological staining**	**Suitability for live imaging**	**Cell reporter system**
Alizarin Red S	Alizarin sulfonic acid sodium salt	+	+ (binds carbonates)	yes	no (requires fixation)	no
Calcein	Fluorescein complex	++	+ (binds Cu^{++}, Zn^{++}, Al^{++})	yes	yes	no
bFA-AF488	bovine fetuin-A labeled with Alexa Fluor™ 488 NHS ester	+++	binds nascent apatite	yes	yes	no
pm-mFA-mEmerald	murine fetuin-A coupled to a GFP derivative	+++	binds nascent apatite	yes	yes	yes
pm-mFA-mRuby3	murine fetuin-A coupled to an GFP derivative	+++	binds nascent apatite	yes	yes	yes

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