

Supplementary information for:

**Leukemia-associated mutations within the NOTCH1 heterodimerization
domain fall into at least two distinct mechanistic classes**

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FIG. S1. HD domain mutations do not affect sensitivity to ligand. Stable cell lines expressing NOTCH1-GAL4 with the indicated mutations were cocultured with OP9 cells or OP9 cells stably expressing Delta-like1. After a day of coculture, lysates were prepared and luciferase assays were performed. Gray bars: OP-9 cells without ligand; black bars: OP-9 cells expressing delta-like 1.

FIG. S2. Treatment with the GSI inhibitor DAPT abrogates the stimulatory effects of HD domain mutations in full-length NOTCH1. Immediately following transfection of U2OS cells with NOTCH1 expression plasmids encoding the indicated mutations, cells were treated with 1 μ M DAPT (+) or carrier alone (-). Transcriptional activation assays were performed 44-48 hr after transfection, as described in FIG. 3.

FIG. S3. Serial dilutions of sHD immunoprecipitates. Conditioned media from 293T cells expressing secreted sHDs were immunoprecipitated with anti-FLAG antibody as described in FIG. 5. A dilution series was loaded on an SDS-PAGE gel and the polypeptides were detected with antibodies to the FLAG and HA epitope tags.

FIG. S4. Representative repeat evaluation of the P12 mutation in the urea sensitivity assay. Conditioned media from 293T cells expressing sHD with the

P12 mutation were treated as in FIG. 6, but with urea concentrations up to 6M.

The P12 mutant is reproducibly (N=4) as stable or only marginally less stable than the native sHD.

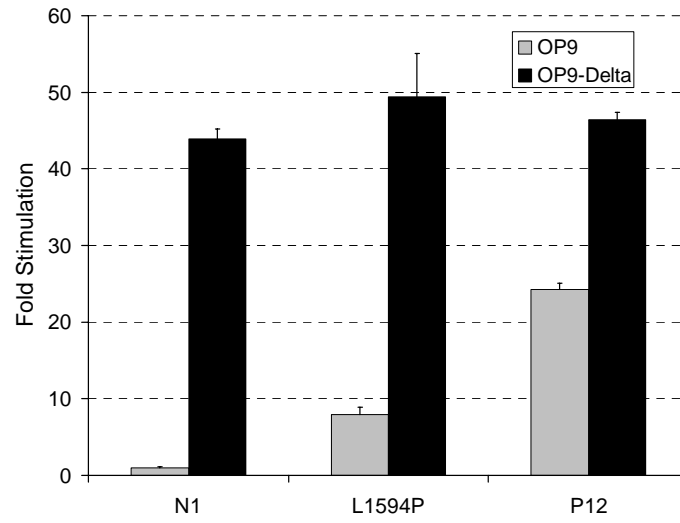


Figure S1

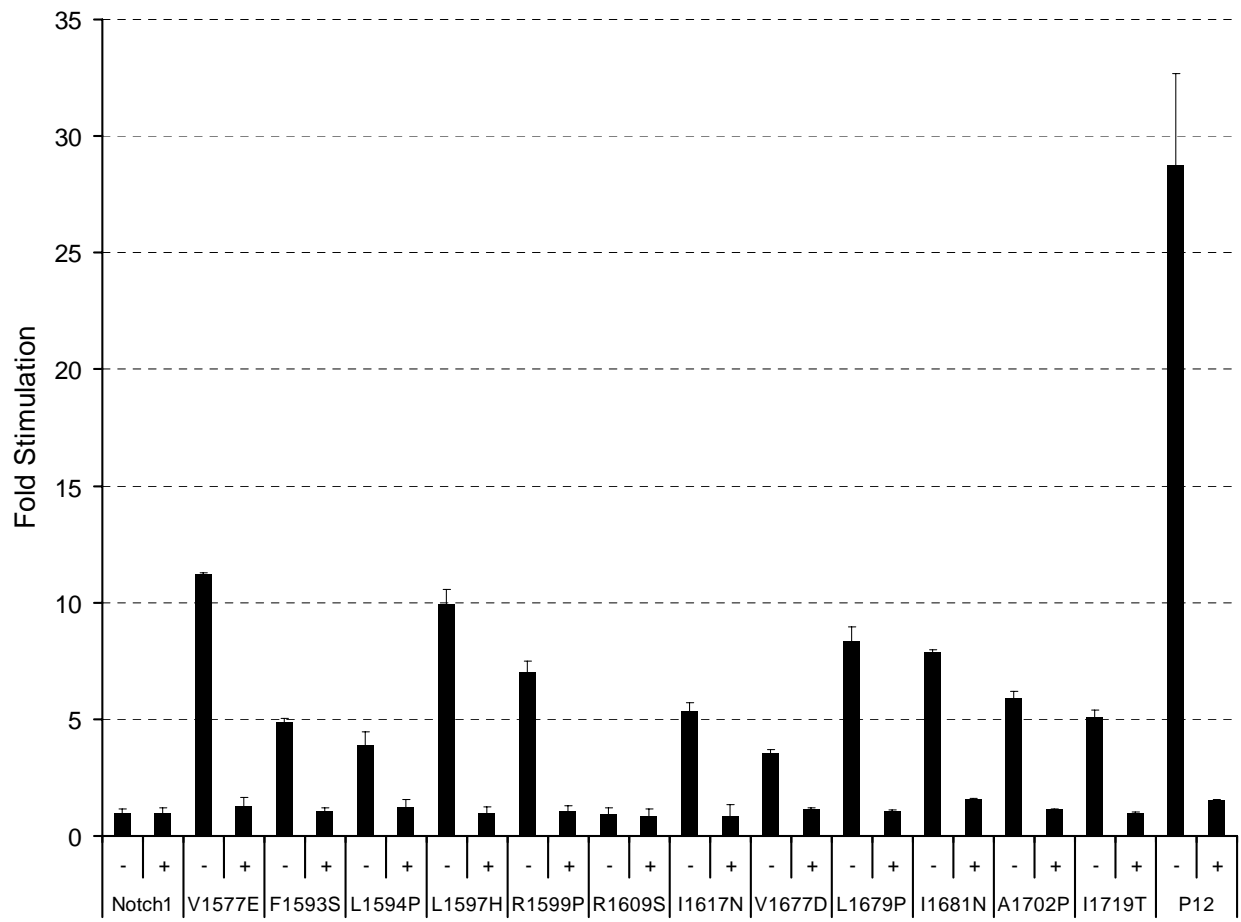


Figure S2

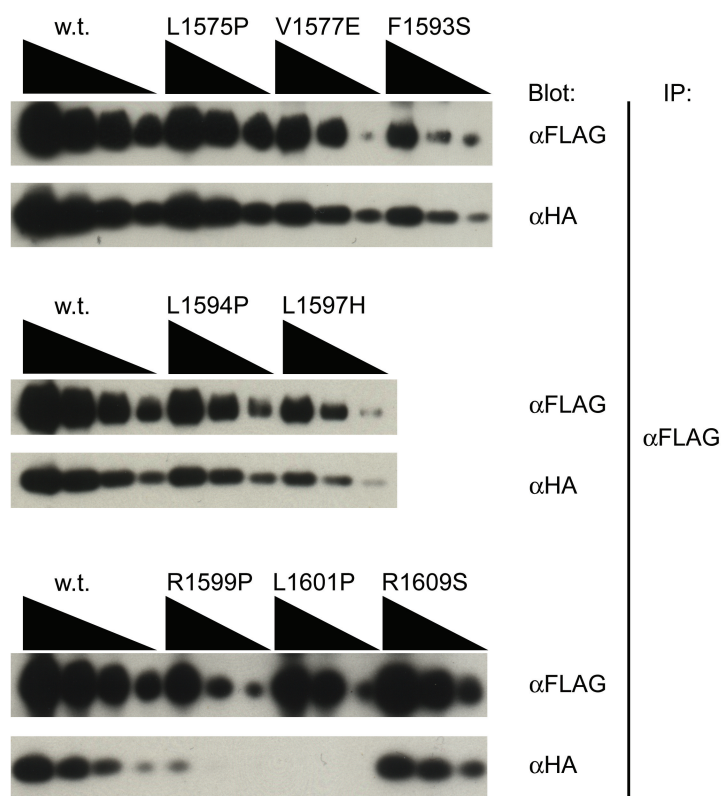


Figure S3

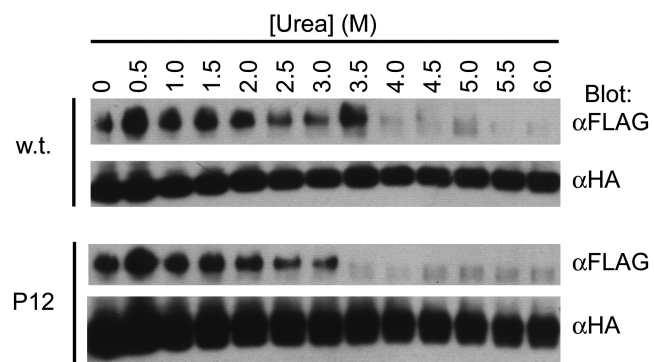


Figure S4