Supplementary material for:

ARTD1 regulates cyclin E expression and consequently cell-cycle re-entry and G_1/S progression in T24 bladder carcinoma cells

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Supplementary Figure 1.

A) Experimental setup of cell cycle analysis in T24 cells. T24 cells were seeded and transfected on the next day at 70%-80% confluence. After 3 days of growth, cells reached confluence and entered the G0 phase. Upon splitting, cells re-initiated the cell cycle and samples for flow cytometry analysis, western blot analysis, qPCR analysis and ChIP analysis were taken at the indicated time points.

B) mRNA expression of *ARTD1* during the cell cycle after siMock and siARTD1 treatment.

C) Western blot analysis of total Rb protein during cell cycle progression in synchronized siMock and siARTD1-treated samples.

D) Quantitative analysis of BrdU incorporation assay in synchronized siMock or siARTD1 treated cells. 300 cells were counted for each condition and time point.

Supplementary Figure 2.

A) Western blot analysis of Chk1-phosphorylation during the cell cycle re-initiation after siMock (M) or siARTD1 (A_1) treatment at the indicated time points (T24* and Hela = corresponding cells treated with 10 µM etoposide).

B) Staining of γH2AX after knockdown of ARTD1. Cells were seeded and, one day later, transfected with siRNA against ARTD1 or a scrambled siRNA. At confluency, cells were also treated with 1 mM H_2O_2 for 10 min as a control reaction and all cells fixed and stained.

C) Tail formation assay of T24 cells after knockdown of ARTD1. Cells were seeded, transfected with the corresponding siRNA and grown until confluency before performing the comet assay.

D) Western blot analysis of cyclin A and cyclin B in synchronized siMock and siARTD1-treated cells.

E) Western blot analysis of cyclin E in shARTD1 and shMock-treated T24 cells in the G_0 phase of the cell cycle.

Supplementary Figure 3.

A-C) qPCR analysis of *c-myc* (A), *miR15* (B) and *miR16* (C) expression levels in siMock and siARTD1 treated cells (n=2-3, t-test).

D) Corresponding western blot to the FACS analysis to detect cyclin E and ARTD1 protein levels.

Supplementary Table 1. List of primers used in this study.

Gene (human)		Sequence (5'-3')
ARTD1	Forward	AGCACGCTTCACATATCAGC
	Reverse	GGTCCCAAGAGGAACGTCTA
p27 (Kip1)	Forward	AGCGACCTGCAACCGACGATTC
	Reverse	TGACGTCTTCTGAGGCCAGGCT
Cyclin E	Forward	GCAGGGAGCGGGATGCGAAG
	Reverse	CTGGTCCCTCGCCGTCCTGT
E2F-1	Forward	GACCGTAGGTGGGATCAGCCCT
	Reverse	CAGCGGTTCTTGCTCCAGGCT
Cyclin A	Forward	GCGCTCCAAGAGGACCAGGAGAAT
	Reverse	CTTAAGGGGTGCAACCCGTCTCGT
Cyclin B	Forward	GCTCCGAGTCACCAGGAACTCGAA
	Reverse	GGGCCGTAGGAACGCGCTTT
CDK2	Forward	GACCCTGTGGTACCGAGCTCCTG
	Reverse	CGGCGAGTCACCATCTCAGCAAAG
Cyclin D1	Forward	CGCAAACACGCGCAGACCTT
	Reverse	AGGCAGTCCGGGTCACACTTGA
miR16	Forward	ACCTTACTTCAGCAGCACAGT
	Reverse	AGTGCCTTAGCAGCACGTAA
miR15	Forward	GAGGCAGCACAATATGGCCT
	Reverse	CCTTGGAGTAAAGTAGCAGCAC
TBP	Forward	TGCACAGGAGCCAAGAGTGAA
	Reverse	CACATCACAGCTCCCCACCA
C-myc	Forward	AGCAGCGACTCTGAGGAGGAAC
	Reverse	ACCAGTGGGCTGTGAGGAGGTT
Cyclin E promoter region	Forward	AGTGAGAGATGGGGTGCAAG
	Reverse	TTGGGTCGTTCATTCATTCA
non-coding region	Forward	GTGGGACAGCCAGACACCACG
	Reverse	TGCTGCAGAAACCGGGTCAGTC