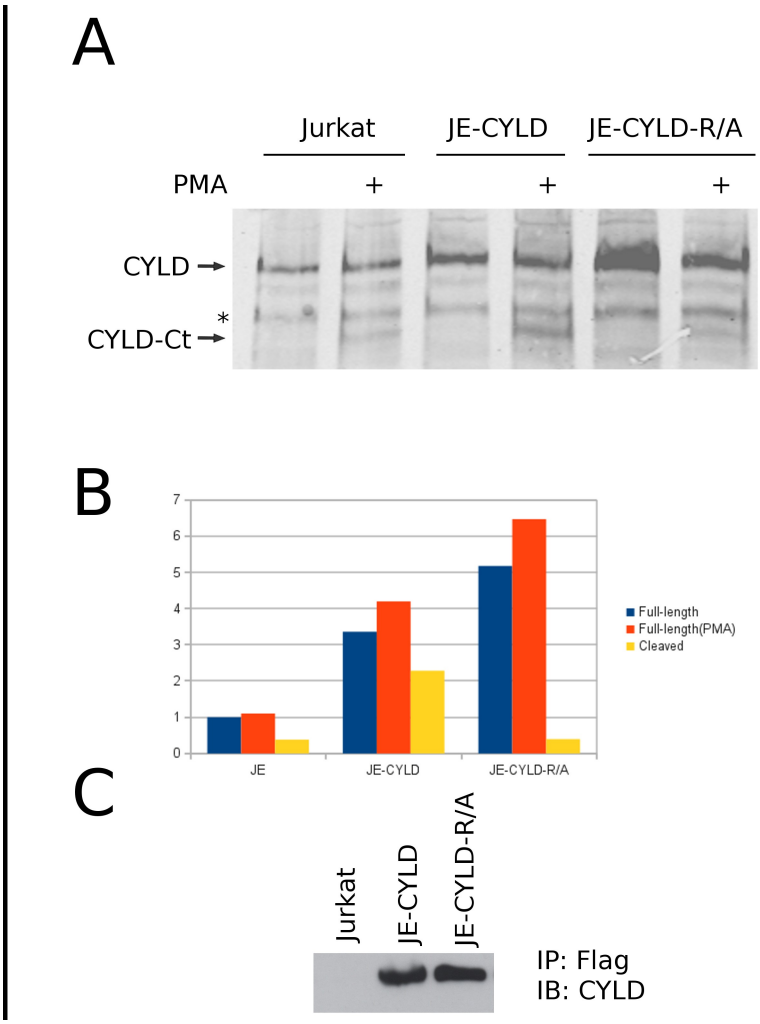
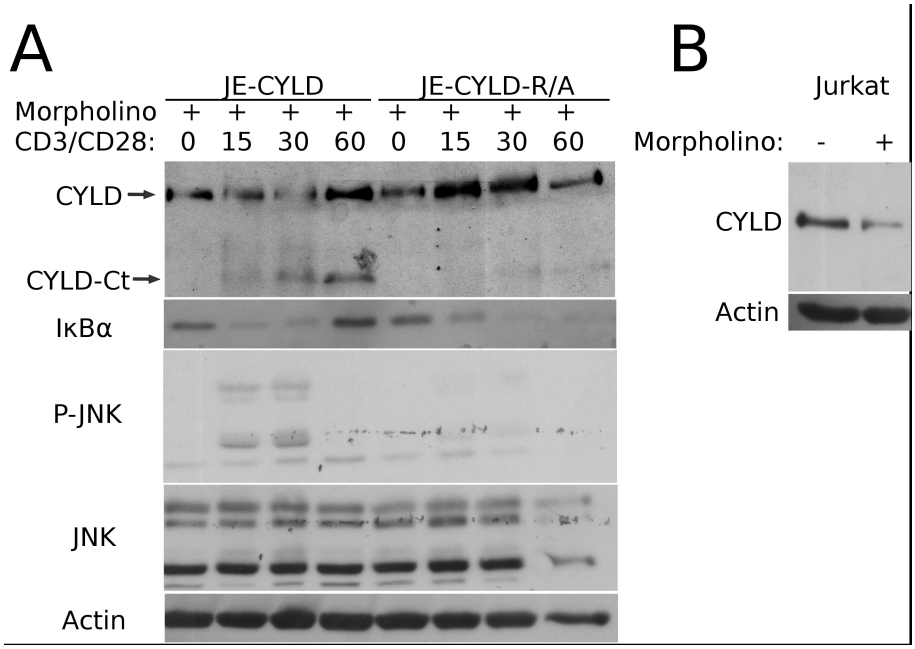
## Supplementary information

### Figure S1



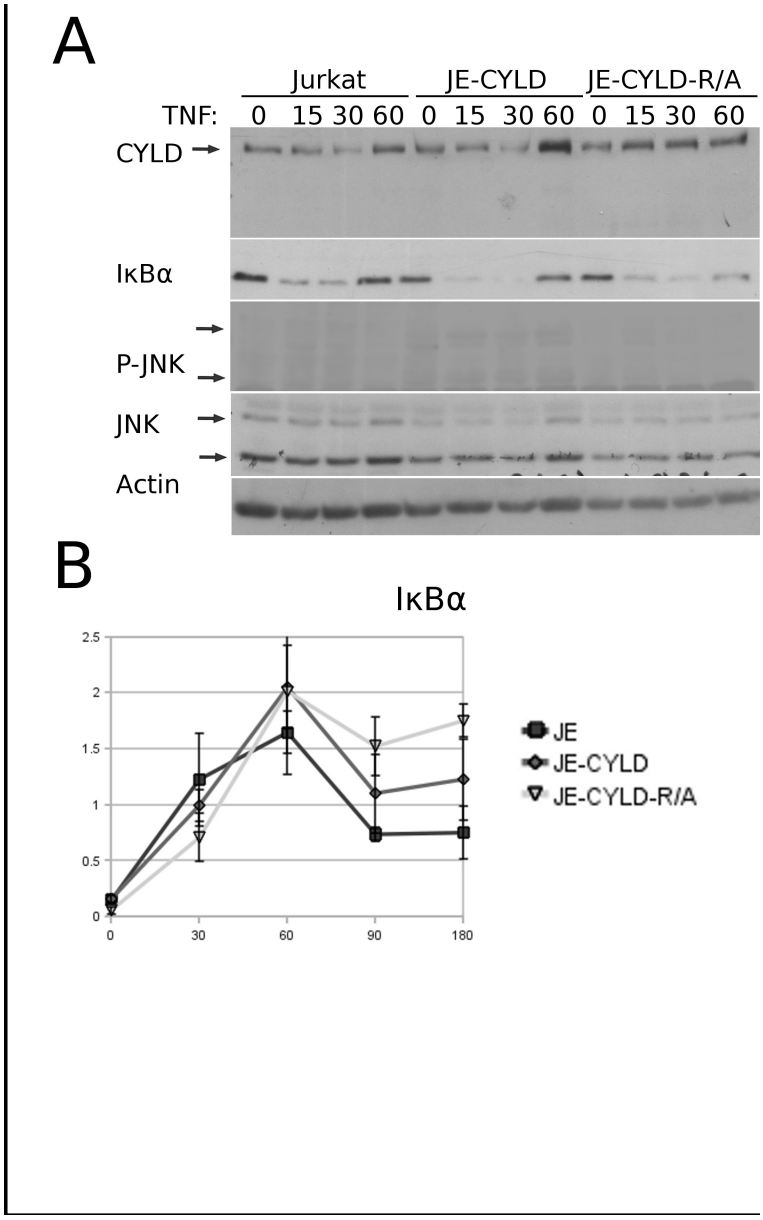

Expression levels of CYLD in parental Jurkat and cells transfected with Flag-tagged wild type CYLD or CYLD-R324A. (**A**) Cells were stimulated with PMA for 1 h as indicated and analyzed for CYLD using immunoblotting with anti-CYLD. The asterisk indicates a non-specific band. (**B**) Intensity of the CYLD bands from the immunoblot shown in (A) was quantified with an Odyssey infrared imaging system (LI-COR Biosciences) and normalized against the non-specific band indicated with an asterisk. CYLD expression in unstimulated Jurkat cells was set as '1'. (**C**) Specific detection of transfected CYLD. CYLD was immunoprecipitated (IP) with anti-Flag and revealed by immunoblotting (IB) with anti-CYLD.

**Figure S2**




JNK and NF-κB signaling in Jurkat cells stably transfected with wild type CYLD or CYLD-R324A and silenced for endogenous CYLD. (A) Immunoblot analysis of CYLD, IκBα, phospho-JNK (P-JNK), JNK and β-actin from cells that are treated for the indicated times (min) with anti-CD3 plus anti-CD28. Endogenous CYLD was silenced by transfection with a morpholino targeting the CYLD 5'UTR two and three days prior to stimulation. (**B**) Endogenous CYLD expression in Jurkat cells transfected with CYLD specific morpholinos (+) or control morpholino (-) as mentioned in (A).

**Figure S3**




TNF-induced signaling in Jurkat cells transfected with wild type CYLD or CYLD-R324A. (**A**) Immunoblot analysis of CYLD, IκBα, phospho-JNK (P-JNK), JNK and β-actin from cells that are treated for the indicated times (min) with 1000 IU/ml human TNF. (**B**) IκBα expression was determined by Q-PCR and normalized against the two housekeeping genes HPRT1 and PPIA.