Supplemental Figure 1. FN production is attenuated in KRA2 cell clones compared with CCL39 cells (A) Immunolabeling of secreted (left) and total (right) fibronectin in NHE1-expressing CCL39 fibroblasts, which are parental cells for NHE1-deficient PS120 cells. (left, live cell labeling, red: extracellular FN, blue: Hoechst staining for nuclei) (right, fixed cell labeling, green: total FN, blue: Hoechst staining for nuclei). (B) Immunolabeling of total FN in the indicated KRA2 clones confirms attenuated FN production is not a clonal variation (green: total FN, blue: Hoechst staining for nuclei).

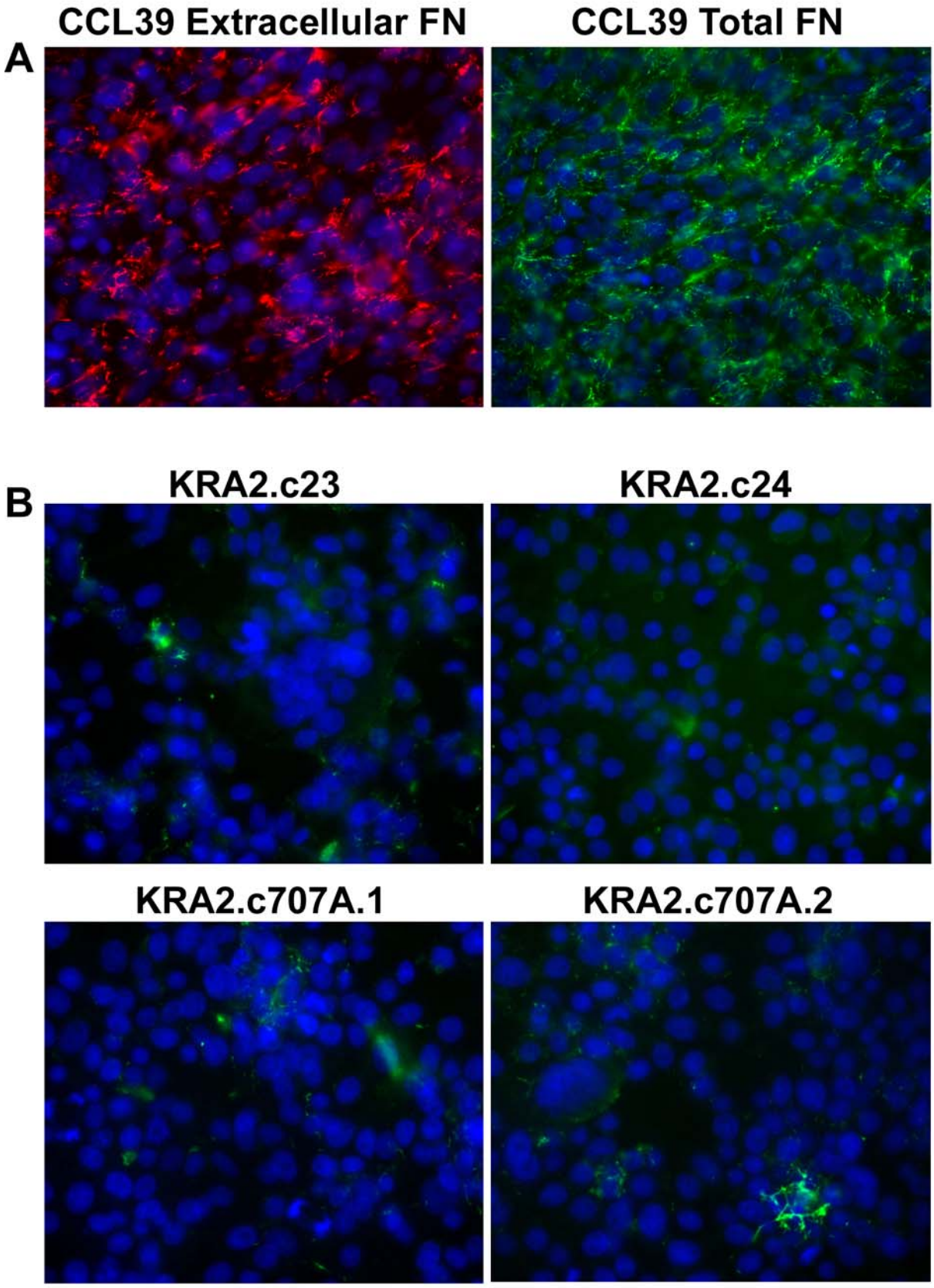
Supplemental Figure 2. KRA2 cells do not assemble a matrix of exogenous FN. Fluorescence and phase images of KRA2 and WT cells maintained in medium containing FITC-labeled FN (15 μg/ml) (Bar, 20 μm).

Supplemental Figure 3. β1 integrin expression is not decreased in KRA2 cells. Representative data from flow-cytometry analysis showing surface expression of β1 integrin in WT and KRA2 cells at 0 and 5 μg/ml anti-β1 9EG7 antibody.

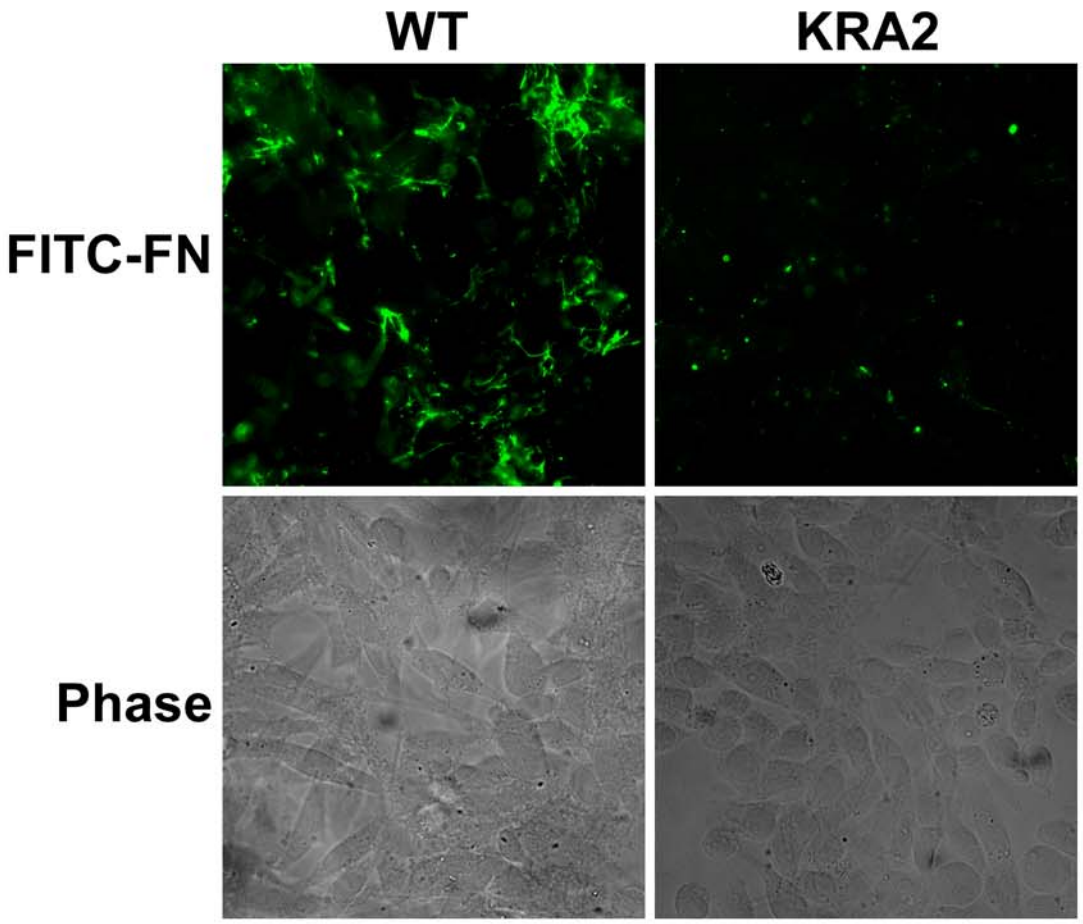
Supplemental Figure 4. FN binding is not decreased in KRA2 cells. (A) Representative data from flow-cytometry analysis showing integrin affinity and FN binding of WT and KRA2 cells with FITC-labeled FN at 0 and 450 nM. (B) Integrin affinity to 112.5 nM FITC-FN is inhibited by incubation with increasing concentrations of an integrin antagonist RGD fragment. Data are means of 3 separate binding assays.

Supplemental Figure 5. KRA2 cells have delayed autophosphorylation of FAK. (A) Time-dependent increase in autophosphorylation of FAK after plating cells on FN (10 μg/ml) determined by immunoblotting cell lysates with antibodies to phosphorylated FAK-Y397 is delayed in KRA2, E266I, and PS120 cells compared with the WT cells. (B) Abundance of FAK autophosphorylation relative to total FAK, determined by immunoblotting, after plating WT and KRA2 cells for the indicated times on FN. Data represent means ± s.e.m. of 5 separate cell preparations.

Supplemental Figure 6. Inhibition of TGF-β-RI inhibits FN production. Immunolabeling of extracellular FN in live WT and KRA2 cells treated with the TGF-β-RI inhibitor SB-431542 (10 mM) in the absence and presence of TGF-β. (green: extracellular FN, blue: Hoechst staining for nuclei).



Supplemental Fig. 1. Karydis et al.



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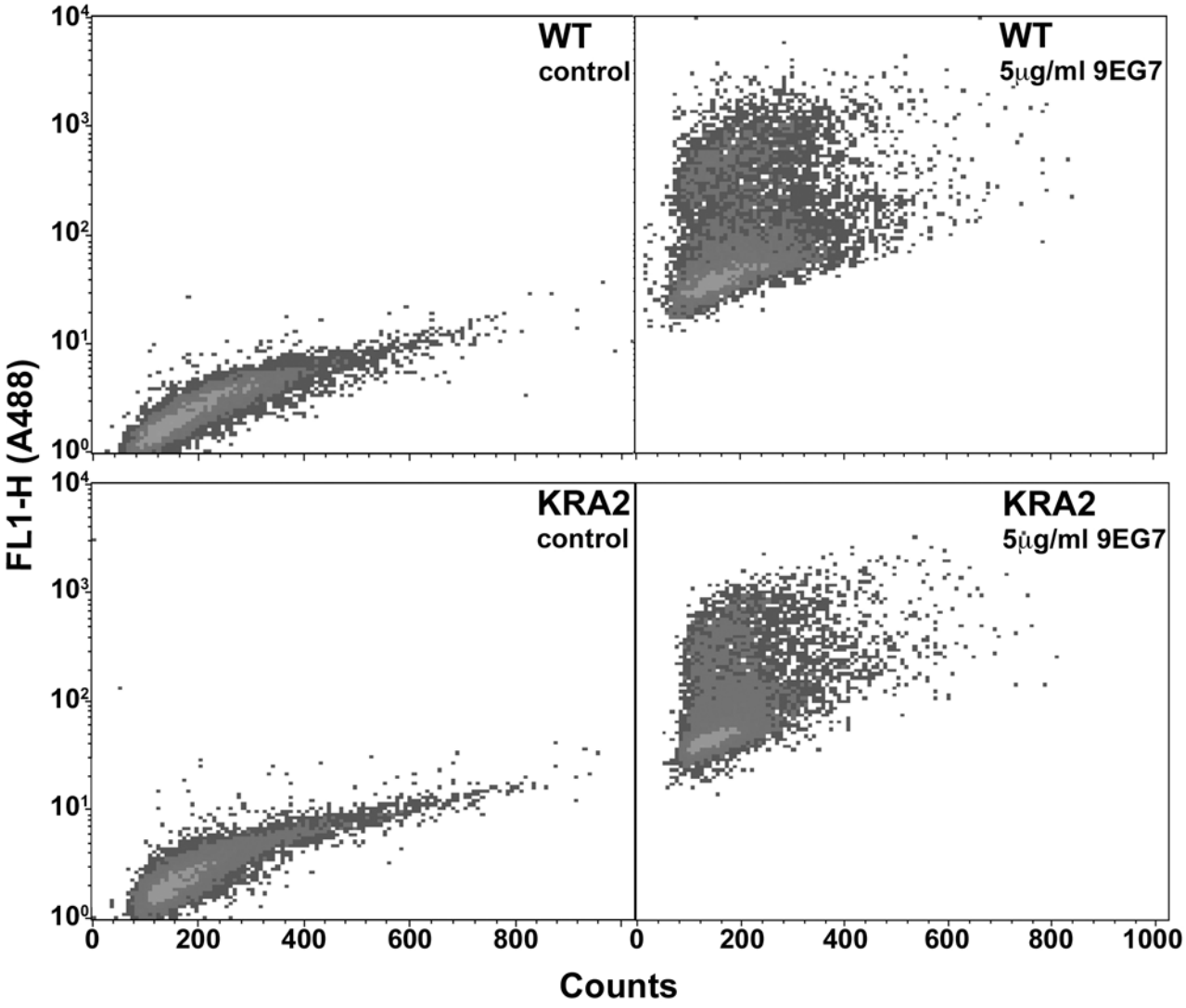

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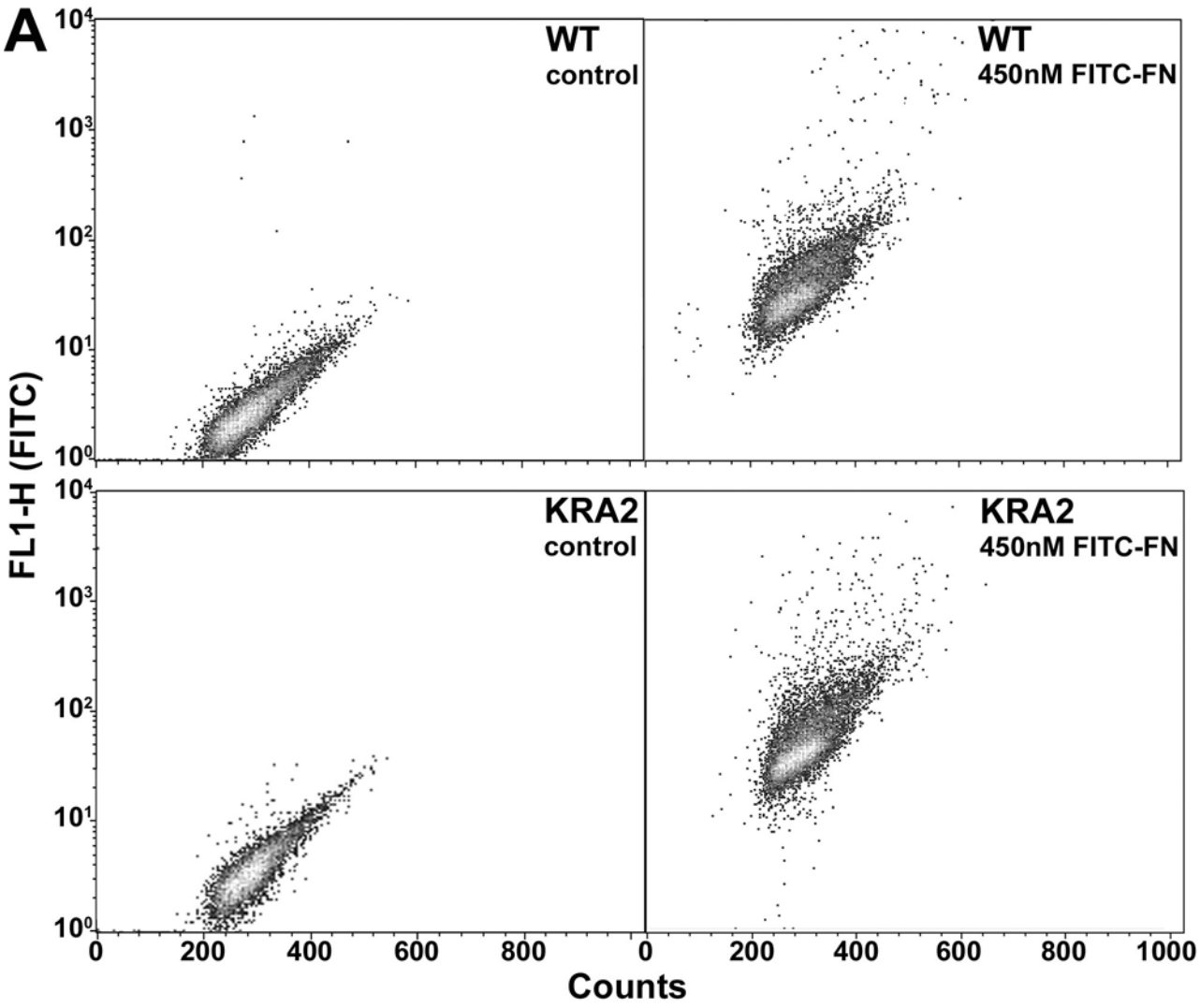
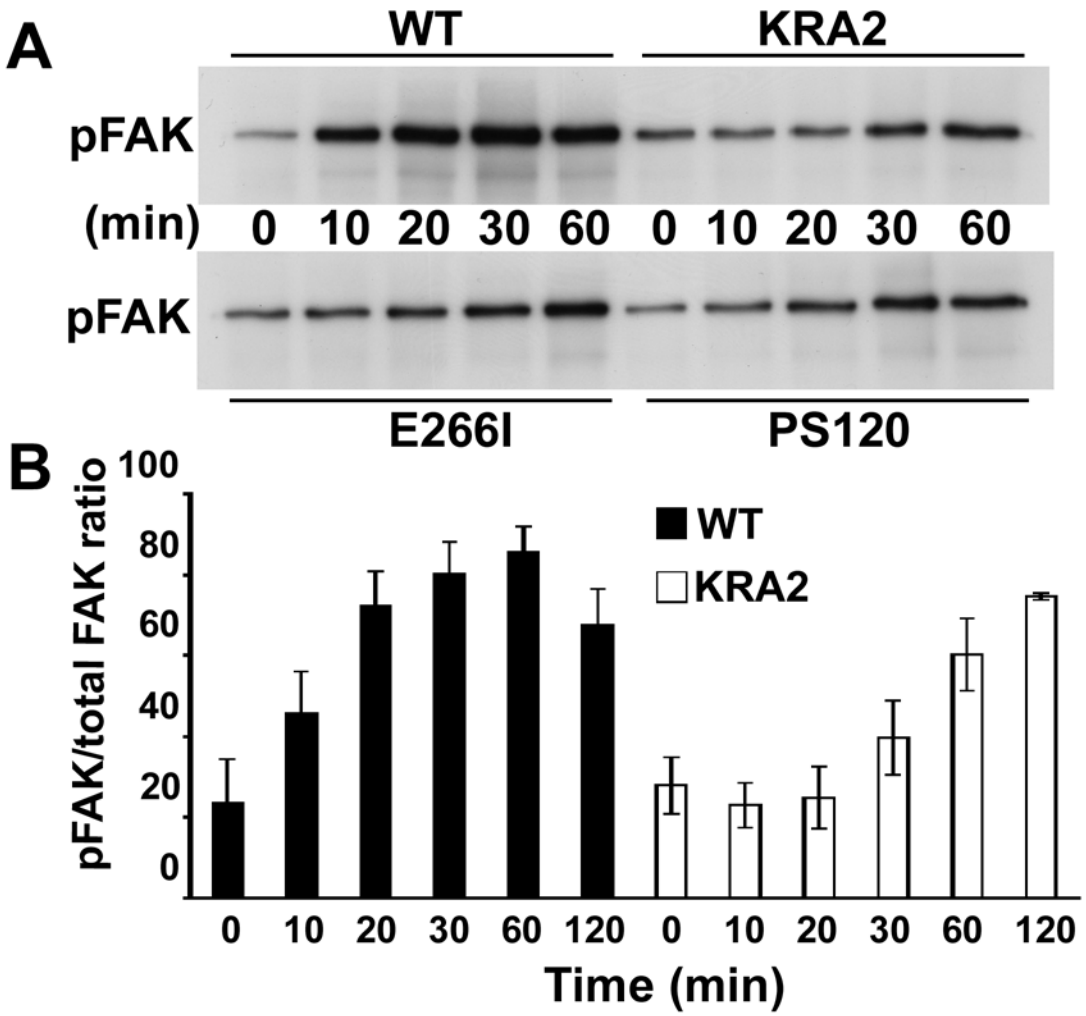
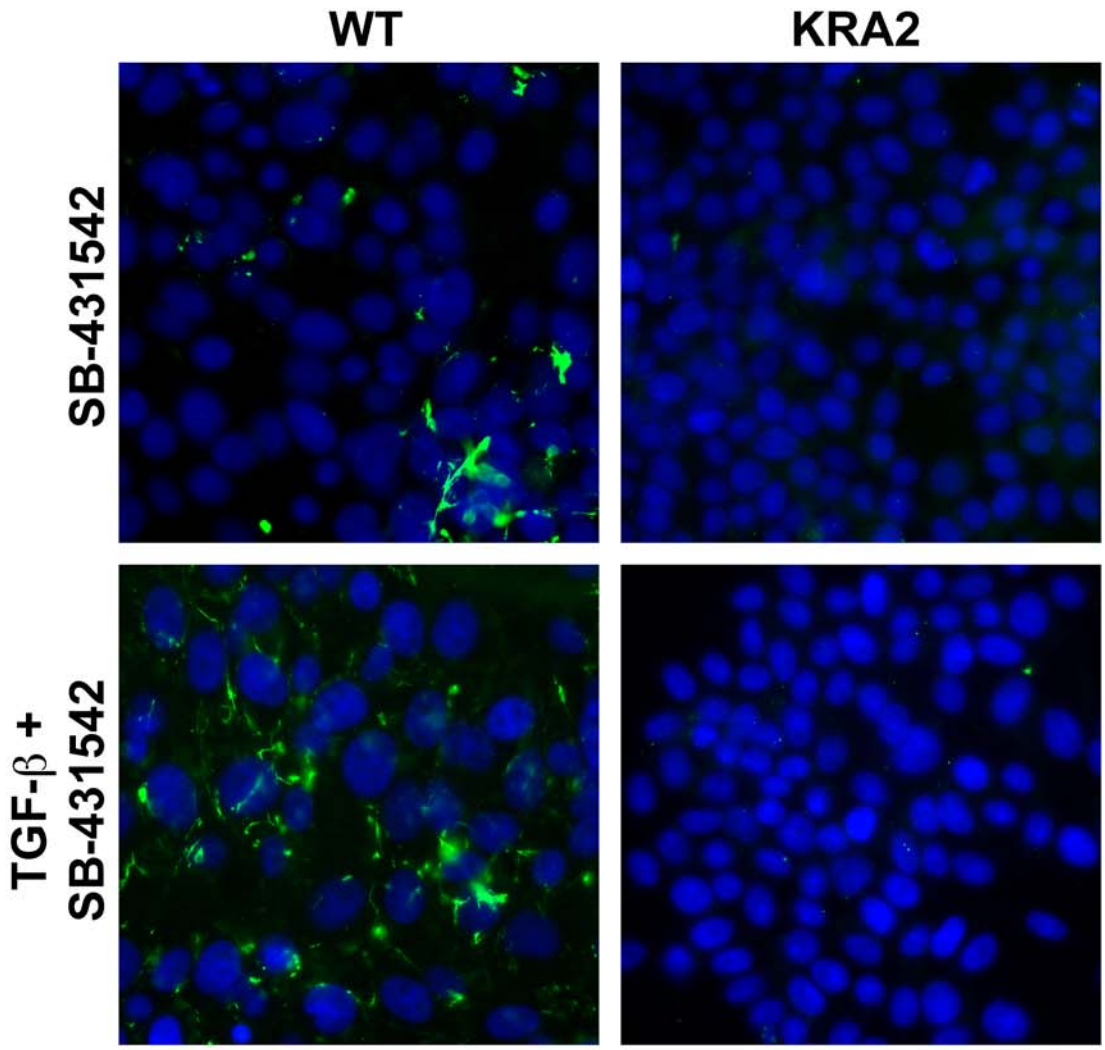


Supplemental Figure 4. Karydis et al.



Supplemental Figure 5. Karydis et al.



Supplemental Figure 6. Karydis et al.