Supplementary Material – Monteiro-Reis *et al*, Journal of Clinical Medicine, 2020
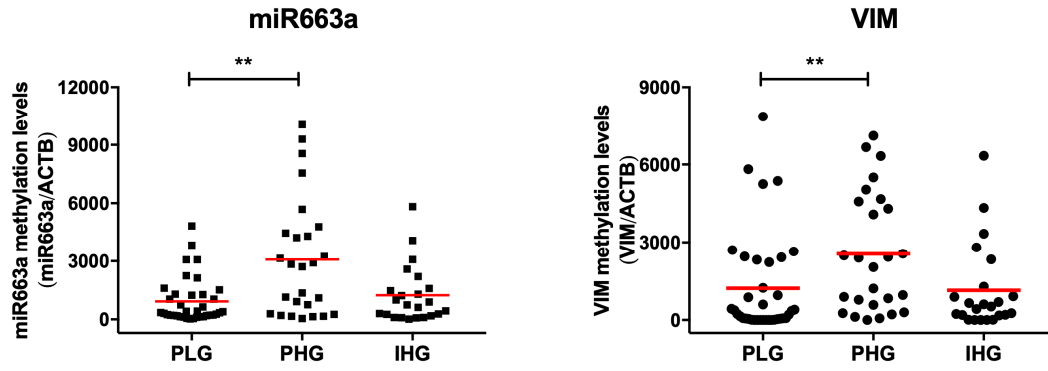
Supplementary Table S1 – Sequences of the primers and probes used in the quantitative methylation-specific PCR experiments.
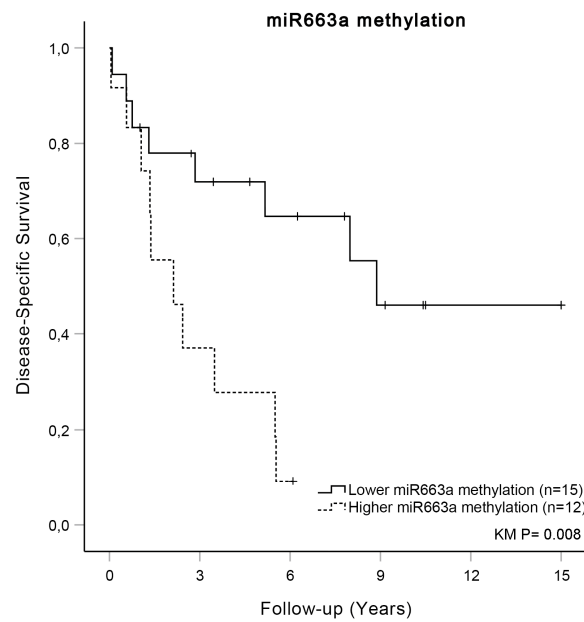
Primer Set		Sequence	Product size, bp	$T_{Annealing}$ ºC
ACTB_QMSP	**Forward Primer**	5' TGGTGATGGAGGAGGTTTAGTAAG T 3'	133	60
	Reverse Primer	5' AACCAATAAAACCTACTCCTCCCTTAA 3'		
	Probe	5' CY5 - ACCACCACCCAACACACAATAACAAACACA - MGB 3'		
miR663a_QMSP	**Forward Primer**	5' GGGATAGCGAGGTTAGGTC 3'	101	60
	Reverse Primer	5' CATTCGTAACGAATAAAACCC 3'		
	Probe	5' VIC - CTCTTCCTCGCCTACG - MGB 3'		
VIM_QMSP	**Forward Primer**	5' TTCGGGAGTTAGTTCGCGTT 3'	108	60
	Reverse Primer	5' ACCGCCGAACATCCTACGA 3'		
	Probe	5' FAM - TCGTCGTTTAGGTTATCGT - MGB 3'		

Supplementary Table S2 – Performance of *VIM*me, *miR663a*me, and *VIM*me-*miR663a*me panel for the detection of bladder cancer in Tissues and Testing Cohort (urines). (PPV – positive predictive value; NPV – negative predictive value)

Samples	Biomarker Performance	*VIM*me (%)	*miR663a*me (%)	*miR663a*me-*VIM*me (%)
Tissues	Sensitivity	94.4	76.6	96.3
	Specificity	94.1	94.1	88.2
	PPV	99.0	98.8	98.1
	NPV	72.7	39,0	78.9
	Accuracy	94.4	79.0	95.2
Testing Set (Urines)	Sensitivity	78.6	82.1	92.6
	Specificity	96.4	82.1	75.0
	PPV	97.1	87.3	80.6
	NPV	75.0	75.4	90.0
	Accuracy	85.7	82.1	84.3

Supplementary Figure S1 – Distribution of *miR663a*me and *VIM*me levels in bladder carcinoma (BlCa; n=94) tissue samples categorized by grade. Mann-Whitney U test, **P<0.01. (PLG – Papillary Low Grade; PHG - Papillary High Grade; IHG – Invasive High Grade).

Supplementary Figure S2 – Kaplan-Meyer (KM) curves representing disease-specific survival according to *miR663a*me status.