

## **SUPPORTING INFORMATION**

### **Identification of specific inhibitors of human RAD51 recombinase using high-throughput screening**

Fei Huang<sup>1</sup>, Nuzhat A. Motlekar<sup>2</sup>, Chelsea M. Burgwin<sup>1</sup>, Andrew D. Napper<sup>2</sup>, Scott L. Diamond<sup>2,3</sup> and Alexander V. Mazin<sup>1\*</sup>

*<sup>1</sup>Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102, USA; <sup>2</sup>Penn center for Molecular Discovery, University of Pennsylvania, Philadelphia, PA 19102, USA; <sup>3</sup>Department of Genetics, University of Pennsylvania, Philadelphia, PA 19104, USA .*

#### **CONTENT:**

#### **Supplementary Tables 1-3**

\*Corresponding Author:

Alexander Mazin, Ph. D.

Drexel University College of Medicine

Department of Biochemistry and Molecular Biology

245 N 15<sup>th</sup> Street, MS 497, NCB, Room 10103

Philadelphia, PA 19102-1192

Tel: 215-762-7195; Fax: 215-762-4452

E-mail: [amazin@drexelmed.edu](mailto:amazin@drexelmed.edu)

**Supplementary Table 1. The effect of selected compounds (100  $\mu$ M) on the D-loop formation promoted by RAD51**

Compounds	Joint Molecules (%)	DNA binding <sup>a</sup>
Control	30.3 $\pm$ 1.9	
A03	5.4 $\pm$ 1.9	
A04	1.4 $\pm$ 0.1	
A05	1.6 $\pm$ 0.2	+
A06	3.5 $\pm$ 0.1	+
A07	29.6 $\pm$ 0.1	
A08	10.0 $\pm$ 0.8	
A09	32.9 $\pm$ 3.5	
A10	6.7 $\pm$ 2.5	
A11	10.2 $\pm$ 1.7	
A12	19.2 $\pm$ 3.4	
A13	27.8 $\pm$ 3.8	+
A14	3.8 $\pm$ 0.1	+
A15	4.2 $\pm$ 0.7	+
B01	11.0 $\pm$ 2.3	
B02	6.0 $\pm$ 0.5	
B04	24.8 $\pm$ 0.1	
B05	20.1 $\pm$ 0.8	

<sup>a</sup>DNA binding was determined by the fluorescent intercalator (ethidium bromide) displacement assay.

**Supplementary Table 2. The IC<sub>50</sub> values for selected RAD51 inhibitors**

Compound	IC <sub>50</sub> (RAD51) (μM)	IC <sub>50</sub> (RecA) (μM)	IC <sub>50</sub> (RecA)/ IC <sub>50</sub> (RAD51)
A03	33.2	187.3	5.6
A04	5.0	5.7	1.1
A10	26.6	35.3	1.3
B02	27.4	>250	n/a
B02-3a	15.3	>100	n/a
B02-3b	27.3	>100	n/a

**Supplementary Table 3. Sequences of the oligonucleotides used in this study**

N	Length, nt	Sequence (5'→3')
25	48	GCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAA AAGGA
25-FLU <sup>a</sup>	47	FLU-CAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATC AAAAGGA
26-BHQ1 <sup>b</sup>	47	TCCTT TTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGC TTAATTG-BHQ1
374	48	G TTCAGTCAGTGCTCGATATGCGGTGTGAATTACGGCTCAGTTG CCTA
90	90	CGGGTGTCTGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGT ACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGT
71	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATTC AGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAA AGGA
169	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATT GCTGAATCTGGTGCTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
170	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATT GCTAAATCTGGTGCTGTAGGTCAACATGTTGTAAATATGCAGCTA AAG
171	63	ACAGCACCAGATTTAGCAATTAAGCTCTAAGCCATCCGCAAAAAT GACCTCTTATCAAAAGGA

<sup>a</sup>FLU = Fluorescein

<sup>b</sup>BHQ1 = Black Hole Quencher 1