Hövelmeyer et al., http://www.jem.org/cgi/content/full/jem.20070318/DC1

SUPPLEMENTAL MATERIALS AND METHODS

The generation of *CYLD*$^{ex7/8}$ mice. The *CYLD*$^{ex7/8}$ mouse strain was generated using standard gene targeting techniques (1). In brief, a *Cyld*-targeting vector flanking exon 7 by loxP sites was generated by inserting the short arm of homology, the exon 7, and the long arm of homology into the *pRapidFlirt* (unpublished data) containing the loxP sites, the FRT-flanked neomycine resistance gene, and the TK gene from herpes simplex as negative selection markers. The 2.5-kb short arm was amplified from C57BL/6 genomic DNA via PCR, digested with BglII, and subsequently cloned into the BamHI site of *pRapidFlirt*. The resulting plasmid was used for the insertion of the 600-bp loxP-flanked sequence into the SbfI site, consisting of a part of intron 7, the exon 7, and part of intron 8. This 600-bp fragment was PCR amplified from C57BL/6 genomic DNA, digested with SbfI, and cloned into the *pRapidFlirt*. The resulting plasmid was used for the finalization of the *CYLD*$^{ex7/8}$ targeting vector by inserting the long arm into the XhoI site. The long arm was amplified by PCR from C57BL/6 genomic DNA. All PCR fragments and plasmids were verified by sequencing. The targeting vector was then linearized and electroporated into V6.5 embryonic stem cells. Recombinant ES cells were identified by Southern blot analysis and were injected into CB20 blastocysts to generate the *CYLD*$^{neo/+}$ mice. In addition, this clone was used for in vitro deletion of the loxP-flanked exon 7 and the neomycine resistance gene by Cre-mediated recombination transfecting with a Cre-expressing plasmid. One of the Cre-deleted clones was injected into tetraploid blastocysts to generate germline *CYLD*$^{ex7/8/wt}$ mice. All mice were backcrossed to C57BL/6 mice 10 times and kept in a specific pathogen-free barrier.

Induced class switch recombination. Using MACS beads, B cells were isolated from wild-type, as well as CYLD$^{ex7/8}$ mice. The B cells were incubated for 5 d in the presence of 10 μg/ml LPS and 20 ng/ml IL-4. Thereafter, the B cells were analyzed for the expression of IgG1 by flow cytometry.

REFERENCE

1. Kuhn, R., and F. Schwenk. 1997. Advances in gene targeting methods. *Curr. Opin. Immunol.* 9:183–188.