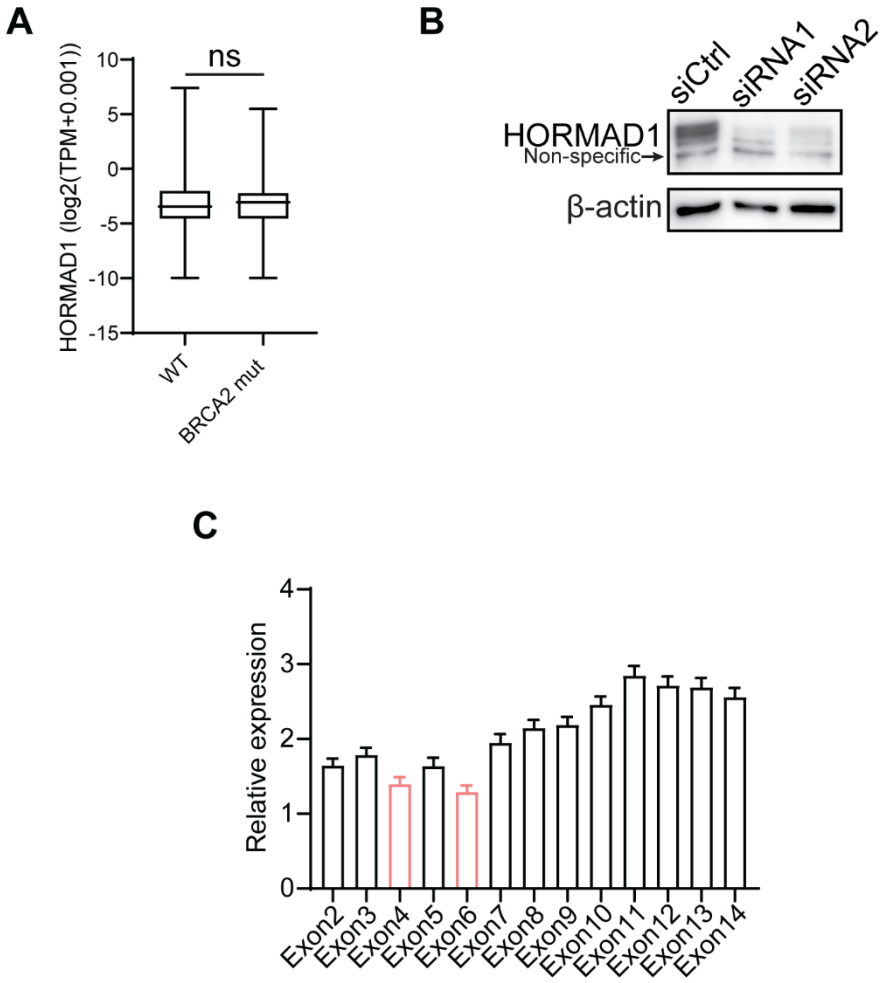

Figure S1. Expression and splicing analysis of HORMAD1.

(A) Boxplot showing HORMAD1 mRNA expression (log2 TPM) in BRCA2 wild-type (WT, n = 765) and BRCA2-mutant (BRCA2 mut, n = 20) breast cancer samples. Data are presented as mean ± SEM, Student's t-test. No significant difference was observed.

(B) Western blot analysis of HORMAD1 expression in HCC1395 cells transfected with control siRNA (siCtrl) or two independent siRNAs targeting HORMAD1 (siRNA1 and siRNA2). The lower band represents a non-specific signal. β-actin was used as a loading control.

(C) HORMAD1 exon expression in breast cancer patient TCGA dataset showing that exon 4 and exon 6 exhibit relatively lower expression compared to other exons, suggesting potential exon skipping events.

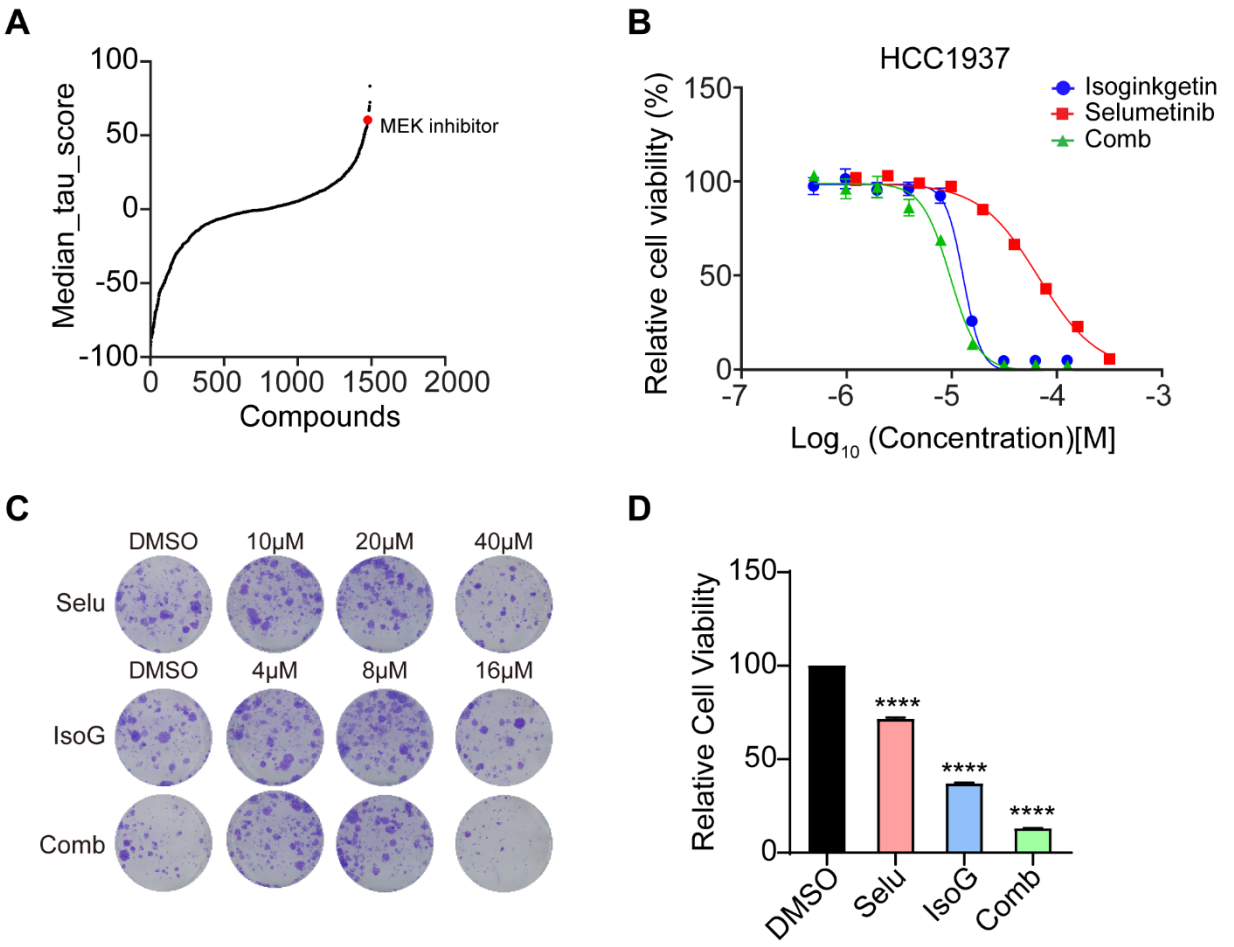

Figure S2. MEK inhibitors and splicing inhibition synergistically suppress BRCA1-mutant breast cancer cell growth.

(A) Connectivity Map (cMAP) analysis based on the differential gene expression signature of BRCA1-mutant versus wild-type breast cancer identified MEK inhibitors as top candidate compounds predicted to reverse the BRCA1-mutant expression profile.

(B) Dose-response curves showing the effects of selumetinib (Selu), isoginkgetin (IsoG), and their combination (Comb) on cell viability in BRCA1-mutant HCC1937 cells, as measured by CCK8 assay.

(C) Representative colony formation assays of HCC1937 cells treated with selumetinib, isoginkgetin, or their combination at the indicated concentrations.

(D) Quantification of colony formation assays shown in (C). Data are presented as mean ± SEM. **** $p < 0.0001$ compared with DMSO control (two-tailed Student's t-test).

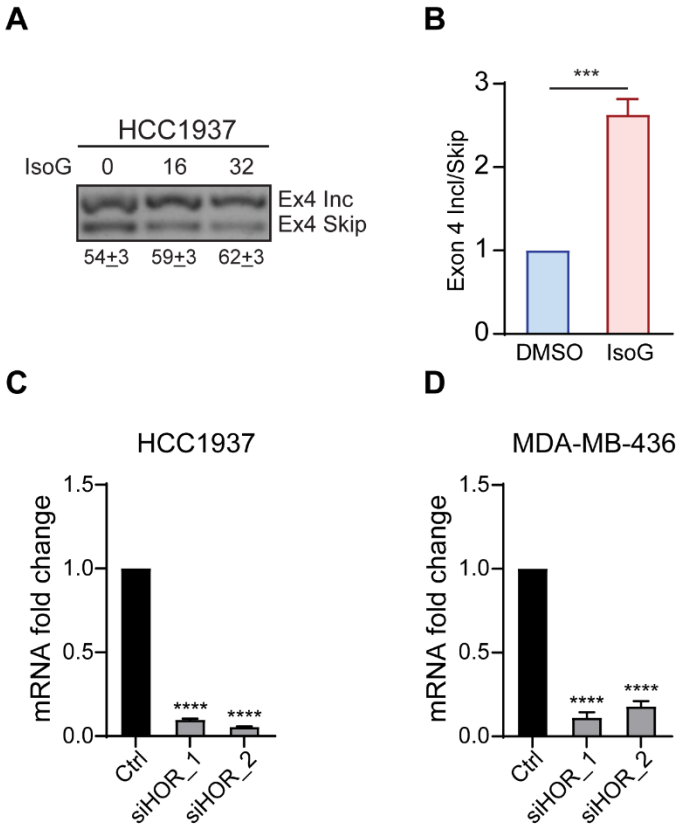

Figure S3. Isoginkgetin promotes HORMAD1 exon 4 inclusion.

(A) Representative Semi-quantitative RT-PCR showing that Isoginkgetin treatment increases HORMAD1 exon 4 inclusion in HCC1937 cells. The corresponding quantification is derived from n = 3 independent experiments.

(B) qRT-PCR analysis confirming the promotion of HORMAD1 exon 4 inclusion upon Isoginkgetin treatment. Error bars represent mean ± SEM. n = 3 independent experiments. Statistical significance was assessed using unpaired two-tailed Student's t-test, *** $p < 0.001$.

(C-D) Quantitative RT-PCR analysis of HORMAD1 mRNA levels in HCC1937 cells (C) and MDA-MB-436 (D) transfected with control siRNA or two independent HORMAD1-targeting siRNAs. Error bars represent mean ± SEM. n = 3 independent experiments. Statistical significance was assessed using unpaired two-tailed Student's t-test, **** $p < 0.0001$. No multiple comparison correction was applied.

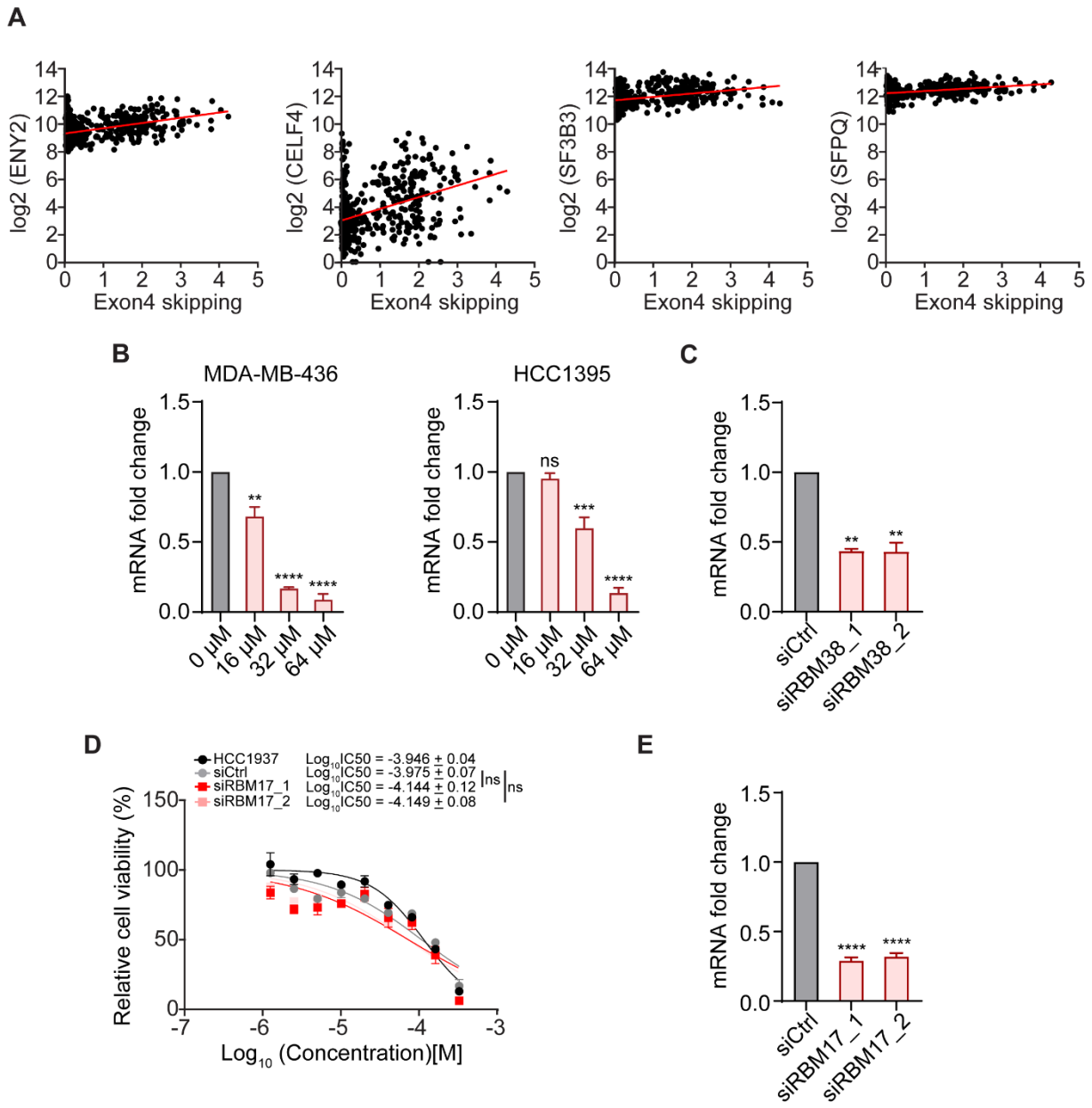

Figure S4. Correlations between RBPs and HORMAD1 exon 4 skipping and downregulation of RBM38 by splicing inhibition.

(A) Scatter plots showing the correlation between HORMAD1 exon4 skipping and selected RBPs in TCGA BRCA dataset.

(B) Quantitative RT-PCR analysis showing a dose-dependent decrease in RBM38 mRNA levels in MDA-MB-436 and HCC1395 cells treated with increasing concentrations of Isoginkgetin. Data represents the mean ± SEM of n = 3 independent experiments. Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant.

(C) qRT-PCR analysis showing RBM38 mRNA levels in HCC1937 cells transfected with siCtrl or siRBM38. Data represents the mean ± SEM of n = 3 independent experiments. Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test ** $p < 0.01$.

(D) Dose-response curves of HCC1937 cells transfected with siCtrl or siRBM17 and treated with Selumetinib for 72 h. Cell viability was determined by CCK-8 assay. Data are plotted as mean ± SEM. Statistical significance was evaluated by comparing the $\log_{10}$IC50 values using One-way ANOVA; ns, no significance.

(E) qRT-PCR analysis showing RBM17 mRNA levels in HCC1937 cells transfected with siCtrl or siRBM17. Data represents the mean ± SEM of three independent experiments. Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test, **** $p < 0.0001$.

Supplementary Table 1 Key resource table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
RBM38 Polyclonal antibody	proteintech	16435-1-AP
HORMAD1 Polyclonal Antibody	Abcam	PA5-99637
Anti-Rabbit IgG H&L	MCE	HY-P8001
Anti-Mouse IgG H&L	MCE	HY-P8004
beta Actin Mouse Antibody	HUABIO	EM21002
Chemicals		
Olaparib	Aladdin	AZD2281
Isoginkgetin	MCE	HY-N2117
Selumetinib	MCE	HY-50706
Critical commercial assays		
Opti-MEM	Thermo Fisher Scientific	#31985062
Lipofectamine 3000	Thermo Fisher Scientific	L3000015
Experimental models: Cell lines		
MCF-10A	ATCC	CRL-10317
CAL-51	MeisenCTCC	CTCC- 007-0267
EMT-6	ATCC	CRL-2755
4T1	ATCC	CRL-2539
MDA-MB-468	Stem Cell Bank	BFN60700349
MDA-MB-436	MINGZHOUBIO	MZ-0235
MDA-MB-231	ATCC	70029549
SUM159	MINGZHOUBIO	MZ-2427
SUM149	MeisenCTCC	CTCC-001-0949
HCC1937	MeisenCTCC	CTCC-004-0226
HCC1395	MeisenCTCC	CTCC-003-0106
RBM38 KO HCC1937	This study	N/A
Oligonucleotides		
siRNA targeting HORMAD1 #1:5'-CCAUGAGUGCACUGGUAUUTT-3'	This study	N/A
siRNA targeting HORMAD1 #2:5'-GCAUUCUCCUCAUUCGCAATT-3'	This study	N/A
siRNA targeting HORMAD1 #3:5'-GGACAAAGAUGUAGAAGAUTT-3'	This study	N/A
siRNA targeting RBM38 #1:5'-AAAUAGUUCUCAGUCAUUCTT-3'	This study	N/A
siRNA targeting RBM38 #2:5'-UUAACUUCUUGCUUUCAGGTT-3'	This study	N/A

sgRNA targeting RBM38 #1: 5′-CACCGACACTACCGACGCCTCGCTC-3′	This study	N/A
sgRNA targeting RBM38 #2: 5′-CACCGACCTTGATCCAGCGGACTTA-3′	This study	N/A
sgRNA targeting RBM38 #3: 5′-CACCGCACGTTCACCAAGATCTTCG-3′	This study	N/A
Primers for RT-qPCR		
HOR Exon3For: 5′-AGTGCACTGGTATTTCCC -3′	This study	N/A
HOR Exon5Rev: 5′-GCATCATAACATCCTAGCA -3′	This study	N/A
HOR Exon5For: 5′-TGCTAGGATGTTATGATGC-3′	This study	N/A
HOR Exon8Rev: 5′-GAGTGGTCCATTATTGGTG -3′	This study	N/A
18s For: 5′-GTAACCCGTTGAACCCCATT -3′	This study	N/A
18s Rev: 5′-CCATCCAATCGGTAGTAGCG -3′	This study	N/A
RBM38 For: 5′-CCACCTTGATCCAGCGGACTTA -3′	This study	N/A
RBM38 Rev: 5′-GCGTGTACTCAATGTAGGGCGA-3′	This study	N/A
Software and algorithms		
GraphPad Prism 10	GraphPad	https://www.graphpad.com
ImageJ	National Institutes of Health	https://imagej.net/ij/download.html