

## Description of Additional Supplementary Files

**Supplementary Data 1.** Thymocyte expression (transcript per million (TPM)) matrix. Related to Fig. 1b, 1c, 6b, 8a, 8b, 8c. Supplementary Fig. 2c, 7a and 8c.

**Supplementary Data 2.** Sleuth output from a likelihood ratio test ( $\sim$ Celltype vs  $\sim$ 1, two-tailed). Related to Supplementary Fig. 2c.

**Supplementary Data 3.** Male vs Female log<sub>2</sub>FC, Wald Test (two-tailed) p-values and BH-corrected q-values from Sleuth on combined thymocyte data. Positive values indicate female-bias, negative values indicate male-bias; related to Fig. 1e, 1f, 2c & Supplementary Fig. 3a, 3c, 3d.

**Supplementary Data 4.** Male vs Female log<sub>2</sub>FC, Wald Test (two-tailed) p-values and BH-corrected q-values from Sleuth on thymocyte subpopulations data. Positive values indicate female-bias, negative values indicate male-bias; related to Fig. 2a.

**Supplementary Data 5.** log<sub>2</sub>FC and empirical Bayes quasi-likelihood F-test (two-tailed) P-values obtained from edgeR (on STAR aligned and featureCounts processed data) for method comparison; related to Supplementary Fig. 3b (referred to as reference alignment).

**Supplementary Data 6.** Male vs Female log<sub>2</sub>fc, Wald Test (two-tailed) p-values and BH-corrected q-values from Sleuth for method comparison; related to Supplementary Fig. 3b (referred to as pseudo alignment).

**Supplementary Data 7.** Thymocyte allele-specific expression for positions on the X-chromosome in Female 3; related to Fig. 3c, 3d, 3e, 4b, 4c, Supplementary Fig. 4b, 4c & 5.

**Supplementary Data 8.** Female 4 Smart-seq2 allele-specific expression; related to Fig. 5a, 5b, 5c, Supplementary Fig. 6a, 6b, 6c and 6d.

**Supplementary Data 9.** Clustering results for each cell based on allelic expression of *XIST* and inactive genes (see methods under *Analysis of Smart-seq2* for details on how the clustering was performed); related to Fig. 5a, 5b, 5c, Supplementary Fig. 6a, 6b, 6c and 6d.

**Supplementary Data 10.** Gene, allele-specific expression (ASE) category (mean ASE across thymocyte subtypes  $< 0.4$  = escape, ASE  $> 0.4$  = inactive, no data = NA), Methylation\_category (TSS -500 methylation  $< 0.25$  = low, 0.25-0.75 = intermediate,  $> 0.75$  = high) and sex-bias expression (log<sub>2</sub>FC, positive values indicate female-bias, negative values indicate male-bias); related to Fig. 7a.

**Supplementary Data 11.** gene, allele-specific expression categorization (see methods for detailed explanation of categories) in either thymocytes or previous assessments and comparison between classifications; related to Fig. 7b.