Functional analyses of *RUNX3* and *CaMKIINα* in ovarian cancer cell lines reveal tumor-suppressive functions for *CaMKIINα* and dichotomous roles for *RUNX3* transcript variants.

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Supplementary Figures S1-S5
Supplementary Figure S1. Gene expression of the transgenes in A2780 and SKOV3 cells normalized against the housekeeping gene Actin b and HPRT. SKOV3 data are additionally normalized to the parental cells. (a) Relative expression of CaMKIIα in parental, CIS-resistant A2780 cells and CaMKIIα clones. (b) Relative expression of CaMKIIα in parental, CIS-resistant SKOV3 cells and the CaMKIIα clones showing an overexpression above the control cells. (c, e) Relative level of RUNX3 TV1 (c) and TV2 (e) in A2780 cells confirmed an overexpression compared to parental and CIS-resistant cells. (d, f) Relative expression of RUNX3 TV1 (d) and TV2 (f) in SKOV3 cells compared to parental, CIS-resistant and empty vector control cells.
Supplementary Figure S2. Correlation of the CaMKIIα overexpression and the cisplatin sensitivity in A2780 (a) and in SKOV3 cells (b). No association between the responsiveness towards cisplatin and CaMKIIα level was seen.
Supplementary Figure S3. Exemplary pictures of the migratory behavior of cells under CaMKIIα and RUNX3 expression in the wound healing assay. Parental cell data for each cell line are identical for all 3 transcripts analyzed. Overview of observed migratory properties in A2780 cells (a) and SKOV3 cells (b) under CaMKIIα expression. A reduced wound closure was achieved in CaMKIIα single cell clones compared to control cells. The A2780 cells expressing RUNX3 TV1 (c) showed an increase in the cellular migration while A2780 cells expressing RUNX3 TV2 (e) experienced an inhibition of migration. In SKOV3 cells (d) both transcript variants led to a reduction of the wound closure but a distinct difference between the two variants was observed. In concordance to A2780 data RUNX3 TV2 reduced the migratory ability stronger than TV1.
Supplementary Figure S4. Raw data of the colony-formation assay using controls and cells under CaMKIINα and RUNX3 expression. Graphs showing the data of all tested CaMKIINα single cell clones of A2780 (a) and SKOV3 cells (b) compared to parental, CIS-resistant and empty vector control cells. An overall decrease in the ability to form colonies was observed. The absolute OD measurements of A2780 cells expressing RUNX3 TV1 (c) and RUNX3 TV2 (e) reflect an inhibitory effect of both RUNX3 transcript variants on the colony forming ability in comparison to the control cells. While in SKOV3 cells the expression of TV1 led to no change, a decline of the colony number was measured when TV2 was overexpressed (d).
Supplementary Figure S5. Data of the MTT assay normalized to values at 2 h reflect the number of cells under CaMKIINα and RUNX3 expression at 4 distinct time points. (a, b) Graphs showing the data of all tested CaMKIINα single cell clones of A2780 (a) and SKOV3 cells (b) compared to parental, CIS-resistant and empty vector control cells. Overall a decrease in the proliferation rate was observed. The absolute OD measurements of A2780 cells (c) and SKOV3 cells (d) expressing RUNX3 TV1 revealed a lower proliferation in comparison to the empty vector control cells. The overexpression of RUNX3 TV2 resulted just in A2780 cells (e; 2/3 clones)) but not in SKOV3 cells (d) to a slight increase in the proliferation of tested single cell clones.