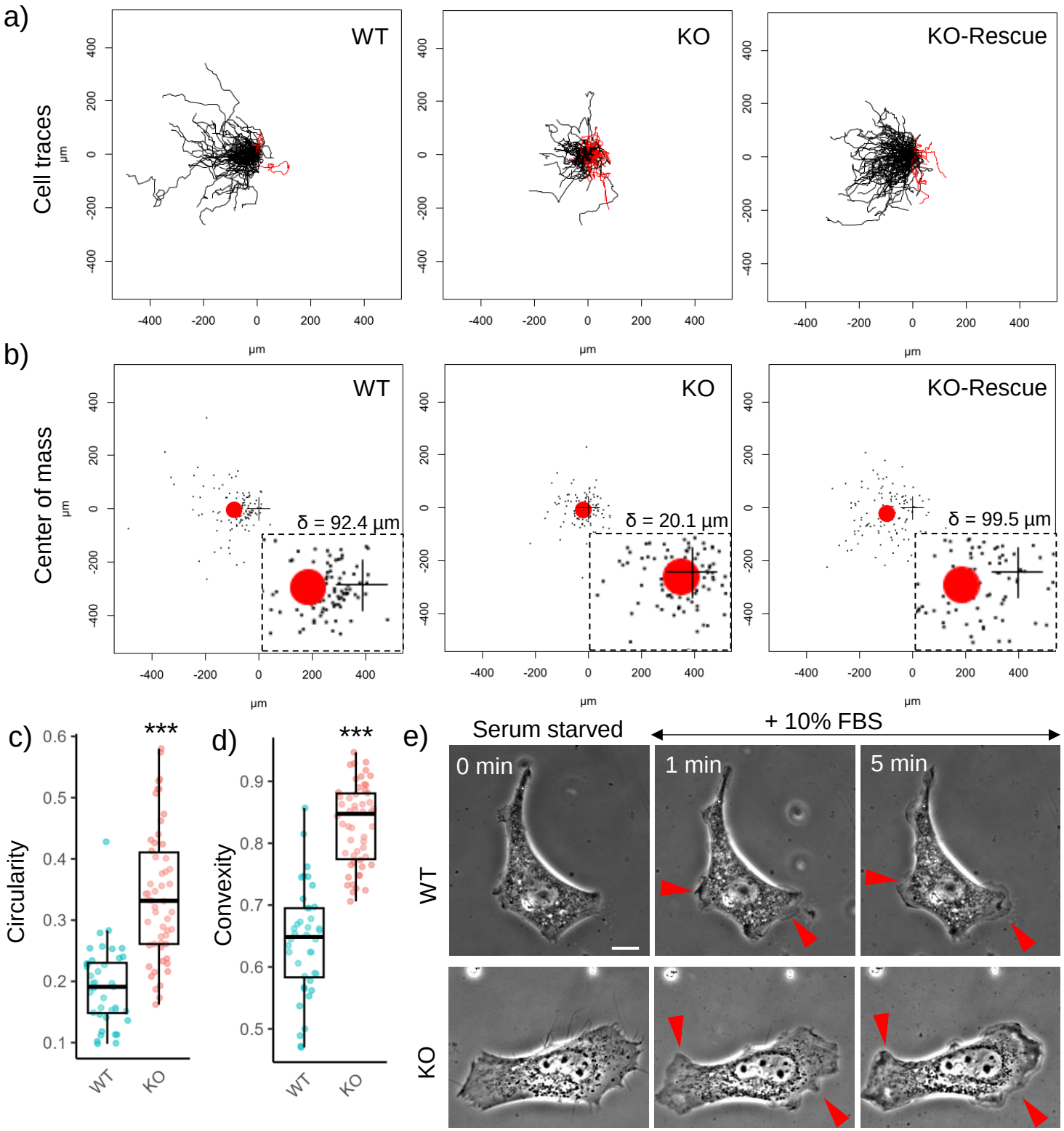
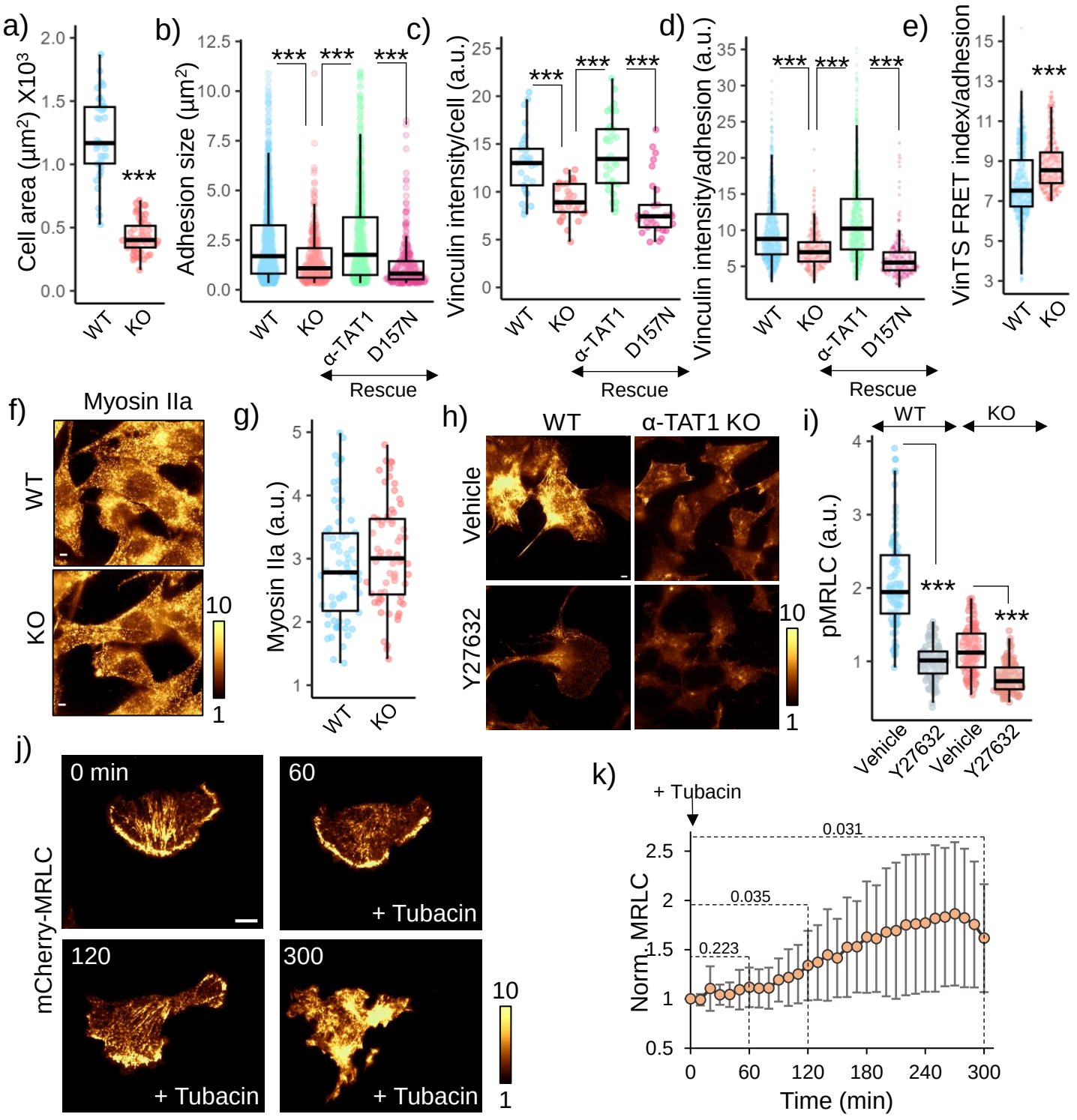
Supplementary Figure 1



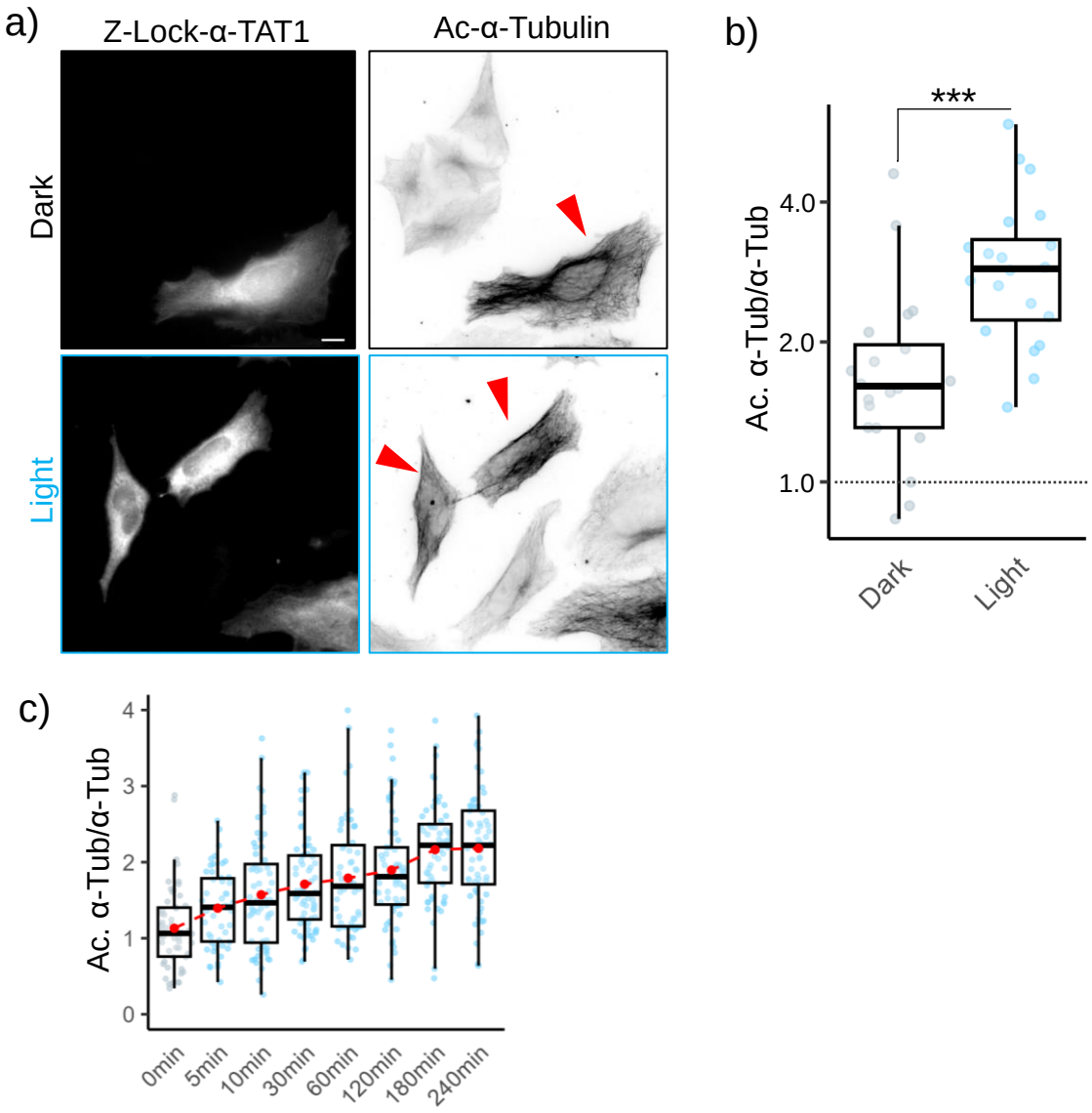
Supplementary Figure S1. a) Tracks of WT, α-TAT1 KO and KO-rescue with α-TAT1 MEFs in chemotaxis assay, n = 120 cells (40 each from three independent experiments); b) Final location of individual cells (black dots) and the center of mass of all the cells (red circle) in chemotaxis assay, origin is indicated by "+", distance between origin and center of mass (δ) is shown above the inset; c) Circularity and d) Convexity of WT α-TAT1 KO MEFs (WT: 40 and KO: 54 cells); e) Morphological changes in serum-starved WT and α-TAT1 KO MEFs on addition of 10% FBS, induced protrusions are indicated with red arrowheads, scale bar: 10 µm. ***: $p<0.001$

Supplementary Figure 2



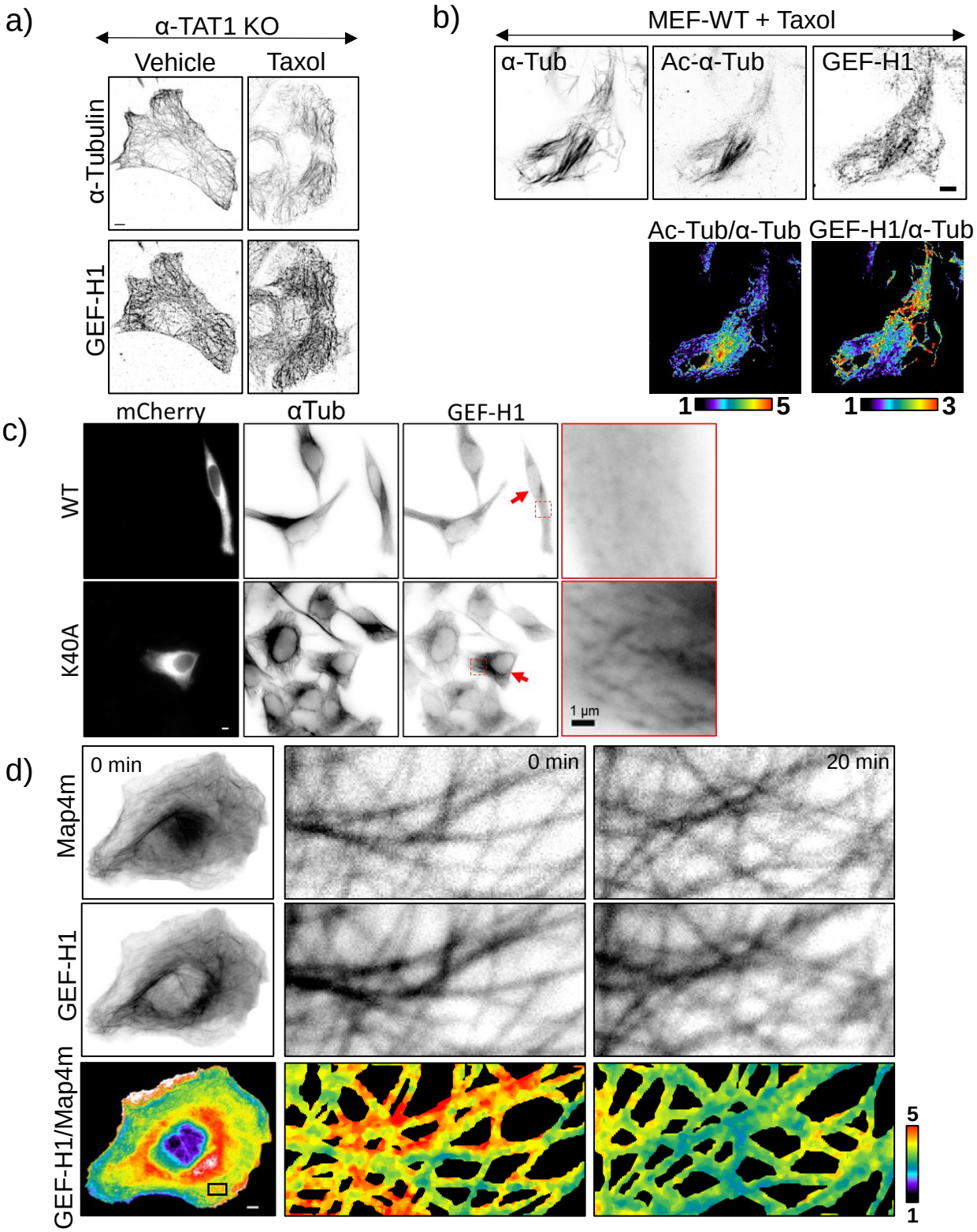
Supplementary Figure S2. a) Cell areas of WT and α-TAT1 KO MEFs (WT: 40, KO: 54 cells); b) adhesion sizes, c) average vinculin intensity per cell, d) average vinculin intensity per adhesion in WT, α-TAT1 KO, rescue-WT and rescue-D157N MEFs (WT: 20, KO: 17, rescue-WT: 16, rescue-D157N: 22 cells); e) VinTS FRET index per adhesion in WT and α-TAT1 KO MEFs (WT:: 18, KO: 16 cells); f), g) Myosin IIa levels in WT and α-TAT1 KO MEFs (WT: 69, KO: 65 cells); h), i) Phospho-MRLC levels in WT and α-TAT1 KO MEFs treated with vehicle or 10 μM Y-27632 (WT-vehicle: 88, WT-Y27632: 91, KO-vehicle: 89, KO-Y27632: 98 cells); j) TIRF images of and k) changes in fluorescence intensity of mCherry-MRLC in WT MEFs on tubacin treatment, 12 cells, mean ± 95% C.I.; scale bar: 10 μm. ***: p<0.001

Supplementary Figure 3



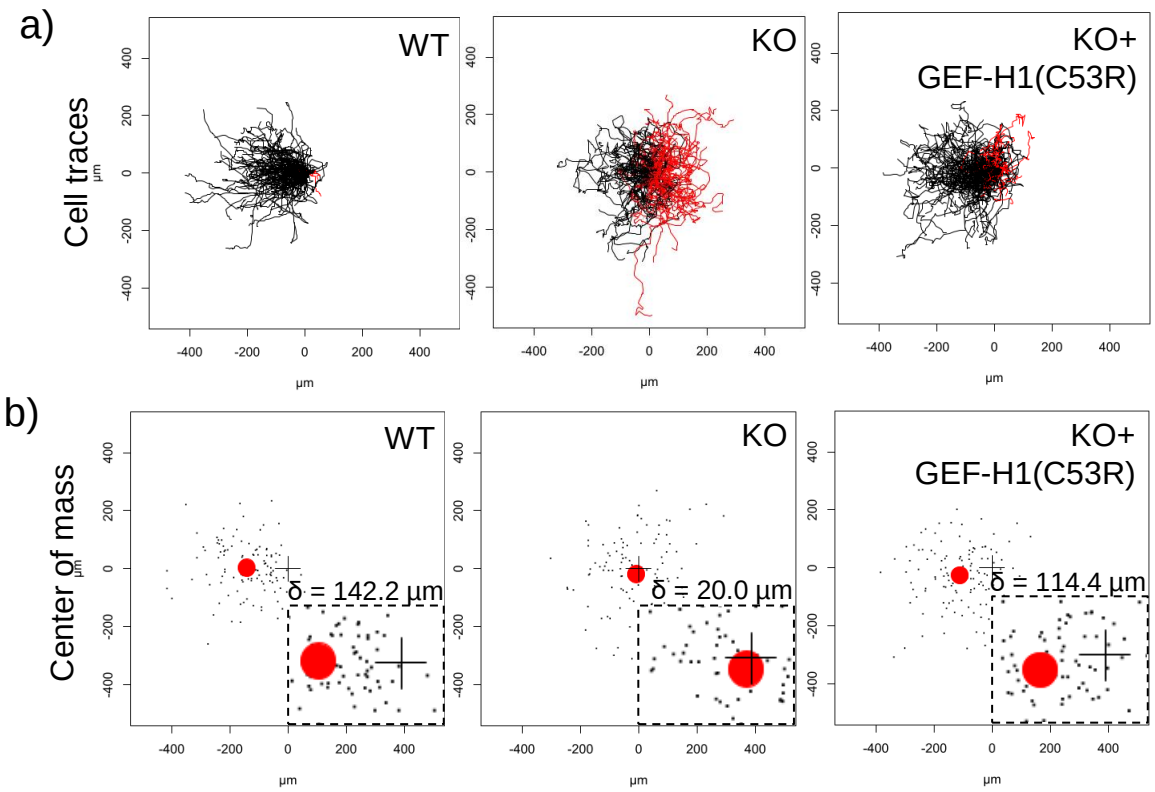
Supplementary Figure S3. a) Microtubule acetylation levels in HeLa cells exogenously expressing mCherry-Z-Lock-α-TAT1, kept in dark or exposed to blue light for 2 hours, red arrowheads indicate transfected cells; scale bar: 10 μm; b) Microtubule acetylation levels in Hela cells expressing mCherry-Z-Lock-α-TAT1 in dark or after blue light exposure, normalized against acetylation levels in non-transfected cells; c) Temporal changes in acetylated microtubules (normalized against total α-Tubulin) on blue light stimulation of HeLa cells stably expressing mVenus-optoTAT V2 (0 min: 54, 5 min: 50, 10 min: 61, 30 min: 66, 60 min: 61, 120 min: 62, 180 min: 60 and 240 min: 61 cells), red dots indicate the mean values; note: time scale is not linear.

Supplementary Figure 4



Supplementary Figure S4. a) Immunostaining against α-Tubulin and GEF-H1 α-TAT1 KO MEFs treated with vehicle (DMSO) or 100 nM Taxol overnight; b) Immunostaining against α-Tubulin, acetylated α-Tubulin and GEF-H1 in WT MEFs treated with 100 nM Taxol overnight; c) Immunostaining against α-Tubulin and GEF-H1 in HeLa cells expressing mCherry-α-Tubulin or mCherry-α-Tubulin(K40A) (lower panels), transfected cells are indicated with red arrowheads, insets are magnified on the right panel; d) Changes in mCherry-GEF-H1/mVenus-MAP4m signal in HeLa cells expressing miRFP703-optoTAT on blue light stimulation, inset is magnified in the right panels; Scale bar: 10 µm or as indicated.

Supplementary Figure 5



Supplementary Figure S5. a) Tracks of WT, α-TAT1 KO and KO-rescue with mCherry-GEF-H1(C53R) MEFs in chemotaxis assay, n = 120 cells (40 each from three independent experiments); b) Final location of individual cells (black dots) and the center of mass of all the cells (red circle) in chemotaxis assay, origin is indicated by "+", distance between origin and center of mass (δ) is shown above the inset.