# Supplemental Figure S1

## A

```
DUXC and DUXC-ALT HD1 alignment
DUXC       PRRRRLVLTASQKGALQAFFQKNPYPSITAREHLARELAISESRIQ-------------  46

           PRRRRLVLTASQKGALQAFFQKNPYPSITAREHLARELAISESRIQ

DUXCALT    PRRRRLVLTASQKGALQAFFQKNPYPSITAREHLARELAISESRIQCRGSLRMGPLVRRM  60


DUXC       ------------------------------------------------VWFQNQRTRQLRQS  60

                                                           VWFQNQRTRQLRQS

DUXCALT    TPVSSVFLVSQSASAPYRGLQQELHPLPMRMTKEEMTLVPMPSVVWFQNQRTRQLRQS  118
```
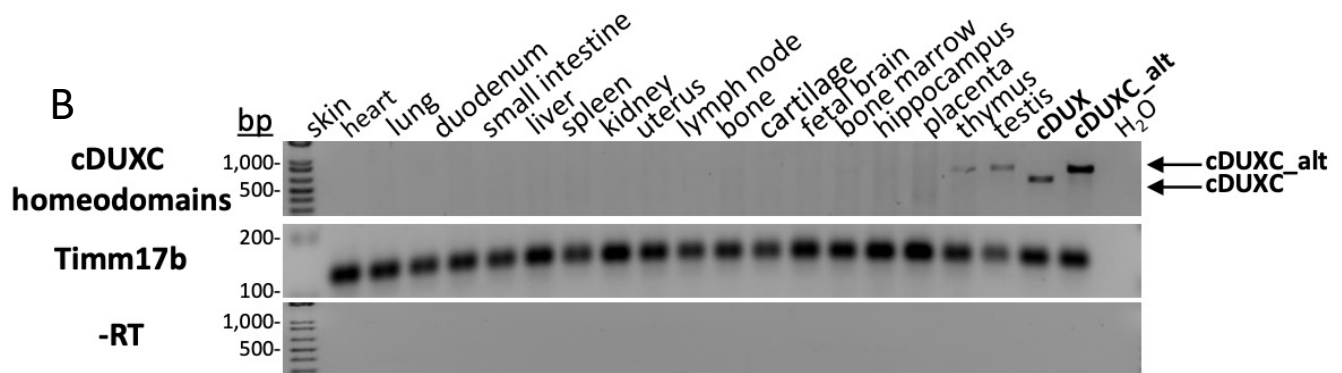
## B



**Figure S1**. **A.** Sequence alignment of the HD1 domain regions of canine DUXC and DUXC-ALT. **Lower panels**: HD1 and HD2 protein alignments. B. RT-PCR of RNA from different canine tissues using primers that will amplify both DUXC and DUXC-ALT shows expression of DUXC-ALT in thymus and testis.

## Supplemental Figure S2

**Homeodomain 1**

```
Mouse   RRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDCRIRVWFQNRRNRSGEEG

          RRR+ VW    Q +AL + F++  Y      R+ LA+ +G+ + R+++WFQN R+R    +   60% Sim

Human   GRRRLVWTPSQSEALRACFERNPYPGIATRERLAQAIGIPEPRVQIWFQNERSRQLRQH

          RRRLV T SQ  AL+A F++NPYP I  RE LA+ + I E R+Q+WFQN+R+RQLRQ  75% Sim

Dog     PRRRLVLTASQKGALQAFFQKNPYPSITAREHLARELAISESRIQVWFQNQRTRQLRQS
```


**Homeodomain 2**

```
Mouse   GRRPRTRLTSLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRRARRRHRR

          GRR RT +T  Q  +L +AFE++   PG A REELAR+TGLPE  I IWFQNRRAR    +   70% Sim

Human   GRRKRTAVTGSQTALLLRAFEKDRFPGIAAREELARETGLPESRIQIWFQNRRARHPGQG

          GRRKRT+++ SQT++LL+AFE++RFPGI   RE LAR+TGLPE+RIQ+WFQNRRARHPGQ  92% Sim

Dog     GRRKRTSISASQTSILLQAFEEERFPGIGMRESLARKTGLPEARIQVWFQNRRARHPGQS
```


**Figure S2. The canine DUXC homeodomains show higher similarity to the human than does the mouse.** HD1 alignments show that the canine has 75% similarity to the human, whereas the similarity of human to mouse is 60%. For HD2, canine is 92% similar to human, whereas mouse is 70% similar to human.
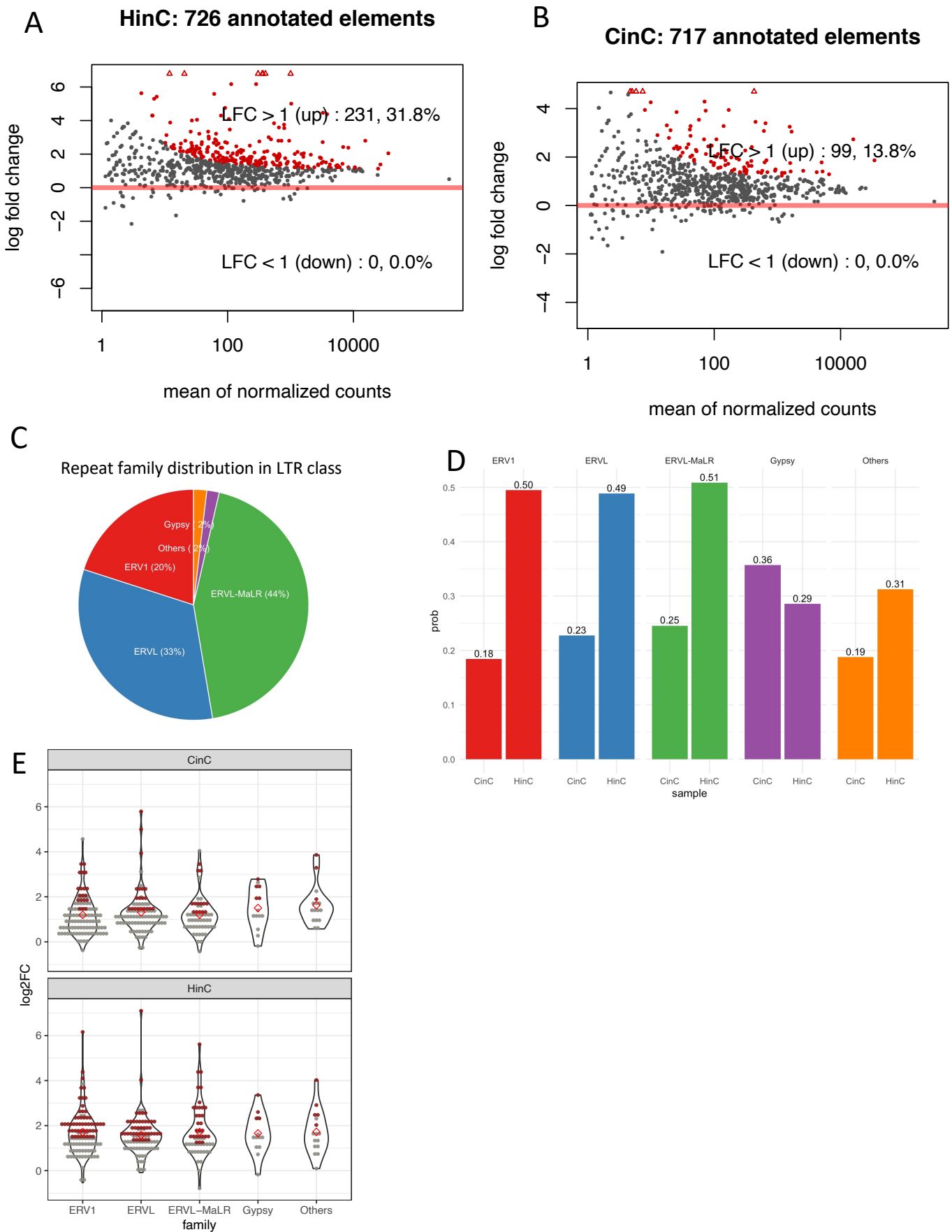
# Supplemental Figure S3



**A** — HinC: 726 annotated elements

LFC > 1 (up) : 231, 31.8%

LFC < 1 (down) : 0, 0.0%

**B** — CinC: 717 annotated elements

LFC > 1 (up) : 99, 13.8%

LFC < 1 (down) : 0, 0.0%

**C** — Repeat family distribution in LTR class

Gypsy 2%
Others ( 2%)
ERV1 (20%)
ERVL-MaLR (44%)
ERVL (33%)

**D**

| sample | ERV1 | ERVL | ERVL-MaLR | Gypsy | Others |
|--------|------|------|-----------|-------|--------|
| CinC | 0.18 | 0.23 | 0.25 | 0.36 | 0.19 |
| HinC | 0.50 | 0.49 | 0.51 | 0.29 | 0.31 |

**E**

CinC / HinC

log2FC — family: ERV1, ERVL, ERVL–MaLR, Gypsy, Others

**Figure S3**. See legend on next page.

**Figure S3**. **Repeat element different analysis and enriched repeat families .** (A) A traditional MA plot to show the expression of 726 repeat elements in canine muscle cells expressing DUX4 (HinC) compared to a control luciferase vector. The x axis is the mean of normalized counts of each repeat element in the two conditions and y axis is the $log_2$ fold change of each element in the two conditions. The red dots represent the differentially expressed repeat elements with adjusted p-value < 0.05 corresponding $\mathcal{H}_0 : |logFC| \leq 0.5$. (B) Similar to A showin the expression of 717 repeat elements in canine muscle cells expressing canine DUXC and luciferase (CinC). (C) The repeat family distribution in LTR class. (D) The percentage of differentially expressed elements within each LTR repeat class in HinC and CinC. (D) A violin plot to show the $log_2$ expression of each element in the LTR families, with red dots indicating the element is differentially expressed.

# Supplemental Figure S4



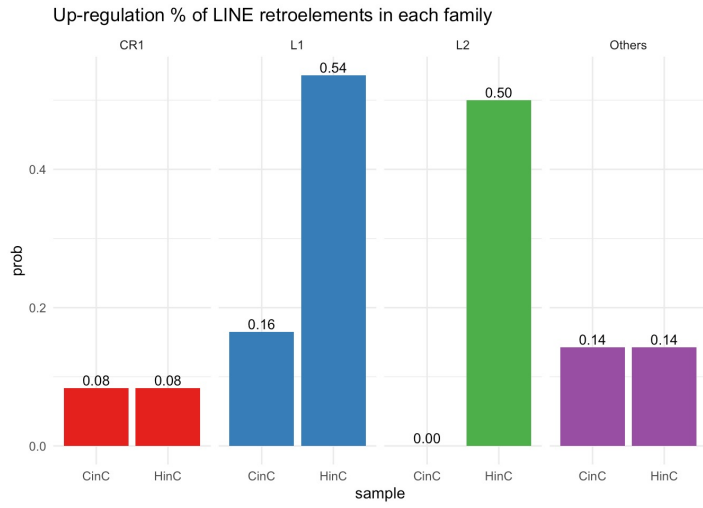Up-regulation % of LINE retroelements in each family

**Figure S4.** A bar plot to show the percentage of up-regulated retroelements in the LINE sub-families of CR1, L1, and L2.