

Coffill and Lee *et al.* Supplemental Information

Molecular dynamics

Initial structures for molecular dynamics (MD) simulations were taken from the Protein Data Bank (PDB). Human p53 transactivation domain peptide (residues 17-29) and nutlin-3a were extracted from the PDB structures 1YCR (Kussie et al. 1996) and 4J3E (Vu et al. 2013) respectively and modeled onto the human HDM2^{Nterm} structure (residues 17-112) taken from the PDB structure 3JZR (Phan et al. 2010). Using PyMOL (DeLano 2002), the human p53 peptide was extended by one residue at its N-terminus and then capped by acetyl and amide groups, while HDM2^{Nterm} was capped at its N- and C-termini by acetyl and N-methyl groups respectively. Complexes of HDM2^{Nterm} with lamprey p53, lamprey p53 G22L mutant and human p53 triple alanine mutant (F19A, W23A, L26A) peptides were generated by mutating the human p53 peptide to the appropriate sequence. The mutations were performed by keeping the peptide backbone fixed and using the tleap module of AMBER 12 (Case et al. 2012) to add the side chains of the mutated residues. Residue protonation states were determined by PDB 2PQR (Dolinsky et al. 2004). The LEaP program in the AMBER 12 package was then used to solvate each system with TIP3P (Jorgensen et al. 1983) water molecules in a periodic truncated octahedron box, such that its walls were at least 10 Å (12 Å and 15 Å for the unbound peptides and for nutlin-3a, respectively) away from the HDM2^{Nterm} complex and for neutralization of charges with either sodium or chloride ions.

Three independent explicit-solvent MD simulations were carried out on each of the complexes of HDM2^{Nterm} with human p53, human p53 triple alanine mutant, lamprey p53, lamprey p53 G22L mutant and nutlin-3a, as well as the unbound forms of HDM2^{Nterm}, p53 peptides and nutlin-3a. Energy minimizations and MD simulations were carried out by the

PMEMD module of AMBER 12, using the ff99SB force field (Hornak et al. 2006) for the protein and peptides and the generalized AMBER force field (Wang et al. 2004) for nutlin-3a. All bonds involving hydrogen atoms were constrained by the SHAKE algorithm (Ryckaert et al. 1977), allowing for a time step of 2 fs. Nonbonded interactions were truncated at 9 Å while electrostatic interactions were treated by the particle mesh Ewald method (Darden et al. 1993). Energy minimization was carried out using the steepest descent algorithm for 500 steps, followed by the conjugate gradient algorithm for another 500 steps. Each system was then heated gradually to 300 K over 50 ps at constant volume before equilibration at a constant pressure (1atm) for another 50 ps. Weak harmonic positional restraints with a force constant of 2.0 kcal mol⁻¹ Å⁻² were imposed on the heavy atoms of the solute during the minimization and these two equilibration steps. Subsequent unrestrained equilibration (2 ns) and production (100 ns) runs were carried out at 300 K and 1 atm. The temperature was maintained using a Langevin thermostat (Izaguirre et al. 2001) with a collision frequency of 2 ps⁻¹ while the pressure was maintained by a Berendsen barostat (Berendsen et al. 1984) with a pressure relaxation time of 2 ps.

Binding free energy calculations

Binding free energies for HDM2^{Nterm} complexes were calculated with the molecular mechanics/generalized Born surface area (MM/GBSA) method (Srinivasan et al. 1998). All programs used for MM/GBSA calculations are from AMBER 12. 200 equally-spaced snapshot structures were extracted from the last 10-30 ns of each of the trajectories, depending on when equilibration of the systems occurred (determined from their root mean square deviation plots), and their molecular mechanical energies calculated with the sander module. The polar contribution to the solvation free energy was calculated by the pbsa (Luo et al. 2002) program using the modified generalized Born (GB) model described by Onufriev

et al. (Onufriev et al. 2004) while the nonpolar contribution was estimated from the solvent accessible surface area (SASA) using the molsurf (Connolly 1983) program with $\gamma = 0.0072$ kcal \AA^{-2} and β set to zero. The nmode program was used to estimate entropies (Brooks et al. 1995). Due to its computational expense, only 50 equally-spaced snapshots from the equilibrated portion of the trajectories were used for entropic analysis. Replica exchange MD simulations were carried out on the free peptides using standard protocols (Lama et al. 2013).

Binding free energy decomposition

The contribution of each peptide residue to the binding free energy was computed using the free energy decomposition method (Gohlke et al. 2003) on the same 200 snapshot structures used for MM/GBSA analysis. Similar to the MM/GBSA calculations, the molecular mechanical energies and polar contribution to solvation free energy were computed by the sander module and pbsa program using the modified GB model described by Onufriev *et al.* (Onufriev et al. 2004) respectively. The nonpolar contribution to solvation free energy was estimated from the SASA using the ICOSA method (Rarey et al. 1996).

Bio-layer Interferometry (BLI) assay

The affinity of HDM2^{Nterm} binding to the Lamprey peptides was determined using the BLItz (ForteBio, USA) system. The purified HDM2^{Nterm} proteins were buffer exchanged into the kinetics buffer (PBS + 0.05% Tween-20) prior to the experiment. Biotinylated human and lamprey peptides were immobilized at a concentration of 2.5 μM on the streptavidin biosensors (ForteBio) which were pre-hydrated in PBS + 5% DMSO for at least 10 minutes. The unbound biotinylated peptides were washed off in the same buffer. The loaded sensors were equilibrated in the kinetics buffer before immersing them into the various titrations of HDM2^{Nterm} (3x serial dilutions from 250 μM) over 120 seconds and then immersed into the

kinetics buffer for dissociation. A blank uncoated sensor reference (without biotinylated peptide) was carried out in 250 μ M HDM2^{Nterm} to ensure no/low binding of HDM2^{Nterm} to the uncoated sensor and a coated sample reference (peptide but without HDM2^{Nterm} protein) was measured as a background binding control. Data analysis was performed using a global fit in the BLItz Pro software to calculate the K_D value.

Amino acid sequence similarity (Table 1)

The amino acid sequence similarities between human and lamprey proteins were determined using pairwise sequence alignment tools (http://www.ebi.ac.uk/Tools/psa/emboss_needle/) (Rice et al. 2000; Li et al. 2015).

Table S1. Computed binding free energies (kcal/mol) of HDM2^{Nterm} complexes.

Ligand	Peptide sequence, if applicable	ΔH_{bind}	$T\Delta S_{\text{bind}}$	ΔG_{bind}
nutlin-3a	N.A.	-82.36	-24.08	-58.29
Hp53 ¹⁶⁻²⁹	Ac-QETFSDLWKLLPEN-NH ₂	-80.39	-37.21	-43.18
Hp53 ^{16-29(AAA)}	Ac-QETASDLAKLAPEN-NH ₂	-79.30	-40.37	-38.92
Lp53 ¹²⁻²⁵	Ac-VDDFDRVWQGGVGL-NH ₂	-80.61	-40.81	-39.80
Lp53 ^{12-25(G22L)}	Ac-VDDFDRVWQGLVGL-NH ₂	-85.63	-37.48	-48.15

Table S2. Experimental determination of binding affinities for peptide and HDM2^{Nterm} complexes

Peptide	Sequence	K_D (μM) ^A	K_D (μM) ^B
Hp53 ¹⁶⁻²⁹	QETFSDLWKLLPEN	2.7 ± 0.5	1.3
Hp53 ^{16-29(AAA)}	QETASDLAKLAPEN	nd	nd
Lp53 ¹²⁻²⁵	VDDFDRVWQGGVGL	nd	nd
Lp53 ^{12-25(G22L)}	VDDFDRVWQGLVGL	41 ± 3	33

^A determined by Fluorescence Anisotropy. Data are averages of at least four replicates \pm SEM.

^B determined by Bio-layer Interferometry (BLI) assay.

nd = not determined due difficulty fitting curve to weak binding data

Table S3. Peptides synthesized for Fluorescence Anisotropy and Bio-layer Interferometry (BLI) assay

Peptide	Sequence	Organism	Reference sequence	Literature reference
FAM-12.1	5 (6) -FAM-RFMDYWEGL-NH ₂	Human	-	(Bottger et al. 1997)
Hs-p53 ¹⁶⁻²⁹	Ac-QETFSDLWKLLPEN-NH ₂ Biotin-SGSG- QETFSDLWKLLPEN-NH ₂		NP_000537.3	
Hs-p53 ^{16-29(AAA)}	Ac-QETASDLAKLAPEN-NH ₂ Biotin-SGSG- QETASDLAKLAPEN-NH ₂			
Lj-p53 ¹²⁻²⁵	Ac-VDDFDRVWQGGVGL-NH ₂ Biotin-SGSG- VDDFDRVWQGGVGL-NH ₂	Lamprey	KT960978	
Lj-p53 ^{12-25(G22L)}	Ac-VDDFDRVWQGLVGL-NH ₂ Biotin-SGSG- VDDFDRVWQGLVGL-NH ₂			

5(6)FAM = mixed isomers 5-(and 6-)carboxyfluoresceine; Ac = acetyl; NH₂ = amide

Table S4. Details of plasmids used in this study

Plasmid	Amino Acids	Tag	Vector	N'/C'	Organism	Reference sequence	Literature reference
Hs-p53	1-393	-	pcDNA3	-	Human	NP_000537.3	
Hs-p53	1-393	3xFLAG 6xHIS	pCI-neo	N'			(Coffill et al. 2012)
Hs-p53Δ	1-355	-	pcDNA3	-			
HDM2	1-491	-	pCMV	-		Q00987	
HDM2 ^{Nterm}	1-125	HA	pcDNA3	C'			
GST-HDM2 _{Nterm}	1-125 or 6-125	GST-precision	pGEX-6P-1	N'			(Bottger et al. 1997; Brown et al. 2013; Chee et al. 2014)
Hs-RPL11	1-178	FLAG	pcDNA3	N'		NP_000966.2	
Hs-RPL5	1-297	FLAG	pcDNA3	N'		NP_000960.2	
Lj-p53	1-428	3xFLAG 6xHIS	pCI-neo	N'	Lamprey	KT960978	
Lj-p53	1-428	FLAG	pcDNA3	N'			
Lj-p53(G22L)	1-428 (G22L)	FLAG	pcDNA3	N'			
Lj-p53Δ	1-390	FLAG	pcDNA3	N'			
Lj-Mdm2	1-603	HA	pXJ40	N'		KT960981	
Lj-Mdm2(C464A) (human #)	1-603(C576A)	HA	pXJ40	N'			
Lj-Mdm2	1-603	HA	pCMV	C'			
Lj-Mdm2	1-603	HA	pcDNA3	C'			
Lj-Mdm2 ^{Nterm}	1-106	HA	pcDNA3	C'			
Lj-Mdm4	1-280	myc	pCMV	N'		KT960982	

Coffill and Lee *et al.* Supplemental Figure Legends

Figure S1. Alignment of p53

Alignment of p53 protein sequences from lamprey (GenBank accession number KT960978); elephant shark (Eshark) (G9J1L8); zebrafish (P79734); *Xenopus laevis* (frog) (P07193); chicken (P10360); mouse (NP_035770.2) and human (P04637). Alignments were carried out using Clustal Omega (Goujon *et al.* 2010; Sievers *et al.* 2011) and Jalview (Waterhouse *et al.* 2009).

Figure S2. Isoforms of p53

Alignment of p53 isoform protein sequences from human (Hs), zebrafish (Dr) and lamprey (Lj): Hs-p53 (P04637); Hs- Δ 40p53 (NP_001119590.1); Hs- Δ 133p53 (NP_001119587.1); Hs- Δ 160p53 (NP_001263626.1); Dr-p53 (P79734); Dr- Δ 18p53 (see (Davidson *et al.* 2010)); Dr- Δ 113p53 (see (Marcel *et al.* 2011)); Lj-p53 (KT960978); Lj- Δ 27p53; Lj- Δ 30p53; Lj- Δ 108p53.

Figure S3. Synteny of *Tp53*, *Tp63* and *Tp73* genes

Tp53, *Tp63* and *Tp73* gene loci in human, coelacanth and lamprey. Genes that are colored indicate genes that show conserved synteny. The orientation of the pentagons (genes) denotes the direction of transcription and circles represent end of scaffold.

Figure S4. Intron positions of Lj-p53, Lj-p63 and Lj-p73

Alignment of Lj-p53, Lj-p63 and Lj-p73 proteins indicating the positions of introns. The alignment was generated using Clustal Omega. The phase of each intron is indicated by color: yellow (phase 0), green (phase 1), red (phase 2).

Figure S5. Alignment of p63

Alignment of p63 protein sequences from lamprey (GenBank accession number KT960979); elephant shark (Eshark) (G9J1L9); zebrafish (A7YYJ7); *Xenopus tropicalis* (frog) (F6ZGN7); chicken (F1N8Z7); mouse (O88898) and human (Q9H3D4).

Figure S6. Alignment of p73

Alignment of p73 protein sequences from lamprey (GenBank accession number KT960980) elephant shark (Eshark) (G9J1M0); zebrafish (B0S576); *Xenopus tropicalis* (frog) (F6TKT0); chicken (XP_417545.3); mouse (Q9JJP2) and human (O15350).

Figure S7. Gene structure of *Tp63* and *Tp73*

Gene structure and isoforms of *Lj-Tp63* (A) and *Lj-Tp73* (B). Coding exons are designated by open boxes and non-coding exons by shaded boxes. The transcription start site is indicated by an arrow. The sizes of 5' introns are labelled in (A). The longest isoform of *Tp63* has an alternative 5' splice site at the first intron compared with the other two shorter isoforms. The figure is not drawn to scale.

Figure S8. Isoforms of p63

Alignment of p63 isoform protein sequences from human (Hs) and lamprey (Lj): Hs-TAp63 α (NP_003713.3); Hs-TAp63 β (NP_001108450.1); Hs-TAp63 γ (NP_001108451.1); Hs- Δ Np63 α (NP_001108452.1); Hs- Δ Np63 β (NP_001108453.1); Hs- Δ Np63 γ (NP_001108454.1); Lj-p63_A (KT960979); Lj-p63_B and Lj-p63_C.

Figure S9. Alignment of Mdm2

Alignment of Mdm2 protein sequences from lamprey (GenBank accession number KT960981) elephant shark (Eshark) (G9J1M1); zebrafish (Q561Z0); *Xenopus laevis* (frog) (P56273); chicken (F1NGX6); mouse (P23804) and human (Q00987).

Figure S10. Alignment of Mdm RING

Alignment of Mdm2 and Mdm4 protein sequences with HMD2 (Q00987); HDM4 (O15151); Lj-Mdm2 (KT960981) and Lj-Mdm4 (KT960982). Arrows denote the amino acid residues required for either p53 ubiquitination and/or degradation (Fang et al. 2000; Dolezelova et al. 2012).

Figure S11. Alignment of Mdm4

Alignment of Mdm4 protein sequences from lamprey (GenBank accession number KT960982) elephant shark (Eshark) (G9J1M2); zebrafish (Q7ZUW7); *Xenopus tropicalis* (frog) (B5DFR1); chicken (E1C4B0); mouse (O35618) and human (O15151).

Figure S12. Synteny of *Mdm2* and *Mdm4* genes

Mdm2 and *Mdm4* gene loci in human, coelacanth, elephant shark and lamprey. Genes that are colored indicate genes that show conserved synteny. The orientation of the pentagons (genes) denotes the direction of transcription and circles represent end of scaffold.

Figure S13. Averaged binding free energy contributions of peptide residues in the complexes of HDM2^{Nterm} with Hs-p53¹⁶⁻²⁹ (blue), Lj-p53¹²⁻²⁵ (red) and Lj-p53^{12-25(G22L)} mutant (black).

Figure S14. Snapshots of free peptides from Replica exchange molecular dynamics simulations.

Figure S15.

(A) Western blot showing *in vitro* translation (IVT) and immunoprecipitation (IP) of lamprey p53 (top panel) by Lj-Mdm2, which were used as bait. Input levels can be seen in the lower panel. (B) Western blot of Lj-p53 levels following co-transfection with various Lj-Mdm2 expressing constructs. Lanes: (1) Lj-p53; (2) Lj-p53 with MG132; (3) Lj-p53 + HA-Lj-Mdm2; (4) Lj-p53 + HA-Lj-Mdm2 with MG132; (5) Lj-p53 + Lj-Mdm2-HA; (6) Lj-p53 + Lj-Mdm2-HA with MG132; (7) Lj-p53 + Lj-Mdm2-HA + myc-Lj-Mdm4; (8) Lj-p53 + Lj-Mdm2-HA + myc-Lj-Mdm4 with MG132; (9) Lj-p53 + myc-Lj-Mdm4; (10) Lj-p53 + myc-Lj-Mdm4 with MG132. Lj-Mdm2 levels can be seen in the upper panel and the loading control can be found in the lower panel.

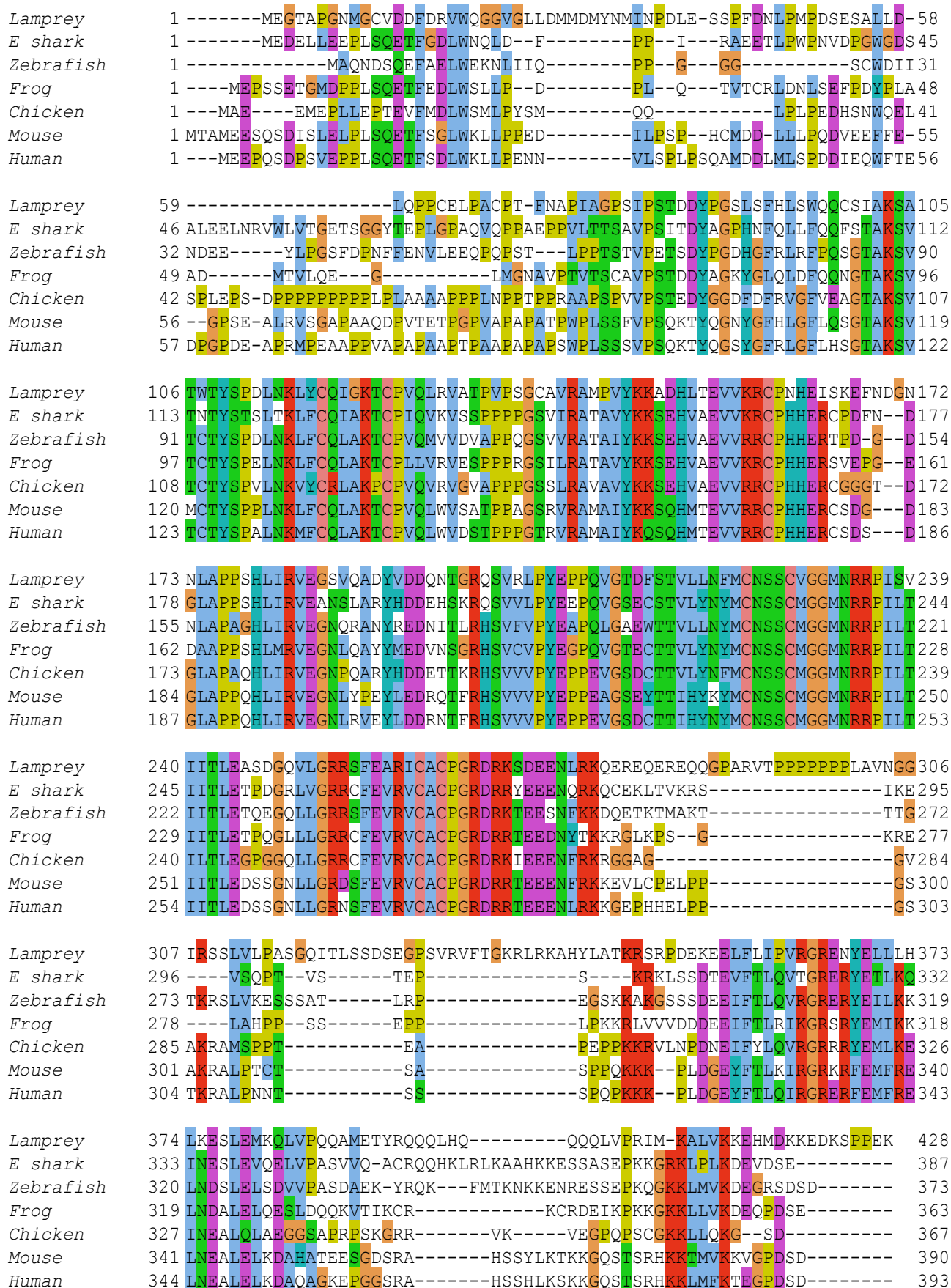
Figure S16.

(A) A surface presentation (pink) of the human crystal structure of HDM2^{Nterm} (residues 25–109) in complex with Nutlin (B) A surface presentation (green) of a homology model of the p53-binding region of Lj-Mdm2^{Nterm}, in complex with Nutlin. Models were generated based on the HDM2^{Nterm} structure (residues 17-112) from the PDB structure 3JZR (Phan et al. 2010).

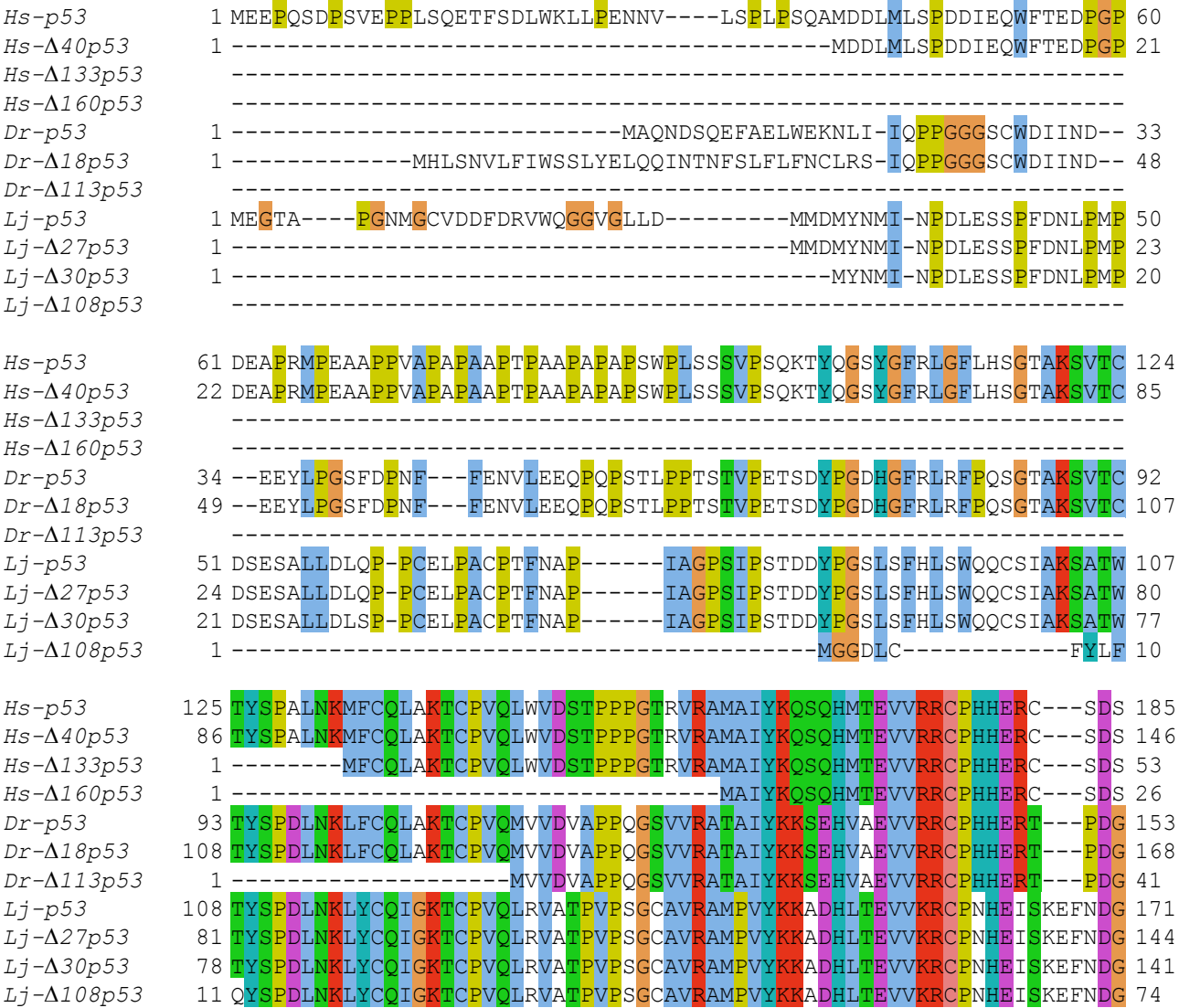
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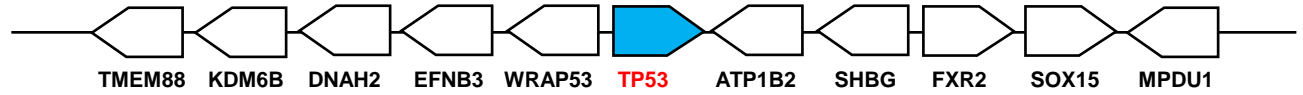
Coffill and Lee et al. Fig S1



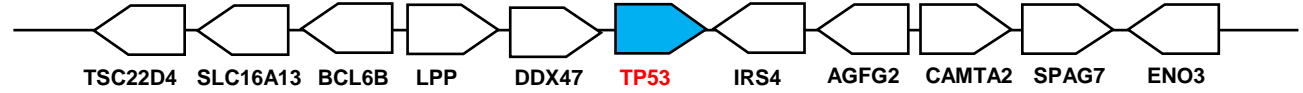
Coffill and Lee *et al.* Fig S2

TP53 locus

Human chr17



Coelacanth Scaffold_JH127111

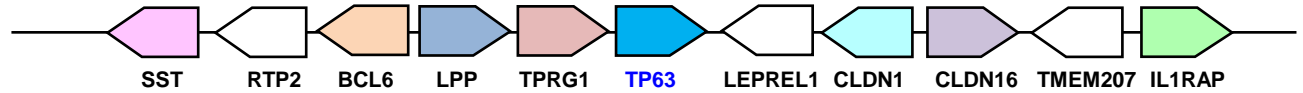


Jlamprey Scaffold_216

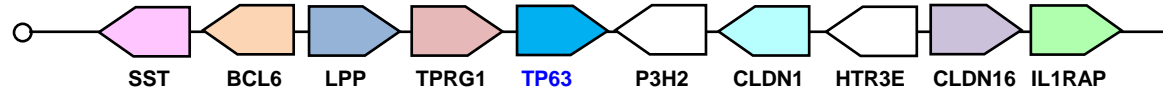


TP63 locus

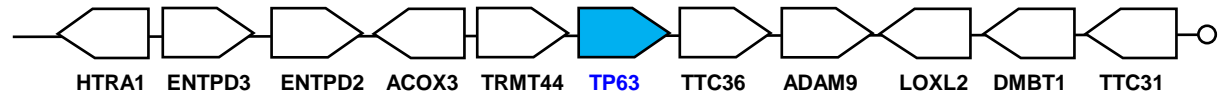
Human chr3



Coelacanth Scaffold_JH126687

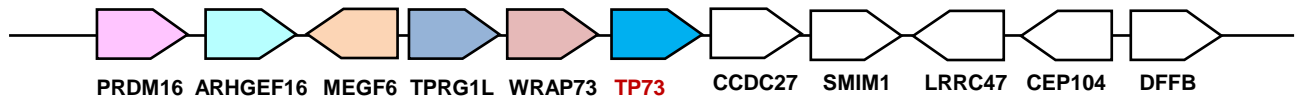


Jlamprey Scaffold_21

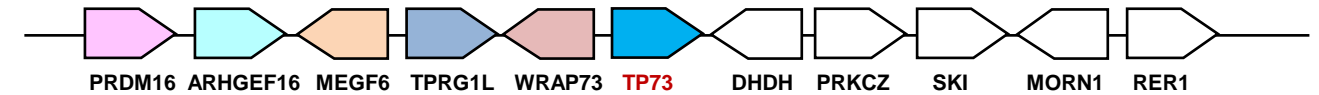


TP73 locus

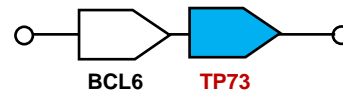
Human chr1

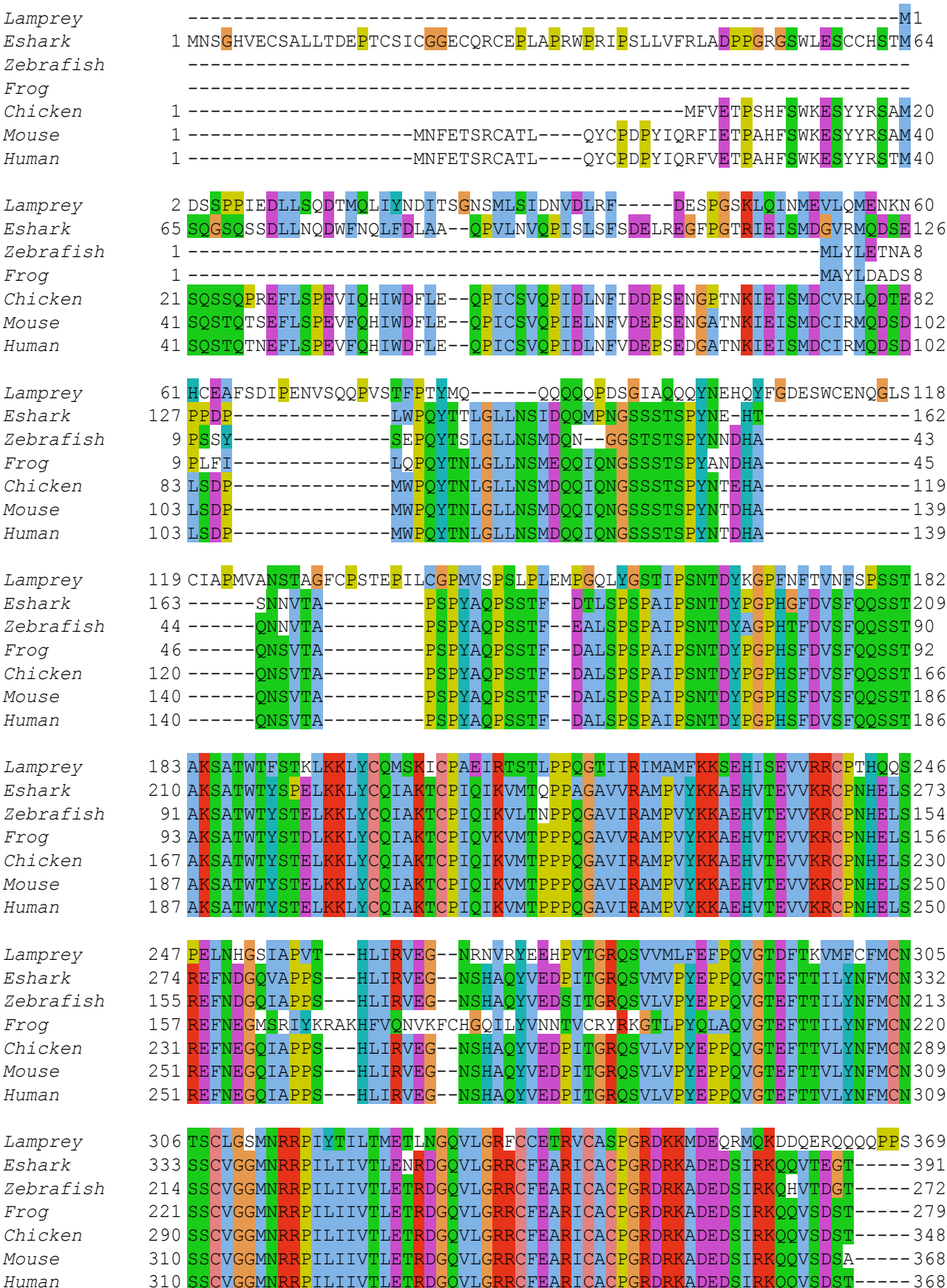


Elephant shark Scaffold_93

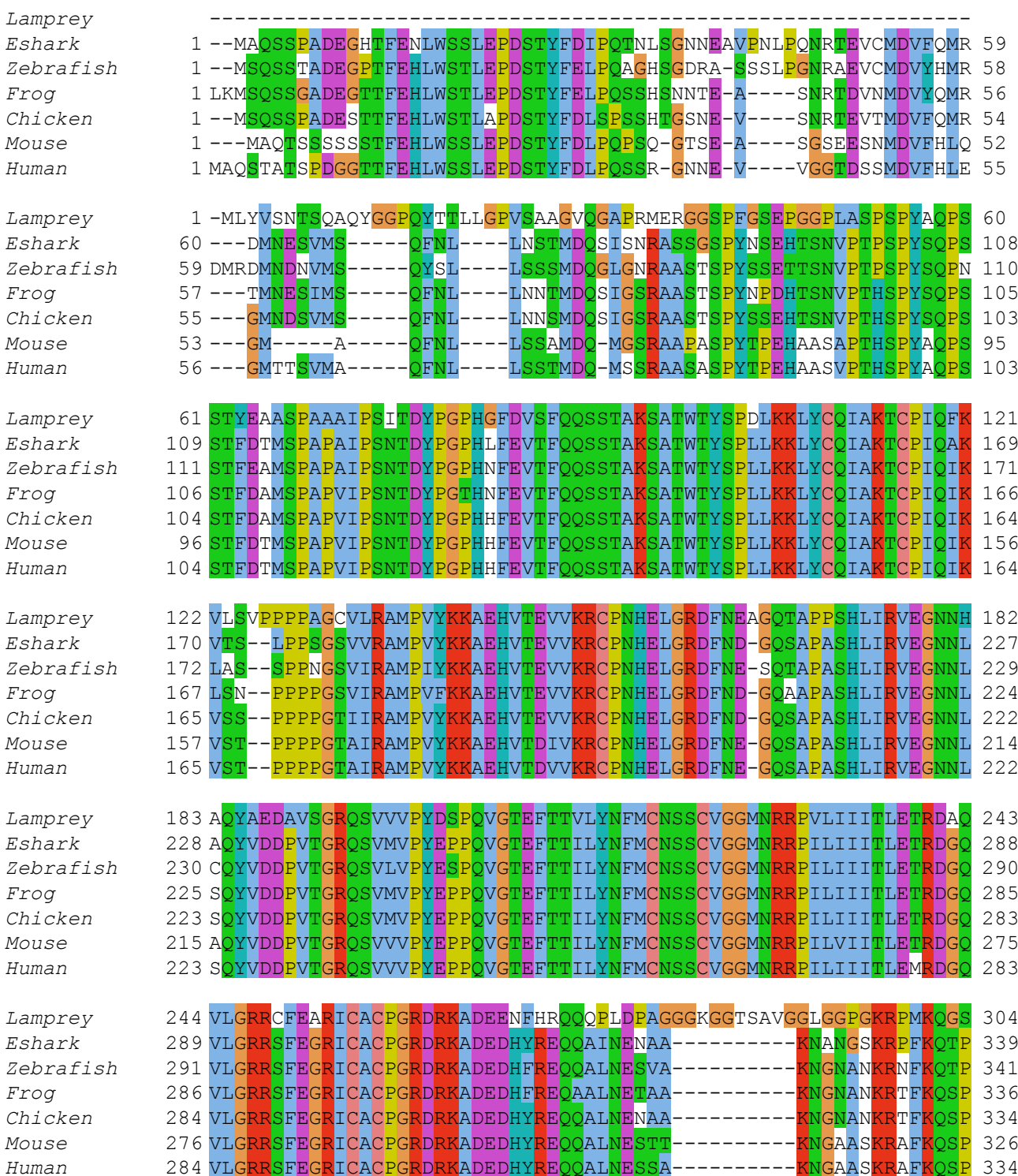


Jlamprey Scaffold_926





Coffill and Lee *et al.* Fig S5, Panel A

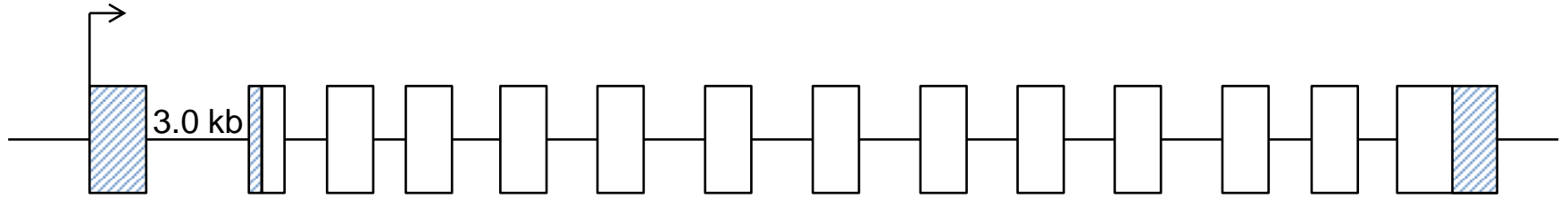


Coffill and Lee *et al.* Fig S6, Panel A

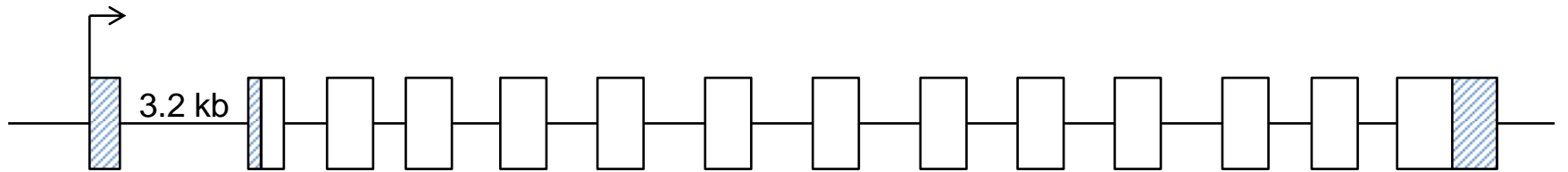
<i>Lamprey</i>	305	PGLH-TSSG	TKKRR	LGDDDV	YVYPV	HGREN	YEVLM	KIKES	LELS	SQFV	PSGAVE	AYRQ	QQQQ	364
<i>Eshark</i>	340	PGAQ	QPTAG	IKKRR	HGDEE	FFYP	PVRG	RENFE	ILMK	IKES	LELM	ELVP	QQLID	SYRQ
<i>Zebrafish</i>	342	TNIT	GPSIN	IKKRR	HGEE	EYYP	PVRG	RENFD	ILMK	IKDS	LELVE	FVPP	QQLVD	SYRQ
<i>Frog</i>	337	PSV	PSMGS	NIKKRR	HGEDE	IFYIP	PVRG	RENFE	ILMK	IKES	LELVEL	VLP	QQLVD	SYRQ
<i>Chicken</i>	335	QAI	PALGP	VKKRR	HGEE	EYYP	PVRG	RENFE	ILMK	IKES	LELVEL	VLP	QQLVD	SYRQ
<i>Mouse</i>	327	PAI	PALGT	NVKKRR	HGDE	DMFY	MHV	RGREN	FEIL	MKV	KESE	LELM	ELVP	QPLVD
<i>Human</i>	335	PAV	PALG	AVKKRR	HGDED	TYYL	QV	RGREN	FEIL	MKL	KESE	LELM	ELVP	QPLVD
<i>Lamprey</i>	365	LFQQ	PCDL	FPPSL	LRP	CLQS	QLPY	PGP	PL	MGPK	LPSV	SQFVG	-----	HGAMG
<i>Eshark</i>	401	QLL	QRQQ	-QASS	AYGA	-VNPP	MNKIP	-----	NV	NKL	PSV	NQLV	GPS	QHSP
<i>Zebrafish</i>	403	LLQR	ONH	VAS	PSSY	GT--	LNN	MNKI	HG--	PI	SKL	PSV	NQLV	TQQT
<i>Frog</i>	398	LLQR	O	THL	QST	TSSY	GP-	VLSP	PMN	KLHG	---	GINK	LPSV	NQLV
<i>Chicken</i>	396	LLQR	ON	BLQ	T	PSSY	GP-	VLSP	PMN	KAHG	---	GINK	LPSV	NQLV
<i>Mouse</i>	388	QLL	QR	PSH	LQ	PSSY	GP-	VLSP	PMN	KVHG	---	GVN	LPSV	NQLV
<i>Human</i>	394	QLL	QR	PSH	LQ	PSSY	GP-	VLSP	PMN	KVHG	---	GMN	LPSV	NQLV
<i>Lamprey</i>	414	TPML	NGHT	-----	GLH	GD	LN	GV	SST	QL-H	LPT	PTH	CTP	PPP
<i>Eshark</i>	455	PTLM	NHH	HHHH	YMP	PPNG	NM	GGN	PS	SQTAM	GPT	SHCT	PPPP	YNAD
<i>Zebrafish</i>	458	ANML	GG	-----	HHM	Q	SNG	DV	NGA	HQS	SQ	--IV	ST	SHCT
<i>Frog</i>	454	PSML	N	---	H--	PLQ	T	NGE	M	NGA	HSS	SQ	--MV	SG
<i>Chicken</i>	453	PGML	N	---	H--	PMQ	P	NGE	M	NGG	HSS	SQ	--MV	SG
<i>Mouse</i>	444	SGML	N	---	HGH	S	M	P	ANG	E	M	NGG	HSS	SQT
<i>Human</i>	450	PGML	NN	---	HGH	A	V	P	ANG	E	M	SSS	HSA	SQ
<i>Lamprey</i>	466	VDF	FT	SQ	GLQ	YANE	V	AQ	L	S	PQ	D	L	E
<i>Eshark</i>	516	LEY	FT	SQ	GLQ	TMY	H	LQ	N	L	S	M	E	D
<i>Zebrafish</i>	512	IDY	FT	SQ	GLQ	S	V	Y	H	LQ	N	L	S	M
<i>Frog</i>	508	IEY	FT	SQ	GLQ	N	I	Y	H	LQ	N	L	S	M
<i>Chicken</i>	507	IDY	FT	SQ	GLQ	N	I	Y	H	LQ	N	L	S	M
<i>Mouse</i>	500	IEC	FT	SQ	GLQ	S	I	Y	H	LQ	N	L	S	M
<i>Human</i>	506	IEY	FT	SQ	GLQ	S	I	Y	H	LQ	N	L	S	M
<i>Lamprey</i>	527	LQQ	HQQ	HQQ	QQQQ	QQQQ	QQQQ	QQQQ	QQ	QLL	QR	AS	GGG	LG
<i>Eshark</i>	570	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>Zebrafish</i>	565	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>Frog</i>	562	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>Chicken</i>	561	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>Mouse</i>	553	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>Human</i>	560	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>Lamprey</i>	588	VHFT	LR	Q	T	VQ	M	PR	-----	HD	W	V	E	Y
<i>Eshark</i>	592	VHFR	VR	R	H	T	I	T	P	N	R	G	-----	DD
<i>Zebrafish</i>	591	VHFR	VR	R	H	T	I	T	P	N	R	G	PA	---
<i>Frog</i>	588	VHFR	VR	R	H	T	I	T	P	N	R	G	GG	---
<i>Chicken</i>	587	VHFR	VR	R	H	T	I	T	P	N	R	G	A	---
<i>Mouse</i>	579	VHFR	VR	R	H	T	I	T	P	N	R	G	G	A
<i>Human</i>	586	VHFR	VR	R	H	T	I	T	P	N	R	G	G	P

Coffill and Lee *et al.* Fig S6, Panel B

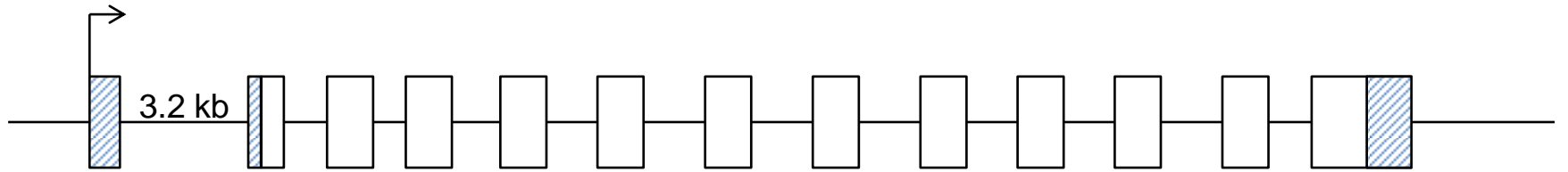
A
Lamprey *Tp63* isoform (3.1 kb)



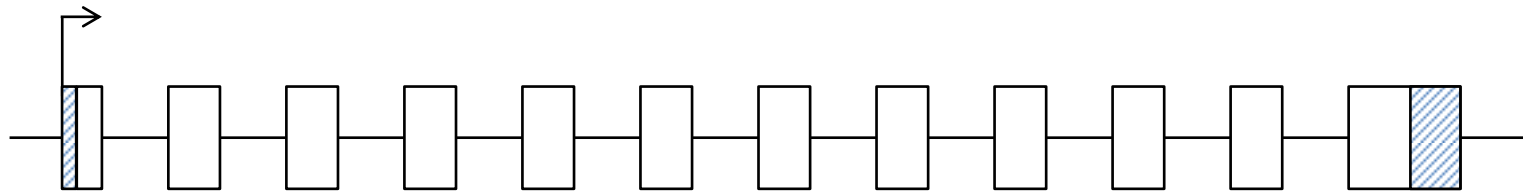
Lamprey *Tp63* isoform (2.8 kb)

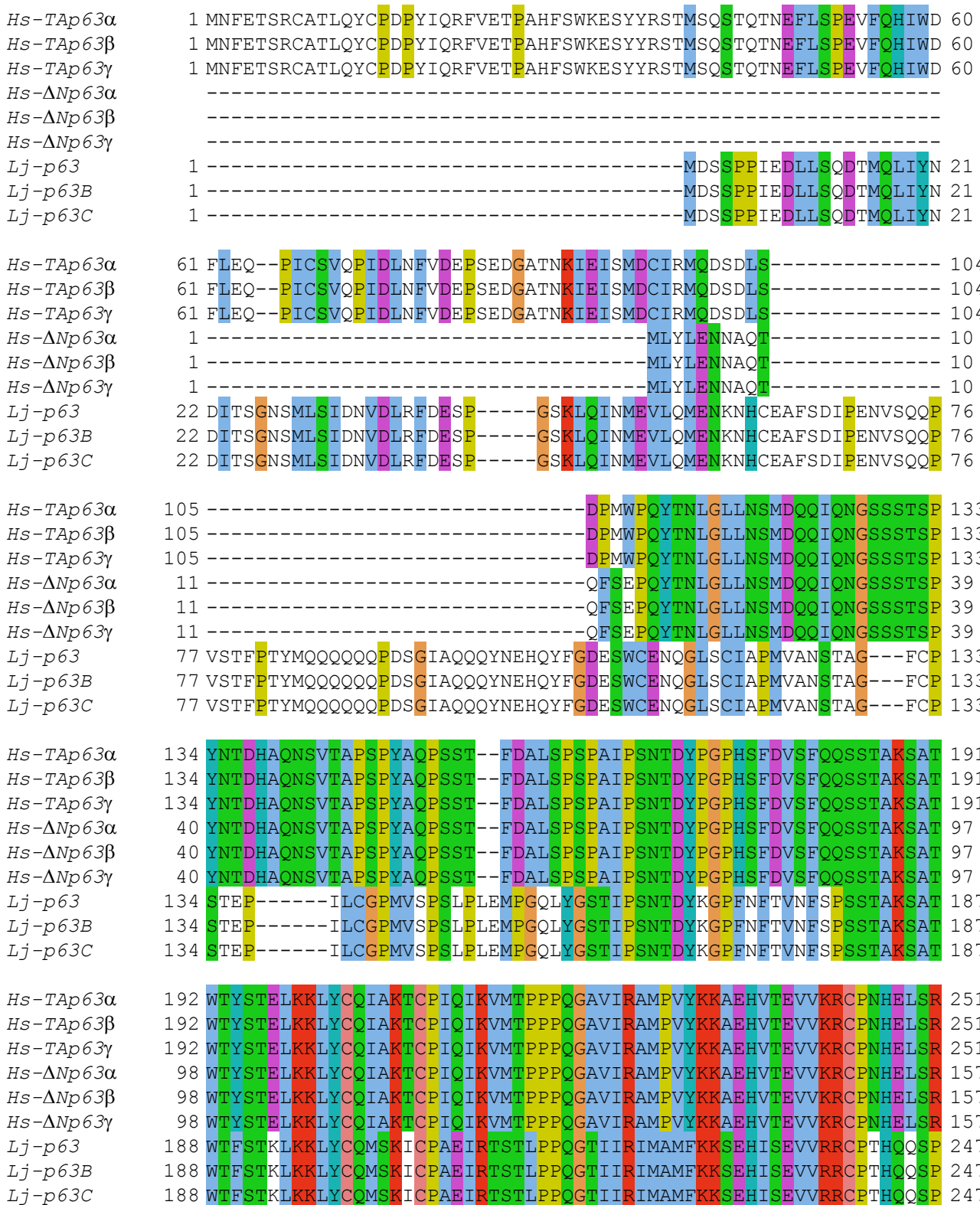


Lamprey *Tp63* isoform (2.6 kb)



B
Lamprey *Tp73* isoform (2.8 kb)





Coffill and Lee *et al.* Fig S8, Panel A

<i>Hs-TAp63α</i>	252	EFNEGQIAPPSHLIRVEGN	SHAQYVEDPITGRQSVLVPYEP	PQVGTEFTTVLYNFMCNSS	311
<i>Hs-TAp63β</i>	252	EFNEGQIAPPSHLIRVEGN	SHAQYVEDPITGRQSVLVPYEP	PQVGTEFTTVLYNFMCNSS	311
<i>Hs-TAp63γ</i>	252	EFNEGQIAPPSHLIRVEGN	SHAQYVEDPITGRQSVLVPYEP	PQVGTEFTTVLYNFMCNSS	311
<i>Hs-ΔNp63α</i>	158	EFNEGQIAPPSHLIRVEGN	SHAQYVEDPITGRQSVLVPYEP	PQVGTEFTTVLYNFMCNSS	217
<i>Hs-ΔNp63β</i>	158	EFNEGQIAPPSHLIRVEGN	SHAQYVEDPITGRQSVLVPYEP	PQVGTEFTTVLYNFMCNSS	217
<i>Hs-ΔNp63γ</i>	158	EFNEGQIAPPSHLIRVEGN	SHAQYVEDPITGRQSVLVPYEP	PQVGTEFTTVLYNFMCNSS	217
<i>Lj-p63</i>	248	ELNHGSIAPVTHLIRVEGN	RNRVRYEEHPVTGRQSVVMLF	FEFPQVGTDFTKVMFCFMCNTS	307
<i>Lj-p63B</i>	248	ELNHGSIAPVTHLIRVEGN	RNRVRYEEHPVTGRQSVVMLF	FEFPQVGTDFTKVMFCFMCNTS	307
<i>Lj-p63C</i>	248	ELNHGSIAPVTHLIRVEGN	RNRVRYEEHPVTGRQSVVMLF	FEFPQVGTDFTKVMFCFMCNTS	307
<i>Hs-TAp63α</i>	312	CVGGMNRRLPILIIVTLE	TRDGOVLGRRCFEARICAC	PGRDRKADEDSIRKQQVSD	STK-- 369
<i>Hs-TAp63β</i>	312	CVGGMNRRLPILIIVTLE	TRDGOVLGRRCFEARICAC	PGRDRKADEDSIRKQQVSD	STK-- 369
<i>Hs-TAp63γ</i>	312	CVGGMNRRLPILIIVTLE	TRDGOVLGRRCFEARICAC	PGRDRKADEDSIRKQQVSD	STK-- 369
<i>Hs-ΔNp63α</i>	218	CVGGMNRRLPILIIVTLE	TRDGOVLGRRCFEARICAC	PGRDRKADEDSIRKQQVSD	STK-- 275
<i>Hs-ΔNp63β</i>	218	CVGGMNRRLPILIIVTLE	TRDGOVLGRRCFEARICAC	PGRDRKADEDSIRKQQVSD	STK-- 275
<i>Hs-ΔNp63γ</i>	218	CVGGMNRRLPILIIVTLE	TRDGOVLGRRCFEARICAC	PGRDRKADEDSIRKQQVSD	STK-- 275
<i>Lj-p63</i>	308	CLGSMNRRLPIYTIL	TMETLNGOVLGRFCCE	TRVCASPRDKKMDHQ	RMOKDDQERQQQPF 367
<i>Lj-p63B</i>	308	CLGSMNRRLPIYTIL	TMETLNGOVLGRFCCE	TRVCASPRDKKMDHQ	RMOKDDQERQQQPF 367
<i>Lj-p63C</i>	308	CLGSMNRRLPIYTIL	TMETLNGOVLGRFCCE	TRVCASPRDKKMDHQ	RMOKDDQERQQQPF 367
<i>Hs-TAp63α</i>	370	-----NGD-----			372
<i>Hs-TAp63β</i>	370	-----NGD-----			372
<i>Hs-TAp63γ</i>	370	-----NGD-----			372
<i>Hs-ΔNp63α</i>	276	-----NGD-----			278
<i>Hs-ΔNp63β</i>	276	-----NGD-----			278
<i>Hs-ΔNp63γ</i>	276	-----NGD-----			278
<i>Lj-p63</i>	368	PSPTTQNSPTTQNS	PPTQNSPTTQNA	QSVQQYPTLAE	AETQTSQPTNQPPQAQFVEHTP 427
<i>Lj-p63B</i>	368	PSPTTQNS-----	PPTQNA	QSVQQYPTLAE	AETQTSQPTNQPPQAQFVEHTP 415
<i>Lj-p63C</i>	368	PSPTTQNS-----	PPTQNA	QSVQQYPTLAE	AETQTSQPTNQPPQAQFVEHTP 415
<i>Hs-TAp63α</i>	373	-----GTKRP-----	FRQNT	HGIQMTS--I--	KKRRSPDDELLYLPVRGR 408
<i>Hs-TAp63β</i>	373	-----GTKRP-----	FRQNT	HGIQMTS--I--	KKRRSPDDELLYLPVRGR 408
<i>Hs-TAp63γ</i>	373	-----GTKRP-----	FRQNT	HGIQMTS--I--	KKRRSPDDELLYLPVRGR 408
<i>Hs-ΔNp63α</i>	279	-----GTKRP-----	FRQNT	HGIQMTS--I--	KKRRSPDDELLYLPVRGR 314
<i>Hs-ΔNp63β</i>	279	-----GTKRP-----	FRQNT	HGIQMTS--I--	KKRRSPDDELLYLPVRGR 314
<i>Hs-ΔNp63γ</i>	279	-----GTKRP-----	FRQNT	HGIQMTS--I--	KKRRSPDDELLYLPVRGR 314
<i>Lj-p63</i>	428	QVPKSSSGSSQPP	SGEPTSDTSSQG	CCKVVDMLMIS	SNVGNKRPSDQEDIFPLLVOGR 487
<i>Lj-p63B</i>	416	QVPKSSSGSSQPP	SGEPTSDTSSQG	CCKVVDMLMIS	SNVGNKRPSDQEDIFPLLVOGR 474
<i>Lj-p63C</i>	416	QVPKSSSGSSQPP	SGEPTSDTSSQG	CCKVVDMLMIS	SNVGNKRPSDQEDIFPLLVOGR 474
<i>Hs-TAp63α</i>	409	ETYEMLLKIKESLE	LMQYLPQHTIETYR	QQQQQHQHLLQK	QTSIQSP----- 456
<i>Hs-TAp63β</i>	409	ETYEMLLKIKESLE	LMQYLPQHTIETYR	QQQQQHQHLLQK	QTSIQSP----- 456
<i>Hs-TAp63γ</i>	409	ETYEMLLKIKESLE	LMQYLPQHTIETYR	QQQQQHQHLLQK	HLLSACFARNELVEPRRETP 468
<i>Hs-ΔNp63α</i>	315	ETYEMLLKIKESLE	LMQYLPQHTIETYR	QQQQQHQHLLQK	QTSIQSP----- 362
<i>Hs-ΔNp63β</i>	315	ETYEMLLKIKESLE	LMQYLPQHTIETYR	QQQQQHQHLLQK	QTSIQSP----- 362
<i>Hs-ΔNp63γ</i>	315	ETYEMLLKIKESLE	LMQYLPQHTIETYR	QQQQQHQHLLQK	HLLSACFARNELVEPRRETP 374
<i>Lj-p63</i>	488	ENFEILKKIKESLE	LMRMLPKD	TNVVLRNLQ	QKRYMMELRGRPASLS----- 534
<i>Lj-p63B</i>	475	ENFEILKKIKESLE	LMRMLPKD	TNVVLRNLQ	QKRYMMELRGRPASLS----- 521
<i>Lj-p63C</i>	475	ENFEILKKIKESLE	LMRMLPKD	TNVVLRNLQ	QKRYMMELRGRPASLS----- 521

Coffill and Lee *et al.* Fig S8, Panel B

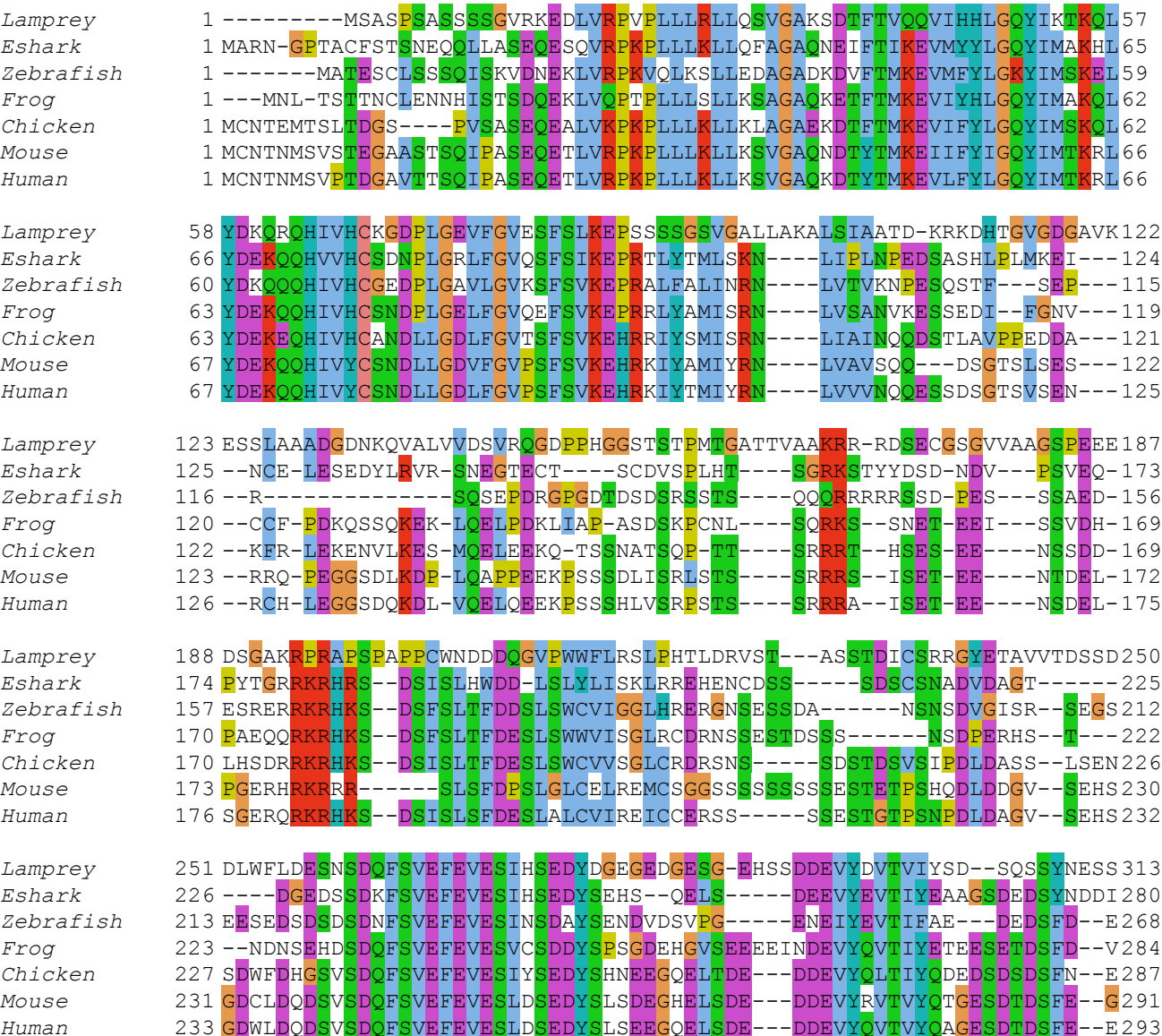
Hs-TAp63 α 457 ----SSYGNSSPPLNKMNSMNKLP---SVSQLINPQQRNALPFTTIIPDGMGAN--IPMMGTHM 510
Hs-TAp63 β 457 ----SSYGNSSPPLNKMNSMNKLP---SVSQLINPQQRNALPFTTIIPDGMGAN--IPMMGTHM 510
Hs-TAp63 γ 469 KQSDVFFRHSKPPNRSVYP----- 487
Hs- Δ Np63 α 363 ----SSYGNSSPPLNKMNSMNKLP---SVSQLINPQQRNALPFTTIIPDGMGAN--IPMMGTHM 416
Hs- Δ Np63 β 363 ----SSYGNSSPPLNKMNSMNKLP---SVSQLINPQQRNALPFTTIIPDGMGAN--IPMMGTHM 416
Hs- Δ Np63 γ 375 KQSDVFFRHSKPPNRSVYP----- 393
Lj-p63 535 -----RHGSEC-----SQEVLINGSQGS-----SSAGVGSSSGVAQNGLTV 569
Lj-p63B 522 -----RHGSEC-----SQEVLINGSQGS-----SSAGVGSSSGVAQNGLTV 556
Lj-p63C 522 -----RHGSEC-----SQEVLINGSQGS-----SSAGVGSSSGVAQNGLTV 556

Hs-TAp63 α 511 PMAGDMNGL--SPTQALPPPLSMPSTSHCTPPPPYPFTDCSIVSFLARLGCSSCLDYFTTQ 568
Hs-TAp63 β 511 PMAGDMNGL--SPTQALPPPLSMPSTSHCTPPPPYPFTDCSIVRIWQV----- 555
Hs-TAp63 γ ----- 555
Hs- Δ Np63 α 417 PMAGDMNGL--SPTQALPPPLSMPSTSHCTPPPPYPFTDCSIVSFLARLGCSSCLDYFTTQ 474
Hs- Δ Np63 β 417 PMAGDMNGL--SPTQALPPPLSMPSTSHCTPPPPYPFTDCSIVRIWQV----- 461
Hs- Δ Np63 γ ----- 461
Lj-p63 570 VEANGSNGPPRSPDGDSPPA--WP---ETLPDSSKPQNTISNFLKQMDCFEFLENFTSR 623
Lj-p63B 557 VEANGSNGPPRSPDGDSPPA--WP---ETLPDSSKPQNTISNFLKQMDCFEFLENFTSR 610
Lj-p63C 557 VEANGSNGPPRSPDGDSPPA--WP---ETLPDSSKPQNTISNFLKQMDCFEFLENFTSR 610

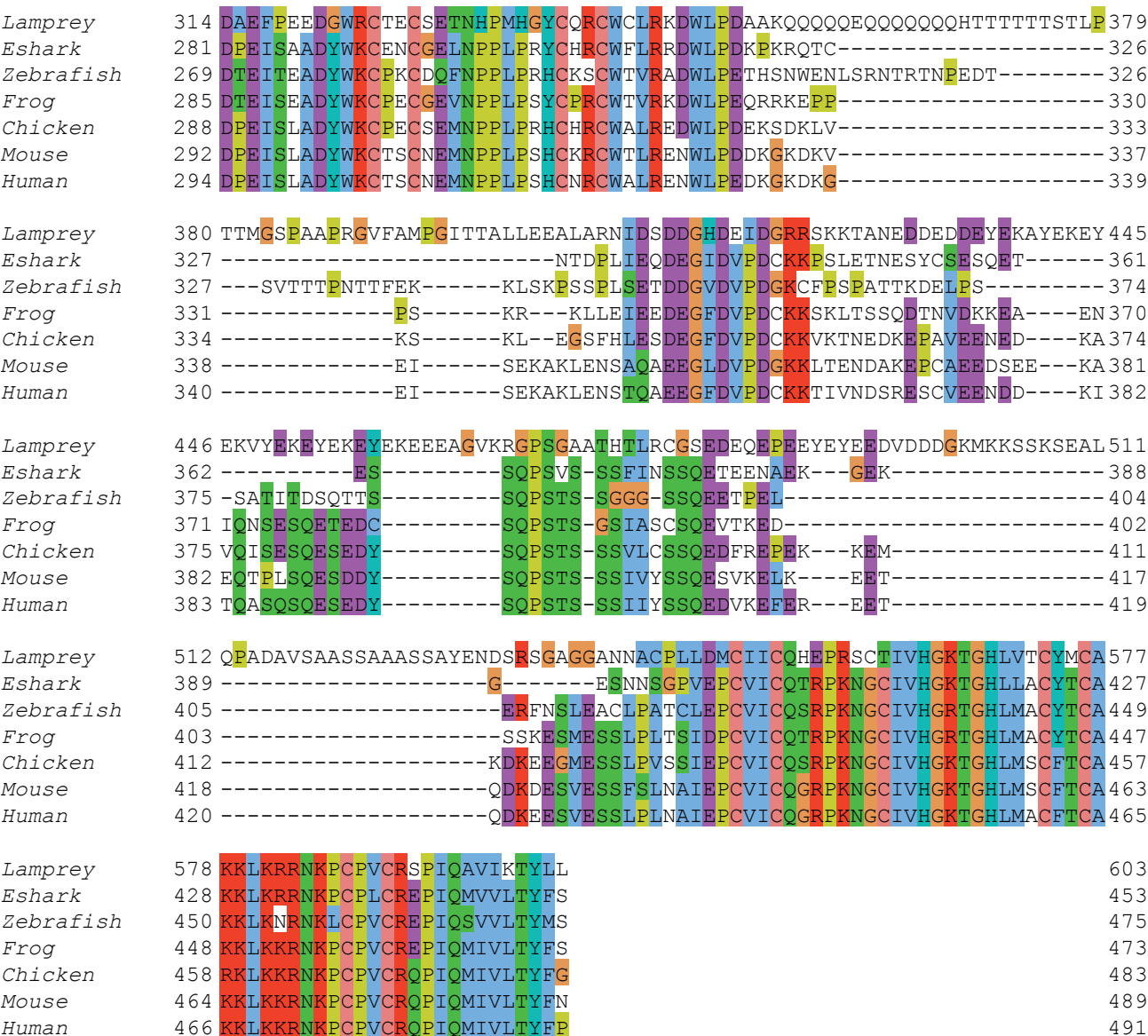
Hs-TAp63 α 569 GLTIIYQIEHYSMDDLASLKIPEQFRHAIWKGILDHRQLHEFSSPSHLLRTPSSASTVSV 628
Hs-TAp63 β ----- 628
Hs-TAp63 γ ----- 628
Hs- Δ Np63 α 475 GLTIIYQIEHYSMDDLASLKIPEQFRHAIWKGILDHRQLHEFSSPSHLLRTPSSASTVSV 534
Hs- Δ Np63 β ----- 534
Hs- Δ Np63 γ ----- 534
Lj-p63 624 GLLSLQQLKNFTLQDLDKLGVPEAKRQLLSG IQEHRVSTTIFSKTTLKRS PSEETVHLS 683
Lj-p63B 611 GLLSLQQLKNFTLQDLDKLGVPEAKRQLLSG IQEHRVSTTIFSKTTLKRS PSEETVHLS 670
Lj-p63C 611 GLLSLQQLKNFTLQVSS-FGRFHANYVFFFTYTGLGFI FKL----- 650

Hs-TAp63 α 629 GSSETRGERVI-----DAVRFTLRQTISFP PPRD 656
Hs-TAp63 β ----- 656
Hs-TAp63 γ ----- 656
Hs- Δ Np63 α 535 GSSETRGERVI-----DAVRFTLRQTISFP PPRD 562
Hs- Δ Np63 β ----- 562
Hs- Δ Np63 γ ----- 562
Lj-p63 684 PEDDDNGIDTKDGI RAKVFPALQASGNLSSVSNHSHNSGISKYIFRVHARQTIYLP RSG 743
Lj-p63B 671 PEDDDNGIDTKDGI RAKVFPALQASGNLSSVSNHSHNSGISKYIFRVHARQTIYLP RSG 730
Lj-p63C ----- 730

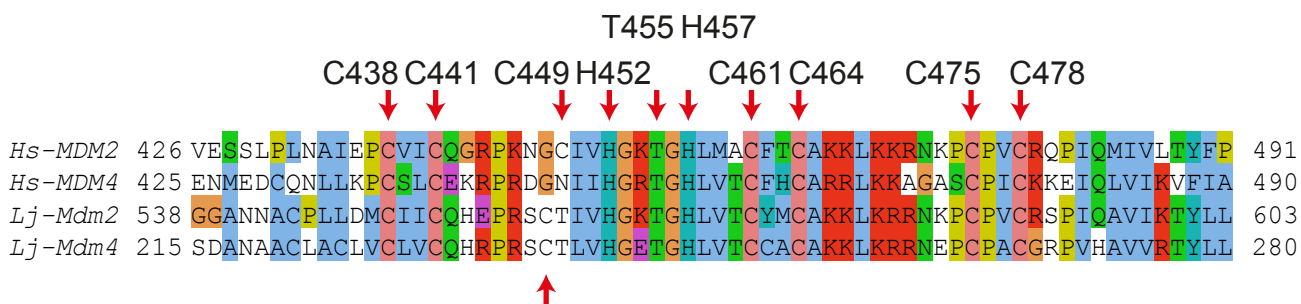
Hs-TAp63 α 657 EWNDFNFMDARRNKQQRICEEGE----- 680
Hs-TAp63 β ----- 680
Hs-TAp63 γ ----- 680
Hs- Δ Np63 α 563 EWNDFNFMDARRNKQQRICEEGE----- 586
Hs- Δ Np63 β ----- 586
Hs- Δ Np63 γ ----- 586
Lj-p63 744 SWGGSSSL-EVPRSKMRKLSSCS DTEEEEERLE 774
Lj-p63B 731 SWGGSSSL-EVPRSKMRKLSSCS DTEEEEERLE 761
Lj-p63C ----- 761



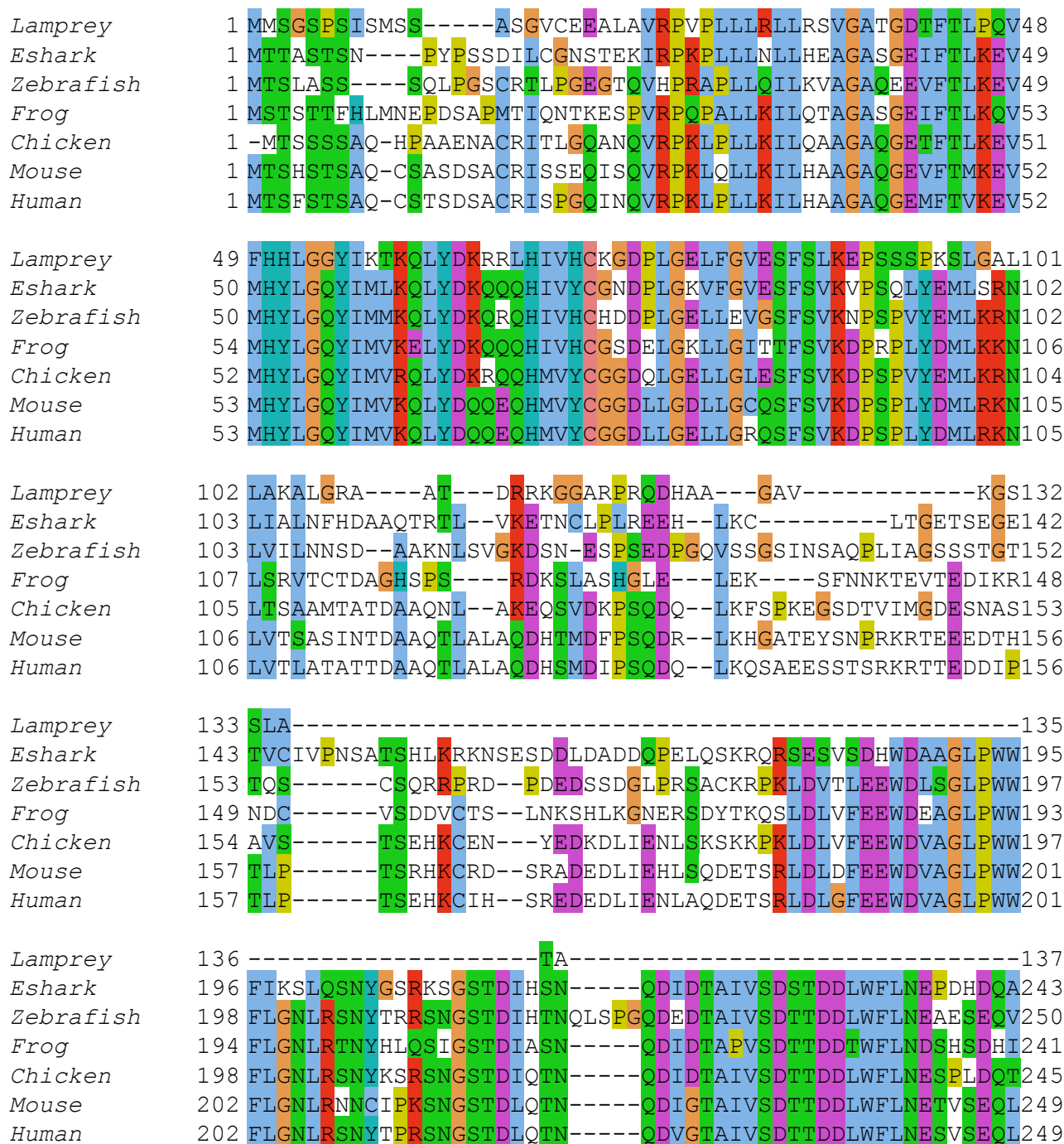
Coffill and Lee *et al.* Fig S9, Panel A



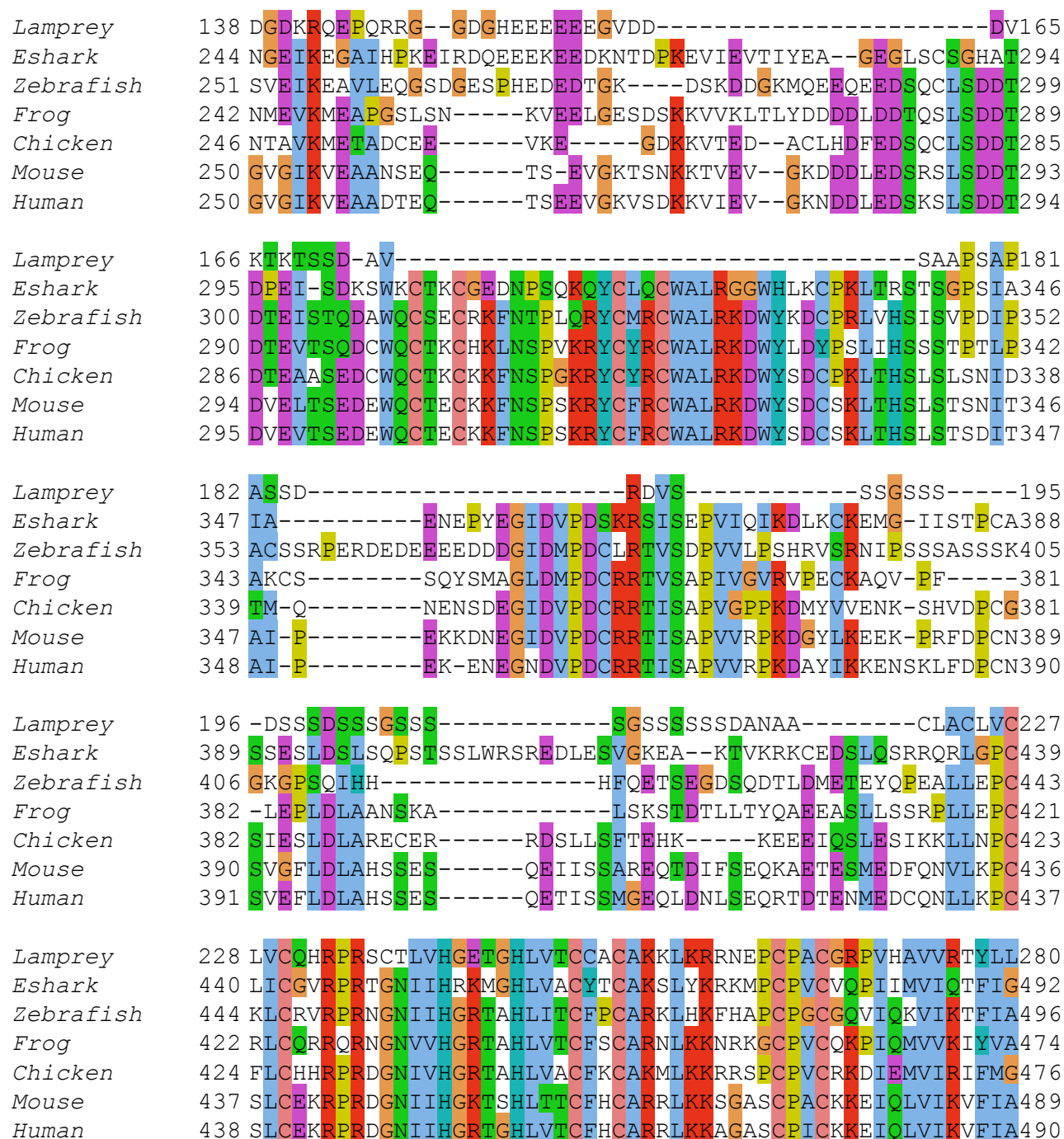
Coffill and Lee *et al.* Fig S9, Panel B



Coffill and Lee *et al.* Fig S10

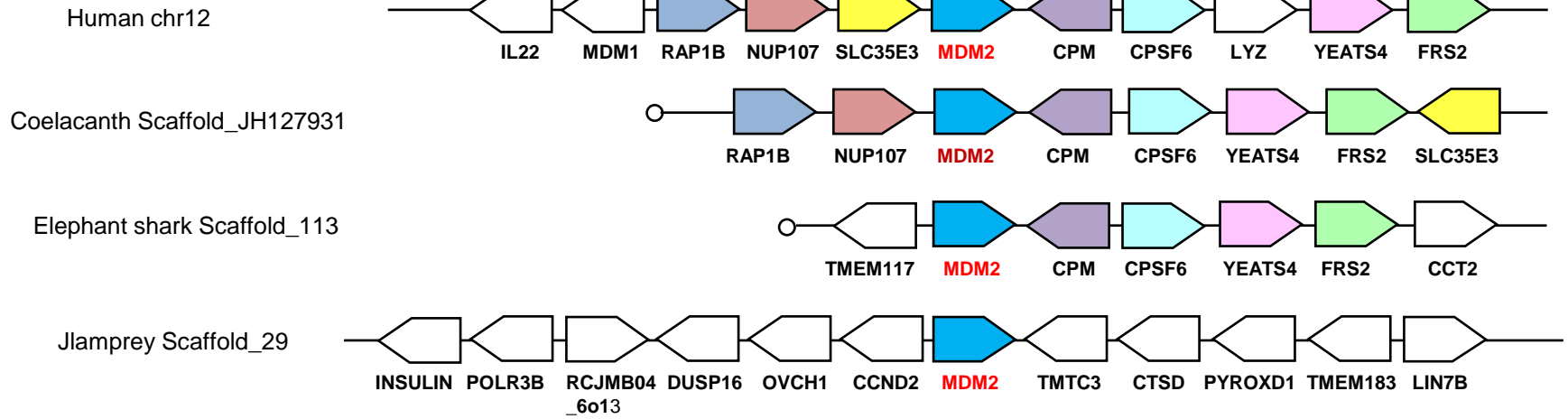


Coffill and Lee *et al.* Fig S11, Panel A

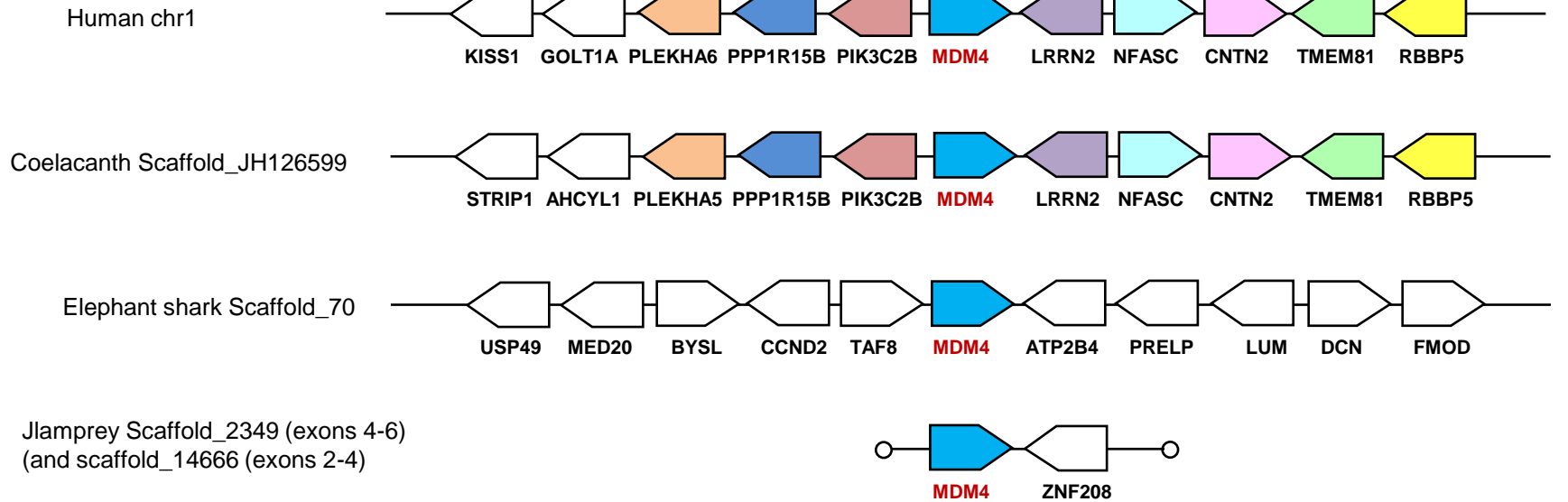


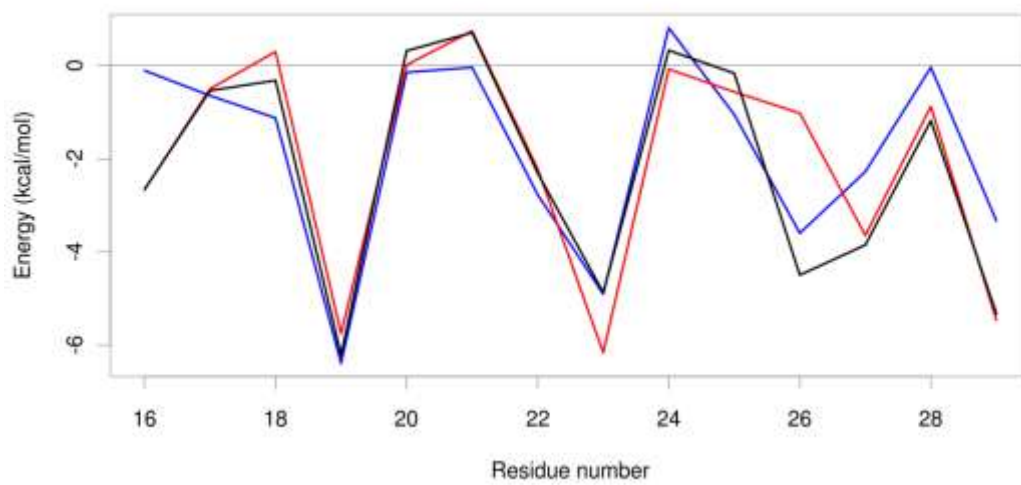
Coffill and Lee *et al.* Fig S11, Panel B

Mdm2 locus



Mdm4 locus





Coffill and Lee *et al.* Fig S13

A

S¹⁵QETFSDLWKLL²⁶PEN
Hp53¹⁶⁻²⁹

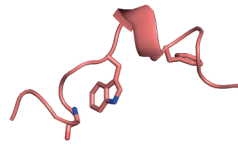
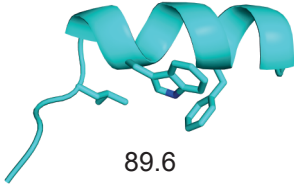
B

C¹¹VDDFDRVWQGG²²VGL
Lp53¹²⁻²⁵

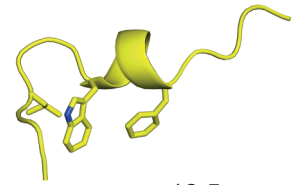
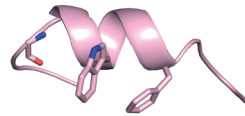
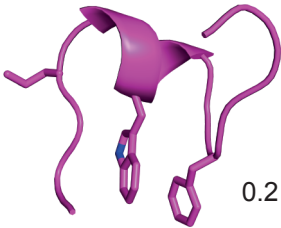
C

C¹¹VDDFDRVWQGL²²VGL
Lp53^{12-25(G22L)}

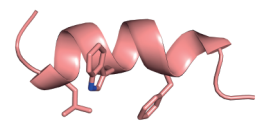
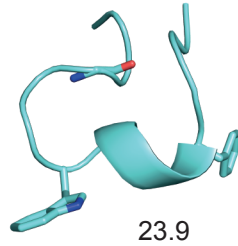
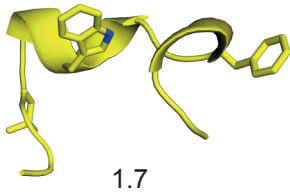
1



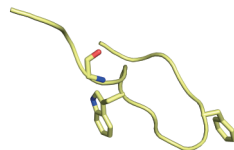
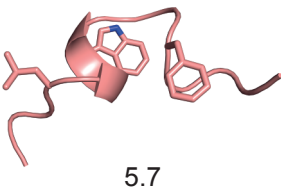
2



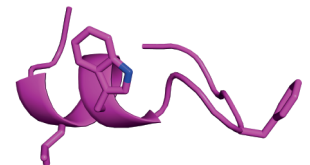
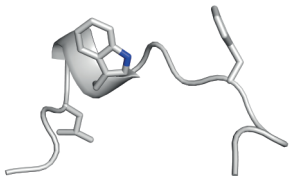
3



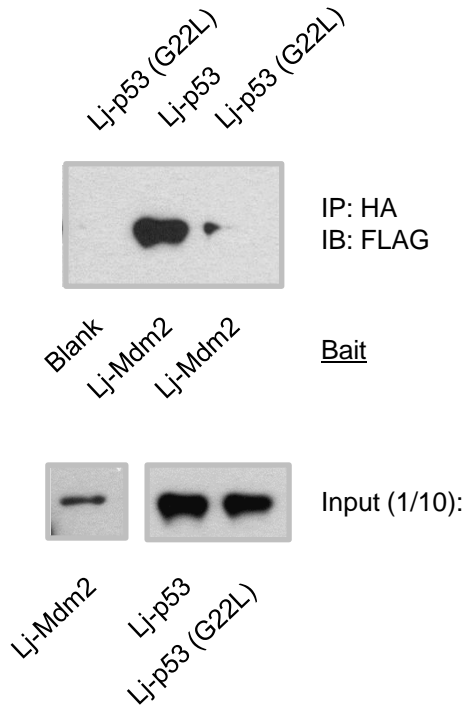
4



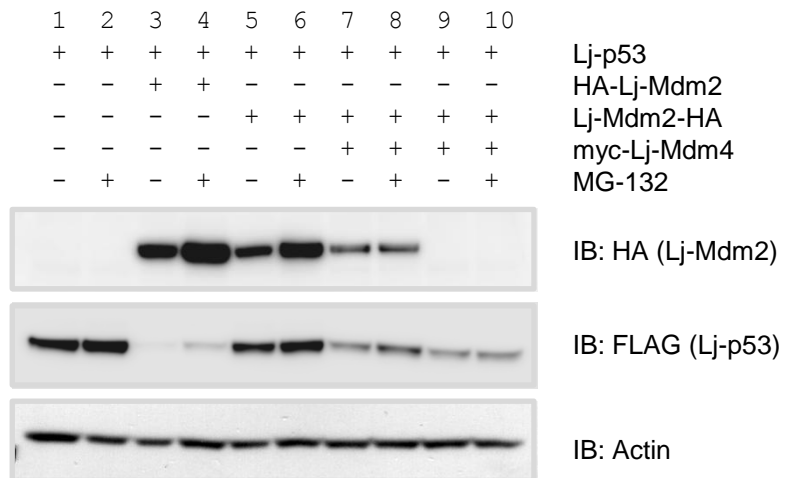
5



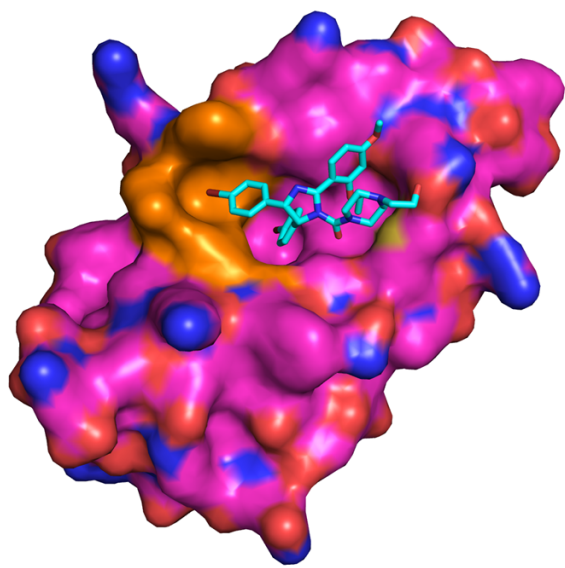
A



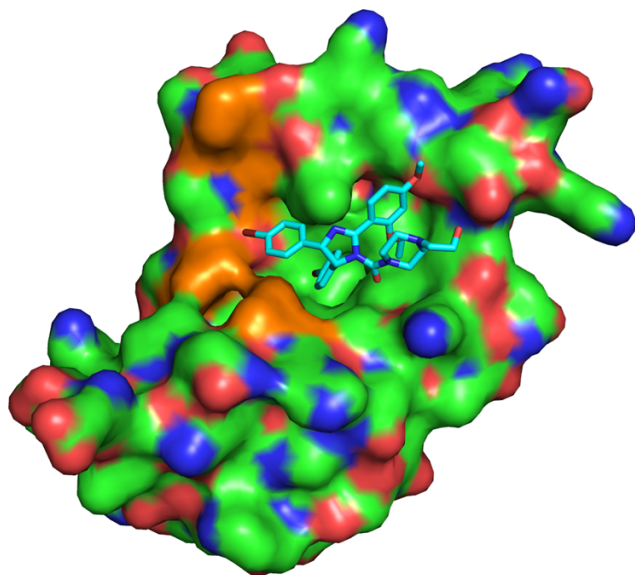
B



A



B



Coffill and Lee *et al.* Fig S16