

## **Supplementary Material**

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### **1) Details of the phylogenetic analysis**

Phylogenetic relationships of the human bHLH domains were inferred by the Neighbor-Joining method using the Jones-Taylor-Thornton amino-acid replacement model, implemented with the PHYLIP package ([evolution.genetics.washington.edu/phylip.html](http://evolution.genetics.washington.edu/phylip.html)).

Sequences were taken from:

- a) Homo sapiens.*
- b) Ciona intestinalis.*
- c) Drosophila melanogaster.*
- d) Saccharomyces cerevisiae.*
- e) Kluyveromyces lactis.*
- f) Schisosaccharomyces pombe.*
- g) Neurospora crassa.*

The gene and ORF names for *Homo sapiens* and *Drosophila melanogaster* were taken from (Ledent *et al.*, 2002). The sequences of *Ciona intestinalis*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Schisosaccharomyces pombe*, *Neurospora crassa* were identified by scanning ORF lists of their genomes, using Hidden Markov Models. The names correspond to their ORFs.

The multiple alignment of the bHLH domains was performed by clustalw (Tompson et al., 1994) and edited manually.

The coloured branches were used to reconstruct a summary of the tree shown in Figure 2a of the paper. That summary tree in Figure 2a is a cladogram and does not represent distances, contrary to the Neighbour Joining tree shown here.

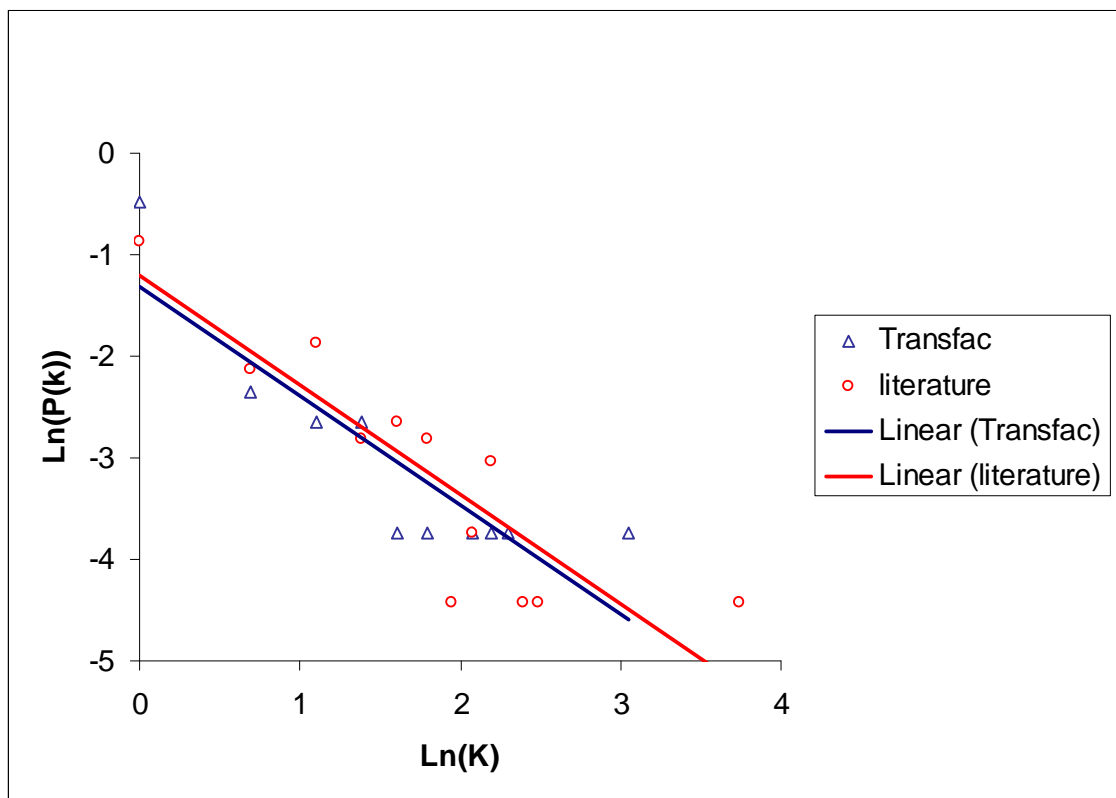
#### References

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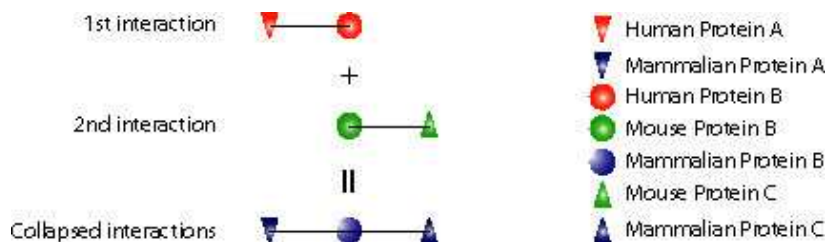
## 2) Details of the scale-free network analysis

For the mammalian bHLH protein-interaction networks, we calculated the logarithm of the frequency of nodes with  $K$  interactions,  $\text{Ln}(P(K))$ , and plotted this against the logarithm of the number of interactions,  $\text{Ln}(K)$ . The plot of  $\text{Ln}(P(K)) - \text{Ln}(K)$  shows clearly that the bHLH protein interaction network is scale-free, because the distribution of connectivity decays as a power-law ( $P(K) \sim K^{-\gamma}$ , where  $\gamma=1$ ). Moreover, the interactions that were extracted solely from Transfac were sufficient to display the scale-free nature of the network.



### 3) Protein-protein interactions

We extracted protein interactions for *Drosophila* and mammalian members of the bHLH class, mediated through the Basic Region - Helix-Loop-Helix domain. Protein interactions for mammals were extracted for both human and murine orthologues, due to their high amino-acid sequence identity (>80%). For example, the human A-factor<sub>(human)</sub> – B-factor<sub>(human)</sub> and mouse B-factor<sub>(mouse)</sub> – C-factor<sub>(mouse)</sub> interactions were collapsed in the mammalian A-factor<sub>(mammalian)</sub> – B-factor<sub>(mammalian)</sub> – C-factor<sub>(mammalian)</sub> interactions.



Protein interactions were initially extracted automatically from the TRANSFAC database (public release v4; Matys *et al.*, 2003). Later, this dataset was significantly enhanced by manual searches of PubMed ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). The list of *Drosophila*, murine, and human bHLH genes reported by Ledent *et al.* (2002) was used as our primary data set. A total of 78 mammalian genes connected by 127 interactions were retrieved. Seventeen of these interactions have been verified for the *Drosophila* orthologues as well and have been highlighted in Figure 2c with thicker lines. Most interactions have been identified by co-immunoprecipitation, Electrophoretic Mobility Shift Assay (EMSA), and Yeast Two Hybrid (Y2H) assays.

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#### 4) List of protein-protein interactions

##### a) Mammals

##### i) E-protein Subnetwork interactions

E2A interacts with:

ABF-1	Massari et al., 1998
AP-4	Dear et al., 1997
BETA3	Peyton et al., 1996
BHLHB4	Bramblett et al., 2002
BHLHB5	Xu et al., 2002
Capsulin	Lu et al., 1998
cHairy1	Leimeister et al., 2000
cMesol	Buchburger et al., 1998
Dermo-1	Dear et al., 1997
dHAND	Yoon et al., 2000
E2A	Dear et al., 1997
E2-2	Yoon et al., 2000
eHAND	Firulli et al., 2000; Yoon et al., 2000
Eip-1 (HES)	Dear et al., 1997
Hash-1	Persson et al., 2000
HEB	Dear et al., 1997
HEN-1	Brown & Baer, 1994
Hey2	Leimeister et al., 2000
Id-1	Langlands et al., 1997
Id-2	Yoon et al., 2000; Langlands et al., 1997
Id-3	Yoon et al., 2000; Langlands et al., 1997
Id-4	Jogi et al., 2002

Lyl-1	Miyamoto et al., 1996
Mash-1	Gradwohl et al., 1996; Li et al., 1995; Persson et al., 2000; Yoon et al., 2000
Mash-2	Johnson et al., 1992
Mash-3	Yoshida et al., 2001
Math-1	Yoon et al., 2000; Akazawa et al., 1995
Math-2	Shimizu et al., 1995
mesogenin1	Yoon et al., 2000
Mist-1	Lemercier et al., 1998
MRF-4/myf6	Lin & Konieczny, 1992; Yoon et al., 2000
Myf-5	Braun et al., 1990
MyoD	Dear et al., 1997; Li et al., 1995; Petropoulos & Skerjanc, 2000
Myogenin	Chakraborty et al., 1991; Li et al., 1995
NeuroD	Mutoh et al., 1997
NeuroD2	Farah et al., 2000
Ngn-2	Gradwohl et al., 1996
N-twist	Verzi et al., 2002
OUT(mouse)	Narumi et al., 2000
Paraxis	Dear et al., 1997; Quertermus et al., 1994; Yoon et al., 2000
Scleraxis	Dear et al., 1997, Li et al., 1995; Cserjesi et al., 1995; Carlberg et al., 2000
Stra-13	Dear et al., 1997
Tal-1	Dear et al., 1997; Hsu et al., 1994
Tal-2	Xia et al., 1994; Li et al., 1995
Twist	Hamamori et al., 1997
USF	Dear et al., 1997

E2-2 interacts with:

ABF-1	Massari et al., 1998
Capsulin	Lu et al., 1998
Hash-1	Persson et al., 2000
Id-1	Langlands et al., 1997

Id-2	Langlands et al., 1997
Id-3	Langlands et al., 1997
Id-4	Jogi et al., 2002
Mash-3	Yoshida et al., 2001
MyoD	Petropoulos & Skerjanc, 2000

HEB interacts with:

ABF-1	Massari et al., 1998
Capsulin	Lu et al., 1998
Id-1	Langlands et al., 1997
Id-2	Langlands et al., 1997
Id-3	Langlands et al., 1997
Mash-3	Yoshida et al., 2001
MyoD	Petropoulos & Skerjanc, 2000

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## ii) Arnt-network interactions

Arnt interacts with:

Ahr	Hahn, 2002; Swanson, 2002
AhrR	Hahn, 2002
BMAL1	Hogenesch et al., 1997
Hif2a	Hogenesch et al., 1997; Gu et al., 2000; Swanson, 2002
Hif1a	Hogenesch et al., 1997; Gu et al., 2000; Swanson, 2002
Hif3a	Gu et al., 2000
NPAS1	Hogenesch et al., 1997; Gu et al., 2000
PER	Hogenesch et al., 1997

Sim1	Swanson, 2002; Chrast et al., 1997
Sim2	Swanson, 2002; Chrast et al., 1997
Hey1	Chin et al., 2000
Hey2	Chin et al., 2000; Swanson, 2002

Arnt2 interacts with:

Hif2a	Gu et al., 2000
Hif1a	Gu et al., 2000
Hif3a	Gu et al., 2000
Sim1	Chrast et al., 1997
Sim2	Chrast et al., 1997

BMAL-1 interacts with:

Dec1	Honma et al., 2002
Dec2	Honma et al., 2002
HIF1a	Gu et al., 2000
Arnt	Hogenesch et al., 1997

BMAL2 interacts with:

HIF1a	Hogenesch et al., 2000
CLOCK	Hogenesch et al., 2000
NPAS2	Hogenesch et al., 2000

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### iii) Max network interactions

Max interacts with:

c-Myc	Luscher, 2001
L-Myc	Luscher, 2001
Mad1	Luscher, 2001
Mad3	Luscher, 2001
Mad4	Luscher, 2001
Max	Luscher, 2001
Mga	Luscher, 2001
Mnt	Luscher, 2001

Mxi1            Luscher, 2001

N-Myc           Luscher, 2001

Mlx interacts with:

Mad1            Luscher, 2001

Mad4            Luscher, 2001

Mlx              Luscher, 2001

Mnt              Luscher, 2001; Baudino & Cleveland, 2001

MondoA         Luscher, 2001

Wbscr14        Luscher, 2001

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#### **iv) Other Interactions**

dHAND interacts with:

dHAND         Firulli et al., 2000

E2A             Firulli et al., 2000

eHAND         Firulli et al., 2000

Hey1            Firulli et al., 2000

Hey2            Firulli et al., 2000

HeyL            Firulli et al., 2000

eHAND interacts with:

Ash1		Firulli et al., 2000
dHAND		Firulli et al., 2000
E2A		Firulli et al., 2000
eHAND		Firulli et al., 2000
Hey1		Firulli et al., 2000
Hey2		Firulli et al., 2000
HeyL		Firulli et al., 2000
MyoD		Firulli et al., 2000

Other dimers:

Abf1	Id2	Wong et al., 2001
Ap4	Ap4	Hu et al., 1990
Ash3	Ash3	Yoshida et al., 2001
Ash3	MyoD	Yoshida et al., 2001
cHairy1	cHairy1	Leimeister et al., 2000
cHey1	cHairy1	Leimeister et al., 2000
cHey1	cHey1	Leimeister et al., 2000
cHey1	cHey2	Leimeister et al., 2000
cHey2	cHairy1	Leimeister et al., 2000
cHey2	cHey2	Leimeister et al., 2000
Dec1	Dec1	St-Pierre et al., 2002
Dec1	Ash1	Boudjelal et al., 1997
Hes1	Id1	Jogi et al., 2002
Hes1	Id2	Jogi et al., 2002
Hes1	Id3	Jogi et al., 2002
Hes1	Id4	Jogi et al., 2002
Id1	Myf6	Langlands et al., 1997
Id1	Myogenin	Langlands et al., 1997
Id2	Myf6	Langlands et al., 1997
Id2	Myogenin	Langlands et al., 1997

Id3	Myf5	Langlands et al., 1997
Id3	Myf6	Langlands et al., 1997
Id3	MyoD	Langlands et al., 1997
Id3	Myogenin	Langlands et al., 1997
Id4	Hes1	Jogi et al., 2002
Id4	MyoD	Jogi et al., 2002
Mist1	Mist1	Lemercier et al., 1998
Mist1	Myf6	Lemercier et al., 1998
Mist1	MyoD	Lemercier et al., 1998
Myf5	Id1	Langlands et al., 1997
Myf5	Id2	Langlands et al., 1997
MyoD	Id	Benezra et al., 1990; Puri & Sartorelli, 2000
MyoD	Id1	Langlands et al., 1997
MyoD	Id2	Langlands et al., 1997
Ngn2	Ash1	Gradwohl et al., 1996
Nsc1	Nsc1	Brown & Baer, 1994
Twist	Twist	Hamamori et al., 1997
Usf1	Usf1	Dhar & Taneja, 2001
Usf2	Usf2	Dhar & Taneja, 2001
Usf1	Usf2	Dhar & Taneja, 2001

All 4 Mitf family genes interact among them      Hemesath et al., 1994

All 4 COE interact among them      Wang et al., 2002 ; Wang et al., 1997



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#### **b) *Drosophila melanogaster* bHLH protein interactions**

A Group - Da subnetwork interactions

Da interacts with

Ac	Cabrera and Alonso, 1991
Sc	Cabrera and Alonso, 1991
Lsc	Cabrera and Alonso, 1991
Emc	Alifragis et al., 1997; Cabrera et al., 1994; Giot et al., 2003
Twist	Castanon et al., 2001
Ato	Jarman et al., 1993

nau	Zhang et al., 1999
amos	Huang et al., 2000
Scl	Giot et al., 2003
Fer3	Giot et al., 2003
m5	Alifragis et al., 1997
m7	Alifragis et al., 1997
m8	Alifragis et al., 1997
mb	Alifragis et al., 1997
mg	Alifragis et al., 1997

#### C Group - Tango subnetwork interactions

Tango interacts with:

ss	Emmons et al., 1999
Sim	Sonnenfeld et al., 1997
Sima	Sonnenfeld et al., 1997
Trh	Sonnenfeld et al., 1997

Cycle interacts with:

Clock	Bae et al., 2000
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#### B Group - Max network interactions

dMax interacts with:

dmyc	Gallant et al., 1996; Giot et al., 2003
dMnt	Orian et al., 2003; Giot et al., 2003

dMlx interacts with:

dMondoA	Ayer (personal communication); Giot et al., 2003
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Other dimers:

Emc	Ac	Cabrera et al., 1994; Alifragis et al., 1997
Emc	Sc	Cabrera et al., 1994; Alifragis et al., 1997; Giot et al., 2003
Emc	Lsc	Cabrera et al., 1994
Emc	Nscl	Giot et al., 2003
mb	mb	Alifragis et al., 1997
mb	mg	Alifragis et al., 1997
mb	md	Alifragis et al., 1997
mb	m3	Alifragis et al., 1997
mb	m5	Alifragis et al., 1997
mb	m7	Alifragis et al., 1997
mb	m8	Alifragis et al., 1997
mg	mg	Alifragis et al., 1997
mg	md	Alifragis et al., 1997
mg	m3	Alifragis et al., 1997
mg	m5	Alifragis et al., 1997
mg	m7	Alifragis et al., 1997
mg	m8	Alifragis et al., 1997
md	md	Alifragis et al., 1997
md	m3	Alifragis et al., 1997
md	m5	Alifragis et al., 1997
md	m7	Alifragis et al., 1997
md	m8	Alifragis et al., 1997
m3	m5	Alifragis et al., 1997
m5	m5	Alifragis et al., 1997
m6	m7	Alifragis et al., 1997
m7	m8	Alifragis et al., 1997
m7	m8	Alifragis et al., 1997
m7	NSCL	Giot et al., 2003

m8	m8	Alifragis et al., 1997
ato	mb	Alifragis et al., 1997
ato	m7	Alifragis et al., 1997
ato	m3	Alifragis et al., 1997
Ac	mb	Alifragis et al., 1997
Ac	mg	Alifragis et al., 1997
Ac	m3	Alifragis et al., 1997; Giot et al., 2003
Ac	m7	Alifragis et al., 1997
Sc	m3	Alifragis et al., 1997
Sc	m7	Alifragis et al., 1997
Sc	mb	Alifragis et al., 1997
Sc	mg	Alifragis et al., 1997
Ase	m3	Alifragis et al., 1997
Ase	m7	Alifragis et al., 1997
Ase	mb	Alifragis et al., 1997
Ase	mg	Alifragis et al., 1997
Twist	Twist	Castanon et al., 2001
Hey	dpn	Giot et al., 2003
tap	mb	Giot et al., 2003
tap	m5	Giot et al., 2003

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