18501	AGGACCCTGA TATATA	AGGGT CAAGATAAT	T TACATGGCT	G GCCTAAATGA	AATTGACTTA	ATAATTGGTA	AAACAAGTTA	ATCACTTGTG	CATTTCTCTT
	TCCTGGGACT ATATAT	ICCCA GTTCTATTA	A ATGTACCGA	C CGGATTTACT	TTAACTGAAT	TATTAACCAT	TTTGTTCAAT	TAGTGAACAC	GTAAAGAGAA
+1	Ser Ser Arg Gin I	Leu Ala Ser Arg F	ne His Glu Gln	Phe lle Val A	rg Glu Asp Leu	Met Gly Leu -	Ala lle Gily Th	r His Gly Ala	Asn lle Gin Gin-
18601	CAGAGTTCAA GGCAGO	CTTGC CTCAAGATT	T CATGAACAG	I TTATEGTAEG	AGAAGATCTG	ATGGGTCTAG	CTATTGGTAC	TCATGGTGCT	AATATTCAGC
	GTCTCAAGTT CCGTCG	GAACG GAGTTCTAJ	a <mark>gtacttgt</mark> ei	A AATAGCATGC	TCTTCTAGAC	TACCCAGATC	GATAACCATG	AGTACCACGA	TTATAAGTCG
+1	Gin Ala Arg Lys Val Pr	ro Giy Val Thr Ala	lle Asp Leu A	Asp Glu Asp Thr	Cys Thr Phe	His lle Tyr Gi	y Giu		
18701	AAGCTAGAAA AGTACO	CTGGG GTCACTGCT	A TTGATTTAG	A TGAGGATACC	TGCACATTTC	ATATTTATGG	AGAG GTAAAT	ATTTCACTAT	AAAATCCAAT
	TTCGATCTTT TCATGO	GACCC CAGTGACG	T AACTAAATC	r actectatge	ACGTGTAAAG	TATAAATACC	TCTCCATTTA	TAAAGTGATA	TTTTAGGTTA
18801	TGCCGCCCCC TCAGAG	БААБС АААТАААС <i>І</i>	G TATTTCAAA?	r gccaattttt	TTTTGCCTTG	ATACTACACC	CACTCACCCT	TCTTTATTAG	GATCAAGATG
	ACGGCGGGGGG AGTCTC	CTTCG TTTATTTGI	C ATAAAGTTTI	A CGGTTAAAAA	AAAACGGAAC	TATGATGTGG	GTGAGTGGGA	AGAAATAATC	CTAGTTCTAC
18901	CAGTCAAAAA GGCTAG	GAAGC TTTCTGGA	T TTGCTGAAG	A TGTCATACAA	GTTCCACGAA	ATTTAGTAGG	TAAGTCAAAA	ATAACTATTG	ATATATACCA
	GTCAGTTTTT CCGATC	CTTCG AAAGACCTT	A AACGACTTC	Г АСАБТАТБТТ	CAAGGTGCTT	TAAATCATCC	ATTCAGTTTT	TATTGATAAC	TATATATGGT

b

18501	AGGACCCTGA TATATAGGGT CAAGATAATT TACATGGCTG GCCTAAATGA AATTGACTTA ATAATTGGTA AAACAAGTTA ATCACTTGTG CATTTCCTT TCCTGGGACT ATATATCCCA GTTCTATTAA ATGTACCGAC CGGATTTACT TTAACTGAAT TATTAACCAT TTTGTTCAAT TAGTGAACAC GTAAAGAGA.	Г À
+1	Ser Ser Arg Gin Leu Ala Ser Arg Phe His Giu Gin Phe Ile Val Arg Giu Asp Leu Met Giy Leu Ala Ile Giy Thr His Giy Ala Asn Ile Gin (Gin:
18601	CAGAGTTCAA GECAGETTEE CTCAAGATTT CATGAACAGT TTATEGTACG AGAAGATETE ATGGETETAG CTATTEGTAE TCATGETEET AATATTCAG	ĉ
	GTCTCAAGTT CCGTCGAACG GAGTTCTAAA GTACTTGTCA AATAGCATGC TCTT <mark>CTAGAC TACCCAGATC GATAACCATG AGTACCACGA TTATAAGTC</mark>	5
+1	-Gin Ala Arg Lys Val Pro Giy Val Thr Ala lle Asp Leu Asp Giu Asp Thr Cys Thr Phe His lle Tyr Giy Giu	
18701	AAGCTAGAAA AGTACCTGGG GTCACTGCTA TTGATTTAGA TGAGGATACC TGCACATTTC ATATTTATGG AGAGGTAAAT ATTTCACTAT AAAATCCAA'	Г
	TTCGATCTTT TCATGGACCC CAGTGACGAT AACTAAATCT ACTCCTATGG ACGTGTAAAG TATAAATACC TCTCCATTTA TAAAGTGATA TTTTAGGTT.	A
18801	TGCCGCCCCC TCAGAGAAGC AAATAAACAG TATTTCAAAT GCCAATTTTT TTTTGCCTTG ATACTACACC CACTCACCCT TCTTTATTAG GATCAAGATG	3
	ACGGCGGGGG AGTCTCTTCG TTTATTTGTC ATAAAGTTTA CGGTTAAAAA AAAACGGAAC TATGATGTGG GTGAGTGGGA AGAAATAATC CTAGTTCTAC	5
18901	CAGTCAAAAA GGCTAGAAGC TTTCTGGAAT TTGCTGAAGA TGTCATACAA GTTCCACGAA ATTTAGTAGG TAAGTCAAAAA ATAACTATTG ATATATACC.	A
	GTCAGTTTTT CCGATCTTCG AAAGACCTTA AACGACTTCT ACAGTATGTT CAAGGTGCTT TAAATCATCC ATTCAGTTTT TATTGATAAC TATATAGG	Г

Supplementary Figure 1 Sequence comparison at the *Fmr1* locus. a, Wildtype: green highlight indicates exon 8; yellow highlight with red text indicates ZFN target site within exon 8; pink highlight indicates exon 9. b, *Fmr1* KO: a 122bp deletion of the intron 7/exon8 junction occurred at 18533bp-18654bp, indicated by blue highlight. Amino acid sequence above the sequence shows the reading frame in the wild type gene. NCBI Gene ID: 24948, RGSC 3.4 assembly.

a	
+2	lle Arg Asp Ser Gly Ala Lys Pro Val Met Val Tyr Ile His Gly Gly SerTyr
9701	GGAAGGGCAT CCTACCTAGC TTGTGTCTCA CCCCTTCTCC TTACAGACAT CCGAGACAGT GGTGCTAAAC CTGTCATGGT CTACATCCAC GGAGGCTCTT CCTTCCCGTA GGATGGATCG AACACAGAGT GGGGAAGAGG AATGTCTGTA GGCTCTGTCA CCACGATTTG GACAGTACCA GATGTAGGTG CCTCCGAGAA
+2	-TyrMet Gilu Gily Thr Gily Asn Met lle Asp Gily Ser Val Leu Ala Ser Tyr Gily Asn Val lle Val lle Thr Leu Asn Tyr Arg Val Gily Val Leu
9801	ACATGGAAGG AACAGGCAAC ATGATTGACG GCAGGGTTCT TGCAAGTTAT GGCAACGTCA TCGTCATCAC ACTCAACTAC CGGGTCGGGG TGCTAGGTAT TGTACCTTCC TTGTCCGTTG TACTAACTGC CGTCGCAAGA ACGTTCAATA CCGTTGCAGT AGCAGTAGTG TGAGGTTGATG GCCCAGCCCC ACGATCCATA
9901	$ \begin{array}{c} \texttt{GGTTCTCTGC} \texttt{CAGGTGCCTA} \texttt{GGAGGAAGGC} \texttt{TG}\texttt{GCTTTGAA} \texttt{AGGGGGAGGA} \texttt{AGGAATCCCA} \texttt{GGGAAGTCAA} \texttt{CTAGGCAGT} \texttt{CCTAGTTCTT} \texttt{TAGTTGCTGT} \\ \texttt{CCAAGGAGCG} \texttt{GTCCACGGAT} \texttt{CCTCCTTCCG} \texttt{ACCGAAACTT} \texttt{TCCCCCTCCT} \texttt{TCCTTAGGGT} \texttt{CCTTCAGTT} \texttt{GAGTCCGTCA} \texttt{GGATCAAGAA} \texttt{ATCAACGACA} \\ \end{array} $
b	
+2	lle Arg Asp Ser Gly Ala Lys Pro Val Met Val Tyr Ile His Gly Gly Ser Tyr-
9701	GGAAGGGCAT CCTACCTAGC TTGTGTCTCA CCCCTTCTCC TTACAGACAT CCGAGACAGT GGTGCTAAAC CTGTCATGGT CTACATCCAC GGAGGCTCTT CCTTCCCGTA GGATGGATCG AACACAGAGT GGGGAAGAGG AATGTCTGTA GGCTCTGTCA CCACGATTTG GACAGTACCA GATGTAGGTG CCTCCGAGAA
+2	-TyrMet Glu Gly Thr Gly Asn Met lle Asp Gly Ser Val Leu Ala Ser Tyr Gly Asn Val lle Val lle Thr Leu Asn Tyr Arg Val Gly Val Leu
9801	ACATGGAAGG AACAGGCAAC ATGATTGACG GCAGCGTTCT TGCAAGTTAT GGCAACGTC <mark>A TCGTCATCAC ACT</mark> CAACTAC CGGGTCGGGG TGCTAGGTAT TGTACCTTCC TTGTCCGTTG TACTAACTGC CGTCGCAAGA ACGTTCAATA CCGTTGCAC <mark>T AGCAGTAGTG TGA</mark> GTTGATG GCCCAGCCCC ACGATCCATA
9901	GGTTCTCTGC CAGGTGCCTA GGAGGAAGGC T <mark>O</mark> GCTTTGAA AGGGGGAGGA AGGAATCCCA GGGAAGTCAA CTCAGGCAGT CCTAGTTCTT TAGTTGCTGT CCAAGAGACG GTCCACGGAT CCTCCTTCCG A <mark>C</mark> CGAAACTT TCCCCCTCCT TCCTTAGGGT CCCTTCAGTT GAGTCCGTCA GGATCAAGAA ATCAACGACA

Supplementary Figure 2 Sequence comparison at the *Nlgn3* locus. a,Wildtype: green highlight indicates exon 5; yellow highlight with red text indicates ZFN target site within exon 5. b, *Nlgn3* KO: a 58bp deletion of the exon5/intron 5 junction occurred at 9874bp-9931bp, indicated by blue highlight. Amino acid sequence above the sequence shows the reading frame in the wild type gene. NCBI Gene ID: 171297, RGSC 3.4 assembly.

a

	tail pull	nape contact	wrestle	box	chase	other contact	sniff/follow	climb	pins
Fmr1 KOs	0.50±0.33	10.38±2.39	5.38±1.65	1.13±0.30	2.00±1.03	16.63±1.89	20.88±2.91	4.25±0.59	3.00±1.23
<i>Fmr1</i> WT littermates	1.22±0.36	18.89±2.88	12.78±3.31	2.33±0.58	3.78±0.88	22.11±1.99	19.22±3.05	3.22±0.62	9.78±3.17
Nlgn3 KOs	0.50±0.31	14.50±3.79	3.20±0.99	1.30±0.52	0.50±0.27	23.00±2.10	9.00±1.24	1.80±0.76	$0.60{\pm}0.40$
<i>Nlgn3</i> WT littermates	1.36±0.65	15.55±2.00	8.91±1.99	3.00±0.52	0.73±0.30	26.09±2.36	8.82±1.64	1.45±0.62	6.45±1.56

Supplementary Table 1| Frequency of direct social interaction behaviors

NON-social odor tests

Social odor tests



Supplementary Figure 3| Olfaction control for *Fmr1* KO rats. a, *Fmr1* KOs and WT littermates spent significantly greater time at odorant holes [(time at odorant holes/time at any hole) x 100%] than chance (3/16=18.75%, represented by dashed line) for non-social and social odor tests and did not differ between genotypes for non-social (*P*=0.980) or social (*P*=0.508) odor. b, *Fmr1* KOs did not differ in latency to sniff odorant hole for non-social (*P*=0.366) or social (*P*=0.075) cues. c, All rats spent more time at the novel odorant hole than familiar odorant hole for the non-social (WT: *P*<0.001, *Fmr1* KO: *P*=0.001) and social (WT: *P*=0.002, *Fmr1* KO: *P*=0.004) test.

NON-social odor tests

Social odor tests



Supplementary Figure 4 Olfaction control for Nlgn3 KO rats. a, *Nlgn3* KOs spend significantly greater time at odorant holes [(time at odorant holes/time at any hole) x 100%] than chance (3/16=18.75%, *represented by dashed line*) for non-social (P=0.211) or social (P=0.397) odor tests. b, *Nlgn3* KOs did not differ in latency to sniff odorant hole for non-social (P=0.787) or social (P=0.189) cues. c, All rats spent more time at the novel odorant hole than familiar odorant hole for the non-social (WT: P=0.045, *Nlgn3* KO: P=0.001) and social (WT: P=0.003, *Nlgn3* KO: P=0.004) test.



Supplementary Figure 5| **Exemplary polysufone chewing by** *Nlgn3* **KO rats.** *Nlgn3* heterozygous females chewed all the way through the plastic polysufone water bottle in a 3 day period (upper left). Juvenile *Nlgn3* KO males showed initial evidences of plastic chewing in a single overnight period (lower left).



Supplementary Figure 6 | *Fmr1* KO and *Nlgn3* KO rats have altered sensorimotor gating behavior and *Nlgn3* KO rats exhibit decreased anxiety. **a**, Total distance traveled in an automated open field activity assay. **b**, Duration that rats spent in the open or closed portions of an elevated zero maze. **c**, Number of head dips over the side of the open sections of the elevated zero maze. **d**, Mean amplitude (arbitrary units) of the startle response to an auditory stimulus. **e**, Prepulse of 4, 8, or 12 dB above background preceded the startle-eliciting stimulus, mean inhibition of startle for each prepulse is shown. **f**, Percent of time freezing when exposed to a previously shock-paired context and shock-paired cue. All data are mean \pm s.e.m., **P*≤0.05, ***P*≤0.01