Supplementary Table 2 Conservation of helicase motifs.

Motif Lobe1	Structural Conservation between SF1/SF2	Function	Structural conservation in Rad54	Comment
I	Conserved in SF1 and SF2	Nucleotide binding	Yes	In our dnRad54ΔN crystals, a sulfate group is bound by the lysine side chain (Lys183) and backbone amide groups (Gly180 and Gly182), and these interactions are equivalent to those helicases make to the γ-phosphate of ATP. This sulfate group also interacts with motif VI.
Ia	Conserved in SF1 and SF2	DNA binding	Yes	The motifs contains exposed backbone amide groups at the N-termini of a helix binding to the ribose and phosphate groups. The Rad54 structure has a one amino-acid truncation of the helicase motif Ia. The truncation is a conserved feature of SWI2/SNF2 proteins (Snf2 hmm of PlSTl), and the Rad54 structure preserves the exposed backbone amide and side chain hydroxyl groups associated with DNA-binding.
TxGx	Conserved in SF1 and SF2	Nucleotide binding	Yes	Like motif Ia, the TxGx motif is also conserved in the SWI2/SNF2 family (Snf2 hmm TSYEl), and is part of the previously defined Snf-A motif.
II	Conserved in SF1 and SF2	Nucleotide binding	Yes	The motif itself is conserved. Immediately after motif II, Rad54 contains two residues that are highly conserved in the Snf2 hmm (DEgHrLKN; higher conservation in uppercase letters) but not in SF1/2 helicases. Lys296 points towards the inter-lobe cleft, and Asn297 has a structural role at a kink in helix α13.
III	Divergent in SF1 and SF2	Nucleotide binding and possible ATP hydrolysis sensor	Rad54 is 'SF2-like'	In Rad54, motif III has a sequence and structure similar to SF2 helicases (Fig. 2C and Fig. 5A), but contains additional residues conserved in SWI2/SNF2 proteins (Snf2 hmm: TGTPlQN). These additional residues interact with the SWI2/SNF2 specific HD1 and HD2 domains. Gln323 interacts with the SWI2/SNF2-specific HD1, and Asn324 is solvent-exposed in the inter-lobe cleft.
Lobe2				
IV	Slightly divergent in SF1 and SF2	DNA binding	Rad54 is 'SF2-like'	In the structure, motif IV is located adjacent to the Snf-D motif, which we defined earlier based on the human Snf2 hmm. Snf-D occurs between β13 and α23 (Fig. 2C Anand Fig. 5A), in a region that differs between the SF1 and SF2 families.

				The conformation of Snf-D is similar to a corresponding region of the HCV NS3 helicase, where an arginine side chain contacts DNA [28]. Although Rad54 does not have an arginine at the same position, the side chain of Arg546 (Snf-D motif: RLDGstkiseR) is positioned similarly.
V	Slightly divergent in SF1 and SF2	DNA binding	Rad54 is 'SF2-like'	Motif V interacts with DNA through basic residues, serine/threonine residues and exposed backbone amide groups (Fig. 5A,B) [28,30]. Rad54 and the remainder of the SWI2/SNF2 proteins (Snf2 hmm: STRAG) have a conserved lysine/arginine (Lys568), two serine/threonine (Ser566 and Ser567) and a glycine (Gly570) residue present. Ser566 and Gly570 have structural roles in the formation of a 3.10 helix, while Ser557 and Lys558 are solvent exposed, and could in principle interact with DNA in a manner analogous to SF1 helicases.
VI	Slightly divergent in SF1 and SF2	Interlobe communication	Rad54 is 'SF2-like'	Motif VI is thought to mediate inter-lobe communication. It is characterized by an arginine that is conserved in both SF1 and SF2 helicase families. This arginine binds to the γ-phosphate of ATP in the SF1 helicase PcrA, and it is thought that it may have an analogous role in SF2 helicases as well. Rad54 has an equivalent arginine (Arg600) conserved in SWI2/SNF2 proteins (Snf2 hmm: QaqdRaHR). In the structure, Arg600 and Arg603 interact with the sulfate ion bound in the ATP γ-phosphate-binding site of motif I in a manner analogous to that seen in the PcrA structure.