Electronic Supplementary Information 2

Royall AH, Frankenberg S, Pask AJ, Holland PWHH.
Of eyes and embryos: subfunctionalisation of the CRX homeobox gene in mammalian evolution.
Proceedings of the Royal Society B.
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(A) SRA identifiers for mouse preimplantation transcriptome datasets.

(B) Maximum-likelihood tree of CRX protein sequences from across metazoans shows divergence of sequence in eutherian mammals.

(C) Homeodomains of mouse ETCHbox genes do not show compensatory changes for the amino acids substituted in eutherian CRX homeodomain.

(D) RT-PCR for *CRX* expression in fat-tailed dunnart adult tissues.

(E) Immunocytochemistry staining for V5-tagged CRX proteins in MEFs shows nuclear localisation and demonstrates correct translation frame.

(F) TPM for ectopically expressed genes and chemical selection gene (puromycinR) shows there is no discrepancy in the levels of ectopic expression between samples, and that there is no endogenous expression of mouse *Crx* in the MEFs used.

(G) Mouse preimplantation embryo transcriptome profiles generated using MFuzz.

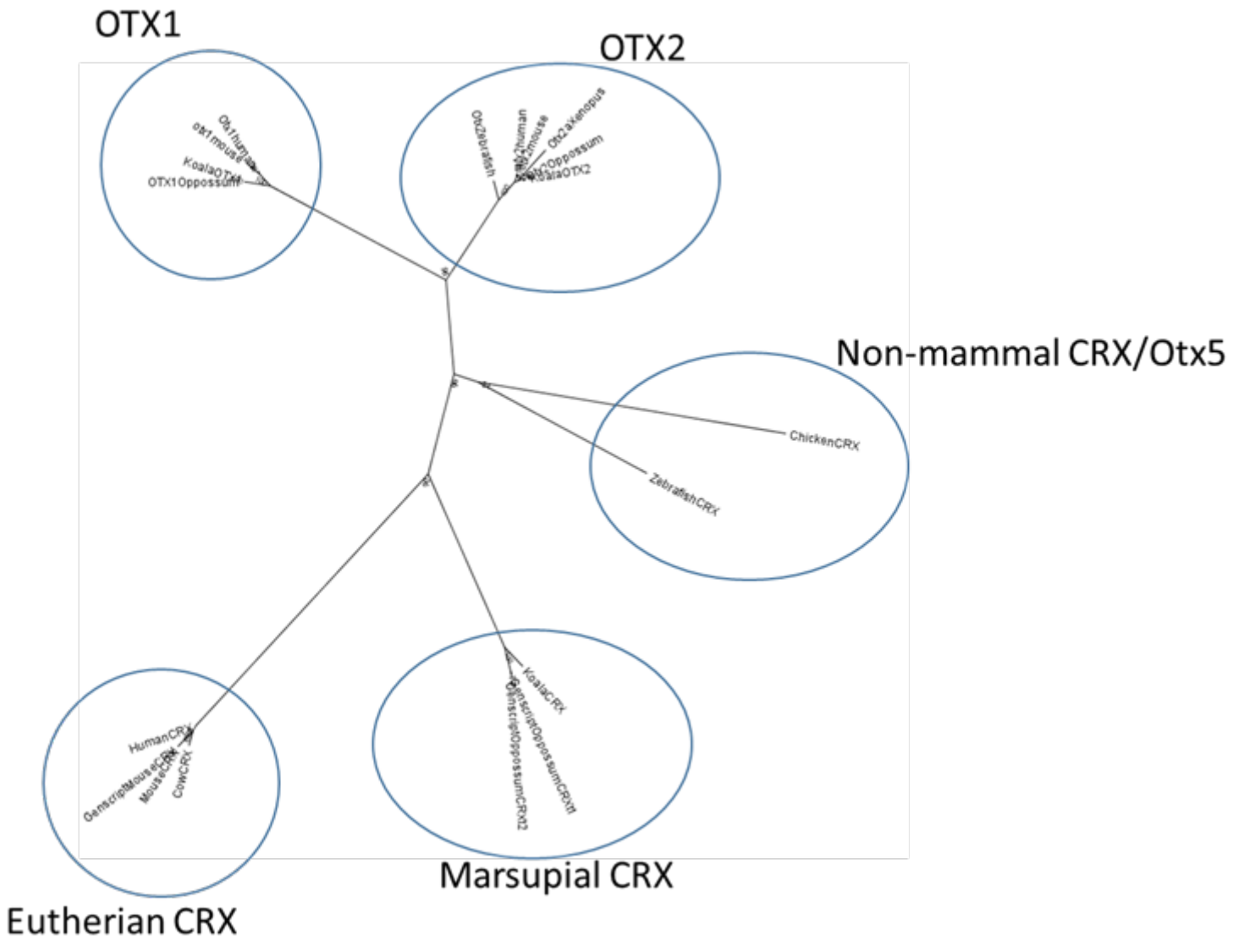
A

SRA identifiers for mouse preimplantation transcriptome datasets.

Species	Tissue	SRA study	Run ID
Mmus	Oocyte	SRP03454	SRR1051273
Mmus	Oocyte	SRP03454	SRR1051274
Mmus	Oocyte	SRP03454	SRR1051275
Mmus	Oocyte	SRP03454	SRR1051276
Mmus	Oocyte	SRP03454	SRR1051277
Mmus	Zygote	SRP03454	SRR1567937
Mmus	Zygote	SRP03454	SRR1051279
Mmus	Zygote	SRP03454	SRR1051278
Mmus	2Cell	SRP03454	SRR1051282
Mmus	2Cell	SRP03454	SRR1051281
Mmus	2Cell	SRP03454	SRR1051280
Mmus	4Cell	SRP03454	SRR1567921
Mmus	4Cell	SRP03454	SRR1567920
Mmus	4Cell	SRP03454	SRR1567919
Mmus	8Cell	SRP03454	SRR1567924
Mmus	8Cell	SRP03454	SRR1567923
Mmus	8Cell	SRP03454	SRR1567922
Mmus	Morula	SRP03454	SRR2048257
Mmus	Morula	SRP03454	SRR2048256
Mmus	Morula	SRP03454	SRR2048255
Mmus	Blastocyst	SRP03454	SRR1567925
Mmus	Blastocyst	SRP03454	SRR1567926
Mmus	Blastocyst	SRP03454	SRR1567927
Mmus	Blastocyst	SRP03454	SRR1567928
Mmus	E7.5	SRP03776	SRR1168503

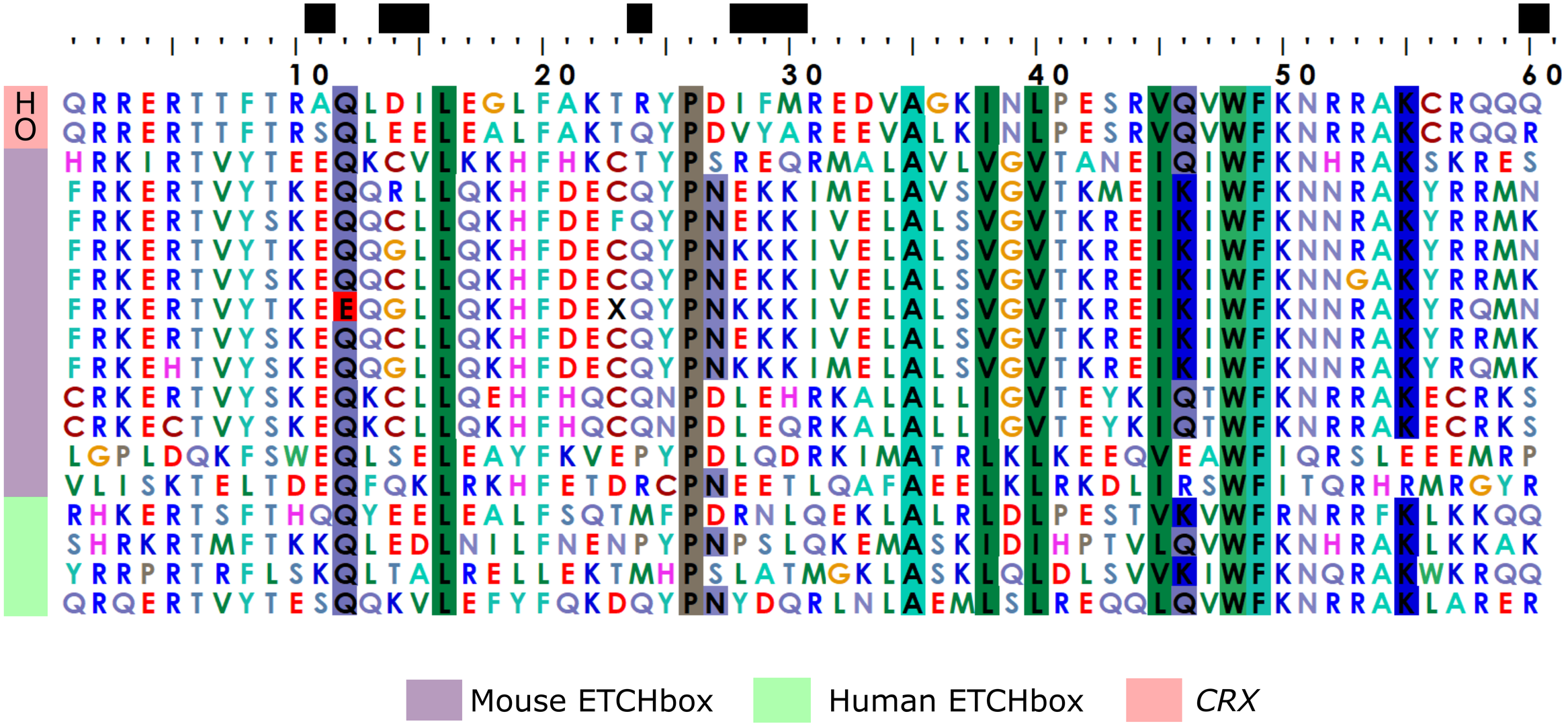
B

Maximum-likelihood tree of CRX protein sequences from across metazoans shows divergence of sequence in eutherian mammals.



C

Homeodomains of mouse ETCHbox genes do not show compensatory changes for the amino acids which are lost in eutherian *CRX* homeodomain (black boxes). H = Human *CRX*, O = Opossum *CRX*.



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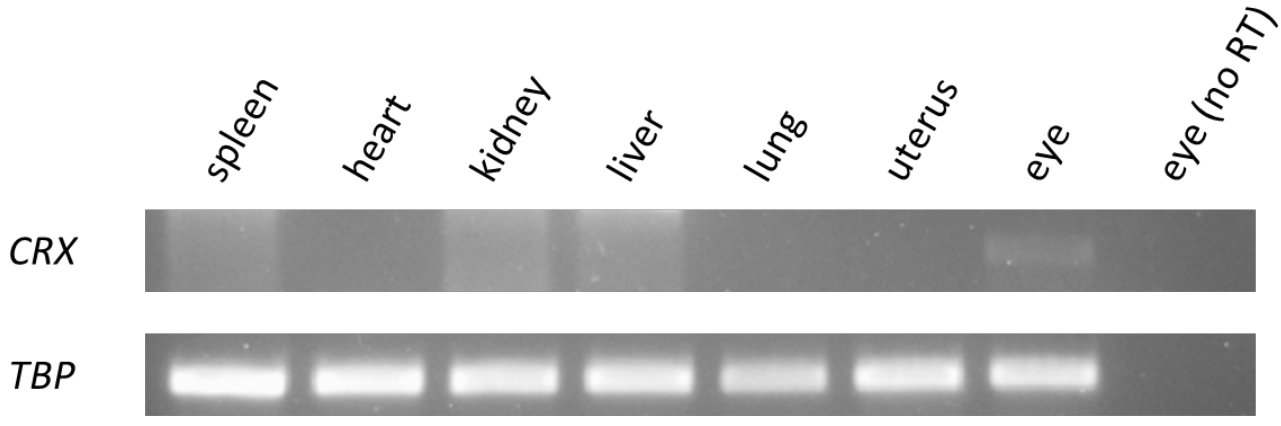
Reverse-Transcriptase PCR for *CRX*expression in fat-tailed dunnart adult tissues.



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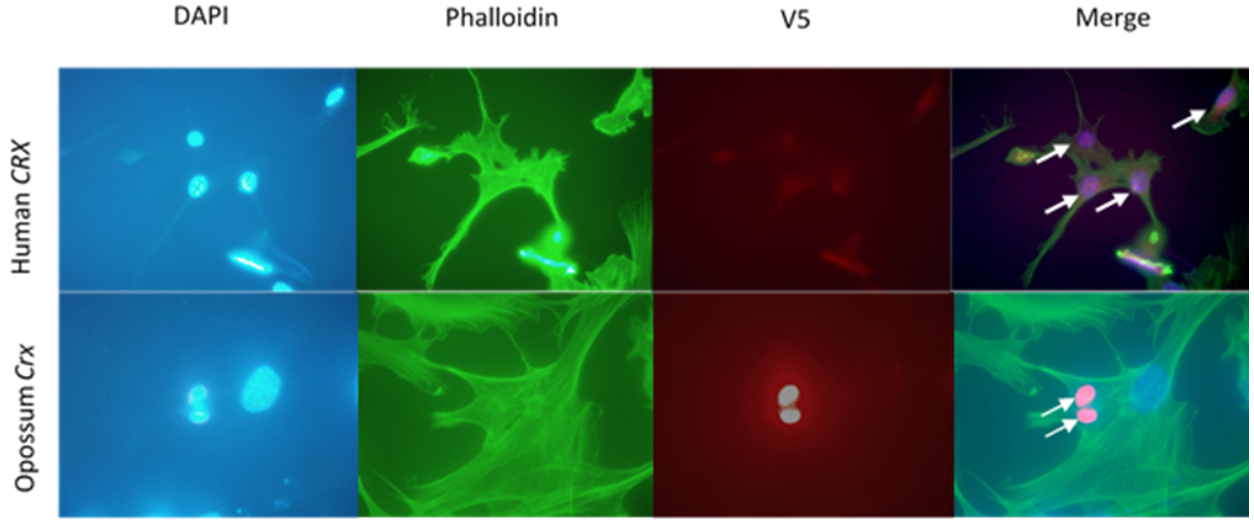
Metatherian and eutherian CRXectopic expression in MEFs is localised to the nucleus

The Opossum (*Monodelphis domestica*) is often used as a tool for investigating the evolution of mammals as it is thought to possess ancestral characteristics of the early metatherians. In order to study the role of *Crx* in regulating the transcriptome, and whether there is any distinction be tween the effect of metatherian and eutherian *Crx*, we ectopically expressed either Monodelphis domestica or human *Crx* genes in primary mouse cells and carried out Poly-A RNA-sequencing.

As homeodomain proteins such as *Crx* function as transcription factors, it is important to confirm that the ectopically expressed protein localises to the nucleus. To test this we ectopically expressed a both proteins of interest with a V5 tag in primary MEFs and visalised the cellular localisation of the fusion protein throu gh immunistaining against the tag. It is particularly important in this case to confirm the sub -cellular protein localisation following over -expression as we are studying genes in the cells of a foreign species meaning it cannot necessarily be assumed that modifications and mechanisms that confer nuclear localisation are transferable between species. The evident nuclear localisation of both proteins in a heterologous system implies that the assumed *in vivo* function may be recapitulated in our experimental system.



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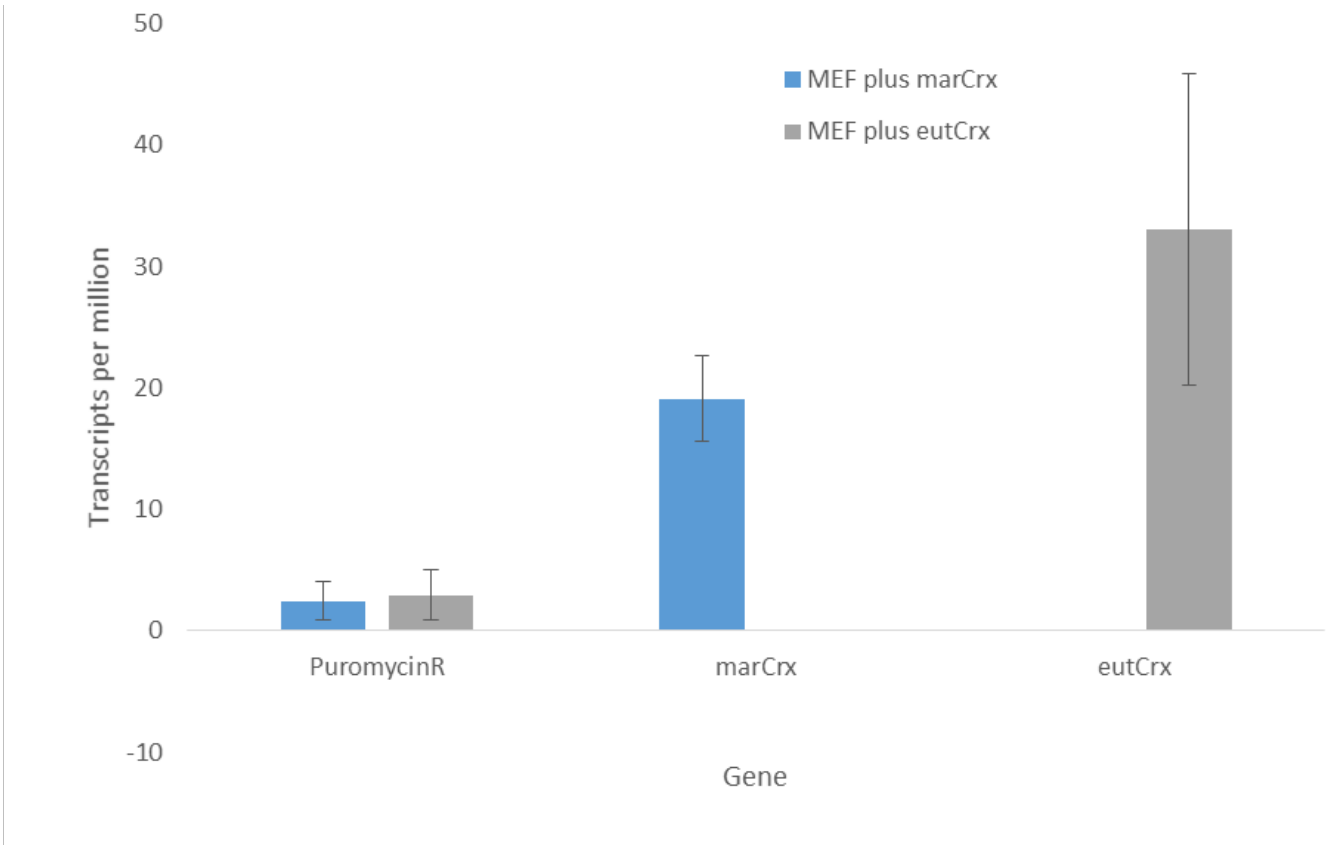
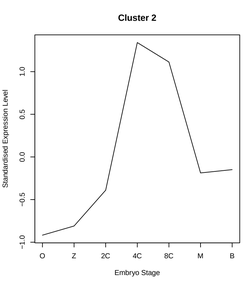
TPM for ectopically expressed genes and chemical selection gene (puromycinR) shows there is no discrepancy in the levels of ectopic expression between samples, and that there is no endogenous expression of mouse *Crx*in the MEFs used.

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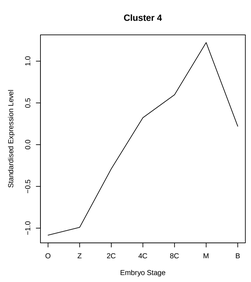
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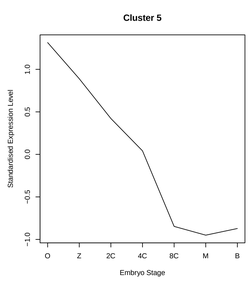
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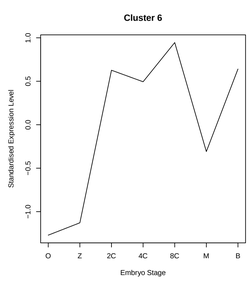
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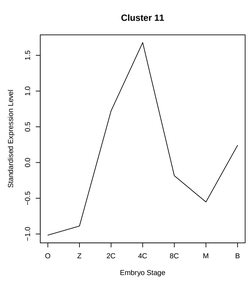
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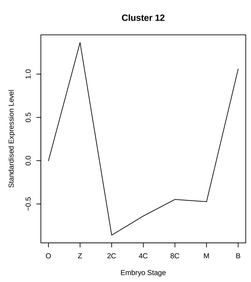
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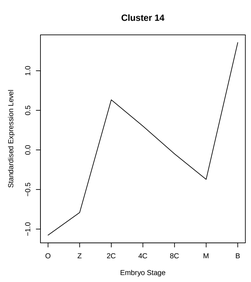
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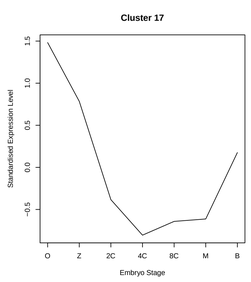
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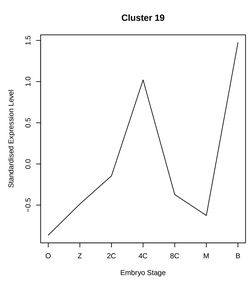
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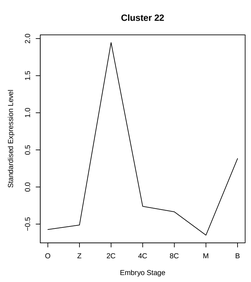
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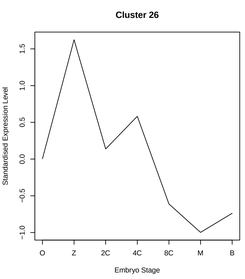
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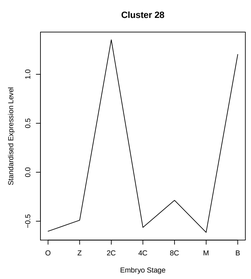
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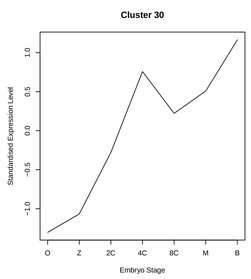
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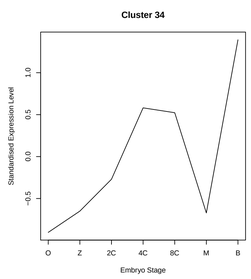
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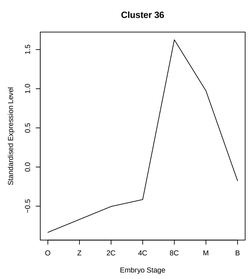
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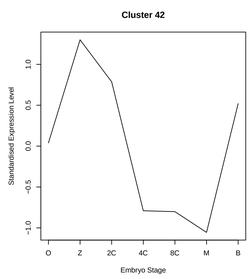
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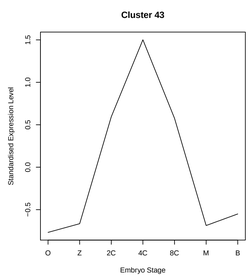
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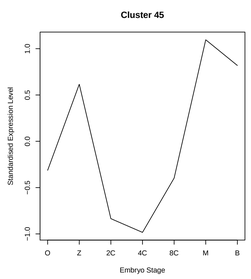
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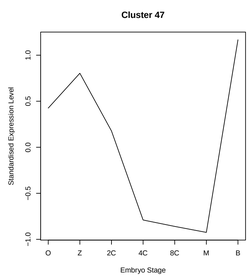
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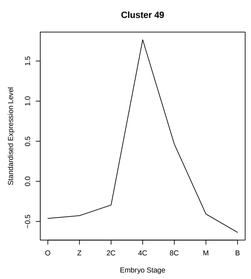
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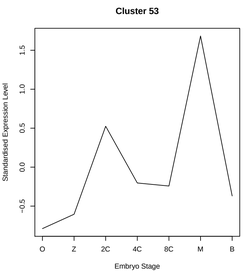
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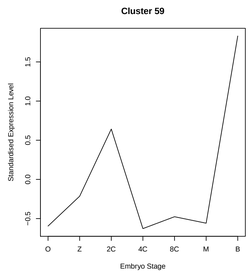
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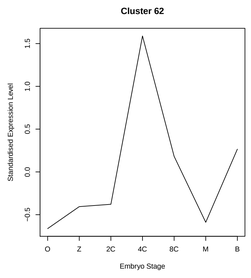
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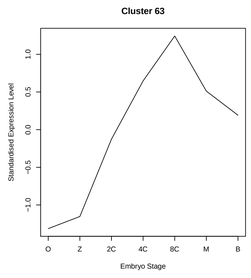
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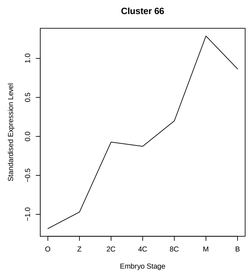
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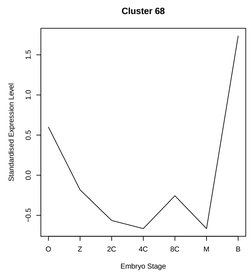
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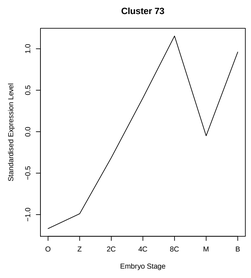
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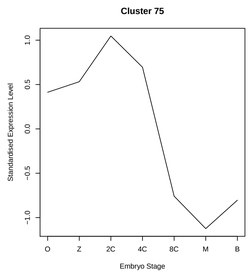
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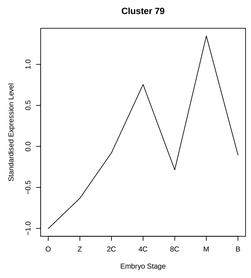
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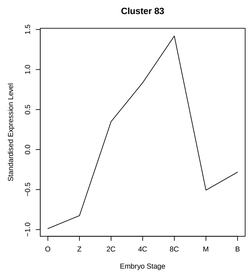
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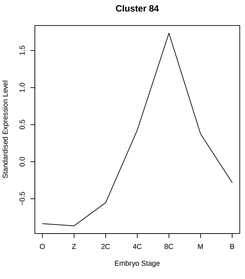
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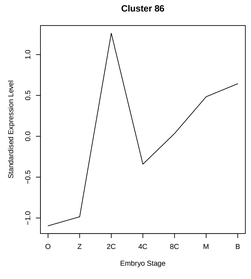
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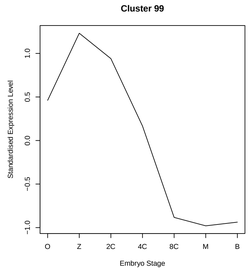
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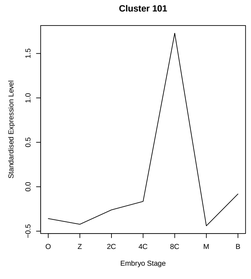
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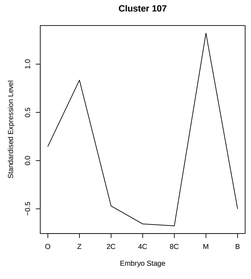
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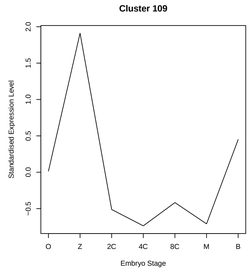
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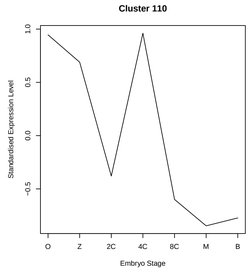
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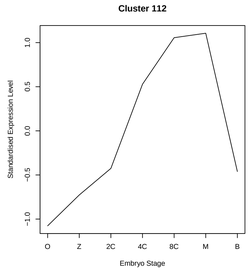
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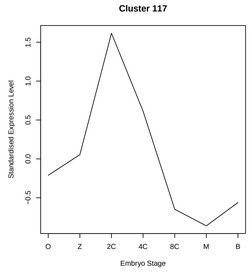
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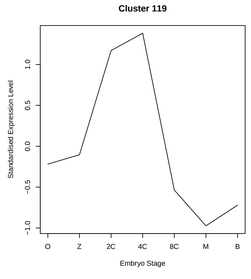
Embryo Stage
O Z 2C 4C 8C M B


Cluster 119
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 120
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 122
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 124
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 126
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 129
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 131
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 137
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 138
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 139
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 141
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 142
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 145
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 146
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 149
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 201
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 202
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 203
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 204
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 205
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 206
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 207
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 208
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 209
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 210
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 211
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 212
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 213
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 214
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 215
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 216
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 217
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 218
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 219
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 220
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 221
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 222
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 223
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 224
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 225
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 226
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 227
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 228
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 229
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 230
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B

Cluster 231
Standardised Expression Level
Embryo Stage
O Z 2C 4C 8C M B