



**Supplemental Figure 3. NADH dehydrogenase activity staining (A) and immunoblotting (B) of BN-PAGE gels of membrane preparations from *E. coli*.** Membranes from the wild-type (*WT*), NuoI knock-out (*KO*), NuoI knock-out revertant (*KO-rev*), and NuoI point mutants (*KO-rev*) were compared. The location of the NDH-1 band is marked. The dodecyl- $\beta$ -maltoside concentration was 1%. The electrophoresis was performed as described under “Experimental Procedures.” For NADH dehydrogenase activity staining, the gels were incubated with 2.5 mg/ml *p*-nitroblue tetrazolium and 150  $\mu$ M NADH for 1 h at 37  $^{\circ}$ C. The reaction was stopped by 7% acetic acid. For immunoblotting, after BN-PAGE, the *E. coli* membrane proteins were electrotransferred onto nitrocellulose membranes. Subsequently, the affinity-purified NuoB antibody were used for the immunostaining of the membranes.