

High-throughput *de novo* screening of receptor agonists with an automated single-cell analysis and isolation system

Author names

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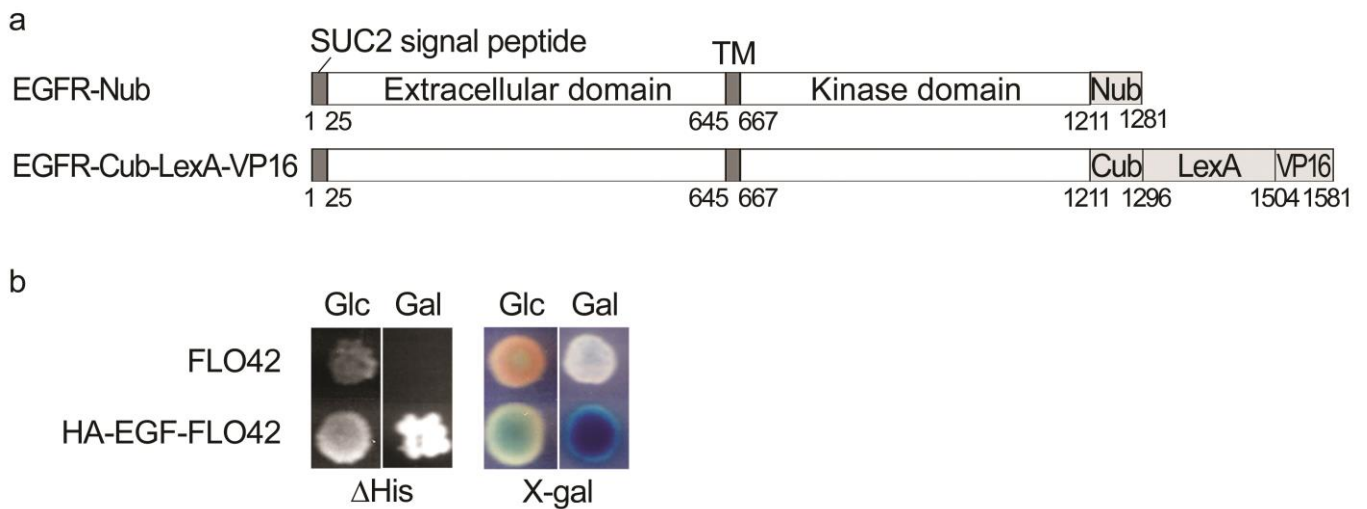
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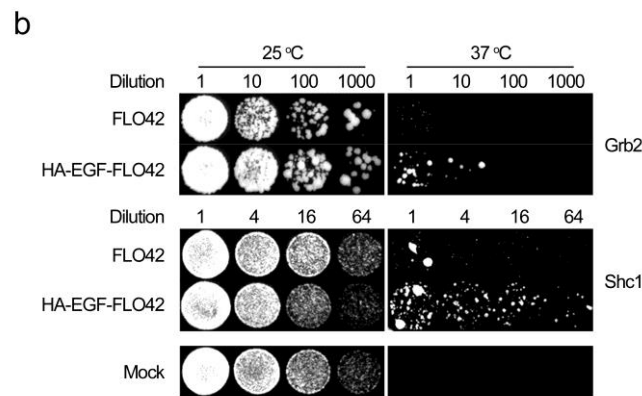
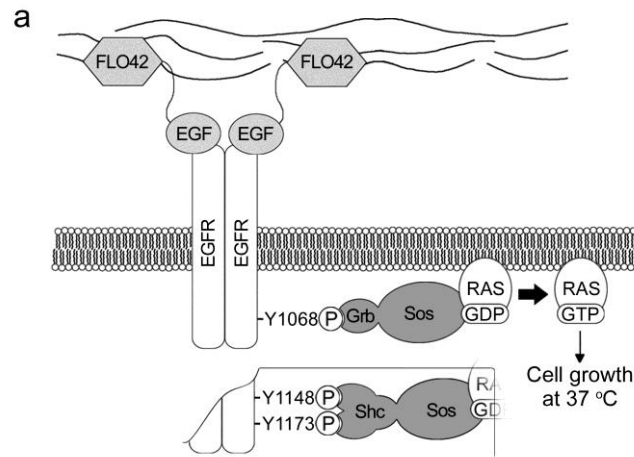
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Supplementary Information



Supplementary Figure S1. EGF-dependent homo-oligomerization of the EGFR in the yeast plasma membrane. (a) Molecular organizations of EGFRs in a split-ub assay. *TM*, transmembrane domain; *Nub*, N-terminal half of ub; *Cub*, C-terminal half of ub; *LexA*, DNA-binding domain of LexA; *VP16*, transcription-activating domain of VP16. **(b)** Split-ub assay. The yeast NMY51 strain co-expressing EGFR-Nub, EGFR-Cub-LexA-VP16 and either FLO42 or HA-EGF-FLO42 was spotted onto an SD plate (pH 7) containing glucose (*Glc*, for repression of *GAL1* promoter) or galactose (*Gal*, for induction of *GAL1* promoter). Δ *His*, histidine-depleted SD medium; *X-gal*, X-gal-containing SD medium.



Supplementary Figure S2. Functional interaction of the EGFR with Grb2 and Shc1 in yeast cells. **(a)** Schematic drawing of the EGF signaling pathway reconstituted in yeast cells. N-terminal human Sos-fused forms of human adaptor proteins, Grb2 (*Grb*) and Shc1 (*Shc*), interact with phospho-EGFR in yeast cells. The membrane-recruited Sos is a guanine nucleotide-exchanging factor for human Ras, which converts yeast RAS from the GDP (inactive) form to the GTP (active) form and complements thermo-sensitive CDC25^{TS} in the yeast *cdc25h* strain, thereby allowing yeast cells to grow at 37 °C. **(b)** Complementation of thermo-sensitivity of the yeast *cdc25h* strain by EGF-dependent activation of the RAS signaling pathway. The yeast *cdc25h* strain co-expressing EGFR-V5 and either FLO42 or HA-EGF-FLO42 was spotted onto an SD plate and then incubated at 25 °C or 37 °C. *Grb2* and *Shc1* indicate the *cdc25h* strains expressing Sos-Grb2 and Sos-Shc1, respectively.

Supplementary Table S1. Nucleotide sequences of secondary helix-coding region for EGFR agonist candidates

Clone no.	nucleotide sequences*
1	AAGCTGTATTCGTTGAAAATGAAGCTACATAAGTTGAAAGCT
2	AAGCTGGATCCGTTGAAAATTAAAGCTAGAGTCGTTGAAAGCT
3	AAGCTGTCTTCGTTGAAAGCTAAGCTATCTCATTTGAAAGCT
4	AAGCTGCCTAAGTTGAAACATAAGCTACCGACGTTGAAAGCT
5	AAGCTGAATCGTTTGAAACATAAGCTATCGTTTTTGAAAGCT
6	AAACTGGATCCGTTGAAAATTAAAGCTAGAGTCGTTGAAAGCT
7	AAGCTGATTTGTTTGAAACATAAGCTAACGCCTTTGAAAGCT
8	AAGCTGACTCCTTTGAAAAGAAGCTAACTGCTTTGAAAGCT
9	AAGCTGGATCCTTTGAAAATTAAAGCTAGAGTCGTTGAAAGCT
10	AAGCTGGATCCGTTGAAAATTAAAGCTAGAGTCGTTGAAAGCT
11	AAGCTGACTCCTTTGAAAAGAAGCTAACTGCTTTGAAAGCT
12	AAGCTGACTCCTTTGAAAAGAAGCTAACTGCTTTGAAAGCT
13	AAGCTGCCGCTTTTGAAATCTAAGCTATTGTCTTTGAAAGCT

* Identified nucleic acids are shown in red.

Supplementary Table S2. Amino acid sequences of EGFR agonist candidates

Clone no.	Amino acid sequences*	Notes
1	KL Y SLK M KL H KLKA	No agonistic activity
2	KL D PLK I KL E SLKA	Identical to clones 6, 9 and 10
3	KL S SLK A KL S H L KA	
4	KL P KL H KL P T L KA	No agonistic activity
5	KL N RLK H KL S FLKA	
7	KL I CLK H KL T PLKA	
8	KL T PLK K KL T ALKA	Identical to clones 11 and 12
13	KL P LLK S KL L SLKA	

* Identified amino acid residues are shown in red.

Supplementary Table S3. Amino acid sequences of EGFR mutants and HLH peptides*

Clone name	Amino acid sequences	Ref.
Human EGF	NSDSECPLSHD ¹⁰ GYCLH ²⁰ DGVCMYIEALDKYACNCVVG ³⁰ YIGER RCQ ⁴⁰ YRD L ⁵⁰ KWWELR	37–39
G12Q	-----Q-----	39
Y13W	-----W-----	39
H16D	-----D-----	39
Clone 114	SRG-K--P-----QG-----R-----A-----T--GR-	40
m28	-----G-----K-V-R-----T--GP-	41
m123	--Y----P-Y-----R-----S-----A-----R--GR-	41
HLH	QAW <u>AELAALEMELAALE</u> ¹⁰ <u>GGGGGGG</u> ²⁰ <u>KL--LK-KL--LKA</u> ³⁰ <u>GGGS</u> ⁴⁰	
2	DP I ES	
3	SS A SH	
5	NR H SF	
7	IC H TP	
8	TP K TA	
13	PL S LS	

*Replaced amino acid residues in enhanced EGF mutants (G12Q, Y13W, H16D, clone 114, m28, m123) are shown. Essential amino acids for EGFR binding are indicated in red. Boxes and underline in HLH sequence indicate α -helix region and loop region, respectively.