

Supporting Information

DDX3X helicase inhibitors as new strategy to fight West Nile Virus infection

Annalaura Brai,^{1,2} Francesco Martelli,³ Valentina Riva,⁴ Anna Garbelli,⁴ Roberta Fazi,¹

Claudio Zamperini,^{1,2} Alessandro Pollutri,¹ Lucia Falsitta,¹ Stefania Ronzini,¹ Laura

Maccari,² Giovanni Maga,⁴ Simone Giannecchini³ and Maurizio Botta^{1,2,5,}*

¹Dipartimento Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via A.

De Gasperi 2, I-53100 Siena, Italy. ²Lead Discovery Siena S.r.l., Castelnuovo Berardenga,

I-53019 Siena, Italy. ³Department of Experimental and Clinical Medicine, University of

Florence, I-50134 Florence, Italy. ⁴Istituto di Genetica Molecolare, IGM-CNR, Via

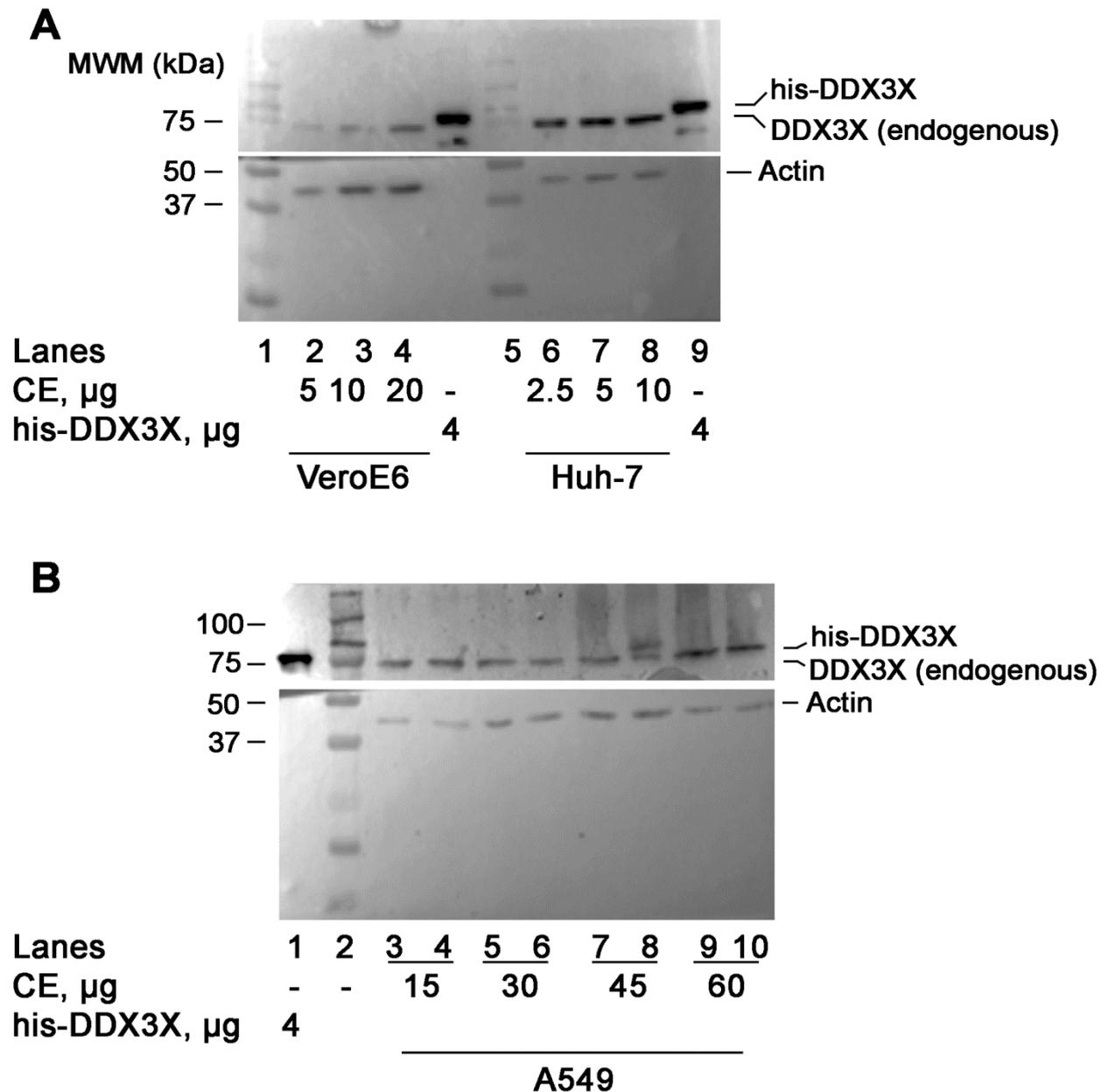
Abbiategrosso 207, I-27100 Pavia, Italy. ⁵Biotechnology College of Science and

Technology, Temple University, Biolife Science Building, Suite 333, 1900 N 12th Street,

Philadelphia, Pennsylvania 19122.

Table of contents:

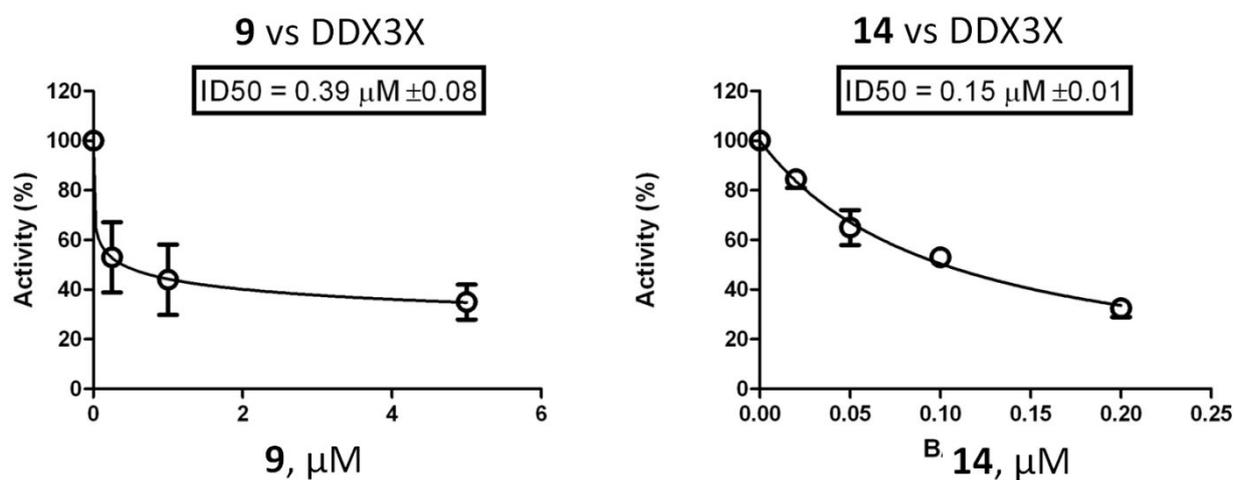
Representative Western blot experiments for the quantification of DDX3X in cell extracts.....	S3
Representative anti-enzymatic curves.....	S4
Anti-WNV dose-response and cytotoxicity curves.....	S5



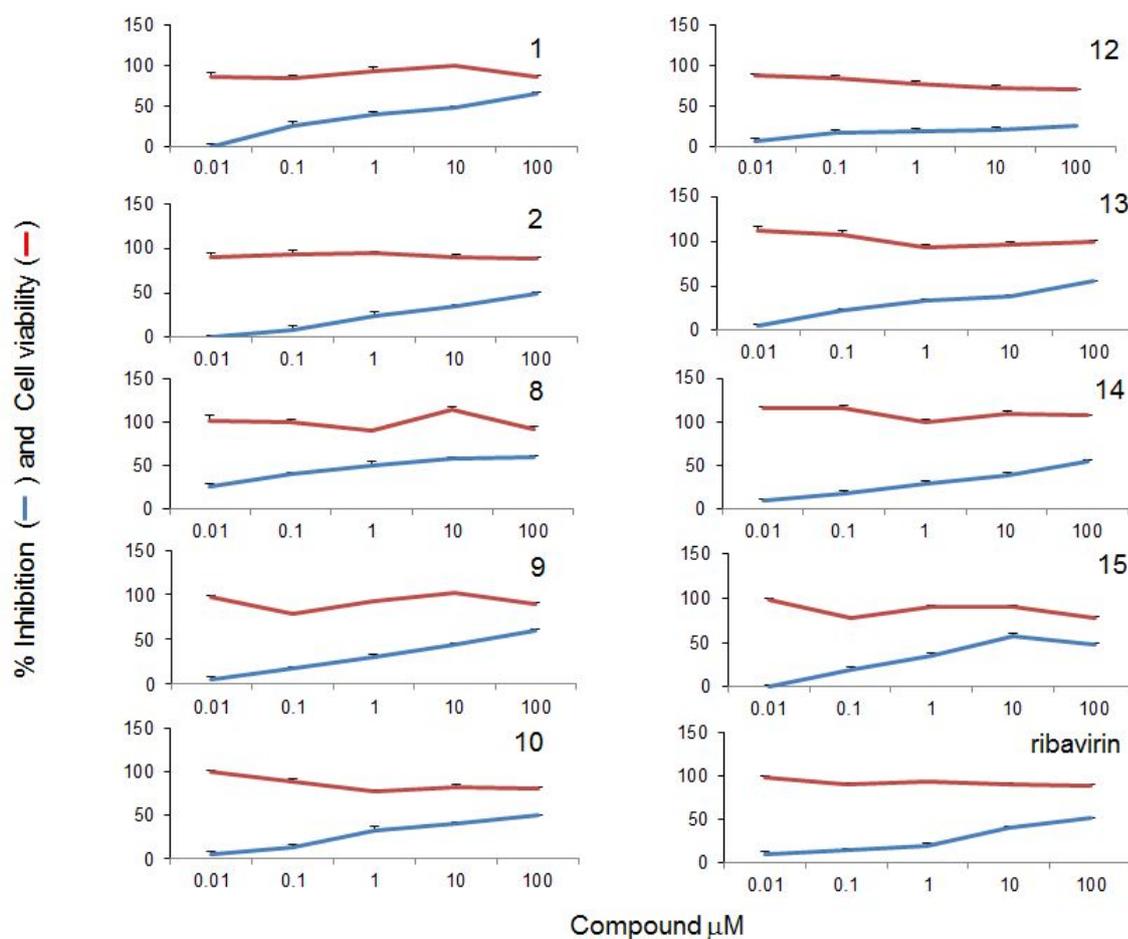
Supplementary Figure 1. Representative Western blot experiments for the quantification of DDX3X in cell extracts (CE). A. Increasing amounts of VeroE6 (lanes 2-4) or Huh-7 (lanes 6-8) total extracts were transferred to a nitrocellulose membrane after SDS-PAGE separation. The membrane was cut immediately above the 50 kDa molecular weight marker (MWM), and the upper half was subjected to immune detection with anti human DDX3X polyclonal antibodies, while the lower half was probed with anti-Actin antibodies, as loading control. Lanes 1, 5: MWM; lane 9: recombinant his-tagged human DDX3X as positive control. B. Increasing amounts in duplicate of A549 (lanes 3-10) total extract were transferred to a

nitrocellulose membrane after SDS-PAGE separation. The membrane was processed as described in panel A. Lane 1: recombinant his-tagged human DDX3X as positive control; lane 2: MWM.

The presence of the double his-tag gives a slower electrophoretic mobility of the recombinant DDX3X with respect to the untagged endogenous protein in both panels.



Supplementary Figure (2). Increasing doses of the compounds **9** and **14**, of Table (1) of the main manuscript file, were incubated in the presence of DDX3X in the fluorescence-based FRET helicase assay under the conditions described in the Methods section. Fluorescence measured in parallel control reactions in the absence of inhibitors were taken as 100% of enzymatic activity. Data are the mean \pm S.D. of three replicates. ID50 values were calculate as described in the Methods section.



Supplementary Figure (3). Kinetic of Antiviral Activities and cytotoxicity of selected compounds against WNV infected Huh-7 cells. WNV infection of Huh7 cells at MOI of 0.1 in presence of compounds at indicated concentration was assayed with the viral plaque reduction assay. Cell viability of Huh7 cells in the same condition in absence of virus infection was assessed with MTT assay. Data represent means \pm standard deviation.