



**Additional File 4. Aggregated and soluble SOD1 enter the cytosol of NSC-34 cells.** NSC-34 cells were treated with soluble or aggregated wt and G93A SOD1 proteins for 120 minutes. Cells were fixed and then permeabilized with Triton-x100 (0.5%) or digitonin (10  $\mu$ M). Cells were labeled with Alexa-633 conjugated to SA (green; to detect biotinylated SOD1) and Red Dot 2 (red; to label nuclei). Images are laser scanning confocal microscopy. In addition, cells were treated with RAP-GST for 120 min, fixed, permeabilized (Triton-x100 or digitonin) and then stained with an anti-GST antibody. RAP-GST remained in the endomembrane system and was not detected after digitonin permeabilization.