nature portfolio

https://doi.org/10.1038/s41586-024-08207-0

Supplementary information

NK2R control of energy expenditure and feeding to treat metabolic diseases

In the format provided by the authors and unedited

Extended figure legends

Figure 1. NK2R agonism is genetically and functionally linked to cardiometabolic protection.

For **e** the line represents the median, the edges of the box are Q1 and Q3, respectively, the Whiskers are the largest and the smallest values within the border for outliers, which is 1.5xIQR. Ordinary one-way ANOVA with Dunnett's post hoc test, significance shows post hoc test, **e, r**, for **r** vehicle vs treatment; unpaired two-tailed *t*-test of AUC from first injection, **h, o**; repeated measures two-way ANOVA with Geisser-Greenhouse correction, significance indicates treatment effect, **i, j, l**; unpaired two-tailed t-test, **k**; repeated measures two-way ANOVA with Geisser-Greenhouse correction, significance indicate time and treatment effect, **p**; repeated measures two-way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparisons test, significance shows post hoc test between treatments on last day, **q**.

Figure 2. Development and characterization of first-in-class selective, long-acting NK2R agonists.

Unpaired two-tailed *t*-test of AUC for 48 hours after injection, **f-j, m-q**; unpaired two-tailed *t*-test, **k, r, w**; repeated measures two-way ANOVA with Geisser-Greenhouse correction, significance shows treatment effect, **s, t, v**; ordinary one-way ANOVA with Tukey's post hoc test, significance shows post hoc test for treatment effects, **s** (AUC graph).

Figure 3. Improvement of systemic energy homeostasis by long-acting NK2R agonism

Repeated measures two-way ANOVA with Geisser-Greenhouse correction, significance indicates treatment effect, **a, b, i, j, u, v**; repeated measures two-way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparisons test, significance shows post hoc test at last time point between treatments, **c, l**; repeated measures two-way ANOVA with Sidak's multiple comparisons test, significance shows post hoc test between treatments, **d-g**; unpaired two-tailed *t*-test, **h, k**; ordinary one-way ANOVA with Tukey's multiple comparison test, significance indicates post hoc test, **n**; repeated measures two-way ANOVA with Sidak's multiple comparison test, significance shows

post hoc test for time effects within groups, **o**; ordinary one-way ANOVA with Tukey's post hoc test of AUC for 48 hours after injection, significance indicates post hoc test for treatment effects, **p-r, t**; one-way ANCOVA with Bonferroni multiple comparisons test, significance indicates post hoc test, **s**.

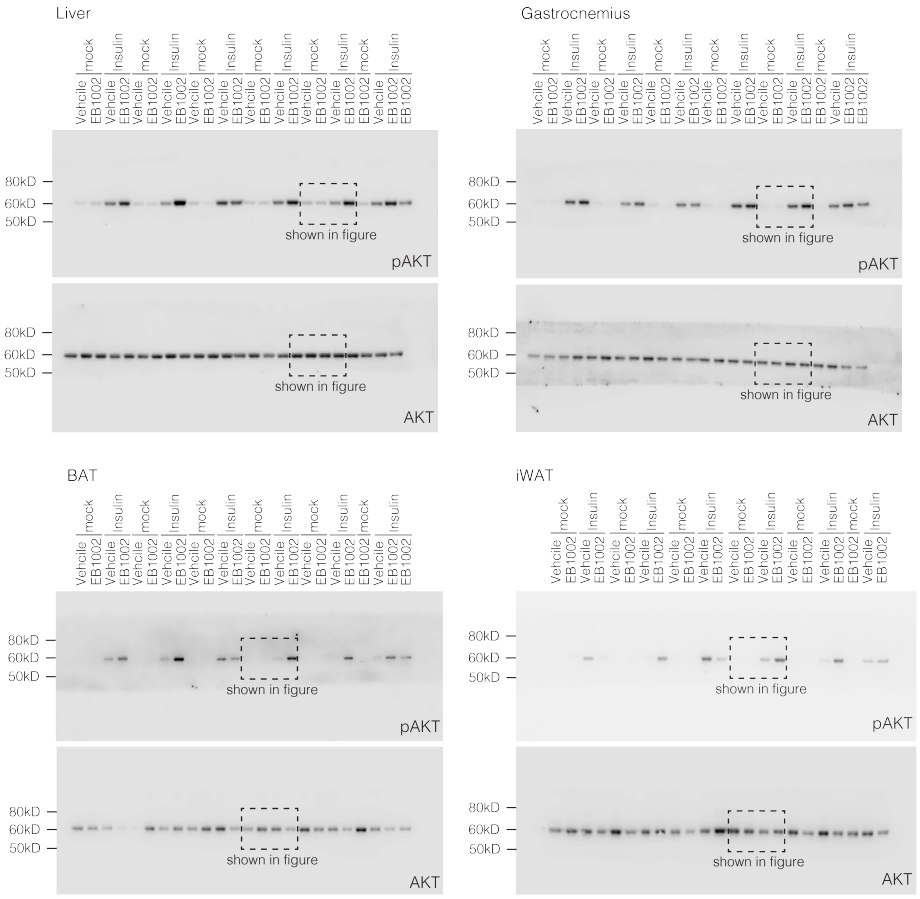
Figure 4. Distinct central and peripheral actions of long-acting NK2R agonism.

DVC - dorsal vagal complex, NTS - nucleus of the solitary tract, AP - area postrema, DMV - dorsal motor nucleus of the vagus, IEG - immediate early genes, AI - agranular insular area, VISC - visceral area, BST - bed nuclei of the stria terminalis, SI - substantia innominate, ACB - nucleus accumbens, STRd - striatum dorsal region, CEA - central amygdalar nucleus, SNc - substantia nigra, compact part, VTA - ventral tegmental area, DR - dorsal nucleus raphe, PAG - periaqueductal gray, LHA - lateral hypothalamic area, PSTN - parasubthalamic nucleus, TU - tuberal nucleus, ZI - zona incerta, PVT - paraventricular nucleus of the thalamus, VPMpc - ventral posteromedial nucleus of the thalamus, parvicellular part, RPA - nucleus raphe pallidus. Unpaired two-tailed *t*-test of AUC for 48 hours after injection, **b, c**; unpaired two-tailed *t*-test, **d, g, i**; repeated measures two-way ANOVA with Sidak's multiple comparison test, significance shows post hoc test for time effects within groups, **e**; ordinary one-way ANOVA with Tukey's multiple comparison test, significance shows post hoc test, **h, o**; repeated measures two-way ANOVA with Geisser-Greenhouse correction, significance indicates treatment effect at last time point, **n**; multiple unpaired t-test with two-stage lineat step-up procedure of Bejamini, Krieger and Yekutieli adjusted P value (FDR < 0.05), **q, r**. For **l, q**: red colour indicates *P < 0.05, for **r**: all shown brain regions are statically different between vehicle and EB1002 (P < 0.05).

Figure 5. NK2R agonism safely counteracts cardiometabolic disease in diabetic, obese nonhuman primates.

Repeated measures one-way ANOVA with Geisser-Greenhouse correction, **b-d, l**; repeated measures two-way ANOVA with Geisser-Greenhouse correction, **f-k, n, o**.

Scans for Extended Data Figures 2s-v



Scans for Extended Data Figure 3y