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Supplementary Materials for

Skin-specific antibodies neutralizing mycolactone toxin during the spontaneous healing of *Mycobacterium ulcerans* infection

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Supplementary Materials and Methods

Table S1. HRP-conjugated secondary antibodies used in this study.

Name of antibodies	Suppliers/references
Sheep anti-mouse IgG	GE-Healthcare; NA931
Goat anti-mouse IgG1	Invitrogen; A10551
Goat anti-mouse IgG2a	Invitrogen; A10685
Goat anti-mouse IgG2b	Invitrogen; M32507
Goat anti-mouse IgG3	Invitrogen; M32707
Goat anti-mouse IgG/IgA/IgM (H+L)	Invitrogen; A10668
Rat anti-mouse IgA	Southern Biotech; 1165-05
Rat anti-mouse IgM	Southern Biotech; 1139-05

Table S2. Primer sequences used in this study.

Primer	Sequence (5’ 3’)	Reference
Actin-Forward	AGC CAT GTA CGT AGC CAT CC	Lefèvre et al. 2013
Actin-Reverse	CTC TCA GCT GTG GTG GTG AA	Lefèvre et al. 2013
IgM-Forward	TGT GTG GAA GAC TGG AAT AAC	This study
IgM-Reverse	ACA GCA GGT GGATGT TTG	This study
IgA-Forward	GAC ACC TTA ACT GGC ACA A	This study
IgA-Reverse	CAG CAC TTC TTT AGG GTT GA	This study
IgG-Forward	GAC CAT CCG TCT TCA TCT TC	This study
IgG-Reverse	ATC CTC GCT CAC ATC CA	This study

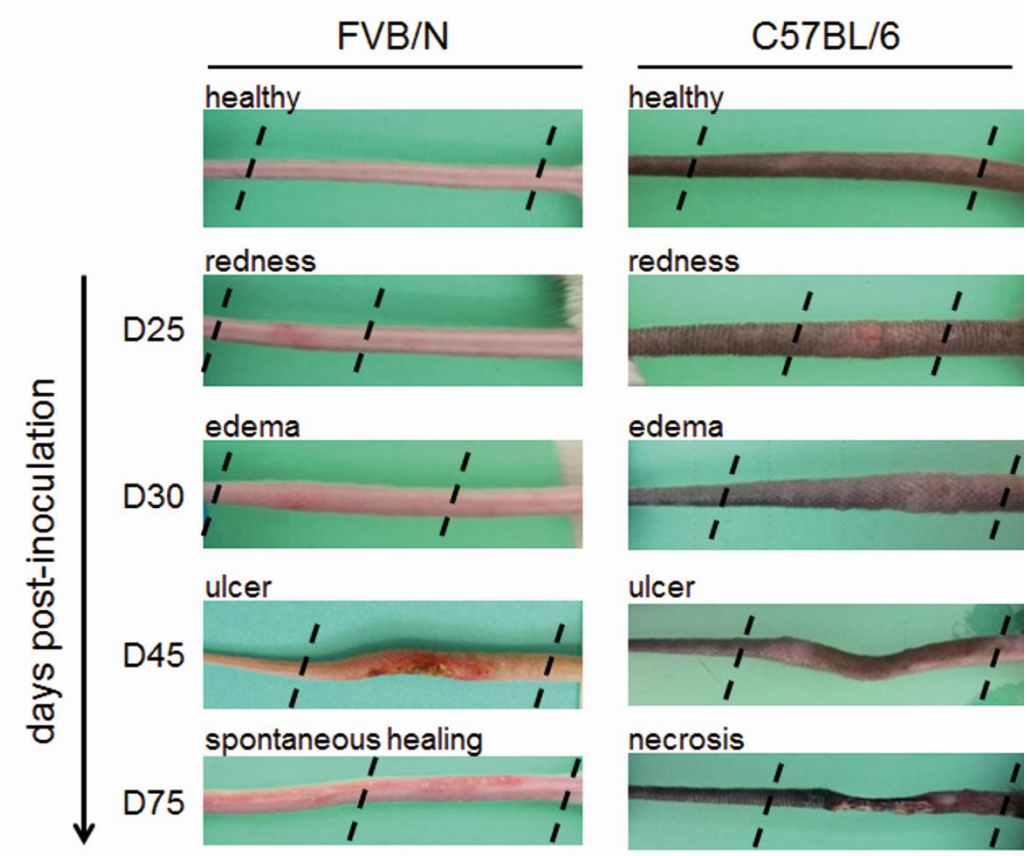

Fig. S1. Macroscopic appearance of lesions in FVB/N and C57BL/6 mice infected with *M. ulcerans*. In both models, lesion development followed the same time course: redness, edema and ulcer occurred 25, 30 and 45 days, respectively, after inoculation with 10^4 bacilli. On day 75, FVB/N mice displayed spontaneous healing, whereas the lesions of C57BL/6 mice had progressed to irreversible necrosis. Excisions were performed on either side of the lesions (at the locations indicated by the dashed lines), and the same amount of skin was excised from healthy tissues as a control. Photo credit: Lucille Esnault, U1232 CRCINA, Institut National de la Santé et de la Recherche Médicale (INSERM), Université de Nantes, Université d'Angers, Angers, France

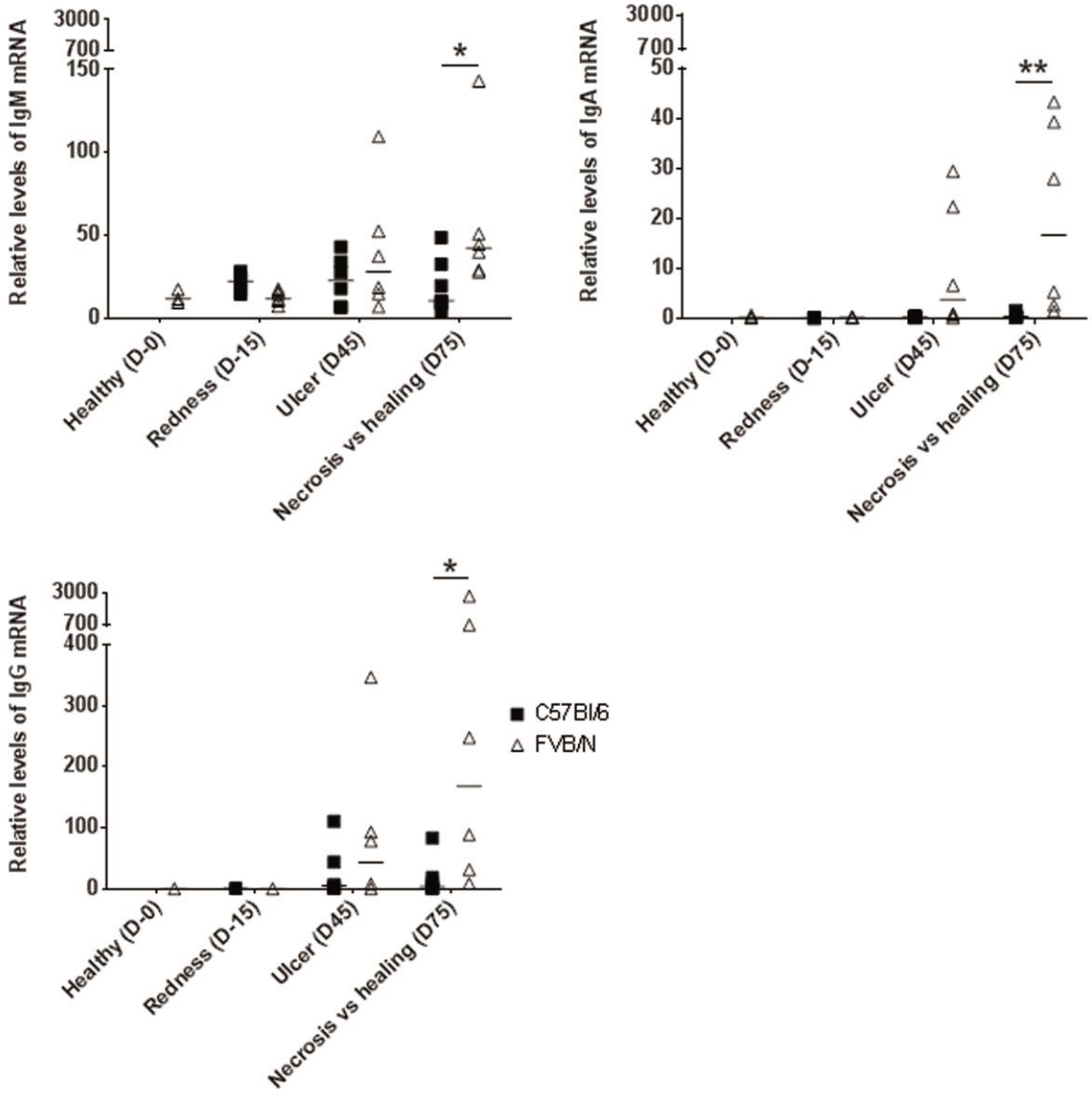

Fig. S2. Ig gene expression in the cutaneous tissues of mice during the course of *M. ulcerans* infection. We evaluated mRNA levels for IgM, IgA and IgG in the two mouse strains at different time points during infection (healthy (n=3 for FVB/N), redness, ulcer, necrosis or healing (n=6 or 7 for FVB/N and C57BL/6 mice) corresponding to 0, 15, 45 and 75 days post inoculation, respectively) by quantitative RT-PCR. Results are expressed as the level of mRNA relative to actin mRNA levels. * $p < 0.05$ and ** $p < 0.01$ (Comparison of gene expression in FVB/N and C57BL/6 mice at each stage of the disease in a Mann Whitney U test).

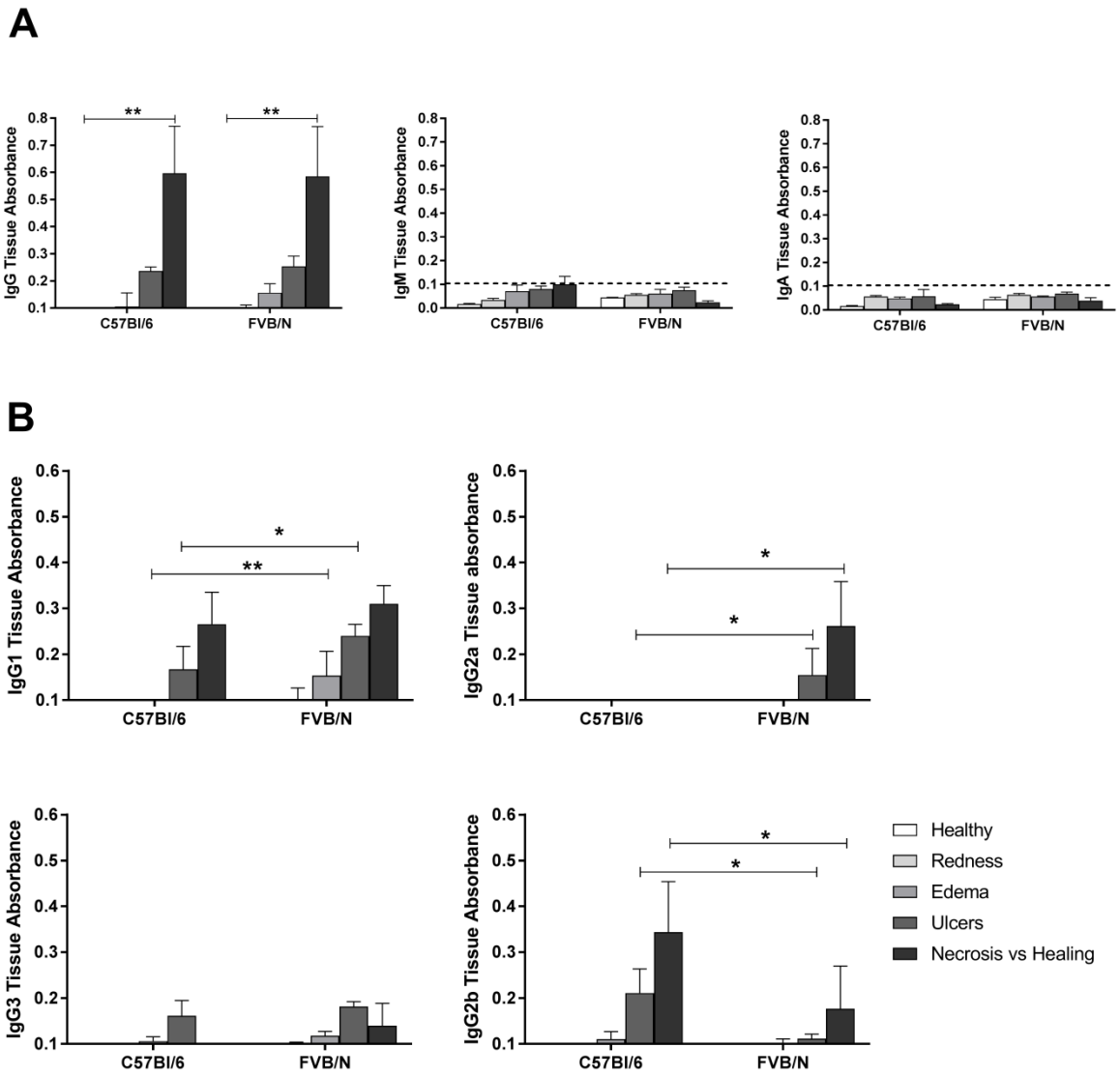

Fig. S3. Detection of cutaneous IgG binding to *M. ulcerans* lysate by quantitative ELISA. *M. ulcerans* lysate was investigated by qualitative ELISA. The immunoglobulin concentrations of cutaneous tissue samples (n=5 for each strain) were normalized. **A**. Detection of IgG, IgM and IgA and **B**. IgG1, IgG2a, IgG3 and IgG2b recognizing the *M. ulcerans* lysate in cutaneous tissue from FVB/N and C57BL/6 mice at various stages of infection (healthy, redness, ulcer, necrosis or healing). The detection limit was an absorbance of 0.1. The histograms show the means ± SD. *p <0.5, ** p < 0.01 (Mann Whitney U test).

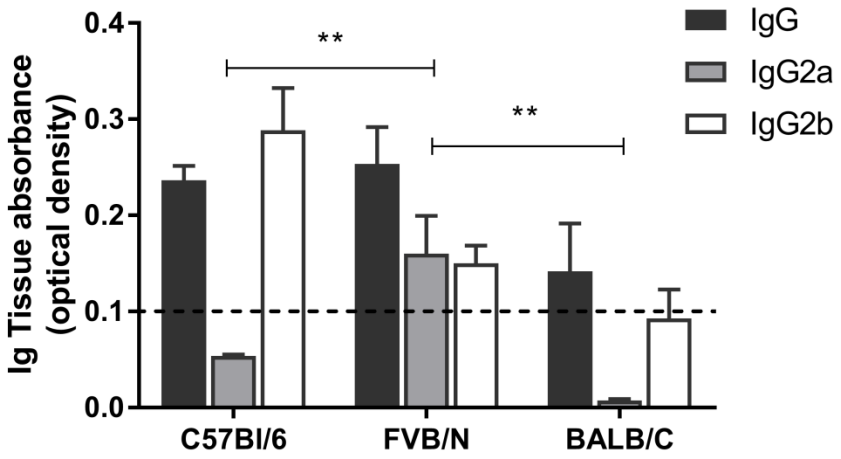

Fig. S4. Cutaneous IgG2a and IgG2b binding mycolactone from C57BL/6, FVB/N, and BALB/c mice at the ulcerative stage. Immunoglobulin concentrations were normalized in cutaneous tissue samples from C57BL/6, FVB/N and BALB/c mice (*n*=5 for each strain). The IgG2a response in the three mouse strains was evaluated by qualitative ELISA. The detection limit was an absorbance of 0.1. The histograms show the means ± SD. ** $p < 0.05$ (Mann Whitney *U* test).

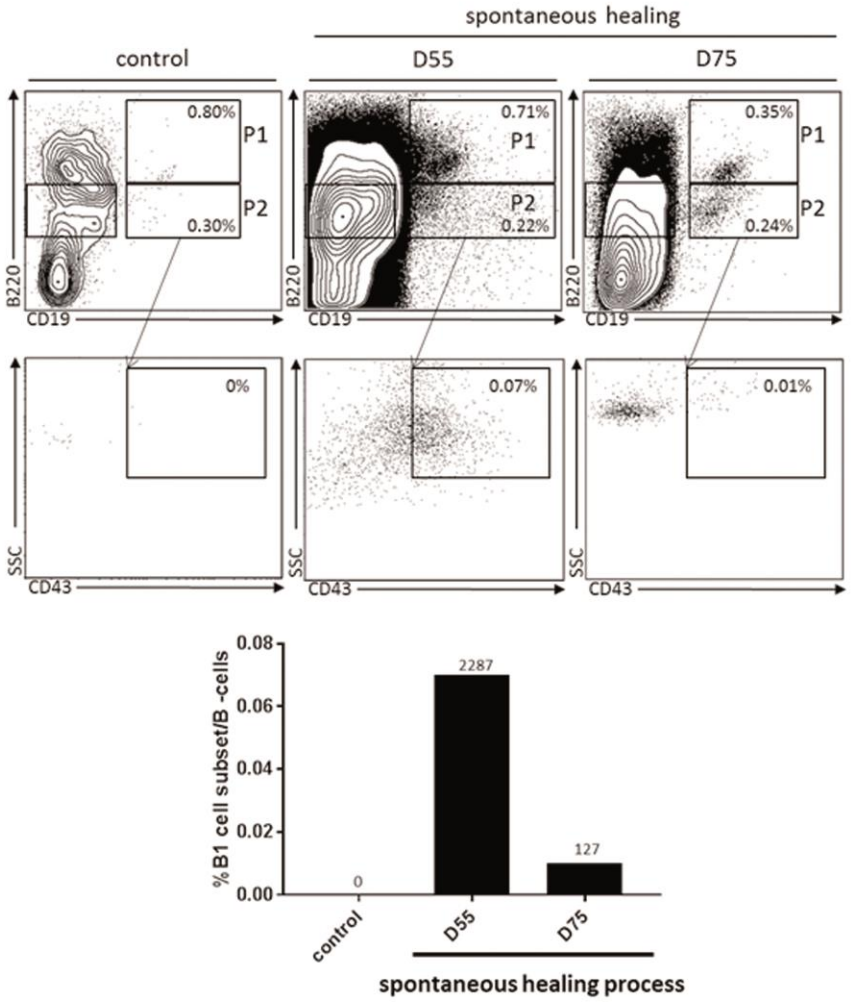

Fig. S5. Quantification of local B1-like subset of B cells in the skin during *M. ulcerans* infection. Cells were analyzed by four-color flow cytometry with the specific staining of total lymphoid cells ($CD45^+$), B cells (population P1 = $CD45^+$, $CD19^+$, $B220^+$), plasmablasts (population P2 = $CD45^+$, $CD19^+$, $B220^{int}$), plasma cells (population P3 = $CD45^+$, $CD19^-$, $B220^{int}$, $CD138^+$) and the B1 subset of B cells ($CD45^+$, $CD19^+$, $B220^{int}$, $CD43^+$)

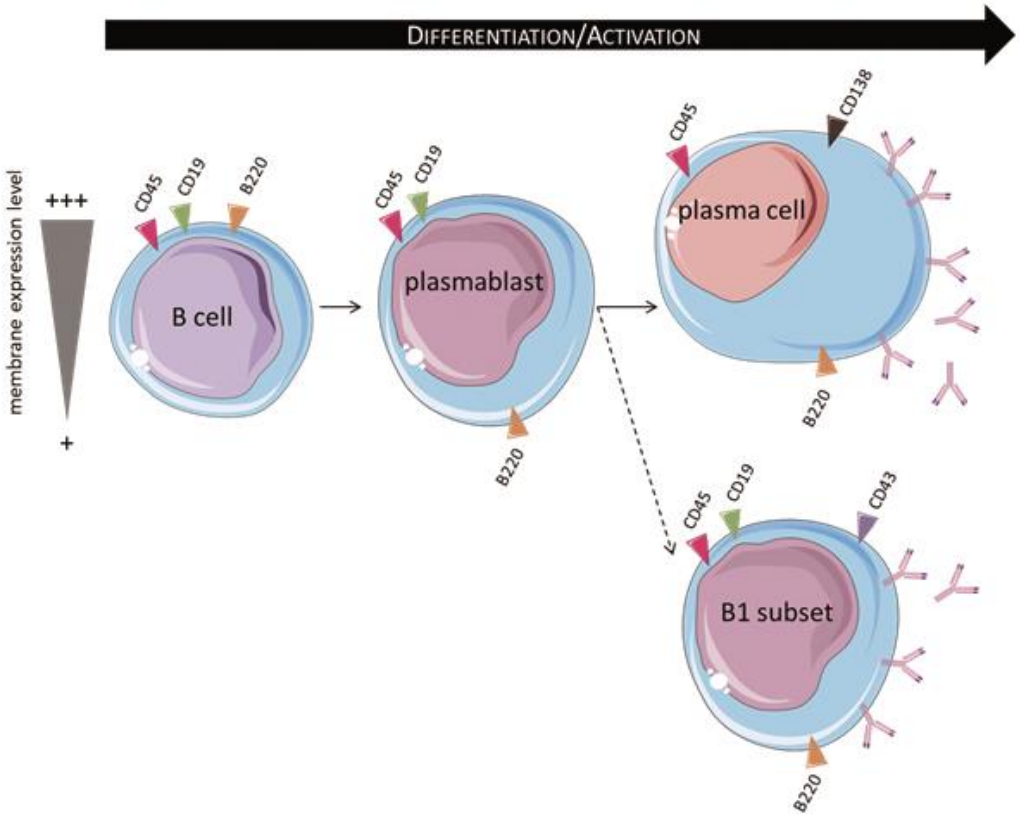

Fig. S6. Markers of murine B cell subsets used for flow cytometry analysis. B cells (the most immature subset) express CD45, CD19 and B220 surface molecules at high levels. Plasmablasts, corresponding to the intermediate differentiation stage, express CD45 and CD19 at high levels and B220 at lower levels. Finally, plasma cells (antibody-producing cells) express CD45 and CD138 at high levels, but have lost CD19 expression and express B220 at low levels. B1 is a specific subset of B cells more frequent at peripheral sites than in the blood. The cells of this subset express CD45, CD19 and CD43 at high levels and B220 at lower levels. Marker selection was based on (*26*, *38*, *39*).