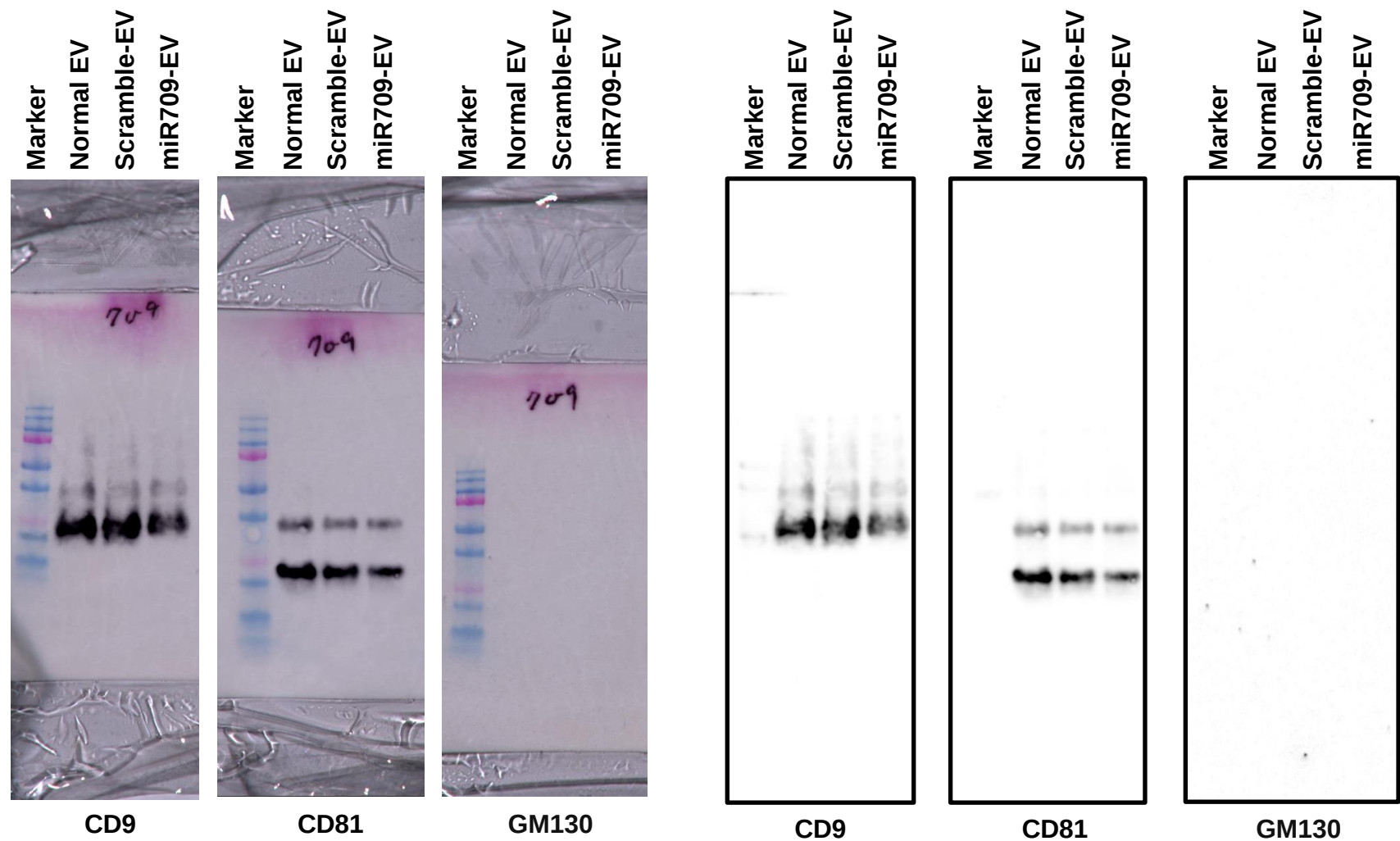
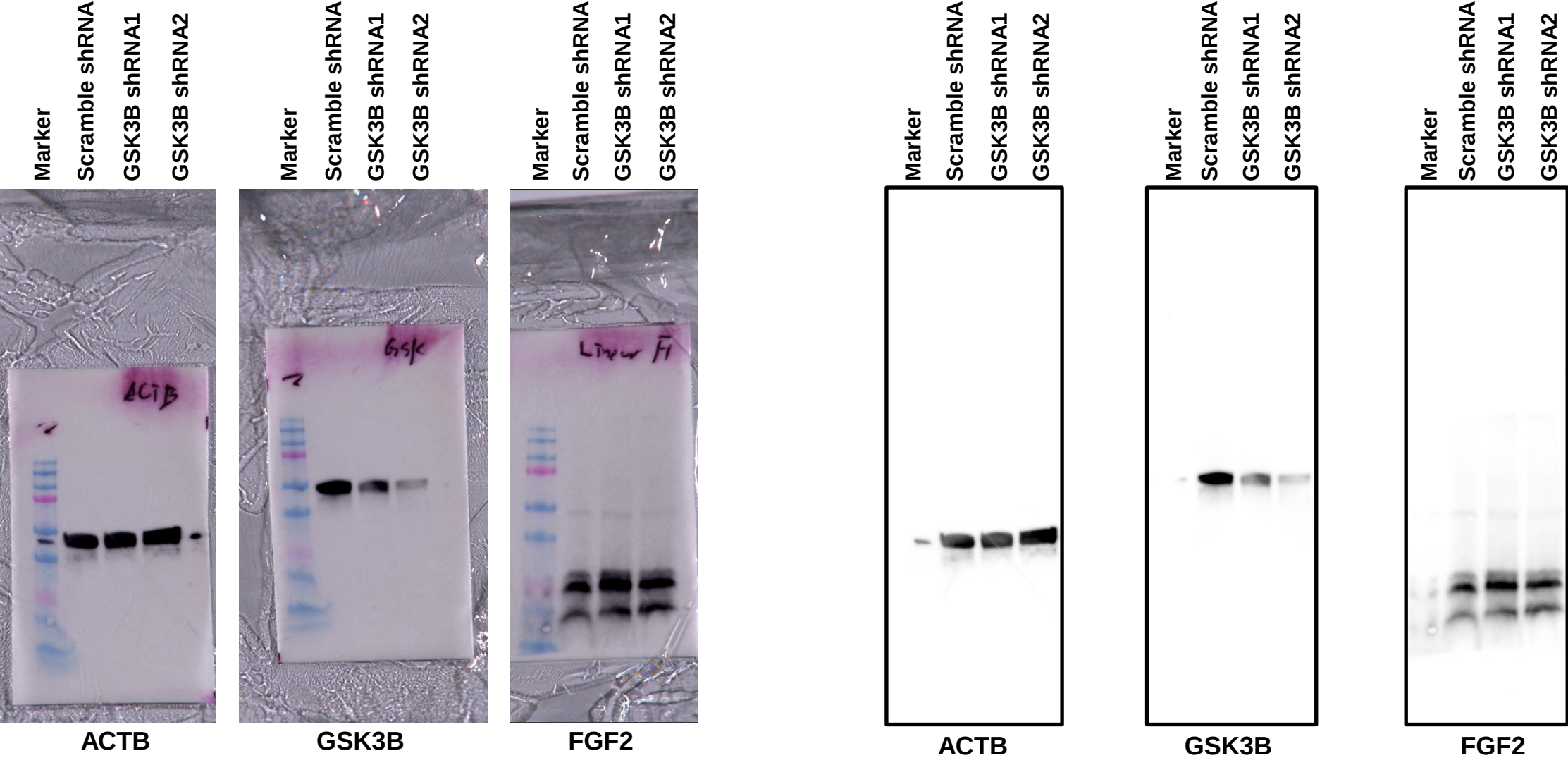


Supplementary Figure 1

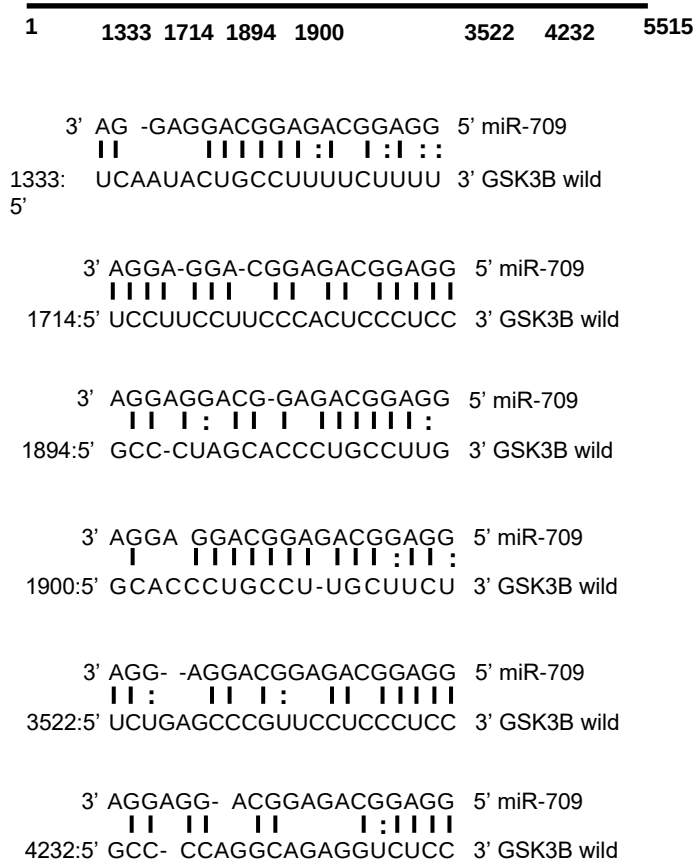


Supplementary Figure 2



Supplementary Figure 3

Mouse GSK3B 3'UTR ENSMUST00000023507.13

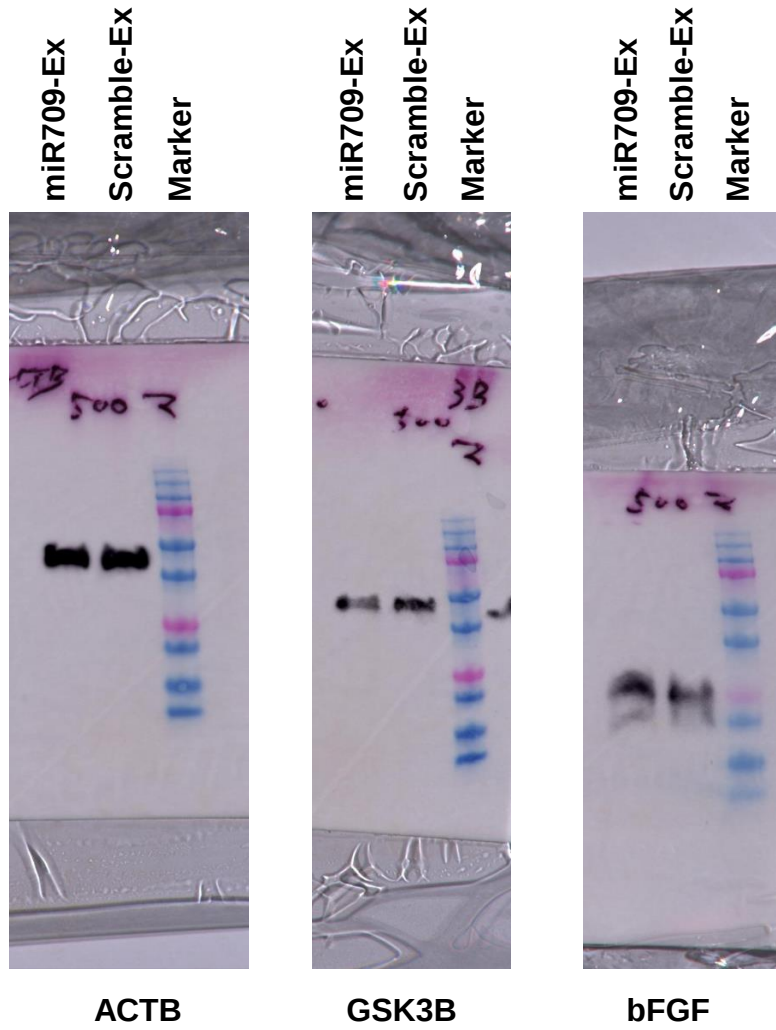


Supplementary Figure 4

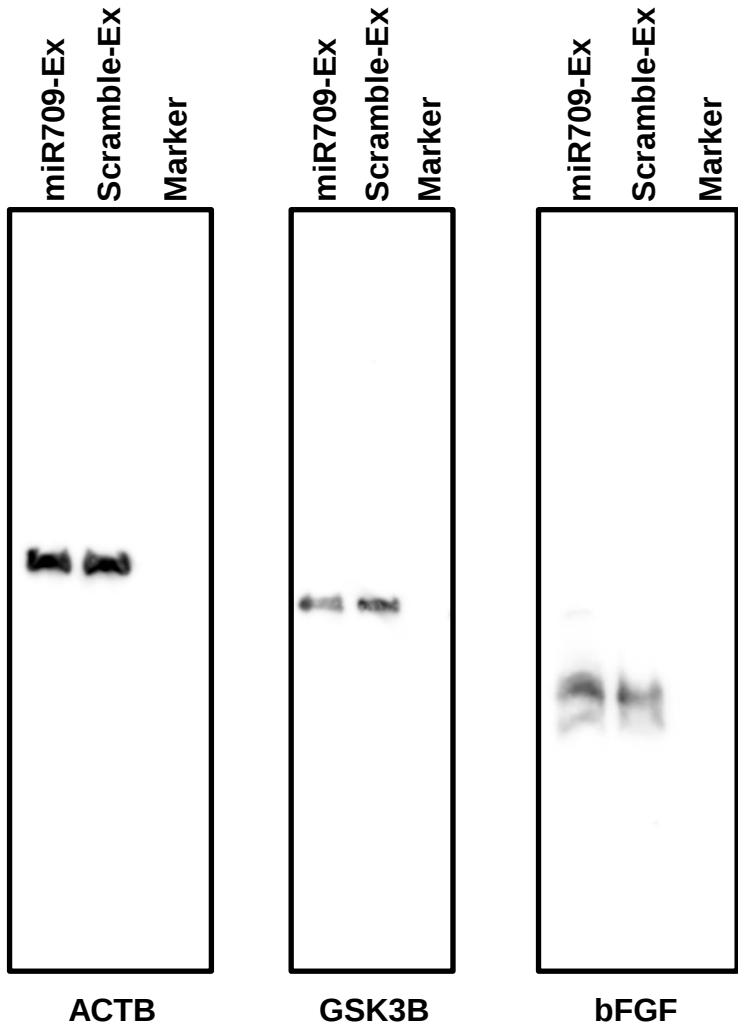
3' AGGAGGACGGA**GACGGAG**G 5' miR-709
 |||||
342:5' AAACCAGACUCCUGCCUCA 3' GSK3B mouse
324:5' AACAGGACUCCUGCCUCA 3' GSK3B human

Supplementary Figure 5

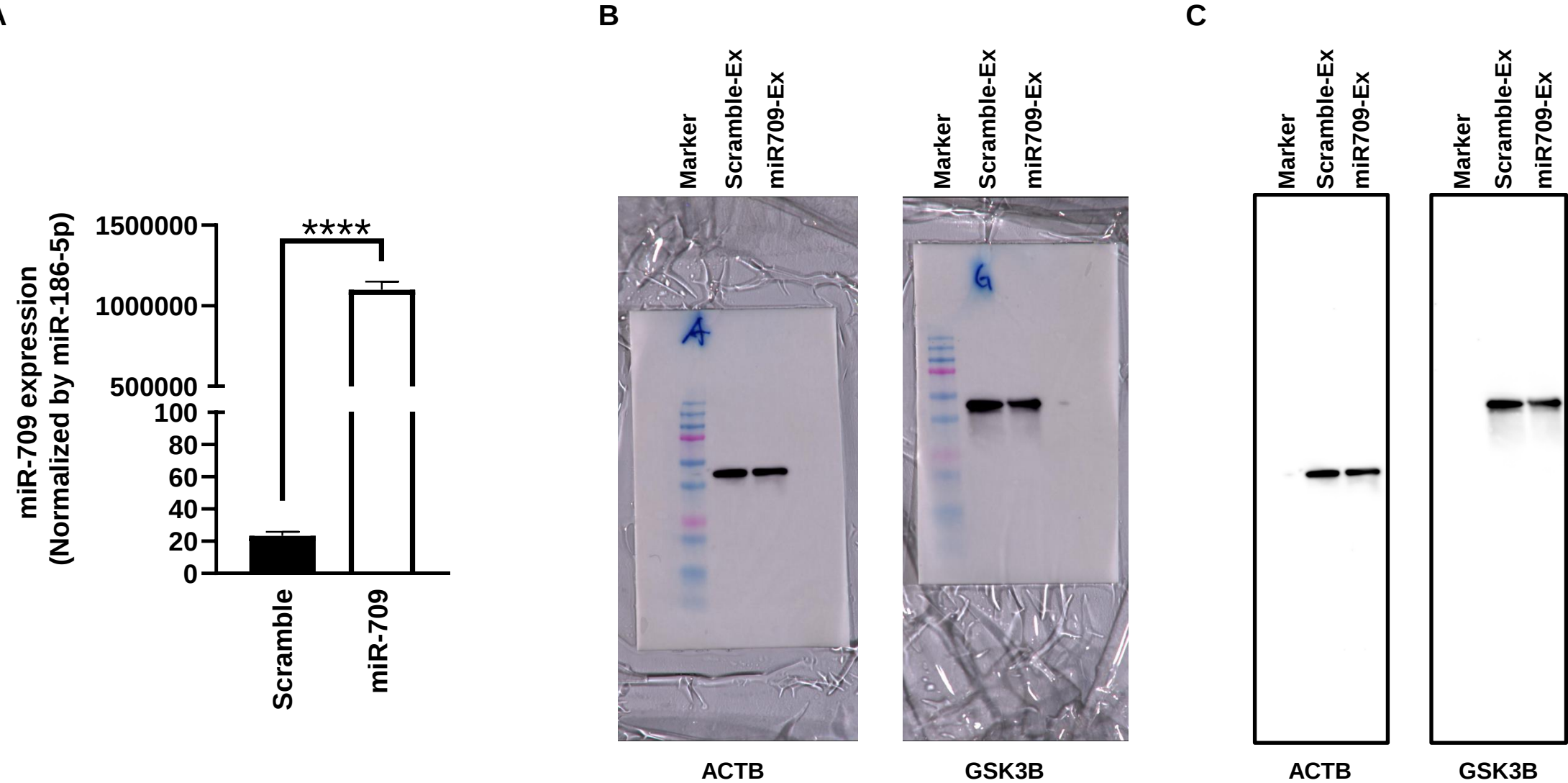
A



B



Supplementary Figure 6



Supplementary Figure legends

Supplementary Figure 1

Raw data for Figure 2A. These figures represent full-length blots with and without colorimetric markers (left and right sides). The exposure was captured in the automatic mode.

Supplementary Figure 2

Raw data for Figure 5C. These figures represent full-length blots with and without colorimetric markers (left and right sides). Figure 5C was excised from the western blot data with the colorimetric markers presented in this figure (Supplementary Figure 2); however, Figure 5C did not undergo any image processing. The exposure in the figures on the right was captured in automatic mode without colorimetric markers.

Supplementary Figure 3

Mouse GSK3B 3'-UTR sequences (ENSMUST00000023507.13) and complementary miR-709-binding sequences. The miR-709-binding sites at six sites in the mouse GSK3B 3'-UTR were predicted using RNA22 v2 miRNA target detection. The upper sequence corresponds to that of miR-709, whereas the lower sequence corresponds to the target sequence of mouse GSK3B.

Supplementary Figure 4

miR-709-binding sites in the mouse and human GSK3B 3'-UTR, as predicted by TargetScan. The upper sequence corresponds to miR-709, the middle sequence is the mouse 3'-UTR sequence of GSK3B, and the bottom sequence is the human 3'-UTR sequence of GSK3B.

Supplementary Figure 5

miRNA-709-encapsulating EVs upregulate GSK3B and FGF2 expression in HAoECs. GSK3B and FGF2 protein levels were measured by western blot analysis in HAoECs three days after co-culture with miRNA-709-encapsulating EVs. ACTB was used as an internal control. A and B: GSK3B and FGF2 protein levels in HAoECs three days after co-culture with miRNA-709-encapsulating EVs. A: Full-length blots with colorimetric markers are shown. B: Full-length blots without colorimetric markers.

Supplementary Figure 6

EVs directly transfected with miR-709 downregulated GSK3B protein in 293T cells. A: miRNA mimics were directly transfected into EVs isolated from the supernatant of 293T cells, and the expression of miRNA mimics was analyzed by qPCR. The miR-709 expression levels were

normalized to those of miR-186-5p. B: Full-length blots with colorimetric markers. C: Full-length blots without colorimetric markers. GSK3B protein levels were measured using western blot analysis in 293T cells. ACTB was used as an internal control.

Supplementary Table 1. Sequences of the miRNAs identified by the first screening.

Number	miRNA accession number chromosome range	Primer sequence 5' → 3'	Enzyme	PCR product	2nd screening
No1	mmu-miR-135a-1-3p MIMAT0004531 chr9:106153824-106154513	GAATTCcagtccttaggaacacacacaggag	EcoRI	294	0
		GGATCCagccctcttgtccacatcagag	BamHI		
No2	mmu-miR-365-1-5p MIMAT0017077 chr16:13453540-13454226	GAATTCcctcttttagtttggctctctgtg	EcoRI	286	2
		GGATCCatgtgccatctttttattcaccact	BamHI		
No3	mmu-miR-665-3p MIMAT0003733 chr12:109586014-109586707	CTCGAGgatggggtctttgttactagagcag	XhoI	294	0
		GAATTCcattgtgctactgaagtgtctgtg	EcoRI		
No4	mmu-miR-677-3p MIMAT0017246 chr10:128084986-128085663	GAATTCagagatgagtggtgagcaggagatt	EcoRI	279	0
		GGATCCcaaattaaacagccttcaaaaatg	BamHI		
No5	mmu-miR-744-5p MIMAT0004187 chr11:65734433-65735132	GAATTCatgtgtgctcattacattttgtctg	EcoRI	295	0
		GGATCCagagatggaataaaccaacagaagc	BamHI		
No6	mmu-miR-1893 MIMAT0007879 chr18:6490264-6490946	GAATTCaactccggttctccatgatcc	EcoRI	238	0
		GGATCCagttaacacgtaccgactcctcttc	BamHI		
No7	mmu-miR-2136 MIMAT0011212 chr9:104425813-104426487	GAATTCgggaattttgtggttagattttcc	EcoRI	285	0
		GGATCCcagacctctatcaattgactgctt	BamHI		
No8	mmu-miR-3968 MIMAT0019352 chr11:115447661-115448360	CTCGAGctatgcctcaggatactgcttgagt	XhoI	293	0
		GAATTCgtcgcaacatcgctctacctgct	EcoRI		
No9	mmu-miR-5099 MIMAT0020606 chr12:36815905-36816578	CTCGAGttccacatctctcttccacca	XhoI	392	1
		GGATCCcctgccttccatacacca	BamHI		
No10	mmu-miR-5122 MIMAT0020630 chr4:133369476-133370164	GAATTCgtctctcttcttacctgacacttc	EcoRI	269	0
		GGATCCagcaacacaccctcttaagac	BamHI		
No11	mmu-miR-5131 MIMAT0020642	GAATTCattataaagtccgggccgagag	EcoRI	274	1
		GGATCCagccaggtggtcaggtc	BamHI		

	chr14:45657661-45658353				
No12	mmu-miR-5620-5p MIMAT0022367 chr7:7298591-7299246	GAATTCgcaagtgtctgaagaagagaacagg	EcoRI	263	0
		GGATCCaagtagcgggcagtgatttct	BamHI		
No13	mmu-miR-6239 MIMAT0024860 chr14:117953443-117954147	GAATTCgagcagattggtgaaagtcaaaca	EcoRI	300	0
		GGATCCgtcttgtgtgcagtaaaaactg	BamHI		
No14	mmu-miR-6378 MIMAT0025124 chr3:34922245-34922951	GAATTCgaggctgagtttagaactgaaaaca	EcoRI	266	0
		GGATCCcgagaatgaaggcagttgaatagtt	BamHI		
No15	mmu-miR-6914-5p MIMAT0027728 chr10:128382538-128383206	CTCGAGacatcaaacacaggtccagagat	XhoI	218	0
		GAATTCatcagcaagtacctgaggagagaag	EcoRI		
No16	mmu-miR-6937-5p MIMAT0027774 chr12:28679025-28679689	CTCGAGggcttgctcttgacacttttaat	XhoI	254	0
		GAATTCgaagagtaatcatgtcccccttctc	EcoRI		
No17	mmu-miR-6980-5p MIMAT0027862 chr18:37990587-37991245	GAATTCagtatttgtgtccgcacagagagt	EcoRI	292	0
		GGATCCctcactctgtacagtgcagctc	BamHI		
No18	mmu-miR-6987-5p MIMAT0027876 chr19:5678704-5679376	GAATTCctctgttttcttctctctctcc	EcoRI	299	0
		GGATCCgctcctgtagactaccataccttg	BamHI		
No19	mmu-miR-6989-5p MIMAT0027880 chr19:6346180-6346844	GAATTCagcagaagcaataactaggacacc	EcoRI	288	0
		GGATCCcagcaacacattgttcacagtag	BamHI		
No20	mmu-miR-6991-5p MIMAT0027884 chr19:7422273-7422942	GAATTCgaggatgaaggctgtagagagac	EcoRI	325	0
		GGATCCgccgtcgtagtcacatcaagtattc	BamHI		
No21	mmu-miR-6999-5p MIMAT0027900 chr2:91944570-91945230	GAATTCggcctaaggaggctacatttcatt	EcoRI	296	0
		GGATCCcacatatactcacactccagtcaca	BamHI		
No22	mmu-miR-7020-5p MIMAT0027944 chr4:139643742-139644411	GAATTCggctgctttacctgaggatatgac	EcoRI	283	1
		GGATCCgctgcagcgtctctctgtattatc	BamHI		
No23	mmu-miR-7042-5p MIMAT0027988	GAATTCtgaggaatcggaatgtagaaag	EcoRI	218	0
		GGATCCcaacaatatctcatcagcttcac	BamHI		

	chr6:113706908-113707565				
No24	mmu-miR-7044-5p	GAATTCatagccactatacccatccttttc	EcoRI	253	0
	MIMAT0027992	GGATCCgacagcagcaagcttgtatcatt	BamHI		
	chr6:118084892-118085563				
No25	mmu-miR-7047-5p	CTCGAGgcaggaagcttaggcctgga	XhoI	227	0
	MIMAT0027998	GAATTCagttgtcatccagcagaatcatgt	EcoRI		
	chr7:24987304-24987967				
No26	mmu-miR-7036b-5p	GAATTCgtctagtgcttgcctccgagt	EcoRI	237	1
	MIMAT0029808	GGATCCgtcagtttttaaagagccgacaag	BamHI		
	chr5:34573851-34574513				
No27	mmu-miR-7658-5p	GAATTCccagcctcaactccatagagaagta	EcoRI	262	2
	MIMAT0029822	GGATCCgccctaacttggtagagaagaca	BamHI		
	chr4:156229759-156230415				
No28	mmu-miR-7662-3p	CTCGAGgctaggcaaggagagaaaaatg	XhoI	210	0
	MIMAT0029831	GAATTCagatcaacagttcccacctacctg	EcoRI		
	chr10:62193867-62194527				
No29	mmu-miR-7672-5p	GAATTCcacggtctgaatgggtctgtga	EcoRI	299	0
	MIMAT0029850	GGATCCctctggctcctctgcatgtttt	BamHI		
	chr14:26669247-26669908				
No30	mmu-miR-8110	GAATTCaatcactcggactttctgtttgac	EcoRI	287	1
	MIMAT0031416	GGATCCgacctgtagtagagaagaagctacc	BamHI		
	chr8:89024435-89025131				
No31	mmu-miR-8112	CTCGAGacttcccctcaggaagtcagg	XhoI	205	0
	MIMAT0031418	GAATTCggcgggaactcacttctct	EcoRI		
	chr6:71271371-71272101				
No32	mmu-miR-211-3p	GAATTCgtcctaagaatcagatcttgggtgga	EcoRI	228	0
	MIMAT0017059	GGATCCacttcatttcagaagcagagaaga	BamHI		
	chr7:64205506-64206211				
No33	mmu-miR-671-5p	GAATTCctgttctacaatgctgactacctg	EcoRI	222	1
	MIMAT0003731	GGATCCcgacaacgggtgtacagttcct	BamHI		
	chr5:24591814-24592511				
No34	mmu-miR-709	GAATTCagggtagccttgaactcagagattt	EcoRI	278	1
	MIMAT0003499	GGATCCaaaacggactcctctaggtgttct	BamHI		
	chr8:84085799-84086486				
No35	mmu-miR-1247-3p	GAATTCctctcccactagggttagctgtt	EcoRI	264	1
	MIMAT0014801	GGATCCctaaaaagcctcagcacagttc	BamHI		

	chr12:110277748-110278429				
No36	mmu-miR-1894-3p	GAATTCcaatcgggtctcttaaattccca	EcoRI	397	0
	MIMAT0007878	GGATCCtttctctgcccatctcttacc	BamHI		
	chr17:35917589-35918269				
No37	mmu-miR-3154	GAATTCcgggttgacactaggtaggagt	EcoRI	287	0
	MIMAT0035714	GGATCCgtagacggagtgaggatctctctg	BamHI		
	chr2:32317965-32318643				
No38	mmu-miR-5132-5p	GAATTCgtgccgttctacaaagtgatg	EcoRI	259	1
	MIMAT0020643	GGATCCgtgaggatgtgaacgaggtcag	BamHI		
	chrX:74023228-74023898				
No39	mmu-miR-5627-5p	GAATTCaagtgagtcgacgatttctctac	EcoRI	235	1
	MIMAT0022385	GGATCCctaagggtgagacctgagtttctg	BamHI		
	chr12:44210013-44210673				
No40	mmu-miR-6236	GAATTCccataccctttctttccactacct	EcoRI	289	0
	MIMAT0024857	GGATCCctgggaagacaccaacagtaactt	BamHI		
	chr9:110280987-110281709				
No41	mmu-miR-6244	GAATTCaaaaatcttctctcggtgtcctg	EcoRI	278	2
	MIMAT0024864	GGATCCcatttctgattcccgactctatgat	BamHI		
	chr9:52115323-52116039				
No42	mmu-miR-7005-5p	GAATTCaccctgggtctcttctatgacttc	EcoRI	269	1
	MIMAT0027914	GGATCCatctccagcatgttatctgtgtcta	BamHI		
	chr2:180179455-180180123				
No43	mmu-miR-7653-5p	GAATTCgaacgggtgtccccaactgtc	EcoRI	243	0
	MIMAT0029812	GGATCCatacaatggagtggtgaaagagac	BamHI		
	chr11:78178527-78179187				

Notes: Uppercase letters represent the restriction enzyme sites. Upper rows in each primer sequence represent the forward primers, while lower rows in the primer sequence represent the reverse primers.

Supplementary Table 2. Primer sequences used for the undertaking of quantitative PCR.

Gene name	Forward primer 5' → 3'	Reverse primer 5' → 3'
mouse ACTB	ggtgtgatggtgggaatgg	tcaggatacctctcttgcctgg
mouse FGF2	gcgacccacacgtcaaactac	gcacacactcccttgatagacac
human ACTB	gctcctcctgagcgcaag	catctgctggaaggaggaca
human FGF2	agcgaccctcacatcaagc	acggtagcacacactccttg
human BMX	ctgaaaagtagcagcagcagtg	ccatgaaatcttgagggtgtg

Supplementary Table 3. Oligo sequences used for the construction of the 3'UTR plasmids for human GSK3B.

Plasmid's name	Oligo sequence 5' → 3'
3'UTR 1694 wild	AAACTAGCGGCCGCTAGTTCCTTCCCCACATGCCTTCT
	CTAGAAAAAGGAAAGGAAAAATAGATGACTAGCGGCCGCTAGTTT
3'UTR 1694 mutated	AAACTAGCGGCCGCTAGTTCCTTCCCCACATACTTTCT
	CTAGAGAAAGTATGTGGGGAAAGAACTAGCGGCCGCTAGTTT
3'UTR 1774 wild	AAACTAGCGGCCGCTAGTTCACCCTCCTCATTGCCACCT
	CTAGAGGTGGCAATGAGGAGGGTGAAGTACTAGCGGCCGCTAGTTT
3'UTR 1774 mutated	AAACTAGCGGCCGCTAGTTTACCCTCTTCATCGTCACCT
	CTAGAGGTGACGATGAAGAGGGTAAACTAGCGGCCGCTAGTTT
3'UTR 2899 wild	AAACTAGCGGCCGCTAGTTCCTTTATCCATGTCTCCT
	CTAGAGGAGACATGGATAAAGGAACTAGCGGCCGCTAGTTT
3'UTR 2899 mutated	AAACTAGCGGCCGCTAGTTCCTTTATCCACGTCCCCT
	CTAGAGGGGACGTGGATAAAGAACTAGCGGCCGCTAGTTT
3'UTR 3248 wild	AAACTAGCGGCCGCTAGTCCTTCCATGCTCTCTCTCTT
	CTAGAAGAGAGAGAGAGCATGGAAGGACTAGCGGCCGCTAGTTT
3'UTR 3248 mutated	AAACTAGCGGCCGCTAGTCCTTTCATACTCCCTCTCCCTT
	CTAGAAGGGAGAGGGAGTATGAAAGGACTAGCGGCCGCTAGTTT
3'UTR 4864 wild	AAACTAGCGGCCGCTAGTACCCAACCCTCTTCCTTCT
	CTAGAGAAGGAAGAGGGTTGGGTACTAGCGGCCGCTAGTTT
3'UTR 4864 mutated	AAACTAGCGGCCGCTAGTACTCAACCTTTTCTTTCT
	CTAGAGAAAGAAAAAGGTTGAGTACTAGCGGCCGCTAGTTT

Notes: Upper rows in each set of oligo sequences represent the forward oligo sequences, while lower rows in each set of oligo sequences represent the reverse oligo sequences.

Supplementary Table 4. Target sequence and oligo sequences used for the construction of the plasmids.

Name	Oligo sequence 5' → 3'
Scramble shRNA	GATCC <u>gtgatactgttgcagctgctgac</u> CTTCCTGTCAGAgatcagcagctgacaacagtatcacTTTTTG
	AATTCAAAAA <u>gtgatactgttgcagctgctgac</u> TCTGACAGGAAGgatcagcagctgacaacagtatcacG
GSK3B shRNA1	GATCC <u>tcctgatactgctgtattaaaac</u> CTTCCTGTCAGAgttttaatacagcagtatcaggaTTTTTG
	AATTCAAAAA <u>tcctgatactgctgtattaaaac</u> TCTGACAGGAAGgtttttaatacagcagtatcaggaG
GSK3B shRNA2	GATCC <u>cccaatgtttcgatatctgttc</u> CTTCCTGTCAGAgaacagatatacgaacattgggTTTTTG
	AATTCAAAAA <u>cccaatgtttcgatatctgttc</u> TCTGACAGGAAGgaacagatatacgaacattgggG

Notes: Upper rows in each oligo sequence set represent the forward oligo sequences, while the lower rows in each oligo sequence set represent the reverse oligo sequences. Underlined solid lines highlight the sense sequences of the target genes, while underlined dot lines highlight the antisense sequence of the target genes.