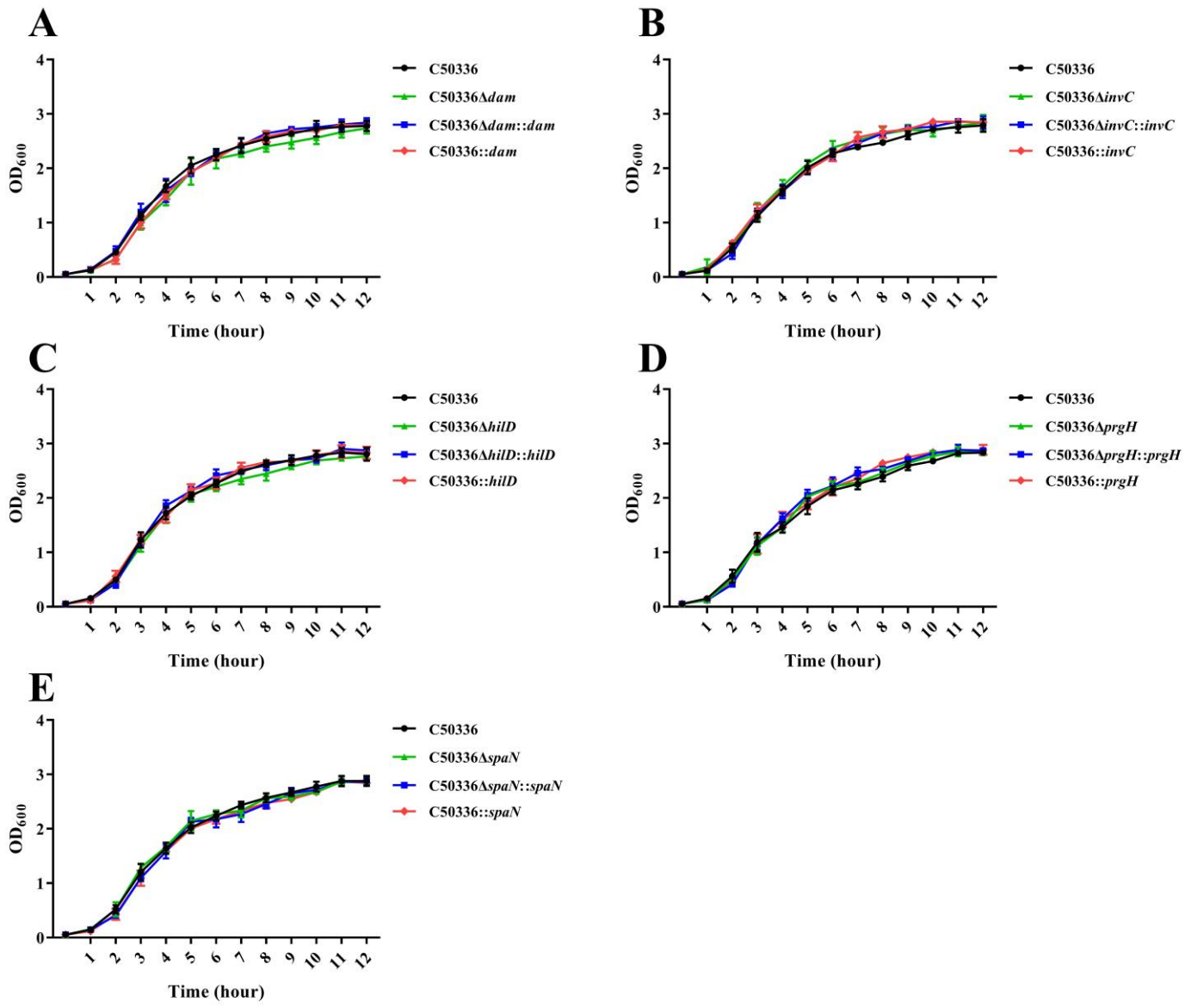
# Supplemental Information

**Essential role of *Salmonella* Enteritidis DNA adenine methylase in modulating inflammasome activation**

Yaxin Guo, Dan Gu, Tingting Huang, Liyan Cao, Xinyu Zhu, Yi Zhou, Kangru Wang, Xilong Kang, Chuang Meng, Xinan Jiao, Zhiming Pan

# Figure S1


A
B
C
D
E
OD600
Time (hour)
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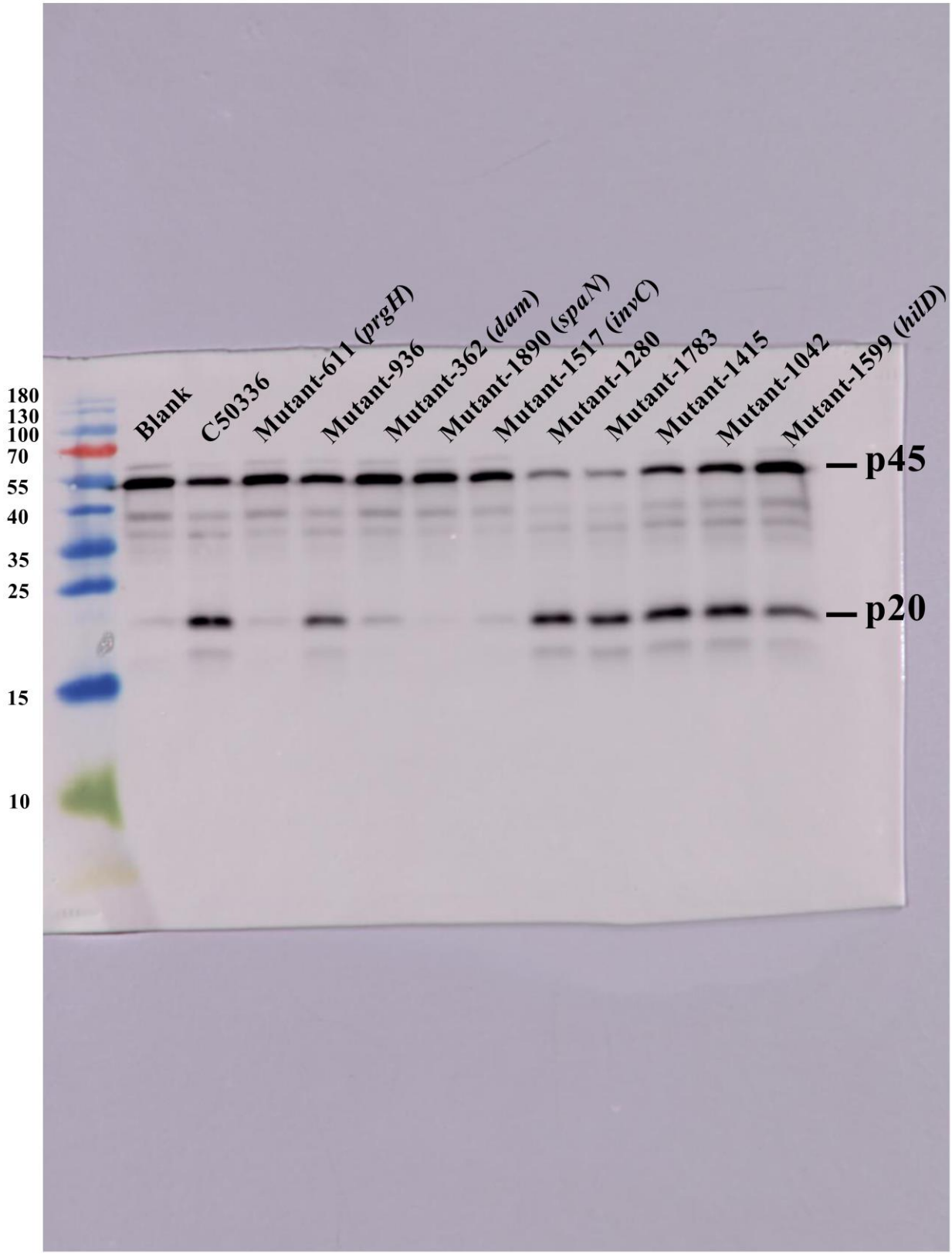
C50336Δdam
C50336Δdam::dam
C50336::dam
C50336ΔinvC
C50336ΔinvC::invC
C50336::invC
C50336ΔhilD
C50336ΔhilD::hilD
C50336::hilD
C50336ΔprgH
C50336ΔprgH::prgH
C50336::prgH
C50336ΔspaN
C50336ΔspaN::spaN
C50336::spaN

# Figure S2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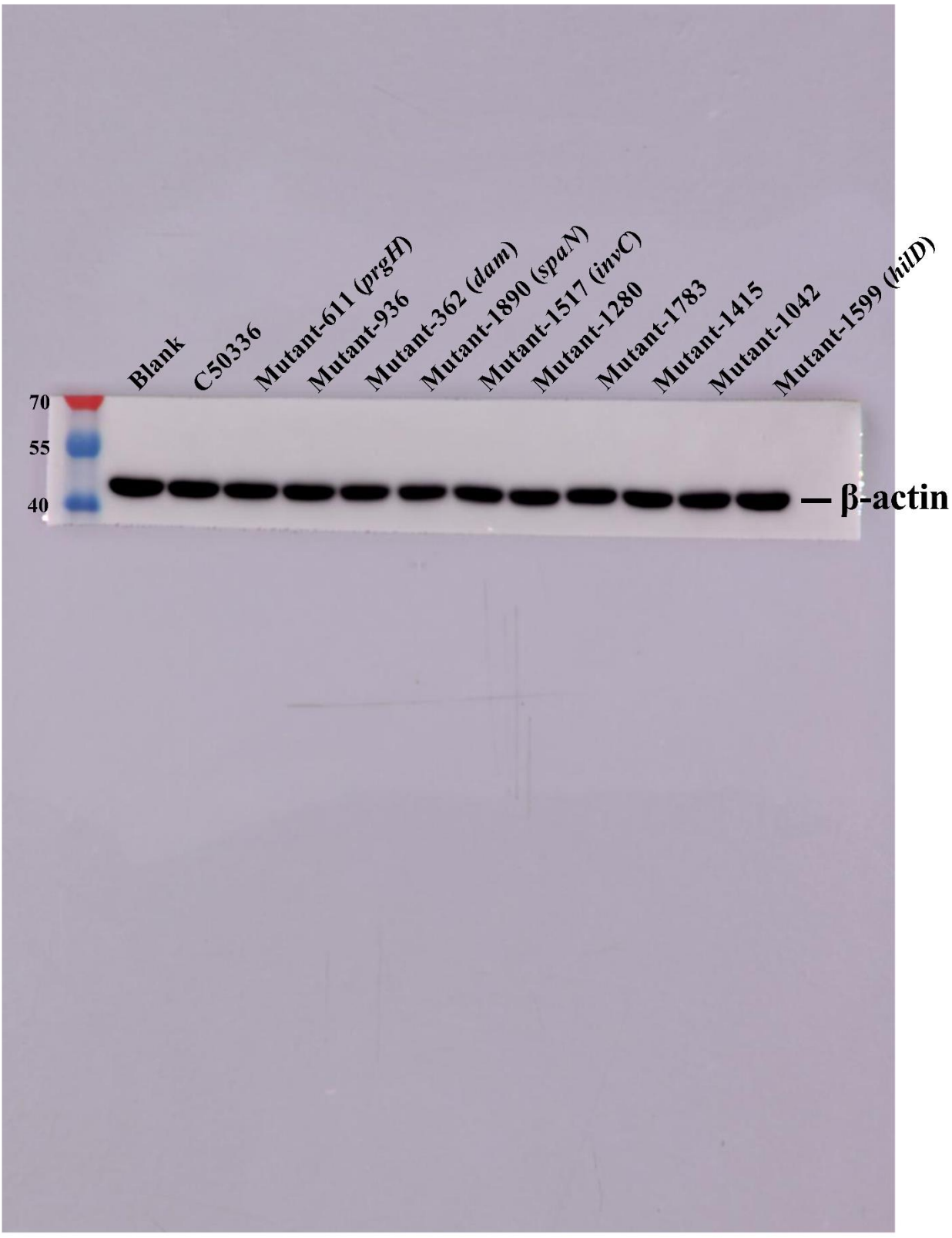
C50336
Mutant-611 (prgH)
Mutant-936
Mutant-362 (dam)
Mutant-1890 (spaN)
Mutant-1517 (invC)
Mutant-1280
Mutant-1783
Mutant-1415
Mutant-1042
Mutant-1599 (hilD)
180
130
100
70
55
40
35
25
15
10
p45
p20

# Figure S2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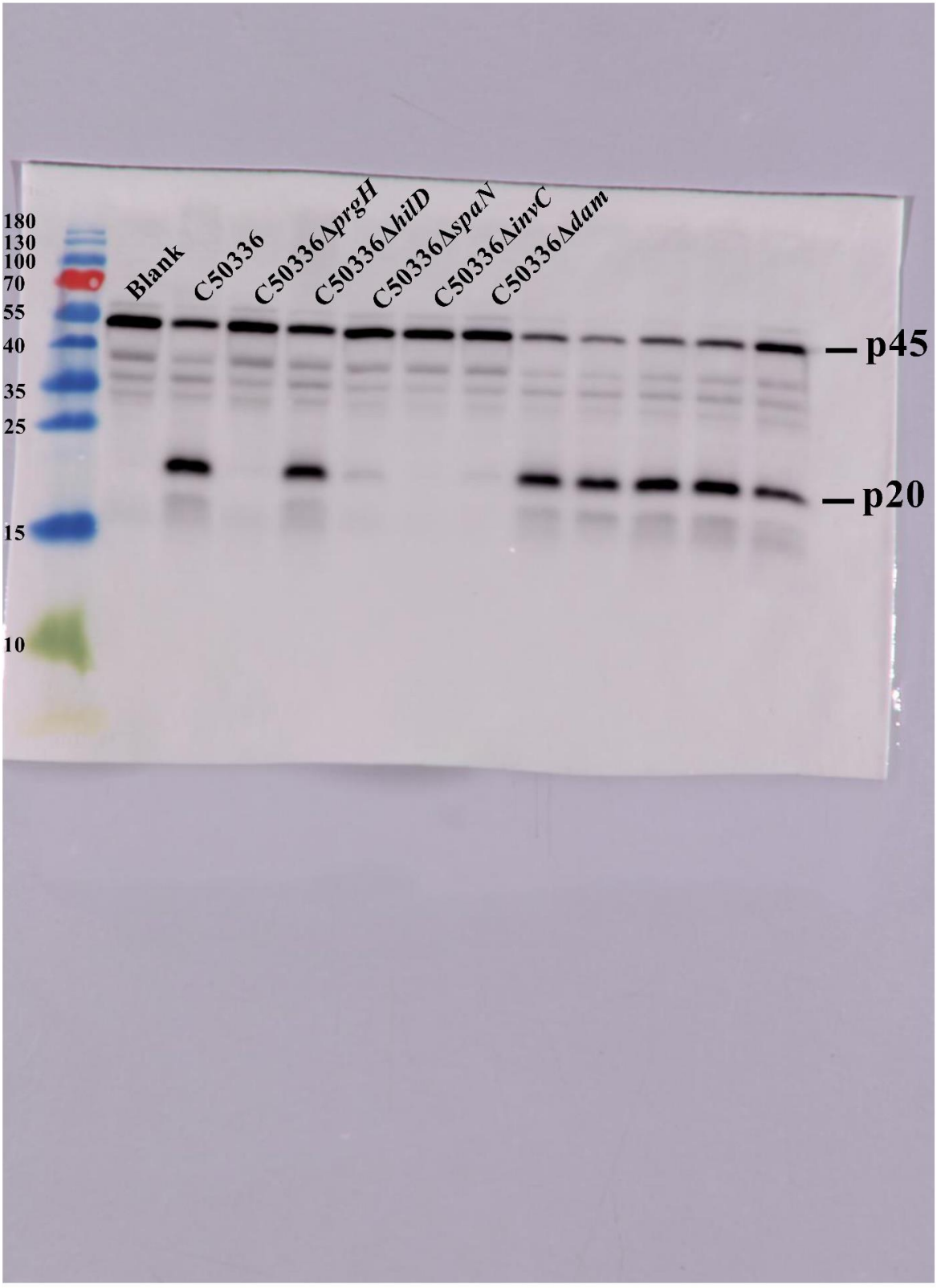
Mutant-611 (prgH)
Mutant-936
Mutant-362 (dam)
Mutant-1890 (spaN)
Mutant-1517 (invC)
Mutant-1280
Mutant-1783
Mutant-1415
Mutant-1042
Mutant-1599 (hilD)
70
55
40
β-actin

# Figure S3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130
100
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55
40
35
25
15
10
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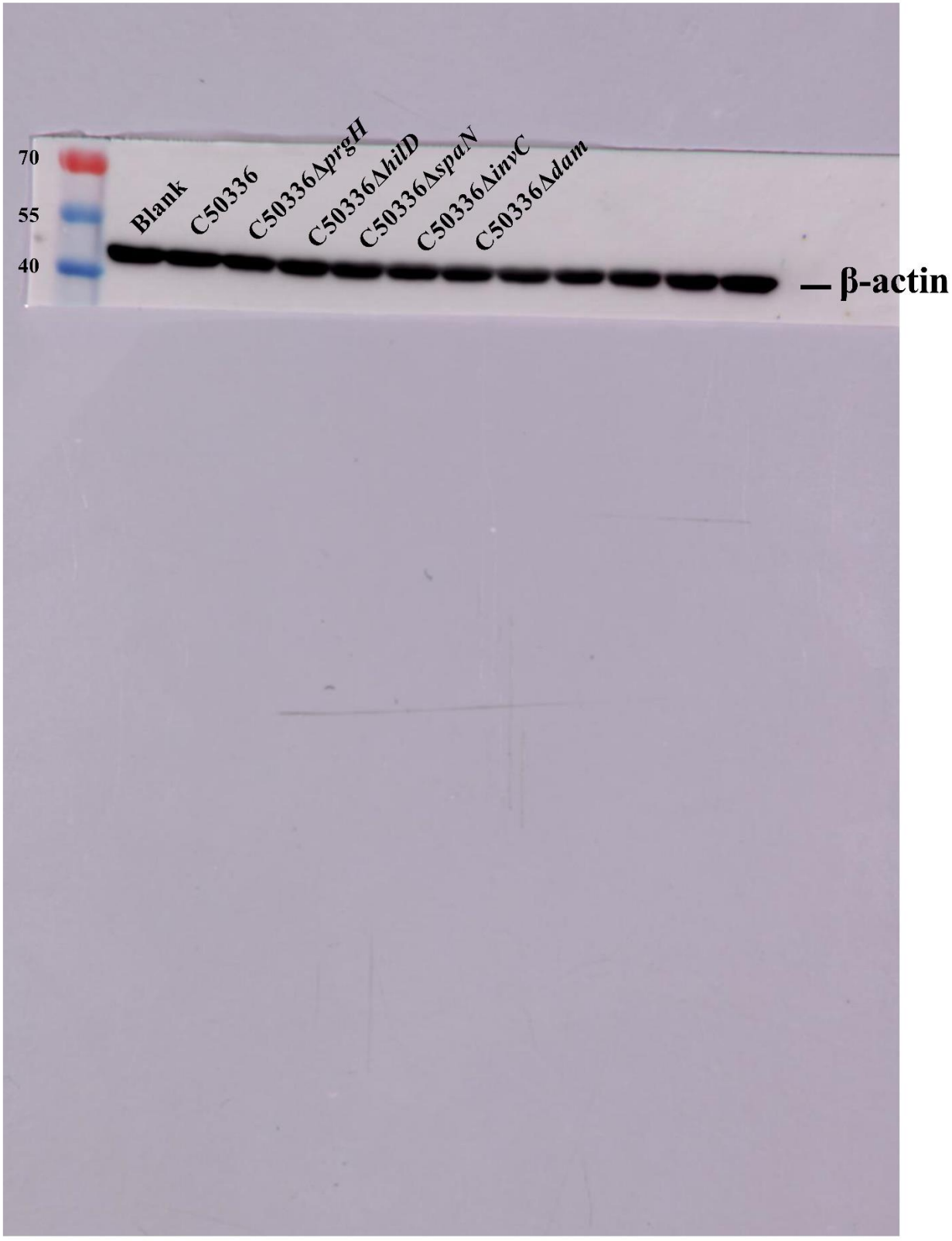
C50336ΔspaN
C50336ΔinvC
C50336Δdam
p45
p20

# Figure S3


70
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40
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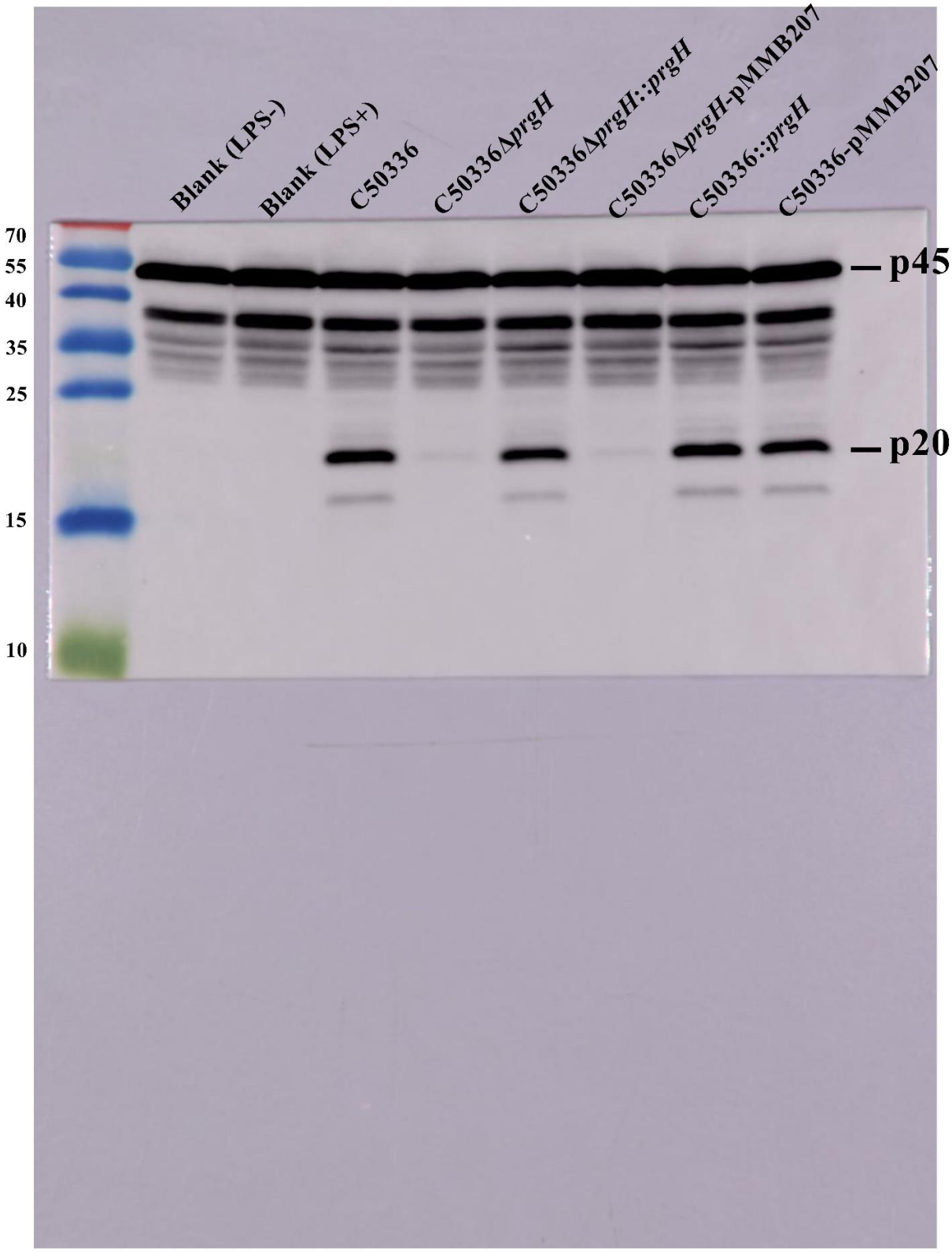
C50336Δdam
β-actin

# Figure S4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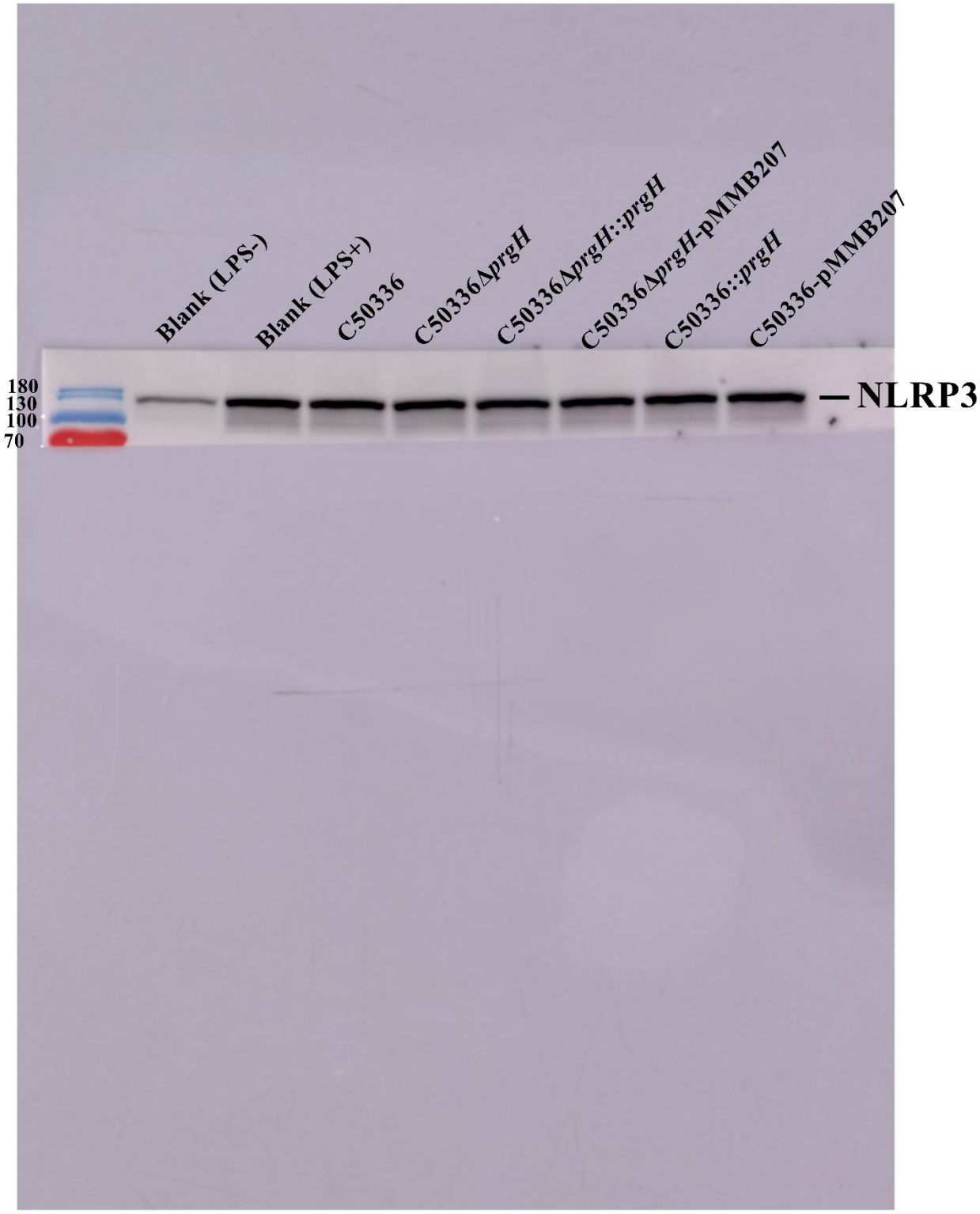
C50336ΔprgH::prgH
C50336ΔprgH-pMMB207
C50336::prgH
C50336-pMMB207
70
55
40
35
25
15
10
p45
p20

# Figure S4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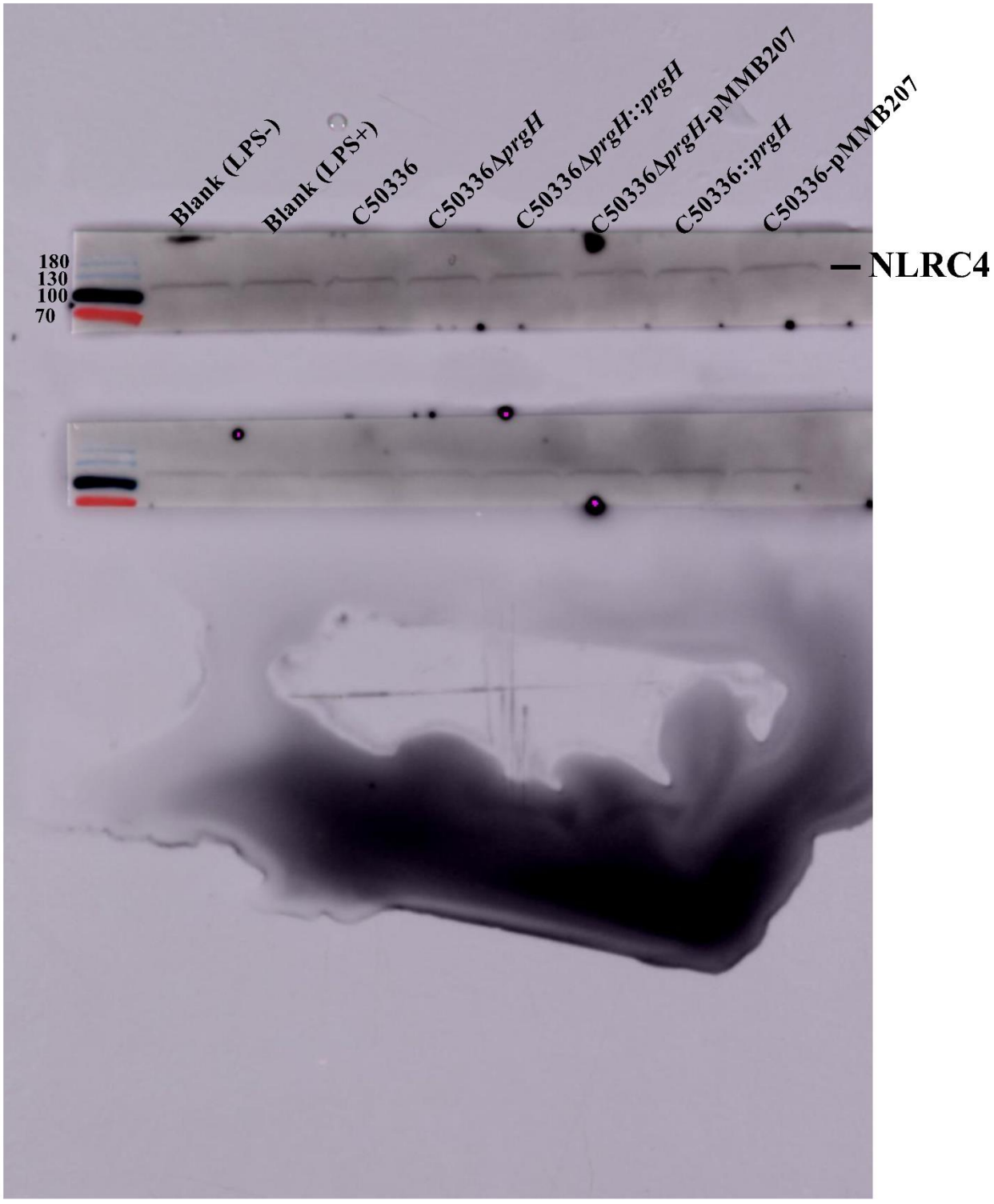
C50336::prgH
C50336-pMMB207
180
130
100
70
NLRP3

# Figure S4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C50336ΔprgH::prgH
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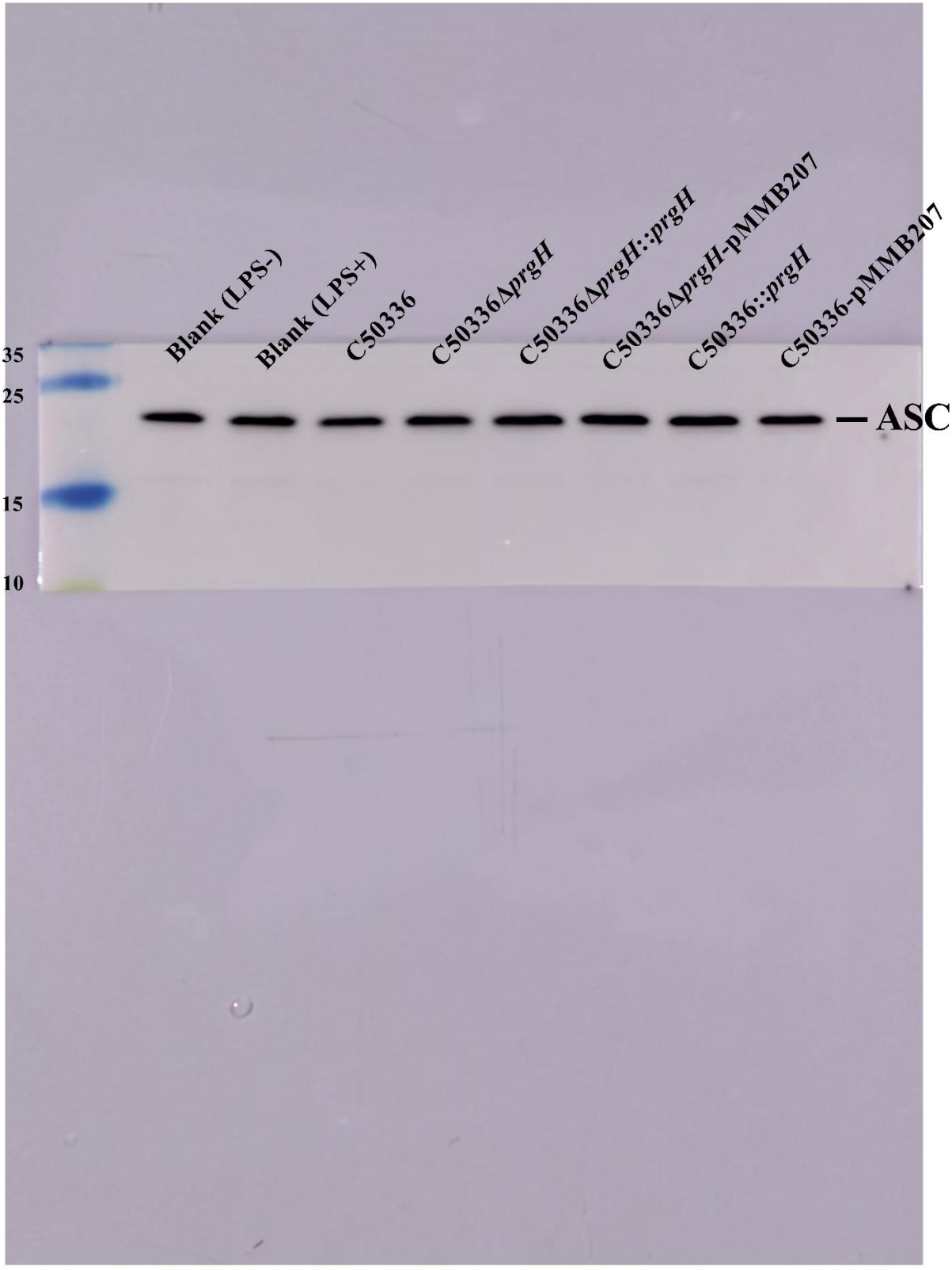
C50336::prgH
C50336-pMMB207
180
130
100
70
NLRC4

# Figure S4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C50336ΔprgH::prgH
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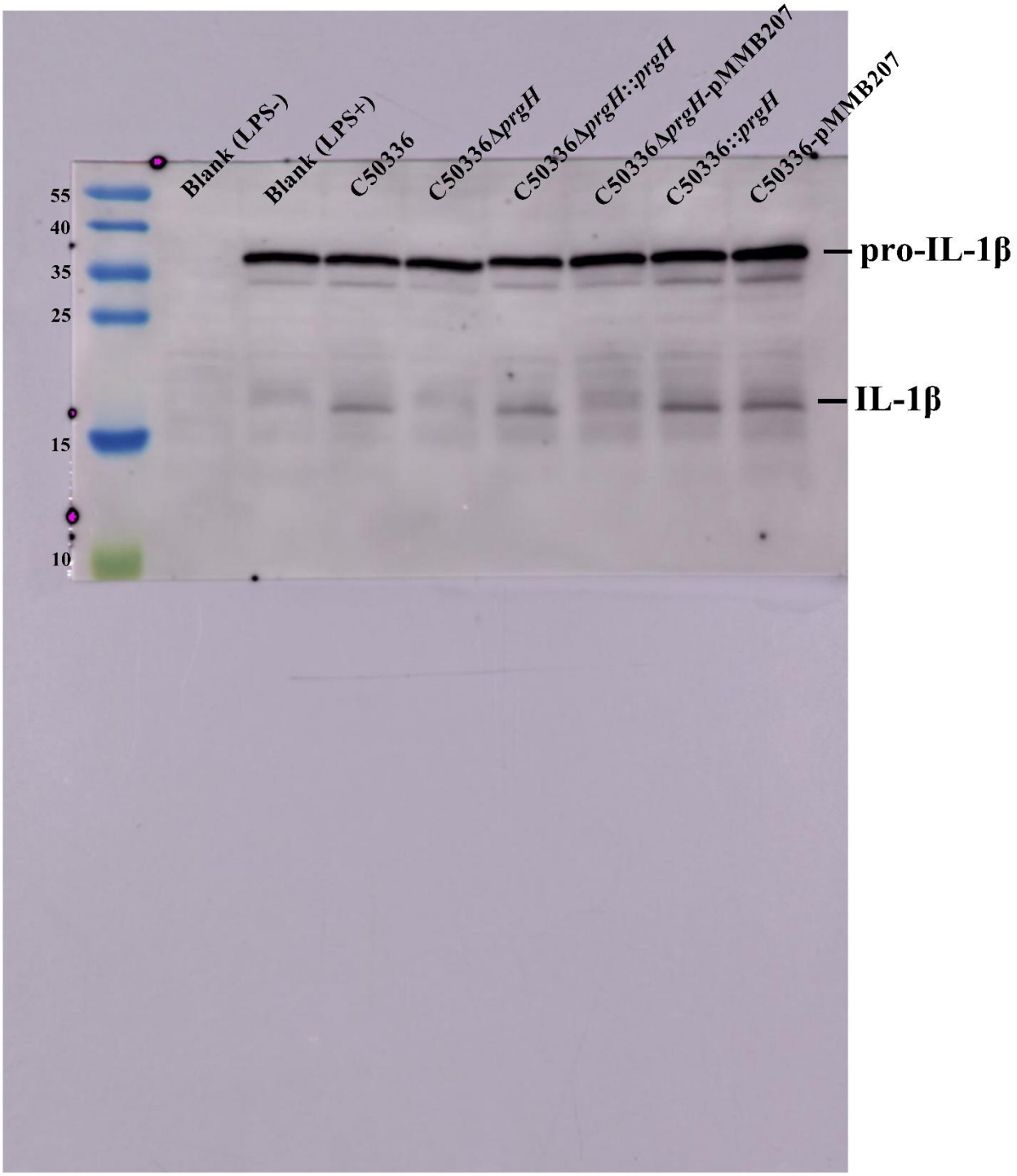
C50336::prgH
C50336-pMMB207
35
25
15
10
ASC

# Figure S4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C50336ΔprgH::prgH
C50336ΔprgH-pMMB207
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C50336-pMMB207
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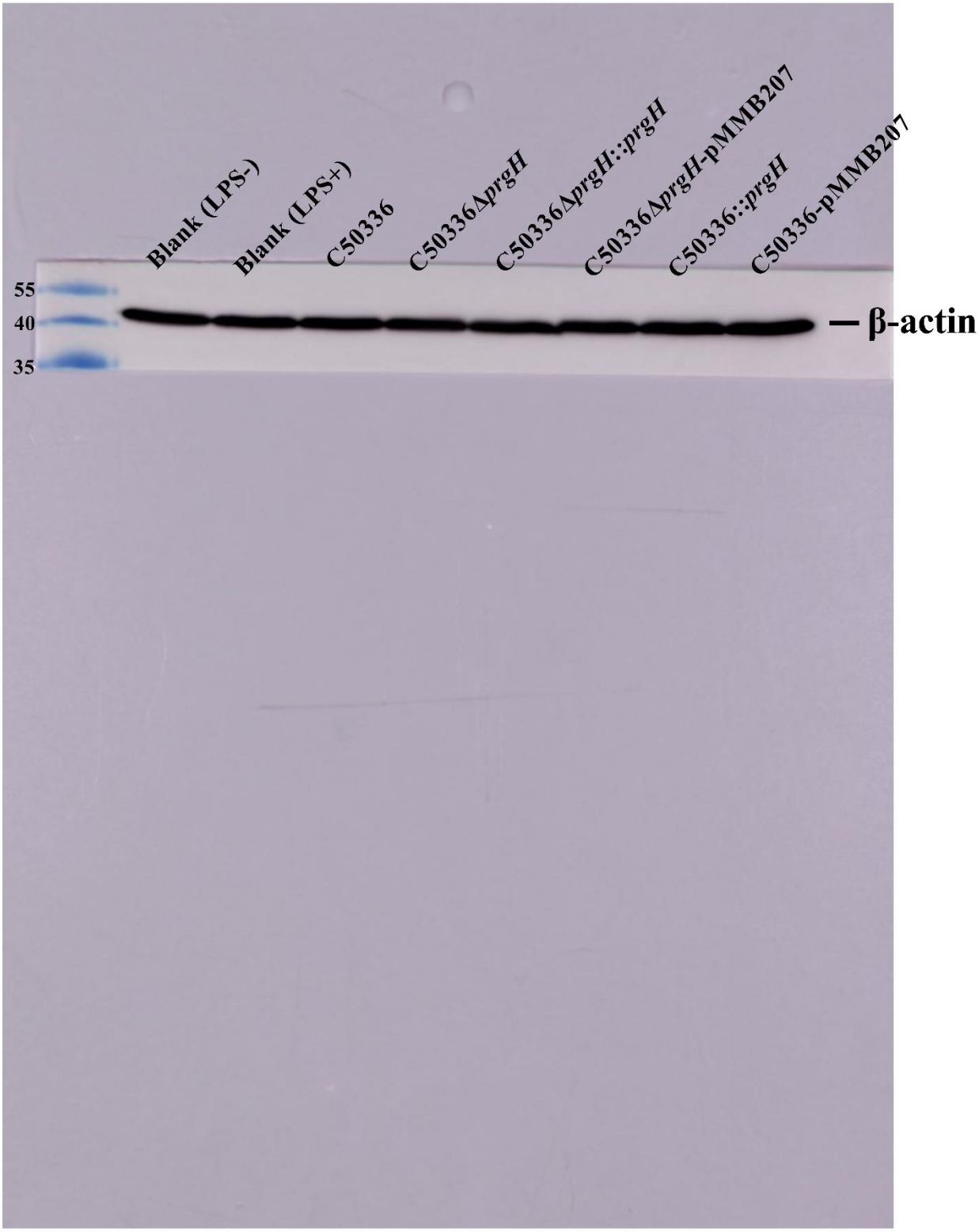
40
35
25
15
10
pro-IL-1β
IL-1β

# Figure S4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C50336ΔprgH::prgH
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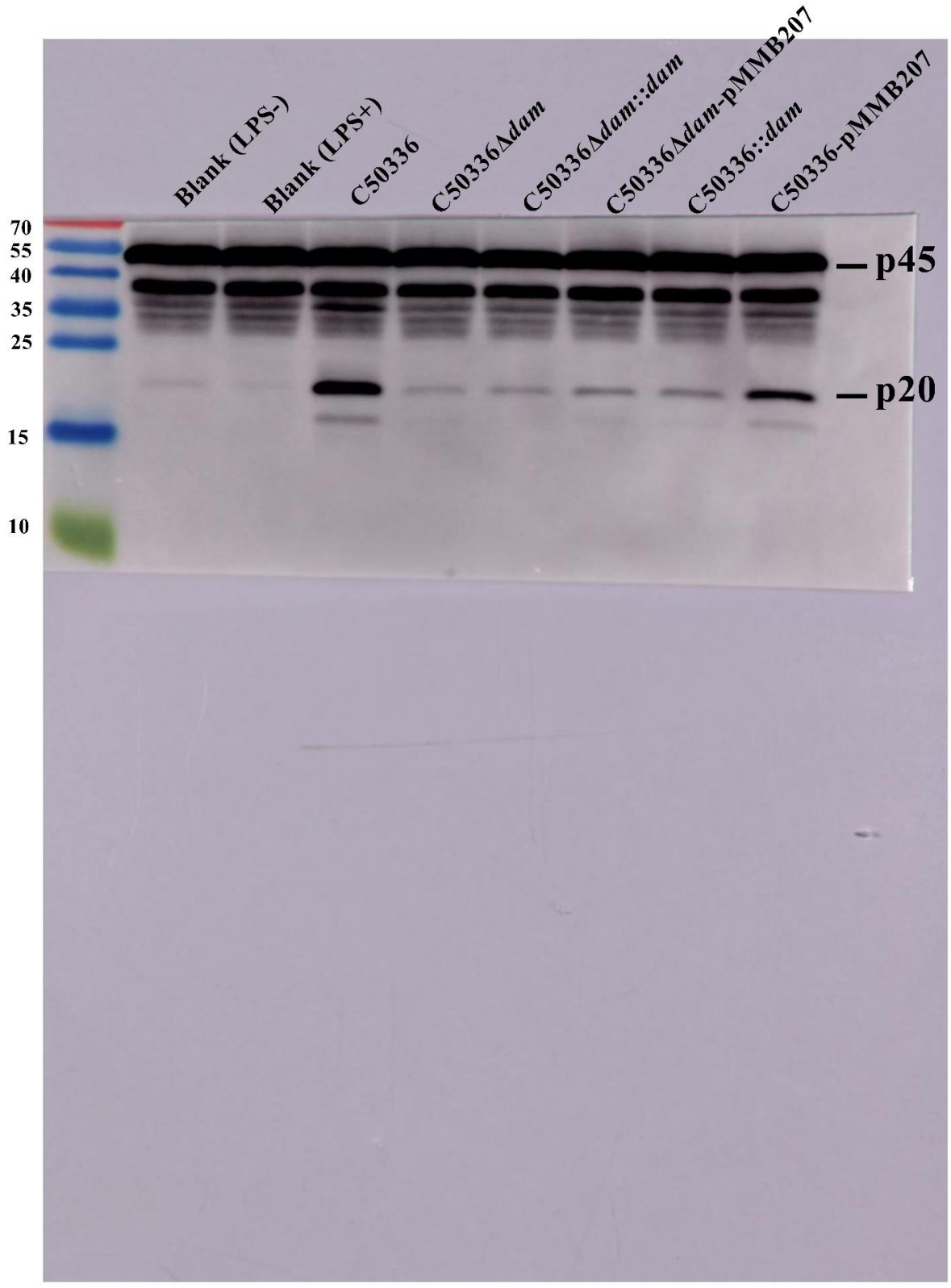
C50336::prgH
C50336-pMMB207
55
40
35
β-actin

# Figure S5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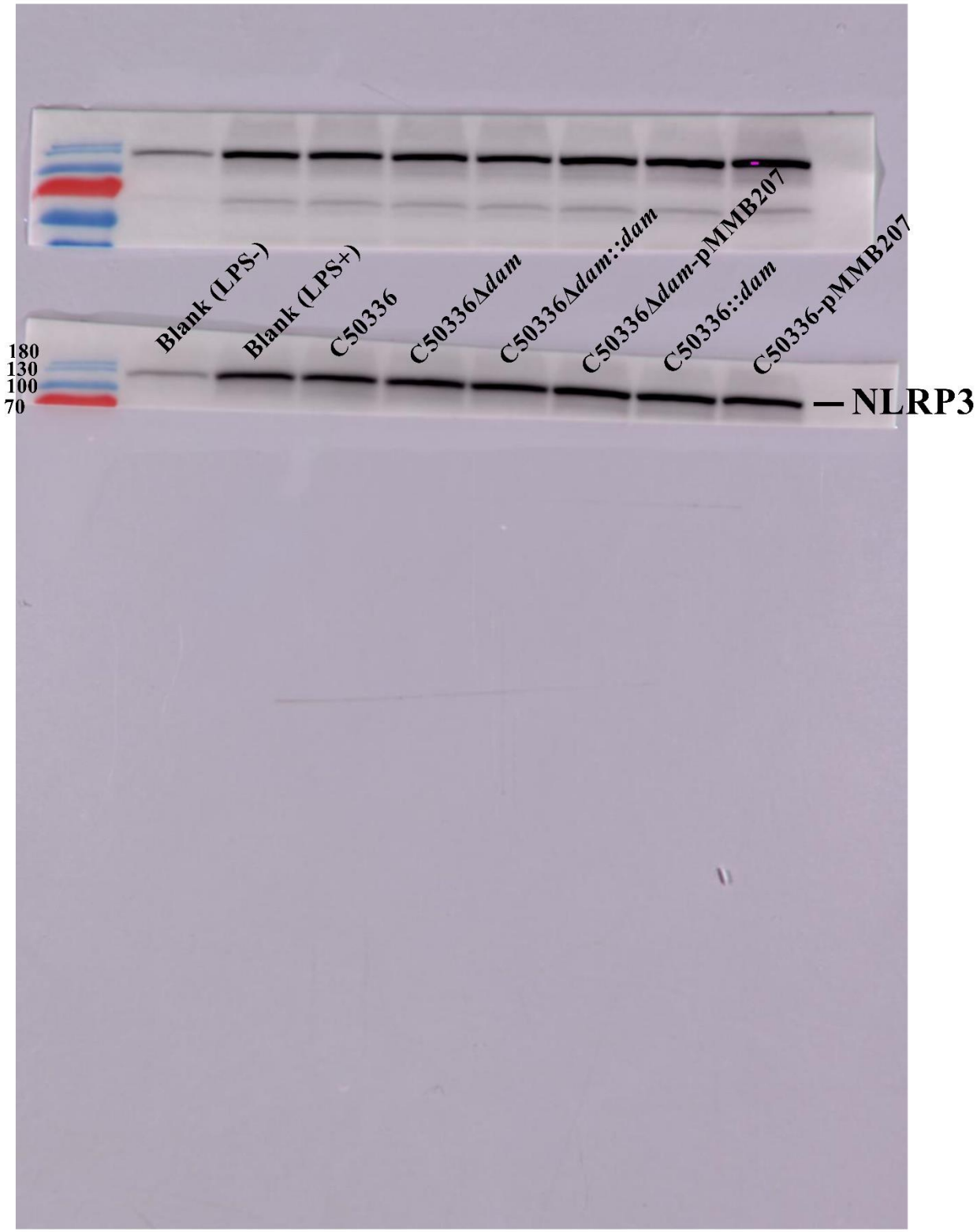
C50336Δdam
C50336Δdam::dam
C50336Δdam-pMMB207
C50336::dam
C50336-pMMB207
70
55
40
35
25
15
10
p45
p20

# Figure S5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Blank (LPS+)
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C50336Δdam
C50336Δdam::dam
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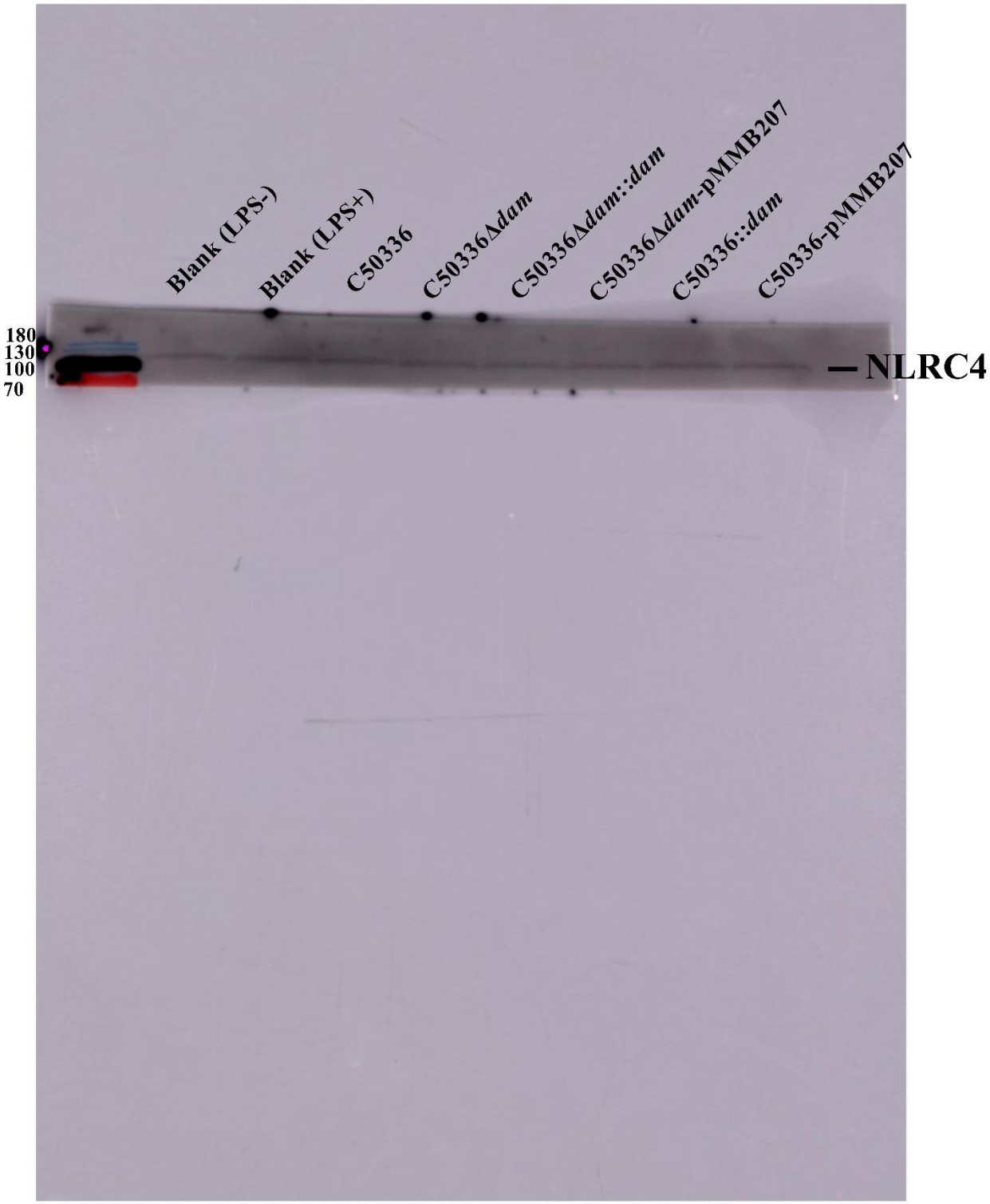
C50336::dam
C50336-pMMB207
180
130
100
70
NLRP3

# Figure S5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Blank (LPS+)
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C50336Δdam
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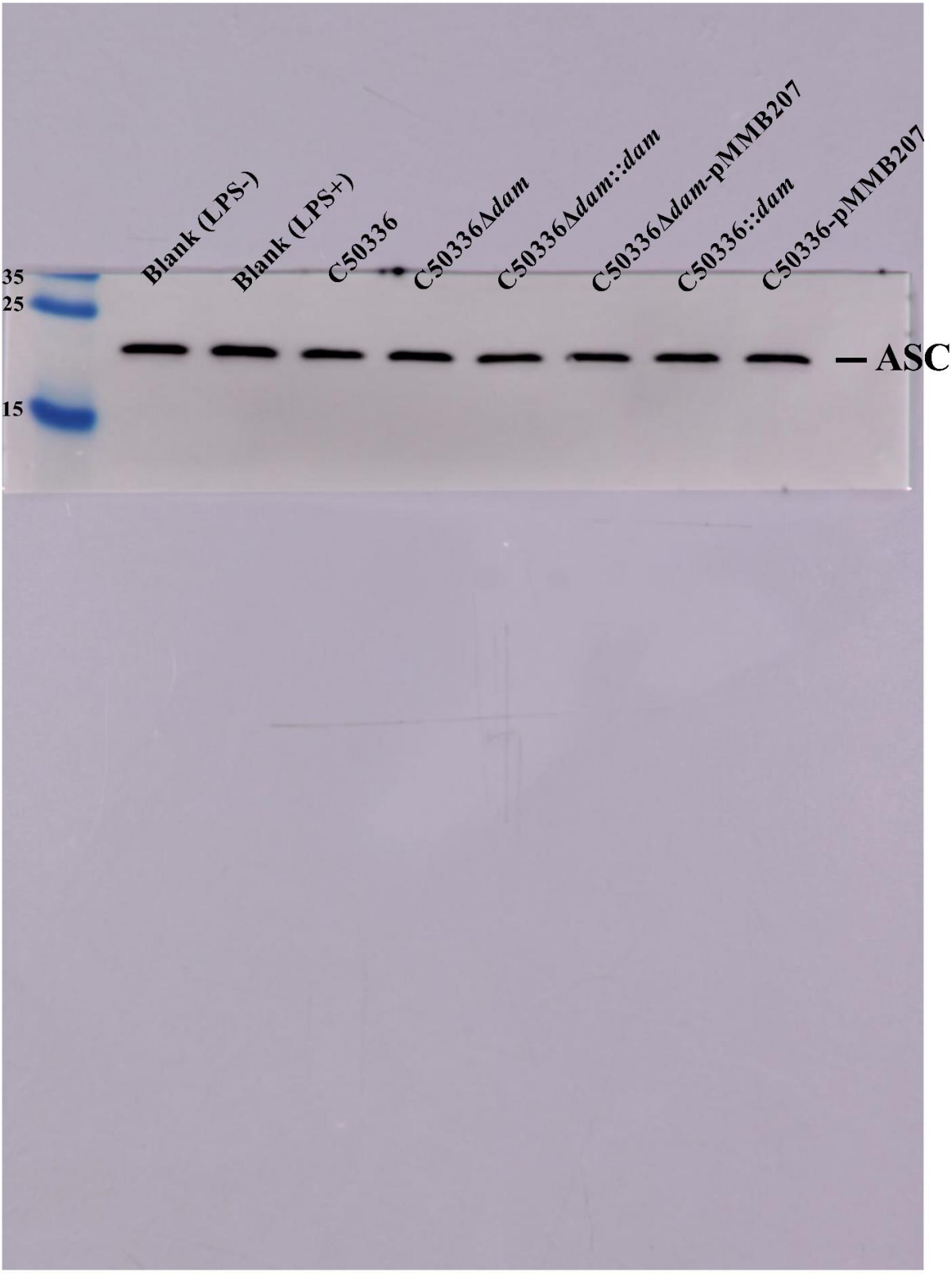
C50336Δdam-pMMB207
C50336::dam
C50336-pMMB207
180
130
100
70
NLRC4

# Figure S5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C50336Δdam-pMMB207
C50336::dam
C50336-pMMB207
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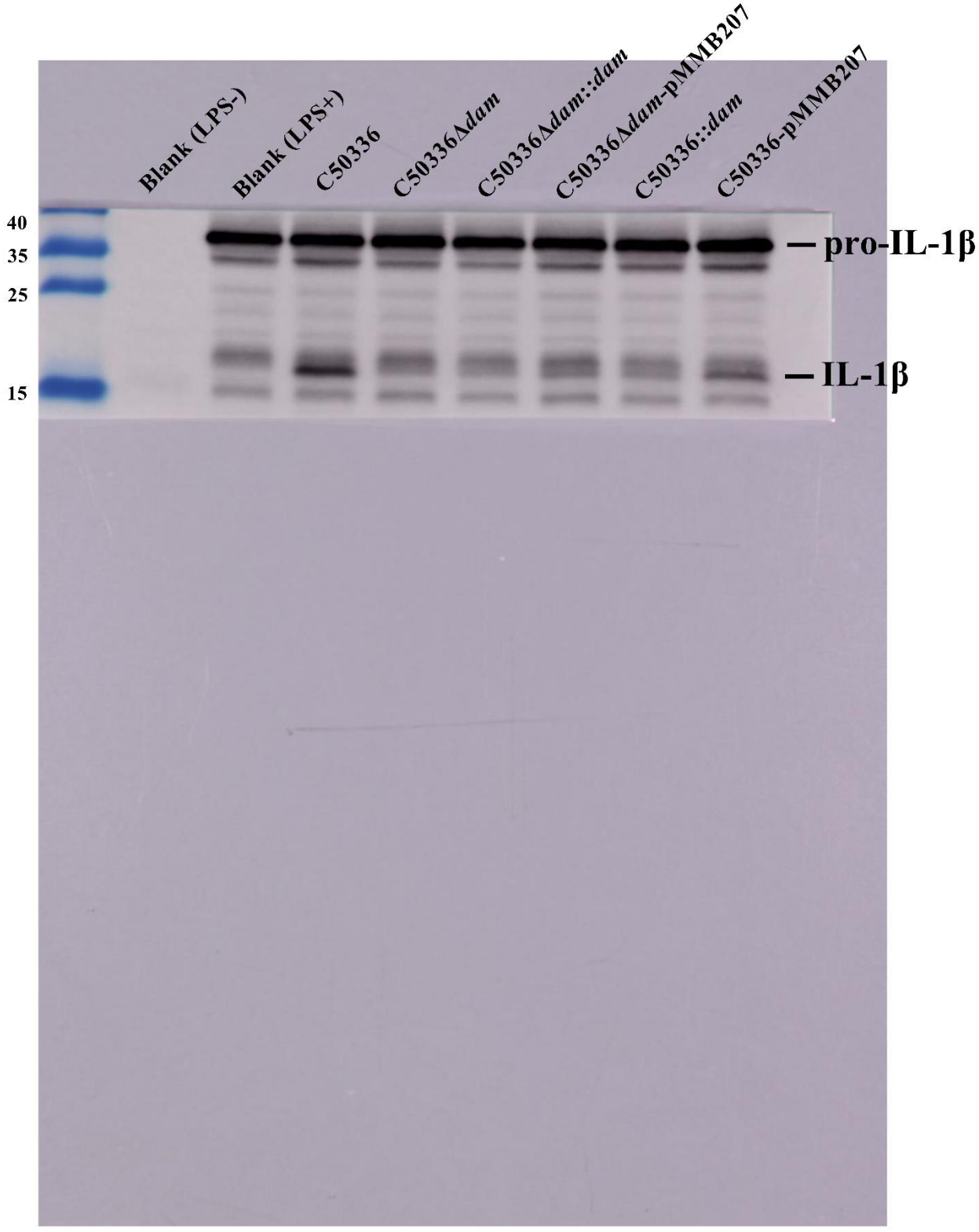
25
15
ASC

# Figure S5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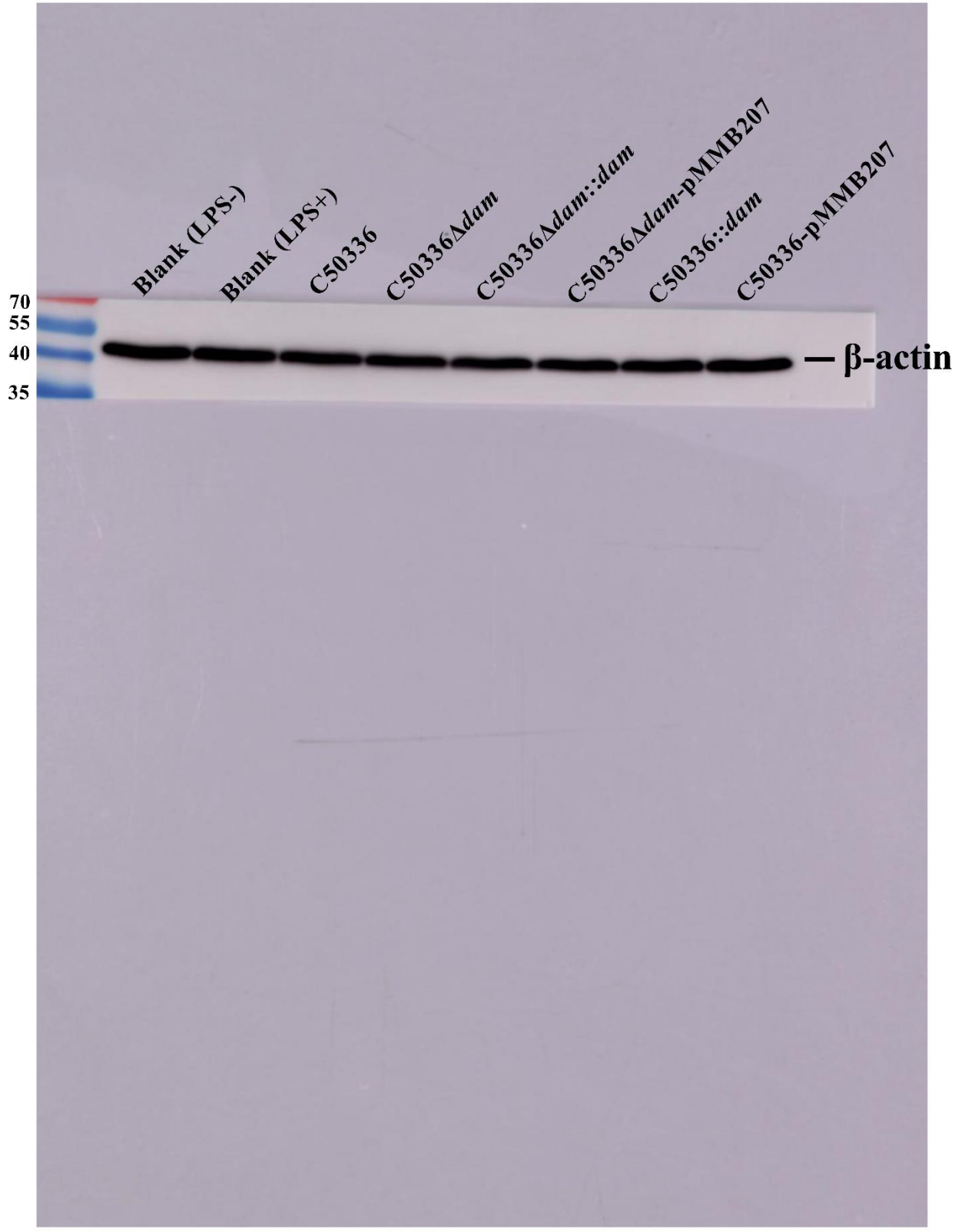
C50336::dam
C50336-pMMB207
40
35
25
15
pro-IL-1β
IL-1β

# Figure S5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C50336::dam
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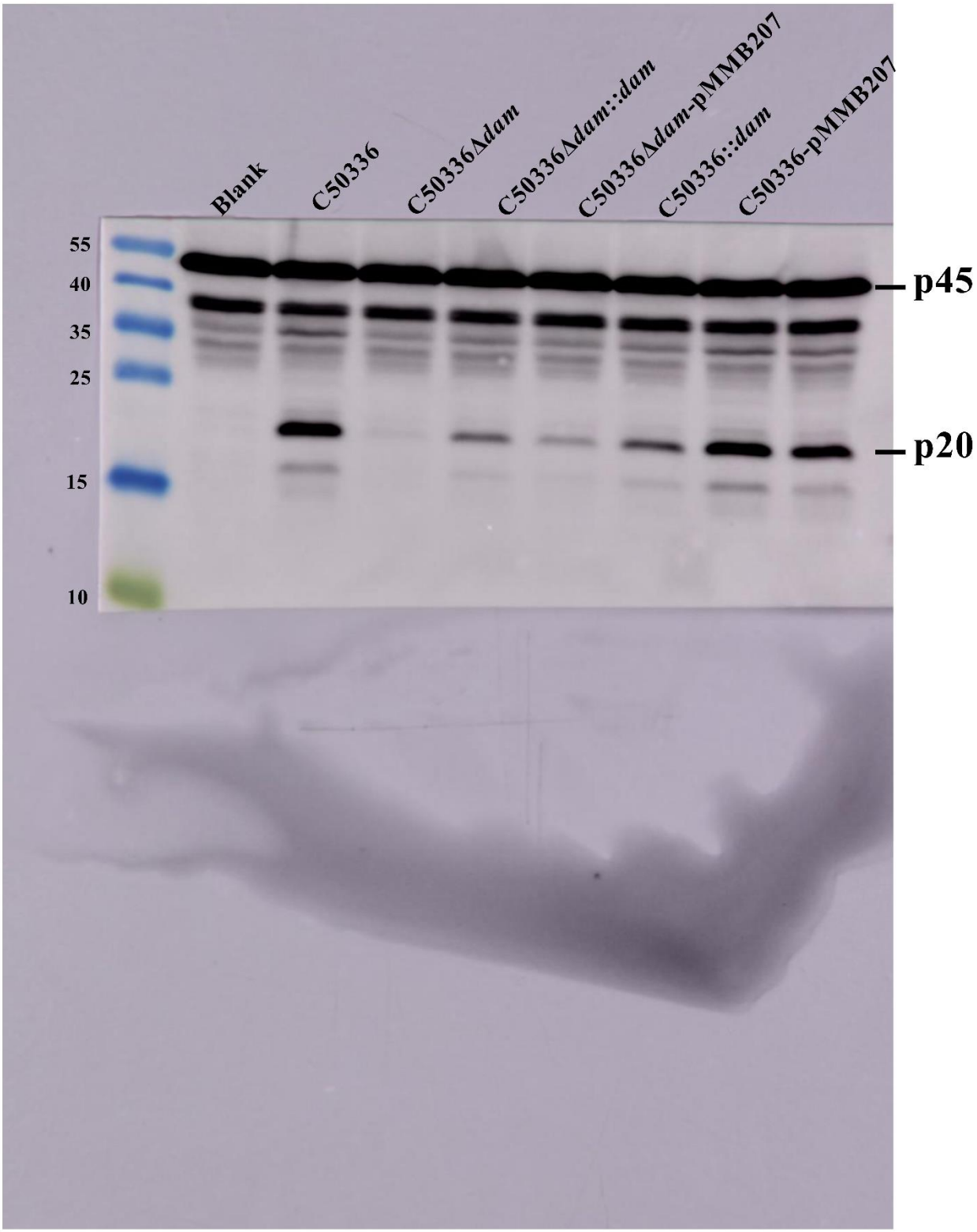
70
55
40
35
β-actin

# Figure S6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C50336-pMMB207
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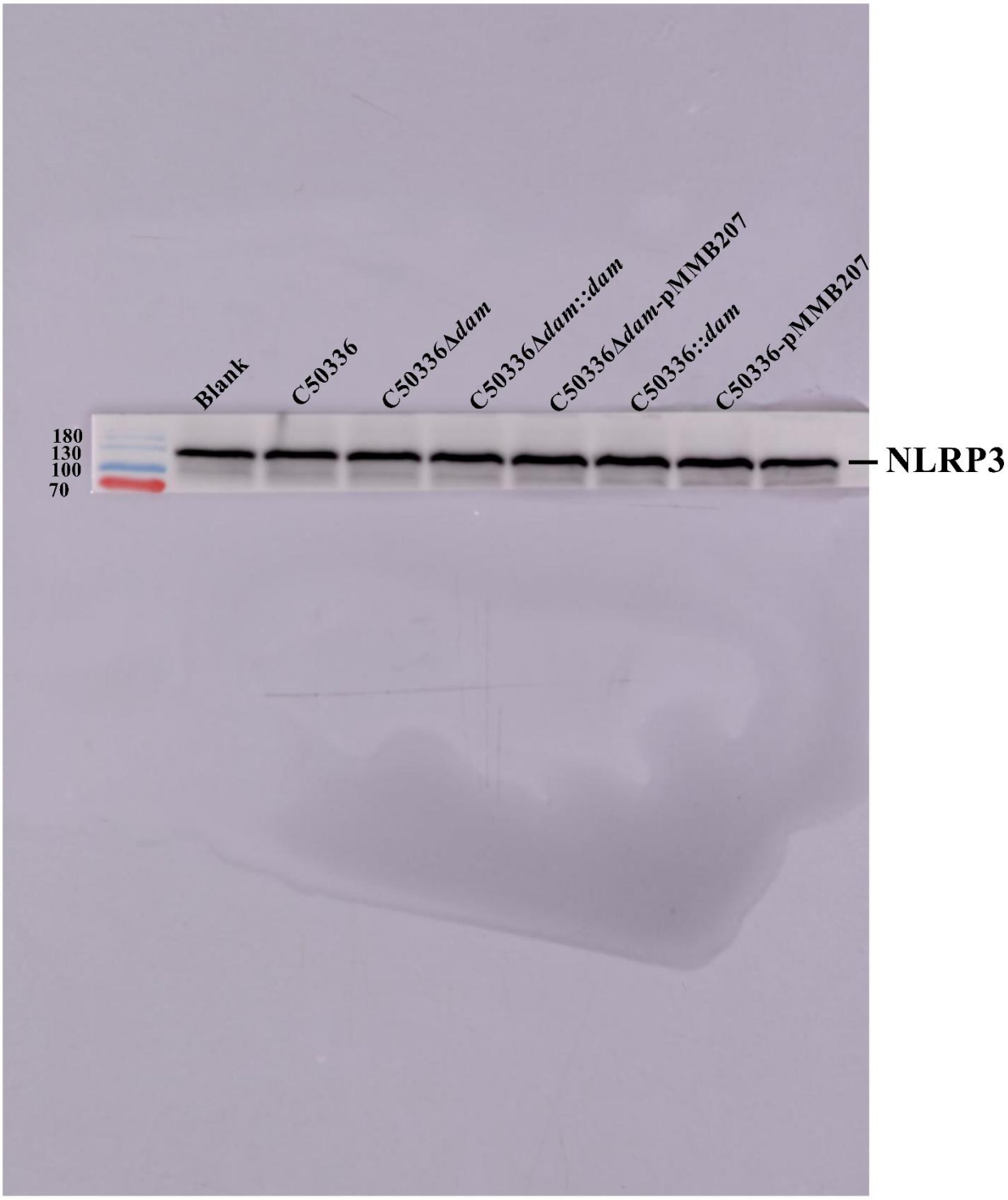
10
p45
p20

# Figure S6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C50336Δdam
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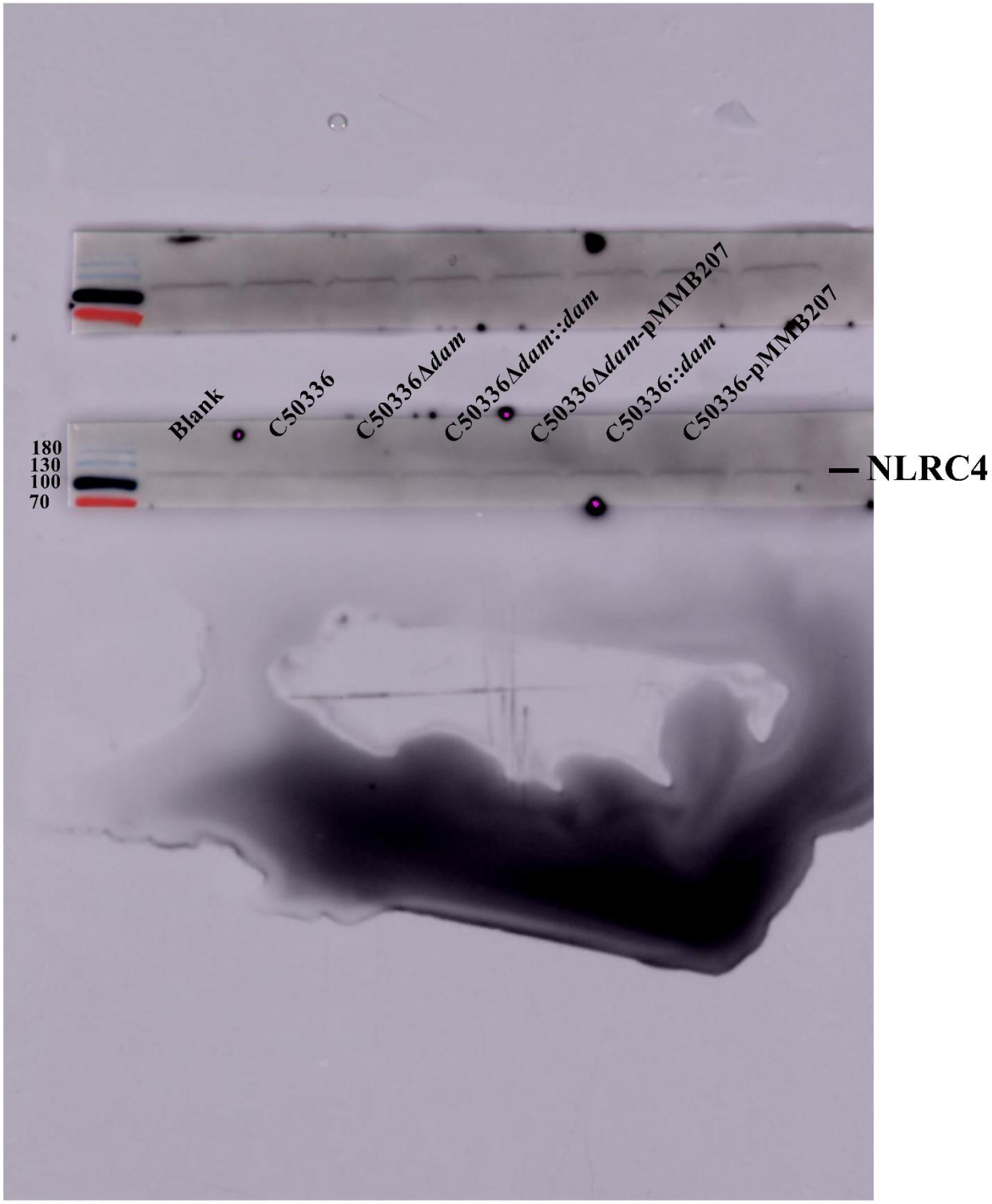
C50336::dam
C50336-pMMB207
180
130
100
70
NLRP3

# Figure S6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C50336::dam
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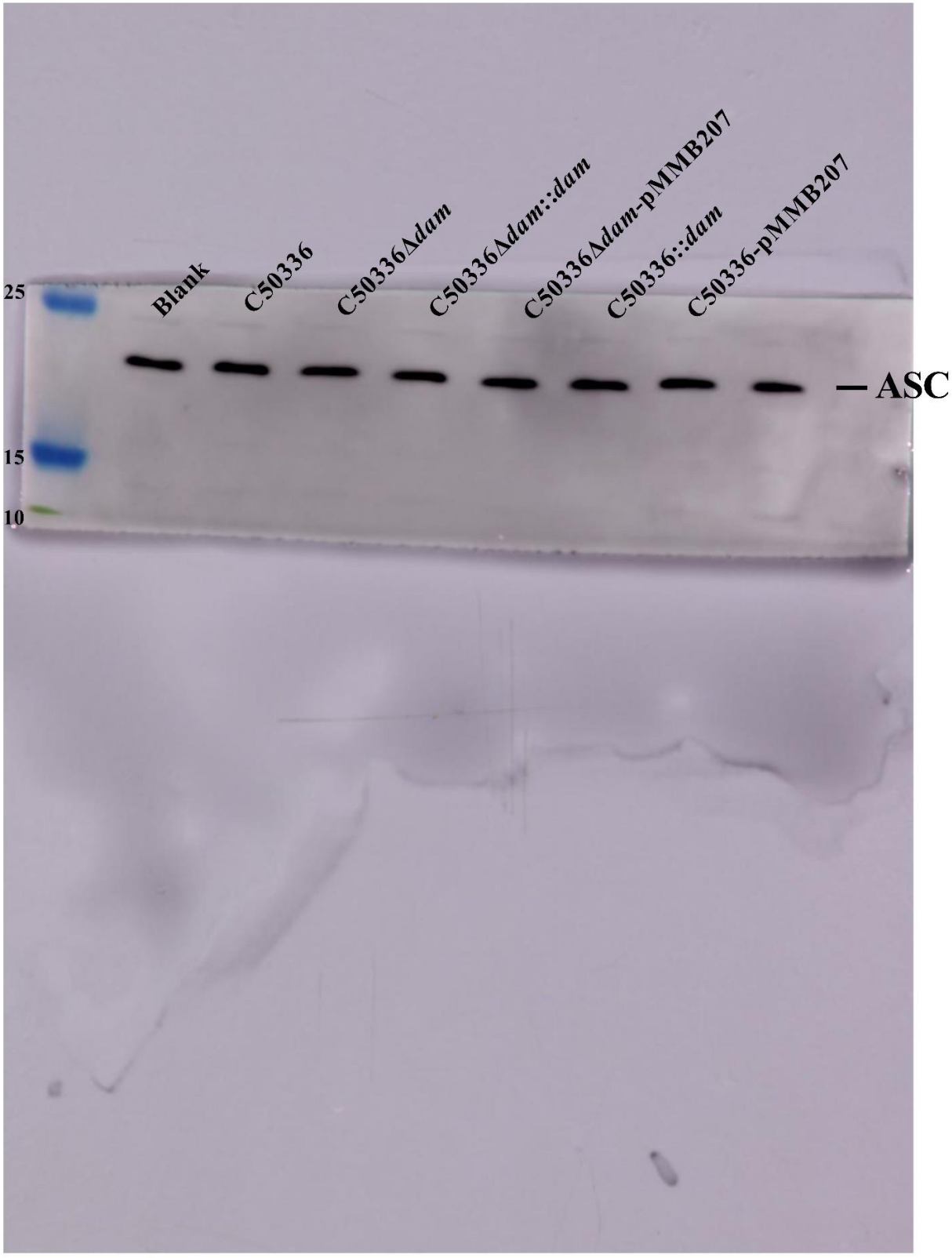
100
70
NLRC4

# Figure S6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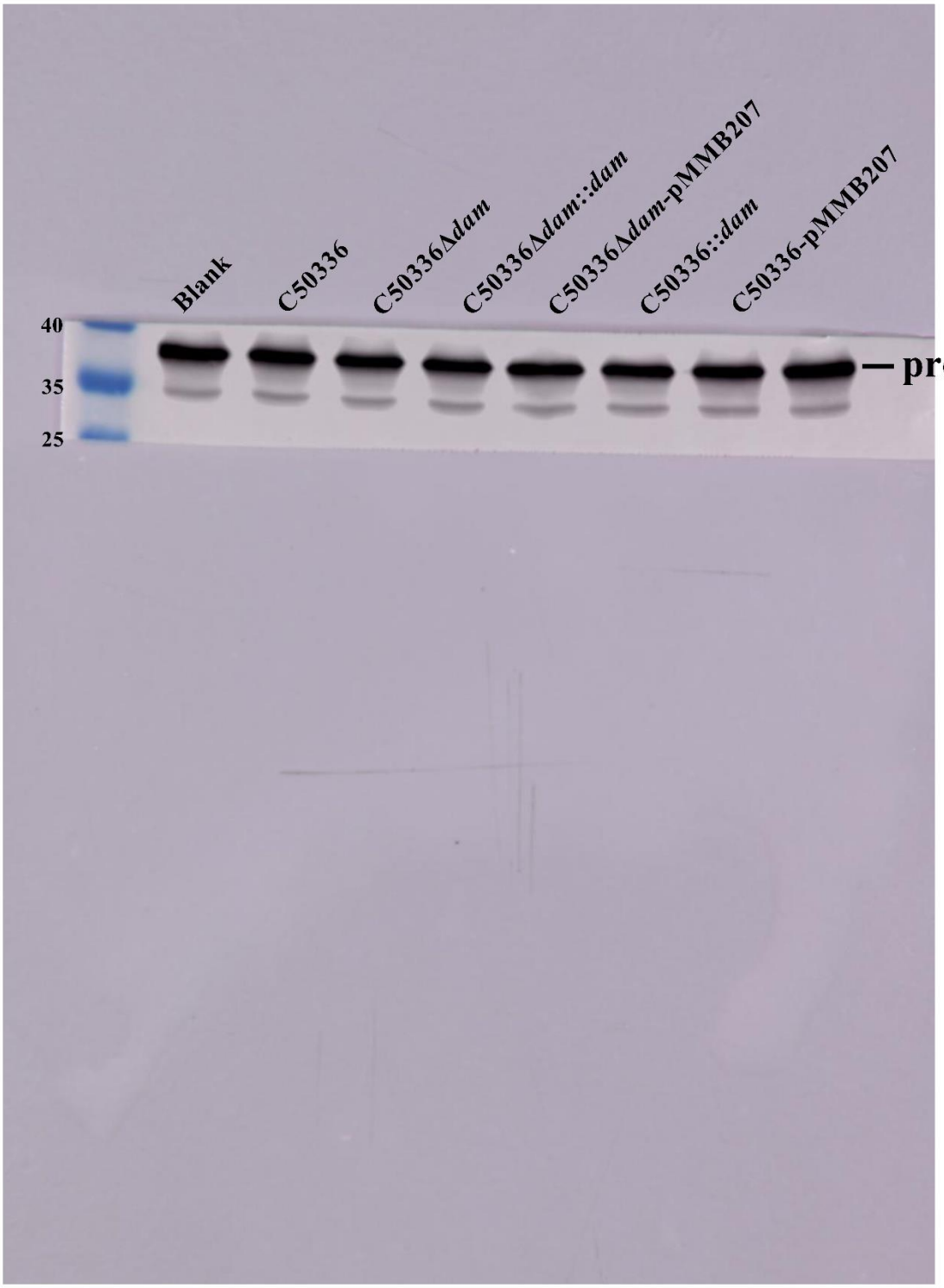
C50336-pMMB207
25
15
10
ASC

# Figure S6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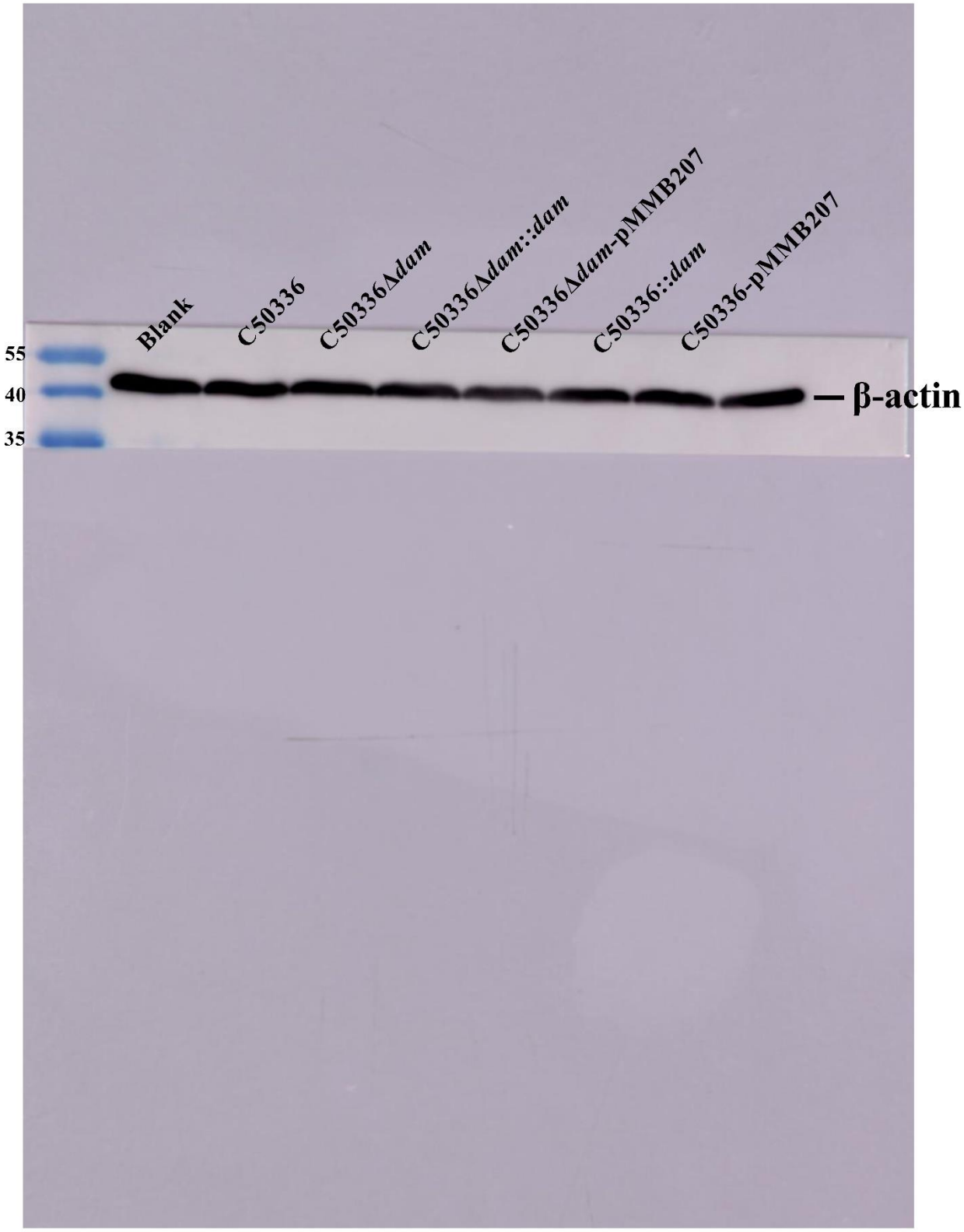
35
25
pro-IL-1β

# Figure S6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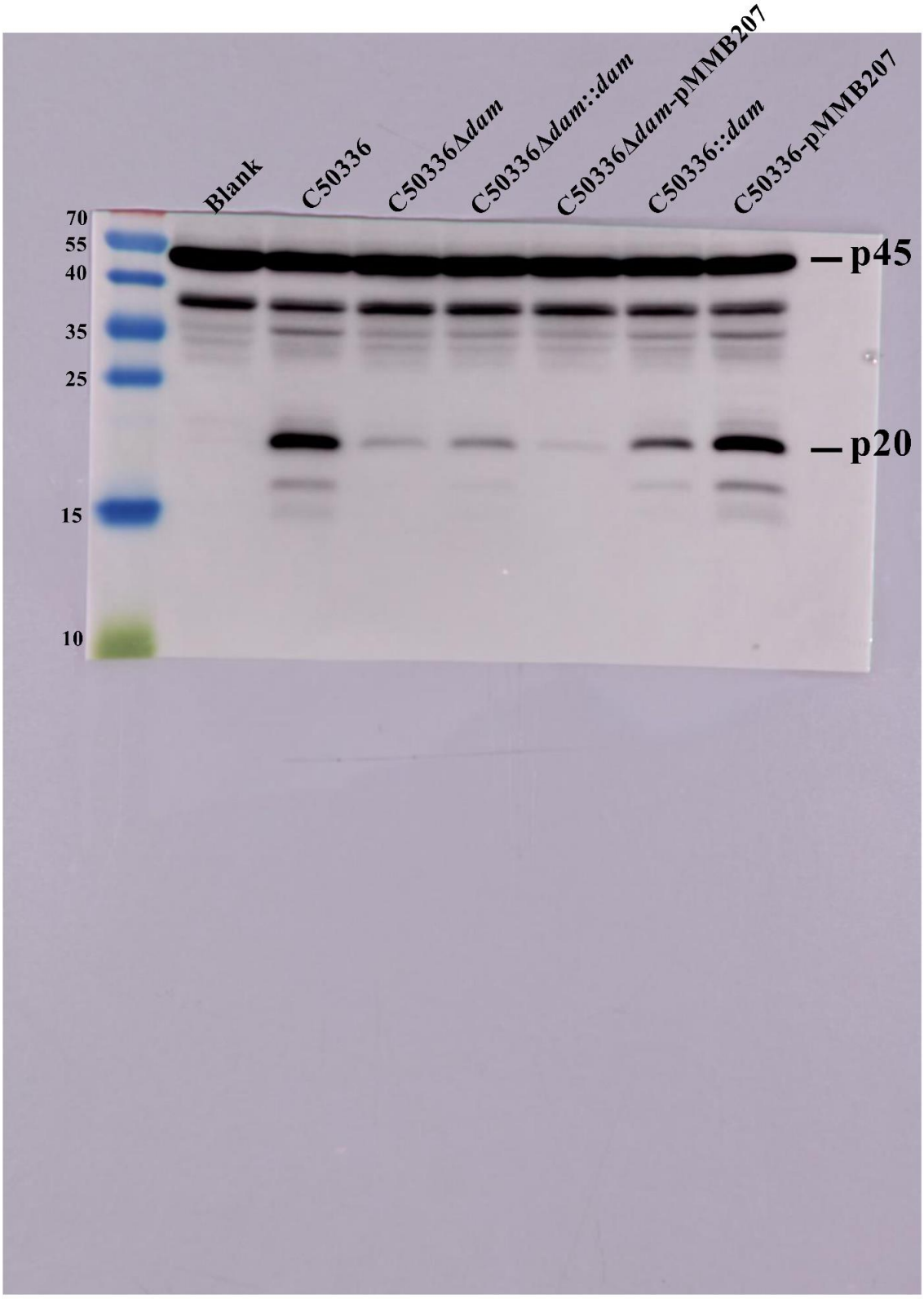
40
35
β-actin

# Figure S7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35
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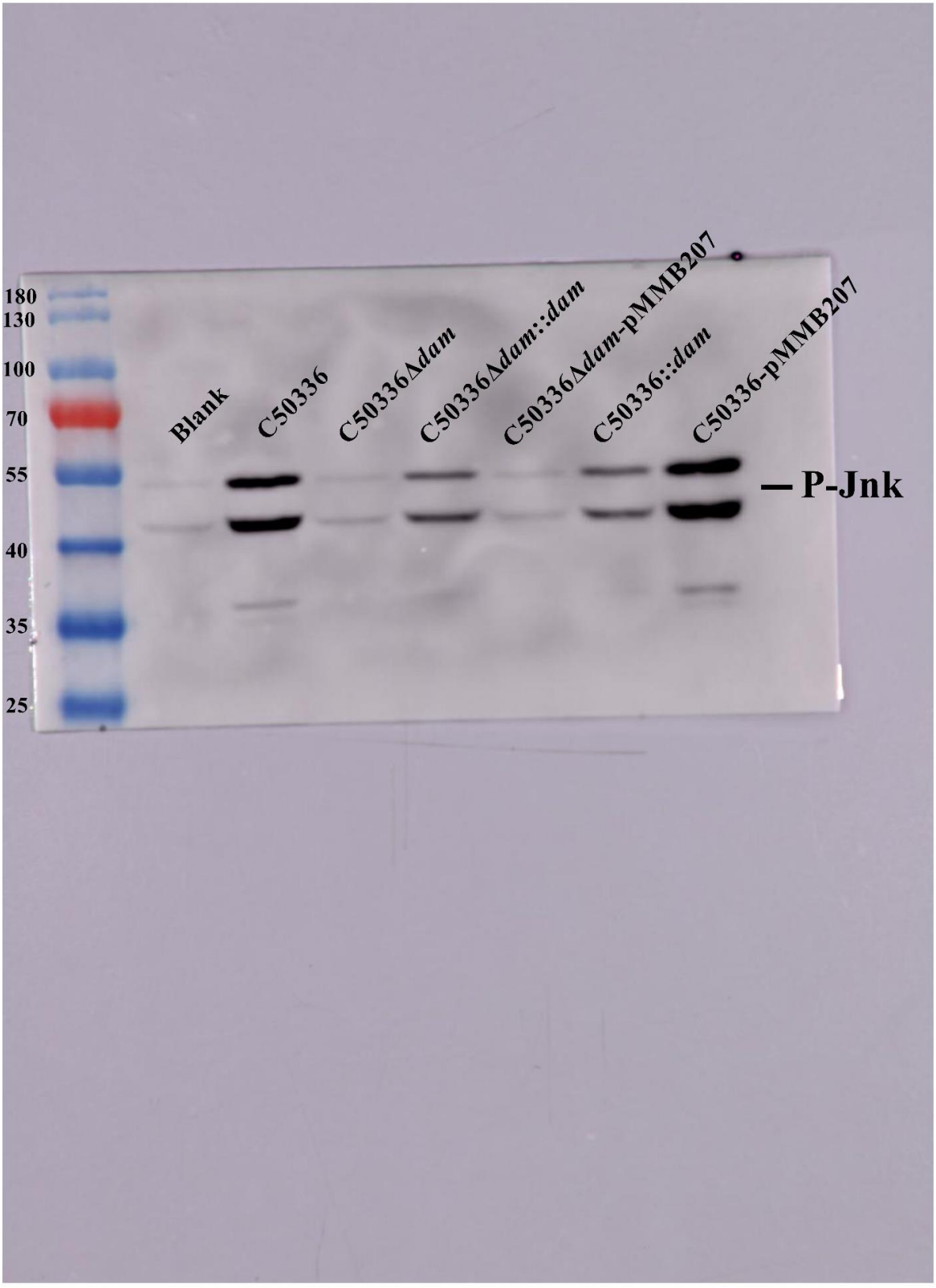
15
10
p45
p20

# Figure S7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100
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C50336::dam
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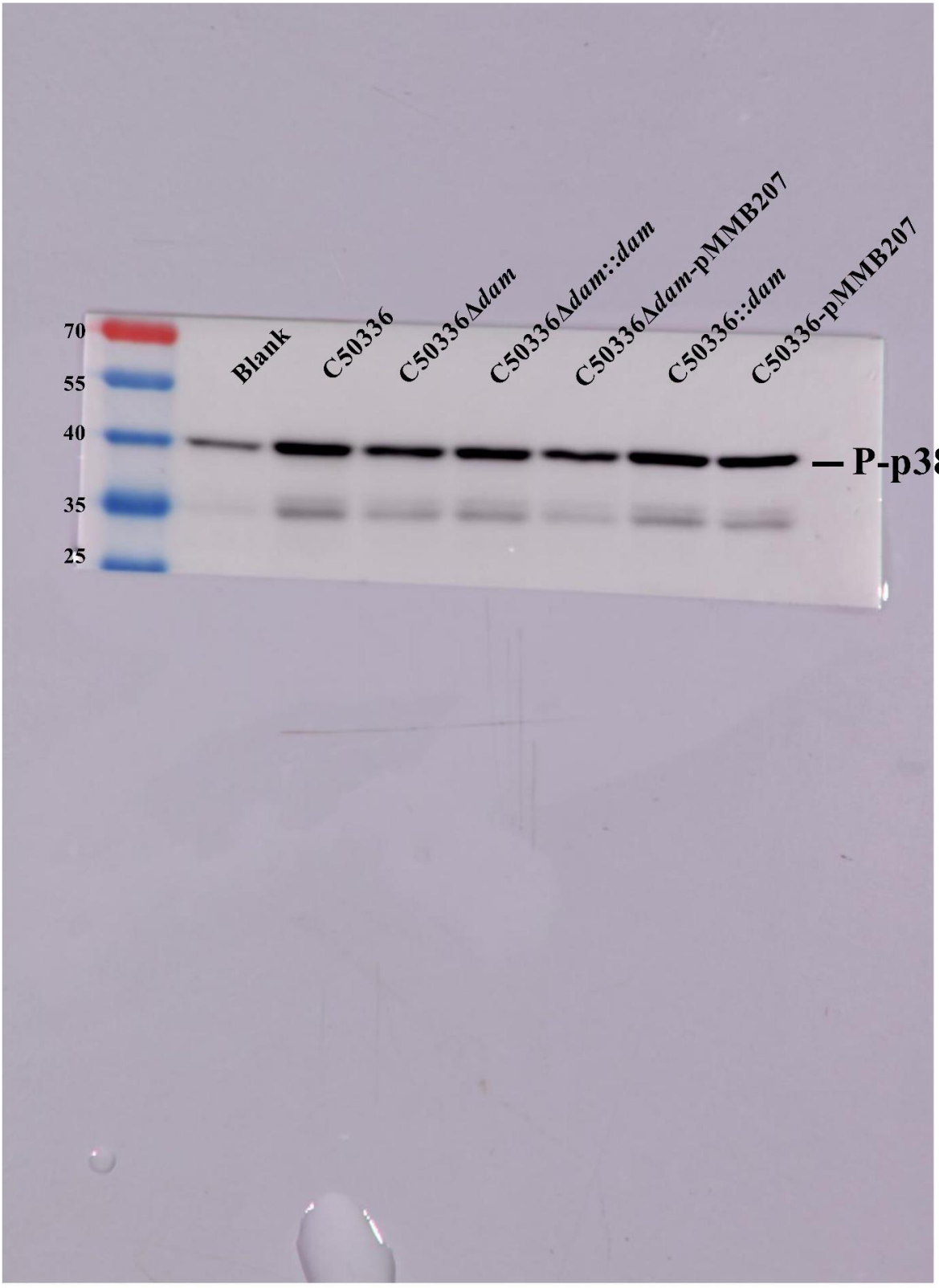
— P-Jnk

# Figure S7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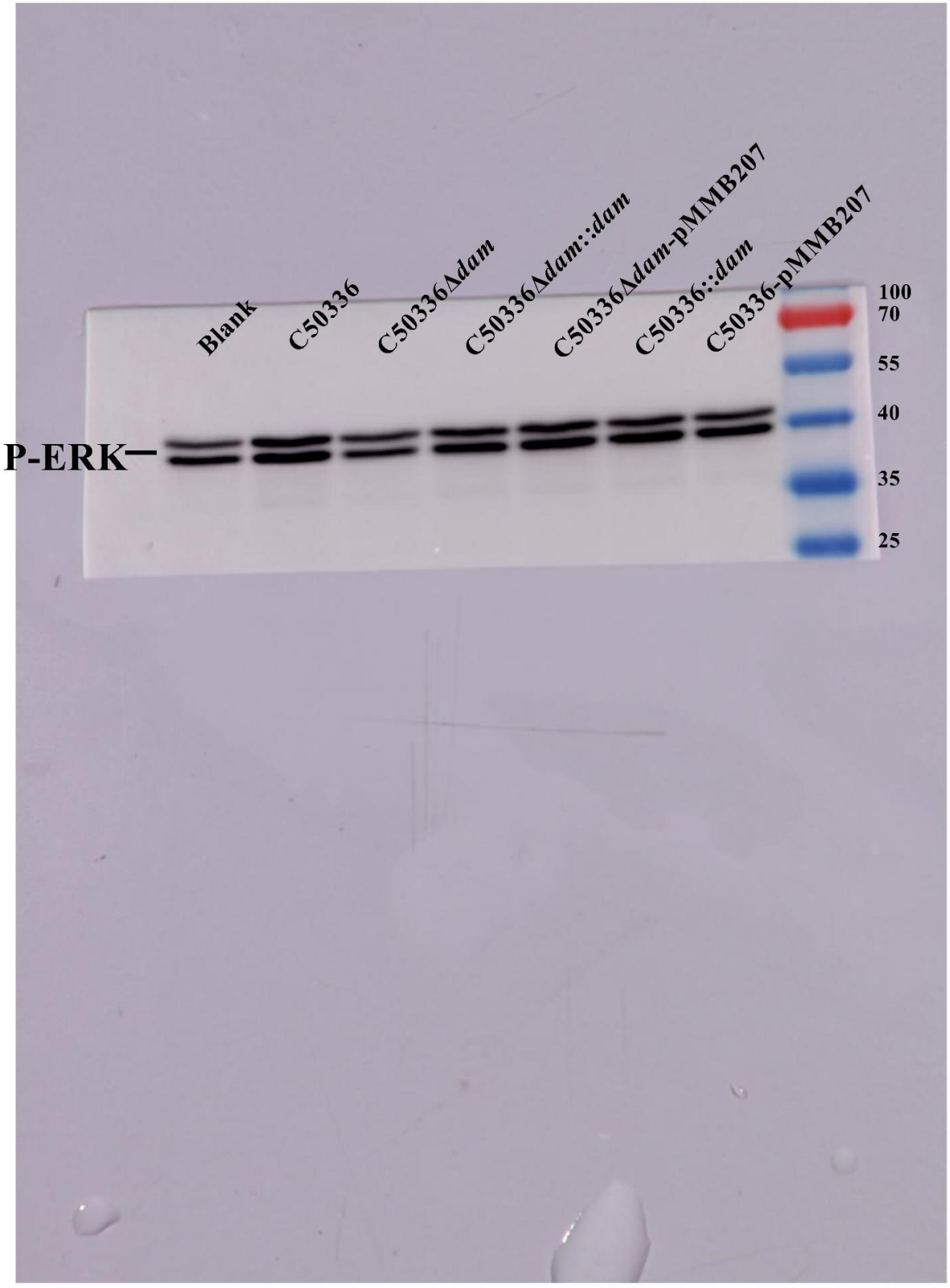
P-p38

# Figure S7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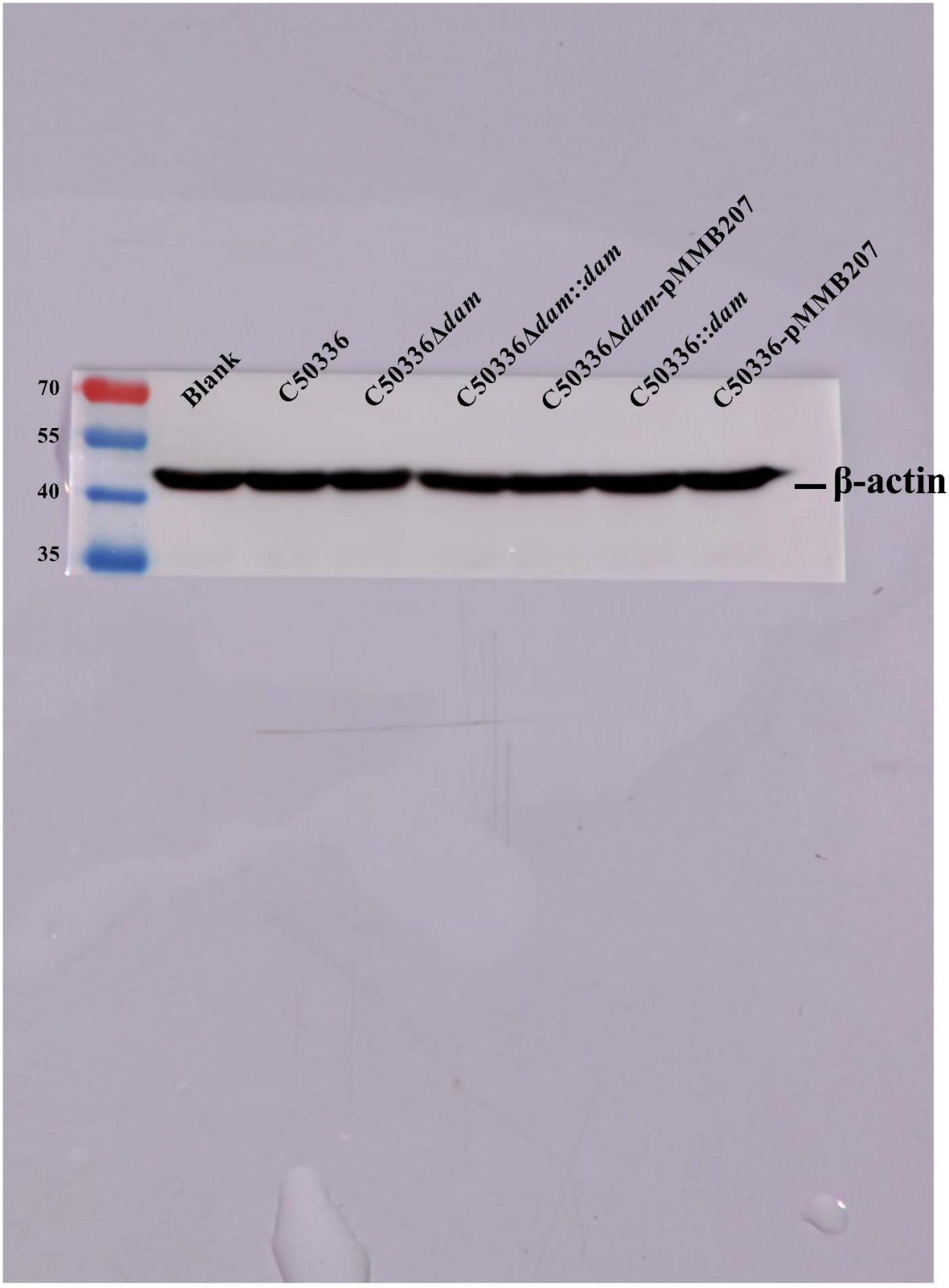
25

# Figure S7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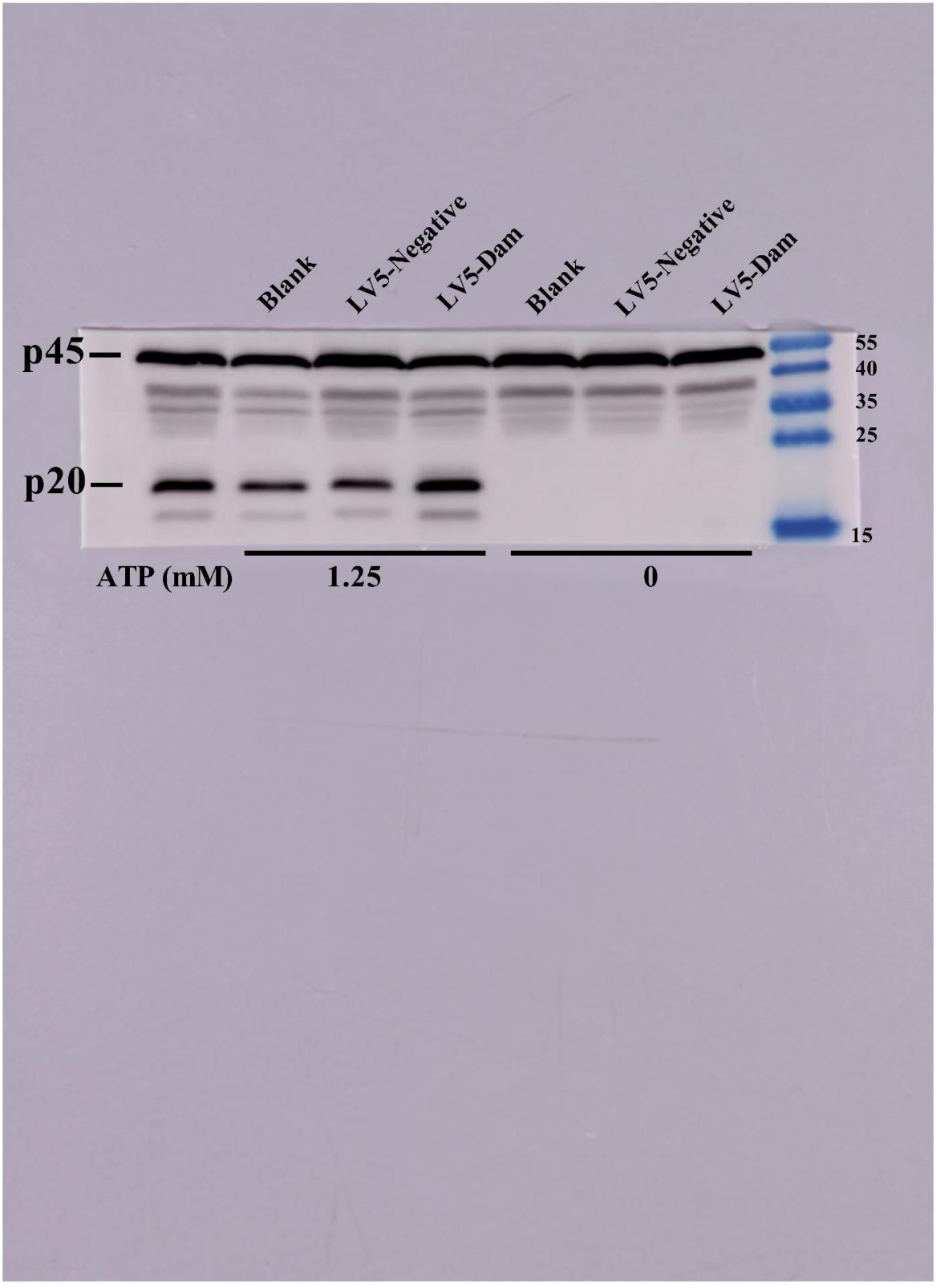
35
β-actin

# Figure S8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LV5-Dam
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LV5-Dam
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p20
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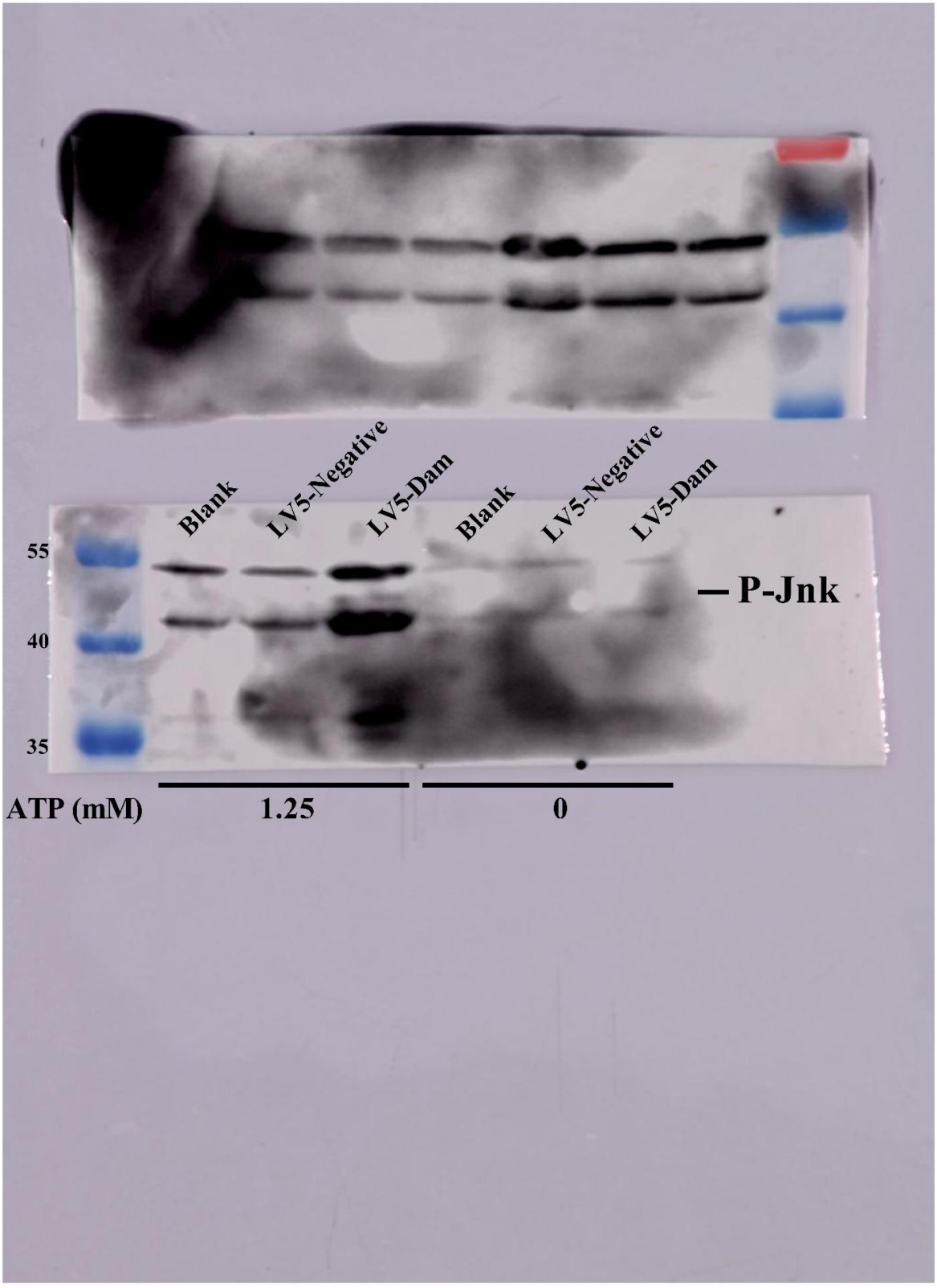
35
25
15
ATP (mM)
1.25
0

# Figure S8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LV5-Negative
LV5-Dam
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LV5-Negative
LV5-Dam
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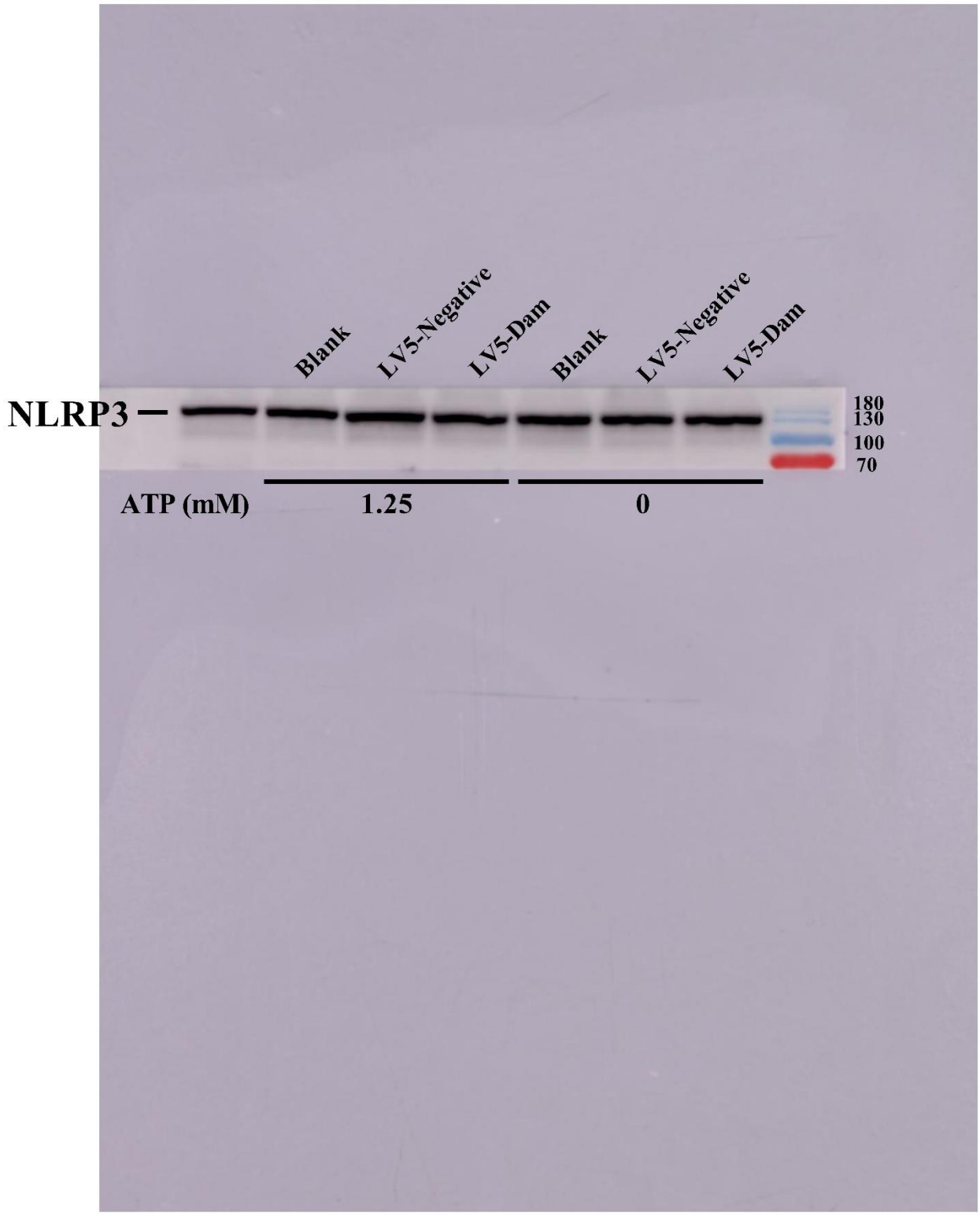
40
35
— P-Jnk
ATP (mM)
1.25
0

# Figure S8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LV5-Negative
LV5-Dam
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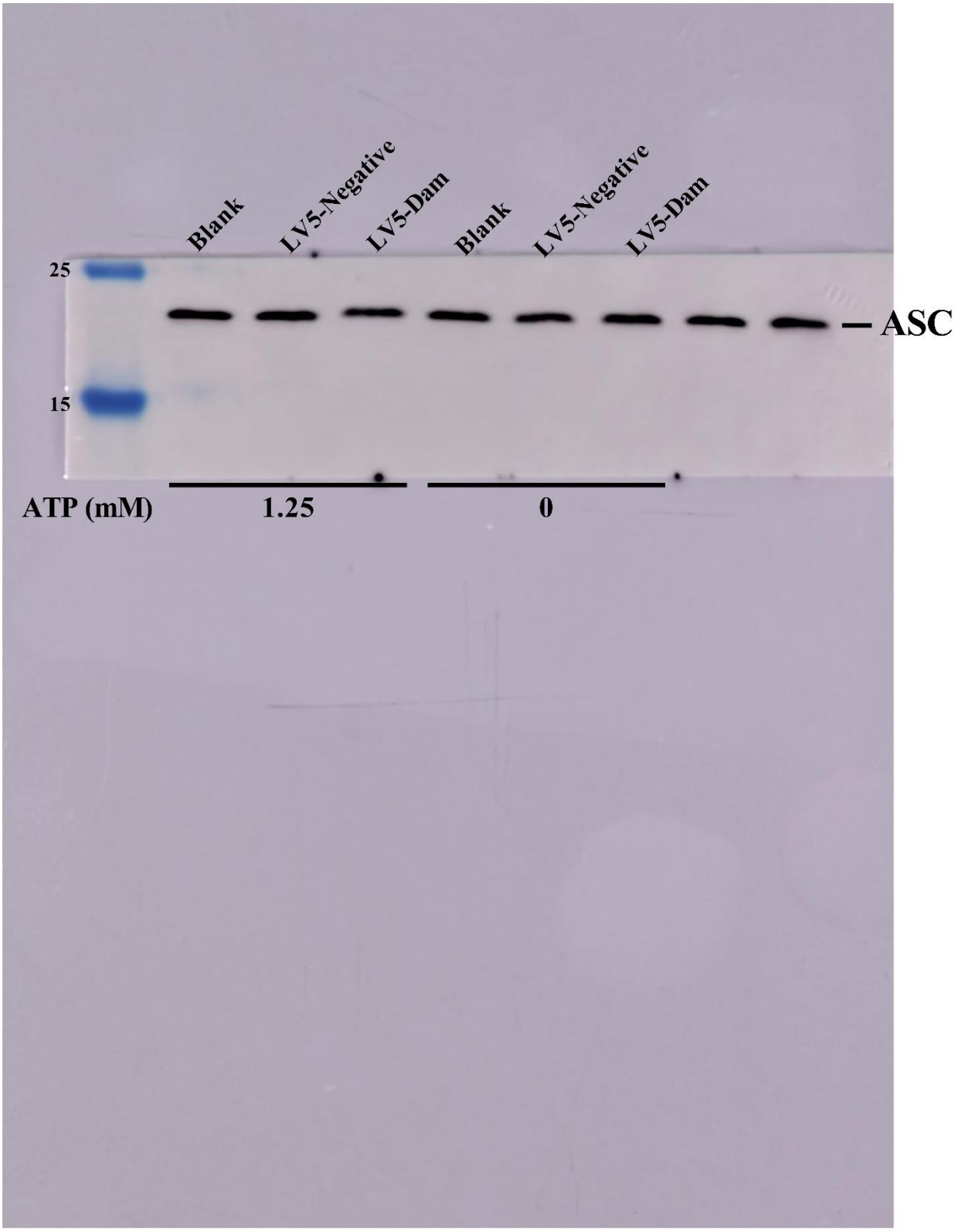
LV5-Negative
LV5-Dam
NLRP3
180
130
100
70
ATP (mM)
1.25
0

# Figure S8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LV5-Dam
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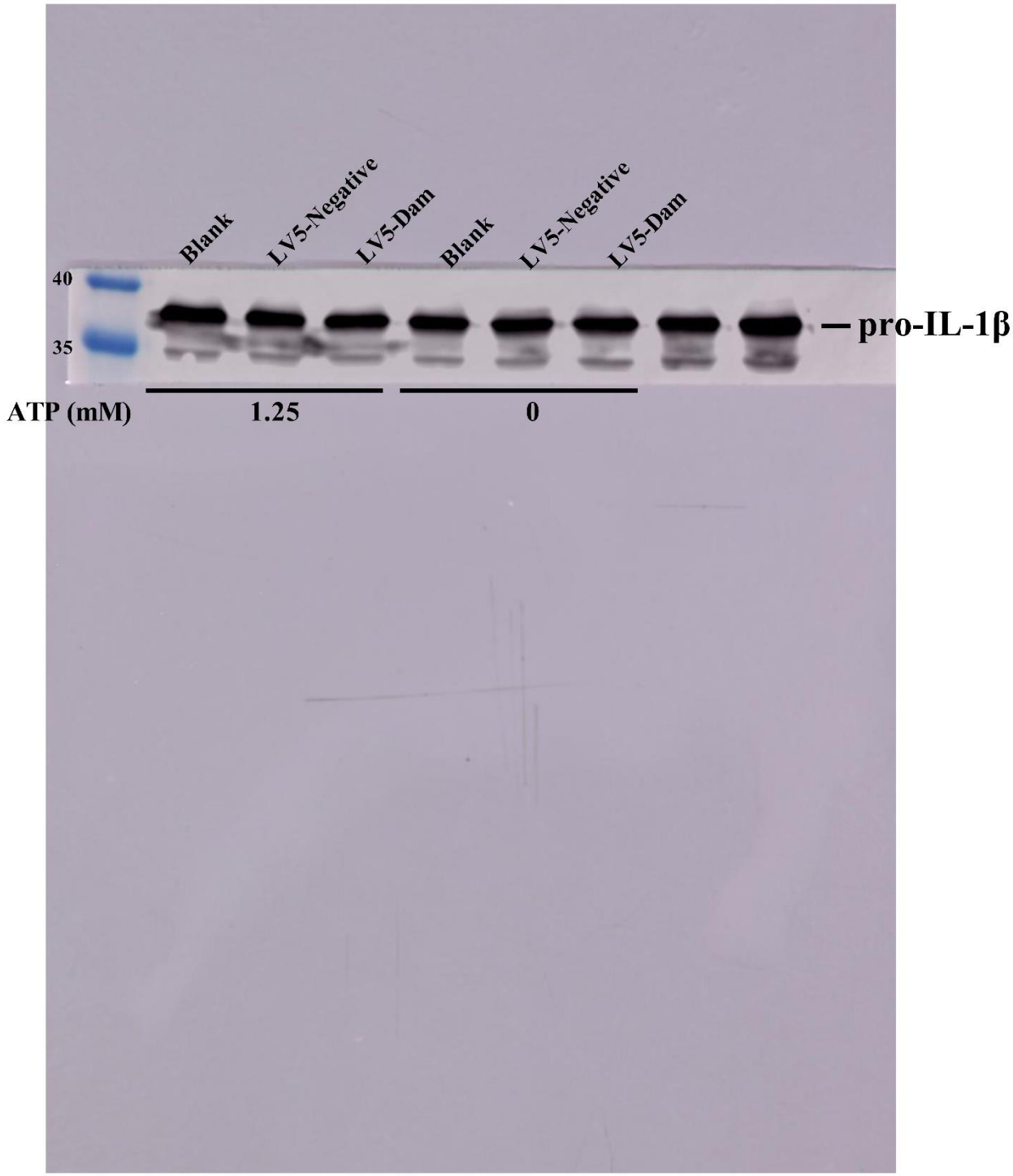
LV5-Negative
LV5-Dam
25
15
ASC
ATP (mM)
1.25
0

# Figure S8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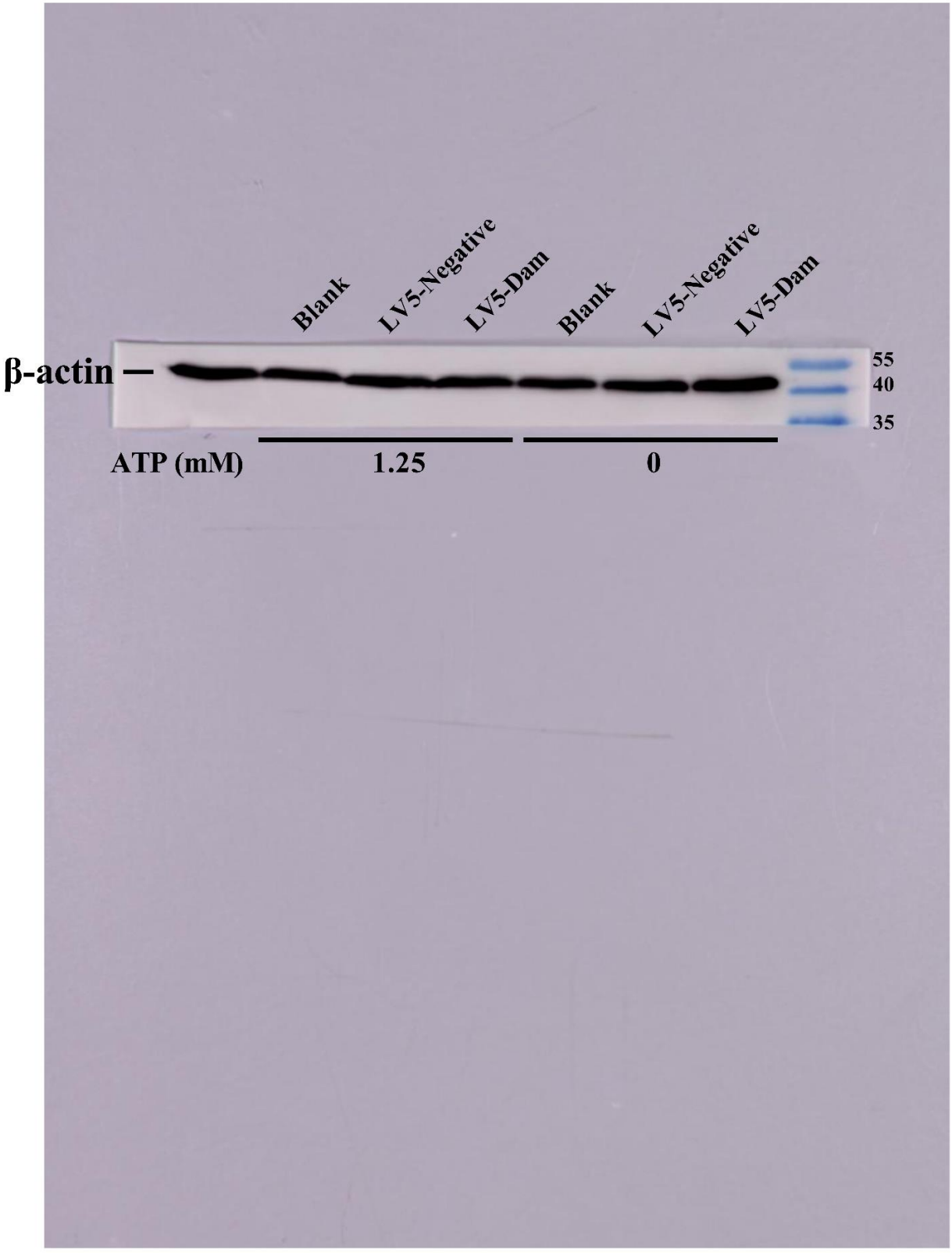
LV5-Negative
LV5-Dam
Blank
LV5-Negative
LV5-Dam
40
35
pro-IL-1β
ATP (mM)
1.25
0

# Figure S8


Blank
LV5-Negative
LV5-Dam
Blank
LV5-Negative
LV5-Dam
β-actin
55
40
35
ATP (mM)
1.25
0

**Supplemental figure legends**

**Fig. S1** Growth curves of *dam* (**a**), *invC* (**b**), *hilD* (**c**), *prgH* (**d**), and *spaN* (**e**) gene deletion mutants, complementation, and overexpression strains. Bacteria were grown in liquid LB medium at 37 ℃ for 12 h with agitation, and the $OD_{600}$ values of triplicate cultures in LB medium were determined in 1-h intervals.

**Fig. S2** Two rounds of screening to identify the genes involved in regulating inflammasome activation *in vitro*. J774A.1 cells were pre-treated with LPS (1 μg/mL, 5 h) and then infected with candidate transposon mutants at an MOI of 20 for 4 h, uninfected cells was used as a negative control (Blank). The activation of caspase-1 (p20) was examined via western blot. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.

**Fig. S3** Deletion mutants of *dam*, *invC*, *prgH*, and *spaN* failed to induce inflammasome activation. J774A.1 cells were pre-treated with LPS (1 μg/mL, 5 h) and then infected with WT strain C50336 and *dam*, *invC*, *hilD*, *prgH*, and *spaN* gene deletion mutants at an MOI of 20 for 4 h, uninfected cells was used as a negative control (Blank). The activation of caspase-1 (p20) was examined via western blot. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.

**Fig. S4** Deletion of *prgH* did not influence the synthesis of inflammasome components. C57BL/6 BMDMs were pre-treated with LPS (1 μg/mL) for 5 h (untreated and uninfected BMDMs was used as a negative control, Blank LPS-), and then infected with C50336, C50336Δ*prgH*, C50336Δ*prgH*::*prgH*, C50336Δ*prgH*-pMMB207, C50336::*prgH*, or C50336-pMMB207 at an MOI of 20 for 4 h, uninfected BMDMs

was used as another negative control (Blank LPS+). Bacteria bearing pMMB207 plasmids were cultured with IPTG (0.5 mM). The expression of caspase-1, NLRP3, NLRC4, ASC, and pro-IL-1β were analyzed by immunoblotting. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.

**Fig. S5** Overexpression of Dam inhibited inflammasome activation. C57BL/6 BMDMs were pre-treated with LPS (1 μg/mL) for 5 h (untreated and uninfected BMDMs was used as a negative control, Blank LPS-), and then infected with C50336, C50336Δ*dam*, C50336Δ*dam*::*dam*, C50336Δ*dam*-pMMB207, C50336::*dam*, or C50336-pMMB207 at an MOI of 20 for 4 h, uninfected BMDMs was used as another negative control (Blank LPS+). Bacteria bearing pMMB207 plasmids were cultured with IPTG (0.5 mM). The expression of caspase-1, NLRP3, NLRC4, ASC, and pro-IL-1β were analyzed by immunoblotting. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.

**Fig. S6** The ability of the *dam* complementation strain and overexpression strain cultured without IPTG to activate the inflammasome was improved. C57BL/6 BMDMs were pre-treated with LPS (1 μg/mL) for 5 h (untreated and uninfected BMDMs was used as a negative control, Blank LPS-), and then infected with C50336, C50336Δ*dam*, C50336Δ*dam*::*dam*, C50336Δ*dam*-pMMB207, C50336::*dam*, or C50336-pMMB207 at an MOI of 20 for 4 h uninfected BMDMs was used as another negative control (Blank LPS+). Bacteria bearing pMMB207 plasmids were cultured without IPTG. The expression of caspase-1, NLRP3, NLRC4, ASC, and pro-IL-1β were analyzed by immunoblotting. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.

**Fig. S7** The deletion of Dam blocked the Jnk pathway in infected J774A.1 cells. J774A.1 cells were pre-treated with LPS (1 μg/mL, 5 h) and then infected with C50336, C50336Δ*dam*, C50336Δ*dam*::*dam*, C50336Δ*dam*-pMMB207, C50336::*dam*, or C50336-pMMB207 at an MOI of 20 for 4 h, uninfected cells was used as a negative control (Blank). Bacteria bearing pMMB207 plasmids were cultured without IPTG. The activation of caspase-1, phosphorylated Jnk (P-Jnk), phosphorylated p38 (P-p38), and phosphorylated ERK1/2 (P-ERK1/2) were analyzed by immunoblotting. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.

**Fig. S8** Dam independently promoted NLRP3 inflammasome activation. J774A.1 cells were transduced with LV5-Dam or LV5-negative lentivirus, untreated cells was used as a negative control (Blank). Cells were then pre-treated with LPS (1 μg/mL, 5 h) and stimulated with or without ATP (1.25 mM) for 1 h. The expression of caspase-1, NLRP3, ASC, pro-IL-1β, and P-Jnk were analyzed by immunoblotting. β-actin was blotted as a loading control. Molecular mass markers in kDa are indicated on the left.