

Met-HER3 crosstalk supports proliferation via MPZL3 in *MET*-amplified cancer cells

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Supplementary material

Supplementary Table 1: Antibodies

Antibody	Supplier	Catalogue number	Dilution
pMET Y1234/5 (D26) XP	Cell Signaling	3077	1:1000
MET	In-house	148	1:1000
pAKT S473 (D7F10) XP	Cell Signaling	9018	1:2000
AKT (40D4)	Cell Signaling	2920	1:1000
pERK1/2 T202/Y204	Cell Signaling	9101	1:2000
ERK1/2 (3A7)	Cell Signaling	9107	1:1000
pHER2 (Y1221/1222)	Cell Signaling	2243	1:1000
HER2	Cell Signaling	2165	1:1000
pHER3(Y1289)	Cell Signaling	4791	1:500
pHER3 (Y1222)	Cell Signaling	4784	1:1000
HER3 (D22C5) XP	Cell Signaling	12708	1:1000
pSTAT3(Y705)	Cell Signaling	9145	1:1000
STAT3	Cell Signaling	8768	1:1000
pEGFR(Y1173)	Santa Cruz	4407	1:1000
EGFR	Cell Signaling	4267	1:1000
pY100	Cell Signaling	9411	1:1000
MPZL3	ProteinTech	25513-1-AP	1:1000
Beta-Actin (AC-15)	Sigma	A5441	1:4000
M2-flag	Sigma	F3165	1:2000
V5	Abcam	ab27671	1:1000
Alpha-tubulin (B512)	Sigma	T5168	1:2000
anti-rabbit-HRP	Cell Signaling	7074	1:10000
anti-mouse IRDye 680 nm	Mandel Scientific	LIC-926-68070	1:10000
anti-rabbit IRDye 800 nm	Mandel Scientific	LIC-926-32211	1:10000

Supplementary Table 2: siRNA duplex sequences

Pooled siRNA duplex sequences (sense)
GCAGUGGGAUUCGAGAAGUG
AGAUUGUGCUCACGGGACA
GUGGAUUCGAGAAGUGACA
GCGAUGCUGAGAACCAAUA

Supplementary table 3: shRNA clone sequences

shRNA	Clone	Expressed sequence
pLKO (vector)	SHC001	
shCtl	SHC002	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTC TTCATCTTGTTGTTTTTTG
shHER3 A	TRCN0000194972	CCGGCTTCGTCATGTTGAACTATAACTCGAGTTATAGTTCA ACATGACGAAGTTTTTTG
shHER3 B	TRCN0000199364	CCGGCCTGTGCATGTGCTCTTATTGCTCGAGCAATAAGAGC ACATGCACAGGTTTTTTG
shMPZL3 A	TRCN0000137252	CCGGGCAGCCACACAGTATCAATATCTCGAGATATTGATAC TGTGTGGCTGCTTTTTTTG
shMPZL3 B	TRCN0000167937	CCGGCCAGGGTGTTTATATCGTCTTCTCGAGAAGACGATAT AAACACCCTGGTTTTTTG
shMPZL3 C	TRCN0000167873	CCGGGAGATCATCAGTAAAGACTTTCTCGAGAAAGTCTTTA CTGATGATCTCTTTTTTTG

Supplementary table 4: MPZL3 clone sequence

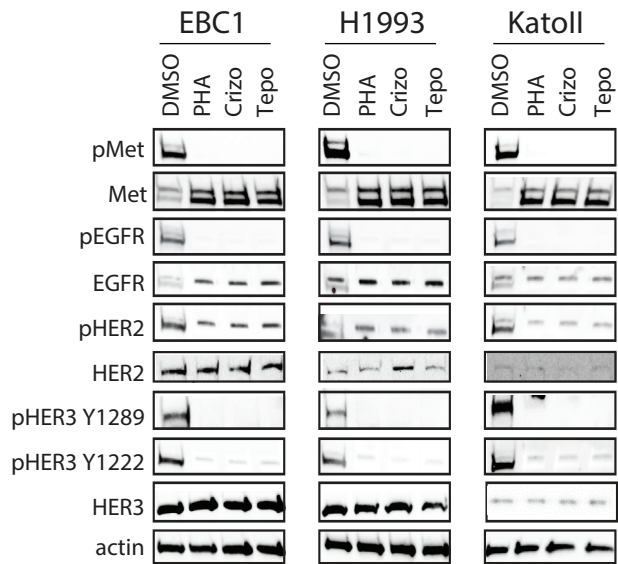
shRNA	Clone	Expressed sequence
pLKO (vector)	SHC001	
shCtl	SHC002	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTC TTCATCTTGTTGTTTTTTG
shHER3 A	TRCN0000194972	CCGGCTTCGTCATGTTGAACTATAACTCGAGTTATAGTTCA ACATGACGAAGTTTTTTG
shHER3 B	TRCN0000199364	CCGGCCTGTGCATGTGCTCTTATTGCTCGAGCAATAAGAGC ACATGCACAGGTTTTTTG
shMPZL3 A	TRCN0000137252	CCGGGCAGCCACACAGTATCAATATCTCGAGATATTGATAC TGTGTGGCTGCTTTTTTTG
shMPZL3 B	TRCN0000167937	CCGGCCAGGGTGTTTATATCGTCTTCTCGAGAAGACGATAT AAACACCCTGGTTTTTTG
shMPZL3 C	TRCN0000167873	CCGGGAGATCATCAGTAAAGACTTTCTCGAGAAAGTCTTTA CTGATGATCTCTTTTTTTG

Supplementary table 5: Sequences for RT-qPCR primers

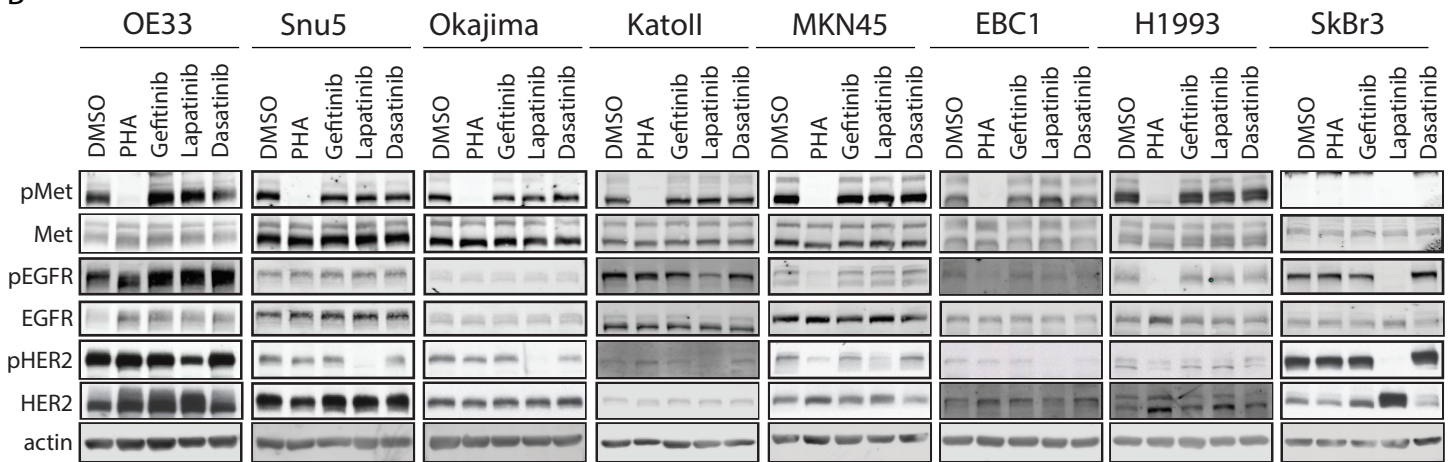
Gene	Primers	
<i>ERBB3</i>	forward	GGGGAGTCTTGCCAGGAG
	reverse	CATTGGGTGTAGAGAGACTGGAC
<i>MPZL3</i>	forward	ATTCCCATGACAGAGCTAACAG
	reverse	GCACAAAGACAAGGATGGAAAG
<i>NYNRIN</i>	forward	CTGAGCTGAGACCAGGATTAAG
	reverse	GTTGGAGAGAATGTGGGTGTAG
<i>LGR6</i>	forward	CTCTTCCCTTTCCTCTC
	reverse	CTGAGTTTTGGTTGTATTTG
<i>BHLHE41</i>	forward	AGAGGAAACGAACAGCAGTTGA
	reverse	TAGGTATCCTTGGTGTCGTCTCG
<i>RAMP1</i>	forward	TGGAGCCTTGGGACAGA
	reverse	GGCTTCCAGGTTAATACCAGAG
<i>SPNS2</i>	forward	CCATCTTCATCTGCCTGATCTT
	reverse	CAGTGATGGCCAGTTAGAAA
<i>PPM1K</i>	forward	TTTCCAAACCAACAGGCAAAG
	reverse	CCTCCCAAAGTGCTAGGATTAC
<i>RPLP0</i>	forward	CTCAACATCTCCCCCTTCTC
	reverse	GACTCGTTTGTACCCGTTGA
<i>GAPDH</i>	forward	GCACCAGGTGGTCTCCTCT
	reverse	TGACAAAGTGGTCGTTGAGG
<i>HPRT</i>	forward	TGATAGATCCATTCCCTATGACTGTAGA
	reverse	CAAGACATTCTTCCAGTTAAAGTTG

Supplementary Figure 1

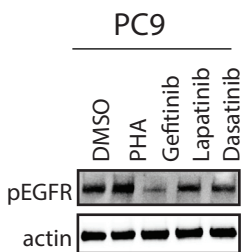
A



B

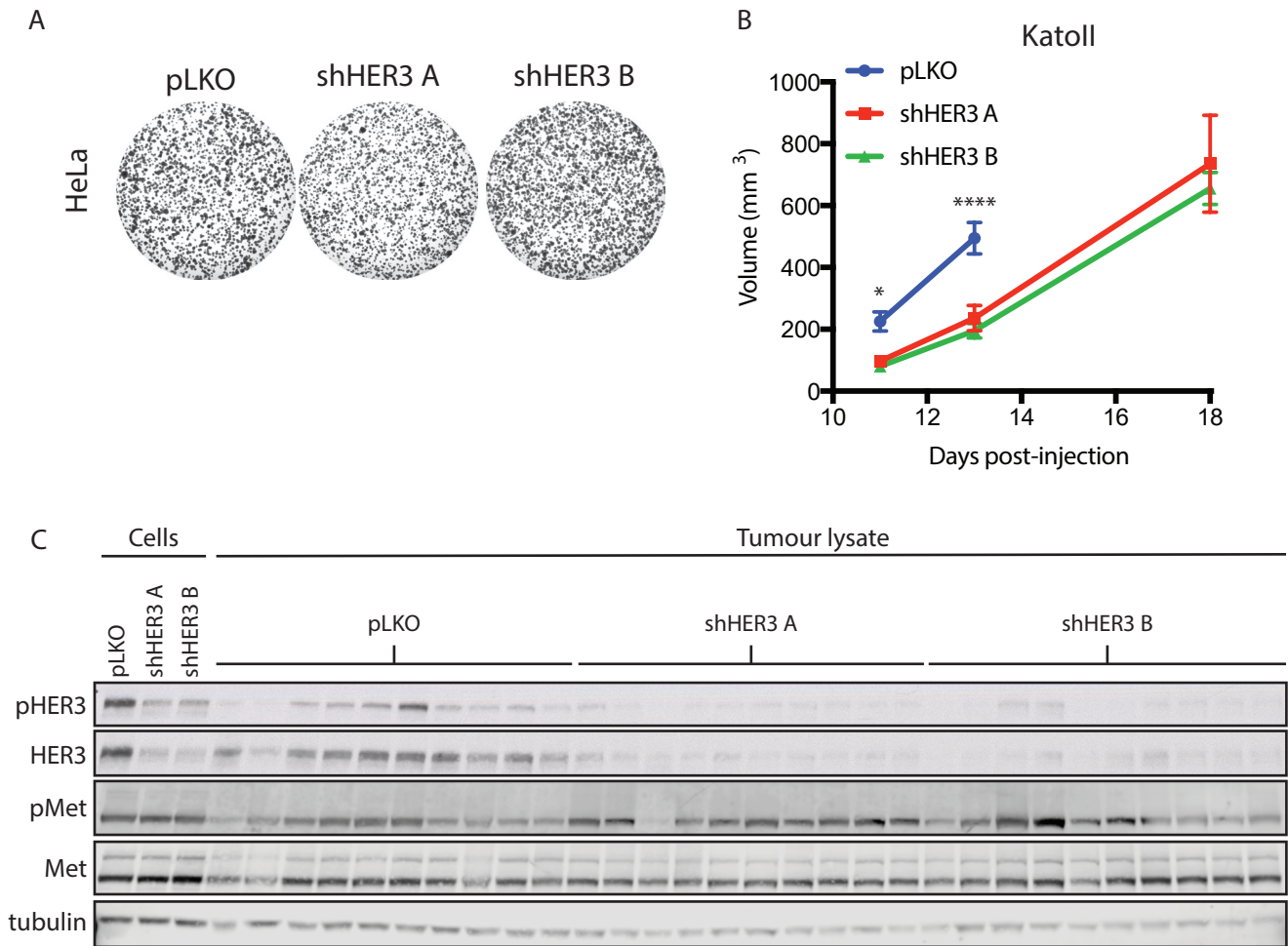


C



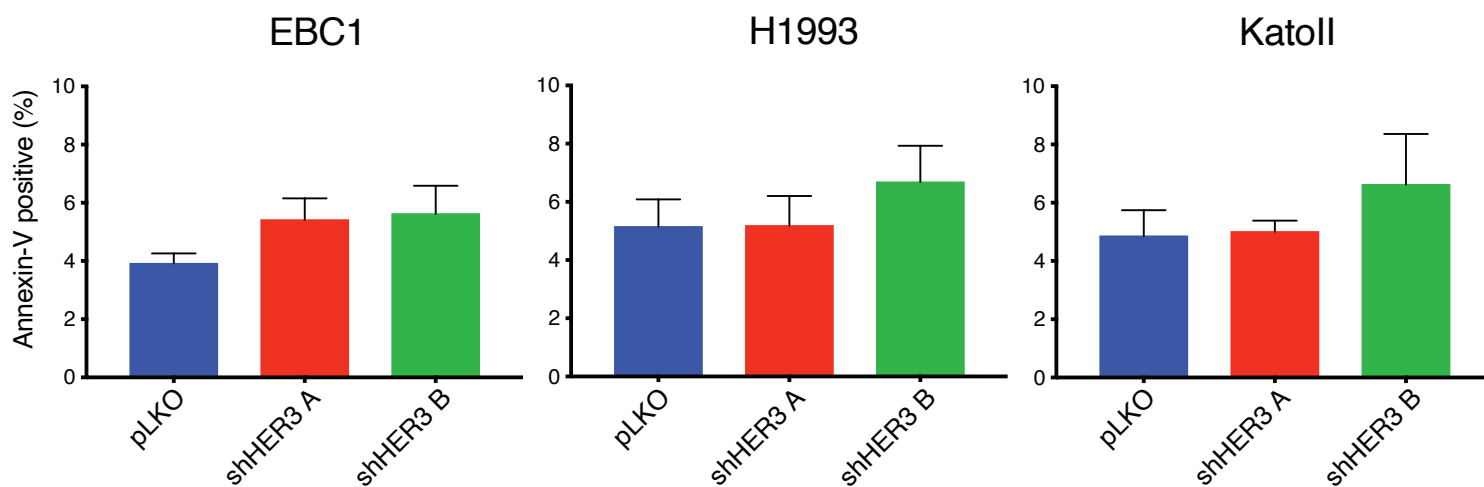
Supplementary Fig. 1 Controls for multiple kinase inhibitors used in MET-amplified cells. Controls for multiple kinase inhibitors used in MET-amplified cells. (A) Western blot analysis of the phosphorylation of Met (Tyr1234/35), EGFR (Tyr1173), HER2 (Tyr1221/22), HER3 (Tyr1289), and HER3 (Tyr1222) in the indicated cell lines treated with the Met inhibitors: PHA (0.5 μ M); Crizotinib (Crizo) (0.1 μ M); and Tepotinib (Tepo) (0.1 μ M), for 1 hour (n=2). (B) Western blot analysis of the phosphorylation of Met (Tyr1234/35), EGFR (Tyr1173) and HER2 (Tyr1221/22) in the indicated cell lines treated with PHA (0.5 μ M); the EGFR inhibitor, gefitinib (1 μ M); the HER2/EGFR inhibitor lapatinib (1 μ M); or the Src and Abl family kinase inhibitor, dasatinib (0.1 μ M), for 1 hour (n=3). (C) Western blot analysis of the phosphorylation of EGFR (Tyr1173) in PC9 cells treated with PHA (0.5 μ M); the EGFR inhibitor, gefitinib (1 μ M); the HER2/EGFR inhibitor lapatinib (1 μ M); or the Src and Abl family kinase inhibitor, dasatinib (0.1 μ M), for 1 hour (n=2).

Supplementary Figure 2



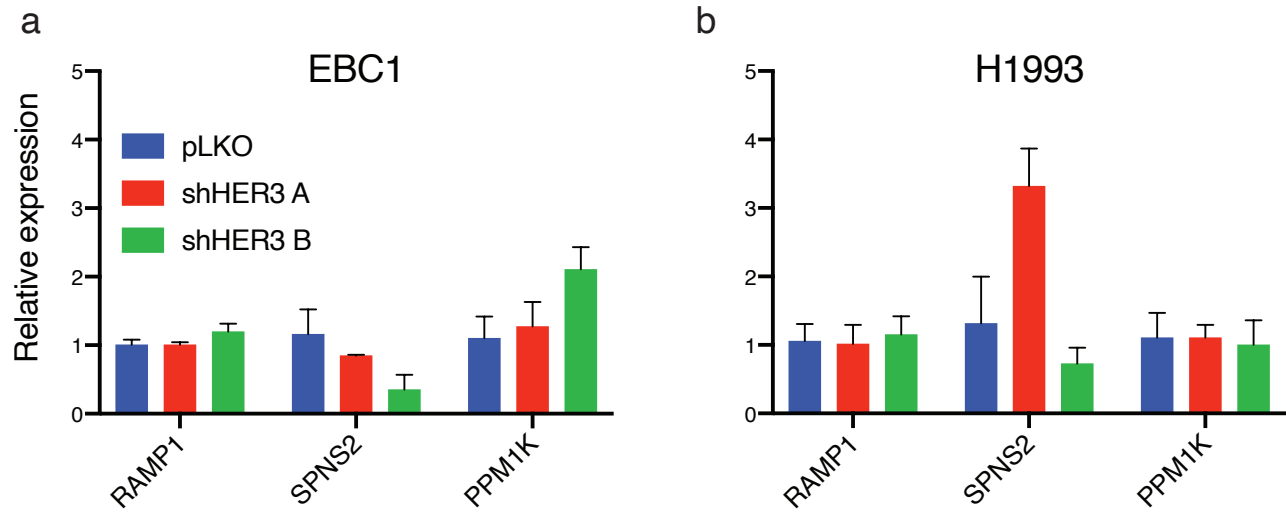
Supplementary fig. 2 Growth of shRNA-treated cells in vitro and in vivo. (a) HeLa cells transduced with shRNA targeting HER3 form colonies at the same rate as controls (pLKO (n=3)). (b) Tumour volumes of Katoll-derived xenografts shown in figure 2e at both points measured before collection (n=10). (c) Western blot analysis of total and phosphorylated Met and HER3 in Katoll tumours at end-point. *, $P < 0.05$, ****, $P < 0.0001$.

Supplementary Figure 3



Supplementary Fig. 3 Apoptosis in shRNA-treated cells. Analysis of annexin-V positivity by flow cytometry in EBC1, H1993 and Katoll cells transduced with shRNA targeting HER3 or control (pLKO).

Supplementary Figure 4



Supplementary Fig. 4 Gene expression analysis of upregulated transcripts from Katoll RNA sequencing in EBC1 and H1993 cells. Measurement of the relative amounts of RAMP1, SPNS2 and PPM1K genes in the indicated cell lines by RT-qPCR

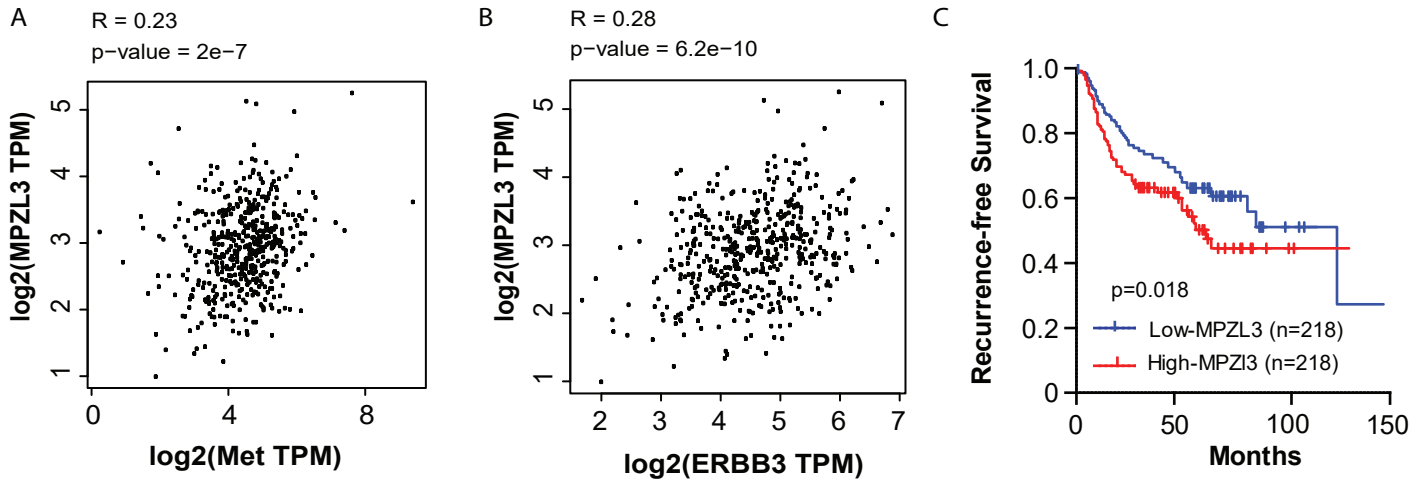


Fig. S5 MPZL3 levels correlate with Met and ERBB3 expression and are associated with poor clinical outcome in Lung squamous cell carcinomas. (a-b) Correlation between the indicated genes in LUSC tumors, estimated using Pearson's correlation (n=436). (c) Recurrence-free survival in LUSC (n=436). Data from Gepia2. Student's t-test; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Figure 1A

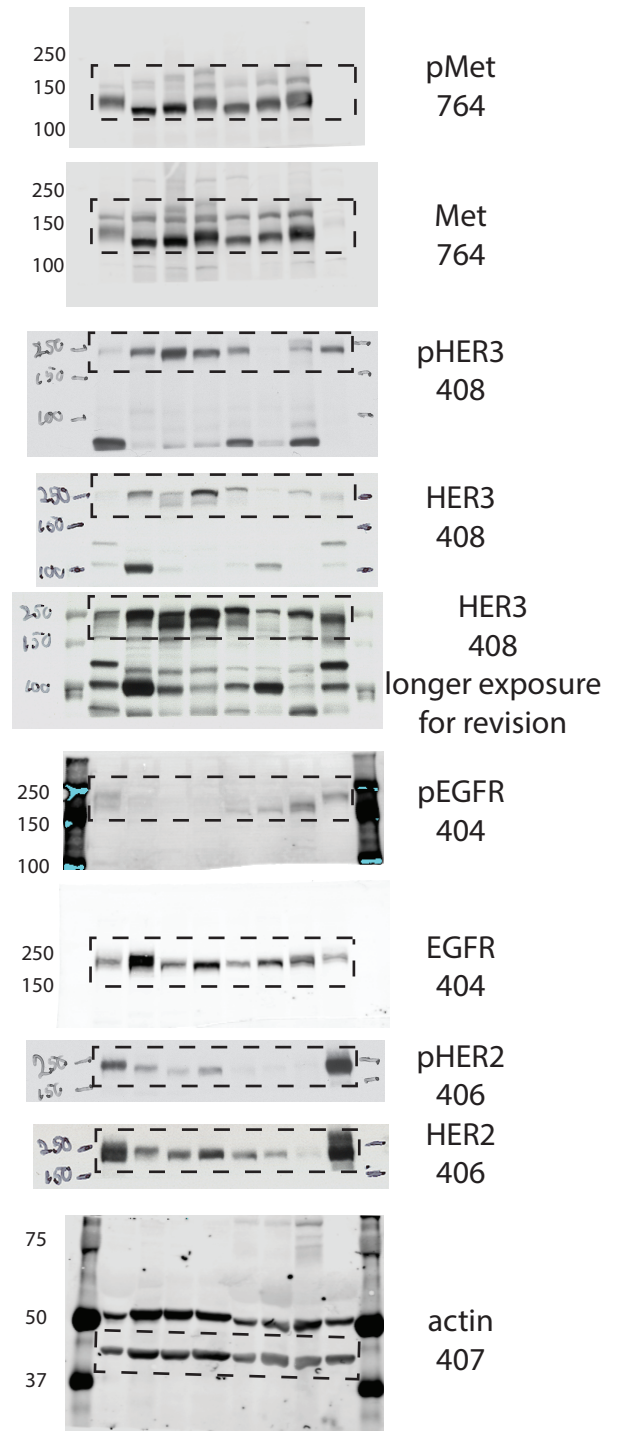
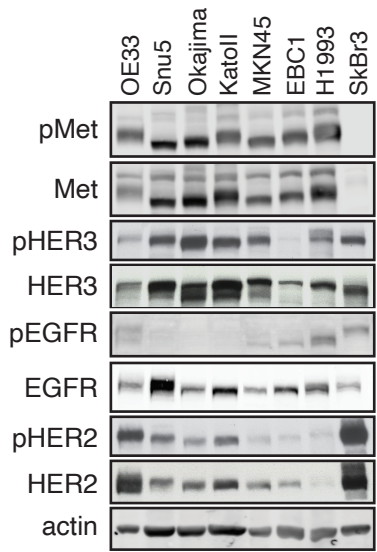


Figure 1B

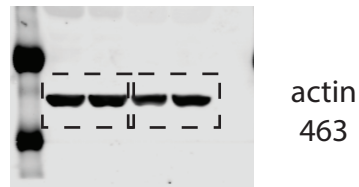
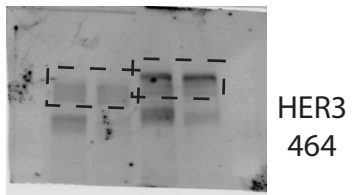
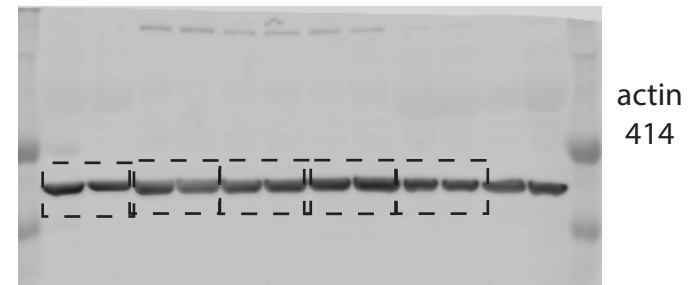
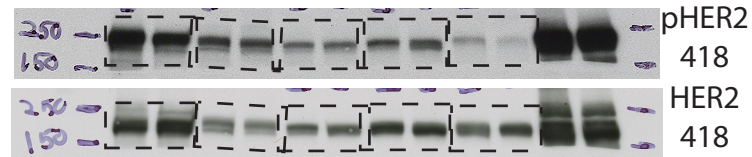
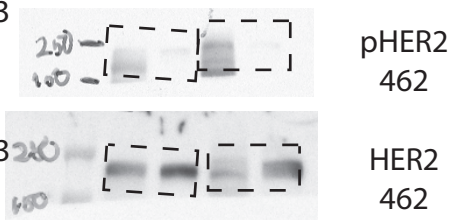
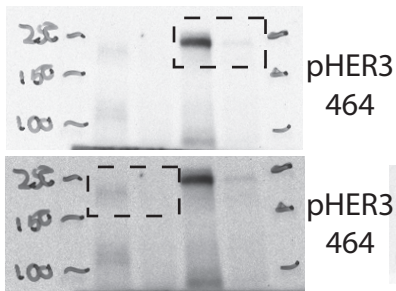
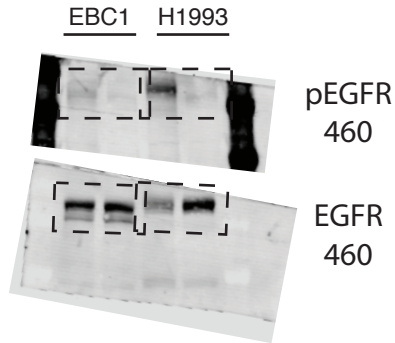
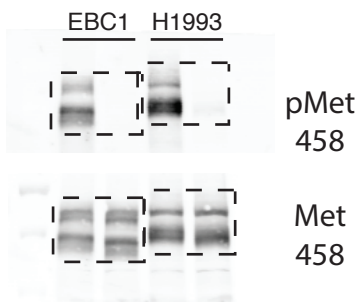
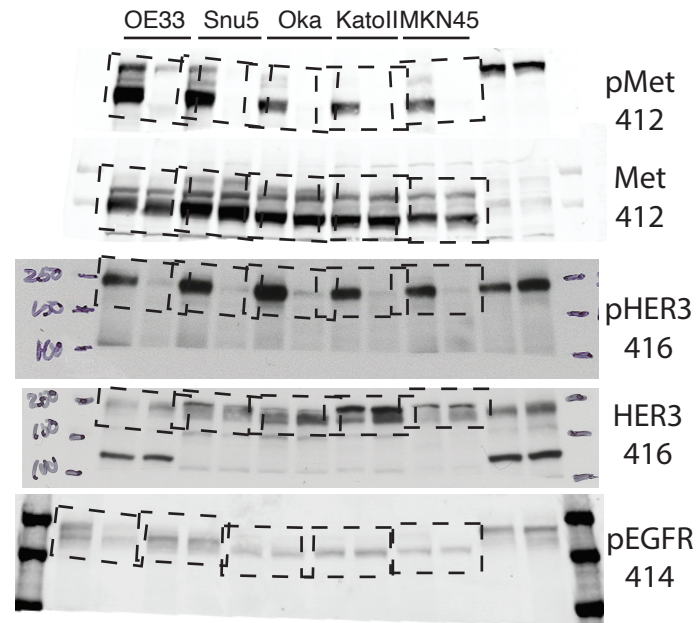
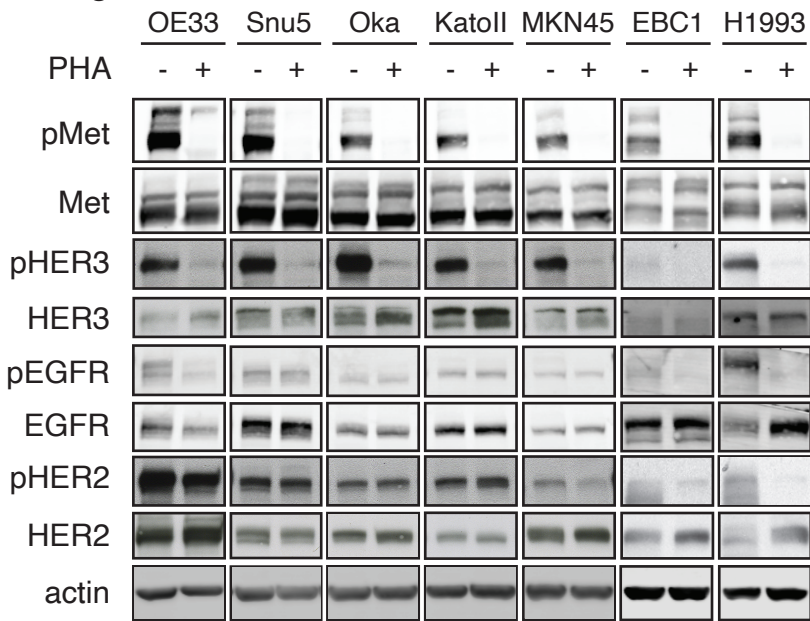


Figure 1C

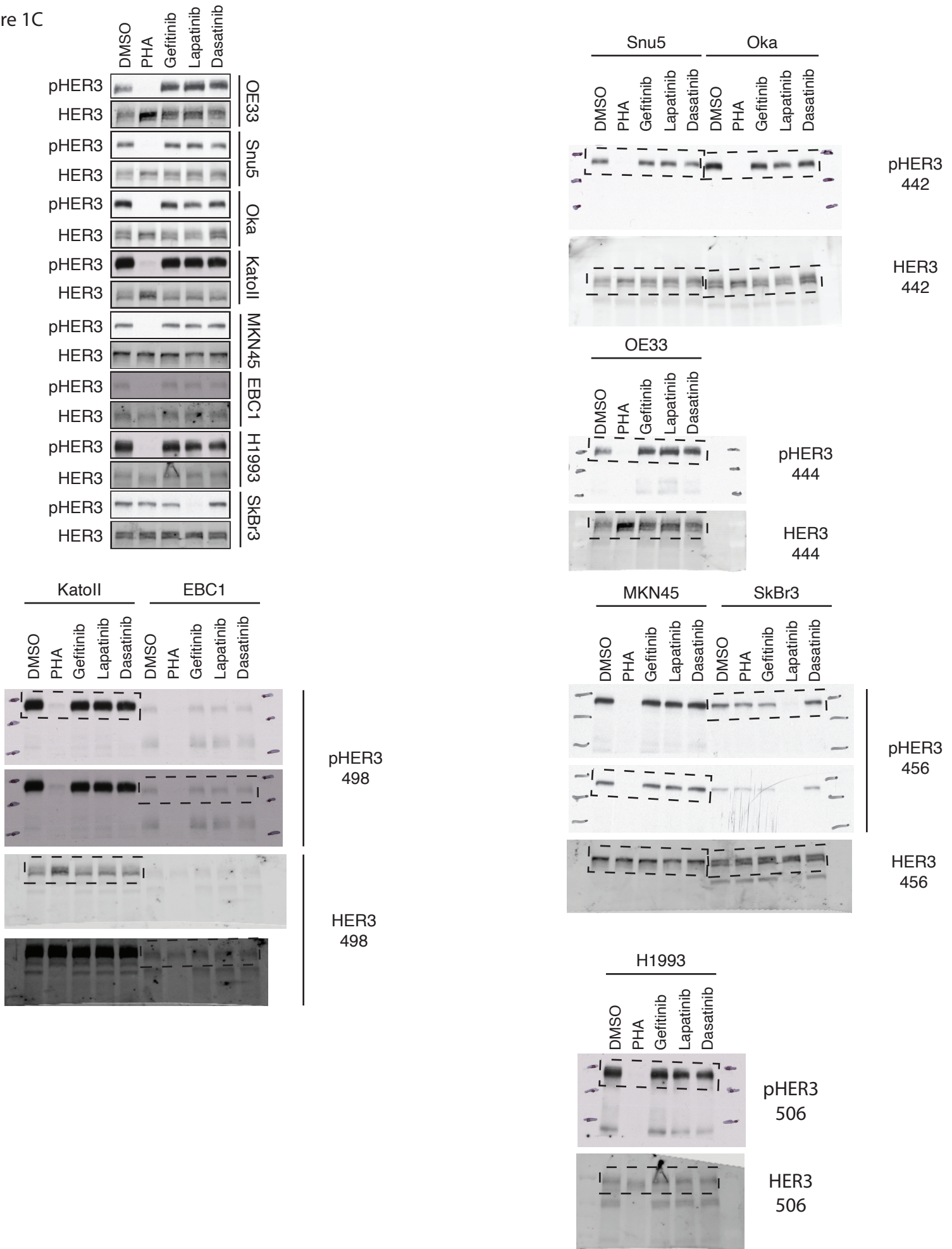
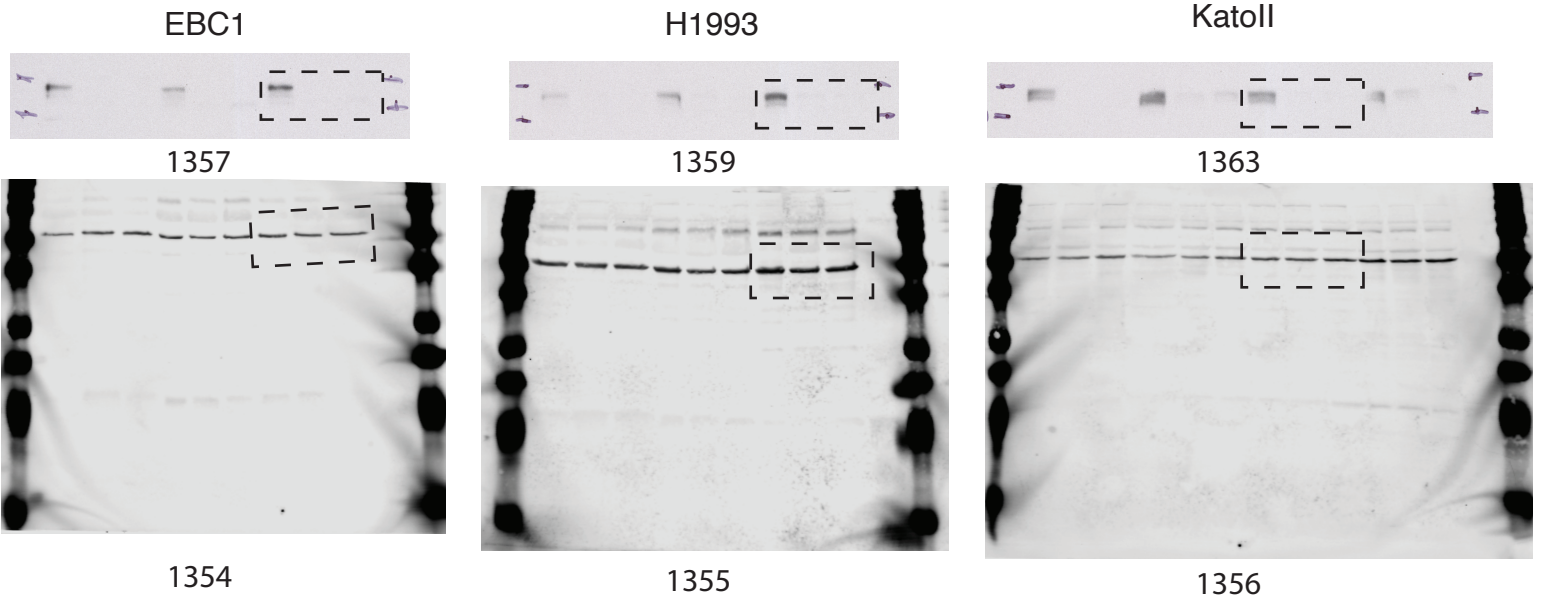
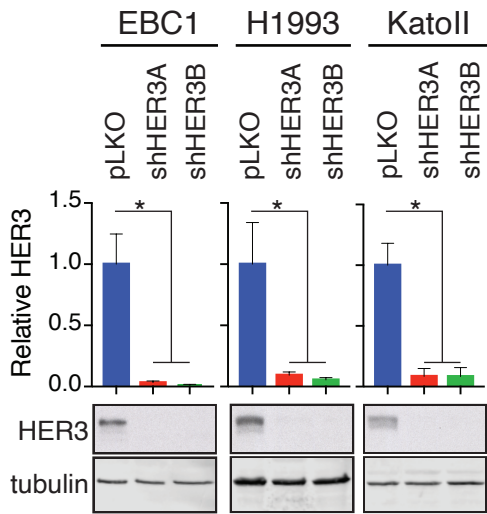
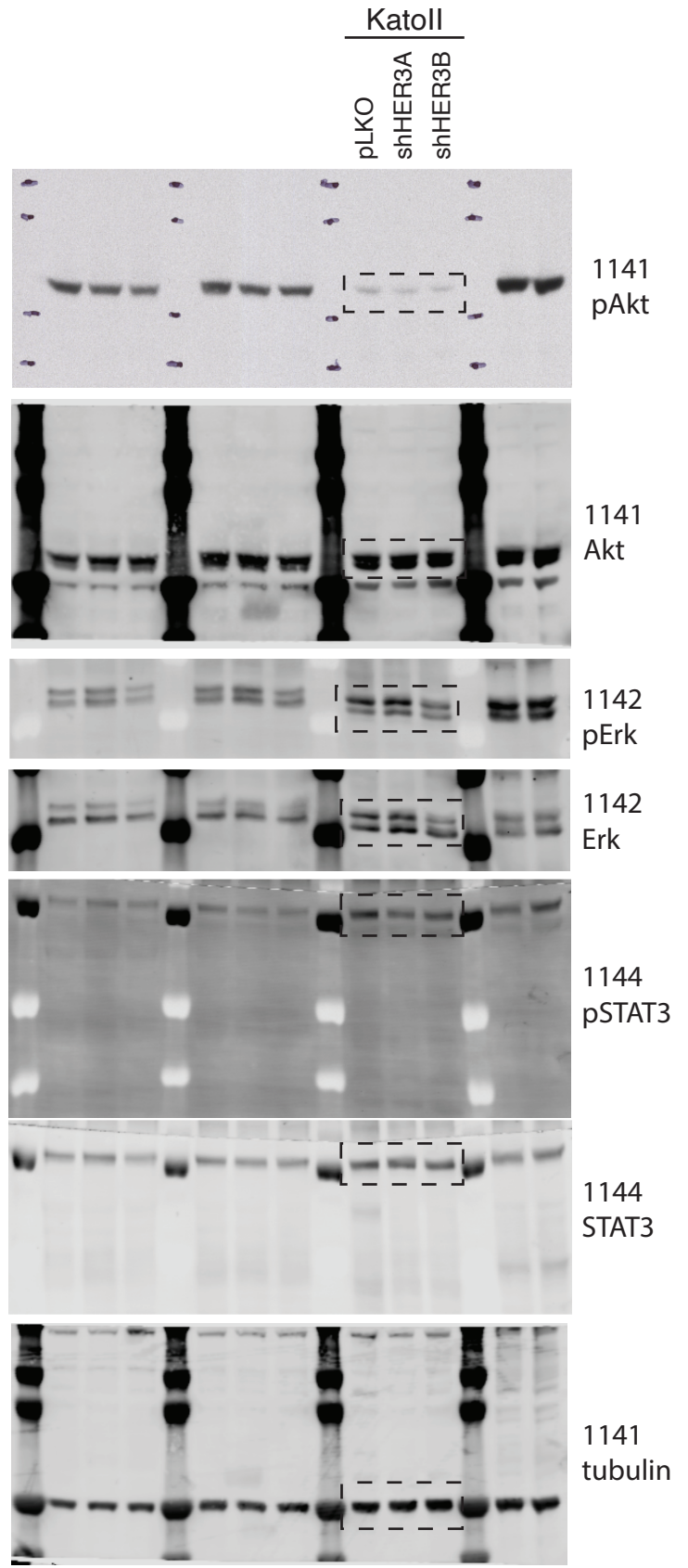
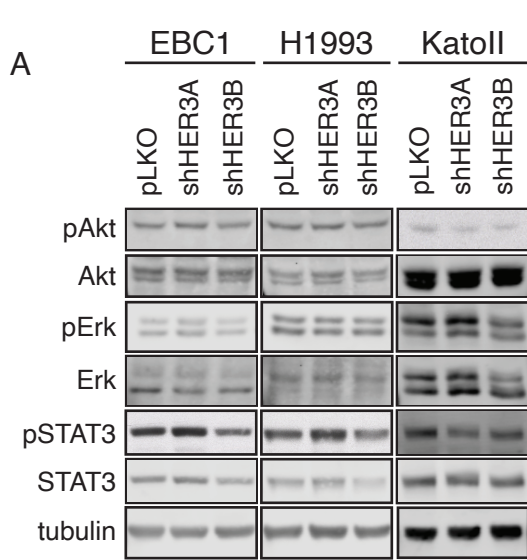


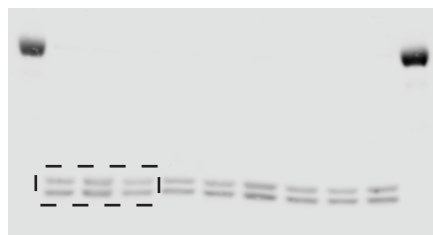
Figure 2A



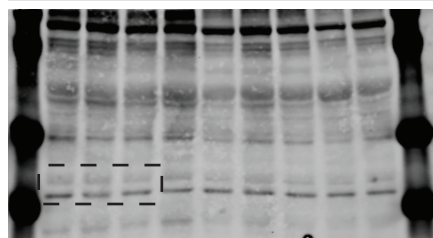


EBC1

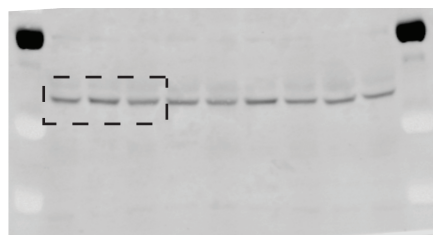
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shHER3B



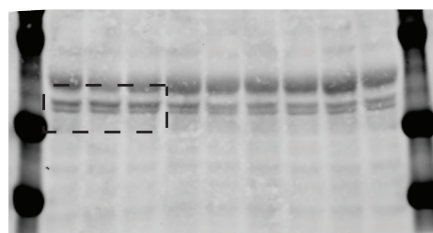
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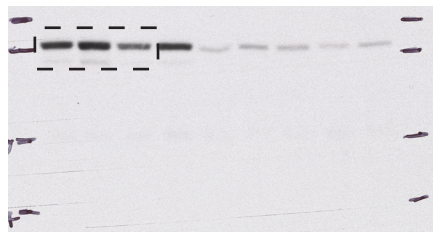
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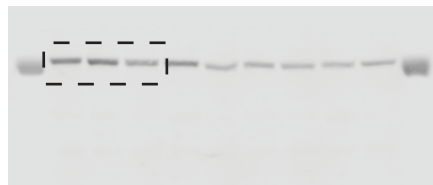
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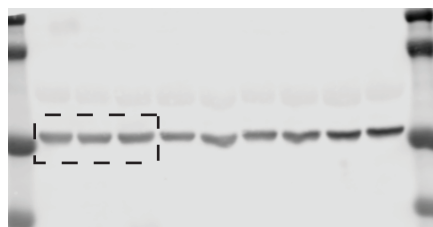
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879
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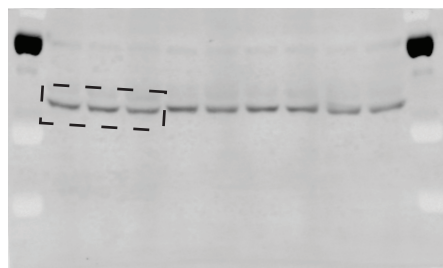
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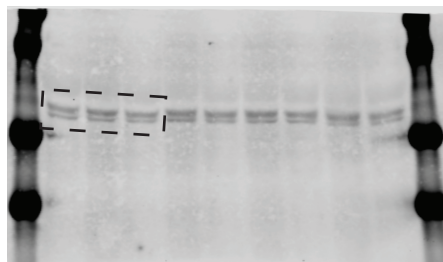
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H1993

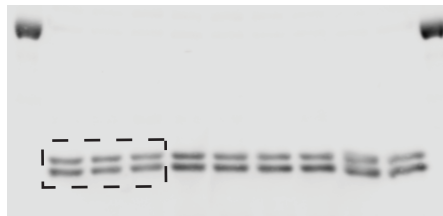
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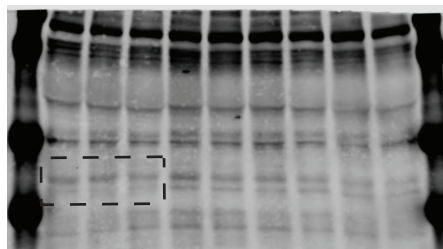
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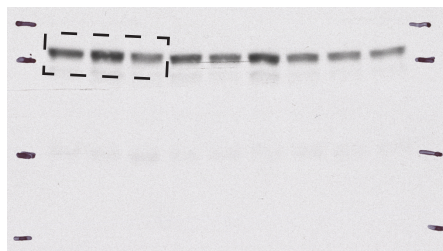
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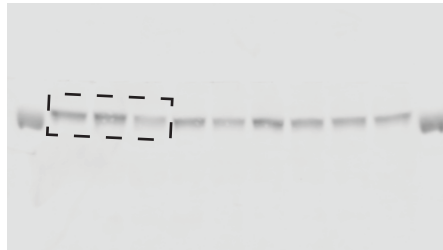
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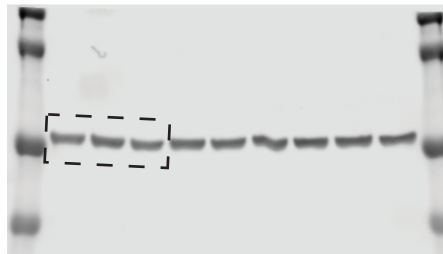
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887
STAT3



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Figure 4A

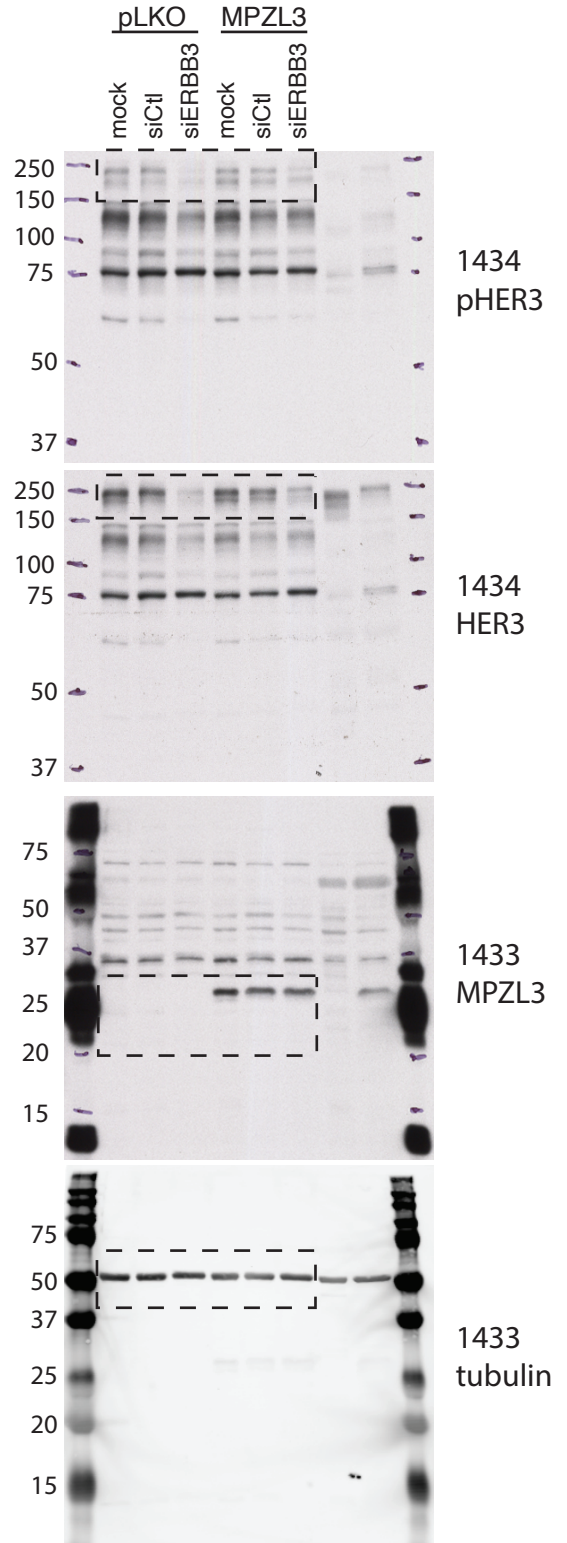
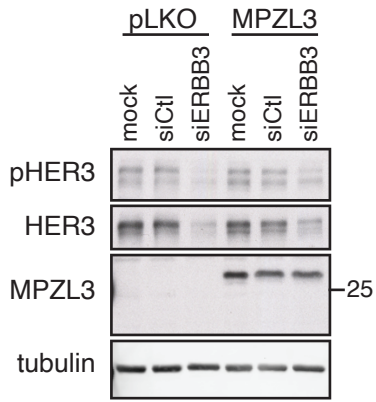


Figure 4E

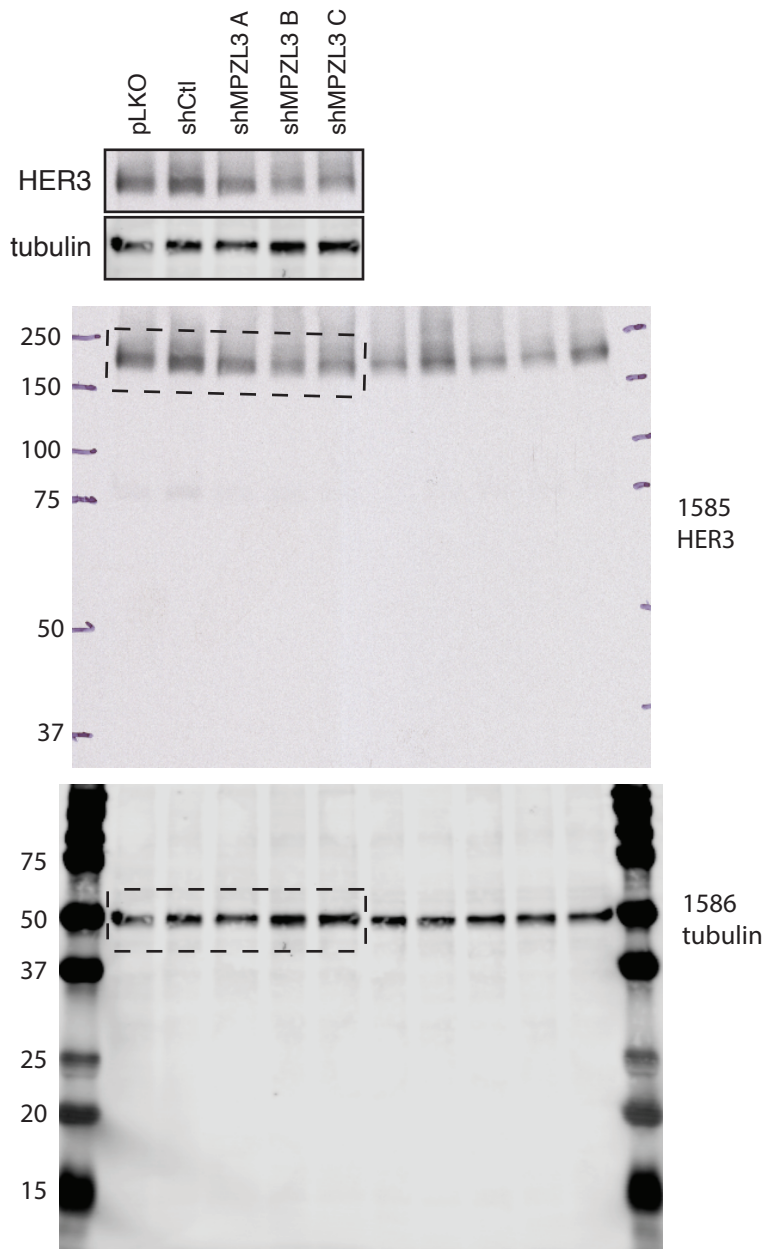


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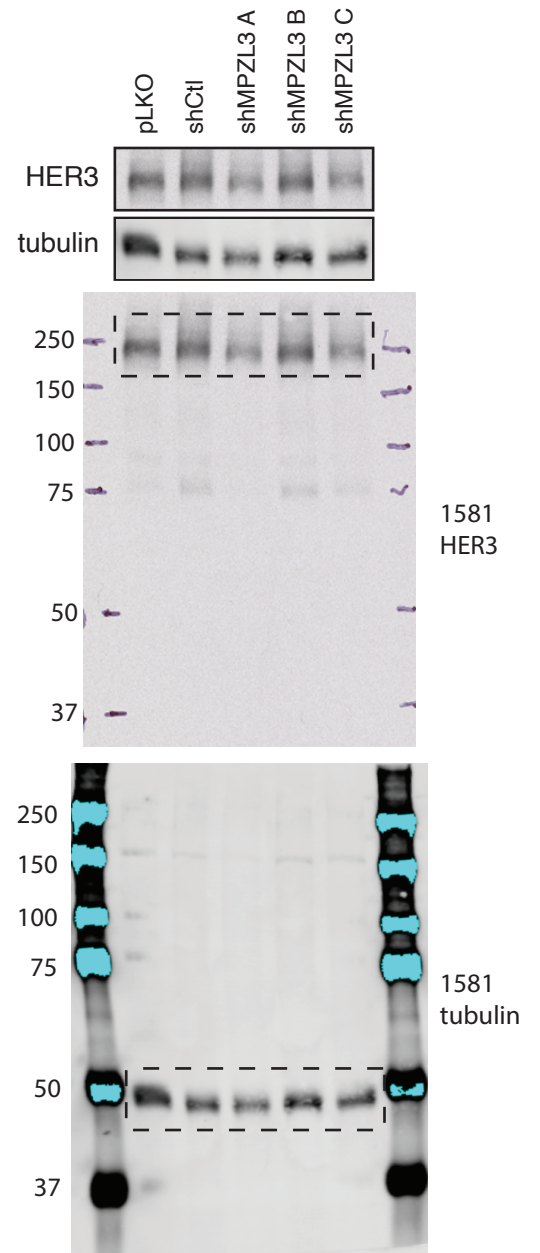


Figure 5A

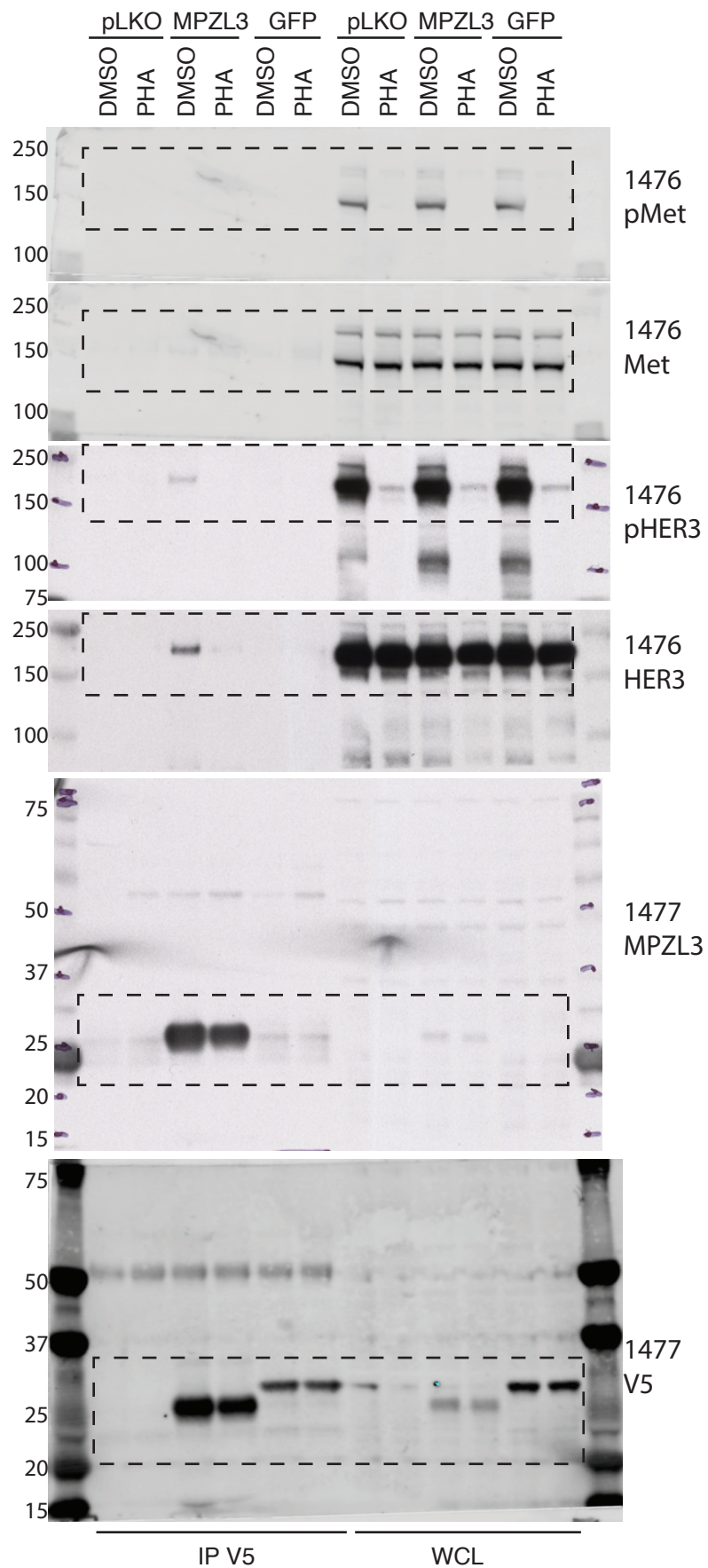
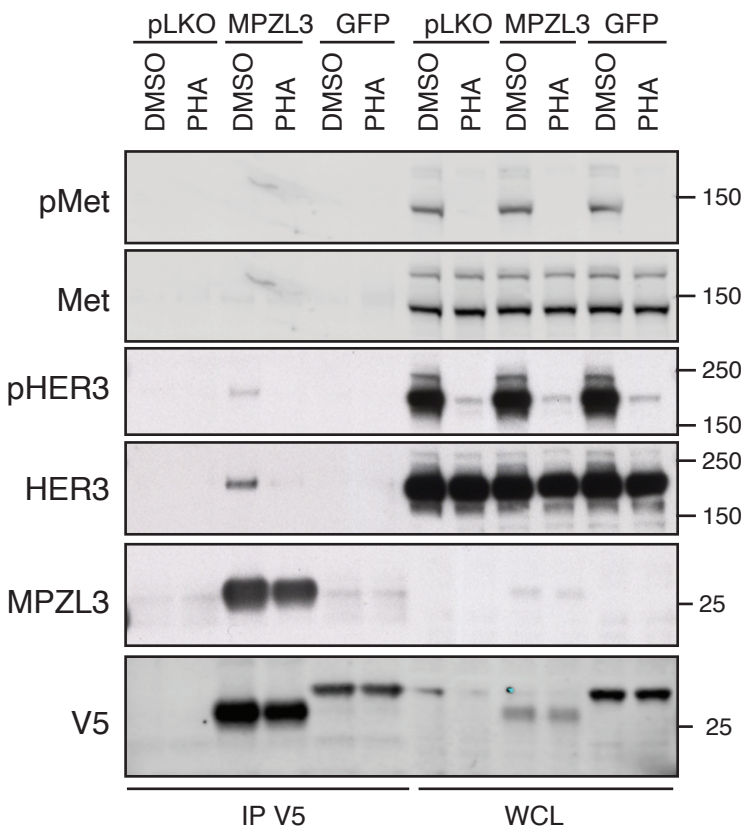


Figure 5B

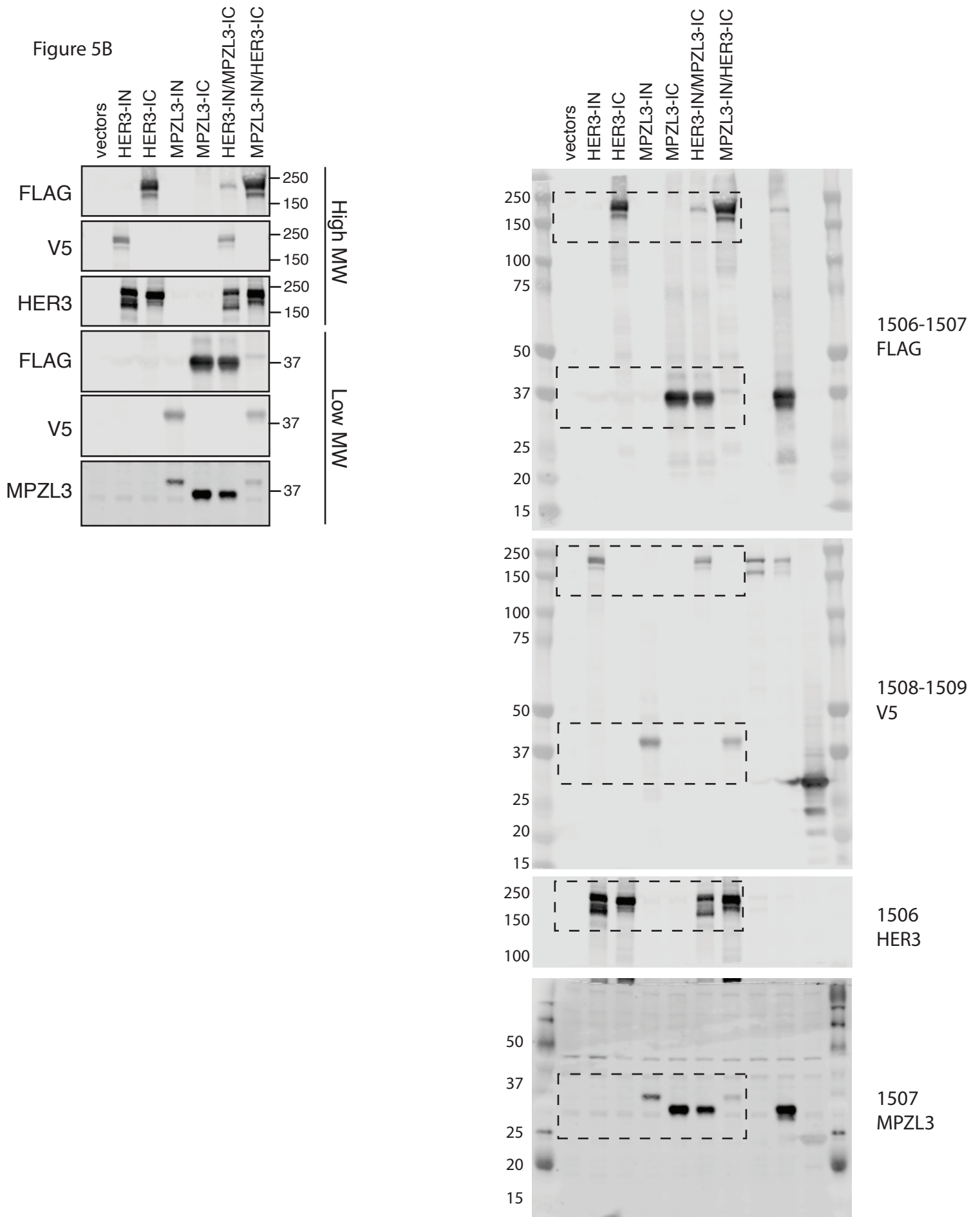
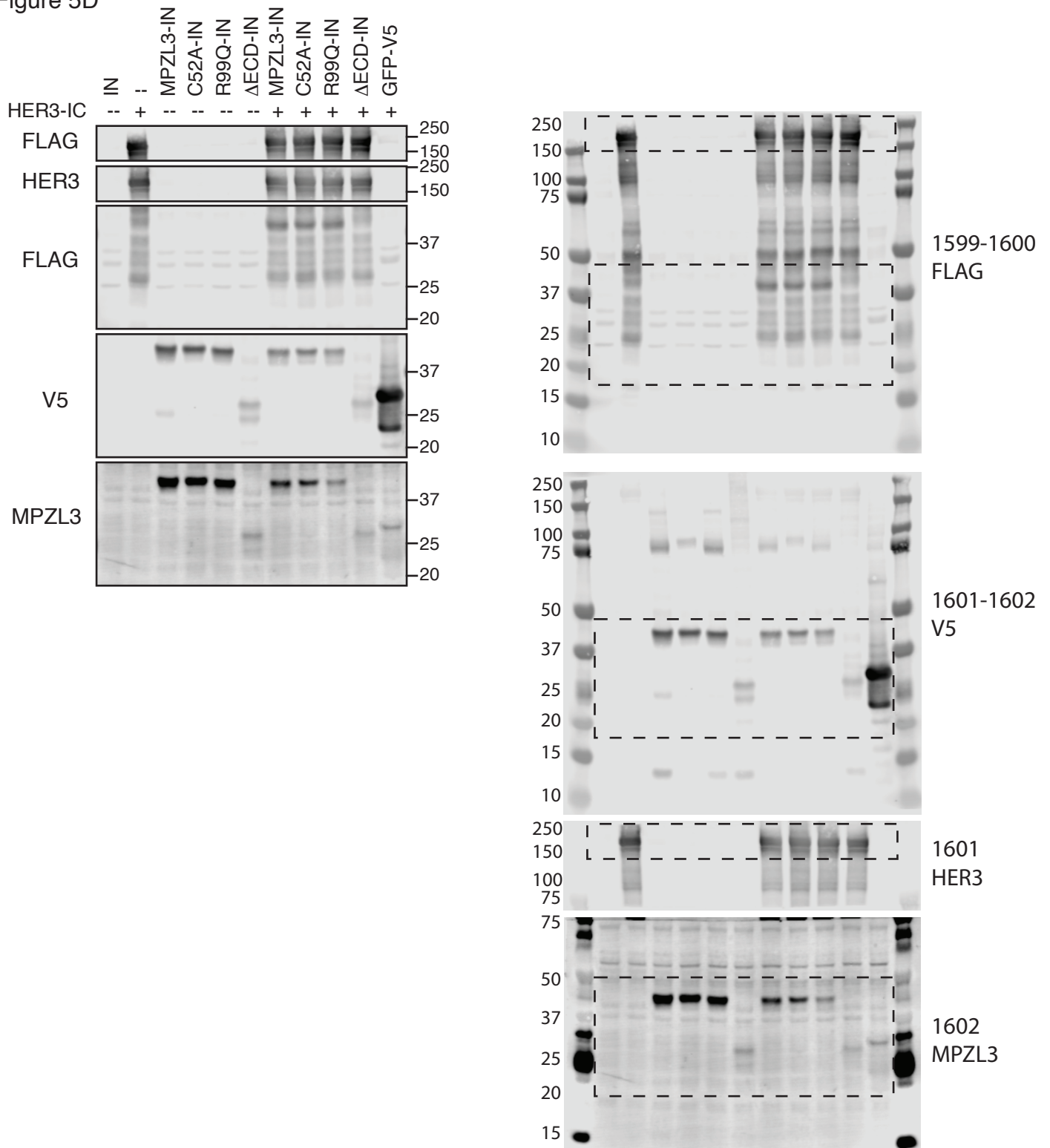


Figure 5D



FigureS1A

A

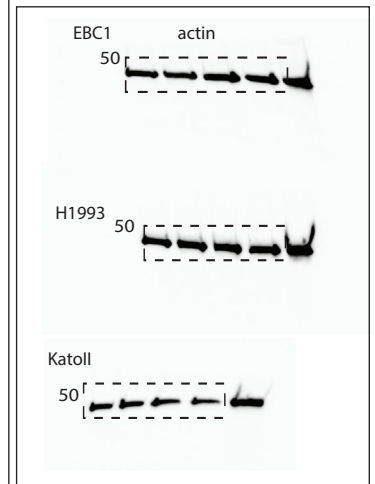
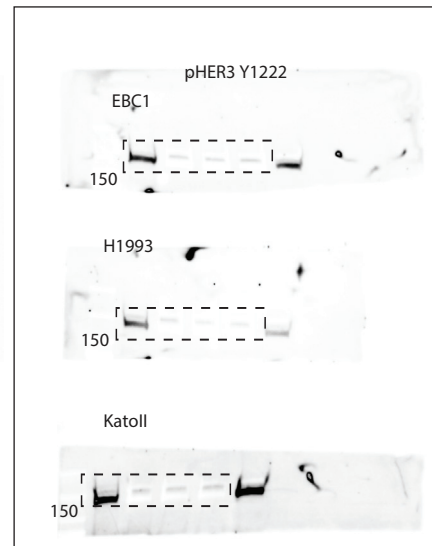
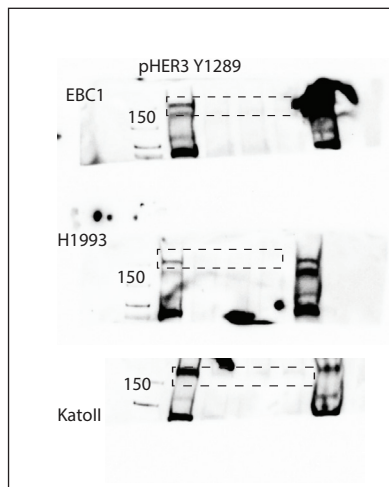
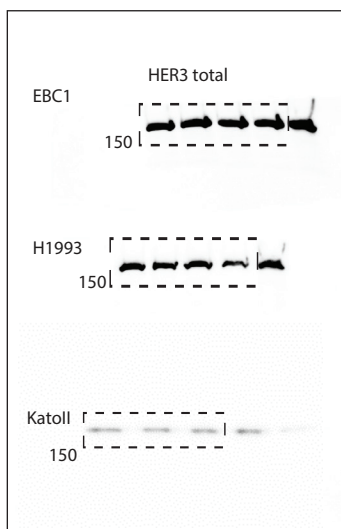
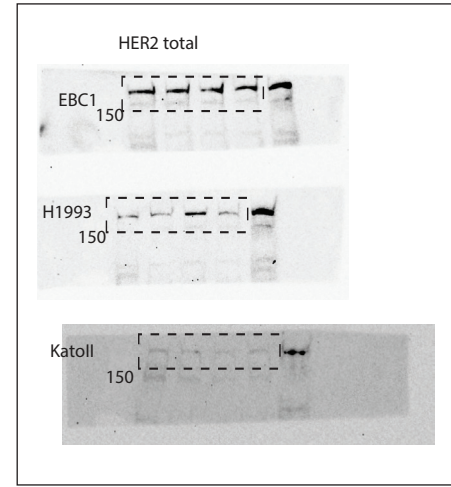
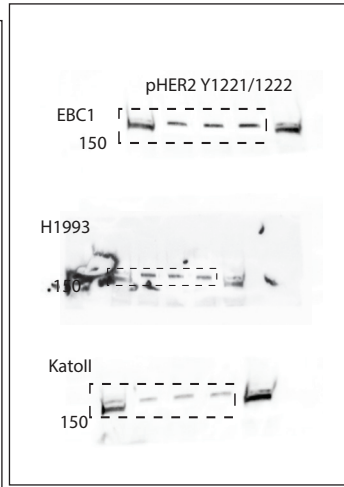
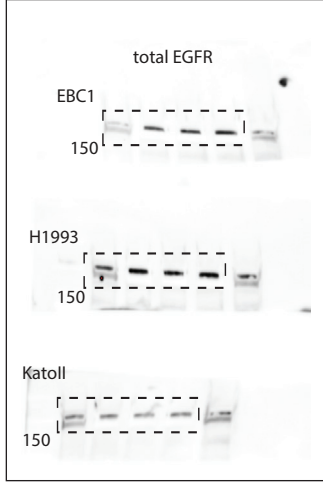
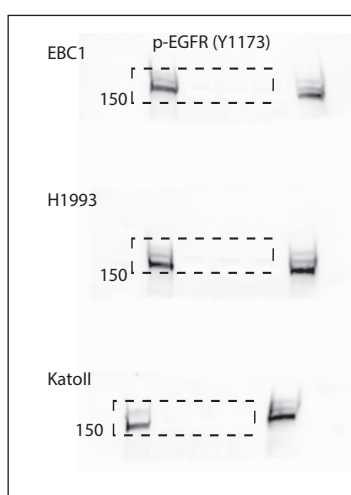
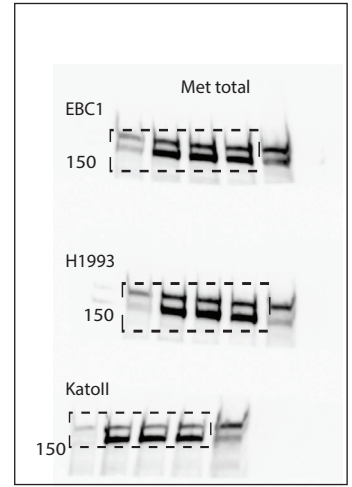
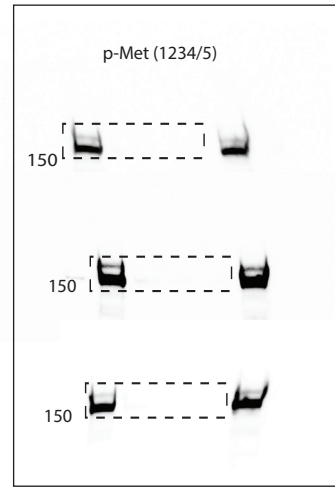
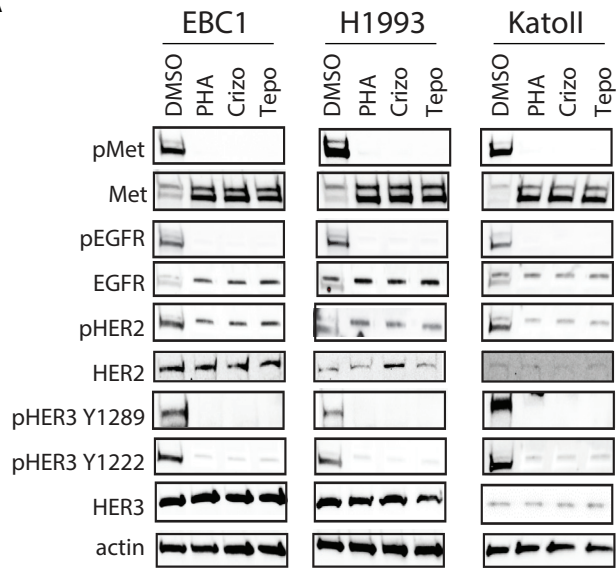


Figure S1b

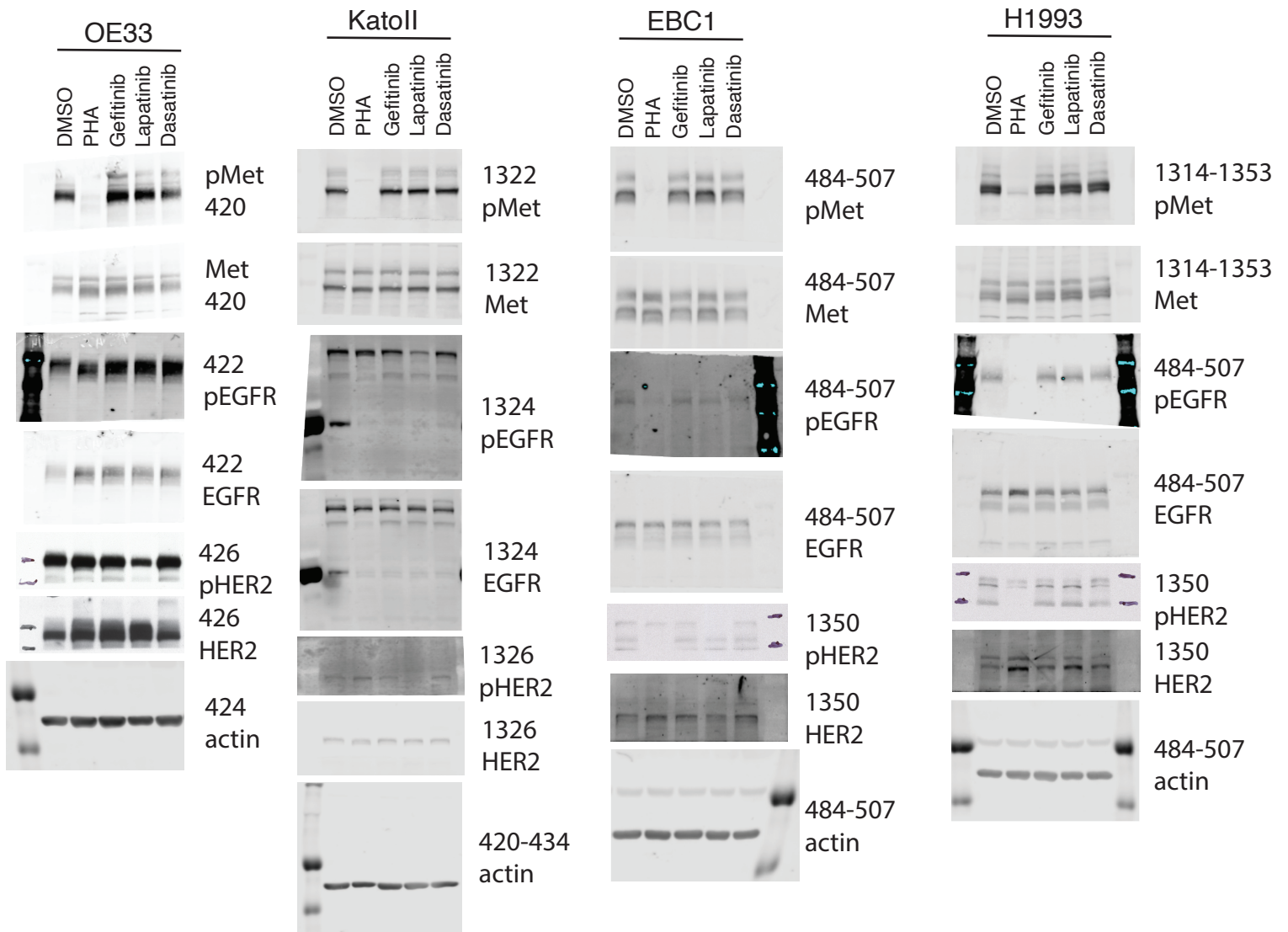
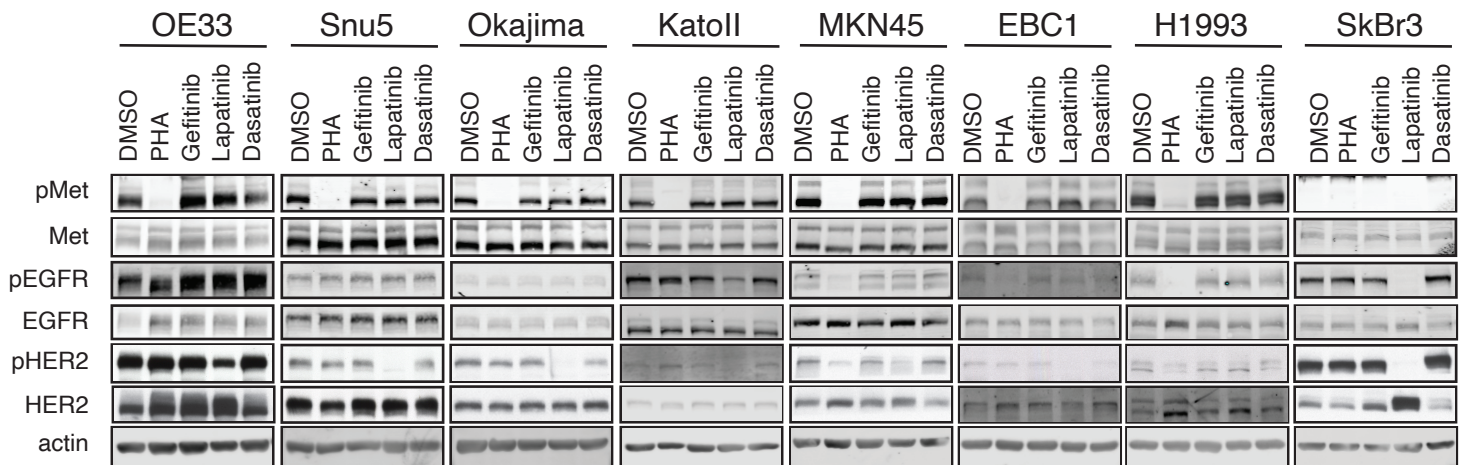
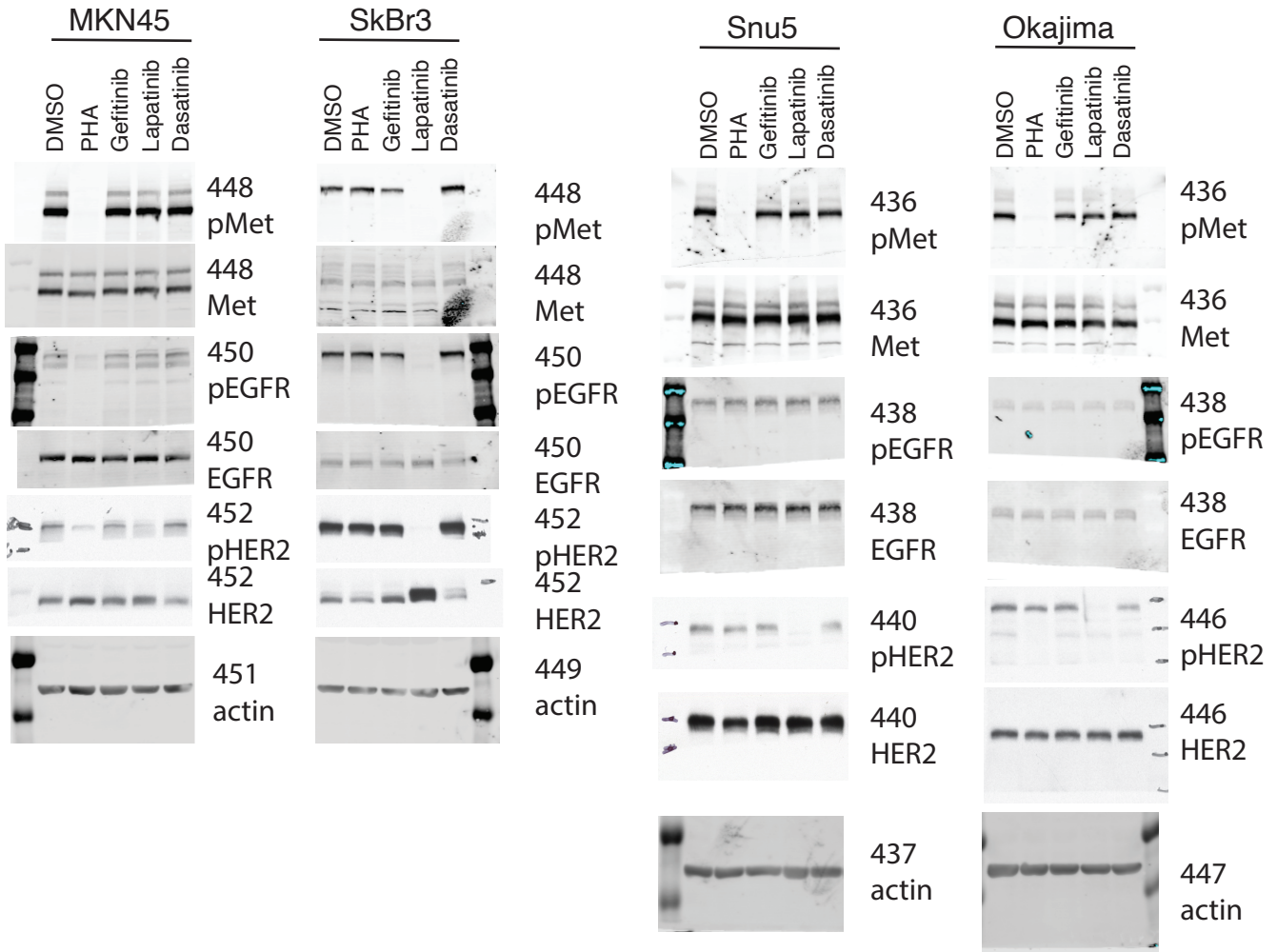
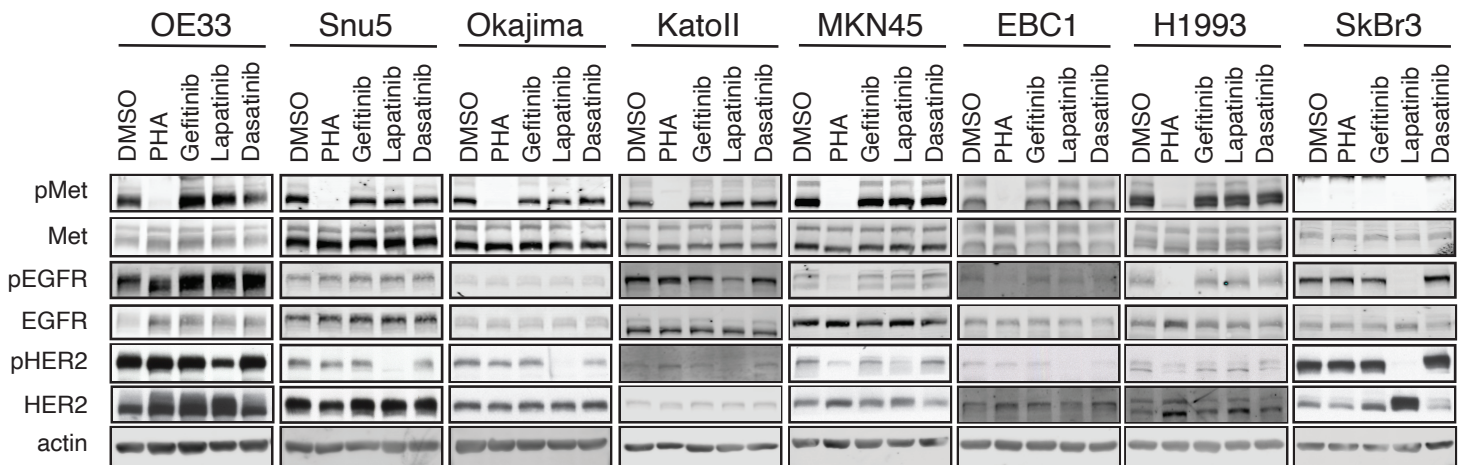


Figure S1b



Supplementary Figure 1

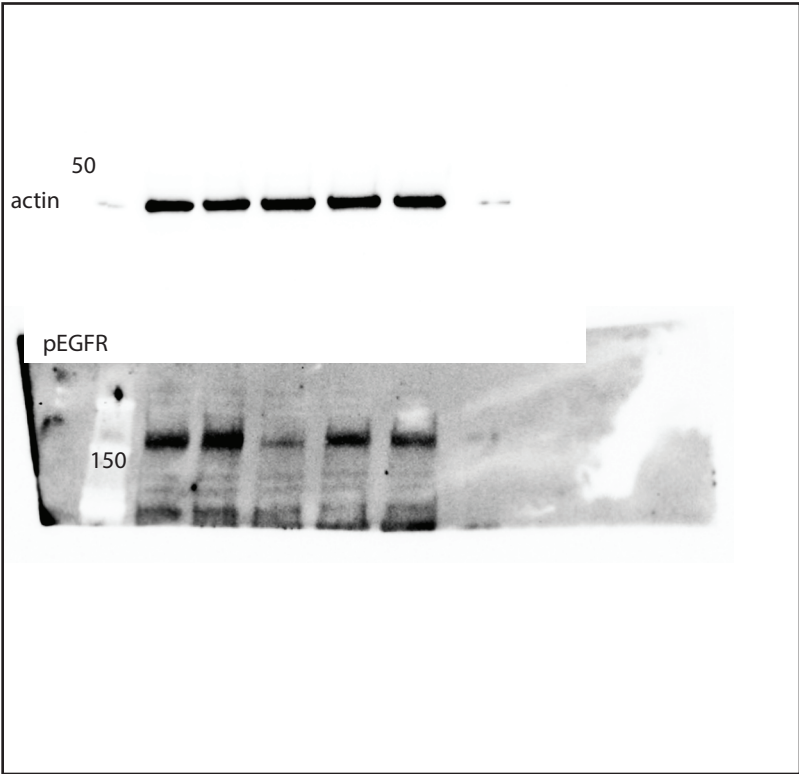
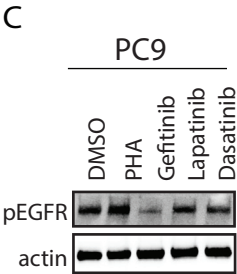
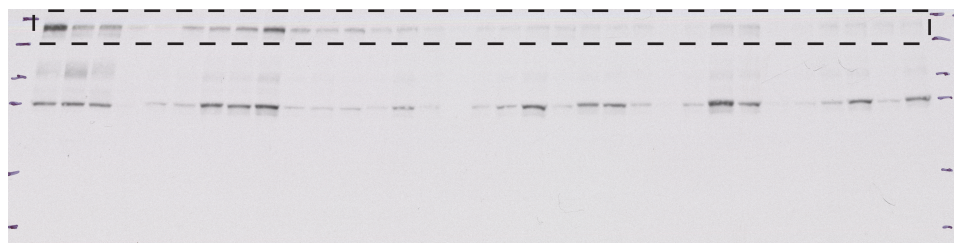
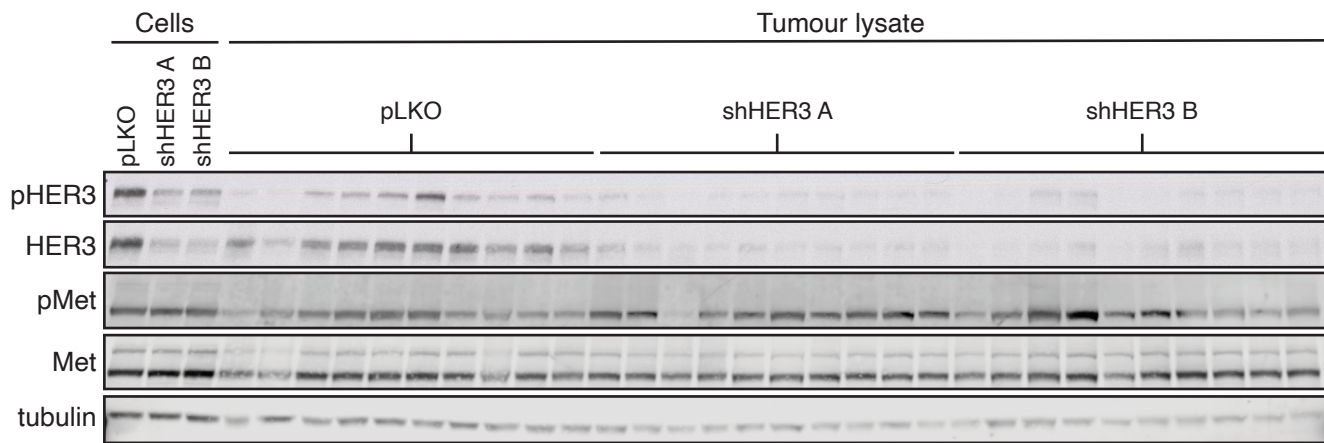
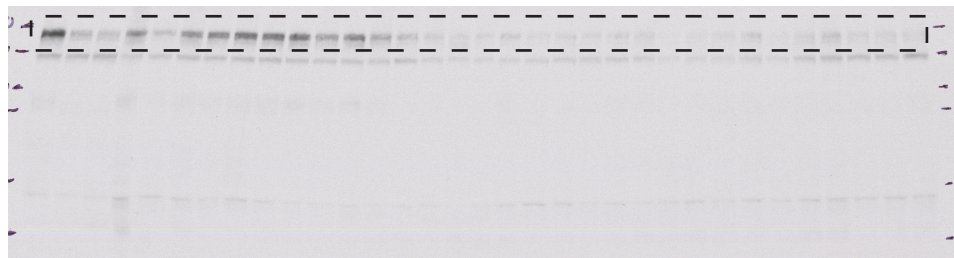


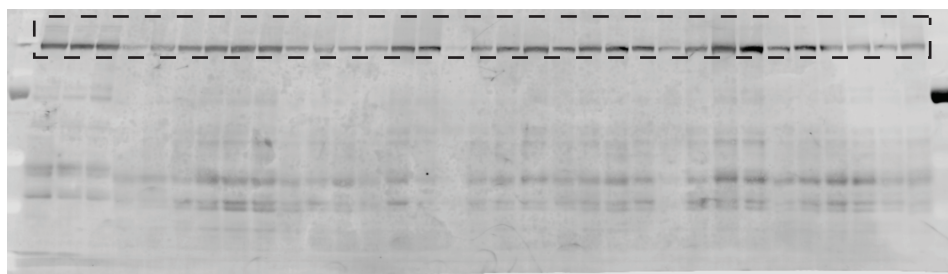
Figure S2



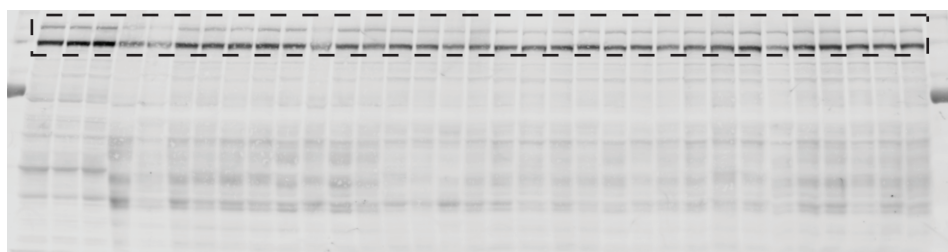
1373
pHER3



1373
HER3



1374
pMet



1374
Met



1373
tubulin