Figure S1. Western blot analyses of human ARMS and ERMS cell lines. (A-C) Representative Western blot analyses (n=2) of (A) secreted WNT5A protein levels in supernatant of L-cells, WNT3A- or WNT5A-producing fibroblasts (WNT3A CM or WNT5A CM, respectively) or DMEM supplemented with 200 ng/ml recombinant WNT5A (rWNT5A) using the mAbrabbit anti-Wnt5a antibody #2530 from Cell Signaling Technologies (A, top). As also done for Fig. S1B (see below) and Fig. 1E, we measured β-Actin in the respective whole cell lysates loaded on another gel (A, bottom). (B) WNT5A protein levels in the supernatant (top) and in whole cell lysates (bottom) of RMS tumor cell lines after transient WNT5A overexpression and (C) typical WNT5A receptors levels in RMS cell lines. β-Actin detection served as loading control for whole cell lysates in (B, bottom) and in (C). Please note that the ordering of the cell lines in (C) is different in the left and right panel and that the translocation negative ARMS cell line FLOH1 (a gift from EwaKoscielniak, Stuttgart, Germany) was included.
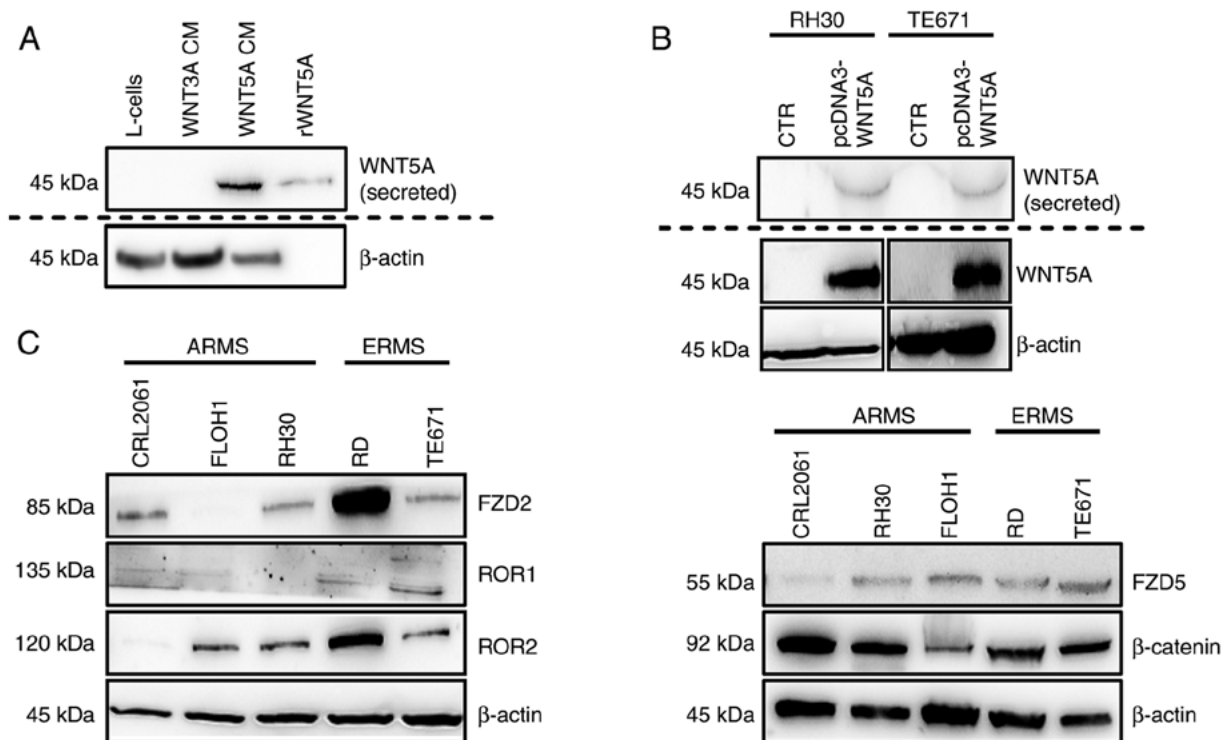
Figure S2. Comparison of WNT5A expression levels and metabolic activity of individual WNT5AOE and WNT5AKD RMS clones. (A) qRT-PCR-based analyses of relative WNT5A expression and (B) MTT assay-based measurement of the cell viability/ metabolic activity of 3 or 2 individual batches of the human ARMS cell line RH30 and ERMS cell lines RD and TE671 stably overexpressing WNT5A (OE1, OE2, OE3) or stably expressing a WNT5A shRNA to induce a WNT5A knockdown (KD1, KD2), respectively. Values of OE and KD cells were compared to values of respective control cell lines (CTR), which were transduced with the respective empty vectors (pBabe or pGIPZ). Please note that the values of CTR were set to 1 in (A) and (B) and that all cell batches were analyzed in 3 independent expriments each measured in duplicates. In (A) the CTR values are indicated as dotted lines to ensure greater clarity. (B) shows fold change cell viability compared to the respective CTRs.Bars: mean + SD. All clones showed almost identical WNT5A expression levels or cell viability/metabolic activity. Statistical analysis was done by Student's t-test. *indicates significance compared to CTR. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
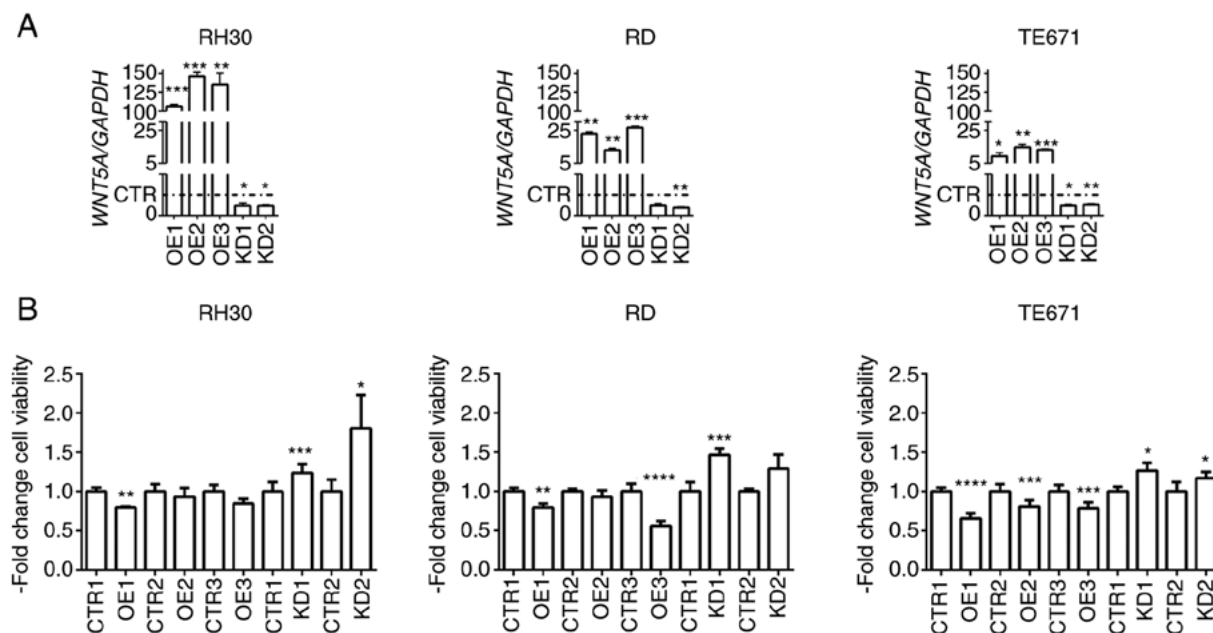
Figure S3. Analysis of cellular viability and proliferation of WNT5AOE and WNT5AKD RMS cell lines. (A) WST-1 assay-based measurement of the cell viability activity, of the human ARMS cell line RH30 and ERMS cell lines RD and TE671 stably overexpressing WNT5A (OE) or stably expressing a WNT5A shRNA to induce a WNT5A knockdown (KD). Cell lines transduced with the respective empty vectors (pBabe or pGIPZ) served as controls (CTR). (B) BrdU incorporation and (C) WST-1 assay of the respective parental cells incubated with or without 200 ng/ml rWNT5A. (A-C)CTR values were set to 100%. Bars: mean + SD. Data represent mean + SD of at least three independent experiments, each measured in duplicates. Statistical analysis in (A) was done by one-way ANOVA and that in (B) and (C) by Student's t-test. *indicates significance compared to CTR; #indicates significance between OE and KD. **$P<0.01$, ***/###$P<0.001$, ****/####$P<0.0001$.
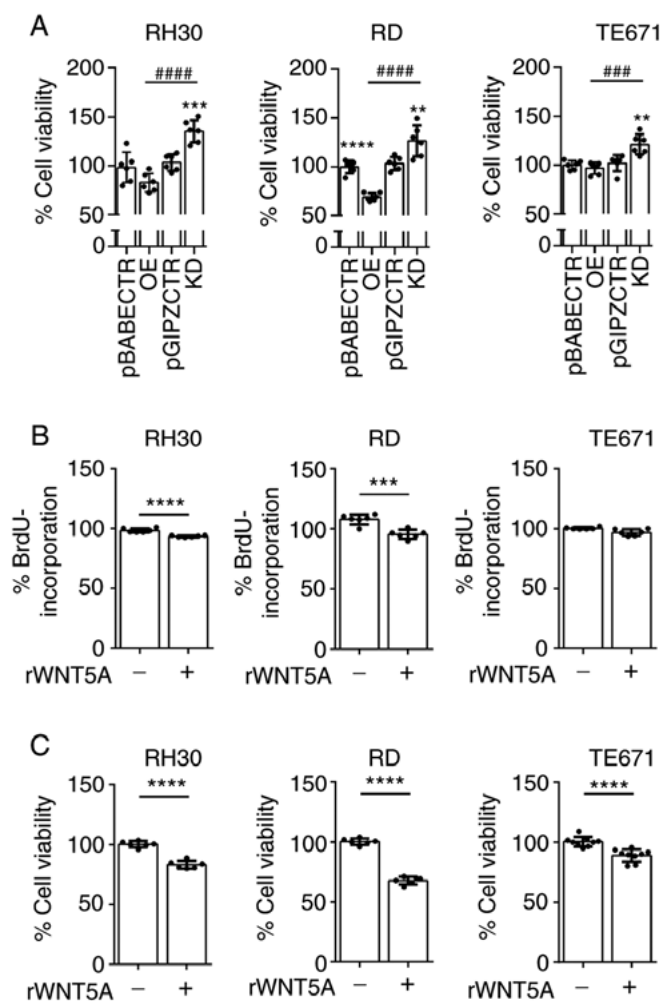
Figure S4. Comparison of cellular proliferation, cell viability and expression of muscle differentiation markers of pBABE and pGIPZ transduced control cell lines and respective parental cell lines. (A) qRT-PCR analyses of WNT5A expression, (B) BrdU and (C) cell viability (WST) assays and (D) Western blot analyses of MYOD, DES and MYOG in RH30, RD and TE671 cells stably transduced with pBABE (pBABECTR) or pGIPZ (pGIPZCTR) and in the respective parental cells (CTR). Values of parental cells were set to 1 in (A) and to 100% in (B-C). (A) Gene expression levels were normalized to GAPDH expression levels. Data represent mean + SD of three independent experiments, each measured in duplicates. (B) and (C) data represent mean+ SD of three independent experiments, each measured in triplicates. Statistical analysis was done by one-way ANOVA. *indicates significance compared to CTR; **P<0.01.