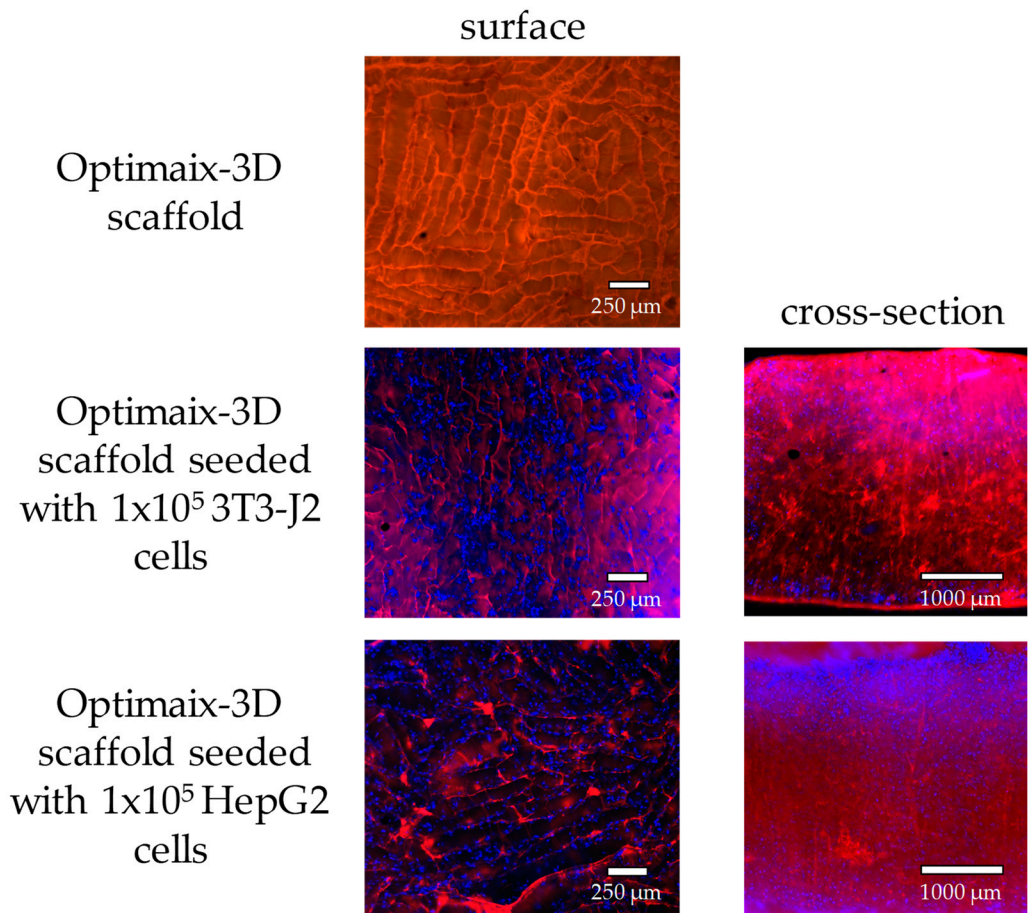**Supplementary Materials:**

Figure S1. Representative pictures of the Optimaix-3D scaffold. The scaffold matrix was stained with SRB, the nuclei of seeded 3T3-J2/ HepG2 cells were stained with HOECHST 33342. Pictures of the scaffold surface are shown in 40-fold magnification, the cross-section pictures were shown in 20-fold magnification.
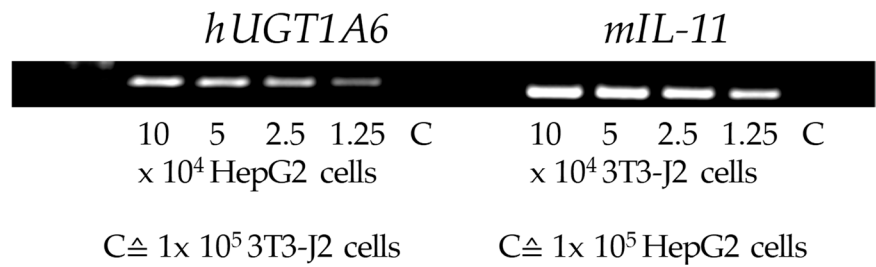

Figure S2. Optimization of the hUGT1A6 and the mIL-11 primer, PCRs were optimized to ensure that the PCR products are in the logarithmic phase. Additionally, species- specificity of the used primers was guaranteed by testing the highest concentration of the respective other cell type.
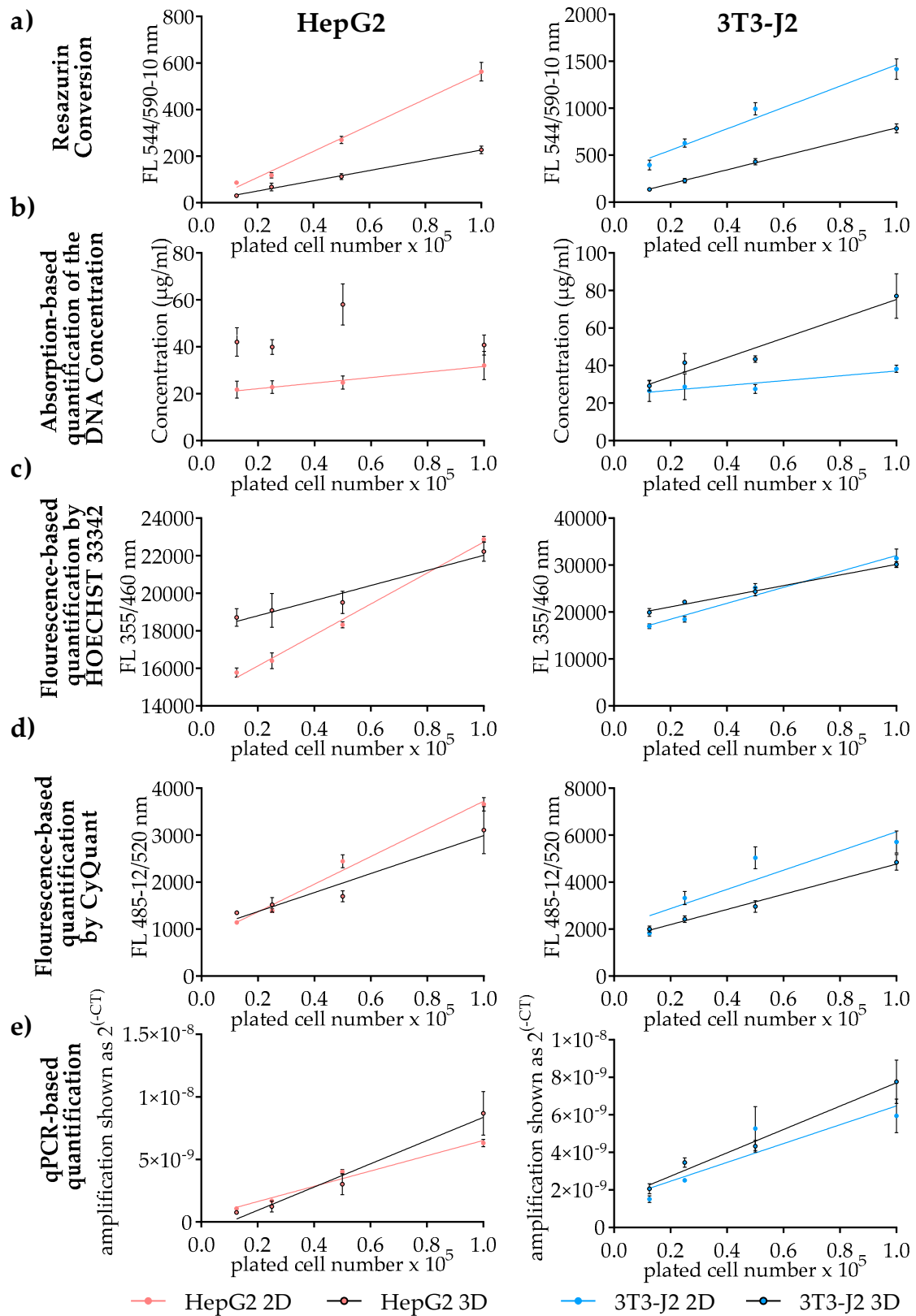
Figure S3. Comparison of different approaches for 2D and 3D scaffold cell culture quantification. a) Results of resazurin conversion, b) Absorption-based DNA quantification, c+d) Fluorescence-based DNA measurement using Hoechst 33342 and CyQuant, and e) Quantification of absolute cell numbers by qPCR with cell-type specific primers. HepG2 and 3T3-J2 cells were plated as mono-cultures in 2D and 3D using different cell numbers. Data measured and the corresponding linear regressions are shown separated by cell type for each quantification technique. N = 3, n = 2; mean ± SEM.
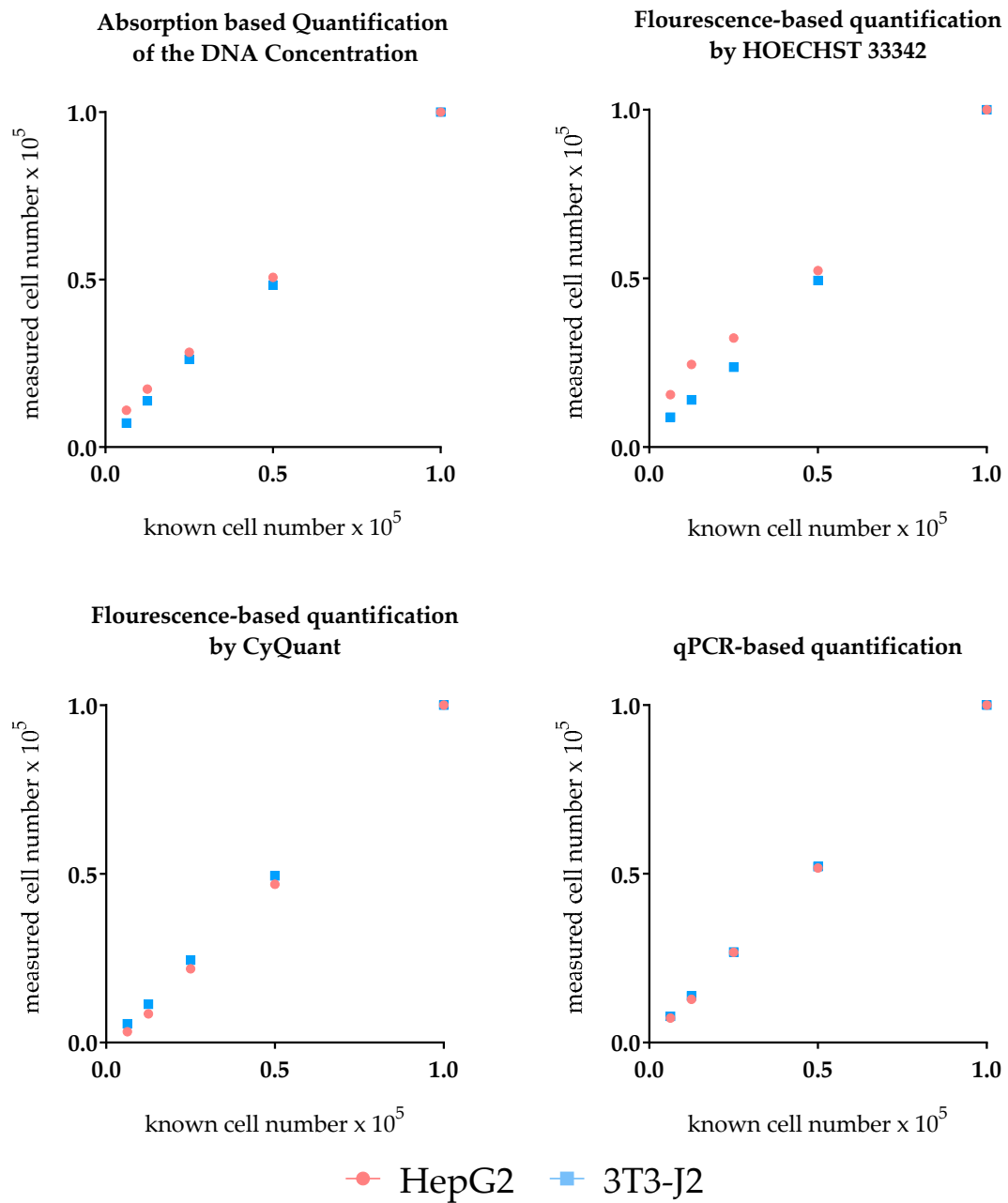
Figure S4. Standard curves of the different DNA based approaches used for calculation of the limit of detection, limit of quantitation and the sensitivity of each method.
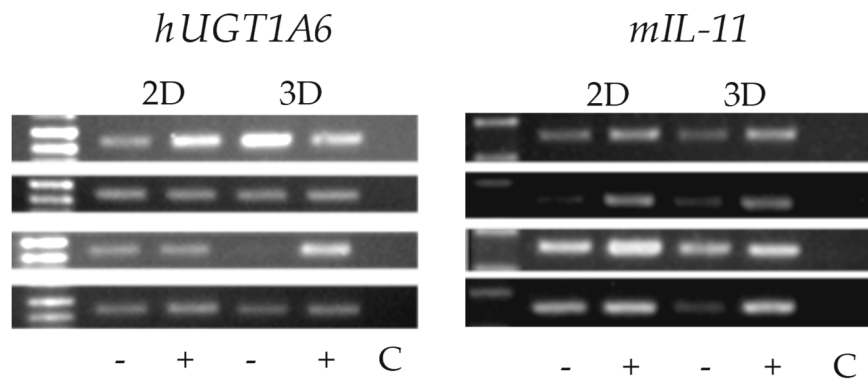
Figure S5. Gel pictures of all four runs of the co-culture experiment analyzed via conventional PCR showing co-cultures [+], monocultures [-], and additionally a negative control (c) consiting of DNA from the 2D monoculture of the other cell line. The pUC19/Msp I marker was used for *hUGT1A6* and for *mIL-11* the Bioline Hyperladder II was used.