## Supplementary Information

### Supplementary Results

*Identification of phages specific for M. abscessus GD82.*

*M. abscessus* GD82 is closely related to *M. abscessus* GD01 with only 44 single nucleotide polymorphisms in the ~99% of shared nucleotide positions; we note that GD82 carries a Cluster MabC prophage that GD01 lacks[20]. Phages used for screening for *M. abscessus* GD82 susceptibility were chosen based on prior studies relating phage genomic information and host range[7,21], and screening many *M. abscessus* clinical isolates[20]. *M. abscessus* GD82 was found to be sensitive to the same three phages used previously to treat an *M. abscessus* infection, Muddy, BPsΔ*33*HTH_HRM10, and ZoeJΔ*45*[22] (Fig. 1c); *M. abscessus* GD82 is also sensitive to several other phages in the screen (Fig. 1c). These include another host range mutant of BPs (BPsΔ*33*HTH_HRM$^{GD03}$) and a host range mutant of Muddy (Muddy_HRM$^{GD04}$). Several other phages grouped in Cluster K – in addition to ZoeJ – infect *M. abscessus* GD82 well including FionnbharthΔ*43*Δ*45* (Subcluster K4), CrimDΔ*41-43* (Subcluster K1) and Mufasa (Subcluster K2). The choice of the phages to use for the cocktail was based on those used previously[7], and avoiding phages from the same cluster, which are likely to have a higher propensity for similar infection and therefore similar resistance profiles.

*Post phage treatment M. abscessus isolates.*

Phage susceptibility testing of *M. abscessus* isolates collected post phage treatment (*M. abscessus* GD82_M1 – GD82-M6) are fully sensitive to phages Muddy and

BPsΔ*33*HTH_HRM10, and the strain sensitivity profiles are consistent with these isolates being the same or similar genomically to the pre-treatment GD82 isolate. However, the post-treatment isolates at month-2, month-3, month-4 and month-6 show substantial reduction in plating of ZoeJΔ*45*, although much less so for the month-5 isolate. The genetic basis of this variation is not known, although we note that *in vitro* isolated mutants resistant to a similar cocktail using *M. abscessus* GD01 yielded two strains containing a fragment of ZoeJΔ*45* DNA integrated into the chromosome. A similar explanation could account for the resistant profile and its seeming instability.

*Phage neutralization*

Phage neutralization depends substantially on the amount of serum used in the assay, and the time of incubation. In the standard assay, using 10 µl serum was incubated with phage in a 100 µl total volume, incubated for 2, 4 and 24 hours, and sample removed and plated on *M. smegmatis* to determine titers. In this assay, the one-month post-treatment serum shows about a 100-fold reduction in titer of phage Muddy after two-hours incubation (Fig. 2c, Fig. S4), but this increased to about a 1000-fold reduction with 4 hours incubation, and about a $10^6$-fold reduction after 24 hours incubation (Fig. 2c, Fig. S4). However, if 10-fold less serum is used, then the one-month post-treatment serum only reduces Muddy plaquing by about 10-fold after 24 hours incubation and there is a similar reduction 48 hours incubation (data not shown). We note that the conditions used previously to test for neutralization of the previously treated patient[7] used 10 µl serum and a two-hour incubation showed no neutralization at all, whereas the samples used here 2-6 months post-treatment give near complete inhibition of Muddy plaquing.

*Phage resistant mutants isolated in vitro*

Challenges of cultures containing $10^8$ CFU *M. abscessus* GD82 with phages at a multiplicity of infection of 10, revealed a small number of survivors recovered on solid media, with a greater number from ZoeJ∆*45* challenge than the other phages (Extended Data Fig. 4a). We were not able to grow any survivors from the BPs∆*33*HTH_HRM10 challenge but propagated 3-4 survivors from the other selections. On re-testing for phage susceptibility, several distinct phenotypes were observed (Extended Data Fig. 4b). For the survivors of the Muddy challenge (GD82-M_RM1, GD82-M_RM3, and GD82-M_RM4), RM1 and RM4 have similar phenotypes (and could be siblings) with complete sensitivity to Muddy and BPs∆*33*HTH_HRM10, with a mild reduction of plating of ZoeJ∆*45*. In contrast, GD82-M_RM3 has reduced sensitivity to Muddy but is fully infected by BPs∆*33*HTH_HRM10 and ZoeJ∆*45* (Extended Data Fig. 4b).

Similarly, the three survivors of ZoeJ∆45 challenge (GD82-Z_RM1, GD82-Z_RM3, and GD82-Z_RM4) all remain sensitive to Muddy, and GD82-Z_RM3 and GD82-Z_RM4 are also sensitive to BPs∆*33*HTH_HRM10 and ZoeJ∆*45*. In contrast, GD82-Z_RM1 is fully resistant to ZoeJ∆*45* (Extended Data Fig. 4b). Finally, four survivors (GD82-B/M/Z-RM1, GD82-B/M/Z-RM2, GD82-B/M/Z-RM3 and GD82-B/M/Z-RM4) were recovered and tested from a challenge with all three phages in the cocktail, and again two distinct profiles were observed. GD82-B/M/Z-RM1, GD82-B/M/Z-RM2, and GD82-B/M/Z-RM4 (possibly siblings) have full sensitivity to BPs∆*33*HTH_HRM10 and Muddy, and a partial reduction in ZoeJ∆*45* plaquing. GD82-B/M/Z-RM3 is sensitive to BPs∆*33*HTH_HRM10 and Muddy, but resistant to ZoeJ∆*45*.

It is notable in these experiments that survival in these challenges is not always accompanied by a stable resistance profile. For example, all of the survivors from a challenge with the cocktail are fully sensitive to Muddy (Extended Data Fig. 4b). It is not clear whether these survived the

challenge though phenotypic avoidance, such as within a clump of cells that blocked phage access, or whether it is a transient genetic change that enable survival but is rapidly lost when grown and re-tested.

*Western blots of phage proteins.*

The protein profiles of phages Muddy, BPsΔ, and ZoeJΔ each show a major protein species in the 21-25 kDa size range that is consistent with being the major tail tube subunit (predicted sizes are 22.6 kDa for the Muddy, 21.8 kDa for BPs and 22.2 kDa for ZoeJ tail tube proteins). Both phages encode major capsid subunits (predicted sizes 34.9 kDa for Muddy, 33.5 kDa for BPs, and 31.8 kDa for ZoeJ) that are covalently crosslinked and interlinked, as has been described previously for phage L5[23]. As a consequence, most of the capsid subunits to do not enter a polyacrylamide gel, with the exceptions being incompletely crosslinked or incompletely interlinked pentameric and hexameric capsomers. These have expected sizes of 175 kDa and 210 kDa for Muddy, 167 and 200 kDa for BPs, and 160 and 191 kDa for ZoeJ, as seen in Fig. 2d. Each phage contains 15-18 additional head and tail proteins present at lower abundance.

**Supplementary References**


21. Jacobs-Sera, D. *et al.* On the nature of mycobacteriophage diversity and host preference. *Virology* **434**, 187–201 (2012).
22. Dedrick, R. M. *et al.* Mycobacteriophage ZoeJ: A broad host-range close relative of mycobacteriophage TM4. *Tuberculosis* **115**, 14–23 (2019).
23. Hatfull, G. F. & Sarkis, G. J. DNA sequence, structure and gene expression of mycobacteriophage L5: a phage system for mycobacterial genetics. *Mol. Microbiol.* **7**, 395–405 (1993).

**Supplementary Figure Legends**

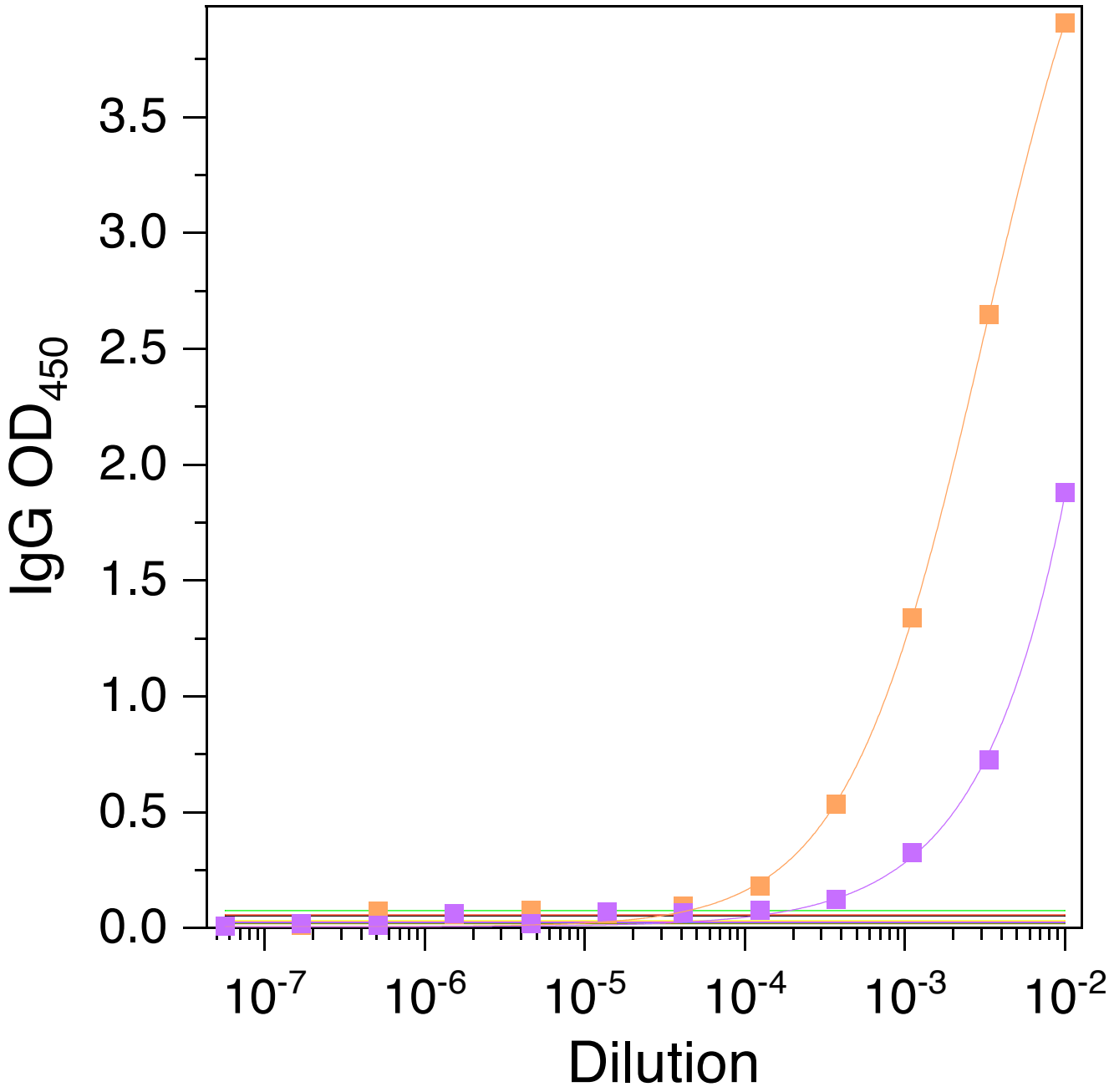
**Figure S1. Extra ELISA controls.** ELISA curves and logistic fits for IgG responses to Muddy (orange squares) and ZoeJ (purple squares) in sera from rabbits immunized with those phages. These curves serve as positive controls, though the antibody titers in these controls are lower than those observed in patient sera. The horizontal lines at the bottom are the average signal observed from the secondary antibodies when no serum is used. In particular, the black and red lines are from Muddy-coated wells probed with anti-human IgG and anti-rabbit IgG secondary antibodies, respectively. Similarly, the green and blue lines are for BPs-coated wells, the teal and magenta lines are for ZoeJ-coated wells, and the yellow and olive lines are for uncoated wells. All of these signals are negligible, and indistinguishable from signals using most-diluted sera in Figure 2b).

**Figure S2. Extra Western blot controls.** Coomassie-stained gel and Western blots for protein ladder, an empty lane, phage Muddy, and non-therapeutic and unrelated phage phiFW2. Western blots are probed with either no human serum (central panel) or a 1:1000 dilution of the patient's serum 3 months after the start of therapy (right panel), then detected with Goat Anti-Human IgG Fc (HRP). Nothing is detected in blot with no primary serum, and the blot probed with the month 3 serum shows reactivity only to proteins in the Muddy positive control lane.

**Figure S3. Raw data for Figure 1e**. Phage titrations are shown for replicate experiments (**A** and **B, C, D**) with 10-fold serial dilutions plates on *M. smegmatis* $mc^2155$. In panels A and B multiple phages are tested, but only those used in Fig. 1e are labeled and boxed. Strains used are indicated, with the time of collection, i.e., the sample of *M. abscessus* GD82 collected one month after start of phage treatment is labeled GD82-M1.

**Figure S4. Raw data for Figure 2c.** Panels **A** and **B** show independent experiments measuring neutralization of phage infection by patient sera. Patient sera were incubated with phage lysates for 2 hours, 4 hours, and 24 hours, as indicated, 10-fold serially diluted, and plated on lawns of *M. smegmatis*. The pre-phage (Pre) serum, no-serum phage control (PC) and sera from months 1-6 (M1-M6) post-treatment are shown.

**Figure S5. Images of unprocessed gels and blots.** Unprocessed images of **a,** Western blots of IgG responses to phages Muddy (M) and BPsΔ*33*HTH_HRM10 (B) with serum dilutions of 1:1000. **b**, Western blots of IgM responses to Muddy and BPsΔ*33*HTH_HRM10 with serum dilutions of 1:1000 **c**, Western blots of IgG responses to Muddy, BPsΔ*33*HTH_HRM10, and ZoeJΔ45 with serum dilutions of 1:100. **d**, Coomassie-stained gel slice of Muddy, BPsΔ*33*HTH_HRM10 and ZoeJΔ45.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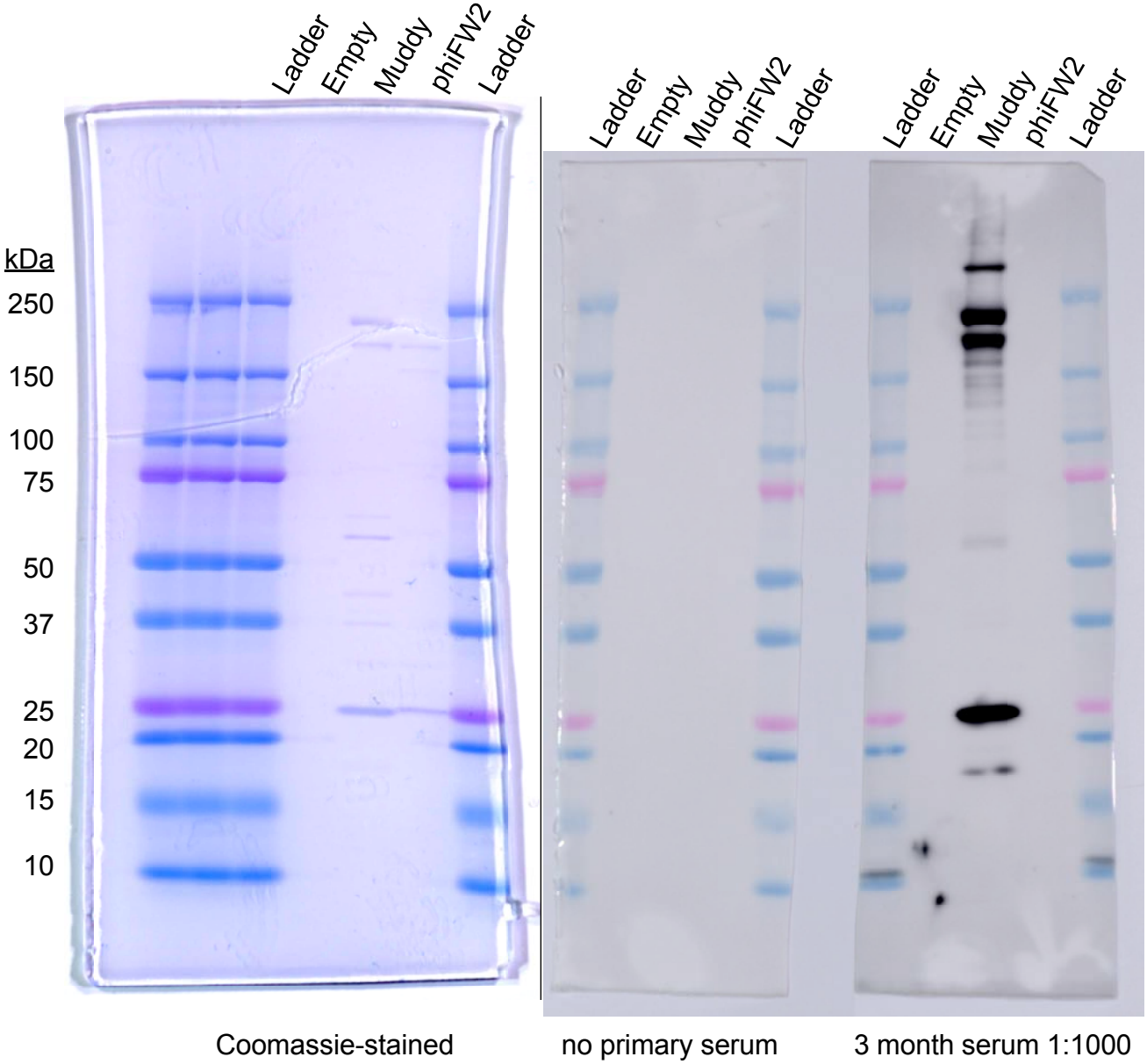
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10-7
10-6
10-5
10-4
10-3
10-2
Dilution

Figure S1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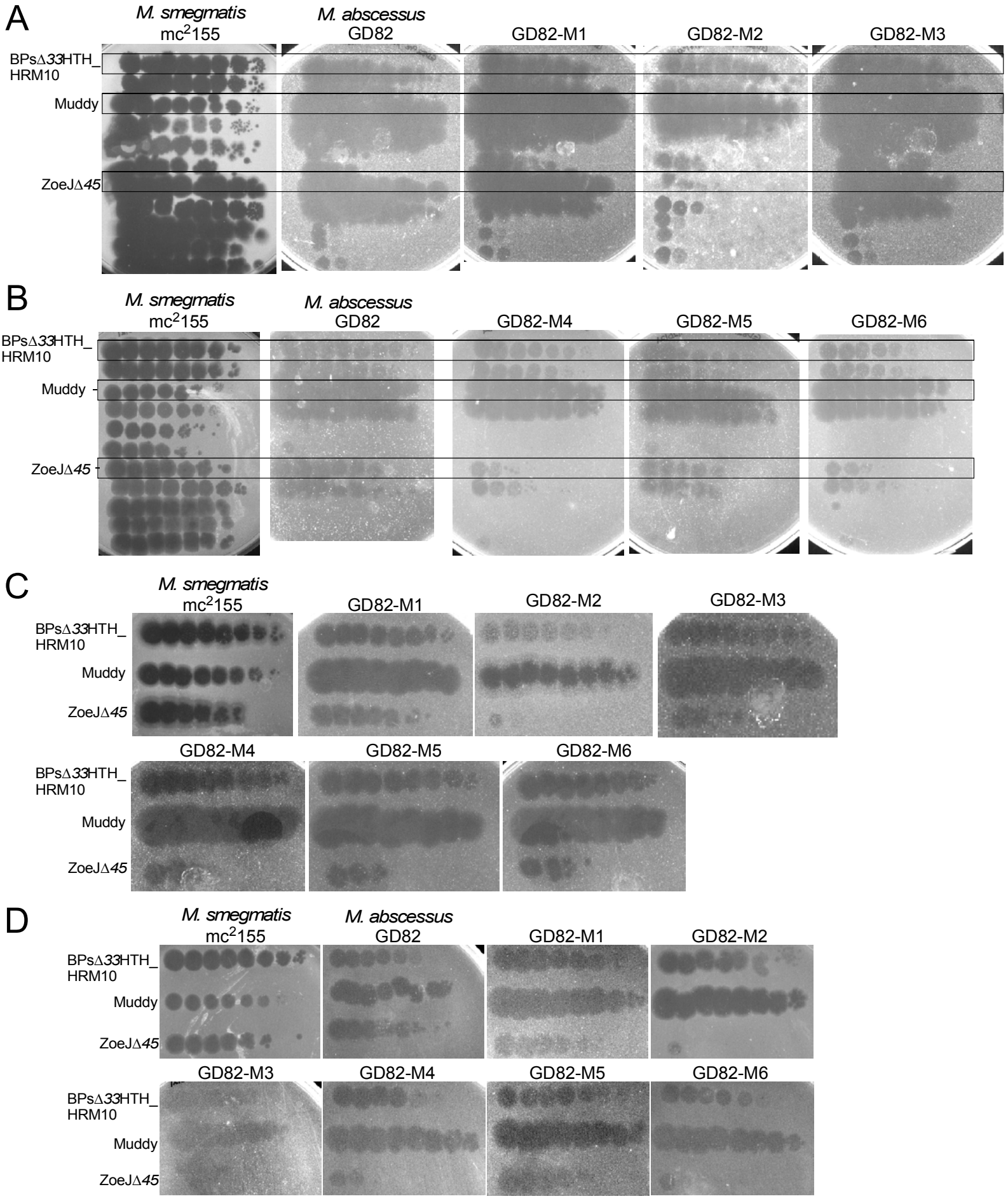
Empty
Muddy
phiFW2
Ladder
kDa
250
150
100
75
50
37
25
20
15
10
Ladder
Empty
Muddy
phiFW2
Ladder
Ladder
Empty
Muddy
phiFW2
Ladder
Coomassie-stained
no primary serum
3 month serum 1:1000

Figure S2


A
M. smegmatis mc²155
M. abscessus GD82
GD82-M1
GD82-M2
GD82-M3
BPsΔ33HTH_HRM10
Muddy
ZoeJΔ45
B
M. smegmatis mc²155
M. abscessus GD82
GD82-M4
GD82-M5
GD82-M6
BPsΔ33HTH_HRM10
Muddy
ZoeJΔ45
C
M. smegmatis mc²155
GD82-M1
GD82-M2
GD82-M3
BPsΔ33HTH_HRM10
Muddy
ZoeJΔ45
GD82-M4
GD82-M5
GD82-M6
BPsΔ33HTH_HRM10
Muddy
ZoeJΔ45
D
M. smegmatis mc²155
M. abscessus GD82
GD82-M1
GD82-M2
BPsΔ33HTH_HRM10
Muddy
ZoeJΔ45
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Muddy
ZoeJΔ45

Figure S3

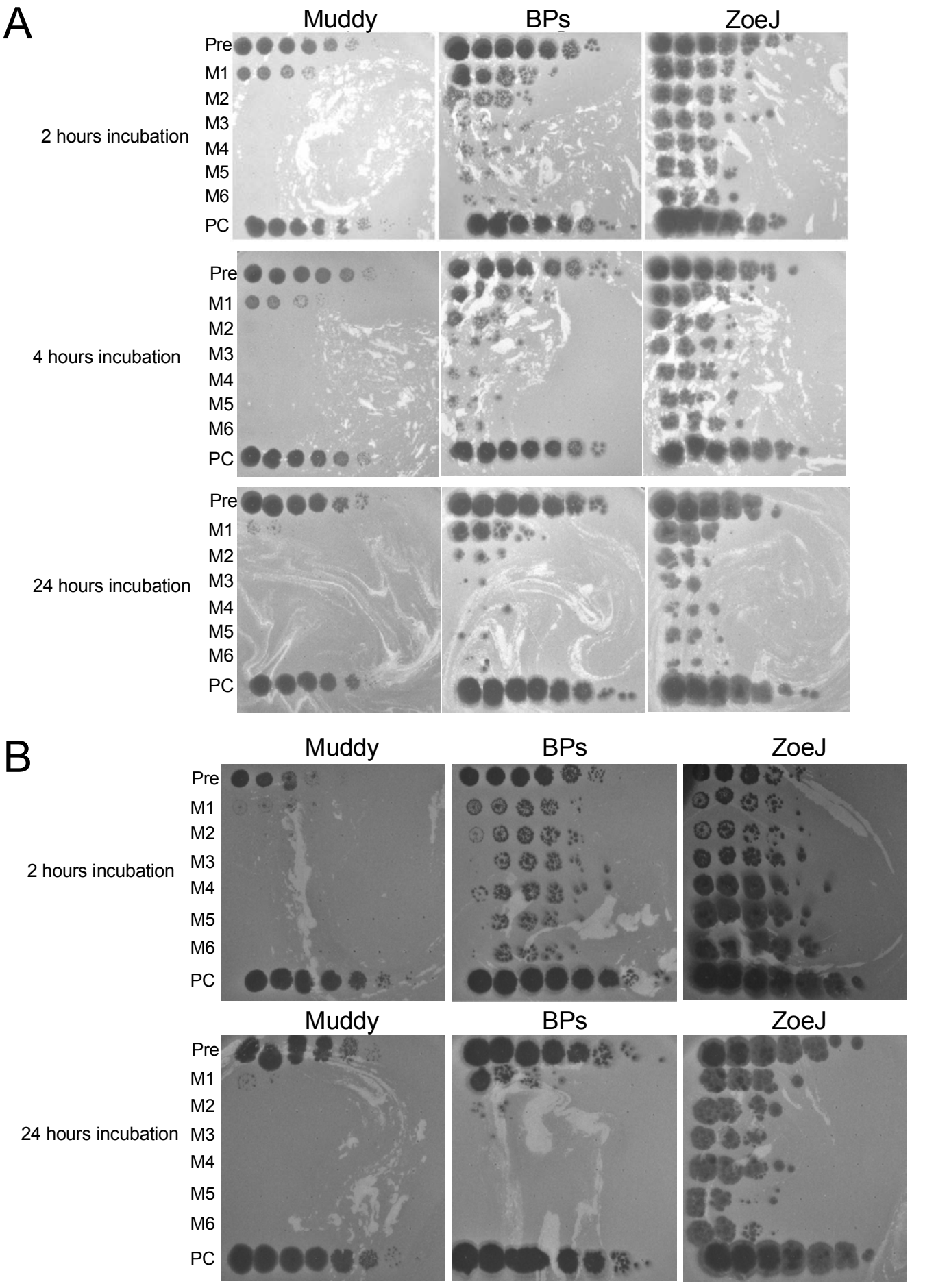
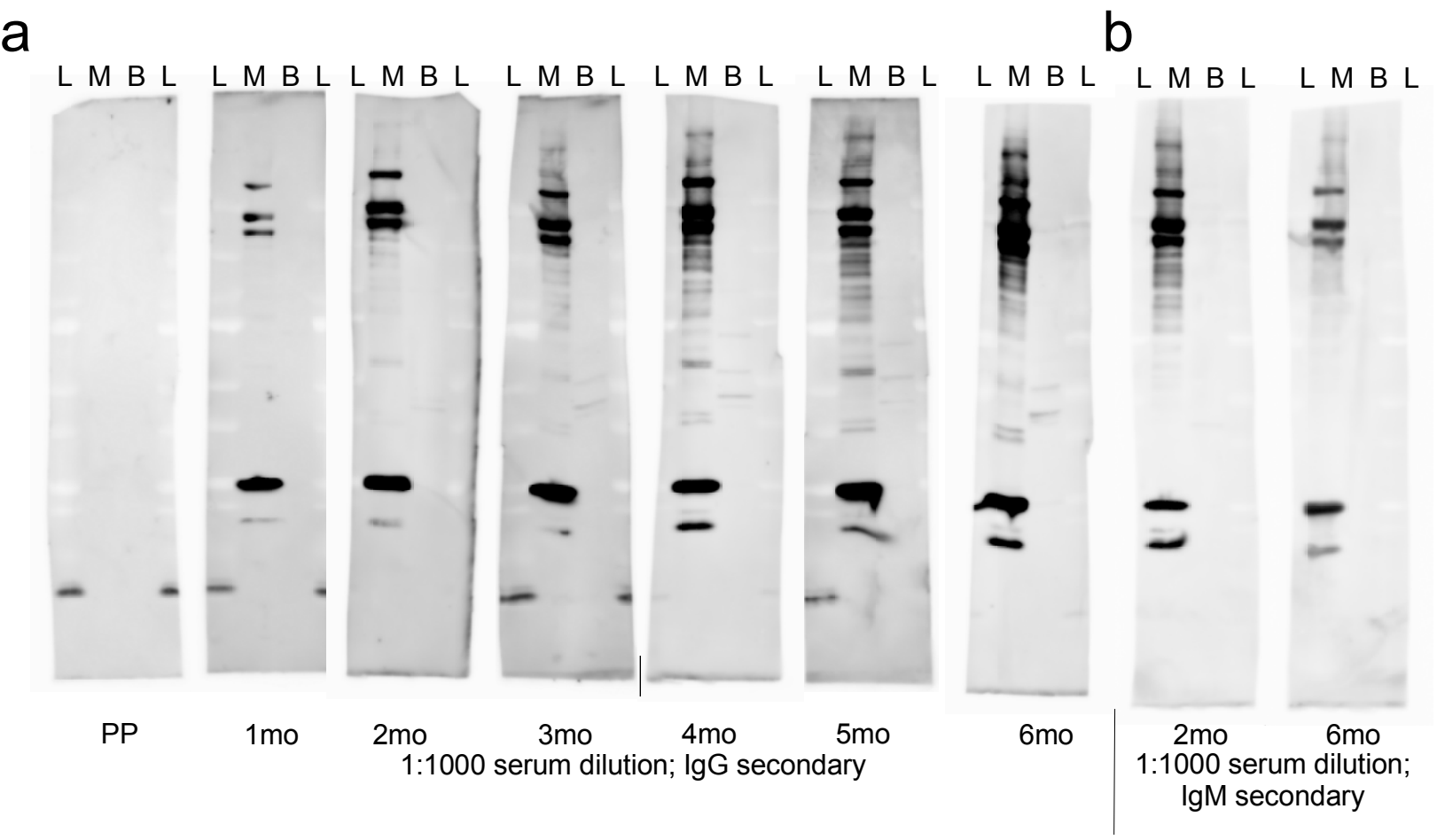

A
Muddy
BPs
ZoeJ
2 hours incubation
4 hours incubation
24 hours incubation
Pre
M1
M2
M3
M4
M5
M6
PC
B
Muddy
BPs
ZoeJ
2 hours incubation
24 hours incubation
Pre
M1
M2
M3
M4
M5
M6
PC

Figure S4


a
b
L M B L
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2mo
3mo
4mo
5mo
6mo
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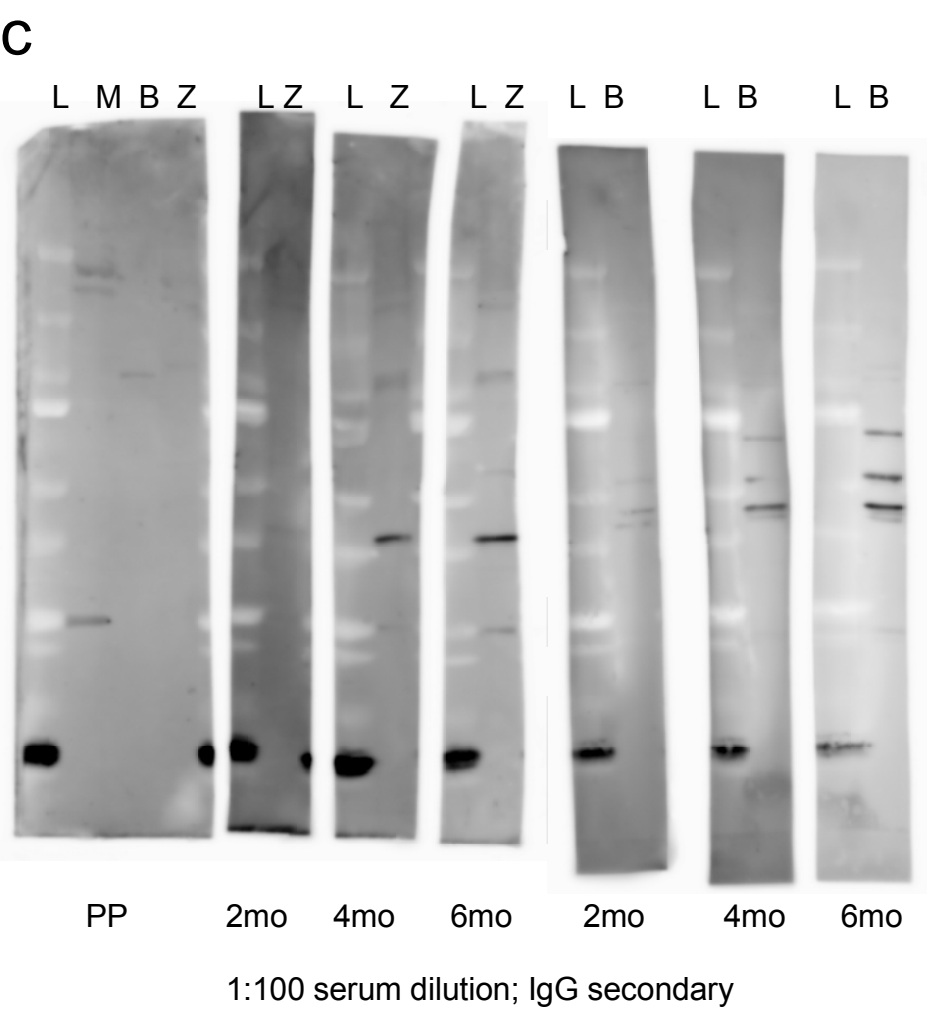
2mo
6mo
1:1000 serum dilution;
IgM secondary


c
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L Z
L Z
L Z
L B
L B
L B
PP
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4mo
6mo
2mo
4mo
6mo
1:100 serum dilution; IgG secondary

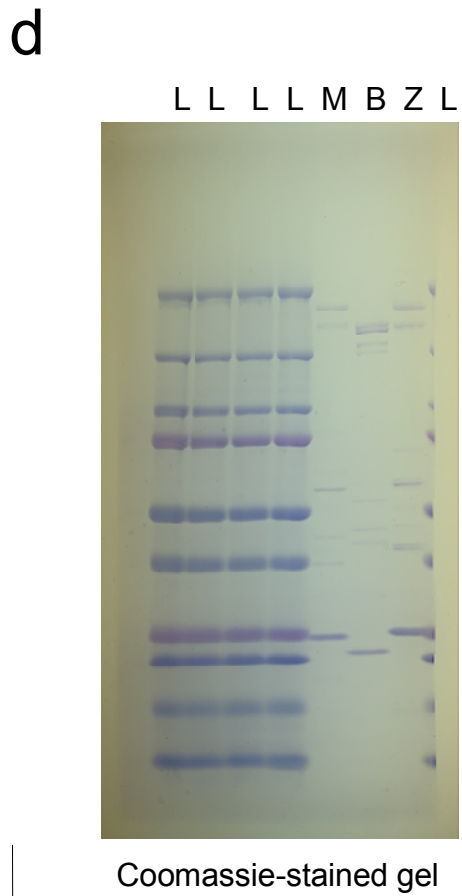
d
L L L L M B Z L
Coomassie-stained gel

Figure S5