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 (1 atm) 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\(^{\text{Nterm}}\) 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 Å$^{-2}$ and $\beta$ 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$^{\text{Nterm}}$ binding to the Lamprey peptides was determined using the BLItz (ForteBio, USA) system. The purified HDM2$^{\text{Nterm}}$ 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 2.5µM 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$^{\text{Nterm}}$ (3x serial dilutions from 250µM) 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\(\mu\)M HDM2\(^{\text{Nterm}}\) to ensure no/low binding of HDM2\(^{\text{Nterm}}\) to the uncoated sensor and a coated sample reference (peptide but without HDM2\(^{\text{Nterm}}\) 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<sup>Nterm</sup> complexes.

| Ligand     | Peptide sequence, if applicable | ΔH<sub>bind</sub> | TΔS<sub>bind</sub> | ΔG<sub>bind</sub> |
|------------|---------------------------------|-------------------|--------------------|-------------------|
| nutlin-3a  | N.A.                            | -82.36            | -24.08             | -58.29            |
| Hp53<sup>16-29</sup> | Ac-QETFSDLWKLLPEN-NH<sub>2</sub> | -80.39            | -37.21             | -43.18            |
| Hp53<sup>16-29(AAA)</sup> | Ac-QETASDLAKLAPEN-NH<sub>2</sub> | -79.30            | -40.37             | -38.92            |
| Lp53<sup>12-25</sup> | Ac-VDDFDRVWQGGVGL-NH<sub>2</sub> | -80.61            | -40.81             | -39.80            |
| Lp53<sup>12-25(G22L)</sup> | Ac-VDDFDRVWQGLVGL-NH<sub>2</sub> | -85.63            | -37.48             | -48.15            |

### Table S2. Experimental determination of binding affinities for peptide and HDM2<sup>Nterm</sup> complexes

| Peptide     | Sequence            | K<sub>D</sub> (µM)<sup>A</sup> | K<sub>D</sub> (µM)<sup>B</sup> |
|-------------|---------------------|-------------------------------|-------------------------------|
| Hp53<sup>16-29</sup> | QETFSDLWKLLPEN       | 2.7 ± 0.5                     | 1.3                           |
| Hp53<sup>16-29(AAA)</sup> | QETASDLAKLAPEN      | nd                           | nd                           |
| Lp53<sup>12-25</sup> | VDDFDRVWQGGVGL      | nd                           | nd                           |
| Lp53<sup>12-25(G22L)</sup> | VDDFDRVWQGLVGL     | 41 ± 3                       | 33                           |

<sup>A</sup> determined by Fluorescence Anisotropy. Data are averages of at least four replicates ± SEM.

<sup>B</sup> 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-RFMDYWEGLNH$_2$      | Human    | -                  | (Bottger et al. 1997)|
| Hs-p53$^{16-29}$ | Ac-QETFSDLWKLLPEN-NH$_2$                     |          | NP_000537.3        |                      |
|             | Biotin-SGSG-QETFSDLWKLLPEN-NH$_2$ |          |                    |                      |
| Hs-p53$^{16-29$(AAA) | Ac-QETASDLAKLAPEN-NH$_2$                  |          |                    |                      |
|             | Biotin-SGSG-QETASDLAKLAPEN-NH$_2$ |          |                    |                      |
| Lj-p53$^{12-25}$ | Ac-VDDFDRVWQGGVGL-NH$_2$                   | Lamprey  | KT960978           |                      |
|             | Biotin-SGSG-VDDFDRVWQGGVGL-NH$_2$ |          |                    |                      |
| 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; NH$_2$ = 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 (3xFLAG)  | 1-393       | 3xFLAG  | pCI-neo    | N'    | Human    | (Coffill et al. 2012) |
| Hs-p53Δ          | 1-355       | -       | pcDNA3     | -     | Human    | Q00987             |                      |
| HDM2             | 1-491       | -       | pCMV       | -     | Human    |                    |                      |
| HDM2<sup>Nterm</sup> | 1-125      | HA      | pcDNA3     | C'    | Human    |                    | (Bottger et al. 1997; Brown et al. 2013; Chee et al. 2014) |
| GST-HDM2<sup>Nterm</sup> | 1-125 or 6-125 | GST-precision | pGEX-6P-1 | N'    | Human    |                    |                      |
| Hs-RPL11         | 1-178       | FLAG    | pcDNA3     | N'    | Human    | NP_000966.2        |                      |
| Hs-RPL5          | 1-297       | FLAG    | pcDNA3     | N'    | Human    | NP_000960.2        |                      |
| Lj-p53           | 1-428       | 3xFLAG  | pCI-neo    | N'    | Lamprey  | KT960978           |                      |
| Lj-p53           | 1-428       | FLAG    | pcDNA3     | N'    | Lamprey  |                    |                      |
| Lj-p53(G22L)     | 1-428 (G22L)| FLAG    | pcDNA3     | N'    | Lamprey  |                    |                      |
| Lj-p53Δ          | 1-390       | FLAG    | pcDNA3     | N'    | Lamprey  |                    |                      |
| Lj-Mdm2          | 1-603       | HA      | pXJ40      | N'    | Lamprey  | KT960981           |                      |
| Lj-Mdm2(C464A)   | 1-603(C576A)| HA      | pXJ40      | N'    | Lamprey  |                    |                      |
| Lj-Mdm2          | 1-603       | HA      | pCMV       | C'    | Lamprey  |                    |                      |
| Lj-Mdm2          | 1-603       | HA      | pcDNA3     | C'    | Lamprey  |                    |                      |
| Lj-Mdm2<sup>Nterm</sup> | 1-106   | HA      | pcDNA3     | C'    | Lamprey  |                    |                      |
| Lj-Mdm4          | 1-280       | myc     | pCMV       | N'    | Lamprey  | KT960982           |                      |
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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-Δ40p53 (NP_001119590.1); Hs-Δ133p53 (NP_001119587.1);
Hs-Δ160p53 (NP_001263626.1); Dr-p53 (P79734); Dr-Δ18p53 (see (Davidson et al. 2010)); Dr-
Δ113p53 (see (Marcel et al. 2011)); Lj-p53 (KT960978); Lj-Δ27p53; Lj-Δ30p53; Lj-Δ108p53.

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-TAp63α
(NP_003713.3); Hs-TAp63β (NP_001108450.1); Hs-TAp63γ (NP_001108451.1);
Hs-ΔNp63α (NP_001108452.1); Hs-ΔNp63β (NP_001108453.1); Hs-ΔNp63γ
(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-p53^{16-29} (blue), Lj-p53^{12-25} (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 9 + 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 S3
| Organism   | Sequence  | Identity  |
|------------|-----------|-----------|
| Lamprey    | MNSHVECSALTDEITCSICTGECQRCHELARWPRIPSSLVFRLAPPGGRSMWLCCHTM64 | 100% |
| Eshark     | MFSVSTSHSWKESYRYSAM20 | 99%  |
| Zebrafish  | MFSVSTSHSWKESYRYSAM40 | 99%  |
| Frog       | MFVETSHSWKESYRYSAM40 | 99%  |
| Chicken    | MNFETSRCATL--QYCPDLTIQRPISTPAHESWKEYRYSAM40 | 99%  |
| Mouse      | MNFETSRCATL--QYCPDLTIQRPISTPAHESWKEYRYSAM40 | 99%  |
| Human      | MNFETSRCATL--QYCPDLTIQRPISTPAHESWKEYRYSAM40 | 99%  |
| Lamprey    | DSSPPIEDDLKQDTMILITNIDTSGNSMLESINQHDNVDIF--DEPSGSQ toes | 89%  |
| Eshark     | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Zebrafish  | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Frog       | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Chicken    | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Mouse      | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Human      | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Lamprey    | DSSPPIEDDLKQDTMILITNIDTSGNSMLESINQHDNVDIF--DEPSGSQ toes | 89%  |
| Eshark     | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Zebrafish  | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Frog       | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Chicken    | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Mouse      | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Human      | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Lamprey    | DSSPPIEDDLKQDTMILITNIDTSGNSMLESINQHDNVDIF--DEPSGSQ toes | 89%  |
| Eshark     | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Zebrafish  | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Frog       | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Chicken    | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Mouse      | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |
| Human      | SQGQSSDLPNQDENLQPLELPPIAIPPSSDEPGEPIEIBIMSMDVRLOQDEB2 | 89%  |

Coffil and Lee et al. Fig S5, Panel A
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 S7
Coffill and Lee et al. Fig S8, Panel A
Coffill and Lee et al. Fig S8, Panel C
Coffill and Lee et al. Fig S10
| Species   | Sequence  |
|-----------|-----------|
| Lamprey   | MMSGSPS1MSMSS-----ASGVCEELAVRPVFLLLRLLRSVGATGD1F1LPPQV48 |
| Eshark    | MTIASTSN-----EYPSSDLIDCNSTEKRPRPKLLINLHAGEAGSEIFLTKEV49 |
| Zebrafish | MTIALASS-----QLPGSCRTLLPGEOTVHPAPPLQILKLVAGAEEVFTLKEV49 |
| Frog      | MTSST1FHLNQEDSMATQINTKESVRPQPOLKLLQIALAQAGSEIFLTQK53 |
| Chicken   | MTSST1SFHLNQEDSMATQINTKESVRPQPOLKLLQIALAQAGSEIFLTQK53 |
| Mouse     | 1MTSHSTSAQ-C1ASDSACRISSEQI5SQRPPLQQLLKILHAGAEvFTMKEV52 |
| Human     | 1MTSHSTSAQ-C1ATS1ASACRISSEQI5SQRPPLQQLLKILHAGAEvFTMKEV52 |

| Species   | Sequence  |
|-----------|-----------|
| Lamprey   | 49FHHLLGYYIKKTKOLYYDRRLHHIVHCKGDPLGELFGVESFLKEPSPPSSPKSLGA101 |
| Eshark    | 50MHLGQYIMLKOLYYDKOQHIVCHGNDPLGKVGEVESFLKEPSPPSLQYEMSLR102 |
| Zebrafish | 50MHYLGQYIMMKOLYYDKQKIRHIVCHCDDPLGELLEGVESFLKPSPPVMEMLRK102 |
| Frog      | 54MHLGQYIMMKOLYYDKOQHIVCHGNDPLGKVGEVESFLKEPSPPSLQYEMSLR106 |
| Chicken   | 52MHLGQYIMVKQOLYYDKQKIRHIVCHCDDPLGELLEGVESFLKPSPPVMEMLRK104 |
| Mouse     | 53MHLGQYIMVKQOLYYDKQKIRHIVCHCDDPLGELLEGVESFLKPSPPVMEMLRK105 |
| Human     | 53MHLGQYIMVKQOLYYDKQKIRHIVCHCDDPLGELLEGVESFLKPSPPVMEMLRK105 |

| Species   | Sequence  |
|-----------|-----------|
| Lamprey   | 102LAKALGRA-----AK-----DARKKQGARPFDHAA-----GAV----------KGS132 |
| Eshark    | 103LIALNFDAAOQTTL-----VKEETCLPLREHH-----LKC----------LTGETSEG142 |
| Zebrafish | 103LVIHLNNSD-----AKANLSVGDNSL---ESPSEDPCQVSSGSINSAQPLAAGSSSTG152 |
| Frog      | 107LSVTCTDAHGS-----RDK3LASHGLP-----LEK----------SPNKTEVTD1IKR148 |
| Chicken   | 105LTSAMATDAAQQL-----AQEKSQVDKSPQQQQ----LKFSPEKESDVTYIMDESNAS153 |
| Mouse     | 106LVTSASINTDAAQTLAODHMDPSQODR-----LKHGATEYSNPKRTTEDD16H156 |
| Human     | 106LVTSATATDAAQTLAODHMDPSQODR-----LQKASEEESTRSRKRTTEDD16H156 |

| Species   | Sequence  |
|-----------|-----------|
| Lamprey   | 133SLA------------------------------------------135 |
| Eshark    | 143TVICVPNSAASHLKRKNSEDDLADDDQPELQSKRQSERVESVSHDWAAGLPWW195 |
| Zebrafish | 153IQ------CSQRKPRD-----PDEDSDSLGERAACKRPVLDVTEEWVLGGL197 |
| Frog      | 149NDC------VSDDVCTS-----LNKSHLKNERQDYTKQSLDVFEEWDEA199 |
| Chicken   | 154AVS------TSRHKCEN-----YEDKDLINLKSKKPQLDVFEEWAVG1LPPW197 |
| Mouse     | 157TLF------TSRHKCD-----SRADEDLHQLQDETSRDLDFEEWAVGLPPW201 |
| Human     | 157TLF------TSRHKCD-----SRADEDLHQLQDETSRDLDFEEWAVGLPPW201 |

| Species   | Sequence  |
|-----------|-----------|
| Lamprey   | 136---------A-----------------------------------137 |
| Eshark    | 196FIKLSQNYGKRKSGSTDHHS-----DDIDTAIVSDSTDDWLFWLNEPDHDAQ243 |
| Zebrafish | 198FLGNHNSNYTNRSSGSTDIHTQOLSQGDEDITAVISDDSTDDWLFWLNEPEEQ250 |
| Frog      | 194FLGNLRTNYHLQGSTDASN-----DDIDTAIVSDSTDDWLFWLNEPDHDAQ241 |
| Chicken   | 198FLGNLRTNYKRKSGSTDQTN-----DDIDTAIVSDSTDDWLFWLNEPEEQ245 |
| Mouse     | 202FLGNHNSNYTNRSSGSTDIHTQTN-----DDIDTAIVSDSTDDWLFWLNETEQ249 |
| Human     | 202FLGNHNSNYTNRSSGSTDIHTQTN-----DDIDTAIVSDSTDDWLFWLNETEQ249 |

Coffil and Lee et al. Fig S11, Panel A
Mdm2 locus

Human chr12

Coelacanth Scaffold_JH127931

Elephant shark Scaffold_113

Jlamprey Scaffold_29

Mdm4 locus

Human chr1

Coelacanth Scaffold_JH126599

Elephant shark Scaffold_70

Jlamprey Scaffold_2349 (exons 4-6) (and scaffold_14666 (exons 2-4)

Coffill and Lee et al. Fig S12
Coffill and Lee et al. Fig S13
Coffill and Lee et al. Fig S14
A

IP: HA
IB: FLAG

B

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|----|
|   | + | + | + | + | + | + | + | + | + | +  |
|   | - | - | + | + | - | - | - | - | - | -  |
|   | - | - | - | - | + | + | + | + | + | +  |
|   | - | - | - | - | - | + | + | + | + | +  |
|   | - | + | - | + | - | + | - | + | - | +  |

Lj-p53
HA-Lj-Mdm2
Lj-Mdm2-HA
myc-Lj-Mdm4
MG-132

IB: HA (Lj-Mdm2)
IB: FLAG (Lj-p53)
IB: Actin

Coffill and Lee et al. Fig S15
