The delta subunit of rod-specific photoreceptor cGMP phosphodiesterase (PDE6D) contributes to hepatocellular carcinoma progression

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Supplementary Material

Figure 1. PDE6D-mediated ERK-activation in HCC. (A) Exemplary image (right side) and densitometric analysis (left side) of ERK-activation (pERK/ERK-levels) (Western blot analysis) in Hep3B cells after si-RNA-mediated PDE6D-suppression (*: p < 0.05 vs control). (B) Western blot analysis (exemplary image representing two independent experiments) revealing time-dependent recombinant Fibroblast-growth-factor (FGF)-induced ERK-activation (pERK/ERK-levels) in starved HCC cells (Hep3B) (cells were starved for 24 hours prior to stimulation with FGF) was prevented by co-treatment with the pharmacologic inhibitor of the PDE6D-KRAS-interaction deltarasin (5 µM). (C) Phospho-ERK (pERK) and PDE6D levels after forced PDE6D overexpression in sorafenib-resistant HCC cells (Hep3B).
Figure S2. Snail protein expression after TGF-β1-mediated stimulation of HCC cells. Densitometric analysis of Snail protein expression levels (Western blot analysis) in PLC cells that were stimulated with different doses (0, 1, 5 ng/ml) of recombinant human TGF-β1 for 72 hours, with or without co-treatment with 15 μM of the TGF-β-receptor-1 (TGFBR1) inhibitor LY2157299 ("galunisertib") (the densitometric values represent two independent Western blot analysis) (*: p < 0.05 vs control; ns: non-significant vs control).

Figure S3. KRAS expression and membrane localization in human HCC tissues as correlated with PDE6D expression and cytoplasmatic localization. (A) Tissue micro array analysis of PDE6D expression levels (high, medium, low) in human HCC tissues correlated with KRAS expression (high, low). (B) Tissue micro array analysis comparing KRAS membrane staining ("yes" vs "no") in human HCC tissues with ("yes") and without ("no") cytoplasmatic localization pattern of PDE6D.
Figure 4. Potential protein-interactions of PDE6D and PDE6D-interactome dependent pathways. (A) Analysis of protein-protein interaction datasets was performed using the "Harmonizome" database. The table depicts potential PDE6D-interacting proteins that were derived from low-throughput or high-throughput studies from the following databases: Reactome, NCI Pathways, PhosphoSite, HumanCyc, HPRD, PANTHER, DIP, BioGRID, IntAct, BIND, Transfac, MiRTarBase, Drugbank, Recon X, Comparative Toxicogenomics Database, and KEGG. The table also depicts information about cellular localization, HCC-relation according to literature (Pubmed-IDs) and related pathways/functions. The black arrows mark nuclear proteins. (B) String database and DAVID bioinformatics database-derived graphical illustration (top panel) as well as Gene enrichment based Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (bottom panel) of the PDE6D-interactome.
Figure S5. Potential transcription factor interactions of the PDE6D-interactome. Gene enrichment analysis using the "Enrichr" database depicting significantly enriched transcription factor terms for the 34 PDE6D-interacting protein list (Figure S1) in the "Enrichr Submissions TF-Gene Cooccurrence" dataset (A) and the "Transcription Factor PPIs" dataset (B).