SUPPLEMENTARY FIGURE LEGENDS

FIG. S1. Stat3 level directly correlates with SrcY527F-cell's ability to produce podosome and digest ECM. (a-d) SrcY527F-cells co-expressing shStat3 were immunostained for Stat3. F-actin was stained with FITC-phalloidin. shRNA-mediated knockdown of Stat3 (marked with white arrowheads) as identified by decreased Stat3 staining resulted in diminished ability of SrcY527F-cells to produce podosomes and rosettes. (e-h) SrcY527F cells stably transduced with Stat3-targeted shRNAs were used to study the effect of Stat3 knockdown on Src-induced ECM digestion. Cells were cultured on gelatin coated coverslips layered with TRITC-Fibronectin for 7 hours. Cells expressing shStat3, as monitored by reduced Stat3 staining (marked with arrowheads), also show greatly reduced capacity to degrade ECM. Scale bars represent 20 μm.

FIG. S2. Stat3 colocalization to podosome structures. SMC and 3T3 cells stably expressing SrcY527F were immunostained with the antibodies as shown. Phalloidin-350 was used to label F-actin. Scanning laser confocal microscopy (a-h) showing x/z profiles (inset), were taken at the line drawn through the cells in respective panels. Each z axis is 4.6 μm in depth. Both Stat3 and Stat3-pY705 are present in the podosome forming actin columns (a and b) as well as Stat3 probed by either rabbit (a-f) or mouse (g-h) antibodies shows similar tendency. Scale bars represent 20 μm.

FIG. S3. Effect of Src/Stat3/p53 on cell migration induced by scratched wound. Stable cell lines as indicated were generated for both SMC (a) and 3T3 (b) cells. In a scratch induced wound healing assay the initial wound gap (at 0 hour) and the residual gap (after 15 hours), at the same location, for each cell type are shown (denoted with dotted lines). The photomicrographs shown were generated through time lapse photography and are representative of three independent experiments. Scale bars represent 100 μm.

FIG. S4. Src, through the activation of Stat3, suppresses p53 and p53-inducible, podosome-antagonist, caldesmon. SMC-SrcY527F (a, c and e-h) 3T3-SrcY527F (b and d) cells were cultured on Fibronectin-coated coverslips. Cells were either treated with vehicle (DMSO) or with 20 μMole of Src kinase inhibitor (PP2) as indicated. (a-d) Inhibition of Src function resulted in increased p53 level, prominent stress fibers and abolished podosome structures in both cell types compared to the DMSO-treated control cells which contain many podosomes and rosettes but few stress fibers. (e and f) SMC-SrcY527F cells predominantly showing nuclear Stat3 (transcriptionally active form of Stat3) staining (e); blocking Src function with PP2 resulted in cytoplasmic sequestration of Stat3 (transcriptionally inactive form of Stat3) with concomitant increase in p53 levels as revealed by stronger nuclear p53 staining (f). (g and h) SMC-SrcY527F cells showing strong nuclear Stat3 staining, less caldesmon-labeled stress fibers and numerous podosomes/rosettes (g); however inhibition of Src-kinase activity leads to diffuse Stat3 expression, prominent caldesmon-labeled stress fibers, and greatly reduced number of podosomes/rosettes (h). (i and j) SrcY527-cells transfected with wtp53, also treated with doxorubicin to over-activate p53. Cells showing robust p53 activation (indicated with arrowheads) concomitantly express prominent, caldesmon-decorated, stress fibers. (k and l) SrcY527-cells were transfected with wild type caldesmon expression construct (EGFP-Cald) and monitored for podosome formation. Cells expressing EGFP-Cald (marked with arrowheads) produce highly reduced number of podosome/rosettes compared to their non-transfected counterpart.

FIG. S5. p53 mutant fails to suppress Stat3 activation in cancer cells.

Prostate cancer cell line, LNCaP and breast cancer cell line, MCF-7 both contain wild-type-p53 were compared with their respective counterparts, Du145 and MDA-MB-231, which have

mutant-p53. (a) Western blot analysis shows that mutant-p53 cell lines tolerate high levels of p53 compared to the wild-type-p53 cell lines, which contain low levels of p53. The mutant-p53 fails to suppress activation of Stat3 (Stat3 pY705). GAPDH was used as a loading control.

FIG. S1. Stat3 level directly correlates with SrcY527F cell's ability to produce podosome and digest ECM

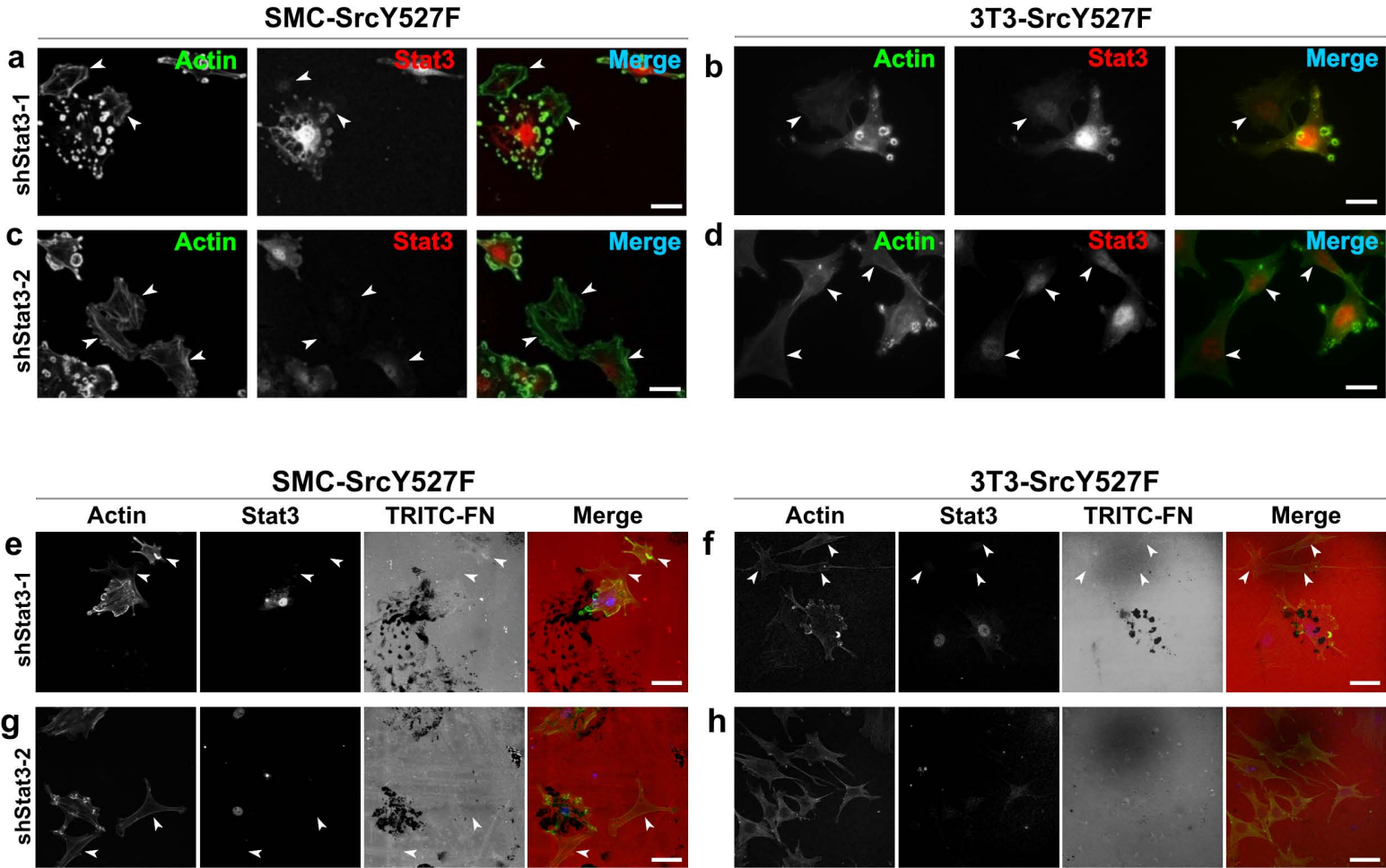


FIG. S2. Stat3 colocalization to podosome structures.

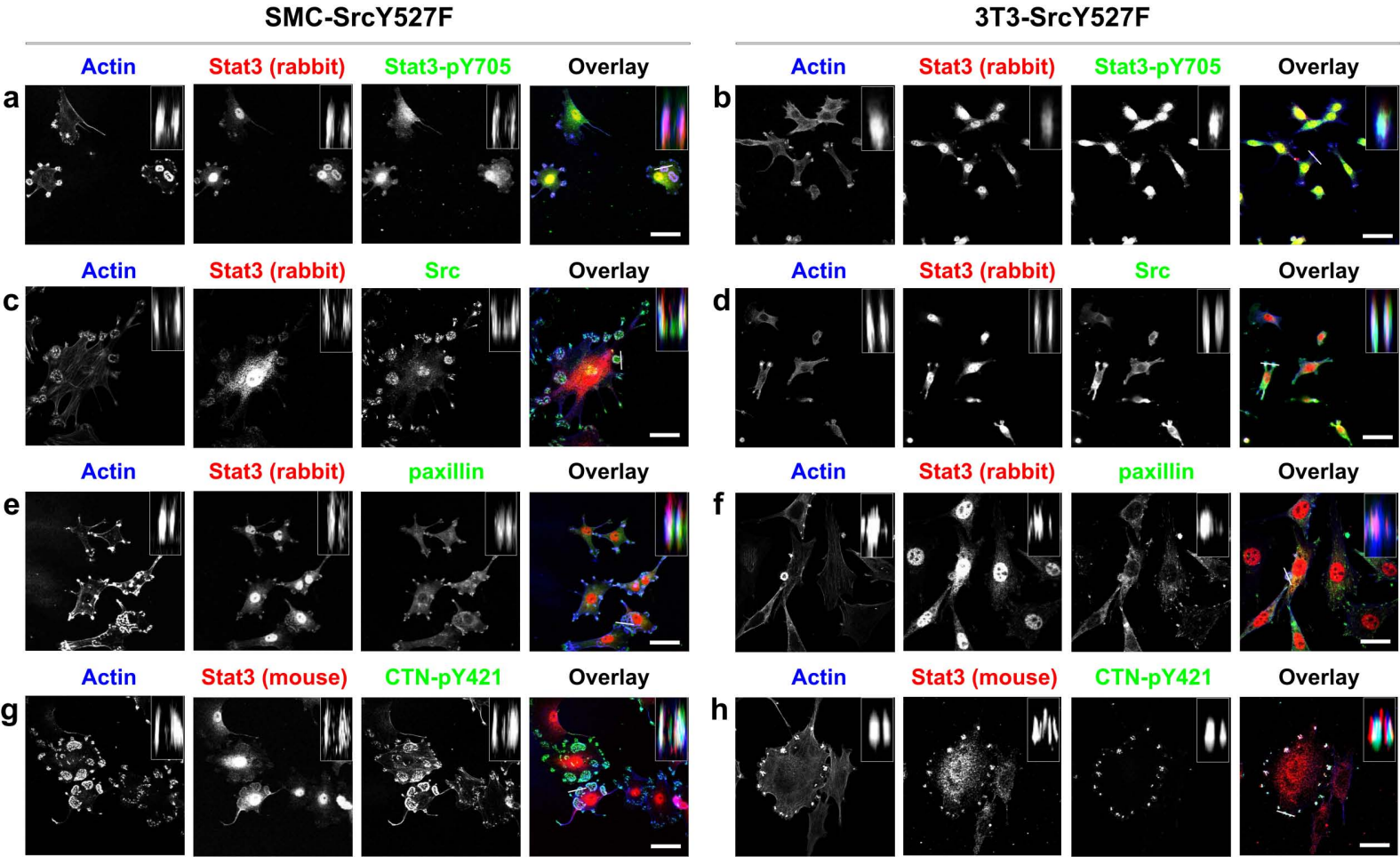
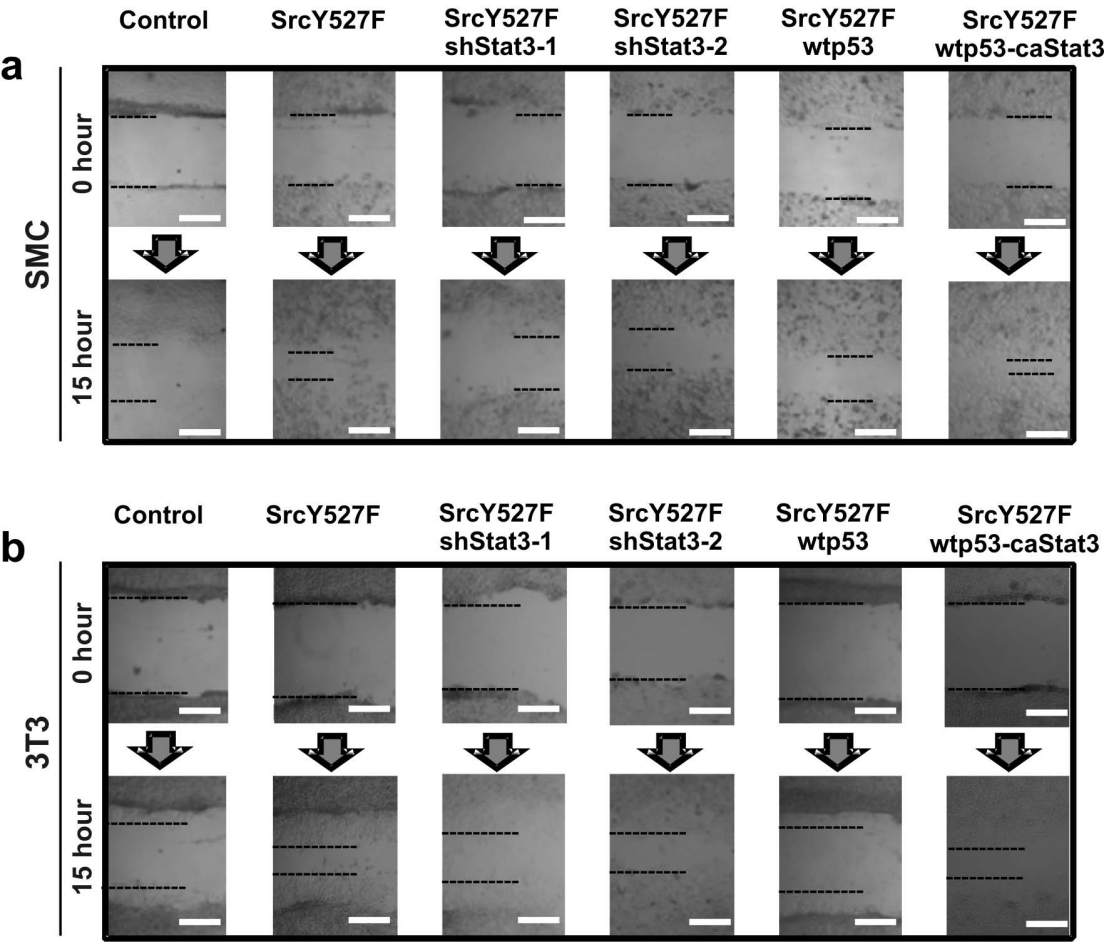


FIG. S3. Effect of Src/Stat3/p53 on cell migration induced by scratched wound.



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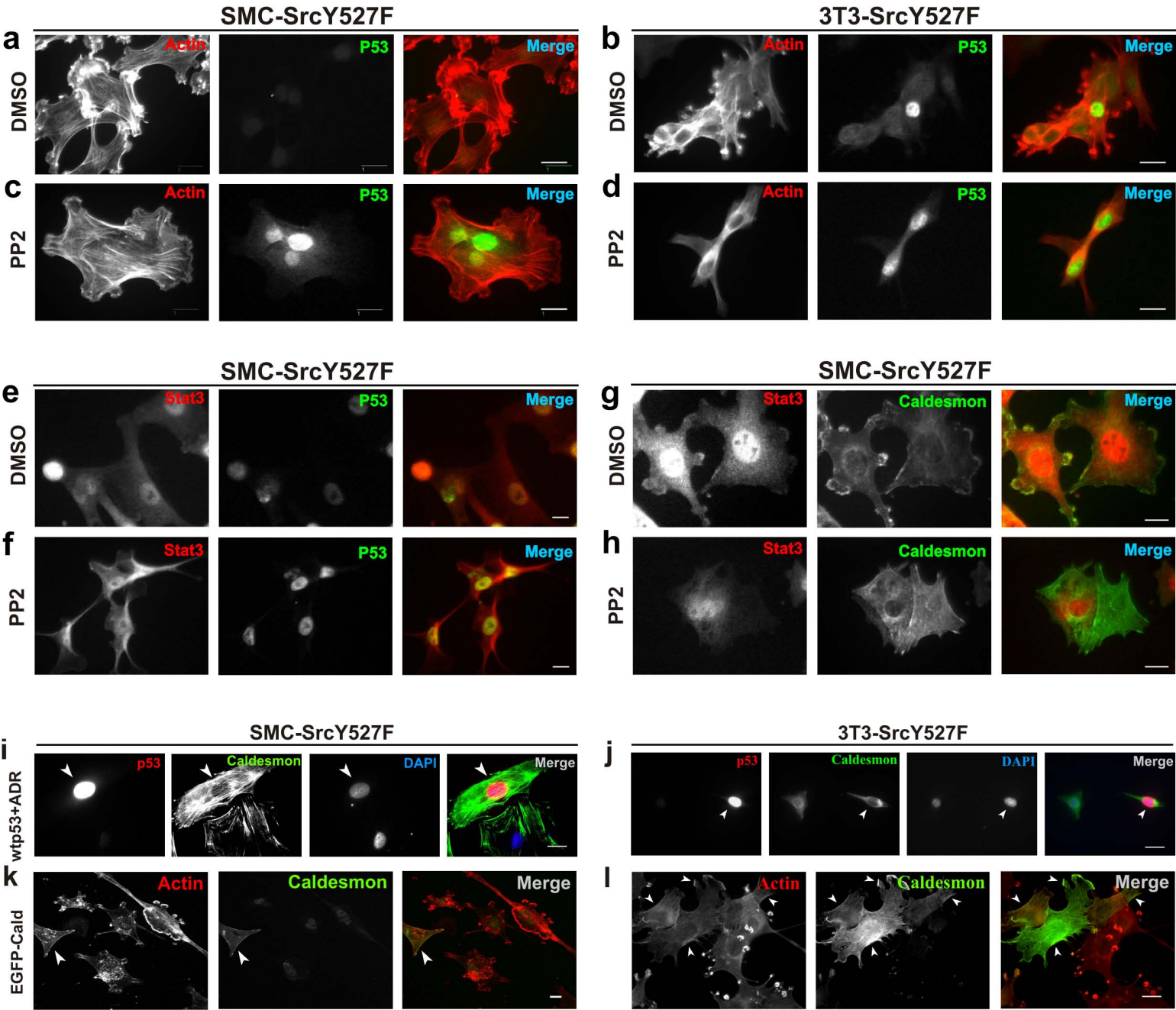

FIG. S5. p53 mutant fails to suppress Stat3 activation in cancer cells.