

**TEMPORAL EXPRESSION OF PLURIPOTENCY-ASSOCIATED TRANSCRIPTION FACTORS
IN CATTLE AND SHEEP PREIMPLANTATION EMBRYOS**

P.G.C. Silva, M.T. Moura, R.L.O. Silva, P.S. Nascimento, J.B. Silva, J.C. Ferreira-Silva, L.F.

Cantanhêde, M.S. Chaves, A.M. Benko-Iseppon, M.A.L. Oliveira

Supplementary Material

Supplementary Table S1. Cattle (*Bos taurus*) and sheep (*Ovis aries*) *in vitro* embryo production efficiency by *in vitro* fertilization (IVF) and parthenogenetic activation (PA).

Species	Oocytes (n)	Method	Cleavage n (%)	Morulae n (%)	Blastocysts n (%)
<i>Ovis aries</i>	156	PA	108/126 (85.7)	67/110 (60.9)	21/63 (33.0)
<i>Bos taurus</i>	122	IVF	122/191 (63.8)	78/138 (56.2)	28/52 (53.8)

Supplementary Table S2. Parameters from reverse transcriptase quantitative PCR (RT-qPCR) reactions for eight pluripotency-associated transcription factors in sheep (*Ovis aries*) and cattle (*Bos taurus*) eggs and preimplantation embryos.

Gene	Blank (Cq/Melting) (S-C)		Eggs (Cq/Melting) (S-C)		Cleaved (Cq/Melting) (S-C)		Morula (Cq/Melting) (S-C)		Blastocyst (Cq/Melting) (S-C)	
	(S-C)	(S-C)	(S-C)	(S-C)	(S-C)	(S-C)	(S-C)	(S-C)	(S-C)	(S-C)
GAPDH	0/0	00/00	28/20	85/86	22/23	85/86	24/23	85/86	18/22	85/85
SDHA	0/0	00/00	31/23	88/88	27/26	88/88	23/24	88/88	22/24	88/88
TBP	0/0	00/00	28/27	81/80	25/31	81/80	26/28	81/00	25/29	81/80
-RT	0/0	00/00	0/0	00/00	0/0	00/00	0/0	00/00	0/0	00/00
CMYC	0/0	00/00	0/28	00/86	30/29	86/86	38/31	00/87	30/29	86/86
DAX1	0/0	00/00	29/22	81/82	28/26	81/82	29/0	82/00	30/29	82/82
GLIS1	0/0	00/00	0/32	00/00	33/0	00/00	0/0	00/00	32/0	00/00
KLF5	32/33	74/74	33/26	00/90	26/30	88/74	22/26	88/90	20/25	88/90
OCT4	35/0	86/00	29/21	86/85	24/24	86/86	21/21	86/86	19/21	86/86
RONIN	0/31	00/73	28/20	90/90	23/23	90/90	24/26	90/90	23/25	90/73
ZFP281	0/0	00/00	31/24	81/81	26/27	81/81	24/24	81/81	22/24	81/81
ZFX	0/30	00/80	30/21	00/80	26/26	81/81	26/26	81/81	24/26	81/81

C: cattle. Cq: quantification cycle. S: Sheep.

Supplementary Table S3. Protein size, isometric point, molecular weight, and subcellular localization for eight pluripotency-associated transcription factors in sheep (*Ovis aries*) and cattle (*Bos taurus*).

Gene	Species	Isoform	Start*	Stop**	Size (Aa)	IP	MW	Subcellular Localization
CMYC	<i>O. aries</i>	-	209	1528	439	5.31	48.47	Nucleus
	<i>B. taurus</i>	X1	707	2026	439	5.21	48.38	Nucleus
	<i>B. taurus</i>	X2	119	1438	439	5.21	48.38	Nucleus
DAX1	<i>O. aries</i>	X1	<1	1149	382	6.27	41.48	Cytoplasm & Nucleus
	<i>O. aries</i>	X2	238	1653	471	6.75	51.56	Cytoplasm & Nucleus
	<i>B. taurus</i>	-	22	1437	471	6.52	51.60	Cytoplasm & Nucleus
GLIS1	<i>O. aries</i>	X1	3063	4940	625	8.48	66.05	Nucleus
	<i>O. aries</i>	X2	5879	8182	767	8.58	81.94	Nucleus
	<i>O. aries</i>	X3	5879	7729	616	7.89	65.54	Nucleus
	<i>B. taurus</i>	-	97	2487	796	8.11	84.36	Nucleus
KLF5	<i>O. aries</i>	X1	286	1650	454	8.54	50.47	Nucleus
	<i>O. aries</i>	X2	170	1270	366	8.77	41.19	Nucleus
	<i>B. taurus</i>	-	304	1668	454	8.54	50.47	Nucleus
OCT4	<i>O. aries</i>	-	4	1083	359	5.99	38.25	Nucleus
	<i>B. taurus</i>	-	<1	1083	360	5.56	38.33	Nucleus
RONIN	<i>O. aries</i>	X1	271	1182	303	8.96	33.01	Nucleus
	<i>O. aries</i>	X2	<1	921	306	9.42	32.97	Nucleus
	<i>B. taurus</i>	-	49	960	303	8.96	33.05	Nucleus
ZFP281	<i>O. aries</i>	X1	1309	3117	602	8.42	65.62	Nucleus
	<i>O. aries</i>	X2	163	2784	873	8.67	93.23	Nucleus
	<i>O. aries</i>	X3	152	2773	873	8.67	93.23	Nucleus
	<i>B. taurus</i>	-	30	2570	846	9.78	90.65	Nucleus
ZFX	<i>O. aries</i>	X1	1124	<3526	800	5.63	90.16	Nucleus
	<i>O. aries</i>	X2	<1	2403	800	5.63	90.16	Nucleus
	<i>O. aries</i>	X3	1107	<3431	774	5.81	87.32	Nucleus
	<i>O. aries</i>	X4	1306	3630	774	5.81	87.32	Nucleus
	<i>O. aries</i>	X5	129	>2531	800	5.63	90.16	Nucleus

Gene	Species	Isoform	Start*	Stop**	Size (Aa)	IP	MW	Subcellular Localization
	O. aries	X6	378	>2780	800	5.63	90.16	Nucleus
	O. aries	X7	215	>2539	774	5.81	87.32	Nucleus
	O. aries	X8	129	2390	753	5.45	84.98	Nucleus
	O. aries	X9	434	>2836	800	5.63	90.16	Nucleus
	O. aries	X10	172	2574	800	5.63	90.16	Nucleus
	O. aries	X11	167	>2569	800	5.63	90.16	Nucleus
	O. aries	X12	48	>2450	800	5.63	90.16	Nucleus
	O. aries	X13	566	>2890	774	5.81	87.32	Nucleus
	B. taurus	-	26	2428	800	5.66	90.60	Nucleus

Aa: Aminoacid. Ip: Isoelectric point. MW: Molecular weight. *Initial nucleotide for protein synthesis. **Last nucleotide for protein synthesis.

Supplementary Figure S1. Melting values from reverse transcriptase quantitative PCR (RT-qPCR) for eight pluripotency-associated transcription factors in sheep (*Ovis aries*) and cattle (*Bos taurus*) eggs and preimplantation embryos.

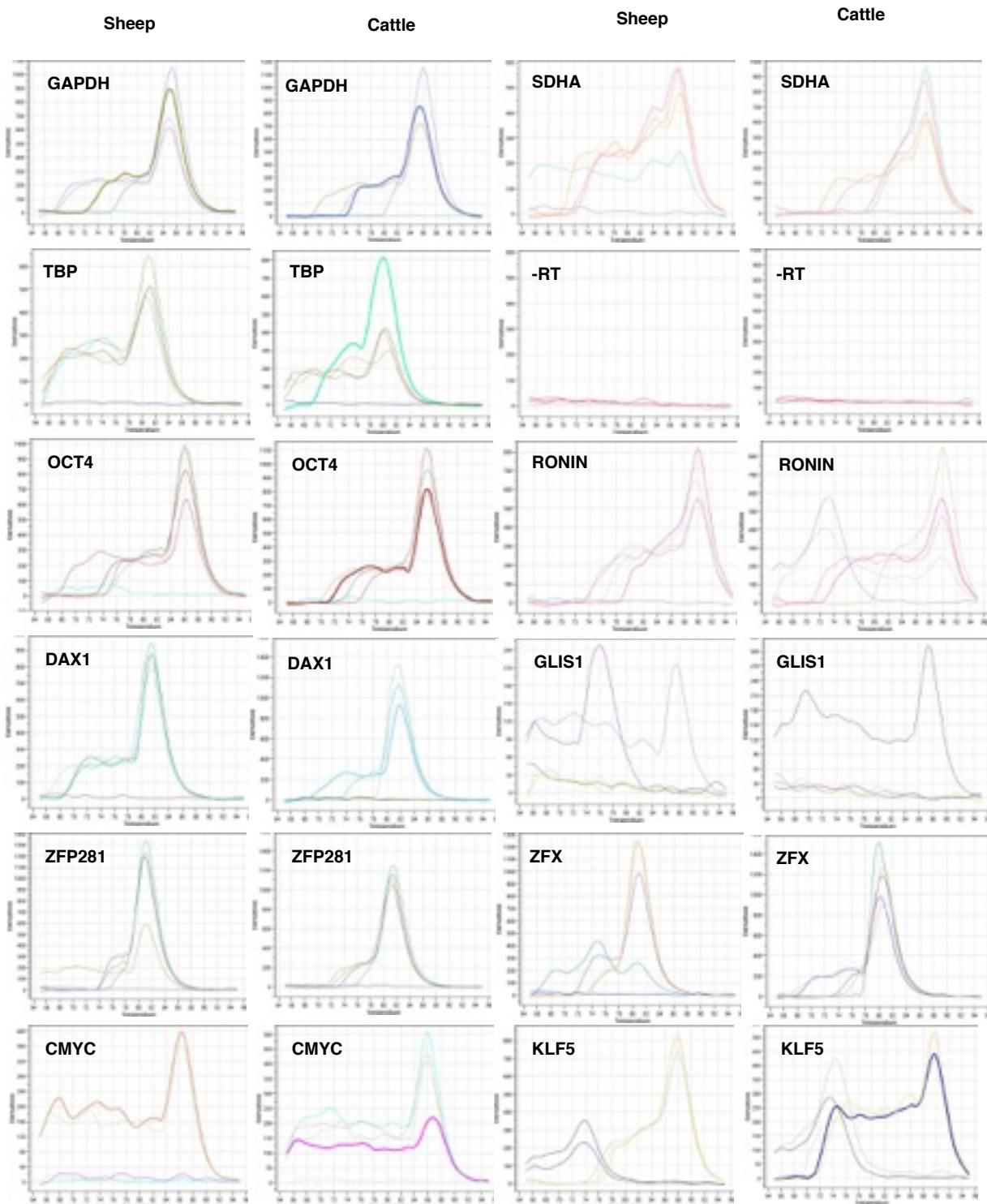
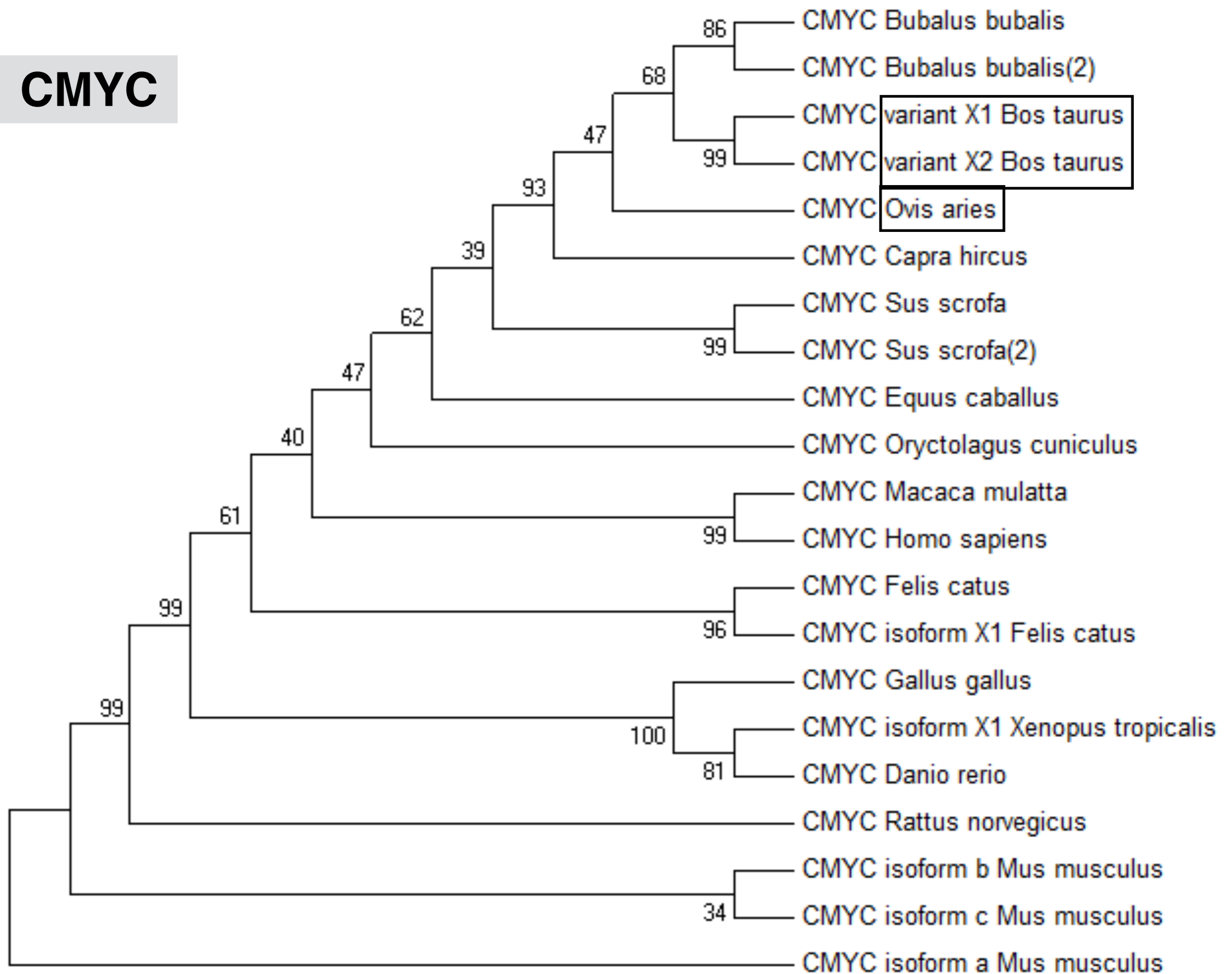
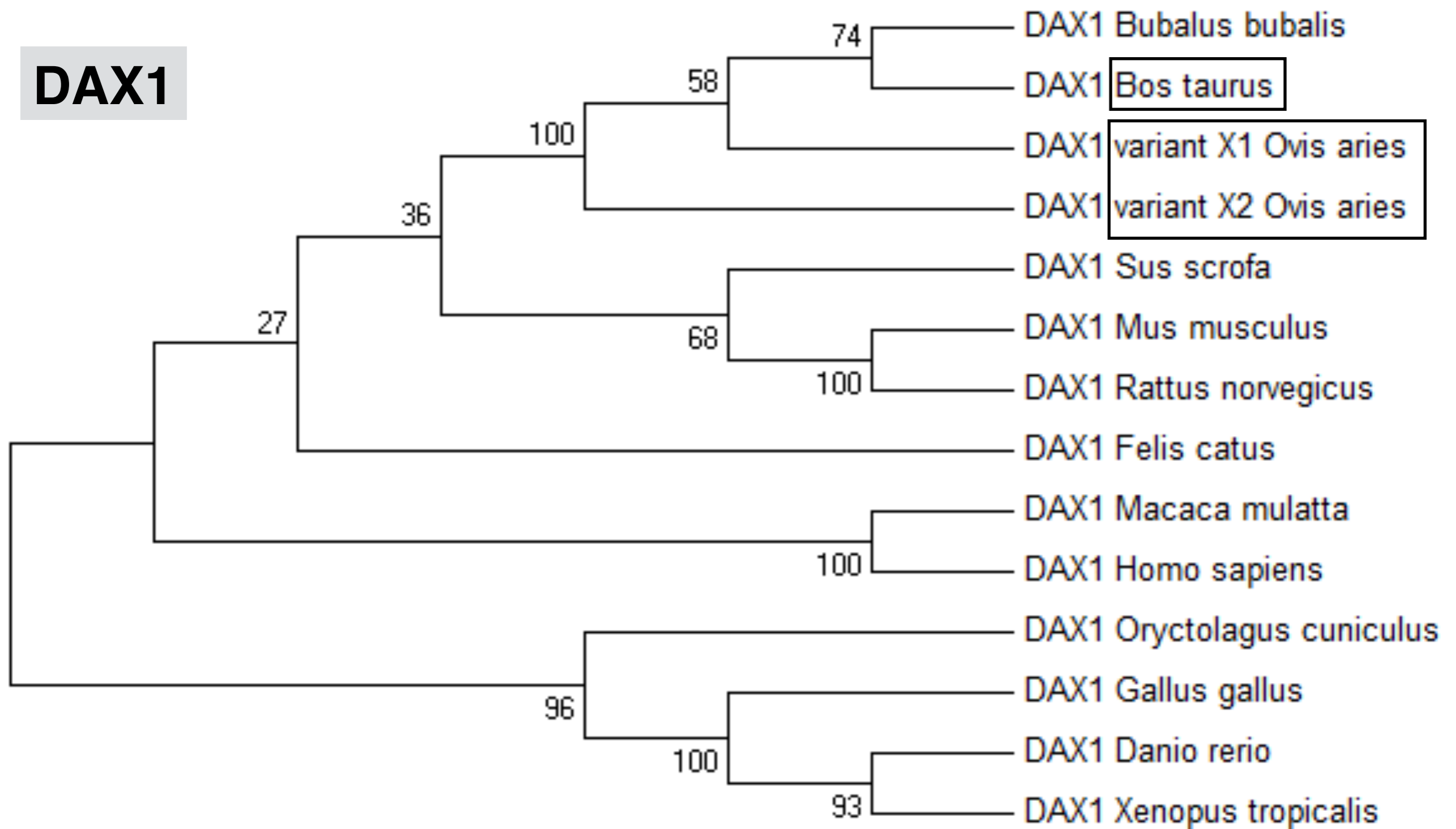


Fig. S2. Phenetic analysis of eight pluripotency-associated transcription factors in ten selected species. Sheep (*Ovis aries*) and cattle (*Bos taurus*) sequences are outlined.

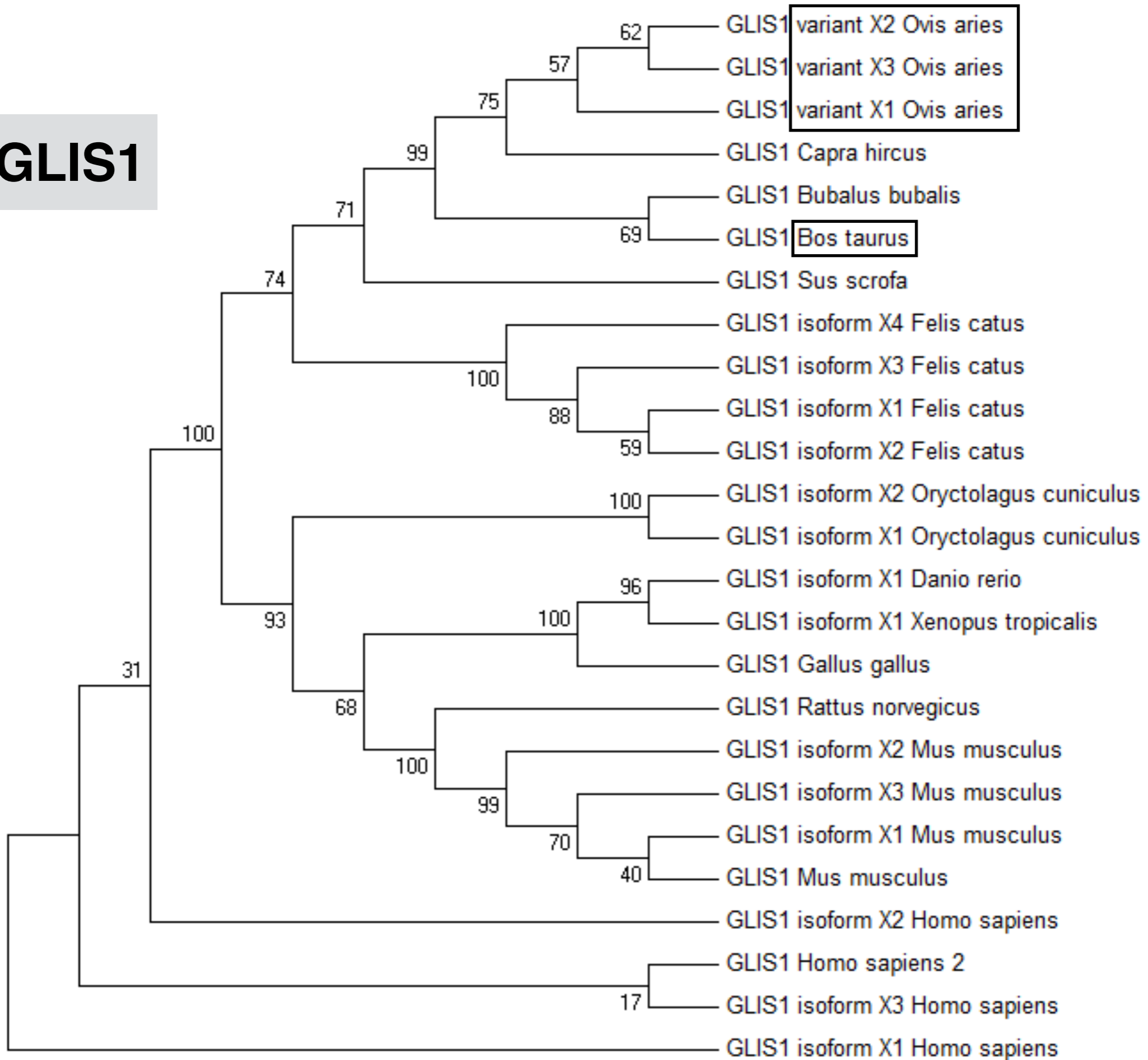
CMYC



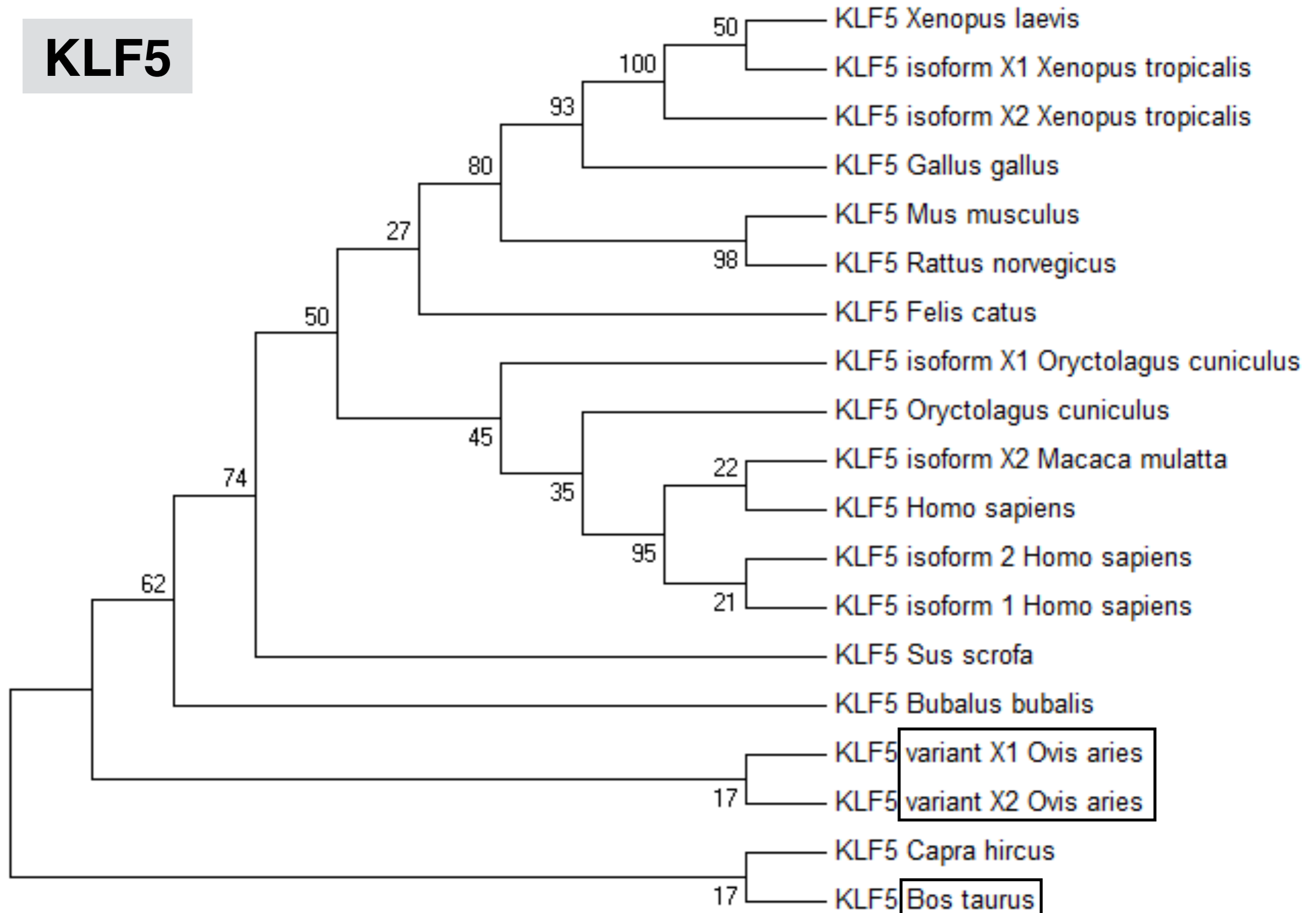
DAX1



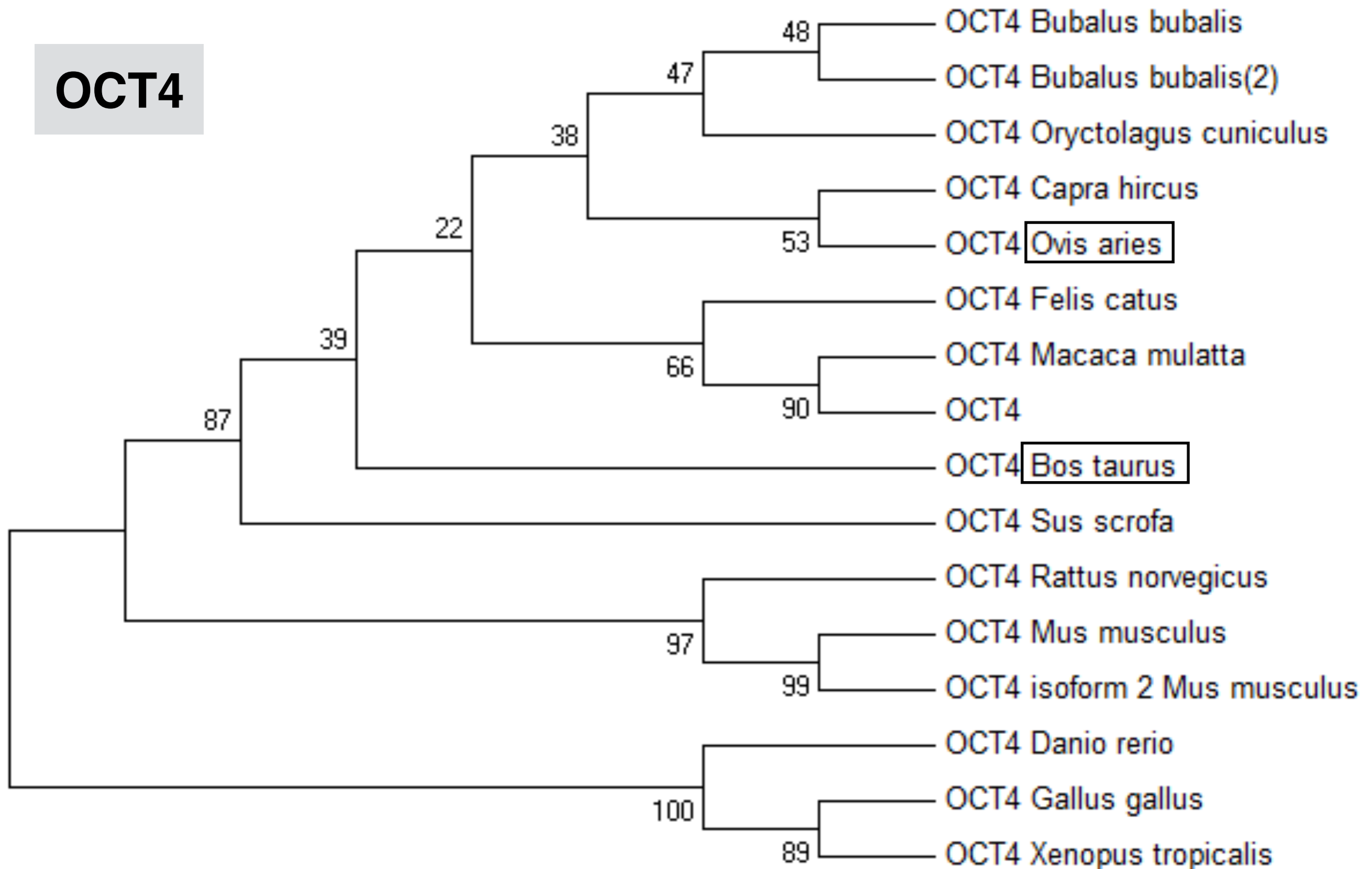
GLIS1



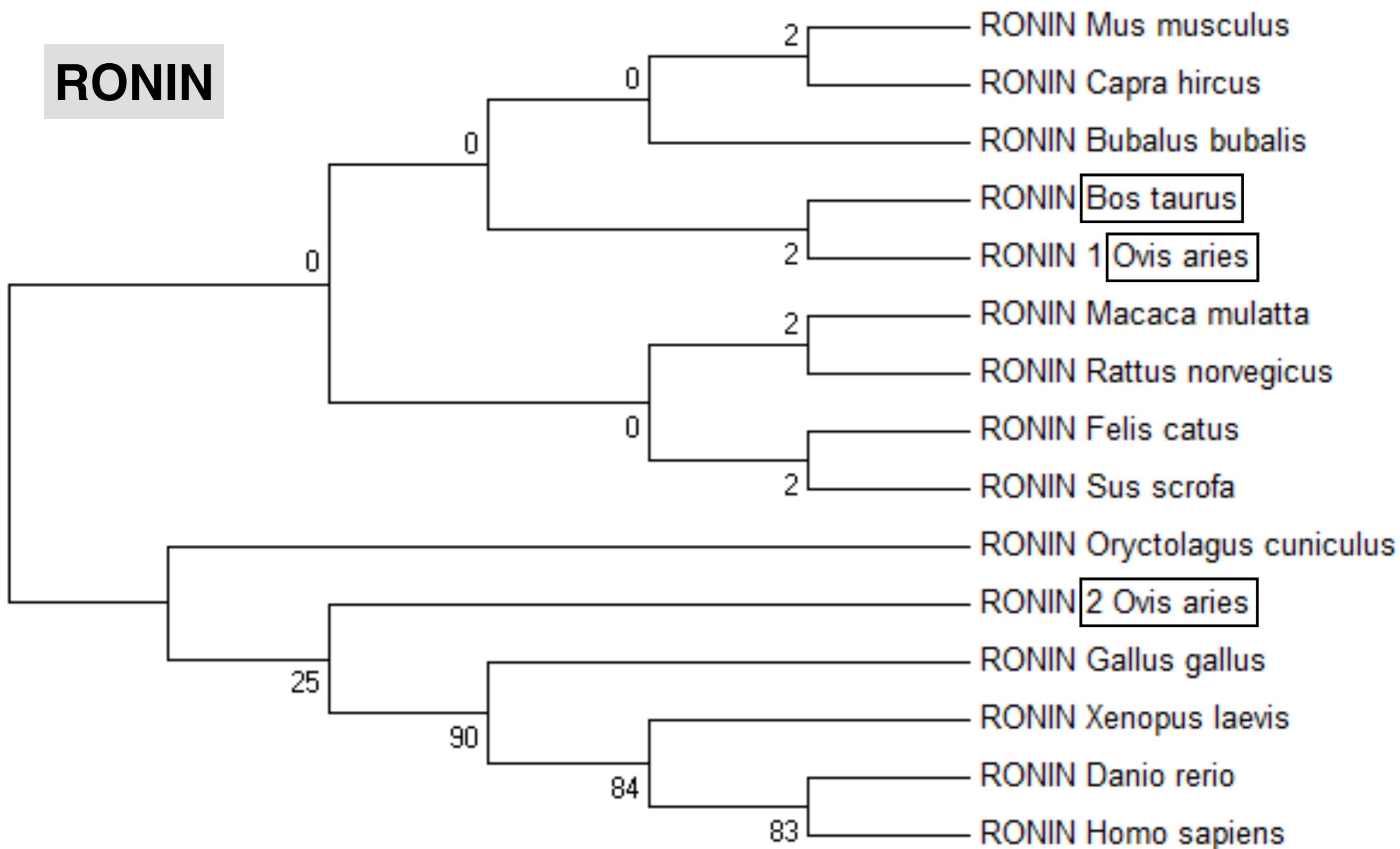
KLF5



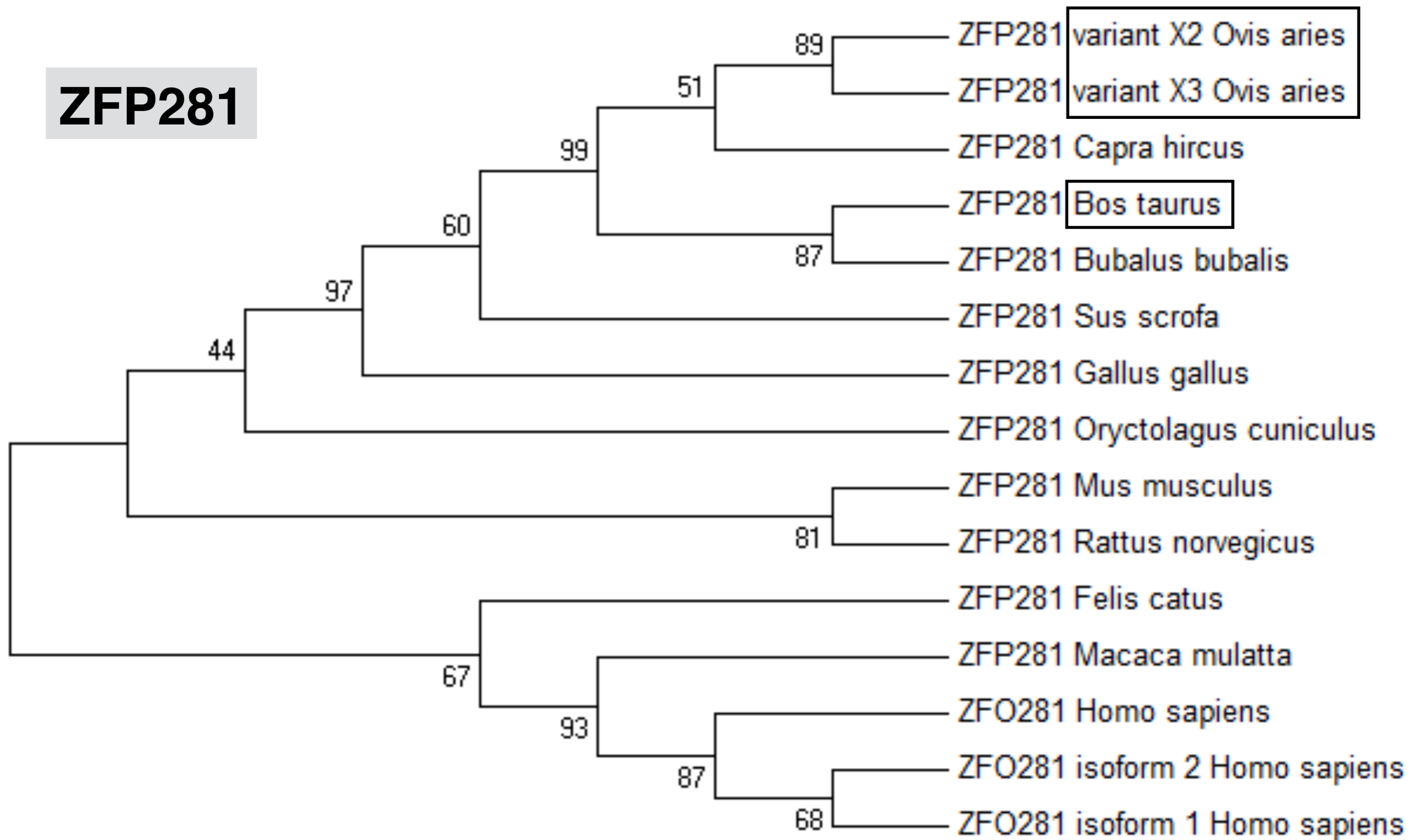
OCT4



RONIN



ZFP281



ZFX

