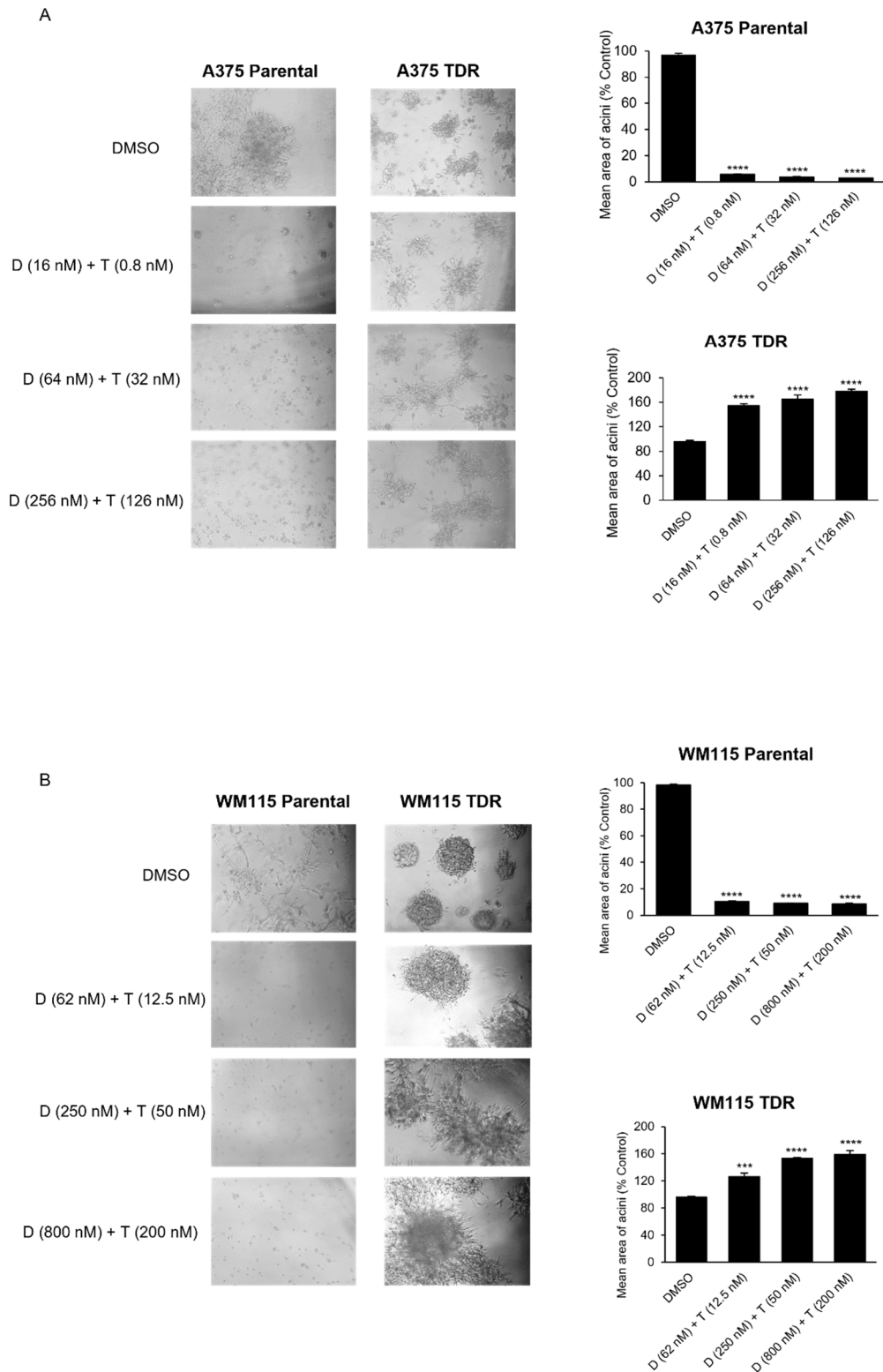


*Supplementary Files*

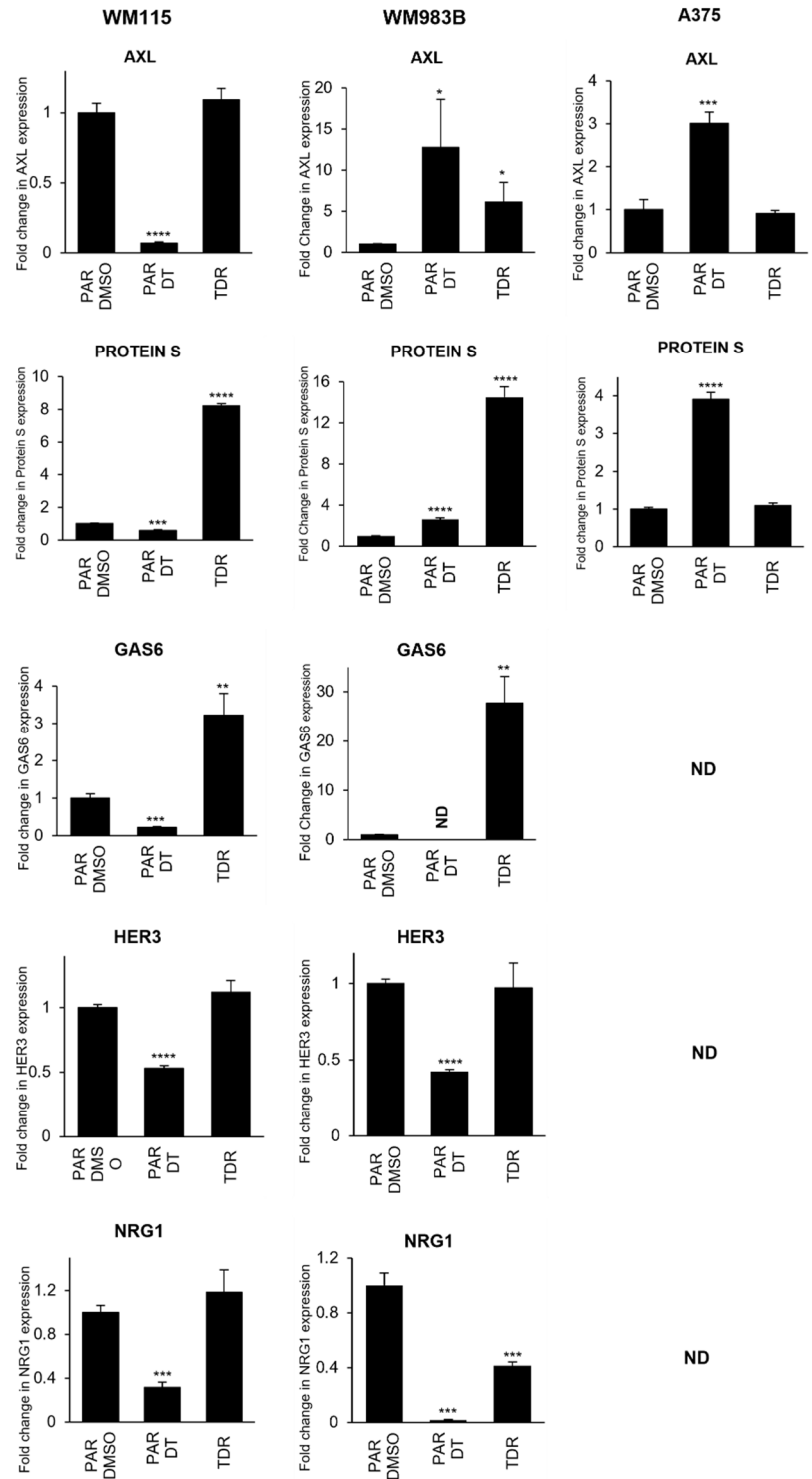
# **IGF1R/IR Mediates Resistance to BRAF and MEK Inhibitors in BRAF-Mutant Melanoma**

**Hima Patel, Rosalin Mishra, Nour Yacoub, Samar Alanazi, Mary Kate Kilroy and Joan T. Garrett**

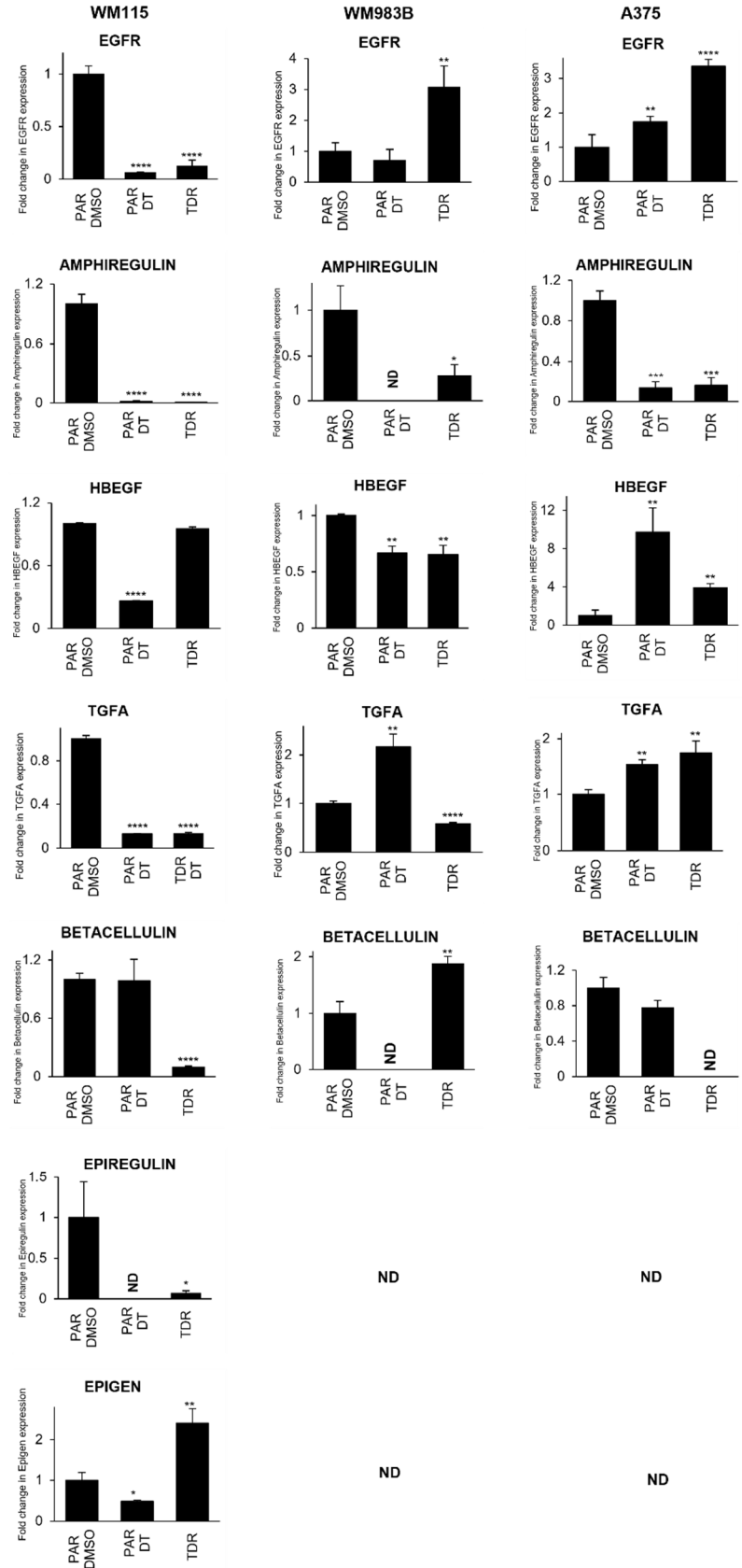


**Figure S1.** Dabrafenib and trametinib inhibit growth of parental cells but not TDR cells. (A) A375 and (B) WM115 Parental and TDR cells (2 x 10<sup>4</sup> cells/well) were seeded on matrigel basement membrane and treated with indicated drugs every second day. 5 pictures of different areas of each well were captured on day 21 (left panel) at 10× magnification. Quantification of mean area of acini (Right panel).\*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  by two-tailed Student's t-test compared to DMSO group.

A

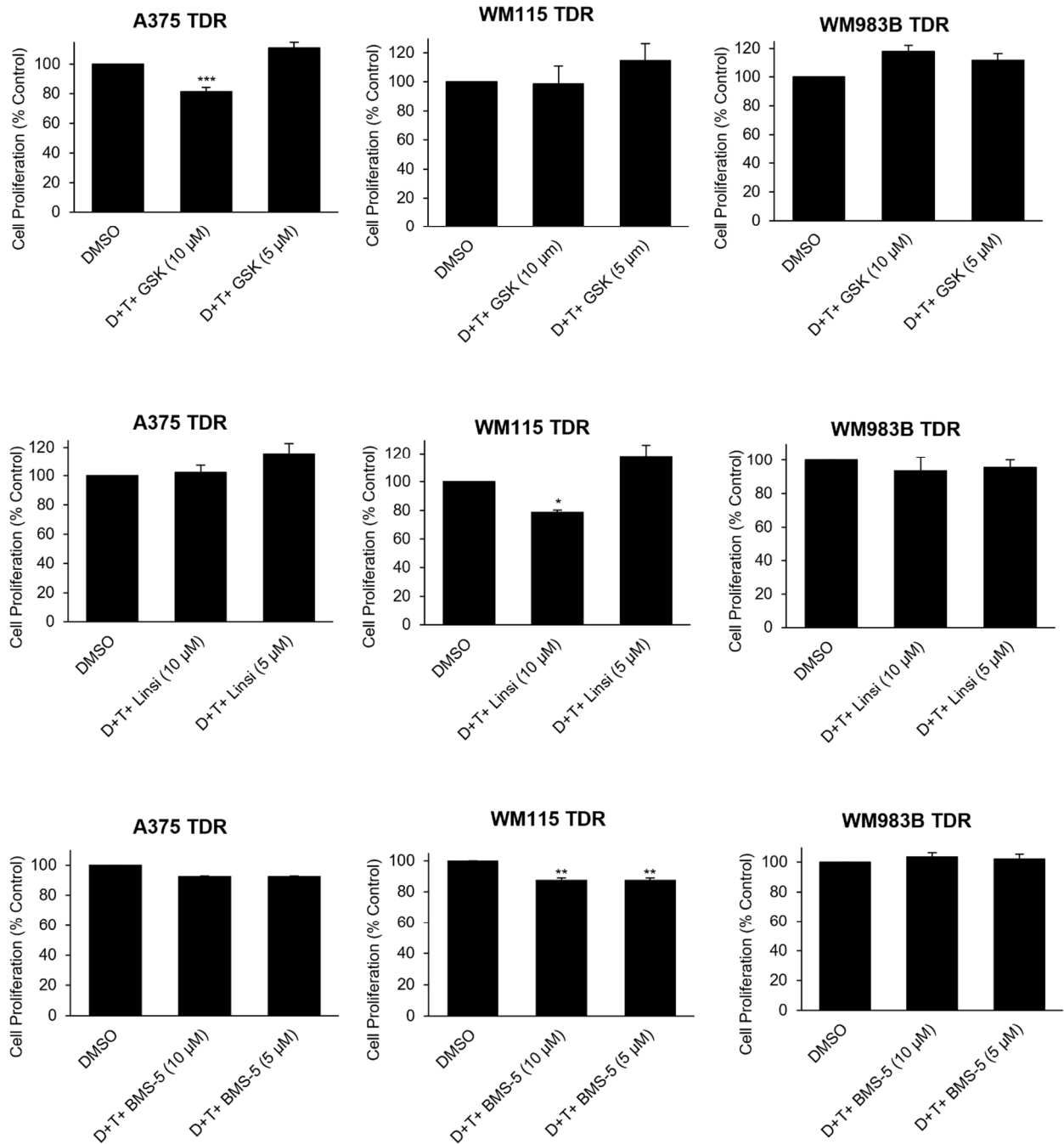


B



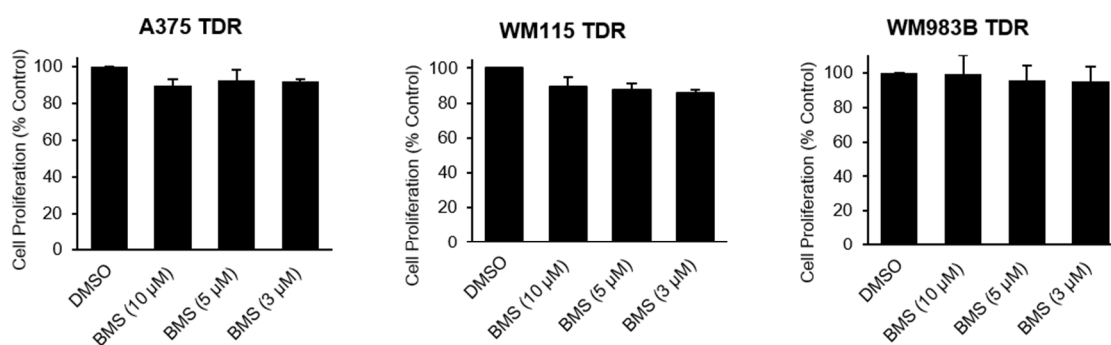
**Figure S2.** mRNA expression of AXL, HER3, EGFR and their ligands in parental and TDR cells. Quantification of Actin- normalized mRNA levels using real-time quantitative PCR for (A) AXL, PROTEIN S, GAS6, HER3, NRG1 and (B) EGFR, AMPHIREGULIN, HBEGF, TGFA, BETACELLULIN, EPIREGLIN, EPIGEN for parental cells treated with DMSO or dabrafenib

(D) + trametinib (T) (WM115: D: 800 nM, T: 200 nM; WM983B: D: 2.4  $\mu$ M, T: 500 nM; A375: D: 250 nM, T: 12.5 nM) and TDR following 24 hours of treatment. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  by two-tailed Student's  $t$ -test. ND: Not detectable.

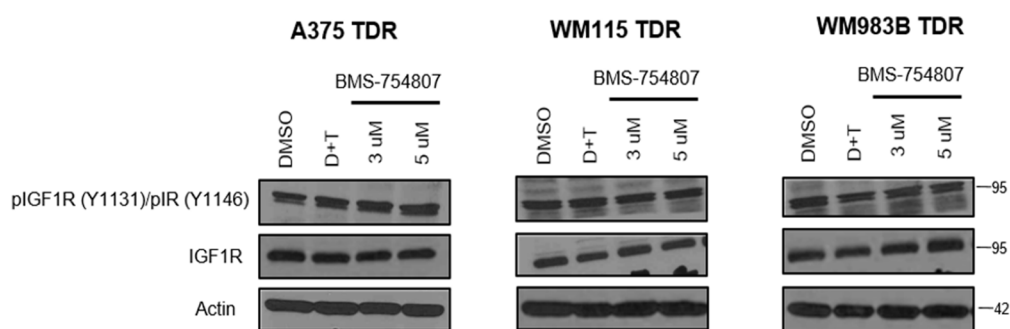


**Figure S3.** Effect of Linsitinib, BMS-536924 and GSK1838705A on TDR cell proliferation. TDR cells (WM115, WM983B and A375;  $1 \times 10^4$  cells/well) plated for 24 h and treated with DMSO, dabrafenib (D) and trametinib (T) (WM115: D: 800 nM, T: 200 nM; WM983B: D: 2.4  $\mu$ M, T: 500 nM; A375: D: 250 nM, T: 12.5 nM) in combination with Linsitinib (Linsi), BMS-536924 (BMS-5) and GSK1838705A (GSK) (5  $\mu$ M and 10  $\mu$ M) and treated with MTT. The intensities are represented as mean, Error bars: SEM \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  by two-tailed Student's  $t$ -test compared to DMSO group.

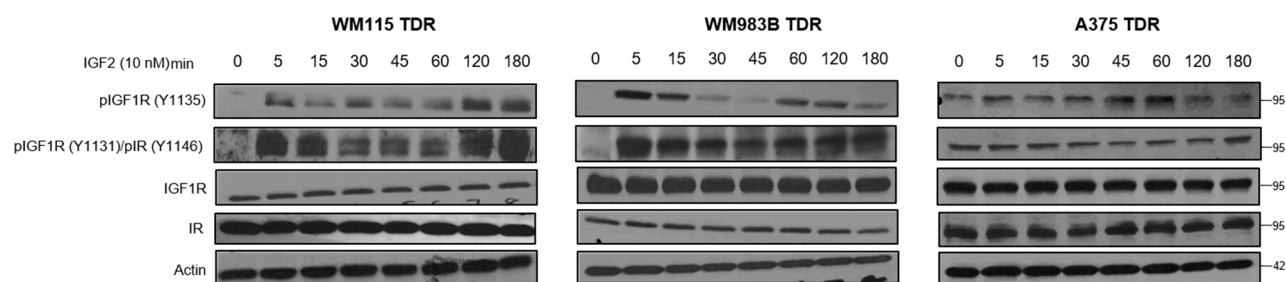
A



B



**Figure S4.** BMS-754807 does not inhibit proliferation and p-IGF1R/p-IR expression in TDR cells. **(A)** TDR cells (WM115, WM983B and A375) were plated and treated with DMSO, BMS-754807 (BMS; 10  $\mu$ M, 5  $\mu$ M or 3  $\mu$ M) for 72 h. Cells were treated with MTT for 4 h and absorbance was read at 570 nm. Data are presented as mean  $\pm$  SEM. **(B)** Immunoblots for A375, WM115 and WM983B TDR cells treated with DMSO, dabrafenib (D) and trametinib (T) (WM115 TDR: D: 800 nM, T: 200 nM; WM983B TDR: D: 2.4  $\mu$ M, T: 500 nM; A375 TDR: D: 250 nM, T: 12.5 nM), or BMS-754807 (5  $\mu$ M or 3  $\mu$ M) for 24 h. Whole cell lysates were analyzed for western blot using indicated antibodies. Actin used as loading control.



**Figure S5.** IGF2 dependent activation of IGF1R and IR in TDR cells. TDR cells were serum starved for 24 h and treated with IGF2 (10 nM) for indicated time. Whole cell lysates were analyzed for western blot using indicated antibodies. Actin used as loading control.

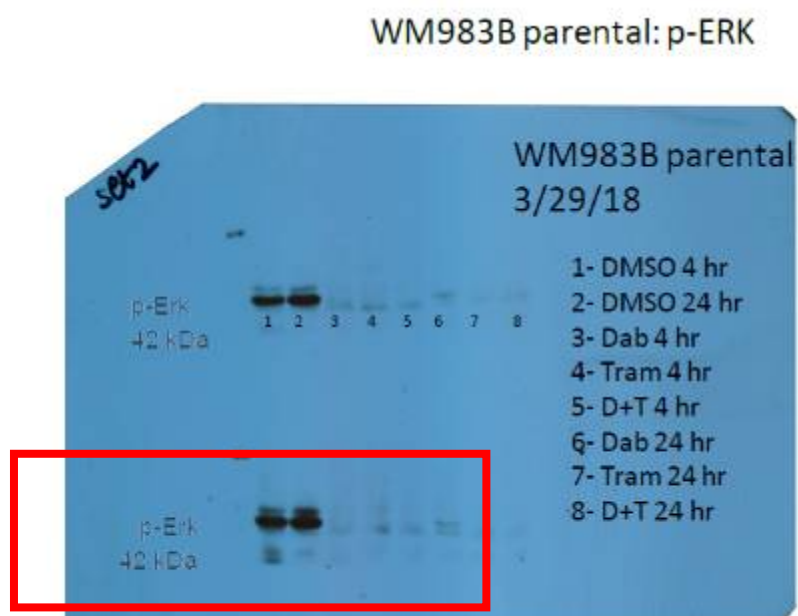
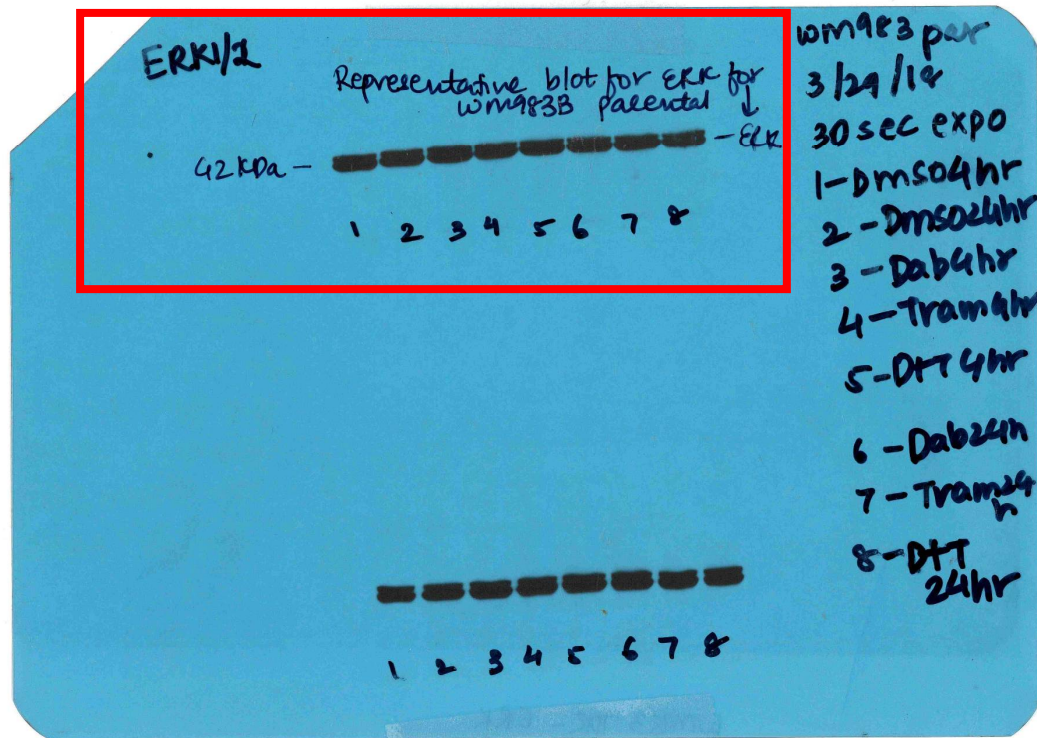
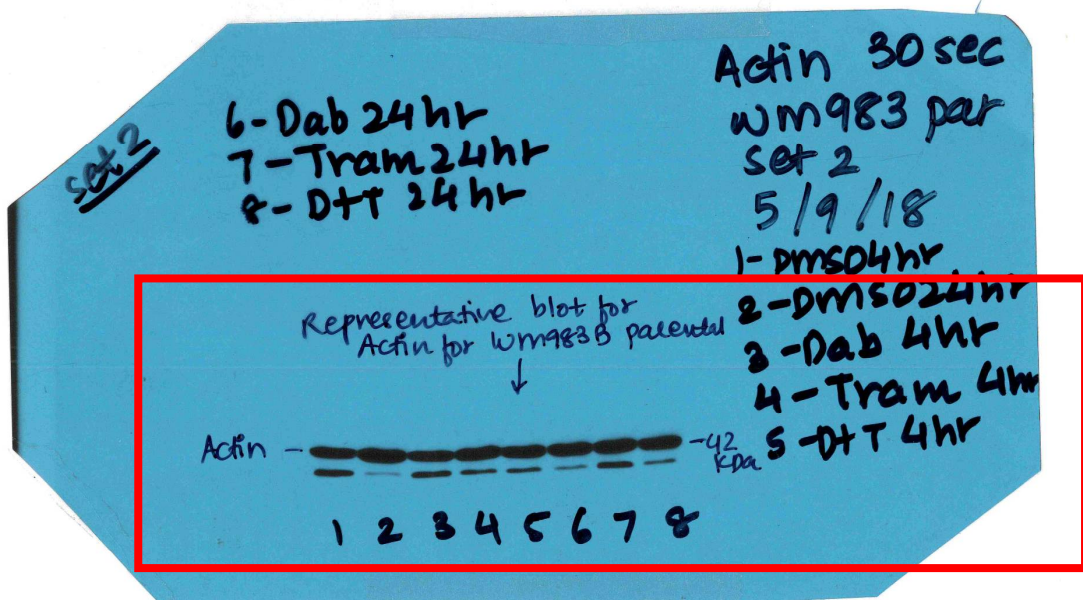


Figure 1D  
WM983B parental - ERK

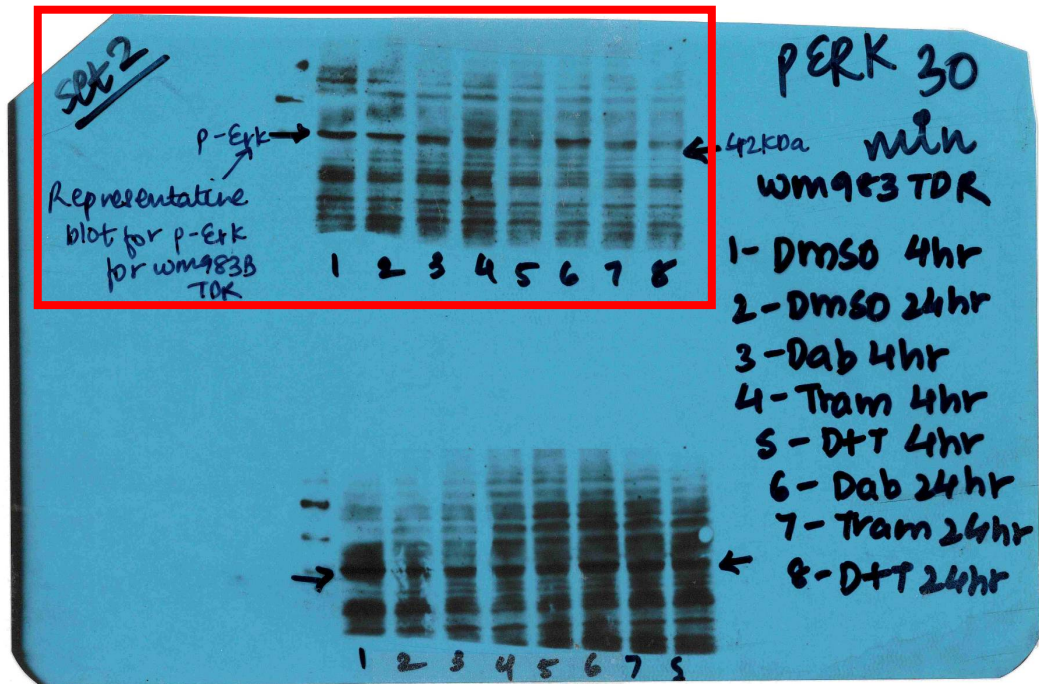


WM983B parental - Actin

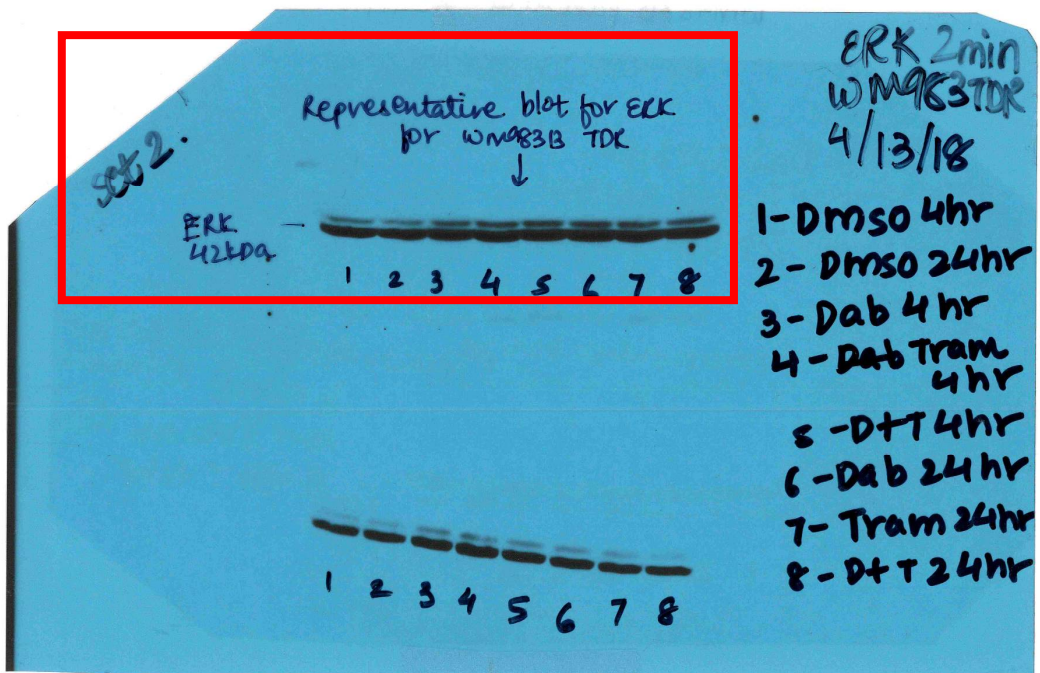




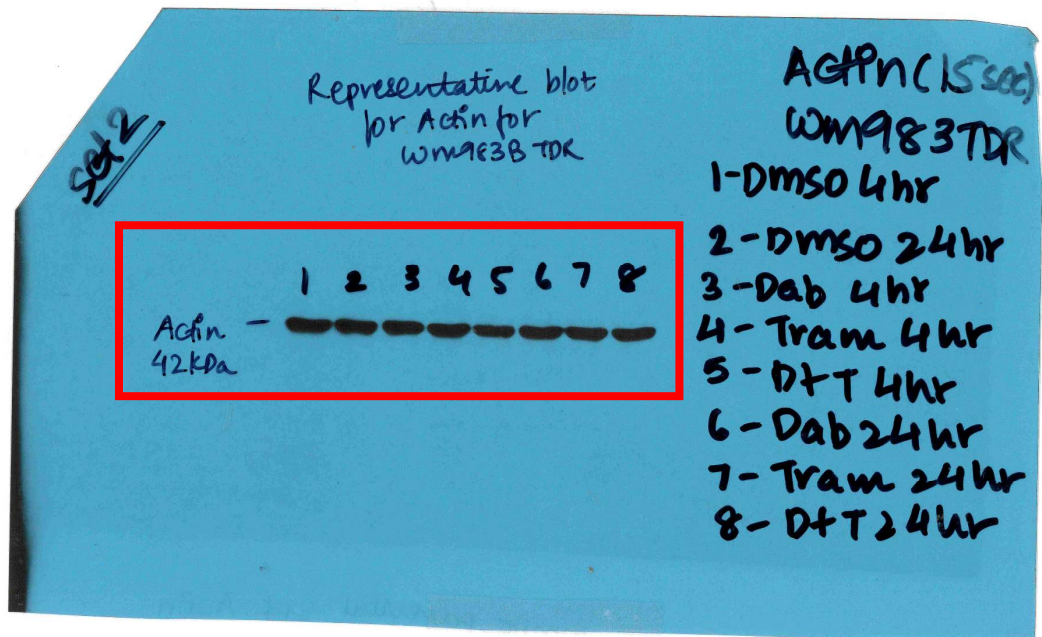
WM983 TDR - p-Erk



WM983 TDR - ERK

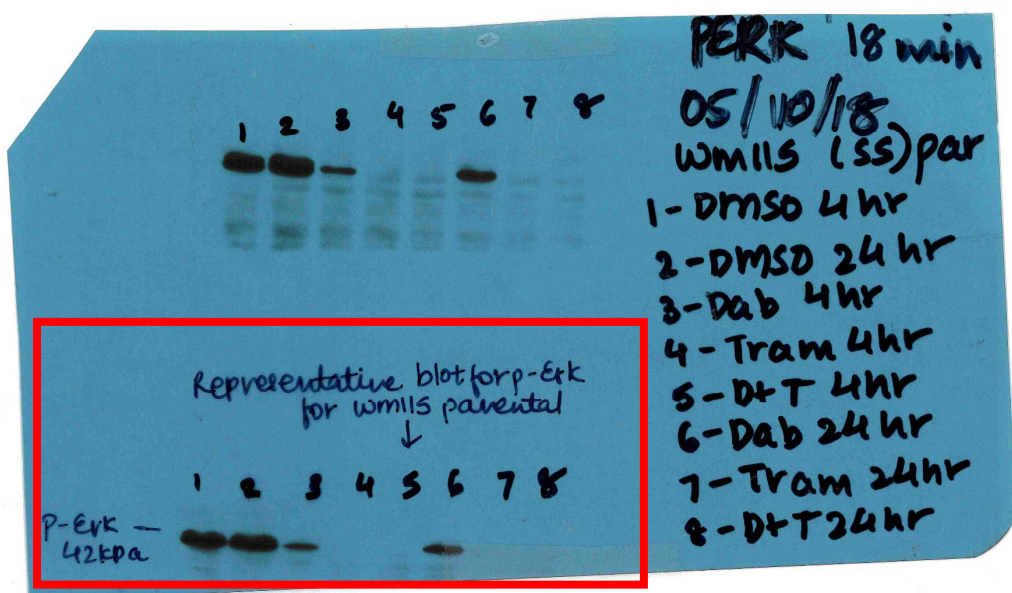


WM983 TDR- Actin

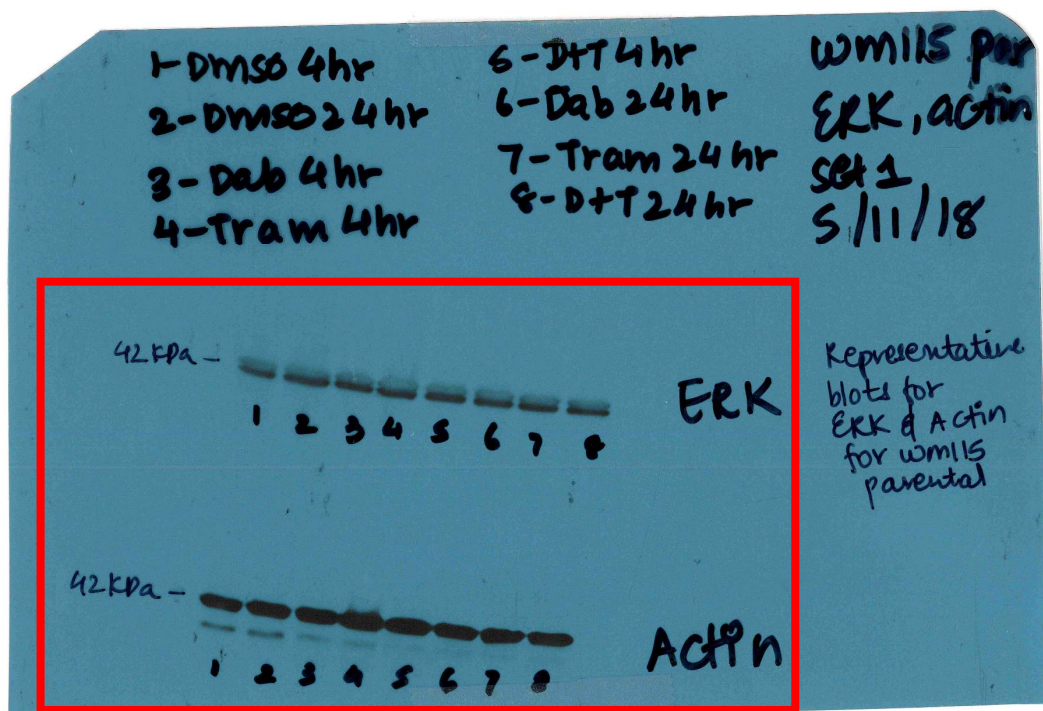




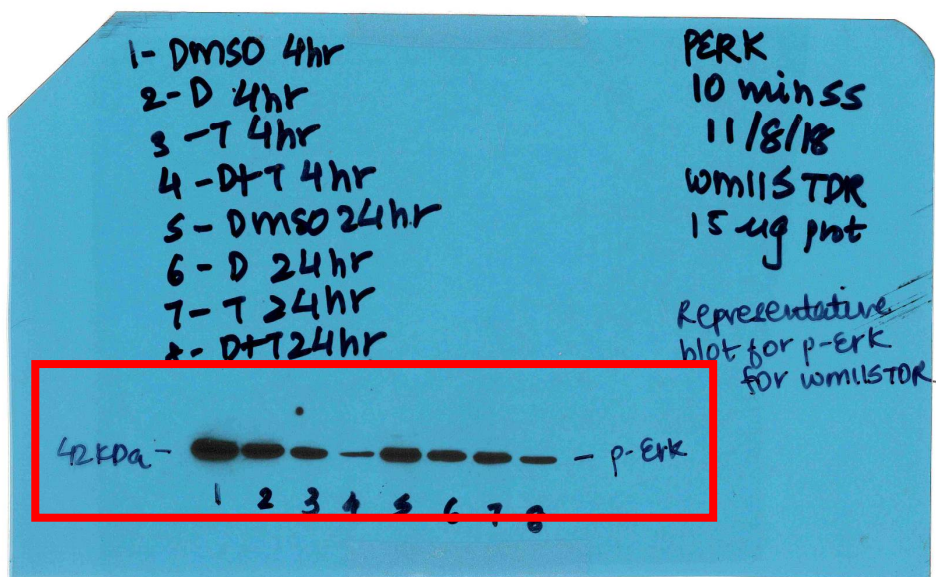
wm115 parental p-Erk



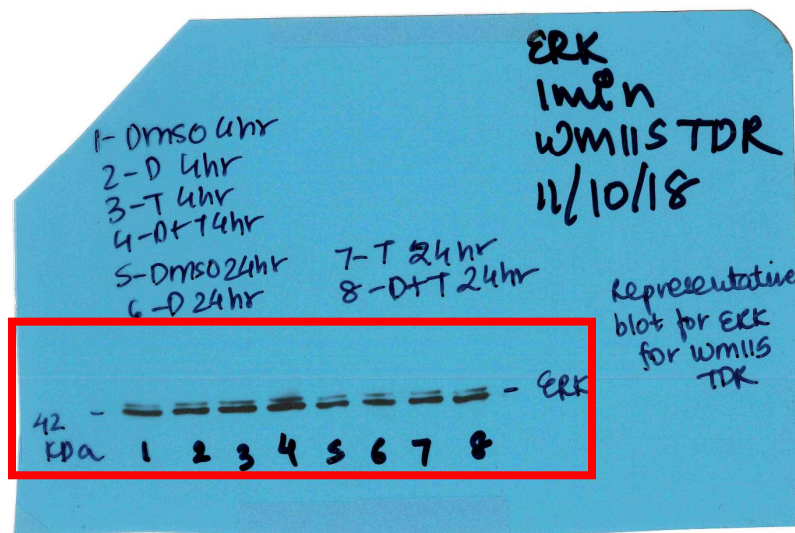
wm115 parental - Erk, Actin



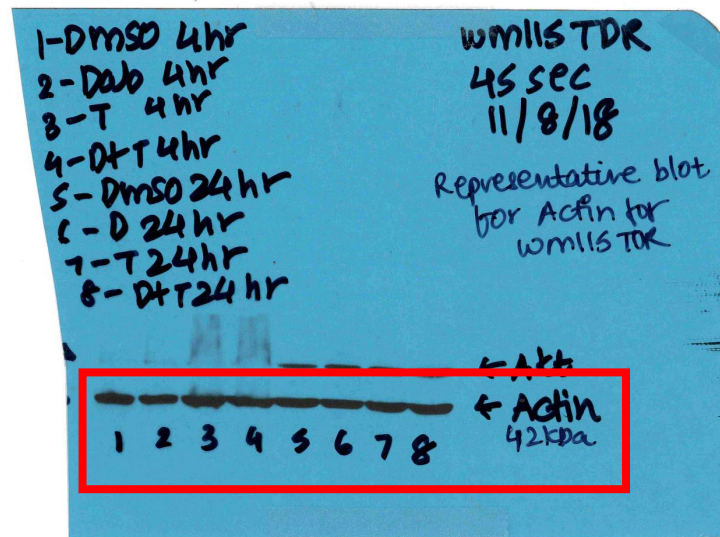
Wm115 TDR: p-Erk



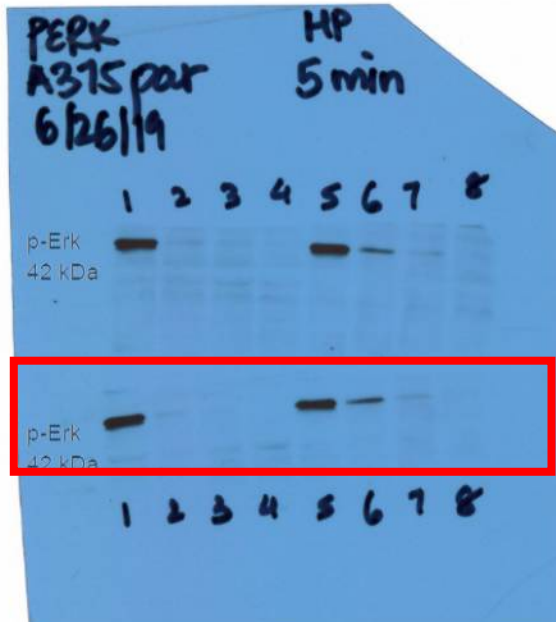
Wm115 TDR: ERK



Wml15TOR: Actin



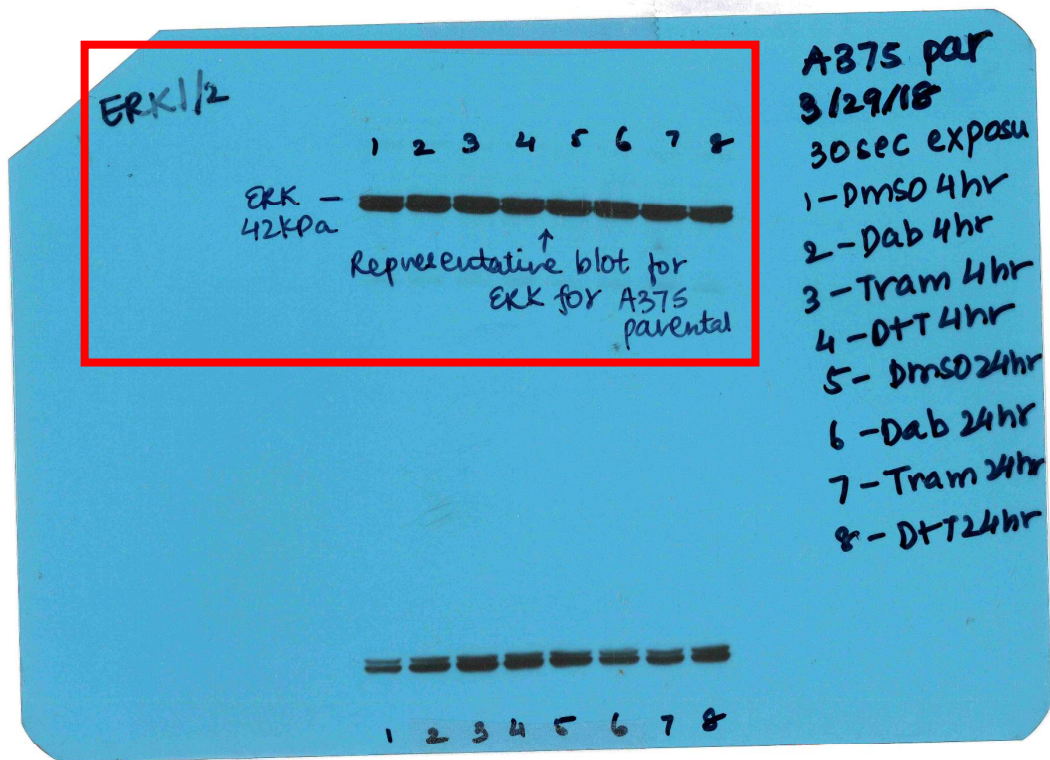
A375 parental: p-ERK



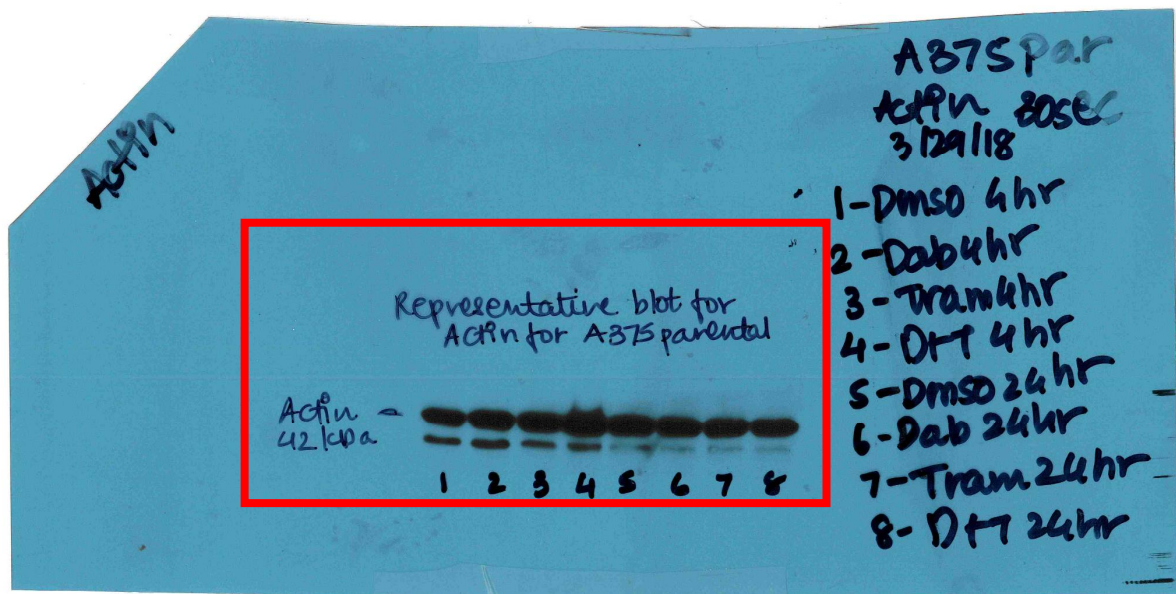
- 1 - DMSO 4 hr
- 2 - Dab 4 hr
- 3 - Tram 4 hr
- 4 - D+T 4 hr
- 5 - DMSO 24 hr
- 6 - Dab 24 hr
- 7 - Tram 24 hr
- 8 - D+T 24 hr



A375 parental: erk



A375 parental: Actin



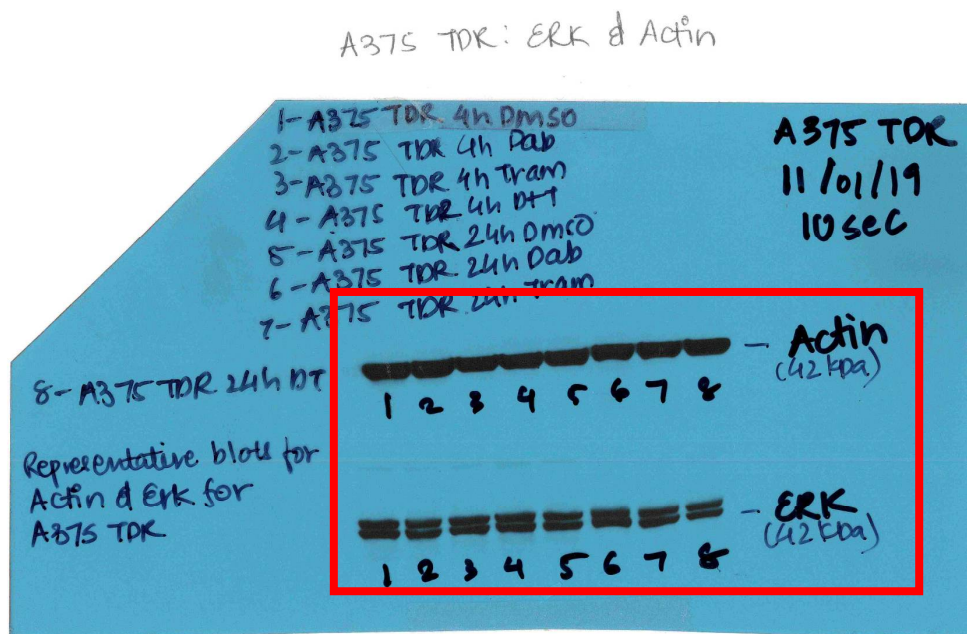
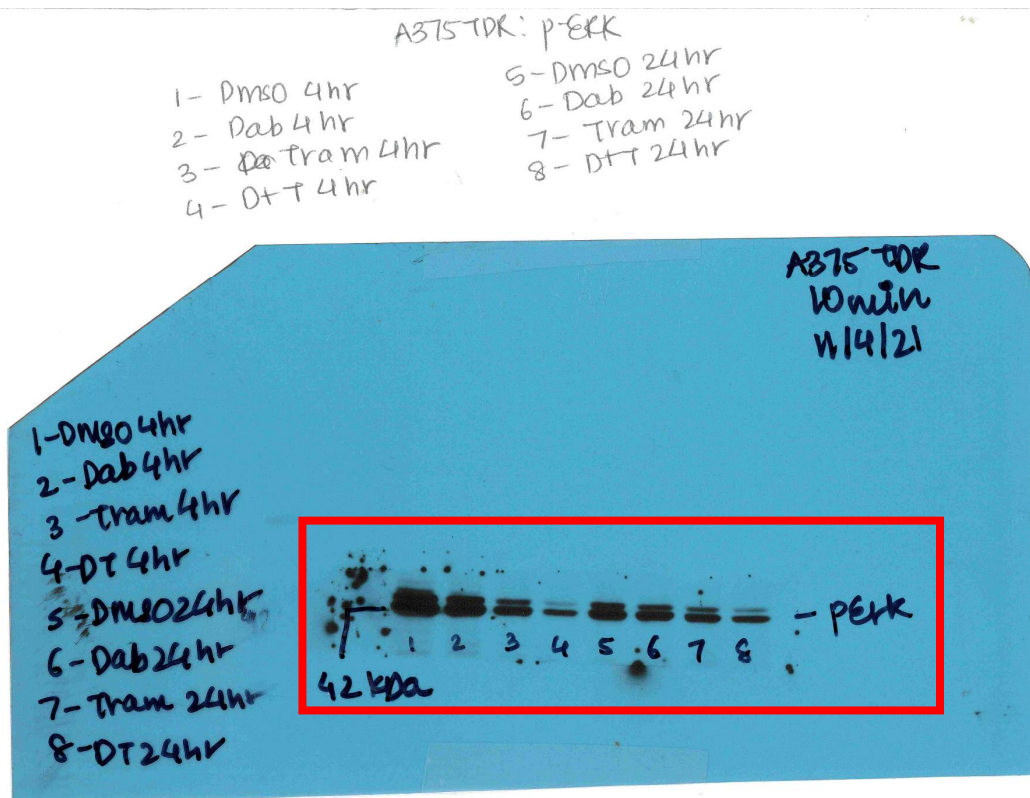
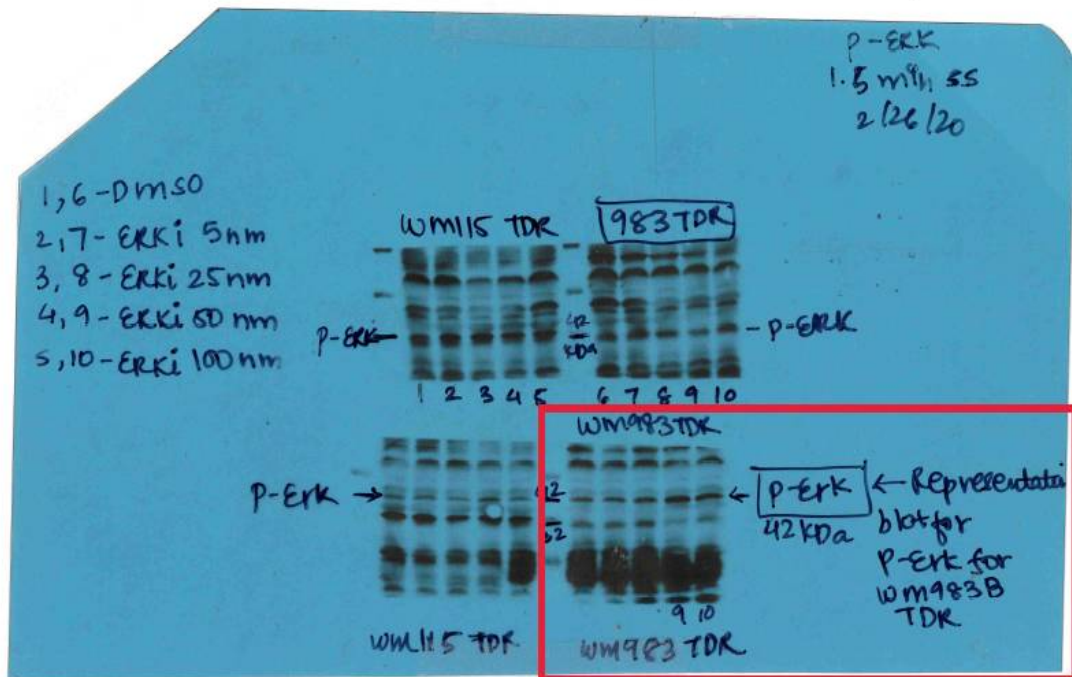


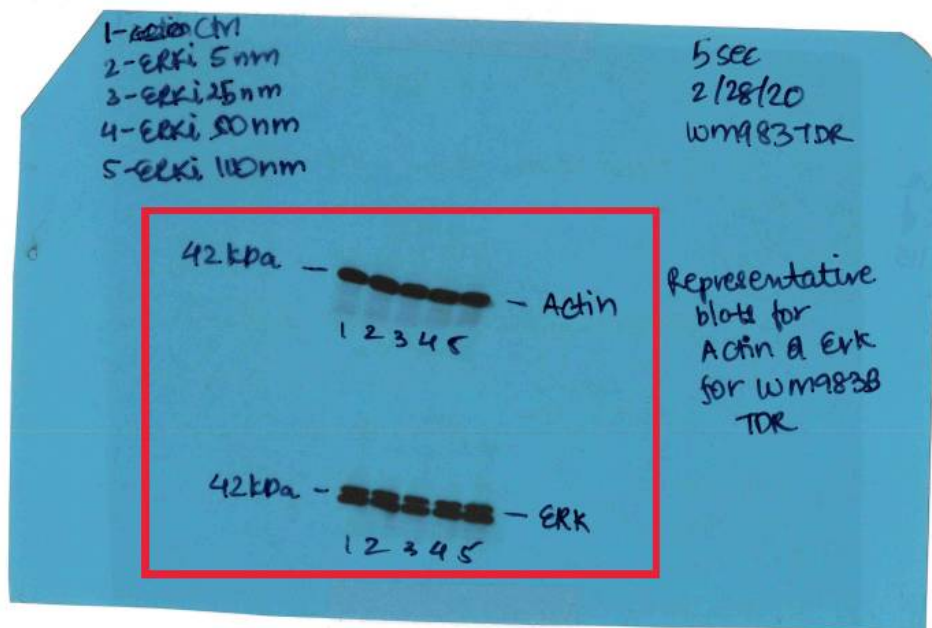
Figure S6. Whole blots for western blots in Figure 1D.



WM983B TDR: p-Erk

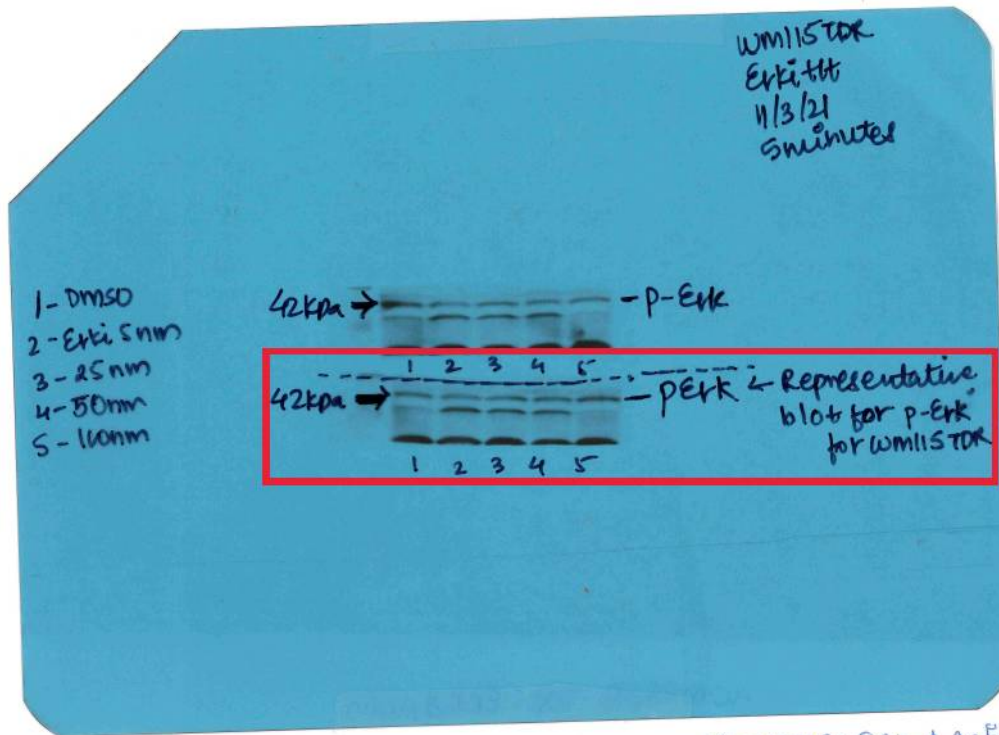


WM983B TDR: Erk & Actin



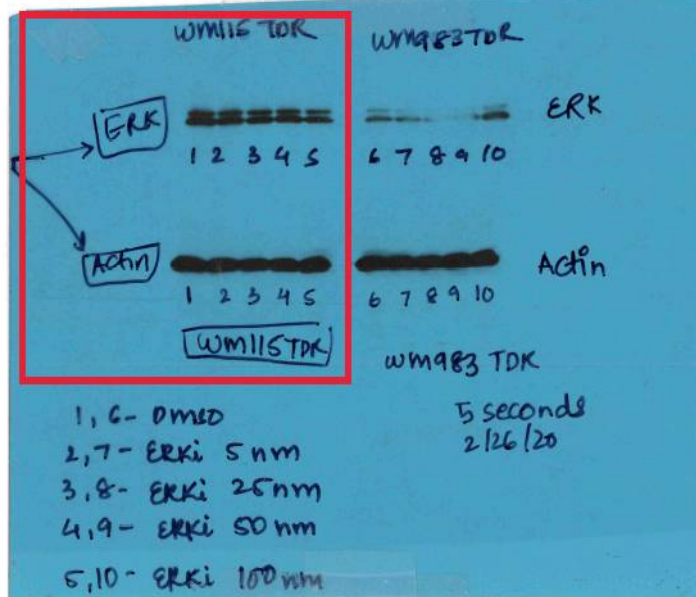


WM115 TDR: p-Erk



WM115 TDR: ERK & Actin

Representative blots for ERK & Actin for WM115 TDR



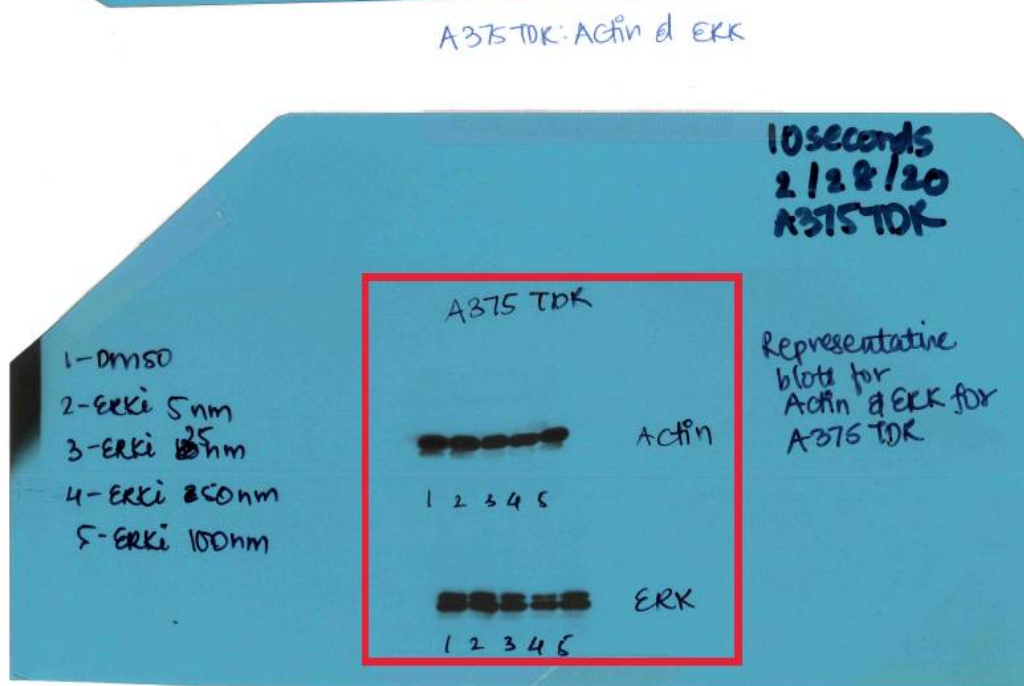
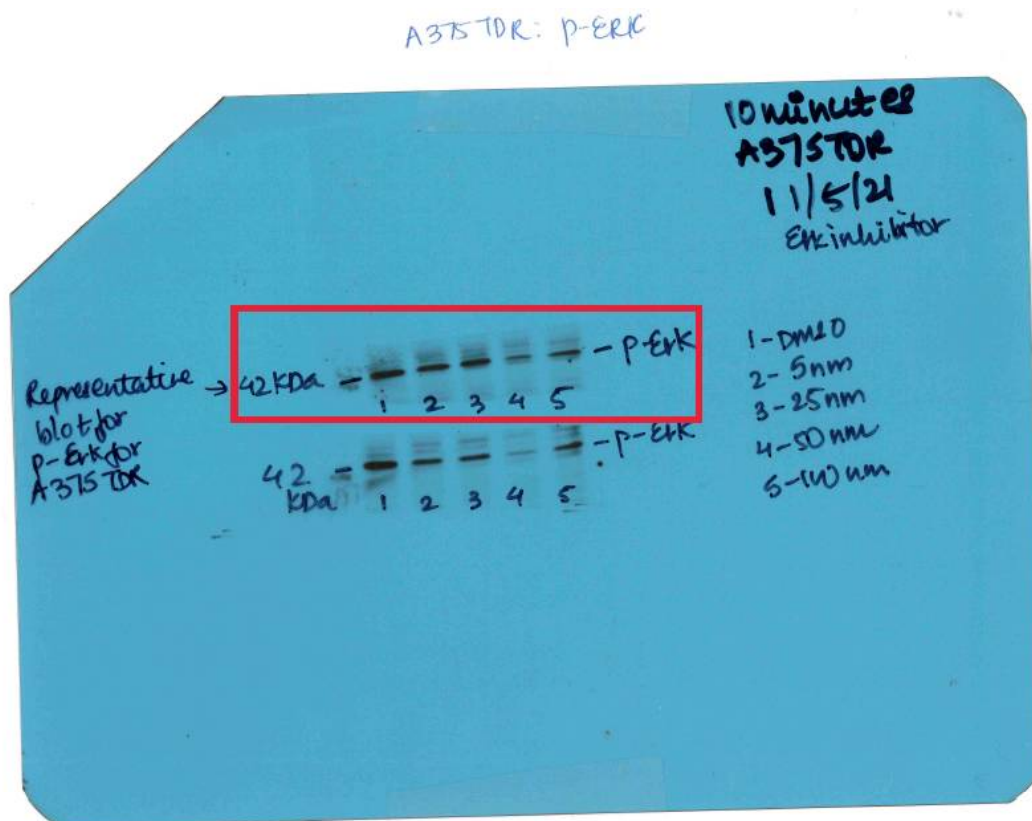
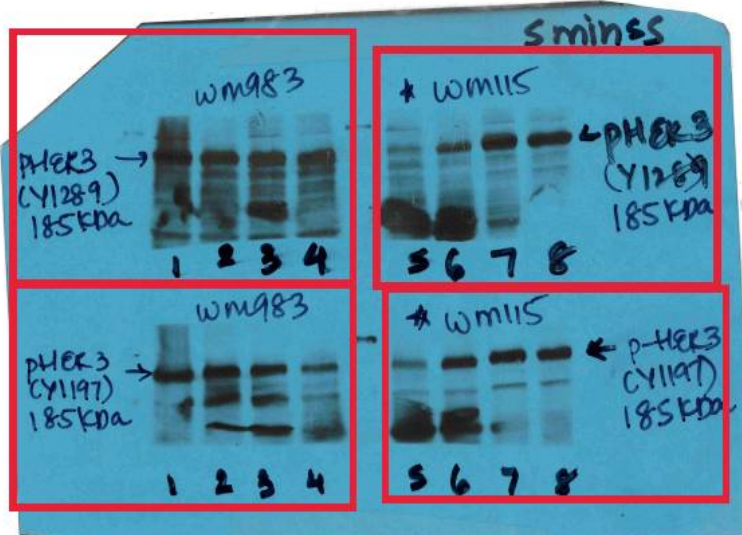


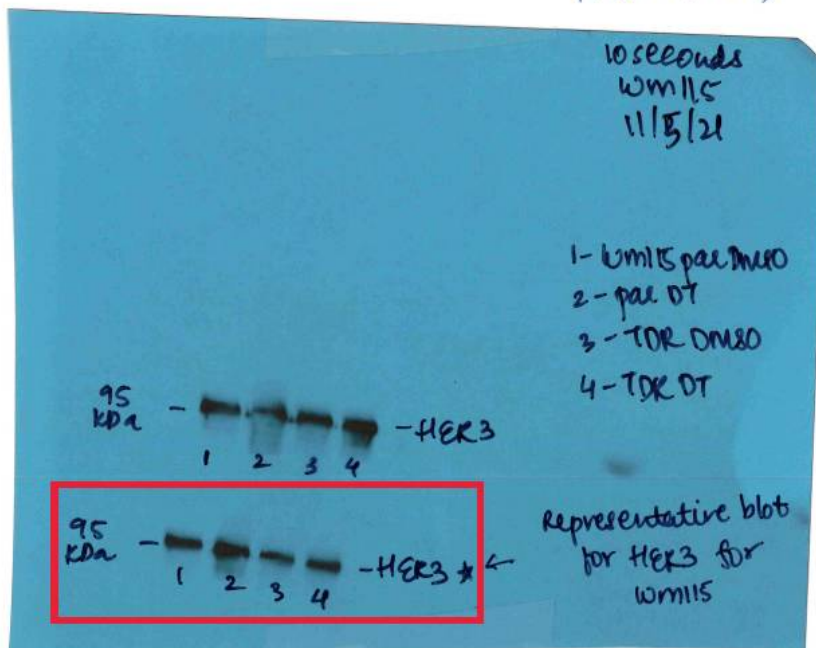
Figure S7. Whole blots for western blots in Figure 1E.

wm115: p-HER3(Y1289) & p-HER3(Y1197)  
 wm983: p-HER3(Y1289) & p-HER3(Y1197)



5- wm115 pal DMUO  
 6- wm115 pal DT  
 7- wm115 TOR DMUO  
 8- wm115 TOR DT

\* Representative blot for p-HER3(Y1289) & p-HER3(Y1197) for wm115; p-HER3(Y1289) for wm983  
 p-HER3(Y1197)



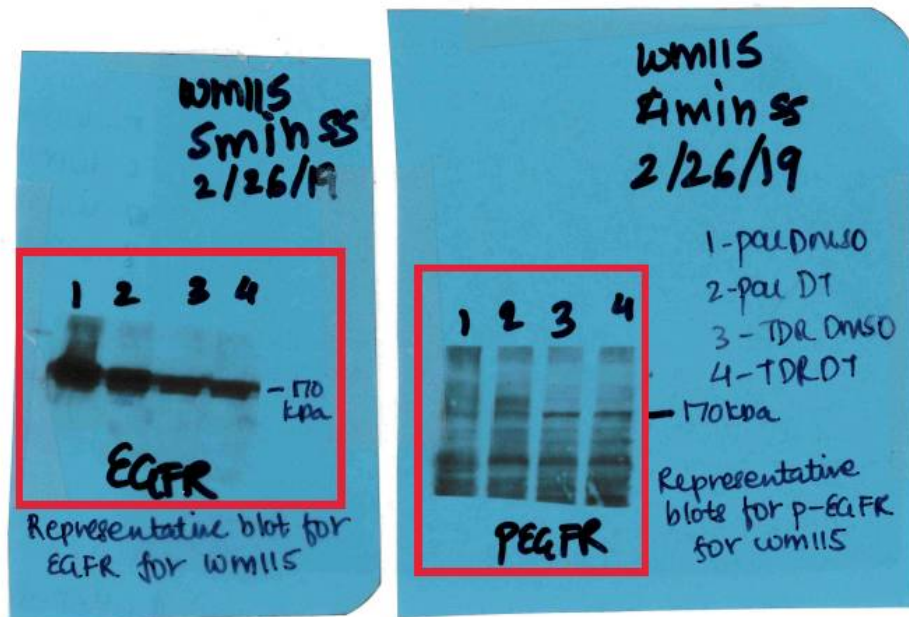
wm115: HER3

1- wm115 pal DMUO  
 2- pal DT  
 3- TOR DMUO  
 4- TOR DT

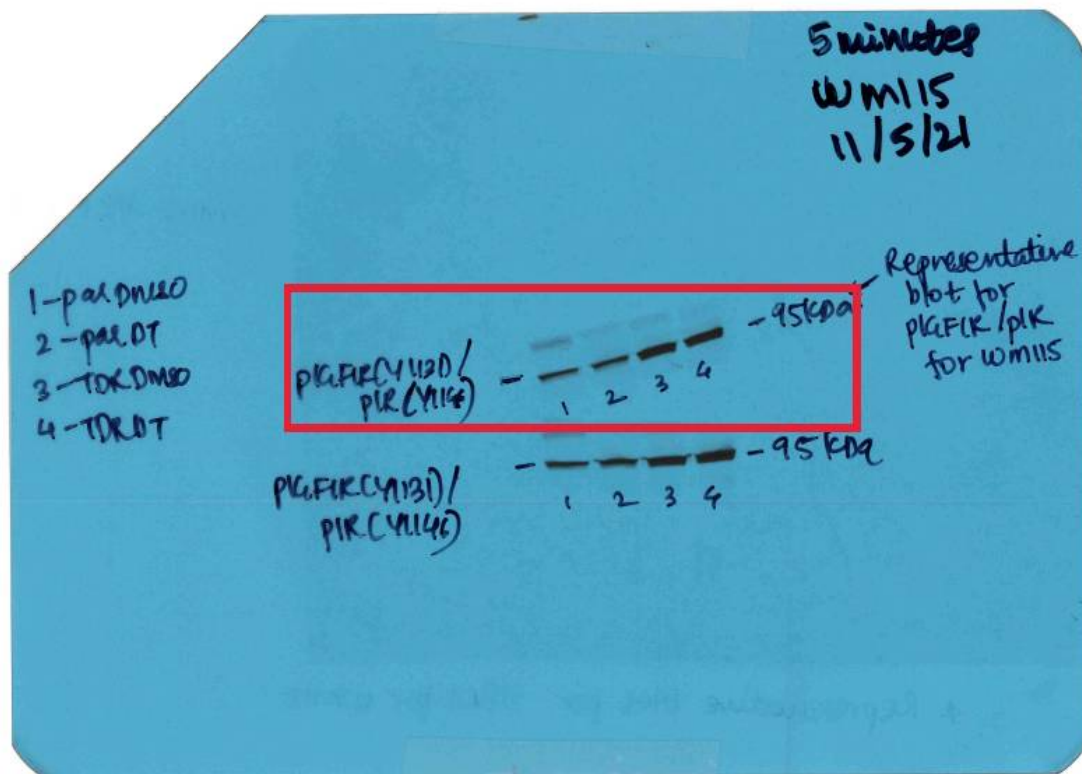
\* Representative blot for HER3 for wm115



WM115: p-EGFR & EGFR



WM115: p-EGFR (Y1131) / p-IR (Y1146)

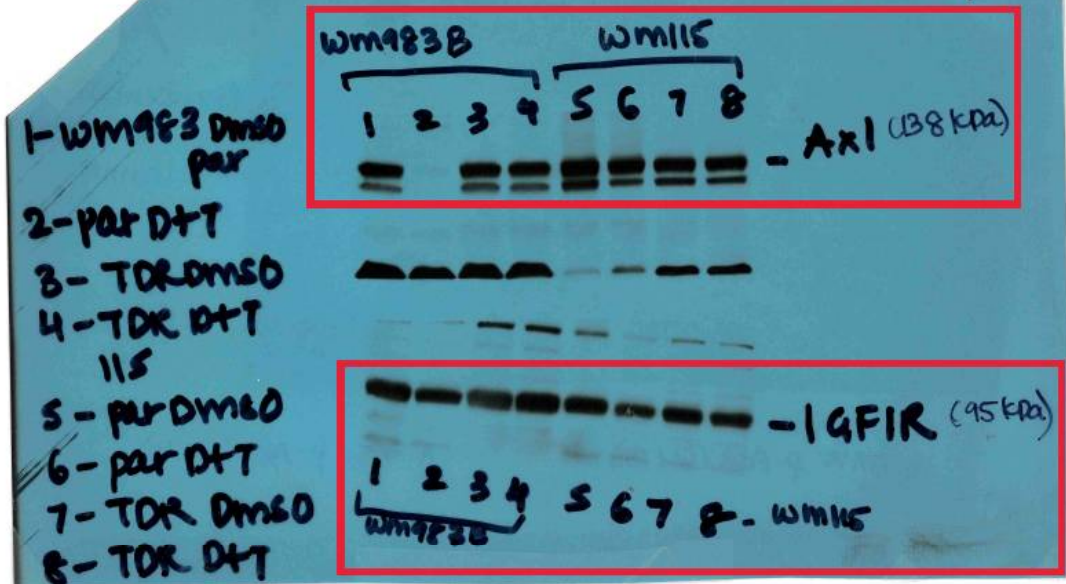


WM983B: Ax1; IGF1R

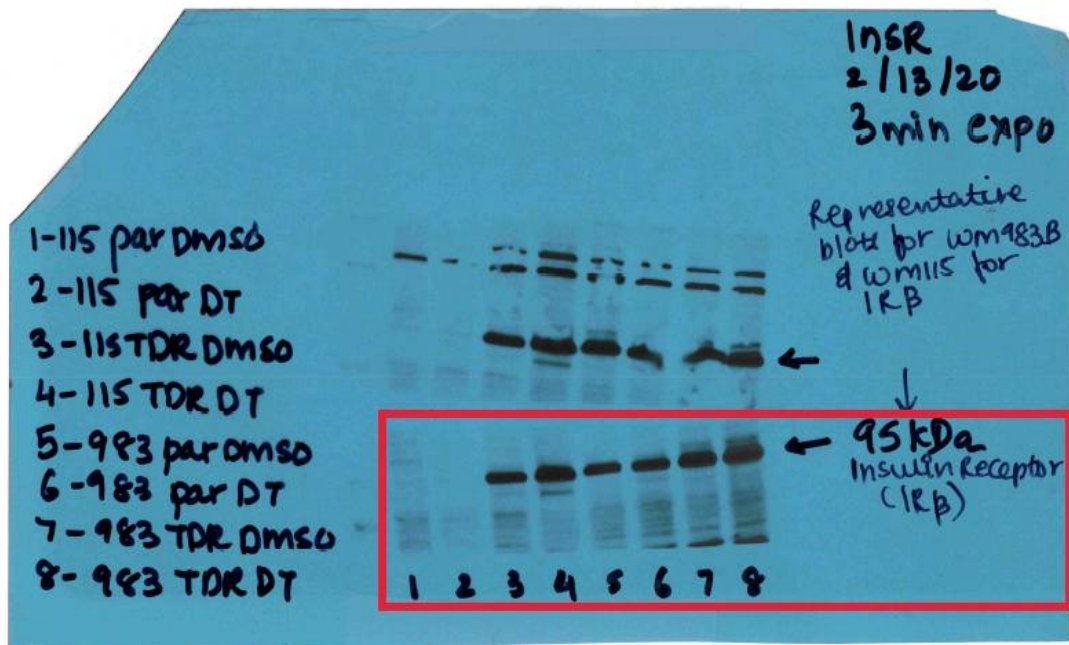
WM115: Ax1; IGF1R

8/24/19

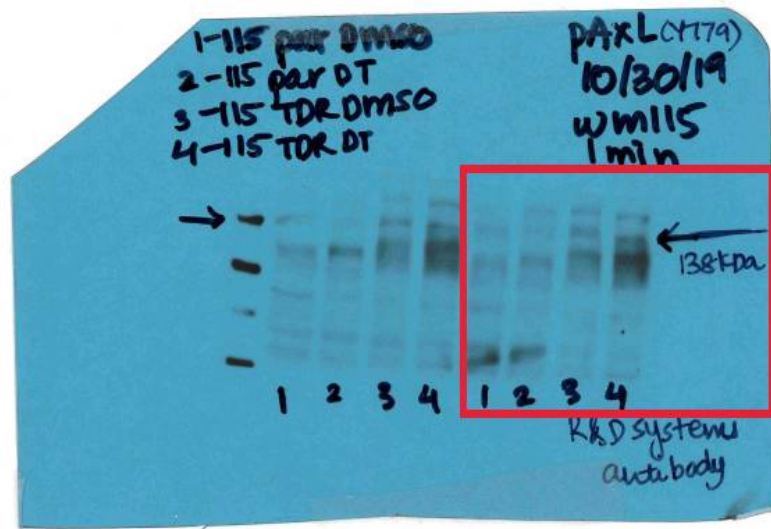
5min exp



↑ Representative blot for WM983B (Ax1 & IGF1R) and WM115 (Ax1 & IGF1R)



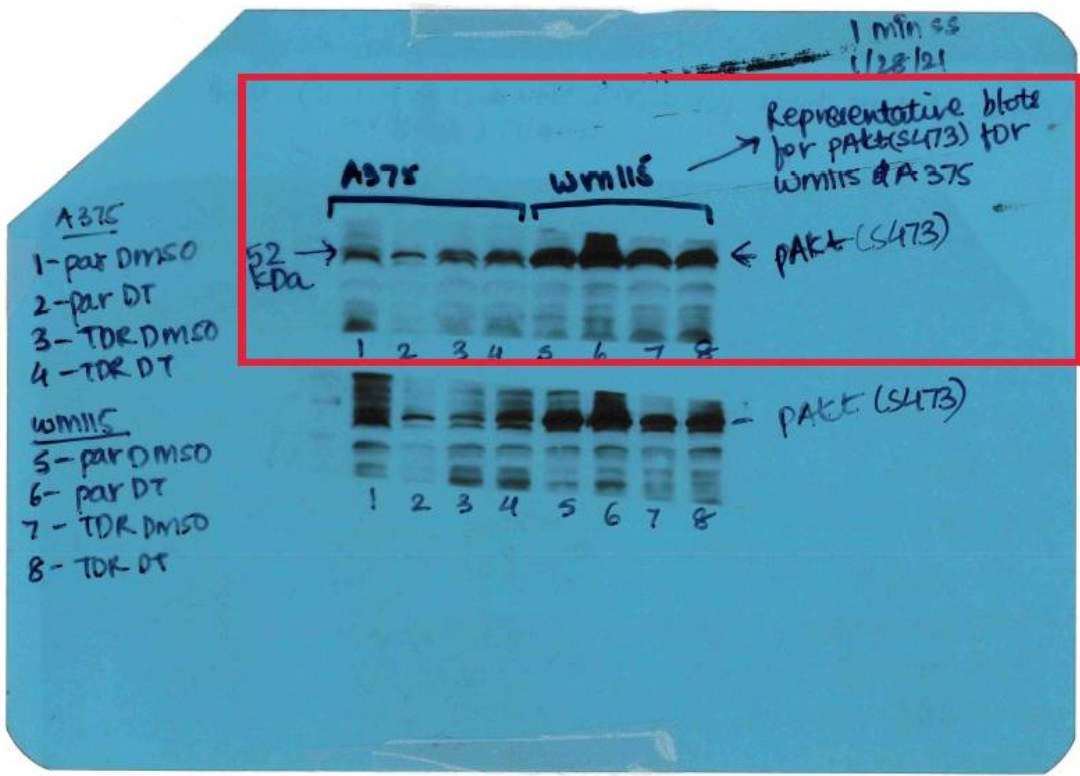
WM115: P-AXL (Y179)



Representative blot for P-AXL (Y179) for WM115.

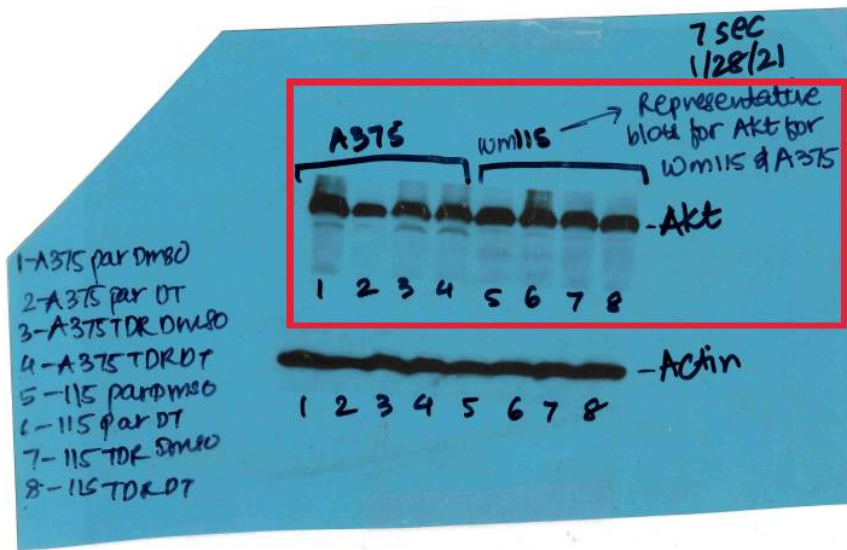
WM115: P-Akt (S473)

A375: P-Akt (S473)

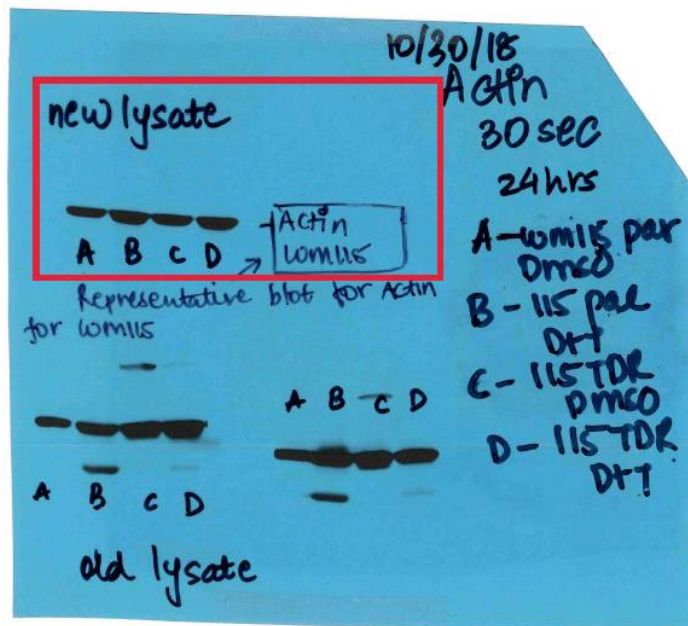




wm115: Akt ; A375: Akt



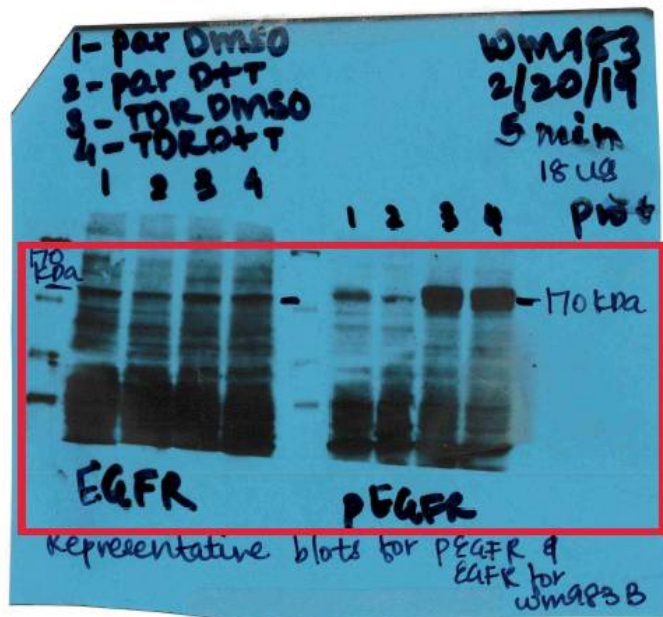
wm115: Actin



WM983B: HER3



WM983B: p-EGFR & EGFR



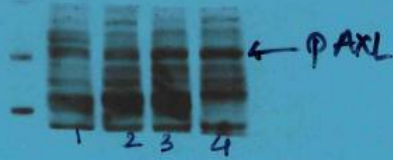


WM983B : p-AXL(Y779)

Representative blot for  
pAXL(Y779) for WM983B

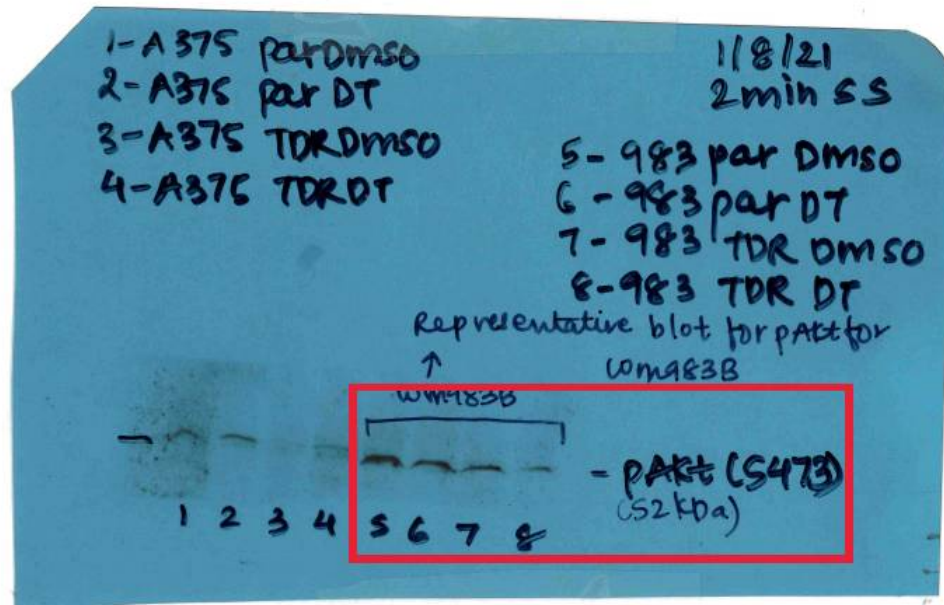


1- pal DMSO  
2- pal DT  
3- TOR DMSO  
4- TOR DT

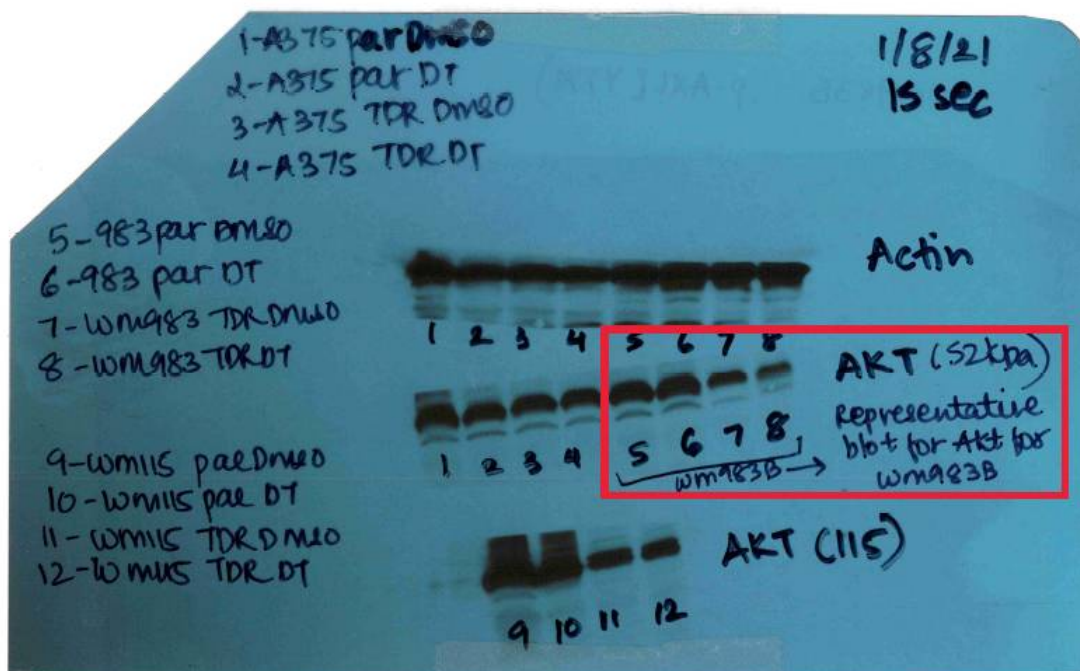


WM983  
11/7/19  
2.5 min  
R8 D systems alb

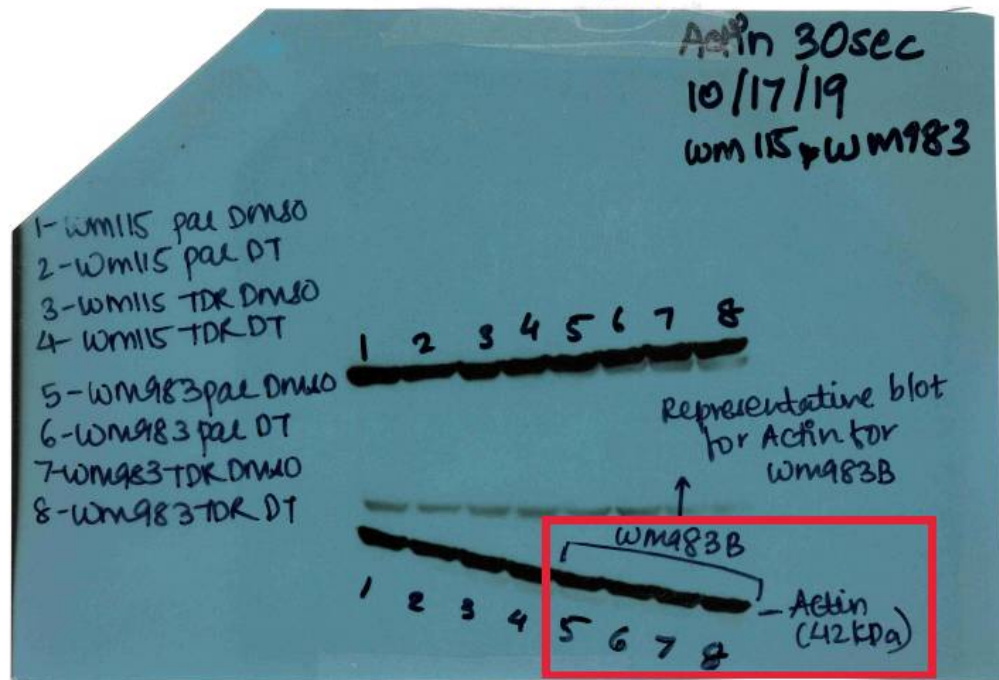
WM983B: p-Akt (S473)



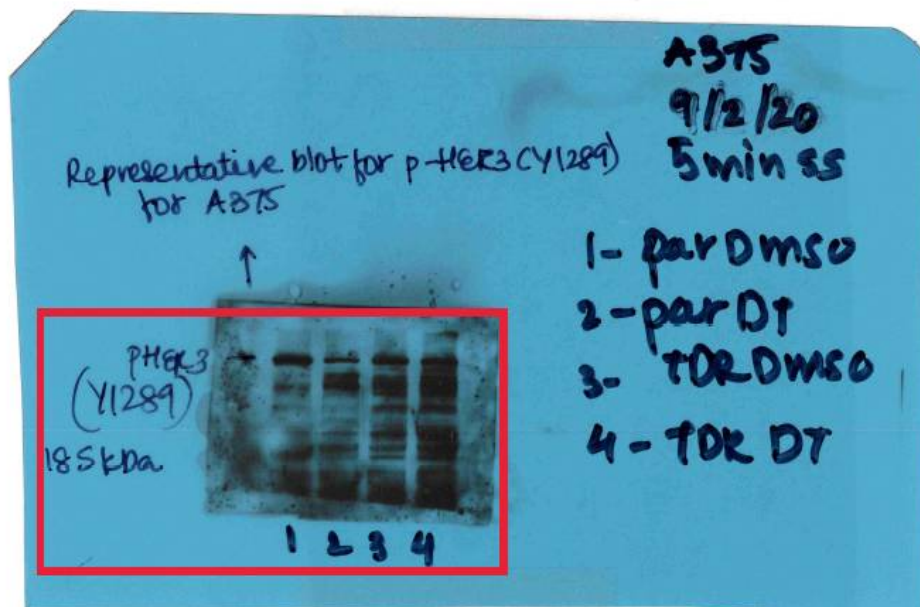
WM983B: Akt



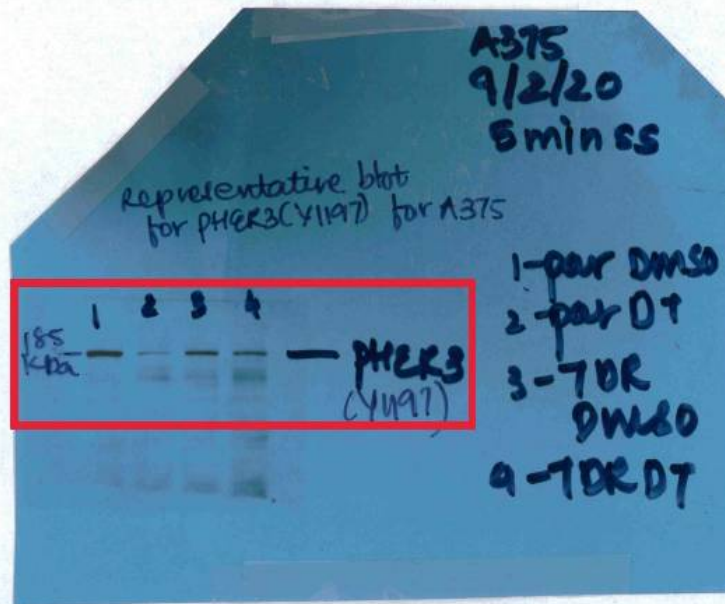
WM983B: Actin



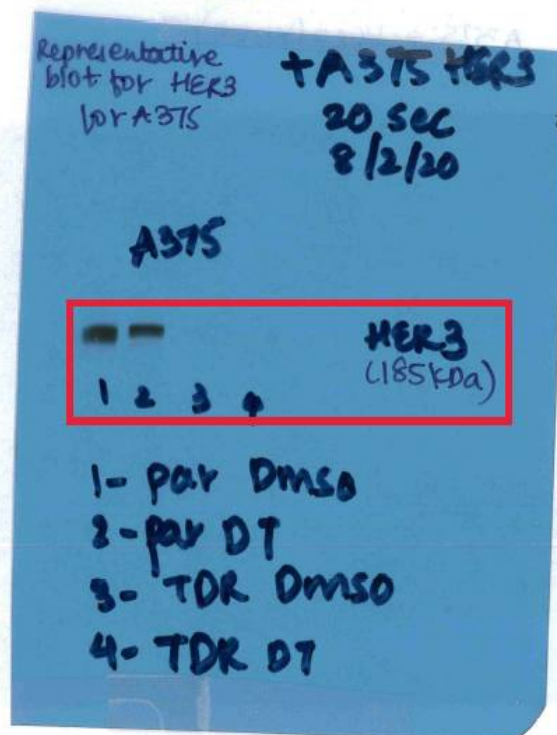
A375: p-HER3 (Y1289)



A375: p-HER3 (Y1197)

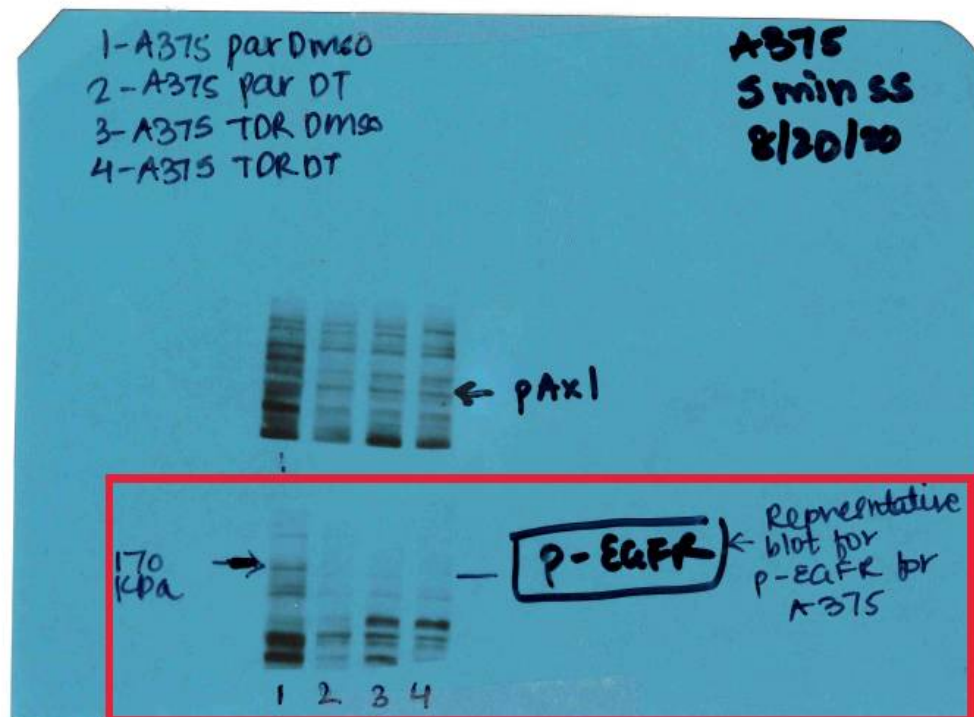


A375: HER3

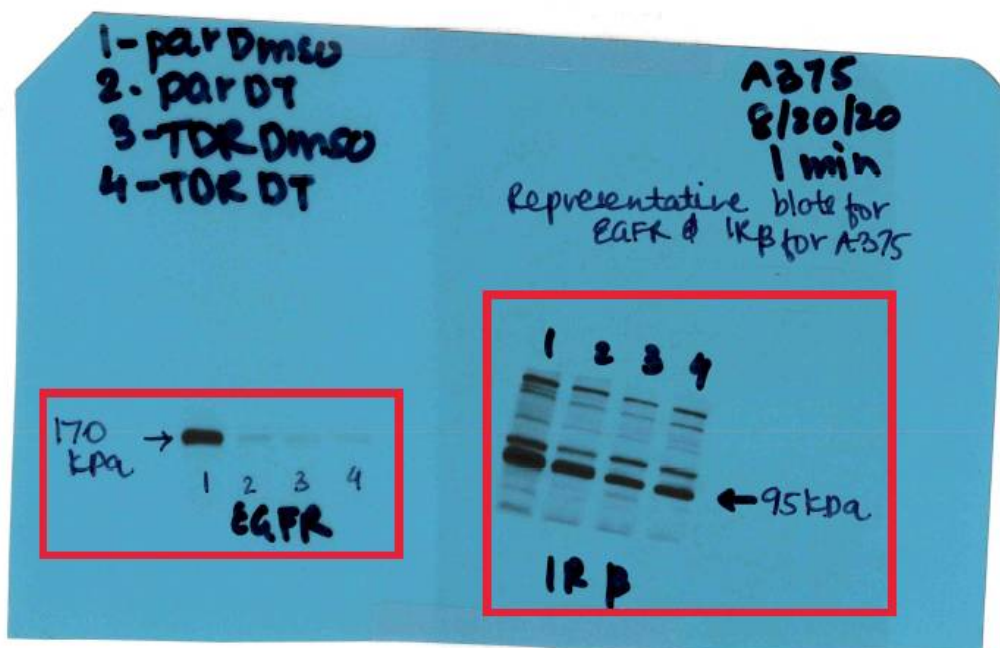




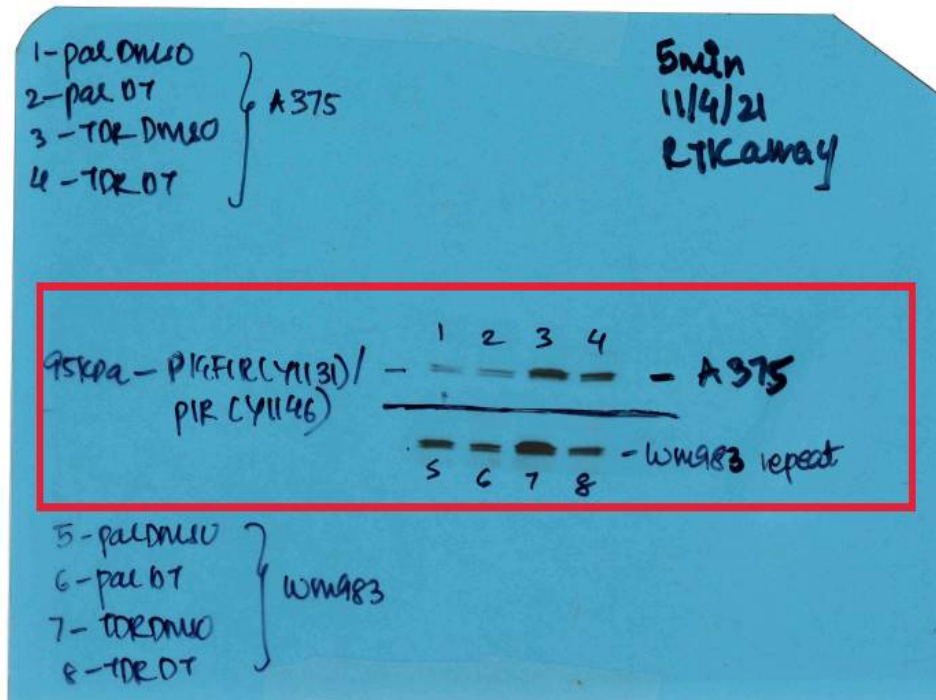
# A375pEGFR



## A375: EGFR & IRP



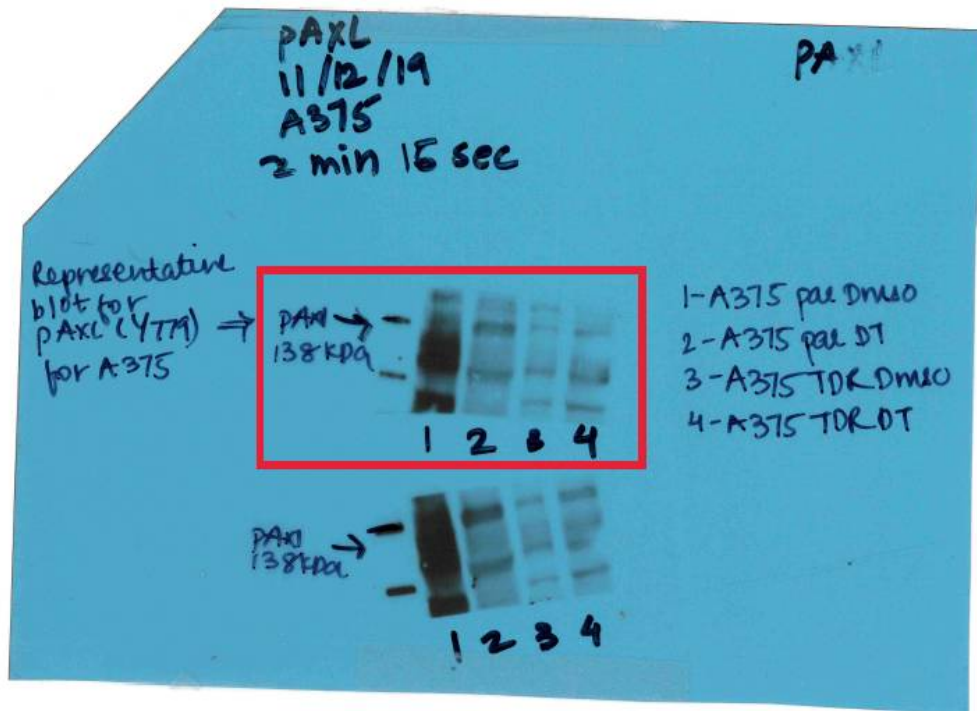
A375 and WM983B p-IGF1R(Y1131) / p-IR(Y1146)



A375:IGF1R



A375: pAXL



A375: AXL & Actin

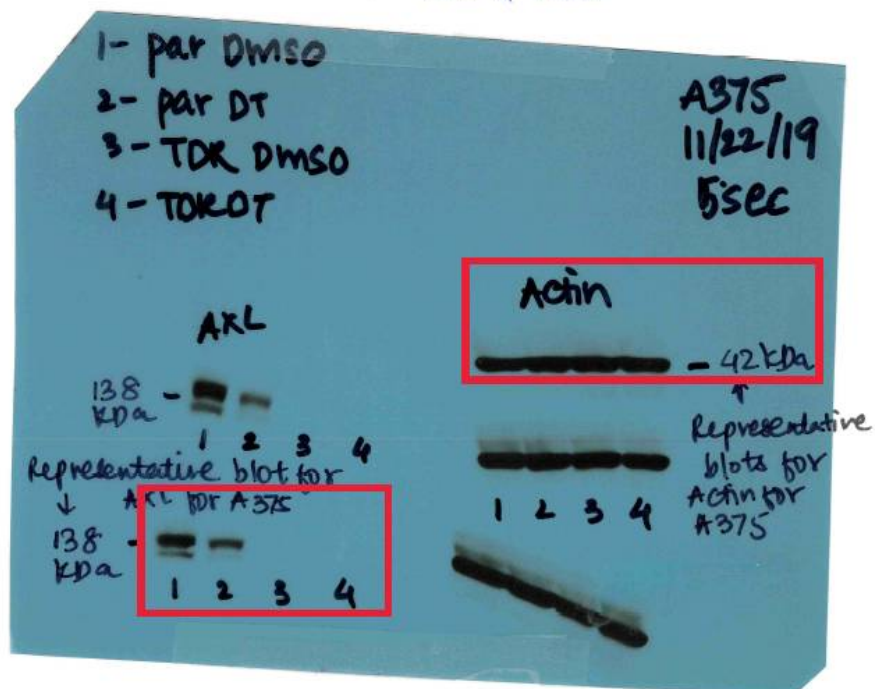
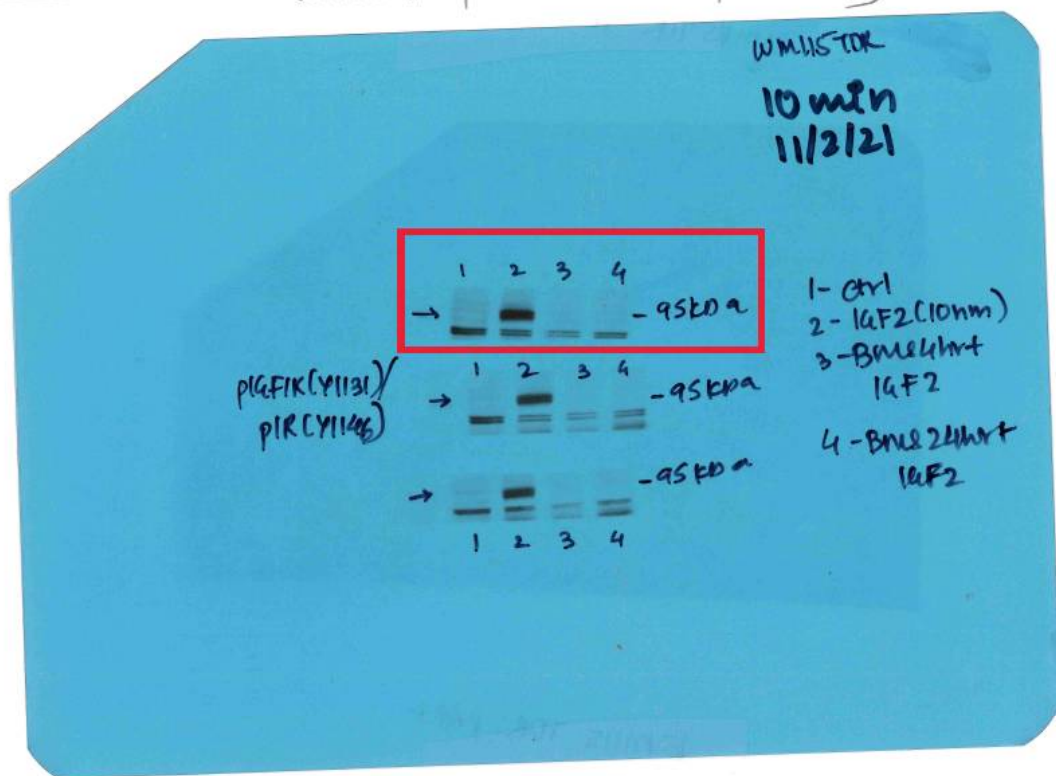


Figure S8. Whole blots for western blots in Figure 2C.

Figure 4D

WM115 TOR: p-I $\alpha$ FIK(Y1131) / PIR(Y1146)

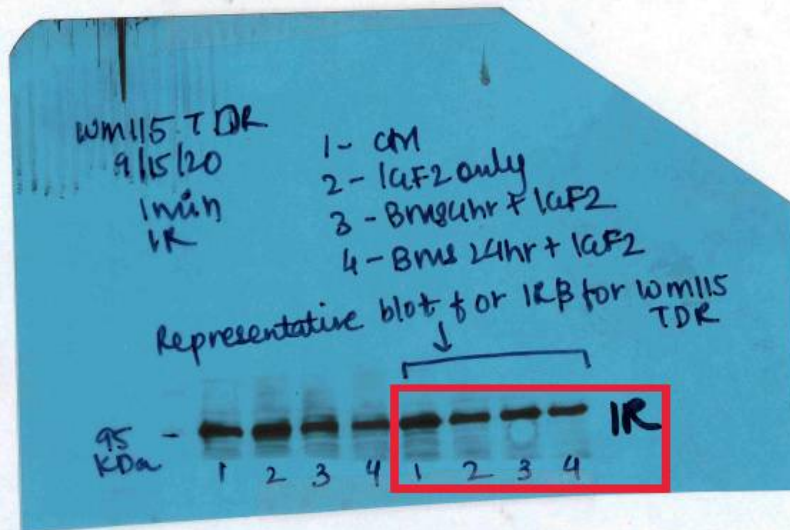


WM115 TOR: I $\alpha$ FIK

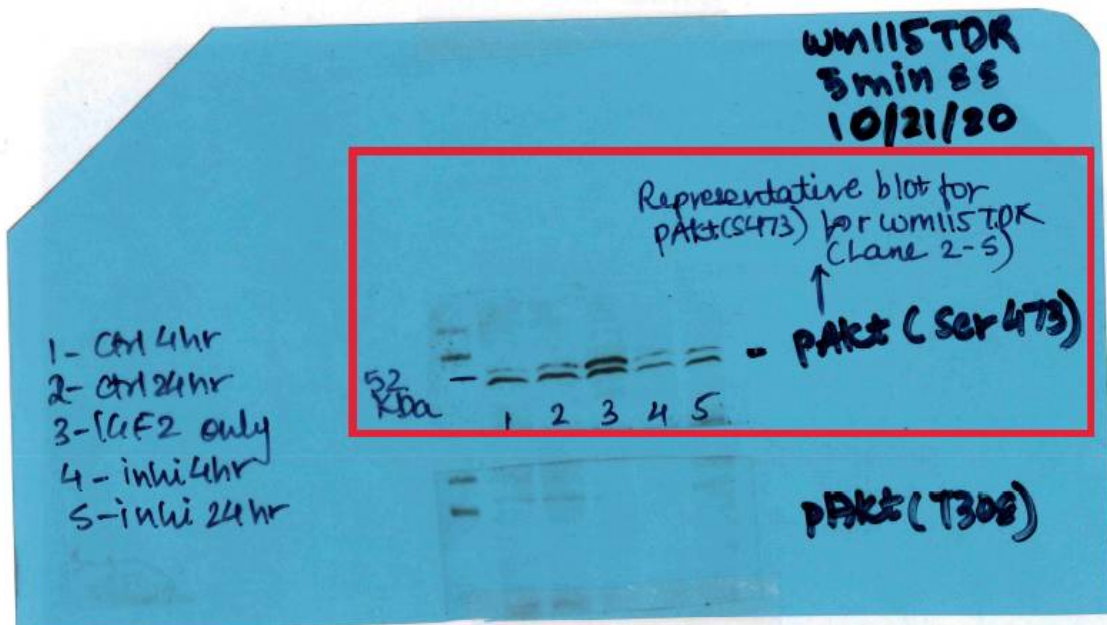




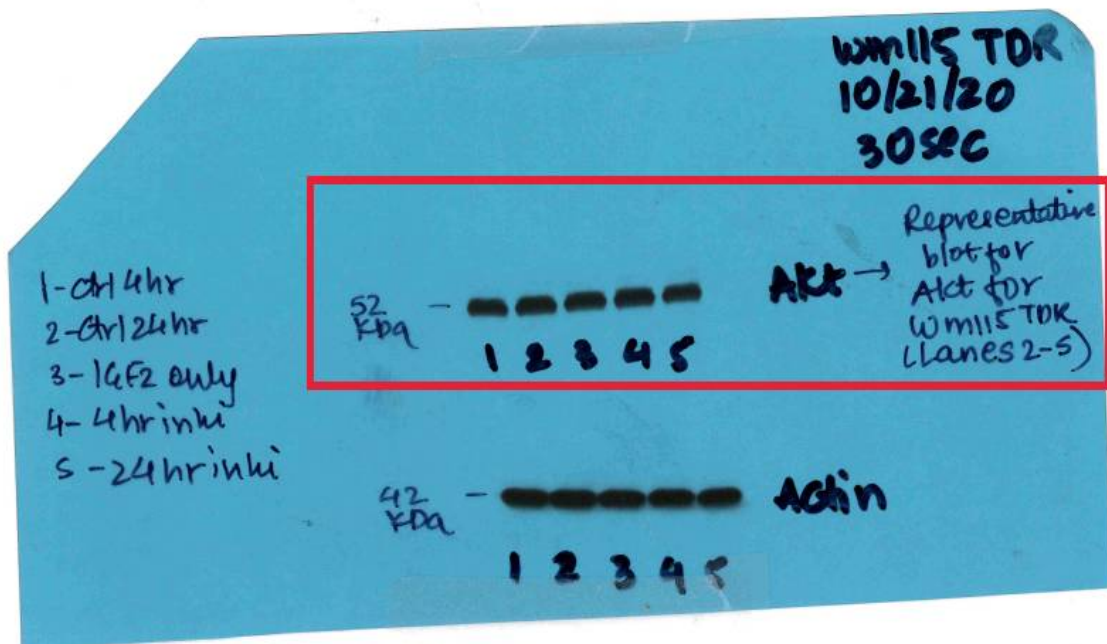
WM115 TDR: IR



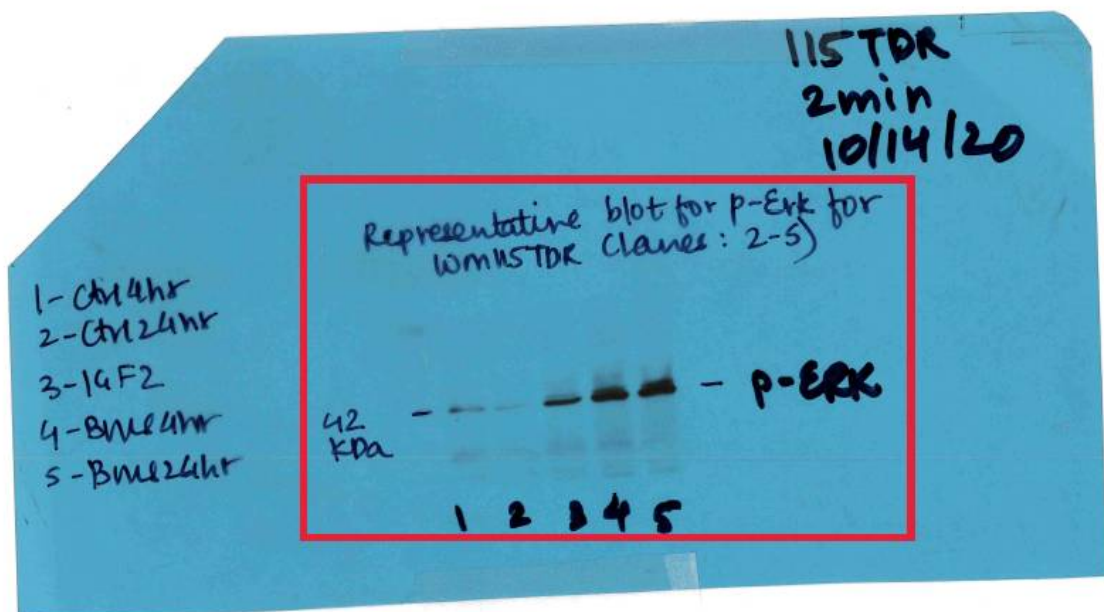
WM115 TDR: PAKT



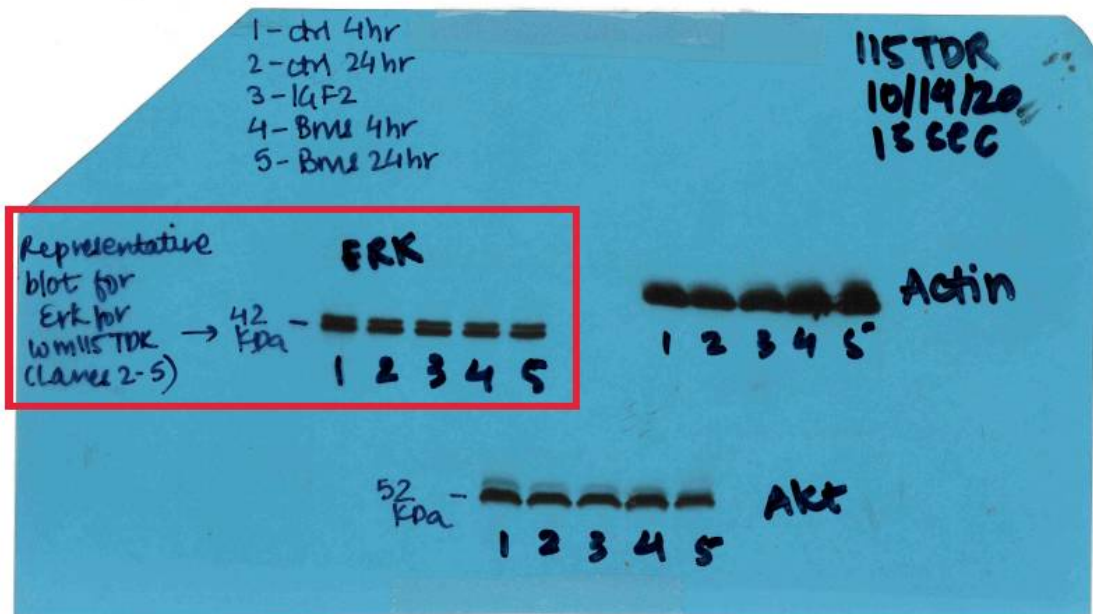
WM115 TDR: ~~phospho~~ Akt



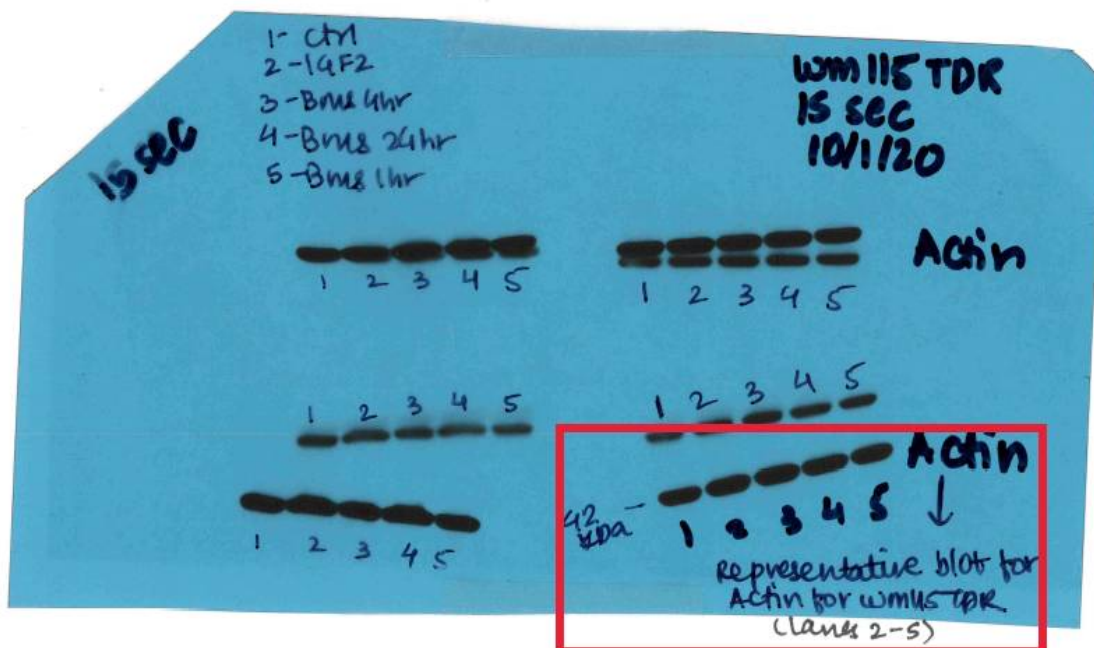
WM115 TDR: p-Erk



Wm115-TDK: ERK

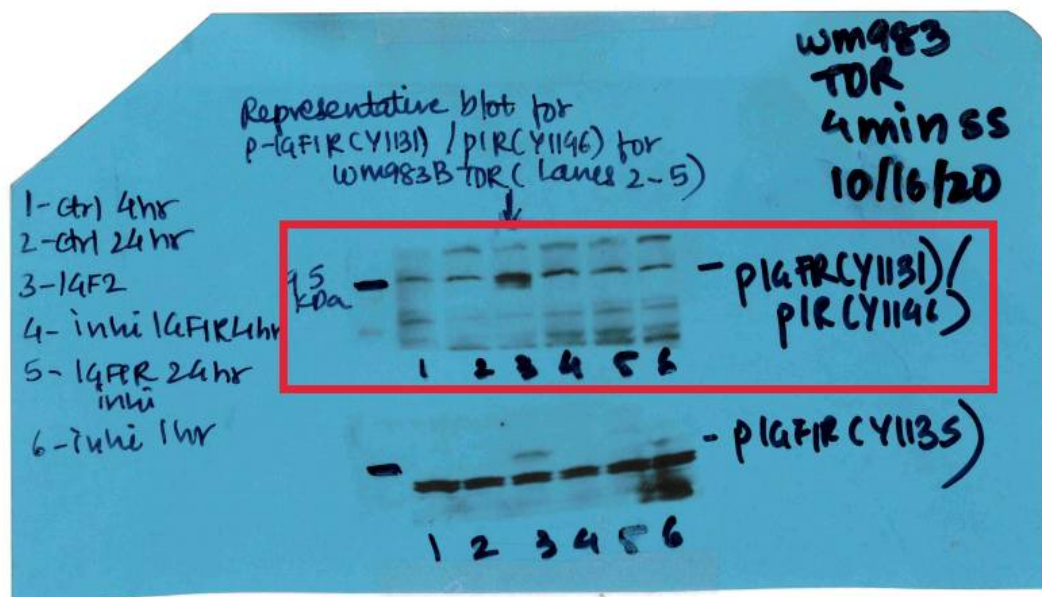


Wm115-TDK: Actin

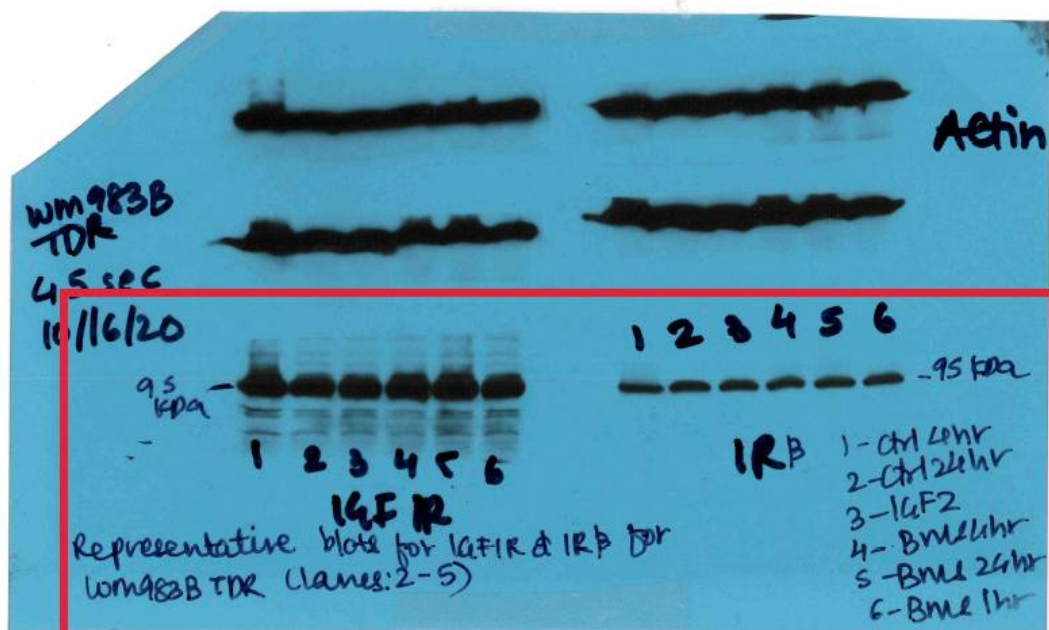




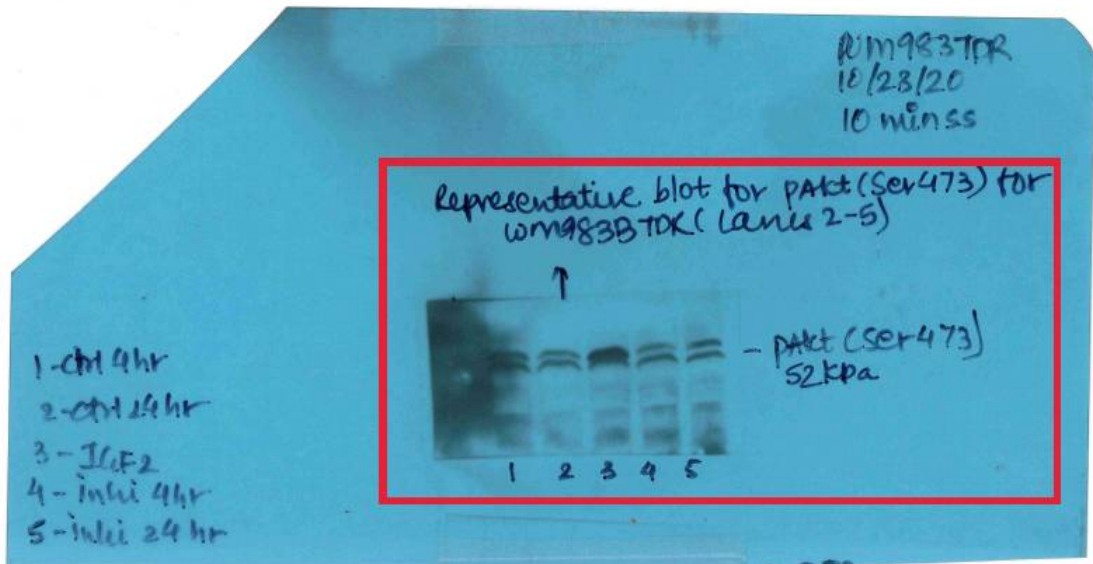
WM983TDR: pIGFIR(Y1131) /  
PIR(Y1146)



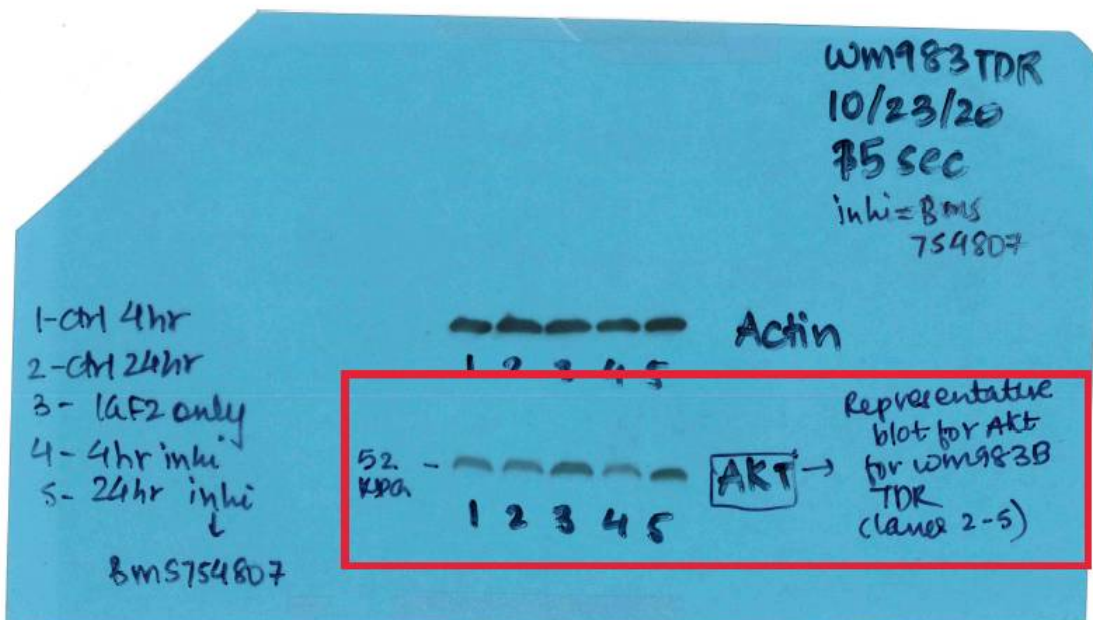
WM983B TDR: IGFIR & IR



WM983TDR: p-Akt

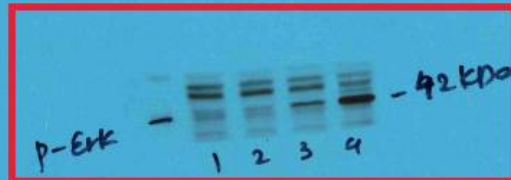


WM983B TDR: AKT



WM983B TOR: P-ERK

WM983B TOR  
10 min  
11/2/21



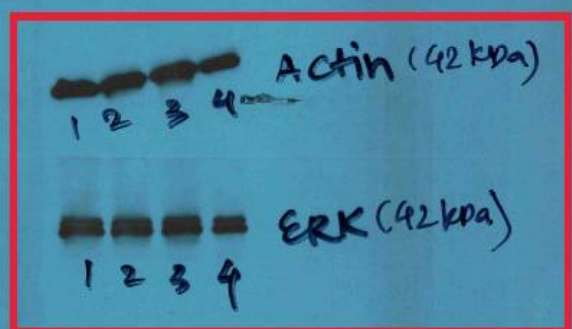
1-ctrl  
2-IGF2 (10nm)  
3-BMS4hr + IGF2  
4-BMS24hr + IGF2

WM983B TOR: ERK & Actin

Representative blots for Actin & ERK  
for WM983B TOR

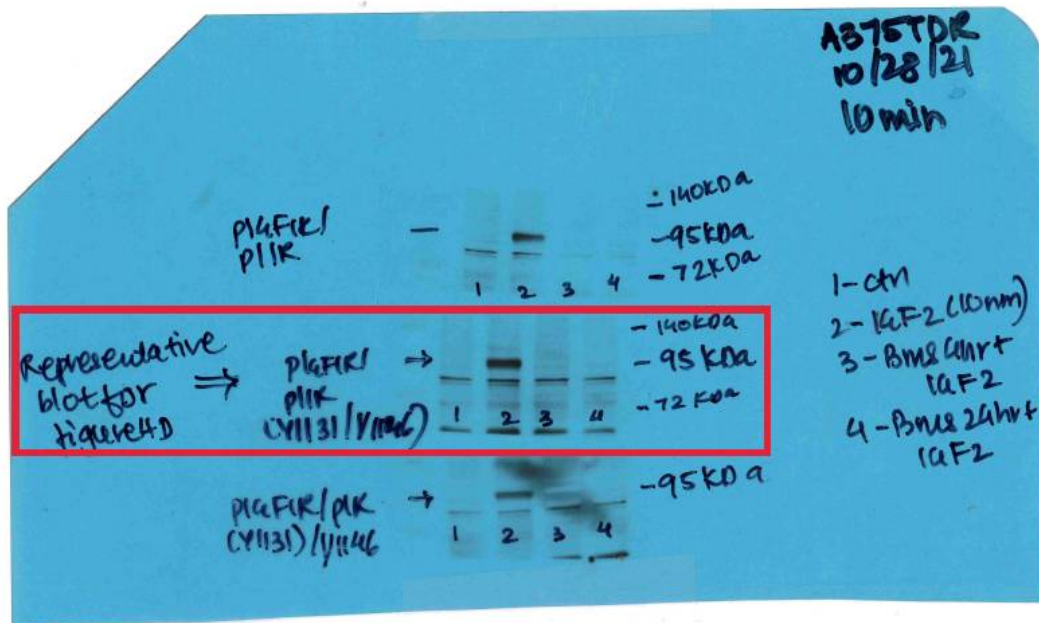
983B TOR  
5sec  
10/30/20

1=ctrl 24hr  
2-IGF2  
3-Inhi 4hr  
4-Inhi 24hr  
BMS-754807

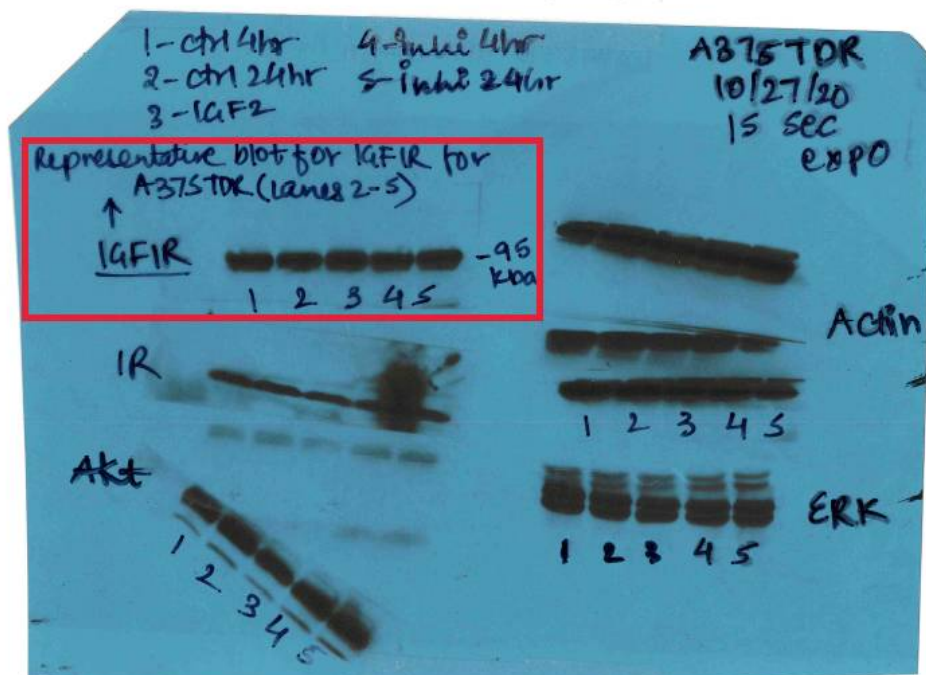




A375TDR: pIGFIR(Y1131)/  
PIR(Y1146)



A375TDR: IGFIR



A375TOR: IR

1- Ctrl  
2- IGF2 only  
3- Bms 4hr + IGF2  
4- Bms 24hr + IGF2

Bms

95 kDa 1 2 3 4

Representative blot for IR for A375TOR

95 kDa 1 2 3 4

A375TOR  
1min.  
Insulin  
receptor

A375TOR: p-Akt

A375TOR  
10/27/20  
1min ss

PAK1-  
S473

1 2 3 4 5

Representative blot for PAK1 (S473) for A375TOR (lanes 2-5)

PAK1-  
S473

1 2 3 4 5

52 kDa

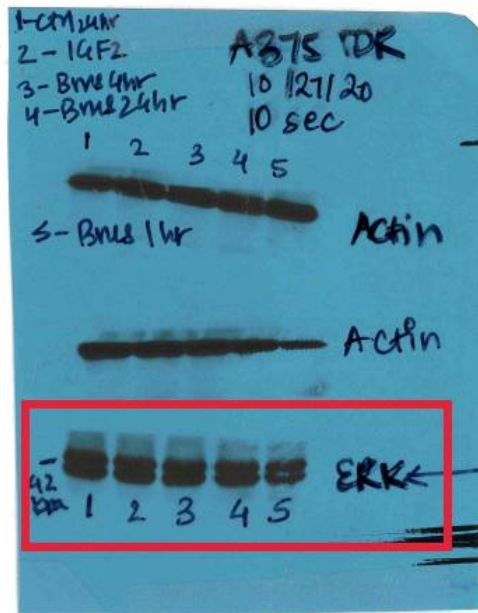
PERK

1 2 3 4 5

1- 4hr Ctrl  
2- 24hr Ctrl  
3- IGF2  
4- 4hr insu  
5- 24hr insu

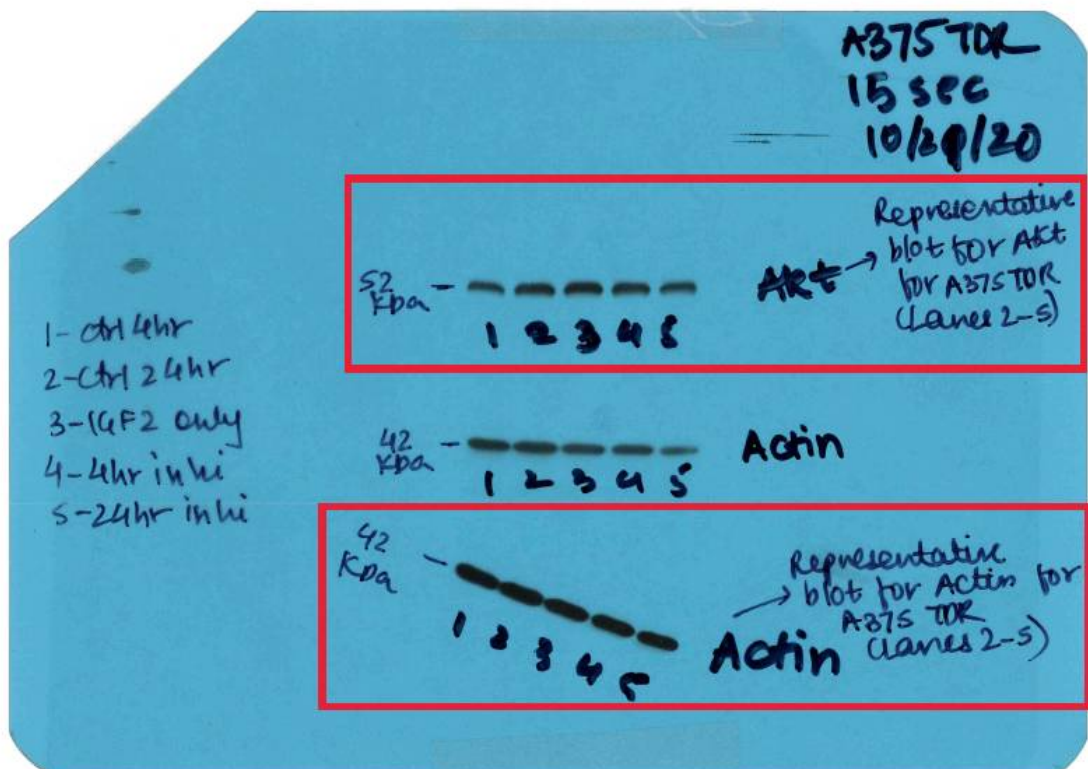


A375 TOR: ERK



Representative blot for ERK for A375 TOR (lanes 1-4)

A375 TOR: Akt & Actin



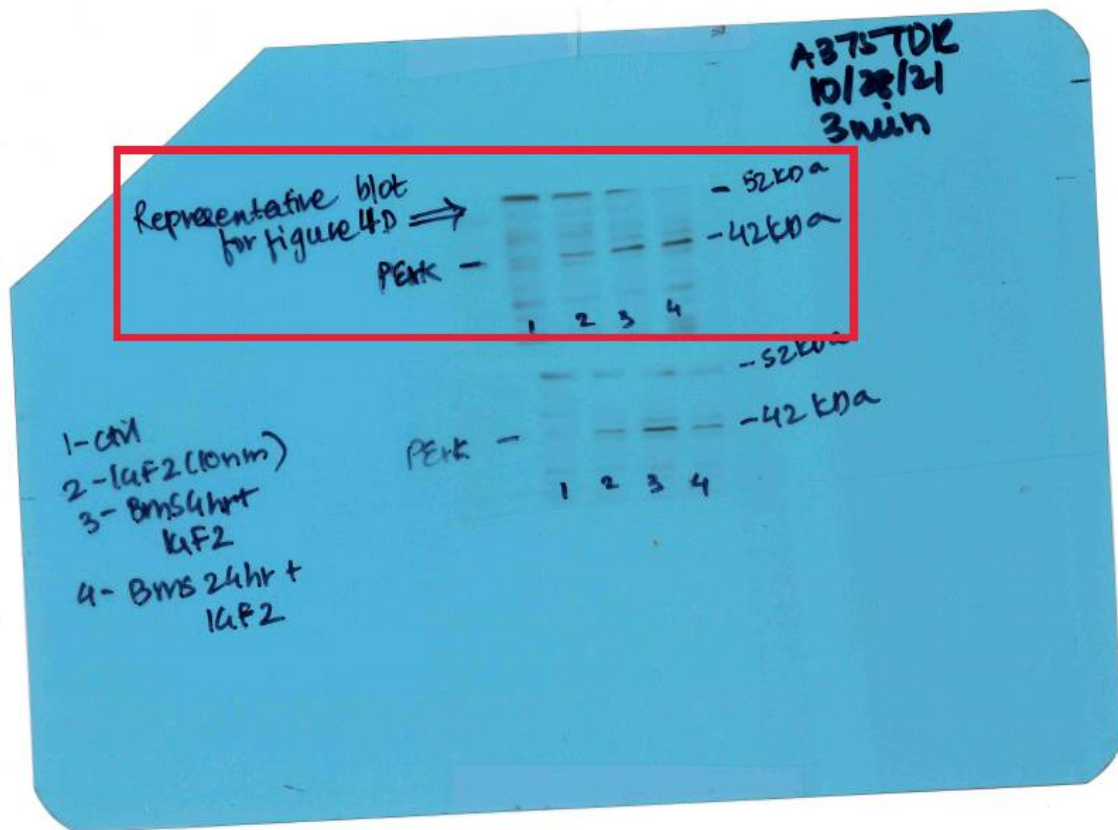


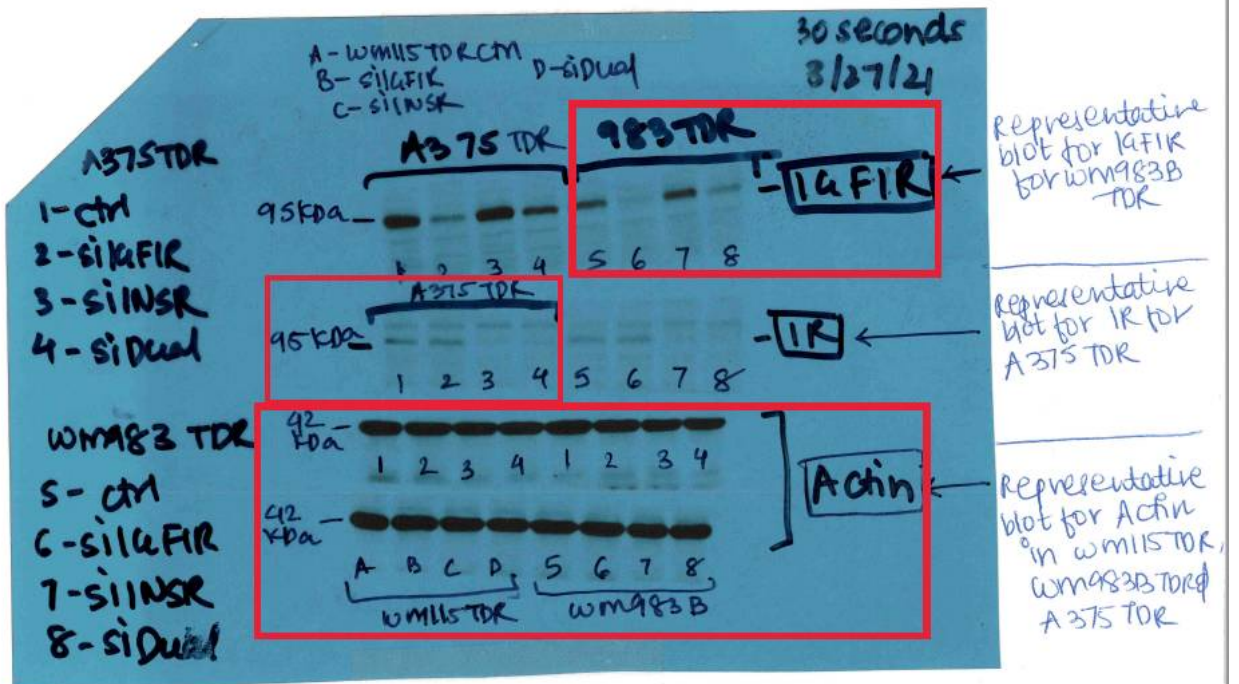
Figure S9. Whole blots for western blots in Figure 4D.

WM115 TDR: IGF1R & IR

Representative blot for IGF1R & IR in WM115 TDR

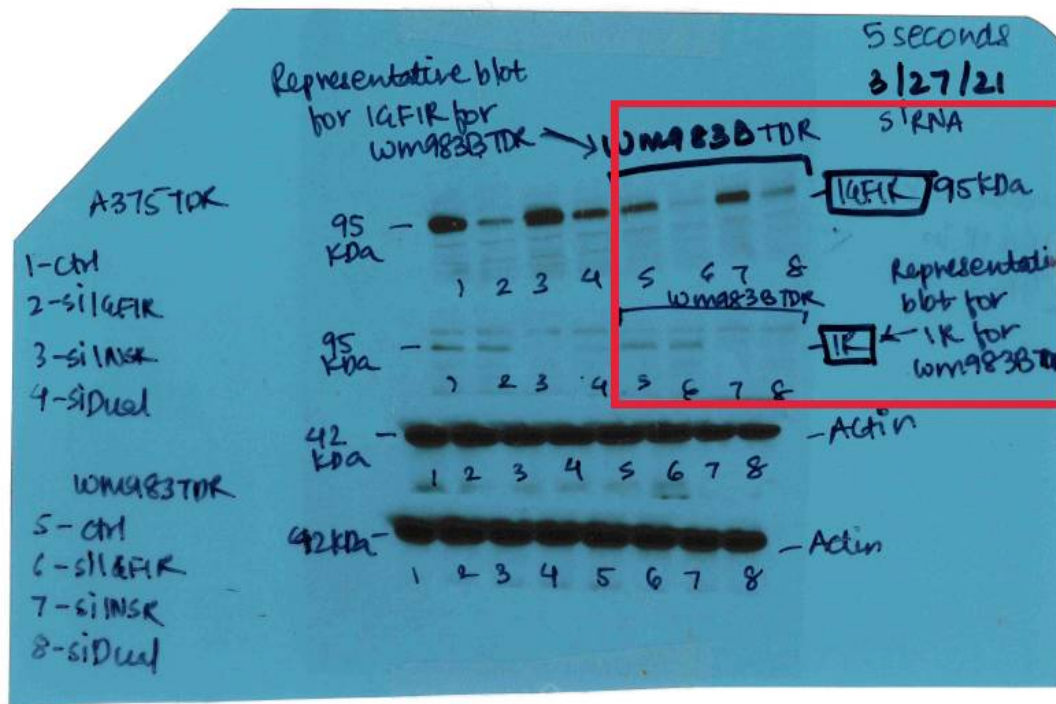


WM115 TDR: Actin; WM983B TDR: Actin, IGF1R; A375 TDR: IR, Actin





Wm9838 TDR: IGF1R & IR



A375 TDR: IGF1R & IR

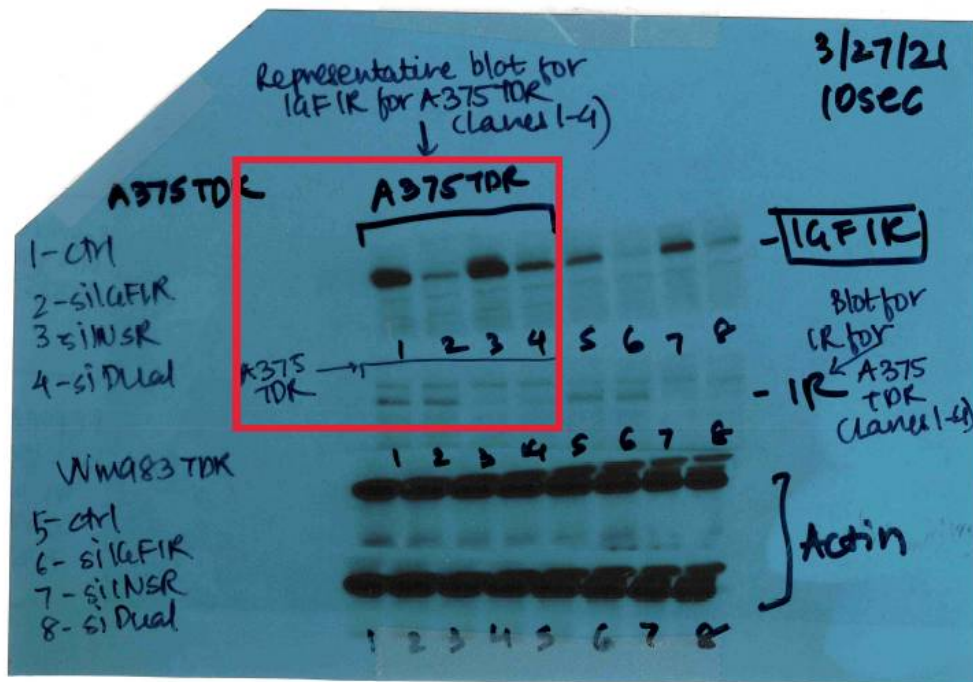
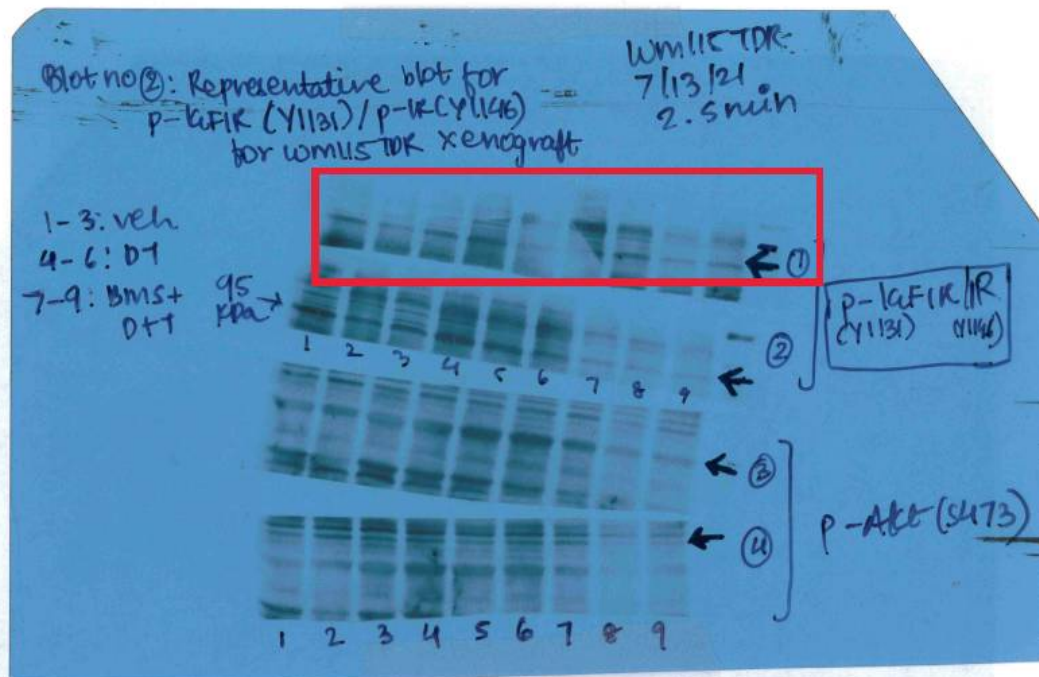


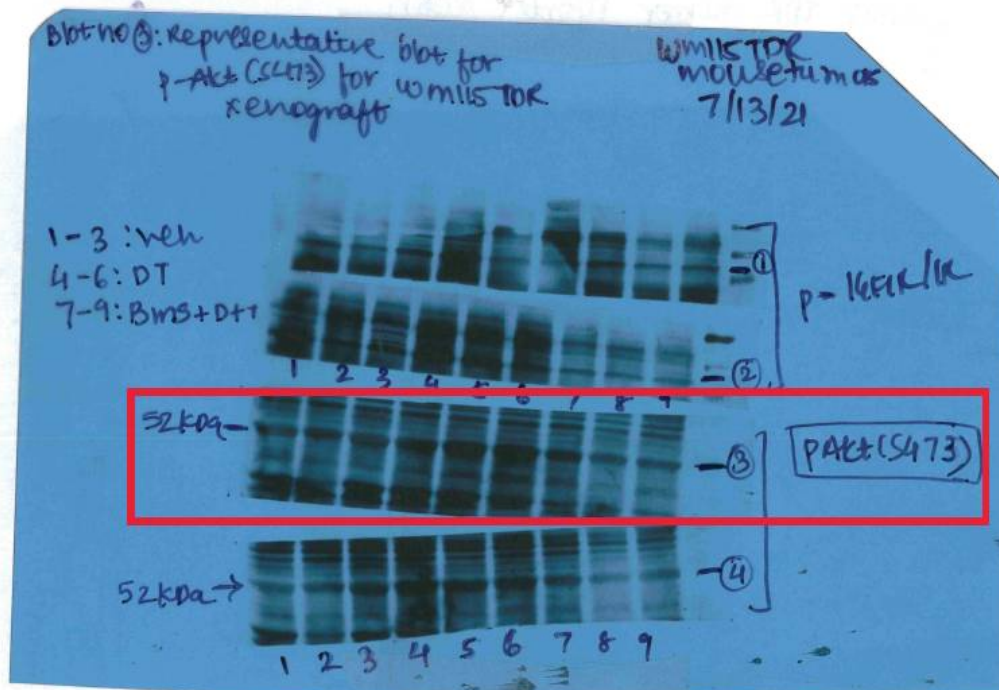
Figure S10. Whole blots for western blots in Figure 5A.



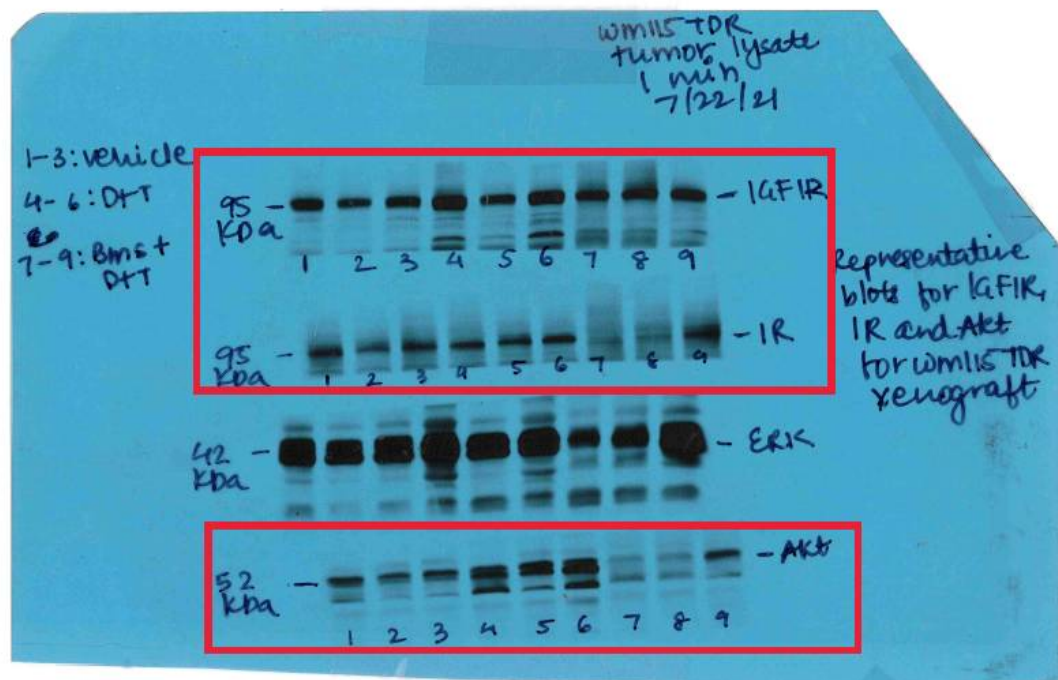
Figure 6  
Wm115 TDR tumor lysate: p-IaFIR / p-IK



Wm115 TDR tumor lysate: p-Akt



WM115 TDR tumor lysate: IgFIR, IR & Akt



WM115 TDR tumor lysate: Actin

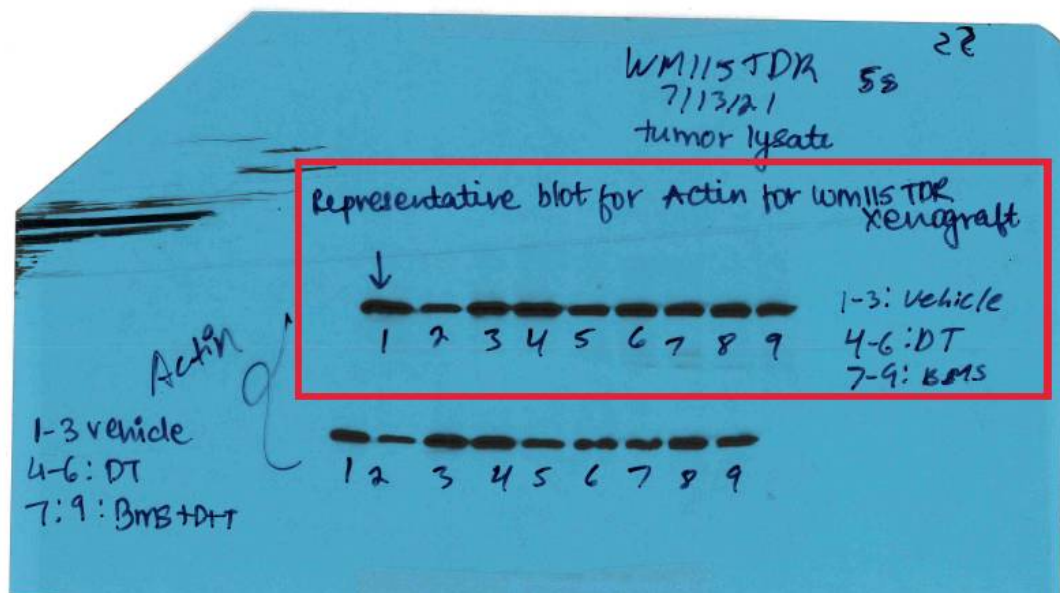


Figure S11. Whole blots for western blots in Figure 6E.

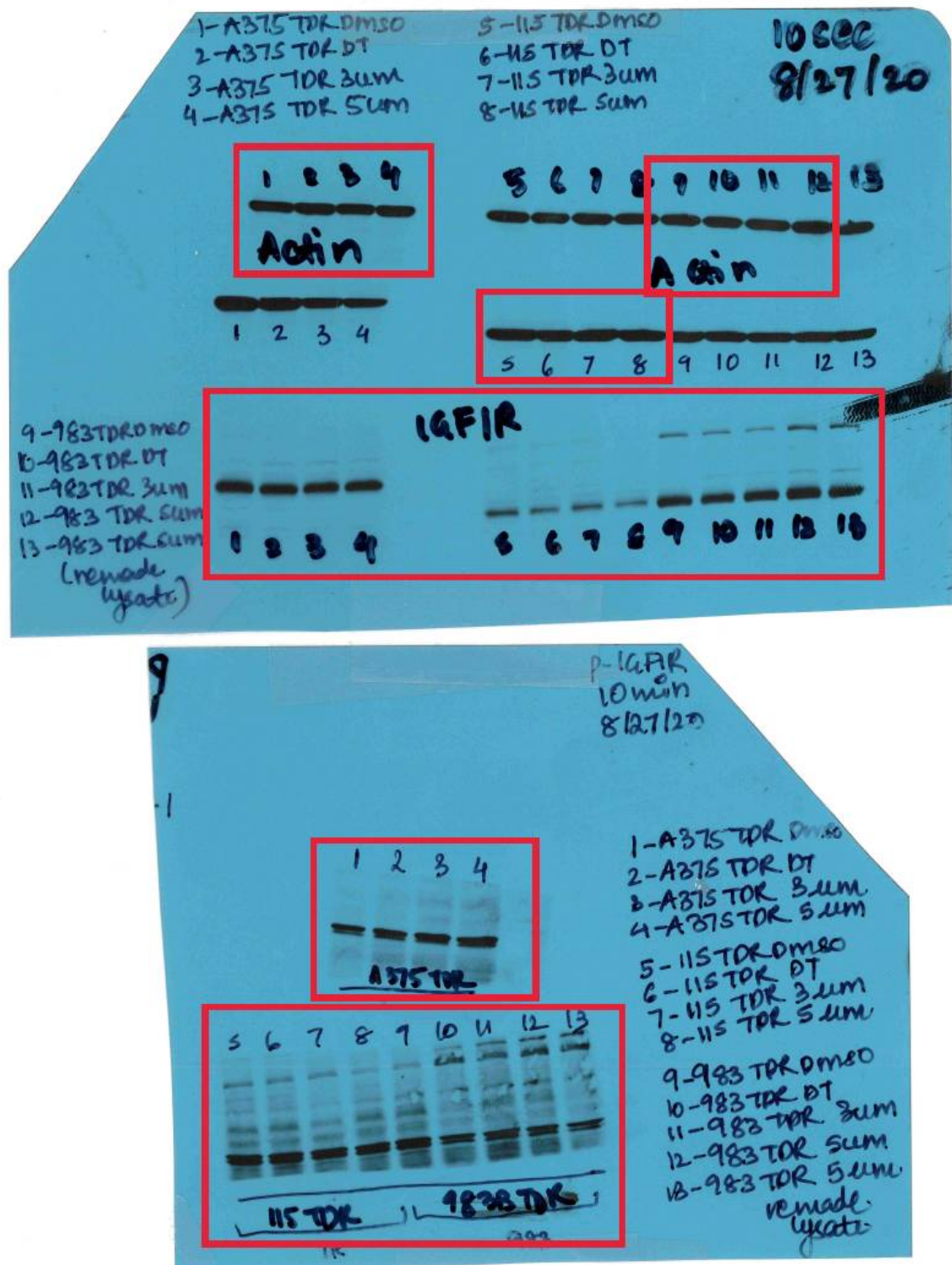
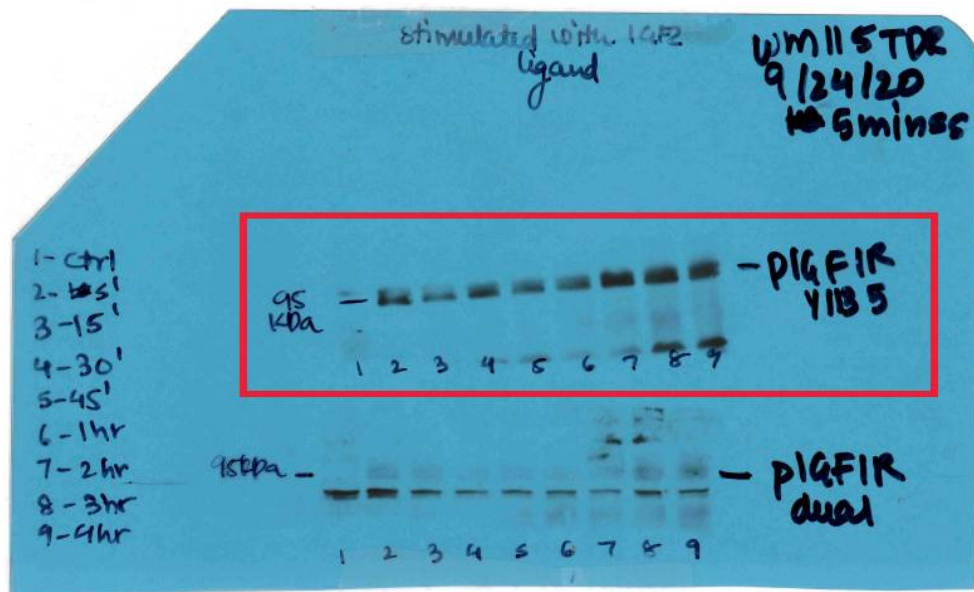


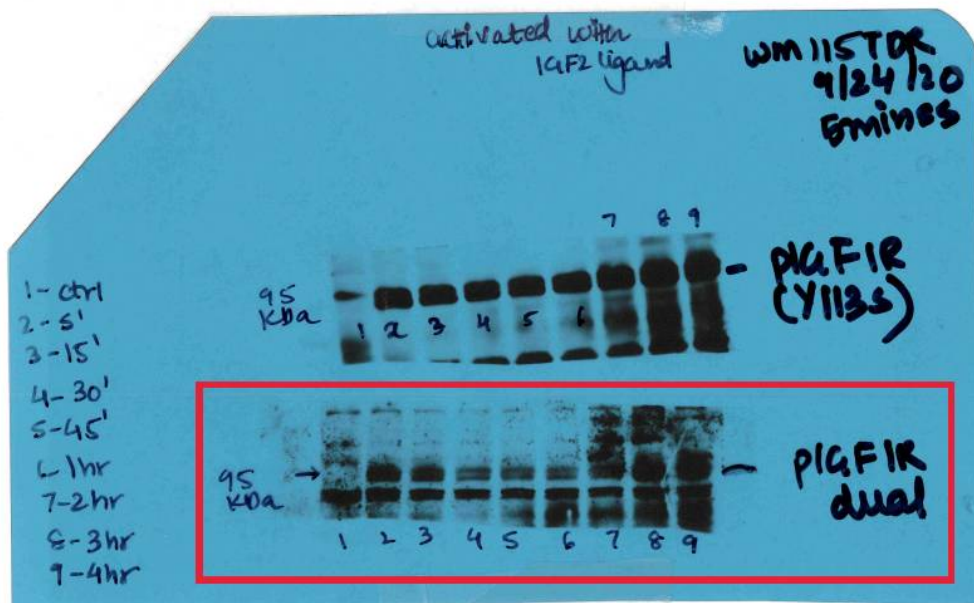
Figure S12. Whole blots for western blots in Figure S4.



Supplementary Figure 4  
WM115 TDR: p-IGFIR (Y1135)

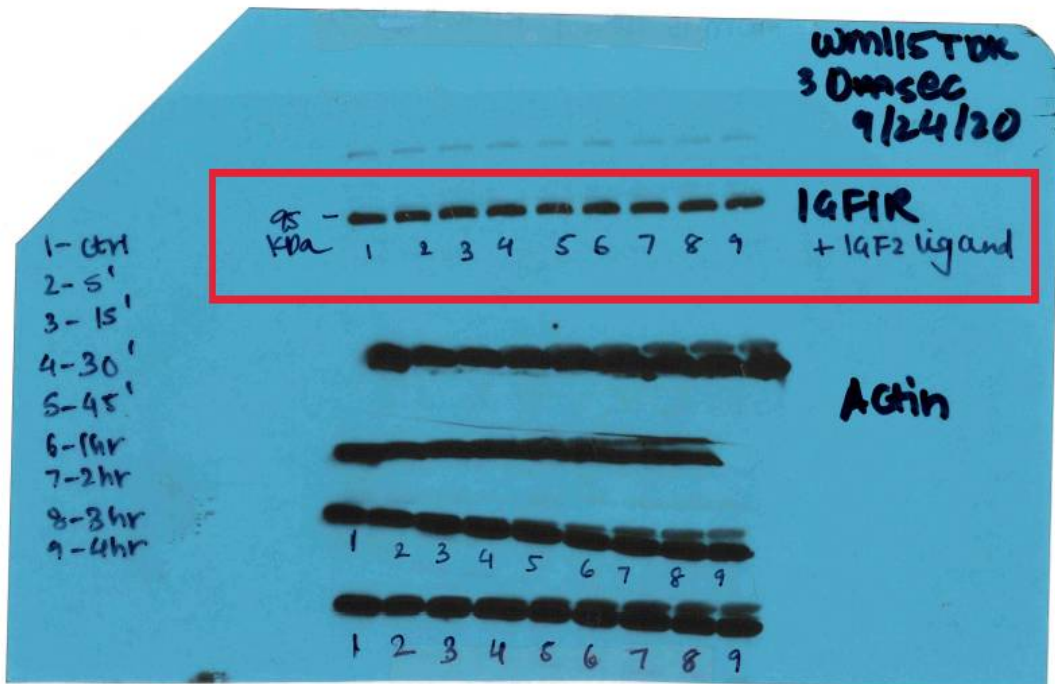


WM115 TDR: P-IGFIR (Y1131) / PIR (Y1146)

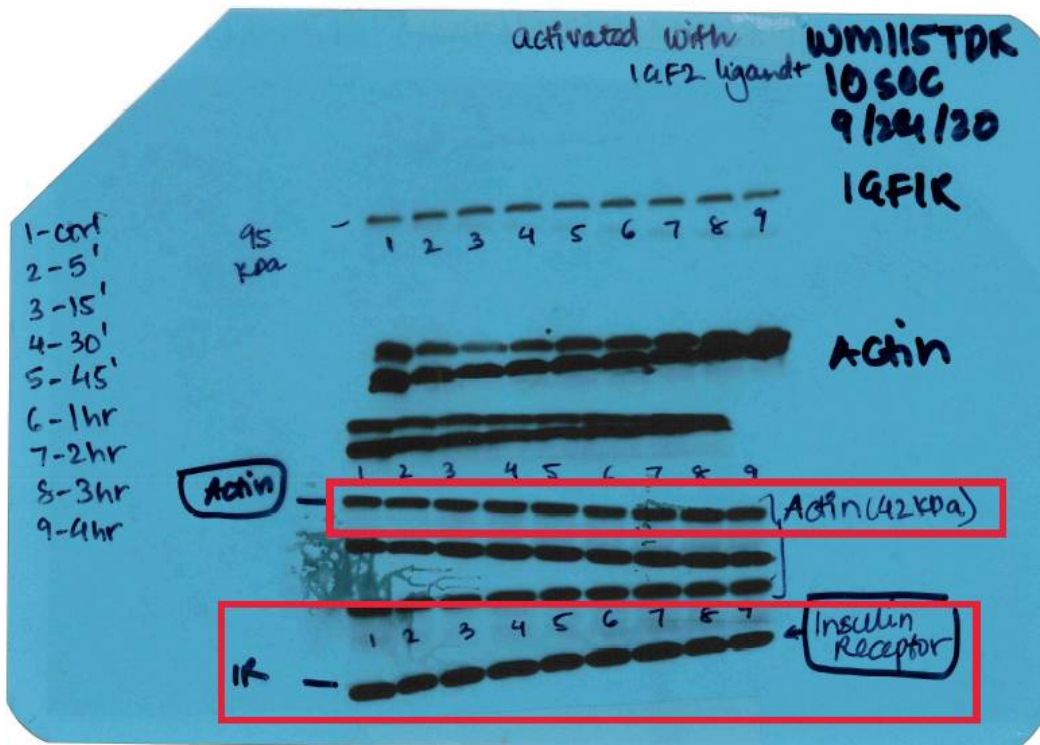




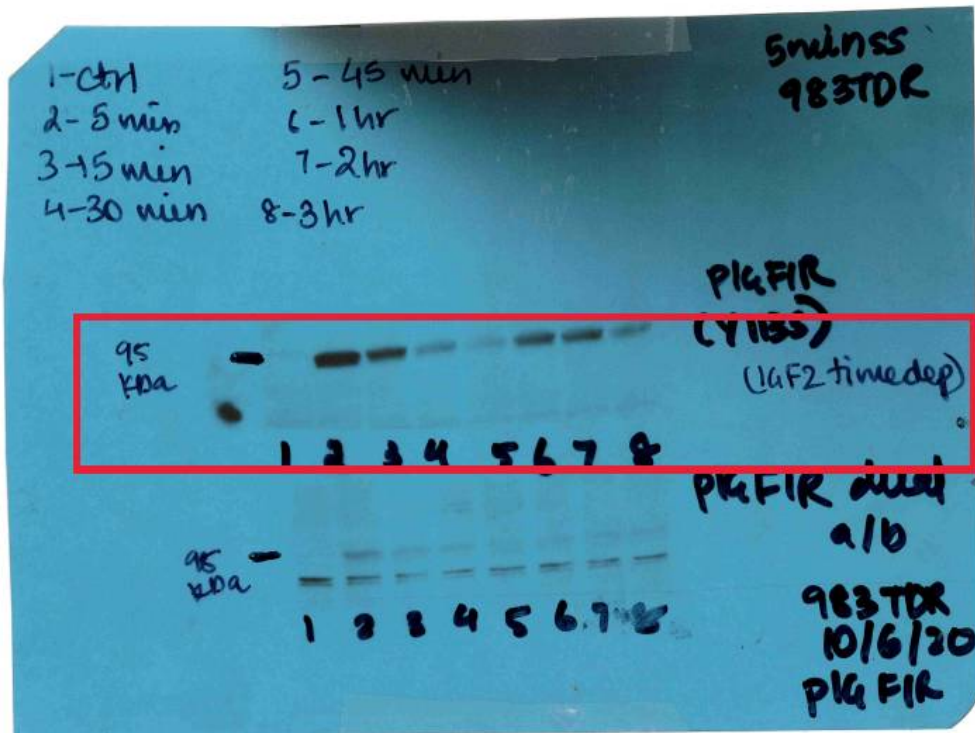
WM115 TDR: IGF1R



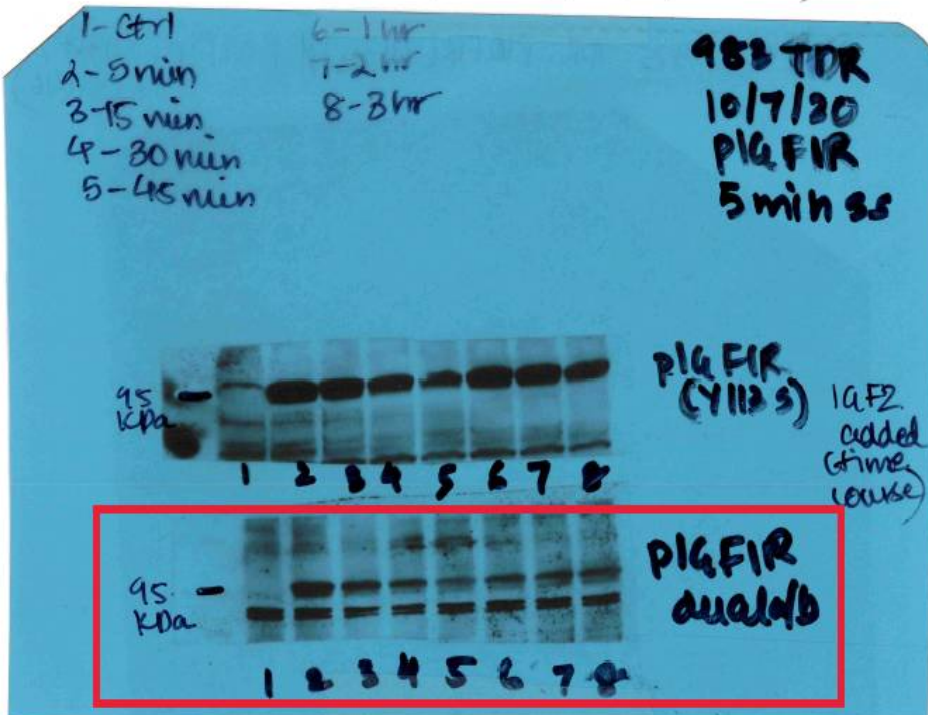
WM115 TDR: Actin & IR



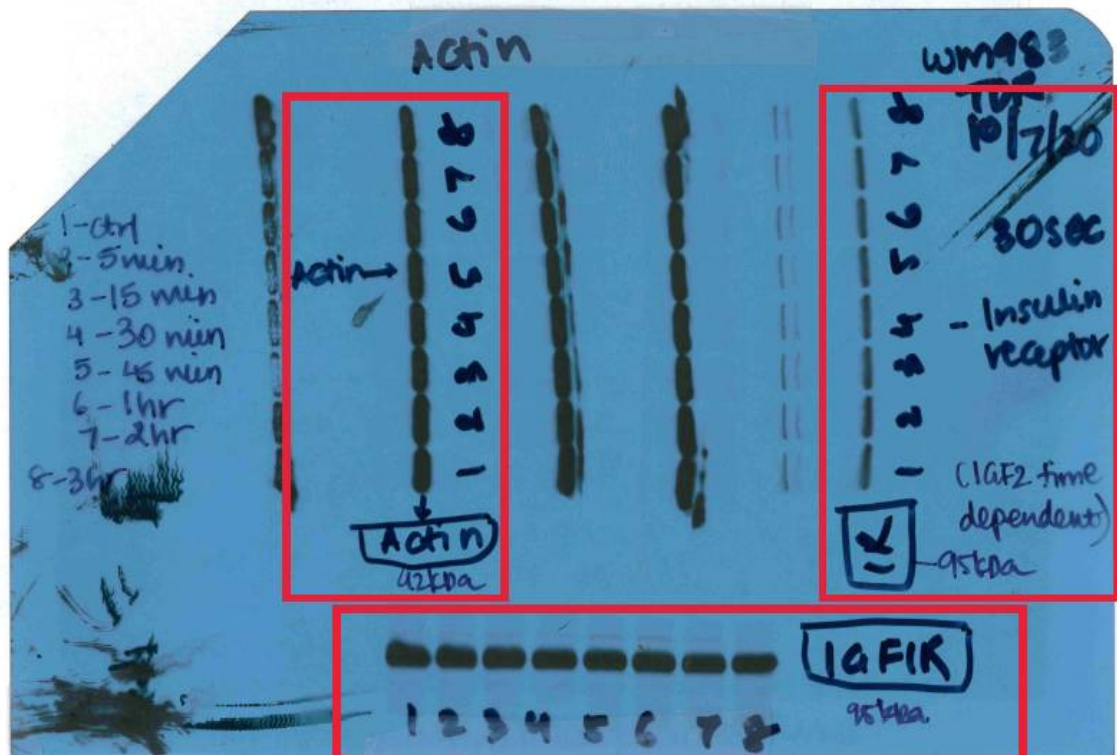
WM983B TDR: P-IGFIR (Y1135)



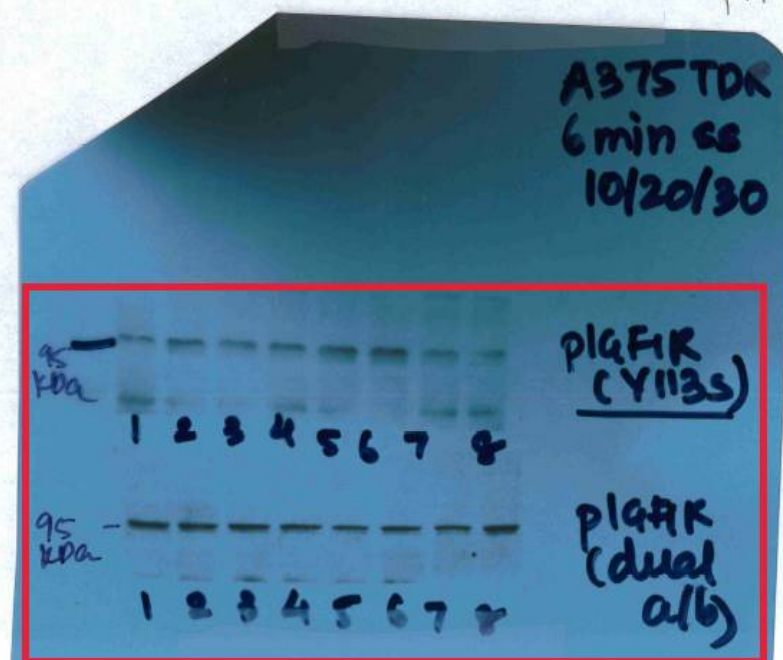
WM983B TDR: P-IGFIR (Y1135)/PIR (Y1146)



WM983B TDR: IR, IGFIR & Actin



A375 TDR: PI3K (Y1135) / P-IGFIR (Y1131) / PIR (Y1146)





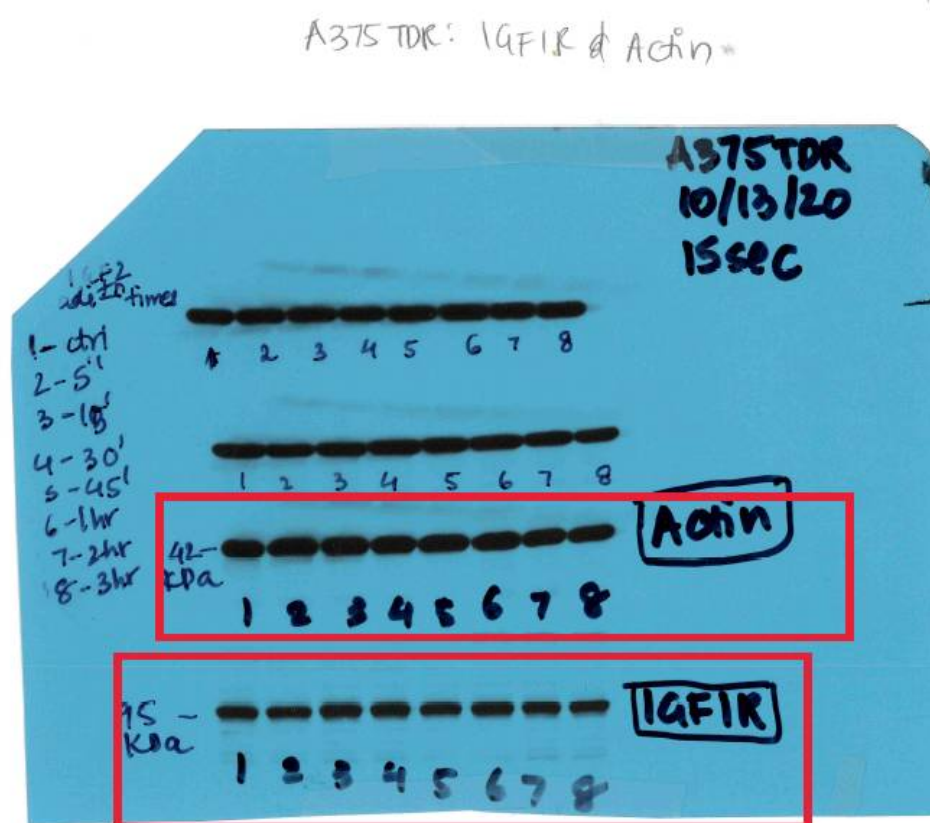
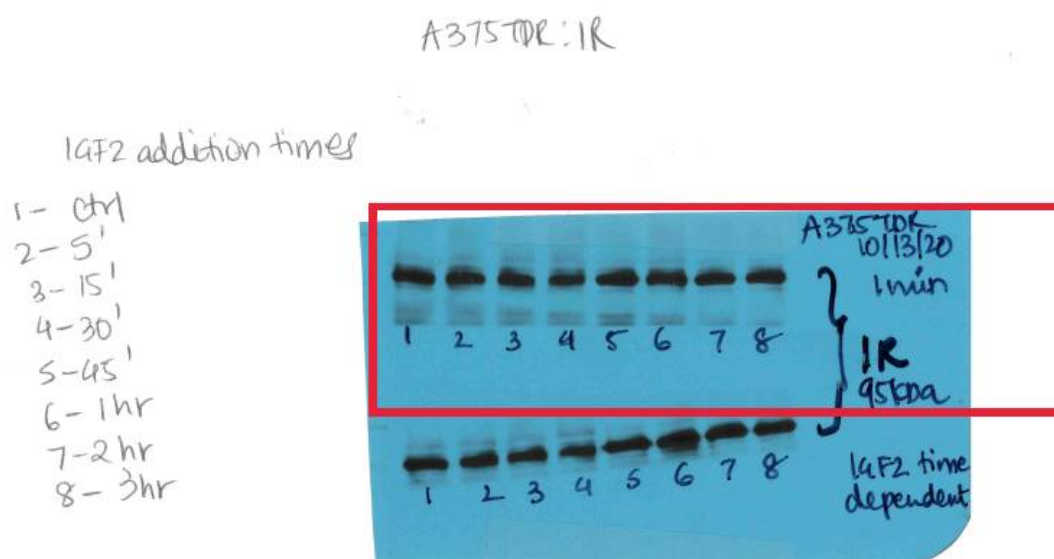


Figure S13. Whole blots for western blots in Figure S5.



**Table S1.** List of antibodies.

<b>Antibody</b>	<b>Source</b>	<b>Catalog number</b>
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Cell Signaling Technology	9101
p44/42 MAPK (Erk1/2)	Cell Signaling Technology	9102
$\beta$ -Actin	Cell Signaling Technology	4970
Phospho-Akt (Ser473)	Cell Signaling Technology	4060
Akt	Cell Signaling Technology	9272
Phospho-HER3/ErbB3 (Tyr1289)	Cell Signaling Technology	4791
Phospho-HER3/ErbB3 (Tyr1197)	Cell Signaling Technology	4561
HER3/ErbB3	Cell Signaling Technology	12708
Phospho-EGF Receptor (Tyr1068)	Cell Signaling Technology	2234
EGF Receptor	Cell Signaling Technology	4267
IGF-I Receptor $\beta$	Cell Signaling Technology	3027
Phospho-IGF-I Receptor $\beta$ (Tyr1135)	Cell Signaling Technology	3918
Insulin Receptor $\beta$	Cell Signaling Technology	3025
Phospho-IGF-I Receptor $\beta$ (Tyr1131)/Insulin Receptor $\beta$ (Tyr1146)	Cell Signaling Technology	3021
Phospho-Axl (Y779)	R&D Systems	AF2228
Axl	Cell Signaling Technology	8661

**Table S2.** List of RT-PCR primers.

<b>Gene</b>	<b>Forward Primer Sequence</b>	<b>Reverse Primer Sequence</b>
IGF1R	TCGACATCCGCAACGACTATC	CCAGGGCGTAGTTGTAGAAGAG
IGF1	TCGGTAACTGACTTGAATGTCCA	TCCTTTTCGCTTCCCTGTTTT
IGF2	GGGCAAGTTCTTCCAATATGA	TCACTTCCGATTGCTGGC
INSR	AAAACGAGGCCCGAAGATTTC	GAGCCCATAGACCCGGAAG
NRG1	GCTTCATGGTGAAAGACCTTTCA	ATTACGTAGTTTTGGCAGCGATC
HER3	GGTGATGGGGAACCTTGAGAT	CTGTCACTTCTCGAATCCACTG
AXL	GTGGGCAACCCAGGGAATATC	GTAAGTGTCCCGTGTCTCGGAAAG
GAS6	GGTAGCTGAGTTTGACTTCCG	GACAGCATCCCTGTTGACCTT
PROTEIN S	TCCTGGTTAGGAAGCGTCGT	CCGTTTCCGGGTCATTTTCAAA
ACTIN	AAGGAGCCCCACGAGAAAAAT	ACCGAACTTGCAATTGATTCCAG
EGFR	AGCTAACCATTATGGCAACA	AGTTTTCAGTGAGTCAGCTCCAT
HBEGF	GAAAGACTTCCATCTAGTCACAAAGA	GGGAGGCCCAATCCTAGA
TGFA	GGACAGCACTGCCAGAGA	CAGGTGATTACAGGCCAAGTAG
BETACELLULIN	TGCCCAAGCAATACAAGC	CGTCTGCTCGGCCACC
AMPHIREGULIN	ATATCACATTGGAGTCACTGCCCA	GGGTCCATTGTCTTATGATCCAC