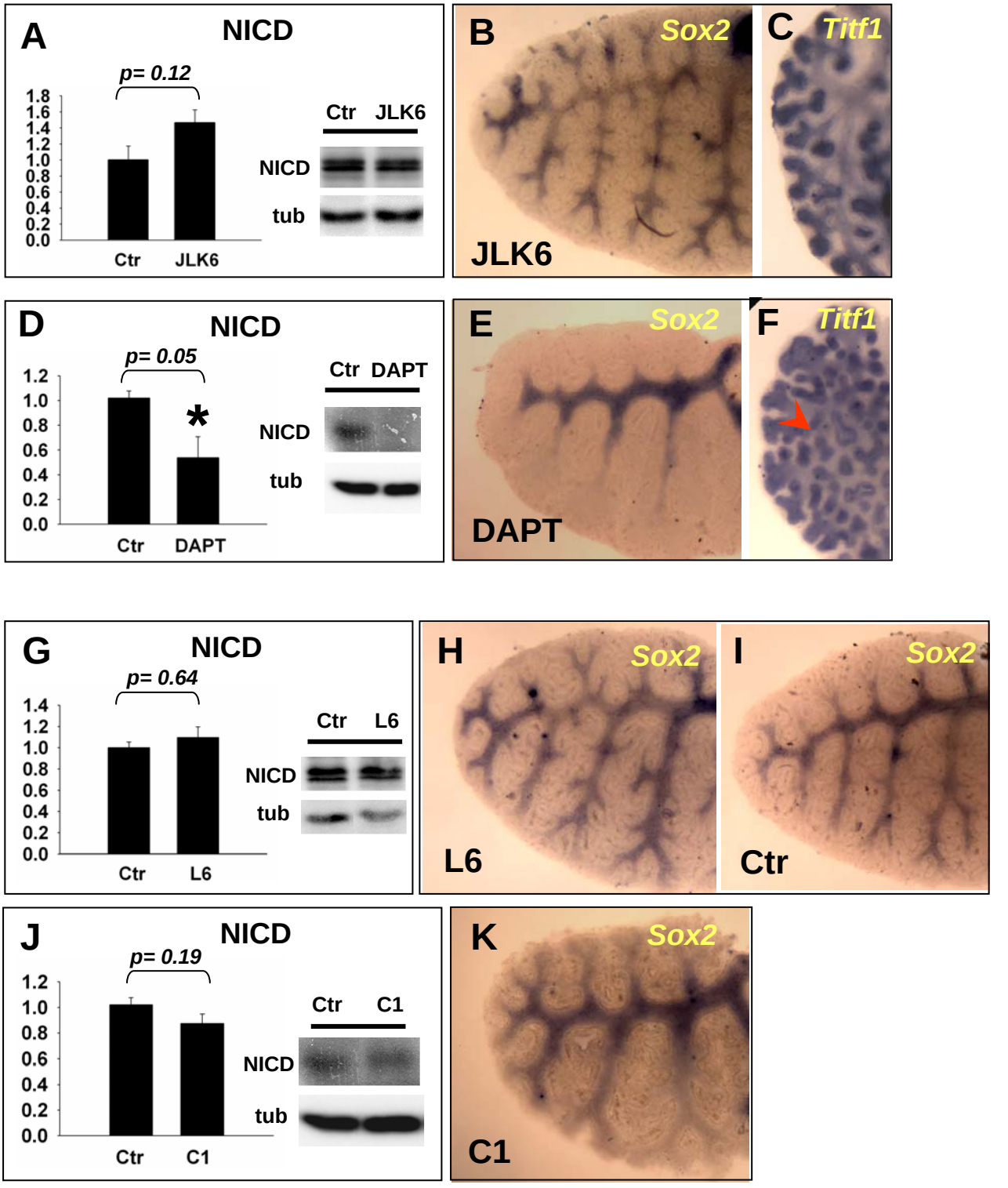
LEGEND SUPPLEMENTARY FIGURE

Supplementary Fig. 1. Effects of the gamma-secretase inhibitors JLK6 (10uM) , DAPT (50 uM), L685458 (L6, 25uM) and Compound 1 (C1, 50uM) in NICD expression and proximal-distal patterning of the developing lung , as assessed by Western blot (**A,D,G,J**) and in situ hybridization of *Sox2* (**B,E,H,I,K**) and *Titf1* (**C,F**). Treatment with JLK6 **(A-C)** (5, 10, 25uM; (27)) , a gamma-secretase inhibitor that does not block Notch cleavage, showed no significant decrease in NICD expression or altered distribution of *Sox2* and *Titf1* mRNAs and did not elicit ectopic budding, effects typically seen in DAPT-treated cultures **(D-F,** arrowhead depicts ectopic buds**)**.Treatment with L685458 **(G-I)** (1, 10, 25, 50 uM) or Compound 1 **(J-K)** (25, 50, 100uM) , gamma-secretase inhibitors known to interfere with Notch cleavage in other biological systems (4,28), did not significantly alter expression of NICD, *Sox2* (compare with control in I) and *Titf1* (not shown), even at the highest concentrations in which some toxicity started to be observed. Thus, in our system DAPT was the only gamma secretase inhibitor that could efficiently disrupt Notch signaling, consistent with its wide use as the drug of choice for Notch inhibition. The altered budding and expression of *Sox2, Titf1* correlated with the decrease in NICD, suggesting that the changes in lung pattern we observed may occur selectively under conditions in which gamma-secretase cleavage of Notch is disrupted. **(A-K)** E11.5 lungs cultured for 48h (as in Methods). (**A,D,G,J**) graphs depict mean, standard error and p value (6 lungs per condition; asterisks, statistically significant at $p = 0.05$; Student T-test; representative gel (NICD; tub, tubulin). (**B, C, E, F, H,I,K**) n> 6 lungs per condition.


A
NICD
p= 0.12
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ctr
JLK6
Ctr JLK6
NICD
tub
B
Sox2
JLK6
C
Titf1
D
NICD
p= 0.05
*
Ctr
DAPT
Ctr DAPT
NICD
tub
E
Sox2
DAPT
F
Titf1
G
NICD
p= 0.64
Ctr
L6
Ctr L6
NICD
tub
H
Sox2
L6
I
Sox2
Ctr
J
NICD
p= 0.19
Ctr
C1
Ctr C1
NICD
tub
K
Sox2
C1