Supplementary Material

Gprc5a deletion enhances the transformed phenotype in normal and malignant lung epithelial cells by eliciting persistent Stat3 signaling induced by autocrine Lif

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Running Title: Stat3 activation in *Gprc5a* knockout lung cells

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Supplementary Figure Legends

Supplementary Figure S1. Autocrine Lif mediates Stat3 activation in MDA959 lung tumor cells. *A*, cells were treated with AG490 (30 μM) or DMSO in DMEM/F12 medium for 24 hours and extracted and analyzed by immunoblotting using antibodies against Stat3 and pStat3. *B*, MDA959 cells were cultured in DMEM/F12 for 24 hours and then the medium was collected and analyzed for secreted Lif by ELISA. DMEM/F12 medium without conditioning by cells was used as a control. Triplicate samples were analyzed and the data are presented as Mean ± SD. *, $P < 0.05$. *C*, cells were starved for one hour in DMEM/F12 medium without serum then treated with

conditioned medium from 24-hour cultures of MDA959 cells (CM959) or with conditioned medium preincubated with neutralizing antibody against Lif or with normal goat IgG (both at 30 μg/ml) for one hour. The cells were then used for analysis of Stat3 activation by immunoblotting as in A.

Supplementary Figure S2. . Inhibition of Stat3 activation increases stress-induced apoptosis and inhibits colony formation in MDA959 lung tumor cells. *A*. MDA959 cells transfected with vector or dominant negative Stat3(Y705F) were starved for 48 hours in serum-free medium then the cells'total proteins were extracted and analyzed by immunoblotting using the indicated antibodies. *B*, *left*, MDA959 cells transfected with vector or Stat3(Y705F) were starved for 48 hours then harvested and subjected to analysis of apoptosis. *Right*, MDA959 cells transfected with vector or Stat3(Y705F) were suspended in Matrigel and analyzed for colony formation after two weeks. *C*, *left*, MDA959 cells were treated with AG490 (30 μM) or DMSO in DMEM/F12 medium without serum for 48 hours then harvested and subjected to analysis of apoptosis. *Right*, MDA959 cells were suspended in Matrigel with AG490 (30 μM) or DMSO and analyzed for colony formation after two weeks. Data were derived from assays performed in triplicates and are presented in bar graph as mean ± SD. *, $P < 0.05$.

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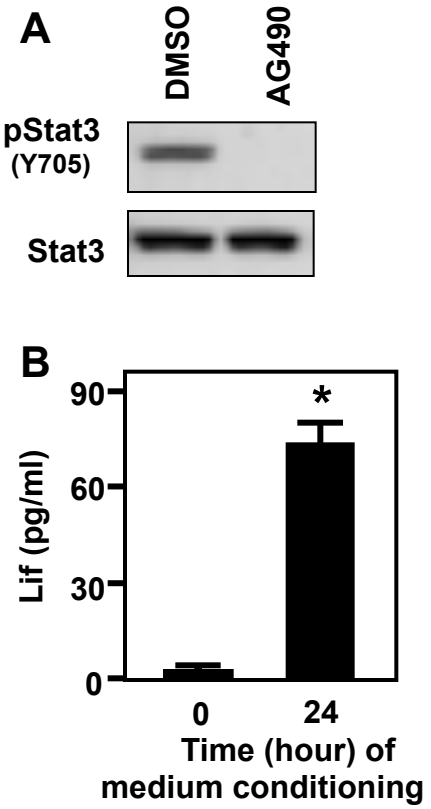

Fig. S2

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