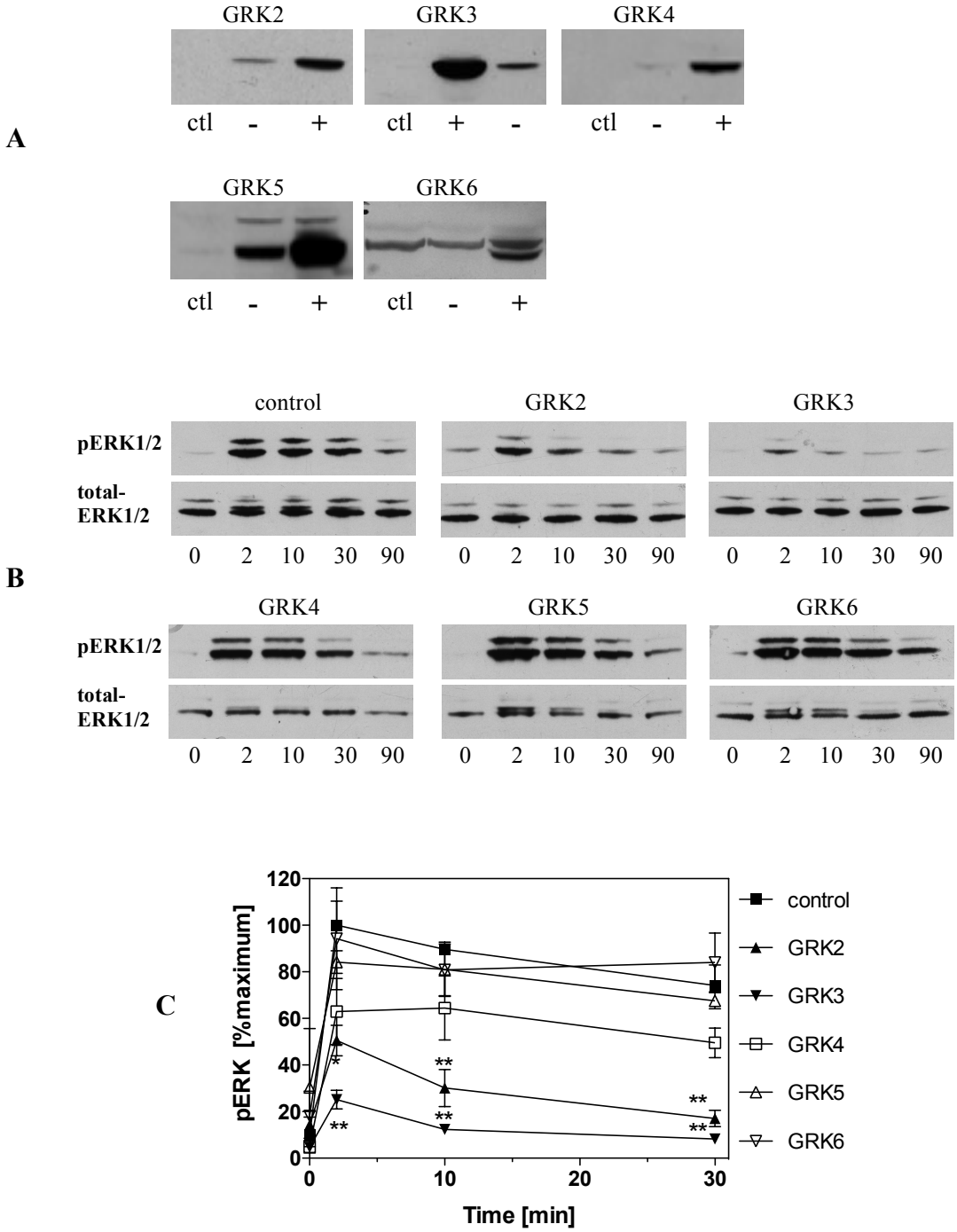

Fig. S1. Desensitization of ERK1/2 activation by GRKs 2-6. *A*, confluent monolayers of HEK 293 cells on 24-well plates stably expressing GRKs 2-6 were lysed and proteins (~20 µg per lane) were immunoblotted as described under *Experimental Procedures*. GRKs 2-6 were detected with specific antibodies obtained from Santa Cruz Biotechnology (Heidelberg). Overexpression of GRKs was induced (+) or not (-) with 0.5 µg/ml tetracycline. The control represents the endogenous level of the respective GRK in HEK 293 cells. *B*, cells expressing various GRKs or not (control) were transiently transfected with B_2Rwt_H and induced with tetracycline. 48 h later the cells were stimulated at 37°C with 1 µM BK for the indicated times. After cell lysis phospho-ERK1/2 and total-ERK1/2 were determined via immunoblotting. *C*, respective bands were quantified by scanning densitometry. The phosho-ERK1/2 signals were normalized for total-ERK1/2 and are given in percentage of the activation after 2 min of control (=100%). Dunnett-Test. *:$p<0.05$, **:$p<0.01$.

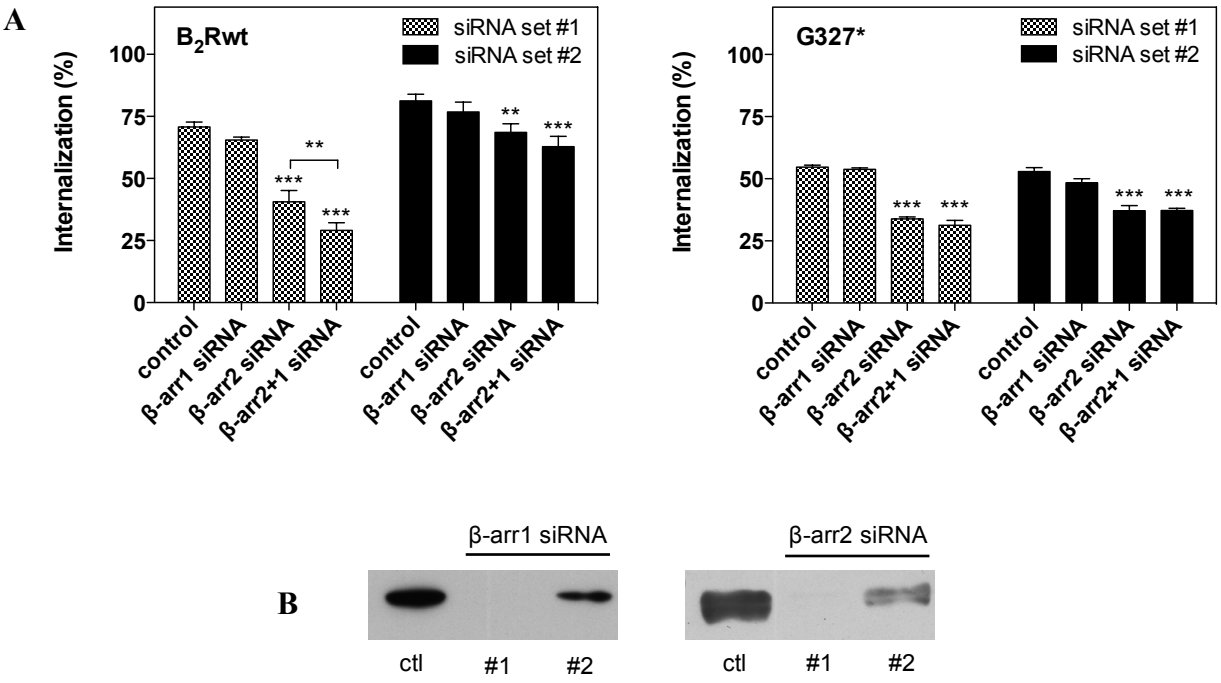

Fig. S2. Knockdown efficiency of two different sets of siRNAs (#1 and #2). *A*, HEK 293 cells stably expressing B_2Rwt or G327* were transfected with the indicated siRNAs. 72 h later internalization was determined with 5 nM [^{3}H]BK (B_2Rwt) or 1 nM [^{3}H]BK (G327*) after 5 min (B_2Rwt) or 15 min (G327*) as described in *Experimental Procedures*. Significance was determined by one-way ANOVA using Bonferroni`s multiple comparison test. **: $p<0.01$, ***: $p<0.001$. *B*, representative immunoblot of β-arrestin levels overexpressed in HEK 293 cells, silenced with the indicated siRNAs and probed with the respective antibodies for β-arrestin1 and 2.