Supplementary S3: apoptosis is not activated in myotubes of D7E or WTA clones

Myotubes of WTA, D7E or IM2 parental were subjected to TUNNEL assay (ROCHE) (A) or immunofluorescence with anti-cleaved caspase 3 (B). (A) The nuclear localization of fluorerescein-dUTP, which marks apoptotic cells was studied in the merge image with bright filed. Nuclear TUNEL staining was detected only after DNaseI treatment was served as a positive control. (B) The anti cleaved Caspase-3 antibody (R&D systems) was visualized with Alexa 594 and PABPN1-FLAG with Alexa 488. Nuclei were counter stained with DAPI. Together these experiments do not reveal activation of apoptosis in myotubes expressing WT or expPAPBN1. For the TUNEL staining four-days fused myotubes were labeled with UTP-fluorescein (Cell Death Detection Kit; Roche) for 15 minutes. Cells were washed with PBS, fixed with 2% formaldehyde and were imaged with 485-535nm filter. DNaseI treated cells (according to the manufacture’s protocol) were used as positive control.