

## Supplementary

### 1 Wildtype and mutated untranslated region sequences for *SLC35F2* and *BCL9L* genes

All 5' and 3' untranslated region (UTR) sequences and the position of mutations for both *SLC35F2* and *BCL9L* were identified according to the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) assembly platform. The single point mutations are emphasized in red colour.

#### Wildtype sequence of 5' UTR *SLC35F2* gene

```
>hg38_knownGene_uc001pq.3 range=chr11:107858768-107859188 5'pad=0 3'pad=0
strand=- repeatMasking=none
actgcttctaactcactgctcactcaccgcttcttagcttaggaatcacctacaataacctcgtgtcactgtatcttgtgattcaaacaa
ggaaccctaaaacctaagggcactggtgcccgtcaacaataaggctcggctgccaagacgaaaaaaaaaattgcccttt
ctcctcctgccaccctggaggtgccagggtgtaactgcaaatgtattgtctggccttagggcttgacagttaatgattggtcgctga
aggagcaggagcaggagcagaggggacgcgagcaagcgagcagggagggagcgcagggccagagcggagcccc
agccggccgcacaggcaccgctcccgtctccgggacccggcacttcgggggtttgggcgccggagacgcgcaagggcg
ccg
```

#### Mutated sequence of 5' UTR *SLC35F2* gene

C>G mutation is at the position 107868920 according to UCSC browser, GRCh38/hg38.

```
>hg38_knownGene_uc001pq.3 range=chr11:107858768-107859188 5'pad=0 3'pad=0
strand=- repeatMasking=none
actgcttctaactcactgctcactcaccgcttcttagcttaggaatcacctacaataacctcgtgtcactgtatcttgtgattcaaacaa
ggaaccctaaaacctaagggcactggtgcccgtcaacaataaggctcggctgccaagacgaaaaaaaaaattgcccttt
ctcctcctgccaccctggaggtgccagggtgtaactgcaaatgtattgtctggccttagggcttgacagttaatgattggtcgctga
aggagcaggagcaggagcagaggggacgcgagcaagcgagcagggagggagcgcagggccagagcggagcccc
agccggccgcacaggcaccgctcccgtctccgggacccggcacttcgggggtttgggcgccggagacgcgcaagggcg
ccg
```

#### Wildtype sequence of 3' UTR *SLC35F2* gene

```
>hg38_knownGene_uc001pq.3 range=chr11:107790991-107792614 5'pad=0 3'pad=0
strand=- repeatMasking=none
ctggagaagatggcgcacccatccccaccaggataaagcagagcctgctgcctgctgaggggacacttgggaaatagca
gactagaatggacacttacaaccctgggttgatccagtggtaggttttagaaaggaacactgaacaaaagtcaaaagtag
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tgatgtggcagaaaacgggtgggtcagccctgaaaagcttgagatataagtgtgactatacaagcattaaactggagtg
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cagctccaggctcactctccaacagttttcagagctccatataatgtagatgccatcctttctaaaaacttctcacgacctcctgga
```

atattcctattgatctcattttatttagcatcagctcaagaaactaagtcttagtgacagtatcacaacaagaaaaagcttggtttta  
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 ccagctgttaaaagacaatgctattactacttctatcaaaaaactgtaatgccctgtgattttatgatacttctcaatacaaagtgtta  
 atatgtgcatcagataataacaaccaaaaaatgccacttcagaaaactgtatgtaaatttttgaacaatgtaaaaaagaaa  
 tggggagtaagtgttcacatcattaaaaggcttgaattcatggaataca

#### Mutated 1 sequence of 3' UTR *SLC35F2* gene

C>T mutation is at position 10779226 according to UCSC browser, GRCh38/hg38.

>hg38\_knownGene\_uc001pj.3 range=chr11:107790991-107792614 5'pad=0 3'pad=0  
 strand=- repeatMasking=none

ctggagaagatggcgcacccatccccaccaggataaagcagagcctgctgctgctgaggggacacttggggaatagca  
 gactgagaatggacactctacaaccctgggttgatccagtggttaggttttagaaaggaacactgaacaaagttcaaaagtag  
 aaataccaaaatagagcagaaaccaagctaagagtgtgttctgtgttgaggaccaatatctgtcgtgttgaggagatgaggtg  
 cagactccacactgggtcttagaggcaccagtgaggaaagcaggactggggagagatccaggactgagccagcctcaggc  
 cagggcgggaaagcaggctgtccaggctgggctggtggtcaggaacaggggacgctacgggtggctctggagtacgtttcct  
 tgtcagatgctcaaggttttttttttttttggctgaggcagttattgtccacaggccttgggtacagatgaggccttggggctgact  
 gcatgtggcagaaaacgggtgggtcagccctgaaaagtcttgagataaagtgtgagtactatacaagcattaaactggagtgc  
 agtaaacctaggaacctcccacaattgtttttgtttgtttgagatgcagagtctagagtgcagtggtgcaagctcagctactgta  
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 cagtcccaggctcactctccaacagtttcagagctccatataatgtagatgccatccttctaaaaacttctcacgacctcctgga  
 atattcctattgatctcattttatttagcatcagctcaagaaactaagtcttagtgacagtatcacaacaagaaaaagcttggtttta  
 taactggtaaaaacaagaaaagatttcatcaaaatgaaaatataaaattaatcatttctaccaaagagatgcctgggagcct  
 ccagctgttaaaagacaatgctattactacttctatcaaaaaactgtaatgccctgtgattttatgatacttctcaatacaaagtgtta  
 atatgtgcatcagataataacaaccaaaaaatgccacttcagaaaactgtatgtaaatttttgaacaatgtaaaaaagaaa  
 tggggagtaagtgttcacatcattaaaaggcttgaattcatggaataca

#### Mutated 2 sequence of 3' UTR *SLC35F2* gene

C>G mutation is at the position 10779529 according to UCSC browser, GRCh38/hg38.

>hg38\_knownGene\_uc001pj.3 range=chr11:107790991-107792614 5'pad=0 3'pad=0  
 strand=- repeatMasking=none

ctggagaagatggcgcacccatccccaccaggataaagcagagcctgctgctgctgaggggacacttggggaatagca  
 gactgagaatggacactctacaaccctgggttgatccagtggttaggttttagaaaggaacactgaacaaagttcaaaagtag  
 aaataccaaaatagagcagaaaccaagctaagagtgtgttctgtgttgaggaccaatatctgtcgtgttgaggagatgaggtg  
 cagactccacactgggtcttagaggcaccagtgaggaaagcaggactggggagagatccaggactgagccagcctcaggc  
 cagggcgggaaagcaggctgtccaggctgggctggtggtcaggaacaggggacgctacgggtggctctggagtacgtttcct  
 ctgtcagatgctcaaggttttttttttttttggctgaggcagttattgtccacaggccttgggtacagatgaggccttggggctgac  
 tgcagatgtggcagaaaacgggtgggtcagccctgaaaagtcttgagataaagtgtgagtactatacaagcattaaactggagtg  
 cagtaaacctaggaacctcccacaattgtttttgtttgtttgagatgcagagtctagagtgcagtggtgcaagctcagctactgta  
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 aaaacatttctccatcactcattccaggctccagagcaaaactctctgctcctcggagcctgtgacactggctatgtgtccacagttt  
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 atattcctattgatctcattttatttagcatcagctcaagaaactaagtcttagtgacagtatcacaacaagaaaaagcttggtttta  
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ccagctgttaaagacaatgctattactacttcttatcaaaaatctgtaatgccctgtgattttatgatacttctcaatacaaagtgta  
 atatgtgcatcagtataataacaaccaacaaaatgccacttcagaaaactgtatgtaaatttttgaacaatgtaaaaaagaaa  
 tggggagtaaggttcacatcattaaaaggctttgaattcatggaataca

### Mutated 3 sequence of 3' UTR *SLC35F2* gene

It contains two mutations. C>T mutation is at position 10779226 and C>G mutation is at the position 10779529 according to UCSC browser, GRCh38/hg38.

>hg38\_knownGene\_uc001pj.3 range=chr11:107790991-107792614 5'pad=0 3'pad=0  
 strand=- repeatMasking=none

ctggagaagatggcgcacccatccccaccaggataaagcagagcctgctgcctgctgaggggacacttggggaaatagca  
 gactgagaatggacacttacaacctgggttgatccagtggttaggttttagaaaggaacactgaacaaagtcaaaagtag  
 aaataccaaaatagagcagaaaccaagctaagagtggttctgtgttgaggaccaatatctgtctgttgaggagatgaggtg  
 cagactccacactgggtcttagaggcaccagtggaagcaggactggggagagatccaggactgagccagcctcaggc  
 cagggcgggaaagcaggctgtccaggctgggcttggtggtcaggaacaggggcagctacgggtgctctggagtacgttctc  
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 atatgtgcatcagtataataacaaccaacaaaatgccacttcagaaaactgtatgtaaatttttgaacaatgtaaaaaagaaa  
 tggggagtaaggttcacatcattaaaaggctttgaattcatggaataca

### Wildtype sequence of 5' UTR *BCL9L* gene

>hg38\_knownGene\_uc001pug.5 range=chr11:118909940-118910904 5'pad=0 3'pad=0  
 strand=- repeatMasking=none

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 cagcaccggggctcgaagctcttgcggctggcgcgcgccagcccctggcgggactgtccgtgtgtctgtccgcgcgcg  
 gggcctggagaagcagctgcagctcctgtccgcctcccgtcgcctcgtggtgtcgtcgtcgtcgtcctcctccgccc  
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 gagccagcgttactggtctccagaagagcccagctgcagccccggggccccgcccaggcctcgtcgtcccggggcctgc  
 tgggatgggcacgggctaggcctcagagctggggacggggcggggcgtggcccaaccggggccccctccacggctggag  
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 gggagacctcacagccgccacacggcaccttctaccttagccttttagtgaattctgtctctgcccggcctcggggcgagg  
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 cccccctcccaagtcacgagtttctcttggggctgtgtcgtcagtcctgctccagtagctacagtagtacctggtgggccccctggg  
 acgcacagggccgtagccccactgtgtgtgggagcc

Mutated sequence of 5' UTR *BCL9L* gene

It contains two mutations. G>T mutation is at the position 118909968 and A>T is mutation at the position 118910162 according to UCSC browser, GRCh38/hg38.

```
>hg38_knownGene_uc001pug.5 range=chr11:118909940-118910904 5'pad=0 3'pad=0
strand=- repeatMasking=none
cgtgctgtcttctccctgtccacgtgtgagctgtgagtggtgtgagtcagagttcgggtgtctgtgggtctctgagcctctgctgg
cagcaccggggctcgccaagctcttgcggctggcgcgcgccagcccctggcgggactgtccgtgtgtctgtccgcgcgcg
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tcgtaatcctctttccagtgccacccccaccctacagctcctagtccagggtgtgggtcctccttccccctccccctctgac
acccccctcccaagtcacgagtttcttggggctgtgtctgcagctccgtgctccagtaccgagtaacctggctgggcctgggca
cgcacagggcctgagccccactgtgtgtgggagcc
```

Wildtype sequence fragment of 3' UTR *BCL9L* gene

```
>hg38_knownGene_uc001pug.5 range=chr11:118893875-118898414 5'pad=0 3'pad=0
strand=- repeatMasking=none
gctgctgccactacttctatcaagaagggggagaacagttcatctgagtcagtgacacctcccctccagctcctcactccctgag
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```

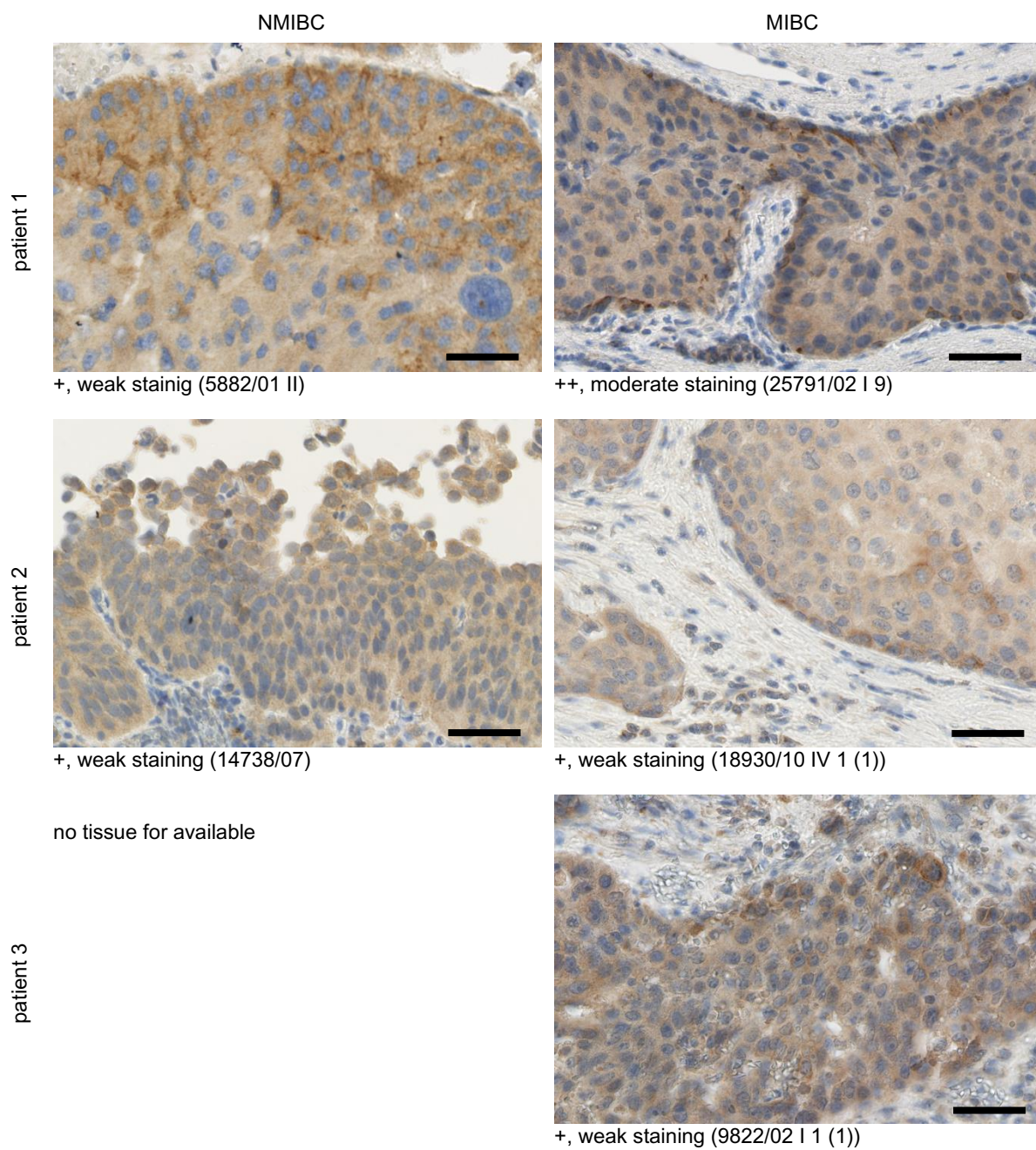
Mutated sequence fragment of 3' UTR *BCL9L* gene

A>T mutation is at the position 118896782 according to UCSC browser, GRCh38/hg38

```
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strand=- repeatMasking=none
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```

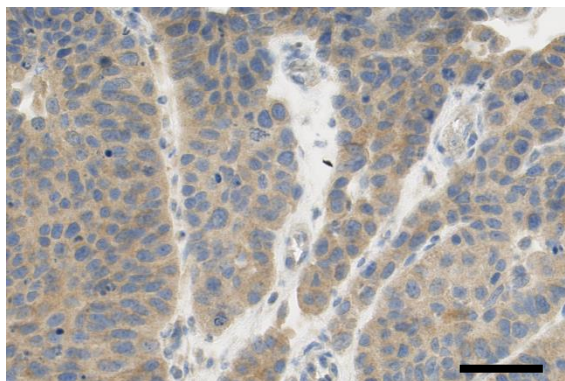
## 2 Supplementary figures

Immunohistochemical staining against SLC35F2 protein.

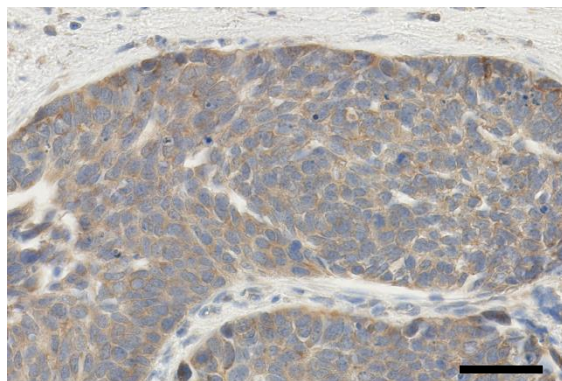




patient 4

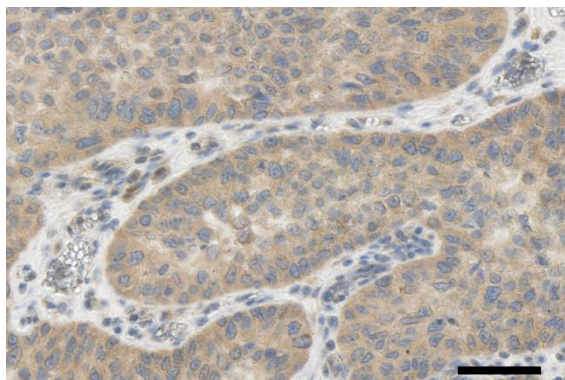


+, weak staining (15320/06 3)

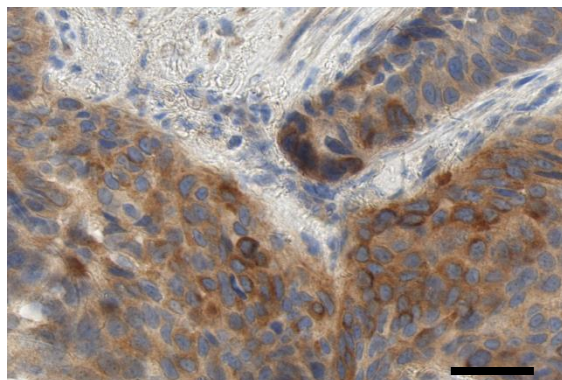


+, weak staining (524/10 II 4)

patient 5

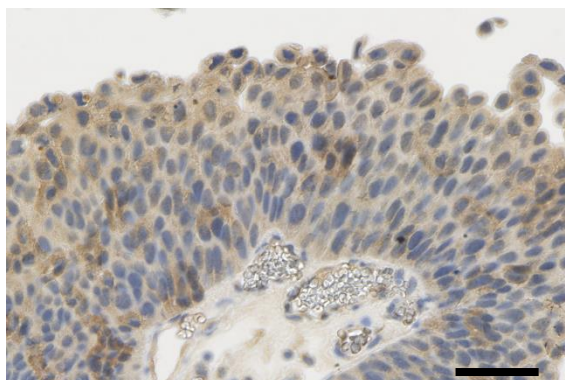


+, weak staining (22541/06 III)

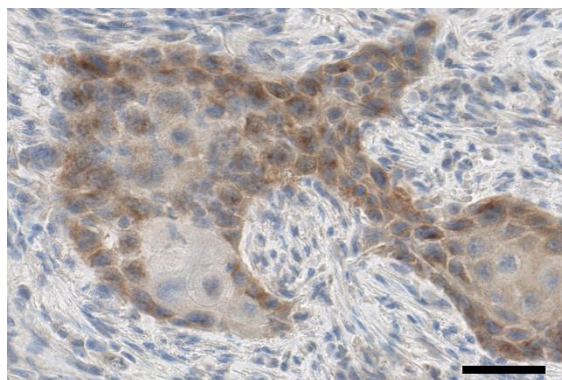


++, moderate staining (16564/09 I)

patient 6

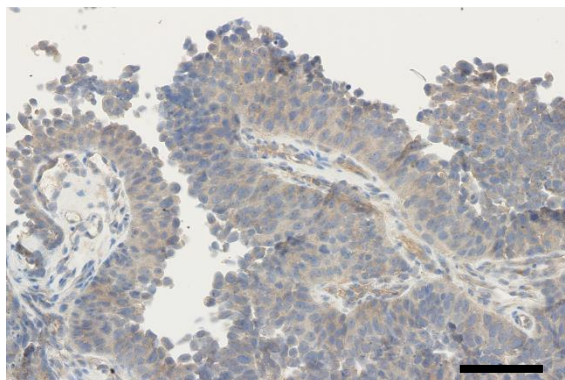


+, weak staining (33312/98 I 1)

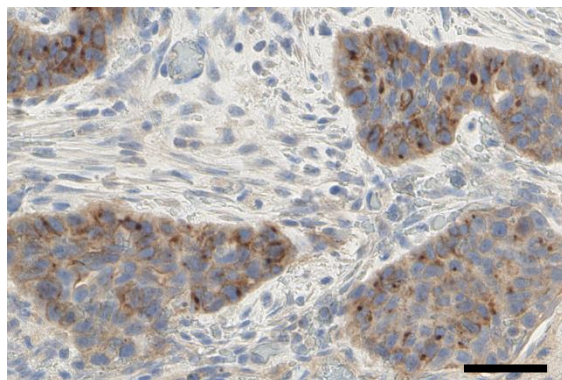


++, moderate staining (4923/05 III 5 (2))

patient 7

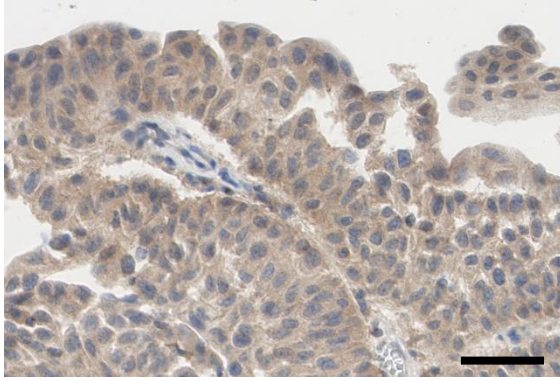


+, weak staining (6642/10 I)

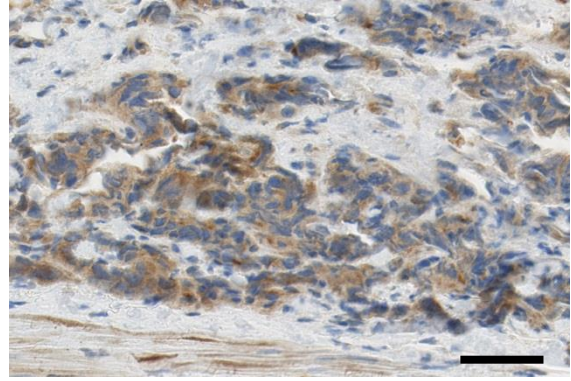


++, moderate staining (2243/14 III 6 (2))

patient 8



+, weak staining (3215/01 III)

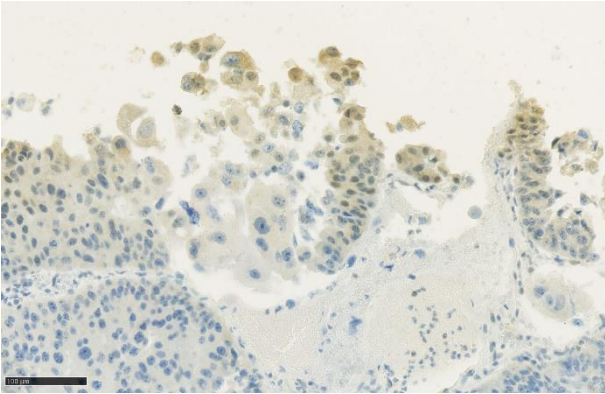
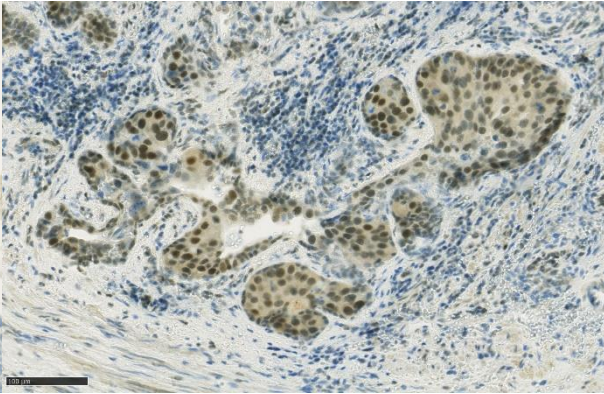
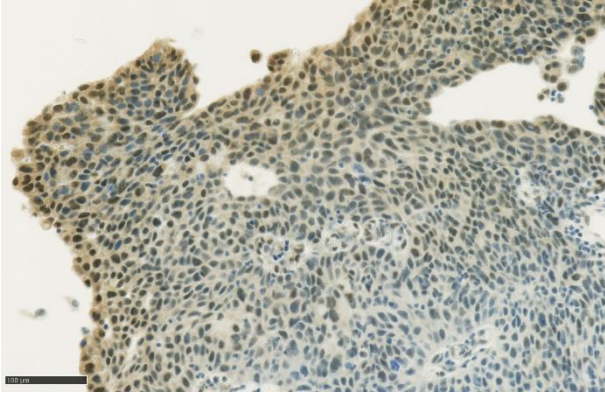
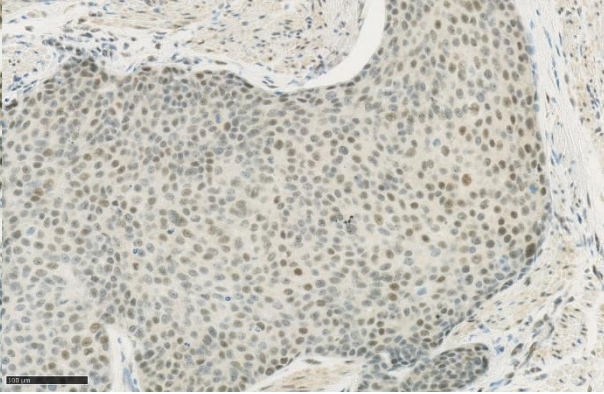
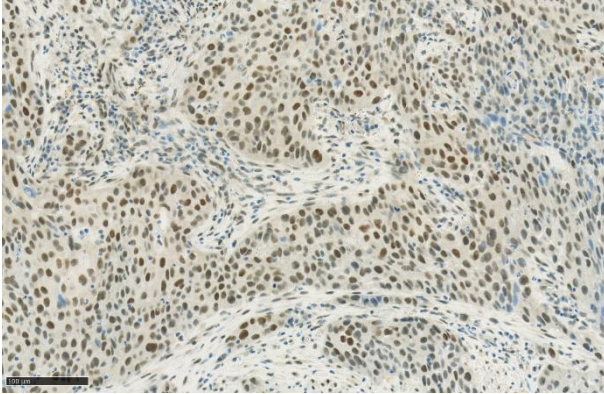


++, moderate staining (7692/06 II)

**Supplementary figure 1. Immunohistochemical staining of SLC35F2 in NMIBC and matched MIBC.** Scale bar = 50  $\mu$ m. Overall average staining intensity of the tumor cells of the whole slide is specified as negative (-), weak (+), moderate (++) and strong (+++). FFPE block number in brackets.

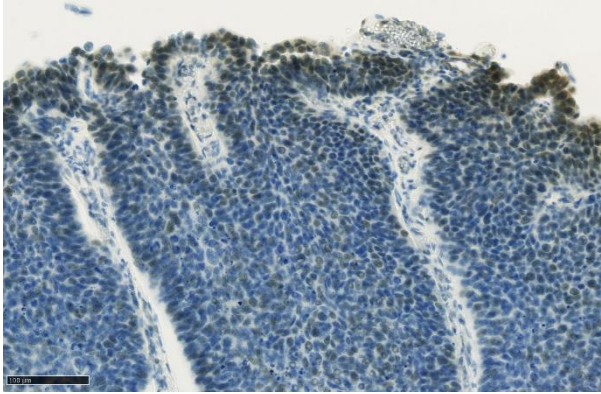


Immunohistochemical staining against BCL9L protein.

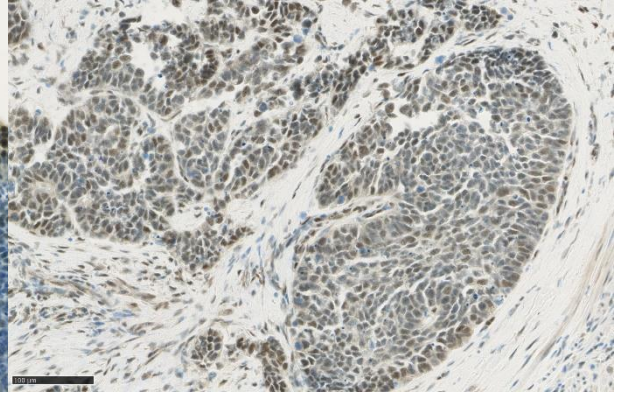
	NMIBC	MIBC
patient 1	 <p>Few peripher tumor cells are weak stained (1% +, H-Score 0.01) (5882/01 II)</p>	 <p>Homogenously weak to strong stained tumor areas (40% +, 50% ++, 10% +++, H-Score 1.7) (25791/02 I 9)</p>
patient 2	 <p>NMIBC-PT Peripheral increasing up to moderate staining (50% +, 20% ++, H-Score 0.9) (8539/06 I 1)</p>	 <p>Homogenously weak stained tumor areas (50% +, H-Score 0.5) (18930/10 IV 1 (1))</p>
patient 3	<p>no IHC for NMIBC available</p>	 <p>Heterogeneously stained tumor with some negative areas (30%, not shown). Positive areas are weak to moderate stained. (40% +, 30% ++, H-Score 1.0) (7145/02 I)</p>



patient 4

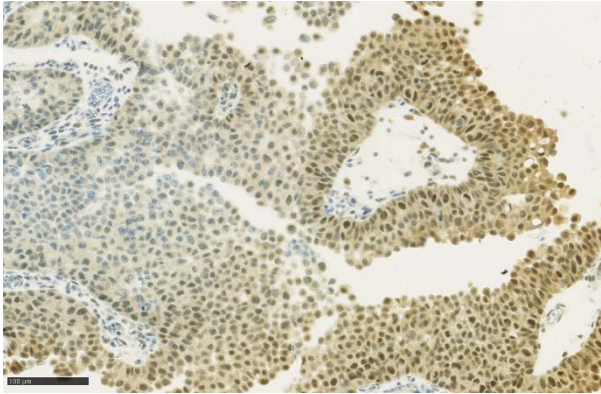


Few peripher tumor cells are weak stained (1% +, H-Score 0.01) (15320/06 3)

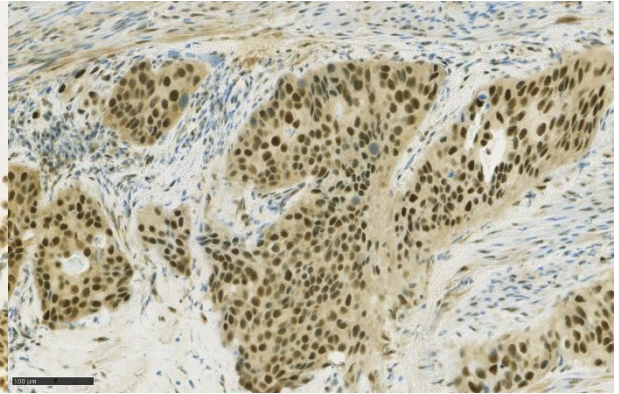


Homogenously negativ to moderate stained MIBC (50% +, 20% ++, H-Score 0.9) (524/10 II 4)

patient 5

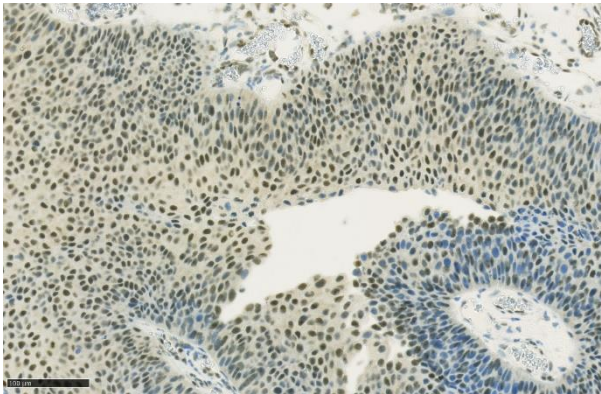


Peripheral increasing up to moderate staining (40% +, 20% ++, H-Score 1.0) (22541/06 III)

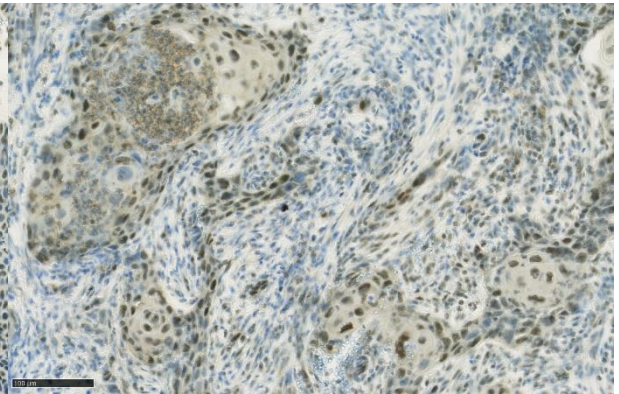


Mainly homogeneously stained tumor with few negative areas (10%, not shown). Positive areas are moderate to strong stained. (30% +, 40% ++, 20% +++, H-Score 1.7) (16564/09 II)

patient 6



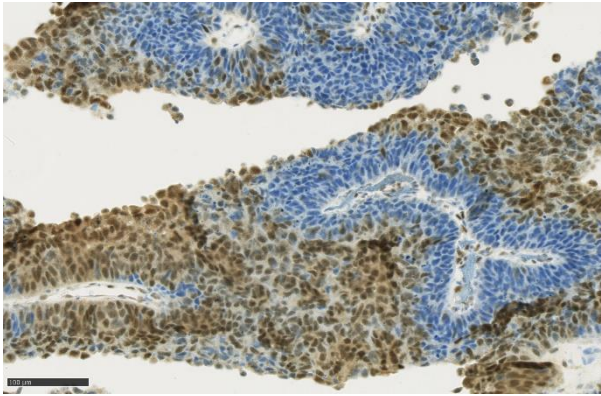
Heterogeneously stained tumor with negative, peripheral increased stained and completely positive tumor areas. (40% +, 40% ++, H-Score 1.2) (33312/98 I 1)



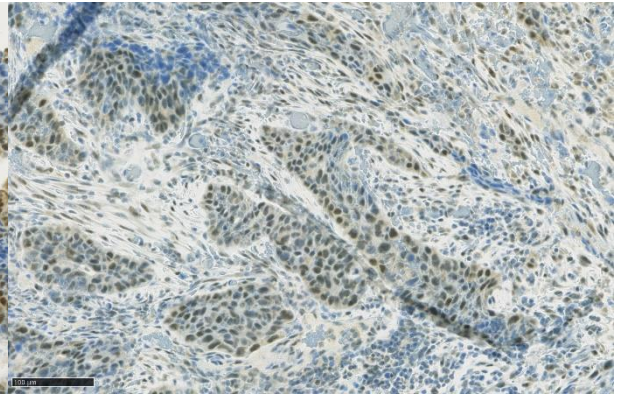
Homogeneously negative to strong stained squamous differentiated tumor. (40% +, 20% ++, 20% +++, H-Score 1.4) (4923/05 III 5 (2))



patient 7

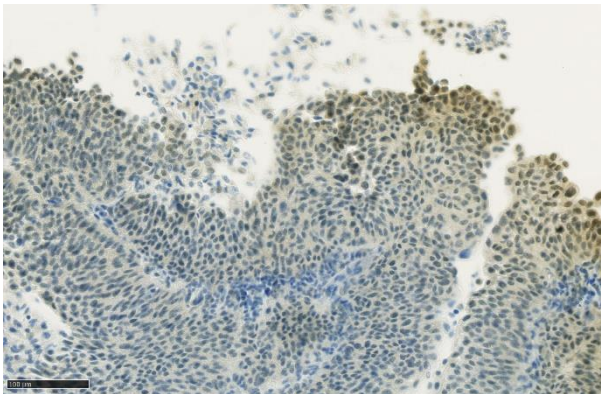


Heterogeneously stained tumor, peripheral increased stained and completely positive tumor areas. (30% +, 40% ++, H-Score 1.1) (6642/10 I)

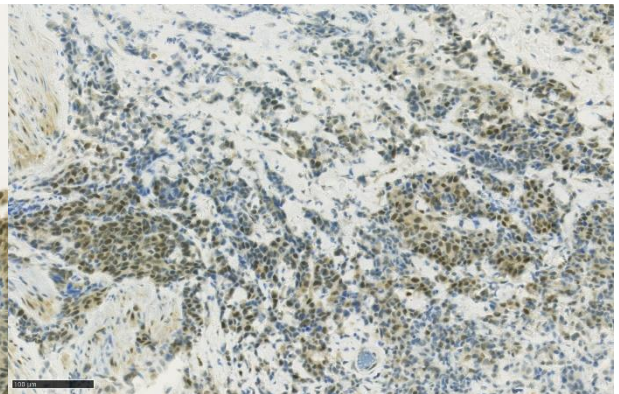


Homogeneously negativ to moderate stained MIBC (50% +, 20% ++, H-Score 0.9) (2243/14 III 6 (2))

patient 8



Only peripheral tumor cells are weak to moderate stained (10% +, 5% ++, H-Score 0.15) (3215/01 III)

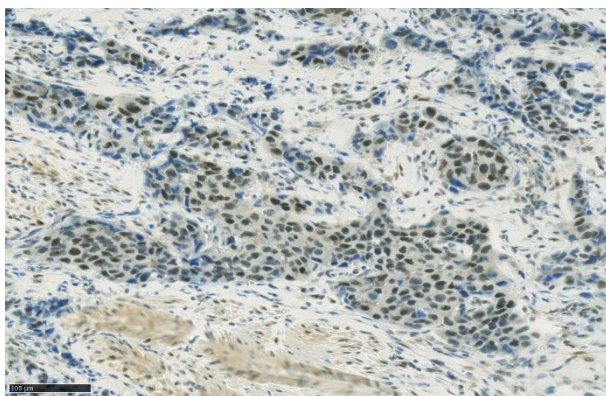


Heterogeneously stained tumor with some negative areas (20%, not shown). Positive areas are weak to moderate stained. (50% +, 30% ++, H-Score 1.1) (7692/06 III)

**Additional samples from MIBC without available NMIBC**

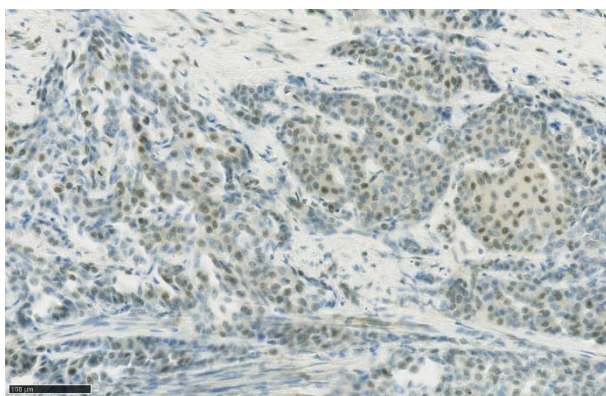
MIBC

MIBC Patient 9



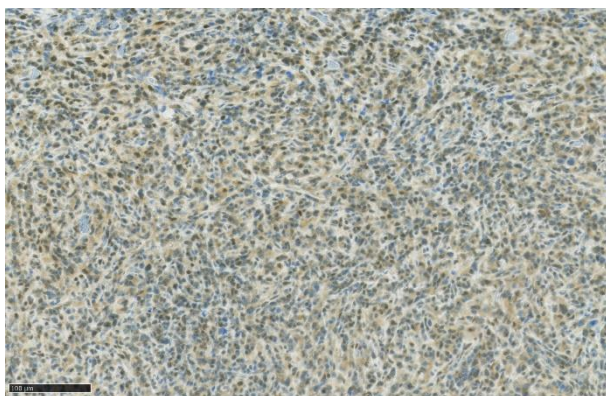
Homogeneously weak to strong stained.  
(60% +, 20% ++, 20% +++, H-Score 1.6) (9267/01 III 1 (1))

MIBC Patient 10



Homogeneously negative to weak stained.  
(50% +, H-Score 0.5) (8481/15 V 14 (2))

MIBC Patient 11



Homogeneously weak to moderate stained tumor cells.  
(60% +, 20% ++, H-Score 1.0) (16117/11 36 (2))

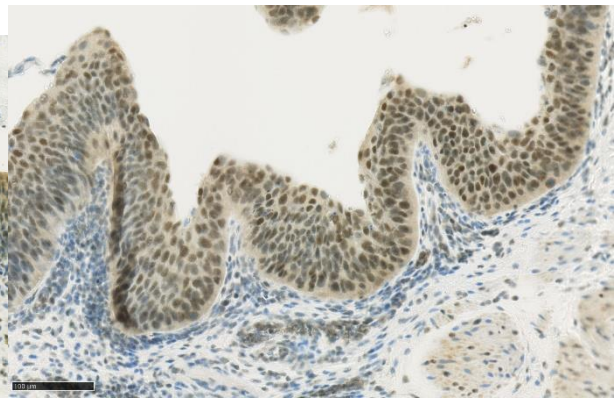
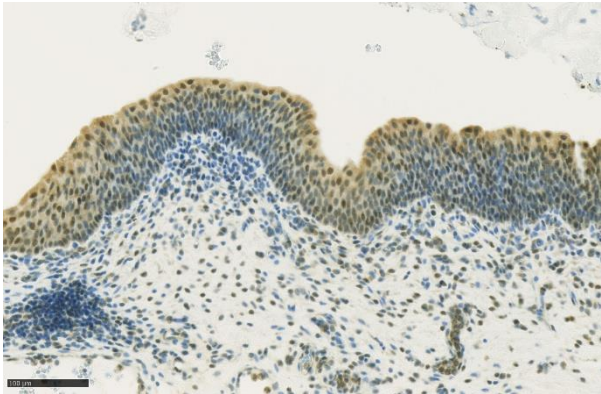


**Normal-like urothelium from tumor samples**

NMIBC

MIBC

Urothelium patient 2



Weak to moderate stained normal urothelial cells in NMIBC.  
(40% +, 20% ++, H-Score 0.8) (14738/07)

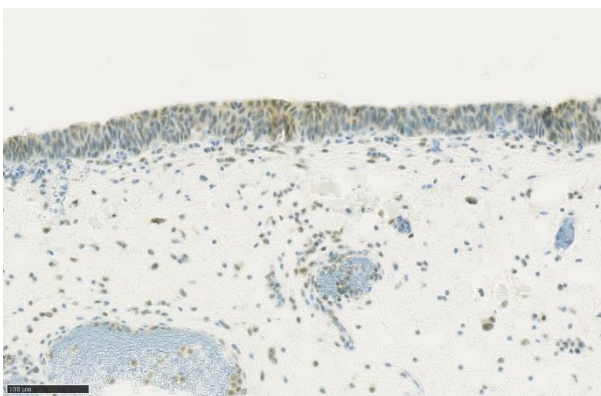
Weak to moderate stained normal urothelial cells in MIBC.  
(80% +, 10% ++, H-Score 1.0) (18930/10 IV 5)

Urothelium patient 3



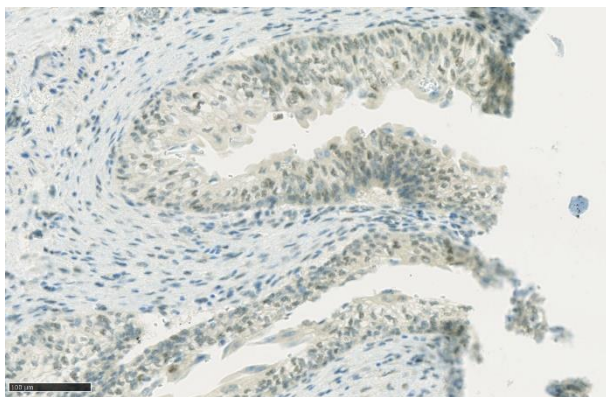
Negativ to weak stained urothelial cells.  
(60% +, H-Score 0.6) (7145/02 I)

Urothelium patient 7



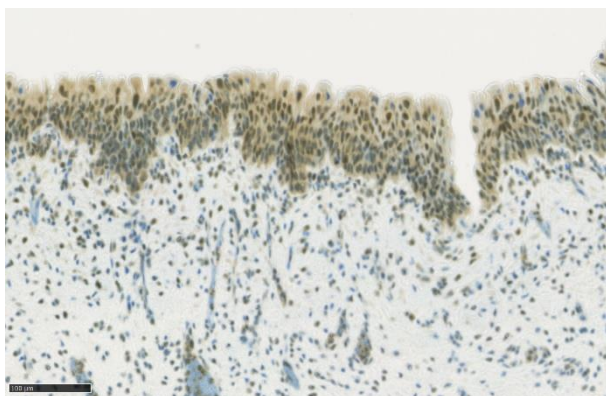
Negativ to weak stained urothelial cells.  
(30% +, H-Score 0.3) (2243/14 III 6 (2))

Urothelium patient 10



Negativ or very weak stained urothelial cells.  
(10% +, H-Score 0.1) (8481/15 V 19)

Urothelium patient 11

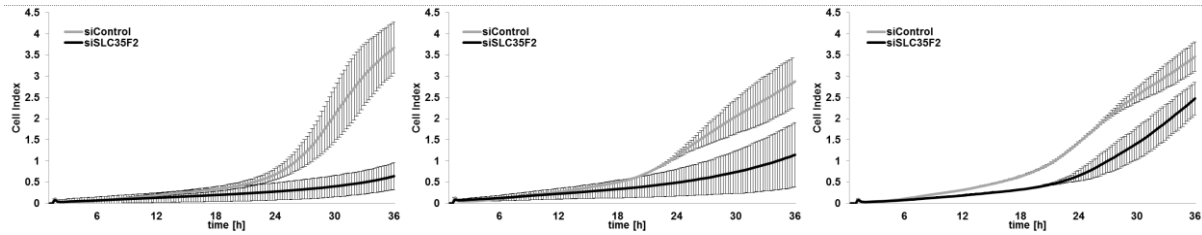


Negativ to moderate stained urothelial cells.  
(70% +, 10% ++, H-Score 0.8) (16117/11 42)

**Supplementary figure 2. Immunohistochemical staining of BCL9L in NMIBC and matched MIBC.** Scale bar = 100  $\mu$ m. Overall average staining intensity of the tumor cells of the whole slide is specified as negative (-), weak (+), moderate (++) and strong (+++). The H-Score is calculated by the formula: 3 x percentage of strong staining + 2 x percentage of moderate staining + percentage of weak staining (Ishibashi et al. 2003). FFPE block number in brackets.

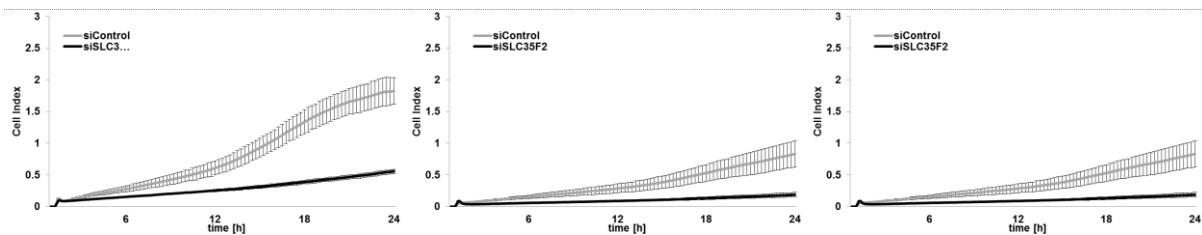
A

## Migration T24



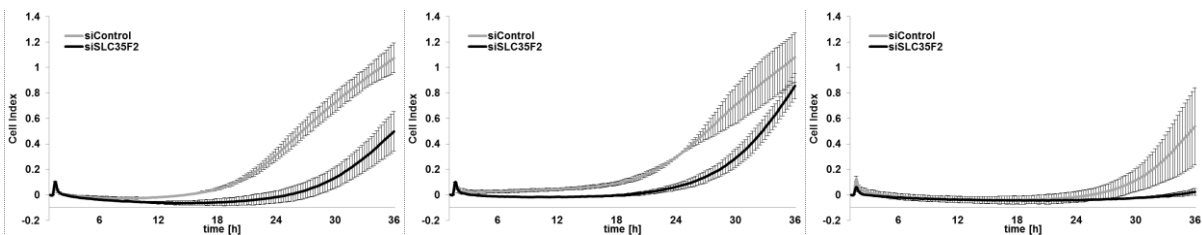
B

## Migration Cal29



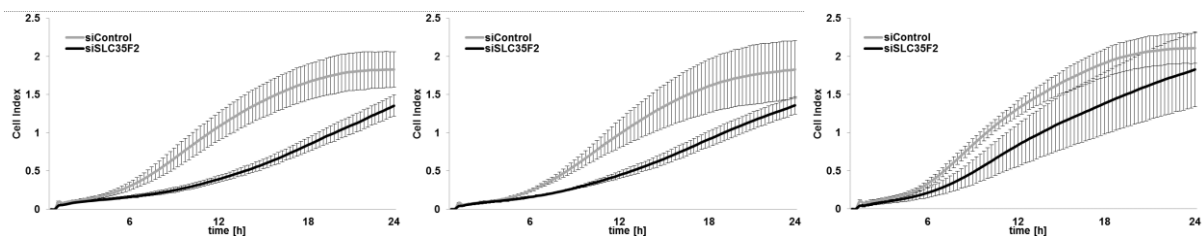
C

## Invasion T24 (1:12 diluted Matrigel)



D

## Invasion Cal29 (1:40 diluted Matrigel)

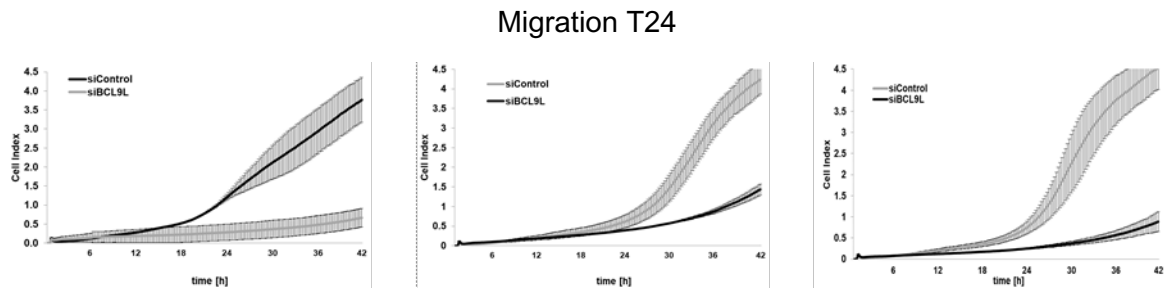


**Supplementary figure 3. siSLC35F2 dependent migration and invasion of bladder cancer cells.**

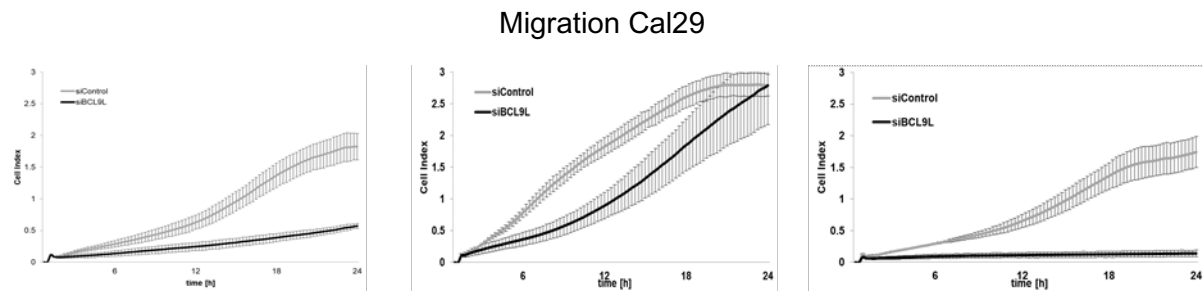
Biological triplicates of xCelligence migration and invasion assay. Migration of Cal29 (A) and T24 (B) is reduced after siRNA knockdown of SLC35F2. Invasion was analyzed using 1:12 diluted matrigel for T24 (C) and using 1:40 diluted matrigel for Cal29. The invasiveness of T24 (C) and Cal29 (D) was significantly reduced after knockdown of SLC35F2 compared to siControl. The cell index reflects the number of cells that pass the membrane between upper and lower chamber. The data are expressed as mean  $\pm$  standard deviation of two technical replicates.



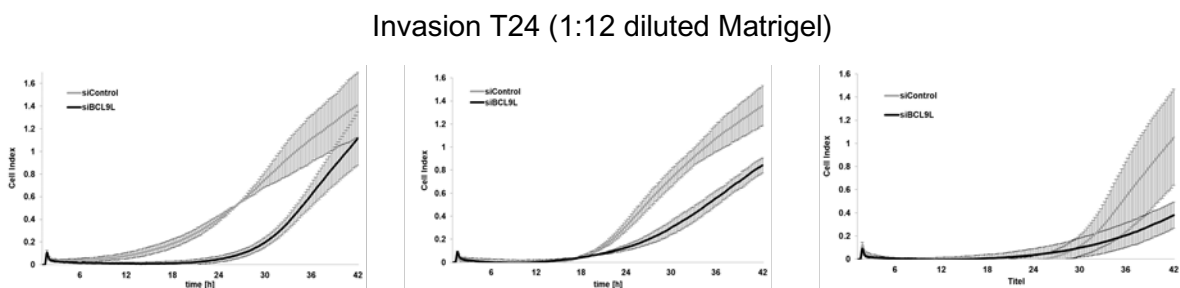
A



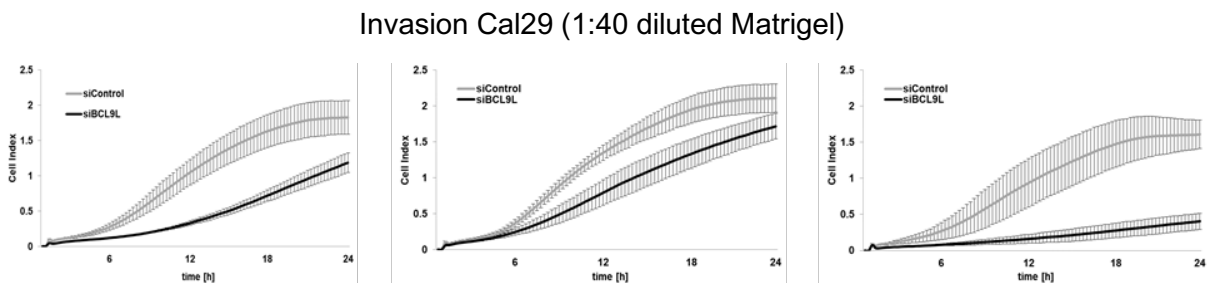
B



C



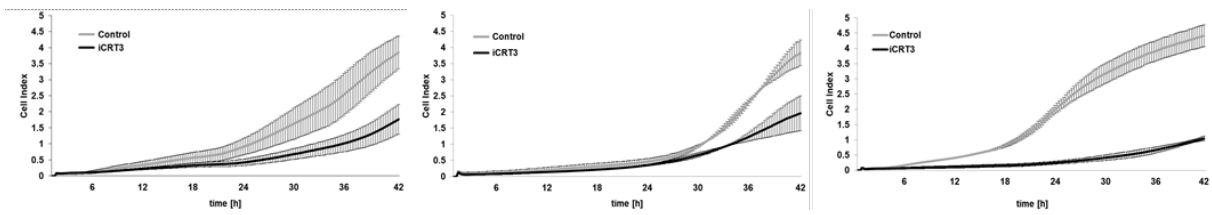
D



**Supplementary figure 4. siBCL9L dependent migration and invasion of bladder cancer cells.** Biological triplicates of xCelligence migration and invasion assay. Migration of Cal29 (A) and T24 (B) is reduced after siBCL9L transfection. Invasion was analyzed using 1:12 diluted matrigel for T24 (C) and using 1:40 diluted matrigel for Cal29. The invasiveness of T24 (C) and Cal29 (D) was significantly reduced after knockdown of BCL9L compared to siControl. The cell index reflects the number of cells that pass the membrane between upper and lower chamber. The data are expressed as mean  $\pm$  standard deviation of two technical replicates.

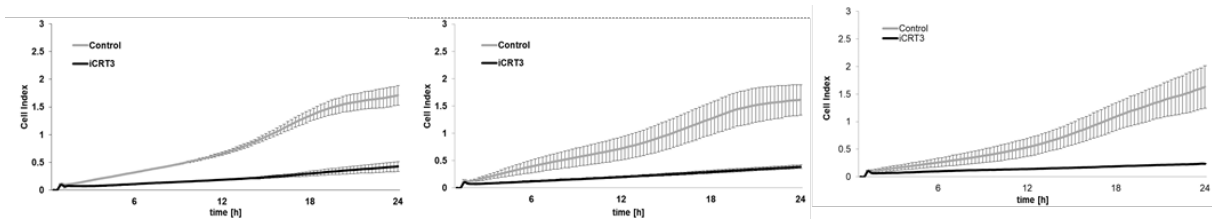
A

## Migration T24



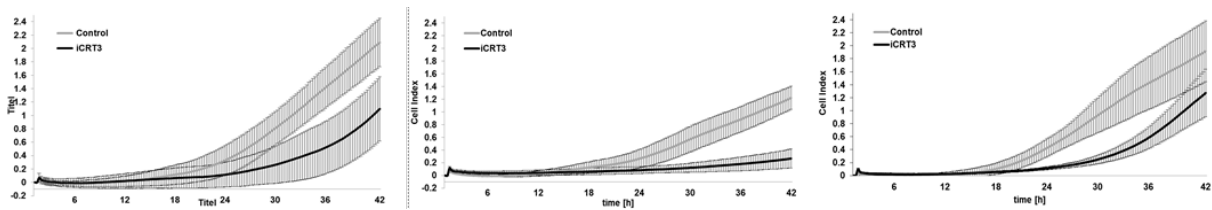
B

## Migration Cal29



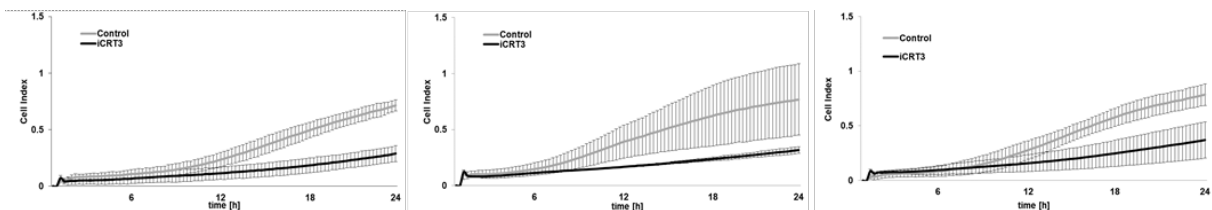
C

## Invasion T24 (1:12 diluted Matrigel)



D

## Invasion Cal29 (1:40 diluted Matrigel)



**Supplementary figure 5. iCRT3 dependent migration and invasion of bladder cancer cells.** Biological triplicates of xCelligence migration and invasion assay. Migration of Cal29 (A) and T24 (B) is reduced after 40  $\mu\text{M}$  treatment compared to DMSO control. Invasion was analyzed using 1:12 diluted matrigel for T24 (C) and using 1:40 diluted matrigel for Cal29. The invasiveness of T24 (C) and Cal29 (D) was significantly reduced after 40  $\mu\text{M}$  treatment compared to DMSO control. The cell index reflects the number of cells that pass the membrane between upper and lower chamber. The data are expressed as mean  $\pm$  standard deviation of three technical replicates.