Crucial role of the Rap G protein signal in Notch activation and leukemogenicity of T-cell acute lymphoblastic leukemia

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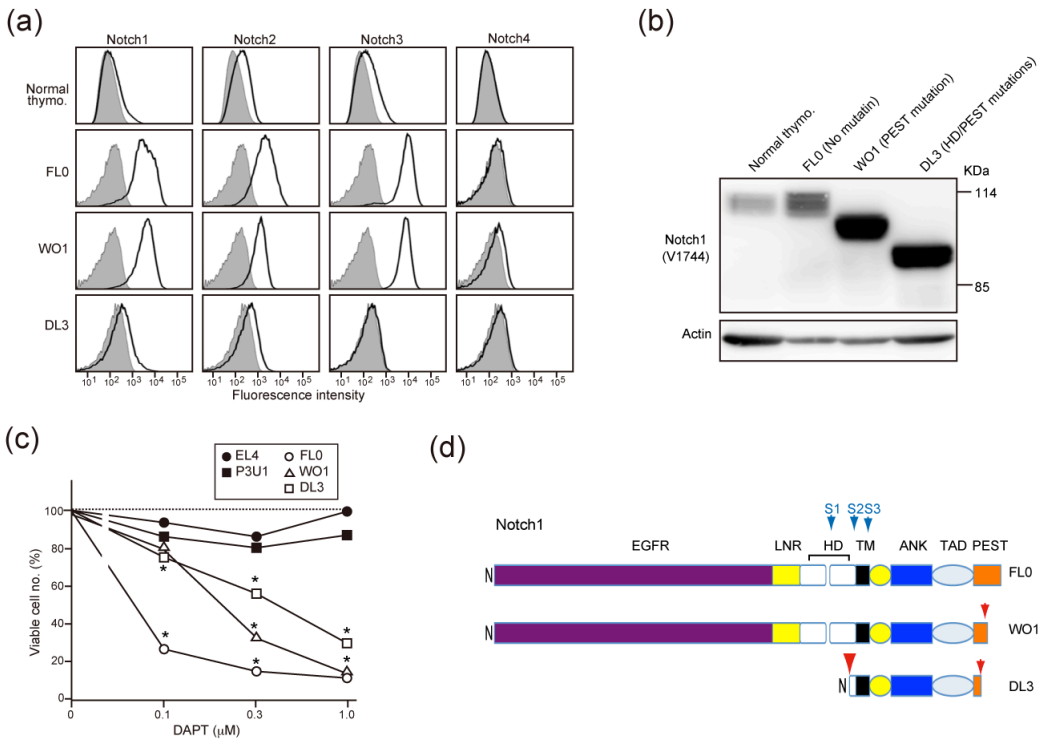
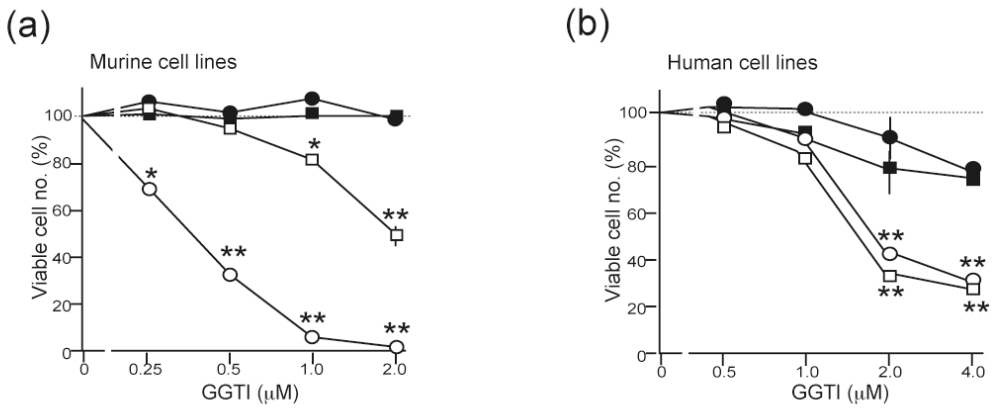
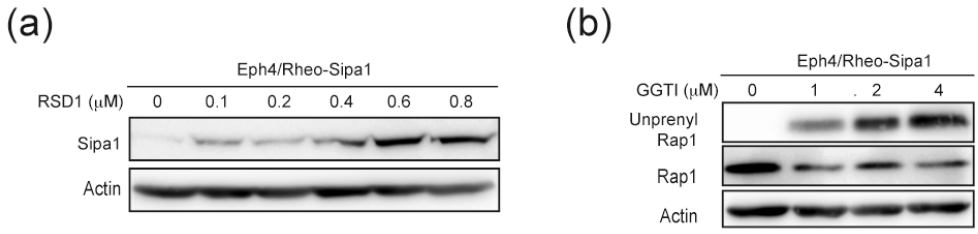


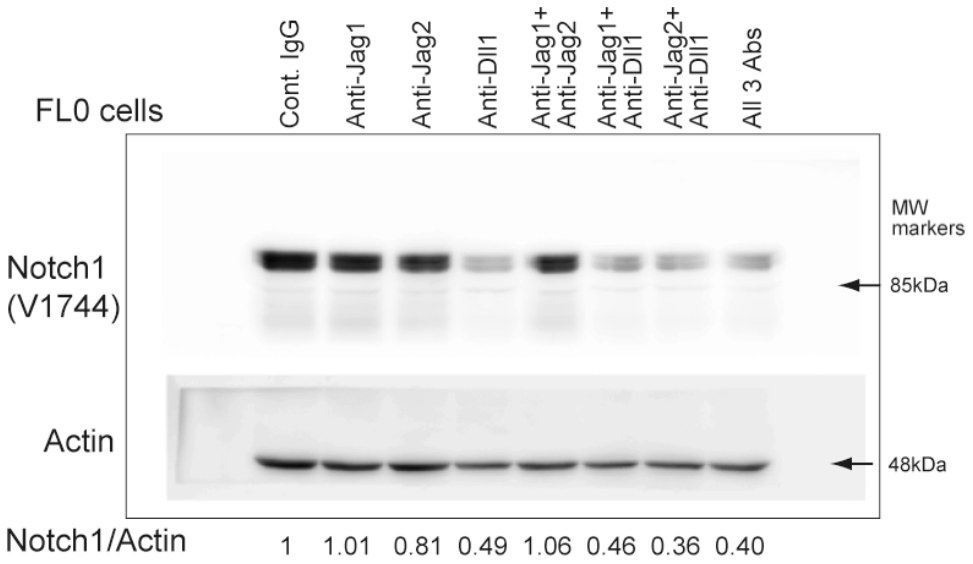
Supplementary Figure S1 | Notch-dependent mouse T-ALL cell lines (a) Expression of cell-surface Notch receptors was analyzed with FACS in normal thymocytes and T-ALL cell lines. The cell lines include FL0 and WO1 derived from T-ALLs developed in the BMT recipients of *Sipa1*$^{-/-}$*C3G-F*$^{+}$HPCs (B6 mice) and DL3 established from spontaneously developed T-ALL in DBA/2 mice. Shaded areas indicate control IgG staining. (b) Cell lysates of normal thymocytes and T-ALL cell lines were immunoblotted with the indicated antibodies. (c) T-ALL (open symbols) and other leukemia (closed symbols) cell lines were cultured at 3×10^4 cells/mL in the absence or presence of varying concentrations of γ-secretase inhibitor (DAPT), and the viable cell numbers were assessed on day 3. Relative cell numbers to control are indicated. *; $p < 0.01$. (d) Schematic presentation of Notch1 in the T-ALL cell lines. FL0 revealed no *Notch1* mutation in the coding region by DNA sequencing, whereas WO-1 and DL3 showed insertional mutations in PEST region, resulting in C-terminal truncation (red arrow). DL3 cell line additionally showed a MuLV-proviral integration at HD region (red arrowhead), leading to the truncation between S1 and S2 sites.



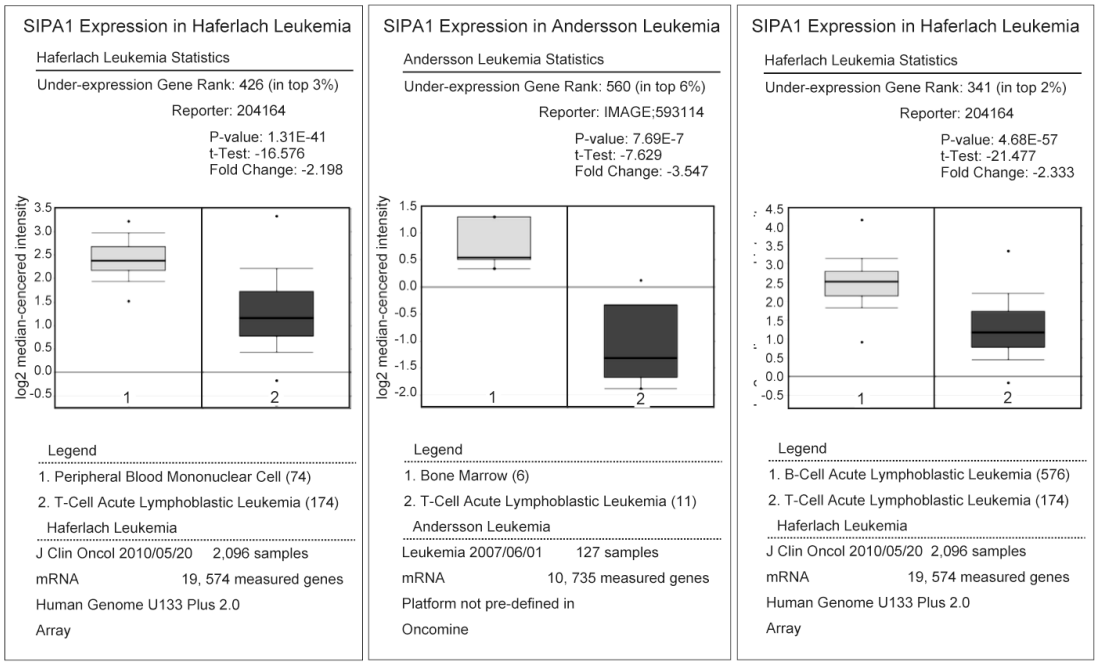
Supplementary Figure S2 | T-ALL lines of mice and humans show higher susceptibility to GGTI than non-T-ALL leukemia cell lines. (a) Mouse T-ALL (open square; WO1, open circle; DL3) and unrelated lymphoma (closed square; EL4, closed circle; P3U1) cell lines were cultured in the absence or presence of GGTI for 3 days, and the viable cell numbers were assessed. *; $p < 0.05$; **; $p < 0.01$. (b) The same experiments with human T-ALL (open square; TALL1, open circle; HPB-ALL) and myeloid leukemia (closed square; U937, closed circle; HL60) cell lines. **$P < 0.01$.



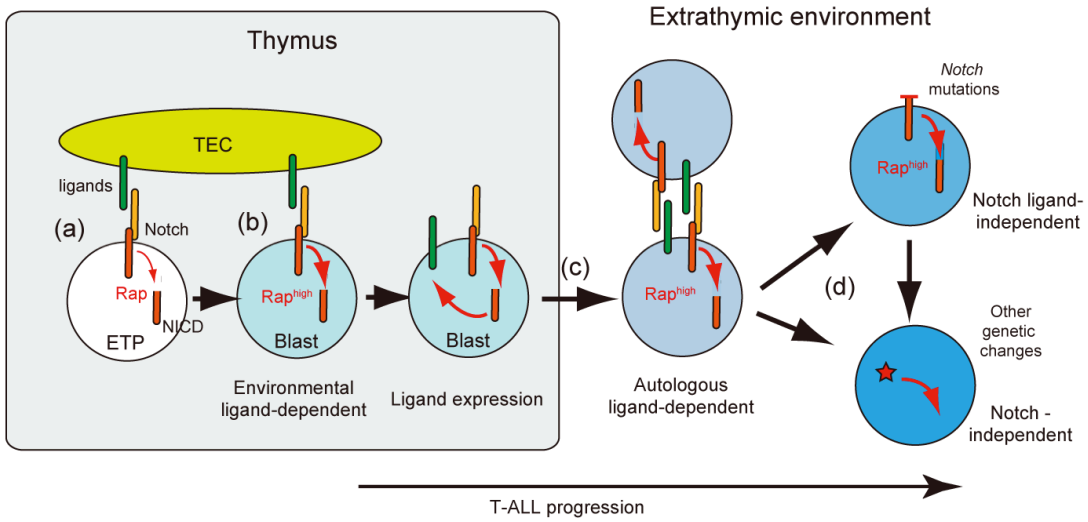
Supplementary Figure S3 | Conditional expression of Sipa1 and the inhibition of Rap prenylation by GGTI in epithelial Eph4/Rheo-Sipa1 cell line. (a, b) Eph4/Rheo-Sipa1 cells were cultured in the absence or presence of varying doses of RSL1 or GGTI for 3 days and were immunoblotted with the indicated antibodies.



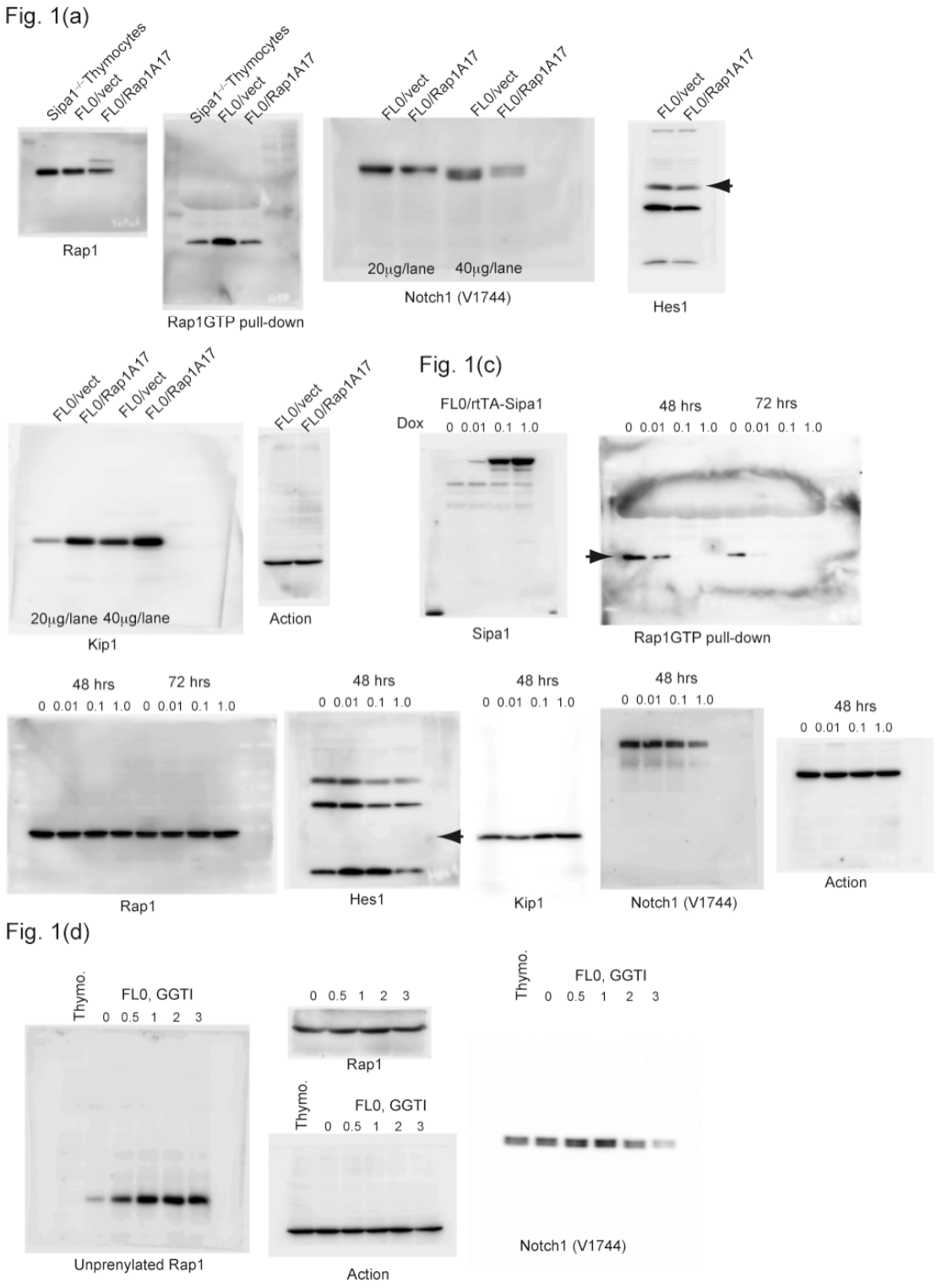
Supplementary Figure S4 | Inhibition of Notch1 activation in FL0 T-ALL cells in the presence of anti-Notch ligand antibodies. FL0 cells were cultured in the presence of monoclonal anti-Notch ligand antibodies (60 μg/mL each) or their mixtures for 1 day, and the lysates were immunoblotted with anti-cleaved Notch1 (V1744) antibody. The signal densities relative to actin are indicated.



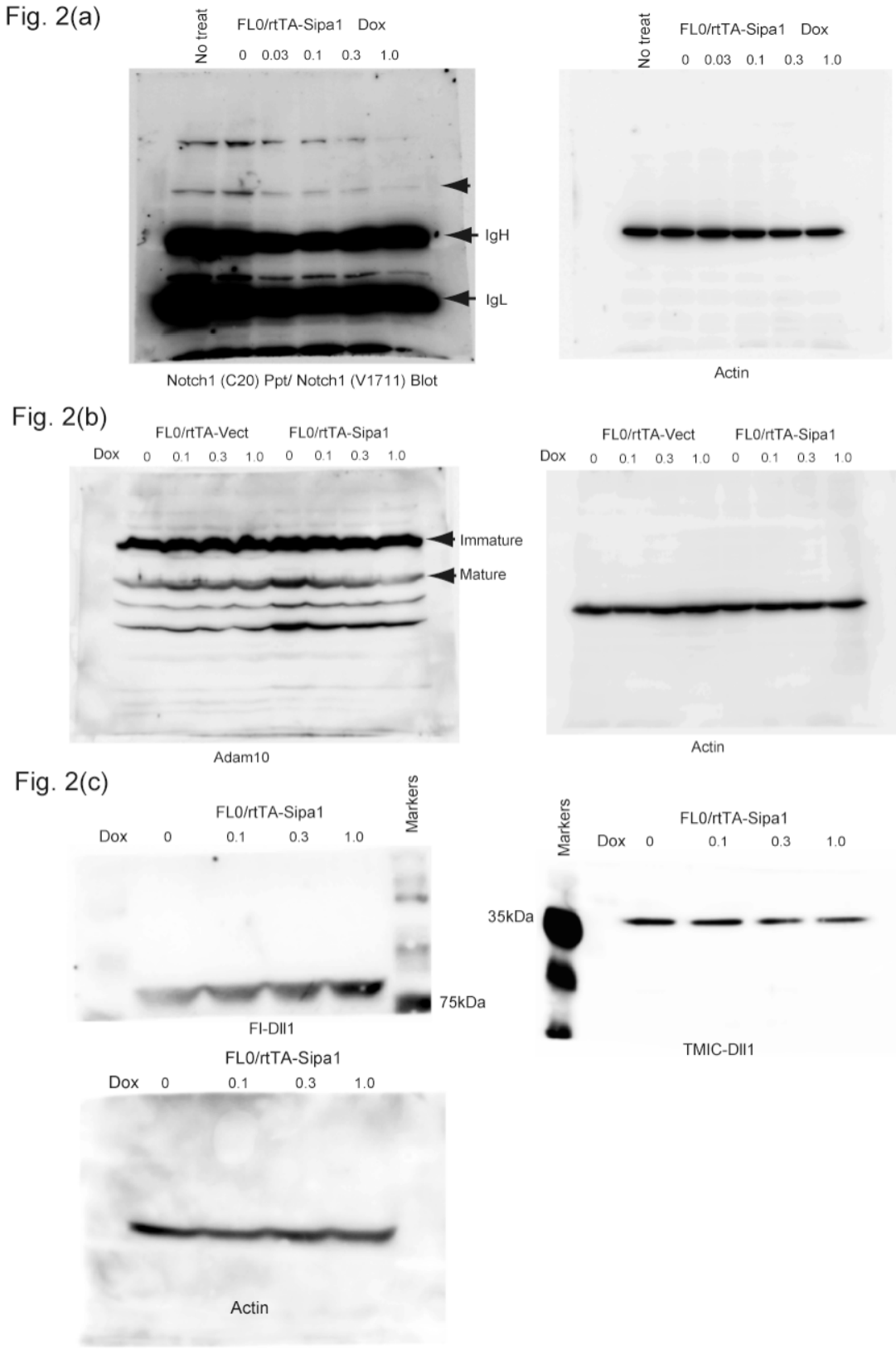
Supplementary Figure S5 | *SIPA1* is significantly underexpressed in human T-ALL. *SIPA1* expression in human T-ALL as compared with normal peripheral mononuclear cells, normal BM cells, and B-ALL was extracted from a meta-analysis of public domain gene expression data (NCBI Gene Expression Omnibus, http://lifesciencedb.jp/geo/).



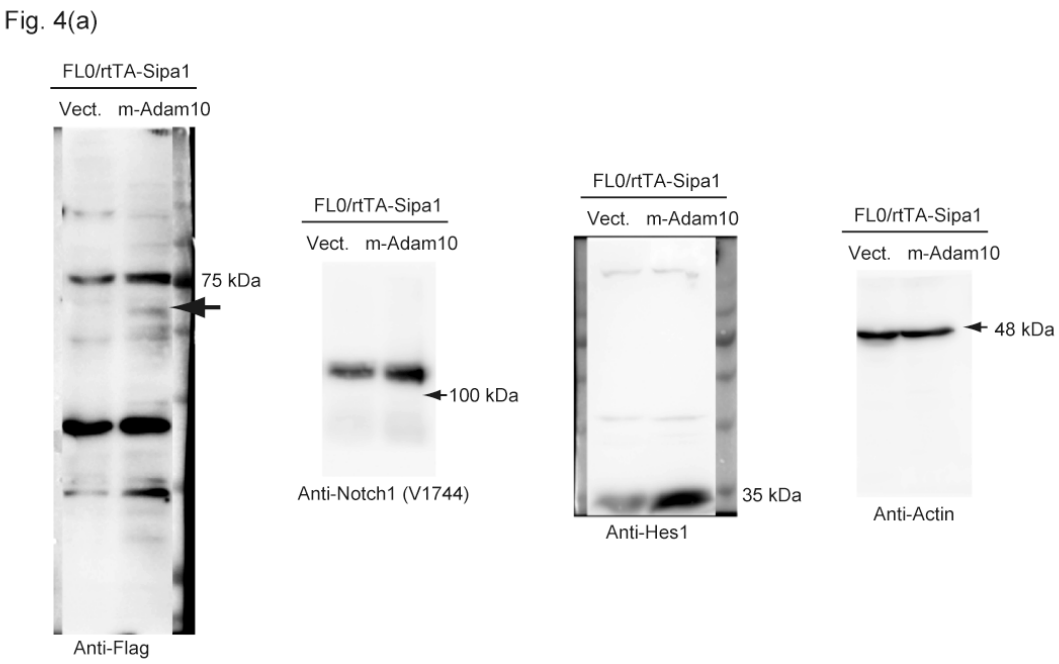
Supplementary Figure S6 | A schematic model for the T-ALL initiation and progression from Rap1^{high} ETPs. Development and proliferation of normal ETPs in the thymus crucially depend on the Notch signal, which is activated via Notch ligand expressed on thymic epithelial cells (TECs). Deregulated Rap signal in ETPs strongly enhances the ligand-mediated Notch activation and proliferation of ETPs (a). Sustained Notch activation results in the development of blast cells, which continue to depend on the ligand-donor cells in the thymic microenvironment (b). Emergence of the blastic subclones expressing functional ligands may promote the leukemic invasion of T-ALL cells in the extrathymic vital organs, due to the cell-autonomous Notch activation and proliferation independent of other ligand-donor cells (c). During their progression, "activating" Notch mutations causing ligand-independent Notch S2 processing or other genetic changes bypassing the Notch signal-dependency per se may follow, leading to the disease aggravation (d).



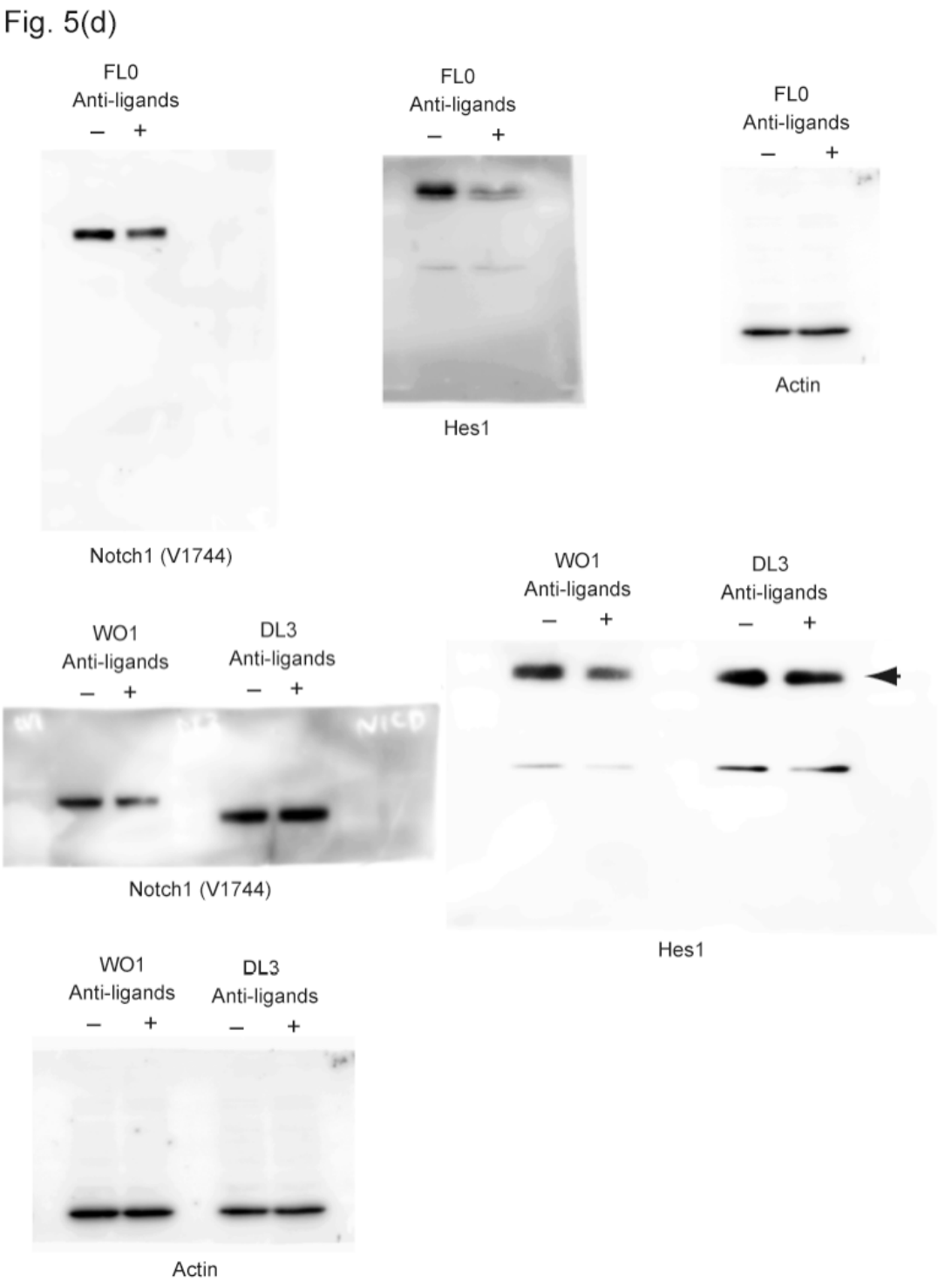
Supplementary Figure S7 | Full-length blots for the data in Figure 1.



Supplementary Figure S8 | Full-length blots for the data in Figure 2.



Supplementary Figure S9 | Full-length blots for the data in Figure 4.



Supplementary Figure S10 | Full-length blots for the data in Figure 5.