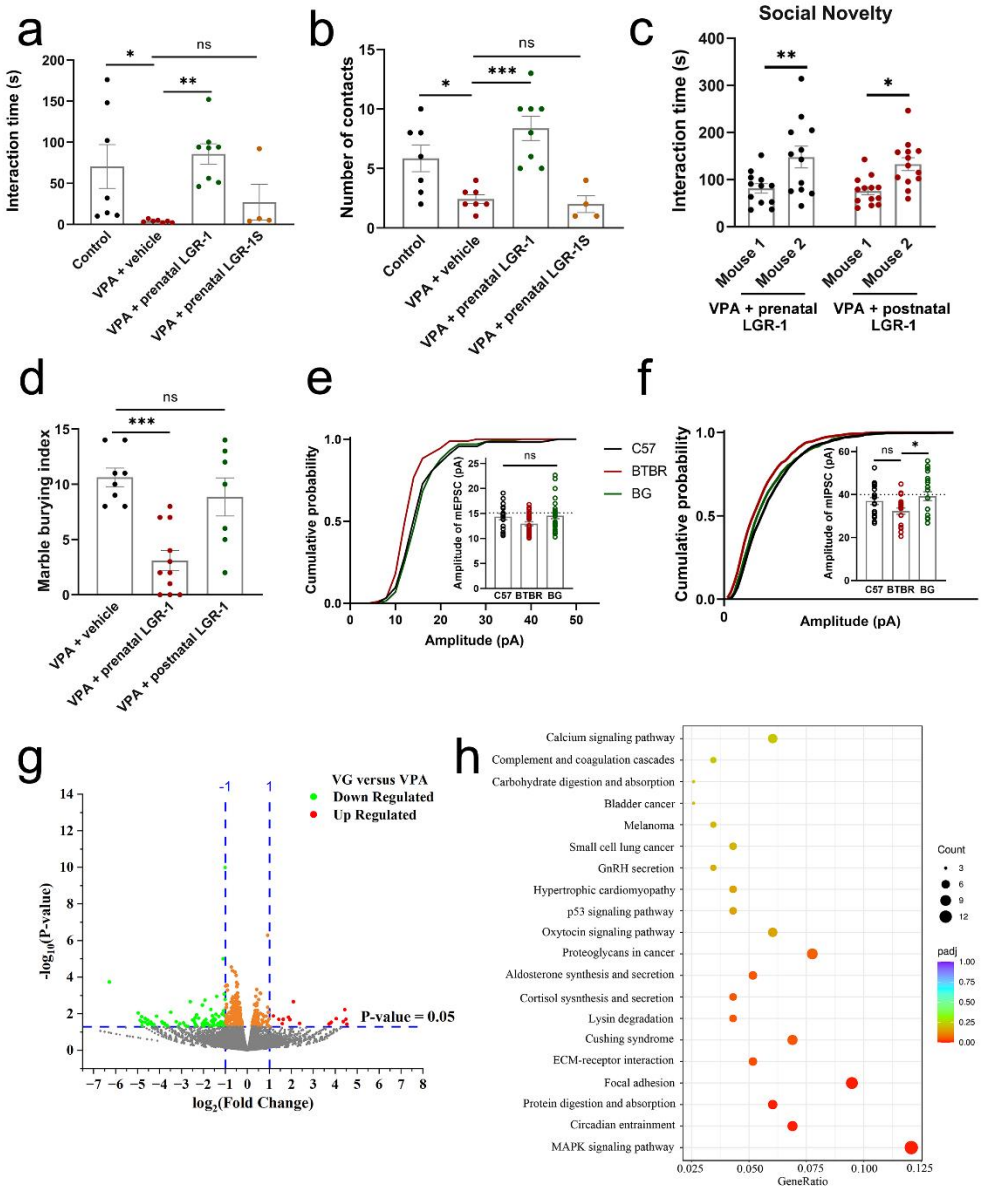
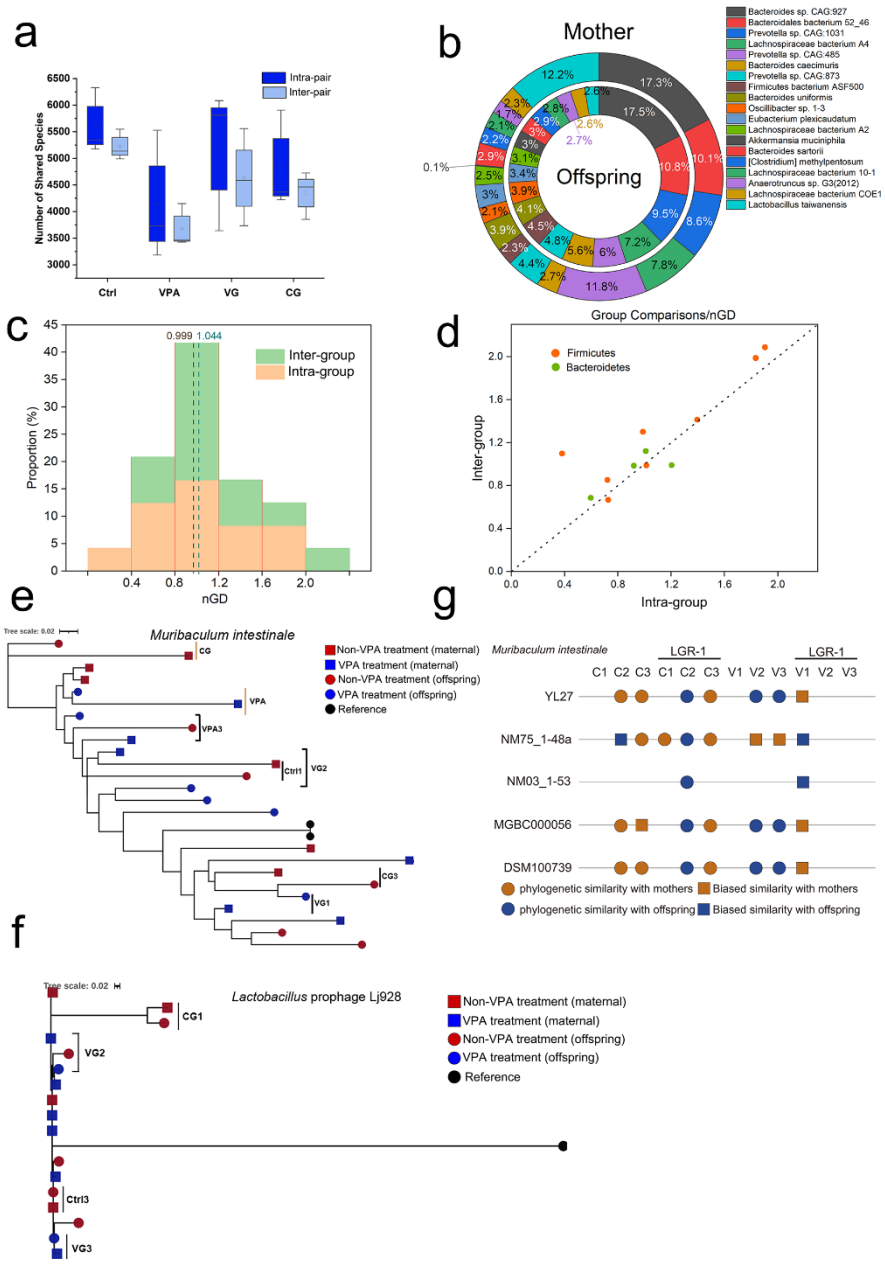
Fig. S1



Supplementary Fig. 1 LGR-1 prenatally attenuates the autism-like aberrations. (**a** and **b**) Reciprocal social interaction of the tested dyad, as presented by the duration (**a**) and number (**b**) of their interactions during 10-min test (*n* = 4-8). **c** Social behavior as assessed in 3-chamber test (*n* = 12-13). Postnatal treatment of LGR-1 lasted from weaning till PNW8. **d** Repetitive behavior as assessed in marble burying test (*n* = 7-11). (**e** and **f**) Amplitude of mEPSC (**e**) and mIPSC (**f**) in neurons residing in medial prefrontal cortex (*n* = 17-28). C57, C57BL/6J; BG, BTBR mice administered with LGR-1 during gestation. **g** Volcano plots depicting the

differentially-expressed genes in response to LGR-1 (n = 3). VPA, VPA injection at E12.5; VG, VPA mice administered with LGR-1 during gestation. **h** KEGG pathways suppressed by LGR-1 treatment relative to VPA group. All data are given as mean ± SEM. Statistical significance was determined by one-way ANOVA or paired t test (**c**). ns, $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

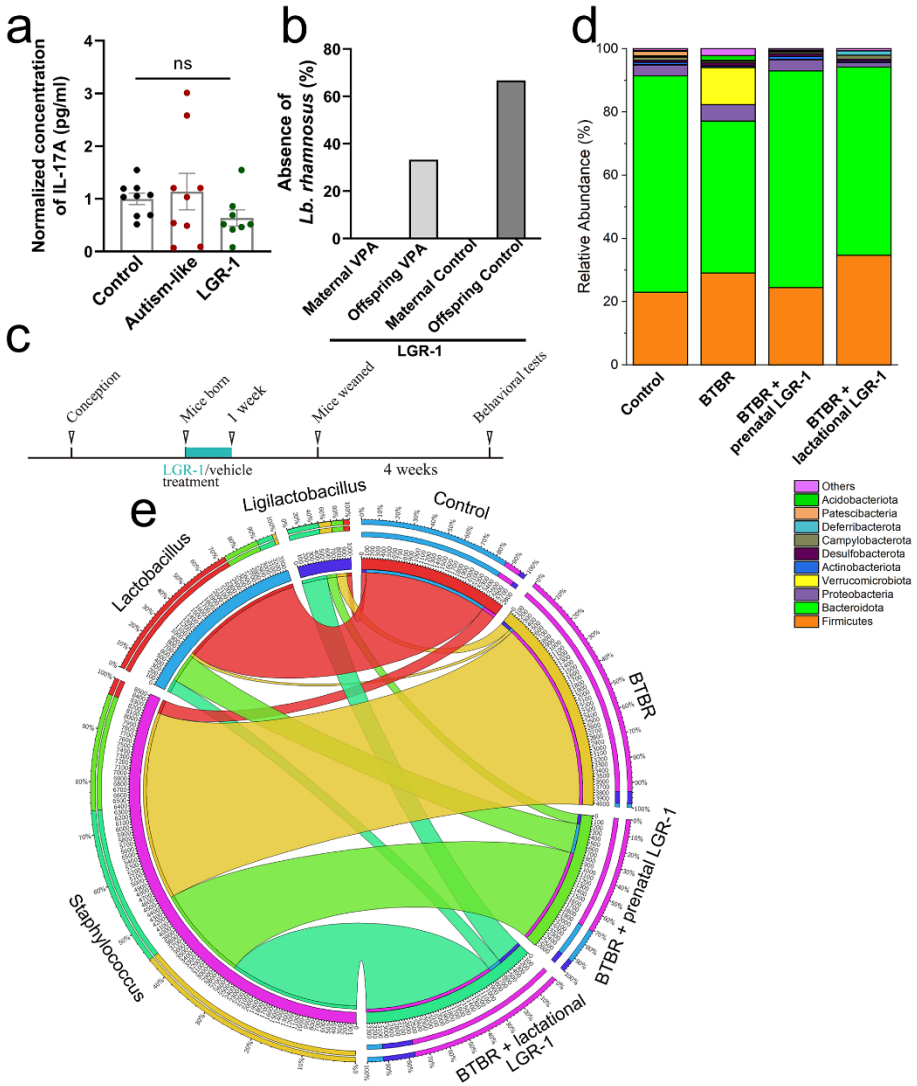
Fig. S2



Supplementary Fig. 2 Microbiota transmission occurs between dams and offspring. a Number of shared species between dams and their offspring (*n* = 3 mother-infant pair for each group). Ctrl, untreated mice; VPA, VPA injection at E12.5; VG, VPA mice orally gavaged with LGR-1 during gestation; CG, control mice orally gavaged with LGR-1. **b** The top 19 species and their proportions in dams and their offspring. **c** Histograms depicting the normalized genetic distances (nGDs) from the

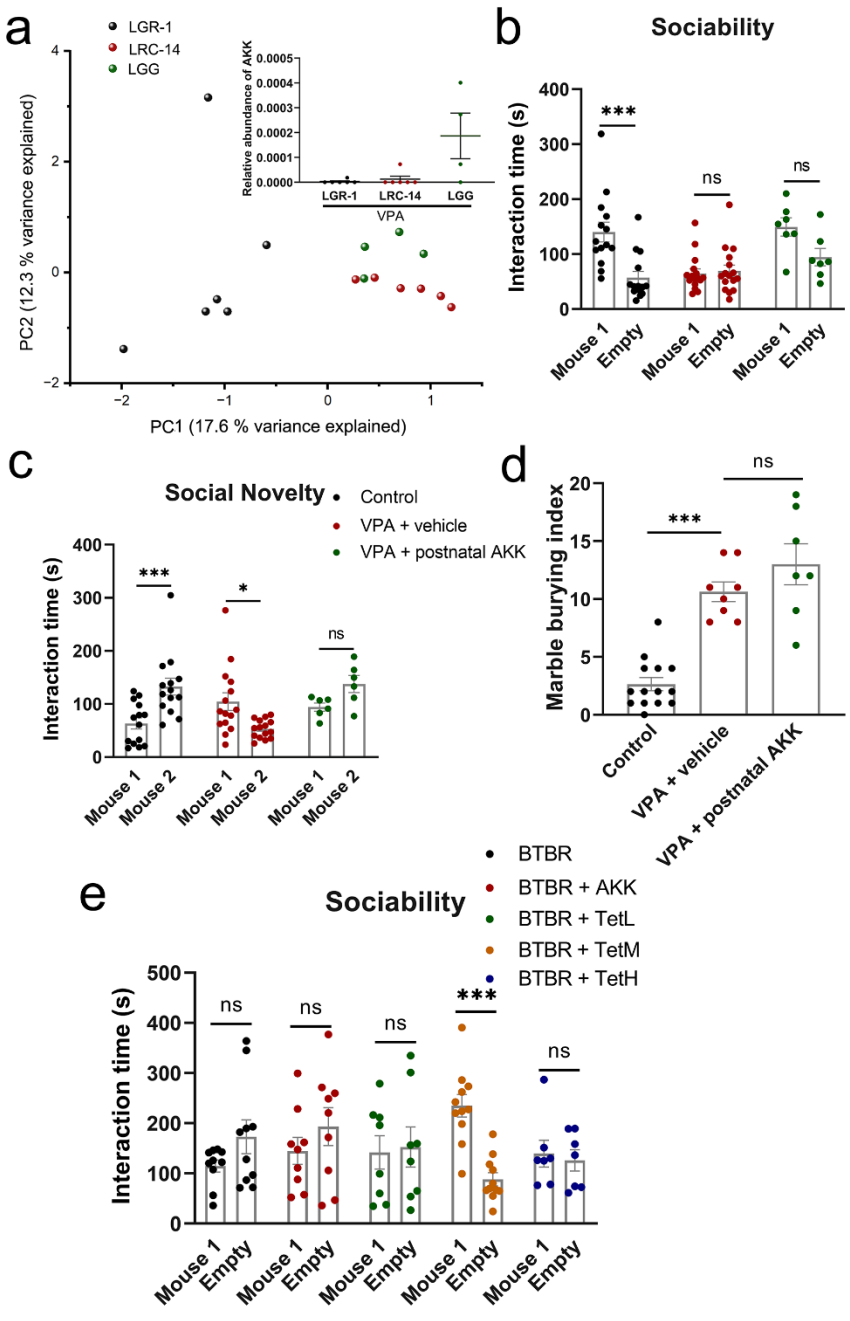
microbes recovered. The average nGDs of intra-group and inter-group were indicated by the respective dashed lines. **d** Scatter plots showing the nGDs from the microbes recovered. Taxa are colored by phylum (Firmicutes, red; Bacteroidetes, green). **e** Maximum likelihood phylogenetic tree of *Muribaculum intestinale*. Nodes are colored by group (red, non-VPA treatment; blue, VPA treatment), and shaped by generation (rectangle, dam; circle, offspring). Mother-infant pairs with adjacent microbial similarity were indicated by black vertical line, and non-mother-infant pairs within the same treatment group were indicated by yellow vertical line. **f** Maximum likelihood phylogenetic tree of *Lactobacillus* prophage Lj928. **g** Map of the distribution of *Muribaculum intestinale* strains in the respective groups. Circle refers to the presence in both dams and offspring but with a preferred enrichment (yellow, mother; blue, offspring), and rectangle refers to the sole presence in either mother (yellow) or offspring (blue). The cutoff value is set as 0.15 of all genetic distances calculated.

Fig. S3



Supplementary Fig. 3 LGR-1 does not act by altering maternal IL-17A or transmit to offspring *per se*. **a** Maternal IL-17A levels in response to LGR-1 treatment (n = 8-9). **b** Percentage of absence of *Lacticaseibacillus rhamnosus* residing in both mothers and offspring (n = 3 mother-infant pair for each group). **c** Schematic of LGR-1 treatment during the first week of lactation (n = 5-8). **d** Relative abundance of microbial taxa on phylum level. The top ten most-enriched taxa were presented. **e** Circos plot describing the composition of the top 5 most-enriched genera in various treatment groups. All data are given as mean ± SEM or single percentage data for (**b**). Statistical significance was determined by one-way ANOVA. ns, $P > 0.05$.

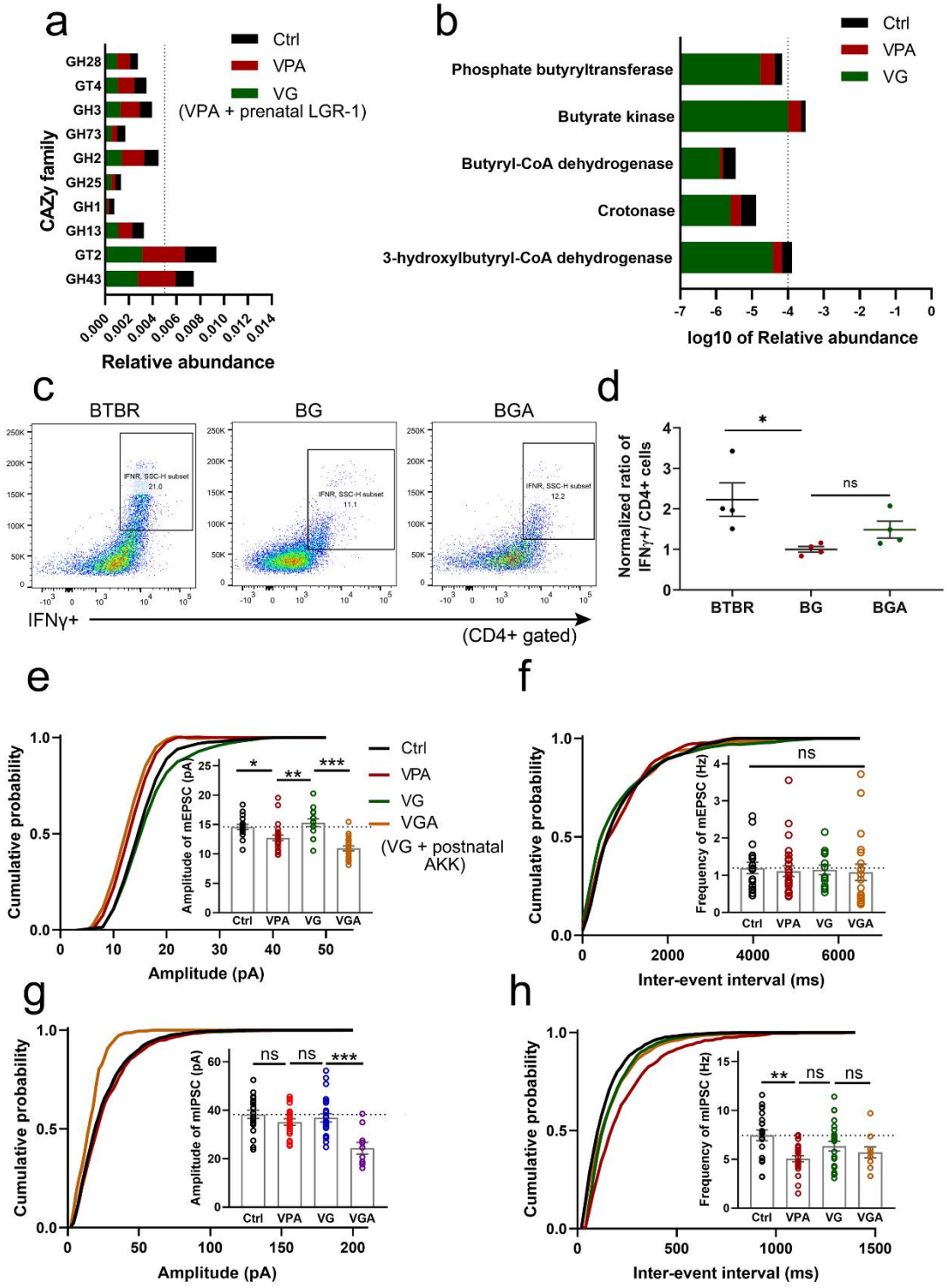
Fig. S4



Supplementary Fig. 4 AKK is involved in the modulation of autism-like behaviors. a PCA analysis for intestinal microbiome of offspring exposed by different strains prenatally (*n* = 4-6). The impact on the AKK level was indicated in the insert. (**b** and **c**) Sociability (**b**) and social novelty (**c**) of mice postnatally fed with AKK as assessed in 3-chamber test (*n* = 6-16). AKK, *Akkermansia muciniphila*. **d** Repetitive behavior as assessed by marble burying test (*n* = 7-14). **e** Sociability of mice subjected to tetracycline treatment under varying doses. Tet, tetracycline; L, low

concentration (1.5 g/l); M, medium concentration (3 g/l); H, high concentration (10 g/l). All data are given as mean ± SEM. Statistical significance was determined by one-way ANOVA (**d**) or two-way ANOVA with post hoc multiple comparisons. ns, $P > 0.05$, $^{*}P < 0.05$ and $^{***}P < 0.001$.

Fig. S5



Supplementary Fig. 5 Gut-brain axis is implicated in the microbe-based intervention. a Stacked chart depicting the distribution of top 10 CAZy (carbohydrate-active enzyme) families. Ctrl, untreated group; V/VPA, VPA treated group; G, LGR-1 treated group prenatally. **b** Stacked chart depicting the distribution

of enzymes related to butyrate synthesis. (**c** and **d**) Flow cytometric analysis of systemic immunity in spleens of 8-week-old male mice (n = 4 mice per group). Representative pseudocolor (**c**) plots of splenic IFNγ-expressing cells were shown. The frequencies (**d**) of $IFN\gamma^+$-expressing cells in the $CD4^+$ population were then normalized against the BTBR mice pretreated with LGR-1 (BG group) and shown in statistical plots. (**e** and **f**) Amplitude (**e**) and frequency (**f**) of mEPSC in neurons residing in medial prefrontal cortex (n = 14-25). A, the postnatal AKK-treated pups prenatally exposed by LGR-1. (**g** and **h**) Amplitude (**g**) and frequency (**h**) of mIPSC in neurons residing in medial prefrontal cortex (n = 10-24). All data are calculated as mean ± SEM and derived. Statistical significance was determined by one-way ANOVA. ns, $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Supplementary Video 1 Representative video clips recording the movement of a VPA-exposed mouse in the second session (sociability) of three-chamber test.

Supplementary Video 2 Representative video clips recording the movement of a VPA-exposed mouse in the third session (social novelty) of three-chamber test.

Supplementary Video 3 Representative video clips recording the movement of an LGR-1-pretreated deficient mouse in the second session of three-chamber test.

Supplementary Video 4 Representative video clips recording the movement of an LGR-1-pretreated deficient mouse in the third session of three-chamber test.