Supplementary Figure 2.

(A) SUMF1 is secreted from the HL3xFS1 clone. Medium (lanes 1 and 2) and cellular pellets (lanes 3 and 4) collected from the HL3xFS1 clone and from control HeLa cells were analyzed by Western blotting using an anti-Flag antibody. Specific signals were found in both samples and not in the controls (cellular extract and medium of HeLa cells). The same filter was decorated with different antibodies: anti-SUMF2, anti-PDI, anti-ERK and anti-beta-tubulin. Antibodies against PDI, which is an ER-resident protein, and against total ERKs, which are abundant cytosolic proteins, detected specific signals in the cellular extracts of HL3xFS1 and HeLa cells exclusively, and not in the corresponding conditioned media. In contrast, using an anti-SUMF2 antibody, there was detection of the specific signal corresponding to SUMF2 in HL3xFS1 and HeLa cellular extracts, and also a faint signal in the conditioned media, meaning that SUMF2 is secreted, as previously shown (Mariappan et al., 2005). These data indicate that SUMF1-3xFlag is actively secreted into the medium from the HL3xFS1 clone, and that other effects due to SUMF1 over-expression causing non-specific secretion of other cytosolic or ER proteins does not occur. (B) Endogenous SUMF1 is secreted from HepG2 cells.
HepG2 cells were cultured for 8 h. Cell extracts and concentrated media were analyzed by Western blotting with an anti-human-SUMF1 antibody.