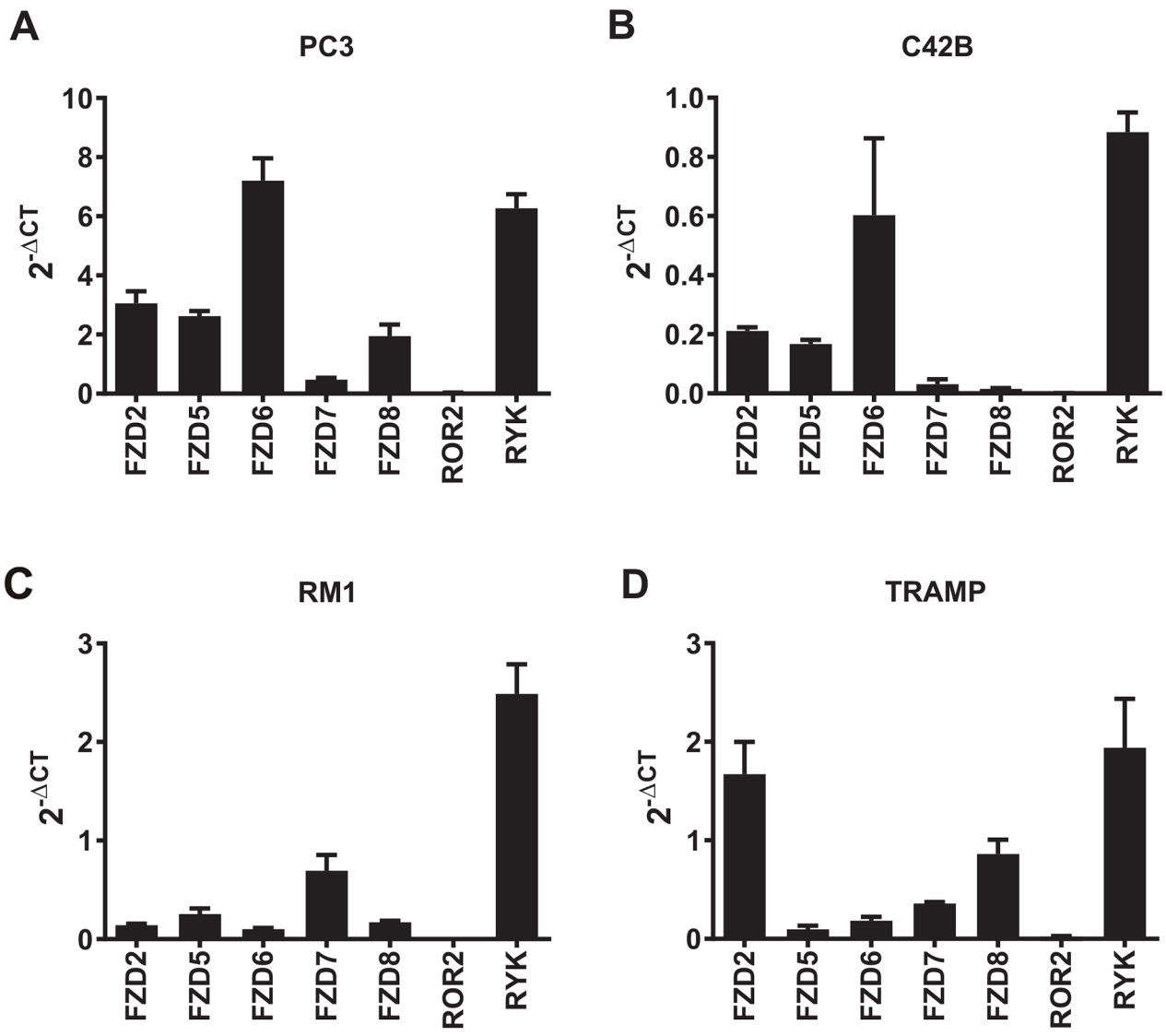
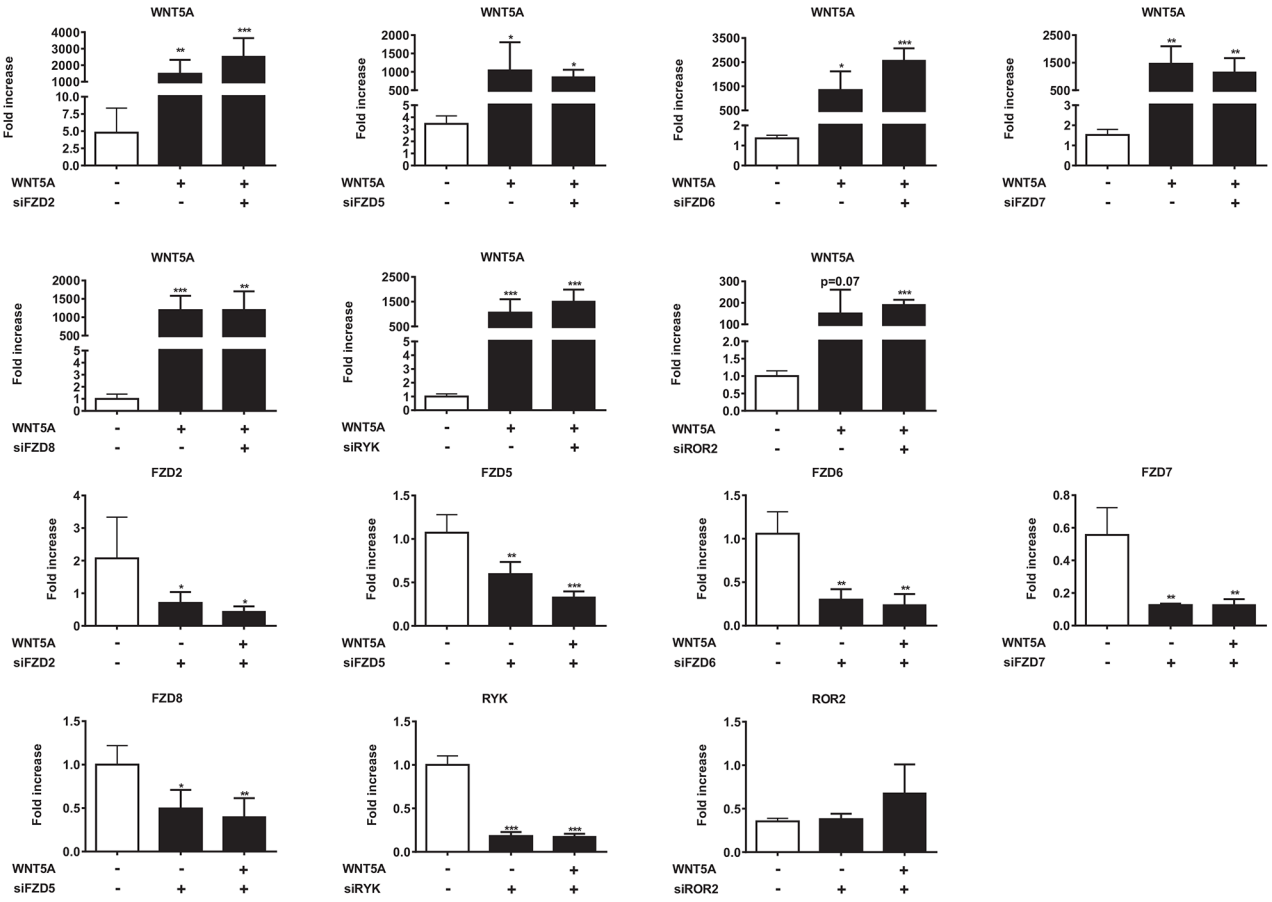
Role of WNT5A receptors FZD5 and RYK in prostate cancer cells

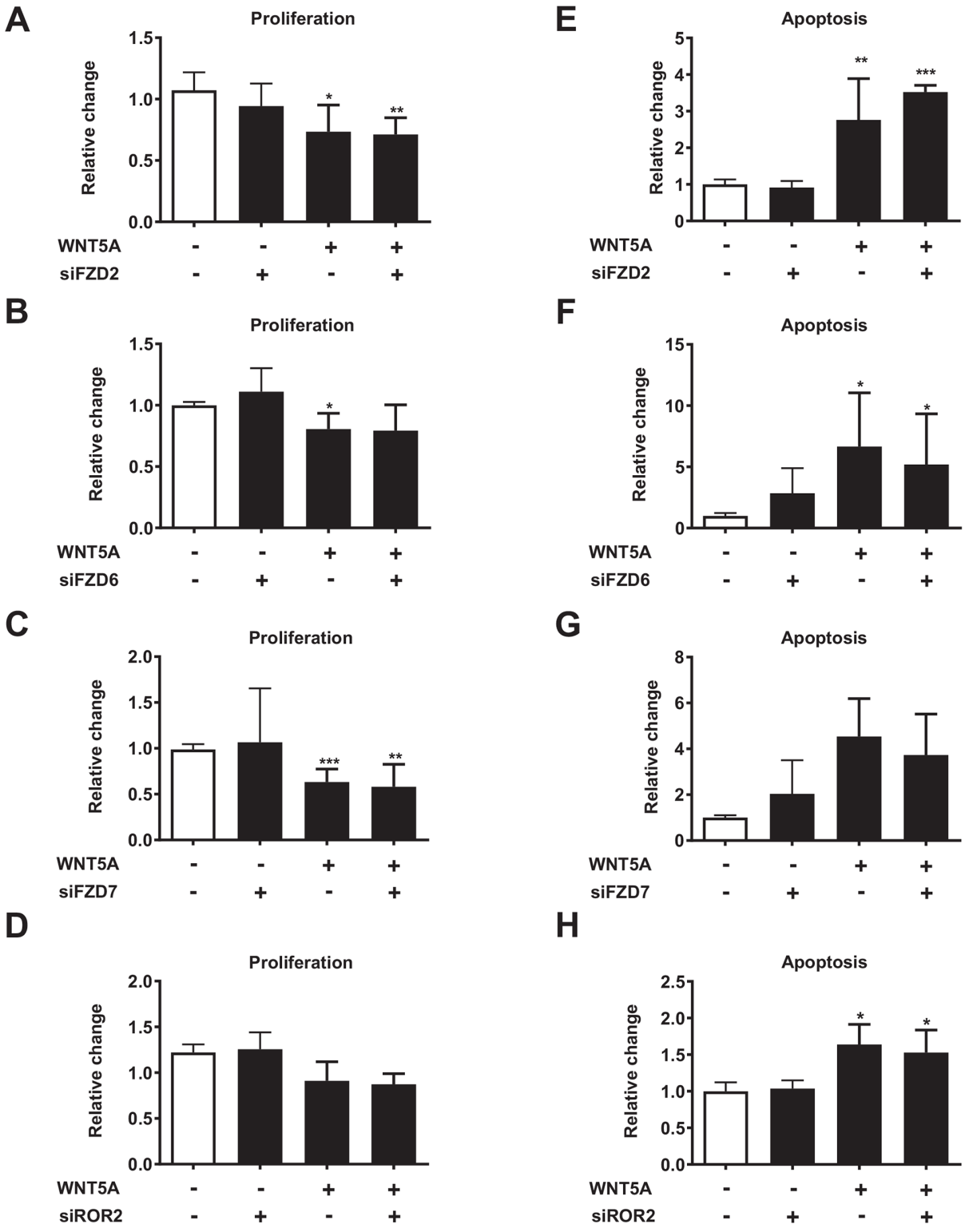
SUPPLEMENTARY MATERIALS



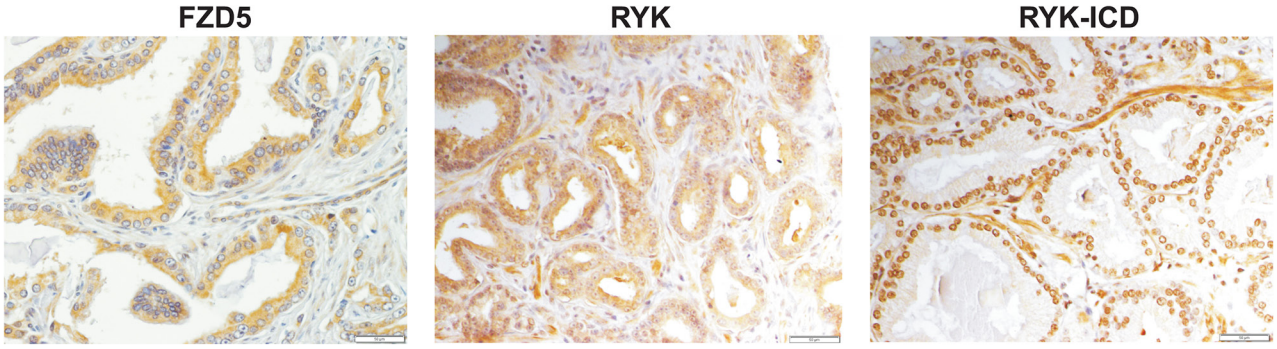
Supplementary Figure 1: Expression of Wnt receptors in human and mouse prostate cancer cell lines. **(A-B)** Expression of FZD2, FZD5, FZD6, FZD7, FZD8, ROR2, RYK in two different human prostate cancer cell lines PC3 and C4-2B. **(C-D)** Expression of Fzd2, Fzd5, Fzd6, Fzd7, Fzd8, Ror2, Ryk in two different murine prostate cancer cell lines RM1 and TRAMP-C2. The mRNA levels of receptor expression were analyzed by qPCR. Data are shown as $2^{-\Delta CT}$ values as mean ± SD.



Supplementary Figure 2: Validation of WNT5A overexpression and Wnt receptor knock-downs. PC3 cells were either single treated using siRNAs (siFZD2, siFZD5, siFZD6, siFZD7, siFZD8, siRYK siROR2) or WNT5A plasmid-DNA (pcDNA3.1WNT5A), or treated with a combination of first siRNA (24 h) and afterwards WNT5A plasmid-DNA (24 h). Gene expression of WNT5A and receptors was analyzed using qPCR and normalized to GAPDH. Data are shown as mean ± SD. $^{*}p<0.05$; $^{**}p<0.01$, $^{***}p<0.001$ vs. control.



Supplementary Figure 3: Effects of FZD2, FZ6, FZD7 and ROR2 knock-down and/or WNT5A overexpression in PC3 cells. Cells were either single treated using siRNAs or WNT5A plasmid-DNA (pcDNA3.1WNT5A), or treated with a combination of first siRNAs (24 h) and afterwards WNT5A plasmid-DNA (24 h). **(A-D)** Proliferation of control (white bars) and treated (black) PC3 cells. Proliferation was measured using a BrdU proliferation assay after 48 h. **(E-H)** Caspase 3/7 activation of control (white bars) and treated (black) PC3 cells. Apoptosis was measured after 48 h with a Caspase Glo® 3/7 assay. Data are shown as mean ± SD. $^{*}p<0.05$; $^{**}p<0.01$, $^{***}p<0.001$. * vs. control.



Supplementary Figure 4: Immunohistochemical staining of prostate cancer tissue with FZD5 and RYK. Images show representative staining for FZD5, RYK, and RYK-ICD (nuclear RYK). Scale bar: 50 μm.

Supplementary Table 1: Sequences of human and mouse primer used in this study

Gene	Primer sequences 5'-3	Gene	Primer sequences 5'-3
Human		**Mouse**	
FZD2	CCCGACTTCACGGTCTACAT CTGTTGGTGAGGCGAGTGTA	FZD2	GACTCGTTTTGCCCGTCTCT ACCGTGAAGAAAGTGGAAGC
FZD5	AGCTAAAATGGCCAGAGCAA AATTCCCCCTGGGAACTATG	FZD5	GACGCCGAGGTTCTGTGTAT TCCTGGGAGTGTAGGTTTGG
FZD6	TTGTTGGCATCTCTGCTGTC CCATGGATTTGGAAATGACC	FZD6	GAGTCCTGTGAGTTCTTCCTGA ATGATTTGTGGTCGCTCCTG
FZD7	CGACGCTCTTTACCGTTCTC GCCATGCCGAAGAAGTAGAG	FZD7	GCTTCCTAGGTGAGCGTGAC AACCCGACAGGAAGATGATG
FZD8	GACACTTGATGGGCTGAGGT CAAATCTCGGGTTCTGGAAA	FZD8	CTGTTAGCTGGCTTCGTGTC TGTAGAGCACGGTGAAGAGG
ROR2	GGGCAACCTTTCCAACTACA CGTTGCTCACATTGCTCACT	ROR2	CTTCTGCCACTTCGTCTTCC TCCAGCACCTCACATTCATC
RYK	TGATCGGTCTTGATGCAGAA CCAGGTGAAGTGCAGGAAAT	RYK	TTAGCCGTTTTGCACCTTCA TACTGGGTCGTCTGGGTAGA
WNT5A	GGACCACATGCAGTACATCG CCTGCCAAAAACAGAGGTGT	WNT5A	CCAACTGGCAGGACTTTCTC GCATTCCTTGATGCCTGTCT
GAPDH	AGCCACATCGCTCAGACAC GCCCAATACGACCAAATCC	β-Actin	GATCTGGCACCACACCTTCT GGGGTGTTGAAGGTCTCAAA

FZD Frizzled; ROR Receptor tyrosine kinase-like orphan receptor; RYK Related to receptor tyrosine kinase.

Supplementary Table 2: Summary of patient characteristics of the cDNA Array (n=48) and the TMA cohort (n=400)

	Characsitics	Median (IQR)
cDNA Array (n=48)	Age at diagnosis (years)	60 (54, 66)
	Staging	**Frequency (%)**
	Healthy	9 (18.75)
	Stage II	18 (37.5)
	Stage III	19 (39.58)
	Stage IV	2 (4.17)
	Gleason score	**Frequency (%)**
	Healthy	9 (18.75)
	< 7	2 (4.17)
	= 7	23 (47.92)
	> 7	14 (29.16)
TMA (n=400)	Age at diagnosis (years)	65 (61, 68)
	Preoperative PSA (ng/ml)	8.6 (5.54, 15.71)
	TNM staging	**Frequency (%)**
	pT2	
		154 (38.5)
	pT4	
	pN0	294 (73.5)
	pN1	106 (26.5)
	Gleason score	**Frequency (%)**
	< 7	78 (19. 5)
	= 7	75 (18.75)
	> 7	247 (61.75)

IQR, Interquartile range; PSA, prostate-specific antigen; TNM, classification of malignant tumors.