

Supplementary Information for Repeated mutation of a developmental enhancer contributed to the human thermoregulatory evolution

Daniel Aldea, Yuji Atsuta, Blerina Kokalari, Stephen Schaffner, Rexxi D. Prasasya, Adam Aharoni, Heather L. Dingwall, Bailey Warder, Yana G. Kamberov

Yana G. Kamberov Email: <u>yana2@pennmedicine.upenn.edu</u>

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Figures S1 to S7 Tables S1 to S4 SI References

2. C. capture from mouse limba 4. Doalf ChP-seq peaks from mouse limba 6. Conserved demonstrationation of the mouse lead of the mouse l	Leg	jend	1. DNas	e I hypersens use limbs at	sitivity E11.5			3. ⊦ Epi	13K2 derm	7Ac fr al ker	m Normal hum tinocytes (NHE	an K)	5. Huma Regions	n Accelerated (HARs)					
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	5	chr1	120795644	120795727						-	160	chr1	121227234	121227766		-			
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9 chr1120796024 120796139 146 chr1121254791 121254850 120811028 1 chr1120811086 120811128 165 chr1121290154 121291057 121291057 2 chr112081565 120816565 120817814 121298130 121298130 121298130 3 chr1120816565 12082517 166 chr11212013485 121304448 121304448 121304541 4 chr12082162 12082517 166 chr1121304385 121304448 121304541 1213147541 1213147541 1213147541 1213147541 1213147541 12131457541 12131457541 <td>8</td> <td>chr1</td> <td>120795919</td> <td>120796012</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>163</td> <td>chr1</td> <td>121254210</td> <td>121254413</td> <td></td> <td></td> <td></td> <td></td> <td></td>	8	chr1	120795919	120796012							163	chr1	121254210	121254413					
C Unit 120001934 120001934 1212910/f 1 chr 120811068 120810680 12129107 2 chr 112081656 12081686 121291134 121291207 3 chr 120817814 12081784 121291207 166 4 chr 12081635 120825171 168 chr 121304341 121304418 5 chr 120842548 12085220 120853227 170 chr 121308131 121304441 6 chr 120842548 120853224 170 chr 121308154 121304448 121344480 6 chr 120859206 12085324 172 chr 121308231 121308154 121304448 7 chr 12086481 12086367 12086367 12086367 12134480 121344480 121344480 1 chr 120868481 120868564 175 chr 121355892 121355844 121370991 121355844 2 chr 120868401 120872807 12087309 178 chr 121371351 121370913 12137091 12137091 6 chr 12088362 12083768 180 chr 121371351 12137166 12137166 12137166 12137166 12137166 12137166 12137166 12137166 12	9	chr1	120796024	120796139							164	chr1	121254791	121254850		-			
2 chini 12016555 120817814 120817814 121208130 121208130 3 chini 12017814 120817814 120817814 121304541 1 4 chini 120825152 120825217 1 167 chini 121304548 121304541 1 6 chini 120825152 12085228 120852828 121304184 12130454 1 1 6 chini 12085208 120859208 120859274 121308154 1	1	chr1	120801934	120801991							165	chr1	121290954	121291077		-			
3 chr1 120817814 120817864 121304388 121304418 121304251 5 chr1 120825152 120825171 120825171 120825171 121307897 121307897 121307897 121307942 1 5 chr1 120359281 120859281 120859281 121307897 121307897 121307897 121307897 121307897 121307897 121307897 121307897 1<	2	chr1	120816565	120816680							167	chr1	121298130	121298192					
4 chr1 12/0825152 12/0825121 100 12/0825121 100 12/0825121 100 100 12/0825121 100 100 12/0825121 100 100 12/0825121 100 100 12/0825121 100 100 12/082521 100 100 12/082521 100 <td>3</td> <td>chr1</td> <td>120817814</td> <td>120817864</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>168</td> <td>chr1</td> <td>121304358</td> <td>121304418</td> <td></td> <td>-</td> <td></td> <td></td> <td></td>	3	chr1	120817814	120817864							168	chr1	121304358	121304418		-			
Communication 120051112 12005120 12130/1997 12130/1997 Communication 120052281 12130821 12130821 12130821 12130821 Communication 1200559281 1200559281 12130821 12130821 12130824 Communication 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 120055927 121355949 177 chr1 120135595 121355949 177 chr1 12035595 121355949 178 chr1 120371361 121371754 180 chr1 120371361 12137166 12137166 12137166 12137166 12137166 12137166 12137183	4	chr1	120825152	120825217	_						169	chr1	121304448	121304541		-			
7 chr1 120589288 120650284 121308271 121308271 121308240 9 chr1 120589781 120589288 120589288 121344380 121344480 9 chr1 120386478 120859747 120589747 120589747 120589747 9 chr1 120386478 120889583 12088458 12135589 12135589 12135589 1 chr1 120387285 120886843 120888563 177 chr1 121355789 121355894 1 2 chr1 120872855 120873099 178 chr1 121371381 121370991 1 4 chr1 12083467 12083465 180 chr1 121371381 12137166 1 6 chr1 120883624 120883624 181 chr1 121371631 12137164 1 1 9 chr1 120883624 120883624 181 chr1 121371981 12137180 1	6	chr1	120842548	120842630							170	chr1	121308031	121308154					
8 chr1 120459288 120459523 173 chr1 121314738 121314732 121314732 9 chr1 120459671 120459747 174 chr1 12134438 121344480 121344480 10 chr1 120458747 176 chr1 12134580 121344480 121344480 12 chr1 120458767 120688650 176 chr1 12135582 121355804 176 2 chr1 120457265 120872684 120872685 120872684 178 chr1 12135582 121356029 121355802 121355802 121355802 121371524 176 176 121371753 121371754 176 176 121371753 121371754 176 176 121371753 121371754 176 176 171 176 121371753 121371754 176 176 171 176 171 177 121371753 121371754 176 176 171 176 171 171 176 171 177 121371753 121371800 171 173 1717 121371753 121371803 1717 171 176 171 1716 121371803 <td>7</td> <td>chr1</td> <td>120859206</td> <td>120859284</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>172</td> <td>chr1</td> <td>121308271</td> <td>121308340</td> <td></td> <td></td> <td></td> <td></td> <td></td>	7	chr1	120859206	120859284							172	chr1	121308271	121308340					
9 chr1 120859071 120859747 10 chr1 120868488 120868554 175 chr1 121344380 121344480 11 chr1 120868488 120868553 176 chr1 121355789 121355884 1 11 chr1 120868853 120868953 177 chr1 121355789 121355894 1 12 chr1 120872907 120873099 179 chr1 121371381 121371991 1 12 chr1 120883467 120883626 181 chr1 121371381 121371666 1 1 12 chr1 120883467 120883624 120883624 183 chr1 121371733 121371764 1 1 12 chr1 120883624 120883624 183 chr1 121371983 121371860 1	8	chr1	120859288	120859523							173	chr1	121314738	121314792		-			
1.1.1.20068350 1.20068350 1.20068350 1.2135789 1.2135584 2 dhr1 120868360 120868360 176 dhr1 12135582 1.2135584 2 dhr1 120868401 120868465 176 dhr1 12135582 1.2135584 4 dhr1 12087285 12087894 178 dhr1 12135582 1.21356849 5 dhr1 12085361 120868465 178 dhr1 12135582 1.2135684 6 dhr1 12085361 12085365 1235784 1.21371651 6 dhr1 12083551 12085364 12085364 1.21371651 1.21371654 6 dhr1 12083552 12086366 180 dhr1 121371731 1.21371666 1.41121371733 1.21371666 6 dhr1 120861261 120886026 184 dhr1 121371731 1.21371843 1.41121371743 8 dhr1 120861261 120886026 184 dhr1 121371630 1.21371843 1.41121371843 9 dhr1 120886126 120886026 184 dhr1 121371630 1.21371843 1.41121371843 9 dhr1 120886126 120886266 184 dhr1 121371630 1.21371843 1.411414 9 dhr1 120861261 12088628 186 dhr1 121371630 1.2138648 1.41141414144 9 dhr1 120867181 120912407	9	chr1	120859671	120859747							174	chr1	121344380	121344480		-			
2 chr1 12068843 120689699 177 chr1 12355822 121355949 121355949 4 chr1 12087285 120872864 178 chr1 121355822 121355949 1 5 chr1 12083407 12083465 180 chr1 121371531 121371524 1 6 chr1 12083455 120883653 120883654 181 chr1 121371531 121371666 1 6 chr1 12083824 120883654 182 chr1 121371731 121371680 1 1 6 chr1 120883624 120886178 120886178 121371731 12137180 1 <t< td=""><td>1</td><td>chr1</td><td>120868560</td><td>120868653</td><td></td><td></td><td></td><td></td><td></td><td></td><td>176</td><td>chr1</td><td>121355789</td><td>121355884</td><td></td><td></td><td></td><td></td><td></td></t<>	1	chr1	120868560	120868653							176	chr1	121355789	121355884					
3 chr1 120872965 12135595 121356029 12135095 4 chr1 20872907 120873099 179 chr1 121371381 121370991 5 chr1 120883467 120883465 120883524 121371351 12137166 12137164 6 chr1 201883264 120883264 120883264 121371731 12137166 12137166 7 chr1 120883624 120883624 120883624 120883624 12137163 12137166 <td>2</td> <td>chr1</td> <td>120868843</td> <td>120868969</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>177</td> <td>chr1</td> <td>121355892</td> <td>121355949</td> <td></td> <td></td> <td></td> <td></td> <td></td>	2	chr1	120868843	120868969							177	chr1	121355892	121355949					
- Umin 12007 2007 12007 3009 121370913	3	chr1	120872585	120872694							178	chr1	121355955	121356029		-			
6 chr1 120883467 120883262 181 chr1 121371531 121371764 7 chr1 120883253 120883864 182 chr1 121371733 121371764 182 chr1 121371733 121371764 8 chr1 120883224 120883824 120883824 121371784 183 chr1 121371733 121371764 9 chr1 120886126 120886126 120886126 121371744 184 chr1 121371985 121370443 1 chr1 120887118 12088724 186 chr1 121379500 121370443 184 chr1 121371985 12138048 2 chr1 120912165 12088724 186 chr1 121388618 121382018 188 chr1 121388612 121380744 3 chr1 120912165 120912409 190 chr1 121388612 121380306 121388648 191 chr1 121390306 121380306 5 chr1 120912409 120912407 190 chr1 121380308 121390306 1	4 5	chr1	120872907	120873009	_						179	chr1	1213/0913	1213/0991		-			
7 chrl 120833553 120833654 121371753 121371753 121371754 9 chrl 12083624 120883624 120883624 121371793 121371763 121371763 9 chrl 120883624 120883624 120886126 121371983 1 1 9 chrl 120886126 120886179 185 chrl 121371983 1 1 1 chrl 120886126 120886179 185 chrl 121379090 121379604 1 1 1 chrl 120886126 12088718 120887284 185 chrl 121387900 121379604 1 1 3 chrl 12088718 120887284 185 chrl 121388618 121388648 1	6	chr1	120883467	120883526							180	chr1	121371531	121371666					
8 chr1 120883824 120883931 1 1 9 chr1 120886022 120886026 1 183 chr1 121371793 121371860 1 0 chr1 120886024 120886026 1 184 chr1 121371983 1 1 1 chr1 120886125 120886265 1 185 chr1 121371983 1 1 2 chr1 12088718 120886245 12088526 1 186 chr1 121371983 1 1 3 chr1 120181265 12091071 1 188 chr1 121381958 12138674 1 1 4 chr1 120912405 120912407 189 chr1 121380182 12138674 1 1 1 1 1 12138674 1 <t< td=""><td>7</td><td>chr1</td><td>120883553</td><td>120883664</td><td></td><td></td><td></td><td></td><td></td><td></td><td>182</td><td>chr1</td><td>121371703</td><td>121371754</td><td></td><td></td><td></td><td></td><td></td></t<>	7	chr1	120883553	120883664							182	chr1	121371703	121371754					
Grin 1 20300022 120380126 1213/1918 1213/1918 1213/1918 1 chr1 1203806126 120380179 185 chr1 1213/1918 1213/1918 1213/1918 1 chr1 1203806126 120380679 185 chr1 1213/1918 1213/1918	8	chr1	120883824	120883931							183	chr1	121371793	121371860		-			
1 1	9	chr1	120886022	120886086							184	chr1	1213/1918	1213/1983		-			
2 chr1 12088718 120887234 187 chr1 12381958 121382018 3 chr1 120910913 120910917 12091243 12388588 1 4 chr1 120912405 120912407 188 chr1 121384058 121388588 1 5 chr1 120912409 120812497 120812497 120812497 121380306 121388588 1 6 chr1 120912527 120912491 190 chr1 121380308 121380306 121380306 1 9 chr1 120912527 120912591 193 chr1 121390308 121390306 121390306 1 9 chr1 120912527 120941870 193 chr1 121390308 121390306 1 9 chr1 120951771 120951867 195 chr1 12393078 1239176 1 1 chr1 120953043 120953044 199 chr1 121393078 12139378 1 3 chr1 120951812 120961812 120961812 120961812 1239377 1 1 19 chr1 12039276 12139277 12399778 121399778 121399078 1213997	1	chr1	120886245	120886526							186	chr1	121379500	121379604					
3 chr1 120910913 120911071 188 chr1 121388400 121388588 1 5 chr1 12091265 120912300 189 chr1 121388612 121388648 1 5 chr1 12091265 120912407 190 chr1 121388612 121388648 1 6 chr1 120912627 120912407 190 chr1 121380861 121388648 1 7 chr1 120912527 120912491 190 chr1 121390308 121390306 1 9 chr1 120941671 120941670 192 chr1 121390508 121390697 1 9 chr1 12095171 120951826 195 chr1 121392525 12139078 1 1 chr1 120953043 120953042 120953043 120953043 120953043 120953043 120953043 120953043 12139378 12139378 12139378 12139378 12139377 1 1 1 1 121392553 121394047 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	2	chr1	120887118	120887234							187	chr1	121381958	121382018					
Grin 1 20912323 120912407 1399 Chi 121388593 121388548 6 chri 1 20912323 120912407 190 chri 121388593 121388744 6 chri 1 20912527 120912547 12091259 121389744 8 chri 1 20912527 120912407 192 chri 121390306 121390300 9 chri 1 20941874 192 chri 121390508 121390507 121390697 9 chri 1 20941874 10051917 120951971 12095197 12139107 12139107 12139107 1 chri 1 20951917 12095197 12095197 12095197 121393078 121393078 2 chri 1 20953043 120953094 196 chri 121393252 121393078 121393078 3 chri 1 20961812 120961845 1209612094 199 chri 121393278 121393078 5 chri 120961394 1209612094 199 chri 121393278 121393972 121393972 5 chri 120961394 120962094 199 chri 121393976 121393972 121394027 6 chri 120961208 120962094 199 chri 121393976 121394027 120 7	3	chr1	120910913	120911071							188	chr1	121388490	121388588		-			
6 chr1 220912409 120912491 191 chr1 12130012 121300306 7 chr1 120912527 120912591 191 chr1 121300308 121390306 9 chr1 120912527 12091291 193 chr1 121390308 121390306 9 chr1 120941871 120951826 193 chr1 121390308 121390306 0 chr1 120951771 120951826 195 chr1 121390270 121390378 1 chr1 120951917 1209513043 120953043 120953043 3 chr1 120951817 120961812 120961812 120961812 20961812 120962094 199 chr1 12139376 121393078 121394027 5 chr1 120961812 1209622094 200 chr1 121393976 121394027 121394027 5 chr1 120961812 120962094 201 chr1 121393976 121394027 121404038 121394027 1	4 5	chr1	120912165	120912320							189	chr1	121388593	121388648					
7 chr1 120912527 120912591 121390308 121390308 121390309 9 chr1 120941703 120941703 120941703 120941703 120941703 120941703 120941703 121390508 1213905097 1213905097 1213905097 1213905097 1213905097 1213905177 1209512917 1209512917 1209513043 120953043 12139053043 12139078 <	6	chr1	120912409	120912498							191	chr1	121390182	121390306					
b chr1 120941703 120941870 121390500 121390697 1 b chr1 120941874 100941874 194 chr1 121390500 121390105 1 b chr1 120951877 120951826 196 chr1 12139270 12139107 12139176 1 c chr1 120951917 120951967 196 chr1 12139270 121393078 1 1 2 chr1 120953191 120953094 196 chr1 12139270 121393078 1 1 3 chr1 120961812 120961864 199 chr1 121393078 121393972 1	7	chr1	120912527	120912591							192	chr1	121390308	121390390		-			
0 chrl 1209-1071 120951217 120951826 195 chrl 121391039 121391036 1 chrl 120951771 120951867 195 chrl 12139107 121391036 1 chrl 120951917 120951867 195 chrl 12139252 1213927 2 chrl 120953043 120953043 120953043 121393078 121393078 3 chrl 120951812 120961812 1209618142 1209618142 1209618142 120962094 199 chrl 121393076 121393072 121394027 1213440233 1213940233 1213940	8	chr1	120941703	120941870					-	-	193	chr1	121390500	121390697		-			
1 chr1 120951017 1209510867 121392525 1213925	0	chr1	120941874	120942147							194	chr1	121390893	121391376					
2 chr1 120953043 120953094 121393078 121393078 3 chr1 120953191 120953282 198 chr1 1213932970 121393078 121393078 4 chr1 120961812 120961864 199 chr1 1213932976 121393078 121393072 5 chr1 120962094 109 chr1 121393376 121394027 109 6 chr1 120962094 200 chr1 121393376 121394027 109 7 chr1 120962094 201 chr1 121393976 121394027 109 6 chr1 1209642043 120964331 203 chr1 121395020 121395189 109 9 chr1 120970137 120970433 203 chr1 121399710 121399770	1	chr1	120951917	120951967							196	chr1	121392525	121392914					
3 chr1 12/0953191 12/0953282 198 chr1 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393389 12/1393530	2	chr1	120953043	120953094							197	chr1	121392970	121393078		-			
Grin 120901012 120901004 121393078 121393078 12139307 Grin 120061045 1209620145 120962073 200 chr1 121393078 121394027 Grin 120064202 12096433 12096433 202 chr1 121393078 121394087 S chr1 120964303 12096431 202 chr1 121399576 121394087 202 chr1 121399578 S chr1 12096433 120964513 203 chr1 121399546 121399678 203 chr1 121399770 D chr1 120983669 120983722 205 chr1 121400220 121400326 202 chr1 121400333 D chr1 121006028 121006124 207 chr1 121400325 121400389 207 chr1 121400385	3	chr1	120953191	120953282							198	chr1	121393389	121393530					
6 chr1 120962096 120962173 201 chr1 121394935 121394998 7 chr1 120964202 120964331 202 chr1 12139502 121395189 9 chr1 120970137 120970243 203 chr1 121399711 121399770 0 chr1 120053669 120963269 120983722 205 chr1 121400326 121399770 0 chr1 120053656 121096022 205 chr1 121400326 121400326 205 chr1 121400328 121400326 2 chr1 121006128 121006124 207 chr1 121400325 121400326 121400326	4 5	chr1	120961812	120961864							199	chr1	121393678	121393972		-			
7 chr1 120964320 120964331 202 chr1 12139502 121395189 203 9 chr1 120970137 120970433 203 chr1 121399711 121399770 204 00 chr1 120983669 120983722 205 chr1 121399710 21399770 205 00 chr1 12005336 121096028 205 chr1 121400328 205 10 chr1 121005386 121006028 205 chr1 121400328 205 12 chr1 121005386 121006028 205 chr1 121400328 205 12 chr1 121400538 121400328 21400389 205	6	chr1	120962096	120962173							201	chr1	121394935	121394998					
8 chr1 12/0964433 12/0964513 203 chr1 12/1399546 12/1399678 1 9 chr1 12/0970137 12/0970243 204 chr1 12/1399711 12/1399770 1 9 chr1 12/10983669 12/0983722 205 chr1 12/1400200 12/1400326 1 9 chr1 12/1005038 12/1400622 205 chr1 12/1400333 12/1400326 1 9 chr1 12/1006028 207 chr1 12/1400333 12/1400326 1 1 9 chr1 12/1400334 12/1400326 207 chr1 12/1400326 1 1	7	chr1	120964202	120964331							202	chr1	121395002	121395189		-			
0 chr11209306369 120983762 204 chr1121399/11 121399/10 0 chr1120983669 120983722 205 chr1121400326 121400326 0 chr1121005936 121006124 205 chr1121400333 121400386 2 chr1121006128 121006124 207 chr1121400235 121400389	8	chr1	120964433	120964513							203	chr1	121399546	121399678		-			
01 chr1 121005936 121006022 206 chr1 121400333 121400389 207 chr1 121400333 121400389 207 chr1 12140000000000000000000000000000000000	9 00	chr1	1209/013/	120970243							204	chr1	121399/11	121399770					
02 cbr1 121006028 121006114 201 207 cbr1 121407825 121407895	01	chr1	121005936	121006022							200	chr1	121400333	121400389					
207 0111121407023 121407003	02	chr1	121006028	121006114							207	chr1	121407825	121407885		-			_
233 chr1 121012716 121012903 208 chr1 121407887 121408024 208 chr1 12140787 121408024 208 chr1 12140787 121408024 208 chr1 12140787 121408024 208 chr1 12140787 1214078	03	chr1	121012716	121012903					-	-	208	chr1	121407887	121408024		-			

B List of En1 Candidates Enhancers (ECEs) tested <i>in v</i>	ivo
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	Coordinates (mm10)	1	2	
ECE	1 120600025-120600904	4		Legend:
ECE	2 120601976-120602486	5,6	En1 promoter	1 Concerved element contained
ECE	3 120604762-120605416	11		within the FCF tested in vivo
ECE	4 120607302-120608054	12,13,14		(see identification number in Extended Data
ECE	5 120621472-120622204	16		
ECE	6 120692742-120693280	30,31,32		2. Reporter expression in En1
ECE	7 120714773-120716154	34,35		positives domain in mouse ventral
ECE	8 120756823-120757766	50,51		limb at P2.5
ECE	9 120760766-120761883	52		Positive
ECE	10 120842070-120843227	66		Negative
ECE	11 120910517-120911428	83		!
ECE	12 120961285-120962463	94,95,96		
ECE	13 120963768-120964907	97,98		
ECE	14 120969875-120970619	99		
ECE	15 121005143-121006490	101,102		
ECE	16 121050854-121051942	110,111,112		
ECE	17 121053456-121054528	113		
ECE	18 121096764-121097826	124,125,126,127	This study	
ECE	19 121154371-121155567	150		
ECE	20 121176848-121178300	153		
ECE	21 121179419-121181061	154,155		
ECE	33 10100060 101004600	156		

C Human derived substitutions relative to chimp/gorilla or to chimp

201,202

ECE23 121394405-121395702

	Human derived substitutions relative to chimp/gorilla	% of the ECE	Human derived substitutions relative to chimp	% of the ECE
ECE2	5	0.93	12	2.24
ECE8	5	0.51	11	1.13
ECE18	13	1.33	24	2.46
ECE20	21	1.51	28	2.01
ECE23	7	0.81	13	1.50

Fig. S1. Genomic location and characteristics of conserved elements and Engrailed 1 Candidate Enhancers (ECE). (A) Coordinates (mm10) of 209 conserved elements within the EN1 TAD identified by phastCons across placental mammals. Each element has a corresponding identifier (N). Criteria used to prioritize conserved elements: overlap with published datasets of epigenomic marks associated with enhancer presence (columns 1-3) **(1, 2)**; overlap with DEAF1 ChIP-seq peaks, which is a transcription factor we recently showed positively regulates Engrailed 1 in human and mouse keratinocytes (column 4) **(3)**; overlap with annotated human accelerated regions (HARs) (column 5) **(4–6)**. Overlap is indicated in red. Prioritized conserved elements which were used as kernels for ECEs are highlighted in orange in column 6. **(B)** Genomic coordinates

1a)

(mm10) of top 23 ECEs tested in mouse transgenic assays. Conserved elements (N) contained within each ECE are listed in column 1. ECEs that induced eGFP-positive clones within the En1-positive expression domain (basal keratinocytes of volar limb) are indicated by orange color in column 2. (C) Human derived substitutions relative to chimp/gorilla, or to chimp are listed as absolutes numbers or percentages calculated with respect to the total length in nucleotides of each positive ECE. ECE18 is boxed in red.



A Enhancer activity of positive ECEs in mouse ventral limb

B Activity of positive ECEs in mouse footpad



C ECE18 enhancer activity in mouse dorsal limb



Fig. S2. Enhancer activity of positives ECEs in transgenic mouse skin. In all panels representative images of GFP antibody-stained sections of limbs from lentiviral-mediated

transgenic mice. Cross-sections of stained ventral mouse limbs (**A**), and footpads (**B**) at P2.5 stage of positives ECEs identified in this study are shown. The eccrine glands of the footpads in (**B**) (red arrow) are undergoing differentiation as evidenced by their invagination into the dermal layer. (**C**) Representative images of P2.5 dorsal distal autopod skin from mouse, chimpanzee and human ECE18 lentiviral-mediated transgenic mice. The large hair follicles (blue arrow) which like eccrine glands derive from basal keratinocytes during development (**7**, **8**), and are characteristic of dorsal skin are also present. eGFP expression was detected by anti-GFP antibody and HRP/DAB coupled immunohistochemistry. GFP positive clones (black arrow). Control images from transgenic skin infected with lentivirus carrying minimal promoter and eGFP-reporter cassette alone.



A Multiz alignment of placental mammals centered on human ECE18

B Bidirectional luciferase lentiviral vector



C Coordinates of ECE18 homologs and others fragments tested in vitro

	[ECE18	ECE18-FragA	ECE18-FragB	2xHAR20	HAR19	HAR80
Species (build)	Chr	Coordinates	Coordinates	Coordinates	Coordinates	Coordinates	Coordinates
Mouse (mm10)	Chr1	121096764-121097826	121096764-121097440	-	-	-	-
Human (hg38)	Chr2	118309555-118310531	118309932-118310531	118309555-118310154	118309583-118310193	118309977-118310085	118309771-118309961
Chimp (panTro6)	Chr2B	4758321-4759295	4758698-4759295	-			
Gorilla (gorGor4)	Chr2B	-	7012616-7013213	-			
Macaque (rheMac10)	Chr12	12819060-12820038	12819060-12819658	-			
Marmoset (calJac3)	Chr6	92356458-92357434	-	2			
Bushbaby (otoGar3)	GL873578	3870683-3871687	-	3			
Cat (felCat9)	C1	122367966-122368951	122367966-122368592	- 1			

D Human ECE18^{FragA} enhancer activity in mouse limb



E Activity of ECE18^{FragA} homologs







H Activity of chimeric ECE18



18	5'-human-DNA	3'-chimp-DNA
ECE	Coordinates (hg38)	Coordinates (panTro6)
imp nera	Chr2:118309555-118310217	Chr2B : 4758984-4759295
년 년	5'-chimp-DNA	3'-human-DNA
mar	Coordinates (panTro6)	Coordinates (hg38)
로	Chr2B : 4758321-4758983	Chr2 : 118310218-118310531

Coordinates of human ECE18^{FragA} derived variants



J Effect of deleting human specific insertions on hECE18 $^{\mbox{\tiny fragA}}$ activity







L Multiz alignment of placental mammals at SP1^A and SP1^B motifs

						SP1	۹.																		S	P1 ^B					
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Chimp										т											А									С	
Gorilla										т											А		С								
Orangutan										Т							Т				A										
Gibbon										Т											A I										
Rhesus										т											1.1										
Crab-eating_macaque										т											1.1										
Baboon										т											i - i										
Green_monkey										Т											1.1										
Marmoset										Т											A		С	т							
Squirrel_monkey	С				2					Ţ							1				Α		С	Т	1	1	1			C	
Bushbaby					Т					T				1			1				i : i				T	C	T			С	
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Dolphin					т					т				G	т						A				т	A	Т				
Killer_whale					т					т				G	т		G				А				т	A	Т				
Tibetan_antelope			С		т					т				т			т				А				т	A	Т				
Cow			С		т					Т							т				A	•			т	A	т				
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Microbat	С				G								С		С	G									т	A	т			G	
Big_brown_bat	С				G								С		С	A							G			A	т				
Hedgehog			С		т					Т					т		т		т	G	i, i			A	т	A	Т			С	
Shrew			С		G					Т											С				т	A	Т			G	
Star-nosed_mole			С		т					т			Т								А				т	A	Т				
Elephant			С		т					т		G							A		А				т	A	Т			С	
Cape_elephant_shrew		A		т	т					Т	A	G	G		A			Т	Т		A				A	т	G			С	
Manatee			С		Т					Т		G							Т		A				Т	A	Т				
Cape_golden_mole			G		Т				:	Т		G									A		A		Т	A	Т			С	
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Fig. S3. Mapping ECE18 enhancer activity. (A) Multiz alignment of placental mammals centered on human ECE18 (hECE18). hECE18 was split into two fragments hECE18^{FragA} and hECE18^{FragB}. 2xHAR20 (dark grey) is a merged element that contains HACNS56, HAR19 and HAR80 (light grey) (6, 9, 10). Conserved elements used as kernels are (#124, #125, #126 and #127) shown in dark

blue. (B) Modified Stagia3 bidirectional luciferase lentiviral reporter vector used to test enhancer activity in cultured keratinocytes. LTR indicates long terminal repeat; ψ , packaging signal; RRE, rev response element; cPPT, central polypurine tract; SV40 polyA, simian vacuolating virus 40 polyadenylation signal; HSV TK promoter, Herpes simplex virus thymidine kinase promoter; cHS4core, insulator core derived from the chicken CHS4 element; Sequence to test; WPRE, woodchuck posttranscriptional regulatory element; bGH polyA, bovine growth hormone polyadenylation signal. (C) Coordinates of ECE18 mammalian homologs and fragments tested in this study. Genome builds are indicated. (D) Representative images of GFP antibody-stained sections from P2.5 forelimbs of hECE18^{FragA} lentivirus-mediated transgenic mouse. GFP positive clones are visualized by HRP-DAB coupled immunohistochemistry so positive clones appear brown (black arrows). Dorsal hair follicle (blue arrow). (E) Comparative quantitative activity of mammalian ECE18^{FragA} orthologs in cultured human GMA24F1A keratinocytes. Fold change normalized luciferase activity relative to Control (empty vector) is plotted. (F) Allele frequencies of polymorphic human variants rs56967129, rs146778681, rs769072620, rs529226880 are shown. Allele frequencies obtained from the 1000 Genomes Project Phase 3, gnomAD genomes r3.0 and UK10K datasets (11-13). (G) Fold change normalized luciferase induction relative to Control (empty vector) by hECE18^{FragA} containing minor allele at rs56967129, rs146778681, rs769072620 and rs529226880. (H) Fold change luciferase quantitative activity of hECE18 human: chimp chimeric enhancers relative to Control (empty vector). Maps of the human-chimp chimeric enhancers are shown (box). (I) Genomic location and alignments of derived human single nucleotide substitutions (A-J), derived human-specific insertions (i and ii), and modern human polymorphic variants (rs56967129, rs146778681, rs769072620, rs529226880) within hECE18^{FragA} are shown. Coordinates in hg38. (J) Fold change normalized luciferase activity of hECE18^{FragA} after deletion of derived human insertions i and ii. (K) Fold change luciferase activity relative to Control (empty vector) upon mutagenesis of hECE18 human derived variants A-J alone or all together to ancestral ape base. (L) Multiz alignments of placental mammals centered on the SP1^A and SP1^B motifs. A or B human derived variants are highlighted in red and pink, respectively. Human variants rs146778681 and rs769072620 are indicated. In panels (E, G, H, J, K) each point represents an

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individual biological replicate and the median (line), 25%-75% percentiles (box bounds) and min and max (whiskers) are plotted. Significance determined by one-way *ANOVA* and Tukey's-adjusted *P*-values are shown in heatmaps. All assays performed in cultured human GMA24F1A keratinocytes.



Fig S4. Effect of hECE18 repression on *INSIG2* and *CCDC93* expression in human keratinocytes. INSIG2 and CCDC93 are the only two protein-coding genes located within the EN1 topological associated domain (TAD). (A) Fold change expression of *INSIG2* and *CCDC93* upon dCas9-KRAB repression of hECE18 was assessed by qRT-PCR and calculated relative to dCAS9-KRAB transduction alone. Cr1 and Cr2 guide RNAs target hECE18. In graphs each point represents an individual biological replicate, median (line), 25%-75% percentiles (box bounds) and min and max (whiskers) are plotted and significance by one-way ANOVA. Tukey-adjusted P-values are reported. ** P<0.01, * P<0.05. Data normalized to the human β -ACTIN gene. Experiments performed in cultured human GMA24F1A keratinocytes.



A Generation of an ECE18 knock-out mouse (mECE18^{del})







Fig. S5. Generation and characterization of volar phenotypes of ECE18 knock-out mouse.

(A) Generation of an ECE18 knock-out mouse (mECE18^{del}) by CRISPR-Cas9 mediated genome editing. CRISPR-Cas9 target sequence and genotyping strategy are shown. Correct deletion junctions were confirmed by Sanger sequencing of F1 pups. (B) Normalized ratio of C57BL/6J : FVB/N allelic expression of En1 from P2.5 volar forelimb of wildtype (C57BL/6J : FVB/N) and

mECE18del (C57BL/6J^(mECE18del) : FVB/N) F1 hybrid mice. Ratios are normalized to the allelic ratio in F1 genomic DNA. Each point represents the mean value across three technical replicates for three or four biological samples consisting of pooled P2.5 volar skins from both forelimbs of three mice. **(C)** Quantification of the number of eccrine glands in the forelimb IFP of adult wildtype (+/+), mECE18^{del} heterozygous (+/mECE18^{del}) and homozygous mECE18^{del}/ mECE18^{del} mice. Each point represents the average number of IFP eccrine glands across both forelimbs of an individual mouse. The total number of animals analyzed per genotype (n). In panel **(B)** significance was assessed by a student's T-test (two-tailed). In panel **(C)** significance assessed by one-way *ANOVA*. Tukey-adjusted *P*-values are shown in a heatmap and the median (line), 25%-75% percentiles (box bounds) and min and max (whiskers) are plotted. N.S., not significant.



A Generation of a human ECE18 knock-in (hECE18^{KI}) mouse

B Genotyping of human ECE18 knock-in (hECE18^{KI}) mouse

											Primers for genotyping
ID#	1	2	3	-	1	2	3			8	Sequence
				11 11				1 : KI (hECE18 ^{KI} /hECE18 ^{KI}) 2 : bet (+/hECE18 ^{KI})	hECE18 ^{ki} allele	F3 R3	5'-GGGGAGATCGGAGATAGCAG-3' 5'-CCCGCTGAGAAAACAAGTGA-3'
1 Kb →			•	IIIIIIII	•	-	-	3 : WT (+/+)	Mouse allele	F8 R7	5'-GGCAGGGAGATGGGAGATAGC 5'-CCACAATGGAATCCGTCATGC-3

		• •	
	_	Sequence	Band size
CE18 ^{ki}	F3	5'-GGGGAGATCGGAGATAGCAG-3'	334 bp
ele	R3	5'-CCCGCTGAGAAAACAAGTGA-3'	
use	F8	5'-GGCAGGGAGATGGGAGATAGC-3'	793 bp
Ie	R7	5'-CCACAATGGAATCCGTCATGC-3'	



C Strategy to confirm single homologous integration of human ECE18 into mouse genome

Fig. S6. Generation of human ECE18 knock-in (hECE18^{KI}) mice. (A) Detailed overview of the generation of a human ECE18 knock-in mouse (hECE18^{KI}). CRISPR-Cas9 technology was used

to replace the endogenous mouse ECE18 with the orthologous human ECE18 sequence. A single knock-in founder mouse (ID #21) was identified and bred to a C57BL/6J male generate F1 pups. F1 pups were screened to confirm transmission of the knock-in allele and correct targeting in 2 pups validated to have correct junctions and insert sequence were used to generate two hECE18^{KI} lines. Each founder F1 mouse was bred onto C57BL/6J for two more generations prior to phenotypic analyses. Phenotypic analyses reported are based on progeny derived from both F1 founder lines at the N3 generation. CRISPR-Cas9 targets sequence, and primers used are listed. (B) Representative agarose gel for genotyping hECE18^{KI} mice. Sequences of genotyping primers used are shown. (C) Schematic of secondary Southern and long-range PCR/species specific restriction digest strategies to validate single homologous integration of hECE18 into hECE18^{KI} mice. KpnI and NotI sites are shown. (D) Genotyping PCR to determine genotype of mice used as source of genomic DNA for the southern blot in E and long-range PCR in F. Primers for genotyping listed in B and are species specific. (E) Autoradiograph of genomic southern blot using P32 labelled Probe 1 is shown. Sequences of primers used to amplify Probe 1 are shown and Probe target sequence is shown in C. Due to sequence conservation, probe against ECE18 (Probe1) cannot distinguish between mouse and human sequence, however hECE18 contains a unique Notl site that is not present in the orthologous mouse ECE18 genomic sequence. Thus, digest with NotI was used to validate integration of hECE18 in KI and het mouse genomic DNA. Presence of uncut KpnI fragment in KI and het lanes is due to incomplete NotI digest of genomic DNA. This is likely the result of partial CpG methylation of the hECE18 sequence, which blocks Notl cleavage, and is consistent with the observation that the enhancer is inactive in some tissues used as the source material for genomic DNA (tail biopsies). (F) A secondary strategy was used in F to confirm identify of sequence within swapped region. Enzymatic digestion of the long-range PCR amplified DNA fragment from WT, KI (hECE18^{KI}/ hECE18^{KI}) and het (+/hECE18^{KI}) mice. Primers used to amplify the genomic DNA fragments are listed. NotI enzyme cut uniquely in the human fragment. WT, wild type. KI, hECE18 knock-in homozygote. het, hECE18 KI heterozygote.

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Fig. S7. Effect of hECE18^{KI} and En1^{KO} alleles on mouse *En1* **expression and interfootpad eccrine gland number. (A)** Spatial *En1* mRNA expression in Control (+/+) and hECE18^{KI} (hECE18^{KI}/hECE18^{KI}) mice. Representative images of the ventral limb, and the hairy dorsal limb at P2.5 stage is shown for both genotypes. *En1* mRNA transcripts are detected using anti-DIG alkaline

phosphatase coupled antibody and appear in purple. **(B)** Effect of hECE18 on IFP eccrine gland number in a wildtype genetic background. IFP eccrine gland number in the forelimb adult wildtype (+/+), +/hECE18^{KI} and hECE18^{KI}/hECE18^{KI} mice is plotted. **(C)** Effect of En1^{KO} allele on eccrine gland number. The number of eccrine glands in the forelimb IFP of adult +/+; +/+, En1^{KO}/+; +/+, and En1^{KO}/+; +/hECE18^{KI} mice is plotted. Values for animals carrying En1^{KO} are also reported in the main text in Fig.3g. In panels **(B, C)** each point represents the average number of IFP eccrine glands across both forelimbs of an individual mouse and median (line), 25%-75% percentiles (box bounds) and min and max (whiskers) are plotted. The total number of animals analyzed per genotype (n). Significance was assessed by one-way *ANOVA* and Tukey-adjusted *P*-values are reported in heatmaps. +, wildtype allele. KI, knock-in. KO, knock-out. N.S., not significant.

ECE	Forward sequence	Reverse sequence
mouse-ECE1	accaattgctcgaggGTGCATTTATCATCCTATTACT	gtcaagcttccattatatagTTACACAGAGGCTAGATTGG
mouse-ECE2	tcgatagtcgaccaattgctcgaggCCCAAATCCCTGCCGCTTCT	cccttgctcaccatggtggcTCTTTGCTTTTTTGCAGGGAGAGCGC
mouse-ECE3	accaattgctcgaggCCTTTGCTGGAGCACCTA	gtcaagcttccattatatagGTCACTCCTCCACATTAA
mouse-ECE4	accaattgctcgaggCAATCAGGGCGACAG	gtcaagcttccattatatagTGGTCGCCACACAGTA
mouse-ECE5	accaattgctcgaggGAGAACTGCCTGTCCCGT	gtcaagcttccattatatagCACCAGGCTCTGTATGTA
mouse-ECE6	cgaccaattgctcgaggCTAGGAGACAGCCTTCCTGGAGG	cggtcaagcttccattatatagTGGCATGCCCTGCAAGTCTGAC
mouse-ECE7	accaattgctcgaggGTACTCACTGGATCTGAA	gtcaagcttccattatatagACTGCCTGAGTCATCTCC
mouse-ECE8	accaattgctcgaggATAACTGAGTTTGGTTTA	gtcaagcttccattatatagTGCTCAGCCCCTCCTCAG
mouse-ECE9	accaattgctcgaggACTGAGCTACATCCGTGG	gtcaagcttccattatatagTCTGTCATATACTTCTCG
mouse-ECE10	accaattgctcgaggCAGAACCTTCTGTATTTA	gtcaagcttccattatatagCAAGTGCTCTCTCACAGG
mouse-ECE11	agtcgaccaattgctcgaggCTCAGTGAGTCTCTGACAAGCC	tccggtcaagcttccattatatagGTGATTCTCTGACCTCTGCATGC
mouse-ECE12	accaattgctcgaggGCTCCTCTTTCCCAACAA	gtcaagcttccattatatagCATGATTGTCACTGGTCC
mouse-ECE13	accaattgctcgaggCTTATTGATTGGATATATG	gtcaagcttccattatatagCCAGTGAGCATAGCAGTGG
mouse-ECE14	agtcgaccaattgctcgaggTGAGGACCTGAGTTCAAATCCC	tccggtcaagcttccattatatagCCATATAGACAGGCACATGCAC
mouse-ECE15	accaattgctcgaggCCTTTGCAGACCTAGAAT	gtcaagcttccattatatagGGAGAACAAACCACCCAG
mouse-ECE16	agtcgaccaattgctcgaggCCATCCTGAGTCAAGGGCGG	gtcaagcttccattatatagCTTCAGAACTCAGAGTAGGGTCAAGC
mouse-ECE17	accaattgctcgaggCATGCATTATATTTACATCA	gtcaagcttccattatatagGGAAGTACCAATGAATCACA
mouse-ECE18	agtcgaccaattgctcgaggTGTGTTTGCCTCCTACAATGGGAGGATA	gtcaagcttccattatataGCTGAAGTTTTCCTTTCTGTTTACCAGGAAG
chimp-ECE18	accaattgctcgaggCCTGTTTGCCTCCCATAATA	gtcaagcttccattatatagCAGAAGTTTTCTTTTCTGTT
human-ECE18	accaattgctcgaggCCTGTTTGCCTCCCATAATA	gtcaagcttccattatatagCAGAAGTTTTCTTTTCTGTT
mouse-ECE19	agtcgaccaattgctcgaggTGTTGGAATAAGGGCACACC	tccggtcaagcttccattatatagGACACTGCTGCCTTCTCTATTC
mouse-ECE20	accaattgctcgaggCACATTCAAGGTCAATG	gtcaagcttccattatatagGCAGCAGTGAGTGTG
mouse-ECE21	accaattgctcgaggCACAATTGCCTCTTTTAGGT	gtcaagcttccattatatagCAAGGATGTTTTCAAATTAG
mouse-ECE22	agtcgaccaattgctcgaggCCTGACCTATCTGCCATCTCC	tccggtcaagcttccattatatagGCCTACACGTTAAGCAATAGGGT
mouse-ECE23	accaattgctcgaggTCTCTGCATAACAGCCCT	gtcaagcttccattatatagGCACTTTAATCACTAAGC

Table S1: Primers used to subclone ECEs in mouse transgenic assays#

*Lower case sequence indicates homology arms to Stagia3 vector

ECE	Forward sequence	Reverse sequence
cat-ECE18	agagatttagaatgacaggcGAGGGATAGGAATAGAAAAGGCCC	tcaagcttccattatatagaattccAAGTTTTCTTTGCTGTTTACCGG
cat-ECE18-FragA	gaaagagagatttagaatgacaggcCAACATATCGGGCTTACAAATTATC	tcaagcttccattatatagaattccAAGTTTTCTTTGCTGTTTACCGG
mouse-ECE18	agagatttagaatgacaggcTGTGTTTGCCTCCTACAATGGG	gcttccattatatagaattccCTGAAGTTTTCCTTTCTGTTTACCAGG
mouse-ECE18-FragA	gaaagagagatttagaatgacaggcCAACATATCGCGCTTACAAATTATC	gcttccattatatagaattccCTGAAGTTTTCCTTTCTGTTTACCAGG
bushbaby-ECE18	agagatttagaatgacaggcCGTGTTTGCCTCCCACAATAAGAGG	cttccattatatagaattccGGAAAGTTTTCTTTTCAGTTTACCAAGGAG
marmoset-ECE18	agagatttagaatgacaggcCCTGTTTGCCTCCCACAATAAGAG	cttccattatatagaattccCAAAAGTTTTCTTTTCTGTTTACCGGGG
macaque-ECE18	agagatttagaatgacaggcCCTGTTTGCCTCCCATAGTAAGAGG	agcttccattatatagaattccCAAAAGTTTTCTTTTCTGTTTGTCGGGG
macaque-ECE18-FragA	agagatttagaatgacaggcCAACATATCGCGCTTACAAATTAT	agcttccattatatagaattccCAAAAGTTTTCTTTTCTGTTTGTCGGGG
gorilla-ECE18-FragA	agagatttagaatgacaggcCAACATATCGCGCTTACAAATTAT	cttccattatatagaattccCAGAAGTTTTCTTTTCTGTTTACCGGG
chimp-ECE18	agagatttagaatgacaggcCCTGTTTGCCTCCCATAATAAGAGG	cttccattatatagaattccCAGAAGTTTTCTTTTCTGTTTACCGGG
chimp-ECE18-FragA	agagatttagaatgacaggcCAACATATCGCGCTCGCAAA	cttccattatatagaattccCAGAAGTTTTCTTTTCTGTTTACCGGG
human-ECE18	agagatttagaatgacaggcCCTGTTTGCCTCCCATAATAAGAGG	cttccattatatagaattccCAGAAGTTTTCTTTTCTGTTTACCGGG
human-ECE18-fragA	agagatttagaatgacaggcCAACATATCGCGCTTACAAATTAT	agcttccattatatagaattccCAGAAGTTTTCTTTTCTGTTTACCGGGGAGGGGAG
human-ECE18-fragB	agagatttagaatgacaggcCCTGTTTGCCTCCCATAATAAGAGG	gtcaagcttccattatatagaattccTTGTTTCTCAGCGGGCCCG

 Table S2: Primers used to clone ECE18 orthologs into bidirectional luciferase reporter vector#

*Lowercase sequence indicates homology arm to vector.

Table S3: Primers used mutagenesis of ECE18#

	h
ECE	Unique primer
hECE18-Mut-A	GGAAATGAAAATCTTCTCCGCCTtTCACGTCGCCGCTGCG
hECE18-Mut-B	CGCCTCTCACGTCGCCaCTGCGCCTTCAAATCCTCTGC
hECE18-Mut-C	CCTCACCTAATGCAAATGGAaCGGAGGCCTCTGTTATTGTATT
hECE18-Mut-D	CCTCACCTAATGCAAATGGAGtGGAGGCCTCTGTTATTGTATT
hECE18-Mut-E	TTAATCCGGCTTAGCtCGCTTAATGATGCCA
hECE18-Mut-F	AATCCGGCTTAGCCCaCTTAATGATGCCACT
hECE18-Mut-G	ATCGGGCCCGCTGAGAAAAtAAGTGACACAAAAAGTGGGCG
hECE18-Mut-H	CGCCCGCGATGGCGCtGATGGCTGATGCCGCGATTACGCC
hECE18-Mut-I	CTATCTCCGATCTCCCCGCCaGGTTTTTCATACTGATATTCTTTGCACCCC
hECE18-Mut-J	GGTTTTTCATACTGATATTaTTTGCACCCCGTAACACAGG
hECE18-FragA-rs56967129 C>T	TTTCATACTGATATTtTTGCACCCCGTAAC
hECE18-FragA-rs146778681 T>A	AAAATCTTCTCCGCCaCTCACGTCGCCGCTG
hECE18-FragA-rs769072620 C>G	CTCCGCCTCTCACGTgGCCGCTGCGCCTTC
hECE18-FragA-rs769072620 C>A	CTCCGCCTCTCACGTaGCCGCTGCGCCTTCA
hECE18-FragA-rs529226880 C>T	CCTTCAAATCCTCTGtGAGCAGACTGGCCTC
hECE18-del-insertion_i	CGGCCCCCTCCCGGGCTCCCCCGGTAAACAGAAAAGA
hECE18-del-insertion_ii	GAAGAGATTTATATTTTTTTGTTGTCAGGAAATGAACAAA
gECE18-Mut1	GCGGAGAAGTCTATgAACTACTTCCATTAAAATGC
gECE18-Mut2	CGCCGCCGCGATGGCGCtGgTGGCTGATGCCGCG
gECE18-Mut3	TGAAGAGATTTATATTTTTTGTTGTCAGGAAATG
gECE18-Mut4	CTTCTCCGCCTtTCACGTCGCCGCCGCGCCTTCAA
gECE18-Mut5	CACCGGCCCCtTCCCGGGCTCCCTCCCCGGTAAAC
hECE18_FragA-del_in_human_SP1A	CCCGTAACACAGGAAATGAAAATCTCACGTCGCCGCTGCGCCTTC
hECE18_FragA-del_in_human_SP1B	CTTCTCCGCCTCTCACGTCGCCAAATCCTCTGCGAGCAGACTGG
mECE18_FragA-add_in_mouse_hSP1A_B	GAAATGAAAATCTTCTCCGCCTCTCACGTCGCCGCTGCGCCTTCAAATCCTCTGCGAACA
mECE18_FragA-add_in_mouse_hSP1A	TGAAAATCTTCTCCGCCTCTCAGTCTCCACTGTATCTCCAAATCC
mECE18_FragA-add_in_mouse_hSP1B	CAGGAAATGAAAATCTTCTCCATTCACGTCGCCGCTGCGCCTTCAAATCCTCTGCGAACA
Chimera-5'-human-chimp-3'-F	CGCCGATGGCTGATGCCGCGATTACGCCGGCGGGGGGGGCGGCCCGC
Chimera-5'-human-chimp-3'-R	GCCGCCCCGCCGGCGTAATCGCGGCATCAGCCATCGGCGCCATC
Chimera-5'-chimp-human-3'-F	CGCTGATGGCTGATGCCGCGGATTACGCCGGCGGGGGGGG
Chimera-5'-chimp-human-3'-F	GCCGCCCCGCCGGCGTAATCGCGGCATCAGCCATCAGCGCCAT

[#]Lowercase nucleotide indicates the base changed in mutagenesis.

Table S4: ChIP-qPCR and qRT-PCR primer sequences	
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Name	Specie	Forward sequence	Reverse sequence
SP1A/B (hECE18)	Human	TGAAAATCTTCTCCGCCTCTCACG	CCAGTCTGCTCGCAGAGGAT
HBG2 promoter	Human	CCAAGGTCATGGATCGAGTT	ACACTGTGACAGCTGGGATG
En1	Mouse	GTGGTCAAGACTGACTCACAGC	GCTTGTCTTCCTTCTCGTTCTT
Rpl13a	Mouse	CAGTGCGCCAGAAAATGC	GAAGGCATCAACATTTCTGGAA
EN1	Human	TTCGGATCGTCCATCCTCC	GCTCCGTGATGTAGCGGTTT
INSIG2	Human	TTGCTGGAGGCATAACAATGGG	TGCCTTCTTCATTCCTGATGAGATT
CCDC93	Human	TGAACGACCAGTACTTGGAGCTG	GGATGTTCAGGAGGCCTTCG
Beta-ACTIN	Human	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

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