Additional file 5: Figure S5 related to Figure 5
Figure S5. Effect of small molecule inhibitors on signalling, PH-Akt-GFP translocation, phospho-Akt/phospho-PDK1 and BMPRII localisation. (A) Effect of PI3K specific and BMP type I receptor specific inhibitors on BMP2 signaling. Western Blot analysis showing BMP2-dependent phosphorylation of Akt-Thr303, Akt-Ser472 and Smad1/5/8. Cells were pre-treated for 1 hour with inhibitors LY-294002 [10µM], PI103 [8nM] and LDN-193189 [0.5µM] and stimulated with BMP2 [10nM] for 60 minutes. The effect of small molecule inhibition on BMP2 induced signalling was compared to a DMSO treated control. (B) BMP2-dependent GFP-PH-Akt translocation. Immunofluorescence of C2C12 cells transfected with GFP-PH-Akt. Upper panel shows stills of a 45 minutes time laps series of BMP2 [10nM] stimulated C2C12 cells transfected with GFP-tagged PH domain of Akt. White arrowheads point towards increased GFP signal accumulation. The lower panel shows GFP-PH-Akt translocation upon 45 minutes BMP2 stimulation [10nM] compared to GFP-PH-Akt translocation when cells were pre-treated with PI103 [8nM]. White arrowheads point towards areas of intense GFP signal. Scale bars represent 20 µm. (C) BMP2 induces phospho Akt and phospho PDK1 at the leading edge. Immunofluorescence showing localization of phospho-Akt Thr308 and phospho PDK1-Ser241 together with F-actin in C2C12 cells upon BMP2 [10nM] stimulation for 60 minutes. Protrusive regions with cortical actin are magnified. BMP2-induced localization of cortical actin and phospho-Akt Thr308 as well as phospho-PDK1 were compared to cells pre-treated with the class Ia PI3K selective inhibitor PI103 [8nM]. Arrowheads point towards co-localization of phospho-Akt Thr308 and cortical actin. Scale bars represent 20µm. (D) Confocal analysis of the localisation of endogenous BMPRII-LF in C2C12 cells upon 10nM BMP2 treatment for indicated time using an antibody which detects the c-terminal tail of BMPRII-LF. (Cell Signaling Tec. #6979). Cyclohexamide [10mg/ml] treatment was performed 6 hours and
0.5µM LDN193189 1 hour prior to stimulation. Arrows indicate for ruffle associated endogenous BMPRII. Scale bars indicate 10µM.