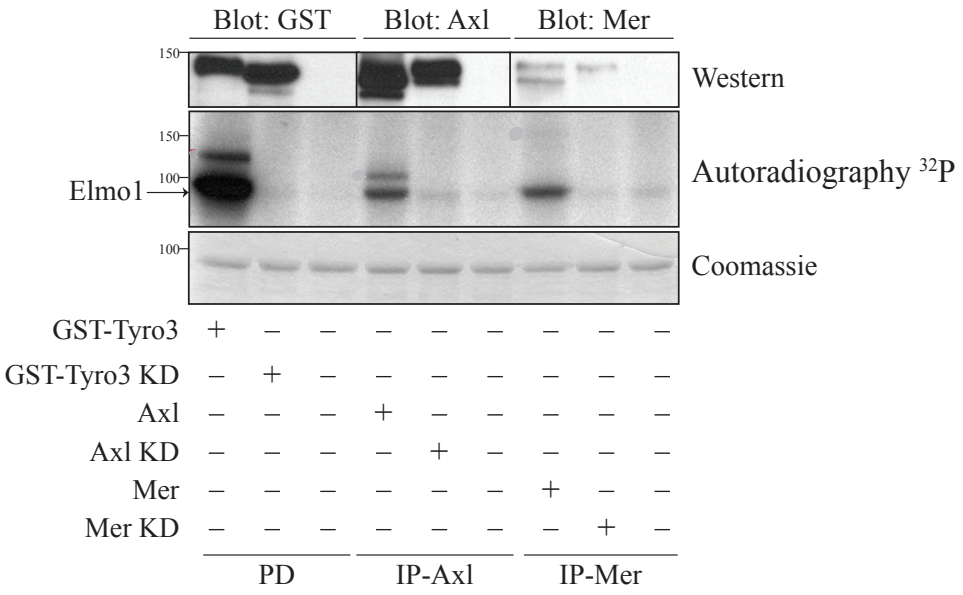
A



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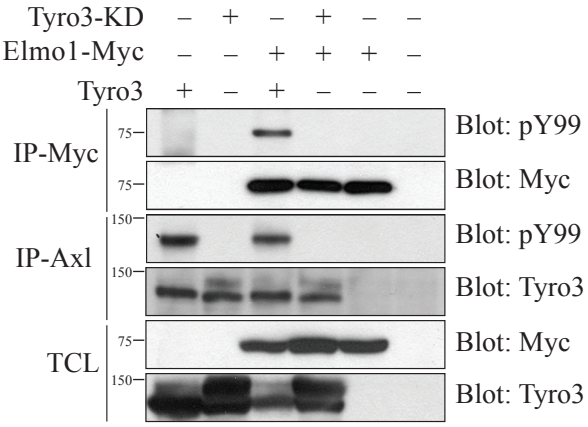

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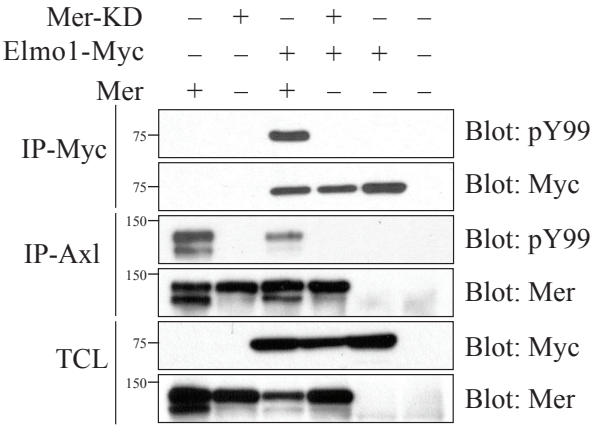

D

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hElmo1 664 IWTDGLNALLGKDMMSDLTRNDLDTLLSMEIKLRLLDLENIQIPDAPPPIPKEPSNYDFVYDCN---- 727
hElmo2 657 IWIDGLSALLGKDMSSELTKSDLDTLLSMEMKLRLLDLENIQIPEAPPPIPKEPSSYDFVYHYG---- 720
hElmo3 706 LWTDGLSALLGSPMGSEQTRLDLEQLLTMETKLRLLELENVPIPERPPPVPPPPTNFNFCYDCSIAEP 773
           :* ***.****. * *: *: **: **:** *****:***: **: ***:*  *:.::* *. .
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Elmo 1
Mus Musculus                  648 LDTLLSMEIKLRLLDLENIQIPDAP--PPIPKEPSNYDFVY 724
Rattus Norvegicus             648 LDTLLSMEIKLRLLDLENIQIPDAP--PPIPKEPSNYDFVY 724
Homo Sapiens                  648 LDTLLSMEIKLRLLDLENIQIPDAP--PPIPKEPSNYDFVY 724
Bos Taurus                    648 LDTLLSMEIKLRLLDLENIQIPDAP--PPIPKEPSNYDFVY 724
Gallus Gallus                 648 LDTLLSMEIKLRLLDLENIQIPDAP--PPIPKEPSNYDFVY 724
Drosophila Melanogaster       646 FDTLLSMEIKLRLLDTEGVDISKDP--PPIPEDPENYDFCF 722
Caenorhabditis Elegans        653 VERMLKMELRVRLLNVKLTNPEEKPEIPPIPDDIKSFISKF 731
                                  .: :*.**:::***: :  :  . *  ****.: ..:   :

Elmo2
Mus Musculus                   633 LDTLLSMEMKLRLLDLENIQIPEAP--PPVPKEPSSYDFVYHYG 720
Rattus Norvegicus              645 LDTLLSMEMKLRLLDLENIQIPEAP--PPVPKEPSSYDFVYHYG 732
Homo Sapiens                   633 LDTLLSMEMKLRLLDLENIQIPEAP--PPIPKEPSSYDFVYHYG 720
Bos Taurus                     633 RDTLLSMEMKLRLLDLENIQIPEAP--PPVPKEPSSYDFVYHYG 720
Gallus Gallus                  633 LDTLLSMEMKLRLLDLENIQIPEAP--PPIPKEPSSYDFVYHYG 720
Drosophila Melanogaster        638 FDTLLSMEIKLRLLDTEGVDISKDP--PPIPEDPENYDFCFES- 724
Caenorhabditis Elegans         645 VERMLKMELRVRLLNVKLTNPEEKPEIPPIPDDIKSFISKF--- 731
                                    : :*.**:::***: :  :  : *  **:*.: ..:   :
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Figure S1. Elmo phosphorylation by the TAM receptors. (A) Lysates of transfected HEK293T cells with indicated plasmids were either immunoprecipitated with antibodies anti-Axl or anti-Mer or glutathione-precipitated with GST-Tyro3 and were incubated with 5μg of GST-Elmo1 and γ32-ATP. The expression of the proteins were analyzed by Coomassie staining and the phosphorylation by autoradiography (n=3). (B-C) Lysates of HEK293T cells transfected with the indicated plasmids were immunoprecipitated with an antibody against the Myc-epitope (Elmo1) and with an antibody against Tyro3 (B) or Mer (C). The phosphorylation and expression levels of Elmo1, Tyro3 and Mer were analyzed via immunoblotting with anti-Myc (Elmo1) and anti-Tyro3 (B) or anti-Mer (C) antibodies, respectively. (D) Elmo protein sequence alignment in different species near identified phosphorylation sites Y720 (highlighted in green) and Y724 (highlighted in yellow).

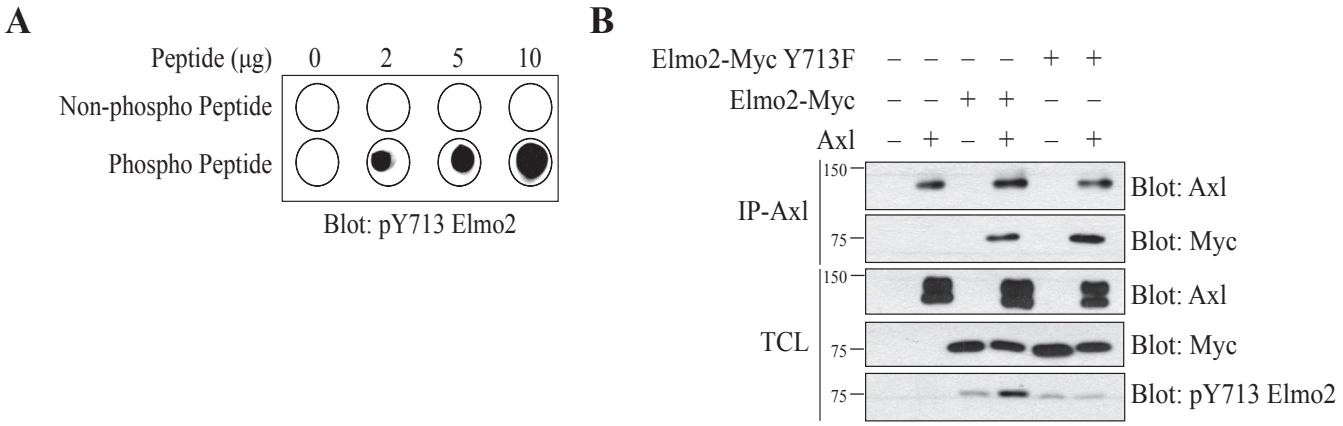


Figure S2. Specificity of pY713 Elmo2 antibody. (A) The purified tyrosine 713 phosphospecific antibody was used for dot blot analysis of increasing amounts (0-10μg) of the phosphorylated and the non-phosphorylated immunogenic peptides used for affinity purification of the serum. (B) Elmo2 phospho-mutant Y720F is able to bind Axl and diminish phosphorylation detected by Elmo2 phospho-specific antibody anti-pY713. Lysates of HEK293T cells transfected with the indicated plasmids were co-immunoprecipitated with an antibody against Axl. The co-precipitation and expression levels of the Axl proteins and Elmo2 were analyzed via immunoblotting with anti-Myc (Elmo2) and anti-Axl antibodies, respectively. Phosphorylation of Elmo2 is detected by immunoblotting using anti-pY713 Elmo2 antibody.

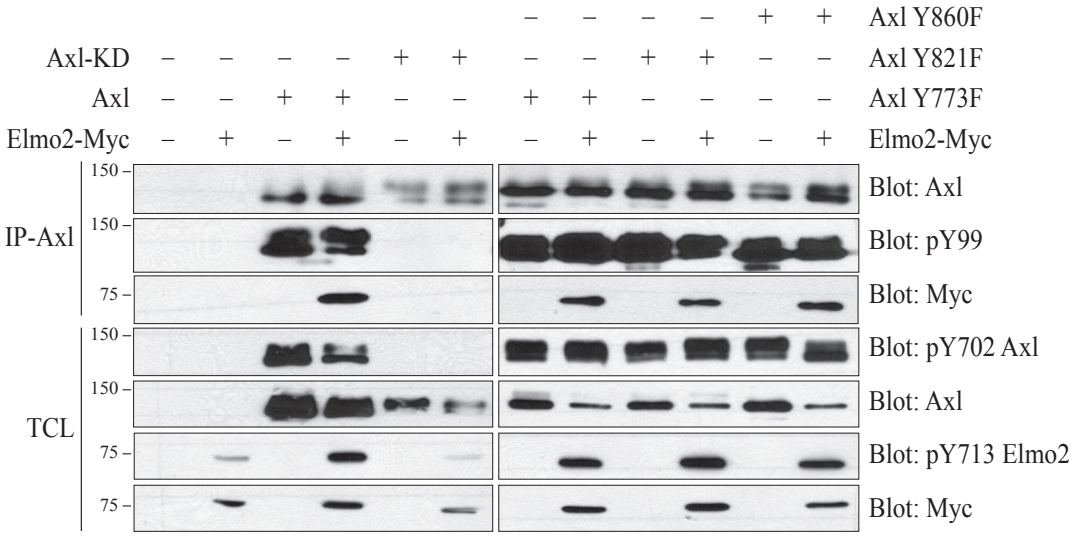


Figure S3. Tyrosine 773, 821 and 860 in Axl C-terminal are not required for Elmo2 binding and phosphorylation. Lysates of HEK293T cells transfected with the indicated plasmids were immunoprecipitated with an antibody against Axl. The phosphorylation and expression levels of Elmo2, Axl and its mutants were analyzed via immunoblotting with anti-Myc (Elmo2), anti-Axl, anti-pY713 Elmo2, anti-pY99 and anti-pY702 Axl.

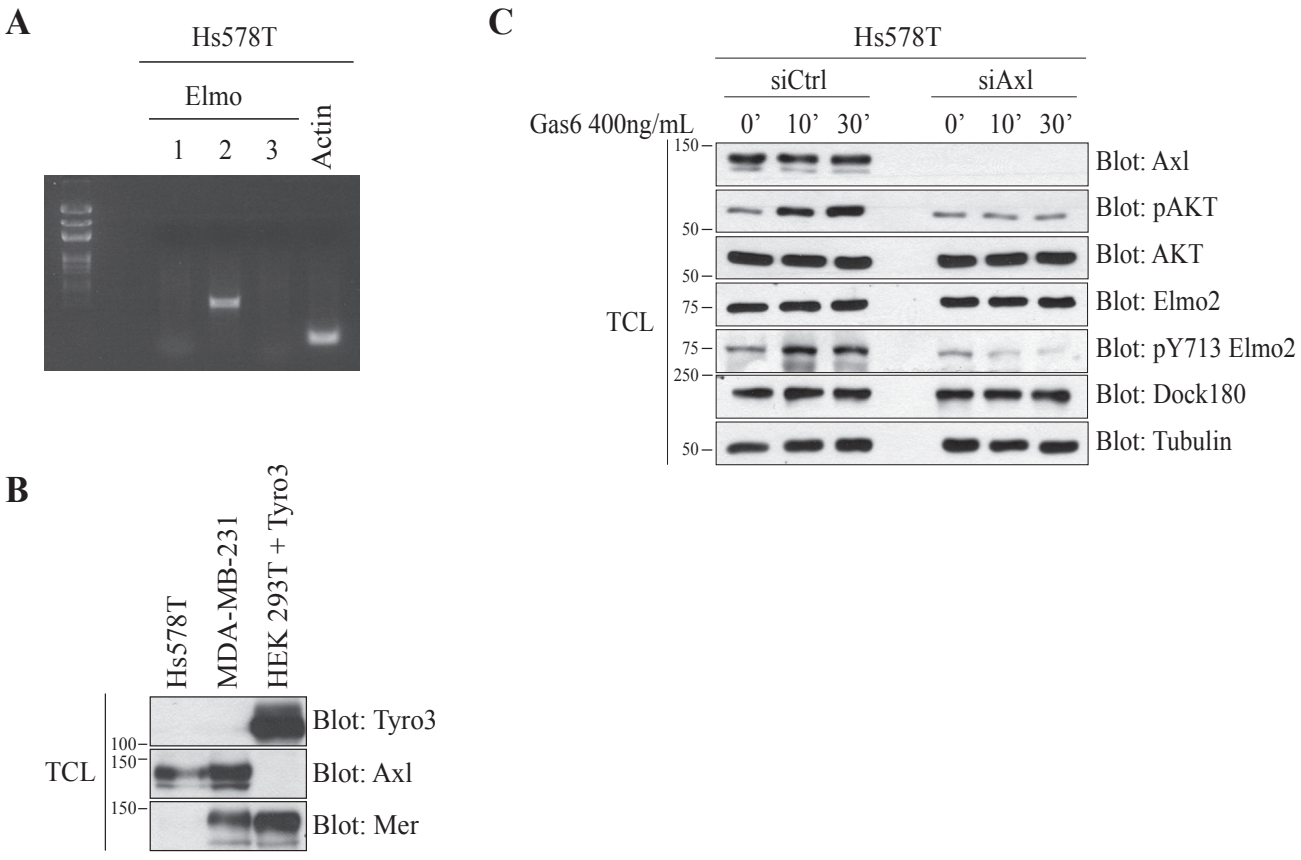
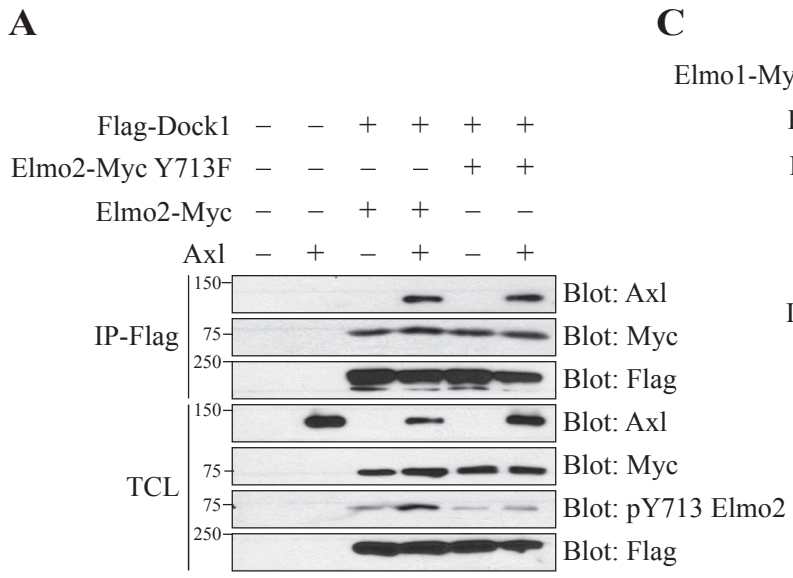
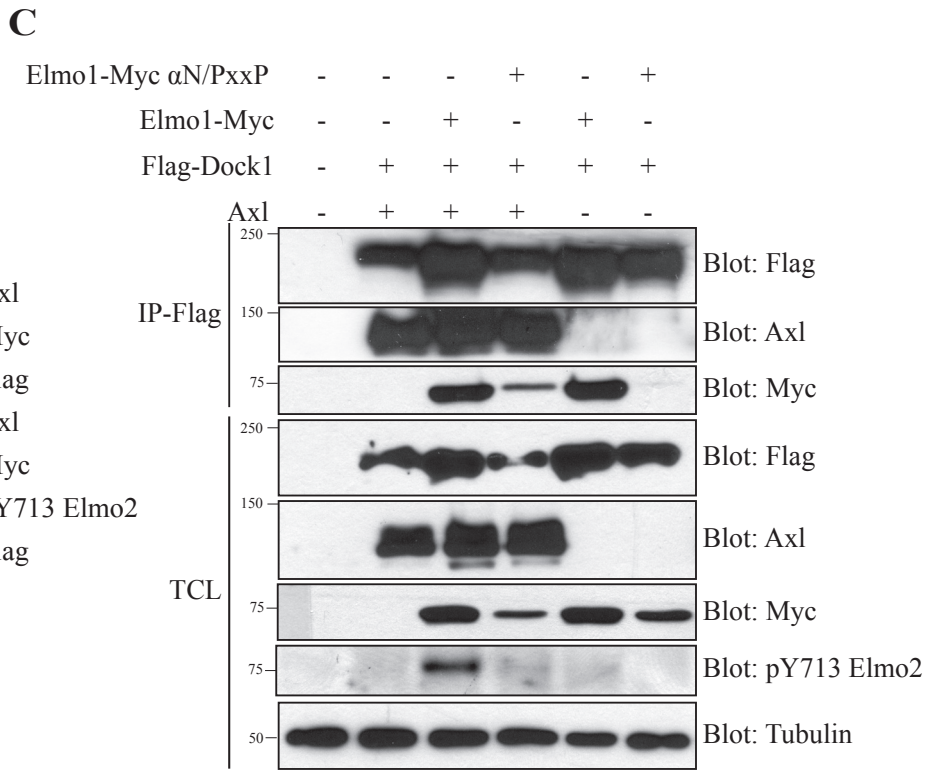


Figure S4. Expression Profile of the TAM receptors and Elmo proteins in MDA-MB-231 and Hs578T cells. (A) RT-PCR analysis on RNA extracted from Hs578T cells using specific primers for Actin, Elmo1, Elmo2 and Elmo3 (Table 3) (B) Lysates of MDA-MB-231 cells, Hs578T cells and trasfected HEK293T cells were analyzed for Axl, Tyro3 and Mer expression via immunoblotting using anti-Axl, anti-Tyro3 and anti-Mer. (C) Phosphorylation of Elmo2 in Hs578T cells is dependent on Axl expression. Hs578T cells were transfected with 100nM NON-targeting or ON-target smart pool Axl siRNA prior to being treated with 400ng/mL of Gas6 for the indicated time points. Cell lysates were analyzed via immunoblotting with anti-pAKT, anti-AKT, anti-Axl, anti-Elmo2, anti-pY713 Elmo2, anti-Dock180 and anti-Tubulin antibodies.





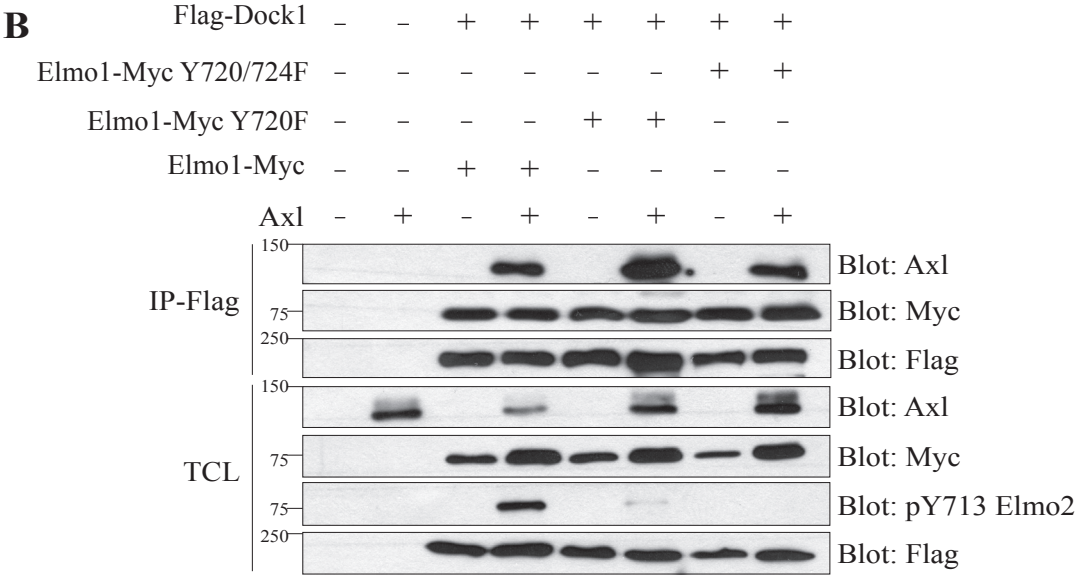


Figure S5. Axl forms a complex with Elmo/Dock1. (A-B) Lysates of HEK293T cells transfected with the indicated plasmids were co-immunoprecipitated with an antibody against Flag-Dock180. The co-precipitation and expression levels of the Axl, Elmo1 (B), Elmo2 (A) and Dock180 were analyzed via immunoblotting with anti-Myc (Elmo), anti-Axl and anti-Flag antibodies. Phoshorylated Elmo in the lysates is detected with the antibody anti-pY713. (C) Lysates of HEK293T cells transfected with the indicated plasmids were co-immunoprecipitated with an antibody against Flag-Dock1. The co-precipitation and expression levels of the Axl, Elmo1, Tubulin and Dock1 were analyzed via immunoblotting with anti-Myc (Elmo), anti-Axl, anti-Tubulin and anti-Flag antibodies. Phoshorylated Elmo in the lysates is detected with the antibody anti-pY713 Elmo2.

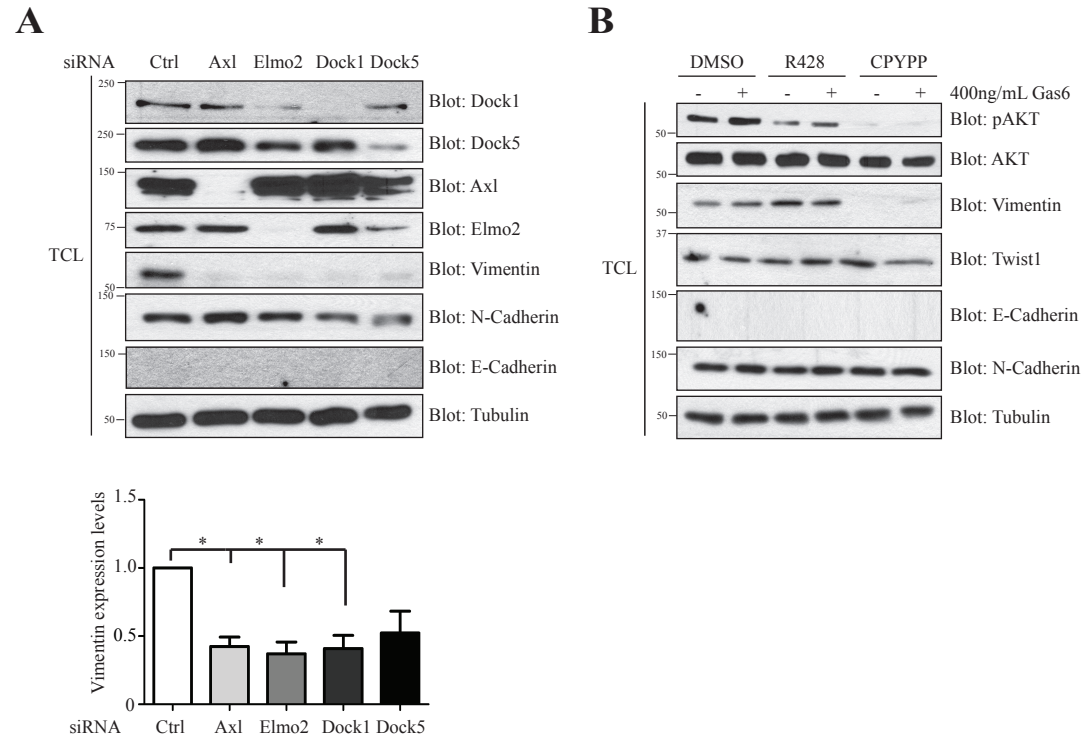


Figure S6. Inhibition of activation or knockdown of Axl, Elmo2, Dock1 and Dock5 alter Vimentin Expression. (A) MDA-MB-231 cells were transfected with either 100nM NON-targeting siRNA, 100nM ON-target Axl siRNA, 60nM ON-target Elmo2 siRNA or 200nM ON-target Dock1 or Dock5 siRNA. The expression levels of Axl, Elmo2, Dock1, Dock5, N-Cadherin, E-Cadherin, Vimentin and Tubulin were analyzed via immunoblotting with anti-Axl, anti-Elmo2, anti-Dock1, anti-Dock5, anti-N-Cadherin, anti-E-Cadherin, anti-Vimentin and anti-Tubulin antibodies. The expression levels of Vimentin were quantified by the software Image J (n=3). Data are shown as mean ± SEM; *$p < 0.05$; one-way ANOVA. (B) Serum-starved MDA-MB-231 cells were treated with 100μM CPYPP or with 1μM R428 for 1hr followed by 400ng/mL Gas6 for 20min. Lysates were then analyzed for expression of Twist1, N-Cadherin, E-Cadherin, Vimentin, p-AKT, AKT and Tubulin via immunoblotting with anti-Twist1, anti-N-Cadherin, anti-E-Cadherin, anti-Vimentin, anti-pAKT, anti-AKT and anti-Tubulin antibodies.

Table S1 : GST-kinase library (List of human protein kinases in the GST-kinase expression library used in Fig. 1 to screen.

Gene Name	Gene Description
ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)
ACVR1	activin A receptor, type I
ACVR1B	activin A receptor, type IB
ADCK4	aarF domain containing kinase 4
ADRBK1	adrenergic, beta, receptor kinase 1
AKT1	v-akt murine thymoma viral oncogene homolog 1
ALS2CR2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2 (ALS2CR2)
ARAF	v-raf murine sarcoma 3611 viral oncogene homolog
AURKB	aurora kinase B (AURKB), mRNA
BLK	B lymphoid tyrosine kinase (BLK), mRNA
BMP2K	BMP2 inducible kinase
BMX	BMX non-receptor tyrosine kinase
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BRD2	bromodomain containing 2
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)
BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)
C9orf96	chromosome 9 open reading frame 96
CAMK1G	calcium/calmodulin-dependent protein kinase IG
CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma
CAMK4	calcium/calmodulin-dependent protein kinase IV
CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2
CAMKV	CaM kinase-like vesicle-associated
CDC2	cell division cycle 2, G1 to S and G2 to M
CDC2L6	cell division cycle 2-like 6 (CDK8-like)
CDK10	cyclin-dependent kinase (CDC2-like) 10
CDK2	cyclin-dependent kinase 2
CDK3	cyclin-dependent kinase 3
CDK4	cyclin-dependent kinase 4
CDK6	cyclin-dependent kinase 6

CDK7	cyclin-dependent kinase 7 (MO15 homolog, Xenopus laevis, cdk-activating kinase)
CDKL5	cyclin-dependent kinase-like 5
CHEK2	CHK2 checkpoint homolog (S. pombe)
CLK1	CDC-like kinase 1
CLK3	CDC-like kinase 3
CLK4	CDC-like kinase 4
CSNK1A1L	casein kinase 1, alpha 1-like
CSNK1D	casein kinase 1, delta
CSNK1E	casein kinase 1, epsilon
CSNK1G2	casein kinase 1, gamma 2
CSNK2A1	casein kinase 2, alpha 1 polypeptid
DCAMKL2	doublecortin and CaM kinase-like 2
DLCK1	doublecortin and CaM kinase-like 1 (DCAMKL1)
DMPK	dystrophia myotonica-protein kinase
DYRK1B	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B
DYRK2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2
DYRK4	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4
EEF2K	eukaryotic elongation factor-2 kinase
EIF2AK1	eukaryotic translation initiation factor 2-alpha kinase 1
EIF2AK2	eukaryotic translation initiation factor 2-alpha kinase 2
FASTK	Fas-activated serine/threonine kinase
FASTKD2	FAST kinase domains 2
FES	feline sarcoma oncogene
FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
FRK	fyn-related kinase
FYN	FYN oncogene related to SRC, FGR, YES
GRK5	G protein-coupled receptor kinase 5
GRK6	G protein-coupled receptor kinase 6
GSK3A	glycogen synthase kinase 3 alpha
HCK	hemopoietic cell kinase
HIPK1	homeodomain interacting protein kinase 1
HIPK4	homeodomain interacting protein kinase 4

ICK	intestinal cell (MAK-like) kinase
ILK	integrin-linked kinase
IRAK3	interleukin-1 receptor-associated kinase 3
IRAK4	interleukin-1 receptor-associated kinase 4
JAK2	Janus kinase
KSR2	kinase suppressor of ras
LATS1	LATS, large tumor suppressor, homolog 1 (Drosophila)
LYK5	protein kinase LYK5
MAP2K1	mitogen-activated protein kinase kinase 1
MAP2K2	mitogen-activated protein kinase kinase 2
MAP2K3	mitogen-activated protein kinase kinase 3
MAP2K4	mitogen-activated protein kinase kinase 4
MAP2K6	mitogen-activated protein kinase kinase 6
MAP3K11	mitogen-activated protein kinase kinase kinase 11
MAP3K14	mitogen-activated protein kinase kinase kinase 14
MAP3K8	mitogen-activated protein kinase kinase kinase 8
MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2
MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5
MAPK10	mitogen-activated protein kinase 10
MAPK11	mitogen-activated protein kinase 11
MAPK12	mitogen-activated protein kinase 12
MAPK13	mitogen-activated protein kinase 13
MAPK3	mitogen-activated protein kinase 3
MAPK8	mitogen-activated protein kinase 8
MAPK9	mitogen-activated protein kinase 9
MARK2	MAP/microtubule affinity-regulating kinase 2
MARK3	MAP/microtubule affinity-regulating kinase 3
MAST2	microtubule associated serine/threonine kinase 2
MKNK1	MAP kinase interacting serine/threonine kinase 1
MYLK2	myosin light chain kinase 2, skeletal muscle
NEK2	NIMA (never in mitosis gene a)-related kinase 2
NEK3	NIMA (never in mitosis gene a)-related kinase 3

NEK4	NIMA (never in mitosis gene a)-related kinase 4
NEK6	NIMA (never in mitosis gene a)-related kinase 6
NEK8	NIMA (never in mitosis gene a)-related kinase 8
NLK	nemo-like kinase
NRBP1	nuclear receptor binding protein 1
NUAK1	NUAK family, SNF1-like kinase, 1 (ARK5)
NUAK2	NUAK family, SNF1-like kinase, 2 (SNARK)
OXSR1	oxidative-stress responsive 1
PAK1	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)
PAK2	p21 (CDKN1A)-activated kinase 2
PAK4	p21 (CDKN1A)-activated kinase 4
PAK6	p21 (CDKN1A)-activated kinase 6
PAK7	p21 (CDKN1A)-activated kinase 7
PBK	PDZ binding kinase
PCTK1	PCTAIRE protein kinase 1
PCTK2	PCTAIRE protein kinase 2
PCTK3	PCTAIRE protein kinase 3
PDIK1L	PDLIM1 interacting kinase 1 like
PFTK1	PFTAIRE protein kinase 1
PIM1	pim-1 oncogene
PIM3	pim-3 oncogene
PKMYT1	protein kinase, membrane associated tyrosine/threonine 1
PKN3	protein kinase N3
PLK1	polo-like kinase 1 (Drosophila)
PLK2	polo-like kinase 2 (Drosophila)
PLK4	polo-like kinase 4 (Drosophila)
PNCK	pregnancy upregulated non-ubiquitously expressed CaM kinase
PRKACG	protein kinase, cAMP-dependent, catalytic, gamma
PRKAG1	protein kinase, AMP-activated, gamma 1 non-catalytic subunit
PRKC1B	protein kinase C, beta 1
PRKCA	protein kinase C, alpha
PRKCH	protein kinase C, eta

PRKCI	protein kinase C, iota
PRKCZ	protein kinase C, zeta
PRKD2	protein kinase D2
PRKRA	protein kinase, interferon-inducible double stranded RNA dependent activator
PRKX	protein kinase, X-linked
PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B (yeast)
PSKH1	protein serine kinase H1
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
RAGE	renal tumor antigen
RIOK1	RIO kinase 1 (yeast)
RIOK2	RIO kinase 2 (yeast)
RIPK2	receptor-interacting serine-threonine kinase 2
RIPK3	receptor-interacting serine-threonine kinase 3
RPS6KA1	ribosomal protein S6 kinase, 90kDa, polypeptide 1
RPS6KL1	ribosomal protein S6 kinase-like 1
SCYL3	SCY1-like 3
SGK2	serum/glucocorticoid regulated kinase 2
SGK3	serum/glucocorticoid regulated kinase 3
SNF1LK	SNF1-like kinase
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)
SRPK1	SFRS protein kinase 1
SRPK2	SFRS protein kinase 2
STK11	serine/threonine kinase 11
STK16	serine/threonine kinase 16
STK17A	serine/threonine kinase 17a
STK17b	serine/threonine kinase 17b
STK19	serine/threonine kinase 19
STK25	serine/threonine kinase 25
STK31	serine/threonine kinase 31
STK32A	serine/threonine kinase 32A
STK32B	serine/threonine kinase 32B
STK33	serine/threonine kinase 33

STK36	serine/threonine kinase 36 (fused homolog, Drosophila)
STK38	serine/threonine kinase 38
STK38L	serine/threonine kinase 38 like
STK40	serine/threonine kinase 40
SYK	spleen tyrosine kinase
TAF1	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa
TBK1	TANK-binding kinase 1
TESK1	testis-specific kinase 1
TLK1	tousled-like kinase 1
TNK2	Similar to activated p21cdc42Hs kinase
TP53RK	TP53 regulating kinase
TSSK1B	testis-specific serine kinase 1B
TSSK2	testis-specific serine kinase 2
TSSK6	testis-specific serine kinase 6
TTBK2	tau tubulin kinase 2
TTK	TTK protein kinase
TYK2	tyrosine kinase 2
TYRO3	TYRO3 protein tyrosine kinase
UHMK1	U2AF homology motif (UHM) kinase 1
ULK4	unc-51-like kinase 4 (C. elegans)
VRK1	vaccinia related kinase 1
WEE1	WEE1 homolog (S. pombe)
YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1
YSK4	yeast Sps1/Ste20-related kinase 4 (S. cerevisiae)

Table S2 : List of kinases capable of phosphorylating Elmo1 identified in the screen for kinases.

Identified Kinase	Synonym	Kinase type	Validation IRCM
BLK (B-Lymphocyte Kinase)	p55-BLK	Non-receptor Tyr Kinase	Not tested
CAMKK2 (CaM-kinase kinase 2)	Calcium/calmodulin-dependent protein kinase, CaMKK beta and KKCC2	Non-receptor Ser/Thr Kinase	Not tested
DCAMKL1	DCDC3A, DCLK1, DCAK1, CAM kinase-like 1 and KIAA0369	Non-receptor Ser/Thr Kinase	Negative
PFTAIRE1 (PFTAIRE protein kinase 1)	KIAA0834, PFT1 and PFTK1	Non-receptor Ser/Thr Kinase	Negative
PRP4 (pre-mRNA processing factor 4)	CBP143, KIAA0536, Pre-mRNA protein kinase, PRP4B, PRP4H, PRP4K, PRP4M, and PRPF4B	Non-receptor Ser/Thr Kinase	Not tested
TTBK2 (Tau-tubulin kinase 2)		Non-receptor Ser/Thr Kinase	Non tested
Tyro3 (Tyrosine kinase gene 3)	Sky, Brt (mouse), Rse, Etk-2 (mouse), Rek (chicken), DTK(mouse) and Tif	Receptor Tyrosine Kinase	Positive

Table S3: Primers used for different procedures

Procedure	Forward	Reverse
RT-PCR Human *β-Actin*	TGATGGTGGGCATGGGTCAGAA	TCCATGTCGTCCCAGTTGGTGA
RT-PCR Human *Elmo1*	CACGATCACAGTGCAGA	CAACTTTCAGCCCCTAGCTG
RT-PCR Human *Elmo2*	CGTTGCCAAACCCAGAGTAT	TGGAGGTGTGAGATGAGCTG
RT-PCR Human *Elmo3*	TGACGCACTCTGAGCGTTAC	CAAGGTCACACTCTCCAGCA
To generate Axl-KD (K561M)	CTCAAGGTCGCTGTGATGACCATGA AAATTGCC	GGCAATTTTCATGGTCATCACAGCG ACCTTGAG
To generate Elmo1 Y352F	GAGAAACGCAAGTCCATGTTCACTC GGGATTATAAAAAAC	GTTTTTTATAATCCCGAGTGAACATG GACTTGCGTTTCTC
To generate Elmo1 Y720F	CCCAAGGAACCTAGCAACTTTGACT TTGTCTATGACTGTAACTG	CAGTTACAGTCATAGACAAAGTCAA AGTTGCTAGGTTCCTTGGG
To generate Elmo1 Y724F	CCCAAGGAACCTAGCAACTATGACT TTGTCTTTGACTGTAACTG	CAGTTACAGTCAAAGACAAAGTCAT AGTTGCTAGGTTCCTTGGG
To generate Elmo1 Y720/724F	CCCAAGGAACCTAGCAACTTTGACT TTGTCTTTGACTGTAACTG	CAGTTACAGTCAAAGACAAAGTCAA AGTTGCTAGGTTCCTTGGG