SUPPLEMENTAL FIGURE LEGENDS

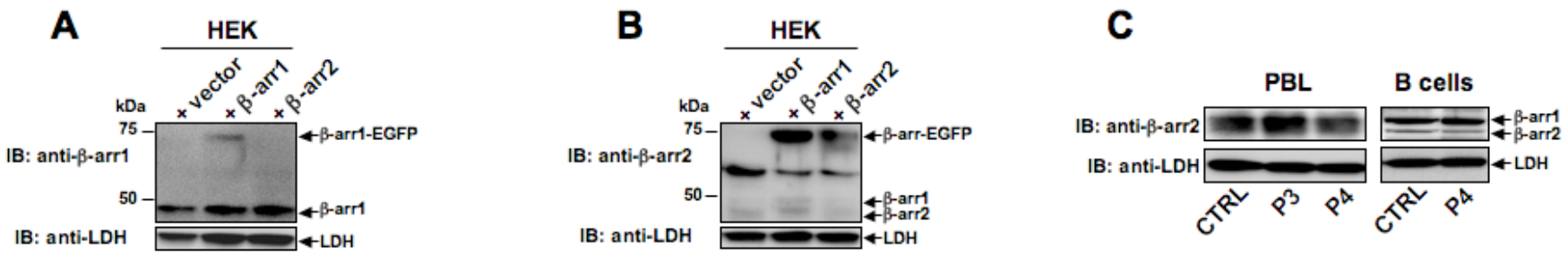
Supplemental Figure 1 : Specificity of anti-β-arrestin Abs and levels of β-arrestin in WHIM^{wt} leukocytes. (A and B) HEK 293T cells were transiently transfected using the reagent FuGene 6 (Roche) with 1 μg pN1-EGFP (vector), pβ-arrestin1-EGFP (β-arr1) or pβ-arrestin2-EGFP (β-arr2). Two days after transfection, β-arrestin expression levels were assessed by IB. **(A)** Protein extracts (40 μg/lane) were probed with a rabbit anti-human β-arrestin1 mAb. The specificity of the Ab for β-arrestin1 is revealed, first, by the detection in all samples of a band with a molecular weight close to that expected for the endogenous β-arrestin1 (~ 47 kDa), second, by the detection of the ectopically expressed EGFP-tagged β-arrestin1 (~ 70 kDa) only in lysates from cells transfected with the corresponding cDNA, and finally, by the absence of detection of either the endogenous β-arrestin2 or the ectopically expressed EGFP-tagged β-arrestin2. **(B)** Blotting of protein extracts (20 μg/lane) with a rabbit anti-serum to β-arrestin2, that cross-reacts with β-arrestin1, revealed in all cell lysates two bands with molecular weights close to those expected for the endogenous β-arrestin 1 and 2 proteins (~ 47 and ~ 46 kDa, respectively), and only in lysates from cells transfected with either *β-arrestin 1 or 2* cDNA a band corresponding to the ectopically expressed EGFP-tagged β-arrestin 1 or 2 (~ 70 kDa). LDH (~ 35 kDa) was used as a loading control. **(C)** The steady-state levels of β-arrestin proteins in leukocytes (PBL) from P3 and P4 (left panel) and in EBV-B cells from P4 (right panel) were compared to those obtained in cells from healthy individuals (CTRL). IB of protein extracts (20 μg/lane) using the anti-β-arrestin2 rabbit Ab revealed endogenous β-arrestin 1 and 2. Results are representative out of 2 **(A and B)** or 3 **(C)** independent determinations.

Supplemental Figure 2 : Specificity of anti-GRK Abs. (A) Protein extracts (20 μg/lane) from HEK 293T cells transfected with 1 μg of either pcDNA1 (vector) or one of the *GRK* encoding vector (*GRK-2*, *-3* or *-6*) were analyzed by IB using an anti-human GRK-2, -3 or -6 Ab. LDH was used as a loading control. Transfection of *GRK* cDNA resulted in a selective increase of each GRK protein over the basal level and at the expected molecular mass (~ 80 kDa for GRK-2 or -3 and ~ 65 kDa for GRK-6). Additionally, ectopic expression of GRK did not alter the endogenous expression of other GRKs. These data provided compelling evidence regarding the selectivity of the anti-human GRK-2, -3 or -6 Ab for the kinase they were raised against. **(B)** Selective decrease in GRK-3 proteins in P3 fibroblasts. Equivalent amounts (30 μg) of protein extracts from healthy (CTRL#1) and P3 fibroblasts were separated by running a 10% Tris-glycine polyacrylamide gel until the 50 kDa molecular weight marker reached the bottom of the gel. Probing with a mouse anti-rat GRK-2/-3 mAb revealed a doublet corresponding to GRK-2 and GRK-3 proteins in CTRL#1 cells, but not in P3 ones. Immunodetection of GRK-6 using a rabbit anti-human GRK-6 Ab was used as a loading control. **(C and D)** Efficiency of *GRK* overexpression in fibroblasts from CTRL#1, P3 and P4 subjects **(C)** or in leukocytes (PBL) from a healthy subject **(D)** following nucleoporation with 5 μg of either pcDNA1 (vector) or *GRK-2*, *-3*, *-5* or *-6* construct. 15h after transfection, whole cell protein extracts (10 μg/lane) were analyzed by IB using an anti-GRK-2/-3 or -GRK-5/-6 mAb or an anti-GRK-2, -3, -6 or -β-arrestin2 Ab. These data confirmed the selectivity of each Ab for the kinase they were raised against and also indicated that the ectopically expressed GRK reached similar levels in control and patient cells that largely exceeded the endogenous ones. Results are representative out of 2 **(A, B and D)** or >5 **(C)** independent determinations.

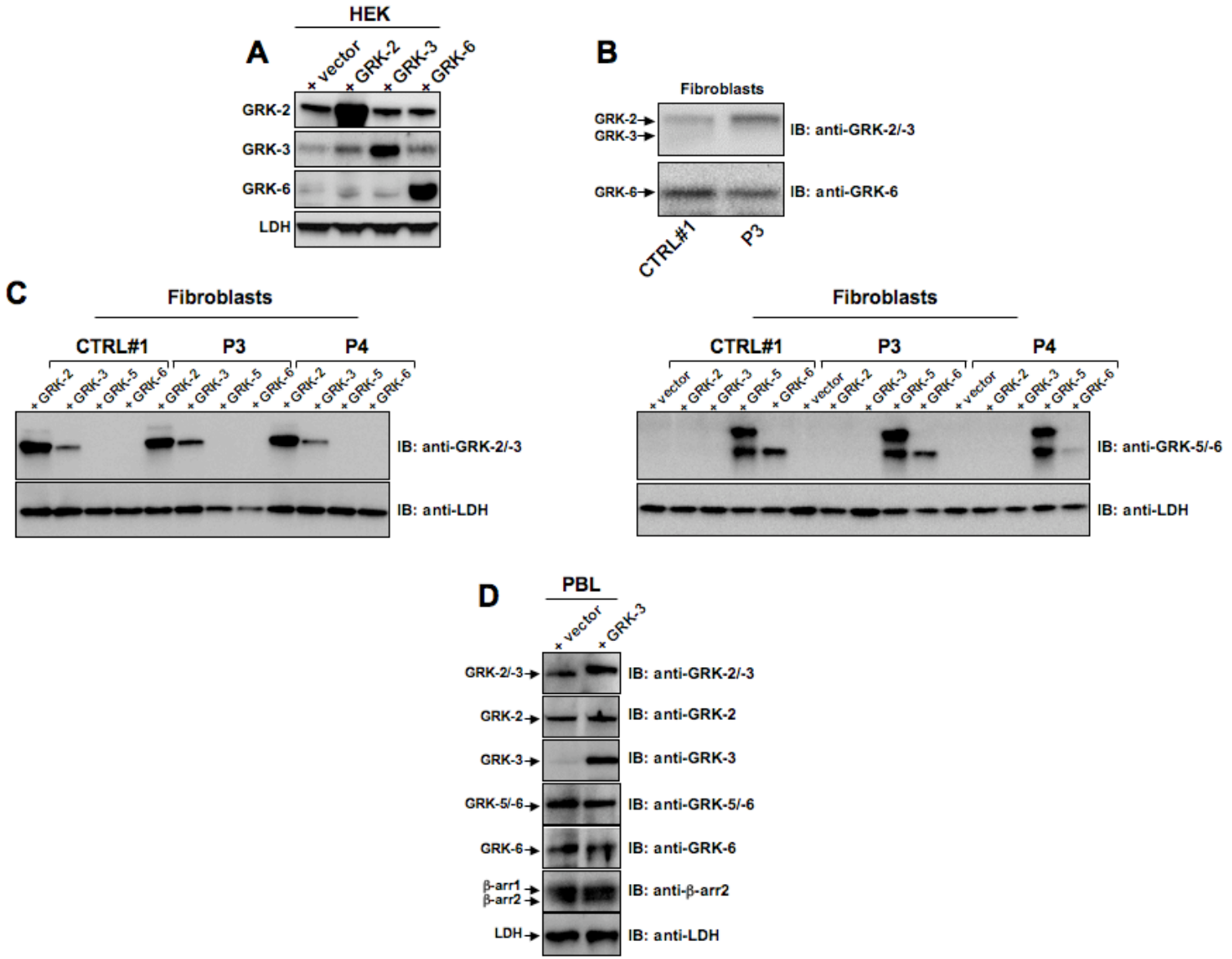
Supplemental Figure 3 : Analysis of *GRK* transcript levels in P4 leukocytes. The steady-state levels of *GRK-2*, *-3* and *-6* mRNAs in leukocytes (PBL) from P4 were compared by real-time PCR to those obtained in leukocytes from family members (I-1 and II-1). Results are means ± SD of 3 independent experiments performed in triplicate.

SUPPLEMENTAL FIGURES

Supplemental Figure 1-Balabanian et *al.*



Supplemental Figure 2-Balabanian et *al.*



Supplemental Figure 3-Balabanian et *al.*