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Supplemental Data

Article

Persistently Activated Stat3

Maintains Constitutive NF-κB Activity in Tumors

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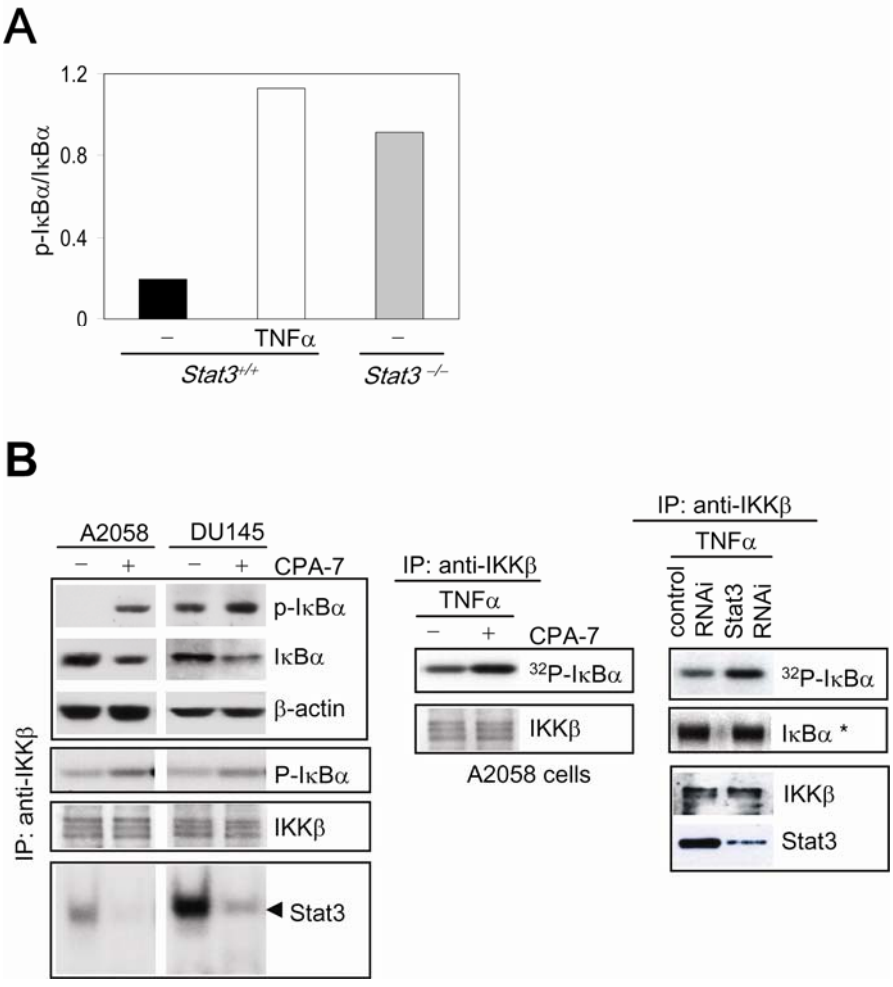

Figure S1.

Figure S1. Stat3 Inhibits IKK Activity in Both Splenic and Tumor Cells

(A) Ablation of Stat3 in hematopoietic systems elevates IKK activity. IKK activity was determined by ELISA as the ratio of p-IκBα to IκBα levels in splenic cells isolated from *Stat3+/+* and *Stat3−/−* mice. TNFα stimulation was used as a positive control.

(B) Inhibition of Stat3 either by a selective inhibitor, CPA-7, or Stat3 siRNA, increases IKKβ activity in tumor cells. Top left: cells were treated with CPA-7, followed by immunoprecipitation with anti-IKKβ. Western blotting analysis was used to determine p-IκBα and IκBα levels. Lower left: in vitro IP-kinase assays were performed in A2058 and DU145 cancer cells using the recombinant IκBα protein as the substrate. Middle and right panels: tumor cells were treated with Stat3 inhibitor (middle) or siRNA (right) then TNFα to induce IKKβ activity. *IκBα levels were determined by Coomassie staining.

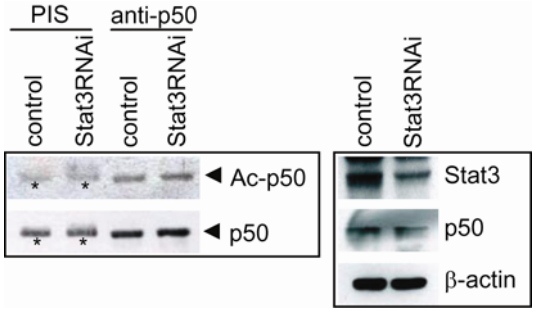

Figure S2. Effects of Stat3 Knockdown on p50 Acetylation

A2058 cells were transfected with the indicated siRNA. After immunoprecipitation with anti-p50, nuclear level of acetylated p50 protein was detected using anti-acetyl-lysine antibody (left panel). * indicates IgG heavy chain (approximately 55 kDa). Stat3 inhibition by siRNA is shown in the right panel.

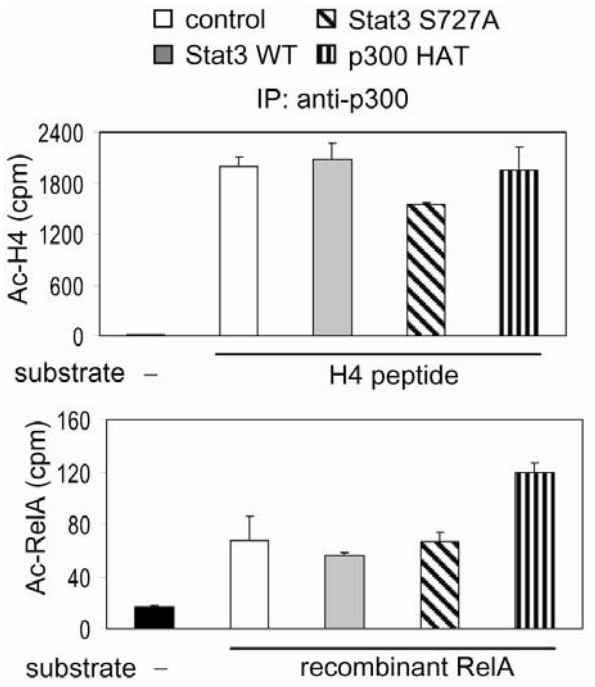

Figure S3. p300 Enzymatic Activity Is Not Affected by Stat3 Mutation on Phosphorylation Sites

B16 cells were transfected either with WT or phosphorylation mutant of Stat3. Forty-eight hours after transfection, p300 was enriched by immunoprecipitation and p300 immune complexes were assayed for enzymatic activity on substrates, as indicated. Data are mean ± SEM (n = 2).

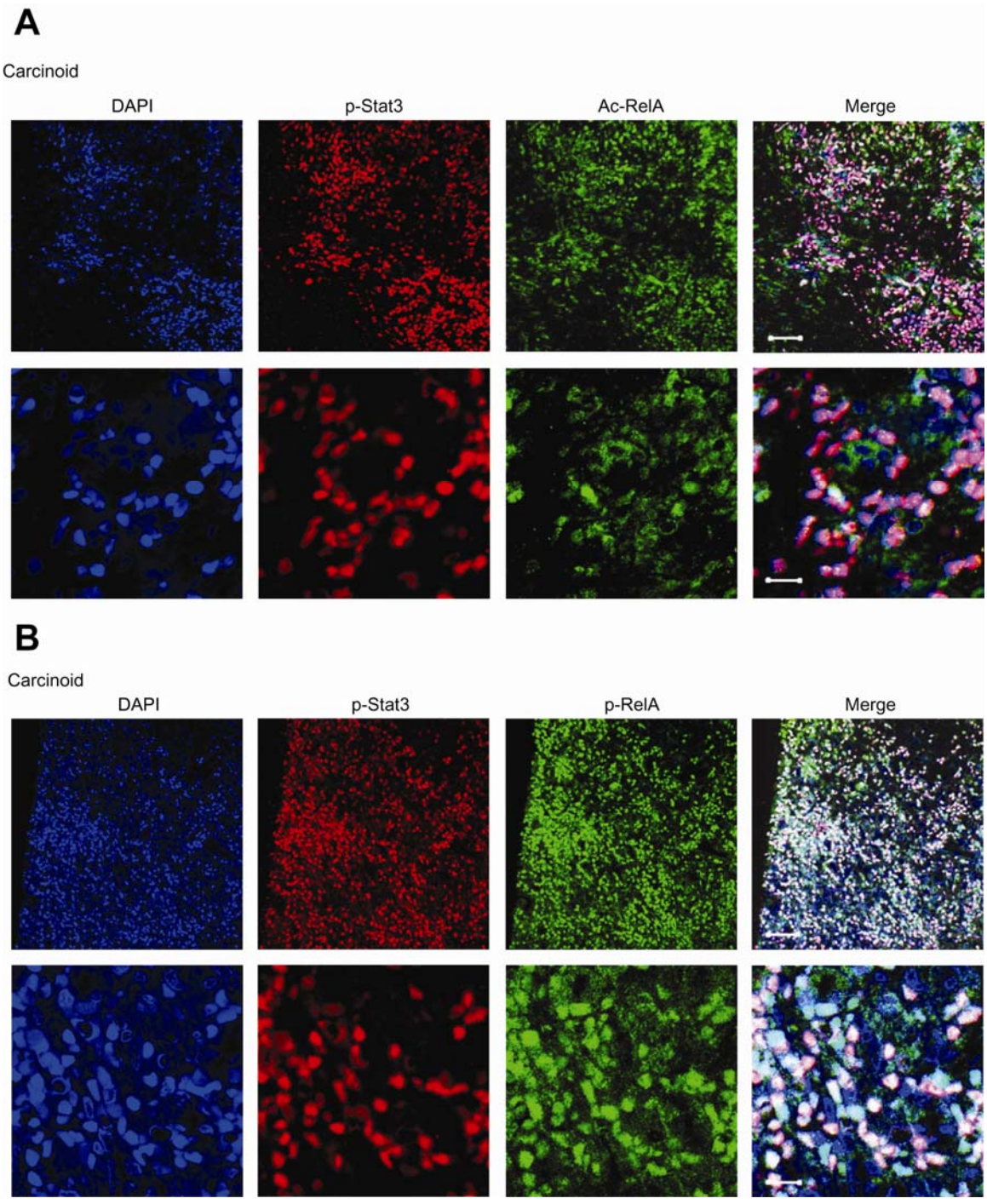
A
Carcinoid
DAPI
p-Stat3
Ac-RelA
Merge
B
Carcinoid
DAPI
p-Stat3
p-RelA
Merge

Figure 4.

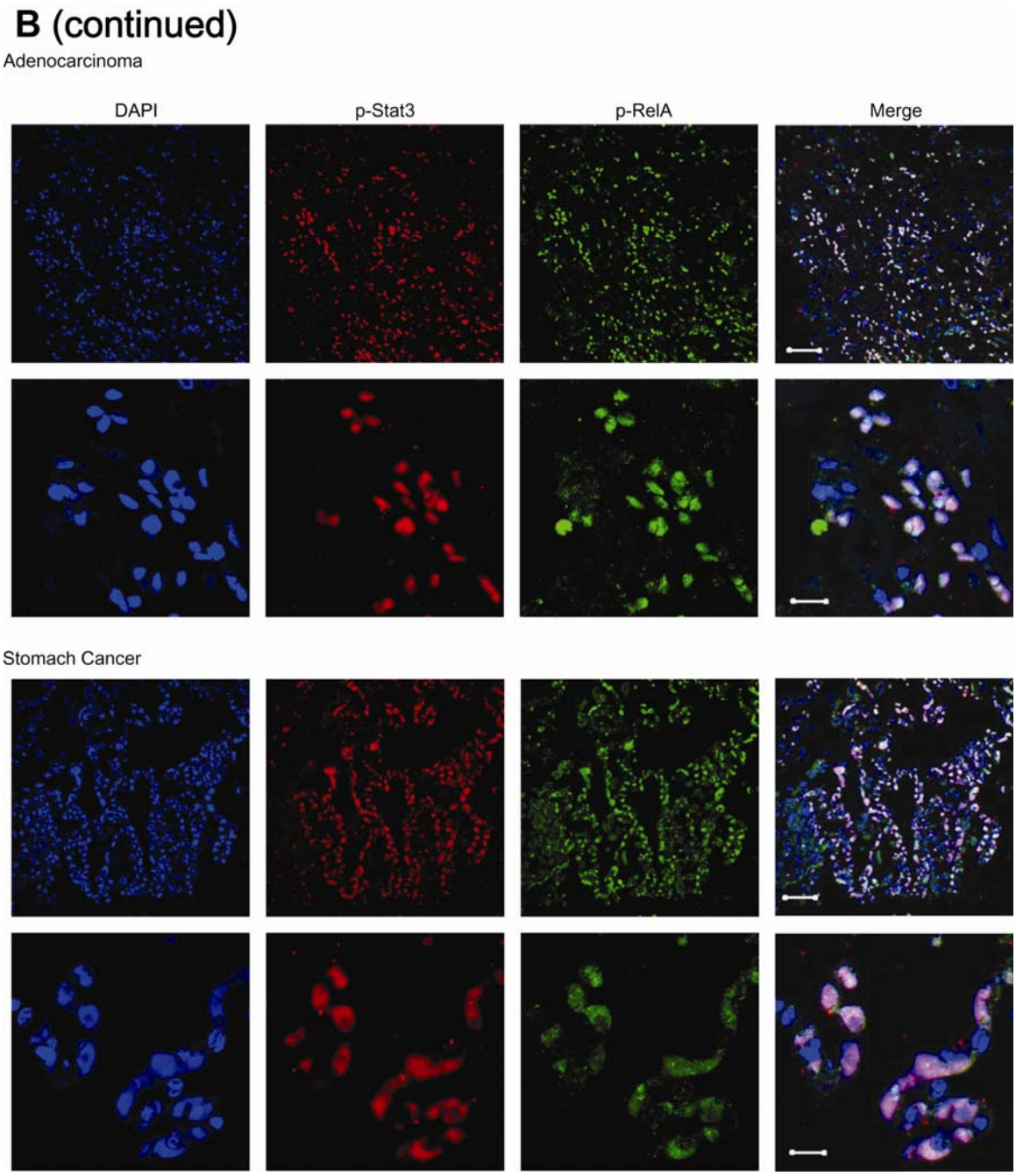

Figure 4. (continued)

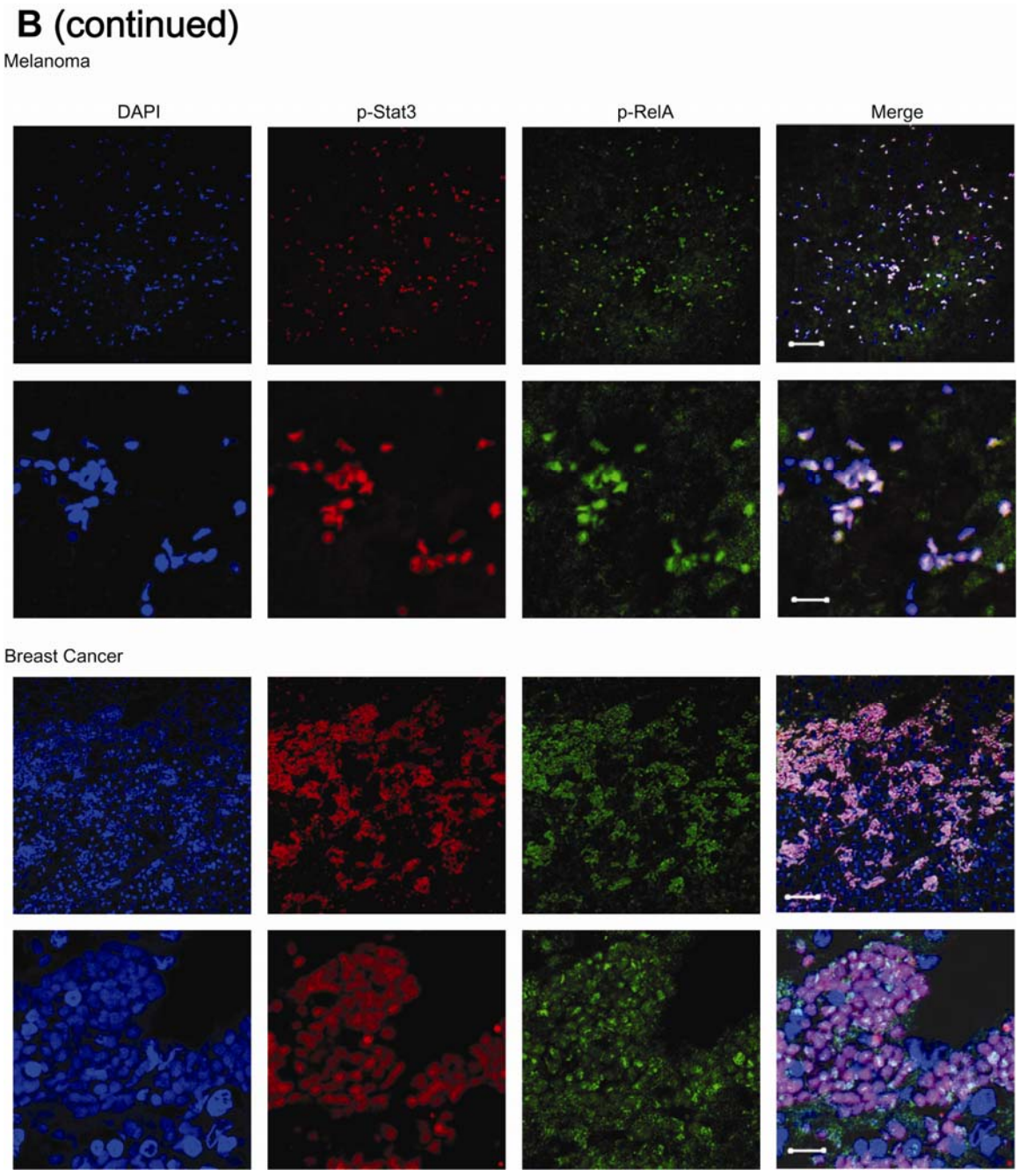

Figure 4. (continued)

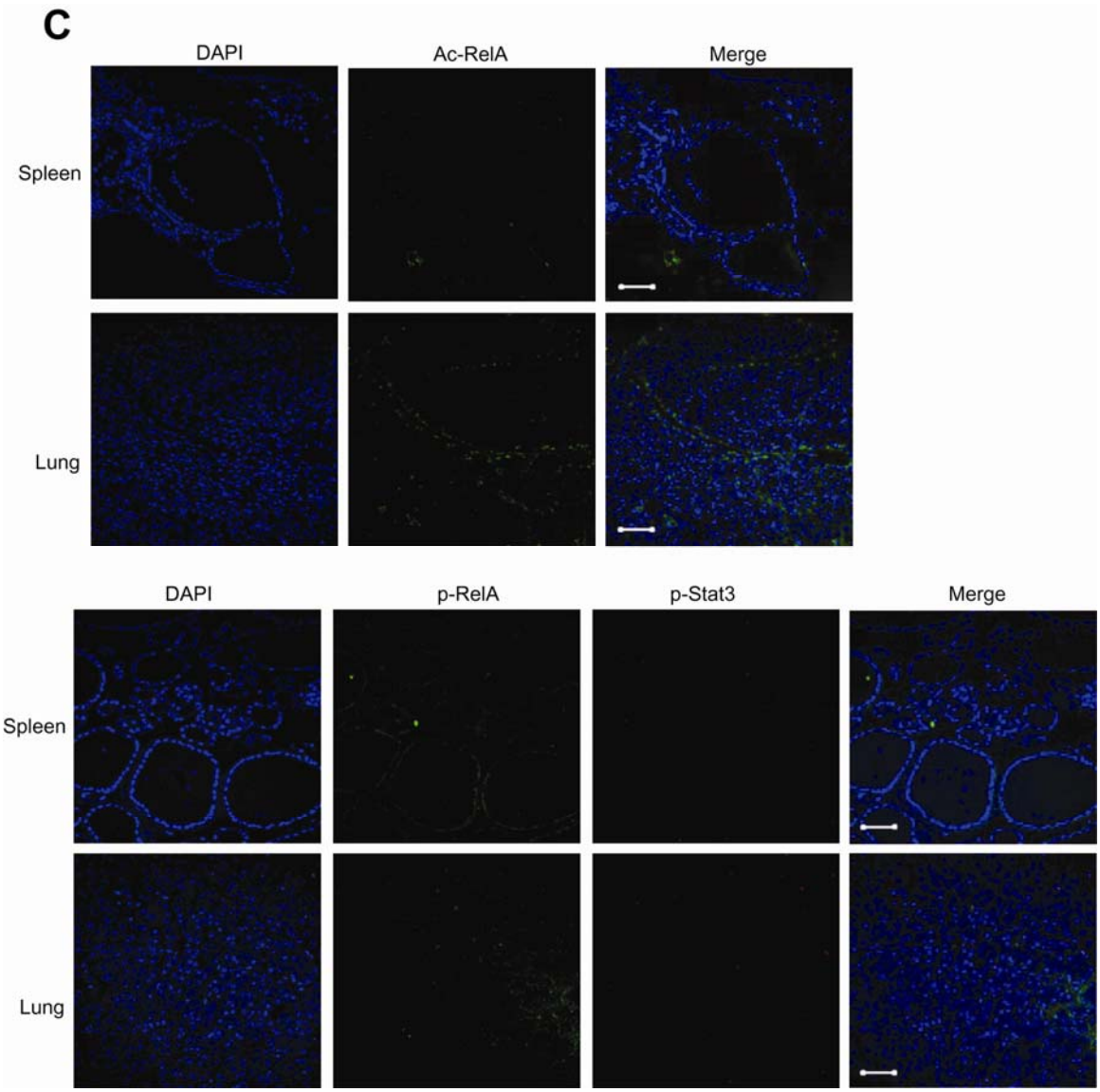

Figure S4. Acetylated RelA Is Prominently Present in Tumors, and Activated Stat3 and RelA Colocalize in the Nuclei of Tumors

(A) Sections of human carcinoid tumors were stained with the indicated antibodies, followed by confocal microscopic analyses. Lower panels are enlarged images of top. Upper panel: Scale = 40μm. Lower: Scale = 10μm.

(B) Nuclear colocalization of p-Stat3 and p-RelA were prominent in human tumors. Upper panel: Scale = 40μm. Lower: Scale = 10μm.

(C) Little acetylated RelA, p-Stat3 and p-RelA were detected in these sections of normal human tissues, stained at the same time as the slides shown above and in Figure 7. Shown are serial sections of normal human spleen and lung tissues. Scale = 40μm.