

1 **Supplementary Information**

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3 **The interaction of DNA repair factors ASCC2 and ASCC3 is**  
4 **affected by somatic cancer mutations**

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6 Junqiao Jia<sup>1</sup>, Eva Absmeier<sup>1,5</sup>, Nicole Holton<sup>1</sup>, Agnieszka J. Pietrzyk-Brzezinska<sup>1,6</sup>, Philipp  
7 Hackert<sup>2</sup>, Katherine E. Bohnsack<sup>2</sup>, Markus T. Bohnsack<sup>2,3</sup>, Markus C. Wahl<sup>1,4,\*</sup>

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9 <sup>1</sup> Freie Universität Berlin, Laboratory of Structural Biochemistry, Takustraße 6, D-14195  
10 Berlin, Germany

11 <sup>2</sup> University Medical Centre Göttingen, Department of Molecular Biology, Göttingen,  
12 Germany

13 <sup>3</sup> Georg-August-Universität, Göttingen Center for Molecular Biosciences, Göttingen,  
14 Germany

15 <sup>4</sup> Helmholtz-Zentrum Berlin für Materialien und Energie, Macromolecular Crystallography,  
16 Albert-Einstein-Straße 15, D-12489 Berlin, Germany

17 <sup>5</sup> Present address: MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus,  
18 Francis Crick Avenue, Cambridge, CB2 0QH, United Kingdom

19 <sup>6</sup> Present address: Institute of Molecular and Industrial Biotechnology, Faculty of  
20 Biotechnology and Food Sciences, Lodz University of Technology, Lodz, 90-924, Poland

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22 \* Correspondence to: markus.wahl@fu-berlin.de

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24 **Supplementary Tables**25 **Supplementary Table 1: Crystallographic data.**

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| Data collection                         |                      |
|---|----------------------|
| Wavelength [Å]                          | 0.97957              |
| Space group                             | I4                   |
| Unit cell parameters<br>a (= b), c [Å]  | 158.1, 69.4          |
| Resolution [Å] <sup>a</sup>             | 50.0-2.7 (2.86-2.70) |
| Reflections                             |                      |
| Total                                   | 162,207 (26,160)     |
| Unique                                  | 45,845 (7,271)       |
| Multiplicity                            | 3.5 (3.6)            |
| Completeness [%]                        | 99.0 (97.8)          |
| Mean <i>I</i> / $\sigma(I)$             | 5.89 (0.47)          |
| $R_{merge}(I)$ [%] <sup>(b)</sup>       | 16.4 (243.5)         |
| $R_{meas}(I)$ [%] <sup>(c)</sup>        | 19.3 (285.9)         |
| $CC_{1/2}$ [%] <sup>(d)</sup>           | 99.5 (13.9)          |
| Phasing                                 |                      |
| Resolution [Å]                          | 42.0-2.8             |
| Number of selenium atoms                | 6                    |
| Figure of merit                         | 0.2                  |
| BAYES-CC                                | 22.9                 |
| Refinement                              |                      |
| Resolution [Å] <sup>a</sup>             | 42.0-2.7 (2.76-2.70) |
| Reflections                             |                      |
| Unique                                  | 23,723 (2,342)       |
| Test set [%]                            | 4.6 (4.6)            |
| $R_{work}$ [%] <sup>(e)</sup>           | 20.4 (33.5)          |
| $R_{free}$ [%] <sup>(f)</sup>           | 24.7 (34.8)          |
| Contents of A.U. <sup>(g)</sup>         |                      |
| Non-H atoms                             | 4,784                |
| Protein residues/atoms                  | 584/4,729            |
| Water oxygens                           | 55                   |
| Mean B factors [Å <sup>2</sup> ]        |                      |
| Wilson                                  | 86.1                 |
| Model atoms                             | 96.0                 |
| Rmsd <sup>(h)</sup> from ideal geometry |                      |
| Bond lengths [Å]                        | 0.007                |
| Bond angles [°]                         | 0.92                 |
| Model quality <sup>(i)</sup>            |                      |
| Overall score                           | 1.9                  |
| Clash score                             | 11.0                 |
| Ramachandran favored [%]                | 95.2                 |
| Ramachandran outliers [%]               | 0.7                  |
| PDB ID                                  | 6YXQ                 |

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28 <sup>a</sup> Values in parentheses refer to the highest resolution shell.29 <sup>b</sup>  $R_{merge}(I) = \sum_h \sum_i |I_{ih} - \langle I_h \rangle| / \sum_h \sum_i I_{ih}$ , in which  $\langle I_h \rangle$  is the mean intensity of symmetry-equivalent  
30 reflections *h* and  $I_{ih}$  is the intensity of a particular observation of *h*.<sup>55</sup>31 <sup>c</sup>  $R_{meas}(I) = \sum_h [N / (N-1)]^{1/2} \sum_i |I_{ih} - \langle I_h \rangle| / \sum_h \sum_i I_{ih}$ , in which  $\langle I_h \rangle$  is the mean intensity of symmetry-  
32 equivalent reflections *h*,  $I_{ih}$  is the intensity of a particular observation of *h* and *N* is the number of  
33 redundant observations of reflection *h*.<sup>55</sup>34 <sup>d</sup>  $CC_{1/2} = (\langle I^2 \rangle - \langle I \rangle^2) / (\langle I^2 \rangle - \langle I \rangle^2 + \sigma_\epsilon^2)$ , in which  $\sigma_\epsilon^2$  is the mean error within a half-dataset.<sup>55</sup>

- 35 e  $R_{work} = \sum_h |F_o - F_c| / \sum F_o$  (working set, no  $\sigma$  cut-off applied).
- 36 f  $R_{free}$  is the same as  $R_{work}$ , but calculated on the test set of reflections excluded from refinement.
- 37 g A.U. – asymmetric unit.
- 38 h Rmsd – root-mean-square deviation
- 39 i Calculated with MolProbity<sup>56</sup>.
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41 **Supplementary Table 2: PCR primers.**

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| Primer name <sup>a</sup>          | Sequence  |
|-----------------------------------|---|
| Slic-sumo-F                       | CCACCATCGGGCGCGGATCCATGCACCATCACCATCACCA            |
| Slic-sumo-R                       | ATGTCGTAATCGCTCATGGGGCCCGGTTTGTTCCTGGT              |
| Slic-ASCC3 <sup>FL</sup> -F       | ACCAGGAACAAACCGGCGGCCCCATGAGCGATTACGACAT            |
| Slic-ASCC3 <sup>FL</sup> -R       | TGGTGATGGTGATGGTGCATGGATCCGCGCCCGATGGTGG            |
| pFL-ASCC3 <sup>NTR</sup> -F       | GGTTTTGAATTCATGGCACTTCCAAGGCTTAC                    |
| pFL-ASCC3 <sup>NTR</sup> -R       | GGTTTTAAGCTTTTAGATCTTCTCTACATCAGCATCC               |
| pFL-ASCC3 <sup>HR</sup> -F        | TCTTTATTTTCAGGGCGCCGAATTCCAACCTCACGTTTACGACTCAC     |
| pFL-ASCC3 <sup>HR</sup> -R        | TCCTCTAGTACTTCTCGACAAGCTTTTATTTACAGGGCGAGGTCGGT     |
| pFL-ASCC3 <sup>NC</sup> -F        | TCTTTATTTTCAGGGCGCCGAATTCCAACCTCACGTTTACGACTCAC     |
| pFL-ASCC3 <sup>NC</sup> -R        | TCCTCTAGTACTTCTCGACAAGCTTTTATGTATGTGGTGGATGCCTCTCTG |
| pFL-ASCC3 <sup>1-207</sup> -F     | GGTTTTGAATTCATGGCACTTCCAAGGCTTAC                    |
| pFL-ASCC3 <sup>1-207</sup> -R     | GGTTTTAAGCTTTTA TGCCTCTTGCAGGTGCTCA                 |
| pFL-ASCC3 <sup>1-197</sup> -F     | GGTTTTGAATTCATGGCACTTCCAAGGCTTAC                    |
| pFL-ASCC3 <sup>1-197</sup> -R     | GGTTTTAAGCTTTTACTTGTAGTCGAGGCTGATGGT                |
| pFL-ASCC3 <sup>1-160</sup> -F     | GGTTTTGAATTCATGGCACTTCCAAGGCTTAC                    |
| pFL-ASCC3 <sup>1-160</sup> -R     | GGTTTTAAGCTTTTATCTGTACCCGTGCTCCTTC                  |
| pFL-ASCC2 <sup>FL</sup> -F        | TCTTTATTTTCAGGGCGCCGAATTCATGCCAGCTCTTCCAACCTGGAT    |
| pFL-ASCC2 <sup>FL</sup> -R        | TCCTCTAGTACTTCTCGACAAGCTTTTAAGAGGGAATCATGCCTTTTCGAG |
| pUCDM-ASCC2 <sup>1-434</sup> -F   | GGTTTTGGATCCTATGCCAGCTCTTCCAACCTGG                  |
| pUCDM-ASCC2 <sup>1-434</sup> -R   | GGTTTTTCTAGATTAGGCTGTCACGGTTACACCG                  |
| pFL-ASCC3 <sup>HR-K505N</sup> -F  | CCCCTACGGGAGCTGGAAATACTAACATCGCTATG                 |
| pFL-ASCC3 <sup>HR-K505N</sup> -R  | CATAGCGATGTTAGTATTTCCAGCTCCCGTAGGGG                 |
| pFL-ASCC3 <sup>HR-K1355N</sup> -F | GTGCTCCAACAGGTAGTGAAATACAGTAGCTGCT                  |
| pFL-ASCC3 <sup>HR-K1355N</sup> -R | AGCAGCTACTGTATTTCCAACCTGTTGGAGCAC                   |
| pFL-ASCC3 <sup>HR-D611A</sup> -F  | CAGGCTGCTGATTCTCGCTGAAGTACACCTCTTGC                 |
| pFL-ASCC3 <sup>HR-D611A</sup> -R  | GCAAGAGGTGTACTTCAGCGAGAATCAGCAGCCTG                 |
| pFL-ASCC3 <sup>HR-D1453A</sup> -F | AACGATCCTGATCATTGCCGAGATCCACCTGTTGG                 |
| pFL-ASCC3 <sup>HR-D1453A</sup> -R | CCAACAGGTGGATCTCGGCAATGATCAGGATCGTT                 |
| pETM11-ASCC3 <sup>NTR</sup> -F    | GGTTTTCCATGGCACTTCCAAGGCTTAC                        |
| pETM11-ASCC3 <sup>NTR</sup> -R    | GGTTTTAAGCTTTTAGATCTTCTCTACATCAGCATCC               |
| pETM11-ASCC3 <sup>1-161</sup> -F  | GGTTTTCCATGGCACTTCCAAGGCTTAC                        |
| pETM11-ASCC3 <sup>1-161</sup> -R  | GGTTTAAGCTTTTAAACTCTGTCACCGTGCTC                    |
| pETM11-ASCC3 <sup>16-197</sup> -F | GGTTTCCATGGCGGTAACGAAGCAGGATAACTACA                 |
| pETM11-ASCC3 <sup>16-197</sup> -R | GGTTTTAAGCTTTTACTTGTAGTCGAGGCTGATGGT                |
| pETM11-ASCC3 <sup>42-197</sup> -F | GGTTTCCATGGCGGACCTTGGACTGACATGGAA                   |
| pETM11-ASCC3 <sup>42-197</sup> -R | GGTTTTAAGCTTTTACTTGTAGTCGAGGCTGATGGT                |
| pETM11-ASCC2 <sup>1-434</sup> -F  | GGTTTCCATGGCGATGCCAGCTCTTCCAACCTGG                  |
| pETM11-ASCC2 <sup>1-434</sup> -R  | GGTTTTAAGCTTTTAGGCTGTCACGGTTACACCG                  |

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44 <sup>a</sup> F, forward; R, reverse

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**Supplementary Table 3: Synthetic genes sequences<sup>a</sup>.**

Synthetic gene sequence for expression of ASCC3<sup>FL</sup>

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ATGGCTCTGCCTAGACTGACTGGTGCCCTGCGCTCTTTTCAGCAACGTCACCAAGCAGGACAACACTACAACGA
AGAAAGTCGCCGACCTGAAGATCAAGCGCTCTAAGCTGCACGAACAGGTCCTGGACTTGGGACTGACCTGGA
AGAAGATCATCAAGTTCCTCAACGAGAAGCTCGAGAAGTCCAAGATGCAGTCCATCAACGAGGACCTGAAG
GACATCCTGCACGCCGCCAAGCAGATCGTCGGAAGTACAACGGAAGGGAAGCCATCGAATCTGGTGCCG
CCTTCCCTGTTTCATGACCTTCCACCTCAAGGACTCCGTCGGACACAAAGAACTAAGGCCATCAAGCAGATGT
TCGGCCCCCTTCCATCTTCTCTGCTACCGCTGCTTGAACGCCACCAACCGCATCATCTCCCCTTCTCTC
AGGACGACCTGACCGCTCTGGTCCAGATGACCGAAAAAGAACACGGCGACAGGGTGTCTTCGAAAGAA
CCTGGCCTTCTCTTTCGACATGCACGACCTGGACCCTTTCGACGAACTGCCATCAACGGCGAAACCCAAA
AGACCATCTCTCTGGACTACAAGAAGTTCTTGAACGAGCACCCTCAAGAGGCTTGCACCCCTGAACTGAAG
CCCGTCGAAAAGACCAACGGATCTTTCCTGTGGTGGCAGGTCGAAAAGTACCTGAACTCTACCCTGAAAGA
GATGACTGAAGTCCCTCGCGTCGAGGACTTGTGCTGCACCCTGTACGACATGCTGGCCTTATCAAGTCTG
GCGACGAGCTGCAGCAACTGTTTCAAGTCTGGGACCCGAAAGGACTGGAATGATCGATCGCAGGACTGCT
GCAGAACCGCATTACTATCGTGGACCGCTTCTGAACTCCTTAACGACCACAGGTTCCAGGCTCTGCAAGA
CAACTGCAAGAAGATCCTGGGAGAAAACGCCAAGCCTAACTACGGATGCCAAGTACCATCCAGTCTGAGC
AAGAAAAGCAGCTGATGAAGCAGTACCGCCGCGAAGAAAAGAGGATCGCCAGGCGAGAAAAGAAAGCCGG
CGAAGATCTGGAAGTCTCTGAAGGACTGATGTGCTTCGACCCCAAAGAACTGCGCATCCAGCGCGAACAGG
CTCTCCTGAACGCTCGCTCTGTCCCCATCTTGTCTCGTCAGAGGGACGCCGACGTCGAGAAGATTACTAC
CCTCACGCTACGACAGCCAGGCCGAAGCCATGAAGACTGCGCTTTCATTGCCGTTGCCAAGATGATCCT
GCCTGAGGGAATCCAGAGGAAAACAACAAGCTGTACGAGGAAGTGCATCCCTACTCTGAACCCATGC
CTCTGTCTTTTCGAAAGAGAAGCCCGTGTACATCCAGGACTTGGACGAAATCGGACAGCTGGCCTTCAAGGGA
ATGAAGAGGCTGAACCGCATCCAGTCCATCGTGTTCGAAACCGCCTACAACACCAACGAGAATGCTGATC
TGCGCCCCCTACCGGTGCCGGCAAGACCAACATTGCTATGCTGACCGTCTGCACGAAATCAGGCAGCACTT
CCAGCAGGGTGTCAAGAAAGAACGAGTCAAGATCGTCTACGTCGCCCTATGAAGGCCCTGGCCGCTG
AAATGACCGACTACTTCTCTAGGCGCCTCGAACCCTTGGGAATCATCGTCAAAGAACTCACCGGCGACATGC
AGCTGTCTAAGTCTGAAATCCTGAGGACCCAGATGCTGGTCAACCCTCTGAAAAGTGGGACGTCGTCACC
CGCAAGTCTGCGGAGATGTCGCCCTGTCTCAGATCGTCAAGCTGCTGATCCTGGACGAAAGTCCACCTGTT
GCACGAGGACGAGGACCCGTCCTGGAATCTATCGTTCGCTAGGACCCTGAGGCAGGTCGAATCTACCCAGT
CTATGATCCGCATCCTGGGACTGAGCGCTACCCTGCCAACTACTTGGACGTCGCCACCTTCTGACGTCAC
ACCCCTACATCGGACTGTTCTTTCGACGGACGCTTCCGTCCTGTGCCTCTGGGACAGACCTTCTCGGA
ATCAAGTGCGCCAACAAGATGCAACAGCTGAACAACATGGATGAAGTCTGCTACGAGAACGTCCTGAAGCAA
GTCAAGGCCGGACACCAAGTCATGGTGTTCGTCACGCTAGGAACGCCACCGTCAGGACCCGCTATGTCTCT
GATCGAAGCGGCCAAGAAGTGTGGACACATCCCATTCTTCTTCCAACTCAAGGACACGACTACGTCCTGGC
CGAAAAGCAGTCCAGAGGTCTAGGAACAAGCAAGTCCCGGAGCTGTTCCCGCAGGATTCTCTATTACC
ACGCCGGAATGCTGCGCCAGGACCCGTAACCTGATCGAAAACCTGTTCTCTAACGGACACATCAAGGTTCTC
GTCTGCACCCGCCACCTTGGCCTGGGGAGTTAACTTACCTGCTCACGCCGTCATCATCAAGGGAACCCAGAT
CTACGCTGCCAAGCGCGGTTCTTTCGTCGACCTGGGAATCCTCGACGTCATGCAAATCTTCGGACGCGCCG
GAAGGCCCCAGTTCGACAAATTCGAGAAAGGCATCATCATCAACCTCACGACAAGCTGTCTCACTACCTGA
CCTTGTGACCCAGAGGAACCCCTATCGAATCTCAGTTCTTGGAAAGCCTGGCCGACAACCTGAACGCCGAA
ATCGCTTTGGGAACCGTCACCAACGTCGAAGAGGCCGTCGAAGTGGATCTCTTACACCTACCTGTACGTCCT
ATGAGGCTAACCTCTGGCTACGGAATCTCTACAAGGCCTACCAGATCGACCCACCTTGGGAAGCA
CCGCGAACAGCTGGTCTATCGAAGTCCGACGCAAGCTGGACAAGGCCAGATGATCCGCTTCAAGAAAGG
ACCGGATACTTCTCTCCACCGACCTCGGACGTACCGCCTCTCACTACTACATCAAGTACAACACTATCGAAA
CCTTCAACGAGTTGTTTCGACGCCACAAGACCGAAGGCCGACATTTTCGCCATCGTGTCTAAGGCCGAAGAG
TTCGACCAGATCAAAGTCCGTGAAGAGGAAATCGAGGAACTGGATAACCCTGCTGTCTAATTCTGCGAACTG
TCTACCCCTGGCCGAGTCGAAAACCTTACGGAAAGATCAACATCCTGCTCCAGACCTACATCTCCCGCGGA
GAAATGGACTCTTCTCTCTGATCTCTGACTCCGCCTACGTGGCCGAGAACGCTGCTAGAATCGTCAGGGCT
CTGTTGAGATCGCCCTGAGAAAAGCGTTGGCCACCATGACCTACCCTGCTGAACTGTCTAAAGTCATC
GACAAGAGGCTGTGGGGATGGCCCTCTCCACTGAGGCAGTTCTCTATCTGCTCCTCCTCACATCCTGACCAG
GCTGGAAGAGAAGAAGCTGACCGTTGACAAGCTGAAAGACATGCGCAAGGACGAGATCGGTACATCCTCC
ACCAGTCAACATCGGCCTGAAAGTCAAGCAGTGTGTCCATCAGATCCCAGCGTCATGATGGAAGCCTCTA
TCCAGCCTATCACCAGAACCGTCTGAGGGTCAACCCTGTCTATCTACGCCGACTTCACCTGGAACGACCAG
GTGCACGGAACCGTTGGAGAACCCTGGTGGATCTGGGTGCAAGATCCCACCAACGATCACATCTACCACTC
CGAATACTTCTCGCGCTGAAGAAGCAAGTTATCTCAAAGAAGCCAGCTGCTGGTTTTCACTATCCCTATC
TTCGAACCCCTGACAGTACTACATCCGCGCTGTTTTCTGACCGCTGGCTGGGAGCTGAAGCCGCTGTG
CATCATCAACTTCCAACACCTCCTGCCAAGAAAGCCACCTCCACACACCGAGCTGTTGGAGTTGAGCC
TCTGCCAATCACCCTCTCGGATGCAAGGCTTACGAGGCCCTGTACAACCTCAGCCACTTCAACCCCGTCCA
GACTCAGATCTTCCACACCTTGTACCACACCGACTGCAACGTCCTGCTGGGTGCTCCTACCGGATCTGGAAA
GACCGTGCCTGCCGAACTGGCTATCTTCCGCGTGTTCACAAGTACCCACCTCTAAGGCTGTCTACATTGC
CCCACTGAAGGCTCTCGTCAGGGAACGCATGGACGACTGAAAGTCCGCATCGAGGAAAAGCTGGGCAAG
AAAGTATCGAGCTGACCGGCGACGTCACCCCTGACATGAAGTCTATCGCCAAGGCCGACCTCATCGTCA
TACCCAGAAAAATGGGACGGTGTCTCTCGCTTGGCAGAACCGTAACTACGTCCAGCAAGTGACCATCCT
GATCATCGACGAAATCCACTTGTCTCGGAGAAGAAAGGGACCTGTCTCGAAGTATCGTCAGCCGCACCA
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ACTTCATCTCTAGCCACACCGAAAAGCCCGTCCGTATCGTCCGACTCTCTACCGCCTTAGCTAACGCCAGAG  
ACTTGCCGACTGGCTGAACATCAAGCAAATGGGCTGTCAACTTCCGTCCATCTGTCAGGCCCGTGCCA  
CTGGAAGTCCACATCCAAGGATTCCCCGACAGCACTACTGCCCCAGGATGGCCTCAATGAACAAGCCTGC  
CTTCCAGGCCATCCGCTCTCACTCTCCAGCTAAGCCTGTCTGATCTTCGTCAGCTCTCGCAGGCAGACCA  
GGCTCACCGCCTTGAATTGATCGCTTTCTGGCCACCGAAGAGGACCCCAAGCAGTGGCTCAACATGGAC  
GAACGCGAGATGGAAAACATCATTGCCACCGTCCCGGACTCTAACCTCAAGCTGACCCTGGCTTTCGGAATC  
GGAATGCACCACGCTGGACTGCACGAGAGGGACAGAAAGACTGTGCAAGAACTTTTCGTAAGTGAAGGT  
CCAGGTGCTGATCGCCACCTCTACTCTGGCTTGGGGTGTCAACTTCCCCGCTCACCTGGTTATCATCAAAGG  
CACCGAGTACTACGACGAAAGACCAGGCGCTACGTTGACTTCCCCATCACCGACGTCTCCAGATGATGG  
GTAGAGCTGGACGCTCTCAGTTCGACGACCAAGGCAAGGCTGTCATCCTGGTCCACGACATCAAGAAAGAC  
TTCTACAAGAAATTCCTCTACGAGCCCTTCCCAGTCAATTTCCCTGCTGGGAGTCTTGAGCGACCACCTC  
AACGCTGAAATTGCCGGCGGAACCATCACTTCTAAGCAGGACGCCCTCGACTACATCACCTGGACTTACTTC  
TTCCGCGCCTGATCATGAACCCCTTACTACAACCTGGGCGACTGTCCCACGACTGTCCCACAAAGTTC  
CTGTCTAACCTCATCGAAAAGTCCCTCATCGAGCTGGAAGTGTCTACTGCATCGAGATCGGAGAGGACAAC  
CGCTCTATCGAGCCCCTGACCTACGGAAGGATCGCTCCTACTACTACCTGAAGCACCAGACCGTCAAGATG  
TTCAAGGACCGCTGAAGCCTGAATGCTCTACCGAAGAAGTGTGTCCATCCTGTCCGACGCTGAAGAGTA  
CACCGACCTGCCAGTCAGGCACAACGAGGATCACATGAACTCTGAACTGGCCAAGTGCCTGCCTATCGAGT  
CTAACCTCACTCCTTCGACTCTCCCCACACCAAGGCTCACCTTTGCTGCAGGCTCACTTGTCTAGGGCCA  
TGCTGCCTTGTCTGACTACGACACCGACACCAAGACCGTGTGGACCAAGCTCTCAGAGTCTGCCAGGCT  
ATGTTGGACGTGGCCGCAATCAAGGATGGCTCGTCAACCTCAACATCACCAACCTGATCCAGATGGTC  
ATCCAAGGCCGCTGGTTGAAGGACTCCTCTCTGCTCACCTGCCTAACATCGAAAACACCACCTCCACCTC  
TTCAAGAAATGGAAGCCCATCATGAAGGGACCTCACGCCAGAGGAAGGACCTCCATCGAGTCTCTGCCCGA  
ACTGATCCACGCTGCGGAGGAAAGGACCAGTTTTCTCTTATGGTCAATCCGAACCTCACGCGCTAA  
GACTAAGCAGGCTTGAAGTTCCTCTCTCACTTGCCTGTCATCAACGTCCGCATCTCCGTCAAAGGATCTTG  
GGACGACTTGGTGAAGGACATAACGAGCTGTCTGTCAAGCCTGACCGCCGACAAAAGGGACGACAAC  
AAATGGATCAAGCTCCACGCTGACCAAGAGTACGTTGCAAGTCTCTCTGCAGCGCGTCCACTTCGGATT  
CACAAGGGAAAGCCGAATCTGCGCTGTACCCCTAGGTTCCCAAGTCAAAGGACGAAGGCTGGTTCTCT  
GATCCTCGGAGAGGTGACAAGCGGAGCTGATCGCTCTAAGCGCGTCCGTTACATCCGCAACCACCACG  
TTGCCAGCCTGTCTTTCTACACCCCTGAGATCCCCGGACGCTACATCTACACCTCTACTTCATGAGCGACT  
GCTACCTGGGACTCGACCAGCAGTACGACATCTACCTGAACGTCACCCAGGCTTCTCTGTCTGCTCAAGTCA  
ATACCAAGGTGTCGACTCTCTGACCGACCTGGCTCTGAAGTAA

Synthetic gene sequence for expression of ASCC3 fragments

ATGGCACTTCCAAGGCTTACGGGTGCTTTGAGAAGTTTCAGCAACGTAACGAAGCAGGATAACTACAACGAG  
GAAGTGGCAGACTTGAAGATCAAGCGAAGCAAGCTGCACGAACAAGTACTTGACCTTGGACTGACATGGAA  
GAAGATCATCAAGTTCCTCAACGAGAAGCTCGAAAAGTCCAAGATGCAGTCCATCAACGAGGACCTCAAGGA  
CATCCTCCACGCAGCAAAACAGATCGTGGGTACAGATAACGGTAGAGAGGCTATTGAGTCAGGTGCAGCATT  
CCTTTTCATGACCTTCCACCTGAAGGACAGTGTAGGTACAAGGAAACAAAGGCAATCAAGCAGATGTTCCG  
TCCTTTCCCTAGTTCCAGCGCAACAGCAGCTTGTAAACGCAACAACAGAATCATCTCGCACTTCAGTCAGGA  
CGACTGACAGCACTTGTACAAATGACAGAGAAGGAGCACGGTGACAGAGTTTTCTTCGGTAAAAACCTCG  
CTTTTCAGTTTCGACATGCACGACCTTGACCATTTCGATGAGTTGCCAATCAACGGAGAAACACAGAAAACCA  
TCAGCCTCGACTACAAGAAGTTTCTCAATGAGCACCTGCAAGAGGCATGTACACCAGAGTTGAAGCCAGTG  
GAGAAAACCTAACGGATCATTCTGTGGTGTGAGGTGGAAAAGTACCTCAACAGTACCCTGAAGGAGATGACT  
GAGGTGCCAAGGGTTGAAGATCTTTGTTGTACCCTTTACGACATGCTCGCTAGTATCAAGTCCGGGTGACGAA  
CTTCAGGACGAAGTTCGAACTGCTGGGTCCAGAAGGACTTGAATTGATCGAAAAGTTGCTCCAGAACAG  
GATAACCATCGTGGACAGATTCTTAACCTCAGCAACGACCACAGGTTCCAAGCTCTTCAGGATAACTGCAA  
GAAGATCCTGGGTGAAAACGCTAAGCCTAATACGGATGCCAAGTACTATCCAGTCAGAGCAAGAGAAGCA  
GCTTATGAAGCAGTACAGAGTGAAGGAGAAGGATCGCTAGGAGGGAAAAGAAAGCTGGTGAAGATCTGG  
AAGTAAAGTGAAGGTTTGTATGTCTTCGACCCAAAGGAAGTGAAGTCCAGAGAGAACAGGCTCTGCTTAA  
GCTAGATCAGTGCCAATCCTGTCAAGACAAAAGGATGCTGATGTAGAGAAGATCCACTACCCTCACGTTTAC  
GACTCACAGGCTGAAGCTATGAAAACATCGGCTTTTCATCGCTGGTGTGCTAAGATGATCCTGCCTGAAGGAATC  
CAAAGAGAAAACAACAAGCTCTACGAGGAAGTCCGTATCCCTACTCGGAACCTATGCCACTGTCTTTCGAA  
GAGAAGCCTGTGTACATCCAGGACTTGGACGAAATCGGACAACTTGCTTTCAGGGTATGAAGAGGCTGAAC  
CGTATCCAGTCAATCGTGTTCGAAACGGCTTACAATACCAACGAGAATATGCTGATCTGCGCCCTACGGGA  
GCTGGAAAAACCTAACATCGCTATGCTGACTGACTGCACGAAATCAGACAACATTTCCAGCAGGGAGTAATCA  
AGAAGAACGAGTTCAAGATCGTGTACGTGGCCCTATGAAAGCCCTGGCTGCTGAAATGACGGATTACTTCA  
GCAGGAGACTGGAACCTTTGGGAATCATCGTAAAGGAAGTGCAGGAGACATGCAGTTGAGCAAGTCAGAA  
ATCCTGAGGACTCAGATGCTCGTACTACTCCGGAATAAGGGACGTTGTAACCTGTAAGTCAAGTGGGAGAT  
GTAGCTCTGTCTCAGATTGTCAGGCTGCTGATTCTCGATGAAGTACACCTCTTGCACGAAGATCGAGGTCCG  
GTTTTAGAGTCTATTGTGCTCGTACTCTCCGTCAAGTTGAATCTACTCAGTCTATGATTTCGCATACTCGGACT  
CTCTGCGACTTCCCAATTACCTTGACGTTGCCACTTTCTTTCGACGTTAATCCGTACATTGGCCTGTTCTTTT  
TCGATGGCAGATCCGTCGCTCCGTTCCCTTAGGACAAACCTTCTCGGAATTAAGTGCACCAATAAGATGCAG  
AGTTGAACAACATGGACGAGGTTTGTCTAGCAAAAAGTCTCAACAGGTCAAAAGCCGGTCAAGTATGATGG  
TCTTTGTCCACGCGCGAAATGCCACTGTTCAAGCCGCGATGCTCTTAATTGAACGTGCCAAAAACTGTGGTC  
ATATACCGTTCTTTTCCCACTCAAGGTCATGACTACGTTCTCGCCGAAAACAGGTTCAACGATCTCGAAA  
TAAGCAGGTCCGTGAAGTCTTTCCCGATGTTTTCTATTACCACGCCGGTATGTTGCGTCAAGATCGTAA  
TTGGTCGAAAACCTTCTCTAACGGCCATATTAAGGTCTCGTCTGCACTGCGACTTTAGCCTGGGGCGCTC

AATTTGCCGCCCATGCCGTCATTATCAAAGGCACTCAAATATATGCCGCCAAACGTGGCTCTTTTGTGGATT  
TGGGCATATTAGATGTGATGCAAATTTTTGGCCGCGCGGGCCGCCGCAATTTGACAAATTTGGCGAAGGCA  
TTATTATAACTACCCATGATAAACTGTCTCATTACCTCACCCCTCCTCACCCAACGCAATCCCATTGAATCCCAAT  
TTTTGGAATCCTTGGCGGATAACTTGAATGCCGAAATGCCCTCGGCACCGTCAACCAATGTCGAAGAAGCCG  
TGAAATGGATTTCTATACCTACTTGTACGTGCGAATGCGTGCGAATCCCTTAGCCTATGGCATTCCCATAAA  
GCGTATCAAATAGACCCACCTTACGCAAACACCGCAACAATTAGTCATTGAGGTCGGACGCAAATGGATA  
AAGCGCAAATGATACGCTTCGAGGAACGCACCGGCTATTTTTCCAGCACCGATTTGGCCGACCGCCAGC  
CATTATTACATAAAAATAATACGATTGAAACCTTCAACGAGTTATTCGACGCCATAAAAACCGAGGGCGACATT  
TTCGCCATTGTCAGCAAAGCCGAGGAGTTTGACCAAATCAAAGTCCGCGAGGAAGAGATTGAGGAGTTAGA  
CACGTTATTATCGAATTTTTGCGAGTTATCGACGCCGGCGGCGTGGAAAACAGCTACGGAAAGATCAACAT  
CCTGCTCCAGACCTACATCAGCAGGGGTGAAATGGACAGTTTCTCGCTGATCAGTGACAGCGCTTACGTTGC  
ACAAAACGCAGCTAGAATCGTAAGGGCTCTTTTTGAGATCGCACTTAGAAAAGCGTTGGCCAACAATGACATA  
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TTTTATACCCCGGAAATTCGGGGCCGCTATATATATACCTCTATTTTATGTCAGACTGCTACCTCGGCCTCGA  
TCAACAATATGACATATATCTGAATGTACCCAAGCGTCACTCTGCCCCAAGTGAATACCAAAGTCAAGTCACTGACT  
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Synthetic gene sequence for expression of ASCC2

ATGCCAGCTCTTCCACTGGATCAGTTGCAGATCACACACAAAGATCCTAAGACGGGAAAGCTGAGGACTAGC  
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CAGTCTCGTGAAACAACCTCAGGAAAAGAAGAAAAAAGAAGCGAATAAAGCGACTCGTGCCAATCACAATCGC  
CGTACTATGGCCGATCGCAAACGCTCGAAAGGCATGATTCCCTCTTAA

48

49 <sup>a</sup> Expression of ASCC3<sup>FL</sup> and ASCC3 fragments was based on different synthetic gene sequences.

50



51 **Supplementary Table 4: Nucleic acids used in helicase assays<sup>a</sup>.**

52

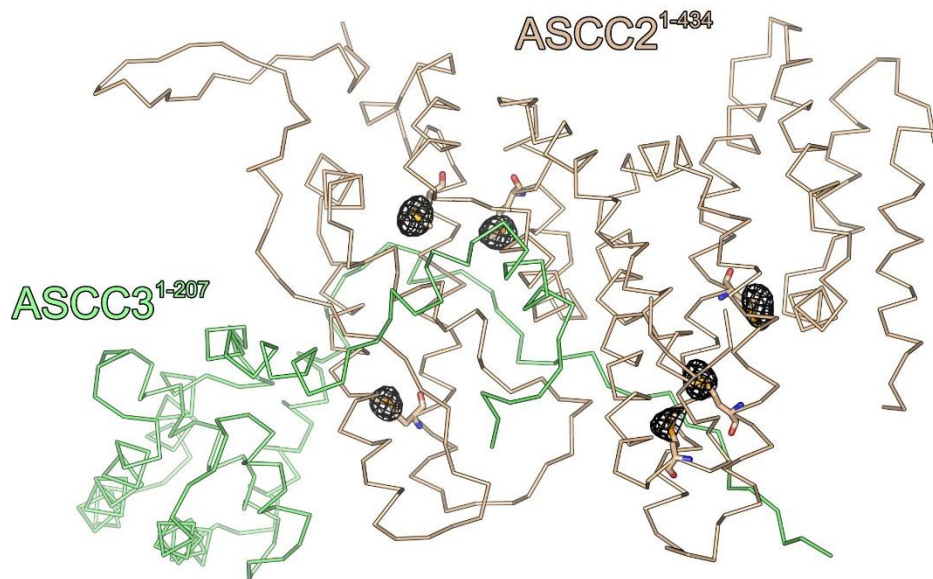
| 3'-overhang DNA  |
|--|
| (Atto 540 Q)5'- <b>GGCCGCGAGCCG</b> GAAATTTAATTATAAACCAGACCGTCTCCTC-3' |
| 5'- <b>CGGCTCGCGGCC</b> -3'(Alexa Fluor 488)                           |
| 5'-overhang DNA  |
| 5'-CTCCTCTGCCAGACCAAATATTAATTTAAAG <b>GCCGAGCGCCGG</b> -3'(Atto 540 Q) |
| (Alexa Fluor 488)5'- <b>CCGGCGCTCGGC</b> -3'                           |
| 3'-overhang RNA  |
| (Atto 540 Q)5'- <b>GGCCGCGAGCCG</b> GAAAUUUAAUUUAUAACCAGACCGUCUCCUC-3' |
| 5'- <b>CGGCUCGCGGCC</b> -3'(Alexa Fluor 488)                           |

53

54 <sup>a</sup> Duplex regions in bold.

55

56 **Supplementary Figures**



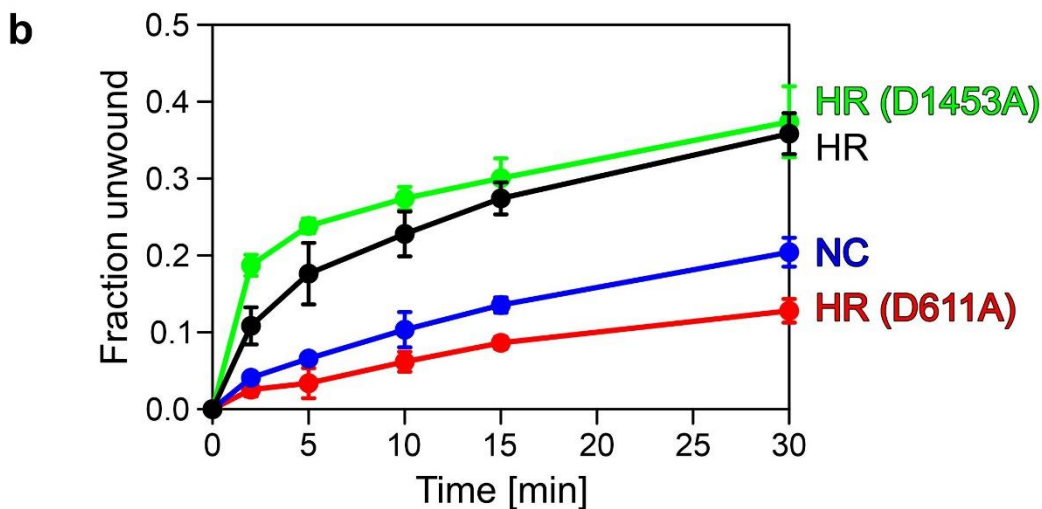
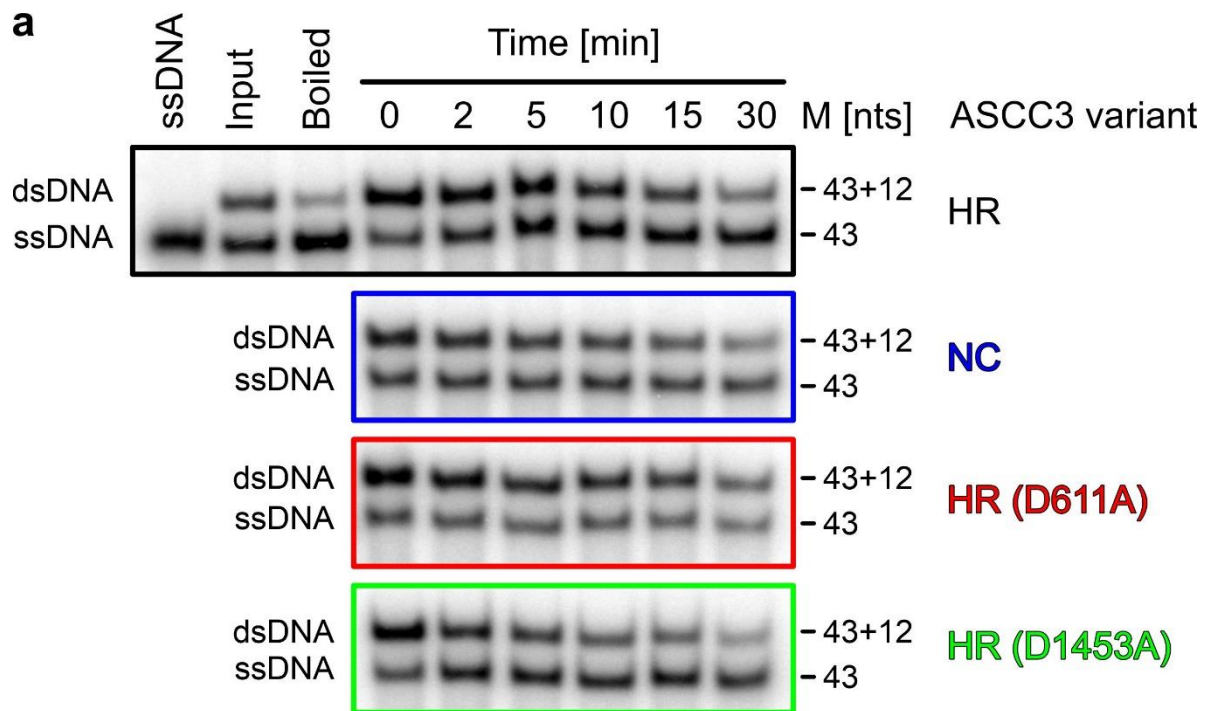
57

58

59 **Supplementary Figure 1: Anomalous difference Fourier map.**

60 Anomalous difference Fourier map contoured at the 5  $\sigma$  level (gray mesh), showing the  
61 positions of selenium atoms in the side chains of SeMet residues (shown as sticks). View as  
62 in Fig. 1d,f, left. ASCC2<sup>1-434</sup>, beige; ASCC3<sup>1-207</sup>, lime green.

63



64

65

66 **Supplementary Figure 2: Gel-based unwinding assay.**

67 **a**, Gels showing time courses of unwinding of a 3'-overhang DNA substrate (long strand

68 radiolabeled; same sequence as used in stopped-flow/fluorescence-based unwinding assays;

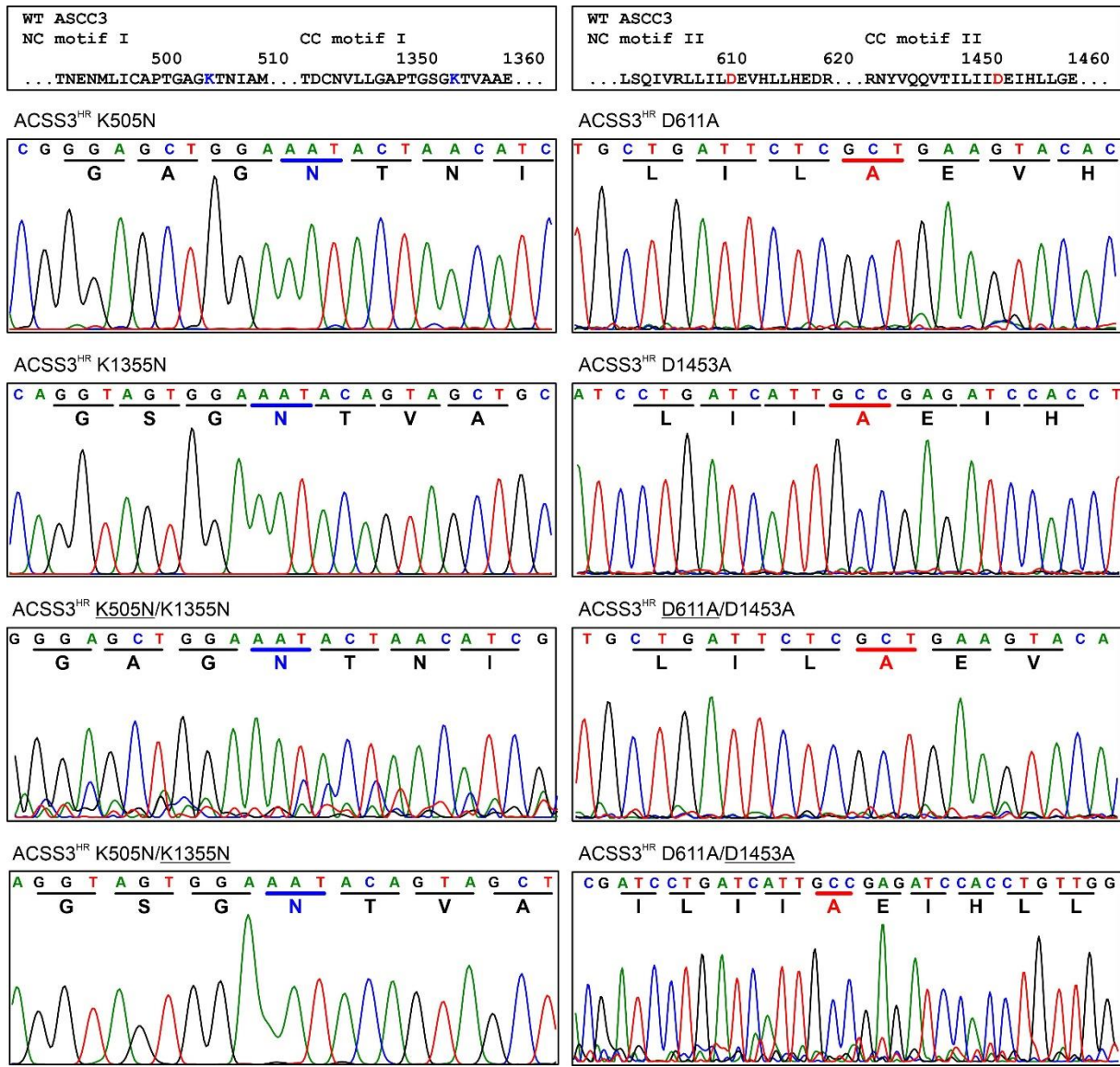
69 Supplementary Table 4) by the ASCC3 variants indicated on the right. HR / NC / HR (D611A)

70 / HR (D1453A), ASCC3 variants as defined in the text. Bands representing double-stranded

71 (ds) and single-stranded (ss) DNA are labeled on the left. The DNA input contained a mixture

72 of dsDNA and ssDNA. Boiled, DNA sample heated to 95 °C for 5 minutes before gel analysis.  
73 M [nts], molecular weight marker (number of nucleotides). Lanes representing ASCC3<sup>HR</sup> and  
74 ASCC3<sup>NC</sup> were run on the same gel, and lanes representing ASCC3<sup>HR,D611A</sup> and  
75 ASCC3<sup>HR,D1453A</sup> were run on the same gel, and gels were processed in parallel. Each gel  
76 contained marker lanes “ssDNA”, “Input” and “Boiled”, identifying running positions of dsDNA  
77 and ssDNA, but are only shown for the first panel. Complete gels are shown in the source data  
78 provided for this paper. To facilitate comparison, relevant gel sections are displayed below  
79 each other. **b**, Quantification of the data shown in **(a)**. Data represent means  $\pm$  SD of n=3  
80 independent experiments, using the same biochemical samples.

81



82

83

84 **Supplementary Figure 3: Sequencing results.**

85 Sequencing results documenting the presence of the intended mutations and the encoded

86 residues in the baculoviruses expressing ASCC3<sup>HR</sup> variants for helicase activity assays shown

87 in Fig. 6b-d. Top panel, motif I (left) and motif II (right) sequences in WT ASCC3; conserved K

88 and D residues highlighted blue and red, respectively. The ASCC3<sup>HR</sup> variants encoded by the

89 sequenced baculoviruses are indicated above the panels. For baculoviruses encoding

90 ASCC3<sup>HR</sup> variants with double residue substitutions, sequencing results for the underlined

91 substitution is displayed in the respective windows. NC, N-terminal helicase cassette; CC, C-  
92 terminal helicase cassette.