

Supplementary Information

Specific Inhibition of FGF5-induced Cell Proliferation by RNA Aptamers

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Figure S1. Immunoblotting image of phospho-FGFR1 in FGF5-stimulated NIH3T3 cells in the presence of F5f1 (lanes 3–6) or random RNA (lanes 7–10).

Figure S2. SPR sensorgrams of FGF5 binding to the aptamers.

Figure S3. SPR sensorgrams of nonspecific binding of FGF5 to the random RNA.

Figure S4. Pairwise sequence alignment of FGF5 and FGF4 (a), FGF6 (b), FGF1 (c) or FGF2 (d).

Figure S5. Sequences and predicted secondary structures of aptamers against FGF2, FGFR1, and FGFR3.

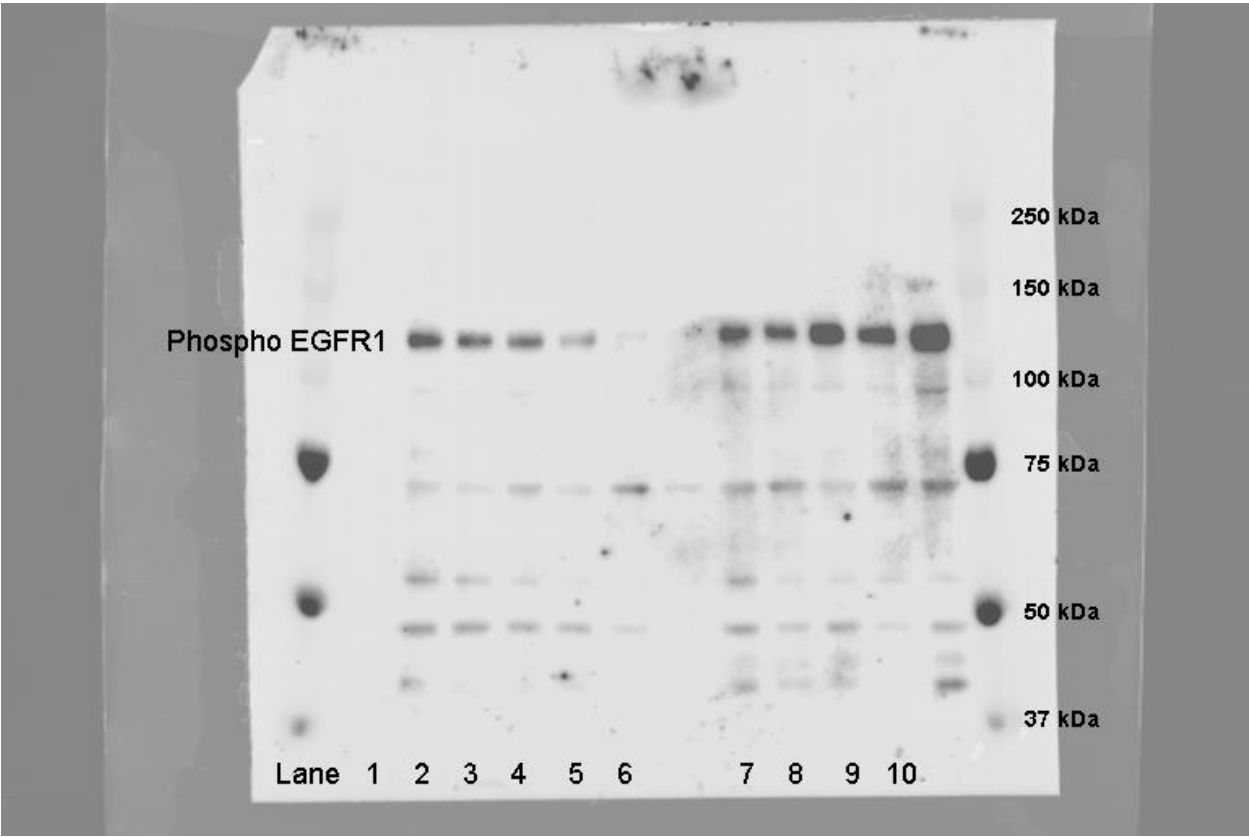


Figure S1. Immunoblotting image of phospho-FGFR1 in FGF5-stimulated NIH3T3 cells in the presence of F5f1 (lanes 3–6) or random RNA (lanes 7–10).

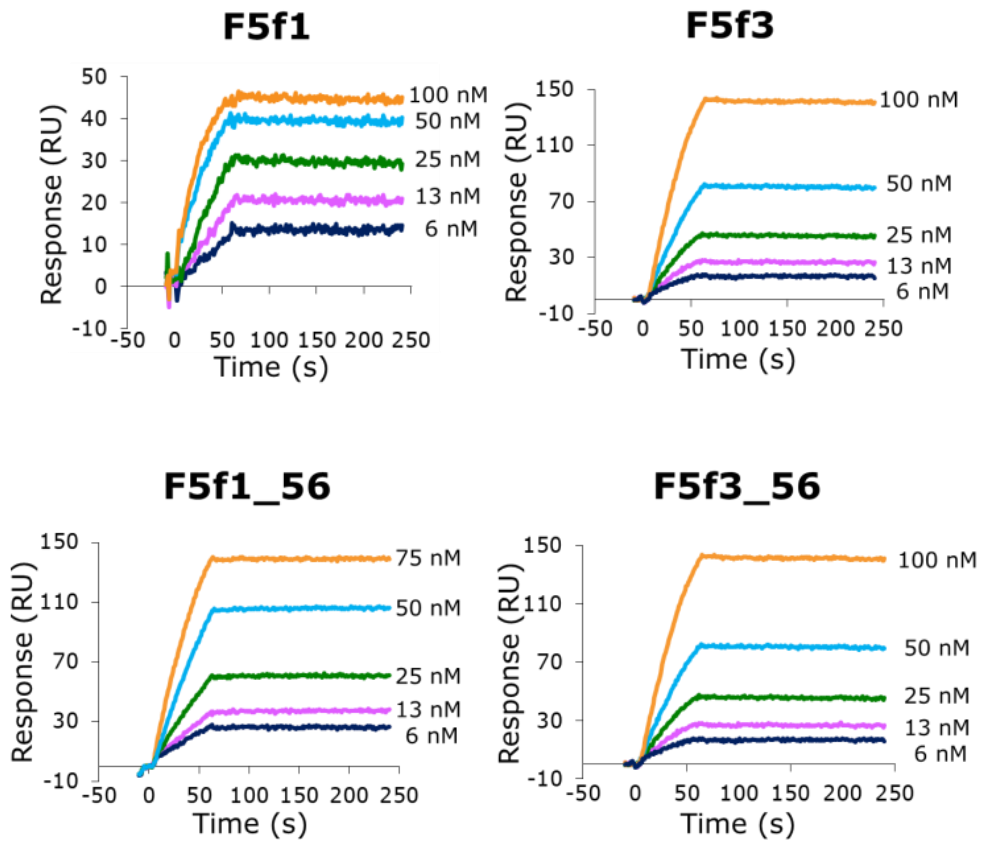


Figure S2. SPR sensorgrams of FGF5 binding to the aptamers. The aptamers F5f1, F5f3, F5f1_56, or F5f3 were immobilized on the sensor chips and various concentrations of FGF5 in SPR running buffer was injected for 60 s and dissociated for 180 s.

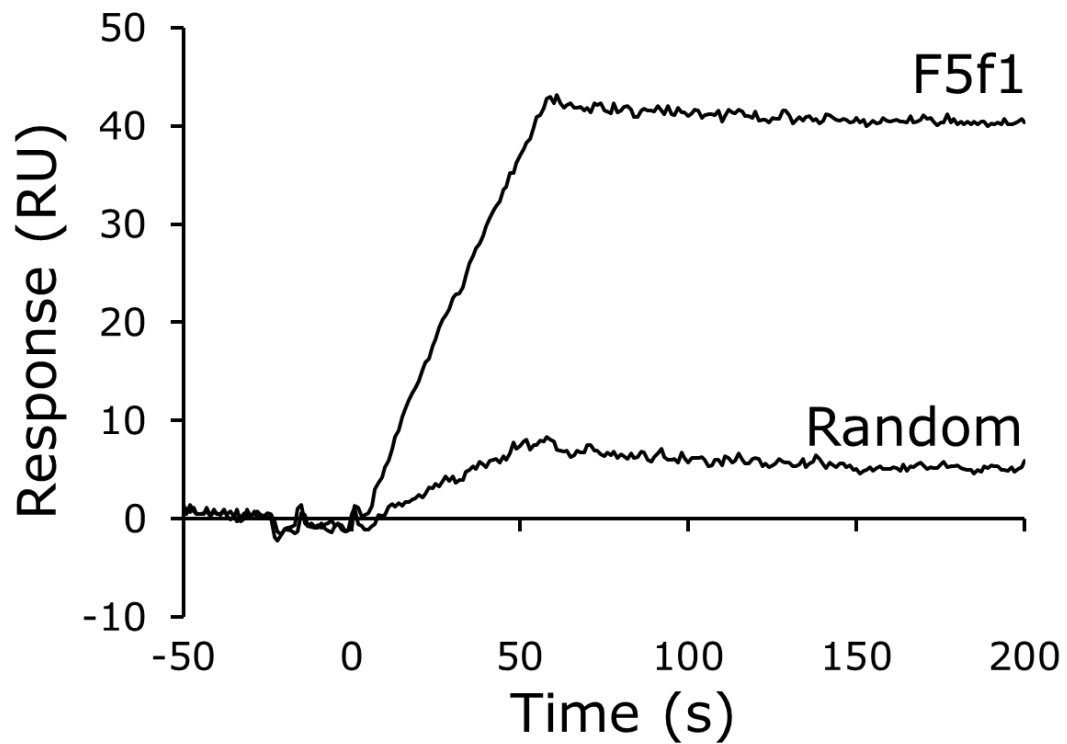


Figure S3. SPR sensorgrams of nonspecific binding of FGF5 to the random RNA. The random RNA pool and aptamers were immobilized to approximately 100 RU on the sensor chip and FGF5 in SPR running buffer was injected for 60 s and dissociated for 140 s.

a

Identity 29.0%
Similarity 40.3%

FGF5	1	-----MSLSFLLLLFFSHLILSAWAHGEKRLAPKGGQPPAATDRNPR	42
		: ...: : : : : : : : ...	
FGF4	1	MSGPGTAAVALLPAVLL---ALLAPWA-----GR-GGAAAPTAPN	36
FGF5	43	GS-----SSRQSSSSAMSS--SASSSPAASLGSQGSGLQSSFWSPSG	85
		: ...: :	
FGF4	37	GTLEAELERRWESLVALSLARLPVAAQPKEAAVQSGAG-----DYLLGI	80
FGF5	86	RRTGSLYCRVIGFHLQIYDPGKVNGSHEANMLSVLEIFAVSQGIVGIRG	135
		: ...: :	
FGF4	81	KRLRRLYCNVIGFHLQALPDGRI GGAHADTRDLSLELSPVERGWSIFG	130
FGF5	136	VFSNKFLAMSKKGLHASAKFTDDCKFRERFQENSYNTYASAIHREKTG	185
		: ...: :	
FGF4	131	VASRFVAMSSKGLYGSPFFTDECTFKEILLPNNYNAYESYKY-----	174
FGF5	186	REWYVALNKRKAKRGCSRVKQHIISTHFLPRFKQSEQPELSFTVTVPE	235
		...: :	
FGF4	175	PGMFIALSKNGTKKG--NRVSPTMKVTHFLPRL-----	206
FGF5	236	KKKPPSPIKPKIPLSAPRKNNTNSVKYRLKFRFG	268
FGF4	207	-----	206

b

Identity 30.2%
Similarity 40.6%

FGF5	1	MSLSFLLLLFFS---HLILSAWAHGEKRLAPKG-----QPGPAATDRN	40
		: ...: :	
FGF6	1	MALGQKLFITMSRAGRLQGLWA---LVFLGILVGMVVPSPAGTRAN	45
FGF5	41	-----PRGSSSRQSSSSAMSSSSASSSPAASLGSQGSGLQSSFWSPSG	85
		: ...: :	
FGF6	46	NTLLDSRWGTLRSRA-----GLAGEIAGVNWESG	77
FGF5	86	-----RRTGSLYCRVIGFHLQIYDPGKVNGSHEANMLSVLEIFAVSQGI	130
		: ...: :	
FGF6	78	YLVGIKRQRRLYCNVIGFHLQVLPDGRISGTHEENPYSLLEISTVERGV	127
FGF5	131	VGIRGVFSNKFLAMSKKGLHASAKFTDDCKFRERFQENSYNTYASAIHR	180
		: ...: :	
FGF6	128	VSLFGVRSALFVAMNSKGRLYATPSFQECKFRETLLPNNYNAYESDLYQ	177
FGF5	181	TEKTGREWYVALNKRKAKRGCSRVKQHIISTHFLPRFKQSEQPELSFT	230
		: ...: :	
FGF6	178	GT-----YIALSKYGRVKRG--SKVSPIMTVTHFLPRI-----	208
FGF5	231	VTVPEKKKPPSPIKPKIPLSAPRKNNTNSVKYRLKFRFG	268
FGF6	209	-----	208

Figure S4. Pairwise sequence alignment of FGF5 and FGF4 (a), FGF6 (b), FGF1 (c) or

FGF2 (d). Sequence alignments were performed using EMBL-EBI search and sequence analysis

tool (<https://www.ebi.ac.uk/Tools/psa/>)¹.

c

Identity 20.7%
Similarity 30.0%

FGF5	1	MSLSFLLLLFFSHLILSAWAHGEKRLAPKQPGPAATDRNPRGSSSRQSS	50
FGF1	1	-----	0
FGF5	51	SSAMSSSSASSSPAASLGSQSGLEQSSFWSPSG--RRTGSLYCRVGI GF	99
FGF1	1	---MAEGEITTFALT-----EKFNLPPGNYKKPKLLYCSNG--GH	36
FGF5	100	HLQIYPDGKVNGSHEANMLSV--LEIFAVSQGIVGIRGVFNSKFLAMSKKG	148
FGF1	37	FLRILPDGTVDGTRDRSDQHIQLQLSAESVGEVYIKSTETGQYLAMD TDG	86
FGF5	149	KLHASAKFTDDCKFRERFQENSYNTYASAIHRTEKTGREWYVALNKRGA	198
FGF1	87	LLYGSQTPNEECLFLERLEENHYNTYISKKH--AEK--NMFVGLKKN GSC	132
FGF5	199	KRGCSPRVKQHIETHFLPRFKQSEQPELSFTVTVPEKKPPSP IKPKIP	248
FGF1	133	KRG--PRTHYGQKAILFLPLPVSSD-----	155
FGF5	249	LSAPRKNTNSVKYRLKFRFG	268
FGF1	156	-----	155

d

Identity 21.5%
Similarity 31.7%

FGF5	1	-----	0
FGF2	1	MVGVGGGDVEDVTPRPGGCQISGRGARGCNGIPGAAAWAALPRRRPRRH	50
FGF5	1	-----MSLSFLLLLFFSHLILSAWAHGEKRLA	27
FGF2	51	PSVNPRSRAAGSPRTRGRRTEERPSSGSR-----GDRGRGRAL	88
FGF5	28	PKGQPGPAATDRNP-----RGSS-----SRQSSSSAMSSSS	58
FGF2	89	PGGRLGGRGRGRAPERVGGRRGRGTAAPRAAPAARGSRPGPAGTMAAGS	138
FGF5	59	ASSSPAASLGSQSGLEQSSFWSPSG--RRTGSLYCRVGI GFHLQIYPD	106
FGF2	139	ITTLPALP--EDGGSG---AF--PPGHFKDPKRL YCKNG--GFFLR IHPD	179
FGF5	107	GKVNGSHEANMLSV--LEIFAVSQGIVGIRGVFNSKFLAMSKKGLHASAK	155
FGF2	180	GRVDGVREKSDPHIKLQLQAEERGVVSIKGVGANRYLAMKEDGRLLASKC	229
FGF5	156	FTDDCKFRERFQENSYNTYASAIHRTEKTGREWYVALNKRGA KRGCSPR	205
FGF2	230	VTDECEFFERLESNNYNTYRSRKYTS-----WYVALKRTGQYKLG--SK	271
FGF5	206	VKQHIETHFLPRFKQSEQPELSFTVTVPEKKPPSP IKPKIPLSAPRKN	255
FGF2	272	TGPGQKAILFLPMSAKS-----	288
FGF5	256	TNSVKYRLKFRFG	268
FGF2	289	-----	288

Figure S4 (continued). Pairwise sequence alignment of FGF5 and FGF4 (a), FGF6 (b), FGF1 (c) or FGF2 (d).

a

Anti-FGF2 aptamer

5'-GGGAUACUAGGGCAUUAUGUUACCAGUGUAGUCCC-3'

Anti-FGFR1 aptamer

5'- GGAGGGAAAAGTTATCAGGTCAAACGTCTTTATGGCTGGGGATGGTGTG
GGTTGTTTTGATTAGTTTTGGAGTACTCGCTCC-3'

Anti-FGFR3 aptamer

5'-AGCUCCAGAAGAUAAAUUACAGGCAGAGGCUGACGUAAACAGACAUU
GAUGGGACCCACCCUUCGCGUGGCAACAACUAGGAUACUAUGACCCC-3'

b

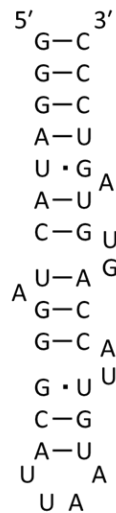


Figure S5. Sequences and predicted secondary structure of aptamers against FGF2, FGFR1, and FGFR3. (a) The sequences of the aptamers. All C and U of the anti-FGF2 aptamer and the anti-FGFR3 aptamer are 2'-fluoro modified. The anti-FGFR1 aptamer is DNA aptamer. (b) The secondary structure of the anti-FGF2 aptamer was predicted using the CentroidFold program (<http://rtools.cbrc.jp/centroidfold/>)².

References

1. Madeira, F. *et al.* The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **47**, W636-W641, doi: 10.1093/nar/gkz268 (2019).
2. Sato, K., Hamada, M., Asai, K. & Mituyama, T. CENTROIDFOLD: a web server for RNA secondary structure prediction. *Nucleic Acids Res.* **37**, W277-280, doi:10.1093/nar/gkp367 (2009).