Thyroid hormone inhibits growth of hepatoma cells through induction of miR-214

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Running title: miR-214 regulated by thyroid hormone

Keywords: microRNA-214; PIM-1; thyroid, receptor, hepatoma

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Supplementary Information

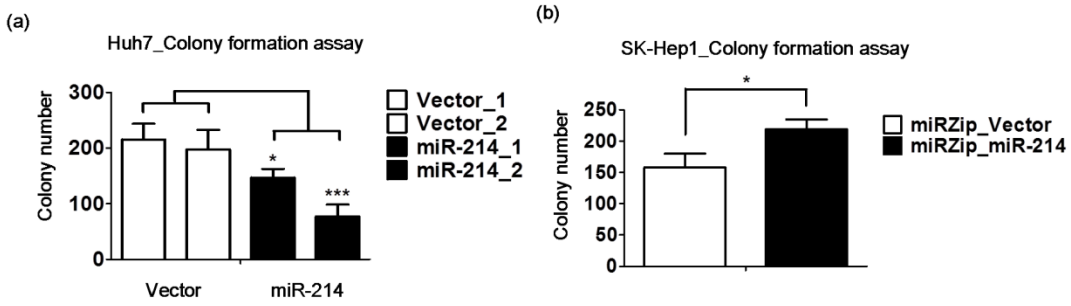


Fig.S1 miR-214 inhibits cell proliferation

Stable expression miR-214 Huh7 cell lines were established by transfect with miR-214 plasmid. (a) Huh7 hepatoma cell lines proliferation capacity was measured by Colony assay. Stable knockdown of miR-214 in SK-Hep-1 cell lines were established by infection the lentivirus of miRZip-miR-214 (Anti-miR-214). (b) SK-Hep-1 hepatoma cell lines proliferation capacity were measured by Colony assay.

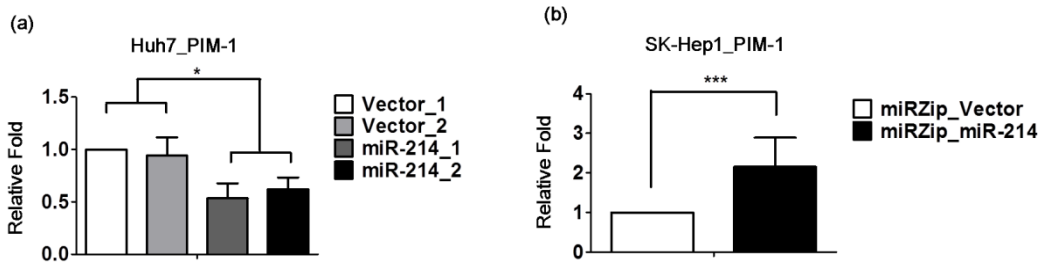


Fig.S2 PIM-1 is the target gene of miR-214

(a) PIM-1 protein levels in Huh7 stable expression and (b) SK-Hep-1 stable knockdown miR-214 cells measuring by western blot. β-actin was used as a loading control. Data are presented as means± s.d. of three independent experiments (*p < 0.05; **p<0.005; ***p<0.001 v.s. Vector).

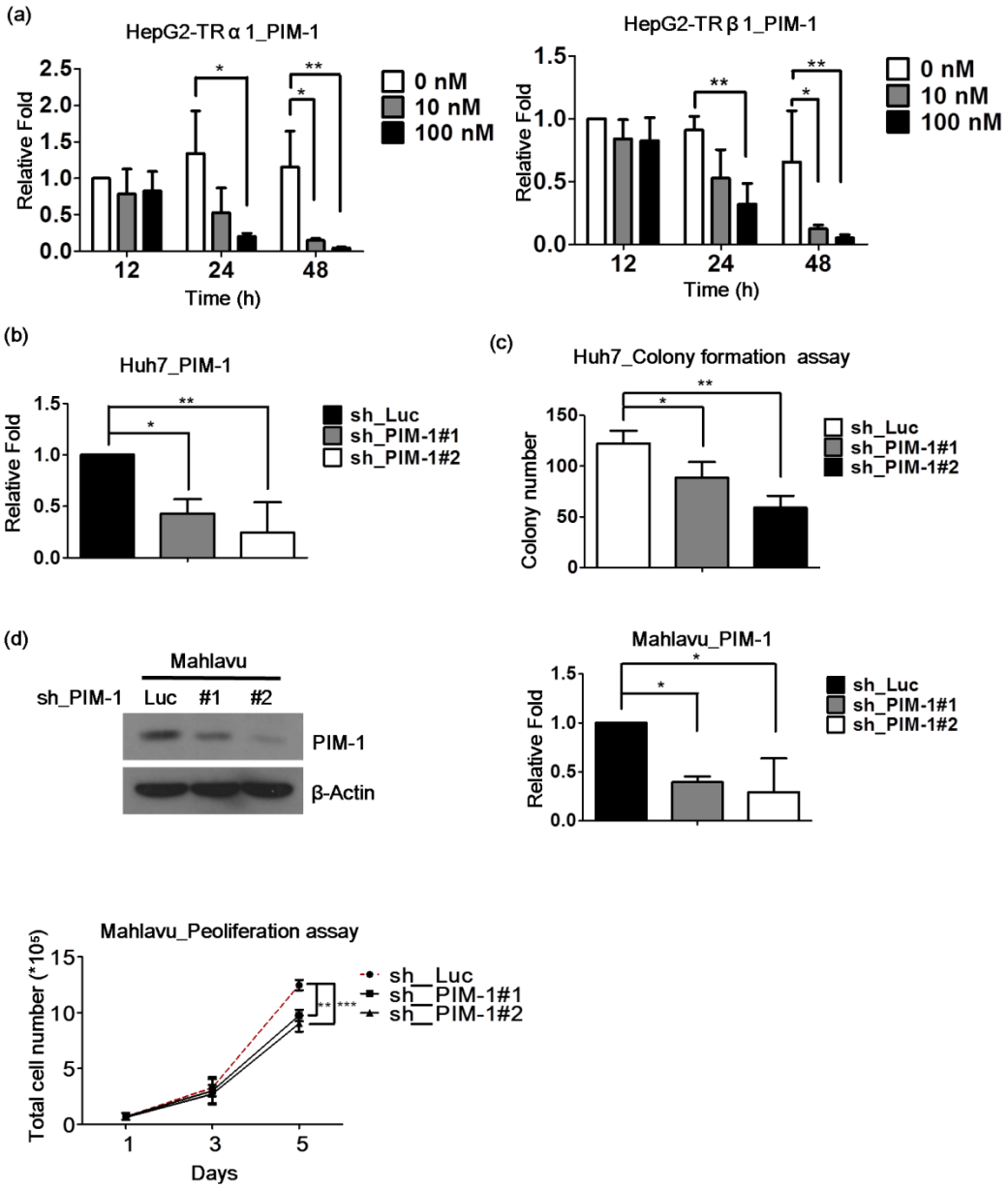


Fig.S3 PIM-1 is downregulated by T_3 and promotes cell proliferation

(a) HepG2-TR cell lines were treated with 0-100 nM T_3 for 12-48 hrs, and PIM-1 protein levels were measured by western blot. (b) Stable knockdown PIM-1 in Huh7 cell lines were established by transfect with shRNA PIM-1 plasmids. (c) Huh7 hepatoma cell lines proliferation capacity was measured by colony formation assay. (d) Stable knockdown PIM-1 in Mahlavu cell lines were established by transfect with shRNA PIM-1 plasmids. β-actin was used as a loading control. (e) Mahlavu hepatoma cell lines proliferation capacity was measured by proliferation assay. Data are

presented as means± s.d. of three independent experiments (*p < 0.05; **p<0.005 v.s. control).

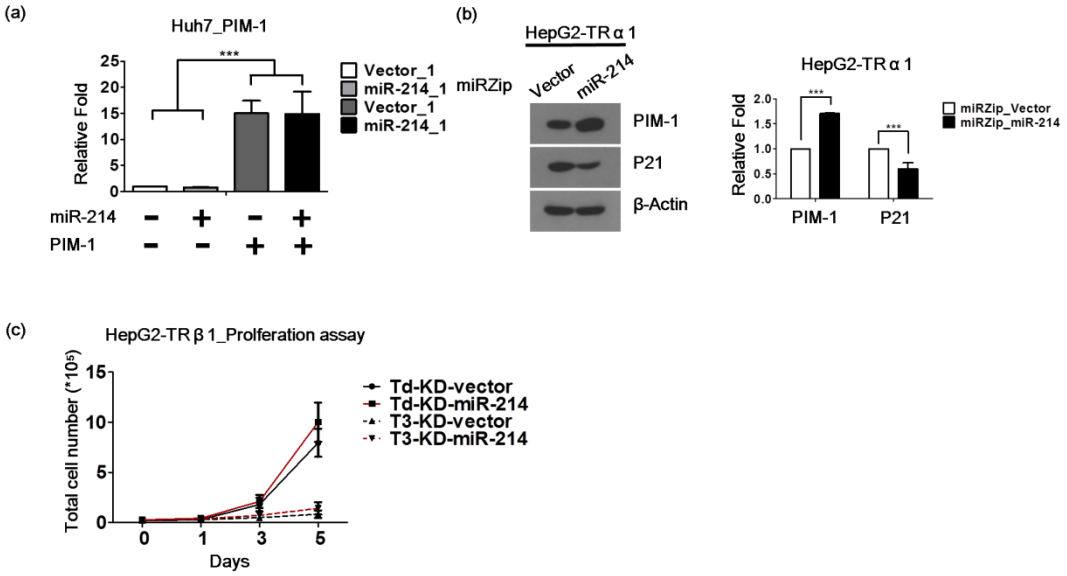
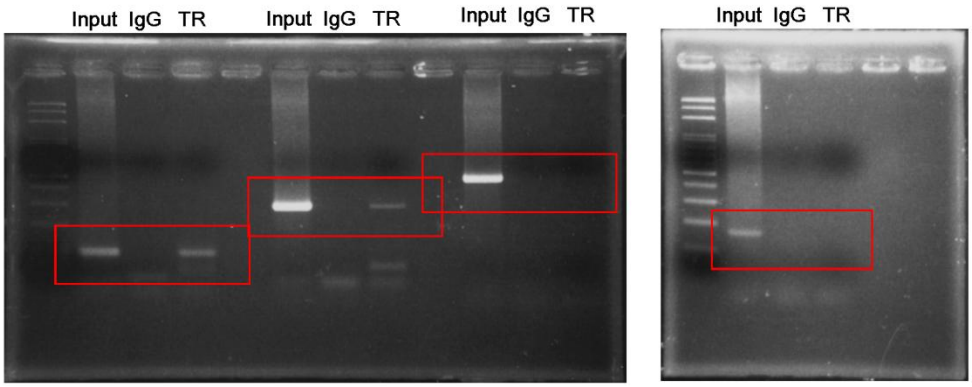


Fig.S4 T_3 inhibits cell proliferation through upregulation miR-214 via modulation of the PIM-1 pathway

(a) Re-expression of PIM-1 in miR-214 stable Huh7 cell lines, and PIM-1 protein levels were measuring by western blot. (b) PIM-1 and p21 protein expression were measuring in stable knockdown miR-214 HepG2-TRα1 cell lines by western blot. β-actin was used as a loading control. (c) HepG2-TRβ1 depletion of miR-214 cell lines were treated with 0-10 nM T_3, and proliferation capacity was measured. Data are presented as means± s.d. of three independent experiments (*P<0.05;**P<0.01 v.s. Vector).

Fig.1e



-5460~-5361 -4560~-4261 -3460~-2961 GAPDH

Fig.3 (b)

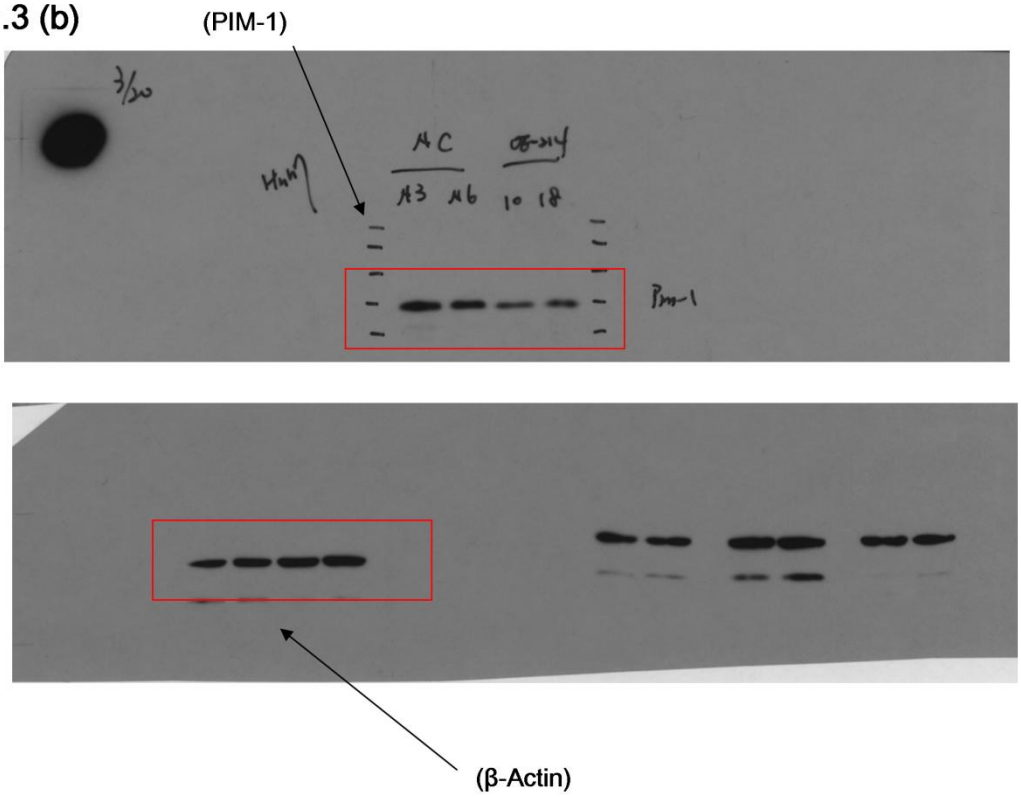


Fig.3 (c)

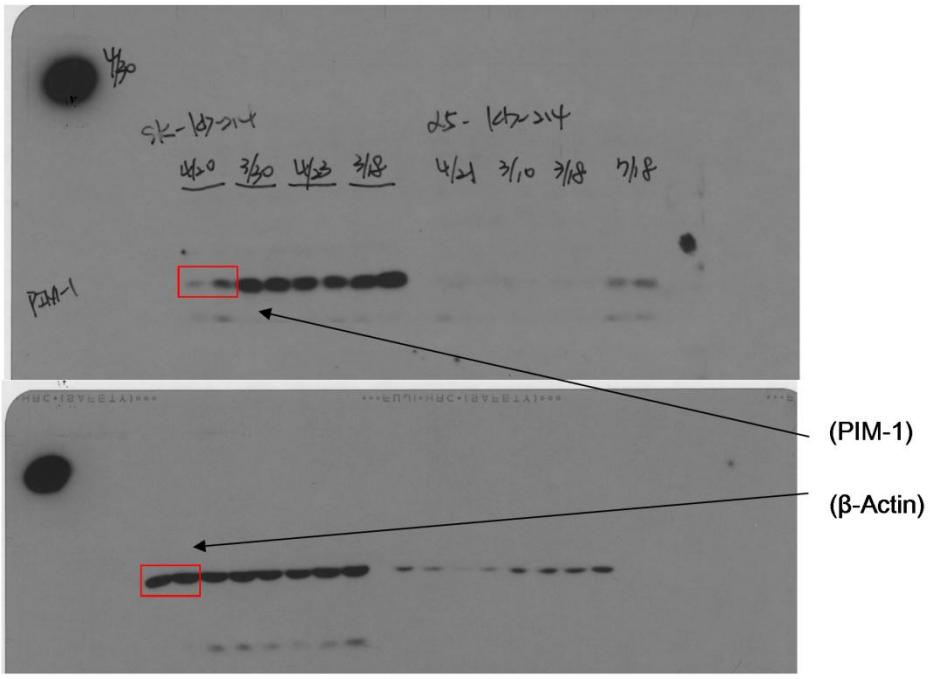


Fig.3 (d)

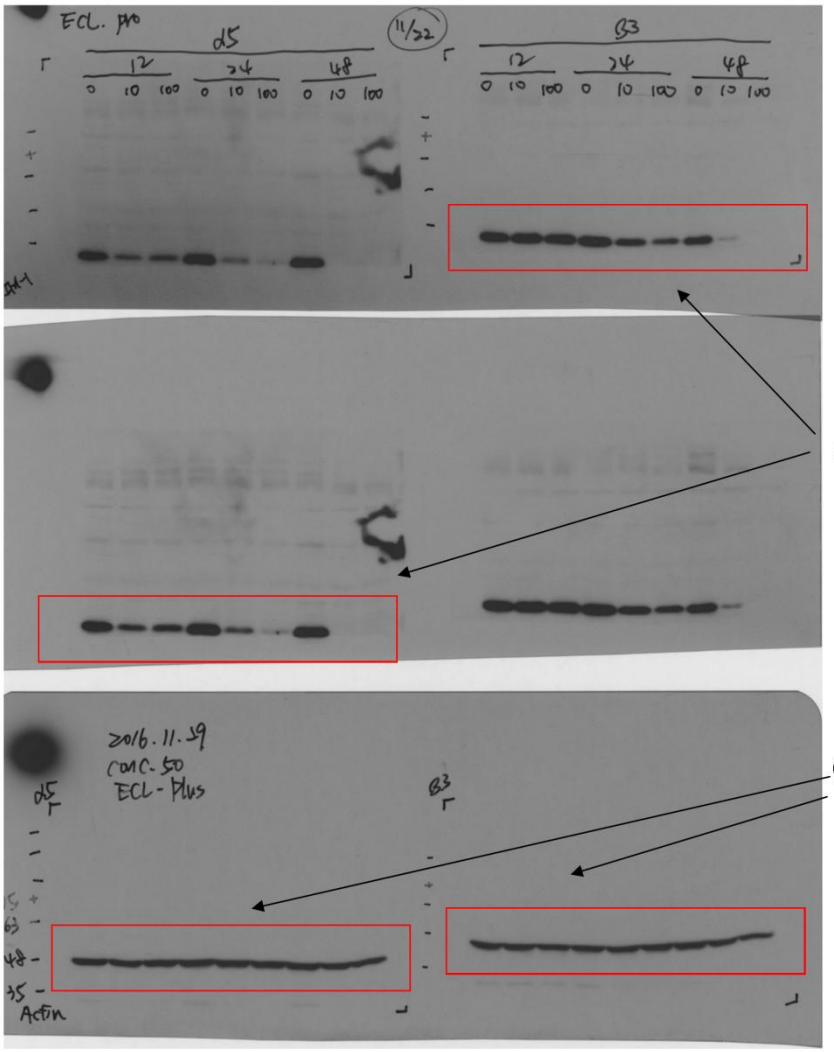

ECL. PIM
11/22
12
24
48
0 10 100
(PIM-1)
2016.11.29
CONC-50
ECL-Plus
(β-Actin)
Actin

Fig.3 (e)

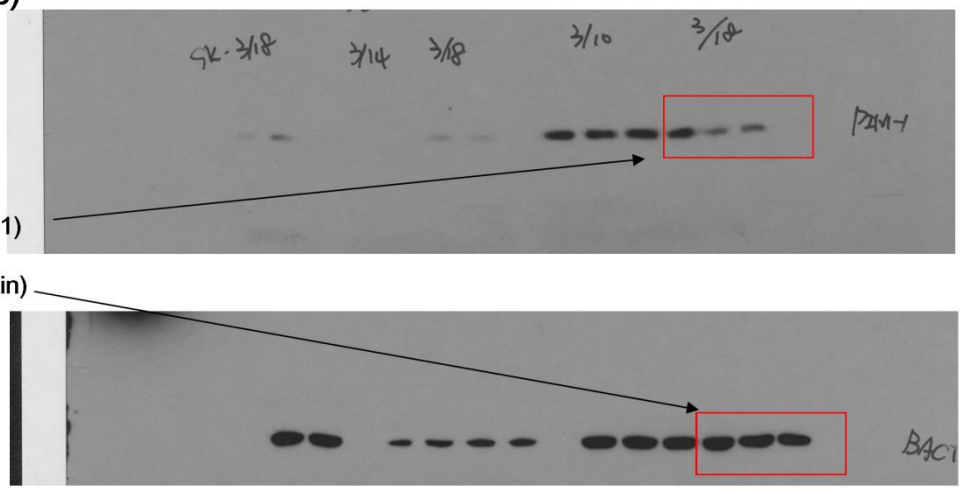

3/18
3/14
3/18
3/10
3/18
PIM-1
(PIM-1)
(β-Actin)

Fig.4 (a)

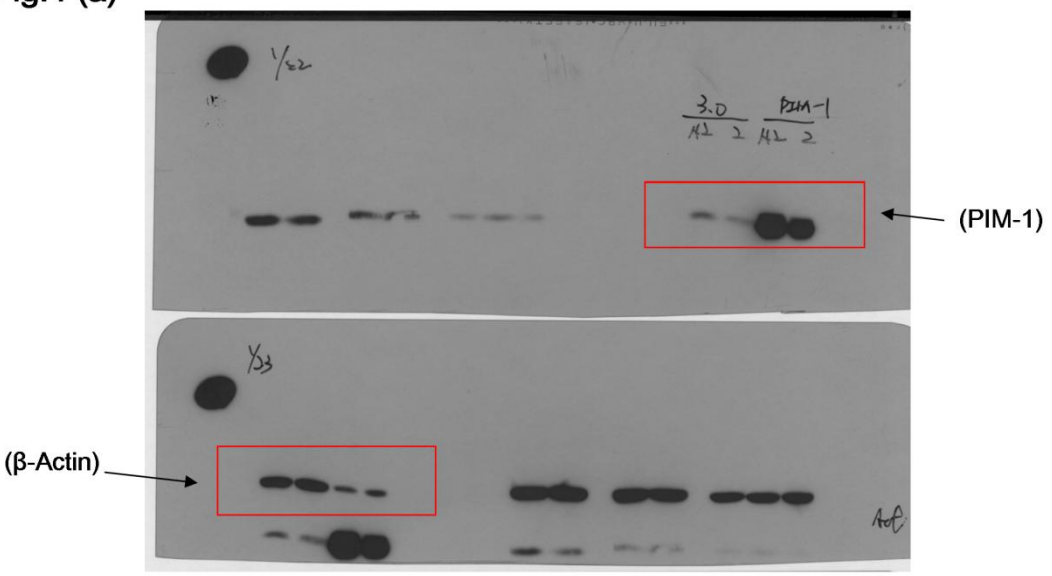


Fig.4 (c)

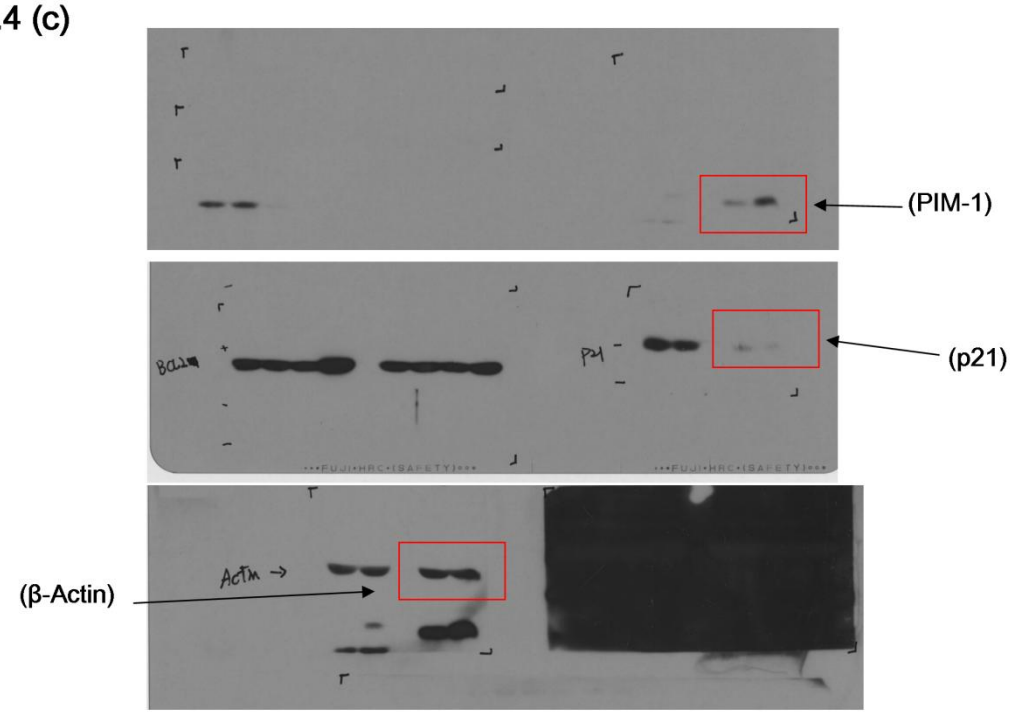


Fig.4 (d)

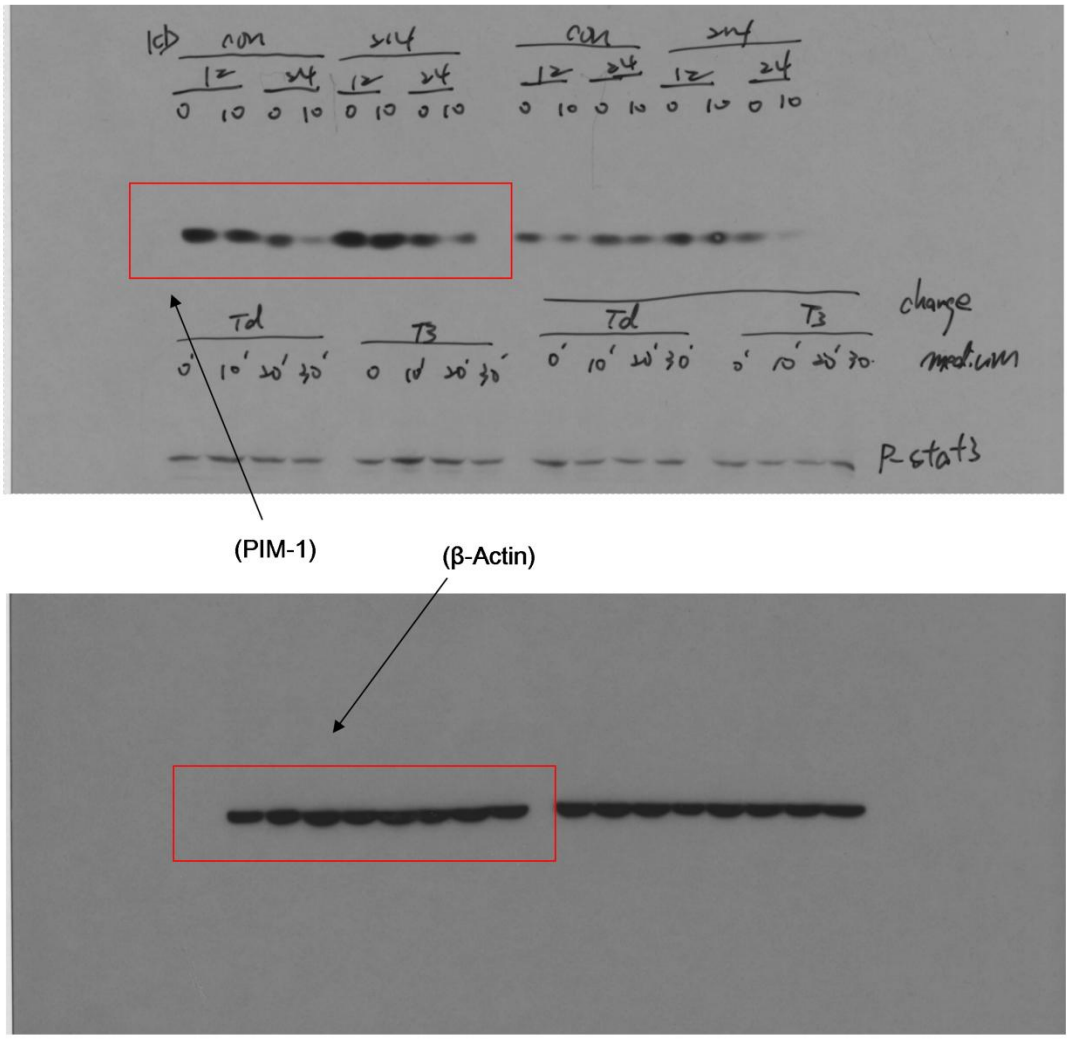


Fig.5 (b)

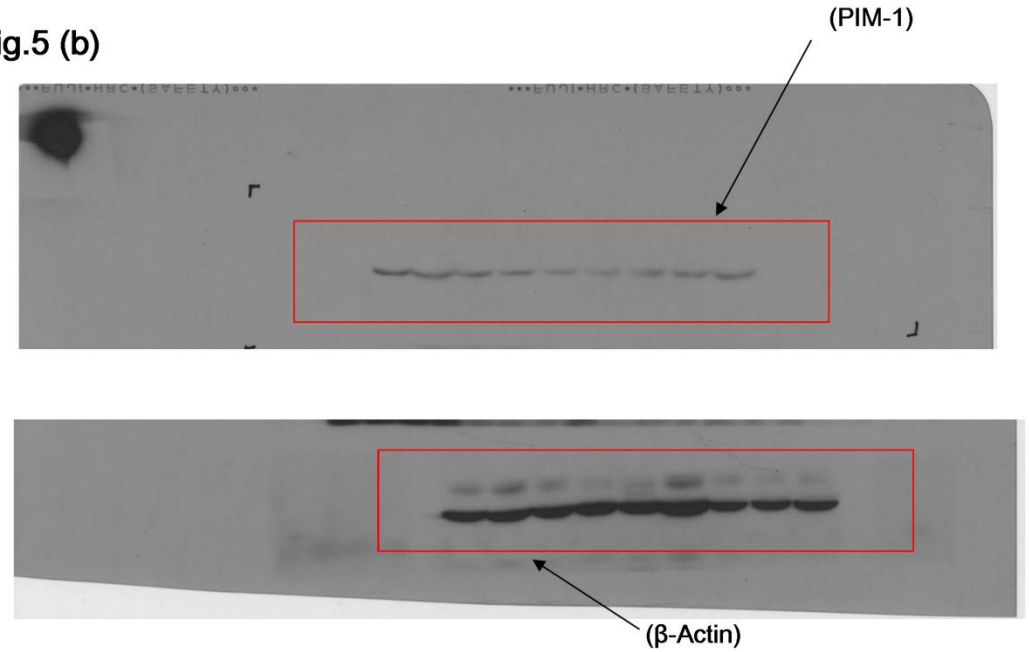


Fig.5 (c)

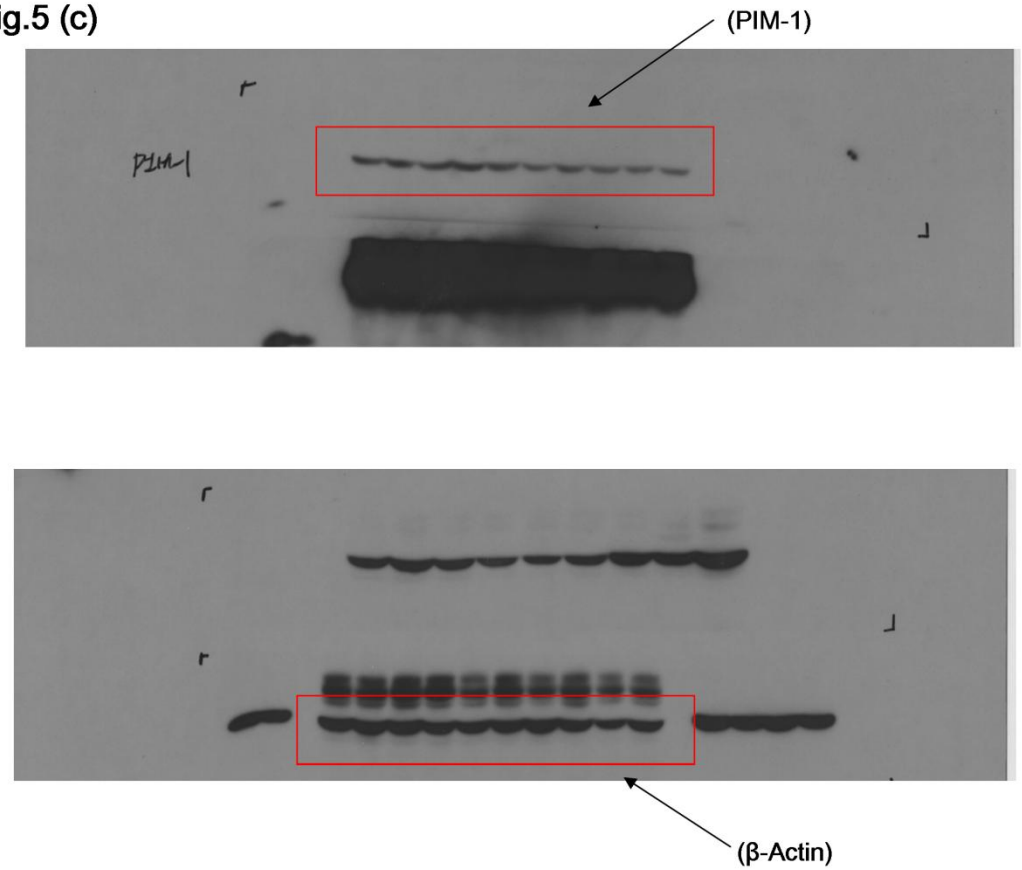


Fig.5 (g)

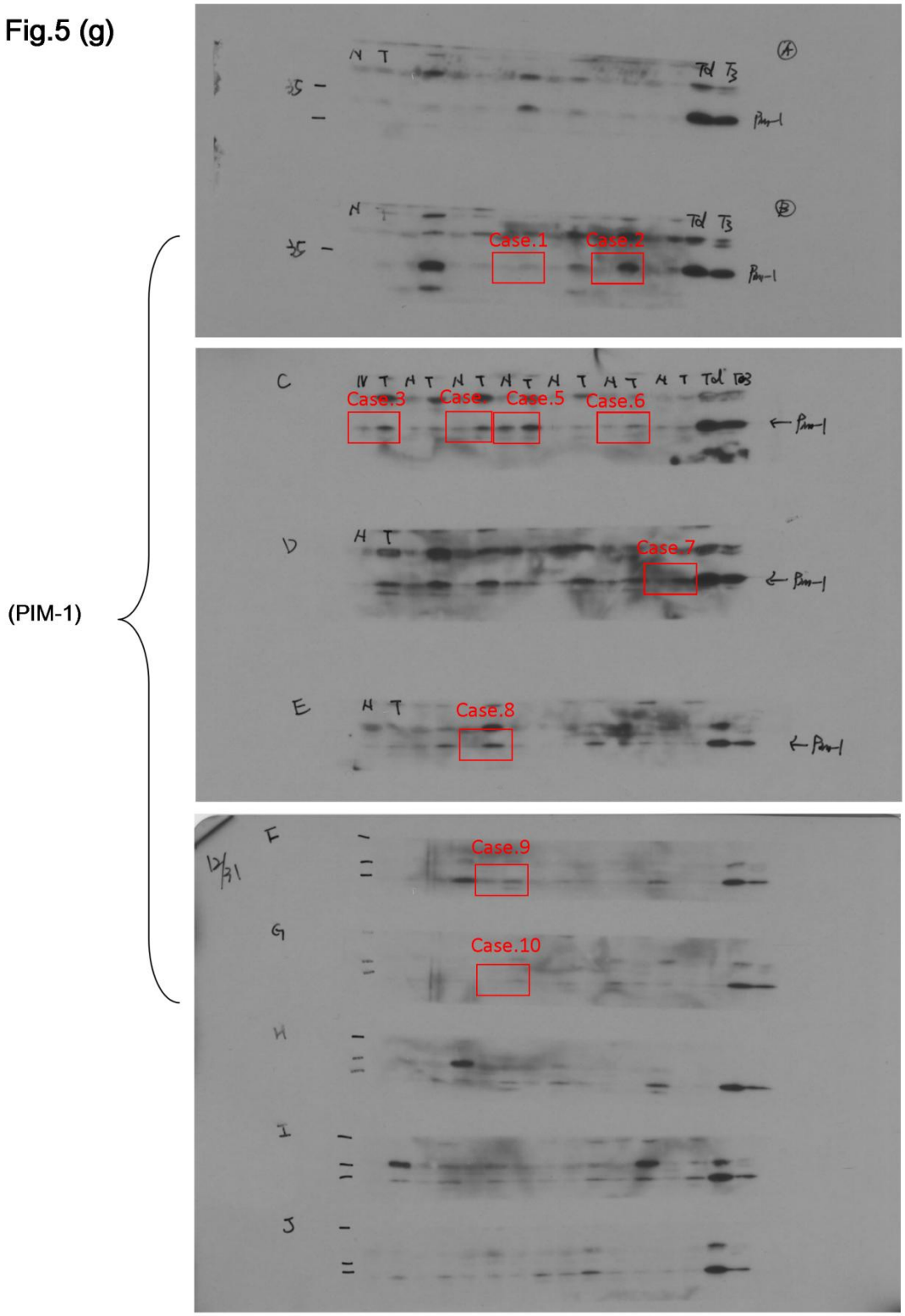

(PIM-1)
Case.1
Case.2
Case.3
Case.4
Case.5
Case.6
Case.7
Case.8
Case.9
Case.10

Fig.5 (g)

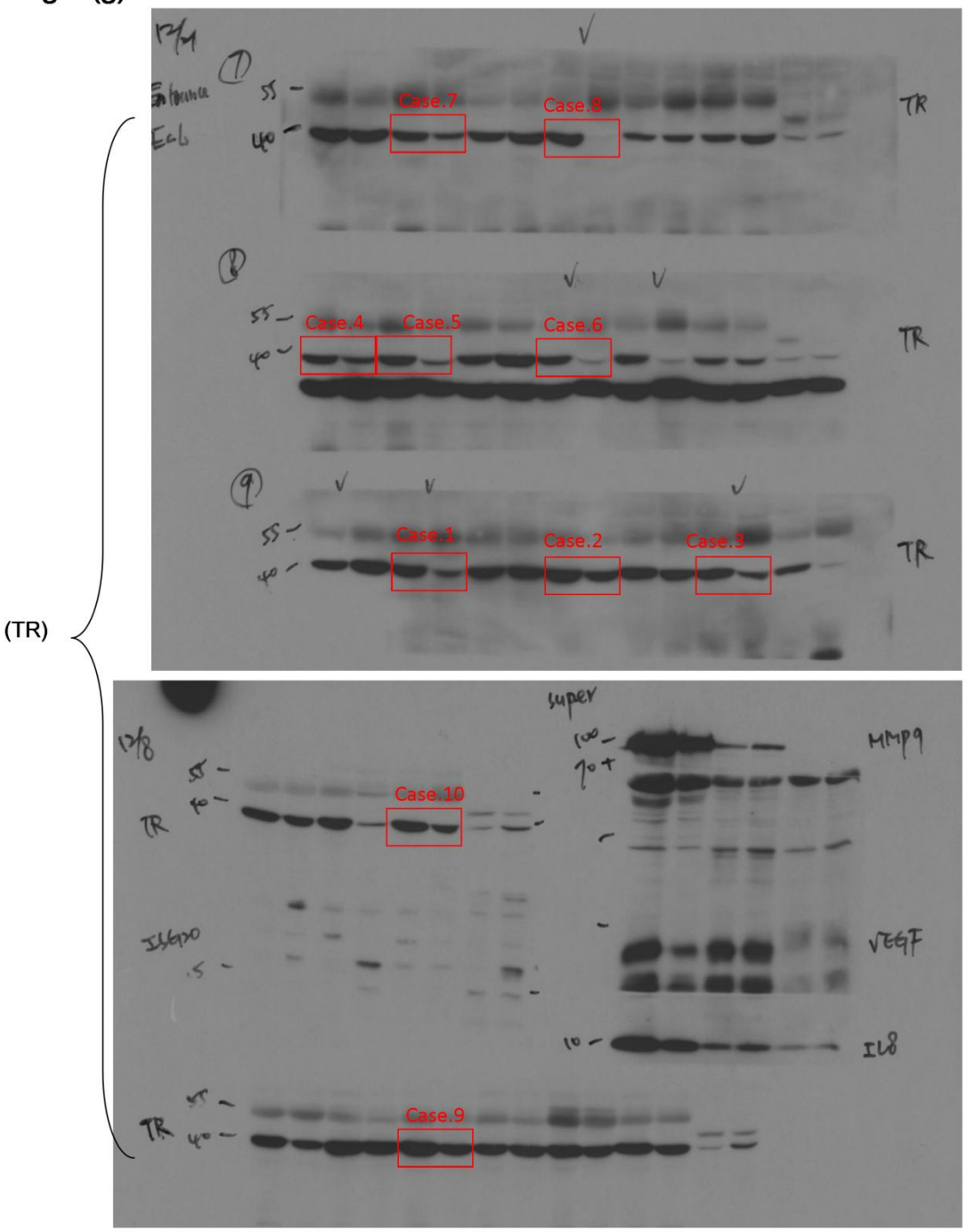

(TR)
Case.7
Case.8
Case.4
Case.5
Case.6
Case.1
Case.2
Case.3
Case.10
Case.9
TR
MMP9
VEGF
IL8
super

Fig.5 (g)

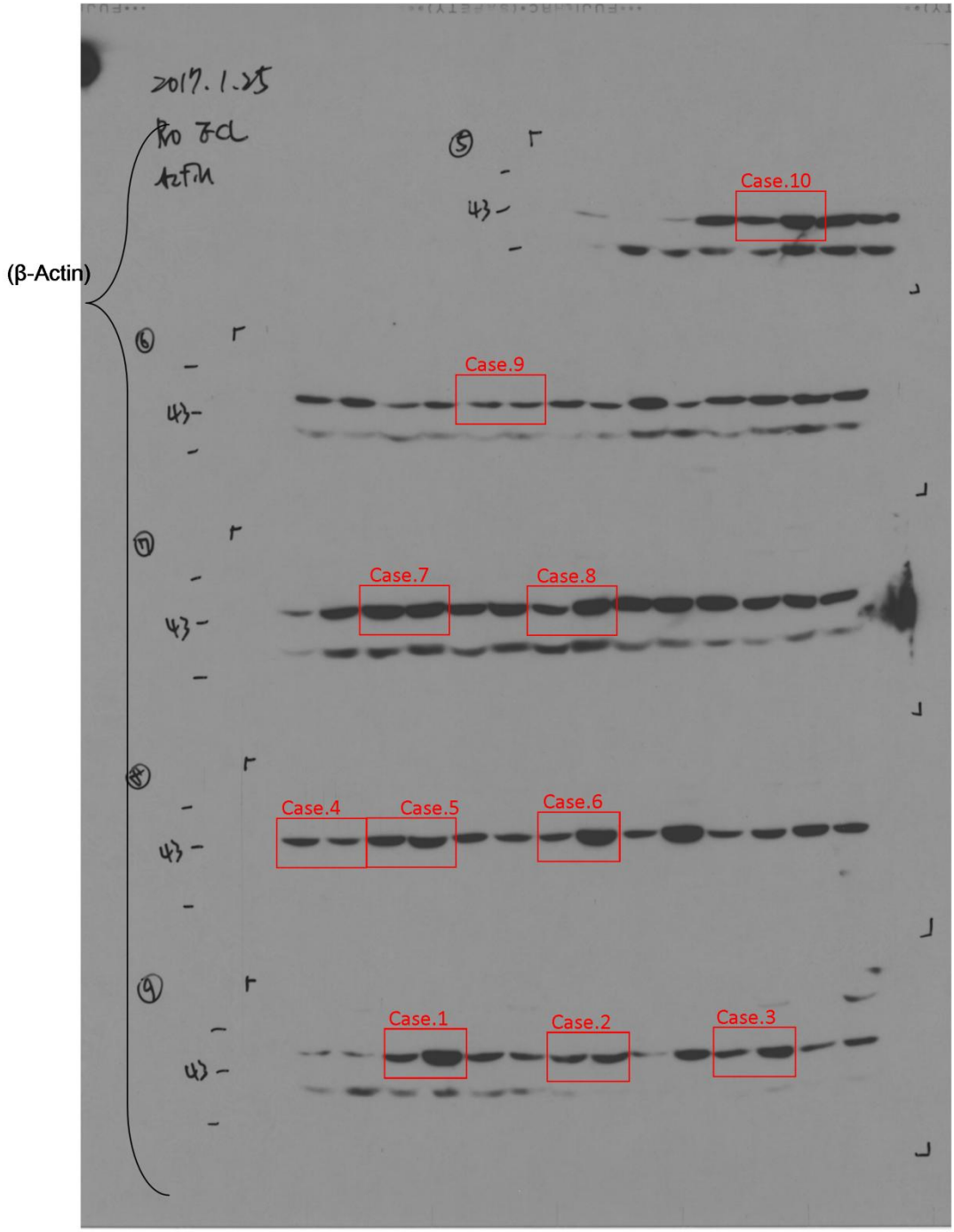

(β-Actin)
Case.10
Case.9
Case.7
Case.8
Case.4
Case.5
Case.6
Case.1
Case.2
Case.3

Fig.S3

(d) (PIM-1, β-Actin)

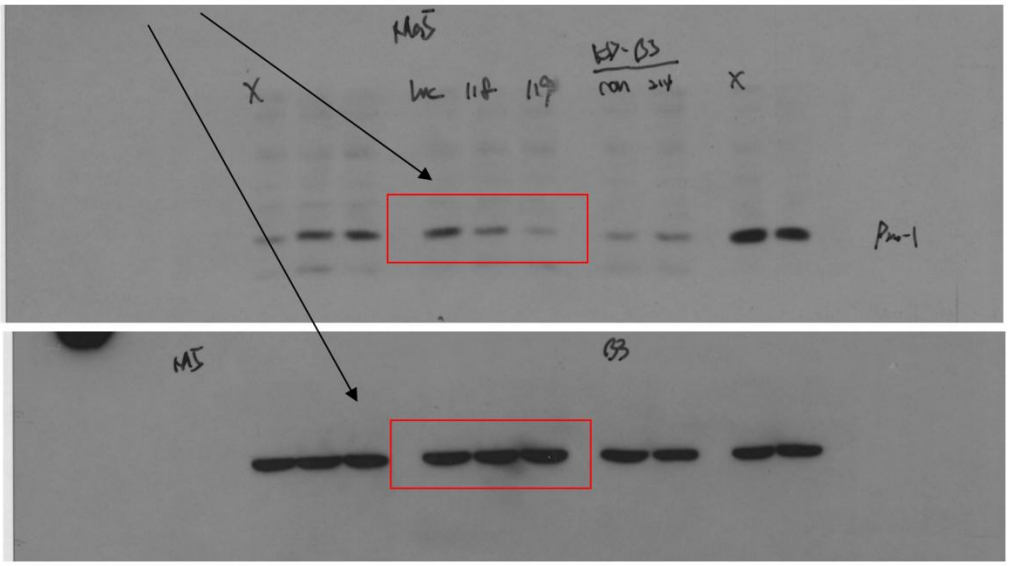


Fig.S3

(b)