SUPPLEMENTARY MATERIAL

Figure 1: Chromatograms of SEC to confirm purity of each ASNase type II, WT and mutants S206C and P40S. SEC was performed in Superdex 200 Increase 10/300 GL column and eluted using 50 mM Tris HCl and 100 mM glycine pH 7.4 with a flow rate of 1 mL/min on 5 column volume.

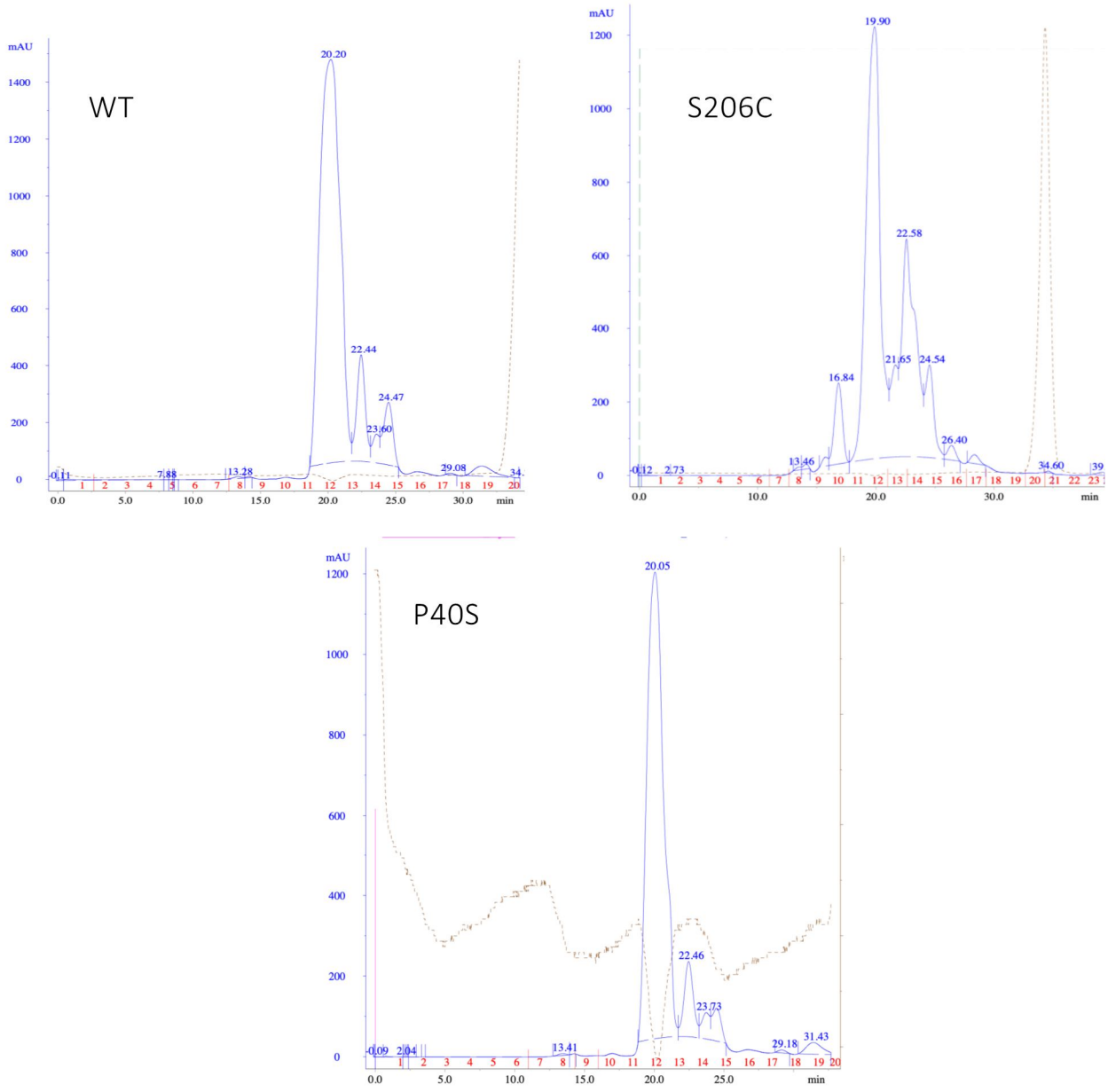

Figure 2: SDS-PAGE gels (14%) to confirm purity of each ASNase type II, WT and mutants S206C and P40S. Elution fraction #12 was used for further tests.

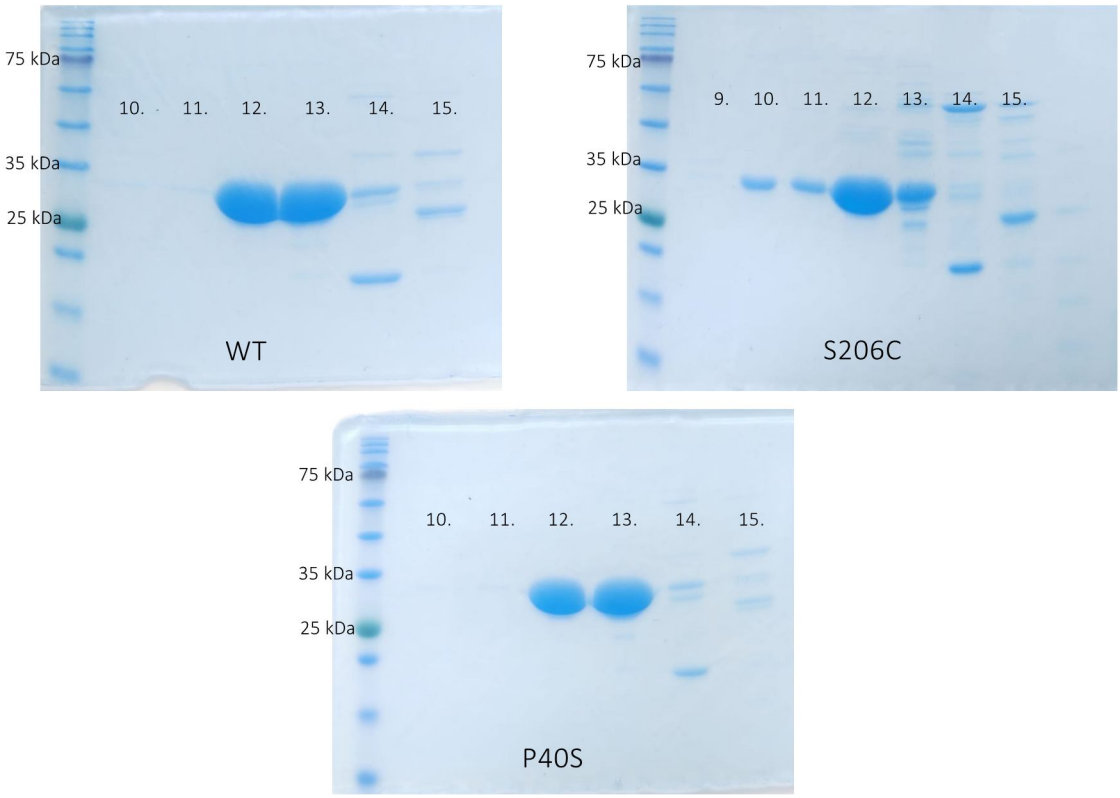

Figure 3: Enzyme stability on human serum (HS). Enzymes were incubated at 37 °C on PBS 1x and on 10% HS up to 96 hours and ASNase activity was measured every 24 hours with Nessler´s reagent. Statistical analyses were significant from 72 hours (A) and 96 hours (B). Standard deviation (SD) is shown by vertical bar, n=2, *: p value <0.05, **: p value <0.005, ***: p value <0.0005.

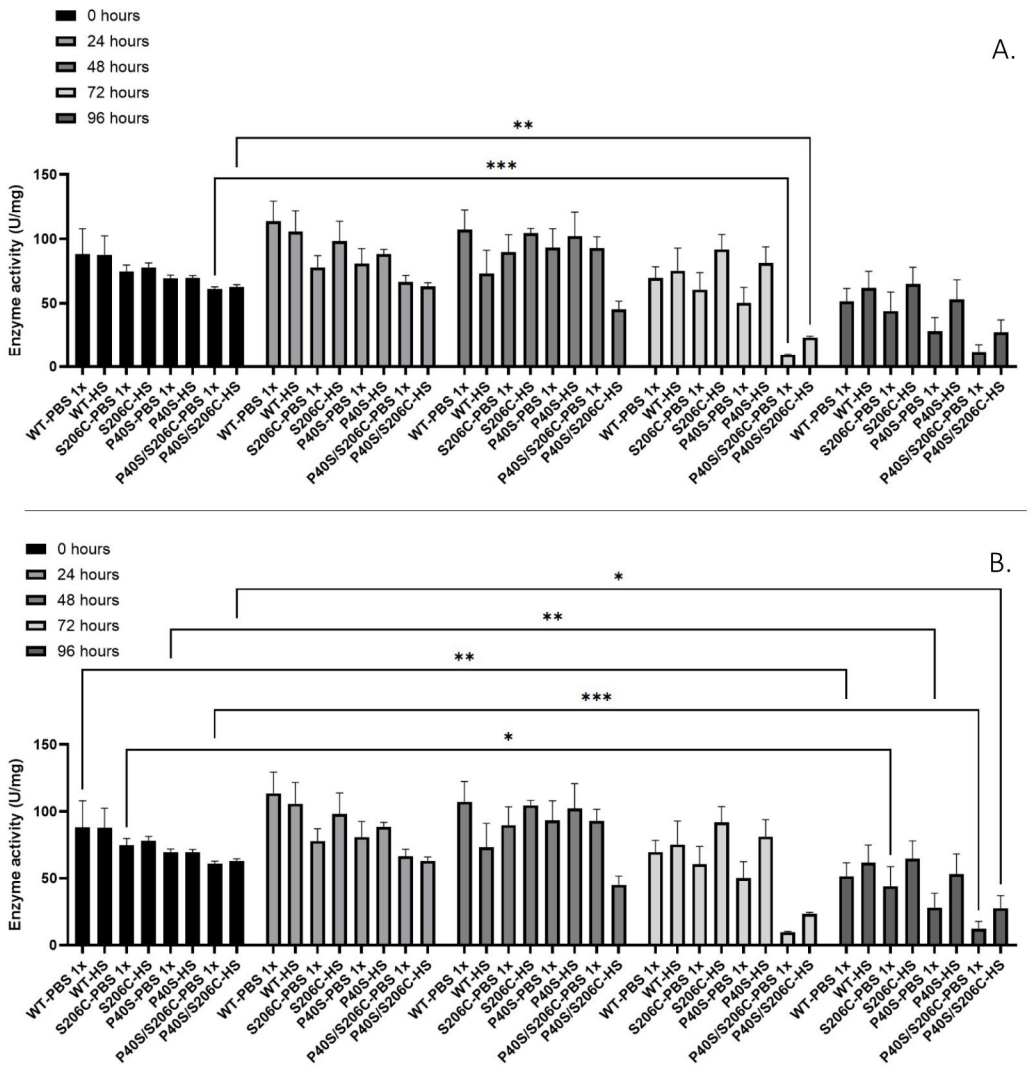

Figure 4: Haematoxylin & Eosin stain of liver, kidney and heart of all enzyme groups. Arrows show microvesicular steatosis on liver, acute tubular necrosis on kidney and mild intercellular edema on heart.