

1 **Cross-species analysis of melanoma enhancer logic using** 2 **deep learning**

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26 **Abstract**

27 Genomic enhancers form the central nodes of gene regulatory networks by harbouring combinations of
28 transcription factor binding sites. Deciphering the combinatorial code by which these binding sites are
29 assembled within enhancers is indispensable to understand their regulatory involvement in establishing
30 a cell's phenotype, especially within biological systems with dysregulated gene regulatory networks,
31 such as melanoma. In order to unravel the enhancer logic of the two most common melanoma cell states,
32 namely the melanocytic and mesenchymal-like state, we combined comparative epigenomics with
33 machine learning. By profiling chromatin accessibility using ATAC-seq on a cohort of 27 melanoma
34 cell lines across six different species, we demonstrate the conservation of the two main melanoma states
35 and their underlying master regulators. To perform an in-depth analysis of the enhancer architecture,
36 we trained a deep neural network, called DeepMEL, to classify melanoma enhancers not only in the
37 human genome, but also in other species. DeepMEL revealed the presence, organisation and positional
38 specificity of important transcription factor binding sites. Together, this extensive analysis of the
39 melanoma enhancer code allowed us to propose the concept of a core regulatory complex binding to
40 melanocytic enhancers, consisting of SOX10, TFAP2A, MITF and RUNX, and to disentangle their
41 individual roles in regulating enhancer accessibility and activity.

42 Introduction

43 A cell's phenotype arises from the expression of a unique set of genes, which is regulated through the
44 binding of transcription factors (TFs) to cis-regulatory elements, such as promoters and enhancers.
45 Deciphering gene regulatory programs entails understanding the network of transcription factors and
46 cis-regulatory elements that governs the identity of a given cell type; as well as understanding how the
47 specificity of such a network is encoded in the DNA sequence of genomic enhancers. Enhancers harbor
48 combinations of binding sites for TFs, through which transcription of nearby target genes is regulated^{1,2}.
49 The chromatin around enhancers is typically enriched for acetylation of histone H3 at lysine 27
50 (H3K27ac) and H3 monomethylation at K3 (H3K4me1), allowing enhancer identification through
51 ChIP-seq for these specific histone marks¹. In addition, profiling accessible chromatin via DNase I
52 hypersensitive sequencing (DNase-seq) or via the Assay for Transposase-Accessible Chromatin using
53 sequencing (ATAC-seq) represents a useful approach for identifying putative enhancers^{3,4}. Indeed,
54 active enhancers are typically depleted of one or more nucleosomes, due to the binding of TFs. Initial
55 changes in DNA accessibility can be facilitated through a special class of TFs that bind with high
56 affinity to their recognition sites and that have a long residence time at the enhancer; sometimes referred
57 to as pioneer TFs^{4,5}. By displacing nucleosomes or thermodynamically outcompeting nucleosome
58 binding they allow other TFs to co-bind, thereby further stabilising the nucleosome depleted region
59 and/or actively enhancing transcription of target genes^{6,7}. As the presence and architecture of TF binding
60 sites within enhancers determine which TFs can bind with high affinity, understanding this 'enhancer
61 logic' can help interpreting the functional role of enhancers within a gene regulatory network. Several
62 techniques exist to study the enhancer code, including (1) motif discovery tools, in which position-
63 weight matrices of TF binding sites are used to calculate their enrichment in sets of co-regulated regions
64 or co-expressed genes^{8,9}; (2) comparative genomics, by exploiting cross-species data to identify
65 conserved and therefore possible important (parts of) enhancers¹⁰⁻¹²; (3) genetic screens to measure the
66 effect of mutations on enhancer activity^{13,14}; and (4) machine learning techniques, where mathematical
67 models are trained to recognise specific patterns in enhancers and help to classify them¹⁵. Particularly
68 the latter has seen a strong boost the last years, with the advent of large training sets derived from
69 genome-wide profiling. Three pivotal methods based on deep learning include DeepBind¹⁶, DeepSEA¹⁷
70 and Basset¹⁸, the first convolutional neural networks (CNNs) applied to genomics data¹⁹. Since their
71 emergence in the genomics field, machine learning techniques, and especially CNNs, have been applied
72 to model a range of regulatory aspects, including TF binding sites²⁰, DNA methylation²¹ and 3D
73 chromatin architecture²², by exploiting large epigenomics datasets.

74
75 Deciphering gene regulation and the underlying enhancer code is not only important during dynamic
76 processes such as development, but also in disease contexts such as cancer, where gene regulatory
77 networks are typically dysregulated due to mutations. Melanoma is a type of skin cancer which mostly
78 develops from a buildup of UV-induced mutations in melanocytes, the pigment-producing cells in the
79 skin²³. Particularly in this cancer type, gene expression is dysregulated and highly plastic, giving rise to
80 two main melanoma cell states: the melanocytic (MEL) state, which still resembles the cell-of-origin,
81 i.e. the melanocyte, expressing high levels of the melanocyte-lineage specific transcription factors
82 MITF, SOX10 and TFAP2, as well as typical pigmentation genes such as *DCT*, *TYR*, *PMEL*, and
83 *MLANA*; and the mesenchymal-like (MES) state, in which the cells are more invasive and therapy
84 resistant, expressing low levels of MITF and SOX10, and high levels of genes involved in TGFbeta
85 signaling and epithelial-to-mesenchymal transition (EMT)-related genes²⁴⁻²⁸. These transcriptomic
86 differences have also been studied at the epigenomics level, with AP-1 and TEAD factors as master
87 regulators of the MES state and binding sites for SOX10 and MITF significantly enriched in MEL-
88 specific regulatory regions²⁷⁻²⁹. However, it remains unclear how these regulatory states are encoded in

89 particular enhancer architectures, and whether such architectures are evolutionary conserved. Besides
90 human cell lines and human patient-derived cultures, several animal models have been established in
91 melanoma research, including mouse, pig, horse, dog and zebrafish³⁰⁻³⁴. Although these models are
92 widely used, it is unknown whether their enhancer landscapes and regulatory programs are conserved
93 with human.

94
95 Here, we combine comparative regulatory genomics with machine learning to investigate enhancer
96 logic in melanoma. Through epigenomic profiling of 27 melanoma cell lines across six species, we
97 examine the conservation of the two main melanoma states and underlying master regulators. By
98 training a deep neural network, called DeepMEL, on topic models derived from the human cell lines,
99 we were able to classify not only human melanoma enhancers, but also regulatory regions in the other
100 species. DeepMEL revealed high-confidence TF binding sites for the different melanoma states, how
101 they are positioned within melanoma enhancers, and where they are placed with respect to the central
102 enhancer nucleosome. This in-depth analysis of the melanoma enhancer code allowed us to propose a
103 mechanistic model of TF binding in MEL melanoma enhancers. Finally, by exploiting the deep layers
104 of our model, we are able to identify causal mutations for melanoma enhancer loss and gain through
105 evolution, not only affecting enhancer accessibility but also activity.

106 Results

107 Melanoma chromatin accessibility landscapes are conserved across species

108 To study the conservation of melanoma cell states and underlying enhancer logic, we performed
109 (Omni)ATAC-seq on a cohort of melanoma cell lines across six species, obtaining accessible chromatin
110 landscapes of a total of 27 samples (Fig. 1a). These include 17 human patient-derived cultures (“MM
111 lines”)^{27,35}, one mouse cell line³⁶, one cell line derived from the pig melanoma model MeLiM
112 (“MeLiM”)³⁰, two horse melanoma lines derived from a Grey Lipizzaner horse (“HoMel-L1”) and from
113 an Arabian horse (“HoMel-A1”)³³, two dog melanoma cell lines (“Cesar” and “Bounty”)³⁷ and four
114 melanoma lines established from zebrafish (“ZMEL1”, “EGFP-121-1”, “EGFP-121-5” and “EGFP-
115 121-3”)^{38,39}. Per sample, between 65,475 and 176,695 ATAC-seq peaks were observed (Fig. S1a),
116 including regions that are accessible across all six species in this study and thus conserved (e.g. *TCF7L2*
117 promoter), peaks that are only accessible in the mammalian lines (i.e. in human, mouse, pig, horse and
118 dog lines) (e.g. *ST3GAL2* promoter) and species-specific peaks (e.g. the human-specific *NMNAT1*
119 intronic enhancer) (Fig. 1a). Interestingly, unsupervised clustering of the 17 human lines grouped the
120 samples into two distinct clusters (Fig. S1b), which correspond to the two main cell states in human
121 melanoma, i.e. the melanocytic state (MEL) and mesenchymal-like state (MES), as was confirmed for
122 twelve of the lines by RNA-seq data using established MEL and MES gene signatures (Fig. S1c)²⁷.
123 Indeed, regulatory regions near MEL-specific genes such as *SOX10* were accessible in human lines in
124 the MEL state (MM001, MM011, MM031, MM034, MM052, MM057, MM074, MM087, MM118,
125 MM122 and MM164), whereas they were closed in MES melanoma lines (MM029, MM047, MM099,
126 MM116, MM163, and MM165) (Fig. 1b). In addition, this classification was in agreement with previous
127 work where respectively nine and ten of these lines were clustered based on epigenomic data (using
128 OmniATAC-seq, and H3K27ac ChIP-seq and FAIRE-seq, respectively)^{27,28}. Of note, similarly as in
129 Wouters et al., we observed inter-cell line heterogeneity within the states, especially within the
130 melanocytic state (Fig. S1b).

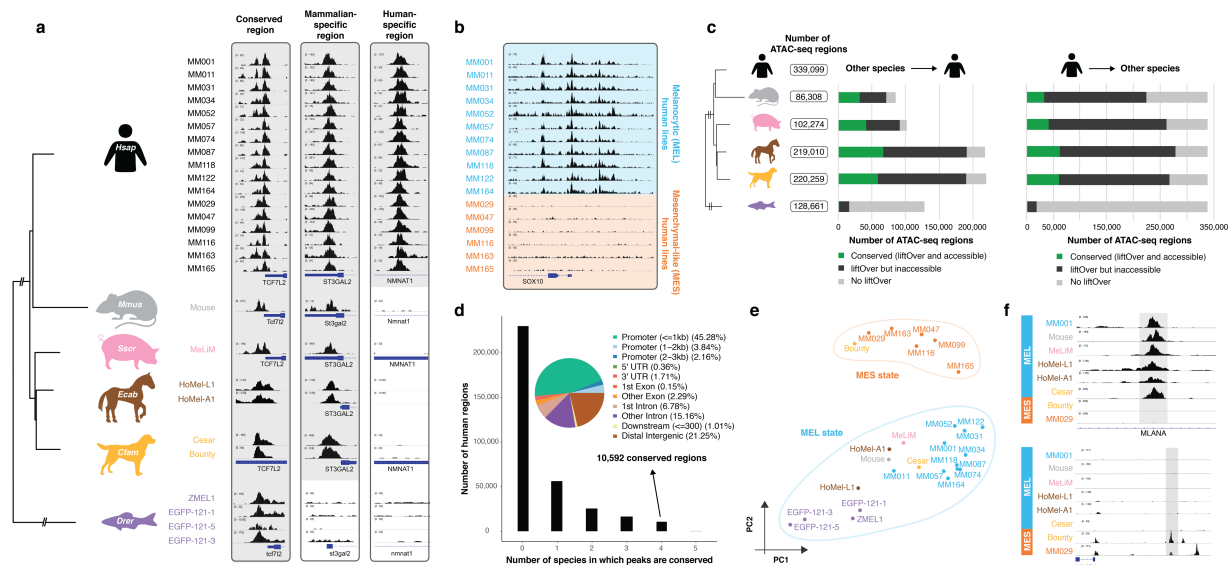
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132 To examine whether the two main melanoma states were conserved in the other species of our cohort,
133 we first identified conserved regulatory regions using the liftOver tool⁴⁰ to compare genomic positions.
134 Between 1.1% and 40.9% of the ATAC-seq regions in non-human lines were conserved in human, i.e.
135 convertible to human coordinates and accessible in human; and between 0.9% and 18.4% of the human
136 peaks were conserved in the other species (Fig. 1c). Note that the most distant species in our cohort, i.e.
137 zebrafish (last common ancestor ~340 million years ago⁴¹), has the smallest proportion of conserved
138 regions (1.1%), as expected. Accordingly, we identified 10,592 regulatory regions conserved across the
139 mammalian species, and, when including zebrafish, 116 conserved regions across all six studied species
140 (Fig. 1d). Nearly half of the 10,592 conserved mammalian regions were promoters within 1 kb of a
141 transcription start site (Fig. 1d). Indeed, high conservation of proximal promoters has previously been
142 reported, which is partially due to their position near the transcription units, making them evolutionarily
143 more stable compared to more distal regulatory elements¹². In each of the mammalian species, the
144 10,592 conserved regions were more accessible compared to all ATAC-seq regions and, in addition,
145 these conserved regions show a higher ChIP-seq signal for H3K27ac in human, a mark for active
146 regulatory regions⁴² (Fig. S1d,e).

147
148 Next, to test how closely related the different melanoma lines are at the epigenomic level, we clustered
149 the lines using the identified conserved regions. Clustering of all mammalian samples based on the
150 accessibility of the 10,592 conserved mammalian regions (Fig. S1g,h) or of all samples using the 116

151 globally conserved regions (Fig. 1e, Fig. S1f), revealed again two main clusters. One cluster contained
 152 all human MEL samples together with 9 of the 10 non-human lines, indicating that most of the non-
 153 human cell lines are epigenomically similar to human MEL lines. On the other hand, the second cluster
 154 consisted of all human MES samples together with the dog cell line ‘Bounty’. Based on this co-
 155 clustering of melanoma lines, we can state that all non-human cell lines are in the MEL state, except
 156 for the dog line ‘Bounty’ which belongs to the MES state. Indeed, known MEL regulatory regions such
 157 as the intronic enhancer of *MLANA*, a MEL-specific gene involved in melanosome biogenesis⁴³, are
 158 accessible in all mammalian lines, except for the MES human lines and the dog line Bounty; whereas
 159 the opposite is true for an enhancer upstream of *MMP3*, a gene which increases metastatic potential in
 160 melanoma cell lines⁴⁴ (Fig. 1f).

161

162 In conclusion, by using ATAC-seq on a panel of 27 melanoma lines across six species, conserved
 163 regulatory regions could be identified. These regions allowed clustering of the melanoma samples into
 164 two groups which correspond to the two main melanoma cell states, indicating conservation of the MES
 165 melanoma state in dog and the MEL melanoma state in pig, mouse, horse, dog and even zebrafish
 166 melanoma samples.
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170 **Figure 1. Comparative epigenomics reveals conservation of two main melanoma states.** **a**, Evolutionary
 171 relationship between the six studied species, represented by a phylogenetic tree (NCBI taxonomy tree). ATAC-
 172 seq profiles of the 27 melanoma cell lines are shown for a conserved region (*TCF7L2* promoter), a mammalian-
 173 specific region (*ST3GAL2* promoter) and a human-specific region (*NMNAT1* intronic enhancer). **b**, ATAC-seq
 174 profiles of the human melanoma lines for the *SOX10* locus. Lines are coloured by the melanocytic (MEL, in blue)
 175 or mesenchymal-like (MES, in orange) melanoma state. **c**, (left) Total number of ATAC-seq regions observed
 176 across all samples of a species, (middle) coloured based on their liftOver (at least 10% of bases must remap) and
 177 conservation status compared to human. (right) Similar graph for the conservation of the 339,099 human regions
 178 in each of the other species. **d**, Number of human regions that are conserved with 0 (i.e. human-specific) to 5
 179 different species. ChIPseeker results are shown for the 10,592 human regions that are conserved across all
 180 mammalian species. **e**, Melanoma cell lines cluster into two groups, linked to the MEL and MES melanoma states
 181 as shown in a PCA plot based on 116 conserved regions across all six species. **f**, ATAC-seq profiles of MEL and
 182 MES lines of different species for an intronic *MLANA* enhancer and the upstream region of *MMP3*.

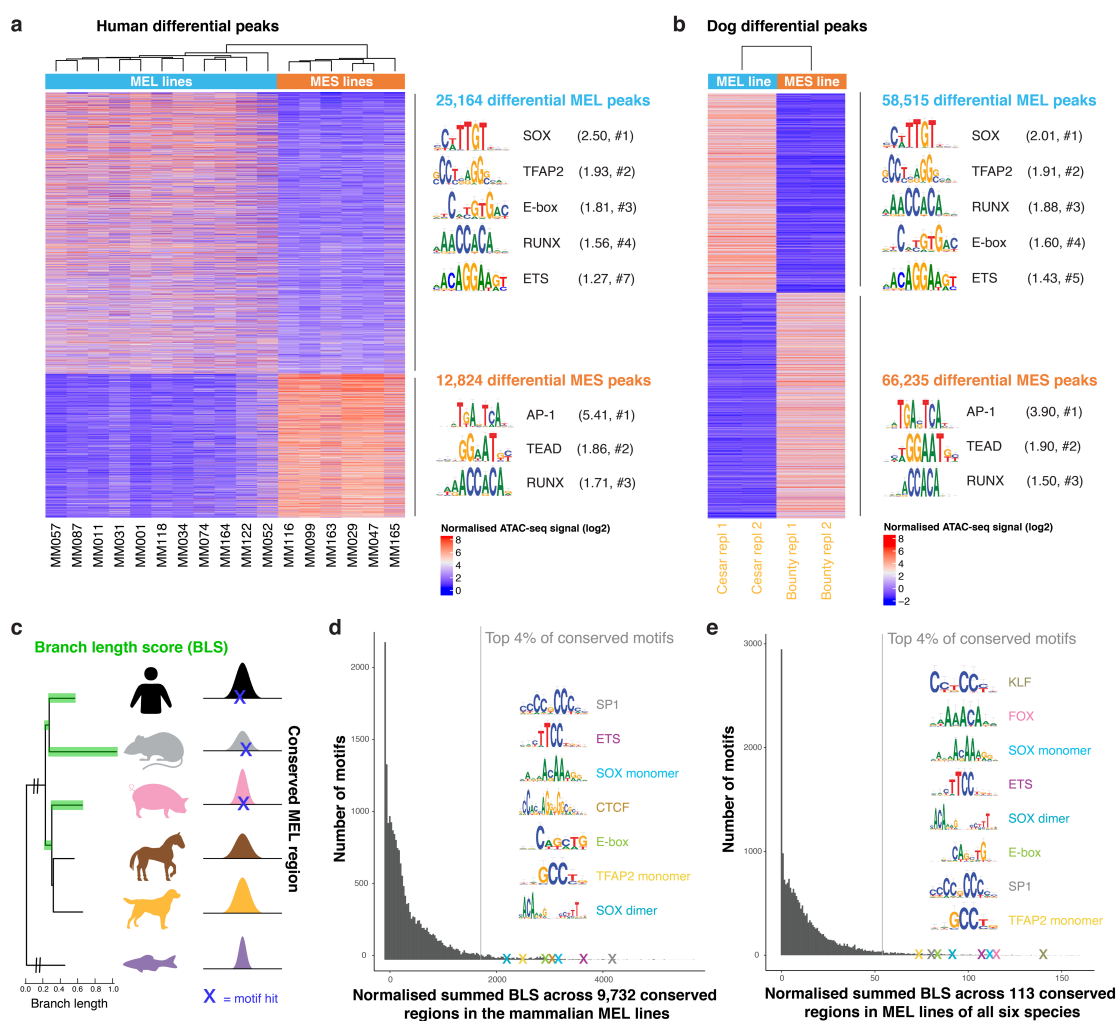
183 **Conserved transcription factor motifs determine state-specific enhancers**

184 Next, we wanted to investigate whether the conserved MEL and MES states are controlled by similar
185 master regulators across different species. First, we performed an evolutionary comparison of
186 differential transcription factor binding sites between MEL and MES cell lines in human and dog, as
187 these were the only two species in our cohort for which cell lines of both states were available.
188 Differential peak calling between the human MEL and MES lines revealed significant enrichment of
189 SOX, TFAP2, MITF, RUNX and ETS TF binding motifs in the 25,164 differential MEL human peaks
190 ($\log_2FC > 2.5$ and $pAdj < 0.0005$; complete Homer output in Supplementary Table 1) (Fig. 2a). Indeed,
191 SOX10, TFAP2 and MITF are among the previously reported master regulators of the MEL state^{24,27-}
192 ²⁹. The 12,824 human differential MES regions were significantly enriched for binding motifs for
193 transcription factors of the AP-1 family and TEADs (Fig. 2a), known regulators in human MES
194 melanoma lines²⁷. To examine the conservation of these master regulators in dog melanoma, we
195 contrasted the two dog lines. Interestingly, the 58,515 peaks specific to the MEL dog line Cesar were
196 significantly enriched for similar TF binding motifs as the human differential MEL peaks, i.e. SOX,
197 TFAP2, RUNX, MITF and ETS motifs, and even the order of the enriched TF families was comparable
198 (Fig. 2b). The same was true for the motifs enriched in the MES-specific human and dog regions (Fig.
199 2b). Note that the difference in the number of differentially accessible regions between dog and human
200 is likely due to the variability between human samples that are used as replicates, while for dog we used
201 two technical replicates of the same cell line. Altogether, these observations indicate that the MEL and
202 MES melanoma cell states are conserved in dog and that they are likely governed by the same master
203 regulators, based on the concordance of motif enrichment for SOX10, MITF, TFAP2 and ETS factors;
204 and for AP-1 and TEAD TFs for the MEL and MES state respectively.

205
206 To further verify the importance of the MEL-specific master regulators in MEL cell lines of the
207 remaining four species, we applied a different strategy since we could not contrast MEL and MES lines
208 for horse, pig, mouse and zebrafish. Therefore, we focused on 9,732 regions that were conserved across
209 all mammalian MEL lines to identify conserved TF binding sites. Note that this number differs from
210 the 10,592 conserved regions defined above as only the MEL lines were used here. We scanned the
211 9,732 conserved regions using our library of 20,003 TF position-weight matrices (PWMs) and used a
212 branch length score (BLS) to calculate the level of evolutionary conservation of each TF binding motif
213 (Fig. 2c), a strategy applied before in other systems^{7,45}. Among the 4% most conserved motifs were
214 SP1, ETS, SOX (both monomer and dimer motifs), CTCF, MITF and TFAP2 motifs (Fig. 2d). Notably,
215 the top conserved motifs were members of the SP/KLF TF family, which bind to GC-rich motifs in
216 promoters⁴⁶. Indeed, 47% of the 9,732 conserved regions in mammalian MEL lines were proximal
217 promoters (≤ 1 kbp from TSS). BLS scoring on the remaining 5,196 more distal conserved regions
218 showed no longer conservation SP1/KLF TF motifs, but just conservation of the previously identified
219 TF binding motifs for TFAP2A, MITF, SOX10, CTCF and ETS factors (Fig. S1i), indicating that distal
220 regions, such as enhancers, mostly contain the state-specific TF binding motifs. Interestingly, when we
221 included zebrafish ATAC-seq regions, only 113 regions were conserved in the MEL cell lines across
222 all six species, but BLS scoring still revealed SOX, ETS, MITF and TFAP2 motifs among the most
223 conserved motifs in MEL lines (Fig. 2e). Note that we did not perform any contrast of MEL versus
224 MES lines prior to the BLS analyses and that these motifs were identified by just focusing on the
225 conserved regions in MEL melanoma lines.

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227 Altogether, two independent strategies of motif analysis suggest that melanoma enhancer logic is
228 conserved across species and that the MEL state is governed by conserved master regulators including
229 SOX10, MITF, TFAP2A and ETS.

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Figure 2. Conservation of binding motifs of master regulators of MEL and MES melanoma states. a, b, Heatmap of differential ATAC-seq regions when comparing (a) human MEL versus human MES lines and (b) the MEL dog line ‘Cesar’ versus the MES dog line ‘Bounty’ (two biological replicates each), coloured by normalised ATAC-seq signal. Enriched TF binding motifs in the differential peaks were identified via Homer⁴⁷ and the first logo of enriched TF families is shown. The ratio of the percentage of target sequences with the motif and the percentage of background sequences with the motif is indicated between brackets, as well as the rank of the TF class within the Homer output (#). **c,** Schematic overview of cross-species motif analysis using the branch length score (BLS) as a measure for the evolutionary conservation of a motif hit (for 20,003 TF position-weight matrices) across conserved regions. The BLS was summed across a set of conserved regions, i.e. the higher the BLS score, the more conserved the motif is in that specific set of regions. **d, e,** Histogram of the normalised summed BLS score for 20,003 motifs on (d) 9,732 conserved regions across the mammalian MEL lines and on (e) 113 conserved regions across MEL lines of all six species. The first hit of the top recurrent TF binding motifs within the top 4% conserved motifs is indicated as a cross and is accompanied by the logo of the motif.

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Deep neural network DeepMEL reveals nucleotide-resolution enhancer logic

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While motif enrichment can predict candidate regulators, we sought to build a more comprehensive model of the MEL enhancers, that would allow cross-species predictions and in-depth analysis of enhancer architecture. To this end, we trained a deep learning (DL) model on human ATAC-seq data. First, to construct an unsupervised training set, we clustered all 339,099 human ATAC-seq peaks using cisTopic⁴⁸ (see Methods) into 24 topics (Fig. 3a, Fig. S2a,b). This provided a more nuanced

251 classification, with topic 4 representing the MEL enhancers being accessible across all MEL samples;
252 and topic 7 representing the MES enhancers that are accessible in the MES samples (Fig. 3a, Fig. S2c).
253 In addition, we found two topics containing regions that are generally accessible across all cell lines
254 (topic 1 and topic 19) (Fig. 3a, S2c), and which were highly enriched for proximal promoters (Fig. S2d)
255 and for known promoter-specific TF binding motifs linked to SP1 and NFY TF families (Fig. S2c)^{46,49}.
256 Other topics were more specific to one or a small group of cell lines. For instance, topic 22 contained
257 regions that were mostly accessible in MM057, MM074 and MM087 (Fig. 3a). These particular lines
258 have previously been reported as an ‘intermediate’ (INT) sub-state of the MEL state, governed by a
259 mixed MEL-MES GRN²⁸. We verified the biological relevance of these topics by investigating nearby
260 target genes using GREAT⁵⁰. Genes near topic 4 regions are significantly enriched for Gene Ontology
261 (GO) terms such as pigmentation (FDR=1.95e-8) and neural crest cell differentiation (FDR=4.26e-7),
262 whereas genes near topic 7 regions were more mesenchymal-like as they are enriched for GO terms
263 involved in cell-cell adhesion (1.56e-13). Next, we performed motif discovery on the top regions
264 assigned to each topic. SOX, ETS, TFAP2 and MITF motifs were enriched in regions of the MEL-
265 specific topic 4 and AP-1 in the MES-specific topic 7 (Fig. S2c), confirming our findings from the
266 supervised differential peak calling discussed above (Fig. 2a). An example topic 4 region in the
267 promoter of the SOX10 target gene *MIA*⁵¹ is shown in Figure 3b, as well as two topic 7 regions upstream
268 of *SERPINE1*, a gene expressed in metastatic melanoma⁵².

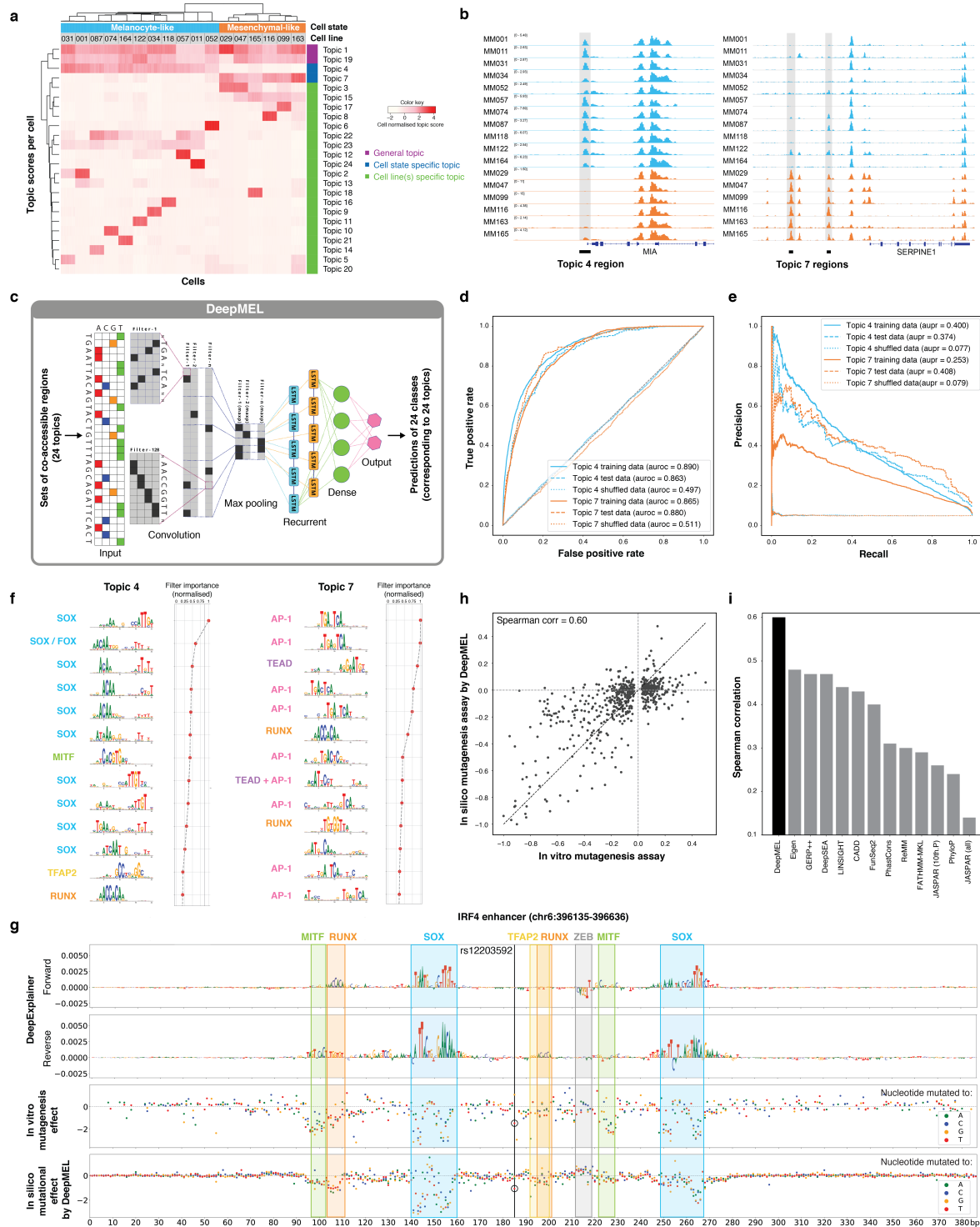
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270 Using the 24 topics as classes, we trained a multi-class, multi-label classifier using a neural network,
271 called “DeepMEL” (Fig. 3c). As input, we used the forward and reverse complement of 500 bp enhancer
272 sequences centered on the ATAC-seq summit. As topology, we used the DanQ CNN-RNN hybrid
273 architecture⁵³ consisting of 4 main layers: a convolution layer to discover local patterns in sequential
274 data, followed by a max-pooling layer to reduce the dimensionality of the data and generalise the model
275 effectively, a bidirectional recurrent layer (LSTM) to detect long-range dependencies of the local
276 patterns discovered in the first layer, and finally a fully-connected (dense) layer just before the output
277 layer to help the classification after the feature extraction layers (Fig. 3c). After successful training of
278 DeepMEL (auroc = 0.863 and aupr = 0.374 on test data for topic 4 regions) (Fig. 3d,e, Fig. S3a), we
279 used the weights of neurons from the convolutional filters to extract local patterns learned by the model.
280 We transformed these convolution filters into PWMs and found the importance of each filter for each
281 topic (see Methods and Supplement). Intriguingly, filters that represent SOX, MITF, TFAP, and RUNX
282 motifs were most relevant for the MEL-specific topic 4 and filters that represent AP-1, TEAD and
283 RUNX binding sites were assigned to the MES-specific topic 7 (Fig. 3f). Thus, DeepMEL learned the
284 relevant features *de novo* from the sequence. DeepMEL can be used to score and classify any given
285 DNA sequence of 500 bp. For instance, when re-entering all ATAC-seq peaks of the MEL line MM001
286 in the model, it classified 3,885 regions as MEL-specific (topic 4 scores above threshold of 0.16 (see
287 Methods)). These regions were indeed highly accessible in MEL lines and closed in MES lines, and
288 interestingly, were also accessible in human melanocytes (Fig. S3b,c). Importantly, this indicates that
289 these MEL-specific regions in melanoma are not cancer-specific but already accessible in their cell-of-
290 origin, i.e. the melanocytes, and that we potentially can extrapolate the observations on this topic to
291 melanocyte enhancers. Although in the remainder of this work we will score accessible regions to
292 identify functional enhancers, it is also possible to score the entire genome, without filtering for ATAC-
293 seq peaks. This may be useful for species where no ATAC-seq data of melanoma or melanocytes is
294 available. Such a scoring yields high precision and recall (69% and 86% respectively, Fig. S3d).

295
296 In order to examine the TF binding site architecture within enhancers, we used a model interpretation
297 tool, DeepExplainer^{54,55}, which does backpropagation of the activation differences⁵⁶, to visualise the
298 importance of each nucleotide in an enhancer with respect to the predicted enhancer class. For instance,

299 in a MEL enhancer located on the 4th intron of *IRF4*, nucleotides important for classifying this enhancer
300 as topic 4 form motifs for SOX10, MITF, TFAP and RUNX factors (Fig. 3g top two rows). Indeed,
301 SOX10 binding has been reported on this location⁵⁷. Another example is given for a region of topic 22,
302 the topic specific to the INT MEL subpopulation, where SOX10 and AP-1 co-exist within the same
303 enhancer, indicating that these cell lines also contain properties of a mixture between the MEL and
304 MES state at the epigenomic level (Fig. S3e,f).

305
306 Importantly, it is known that enhancer accessibility does not directly translate to enhancer activity¹. To
307 test whether the same TF binding motifs were contributing to the activity of MEL enhancers, we used
308 the *IRF4* enhancer as case study. For this enhancer, Kircher et al.¹⁴ performed saturation mutagenesis
309 followed by an *in vitro* massively parallel reporter assay (MPRA), testing the effect of every possible
310 single nucleotide mutation on enhancer activity (Fig. 3g, 3th row). The most deleterious mutations
311 coincided with the SOX, E-box and RUNX-like motifs that were predicted by DeepMEL, indicating
312 that the predicted motifs are also contributing to enhancer activity, as their disruption reduced enhancer
313 activity *in vitro*. To further examine how well DeepMEL can predict the *in vitro* MPRA effect, we
314 measured the effect on the topic 4 DL score of each single nucleotide mutation *in silico* (Fig. 3g, bottom
315 row). Interestingly, mutations that have the strongest *in silico* effect overlapped with predicted TF
316 binding motifs, and more intriguingly, also the magnitude of the effect highly correlated with the *in*
317 *vitro* mutations (Spearman correlation of 0.60) (Fig. 3g,h), even though DeepMEL was trained only on
318 binary accessibility data (i.e. binary topics of co-accessible regions). These observations indicate that,
319 although the DeepMEL was trained to predict enhancer accessibility, it is also a good predictor of
320 enhancer activity of this specific enhancer. Notably, our DeepMEL performed best in predicting the *in*
321 *vitro* mutagenesis on the *IRF4* enhancer activity compared to other classifiers and deep learning models
322 that were benchmarked in Kircher et al.¹⁴ (CAGI challenge, 2018) (Fig. 3i). Interestingly, enhancer
323 accessibility and activity were not only influenced by mutations that break a motif for an activating TF,
324 but also by the creation of a repressor binding motif. This was the case for a C-to-T mutation that
325 coincided with a SNP involved in freckles, brown hair and high sensitivity of the skin to sun exposure
326 (rs12203592, SNPedia) (Fig. 3g). This SNP creates a ZEB/SNAI-like motif that negatively contributes
327 to the MEL topic score of this enhancer (Fig. S3g). A similar motif was also found to decrease the MEL
328 prediction in the wild-type sequence (Fig. 3g, “ZEB”, letters facing downwards) and mutating this motif
329 increased the topic 4 prediction score, indicating that the ZEB/SNAI-like TF binding motif (CAGGT)
330 may function as a repressor for the MEL state. Indeed, ZEB factors have been reported to act as
331 transcriptional repressors by interaction with the corepressor CtBP⁵⁸, and mutations in the binding motif
332 of the transcriptional repressor SNAI2 have been shown to increase chromatin accessibility¹¹. Note that
333 the ability of DeepMEL to predict the effect of mutations on enhancer accessibility (and activity) raises
334 the opportunity to apply DeepMEL to predict enhancer mutations that affect chromatin accessibility in,
335 for instance, personalised cancer genomes; as we did in our companion paper for phased melanoma
336 genomes of a total of 10 patient-derived melanoma cultures (Kalender Atak et al., 2019).

337
338 In conclusion, our DL model DeepMEL, trained on topics of human co-accessible regions, is performant
339 in classifying melanoma regulatory regions into different classes based on purely the DNA sequence.
340 Interestingly, features learned by DeepMEL corresponded to TF binding motifs of master regulators of
341 specific classes. These motifs could also be located and visualised within regions using a model
342 interpretation tool, allowing examination of the motif architecture within specific enhancers and
343 predicting the effect of mutations on enhancer accessibility.



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Figure 3. DeepMEL classifies melanoma enhancers and predicts important TF binding motifs. **a**, Cell-topic heatmap of cisTopic applied to 339,099 ATAC-seq regions across the 17 human melanoma lines, coloured by normalised topic scores. 24 topics or sets of co-accessible regions are found, including general topics, cell state specific topics and cell line(s) specific topics. **b**, Example regions of a MEL-specific (topic 4) region near *MIA* and MES-specific (topic 7) regions upstream of *SERPINE1*. **c**, Schematic overview of DeepMEL. 24 sets of co-accessible regions were used as input for training of a multi-class multi-label neural network. **d**, **e**, (d) Receiver operating characteristic curve and (e) precision-recall curve for DeepMEL on training, test and shuffled data of topic 4 and topic 7 regions. **f**, Top 13 enriched filters learned by DeepMEL to classify regions as MEL (topic 4) or MES (topic 7). Filters were converted to logos and accompanied by the candidate TF binding motif names, as identified by TomTom comparison⁵⁹. Normalised filter importance is shown per filter. **g**, Example of a MEL-

355 predicted enhancer near *IRF4*. (first and second row) DeepExplainer view of the forward and reverse strand are
356 shown, with the height of the nucleotides indicating the importance for prediction of the MEL enhancer. SOX,
357 MITF, TFAP2, ZEB-like and RUNX-like motifs within the enhancer are highlighted. (third row) *In vitro* effect
358 of point mutations on enhancer activity as measured by MPRA¹⁴. Colours represent the nucleotide to which the
359 wild type nucleotide is mutated. (bottom row) *In silico* effect of point mutations as predicted by DeepMEL. The
360 location of SNP rs12203592 is highlighted by a black vertical line and the *in vitro* and *in silico* point mutations
361 that generate the SNP are encircled. **h**, Correlation between the *in vitro* mutational effects on the *IRF4* enhancer
362 compared to the *in silico* mutagenesis predictions. **i**, Performance of variant effect prediction of several previously
363 tested models on the *IRF4* enhancer case¹⁴.

364 **Cross-species scoring identifies orthologous melanoma enhancers**

365 Next, we wanted to use the human-trained DL model DeepMEL for predicting MEL and MES
366 enhancers in other species. We started with the dog genome as a test case, because the differential
367 ATAC-seq peaks between the MEL (Cesar) and MES (Bounty) dog cell lines could be used as true
368 positives. DeepMEL reached an area under the receiver operating characteristic (auROC) of 0.979 for
369 predicting MEL regions (as topic 4) versus MES regions (as topic 7) in dog, which approximates the
370 model's performance for classifying human MEL and MES differential regions (auROC = 0.987), and
371 this accuracy is significantly higher compared to using cis-regulatory module (CRM) scoring with
372 PWMs (Fig 4a,b,c). Having confirmed that the human model can identify enhancers in the dog
373 epigenome, we predicted MEL and MES enhancers across all six species. This yielded between 2,093
374 and 5,400 MEL enhancers, and between 7,459 and 10,743 MES enhancers, in samples of the MEL and
375 MES state respectively (Fig. 4d, S4c). Interestingly, although the total number of accessible regions in
376 the genome varies between cell lines and species (Fig. 4d, numbers between brackets), for all MEL cell
377 lines around 2.5% of the accessible regions were predicted MEL enhancers. Note that the majority of
378 these enhancers could not have been detected using whole genome alignments (liftOver) (Fig. 4b,c, Fig
379 S4a-d).

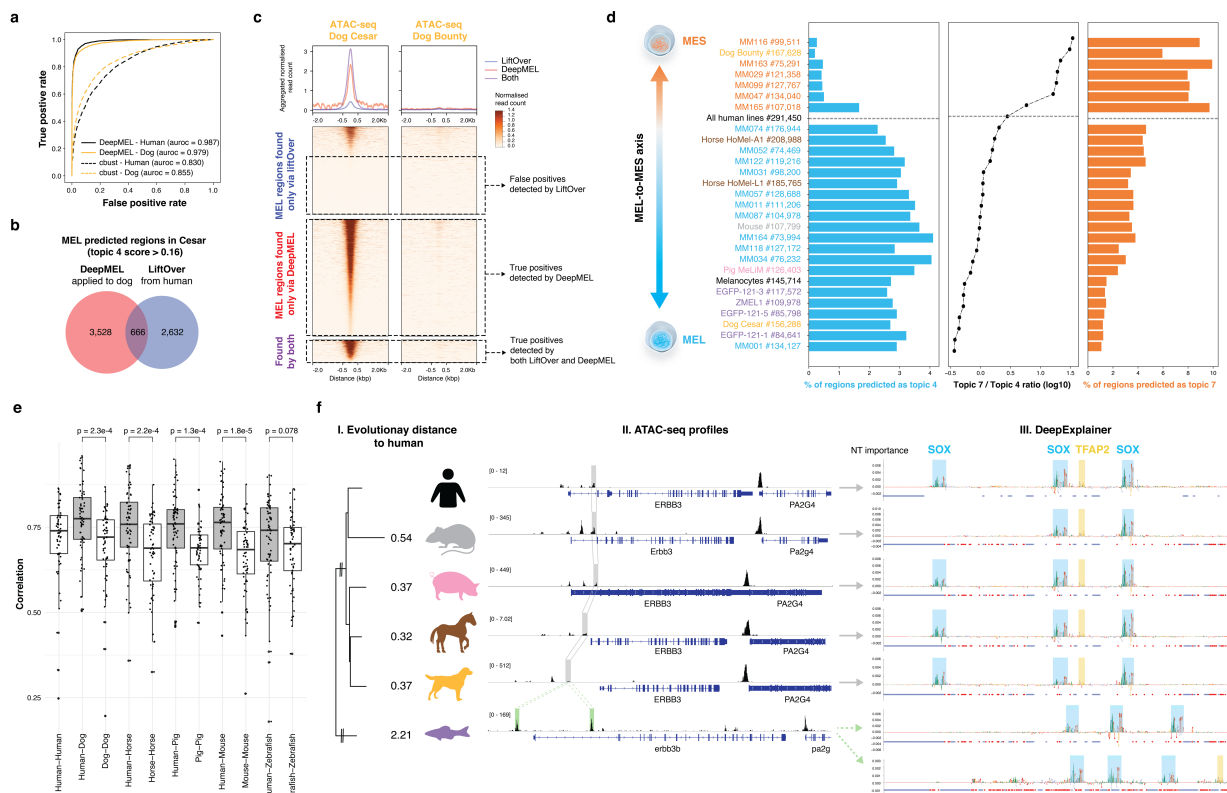
380
381 Having identified high-confidence MEL enhancers genome-wide across 6 species, as a combination of
382 ATAC-seq peaks and high topic 4 prediction scores, we analysed their distribution with respect to
383 orthologous genes, and their evolutionary divergence. Particularly, we looked at enhancers located near
384 a set of 379 human genes that are specifically expressed in the MEL state (derived from RNA-seq data
385 across a cohort of twelve MM lines (see Methods)). Of these 379 genes, 217 (67%) had at least one
386 MEL-predicted enhancer within a locus of 200kb up- and downstream of the gene (the MEL cell line
387 MM001 was used for this analysis). Between 70-85% of the orthologous MEL genes in other species
388 had at least one MEL enhancer nearby (Fig. S4e). Note that only a small subset of these enhancers could
389 have been found using liftOver (2-43% depending on the species). Of these genes, 32 form a core set
390 of conserved genes throughout all species, each having a MEL enhancer, including zebrafish. Examples
391 of genes in the core set are *MITF*, *PMEL* and *TYRP1*, genes known to be involved in melanocyte
392 development, melanosome formation and melanin production⁶⁰.

393
394 A long-standing question in enhancer studies is how to compare enhancers with each other, if their
395 sequences do not align^{61,62}. Here we tackle this question by using the dense layer of DeepMEL as a
396 reduced dimensional space to calculate the correlation between enhancers. Using this measure we found
397 that MEL-predicted enhancers in proximity of homologous MEL genes are significantly more similar
398 to each other compared to MEL-predicted enhancers in proximity of different MEL genes within the
399 same species (Fig. 4e), indicating that MEL enhancers near orthologous genes are indeed orthologous
400 enhancers. Note that the correlation of orthologous MEL enhancers approximated or even surpassed the
401 correlation of redundant (or shadow enhancers⁶³) linked to the same MEL gene in a species (Fig. S4f).

402 Lastly, we studied an example of a MEL enhancer in more detail, namely the enhancer near *ERBB3*.
 403 DeepMEL predicts a MEL enhancer upstream or intronic of *ERBB3* in each of the mammalian species,
 404 which were also found by liftOver of the human *ERBB3* enhancer (Fig. 4f II). However, in the zebrafish
 405 genome, liftOver was unable to identify the homologous region, whereas DeepMEL predicted two MEL
 406 enhancers, one upstream of the TSS of *erbb3b* and another in the first intron. Both zebrafish enhancers
 407 were highly correlated with the human ERBB3 enhancer (deep layer pearson correlation of 0.812 and
 408 0.797 for the upstream and intronic zebrafish enhancer, respectively), suggesting that both enhancers
 409 are orthologous to the human *ERBB3* enhancer. Applying DeepExplainer to the multiple-aligned
 410 sequences revealed a conserved motif architecture in the orthologous mammalian *ERBB3* enhancers
 411 containing each three SOX motifs and one TFAP2 motif (Fig. 4f III). Note that in mouse, one SOX
 412 binding site was lost, mouse is also the mammalian species that is most distant from human, among the
 413 included species in this study (Fig. 4f I). The two zebrafish enhancers contain several SOX motifs,
 414 however with different inter-motif distances. The two zebrafish enhancers have a highly similar motif
 415 architecture, suggesting that they arose by duplication from a common ancestor enhancer.

416
 417 In conclusion, we showed that DeepMEL is able to identify MEL- and MES-specific enhancers in
 418 different species, which allows studying evolutionary events and enhancer logic within orthologous
 419 enhancers, even in distant species such as zebrafish.

420



421
 422 **Figure 4. Human-trained deep learning model on cross-species ATAC-seq data.** a, DeepMEL performs well
 423 in classifying MEL and MES differential peaks in human and dog, and outcompetes Cluster-Buster (cbust). b,
 424 Venn diagram of the number of topic 4 (MEL-specific) regions predicted by DeepMEL in the dog line ‘Cesar’
 425 and of dog regions found by liftOver of the human MEL regions. c, Heatmaps of ATAC-seq signal of the dog
 426 lines ‘Cesar’ and ‘Bounty’ on MEL-predicted regions found via liftOver (blue), MEL regions predicted by
 427 DeepMEL (red) and MEL regions identified by both methods (purple). Heatmaps are coloured by normalised read
 428 counts and ordered according to the ATAC-seq signal in ‘Cesar’. Aggregation plots are shown on top. d,
 429 Percentage of MEL and MES predicted ATAC-seq regions across all samples in our cohort and in human

430 melanocytes. Samples are ordered according to the MEL-MES axis by using the ratio of the number of MES /
431 MEL predicted regions. e, Pearson correlation of deep layer scores between MEL-predicted regions of orthologous
432 MEL genes between human and another species ('Human-Species') or between MEL-predicted regions near
433 different MEL genes within one species ('Species-Species'). f, (I) Evolutionary distance between human and other
434 species in branch length units. (II) ATAC-seq profiles of the *ERBB3* locus in the six different species. MEL-
435 specific enhancers that were predicted by DeepMEL and that were also found via liftOver of the human MEL
436 enhancer are highlighted in grey, whereas MEL-predicted regions only found by DeepMEL are highlighted in
437 green. (III) DeepExplainer plots are shown for the multiple-aligned MEL-predicted *ERBB3* enhancers, for
438 zebrafish the first and second row represent the DeepExplainer plots of the upstream and intronic enhancer,
439 respectively. SOX and TFAP2 motifs formed by important nucleotides are highlighted. Red and blue dots
440 represent point and indels mutations, respectively.

441 **Motif architecture of the MEL enhancer**

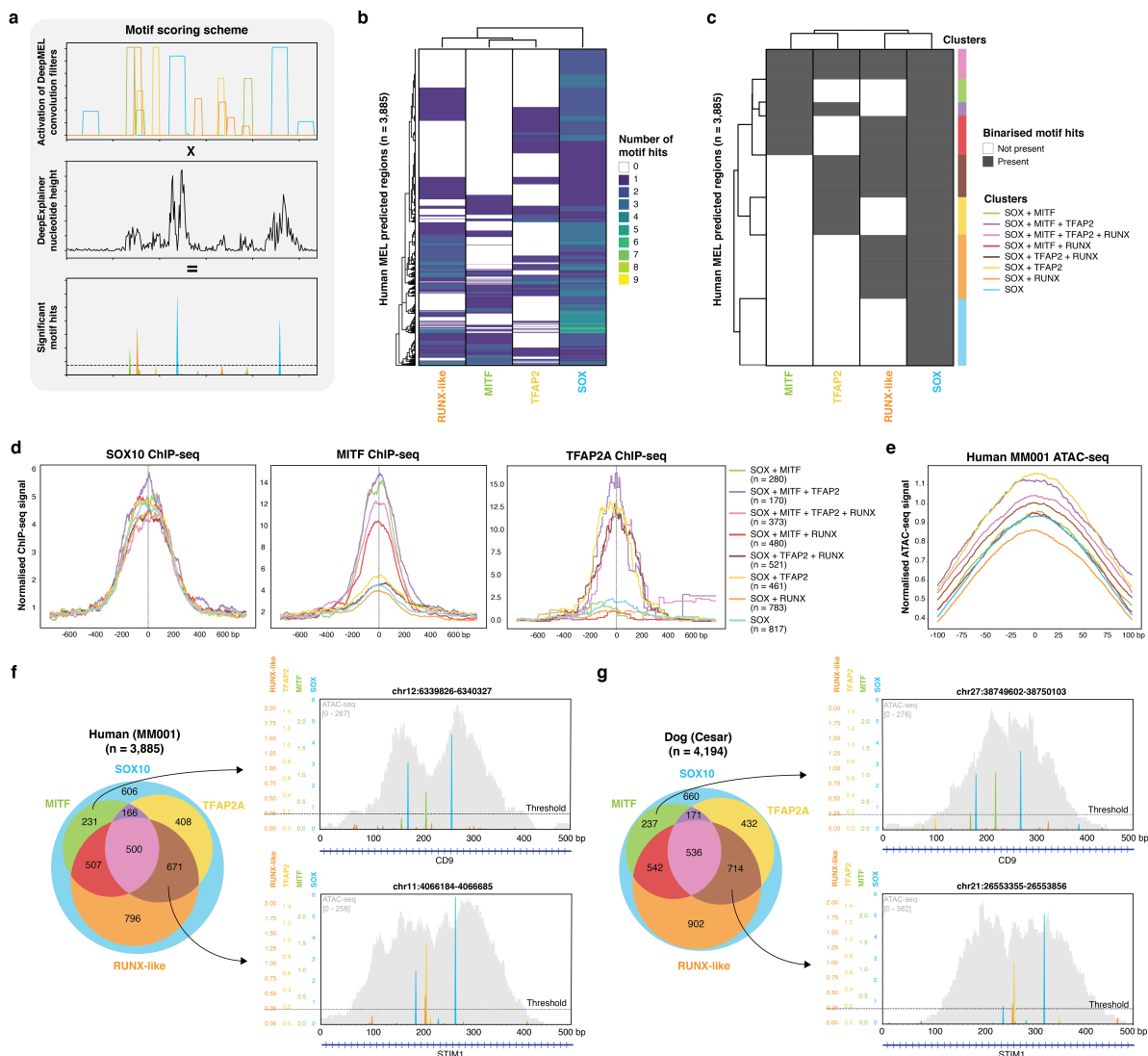
442 To study the architecture of MEL enhancers in more detail, including motif composition, motif order
443 and distance, and relationships to the nucleosome position, we set out to obtain high-confidence motif
444 annotations in each of the 3,885 MEL enhancers in human (MM001, the most MEL-like human cell
445 line), for each of the predicted core regulatory factors (SOX10, MITF, TFAP2A, RUNX). To achieve
446 this, we devised an improved motif scoring method that obtains precise positions of TF binding motifs
447 by multiplying DeepMEL activation scores of convolutional filters (i.e. motifs) with the DeepExplainer
448 profile on each enhancer (Fig. 5a)⁶⁴. A motif hit is predicted as significant when its importance is above
449 a motif-specific threshold which was determined by using all regions as background (see Methods).

450
451 The first remarkable observation was that each MEL enhancer contains at least one SOX10 motif hit,
452 and often two or more (Fig 5b). This suggests that SOX10 plays a central role in MEL enhancer
453 accessibility. Indeed, knock-down of SOX10 in MM001 significantly decreases the accessibility of
454 MEL enhancers (Fig. S5a), and the regions that close after SOX10-KD are highly enriched for SOX
455 motifs (NES = 28.5), possibly revealing a pioneering-role of SOX10 in MEL enhancers. Pioneer factors
456 can access their binding sites on nucleosomal DNA, thereby directly or indirectly displacing the
457 nucleosome, which results in the accessibility of the region⁵. Next to SOX, a combination of one or
458 multiple TFAP2, MITF or RUNX-like motifs was present in 84% of the MEL-predicted enhancers. To
459 facilitate a systematic study of the MEL enhancer logic, we binarised the motif-region matrix to simplify
460 the region clustering (Fig 5c). We obtained 8 different enhancer classes, each with a different motif
461 composition (Fig. 5c). As validation of the clusters and the predicted TF binding sites, we used human
462 ChIP-seq data of SOX10, MITF and TFAP2A in melanoma or melanocytes^{65,66} (Fig. 5d). All clusters
463 were indeed highly bound by SOX10, validating the prevalent SOX10 motif in all MEL enhancers. In
464 contrast, MITF ChIP-seq data revealed that MITF binds more to enhancer classes with MITF motifs
465 compared to regions lacking a significant MITF motif. Similarly, only enhancers containing at least one
466 TFAP2 motif were bound by TFAP2A. Interestingly, regions containing a TFAP2A motif, next to the
467 SOX10 motif(s) and possible others, showed a modest increase in accessibility (Fig. 5e), which could
468 be in line with the previously described role of TFAP2A as a stabiliser of nucleosome-depleted regions⁶.
469 The opposite was true for regions containing RUNX-like TF binding sites, as these were found to be
470 less accessible compared to regions containing only SOX10 motifs, suggesting a repressive role of
471 RUNX factors. The presence of a MITF site did not seem to alter the accessibility of enhancers
472 compared SOX-only enhancers, but did increase H3K27ac signal (Fig. S5b), possibly indicating that
473 MEL enhancers bound by MITF are more active.

474
475 To validate these MEL enhancer classes in other species, we applied the same motif scoring and
476 binarisation to DeepMEL-predicted MEL regions in the other species in our cohort. Interestingly, MEL

477 enhancers in other species also clustered into the same 8 clusters, with a similar distribution of regions
 478 per cluster (Fig. 5f,g, Fig. S5c). To test the conservation of the clusters, we used liftOver to compare
 479 the classification of enhancers across species. Although identifying orthologous sequences via whole
 480 genome alignment is not always correct, as shown above, a general trend was observed where the
 481 regions of a human cluster correspond to the same cluster in the other species (Fig. S5d), indicating
 482 conservation of the MEL enhancer clusters across species. For instance, the dog-orthologs of two human
 483 MEL enhancers belonging to either the cluster containing SOX10 and MITF binding sites (intronic
 484 enhancer of *CD9*) or to the cluster containing SOX10, TFAP2A and RUNX-like motifs (intronic
 485 enhancer of *STIM1*) (Fig. 5f) were part of the corresponding clusters in dog (Fig. 5g). In these examples
 486 we observed preserved spacing of around 80 bp between the two SOX10 binding sites within the
 487 enhancers, to which we will come back further below.

488
 489 Altogether, these data suggest a COre Regulatory Complex (CoRC)⁶⁷ of SOX10, TFAP2A, MITF and
 490 RUNX factors in regulating melanoma MEL enhancers, encoded by a mixed enhancer model⁶⁸, with
 491 high flexibility in the combination of binding sites for these four TFs, but with some rigidity (or
 492 hierarchy) in the code as at least one SOX10 binding site is required.
 493



494
 495 **Figure 5. COre Regulatory Complex of MEL melanoma enhancers.** a, Schematic overview of motif scoring
 496 method in which extended convolutional filter hits from DeepMEL are multiplied by DeepExplainer profiles to

497 yield significant motif hits. **b**, Heatmap of the number of significant SOX, TFAP2, MITF and RUNX-like motif
498 hits on the 3,885 MEL predicted regions in the human cell line MM001. **c**, Binarised heatmap of significant SOX,
499 TFAP2, MITF and RUNX-like motif hit(s) on the 3,885 MEL predicted regions in the human cell line MM001.
500 Eight region clusters can be distinguished, representing different combinations of significant motifs present in the
501 enhancers. **d**, Aggregation plot of normalised ChIP-seq signal of SOX10 (left), MITF (middle) and TFAP2A
502 (right) on the human enhancer clusters. **e**, Aggregation plot of normalised ATAC-seq signal of MM001 on the
503 human enhancer clusters. **f, g**, Venn diagram representation of regions clusters on (**f**) the 3,885 regions predicted
504 as MEL in human (in MM001) and (**g**) the 4,194 MEL-predicted regions in dog (in Cesar). In addition, example
505 MEL-predicted regions in human and dog are shown for two of the region clusters: an intronic *CD9* enhancer as
506 representative for the SOX10 + MITF cluster and an intronic *STIM1* enhancer containing SOX10, TFAP2 and
507 RUNX motif hits.
508

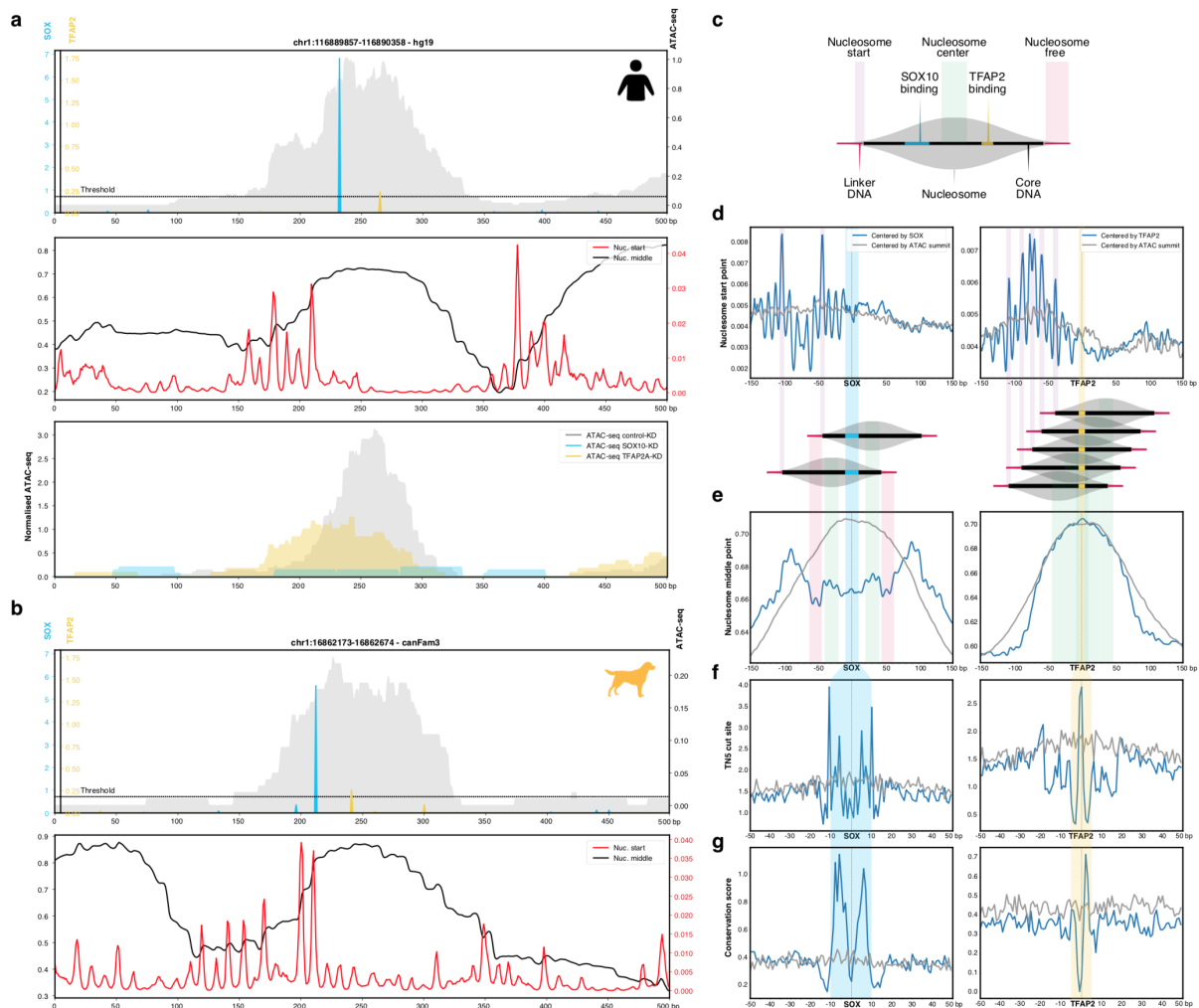
509 **SOX10 functions as pioneer and TFAP2A as stabiliser in melanoma MEL** 510 **enhancers**

511 As previous results suggested a pioneering and stabiliser function for SOX10 and TFAP2A respectively,
512 we wanted to further investigate these putative roles and how they are mechanistically affecting
513 chromatin accessibility. First, we analysed the location of binding sites relative to the position of the
514 nucleosome, focusing on MEL enhancers that contain a combination of SOX10 and TFAP2A sites (Fig.
515 6a,b). We predicted the nucleosome start and middle point using a previously published model⁶⁹.
516 Interestingly, we observed that SOX10 binding sites are situated within the borders of the nucleosome,
517 near the former nucleosome start point, whereas TFAP2A binding occurs preferentially near the center
518 of the nucleosome (Fig. 6a,b). Note that KD of TFAP2A halved the accessibility of this specific human
519 region, whereas SOX10-KD completely abolished the ATAC-seq peak (Fig. 6a), indicating that SOX10
520 is necessary for accessibility, and that TFAP2A further increases the accessibility, which is in line with
521 our previous observations (Fig. 5e, S5a).
522

523 These example enhancers raised an interesting positional preference of SOX10 and TFAP2A. To assess
524 whether this occurs globally we centered human MEL enhancers on the SOX10 and TFAP2A motif hits
525 and calculated the aggregated location of the nucleosome start and middle point (Fig. 6c,d,e).
526 Interestingly, SOX10 had a consistent preference for binding within the nucleosome borders, around 40
527 bp away from the nucleosome start point (Fig. 6c,d). Since in chromatinised DNA, 146 bp of DNA
528 sequence is wrapped around the nucleosome, we anticipated the nucleosome middle point to be situated
529 ~35 bp (= 146 bp / 2 - 40 bp) away from the SOX10 motif, which was indeed the case (Fig. 6e). Other
530 pioneering factors have also been shown to bind near the borders of the nucleosome, such as FOX
531 factors which bind around 60 bp from the center of the nucleosome, displacing linker histones and
532 destabilising the central nucleosome^{6,70}. On the other hand, when centering the MEL regions based on
533 the TFAP2A motif, we did not observe a strong preference in the location of the nucleosome start point
534 relative to the TFAP2A binding site (Fig. 6d), but in fact TFAP2A was consistently binding in a wide
535 range on and around the nucleosome middle point (Fig. 6e). Stabilisers, such as NFIB, are known to
536 directly compete with the central nucleosomes to stabilise the accessible chromatin configuration^{6,71}.
537 Centering based on the SOX10 motif hit revealed protection of Tn5 cutting on the conserved nucleotides
538 of the dimer (Fig 6f,g). Similarly, protection and conservation was observed on important nucleotides
539 in the TFAP2A dimer. We did not observe strong positional preferences of MITF and RUNX motifs
540 relative to the nucleosome start or middle point (Fig. S6).
541

542 Altogether these data highly suggest that SOX10 functions as a pioneer in the CoRC of MEL enhancers,
543 leading to their accessibility by binding to the central nucleosome, near the nucleosome start point. On

544 the other hand, TFAP2A appears to act as stabiliser of SOX-dependent nucleosome depleted regions by
 545 binding around the nucleosome middle point, possibly going in competition with the central
 546 nucleosome.
 547



548 **Figure 6. Positional specificity of SOX10 and TFAP2A in MEL melanoma enhancers.** **a.** (top) Example
 549 human MEL-predicted enhancer containing significant SOX10 and TFAP2 motifs. The ATAC-seq signal is
 550 shown in grey. (middle) Imputed nucleosome start and middle point profiles. (bottom) ATAC-seq profiles of
 551 MM001 in control condition, after 72 h of SOX10 knock-down or TFAP2A knock-down. **b.** (top) Example dog
 552 MEL-predicted enhancer containing significant SOX and TFAP2 motifs. The ATAC-seq peak is shown in grey.
 553 (bottom) Imputed nucleosome start and middle point profiles. **c.** Schematic overview of nucleosome structure
 554 explaining the colours used in **(d,e,f,g)**. **d,e,f,g.** Nucleosome start point **(d)**, nucleosome middle point **(e)**, Tn5 cut
 555 site **(f)**, phyloP conservation score profiles **(g)** on MEL-predicted regions containing one SOX10 (left) or one
 556 TFAP2 motif (right) next to possible other motifs, where the regions are either centered on the ATAC-seq summit
 557 (grey) or on the SOX10 or TFAP2 motif (blue). SOX10 binding is enriched around 40 bp away the nucleosome
 558 start point, as is clear by the two peaks in the nucleosome start profile **(d)** that are situated respectively ~40 and
 559 ~110 bp away from the beginning of the SOX10 motif (which is 20 bp long), reflecting SOX binding at either
 560 side of the nucleosome as shown by the illustration.
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563

564 DeepMEL predicts evolutionary changes in MEL enhancer accessibility 565 and activity

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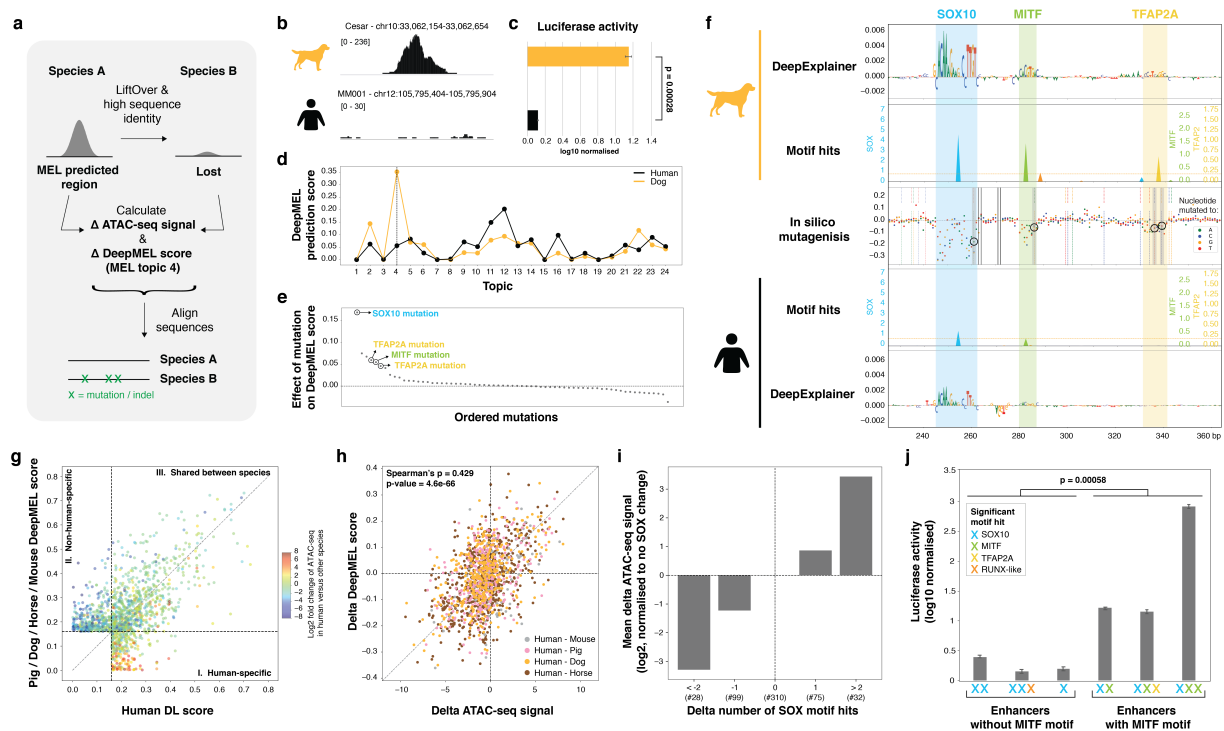
567 Next, we wanted to further validate our findings on the MEL enhancer logic using comparative
568 genomics. This allowed us, in addition, to test how turnover of TF binding sites affects enhancer
569 accessibility and function. To this end, we compared pairs of MEL enhancers that are homologous but
570 only accessible in one of the species, to investigate which mutations cause the collapse of a MEL
571 enhancer during evolution (Fig. 7a). We focused only on pairs of highly probable orthologous enhancers
572 by requesting a stringent liftOver score (minimum of 99% of the bases must remap) and high sequence
573 identity (at least 80% of the bases must be identical). We calculated the loss in ATAC-seq signal and
574 in DeepMEL score, and aligned the sequence pairs to determine point mutations and indels between the
575 homologous sequences (Fig. 7a). For example, an enhancer upstream of *APPL2* is predicted as MEL
576 enhancer in the MEL dog line Cesar (topic 4 DL score of 0.35), whereas the orthologous enhancer in
577 human was completely closed (Fig. 7b). Interestingly, not only the accessibility of the human homolog
578 was lost, but also the activity, as we confirmed by a luciferase assay (Fig. 7c). Importantly, the
579 DeepMEL score for this enhancer was seven times lower in human than in dog, falling below the topic
580 4 significance threshold of 0.16, indicating that the model detected critical changes in the human
581 enhancer sequence that could explain the loss of this MEL enhancer. To determine which mutations
582 were causal for the loss in accessibility (and activity), we calculated the effect on the MEL prediction
583 score of each detected point mutation between the dog and human sequence, via *in silico* mutating the
584 dog sequence (see Methods, similar as in the *IRF4* enhancer above). Several mutations seemed to alter
585 the DL score (Fig. 7e,f). To pinpoint the functional effect of each mutation, we plotted DeepExplainer
586 profiles and significant motif hits for CoRC factors on the original dog and human sequence (Fig. 7f).
587 The functional dog enhancer contained a SOX10, MITF and TFAP2A binding site, which (almost)
588 disappeared in the non-functional human homologous sequence. The losses could be explained by one
589 T-to-A mutation in the SOX10 motif, one A-to-G mutation in the MITF motif and two mutations in the
590 TFAP2A motif (Fig. 7f, encircled mutations). The SOX10 motif mutation had the strongest effect, as it
591 caused a 45% drop in the MEL-prediction score (Fig. 7e).

592

593 Next, we performed this analysis on a larger scale, to globally study evolutionary changes in
594 accessibility of orthologous MEL enhancers between human and each of the other mammalian species
595 in our cohort. Firstly, we compared the topic 4 DeepMEL score for each pair of orthologous MEL
596 enhancers and observed that regions predicted as MEL in human but not in the other species were indeed
597 more accessible in human (Fig. 7g, I); in contrast, regions that were only predicted as MEL enhancers
598 in a non-human species were lowly accessible in human (Fig. 7g, II). Orthologous regions that were
599 predicted as MEL enhancer in both human and another mammalian species were similarly accessible
600 in both species (Fig. 7g, III). In fact, DeepMEL proved to be a good predictor for evolutionary changes
601 in accessibility, displaying a high correlation between the delta accessibility and the delta MEL
602 DeepMEL score between orthologous regions (Spearman's correlation of 0.429) (Fig. 7h).
603 Interestingly, we noticed that among the four CoRC factors, mostly the disruption or gain of one or
604 more SOX10 binding sites between orthologous enhancers quantitatively altered the ATAC-seq signal
605 in a negative and positive way, respectively (Fig. 7i, Fig. S7a), indicating that SOX10 mutations are
606 most causal for changes in MEL enhancer accessibility. Indeed, in the example *APPL2* enhancer
607 presented above, a detrimental mutation in the SOX10 binding site had the strongest effect on the MEL
608 DeepMEL score (Fig. 7e,f), and thus likely, the most impact on not only the loss of enhancer
609 accessibility in human (Fig. 7b), but also on the loss of enhancer activity (Fig. 7c). However, this was
610 not the case for all MEL enhancers. For instance, an intronic enhancer of *KIF1B* was accessible and

611 predicted as MEL in human, but not in dog (Fig. S7b,d). Although the human region was accessible
 612 and predicted as MEL, both the dog and the human enhancer showed no strong activity in a luciferase
 613 assay (Fig. S7c). A deeper look at the enhancer code revealed that this human enhancer only contained
 614 two significant SOX10 binding sites, but none of the other three CoRC players (Fig. S7e,f).
 615 Interestingly, by testing the activity of a total of six human or dog MEL-predicted enhancers, we could
 616 distinguish two groups: enhancers that were only accessible and showed little activity ($n = 3$), or
 617 enhancers that were both accessible and significantly more active ($n = 3$) (Fig. 7j). Profiling
 618 DeepExplainer and significant motif hits revealed that the enhancers in the latter group all contained at
 619 least one significant MITF binding site, while none of the enhancer in the former group did. Although
 620 the number of tested enhancers is small, this trend, together with the fact that MEL enhancers containing
 621 a MITF binding site showed increased H3K27ac signal (Fig. S5b), indicates that MITF could function
 622 as activator in MEL enhancers. Indeed, MITF has been shown to activate genes involved in
 623 pigmentation by recruitment of co-factors and chromatin remodelling complexes⁷² and was previously
 624 classified as a TF involved in co-factor recruitment and activation based on its motif distribution in
 625 nucleosome depleted regions⁶. Importantly, note that SOX10 binding is insufficient but appears
 626 necessary for enhancer activity, as mutations in SOX10 binding sites disrupted enhancer activity in the
 627 *IRF4* (Fig. 3g).
 628

629 In conclusion, DeepMEL provides a suitable platform to study the effect of evolutionary mutations on
 630 MEL enhancer accessibility and, in some cases, activity across species. Together, these results validate
 631 that SOX10 is crucial for enhancer accessibility in MEL enhancers, and necessary but insufficient for
 632 MEL enhancer activity, as activity appeared to be mainly dependent on MITF binding.
 633



634 **Figure 7. Predicting causal mutations of evolutionary changes in MEL enhancers.** a, Homologous (identified
 637 by stringent liftOver and high sequence identity) MEL enhancers that are accessible and predicted as MEL in one
 638 species and that lose accessibility in another are used to identify deleterious cis-regulatory mutations by
 639 calculating the delta ATAC-seq signal and delta DeepMEL score for the MEL-specific topic (topic 4). b, c,
 640 Example region upstream of *APPL2* that is (b) accessible and active (c) in the MEL dog line Cesar but not in

641 human MEL lines (ATAC-seq profiles of Cesar and MM001 shown here). Luciferase activity in MM001 is shown
642 relative to renilla signal and is log₁₀ transformed. P-value was determined using Student's t-test and the error bars
643 represent the standard deviation. **d**, DeepMEL prediction score of each of the 24 topics for the dog and human
644 sequence. The dog sequence is predicted as MEL enhancer (topic 4 score > 0.16), whereas this is not the case for
645 the human sequence. **e**, The effect on topic 4 DeepMEL score on the dog sequence when *in silico* simulating each
646 of the single detected point mutations between the dog and human sequence. **f**, DeepExplainer plots and motif
647 hits for SOX10, MITF and TFAP2A are shown for part of the 500 bp dog and human sequence. In the middle, the
648 effect of each possible point mutation between the dog and human sequence on the MEL DeepMEL was *in silico*
649 calculated and is represented by coloured dots depending on the nucleotide the original dog nucleotide was *in*
650 *silico* mutated to. Truly existing point mutations between the dog and human sequence (as observed by alignment
651 of the sequence via Needle) are highlighted by vertical dashed lines (the colour indicates the original dog base
652 (top dashed line) and the human base (bottom dashed line)). Four mutations that decrease the motif score of the
653 SOX10, MITF and TFAP2A motifs are highlighted by a grey box and are encircled. **g**, Scatter plot of the
654 DeepMEL prediction score for topic 4 in human and in another non-human mammalian species of pairs of
655 homologous sequences. Only enhancers predicted as MEL-specific by DeepMEL (topic 4 score > 0.16) in at least
656 one of the species are used here. Enhancers are represented by a dot and are coloured by the log₂ fold change in
657 ATAC-seq signal between human and the other species. In the first quadrant (I) enhancers that are predicted as
658 MEL in human but not in the other species are shown; in quadrant (II) MEL enhancers of non-human species that
659 are not predicted as MEL in human; and the third quadrant (III) contains enhancers that are MEL-predicted in
660 both species. **h**, Scatter plot of the delta ATAC-seq signal and delta DeepMEL prediction score for topic 4 of pairs
661 of homologous enhancers between human and another mammalian species. Dots are colored depending on the
662 species the human homolog was compared to. **i**, Barplot showing the mean effect on the log₂ delta ATAC-seq
663 signal of a non-human region compared to the human homolog depending on the number of SOX10 motif hits
664 lost or gained. Only regions having no change in the number of significant TFAP, MITF and RUNX motifs hits
665 were used. The y-axis is normalised to the category with no changes in the number of significant SOX10 motif
666 hits. The number of regions in each of the categories is mentioned (#). **j**, Luciferase assay on six human or dog
667 enhancers. Significant motif hits per enhancer are shown with coloured crosses. Luciferase activity in MM001 is
668 shown relative to renilla signal and is log₁₀ transformed. P-values were determined using Student's t-test and the
669 error bars represent the standard deviation over three biological replicates.

670

671 Discussion

672 Here, we present an in-depth study of melanoma enhancer logic, especially in enhancers specific to the
673 MEL state, by exploiting both cross-species data and machine learning. Although the MEL and MES
674 melanoma cell state have been studied extensively on a transcriptomic and epigenomic level, the
675 combinatorial code of binding sites of their regulatory factors in state-specific enhancers has not yet
676 been explored. Understanding the enhancer logic and the mechanism by which TFs bind and direct
677 active enhancers will become increasingly important, as it will be essential for the development of new
678 therapies that either influence cell state-specific enhancer functions; for the use of (synthetic) enhancers
679 in a targeted way, i.e. enhancer therapy^{73,74}; or to prioritise non-coding variants in whole genome
680 sequencing studies of personal or cancer genomes (see our companion paper).

681
682 Predicting enhancers and determining their functional role within gene regulatory networks has been an
683 active field for years. Classically, ChIP-seq¹, motif discovery tools^{1,8}, genetic screens^{13,14} and
684 comparative genomic studies¹⁰⁻¹² have proven useful to reach this goal. For instance Villar et al.
685 uncovered enrichment of CEBPA motifs in highly conserved liver enhancers by performing a
686 comparative genomic analysis in 20 mammalian species; and Prescott et al. identified a novel
687 ‘coordinator’ motif predictive of species-biased cranial neural crest enhancers between human and
688 chimp. Despite the well-established power of cross-species approaches, to our knowledge, a large
689 comparative epigenomics study in melanoma has not yet been conducted, although several non-human
690 models are commonly used in melanoma research³⁴. These have either been studied on an intra-species
691 level^{33,75-80}; in relation to human melanoma at the level of marker genes³⁰, morphology and
692 pharmacological sensitivity³², transcriptome⁸¹; or across three species in the context of genomic
693 landscapes⁸². Here, we conducted a comparative epigenomics study in melanoma across six species,
694 allowing us to demonstrate, for the first time, the conservation of not only the MEL cell state (and the
695 MES cell state in dog), but also the conservation of the underlying master regulators, based on
696 enrichment of TF binding sites within differential MEL and MES peaks and within conserved MEL
697 enhancers.

698
699 Although their proven advantages, sequence-based comparative approaches have limited power to
700 identify orthologous regulatory regions in distant species, in part because of the rapid evolution of distal
701 enhancers^{83,84}. Methods, such as enhancer element locator (EEL), try to tackle this question by aligning
702 TF binding sites to identify conserved enhancer elements⁸⁵, or by calculating the co-occurrence of
703 sequence patterns⁶¹. However, these methods are either supervised as they require user-provided PWMs
704 ⁸⁵ or are difficult to extract the important biologically-relevant features from⁶¹. In addition, the
705 identification and exact localisation of important (*de novo*) TF binding sites within enhancers is
706 complex as motif discovery tools are often dependent on user-provided databases and motif-specific
707 thresholds. Recently, deep learning approaches, which are commonly used in disciplines such as speech
708 recognition and image analysis, found their way into the regulatory genomics field to overcome these
709 concerns¹⁵, but have, to our knowledge, not yet been applied to evolutionary enhancer studies. As deep
710 learning models, such as DeepBind, are particularly powerful in learning complex patterns by
711 leveraging large epigenomics datasets, they are well suited to function as *de novo* motif detectors, as
712 well as to uncover more complex sequence features at higher-level layers that capture the internal
713 structure^{15,16}. By designing DeepMEL, a multi-class multi-label neural network trained on melanoma-
714 specific human regulatory topics of co-accessible regions, and by using the model interpretation tool
715 DeepExplainer^{54,55}, we were able to perform a thorough and unsupervised analysis of important TF
716 binding sites in melanoma enhancers. Specifically, in MEL enhancers, our data suggests conserved co-
717 binding of a Core Regulatory Complex of four main transcription factors, consisting of SOX10,

718 TFAP2A and MITF. DeepMEL also finds motifs for RUNX factors, but their role in the melanocyte or
719 melanoma is less clear. Evidence for co-binding of SOX10, MITF, and TFAP2A was previously
720 observed by enrichment of both MITF and TFAP2A motifs in SOX10 ChIP-seq data in melanoma
721 cells⁶⁵. To predict the precise location and the significance of these TF binding motifs, we designed a
722 new motif scoring scheme by multiplying DeepMEL convolution filters with DeepExplainer
723 profiles^{54,55}. We observed high flexibility in the organisation of TF binding sites of the CoRC since
724 eight different modalities were found, formed by all permutations of the CoRC factors, with the
725 exception that all MEL enhancers contained at least one SOX10 binding site. MEL enhancers adhere to
726 a ‘mixed modes enhancer’ model, a billboard-like model with mostly high flexibility in the TF motif
727 organisation, except for the ever-present SOX10 binding sites⁶⁸. Other cross-species studies of
728 enhancers have used ChIP-seq against TFs to examine conserved and divergent enhancers^{10,86,87}. Here
729 we avoid the necessity of cross-species ChIP-seq data, as we approximate this by combining ATAC-
730 seq and DeepMEL to characterise, in an unsupervised way, the conservation and divergence of
731 enhancers linked to several melanoma master regulators

732

733 It is well recognised that distinct functional classes of TFs exist, with respect to enhancer binding.
734 Pioneer TFs, such as OCT4, SOX2, GRHL, and FOXA1, are able to bind nucleosomal DNA, leading
735 to displacement of the nucleosome and facilitating the binding of other TFs to the accessible
736 enhancer^{5,7,68}. SOX2, for example, was shown to bind nucleosomal DNA *in vitro* and associate with
737 closed chromatin⁸⁸⁻⁹⁰. SOX2 and other SOX factors have a HMG domain that interacts with the minor
738 groove of the DNA, causing the DNA to bend in a 60-70° angle, a property that has been suggested to
739 contribute to the pioneering activity of SOX2, and possibly of other SOXs⁹¹. There is still some dispute
740 on the pioneering properties of SOX TFs, as another study classified SOXs as ‘migrant TFs’, i.e. non-
741 pioneering TFs that only bind sporadically to (non)-chromatinised DNA⁹². Nonetheless, we find strong
742 evidence for a pioneering function of SOX10 in MEL melanoma cells. Our current and previous study²⁹
743 have shown that knock-down of SOX10 induces closure of SOX10-bound ATAC-seq peaks containing
744 a SOX10 motif. In fact, DeepMEL predicts SOX10 binding sites as essential for MEL enhancer
745 accessibility. SOX10 is known to engage with open chromatin, as 98% of SOX10 ChIP-seq peaks
746 overlap with DNase-seq sites⁵⁷ and, in addition, SOX10 has been shown to physically interact with
747 BRG1, a subunit of the SWI/SNF chromatin remodeling complex, in differentiating melanocytes⁹³.
748 Altogether, this supports the pioneering role of SOX10 in melanocytic melanomas. Notably, especially
749 the binding of SOX10 *dimers* appeared important for MEL enhancer accessibility as eight of the ten
750 enriched SOX10 DL filters in topic 4 represent a SOX10 dimer motif rather than a monomeric motif.
751 This is further supported by the fact SOXE proteins, such as SOX10, are known to form homo- and
752 heterodimers with other SOXE factors⁹⁴. In addition, a study on SOX9, another member of the SOXE
753 TF family, showed that dimerisation of SOX9 was necessary to remodel the chromatin of a *Col2a1*
754 enhancer and to, eventually, allow its activation⁹⁵. Interestingly, we also detected a positional specificity
755 for the SOX10 dimer binding sites as they are mainly localised within the nucleosomal DNA, around
756 40 bp inwards from the nucleosome start point. Although the findings from Zhu et al. support the
757 binding of SOX(10) proteins inside the nucleosome borders, they observe an enrichment of SOX10
758 binding towards the dyad of the nucleosome, more towards the center compared to our results reveal.
759 Therefore, further investigations of SOX10 binding to chromatinised DNA might improve the
760 resolution of the exact location of this TF with relation to the nucleosome start and middle point.

761

762 Next to pioneer factors, other functional classes of TFs exist, including factors that stabilise the
763 accessibility of the nucleosome depleted regions. TFAP2A was previously classified as such a
764 chromatin stabiliser⁶. Indeed, evolutionary divergence from the TFAP2A consensus motif correlates
765 with loss of chromatin accessibility and H3K27ac ChIP-seq signal¹¹. These reports support our

766 observations of TFAP2A as a stabiliser of SOX10-dependent accessible MEL enhancers, likely due to
767 direct competition of TFAP2A with the nucleosome, as TFAP2A binding sites were highly enriched at
768 the predicted center of the central nucleosome. The dependence of SOX10 for opening MEL enhancers
769 prior to TFAP2A binding is in line with the reported classification of TFAP2A as a ‘settler’, a TF whose
770 binding depends predominantly on the accessibility of the chromatin at their binding sites⁹².

771
772 Besides classifying accessible (orthologous) regions and predicting important TF motifs within them,
773 DeepMEL is an accurate predictor of the effect of mutations on enhancer accessibility and, for some
774 enhancers, also the activity. This was for instance the case for the *IRF4* MEL enhancer, where
775 DeepMEL performed best among the computational methods tested in Kircher et al.. Note however,
776 that the other models in the benchmark were trained to predict the activity of a total of 20 regulatory
777 regions ranging across different cell types; whereas our DL model is specialised for melanoma
778 regulatory regions. This demonstrates the value of using case-specific training data, such as the data set
779 generated in this study for melanoma. Interestingly, not all predicted MEL enhancers were in fact active.
780 Luciferase assays on a total of six MEL enhancers suggest that SOX10 alone is sufficient for enhancer
781 accessibility, but not for enhancer activity, as MITF binding seems to be needed to activate SOX10-
782 dependent melanoma enhancers. The study of Fufa et al. supports this hypothesis, as activating SOX10-
783 regions in mouse melanocytes showed significant enrichment of E-box motifs (bound by the bHLH
784 protein family, which includes MITF), indicating that it might cooperate with SOX10 to execute
785 melanocyte-specific gene activation. In addition, MITF was previously classified as a TF involved in
786 co-factor recruitment and activation^{6,72}. Although SOX10 binding is not sufficient for enhancer activity,
787 it is necessary, as disruption of the SOX10 binding site in the *IRF4* enhancer had a strong effect on
788 activity, probably due to the reappearance of the central nucleosome. Also in an enhancer located about
789 15 kb upstream of the MEL-specific gene tyrosinase in mouse, both Sox10 and Mitf binding sites were
790 required for activity⁹⁶. This mode of action is also present in other cell types, such as epithelial cells in
791 *Drosophila*, where Grainyhead acts as pioneer TF and is necessary for both accessibility and activity of
792 epithelial enhancers, but not sufficient for their activity; where it was suggested that the TF Atonal, also
793 a bHLH factor like as MITF, could function as activator of Grh-dependent enhancers⁷. Note that the
794 human and pig predicted MEL enhancers were also accessible in human and pig melanocytes,
795 respectively, indicating that we possibly could extend these observations on the MEL enhancer logic to
796 enhancers in melanocytes.

797
798 In conclusion, the combination of comparative epigenomics with deep learning allowed us to perform
799 an in-depth analysis of the melanoma enhancer logic. This work presents an overall framework which
800 can be applied to decipher the enhancer logic in a cell type or cell state of interest, starting from the
801 generation of an extensive cell type-specific (cross-species) epigenomics dataset, all the way through
802 the training and exploitation of a deep neural network to decode enhancer features across species, and
803 to utilise it to assess the impact of cis-regulatory variation.

804 **Methods**

805 **Cell culture**

806

807 *Human melanoma cell lines*

808 Human melanoma cultures (“MM lines”) are short-term cultures derived from patient biopsies^{27,35}
809 (Gembaraska et al., 2012; Verfaillie et al., 2015). Cells were cultured at 37°C with 5% CO₂ and were
810 maintained in Ham's F10 nutrient mix (Thermo Fisher Scientific) supplemented with 10% fetal bovine
811 serum (FBS; Invitrogen) and 100 µg ml⁻¹ penicillin/streptomycin (Thermo Fisher Scientific).

812 *Zebrafish melanoma cell lines*

813 Experiments were performed as outlined by Ceol et al.⁹⁷. Briefly, 25 pg of MCR:EGFP were
814 microinjected together with 25 pg of Tol2 transposase mRNA into one-cell Tg(BRAFV600E);p53^{-/-};
815 mitf^{-/-} zebrafish embryos. Embryos were scored for melanocyte rescue at 48-72 hours post-fertilisation,
816 and equal numbers were raised to adulthood (15-20 zebrafish per tank), and scored weekly (from 8-12
817 weeks post-fertilization) or bi-weekly (> 12 weeks post-fertilization) for the emergence of raised
818 melanoma lesions³¹. For *in vitro* culture, large tumors were isolated from MCR/MCR:EGFP (14-28
819 weeks post-fertilization). Zebrafish were maintained under IACUC-approved conditions. Zebrafish
820 primary melanoma ZMEL1 cell line was previously described^{38,39} and EGFP 121-1, EGFP 121-2, EGFP
821 121-3, EGFP 121-5, were generated as described^{98,99}. All cell lines were cultured in DMEM medium
822 (Life Technologies) supplemented with 10% heat-inactivated FBS (Atlanta Biologicals), 1X
823 GlutaMAX (Life Technologies) and 1% Penicillin-Streptomycin (Life Technologies), at 28°C, 5% CO₂.
824 Zebrafish melanoma lines were authenticated by qPCR and Western for EGFP transgene expression,
825 and periodically checked for mycoplasma using the Universal Mycoplasma Detection Kit (ATCC).

826

827 *Horse melanoma cell lines*

828 The horse cell lines HoMel-L1 and HoMel-A1 are melanoma cell lines derived from a Lipizzaner
829 stallion and Shagya-Arabian mare respectively and were established in Seltenhammer et al.. Cells were
830 cultured at 37°C with 5% CO₂ in Roswell Park Memorial Institute (RPMI) medium (Thermo Fisher
831 Scientific) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 1%
832 penicillin/streptomycin (Thermo Fisher Scientific).

833 *Pig melanoma and melanocyte cell lines*

834 Both the immortal line of pigmented melanocytes (PigMel) and the primary melanoma cell line
835 (MeLiM) were previously derived^{30,100}. PigMel cells were cultured at 37°C with 10% CO₂ in MEM
836 medium supplemented with 1X MEM non essential amino acids (Thermo Fisher Scientific), 10mM Na
837 pyruvate, 2mM glutamine, 100U/ml penicilin/streptomycin (Thermo Fisher Scientific), 10% FCS and
838 3,7g/ml Na bicarbonate. MeLiM cells were cultured in DMEM high glucose (Thermo Fisher Scientific),
839 10% FCS, Pen/Strep, 5% CO₂.

840 *Dog melanoma cell lines*

841 The dog cell lines Bounty and Cesar were established by Aline Primot³⁷, and were derived from an
842 uveal melanoma from a Beagle crossed dog and an oral melanoma from the palate from a Shih-tzu,
843 respectively. Cells were cultured at 37°C with 5% CO₂ in Ham's F-12 Nutrient Mixture medium
844 (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 1%
845 penicillin/streptomycin (Thermo Fisher Scientific).

846 *Mouse melanoma cell lines*

847 The mouse melanoma cell line was generated as described in³⁶. Cells were cultured at 37°C with 5%
848 CO₂ in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific) supplemented with
849 10% fetal bovine serum (FBS; Invitrogen) and 1% penicillin/streptomycin (Thermo Fisher Scientific).

850 **Knock-down experiments**

851 SOX10, TFAP2A and the control knockdown were performed in MM001 using a SMARTpool of four
852 siRNAs against, respectively, SOX10 (SMARTpool: ON-TARGETplus SOX10 siRNA, number
853 L017192-00-0005, Dharmacon), TFAP2A (SMARTpool: ON-TARGETplus TFAP2A siRNA, number
854 L-006348-02-0005, Dharmacon) and a negative control pool (ON-TARGETplus non-targeting pool,
855 number D-001810-10-05, Dharmacon) at a concentration of 20 nM for SOX10-KD, and 40 nM for
856 TFAP2A-KD and the control using as medium Opti-MEM (Thermo Fisher Scientific) and omitting
857 antibiotics. The cells were incubated for 72 h before processing.

858 **OmniATAC-seq data generation, data processing and follow-up analyses**

859
860 *OmniATAC-seq on mammalian lines*

861
862 OmniATAC-seq was performed as described previously¹⁰¹. Cells were washed, trypsinised, spun down
863 at 1000 RPM for 5 min, medium was removed and the cells were resuspended in 1 mL medium. Cells
864 were counted and experiments were only continued when a viability of above 90% was observed.
865 50,000 cells were pelleted at 500 RCF at 4°C for 5 min, medium was carefully aspirated and the cells
866 were washed and lysed using 50 uL of cold ATAC-Resuspension Buffer (RSB) (see Corces et al. for
867 composition) containing 0.1% NP40, 0.1% Tween-20 and 0.01% digitonin by pipetting up and down
868 three times and incubating the cells on ice for 3 min. 1 mL of cold ATAC-RSB containing 0.1% Tween-
869 20 was added and the eppendorf was inverted three times. Nuclei were pelleted at 500 RCF for 10 min
870 at 4°C, the supernatant was carefully removed and nuclei were resuspended in 50 uL of transposition
871 mixture (25 uL 2x TD buffer (see Corces et al. for composition), 2.5 uL transposase (100 nM), 16.5 uL
872 DPBS, 0.5 uL 1% digitonin, 0.5 uL 10% Tween-20, 5 uL H₂O) by pipetting six times up and down,
873 followed by 30 minutes incubation at 37°C at 1000 RPM mixing rate. After MinElute clean-up and
874 elution in 21 uL elution buffer, the transposed fragments were pre-amplified with Nextera primers by
875 mixing 20 uL of transposed sample, 2.5 uL of both forward and reverse primers (25 uM) and 25 uL of
876 2x NEBNext Master Mix (program: 72°C for 5 min, 98°C for 30 sec and 5 cycles of [98°C for 10 sec,
877 63 °C for 30 sec, 72°C for 1 min] and hold at 4°C). To determine the required number of additional
878 PCR cycles, a qPCR was performed (see Buenrostro et al.³ for the determination of the number of extra
879 cycles). The final amplification was done with the additional number of cycles, samples were cleaned-
880 up by MinElute and libraries were prepped using the KAPA Library Quantification Kit as previously
881 described¹⁰¹. Samples were sequenced on a HiSeq4000 or NextSeq500 High Output chip.

882 *ATAC-seq on zebrafish lines*

883 50,000 cells per line were lysed and subjected to a tagmentation reaction and library construction as
884 described in Buenrostro et al.³. Libraries were run on an Illumina HiSeq 2000.

885

886 *Data processing of human melanoma baseline OmniATAC-seq samples*

887 Paired-end reads were mapped to the human genome (hg19-GenCode v18) using bowtie2 (v2.2.6).
888 Mapped reads were sorted using SAMtools (v1.8) and duplicates were removed using Picard

889 MarkDuplicates (v1.134). Reads were filtered by removing mitochondrial reads and filtering for Q>30
890 using SAMtools. Bam files of technical replicates of the same cell line were merged at this point using
891 samtools merge. Peaks were called using MACS2 (v2.1.2) callpeak using the parameters -q 0.05, --
892 nomodel, --call-summits, --shift -75 --keep-dup all and --extsize 150 per sample. Blacklisted regions
893 (ENCODE) and peaks overlapping with alternative chromosomes and chrM were removed. Summits
894 were extended by 250bp up- and downstream using slopBed (bedtools; v2.28.0), providing human
895 chromosome sizes. Peaks were normalised for the library size using a custom script and overlapping
896 peaks were filtered using the peak score by keeping the peak with the highest score. For visualisation
897 in IGV, normalised bigWigs were made by bamCoverage (DeepTools, v3.3.1), using as parameters --
898 normalizeUsing None, -bl EncodeBlackListedRegions --effectiveGenomeSize 2913022398 and as
899 scaling parameter (-scaleFactor) 1/(RIP/1E6), where the RIP stands for the number of reads in peaks.

900 *Data processing of non-human (Omni)ATAC-seq samples, and of human SOX10 and TFAP2A knock-*
901 *down OmniATAC-seq data*

902 Adapter sequences were trimmed from the fastq files using fastq-mcf (as part of eautils; v1.05) and the
903 read quality was checked using FastQC (v0.11.8). Reads were mapped using STAR (v2.5.1b) (for the
904 zebrafish samples paired-end reads were mapped) to the genome which were downloaded from UCSC
905 (<http://hgdownload.cse.ucsc.edu/goldenPath/>) (for human: hg19-Gencode v18; for dog: canFam3; for
906 horse: equCab2; for pig: susScr11; for mouse: mm10; for zebrafish: danRer10) and by applying the
907 parameters --alignIntronMax 1 and --alignIntronMin 2. Mapped reads were filtered for quality using
908 SAMtools (v1.2) view with parameter -q4, sorted with SAMtools sort and indexed using SAMtools
909 index. Peaks were called using MACS2 (v2.1.2) callpeak using the parameters -q 0.05, --nomodel, --
910 call-summits, --shift -75 --keep-dup all and with the genome size for the correct species in --g, and this
911 for each sample per species separately. Summits were extended by 250bp up- and downstream using
912 slopBed (bedtools; v2.28.0), providing the chromosome sizes for the specific species. Per sample, peaks
913 were normalised for the library size using a custom script and overlapping peaks were filtered using the
914 peak score (keeping the highest scoring peak). Normalised bedGraphs were produced by
915 genomeCoverageBed (as part of bedtools; v2.28.0) using as scaling parameter (-scale) 1E6/(number of
916 non-mitochondrial mapping reads). BedGraphs were converted to bigWigs by the bedtools suit
917 functions bedSort to sort the bedGraphs, followed by bedGraphToBigWig to create the bigWigs, which
918 were used in IGV for visualisation.

919 *Homer on human and dog differential accessible peaks*

920 First, merged bed files of human and dog ATAC-seq regions were converted to gff format. Count
921 matrices were produced by featureCounts (v1.6.5) using these gff files and bam files of 5 MEL and 5
922 MES lines for human, and gff and bam files of Cesar and Bounty for dog. Differential peaks were
923 identified using DESeq2 (v1.22.2, R v3.5.2) with a log2FC higher than 2 and a pAdj lower than 0.0005.
924 Homer⁴⁷ was performed on the differential regions using findMotifsGenome.pl, providing the
925 differential regions as a bed file and a fasta file of the human or dog genome, with parameters -mask, -
926 size give and -len 6,8,10,11,12,17,18.

927 *Defining sets of conserved ATAC-seq regions*

928 Accessible regions of non-human species were converted to hg19 coordinates using liftOver (Kent-
929 tools) by providing the appropriate liftOver chain (UCSC) and allowing a -minMatch=0.1. LiftOvered
930 regions were intersected with accessible peaks in human (accessible peaks of 5 MEL MM lines) using
931 intersectBed (bedtools, v2.28.0) with -f 0.6 and to define set of conserved regions across species, e.g.
932 conserved regions in across the six species were identified by the intersection of all liftOver bedfiles of

933 non-human species with the human accessible regions, maintaining only the coordinates with which all
934 six species overlapped.

935 *Clustering of species based on conserved ATAC-seq regions*

936 Per species, a count matrix was made on the conserved ATAC-seq regions (conserved in all mammalian
937 species or in all six species, as described above) by featureCounts (v1.6.5) using a gff file of the
938 conserved regions in the coordinates of the specific species and bam files for the specific species. Count
939 matrix of different species were merged and the final count matrix was CPM normalised (edgeR
940 v3.22.5, R v3.5.2), followed by quantile normalisation. A principal component analysis (PCA) on the
941 normalised count matrix was performed using irlba (v2.3.3, R v3.5.2) and the first two principal
942 components were used for visualisation.

943 **Branch length scoring across species**

944 Conserved ATAC-seq regions were identified as described above, and for each of the species, the set
945 of conserved regions was converted to the coordinate system per species and fasta sequences were
946 retrieved. All sequences were scored with our collection of 20,003 motifs using Cluster-Buster¹⁰² with
947 parameters -m 0, -c 0 and -r 10000. For each motif, the highest CRM score per conserved sequence was
948 used to calculate the BLS across species according to (ref). The branch length was taken from the
949 phylogenetic data from <http://hgdownload.cse.ucsc.edu/goldenpath/hg19/phyloP100way/> (UCSC). The
950 sum of the BLSs for all the conserved sequences across the mammalian or all six species was used as a
951 total score for each motif. We normalised these scores by performing BLS on a shuffled variant of all
952 sequences by shuffleseq (EMBOSS, v6.6.0.0), keeping the same base-pair compositions and sequence
953 lengths, and subtracting the shuffled BLS from the true BLS pre motif. This corrected BLS per motif
954 represents the conservation of the motif across a set of conserved regions across a set of species.

955 **cisTopic analysis to obtain sets of co-accessible regions in human OmniATAC-seq data**

956 To apply cisTopic²⁹, a tool for single-cell ATAC-seq analysis, we first simulated single cells from our
957 bulk OmniATAC-seq data on the 17 human melanoma lines via bootstrapping. Per cell line, 50
958 simulated single cell bam files were generated containing each 50,000 random reads that were
959 bootstrapped from the bulk bam files. These simulated single cell bam files were provided as input for
960 cisTopic (v0.2.0, R v3.4.1), together with the merged regions across all 17 samples, after removing
961 blacklisted regions (ENCODE). We ran cisTopic (parameters: $\alpha=50/T$, $\beta=0.1$, burn-in
962 iterations = 500, recording iterations = 1,000) for models with a number of topics between 2 and 30 (2
963 by 2). The best model, containing 24 topics, was selected on the basis of the highest log-likelihood.
964 Topics were binarised using a probability threshold of 0.995, and performed motif enrichment analysis
965 with cisTarget⁸.

967 **Deep Learning**

968 *Data preparation*

969 Regions, which were obtained after peak calling for each baseline (as explained in *Data processing of*
970 *human melanoma baseline OmniATAC-seq samples*), were merged into one bed file and overlapping
971 regions were removed via custom script. Before intersecting this merged peak file with topics to label
972 each region, regions were augmented in order to have more training data for DeepMEL by extending
973 them to 700 bp and sliding a 500 bp window over them with a 10 bp stride, which meant that each 500
974 bp augmented region still contained the ATAC-seq summit. Each augmented region had at least 400 bp
975 overlap with its origin. This augmented master region file was intersected with each topic file separately
976 via bedtools and each region was labelled with the topic number if there was an at least 60% overlap.
977

978 If regions overlapped with multiple topics, we assigned multiple labels to them, allowing for a multi-
979 label and multi-class DL model. The average number of regions in each topic was 1,498 (35,940 in
980 total). After the augmentation and intersection, there were 696,654 regions for training in total,
981 excluding 58,086 chr2 regions for testing.

982 *The DeepMEL model architecture and training parameters*

983

984 The DeepMEL architecture was built by using mainly 4 layers between input and output layer; Conv1D
985 layer (with 128 filters, kernel_size as 20, strides as 1, and activation as relu), MaxPooling1D layer (with
986 pool_size as 10 and strides as 10), TimeDistributed Dense layer together with Bidirectional LSTM layer
987 (with 128 units, dropout as 0.1, recurrent_dropout as 0.1), and Dense layer (with 256 units and activation
988 as relu). After MaxPooling1D, Bidirectional LSTM, and Dense layer Dropout was used as 0.2, 0.2, and
989 0.4 respectively. The DeepMEL takes one-hot encoded (500bp x 4 nucleotide) forward and reverse
990 strand of the region, passes them separately through the model and takes the average of the activations
991 of the neurons in the final Dense layer (24 units corresponding to 24 topics with sigmoid activation)
992 with the *average* function in order to make the final prediction. The model was compiled using Adam
993 optimizer with 0.001 learning rate. In order to make the model multi-label classifier, sigmoid activation
994 function was used at the end of the final layer of the model and binary cross entropy loss function was
995 used. The model was trained for 2 epochs with 128 batch size, which took 67 minutes. Keras 2.2.4¹⁰³
996 with tensorflow 1.14.0¹⁰⁴ was used. A Tesla P100-SXM2-16GB GPU was used for training on VSC
997 servers (Flemish Supercomputer Center).

998

999 *Performance evaluation*

1000 The performance of the model was evaluated for each topic separately since it was a multi-label
1001 classifier. The area under the Receiver Operator Characteristic curve (auROC) and the Precision Recall
1002 curve (auPR) were calculated for training (regions on all chromosomes except chr2), test (regions on
1003 chr2), and label-shuffled regions.

1004 *Converting convolution filters to PWMs, filter-topic assignment, and filter-annotation*

1005 After the model was trained, the filters of the convolution layer were converted into PWMs by the
1006 following strategy: (i) 4,000,000 unique 20bp-long (size of the filters) sequences were randomly
1007 generated. (ii) The activation score of each filter for each sequence was calculated and the top 100
1008 sequence were selected. (iii) A count matrix was generated from these 100 sequences obtained for each
1009 filter. (iv) Finally, the count matrices were converted into PWMs. In order to assign the filters to topics,
1010 a similar strategy that is mentioned in Basset¹⁸ was used. The activation score of the filter was separately
1011 set to its mean activation score over all sequences, then the loss/accuracy score on the prediction was
1012 calculated for each class. Filters were ordered based on their effect on a certain topic. After the filters
1013 were converted into PWMs, Tomtom⁵⁹ motif annotation tool was used together with using a curated
1014 collection of more than 22,000 PWMs in order to annotate the DL features to known motifs. The cutoff
1015 for the q-value was set to 0.3.

1016 *DeepExplainer*

1017 Among 35,940 topic regions, 500 of them were randomly selected to initialise DeepExplainer⁵⁴.
1018 Importance score for each position of the sequence of interest was calculated with respect to any of the
1019 24 classes. The hypothetical importance score, which is obtained from the DeepExplainer output, was
1020 multiplied by the one-hot encoded matrix of the sequence. Finally, the 500 bp sequences were visualised

1021 by adjusting the nucleotide heights based on their importance score by using modified viz_sequence
1022 function from the DeepLift¹⁰⁵ repository.

1023 *In silico saturation mutagenesis on IRF4*

1024 By changing each nucleotide on a 500 bp sequence into three other nucleotides, 1,500 sequences that
1025 contain only one mutation compared to initial sequence were generated and scored by the model. The
1026 delta prediction score for each mutation was calculated for each class by comparing the final prediction
1027 score relative to the prediction score for the initial sequence. The *IRF4* enhancer (chr6:396,143-
1028 396,593) used in *in vitro* saturation mutagenesis assay is also covered by one of our MEL enhancers
1029 predicted as topic 4 (chr6:396,135-396,636). *In silico* saturation mutagenesis assay on this region was
1030 done using the delta prediction score of topic 4 and a Pearson correlation was calculated on overlapping
1031 nucleotides between the *in silico* and *in vitro* assays (451 bp).

1032 *Motif scoring method and centering regions*

1033 Using only the filters identified from the convolutional layer is not sufficient to localise significant
1034 motif hits on MEL enhancers since it does not necessarily mean that when the activation of a filter
1035 passes the activation threshold, that the filter has an effect on the final classification for a position in
1036 the sequence. Also using only the DeepExplainer importance scores is not sufficient either since it is
1037 not able to precisely detect the exact location, size, and the name of the motif hit. In order to overcome
1038 this problem, activation scores of the filters on each sequence were multiplied by the DeepExplainer
1039 importance scores. Then, a threshold was calculated for each motif by comparing MEL and MES
1040 enhancers after the output of the multiplication was normalised. This approach yielded significant motif
1041 hits with their precise location.

1042 *Nucleosome positioning*

1043 Nucleosome start and middle point predictions were calculated by using an executable nucleosome
1044 prediction tool called Kaplan_v3⁶⁹ that takes only the DNA sequence and calculates the nucleosome
1045 positioning for each nucleotide. In order to get more precise results, as the authors of Kaplan_v3
1046 suggest, enhancers were extended 3 kb from both ends. After obtaining the predictions, the middle 500
1047 bp part of the 6.5kb nucleosome prediction score was used.

1048 *Tn5 footprinting*

1049 Footprint of the Tn5 was determined by inferring Tn5 cut sites with a custom script that takes bam file
1050 and locates the Tn5 cut site deduced from the start point of each read resulted from the ATAC
1051 sequencing.

1052

1053 **AUROC on human and dog of DL and Cluster-Buster**

1054 To the performance of the model to discriminate between MEL and MES regions in human and dog
1055 was performed by scoring the top 5,000 differential MEL and MES regions in human and dog (described
1056 above) by DeepMEL and calculating precision of correct assignment (i.e. topic 4 score for the MEL
1057 regions and topic 7 scores for the MES regions). The performance of DeepMEL was compared with the
1058 motif scoring tool Cluster-Buster¹⁰² by scoring the same sets of regions with Cluster-Buster by using a
1059 merged motif file of (some of) the top filters identified by the model in either topic 4 or topic 7, and by
1060 using the obtained CRM score to estimate the performance of Cluster-Buster.

1061

1062

1063 **Identification of homologous MEL genes and enhancers**

1064 To identify genes differentially expressed in human MEL cell lines, we performed DESeq2 (v1.22.2, R
1065 v3.5.2) on 7 MEL (MM031, MM034, MM057, MM074, MM087, MM118, MM164) and 5 MES
1066 (MM029, MM099, MM116, MM163, MM165) human lines. 379 genes were found differentially
1067 expressed in MEL lines ($\log_2FC > 2.5$ and $adjP < 0.005$). We converted the gene symbols to Ensemble
1068 gene IDs using biomaRt (v2.38.0, R v3.5.2) and found back the genomic locations of the genes using
1069 GenomicFeatures (v1.34.8, R v3.5.2). We searched for MEL enhancers in the extended gene loci, by
1070 extending the genomic locations 200 kbp upstream and downstream of the start and the end of the gene
1071 and using bedtools intersect (v2.28.0) to intersect the extended loci with the MEL-predicted regions in
1072 MM001. For the human differential MEL genes with at least one MEL-predicted peak in their extended
1073 gene locus, the homologous genes in the other six species was identified by using biomaRt to convert
1074 the human Ensemble gene IDs to Ensemble gene IDs of the other species. Again GenomicFeatures was
1075 used to get the genomic locations of the genes in the different species. Next, we identified the MEL
1076 enhancers per species that were intersection with the extended gene loci of each of the homologous
1077 genes in that specific species using bedtools intersect. liftOver -minMatch=0.1 was used to calculate
1078 the number of these regions that could be identified by performing coordinate conversion.

1079

1080 **Correlation of MEL enhancers using deep layers of DeepMEL**

1081 Conserved MEL enhancers in the extended loci of conserved MEL genes across the six species were
1082 scored by the DeepMEL. By taking the activation scores of the neurons on the Dense layer, which
1083 comes before the final output layer and harbours the characteristics and the contents of the enhancers
1084 coming from previous feature extraction layers, a matrix was generated consisting of a score for 256
1085 nodes for each of the regions. A pearson correlation was generated to calculate the pairwise similarity
1086 between each of the regions.

1087

1088 **Genome-wide prediction of MEL enhancers**

1089 (Soft)-masked genomes where downloaded from UCSC for Homo sapiens (human, hg19), Equus
1090 caballus (horse, equCab2), Sus scrofa (pig, susScr11), Canis lupus familiaris (dog, canFam3), Mus
1091 musculus (mouse, mm10), Danio rerio (zebrafish, danRer10), Ciona intestinalis (ci3), Caenorhabditis
1092 elegans (cel1) and Saccharomyces cerevisiae (sacCer3). The first chromosome of each species was
1093 tiled with a sliding window of 500 bp and a 100 bp shift using bedtools makewindows (v2.28.0). Tiles
1094 containing 'N' were deleted and the remaining tiles were scored by DeepMEL. The number of MEL-
1095 predicted tiles (topic 4 score > 0.16) was divided by the number of genes per species to yield an estimate
1096 of the content of the MEL-enhancer code in each genome.

1097

1098 **Mutations in orthologous enhancers across species**

1099 We defined highly-probable orthologous MEL enhancers between human and another species as
1100 regions that were predicted as MEL in one species and for which there was a stringent liftOver (liftOver
1101 -minMatch=0.995) and high sequence identity (more than 80% after pairwise alignment via needle
1102 (EMBOSS, v6.6.0.0), using parameters -gapopen 10.0 -gapextend 0.5) in the other species. Note that
1103 also the reverse complement of the regions was checked here. Delta ATAC-seq scores were calculated
1104 for the pairs of orthologous regions by making a count matrix using featureCounts (v1.6.5) on the
1105 regions and the bam file of a sample of the species, and by normalising this count matrix using the
1106 library size according to the bam file used, followed by dividing the counts of the two species (human
1107 counts / non-human counts) after adding a pseudocount. Mutations were identified by alignment via
1108 needle, using parameters -gapopen 10.0 -gapextend 0.5.

1109

1110

1111 **Luciferase assay**

1112

1113 Six MEL-predicted enhancers (3 in the dog line Cesar and 3 in the human line MM001) were
1114 synthetically generated and cloned into a pTwist ENTR plasmid (Twist Bioscience) via Twist
1115 Bioscience. Regions were transferred from the Gateway entry clone into the destination vector
1116 (pGL4.23-GW, Addgene) via an LR reaction by mixing 2 ul of the entry clones (100 ng/ul) with 1ul of
1117 the destination plasmid (150 ng/ul), 1 ul TE buffer and 1 ul LR enzyme (LR Clonase II Plus enzyme
1118 mix, Thermo Fisher Scientific), and incubation at 25°C for 1 hour. Afterwards, 1ul of proteinase K
1119 (Thermo Fisher Scientific) was added and reactions were incubated at 25°C for 10 min. 3ul of each LR
1120 reaction was transformed into 50 ul of Stellar competent cells (Takara Bio) via heatshock, 200ul of SOC
1121 medium was added and incubated for 1 hour in a shake incubator at 37°C, before plating the transformed
1122 cells on LB agar plates with 1/1000 carbenicillin and incubation overnight at 37°C. One colony per
1123 construct was grown overnight in a shake incubator at 37°C before plasmid extraction using the
1124 NucleoSpin Plasmid Transfection-grade kit (Macherey-Nagel). For each construct three biological
1125 replicates were performed by transfecting the plasmids into 80% confluent cells of MM001 in a 24 well
1126 plate. Per transfection, 400ng of the construct was transfected together with 40ng of Renilla plasmid
1127 (Promega) using lipofectamine 2000 (Thermo Fisher Scientific). Luciferase activity of each construct
1128 was measured using the Dual-Luciferase Reporter Assay (Promega) according to the manufacturer's
1129 instructions. Luciferase activity was normalised against the Renilla luciferase activity.

1130 **Publicly available data used in this work**

1131 SOX10 ChIP-seq and MITF ChIP-seq data on the 501Mel melanoma cell lines were downloaded as
1132 raw fastq files from NCBI's Gene Expression Omnibus through GEO accession number GSE61965⁶⁵
1133 and were mapped to the human genome using Bowtie2 (v2.1.0) and peaks were called by MACS2
1134 (v2.1.1). TFAP2A ChIP-seq data on human primary melanocytes from neonatal foreskin was retrieved
1135 from Seberg et al. (GSE67555) as a bed file, which was converted to a bedGraph and BigWig using the
1136 peak height from the bed file. H3K27ac-seq and H3K27me3 ChIP-seq data for MM001 (GSE60666);
1137 and RNA-seq data (data for MM031, MM034, MM057, MM074, MM087, MM099 and MM118 was
1138 downloaded from GSE60666; data for MM029, MM116, MM0163, MM164, adn MM165 from
1139 GSE134432) were processed as mentioned in Verfaillie et al.. OmniATAC-seq data for the human lines
1140 MM001, MM011, MM029, MM031, MM047, MM074, MM057, MM087 and MM099 were obtained
1141 through GSE134432²⁸ and were processed as described above in 'Data processing human melanoma
1142 baseline OmniATAC-seq samples'; which was also the case for ATAC-seq data from normal human
1143 melanocytes on foreskin (NHM1), which were downloaded as raw fastq files from GSE94488
1144 (GSM2476338)¹⁰⁶. ATAC-seq data from *C. elegans* and *S. cerevisiae* were downloaded as raw fastq
1145 files from GSE114439 (SRR7164221)¹⁰⁷ and GSE66386 (SRR1822137)¹⁰⁸, respectively, and were
1146 mapped paired-end using STAR (v2.5.1b) to *ce11* and *sacCer3*, respectively, before calling peaks using
1147 MACS2 (v2.1.2) with -q 0.05, extending the peaks 250bp up- and downstream of the summit and
1148 filtering out overlapping peaks based peak height. The MPRA data on the *IRF4* enhancer was
1149 downloaded from <https://mprs.gs.washington.edu/satMutMPRA/> and was processed as described
1150 above.

1151 **Data availability**

1152 The data generated for this study have been deposited in NCBI's Gene Expression Omnibus and are
1153 accessible through GEO Series accession number GSE142238. This includes OmniATAC-seq data of
1154 eight human melanoma cell lines, two dog melanoma cell lines, two horse melanoma cell lines, one pig

1155 melanoma cell line, one pig melanocyte cell lines and one mouse melanoma cell line; ATAC-seq data
1156 of four zebrafish cell lines and OmniATAC-seq data of SOX10 and TFAP2A knock-down in the human
1157 melanoma cell line MM001.

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1170

1171 **Author contributions**

1172

1173 L.M., I.I.T. and S.A. conceived the study. L.M. performed the experimental work for the mammalian
1174 OmniATAC-seq dataset with the help of L.V.A, S.M., V.C and J.W.. M.F., E.v.R. and L.Z. established
1175 and maintained the zebrafish cell lines and performed ATAC-seq on these. G.E.M. maintained and
1176 provided the pig cell lines. A.P. and E.C. established and provided the dog cell lines. P.K. established
1177 and provided the mouse melanoma cell line. M.S. established, maintained and provided the horse cell
1178 lines. G.E.G. established and provided the human cell lines. L.M. performed the experimental work and
1179 analysis of the luciferase assays together with D.M. L.M. performed the bioinformatic analyses of the
1180 OmniATAC-seq dataset. I.I.T. established the neural network and performed all bioinformatic analyses
1181 regarding the model. L.M., I.I.T., J.W. and S.A. wrote the manuscript.

1182 **References**

- 1183 1. Shlyueva, D., Stampfel, G. & Stark, A. Transcriptional enhancers: From properties to genome-
1184 wide predictions. *Nat. Rev. Genet.* 15, 272–286 (2014).
- 1185 2. Vernimmen, D. & Bickmore, W. A. The Hierarchy of Transcriptional Activation: From
1186 Enhancer to Promoter. *Trends Genet. TIG* 31, 696–708 (2015).
- 1187 3. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of
1188 native chromatin for fast and sensitive epigenomic profiling of open chromatin , DNA-binding
1189 proteins and nucleosome position. *Nat. Methods* 10, (2013).
- 1190 4. Klemm, S. L., Shipony, Z. & Greenleaf, W. J. Chromatin accessibility and the regulatory
1191 epigenome. *Nat. Rev. Genet.* 20, 207–220 (2019).
- 1192 5. Zaret, K. S. & Carroll, J. S. Pioneer transcription factors: establishing competence for gene
1193 expression. *Genes Dev.* 25, 2227–2241 (2011).
- 1194 6. Grossman, S. R. et al. Positional specificity of different transcription factor classes within
1195 enhancers. *Proc. Natl. Acad. Sci. U. S. A.* 115, E7222–E7230 (2018).
- 1196 7. Jacobs, J. et al. The transcription factor Grainy head primes epithelial enhancers for
1197 spatiotemporal activation by displacing nucleosomes. *Nat. Genet.* 50, 1011–1020 (2018).
- 1198 8. Imrichová, H., Hulselmans, G., Kalender Atak, Z., Potier, D. & Aerts, S. i-cisTarget 2015
1199 update: generalized cis-regulatory enrichment analysis in human, mouse and fly. *Nucleic Acids*

- 1200 Res. 43, W57–W64 (2015).
- 1201 9. Janky, R. et al. iRegulon: From a Gene List to a Gene Regulatory Network Using Large Motif
1202 and Track Collections. *PLoS Comput. Biol.* 10, (2014).
- 1203 10. Ballester, B. et al. Multi-species, multi-transcription factor binding highlights conserved control
1204 of tissue-specific biological pathways. *eLife* 3, e02626 (2014).
- 1205 11. Prescott, S. L. et al. Enhancer divergence and cis-regulatory evolution in the human and chimp
1206 neural crest. *Cell* 163, 68–83 (2015).
- 1207 12. Villar, D. et al. Enhancer evolution across 20 mammalian species. *Cell* 160, 554–566 (2015).
- 1208 13. Gasperini, M. et al. A Genome-wide Framework for Mapping Gene Regulation via Cellular
1209 Genetic Screens. *Cell* 176, 377–390.e19 (2019).
- 1210 14. Kircher, M. et al. Saturation mutagenesis of twenty disease-associated regulatory elements at
1211 single base-pair resolution. *Nat. Commun.* 10, 3583 (2019).
- 1212 15. Park, Y. & Kellis, M. Deep learning for regulatory genomics. *Nat. Biotechnol.* 33, 825–826
1213 (2015).
- 1214 16. Alipanahi, B., DeLong, A., Weirauch, M. T. & Frey, B. J. Predicting the sequence specificities of
1215 DNA- and RNA-binding proteins by deep learning. *Nat Biotechnol* 33, 831–838 (2015).
- 1216 17. Zhou, J. & Troyanskaya, O. G. Predicting effects of noncoding variants with deep learning-
1217 based sequence model. *Nat. Methods* 12, 931–4 (2015).
- 1218 18. Kelley, D. R., Snoek, J. & Rinn, J. L. Basset: learning the regulatory code of the accessible
1219 genome with deep convolutional neural networks. *Genome Res.* 26, 990–999 (2016).
- 1220 19. Eraslan, G., Avsec, Ž., Gagneur, J. & Theis, F. J. Deep learning: new computational modelling
1221 techniques for genomics. *Nat. Rev. Genet.* 20, 389–403 (2019).
- 1222 20. Wang, M., Tai, C., E, W. & Wei, L. DeFine: deep convolutional neural networks accurately
1223 quantify intensities of transcription factor-DNA binding and facilitate evaluation of functional
1224 non-coding variants. *Nucleic Acids Res.* 46, e69 (2018).
- 1225 21. Angermueller, C., Lee, H. J., Reik, W. & Stegle, O. DeepCpG: accurate prediction of single-cell
1226 DNA methylation states using deep learning. *Genome Biol.* 18, 67 (2017).
- 1227 22. Schreiber, J., Libbrecht, M., Bilmes, J. & Noble, W. S. Nucleotide sequence and DNaseI
1228 sensitivity are predictive of 3D chromatin architecture.
1229 <http://biorxiv.org/lookup/doi/10.1101/103614> (2017) doi:10.1101/103614.
- 1230 23. Shain, A. H. & Bastian, B. C. From melanocytes to melanomas. *Nat Rev Cancer* 16, 345–358
1231 (2016).
- 1232 24. Hoek, K. S. et al. Metastatic potential of melanomas defined by specific gene expression
1233 profiles with no BRAF signature. *Pigment Cell Res.* 19, 290–302 (2006).
- 1234 25. Hoek, K. S. et al. In vivo switching of human melanoma cells between proliferative and invasive
1235 states. *Cancer Res.* 68, 650–656 (2008).
- 1236 26. Rambow, F., Marine, J.-C. & Goding, C. R. Melanoma plasticity and phenotypic diversity:
1237 therapeutic barriers and opportunities. *Genes Dev.* 33, 1295–1318 (2019).
- 1238 27. Verfaillie, A. et al. Decoding the regulatory landscape of melanoma reveals TEADS as
1239 regulators of the invasive cell state. *Nat. Commun.* 6, 6683–6683 (2015).
- 1240 28. Wouters, J. et al. Single-cell gene regulatory network analysis reveals new melanoma cell states
1241 and transition trajectories during phenotype switching.
1242 <http://biorxiv.org/lookup/doi/10.1101/715995> (2019) doi:10.1101/715995.
- 1243 29. Bravo González-Blas, C. et al. cisTopic: cis-regulatory topic modeling on single-cell ATAC-seq
1244 data. *Nat. Methods* 16, 397–400 (2019).
- 1245 30. Egidy, G. et al. Transcription analysis in the MeLiM swine model identifies RACK1 as a
1246 potential marker of malignancy for human melanocytic proliferation. *Mol. Cancer* 7, 34 (2008).
- 1247 31. van Rooijen, E., Fazio, M. & Zon, L. I. From fish bowl to bedside: The power of zebrafish to

- 1248 unravel melanoma pathogenesis and discover new therapeutics. *Pigment Cell Melanoma Res.*
1249 30, 402–412 (2017).
- 1250 32. Segaoula, Z. et al. Isolation and characterization of two canine melanoma cell lines: new models
1251 for comparative oncology. *BMC Cancer* 18, 1219 (2018).
- 1252 33. Seltenhammer, M. H. et al. Establishment and characterization of a primary and a metastatic
1253 melanoma cell line from Grey horses. *Vitro Cell. Dev. Biol. - Anim.* 50, 56–65 (2014).
- 1254 34. van der Weyden, L. et al. Cross-species models of human melanoma. *J. Pathol.* 238, 152–165
1255 (2016).
- 1256 35. Gembarska, A. et al. MDM4 is a key therapeutic target in cutaneous melanoma. *Nat. Med.* 18,
1257 1239–47 (2012).
- 1258 36. Dankort, D. et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat.*
1259 *Genet.* 41, 544–552 (2009).
- 1260 37. Cani-DNA biobank. Selected canine abstracts from the Companion Animal Genetic Health
1261 conference 2018 (CAGH 2018): Canine Genetics and Epidemiology: Edinburgh, Scotland. 14-
1262 15 May 2018. *Canine Genet. Epidemiol.* 5, 7, s40575-018-0062-z (2018).
- 1263 38. White, R. M. et al. Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell*
1264 *Stem Cell* 2, 183–189 (2008).
- 1265 39. White, R. M. et al. DHODH modulates transcriptional elongation in the neural crest and
1266 melanoma. *Nature* 471, 518–522 (2011).
- 1267 40. Meyer, L. R. et al. The UCSC Genome Browser database: extensions and updates 2013. *Nucleic*
1268 *Acids Res.* 41, D64–D69 (2012).
- 1269 41. Amores, A., Catchen, J., Ferrara, A., Fontenot, Q. & Postlethwait, J. H. Genome evolution and
1270 meiotic maps by massively parallel DNA sequencing: spotted gar, an outgroup for the teleost
1271 genome duplication. *Genetics* 188, 799–808 (2011).
- 1272 42. Creyghton, M. P. et al. Histone H3K27ac separates active from poised enhancers and predicts
1273 developmental state. *Proc. Natl. Acad. Sci. U. S. A.* 107, 21931–21936 (2010).
- 1274 43. De Mazière, A. M. et al. The melanocytic protein Melan-A/MART-1 has a subcellular
1275 localization distinct from typical melanosomal proteins. *Traffic Cph. Den.* 3, 678–693 (2002).
- 1276 44. Shoshan, E. et al. NFAT1 Directly Regulates IL8 and MMP3 to Promote Melanoma Tumor
1277 Growth and Metastasis. *Cancer Res.* 76, 3145–3155 (2016).
- 1278 45. Stark, A. et al. Discovery of functional elements in 12 *Drosophila* genomes using evolutionary
1279 signatures. *Nature* 450, 219–232 (2007).
- 1280 46. Dynan, W. S. & Tjian, R. The promoter-specific transcription factor Sp1 binds to upstream
1281 sequences in the SV40 early promoter. *Cell* 35, 79–87 (1983).
- 1282 47. Heinz, S. et al. Simple combinations of lineage-determining transcription factors prime cis-
1283 regulatory elements required for macrophage and B cell identities. *Mol. Cell* 38, 576–589
1284 (2010).
- 1285 48. Bravo González-Blas, C. et al. cisTopic: cis-regulatory topic modeling on single-cell ATAC-seq
1286 data. *Nat. Methods* (2019) doi:10.1038/s41592-019-0367-1.
- 1287 49. Maity, S. N. & de Crombrughe, B. Role of the CCAAT-binding protein CBF/NF-Y in
1288 transcription. *Trends Biochem. Sci.* 23, 174–178 (1998).
- 1289 50. McLean, C. Y. et al. GREAT improves functional interpretation of cis-regulatory regions. *Nat.*
1290 *Biotechnol.* 28, 495–501 (2010).
- 1291 51. Graf, S. A., Busch, C., Bosserhoff, A. K., Besch, R. & Berking, C. SOX10 promotes melanoma
1292 cell invasion by regulating melanoma inhibitory activity. *J. Invest. Dermatol.* 134, 2212–2220
1293 (2014).
- 1294 52. Klein, R. M., Bernstein, D., Higgins, S. P., Higgins, C. E. & Higgins, P. J. SERPINE1
1295 expression discriminates site-specific metastasis in human melanoma. *Exp. Dermatol.* 21, 551–

- 1296 554 (2012).
- 1297 53. Quang, D. & Xie, X. DanQ: a hybrid convolutional and recurrent deep neural network for
1298 quantifying the function of DNA sequences. *Nucleic Acids Res.* 44, e107 (2016).
- 1299 54. Lundberg, S. M. & Lee, S.-I. A Unified Approach to Interpreting Model Predictions. in
1300 *Advances in Neural Information Processing Systems 30* (eds. Guyon, I. et al.) 4765–4774
1301 (Curran Associates, Inc., 2017).
- 1302 55. Lundberg, S. M. et al. Explainable AI for Trees: From Local Explanations to Global
1303 Understanding. *ArXiv190504610 Cs Stat* (2019).
- 1304 56. Avsec, Ž. et al. Deep learning at base-resolution reveals motif syntax of the cis-regulatory code.
1305 <http://biorxiv.org/lookup/doi/10.1101/737981> (2019) doi:10.1101/737981.
- 1306 57. Fufa, T. D. et al. Genomic analysis reveals distinct mechanisms and functional classes of
1307 SOX10-regulated genes in melanocytes. *24*, 5433–5450 (2015).
- 1308 58. Postigo, A. A. & Dean, D. C. ZEB represses transcription through interaction with the
1309 corepressor CtBP. *Proc. Natl. Acad. Sci. U. S. A.* 96, 6683–6688 (1999).
- 1310 59. Gupta, S., Stamatoyannopoulos, J. A., Bailey, T. L. & Noble, W. S. Quantifying similarity
1311 between motifs. *Genome Biol.* 8, R24 (2007).
- 1312 60. D’Mello, S. A. N., Finlay, G. J., Baguley, B. C. & Askarian-Amiri, M. E. Signaling Pathways in
1313 Melanogenesis. *Int. J. Mol. Sci.* 17, (2016).
- 1314 61. Arunachalam, M., Jayasurya, K., Tomancak, P. & Ohler, U. An alignment-free method to
1315 identify candidate orthologous enhancers in multiple *Drosophila* genomes. *Bioinforma. Oxf.*
1316 *Engl.* 26, 2109–2115 (2010).
- 1317 62. Cliften, P. F. et al. Surveying *Saccharomyces* genomes to identify functional elements by
1318 comparative DNA sequence analysis. *Genome Res.* 11, 1175–1186 (2001).
- 1319 63. Hong, J.-W., Hendrix, D. A. & Levine, M. S. Shadow enhancers as a source of evolutionary
1320 novelty. *Science* 321, 1314 (2008).
- 1321 64. Shrikumar, A. et al. TF-MoDISco v0.4.2.2-alpha: Technical Note. *ArXiv181100416 Cs Q-Bio*
1322 *Stat* (2019).
- 1323 65. Laurette, P. et al. Transcription factor MITF and remodeller BRG1 define chromatin
1324 organisation at regulatory elements in melanoma cells. *eLife* 2015, 1–40 (2015).
- 1325 66. Seberg, H. E. et al. TFAP2 paralogs regulate melanocyte differentiation in parallel with MITF.
1326 *PLOS Genet.* 13, e1006636 (2017).
- 1327 67. Arendt, D. et al. The origin and evolution of cell types. *Nat. Rev. Genet.* 17, 744–757 (2016).
- 1328 68. Long, H. K., Prescott, S. L. & Wysocka, J. Ever-Changing Landscapes: Transcriptional
1329 Enhancers in Development and Evolution. *Cell* 167, 1170–1187 (2016).
- 1330 69. Kaplan, N. et al. The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*
1331 458, 362–366 (2009).
- 1332 70. Iwafuchi-Doi, M. et al. The Pioneer Transcription Factor FoxA Maintains an Accessible
1333 Nucleosome Configuration at Enhancers for Tissue-Specific Gene Activation. *Mol. Cell* 62, 79–
1334 91 (2016).
- 1335 71. Denny, S. K. et al. Nfib Promotes Metastasis through a Widespread Increase in Chromatin
1336 Accessibility. *Cell* 166, 328–342 (2016).
- 1337 72. Kawakami, A. & Fisher, D. E. The master role of microphthalmia-associated transcription factor
1338 in melanocyte and melanoma biology. *Lab. Invest.* 97, 649–656 (2017).
- 1339 73. Hamdan, F. H. & Johnsen, S. A. Perturbing Enhancer Activity in Cancer Therapy. *Cancers* 11,
1340 (2019).
- 1341 74. Johnson, L. A., Zhao, Y., Golden, K. & Barolo, S. Reverse-engineering a transcriptional
1342 enhancer: a case study in *Drosophila*. *Tissue Eng. Part A* 14, 1549–1559 (2008).
- 1343 75. Hitte, C. et al. Genome-Wide Analysis of Long Non-Coding RNA Profiles in Canine Oral

- 1344 Melanomas. *Genes* 10, 477 (2019).
- 1345 76. Jiang, L. et al. Constitutive activation of the ERK pathway in melanoma and skin melanocytes
1346 in Grey horses. *BMC Cancer* 14, 857 (2014).
- 1347 77. Kaufman, C. K. et al. A zebrafish melanoma model reveals emergence of neural crest identity
1348 during melanoma initiation. *Science* 351, aad2197–aad2197 (2016).
- 1349 78. Rambow, F. et al. Identification of differentially expressed genes in spontaneously regressing
1350 melanoma using the MeLiM Swine Model: Differential gene expression in swine melanoma.
1351 *Pigment Cell Melanoma Res.* 21, 147–161 (2008).
- 1352 79. Rosengren Pielberg, G. et al. A cis-acting regulatory mutation causes premature hair greying
1353 and susceptibility to melanoma in the horse. *Nat. Genet.* 40, 1004–1009 (2008).
- 1354 80. Sundström, E. et al. Identification of a melanocyte-specific, microphthalmia-associated
1355 transcription factor-dependent regulatory element in the intronic duplication causing hair
1356 greying and melanoma in horses: A melanocyte-specific regulatory element in the duplicated
1357 sequence causing greying and melanoma in horses. *Pigment Cell Melanoma Res.* 25, 28–36
1358 (2012).
- 1359 81. Rahman, Md. M. et al. Transcriptome analysis of dog oral melanoma and its oncogenic analogy
1360 with human melanoma. *Oncol. Rep.* (2019) doi:10.3892/or.2019.7391.
- 1361 82. Wong, K. et al. Cross-species genomic landscape comparison of human mucosal melanoma with
1362 canine oral and equine melanoma. *Nat. Commun.* 10, 353 (2019).
- 1363 83. Dermitzakis, E. T. & Clark, A. G. Evolution of transcription factor binding sites in Mammalian
1364 gene regulatory regions: conservation and turnover. *Mol. Biol. Evol.* 19, 1114–1121 (2002).
- 1365 84. Lindblad-Toh, K. et al. A high-resolution map of human evolutionary constraint using 29
1366 mammals. *Nature* 478, 476–482 (2011).
- 1367 85. Hallikas, O. et al. Genome-wide prediction of mammalian enhancers based on analysis of
1368 transcription-factor binding affinity. *Cell* 124, 47–59 (2006).
- 1369 86. He, Q. et al. High conservation of transcription factor binding and evidence for combinatorial
1370 regulation across six *Drosophila* species. *Nat. Genet.* 43, 414–420 (2011).
- 1371 87. Paris, M. et al. Extensive divergence of transcription factor binding in *Drosophila* embryos with
1372 highly conserved gene expression. *PLoS Genet.* 9, e1003748 (2013).
- 1373 88. Soufi, A., Donahue, G. & Zaret, K. S. Facilitators and impediments of the pluripotency
1374 reprogramming factors' initial engagement with the genome. *Cell* 151, 994–1004 (2012).
- 1375 89. Soufi, A. et al. Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate
1376 reprogramming. *Cell* 161, 555–568 (2015).
- 1377 90. Zhu, F. et al. The interaction landscape between transcription factors and the nucleosome.
1378 *Nature* 562, 76–81 (2018).
- 1379 91. Hou, L., Srivastava, Y. & Jauch, R. Molecular basis for the genome engagement by Sox
1380 proteins. *Semin. Cell Dev. Biol.* 63, 2–12 (2017).
- 1381 92. Sherwood, R. I. et al. Discovery of directional and nondirectional pioneer transcription factors
1382 by modeling DNase profile magnitude and shape. *Nat. Biotechnol.* 32, 171–178 (2014).
- 1383 93. Marathe, H. G. et al. BRG1 interacts with SOX10 to establish the melanocyte lineage and to
1384 promote differentiation. *Nucleic Acids Res.* 45, 6442–6458 (2017).
- 1385 94. Kamachi, Y. & Kondoh, H. Sox proteins: regulators of cell fate specification and differentiation.
1386 *Dev. Camb. Engl.* 140, 4129–4144 (2013).
- 1387 95. Coustry, F. et al. The dimerization domain of SOX9 is required for transcription activation of a
1388 chondrocyte-specific chromatin DNA template. *Nucleic Acids Res.* 38, 6018–6028 (2010).
- 1389 96. Murisier, F., Guichard, S. & Beermann, F. The tyrosinase enhancer is activated by Sox10 and
1390 Mitf in mouse melanocytes. *Pigment Cell Res.* 20, 173–184 (2007).
- 1391 97. Ceol, C. J. et al. The histone methyltransferase SETDB1 is recurrently amplified in melanoma

- 1392 and accelerates its onset. *Nature* 471, 513–517 (2011).
- 1393 98. Heilmann, S. et al. A Quantitative System for Studying Metastasis Using Transparent Zebrafish.
1394 *Cancer Res.* 75, 4272–4282 (2015).
- 1395 99. Wojciechowska, S., van Rooijen, E., Ceol, C., Patton, E. E. & White, R. M. Generation and
1396 analysis of zebrafish melanoma models. *Methods Cell Biol.* 134, 531–549 (2016).
- 1397 100. Julé, S., Bossé, P., Egidy, G. & Panthier, J.-J. Establishment and characterization of a normal
1398 melanocyte cell line derived from pig skin. *Pigment Cell Res.* 16, 407–410 (2003).
- 1399 101. Corces, M. R. et al. An improved ATAC-seq protocol reduces background and enables
1400 interrogation of frozen tissues. *Nat. Methods* 14, (2017).
- 1401 102. Frith, M. C., Li, M. C. & Weng, Z. Cluster-Buster: Finding dense clusters of motifs in DNA
1402 sequences. *Nucleic Acids Res.* 31, 3666–3668 (2003).
- 1403 103. Chollet, F. & others. Keras. (2015).
- 1404 104. Abadi, M. et al. TensorFlow: Large-Scale Machine Learning on Heterogeneous Distributed
1405 Systems. ArXiv160304467 Cs (2016).
- 1406 105. Shrikumar, A., Greenside, P. & Kundaje, A. Learning Important Features Through Propagating
1407 Activation Differences. ArXiv170402685 Cs (2017).
- 1408 106. Fontanals-Cirera, B. et al. Harnessing BET Inhibitor Sensitivity Reveals AMIGO2 as a
1409 Melanoma Survival Gene. *Mol. Cell* 68, 731-744.e9 (2017).
- 1410 107. Jānes, J. et al. Chromatin accessibility dynamics across *C. elegans* development and ageing.
1411 *eLife* 7, (2018).
- 1412 108. Schep, A. N., Wu, B., Buenrostro, J. D. & Greenleaf, W. J. chromVAR: inferring transcription-
1413 factor-associated accessibility from single-cell epigenomic data. *Nat. Methods* 14, (2017).