Supplementary Information

Localized regulation of cell junction mRNAs is required for epithelial cell integrity

Ashley Chin[1,2], Jonathan Bergeman[1], Laudine Communal[3,4], Seda Barutcu[1], Jonathan Boulais[1], Gene W. Yeo[5-7], Anne-Marie Mes-Masson[3,4,8], Eric Lécuyer[1,2,9*]

1- Institut de Recherches Cliniques de Montréal (IRCM), Montréal, Canada;

2- Division of Clinical and Translational Research, McGill University, Montréal, Canada;

3- Institute du Cancer de Montréal, Montréal, Canada;

4- Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montréal, Canada

5- Department of Cellular and Molecular Medicine, University of California San Diego (UCSD), La Jolla, CA, USA;

6- Stem Cell Program, UCSD, Sanford Consortium for Regenerative Medicine, La Jolla, CA, USA;

7- Institute for Genomic Medicine, UCSD, La Jolla, CA, USA;

8- Department of Medicine, Université de Montréal, Montréal, Canada;

9- Department of Biochemistry and Molecular Medicine, University of Montreal, Montréal, Canada.

*Corresponding author:

Dr. Eric Lécuyer

Director of RNA Biology, IRCM

110 Pine Avenue West

Montreal, Canada, H2W 1R7

eric.lecuyer@ircm.qc.ca

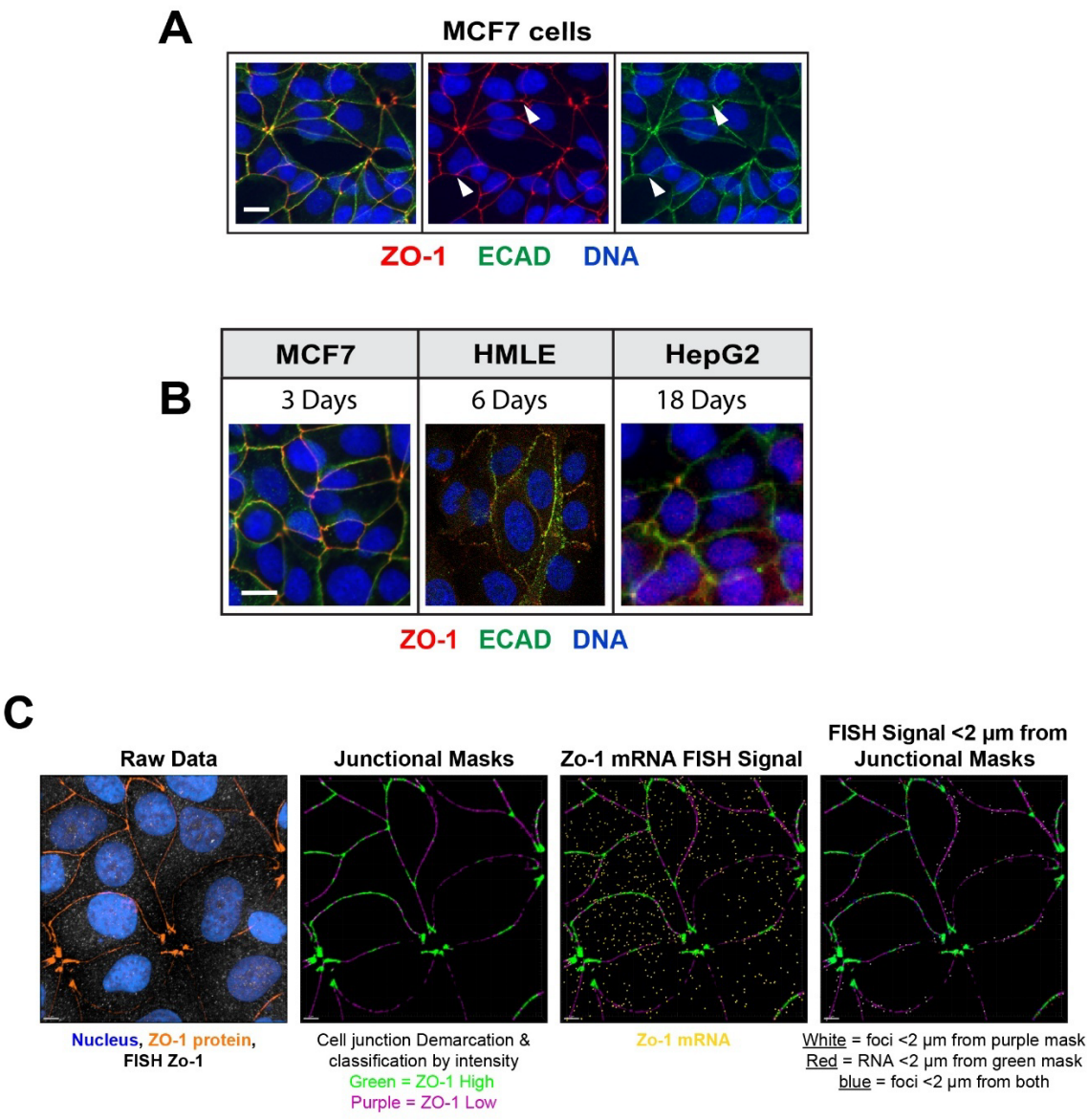
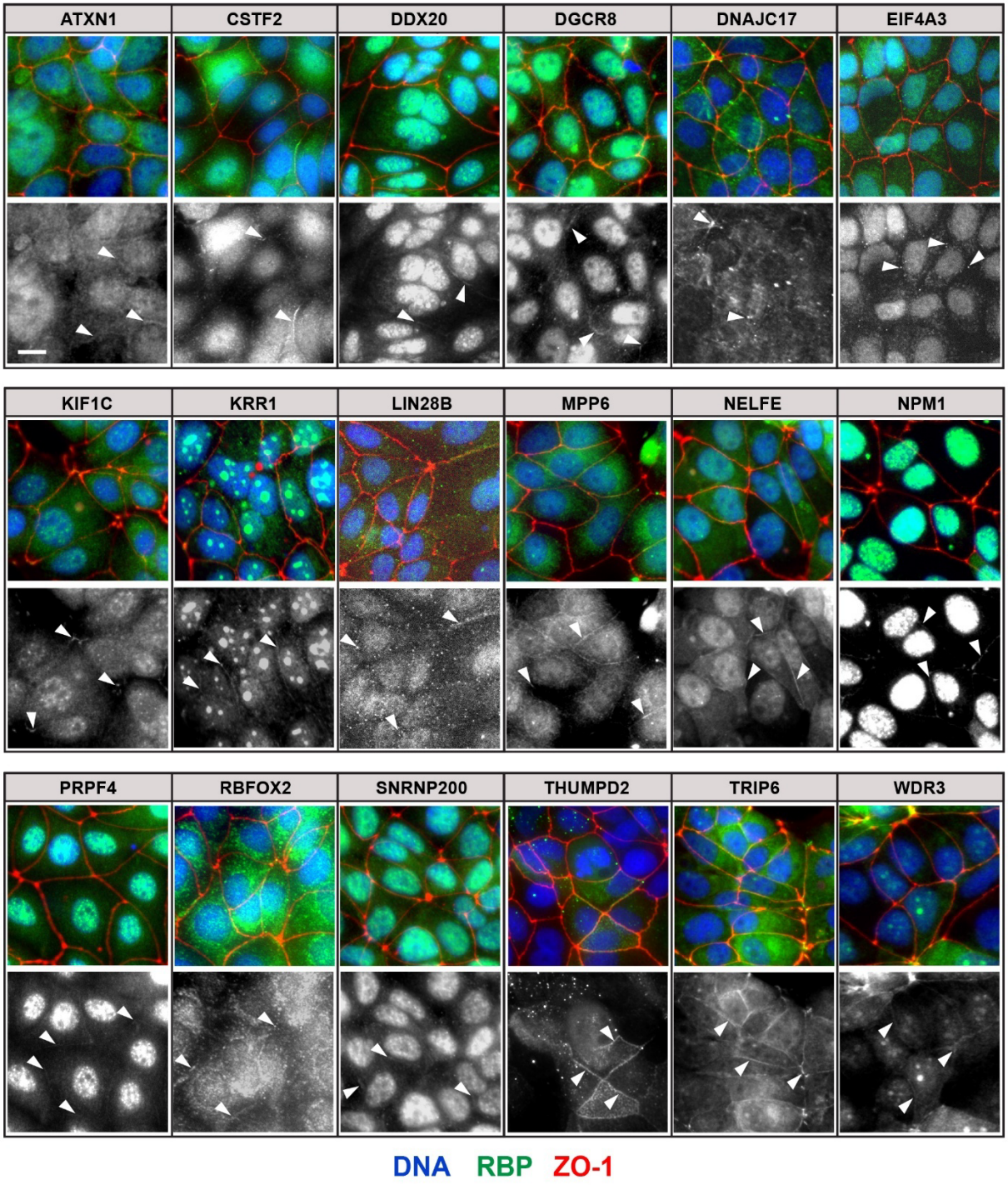


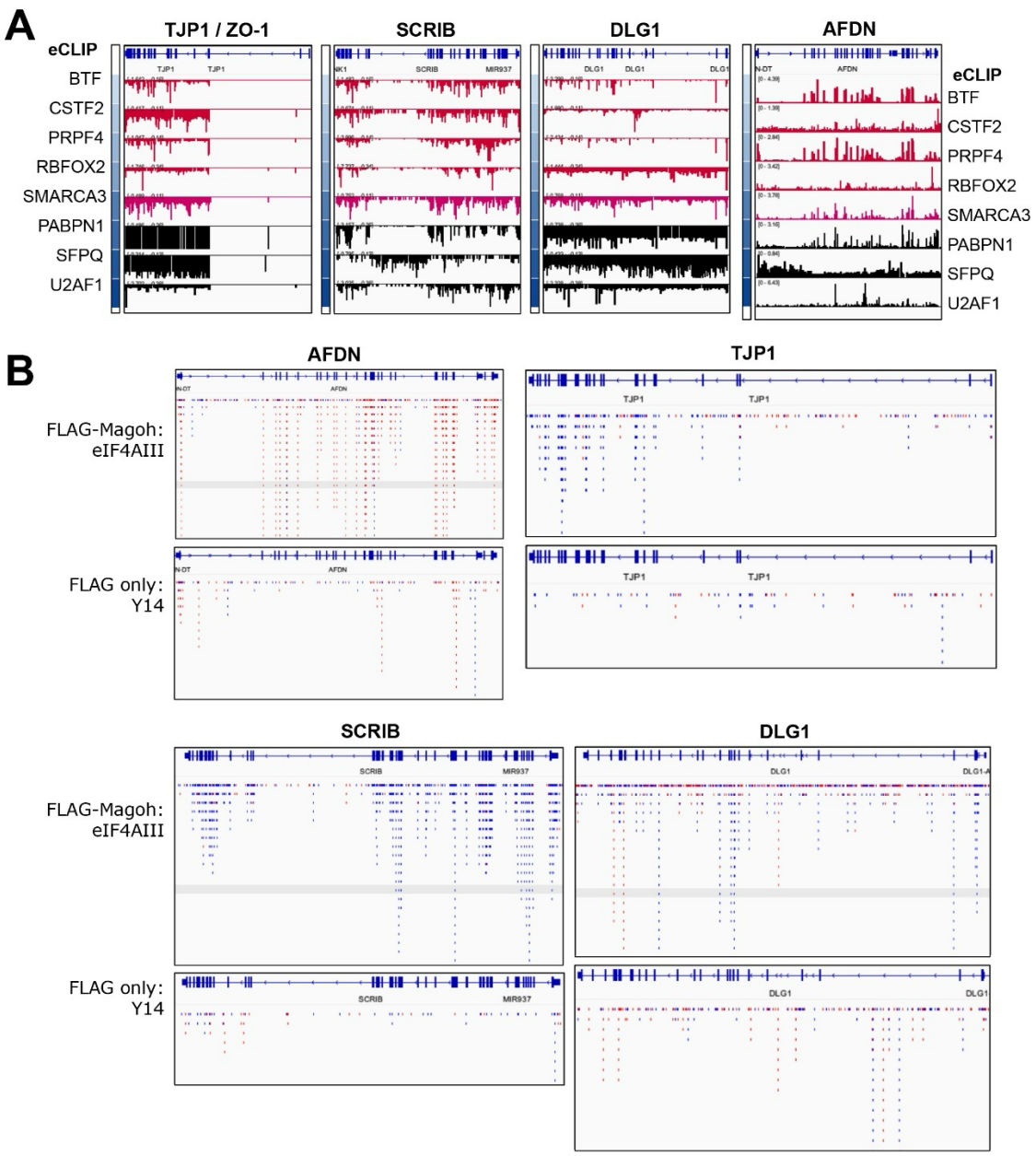
Figure S1. Time course of ZO-1 marker labeling in epithelial cellular models and schematic of image analysis approach to quantify mRNA foci localized to cell junction regions.

(A) Representative IF images of ZO-1 (red) and ECAD (green) co-labeling in MCF-7 cells at three days post-seeding. Arrowhead with star points to tri-cellular enrichment in ZO-1. Blue, DNA. Scale bar represents 30µm. **(B)** Representative IF of ZO-1 (red) and ECAD (green) proteins in MCF7, HEPG2 or HMLE cells at the indicated number of days post-seeding. Blue, DNA. Scale bar represents 30µm. **(C)** Example of image analysis approach to quantify the number of smiFISH mRNA foci detected in proximity to junctional markers. Using Imaris software, marker-labeled cell junction are identified and segmented using the Surface tool. For Figure 1E and 1F only, these segmented junctions were further sub-classified according to their intensity of ZO-1 expressoin (high or low).

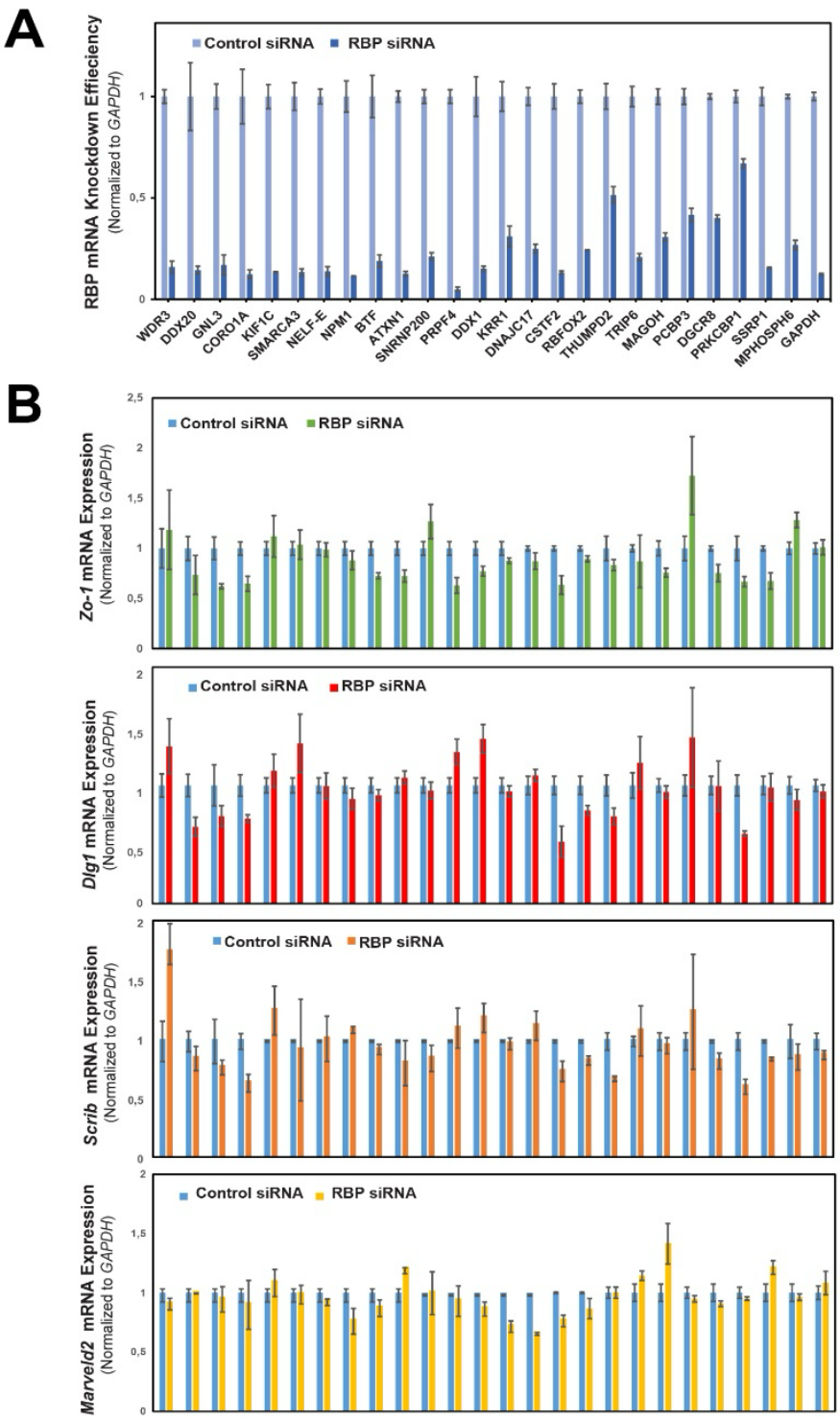


<u>Figure S2.</u> Examples of RBPs exhibiting localization to cell-cell junction regions.

Representative images for candidate RBPs (green) with cell junction IF patterns in relation to ZO-1 protein (red) in MCF7 cells. Arrowheads illustrate areas of co-localization with ZO-1. Blue, DNA. Scale bar represents 20 µm.



<u>Figure S3.</u> Genome browser visualization of eCLIP and RIPiT sequencing reads across candidate cell junction genes. (A) Genome-mapped reads stemming from eCLIP data for the indicated RBPs were obtained from the ENCORE database as bigwig files and visualized by IGV browser with an autoscale for individual eCLIP samples across the indicated genes. **(B)** Mapped read counts for the indicated RIPiT specimens, including the tandem purification of FLAG-MAGOH followed by eIF4AIII (FLAG-Magoh:eIF4AIII) or the negative control FLAG only purification followed by Y14, are visualized across the 4 genes encoding junctional in the IGV browser. The high read count regions were cropped to visualize the background and low read-count regions. Note the much higher density of positive strand reads in the samples from tandem FLAG-Magoh:eIF4AIII purifications compared to control FLAG only-1Y14 purifications.



<u>Figure S4.</u> RT-qPCR gene expression analysis of cells transfected with siRNAs targeting the mRNAs of specific cell junction localized RBPs. RT-qPCRs were conducted on total RNA extracted from MCF7 cells transfected with control scrambled siRNA or with siRNAs targeting the mRNAs of specific RBPs. Histograms reveal the relative expression level (normalized to *GAPDH* mRNA) of **(A)** mRNA transcripts for the targeted RBPs, or **(B)** mRNAs encoding cell junction/polarity associated transcripts, including *Zo-1*, *Dlg1*, *Scrib* and *Marveld2*.

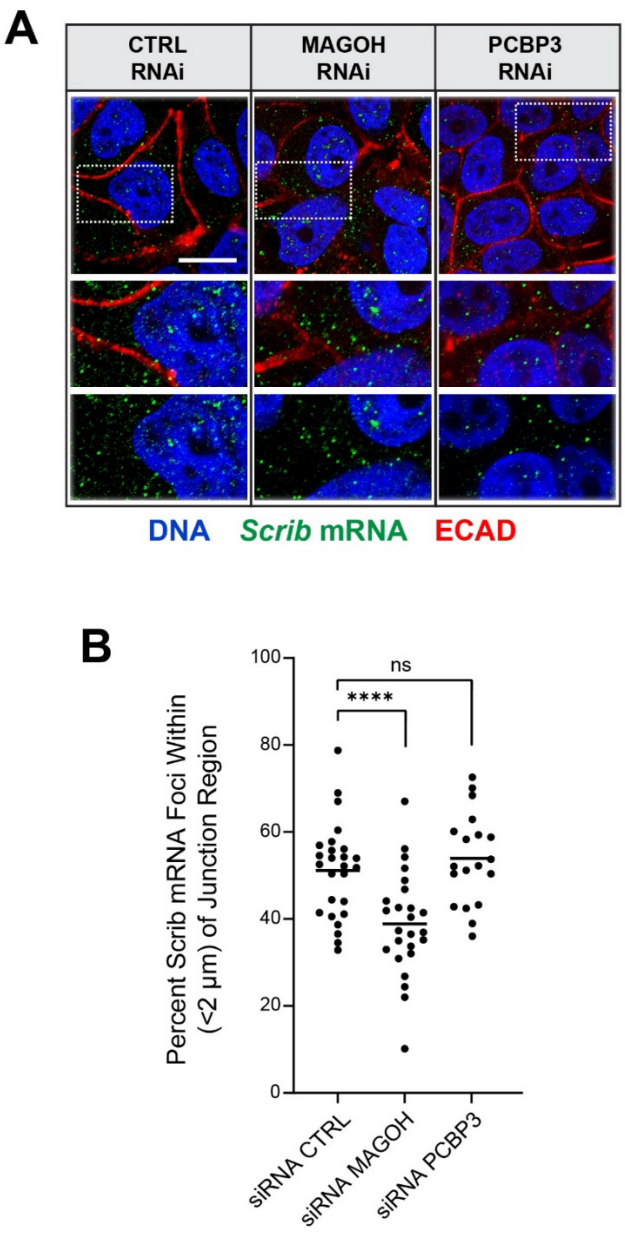


Figure S5. Impact of MAGHO and PCBP3 knock-downs on *Scrib* mRNA localization to junctional regions. (A) Immuno-smFISH co-labeling of *Scrib* mRNA (green) and ECAD protein (red) in MCF-7 cells transfected with scrambled siRNA control (CTRL), or siRNAs targeting MAGOH or PCBP3. Blue, DNA. Scale bar represents 20µm. **(B)** Quantifications of immuno-smFISH labeling as conducted in (A) revealing the percentage of *Scrib* mRNA foci that localize in proximity (<2µm) to junctional regions. Statistical significance was assessed by two-tailed t-tests; **** denotes a p-value of <0.0001; 'ns' denotes non-significance.

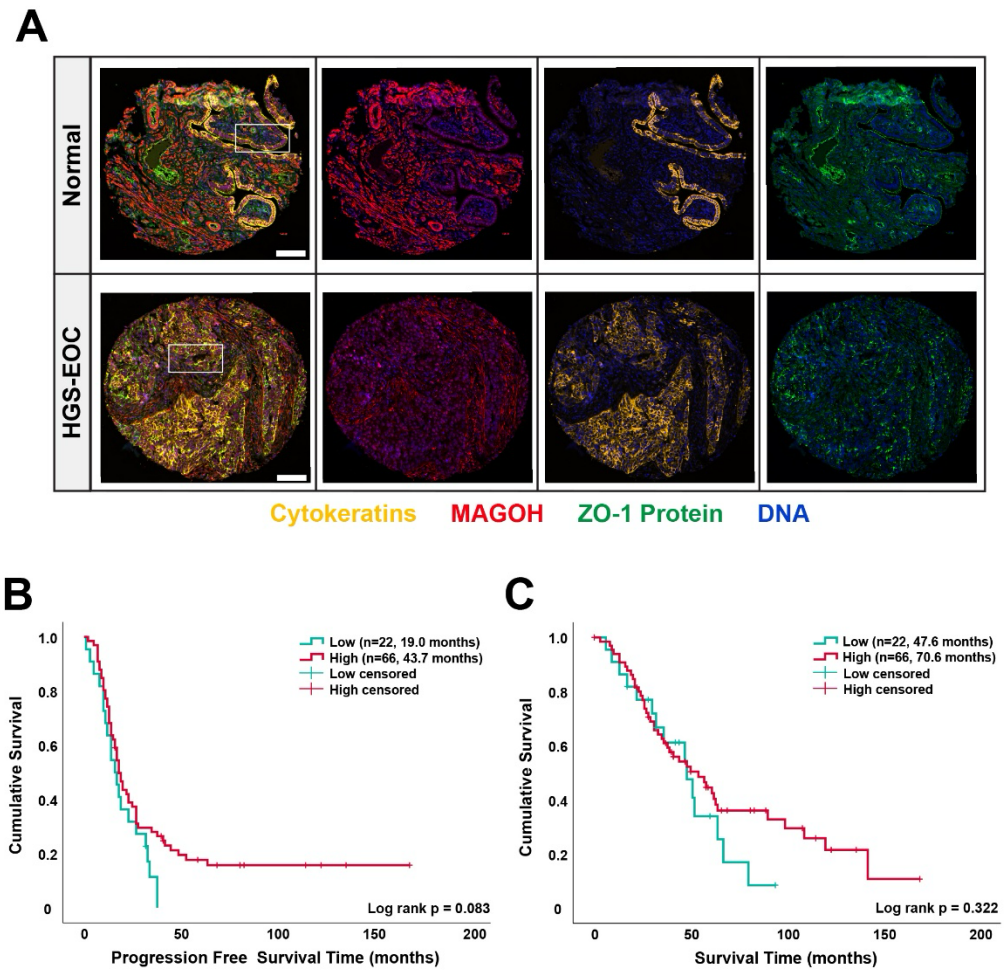


Figure S6. Visualization of ZO-1 and MAGOH protein expression in tissue microarrays of a discovery cohort of ovarian cancer specimens.

(A) Examples of IF results obtained for ZO-1 and MAGOH proteins on TMA panels consisting of surgically extracted human ovarian tissues, including normal fallopian tubes (n=15) and HGS-EOC tumor specimens (n=101) **.** Normal tissues reveal that MAGOH expression is expressed in both epithelium and surrounding stroma, with organized Cytokeratins and ZO-1 staining at the highly polarized epithelium. However, a general disorganization of these three markers is observed in HCS. White squares denote the enlarged view of the selected region shown in **Figure 7D**. DAPI (blue), MAGOH (red), Cytokeratins (yellow), ZO-1 (green) and DAPI staining are shown. Scale bars represent 100µM. **(B)** Kaplan-Meier curves of MAGOH expression and its association with progression-free survival. MFI was used as measure of expression for markers, which was dichotomized by the median for MAGOH by the 25th percentile cutoff, into groups belonging to low (n = 22) or high expression (n = 66). P-value of 0.083 was observed by Mantel-Cox analysis. **(C)** Kaplan-Meier curves of MAGOH expression and its association with survival time. MFI was quantified for markers, which was separated into groups as above. P-value of 0.322 was observed by Mantel-Cox analysis.

Table. S1. General information on candidates identified from RBP-focused screen which exhibited cell junction-like distribution patterns. Table detailing description of proteins identified, signal strength and localization attributes in MCF7.

Mammalian RBP	Description	Junctional Signal	Nuclear Localization	Cytoplasmic Localization
ATXN1	Spinocerebellar ataxia type protein	Weak	Yes	Yes
CORO1A	Coronin, actin binding protein	Weak	Yes	Yes
CSTF2	Cleavage stimulation factor subunit	Weak	Yes	Yes
DDX20	DEAD box polypeptide	Weak	Yes	Yes
DGCR8	Microprocessor complex subunit	Strong	Yes	Yes
DNAJC17	DnaJ homolog	Weak	Yes	Yes
EIF4A3	Component of EJC	Weak	Yes	Yes
GNL3	Guanine nucleotide binding protein-like	Weak	Yes	Yes
KIF1C	Kinesin family member	Weak	Yes	Yes
KRR1	Small subunit processome component	Weak	Yes	Yes
LIN28B	Suppressor miRNA biogenesis	Weak	Yes	Yes
MAGOH	Component of EJC	Strong	Yes	Yes
MPP6	M-phase phosphoprotein	Strong	Yes	Yes
NELF-E	Negative elongation factor complex	Strong	Yes	Yes
NPM1	Nucleophosmin	Strong	Yes	No
PCBP3	Poly(RC) binding protein	Strong	Yes	Yes
PRKCBP1	Zinc finger, MYND-type	Strong	Yes	Yes
PRPF4	Pre-mRNA processing factor	Weak	Yes	Yes
RBFOX2	Fox-1 homolog	Weak	Yes	Yes
SNRNP200	Small nuclear ribonucleoprotein	Weak	Yes	Yes
SSRP1	Structure specific recognition protein	Strong	Yes	Yes
THUMPD2	THUMP domain containing protein	Weak	Yes	Yes
TRIP6	Thyroid hormone receptor interactor	Strong	Yes	Yes
WDR3	WD repeat domain	Strong	Yes	No

Table. S2. Primers used for RIP-RTqPCR assays. A list showing the multiple primer sets utilized for testing association of RBP candidates with selected cell junction mRNAs when compared to housekeeping controls.

Primer ID	Sequence
Zo1_1_Fw	ggaggtagaacgaggcatc
Zo1_1_Rv	gagcggacaaatcctctctg
Zo1_2_Fw	ttgcaatatctggtggacg
Zo1_2_Rv	tgaaactccgttaaccattgc
Zo1_3_Fw	aaagagatgaacgggctacg
Zo1_3_Rv	ggggaggcctatcgtgtg
Scrib_1_Fw	agctgatcctcacggagaac
Scrib_1_Rv	ctcaaggagaggacgctgag
Scrib_2_Fw	agcccctgaagctggactac
Scrib_2_Rv	ctaggggagcagggagattc
Scrib_3_Fw	ctcactgacctgctgctgtc
Scrib_3_Rv	aggttctccgtgaggatcag
Dlg1_1_Fw	caacccacacattggagatg
Dlg1_1_Rv	cctgcttctttcaacgcttc
Dlg1_2_Fw	cgttaatggcacagatgcag
Dlg1_2_Rv	catctccaatgtgtgggttg
Dlg1_3_Fw	aacgagcccgattaaaaac
Dlg1_3_Rv	ttcactatcgctggcattag
Afdn_1_Fw	aatgggccttagcattgttg
Afdn_1_Rv	accagacttcgtccatccac
Afdn_2_Fw	gaatgctgtcctctcccaag
Afdn_2_Rv	ttcccgatcatctttgttcc
Afdn_3_Fw	caaagctttaccgccttcag
Afdn_3_Rv	tcacagtgaccactccatcc
Rps16_1_Fw	tgcagtctgtgcaggtcttc
Rps16_1_Rv	cgttcaccttgatgagacc

Rps16_2_Fw	ggcaatggtctcatcaagg
Rps16_2_Rv	accacccttacacggacac
Rps16_3_Fw	tggtgtagacatccgtgtcc
Rps16_3_Rv	ttcttggaagcctcatccac
Ppia_1_Fw	cctaaagcatacgggtcctg
Ppia_1_Rv	ggcctccacaatattcatgc
Ppia_2_Fw	agggttcctgctttcacag
Ppia_2_Rv	caggacccgtatgctttagg
Ppia_3_Fw	ggcctggataccaagaagtg
Ppia_3_Rv	gtctttgggaccttgtctgc

Table. S3. Primers used for testing siRNA efficiency. A list showing the primer sequences used for testing knockdown of RBP candidates.

Primer ID	Sequence
WDR3_Fw	ggggttggttctgttgtcag
WDR3_Rv	ttgattttcttggggtctgg
DDX20_Fw	tacattggggctgacagtga
DDX20_Rv	cccaatccacacattcttcc
GNL3_Fw	gccagagatcctcttggttg
GNL3_Rv	tgaggctctgaacaccactg
CORO1A_Fw	ccttctttgaccctgacacc
CORO1A_Rv	tggcgatctcacacttgttc
KIF1C_Fw	tcacccaccacacataatgg
KIF1C_Rv	tctcctcccatgtctcgttc
HLTF_Fw	accagtgaaaaggcagatgg
HLTF_Rv	gcgggagctagacaattctg
NELF-E_Fw	ccgttccagaggagcatatc
NELF-E_Rv	ttcatagccccagtcaaagc
NPM1_Fw	ttgttgaagcagaggcaatg
NPM1_Rv	aatatgcactggccctgaac
ATXN-1_Fw	gtgaagcctccccttctacc
ATXN-1_Rv	gtggtctgaatgaccgtgtg
SNRNP200_Fw	agaagctggagctgcagaag
SNRNP200_Rv	tgtgagatgaaggcttgcag
PRPF4_Fw	agtcagattggggatgatcg
PRPF4_Rv	tgcaatcaggaacagaccag
KRR1_Fw	tttggaggagagcagtttcg
KRR1_Rv	caaacagtcatgctgccttc
DNAJC17_Fw	ccacccagacaaaaatccag
DNAJC17_Rv	cttgcttcttggctttcctg
RBFOX2_Fw	aagcccagtagttggagctg
RBFOX2_Rv	tcttcctgataggggcactg
THUMPD2_Fw	ccaggaagttggttgaatgc
THUMPD2_Rv	cagctggcagtccctattc
TRIP6_Fw	atgagctggataggctgacg
TRIP6_Rv	atccccaaccacatcttctc
MAGOH_Fw	aaagcgtgatggaggaact
MAGOH_Rv	ccttctggatccttggattg
PCBP3_Fw	atttgaccaagctccaccag
PCBP3_Rv	gactggcccatcagattttg

DGCR8_Fw	catcctgcacgagtacatgc
DGCR8_Rv	tcagggatgaggatttccag
SSRP1_Fw	gccaaactcgctaccacttc
SSRP1_Rv	atcttgcggtttaccagtgc
MPHOSPH6_Fw	gccagaaagagagaccatgc
MPHOSPH6_Rv	cctggggctttaagaacatc
GAPDH_Fw	atgttcgtcatgggtgtg
GAPDH_Rv	ggtgctaagcagttggtgg
ZO-1_Fw	ggaggtagaacgaggcatc
ZO-1_Rv	gagcggacaaatcctctctg
DLG1_Fw	gaggtacggacaacccacac
DLG1_Rv	cctgcttctttcaacgcttc
SCRIB_Fw	agctgatcctcacggagaac
SCRIB_Rv	ctcaaggagaggacgctgag