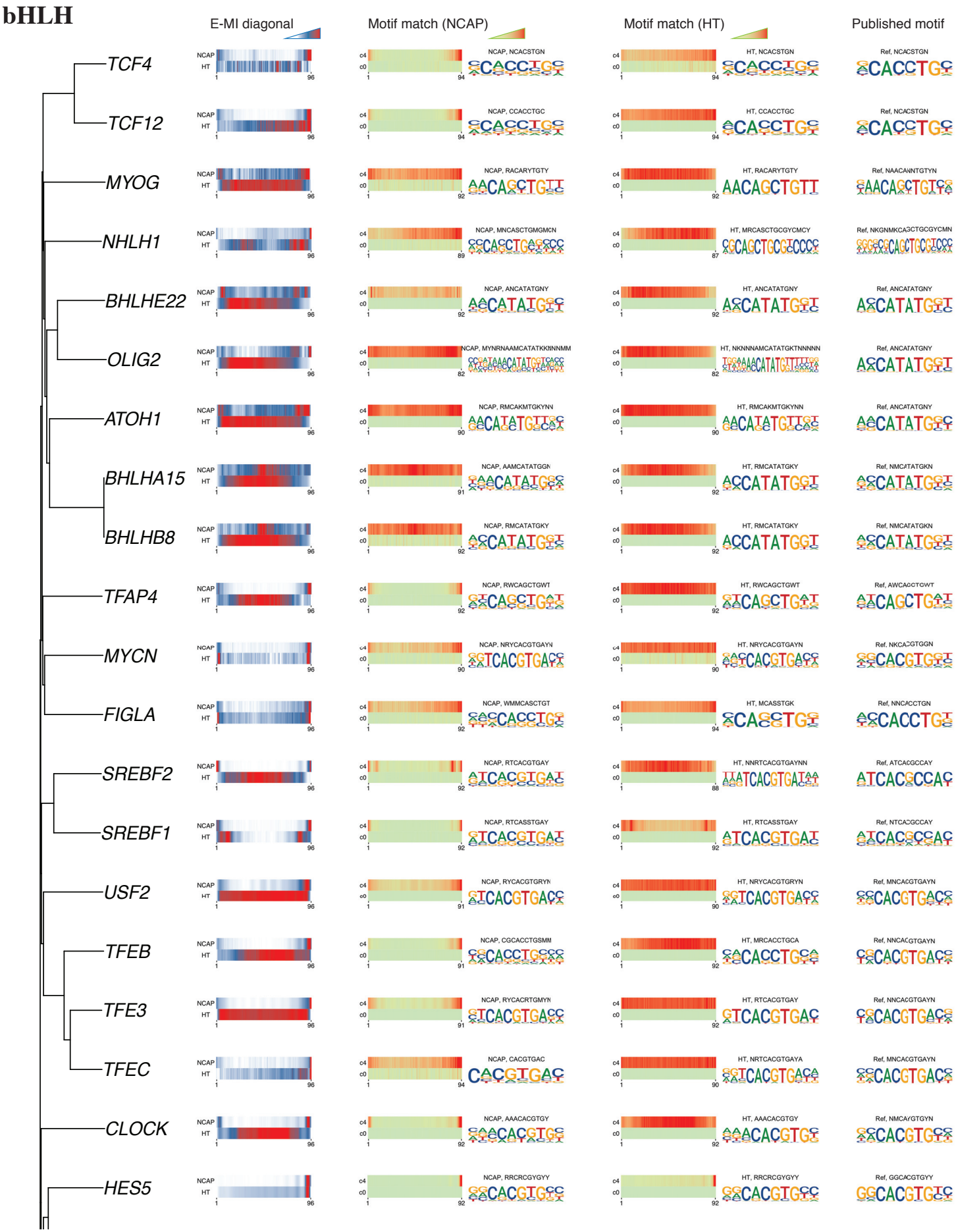
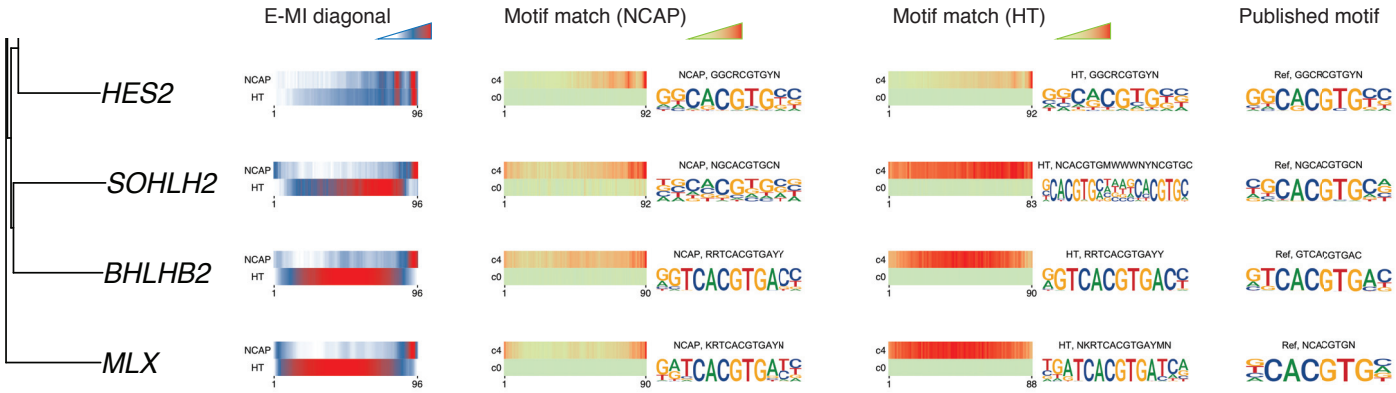
Supplementary Data 1 | TFs' binding specificities in NCAP-SELEX and HT-SELEX libraries. The phylogenetic tree was generated based on the amino acid sequences of the TF DBDs. The primary motifs were curated for lig147 NCAP-SELEX and HT-SELEX libraries with manually curated seeds (see **Method** for details). As a nucleosome wraps 147 bp DNA, we expect no flanking free DNA presents in the lig147 NCAP-SELEX. Motif matching results for each TF's cycle 4 and cycle 0 libraries were illustrated. The published motifs were from our previous curations[1,2].

In the absence of nucleosome, some TFs still show a biased distribution of motif match towards one end or both ends of the ligand. This bias potentially originates from the fixed adaptors of the SELEX ligand. The 10 bp periodic preference for some TFs in HT-SELEX (e.g. ETS factors) likely emerge from quenching of the vibrational modes of DNA[3]. The asymmetry of the ligand due to the fixed linker sequences most likely results in this pattern occurring at specific positions of the ligand. Such pattern of ETS is less obvious in NCAP-SELEX because nucleosome decreased the overall binding.

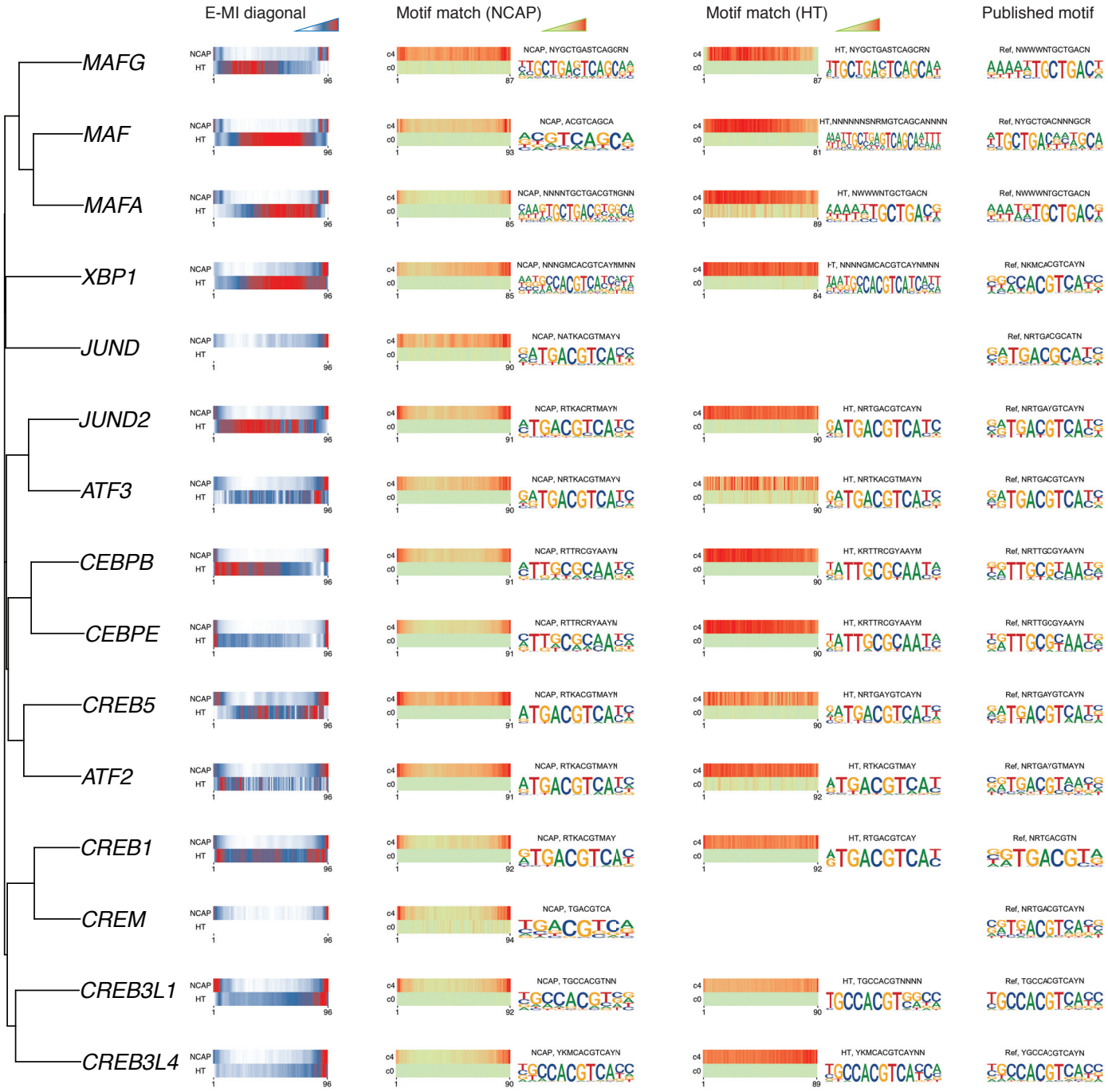
1. Yin, Y. *et al*. Impact of cytosine methylation on DNA binding specificities of human transcription factors. *Science* **356**, eaaj2239 (2017).
2. Jolma, A. *et al*. DNA-binding specificities of human transcription factors. *Cell* **152**, 327-339 (2013).
3. Kim, S. et al. Probing Allostery Through DNA. Science **339**, 816-819 (2013).


bHLH
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
TCF4
TCF12
MYOG
NHLH1
BHLHE22
OLIG2
ATOH1
BHLHA15
BHLHB8
TFAP4
MYCN
FIGLA
SREBF2
SREBF1
USF2
TFEB
TFE3
TFEC
CLOCK
HES5
NCAP
HT

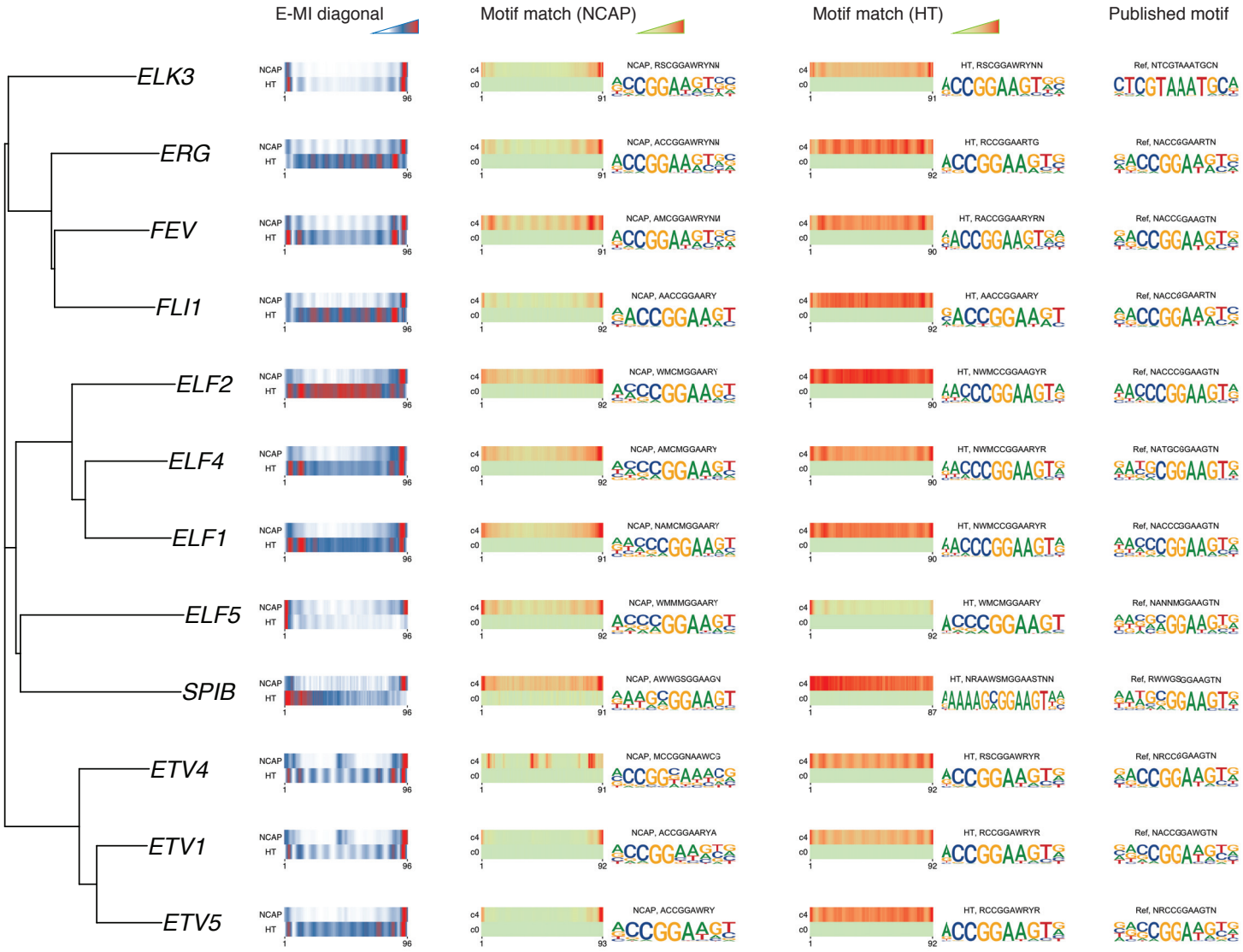
bHLH


E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
HES2
SOHLH2
BHLHB2
MLX
NCAP
HT
NCAP, GGCRCGTGYN
HT, GGCRCGTGYN
Ref, GGCRCGTGYN
NCAP, NGCACGTGCN
HT, NCACGTGMWWWNYNCGTGC
Ref, NGCACGTGCN
NCAP, RRTCACGTGAYY
HT, RRTCACGTGAYY
Ref, GTCACGTGAC
NCAP, KRTCACGTGAYN
HT, NKRTCACGTGAYMN
Ref, NCACGTGN

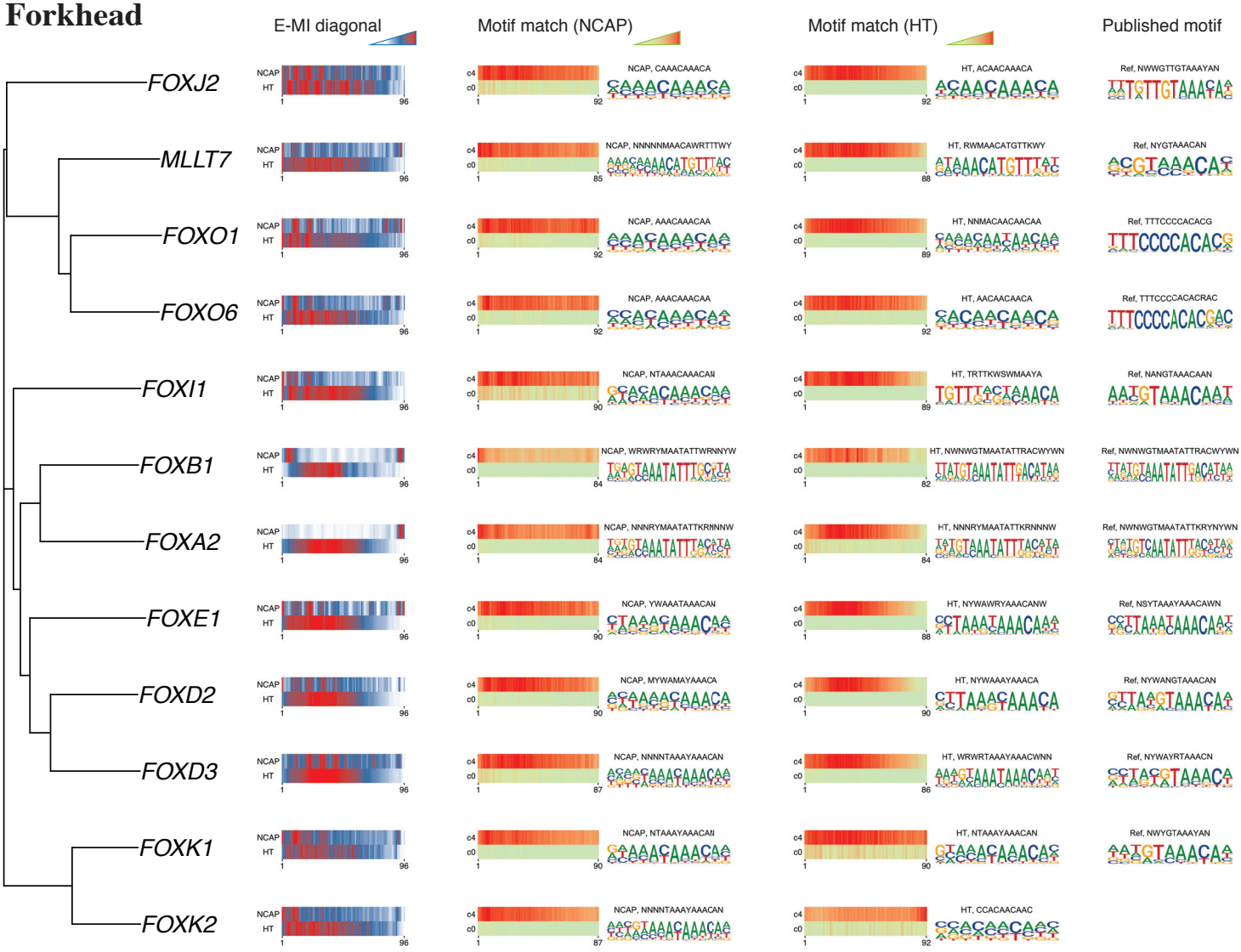
bZIP

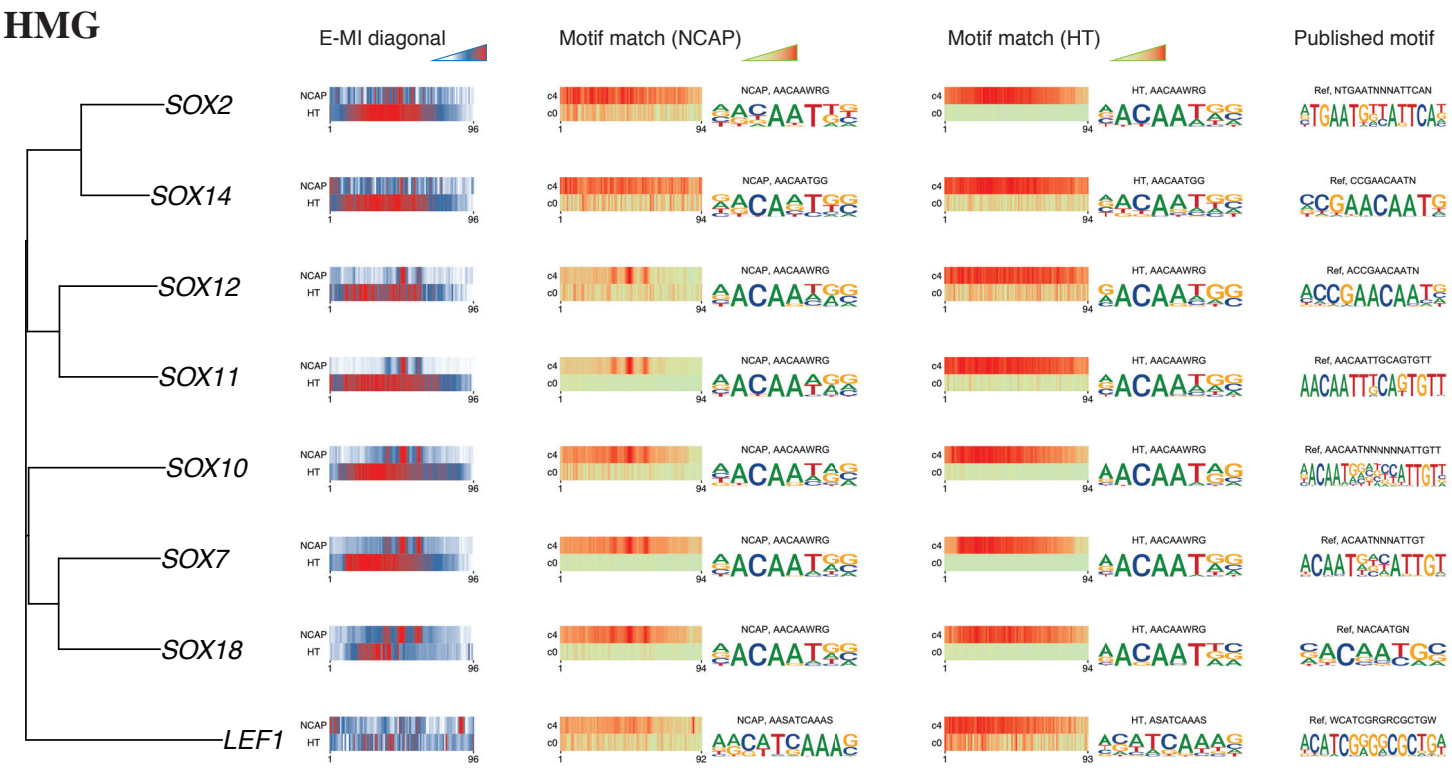

E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
MAFG
MAF
MAFA
XBP1
JUND
JUND2
ATF3
CEBPB
CEBPE
CREB5
ATF2
CREB1
CREM
CREB3L1
CREB3L4
NCAP
HT
NCAP, NYGCTGASTCAGCRN
HT, NYGCTGASTCAGCRN
Ref, NWWWNTGCTGACN
NCAP, ACGTCAGCA
HT,NNNNNNSNRMGTCAGCANNNN
Ref, NYGCTGACNNNGCR
NCAP, NNNNTGCTGACGTNGNN
HT, NWWWNTGCTGACN
Ref, NWWWNTGCTGACN
NCAP, NNNGMCACGTCAYNMNN
HT, NNNNGMCACGTCAYNMNN
Ref, NKMCACGTCAYN
NCAP, NATKACGTMAYN
Ref, NRTGACGCATN
NCAP, RTKACRTMAYN
HT, NRTGACGTCAYN
Ref, NRTGAYGTCAYN
NCAP, NRTKACGTMAYN
HT, NRTKACGTMAYN
Ref, NRTGACGTCAYN
NCAP, RTTRCGYAAYN
HT, KRTTRCGYAAYM
Ref, NRTTGCGYAAYN
NCAP, RTTRCRYAAYN
HT, KRTTRCGYAAYM
Ref, NRTTGCGYAAYN
NCAP, RTKACGTMAYN
HT, NRTGAYGTCAYN
Ref, NRTGAYGTCAYN
NCAP, RTKACGTMAYN
HT, RTKACGTMAY
Ref, NRTGAYGTMAYN
NCAP, RTKACGTMAY
HT, RTGACGTCAY
Ref, NRTGACGTN
NCAP, TGACGTCA
Ref, NRTGACGTCAYN
NCAP, TGCCACGTNN
HT, TGCCACGTNNNN
Ref, TGCCACGTCAYN
NCAP, YKMCACGTCAYN
HT, YKMCACGTCAYNN
Ref, YGCCACGTCAYN

ETS


E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
ELK3
ERG
FEV
FLI1
ELF2
ELF4
ELF1
ELF5
SPIB
ETV4
ETV1
ETV5
NCAP
HT
NCAP, RSCGGAWRYNN
HT, RSCGGAWRYNN
Ref, NTCGTAAATGCN
NCAP, ACCGGAWRYNN
HT, RCCGGAARTG
Ref, NACCGGAARTN
NCAP, AMCGGAWRYNN
HT, RACCGGAARYRN
Ref, NACCGGAAGTN
NCAP, AACCGGAARY
HT, AACCGGAARY
Ref, NACCGGAARTN
NCAP, WMCMGGAARY
HT, NWMCCGGAAGYR
Ref, NACCCGGAAGTN
NCAP, AMCMGGAARY
HT, NWMCCGGAARYR
Ref, NATGCGGAAGTN
NCAP, NAMCMGGAARY
HT, NWMCCGGAARYR
Ref, NACCCGGAAGTN
NCAP, WMMMGGAARY
HT, WMCMGGAARY
Ref, NANNMGGAAGTN
NCAP, AWWGSGGAAGN
HT, NRAAWSMGGAASTNN
Ref, RWWGSGGAAGTN
NCAP, MCCGGNAAWCG
HT, RSCGGAWRYR
Ref, NRCCGGAAGTN
NCAP, ACCGGAARYA
HT, RCCGGAWRYR
Ref, NACCGGAWGTN
NCAP, ACCGGAWRY
HT, RCCGGAWRYR
Ref, NRCCGGAAGTN

Forkhead


E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
FOXJ2
MLLT7
FOXO1
FOXO6
FOXI1
FOXB1
FOXA2
FOXE1
FOXD2
FOXD3
FOXK1
FOXK2
NCAP
HT
NCAP, CAAACAAACA
HT, ACAACAAACA
Ref, NWWGTTGTAAAYAN
NCAP, NNNNNMAACAWRTTTWY
HT, RWMAACATGTTKWY
Ref, NYGTAAACAN
NCAP, AAACAAACAA
HT, NNMACAACAACAA
Ref, TTTCCCCACACG
NCAP, AAACAAACAA
HT, AACAACAACA
Ref, TTTCCCCACACRAC
NCAP, NTAAACAAACAN
HT, TRTTKWSWMAAYA
Ref, NANGTAAACAAN
NCAP, WRWRYMAATATTWRNNYW
HT, NWNWGTMAATATTRACWYWN
Ref, NWNWGTMAATATTRACWYWN
NCAP, NNNRYMAATATTKRNNNW
HT, NNNRYMAATATTKRNNNW
Ref, NWNWGTMAATATTKRYNYWN
NCAP, YWAAATAAACAN
HT, NYWAWRYAAACANW
Ref, NSYTAAAYAAACAWN
NCAP, MYWAMAYAAACA
HT, NYWAAAYAAACA
Ref, NYWANGTAAACAN
NCAP, NNNNTAAAYAAACAN
HT, WRWRTAAAYAAACWNN
Ref, NYWAYRTAAACN
NCAP, NTAAAYAAACAN
HT, NTAAAYAAACAN
Ref, NWYGTAAAYAN
NCAP, NNNNTAAAYAAACAN
HT, CCACAACAAC


HMG
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
SOX2
SOX14
SOX12
SOX11
SOX10
SOX7
SOX18
LEF1
NCAP
HT
NCAP, AACAAWRG
NCAP, AACAATGG
NCAP, AASATCAAAS
HT, AACAAWRG
HT, AACAATGG
HT, ASATCAAAS
Ref, NTGAATNNNATTCAN
Ref, CCGAACAATN
Ref, ACCGAACAATN
Ref, AACAATTGCAGTGTT
Ref, AACAATNNNNNNATTGTT
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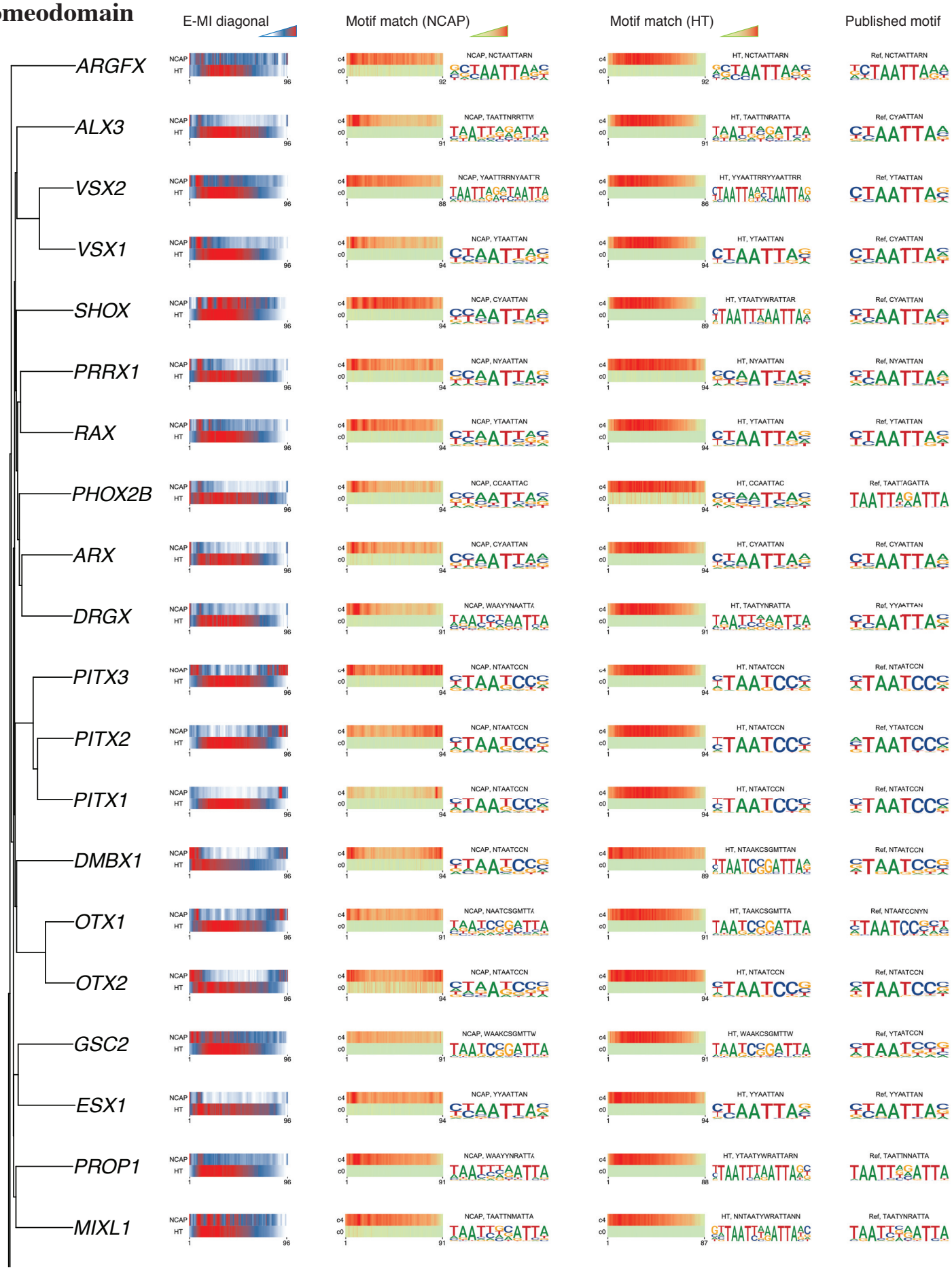
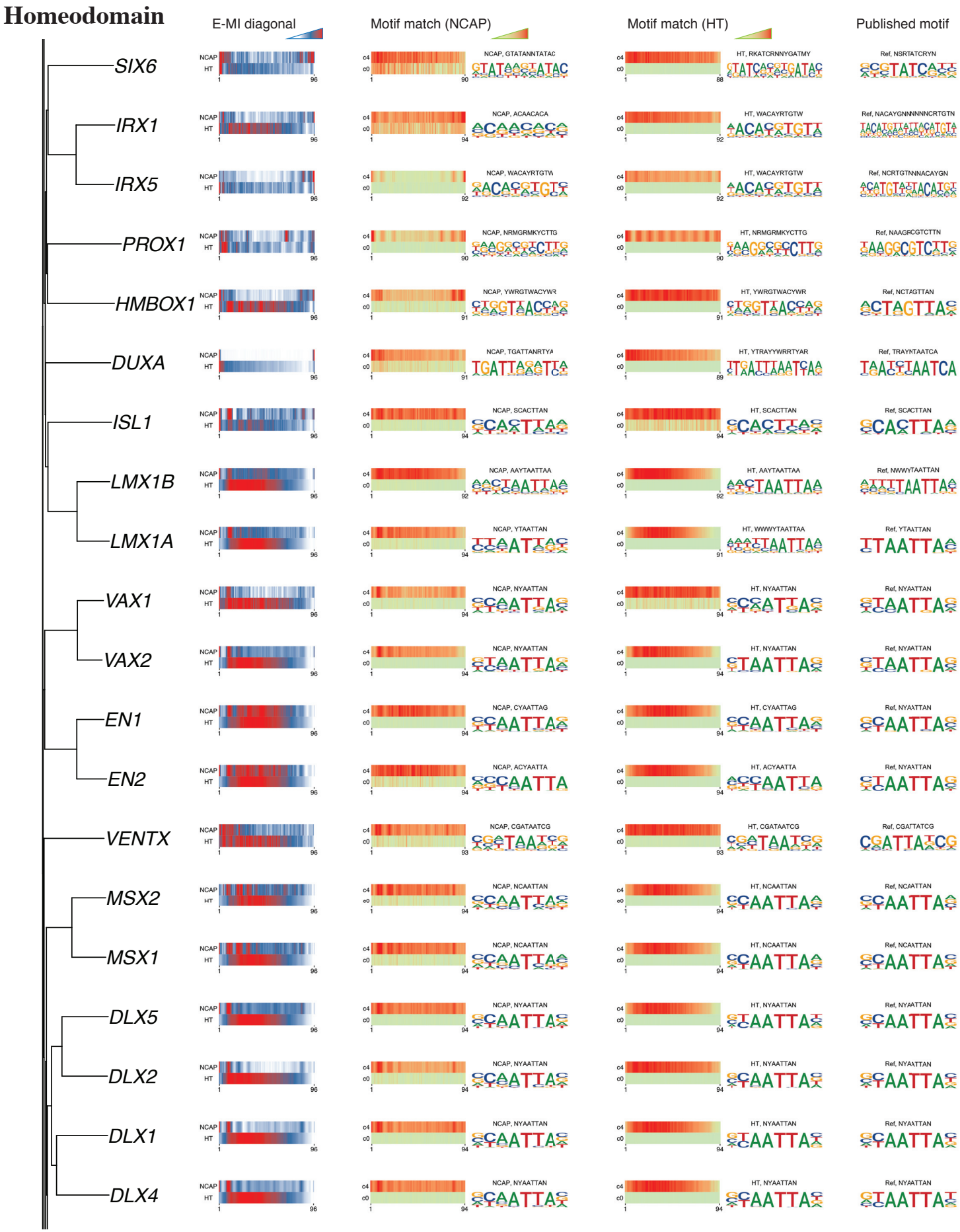
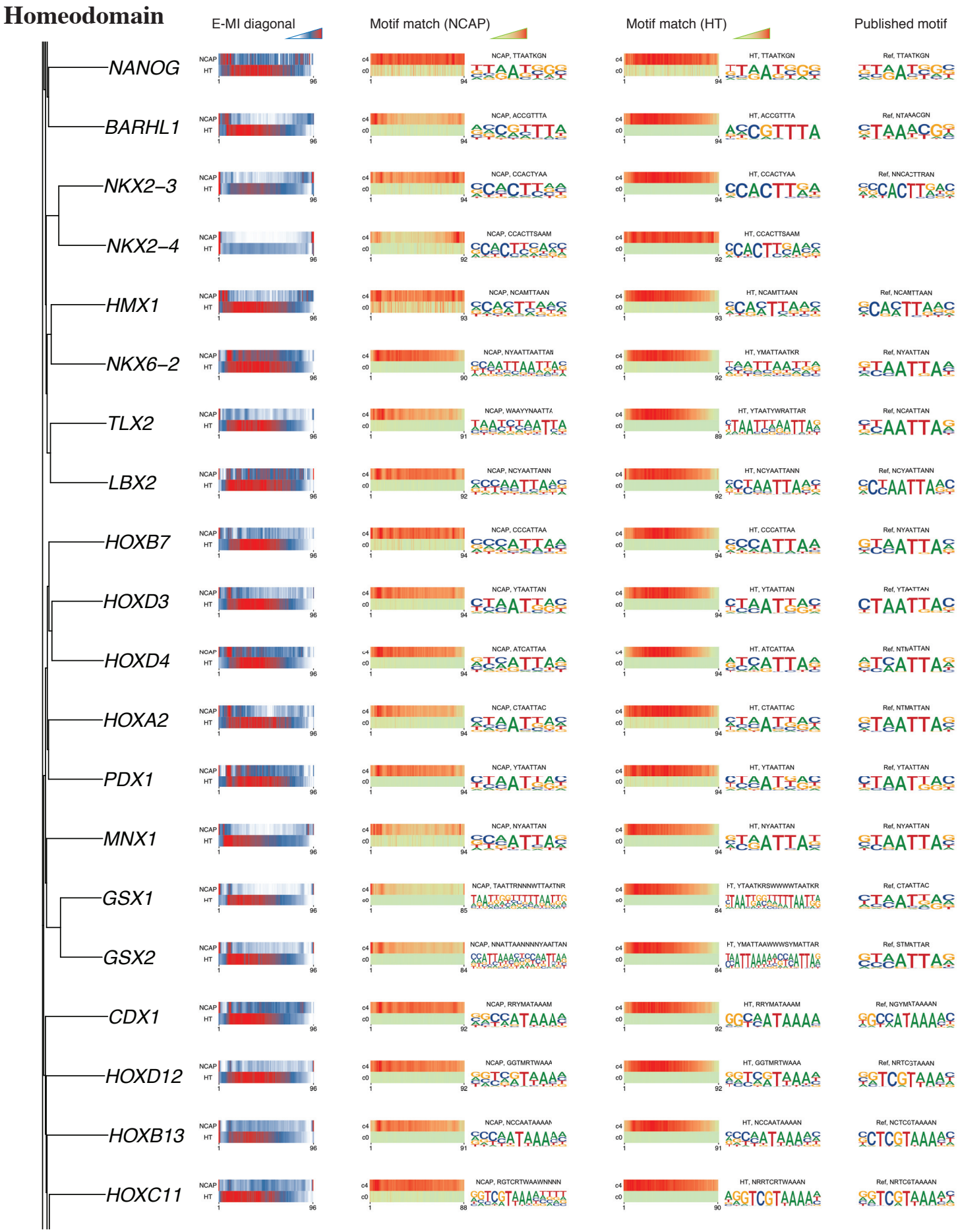
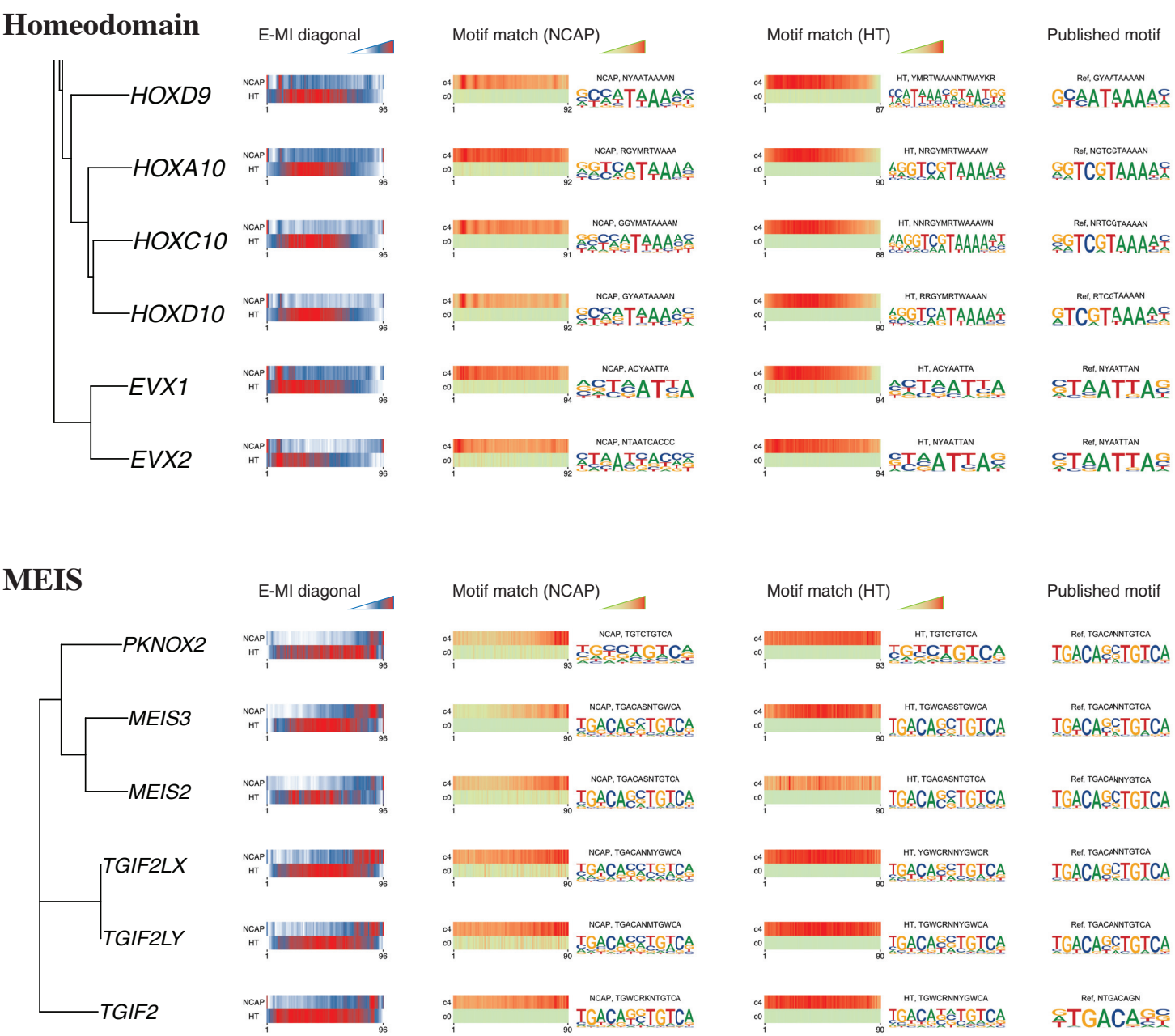
Ref, NACAATGN
Ref, WCATCGRGRCGCTGW

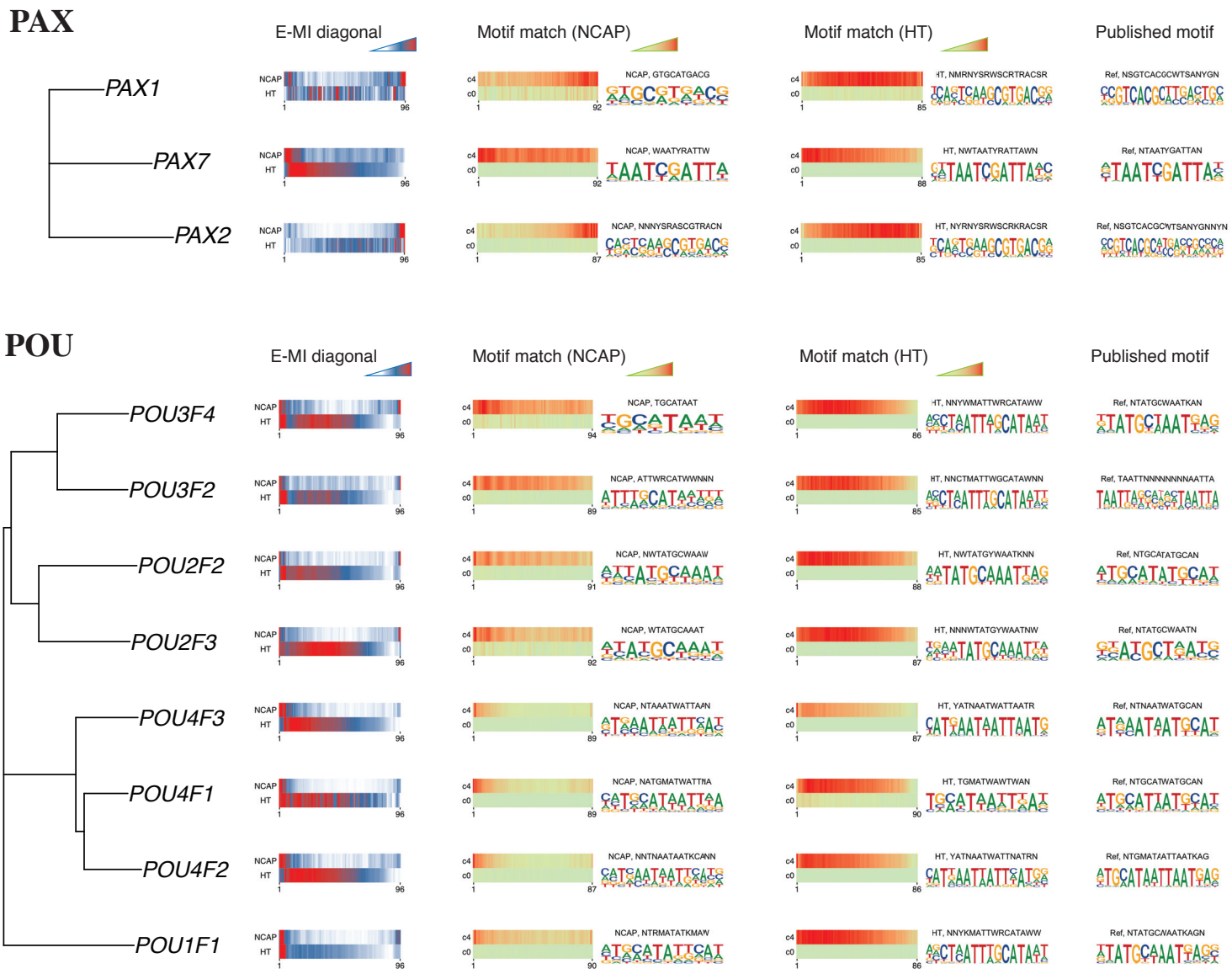
Homeodomain

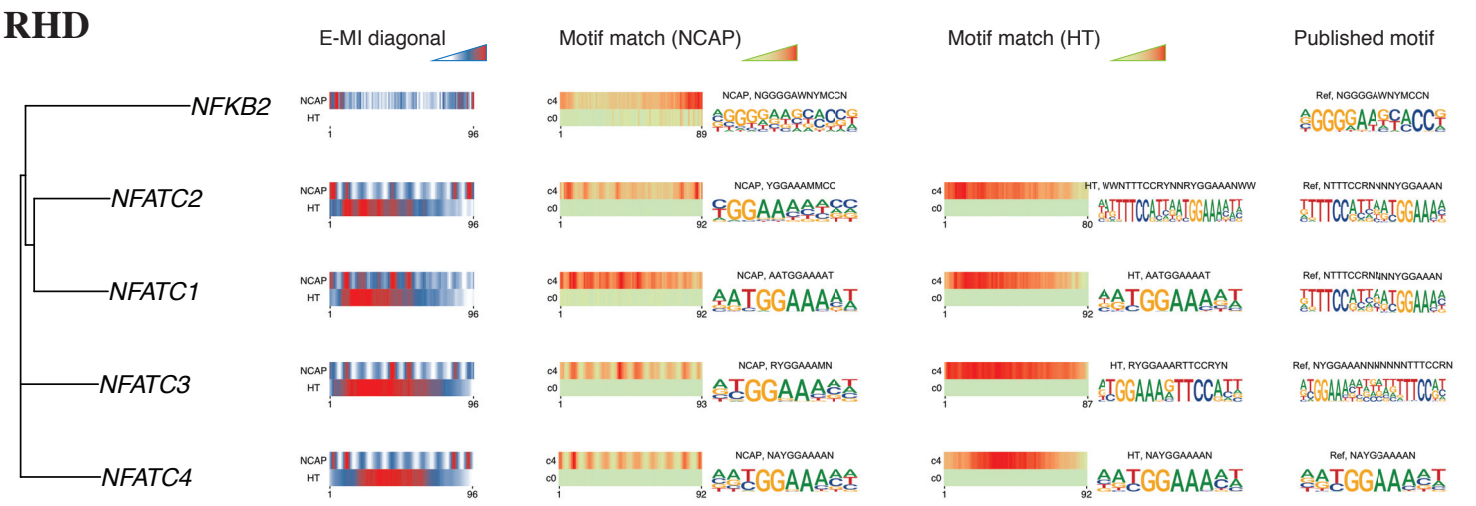


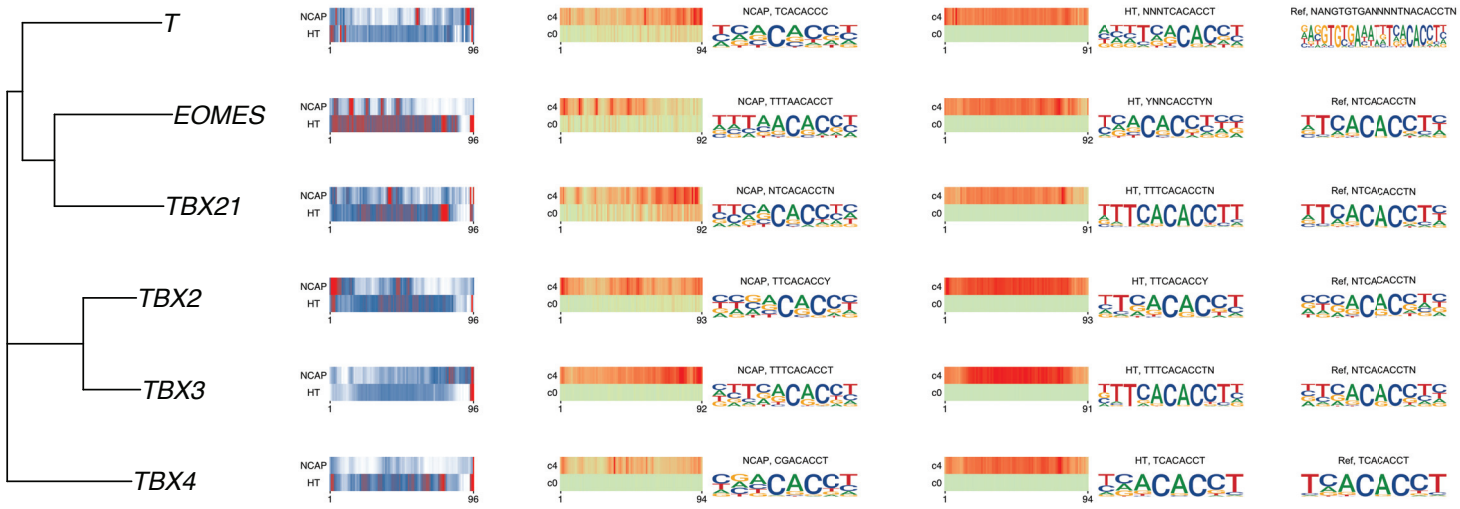

Homeodomain
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
SIX6
IRX1
IRX5
PROX1
HMBOX1
DUXA
ISL1
LMX1B
LMX1A
VAX1
VAX2
EN1
EN2
VENTX
MSX2
MSX1
DLX5
DLX2
DLX1
DLX4


Homeodomain
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
NANOG
BARHL1
NKX2–3
NKX2–4
HMX1
NKX6–2
TLX2
LBX2
HOXB7
HOXD3
HOXD4
HOXA2
PDX1
MNX1
GSX1
GSX2
CDX1
HOXD12
HOXB13
HOXC11

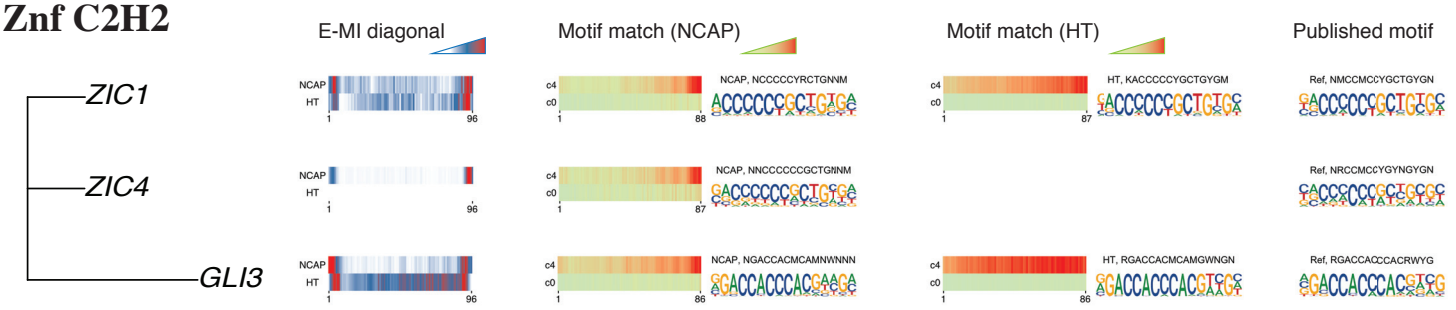

Homeodomain
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
HOXD9
HOXA10
HOXC10
HOXD10
EVX1
EVX2
MEIS
PKNOX2
MEIS3
MEIS2
TGIF2LX
TGIF2LY
TGIF2
Nuclear receptor
THRB
NR4A2
NR3C2


PAX
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
PAX1
PAX7
PAX2
POU
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
POU3F4
POU3F2
POU2F2
POU2F3
POU4F3
POU4F1
POU4F2
POU1F1
RFX
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
RFX1
RFX5
RFX4
RFX2
RFX3

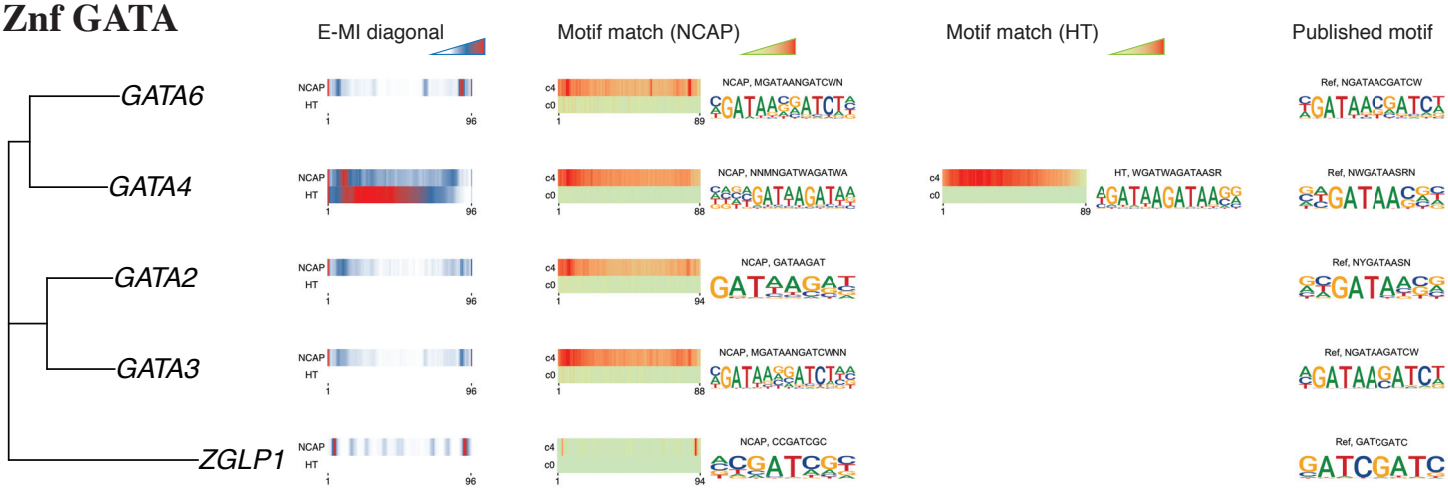

RHD
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
NFKB2
NFATC2
NFATC1
NFATC3
NFATC4
NCAP
HT
Ref, NGGGGAWNYMCCN
Ref, NTTTCCRNNNYGGAAAN
Ref, NTTTCCRNNNNYGGAAAN
Ref, NYGGAAANNNNNNTTTCCRN
Ref, NAYGGAAAAN


T box
E-MI diagonal
Motif match (NCAP)
Motif match (HT)
Published motif
T
EOMES
TBX21
TBX2
TBX3
TBX4
NCAP
HT
Ref, NANGTGTGANNNNTNACACCTN
Ref, NTCACACCTN
Ref, TCACACCT

Znf C2H2



Znf GATA



Other families