SUPPLEMENTARY INFORMATION

A ligand motif enables differential vascular targeting of endothelial junctions between brain and retina

Fenny H.F. Tang, Fernanda I. Staquicini, André A. R. Teixeira, Jussara S. Michaloski, Gislene M. Namiyama, Noemi N. Taniwaki, João C. Setubal, Aline M. da Silva, Richard L. Sidman, Renata Pasqualini, Wadih Arap and Ricardo J. Giordano

ONLINE METHODS

Library construction. Briefly, the fUSE55 vector was prepared in large scale with the Maxiprep kit (Qiagen) followed by two consecutive CsCl equilibrium gradient purifications. Equimolar amounts of oligonucleotides 5′-CACTCGGCCGACGGGGCTTGCNNKNNKNNKNNKNNKNNKNNKTGCGGGGCC GCTGGGGCCGAA-3′ and 5′-TTCGGCCCCAGCGGC-3′ (where N = any nucleotide and K = T or G) were converted to double-stranded DNA with Klenow enzyme (as per the manufacturer, New England Biolabs) and purified on a P500 Maxiprep column (Qiagen). The vector (1 mg) and oligonucleotide insert (40 µg) digested with the restriction enzyme BglI were ligated with T4 DNA ligase (New England Biolabs), and the product was purified in a P500 Maxiprep column and transformed into electrocompetent *Escherichia coli* (MC1061 strain) cells. Bacteria were cultured in Luria-Bertani (LB) broth media supplemented with streptomycin (25 µg/ml) and tetracyclin (20 µg/ml) for ~20 hours at 37^{o}C and 200 rpm, and phage were purified from culture supernatants by the polyethylene glycol/NaCl method (PEG/NaCl) (1,2).

Synthetic peptides. Peptides were synthesized and purified by high-performance liquid chromatography to a purity greater than 95% by Chinese Peptide Company. Two peptides [were used in this study: CFFWKFRWMC and CARAC (referred to as control) (25).

Identification of brain-targeting peptides by phage display *in vivo*. To isolate peptides targeting the brain, animals received the CX8C phage library (10^9 transducing units -TU) by i.v. injection. After 30 minutes in circulation, mice were perfused with 20 mL of Dulbecco's modified Eagle's medium (DMEM) and different regions of the brain (cerebellum, olfactory bulb and hemispheres) were collected. Phage bound to tissue were recovered by tissue homogenization followed by bacterial infection (3), transferred to LB media supplemented with kanamycin (100 µg/ml) and tetracylcin (20 µg/ml) amplified by overnight culture (200 rpm, 37^{o}C) and purified from culture supernatants by the PEG/NaCl method (1,2). Two more successive rounds of selection were then performed by reinjecting i.v. the recovered pools of phage (10^9 TU) into different mice (one for each brain area). After the final round, random bacterial colonies were selected for DNA sequencing to identify phage coding peptides targeting each brain area.

Phage sequencing by Sanger method. Individual bacterial colonies were dispersed in 50 µl in phosphate buffered saline solution (PBS, 10 mM Na2HPO4, 1.8 mM KH2PO4, 2.7 mM KCl and 137 mM NaCl pH 7.4), and 2 µl were used to subject the gene III encoding the random DNA insert to polymerase chain reaction (PCR) using Taq DNA polymerase (Thermo Fisher Scientific), dNTP (25 µM) and specific oligonucleotides (0.8 µM) [5′-GCAAGCTGATAAACCGATACAATT-3′ (forward) and 5′-CCCTCATAGTTAGCGTAACGATCT-3′ (reverse)] in a thermal cycler (Bio-Rad) (94°C for 2 min, then 35 cycles at 94°C for 15 s, 60°C for 20 s, and 72°C for 45 s). DNA sequencing was performed by the Sanger method in the DNA sequencing facility at the Institute of Chemistry, University of São Paulo.

Phage high throughput sequencing. After the third round of selection, by PCR the gene III of all brain-targeting phage isolated from each brain area with specific primers (**Supplementary Data Table S2**). We used four different forward and

reverse primers containing zero to three degenerated bases to add the diversity necessary for amplicon sequencing with the Illumina platform. The primers also contained an overhang corresponding to the sequence recognized by the Nextera XT kit. We used 10^6 phage-transducing units per reaction. Phage were amplified for 15 cycles (melting: 95^{o}C for 30s; annealing: 55^{o}C for 30s; extension: 72^{o}C for 1 min) using Kapa high fidelity polymerase (Kapa Biosystems). The PCR product was then purified in spin columns (QIAGEN) and index adaptors (barcodes) were added using the Nextera XT kit (Illumina) following the manufacturer's instructions. We quantified the libraries by qPCR using the library quantification kit (Kapa Biosystems) by diluted samples to to 4 nM. The DNA was denatured using 0.2M NaOH and heat (95^{o}C for 5 min), and sequenced with the MiSeq Reagent Kit v2 (500 cycles) on an Illumina MiSeq equipment. Paired-end reads were assembled with PEAR (4), and insert sequences were extracted and counted. A total of 838,448 reads were obtained by Illumina using the MiSeq platform. Only sequences with two or more reads were analyzed. The remaining 803,072 reads (hemispheres: 391,523 reads; olfactory bulb: 216,213 reads; cerebellum: 195,336 reads) encoded a total of 3,074 unique peptides. Peptides were then filtered for the motif [FYW][ARKH][FYW], resulting in 1,021 peptides.

Validation of phage homing *in vivo*. Phage homing in vivo to different tissues and organs were performed as described (5) with modifications. Two different protocols were used to determine phage homing: immunofluorescence and colony counting. In both cases, animals were initially deeply anesthetized (Avertin 250 mg/kg) and administered i.v. with 10^9 TU of phage CFFWKFRWMC or Fd-tet insertless control phage (in 100 µL of PBS). To determine homing by immunostraining, phage were allowed to circulate for 30 minutes before animals received 50 µg of Lycopersicon Esculentum (Tomato) Lectin conjugated to Fluorescein isothiocyanate (FITC) (Vector Laboratories) intravenously (6). After an additional 2 minutes circulation, animals were perfused through the heart with 20 ml PBS containing 4% paraformaldehyde (5,6). Individual organs and tissues

(brain, eye including optic nerve, gonad, pancreas, spleen, bladder, stomach, small and large intestines, kidney and liver) were collected, fixed for an additional 1 to 2 hours in PBS containing 4% paraformaldehyde and incubated in PBS containing 30% sucrose overnight. Organs were then embedded in optimum cutting temperature compound (OCT) and frozen (-20 ^{o}C). Special attention was taken with the eyes to preserve as much as possible of the optic nerve, which was then embed in a horizontal orientation. Immunofluorescence was performed on sections 50-µm thick (liver was also stained using 20-µm thick sections). Tissue sections were washed 3 times with PBS, followed by blocking for 2 hours in 5% donkey normal serum diluted in PBS containing 0.3% Triton X-100. Tissue sections were then incubated with rabbit immunoglobulin anti-fd bacteriophage (Sigma-Aldrich) diluted in PBS (1:400) containing 1% donkey normal serum and 0.3% Triton X-100 overnight. Sections were stained for 4 hours with goat anti-rabbit IgG antibody conjugated to Dylight-594 (1:300)(Jackson Immunoresearch). Samples were mounted with VECTASHIELD (Vector Laboratories) and examined in an epifluorescence microscope (Nikon). For retinal whole mount preparation, animals were anesthetized and administered i.v. with 10^{9} TU of phage CFFWKFRWMC or Fd-tet (in 100 µL of PBS). After 30 minutes of blood circulation, animals received 50 µg of Lycopersicon Esculentum (Tomato) Lectin conjugated to Fluorescein isothiocyanate (FITC) (Vector Laboratories) intravenously. Animals were enucleated and the retinas dissected for phage immunoistaining. As control for the experiments we also used animals that were administered i.v. with 10^{9} TU of phage CFFWKFRWMC and 10 minutes later injected with 50 µg of Lycopersicon Esculentum (Tomato) Lectin conjugated to Fluorescein isothiocyanate (FITC) (Vector Laboratories) intravenously. These animals were enucleated without perfusion and retinas dissected for whole mount preparation. Retinas were then fixed with 4% paraformaldehyde (1 hour) and blocked for 2 hours in 5% donkey normal serum diluted in PBS containing 1% Triton X-100. Retinas were incubated with rabbit immunoglobulin anti-fd

bacteriophage (Sigma-Aldrich) diluted in PBS (1:400) containing 1% donkey normal serum and 1% Triton X-100 overnight. Tissues were stained for 4 hours with goat anti-rabbit IgG antibody conjugated to Dylight-594 (1:300)(Jackson Immunoresearch). Samples were mounted with VECTASHIELD (Vector Laboratories) and examined in a DMi8- Leica Microscope. For phage homing quantification by colony count, animals were deeply anesthetized (Avertin 250 mg/kg) and administered intravenously with 10^9 TU of CFFWKFRWMC phage or Fd-tet insertless control phage. After 30 minutes, animals were perfused through the heart with 20 ml DMEM and individual organs and tissues (brain and selected areas, small intestine, kidney, liver, spleen, pancreas and retinas) were collected, weighed and homogenized with a glass Dounce homogenizer (5). Retinas were isolated with a dissection retinal whole mount preparation, animals were anesthetized and administered i.v. with 10^9 TU of phage CFFWKFRWMC or Fd-tet (in 100 µL of PBS). After 30 minutes of blood circulation, animals received 50 µg of Lycopersicon Esculentum (Tomato) Lectin conjugated to Fluorescein isothiocyanate (FITC) (Vector Laboratories) intravenously. Animals were enucleated and the retinas dissected for phage immunoistaining. As control for the experiments we also used animals that were administered i.v. with 10^9 TU of phage CFFWKFRWMC and 10 minutes later injected with 50 µg of Lycopersicon Esculentum (Tomato) Lectin conjugated to Fluorescein isothiocyanate (FITC) (Vector Laboratories) intravenously. These animals were enucleated without perfusion and retinas dissected for whole mount preparation. Retinas were then fixed with 4% paraformaldehyde (1 hour) and blocked for 2 hours in 5% donkey normal serum diluted in PBS containing 1% Triton X-100. Retinas were incubated with rabbit immunoglobulin anti-fd bacteriophage (Sigma-Aldrich) diluted in PBS (1:400) containing 1% donkey normal serum and 1% Triton X-100 overnight. Tissues were stained for 4 hours with goat anti-rabbit IgG antibody conjugated to Dylight-594 (1:300)(Jackson Immunoresearch). Samples were mounted with VECTASHIELD (Vector Laboratories) and examined in DMi8- Leica Microscope.

For phage homing quantification by colony count, animals were deeply anesthetized (Avertin 250 mg/kg) and administered intravenously with 10^9 TU of CFFWKFRWMC phage or Fd-tet insertless control phage. After 30 minutes, animals were perfused through the heart with 20 ml DMEM and individual organs and tissues (brain and selected areas, small intestine, kidney, liver, spleen, pancreas and retinas) were collected, weighed and homogenized with a glass Dounce homogenizer (5). Retinas were isolated with the help of a dissection microscope (1). Tissue homogenates were then suspended in 1 mL of DMEM supplemented with 5% bovine serum albumin (DMEM 5% BSA)(except for retinas, which were re-suspended in 100 µL) with the assistance of a vortex mixer, and washed two times with DMEM 5% BSA. Next, the homogenates were incubated with 1 mL of host bacteria (log-phase *E. coli* K91kan strain; OD_{600} ~2) and serial dilutions were plated onto LB agar containing 40 µg/mL tetracycline and 100 µg/mL kanamycin. Plates were incubated overnight at 37°C. Phage in each tissue sample were quantified by colony count in triplicate plating on the next day. For *in vivo* competition assay, animals were administered with specified synthetic peptides CFFWKFRWMC or CARAC (70 pmol or 200 pmol per animal in 100 µl PBS 10% dimethylsulfoxide [v/v]). After one minute, animals were injected i.v. with 10^8 TU of phage CFFWKFRWMC or Fd-tet insertless control phage (in 100 µL of PBS). After another 30 minutes circulation, mice were perfused through the heart with 20 mL DMEM and individual organs and tissues (brain, eye, liver, kidney, small intestine and pancreas) were collected, weighed and homogenized with a glass Dounce homogenizer. Phage in each tissue sample were quantified by colony count.

Site-Directed Mutagenesis of CFFWKFRWMC Phage Particles. Mutant phage particles displaying alanine-scanning variants of CFFWKFRWMC were prepared by site-directed PCR mutagenesis as described (5). Briefly, oligonucleotide (Thermo scientific) pairs for each alanine-scanning variant of

phage CFFWKFRWMC (concentration 10 nM) (**Supplementary Data Table S3**) were annealed in annealing buffer (10 mM Tris.HCl pH 8.0, 100 mM NaCl, 1 mM EDTA), ligated into BglI-digested fUSE55 vector using T4 DNA ligase(Fermentas) and transformed into electrocompetent *E. coli* MC1061 cells. Phage was purified from overnight cultures in LB containing 25 μg/ml streptomycin and 40 μg/ml tetracyclin by the PEG/NaCl method as described above.

Transmission electron microscopy (TEM). To identify the brain vascular binding site *in vivo* for phage CFFWKFRWMC, we used TEM. BALB/c mice were deeply anesthetized with Avertin (250 mg/Kg) and administered i.v. with 10^{10} TU of CFFWKFRWMC phage or control Fd-tet phage. After 30 minutes, animals were perfused through the heart with 20 ml of fixative solution (2.5% glutaraldehyde:4% paraformaldehyde in 0.1M phosphate monobasic/dibasic buffer, pH 7.4). Brain tissues were dissected and fixed with the same fixative solution overnight and processed for TEM as described (7) with modifications. Tissues were post-fixed with 1% (w/v) osmium tetroxide (OsO_4) and 0.5% uranyl acetate was used as contrast. Samples were dehydrated in acetone series and embedded in Epon resin (EMbed-812 resin) for 72 hours at 60ºC. Semithin sections were stained with 1% methylene blue and examined by light microscopy, to select areas containing blood vessels. Ultrathin sections (100 nm) were stained with uranyl acetate and lead citrate and observed with a JEOL transmission electron microscope (model JEM1011) (JEOL/Massachusetts/USA) operating at 80kV. Images were recorded with a Gatan (model 785 ES1000W Erlangshen camera, Gatan, USA).

Imaging *in vivo* with CFFWKFRWMC phage. Phage particles were labeled with IRDye 800CW and used for imaging *in vivo* as described (8). In brief, 3 μL of RDye 800CW NHS ester (LI-COR) (20 mg/ml) were added to $5x10^{11}$ TU of CFFWKFRWMC or Fd-tet phage in 1 mL of PBS and incubated overnight. Phage

were purified by the PEG/NaCl method. Fluorescent phage localization was evaluated on cohorts of size- matched female immunodeficient BALB/c athymic mice administered i.v. with either IRDye 800CW-labeled CFFWKFRWMC phage or Fd-tet control (insertless) labeled phage (2×10^{10} TU per mouse). Near infrared (NIR) fluorescent images were acquired serially over a 5 to 30 minutes time periods and analyzed using the IVIS Spectrum In Vivo Imaging System (PerkinElmer). For *ex vivo* quantification, After 30 minutes, animals were perfused through the heart with 20 ml DMEM and selected organs (brain, retina and liver) were collected and near infrared (NIR) fluorescent images from these organs were acquired in the Odyssey Infrared Imaging Scanner.

Statistics. All numerical data are expressed as mean ± standard error of the mean (SEM). We analyzed data sets for significance using with 1-way ANOVA or 2-way ANOVA (Prism GraphPad software). P-values of less than 0.05 were considered to be statistically significant.

REFERENCES FOR SI MATERIALS AND METHODS

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3. ➞#8. Staquicini, F.I. *et al.* (2011) Systemic combinatorial peptide selection yields a non- canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. *J Clin Invest.* 121:161-173.

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5. ➞#2. Giordano, R.J. *et al.* (2008) Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J Biol Chem*. 283:29447-29460.

6. ➞#27. Falcon, B.L. *et al.* (2011) Increased vascular delivery and efficacy of chemotherapy after inhibition of platelet-derived growth factor-B. *Am. J. Pathol.* 178:2920-2830.

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8. ➞#24. Dobroff, A.S., *et al.* (2016) Towards a transcriptome-based theranostic platform for unfavorable breast cancer phenotypes. *Proc Natl Acad Sci U S A*. 113:12780–12785.

References 1-8 are in the presentation order in the SI. Numbers after the arrows indicate the same References in the primary text.

Olfactory Bulb

FFWKFRWM	6
FSRKFRWM	2
VWVSFRWS	4
VWFNFRYV	1
IWSRFMWT	1
HGSGDRRP	1
IGVHLIGV	1

Hemispheres

YSFLTSDF	1
FFDAIEFR	1
GLAVGTAD	1
LYVNFAWR	10
YLNWRWSV	1
SVNLVFGS	1
VYMYFGWF	1
FFVFPRWY	2
FFADFRWY	5
MWVAWRWV	1
YFYNYRWW	1
VWANFRWQ	6
VWMNYRWV	1

Cerebellum

FWWKYVYR	1
VYHGFRWR	1
RYIDFRWS	1
VWVDFHWV	2
VWVSFHWV	2
IWVDFRWK	4
FWYGMRWW	1
FWYSYRWI	1
RYSAWKWW	1
WRALNYSP	1
GSDGEVGR	1

Figure S1. Individual phage colonies from each brain area (64 total) were sequenced by Sanger to identify the encoded and displayed peptide. Numbers (right column) indicate the frequence of each peptide. The FRW motif is highlighted (yellow).

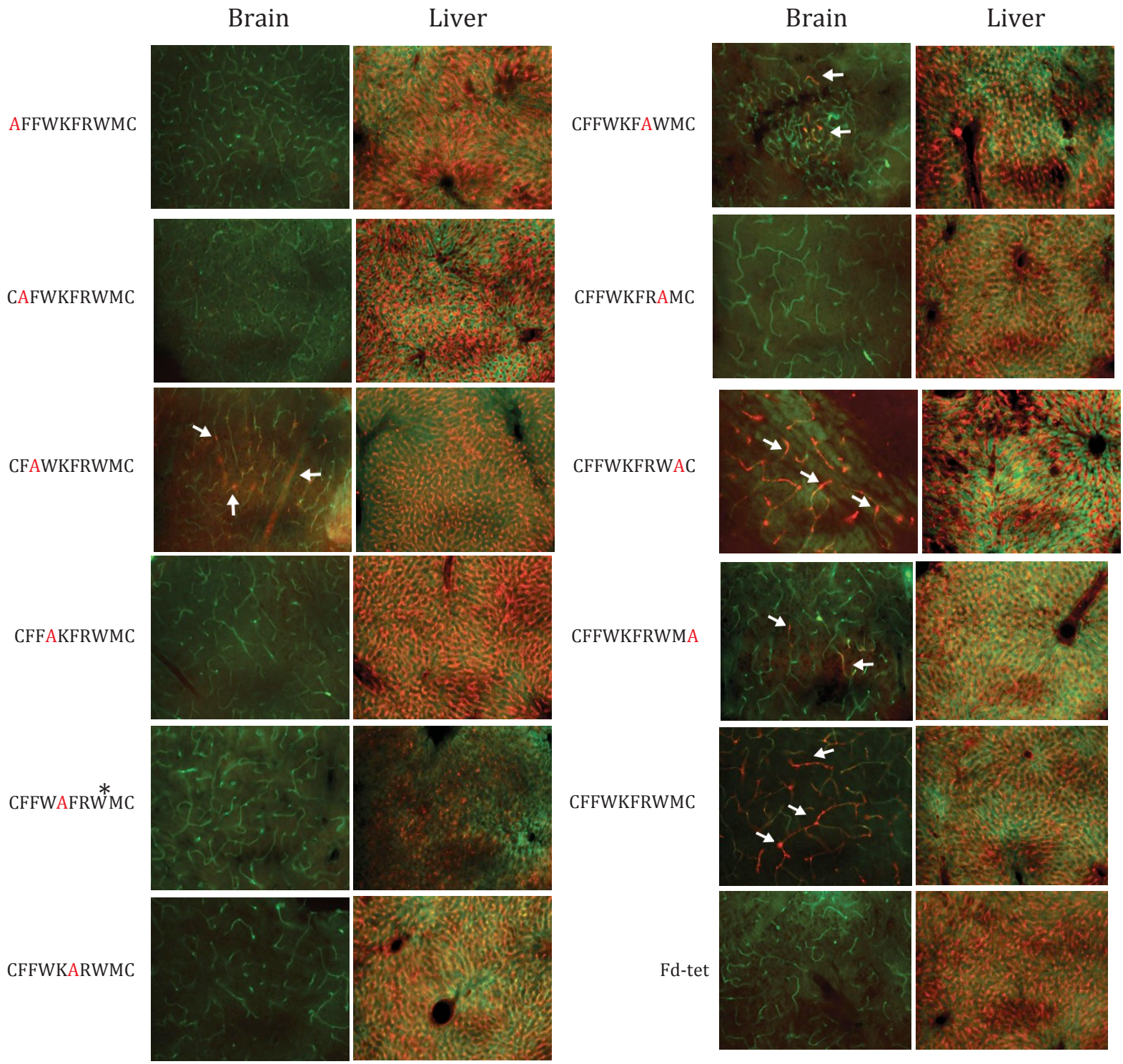

Figure S2. Mutation of certain residues (to alanine) within CFFWKRRWMC abolishes or significantly diminishes (arrows) phage binding to brain blood vessels. Mice were administered with 10^9 TU of each individual phage. After 30 minutes circulation, brain and liver tissue sections were stained with anti-bacteriophage sera (red) or FITC-conjugated *Lycopersicon (Tomato) esculentum* lectin (blood vessels, green). Liver is always positive for phage and was included as phage load control.

(*) Phage CFFWAFRWMC was obtained with extremely low titers and because the liver is almost negative, it indicates suboptimal phage load. Thus, it is difficult to assess the contribution of this residue in peptide CFFWKRRWMC for phage binding to the brain.

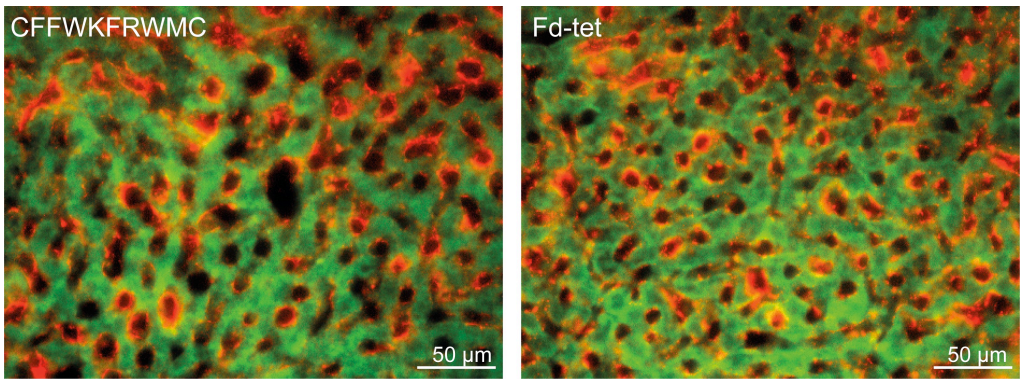

Figure S3. Phage homing to liver. Mice were administered with 10^9 TU of individual phage CFFWKFRWMC and Fd-tet. Liver tissue sections (20-µm thick) were stained with antibacteriophage sera (red) and FITC-conjugated *Lycopersicon* (*Tomato) esculentum* lectin (blood vessels, green).

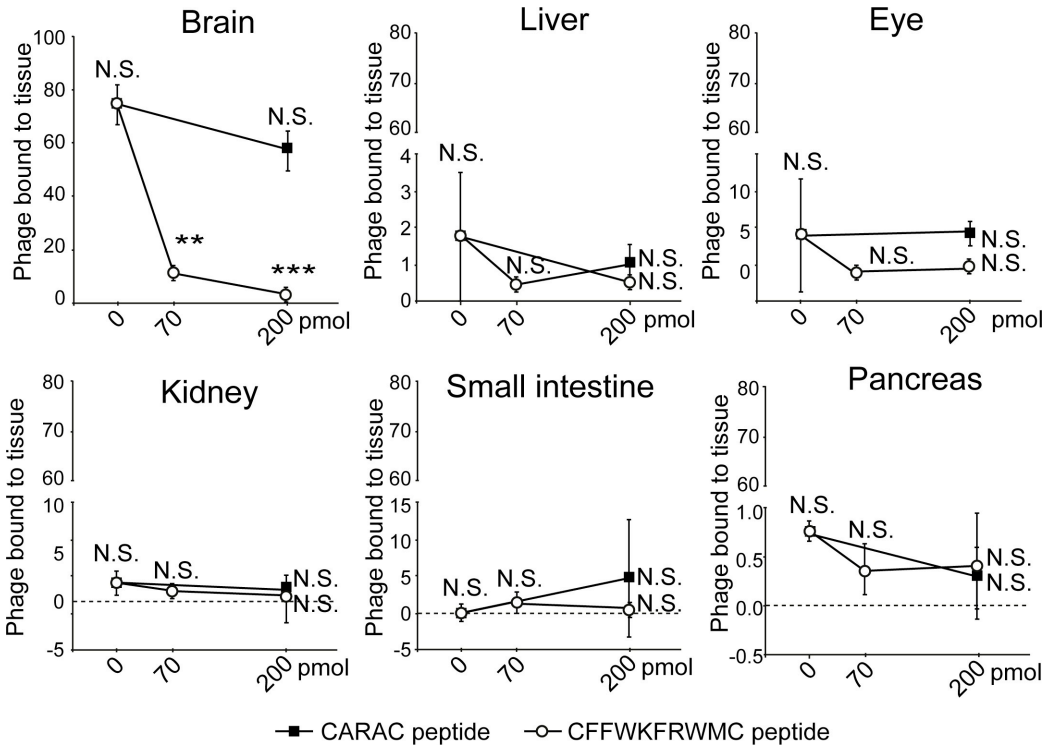

Figure S4. Peptide competition assay. Animals were injected with synthetic peptides (CFFWKFRWMC or a control peptide) before phage administration. Phage homing (in TU/mg) was normalized relative to the control phage (Fd- tet). Bars represent means ± SEM from quadruplicate plating for colony count (N=1 animal per condition, with N=2 retinas or brain and liver halves). Statistical test used, 1-way ANOVA. N.S., not significant; ***, P ≤ 0.0005].

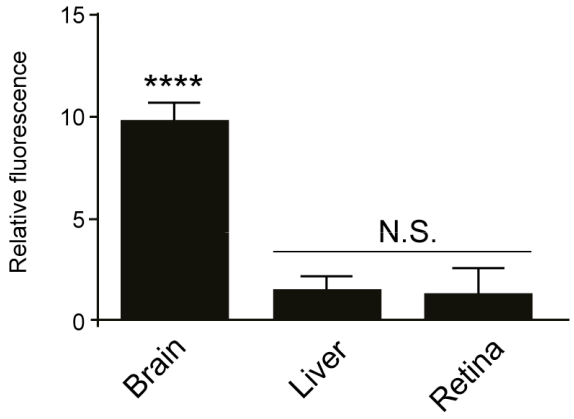

Figure S5. Near-infrared (NIR) fluorescence *ex vivo* quantification following a single intravenous dose of targeted CFFWKFRWMC-displaying phage. Mice were administered with $2x10^{10}$ TU of IRDye 800CW-labeled CFFWKFRWMC phage or Fd-tet control (insertless) labeled phage and after 30 minutes circulation, tissues were dissected and analyzed. Relative fluorescence was normalized relative to the control phage (Fd-tet). Bars represent means ± SEM from duplicate near infrared (NIR) fluorescent images (N=1 animal per condition, with N=2 retinas or brain and liver halves). Statistical test used, 1- way ANOVA. N.S., not significant; ****, P ≤ 0.0001].

Peptide sequence	Frequency (%)
FFWKFRWM	10
LYVNFAWR	10
FFADFRWY	7.6
VWANFRWQ	7.4
VWVSFRWS	5.7
VWMNYRWV	4.1
VWVDFHWV	3.5
IWVDFRWK	3.0
RYIDFRWS	2.8
WYAGLRWY	2.6
YFAAWRWW	2.1
FPFHRNLG	1.4
FYAGYRWV	1.4
FWYSYRWI	1.4
FWWKYVYR	1.2
VWFNFRYV	1.0

Table S1. Most abundant peptides identified by phage display *in vivo* followed by next generation sequencing. Sequence (flanking cysteines were omitted) and frequency of the peptides is shown.

Primer	Sequence
Ill-f5-Fw-0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATTGTCGGCGCAACTATCG Ill-f5-Fw-
1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNCATTGTCGGCGCAACTATCG Ill-f5-Fw-
2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNCATTGTCGGCGCAACTATCG Ill-f5-
Fw-3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNCATTGTCGGCGCAACTATCG Ill-f5-
Rv-0	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAACCACAACGCCTGTAGC Ill-f5-Rv-
1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNCAAACCACAACGCCTGTAGC Ill-f5-Rv-
2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNCAAACCACAACGCCTGTAGC
Ill-f5-Rv-3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCAAACCACAACGCCTGTAGC

Table S2. Primers used for the first PCR amplification during deep sequencing library preparation. Underlined bases correspond to region specific to the fUSE5 vector, followed upstream by the des agenerationnd Nextera XT compatible overhangs.

Primer	Sequence
AFFWKFRWMC-Fw	GGGCTGCGTTTTTTTGGAAGTTTAGGTGGATGTGCGGGGCCGCTG
CAFWKFRWMC-Fw	GGGCTTGCGCGTTTTGGAAGTTTAGGTGGATGTGCGGGGCCGCTG
CFAWKFRWMC-Fw	GGGCTTGCTTTGCGTGGAAGTTTAGGTGGATGTGCGGGGCCGCTG
CFFAKFRWMC-Fw	GGGCTTGCTTTTTTTGCGAAGTTTAGGTGGATGTGCGGGGCCGCTG
CFFWAFRWMC-Fw	GGGCTTGCTTTTTTTTGGGCGTTTAGGTGGATGTGCGGGGCCGCTG
CFFWKARWMC-Fw	GGGCTTGCTTTTTTTTGGAAGGCGAGGTGGATGTGCGGGGCCGCTG
CFFWKFAWMC-Fw	GGGCTTGCTTTTTTTTGGAAGTTTGCGTGGATGTGCGGGGCCGCTG
CFFWKFRAMC-Fw	GGGCTTGCTTTTTTTTGGAAGTTTAGGGCGATGTGCGGGGCCGCTG
CFFWKFRWAC-Fw	GGGCTTGCTTTTTTTTGGAAGTTTAGGTGGGCGTGCGGGGCCGCTG
CFFWKFRWMA-Fw	GGGCTTGCTTTTTTTTGGAAGTTTAGGTGGATGGCGGGGGCCGCTG
AFFWKFRWMC-Rv	CGGCCCCGCACATCCACCTAAACTTCCAAAAAAACGCAGCCCCGT
CAFWKFRWMC-Rv	CGGCCCCGCACATCCACCTAAACTTCCAAAACGCGCAAGCCCCGT
CFAWKFRWMC-Rv	CGGCCCCGCACATCCACCTAAACTTCCACGCAAAGCAAGCCCCGT
CFFAKFRWMC-Rv	CGGCCCCGCACATCCACCTAAACTTCGCAAAAAAGCAAGCCCCGT
CFFWAFRWMC-Rv	CGGCCCCGCACATCCACCTAAACGCCCAAAAAAAGCAAGCCCCGT
CFFWKARWMC-Rv	CGGCCCCGCACATCCACCTCGCCTTCCAAAAAAAGCAAGCCCCGT
CFFWKFAWMC-Rv	CGGCCCCGCACATCCACGCAAACTTCCAAAAAAAGCAAGCCCCGT
CFFWKFRAMC-Rv	CGGCCCCGCACATCGCCCTAAACTTCCAAAAAAAGCAAGCCCCGT
CFFWKFRWAC-Rv	CGGCCCCGCACGCCCACCTAAACTTCCAAAAAAAGCAAGCCCCGT
CFFWKFRWMA-Rv	CGGCCCCCGCCATCCACCTAAACTTCCAAAAAAAGCAAGCCCCGT

Table S3. Oligonucleotides used to prepare different phage particles dalaninecanning variants of CFFWKFRWMC. Mutations sites are indicated (underline).

Supplementary Data Alignment File

Next generation sequencing of all phage recovered from each brain area. A total of 838,448 reads were obtained by Illumina using the MiSeq platform (see online methods for details). The paired-end reads were assembled, inserts trimmed and only sequences with two or more reads were used for further analysis (Dias-Neto *et al*., *PLoS One*.
4(12):e8338, 2009). The remaining 803,072 reads (hemispheres 391,523 reads, olfactory bulb 216,213 reads, cerebellum 195,336 reads) encoded a total of 3,074 unique peptides. Peptides were then filtered using the motif [FYW][ARKH][FYW], resulting in 1,021 peptides.

Alignment file of 1021 peptides containing the [FYW][ARKH][FYW] motif:

----SWYSYRWI----	----VWVHFRWI----	----YFIRYRWM----	----IYLFFRYI----
----MYHNFAWY----	-----YDNFRWIK---	------FFWKFGWM--	----SWMHFRWI----
----YYAFFRWH----	----IWASWRFW----	----VWFNFRFA----	----VYVFWRYV----
----IYSFFAWR----	----RYIDFRWA----	------FFWKFRRM--	----LYVNFAWW----
----FFWMFRWI----	----VYVGWRWS----	----FFLQFRWF----	----SWCNWRWE----
----SWFDFRWH----	------VWFKFRYV--	----FYIHYKWF----	----SYSNWHWW----
----VYLDFKWQ----	----VYLNWRWG----	----VWADFRWY----	----VYNFFRWV----
----VWVSYRWV----	----IYVAFRWR----	----IYVAYKWI----	----RYSGFRWA----
----IYFQYHWV----	----LYVAFKWV----	----FFYMFRWT----	----FFQDFRWW----
----TWVNFRYV----	----YFAAWRWG----	----WYELYRWT----	----LYVNFAWQ----
----VFWKFRWM----	----YFAAWRWW----	----VYILYRWI----	-----YVGFRWLM---
----FYWKFRWM----	----RHIDFRWS----	----FFWRFRWM----	----VRHNFRYL----
----LYANFAWR----	-----RLNFRWVI---	----FWVDFRYT----	----MWCSFRWI----
----VWVSYRWS----	----GYVYFRWV----	----FFLHFRWF----	-----YLNWRWSV---
----VWHSYRWP----	----YFWNFRWW----	----LYVDFAWR----	----MWVAWRWV----
-----YFDWHYVR---	----YFWKFRWM----	----GWVSFRWS----	-----WYGFRYLA---
----MYSAWKWV----	----VFADFRWY----	----WWQFFRWV----	----YCAAWRWW----
----FYSGFRWR----	-----YSGFRYFD---	----FYHGFRWR----	----IYLGFRYY----
----HWAGYRWV----	----GYRDYHWR----	----VWMNYRWA----	----FWHSYRWI----
------YWFAFRYS--	----VWVSFRWC----	---MFDNVFRY-----	----LYVNFAWV----
----EWVSFRWS----	----IWVDFRWT----	----FFSDFRWY----	----IWFNFRYV----
----FYVHFRWL----	----FWDNFRWQ----	----MYDLFRWR----	----IWIDFRWK----
----YFAYFRWV----	----VWSYFRWV----	----RYVLFRWV----	----IWVVFRWK----
----IWFGFRWK----	----VWVDFHWL----	----YFYNYRWW----	-------FFAFPRWY-
----LYVIFAWR----	----YYVDWRYE----	----DYVNFAWR----	----VFVSFRWW----
----FWKDWRWV----	----SLSDFRWH----	----MWFAFRWV----	----VWFDYRWR----
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----SYVYFRWV----	----YFYNYRWV----	----FFADFRWH----	----FFWNFRWR----
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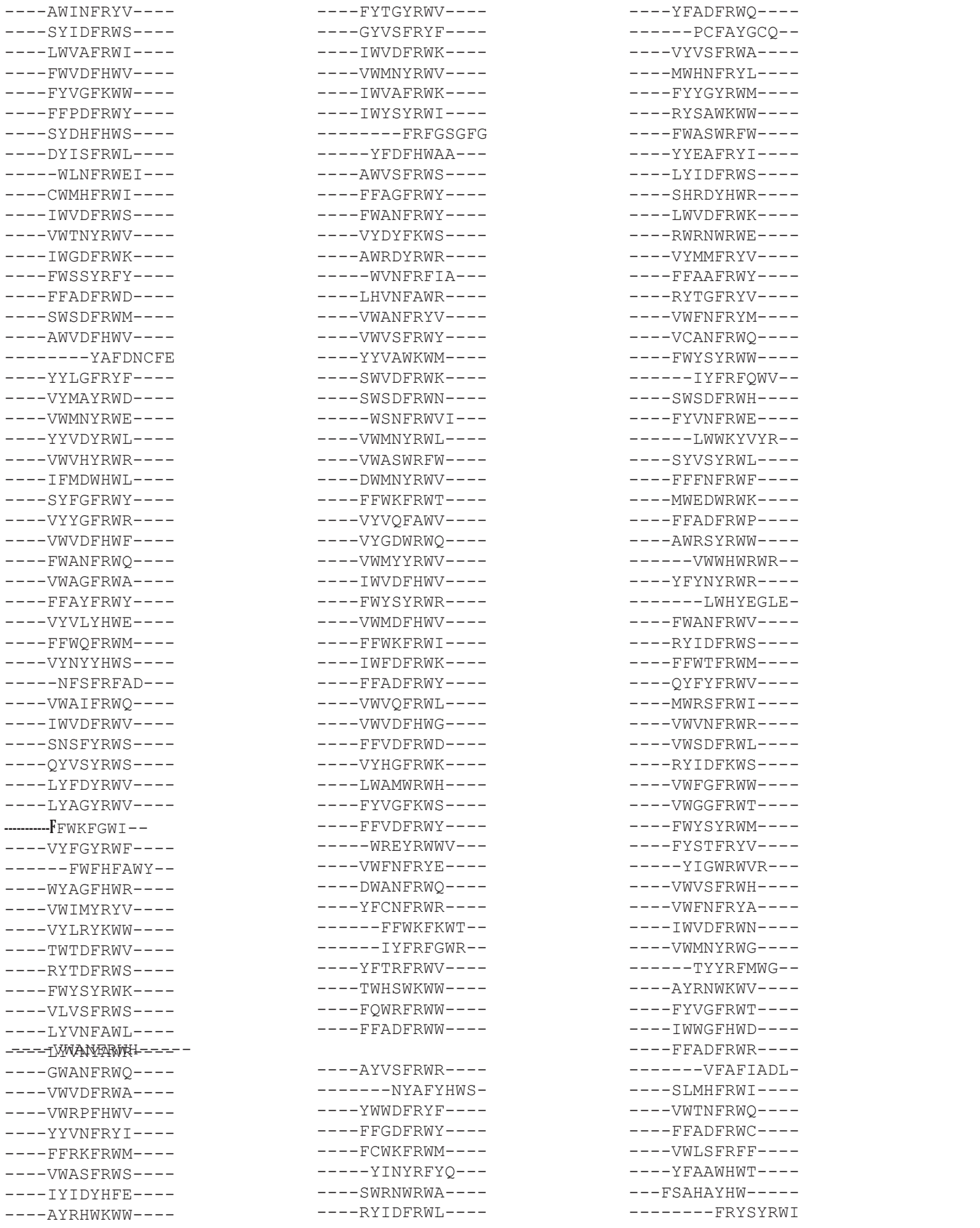
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