Supplementary Figure 1. Mass spectrometry analysis of SUMF1.

Protein extracts from the HL3xFS1 clone and from HeLa recipient cells cultured in conditioned medium collected from the HL3xFS1 clone were immunoprecipitated using an anti-Flag antibody, and fractionated by the SDS-PAGE. The gel was stained with colloidal Coomassie blue. All of the specific protein bands were excised from the gels, in situ deglycosylated with PNGase F, and digested with trypsin. The resulting peptide mixtures were analysed by both MALDIMS and LC-MS/MS and the mass spectrometry data were used to search a non-redundant protein database. SUMF1 was clearly identified in only three bands, corresponding to the three SUMF1 forms, and in particular to bands 1, 2 and 3 in the HL3xFS1 cellular extracts and to bands 1 and 2 in the HeLa recipient cells upon uptake. Due to the presence of the peptide 1-12 at m/z 1215.6, band 1 corresponds to the full length SUMF1 protein. Band 2 lacks the first 33 amino acids, as demonstrated by the exclusive presence of the N-terminal peptide 34-69 at m/z 3235.7. Band 3 corresponds to a further N-terminal truncated form of SUMF1 starting at Tyr70. Bands 1 and 2 were fully glycosylated at Asn141, as shown by the mass signal at m/z 1459.7 that is 1 Da higher with respect to the expected signal, since Asn141 was...
converted into Asp upon PNGase F treatment. In contrast, band 3 showed only a small percentage of glycosylated Asn141, as indicated by the occurrence of the mass signal of the unmodified non-glycosylated peptide 139-151 (m/z 1458.7), along with a faint signal occurring 1 Da higher. The results of the mass spectrometry analysis are summarized in Table 1.