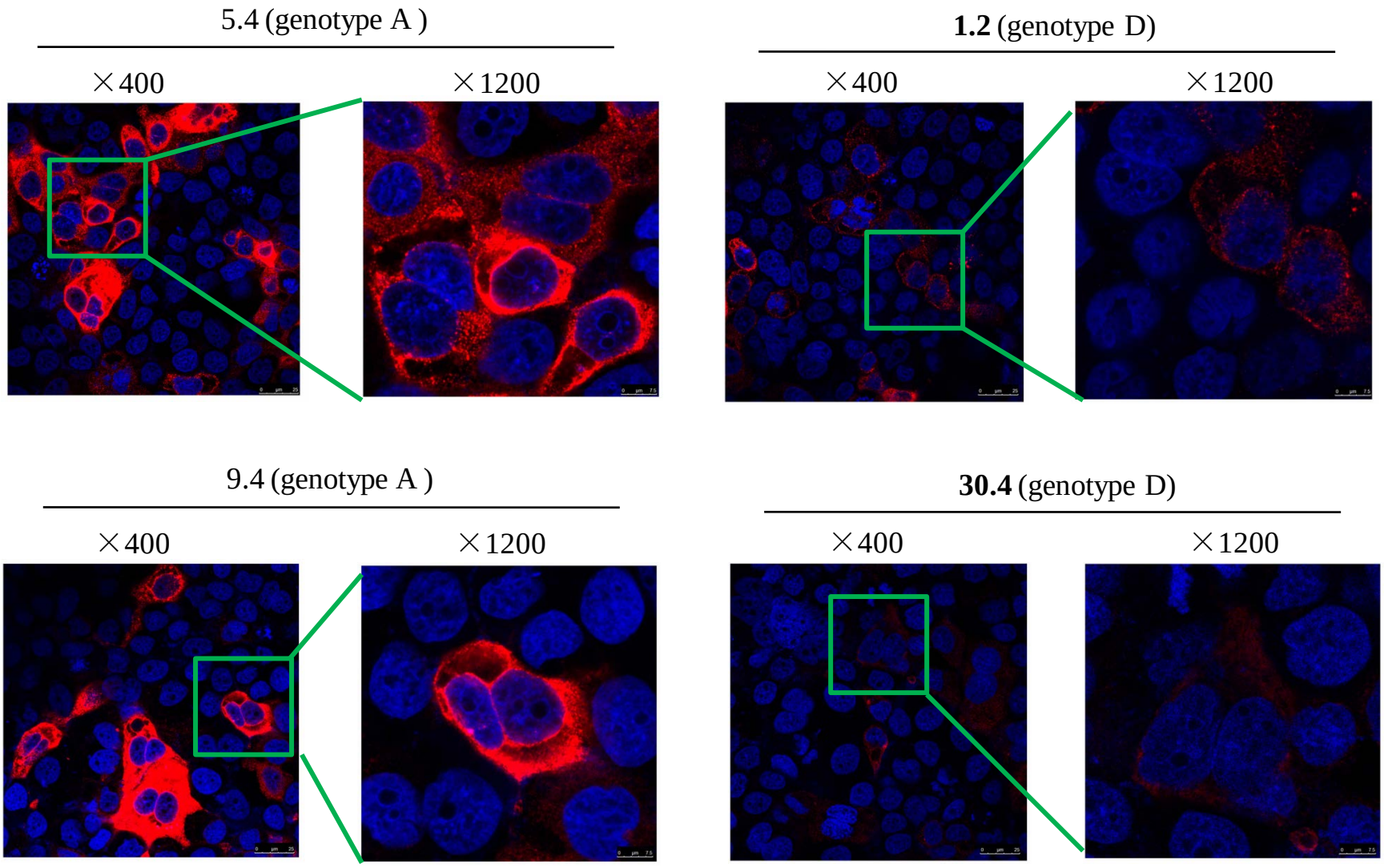
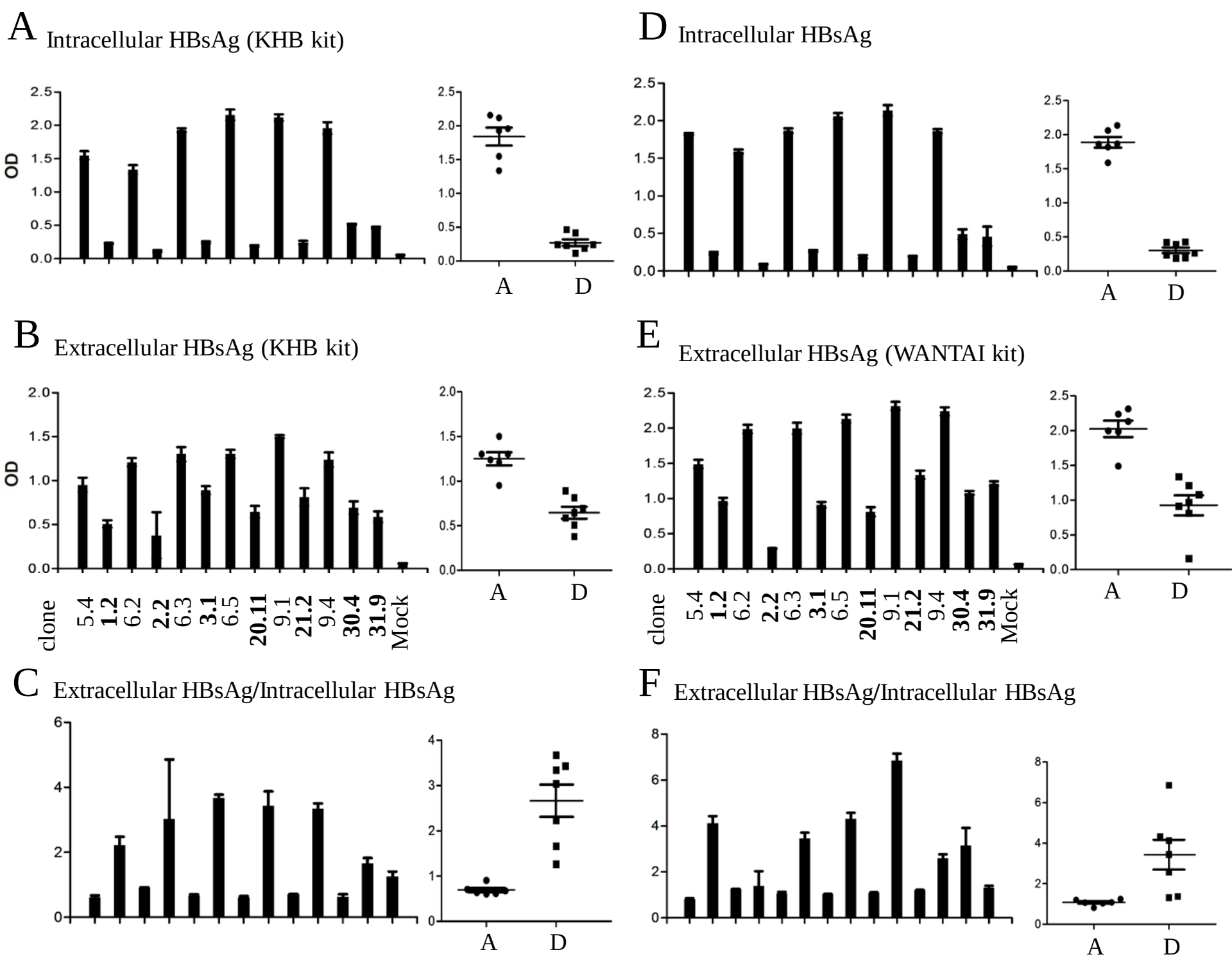
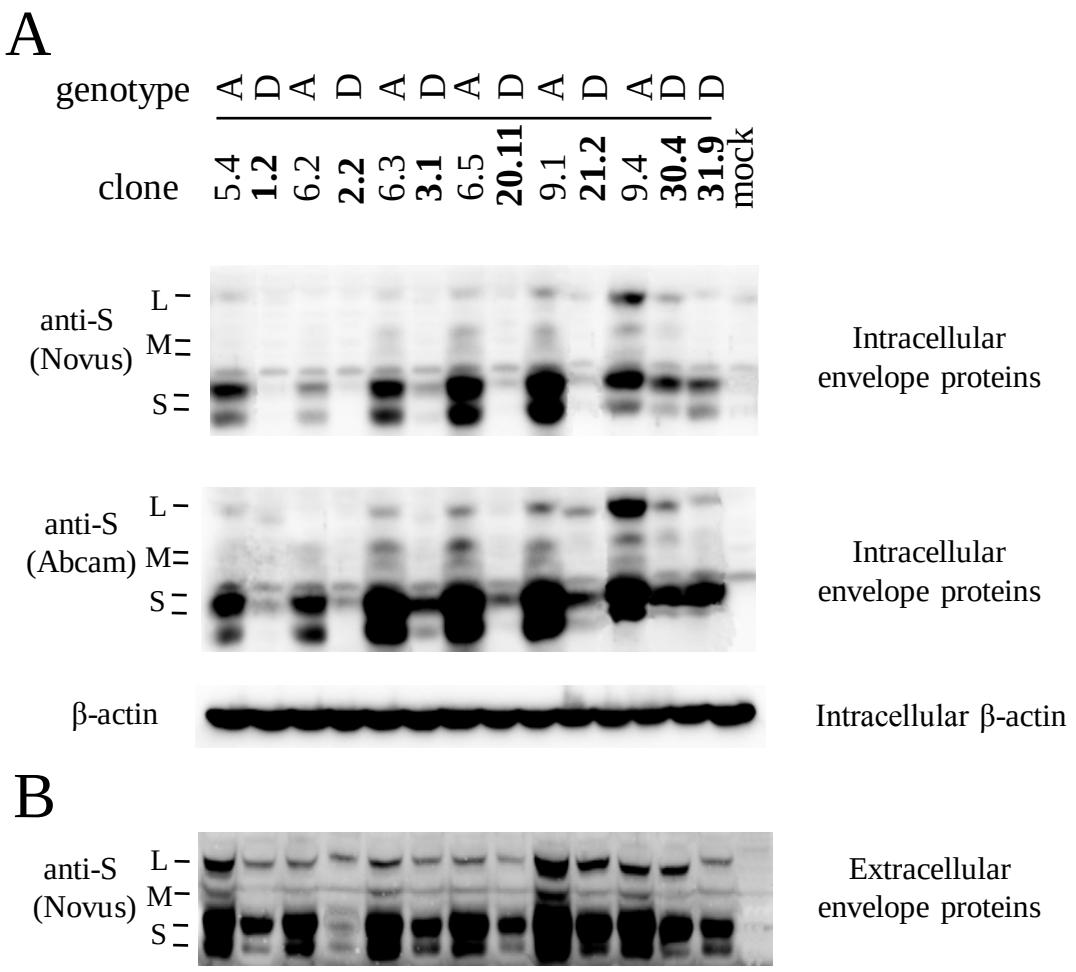


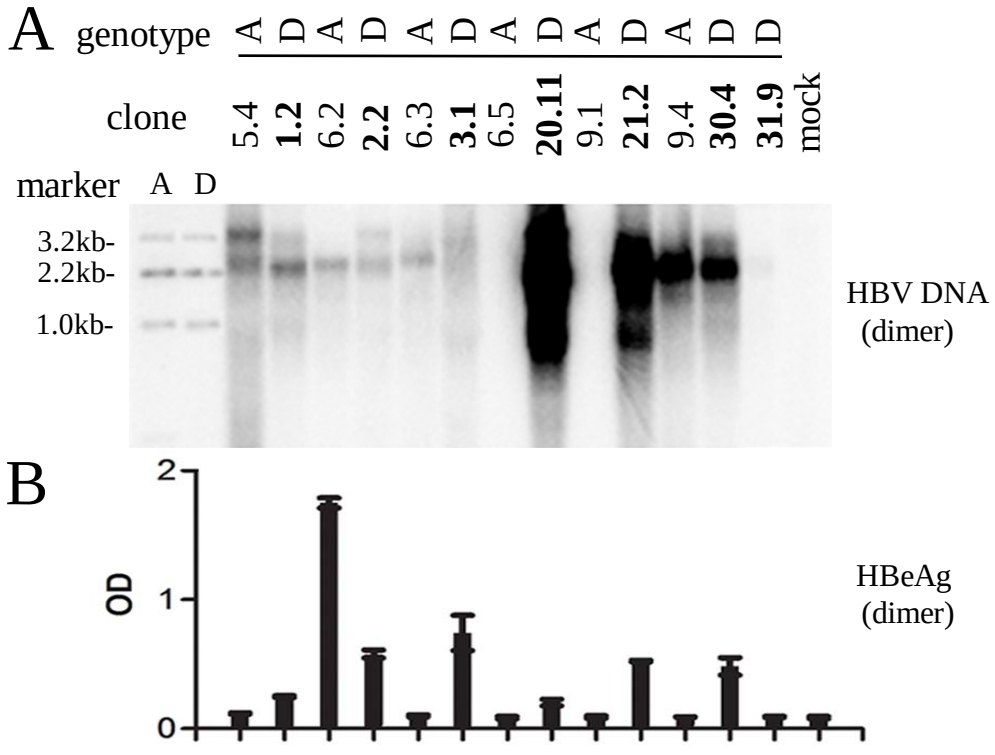
Supplementary Figure 1. Immunofluorescence staining of intracellular S protein. To visualize the S protein expression with single-cell resolution, two genotype A clones (5.4 and 9.4) and two genotype D clones (1.2 and 30.4) were transfected to Huh7 cells. Cells were fixed 5 days later, labeled with Novus anti-S antibody (1:400 dilution), red immunofluorescence antibody (1:500 dilution) and analyzed by confocal laser scanning microscopy. The cell nucleus was stained blue by DAPI. Images are shown at 1x400 (left) and 1x1200 (right) magnifications.



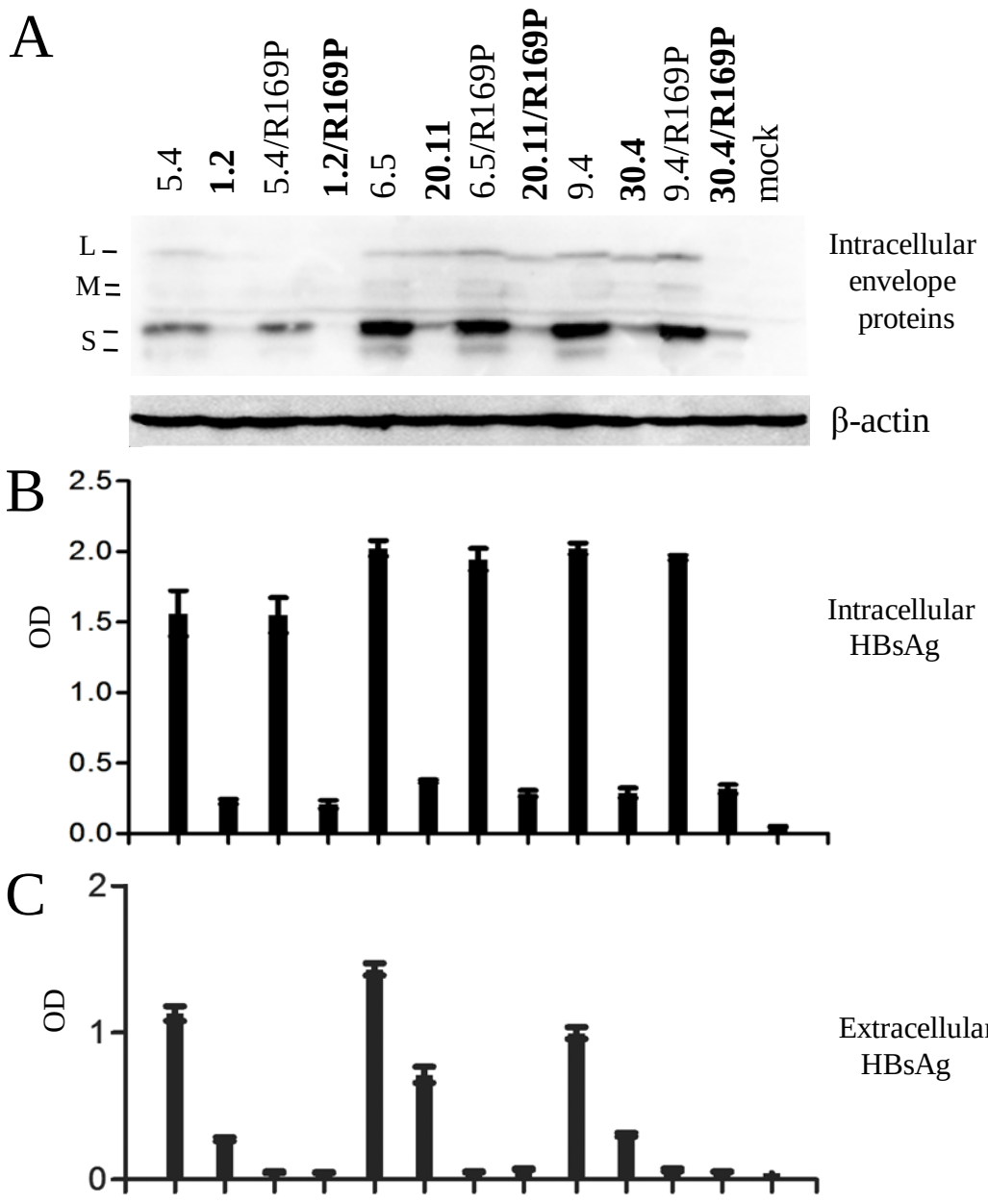
Supplementary Figure 2. Comparison of intracellular and extracellular HBsAg from HepG2 cells transfected with dimeric constructs of genotype A and genotype D. SphI dimers of 6 genotype A clones and 7 genotype D clones were transiently transfected to HepG2 cells, followed by ELISA detection of intracellular HBsAg (diluted 1:800 for KHB kit, 1:400 for Wantai kit) and secreted HBsAg (diluted 1:400 for KHB kit, 1:200 for Wantai kit) 5 days posttransfection (A, B, D and E). Furthermore, the ratio of extracellular HBsAg/intracellular HBsAg was calculated (C and F). The ELISA data for HBsAg were based on 2 independent transfection experiments.



Supplementary Figure 3. Comparison of intracellular and secreted envelope proteins from HepG2 cells transfected with dimeric constructs of genotype A and genotype D. SphI dimers of 6 genotype A clones and 7 genotype D clones were transiently transfected to HepG2 cells, followed by Western blot analysis of intracellular and secreted envelope proteins (A and B). For cell lysate, the same blot was sequentially probed with commercial polyclonal anti-S antibody from Novus, Abcam, and anti-actin antibody for loading control. Positions of L, M, and S proteins are indicated. Western blot analysis of viral envelope proteins from culture supernatant was preceded by PEG precipitation.



Supplementary Figure 4. Comparison of intracellular HBV DNA and secreted HBeAg from Huh7 cells transiently transfected with genotype A and genotype D clones. Huh7 cells were transfected with SphI dimers of the same 13 HBV clones, followed by Southern blot analysis of replicative DNA (A), and ELISA for secreted HBeAg (after 1: 400 dilution) (B). Cloned HBV DNA digested with restriction enzymes (SphI and SphI+XbaI for genotype A, SphI and SphI+XhoI for genotype D) served as size markers for the Southern blot.



Supplementary Figure 5. Impact of blocking HBsAg secretion on intracellular S protein level from 0.7mer expression construct. The R169P mutation was introduced to the 0.7mer L/M/S protein construct for three genotype A clones (plain) and three genotype D clones (bold). The parental 0.7mer construct and the R169P mutant were both transfected to Huh7 cells. (A) Intracellular S protein was detected by Western blot, using β-actin as a loading control. (B) Intracellular HBsAg was detected by ELISA (1:400 dilution). (C) HBsAg in culture supernatant was detected by ELISA (1:200 dilution).