

Re-introduction of type 1 iodothyronine deiodinase in renal cancer cells affects their migration and expression of adhesion-related genes



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Introduction: Type 1 iodothyronine deiodinase (DIO1) is one of the three enzymes regulating bioavailability of thyroid hormones. In contrast to DIO2 and DIO3, the specific cellular role of DIO1 remains controversial. Our previous studies showed that DIO1 expression in clear cell renal cell carcinoma (ccRCC) is decreased, followed by lowered intracellular T3 concentration (Fig.1). In this study we explored how re-introduction of *DIO1* in ccRCC cells affects their migration and proliferation.

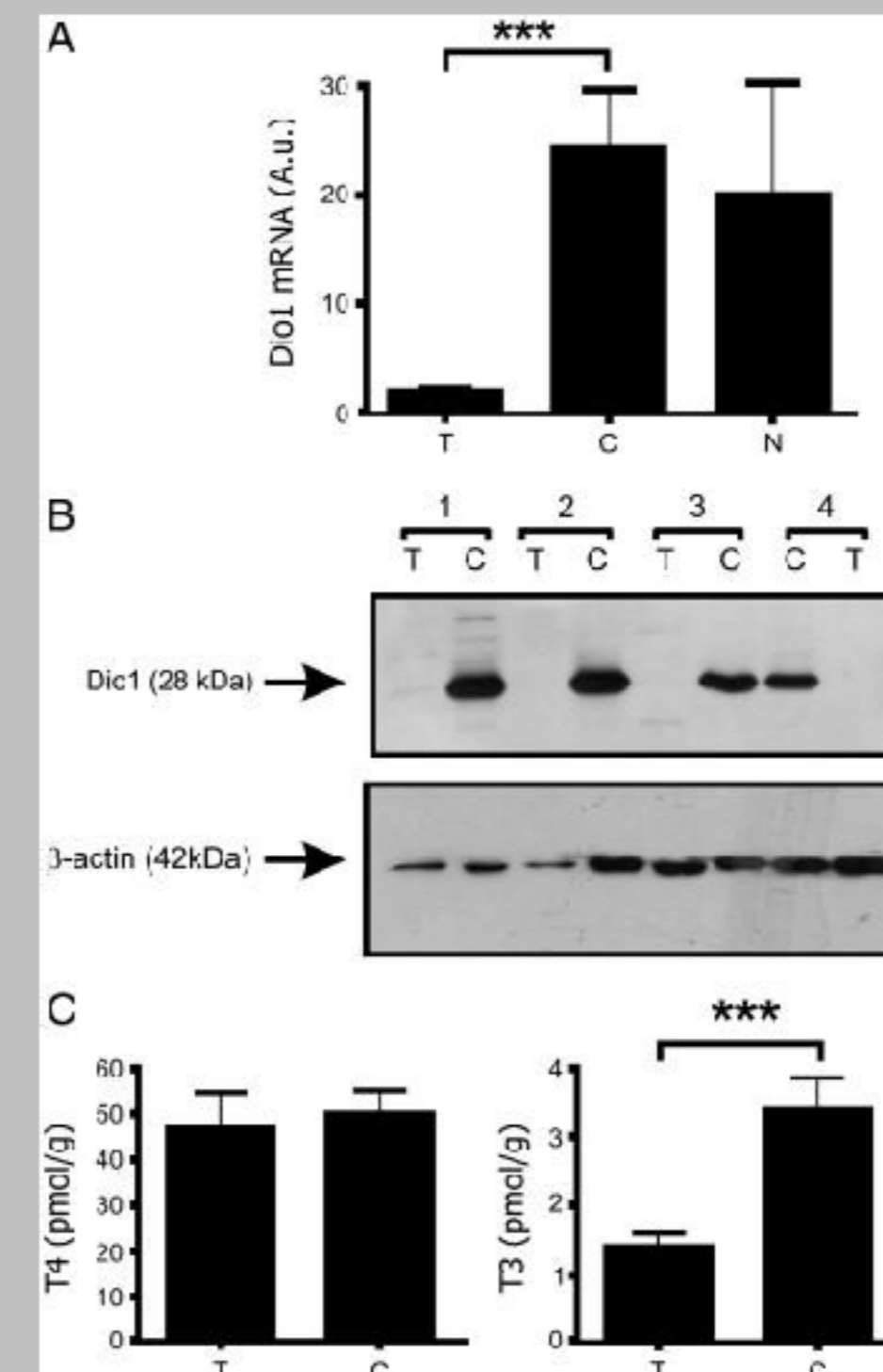


Fig. 1. Expression of *DIO1* and tissue concentrations of thyroid hormones in ccRCC. A. Expression of *DIO1* mRNA in tissue samples. $n = 33$ for T, $n = 33$ for C, $n = 10$ for N. Data are means \pm S.E., $***p < 0.001$. Statistical analysis was performed using a paired *t*-test. B. Western blot showing expression of D1 (28 kDa protein) and β -actin (42 kDa protein) in four representative C and T paired samples. C. Tissue concentrations of T4 and T3, in paired control (C) and ccRCC (T) samples. Data are shown as mean \pm S.E. ($n = 12$ for T, $n = 12$ for C). Statistical analysis was performed using paired *t*-test. $***p < 0.001$. Master A, Wójcicka A, Piekietko-Witkowska A, Bogusławska J, Popławski P, Tański Z, Darras VM, Williams GR, Nauman A. *Biochim Biophys Acta*. 2010 Nov;1802(11):995-1005.

Material: 78 matched pairs of ccRCC tumors and control samples, approved by the local Bioethical Committee. Two ccRCC cell lines KIJ-265T and KIJ-308T.

Methods: ccRCC cell lines (KIJ-265T-pcDNA3-DIO1 and KIJ-308T-pcDNA3-DIO1) with stable re-expression of DIO1 and control cell lines (KIJ-265T-pcDNA3 and KIJ-308T-pcDNA3) stably transfected with empty pcDNA3 vector were generated. The expression of genes involved in adhesion and ECM was analyzed with RT² Profiler™ PCR Array (SABiosciences), RealTime ready Custom Panel (Roche), followed by SYBRGreen/ qPCR validation. Scratch test and Cell Proliferation ELISA, BrdU (Roche) were used for analysis of migration and proliferation, respectively.

Results: Expression of collagen COL1A1 and integrin ITGB2 was elevated in ccRCC tumors compared with control samples (Fig. 2A, Fig. 3A). Re-expression of DIO1 in KIJ-265T (grade IV) and KIJ-308T (grade II) cell lines (Fig. 4) resulted in decreased expression of COL1A1 (by 80% and 66%, respectively) (Fig. 2B and 2C, Tab. 1) and ITGB2 (by 67% and 30%, respectively) (Fig. 3B and 3C; Tab.1). Scratch test revealed that migration of KIJ265 was inhibited by DIO1 expression both in presence or in absence of 1 μ g/ml actinomycin D (proliferation inhibitor) while in KIJ308T DIO1 reduced migration only when proliferation was inhibited (Fig. 5). Proliferation of both cell lines was reduced by DIO1 expression (Fig.6).

Conclusions: We show for the first time that re-expression of DIO1 in renal cancer cells inhibits their proliferation and migration. This effect is probably mediated by DIO1-induced changes in expression of genes involved in cellular adhesion. The specific mechanisms of DIO1 action in ccRCC require further investigation. These results suggest that DIO1 loss in renal cancer may significantly contribute to the process of cancerogenesis.

Tab. 1 DIO1-induced changes in expression of genes involved in cellular adhesion and migration. Changes of gene expression in DIO1-expressing cells are shown as percentage of expression in control cells. Statistically significant changes ($p < 0.05$) are shown in green, $n=3$.

gene	KIJ265T-Dio1	KIJ308T-Dio1
<i>COL1A1</i>	-80%	-66%
<i>ITGB1</i>	0	7%
<i>ITGB2</i>	-67%	-30%
<i>ITGB3</i>	-49%	3%
<i>ITGAV</i>	-12%	12%
<i>ITGA2</i>	-39%	16%
<i>ITGA4</i>	-29%	-23%
<i>ITGA5</i>	-26%	15%

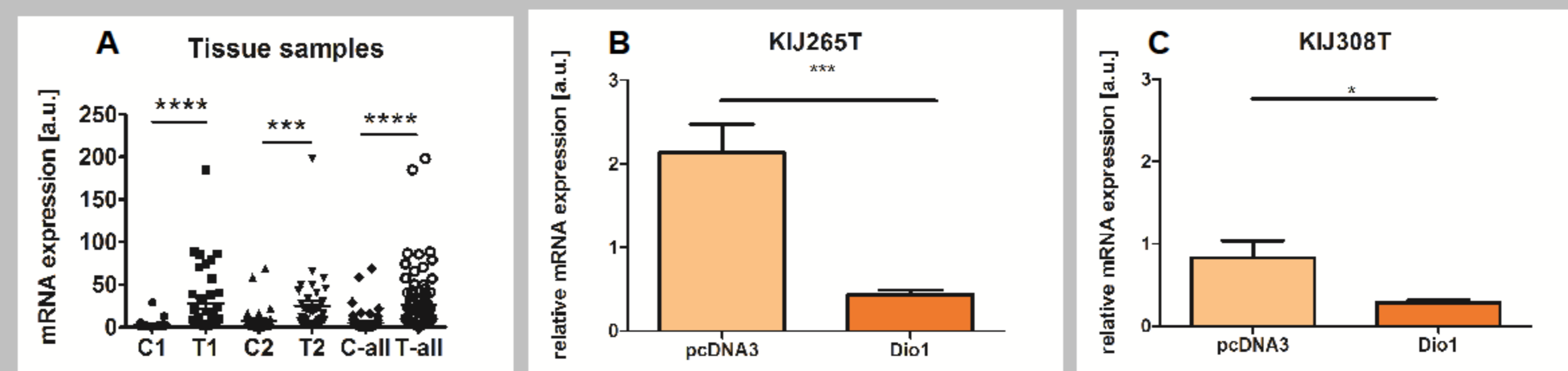


Fig. 2 COL1A1 expression. A - mRNA expression in Tissue samples. T1 – tumor samples from stage I and II tumors ($n=40$), C1 – control samples ($n=40$), T2 - tumor samples from stage III and IV tumors ($n=38$), C2 – control samples ($n=38$), T-all – all tumor samples ($n=78$), C-all - control samples ($n=78$); B - mRNA expression in KIJ265T cell line $n=3$ C - mRNA expression in KIJ308T cell line $n=3$; * $p < 0.05$, $***p < 0.001$; $****p < 0.0001$

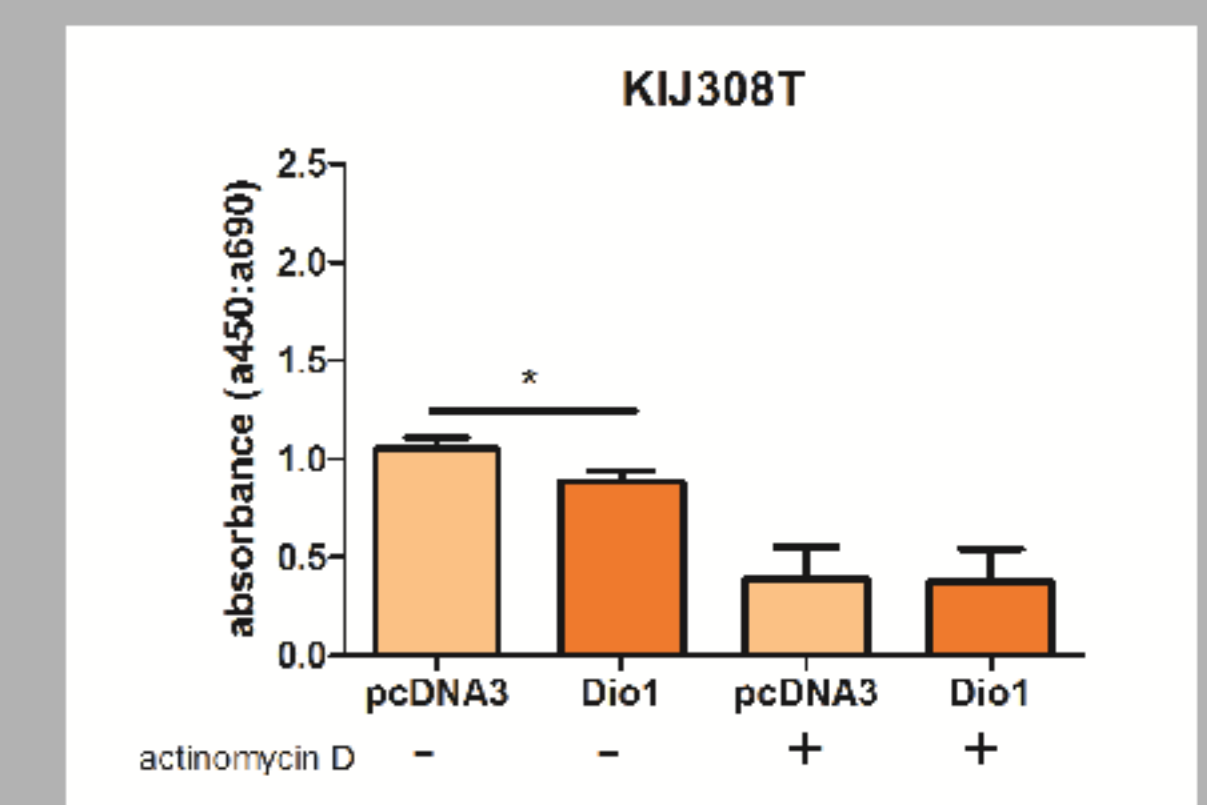
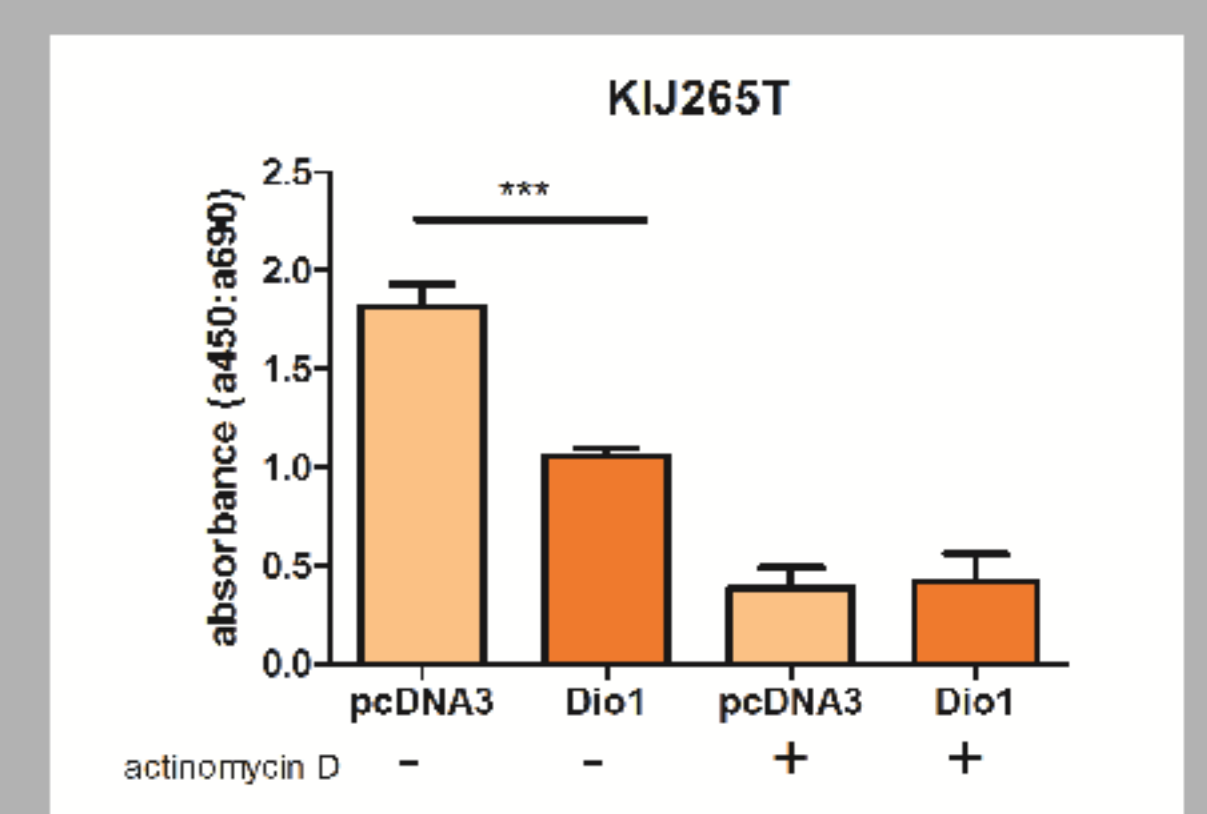


Fig. 6 The effects of DIO1 re-expression on proliferation. The graphs show results of Brdu assay performed in the presence (+) or absence (-) of proliferation inhibitor, actinomycin D. $n=3$ * $p < 0.05$, $***p < 0.001$

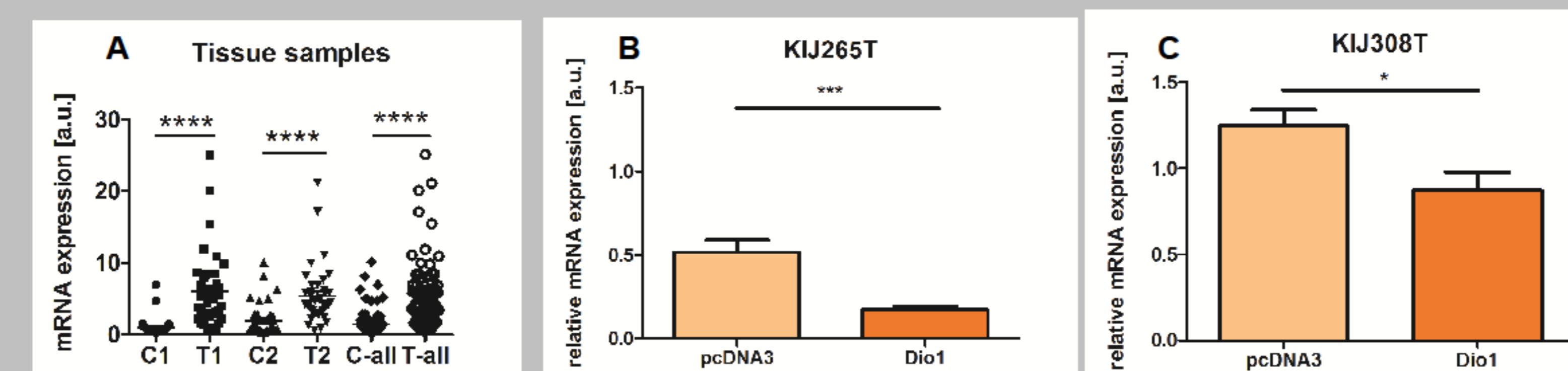


Fig. 3 ITGB2 expression. A - mRNA expression in Tissue samples. T1 – tumor samples from stage I and II tumors ($n=40$), C1 – control samples ($n=40$), T2 - tumor samples from stage III and IV tumors ($n=38$), C2 – control samples ($n=38$), T-all – all tumor samples ($n=78$), C-all - control samples ($n=78$); B - mRNA expression in KIJ265T cell line $n=3$ C - mRNA expression in KIJ308T cell line $n=3$; * $p < 0.05$, $***p < 0.001$; $****p < 0.0001$

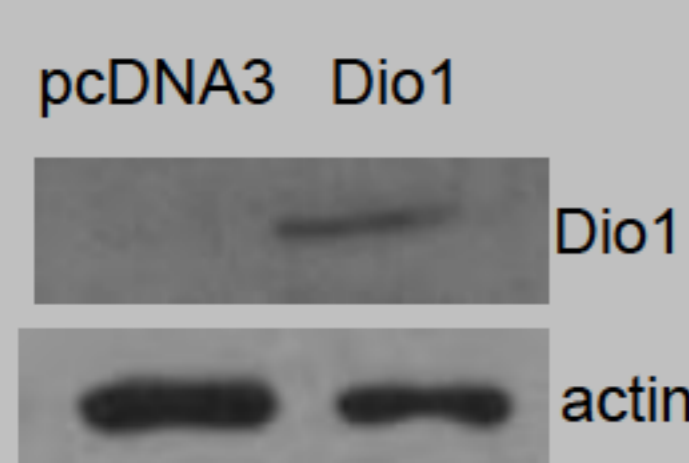
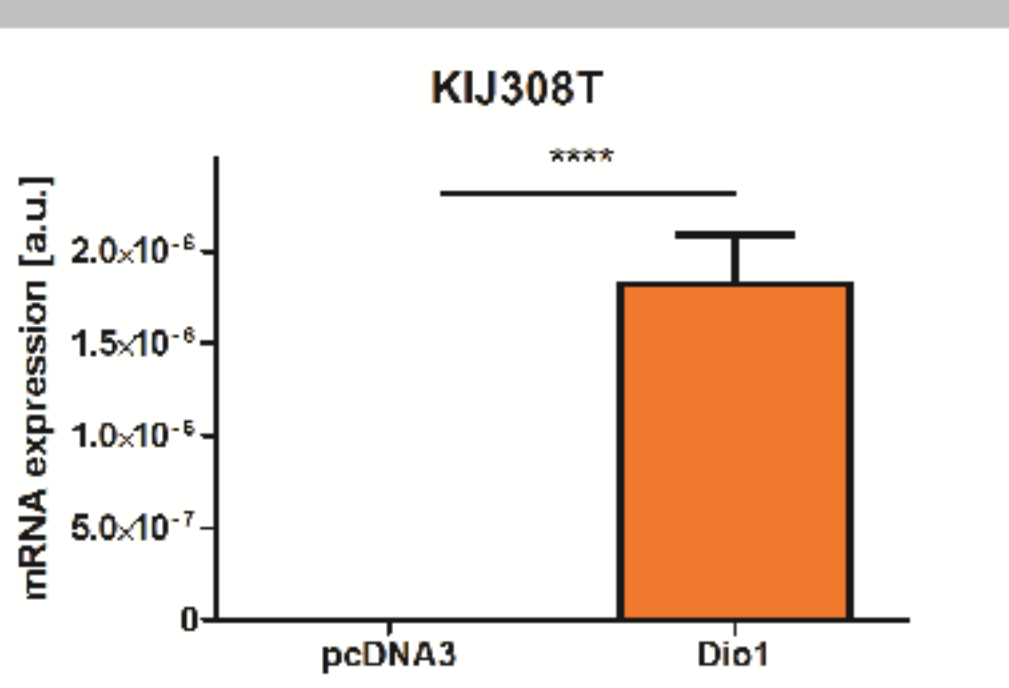
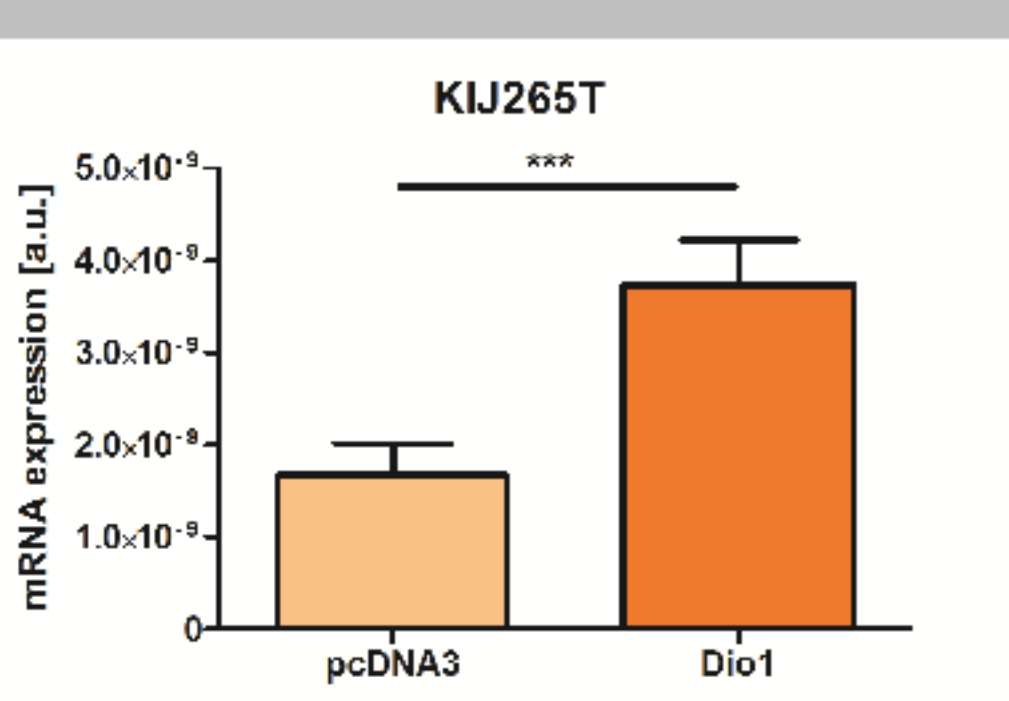


Fig. 4 Expression of type 1 deiodinase in stably transfected ccRCC cell lines (KIJ265T, KIJ308T)
 $n=3$ $***p < 0.001$ $****p < 0.0001$

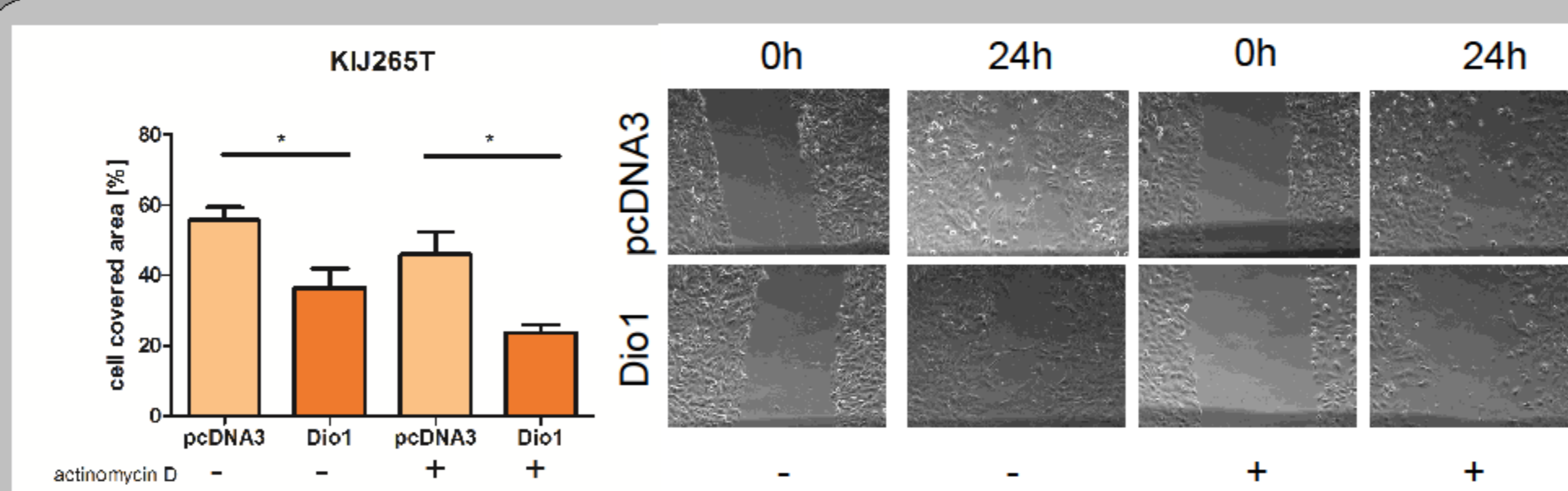


Fig. 5 The effects of DIO1 re-expression on cells migration. The graphs show results of Scratch Assay performed in the presence (+) or absence (-) of proliferation inhibitor, actinomycin D. $n=3$; * $p < 0.05$,

