SUPPORTING INFORMATION

Macrophage protects mycoplasma-infected chronic myeloid leukaemia cells from NK cell killing

Choo et al.

Supplementary Methods

Treatment of CML cells with poly(I:C) or lipopolysaccharide (LPS)

K562 CML cells were seeded at a density of 1 million cells per mL and treated with 10 or 100 ng mL^{-1} LPS (Sigma-Aldrich, St Louis, USA, *E. coli*, O55:B5) for 4 or 16 hours, or with 10 μg mL^{-1} poly(I:C) for 24 hours, according to established protocols[1-3]. The treated cells were seeded at 0.4 million cells per mL for determination of IL-8 production, to serve as an indicator of successful stimulation with LPS or poly(I:C).

Neutralisation of TIMP-1, TGFβ and IL-8

To determine the effect of TIMP-1, TGFβ and IL-8 on CML survival under trio-cultures of Mϕ, NK and CML, neutralising antibodies against TIMP-1 (10 µg mL^{-1}, polyclonal),TGFβ (10 µg mL^{-1}, clone # 9016) or IL-8 (various concentration as stated in the figure, clone # 6217) were added to the cultures. For TIMP-1 and TGFβ1 neutralising antibodies, the optimal concentration was employed for experiment after testing a range of concentrations of neutralising antibodies (0.3, 1, 5, 10 and 20 μg mL^{-1}). All neutralising antibodies and corresponding isotype controls were from R&D Inc, Minneapolis, USA (Supplementary Table 2).

Measurement of phosphorylated CD3ζ by flow cytometry

Following co-culture (of CML, Mϕ and NK), to capture the level of CD3ζ phosphorylation (anti-pY142-CD3ζ, Becton Dickinson, Franklin Lakes, USA) in NK cells, the cells were fixed for 7 minutes with 0.4% paraformaldehyde (PFA) at room temperature, before staining with fixable viability dye to distinguish dead cells and debris. The cells were then fixed again with 4% PFA for 7 minutes at room temperature, and subsequently permeabilised with ice-cold 100% methanol for 10 minutes on ice. After Fc receptor blocking, the cells were stained with anti-CD14-APC (to distinguish Mϕ from NK and CML), and anti-pY142-CD3ζ-PE primary conjugated antibody before acquisition of fluorescence signals by flow cytometry. Level of phosphorylation of CD3ζ was determined based on fold change in median fluorescence intensity (MFI).

1. Ngkelo A, Meja K, Yeadon M, Adcock I, Kirkham PA. LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and Gialpha dependent PI-3kinase signalling. *Journal of inflammation (London, England)* 2012; **9:** 1.
2. Shi H, Guo Y, Liu Y*, et al.* The *in vitro* effect of lipopolysaccharide on proliferation, inflammatory factors and antioxidant enzyme activity in bovine mammary epithelial cells. *Animal nutrition (Zhongguo xu mu shou yi xue hui)* 2016; **2:** 99-104.
3. Suet Ting Tan R, Lin B, Liu Q*, et al.* The synergy in cytokine production through MyD88-TRIF pathways is co-ordinated with ERK phosphorylation in macrophages. *Immunology and cell biology* 2013; **91:** 377-87.

Supplementary Tables

Supplementary table 1. Primer sequences used for general detection of mycoplasma and specific species using polymerase chain reaction.

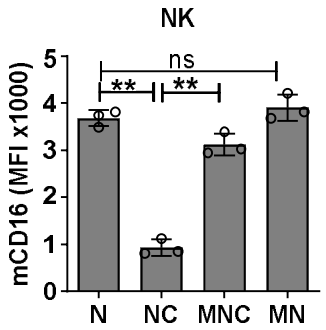
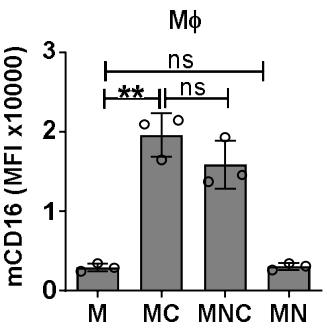
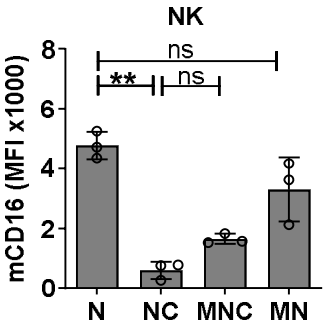
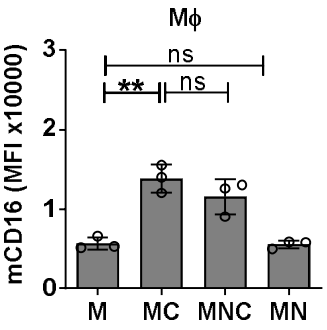
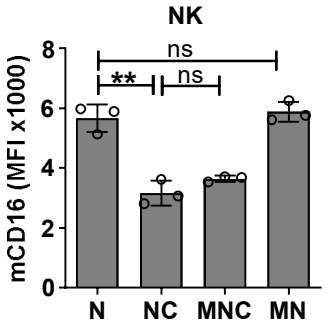
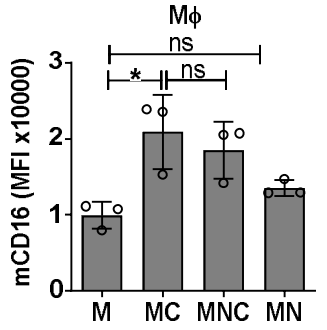
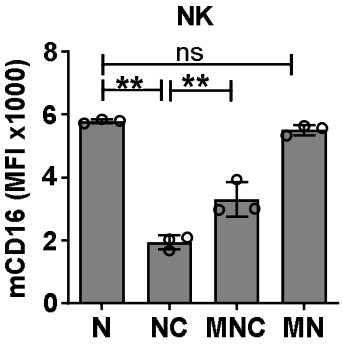
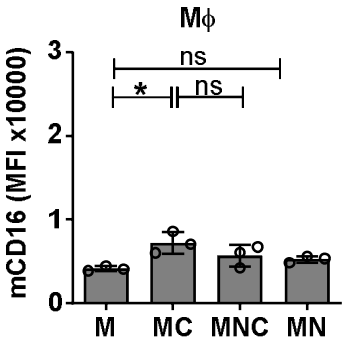
Name	Sequence (5' to 3')	Amplicon size	Citation
General mycoplasma detection	(F1) CGCCTGAGTAGTACGTTCGC (F2) CGCCTGAGTAGTACGTACGC (F3) TGCCTGAGTAGTACATTCGC (F4) TGCCTGGGTAGTACATTCGC (F5) CGCCTGGGTAGTACATTCGC (F6) CGCCTGAGTAGTATGCTCGC (R1) GCGGTGTGTACAAGACCCGA (R2) GCGGTGTGTACAAAACCCGA (R3) GCGGTGTGTACAAACCCCGA	500	Ref. 1
Mycoplasma bovis	(F) TCGTCCGCTGATGCAAGTGC (R) CGTCCGCTGACCTCAAGAA	499	Ref. 2
Mycoplasma arginini	(F) GATTCCGTTGTGAAAGGAGC (R) TCAAGCTTTCGCTC ATTGTG	202	Ref. 3
Mycoplasma fermentans	(F) GGACTATTGTCTAAACAATTTCCC (R) GGTTATTCGATTTCTAAATCGCCT	206	Ref. 4
Mycoplasma hominis	(F) ATACATGCATGTCGAGCGAG (R) CATCTTTTAGTGGCGCCTTAC	170	Ref. 4
Mycoplasma hyorhinis	(F) GATGTAGCAATACATTCAGTAGC (R) AAGTGAAGCTGTGAAGCTC	150	Ref. 5
Mycoplasma orale	(F) TAATCCTGTTTGCTCCCCAC (R) GGAGCGTTTCGTCCGCTAAG	583	Ref. 6
Acholeplasma laidlawii	(F) GATGAGAACTAAGTGTTGGCCATAA (R) CGCTAGAGTCCCCAACTTAATGA	328	Ref. 7

1. Uphoff CC, Drexler HG. Comparative PCR analysis for detection of mycoplasma infections in continuous cell lines. *In Vitro Cell Dev Biol Anim* 2002; **38:** 79-85.
2. Rodriguez JG, Mejia GA, Del Portillo P, Patarroyo ME, Murillo LA. Species-specific identification of Mycobacterium bovis by PCR. *Microbiology* 1995; **141:** 2131-8.
3. Lee DS, Yi TG, Lee HJ*, et al.* Mesenchymal stem cells infected with Mycoplasma arginini secrete complement C3 to regulate immunoglobulin production in B lymphocytes. *Cell death & disease* 2014; **5:** e1192.
4. Choppa PC, Vojdani A, Tagle C, Andrin R, Magtoto L. Multiplex PCR for the detection of Mycoplasma fermentans, M. hominis and M. penetrans in cell cultures and blood samples of patients with chronic fatigue syndrome. *Mol Cell Probes* 1998; **12:** 301-8.
5. Clavijo MJ, Oliveira S, Zimmerman J, Rendahl A, Rovira A. Field evaluation of a quantitative polymerase chain reaction assay for Mycoplasma hyorhinis. *Journal of veterinary diagnostic investigation* 2014; **26:** 755-60.
6. Timenetsky J, Santos LM, Buzinhani M, Mettifogo E. Detection of multiple mycoplasma infection in cell cultures by PCR. *Brazilian journal of medical and biological research* 2006; **39:** 907-14.
7. Molla Kazemiha V, Shokrgozar MA, Arabestani MR*, et al.* PCR-based detection and eradication of mycoplasmal infections from various mammalian cell lines: a local experience. *Cytotechnology* 2009; **61:** 117-124.

Supplementary table 2. List of primary antibodies used.

Antigen	Fluorescence	Clone	Company
Polychromatic flow cytometry			
CD45	APC-Cy7	2D1	BioLegend, San Diego, USA
CD3	FITC	OKT3	BioLegend, San Diego, USA
CD19	FITC	HIB19	BioLegend, San Diego, USA
CD34	PE	561	BioLegend, San Diego, USA
CD117	BV421	104D2	BioLegend, San Diego, USA
25F9	eFluor 660	eBio25F9	Thermo Fisher Scientific, Waltham, USA
CD56	PerCP-eFluor® 710	CMSSB	Thermo Fisher Scientific, Waltham, USA
CD14	APC	61D3	Thermo Fisher Scientific, Waltham, USA
CD14	BV605	M5E2	BioLegend, San Diego, USA
TIMP-1	PE	63515	R&D Inc, Minneapolis, USA
NKp46	PE/Dazzle594	9E2	BioLegend, San Diego, USA
CD16	PE	B73.1	Thermo Fisher Scientific, Waltham, USA
CD16	BV650	3G8	BioLegend, San Diego, USA
CD62L	PE/Dazzle™ 594	DREG-56	BioLegend, San Diego, USA
CD107a	PE	eBioH4A3	Thermo Fisher Scientific, Waltham, USA
IFNγ	eFluor® 450	4S.B3	Thermo Fisher Scientific, Waltham, USA
TNFα	APC-Cy7	MAb11	BioLegend, San Diego, USA
MIP-1α	PE	CR3M	Thermo Fisher Scientific, Waltham, USA
Neutralising antibodies			
TIMP-1	-	Goat polyclonal	R&D Inc, Minneapolis, USA
TGFβ	-	Mouse IgG1 monoclonal, clone #9016	R&D Inc, Minneapolis, USA
IL-8	-	Mouse IgG1 monoclonal, clone #6217	R&D Inc, Minneapolis, USA
Goat IgG	-	-	R&D Inc, Minneapolis, USA
Mouse IgG1	-	Clone #11711	R&D Inc, Minneapolis, USA

Supplementary table 3. Fold-change in sCD16 and mCD16 in NK and Mϕ in duo- and trio-cultures.

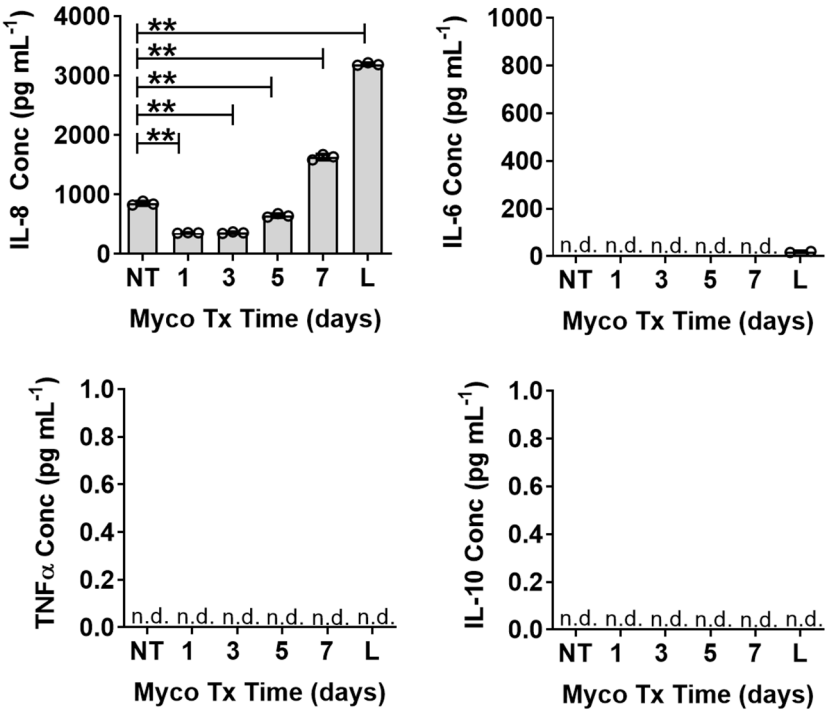
	NK (fold change)[a]			Mϕ (fold change)[b]		
	NC / N	MNC / N	MN / N	MC / M	MNC / M	MN / M
sCD16	↑ 3.12	↑ 10.1	↑ 3.16	↑ 4.14	↑ 9.93	↑ 3.11
mCD16	↓ 3.95	↓ 1.18 (maintained)	1.06 (maintained)	↑ 6.64	↑ 5.38	1.04 (maintained)
mCD16[c]						
Experiment 1						
Experiment 2						
Experiment 3						
Experiment 4						

[a,b] Fold-change is derived through comparison of sCD16 concentration or mCD16 MFI with respect to NK or Mϕ alone culture. Fold-change < 1.2 was considered as no change, viz, the level of mCD16 was maintained.

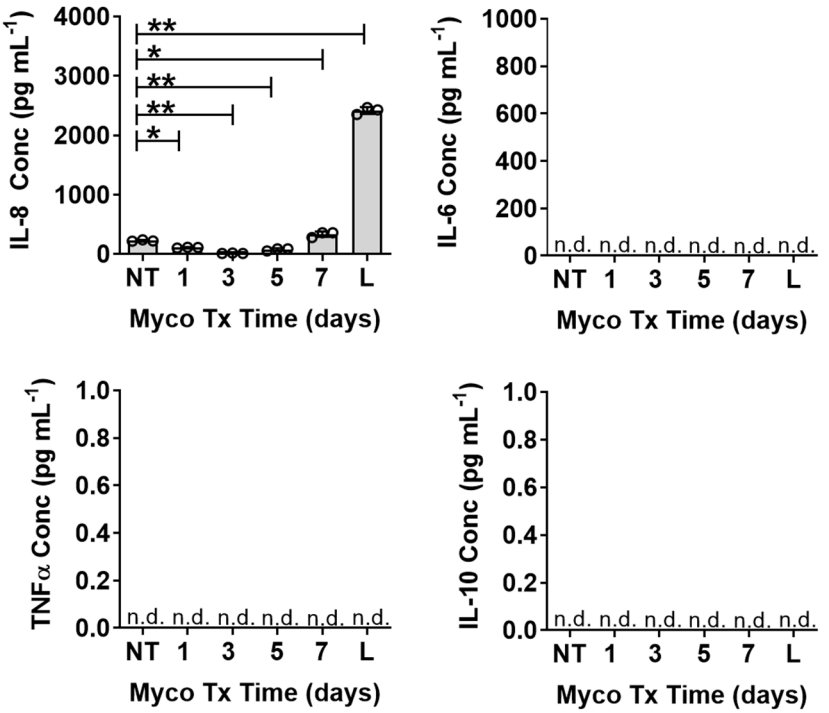
[c]Data from each experiment are presented as mean ± s.d. of 3 replicate cultures. 4 independent experiments, each from different donors are shown. For comparison between multiple groups, 1-way ANOVA was performed with Bonferroni post-hoc analysis. *$P < 0.05$, **$P < 0.01$, ns non-significant

Supplementary Figures

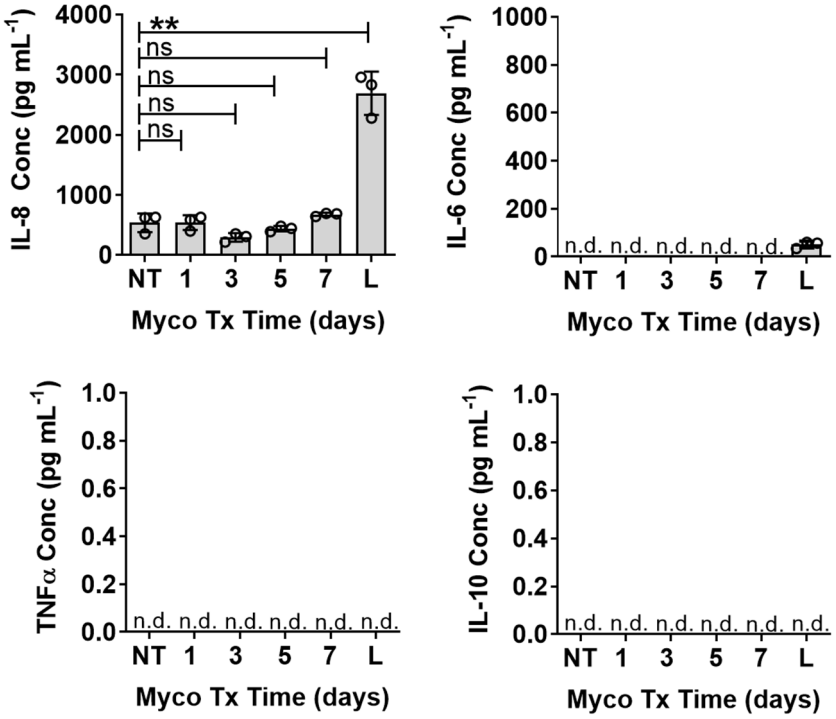
Experiment 1



Experiment 2

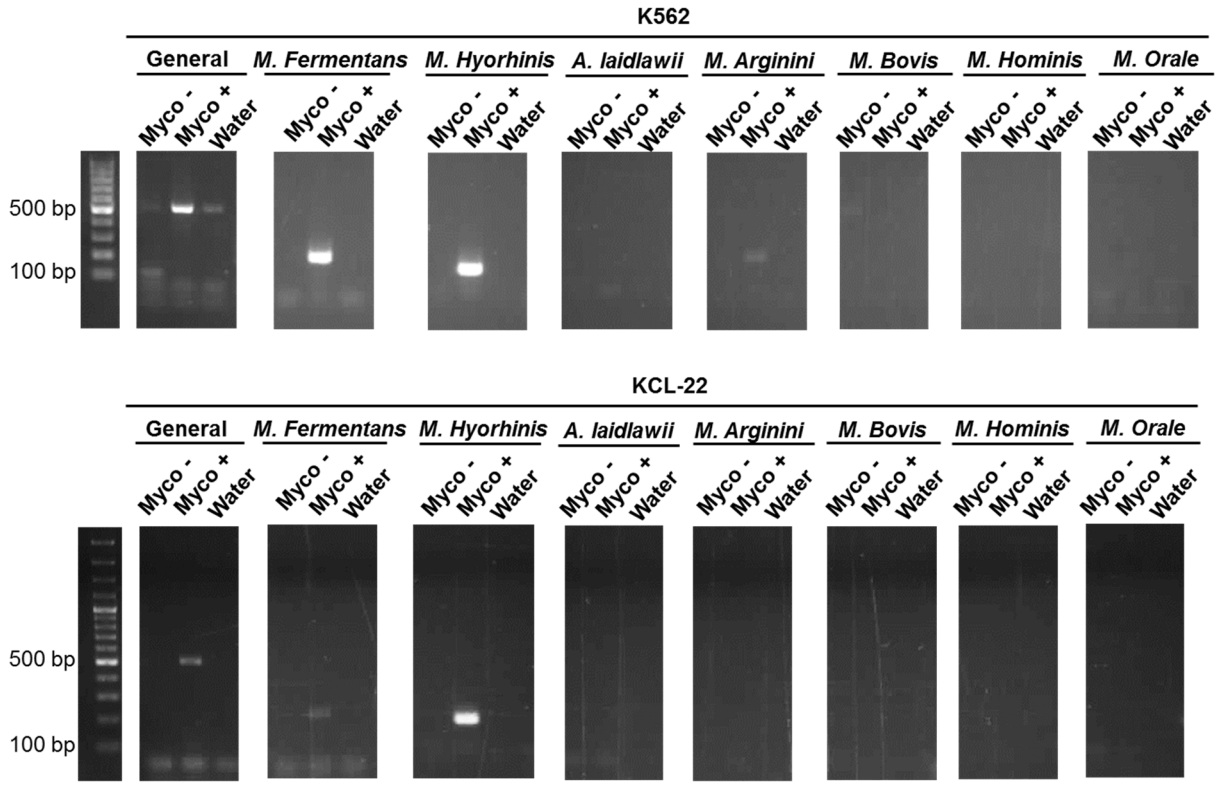


Experiment 3



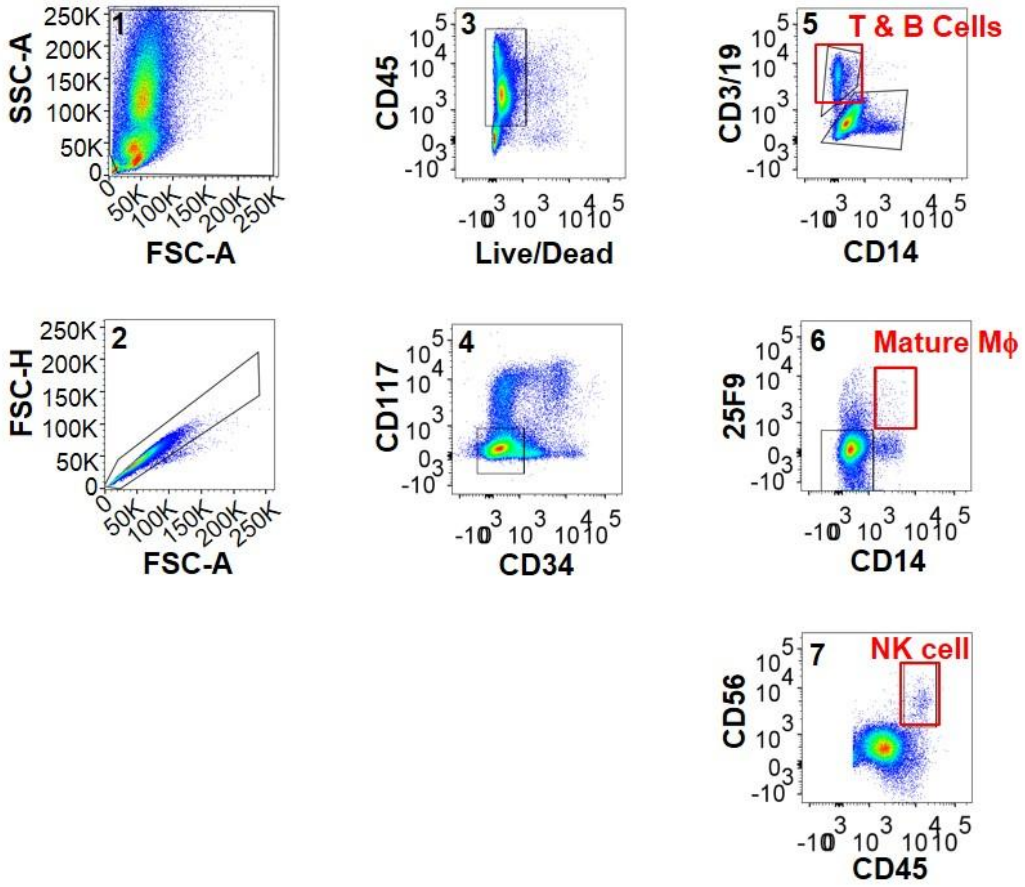
Supplementary figure 1, related to Figure 1b. Increased production of IL-8 by CML with chronic and acute infection of mycoplasma.

Mycoplasma-infected K562 cells were seeded at 1 million cells per mL and incubated overnight. Culture supernatants were tested for presence of IL-8, IL-6, TNFα and IL-10 using ELISA. Data from each experiment are presented as mean ± s.d. of 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$; ** $P < 0.01$; ns, non-significant.

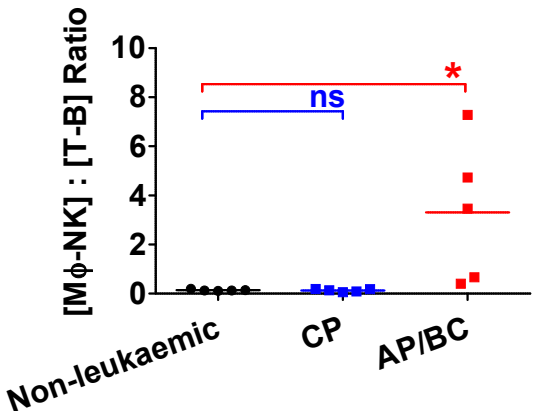


Supplementary figure 2. M. Fermentans and M. Hyorhinis were consistently detected in mycoplasma-infected cultures of K562 and KCL-22. Cell culture supernatants were tested for presence of mycoplasma (general) and the specific species of mycoplasma present via PCR. DNA bands were visualised via UV transillumination (Biorad imager and Syngene Genesnap software) of SYBR safe-stained agarose gel. Both K562 and KCL-22 cultures were mycoplasma-positive based on detection of the 500-bp band for general mycoplasma. Consistently, 206-bp band for Mycoplasma fermentans and 150-bp band for Mycoplasma hyorhinis were detected in cultures of K562 and KCL-22. Although a faint band for Mycoplasma arginini was detected, it was deemed non-specific as it was inconsistent for the three independent experiments performed.

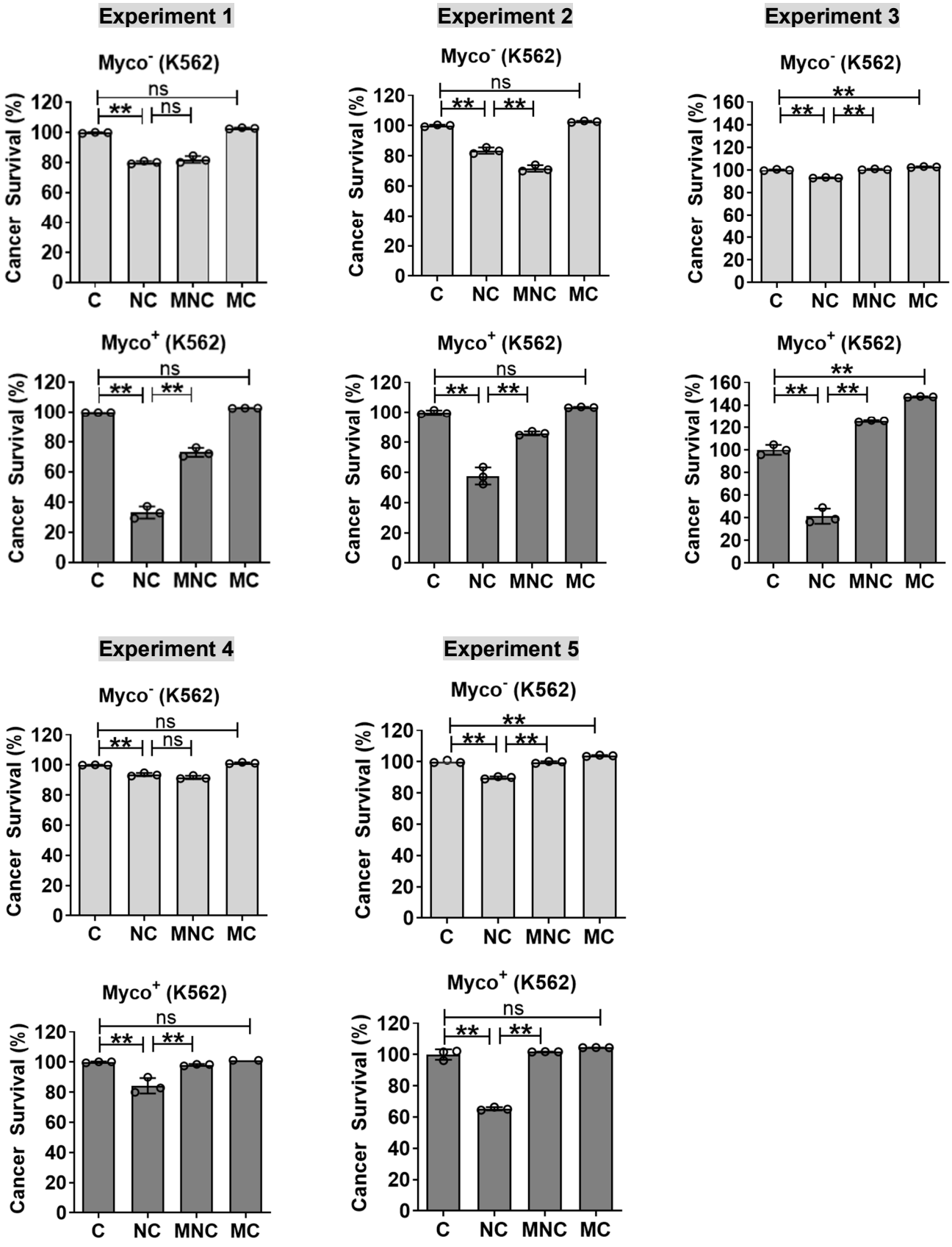
(a)



(b)

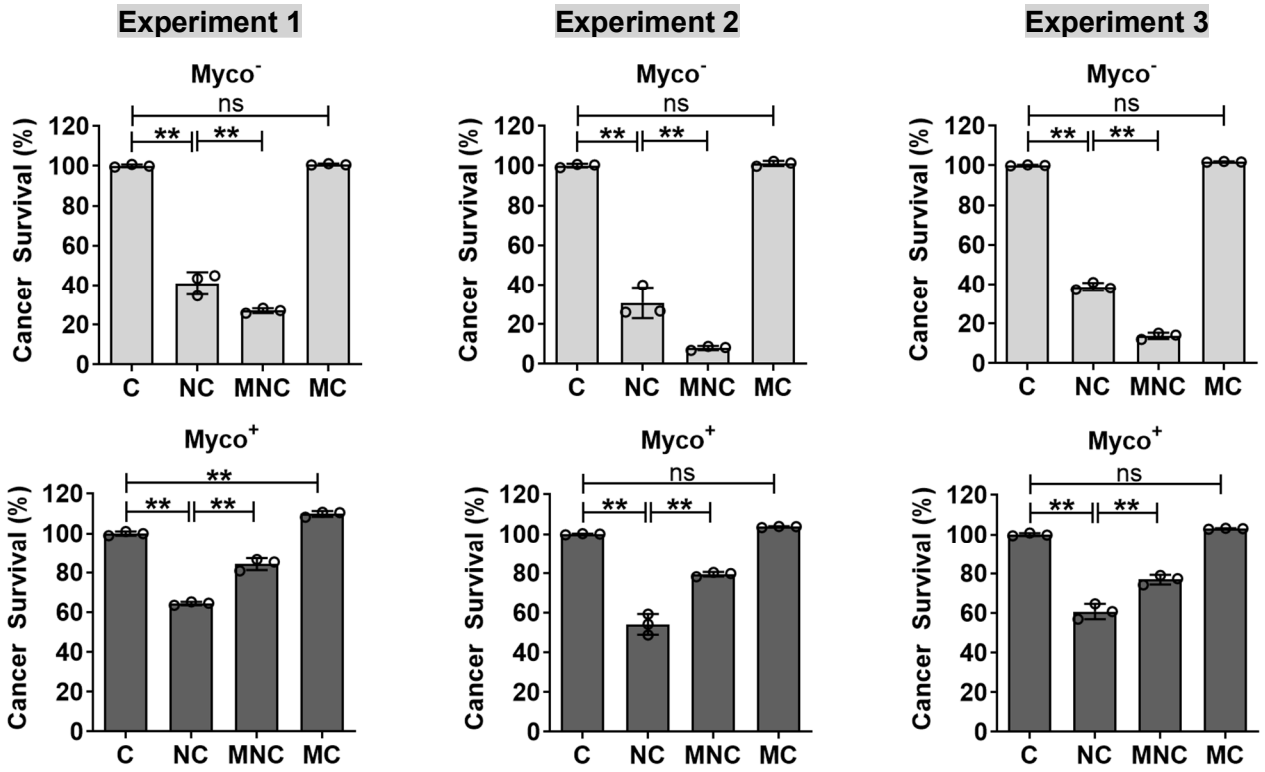


Supplementary figure 3. Increase in the ratio of innate Mϕ-NK to adaptive T-B cells in severe CML. **(a)** Representative flow cytometry dot plots showing flow cytometry gating strategy for delineating mature Mϕ, NK, T and B cells from fresh/frozen CML and non-CML control samples. Sequential gating was used (from plots 1 to 7) for delineating mature monocytes/Mϕ, NK, T and B cells. Mature Mϕ was defined as $CD45^+$ $CD34^-$ $CD117^-$ $CD3/19^-$ $CD14^+$ and $25F9^+$. NK cell was defined as $CD45^+$ $CD34^-$ $CD117^-$ $CD3/19^-$ $CD14^-$ $25F9^-$ $CD56^+$. T and B cells were defined as $CD45^+$ $CD34^-$ $CD117^-$ $CD3/19^+$. **(b)** Graphical representation of [Mϕ-NK] : [T-B] ratio in CML stratified by staging. Non-leukaemia patients n=5; CP, chronic phase patients n=5; AP/BC, accelerated phase/blast crisis patients n=5. Statistical significance was determined using Kruskal-Wallis test with Dunns' post-hoc analysis. * $P < 0.05$; ** $P < 0.01$; ns, non-significant.

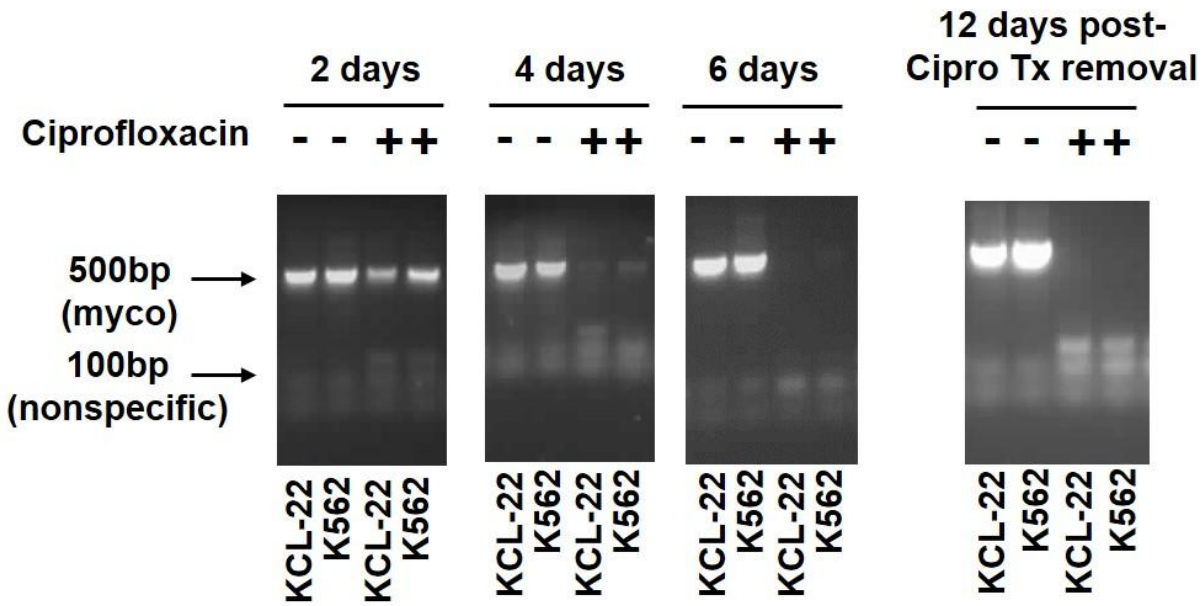


Supplementary figure 4, related to Figure 2c. Mϕ attenuates NK cytotoxicity against mycoplasma-infected CML.

CML survival measured under myco$^+$ and myco$^-$ conditions, upon co-culture with Mϕ, N and normalised to cancer alone control. Data from each experiment are presented as mean ± s.d. of 2-3 replicate cultures. 5 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$; ** $P < 0.01$; ns, non-significant.

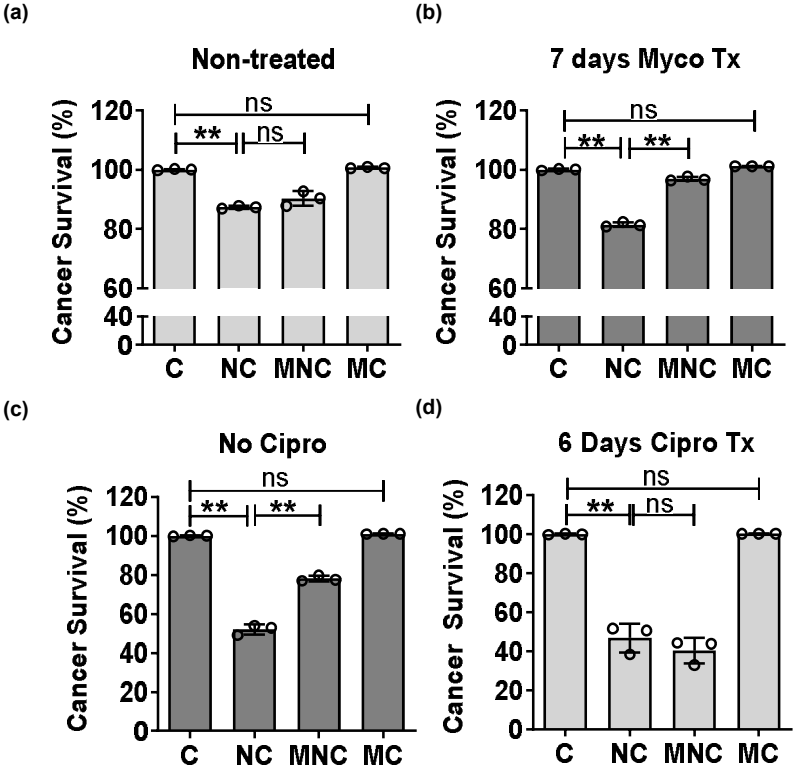


Supplementary figure 5. Mϕ protection of CML against NK cytotoxicity under myco$^+$ condition was also observed for KCL-22 CML cells. NK cell and Mϕ were co-cultured with myco$^+$/myco$^-$ KCL-22. KCL-22 survival was measured based on negative staining for viability dye (FVD) and normalised to cancer alone control. Data from each experiment are presented as mean ± s.d. of 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. *$P < 0.05$, **$P < 0.01$, ns, non-significant. C, CML alone; NC, NK+CML; MNC, Mϕ+NK+CML; MC, Mϕ+CML.

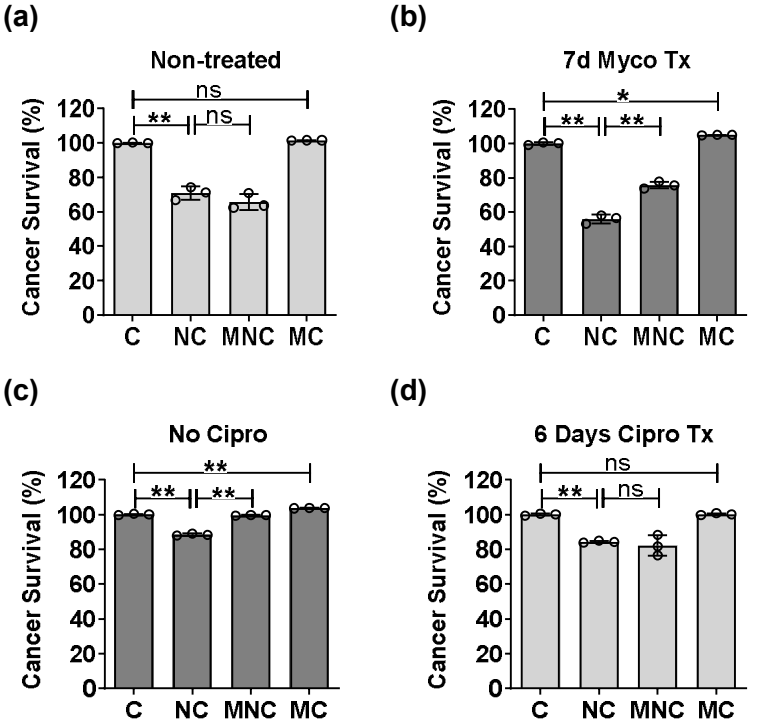


Supplementary figure 6. Optimal ciprofloxacin treatment period of mycoplasma-infected CML culture was 6-days. Myco$^+$ CML cells were treated with 10 μg mL^{-1} ciprofloxacin for 2, 4 and 6 days of treatment. Culture supernatants from ciprofloxacin-treated CML cells were aliquoted for mycoplasma PCR test. Mycoplasma-infected culture was observed to be cleared of mycoplasma by 6-day ciprofloxacin treatment. There was no recurrence of mycoplasma infection 12 days post-ciprofloxacin treatment.

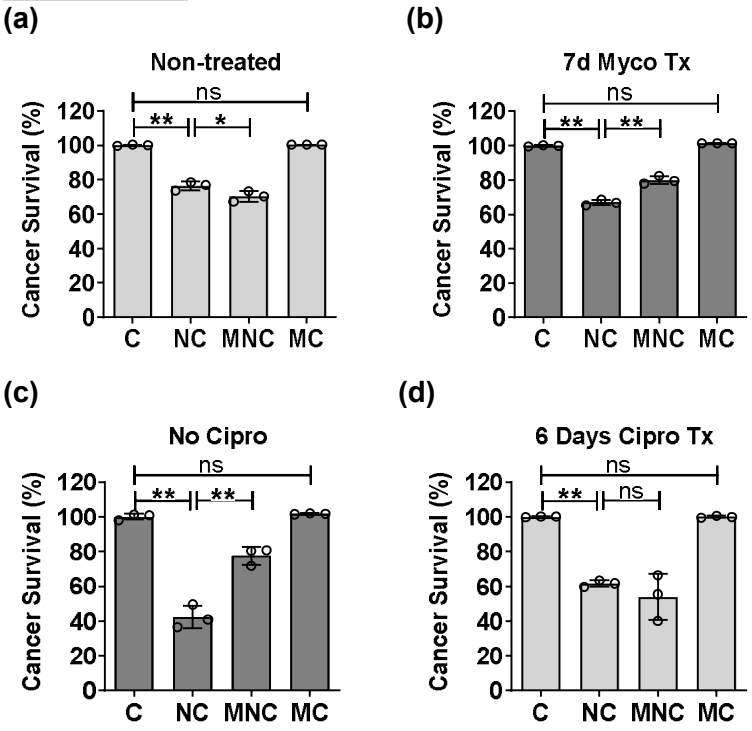
Experiment 1



Experiment 2

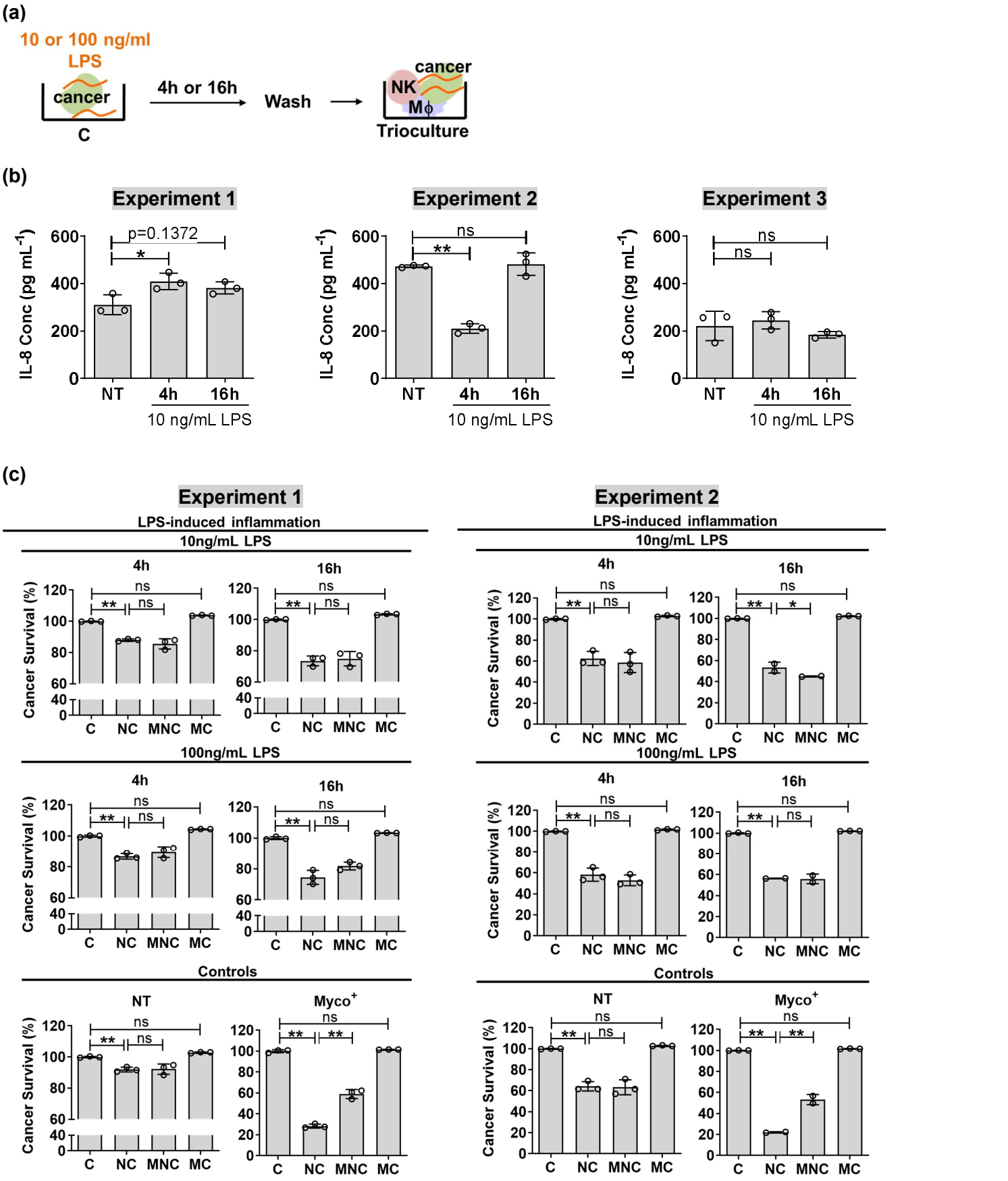


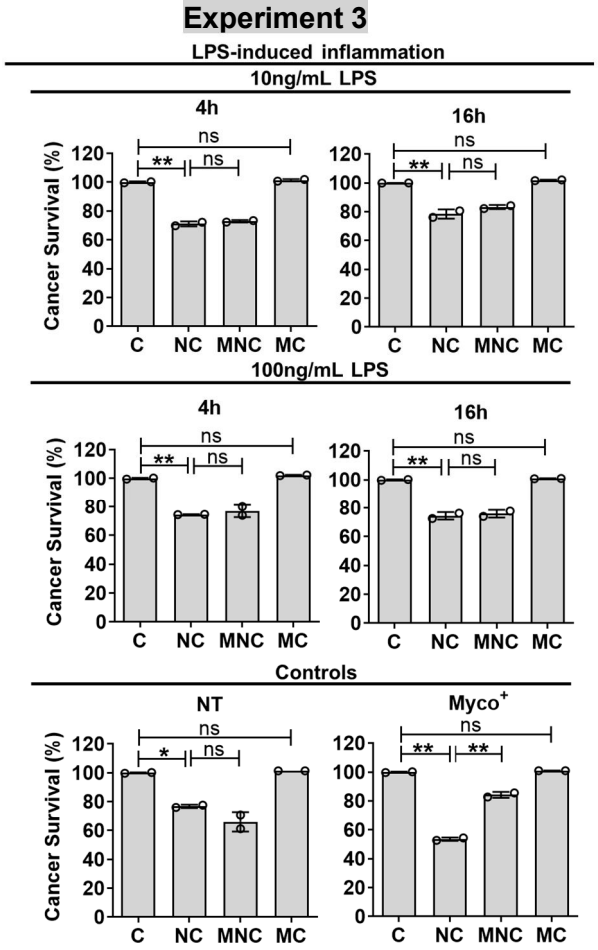
Experiment 3



Supplementary figure 7, related to Figure 3. Mϕ protection of CML is specific to infection-inflammation condition induced by mycoplasma.

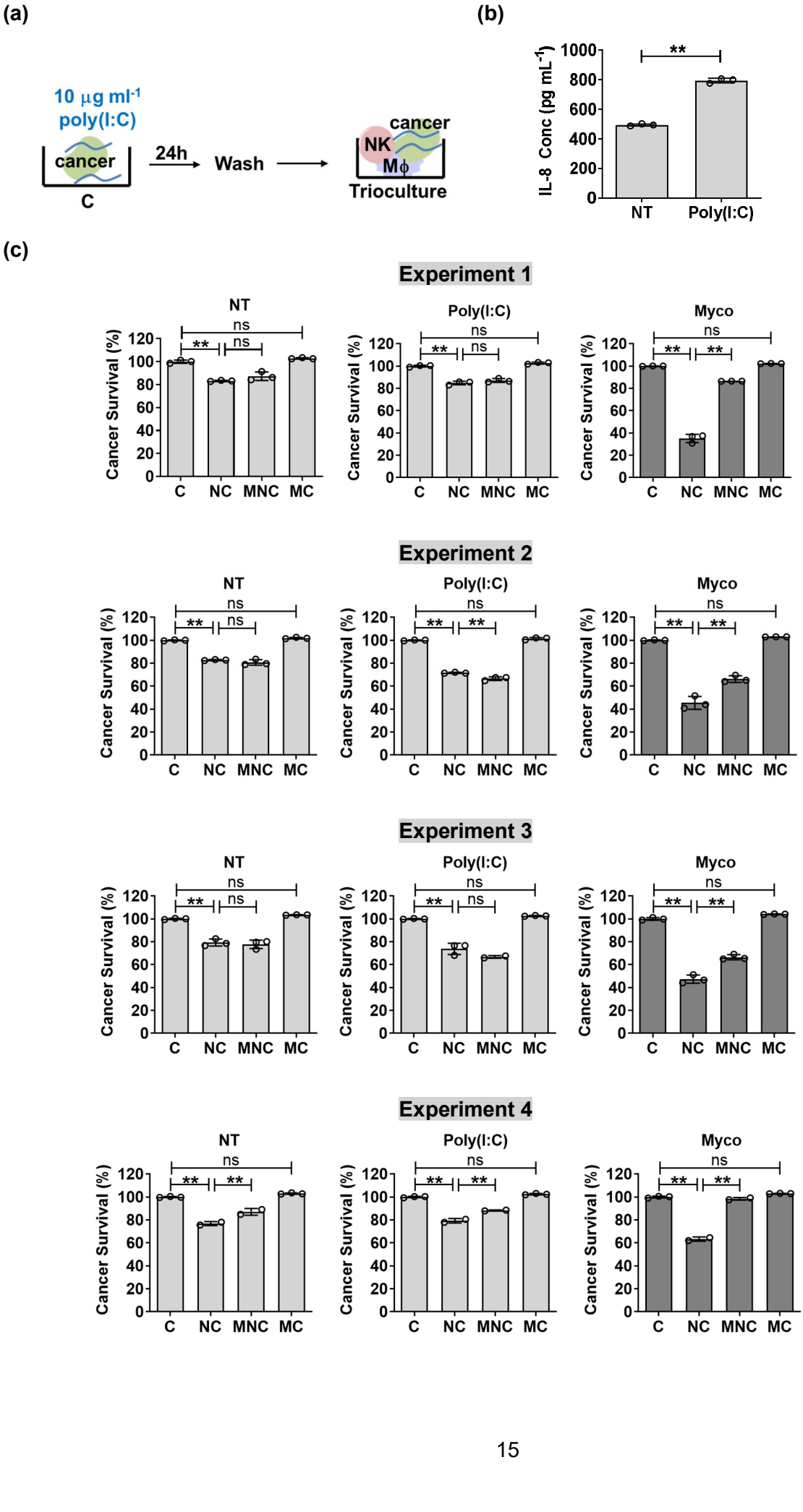
NK and Mϕ were co-cultured with **(a, b)** non-infected CML treated with mycoplasma-containing medium or, **(c, d)** chronically-infected CML treated with 10 μg mL^{-1} ciprofloxacin. CML survival was subsequently measured based on negative staining for viability dye (FVD) and normalised to cancer alone control. Data from each experiment are presented as mean ± s.d. of 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$; ** $P < 0.01$; ns, non-significant.


(a)
10 or 100 ng/ml
LPS
cancer
C
4h or 16h
Wash
cancer
NK
Mϕ
Trioculture
(b)
Experiment 1
Experiment 2
Experiment 3
IL-8 Conc (pg mL-1)
p=0.1372
ns
NT
4h
16h
10 ng/mL LPS
(c)
Experiment 1
Experiment 2
LPS-induced inflammation
10ng/mL LPS
100ng/mL LPS
Controls
4h
16h
NT
Myco+
Cancer Survival (%)
C
NC
MNC
MC

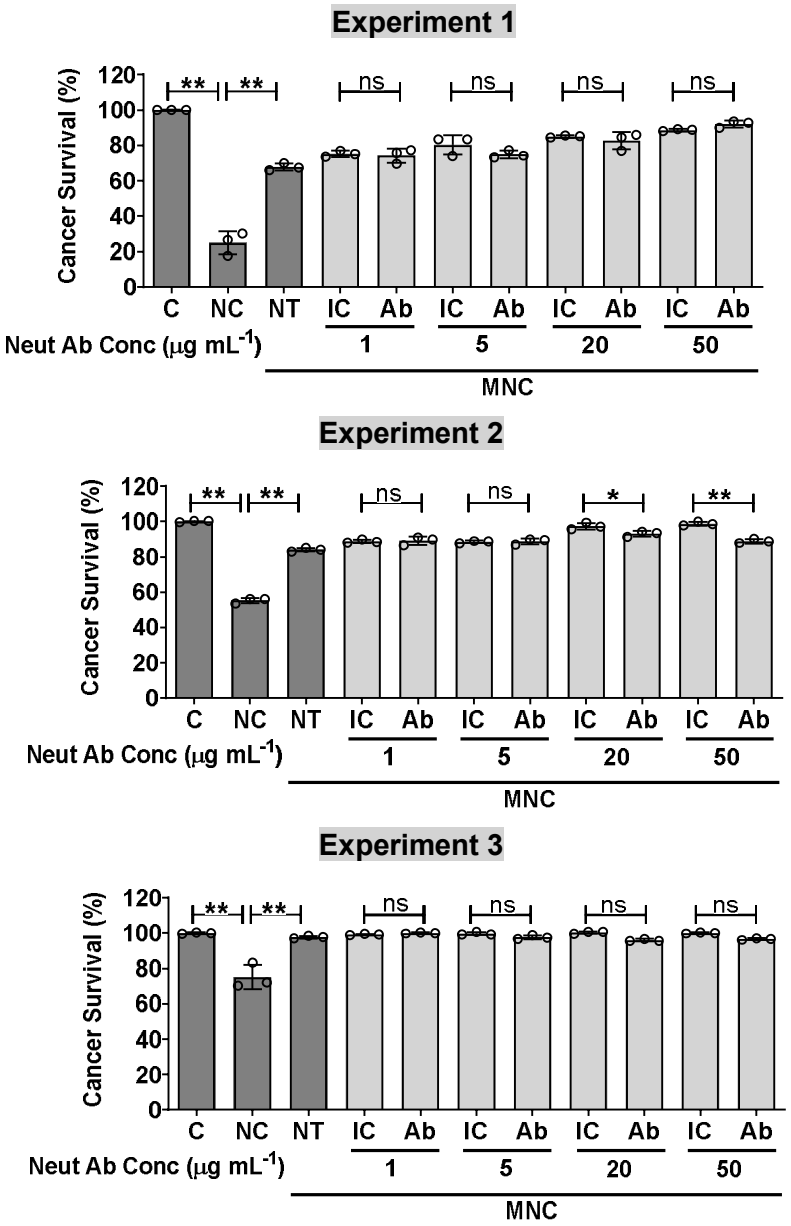


Supplementary figure 8. LPS-induced infection-inflammation condition does not lead to macrophage protection of CML.

(a) Experimental set-up for LPS treatment of CML cells prior to Mϕ, NK and CML trio-culture. Cells were treated for 4 or 16 hours with 10 or 100 ng mL^{-1} LPS and washed prior to the trio-culture. **(b)** LPS-treated cells were seeded at a density of 0.4 million cells per mL. Culture media was measured for IL-8 production. **(c)** CML cell survival measured under each condition, with NT (not treated with LPS) as negative control and mycoplasma$^+$ (myco$^+$) as positive control. CML survival was measured based on negative staining for FVD and normalised to cancer alone control. Data from each experiment are presented as mean ± s.d. from 2 – 3 replicate cultures. For **(b and c)**, 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$, ** $P < 0.01$, ns non-significant. C, CML alone; NC, NK+CML; MNC, Mϕ+NK+CML; MC, Mϕ+CML.


(a)
10 μg ml-1
poly(I:C)
cancer
C
24h
Wash
cancer
NK
Mϕ
Trioculture
(b)
IL-8 Conc (pg mL-1)
1000
800
600
400
200
0
**
NT
Poly(I:C)
(c)
Experiment 1
Experiment 2
Experiment 3
Experiment 4
NT
Poly(I:C)
Myco
Cancer Survival (%)
120
100
80
60
40
20
0
ns
**
C
NC
MNC
MC

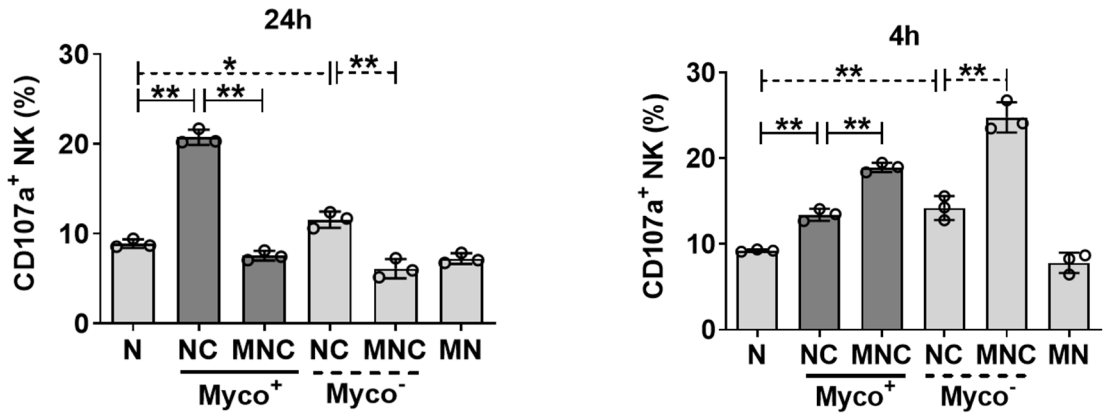
Supplementary figure 9. Macrophages do not protect poly(I:C)-treated CML from NK cytotoxicity. **(a)** Experimental set-up for polyinosinic-polycytidylic acid (poly(I:C)) treatment of CML cells prior to Mϕ, NK and CML trio-culture. Cells were treated for 24 hours with 10 μg mL^{-1} poly and washed prior to the trio-culture. **(b)** Poly(I:C)-treated cells were seeded at a density of 1 million cells per mL. The culture media were tested for IL-8. **(c)** CML cell survival measured under each condition, with NT (not treated) as negative control and $myco^{+}$ as positive control. CML survival was measured based on negative staining for FVD and normalised to cancer alone control. Data from each experiment are presented as mean ± s.d. from 2 – 3 replicate cultures. For **(c)**, 4 independent experiments, each from different donors are shown. Statistical significance was determined using two-tailed Student's *t*-test in **(b)** and 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test in **(c)**. $*P < 0.05$, $**P < 0.01$, ns non-significant. C, CML alone; NC, NK+CML; MNC, Mϕ+NK+CML; MC, Mϕ+CML.



Supplementary figure 10. Increasing dose of IL-8 neutralising antibodies (Ab) added to MNC trio-culture does not influence Mϕ protection of myco$^+$ CML from NK cytotoxicity.
MNC trio-cultures were treated with neutralising antibodies against human IL-8 (Ab) and equal amount of mouse IgG1 isotype control in increasing doses, as indicated. The treated samples were then measured for CML survival based on negative staining for FVD. Data from each experiment are presented as mean ± s.d. from 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$; ** $P < 0.01$; ns, non-significant; C – cancer alone; NC – NK + cancer ; NT - Mϕ + NK + cancer (MNC), without Ab treatment; IC – MNC treated with isotype control; Ab – MNC treated with anti-IL-8 antibody.

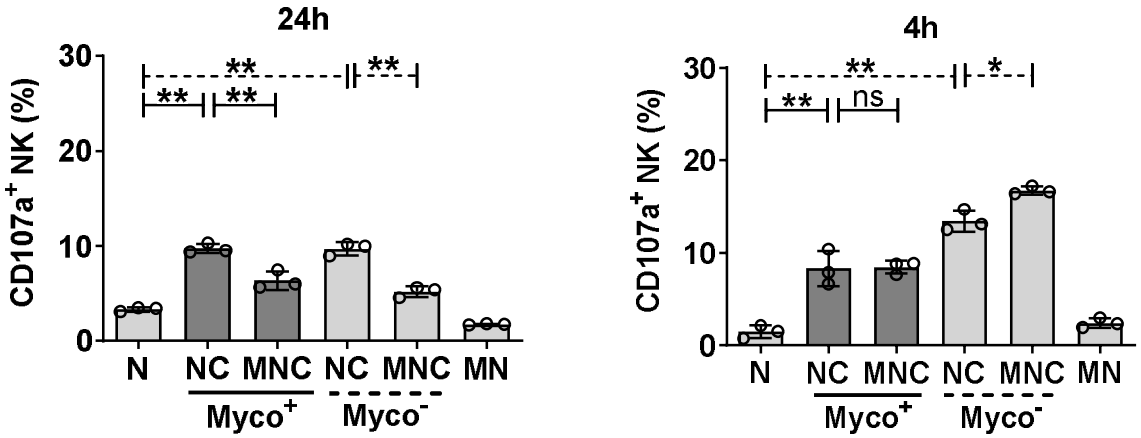
Experiment 1

(a) **(b)**



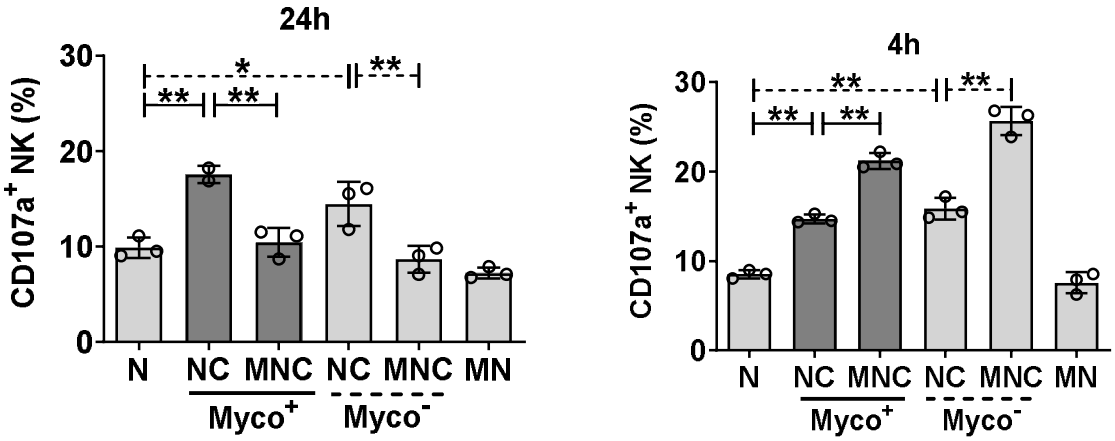
Experiment 2

(a) **(b)**



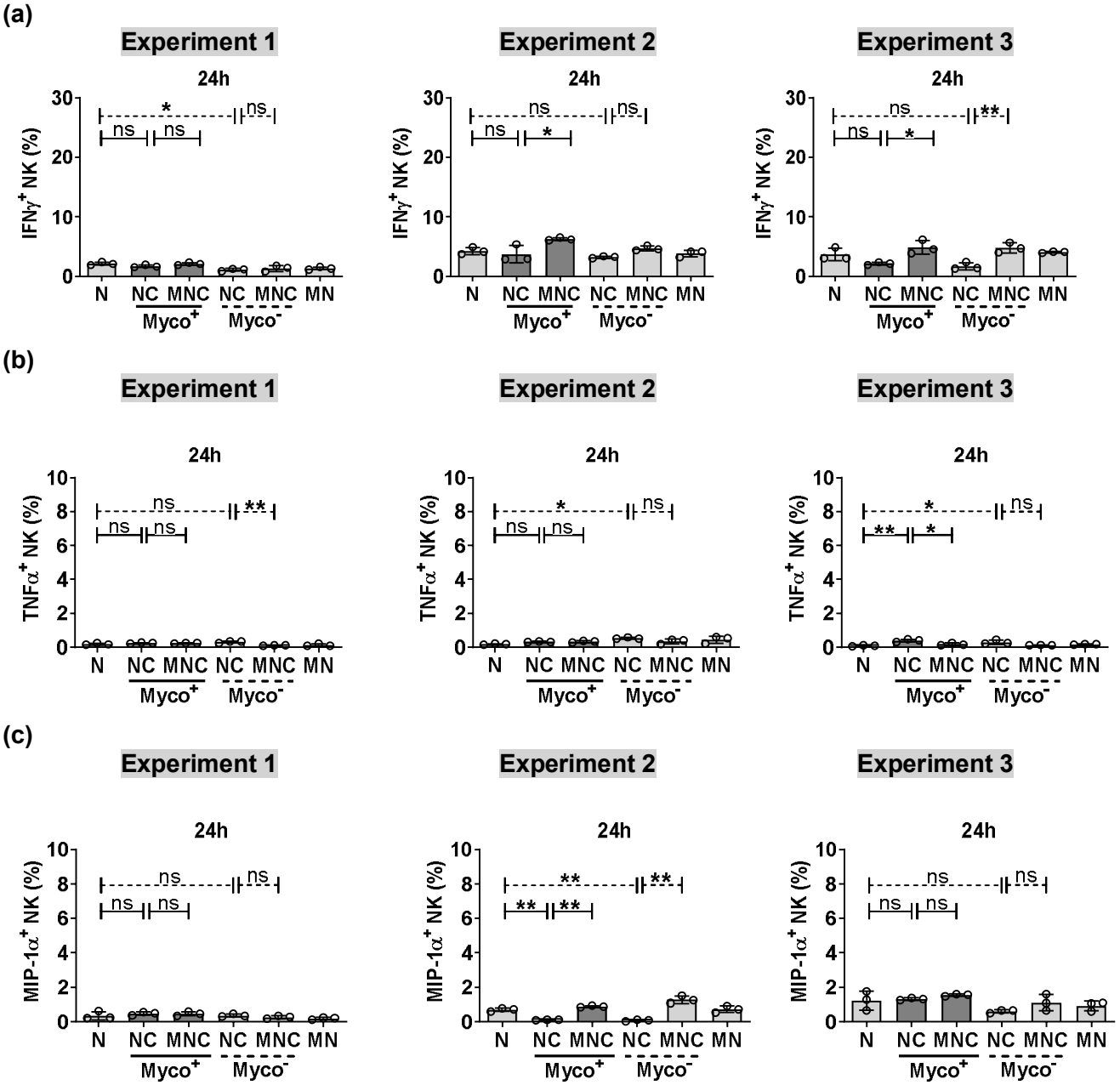
Experiment 3

(a) **(b)**

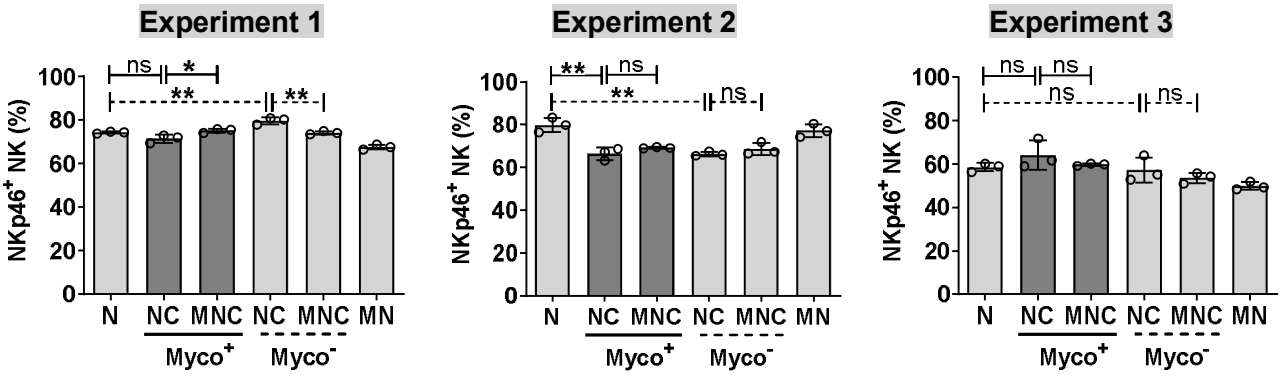


Supplementary figure 11, related to Figure 4. Mϕ suppresses NK degranulation over time in MNC trio-culture.

Mϕ, and myco$^-$ and myco$^+$ CML were incubated in mono-, duo- and trio-cultures according to the experimental strategies outlined in **(a)** (24 hours) and **(b)** (4 hours). CFSE$^-$CD14$^-$CD56$^+$ NK were then gated and CD107a$^+$ NK were determined. Data from each experiment are presented as mean ± s.d. of 3 replicate cultures. 3 independent experiments, each from different donors are shown. Each graph contains data from one of the independent experiments, presented as mean ± s.d. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test, based on the technical replicates within the independent experiment presented. * $P < 0.05$; ** $P < 0.01$; ns, non-significant.



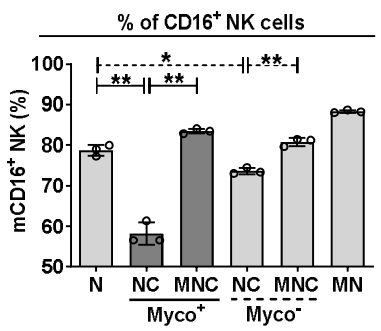
Supplementary figure 12. No significant changes in IFNγ, TNFα and MIP-1α production in NK upon duo- or trio-culture with Mϕ and CML cells. NK, Mϕ, and myco$^-$ and myco$^+$ CML were incubated in mono-, duo- and trio-cultures for 24 hours. Brefeldin was added at the last 4 hours of the co-cultures to ensure that cytokines were retained in the NK for intracellular cytokine staining of **(a)** IFNγ, **(b)** TNFα and **(c)** MIP-1α. Data from each experiment are presented as mean ± s.d. from 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * *P < 0.05*; ** *P < 0.01*; ns nonsignificant. N, NK alone; NC, NK+CML; MNC, Mϕ+NK+CML; MN, Mϕ+NK.



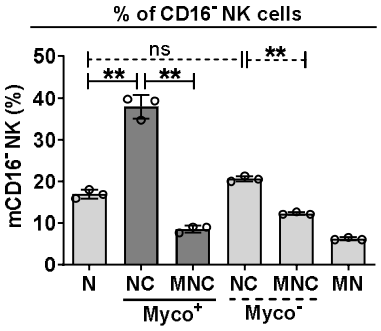
Supplementary figure 13. Marginal changes in the level of activating receptor NKp46 on NK upon co-culture with Mϕ and CML. NK, Mϕ, and myco$^-$ and myco$^+$ CML were incubated in mono-, duo- and trio-cultures. CFSE$^-$CD14$^-$CD56$^+$ NK cells were gated. NKp46 activating receptors level was measured on NK upon co-culture with Mϕ and CML. Data from each experiment are presented as mean ± s.d. from 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * *P < 0.05*; ** *P < 0.01*; ns nonsignificant. N, NK alone; NC, NK+CML; MNC, Mϕ+NK+CML; MN, Mϕ+NK.

Experiment 1

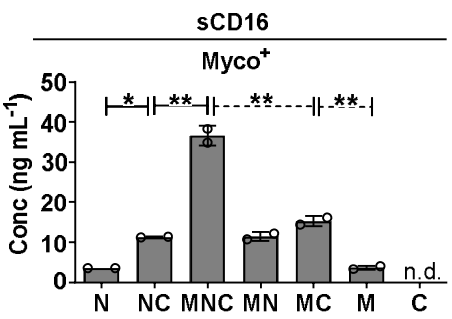
(a)



(b)

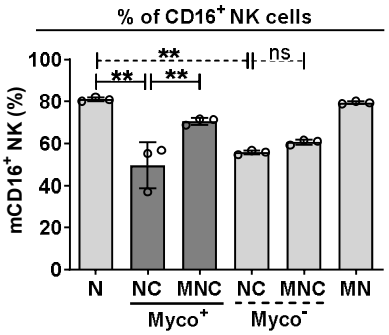


(c)

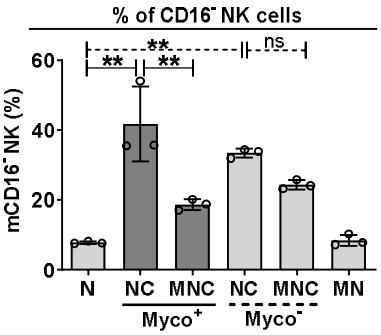


Experiment 2

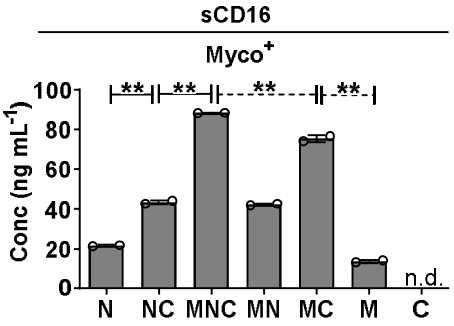
(a)



(b)

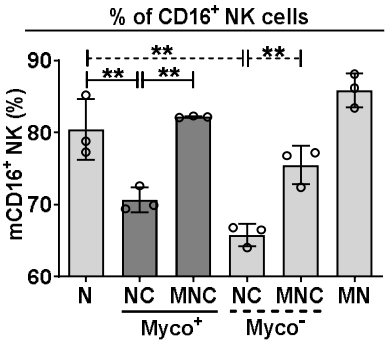


(c)

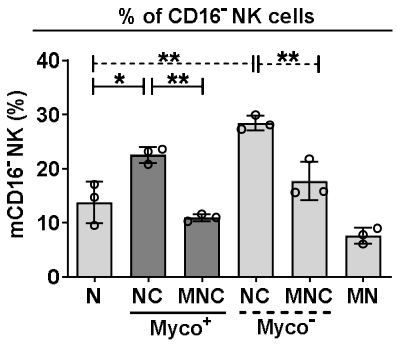


Experiment 3

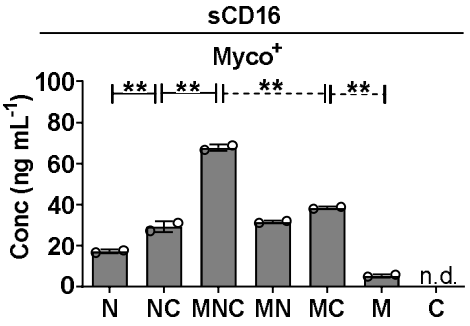
(a)



(b)

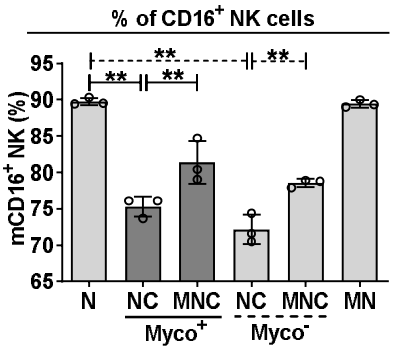


(c)

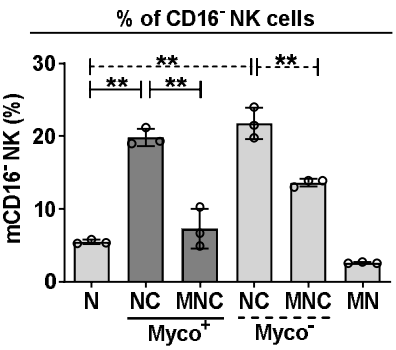


Experiment 4

(a)

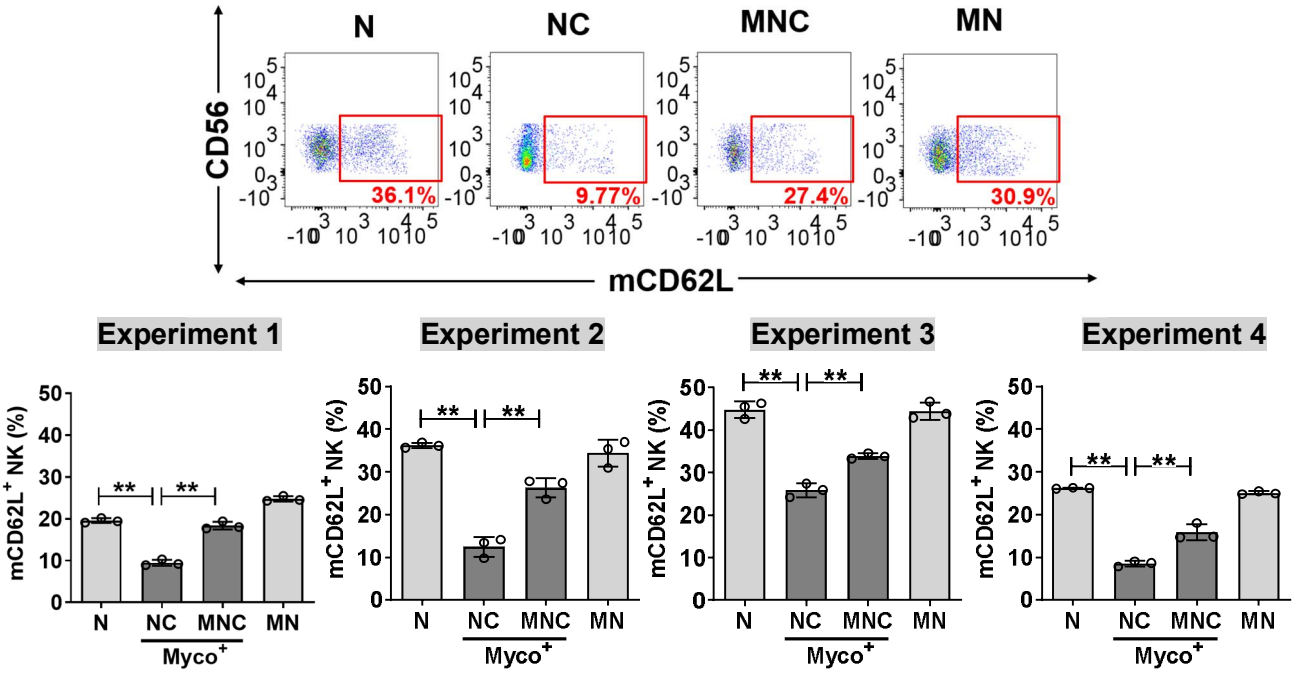


(b)

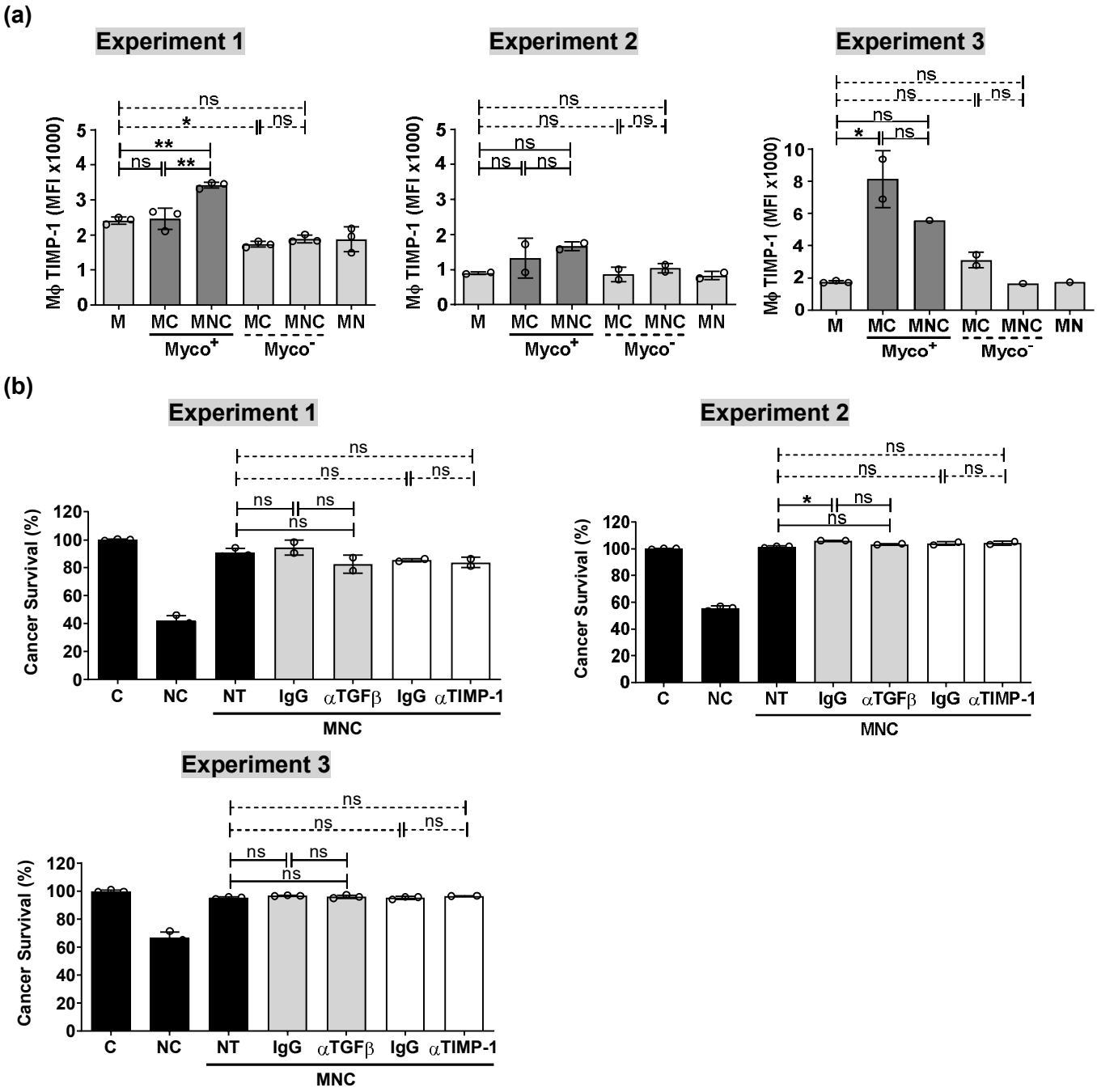


Supplementary figure 14, related to Figure 5. NK mCD16 level is maintained in vitro in MNC trio-culture under infection-inflammation condition of CML cells.

(a-b) NK, Mϕ, and myco$^-$/myco$^+$ CML were incubated in mono-, duo- and trio-cultures. CFSE$^-$CD14$^-$CD56$^+$ NK cells were gated. % of **(a)** CD16$^+$ and **(b)** CD16$^-$ NK were determined on NK upon co-culture with Mϕ and CML. NK mCD16 level was determined specifically on CD56^{dim} NK, which is the cytotoxic counterpart of the NK population. **(c)** NK, Mϕ, and myco$^-$ and myco$^+$ CML were incubated in mono-, duo- and trio-cultures. Culture supernatants were collected and measured for concentration (conc) of soluble CD16 (sCD16) using ELISA. Data from each experiment are presented as mean ± s.d. from 2-3 replicate cultures. 4 independent experiments **(a, b)** or 3 independent experiments **(c)**, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$; ** $P < 0.01$; ns, non-significant.

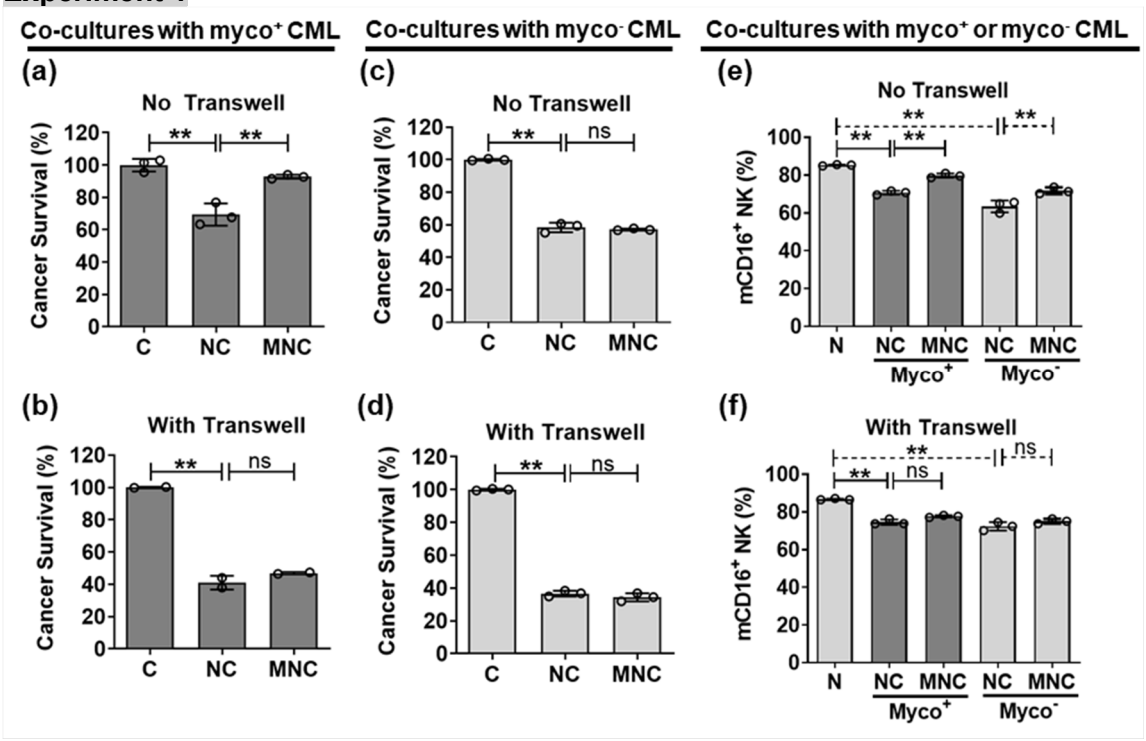


Supplementary figure 15. Attenuation of sheddase activity in MNC trio-cultures under myco$^+$ condition. NK, Mϕ, and myco$^+$ CML were incubated in mono-, duo- and trio-cultures. NK was gated in flow cytometry as CFSE$^-$CD14$^-$CD56$^+$. NK mCD62L level was determined specifically on CD56^{dim} NK, which is the cytotoxic counterpart of the NK population, as indicated in red on the representative flow cytometry dot-plots. Data on percentage of mCD62L$^+$ NK in each experiment are presented as mean ± s.d. from 3 replicate cultures. 4 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. *$P < 0.05$; **$P < 0.01$; ns nonsignificant. N, NK alone; NC, NK+CML; MNC, Mϕ+NK+CML; MN, Mϕ+NK.

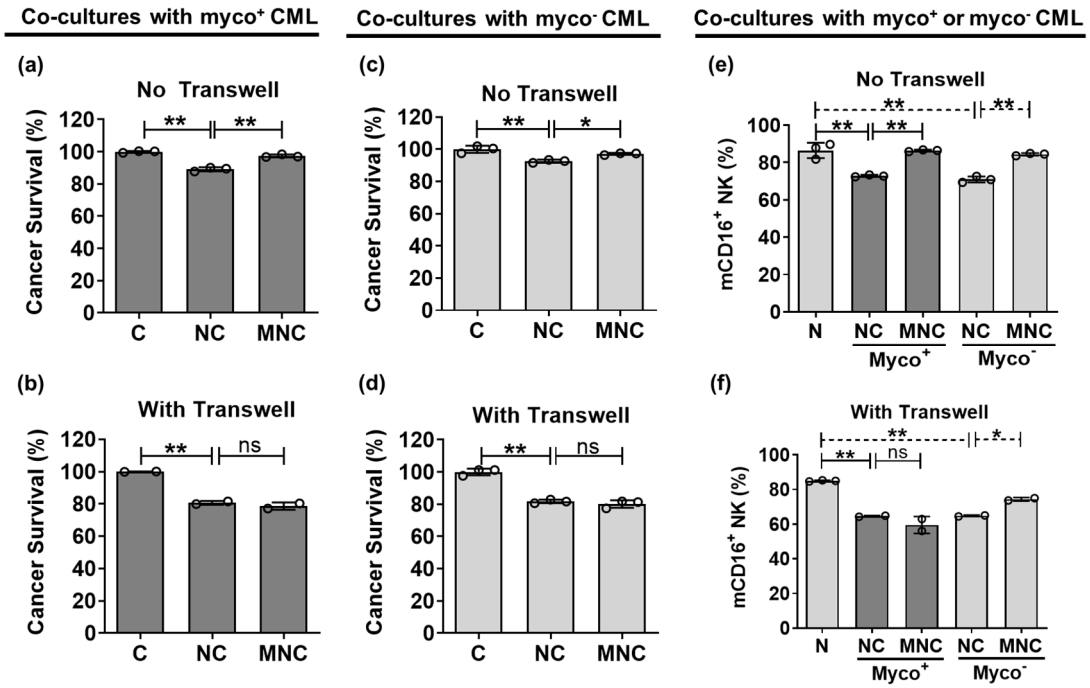


Supplementary figure 16. TIMP-sheddase modulation does not influence CML survival in MNC trio-culture. (a) Intracellular TIMP-1 expression in Mϕ were measured under mono, duo- and trio-cultures. $CFSE^{-}CD14^{+}$ Mϕ were gated in flow cytometry. **(b)** MNC trio-cultures were treated with neutralising antibodies against TGFβ or TIMP-1 and equal amount of IgG isotype control. The treated samples were then measured for CML survival based on negative staining for FVD and normalised to cancer alone control. Data from each experiment are presented as mean ± s.d. from 1-3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * *P < 0.05*; * *P < 0.01*; ns nonsignificant. Mϕ, macrophage alone; N, NK alone; C, CML alone; NC, NK+CML; MNC, Mϕ+NK+CML; MN, Mϕ+NK; NT, non-treated with neutralising antibody; IgG, IgG isotype control.

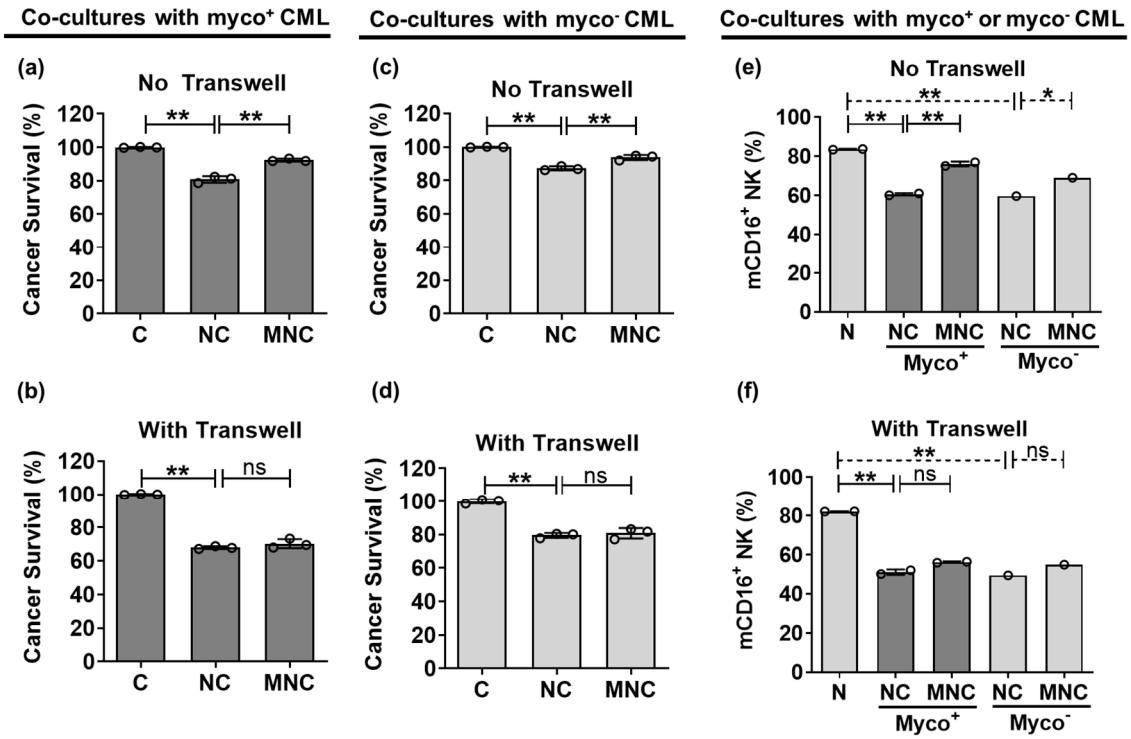
Experiment 1



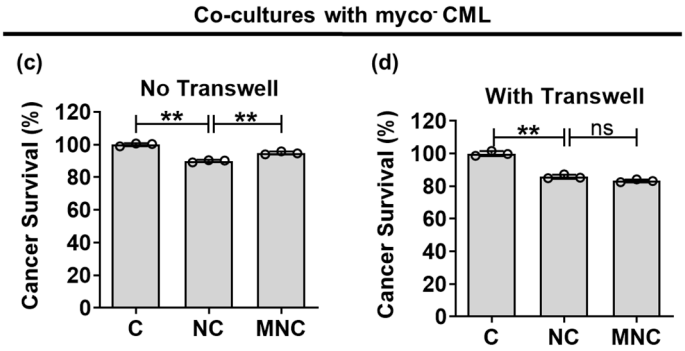
Experiment 2



Experiment 3

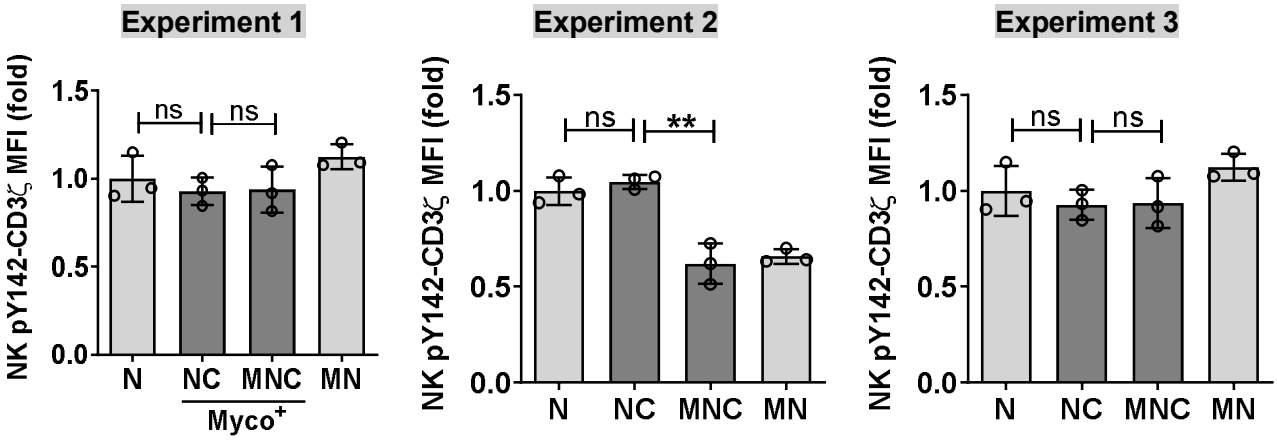


Experiment 4



Supplementary figure 17, related to Figure 6. Mϕ protection of CML from NK cytotoxicity was abrogated when cell-cell contact interactions were disrupted.

(a, b) Mϕ, NK cells and myco+ CML cells were co-cultured in the presence and absence of transwell for 24h. CML survival was then assessed based on negative staining for viability dye and normalising to cancer alone control. Results shown are representative of 3 independent experiments (donors). Data in are presented as mean ± s.d. of 2-3 replicate cultures. **(c, d)** Mϕ, NK cells and myco- CML were assessed as described in (a, b). **(e, f)** Mϕ, NK cells and myco+ or myco- CML were co-cultured in the presence and absence of transwell for 24h and then assessed for percentage mCD16+ NK cells by flow cytometry. Data from each experiment are presented as mean ± s.d. from 1-3 replicate cultures. 3 independent experiments **(a, b, e and f)** or 4 independent experiments **(c, d)**, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * P < 0.05; ** P < 0.01; ns, non-significant.



Supplementary figure 18. No significant changes in NK phosphorylated CD3ζ level. NK, Mϕ, and myco$^+$ CML were incubated in mono-, duo- and trio-cultures. Cells were immediately fixed and permeabilised to capture the level of CD3ζ phosphorylation at tyrosine residue 142 at the end of co-cultures. NK was gated in flow cytometry as CFSE$^-$CD14$^-$. Data from each experiment are presented as mean ± s.d. from 3 replicate cultures. 3 independent experiments, each from different donors are shown. Statistical significance was determined using 1-way ANOVA corrected with Bonferroni's Multiple Comparison Test. * $P < 0.05$; ** $P < 0.01$; ns nonsignificant. N, NK alone; NC, NK+CML; MNC, Mϕ+NK+CML; MN, Mϕ+NK.