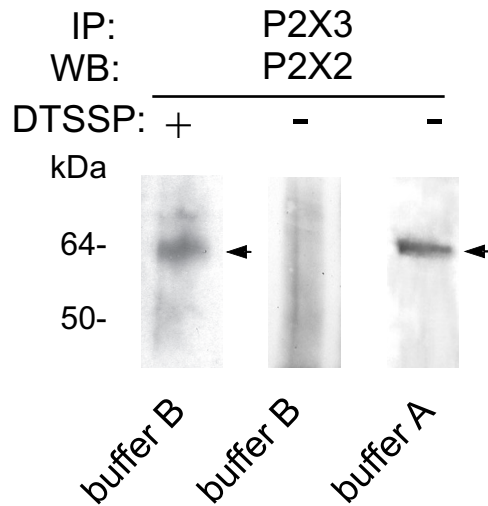




Supplemental Figure 4. DTSSP, a membrane impermeable cross-linker, allows selective detection of heteromeric P2X_{2/3} located at membrane level. Furthermore, this chemical compound gives the possibility of using the strong, denaturing lysis buffer RIPA (buffer B) in order to avoid non-specific contamination during the pull down procedure (left lane). Without DTSSP, strong lysis conditions preclude the possibility of heteromeric receptor detection (middle lane). Co-immunoprecipitation of P2X₃ and P2X₂ subunits can also be performed with a mild buffer (buffer A: 10 mM Tris pH 7.5, 150 mM NaCl, 2 mM EDTA, 1 % TritonX-100, 100 mM NaF, 20 mM sodium orthovanadate and a protease inhibitors cocktail) in the absence of cross-linking: this procedure does not allow distinguishing heteromeric receptors at membrane level from those in intracellular compartments (right lane).