## **Supplementary Information**

## **Rolling Circle Translation of Circular RNA in Living Human Cells**

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Name	Sequence $(5' \text{ to } 3')^*$
4× FLAG RNA	1 GGGA <u>GCCACC AUGG</u> ACUACA AGGACGACGA CGACAAGAUC AUCGACUACA
(129 nt)	51 AGGACGACGA CGACAAGAUC AUCGACUACA AGGACGACGA CGACAAGAUA
	101 AUUGACUAUA AAGACGACGA CGAUAAAGU
8× FLAG RNA	1 GGGA <u>GCCACC AUG</u> GACUACA AGGACGACGA CGACAAGAUC AUCGACUACA
(258 nt)	51 AGGACGACGA CGACAAGAUC AUCGACUACA AGGACGACGA CGACAAGAUA
	101 AUUGACUAUA AAGACGACGA CGAUAAAGUA CUGGACUAUA AAGACGACGA
	151 CGACAAGAUC AUCGACUACA AGGACGACGA CGACAAGAUC AUCGACUACA
	201 AGGACGACGA CGACAAGAUU AUAGACUACA AAGACGACGA CGAUAAAUUC
	251 AACCAGAA
12× FLAG RNA	1 GGGAGCCACC AUGGACUACA AGGACGACGA CGACAAGAUC AUCGACUACA
(387 nt)	51 AGGACGACGA CGACAAGAUC AUCGACUACA AGGACGACGA CGACAAGAUA
	101 AUUGACUAUA AAGACGACGA CGAUAAAGUA CUGGACUAUA AAGACGACGA
	151 CGACAAGAUC AUCGACUACA AGGACGACGA CGACAAGAUC AUCGACUACA
	201 AGGACGACGA CGACAAGAUU AUAGACUACA AAGACGACGA CGAUAAAUUC
	251 AACCAGAUAU CC <mark>GAUUAUAA GGACGACGAC GACAAG</mark> AUCA UCGACUACAA
	301 GGACGACGAC GACAAGAUCA UCGACUACAA GGACGACGAC GACAAGAUAA
	351 UAGAUUACAA GGACGACGAC GAUAAACCCU CUAGAGG

**Table S1**. Sequence of RNA transcripts containing multiple FLAG coding sequences. The first and the last nucleotides are connected in the circularized molecule.

\* The Kozak sequence is underlined. FLAG coding sequences have gray-colored background.

**Table S2**. Template dsDNA sequences for *in vitro* transcription to synthesize RNAs containing multiple FLAG coding sequences.

Name	Sequence (5' to 3')*
4× FLAG DNA	CGCGGATCCTAATACGACTCACTATAGGGAGCCACCATGGACTACAAGGACGACGACGACAA
(155 bp)	GATCATCGACTACAAGGACGACGACGACGACGACGACGACGACGACGACGA
	TAATTGACTATAAAGACGACGACGATAAAGT
8× FLAG DNA	CGCGGATCCTAATACGACTCACTATA <mark>G</mark> GGA <u>GCCACCATG</u> GACTACAAGGACGACGACGACAA
(284 bp)	GATCATCGACTACAAGGACGACGACGACGACGACGACGACGACGACGACGA
	TAATTGACTATAAAGACGACGACGACGATAAAGTACTGGACTATAAAGACGACGACGACAAGATC
	ATCGACTACAAGGACGACGACGACGACGACGACGACGACGACGACGA
	AGACTACAAAGACGACGACGATAAATTCAACCAGAA
12× FLAG DNA	CGCGGATCCTAATACGACTCACTATAGGGA
(413 bp)	GATCATCGACTACAAGGACGACGACGACGACGACGACGACGACGACGACGA
	TAATTGACTATAAAGACGACGACGACGATAAAGTACTGGACTATAAAGACGACGACGACGACAAGATC
	ATCGACTACAAGGACGACGACGACGACGACGACGACGACGACGACGA
	AGACTACAAAGACGACGACGATAAATTCAACCAGATATCCGATTATAAGGACGACGACGACGAC
	AGATCATCGACTACAAGGACGACGACGACGACAAGATCATCGACTACAAGGACGACGACGACAAG
	ATAATA <b>GATTACAAGGACGACGACGATAAA</b> CCCTCTAGAGG

\* T7 promoter sequence is shown in a box with a solid line. The G base highlighted in yellow is the first base incorporated into RNA during transcription. The Kozak sequence is witten in italic and underlined. FLAG-coding sequences are shown in bold letters.

**Table S3.** Sequences of RNA transcripts that code a human growth factor. The corresponding amino acid sequences are shown under the nucleotides in bold letters. Molecular masses of expected translation product are shown in the left column.

Name of RNA			RN	A seq	uence	(5' to	3') ar	nd corr	respon	ding a	amino	acid s	equen	ce*		
FLAG-EGF	G GG	GA GC	CC AC	C AU	IG GA	C UA	C AA	G GA	C GAG	C GAG	C GAC	AAG	AUC	AUC	GAC	UAU
(264 nt)	<i>G</i> дад	A GAC	<b>T</b> GAC	M GAC	D Gaii	Y AAA	GGU	D GGC	D GAC	D TIATI	D AAG	K GAC	I GAC	I GAC	DGAC	Y AAA
	ĸ	D	D	D	D	ĸ	G	G	D	Y	K	D	D	D	D	ĸ
From linear DNA	GCC	AUU	AAU	AGU	GAC	UCU	GAG	UGU	CCC	CUG	UCC	CAC	GAC	GGG	UAC	UGC
From linear RinA,	A	I	N	S	D	S	E	C	P	L	S	H	D	G	Y	C
9,862.8 Da	CUC	CAC	GAC	GGU	GUG	UGC	AUG	UAU V	AUU	GAA	GCA	UUG	GAC	AAG	UAC	GCC
From circular RNA,	UGC	AAC	UGU	GUU	GUU	GGC	UAC	AUC	GGG	GAG	CGC	UGU	CAG	UAC	CGA	GAC
10,205.2 Da/ round	С	N	C	v	v	G	Y	I	G	Е	R	C	Q	Y	R	D
	CUG	AAG	UGG	UGG	GAA	CUG	CGC	CU-								
	L	K	W	W	E	L	R	L	a a 7					3110	<b>a a</b>	
FLAG-IGF1	G GG	$\frac{GC}{\mathbf{z}}$	<u>7</u>	C AU	<u>IG G</u> A	C UA	C AA	G GA	C GAC	GAC	GAC	: AAG	F AUC	AUC T	GAC	UAU V
(315 nt)	AAA	GAC	GAC	GAC	GAU	AAA	GGU	GGC	GAC	UAU	AAG	GAC	GAC	GAC	GAC	AAA
	к	D	D	D	D	к	G	G	D	Y	к	D	D	D	D	к
From linear DNA	GCC	AUU	GGA	CCG	GAG	ACG	CUC	UGC	GGG	GCU	GAG	CUG	GUG	GAU	GCU	CUU
FIOIT IIITEAL KINA,	A	I	G	P	E	T	L	C	G	<b>A</b>	E	L	V	D	A	L
12,004.3 Da	CAG	UUC	GUG	UGU	GGA	GAC	AGG	GGC	000	UAU V	UUC F	AAC	AAG		ACA	GGG
From circular RNA,	¥ UAU	GGC	UCC	AGC	AGU	CGG	AGG	GCG	CCU	CAG	ACA	GGU	AUG	GUG	GAU	GAG
12.346.6 Da/ round	Y (	G	S	S	S	R	R	A	Р	Q	т	G	М	v	D	Е
,	UGC	UGC	UUC	CGG	AGC	UGU	GAU	CUA	AGG	AGG	CUG	GAG	AUG	UAU	UGC	GCA
	C	C	F	R	S	C	D	L	R	R	L	Е	Μ	Y	C	A
	D	CUC	AAG	CCU P	GCC	AAG K	UCA C	GCU	CU-							
	G GG	A GC	CC AC	C AU	IG GA	C UA	C AA	G GA	C GAG	C GAG	C GAC	AAG	AUC	AUC	GAC	UAU
FLAG-IGF2	G GG	A GC	CC AC	CAU	IG GA	C UA	C AA K	G GA	C GAC D	C GAC D	C GAC D	AAG	AUC	AUC I	GAC D	UAU Y
<b>FLAG-IGF2</b> (306 nt)	G GG G AAA	GAC	CC AC A T GAC	C AU M GAC	IG GA D GAU	C UA Y AAA	C AA K GGU	G GA D GGC	C GAC	C GAC D UAU	C GAC D AAG	C AAG K GAC	AUC I GAC	AUC I GAC	GAC D GAC	UAU Y AAA
<b>FLAG-IGF2</b> (306 nt)	G GG AAA K	GAC	CC AC A T GAC D	C AU GAC D	IG GA D GAU D	C UA Y AAA K	C AA K GGU G	G GAO D GGC G	C GAC D GAC D	C GAC D UAU Y	C GAC D AAG K	CCC	AUC I GAC D	AUC I GAC D	GAC D GAC D	UAU Y AAA K
<b>FLAG-IGF2</b> (306 nt) From linear RNA,	G GG G AAA K GCC A	GAC D AUU	CC AC A T GAC D GCU A	C AU GAC D UAC	IG GA D GAU D CGC R	C UA Y AAA K CCC P	C AA K GGU G AGU S	G GAG GGC G GAG E	C GAC D GAC D ACC T	C GAC D UAU Y CUG L	C GAC D AAG K UGC C	AAG K GAC D GGC G	AUC I GAC D GGG GGG	AUC I GAC D GAG E	GAC D GAC D CUG	UAU Y AAA K GUG V
<b>FLAG-IGF2</b> (306 nt) From linear RNA, 11.287.4 Da	G GG G AAA K GCC A GAC	GAC GAC D AUU I ACC	CC AC A T GAC D GCU A CUC	C AU GAC D UAC Y CAG	IG GA D GAU D CGC R UUC	C UA Y AAA K CCC P GUC	C AA K GGU G AGU S UGU	G GAG GGC GGC GAG E GGG	C GAC D GAC D ACC T GAC	C GAC D UAU Y CUG L CGC	C GAC D AAG K UGC C GGC	C AAG K GAC D GGC G UUC	AUC GAC D GGG G UAC	AUC I GAC D GAG E JUC	GAC D GAC D CUG L AGC	UAU Y AAA K GUG V AGG-
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da	G GG G AAA GCC A GAC D	A GC GAC D AUU I ACC T	CC AC GAC D GCU A CUC L	C AU GAC D UAC Y CAG Q	IG GA D GAU CGC R UUC F	C UA Y AAA K CCCC P GUC V	C AA GGU G AGU S UGU C	G GAG GGC G GAG GAG E GGG G	C GAC GAC D ACC T GAC D	C GAC D UAU Y CUG L CGC R	C GAC D AAG K UGC C GGC GGC	C AAG GAC D GGC G UUC F	AUC GAC D GGG G UAC Y	AUC I GAC D GAG E UUC Z	GAC D GAC D CUG L AGC S	UAU Y AAA K GUG V AGG- R
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA,	G GG G AAA K GCC A GAC D	GAC GAC D AUU I ACC T GCA	CC AC GAC D GCU A CUC L AGC	CAG CAG CAG CAG CAG	IG GA D GAU D CGC R UUC F GUG	C UA Y AAA K CCC P GUC V AGC	C AA GGU G AGU S UGU C CGU	G GAG GGC G GAG GAG GGG GGG CGC	GAC D ACC T GAC D AGC	C GAC D UAU Y CUG L CGC R CCU	C GAC D AAG K UGC C GGC GGC GGC	C AAG GAC D GGC G UUC F AUC	AUC I GAC D GGG G UAC Y GUU	AUC I GAC D GAG E UUC F GAG	GAC D GAC D CUG L AGC S GAG	UAU Y AAA GUG V AGG- R UGC
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round	G GG G AAA K GCC A GAC D CCC P UGU	GAC D AUU I ACC T GCA A	CC AC GAC D GCU A CUC L AGC S CGC	CAU GAC D UAC Y CAG Q CGU R	IG GA D GAU D CGC R UUC F GUG GUG	C UA AAA K CCCC P GUC V AGC S GAC	C AA GGU G AGU S UGU C C C GU R C UGU	G GAG GGC GAG GAG GGG GGG CGC R GCC	C GAC D ACC T GAC D AGC S CUC	C GAC D UAU Y CUG CGC R CCU P CUG	C GAC D AAG K UGC C GGC G GGC G G G C	C AAG GAC D GGC G UUC F AUC I	AUC I GAC D GGG G UAC Y GUU V UAC	AUC I GAC D GAG E UUC F GAG E UIGU	GAC D GAC D CUG L AGC S GAG E CCU	UAU Y AAA K GUG V AGG- R UGC C
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round	G GG G AAA GCC A GAC D CCC P UGU C	GAC D AUU I ACC T GCA A UUC F	CC AC GAC D GCU A CUC L AGC S CGC R	CAU GAC D UAC Y CAG CGU R AGC S	IG GA D GAU D CGC R UUC F GUG V UGU C	C UA AAA K CCCC P GUC V AGC S GAC D	C AA GGU G AGU S UGU C CGU R CUG L	G GAG GGC G GAG E GGG G CGC R GCC A	C GAC D GAC D ACC T GAC D AGC S CUC L	C GAC D UAU Y CUG CGC R CCU P CUG L	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E	C AAG GAC D GGC G UUC F AUC I ACG T	AUC GAC D GGG G UAC Y S UAC V UAC Y	AUC I GAC D GAG E UUC F GAG E UGU C	GAC D GAC D CUG L AGC S GAG E GAG E GCU A	UAU Y AAA K GUG GUG V AGG- R UGC C ACC T
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round	G GG G AAA K GCC A GAC D CCC P UGU C CCC	A GC GAC D AUU I ACC T GCA A UUC F GCC	A GAC D GCU A CUC L AGC S CGC R AAG	CAU GAC D UAC Y CAG Q CGU R AGC S UCC	GAU GAU D CGC R UUC F GUG V UGU C GAG	C UA AAA K CCCC P GUC V AGC S GAC D CU-	C AA GGU G AGU S UGU C CGU R CUG L	G GAG GGC GAG E GGG G CGC R GCC A	C GAC D ACC T GAC D AGC S CUC L	C GAC D UAU Y CUG L CGC R CCU P CUG L	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E	AAG GAC D GGC G UUC F AUC I ACG T	AUC GAC D GGG G UAC Y UAC V UAC Y	AUC I GAC D GAG E UUC F GAG E UGU C	GAC D GAC CUG L AGC S GAG GAG GCU A	UAU Y AAA K GUG V AGG- R UGC C ACC T
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round	G GG G AAA K GCC A GAC D CCC P UGU C CCC P	GAC GAC D AUU I ACC T GCA A UUC F GCC A	A GAC D GCU A CUC L AGC S CGC R AAG K	C AU GAC D UAC Y CAG Q CGU R AGC S UCC S	GAU GAU D CGC R UUC F GUG V UGU C GAG E	C UA AAA K CCCC P GUC V AGC S GAC D CU- L	C AA GGU G AGU S UGU C CGU R CUG L	G GAG GGC GAG GAG GGG GGG CGC R GCC A	C GAC D ACC T GAC D AGC S CUC L	C GAC D UAU Y CUG CGC R CCU P CUG L	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E	C AAG GAC D GGC G UUC F AUC I ACG T	AUC GAC D GGG GGG V AC Y S UAC V UAC Y	AUC GAC D GAG E UUC F GAG E UGU C	GAC D GAC L AGC S GAG E GAG CU A	UAU Y AAA K GUG V AGG- R UGC C ACC T
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round FLAG-EGF_stop	G GG G AAA GCC A GAC D CCC P UGU C CCC P G GG	GAC D AUU I ACC T GCA F GCC F GCC A GCC A	A GAC D GCU A CUC L AGC S CGC R AAG K CC AC	CAU GAC D UAC Y CAG CAG CGU R AGC S UCC S CAU	GAU GAU CGC R UUC F GUG V UGU C GAG E GGGA	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA	C AA GGU G AGU S UGU C CGU R CUG L C AA	G GAG GGC GAG GGG GGG CGC R GCC A G GAC	GAC GAC D ACC T GAC D AGC S CUC L	C GAC D UAU Y CUG CGC R CCU P CUG L C GAC	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E	C AAG GAC D GGC G UUC F AUC I ACG T	AUC GAC D GGG G UAC Y UAC Y UAC Y C AUC	AUC GAC D GAG E UUC F GAG E UGU C	GAC D CUG L AGC S S GAG GCU A GAC	UAU Y AAA K GUG V AGG- R UGC C ACC T
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round FLAG-EGF_stop (273 bp)	G GG G AAA K GCC A GAC D C C C C C C C C C C C C C C C C C C	GAC GAC D AUU I ACC T GCA F GCC A GCC A GAC	A GAC D GCU A CUC L AGC S CGC R AAG K CC AC	CAU GAC D UAC Y CAG Q CGU R AGC S UCC S CAU	GAU GAU D CGC R UUC F GUG C GAG E IG GA	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA	C AA GGU G AGU S UGU C CGU R CUG L C AA C AA C AA	G GAG GGC G GGG GGG CGC R GCC A G GAC G GAC	C GAC D GAC D ACC T GAC D AGC S CUC L C GAC	C GAC D UAU Y CUG CGC R CCU P CUG L CUG L C GAC D	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG	C AAG GAC D GGC G UUC F AUC I ACG T C AAG K GAC	AUC GAC D GGG G UAC Y UAC Y UAC Y C AUC I GAC	AUC I GAC D GAG E UUC F GAG C AUC I GAC	GAC D GAC L AGC S GAG E GAG C Q C U A GAC D GAC	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round FLAG-EGF_stop (273 bp)	G GG G AAA K GCC A GAC D CCC P UGU C CCC P G GG AAA K	GAC GAC D AUU I ACC T GCA A UUC F GCC A GCC A GAC D	A GAC A GAC D GCU A CUC L AGC S CGC R AAG K CC AC GAC D	CAG GAC D UAC Y CAG Q CGU R AGC S UCC S CAU M GAC D	GAU GAU D CGC R UUC F GUG C GAG C GAG GAG C GAU D	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA K	C AA GGU G AGU S UGU C CGU R CUG L CUG L C AA C AA GGU G	G GAG GGG GAG GGG GGG CGC R GCC A GGCC A GGC GGC G	GAC GAC D ACC T GAC D AGC S CUC L C GAC D GAC D	C GAC D UAU Y CUG CGC R CCU P CUG L C GAC D UAU Y	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG K	AAG GAC D GGC G UUC F AUC I ACG T C AAG T C AAG D	AUC GAC D GGG G UAC V UAC V UAC Y UAC Y UAC Y UAC Y UAC Y UAC D	AUC GAC D GAG E UUC F GAG C AUC C AUC I GAC D	GAC D GAC L AGC S GAG GAG GAC D GAC D GAC D	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA K
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round FLAG-EGF_stop (273 bp)	G GG G AAA K GCC A GAC D C C C C C C C C C C C C C C C C C C	GAC GAC D AUU I ACC T GCA F GCC F GCC A GAC D AUU	CC AC GAC D GCU A CUC L AGC S CGC R AAG K CC AC GAC D AAU	CAG GAC D UAC Y CAG CAG CGU R AGC S UCC S CAU M GAC D AGU	GAU GAU CGC R UUC F GUG C GAG GAG GAG GAU D GAC	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA K UCU	C AA GGU G AGU S UGU C CGU R CUG CUG L C AA C C AA C GGU G GAG	G GAG GGC GAG GGG GGG CGC R GCC A GCC A GGC G GGC G UGU	GAC GAC D ACC T GAC D AGC S CUC L C GAC D GAC D GAC D CCC	C GAC D UAU Y CUG CGC R CCU P CUG CUG C UAU Y CUG	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG K UCC	AAG GAC D GGC G UUC F AUC I ACG T CACG CAC	AUC GAC D GGG G UAC Y UAC Y UAC Y UAC Y UAC Y GAC I GAC D GAC	AUC GAC D GAG E UUC F GAG C UGU C AUC I GAC I GAC D GGG	GAC D CUG CUG CUG S S GAG GAG CU A GAC D GAC D UAC	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA X UGC
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round FLAG-EGF_stop (273 bp) From linear RNA,	G GG G AAA K GCC A GAC D CCC P UGU C CCC P G GG AAA K GCC A	GAC GAC D AUU I ACC T GCA A UUC F GCC A C GAC D AUU I I	A GAC A GAC D GCU A CUC L AGC S CGC R AAG K CC AC GAC D AAU N	CAU GAC D UAC Y CAG Q CGU R AGC S UCC S CAU M GAC D AGU S	GAU GAU D CGC R UUC F GUG C GAG GAG GAG GAU D GAU D	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA K UCU S	C AA GGU G AGU S UGU C CGU R CUG CUG L C AA C AA GGU G GGU G AG	G GAG GGC GGG GGG GGG CGC R GCC A GGC G GGC G GGC G UGU C	C GAC D GAC D ACC T GAC D AGC S CUC L C GAC D GAC D CCC P	C GAC D UAU Y CUG CGC R CCU P CUG L CUG L UAU Y CUG L	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG K UCC S	CAAG CAC C C C C C C C C C C C C C C C C	AUC GAC D GGG G UAC Y UAC Y UAC Y UAC Y GAC D GAC D	AUC GAC D GAG E UUC F GAG C AUC C AUC I GAC D GGG G	GAC D GAC L AGC S GAG GAG GAC D GAC D GAC D UAC Y	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA K UGC C
FLAG-IGF2 (306 nt)From linear RNA,11,287.4 DaFrom circular RNA,11,629.8 Da/ roundFLAG-EGF_stop (273 bp)From linear RNA,9,862.8 Da	G GG G AAA K GCC A GAC D CCC P UGU C CCC P G GG AAA K GCC A CUC	GAC GAC D AUU I ACC T GCA F GCC A UUC F GCC A C C A C C A C C A C C A C C C C C	CC AC GAC D GCU A CUC L AGC S CGC R AAG CGC R AAG CC AC GAC D AAU N GAC	CAG GAC D UAC Y CAG CAG CGU R AGC S UCC S CAU M GAC D AGU S GGU	GAU GAU CGC R UUC F GUG C GAG GAG GAG GAU GAU GAU GAC D GUG	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA K UCU S UGC C	C AA GGU G AGU S UGU C CGU R CUG CUG L CUG L C AA C GGU GAG GAG E AUG	G GAG GGC GGG GGG GGG GGC R GCC A GGC G GGC G UGU UGU V V	GAC GAC D ACC T GAC D AGC S CUC L C GAC D GAC D GAC D CCC P AUU	C GAC D UAU Y CUG CGC R CCU P CUG L CUG L CUG L CUG L GAA	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG K UCC S GCA	AAG GAC D GGC G UUC F AUC I ACG T CACG K GAC D CAC H UUG	AUC GAC D GGG G UAC Y UAC Y UAC Y UAC Y GAU GAC D GAC D GAC	AUC GAC D GAG E UUC F GAG C UGU C AUC I GAC I GAC G GGG G AAG	GAC D GAC L AGC S GAG GAG GAC D GAC D GAC D UAC Y UAC	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA K UGC C GCC A
FLAG-IGF2(306 nt)From linear RNA,11,287.4 DaFrom circular RNA,11,629.8 Da/ roundFLAG-EGF_stop(273 bp)From linear RNA,9,862.8 DaFrom circular RNA,	G GG G AAA K GCC A GAC D C C C C C C C C C C C C C C C C C C	GAC GAC D AUU I ACC T GCA F GCC A UUC F GCC A C A C A C A C A C A C A C A C A C	CC AC GAC GAC D GCU A CUC L AGC S CGC R AAG CC AC GAC D AAU N GAC D UGU	CAG GAC D UAC Y CAG CGU R AGC S UCC S CAU M GAC D AGU S GGU GUU	GAU GAU CGC R UUC F GUG C GAG GAG GAU GAU D GAU D GAU Q UGU	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA X UCU S UGC C GGC	C AA GGU G AGU S UGU C CGU R CUG CUG CUG L CUG CUG CUG CUG CUG CUG CUG CUG CUG CUG	G GAG GGG GGG GGG GGG CGC R GCC A GGC G GGC G UGU C UGU C UAU Y AUC	C GAC D ACC T GAC D AGC S CUC L C GAC D GAC D CCC P AUU I GGG	C GAC D UAU Y CUG CGC R CCU P CUG L CUG L CUG L UAU Y CUG L GAA E GAG	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG K UCC S GCA A CGC	CACG CACC C C C C C C C C C C C C C C C	AUC GAC D GGG G UAC Y UAC Y UAC Y UAC Y GUU V UAC Y GAC D GAC D GAC D CAG	AUC GAC D GAG E JUC F GAG C C AUC C AUC C GAC G G G G G G G G G G G G G G G G	GAC D CUG CUG CUG S S GAG GAC D GAC D GAC D UAC Y UAC Y CGA	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA K UGC C GCC A GAC
FLAG-IGF2 (306 nt) From linear RNA, 11,287.4 Da From circular RNA, 11,629.8 Da/ round FLAG-EGF_stop (273 bp) From linear RNA, 9,862.8 Da From circular RNA, 9.862.8 Da	G GG G AAA K GCC A GAC D CCC P UGU C CCC P UGU C CCC AAA K GCC A AAA K GCC L UGC C	GAC GAC D AUU I ACC T GCA A UUC F GCC A GCC A C C A C C A C A C A C A C A	CC AC GAC D GCU A CUC L AGC S CGC R AAG K CC AC GAC D AAU N GAC D UGU C	CAG GAC D UAC Y CAG Q CGU R AGC S UCC S UCC S CAU M GAC D AGU S GGU G UU	GAU GAU D CGCC R UUC F GUG C GAG C GAG GAU GAU GAU D GAU D GAU V UGU V	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA X UCU S UGC C GGC G	C AA GGU G AGU S UGU C CGU R CUG CUG CUG CUG CUG CUG CUG CUG CUG CUG	G GAG GGG GAG GGG GGG CGC R GCC A GGC G GGC G UGU C UAU Y AUC I	GAC GAC D ACC T GAC D AGC S CUC L C GAC D GAC D CCC P AUU I GGG G GGG	C GAC D UAU Y CUG CGC R CCU P CUG L CUG L UAU Y CUG L GAA E GAG E	C GAC D AAG K UGC C GGC G GGC G GGC G GAG E C GAC D AAG K UCC S GCA A CGC R	C AAG GAC D GGC G UUC F AUC I ACG T C ACG C C C C C C C	AUC GAC D GGG G UAC V UAC V UAC Y UAC Y GAC GAC D GAC D GAC D CAG	AUC GAC D GAG E UUC F GAG C AUC C AUC C AUC GAC G G G G G G G G G G G G G G G C Y	GAC D GAC L CUG CUG S S GAG GAG C D GAC D GAC D UAC Y CGA R	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA K UGC C GCC A GAC
FLAG-IGF2 (306 nt)From linear RNA, 11,287.4 DaFrom circular RNA, 11,629.8 Da/ roundFLAG-EGF_stop (273 bp)From linear RNA, 9,862.8 DaFrom circular RNA, 9,862.8 Da	G GG G AAA K GCC A GAC D C C C C C C C C C C C C C C C C C C	GAC GAC D AUU I ACC T GCA F GCC A UUC F GCC A UUC F GCC A UUC F GCC A UUC F GCC A UUC F GCC A UUC F GCC A A UUC F GCC A A UUC F GCC A A UU A C A C A C A C A C A C A C A	CC AC GAC D GCU A CUC L AGC S CGC R AAG CGC R AAG CC AC C C AC D AAU N GAC D UGU C UGG	CAG GAC D UAC Y CAG Q CGU R AGC S UCC S CAU M GAC D AGU S GGU GGU UCC S UCC Y UCC S CAU	GAU GAU CGC R UUC F GUG C GAG GAG GAG GAU GAU GAU GAU C C GAU C C GAU C C GAU C C GAU C C GAG C C GAG C C C C C C C C C C C C	C UA AAA K CCCC P GUC V AGC S GAC D CU- L C UA Y AAA X UCU S UGC C GGC C GGC CUG	C AA GGU G AGU S UGU C CGU R CUG CUG CUG L CUG C GGU GAG GAG E AUG M UAC Y CGC	G GAG GGC GGG GGG GGG GGC R GCC A GGC A GGC G UGU UGU UGU UGU Y AUC I UGA	C GAC D ACC T GAC D ACC D AGC S CUC L C GAC D GAC D GAC D CCC P AUU I GGG G G UAG	C GAC D UAU Y CUG CGC R CCU P CUG L CUG L CUG L CUG L GAA E GAG E UAA	C GAC D AAG K UGC C GGC G GGC G GAG E C GAC D AAG K UCC S GCA A CGC R CU-	AAG GAC D GGC G UUC F AUC I ACG T C ACG C C C C C C	AUC GAC GGG G UAC Y UAC Y UAC Y UAC Y GAU GAC D GAC D GAC D CAG Q	AUC GAC D GAG E UUC F GAG C UGU C AUC I GAC D GGG G G AAG K UAC Y	GAC D GAC L AGC S GAG GAG GAC D GAC D GAC D UAC Y UAC Y CGA R	UAU Y AAA K GUG V AGG- R UGC C ACC T UAU Y AAA K UGC C GCC A GAC A

FLAG-EGF weak	G G(	GA CO	GC UU	JU AU	G GA	C UA	C AA	g gao	C GAC	C GAC	GAC	AAG	AUC	AUC	GAC	UAU
	G	R	F	' M	D	Y	K	D	D	D	D	K	I	I	D	Y
kozak	AAA	GAC	GAC	GAC	GAU	AAA	GGU	GGC	GAC	UAU	AAG	GAC	GAC	GAC	GAC	AAA
(264 bp)	ĸ	D	D	D	D	ĸ	G	G	D	Y	ĸ	D	D	D	D	K
(2010)	GCC	AUU	AAU	AGU	GAC	UCU	GAG	UGU	CCC	CUG	UCC	CAC	GAC	GGG	UAC	UGC
	A	I	N	S	D	S	E	С	Р	L	S	H	D	G	Y	С
From linear RNA,	CUC	CAC	GAC	GGU	GUG	UGC	AUG	UAU	AUU	GAA	GCA	UUG	GAC	AAG	UAC	GCC
	L	H	D	G	v	С	Μ	Y	I	E	Α	<b>L</b>	D	K	Y	Α
10,323.3 Da	UGC	AAC	UGU	GUU	GUU	GGC	UAC	AUC	GGG	GAG	CGC	UGU	CAG	UAC	CGA	GAC
From circular RNA	C	N	C	v	v	G	Y	I	G	E	R	С	Q	Y	R	D
	CUG	AAG	UGG	UGG	GAA	CUG	CGC	CU-								
10,796.8 Da/ round	L	к	W	W	Е	L	R	L								

\*Authentic Kozak sequences are underlined. A start codon methionine for linear RNA is shown in red. In-frame stop codons are shown in bold text. Amino acids which would be translated only on circular RNA are shown in italic.

**Table S4.** Template dsDNA sequences for *in vitro* transcription to synthesize RNAs that code a human growth factor. Sense sequences are shown in this table.

Name	Sequence (5' to 3')*
FLAG-EGF**	CGCGGATCCTAATACGACTCACTATA <mark>G</mark> GGA <u>GCCACCATG</u> GACTACAAGGACGACGACGACAA
(290 bp)	GATCATCGACTATAAAGACGACGACGACGATAAAGGTGGCGACTATAAGGACGACGACGACAAAG
	CCATTAATAGTGACTCTGAGTGTCCCCTGTCCCACGACGGGTACTGCCTCCACGACGGTGTG
	TGCATGTATATTGAAGCATTGGACAAGTACGCCTGCAACTGTGTTGTTGGCTACATCGGGGA
	GCGCTGTCAGTACCGAGACCTGAAGTGGTGGGAACTGCGCCT
FLAG-IGF1***	CGCGGATCCTAATACGACTCACTATA <mark>G</mark> GGA <u>GCCACCATG</u> GACTACAAGGACGACGACGACAA
(341 bp)	GATCATCGACTATAAAGACGACGACGACGATAAAGGTGGCGACTATAAGGACGACGACGACAAAG
	CCATTGGACCGGAGACGCTCTGCGGGGCTGAGCTGGTGGATGCTCTTCAGTTCGTGTGGA
	GACAGGGGCTTTTATTTCAACAAGCCCACAGGGTATGGCTCCAGCAGTCGGAGGGCGCCTCA
	GACAGGTATGGTGGATGAGTGCTGCTTCCGGAGCTGTGATCTAAGGAGGCTGGAGATGTATT
	GCGCACCCCTCAAGCCTGCCAAGTCAGCTCT
FLAG-IGF2****	CGCGGATCCTAATACGACTCACTATA <mark>G</mark> GGA <u>GCCACCATG</u> GACTACAAGGACGACGACGACAA
(332 bp)	GATCATCGACTATAAAGACGACGACGACGATAAAGGTGGCGACTATAAGGACGACGACGACGACAAAG
	CCATTGCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGGGGG
	GTCTGTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCC
	TGGCATCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGTG
	CTACCCCGCCAAGTCCGAGCT
FLAG-EGF_stop**	CGCGGATCCTAATACGACTCACTATA <mark>G</mark> GGA <u>GCCACCATG</u> GACTACAAGGACGACGACGACAA
(299 bp)	GATCATCGACTATAAAGACGACGACGACGATAAAGGTGGCGACTATAAGGACGACGACGACGACAAAG
	CCATTAATAGTGACTCTGAGTGTCCCCTGTCCCACGACGGGTACTGCCTCCACGACGGTGTG
	TGCATGTATATTGAAGCATTGGACAAGTACGCCTGCAACTGTGTTGTTGGCTACATCGGGGA
	GCGCTGTCAGTACCGAGACCTGAAGTGGTGGGAACTGCGC <b>TGATAGTAA</b> CT
FLAG-EGF_weak	CGCGGATCC <mark>TAATACGACTCACTATA<mark>G</mark>GG</mark> ACGCTTTATG <b>GACTACAAGGACGACGACGACAA</b>
kozak**	GATCATCGACTATAAAGACGACGACGACGATAAAGGTGGCGACTATAAGGACGACGACGACGACAAAG
(290 bp)	CCATTAATAGTGACTCTGAGTGTCCCCTGTCCCACGACGGGTACTGCCTCCACGACGGTGTG
	TGCATGTATATTGAAGCATTGGACAAGTACGCCTGCAACTGTGTTGTTGGCTACATCGGGGA
	GCGCTGTCAGTACCGAGACCTGAAGTGGTGGGAACTGCGCCT

\* T7 promoter sequence is shown in a box with a solid line. The G base highlighted in yellow is the first base incorporated into RNA during transcription. The Kozak sequence is witten in italic and underlined. FLAG-coding sequences are shown in bold letters. Coding sequences for EGF, IGF-1 and IGF-2 have gray-colored background.

\*\* GenBank: X04571.1

\*\*\* GenBank: A29117.1

\*\*\*\* GenBank: EU622024.1



*Figure* **S1.** Denaturing PAGE analysis of ligation reactions in the preparation of circular RNAs containing multiple FLAG-coding sequences. The gel contained 5% polyacrylamide. The gels were visualized by SYBR Green II staining. (A) Lane 1, **4× FLAG** linear RNA; lane 2, **4× FLAG** linear RNA + guide DNA + T4 RNA ligase 2; lane 3, **12× FLAG** linear RNA; lane 4, **12× FLAG** linear RNA + T4 RNA Ligase 2. (B) Lane 1, **8× FLAG** linear RNA; lane 2, **8× FLAG** linear RNA + guide DNA + T4 RNA; lane 2, **8× FLAG** linear RNA + guide DNA + T4 RNA; lane 2, **8× FLAG** linear RNA + guide DNA + T4 RNA; lane 2.



*Figure S2.* Western blot analysis of the translation product of linear RNAs after purification. RNA (0.48  $\mu$ M) was incubated in rabbit reticulocyte lysate at 30°C for overnight. After incubation, the sample was purified using anti-FLAG antibody-conjugated agarose beads according to the manufacturer's protocol (Wako Pure Chemicals). The anti-FLAG antibody dissociated from the beads was detected at around 25 and 50 KDa of the size marker.

Expected sequences of translated peptides and their molecular mass are shown below.

## From **4**× **FLAG** linear RNA:

MDYKDDDDKI IDYKDDDDKI IDYKDDDDKI IDYKDDDDK (39 a. a.)

Molecular mass: 4,808.0

From **8**× **FLAG** linear RNA: MDYKDDDDKI IDYKDDDDKI IDYKDDDDKI IDYKDDDDKV LDYKDDDDKI IDYKDDDDKI IDYKDDDDKI IDYKDDDDKF NQ (82 a. a.) Molecular mass: 10,068.6

From 12× FLAG linear RNA:

MetDYKDDDDKI IDYKDDDDKI IDYKDDDDKI IDYKDDDDKV LDYKDDDDKI IDYKDDDDKI IDYKDDDDKI IDYKDDDDKF NQISDYKDDD DKIIDYKDDD DKIIDYKDDD DKIIDYKDDD DKPSR (125 a. a.) Molecular mass: 15 268 0

Molecular mass: 15,268.0



**Figure S3.** Immunofluorescent staining of HeLa cells after the transfection of a circular RNA named **4**× **FLAG**. Microscopic imaging of the translation product was performed using anti-FLAG antibody labeled with Alexa Fluor 488 (green). Cells were counterstained with DAPI (4',6-diamidino-2-phenylindole; blue) to image nuclei. Differential interference contrast (DIC) images and the merged image of the three (Merge) are also shown. The 129 nt RNA sequence was chemically synthesized on a DNA/RNA synthesizer with 5'-phosphorylation. After being purified, the linear RNA was circularized using T4 RNA ligase 2 on a short complementary DNA oligo.



**Figure S4.** Denaturing PAGE (6%) analysis of *in vitro* transcribed RNAs and subsequent circularization reactions in the preparation of circular RNAs coding a human growth factor. The RNAs were annealed on a complementary 20-nt DNA oligomer and incubated with T4 RNA ligase 2 at 37°C for 4 h. The gels were visualized by SYBR Green II staining. (A) Lane 1, FLAG-EGF transcribed linear RNA; lane 2, FLAG-EGF transcribed linear RNA + ligase; lane 3, FLAG-IGF1 transcribed linear RNA; lane 4, FLAG-IGF1 transcribed linear RNA + ligase; lane 5, FLAG-IGF2 transcribed linear RNA; lane 6, FLAG-IGF2 transcribed linear RNA + ligase. (B) Lane 1, FLAG-EGF\_stop transcribed linear RNA; lane 2, FLAG-EGF\_stop transcribed linear RNA; lane 2, FLAG-EGF\_stop transcribed linear RNA; lane 2, FLAG-EGF\_weak kozak transcribed linear RNA; lane 2, FLAG-EGF weak kozak transcribed linear RNA + ligase.



*Figure S5.* Circular RNAs which code a human growth factor were translated into peptides of high-molecular weight in an E. coli cell-free expression system. The RNA was translated in a cell-free translation system (PURExpress, New England Biolabs) at 1  $\mu$ M concentration. After being incubated at 37°C for 2 h, 1  $\mu$ L of each reaction mixture was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The peptides were then transferred to a PVDF membrane. Anti-FLAG M2 monoclonal antibody (Sigma-Aldrich) and anti-mouse IgG peroxidase-conjugated antibody (Sigma-Aldrich) were used to visualize the blot. Letters "L" and "C" in the figure stand for linear RNA or circular RNA, respectively.