

Expression of *Dux* family genes in early preimplantation embryos

**Kenta Sugie¹, Satoshi Funaya¹, Machika Kawamura¹, Toshinobu Nakamura²,
Masataka G. Suzuki¹, and Fugaku Aoki^{1*}**

¹Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, 277-8562 Japan.

²Department of Bio-Science, Nagahama Institute of Bio-Science and Technology, Shiga 526-0829, Japan.

**Address correspondence to: Fugaku Aoki, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Seimei-Building 302, 5-1-5 Kashiwanoha, Kashiwa 277-8652, Japan, E-mail: aokif@edu.k.u-tokyo.ac.jp*

Author contributions:

FA conceived and supervised the study; KS and FA designed the experiments; KS performed the experiments; KS, SF, MK, MGS, and FA analyzed the data; and KS and FA wrote the manuscript.

Supplemental Table. S1. Primers and PCR conditions used for RT-PCR

Target gene		Primer	Annealing temperature	Cycle
<i>Dux</i> family	Forward	5'-GCCCTGCTATCAACTTTCAAGAAG-3'	57 °C	36
	Reverse	5'-AGGCTTGCCCTAGGATCCTGAG-3'		
<i>Zfp352</i>	Forward	5'-AAAGCCTTGATCCTCAGGTG-3'	57 °C	36
	Reverse	5'-GCCGAAGAGTTTTTCTGAGG-3'		
<i>Zscan4d</i>	Forward	5'-AAGAGGTGAGGTGGAGGAGT-3'	57 °C	37
	Reverse	5'-AAGGTCTTTTGCTGGTGCCT-3'		
<i>Wsb1</i>	Forward	5'-CTCCAAGGCAAGTCCCTAGC-3'	57 °C	36
	Reverse	5'-TGTAAGGGCGCTCTGTCTG-3'		
<i>Galk1</i>	Forward	5'-GGCAGGGAGCTAATGAGCAA-3'	57 °C	36
	Reverse	5'-GGGCAGCTGACCTCATAGTC-3'		
<i>rabbit α-globin</i>	Forward	5'-GTGGGACAGGAGCTTGAAAT-3'	58 °C	26
	Reverse	5'-GCAGCCACGGTGGCGAGTAT-3'		

Supplemental Table. S2. Primers used for DNA sequencing

Target	Primers	
<i>Dux</i> full length CDS (2025 bps)	Forward	
	M13F (vector origin)	5'-GTAAAACGACGGCCAG-3'
	<i>Dux</i> origin	5'-TCCAGGCCACAGGGTAGAG-3'
		5'-TGGAGACCCTGGTGGCA-3'
		5'-TCCCAAGTGGCACAGCG-3'
	Reverse	
M13R (vector origin)	5'-CAGGAAACAGCTATGAC-3'	
<i>Dux</i> origin	5'-CTCTACCCTGTGGCCTGGA-3'	
	5'-TGCCACCAGGGTCTCCA-3'	
	5'-GGGAAGGCTTGACTCTCTGA-3'	
Part of <i>Dux</i> CDS (31-889 bps)	Forward	
	M13F (vector origin)	5'-GTAAAACGACGGCCAG-3'
	Reverse	
M13R (vector origin)	5'-CAGGAAACAGCTATGAC-3'	


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Dux      CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG1    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG2    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG3    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG4    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG5    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG6    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG7    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG8    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG9    CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG10   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG11   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG12   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG13   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG14   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG15   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG16   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG17   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG18   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG19   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG20   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG21   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG22   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025
DuxG23   CCTCTCACTTCAGAAGAATATCAGACTCTTCTAGATATGCTCTGA 2025

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Supplemental Figure S1. The nucleotide sequences of *Dux* family genes

The *Dux* sequence (top) was referenced from NCBI database. Red letters indicate the mutation sites when compared with the *Dux* sequence.


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Dux      GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR1   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR2   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR3   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR4   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR5   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR6   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR7   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR8   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR9   GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR10  GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR11  GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR12  GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR13  GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
DuxR14  GGTGCCCAGGAGAAC TTGTTGCCACAGGAAGAAGCAGGAAGTACGGGCATGGATACCTCG 660
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Dux      AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR1   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR2   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR3   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR4   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR5   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR6   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR7   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR8   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR9   AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR10  AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR11  AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR12  AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR13  AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720
DuxR14  AGCCCTAGCGACTTGCCCTCCTTCTGCGGAGAGTCCCAGCCTTTCCAAGTGGCACAGCCCC 720

Dux      CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR1   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR2   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR3   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR4   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR5   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR6   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR7   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR8   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR9   CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR10  CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR11  CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR12  CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR13  CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
DuxR14  CGTGGAGCAGGCCAACAAAGAGGCCCCCCACTCGAGCAGGCAACGCAGGCTCTCTGGAACCC 780
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Dux      CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR1   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR2   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR3   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR4   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR5   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR6   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR7   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR8   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR9   CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR10  CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR11  CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR12  CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR13  CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840
DuxR14  CTCC TTGATCAGCTGCTGGATGAAGTCCAAGTAGAAGAGCCTGCTCCAGCCCCCTCTGAAT 840

Dux      TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR1   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR2   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR3   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR4   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR5   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR6   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR7   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR8   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR9   TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR10  TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR11  TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR12  TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR13  TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
DuxR14  TTGGATGGAGACCCTGGTGGCAGGGTGTCATGAAGGTTCCCAGGAGAGCT 889
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Supplemental Figure S2. Nucleotide sequences of *Dux* family mRNA expressed in early preimplantation embryos

The sequences of *Dux* family transcripts (31 to 889 bps) are aligned. The *Dux* sequence (top) was referenced from NCBI database. Red letters indicate the mutation sites when compared with the *Dux* sequence.

Supplemental Fig. S3

Dux 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR1 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR2 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR3 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR4 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR5 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR6 11 SGVARE SRRHRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR7 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR8 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR9 11 SGVARE SRRHRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR10 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR11 11 SGVARE SRRRRKTVWQA GQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR12 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR13 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60
DuxR14 11 SGVARE SRRRRKTVWQAWQEQALLSTFKKKRYLSFKERKELAKRMGVSDC 60

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Dux RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR1 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR2 RIRVWFQNRNRNSG KEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR3 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR4 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR5 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR6 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR7 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR8 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR9 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR10 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRP RGRGMRSSGRRPRTL120
DuxR11 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR12 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR13 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120
DuxR14 RIRVWFQNRNRNSGEEGHASKRSIRGSRRLASPQLQEELGSRPQGRGMRSSGRRPRTL120

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Dux SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR1 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR2 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR3 SLQLRILGQAFERNPRPGFATREELARDT GMPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR4 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR5 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR6 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR7 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR8 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR9 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR10 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR11 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR12 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR13 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180
DuxR14 SLQLRILGQAFERNPRPGFATREELARDTGLPEDTIHIWFQNRARRRHRGRPTAQDQD180

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Dux LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR1 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR2 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR3 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR4 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR5 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR6 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR7 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR8 LL V SQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR9 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR10 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR11 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR12 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR13 LLASQGS DGAPAGPEGREREGAQENLLPQEEAGSTGMDTSSPDLPSFCGESQPFQVAQP 240
DuxR14 LLASQGS DGAPAGPEGREREGAQENLLPQEEA E STGMDTSSPDLPSFCGESQPFQVAQP 240

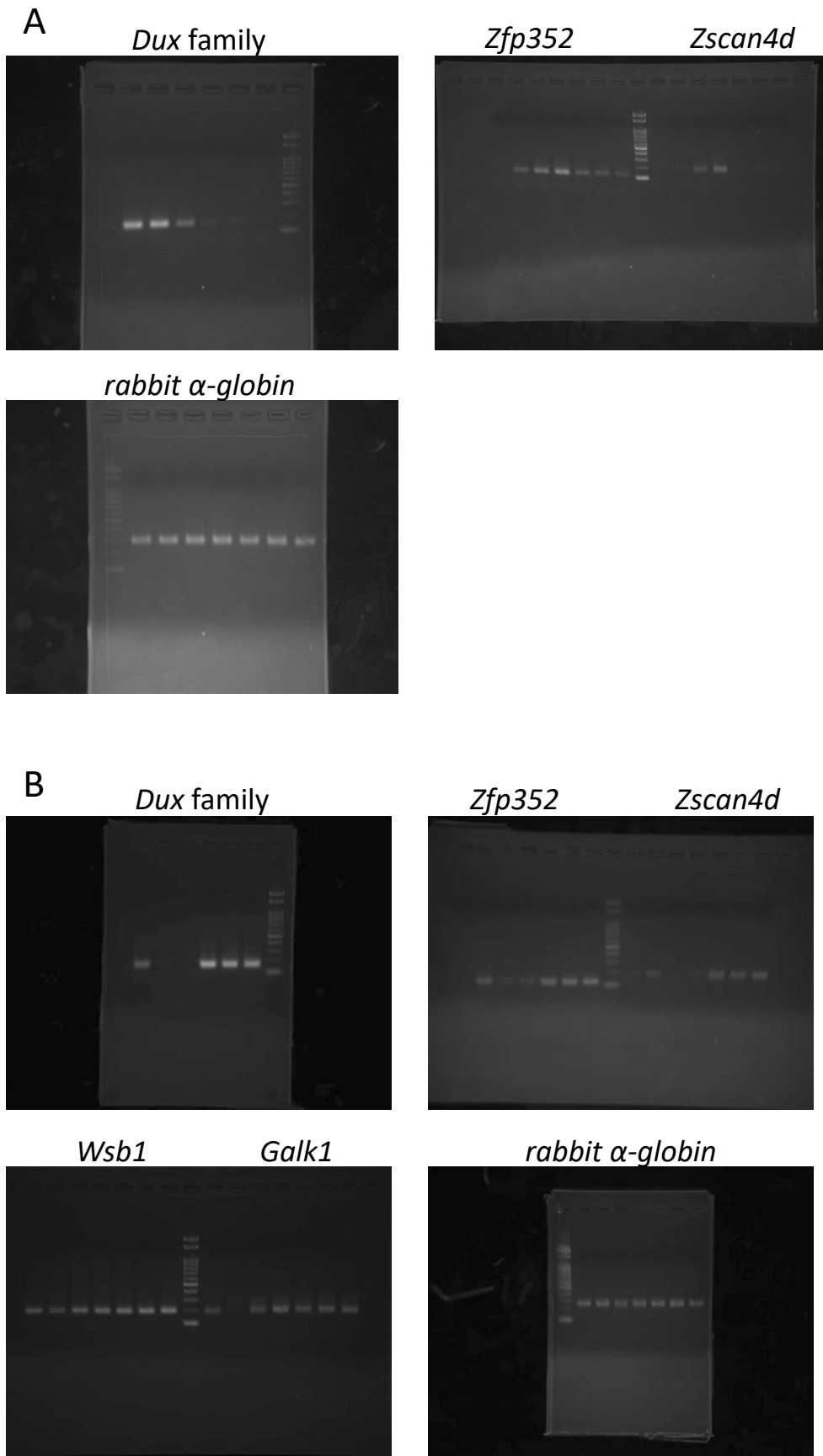
* *

Dux RGAGQQEAPTRAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR1 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR2 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR3 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR4 RGAGQQEAPTRAGNAGSLE TLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR5 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR6 RGAGQQEAPTRAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR7 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR8 RGAGQQEAPTRAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR9 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR10 RGAGQQEAPTRAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR11 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRV YEGSQES 296
DuxR12 RGAGQQEAPT QAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR13 RGAGQQEAPTRAGNAGSLE TLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296
DuxR14 RGAGQQEAPTRAGNAGSLEPLLDQLLDEVQVEEPAPAPLNLDGDPGGRVHEGSQES 296

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Supplemental Figure S3. Amino acid sequences of *Dux* families

The sequences of *Dux* family mRNAs in Supplemental Figure 2 were translated to those of amino acids. Red letters indicate the mutation sites when compared with the *Dux* sequence. Although the nucleotide sequences were different, DuxR1, DuxR7 and R12 code the same amino acid sequence, and so do DuxR4 and DuxR13.



Supplemental Figure S4. Original electrophoresis images

These are uncropped images of electrophoresis gels in Fig. 1A (A) and Fig. 3A (B).