

**Supplementary Materials for**

**PDGFR $\alpha$  signaling regulates Srsf3 transcript binding to affect PI3K signaling and endosomal trafficking**

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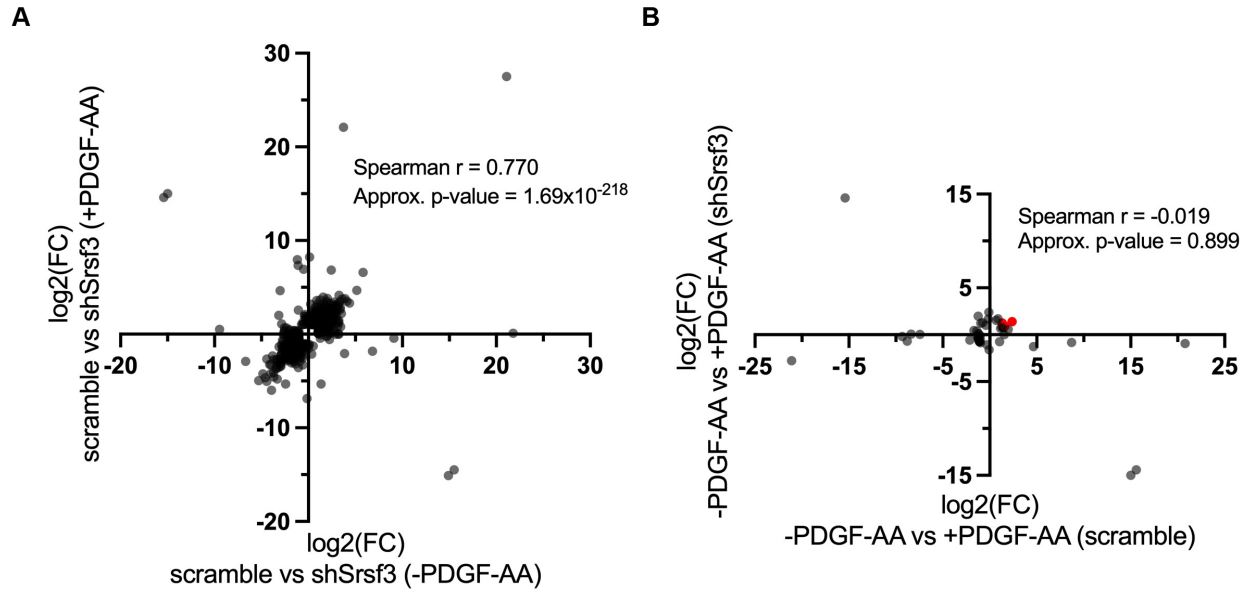
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**The PDF file includes:**

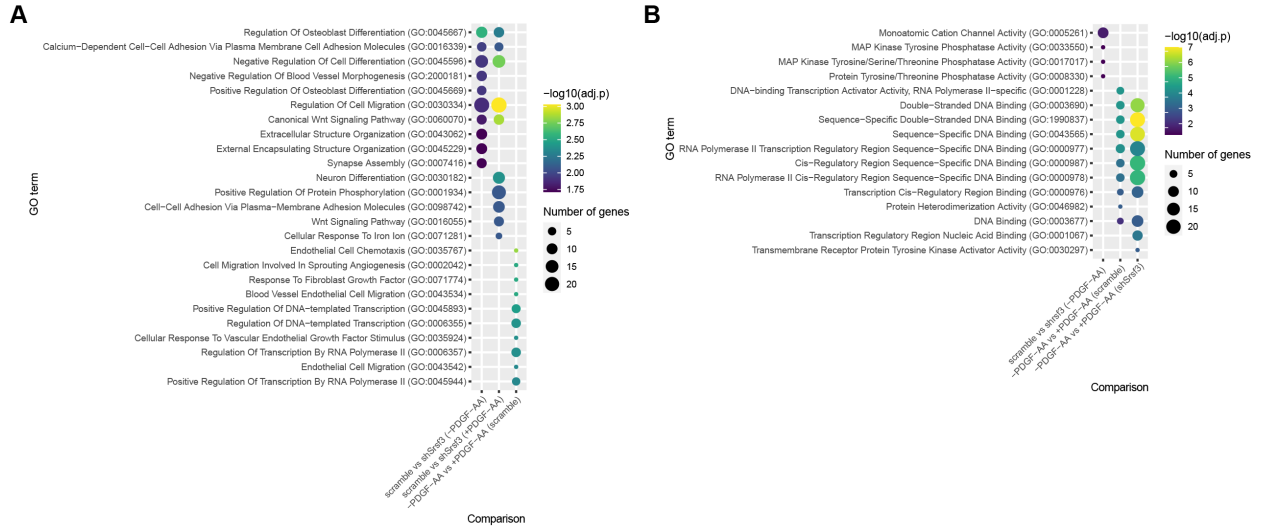
Figs. S1 to S6  
Tables S1, S7 and S16

**Other Supplementary Material for this manuscript includes the following:**

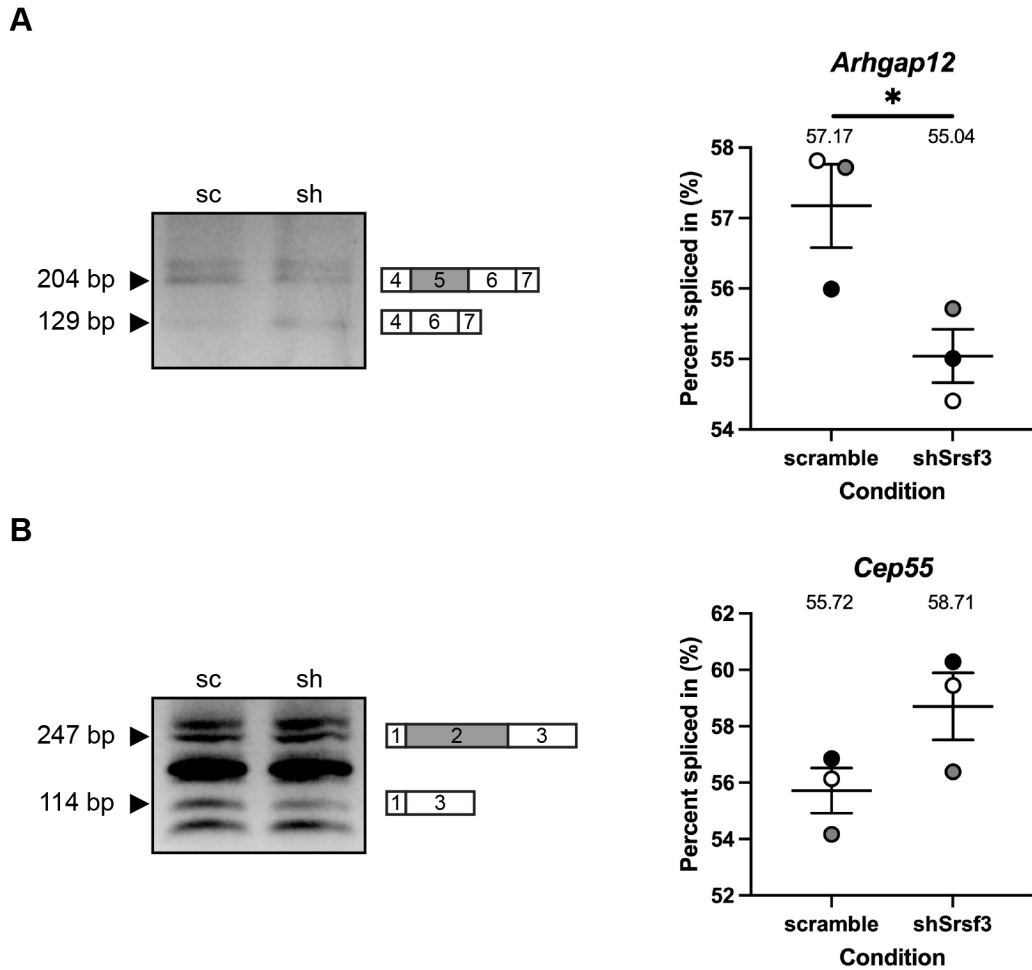
Tables S2-S6 and S8-S15



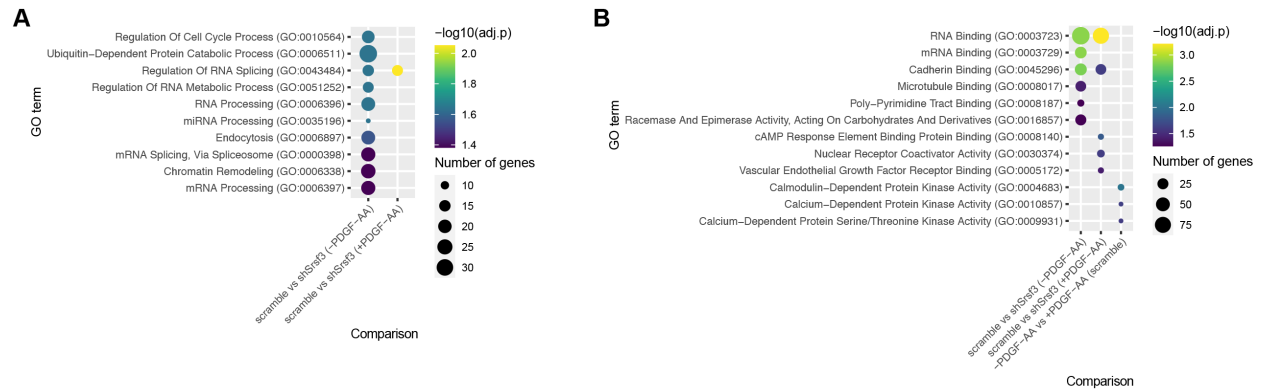
**Figure S1. High correlation of Srsf3-dependent differentially-expressed genes across ligand treatment conditions. (A,B)** Scatter dot plots depicting Srsf3-dependent (A) and PDGF-AA-dependent (B) differentially-expressed genes.  $\log_2(\text{fold change})$  (FC) values represent  $\log_2(\text{shSrsf3 normalized counts}/\text{scramble normalized counts})$  (A) or  $\log_2(+\text{PDGF-AA normalized counts}/-\text{PDGF-AA normalized counts})$  (B). Spearman correlation values and approximate p-values are listed. Immediate early genes are represented in red in B.























**Figure S2. Gene ontology analysis of differentially-expressed genes across treatment comparisons. (A,B)** Bubble plots depicting up to ten of the most significant gene ontology (GO) terms for biological process (A) and molecular function (B) for Srsf3-dependent and PDGF-AA-dependent differentially-expressed genes. Colors correspond to  $-\log_{10}(\text{adjusted p-value})$ ; sizes correspond to number of genes.



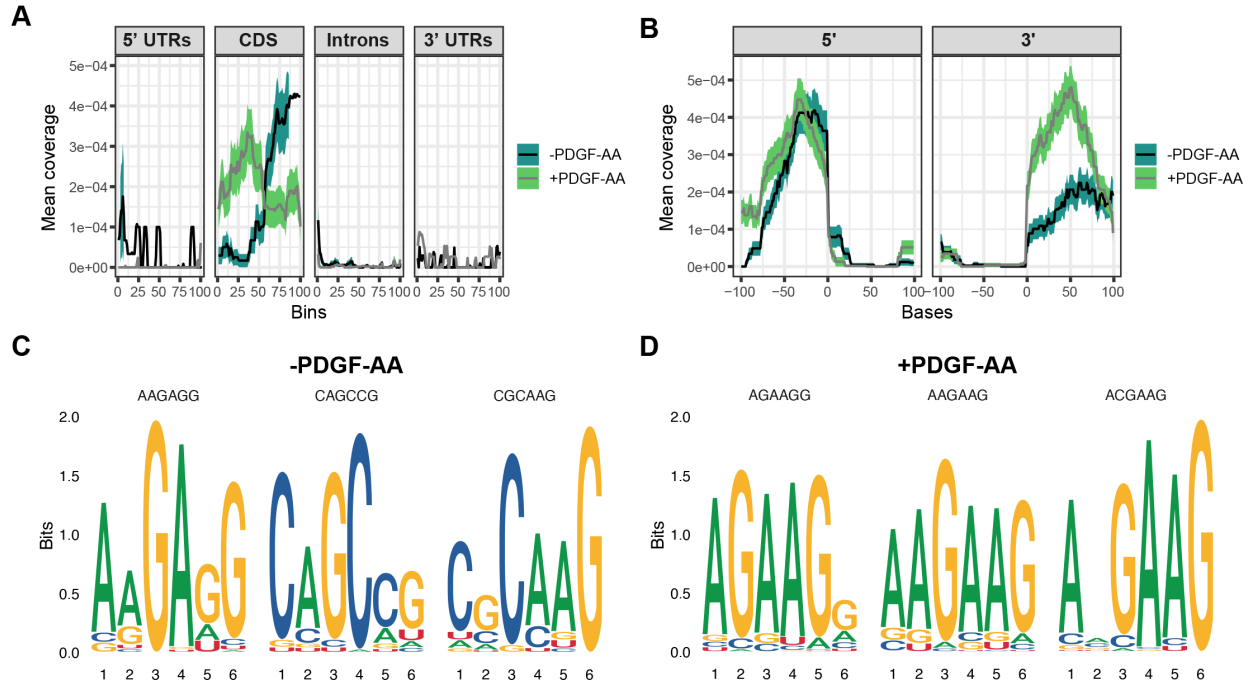
**Figure S3. qPCR validation of differential AS between scramble and shSrsf3 samples.** (A,B) Representative qPCR gels (left) with depictions of differentially alternatively-spliced exon (gray), and upstream and downstream sequences (white) that were assessed by qPCR in scramble (sc) versus shSrsf3 (sh) samples for *Arhgap12* (A) and *Cep55* (B). Scatter dot plots (right) depicting the percent spliced in from  $n = 3$  biological replicates as at left. Data are mean  $\pm$  s.e.m. \*,  $P < 0.05$ . Shaded circles correspond to independent experiments.



**Figure S4. Gene ontology analysis of alternatively-spliced transcripts across treatment comparisons. (A,B)** Bubble plots depicting up to ten of the most significant gene ontology (GO) terms for biological process (A) and molecular function (B) for Srsf3-dependent and PDGF-AA-dependent alternatively-spliced transcripts. Colors correspond to  $-\log_{10}(\text{adjusted p-value})$ ; sizes correspond to number of genes.

-PDGF-AA				+PDGF-AA			
Sequence logo	Occurrence in eCLIP (per 1000 peaks)	Occurrence in control (per 1000 peaks)	p-value	Sequence logo	Occurrence in eCLIP (per 1000 peaks)	Occurrence in control (per 1000 peaks)	p-value
	4095	991	$<2.2 \times 10^{-16}$		1432	465	$<2.2 \times 10^{-16}$
	4281	1209	$<2.2 \times 10^{-16}$		2032	649	$<2.2 \times 10^{-16}$
	1307	555	$3.6 \times 10^{-11}$		2029	726	$<2.2 \times 10^{-16}$
	4239	1135	$<2.2 \times 10^{-16}$		1279	353	$<2.2 \times 10^{-16}$
	602	253	$5.4 \times 10^{-15}$		833	315	$<2.2 \times 10^{-16}$
	4286	1151	$<2.2 \times 10^{-16}$		1273	436	$<2.2 \times 10^{-16}$
	4169	1116	$<2.2 \times 10^{-16}$		1660	663	$<2.2 \times 10^{-16}$
	1233	488	$<2.2 \times 10^{-16}$		1486	636	$7.5 \times 10^{-16}$
	3970	1026	$<2.2 \times 10^{-16}$		1726	631	$<2.2 \times 10^{-16}$
	758	307	$3.0 \times 10^{-10}$		1383	545	$<2.2 \times 10^{-16}$

**Figure S5. PDGFR $\alpha$  signaling influences Srsf3 binding specificity.** Top 10 motifs enriched in eCLIP peaks in the absence (left) or presence (right) of PDGF-AA stimulation with associated *P* values.



**Figure S6. Srsf3 exhibits differential transcript binding upon PDGFR $\alpha$  signaling in the subset of transcripts from the high-confidence, overlapping datasets. (A,B)** Mean coverage of eCLIP peaks within the high-confidence, overlapping datasets across various transcript locations (A) and surrounding the 5' and 3' splice sites (B) in the absence or presence of PDGF-AA stimulation. **(C,D)** Top three motifs enriched in eCLIP peaks within the high-confidence, overlapping datasets in the absence (C) or presence (D) of PDGF-AA stimulation.

**Table S1. RNA-seq sample information.**

Sample	Raw read pairs	Trimmed read pairs for Salmon input	Salmon mapping rate	Trimmed read pairs (125 bp) for STAR input	STAR unique mapping rate
-PDGF-AA scramble_1	47181591	44410442	0.89055	36343779	0.8773
-PDGF-AA scramble_2	54612492	50500367	0.878847	39971864	0.8681
-PDGF-AA scramble_3	69353787	65529075	0.912399	48327896	0.9022
-PDGF-AA shSrsf3_1	91657568	84086217	0.913324	61269254	0.9035
-PDGF-AA shSrsf3_2	77309551	71220292	0.91638	49390634	0.9013
-PDGF-AA shSrsf3_3	42645900	41078549	0.910338	28737018	0.9054
+PDGF-AA scramble_1	71080979	66836059	0.916828	48116755	0.9027
+PDGF-AA scramble_2	69667521	64974624	0.890505	47762451	0.884
+PDGF-AA scramble_3	78680108	72721916	0.911689	52936280	0.9008
+PDGF-AA shSrsf3_1	42776470	41165756	0.914797	28076373	0.9019
+PDGF-AA shSrsf3_2	37944773	35759828	0.908637	23528077	0.8987
+PDGF-AA shSrsf3_3	36391090	34257983	0.911463	26455873	0.8995

**Table S2. DEseq2 output.****Table S3. rMATS output for scramble (-PDGF-AA) versus shSrsf3 (-PDGF-AA) RNA-seq analysis.****Table S4. rMATS output for scramble (+PDGF-AA) versus shSrsf3 (+PDGF-AA) RNA-seq analysis.****Table S5. rMATS output for -PDGF-AA (scramble) versus +PDGF-AA (scramble) RNA-seq analysis.****Table S6. rMATS output for -PDGF-AA (shSrsf3) versus +PDGF-AA (shSrsf3) RNA-seq analysis.****Table S7. eCLIP sample information.**

Sample	Raw read pairs	Trimmed read pairs	Collapsed reads	Reads after removing repetitive elements	Mapped reads	Peaks	Annotated Peaks
-PDGF-AA size-matched input	34303575	22904092	13449745	13358235	17206	6969	6607
-PDGF-AA replicate 1	22983544	18369023	2758371	2758371	440436		
-PDGF-AA replicate 2	15666256	12263540	2742638	2065674	388996		
+PDGF-AA size-matched input	52420337	37454316	13811675	13643948	24275	9075	8623
+PDGF-AA	30105052	23355466	3417325	2845801	872085		



**Table S8. eCLIP output.**

**Table S9. Raw peak counts of eCLIP peaks across various transcript locations.**

**Table S10. Matt output.**

**Table S11. List of transcripts and genes from Venn diagram in Figure 5A.**

**Table S12. High confidence, overlapping dataset output correlating eCLIP with scramble (-PDGF-AA) versus shSrsf3 (-PDGF-AA) rMATS RNA-seq analysis.**

**Table S13. High confidence, overlapping dataset output correlating eCLIP with scramble (+PDGF-AA) versus shSrsf3 (+PDGF-AA) rMATS RNA-seq analysis.**

**Table S14. High confidence, overlapping dataset output correlating eCLIP with -PDGF-AA (scramble) versus +PDGF-AA (scramble) rMATS RNA-seq analysis.**

**Table S15. High confidence, overlapping dataset output correlating eCLIP with -PDGF-AA (shSrsf3) versus +PDGF-AA (shSrsf3) rMATS RNA-seq analysis.**

**Table S16. Primers used in qPCR analysis.**

<b>Transcript</b>	<b>Forward primer (5' to 3')</b>	<b>Reverse primer (5' to 3')</b>
<i>Arhgap12</i>	GGAGACATAGCACCATTTGTG	GCACTGCCCAAGAAGACAAC
<i>Cep55</i>	CCTTTCGGCTCCTTTGAACT	GCAGTGTCTGACTTGGAGCT
<i>Wdr81</i>	GCTTTGTGGACTGCAGGAAG	GCAGGGAACAGACACCAATC