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Supplemental information

DLL3 regulates Notch signaling in small cell lung cancer

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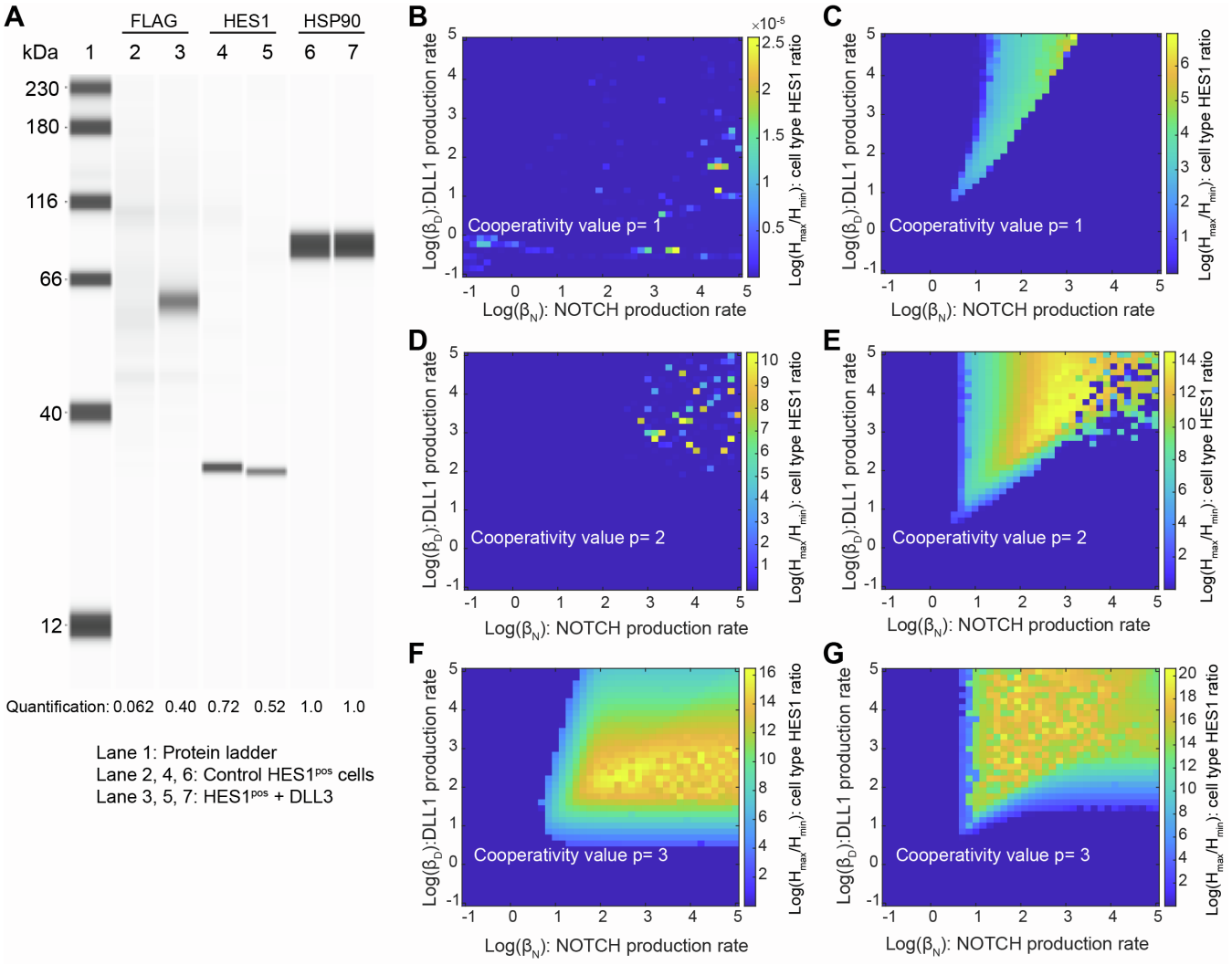

Figure S1, related to Figure 1. Ectopic expression of DLL3 in HES1^{GFP}-positive cells. (**A**) Immunoassay measuring DLL3 and HES1 expression in control HES1^{GFP}-positive cells and the same cells with stable expression of DLL3. HSP90 serves as a loading control. The expected molecular weights of DLL3, HES1, and HSP90 are 65 kDa, 30 kDa, and 90 kDa, respectively. The band intensities were quantified and normalized by those of HSP90. (**B-G**) Log(H_{max}/H_{min}) at steady state were calculated as a function of β_N and β_D without (**B, D, F**) and with (**C, E, G**) mutual inactivation using different values of the feedback loop cooperativity, p. Regions with values greater than 0 (light blue to yellow regions) support patterning, while those with 0 (dark blue) do not. As was shown in the studies by Sprinzak *et al.*, when p = 1 mutual inactivation was required to support patterning in the tested parameter space. Note the different scale in (**B**) ($x10^{-5}$).

Figure	Equations	Parameters	Initial conditions
1E,G,H	1–4	$\gamma = 1$, $k_e = 0.1$, $k_{DR} = 1$, $k_f = 0.1$, $Y_s = 1$, $p = 3$, $k_{RS} = 300000$, $Y_R = 1$, $\beta_N = 20$, $\beta_H = 1000000$, $m = 1$, sigma = 0.1, $k_c = 0.1$, $k_t = 1$, $\beta_D = 0.1$ to 1e5, $\beta_{D3} = 0.1$ to 1e5	Cells initially set in low values of NOTCH, DLL1, and DLL3 with small variability.
1F	1–4	$\gamma = 1$, $k_e = 0.1$, $k_{DR} = 1$, $k_f = 0.1$, $Y_s = 1$, $p = 3$, $k_{RS} = 300000$, $Y_R = 1$, $\beta_D = 15$, $\beta_N = 20$, $\beta_H = 1000000$, $m = 1$, sigma = 0.1, $k_c = 0.1$, $k_t = 1$, $\beta_{D3} = 0, 25, 30$	Cells initially set in low values of NOTCH, DLL1, and DLL3 with small variability.
1I	1–4	$\gamma = 1$, $k_e = 0.1$, $k_{DR} = 1$, $k_f = 0.1$, $Y_s = 1$, $p = 3$, $k_{RS} = 300000$, $Y_R = 1$, $\beta_D = 15$, $\beta_N = 20$, $\beta_H = 1000000$, $m = 1$, sigma = 0.1, $k_c = 0.1$, $k_t = 1$, $\beta_{D3} = 0, 25, 30, 100, 1000$	Cells initially set in low values of NOTCH, DLL1, and DLL3 with small variability.
S1B-G	1–4	$\gamma = 1$, $k_e = 0.1$, $k_{DR} = 1$, $k_f = 0.1$, $Y_s = 1$, $p = 3$, $k_{RS} = 300000$, $Y_R = 1$, $\beta_D = 0.1$ to 1e5, $\beta_N = 0.1$ to 1e5, $\beta_H = 1000000$, $m = 1$, sigma = 0.1, $k_c = 0.1$, $k_t = 1$, $\beta_{D3} = 0$	Cells initially set in low values of NOTCH and DLL1 with small variability.

Table S1, related to Figure 1: Parameter values for mathematical modeling
See equations in Methods and Figure 1.

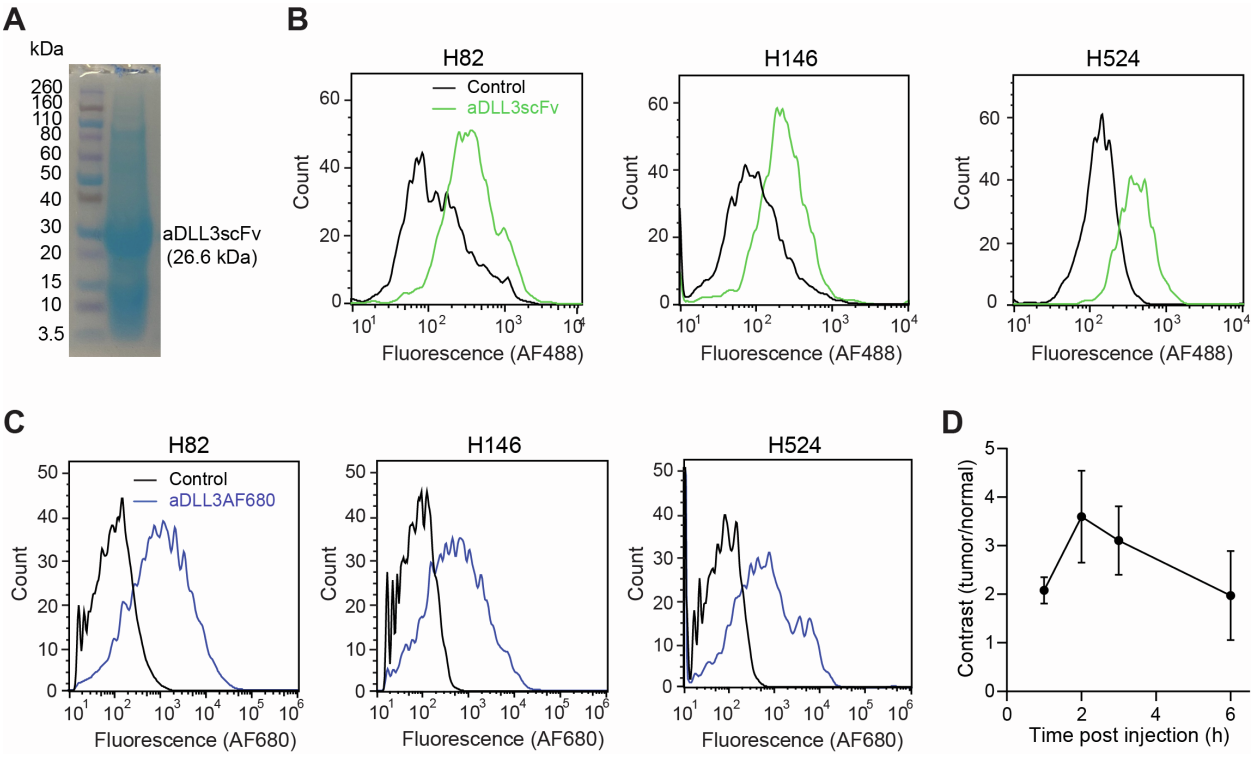

Figure S2, related to Figure 2. Anti-DLL3 scFv binds to human SCLC cells *in vitro* and *in vivo*. (**A**) SDS-PAGE gel stained with SimplyBlue Safe Stain showing aDLL3scFv purified using nickel-NTA affinity column. (**B**) Representative flow cytometry histograms showing binding of human SCLC cells to His-tagged aDLL3scFv, which was detected using rabbit anti-His antibody and AF488 labeled anti-rabbit antibody. (**C**) Representative flow cytometry histograms showing binding of aDLL3AF680 to human SCLC cells. (**D**) Quantification of imaging contrast reported as the ratio of fluorescent signals for tumor versus normal tissue. Error bars represent s.d., n = 3.

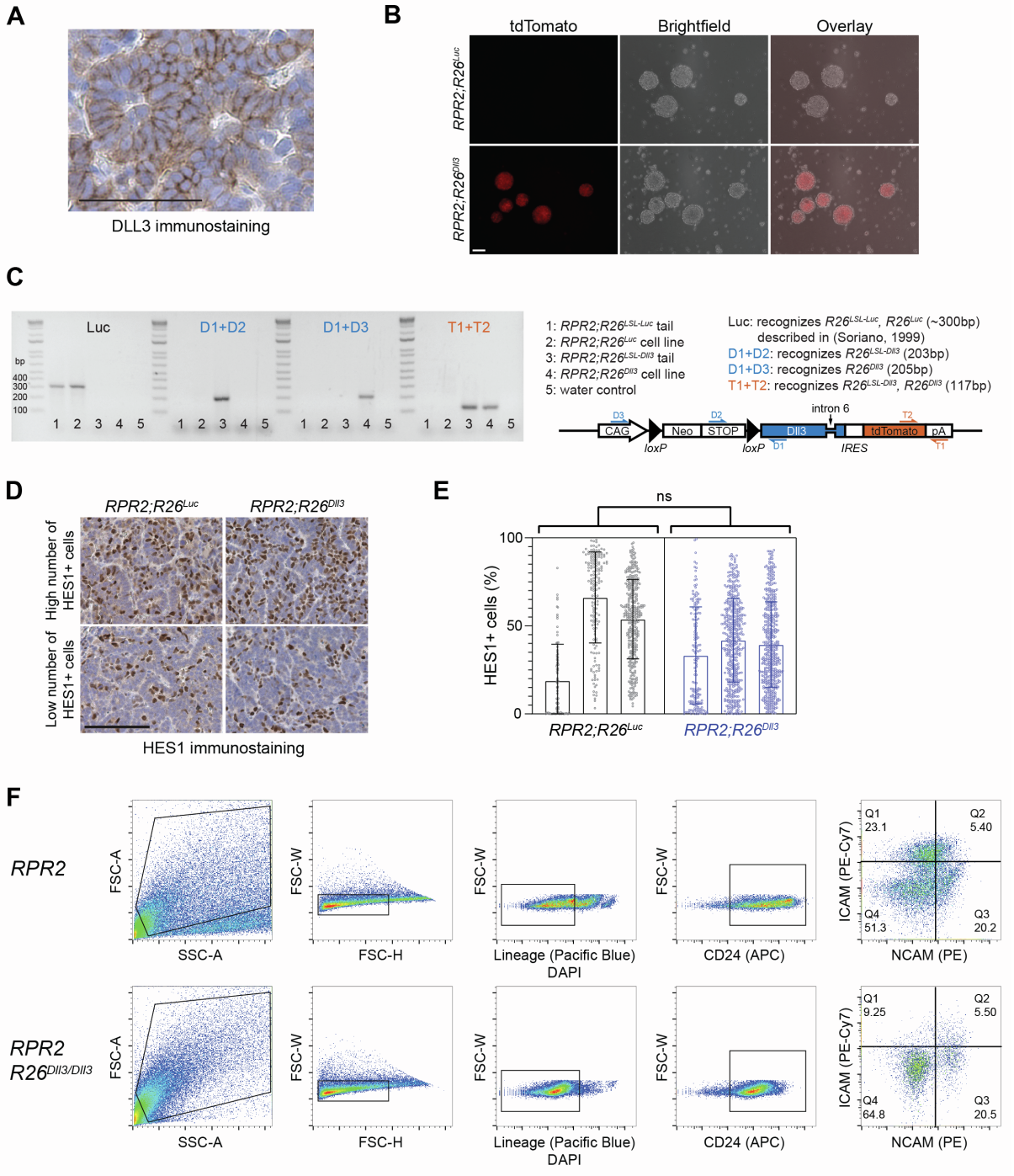

Figure S3, related to Figure 3. **Characterization of a new mouse allele with inducible expression of DLL3.** (**A**) Representative DLL3 immunostaining in an *RPR2;R26*^{Luc} mutant lung tumor section. Scale bar 50μm. (**B**) Representative tdTomato fluorescence and brightfield images of cell lines derived from *RPR2;R26*^{Luc} (top) or *RPR2;R26*^{Dll3} (bottom) mutant lung tumors, scale bar 100μm. (**C**) Genotyping PCR and schematic of allele-specific primer pairs. DNA was extracted from the cell lines shown in (B) and from mouse tails for unrecombined controls. Note that the D1-D3 PCR fragment is too long to be amplified in un-recombined alleles. (**D**) Representative HES1 immunostaining in *RPR2;R26*^{Luc} (left) and *RPR2;R26*^{Dll3} (right) mutant lung tumor sections. Scale bar 100μm. (**E**) Quantification of (D). Each point is a 100 μm diameter region within a tumor, and each bar represents a section from an individual mouse. p=0.597 by nested t-test. Error bars represent SD. (**F**) Flow cytometry strategy to analyze NCAM^{high} ICAM^{low} and NCAM^{low} ICAM^{high} SCLC cells from RPR2;R26^{luc} (top) and RPR2;R26^{Dll3} (bottom) mutant mouse SCLC tumors.

Gene	Sequence
Rb1^{flox} Forward	5'-CCTTGACCATAGCCCAGCAC-3'
Rb1^{flox} Reverse	5'-CTCTAGATCCTCTCATTCTTCCC-3'
p53^{flox} Forward	5'-GAAGACAGAAAAGGGGAGGG-3'
p53^{flox} Reverse	5'-AAGGGGTATGAGGGACAAGG-3'
Rbl2^{flox} Forward	5'-GACTGCTGGTATTAGAACCC-3'
Rbl2^{flox} Reverse	5'-GTGTTGTAACATTCTCGTGGG-3'
R26^{Luc} Forward	5'-AAAGTCGCTCTGAGTTGTTAT-3'
R26^{Luc} Reverse	5'-GCGAAGAGTTTGTCCTCAACC-3'
R26$^{LSL-Dll3}$ Forward	5'-AAGAAAAGCCAGGATCAGCGTCTGG-3'
R26$^{LSL-Dll3}$ Reverse	5'-CACTGCATTCTAGTTGTGGTTTGTCCAAAC-3'
R26^{Dll3} Forward	5'-AAGAAAAGCCAGGATCAGCGTCTGG-3'
R26^{Dll3} Reverse	5'-GGGCAACGTGCTGGTTATTG-3'
tdTomato Forward	5'-TGGAGTGGCAACTTCCAGGG-3'
tdTomato Reverse	5'-ACGGCATGGACGAGCTGTA-3'

Table S2 related to Figure S3 and STAR Methods. Primers for genotyping.