Supplementary Information

AXL/CDCP1/SRC axis confers to acquired resistance to osimertinib in lung cancer

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E-mail: ono@st-mary.ac.jp

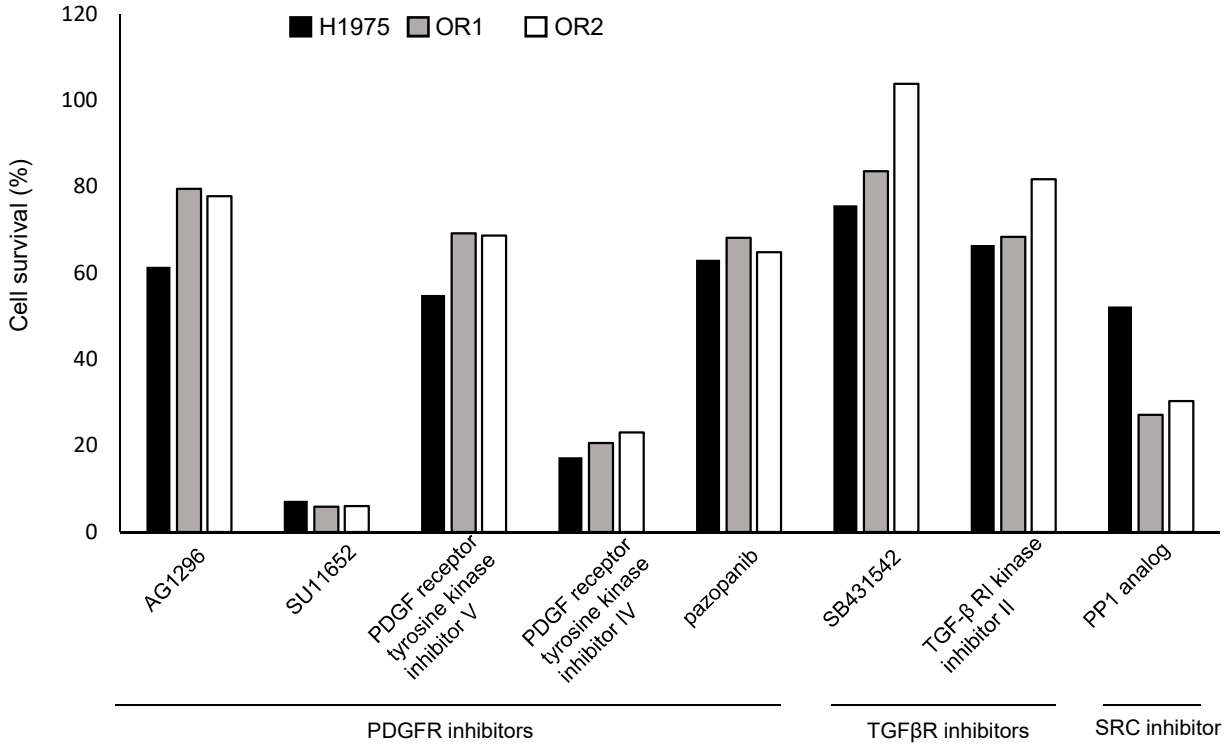


Figure S1. OR1 and OR2 cells could not exhibited high sensitivities to PDGFR and TGFβR inhibitors as compared with the parental cells. The sensitivity to kinase inhibitors was assessed using SCADS inhibitor kit. H1975, OR1, and OR2 cells were treated with kinase inhibitors at 5 μM for 96 hours and subjected to WST assay. Values are expressed as the percentage of the value in the absence of drugs.

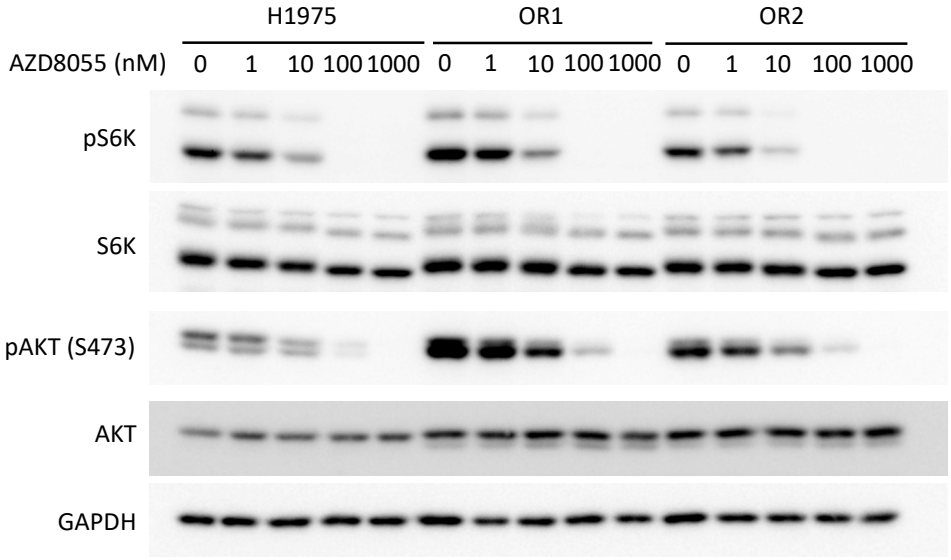


Figure S2. The inhibitory effects of AZD8055 on S6K and AKT activation.
Cells were exposed to AZD8055 for 6 hours.

Table S1. Osimertinib resistant cells show collateral sensitivity to inhibitors of SFK

Drugs	Targets	IC50, μM[1)]		
		H1975	OR1	OR2
Crizotinib	MET, ALK	1.82 (1)	1.38 (0.76)	1.20 (0.66)
PPP	IGF-1R	0.65 (1)	0.54 (0.83)	0.74 (1.14)
Sorafenib	RAF, VEGFR2 PDGFR	4.74 (1)	5.32 (1.12)	5.21 (1.10)
Dasatinib	SRC, ABL, KIT, EPH	0.23 (1)	0.0049 (0.021)	0.0207 (0.09)
PP1	SRC, LCK, FYN, KIT, EGFR	9.60 (1)	1.88 (0.20)	3.07 (0.32)
Saracatinib	SRC, LCK, FYN, KIT, EGFR	3.65 (1)	0.55 (0.15)	0.53 (0.15)
AZD8055	mTORC1, C2	0.048 (1)	0.015 (0.31)	0.016 (0.33)
GSK650394	SGK1/2	65.24 (1)	20.59 (0.32)	53.78 (0.82)
LY294002	PI3K	21.35 (1)	9.79 (0.46)	17.10 (0.80)
AKT inhibitor Ⅳ	AKT	0.19 (1)	0.21 (1.11)	0.28 (1.47)
U0126	MEK1/2	ND[2)]	ND[2)]	ND[2)]
SP600125	JNK1/2, Aurora A	22.30 (1)	51.76 (2.32)	26.83 (1.20)

1) IC_{50} values (μM) are presented as average values of triplicate experiments. The relative drug sensitivity for each drug is presented in parenthesis as the IC50 of OR1/2 normalized to the IC_{50} of H1975 cells.
2) ND: not detectable, no cell toxicity is seen by the drug up to 30 μM

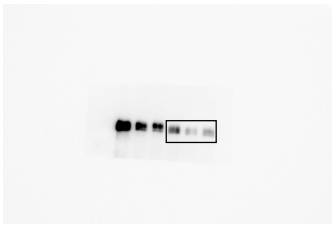
Original western blot images

Figure 1a

pEGFR

pEGFR marker

EGFR

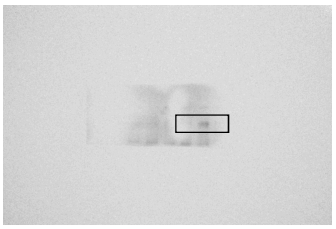


EGFR marker

pHER2

pHER2 marker

HER2



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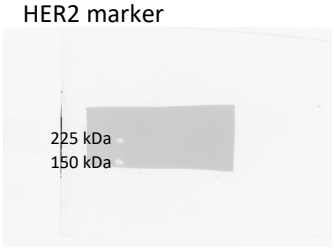

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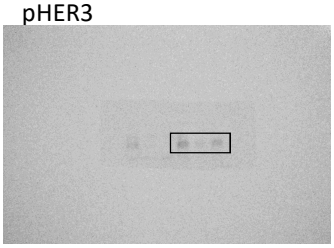

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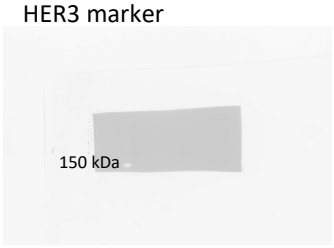
HER3

HER3 marker



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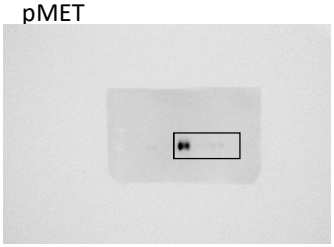

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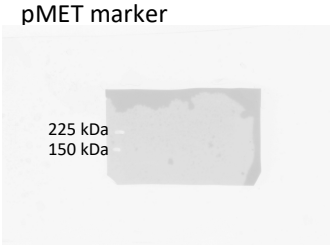

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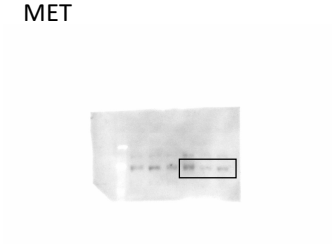

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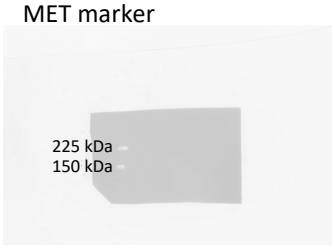

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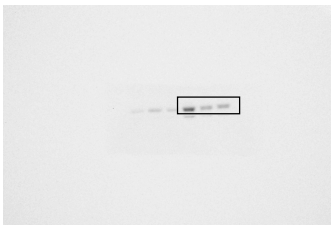

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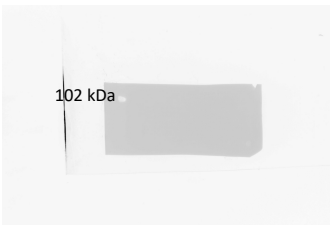

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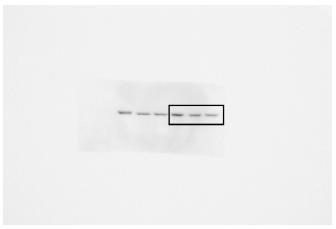

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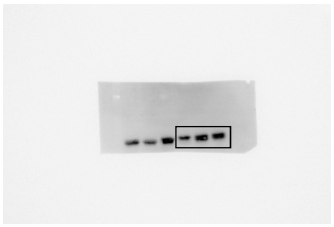

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pAKT(S473)

pAKT (S473) marker

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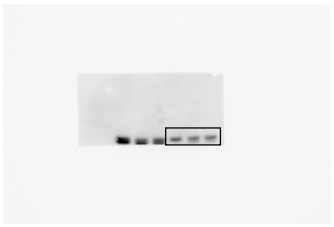

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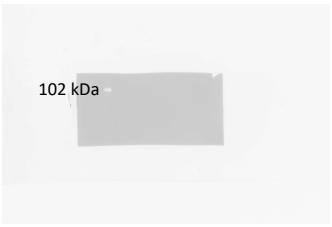

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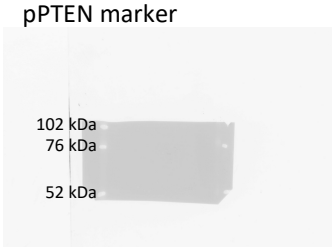

Figure 1a

PTEN

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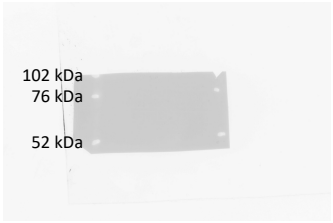

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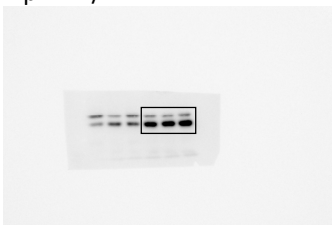

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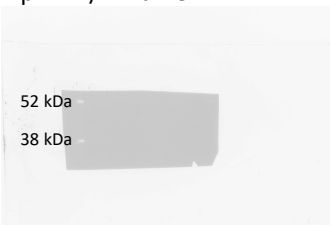

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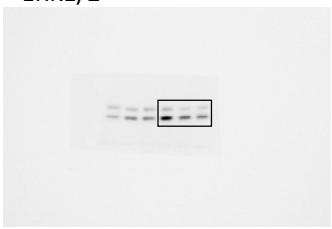

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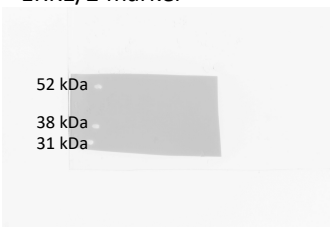

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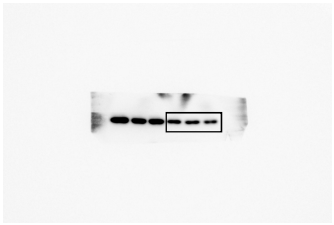

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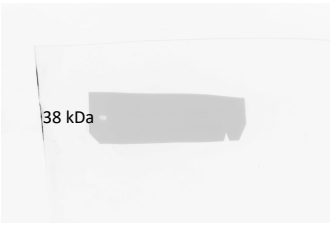
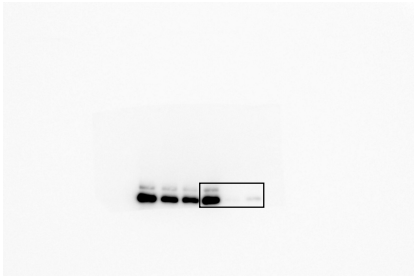
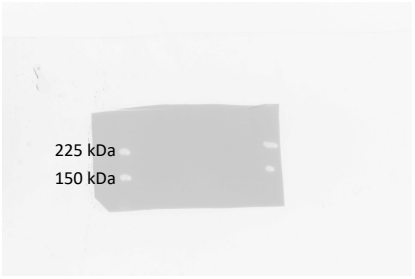


Figure 1b

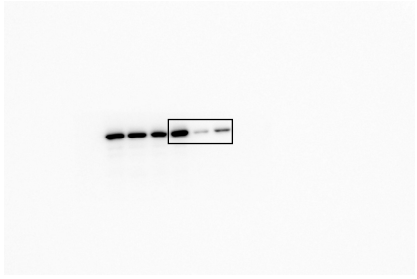
E-cadherin



E-cadherin marker



β-catenin



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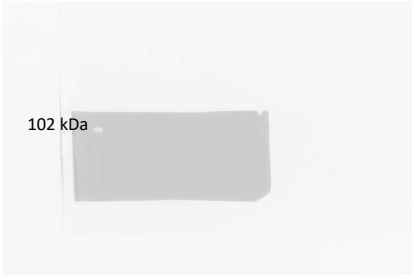

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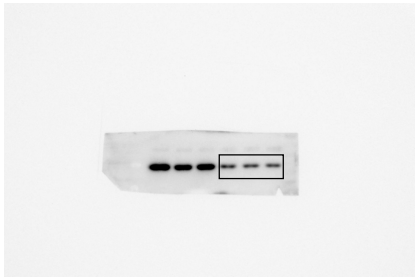

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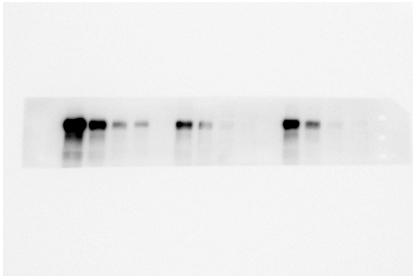
Figure 1c

pEGFR



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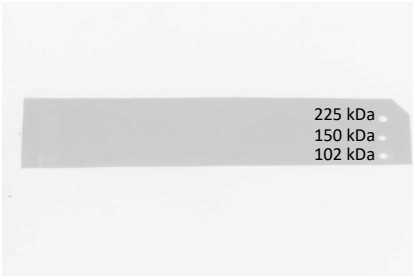

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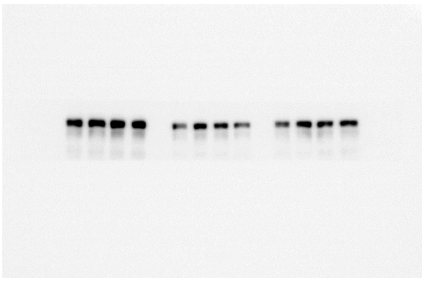

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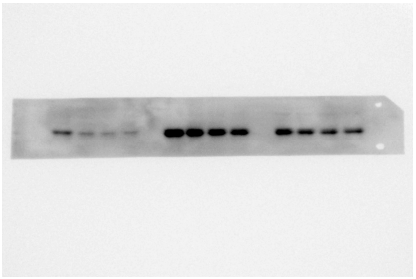

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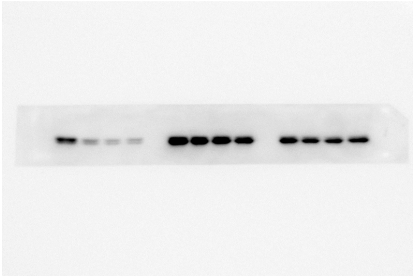

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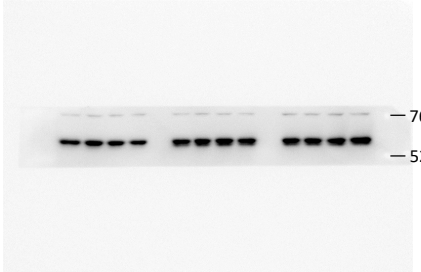

Figure 1c

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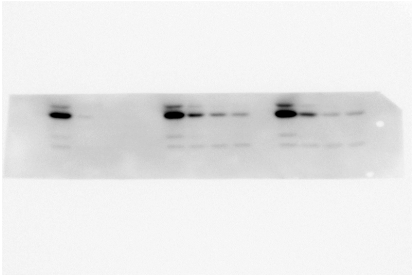

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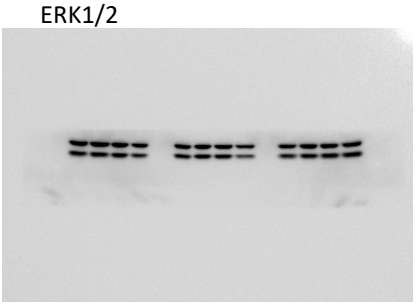
ERK1/2



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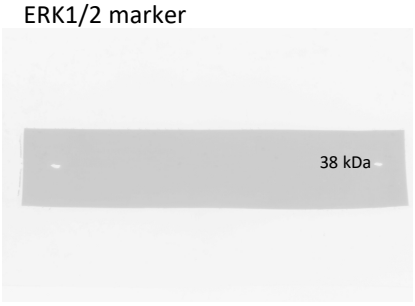

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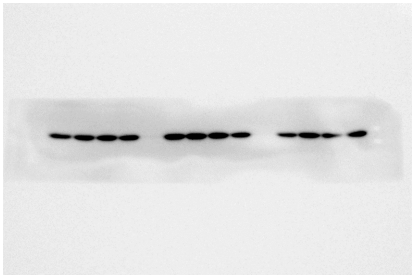

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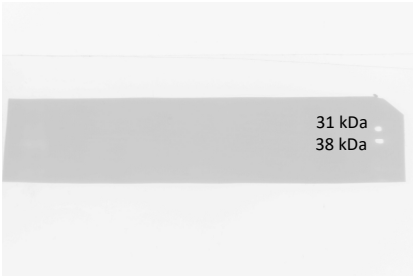
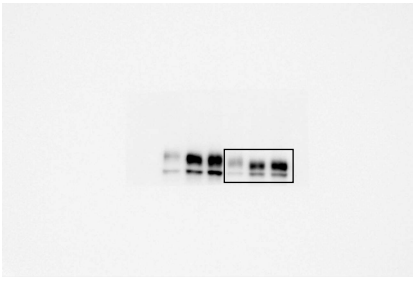


Figure 2a

AXL



AXL marker

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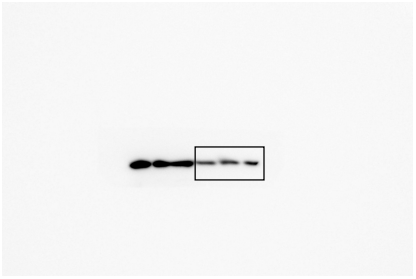

GAPDH marker

Figure 2b

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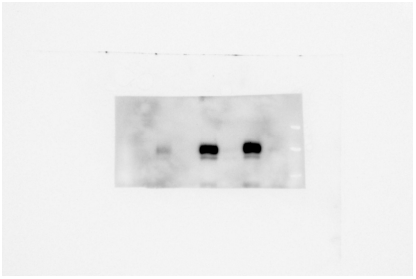

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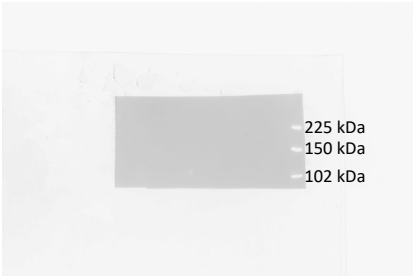
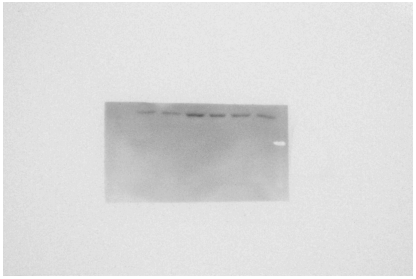


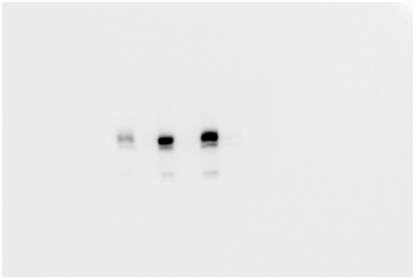
β-actin



β-actin marker

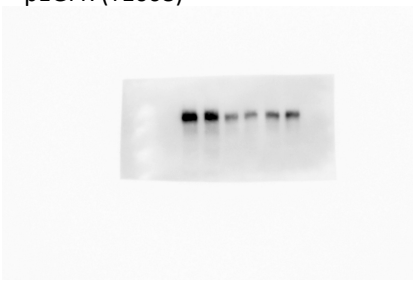
Figure 2d

AXL

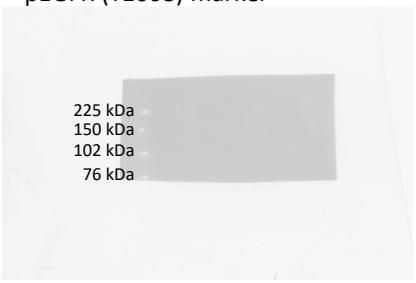


AXL marker

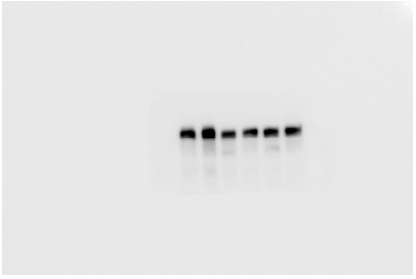
pEGFR (Y1068)



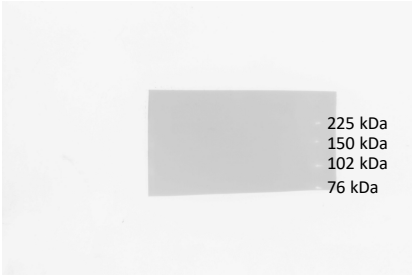
pEGFR (Y1068) marker



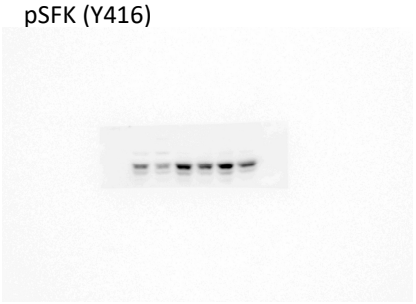
EGFR



EGFR marker

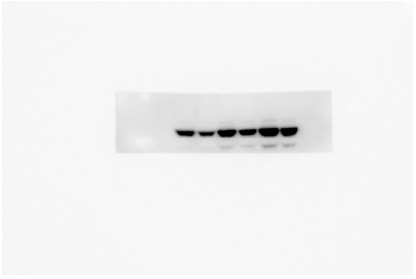


pSFK (Y416)



pSFK (Y416) marker

SRC



SRC marker

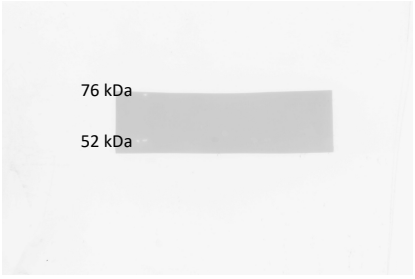
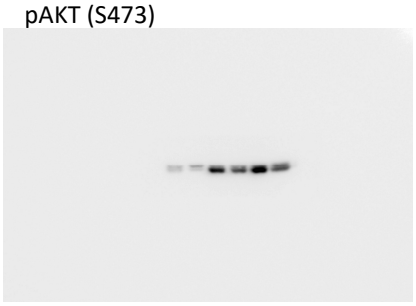
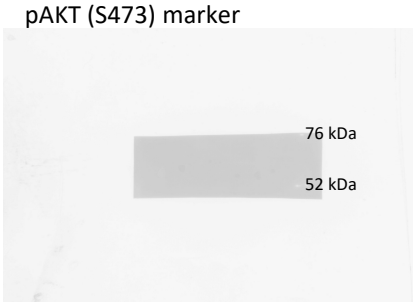


Figure 2d

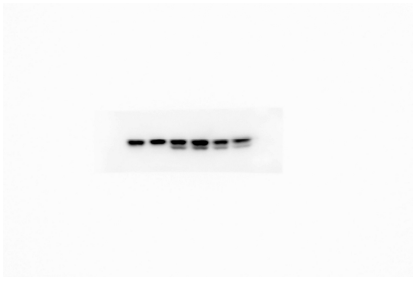
pAKT (S473)



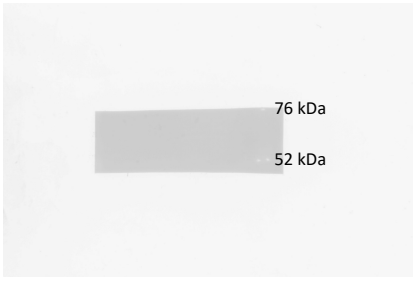
pAKT (S473) marker



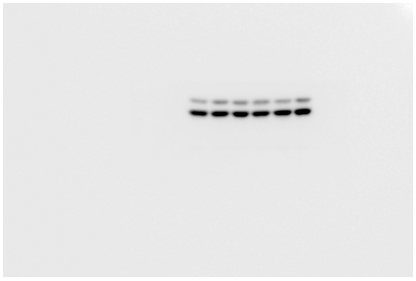
AKT



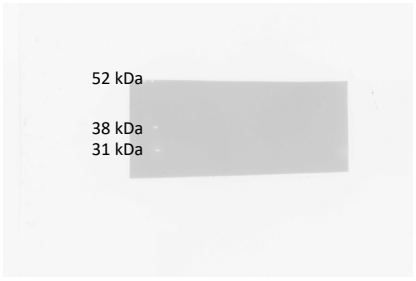
AKT



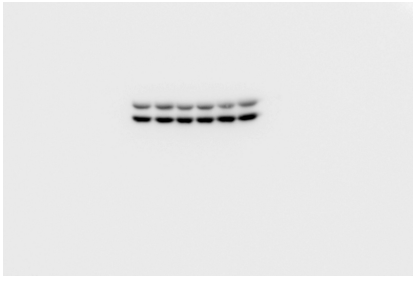
pERK1/2



pERK1/2 marker



ERK1/2



ERK1/2 marker

β-actin

β-actin marker

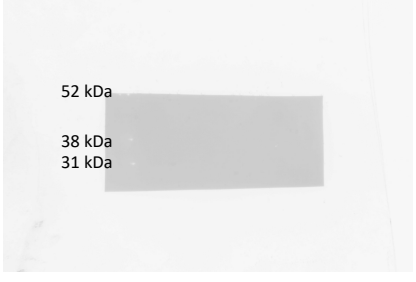
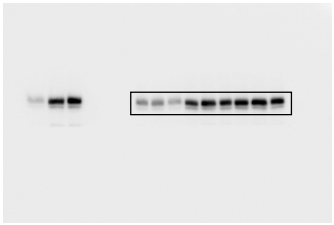
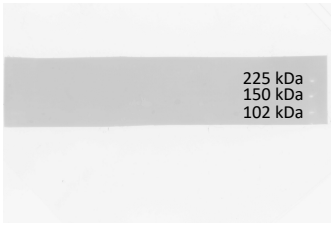


Figure 2f

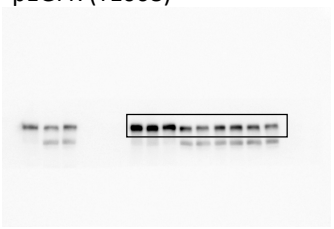
AXL



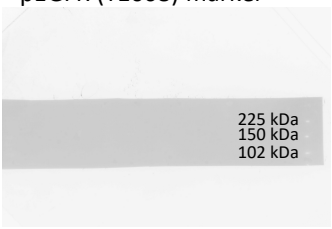
AXL marker



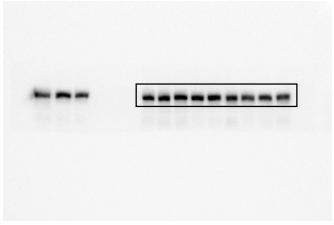
pEGFR (Y1068)



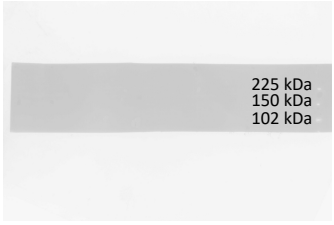
pEGFR (Y1068) marker



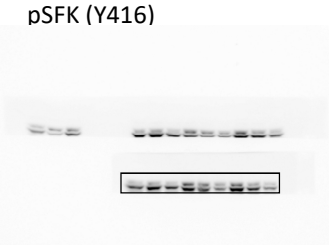
EGFR



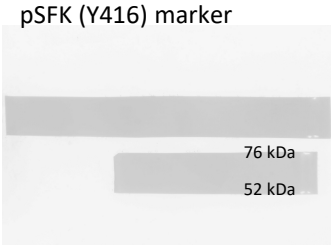
EGFR marker



pSFK (Y416)

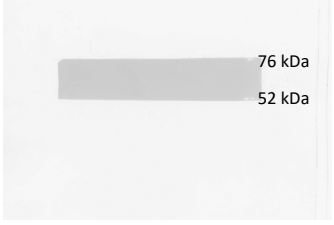


pSFK (Y416) marker

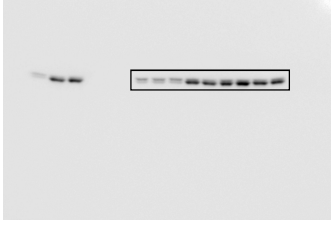


SRC

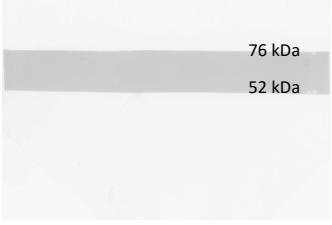
SRC marker



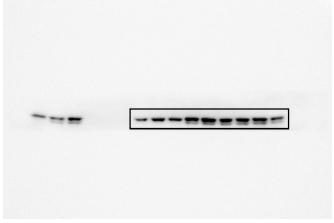
pAKT (S473)



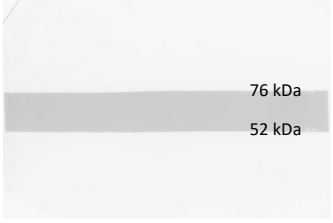
pAKT (S473) marker



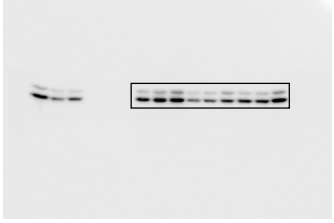
AKT



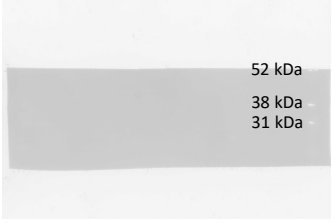
AKT marker



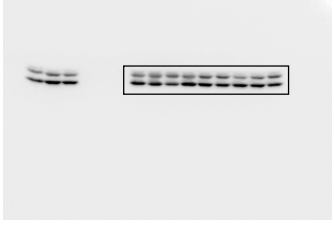
pERK1/2



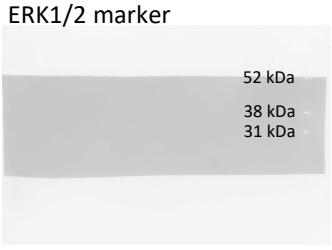
pERK1/2 marker



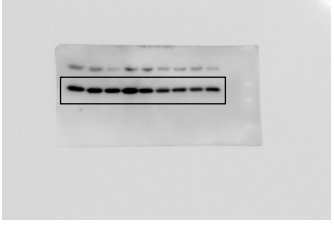
ERK1/2



ERK1/2 marker



GAPDH



GAPDH marker

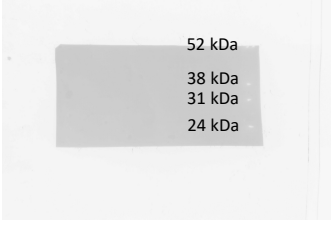
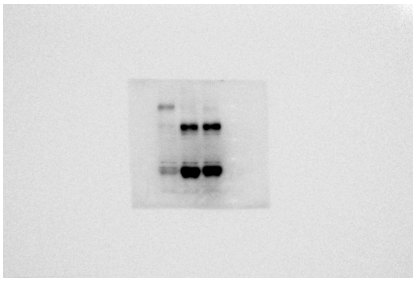
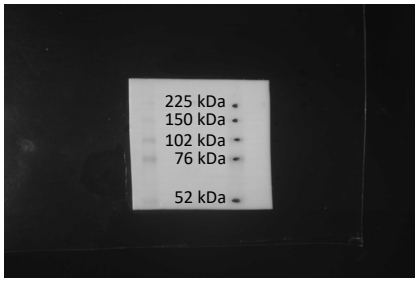


Figure 3a

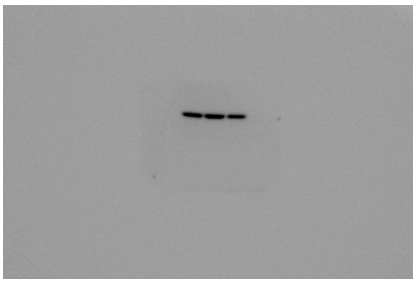
CDCP1



CDCP1 marker



GAPDH



GAPDH marker

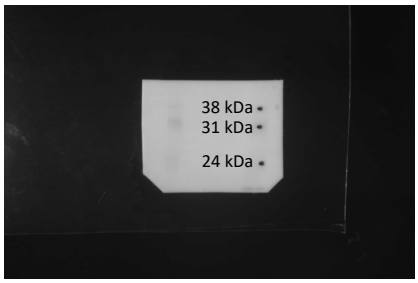
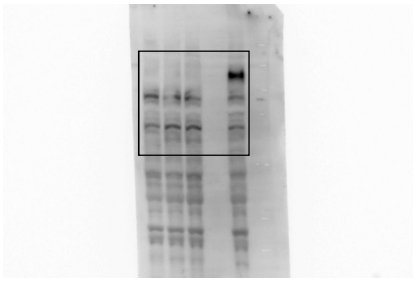
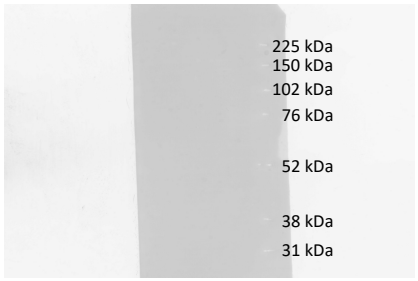


Figure 3b

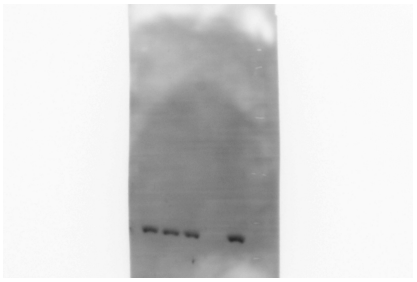
pCDCP1



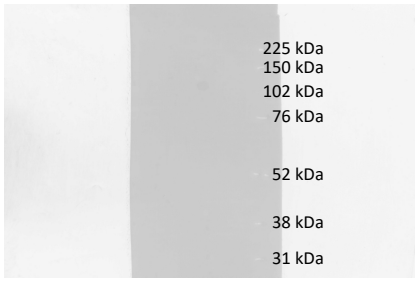
pCDCP1 marker



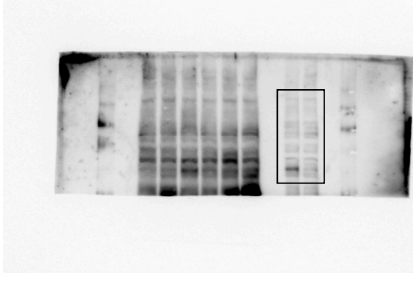
GAPDH



GAPDH marker

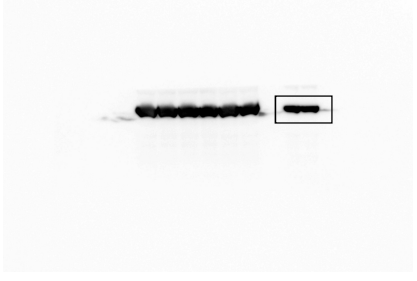


pCDCP1



pCDCP1 marker

β-actin



β-actin marker

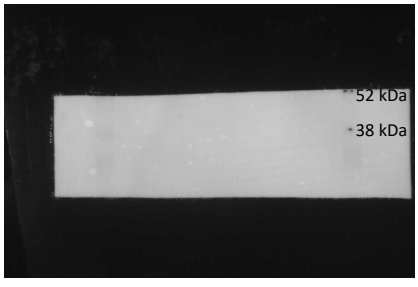
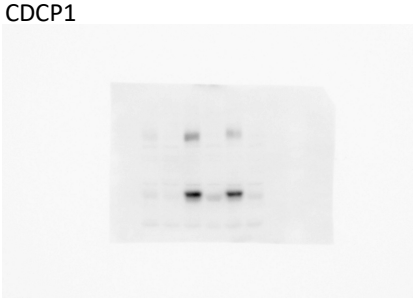
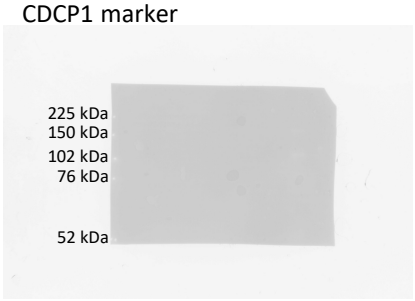


Figure 3d

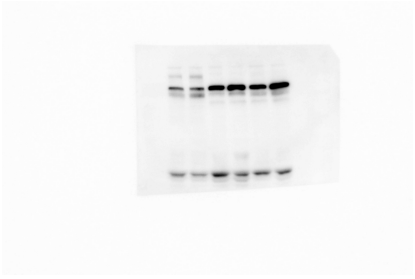
CDCP1



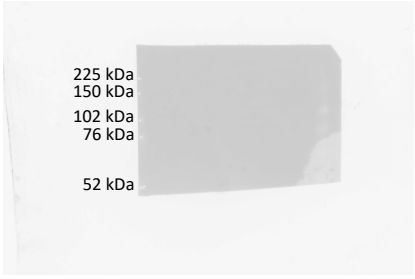
CDCP1 marker



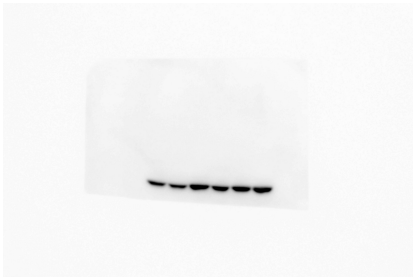
pSFK (Y416)



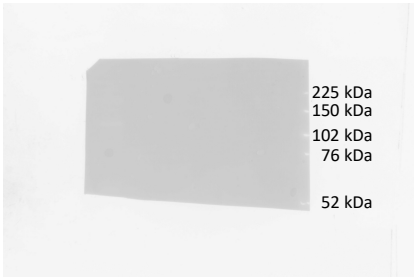
pSFK (Y416) marker



SRC



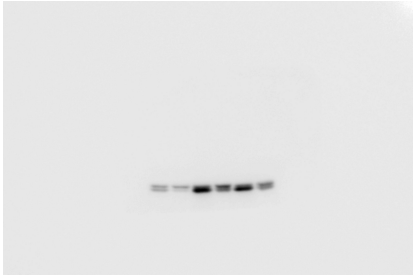
SRC marker



pAKT (T308)

pAKT (T308) marker

pAKT (S473)

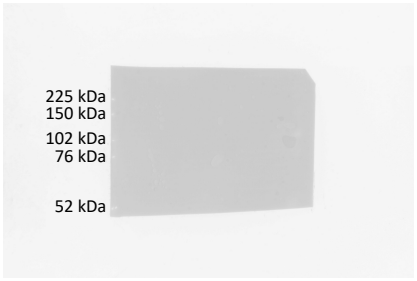


pAKT (S473) marker

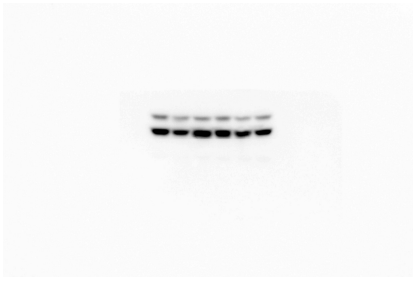
Figure 3d

AKT

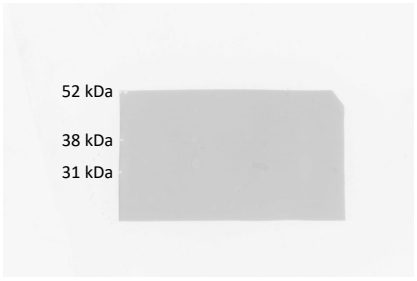
AKT marker



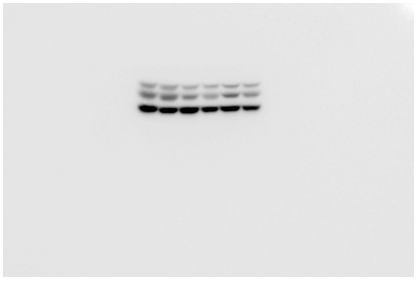
pERK1/2



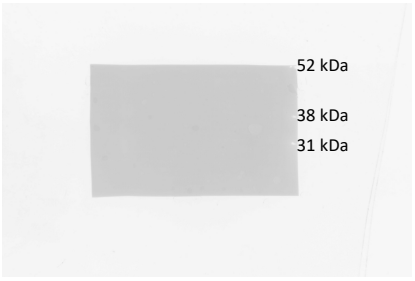
pERK1/2 marker



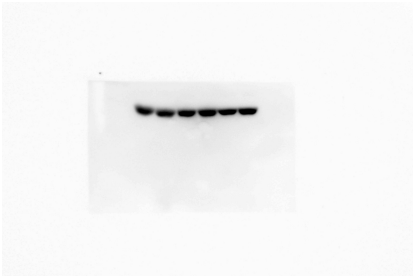
ERK1/2



ERK1/2 marker



β-actin



β-actin marker

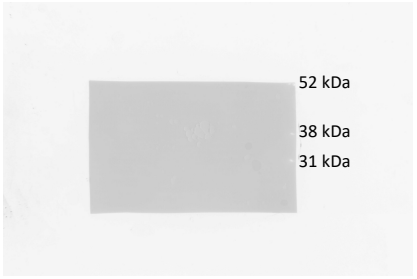
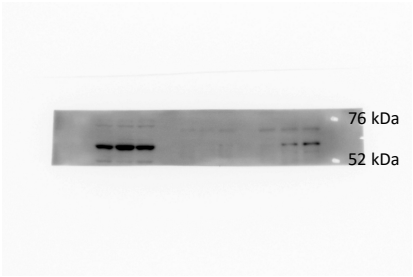


Figure 3e

SRC



CDCP1

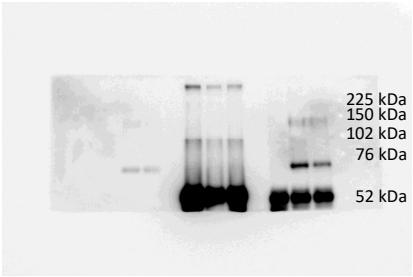
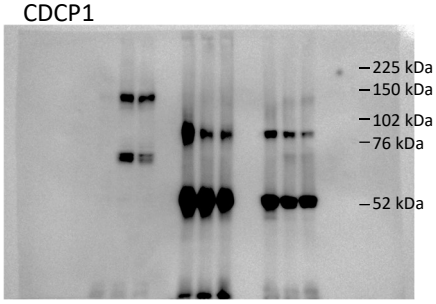


Figure 3f

CDCP1



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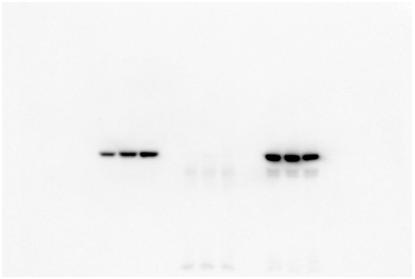
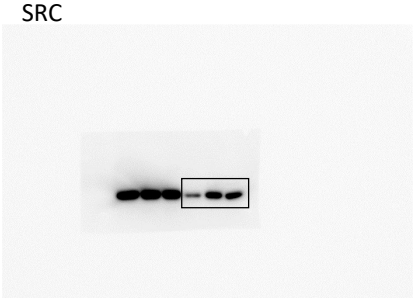


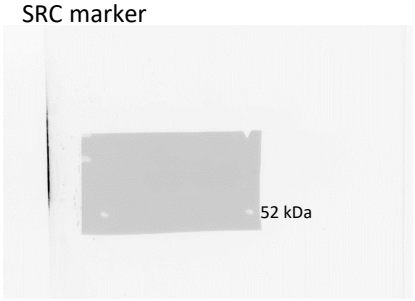
SRC marker

Figure 4a

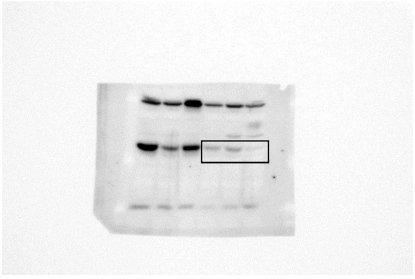
SRC



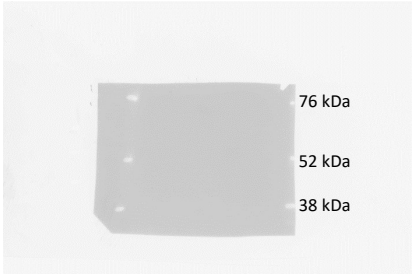
SRC marker



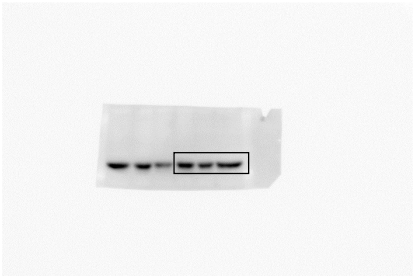
FYN



FYN marker

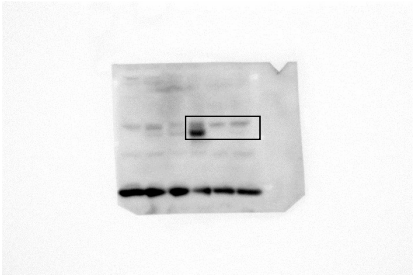


LYN

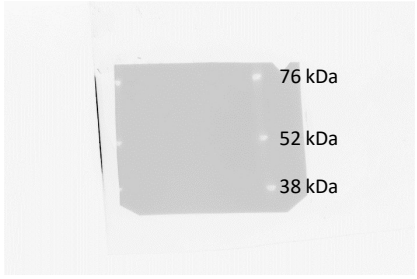


LYN marker

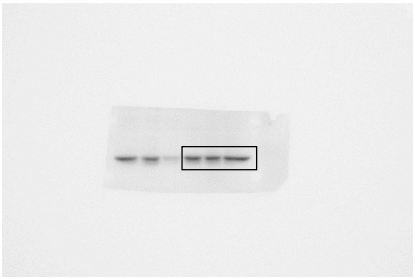
LCK



LCK marker



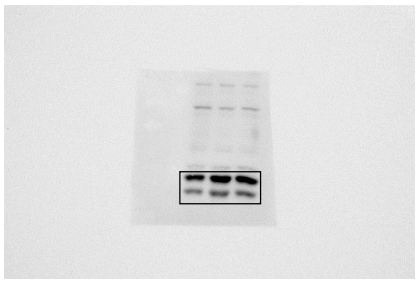
YES



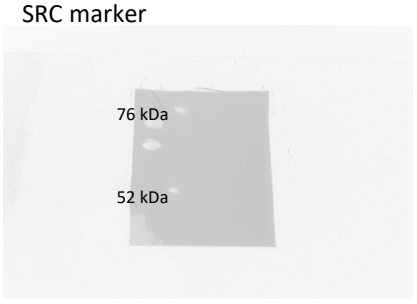
YES marker

Figure 4a

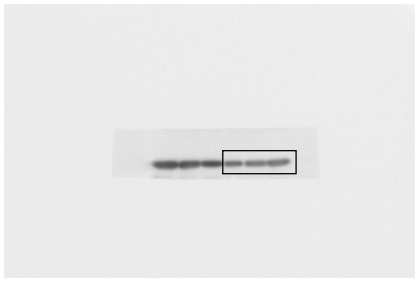
CSK



SRC marker



GAPDH



GAPDH marker

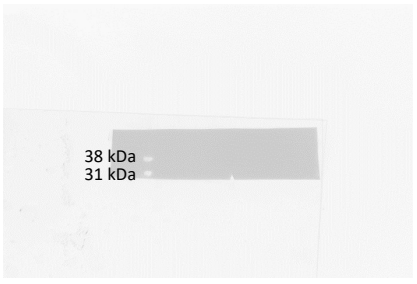
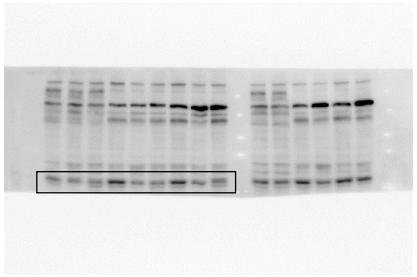
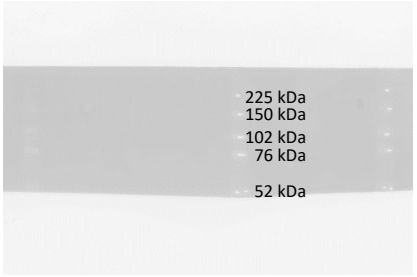


Figure 4c

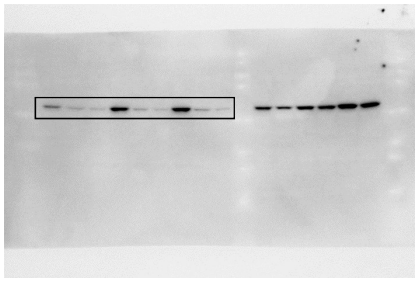
pSFK



pSFK marker

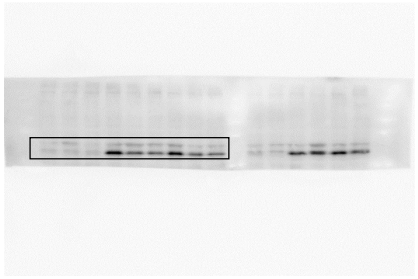


SRC



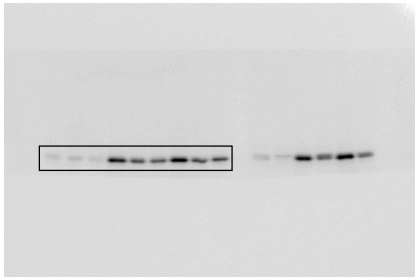
SRC marker

pAKT(T308)

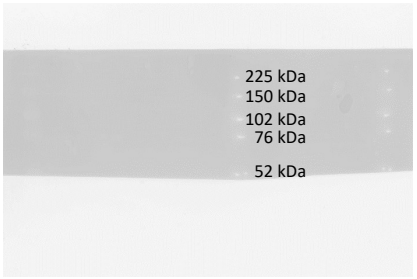


pAKT (T308) marker

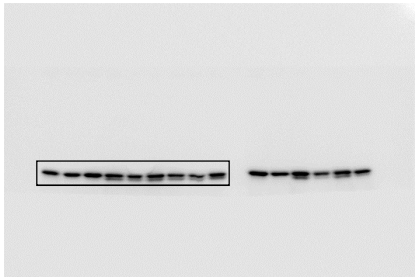
pAKT (S473)



pAKT (S473) marker



AKT



AKT marker

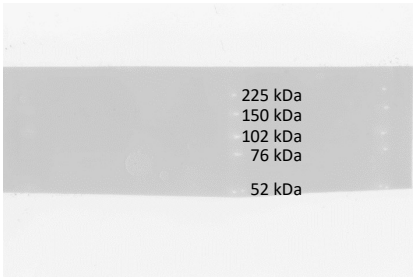
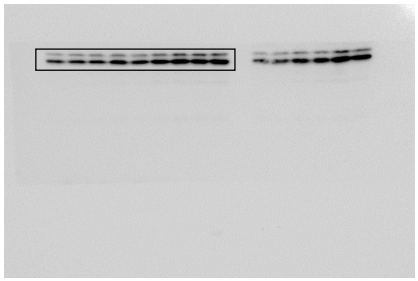


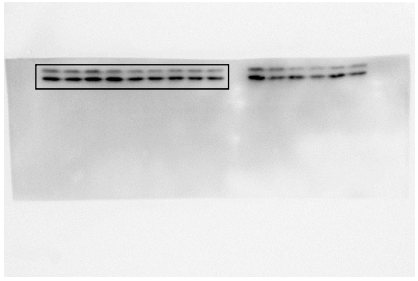
Figure 4c

pERK1/2



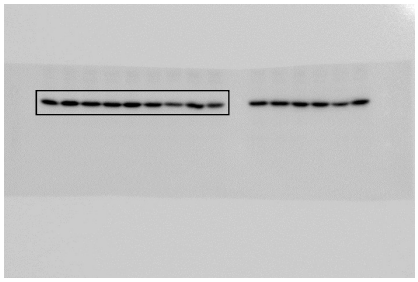
pERK1/2 marker

ERK1/2



ERK1/2 marker

GAPDH



GAPDH marker

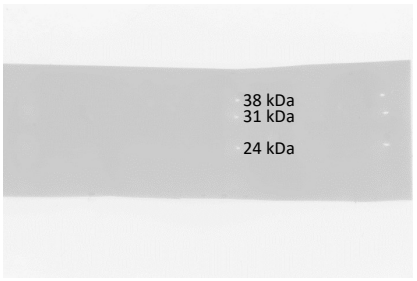
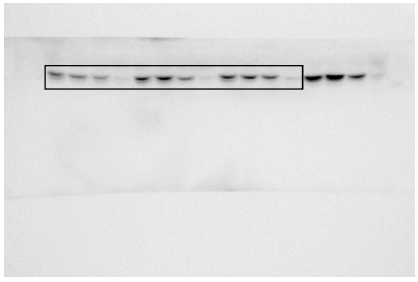
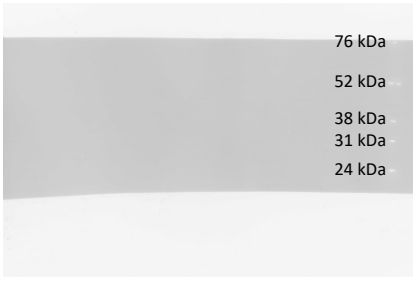


Figure 4e

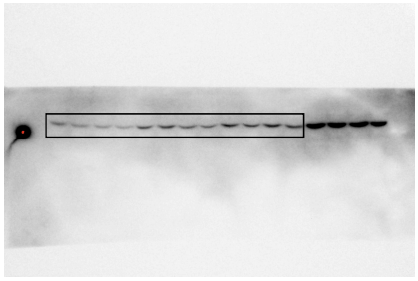
pSFK



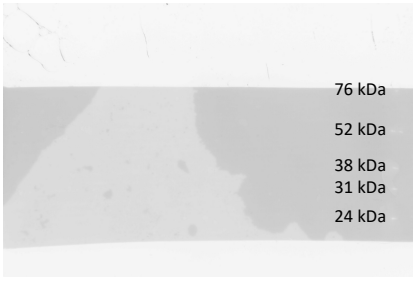
pSFK marker



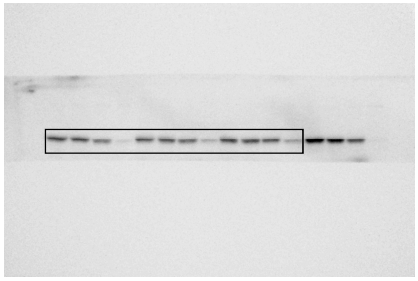
SRC



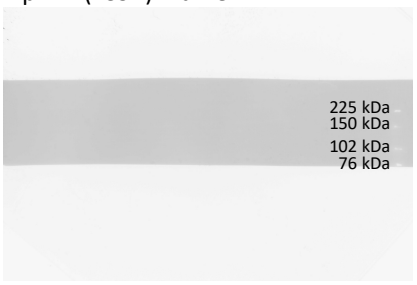
SRC marker



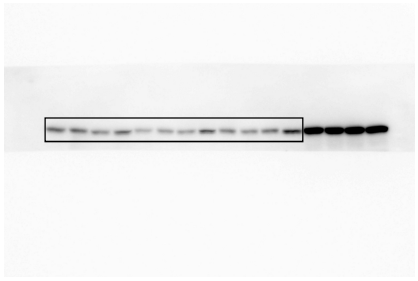
pFAK (Y397)



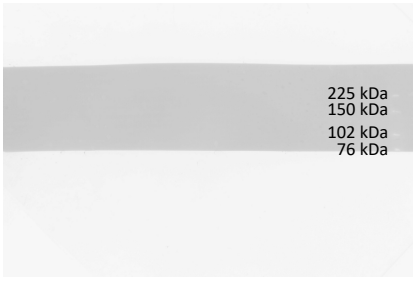
pFAK (Y397) marker



FAK



FAK marker



pAKT (S473)

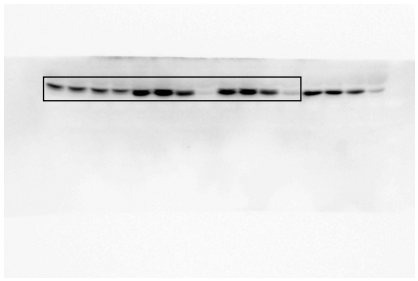
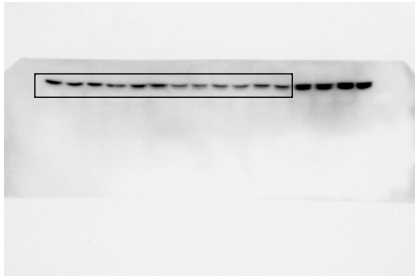
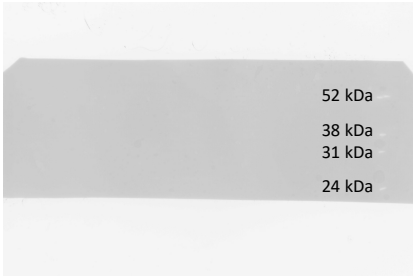


Figure 4e

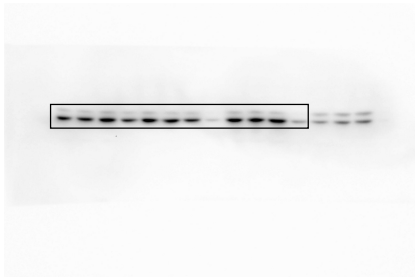
AKT



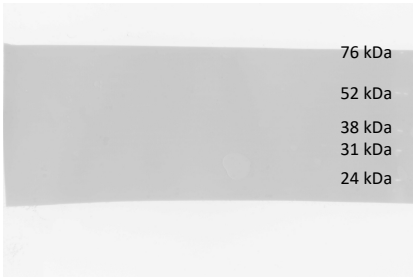
AKT marker



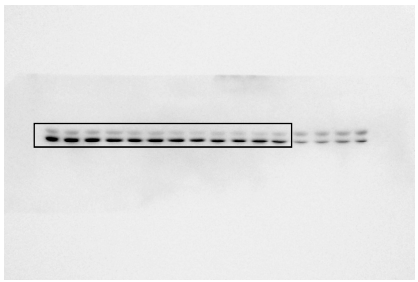
pERK1/2



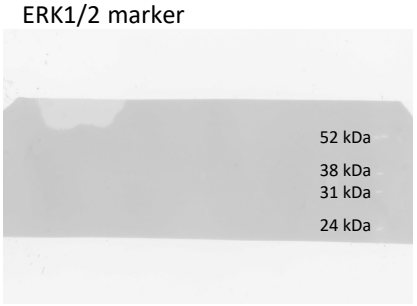
pERK1/2 marker



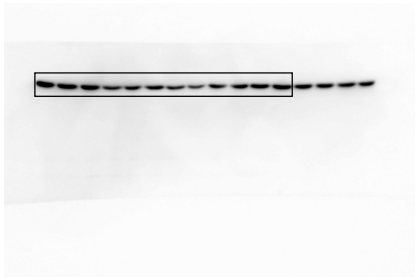
ERK1/2



ERK1/2 marker



α-tubulin



α-tubulin marker

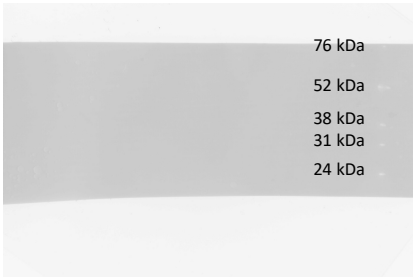
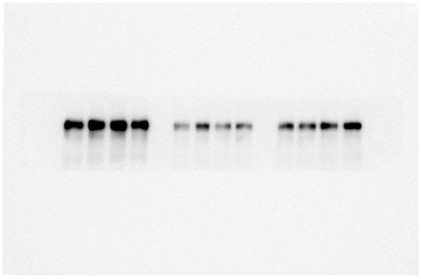
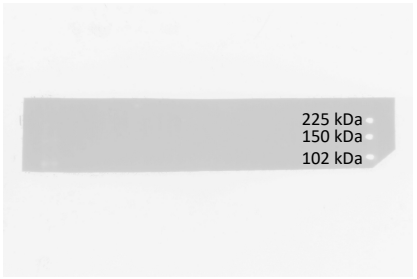


Figure 4g

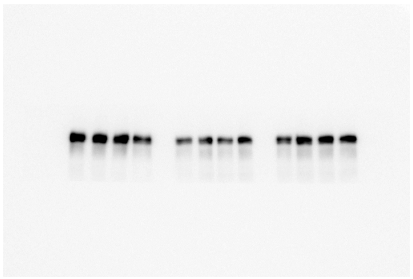
pEGFR (Y1068)



pAKT(S473) marker

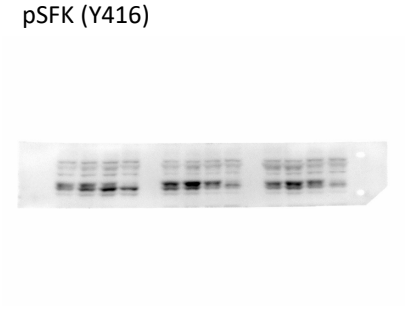


EGFR



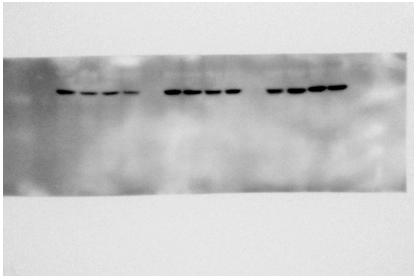
EGFR marker

pSFK (Y416)

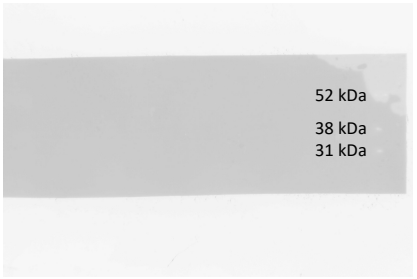


pSFK (Y416) marker

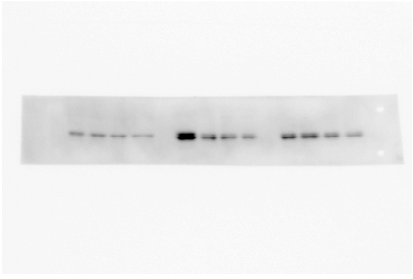
SRC



SRC marker



pAKT (T308)



pAKT(T308) marker

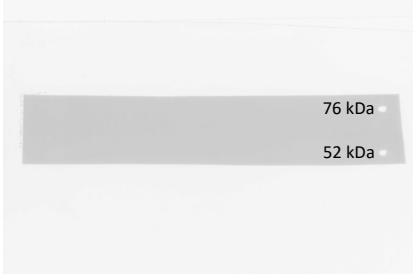
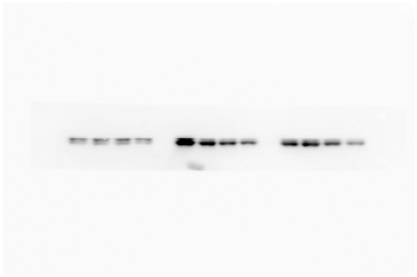


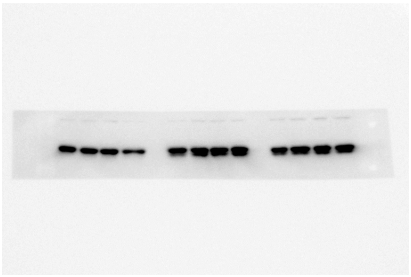
Figure 4g

pAKT (S473)



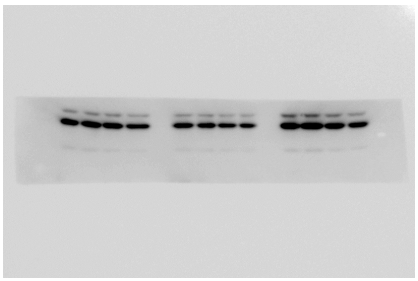
pAKT(S473) marker

AKT



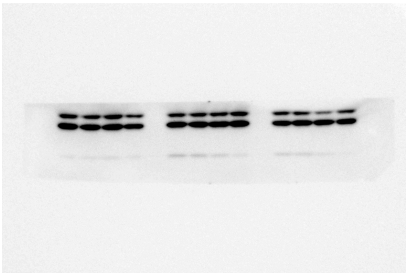
AKT marker

pERK1/2



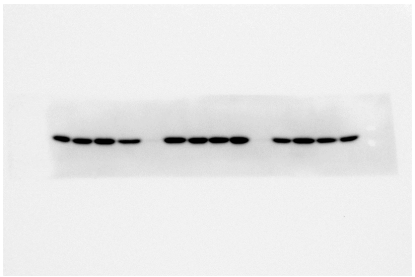
pERK1/2 marker

ERK1/2



ERK1/2 marker

GAPDH



GAPDH marker

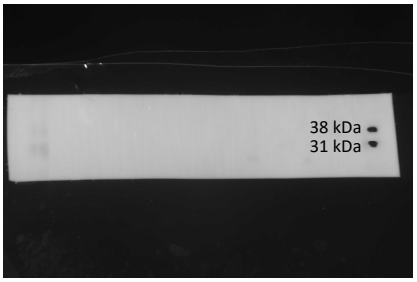
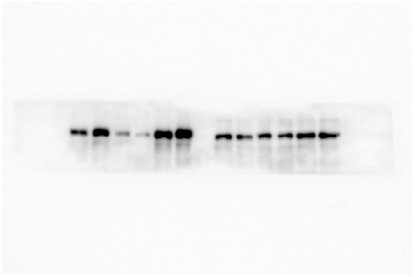
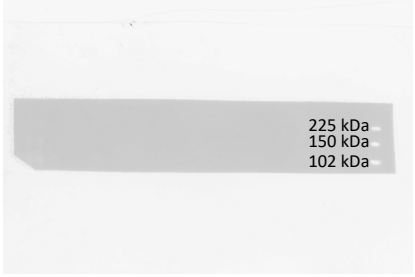


Figure 4i

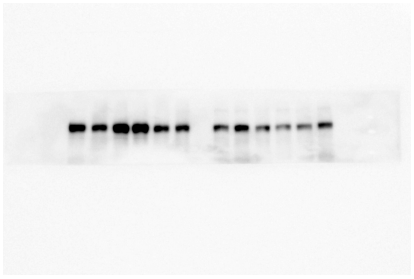
EGFR



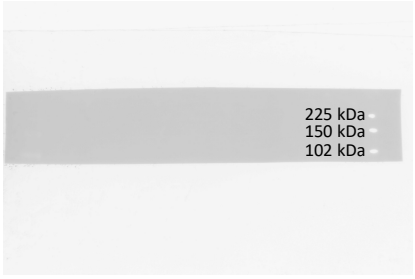
EGFR marker



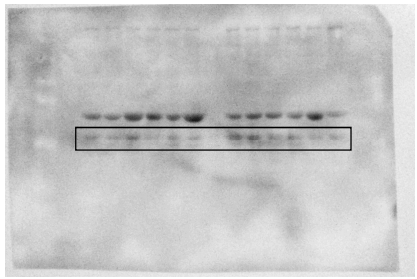
EGFR



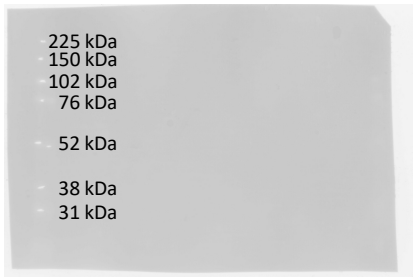
EGFR marker



pSFK



pSFK marker



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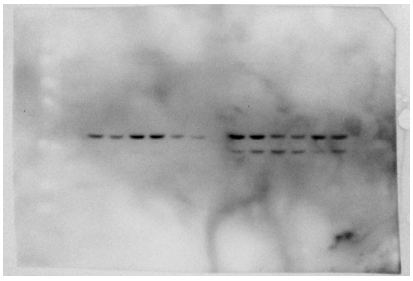
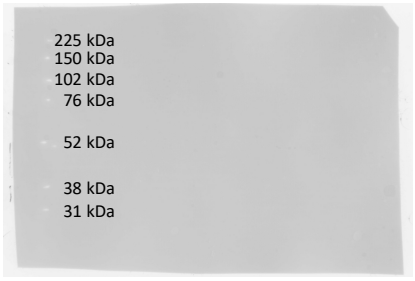
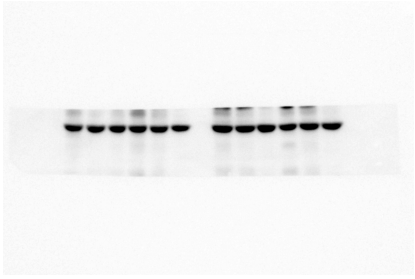


SRC marker



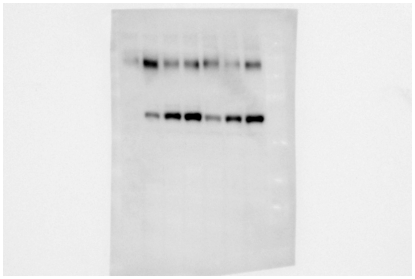
β-actin



β-actin marker

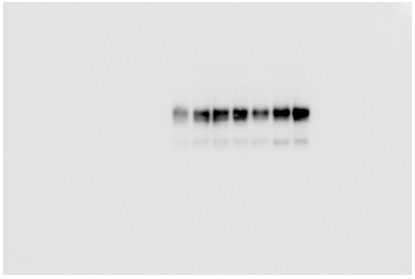
Figure 5c

CDCP1

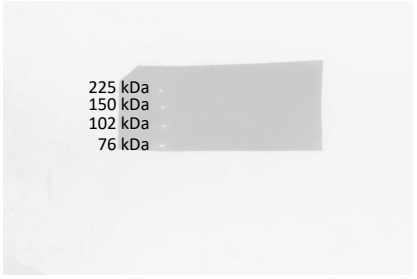


CDCP1 marker

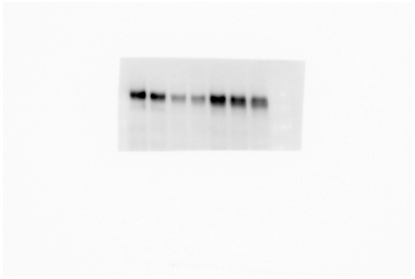
AXL



AXL marker

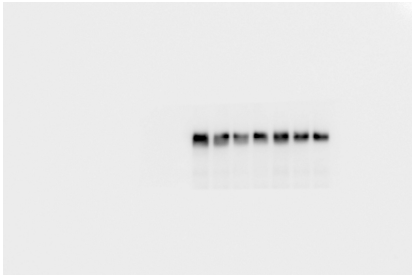


pEGFR

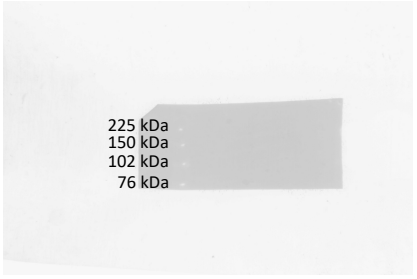


pEGFR marker

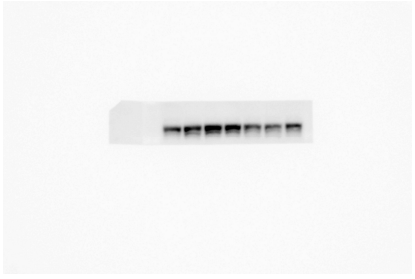
EGFR



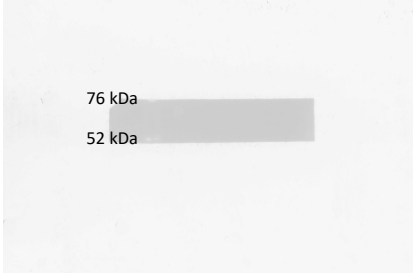
EGFR marker



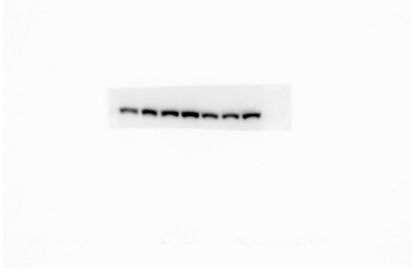
pSFK



pSFK marker



SRC



SRC marker

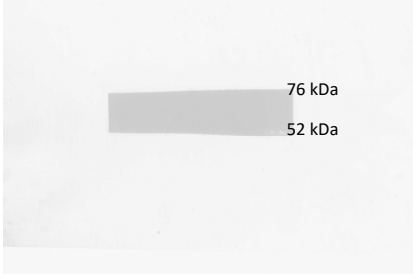
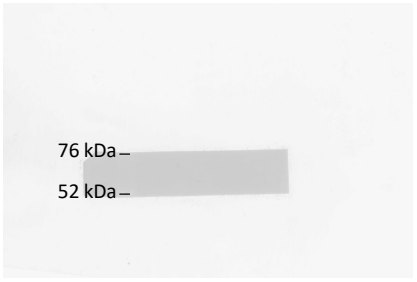


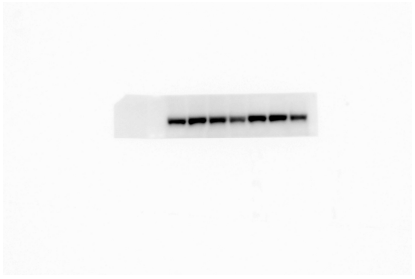
Figure 5c

pAKT

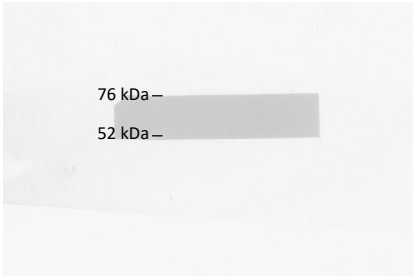
pAKT marker



AKT

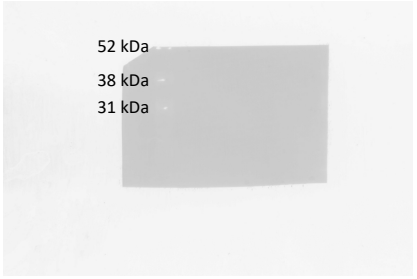


AKT marker



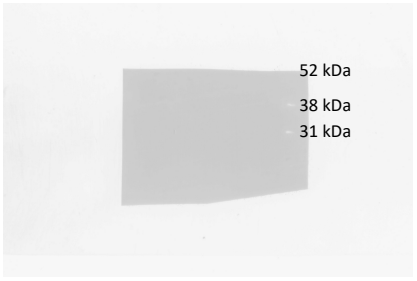
pERK1/2

pERK1/2 marker

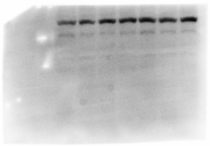


ERK1/2

ERK1/2 marker



β-actin



β-actin marker

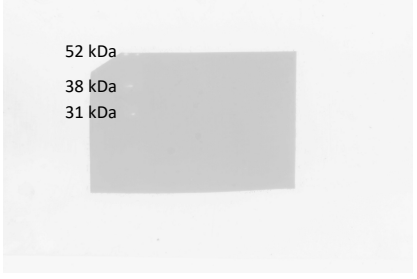
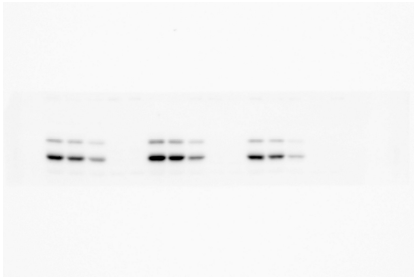
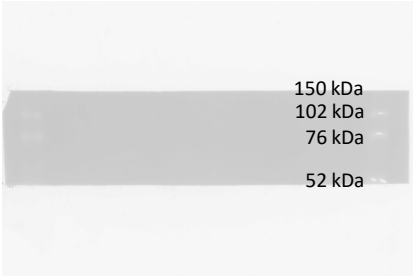


Figure S2

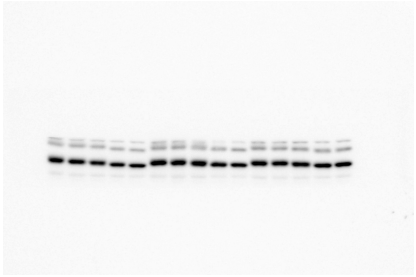
pS6K



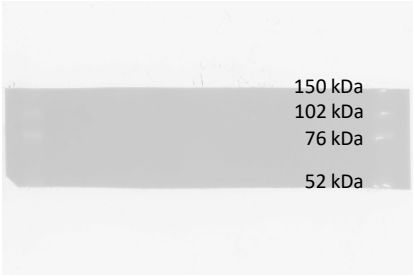
pS6K marker



S6K



S6K marker

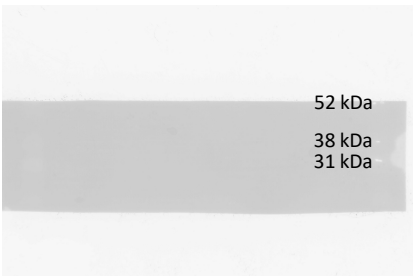


pAKT

pAKT marker

AKT

AKTmarker

GAPDH

GAPDH marker