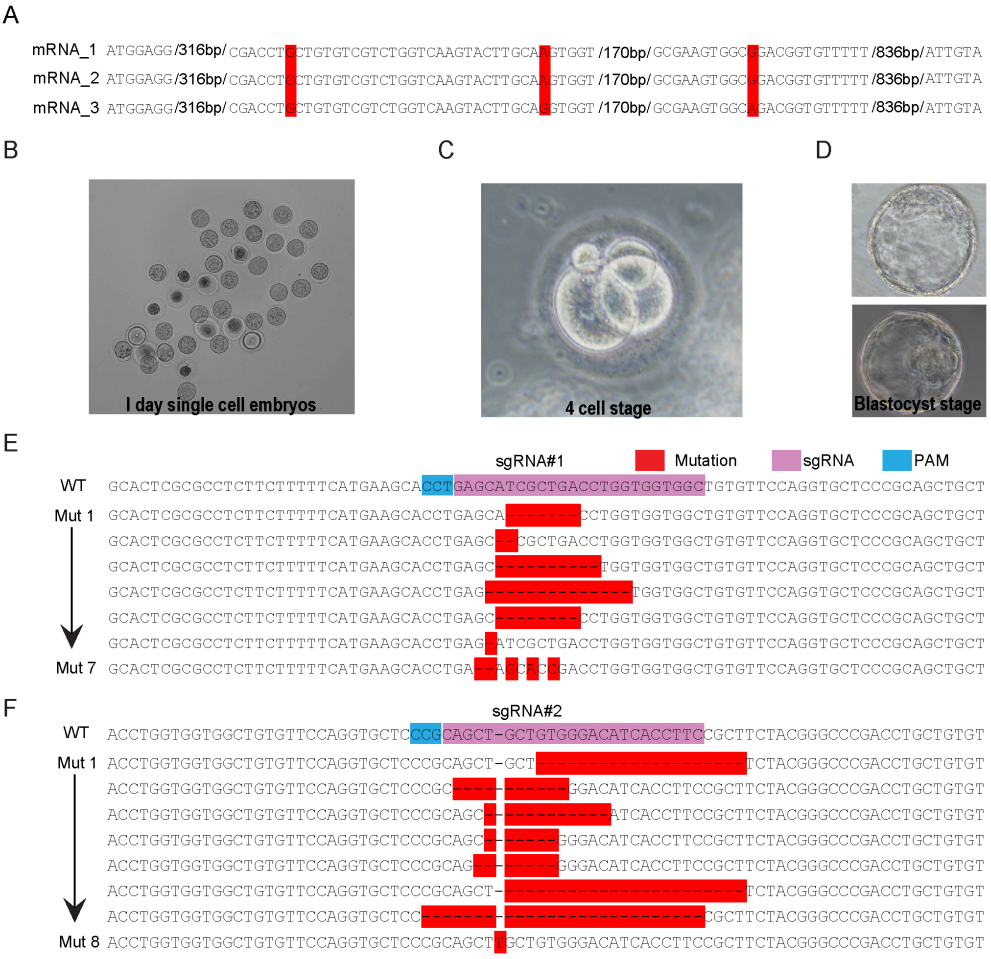

Figure S1. CRISPR mutagenesis and culture of vole embryos; related to Figure 1

A. 3 naturally occurring synonymous substitutions in *Oxtr* exon1 from 4 WT voles.

B. Embryos harvested from female voles prior to pronuclear injection.

C. Embryo at the 4 cell stage 1 day after ribonucleoprotein complex injection.

D. Blastocysts grown in vitro, 4 days after injection.

E. Sequences from individual blastocysts after injection with sgRNA#1.

F. Sequences from individual blastocysts after injection with sgRNA#2.

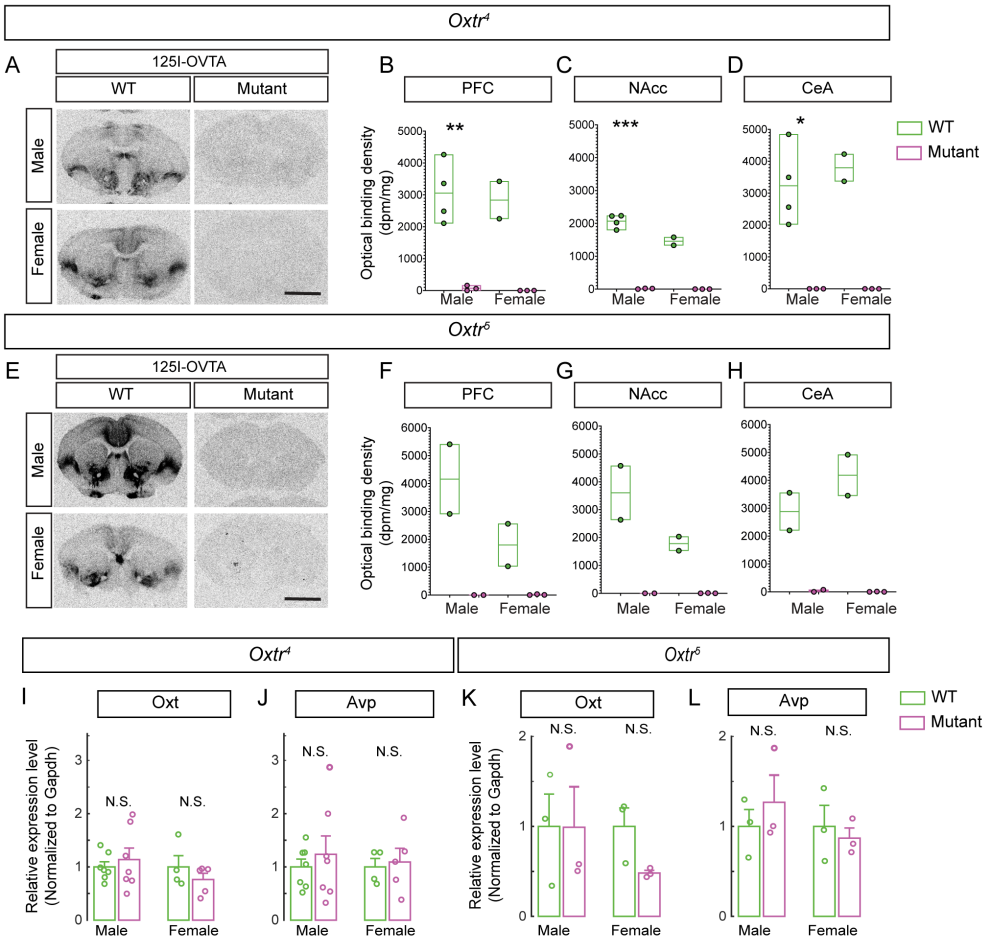

Figure S2. *Oxtr*^{4} and *Oxtr*^{5} translation products do not bind ligand and do not result in changes in transcription of Oxt or Avp; related to Figure 2

A,E. Loss of binding with the competitive agonist 125I-OVTA visualized in coronal sections through the rostral telencephalon of *Oxtr*$^{4-/-}$ (A) and *Oxtr*$^{5-/-}$ (E) voles.

B-D, F-H. Optical density based quantification of binding to 125I-OVTA shows that binding in *Oxtr*$^{4-/-}$ and *Oxtr*$^{5-/-}$ voles is essentially undetectable in PFC (B ,F), NAcc (C, G), and CeA (D, H). Scale bar =5 mm, boxplot depicts max-min, midline denotes mean; n = 4 WT and 3 mutant males, 2 WT and 3 mutant females (B, C, D), 2 WT and mutant males each, 2 WT and 3 mutant females (F, G, H); *p<0.05, **p<0.01, ***p<0.001.

I, J. No compensatory increase in Oxt (I) or Avp (J) mRNA levels in paraventricular nucleus (PVN) of *Oxtr*$^{4-/-}$ voles.

K, L. No compensatory increase in Oxt (K) or Avp (L) mRNA levels in PVN of Oxtr$^{5-/-}$ voles.

Mean ± SEM; n = 7 WT and mutant males each, 4 WT and 5 mutant females (I), 7 WT and 6 mutant males, 4 WT and 5 mutant females (J), 8 WT and mutant males each (2-3 animals were pooled for each point) (K), 9 WT and mutant females each (3 animals were pooled for each point) (L); N.S., not significant.

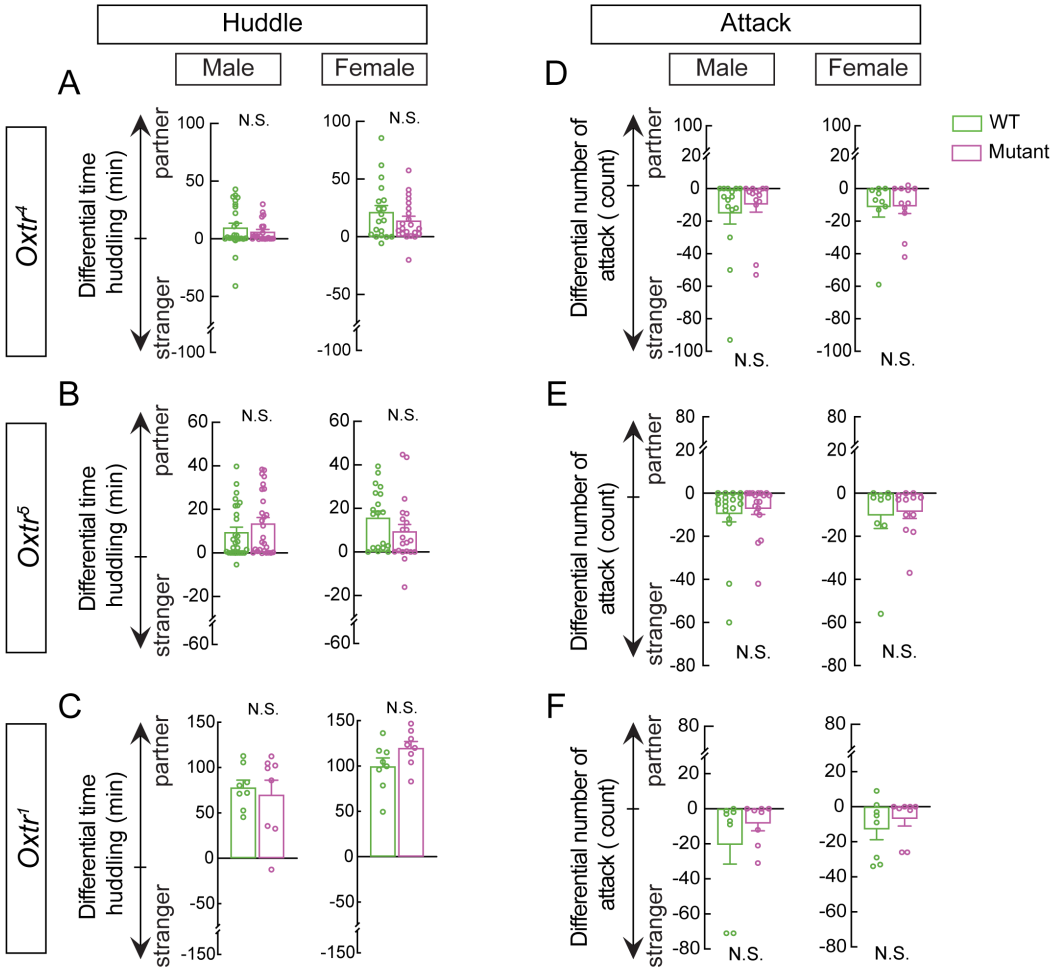

Figure S3. *Oxtr* null voles form pair bonds; related to Figure 3

A-C. No difference in time spent huddling differentially with partner over stranger of opposite sex between WT and *Oxtr*$^{4-/-}$ (A), *Oxtr*$^{5-/-}$ (B), or *Oxtr*$^{1-/-}$ (C) voles. For individual experimental voles, differential time huddling = duration huddling with partner – duration huddling with stranger.

D-F. No difference in number spent differentially attacking stranger of opposite sex between WT, *Oxtr*$^{4-/-}$ (D), *Oxtr*$^{5-/-}$ (E) and *Oxtr*$^{1-/-}$ (F) voles. For individual experimental voles, differential number attacking = number attacking partner – number attacking stranger.

Mean ± SEM; n = 25 WT and 21 mutant males, 19 WT and 21 mutant females (A), 27 WT and mutant males each,19 WT and 21 mutant females (B), 8 WT mutant males and females each (C), 15 WT and 14 mutant males, 9 WT and 11 mutant females (D), 18 WT and mutant males each, 9 WT and 12 mutant females (E); 8WT and mutant males and females each (F); *$p<0.05$, **$p<0.01$, ***$p<0.001$; N.S., not significant.

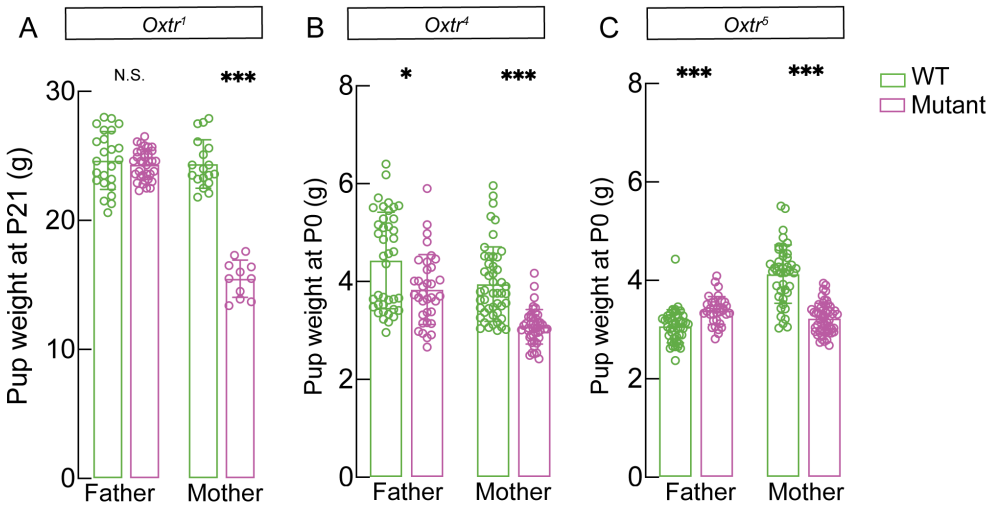

Figure S4. Prairie voles lacking Oxtr have altered weight on day of birth and weaning; related to Figure 4

A. *Oxtr*$^{1-/-}$ fathers weaned pups with no difference in body weight while *Oxtr*$^{1-/-}$ mothers weaned pups with significantly lower body weight compared to WT parents.

B. *Oxtr*$^{4-/-}$ fathers and mothers had pups with lower body weights at birth compared to WT parents

C. *Oxtr*$^{5-/-}$ mothers had pups with lower body weights at birth compared to WT parents, while fathers had pups with increased weight at birth.

Mean ± SEM; n = 24 WT and 35 mutant males, 18 WT and 11 mutant females (A); 40 WT and 35 mutant males, 48 WT and 45 mutant females (B); 45 WT and 34 mutant males each, 42 WT and 53 mutant females (C); *p<0.05, **p<0.01, ***p<0.001; N.S., not significant.