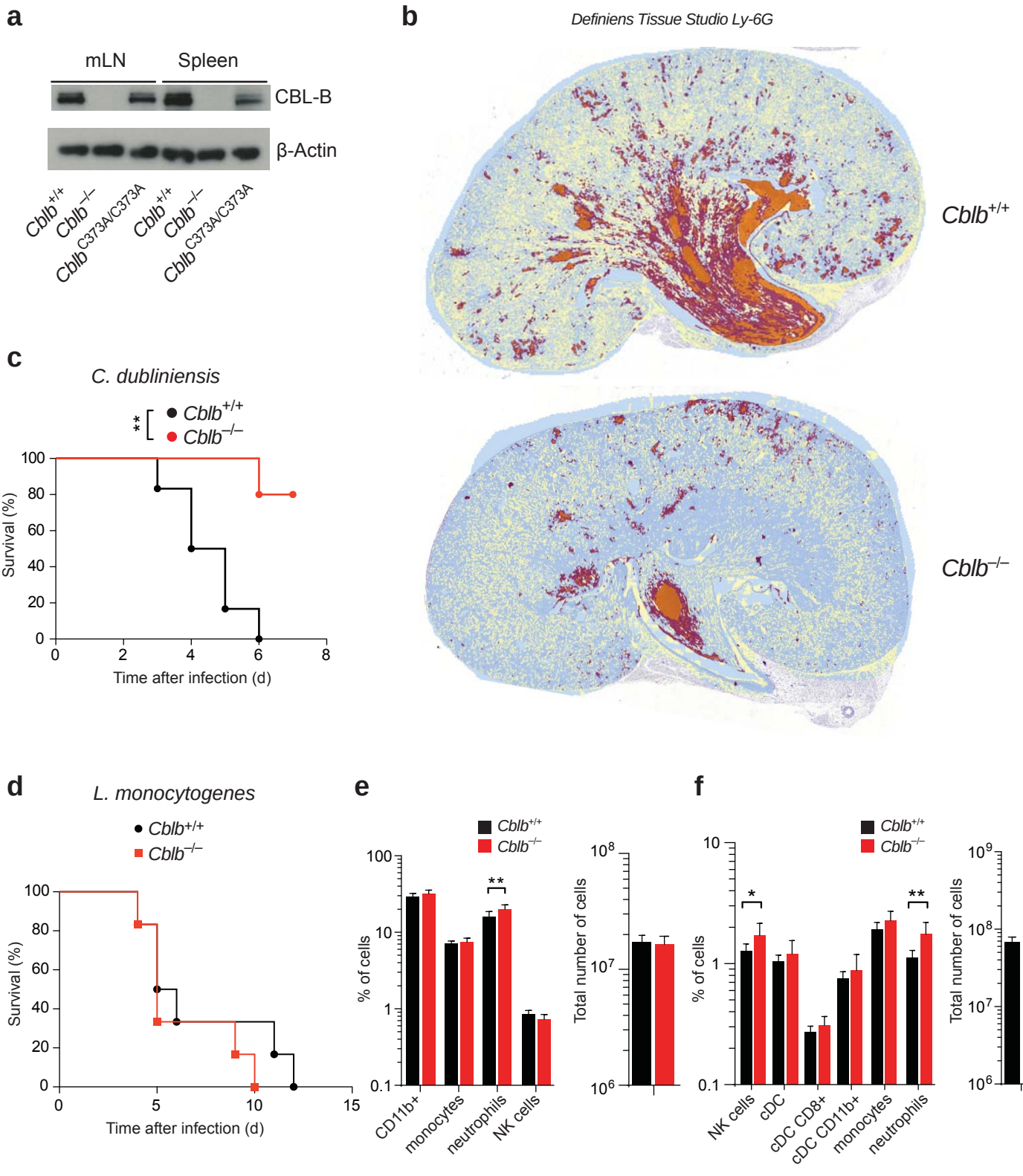
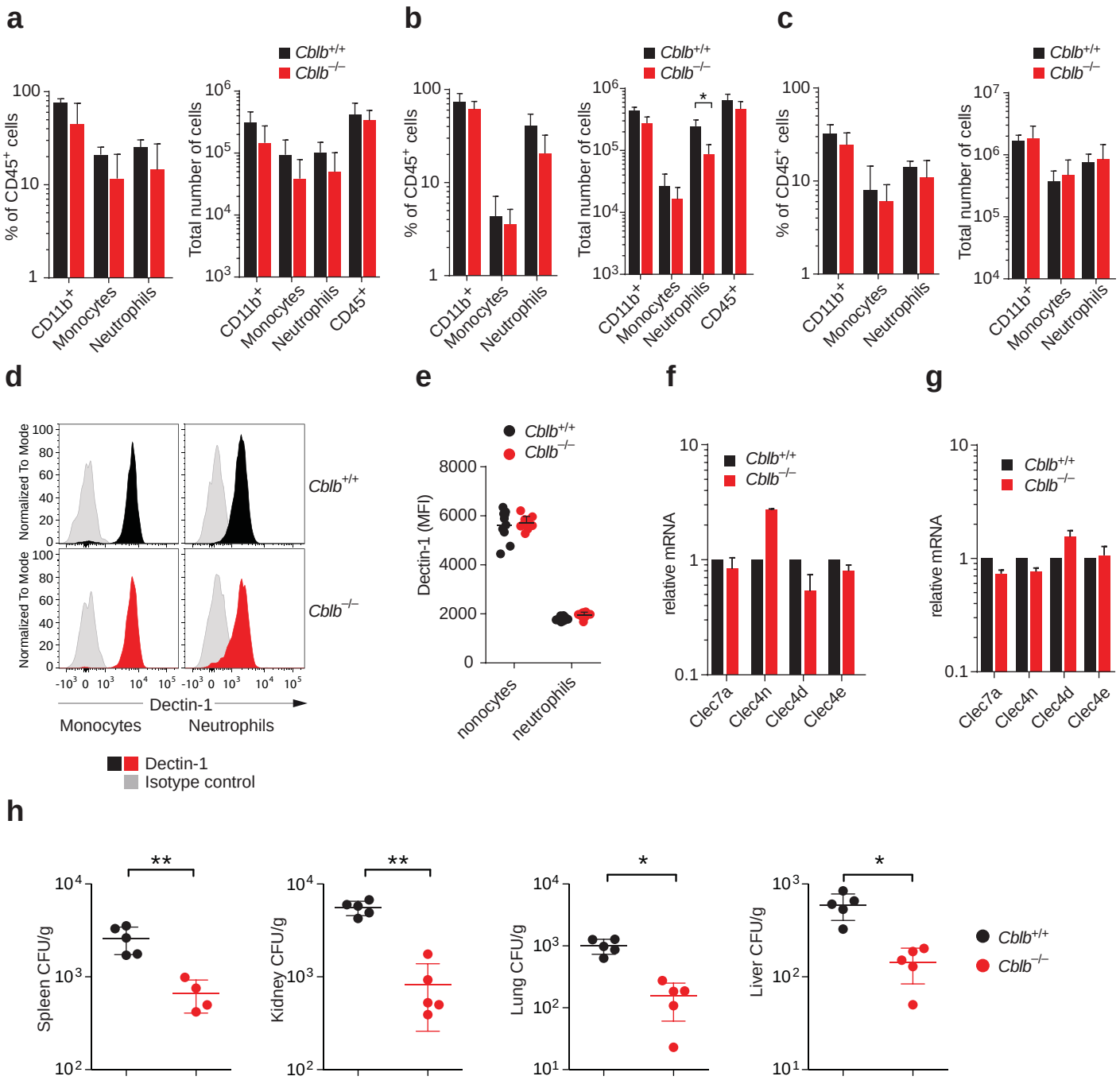
Supplementary Figure 1



Supplementary Figure 1. *Candida ssp.* and *Listeria monocytogenes* infection.

(**a**) Protein levels of CBL-B in lysates prepared from mesenteric lymph nodes (mLN) or spleens of mice of the indicated genotypes as assessed with Western blotting. β-Actin is shown as loading control. (**b**) Representative Definiens Tissue Studio software analysis of Ly-6G stained kidney sections from **Figure 1h**. (**c**) *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ mice were infected intravenously with *C. dubliniensis* (10^6 CFUs) and monitored for the indicated period of time. Plot depicts survival over time after infection (*P* values assessed with log rank test) $n = 5$ for *Cblb*$^{+/+}$ and $n = 6$ for *Cblb*$^{-/-}$ cohort. (**d**) *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ mice were infected intravenously with *L. monocytogenes* (10^5 CFUs) and monitored for the indicated period of time. Plot depicts survival over time after infection. (**e,f**) Frequency of indicated innate immune cell lineages and total immune cell counts of (**e**) femurs or (**f**) spleens of mice of the indicated genotypes as assessed by flow cytometry. $n = 8$ for *Cblb*$^{+/+}$, $n = 11$ for *Cblb*$^{-/-}$ littermates. Data in **e**,**f** are shown as means ± standard deviation. For panels (**a,b,d,e,f**) 1 representative of 3, for panel (**c**) 1 representative of 2 independent experiments is shown. $*P < 0.05$, $**P < 0.01$ as calculated with *Student's t-test,* unless stated otherwise.

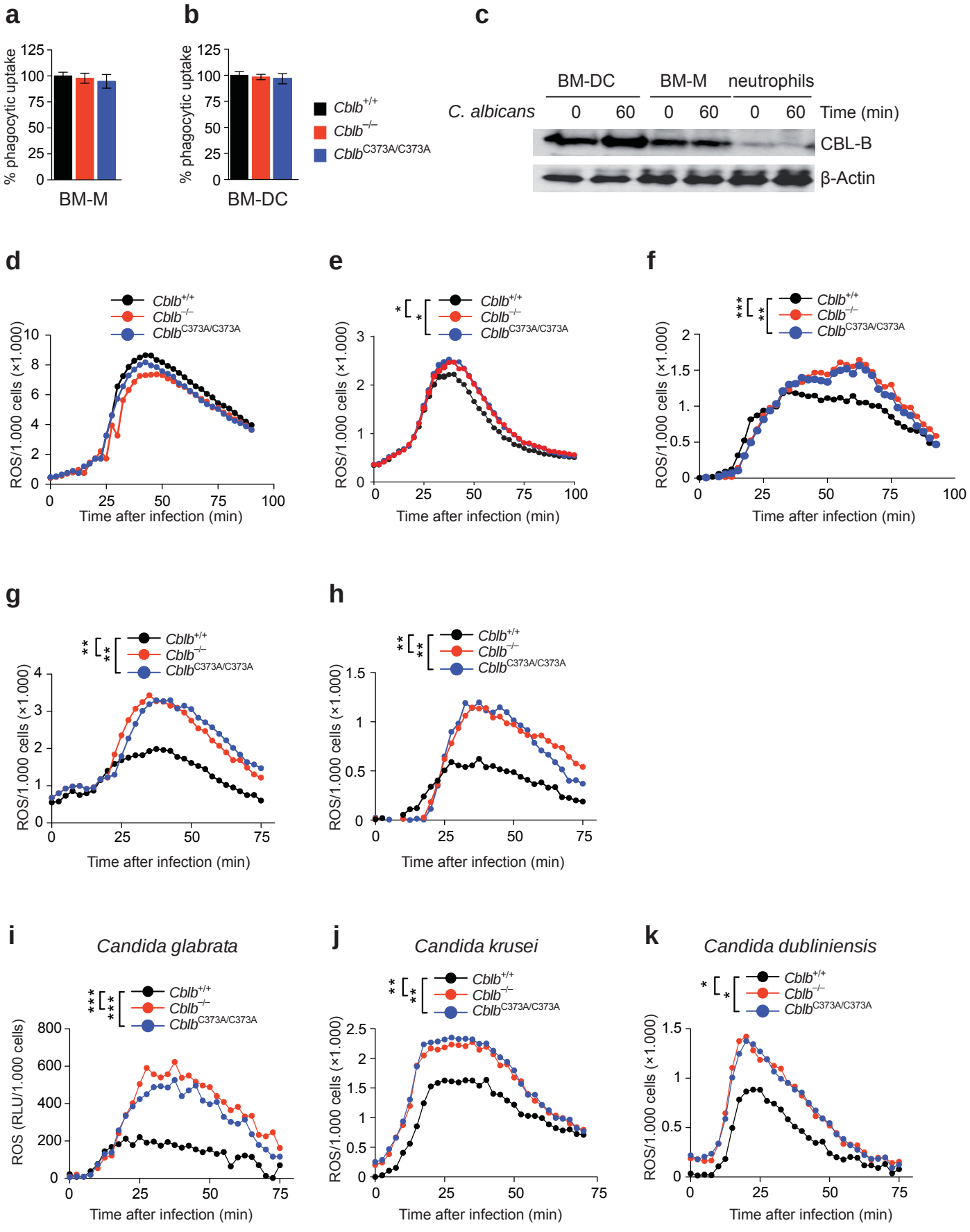
Supplementary Figure 2



Supplementary Figure 2. Immune infiltrations, CLR expression, and early fungal loads.

(**a,b,c**) Cellular recruitment of the indicated immune cell lineages to kidneys (**a**), lungs (**b**), and, livers (**c**) of *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ mice 24 h after infection with *C. albicans* (10^5 CFU/21.5 g body weight). Plots depict percentages of CD45$^+$ hematopoietic cells (left panels) or total numbers of recruited cells per organ (right panels). $n = 5$ for *Cblb*$^{+/+}$, $n = 6$ for *Cblb*$^{-/-}$ mice. (**d,e**) Dectin-1 protein expression on peripheral blood monocytes and neutrophils isolated from *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ mice as assessed by flow cytometry. (**d**) Representative stainings and (**e**) Dectin-1 mean fluorescence intensities (MFI) are shown. Dots in (**e**) represent individual mice. (**f,g**) mRNA expression levels of the indicated C-type lectin receptors in (**f**) bone marrow cells and (**g**) splenocytes of *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ mice as assessed by quantitative PCR. Experiments were done in triplicates. (**h**) *C. albicans* fungal load in depicted organs 24 h after infection, plotted as colony forming units (CFU) / gram organ weight. Each dot represents an individual mouse. For panels (**a,b,c,e,f,g,h**) data are shown as means ± standard deviation. For panels (**a,b,c,f,g**) 1 representative of 3, for panels (**d,e**) 1 representative of 5 independent experiments is shown. *$P < 0.05$, **$P < 0.01$ as calculated with *Student's t-test*.

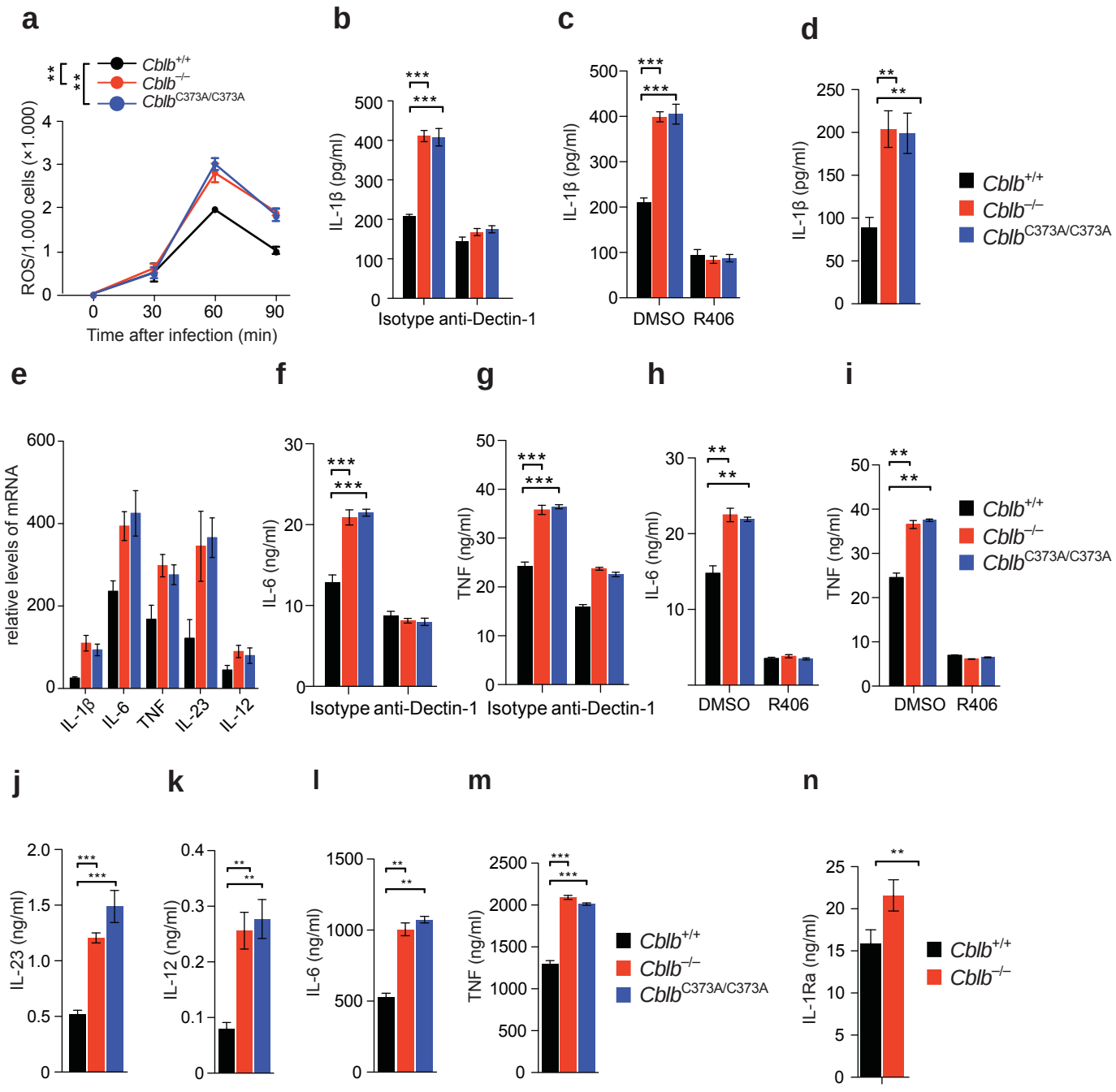
Supplementary Figure 3



Supplementary Figure 3. Phagocytosis and ROS production in response to fungal stimulation.

(**a,b**) Rate of phagocytosis by (**a**) BM-M or (**b**) BM-DC of the indicated genotypes co-cultured with *C. albicans*. (**c**) CBL-B protein expression as assessed by Western blotting from lysates prepared from *C. albicans* stimulated or unstimulated BM-DC, BM-M or bone marrow neutrophils derived from *Cblb*$^{+/+}$ mice. β-Actin blots are shown as loading control. (**d-h**) Reactive oxygen species (ROS) production by (**d**) bone marrow neutrophils, (**e**) BM-M, (**f**) BM-DC, (**g**) bone marrow monocytes, or (**h**) splenic DC co-cultured with *C. albicans* and monitored in real time over the indicated time periods using the luminol assay. Experiments were performed in triplicates. Values are expressed as relative light units per 1.000 cells. (**i-k**) ROS production by BM-DC of the indicated genotypes stimulated with (**i**) *C. glabrata*, (**j**) *C. krusei*, or (**k**) *C. dubliniensis*, monitored and analyzed as in (**d-h**). Data in panels (**a,b**) are shown as means ± standard deviation. *P* values for panel (**a,b**) were calculated with *Student's t-test*, for panels (**d-k**) with two-way ANOVA. For all experiments 1 representative of 3 independent experiments is shown. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

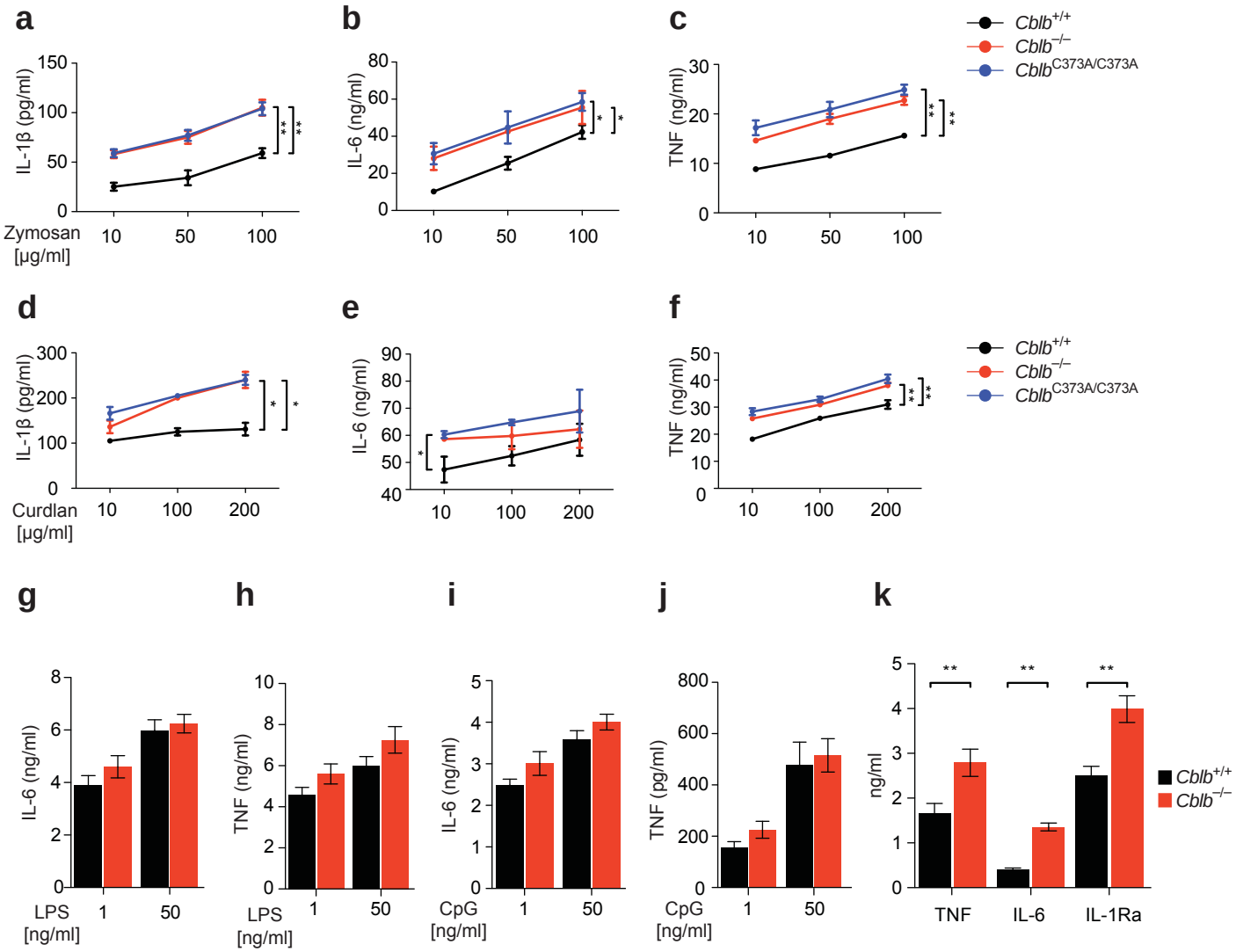
Supplementary Figure 4



Supplementary Figure 4. Cytokine release in response to fungal stimulation.

(**a**) Activation of Caspase-8 in BM-DC of the indicated genotypes monitored at the indicated time points after stimulation with *C. albicans*, using the CaspGLOW assay. (**b,c**) Secretion of IL-1β by BM-DC of the indicated genotypes stimulated with *C. albicans* for 18 h in the presence of (**b**) an anti-Dectin-1, or isotype control antibody, or (**c**) the SYK inhibitor R406, or DMSO as assessed by ELISA. (**d**) Secretion of IL-1β by BM-M upon 18 h co-culture with *C. albicans* as assessed with ELISA. (**e**) Induction of cytokine RNA expression in BM-DC of the indicated genotypes stimulated for 2 h with *C. albicans*, as assessed by quantitative PCR. Experiments were done in triplicates. (**f-i**) Secretion of the indicated cytokines by BM-DC of the indicated genotypes upon 18 h co-culture with *C. albicans* in the presence of (**f,g**) an anti-Dectin-1, or isotype control antibody, or (**h,i**) the SYK inhibitor R406, or DMSO as assessed with ELISA. (**j-n**) Secretion of the indicated cytokines by (**j,k,n**) BM-DC, or (**l,m**) BM-M of the indicated genotypes upon 18 h co-culture with *C. albicans* as assessed with ELISA. Data in all panels are shown as means ± standard deviation. *P* values for all panels except (**a**) were calculated with *Student's t-test*, for panel (**a**) with two-way ANOVA. For panels (**a-d,f-n**) 1 representative of 4, for panels (**e**) 1 representative of 5 independent experiments is shown. $^{**}P < 0.01$, $^{***}P < 0.001$.

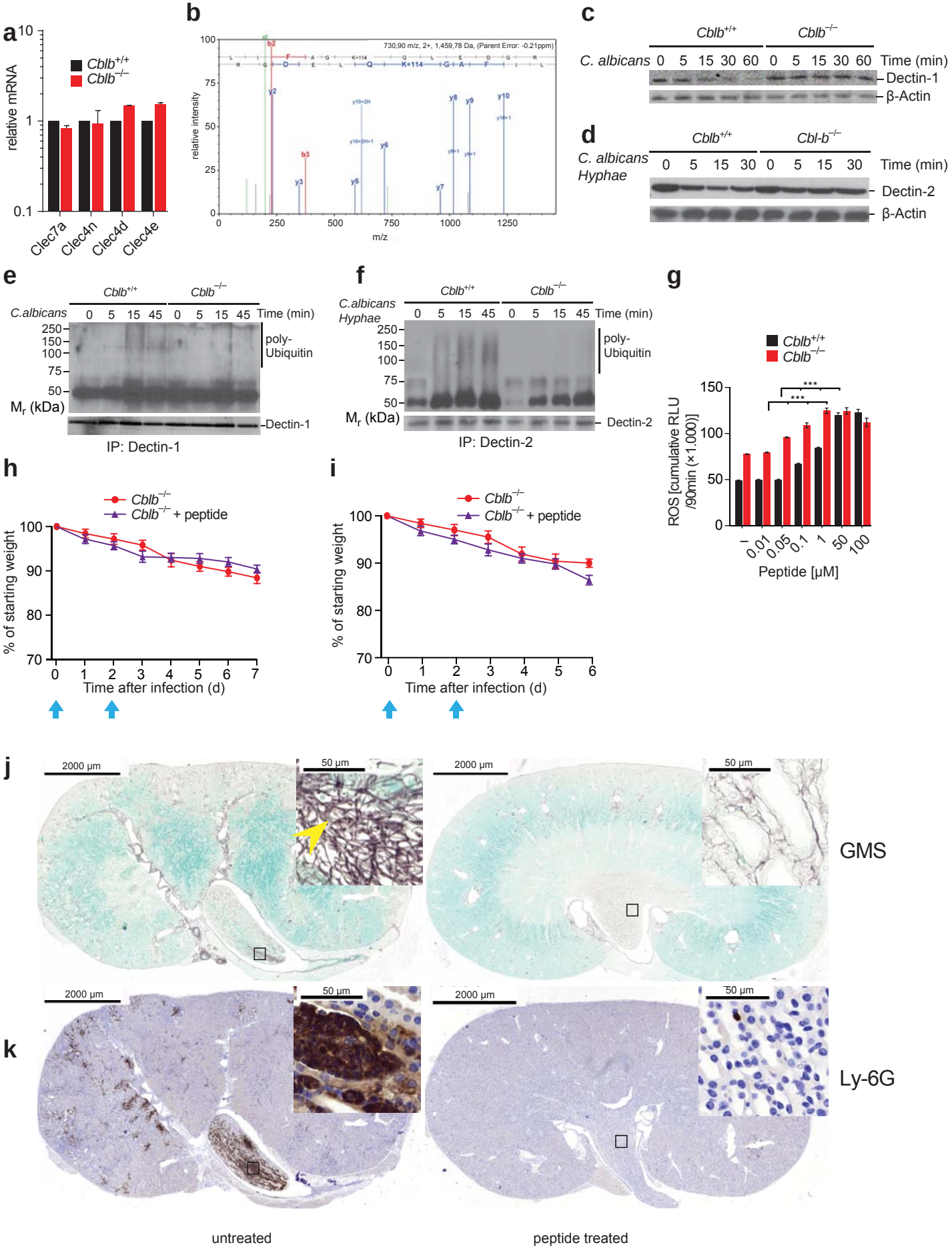
Supplementary Figure 5



Supplementary Figure 5. Zymosan, Curdlan, LPS, CpG, and *C. albicans* triggered cytokine release by BM-DC and intraperitoneal inflammatory infiltrates.

(**a-f**) Secretion of the indicated cytokines by *Cblb*$^{+/+}$, *Cblb*$^{C373A/C373A}$ or *Cblb*$^{-/-}$ BM-DC stimulated with increasing doses of either Zymosan **(a-c)**, or Curdlan **(d-f)** for 18 h, as assessed by ELISA. **(g-j)** Secretion of the indicated cytokines by *Cblb*$^{+/+}$ or *Cblb*$^{-/-}$ BM-DC stimulated with the indicated doses of either LPS **(g,h)**, or CpG **(i,j)** for 18 h, as assessed by ELISA. **(k)** Expression of the indicated cytokines in the intraperitoneal lavages of *Cblb*$^{+/+}$ or *Cblb*$^{-/-}$ mice 24 h after intraperitoneal infection with *C. albicans* as in **Figure 3h-I** as assessed by ELISA. Data in all panels are shown as means ± standard deviation. *P* values as calculated with **(a-f)** two-way ANOVA, or **(k)** *Student's t-test.* For all panels 1 representative of 3 independent experiments is shown. *$P < 0.05$, **$P < 0.01$

Supplementary Figure 6



Supplementary Figure 6. SYK and Dectin-1, -2 ubiquitination and CBL-B inhibitory peptide treatment.

(**a**) mRNA expression levels of the indicated C-type lectin receptors in *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ BM-DC assessed by qPCR. Experiments were done in triplicates. (**b**) Mass spectrometric analysis of the ubiquitin chains generated by *in vitro* ubiquitination by CBL-B. A representative MS/MS spectrum acquired for a peptide derived from K48-linked Ubiquitin chains, using a sample processed identically and in parallel to the sample shown in **Figure 5a**, is shown. Both β-type (red) and γ-type (blue) ions of the selected peptide are indicated. (**c,d**) Immunoblot analysis of (**c**) Dectin-1 and (**d**) Dectin-2 protein expression in *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ BM-M upon infection with (**c**) *C. albicans* or (**d**) *C. albicans* hyphae with an anti-Dectin-1 or anti-Dectin-2 antibody, respectively. β-Actin blots are shown as loading control. (**e,f**) Immunoblot analysis of (**e**) Dectin-1 and (**f**) Dectin-2 ubiquitination in E64-pre-treated *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ BM-M after immunoprecipitation with an anti-Dectin-1 or anti-Dectin-2 antibody, respectively, with an anti-ubiquitin antibody. Dectin-1 and Dectin-2 immunoblots are shown as controls. (**g**) ROS production by *Cblb*$^{+/+}$ and *Cblb*$^{-/-}$ BM-DC co-cultured with *C. albicans* in the presence of the indicated concentrations of the TKB-binding peptide. Plot depicts cumulative ROS production over 75 minutes for the indicated peptide concentrations. Experiments were performed in triplicates. Values are expressed as relative light units per 1.000 cells. (**h,i**) *Cblb*$^{-/-}$ mice were infected intravenously with *C. albicans* (10^5 CFU/21.5g body weight), injected intraperitoneally with (**h**) 100 μg or (**i**) 20 μg of the TKB-binding peptide at day 0 and day 2 after infection, and monitored for weight loss over time after infection as compared to starting weight (*P* values assessed by two-way ANOVA). $n = 5$ for treated and untreated cohorts. (**j,k**) Representative kidney sections from *Cblb*$^{+/+}$ mice treated as in **Figure 6g**, and stained with (**j**) Gomori methenamine silver (GMS) or (**k**) for the neutrophil marker Ly-6G. Yellow arrowhead in (**j**) indicates fungal hyphae. $n = 3$ for peptide treated and untreated cohorts. 4 kidney sections per mouse were analyzed. Data in (**a,g**) are shown as means ± standard deviation. For panel (**a,b**) 1 representative of 4, for panel (**c-k**) 1 representative of 3 independent experiments is shown. ***$P < 0.001$ as calculated with *Student's t-test*.

Antibodies and Primers used in this study

Antibodies

Antigen	Clone	Distributor
Syk/SYK	D1I5Q	Cell Signaling Technologies
phospho Syk	C87C1	Cell Signaling Technologies
Src	36D10	Cell Signaling Technologies
phospho Src	D7F2Q	Cell Signaling Technologies
Plc-γ2	Q-20	Santa Cruz Biotechnology
phospho-Plc-γ2	Tyr1217	Cell Signaling Technologies
NF-κB p65	E498	Cell Signaling Technologies
phospho-NF-κB p65	93H1	Cell Signaling Technologies
Shp-2	C-18	Santa Cruz Biotechnology
phospho-Shp-2	Tyr542	Cell Signaling Technologies
β-Actin	13 E 6	Cell Signaling Technologies
Ubiquitin	P4D1	Santa Cruz Biotechnology
Ly-6G FITC	1A8	BD Biosciences
CD8 Brilliant Violet 605	53-6.7	Biolegend
TCRβ Brilliant violet 421	H57-597	BD Biosciences
NK-1.1 APC	PK136	BD Biosciences
CD19 PE	6D5	BD Biosciences
CD11c FITC	HL3	Biolegend
CD16/32 purified	2.4G2	BD Biosciences
Ly-6C PerCP-Cy5.5	AL-21	BD Biosciences
anti-rabbit Alexa555	polyclonal	Invitrogen
anti-Dectin-1	GE2	Abcam
anti-Dectin-2	217611	R&D Systems

PCR Primers

Target	fwd	rev	amplicon
Clec4n	CTGCCCAAATCACTGGAAGT	GAAATTCTGCTCCGCTTCAG	142bp
Clec7a	GAACCACAAGCCCACAGAAT	TAGGAAGGCAAGGCTGAGAA	95bp
Clec4d	TACGCTGGACGAGAGGAAGT	ACAGGACAGCAGGTCCAAGT	112bp
Clec4e	CAAGTGCTCTCCTGGACGAT	CTGTAAGTTCTGCCCGGAAA	114bp
TNF	CAAAATTCGAGTGACAAGCCTG	GAGATCCATGCCGTTGGC	88bp
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA	140bp
IL-12	GGAAGCACGGCAGCAGAATA	AACTTGAGGGAGAAGTAGGAATGG	159bp
IL-23	GCAGATTCCAAGCCTCAGTC	TTCAACATATGCAGGTCCCA	86bp
IL-1β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA	120bp