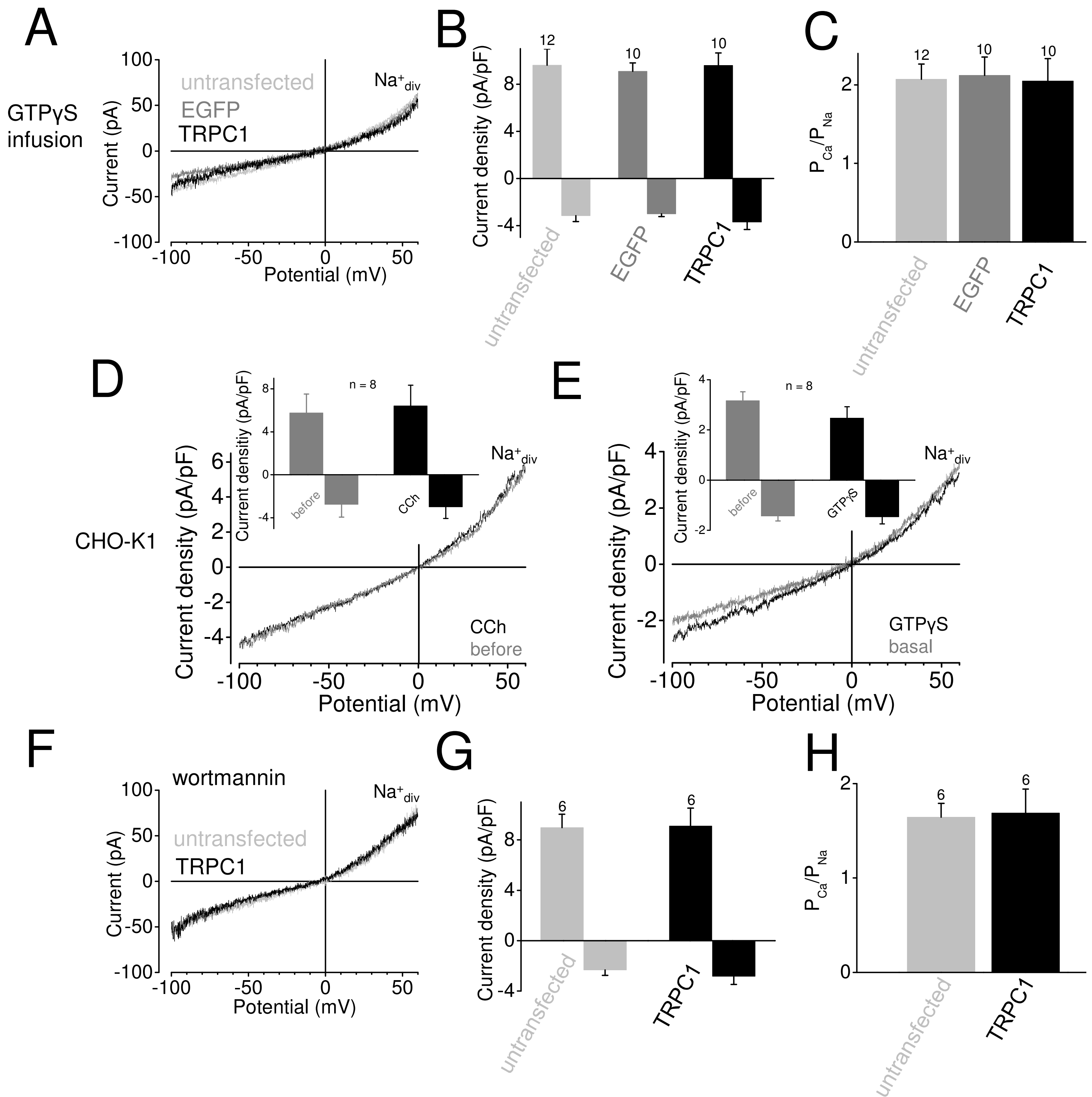
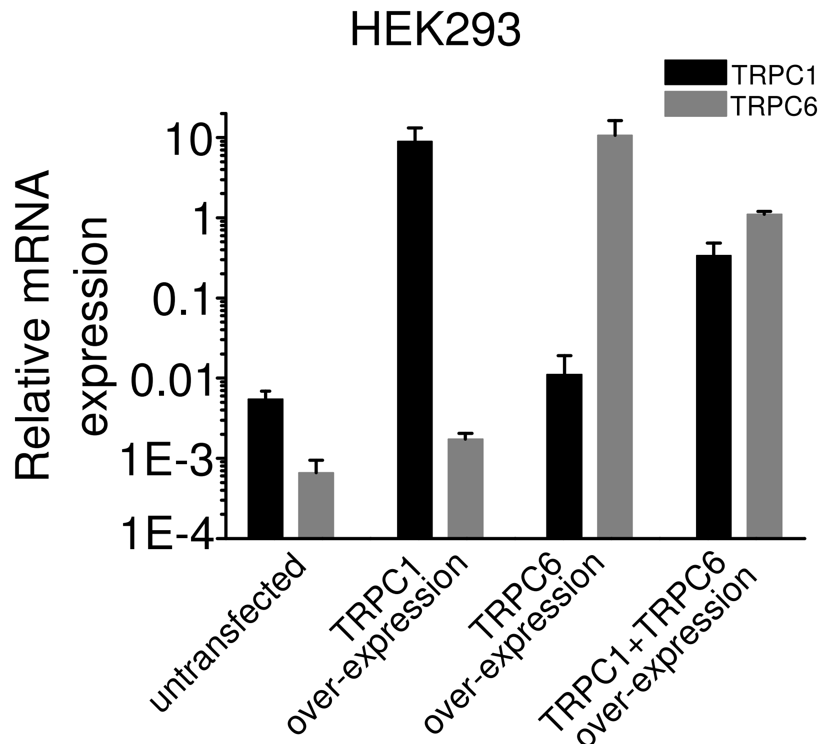
Supplementary Figure 1



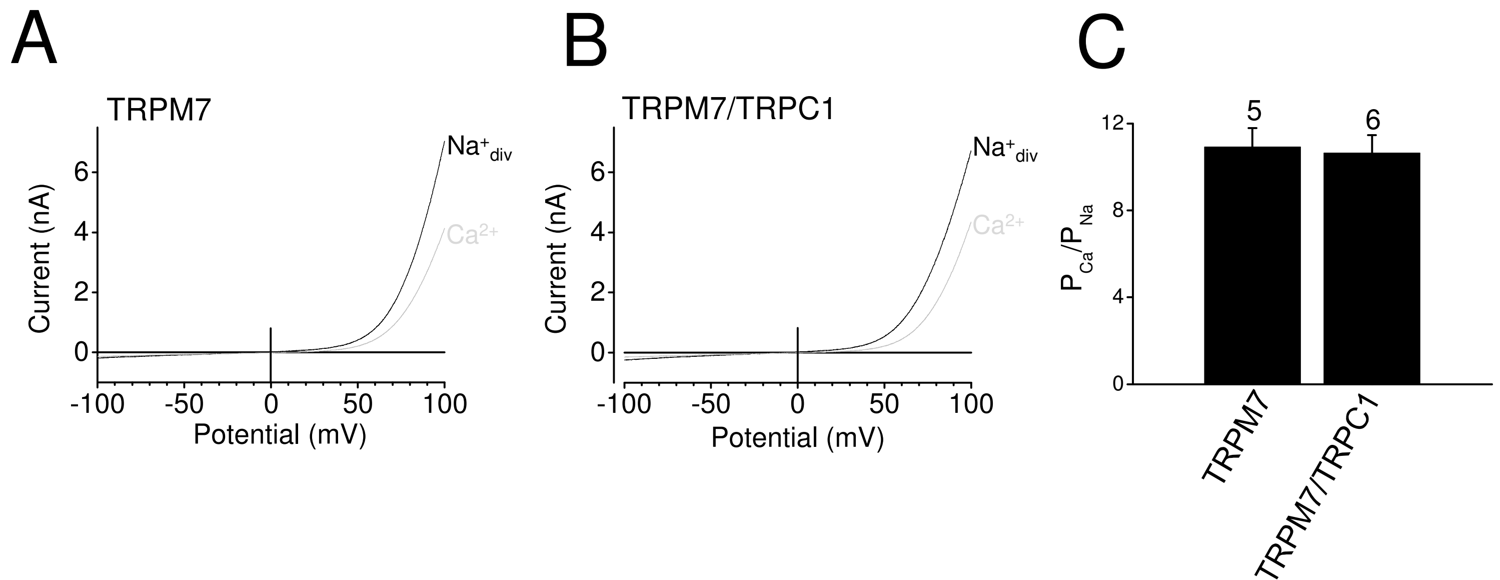
Supplementary Figure S1: TRPC1 is not able to form functional homomeric channels. (*A-H*) Whole-cell recordings from untransfected HEK293 cells and HEK293 cells expressing EGFP alone or TRPC1 and the muscarinic M_5 receptor (M_5R) (*A-C and F-H*) and CHO-K1 cells over-expressing TRPC1 and the M_5R (*D,E*). GTPγS (200 µM) infusions (*A-C,E*), agonist stimulation with 100 µM carbachol “CCh” (*D*) and application of wortmannin (20 µM) (*F,G*) are displayed. Exemplary IV curves (*A,D-F*) in standard bath solution (Na^{+}_{div}) are shown. Analysis of current densities (*B,D,E,G*) and of calcium permeabilities (*C,H*). The numbers over the bars indicate the number of cells measured.

Supplementary Figure 2



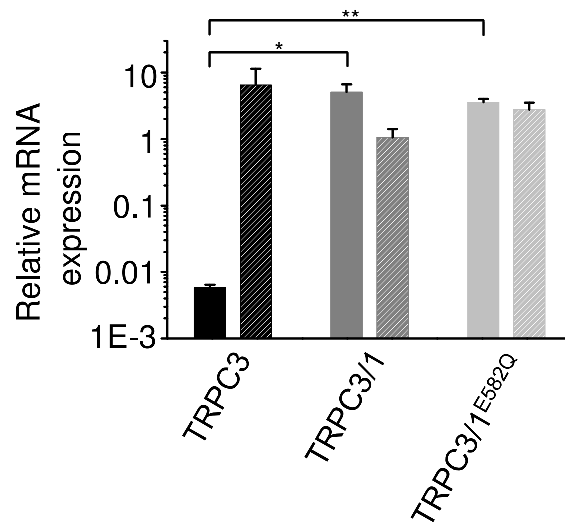
Supplementary Figure S2: Expression levels of TRPC1 and TRPC6 in untransfected and in transiently transfected HEK293 cells. Analysis of three independent quantitative qPCR approaches with untransfected, human TRPC1, human TRPC6 and human TRPC1/ human TRPC6 expressing HEK293 cells. The following primers pairs were used for the amplification of specific fragments from the first strand synthesis: TRPC1, C1for: 5'-ATG GCG CTGA AGG ATGT G-3' and C1rev: 5'-TCC TCC AAA ATC TTT TTA ACC ATA TAA-3', TRPC6, C6for: 5'-ATT TAC TGG TTT GCT CCA TGC-3' and C6rev: 5'-GCA GTC CCA GAA AAA TGG TG-3' and three references hypoxanthin phosphoribosyltransferase 1, Hprt1for (5'-TGA CCT TGA TTT ATT TTG CAT ACC-3'), Hprt1rev (5'-CGA GCA AGA CGT TCA GTC CT-3'), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide, Ywhazfor (5'-GAT CCC CAA TGC TTC ACA AG-3'), Ywhazrev (5'-TGC TTG TTG TGA CTG ATC GAC-3') and succinate dehydrogenase complex, subunit A, Sdhafor (5'-GGA CCT GGT TGT CTT TGG TC-3'), Sdharev (5'-CCA GCG TTT GGT TTA ATT GG-3') giving predicted product sizes of 113 bp for TRPC1, 103 bp for TRPC6, 102 for Hprt1, 130 for Ywhaz and 93 for Sdha.

Supplementary Figure 3



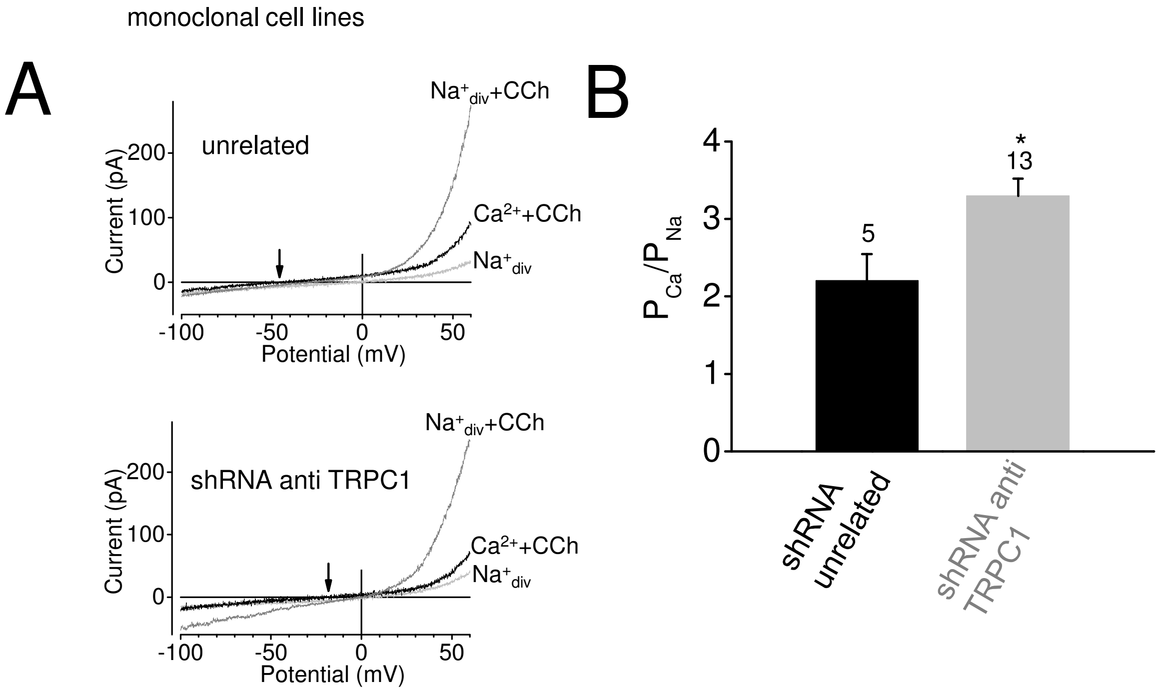
Supplementary Figure S3: TRPC1 is not able to co-assemble with TRPM7. Whole-cell recordings from HEK293 cells expressing TRPM7 alone (*A*) or TRPM7 and TRPC1 (*B*). Current activation was induced by using the following intracellular solution: 130 mM CsCl, 0.635 mM $CaCl_2$ (5.5 nM calculated free [Ca^{2+}]), 10 mM BAPTA, 1 mM HEDTA, and 10 mM HEPES, pH 7.2. IV relations were recorded during voltage ramps from –100 to +100 mV with a slope of 0.5 V/s applied at a frequency of 2 Hz. Exemplary IV relationships in standard bath solution and in 10 mM Ca^{2+} solution (*A,B*) are shown. (*C*) Analysis of the respective calcium permeabilities. The numbers over the bars indicate the number of cells measured.

Supplementary Figure 4



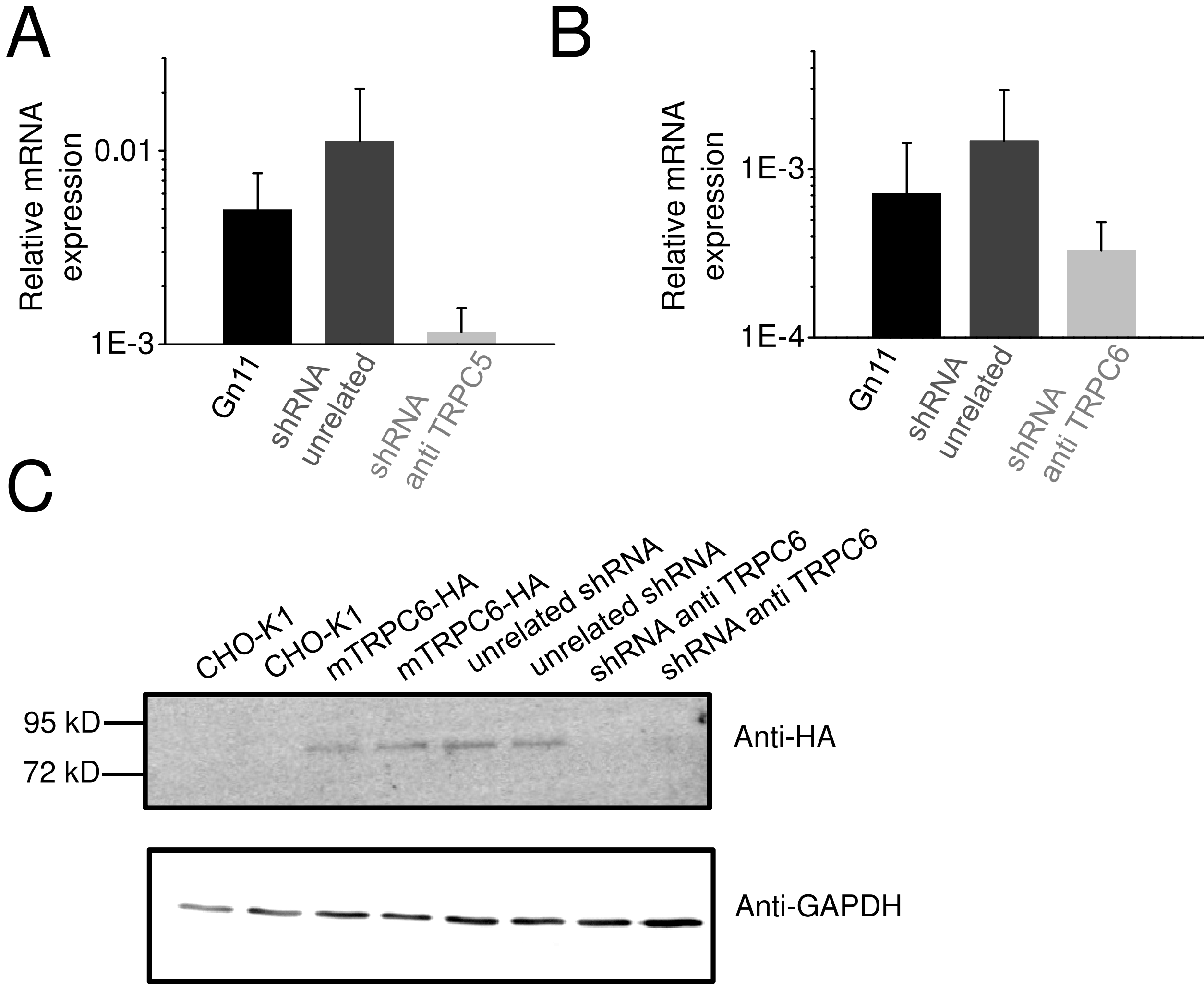
Supplementary Figure S4: qPCR analysis of HEK293 cells transfected with TRPC3 alone, with TRPC3 and TRPC1 and with TRPC3 and the TRPC1 mutant TRPC1^{E582Q}. Analysis of three independent qPCR experiments using human TRPC1 and TRPC3 primers. Relative TRPC1 (*left bars,* uncolored) and relative TRPC3 (*right bars,* shaded) mRNA expression.

Supplementary Figure 5



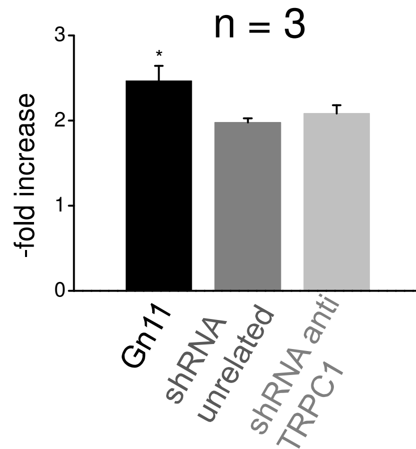
Supplementary Figure S5: Knock-down of TRPC1 in Gn11 cells increases calcium permeability. (*A,B*) Whole-cell measurements of monoclonal cell lines of Gn11 cells stably transfected with unrelated shRNA and with shRNA targeting TRPC1 with exemplary IV relationships before and during agonist stimulation with carbachol (CCh) and in 10 mM Ca^{2+} solution (*A*). (*B*) Analysis of the respective calcium permeabilities.

Supplementary Figure 6



Supplementary Figure S6: Efficiency testing of shRNA targeting mouse TRPC5 and TRPC6. (A,B) Analysis of the relative TRPC5 (A) and TRPC6 (B) mRNA expression in Gn11 wild-type cells and in Gn11 cell lines expressing unrelated control shRNA “shRNA unrelated” or shRNA targeting TRPC5 “shRNA anti TRPC5” (A) or TRPC6 “shRNA anti TRPC6” (B) of three independent experiments each determined in quadruplicates. (C) Western blot analysis of CHO-K1 cells and of CHO-K1 cells stably expressing HA-tagged mouse TRPC6 “mTRPC6-HA” alone, in combination with unrelated control shRNA or with shRNA targeting TRPC6.

Supplementary Figure 7



Supplementary Figure S7: Knock-down of TRPC1 in Gn11 cells did not affect proliferation. Analysis of proliferation assays with polyclonal Gn11 wild-type cells and with monoclonal cell lines of Gn11 cells stably transfected with unrelated shRNA and with shRNA targeting TRPC1.

Supplementary Material and Methods

Proliferation assay

To analyze the effect of TRPC1 knock down on proliferation ability of migrating Gn11 cells, CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) was used according to the manufacturer's description. $2x10^4$ Gn11 cells were seeded in normal growth medium in a 96-well plate and viable cell number was measured right after and 24 hours after seeding of cells. All experiments were performed in triplicate and results are shown as fold increase of cell number compared to wild type Gn11 cells.