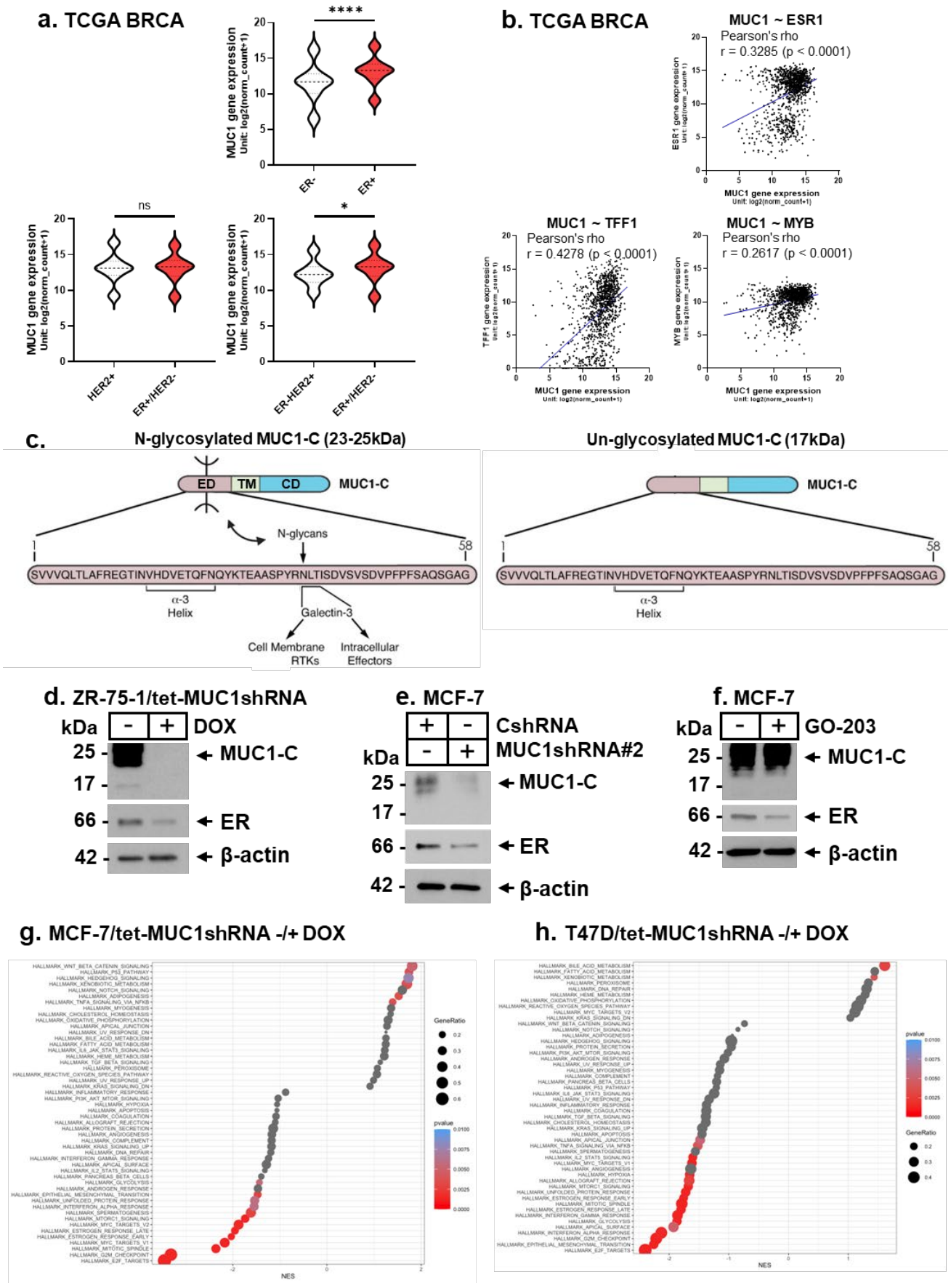
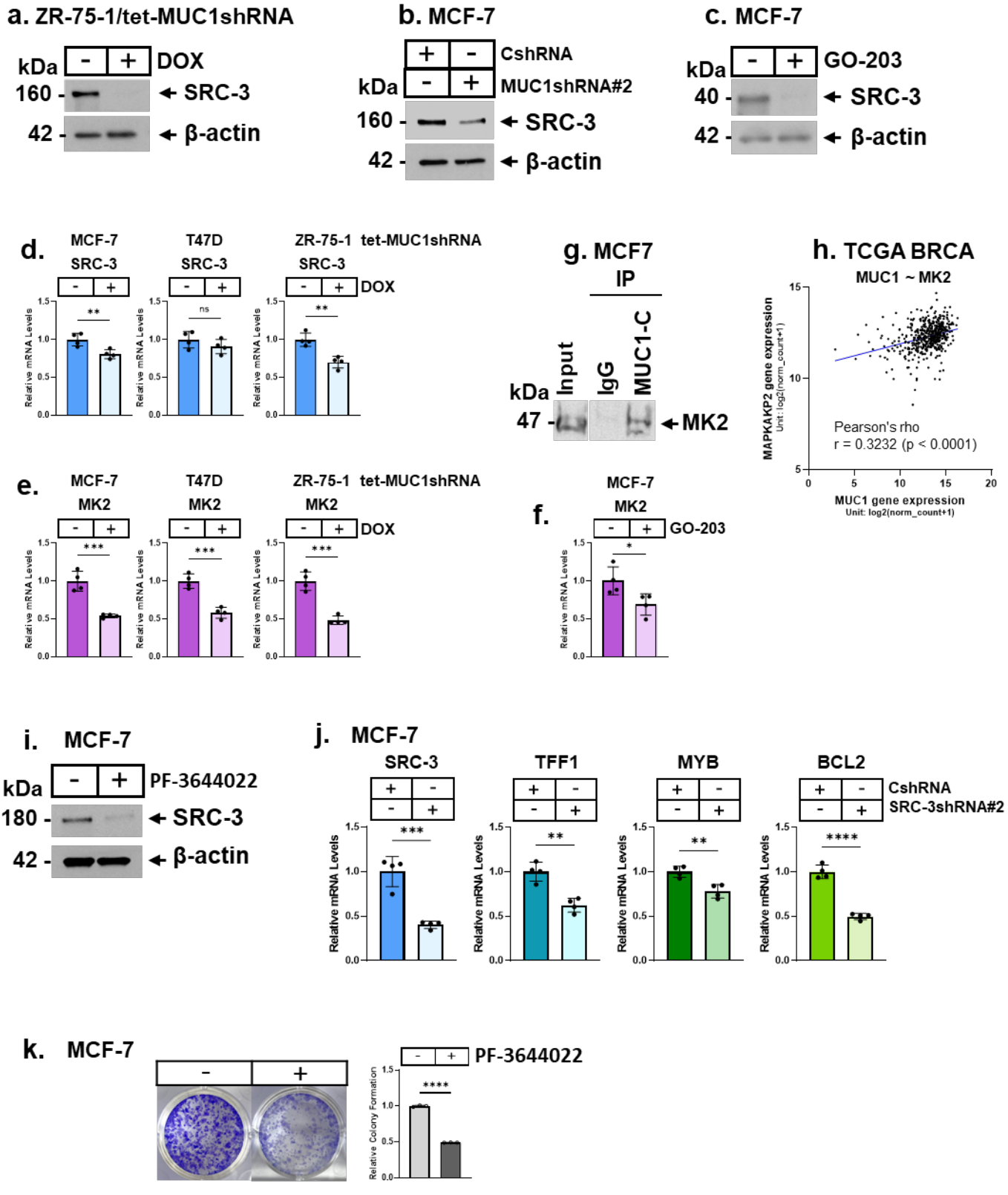
Supplemental Figures

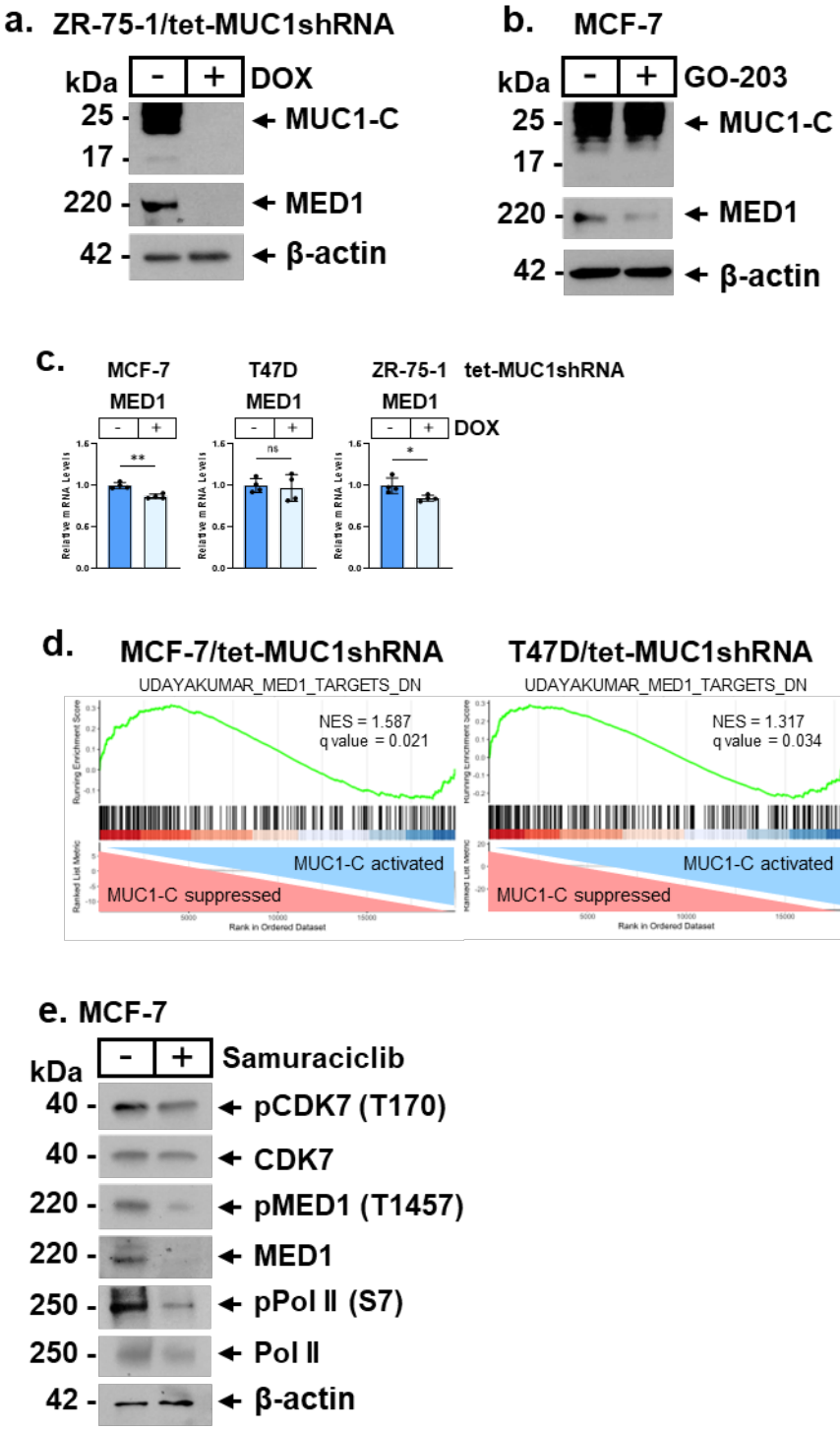


Supplemental Figure S1. Association of MUC1 with ESR1 expression and HALLMARK gene signatures. a. Analysis of the TCGA BRCA dataset for MUC1 expression in (i) ER+ vs ER-, and (ii) HER2+ vs HER2- BCs. **b.** Association of *MUC1* with expression of *ESR1* and ER target genes *TFF1* and *MYB* in the TCGA BRCA dataset. **c.** Schema of MUC1-C structure with the 58 aa extracellular domain (ED), 28 aa transmembrane domain (TD) and 72 aa cytoplasmic domain (CD). Modification of the ED by N-glycosylation at the NLT motif functions as galectin-3 binding site and thereby interactions with RTKs at the cell membrane, as well as intracellular effectors. N-glycosylated MUC1-C is detectable as a ~23-25 kDa glycoprotein (left). Unglycosylated MUC1-C is detectable as a 17 kDa protein (right). The MUC1-C ED alpha-3 helix is the ADC target. **d.** Lysates from ZR-75-1/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were immunoblotted with antibodies against the indicated proteins. **e.** Lysates from MCF-7/CshRNA and MCF-7/MUC1shRNA#2 cells were immunoblotted with antibodies against the indicated proteins. **f.** Lysates from MCF-7 cells treated with 2.5 μM GO-203 for 3 days were immunoblotted with antibodies against the indicated proteins. **g and h.** GSEA of RNA-seq data from MCF-7 (**g**) and T47D (**h**) cells with MUC1-C silencing using the indicated HALLMARK gene signatures.



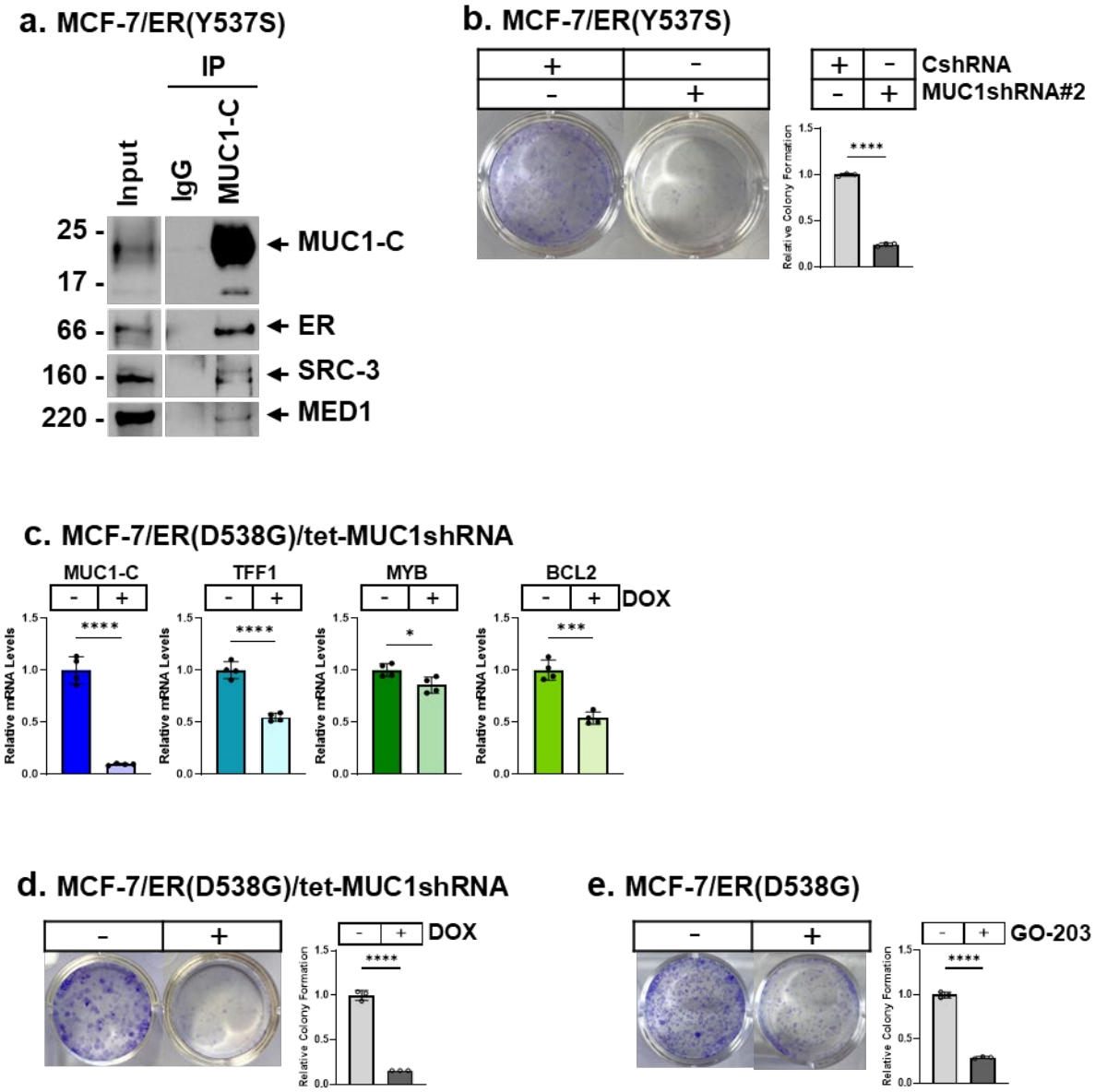
Supplemental Fig. S2. MUC1-C regulates SRC-3 expression. a. Lysates from ZR-75-1/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were immunoblotted with antibodies against the indicated

proteins. **b.** Lysates from MCF-7/CshRNA and MCF-7/MUC1shRNA#2 cells were immunoblotted with antibodies against the indicated proteins. **c.** Lysates from MCF-7 cells treated with 5 μM GO-203 for 4 days were immunoblotted with antibodies against the indicated proteins. **d.** MCF-7/tet-MUC1shRNA, T47D/tet-MUC1shRNA and ZR-75-1/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were analyzed for SRC-3 transcripts by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **e.** The indicated cells expressing tet-MUC1shRNA treated with vehicle or DOX for 7 days were analyzed for MK2 transcripts. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **f.** MCF-7 cells treated with vehicle or 2.5 μM GO-203 for 4 days were analyzed for MK2 transcripts. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **g.** Lysates from E2-stimulated MCF-7 cells were immunoprecipitated with anti-MUC1-C and a control IgG. The input (10% of total lysate) and precipitates were immunoblotted with anti-MK2. **h.** Association of MUC1 and MK2 in the TCGA BRCA dataset. **i.** Lysates from MCF-7 treated with vehicle or 5 μM PF-3644022 for 24 hours were immunoblotted against the indicated proteins. **j.** MCF-7/CshRNA and MCF-7/SRC-3shRNA#2 cells were analyzed for the indicated transcripts by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for CshRNA cells (assigned a value of 1). **k.** MCF-7 cells treated with vehicle or 5 μM PF-3644022 were analyzed for colony formation. Shown are representative photomicrographs of stained colonies (left). The results (mean±SD of three determinations) are expressed as relative colony formation compared to that for vehicle-treated cells (assigned a value of 1)(right).



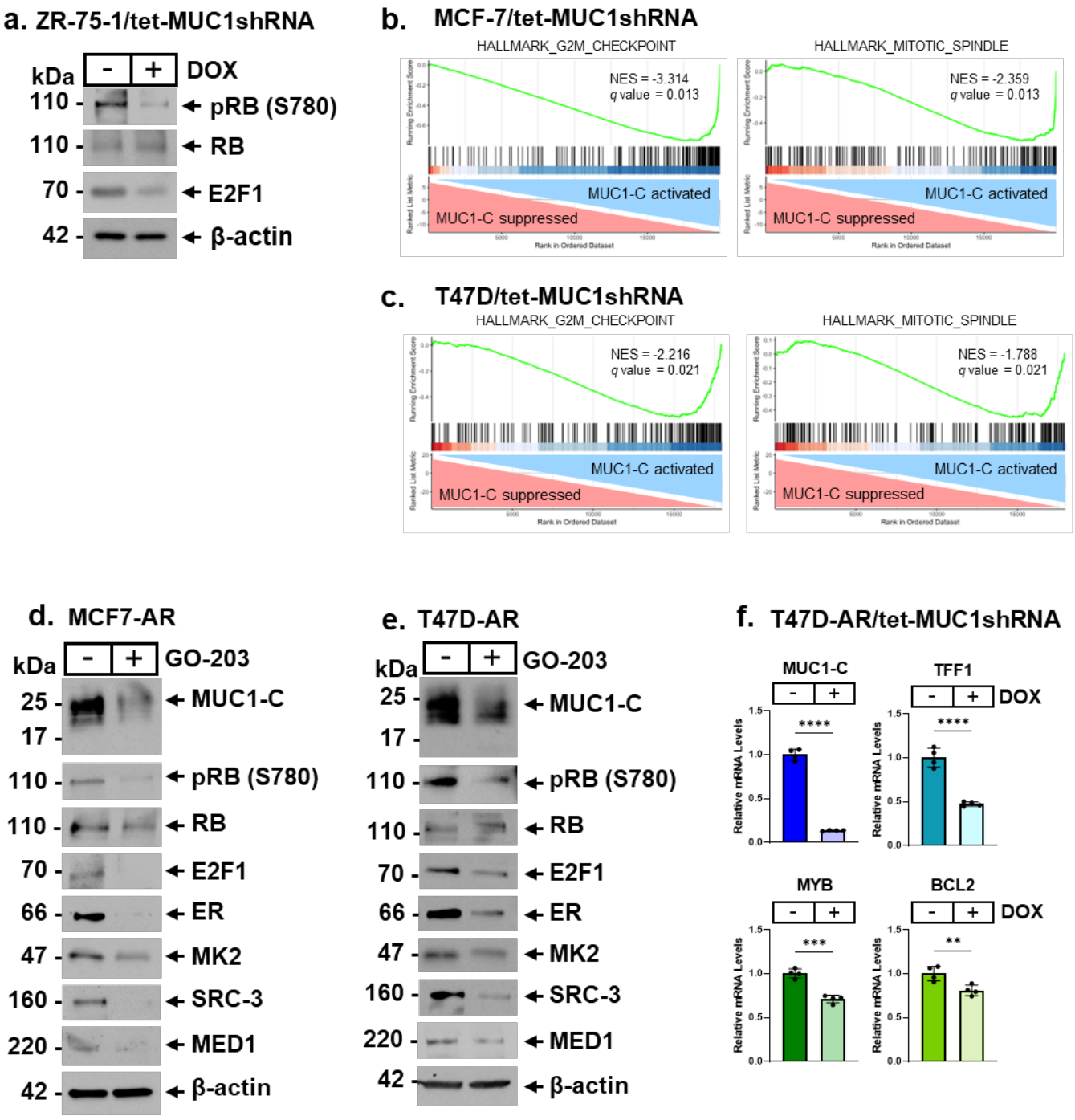
Supplemental Figure S3. MUC1-C regulates MED1 expression. a. Lysates from ZR-75-1/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were immunoblotted with antibodies against the indicated proteins. **b.** Lysates from MCF-7 cells treated with 5 μM GO-203 for 4 days were immunoblotted with antibodies against the indicated proteins. **c.** MCF-7/tet-MUC1shRNA, T47D/tet-MUC1shRNA and ZR-75-1/tet-

MUC1shRNA cells treated with vehicle or DOX for 7 days were analyzed for MED1 transcripts by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **d.** GSEA of RNA-seq data from MCF-7 and T47D cells with MUC1-C silencing using the UDAYAKUMAR MED1 TARGETS DN gene signature. **e.** Lysates from MCF-7 cells treated with vehicle or 1 μM samuraciclib for 48 hours were immunoblotted with antibodies against the indicated proteins.



Supplemental Figure S4. MCF-7(D538G) cells are dependent on MUC1-C for survival. a. Nuclear lysates from MCF-7(Y537S) cells grown in hormone deprived conditions for 2 days and then stimulated with 100 nM E2 for 3 hours were precipitated with anti-MUC1-C or a control IgG. The precipitates and input lysate were immunoblotted with antibodies against the indicated proteins. **b.** MCF-7/ER(Y537S)/CshRNA and MCF-7/ER(Y537S)/MUC1shRNA#2 cells were analyzed for colony formation. Shown are representative photomicrographs of stained colonies (left). The results (mean±SD of three determinations) are expressed as relative colony formation compared to that for CshRNA cells (assigned a value of 1)(right). **c.** MCF-7/ER(D538G)/tet-MUC1shRNA cells treated with

vehicle or DOX for 7 days were analyzed for the indicated transcripts by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **d.** MCF-7/ER(D538G)/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were analyzed for colony formation. Shown are representative photomicrographs of stained colonies (left). The results (mean±SD of three determinations) are expressed as relative colony formation compared to that for vehicle-treated cells (assigned a value of 1)(right). **e.** MCF-7/ER(D538G) cells treated with vehicle or 5 μM GO-203 were analyzed for colony formation. Shown are representative photomicrographs of stained colonies (left). The results (mean±SD of three determinations) are expressed as relative colony formation compared to that for vehicle-treated cells (assigned a value of 1)(right).


a. ZR-75-1/tet-MUC1shRNA
kDa
-
+
DOX
110
pRB (S780)
110
RB
70
E2F1
42
β-actin
b. MCF-7/tet-MUC1shRNA
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q value = 0.013
MUC1-C activated
MUC1-C suppressed
HALLMARK_MITOTIC_SPINDLE
NES = -2.359
q value = 0.013
MUC1-C activated
MUC1-C suppressed
c. T47D/tet-MUC1shRNA
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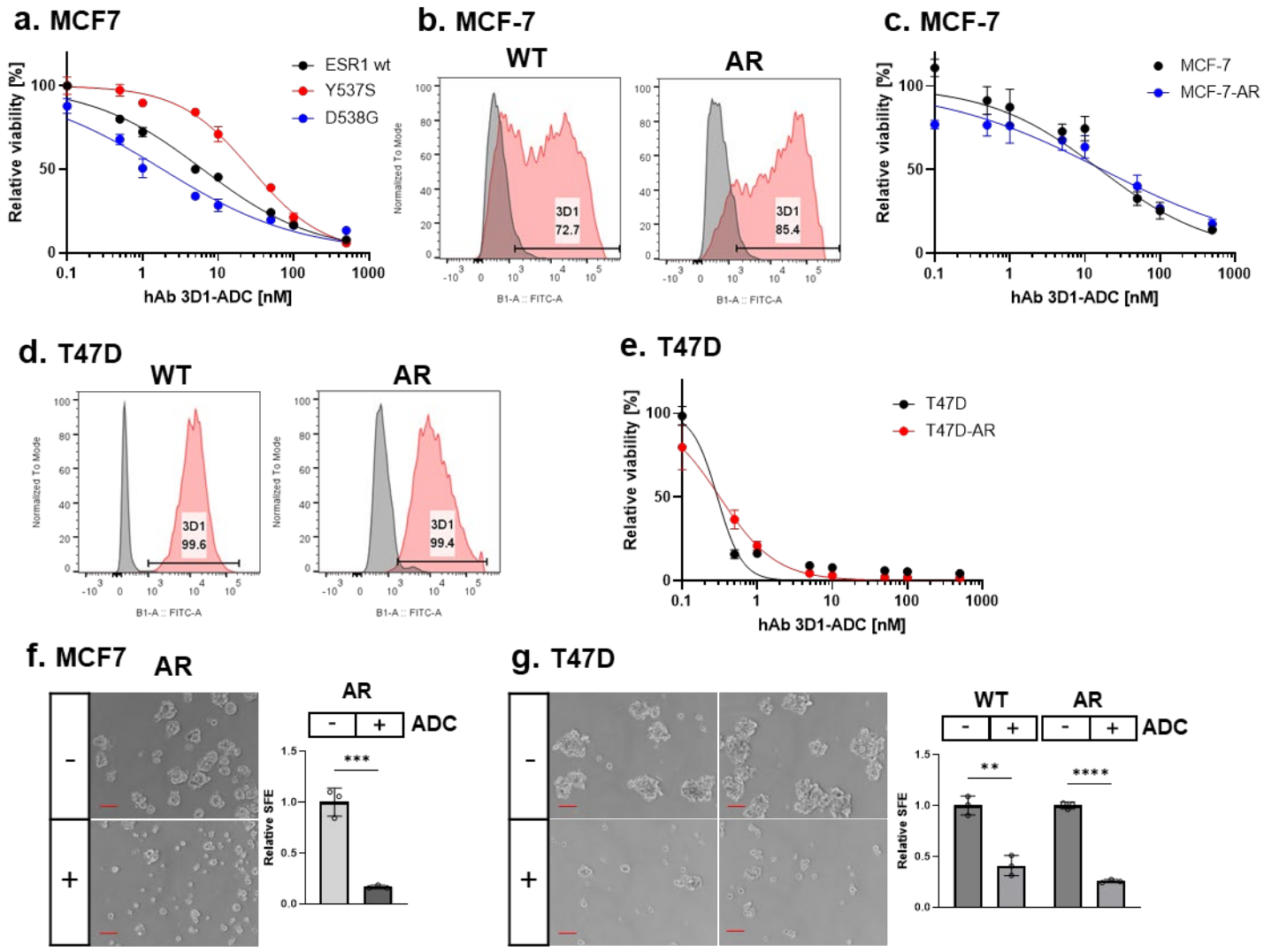
NES = -2.216
q value = 0.021
MUC1-C activated
MUC1-C suppressed
HALLMARK_MITOTIC_SPINDLE
NES = -1.788
q value = 0.021
MUC1-C activated
MUC1-C suppressed
d. MCF7-AR
kDa
GO-203
25
17
MUC1-C
110
pRB (S780)
110
RB
70
E2F1
66
ER
47
MK2
160
SRC-3
220
MED1
42
β-actin
e. T47D-AR
kDa
GO-203
25
17
MUC1-C
110
pRB (S780)
110
RB
70
E2F1
66
ER
47
MK2
160
SRC-3
220
MED1
42
β-actin
f. T47D-AR/tet-MUC1shRNA
MUC1-C
TFF1
DOX
Relative mRNA Levels
****
MYB
BCL2
***
**
g. MCF-7-AR
GO-203
Relative Colony Formation
****
h. T47D-AR
GO-203
Relative Colony Formation
****

Supplemental Figure S5. MCF-7-AR and T47D-AR cells are MUC1-C dependent. a. Lysates from ZR-75-1/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were immunoblotted with antibodies against the indicated proteins. **b and c.** GSEA of RNA-seq data from MCF-7 cells (**b**) and T47D cells (**c**) with MUC1-C silencing using the indicated HALLMARK gene signatures. **d and e.** MCF-7-AR (**d**) and T47D-AR (**e**) cells treated with vehicle or 5 μM GO-203 for 3 days were immunoblotted with antibodies against the indicated proteins. **f.** T47D-AR/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were analyzed for the indicated transcripts by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **g and h.** MCF-7-AR (**g**) and T47D-AR (**h**) cells treated with vehicle or 5 μM GO-203 were analyzed for colony formation. Shown are representative photomicrographs of stained colonies (left). The results (mean±SD of three determinations) are expressed as relative colony formation compared to that for vehicle-treated cells (assigned a value of 1)(right).



Supplemental Figure S6. Cell surface MUC1-C expression and anti-MUC1-C ADC dose-response curves. a. The designated MCF-7 cells were treated with the indicated concentrations of anti-MUC1-C ADC for 7 days and analyzed for cell viability by Alamar Blue staining. The results (mean±SD of three determinations) are expressed as relative cell viability (% control) compared with that for untreated cells. **b.** The designated MCF-7 cells were analyzed for cell surface MUC1-C expression by flow cytometry. **c.** The designated MCF-7 cells were treated with the indicated concentrations of anti-MUC1-C ADC for 7 days and analyzed for cell viability by Alamar Blue staining. The results (mean ± SD of three determinations) are expressed as relative cell viability (% control) compared with that for untreated cells. **d.** The designated T47D cells were analyzed for cell surface MUC1-C

expression by flow cytometry. **e.** The designated T47D cells were treated with the indicated concentrations of anti-MUC1-C ADC for 7 days and analyzed for cell viability by Alamar Blue staining. The results (mean ± SD of three determinations) are expressed as relative cell viability (% control) compared with that for untreated cells. **f and g.** The designated MCF-7 (**f**) and T47D (**g**) cells treated with 100 nM anti-MUC1-C ADC were analyzed for tumorsphere formation. Shown are representative photomicrographs of tumorspheres (left). The results (mean±SD of three determinations) are expressed as relative sphere formation efficiency (SFE) compared to that for control cells (assigned a value of 1)(right).

Supplemental Tables

Supplemental Table S1. Common down-regulated ERGs and LRGs in MCF-7 and T47D cells with MUC1-C silencing.

ERGs	**LRGs**
CA12	CA12
CD44	CD44
ELOVL2	KIF20A
GFRA1	MYB
GREB1	RAB31
MUC1	SLC7A5
MYB	TOP2A
MYBL1	
RAB31	
SLC7A5	
UGCG	

Supplemental Table S2. Primers used for qRT-PCR.

MUC1-C	**FWD**	TACCGATCGTAGCCCCTATG
	REV	CTCACCAGCCCAAACAGG
TFF1	**FWD**	CCCTCCCAGTGTGCAAATAAG
	REV	GAACGGTGTCGTCGAAACAG
MYB	**FWD**	GAAAGCGTCACTTGGGGAAAA
	REV	TGTTCGATTCGGGAGATAATTGG
BCL2	**FWD**	GGTGGGGTCATGTGTGTGG
	REV	CGGTTCAGGTACTCAGTCATCC
SRC-3	**FWD**	AGACGGGAGCAGGAAAGTAAA
	REV	GTAAAAGCGGTCCTAAGGAGTC
MK2	**FWD**	CGCAGTTCCACGTCAAGTC
	REV	GGGCGAATTTCTCCTGGGTC
MED1	**FWD**	CTGGAACGGCTCCATGCAA
	REV	CTTCTCCATGACTTGACGCAC
β-actin	**FWD**	GATGAGATTGGCATGGCTTT
	REV	CACCTTCACCGTTCCAGTTT

Supplemental Table S3. Clinical characteristics of patients with HR+/HER2- BC tumors stained for MUC1-C expression by IHC.

Data are expressed as number (n) and percentage (%) of tumors unless otherwise specified.

a. HR+/HER2- BC surgical specimens (n=18)

Age at surgery (mean ± SD)	53.4 ± 14.0
male/female	0(0) / 18(100)
T is/1/2/3 N 0/1 M 0/1	0(0) / 13(72) / 4(22) / 1(5.6) 18(100) / 0(0) 18(100) / 0(0)
Histology Invasive Ductal Carcinoma/others	20(100) / 0(0)
Neoadjuvant treatment yes/no	0(0) / 18(100)
MUC1-C staining score Apical 0/1+/2+/3+ Membrane 0/1+/2+/3+ Cytoplasm 0/1+/2+/3+	 2(11) / 0(0) / 3(17) / 13(72) 4(22) / 7(39) / 7(39) / 0(0) 1(5.6) / 8(44) / 7(39) / 3(17)

b. ER mutant recurrent/metastatic tumor biopsy specimens (n=11)

Age at biopsy (mean ± SD)	51.2 ± 10.7
male/female	0(0) / 11(100)
ESR1 mutation Y537S / L536R	10(91) / 1(9.1)
Biopsy site skin/lymph node/liver	4(36) / 3(27) / 4(36)
Previous ET yes/no	11(100) / 0(0)
MUC1-C staining score Apical 0/1+/2+/3+ Membrane 0/1+/2+/3+ Cytoplasm 0/1+/2+/3+	 2(18) / 1(9.1) / 2(18) / 6(55) 2(18) / 2(18) / 5(45) / 2(18) 0(0) / 4(36) / 4(36) / 3(27)

c. CDK4/6i refractory tumor biopsy specimens (n=5)

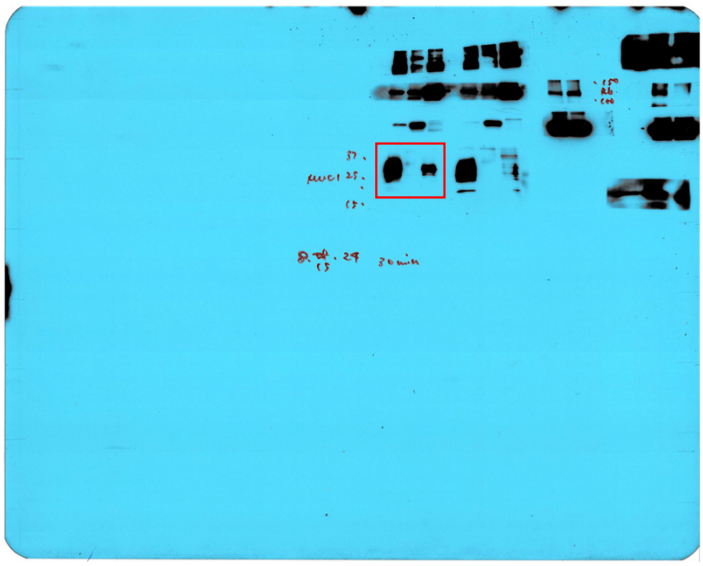
CDK4/6i starting age (mean ± SD)	51.2 ± 10.14
male/female	0(0) / 5(100)
CDK4/6i used Palbociclib/Abemaciclib/sequencial(P→A)	2(40) / 0(0) / 3(60)
ET used in combination with CDK4/6i Fulvestrant/Letrozole	3(60) / 2(40)
CDK4/6i administration duration (median, days)	224
Previous ET yes/no	4(80) / 1(20)
Previous chemotherapy yes/no	3(60) / 2(40)
MUC1-C staining score pre-CDK4/6i Apical 0/1+/2+/3+ Membrane 0/1+/2+/3+ Cytoplasm 0/1+/2+/3+	 1(20) / 0(0) / 1(20) / 3(60) 3(60)/ 0(0) / 1(20) / 1(20) 2(40) / 1(20) / 2(40) /0(0)
MUC1-C staining score post-CDK4/6i Apical 0/1+/2+/3+ Membrane 0/1+/2+/3+ Cytoplasm 0/1+/2+/3+	 0(0) / 2(40) / 2(40) / 1(20) 0(0) / 1(20) / 3(60) / 1(20) 0(0) / 0(0) / 0(0) / 5(100)

Supplemental Figure S7

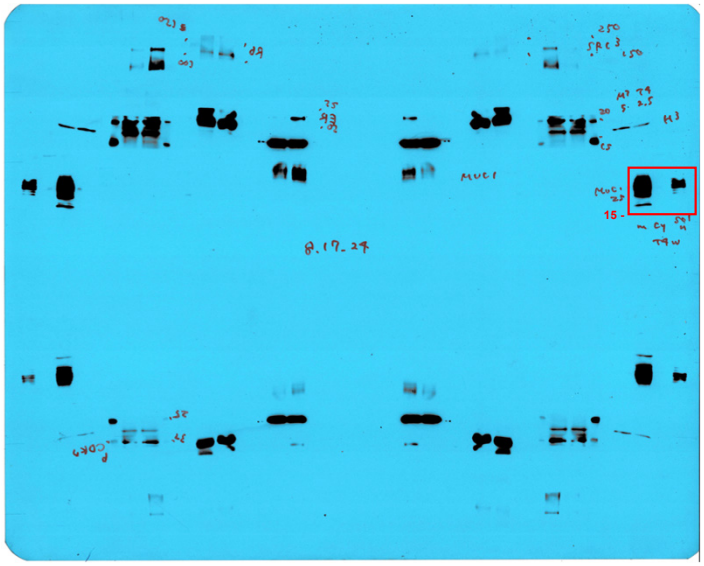
Uncropped images of western blots

Figure 1c

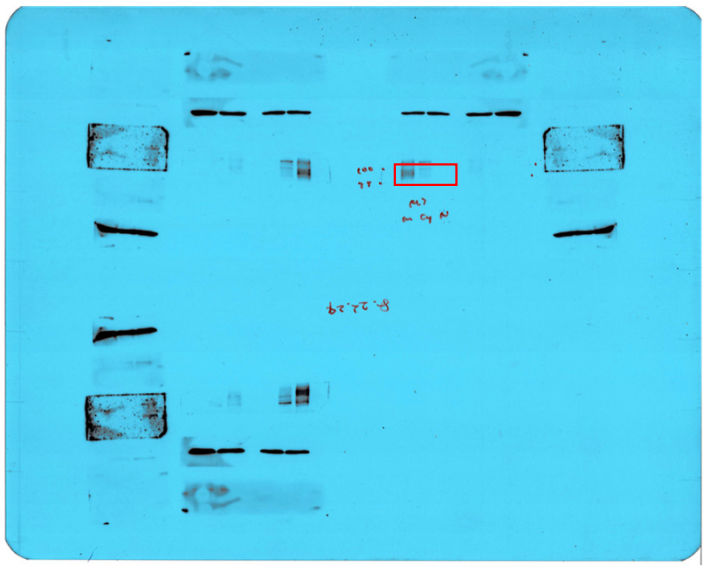
MUC1-C MCF-7



MUC1-C T47D



Na,K-ATPase MCF-7



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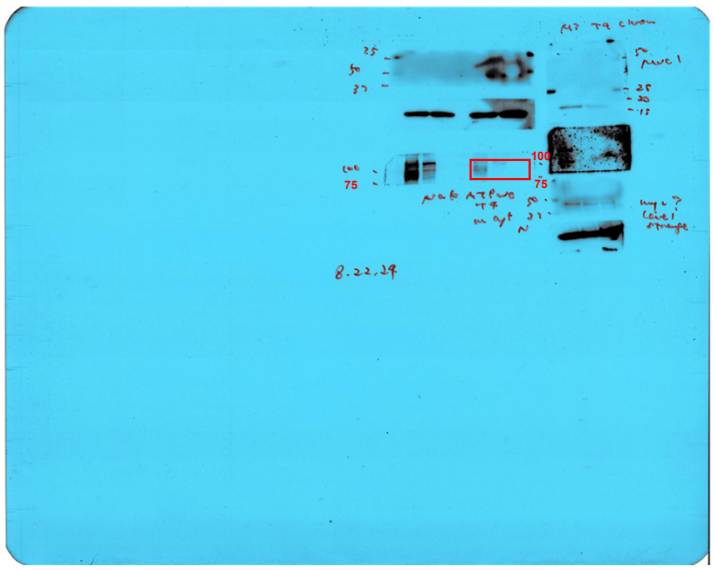


α-tubulin MCF-7 T47D

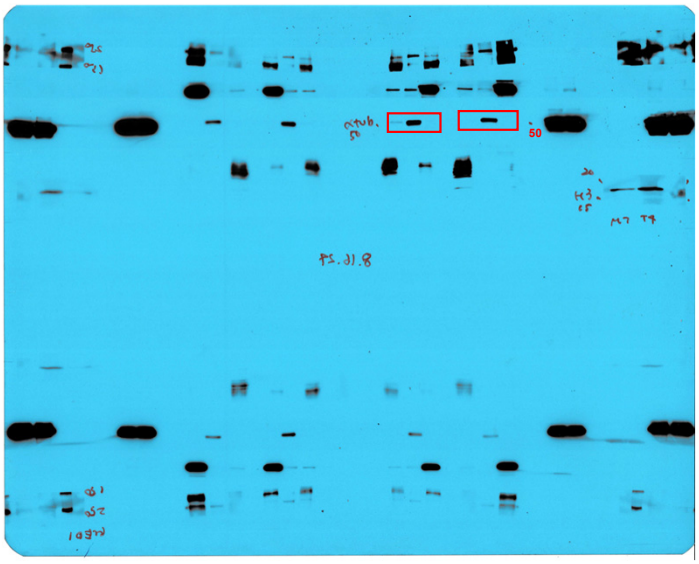
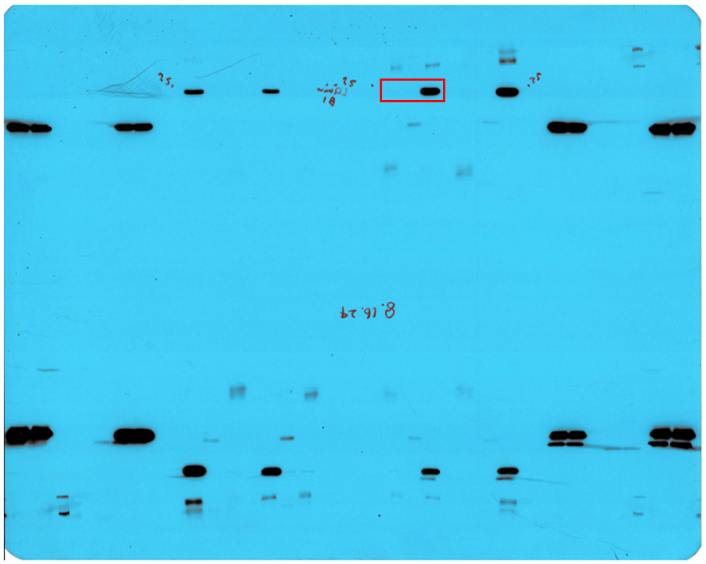


Figure 1c (continued)

LaminB1 MCF-7



LaminB1 T47D

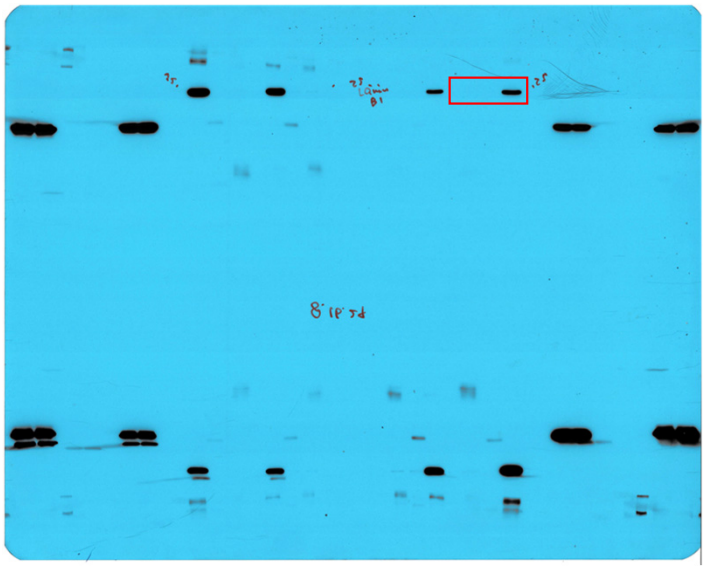
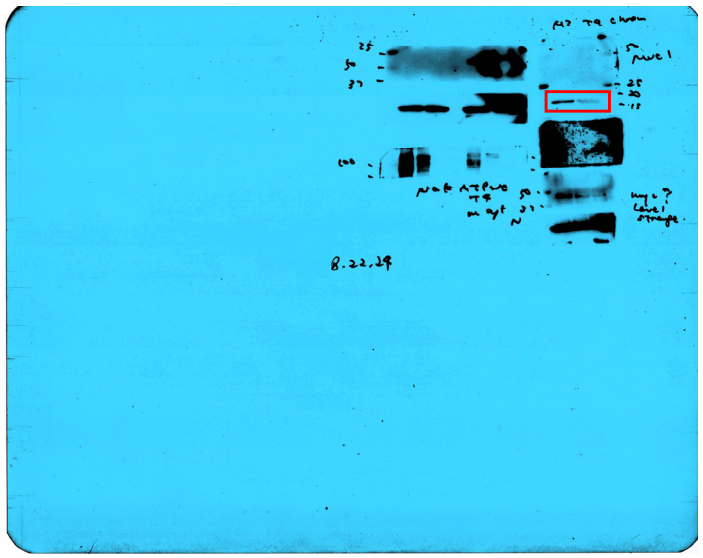


Figure 1d

MUC1-C



H3

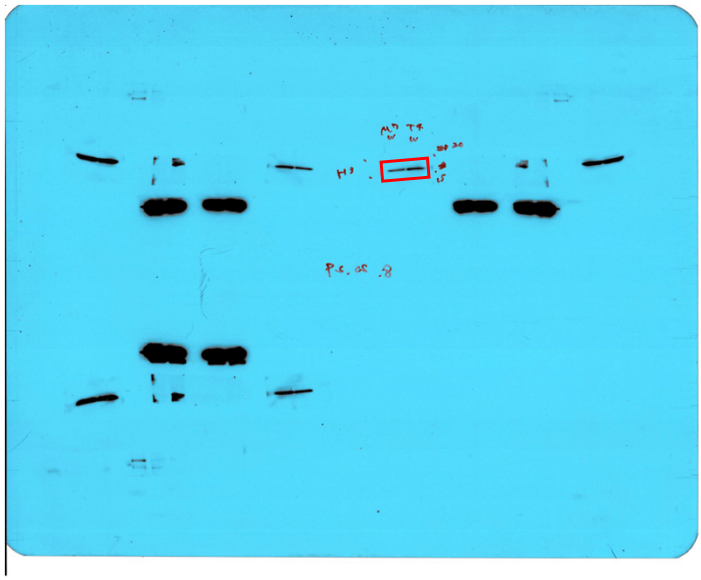
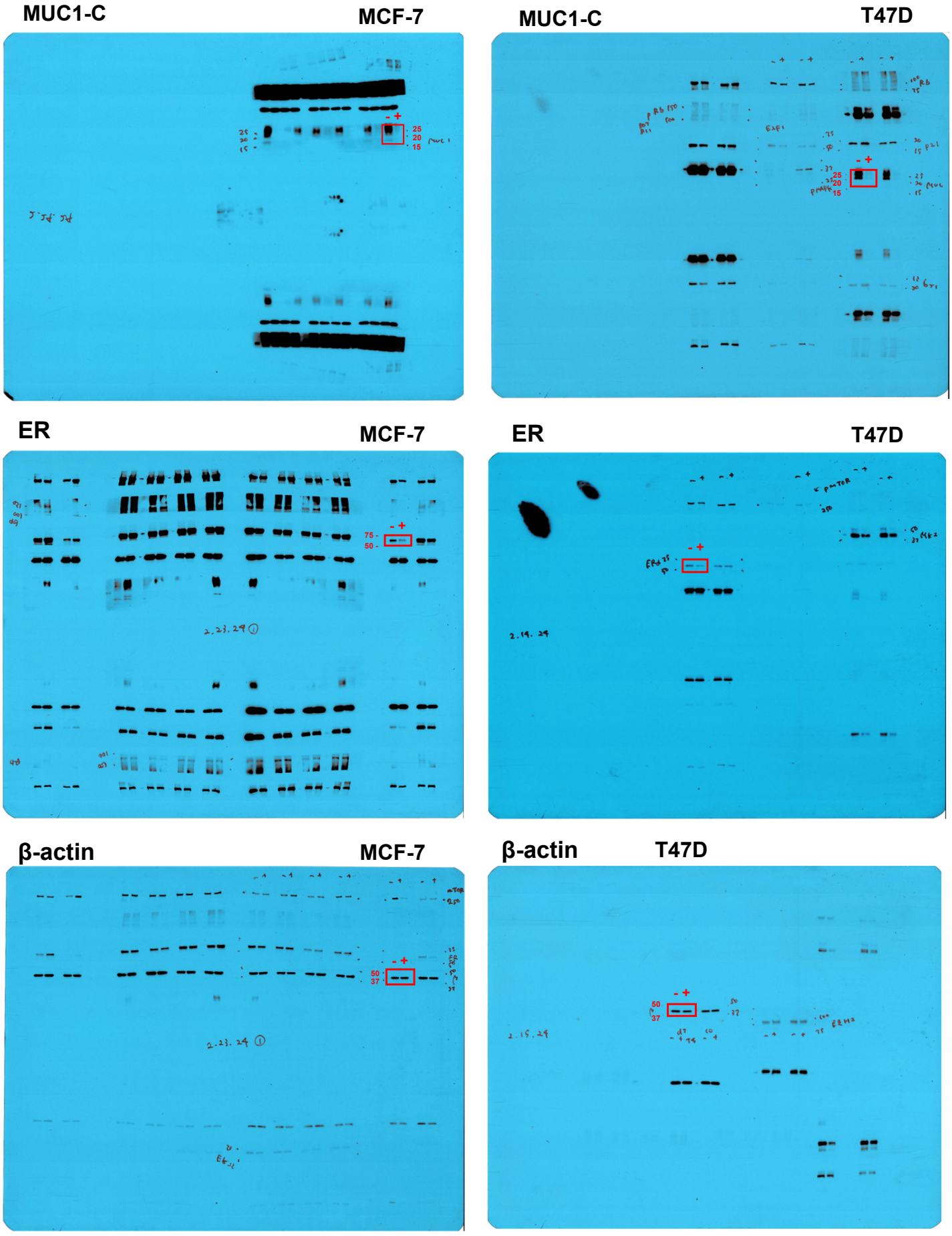


Figure 1e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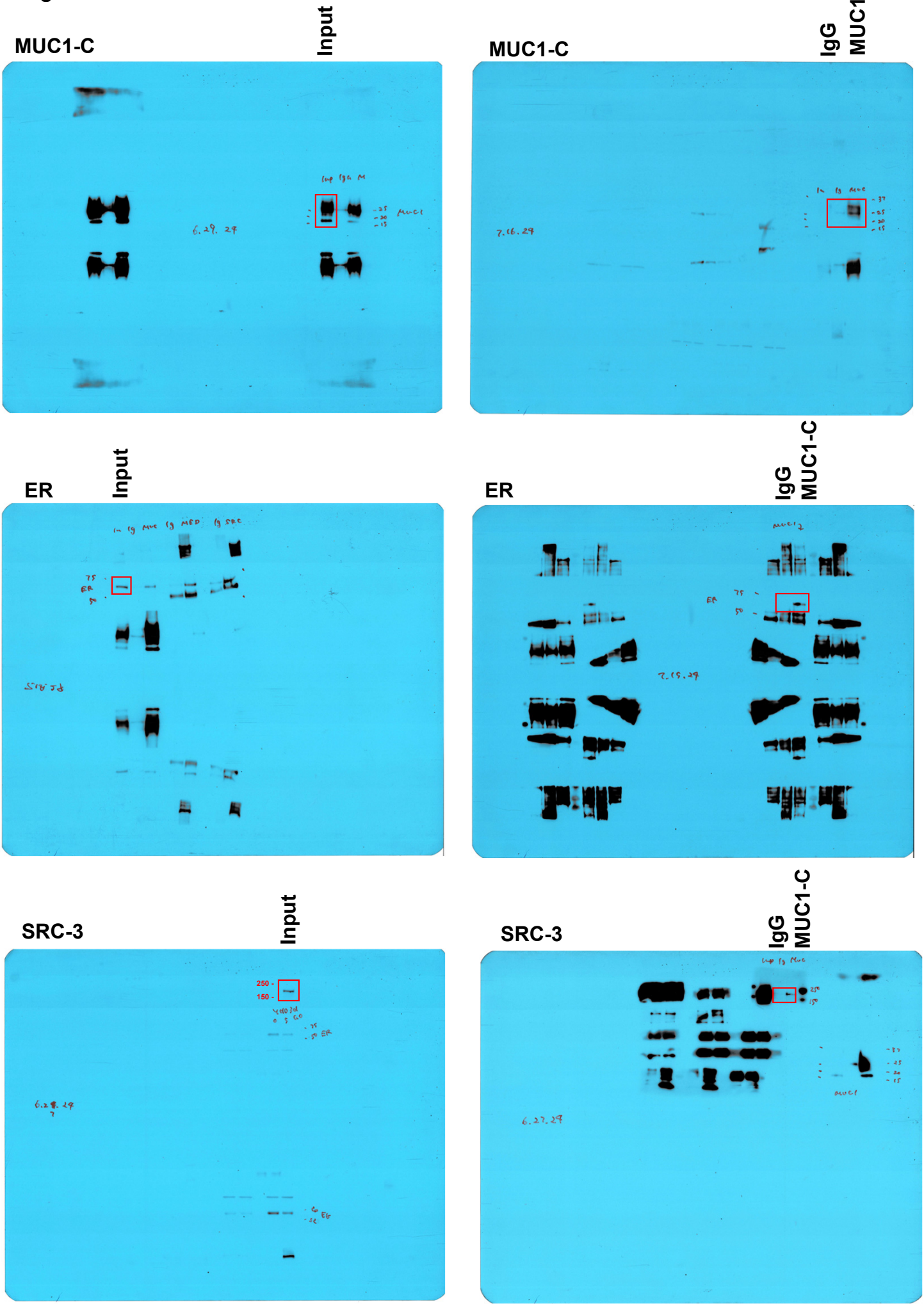
MCF-7
MUC1-C
T47D
ER
MCF-7
ER
T47D
β-actin
MCF-7
β-actin
T47D

Figure 2a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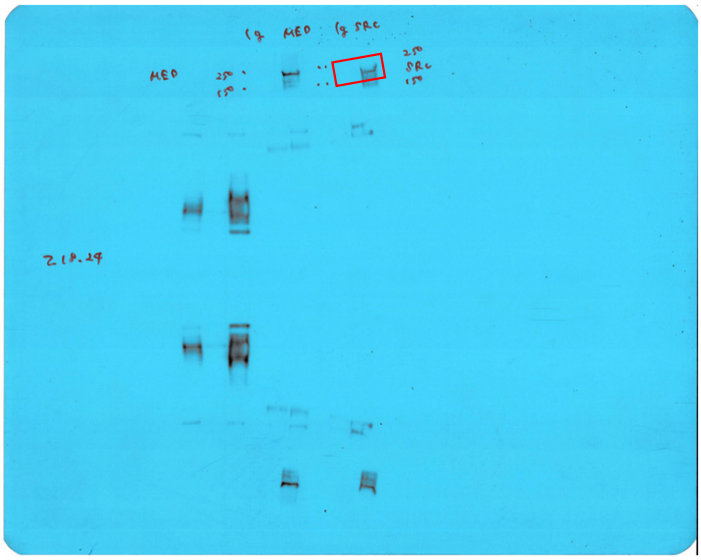
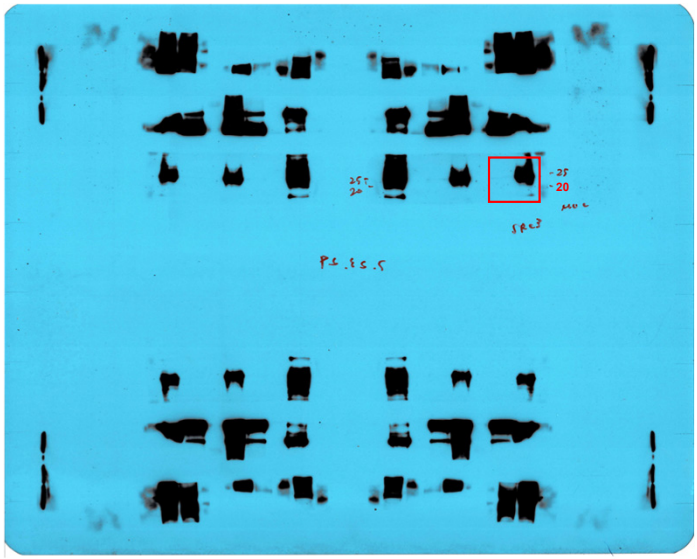
SRC-3
IgG
MUC1-C

Figure 2b

SRC-3



MUC1-C



ER

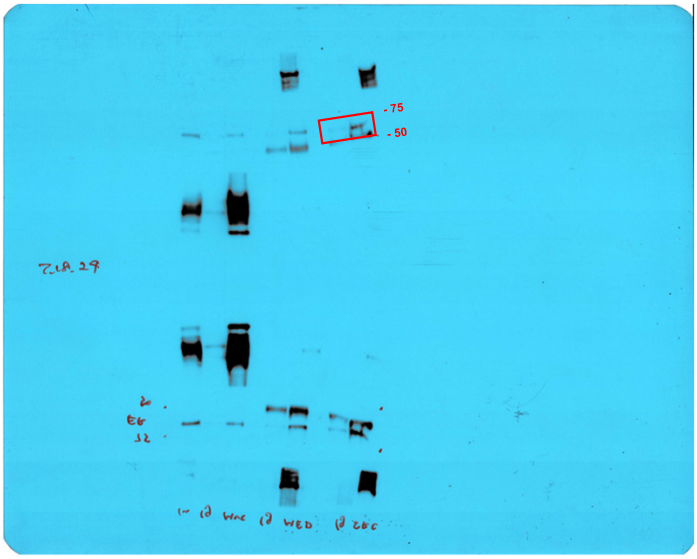


Figure 2c

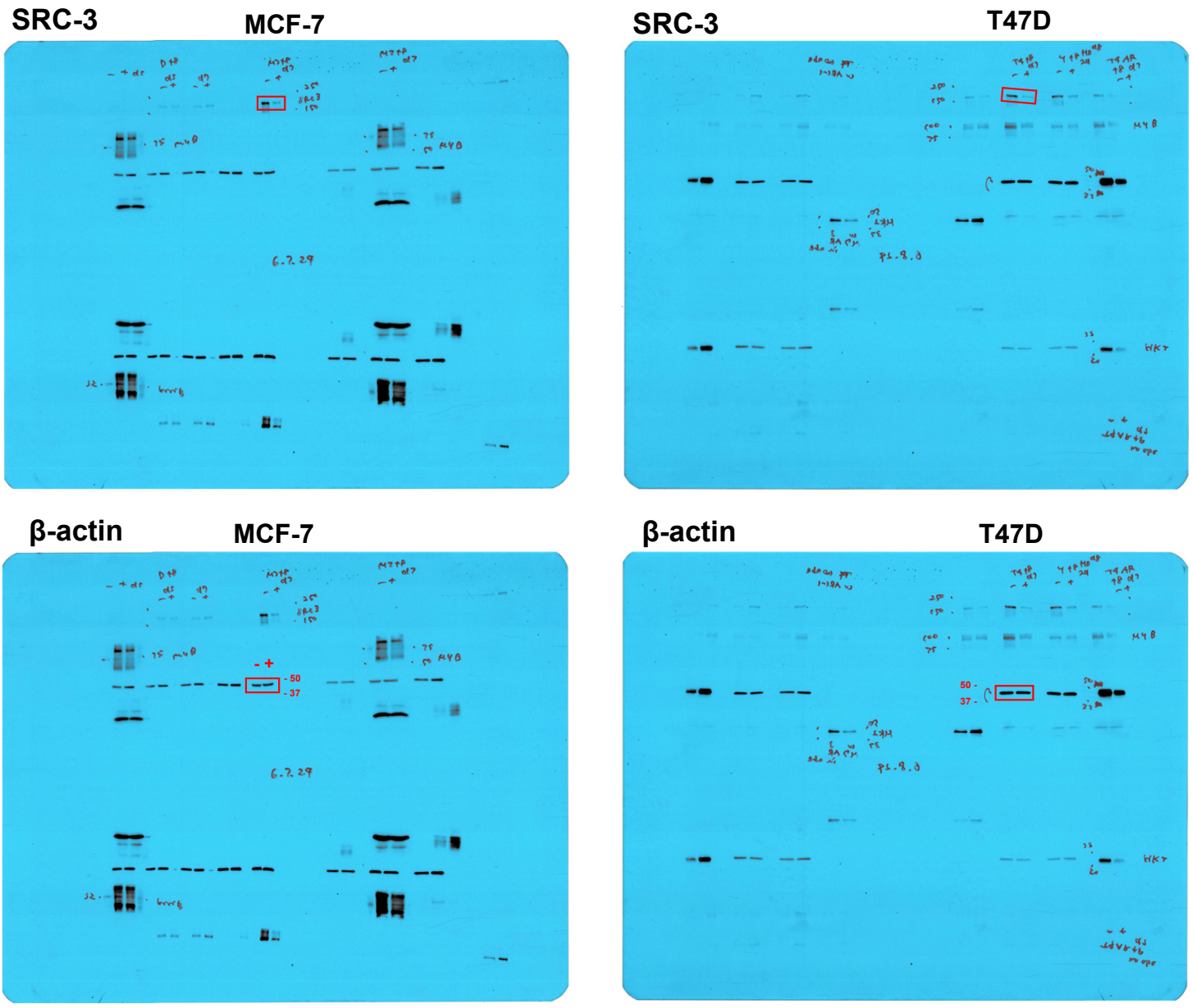
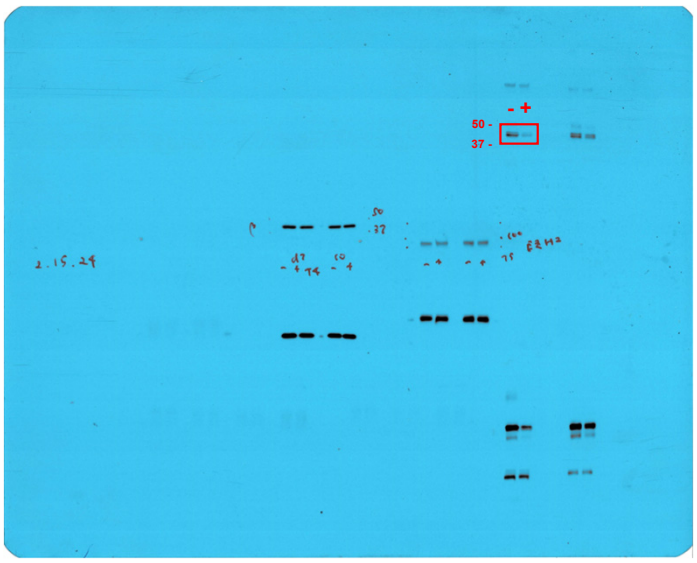
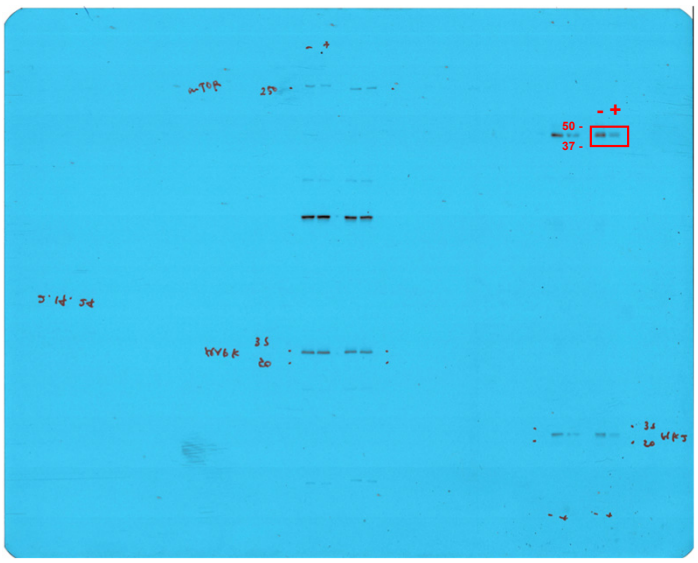


Figure 2d

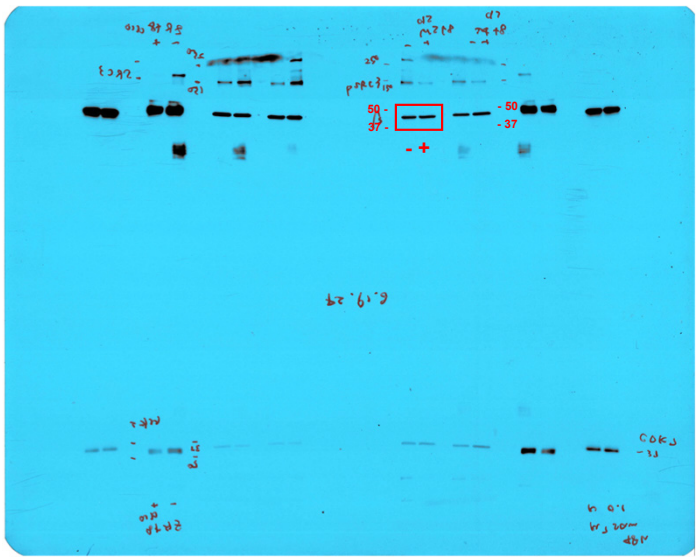
MK2 MCF-7



MK2 T47D



β-actin MCF-7



β-actin T47D

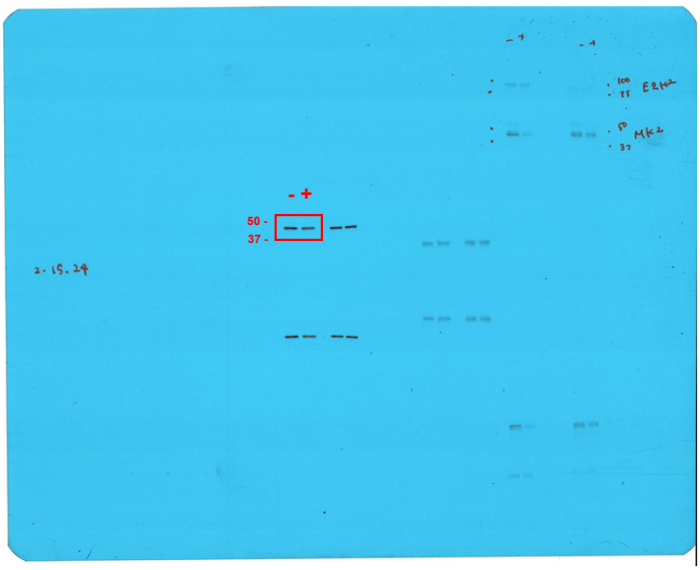
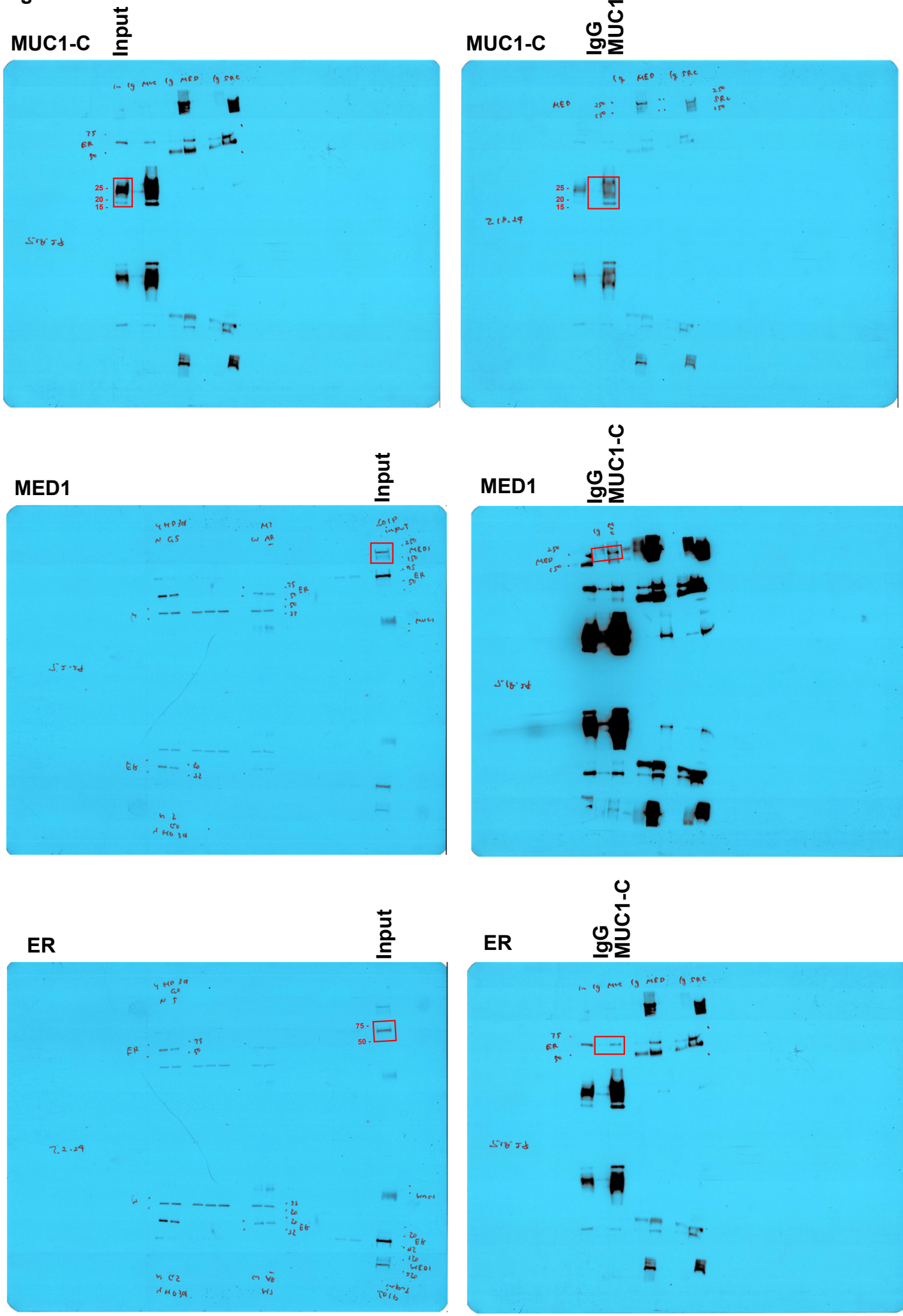


Figure 3a


MUC1-C
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IgG
MUC1-C
MED1
Input
MED1
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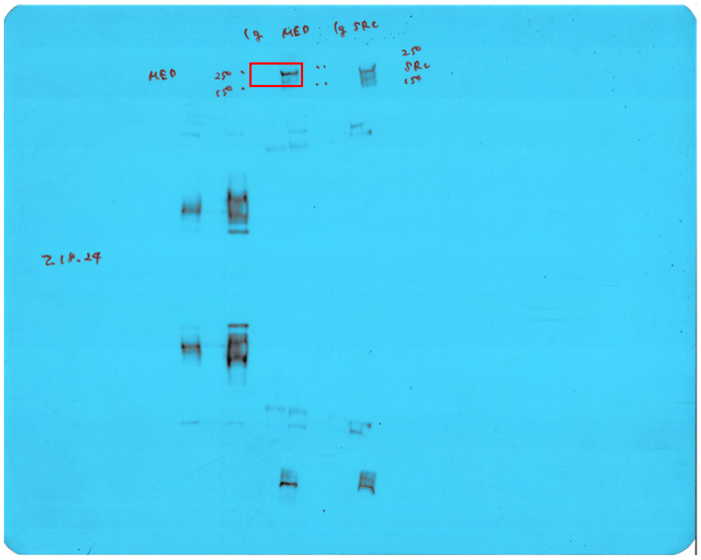
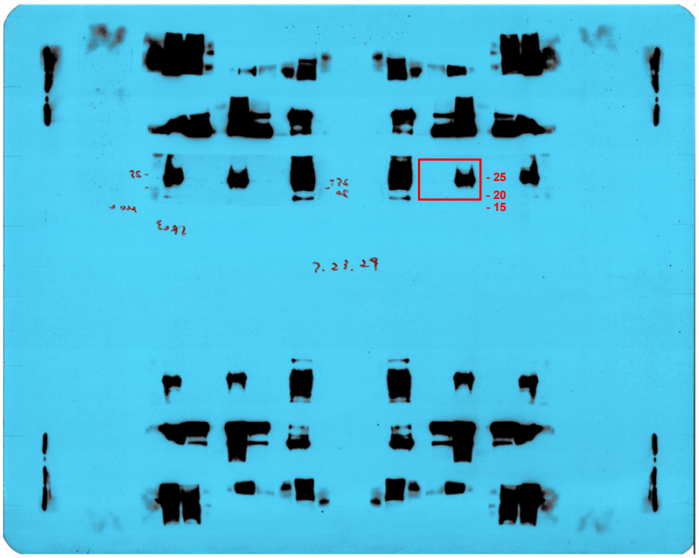
ER
Input
ER
IgG
MUC1-C

Figure 3b

MED1



MUC1-C



ER

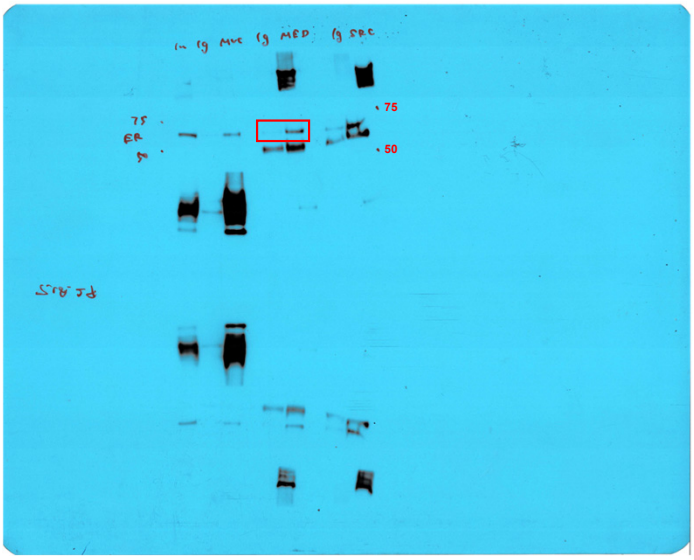
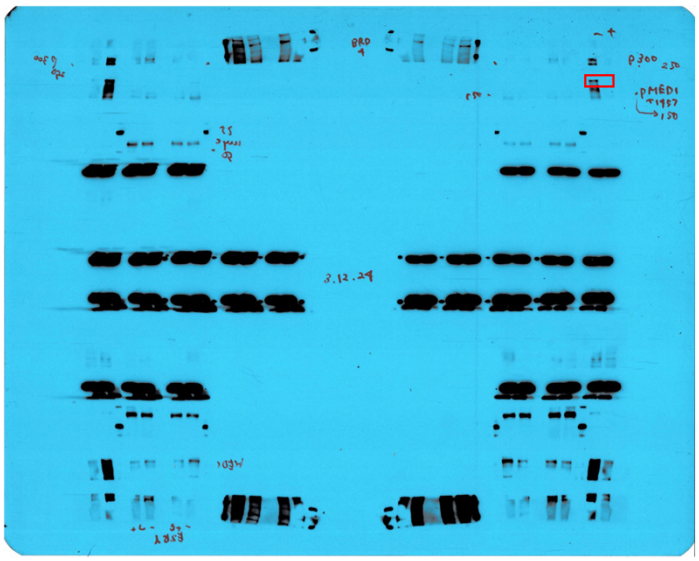
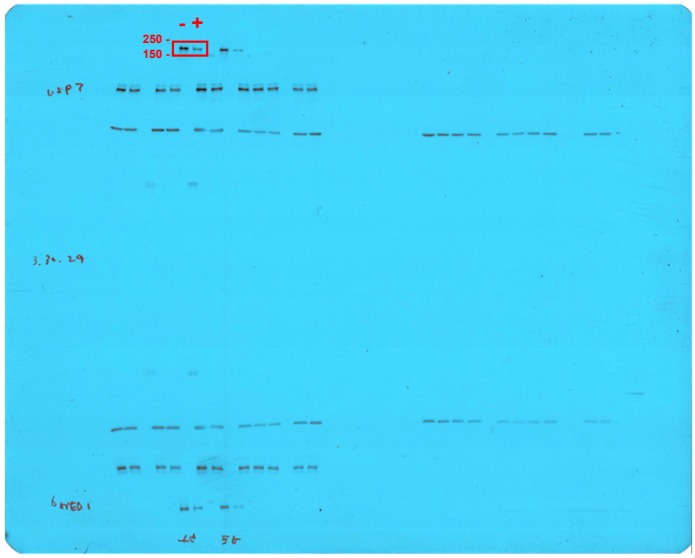


Figure 3c

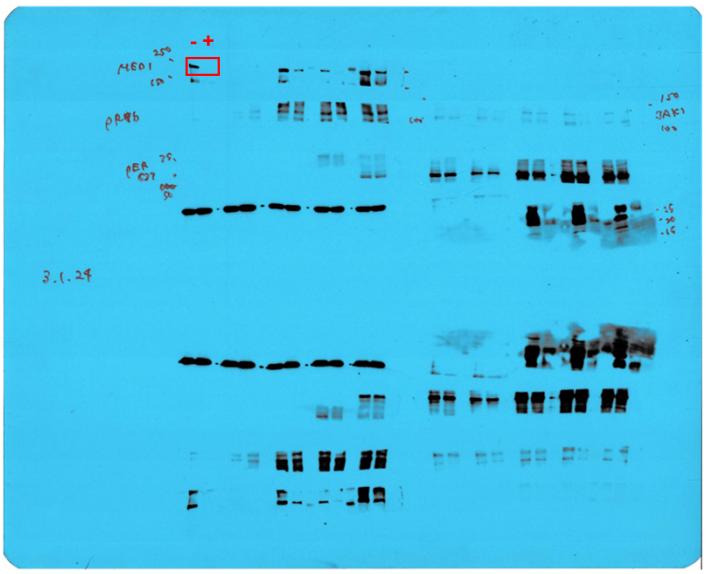
pMED1 (T1457) MCF-7



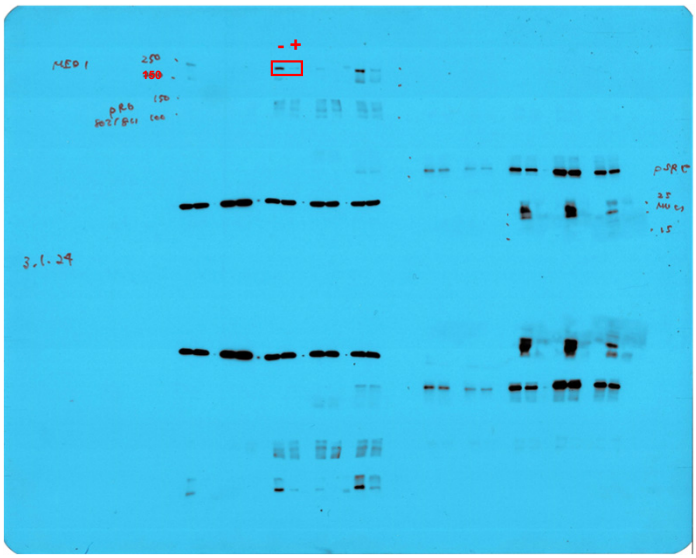
pMED1 (T1457) T47D



MED1 MCF-7



MED1 T47D



β-actin MCF-7 T47D

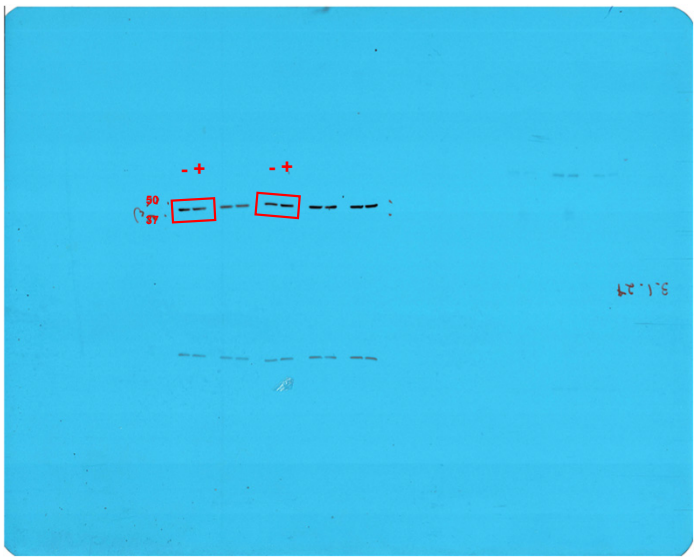
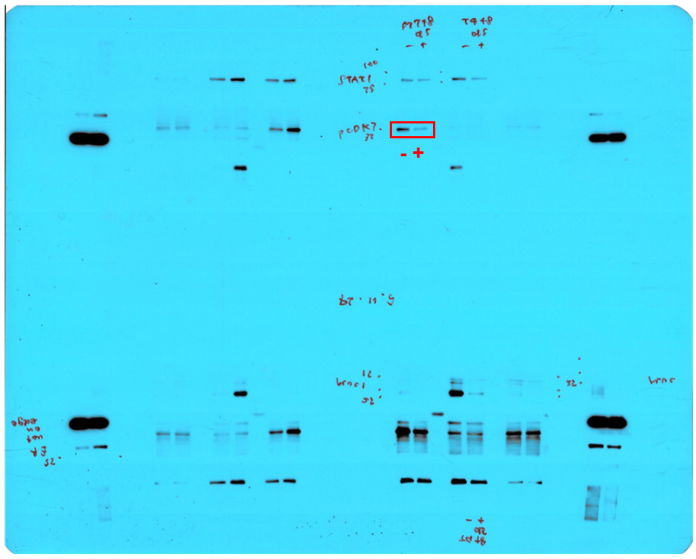
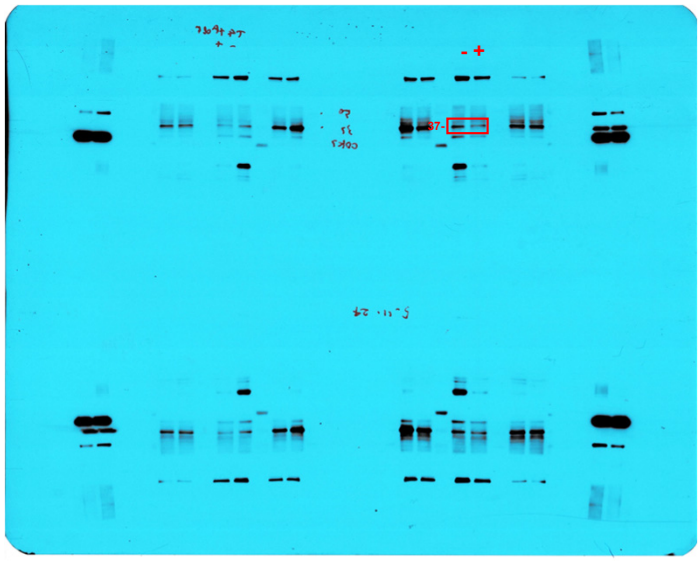


Figure 3d

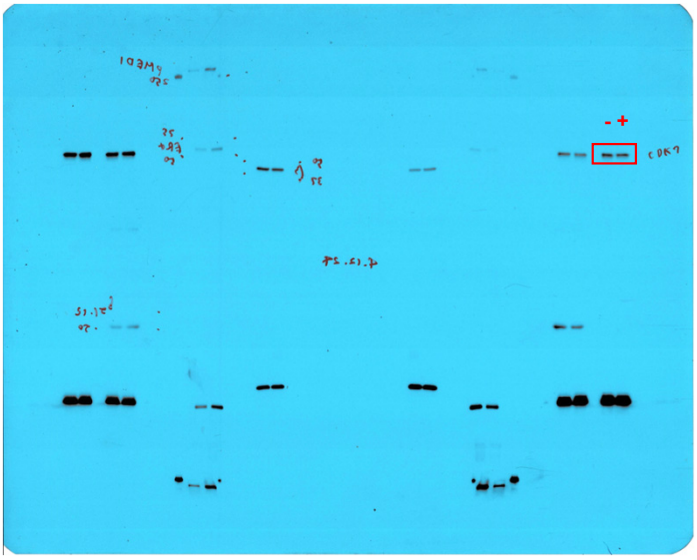
pCDK7(T170) MCF-7



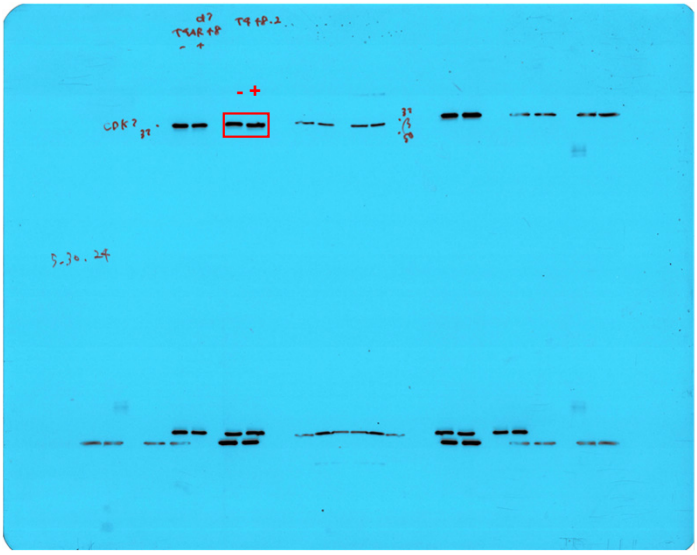
pCDK7(T170) T47D



CDK7 MCF-7



CDK7 T47D



β-actin MCF-7 T47D

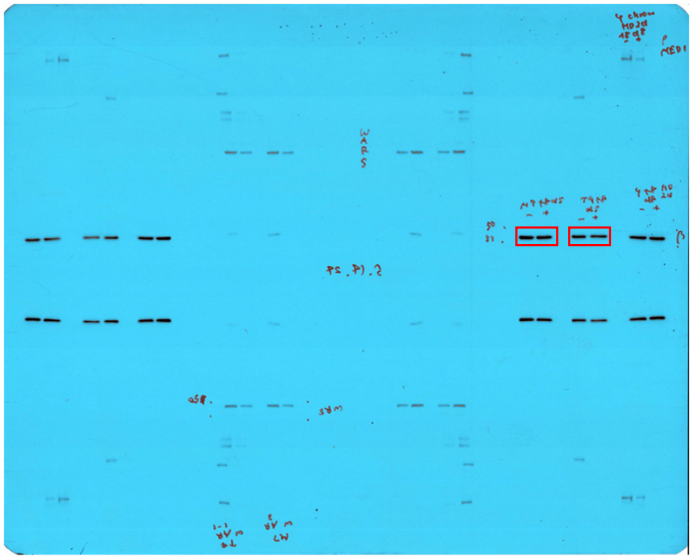


Figure 4a

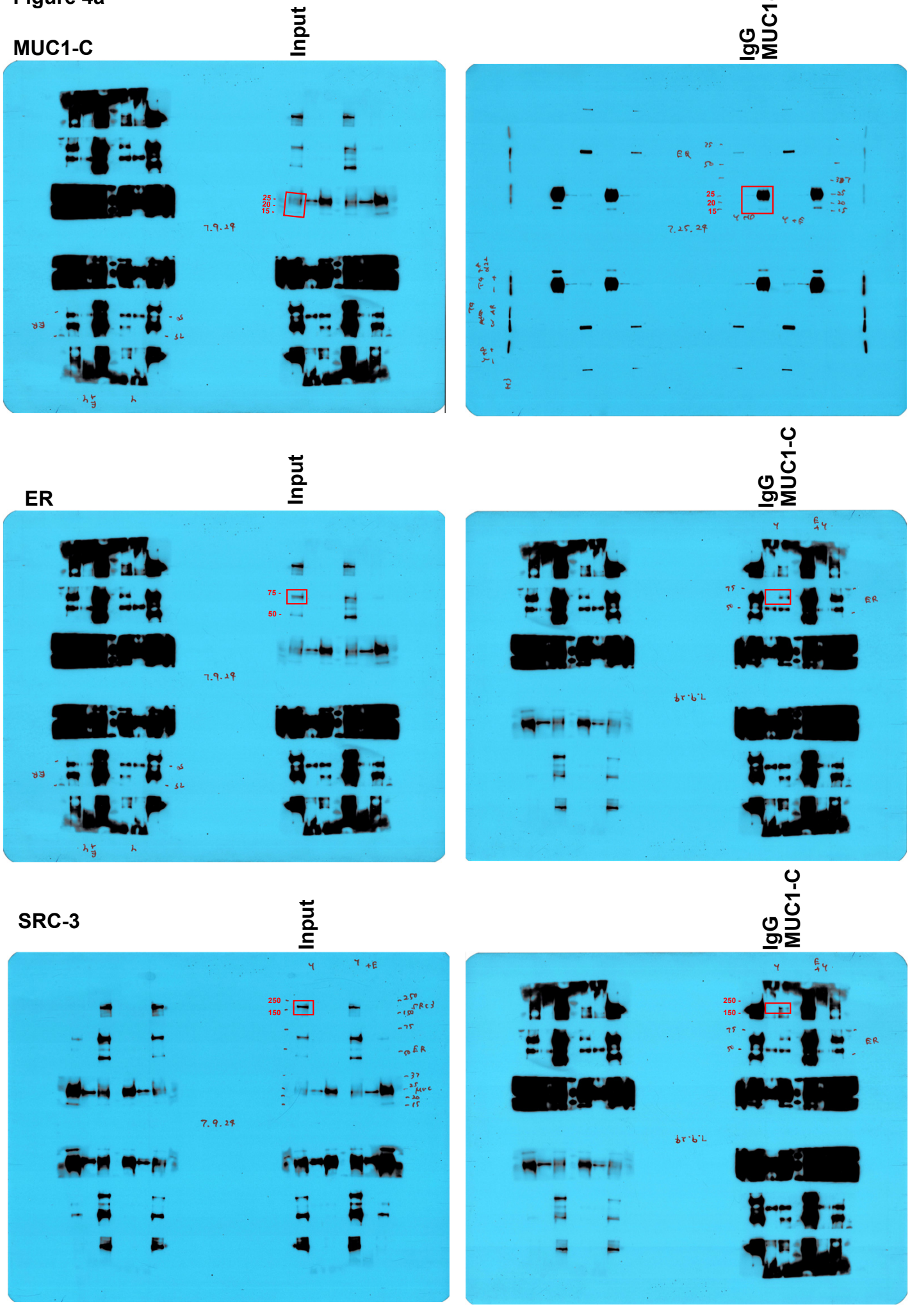

MUC1-C
Input
IgG
MUC1-C
ER
Input
IgG
MUC1-C
SRC-3
Input
IgG
MUC1-C

Figure 4a (continued)

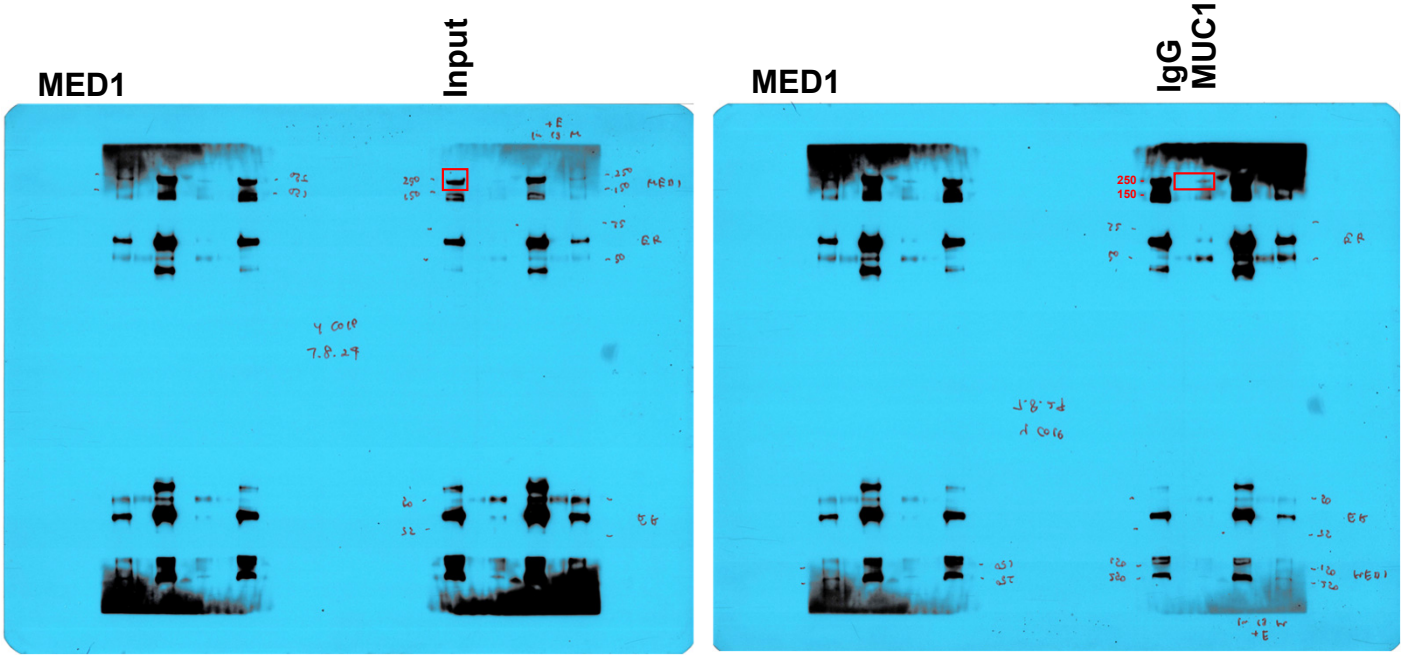
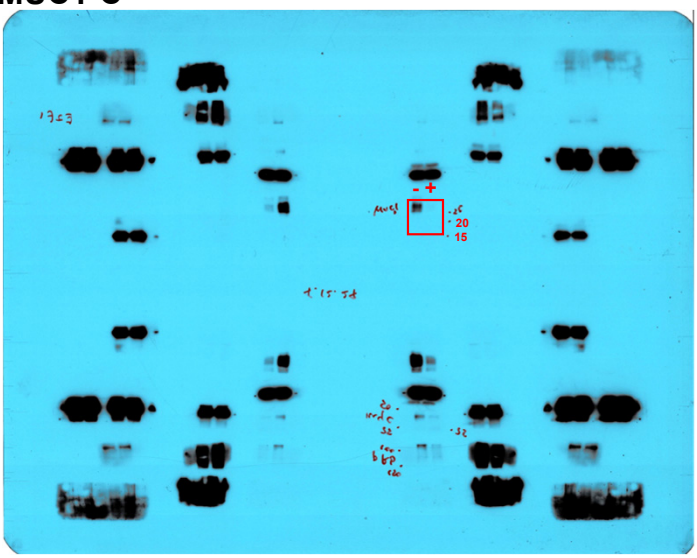
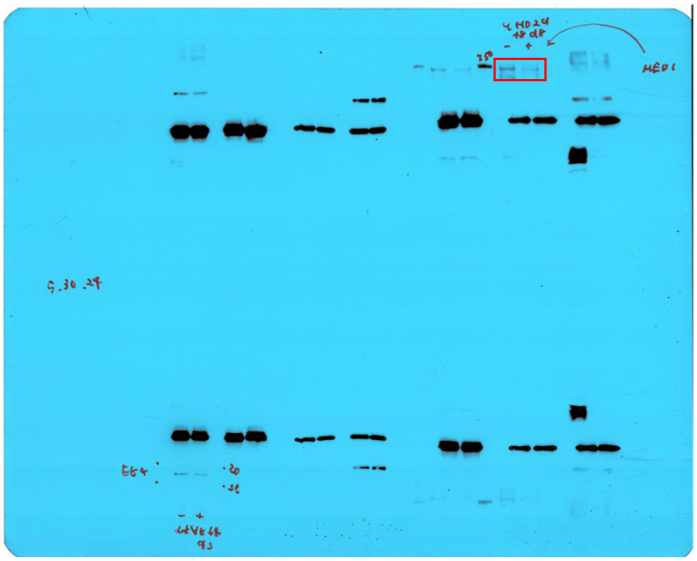


Figure 4b

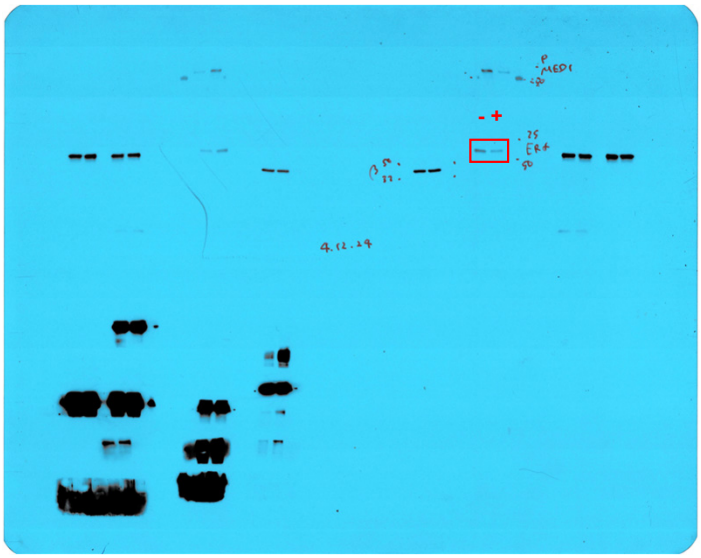
MUC1-C



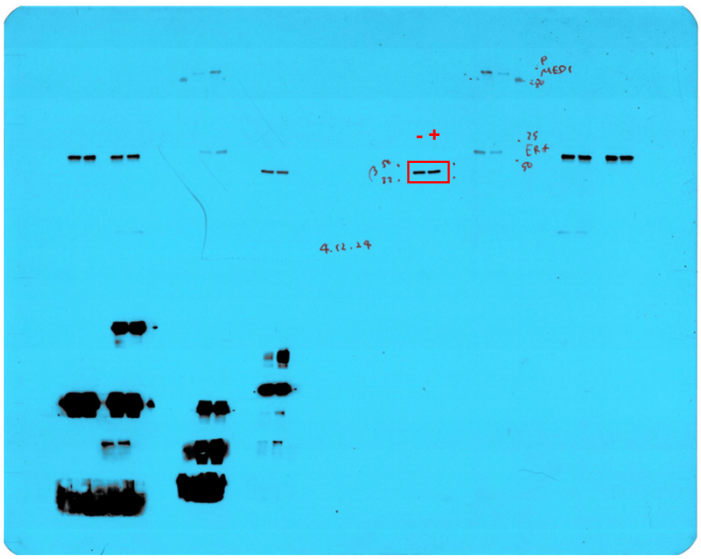
MED1



ER



β-actin



SRC-3

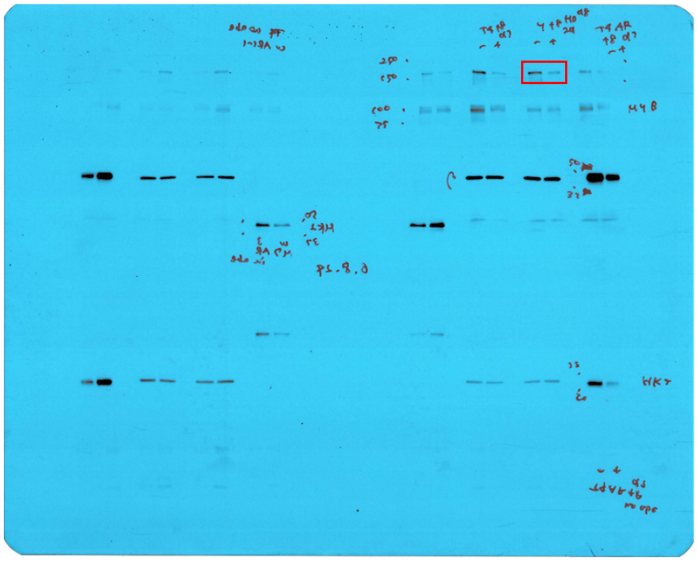
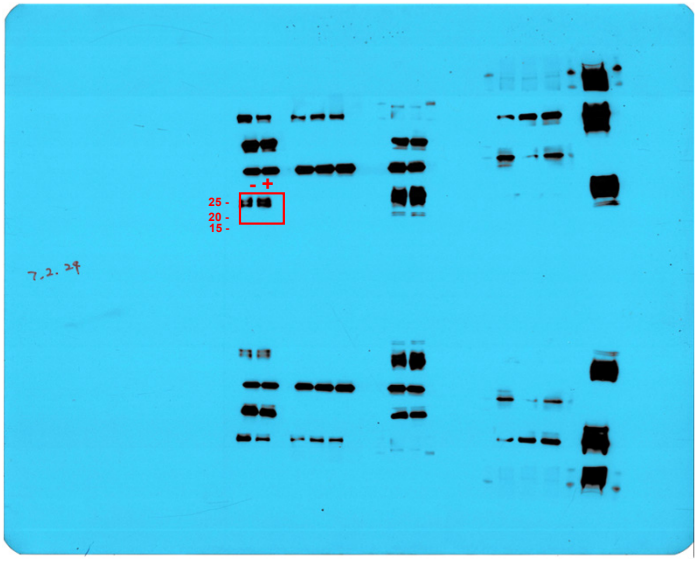
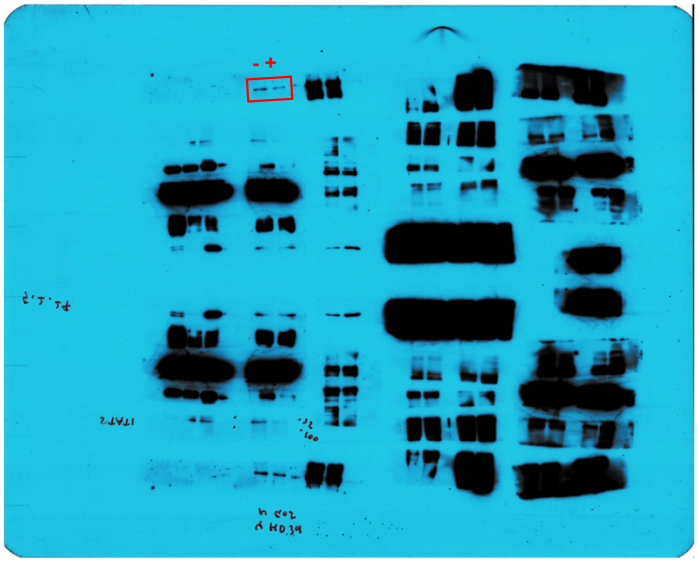


Figure 4c

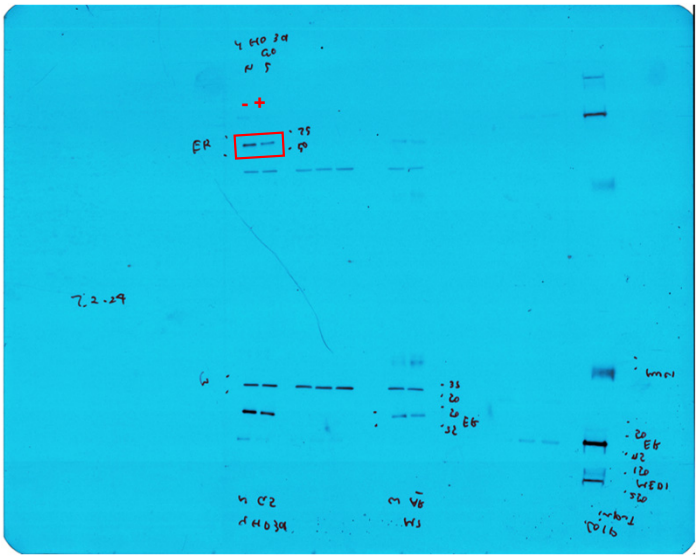
MUC1-C



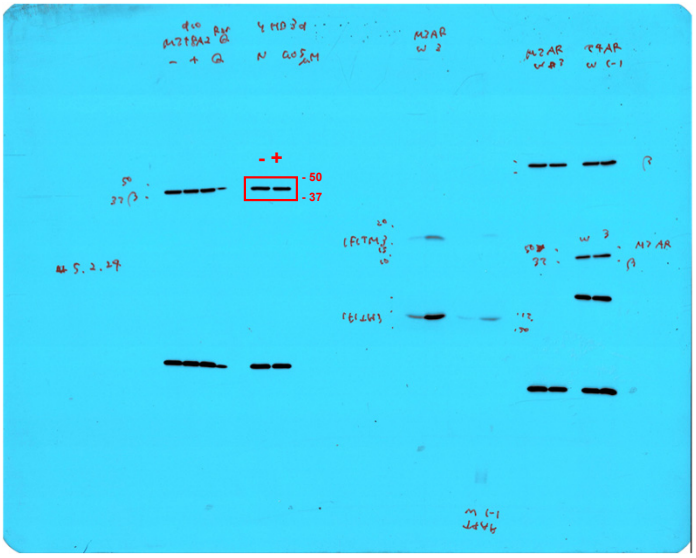
MED1



ER



β-actin



SRC-3

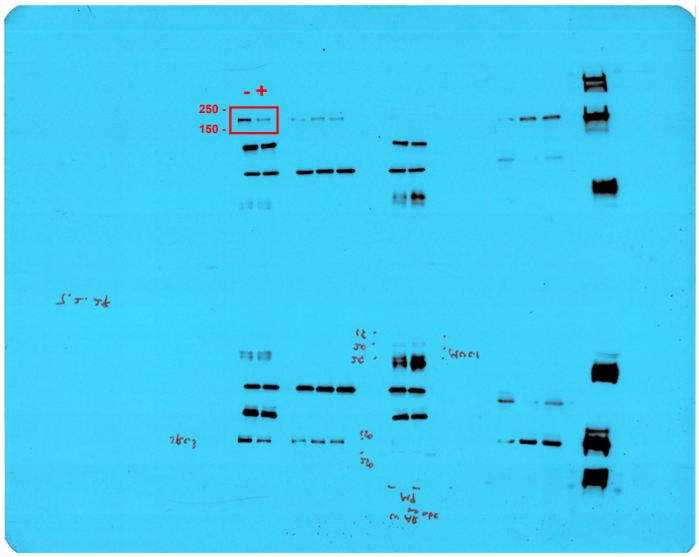
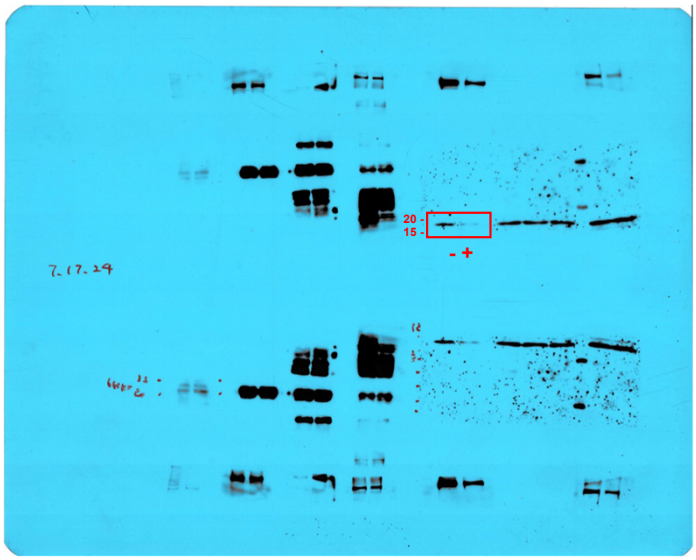
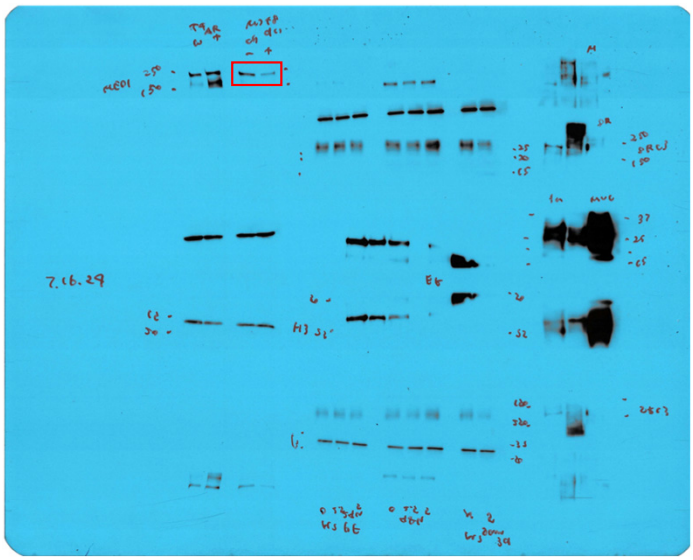


Figure 4d

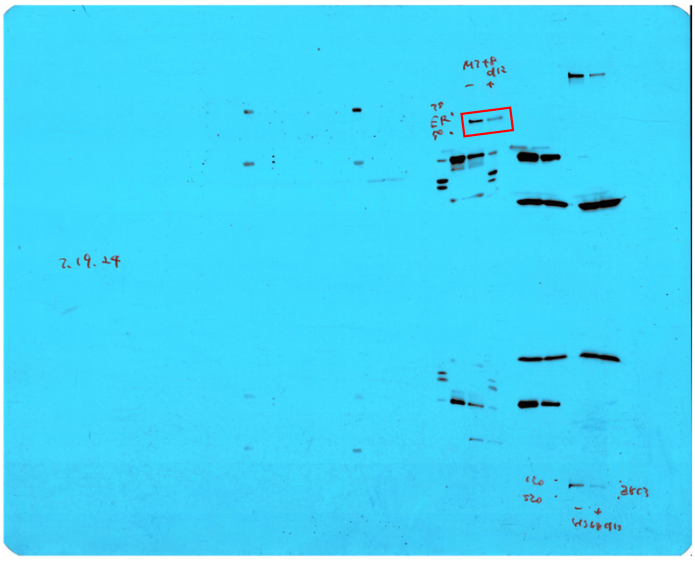
MUC1-C



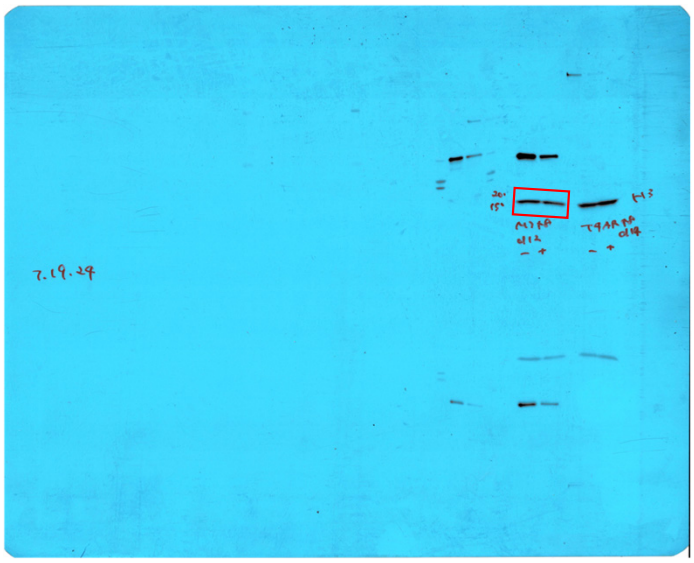
MED1



ER



H3



SRC-3

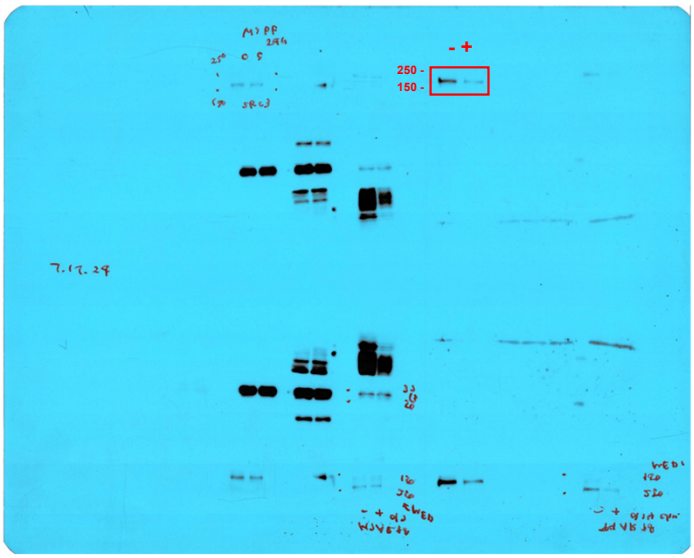
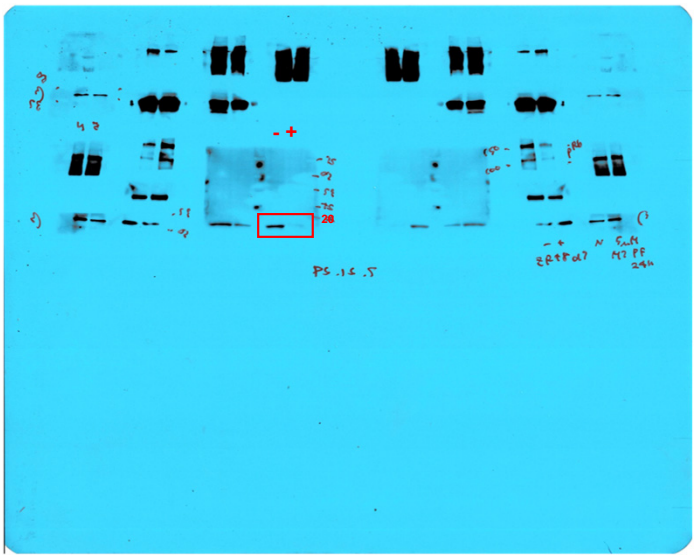
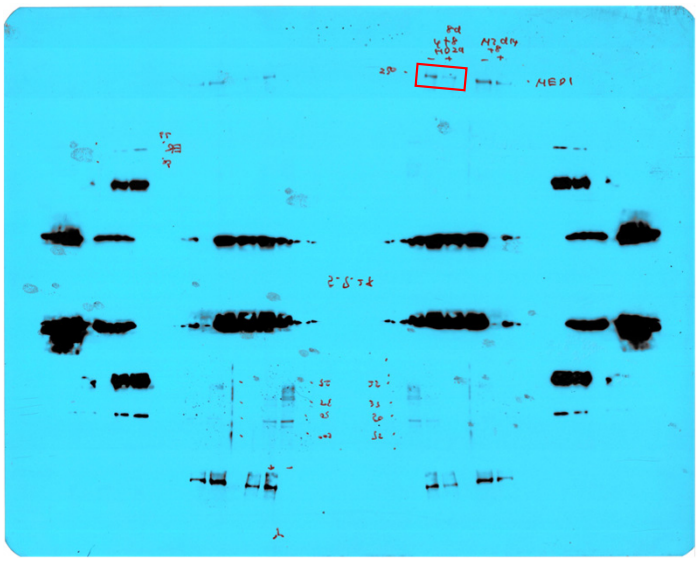


Figure 4e

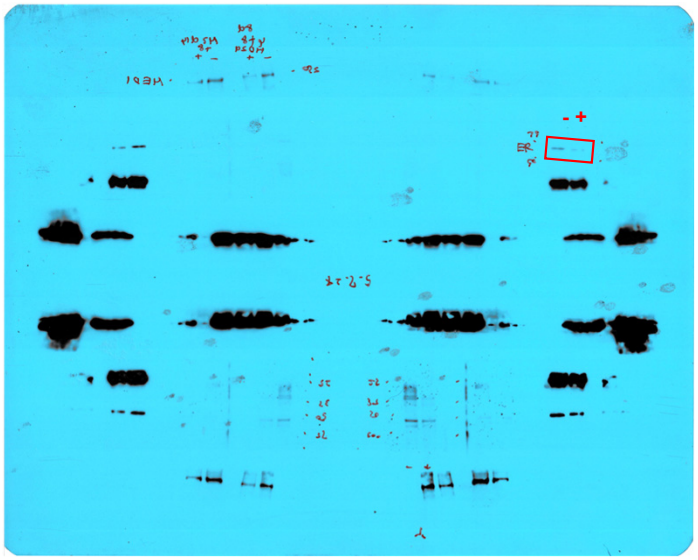
MUC1-C



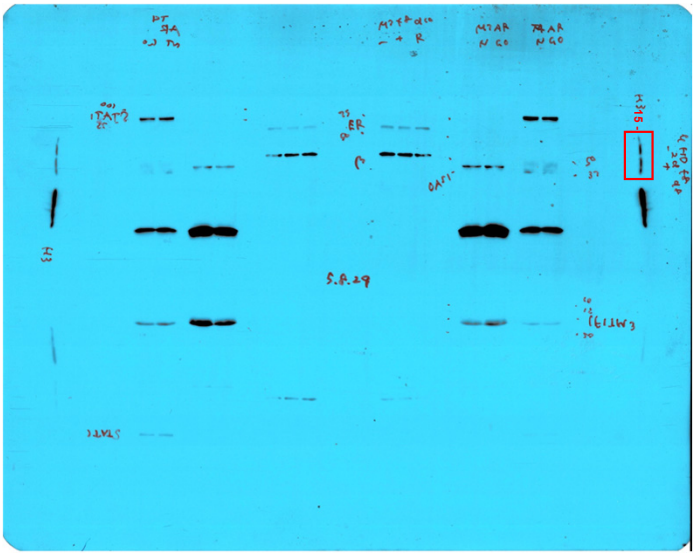
MED1



ER



H3



SRC-3

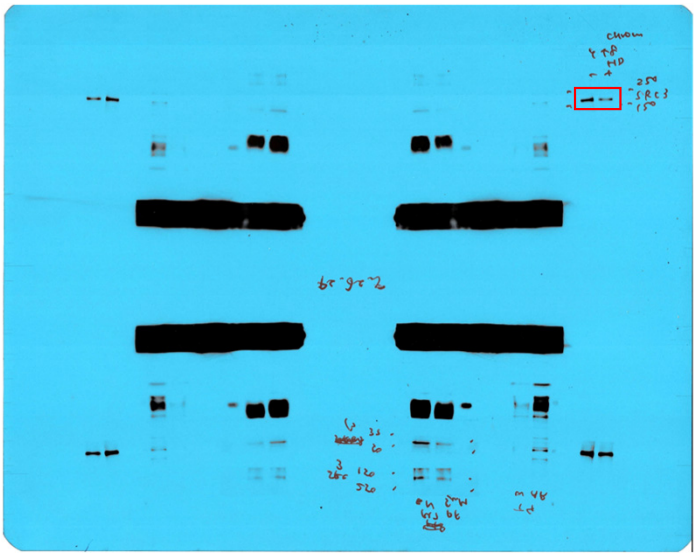
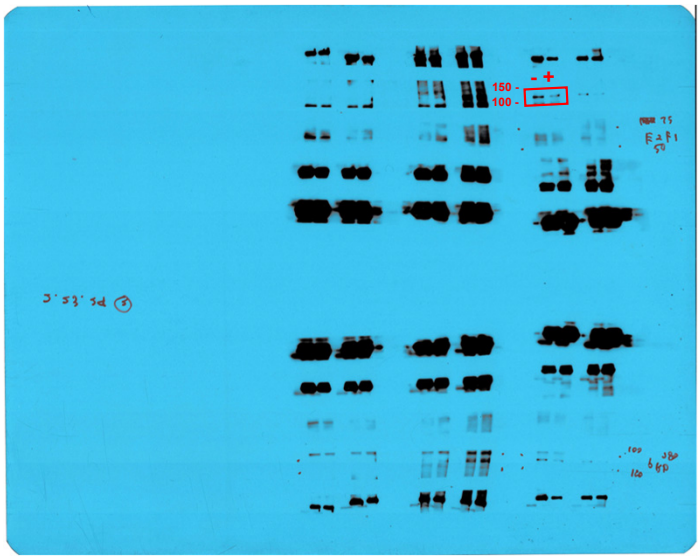
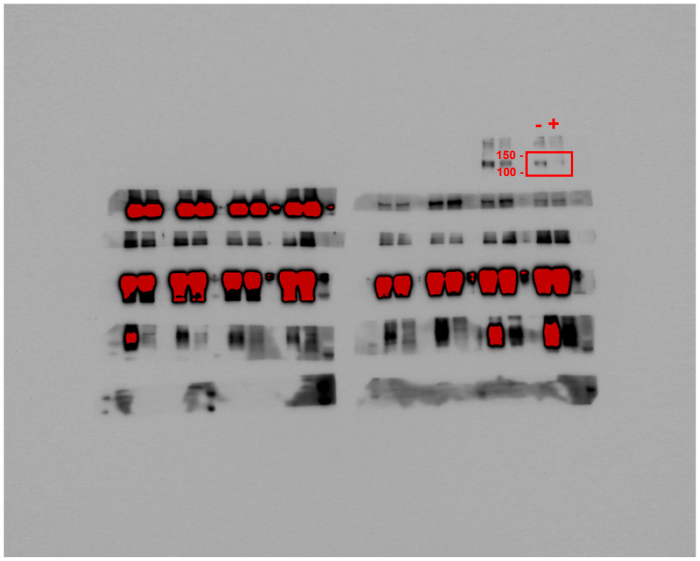


Figure 5a

pRB (S780) MCF-7

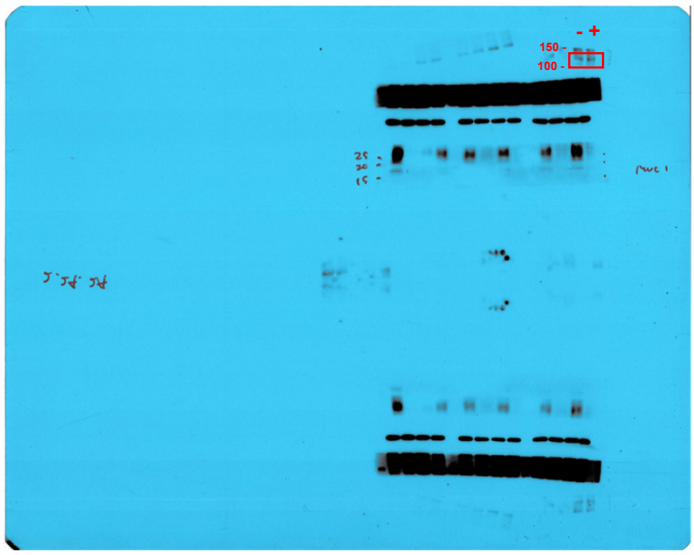


pRB (S780) T47D

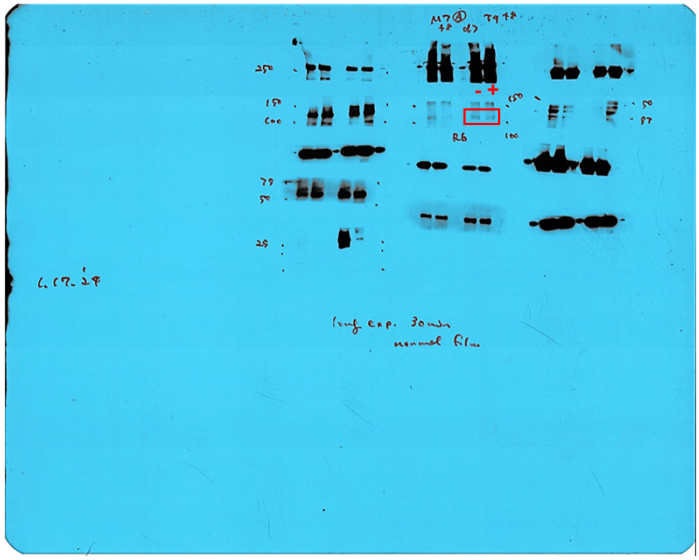


BIORAD ChemiDoc MP developer was used for this blot.
A classical film developer was used for all other blots.

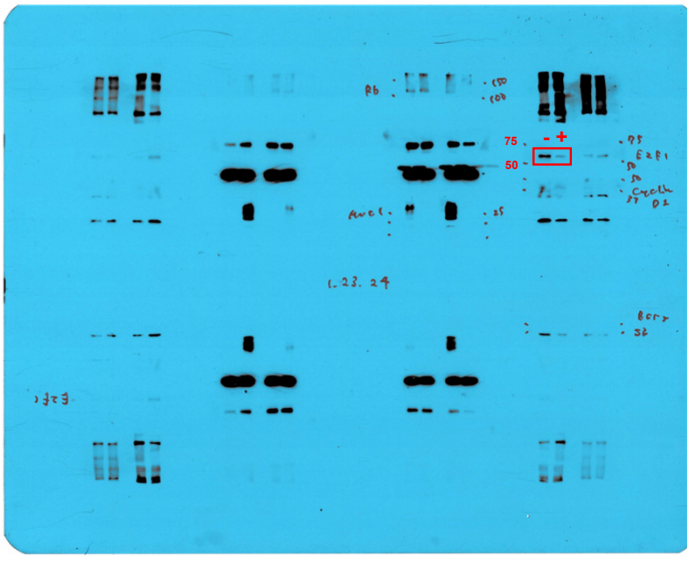
RB MCF-7



RB T47D



E2F1 MCF-7



E2F1 T47D

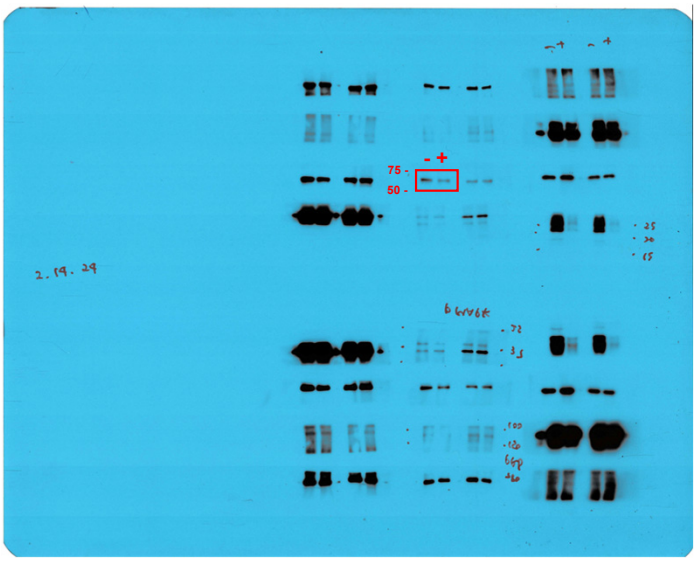
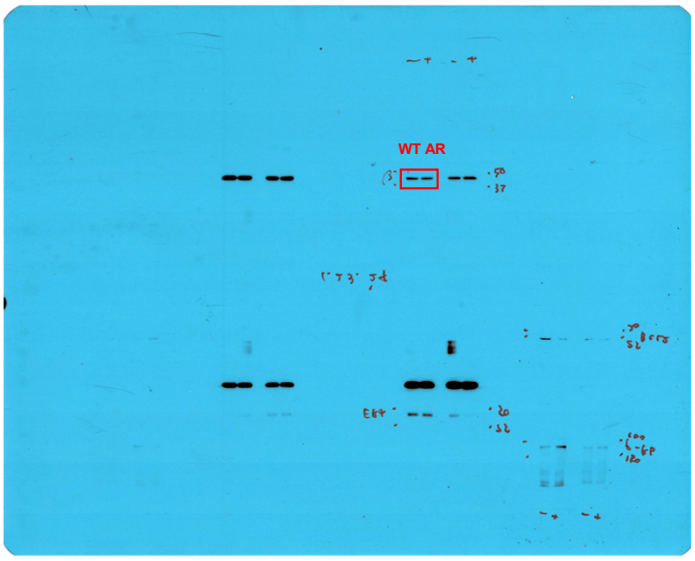


Figure 5a (continued)

β-actin MCF-7



β-actin T47D

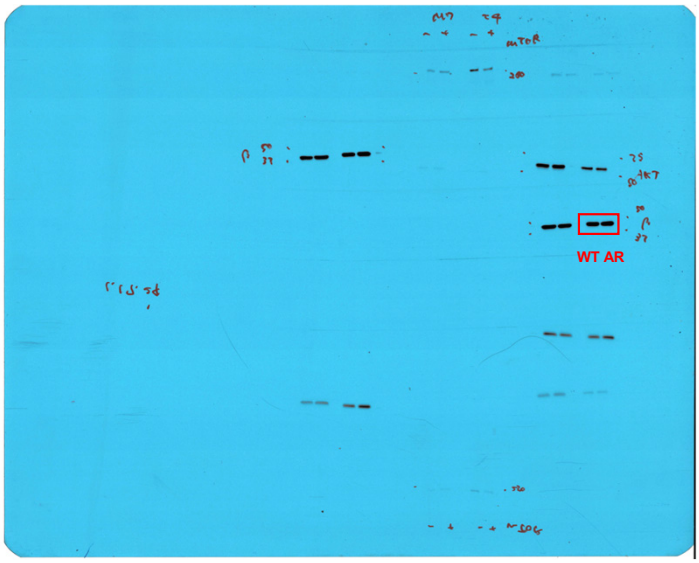
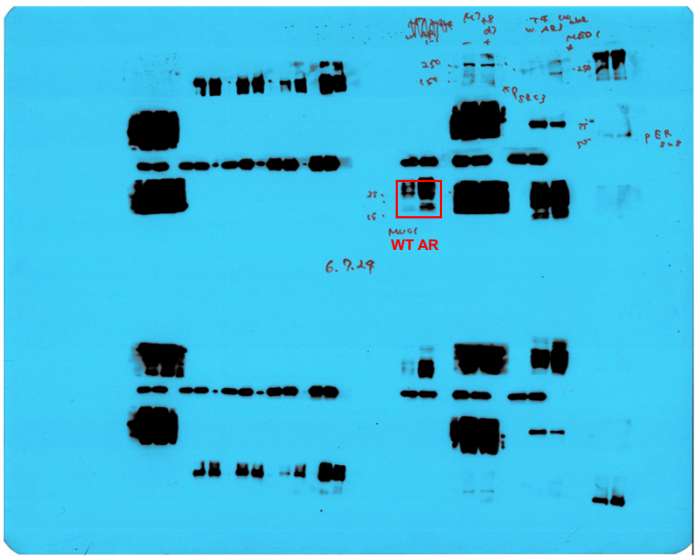
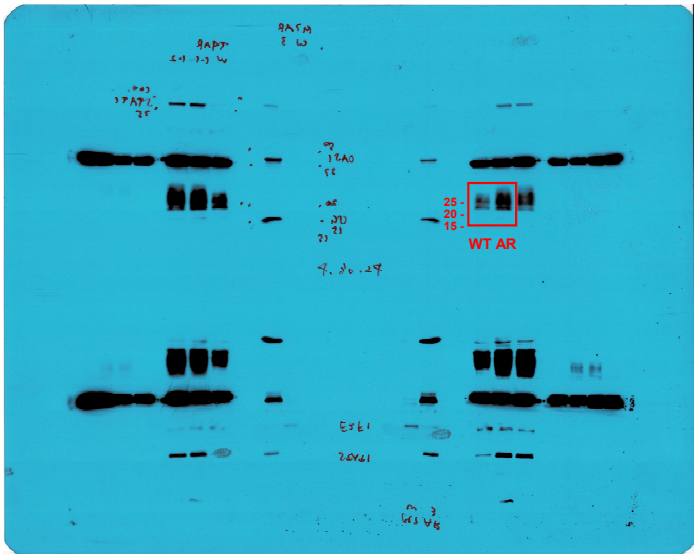


Figure 5d

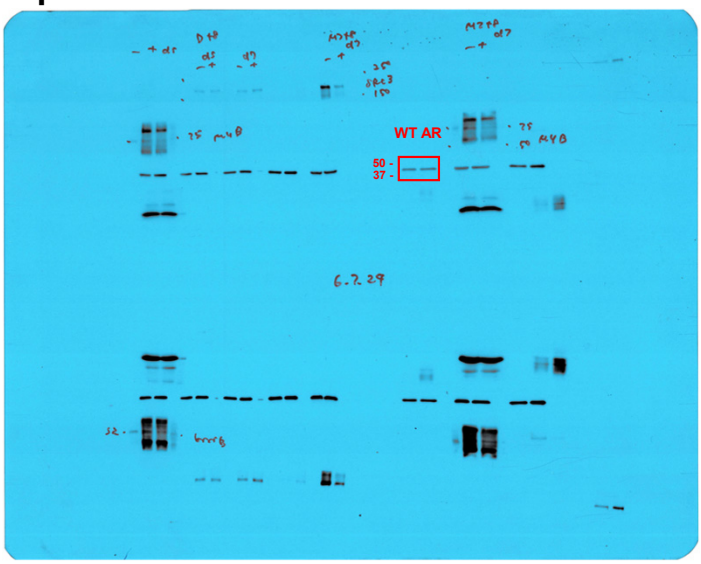
MUC1-C MCF-7



MUC1-C T47D



β-actin MCF-7



β-actin T47D

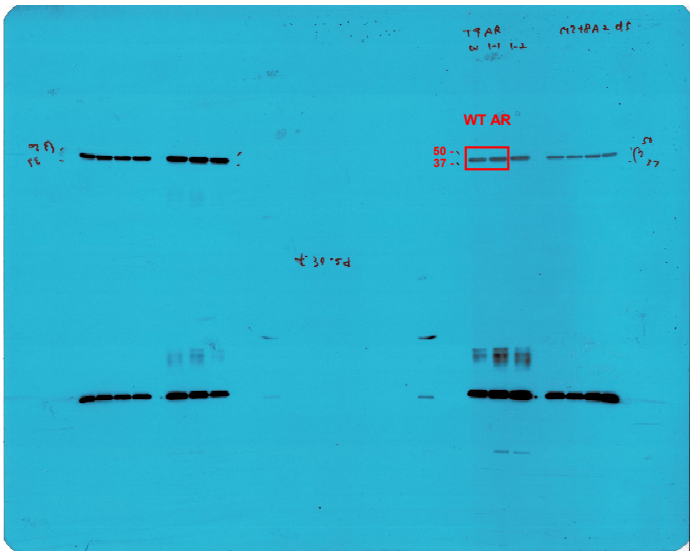


Figure 5e

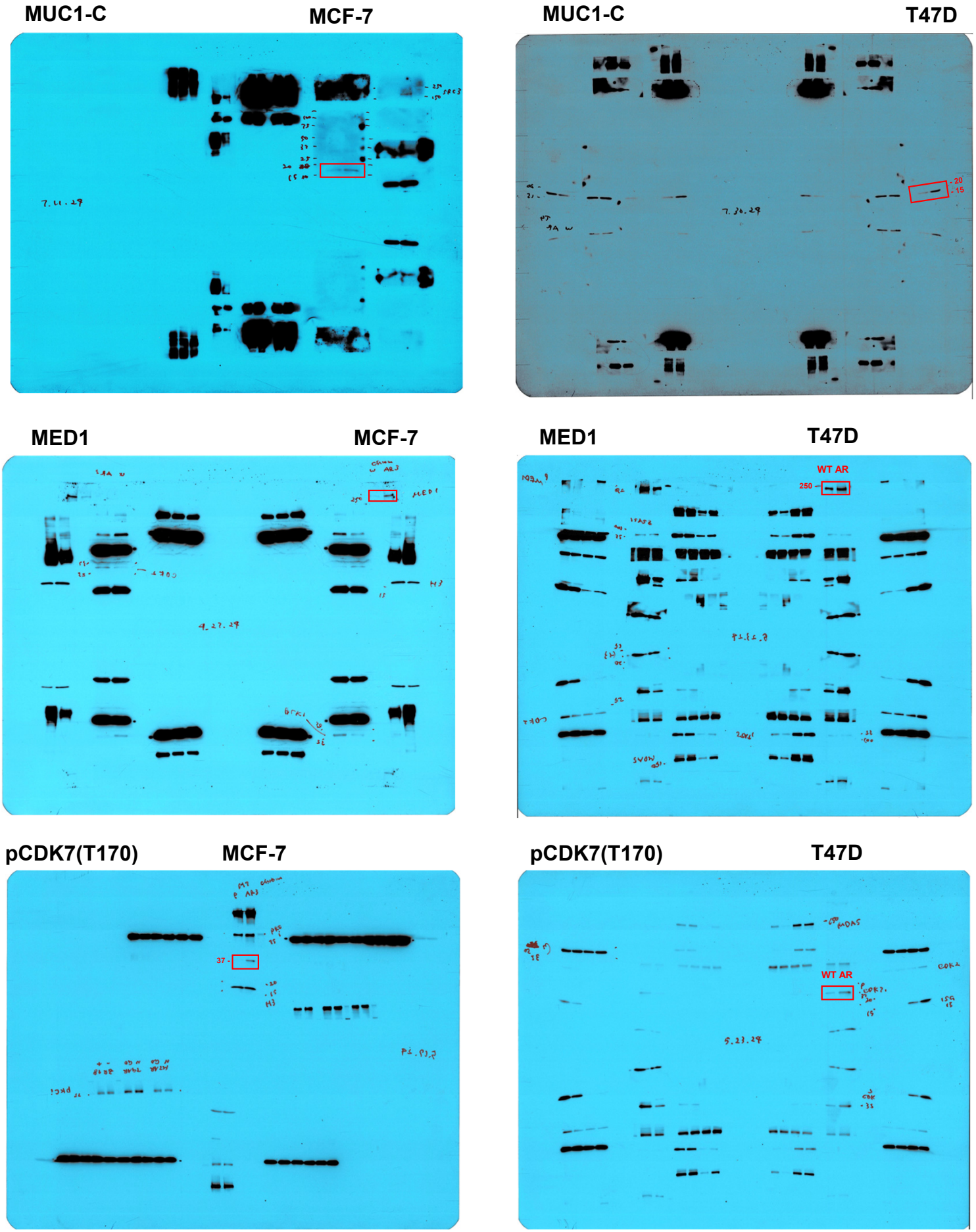
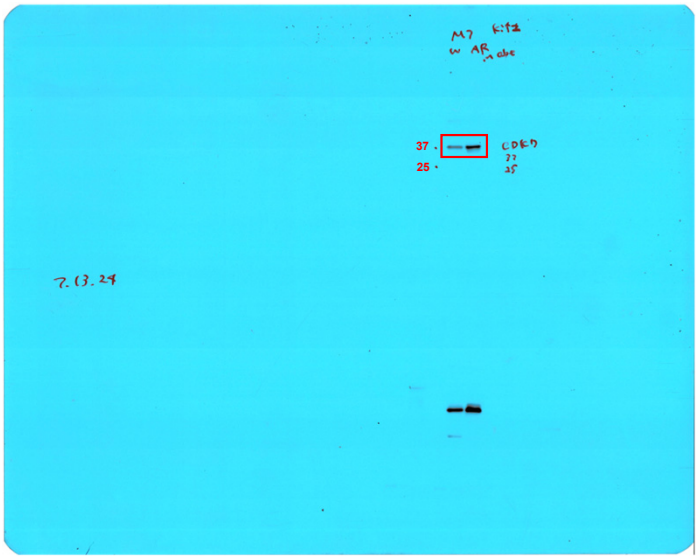
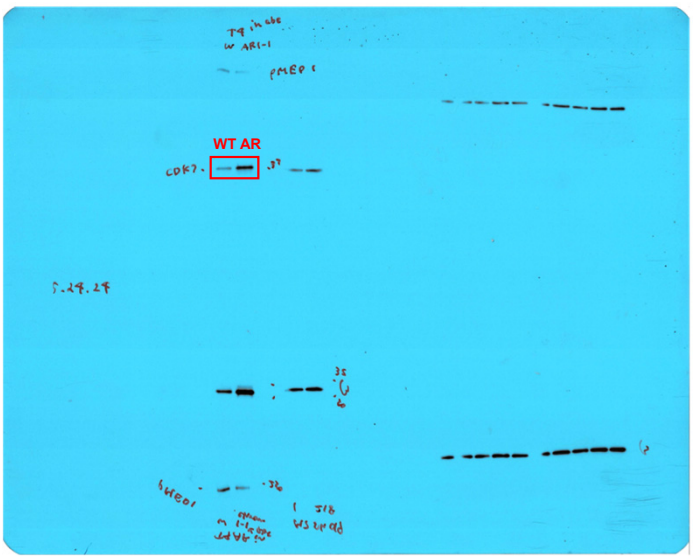

MUC1-C
MCF-7
MUC1-C
T47D
MED1
MCF-7
MED1
T47D
WT AR
250
pCDK7(T170)
MCF-7
pCDK7(T170)
T47D
WT AR

Figure 5e (continued)

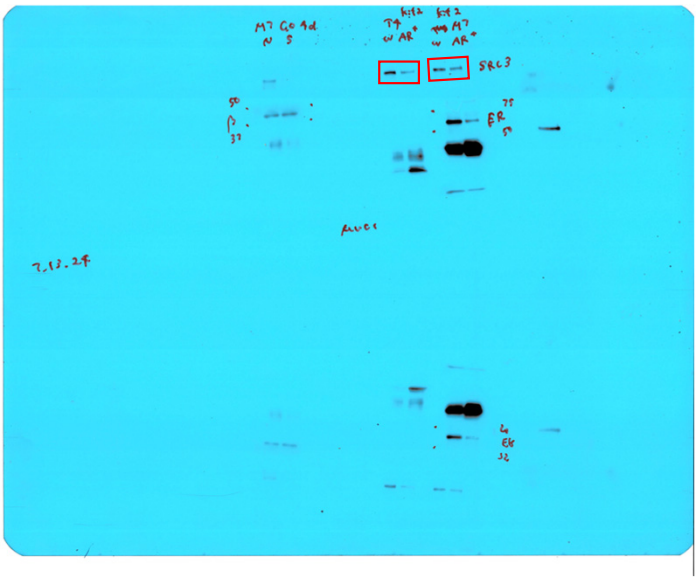
CDK7 MCF-7



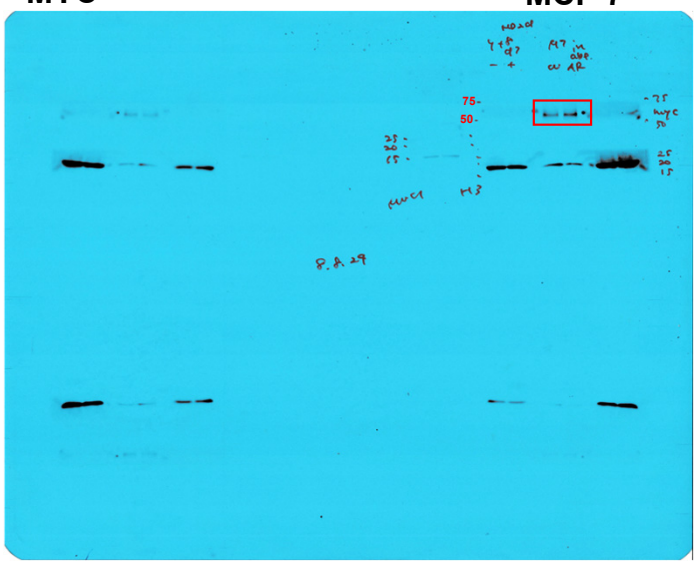
CDK7 T47D



SRC-3 T47D MCF-7



MYC MCF-7



MYC T47D

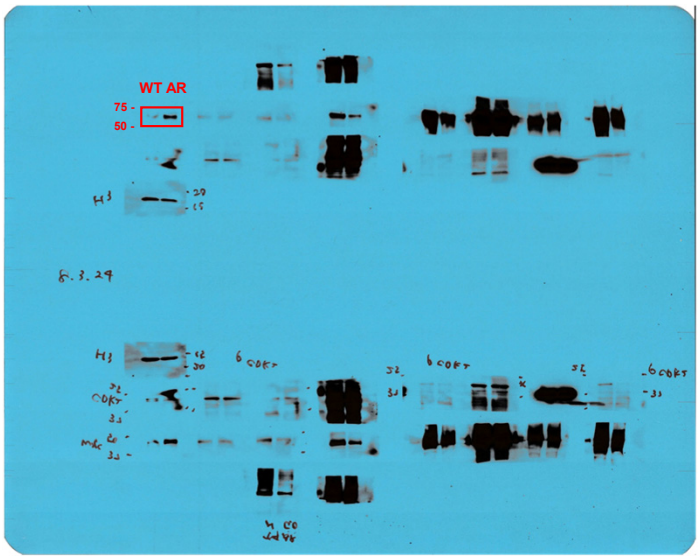


Figure 5e (continued)

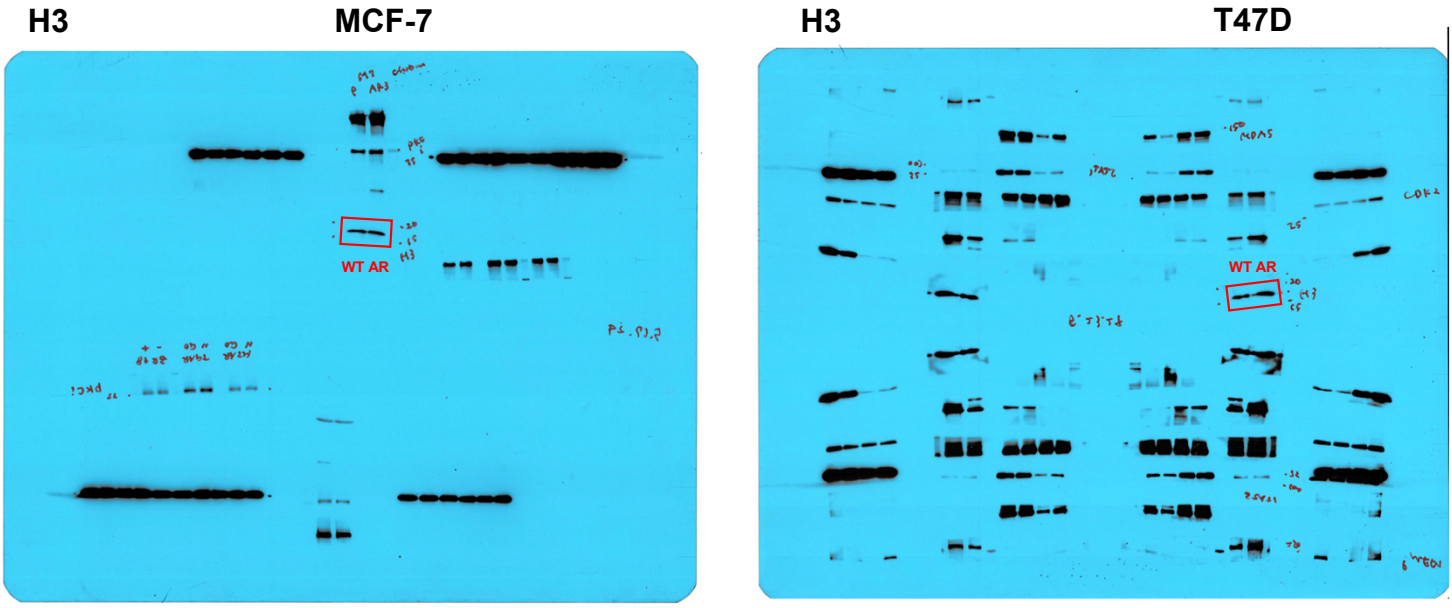
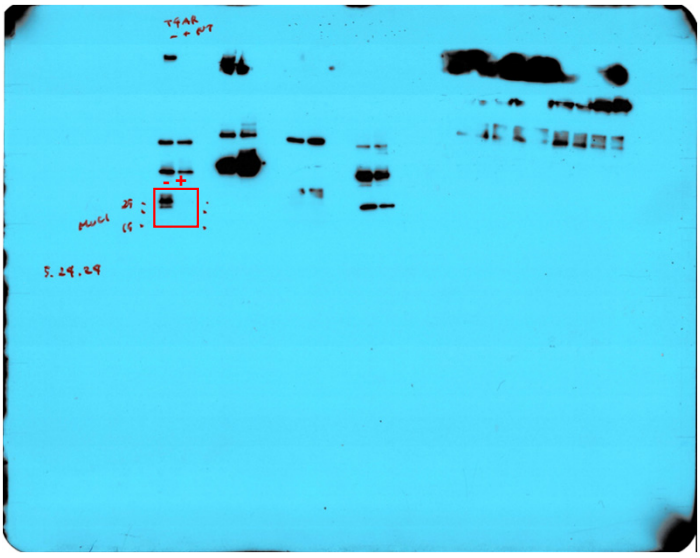
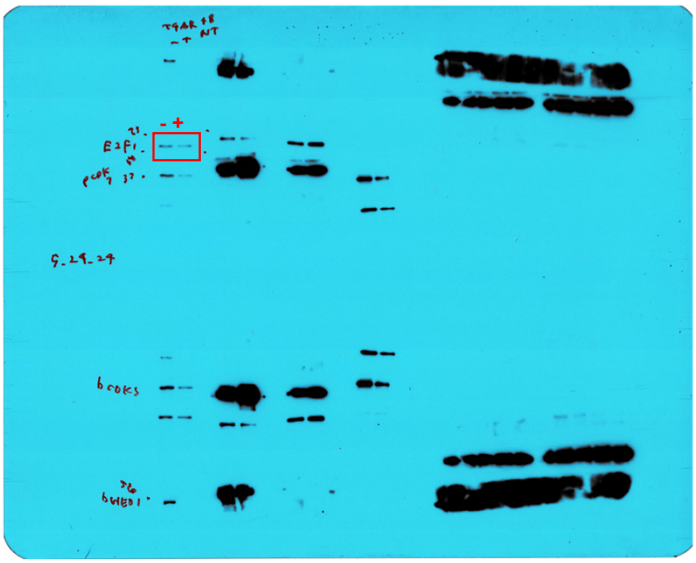


Figure 5f

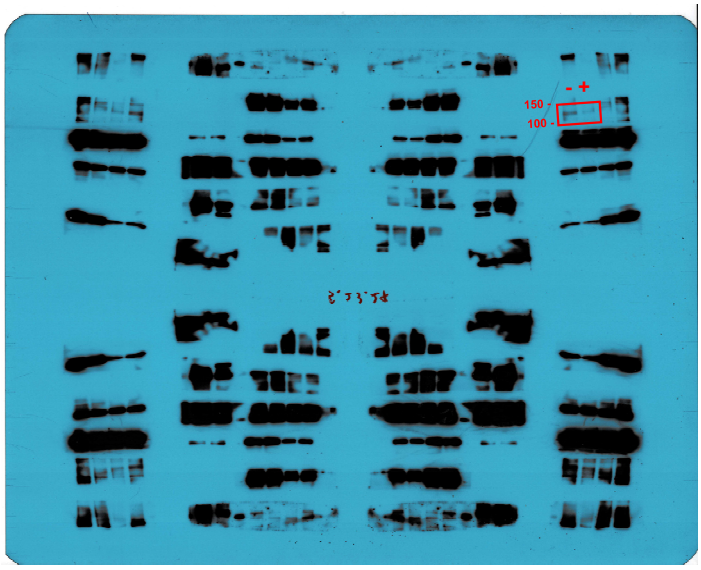
MUC1-C



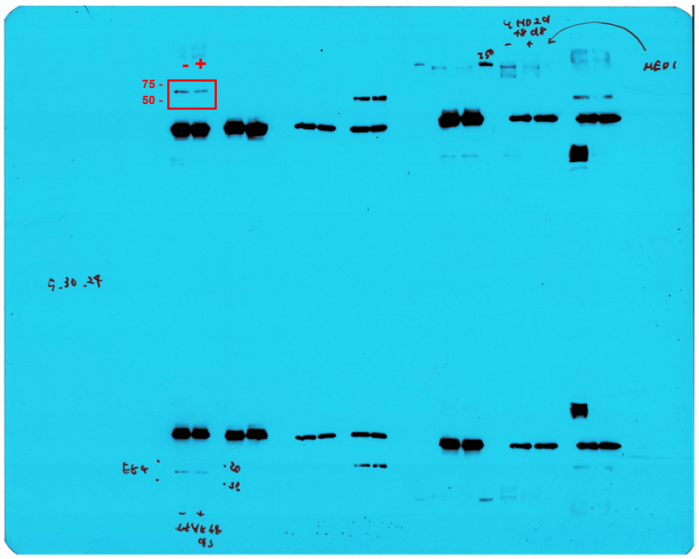
E2F1



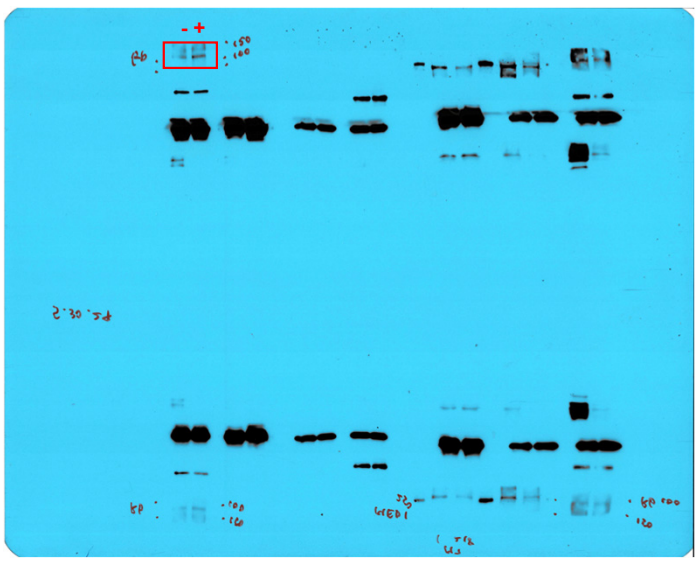
pRB (S780)



ER



RB



MK2

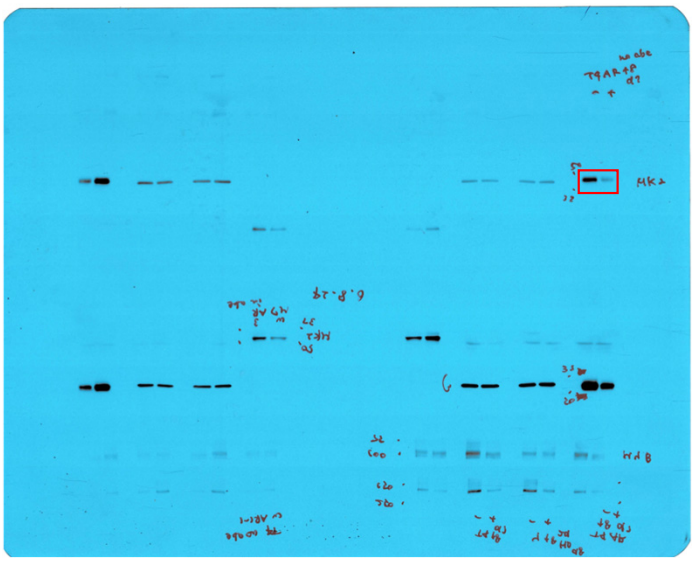
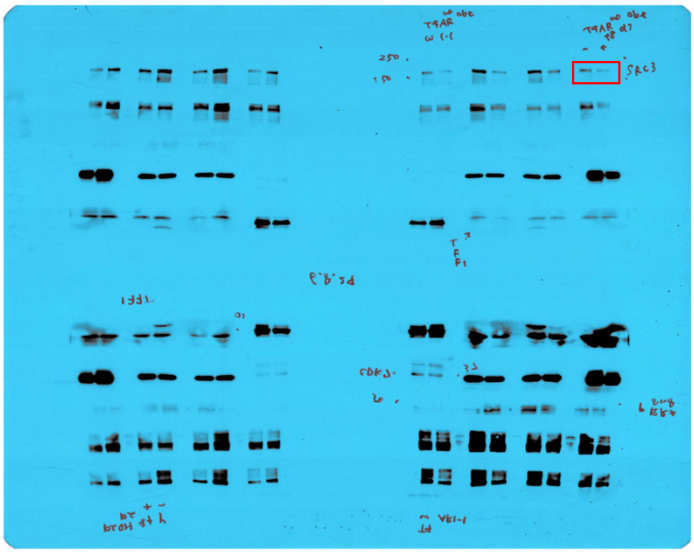
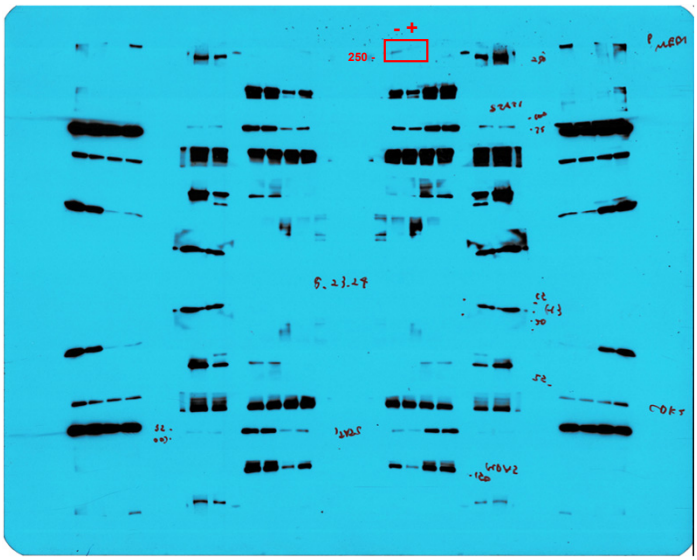


Figure 5f (continued)

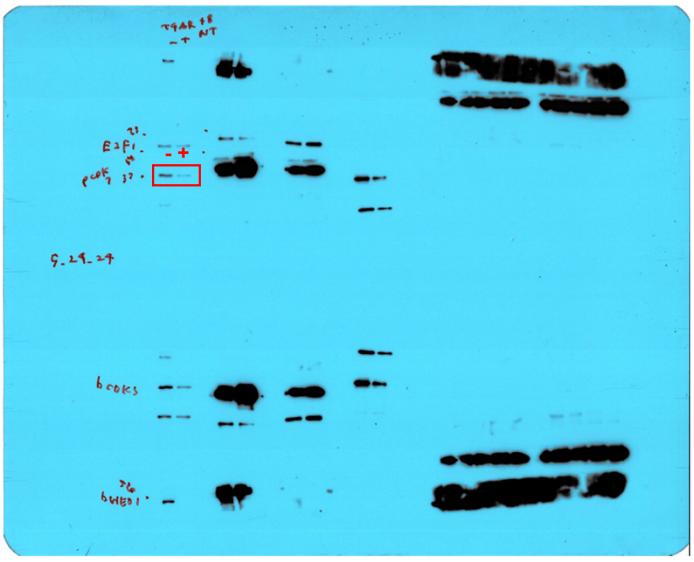
SRC-3



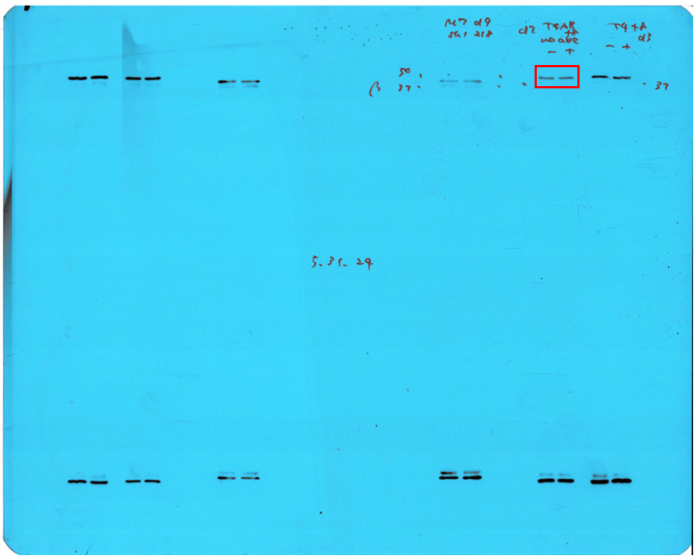
MED1



pCDK7 (T170)



β-actin



CDK7

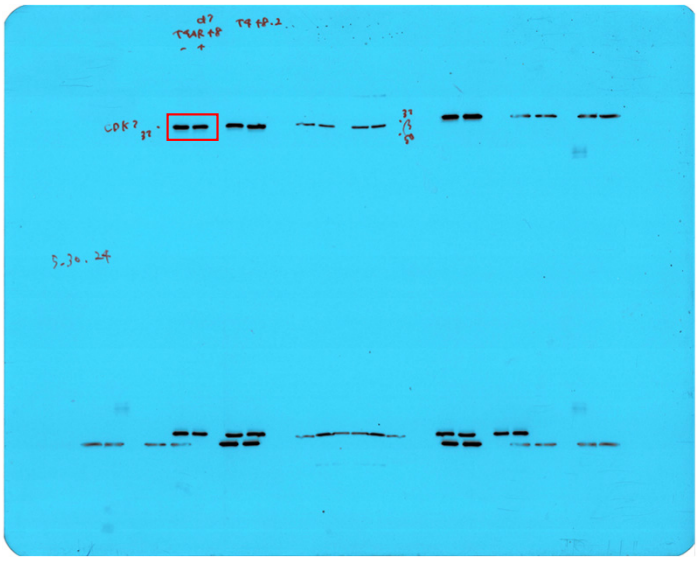
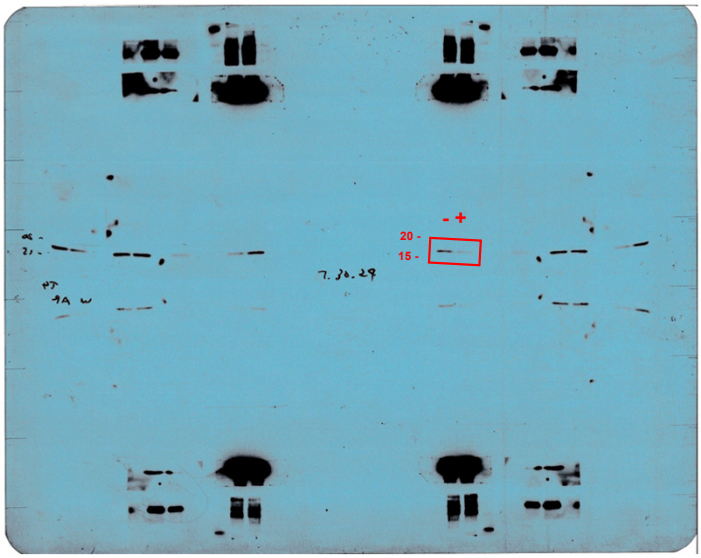
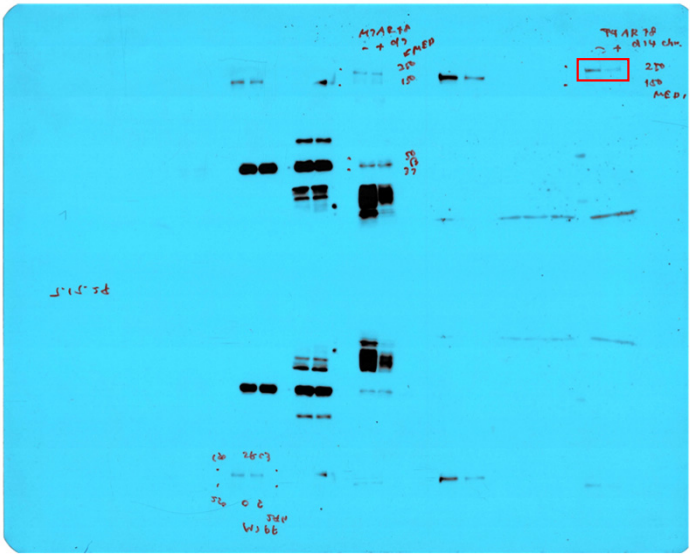


Figure 5g

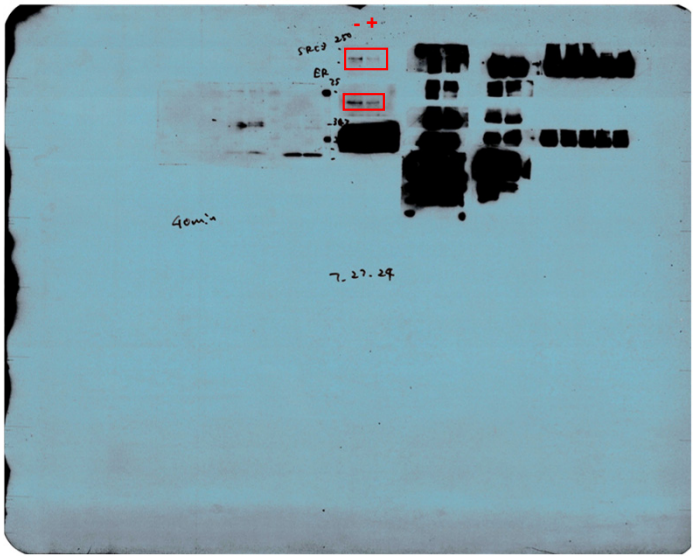
MUC1-C



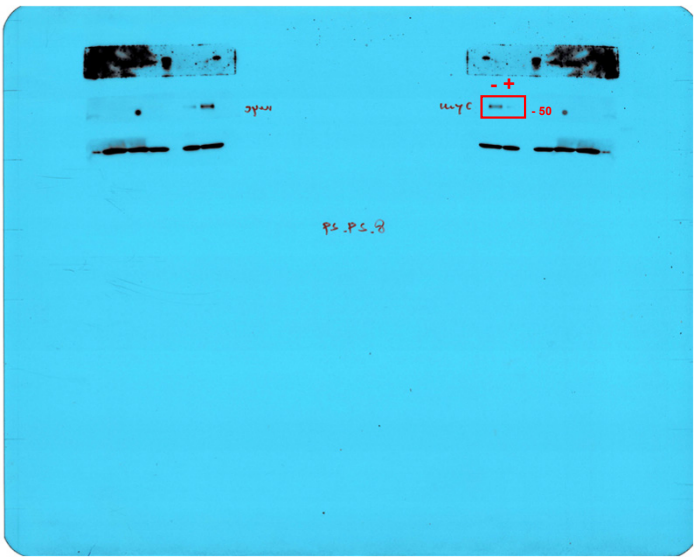
MED1



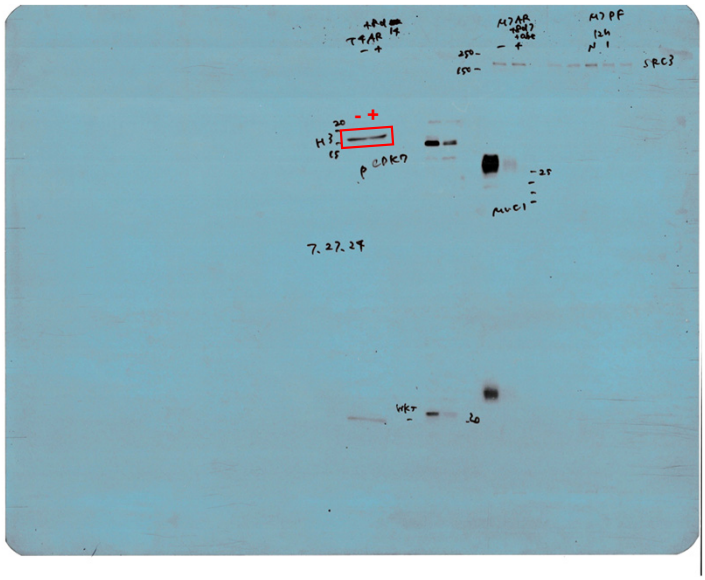
ER SRC-3



MYC



H3



Supplemental Figure S8

Cropped images of western blots

Figure 1c

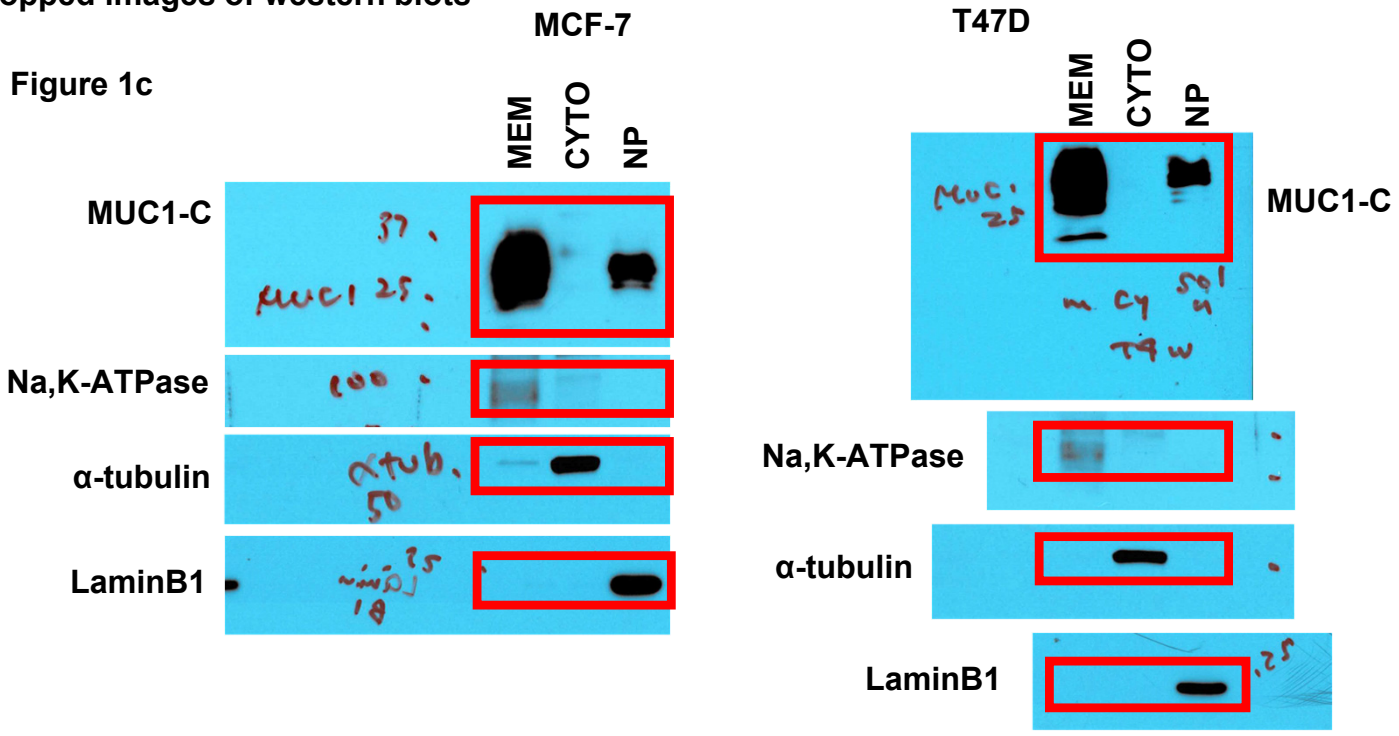


Figure 1d

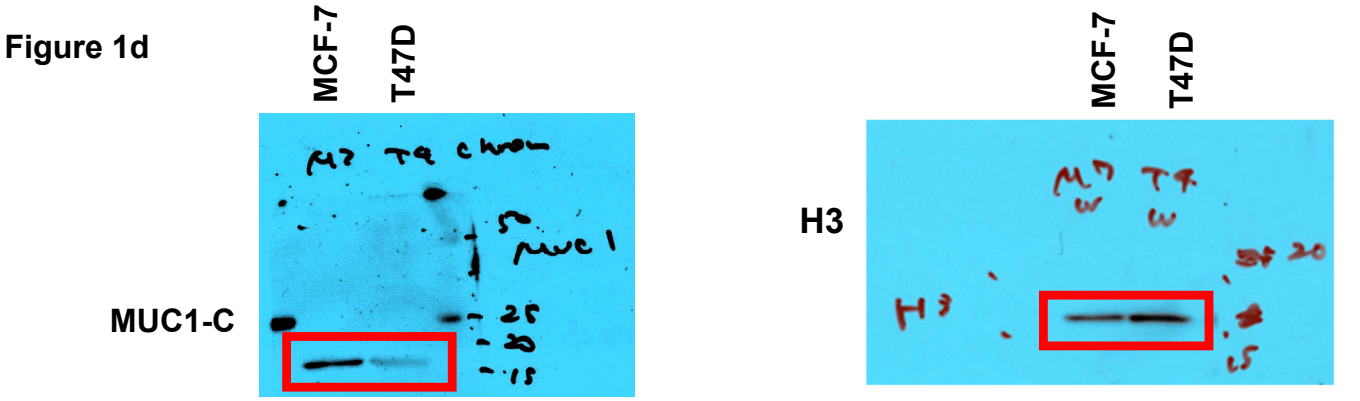


Figure 1e

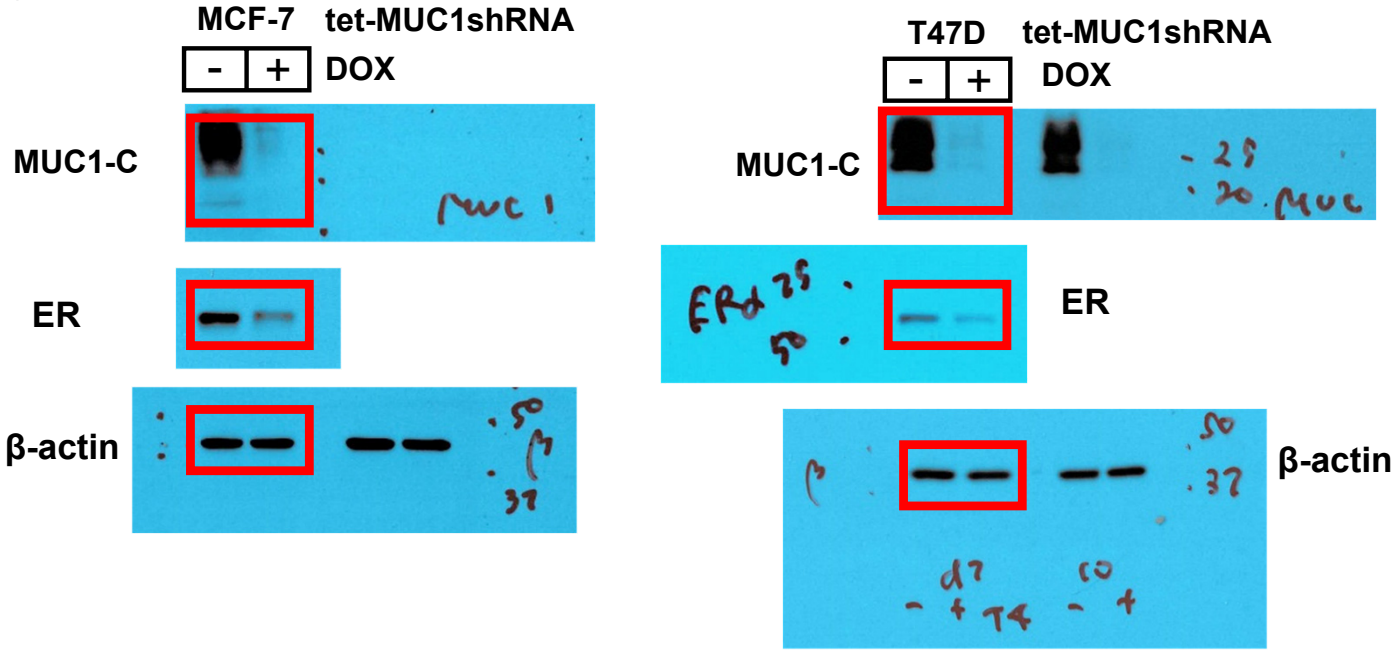


Figure 2a

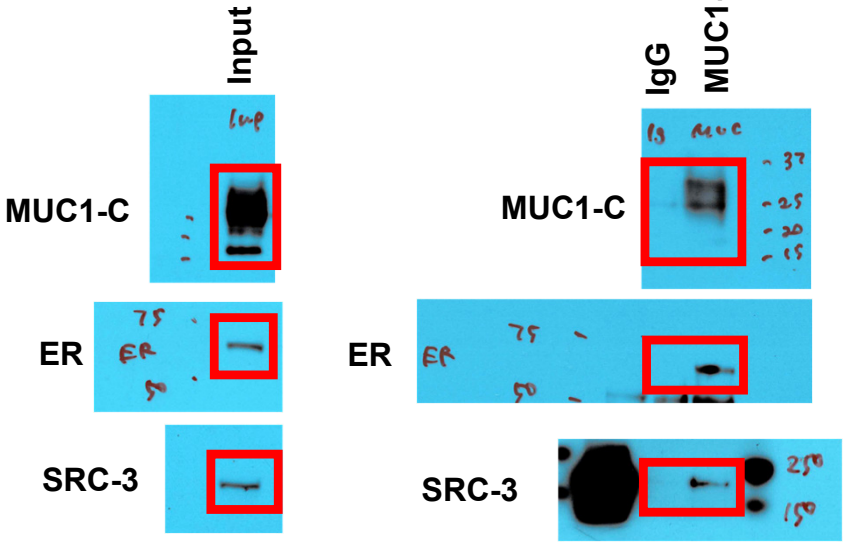


Figure 2b

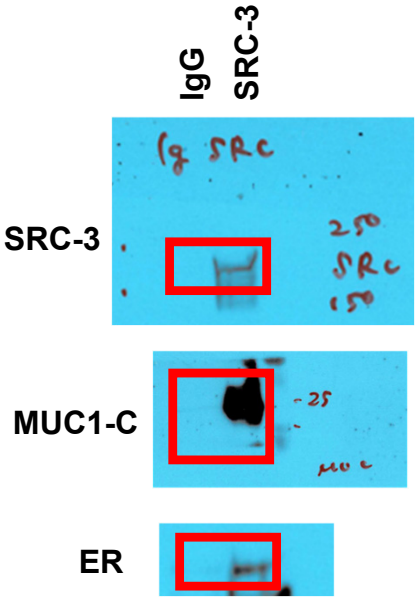


Figure 2c

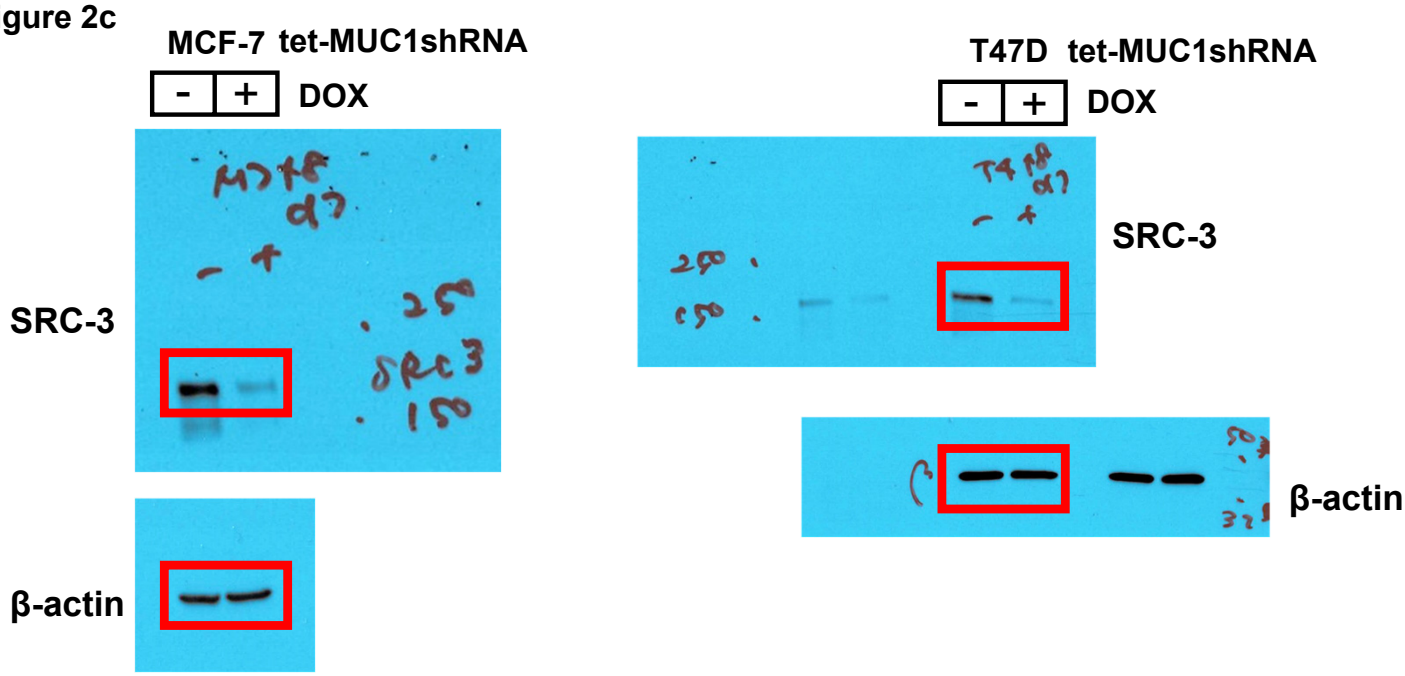


Figure 2d

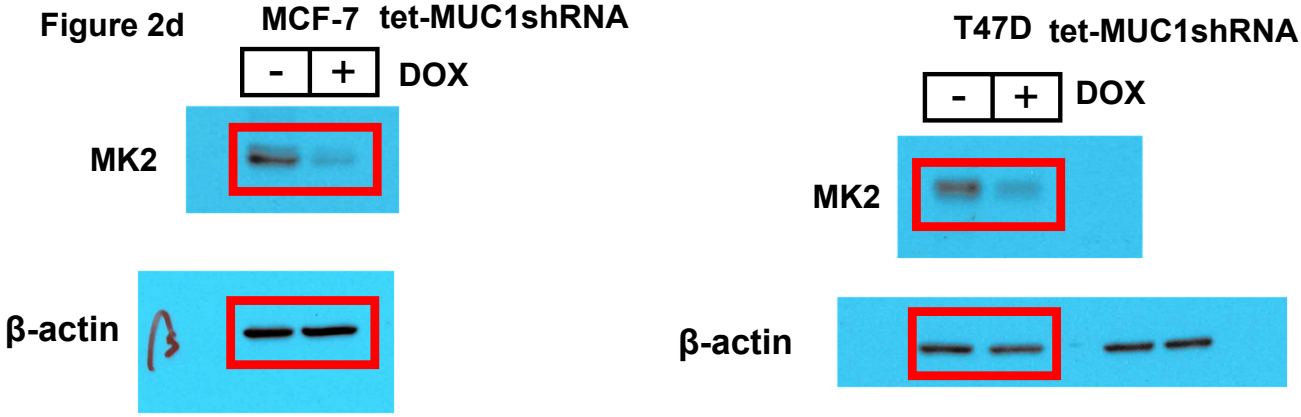


Figure 3a

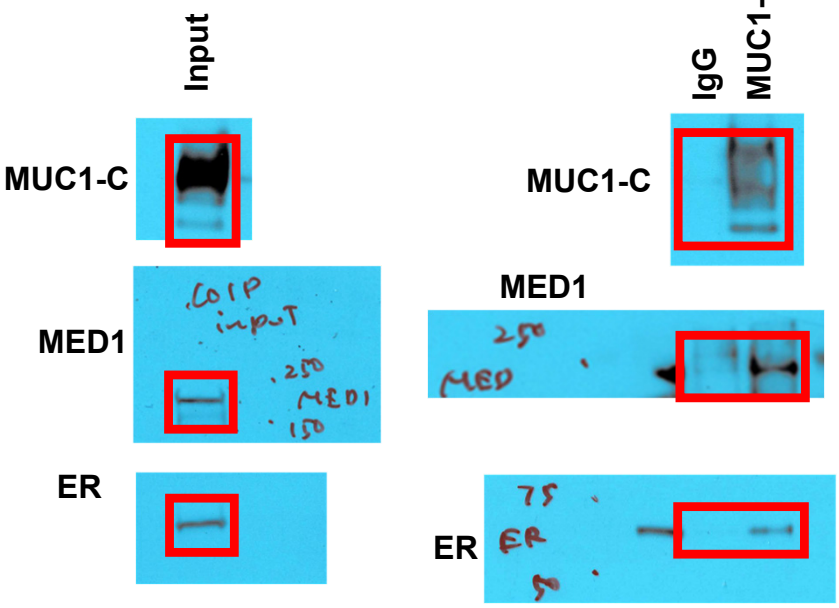


Figure 3b

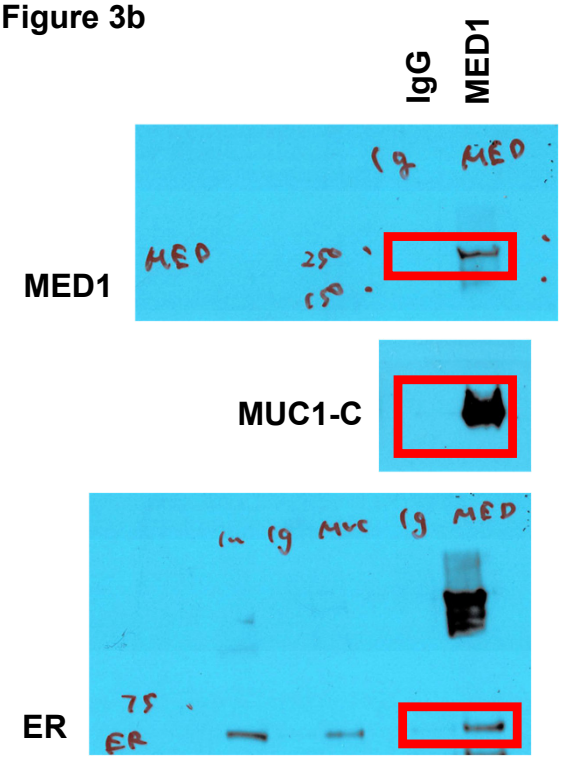


Figure 3c

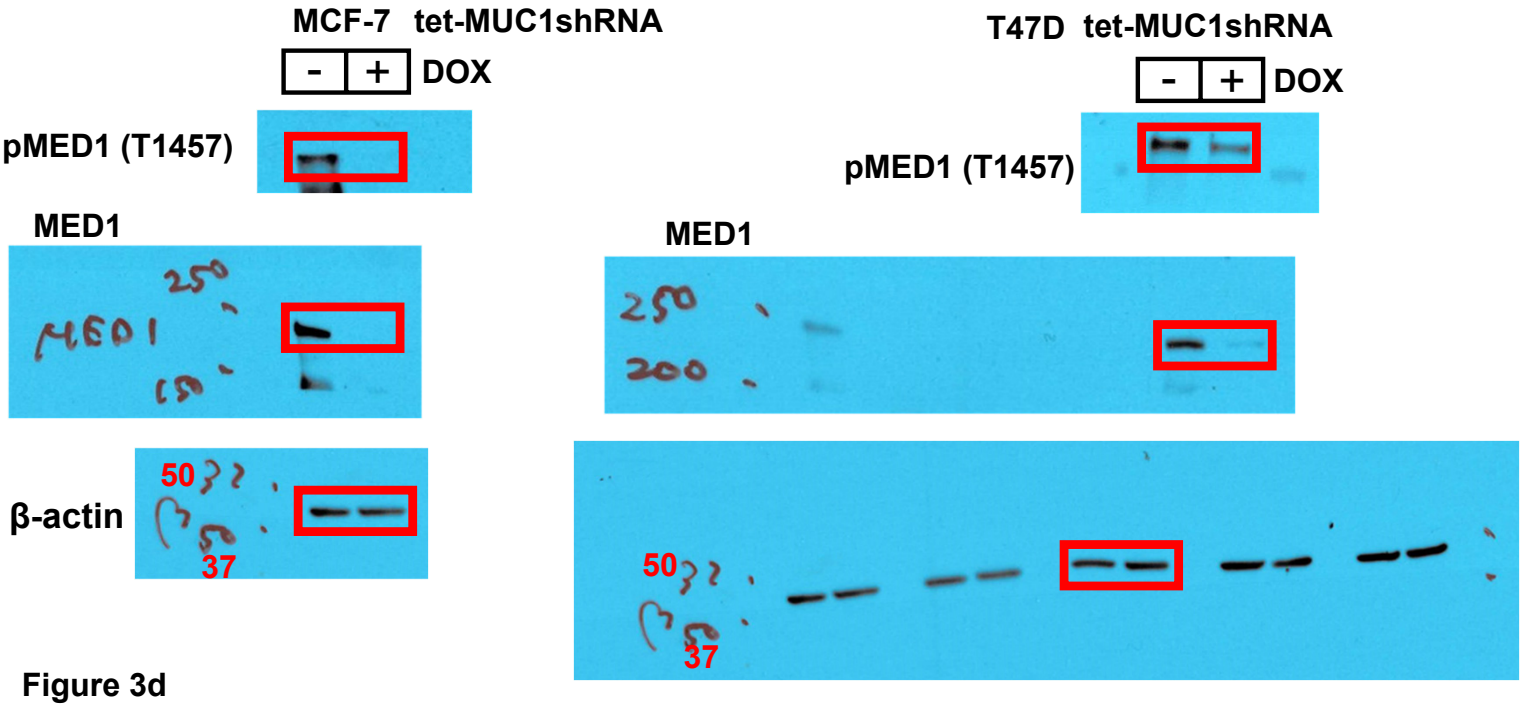


Figure 3d

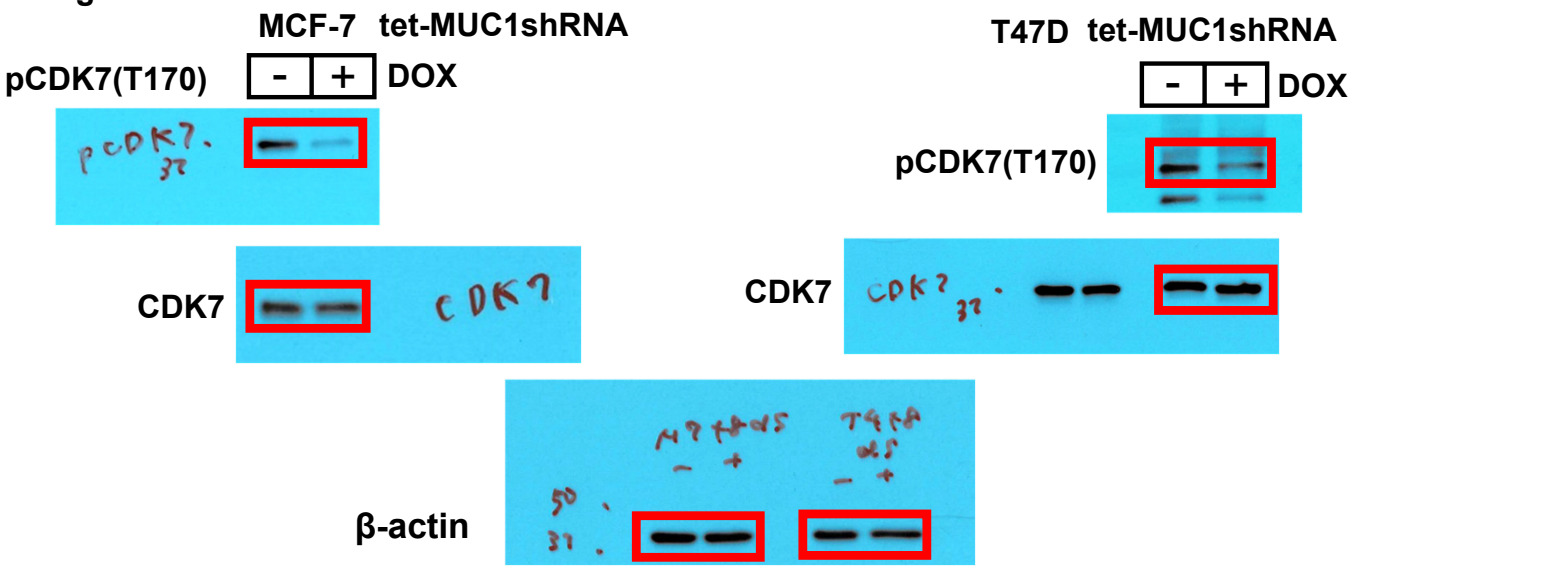


Figure 4a

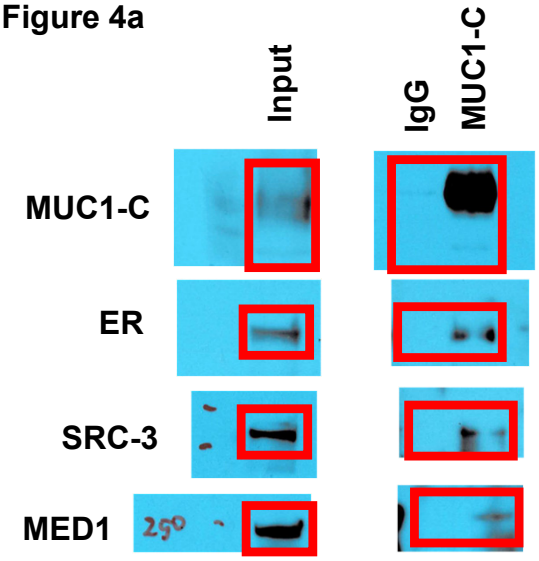


Figure 4b

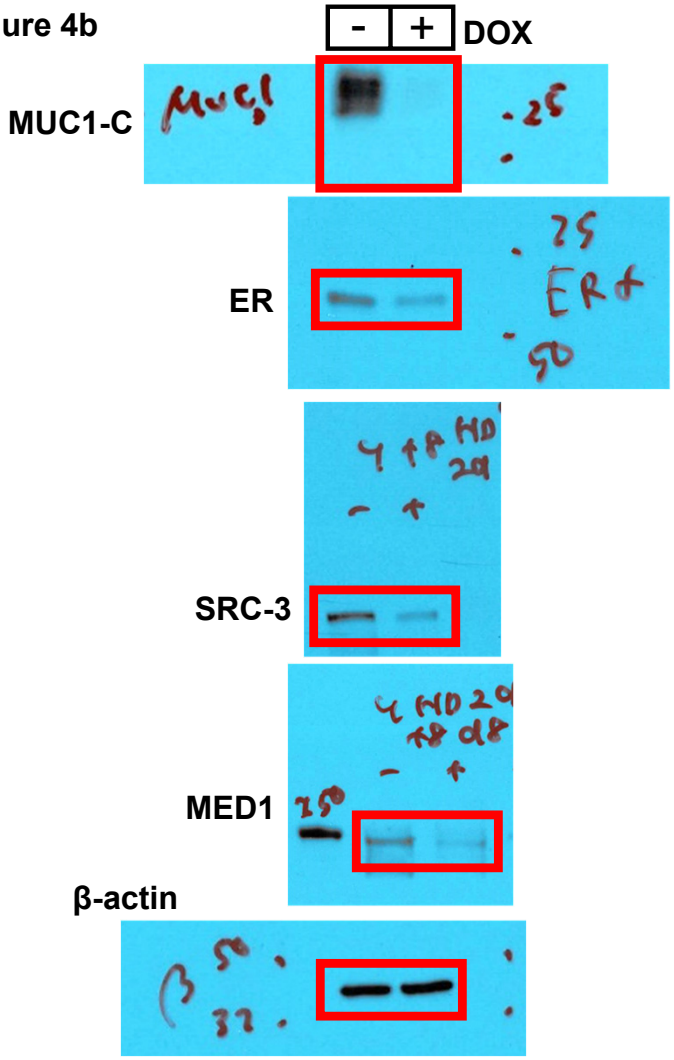


Figure 4c

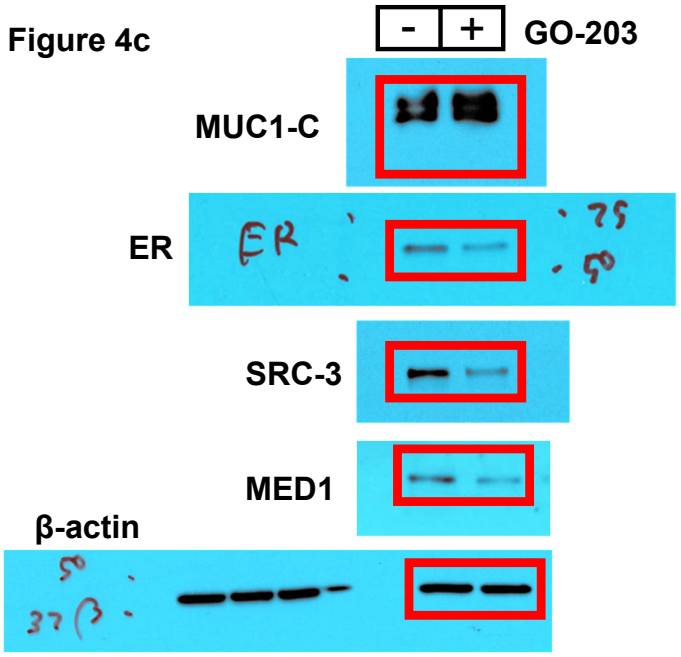


Figure 5a

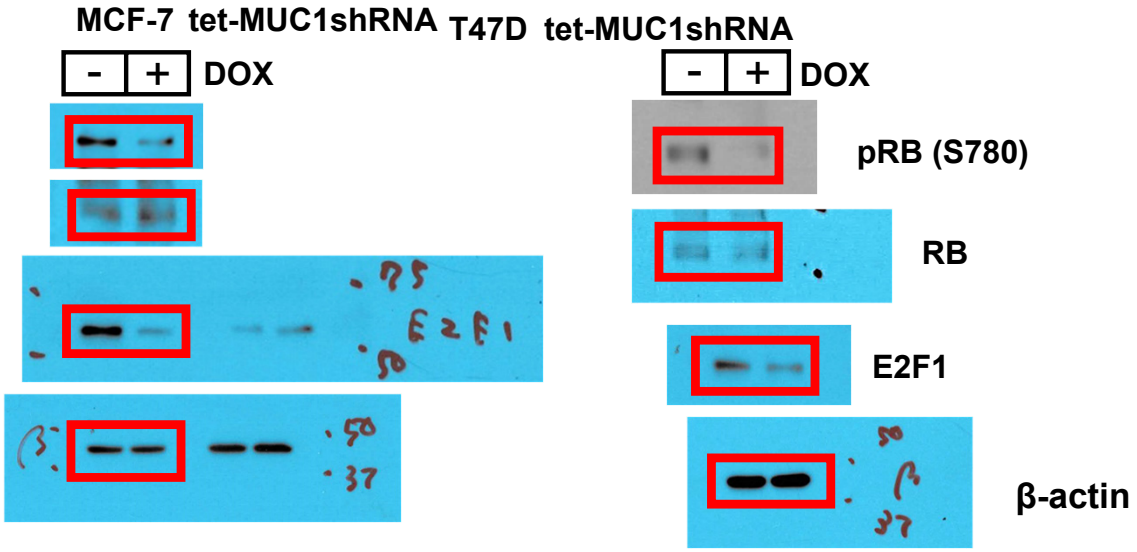


Figure 5d

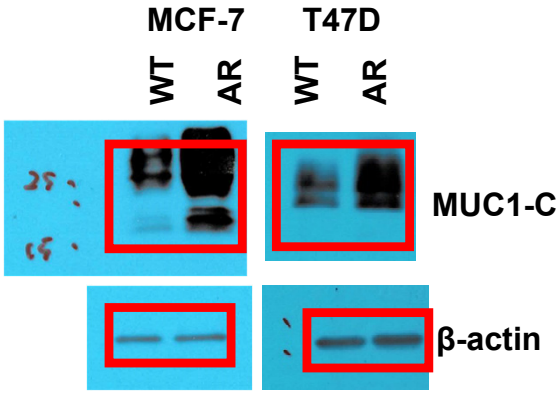


Figure 5e

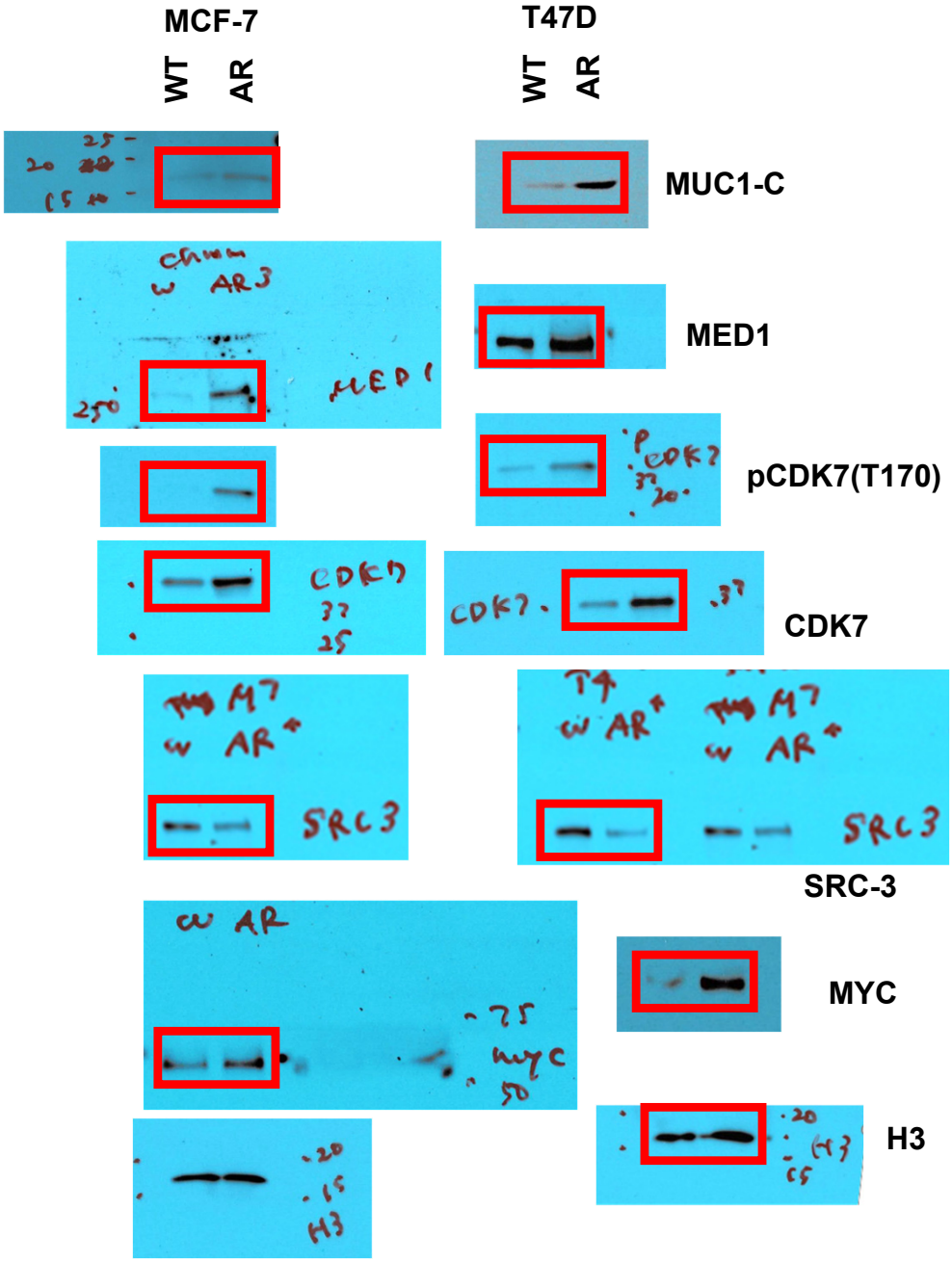


Figure 5f

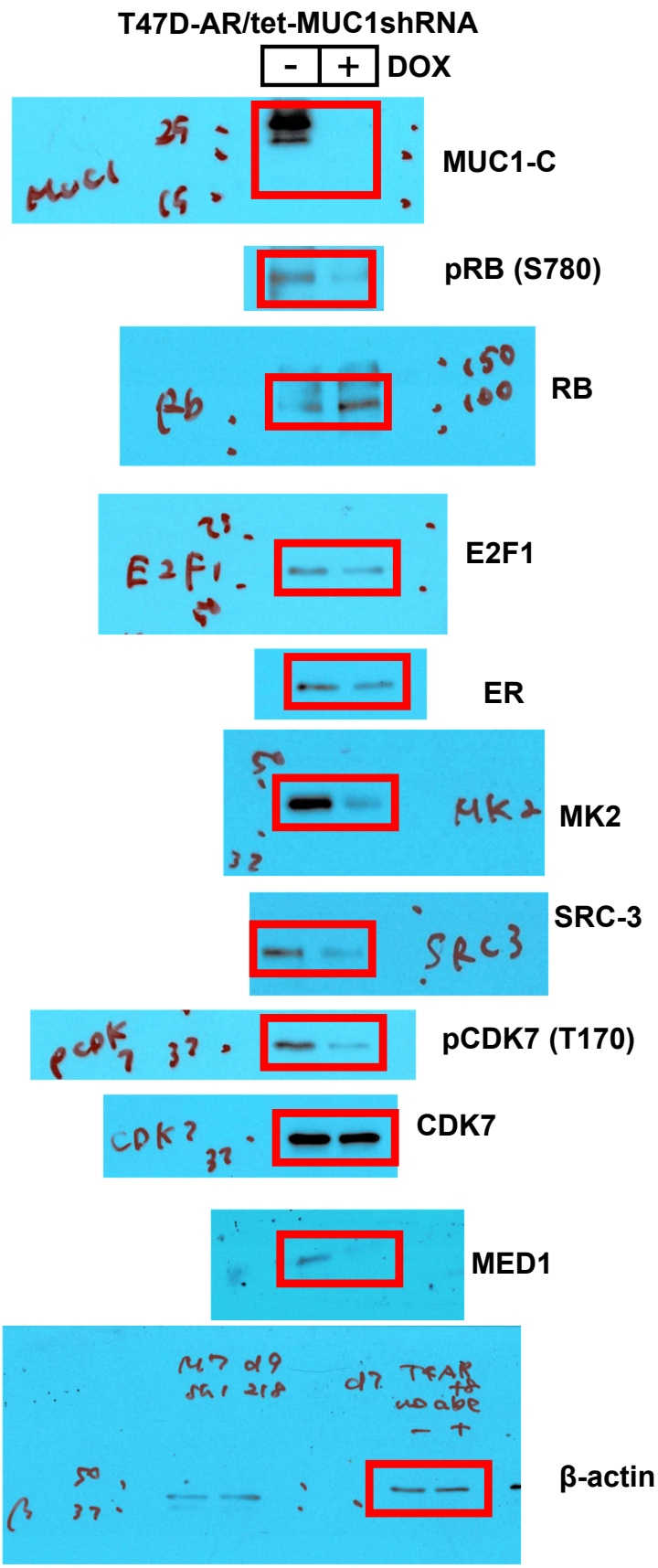


Figure 5g