Supplementary materials for: Anatomy of Mdm2 and Mdm4 in Evolution

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Methods

The sequences for Mdm2 and Mdm4 for the organisms used in this study were obtained from NCBI Protein Database (Pruitt *et al.*, 2014) and UniProt (Consortium, 2015). All sequence alignments were performed with MEGA 7 software (Kumar *et al.*, 2016) using the MUSCLE algorithm (Edgar, 2004). The phylogeny tree was obtained and modified from the TimeTree database (Hedges *et al.*, 2006). Human Mdm2 p53 binding region and p53 alpha helix was obtained from Kussie et al. (1996) (PDB: 1YCR) and while the homology model of adenovirus E1B bound to p53 was generated by MODELLER 9.13 (Sali and Blundell, 1993). Zebrafish husbandry, morpholino injections and immunohistochemistry were performed as described in Chua *et al.* (2015). All zebrafish experiments were conducted within ethical guidelines and with the approval of the Institutional Animal Care and Use Committee, Biomedical Research Council, A*STAR.

Lamprey (*Lethenteron japonicum*) RPL11 protein sequence from the genome sequence initiative of Metha *et al.* (2013) and kindly provided by Byrappa Venkatesh and his team (Mehta *et al.*, 2013): MAAEQGEKKENPMRELRIRKLCLNICVGESGDRLTRAAKVLEQLTGQTPVFSKARYTVRSFGIRRNEKIAVHCTVRG AKAEEILEKGLKVREYELRKSNFSDTGNFGFGIQEHIDLGIKYDPSIGIYGLDFYVVLGRPGFCIADKKRKRGRIGFKHRI FKEEAIRWFQQKYDGVILPGK

Supplementary References

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Supplementary figure legends

Figure S1. Taxonomy tree of the various species studied in this article. Adapted from Tree of Life *(Hedges* et al*., 2006, 2015; Kumar and Hedges, 2011)*.

Figure S2. The four major domains of *Homo sapiens* Mdm2 and Mdm4 and their amino acid positions in the respective polypeptide chains.

Figure S3. Alignment of the WWW sequence around residues 190-210 of human Mdm4, with the tryptophans critical in its autoinhibition highlighted in yellow. The numbers on the right indicate the percentage identity within this region compared to human Mdm4.

Figure S4. Mdm2$^{-/-}$ embryos with the injection of p53 morpholinos were able to survive beyond the expected 3 days post fertilisation, while embryos without p53 morpholino injections did not. The morpholinos were injected as described by Chua *et al*. (2015).

Figure S5. Comparison of the homology model of (**a**) adenovirus E1B (residues 223-285) bound to p53 with (**b**) the crystal structure of human Mdm2 (residues 25-109) bound to p53 (PDB 1YCR). Regions of positive, negative and neutral electrostatic potential on the protein surfaces are shown in blue, red, and white respectively. The sequences at the bottom show the Clustal Omega alignment for the conserved region, with the residues responsible for creating the hydrophobic cleft for p53 interaction highlighted in red.
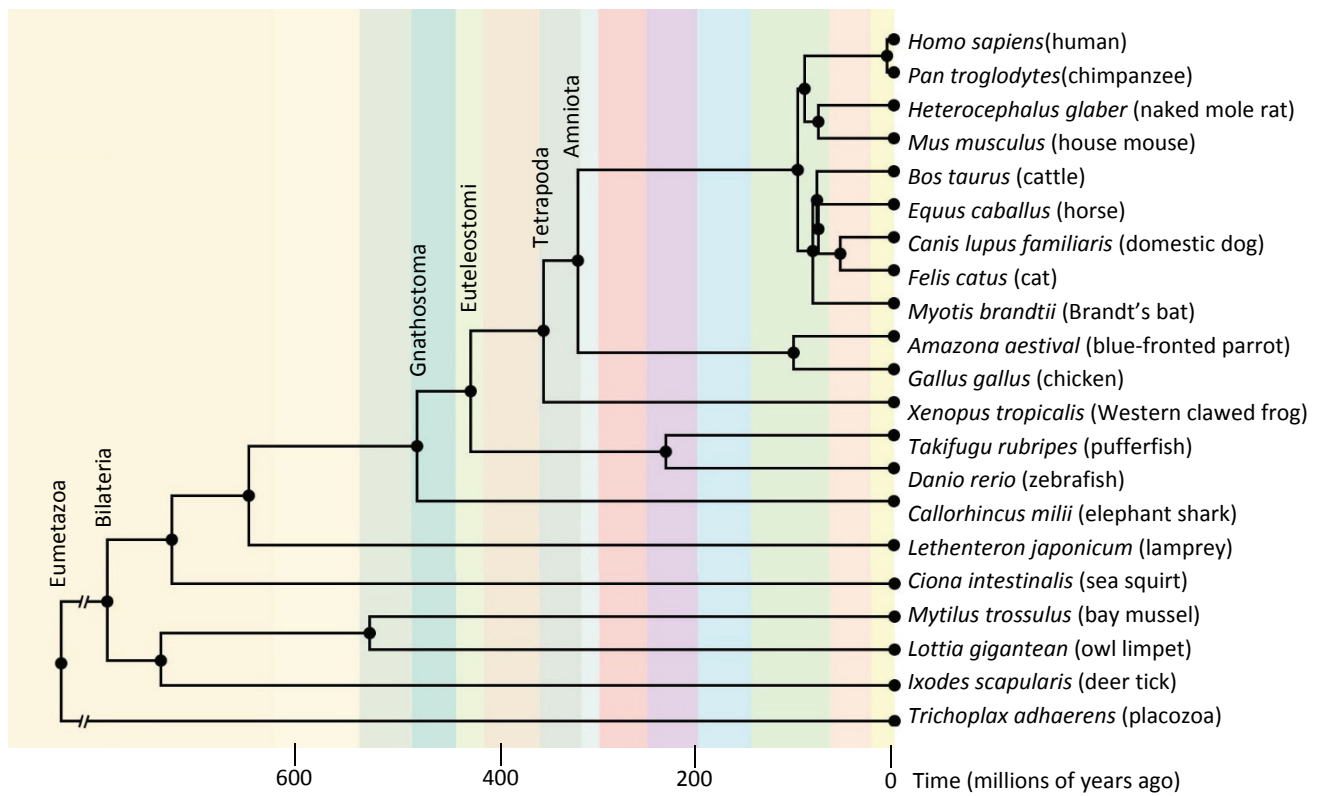
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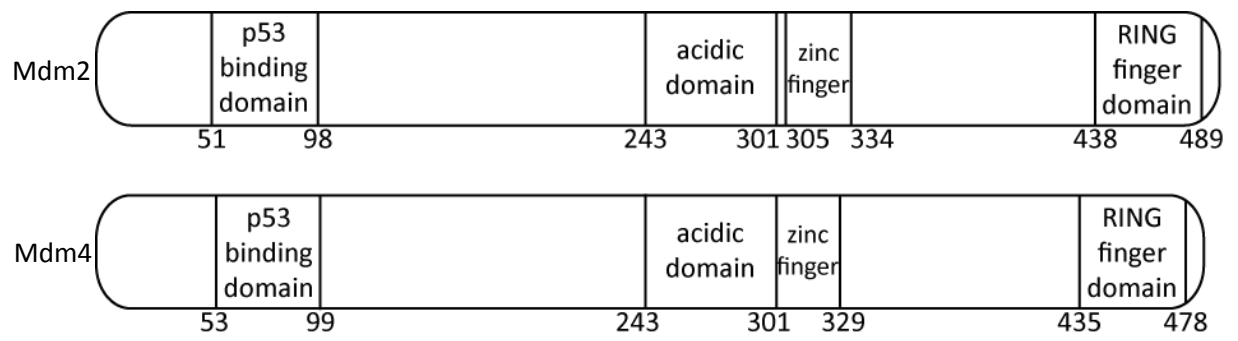
Figure S2. The four major domains of Homo sapiens Mdm2 and Mdm4 and their amino acid positions in the respective polypeptide chains.

	Species	Sequence	
Mdm4	Homo sapiens	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Pan troglodytes	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Canis lupus familiaris	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Felis catus	F E E W D I A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R N N - - - Y	
	Bos taurus	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R N N - - - Y	
	Equus caballus	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R N N - - - Y	
	Heterocephalus glaber	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Myotis brandtii	L E E W D V A G W P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R N S - - - Y	
	Mus musculus	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R N N - - - C	
	Amazona aestiva	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Gallus gallus	F E E W D V A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Xenopus tropicalis	F E E W D E A G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R T N - - - Y	
	Takifugu rubripes	L D E W D L S G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Danio rerio	L E E W D L S G L P <mark>W</mark> - - - - - <mark>W</mark> F L G N L R S N - - - Y	
	Callorhinchus milli	S D H W D A A G L P <mark>W</mark> - - - - - <mark>W</mark> F I K S L Q S N - - - Y	
	Lethenteron japonicum	H E E E E E E G V - <mark>-</mark> - - - - - <mark>-</mark> - - - - - - - - - - - -	
Mdm	Ciona intestinalis	H V S W G S Y H V S <mark>W</mark> G S C H V S <mark>W</mark> G S C H E R A T V E A Y	
	Ixodes scapularis	F F - - - I P G S P <mark>E</mark> - - - - - <mark>-</mark> - - - - - - - - - - - Y	
	Mytilus trossulus	A A P T G R R H R W <mark>-</mark> - - - - - <mark>-</mark> - - - - - - - - - - - V	
	Lottia gigantea	F S D N E S A S Y P <mark>W</mark> - - - - - <mark>Y</mark> F Q V Q V E D E - - - -	
	Trichoplax adhaerens	- - - - - - - - - <mark>F</mark> - - - - - <mark>S</mark> V L E K L K A Q - - - V	

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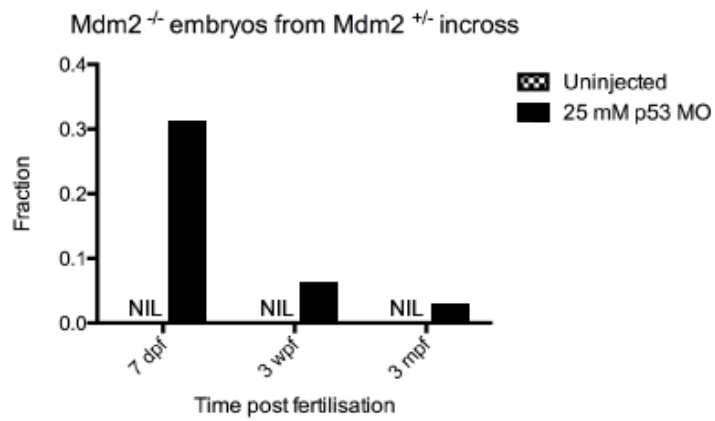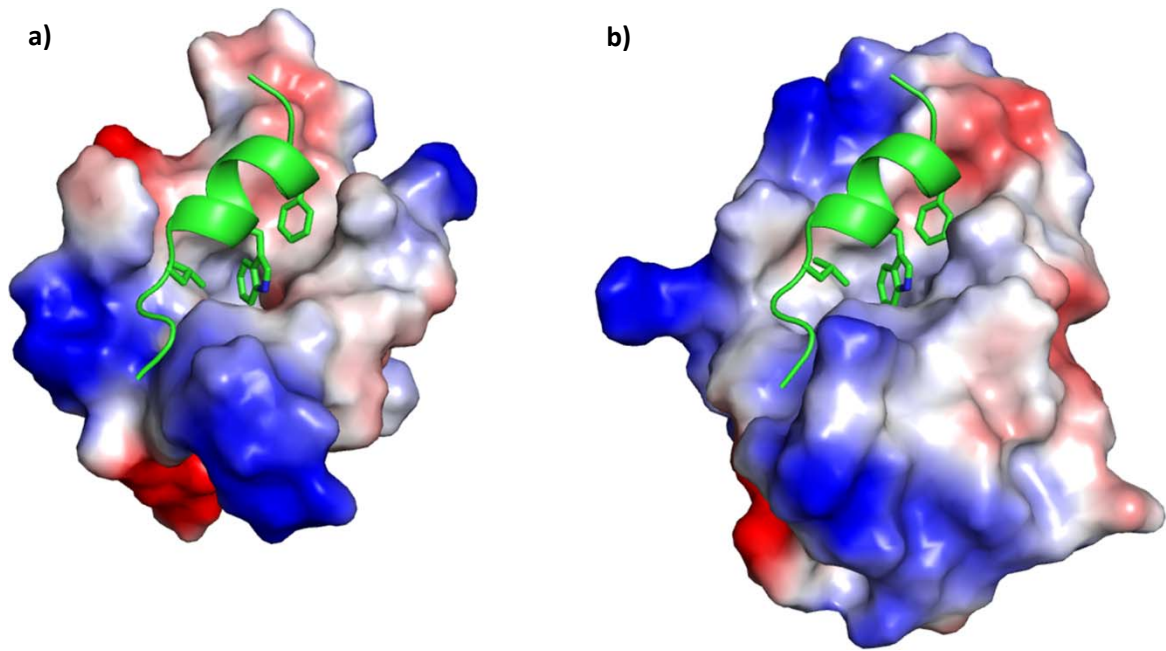
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Homo sapiens Mdm2 53-108   V **L** F Y **L G** Q Y **I M** T K R L **Y D** E K Q **Q** H I **V** Y **C** S N D - L L - - G - D L **F** G V P S **F** S **V** K E H R K **I Y** T M **I** Y R N L V

Adenovirus E1B 228-280   V **L** G M **D G** V V **I M** N V R F **T G** P N F **S** G T **V F L** A N T N L I L H G V S F **Y** G F N N **T** C **V** E A W T D **V R** V - - - - - - -

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