Figure Legends

Figure 1: Cortex moutan, a root ingredient present in the Chinese herbal supplement Zhi Bai Di Huan Wan inhibits HCV infection in a dose dependent manner. Jc1 viruses were premixed with indicated dilutions of extracts of **(A)** Zhi Bai Di Huang Wan or **(B)** selected root extracts present in Zhi Bai Di Huang Wan (Rhemannia, Phellodendron, Poria, Cortex moutan, Fr. Corni, Dioscorea and Alismatis orientalis), and infectivity was assessed by limiting dilution assay ($TCID_{50}$). Titration of Jc1 in the absence of components was performed in parallel as a control. Mean values and standard deviations of three independent experiments are shown. **(C)** Cells were either pretreated with given doses of PGG (pre-infection, I), inoculated with virus in the presence of PGG (co-infection, II), or incubated with PGG subsequent to virus inoculation (post-infection, III). In each case, virus was inoculated for 4 hours and PGG was present during a 4h interval. Unbound viruses and compounds were removed by washing with PBS, and addition of fresh medium. At 48 hours post inoculation, monolayers were washed and lysed. Renilla luciferase activity from cell lysates was measured. Data are shown as percentage of infection in presence of DMEM. Mean values and standard deviations of three independent experiments are shown.

Figure 2: PGG is a pangenotypic HCV cell entry inhibitor that also impedes ZIKV infection. Huh-7.5 cells were inoculated with renilla luciferase reporter Jc1 viruses in the presence of indicated concentrations of PGG added to cells either **(A)** before inoculation of viruses, **(B)** during virus inoculation or **(C)** after virus inoculation. **(D)** Huh-7.5 cells were treated with indicated concentrations of PGG for 4 hours. Thereafter, cells were washed with PBS and fresh medium was added. At 48 hours post-treatment an MTT-assay was performed and OD 570/630 values were normalized to untreated control. (E) Huh-7.5 cells constantly expressing a firefly-luciferase were treated similarly as in (B) with indicated concentrations of PGG. Infection was assessed by detection of renilla luciferase activity. Cell viability is reflected by firefly

luciferase activity in cell lysates. (F) Huh-7.5 cells were infected with given renilla luciferase reporter viruses bearing the structural proteins of indicated genotypes in the presence of increasing concentrations of PGG. After 4 hours incubation, viruses were removed, monolayers were washed with PBS and fresh medium was added. At 48 hours post-infection, monolayers were washed with PBS and lysed. Renilla luciferase activity from cell lysates was measured with a luminometer. Results are shown as percentage of infection in presence of solvent control. **(G)** Vero B4 cells were infected with ZIKV Puerto Rico strain with indicated concentrations of PGG. ZIKV RNA was measured in supernatant of infected cells after 48 hours p.i.. Genome equivalents (GE) were normalized to solvent control. Mean values and standard deviations of two (F) and three (A-E and G) independent experiments are shown.

Figure 3: PGG does not disrupt HCV particle integrity. (A) Undiluted and 1:10 diluted virus stock (renilla luciferase reporter virus) was incubated with PGG at indicated concentrations. After 1 hour, the undiluted virus/PGG mixture was diluted by a factor 1:10 with normal medium and used for infection of Huh-7.5 cells. After 48 hours luciferase activity of cell lysates was measured as a marker of viral infection. As a negative control ITX 5061 (ITX), an HCV receptor antagonist was used at concentrations of 0.5µM (a), 0.05µM (b) or 0.5µM (c). Results are shown as percentage of infection in presence of solvent control. .Mean values and standard deviations of four representative experiments are shown. **(B)** Proteinase K (PK) assay was performed to assess the effect of PGG on the viral envelope. Following incubation of Jc1 with PGG (10 µM) or vehicle control for 4h at room temperature, samples were divided in three parts. One part was treated with PBS, a second part was treated with PK for 1 h at 4°C, and the third part was lysed in 2% triton X-100 prior to PK treatment. Protease-resistant core protein was quantified by ELISA. **(C)** Iodixanol gradient centrifugation of Jc1 particles incubated with PGG (15 µM) or solvent control for 4h at room temperature beforehand. 10 fractions were collected and their infectivity

was measured by $TCID_{50}$. Mean values and standard deviations of four **(A),** three **(B)** or one representative out of three **(C)** independent experiments are shown.

Figure 4: Viral RNA integrity is protected from PGG. (A) Experimental set up of figures **4B** and **4C** to examine the effect of PGG on HCV particle integrity by assessing infectivity and RNA stability. Incubation of Jc1 viruses with PGG (20 µM) or vehicle control was performed for 4 h at 37°C. Afterwards, infectivity of particles was measured by limiting dilution assay ($TCID_{50}$) and RNA levels were measured by qRT-PCR following RNA extraction. Extracted RNA was also used to transfect naïve cells by electroporation. RNA levels in the supernatants of transfected cells were measured at 4 and 72 h post-electroporation. Infectivity of these supernatants was measured by $TCID_{50}$ at 72 h post-electroporation. **(B)** RNA quantification immediately after virus incubation (I) and at 4 and 72 h after electroporation of HCV RNA extracted from treated virions (II and III) was performed by qPCR. Heat inactivation of particles was done in parallel as a treatment control affecting particle and RNA integrity. **(C)** Infectivity levels were measured by $TCID_{50}$ immediately after virus incubation (I) or at 72 h after electroporation with extracted RNA (II).

Figure 5: PGG inhibits early steps of HCV cell entry (A) Huh-7.5 cells were infected with firefly luciferase reporter Jc1 and indicated compounds were added at different time points throughout infection. Virus attachment was performed for 1 h at 4°C. Subsequently, unbound viruses were washed away with PBS and cell monolayers were transferred to 37°C. Attachment inhibitor heparin (50 µg/mL), PGG (6 µM) or endosome acidification inhibitor ConcaA (5 nM) were added either during inoculation (protocol 1) or every 20 min until 3 h after transfer to 37°C (protocol 2-7). Each compound was incubated with the cell monolayers for a total of 4 h before washing and medium exchange. Cells were lysed and firefly luciferase measured 48 h after

inoculation. Values are normalized to solvent control. Curves are plotted until maximum normalized infectivity of 100% was achieved.

Figure 6: PGG primarily inhibits attachment of HCV

(A) Huh7-5 cells were infected with renilla luciferase reporter Jc1 at different time points during treatment with ConcaA. Fusion was triggered by addition of low pH (pH5). PGG was present at different steps of the entry-process. Flunarizin (fusion-inhibitor) and CD81 (HCV receptor antibody) served as controls. Viral infection was determined by lysing the cells 48 hours after infection and measuring luciferase activity. Normalized data represent mean values and standard deviations of three independent experiments**. (B)** Virus binding was assessed by measuring fluorescence of R18-labeled HCV virions attached to cells. Virions were incubated with increasing concentrations of PGG for 10 min at 37ºC. Pre-chilled cell monolayers of Huh-7.5 cells were inoculated with pre-chilled virions for 1 h at 4ºC. After extensive washing with PBS, cells were lysed and measured for R18 fluorescence. Data is shown as percentage of virus binding in presence of vehicle. Mean values and standard deviations of three (A and B) and two (C) independent experiments are shown.

Figure 7: PGG increases potency of HCV inhibitors. Huh-7.5 cells were inoculated with renilla luciferase reporter Jc1 in the presence of increasing concentrations of PGG. After 4 h cell monolayers were washed with PBS and fresh medium containing 600fM of Daclatasvir was added. Cells were lysed 48 h later and renilla luciferase activity was measured. Mean values and standard deviations of three independent experiments are shown.

Figure 8: PGG inhibits HCV entry into primary human hepatocytes and is highly bioavailable in mice. Monolayers of primary human hepatocytes isolated from three donors were inoculated with Jc1 in presence of vehicle control, concanamycin A (ConcaA, 8.2 nM) or PGG (8 µM) at 37ºC. After 6 h, the monolayer of primary human hepatocytes was extensively

washed with PBS and new media was added. The supernatants of these cells were harvested at 48 h post-infection and virus infectivity was determined by $TCID_{50}$ **(A)**. To determine pharmacokinetics *in vivo*, a single dose of PGG (50mg/kg) was administered intraperitoneally to 5 SCID mice. PGG concentration in plasma of mice was determined by LC-MS/MS on indicated days. The dashed line depicts the tissue culture IC_{50} **(B).** For detection of hepatotoxicity in mice, 10 Balb/c mice were treated with a single dose of PGG (50mg/kg) intraperitoneally. Blood samples were drawn at indicated days and were analyzed for ALT-levels. Day 0 thereby reflects pre-treatment levels **(C)**.