

Full Uncropped Gels and Blots

Manuscript

USP13 stabilizes prohibitin 1 to enforce CD14 turnover and restrain TLR4-NLRP3 inflammatory injury in sepsis

Western blot and immunoprecipitation signals in this study were developed by enhanced chemiluminescence and visualized using conventional X-ray film. The original physical X-ray films were retained without physical cropping. The images provided below are digital photographs of the complete original films corresponding to the gel- and blot-based results presented in the manuscript. Images have been oriented for readability where necessary; no individual lanes or bands have been selectively altered. Typed labels outside the film images are provided solely to facilitate correspondence with the manuscript figure panels.

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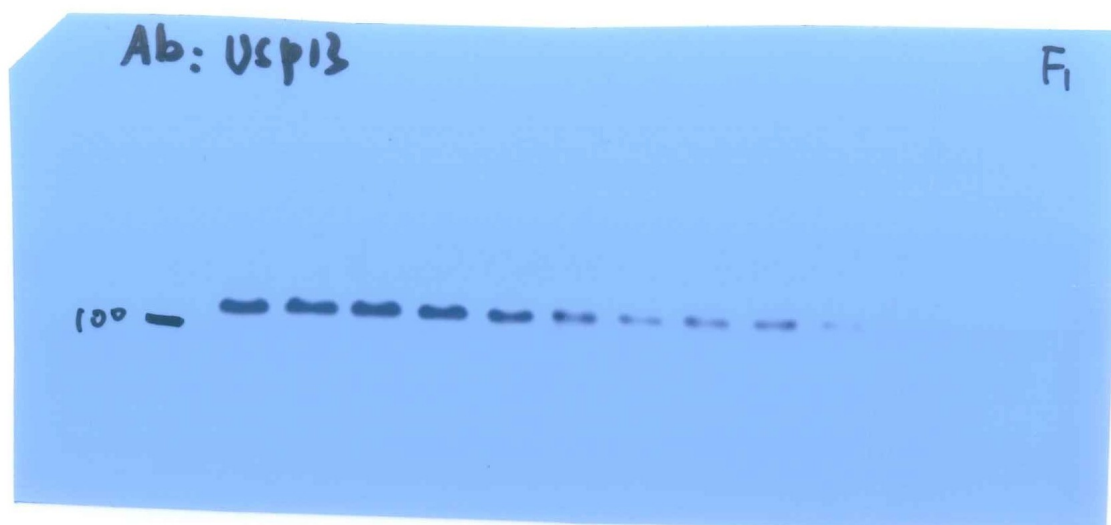
Figure 3, panel D - USP13 suppression after LPS challenge

Experimental system: Mouse lung tissue

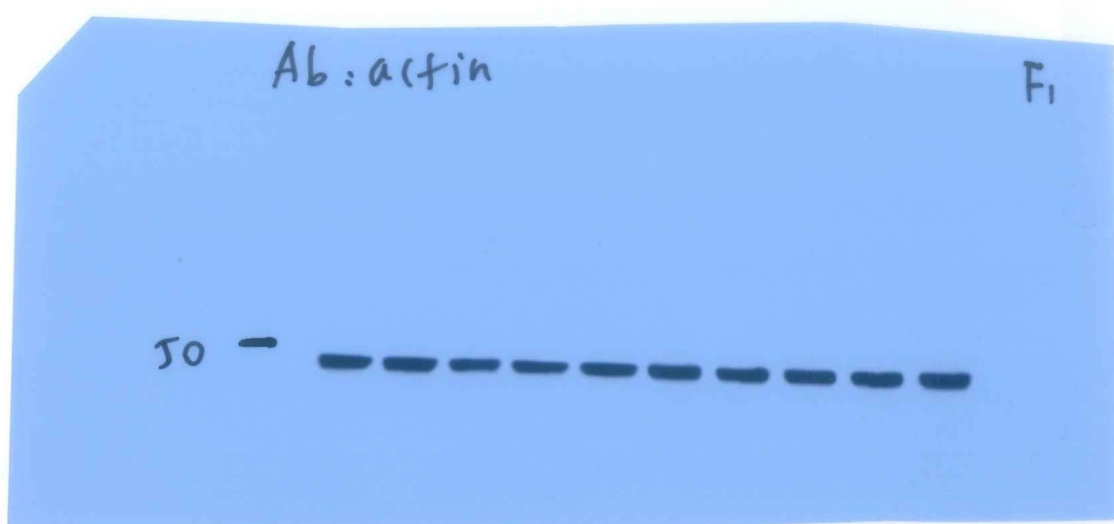
Assay: Western blot

Published targets: USP13; beta-actin

Published groups: PBS / LPS



USP13 - complete original X-ray film



beta-actin - complete original X-ray film

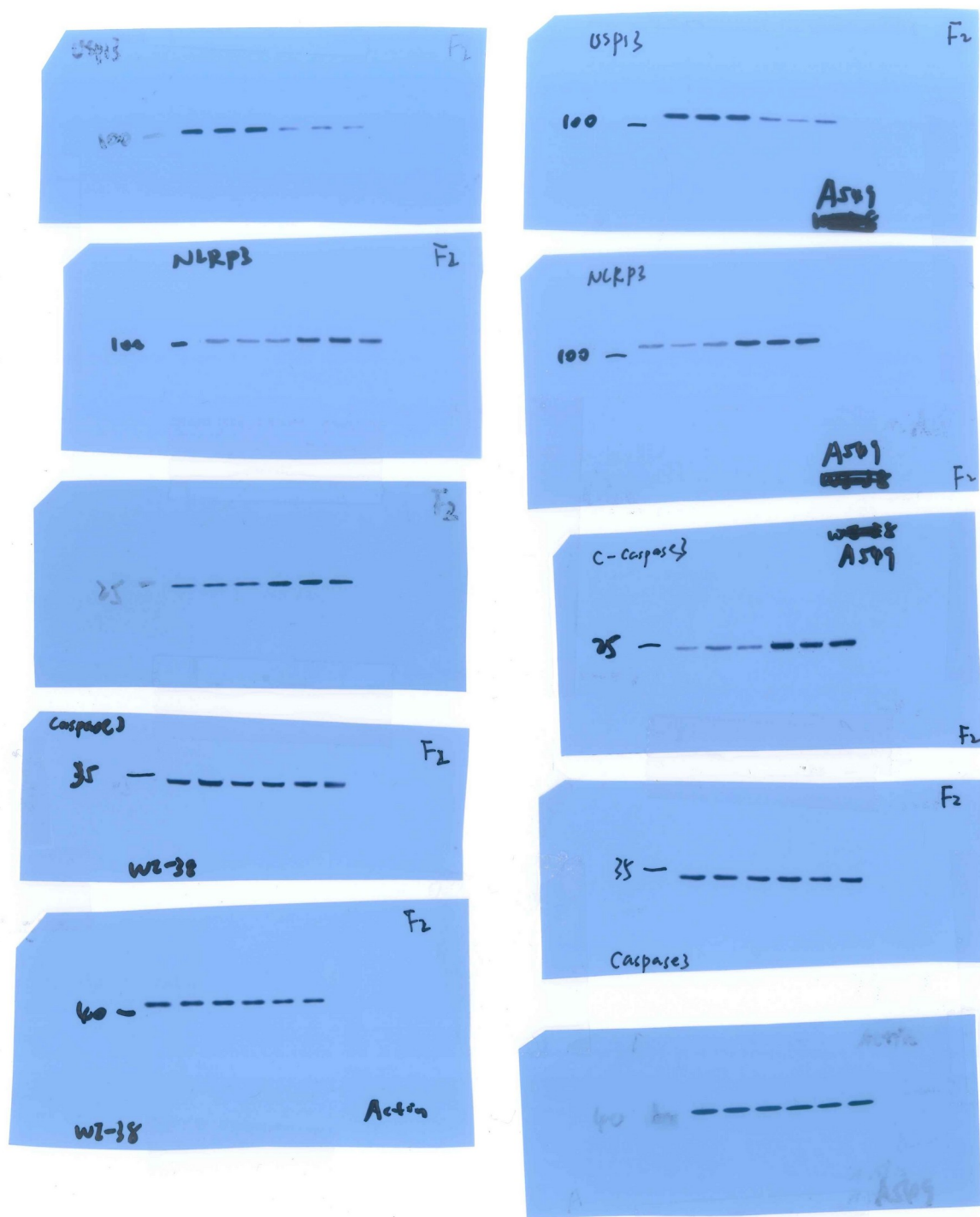
Figure 4, panel C - WI-38 and A549 immunoblots

Experimental system: WI-38 and A549 cells

Assay: Western blot

Published targets per cell line: USP13, NLRP3, cleaved caspase-3, total caspase-3, beta-actin

A549 ~100 kDa source film: USP13.



Complete original photograph. Original films retain historical laboratory annotations.

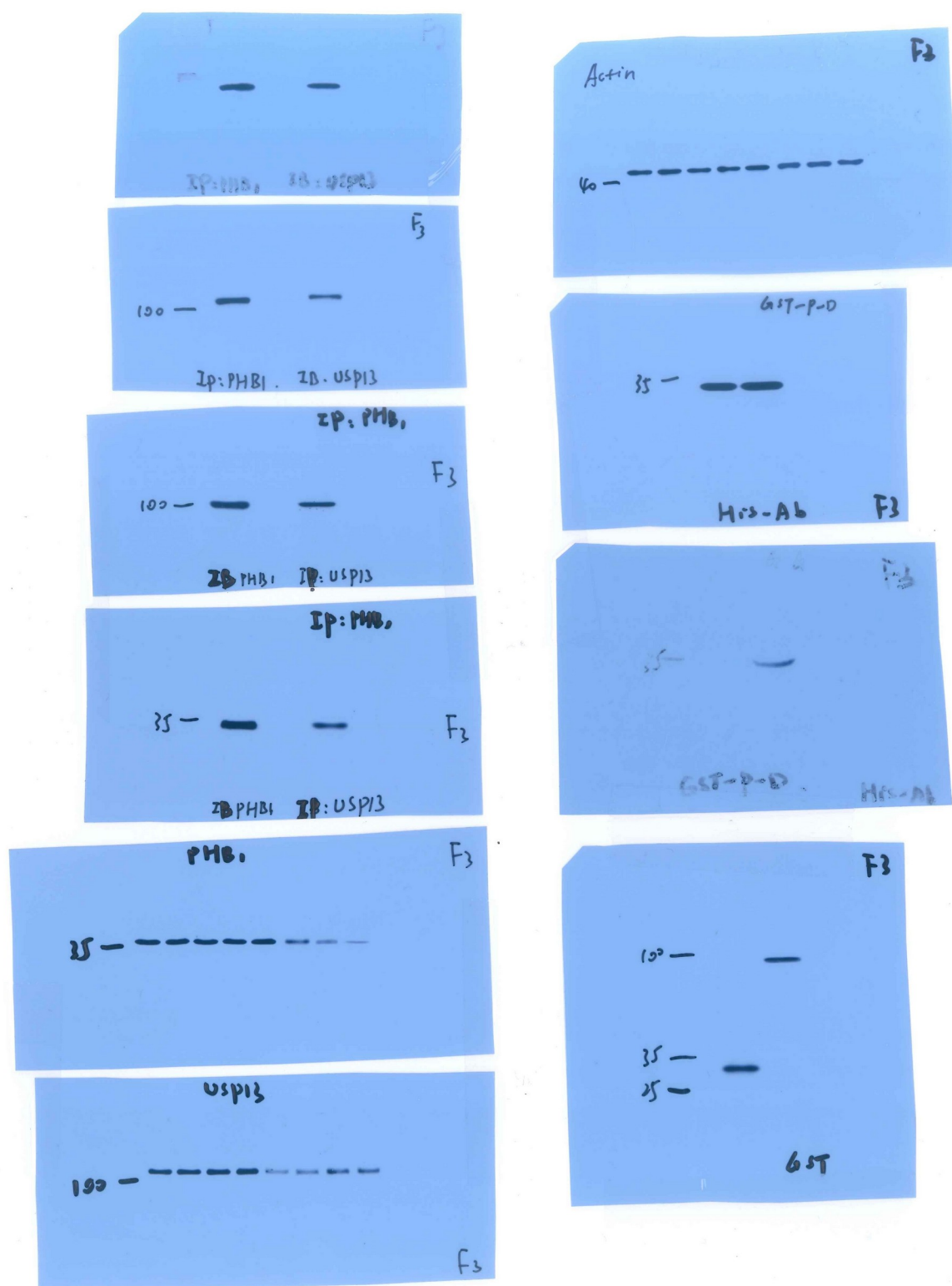
Figure 5, panels A, B and E - interaction and CHX-chase source films

Experimental systems: A549 cells and purified recombinant proteins

Assays: endogenous co-IP; GST pull-down; CHX-chase Western blot

Published targets: PHB1, USP13, His-PHB1, GST/GST-USP13 and beta-actin

The photograph contains separate complete physical films; the films are not visually merged.



Complete original photograph. Original films retain historical laboratory annotations.

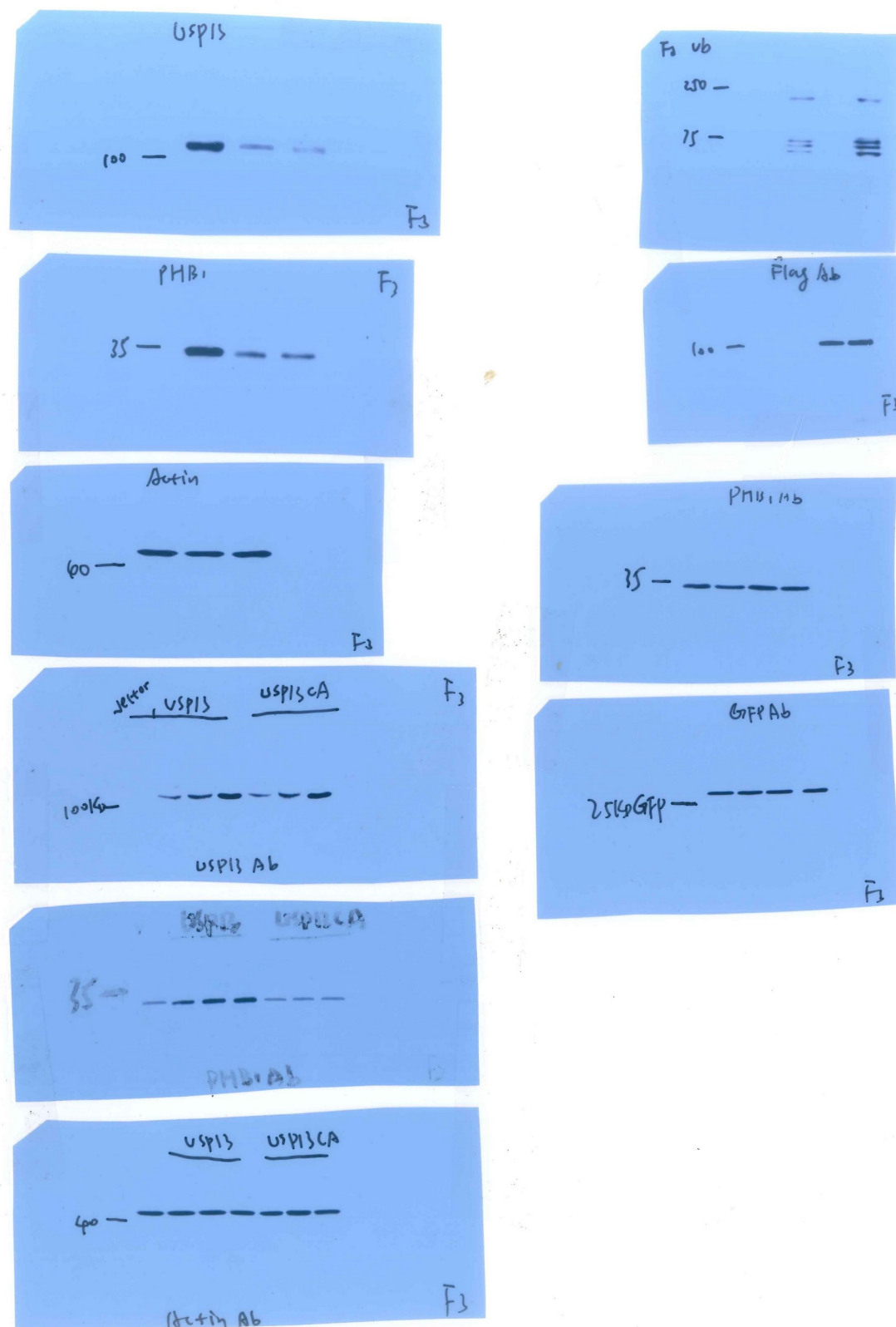
Figure 5, panels C, D and F - dose, knockdown and PHB1 ubiquitination

Experimental system: transfected cells / A549 cells

Assays: Western blot dose series; knockdown validation; denaturing PHB1 ubiquitination assay

Published targets: Flag-USP13, PHB1, beta-actin, ubiquitinated PHB1, GFP

The complete high-molecular-weight ubiquitination film is retained with all lanes and background.



Complete original photograph. Original films retain historical laboratory annotations.

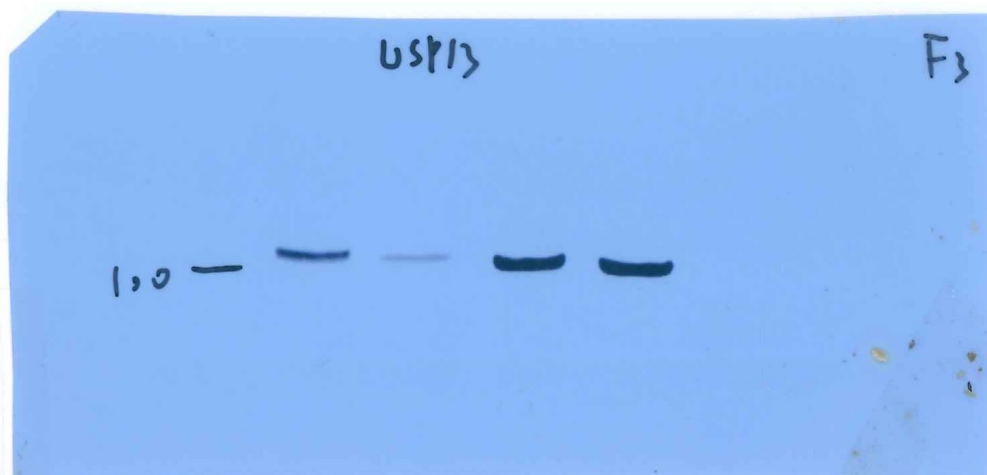
Figure 5, panel G - USP13 rescue experiment

Experimental system: A549 cells

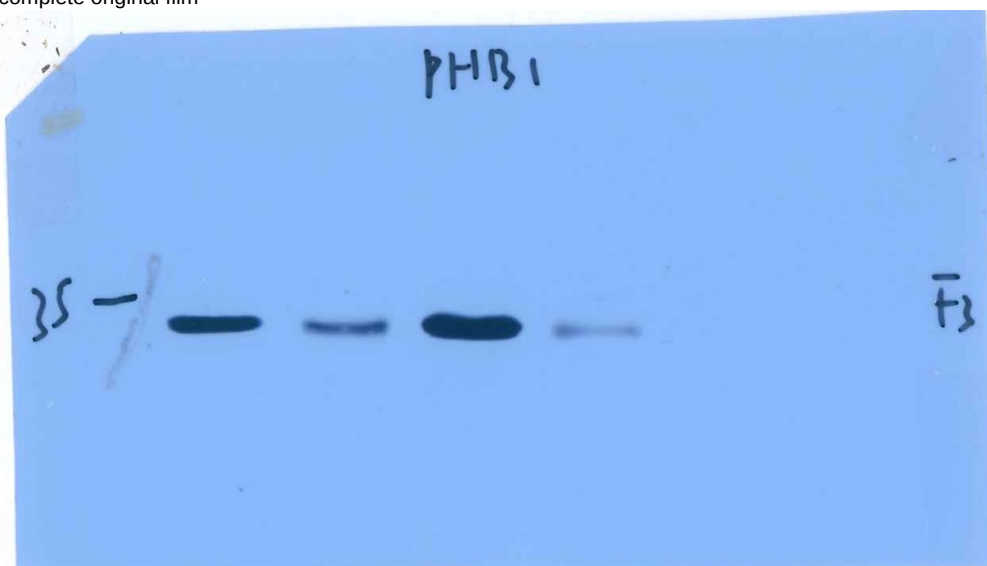
Assay: Western blot rescue experiment

Published groups: pLL3.7 + PCI-neo / shUSP13 + PCI-neo / shUSP13 + Flag-USP13 WT / shUSP13 + Flag-USP13 C345A

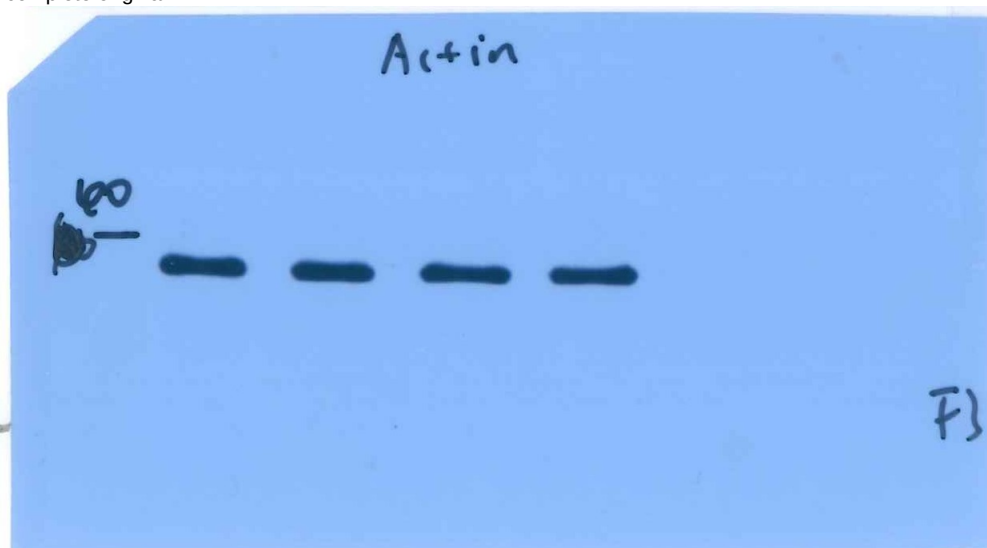
Complete original X-ray films corresponding to Figure 5G are shown below.



USP13 (~100 kDa) - complete original film



PHB1 (~30-35 kDa) - complete original film



beta-actin (~40 kDa) - complete original film

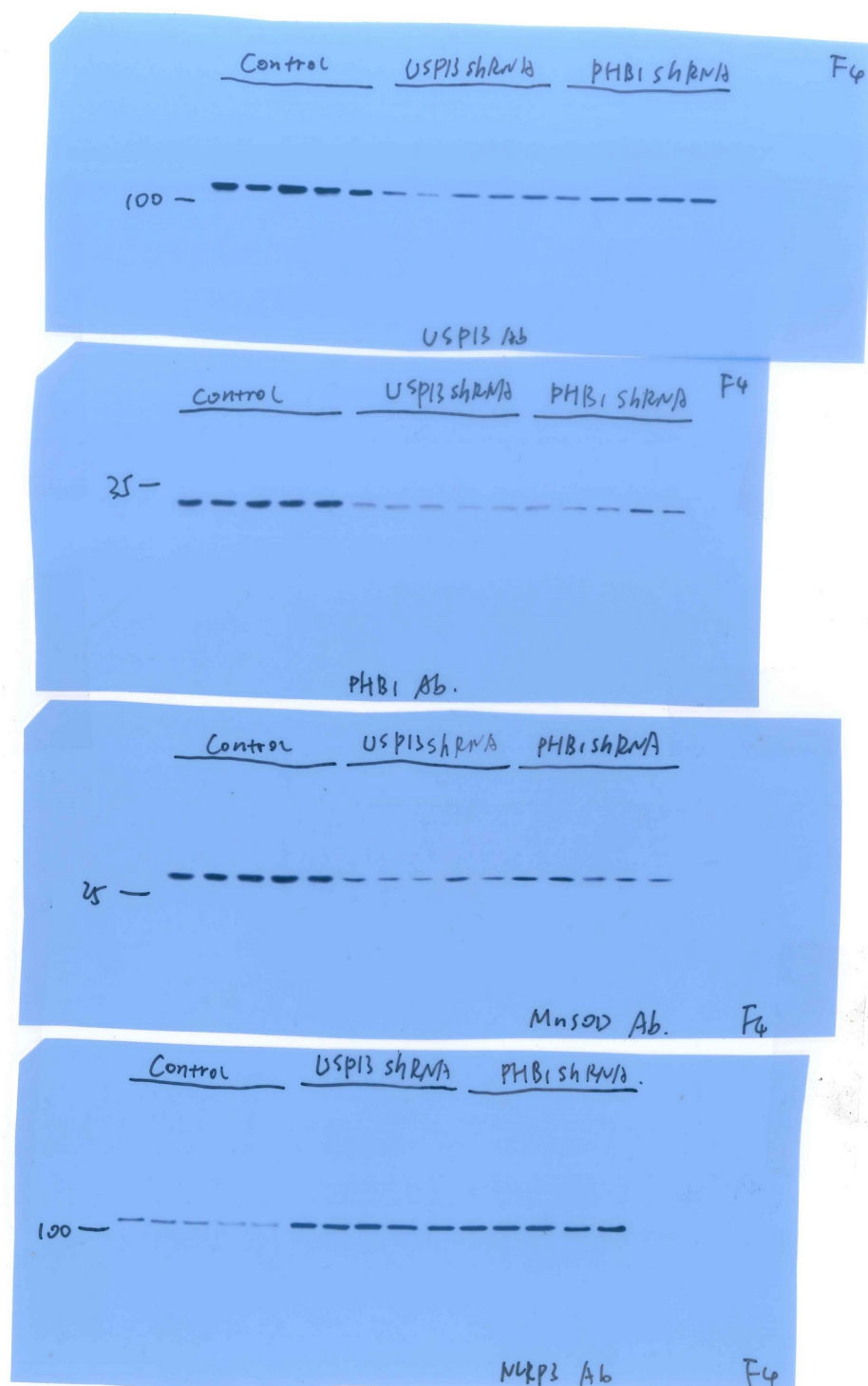
Figure 6, panel C - mouse lung immunoblots (part 1)

Experimental system: Mouse lung homogenates after LPS

Assay: Western blot

Published targets: USP13, PHB1, MnSOD, NLRP3

Published groups: Control / USP13 shRNA / PHB1 shRNA (5 lanes per group; 15 lanes)



Complete original photograph. Original films retain historical laboratory annotations.

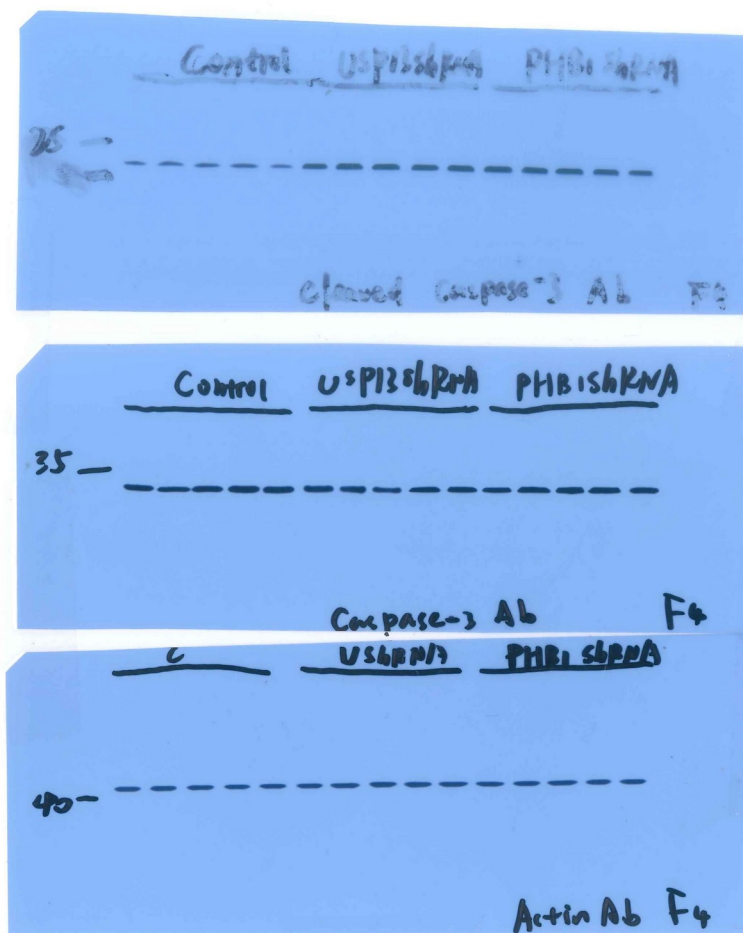
Figure 6, panel C - mouse lung immunoblots (part 2)

Experimental system: Mouse lung homogenates after LPS

Assay: Western blot

Published targets: cleaved caspase-3, total caspase-3, beta-actin

Published groups: Control / USP13 shRNA / PHB1 shRNA (5 lanes per group; 15 lanes)



Complete original photograph. Original films retain historical laboratory annotations.

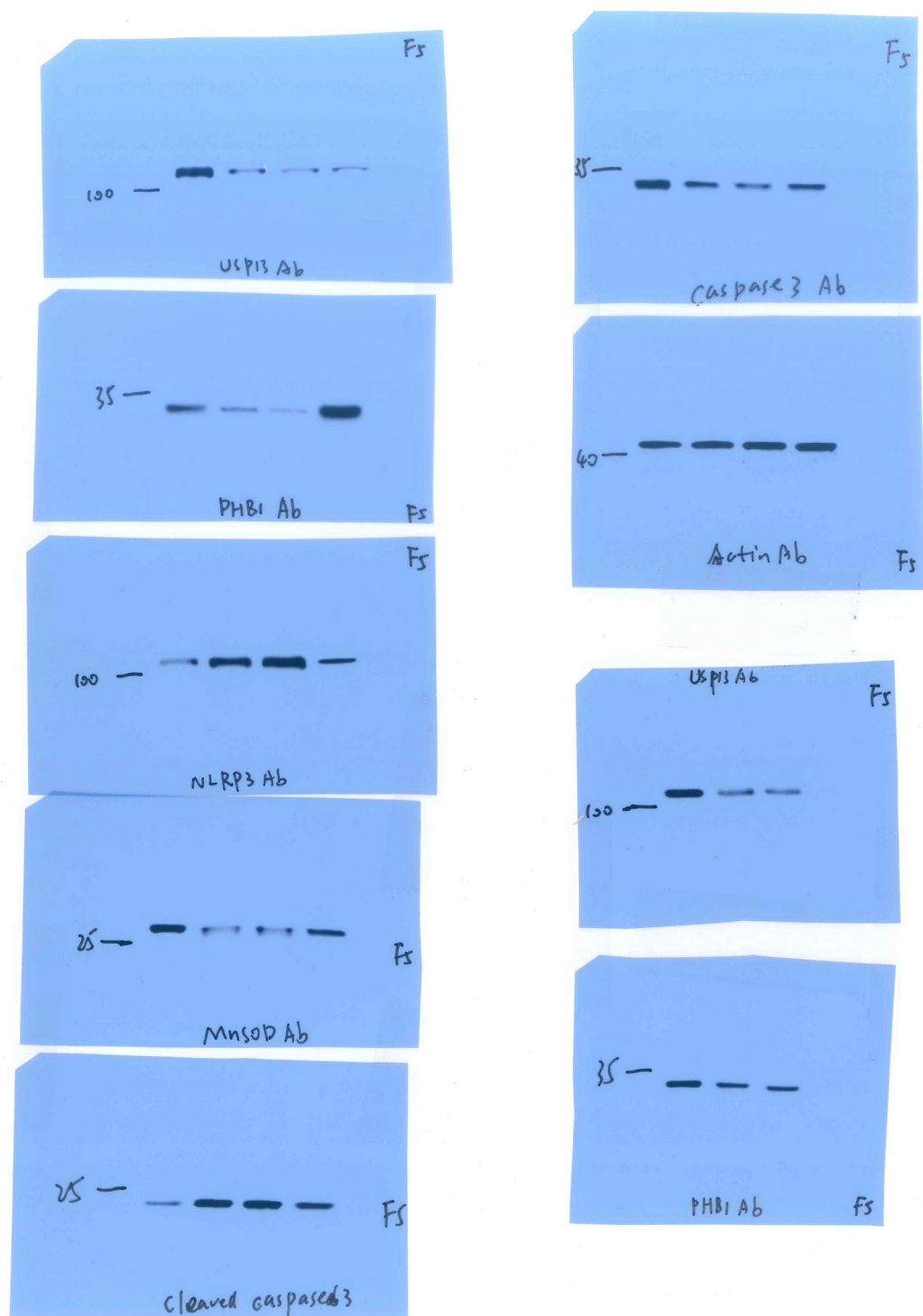
Figure 7, panel C - PHB1 rescue in USP13-deficient mice

Experimental system: Mouse lung homogenates after LPS

Assay: Western blot rescue experiment

Published targets: USP13, PHB1, MnSOD, NLRP3, cleaved caspase-3, total caspase-3, beta-actin

Groups: Control / USP13 shRNA / USP13 shRNA + Empty / USP13 shRNA + PHB1



Complete original photograph. Original films retain historical laboratory annotations.

Figure 9, panels A and B - THP-1 and U937 immunoblots

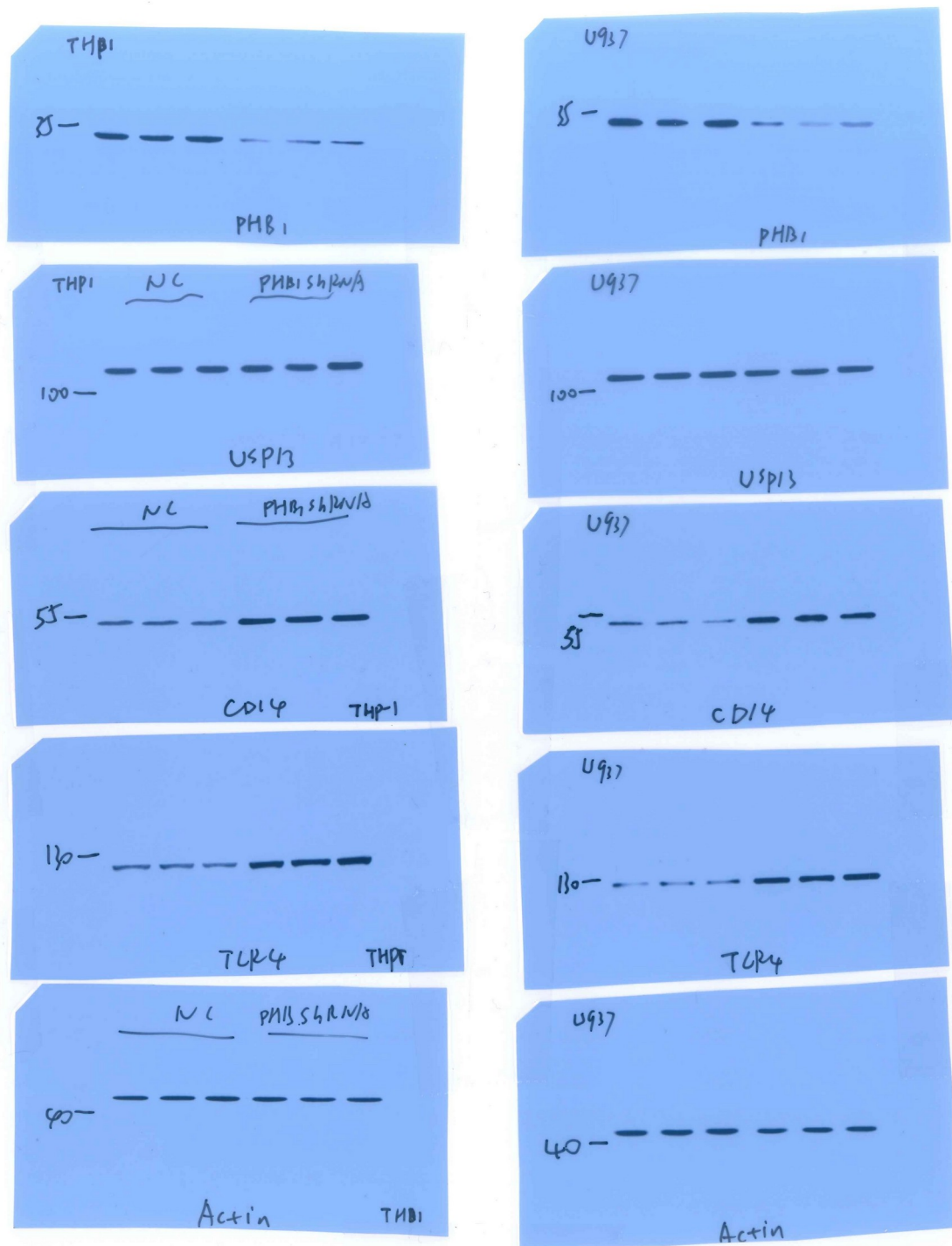
Experimental systems: THP-1 and U937 cells with LPS

Assay: Western blot

Published targets per cell line: PHB1, USP13, CD14, TLR4, beta-actin

Groups: NC (3 lanes) / PHB1 shRNA (3 lanes)

Frg 7. AB



Complete original photograph. Original films retain historical laboratory annotations.

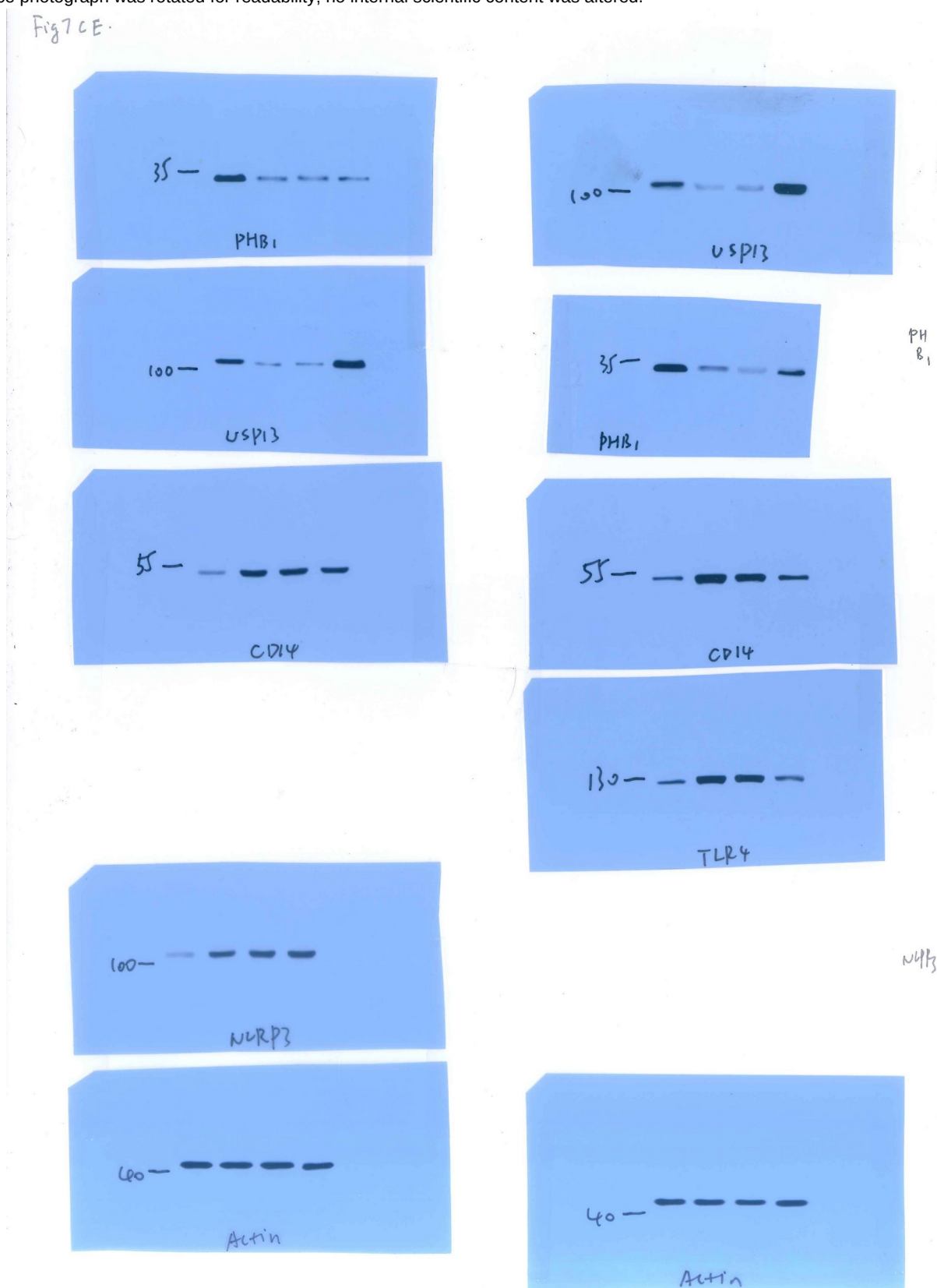
Figure 9, panels C and E - lung-tissue immunoblots

Experimental system: Mouse lung tissue collected 30 h after PCI

Assay: Lung-tissue Western blot

Published targets: PHB1, USP13, CD14, TLR4, NLRP3, beta-actin

The source photograph was rotated for readability; no internal scientific content was altered.



Complete original photograph. Original films retain historical laboratory annotations.

Figure 9, panels C and E - lung-tissue CD14 ubiquitination assays

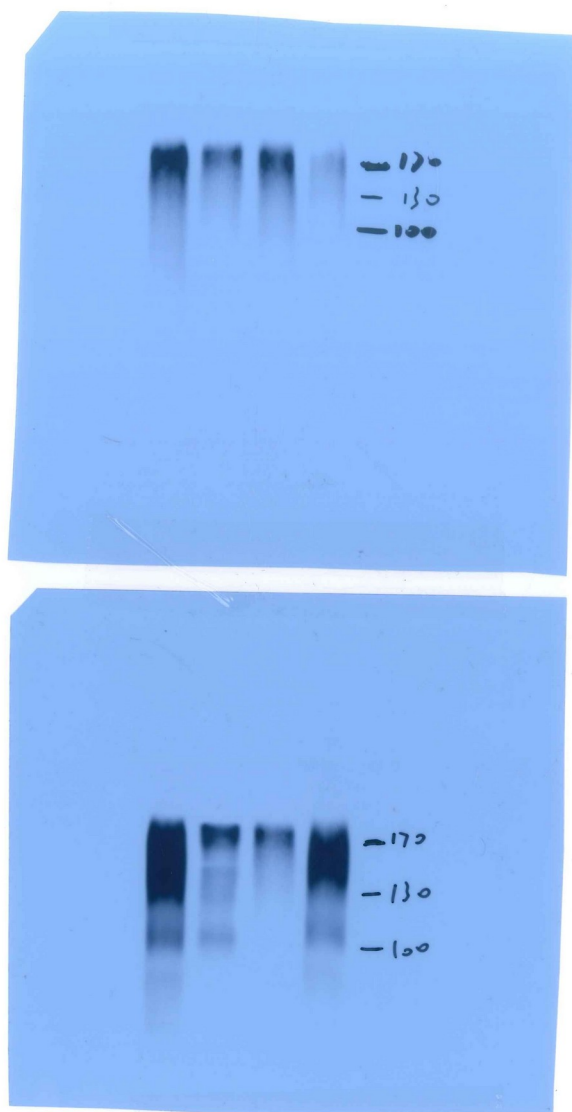
Experimental system: Mouse lung tissue collected 30 h after PCI

Assay: CD14 immunoprecipitation followed by anti-K48-linked ubiquitin immunoblotting

Source: complete original X-ray films

All lanes, high-molecular-weight smear and background signal are retained.

Fig 7E.



Upper film: Figure 9C Ub-CD14; lower film: Figure 9E Ub-CD14.

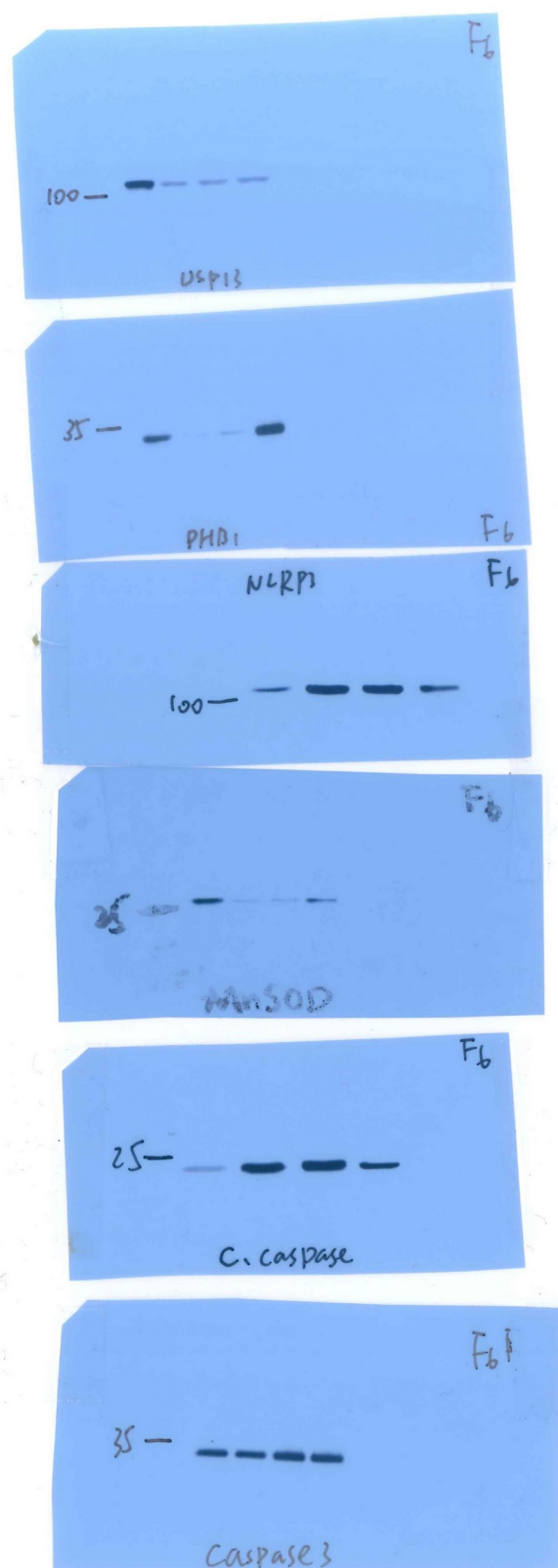
Figure 8, panel C - PCI epistasis immunoblots (part 1)

Experimental system: Mouse lung homogenates after PCI

Assay: Western blot epistasis experiment

Published targets: USP13, PHB1, NLRP3, MnSOD, cleaved caspase-3, total caspase-3

Groups: Control / USP13 shRNA / USP13 shRNA + Empty / USP13 shRNA + PHB1



Complete relevant original films. Physical boundaries and all lanes are retained.

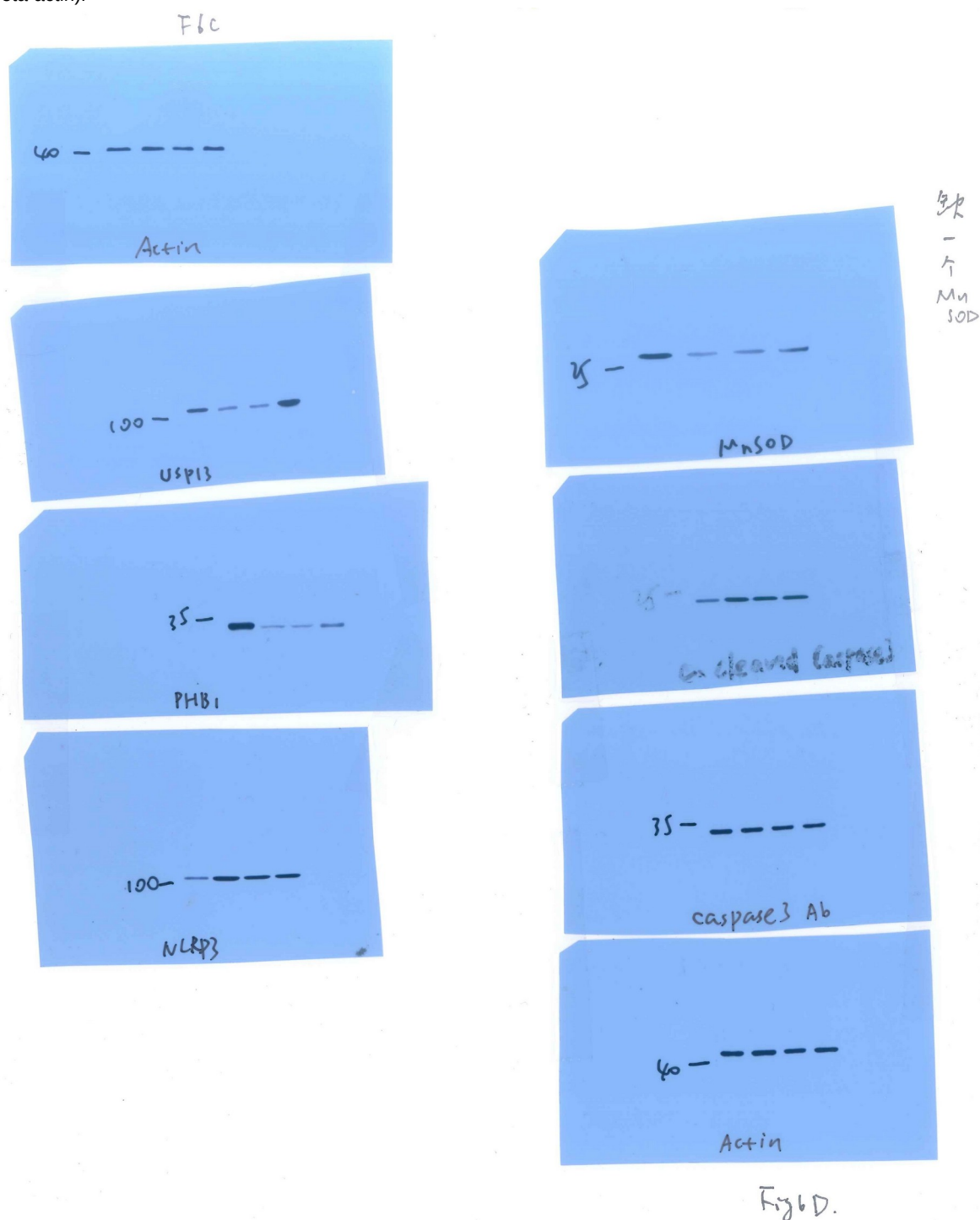
Figure 8, panels C and G - PCI epistasis immunoblots (part 2)

Experimental system: Mouse lung homogenates after PCI

Assay: Western blot epistasis experiments

Figure 8C: USP13-deficiency / PHB1-restoration cohort (beta-actin).

Figure 8G: reciprocal PHB1-deficiency / USP13-overexpression cohort (USP13, PHB1, NLRP3, MnSOD, cleaved caspase-3, total caspase-3, beta-actin).



Complete original photograph. Original films retain historical laboratory annotations.