

Supplemental Table S7
Oligonucleotides and gene blocks employed in this work

Oligo/ gene block ID	Brief description	Sequence
gbEP14	Used to build EEVN391	AGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCACCACCACCATCAC CATCAGCGCGCTTCTACTGATAAGAATGCCCCAACTGACTTAGAAGTCGTTGCTGC CACCCCAACTTCCTTATTGATTTCTGCGGATGCCCCAGCTCACGCTGTGACCGTTT CTCCTGCAGGTAGATACTACCGTATCACCTACGGTGAAACCGGTGGTAATACCCCA GTCCAAGAATTCACAGTCCCAGGTTCCAAGTCCACCGCTACCATCTCCGGTTTGAA ACCAGGTGTCGATTACACCATACCGTCTACGCCGTTACCGGTCTGCGGCGTACGTC CAGCTTCTTCCAAGCCTATCTCTATTAATTACAGAAGTGC GGCCGTCATGAGCAAAG GAGAAGAAGTCTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGAT
gbEP17	TTC-CCA-optimal	TCTAAGCCAATTTCCATTAACCTACAGAACCACCTCTACTAACTTAATTAATAGTGGTA CGGGCCCCAACTTATTGCCAGTCGACGCACGTGGTTTGGAAGCTTTGTTCCAAGGT CCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCAC CACCACCATCACCATCAGCGCGCTTTGCCATCTACTGATAAGAATGCCCCAACTGA CTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGCGGATGCCCCAG CTCACGCTGTGTTGCCAACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTAC GGTGAAACCGGTGGTAATACCCAGTCCAAGAATTC
gbEP18	CTC-CCG; TTC-CCG, 1 inhibitory	TCTAAGCCAATTTCCATTAACCTACAGAACCACCTCTACTAACTTAATTAATAGTGGTA CGGGCCCCAACTTACTCCCGGTCGACGCACGTGGTTTGGAAGCTTTGTTCCAAGGT CCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCAC CACCACCATCACCATCAGCGCGCTTTGCCATCTACTGATAAGAATGCCCCAACTGA CTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGCGGATGCCCCAG CTCACGCTGTGTTGCCAACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTAC GGTGAAACCGGTGGTAATACCCAGTCCAAGAATTC

gbEP19	CTC-CCG, TTC-CCG, 2 inhibitory	TCTAAGCCAATTTCCATTAACTACAGAACCACCTCTACTAACTTAATTAATAGTGGTA CGGGCCCCAACTTACTCCCGGTGACGCACGTGGTTTGGAAGCTTTGTTCCAAGGT CCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCAC CACCACCATCACCATCAGCGCGCTTTGCCATCTACTGATAAGAATGCCCCAACTGA CTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCCCCAG CTCACGCTGTGCTCCCGACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTAC GGTGAAACCGGTGGTAATACCCAGTCCAAGAATTC
gbEP20	CTC-CCG- All inhibitory	TCTAAGCCAATTTCCATTAACTACAGAACCACCTCTACTAACTTAATTAATAGTGGTA CGGGCCCCAACTTACTCCCGGTGACGCACGTGGTTTGGAAGCTTTGTTCCAAGGT CCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCAC CACCACCATCACCATCAGCGCGCTCTCCCGTCTACTGATAAGAATGCCCCAACTGA CTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCCCCAG CTCACGCTGTGCTCCCGACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTAC GGTGAAACCGGTGGTAATACCCAGTCCAAGAATTC
gbEP021	5' UTR YDL224C	AATAATACTTTCAACATTTTCGGTTTGTATTACTTCTTATTCAAATGTAATAAAAGCT AGGAGCCTCGAACCGGTAGTTTTACAGAAAATAGACCCATCTGGTGGCAACACTAA ATCATTGAAATAATAGCAAACAATTGTTTCAAACCTCCTCCCATTTGGATCTCATCTC AATTGTTTTTCTTCTTAGTTAAAAGTTCACGAAATTAGGAAATCTAAGAATCAAAA TATAATCCGCTGTTATTTCTGCGTGTAAGGTTTATCTTCTACTTGTGTCATATTTCA CAAATTATCACTTTGCTAAAACAAGGGACTGACTTCTTCTTTATTTTTTCGAGCATTT TTCAAAAAAGCAGGGTTTTTGAAAACAGGAACCTTAGTTGCCACATCTACGCTGAAA AATAACGCTTAAGGTTTCTCAATTAACAATGGACTACAAGGACGACGATGACAAGAC TAGTGTTTCAAAGGTGAAGAAGATAATATGGCTATTATTAAGAATTTATGAGATTT AAA

gbEP029	RR 0000	TTCTAAGCCAATTTCCATTAACACAGAACACCTCTACTAACTTAATTAATAGTGGT ACGGGGCCCTCACCACCGTCGTCAAGTCGACGCACGTGGTTTGGAAGCTTTGTTCCA AGGTCCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTT CTCACCACCACCATCACCATCAGCGCGCTTTGCCATCTACTGATAAGAATGCCCCA ACTGACTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCC CCAGCTCACGCTGTGCGTCGTACCGTTTCTCCTGCAGGTAGATACTACCGTATCAC CTACGGTGAAACCGGTGGTAATACCCACAGTCCAAGAATTCACAGTCCCAGGTTCCA AGTCCACCGCTACCATCTCCGGTTTGAAACCAGGTGTGCGATTACACCATTACCGTC TACGCCGTTACCGGTGCGTGGCGTACGTGCGTCGTCCAGCTTCTTCCAAGCCTATCTC TATTAATTACAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAAGCTTTTCACTGGAG TTGTCCCAATTCTTGTTGAATTAGATG
gbEP030	RR 1000	TTCTAAGCCAATTTCCATTAACACAGAACACCTCTACTAACTTAATTAATAGTGGT ACGGGGCCCTCACCACCGACGACAAGTCGACGCACGTGGTTTGGAAGCTTTGTTCC AAGGTCCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTT CTCACCACCACCATCACCATCAGCGCGCTTTGCCATCTACTGATAAGAATGCCCCA ACTGACTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCC CCAGCTCACGCTGTGCGTCGTACCGTTTCTCCTGCAGGTAGATACTACCGTATCAC CTACGGTGAAACCGGTGGTAATACCCACAGTCCAAGAATTCACAGTCCCAGGTTCCA AGTCCACCGCTACCATCTCCGGTTTGAAACCAGGTGTGCGATTACACCATTACCGTC TACGCCGTTACCGGTGCGTGGCGTACGTGCGTCGTCCAGCTTCTTCCAAGCCTATCTC TATTAATTACAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAAGCTTTTCACTGGAG TTGTCCCAATTCTTGTTGAATTAGATG
gbEP031	RR 1011	TTCTAAGCCAATTTCCATTAACACAGAACACCTCTACTAACTTAATTAATAGTGGT ACGGGGCCCTCACCACCGACGACAAGTCGACGCACGTGGTTTGGAAGCTTTGTTCC AAGGTCCAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTT CTCACCACCACCATCACCATCAGCGCGCTTTGCCATCTACTGATAAGAATGCCCCA ACTGACTTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCC CCAGCTCACGCTGTGCGACGAACCGTTTCTCCTGCAGGTAGATACTACCGTATCAC CTACGGTGAAACCGGTGGTAATACCCACAGTCCAAGAATTCACAGTCCCAGGTTCCA AGTCCACCGCTACCATCTCCGGTTTGAAACCAGGTGTGCGATTACACCATTACCGTC TACGCCGTTACCGGTGCGTGGCGTACGTGCGACGACGAGCTTCTTCCAAGCCTATCTC TATTAATTACAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAAGCTTTTCACTGGAG TTGTCCCAATTCTTGTTGAATTAGATG

gbEP27	RPL7A	ATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAG CTTCGCGACTTCTGAGAATCATTGCCATCTGTATCTGTATCACTTTGACAACTTCAT CCTTTCGGCCGTATTTCGAATGATAATGCTTCCGGTGGCTATATGCAGCCGGATCA ATCTTCGAACGCTTCGGATCCCCGGGTACCGAGCTCGAATTCACTGGCCGTCGTTT TACAACGTCGTGACTGGGA
gbEP28	RPL7A	ATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAG CTTTGTATAGTTTTGCGCTGTGTGCACATTGTGTGCCTCTCCAATTTGTGTTAAAAA ATGCACGCGTGGTCCGCAACAGTTGCAAAATCAATGATGCCTGCTACCGTCATGCT ATCATGACAAACGATGGCAGGATCCCCGGGTACCGAGCTCGAATTCACTGGCCGT CGTTTTACAACGTCGTGACTGGGA
OBB021:	tL(GAG)Δ::HygroR	GCGTTTGCTTTATTCCCTTACCCAACAGATGAATTCAGCTGAAGCTTCGTACGC
OBB022:	tL(GAG)Δ::HygroR	ATTCTTATTGAAGTGCGTAGATTCAAAAAAATATGCATAGGCCACTAGTGGATCTG
OBB023:	tL(GAG)Δ::HygroR	GCTTCACTTAAATGGCGTATCTTCCTTTTATAAATTGCGTTTGCTTTATTCCCTTACC
OBB024:	tL(GAG)Δ::HygroR	GGTACATAGAAAAAAATGCTATCCTAACAGCCTAATTCTTATTGAAGTGCGTAG
OBB028	confirmation tL(GAG)Δ; +476	CGTATCTCAGACAGAAGTTATCG
OBB029	confirmation tL(GAG)Δ; -276	AGTTGGTAGAGCGTTCGGC
gbBB001	Arg Arg All Optimal OOOO	ACATTCAACTGGTGGTATGGATGAATTATATAAAAAGTTAATTAATAGTGGTACGGG CCCTCACCACAGAAGACAAGTCGACGCACGTGGTTTGGAAGCTTTGTTCCAAGGTC CAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCACC ACCACCATCACCATCAGCGCGCTAGAAGATCTACTGATAAGAATGCCCAACTGAC TTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCCCCAGCT CACGCTGTGAGAAGAACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTACGG TGAAACCGGTGGTAATACCCAGTCCAAGAATTCACAGTCCCAGGTTCCAAGTCCA CCGCTACCATCTCCGGTTTGAAACCAGGTGTCGATTACACCATTACCGTCTACGCC GTTACCGGTCGTGGCGTACGTAGAAGACCAGCTTCTTCCAAGCCTATCTCTATTAAT TACAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCC AATTCTTGTTGAATTAGATGG

gbBB002	Arg Arg 2 inhibitory IOOI	ACATTCAACTGGTGGTATGGATGAATTATATAAAAAGTTAATTAATAGTGGTACGGG CCCTCACCACCGACGACAAGTCGACGCACGTGGTTTGAAGCTTTGTTCCAAGGTC CAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCACC ACCACCATCACCATCAGCGCGCTAGAAGATCTACTGATAAGAATGCCCCAACTGAC TTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCCCCAGCT CACGCTGTGAGAAGAACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTACGG TGAAACCGGTGGTAATACCCCAAGTCCAAGAATTCACAGTCCCAGGTTCCAAGTCCA CCGCTACCATCTCCGGTTTGAAACCAGGTGTGCGATTACACCATTACCGTCTACGCC GTTACCGGTTCGTGGCGTACGTGACGACCAGCTTCTTCCAAGCCTATCTCTATTAA TTACAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCC CAATTCTTGTTGAATTAGATGG
gbBB003	- Arg Arg 3 inhibitory IIIO	ACATTCAACTGGTGGTATGGATGAATTATATAAAAAGTTAATTAATAGTGGTACGGG CCCTCACCACCGACGACAAGTCGACGCACGTGGTTTGAAGCTTTGTTCCAAGGTC CAGGTCCAGGTAGAATCTTTTATCCATACGATGTTCCAGATTACGCCGGTTCTCACC ACCACCATCACCATCAGCGCGCTCGACGATCTACTGATAAGAATGCCCCAACTGAC TTAGAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCCCCAGCT CACGCTGTGCGACGAACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTACGG TGAAACCGGTGGTAATACCCCAAGTCCAAGAATTCACAGTCCCAGGTTCCAAGTCCA CCGCTACCATCTCCGGTTTGAAACCAGGTGTGCGATTACACCATTACCGTCTACGCC GTTACCGGTTCGTGGCGTACGTAGAAGACCAGCTTCTTCCAAGCCTATCTCTATTAA TACAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCC AATTCTTGTTGAATTAGATGG
gbBB004	tL(GAG) with EAH118-2 homology	AGAATTGCACTTTAACATTAATATTGACAAGGAGGAGGGCACCACACAAAAAGTCTA GAGCTCCGGAGTAATTGACTTGATTTTGCAAAGCTTCACTTAAATGGCGTATCTTCC TTTTATAAATTGCGTTTGCTTTATTCCCTTACCCAACAGATGAATTGGTACTCTGGCC GAGTGGTCTAAGGCGTCAGGTGAGGTCCTGATCTCTTCGGAGGCGCGGGTTCAA ACCCGCGGGTATCAATATTTTTTTGAATCTACGCACTTCCCCGGGCTTTTATCCAA GGACATCAGGACTTTCTTAAAGAAAAGATTGGCTAGCTATGCT

gbBB005	OI ArgArg insert with Optimal LeuPro at site 3	GAAGTCGTTGCTGCCACCCCAACTTCCTTATTGATTTCTGGGATGCCCCAGCTCA CGCTGTGTTGCCAACCGTTTCTCCTGCAGGTAGATACTACCGTATCACCTACGGTG AAACCGGTGTAATACCCCAAGTCCAAGAATTCACAGTCCCAGGTTCCAAGTCCACC GCTACCATCTCCGGTTTGAAACCAGGTGTCGATTACACCATTACCGTCTACGCCGT TACCGGTCGTGGCGTACGTCGACGACCAGCTTCTTCCAAGCCTATCTCTATTAATTA CAGAACTGCGGCCGTCATGAGCAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAA TTCTTGTTGAATT
gbBB006	tL(GAG)C33U mutation with EAH118-2 homology	AGAATTGCACTTTAACATTAATATTGACAAGGAGGAGGGCACCACACAAAAAGTCTA GAGCTCCGGAGTAATTGACTTGATTTTGCAAAGCTTCACTTAAATGGCGTATCTTCC TTTTATAAATTGCGTTTGCTTTATTCCCTTACCCAACAGATGAATTGGTACTCTGGCC GAGTGGTCTAAGGCGTCAGGTTGAGGTCCTGATCTCTTCGGAGGCGCGGGTTCAA ACCCCGCGGGTATCAATATTTTTTTGAATCTACGCACTTCCCCGGGCTTTTATCCAA GGACATCAGGACTTTCTTAAAGAAAAGATTGGCTAGCTATGCT
OBB083	PCR confirmation of inserts at LYS2 +213 insertion site	GTCGCTTTGAGTGTATGGGC
OBB084	PCR confirmation of inserts at LYS2 - -108 insertion site	GGGTACGAACCTTGGTAGCG
OBB085	PrimerF1 homolgy to YNCL0001W tP(UGG)	GACAAGGAGGAGGGCACCACACAAAAAGTCTAGAGCTTCTCATAACCTATGTCATC
OBB086	PrimerR1 homolgy to YNCL0001W tP(UGG)	AAGAAAGTCCTGATGTCCTTGGATAAAAGCCCGGGAGGTAAAGAGGGTGAAAGCC
OBB087	PrimerF2 homolgy to OB85+EAH118-2	AGAATTGCACTTTAACATTAATATTGACAAGGAGGAGGGCACC
OBB088	Round R2 homolgy to OBB086, +EAH118-2	AGCATAGCTAGCCAATCTTTTCTTTAAGAAAGTCCTGATGTCCTTGG

gbEP021	5'UTR YDL224C inserted NheI-SpeI cut EBB024 to produce EBB027	AACTAATACTTTCAACATTTTCGGTTTGTATTACTTCTTATTCAAATGTAATAAAAGCT AGGAGCCTCGAACCGGTAGTTTTACAGAAAATAGACCCATCTGGTGGCAACACTAA ATCATTGAAATAATAGCAAACAATTGTTTCAAACCTCCTCCCATTTGGATCTCATCTC AATTGTTTTTTCCTTCTTAGTTAAAAGTTCACGAAATTAGGAAATCTAAGAATCAAAA TATAATCCGCTGTTATTTCTGCGTGTAAGGTTTATCTTCTACTTGTGTCATATTTCA CAAATTATCACTTTGCTAAAACAAGGGACTGACTTCTTTCTTTATTTTTTCGAGCATTT TTCAAAAAGCAGGGTTTTTGAAAACAGGAACCTTAGTTGCCACATCTACGCTGAAA AATAACGCTTAAGGTTTCTCAATTAACAATGGACTACAAGGACGACGATGACAAGAC TAGTGTTTCAAAGGTGAAGAAGATAATATGGCTATTATTAAGAATTTATGAGATTT AAA
OEP302	Cloning FN-ICP-FN region into the URA3 plasmids	AGTGAAAAGTTCTTCTCCTTTGCTCATGACtGC
OEP303	Cloning FN-ICP-FN region into the URA3 plasmids	GACGTTCCAAGAACTTGGAAG
OBB047	Sequence ICPs in URA3 plasmid, 10 bases from NdeI in EBB325	AAGGAGCACAGACTTAGATTGG