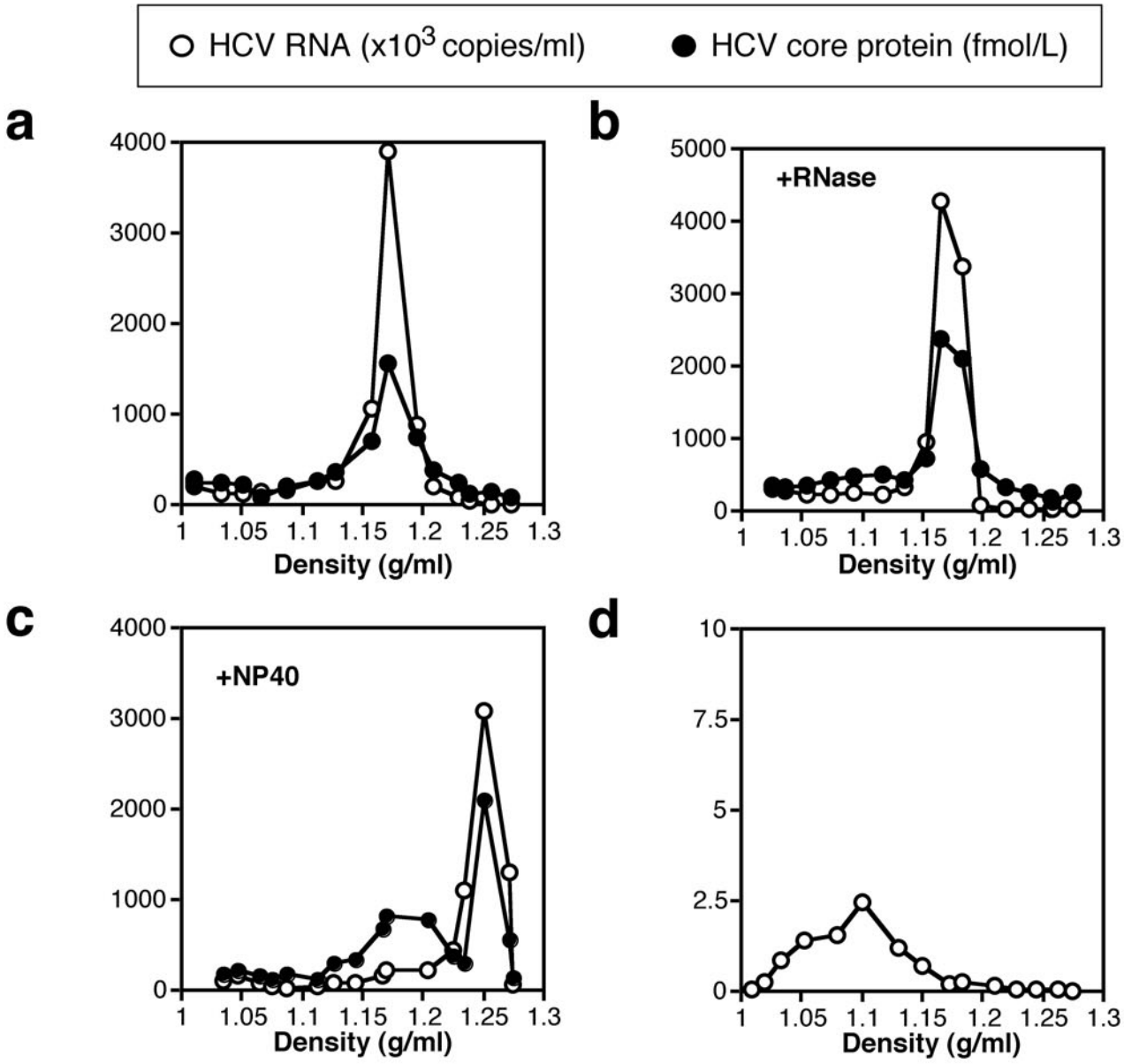
Supplementary Figure 3



Supplementary Fig. 3: Density gradient analysis of culture supernatants of transfected Huh7 cells. Supernatants harvested 6 days after transfection contained core protein at a concentration of 3845.2 fmol/L in case of JFH1 transfection or no core protein in case of transfection with the subgenomic replicon. Collected medium was cleared by centrifugation and passed through a 0.45-μm filter. Filtered media (600 μl) of Huh7 cells transfected with full-length JFH1 RNA (a to c) or subgenomic JFH1 RNA (d) were layered on a stepwise sucrose gradient (60% to 10%, wt/vol). Sixteen fractions were collected from the bottom of the tubes and core protein concentration and HCV RNA titers in each fraction were determined. Culture medium was analyzed either directly after filtration (a, d) or after treatment with RNaseA for 20 min at room temperature (b), or after treatment with 0.25% NP40 for 20 min at 4 ºC prior to centrifugation (c). HCV RNA titer and HCV Core protein concentration are indicated by open and closed circles, respectively. HCV RNA and core protein cosedimented at a density of 1.17 g/ml (a). Treatment of supernatant with RNaseA does not affect particle sedimentation indicating nuclease resistance (b). NP40- treatment resulted in a peak shift for both core and RNA to a density of 1.25 g/ml consistent with removal of a lipid envelope (c). Supernatant from JFH-1 subgenomic replicon RNA-transfected cells contained less HCV RNA and with a density of 1.10 g/ml (d) similar to what has been described recently[6].