

Supplementary Figure 1. PABPN1 depletion does not alter poly(A) RNA content.

Pure cultures of primary mouse myoblasts from limb (L), pharyngeal (Ph) and extraocular (EOM) muscles were transfected with PABPN1-2 or control scrambled siRNA using Lipofectamine 2000 (Invitrogen) in GM according to the manufacturer's instructions. After overnight incubation, medium containing transfection complexes was replaced by fresh medium. Cells were collected after 40h of transfection, total RNA was isolated and samples of total RNA (5 µg) were denatured by mixing with equal volumes of formaldehyde (37%) and 20X SSC and heating at 65°C for 15 min. Duplicate samples were loaded on a nylon membrane under vacuum and RNA was cross-linked to the membrane using ultraviolet light. The membrane was first prehybridized at 42°C for 3 hours in hybridization solution (50% formamide, 2X SSPE, 0.5% SDS, 2X Denhardt's solution, 10 µg/mL denatured salmon sperm DNA. [γ -³²P]ATP-labeled dT₃₀ or 18S (CGGCATGTATTAGCTCTAGAATTACCACAG) probe was then added to hybridization solution containing 10% dextran sulfate and incubated with membranes for 16 hr at 42°C. The membranes were washed sequentially in 1X SSPE/0.1% SDS for 30 min at 42°C and 0.5X SSPE/0.1% SDS for 30 min at 42°C, and subsequently exposed to radiographic film.

