

A double helical motif in OCIAD2 is essential for its localization, interactions and STAT3 activation.

Saloni Sinha¹, Anudeep Venkata B¹ and Maneesha S. Inamdar^{1,2*}

¹Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560064, India;

²Institute for Stem Cell Biology and Regenerative Medicine, GKVK, Bellary Road, Bangalore 560065, India.

Inventory of Supplementary Information

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Supplementary Information

Supplementary Figure legends

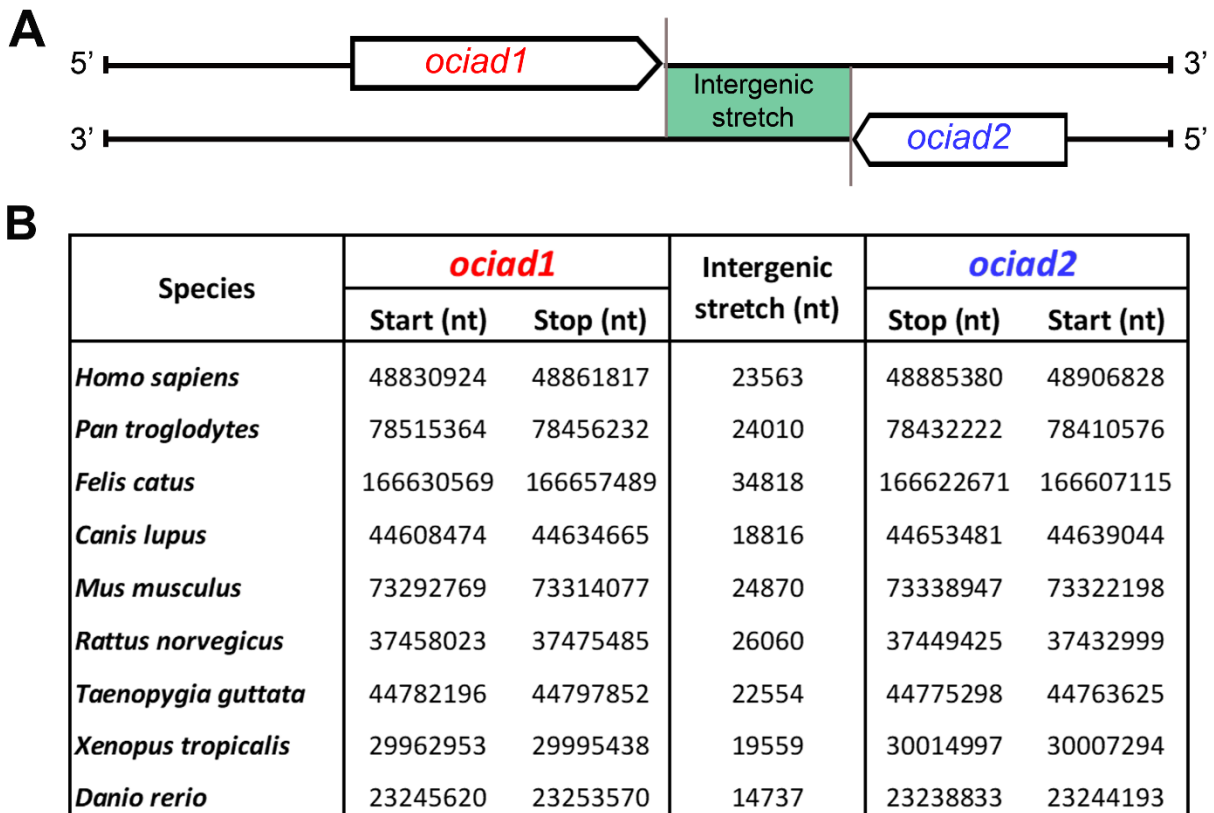


Fig. S1. Comparative analysis of nucleotide positions of *ociad1* and *ociad2* across representative species. (A) Schematic representing the transcriptional direction of *ociad1* and *ociad2*. (B) Table showing the nucleotide (nt) positions of *ociad1* and *ociad2* and the intergenic stretch between them in species where the genes are neighboring.

A

mouse OCIAD2	1	-MASVSTHGNQEK-----SPHLPPLSKQSLLFPCPKSKLHIHRGEI	54
rat OCIAD2	1	-MASVSTHGNQEK-----SHHLPPLSKQSLLFPCPKSKLHIHRGEI	54
human OCIAD2	1	-MASASARGNQDK-----DAHFPFPPSKQSLLFPCPKSKLHIHRAE	54
fish OCIAD2	1	----MSTEGNQSTGGESAVDTKSGRPGWKQFCPIADPRFPRE	56
frog OCIAD2	1	ATSQSLSSGNSEKQGLQSQDKKPCPKSSFMHCPVSN--AHRE	58
human OCIAD1	1	--MNGRAD----FREPN-AE--VPRP-----IPHIGPDYI	46
mouse OCIAD1	1	--MNGRAD----FREPN-AQ--VSRP-----IPDIGGGYI	46
rat OCIAD1	1	--MNGRAD----FREPN-AQ--VSRP-----IPDIGGGYI	46
fish OCIAD1	1	-MSQASSG----FTPAAQVQ--HGSS-----KGAFNSAYI	48
fly OCIAD1	1	-MDSPLNDGS-----H-HPPPH-----APHPLADYQ	44
frog OCIAD1	1	-MAPSPAEE----FSDQQQPA--PHRT-----VQPPGVGYI	48
		: : ** . : * :	
mouse OCIAD2	55	ALPFSLISMLVTQGLVHGYLAANPRFGSLPKVALAGLLGFLGKASY	114
rat OCIAD2	55	ALPFSLISMLVTQGLVHGYLAANPRFGSLPKVALAGLLGFLGKASY	114
human OCIAD2	55	ALPFSLVSMMLVTQGLVYGYLAANSRFGSLPKVALAGLLGFLGKASY	114
fish OCIAD2	57	ALPLSAGSMAVTGGLIYSVWKQSKRFGYFPKLILAGIVGFAV	116
frog OCIAD2	59	ALPISLGSMLVTQGLIYKGYLSRNKRFGSLPKVALAGVLSY	118
human OCIAD1	47	SVPLAATSMMLITQGLISKILSSHPKYGSIPKLILACIMGY	106
mouse OCIAD1	47	SVPLAATSMMLITQGLISKILSSHPKYGSIPKLILACIMGY	106
rat OCIAD1	47	SVPLAATSMMLITQGLISKILSSHPKYGSIPKLILACIMGY	106
fish OCIAD1	49	SLPFSAIAVGITQVLVAKGMLSPSPRFGALPKIAFAGIFGY	108
fly OCIAD1	45	SLPFGTGLGLLAYFGVKNYGLQGHVYKAVPKVVMGVILGY	104
frog OCIAD1	49	SLPISAVSMIVTQGLISRIILTSSRFGSLPKVAFAGLCY	108
		::*.: : : * : : * : : * : : * : : * :	
mouse OCIAD2	115	D----QLRGAGFGPE----HNRHCLLTCECKTRRGLSEKAGSQ	154
rat OCIAD2	115	D----QLRGAGFGPE----HNRFVVM-----	132
human OCIAD2	115	D----QLRGAGFGPQ----HNRHCLLTCEECKIKHGLSEKGD	154
fish OCIAD2	117	GPEFTKAFGGPGFGPGGF--RPGHNHCIVHCEKCKQEAQAAT	165
frog OCIAD2	119	MQ----PLFDAGFVPPFV--KNKGCQHTCEECKTKSAM	164
human OCIAD1	107	NSPLGEALRSGQARRSS--PPGHY----YQKSKYDSSVSG--	147
mouse OCIAD1	107	NSPLGEALRSGELRRSS--PPGHY----TQKPKFDSNVSG--	147
rat OCIAD1	107	NSPLGEALRSGELRRSL--PPGHY----TQKPKYDSNVSG--	147
fish OCIAD1	109	NSPLGEALRQGRLRHVSSEM-----NQPDFDPNSSE--	156
fly OCIAD1	105	NSHLGELLRQRRQGGGVISS-----ITPDENLGRAFTLAP	147
frog OCIAD1	109	NSPLGEALRQGYRNIPTQYPSGTSE--FSDVNPKTASPADG--	154
		:	
mouse OCIAD2	155	-----	154
rat OCIAD2	133	-----	132
human OCIAD2	155	-----	154
fish OCIAD2	166	-----	165
frog OCIAD2	165	-----	164
human OCIAD1	148	-----PAADNIE--MLPHYEPIPFSSSMNESAPTGITDHIVQ-	196
mouse OCIAD1	148	-----PAADNIEKEALPRYEPIPFSSASMNESTPTGITD	198
rat OCIAD1	148	-----PAADNIEKETLPRYEPIPFSSASMNESTPTGITD	198
fish OCIAD1	157	SATESYSSYTSDYTYSTPSQSYETTPFSSGFSDSGPANIR	216
fly OCIAD1	148	YSDEAYQPGRST----SINLDTESRPTLSGLDDIYRPTL	200
frog OCIAD1	155	-----PPSSVYSSHNNSTSDTVFPFSTSLGESSPSGID	205
		-----E-RVPKKEVKVKNKYGDTWDE	245
mouse OCIAD2	155	-----	154
rat OCIAD2	133	-----	132
human OCIAD2	155	-----	154
fish OCIAD2	166	-----	165
frog OCIAD2	165	-----	164
human OCIAD1	197	ITYEELRNKNRESYEVSLTQKTDPSVRPMH-----E-	245
mouse OCIAD1	199	VTYEELRNKNRESYGVTLPHKTDPSVRPMQ-----E-	247
rat OCIAD1	199	VTYEELRNKNRESYGVTLSHKTDPSVRPMQ-----E-	247
fish OCIAD1	217	VLYEELRNKNRENYEVTLPQKNQTMKPKQM-----E	266
fly OCIAD1	201	QSYEDLRRRNREEYSKHQQSPYRPPYPPVAVQRPVEQAQ	257
frog OCIAD1	206	MTYDELRSRNRETYEMAVTQRADAPVRSSL-----D-	254

Fig. S2. Protein sequence alignment of OCIAD1 and OCIAD2 for different species. (A) Multiple sequence alignment of OCIAD1 and OCIAD2 protein sequences from various organisms

(using Clustal-Omega) showing sequence conservation in the N-terminal regions as opposed to the C-terminal regions.

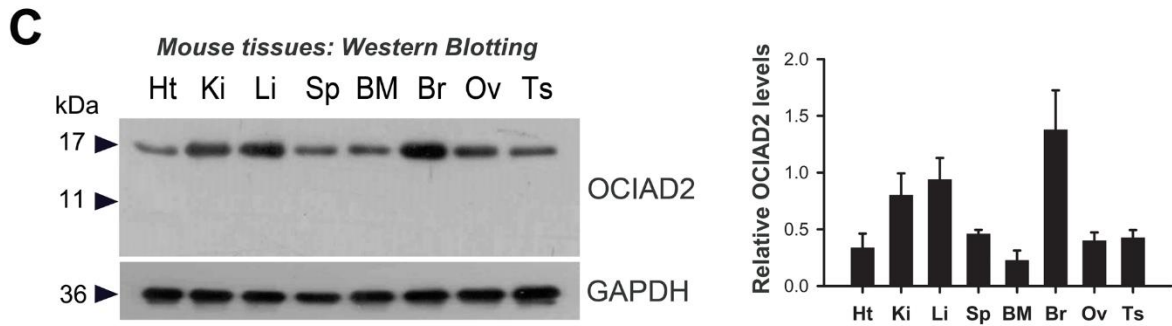
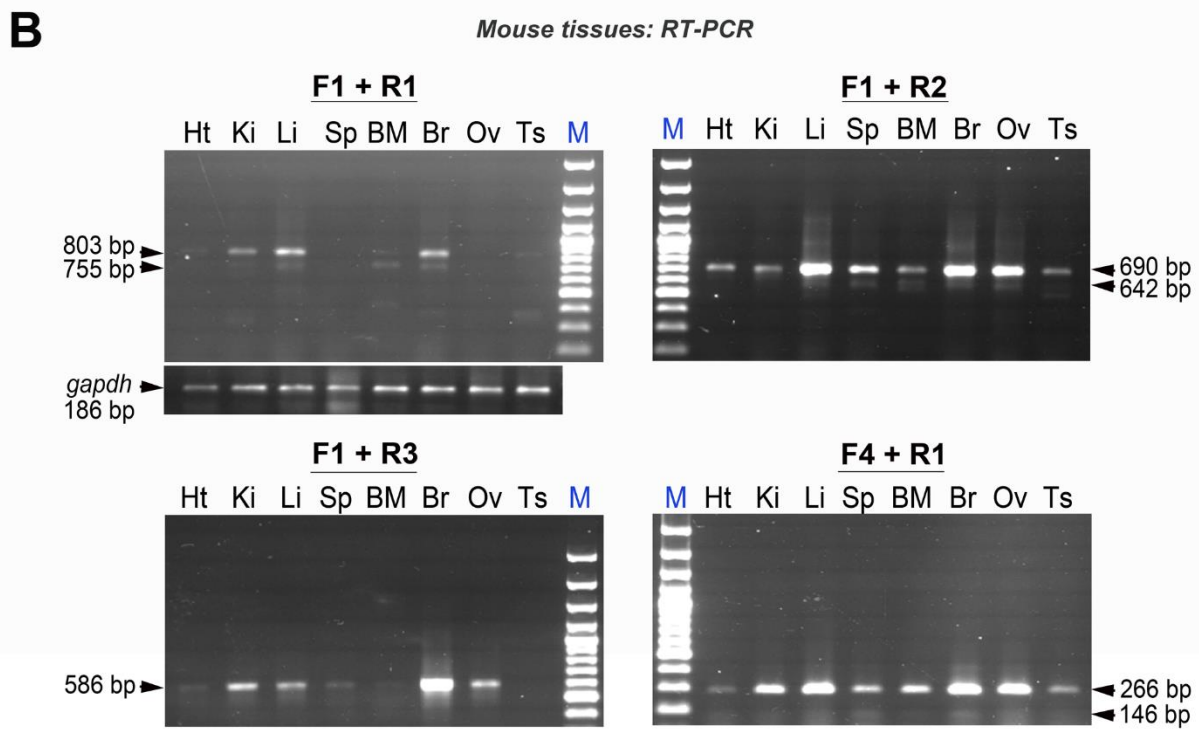
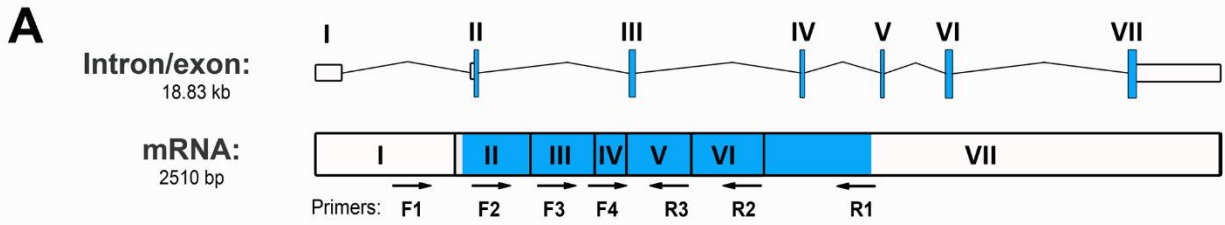


Fig. S3. Analysis of *ociad2* transcript and protein expression in various mouse tissues. (A) Schematic representing the introns (black lines) and exons (boxes) of the mouse *ociad2* gene. Coding regions shown in blue and untranslated regions in white. Exon number indicated in Roman numerals. The arrows below the longest transcript (2510 bp) indicate the position of the forward (F: 1, 2, 3, 4) and reverse (R: 1, 2, 3) primers used for RT-PCRs. (B) Differential expression of *ociad2* transcripts. Different combinations of primer pairs (positions identified in (A)) were used to perform semi-quantitative RT-PCRs on cDNA from mouse tissues as indicated above the gel pictures. A combination of forward and reverse primers used as indicated to amplify 803 bp, 755 bp (F1 + R1); 690 bp, 642 bp (F1 + R2), 586 bp (F1 + R3) and 266 bp, 146 bp (F1 + R4). (C) Western Blotting for detection of OCIAD2 in mouse tissues. Protein size markers (in kDa) are indicated to the left. Tissues used: H, heart; K, kidney; Li, liver; S, spleen; BM, Bone marrow; B, brain; Ov, ovary and Ts, testis. M indicates marker. Data are representative of three independent experiments. Full-length gels and blots are presented in Supplementary Figure S6.

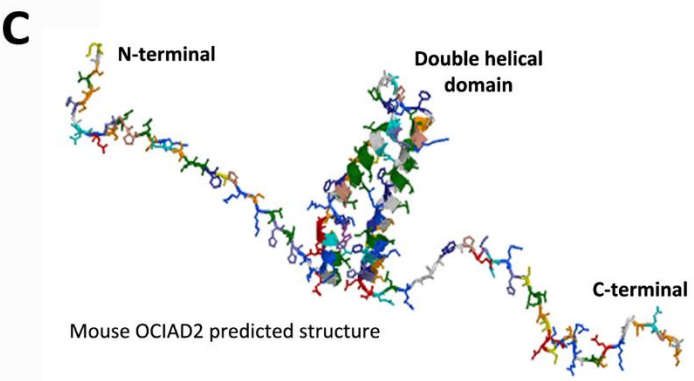
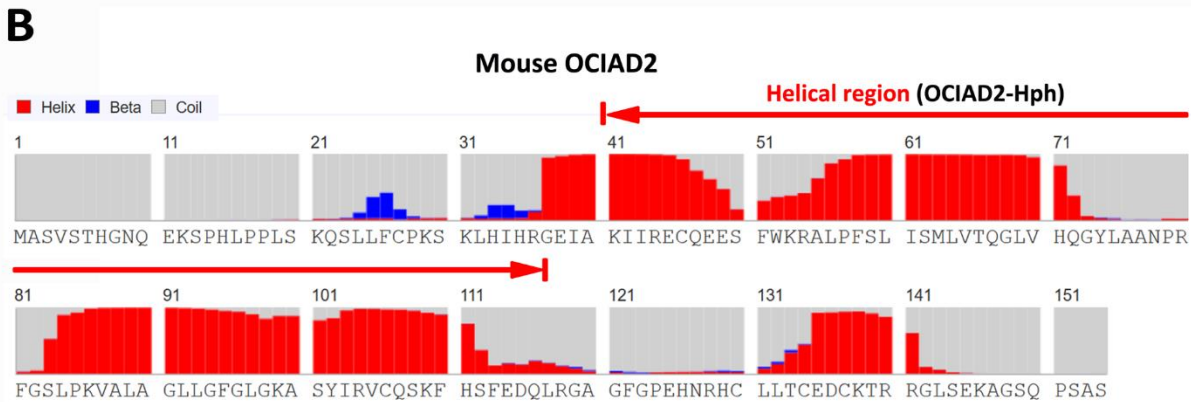
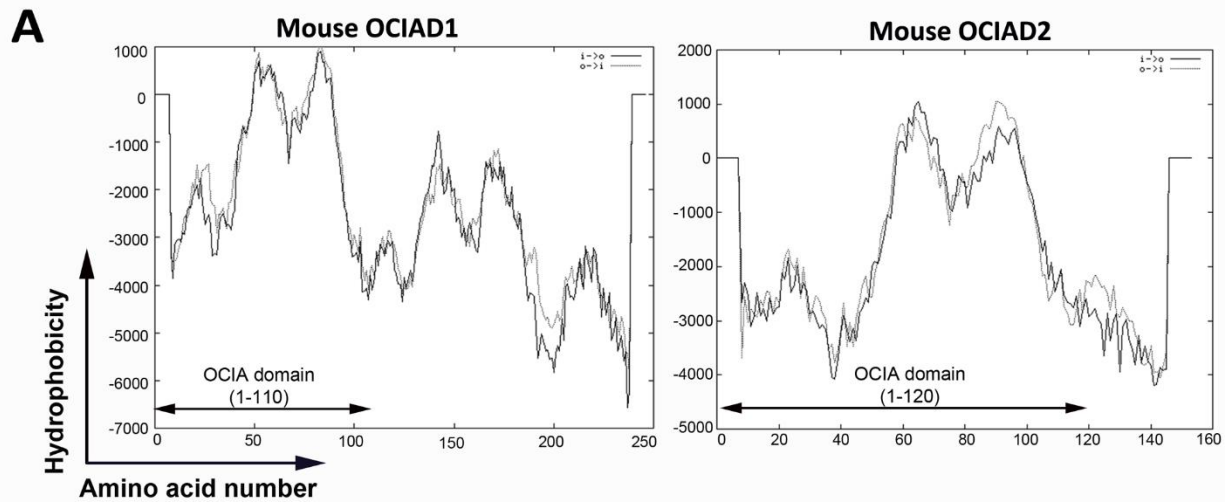


Fig. S4. The central region of OCIAD2 is predicted to contain two alpha helices. (A) Hydrophobicity analysis of mouse OCIAD1 and OCIAD2 (source: TmPred). (B) Secondary structure prediction shows that the hydrophobic region of mouse OCIAD2 (mOCIAD2-Hph) is predomi-

nantly helical (source: RaptorX). (C) Predicted structure of mouse OCIAD2 showing the N-terminal region, double helical domain and the C-terminal region (source: RaptorX). (D) Zoomed view of (C) showing the double helical region ranges from residues phenylalanine 41 to leucine 117.

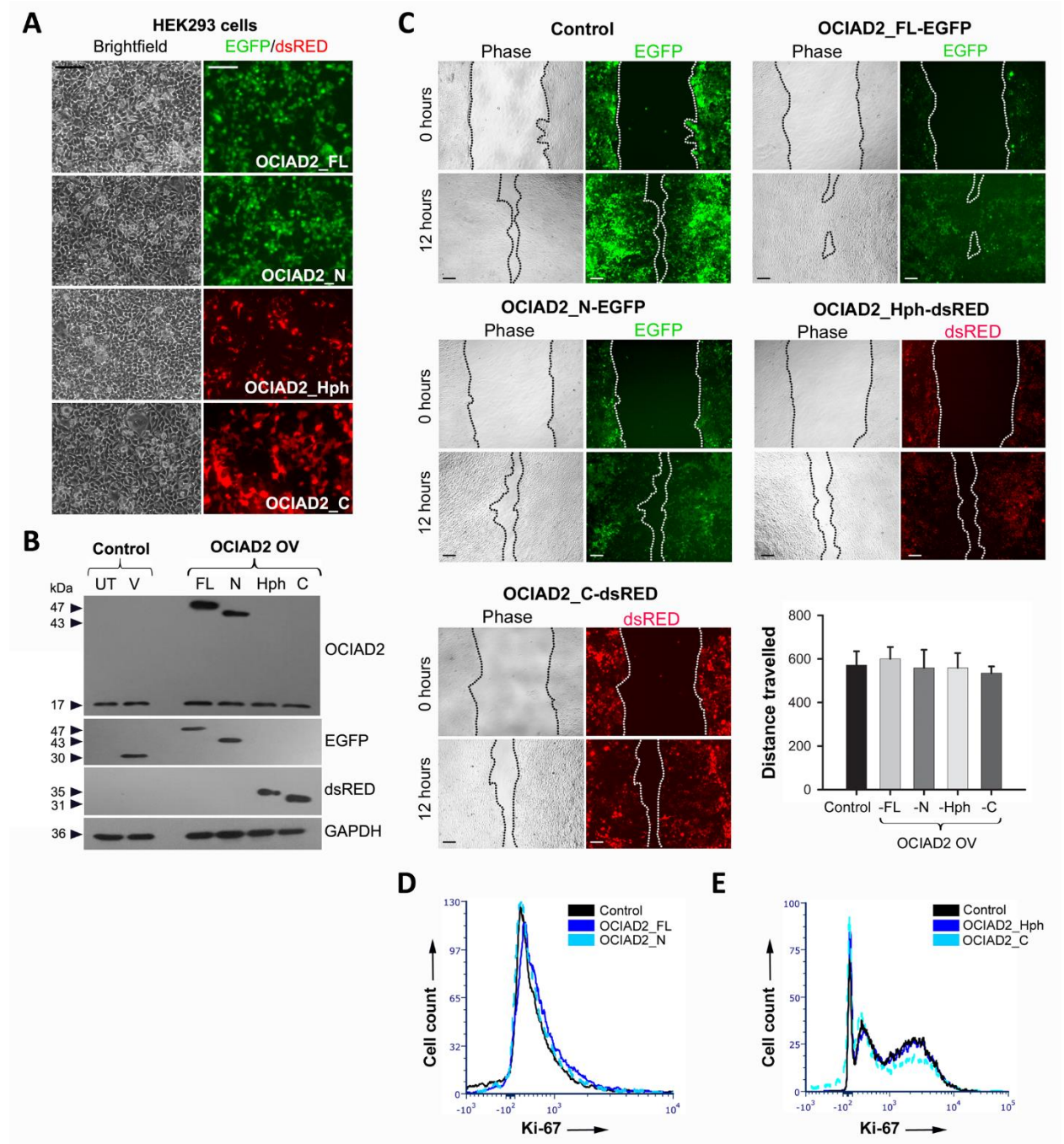


Fig. S5. OCIAD2 overexpression does not influence cell migration or proliferation. (A, B)

Validation of transfection of various OCIAD2 reporter constructs into HEK293 cells by fluorescence imaging and Western Blotting. UT = untransfected, V = empty vector transfected; FL = OCIAD2_FL-EGFP; N = OCIAD2_N-EGFP; Hph = OCIAD2_Hph-dsRED; C = OCIAD2_C-dsRED. Scale bar in (A) is 20 μ m. Full-length blots are presented in Supplementary Figure S6. **(C)** No change observed in migration of HEK293 cells overexpressing various OCIAD2 expression constructs. Monolayers of HEK293 cells transfected with empty vector (control), OCIAD2_FL-EGFP, OCIAD2_N-EGFP, OCIAD2_Hph-dsRED and OCIAD2_C-dsRED were scratched and the degree of recovery was measured at 0, 12 and 24 hours post-wounding. Representative phase and fluorescence images shown for 0 and 12 hour time points, 4X magnification. Measurement and estimation of wound recovery was based on the initial wound size. Graph alongside shows quantification of results obtained from three independent experiments (mean \pm SEM). **(D, E)** Flow cytometry analysis of Ki-67⁺ population in cells overexpressing various OCIAD2 reporter constructs.

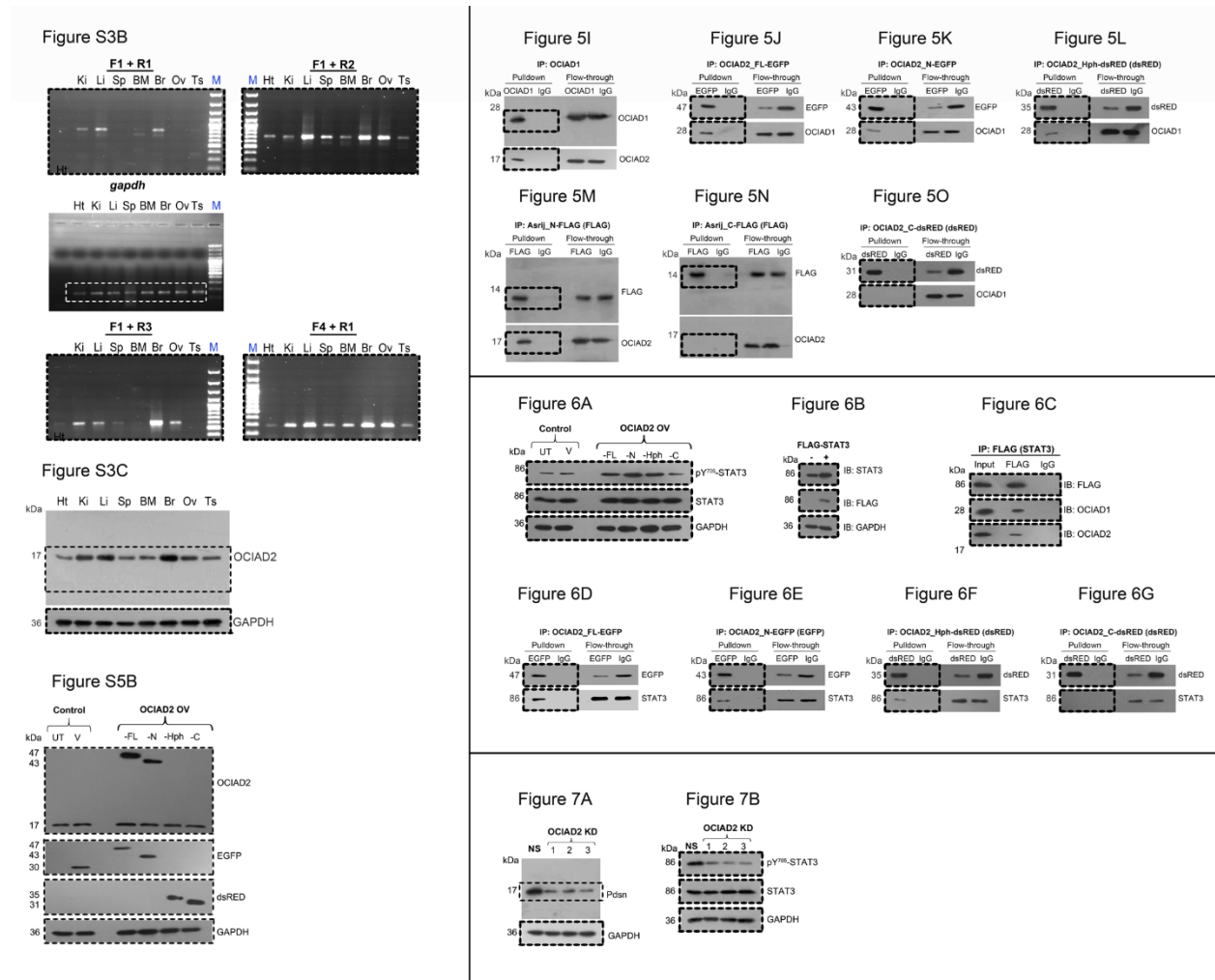


Fig. S6. Uncropped full-length pictures of Western Blotting membranes and DNA gels. Uncropped full-length pictures of Western Blotting membranes and DNA gels presented in the main figures and supplementary figures. Membranes were often cut to enable blotting for multiple antibodies.

Supplementary Table legends

Table S1. List of species and accession IDs of OCIAD proteins used for phylogenetic analysis.

Table S2. Maximum Likelihood fits of 56 different amino acid substitution models. Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (lnL), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+G) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. They are followed by amino acid frequencies (f) and rates of amino acid substitutions (r) for each amino acid pair. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. The analysis involved 106 amino acid sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 93 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2]. Abbreviations: GTR: General Time Reversible; JTT: Jones-Taylor-Thornton; rtREV: General Reverse Transcriptase; cpREV: General Reversible Chloroplast; mtREV24: General Reversible Mitochondrial.

Supplementary References

1. Nei M. and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
2. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.