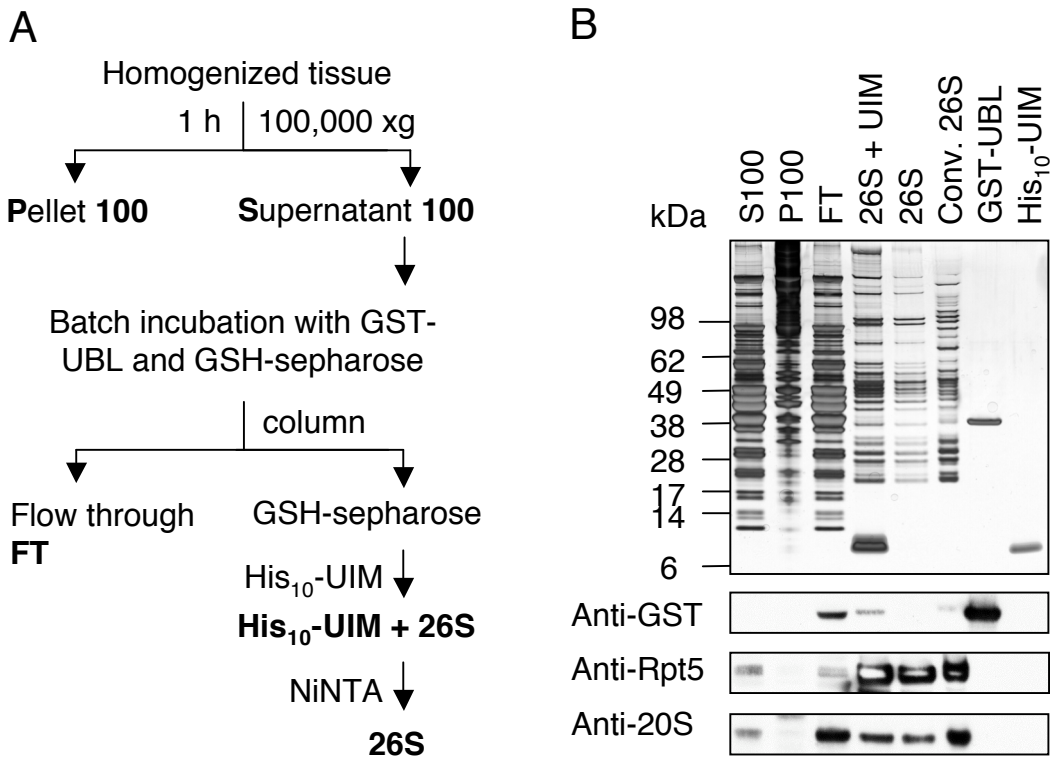
Supplemental Figure 1



Supplemental Figure 1: Ubl-affinity purified 26S contained no trace of GST-UBL or His_{10}-UIM. A) Frozen rabbit muscle was thawed and homogenized in a blender. Cell debris was removed by a low speed spin. The remaining homogenate was cleared from microsomes by ultracentrifugation (1 h 100,000 xg) and the respective microsomal fraction (P100) was discarded. The supernatant (S100) was supplemented with 0.1 mg GST-UBL/ml lysate as well as GSH-sepharose (250 μl sepharose/mg GST-Ubl). After incubation (2 h) the suspension was poured into a column. The flow through (FT) was collected for analysis and the resin extensively washed with buffer. For elution the resin was gently agitated twice in 500 μl buffer containing 2 mg/ml His_{10}-UIM and incubated for 15 min. Subsequently the buffer containing proteasomes and excess His_{10}-UIM was collected and incubated with 100 μl Ni-NTA resin for 15 min. The Ni-NTA was removed by spinning the sample through a 0.22 μm filter and pure proteasomes were retrieved. B) Samples from the purification marked bold in A) were taken and subjected to SDS gel electrophoresis and Western blot. 10 μg of S100, P100 and FT, 1.5 μg of isolated 26S and the corresponding volume of 26S still containing the UIM were loaded. For comparison 1.5 μg of conventionally by multi-step chromatography purified proteasome was loaded (the respective mass spectrometric analysis of these two lanes is presented in figure 2C). 0.5 μg GST-UBL and 3 μg His_{10}-UIM were loaded as controls. The Western blot was performed with antibodies against GST, Rpt5, and 20S as indicated.