Supplementary Information

MERTK activation drives *EGFR*-mutated non-small cell lung cancer resistant to osimertinib

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Patent numbers to XW and SVF describing MRX-2843 are listed here: PCT/US2012/058298, WO2013052417 A1, US2013/641729, US 9273056, ZL2012800568254, Japanese patent number 6316925, Australian patent number 2012318896, European patent number FR2763988, RU 2631655, HK1201256, US2015/790700, US10179133, KR 10-2063098, PCT/ US2015/014573, US 2016/0151372, US 9795606 B2, PCT/US2015/024381, WO2015157128A1, US9555031, US10004755, PCT/US2020/026167, WO2020205967, US2022/0162214, and JPA 2022522539-000000 (to University of North Carolina).

Table S1 Cell line source information.

Cell Line	Source
HBEC-3KT	American Type Culture Collection, Manassas, VA
A549	
H226	
H2009	
PC9	Immuno-Biological Laboratories Co, Japan
COLO699	German Collection of Microorganisms and Cell Culture, Germany
H2228	Drs. John Minna and Adi Gazdar, University of Texas Southwestern Medical Center, Dallas, TX
H4006	
H4011	
H1975	
H1650	

Table S2 Reagent information.

Reagents	Company	Catalog#
Recombinant GAS6 (rGAS6)	R&D Systems	885-GSB
Recombinant human EGF		236-EG-200
Recombinant PROS1 (rPROS1)	Sino Biological	12179-H08H
geneticin	ThermoFisher Scientific	10131027
EGFR siRNA (EGFRsi)		EGFRHSS103114
MERTK siRNA (MERTKsi)		MERTKHSS116030
AXL siRNA (AXLsi)		AXLHSS100897
Non-targeting siRNA (Vsi)	Santa Cruz	sc-37007
Nuclight^{TM}Red lentivirus	Essen Bioscience	4476
Puromycin	TaKaRa	631306

Table S3 Primers used for *EGFR* gene sequencing.

Sequence (5`-3`)
GCCAAGGCACGAGTAACAAGC
TGCCCTGTGCAACGTGGAGA
GTGGTGACAGATCACGGCTC
GACCAAGCAACATGGTCAG
CTGCCTCAGGCCATGAACATC
GAAGCGCACGCTGCGGAGGC
TGAACTACTTGGAGGACCGTC
CAGATAGTCGCCCAAAGTTC
GACGACACCTTCCTCCCAG

Table S4 Antibody information.

Antibody	Catlog #	Clone #	Company	Application
pAKT	9271S	polyclonal	Cell signaling	Primary antibodies for immunoblot
AKT	9272S	polyclonal		
pERK	4376S	20G11		
ERK	9102S	polyclonal		
pEGFR	2234S	polyclonal		
EGFR	2232S	polyclonal		
pS6	4858S	D57.2.2E		
GFP	2555S	Polyclonal		
TYRO3	5585S	D38C6		
GAPDH	2118S	14C10		
TUBULIN	2125S	11H10		
AXL	AF154	Polyclonal	R&D Systems	
GAS6	AF885	Polyclonal		
MERTK	ab52968	Y323	Abcam	
LGALS3	ab2785	A3A12		
pMERTK	p186-749	Polyclonal	Phosphosolutions (1)	
PROS1	A0384	Polyclonal	Dako	
MERTK	MAB8912	125518	R&D Systems	Immunoprecipitation
EGFR	MAB1095	102618		
Goat anti-mouse	170-6516	Polyclonal	Bio-Rad	Secondary antibodies for immunoblot
Goat anti-rabbit	170-6515	Polyclonal		
Donkey anti-goat	sc-2020	polyclonal	Santa Cruz Biotechnology	

Table S5 *EGFR* mutation status and EGFR TKI treatment history for patient samples.

	Pre or post	Prior EGFR TKI Y/N	Relapsed on prior EGFR TKI Y/N	Duration of OSI	*EGFR* mutation status	Mechanism of OSI resistance
Patient 1	pre	Yes	No	18 months	*EGFR* L858R	
	post					Not known
Patient 2	pre	Yes	No	22 months	*EGFR* exon 19 del	
	post					Not known
Patient 3	pre	Yes	No	18 months	*EGFR* exon 19	
	post					de novo *EGFR*-C797S mutation

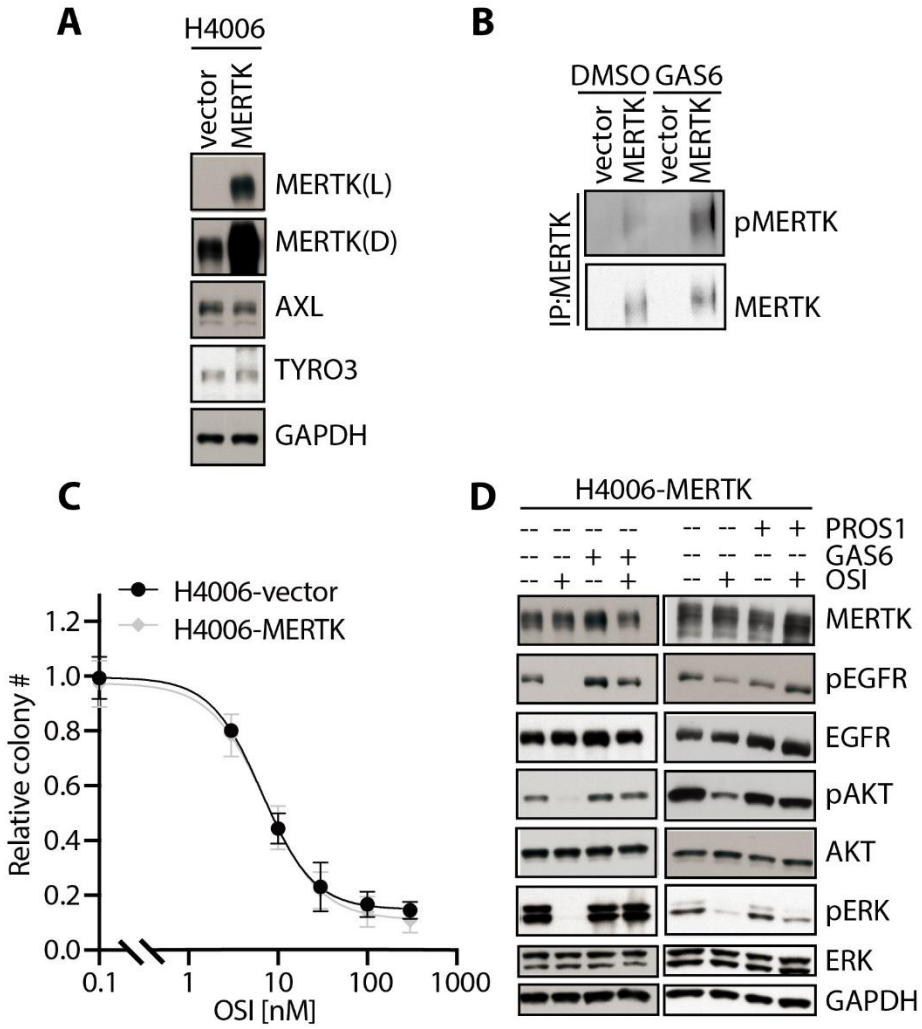


Figure S1 MERTK overexpression is not sufficient to confer OSI resistance. A) H4006 cell lines with MERTK overexpression (MERTK) or empty vector (vector) were established, and expression of TAM receptors was assessed by immunoblot. **B**) H4006-MERTK and H4006-vector cells were serum-starved overnight, then stimulated with or without 50nM GAS6 for 10min and treated with pervanadate and cell lysates were analyzed by immunoblot. **C**) H4006-MERTK and H4006-vector cells were cultured at low density and treated with the indicated concentrations of OSI or vehicle for 8d before cells were fixed and colonies were stained and counted. Mean colony numbers relative to vehicle-treated control cultures and standard deviations from three independent experiments are shown. **D**) H4006-MERTK cells were serum-starved overnight and then treated with DMSO or 100nM OSI for 2h followed by 10min stimulation with or without 50nM GAS6 or 50nM PROS1. Cell lysates were analyzed by immunoblot.

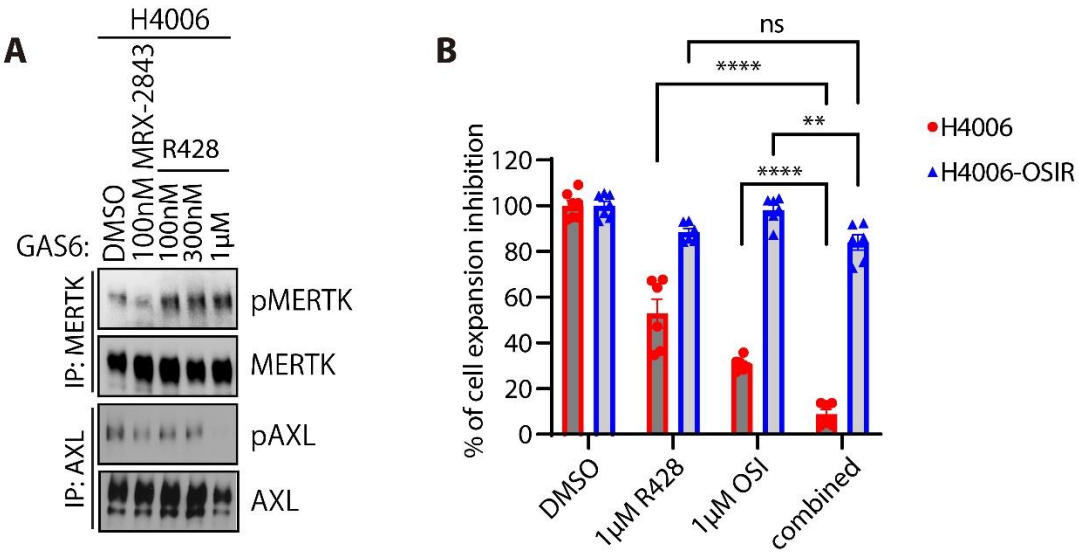


Figure S2 AXL inhibitor R428 sensitizes *EGFR*^{MT} H4006 parental cells to OSI but does not enhance therapeutic efficacy in combination with OSI in OSI resistant (OSIR) H4006 cells. **A)** H4006 cells were serum-starved overnight and then treated with the indicated concentrations of MRX-2843 or R428 for 2h, followed by GAS6 stimulation for 10min. Phosphorylated (denoted by p) and total proteins were detected after pervanadate treatment and immunoprecipitation. **B)** H4006 and H4006-OSIR cells were treated with R428 or OSI alone or combined for 120h and cell numbers were determined using CellTiter Glo reagent. (**, p<0.01; ****, p<0.0001; ns=not significant; one-way ANOVA).

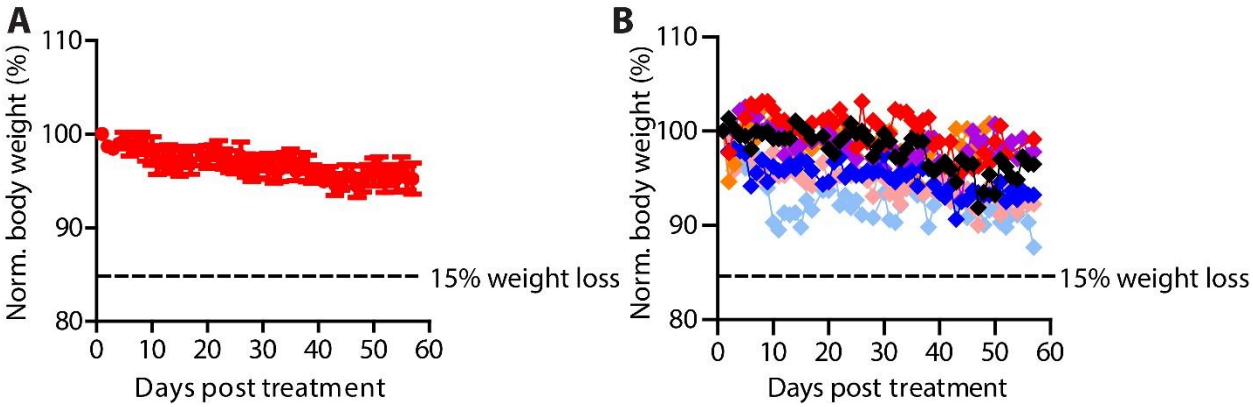
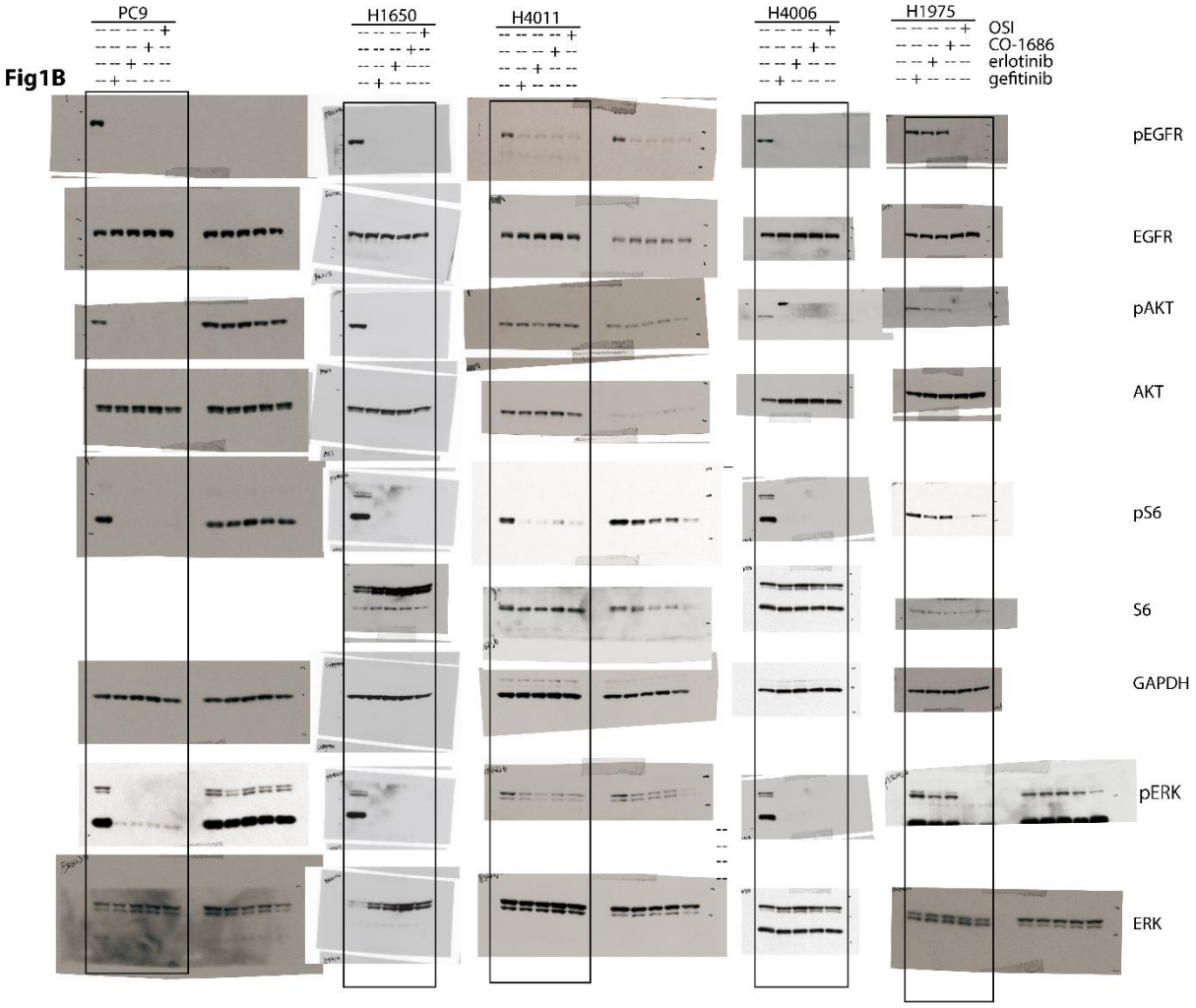


Figure S3 Body weights during treatment with combined OSI and MRX-2843 in mice with H4006 tumor xenografts. A) Mean body weights and standard errors (n=7). **B**) Body weights in individual mice.

1. Zhang W, DeRyckere D, Hunter D, Liu J, Stashko MA, Minson KA, et al. UNC2025, a potent and orally bioavailable MER/FLT3 dual inhibitor. *J Med Chem.* 2014;57(16):7031-41.

Full unedited gel for Figure 1B



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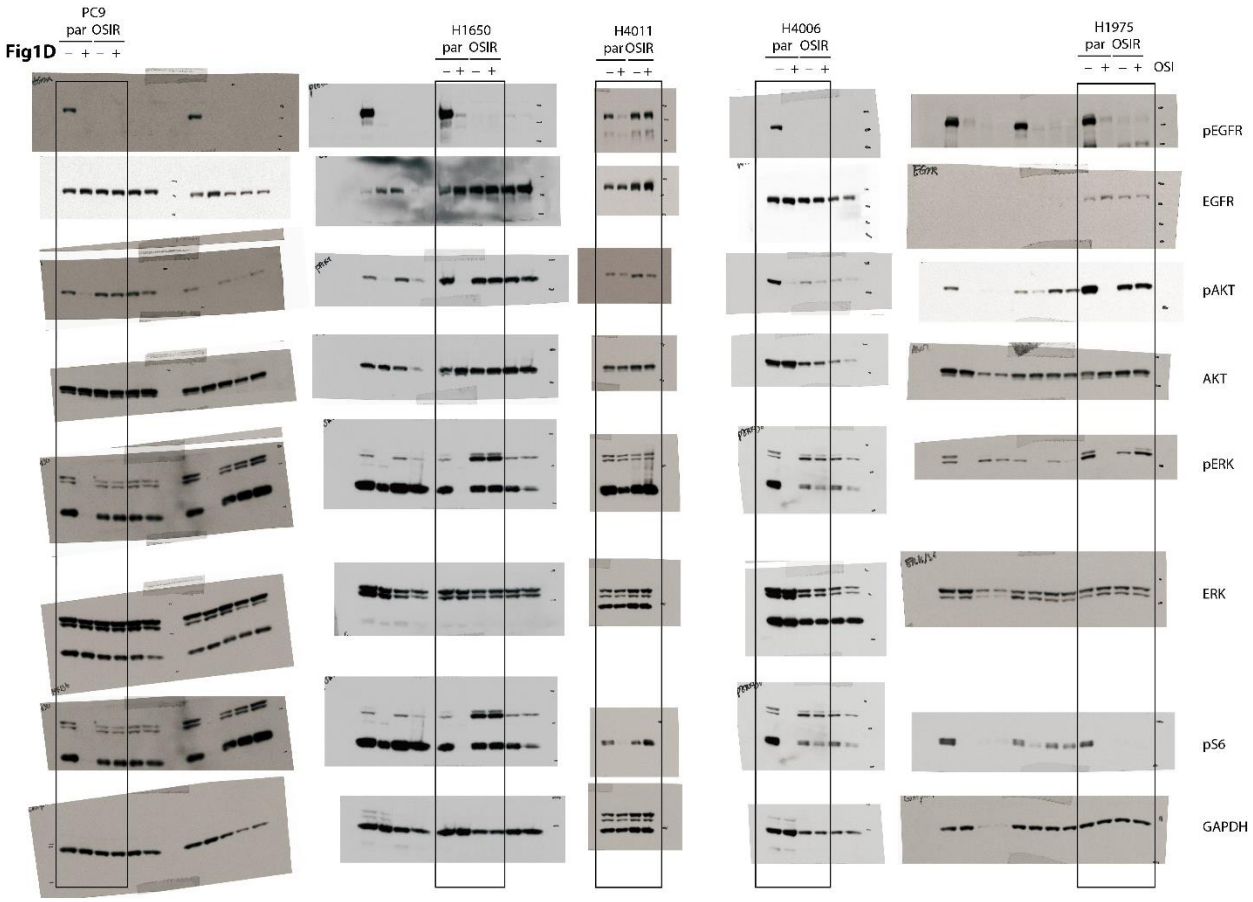

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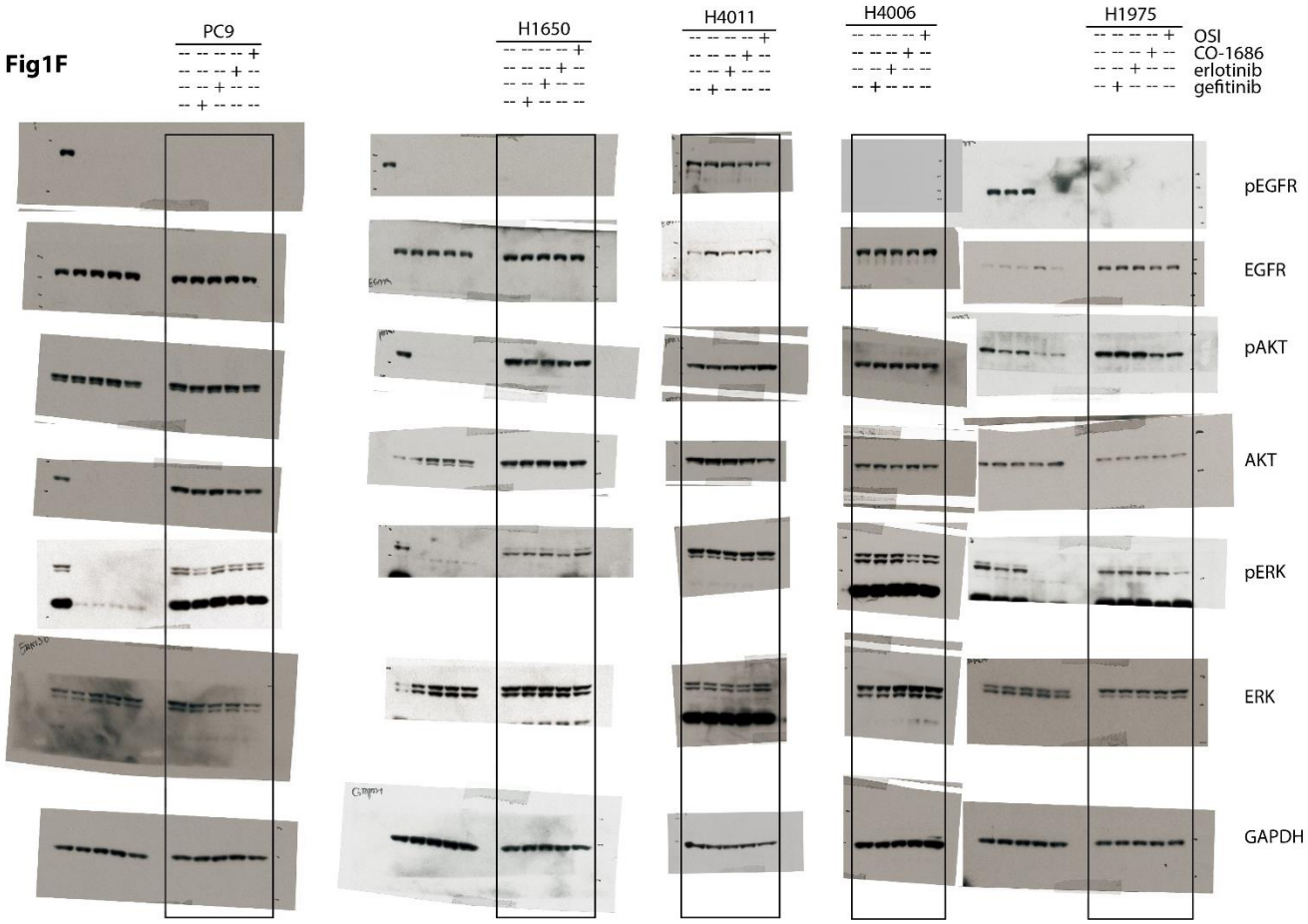

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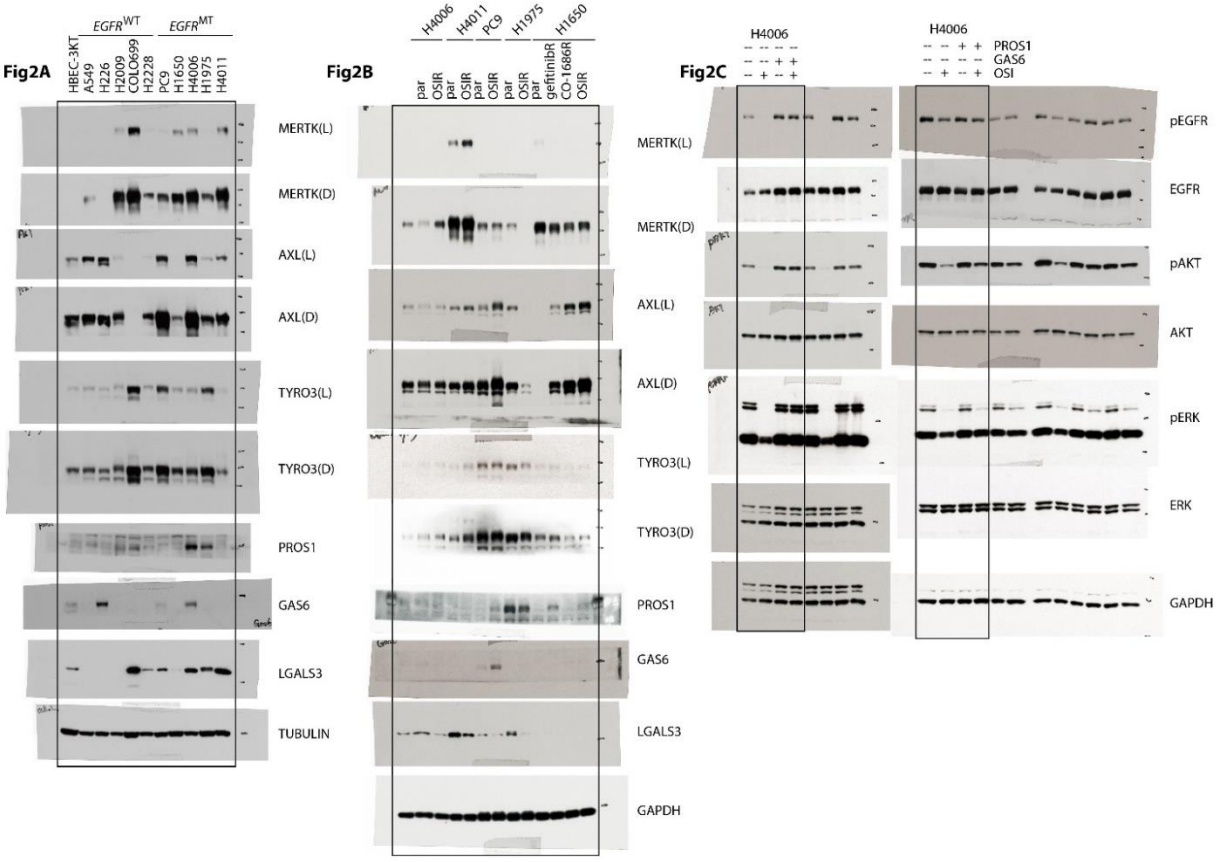

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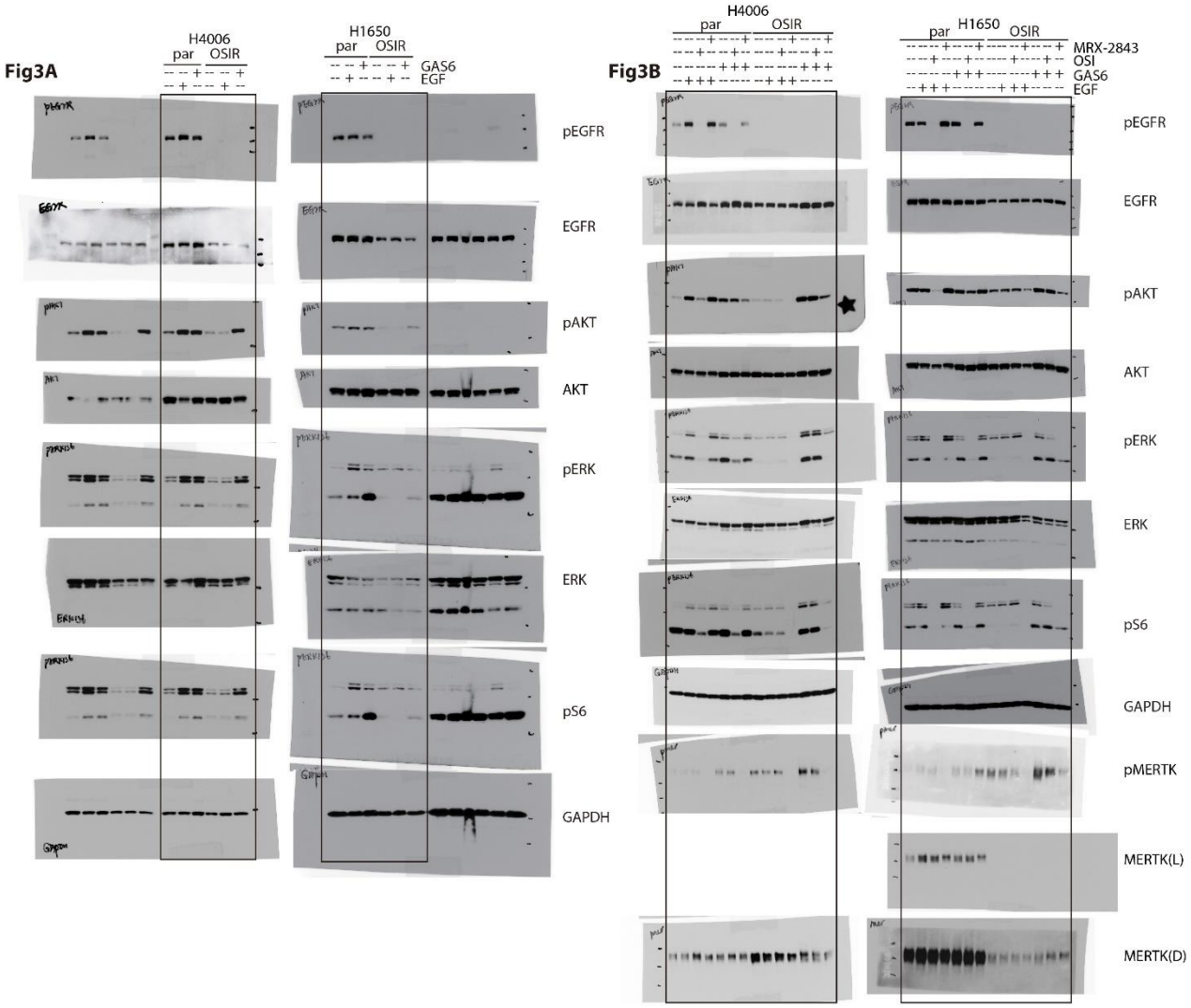

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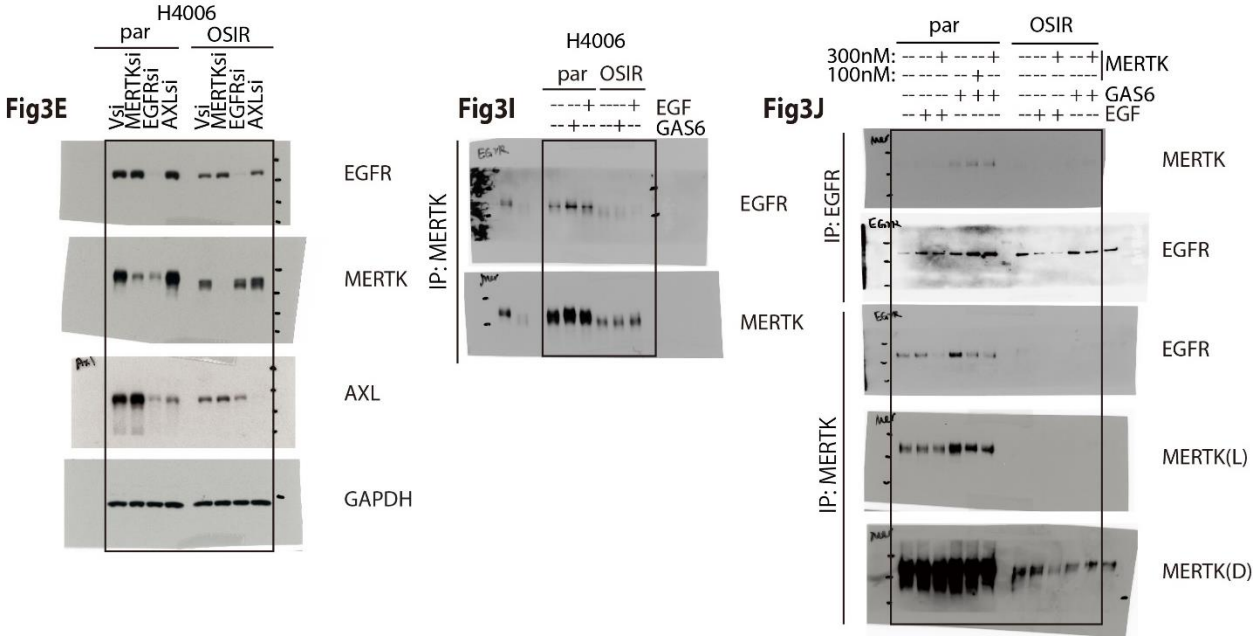

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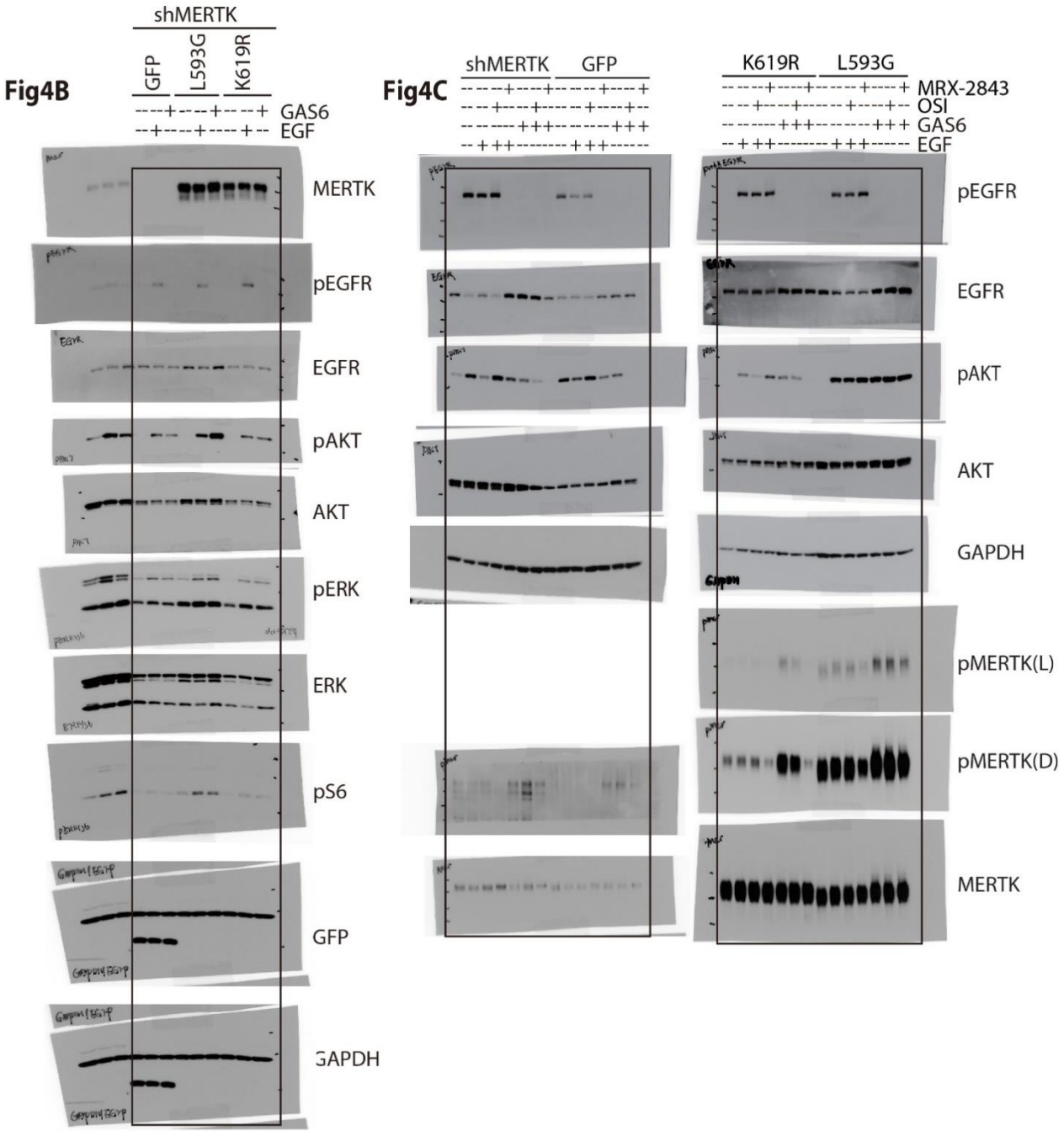

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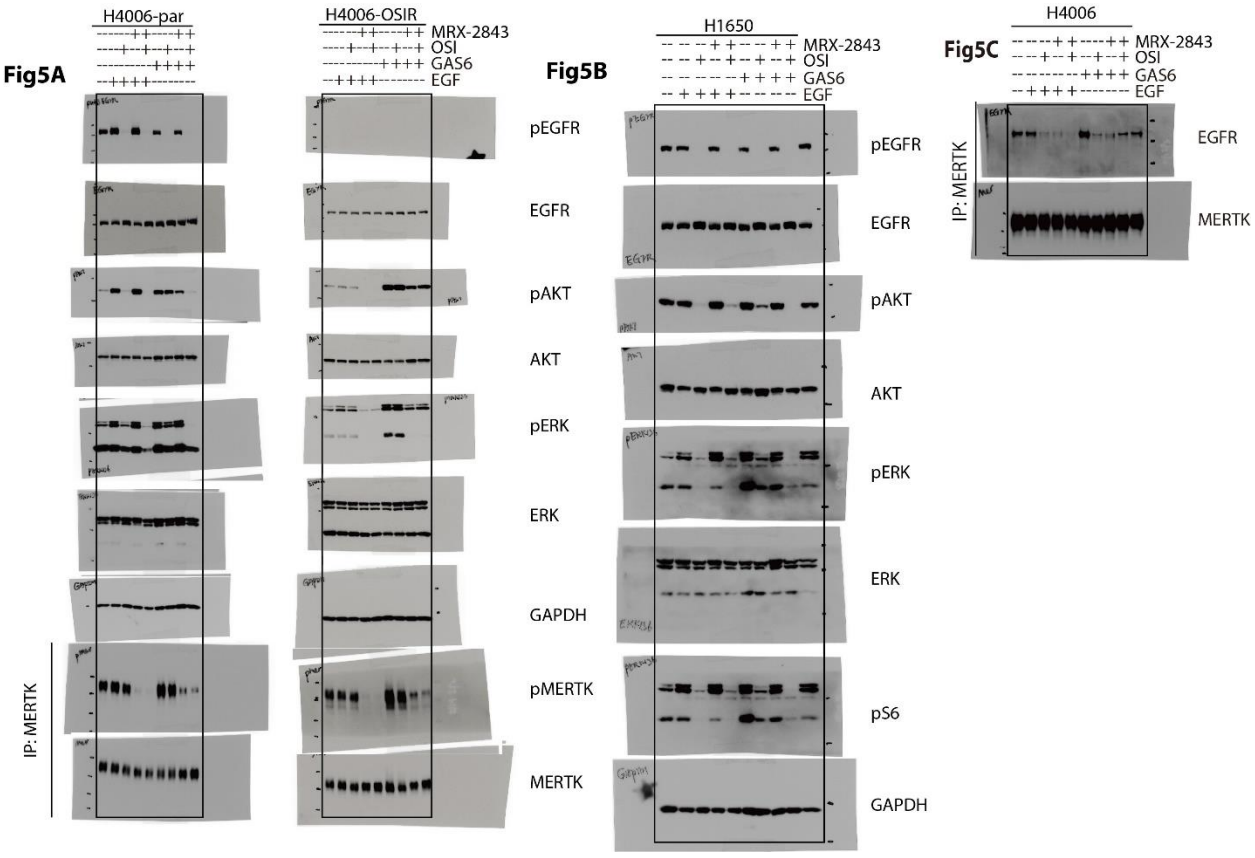

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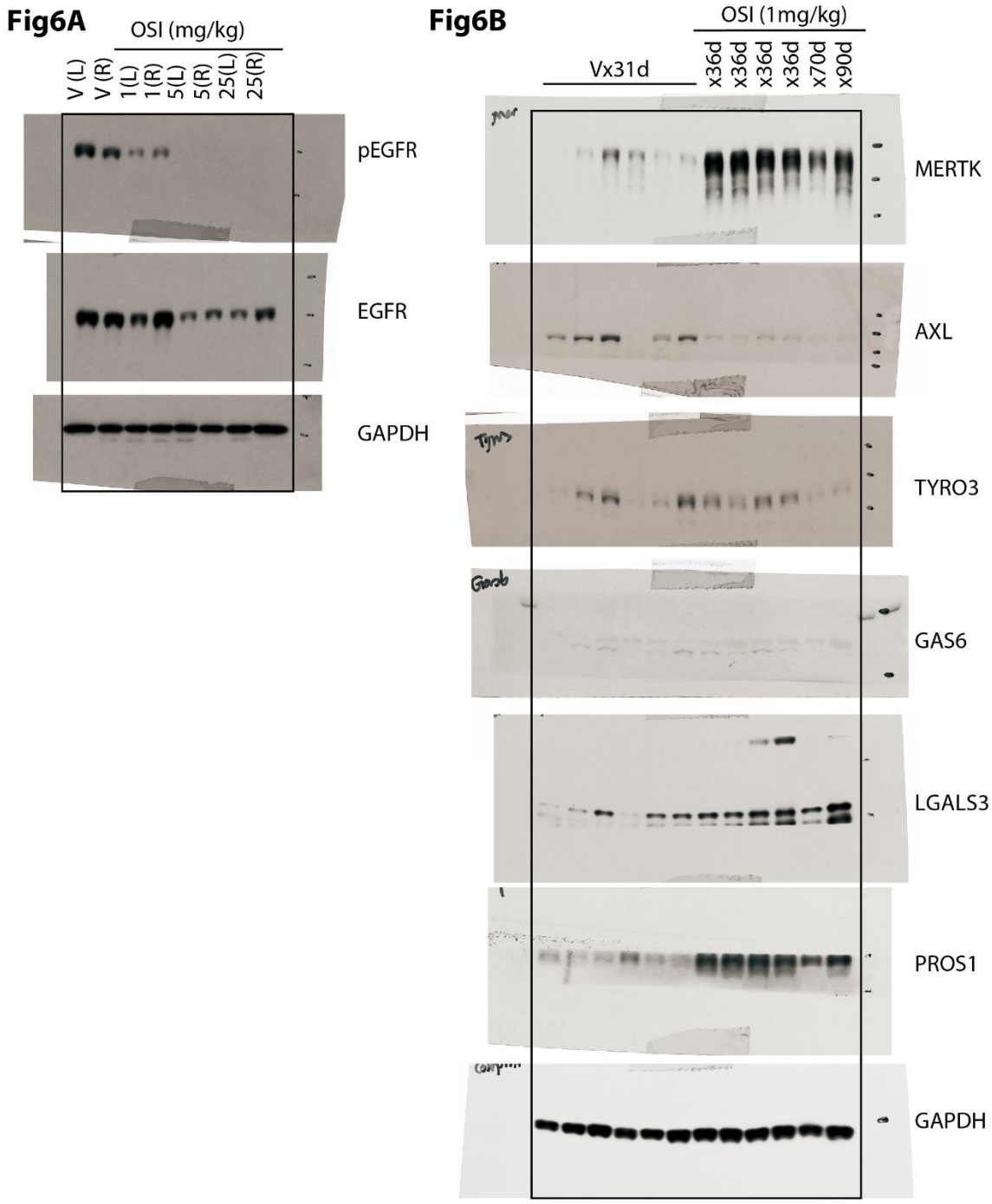

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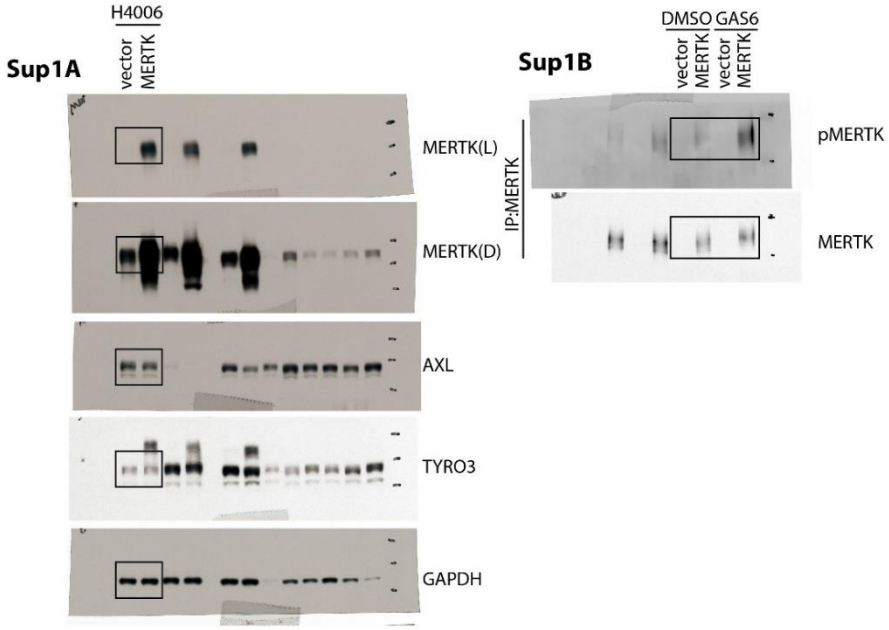

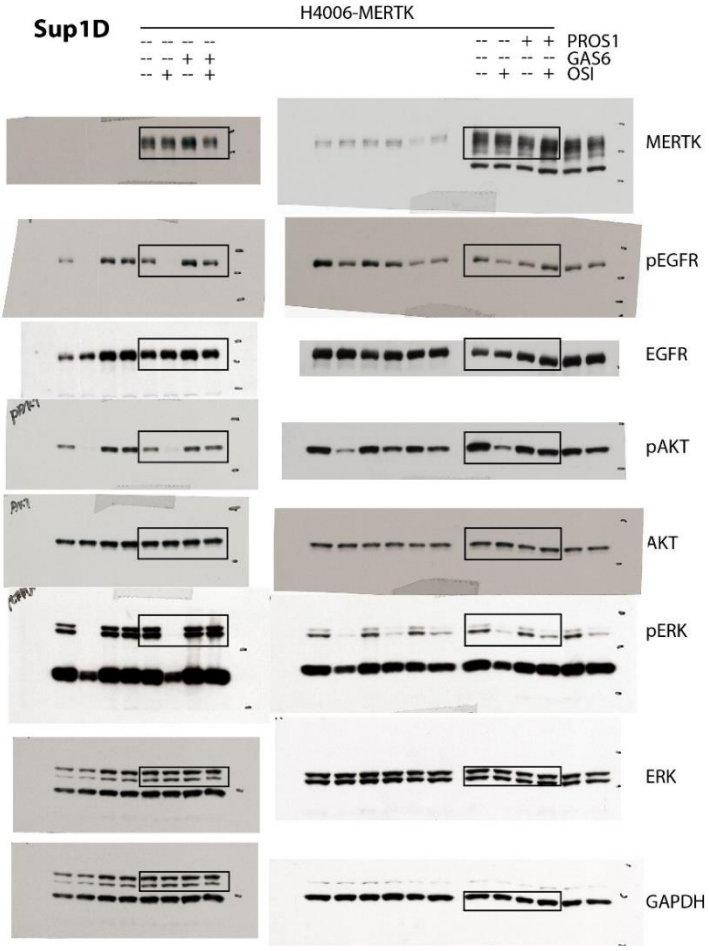

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