

Table S1. The GenBank accession numbers of genes targeted in this study.

Target gene	GenBank accession number
<i>elastase</i>	NM_001011493.1
<i>ets1</i>	NM_001130368.1
<i>ets2</i>	NM_001127061.1
<i>grp78</i>	XM_002941644.1
<i>hhex</i>	NM_204089.1
<i>pat</i>	XM_002940115.1
<i>pdx1</i>	XM_002934019.1
<i>ptf1a/p48</i>	XM_002933135.1
<i>tm4sf4</i>	NM_203627.1
<i>tyrosinase</i>	NM_001103048.1

Table S2. Computationally identified total number of potential off-target sites in *Xenopus tropicalis* genome with up to 5 mismatches to all the loci targeted in this study.

Target ID	Number of mismatch				
	1	2	3	4	5
<i>elastase</i> -T1	0	0	4	45	427
<i>elastase</i> -T2	0	0	2	22	278
<i>ets1</i> -T1	0	0	5	37	578
<i>ets1</i> -T2	0	0	5	59	652
<i>ets1</i> -T3	0	0	0	34	329
<i>ets2</i>	0	0	0	26	354
<i>grp78</i>	0	0	13	78	716
<i>hhex</i>	0	1	24	220	1941
<i>pat</i> -T1	0	0	8	93	822
<i>pat</i> -T2	0	1	9	84	866
<i>pat</i> -T3	0	0	5	89	653
<i>pdx1</i> -T1	0	1	9	76	763
<i>pdx1</i> -T2	0	0	40	278	2459
<i>pdx1</i> -T3	0	1	11	131	936
<i>ptf1a/p48</i>	0	0	4	36	368
<i>tm4sf4</i> -T1	0	0	5	27	307
<i>tm4sf4</i> -T2	0	0	3	48	402
<i>tyrosinase</i>	0	0	2	40	345

Table S3. 119 potential off-target sites examined by T7EI assay and corresponding PCR primers used for T7EI assay.

Site ID	Potential Off Target Site	Coordinate (Start)	Forward PCR Primer	Reverse PCR Primer
<i>hhex-2</i>	GGGCTGGGAGCTGGGGGCGCGG	scaffold_5011:(6508)	TGGTGCCACATCCTGTGGCAC	GGCCCCCCCAGCTCTCCAG
<i>pat-T2-2</i>	GGCACATTCTGATGCACTAGAGG	scaffold_471:(239289)	CTATGAACTCGAGAGCCGCTCT	CCGCATTTCTCCATGATGTTT
<i>pdx1-T1-2</i>	GGGCCATGATTTCTTTTCTGGG	scaffold_163:(1506895)	CCCTCTGGTATGTGCTACACC	ATAGGGCTTTATAGCTTGTGTGC
<i>pdx1-T3-2</i>	GGGAATGCCATTTGCTGGAGAGG	scaffold_82:(2396453)	CAGAAGAGGAAGACAAGTATCAGG	ACTCCTGTTTGTCACTTTTCCAC
<i>pat-T2-3</i>	GTTTCATTCTGATGCAGCAGTGG	scaffold_567:(560009)	TTTACATGGCTGTATGTACCAG	TTTCCCATGAGCATCTAAAGG
<i>pdx1-T1-3</i>	GGGCCACATTTTCATTTTCTAGG	scaffold_75:(1531776)	TCCATCTTTGCTACAGAAAGCC	TTTAGGCCCTTACATAGGAG
<i>pdx1-T3-3</i>	GGGGATGGATTGCTGGTGGGG	scaffold_618:(156398)	CAGCCAGATTGTTTTCTCCAG	GTACCTGAAGCACCCTGCTG
<i>ets1-T1-3</i>	GGATCTGCTTTATGTGGCCTGGG	scaffold_19:(386769)	AGGGTGCATTGAGACACTTTG	GGGTAGCTGGCCTATAAATAGG
<i>pat-T1-3</i>	GGGCTTTAGGCAAAACATTTGGG	scaffold_90:(1242588)	TACCATTTTCATTTAGCAAAGCC	TTAATTTCTTAGAGCCCTTTGAG
<i>elastase-T1-3-1</i>	TGAAGATGGTACCATACTGTGG	scaffold_384:(6780)	ATAATGGATGTTTGTGGAATTGC	ATGATACATTGCTTTACGCTG
<i>elastase-T1-3-2</i>	GGTTATTGGTACCATACTGTGG	scaffold_67:(1803421)	TGGGTATCGCTTTTCCAGACTATTG	GGTTCAACAAGCACCTGATAGC
<i>ets2-4-1</i>	GAGGTGGACTATTACTCTCATGG	scaffold_104:(2007422)	CAAGATGCCTAGATACTGCTGTTG	TGGACCATTAGTGAGCCCTATC
<i>ets2-4-2</i>	GGTCAGGCCTGTTACTGTCAGGG	scaffold_12:(2621242)	CCTCTTCTACACTTCTCCATCTGG	ACAGTTCAGGGGAAATGATGC
<i>ets2-4-3</i>	AGACTGGACTCTTCTCCCATGG	scaffold_12:(2854544)	AACTAATTGATTGGCCATATCGG	TGGTGCCTCTTAAAGAATTTAC
<i>ets2-4-4</i>	AGTCTGCACTCTTAATCTTAAGG	scaffold_13:(3467302)	GGCATAAAGTACAGTCCACCAG	TTTCATACTTTGCAATGTGTCTG
<i>ets2-4-5</i>	GGTCAGTTATCTTACTCTCAGGG	scaffold_132:(2363382)	ATAGAATTTCTTTGAGCCTGCC	GTATGGGGTTTGTCTATTGTTGAT
<i>ets2-4-6</i>	TGTCTGGTTCTTCTCTCAAGG	scaffold_144:(139498)	TATATTTGTAGGGATGACCAGATT	CTATGGGGGTGACAGGGATT
<i>ets2-4-7</i>	GGACTGGATTCTGACTCTTACGG	scaffold_163:(1321382)	TCAGCCAGTGCAAGAAATACTTC	GGCTGGCCTTATCAATTATTCTAC
<i>ets2-4-8</i>	TGTCTGGTTCTTCTCTCAAGG	scaffold_171:(2122408)	CCTAGGATAGGCCTCATAGGAG	AGGTGCAACTACTGCTTAATGGC
<i>ets2-4-9</i>	GGGCTGGCTCTTACTTTAATGG	scaffold_175:(2078354)	CAAGTGGTGCCTGTGTTTGC	TGACAGTGTGTGCTCCTTTG
<i>ets2-4-10</i>	GGTCTAGTCTAATTTCTCTCATGG	scaffold_214:(1626645)	GTAAAGCTGTGACTGAAACACCC	CAGCCTTTCTGGGTTTCAGAG
<i>ets2-4-11</i>	GGCCTGTACTTTTACTCAACAAGG	scaffold_313:(732450)	TATTATACTTATGTTGCAAGTGC	ATTTATTACACCCCTAGTTTGTCTT
<i>ets2-4-12</i>	AGTCTGGCCTCTTACTGTAACGG	scaffold_374:(908485)	TGACACTGTAGGCACAAATATGG	CGTTGCATTTGTTCTTATTCC
<i>ets2-4-13</i>	GGTCTTGTCTCTTACCCTCCAGG	scaffold_435:(862492)	TAAAGTGTGTTTGGTGTCCG	CTCCATTGACTCCATTGCTT
<i>ets2-4-14</i>	GGTCTGGATTCTGACACTTAGG	scaffold_463:(421903)	GTTTACATTTTATTACAGGGCTTG	CTTCTTCCACTGCATAACCTC
<i>ets2-4-15</i>	GGTCTGGTATCTTACCCACAGG	scaffold_482:(377203)	GGGAGACCATCTAGAACAGG	CGGTCTATCTCTCGCTAGTG
<i>ets2-4-16</i>	GGTTGGCATCTTACTACACAGG	scaffold_510:(337303)	GGTACAAACTGGCTCCCTTTC	GACAGATGCAGCAGAGCTTATG
<i>ets2-4-17</i>	GGTCGGCAATCTTACTCTCAGGG	scaffold_518:(816043)	TTCACGTGCATTATAAATCCAAC	GGTCAGTTTGAAGTGTATCTGGAG
<i>ets2-4-18</i>	TGTCTGGCCTCTTCTCTGATGG	scaffold_578:(441317)	GGGCAGAAACCCCTACAAAG	TTAGCTTTATGCAAAAGGCTGG
<i>ets2-4-19</i>	GGTCAGGGTCTTACTCTCAGGG	scaffold_58:(274580)	GTTTCACAAAACAATTCGTCAAGT	GCTAAGTGGAAATCCAAGTTCTC
<i>ets2-4-20</i>	ATTCTTGACTCTTCTCTCAGGG	scaffold_59:(1960990)	CATGTGCTATTCCTAGGAAAGG	CCCCCTTAAATAGTGTCTTCTG
<i>ets2-4-21</i>	GGTCTGGACTGGTAGCTCATGG	scaffold_68:(1440099)	ATTACAGGCCACTGGGCTCTAC	AGACACGCAATGTTTCTCTGG
<i>ets2-4-22</i>	GGTTGGACTTTTACTGGCAAGG	scaffold_7:(5245156)	GTGTTGATGTCTGGTGTGTTCTT	GACCAGCCAAAATAACAGAGG
<i>ets2-4-23</i>	GGGCTGGACTATTACTCAGATGG	scaffold_753:(150)	CGGAAATGAATAGTGTGACTG	CATAGTTGAACTTCTATGGCAGAG
<i>ets2-4-24</i>	GGGCTGGACTATTACTCAGATGG	scaffold_753:(7514)	CAGTTTATAGCCGAAATGAATAG	CCCTGTAAGTTGCCATCTTGTAC
<i>ets2-4-25</i>	GTTCTGGGCTCTTACTTTGAAGG	scaffold_8529:(5756)	GCACAGAAAGAGGGTGCATTTAC	CTCCTGGATTCTCCAAATGGAC
<i>ets2-4-26</i>	GGACTGGGACCTACTCTCATGG	scaffold_917:(200870)	GGGGCATTACACACAGGGGA	CAAGGGGCCACTAACGTATGC
<i>ptf1a/p48-3-1</i>	AGATGACAAATGTGGACTTCTAGG	scaffold_381:(76541)	TTGAACATAAATGTCTCTGGCTG	TTCTTCTTTTGTTCATGGTCC
<i>ptf1a/p48-3-2</i>	GGAAAGAGGATTTGGACTTCTGG	scaffold_47:(3047525)	CTCAGGGTTGCAACTATTTG	TCTTACCCTCTATGCAGGTAAGC
<i>ptf1a/p48-3-3</i>	GGAAAGACCCTGTGCACTTCTGG	scaffold_81:(1528612)	ATTGTTTATGGGAACAATGGCT	AAATAGTAGCGCACAGCACAAG
<i>ptf1a/p48-3-4</i>	GGAGGACGATGTGCACATCTGGG	scaffold_9095:(7960)	CTTCGTCTTTGGAGCCTAGAATC	GCATATGTCTATCGGTGTGTAG
<i>ptf1a/p48-4-1</i>	GGAAAGAGGATTTTACTTCTGG	scaffold_11680:(787)	CAACCAAGATGGCATTTGGAGTTAC	CCTGTTGGAGCTTTGGACTGC
<i>ptf1a/p48-4-2</i>	GGAAAACGCTGTGAACTTTTGG	scaffold_125:(2489873)	GGATGTAAAGTGTGCCATAGGAC	TATCGGTCAATCATCACCTGG
<i>ptf1a/p48-4-3</i>	GGAAAGACAGGTGGACTTAAAGG	scaffold_128:(556723)	TACAAGTGCATGTGTGCAAGG	TAGGTGAAACAGATTTAGCCATG
<i>ptf1a/p48-4-4</i>	TGAAGACAATGATGACTTCTGGG	scaffold_139:(1729414)	ATCCCAGCAACAATTAGGTTTC	TCTTGCAAAACATCAGCAAATC
<i>ptf1a/p48-4-5</i>	GGAAAGCGATGTGGACCTCTGG	scaffold_144:(1611751)	CAGTCTTATAGGGGGTTGGTTC	CGGAAATTACACTACCCTCCAC
<i>ptf1a/p48-4-6</i>	GGATGAGGTTGTGGACTTCCAGG	scaffold_150:(1771007)	TGACGGTGTCTTATTACAAAATG	ACTAGTGGGAAAGCATACTGTAGC
<i>ptf1a/p48-4-7</i>	GGAGGAGGTTCTGGACTTCTGG	scaffold_155:(58578)	AATTATGCCTTGAAATGTTTTCAGC	AGTTGCCTAGTAATCTCCAGAATG
<i>ptf1a/p48-4-8</i>	GGAGGAGGTTCTGGACTTCTGG	scaffold_155:(58617)	TTCACAAGTCTACAAAAGTCAAGC	TACATTTGGATCAATACCTGTGCTA
<i>ptf1a/p48-4-9</i>	GGAAAGTCGTTGTGCACGTCTGG	scaffold_1804:(27886)	AAATCCTAAGCCTTTTTCGCAG	GAGTGCAGCAGTATTACAAAAGAGG
<i>ptf1a/p48-4-10</i>	GGAAAGCCTTAGGACCTCTAGG	scaffold_2:(2433980)	AAACGCTTGGTCTCTCTCTGC	AGACATTTGCTTTTGTCTTGG
<i>ptf1a/p48-4-11</i>	AGGAGACGCTGTGGGCTTCTGG	scaffold_2:(4338728)	TGTTTGTAAACCCGCCAGACC	AACCCTCACTCCACATAGGCTA
<i>ptf1a/p48-4-12</i>	GCAGGACGATGTGAATTTCTGGG	scaffold_212:(1294306)	AGAAGCATAGACTGTGATCTTTGC	CGTGTGTCTTAGCCCTTAAGC
<i>ptf1a/p48-4-13</i>	GTAAGTAGATGTGGACTTATTGG	scaffold_24:(1548124)	ATTAAGACAAAATGACCATGTTGA	AATGGCACTTCTCACCTACAG
<i>ptf1a/p48-4-14</i>	TGTAGACCCTGTGGACTTCTGG	scaffold_242:(750479)	CTATCACCTGGGTGTAATGAAAC	GCAGCAACACTTTCTCAAACAG
<i>ptf1a/p48-4-15</i>	GGAAGAGGATTTTACTTCTGG	scaffold_267:(152383)	TGCAATTTTATTGAGTGAATAAG	GTCTTACCCTCTATGCAGGTAAC
<i>ptf1a/p48-4-16</i>	GGAAGAGGATTTTACTTCTGG	scaffold_268:(337535)	GAAGCATACTTGTAAACCCTCAGG	GTCTTACCCTCTATGCAGGTAAC
<i>ptf1a/p48-4-17</i>	GGAAGAGGATTTTACTTCTGG	scaffold_268:(342264)	AGTGACTGGATTCCCATTTCC	CAGGCATCAACCTGTGTACAAC
<i>ptf1a/p48-4-18</i>	GGAAGAGGATTTTACTTCTGG	scaffold_268:(348088)	TTTACTAGTCTTGGCCGAGGAC	GGGTCTTACCCTCTATGCAGG
<i>ptf1a/p48-4-19</i>	GGAGGAGGATATGAACTTCTAGG	scaffold_274:(172841)	GAAGGATTAGCAGAGGGATGATT	ATCAACCTTTTCTTGGGGCTAT

Site ID	Potential Off Target Site	Coordinate (Start)	Forward PCR Primer	Reverse PCR Primer
<i>ptf1a/p48</i> -4-20	GGAAGAGGATTTTGTACTTCTGG	scaffold_291:(113367)	TTGCAGACTAGCATGTCCACATC	ACACTGCCTGTAGAATGGGAATC
<i>ptf1a/p48</i> -4-21	GGAAGCCCAAGTGCACCTTCTGGG	scaffold_387:(351500)	GATTATTCAGGTGCTGTGAGG	ACAGGGGCTCCCTTTATAACC
<i>ptf1a/p48</i> -4-22	GGAAGATTATGTGCATTTCTGGG	scaffold_408:(152759)	CTAAGGGCAGGTAAGGCTCTCT	CCACAAGGAGGTGATACAGGAGT
<i>ptf1a/p48</i> -4-23	GGAGGCAATATGGACTTCAAGG	scaffold_441:(983968)	GCAGACTCTCGTGCATTATGC	AACATCTGCTGCAAGATAAAAACC
<i>ptf1a/p48</i> -4-24	GGAAGAGGATTTTGTACTTCTGG	scaffold_569:(721734)	TTCTCCATAGTTGTTGAAATGAGTG	CTCCTGTTGGAGCTTTGGACT
<i>ptf1a/p48</i> -4-25	GGAAGAGGATTTGTAGTTCTTGG	scaffold_625:(443803)	GTGCATTCTGGATAATGGGTC	CTCATTCATATATCAGTCCCTGC
<i>ptf1a/p48</i> -4-26	GAAAGAGGATGTGGCTACTTGG	scaffold_63:(205522)	CATCTCCGATCAGCACGTTTC	GTTCTCTTGTTTTGTGTGATATTTT
<i>ptf1a/p48</i> -4-27	GGAAGAGGATTTTGTACTTCTGG	scaffold_6344:(1703)	GTTTGAGAGACCAACATCCCC	ACTGCTCAGCTACCCTGACTG
<i>ptf1a/p48</i> -4-28	GGATGACGATGAAGACTTGTGG	scaffold_649:(10287)	CTTTGCCATGGAGGCTATTG	GTGGTGGCTACTGAAGCAGAC
<i>ptf1a/p48</i> -4-29	GGAATACGTTGTGGAAATTTTGG	scaffold_653:(376103)	ATTTAGTCGCCATAATATGCAGTAC	GTACCGCTGACTACTTCATCCT
<i>ptf1a/p48</i> -4-30	GGAACACTATTTGGACCTCTTGG	scaffold_709:(400270)	CCTGCCCTAAGCTTAGTGTGAC	GGGCGATACAACCTCTGTATTGTG
<i>ptf1a/p48</i> -4-31	GGAGGAAGATGTGGATTTCTTGG	scaffold_742:(350425)	GTAGCAGATTGCATCTACTTCAGTC	GGGATTTGCCATATGTCCATTT
<i>ptf1a/p48</i> -4-32	AGAAGACGATGGGGCTACTGGG	scaffold_782:(110628)	GCAGCAATGATTCAGGACTTC	TGATGTTTTGGGCTTCTATACG
<i>ptf1a/p48</i> -4-33	GGAAGAGGATTTTGTACTTCTGG	scaffold_791:(6875)	ACAGTTTGAGAGAACAACATCCC	ACTGCTCAGCTACCCTGACTG
<i>ptf1a/p48</i> -4-34	GGAAGAAGATGGGGCTACTGGG	scaffold_8389:(8767)	GCAAATAATCGAGCAGAAAATAAG	GGAGAACTAAAGGCTTTAGGACC
<i>ptf1a/p48</i> -4-35	AGAAGACAATATGGATTTCTTGG	scaffold_93:(1041592)	GCAGATCCCAAGAGATATAGTTTG	TGAATCTATTGCCAGTAAAATGC
<i>ptf1a/p48</i> -4-36	GAAAGAGGATGTGGCTACTTGG	scaffold_9648:(349)	GCATAGGGGAGCTGAGATGAAG	GTGTTACTTTGATTTGGCCGC
<i>tyrosinase</i> -3-1	GACCCCGAGTTTCCCTTCACTGG	scaffold_440:(120683)	CACACTGCAGAACTTCATTCAAC	CGTGTGGAAGTACCACAAAAC
<i>tyrosinase</i> -3-2	GGCCTTTAGTTTCCAGTCACTGG	scaffold_99:(2814102)	GTGGGATTTCAAGGTGTGATG	GAATGGAATGCTTCCACCTGC
<i>tyrosinase</i> -4-1	GGCAATCAGTTTCCAATGACAGG	scaffold_10:(1775587)	CTCCTAGATCCCTTGAAATTGAAG	TTTGTGGACATATGAATGTTATTGG
<i>tyrosinase</i> -4-2	GGCTCCAGGTTTACAATCACTGG	scaffold_10:(3396052)	CAAATACAGTCCACCAGAAAGAGC	TAACTTCTCAACCGACACAGATT
<i>tyrosinase</i> -4-3	GACCCTCAGTTTCTGTTCACAAGG	scaffold_101:(2091511)	TTAGAATTGCCAGTGATTAGGGA	CTGGTGAGAAAGCATCTCTGTGA
<i>tyrosinase</i> -4-4	TGGCCTCAGTTTACTTCACTGG	scaffold_102:(757505)	AATGATTGATTAATGGGCACCA	CTTCTTTATAACACTGGCATGCTG
<i>tyrosinase</i> -4-5	CGCCCTCAGTTTCTAGTCACTTGG	scaffold_105:(172804)	GCTTCTGTTCAGAATAACCCAT	TATCTCAATTCTGGCCTTAGGAG
<i>tyrosinase</i> -4-6	GGCCCTTAGTGCCTTACACGGG	scaffold_106:(171699)	GGTGGGGATATCAGGCTAAT	AACAAATGCACATGAATTGATT
<i>tyrosinase</i> -4-7	TGGGGTCAGTTCCCATTCACGGG	scaffold_123:(654169)	GTGGACATGGCCATTTATTGG	CCCCTTAAAGAGACAGTCAGAGAC
<i>tyrosinase</i> -4-8	GTCCTCAGTTTCCATGCAGTGG	scaffold_12888:(1272)	GTCAGGTCGTGTTTTGAGGTTG	GAAATTGATAAAGCATATAAAGGGA
<i>tyrosinase</i> -4-9	GGCCCTCGGTTTCCCGCACAGG	scaffold_137:(2005057)	CGAAAGGCAATGCTTCTGTCA	TGTGGGTTCTGAGCAAGCGTA
<i>tyrosinase</i> -4-10	GCCCTCAGTGTTCATTACAGGGG	scaffold_14:(5389042)	GCAGCTCAGCAGTAAAGTGAGA	AGTTTGCAGTAAATGTAAGCAGAT
<i>tyrosinase</i> -4-11	GTGCCTCAGTTTGCATTCCTTGG	scaffold_143:(518441)	TTTGGTAAAACCTCCATGTTTAGTAT	ATGTAGCGTTGCACATAGTAGCA
<i>tyrosinase</i> -4-12	GGCCTTCAGCTTCCATTGCCTGG	scaffold_149:(202527)	TGCTCCTCTGGGAACAGATGT	CTACAGGATGTGGGCTATGACTAT
<i>tyrosinase</i> -4-13	GCCCTCATGTTCCCTTCACAGG	scaffold_180:(662866)	GGCTGCAATAAATACAATAATGTC	TATAAAATCCCGTTTTTGTCTCC
<i>tyrosinase</i> -4-14	GGCATAACAGTTACCATTCACTGG	scaffold_210:(341070)	GACAGTTCCTGACTCTCTCTGATTA	ACAGCCCTATCTCCTTCATTGG
<i>tyrosinase</i> -4-15	GGCAGTCAGATTCATTACAGCGG	scaffold_228:(627447)	AACAGAAATAAACATGGAGCTTGTA	TGAGACTTCTCAAGGCAAGAG
<i>tyrosinase</i> -4-16	GCCCTTAGTGTCCACTCACTGG	scaffold_27:(1013047)	GTAGCCGTTACGACTTGGCAG	TTAAATCTGTTTTCTCTGGACTT
<i>tyrosinase</i> -4-17	GGGCATCAGTTACCATAACAGG	scaffold_274:(1373068)	AGTTTTCTTAGACGCATGGCAA	GCCCAACGAAAGTGGCTGAT
<i>tyrosinase</i> -4-18	GGCCGTCAGGTTCCATCAACTGG	scaffold_277:(630877)	AGGTAATCAGGGCAATATAGAATAA	CAGTTGTACAAAGCTGTTCTAAATA
<i>tyrosinase</i> -4-19	GAGCCTCAGTTTCTATTCCAGG	scaffold_305:(90979)	GCTGCAATTTGTTTAGTAACGC	GTTTGTGATTTTCTTAAACTTGGC
<i>tyrosinase</i> -4-20	GTCCCTCAGTTTCACTTACAGG	scaffold_33:(2154089)	CAGGGACTGGTTTGCCATCTT	TGTAGCACACAACAACATCAGTAAT
<i>tyrosinase</i> -4-21	GGCCCTCATTTTCTATTACAGG	scaffold_359:(468247)	AGCCAAACCAGATTTATTTCTCA	TAAATGAAATAGTTGCAGAAGACAC
<i>tyrosinase</i> -4-22	TGTCCCTCAGTTTCACTTACAGG	scaffold_409:(662043)	GTGAAATGCACCTTGCACTCTAT	GTGGGTGAATAAATCAGAGTGGTT
<i>tyrosinase</i> -4-23	GTCCCACAGTTTCCATTATTTGG	scaffold_414:(811399)	AGAGTGTGTTGTGTAGATGTATGTA	GCTTGTGTGCCCTTCCCTTCTT
<i>tyrosinase</i> -4-24	GCCCTTAAATGTCATTACACAGG	scaffold_441:(601770)	AAACGTTGCGCCTTTTAAAGTTA	GACAGCAGACTATGGCTACTTACC
<i>tyrosinase</i> -4-25	GGCTCCCATTTTCCATTCCCTGG	scaffold_451:(852715)	ACAGAGAGATTCTGATATTCTGCTT	GCGACAATCTGCAGTTTACAATA
<i>tyrosinase</i> -4-26	GGTGCACAGTTTCCATGCAGTGG	scaffold_459:(263553)	CGTCAAGAACATTGCGGTGAA	AGGATGTCTTCAAACCATAAATCTG
<i>tyrosinase</i> -4-27	GCTCTCTGTTCCCATTCACAGG	scaffold_479:(495121)	ATGGACAGAGATCAAAAGATAGA	GTCCAAGGGAAGGGGACACA
<i>tyrosinase</i> -4-28	GTCTCTCAGTTTCCATGCAGTGG	scaffold_491:(181703)	GTCATGGCTATGGTGTCTAGATTCT	GCTATGCTAATATGAACCATGACCA
<i>tyrosinase</i> -4-29	GTCTCTCAGTTTCCATGCAGTGG	scaffold_491:(194802)	TTTGAGGTTGCACACTTCTCATT	TTCTTGTGACAGACCATAAATCCTT
<i>tyrosinase</i> -4-30	TGCCCTCAGTTTACATGCATGGG	scaffold_5:(5861390)	ATATAGGGAGATGCTCTCAGTTTCAG	TTAGTTAAAATCTTCTTGATGTGCG
<i>tyrosinase</i> -4-31	CGCAATCAGTTGCCATTCACTGG	scaffold_537:(315212)	CCGAGGCAGAAGGACTAGCTAT	CACAGGGAAGAACAGAGGCAT
<i>tyrosinase</i> -4-32	GGCCATCAGTTTCTTTCACACAGG	scaffold_57:(2987708)	AGACATCAGGCTCAGTTACTTGC	ATCACACTAGAATATGGATCACCAC
<i>tyrosinase</i> -4-33	GACAGGCAGTTTCCATTCACTGG	scaffold_570:(345550)	GCCATGAAAGCTTGAGATAAATTC	CGTAGTGTTCGATTTTCAGCAGGT
<i>tyrosinase</i> -4-34	GGCCCTGTGTTTCCAGCCACAGG	scaffold_577:(227920)	CTGTCAAGTTCACTGCCCTGCA	CGCTGTCACGGTAGCCTTATC
<i>tyrosinase</i> -4-35	GTCCCTCCGTTTCCATTTAGAGG	scaffold_64:(3047845)	CTGCTTCTGTAGTATAACTGGTAC	TGTAACACCCTGTAACAGAGAATGG
<i>tyrosinase</i> -4-36	GGCTTTCAGTTTACATTCTCAGG	scaffold_937:(132022)	CAAAATGTCATCTACAATAAATCAG	ATATGACTGAAACAGCCTTGGTGA
<i>tyrosinase</i> -4-37	GGCCTTCAGTCTGCATCACTGG	scaffold_99:(530571)	TTTCACTCCAAGTGGCCTTCA	GCAGCTCAATAAGGTCCATTGTAT
<i>tyrosinase</i> -4-38	GGCCTTCAGTCTGCATCACTGG	scaffold_99:(551360)	TTTCACTCCAAGTGGCCTTCA	GCAGCTCAATAAGGTCCATTGTAT
<i>tyrosinase</i> -4-39	GGCCAGCAGCTCCATTCACTGG	scaffold_992:(42836)	TTGTGACCTTGGAAATGAATTCTGTT	CCTCATTGTGGTGTGGCGT
<i>tyrosinase</i> -4-40	GGCCAGCAGCTCCATTCACTGG	scaffold_992:(62574)	TTGTGACCTTGGAAATGAATTCTGTT	CCTCATTGTGGTGTGGCGT

In a given potential off-target sequence, the red letters indicate the nucleotides that are different from those in the corresponding on-target site illustrated in Table 1.

Table S4. List of PCR primers used to amplify the targeted loci.

Target gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>elastase-T1</i>	GGAGTGGCATCATACTGACTGTC	AAGGGAAGGGATGTAAAAAGAGC
<i>elastase-T2</i>	ATGGTCATCCAATCTGGAAGC	CTGTCCCTTCCGTAATGATGC
<i>ets1-T1</i>	GGTTCGTGTTTGGATAACAAGTACC	AAAAGTATGTTCAACCCAAGCC
<i>ets1-T2</i>	GAATCTGCTTGTTCTTCAGGAAC	GCAGTAATGAGATGGTCACGG
<i>ets1-T3</i>	GAATCTGCTTGTTCTTCAGGAAC	GCAGTAATGAGATGGTCACGG
<i>ets2</i>	CCAAATTAGAAGGCTTCCATGTAG	CAATATTAACAGAGTTGGCACCG
<i>grp78</i>	AAACCCTATTGAATTAGTTGGAGGC	TCCCTTAACATGTGACTCCAAACC
<i>hhex</i>	ATGTTGATTCCGATCTCTCATTTTC	CCCATACAACTGGCAGTTAGTTG
<i>pat-T1</i>	AAATCCATGCTGCATTTACAAG	AACAAGCTGCTCTTATATCTAATGC
<i>pat-T2</i>	CCATATAATTGCATGTGGCATAAC	AAATAACCATCTGCTCTGAGGG
<i>pat-T3</i>	CCATATAATTGCATGTGGCATAAC	AAATAACCATCTGCTCTGAGGG
<i>pdx1-T1</i>	CGTGCAACCACAGCTAAATAGTG	CGGAGTCTGAATATTTGCACC
<i>pdx1-T2</i>	AACAGGTGTCCACTGCCAAG	CCCAGCCTGAGTCTCCTACTC
<i>pdx1-T3</i>	AACAGGTGTCCACTGCCAAG	CCCAGCCTGAGTCTCCTACTC
<i>ptf1a/p48</i>	GCAGAAGCGCAATGCTATG	GGATGAGAAGGAGAAGTTGCC
<i>tm4sf4-T1</i>	GCATGATAACATTAAGGGCACA	CAAATTCCAAAGGCCATCTC
<i>tm4sf4-T2</i>	GGAGGTTAGTGGCTAAGGTAAT	CTGACAACAGCAGGATAAAGAT
<i>tyrosinase</i>	GAGATCCTGCGTGTCATATTGG	CATAGGGTTGGAGCCATTATTC

grp78 88.9% (16/18)
 GACCATGAAGCTGTTGGCTTGGTGTGCTGCTGCCAGCGTATTGCTGCTGATGA Wild-type

GACCATGAAGCTGTTGCCTTGGT---TGCTGGTGTCTGCCAGCGTATTGCTGCTGATGA (Δ2) [x2]
 GACCATGAAGCTGTTGCCTTGGT---CGCTGGTGTCTGCCAGCGTATTGCTGCTGATGA (Δ3, +1)
 GACCATGAAGCTGTTGGCT---GG---CTGGTGTCTGCCAGCGTATTGCTGCTGATGA (Δ6)
 GACCATGAAGCTGTTGCCTTGGT---CTG---CCAGCGTATTGCTGCTGATGA (Δ9) [x3]
 GACCATGAAGCTGTTGCCTTGGT---TGT---CTG---CCAGCGTATTGCTGCTGATGA (Δ10)
 GACCATGAAGCTGTTGC---TGGTGTCTGCCAGCGTATTGCTGCTGATGA (Δ11)
 GACCATGAAGCTGTTGG---TGCTGTCTGCCAGCGTATTGCTGCTGATGA (Δ14)
 ---GTTGCTGGTGTCTGCCAGCGTATTGCTGCTGATGA (Δ48)
 GACCATGAAGCTGTTGCCTTGGTGGTTGGTGTCTGCCAGCGTATTGCTGCTGATG (+1)
 GACCATGAAGCTGTTGCCTTGGTGGTTGGTGTCTGCCAGCGTATTGCTGCTGATG (Δ4, +14)
 GACCATGAAGCTGTTGCCTTGGTGGTTGGTGTCTGCCAGCGTATTGCTGCTGATG (+5) [x2]
 GACCATGAAGCTGTTatagTgcactgctactgcaagtcTGTGCTGCTGCCAGCGT (Δ9, +24)
 GACCATGAAGCTGTTgcctTGGTGTCTGCCAGCGTATTGCTGCTGATGA [x2]

elastase Target 1 100% (20/20)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCATACCTGTTGGGGCTCCCTGATCCCGTG Wild-type

CCCTCCAGTACTGTCTGGTGGCAGTGGTACCATAC---TGTGGGGCTCCCTGATCCCGTG (Δ1)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---CTGTGGGGCTCCCTGATCCCGTG (Δ3)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---A---CTG---GGGGCTCCCTGATCCCGTG (Δ3)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---A---CTG---GGGGCTCCCTGATCCCGTG (Δ3)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ5) [x3]
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---gggaGTGGGGCTCCCTGATCCCGTG (Δ5, +4)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ6, +1)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ6)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ6)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---aacgctaccTGTGGGGCTCC (Δ9)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ11) [x2]
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ14)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ15)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---A---CTG---GGGGCTCCCTGATCCCGTG (Δ15)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TGTGGGGCTCCCTGATCCCGTG (Δ17)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---TgtgctactgTGTGGGGCTC (Δ7, +11)
 CCCTCCAGTACTGTCTGGTGGCAGTGGTACCAC---AC---agggg---TG (Δ18, +1)

elastase Target 2 87.5% (14/16)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCCCTCAGGATGCCAGGTACAATGAT Wild-type

AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCCCTC---CAGGATGCCAGGTACAATGAT (Δ1)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCCCTC---ATTGAGATGCCAGGTACAATGAT (Δ1, +1)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCCCTC---TTCAGGATGCCAGGTACAATGAT (+1)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---AGGATGCCAGGTACAATGAT (Δ5)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---GGATGCCAGGTACAATGAT (Δ5)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---GATGCCAGGTACAATGAT (Δ5)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---tcaatttgt---GCCAGGTACAATGAT (Δ16, +9)
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---GCCAGGTACAATGAT (Δ11) [x2]
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---AGGTACAATGAT (Δ13) [x2]
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---ACAATGAT (Δ13) [x2]
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCC---GAT (Δ19) [x2]
 AGTCCACCATGGTGTGCTGCTGGTGTGATGGAGTCCGTTTCCAGGATGCCAGGTACAATGAT [x2]

ets1 Target 1 33.3% (6/18)
 TTCAGAAGTTCTGTATGAGCGGAGCAGCATTATGTTGGCTGGGGAAGGAATGTTTCTAG Wild-type

TTCAGAAGTTCTGTATGAGCGGAGCAGCATTATG---TGGGAAGGAATGTTTCTAG (Δ5) [x2]
 TTCAGAAGTTCTGTATGAGCGGAGCAGCATTATGTTGG---GGAAGGAATGTTTCTAG (Δ5)
 TTCAGAAGTTCTGTATGAGCGGAGCAGCATT---acagaaCTGGGGAAGGAATGTTTCTAG (Δ8, +6)
 TTCAGAAGTTCTGTATGAGCGGAGCAGCATT---GGAATGTTTCTAG (Δ17)
 TTCAGAAGTTCTGTATGAGCGGAGCAGCATT---GAATGTTTCTAG (Δ20)
 TTCAGAAGTTCTGTATGAGCGGAGCAGCATTATGTTGGCTGGGGAAGGAATGTTTCTAG [x12]

ets2 100% (21/21)
 AGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCAATGATGCTCTTACTTACCT Wild-type

AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---CATGATGCTCTTACTTACTC (Δ1, +3)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---ATGTTATGCTCTTACTTACTC (Δ2) [x2]
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---CATGGTATGCTCTTACTTACTC (Δ2, +5)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---catagcaTGGTATGCTCTTACTC (Δ3, +7)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---ATGTTATGCTCTTACTTACTC (Δ3, +1)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---ATGTTATGCTCTTACTTACTC (Δ4)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTT---C---ATGTTATGCTCTTACTTACTC (Δ5)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---TGGTATGCTCTTACTTACTC (Δ6)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---TGGTATGCTCTTACTTACTC (Δ8)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTT---GGTATGCTCTTACTTACTC (Δ13, +5)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---TggcTaaTccTcaaaaTaaTc (Δ13, +5)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCT---TATGTTCTTACTTACTC (Δ16)
 AGAACAGGCGGTACCAACGGG---T (Δ38)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCTactcATGTTATGCTCTTACTC (+2)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCTcatcATGTTATGCTCTTACTC (+4)
 AGAACAGGCGGTACCAACGGGCTCTGGACTCTTACTCTgttactCATGTTATGCTCTTACTC (+6)

hhex 100% (18/18)
 AGAATAGCCATGCAGTACCCGACCCAGCTCTCTCAGCCCCTGGGGTTCAGTGTCCCCTG Wild-type

AGAATAGCCATGCAGTACCCGACCCAGCTCTCTCAGCCC---CCAGCTCTCTCAGCCCCTGGGGTTCAGTGTCCCCTG (Δ1, +3)
 AGAATAGCCATGCAGTACCCGACCCCTcagctacCAGCTCTCTCAGCCCCTGGGGTTCAGTGTCCCCTG (+7)
 AGAATAGCCATGCAGTACCCAGCTCTCTCAGC---CCTGGGGTTCAGTGTCCCCTG (Δ8) [x9]
 AGAATAGCCATGCAGTAC---CCTCAGCCCCTGGGGTTCAGTGTCCCCTG (Δ14)
 AGAATAGCCATGCAGT---CCTCAGCCCCTGGGGTTCAGTGTCCCCTG (Δ15)
 AGAATAGCCATGCAGTACCCAGC---CCTGGGGTTCAGTGTCCCCTG (Δ16)
 AGAATAGCCATGCAGTAC---TACAGCCCCTGGGGTTCAGTGTCCCCTG (Δ16)
 AGAATAGCCATGC---TCTCAGCCCCTGGGGTTCAGTGTCCCCTG (Δ17)
 AGAATAGCCATGC---AGCCCTGGGGTTCAGTGTCCCCTG (Δ23)
 ---TCTCAGCCCCTGGGGTTCAGTGTCCCCTG (Δ40)

pf1alp48 72.2% (13/18)
 TGGACCCAGACGACTTTTGGAAAGACGATGGGACTTCTGGCCGGTTCAGTCCAAGACT Wild-type

TGGACCCAGACGACTTTTGGAAAGACGAT---CCTGGCCGGTTCAGTCCAAGACT (Δ8)
 TGGACCCAGACGACTTTTGGAAAGACGATGGG---CCGGTTCAGTCCAAGACT (Δ9)
 TGGACCCAGACGACTTTTGGAAAGACGATGG---TGGCCGGTTCAGTCCAAGACT (Δ9) [x4]
 TGGACCCAGACGACTTTTGGAAAGACGAT---TCTGGCCGGTTCAGTCCAAGACT (Δ11)
 TGGACCCAGACGACTTTTGGAAAGACGATGG---TcaagatgTGGTTCAGTCCAAGACT (Δ14, +10)
 TGGACCCAGACGACTTTTGGAAAGACGATGG---ATCCAGACT (Δ20)
 ---TGGCCGGTTCAGTCCAAGACT (Δ28)
 ---TGGCCGGTTCAGTCCAAGACT (Δ52) [x2]
 TGGACCCAGACGACTTTTGGAAAGACGATGG---TGGCCGGTTCAGTCCAAGACT (Δ55)
 TGGACCCAGACGACTTTTGGAAAGACGATGGGACTTCTTGGCCGGTTCAGTCCAAGACT [x5]

pat Target 1 10% (2/20)
 GTAGCATTGGTGGGATATTGGCCGTAAAGCAAAACATTGTGTTTATAGATAAGTTTCT Wild-type

GTAGCATTGGTGGGATATTGGCCGT---GTTTCT (Δ24)
 GTAGCATTGGTGGGATATTGGCCCT---AAGGTTTCT (Δ26)
 GTAGCATTGGTGGGATATTGGCCGTAAAGCAAAACATTGTGTTTATAGATAAGTTTCT [x18]

tm4sf4 Target 1 86.7% (12/19)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCATTACCCCTTATACC Wild-type

TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ2, +5)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ3)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ4) [x2]
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ4)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ4, +4)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ10, +10)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ11, +10)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ13)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ14, +2)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ16)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ27)
 TAACCTGAAAAATGTGTTCTGGTGGTGGCAGTGGTGGCAGTGGCAGTGGCATTACCCCTTATACC (Δ7)

tm4sf4 Target 2 100% (20/20)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGGGACTGCAAAATGCTGTG Wild-type

GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---CTGTGGGACTGCAAAATGCTGTG (Δ2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGTGGGACTGCAAAATGCTGTG (Δ2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGTGGGACTGCAAAATGCTGTG (Δ2, +1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGTGGGACTGCAAAATGCTGTG (Δ3)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGTGGGACTGCAAAATGCTGTG (Δ4)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGTGGGACTGCAAAATGCTGTG (Δ5, +6)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGTGGGACTGCAAAATGCTGTG (Δ6)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TcaTGGGACTGCAAAATGCTGTG (Δ6, +3)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---AGGGACTGCAAAATGCTGTG (Δ6, +3)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGGGACTGCAAAATGCTGTG (Δ6, +13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGGGACTGCAAAATGCTGTG (Δ7, +9)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGGGACTGCAAAATGCTGTG (Δ9, +6)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---GGGACTGCAAAATGCTGTG (Δ10)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---GGGACTGCAAAATGCTGTG (Δ12) [x2]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---GGACTGCAAAATGCTGTG (Δ13) [x2]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---TGCAAATGCTGTG (Δ12)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---CTGCAAAATGCTGTG (Δ14)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACA---AATGCTGTG (Δ24)

tyrosinase 82.4% (14/17)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCCAGTGGGTTGACGATAGAGAGA Wild-type

TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---TTCAGTGGGTTGACGATAGAGAGA (+3)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---GGGTTGACGATAGAGAGA (Δ24) [x3]
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---CTGGGTTGACGATAGAGAGA (Δ4) [x4]
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---GGGTTGACGATAGAGAGA (Δ5)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---GGGTTGACGATAGAGAGA (Δ5) [x2]
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---TGGGTTGACGATAGAGAGA (Δ6)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---TGGGTTGACGATAGAGAGA (Δ6)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---TggggTctcagtttCTGGGTTGACG (Δ6, +15)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---GACGATAGAGAGA (Δ17)
 TCCTGACCAGCTCTGCTACTGGCCCTCAGTTTCCATCC---TGGGTTGACGATAGAGAGA [x3]

Fig. S1. DNA sequencing data show the targeted mutations induced by gRNA/Cas9 targeting the loci illustrated in Fig. 2A. For all the panels, the wild-type sequence is shown at the top with the target site highlighted in yellow and the PAM sequence in blue text. Mutated regions are shaded in gray with red dashes indicating deletions and lowercase letters in red indicating insertions. The numbers in parentheses show the number of deleted (Δ) or inserted (+) base pairs, whereas numbers in square brackets show the frequencies of the mutation in all the sequenced samples.

etsI Target 2 76.9% (10/13)

AACATGCACAGTGTGTTCCG**CCCTCTGAATTCTCTGAACC**CAGCTTCATCACAGAGTCAT Wild-type
AACATGCACAGTGTGTTCCGCCT--GAATTCTCTGAACCAGCTTCATCACAGAGTCAT (Δ2) [x2]
AACATGCACAGTGTGTTCCGC--TGAATTCTCTGAACCAGCTTCATCACAGAGTCAT (Δ4)
AACATGCACAGTGTGTTCC--TGAATTCTCTGAACCAGCTTCATCACAGAGTCAT (Δ6)
AACATGCACAGTGTGTTCC--TGAATTCTCTGAACCAGCTTCATCACAGAGTCAT (Δ7) [x2]
AACATGCACAGTGTGTTCCGCCT--GTGAACCAGCTTCATCACAGAGTCAT (Δ9)
AACATGCACAGTGTGTTCCGCC--GAACCAGCTTCATCACAGAGTCAT (Δ12)
AACATGCACAGTGAATTC--TCTGAACCAGCTTCATCACAGAGTCAT (Δ14)
AACATGCACAGTGTG**gaacagtgtgtt**CTGAATTCTCTGAACCAGCTTCATCACAGAGTC (Δ9,+11)
AACATGCACAGTGTGTTCCGCCCTTGAATTCTCTGAACCAGCTTCATCACAGAGTCAT [x3]

pat Target 2 57.9% (11/19)

CACAGACTCTGAATCCAAAGGCACATTCTGATGCAG**AGTGG**CAGCTCTTGGAGAACA Wild-type
CACAGACTCTGAATCCAAAGGCACATTCTGATGCAG**A**-TGGCAGCTCTTGGAGAACA (Δ2,+1)
CACAGACTCTGAATCCAAAGGCACATTCTGATGCAG--TGGCAGCTCTTGGAGAACA (Δ3) [x2]
CACAGACTCTGAATCCAAAGGCACATTCTGATGC--AGTGGCAGCTCTTGGAGAACA (Δ3)
CACAGACTCTGAATCCAAAGGCACATTCTGAT--GCAGTGGCAGCTCTTGGAGAACA (Δ3) [x3]
CACAGACTCTGAATCCAAAGGCACATTCT--AGTGGCAGCTCTTGGAGAACA (Δ9)
CACAGACTCTGAATCCAAAGGCACATTCTGATGCAGC--TCTTGGAGAACA (Δ9)
CACAGACTCTGAATCCAAAGGCACATTCT--AGTGGCAGCTCTTGGAGAACA (Δ9)
CACAGACTCTGAATCCAAAGGCACATTCT--TGGCAGCTCTTGGAGAACA (Δ11)
CACAGACTCTGAATCCAAAGGCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGAACA [x8]

pdxI Target 2 75% (15/20)

CACCACCATCATCACCAC**CCATCACCAGGGATCCCTCACCC**CCACAGCAAAATGCCATT Wild-type
CACCACCATCATCACCACCAT--CCAGGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ2)
CACCACCATCATCACCACCAT**gag**CCAGGGATCCCTCACCCCCACAGCAAAATGCCA (+3)
CACCACCATCATCACCACCA--CCAGGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ4)
CACCACCATCATCACCACCATCA--GGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ4) [x2]
CACCACCATCATCACCACCC--AGGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ6)
CACCACCATCATCACCACCC--CAGGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ6)
CACCACCATCATCACCACCAT**gatggg**GGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ5,+5)
CACCACCATCATCACCACCAT**gatggg**GGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ6,+6)
CACCACCATCATCACCACCAT**gagctcagat**ACCCAGGGATCCCTCACCCCCACAGCAAAATGCCA (Δ7,+10)
CACCACCATCATCACCACCAT--**gggat**CAGGGATCCCTCACCCCCACAGCAAAATGCCATT (Δ14,+5)
CACCACCATCACCACCA--GCAAAATGCCATT (Δ27) [x2]
gtgagggATCCCTCACCC--CACCAGCAAAATGCCATT (Δ30,+7)
CACCACCATCATCACCAC-- (Δ50)
CACCACCATCATCACCACCA**CCAGGGATCCCTCACCCCCACAGCAAAATGCCATT** [x5]

etsI Target 3 66.7% (10/15)

AATATGAGAATGACTACC**CTTTAGCATTGCTGCGTGACCC**CCTGCAGCCTGAATCTCAGG Wild-type
AATATGAGAATGACTACC**CCCTTTAGCATTGCTGCGTGACCC**CCTGCAGCCTGAATCTCAGG (Δ6)
AATATGAGAATGACTAACCT--TTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG (Δ6) [x2]
AATATGAGAATGACTACC--CATTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG (Δ6) [x2]
AATATGAGAATGACTACT--ATTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG (Δ7)
AATATGAGAATGACTAC--CATTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG (Δ7)
AATATGAGAATGA--CATTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG (Δ11)
--CATTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG (Δ47)
AATATGAGAATGACT**gaccggtac**CA**g**TGCTGCG**g**GACCC**ccg**CA**g**CC**g**ATTCTCA**g** (Δ11,+11)
AATATGAGAATGACTACCCTTTAGCATTGCTGCGTGACCCCTGCAGCCTGAATCTCAGG [x5]

pat Target 3 78.9% (15/19)

GCACATTCTGATGCAGCAGT**GGCAGCTCTTGGAGAACA**GGAGTACATTACCATTCT Wild-type
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGA**A**--GTGGAGTACATTACCATTCT (Δ2)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGA**g**EACAGTGGAGTACATTACCATTCT (Δ2,+2)
GCACATTATGATGCAGCAGTGGCAGCTCTTGGAGA**g**C--TGGAGTACATTACCATTCT (Δ3,+1)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGA**g**ACAE**T**GGAGTACATTACCATTCT (Δ2,+1)
GCACATTCTGATGCAGCAGTGGCAGCTCTT**g**--TACAGTGGAGTACATTACCATTCT (Δ5,+1)
GCACATTCTGATGCAGCAGTGGCAGCTCT--**g**EACAGTGGAGTACATTACCATTCT (Δ6,+1)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGA**g**gt**ttgtt**CA**g**TGGAGTACATTCT (Δ7)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGA**g**--GTGGAGT**gggag**EACATTCA (Δ3,+6)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAGA--GTACATTACCATTCT (Δ9) [x2]
GCACATTCTGATGCAGCAGTGGCAGCTCTTGG--GAGTACATTACCATTCT (Δ11)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAA**g**--TACATTACCATTCT (Δ11) [x2]
GCACATTGTGATGCAGCAGTGGCAGCTCTCG**g**TA**g**TA**oattcogt**ACAGTGGAGTAC (+11)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAA**g**--**gtggagtgagg**AGTGGAGTAC (Δ2,+11)
GCACATTCTGATGCAGCAGTGGCAGCTCTTGGAA**g**AGAGTGGAGTACATTACCATTCT [x4]

pdxI Target 3 45% (9/20)

ACCCAGGGATCCCTCAC**CCCTCACCAGCAAAATGCCATTCC**AGATGACACTGAATCTGCAA Wild-type
ACCCAGGGATCCCTCACCC**CCCTCACCAGCAAAATGCCATTCC**AGATGACACTGAATCTGCAA (Δ4)
ACCCAGGGATCCCTCACCC**CCCTCACCAGCAAAATGCCATTCC**AGCAAAATGCCATTCCAGATGACACTGAATCTG (+3)
ACCCAGGGATCCCTCACCC--AGCAAAATGCCATTCCAGATGACACTGAATCTGCAA (Δ4)
ACCCAGGGATCCCTCA--AATGCCATTCCAGATGACACTGAATCTGCAA (Δ12)
ACCCAGGGATCCCTCAC**tttgc**CA**atg**GCAAAATGCCATTCCAGATGACACTGAATCTG (+5,+7)
ACCCAGGGATCCCTCACCC**CCCTCACCAGGGG**TGCCATTCCAGATGACACTGAATCTG (+7,+9)
AE-CA--**AT**ACCTCACCC**CCCTCACCAGCAAAATGCCATTCC**AGATGACACTGAATCTGCAA (Δ9,+5)
ACCCAGG**GA**--AATGCCATTCCAGATGACACTGAATCTGCAA (Δ19)
ACCCAG--ATGACACTGAATCTGCAA (Δ36)
ACCCAGGGATCCCTCACCC**CCCTCACCAGCAAAATGCCATTCC**AGATGACACTGAATCTGCAA [x11]

Fig. S2. DNA sequencing data show the targeted mutations induced by gRNA/Cas9 targeting the genes shown in Fig. 2H. For all the panels, the wild-type sequence is shown at the top with the target site highlighted in yellow and the PAM sequence in blue text. Mutated regions are shaded in gray with red dashes indicating deletions and lowercase letters in red indicating insertions. The numbers in parentheses show the number of deleted (Δ) or inserted (+) base pairs, whereas numbers in square brackets show the frequencies of the mutation in all the sequenced samples.

ets2 m1 0% (0/19)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x19]

ets2 m3 0% (0/20)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x20]

ets2 m5 0% (0/20)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x20]

ets2 m7 0% (0/20)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x20]

ets2 m9 0% (0/19)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x19]

ets2 m11 0% (0/18)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x18]

ets2 m12 10% (2/20)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ2)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ4) [x18]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC

ets2 m13 53% (10/19)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ2) [x3]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ4, +2)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8) [x2]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ9)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ5, +7)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ18)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ27)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x10]

ets2 m15 18% (3/17)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ5, +12)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ2)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ31) [x14]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC

ets2 m17 18% (3/17)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8) [x2]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8) [x14]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC

ets2 m18 80% (16/20)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ3, +18) [x2]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ2) [x5]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ5)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ24)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ22)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ2) [x2]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ4, +3)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8) [x4]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC

ets2 m19 68% (13/19)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ2) [x5]
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ8)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ9)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ9, +1)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ1, +9)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ10)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ17)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ19)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x6]

ets2 m20 6% (1/16)
AAGAACAGGCGGTACCAACGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC Wild-type
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC (Δ7, +1)
AAGAACAGGCGGTACCAACGGGTCTGGACTCTTACTCTCATGGTATGTGTCTTGTCTCCC [x15]

Fig. S3. DNA sequencing data show the the consequence of single-nucleotide mismatches between the spacer and the protospacer sequences on the Cas9 mediated gene targeting efficiencies as illustrated in Fig. 3A for *ets2* loci. For all the panels with mutations, the wild-type sequence is shown at the top with the target site highlighted in yellow and the PAM sequence in blue text. Mutated regions are shaded in gray with red dashes indicating deletions and lowercase letters in red indicating insertions. For all the panels without mutations, only the wild-type sequence is shown. The numbers in parentheses show the number of deleted (Δ) or inserted (+) base pairs, whereas numbers in square brackets show the frequencies of the mutation in all the sequenced samples.

tm4sf4-T2 m1 0% (0/19)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x19]

tm4sf4-T2 m3 0% (0/16)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x16]

tm4sf4-T2 m5 0% (0/20)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x20]

tm4sf4-T2 m7 0% (0/20)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x20]

tm4sf4-T2 m9 0% (0/18)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x18]

tm4sf4-T2 m11 0% (0/20)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x20]

tm4sf4-T2 m12 95% (19/20)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4) [x5]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ1,+4)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (+7)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ11) [x3]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ11)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ8,+5)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ14)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ25)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ43)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG

tm4sf4-T2 m13 35% (7/20)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2,+2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5,+2)
 CCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ3)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2,+6)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ12)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x13]

tm4sf4-T2 m15 63% (12/19)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2) [x2]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ7)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ12)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ14)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ16)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ17)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ57)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ23)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ25)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x7]

tm4sf4-T2 m17 42% (8/19)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13) [x2]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ6,+6)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ3,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ1,+9)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2,+2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ24)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x11]

tm4sf4-T2 m18 71% (12/17)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ3,+2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ3,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4,+2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5,+10) [x3]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ11)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ14,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x5]

tm4sf4-T2 m19 61% (11/18)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ4,+5)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5,+10) [x6]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ7)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ8,+2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x7]

tm4sf4-T2 m20 63% (12/19)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG Wild-type
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ23) [x3]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ5,+10) [x2]
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ2,+1)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ13)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ1,+3)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ8,+4)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG (Δ10,+8)
 GCGCCATTCAAGCTATCAACGGACTCATAGGAACAATCTGTGGGGACTGCAAAATGCTGTG [x7]

Fig. S4. DNA sequencing data show the consequence of single-nucleotide mismatches between the spacer and the protospacer sequences on the Cas9 mediated gene targeting efficiencies as illustrated in Fig. 3B for *tm4sf4-T2* loci. For all the panels with mutations, the wild-type sequence is shown at the top with the target site highlighted in yellow and the PAM sequence in blue text. Mutated regions are shaded in gray with red dashes indicating deletions and lowercase letters in red indicating insertions. For all the panels without mutations, only the wild-type sequence is shown. The numbers in parentheses show the number of deleted (Δ) or inserted (+) base pairs, whereas numbers in square brackets show the frequencies of the mutation in all the sequenced samples.

***elastase* T1 + *grp78* gRNA**

***elastase* Target 1 100% (16/16)**

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CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACCATACCTGTTGGGGGCTCCCTGATCCCGTGT Wild-type
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACCATAC-TGTGGGGGCTCCCTGATCCCGTG (Δ1)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACCATAC-TagGGGGGCTCCCTGATCCCGTG (Δ3,+2)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACC-CC-GTGGGGGCTCCCTGATCCCGTG (Δ4)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACCAGacagggagtTGGGGGCTCCCTGATC (Δ4,+10)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACC-----TGTGGGGGCTCCCTGATCCCGTG (Δ5)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACCatac-TGTGGGGGCTCCCTGATCCCGTG (Δ5,+4)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGTACCAGacaggtactggagggagatcTGGGG (Δ6,+21)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGaACCATct-gtTGGGGGCTCCCTGATCCCGTG (Δ6,+5)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGggggg---CTGTGGGGGCTCCCTGATCCCGTG (Δ8,+5)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGGT-----GTGGGGGCTCCCTGATCCCGTG (Δ9) [x2]
CCCTCCAGTACCTGCTCTGGTGGCAGT-----TGGGGGCTCCCTGATCCCGTG (Δ14) [x2]
CCCTCCAGTACCTGCTCTGGTGGCAGTT-----GGGGCTCCCTGATCCCGTG (Δ15)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGG-----GCTCCCTGATCCCGTG (Δ16)
CCCTCCAGTACCTGCTCTGGTGGCAGTTGG-----GGCTCCgtgatggctc (Δ26,+11)
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***grp78* 84% (16/19)**

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GACCATGAAGCTGTTTGCCTTGGTGTGGTGTCTGCTGATGACCGTATTTGCTGCTGATGA Wild-type
GACCATGAAGCTGTTTGCCTTGGTGT--CTGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ2)
GACCATGAAGCTGTTTGCCTTGGcc-TTGTGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ3,+2)
GACCATGAAGCTGTTTGCCTT-----GCTGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ6)
GACCATGAAGCTGTTTGCCTTGGc-----TGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ7,+1)
GACCATGAAGCTGTTTGCCTTGGc-----TGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ7,+1)
GACCATGAAGCTGTTTGCCTTGGTGT-----TGCCAGCGTATTTGCTGCTGATGA (Δ9)
GACCATGAAGCTGTTTGCCTTGGTGT-----TGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ10)
GACCATGAAGcCTGTTgGCCTTGGc-----GTCTGCCAGCGTcTTTgTGTgatga (Δ14,+11)
GACCATGcAGcGTTgGCC-----TGgTG-T--CTGCCAGCGcATTGCTGCTGATGA (Δ15,+5)
GACCATGAAG-----GTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ21)
----CA-----TGGTGGTGTCTGCCAGCGTATTTGCTGCTGATGA (Δ25,+1)
GACCATGAAGCTGTTTGCCTTGGTGGTgTTGCTGGTGTCTGCCAGCGTATTTGCTGCTGATG (+1)
GACCATGAAGCTGTTTGCCTTGGTgTTGCTGGTGTCTGCCAGCGTATTTGCTGCTGATG (+1) [x2]
GACCATGAAGCTGTTTGCCTTGGTgaccatgaagctgTTGCTGGTGTCTGCCAGCGTATT (+12)
GACCATGAAGCTGTTTGCCTcttctcactgctaaagcttTTGCTGGTGTCTGCCAGCGTAT (+19)
GACCATGAAGCTGTTTGCCTTGGTGTGCTGGTGTCTGCCAGCGTATTTGCTGCTGATGA [x3]
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Fig. S5. DNA sequencing data show the targeted mutations in *elastase* (the upper panel) and *grp78* (the lower panel) induced by co-injection of Cas9 mRNA together with two gRNAs targeting *elastase*-T1 and *grp78*. For both panels, the wild-type sequence is shown at the top with the target site highlighted in yellow and the PAM sequence in blue text. Mutated regions are shaded in gray with red dashes indicating deletions and lowercase letters in red indicating insertions. The numbers in parentheses show the number of deleted (Δ) or inserted (+) base pairs, whereas numbers in square brackets show the frequencies of the mutation in all the sequenced samples.

