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Supplementary Information

Molecular evolution of peptides by yeast surface display technology

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Supplementary tables

Target	Peptide			
	Before		After	
Mcl-1	Bim BH3	^{N}RPEIWIAQELRRIGDEFNAYYAR^{C}	MB1[b]	^{N}RPEIWIAQE**ID**RIGDE**V**NAYYAR^{C}
Bcl-x_L			XH11[b]	^{N}RPEIW**V**AQEL**K**R**N**GDEFNAYYAR^{C}
Bfl-1			FA1_D3fK[b]	^{N}RPEIWIAQELRR**A**G**KVL**NAYYAR^{C}
Mcl-1			MS1[b]	^{N}RPEIW**MT**Q**G**LRR**L**GDE**I**NAYYAR^{C}
Bcl-x_L			XXA1[b]	^{N}RPEIW**Y**AQ**G**L**K**R**F**GDEFNAYYAR^{C}
Bfl-1	Puma BH3	^{N}QWAREIGAQLRRMADDLNAQYER^{C}	FS1[b]	^{N}QW**V**REI**A**A**G**LR**LA**AD**NV**NAQ**L**ER^{C}
Bfl-1			srt.F10[b]	^{N}**RRVVQ**I**A**A**G**LRR**AG**D**Q**L**EKYG**ER^{C}
Mcl-1			srt.M9[b]	^{N}**RSQY**E**V**I**QE**LI**R**I**G**D**IVL**A**YF**ER^{C}
Bcl-x_L			srt.X7[b]	^{N}Q**PLIWF**GAQLRR**G**AD**EFA**AQ**R**ER^{C}
TCR-42F3	QL9	^{N}QLSPFPFDL^{C}	5F2[a]	^{N}**EEN**PF**W**FD**I**^{C}
			p4B10[a]	^{N}QLS**DV**P**M**DL^{C}
TCR55	HLA-B35	^{N}IPLTEEAEL^{C}	Pep20[a]	^{N}**V**PLTE**D**AEL^{C}
TCR2B4	MCC	^{N}YX(V/I)VP(D/E)---ADLIAYLKQATK^{C}	MCC-velcro[a]	^{N}Y**VV**VP**D**GT---ADLIAYLKQATK^{C}
	Pep17	^{N}YX(V/I)VP(D/E)---ADSLSFFSSSIK^{C}	Pep17-velcro[a]	^{N}Y**VV**VP**D**GT---ADSLSFFSSSIK^{C}
LpIA	LAP1	^{N}IETDKAVLEVPG^{C}	LAP2[c]	^{N}**GF**E**I**DK**VWYDLDA**^{C}

Supplementary table 1. **Amino acid sequences of linear peptides evolved using yeast display technology**. Amino acid sequences of linear peptide with higher affinity are reported. Mutated residues are shown in bold. Legend: a = higher affinity; b = higher specificity; c = higher activity; --- = peptide linker; BH3 = Bcl-2-homology-3 motif; Bim = Bcl-2-like protein 11; Puma = Bcl-2-binding component 3; Mcl-1 = Myeloid cell leukemia sequence 1; Bcl-x_L = B-cell lymphoma-extra large; Bfl-1 = Bcl-2-related protein A1; TCR = T-cell receptors; HLA = human leukocyte antigen; QL9 = peptide epitope of 2-oxogluterate dehydrogenase; MCC = moth cytochrome C; LplA = *Escherichia coli* lipoic acid ligase; LAP = LpIA acceptor peptide; IL-2 = interleukin-2, CD69 = cluster of differentiation 69; * = specificity for single target molecule.

Target	Peptide			
	Before		After	
cetuximab	Md1	^{N}CQFDLSTRRLKC^{C}	Q1V/D3N/S5G /T6I/K10R[a]	^{N}C**V**FN**L**G**I**RRL**R**C
$\alpha_v\beta_3$	lacticin 481	^{N}KGGSGVIHBIAHEANMNAW QFVFAAS^{C}	HVRGDN[a]	^{N}KGGSGVIHBIAHEANMNA**HVRGDN**A AS^{C}
$\alpha_v\beta_3$, $\alpha_v\beta_5$	EETI-II	^{N}GCPRILMRCKQDSDCLAGC VCGPNGFCG^{C}	2.5F[a]	^{N}GCPR**PRGDNPPLT**CKQDSDCLAGCV CGPNGFCG^{C}
$\alpha_v\beta_3$, $\alpha_v\beta_5$			3-4A[a,b]	^{N}GC**PQGRGDWAPTS**CSQDSDCLAGC VC**REARGDMPRT**CG^{C}
$\alpha_v\beta_3$	AgRP	^{N}GCVRLHESCLGQQVPCCDP AATCYCRFFNAFCYCR^{C}	7A[a]	^{N}GCVRLHESCLGQQVPCCDPAATCYC **SGRGDNDLV**CYCR^{C}
$\alpha_{iib}\beta_3$			b3A[a,b]	^{N}GCVRLHESCLGQQVPCCDPAATCYC **VGRGDVRRK**CYCR^{C}
matriptase-1	SOTI-III	^{N}EDKCSPSGAICSGFGPPEQC CSGACVPHPILRIFVCQ^{C}	SOTI Var.1[b,c]	^{N}EDKCSPSGAICSGFGPPEQCCSGACV**L** **NRRA**R**SWR**CQ^{C}
	MCoTI-II	^{N}SGVCPKILKKCRRDSDCPGA CICRGNGYCG^{C}	MCoTI Var.4[b,c]	^{N}**W**GVCPK**V**L**RN**CRRDSDCPGACIC**L**G NGYCG^{C}
CTLA-4	MCoTI-II		MC-CT-010[a]	^{N}**APRC KYSHVP**CRRDSDCPG**K**CICRG NGYCG^{C}

Table 2. Amino acid sequences of cyclic peptides evolved using yeast display technology. Amino acid sequences of selected cyclic peptides are reported. Mutated residues are shown in bold. Legend: a = higher affinity; b = higher specificity; c = higher activity; B = aminobutyric acid (Abu); cetuximab = chimeric (mouse/human) monoclonal antibody targeting EGFR; Md1 = Meditope; $\alpha_y\beta_x$ = integrin receptor family; EETI-II = *Ecballium elaterium* trypsin inhibitor II; AgRP = human Agouti-related protein; SOTI = *Spinacia oleracea* trypsin inhibitor; MCoTI = *Momordica cochinchinensis* trypsin inhibitor; CTLA-4 = cytotoxic T lymphocyte-associated antigen 4.

Target	Peptide			
	Before		After	
human EGFR	Gp2	^{N}KFWATVESSEHSFEVPIYAETLDEALELAEWQYVPAGFEVTRVRP^{C}	GP2-EGFR$_{2.2.3}$[a,b]	^{N}KFWATV**SRGDSYW**FEVP**V**YAETLDEALELAE**W**QY**PMYHIYY**VTRVRP^{C}
lysozyme			GαLys$_{A0.3.3}$[a,b]	^{N}KFWATV**FSYGNL**FEVPIYAETLDEALELAEWQY**SG**A**YEY**VTRVRP^{C}
rabbit IgG			GαRIgG$_{3.2.3}$[a,b]	^{N}KFWATV**HSVHGY**FEVPIYAETLDEALELAEWQY**GN**A**LGY**VTRVRP^{C}
goat IgG			GαGIgG$_{2.2.1}$[a,b]	^{N}KFWATV**YDYDADYY**FEVPIYAETLDEALELAEWQY**YSNHSDYL**VTRVRP^{C}
InsR			Gp2 #5[a]	^{N}KFWATV**DCLYNDTA**FEVP**V**YAETLD**K**ALELAEWQY**DPNYCI**VTRVRP^{C}
KRas, NRas, HRas	aPP	^{N}GPRRPRYPGDDAPVXDLXXFXAXLXXYLXVVA^{C}	225-11[a,b]	^{N}GPRRPRYPGDDA**SIE**DL**HEYW**A**R**L**W****NYL**Y****A**VA^{C}
BoNT/H$_{c}$B	n.a.	n.a.	Bot.3194.4[a]	^{N}AKATAADRMFAELKCKFFKEIGLEVEVREKNGTFICEAR^{C}
H1 HA	n.a.	n.a.	HB1.6394.2.3[a]	^{N}CQEYRFTNPFACQIALEILRDFGYACTVQTINGECRVRCC^{C}

Table 3. Amino acid sequences of peptides with a well-defined tertiary structure evolved using yeast display technology. Amino acid sequences of peptides with well-defined tertiary structure are reported. Mutated residues are shown in bold. Legend: a = higher affinity; b = higher specificity; X = random amino acid; EGFR = epidermal growth factor receptor; IgG = immunoglobulin G; InsR = insulin receptor; Gp2 = T7 phage gene 2 protein; KRas = Kirsten rat sarcoma 2 viral oncogene homolog; NRas = Neuroblastoma Ras viral oncogene homolog; HRas = Harvey rat sarcoma viral oncogene homolog; aPP = avian pancreatic polypeptide; BoNT/B = botulinum neurotoxin B; H1 HA = influenza A H1 haemagglutinin. Note: n.a. = not available.

Supplementary figures

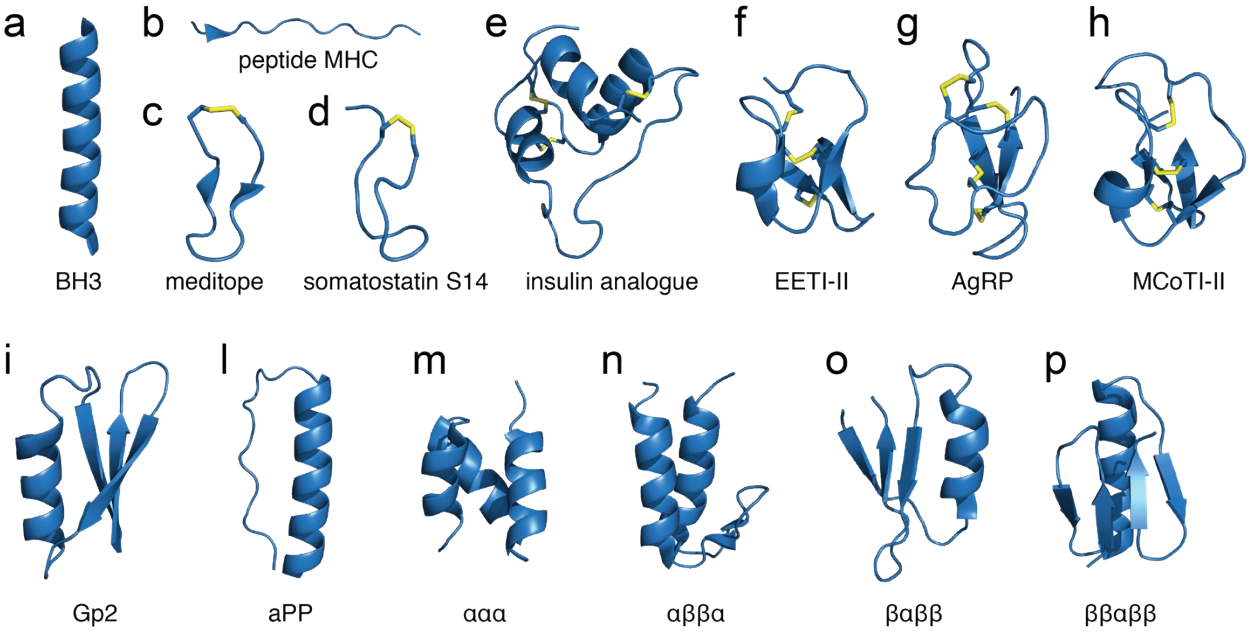

Figure S1. Tridimensional structures of peptides evolved using yeast display technology. a) BH3-like peptide (PDB code: 6E3I; ^{N}QRVVHIAAGLRRTGDQLEAYG^{C}); **b**) peptide loaded on a MHC (PDB code: 6BGA; ^{N}ADSLSFFSSSIK^{C}); **c**) cyclic peptide meditope (PDB code: 4GW1; ^{N}CQFDLSTRRLKC^{C}); **d**) bioactive isoform of the cyclic peptide somatostatin S-14 (PDB code: 2MI1; ^{N}AGCKNFFWKTFTSC^{C}); **e**) single-chain insulin-like peptide (PDB code: 2JZQ; ^{N}FVNQHLCGSDLVEALYLVCGERGFFYTDPTGGGPRRGIVEQCCHSICSLYQLENYCN^{C}); **f**) *Ecballium elaterium* trypsin inhibitor II (EETI-II, PDB code: 2IT7; ^{N}GCPRILMRCKQDSDCLAGCVCGPNGFCG^{C}); **g**) human Agouti-related protein (AgRP, PDB code: 1HYK; ^{N}CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYCRKLGTAMNPCSRT^{C}); **h**)

Momordica cochinchinensis trypsin inhibitor (MCoTI-II, PDB code: 1HA9; ^{N}SGSDGGVCPKILKKCRRDSDCPGACICRGNGYCG^{C}); **i**) minimized T7 phage gene 2 protein (Gp2, PDB code: 2WNM; ^{N}TGSLSVDNKKFWATVESSEHSFEVPIYAETLDEALELAEWQYVPAGFEVTRVRPCVAP K^{C}); **l**) avian pancreatic polypeptide (aPP, PDB code: 1PPT; ^{N}GPSQPTYPGDDAPVEDLIRFYDNLQQYLNVVTRHRY^{C}); **m**) ααα design (PDB code: 5UOI; ^{N}RKWEEIAERLREEFNINPEEAREAVEKAGGNEEEARRIVKKRL^{C}); **n**) αββα design (PDB code: 5UYO; ^{N}HMDVEEQIRRLEEVLKKNQPVTWNGTTYTDPNEIKKVIEELRKSM^{C}); **o**) βαββ design (PDB code: 5UP5; ^{N}TQTQEFDNEEEARKAEKELRKENRRVTVTQENGRWRVTWD^{C}); **p**) ββαββ (PDB code: 5UP1; ^{N}GLVPRGSHMTTVKLGDIKVTFDNPEKAKKYAQKLAKIYQLTVHVHGDTIHVK^{C}). Peptides are shown as cartoon colored in *blue*. Disulfide bonds are shown as *yellow* sticks.