Supplemental Figure Legends

Figure S 1. **Activation of β-catenin/TCF signaling in short-term versus long-term cultures** (**A**) qPCR analysis of Axin2 message from freshly isolated (Day_0) AT2 cells and cells cultured for 2, 7 and 14 days. Mean values from triplicate qPCR measurements +/- standard deviations are shown. (**B**) Immunoblot analysis of AT2 lysates (20μg) from freshly isolated cells (Day_0) and cells plated in culture for 2-14 days. Note that in this experiment, the total level of β-catenin was very different between Day_0 and Day_2 cells (in contrast to Fig. 1D), due to variable contamination by red blood cells that lack β-catenin. Nonetheless, when amounts of AT2 lysates are corrected for the same level of total β-catenin (60μg of Day_0 lysate versus 20μg of Day_2 lysate), the activated form of β-catenin (ABC) is still upregulated relative to the total β-catenin pool (pan-β-catenin). (**C**) Immunofluorescence analysis of rat AT2 cells plated on filters and cultured for 2 or 14 days at an air (apical)/liquid (basolateral) interface. Note that the cells visibly flatten and increase their surface area in these cultures, and the signaling form of β-catenin appears to be more visibly recruited to cell-cell junctions. Scale bar = 10μm. (**D**) The signaling active form of β-catenin is a fraction of the total cellular pool of β-catenin. Immunoblot analysis of AT2 lysate compared with defined amounts (0.003-0.3μg) of recombinant β-catenin reveals that 50μg of AT2 lysate contains ~0.03μg of ABC compared with 0.3μg of total β-catenin.

Figure S 2. **Signaling form of β-catenin is detected in airway but not alveolar epithelial cells. (A)** Immunoblot detection ABC in adult rat lung total homogenate. **(B-G)** Adult rat lung paraffin sections incubated with (**B**) GAM-HRP secondary alone, (**C**) anti-pan-β-catenin (recognizes all forms of β-catenin), (**D&E**), anti-de-phospho-Activated β-catenin (ABC, 8E7), (**F**) anti-de-phospho-GSK-epitope-β-catenin (06-734, Millipore) and (G) anti-Wnt1 and processed for immunohistochemistry. (**H-J**) Immunofluorescence-double labeling from the same rat lung

block using dephospho-β-catenin, ABC and CC10 antibodies.

Figure S 3. **Wnt/β-catenin signaling reporter mouse (Axin2 $^{+/LacZ}$) reveals no baseline activity in adult mouse lung.** Reporter mice (~20gms) were injected (IP) with 0.66mls of normal saline (**A**) or a 0.15M solution of LiCl (**B&C**) for three days. Lungs were harvested and processed for X-gal staining and paraffin embedding using standard procedures as described in Methods. Note that both small and large airways are most sensitive to activation of the Axin2 $^{+/LacZ}$ reporter compared with the alveolar compartment.

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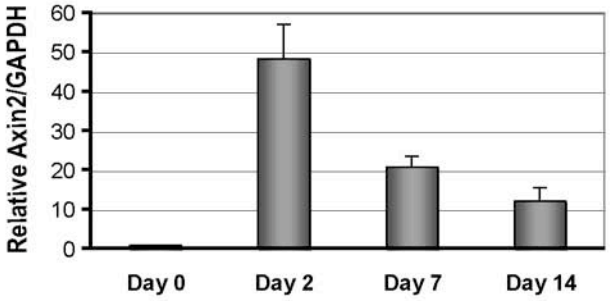
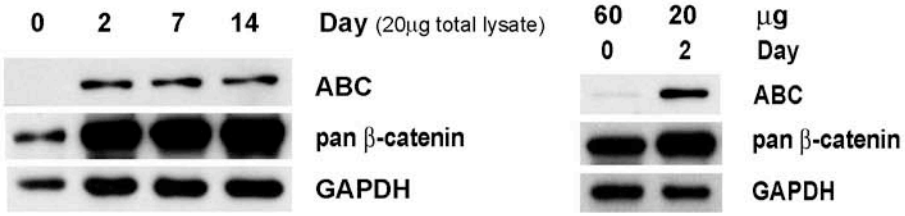
Fig. S1

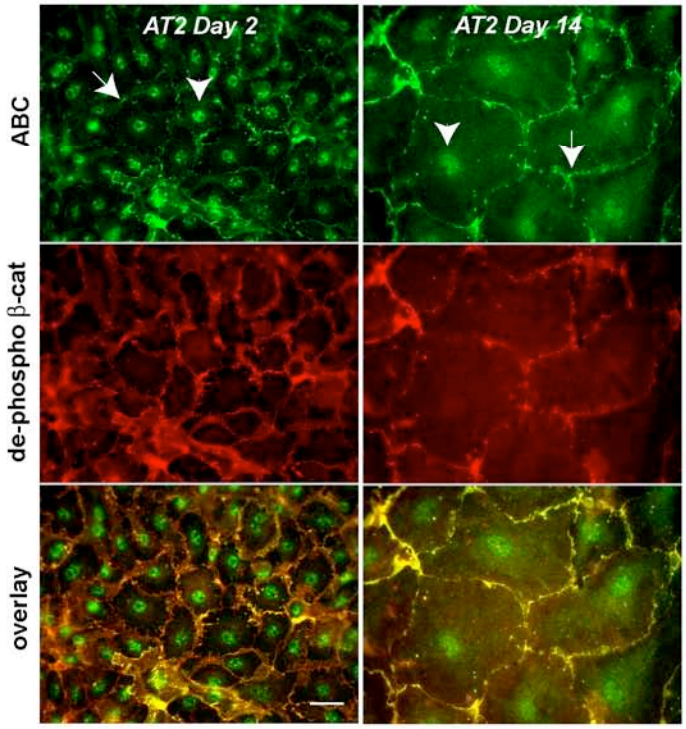
A


Relative Axin2/GAPDH
60
50
40
30
20
10
0
Day 0
Day 2
Day 7
Day 14

B


0
2
7
14
Day (20μg total lysate)
ABC
pan β-catenin
GAPDH
60
20
μg
0
2
Day
ABC
pan β-catenin
GAPDH

C


AT2 Day 2
AT2 Day 14
ABC
de-phospho β-cat
overlay

D

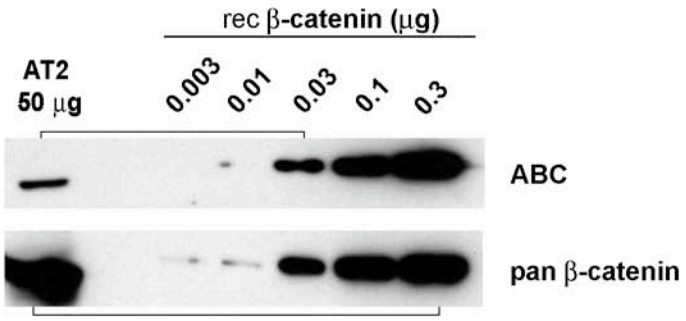

rec β-catenin (μg)
AT2
50 μg
0.003
0.01
0.03
0.1
0.3
ABC
pan β-catenin

Fig. S2

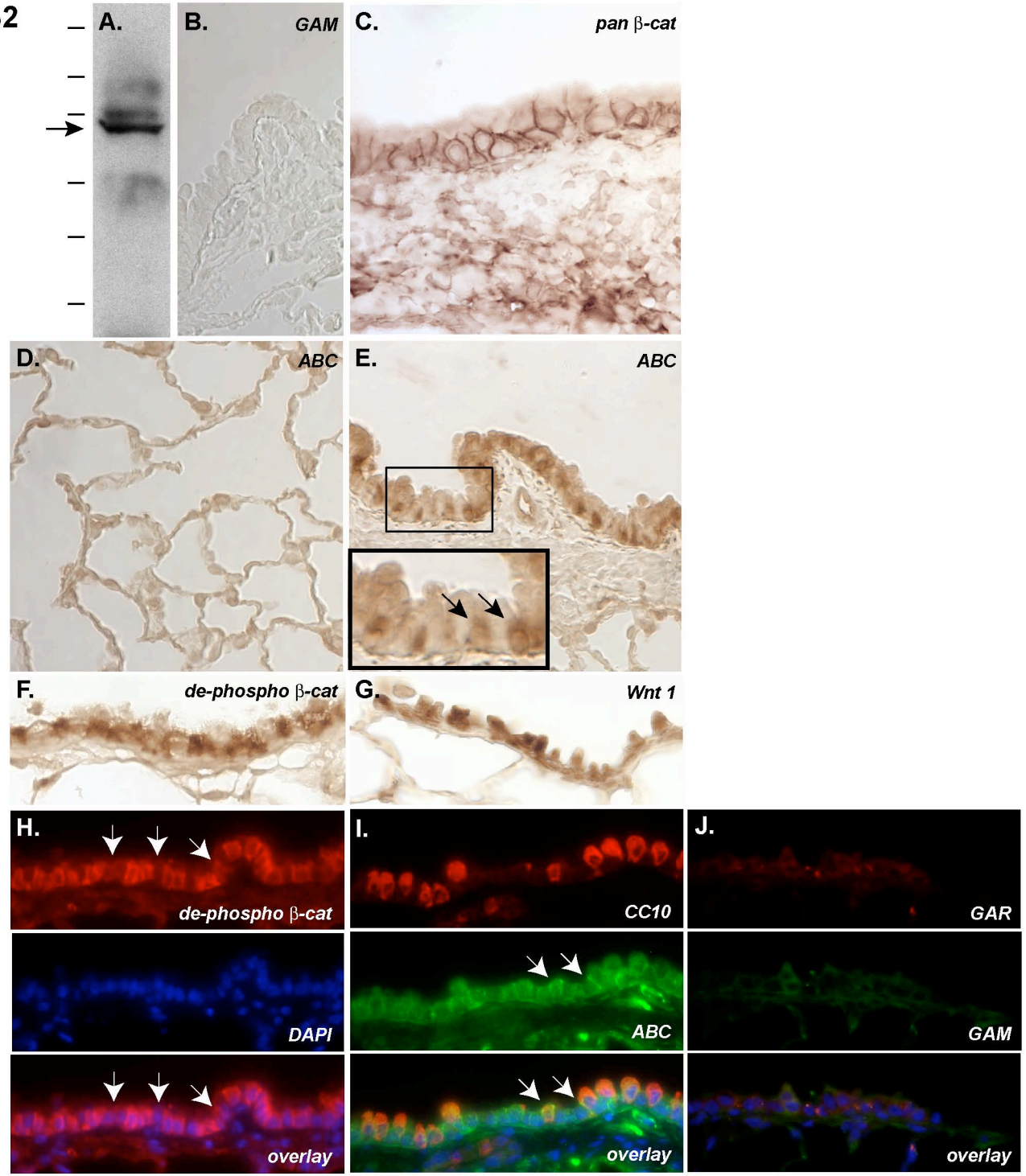

A.
B.
GAM
C.
pan β-cat
D.
ABC
E.
ABC
F.
de-phospho β-cat
G.
Wnt 1
H.
de-phospho β-cat
DAPI
overlay
I.
CC10
ABC
overlay
J.
GAR
GAM
overlay

Fig. S3