Supporting Information

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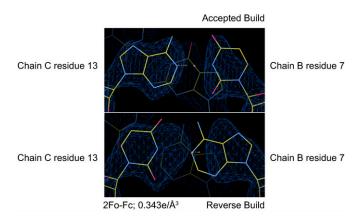


Fig. 51. Assignment of the dsRNA orientation in the hOAS3.DI•dsRNA19 crystal structure. The orientation of the dsRNA was determined by refining the data against build 1 (accepted) and build 2 (reverse). Biased electron density maps are consistent only with build 1.

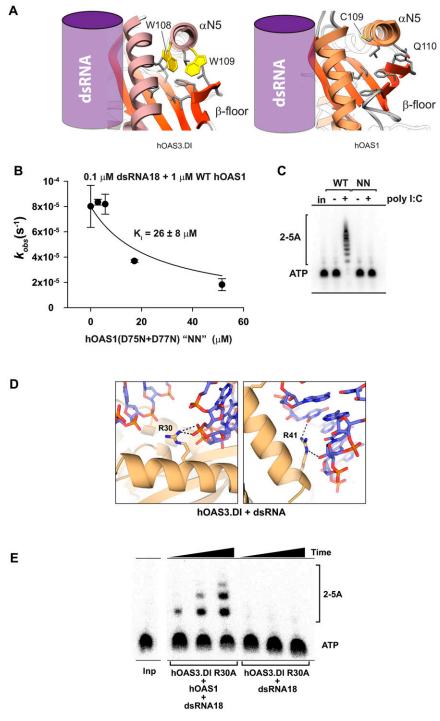


Fig. S2. Structural features of hOAS3.DI. (*A*) Structural comparison shows that helix αN5 of hOAS3.DI (PDB ID code 4S3N) contains two tryptophan side chains W108 and W109 buried in the hydrophobic core. In contrast, helix αN5 of the catalytically active hOAS1 (PDB ID code 4IG8) contains compact side chains in the corresponding positions. This structural difference may explain the lack of movement of αN5 and β-floor in hOAS3.DI. (*B*) Inhibition of WT hOAS1 by the inactive hOAS1(D75N+D77N) mutant. Data points are mean \pm SD of two replicates. K_i is average from two replicates, uncertainty is SE. (*C*) Confirmation that the hOAS1(D75N+D77N) mutant is catalytically inactive. WT or mutant hOAS1 (0.5 μM) were incubated with 1 mM ATP trace-labeled with 32 P-α-ATP, in the absence or presence of 2.5 OD260 poly I:C. (*D*) Mutants of the protein/RNA interface used as a control in Fig. 3*D*. Contacts between hOAS3.DI residues R30 and R41 and dsRNA19 are highlighted. (*E*) Mutation R30A does not render hOAS3.DI constitutively active. Reaction time points were 2 min, 10 min, and 26 min (*Methods*).

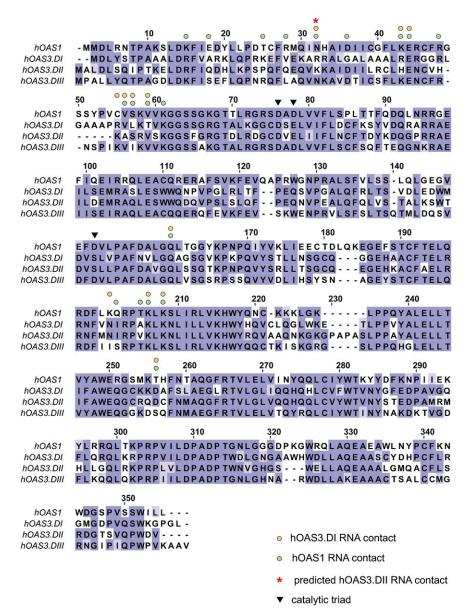


Fig. S3. Multiple sequence alignment of hOAS1 and hOAS3. Protein–dsRNA contacts in complexes of hOAS1 (PDB ID code 4IG8) and hOAS3.DI (PDB ID code 4S3N), as well as the catalytic residues in hOAS1 are marked. Alignments of hOAS3.DII and hOAS3.DII are provided for comparison. A predicted dsRNA contact of hOAS3.DII is marked with a star. The alignment is colored by BLOSUM62 similarity matrix.

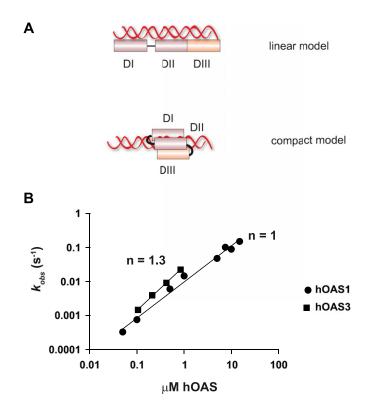


Fig. 54. Two possible arrangements of hOAS3 subunits and cooperativity of hOAS1 and hOAS3. (A) The linear model suggests that hOAS3 binds to a long dsRNA molecule, whereas the compact model suggests that hOAS3 could wrap around a shorter dsRNA fragment. (B) Protein concentration dependence of hOAS1 and hOAS3 activity in the presence of 2.5 OD₂₆₀ poly I:C. Slope of the lines defines the Hill coefficient (n).

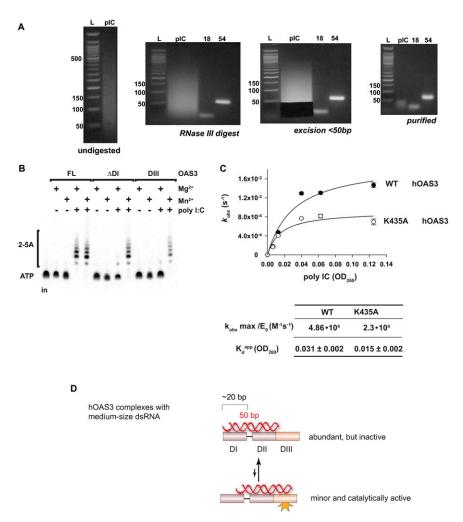


Fig. 55. Preparation of <50-bp poly I:C, and a model for its recognition by hOAS3. (A) Agarose gels (4%) of poly I:C (*Left*), poly I:C digested with RNase III (*Center*), and purified digested poly I:C (*Right*). Long (undigested) poly I:C used in this study ranges from less than 50 bp to ~500 bp, with a median size ~120 bp. Gel markers: L, 50-bp DNA ladder (New England Biolabs); 18, dsRNA18 (*Methods*); 54, a 54 bp in vitro transcribed dsRNA size control. (*B*) Two-hour end point activity assays with full length (FL) and truncated hOAS3 in the presence of Mg^{2+} and Mr^{2+} . We find that ΔDI has robust activity in the presence of Mr^{2+} , confirming that it is properly folded and well behaved. (C) Mutagenesis of a predicted dsRNA-binding contact in domain DII of hOAS3 (see also Fig. S3). Reactions were conducted as in Fig. 4*B*. (*D*) A proposed model for recognition of medium-size poly I:C by hOAS3, which explains the low specific activity with shorter dsRNA.

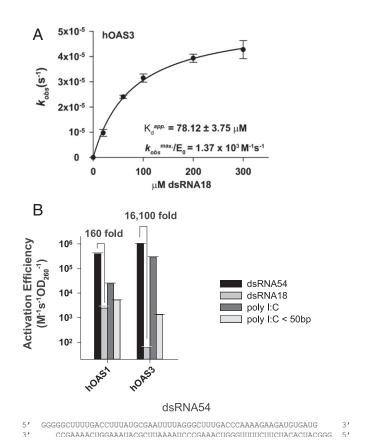


Fig. S6. Activation of hOAS3 by dsRNA18 and dsRNA54. (*A*) 40 nM hOAS3 was incubated with increasing concentrations of dsRNA18 and 2-5A synthesis rates were measured as in Fig. 3 (*Methods*). (*B*) Activation of hOAS1 and hOAS3 by transcribed dsRNA of defined length, dsRNA18 and dsRNA54. The different activation efficiencies of dsRNA18 and dsRNA54 likely reflect differences due to both dsRNA length and sequence. The results with poly I:C shown here for comparison reflect only the effects of dsRNA length. Reactions contained 50 nM hOAS1 and 5 nM hOAS3.

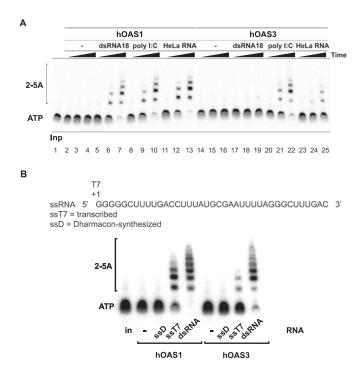


Fig. S7. Detection of endogenous double-stranded RNA in HeLa cells. (A) Time-course (10 s, 5 min, and 30 min) of 2-5A synthesis by hOAS1 (0.5 μM) and hOAS3 (0.1 μM) in the absence or presence of 5 μM dsRNA18, 0.125 OD₂₆₀ poly I:C, or 22.4 OD₂₆₀ total RNA from HeLa cells. (B) Both hOAS1 and hOAS3 are strongly activated by ssRNA transcribed in vitro. Neither is activated by the same ssRNA synthesized chemically. Human OAS1 or hOAS3 (0.1 μM), and 32 P-α-ATP were incubated with 1 μM synthetic 39-mer synthesized by Dharmacon (ssD), the same RNA transcribed with T7 RNA polymerase (ssT7), or a 36-bp dsRNA (dsRNA) generated by annealing two in vitro transcribed reverse complements. The 39 nt vs. 36 bp difference is due to a 3-nt overhang of GGG sequence at the transcription start site.

Table S1. Data collection and refinement statistics

Human OAS3.DI•dsRNA19

	Tidilian OA33.DI•d3KNA13
Data collection	
Space group	P2 ₁
Cell dimensions	
a, b, c, Å	48.7, 104.7, 63.8
α, β, γ , °	90, 98, 90
Resolution, Å	43.82-2.0 (2.1-2.0)*
R-meas, %	6.6 (67.4)
CC(1/2)	99.9 (88.9)
l/ol	21.33 (3.25)
Completeness, %	99.8 (100)
Redundancy	6.27 (6.25)
Refinement	
Resolution, Å	2.0
No. unique reflections	42,802 (5,835)
$R_{\text{work/}} R_{\text{free}}$	0.1818/0.2113
No. atoms	
Protein	2,707
dsRNA19	799
Water	876
B-factors	
Protein	48.06
dsRNA19	84.87
r.m.s. deviations	
Bond lengths, Å	0.007
Bond angles, °	0.997
Ramachandran plot, %	
Favored	92
Allowed	8
Disallowed	0.0

A single crystal was used for data collection.

^{*}Highest resolution shell is shown in parenthesis.