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Supplemental information

Multifaceted role of RNA editing in promoting loss-of-function of PODXL in cancer

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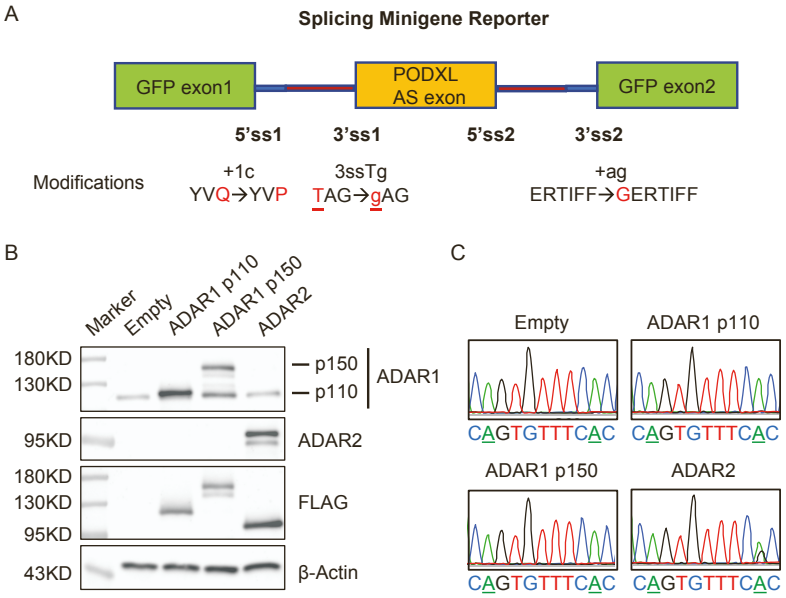

Figure S1. Co-transfection of ADARs with PODXL splicing reporters in HeLa cells, related to Figure 1.

(A) Illustration of different modifications (+1c, +ag, 3ssTg) made to the PODXL splicing reporters. This reporter contains two GFP split-exons that are upstream and downstream of the tested alternative exon. Two insertion modifications (+1c, +ag) were made to the splicing reporter to generate an in-frame transcript when PODXL alternative exon is included. The amino acid changes were indicated for each insertion modification. A T-to-G mutation was introduced to the 3' splice site of the PODXL alternative exon to increase the basal inclusion rate so that it approximately matches the endogenous exon inclusion level.

(B) Western blot showing the overexpression of ADARs in Hela cells. All ADARs are FLAG tagged. For ADAR1 p150 overexpression, the minor bands between p110 and p150 likely represent truncated proteins due to alternative translation initiation. For ADAR2 overexpression, the upper bands represent the FLAG-ADAR2 fusion proteins (see FLAG Western). The lower bands represent ADAR2 proteins without FLAG tagging, which may result from alternative translation start sites in the overexpression constructs.

(C) Sanger sequencing traces to detect the A714G and A722G editing sites (underlined As) on the reporters after co-transfection with the ADARs and the empty control in Hela cells.

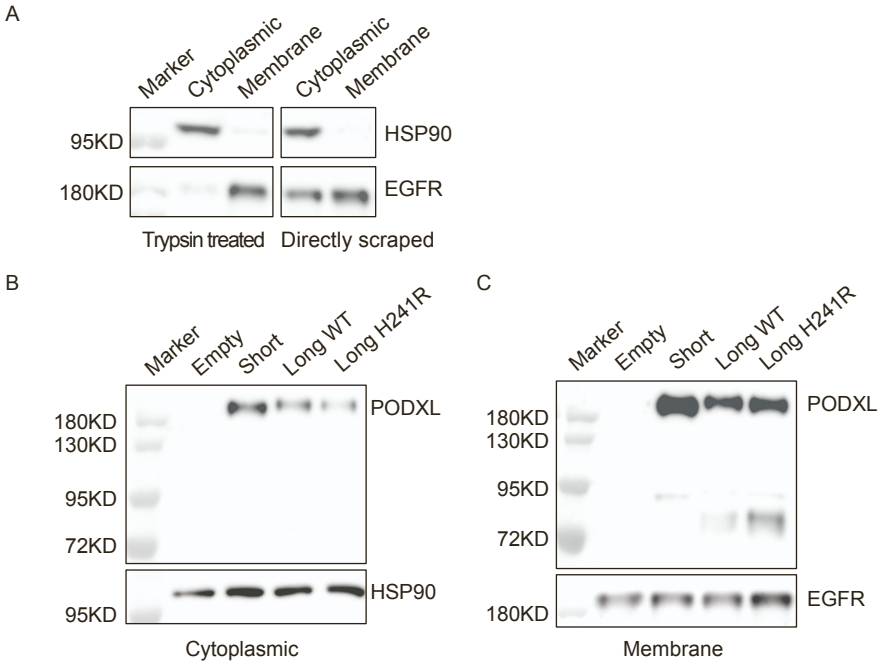

Figure S2. Cellular localizations of PODXL isoforms, related to Figure 2.
(A) Western blot detecting marker genes for cytoplasmic (HSP90) and membrane (EGFR) fractions of wild type A549 cells. Cell fractionations were performed with or without trypsin digestion (see Methods). Trypsin treated: cells treated with trypsin before cell fractionation. Directly scraped: cells directly lysed and scraped from cell culture plates for cell fractionation. Superfluous lanes were deleted and replaced with a space between the trypsin treated and directly scraped groups.
(B-C) Western blot detecting PODXL expression in the cytoplasmic (B) and membrane (C) fractions of A549 cells overexpressing different PODXL isoforms. Cells are directly scraped for cell fractionation.

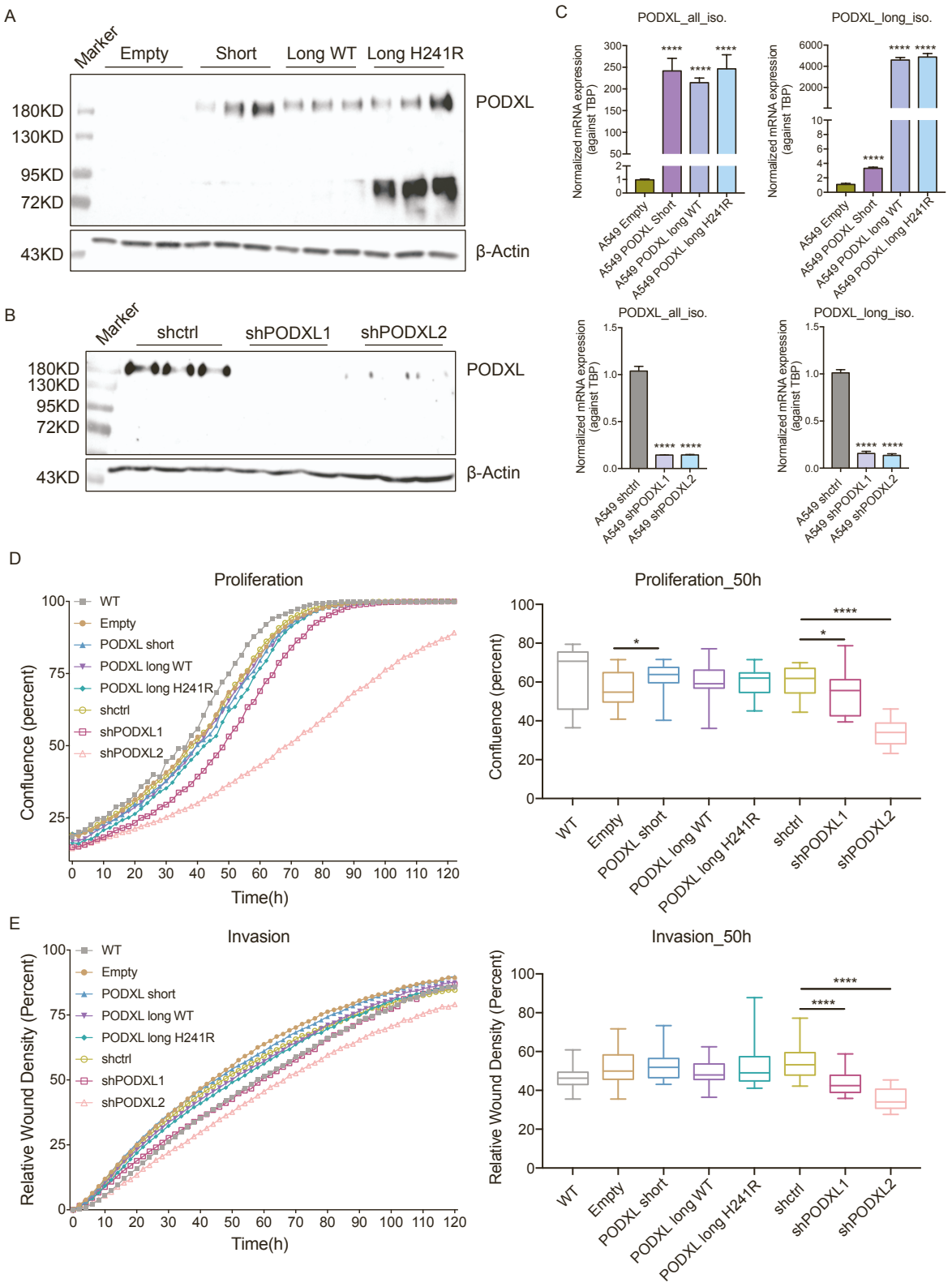

Figure S3. Cell proliferation and invasion assay of A549 cells with PODXL overexpression and knockdown, related to Figure 3.

(A-B) Western blot detecting PODXL overexpression (A) and knockdown (B) in A549 cells. Three biological replicates are shown.

(C) Normalized mRNA expression levels of all PODXL isoforms (PODXL_all_iso.) and the PODXL long isoform (PODXL_long_iso.) in A549 cells with PODXL overexpression or KD, and controls (WT, Empty, shctrl). Three biological replicates are included. Data are plotted as mean ± SEM. The *p*-values were calculated for each cell line compared to the corresponding controls (Empty or shctrl) using Student's t-test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

(D) Left: Cell proliferation curve of the A549 cells with PODXL overexpression or KD, and controls (WT, Empty, shctrl). The plot shows one set of experiment performed with three biological replicates. Right: Quantification of cell proliferation using cell confluence. Data at 50 h post wound creation are shown to examine the possible effect of cell proliferation on cell invasion shown in B. Two independent sets of experiments were performed with three biological replicates included in each experiment. Data are plotted as mean ± SEM. The *p*-values were calculated using Student's t-test (*p < 0.05, ***p < 0.001, ****p < 0.0001).

(E) Left: Cell invasion curve of the A549 cells with PODXL overexpression or KD, and controls (WT, Empty, shctrl). The plot shows one set of experiment performed with three biological replicates. Right: Quantification of cell invasion with relative wound density. Data at 50 h post wound creation are shown, when most cell lines reached around 50% relative wound density. Two independent sets of experiments were performed with three biological replicates included in each experiment. Data are plotted as mean ± SEM. The *p*-values were calculated using Student's t-test (*p < 0.05, ***p < 0.001, ****p < 0.0001).

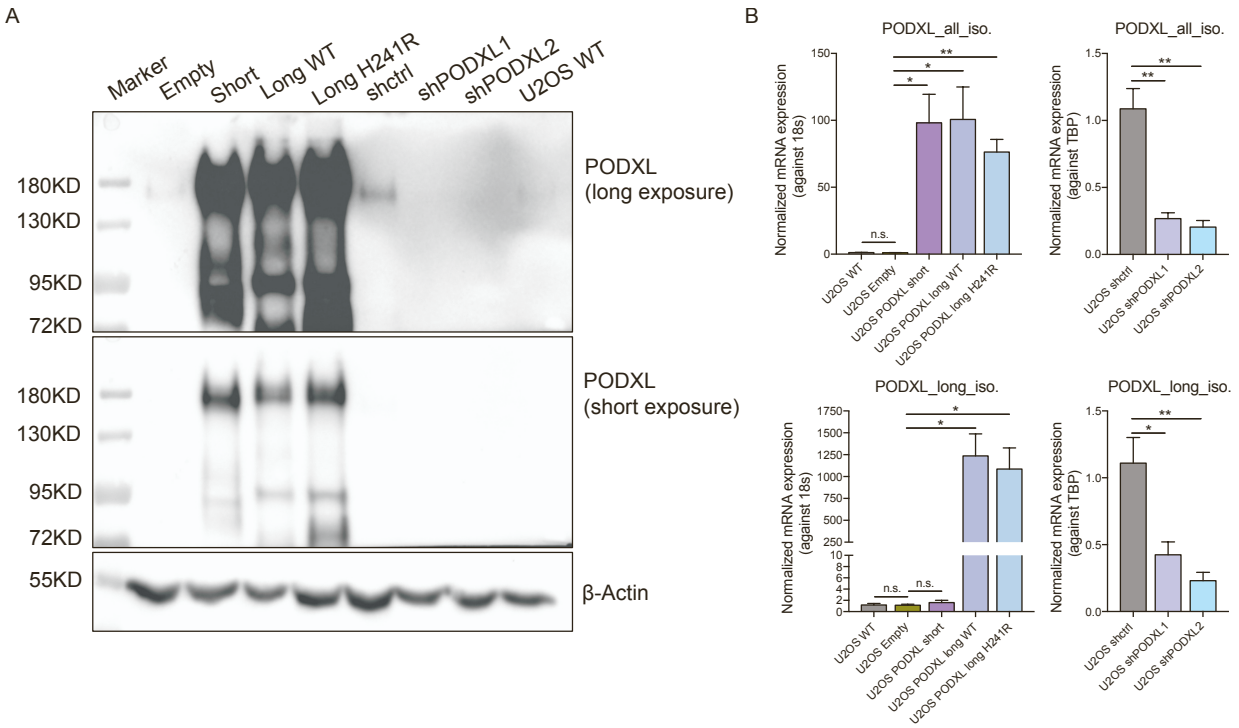

Figure S4. PODXL overexpression and knockdown in U2OS cells, related to Figure 4.
(A) Western blot detecting PODXL overexpression (A) and knockdown (B) in U2OS cells.
(B) Normalized mRNA expression levels of all PODXL isoforms (PODXL_all_iso.) and the PODXL long isoforms (PODXL_long_iso.) in U2OS cells with PODXL overexpression or KD, and controls (WT, Empty, shctrl). Three biological replicates are included. Data are plotted as mean ± SEM. The *p*-values were calculated using Student's t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n.s., not significant).

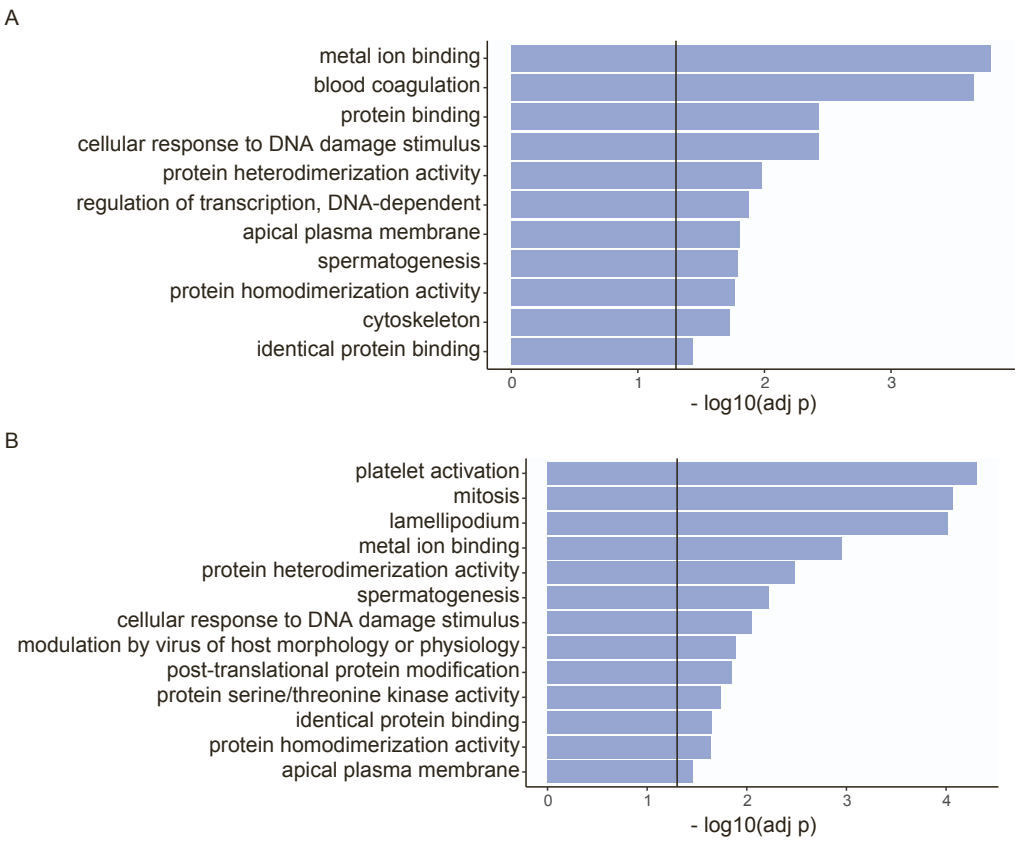

Figure S5. Gene ontology terms enriched in the genes with alternative exons containing RNA editing sites, related to Figure 6.

(A) Exon harboring recoding sites from REDIportal.

(B) Exons harboring any editing sites from REDIportal.

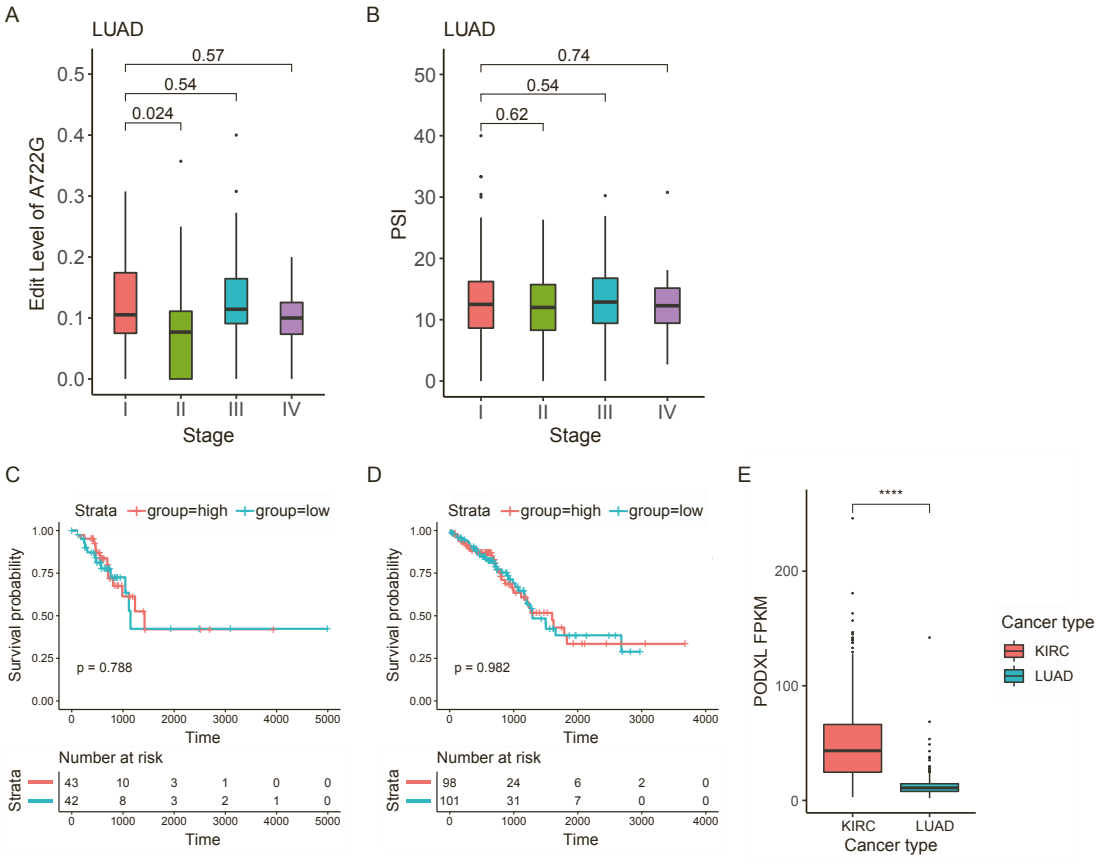

Figure S6. Clinical relevance of PODXL editing and splicing in LUAD, related to Figure 5.
(A) Editing level of the A722G site over stage progression of LUAD. The *p*-values were calculated using Wilcoxon rank sum test and annotated on the plot between each comparasion.
(B) PODXL alternative exon inclusion (measured by PSI) over stage progression of LUAD. The *p*-values were calculated using Wilcoxon rank sum testand annotated on the plot between each comparasion.
(C) Overall survival of LUAD patients seperated by editing levels of the A722G site. Patients were grouped into high (red) and low (blue) groups by editing level tertiles. The *p*-value was calculated by the log-rank test.
(D) Overall survival of LUAD patients seperated by PODXL alternative exon inclusion. Patients were grouped into high (red) and low (blue) groups by PSI tertiles. The *p*-value was calculated by the log-rank test.
(E) PODXL expression level in primary tumors of KIRC and LUAD in TCGA. The *p*-values were calculated using Wilcoxon rank sum test (****p <= 0.0001).

Table S1. Oligonucleotides used in this study, related to STAR Methods

Primers used for PODXL overexpression constructs

name	sequences	notes
PODXL_kozac_AgeI_F	ctaccggtcgccaccATGCGCTGCGCGCTGGCGC	adds kozac sequence
PODXL-EcoRI-R	tggcgaattcTTACTAGAGGTGTGTGTCTTC	
PODXL-A722G-F	ACAGTGTTTCGCCATGTCAGCC	introduces the recoding site mutation
PODXL-A722G-R	GCTGACATGGCGAAACACTGTCTCTAGT	
pLJM1-seq-R	gtggatctctgctgtccctg	plasmid sequencing

Primers used for PODXL shRNA constructs

name	sequences
PODXL_sh1_F (TRCN0000296029)	CCGGAGCCACGTAAGGGACTTTATACTCGAGTATAAAGTCCCTTACGTGGCTTTTTTG
PODXL_sh1_R (TRCN0000296029)	AATTCAAAAAAGCCACGTAAGGGACTTTATACTCGAGTATAAAGTCCCTTACGTGGCT
PODXL_sh2_F (TRCN0000310117)	CCGGACGAGCGGCTGAAGGACAAATCTCGAGATTTGTCCTTCAGCCGCTCGTTTTTTG
PODXL_sh2_R (TRCN0000310117)	AATTCAAAAAACGAGCGGCTGAAGGACAAATCTCGAGATTTGTCCTTCAGCCGCTCGT

Primers for endogenous PODXL isoform detection

name	sequences
PODXL exonb F	CACTTCGACGCATCCTGTG
PODXL exond R	GTAGAGCTGGCTGGCATC

Primers for PODXL splicing minigene constructs

name	sequences
pzw_AgeI_F	tccgctagcgctaccggtc
pzw_HindIII_R	CGCCTGGCaagctttTAAGAC
pzw_5ss1_+1c_F	cgaaggctacgtcccaggtaagtctcgaCGAAACaag
pzw_5ss1_+1c_R	cttGTTTCGtcgagacttacctgggacgtagccttcg
PODXL_HindIII_F	gagaagcttGCCAGGCGTGATGGCTCTG
PODXL_SacII_R	tatccgcggCCAGTGGAATAACCCGGCAAAG
PODXL_doubleA_F	AGAGACAGTGTTTCACCATGTCAGCC
pzw_podxl_3ss1_g9_65_DoubleA_R	CTGACATGGTGAAACACTGTCTCTcCTTGA
pzw_podxl_3ss1_g9_65_A714G_F	TTTCAAGgAGAGACGGTGTTTC
pzw_podxl_3ss1_g9_65_A714G_R	CTGACATGGTGAAACACCGTCTCTcCTTGA
PODXL-A722G-F	ACAGTGTTTCGCCATGTCAGCC
pzw_podxl_3ss1_g9_65_A722G_R	CTGACATGGCGAAACACTGTCTCTcCTTGA
PODXL_doubleG_F	AGAGACGGTGTTTCGCCATGTCAGCC
pzw_podxl_3ss1_g9_65_DoubleG_R	CTGACATGGCGAAACACCGTCTCTcCTTGA
PODXL Alu seqF	TAGCTGGGACTACAGGTGTG
PODXL Alu seqR	ACTTTGGGAGGCCAAGGTG
pzw_3ss2_+ag_SacII_F	TGGccgcggtctctttcttccaggagagcgcaccatcttcttc
pzw_BamHI_R	tccggtggatccttacttgtacagctcgtccatgc

Primers for PODXL isoform detection in splicing minigene

name	sequences
Gexon F1 (gfp)	AGTGCTTCAGCCGCTACCC
Gexon Rv (gfp)	GTTGTACTCCAGCTTGTGCC

Primers for detecting PODXL isoforms via qPCR

name	sequences
PODXL_longiso_qPCR_F	CACTTCGACGCATCCTGTG
PODXL_longiso_qPCR_R	ACTTTGGGAGGCCAAGGTG
PODXL_qPCR_both_F	TGCAGACACCACTACAGTTGC
PODXL_qPCR_both_R	ATGGTCATGTCCCGAGCTTG
18S_qPCR_F	CTCTTAGCTGAGTGTCCCGC
18S_qPCR_R	CTGATCGTCTTCGAACCTCC
TBP_qPCR_F	CAGCAACTTCCTCAATTCCTTG
TBP_qPCR_R	GCTGTTTAACTTCGCTTCCG

Primers for ADAR overexpression constructs

name	sequences
Flag_Fw	CATCGACTACAAGGATGACG
p110 NotI F	AAGGAAAAAAGCGGCCGCAAGCCGAGATCAAGGAGAAAATCTG
ADAR1_BstBI_stop_R	atactgttcgaaCTATACTGGGCAGAGATAAAAGTTCTTTTCCTC
ADAR2_XbaI_R	CCCTCTAGACCGGGCG

ADAR2_EAA1_F	GGCTCTGGTCCCACAGAGGCAAAGGCAGCACTCCATGCTGCTGAGAAGG
ADAR2_EAA1_R	CCTTCTCAGCAGCATGGAGTGCTGCCTTTGCCTCTGTGGGACCAGAGCC
ADAR2_EAA2_F	GGCTCGGGGAGAAACGAGGCGCTTGCCGCGGCCCGGGCTGCGC
ADAR2_EAA2_R	GCGCAGCCCGGGCCGCGGCAAGCGCCTCGTTTCTCCCCGAGCC
ADAR2_E396A_F	CATTAAATGACTGCCATGCAGCAATAATATCTCGGAGATCCTT
ADAR2_E396A_R	AAGGATCTCCGAGATATTATTGCTGCATGGCAGTCATTTAATG
ADAR2_E488Q_F	GACCAAAATAGAGTCTGGTCAGGGGACGATTCCAGTGCG
ADAR2_E488Q_R	CGCACTGGAATCGTCCCCTGACCAGACTCTATTTTGGTC

Primers for PODXL minigene editing detection

name	sequences
EGFP_SacI_F	GCGAGGAGCTCTTCACCGGGG
PODXL_EGFP_R	tggtgcgctcCTGTAATCCCAG