Effect of Amino Acid Substitutions on 70S Ribosomal Binding, Cellular Uptake, and Antimicrobial Activity of Oncocin Onc112

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**Table S1:** Concentrations of unlabeled peptides, ribosome preparations, and Cf-Onc112 used for competitive fluorescence polarization screening experiments. Concentrations of the unlabeled peptides correspond to 70 to 80% of the corresponding IC$_{50}$.

| Bacterium       | IC$_{50}$ [nmol/L] | Unlabeled peptide [nmol/L] | Ribosome [nmol/L] | Cf-Onc112 [nmol/L] |
|-----------------|--------------------|-----------------------------|-------------------|-------------------|
| *E. coli* BW25113 | 189 ± 17           | 150                         | 250               | 20                |
| *A. baumannii* DSM 30008 | 246 ± 11           | 200                         | 250               | 20                |
| *K. pneumoniae* DSM 681 | 119 ± 19           | 80                          | 77                | 6.7               |
| *P. aeruginosa* DSM 1117 | 52 ± 14            | 40                          | 36                | 6.7               |
Table S2: Sequences, monoisotopic masses, MRM transitions and limits of quantitation (LOQ) of peptides in uptake studies with RP-HPLC-ESI-QTOF-MRM. Precursors were [M+6H]^{6+} except for peptides Onc112, Onc245, Onc248, Onc249, Onc251, Onc257, Onc272, and Onc287, where precursor [M+5H]^{5+} was used. Dit, Nty, Nle, Tle, Har, O, and r denote 3,5-diiodotyrosine, 3-nitrotyrosine, nor-leucine, tert-leucine, homoarginine, ornithine, and D-arginine. Onc275, Onc278, an Onc279 were not further considered due to the high LOQ of 6 mg/L.

| Peptide | Sequence | Monoisotopic mass | MRM mass Q3 | LOQ [mg/L] |
|---------|----------|------------------|-------------|------------|
| Onc112  | VDKPPYLPRRPRPrYrYr-NH₂ | 2388.3766 | 512.5539 y_{16}^{4+} | 1          |
| Onc244  | VRKPPYLPRRPRPrYrYr-NH₂ | 2429.4508 | 488.2907 y_{15}^{+}  | 4          |
| Onc245  | VWKPPYLPRRPRPrYrYr-NH₂ | 2459.4290 | 512.5539 y_{16}^{4+} | 4          |
| Onc246  | VDRPPYLPRRPRPrYrYr-NH₂ | 2416.3828 | 464.4702 y_{15}^{+}  | 1          |
| Onc247  | VDKKPYPRLPRRPrYrYr-NH₂ | 2419.4188 | 488.2907 y_{15}^{4+} | 2          |
| Onc248  | VDKFPYLPRRPRPrYrYr-NH₂ | 2438.3923 | 650.7185 y_{15}^{+}  | 1          |
| Onc249  | VDKYPYLPRRPRPrYrYr-NH₂ | 2454.3872 | 650.7185 y_{15}^{3+} | 1          |
| Onc250  | VDKPYLPRRPRPrYrYr-NH₂ | 2438.3923 | 525.0578 y_{16}^{4+} | 1          |
| Onc251  | VDKPYLPRRPRPrYrYr-NH₂ | 2454.3872 | 529.0565 y_{16}^{4+} | 1          |
| Onc252  | VDKPHYLPRRPRPrYrYr-NH₂ | 2428.3828 | 522.5554 y_{16}^{4+} | 1          |
| Onc253  | VDKPYLPRRPRPrYrYr-NH₂ | 2447.4250 | 470.6786 y_{18}^{5}  | 1          |
| Onc254  | VDKPPWLPYRPRRPrYrYr-NH₂ | 2411.3926 | 518.3076 y_{16}^{5+} | 2          |
| Peptide   | Sequence                  | Monoisotopic mass | MRM mass | LOQ [mg/L] |
|-----------|---------------------------|-------------------|----------|------------|
| Onc255    | VDKPFLPRPRPRrLYr-NH\_2    | 2372.3817         | 508.5551 | 1          |
| Onc256    | VDKPHLPRPRPRrLYr-NH\_2    | 2362.3722         | 506.0528 | 1          |
| Onc257    | VDKPP-Dit-PRPRPRrLYr-NH\_2| 2640.1699         | 575.5022 | 1          |
| Onc258    | VDKPP-Nty-PRPRPRrLYr-NH\_2| 2433.3617         | 523.8001 | 1          |
| Onc259    | VDKPPY-Nle-PRPRPRrLYr-NH\_2| 2388.3766        | 512.5539 | 1          |
| Onc260    | VDKPPY-Tle-PRPRPRrLYr-NH\_2| 2388.3766         | 512.5539 | 1          |
| Onc261    | VDKPPYRPRPRPRrLYr-NH\_2   | 2431.3937         | 523.3081 | 1          |
| Onc262    | VDKPPYMMPRPRPRrLYr-NH\_2  | 2406.3331         | 517.0430 | 1          |
| Onc263    | VDKPPYFPRPRPRrLYr-NH\_2   | 2422.3610         | 521.0500 | 1          |
| Onc264    | VDKPPYLPRWRPRrLYr-NH\_2   | 2477.4032         | 534.8105 | 1          |
| Onc265    | VDKPPYLPRPWPRrLYr-NH\_2   | 2418.3548         | 520.0484 | 1          |
| Onc266    | VDKPPYLPRPHPRrLYr-NH\_2   | 2369.3344         | 507.7933 | 1          |
| Onc267    | VDKPPYLPRPKPRrLYr-NH\_2   | 2360.3705         | 505.5523 | 1          |
| Onc268    | VDKPPYLPRPFPRrLYr-NH\_2   | 2379.3439         | 510.2957 | 1          |
| Onc269    | VDKPPYLPRP-Har-PPrLYr-NH\_2| 2402.5030        | 516.0578 | 1          |
| Onc270    | VDKPPYLPRPPRrl-Dit-Nr-NH\_2| 2640.1699       | 575.5022 | 1          |
| Peptide   | Sequence           | Monoisotopic mass | MRM mass | LOQ [mg/L] |
|-----------|--------------------|-------------------|----------|------------|
| Onc271    | VDKPPYLPRPRrI-Nty-Nr-NH₂ | 2433.3617         | 2433.3617 | 1          |
|           |                    |                   | y₁₆⁺     |            |
| Onc272    | VDKPPYLPRPRwPRiYNr-NH₂ | 2477.4032         | 534.8105 | 1          |
|           |                    |                   | y₁₆⁺     |            |
| Onc273    | VDKPPYLPRPRwERiYNr-NH₂ | 2509.3930         | 542.8080 | 4          |
|           |                    |                   | y₁₆⁺     |            |
| Onc274    | VDKPPYLPRPRwRRiYNr-NH₂ | 2536.4515         | 549.5726 | 4          |
|           |                    |                   | y₁₆⁺     |            |
| Onc275*   | VDKPPYLPRPRwwRrIYNr-NH₂ | 2566.4297         | 557.0672 | 6          |
|           |                    |                   | y₁₆⁺     |            |
| Onc276    | VDKPPYLPRPRwKRRiYNr-NH₂ | 2508.4454         | 542.5711 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Onc277    | VDKPPYLPRPRwHRRiYNr-NH₂ | 2517.4093         | 544.8121 | 4          |
|           |                    |                   | y₁₆⁺     |            |
| Onc278*   | VDKPPYLPRPRwIRiYNr-NH₂ | 2493.4345         | 538.8123 | 6          |
|           |                    |                   | y₁₆⁺     |            |
| Onc279*   | VDKPPYLPRPRwFRRiYNr-NH₂ | 2527.4188         | 547.3144 | 6          |
|           |                    |                   | y₁₆⁺     |            |
| Onc280    | VDKPPYLPRPRwLRRiYNr-NH₂ | 2493.4345         | 538.8183 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Onc281    | VDKPPYLPRPRwARiYNr-NH₂ | 2451.3875         | 528.3066 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Onc282    | VDKPPYLPRPRwNRiYNr-NH₂ | 2494.3933         | 539.0581 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Onc283    | VDKPPYLPRPRwVRRiYNr-NH₂ | 2479.4188         | 535.3144 | 4          |
|           |                    |                   | y₁₆⁺     |            |
| Onc284    | VDKPPYLPRPRwGRRiYNr-NH₂ | 2437.3719         | 524.8027 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Onc285    | VDKPPYLPRPRwSRRiYNr-NH₂ | 2467.3824         | 532.3053 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Onc286    | VDKPPYLPRPRwQRiYNr-NH₂ | 2508.4090         | 542.5620 | 2          |
|           |                    |                   | y₁₆⁺     |            |
| Peptide | Sequence                  | Monoisotopic mass | MRM mass  | LOQ [mg/L] |
|---------|--------------------------|-------------------|-----------|------------|
| Onc287  | VDKPPYLPRPRWYr\textsubscript{r}Yr-NH\textsubscript{2} | 2543.4137        | 551.3132  | 2          |
|         |                          |                   | y\textsubscript{16}\textsuperscript{4+}     |            |
| Onc288  | VDKPPYLPRPR\textsubscript{D}r\textsubscript{r}Yr-NH\textsubscript{2} | 2495.3774        | 539.3041  | 2          |
|         |                          |                   | y\textsubscript{16}\textsuperscript{4+}     |            |
| Onc289  | VDKPPYLPRPR\textsubscript{W}r\textsubscript{r}Yr-NH\textsubscript{2} | 2481.3981        | 535.8092  | 2          |
|         |                          |                   | y\textsubscript{16}\textsuperscript{4+}     |            |
| Onc290  | VDKPPYLPRPR\textsubscript{O}r\textsubscript{r}Yr-NH\textsubscript{2} | 2494.3933        | 539.0672  | 2          |
|         |                          |                   | y\textsubscript{16}\textsuperscript{4+}     |            |
Table S3: Ion source and mass analyzer settings applied on the ESI-QTOF-MS operated in MRM-mode to quantify PrAMPs in medium supernatants and bacterial pellets.

| Parameter                  | Setting   |
|----------------------------|-----------|
| Ionization mode            | Positive  |
| Resolution                 | 20000     |
| Capillary                  | 3 kV      |
| Source temperature         | 100 °C    |
| Desolvation temperature    | 250 °C    |
| Sampling cone              | 30 V      |
| Source offset              | 80 V      |
| Cone gas flow              | 50 L/hr   |
| Desolvation gas flow       | 600 L/hr  |
| Trap gas flow              | 2.0 mL/min|
| m/z range                  | 50-2000   |
**Table S4:** Amino acid substitutions in Onc112. Dit, Nty, Nle, Tle, Har, O, and r denote 3,5-diiodotyrosine, 3-nitrotyrosine, nor-leucine, tert-leucine, homoarginine, ornithine, and D-arginine. Substitutions in position 13 are always combined with substitution P12W.

| Position | Original amino acid | Amino acid substitution                      |
|----------|---------------------|---------------------------------------------|
| 1        | V                   | None                                        |
| 2        | D                   | R, W                                        |
| 3        | K                   | R                                           |
| 4        | P                   | F, Y                                        |
| 5        | P                   | F, Y, H, R                                  |
| 6        | Y                   | W, F, H, Dit, Nty                           |
| 7        | L                   | Nle, Tle, R, M, F                           |
| 8        | P                   | None                                        |
| 9        | R                   | None                                        |
| 10       | P                   | W                                           |
| 11       | R                   | W, H, K, F, Har                             |
| 12       | P                   | W                                           |
| 13       | P                   | E, R, W, K, H, I, F, L, A, N, V, G, S, Q, Y, D, T, O |
| 14       | R                   | None                                        |
| 15       | r                   | None                                        |
| 16       | I                   | None                                        |
| 17       | Y                   | Dit, Nty                                    |
| 18       | N                   | None                                        |
| 19       | r                   | None                                        |
Table S5: Minimal inhibitory concentrations (MICs) of Onc112-derivatives determined in 25% MHB2. All peptides were synthesized as C-terminal amides. Dit, Nty, Nle, Tle, Har, O, and r denote 3,5-diiodotyrosine, 3-nitrotyrosine, nor-leucine, tert-leucine, homoarginine, ornithine, and D-arginine.

| Name    | Sequence                  | Net charge | MIC [mg/L] |
|---------|---------------------------|------------|------------|
| Onc112  | VDKPPYLPRPRPRrIYNr        | +6         | 8          |
| Onc244  | VRKPPYLPRPRPRrIYNr        | +8         | 8          |
| Onc245  | VVKPPYLPRPRPRrIYNr        | +7         | 8          |
| Onc246  | VDRPPYLPRPRPRrIYNr        | +6         | 8          |
| Onc247  | VDKKEPPYLPRPRPRrIYNr      | +7         | 8          |
| Onc248  | VDKFPYLPRPRPRrIYNr        | +6         | 8          |
| Onc249  | VDKYPYLPRPRPRrIYNr        | +6         | 8          |
| Onc250  | VDKPFLPRPRPRrIYNr         | +6         | 8          |
| Onc251  | VDKPYLPRPRPRrIYNr         | +6         | 8          |
| Onc252  | VDKHYLPRPRPRrIYNr         | +6         | 8          |
| Onc253  | VDKPYPYLPRPRPRrIYNr       | +7         | 8          |
| Onc254  | VDKPPWLPRPRPRrIYNr        | +6         | 8          |
| Onc255  | VDKPPEPLPRPRPRrIYNr       | +6         | 8          |
| Onc256  | VDKPHLPRPRPRrIYNr         | +6         | 8          |
| Onc257  | VDKPP-Dit-LPRPRPRrIYNr    | +6         | 8          |
| Onc258  | VDKPP-Nty-LPRPRPRrIYNr    | +6         | 8          |
| Onc259  | VDKPPY-Nle-PRPRPRrIYNr    | +6         | 8          |
| Onc260  | VDKPPY-Tle-PRPRPRrIYNr    | +6         | 8          |
| Onc261  | VDKPPYP-FPRPRPRrIYNr      | +7         | 8          |
| Onc262  | VDKPPYM-PRPRPRrIYNr       | +6         | 8          |
| Onc263  | VDKPPYEP-PRPRPRrIYNr      | +6         | 8          |
| Onc264  | VDKPPYLPRPWR-PRPRrIYNr    | +6         | 8          |
| Onc265  | VDKPPYLPRP-WPPRrIYNr      | +5         | 8          |
| Onc266  | VDKPPYLPRP-HPPRrIYNr      | +5         | 8          |
| Onc267  | VDKPPYLPRP-KPPRrIYNr      | +6         | 8          |
| Onc268  | VDKPPYLPRP-FPPRrIYNr      | +5         | 8          |
| Name  | Sequence                  | Net charge | E. coli BW25113 | E. coli MC4100 | E. coli ATCC25922 | K. pneumoniae DSM681 | A. baumannii DSM30008 | P. aeruginosa DSM117 | S. aureus DSM6247 |
|-------|---------------------------|------------|-----------------|----------------|-------------------|----------------------|-----------------------|---------------------|-------------------|
| Onc269 | VDKPPYLPRP-Har-PPrrIYNr    | +5         | 8               | 4              | 16                | 4                    | 32                    | 64                  | 64                |
| Onc270 | VDKPPYLPRP-PRrIYNr-Dit-Nr | +5         | 16              | 8              | 16                | 16                   | 32                    | >128                | >128              |
| Onc271 | VDKPPYLPRP-PRrIYNr-Nty-Nr | +5         | 8               | 8              | 16                | 8                    | 32                    | >128                | >128              |
| Onc272 | VDKPPYLPRP-WPrIrYNr       | +6         | 4               | 4              | 8                 | 4                    | 16                    | 128                 | 64                |
| Onc273 | VDKPPYLPRP-EErIYNr        | +5         | 16              | 8              | 32                | 8                    | 128                   | >128                | >128              |
| Onc274 | VDKPPYLPRP-RrIYNr         | +7         | 8               | 8              | 16                | 2                    | 16                    | 32                  | 16                |
| Onc275 | VDKPPYLPRP-WWrIrYNr       | +6         | 16              | 8              | 8                 | 4                    | 16                    | 64                  | 32                |
| Onc276 | VDKPPYLPRP-KWrIrYNr       | +7         | 8               | 8              | 16                | 4                    | 16                    | 32                  | 16                |
| Onc277 | VDKPPYLPRP-HERrIYNr       | +6         | 16              | 8              | 16                | 2                    | 64                    | 128                 | 64                |
| Onc278 | VDKPPYLPRP-JIrIYNr        | +6         | 32              | 32             | 8                 | 4                    | 16                    | 128                 | 128               |
| Onc279 | VDKPPYLPRP-JFrIrYNr       | +6         | 32              | 32             | 8                 | 4                    | 16                    | 128                 | 64                |
| Onc280 | VDKPPYLPRP-LWrIrYNr       | +6         | 32              | 32             | 8                 | 4                    | 16                    | 128                 | 64                |
| Onc281 | VDKPPYLPRP-AWrIrYNr       | +6         | 16              | 16             | 8                 | 4                    | 32                    | >128                | 128               |
| Onc282 | VDKPPYLPRP-WNrIrYNr       | +6         | 8               | 4              | 8                 | 2                    | 32                    | 128                 | 64                |
| Onc283 | VDKPPYLPRP-WWrIrYNr       | +6         | 16              | 16             | 16                | 4                    | 16                    | 128                 | 64                |
| Onc284 | VDKPPYLPRP-WSrIrYNr       | +6         | 4               | 4              | 8                 | 2                    | 16                    | 64                  | 32                |
| Onc285 | VDKPPYLPRP-SSrIrYNr       | +6         | 8               | 8              | 4                 | 2                    | 32                    | 128                 | 64                |
| Onc286 | VDKPPYLPRP-WSrIrYNr       | +6         | 8               | 8              | 8                 | 4                    | 32                    | 128                 | 64                |
| Onc287 | VDKPPYLPRP-WYrIrYNr       | +6         | 16              | 16             | 8                 | 4                    | 16                    | 128                 | 64                |
| Onc288 | VDKPPYLPRP-WDrIrYNr       | +5         | 8               | 16             | 32                | 4                    | 64                    | >128                | >128              |
| Onc289 | VDKPPYLPRP-TRrIrYNr       | +6         | 8               | 8              | 8                 | 4                    | 16                    | >128                | 64                |
| Onc290 | VDKPPYLPRP-WOrIrYNr       | +7         | 8               | 8              | 8                 | 4                    | 16                    | 32                  | 8                 |
Figure S1: Peptide losses calculated for cultures of *K. pneumoniae* DSM 681 (A), *A. baumannii* DSM 30008 (B) and *P. aeruginosa* DSM 1117 (C). Cell cultures were incubated in 25% MHB2 medium containing one oncocin analog (8 mg/L) for 30 minutes. The peptides were quantified after centrifugation in the supernatant using RP-HPLC-ESI-QTOF-MS (MRM mode). Black, grey, and white bars indicate the peptide loss based on the net positive charge of the peptide, i.e. +7 or +8, +6, and +5, respectively, at neutral pH. Dotted lines indicate the loss determined for Onc112.
Figure S2: Peptide losses in the supernatant after incubation with *S. aureus* DSM 6247. Each bar represents a single experiment. Black, grey, and white bars indicate the net charge of the peptide, i.e. +7 or +8, +6, and +5, respectively, at neutral pH.
Figure S3: Peptide quantities determined in the supernatant (see Figure S1) and the bacterial pellet. Cultures of *E. coli* BW25113 (A), *K. pneumoniae* DSM 681 (B), *A. baumannii* DSM 30008 (C) and *P. aeruginosa* DSM 1117 (D) were grown in 25% MHB2 and incubated with one Onc112 analog for 30 minutes.
Figure S4: Peptide quantities determined in the supernatant (see Figure S2) and the bacterial pellet of *S. aureus* DSM 6247 after incubation with one Onc112 analog for 30 minutes. Each bar represents a single experiment.
Table S6: MIC values, relative FP values, and uptake data (relative loss of peptide in supernatant) used for figure 5. Dit, Nty, Nle, Tle, Har, O, and r denote 3,5-diiodotyrosine, 3-nitrotyrosine, nor-leucine, tert-leucine, homoarginine, ornithine, and d-arginine. E. c., K. p., A. b. and P. a. denote *E. coli* BW25113, *K. pneumoniae* DSM 681, *A. baumannii* DSM 30008 and *P. aeruginosa* DSM 1117.

| Peptide name  | analog          | 1-letter code | MIC [mg/L] | Relative FP [%] | Relative loss [%] |
|---------------|-----------------|---------------|------------|-----------------|------------------|
|               |                 |               | E. c.      | K. p.           | A. b. | P. a. | E. c. | K. p. | A. b. | P. a. | E. c. | K. p. | A. b. | P. a. |
| Onc112        | Onc112          | Onc112        | 8          | 2               | 32    | 64    | 64    | 76   | 76    | 88    | 62    | 45    | 50    | 52    |
| Onc244        | [Asp2Arg]-Onc112| D2R           | 8          | 16              | 8     | 32    | 61    | 78   | 77    | 93    | 75    | 55    | 46    | 60    |
| Onc245        | [Asp2Trp]-Onc112| D2W           | 8          | 16              | 16    | 32    | 67    | 93   | 73    | 96    | 66    | 56    | 55    | 56    |
| Onc246        | [Lys3Arg]-Onc112| K3R           | 8          | 8               | 32    | 64    | 47    | 82   | 61    | 86    | 65    | 35    | 46    | 47    |
| Onc247        | [Pro4Lys]-Onc112| P4K           | 8          | 8               | 16    | 32    | 47    | 71   | 66    | 87    | 80    | 25    | 38    | 53    |
| Onc248        | [Pro4Phe]-Onc112| P4F           | 16         | 16              | 32    | 128   | 61    | 73   | 69    | 91    | 61    | 34    | 32    | 31    |
| Onc249        | [Pro4Tyr]-Onc112| P4Y           | 16         | 16              | 32    | 128   | 65    | 89   | 67    | 94    | 65    | 25    | 26    | 30    |
| Onc250        | [Pro5Phe]-Onc112| P5F           | 16         | 8               | 32    | 128   | 68    | 90   | 83    | 91    | 64    | 36    | 32    | 60    |
| Onc251        | [Pro5Tyr]-Onc112| P5Y           | 16         | 8               | 16    | 128   | 60    | 83   | 62    | 87    | 63    | 19    | 19    | 62    |
| Onc252        | [Pro5His]-Onc112| P5H           | 8          | 8               | 16    | 128   | 54    | 38   | 68    | 86    | 73    | 18    | 13    | 80    |
| Onc253        | [Pro5Arg]-Onc112| P5R           | 8          | 8               | 32    | 64    | 70    | 76   | 72    | 86    | 100   | 42    | 40    | 77    |
| Onc254        | [Tyr6Trp]-Onc112| Y6W           | 8          | 4               | 16    | 64    | 60    | 73   | 71    | 74    | 54    | 23    | 4     | 32    |
| Onc255        | [Tyr6Phe]-Onc112| Y6F           | 16         | 4               | 64    | 128   | 67    | 86   | 63    | 75    | 65    | 17    | 11    | 44    |
| Onc256        | [Tyr6His]-Onc112| Y6H           | 4          | 4               | 32    | 64    | 65    | 61   | 54    | 84    | 80    | 18    | 16    | 33    |
| Onc257        | [Tyr6Dit]-Onc112| Y6Dit         | 64         | >128            | 128   | >128  | 90    | 92   | 94    | 93    | 77    | 40    | 33    | 51    |
| Onc258        | [Tyr6Nty]-Onc112| Y6Nty         | 8          | 4               | 64    | >128  | 54    | 76   | 64    | 80    | 63    | 18    | 5     | 25    |
| Onc259        | [Leu7Nle]-Onc112| L7Nle         | 8          | 4               | 32    | 128   | 65    | 73   | 51    | 84    | 65    | 32    | 41    | 49    |
| Onc260        | [Leu7Tle]-Onc112| L7Tle         | 16         | 4               | 64    | 128   | 67    | 71   | 71    | 76    | 65    | 38    | 43    | 52    |
| Onc261        | [Leu7Arg]-Onc112| L7R           | 8          | 4               | 16    | 32    | 62    | 43   | 55    | 54    | 100   | 31    | 38    | 58    |
| Onc262        | [Leu7Met]-Onc112| L7M           | 16         | 4               | 64    | 128   | 69    | 71   | 77    | 77    | 68    | 15    | 11    | 43    |
| Onc263        | [Leu7Phe]-Onc112| L7F           | 16         | >128            | 128   | >128  | 83    | 88   | 87    | 86    | 69    | 0     | 0     | 12    |
| Onc264        | [Pro10Trp]-Onc112| P10W         | 8          | 4               | 16    | >128  | 71    | 76   | 74    | 82    | 100   | 24    | 27    | 36    |
| Peptide name | analog | 1-letter code | MIC [mg/L] | Relative FP [%] | Relative loss [%] |
|--------------|--------|--------------|------------|-----------------|------------------|
|              |        |   E. c.  |   K. p.  |   A. b.  |   P. a.  |   E. c.  |   K. p.  |   A. b.  |   P. a.  |
| Onc265       | [Arg11Trp]-Onc112 | R11W |  8  |  4  |  16  |  64  |  64  |  77  |  71  |  82  |  43  |  24  |  21  |  32  |
| Onc266       | [Arg11His]-Onc112 | R11H |  8  |  8  |  32  | 128  |  59  |  60  |  65  |  84  |  50  |  16  |   5  |  28  |
| Onc267       | [Arg11Lys]-Onc112 | R11K |  8  |  8  |  64  |  64  |  58  |  73  |  65  |  61  |  66  |   7  |   0  |  44  |
| Onc268       | [Arg11Phe]-Onc112 | R11F | 16  |  4  |  16  | >128 |  59  |  81  |  60  |  81  |  40  |  12  |   0  |  32  |
| Onc269       | [Arg11Har]-Onc112 | R11Har | 8  |  4  |  32  |  64  |  57  |  68  |  55  |  71  |  70  |  27  |  30  |  42  |
| Onc270       | [Tyr17Dit]-Onc112 | Y17Dit | 16  |  16  |  32  | >128 |  66  |  80  |  49  |  82  |  69  |   0  |   3  |   0  |
| Onc271       | [Tyr17Nty]-Onc112 | Y17Nty | 8  |  8  |  32  | >128 |  64  |  68  |  61  |  81  |  48  |  10  |   0  |   1  |
| Onc272       | [Pro12Trp]-Onc112 | P12W |  4  |  4  |  16  |  128 |  66  |  75  |  74  |  88  |  67  |  23  |  22  |  18  |
| Onc273       | [13Glu]-Onc272 | P12W P13E | 16  |  8  |  128 | >128 |  75  |  79  |  71  |  87  | 100  |   0  |   0  |  60  |
| Onc274       | [13Arg]-Onc272 | P12W P13R |  8  |  2  |  16  |  32  |  71  |  82  |  72  |  88  | 100  |  43  |  18  |  27  |
| Onc275       | [13Trp]-Onc272 | P12W P13W |  8  |  4  |  16  |  64  |  75  |  90  |  78  |  70  | 100  |  29  |   6  |   1  |
| Onc276       | [13Lys]-Onc272 | P12W P13K |  8  |  4  |  16  |  32  |  70  |  71  |  76  |  76  | 100  |  36  |  21  |  37  |
| Onc277       | [13His]-Onc272 | P12W P13H | 16  |  2  |  64  |  128 |  64  |  80  |  72  |  85  | 100  |  28  |  26  |  63  |
| Onc278       | [13Ile]-Onc272 | P12W P13I | 32  |  4  |  16  |  64  |  66  |  81  |  72  |  85  | 100  |  31  |  24  |  100 |
| Onc279       | [13Phe]-Onc272 | P12W P13F | 32  |  4  |  8  |  128 |  72  |  86  |  74  |  91  | 100  |  34  |  15  |  41  |
| Onc280       | [13Leu]-Onc272 | P12W P13L | 32  |  4  |  16  |  128 |  75  |  76  |  78  |  87  | 100  |  40  |  23  |  31  |
| Onc281       | [13Ala]-Onc272 | P12W P13A | 16  |  4  |  32  | >128 |  81  |  86  |  74  |  77  | 100  |  33  |  20  |  59  |
| Onc282       | [13Asn]-Onc272 | P12W P13N |  8  |  2  |  32  |  128 |  59  |  71  |  69  |  86  | 100  |  26  |  27  |  100 |
| Onc283       | [13Val]-Onc272 | P12W P13V | 16  |  4  |  16  |  128 |  66  |  72  |  67  |  84  | 100  |  28  |  10  |  100 |
| Onc284       | [13Gly]-Onc272 | P12W P13G |  4  |  2  |  16  |  64  |  70  |  66  |  73  |  87  | 100  |  23  |  14  |  100 |
| Onc285       | [13Ser]-Onc272 | P12W P13S |  8  |  2  |  32  |  128 |  68  |  70  |  75  |  89  | 100  |  25  |  21  |  100 |
| Onc286       | [13Gln]-Onc272 | P12W P13Q |  8  |  4  |  32  |  128 |  71  |  72  |  73  |  89  | 100  |  28  |  27  |  100 |
| Onc287       | [13Tyr]-Onc272 | P12W P13Y | 16  |  4  |  16  |  128 |  70  |  75  |  73  |  90  | 100  |  26  |  13  |  100 |
| Onc288       | [13Asp]-Onc272 | P12W P13D |  8  |  4  |  64  | >128 |  71  |  66  |  71  |  76  |  65  |  23  |  12  |  100 |
| Onc289       | [13Thr]-Onc272 | P12W P13T |  8  |  4  |  16  | >128 |  68  |  64  |  72  |  74  | 100  |  27  |  20  |  100 |
| Onc290       | [13Orn]-Onc272 | P12W P13O |  8  |  4  |  16  |  32  |  75  |  59  |  70  |  75  | 100  |  31  |  16  |  28  |
Table S7: Explanation of the color codes used in Figure 5. Data sets were divided into five groups each represented by one color.

| Color     | MIC values [mg/L] | Relative FP [%] | Relative loss [%] |
|-----------|-------------------|-----------------|------------------|
| Green     | 2-4               | 38-51           | 80-100           |
| Light green | 8                | 52-64           | 60-79            |
| Yellow    | 16-32             | 65-77           | 40-59            |
| Orange    | 64                | 78-90           | 20-39            |
| Red       | >64               | >90             | <20              |