Supporting information for:

In vitro selection of multiple libraries created by genetic code reprogramming to discover macrocyclic peptides that antagonize VEGFR2 activity in living cells

Takashi Kawakami[1], Takahiro Ishizawa[1], Tomoshige Fujino[1], Patrick C. Reid[2], Hiroaki Suga[3], and Hiroshi Murakami[1,*]

[1]*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo,* 3-8-1 Komaba, Meguro-ku, Tokyo, 153-8902, Japan, Tel/Fax: +81-3-5465-8850

[2]*PeptiDream Inc.* 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan, Tel/Fax: +81-3468-9022

[3]*Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan,* Tel/Fax: +81-3-5841-8372

[*]e-mail: murah@bio.c.u-tokyo.ac.jp

Supplementary methods

Chemical synthesis of *N*-acyl-Phe-CME derivatives

N-chloroacetyl-L-phenylalanine cyanomethyl ester (ClAc-L-Phe-CME) and *N*-chloroacetyl-D-phenylalanine cyanomethyl ester (ClAc-D-Phe-CME) were synthesized by the procedure reported before[1, 2]. *p*-(*N*-chloroacetoamido)benzoyl-L-phenylalanine cyanomethyl ester (ClAB-L-Phe-CME) and *p*-(*N*-chloroacetoamido)benzoyl-D-phenylalanine cyanomethyl ester (ClAB-D-Phe-CME) were synthesized by the procedure described elsewhere [3].

Preparation of enhanced flexizyme and initiator tRNA^{fMet}(CAU)

Enhanced flexizyme and initiator tRNA^{fMet}(CAU) were prepared by run-off *in vitro* transcription using T7 RNA polymerase as previously described[1, 4].

Preparation of aminoacyl-tRNA^{fMet}(CAU)

Aminoacyl-tRNA^{fMet}(CAU) charged with ClAc-L-Phe, ClAc-D-Phe, ClAB-L-Phe or ClAB-D-Phe was prepared according to the previously described procedure[1, 2].

Preparation of His-free *N*-α-methyl-His

100 mM of potassium salt of *N*-α-methyl-His (Watanabe Kagaku) dissolved in bicine [pH 9] was treated with 50 mM of acetic anhydride at 37°C for 30 min to make contaminated His acetylated and the reaction was quenched by the addition of acetic acid to make pH of the solution 7-8.

Ribosomal synthesis of model peptides containing a backbone-modified non-proteinogenic amino acid via aaRS-catalyzed *in situ* tRNA aminoacylation

Ribosomal synthesis of model peptides containing a non-proteinogenic amino acid was performed according to the similar procedure described before[1] Translation reaction mixture containing 0.04 μM DNA template, 0.5 mM each Met, Tyr, Lys, 50 μM [^{14}C]-Asp, and 5 mM non-proteinogenic amino acid was incubated for 60 min at 37°C. The products were analyzed by tricine-SDS PAGE and autoradiography (Pharox FX, BIO-RAD). For MALDI-TOF mass analysis, the reaction was carried out with Asp instead of [^{14}C]-Asp, and the translation product was desalted with C-TIP (Nikkyo Technos), eluted with 80% acetonitrile, 0.5% acetic acid saturated with CHCA and analyzed by autoflex II (BRUKER DALTONICS) operated in the linear positive mode.

Preparation of the TRAP system

The TRAP system for proteinogenic amino acid-based peptide library was prepared with 0.25 mM each of proteinogenic amino acids according to the procedure described elsewhere[3]. The protein

concentration in the TRAP system are listed in Supplementary Table 3. The concentration of tRNAs and small molecules in the TRAP system are listed in Supplementary Table 4. Creatine kinase, creatine phosphate and *E. coli* tRNAs were purchased from Roche Diagnostics. Myokinase was purchased from Sigma-Aldrich. The TRAP system for non-proteinogenic amino acid-based peptide library was prepared with the same components as that for proteinogenic amino acid-based peptide library except that 2.5 mM each of His-free *N*-α-methyl-His, *N*-methyl-Phe (Watanabe Kagaku), D-Tyr (Peptide Institute), cycloleucine (Watanabe Kagaku), 10 μM native $tRNA^{Tyr}$ (Sigma-Aldrich) and additional 0.2 μM HisRS were included instead of His, Phe, Tyr, and Val.

VEGFR pull-down assay

VEGFR pull-down assay was performed according to the similar procedure to the TRAP display selection of anti-VEGFR2 macrocyclic peptides. VEGFR-Fc proteins were purchased from R&D systems; VEGFR2-Fc (amino acid residues 1–764 of the extracellular domain of human VEGFR2 fused to the hexa-histidine tagged Fc of human IgG via the IEGRMD peptide), VEGFR1-Fc (amino acid residues 1–687 of the extracellular domain of human VEGFR1 fused to the hexa-histidine tagged Fc of human IgG), and VEGFR3-Fc (amino acid residues 1–776 of the extracellular domain of human VEGFR3 fused to the hexa-histidine tagged Fc of human IgG via the IEGRMD peptide). Each macrocyclic peptide displayed on mRNA was prepared by transcription and translation of the cloned cDNA in the TRAP system containing 1 μM T7 RNA polymerase and 5% volume of the crude PCR mixture containing the template cDNA. The reverse transcription of mRNAs displaying macrocyclic peptides was performed using RNase H-inactivated reverse transcriptase (TOYOBO, ReverTra) at 42°C for 30 min. After quenching the reverse transcription with EDTA and neutralizing the solution with HEPES, the mixture containing cDNA/mRNA-displayed macrocyclic peptides were mixed with the VEGFR immobilized on beads at 25°C for 30 min, followed by thrice washing with HBS-T at 25°C. The selected cDNAs on the beads were eluted at 95°C for 5 min and quantified by quantitative PCR.

Chemical synthesis of selected peptides

All Fmoc amino acid building blocks were purchased from Watanabe Kagaku. All of the selected peptides were synthesized by an automated microwave peptide synthesizer, Liberty1 (CEM), using 0.05 mmol of Rink Amide-ChemMatrix resin (Biotage). Fmoc group was removed with 20% piperidine in DMF. Amino acid coupling was performed with 4 equivalent each of HBTU, HOBt,

and Fmoc amino acid building block, and 8 equivalent of DIEA in DMF. Cycloleucine coupling and amino acid coupling right after *N*-α-methyl-histidine, *N*-methyl-phenylalanine, and cycloleucine were performed with HATU and HOAt instead of HBTU and HOBt. After complete extension of the selected peptides, the *N*-terminal amino group was reacted with 2-chloroacetic acid, HBTU, HOBT, and DIEA at 25°C for 30 min. After washing the resin with DMF, DCM and diethyl ether, peptides were cleaved from the resin and deprotected with TFA/3,6-dioxa-1,8-octanedithiol/TIS/water (97.5:2.5:2.5:2.5). Cleaved peptides were precipitated with diethyl ether and the precipitates were washed with diethyl ether and dried in vacuo. The crude linear peptides were dissolved in DMSO and cyclized by the addition of triethylamine. The macrocyclized peptides were lyophilized to remove triethylamine and dissolved in DMSO to give crude macrocyclic peptides for subsequent screening of VEGFR2 antagonists. All the synthesized peptides were analyzed by MALDI-TOF-MS and HPLC. Crude L1, BL1, BD1, uBL1 and rBL1 peptides were purified by reverse phase-HPLC, lyophilized and dissolved in DMSO.

VEGFR2 phosphorylation assay

HUVEC cells (TaKaRa Bio) were serum starved for 18h and treated with macrocyclic peptides in serum-free media for 15 min followed by 20 ng/mL VEGF (R&D Systems) for 7 min at 37°C and under 5% CO_2. Cells were scraped in cold PBS containing protease inhibitor cocktail and phosphatase inhibitor cocktail and centrifuged to collect cells. The collected cells were lysed in TBS-T containing 1% SDS and 10 mM DTT and the lysates were spotted on PVDF membranes. To detect total VEGFR2, the membranes were blocked with 0.1% skim milk and probed with anti-VEGFR2 antibody (Cell Signaling Technology) in TBST containing 0.1% skim milk and anti-rabbit IgG HRP-conjugated secondary antibody followed by enhanced chemiluminescent detection. To detect phosphorylated VEGFR2, the membranes were blocked with 0.1% BSA and probed with anti-phosphorylated VEGFR2 antibody (Cell Signaling Technology) in Can Get Signal (TOYOBO) and anti-rabbit IgG HRP-conjugated secondary antibody followed by enhanced chemiluminescent detection.

HUVEC proliferation assay

HUVEC cells were plated on 96-well plates coated with gelatin (Sigma-Aldrich) and serum starved for 18h. HUVEC cells were treated with macrocyclic peptides for 30 min followed by 20 ng/mL VEGF (R&D Systems) for 18h in the presence of the macrocyclic peptides at 37°C and under 5%

CO_2. HUVEC proliferation was evaluated by Cell Proliferation ELISA, BrdU (colorimetric) (Roche) according to the manufacture's protocol. Cells were labeled with BrdU in the presence of VEGF and macrocyclic peptides for 4h, then fixed and stained with anti-BrdU antibody conjugated with peroxidase. The peroxidase activity was detected with tetramethyl-benzydin colorimetric substrate.

VEGFR2 binding assay

VEGFR2 binding assay was performed by Octet system using VEGFR2-Fc (R&D systems) immobilized on anti-Human IgG Fc Capture biosensor (ForteBio) according to the manufacture's protocol. The binding assay was performed at 30°C in the following condition: 50 mM Hepes-KOH pH 7.5, 300 mM NaCl, 0.05% Tween20 and 0.001% DMSO. Each step in the binding assay was as follows: immobilization of VEGFR2-Fc to anti-Human IgG Fc Capture biosensor for 30 min, association (binding of peptides to VEGFR2) for 600 s, dissociation for 600 s.

HUVEC angiogenesis assay

Angiogenesis assay was performed by Angiogenesis Kit (KURABO) according to the manufacture's protocol. HUCECs co-cultured with fibroblasts were incubated with 10 ng/mL VEGF and macrocyclic peptides at 37°C and under 5% CO_2. Media containing VEGF and macrocyclic peptides were changed on the 4th, 7th, and 9th days after the first treatment of VEGF and macrocyclic peptides. After 11 days of culture, HUVEC cells were fixed with 70% ethanol, blocked with 1% BSA in PBS and immunostained with anti-CD31 antibody and anti-mouse IgG alkaliphosphatase-conjugated antibody followed by visualization with BCIP colorimetric substrate.

Serum-stability assay

Media containing 10 μM L1 or BL1 peptide were incubated at 37°C and under 5% CO_2 for 24, 48 and 72h. The peptide samples in media were mixed on ice with nD1 peptide used as an internal standard, immediately desalted with C-TIP (Nikkyo Technos), eluted with 80% acetonitrile, 0.5% acetic acid saturated with CHCA and analyzed by MALDI-TOF-MS.

Oligo DNAs

Oligo DNAs were purchased from Greiner Bio-One, Operon or BEX.

T7SD8M2.F44: 5′-ATACT AATAC GACT CACTA TAGGA TTAAG GAGGT GATAT TTATG-3′

G5S-4an16.R36: 5′-CTCCC GCCCC CCGTC CTAGC TACCT CCTCC TCCAC C -3′

G5S-4.R20: 5′-TAGCT ACCTC CTCCT CCACC-3′

SD8NNK8: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_8$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK9: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_9$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK10: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_{10}$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK11: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_{11}$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK12: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_{12}$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK13: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_{13}$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK14: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_{14}$ CAT AAATA TCACC TCCTT AATC-3′

SD8NNK15: 5′-TAGCT ACCTC CTCCT CCACC GCA $(MNN)_{15}$ CAT AAATA TCACC TCCTT AATC-3′

puromycin-DNA linker: 5′-CTCCC GCCCC CCGTC C-$(SPC18)_5$-CC-Puromycin-3′

SPC18: 18-O-Dimethoxytritylhexaethyleneglycol,1-[(2-cyanoethyl)- (N,N-diisopropyl)] -phosphoramidite

References

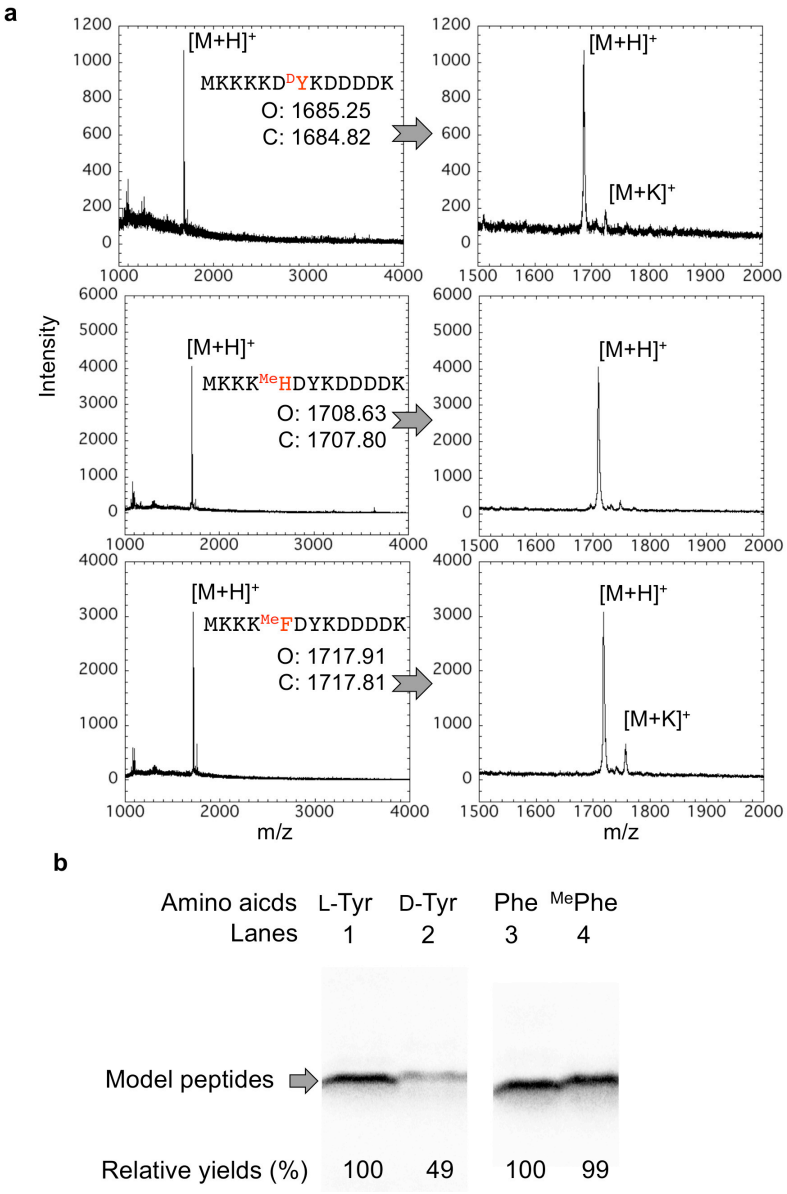
(1) Kawakami, T., Murakami, H., and Suga, H. (2008) Ribosomal synthesis of polypeptoids and peptoid-peptide hybrids, *J. Am. Chem. Soc. 130*, 16861-16863.

(2) Goto, Y., Ohta, A., Sako, Y., Yamagishi, Y., Murakami, H., and Suga, H. (2008) Reprogramming the translation initiation for the synthesis of physiologically stable cyclic peptides, *ACS Chem. Biol. 3*, 120-129.

(3) Ishizawa, T., Kawakami, T., Reid, P. C., and Murakami, H. TRAP display: a high-speed selection method for the generation of functional polypeptides, *J. Am. Chem. Soc., in press*

(4) Murakami, H., Ohta, A., Ashigai, H., and Suga, H. (2006) A highly flexible tRNA acylation

method for non-natural potypeptide synthesis, *Nat. Methods 3*, 357-359.



Supplementary Figure 1. Ribosomal incorporation of backbone-modified non-proteinogenic amino acids into a peptide via aaRS-catalyzed *in situ* tRNA aminoacylation. (a) MALDI-TOF mass spectra of the backbone-modified non-proteinogenic amino acid-containing model peptides. The calculated molecular mass (C:) and observed molecular mass (O:) for singly charged species $[M+H]^{+1}$ are shown in each spectrum. The backbone-modified non-proteinogenic amino acid in each model peptide sequence is highlighted in red. ^{D}Y, D-tyrosine; ^{Me}H, *N*-α-methyl-L-histidine; ^{Me}F, *N*-methyl-L-phenylalanine. The right panels are expanded spectra of the left-panels. (b) Tricine-SDS-PAGE analysis of the expressed model peptides labeled with [^{14}C]-Asp detected by autoradiography.

L-library

		Frequency
L1	ClAc-^{L}FVVVSTDPWVNGLYIDC	11
L2	ClAc-^{L}FVVASTDPWVNGLYIDC	1
L3	ClAc-^{L}FVKTILKLLC	1
L4	ClAc-^{L}FCCAIFLLFLKYLC	1
L5	ClAc-^{L}FTWHRWLREC	1
L6	ClAc-^{L}FRWHPCLILVALATIYC	1
L7	ClAc-^{L}FEIRIWLHSLY	1
L8	ClAc-^{L}FSTLDTHGLHIWSC	1
L9	ClAc-^{L}FSTSLTDIISCQRC	1
L10	ClAc-^{L}FIIYDSWAENFATINC	1

D-library

		Frequency
D1	ClAc-^{D}FLFPIGDLSWVLLPC	19
D2	ClAc-^{D}FRTLILPRSC	1
D3	ClAc-^{D}FTLPPVHSRCLAUC	1
D4	ClAc-^{D}FWNISPSIDWRLFC	1

BL-library

BL1	ClAB-^{L}FIGHYRVKVHPISLERC 14
BL2	ClAB-^{L}FKPDWWAYYDLRHPC 3
BL3	ClAB-^{L}FSIRFWEHYPLHHVC 2
BL4	ClAB-^{L}FDPRWWQFYPLWHTC 1
BL5	ClAB-^{L}FQPLWCHVLRHPLD 1
BL6	ClAB-^{L}FVPAILIYTUIKFLC 1

BD-library

BD1	ClAB-^{D}FKPDWWTYYDLRHPC 12
BD2	ClAB-^{D}FKPDWWTYYDLKHPC 1
BD3	ClAB-^{D}FKPDWWTYYDLWHPC 1
BD4	ClAB-^{D}FVVVSTDPWVNGLYIDC 5
BD5	ClAB-^{D}FVVVSTDPWVNGLYINC 1
BD6	ClAB-^{D}FSIRFWEYYPLHHVC 1
BD7	ClAB-^{D}FSIRFWEHYPLHHVC 1

nL-library

nL1	ClAc-^{L}FTWHRWLREC 9
nL2	ClAc-^{L}FTWHRWLQCC 3
nL3	ClAc-^{L}FITLFLPHTTC 5
nL4	ClAc-^{L}FITLFLPHTTR 1
nL5	ClAc-^{L}FITLFLPHTTC 2
nL6	ClAc-^{L}FTVWDSWAENHLIISC 4

nD-library

nD1	ClAc-^{D}FLFPIGDLSWVLLPC 13
nD2	ClAc-^{D}FLFPIGNLSWVLLPC 1
nD3	ClAc-^{D}FLFPIGNLSWALLPC 1
nD4	ClAc-^{D}FLFPIGDRSWVLLPC 1
nD5	ClAc-^{D}FRTDVWPSIVPVRITPC 1
nD6	ClAc-^{D}FQEVPPKIWSI 1

nBL-library

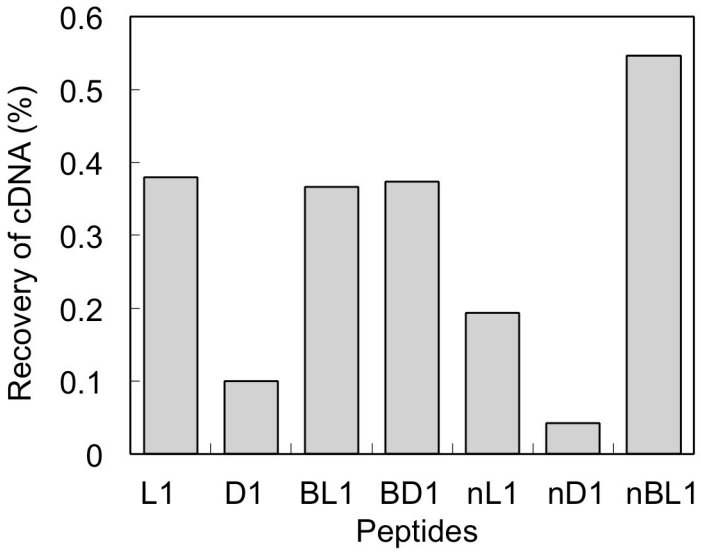
nBL1	ClAB-^{L}FWELKYLNTRYC 12
nBL2	ClAB-^{L}FRTDVWPSIVPVRITPC 1
nBL3	ClAB-^{L}FRTDVWPSIVPVRITPW 1
nBL4	ClAB-^{L}FIGHHRVKVHPISLERC 1
nBL5	ClAB-^{L}FIGHHRVKIHPISLERC 1
nBL6	ClAB-^{L}FIGHHRVKIHPISLEPC 1
nBL7	ClAB-^{L}FIGHYRVKVHPISLERC 1
nBL8	ClAB-^{L}FIGHFRVKVHPISLERC 1
nBL9	ClAB-^{L}FRCSSLTVWQGIC 2
nBL9	ClAB-^{L}FRCSSLIVWQGIC 1

nBD-library

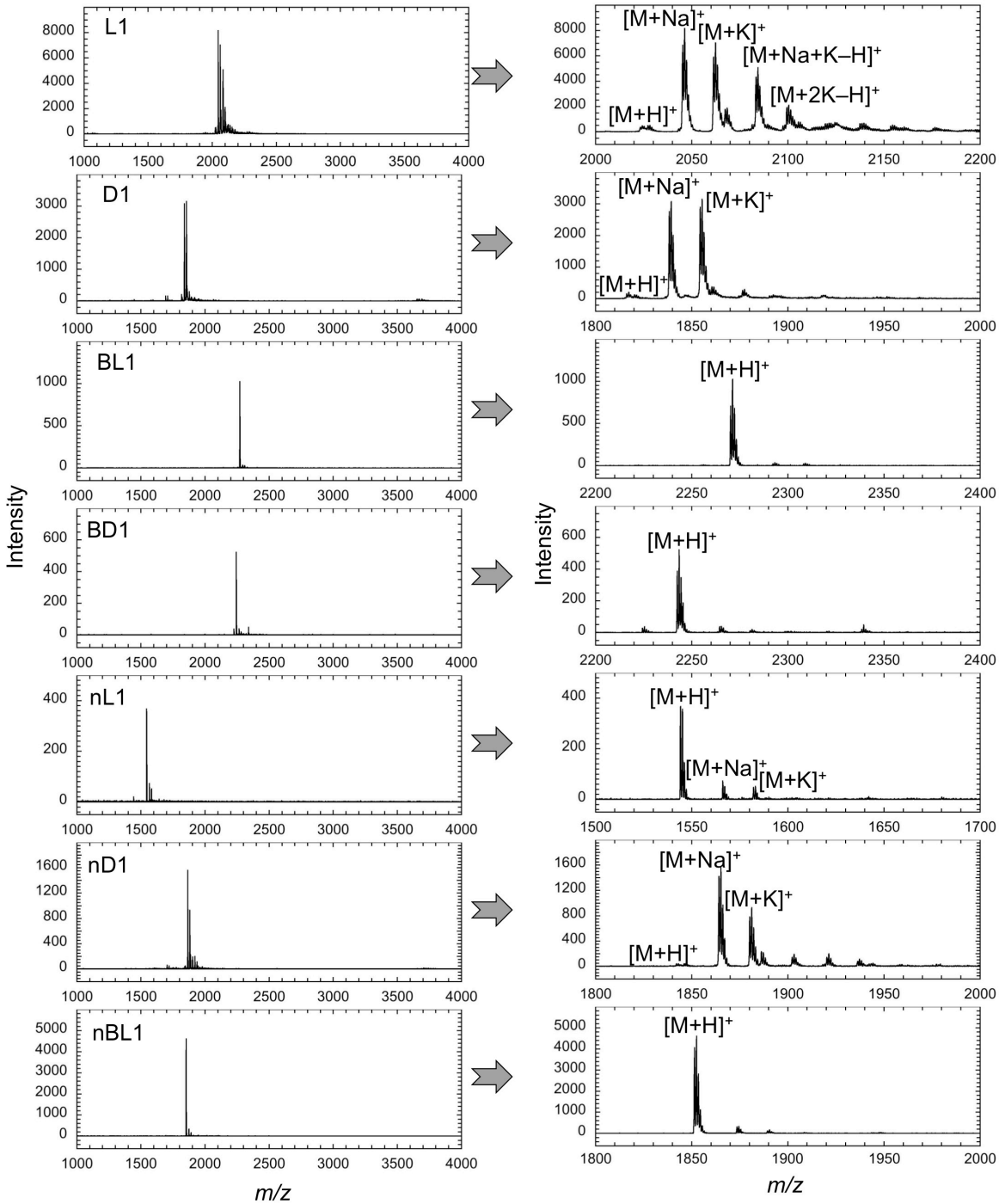
nBD1	ClAB-^{D}FDCGLWRRNIPSNC 6
nBD2	ClAB-^{D}FDCGLWRRNIPSNR 1
nBD3	ClAB-^{D}FDCGLWRRDIPSNC 1
nBD4	ClAB-^{D}FLFPIGDLSWVLLPC 2
nBD5	ClAB-^{D}FWPGTNWWELYPSSHPC 2
nBD6	ClAB-^{D}FAQHTPWWETYPLEHL 1
nBD7	ClAB-^{D}FARNFWEWYPLSHPC 1
nBD8	ClAB-^{D}FSIRFWEHYPLHHVC 1
nBD9	ClAB-^{D}FKPDWWTYYDLRHPC 1

F, ^{Me}Phe; Y, D-Tyr; H, ^{Me}His; V, Cle

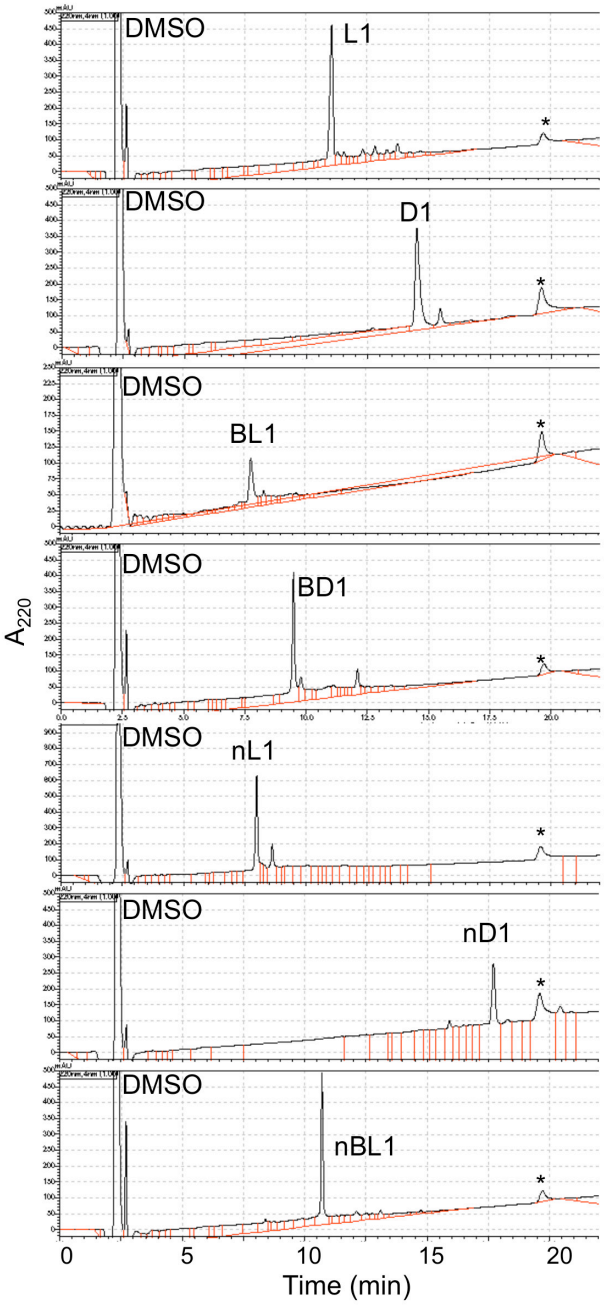
Supplementary Figure 2. Sequences of the cloned macrocyclic peptides selected with VEGFR2. The most abundant sequences selected from the L-, D-, BL-, BD, nL-, nD-, and nBL-libraries (L1, D1, BL1, BD1, nL1, nD1, and nBL1, respectively) are highlighted in yellow. The non-proteinogenic amino acids are in red.



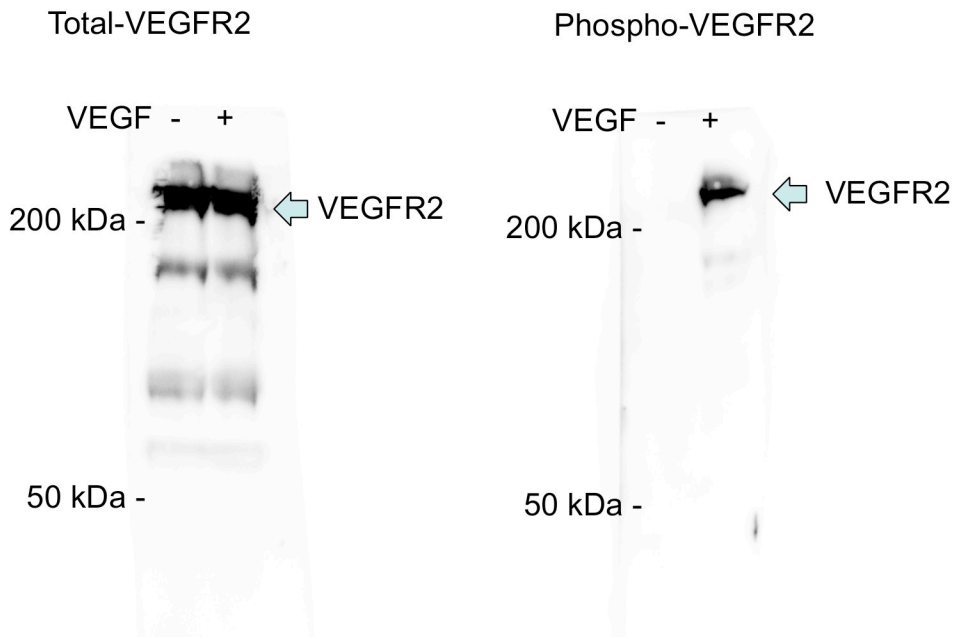
Supplementary Figure 3. VEGFR2 binding of the most abundant macrocyclic peptide selected from each of seven libraries. Binding of VEGFR2 to each macrocyclic peptide displayed on its encoding mRNA/cDNA complex was evaluated by quantification of the cDNA recovered after incubation with immobilized VEGFR2.



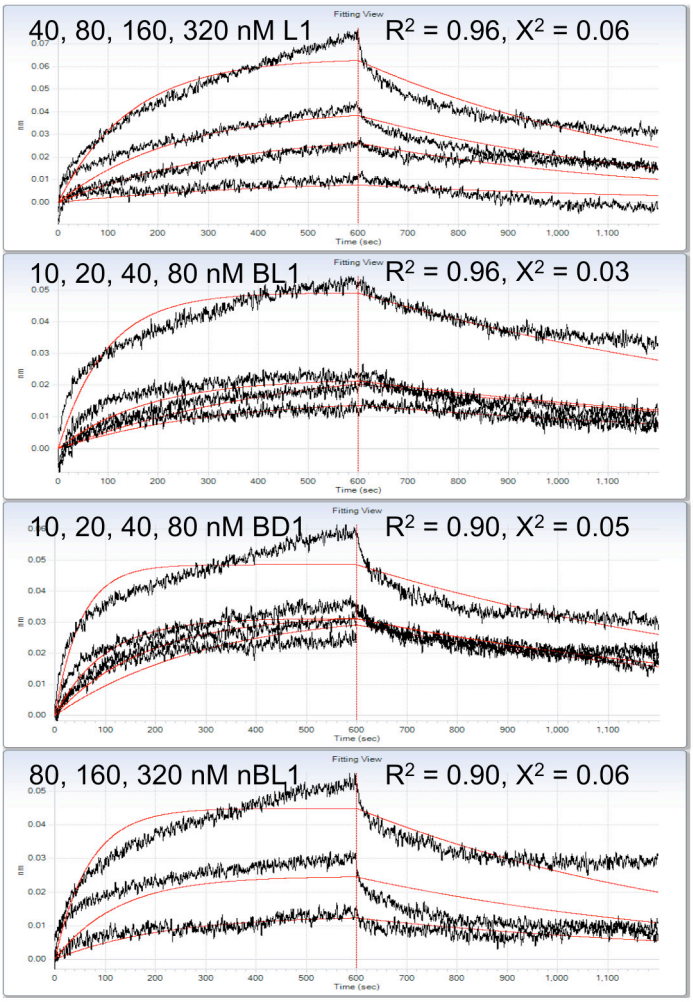
Supplementary Figure 4. MALDI-TOF spectra of chemically synthesized macrocyclic peptides before HPLC purification. The right panels are the expanded spectra of the left panels.



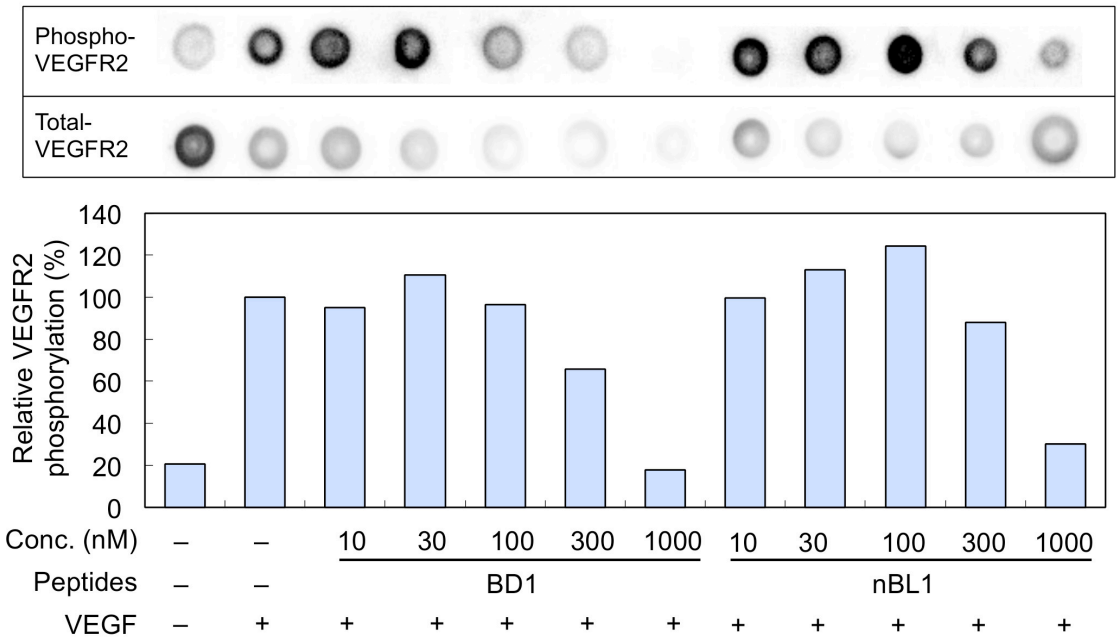
Supplementary Figure 5. HPLC of chemically synthesized macrocyclic peptides before HPLC purification. Asterisks (*) denote an unknown contaminant in the HPLC system observed without injection of peptide samples.



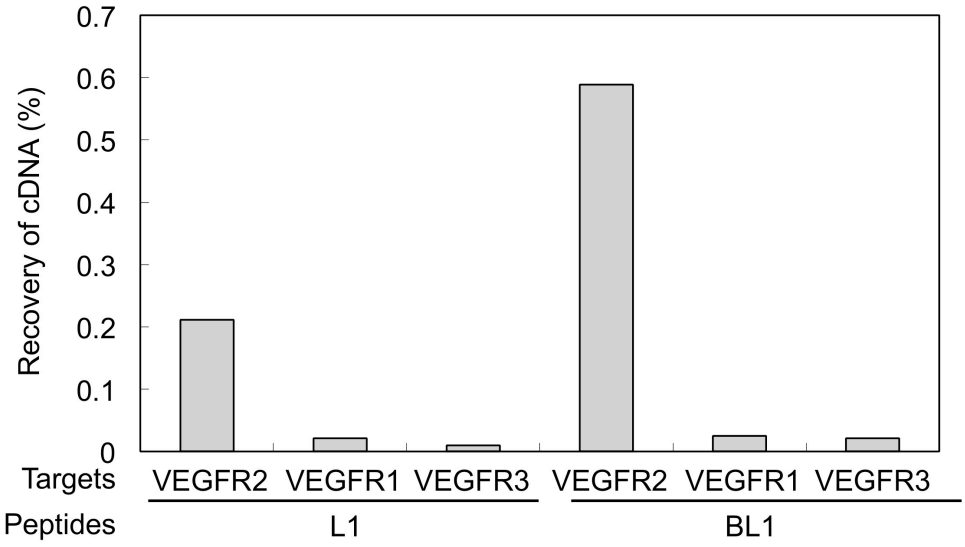
Supplementary Figure 6. Specificity of the anti-VEGFR2 and anti-phosphoVEGFR2 antibodies used in the dot-blot assays. HUVEC lysates with or without VEGF treatment were separated by 7.5% SDS-PAGE and subjected to western blotting with antibodies specific for VEGFR2 and phosphorylated VEGFR2.



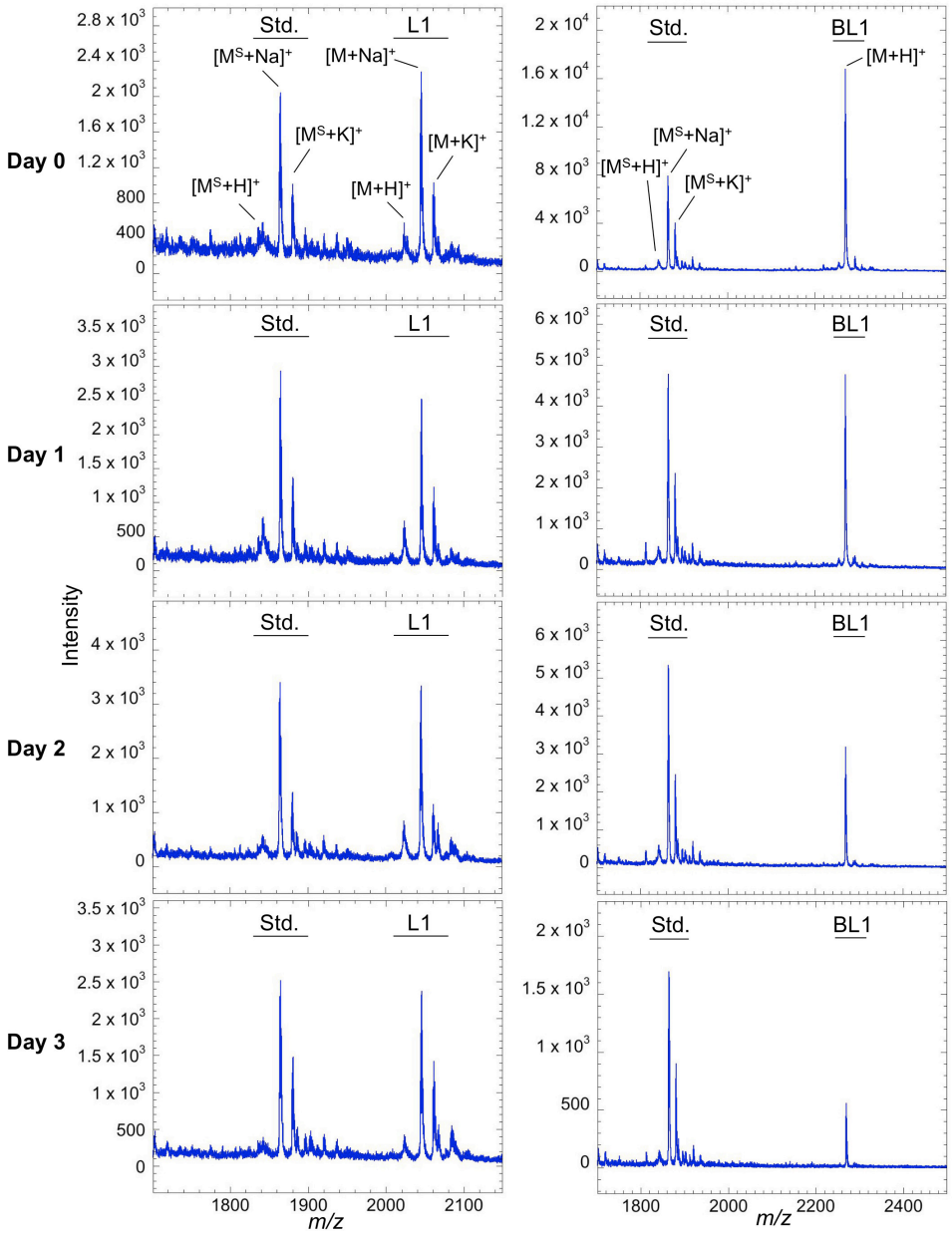
Supplementary Figure 7. Binding kinetics of the macrocyclic peptides determined by the ForteBio system. Fc-fused VEGFR2 was immobilized on Anti-Human IgG Capture Biosensors. All experiments were performed at 30°C, and different concentrations of each macrocyclic peptide were used.



Supplementary Figure 8. Inhibition of VEGFR2 autophosphorylation on HUVEC by the macrocyclic peptides BD1 and nBL1. HUVEC were treated with various concentrations of each peptide for 15 min, followed by treatment with 10 ng/mL VEGF for 7 min. Total VEGFR2 and phosphorylated VEGFR2 were visualized by dot blotting of cell lysates with antibodies specific for VEGFR2 and phosphorylated VEGFR2, respectively. The VEGF control without peptide was defined as 100% phosphorylation.



Supplementary Figure 9. Binding selectivity of the most two potent macrocyclic peptides (L1 and BL1) against VEGFR family proteins. Binding of VEGFR proteins to each macrocyclic peptide displayed on its encoding mRNA/cDNA complex was evaluated by quantification of the cDNA recovered after pull-down of the macrocyclic peptide/mRNA/cDNA complex with immobilized VEGFR proteins.



Supplementary Figure 10. Stability of the most two potent macrocyclic peptides (L1 and BL1) in serum-containing media used in angiogenesis assay. After the incubation of L1 or BL1 peptide in the serum-containing media at 37°C for 1–3 days, the sample was mixed on ice with nD1 peptide used as the internal standard (Std.) and analyzed by MALDI-TOF MS.

Supplementary Table 1. Mass values of chemically synthesized macrocyclic peptides

Cyclic peptide	Calculated mass			Observed mass		
	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$
L1	2022.96	2044.95	2060.92	2023.35	2045.33	2061.4
D1	1815.95	1837.93	1853.91	1816.23	1838.34	1854.42
BL1	2270.19	2292.16	2308.14	2270.26	2292.26	2308.19
BD1	2242	2263.98	2279.95	2242.48	2264.53	2280.4
nL1	1543.74	1565.72	1581.69	1544.47	1566.54	1582.55
nD1	1841.97	1863.95	1879.92	1842.2	1864.19	1880.21
nBL1	1850.87	1872.85	1888.82	1851.48	1873.56	1889.5
rBL1	2270.19	2292.16	2308.14	2271.67	2293.71	2309.7

Supplementary Table 2. Binding properties of the macrocyclic peptides with VEGFR2 determined by the ForteBio system

Cyclic peptide	k_{on} (x10^{6} Ms^{-1})	k_{off} (x10^{-3} s^{-1})	K_d (nM)
L1	0.02	2	94
BL1	0.1	0.9	8
BD1	0.7	1	2
nBL1	0.04	1	33

Supplementary Table 3. Concentration of protein factors and ribosome in the TRAP system.

Name	Concentration (μM)
AlaRS	0.73
ArgRS	0.03
AsnRS	0.38
AspRS	0.13
CysRS	0.02
GlnRS	0.06
GluRS	0.23
GlyRS	0.09
HisRS	0.02
IleRS	0.4
LeuRS	0.04
LysRS	0.11
MetRS	0.03
PheRS	0.68
ProRS	0.16
SerRS	0.04
ThrRS	0.09
TrpRS	0.03
TyrRS	0.02
ValRS	0.02
MTF	0.6
IF1	2.7
IF2	0.4
IF3	1.5
EF-G	0.26
EF-Tu	10
EF-Ts	10
RF2	0.25
RF3	0.17
RRF	0.5
Nucleoside-diphosphate kinase	0.1
Inorganic pyrophosphatase	0.1
T7 RNA polymerase	0.1
Creatine kinase	4 (μg/mL)
Myokinase	3 (μg/mL)
Ribosome	1.2

Supplemantary Table 4. Concentration of small molecules and tRNAs in the TRAP system.

Name	Concentration (mM)
ATP	2
GTP	2
CTP	1
UTP	1
Creatine phosphate	20
Hepes-KOH pH 7.6	50
Potassium acetate	100
Magnesium acetate	12
Spermidine	2
DTT	1
10-formyl-5,6,7,8 tetrahydrofolic acid	0.1
E. coli total tRNA	1.5 (mg/mL)