Schulz et al., http://www.jcb.org/cgi/content/full/jcb.201503128/DC1

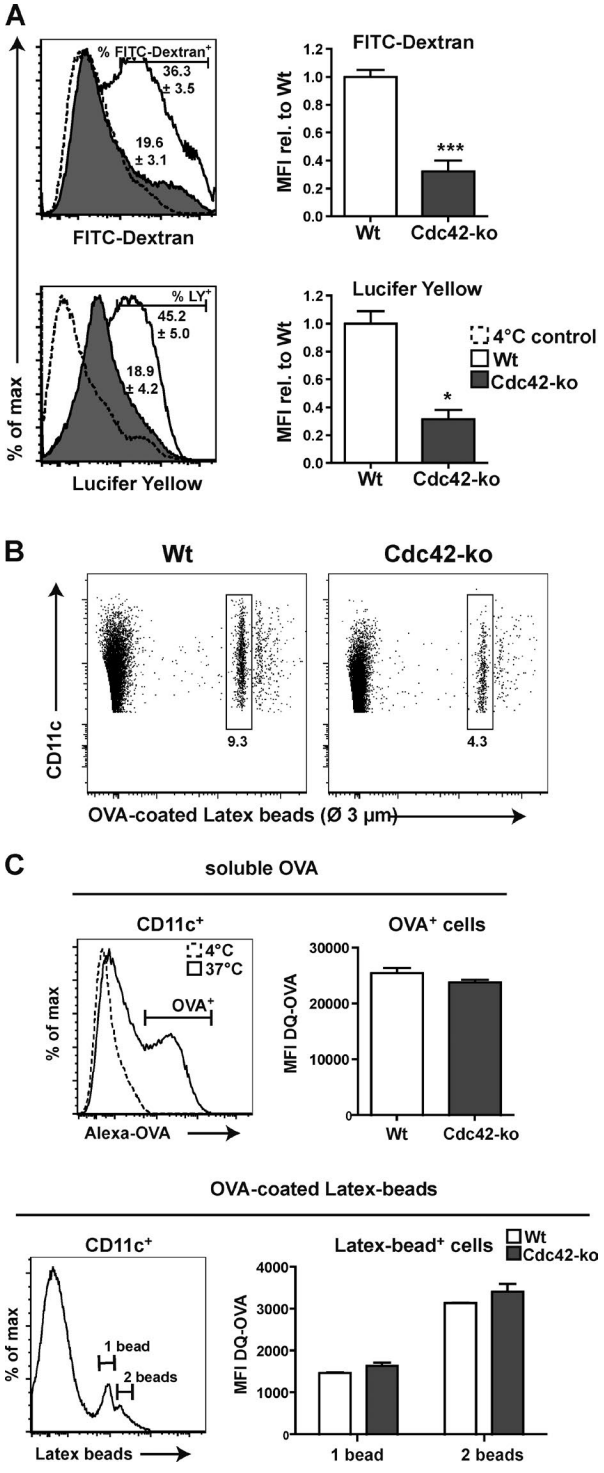

Figure S1. **Cdc42 ko BMDCs show defective Ag uptake and priming.** BMDCs were incubated with limited amounts of the following fluorescent proteins: FITC-dextran and Lucifer yellow (A), OVA-coated latex beads (B), or DQ OVA plus Alexa OVA (C). Numbers on FACS blots in A and C indicate the frequencies of Ag+ cells or frequencies of DCs, which took up a single latex bead in B. Graphs show MFI values gating on CD11c+ cells. Data are from three independent experiments. *n* = 4. (C) BMDCs were incubated with Alexa OVA and DQ OVA for 15 min. Residual protein was removed from culture supernatants, and cells were allowed to process DQ OVA for 15 min. MFI of DQ OVA were obtained from gating on CD11c+Alexa OVA+ cells. Data show one experiment (*n* = 2) out of two with similar results. (D) wt or Cdc42 ko BMDCs were pulsed with 100 pg/ml $OVA_{323–339}$ and then co-cultured with OVA-specific CFSE-labeled OT-II T cells at the indicated DC/T cell ratios. After 4 d, T cell proliferation was determined as CFSE dilution. For gating strategy, see Fig. 1. Graphs show the percentage of divided T cells of one representative experiment out of two. *n* = 2.

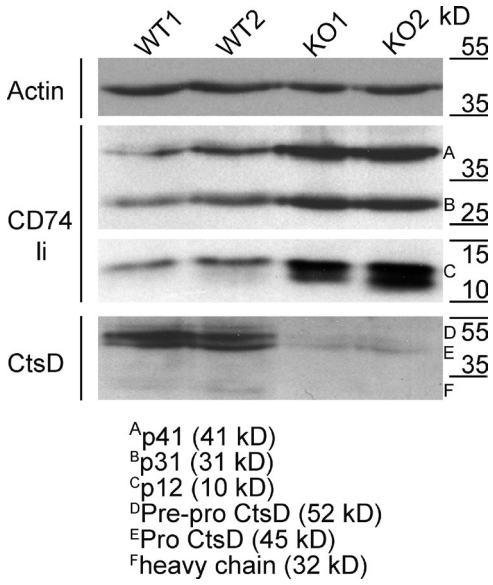

Figure S2. **Lack of Cdc42 causes inefficient degradation of Ii.** BMDC lysates were analyzed by Western blotting using antibodies specific for CTSD or Ii (clone In-1, which recognizes a 41- and 31-kD isoform as well as a 12-kD processing intermediate of CD74). Actin was used as a loading control.

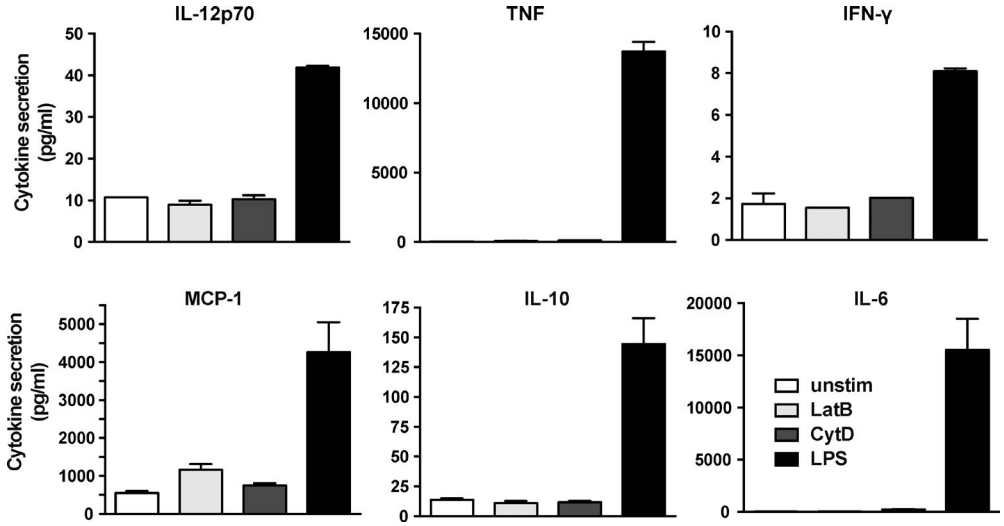

Figure S3. **Treatment with actin inhibitors does not induce maturation of BMDCs.** To exclude an effect on DC maturation, supernatants of DCs pulsed with inhibitors were analyzed using a cytometric bead array (Mouse Inflammation kit; BD). Supernatants were tested for proinflammatory cytokines, and LPS-treated culture supernatant served as a positive control. Bar graphs show one of two independent experiments performed in duplicates. Error bars represent mean ± SEM.

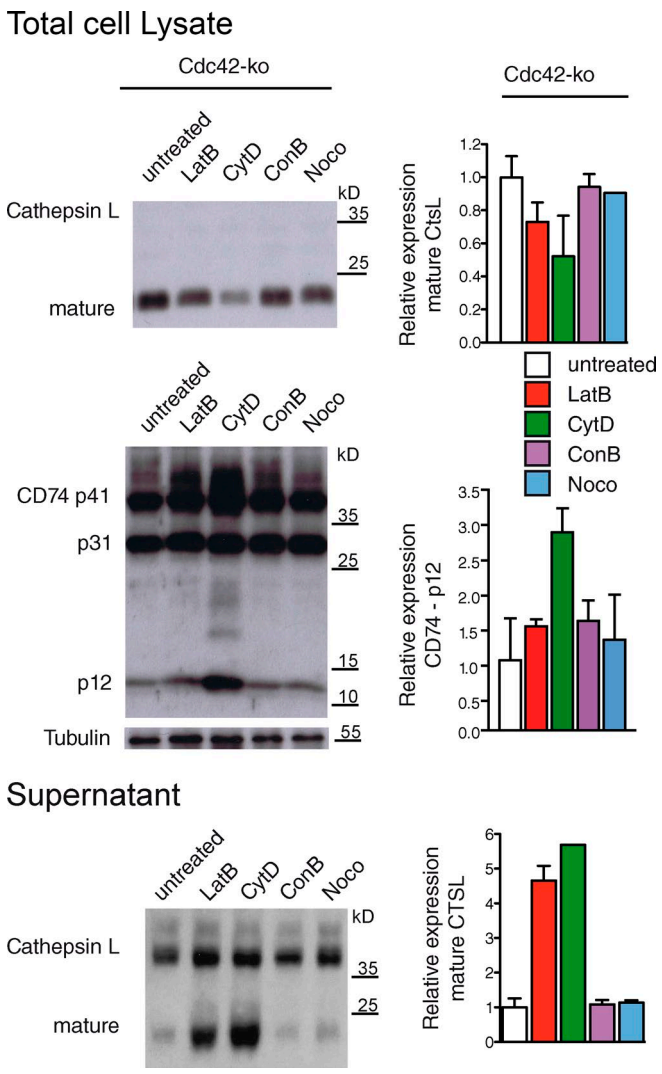

Figure S4. **Cdc42 ko BMDCs can be further inhibited by actin inhibitors.** TCLs (top) and supernatants (bottom) of Cdc42 ko BMDCs were analyzed by Western blotting for CTSL and Ii (clone In-1). Tubulin served as a loading control. Bar graphs show a quantification of protein expression levels from two independent experiments (n = 2) normalized to tubulin. Error bars represent mean ± SEM.

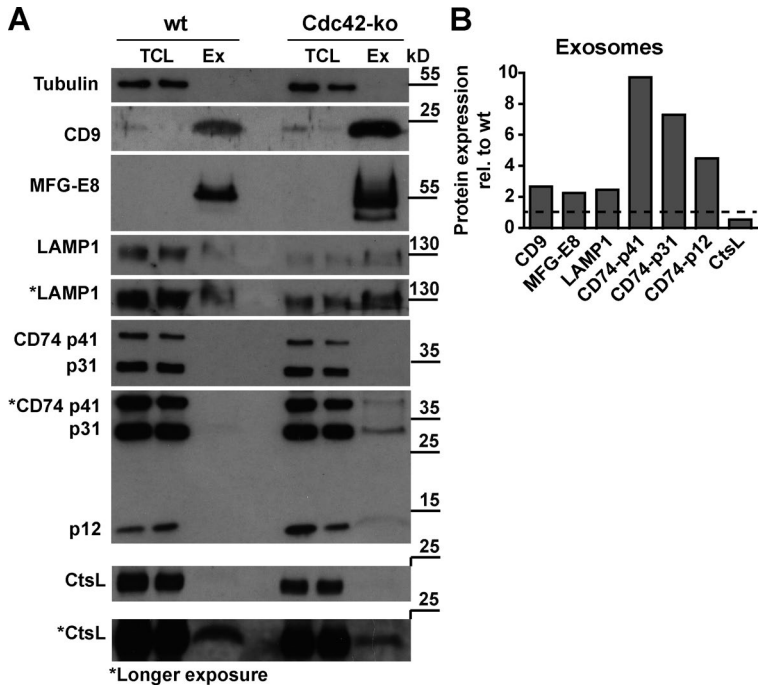

Figure S5. **Exosomes secreted from Cdc42 ko BMDCs accumulate LAMP-1 and Ii in addition to classical exosomal proteins.** (A) TCLs and exosomes (Ex) from wt and Cdc42 ko BMDCs were analyzed by Western blotting for LAMP-1, Ii (clone In-1), and CTSL. The exosomal fraction contains exosomes isolated from two biological replicates. Tubulin served as a loading control for TCLs, and CD9 and MFG-E8 were used as markers for exosomes. Equal amounts of proteins were loaded onto each lane. (B) Bar graph shows a quantification of protein expression levels from one (n = 2) out of two independent experiments (n = 4). The protein contents of exosomes secreted from Cdc42 ko DCs were normalized to exosomal protein contents secreted by wt BMDCs (dashed line).

Table S1 is provided as an Excel file and contains GO term analysis cellular component lysosome (GO: 0005764).

Table S2 is provided as an Excel file and shows the GO term Molecular Function Actin binding proteins (GO: 0003779) analysis.

Table S3 is provided as an Excel file and shows GO term Molecular Function Microtubule binding proteins (GO:0008017).

Table S4 is provided as an Excel file and contains proteins that showed >1.5-fold regulation in both replicates for loading to DAVID (http://david.abcc.ncifcrf.gov/home.jsp) for functional annotation clustering.

Table S5 is provided as an Excel file and shows a list of proteins in annotation clusters with an enrichment score >1 from DAVID functional annotation analysis.