**Fig. S1. Twist-1 inhibits muscle cell differentiation.** A) Newborn human myoblasts were transfected with an adenovirus that expressed human Twist-1 (AdT) and left to differentiate for 6 days. Myotubes were fixed and stained with MyHC and nucleus-directed Hoechst. AdT transduction caused inhibition of muscle cell differentiation compared to cells transfected with a control adenovirus (AdC) or compared to untransfected cells (Scale bar, 200 μm). Inhibition of muscle cell differentiation by AdT compared to untransfected cells or to cells transfected with AdC is shown by the calculated fusion index. B) Foetus human myoblasts were transfected with siRNA Twist-1 and left to differentiate for 6 days. Myotubes were fixed and stained with MyHC and nucleus-directed Hoechst. Twist-1 siRNA transfection caused an increase of muscle cell differentiation compared to cells transfected with a control siRNA (siRNA negative) or compared to untransfected cells (Scale bar, 200 μm). The increase of muscle cell differentiation by Twist-1 siRNA compared to untransfected cells or to cells transfected with siRNA negative is shown through the calculated fusion index.
Fig. S2. Overexpression or downregulation of miR-206 alter the endogenous level of Twist-1. A) Endogenous levels of miR-206 in foetus myoblasts were increased following transfection with miR-206 mimic as detected by Real-time PCR. B) 14-
week-old foetus myoblasts were transfected with miR-206 mimic, fixed 48 hours following initiation of differentiation and stained with the Twist-1 and nucleus-directed Hoechst. miR-206 mimic decreased Twist-1 levels, compared to transfection with the negative control or untransfected cells (Scale bar, 100 μm). C) Endogenous levels of miR-206 in the Newborn myoblasts were decreased following the transfection with miR-206 antagonir (amiR-206) as detected by Real-Time PCR. D) Newborn myoblasts were transfected with miR-206 antagoniR (amiR-206), fixed 48 hours following initiation of differentiation and stained with the Twist-1 and nucleus-directed Hoechst. amiR-206 increased Twist-1 levels, compared to transfection with the anti-miR control or untransfected cells (Scale bar, 100 μm).
**Fig. S3. MyoD overexpression induces miR-206 levels.** A) Transduction of 14-week-old foetus human myoblasts with an adenovirus which expresses MyoD (AdM) caused an increase in cellular MyoD levels, accompanied by an increase in the myogenesis markers muscle actin and troponin compared to untransfected cells or to cells transduced with a control adenovirus (AdC). B) Similarly, AdM transduction caused an increase in the endogenous miR-206 compared to untransfected cells or to cells transduced with a control adenovirus (AdC). Experiments were performed in triplicate, and the data represent the mean ± S.D. * represents a significance of (p <0.01) using Student’s t test.
Fig. S4. MyoD and Twist-1 levels are decreased and increased, respectively in DM1 cells compared to healthy cells. Protein analysis by Western blotting of 3 different DM1 and healthy cell lines (A-C) showed that MyoD protein levels were decreased in DM1 cells compared to the healthy cells, whereas Twist-1 protein levels were increased in DM1 cells compared to healthy ones.