Supplemental Figure Legends

Figure S1: X-linked transcription factors downstream target genes are enriched on the X chromosome. For each transcription factor grouped by their chromosomal location, the proportion of their targets on the X (A, B), 2L (C, D), 2R (E, F), 3L (G, H), and 3R (I, J) are indicated with boxplots. Separate plots were made considering the genes analyzed for gene expression in this study (A, C, E, G, I) and all the genes represented in the regulatory network (B, D, F, H, J).

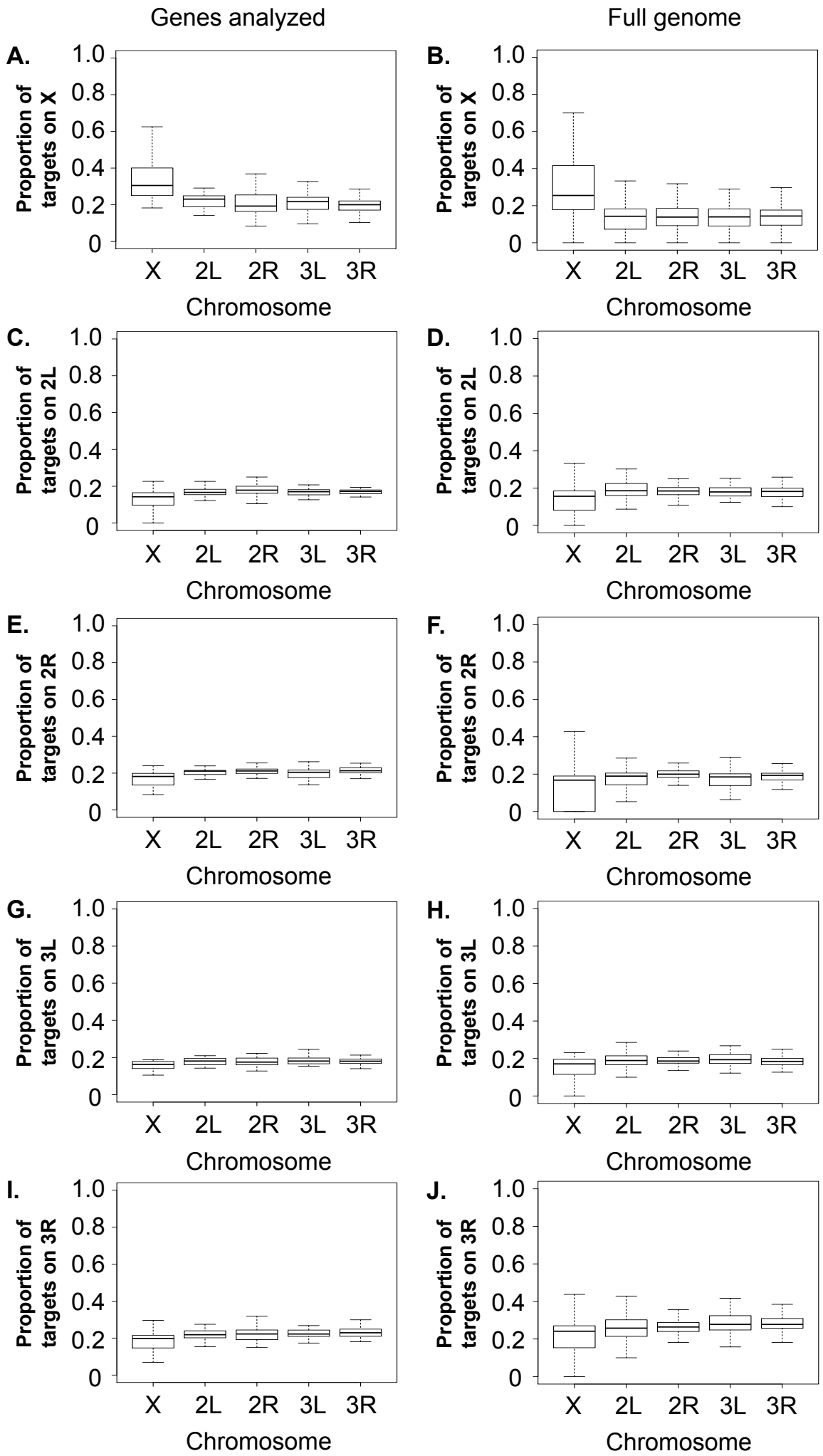
Figure S2: Upstream regulators of target genes on the X chromosome are enriched on the X chromosome. For each target gene grouped by their chromosomal location, the proportion of their regulators on the X (A, B), 2L (C, D), 2R (E, F), 3L (G, H), and 3R (I, J) are indicated with boxplots. Separate plots were made considering the genes analyzed for gene expression in this study (A, C, E, G, I) and all the genes represented in the regulatory network (B, D, F, H, J).

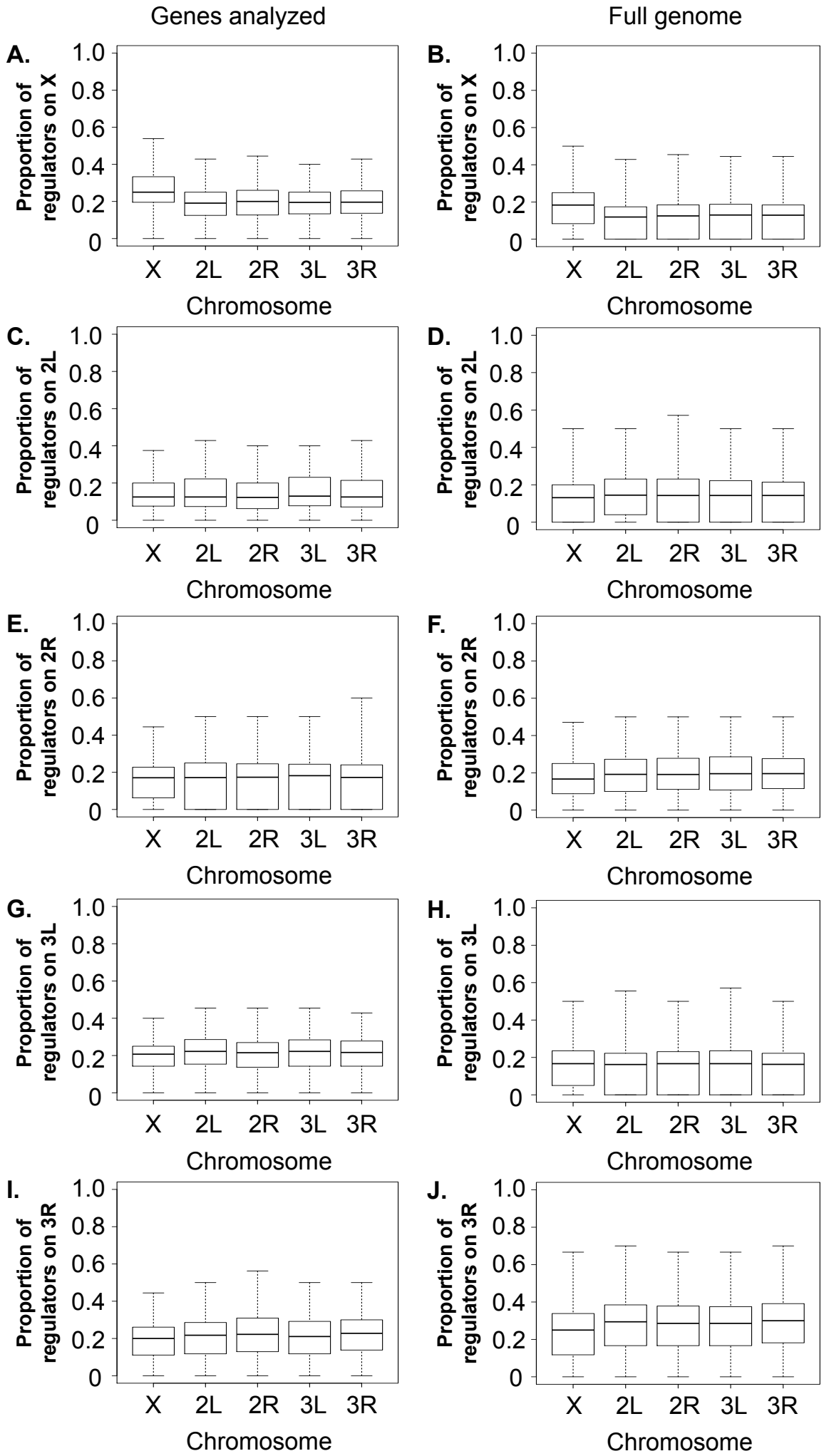
Figure S3: Faster-X divergence of transcription factor DNA sequences. Percent sequence divergence is shown for coding (**A**) and noncoding (**B**) regions of each of the 46 X-linked and 167 autosomal transcription factors analyzed in all three comparisons (*mel-mel*, *sim-sech*, *mel-sim*). Maximum likelihood estimates of (**C**) nonsynonymous (*dN*) and (**D**) synonymous changes (*dS*) as well as (**E**) the ratio of nonsynonymous to synonymous changes (*dN/dS*) are shown. Boxplots show the distribution of these values separately for genes on the X chromosome and autosomes with the median as well as 25^{th} and 75^{th} percentiles. Statistical significance of the difference in median values between X-linked and autosomal genes was determined using Mann-Whitney U tests, where **NS** indicates not significant, * indicates $P \leq 0.05$, ** indicates $P \leq 0.001$, and *** indicates $P \leq 1 \times 10^{-4}$.

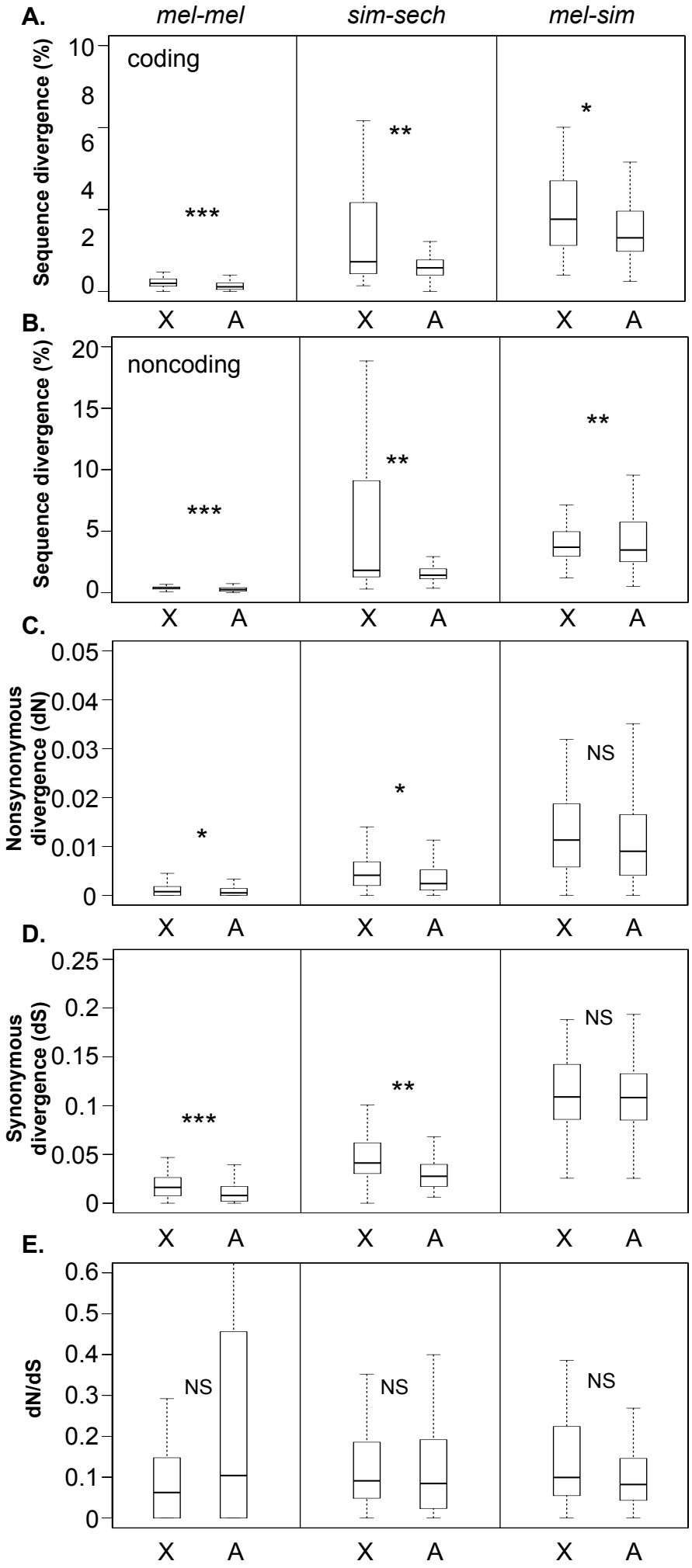
Figure S4: Correlation between the proportion of X-linked regulators and expression, *cis* and *trans* divergence. For each target gene grouped by their chromosomal location, the proportion of their regulators on the X chromosome is plotted against **A,** the magnitude of expression divergence (abs(log2(strain1/strain2))), **B,** the magnitude of *cis*-regulatory divergence (abs(log2(allele 1/allele 2))), and **C,** the magnitude of *trans*-regulatory divergence (abs(log2(species 1/species 2)) - abs(log2(allele 1/allele 2))). Best-fit lines are indicated in each plot. Pearson's correlation coefficients (ρ) and *P*-values indicating the significance of these correlations from permutation tests are shown.

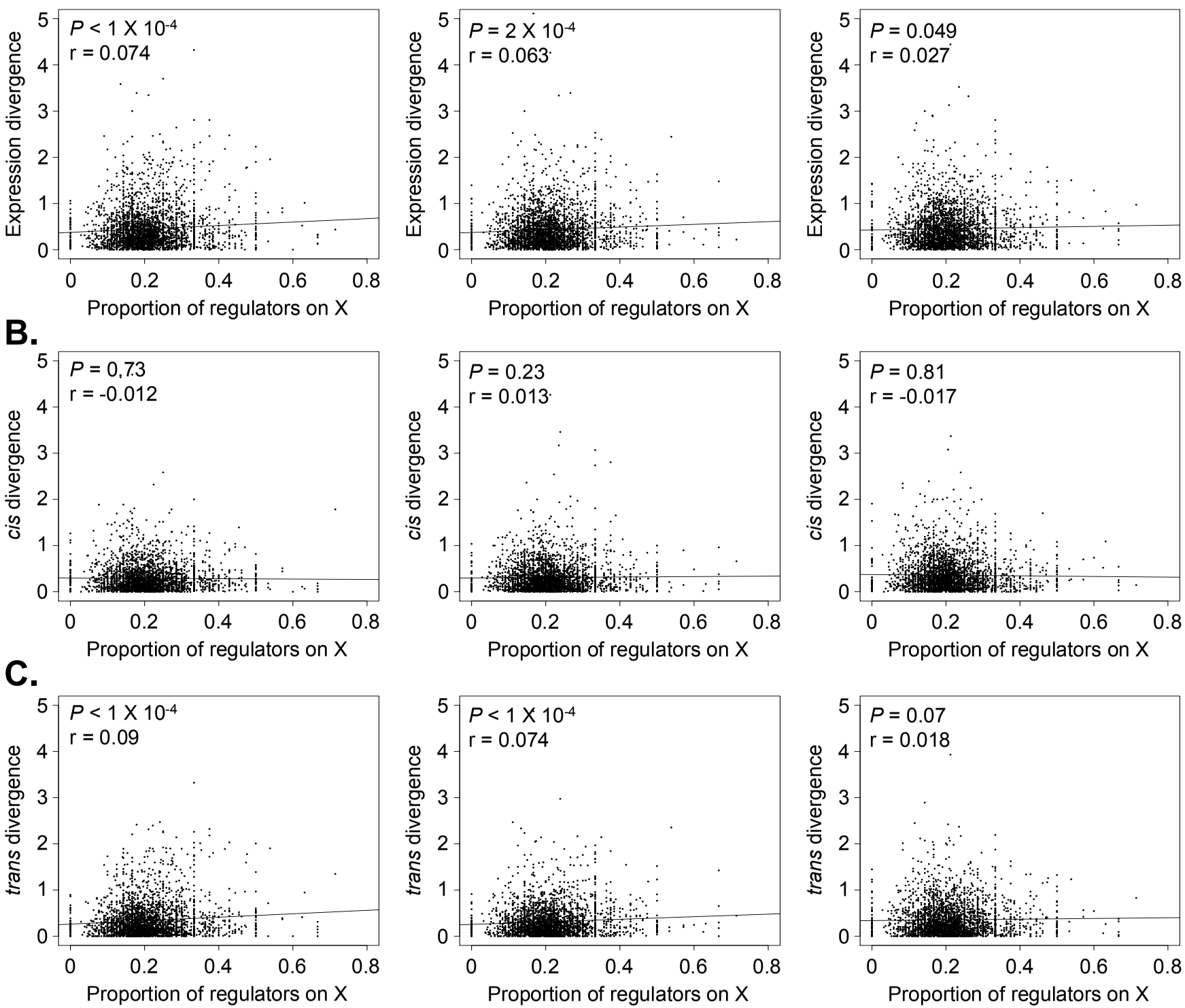
Supplemental Table Legends

Table S1: Results from statistical tests: The results of all statistical tests reported in the main text are shown inculding median values for X and autosomal genes, sample sizes and associated P-values. The figure where each test was presented is indicated. NAs are used where the data shown were single values.


Genes analyzed
Full genome
A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
Proportion of targets on X
Proportion of targets on 2L
Proportion of targets on 2R
Proportion of targets on 3L
Proportion of targets on 3R
0
0.2
0.4
0.6
0.8
1.0
X
2L
2R
3L
3R
Chromosome


Genes analyzed
Full genome
A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
Proportion of regulators on X
Proportion of regulators on 2L
Proportion of regulators on 2R
Proportion of regulators on 3L
Proportion of regulators on 3R
1.0
0.8
0.6
0.4
0.2
0
X
2L
2R
3L
3R
Chromosome


A.
mel-mel
sim-sech
mel-sim
coding
Sequence divergence (%)
10
8
6
4
2
0
***
**
*
X
A
B.
noncoding
Sequence divergence (%)
20
15
10
5
0
***
**
**
C.
Nonsynonymous divergence (dN)
0.05
0.04
0.03
0.02
0.01
0
*
*
NS
D.
Synonymous divergence (dS)
0.25
0.2
0.15
0.1
0.05
0
***
**
NS
E.
dN/dS
0.6
0.5
0.4
0.3
0.2
0.1
0
NS
NS
NS


A.
mel-mel
sim-sech
mel-sim
P < 1 X 10-4
r = 0.074
P = 2 X 10-4
r = 0.063
P = 0.049
r = 0.027
B.
P = 0.73
r = -0.012
P = 0.23
r = 0.013
P = 0.81
r = -0.017
C.
P < 1 X 10-4
r = 0.09
P < 1 X 10-4
r = 0.074
P = 0.07
r = 0.018
Expression divergence
cis divergence
trans divergence
Proportion of regulators on X
0
0.2
0.4
0.6
0.8
1
2
3
4
5