Supplemental Figures

Compartment-specific and ELAVL1-coordinated regulation of intronic polyadenylation isoforms by doxorubicin

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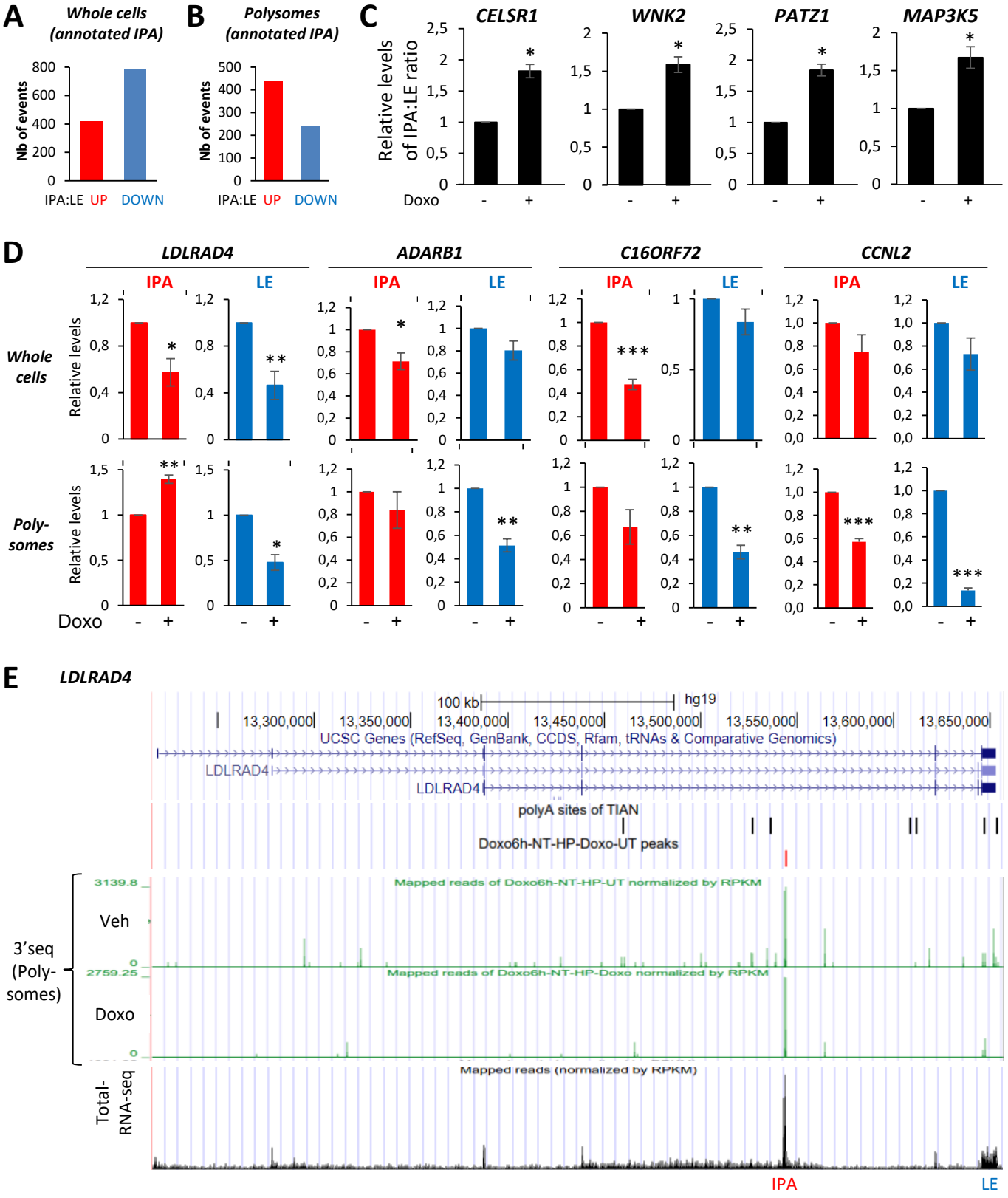
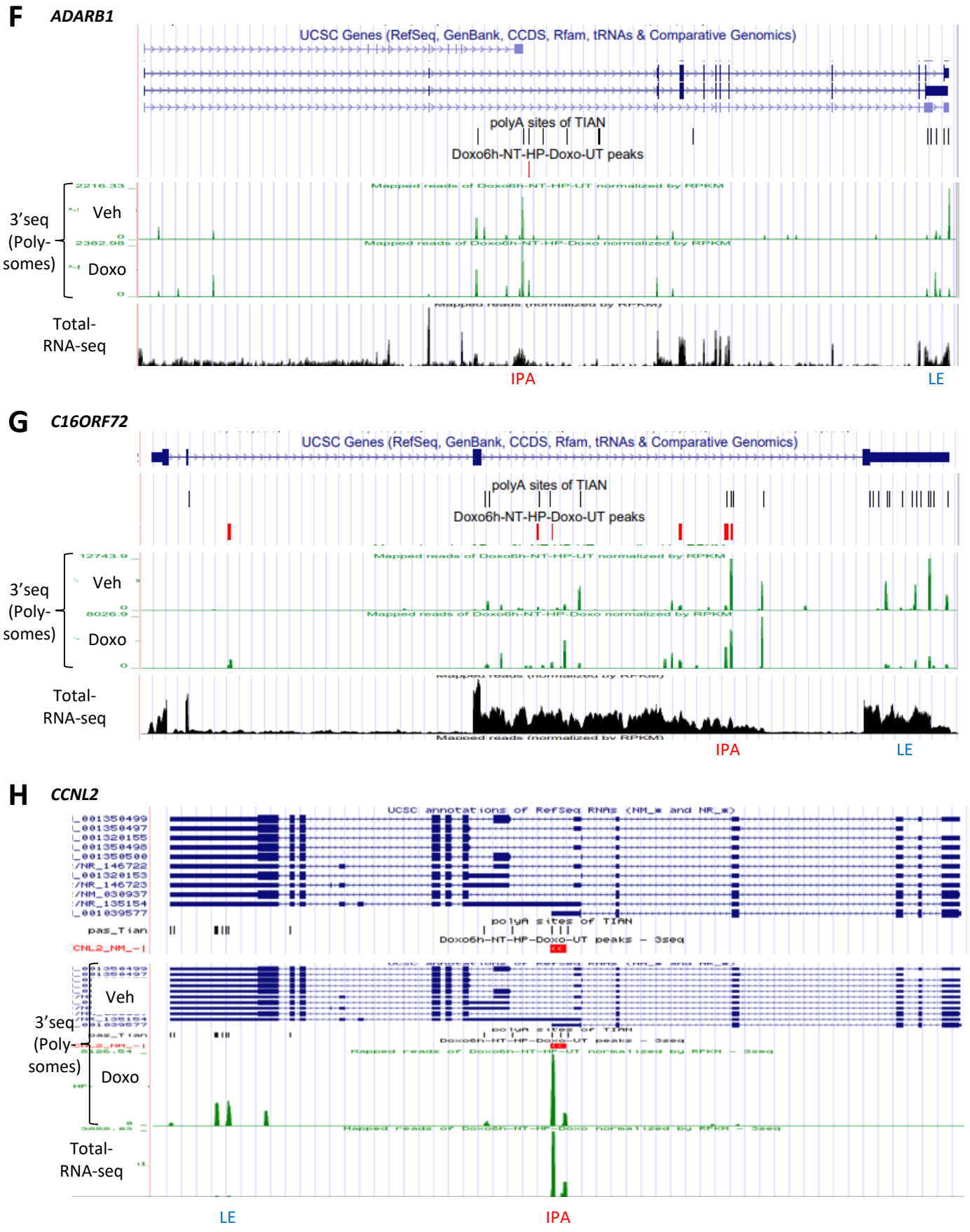
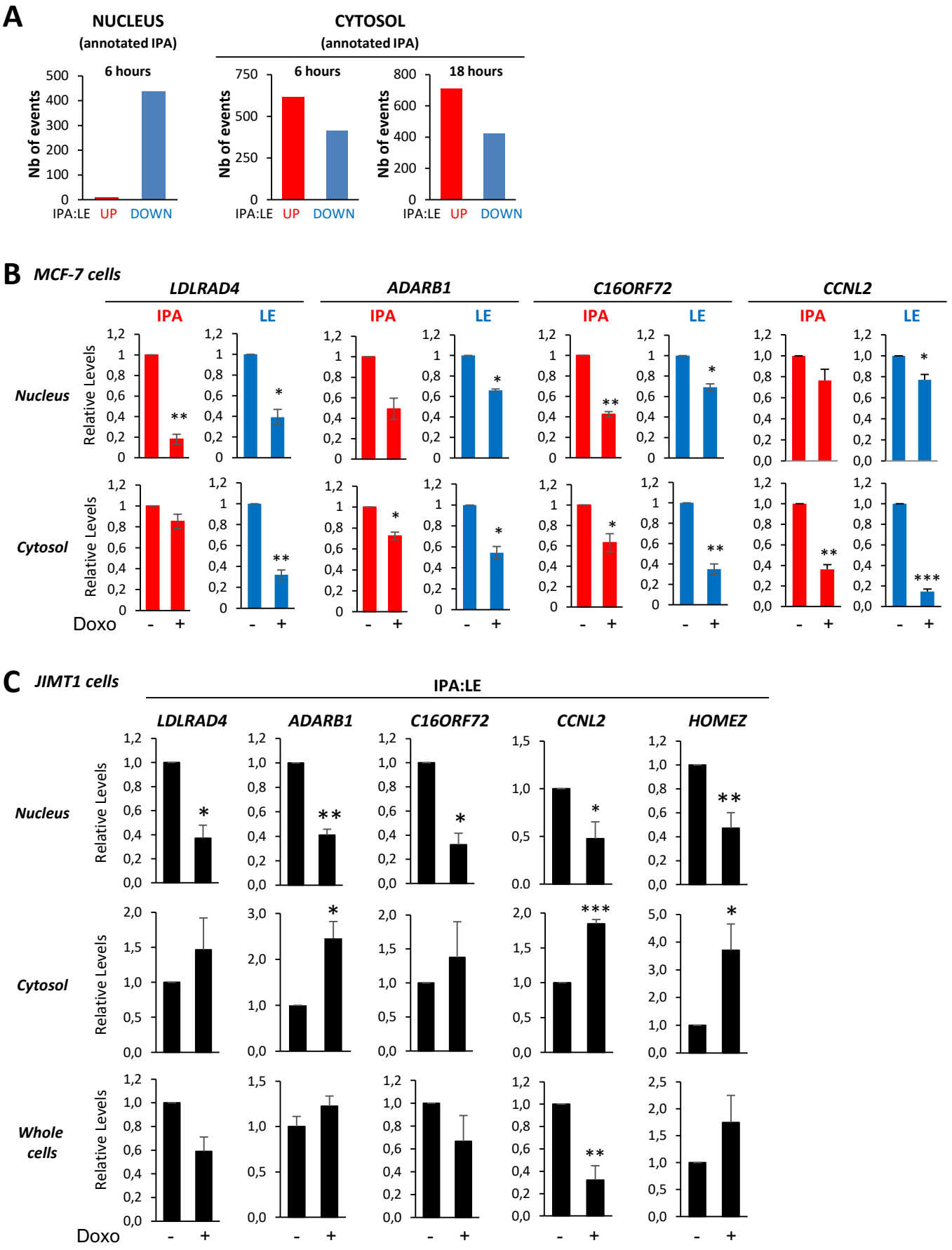




Supplemental Figure S1: A-B, Identification of IPA isoforms regulated by Doxo relative to matched last-exon isoform (IPA:LE ratio) by 3'-seq on whole MCF-7 cells (transcriptome) and polysomes (translatome). Only IPA isoforms corresponding to annotated IPA sites are shown. **C**, RT-qPCR analysis of IPA:LE isoform ratio regulation by Doxo in the indicated genes in whole cells. **D**, RT-qPCR analysis of IPA and LE isoforms regulation by Doxo in the indicated genes in whole cells and in polysomes. **E-H**, Visualization of 3'-seq and total-RNA-seq data for the indicated genes in the UCSC genome browser.

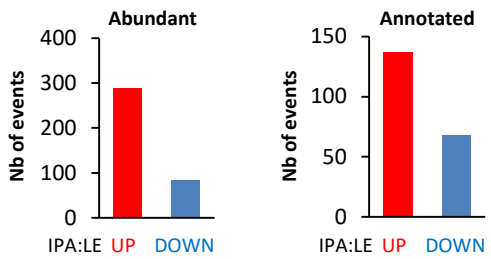

A
NUCLEUS
(annotated IPA)
6 hours
Nb of events
IPA:LE UP DOWN
CYTOSOL
(annotated IPA)
6 hours
18 hours
B MCF-7 cells
LDLRAD4
ADARB1
C16ORF72
CCNL2
IPA
LE
Nucleus
Cytosol
Relative Levels
Doxo
C JIMT1 cells
IPA:LE
LDLRAD4
ADARB1
C16ORF72
CCNL2
HOMEZ
Nucleus
Cytosol
Whole cells
Relative Levels
Doxo

D

IPA:LE regulation	Function	Term	Count	%	Fold Enrichment	PValue	FDR
Cyto IPA:LE up	other	GO:0005813~centrosome	53	5,70	2,6	3E-10	3E-08
Cyto IPA:LE up	other	GO:0003682~chromatin binding	45	4,90	2,4	2E-07	7E-05
Cyto IPA:LE up	other	GO:0000775~chromosome, centromeric region	14	1,50	5,2	2E-06	1E-04
Cyto IPA:LE up	other	GO:0005694~chromosome	18	1,90	3,6	8E-06	5E-04
Cyto IPA:LE up	other	GO:0016605~PML body	15	1,60	3,3	2E-04	8E-03
Cyto IPA:LE up	cell cycle	GO:0007062~sister chromatid cohesion	17	1,80	3,4	4E-05	2E-02
Cyto IPA:LE up	other	GO:0005814~centriole	15	1,60	2,8	8E-04	3E-02
Cyto IPA:LE up	cell cycle	GO:0030496~midbody	16	1,70	2,6	1E-03	4E-02
Cyto IPA:LE up	DDR	GO:0002039~p53 binding	11	1,20	3,4	1E-03	7E-02
Cyto IPA:LE up	cell death	GO:2001237~negative regulation of extrinsic apoptotic signaling pathway	9	1,00	4,9	4E-04	9E-02
Cyto IPA:LE up	DDR	GO:0006281~DNA repair	25	2,70	2,2	5E-04	1E-01
Cyto IPA:LE up	cell cycle	GO:0000922~spindle pole	13	1,40	2,5	5E-03	1E-01
Cyto IPA:LE up	cell cycle	GO:0000776~kinetochore	10	1,10	2,6	1E-02	2E-01
Cyto IPA:LE up	other	GO:0005815~microtubule organizing center	15	1,60	2,1	1E-02	2E-01
Cyto IPA:LE up	cell cycle	GO:0000777~condensed chromosome kinetochore	10	1,10	2,4	2E-02	3E-01
Cyto IPA:LE up	DDR	GO:0006974~cellular response to DNA damage stimulus	21	2,30	2,1	3E-03	4E-01
Cyto IPA:LE up	DDR	hsa03440:Homologous recombination	5	0,50	4,3	3E-02	4E-01
Cyto IPA:LE up	DDR	hsa03460:Fanconi anemia pathway	7	0,80	3,3	2E-02	4E-01
Cyto IPA:LE up	cell death	GO:0031264~death-inducing signaling complex	3	0,30	9,1	4E-02	4E-01
Cyto IPA:LE up	cell death	GO:0031265~CD95 death-inducing signaling complex	3	0,30	10,6	3E-02	4E-01
Cyto IPA:LE up	cell death	GO:0097342~ripoptosome	3	0,30	9,1	4E-02	4E-01
Cyto IPA:LE up	cell death	GO:1902043~positive regulation of extrinsic apoptotic signaling pathway via death domain receptors	5	0,50	7,3	4E-03	5E-01
Cyto IPA:LE up	cell death	GO:2000811~negative regulation of anoikis	5	0,50	6	8E-03	7E-01
Cyto IPA:LE up	other	GO:0019827~stem cell population maintenance	8	0,90	3,3	1E-02	8E-01
Cyto IPA:LE up	cell cycle	GO:0006260~DNA replication	14	1,50	1,9	4E-02	1E+00
Cyto IPA:LE up	cell cycle	GO:0007064~mitotic sister chromatid cohesion	4	0,40	5,9	3E-02	1E+00
Cyto IPA:LE up	cell cycle	GO:0048478~replication fork protection	3	0,30	10,3	3E-02	1E+00
Cyto IPA:LE up	cell cycle	GO:0090267~positive regulation of mitotic cell cycle spindle assembly checkpoint	3	0,30	8,8	4E-02	1E+00
Cyto IPA:LE up	DDR	GO:0000724~double-strand break repair via homologous recombination	9	1,00	2,5	3E-02	1E+00
Cyto IPA:LE up	DDR	GO:0000731~DNA synthesis involved in DNA repair	6	0,60	3,5	3E-02	1E+00
Cyto IPA:LE up	DDR	GO:0032508~DNA duplex unwinding	7	0,80	3,3	2E-02	1E+00
Cyto IPA:LE up	DDR	GO:0045910~negative regulation of DNA recombination	3	0,30	12,3	2E-02	1E+00
Cyto IPA:LE up	cell death	GO:0043065~positive regulation of apoptotic process	24	2,60	1,6	2E-02	1E+00
Cyto IPA:LE up	cell death	GO:1901216~positive regulation of neuron death	4	0,40	4,8	5E-02	1E+00
Cyto IPA:LE up	cell death	h_fasPathway:FAS signaling pathway (CD95)	6	0,60	3,1	4E-02	1E+00
Cyto IPA:LE up	other	GO:0000732~strand displacement	5	0,50	3,9	4E-02	1E+00
Cyto IPA:LE up	other	GO:0007249~I-kappaB kinase/NF-kappaB signaling	8	0,90	2,7	3E-02	1E+00
Cyto IPA:LE up	other	GO:0008285~negative regulation of cell proliferation	29	3,10	1,5	3E-02	1E+00
Cyto IPA:LE down	cell cycle	GO:0051301~cell division	25	6,00	3,1	2E-06	3E-03
Cyto IPA:LE down	cell cycle	GO:0030496~midbody	11	3,00	3,9	5E-04	3E-02
Cyto IPA:LE down	other	GO:0005813~centrosome	22	5,00	2,4	5E-04	3E-02
Cyto IPA:LE down	other	GO:0005815~microtubule organizing center	12	3,00	3,6	5E-04	3E-02
Cyto IPA:LE down	other	GO:0005694~chromosome	9	2,00	3,9	2E-03	8E-02
Cyto IPA:LE down	cell cycle	GO:0005819~spindle	9	2,00	3,4	5E-03	2E-01
Cyto IPA:LE down	cell cycle	GO:0007067~mitotic nuclear division	16	4,00	2,8	6E-04	3E-01
Cyto IPA:LE down	other	GO:0000784~nuclear chromosome, telomeric region	8	2,00	2,8	2E-02	6E-01
Cyto IPA:LE down	cell cycle	GO:0007049~cell cycle	12	3,00	2,4	1E-02	1E+00
Cyto IPA:LE down	cell cycle	GO:0007062~sister chromatid cohesion	7	2,00	3	3E-02	1E+00
Cyto IPA:LE down	DDR	GO:0000077~DNA damage checkpoint	4	1,00	5,8	3E-02	1E+00
Cyto IPA:LE down	DDR	GO:0006281~DNA repair	12	3,00	2,2	2E-02	1E+00
Cyto IPA:LE down	DDR	GO:0032205~negative regulation of telomere maintenance	3	1,00	32,7	3E-03	1E+00
Cyto IPA:LE down	DDR	hsa03460:Fanconi anemia pathway	5	1,00	4,2	3E-02	1E+00
Cyto IPA:LE down	cell death	GO:0070266~necroptotic process	4	1,00	9,7	8E-03	1E+00
Cyto IPA:LE down	cell death	h_tnfr2Pathway:TNFR2 Signaling Pathway	3	1,00	8,5	4E-02	1E+00
Nuc IPA:LE down	other	GO:0016605~PML body	4	4,00	8,2	1E-02	4E-01
Nuc IPA:LE down	cell cycle	GO:0006260~DNA replication	4	4,00	5	5E-02	1E+00
Nuc IPA:LE down	cell cycle	GO:0007049~cell cycle	5	5,00	4,4	3E-02	1E+00
Nuc IPA:LE down	DDR	GO:0034644~cellular response to UV	3	3,00	13,2	2E-02	1E+00
Nuc IPA:LE down	DDR	GO:1901796~regulation of signal transduction by p53 class mediator	4	4,00	6,2	3E-02	1E+00
Nuc IPA:LE down	other	GO:0051865~protein autoubiquitination	3	3,00	11,6	3E-02	1E+00

Supplemental Figure S2: A, Identification of annotated IPA isoforms that are regulated by Doxo relative to matched last-exon isoform (IPA:LE ratio) by 3'-seq on the nucleus and cytosol of MCF-7 cells at the indicated time points. **B**, RT-qPCR analysis of IPA and LE isoforms regulation by Doxo in the indicated genes in the nucleus and cytosol of MCF-7 cells. **C**, RT-qPCR analysis of IPA:LE isoform ratio regulation by Doxo in the indicated genes in the nucleus, cytosol and whole cells in the JIMT1 cell line. **D**, Gene Ontology terms enriched in the three main patterns of IPA:LE regulation patterns identified in Fig. 2A and that are related to the DDR.

A



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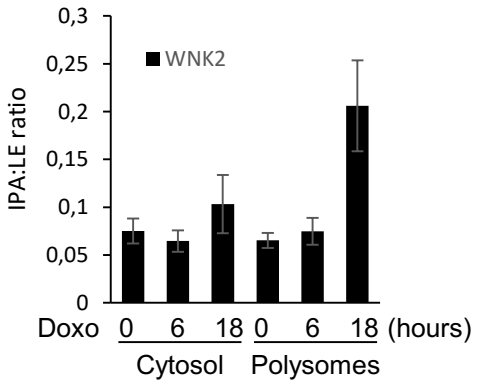

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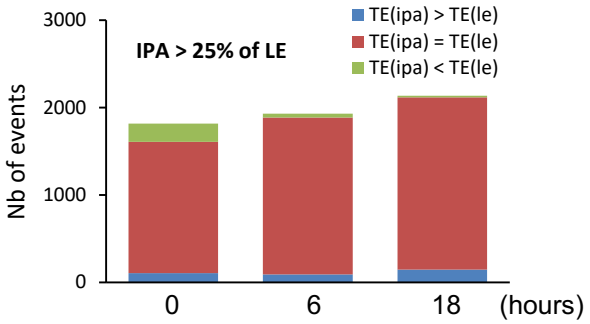
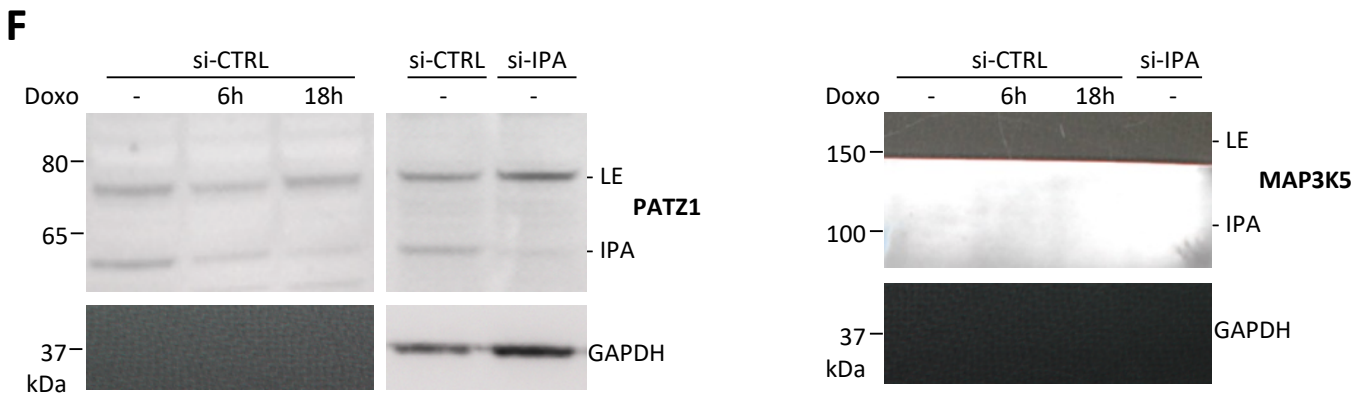


D

Term	Count	%	Fold Enrichment	PValue	FDR
GO:0006351~transcription, DNA-templated	67	25	2,5	9E-13	1E-09
GO:0003676~nucleic acid binding	43	16	3,2	2E-11	8E-09
GO:0006355~regulation of transcription, DNA-templated	55	21	2,7	2E-11	1E-08
GO:0005634~nucleus	118	44	1,6	9E-10	2E-07
GO:0005654~nucleoplasm	74	28	2,0	4E-09	4E-07
GO:0046872~metal ion binding	59	22	2,1	3E-08	6E-06
GO:0005622~intracellular	40	15	2,2	3E-06	2E-04
GO:0003677~DNA binding	47	18	2,1	2E-06	2E-04
GO:0005813~centrosome	20	7	3,5	5E-06	3E-04
GO:0000775~chromosome, centromeric region	8	3	10,5	1E-05	5E-04
GO:0003700~transcription factor activity, sequence-specific DNA binding	32	12	2,5	6E-06	5E-04
GO:0005694~chromosome	9	3	6,4	8E-05	3E-03
GO:0016607~nuclear speck	12	4	4,5	8E-05	3E-03
GO:0005515~protein binding	146	54	1,2	3E-04	2E-02
GO:0000978~RNA polymerase II core promoter proximal region sequence-specific DNA binding	15	6	3,1	3E-04	2E-02
GO:0000776~kinetochore	7	3	6,5	7E-04	2E-02
GO:0005814~centriole	8	3	5,3	8E-04	2E-02
GO:0007062~sister chromatid cohesion	9	3	6,4	8E-05	3E-02
GO:0003682~chromatin binding	15	6	2,8	9E-04	4E-02
GO:0045171~intercellular bridge	5	2	8,5	3E-03	7E-02
GO:0000922~spindle pole	7	3	4,8	3E-03	7E-02
GO:0018024~histone-lysine N-methyltransferase activity	5	2	9,5	2E-03	8E-02

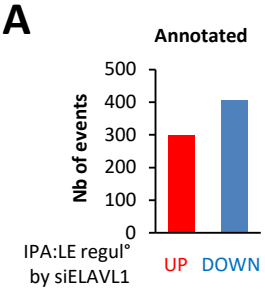
E

Term	Count	%	Fold Enrichment	PValue	FDR
GO:0005654~nucleoplasm	59	36	2,6	1,E-12	2,E-10
GO:0000122~negative regulation of transcription from RNA polymerase II promoter	24	15	3,8	6,E-08	6,E-05
GO:0005634~nucleus	70	43	1,6	1,E-05	1,E-03
GO:0006351~transcription, DNA-templated	38	23	2,2	3,E-06	1,E-03
GO:0005515~protein binding	101	62	1,3	9,E-06	3,E-03
GO:0045944~positive regulation of transcription from RNA polymerase II promoter	24	15	2,8	1,E-05	4,E-03
GO:0003682~chromatin binding	14	9	4,2	3,E-05	4,E-03
GO:0005737~cytoplasm	63	38	1,5	4,E-04	3,E-02
GO:0018024~histone-lysine N-methyltransferase activity	5	3	15,0	3,E-04	3,E-02
GO:0003700~transcription factor activity, sequence-specific DNA binding	19	12	2,3	1,E-03	8,E-02
GO:0003677~DNA binding	27	16	1,9	2,E-03	8,E-02
GO:0003714~transcription corepressor activity	8	5	4,6	2,E-03	8,E-02



Supplemental Figure S3: A, Identification of IPA isoforms that are regulated by Doxo relative to matched last-exon isoform (IPA:LE ratio) by 3'-seq on polysomes of MCF-7 cells treated with Doxo or vehicle for 18 hours. Data for abundant and annotated IPA isoforms are shown. **B**, RT-qPCR analysis of IPA:LE isoform ratio regulation by Doxo in the *WNK2* gene in the cytosol and polysomes at 0, 6 or 18 hours of Doxo treatment. **C**, Relative translation efficiency (TE) of IPA and LE isoforms measured by 3'-seq at different time points of Doxo treatment. Only IPA isoforms whose abundance equalizes at least 25% of matched LE isoforms are shown. **D-E**, Gene Ontology terms enriched in the 344 and 197 genes identified in Fig. 3D (**D**) and Fig. 3A (**E**), respectively. **F**, Western blot analysis of MCF-7 cells using antibodies against N-terminal regions of PATZ1 and MAP3K5 (and GAPDH as a loading control). For both genes, we detected a band at the expected size that was decreased by IPA-targeting siRNAs (pool of siIPA#1 and #2 from Fig. 3E), providing evidence for the existence of these IPA isoforms at the protein level. For PATZ1, the IPA isoform was nearly as abundant as the LE isoform in untreated cells and at 6 hours of Doxo treatment and became relatively minor at 18 hours. For MAP3K5, we could not detect the LE isoform; the IPA isoform was abundant at 6 hours of Doxo treatment and declined at 18 hours. For both IPA isoforms, whether their protein abundance may be increased by Doxo at some time point or may be limited by protein degradation remains to be determined.

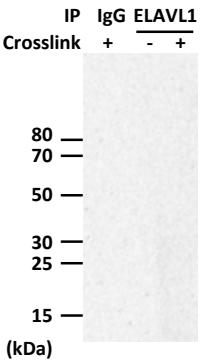
A



B

Term	Count	%	Fold Enrichment	PValue	FDR
GO:0005515~protein binding	860	57	1,2	5,E-21	5,E-18
GO:0005654~nucleoplasm	337	22	1,6	9,E-20	6,E-17
GO:0005829~cytosol	378	25	1,5	6,E-18	2,E-15
GO:0005737~cytoplasm	522	35	1,3	2,E-13	4,E-11
GO:0005524~ATP binding	199	13	1,7	1,E-13	6,E-11
GO:0004674~protein serine/threonine kinase activity	72	5	2,4	5,E-12	2,E-09
GO:0005634~nucleus	517	34	1,2	7,E-10	1,E-07
GO:0005813~centrosome	71	5	2,2	8,E-10	1,E-07
GO:0006468~protein phosphorylation	80	5	2,2	3,E-11	1,E-07
GO:0051056~regulation of small GTPase mediated signal transduction	34	2	3,2	3,E-09	6,E-06
GO:0005096~GTPase activator activity	49	3	2,2	3,E-07	8,E-05
GO:0004672~protein kinase activity	58	4	2,0	3,E-07	8,E-05
GO:0043547~positive regulation of GTPase activity	83	6	1,8	7,E-08	9,E-05
GO:0017137~Rab GTPase binding	29	2	2,7	2,E-06	4,E-04
hsa04120:Ubiquitin mediated proteolysis	28	2	2,8	2,E-06	4,E-04
GO:0016874~ligase activity	45	3	2,1	4,E-06	6,E-04
GO:0061630~ubiquitin protein ligase activity	34	2	2,3	1,E-05	2,E-03
GO:0005856~cytoskeleton	53	4	1,9	2,E-05	2,E-03
GO:0005794~Golgi apparatus	101	7	1,5	2,E-05	2,E-03
GO:0005085~guanyl-nucleotide exchange factor activity	25	2	2,7	2,E-05	2,E-03
GO:0005815~microtubule organizing center	28	2	2,4	4,E-05	3,E-03
GO:0004842~ubiquitin-protein transferase activity	49	3	1,9	3,E-05	3,E-03
GO:0043231~intracellular membrane-bounded organelle	70	5	1,6	5,E-05	4,E-03
GO:0005913~cell-cell adherens junction	45	3	1,8	1,E-04	9,E-03
GO:0005089~Rho guanyl-nucleotide exchange factor activity	18	1	2,9	9,E-05	9,E-03
GO:0004386~helicase activity	19	1	2,8	1,E-04	9,E-03
GO:0035091~phosphatidylinositol binding	19	1	2,8	1,E-04	9,E-03
GO:0000139~Golgi membrane	71	5	1,6	2,E-04	1,E-02
GO:0008270~zinc ion binding	128	9	1,4	1,E-04	1,E-02
GO:0016301~kinase activity	37	2	1,9	2,E-04	1,E-02
GO:0035023~regulation of Rho protein signal transduction	20	1	3,1	2,E-05	1,E-02
GO:0005802~trans-Golgi network	24	2	2,3	3,E-04	1,E-02
GO:0004702~receptor signaling protein serine/threonine kinase activity	14	1	3,3	2,E-04	1,E-02
hsa04360:Axon guidance	23	2	2,5	1,E-04	2,E-02
GO:0005643~nuclear pore	16	1	2,9	3,E-04	2,E-02
GO:0043021~ribonucleoprotein complex binding	10	1	4,2	4,E-04	3,E-02
GO:0018105~peptidyl-serine phosphorylation	25	2	2,5	4,E-05	3,E-02
GO:0007030~Golgi organization	18	1	3,0	6,E-05	3,E-02
GO:0035556~intracellular signal transduction	56	4	1,7	6,E-05	3,E-02
GO:0016192~vesicle-mediated transport	28	2	2,3	6,E-05	3,E-02
GO:0005086~ARF guanyl-nucleotide exchange factor activity	9	1	4,5	5,E-04	3,E-02
GO:0046872~metal ion binding	204	14	1,2	6,E-04	4,E-02
GO:0005545~1-phosphatidylinositol binding	8	1	5,0	6,E-04	4,E-02
GO:0032266~phosphatidylinositol-3-phosphate binding	10	1	3,9	6,E-04	4,E-02
GO:0030123~AP-3 adaptor complex	6	0	7,1	8,E-04	4,E-02
GO:0030027~lamellipodium	25	2	2,0	1,E-03	5,E-02
GO:0005912~adherens junction	12	1	3,1	1,E-03	5,E-02
GO:0019898~extrinsic component of membrane	16	1	2,5	1,E-03	5,E-02
GO:0031965~nuclear membrane	32	2	1,8	1,E-03	5,E-02
GO:0051010~microtubule plus-end binding	6	0	6,9	1,E-03	5,E-02
GO:0016925~protein sumoylation	23	2	2,5	1,E-04	5,E-02
GO:0045893~positive regulation of transcription, DNA-templated	66	4	1,6	1,E-04	5,E-02
GO:0015629~actin cytoskeleton	30	2	1,8	3,E-03	8,E-02
GO:0005938~cell cortex	20	1	2,1	3,E-03	8,E-02
GO:0051015~actin filament binding	22	1	2,1	2,E-03	9,E-02

C



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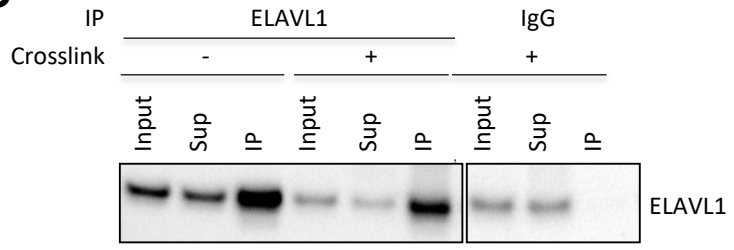

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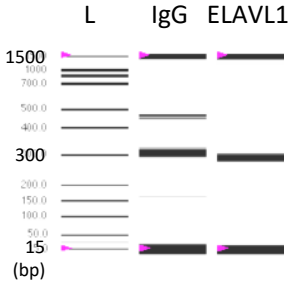
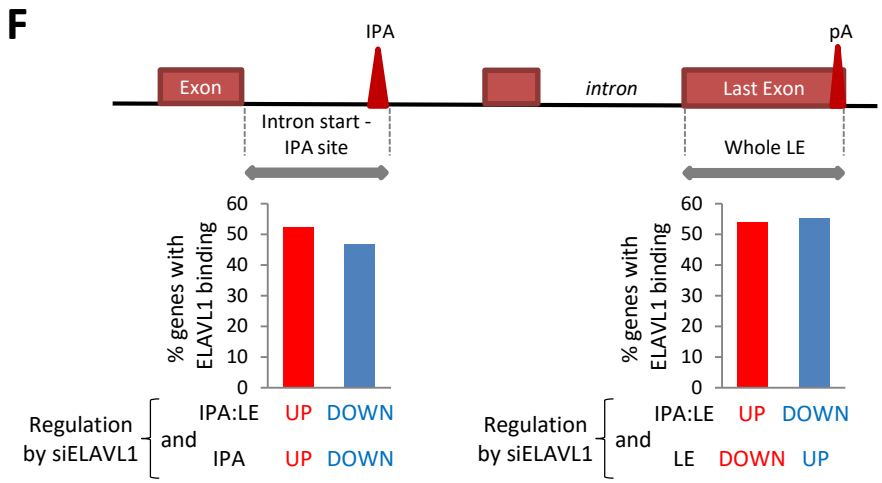
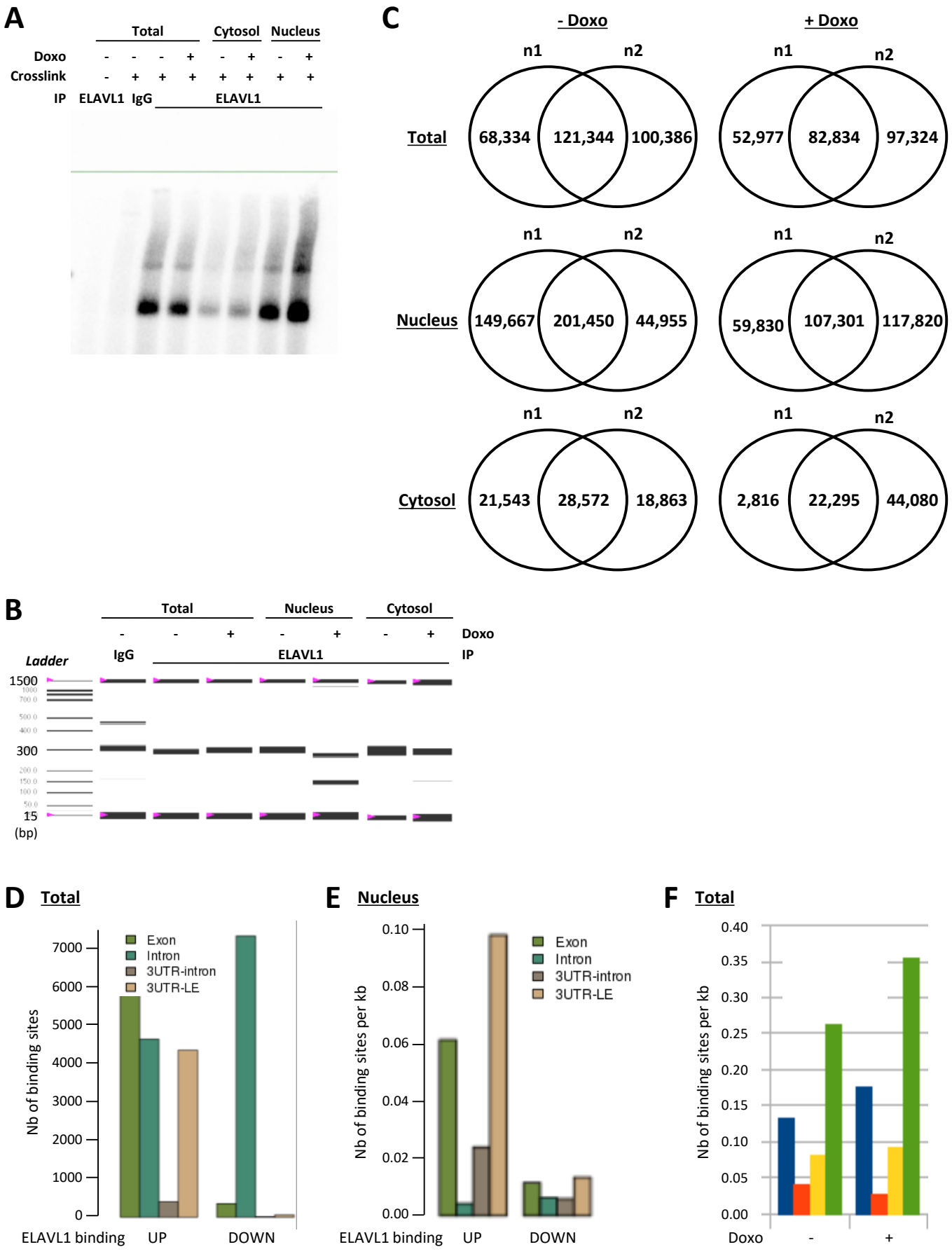


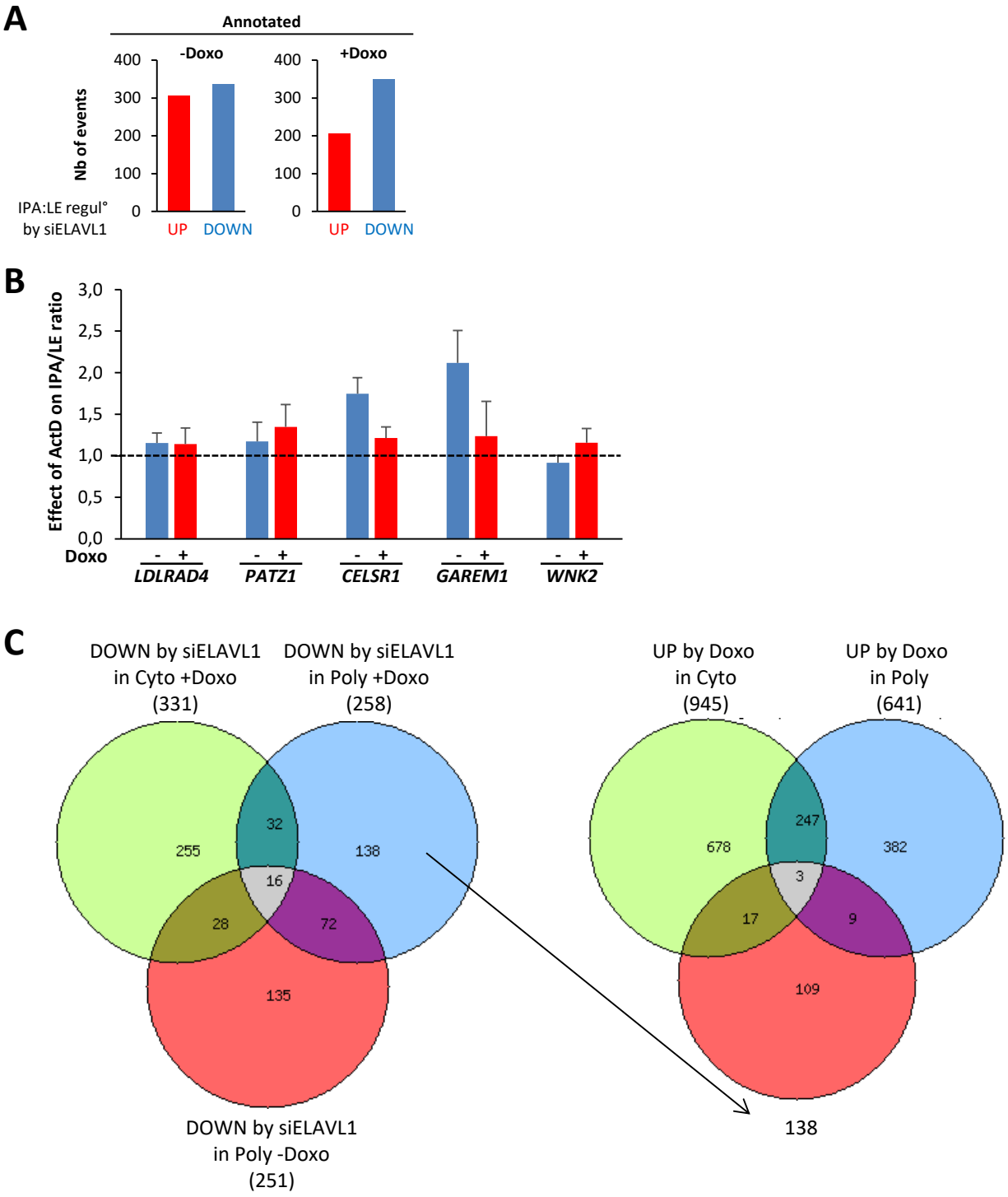


Supplemental Figure S4: A, Identification by 3'-seq on whole MCF-7 cells of annotated IPA isoforms, for which the IPA:LE ratio is regulated by siELAVL1 when compared to siCTRL. **B**, Gene Ontology terms enriched in the genes with IPA:LE isoform ratio regulation by siELAVL1, which were identified in Fig. 4B. In red, GO terms that were also found in Fig. S3 D-E. **C**, Gel electrophoresis of RNA-protein complexes from MCF-7 whole cell lysates (UV crosslinked or not) labelled with γ-^{32}P ATP after immunoprecipitation with either anti-ELAVL1 or total mouse immunoglobulins G (IgG). **D**, Western blot analysis of ELAVL1 protein levels in MCF-7 whole cell lysates (UV crosslinked or not) either before (input) or after immunoprecipitatation with ELAVL1 or total mouse IgG. Sup, supernatant. IP, immunoprecipitate. **E**, iCLIP libraries obtained from MCF-7 whole cell lysates with either anti-ELAVL1 or total mouse IgG and run on Experion automated electrophoresis system. L, ladder. bp, base pairs. **F**, Fraction of genes that have an ELAVL1 binding site in the IPA region, among the genes that have their IPA isoform levels either up- or down-regulated by siELAVL1 (left panel). Fraction of genes that have an ELAVL1 binding site in the LE region, among the genes that have their LE isoform levels either up- or down-regulated by siELAVL1 (right panel).



Supplemental Figure S5: A, Gel electrophoresis of RNA-protein complexes labelled with γ-^{32}P ATP after ELAVL1 immunoprecipitation of whole cell, nuclear or cytosolic lysates from MCF-7 cells treated with Doxo or vehicle. Also showing controls without UV crosslink or with total mouse IgG. **B**, iCLIP libraries obtained with either anti-ELAVL1 or total mouse IgG and with either whole cell, nuclear or cytosolic lysates from MCF-7 cells treated with Doxo or vehicle. Libraries were run on Experion automated electrophoresis system. bp, base pairs. **C**, Venn diagrams comparing the peaks found in two biological

replicates of ELAVL1 iCLIP in the indicated conditions and fractions of MCF-7 cells. **D**, Number of Doxo up- and down-regulated ELAVL1 binding sites from total cells in exons, introns, 3'UTRs overlapping introns, and 3'UTRs overlapping the last exon of genes. **E**, Density of Doxo up- and down-regulated ELAVL1 binding sites in nucleus in exons, introns, 3'UTRs overlapping introns, and 3'UTRs overlapping the last exon of genes. **F**, Density of ELAVL1 binding sites from vehicle-treated and Doxo-treated total cells in exons, introns, 3'UTRs overlapping introns, and 3'UTRs overlapping the last exon of genes.



Supplemental Figure S6: A, Identification of annotated IPA isoforms, for which the IPA:LE ratio is regulated by siELAVL1 when compared to siCTRL, by 3'-seq analysis on cytosol of MCF-7 cells grown either without or with Doxo. **B**, RT-qPCR analysis of the IPA:LE isoform ratio for the five indicated genes in MCF-7 cells treated with or without Doxo for 6 hours and then with or without actinomycin D for another 6 hours. **C**, 3'-seq analysis of IPA:LE isoform ratio regulation by ELAVL1 and Doxo in cytosol and polysomes. Left, Venn diagram comparing genes with IPA:LE down-regulation by ELAVL1 depletion in Doxo-treated cytosol and polysomes and in untreated polysomes. Right, Venn diagram comparing genes with IPA:LE down-regulation by ELAVL1 depletion and IPA:LE up-regulation by Doxo. (These Venn diagrams were made with gene symbols, while those of Fig. 3A were made with IPA sites.) To determine whether ELAVL1 may mediate isoform-specific translational regulation by Doxo (*e.g.*, IPA:LE ratio up-regulation in polysomes but not cytosol as described in Fig. 3A), 3'-seq analyses on cytosol and polysomes of Doxo-treated and untreated cells transfected with siCTRL (shown in Fig. 3A) were carried out in parallel in cells transfected with siELAVL1. Through these analyses, we identified 138 genes with IPA:LE ratio down-regulation by ELAVL1 depletion, specifically in polysomes (not cytosol) of Doxo-treated (not untreated) cells (left panel). However, only 9 of these 138 events corresponded to IPA:LE events that were up-regulated by Doxo in polysomes and not cytosol (right panel). These data

suggest that in most cases, IPA:LE ratio up-regulation events found in polysomes but not cytosol in response to Doxo are not mediated by ELAVL1.