

Supplementary information, Table S4. sgRNA sequences and primers

sgRNA sequences

Locus	Sequence (5'-3')	Figure
<i>GFP-A</i>	AAGGGCGAGGAGCTGTTACCCGG	1
<i>GFP-B</i>	CTGAAGTTCATCTGCACCACCGG	1
<i>GFP-C</i>	GGAGCGCACCATCTTCTCAAGG	1
<i>GFP-D</i>	GGTGAACCGCATCGAGCTGAAGG	1
<i>Tyr-A</i>	GGGAAGGGTTACTCAGAGTCAGG	2
<i>Tyr-B</i>	GCGAAGGCACCGCCCTCTTTTGG	2
<i>Tyr-C</i>	CCAGAAGCCAATGCACCTATCGG	2
<i>Tyr-D</i>	CTTCATAACATCCAAGGATCTGG	2
<i>Tyr-E</i>	TACAGCTACCTCCAAGAGTCAGG	2
<i>Tyr-F</i>	CAATGTGGGTAACCTCTTTGGGG	2
<i>Tet1-A</i>	GGCATGCTGGACTTCATTCTCGG	3
<i>Tet1-B</i>	GATGTCCATGCCGGTTACACAGG	3
<i>Tet1-C</i>	GAAGCCAGAGGCCACCTCACAGG	3
<i>Tet2-A</i>	CAGGGAGCAAGAGATTCCGAAGG	3
<i>Tet2-B</i>	TCAGTCCTCCACTCTCAGACCGG	3
<i>Tet2-C</i>	GTGAACCAAGGACCGTCTCCAGG	3
<i>Tet3-A</i>	CCCTACTTCCACAGAGCTCAGG	3
<i>Tet3-B</i>	GCCTGTTAGGCAGATTGTTCTGG	3
<i>Tet3-C</i>	ACAAGCTGGAGGAGCTCATCCGG	3
<i>Zfy1-A</i>	GGATAGTGACCAGATTGTTGTGG	4
<i>Zfy1-B</i>	CCTGAGCAAGTTCTCAATTTAGG	4
<i>Ubely1-A</i>	GAAACCCCTACTTGCCTCGCTGG	4
<i>Ubely1-B</i>	CTCAGCACAGCAGGCCTCCTCGG	4
<i>Kdm5d-A</i>	GATGGTACCTACAGAAGTTGTGG	4
<i>Kdm5d-B</i>	GGACTTATCTCCTGAAGAAAAGG	4
<i>Eif2s3y-A</i>	ACAATTGGTCATGTTGCTCATGG	4
<i>Eif2s3y-B</i>	AGGAATATTACCATAAACTTGG	4
<i>Ddx3y-A</i>	GTCTGTGATAAGGACAGTTCAGG	4
<i>Ddx3y-B</i>	GTGATCGTGGAAGTGGATCCAGG	4
<i>Usp9y-A</i>	ACTCTGGCTCTGTGTTCCAGG	4
<i>Usp9y-B</i>	AGGATTAGACTACCTTTGGAAGG	4
<i>Sry-A</i>	GCATTTATGGTGTGGTCCCGTGG	4
<i>Sry-B</i>	CGAAAAAAGGCCCTTTTTCCAGG	4
<i>Erdr1-A</i>	ACGGACGACTCCACAAGGTAGG	4
<i>Erdr1-B</i>	TGGCACATACATCTTGACCGCGG	4
<i>Prri2-A</i>	GATGGCAGCCAGCAGCTCTGAGG	5
<i>Prri2-B</i>	GGCCAAGCTCTTAAGCATCGTGG	5
<i>Prri2-C</i>	GCCTCCTGCGTCATCAACTTAGG	5
<i>Prri2-D</i>	CAGGCAGGGGAGGAATGGAACCGG	5

<i>ArntL-A</i>	GCTGGCCACCCACAAAGATGGGG	5
<i>ArntL-B</i>	GCAGTCGTCCAATTGCAACGAGG	5
<i>ArntL-C</i>	GTTTCTCGGCACGCGATAGATGG	5

Primer sequences used to generate the templates for sgRNA *in vitro* transcriptions.

Locus	Sequence (5'-3')	Figure
<i>GFP-A</i>	TAATACGACTCACTATAGGGAAGGGCGAGGAGCTGTTTCACGTTTATAGAGCTAGAAATAG	1
<i>GFP-B</i>	TAATACGACTCACTATAGGGCTGAAGTTCATCTGCACCACGTTTATAGAGCTAGAAATAG	1
<i>GFP-C</i>	TAATACGACTCACTATAGGGGGAGCGCACCATCTTCTTCAGTTTATAGAGCTAGAAATAG	1
<i>GFP-D</i>	TAATACGACTCACTATAGGGGGTGAACCGCATCGAGCTGAGTTTATAGAGCTAGAAATAG	1
<i>Tyr-A</i>	TAATACGACTCACTATAGGGGGGAAGGGTTACTCAGAGTCGTTTATAGAGCTAGAAATAG	2
<i>Tyr-B</i>	TAATACGACTCACTATAGGGGGCAAGGCACCGCCCTCTTTGTTTTAGAGCTAGAAATAG	2
<i>Tyr-C</i>	TAATACGACTCACTATAGGGCCAGAAGCCAATGCACCTATGTTTTAGAGCTAGAAATAG	2
<i>Tyr-D</i>	TAATACGACTCACTATAGGGCTTCATAACATCCAAGGATCGTTTATAGAGCTAGAAATAG	2
<i>Tyr-E</i>	TAATACGACTCACTATAGGGTACAGCTACCTCCAAGAGTCGTTTATAGAGCTAGAAATAG	2
<i>Tyr-F</i>	TAATACGACTCACTATAGGGCAATGTGGGTAACCTCTTTGGTTTTAGAGCTAGAAATAG	2
<i>Tet1-A</i>	TAATACGACTCACTATAGGGGGCATGCTGGACTTCATCTGTTTATAGAGCTAGAAATAG	3
<i>Tet1-B</i>	TAATACGACTCACTATAGGGGATGTCCATGCCGTTTACACGTTTATAGAGCTAGAAATAG	3
<i>Tet1-C</i>	TAATACGACTCACTATAGGGGAAGCCAGAGGCCACCTCACGTTTATAGAGCTAGAAATAG	3
<i>Tet2-A</i>	TAATACGACTCACTATAGGGCAGGGAGCAAGAGATCCGAGTTTATAGAGCTAGAAATAG	3
<i>Tet2-B</i>	TAATACGACTCACTATAGGGTCAGTCCTCCACTCTCAGACGTTTATAGAGCTAGAAATAG	3
<i>Tet2-C</i>	TAATACGACTCACTATAGGGGTGAACCAAGGACCGTCTCCGTTTATAGAGCTAGAAATAG	3
<i>Tet3-A</i>	TAATACGACTCACTATAGGGCCCTACTTCCACAGAGCCTCGTTTATAGAGCTAGAAATAG	3
<i>Tet3-B</i>	TAATACGACTCACTATAGGGGCCTGTAGGCAGATTGTTCGTTTATAGAGCTAGAAATAG	3
<i>Tet3-C</i>	TAATACGACTCACTATAGGGACAAGCTGGAGGAGCTCATCGTTTATAGAGCTAGAAATAG	3
<i>Zfy1-A</i>	TAATACGACTCACTATAGGGGGATAGTGACCAGATTGTTGGTTTTAGAGCTAGAAATAG	4
<i>Zfy1-B</i>	TAATACGACTCACTATAGGGCCTGAGCAAGTTCTCAATTTGTTTTAGAGCTAGAAATAG	4
<i>Ube1y1-A</i>	TAATACGACTCACTATAGGGGAAACCCCTACTTGCCTCGCGTTTTAGAGCTAGAAATAG	4
<i>Ube1y1-B</i>	TAATACGACTCACTATAGGGCTCAGCACAGCAGGCCTCCTGTTTTAGAGCTAGAAATAG	4
<i>Kdm5d-A</i>	TAATACGACTCACTATAGGGGATGGTACCTACAGAAGTTGGTTTTAGAGCTAGAAATAG	4
<i>Kdm5d-B</i>	TAATACGACTCACTATAGGGGGACTTATCTCCTGAAGAAAGTTTTAGAGCTAGAAATAG	4
<i>Eif2s3y-A</i>	TAATACGACTCACTATAGGGACAATTGGTCATGTTGCTCAGTTTTAGAGCTAGAAATAG	4
<i>Eif2s3y-B</i>	TAATACGACTCACTATAGGGAGGAATATTACCATAAACTGTTTTAGAGCTAGAAATAG	4
<i>Ddx3y-A</i>	TAATACGACTCACTATAGGGGTCTGTGATAAGGACAGTTCGTTTTAGAGCTAGAAATAG	4
<i>Ddx3y-B</i>	TAATACGACTCACTATAGGGGTGATCGTGGAAGTGGATCCGTTTTAGAGCTAGAAATAG	4
<i>Usp9y-A</i>	TAATACGACTCACTATAGGGACTCTGGCTCTGTGTTCCCGTTTTAGAGCTAGAAATAG	4
<i>Usp9y-B</i>	TAATACGACTCACTATAGGGAGGATTAGACTACCTTTGGAGTTTTAGAGCTAGAAATAG	4
<i>Sry-A</i>	TAATACGACTCACTATAGGGGCATTTATGGTGTGGTCCCGTTTTAGAGCTAGAAATAG	4
<i>Sry-B</i>	TAATACGACTCACTATAGGGCGAAAAAAGGCCCTTTTTCCGTTTTAGAGCTAGAAATAG	4
<i>Erd1-A</i>	TAATACGACTCACTATAGGGACGGACGGACTCCACAAGGTGTTTTAGAGCTAGAAATAG	4
<i>Erd1-B</i>	TAATACGACTCACTATAGGGTGGCACATACATCTTGACCGTTTTAGAGCTAGAAATAG	4
<i>Prrt2-A</i>	GAAATTAATACGACTCACTATAGGGCTGCAGCAGCTGGTTGAGGGTTTTAGAGCTAGAAATAGC	5

<i>Prrt2-B</i>	GAAATTAATACGACTCACTATAGGGGCCAAGCTCTTAAGCATCGGTTTTAGAGCTAGAAATAGC	5
<i>Prrt2-C</i>	GAAATTAATACGACTCACTATAGGGCCTCCTGCGTCATCAACTTGTTTTAGAGCTAGAAATAGC	5
<i>Prrt2-D</i>	GAAATTAATACGACTCACTATAGGCAGGCAGGGGAGGAATGGAAGTTTTAGAGCTAGAAATAGC	5
<i>ArntL-A</i>	TAATACGACTCACTATAGGGGCTGGCCACCCACAAAGATGGTTTTAGAGCTAGAAATAG	5
<i>ArntL-B</i>	TAATACGACTCACTATAGGGGCAGTCGTCGAATTGCAACGGTTTTAGAGCTAGAAATAG	5
<i>ArntL-C</i>	TAATACGACTCACTATAGGGGTTTTCTCGGCACGCGATAGAGTTTTAGAGCTAGAAATAG	5
sgRNA-R	AAAAGCACCGACTCGGTGCC	
<i>Cas9</i> mRNA	F TAATACGACTCACTATAGGGAGATTCAGGTTGGACCGGTG R GACGTCAGCGTTCGAATTGC	

Primers used for genotyping.

Locus	Sequence (5'-3')	Product (bp)	Figure
<i>Tyr</i>	Outer F: GTTATCCTCACACTACTTCTG	807	2
	Outer R: GTAATCCTACCAAGAGTCTCA		
	Inner F: TCCTCACACTACTTCTGATG	788	2
	Inner R: GTCTCAAGATGGAAGATCAC		
<i>Eif2s3y</i>	Outer F: ATCACAGGAATCTGGAAGC	834	4
	Outer R: CTACTAAGCAACTGTCCATC		
	Inner F: CAGGCTCACAGAGGTCTA	514	4
	Inner R: ACTCATCAGGTGTACTACTTC		
<i>Zfy1</i>	Outer F: CACAATAAGAAATATATGC	799	4
	Outer R: CAGATAATCCTCACAGTTGATTTGT		
	Inner F: GTTACATAACCTAAAAGTAGAC	575	4
	Inner R: TATAGGATCAGTGATGACTTCTG		
<i>Ubel1y1</i>	Outer F: GCAAAGGCACAGATTAGAC	769	4
	Outer R: GGTTGTCAGTAGCAGGAG		
	Inner F: TGGAGGCATTGTCAGTCA	625	4
	Inner R: ATGGCATCAGAAGCACAC		
<i>Kdm5d</i>	Outer F: GCAGGCTACACAGGAGTA	491	4
	Outer R: AGGGACAGTAACAGGCATA		
	Inner F: TTGGTGAGATGGCTGACT	457	4
	Inner R: GGACAGTAACAGGCATATGA		
<i>Ddx3y</i>	Outer F: CACTATACTACCAAGCCACAT	684	4
	Outer R: CCAAGACATCCAATCTCATAAG		
	Inner F: TTCTCACAATGTAGCTCAGT	512	4
	Inner R: AGGTAAGTAGGTAGACAGATTC		
<i>Usp9y</i>	Outer F: AGATGTAAGTTCAGCTAGGT	875	4
	Outer R: CACACTTTCTACTAACAGTCAC		
	Inner F: GTTGGGTATGTCTTTAGGTT	702	4

	Inner R: CATGAAGGACAACAGGTAA		
<i>Sry</i>	Outer F: GCACATTTTGGTCAGTGGCT	632	4
	Outer R: GCTCTACTCCAGTCTTGCCT		
	Inner F: GTTCAGCCCTACAGCCACATG	355	4
	Inner R: GCAGGCTGTAAAATGCCACTC		
<i>Prrt2- Exon3</i>	Outer F: GGAGGGATATGGAAACACAT	937	5
	Outer R: GATGCCAAGACAATGGAAGT		
	Inner F: TTTCCACCTGATCCCTTCTG	776	5
	Inner R: GAGGTCCAGAGAATTTGGGA		
<i>ArntL- Exon 13</i>	Outer F: CCAGTACCTCCAATTTCACTTC	1117	5
	Outer R: CAGAGACTCACATCACTTGTC		
	Inner F: ATACATTCTAGTCCCTCAGC	950	5
	Inner R: GGTACAATCACAGCTTACTG		

Primers used for off-target analysis.

sgRNA	Locus	Sequence	Product (bp)	
sgRNA-Tyr-B	chr17:57065718	F: CCATTACCTCATAGGACCT R: GTTGCTGATCTGAAGAAGTG	617	
	chr6:138140489	F: GGTATTCACAGCAATAGAGC R: GCAGTGACTTCTCATCTTGT	777	
	chr4:138750107	F: TTCTCTATCTGAGGCATCTG R: GACTCTACTGTTGTAGGACC	701	
	chr2:32395585	F: AACTCCTGCCCTACTTAGAAG R: CAATGATGACCTGTAACCTCGTG	748	
	chr9:23096850	F: AAGATGCCTCCTGTTCTGAAG R: CAATCCATCATGCCTCTGAAG	671	
	chr17:24453551	F: CTGTCTGGAACCTCACTCTAT R: GACATTGGTTCTCAGACTTCC	764	
	chr9:96832929	F: GCTAATGCTAAGCCCTAACTG R: CATTCTCTGTGTCTGACTCTG	633	
	chr15:79169441	F: GTATATGTGTATGCCTGCGTC R: ACAGAGTGAGTTCTAGGACAG	1075	
	chr4:86233378	F: AGTGCGATACCTTACTGTCTC R: CATGTAGCAGCCTAGTTCTTC	659	
	chr6:115975395	F: GATACTTCTGGTCTTCAGCAG R: CATCCACAGCAGATACTAAGG	625	
	sgRNA-Tyr-C	chr8:73203311	F: GCCTGTAATGATTGGACTGT R: GGACCTAACTATTGTGGGAAG	760
		chr10:55107975	F: CATAGACTGTAGGACCAAGAC R: ACCCTTACCACCTGTTTATG	625
		chr10:116978236	F: AGGAGCTGCATCATCTTATG	680

	R: ACAATCTGGAAGGCGAATAG	
chr10:124330321	F:CAACCTCAAGAGTCAGACAAG R:CCAGCAGTGAATTTCCATCAG	731
chr17:8532910	F:CTGTGTATTTGTGGGACCTATG R:GATTAGTCCATCAGAAGCAGAG	748
chr16:57712501	F:GTCCTCTACTATTCTGTGTCTG R:GATTGGTCTCTGTATAGTTGGC	639
chr17:44031368	F:CCAATGACATACTCAGTGCTC R:AGAGATGAGAGTTGGAGACAG	743
chr3:6828323	F:CTCTACTTCCACTGAGGGTAC R:GGACAATTCTCTTGGTTCTCC	703
chr11:28415232	F:TAACCTACCTTACCACCATCC R:GCCTATTGACCAGAGTTAGTG	718
chr1:33799882	F:TAGTGTCACCTAGTAGAGTCC R:GAGTAGCCAGTATGCTTAACC	857
chr15:74536351	F: AGACAGAGGAAGCATTGAG R: CTCCAGGTCATACTGAG	516
chr18:43788370	F: CCTGGATATGGCAGTCTCTA R: CTGATTCTCTGAGAGGTGGTA	957
chr15:61581619	F: GTGAAGGACTCAGGTTACTA R: ATGATACAGTGGTAGCAGAC	799
chr13:39330053	F:CATGGCTCAGGTACATGCTATGA R:GGGCTCCGATGGTGCCTGTC	998
chr14:80206603	F:GTGGGCACAGTTGCTGATGAT R:GCAGTTCCAACCAATGCTCAC	752
chr5:36335565	F:ATGAATCAGACCTTGCCCGTCAC R:CATGCTGGGTGAAGGCAAGATC	653
chr8:85681387	F:TGGACCATTAGACAGTGGGAGA R:AGGCAGAAGAGGGTGAGTGGT	941
chr3:98944708	F:TACCTTGGGTCTTATGGTTGTT R:TTGGCAGTAGTTGATGAGGAGAAGT	766
chr6:42584574	F:TCTGTAAGGACACGAGTTAGGGATC R:TTTGCTCCTATGACCTGGGTTCC	893
chr10:18410911	F:GTACTACTTGAATTAGCAGCCACTGC R:AAGTAGCCATGCAAAGCCAAGC	694
chr8:108702504	F: GCTTTGATGTACTGGGAATC R: GCTTAGAATGGAGGTCACCT	757
chrX:130677312	F: GACAGAAGGATGAGGAATGA R: CCAACCACTATTGAACTCAC	700
chr3:130295069	F: CTTGTCATAGCAGTGTCTG R: GCTCCTTGAGAGTCCATTAG	643
chr7:123982528	F:TAGCCCGCAGTGCCTCCACAG R:CTAGTCGCGGCGGCACCTGCTC	766

sgRNA-Tyr-D

sgRNA-Tyr-E

	chr3:133082201	F:CCTGCATCCAGCTAAATGGCACA R:AGGCTGCCATACCTCCAACAA	1045
	chrX:147311924	F:CAGCCAAGGTGGGCACAAGGT R:CAGGCATCCTCACAGGAAATTATGG	830
	chr1:168151597	F:TGAGCATGTCTGACTTCCCAGTT R:AAGGTTACATTATGCCTCAGTTGT	745
	chr5:50071052	F:CAGGGACAGCATCCAGGTTTAT R:GTGCCATCAATCACTAAGGCAAATC	782
	chr2:7777791	F:AAGCTGAAAGTGACTGGGAGA R:CTGGGTGTATTTGTGCCTGTGT	973
	chr10:84425609	F:CATTTGTCAAACAGGAGCCACG R:TGGTAATGAGGTAGGGACAGGC	807
sgRNA- <i>Prrt2</i> -B	chr16:19639092	F:GGTAGTATTGCCAGATCCAA R:CGGGTTCAAGTGATTCTTCTG	631
	chr8:8862302	F:GTTGAGGATCACTGTTCTACC R:CTTGTCTGTTTGTAGGTTGG	763
	chr7:142439417	F:ACAGACCTACAGAAGTGACTC R:GCACGGAATCTATACAGAGAC	677
	chr16:18400530	F:ACAGACCTACAGAAGTGACTC R:CAGAGACAGGCTAAGATATTGC	663
	chr3:1322473581	F:GAGTGTCTTGATGGGCTAGA R:CTCTGTGATTCTGGTATCTG	967
	chr7:9980144	F:ACAAGTCTGGAGATTCCTTC R:GATGTTCTTGGTGTGATTC	937
	chr10:63847987	F:CCCACCATCAACCTAGATTG R:GTCCTCACAGAACTCTCATT	706
	chr8:42993888	F:CCAGAGCAGTTACTTCAGAAG R:GGCTAGTGGTTACAGTATTGG	944
	chr13:43492573	F:GCACAGACCTAGCTAATCTCG R:CTTCCTTCCTTCCTTCCTCC	806
	chr14:3502008	F:GGACATGGATGAAGTTGGAAG R:TCAGTAATCTACAGAGGAGGTG	696
	chr20:61736084	F:ATGACCTAATCCATGCTCCAG R:GAACTCCTGACCTCAGATGAT	890
	chr5:72228276	F:CAATTCAGTGGGAAGTATAGGG R:GACACAGTTGTTACCATCTAC	882
sgRNA- <i>Prrt2</i> -C	chr7:83976766	F:CCTAAACTCCCTTGACTTCTG R:CCTGTTGTAACTCACTTCCTG	958
	chr1:190035638	F:GATACAGACTCAGAGGAATGG R:CAGGAGGTCTTCATCTTCAAC	703
	chr9:69194244	F:GGTAGGCAAGTATGGTAAAGG R:GGATTGTATCCTGGTGACTTC	605
	chr3:19342682	F:GATCACCTGAGGTTAGGAGTT	708

		R:CAGATTCTAGCTGAGTGGATG	
	chr15:40107119	F:CCTCATCATCTTCATGTCGAG	792
		R:CATCTACTCACTTCCTCTAGC	
	chr20:61058621	F:GGGAAGTGAATGAACTGATGG	700
		R:CCTGAAGTGAGGCAATAAGAG	
	chr14:112810197	F:GAGAACAGTGTCCCTTGATGAG	897
		R:GGAGGTAGAGGTTGTAGTGAG	
	chr10:85811043	F:CAACAGACAAGGCTAACTCAG	637
		R:GACAGAGTGAGACTCCATTAC	
	chr3:88667957	F:GGTAAGTCTGGAACCAATAGG	635
		R:CAGTCAAGCAGTATCTCTGAG	
	chr7:96903885	F:CTCCTTACATCTCATTGGTCAG	970
		R:CTATCCATCCTTCGTGATCTG	
	chr3:10010150	F:CTCATCCTGTTCTACACCAAG	665
		R:GAGTAAGGGAATCCATCTCTC	
	chr2:37733632	F:CCTACTATACACAAGGTACTGG	794
		R:CTAGAGGAAGACTGCTAAGTG	
	chr12:127960318	F:GCTCACCATATCCAACCTCATACT	905
		R:CTCCATCCACTCTCCATCTAT	
sgRNA-Prnt2-D	chr5:116240088	F:CGGTTCTCTCCACTAACATTC	765
		R:GTTAGAGGCATCAGGGATTTTC	
	chr5:151113455	F:CCCACCATCAACCTAGATTTG	706
		R:GTCCTCACAGAACTCTCATTC	
	chr1:62103725	F:GTGTGGACTGAGTTCTATAAGG	746
		R:GATCACTTGAGGTCAGGAGTT	
	chr1:146757199	F:GGCAGCACTTGGCATTTAACC	760
		R:CTAGGCTCGGCTTGGATGTTC	
	chr2:141699162	F:GCAGAGATCAGTTGCTTGTTTC	638
		R:CTGGGCTAGTGTTCTTAATGG	

Primers used for validation of the off-target site from whole-genome analysis.

sgRNA-Prnt2-C	chr4:146185910	F:AAGGATACAAGGATCTCAGG	999
		R:ACCACTCCGAAGATAATAGC	