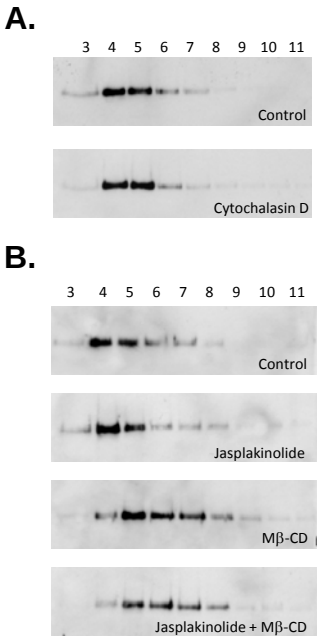

Supplementary Figure 4. Effect of actin cytoskeleton manipulations on the lipid raft distribution of the P2X1 receptor. A. Sucrose density centrifugation and Western blotting shows the P2X1 receptor is associated with buoyant lipid rafts. Blots show samples from successive 1 ml fractions taken following sucrose density centrifugation (for methods see Vial & Evans, 2005, J. Biol. Chem. 280, 30705-30711). The P2X1 receptor is detected predominantly in the buoyant lipid fractions towards the top of the gradient (fractions 4-5) as described previously. Treatment with cytochalasin D (500nM 1hr) had no effect on the distribution of the P2X1 receptor. Western blotting representative of 3 separate experiments. **B.** Representative blots of P2X1 receptor distribution after treatment with jasplakinolide (Jasp, 30 nM, 1hr), Methyl-β-cyclodextrin (Mβ-CD) (10 mM, 1 hr) or jasplakinolide together with Methyl-β-cyclodextrin (Jasp, 30 nM, Mβ-CD 10 mM 1hr) following pre-treatment with jasplakinolide (Jasp, 30 nM, 1hr). Western blotting representative of 4 separate experiments. Jasplakinolide had no effect on the association of the P2X1 receptor in the buoyant lipid rafts. However cholesterol depletion with Mβ-CD resulted in a redistribution of the P2X1 receptor to lower fractions (6-8) as shown previously (Vial & Evans, 2005, J. Biol. Chem. 280, 30705-30711). This movement of the P2X1 receptor by Mβ-CD was unaffected by jasplakinolide pre-treatment.