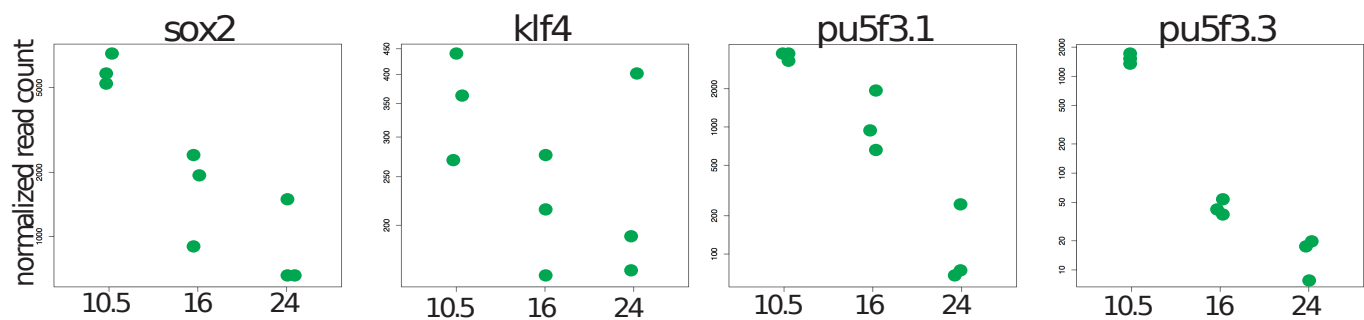
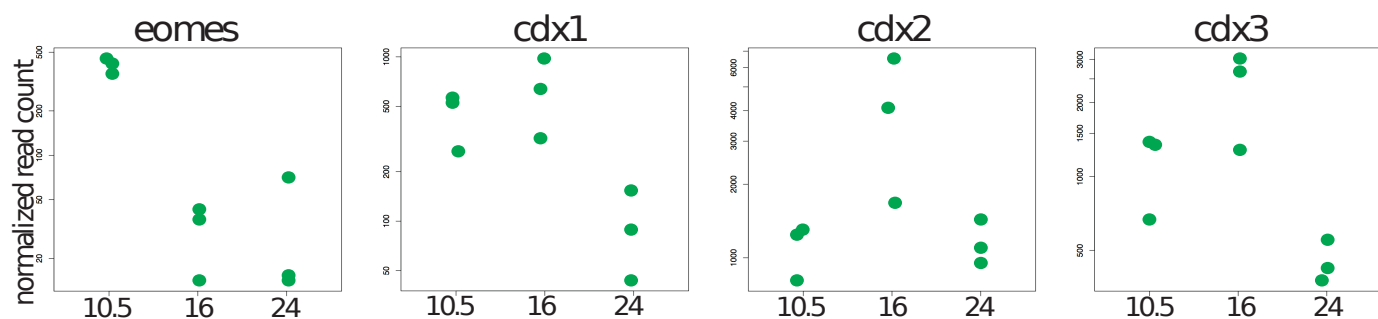


Figure S1

A Pluripotency associated



B Mesendoderm



C Ectoderm

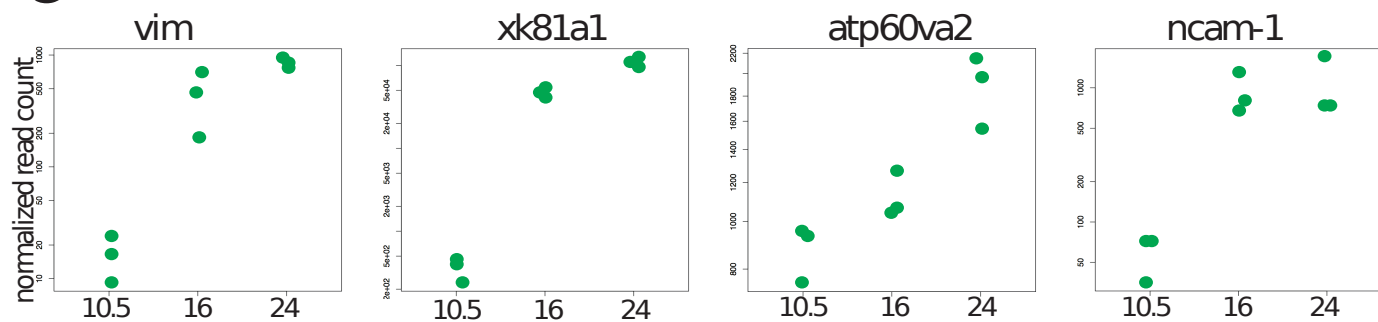
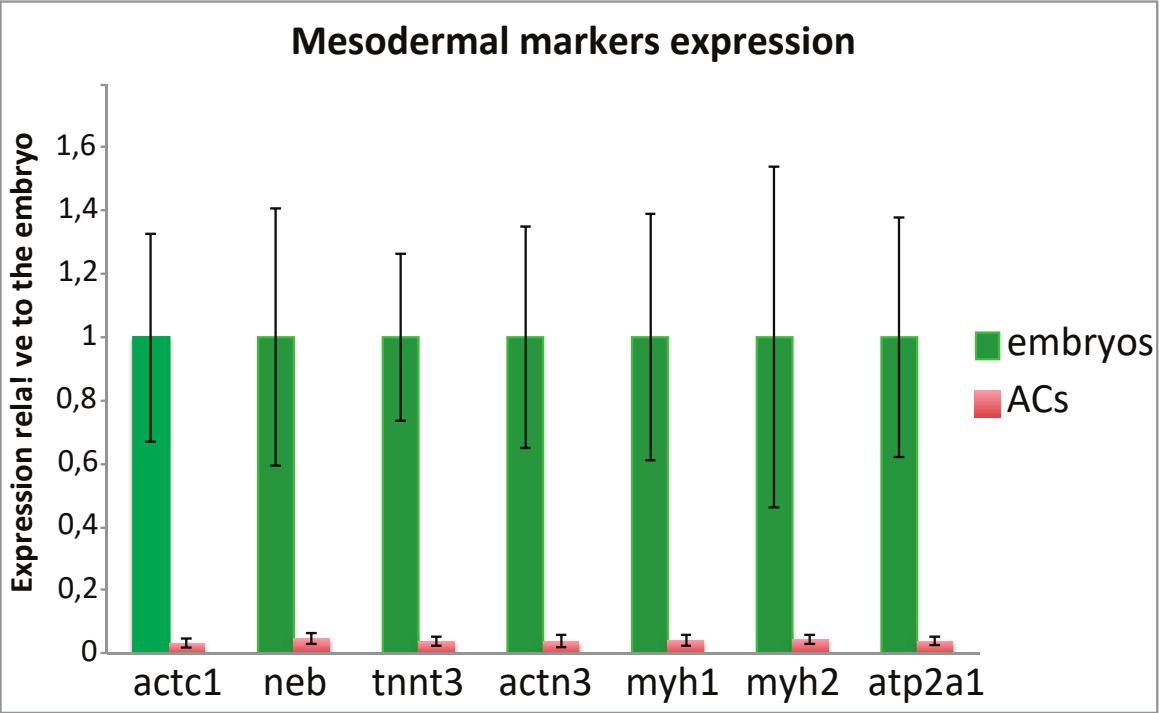


Figure S2

A



B

Gene name	Embryo/AC fold-difference
actc1	31
neb	21
tnnt3	26
actn3	26
myh1	24
myh2	22
atp2a1	25
average difference	25,03

Figure S3

Rfx2

```
XM_012955542.2  NONSDSGSDSATSVLRFTSASAAQPVQPVASQORSLQASGSAPEGAHQGQISVPRAH
XM_012955540.2  NONSDSGSDSATSVLRFTSASAAQPVQPVASQORSLQASGSAPEGAHQGQISVPRAH
NM_001123483.1  *****
XM_012955542.2  OLOQOVQTVQHVVPAAQVQVVEGDVTYTNCAIRTAISYNAAQIYAPSSGSSYFDSQGGGA
XM_012955540.2  OLOQOVQTVQHVVPAAQVQVVEGDVTYTNCAIRTAISYNAAQIYAPSSGSSYFDSQGGGA
NM_001123483.1  ----QVQTVQHVVPAAQVQVVEGDVTYTNCAIRTAISYNAAQIYAPSSGSSYFDSQGGGA
*****
XM_012955542.2  QVTVVSSPTAIPBSHWGIAMDVSGQIISSSGAYLIHGGLNSRHTFSHTSRTPATL
XM_012955540.2  QVTVVSSPTAIPBSHWGIAMDVSGQIISSSGAYLIHGGLNSRHTFSHTSRTPATL
NM_001123483.1  *****
XM_012955542.2  EMAIENLQKNBEGITSHKSSLLNSHLQWLLDNVETABGVSLPSSSLYNYLRHCQDHKLD
XM_012955540.2  EMAIENLQKNBEGITSHKSSLLNSHLQWLLDNVETABGVSLPSSSLYNYLRHCQDHKLD
NM_001123483.1  *****
XM_012955542.2  VNAASFGKILRSVFMGLRTRRLGTRGNSKYHYGIRLKPDSPLNRLQDQTYMAIRQPI
XM_012955540.2  VNAASFGKILRSVFMGLRTRRLGTRGNSKYHYGIRLKPDSPLNRLQDQTYMAIRQPI
NM_001123483.1  *****
XM_012955542.2  HQKQ--RPAQKMDGNGENTANSQHTSPESQVAASQHQHQFIDTAHVFPDFPEDLGNV
XM_012955540.2  HQKQRYRPAQKMDGNGENTANSQHTSPESQVAASQHQHQFIDTAHVFPDFPEDLGNV
NM_001123483.1  HQKQRYRPAQKMDGNGENTANSQHTSPESQVAASQHQHQFIDTAHVFPDFPEDLGNV
*****
XM_012955542.2  LLPEGITMTDINKLQIMYRHCACATIDVVMNLQFYQIEKLAQFWMNSKSSPGSGNPNNS
XM_012955540.2  LLPEGITMTDINKLQIMYRHCACATIDVVMNLQFYQIEKLAQFWMNSKSSPGSGNPNNS
NM_001123483.1  LLPEGITMTDINKLQIMYRHCACATIDVVMNLQFYQIEKLAQFWMNSKSSPGSGNPNNS
*****
```

Tp63

```
XM_004914377.3  VMTFPFQGAUVRAHPVYKKAHVTEVVKRCPNHLSREFNEGQIAPPSHLIRVEGNSHAQ
XM_012963343.1  VMTFPFQGAUVRAHPVYKKAHVTEVVKRCPNHLSREFNEGQIAPPSHLIRVEGNSHAQ
XM_012963344.2  VMTFPFQGAUVRAHPVYKKAHVTEVVKRCPNHLSREFNEGQIAPPSHLIRVEGNSHAQ
XM_004914376.3  VMTFPFQGAUVRAHPVYKKAHVTEVVKRCPNHLSREFNEGQIAPPSHLIRVEGNSHAQ
XM_004914374.2  VMTFPFQGAUVRAHPVYKKAHVTEVVKRCPNHLSREFNEGQIAPPSHLIRVEGNSHAQ
*****
XM_004914377.3  YVEDPIGRQSVLVPTPEPQVCTEFTTILYFMNCSSCGVMNRRLPILIVLETRDQV
XM_012963343.1  YVEDPIGRQSVLVPTPEPQVCTEFTTILYFMNCSSCGVMNRRLPILIVLETRDQV
XM_012963344.2  YVEDPIGRQSVLVPTPEPQVCTEFTTILYFMNCSSCGVMNRRLPILIVLETRDQV
XM_004914376.3  YVEDPIGRQSVLVPTPEPQVCTEFTTILYFMNCSSCGVMNRRLPILIVLETRDQV
XM_004914374.2  YVEDPIGRQSVLVPTPEPQVCTEFTTILYFMNCSSCGVMNRRLPILIVLETRDQV
*****
XM_004914377.3  LGRKCFEARIACACGRDRKADSDIRKQVSDSTKNGEAFROSTHGIQNTSIKKRSPDD
XM_012963343.1  LGRKCFEARIACACGRDRKADSDIRKQVSDSTKNGEAFROSTHGIQNTSIKKRSPDD
XM_012963344.2  LGRKCFEARIACACGRDRKADSDIRKQVSDSTKNGEAFROSTHGIQNTSIKKRSPDD
XM_004914376.3  LGRKCFEARIACACGRDRKADSDIRKQVSDSTKNGEAFROSTHGIQNTSIKKRSPDD
XM_004914374.2  LGRKCFEARIACACGRDRKADSDIRKQVSDSTKNGEAFROSTHGIQNTSIKKRSPDD
*****
XM_004914377.3  EVLYLPLVKGREIYEMLLKIESLEIMQFLPQHTIESYRQOQHLLQKQDSLSQSNMGPR
XM_012963343.1  EVLYLPLVKGREIYEMLLKIESLEIMQFLPQHTIESYRQOQHLLQKQDSLSQSNMGPR
XM_012963344.2  EVLYLPLVKGREIYEMLLKIESLEIMQFLPQHTIESYRQOQHLLQKQDSLSQSNMGPR
XM_004914376.3  EVLYLPLVKGREIYEMLLKIESLEIMQFLPQHTIESYRQOQHLLQKQDSLSQSNMGPR
XM_004914374.2  EVLYLPLVKGREIYEMLLKIESLEIMQFLPQHTIESYRQOQHLLQKQDSLSQSNMGPR
*****
XM_004914377.3  NGRSDSTFTIQHWKRNPNHSFNYMBQTLKHSPIQDLDRETYLNRSSLPQSSSGSTSP
XM_012963343.1  -----SSLPQSSSGSTSP
XM_012963344.2  -----SSLPQSSSGSTSP
XM_004914376.3  -----SSLPQSSSGSTSP
XM_004914374.2  NGRSDSTFTIQHWKRNPNHSFNYMBQTLKHSPIQDLDRETYLNRSSLPQSSSGSTSP
*****
XM_004914377.3  PLGKNNMNNKLPVSQGLMFPQGRNSLTPNAMS DGHGANIPMSTHMPHTSDNLGLESQ
XM_012963343.1  PLGKNNMNNKLPVSQGLMFPQGRNSLTPNAMS DGHGANIPMSTHMPHTSDNLGLESQ
XM_012963344.2  PLGKNNMNNKLPVSQGLMFPQGRNSLTPNAMS DGHGANIPMSTHMPHTSDNLGLESQ
XM_004914376.3  PLGKNNMNNKLPVSQGLMFPQGRNSLTPNAMS DGHGANIPMSTHMPHTSDNLGLESQ
XM_004914374.2  PLGKNNMNNKLPVSQGLMFPQGRNSLTPNAMS DGHGANIPMSTHMPHTSDNLGLESQ
*****
XM_004914377.3  LPPSLSLPSTSHCTPPPPPPFSDCSIASHLG-----L
XM_012963343.1  LPPSLSLPSTSHCTPPPPPPFSDCSIASHLG-----L
XM_012963344.2  LPPSLSLPSTSHCTPPPPPPFSDCSIASHLG-----L
XM_004914376.3  LPPSLSLPSTSHCTPPPPPPFSDCSIASHLG-----L
XM_004914374.2  LPPSLSLPSTSHCTPPPPPPFSDCSIASHLG-----L
*****
XM_004914377.3  DSTPSNPQVHAY-----CQVKERREFEIGMF
XM_012963343.1  DSTPSNPQVHAY-----CQVKERREFEIGMF
XM_012963344.2  DSTPSNPQVHAY-----CQVKERREFEIGMF
XM_004914376.3  ASLKPQDFRHAIWGIMHEKQHDPSTPHLLKTTSSASTVSQGSNEPAGERVIDAVRF
XM_004914374.2  ASLKPQDFRHAIWGIMHEKQHDPSTPHLLKTTSSASTVSQGSNEPAGERVIDAVRF
*****
XM_004914377.3  ILSCLLYHPGPEHLL-----SD
XM_012963343.1  ILSCLLYHPGPEHLL-----SD
XM_012963344.2  LKHSPT--FHSWHP-----
XM_004914376.3  TLRCGTIEFFPRDMDNFFDLSTRNKQQRKEEGE
XM_004914374.2  TLRCGTIEFFPRDMDNFFDLSTRNKQQRKEEGE
*****
```

Mcidas

```
XM_002941110.4  MQNRRKAFDKLCPNTMPDLSRRMGRQSKTEKLHKNIPTWGPVTIIVDPSSVDSAL
XM_04910354.3  MQNRRKAFDKLCPNTMPDLSRRMGRQSKTEKLHKNIPTWGPVTIIVDPSSVDS-AL
XM_04910355.3  -----MAVQMCYTHFPTTG
*****
XM_002941110.4  ATIDWQSLADCNVIGQDAAGGALTEQ000GHCILPGESDFDQEFDAVDQFIADQPSIM
XM_04910354.3  ATIDWQSLADCNVIGQDAAGGALTEQ000GHCILPGESDFDQEFDAVDQFIADQPSIM
XM_04910355.3  -YDM-----GQCLPCGESDFDQEFDAVDQFIADQPSIM
*****
XM_002941110.4  QPGLGSSGFQJPCNGSVFEPFCMTDTLQPOPDHLLPISQVOSTPSTQYNRDVAHNBQKALG
XM_04910354.3  QPGLGSSGFQJPCNGSVFEPFCMTDTLQPOPDHLLPISQVOSTPSTQYNRDVAHNBQKALG
XM_04910355.3  QPGLGSSGFQJPCNGSVFEPFCMTDTLQPOPDHLLPISQVOSTPSTQYNRDVAHNBQKALG
*****
XM_002941110.4  DALVENNQIQQVSLTEKQEEIASLKEKNITQNELANQAKHLASVLDLMLKERPKHSSGATQ
XM_04910354.3  DALVENNQIQQVSLTEKQEEIASLKEKNITQNELANQAKHLASVLDLMLKERPKHSSGATQ
XM_04910355.3  DALVENNQIQQVSLTEKQEEIASLKEKNITQNELANQAKHLASVLDLMLKERPKHSSGATQ
*****
XM_002941110.4  GRLEVKRSLEDFYPQSNFEDSTQVDEILREISKKCNALMAGNELSEKRPLEPMDTNGW
XM_04910354.3  GRLEVKRSLEDFYPQSNFEDSTQVDEILREISKKCNALMAGNELSEKRPLEPMDTNGW
XM_04910355.3  GRLEVKRSLEDFYPQSNFEDSTQVDEILREISKKCNALMAGNELSEKRPLEPMDTNGW
*****
XM_002941110.4  QEEGITIKMCGAFNGLTSTCLNSVNLGTELEEDVSFTSISKEHSTINTLAFPGCNAP
XM_04910354.3  QEEGITIKMCGAFNGLTSTCLNSVNLGTELEEDVSFTSISKEHSTINTLAFPGCNAP
XM_04910355.3  QEEGITIKMCGAFNGLTSTCLNSVNLGTELEEDVSFTSISKEHSTINTLAFPGCNAP
*****
XM_002941110.4  TIRTAAGGVKFRWVPH
XM_04910354.3  TIRTAAGGVKFRWVPH
XM_04910355.3  TIRTAAGGVKFRWVPH
*****
```

Grl1

```
XM_004914477.3  -----MMSINGDESA
XM_002938890.4  MTQDYDNKRPLVLQNDGLYQQRSTYSDEDAWKSFLNPLTAATKAMSINGDESA
XM_012963461.2  MTQDYDNKRPLVLQNDGLYQQRSTYSDEDAWKSFLNPLTAATKAMSINGDESA
XM_002938889.4  MTQDYDNKRPLVLQNDGLYQQRSTYSDEDAWKSFLNPLTAATKAMSINGDESA
XM_04914473.3  MTQDYDNKRPLVLQNDGLYQQRSTYSDEDAWKSFLNPLTAATKAMSINGDESA
*****
XM_004914477.3  LGLLYDYKVPFRERPSAAKQEHHDQEHNNKGLPQINEALLSENQVGLKTVFPFI
XM_002938890.4  LGLLYDYKVPFRERPSAAKQEHHDQEHNNKGLPQINEALLSENQVGLKTVFPFI
XM_012963461.2  LGLLYDYKVPFRERPSAAKQEHHDQEHNNKGLPQINEALLSENQVGLKTVFPFI
XM_002938889.4  LGLLYDYKVPFRERPSAAKQEHHDQEHNNKGLPQINEALLSENQVGLKTVFPFI
XM_04914473.3  LGLLYDYKVPFRERPSAAKQEHHDQEHNNKGLPQINEALLSENQVGLKTVFPFI
*****
XM_004914477.3  VVPLAQVDRKRGHLLTPDTTAAVITSILPANH IKTESQSHCSFVGLGSVFHTEPTEIRIVT
XM_002938890.4  VVPLAQVDRKRGHLLTPDTTAAVITSILPANH IKTESQSHCSFVGLGSVFHTEPTEIRIVT
XM_012963461.2  VVPLAQVDRKRGHLLTPDTTAAVITSILPANH IKTESQSHCSFVGLGSVFHTEPTEIRIVT
XM_002938889.4  VVPLAQVDRKRGHLLTPDTTAAVITSILPANH IKTESQSHCSFVGLGSVFHTEPTEIRIVT
XM_04914473.3  VVPLAQVDRKRGHLLTPDTTAAVITSILPANH IKTESQSHCSFVGLGSVFHTEPTEIRIVT
*****
XM_004914477.3  FDRTVPSDHTFNSQPPNSQRTPTDSTFSETYKEDVPEVFPDPLSRMGSNSEDYVFD
XM_002938890.4  FDRTVPSDHTFNSQPPNSQRTPTDSTFSETYKEDVPEVFPDPLSRMGSNSEDYVFD
XM_012963461.2  FDRTVPSDHTFNSQPPNSQRTPTDSTFSETYKEDVPEVFPDPLSRMGSNSEDYVFD
XM_002938889.4  FDRTVPSDHTFNSQPPNSQRTPTDSTFSETYKEDVPEVFPDPLSRMGSNSEDYVFD
XM_04914473.3  FDRTVPSDHTFNSQPPNSQRTPTDSTFSETYKEDVPEVFPDPLSRMGSNSEDYVFD
*****
XM_004914477.3  SVAGNNFETLEASKSRLPKFGDSTMTYLNKQGYPTTLKEIGNKGIIHHPISKVRSVM
XM_002938890.4  SVAGNNFETLEASKSRLPKFGDSTMTYLNKQGYPTTLKEIGNKGIIHHPISKVRSVM
XM_012963461.2  SVAGNNFETLEASKSRLPKFGDSTMTYLNKQGYPTTLKEIGNKGIIHHPISKVRSVM
XM_002938889.4  SVAGNNFETLEASKSRLPKFGDSTMTYLNKQGYPTTLKEIGNKGIIHHPISKVRSVM
XM_04914473.3  SVAGNNFETLEASKSRLPKFGDSTMTYLNKQGYPTTLKEIGNKGIIHHPISKVRSVM
*****
XM_004914477.3  VVPADKSRBDQLRWKYNHMSRQHTAKQRCID IADYKESFNTISNIEETAYNAISFTWDL
XM_002938890.4  VVPADKSRBDQLRWKYNHMSRQHTAKQRCID IADYKESFNTISNIEETAYNAISFTWDL
XM_012963461.2  VVPADKSRBDQLRWKYNHMSRQHTAKQRCID IADYKESFNTISNIEETAYNAISFTWDL
XM_002938889.4  VVPADKSRBDQLRWKYNHMSRQHTAKQRCID IADYKESFNTISNIEETAYNAISFTWDL
XM_04914473.3  VVPADKSRBDQLRWKYNHMSRQHTAKQRCID IADYKESFNTISNIEETAYNAISFTWDL
*****
XM_004914477.3  NDEGKVFISVNCLSDFSSQGVKGLPLMLQIDTYSYNNRKNPVHRAQCQIKVFCRGA
XM_002938890.4  NDEGKVFISVNCLSDFSSQGVKGLPLMLQIDTYSYNNRKNPVHRAQCQIKVFCRGA
XM_012963461.2  NDEGKVFISVNCLSDFSSQGVKGLPLMLQIDTYSYNNRKNPVHRAQCQIKVFCRGA
XM_002938889.4  NDEGKVFISVNCLSDFSSQGVKGLPLMLQIDTYSYNNRKNPVHRAQCQIKVFCRGA
XM_04914473.3  NDEGKVFISVNCLSDFSSQGVKGLPLMLQIDTYSYNNRKNPVHRAQCQIKVFCRGA
*****
XM_004914477.3  ERKIRDEERKQSKRGKCEPSSQINTFDQVKGVLPPSHKRRDITVTFKPMOLDQTPVLF
XM_002938890.4  ERKIRDEERKQSKRGKCEPSSQINTFDQVKGVLPPSHKRRDITVTFKPMOLDQTPVLF
XM_012963461.2  ERKIRDEERKQSKRGKCEPSSQINTFDQVKGVLPPSHKRRDITVTFKPMOLDQTPVLF
XM_002938889.4  ERKIRDEERKQSKRGKCEPSSQINTFDQVKGVLPPSHKRRDITVTFKPMOLDQTPVLF
XM_04914473.3  ERKIRDEERKQSKRGKCEPSSQINTFDQVKGVLPPSHKRRDITVTFKPMOLDQTPVLF
*****
XM_004914477.3  IPDVHFANLQRTHTVLP IAPDEMGEGLSPGKMKRVFSPREEDTAPAKLRVDEPKRVL
XM_002938890.4  IPDVHFANLQRTHTVLP IAPDEMGEGLSPGKMKRVFSPREEDTAPAKLRVDEPKRVL
XM_012963461.2  IPDVHFANLQRTHTVLP IAPDEMGEGLSPGKMKRVFSPREEDTAPAKLRVDEPKRVL
XM_002938889.4  IPDVHFANLQRTHTVLP IAPDEMGEGLSPGKMKRVFSPREEDTAPAKLRVDEPKRVL
XM_04914473.3  IPDVHFANLQRTHTVLP IAPDEMGEGLSPGKMKRVFSPREEDTAPAKLRVDEPKRVL
*****
```

Figure S4

A

B



Type your gene or transcript name here:

gene	transcript	avgFPKM_10	avgFPKM_16	avgFPKM_24
rfx2	XM_012955542.2	11.37	11.22	5.97
rfx2	XM_012955540.2	41.09	45.28	16.33
rfx2	NM_001123483.1	45.05	53.07	25.74

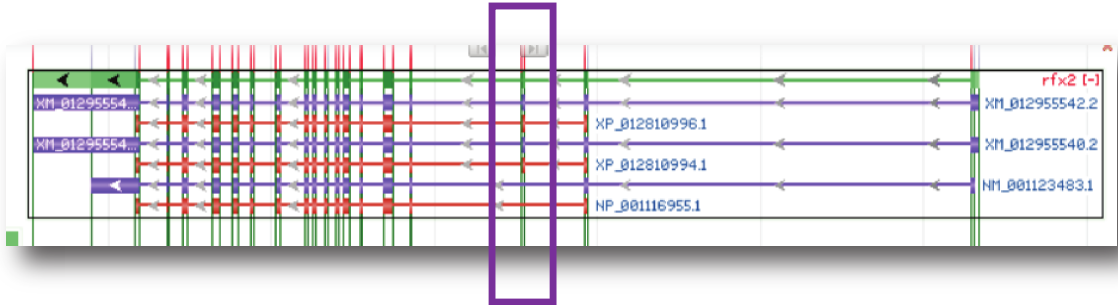
XM_012955542.2	GAGAAACCAGCATGCAGAATTCAGACAGTGGATCAGACTCTGCAACATCTGTTGCTCTAA
XM_012955540.2	GAGAAACCAGCATGCAGAATTCAGACAGTGGATCAGACTCTGCAACATCTGTTGCTCTAA
NM_001123483.1	GAGAAACCAGCATGCAGAATTCAGACAGTGGATCAGACTCTGCAACATCTGTTGCTCTAA

XM_012955542.2	GGACATCAGCATCAGCACAAGCTCCCGTAGTGCAGCCCGTACCAGCATCTCAACAGAGAA
XM_012955540.2	GGACATCAGCATCAGCACAAGCTCCCGTAGTGCAGCCCGTACCAGCATCTCAACAGAGAA
NM_001123483.1	GGACATCAGCATCAGCACAAGCTCCCGTAGTGCAGCCCGTACCAGCATCTCAA-----

XM_012955542.2	GTTTGCTCTCAAGCATCTGGCTCTGCTCCCAAAGGGGCACATGGTCAGGGTATCTCTGTAC
XM_012955540.2	GTTTGCTCTCAAGCATCTGGCTCTGCTCCCAAAGGGGCACATGGTCAGGGTATCTCTGTAC
NM_001123483.1	-----

XM_012955542.2	CCAGAGCCACCAAGTTACAGCAGCAGGTCCAGACTGTCCAGCATGTCTACCCAGCTCAAG
XM_012955540.2	CCAGAGCCACCAAGTTACAGCAGCAGGTCCAGACTGTCCAGCATGTCTACCCAGCTCAAG
NM_001123483.1	-----CAGGTCCAGACTGTCCAGCATGTCTACCCAGCTCAAG

C



The *Xenopus* Animal Cap Transcriptome – Building a Mucociliary Epithelium

Alessandro Angerilli¹, Pawel Smialowski^{2,3} and Ralph A.W. Rupp^{1,*}

1 Molecular Biology Division, Biomedical Center, Ludwig-Maximilians-University München, D-82152 Martinsried

2 Bioinformatic Core Facility, Biomedical Center, Ludwig-Maximilians-University München, D-82152 Martinsried

3 Helmholtz Zentrum München, Institute of Stem Cell Research, Ingolstädter Landstraße 1, D-85764 Neuherberg-München

Supplementary Data Set

qRT/PCR analysis

Total RNA of 15 embryos was extracted using Trizol (Ambion) and phenol/chloroform extracted. The RNA was precipitated with 70% Isopropanol and cleaned using the RNeasy Cleanup Kit (Qiagen) including DNaseI-on-column digestion. For qPCR analysis 1 µg of total RNA was reverse-transcribed with the DyNAmo CDNA Sythesis Kit (Biozym). The PCR amplification was performed in technical triplicates with the Fast SYBR Green Master mix (Applied Biosystems) in a Lightcycler 480 II (Roche). Primer sequences are given in Table S12. Relative gene expression is normalized to ODC and to the embryo.

Ectodermal purity of RNA-Seq data

AC explants can become contaminated with adjacent mesodermal cells during manual dissection. To obtain a quantitative estimate of such a contamination, we have measured by qRT/PCR the transcript levels of seven structural muscle genes both in embryos and in the very same AC samples, used for the RNA-Seq analysis. The selected genes are expressed at very high levels in *Xenopus tropicalis* (e.g. *actc1*: ~3x10¹⁰ TPE at NF24; Xenbase) and therefore represent sensitive means to evaluate a contamination in our data set. The normalized mRNA levels for these muscle genes are on average 25-fold higher in embryos, compared to explants (Supplementary Figure S4).

In addition, one has to consider the cellular dilution factor for these transcripts in total embryonic RNA. Based on data for the closely related *X. laevis*, a hatched tadpole (NF37/38) contains about 90 somites in total, each consisting of 35 cells or less (Xenbase, XAO ID: 0000058; A.G. Jacobson 1988, PMID: 3077109). In tailbud embryos, the endpoint of our analysis (NF24), about 30 somites have been formed (Nieuwkoop and Faber, 1994), with the presegmented mesoderm lacking behind in differentiation and muscle gene expression. As a conservative estimate (not all somitic cells become muscle), an embryo contains between 1050 (NF24) and 3150 myocytes (NF37/38). Furthermore, based on independent and consenting DNA measurements, a tailbud stage embryo consists of about $1,0 - 1,5 \times 10^5$ cells in total (Thiebaud & Fischberg 1977, PMID: 837803; Dawid 1965, PMID: 5892910). Based on these numbers, the cellular expression levels of striated muscle genes, as shown in Figure S4, are estimated for the embryo to be diluted between 32- and 143-fold. Combined with the 25-fold lower expression of these genes in the ACs, the degree of contamination with muscle-derived mRNAs is estimated to be around 0.1%.

Supplementary Figures:

Figure S1: Temporal expression of germ layer-specific regulatory genes. Expression of selected potential pluripotency associated genes (A), mesendodermal genes (B) and ectodermal genes (C) was plotted at the three developmental stages as normalized read counts. Note the different y-axis scales.

Figure S2: mRNA expression of striated muscle markers in embryos and ACs

To evaluate possible mesodermal contamination in AC samples, qRT/PCR analysis was carried out for seven, highly expressed striated muscle genes in age-matched embryos and the same ACs used for RNA-Seq. A) Relative expression levels normalized to *odc* and to the embryo. B) Individual and average difference in mRNA abundance between embryonic and AC-derived RNA samples.

Figure S3: RNA splice isoforms with predicted coding differences. Alignment of the coding sequence of selected splice variants for *rfx2*, *mcidas*, *tp63* and *grhl1*, represented by the unique NCBI transcripts as indicated. The alignment was

generated using MUSCLE (EMBL-EBI). For practical reasons not all splicing variants from these gene were aligned. The graph is showing only the regions displaying heterogeneity in the open reading frame.

Figure S4: Predicted RFX2 protein variants. Panel A) Rfx2 splicing isoforms and their relative expression retrieved from the Web Tool „Skin_differentiation“. A red arrow indicates the Refseq transcript. B) Nucleotide alignment of the identified isoforms highlighting a variable region. C) Transcript and protein models derived from NCBI. A magenta square marks an alternative exon that is skipped in the Refseq transcript.

Supplementary Tables:

Table S1: Expression of conserved pluripotency genes derived from ref. 40. Highlighted in orange are genes whose expression decreases over development.

Table S2: Gene expression in animal caps compared to the embryo at neurula stage (NF16) with a cutoff $p < 0.01$.

Table S2: Gene expression in animal caps compared to the embryo at neurula stage (NF16) with a cutoff $p < 0.01$.

Table S3: Genes that are differentially expressed between the analyzed stages with a cutoff $p < 0.01$. The table is composed of 3 sections, one for each pairwise comparison.

Table S4: Validation of reported epidermal marker genes.

Our gene lists of differentially expressed AC genes from Tables S2 and S3 were compared to published gene signatures of epidermal cell types. Section “Briggs 1”: Potential cell fate regulators derived from Data S3 of ref. 41. Section “Briggs 2”: Marker genes of epidermal cell types derived from Data S4 of ref. 41. Note: „Xetrov“ RNA identifiers cannot be matched to *Xenopus tropicalis* genome annotation version 9.0. (28). Section “not expressed/not modulated” details genes not expressed and genes, whose expression does not change significantly during the time course.

Section “Quigley”: upregulated Core MCC genes from ref. 42. The overall coverage of the core MCC genes in our data is therefore 84% (see accompanying graph in this table section).

Table S5: Expression of RNA splice variants in ACs compared to the whole embryo at NF16 with a cutoff $p < 0.01$.

Table S6: RNA splice variants that are differentially expressed between the analysed stages with a cutoff $p < 0.01$. Gene ID and transcript ID (=trID) follow NCBI transcript nomenclature in annotation release 103. The table is composed of 3 sections, one for each comparison.

Table S7: Genes that have at least 2 splicing variants, one of which is prevalent in ACs and another one that is prevalent in the embryo with a p-value threshold of 0.05. Transcripts from *cpne1* and *evpl* genes are top responders on this list.

Table S8: Repetitive DNA elements that are differentially expressed between ACs and the embryo at NF10.5 with a cutoff $p < 0.01$.

Table S9: Repetitive elements that are differentially expressed between the analyzed stages with a cutoff $p < 0.01$. The table is composed of 3 sections, one for each comparison.

Table S10: Circular RNAs expressed at each given time point in each single biological replicate. The ones, analysed in Figure 7, are highlighted in grey. The table is composed of 3 sections, one for each developmental stage.

Table S11: Transcription factors list generated using the Mogrify algorithm. Each TF is ranked based on its individual weight on the entire transcriptome (column ii). Neg_Rank lists TFs specific for the younger stage in the comparison, Pos_Rank lists those that are specific for the older stage in each pairwise comparison. V1 - gene name, V2 - ENSEMBLE identifier number, SumAbs - value from STRING analysis, V8 - value calculated from the log2 fold change of expression, V8i, sumAbsi - relative rankings, ii - combined ranking of a TF.

Table S12: Oligonucleotide primer sequences used in this paper: blue - primers used for the circularRNA analysis; green - primers used to clone the RNA splice isoform-specific probes; yellow - primers used to clone the probes for repetitive DNA elements, orange primers used for qPCR.

Supplementary Movies:

Movie S1: NF24 AC

Movie S2: NF24 Embryo