**Supporting information**

# KnowVolution of the polymer binding peptide LCI for polypropylene binding

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**Table S1.** Primer sequences.

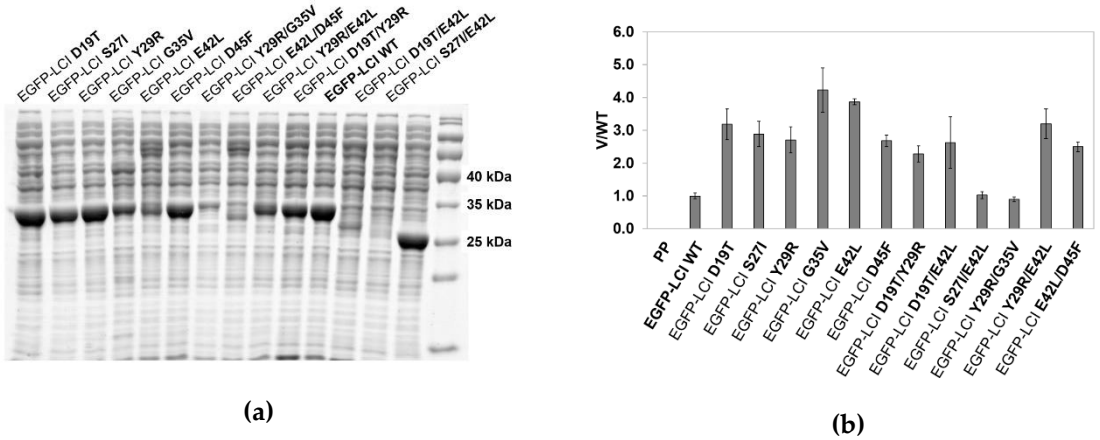
| Primer | Sequence 5′ → 3′ |
|---|---|
| F-epLCI | GCCGAAAATCTGTATTTTCAGGGT |
| R-epLCI | GTCGACGGAGCTCGAATTCTTA |
| F-K3-SSM | GTTGCGCCATTNNKCTGGTTCAGAG |
| R-K3-SSM | CTCTGAACCAGMNNAATGGCGCAAC |
| F-P8-SSM | CTGGTTCAGAGCNNKAATGGTAATTTTGCAGC |
| R-P8-SSM | GCTGCAAAATTACCATTMNNGCTCTGAACCAG |
| F-N9-SSM | CTGGTTCAGAGCCCGNNKGGTAATTTTGCAGC |
| R-N9-SSM | GCTGCAAAATTACCMNNCGGGCTCTGAACCAG |
| F-D19-SSM | GCAGCAAGCTTTGTTCTGNNKGGCACCAAATGG |
| R-D19-SSM | CCATTTGGTGCCMNNCAGAACAAAGCTTGCTGC |
| F-I24-SSM | GGATGGCACCAAATGGNNKTTCAAAAGC |
| R-I24-SSM | GCTTTTGAAMNNCCATTTGGTGCCATCC |
| F-Y29-SSM | CTTCAAAAGCAAANNKTATGACAGCAGC |
| R-Y29-SSM | GCTGCTGTCATAMNNTTTGCTTTTGAAG |
| F-D31-SSM | GCAAATACTATNNKAGCAGCAAAGGTTATTGGGTGGGT |
| R-D31-SSM | ACCCACCCAATAACCTTTGCTGCTMNNATAGTATTTGC |
| F-S33-SSM | GCAAATACTATGACAGCNNKAAAGGTTATTGGGTGGGT |
| R-S33-SSM | ACCCACCCAATAACCTTTMNNGCTGTCATAGTATTTGC |
| F-G35-SSM | CTATGACAGCAGCAAANNKTATTGGGTGGGT |
| R-G35-SSM | ACCCACCCAATAMNNTTTGCTGCTGTCATAG |
| F-I40-SSM | TGGGTGGGTNNKTATGAAGTGTGG |
| R-I40-SSM | CCACACTTCATAMNNACCCACCCA |
| F-E42-SSM | TGGGTGGGTATTTATNNKGTGTGGGATCGC |
| R-E42-SSM | GCGATCCCACACMNNATAAATACCCACCCA |
| F-W44-SSM | GTATTTATGAAGTGNNKGATCGCAAATAAG |
| R-W44-SSM | CTTATTTGCGATCMNNCACTTCATAAATAC |
| F-D45-SSM | GAAGTGTGGNNKCGCAAATAAGAATTCGAGCTCCG |
| R-D45-SSM | CGGAGCTCGAATTCTTATTTGCGMNNCCACACTTC |
| F-Y29R | CTTCAAAAGCAAACGTTATGACAGCAGC |
| R-Y29R | GCTGCTGTCATAACGTTTGCTTTTGAAG |
| F-G35V | CTATGACAGCAGCAAAGTGTATTGGGTGGGT |
| R-G35V | ACCCACCCAATACACTTTGCTGCTGTCATAG |
| F-Y29/G35 | CTTCAAAAGCAAAMVWTATGACAGCAGCAAANNKTATTGGGTGGGT |
| R-Y29/G35 | ATACCCACCCAATAMNNTTTGCTGCTGTCATAWBKTTTGCTTTTGAAG |

**Table S2.** Summary of binding performance and amino acid substitutions found in improved EGFP-epLCI variants screened for improved PP binding in presence of 1 mM Triton X-100. Potential beneficial positions are underlined.

| Variant | V/WT | Substitutions |
|---|---|---|
| LCI-M1-PP | 3.4 ± 0.8 | I24T Y29H E42K |
| LCI-M2-PP | 2.5 ± 0.2 | D31V E42G |
| LCI-M3-PP | 4.1 ± 0.5 | D31V S32C D45V |
| LCI-M4-PP | 3.6 ± 0.2 | K3R P8Q N9K G10C D19G I24T S27G G35D W44R D45V |
| LCI-M5-PP | 2.9 ± 0.4 | Q6H Y29F I40T D45A |
| LCI-M6-PP | 2.6 ± 0.2 | P8L S15R S27C D45G |
| LCI-M7-PP | 2.6 ± 0.8 | W23R S33T Y36C |
| LCI-M8-PP | 2.6 ± 0.4 | L4Q K34R E42V |
| LCI-M9-PP | 2.6 ± 0.3 | I2F K3Q N11S D19G G35C K47R |
| LCI-M10-PP | 2.5 ± 0.1 | F16L I24S I40T D45Y |

**Table S3.** LCI key positions and identified amino acid substitutions for improved PP binding.

| Position | Variant | V/WT |
|---|---|---|
| K3 | K3W | 1.3 ± 0.1 |
| P8 | P8R | 1.6 ± 0.3 |
| D19 | D19V | 2.5 ± 0.5 |
| | D19T | 2.5 ± 0.5 |
| | D19R | 2.4 ± 0.4 |
| I24 | I24G | 1.4 ± 0.1 |
| | I24L | 1.3 ± 0.2 |
| S27 | S27V | 2.1 ± 0.2 |
| | S27I | 1.7 ± 0.2 |
| | S27A | 1.5 ± 0.2 |
| Y29 | Y29R | 3.2 ± 0.5 |
| | Y29C | 3.0 ± 0.2 |
| | Y29K | 2.8 ± 0.4 |
| D31 | D31R | 3.1 ± 0.4 |
| | D31T | 3.0 ± 0.3 |
| | D31A | 2.9 ± 0.1 |
| | D31L | 2.9 ± 0.2 |
| | D31S | 2.7 ± 0.3 |
| G35 | G35W | 3.8 ± 0.5 |
| | G35V | 3.7 ± 0.4 |
| | G35Y | 3.1 ± 0.4 |
| | G35C | 2.4 ± 0.3 |
| | G35R | 2.2 ± 0.2 |
| I40 | I40W | 2.1 ± 0.7 |
| | I40S | 2.0 ± 0.4 |
| E42 | E42L | 2.9 ± 0.5 |
| | E42I | 2.3 ± 0.3 |
| D45 | D45F | 2.3 ± 0.3 |
| | D45L | 2.3 ± 0.2 |
| | D45H | 2.1 ± 0.3 |




**(a)** **(b)**

**Figure S1.** Expression and performance of PP-binding peptide LCI variants. SSM variants LCI Y29R and LCI G35V and generated recombination variants LCI Y29R/G35V were produced in MTP and resulting CFE was used for SDS-PAGE to evaluate expression level (a) and in ABBA screening system to evaluate binding performance (b).

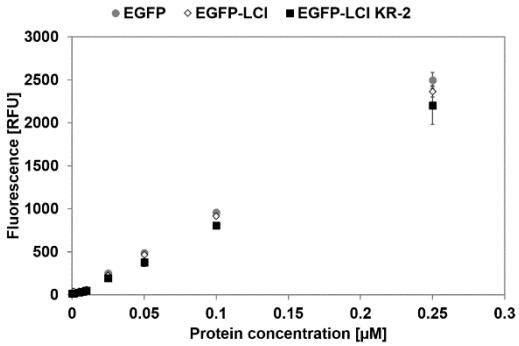



**Figure S2.** Fluorescence of EGFP (grey), EGFP-LCI (white), and EGFP-LCI KR-2 in the protein concentration range of 0.001-0.25 µM. The fluorescence was detected with 96-well MTP reader FLUOstar Omega (BMG LABTECH GmbH, Ortenberg, Germany) (excitation (ex.) 485 nm, emission (em.) 520 nm, gain 750, 35 reads/well).

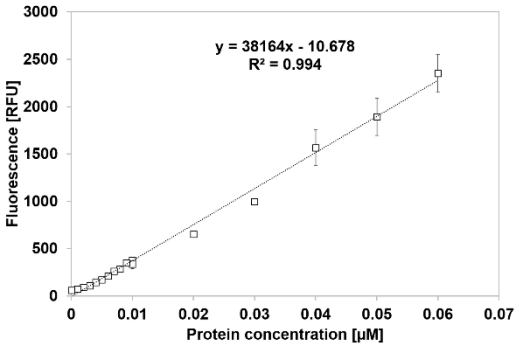



**Figure S3.** Quantification of fluorescence intensity of EGFP-LCI (concentrations: 0-0.06 µM). Detection was performed with FLUOstar Omega (exc. 485 nm, em. 520 nm, gain 1000). Each concentration was determined in triplicates. Error bars indicate the standard deviation.

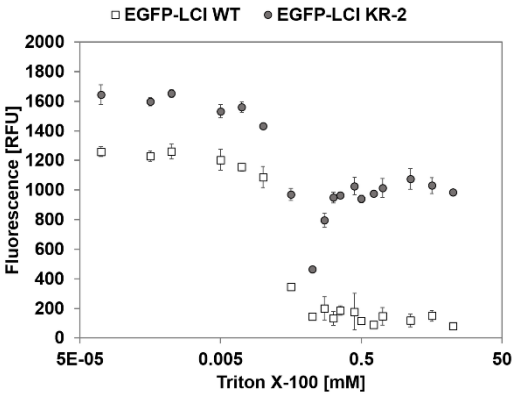



**Figure S4.** PP-binding of EGFP-LCI WT (white) and EGFP-LCI KR-2 (grey) after selection with nonionic surfactant Triton X-100 (pH 8.0, 0.0001-10 mM).