Supporting Information

Selection of A Full Agonist Combinatorial Antibody that Rescues Leptin Deficiency In Vivo

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Supplementary Figures and Tables:
Figure S1. Characterization of agonist activity of combinatorial antibody ligands. a) Schematic illustration of a functional selection system for combinatorial antibody library based on STAT3 phosphorylation reporter gene assay; b) FACS sorting results of activated reporter cells infected with lentivirus containing a focused combinatorial antibody library; c) Sequence alignment of Ab06 and Ab11 in the CDR regions; d) Binding (left) and activation
(right) of scFv and full IgG to hLepR expressed reporter cell lines were analyzed by flow-cytometry; e) SDS- and native- PAGE analyses of H6, leptin, hLepR-ECD, and hLepR-ECD complexed with H6, leptin, or both at different ratios.

**Figure S2. Affinity maturation of combinatorial antibodies using yeast surface display.**

**a)** Selection scheme of antibody affinity maturation using recombinant hLepR- and mLepR-ECD on yeast surface; **b)** Yeast sorting results of each selection round.
**Figure S3. Affinity validation of combinatorial antibodies.**

a) Dose-dependent activation of LepR by leptin and high affinity agonist antibody candidates (H1-H7) via chemiluminescent detection of STAT3 phosphorylation in luciferase reporter cell lines (Luminescence); b) Dose-dependent activation of LepR by leptin and high affinity agonist antibody candidates (H1-H7) via leptin-dependent cell proliferation of murine IL-3 dependent Ba/F3 pre-B cells (OD490, absorption at wavelength 490 nm); c) Yields of scFv antibodies in 293F freestyle expression system. Data are shown as means ± SEM (error bars).
Figure S4. Competitive ELISA assay for the binding of leptin and antibody against hlepR-ECD. Blocking of 20nM hLepR-Fc binding to coated leptin with different concentrations of leptin or antibody. Dose dependent competitive inhibition curve was fitted and IC50 of each ligand was calculated. All of the data are from three different experiments and shown as mean±SEM (error bars). N/A represents not applicable.
Figure S5. Pharmacokinetic studies and animal tissue samples analyses. a) A quick PK studies of leptin and antibody in wild type mice by intravenous injection. Leptin or antibody amounts were estimated at indicated time points based on Ba/F3 reporter cell proliferation assay; b) & c) Weight and weight ratio of adipose tissues in mice after leptin and H6 treatments, respectively; d) & e) Weight and weight ratio of livers in mice after leptin and H6 treatments, respectively. All of the data are shown as means ± SEM (error bars, n=8) by ANOVA, in which ns represents not significant. *P value <0.05, **P value < 0.01, ***P value < 0.001.
Figure S6. Antibody agonistic activity validation in wide type and db/db mice. a) & c) Daily body weight and food intake of treated wild type C57BL mice for two weeks; b) & d) Daily body weight and food intake of treated db/db mice. H6 group (n=5, 5.0 mg/kg, qod) and vehicle control group (n=5, 5.0 ml/kg, qod) contained 6 mice each. All of the data are shown as means ± SEM (error bars) by ANOVA, in which ns represents not significant. *P value <0.05, **P value < 0.01, ***P value < 0.001.
Table S1. Phage panning protocols for *hLepR*-focused combinatorial antibody libraries enrichment

| Panning method | Antigen                     | Elution             |
|----------------|-----------------------------|---------------------|
| POOL-1         | *hLepR*-ECD-Fc dimer        | glycine·HCl (pH2.0) |
| POOL-2         | *hLepR*-ECD-Fc dimer        | 1 mg/mL leptin      |
| POOL-3         | *hLepR*-ECD-6xHis monomer   | glycine·HCl (pH2.0) |
Table S2. Amino acid sequences of five truncated motifs of hLepR-ECD

| Motifs | Amino acid sequences |
|--------|----------------------|
| NTD    | FNLSYPITPWRFKLSCMPPNSTYDYLPLLPGSKNTSNSNGHYETAVEPKFNSSGTHFSNLSKTTFHCRCFRSEQDRNCSCLDADNIEKTFVSTVNSLVFQ |
| CRH1   | QIDANWNIQCWLKGDKLKFICYVESLFKNLRNFRNYKVHLYVLPEVLEDSPLOVPQKGSFQMVHCNCSVHECCCLVPVPTAKLNDTLMCLKITSQGVIFQSPLMVSQPINMVKPDPPLGLHMEITDDGNLKISWSSPLVPFPLQYQVVKYSENSTTVIREADKIVSATSLLVIDPSILPGSSSTVYQVRGKRLDGPGIWSWSTPRVFTTQDV |
| IgD    | IYFPKILTSVSNVSFHICYIYKKENKVIPSKEIVVWMNLAEKIPQSQYDVVSVDHSVKTFFNLNETKPRGKFTYDAVYCCNEHECHIRYAELV |
| CRH2   | IDVNI NISCETDGYLTKMTCRWSTSTIQSLAESTLQLRYHRSSLYCSDIPSIHPISEPKDCYQLSDGFYECIFQPIFILSSGYTMWIRNHLSLSLDSPPTCVLPDSVVKLPSSVKAETINIGFLKISWEKPVPENLQFQIRYGLSQKEVQWKMZEVEYDAKSKVSLPVDPDLCAVAYAVQVRCKRLDGPGIWSNWSNPDAYTVMD |
| FNIII  | IKVPMRGPEFRIINGDMKKEKNTLLWKPLMKNDLSLCVQRYVINHHTSCNGTWSEDVGNHTKFIFLMTQLWQAHTVLAINSIGASYVANFNLTWSPMKTVNIVQSLSAYLNSSCIVSVWILSPDYKLMYFIIEWKLNEDGEIKWLRISSSVKKYYIHDFIFIEKYQUFSLYPIFMESVGGPKIINSFTQDDIEKHQSD |