

Strain-dependent tumor microenvironment

Table S1. List of genes (n = 75) differentially upregulated (q < 0.05) in FVB versus C57BL/6

C1rb	Gm8615	H2-Ea-ps	Il17b	Kcns1
Serpina1b	Chil1	Gm8909	Gm8580	D030025P21Rik
4933432I03Rik	Gm5801	Xlr3c	H2-Q2	Fmmpd3
AA792892	Sorcs3	Mrgprd	Serpina3k	Mup5
Madcam1	Apol11b	Trim34b	Ptpv	C1s2
Cyp4f37	4930565N06Rik	9830107B12Rik	A930015D03Rik	Zfp949
H2-Q1	C920025E04Rik	Eno1b	Gdpd3	Olfr1251
Fam196a	Mir6909	Tekt4	Tpte	C4a
Slc6a3	Zfp605	Cdh16	Gm17746	Mmel1
Gm3558	Tmod4	Mybph	H2-Q10	Ibsp
Hal	Slc24a1	Nlrp5	Dlx4	Gm10653
Slco4c1	H2-T3	Art2a-ps	Abhd1	AI506816
Pcdhb7	Tspan10	Dmp1	Vash2	Snord49b
Ppy	Mir7063	Apom	Dupd1	C330046G13Rik
Tbr1	1700008C04Rik	Scn11a	Tcstv3	Myh7b

Table S2. List of genes (n = 73) differentially downregulated (q < 0.05) in FVB versus C57BL/6

Afm	Gm14305	Mir7688	Fam47e	Mucl1
Gm14393	2310003N18Rik	Olfr1034	Rbmxl2	Gzmk
D730005E14Rik	4930481A15Rik	Tmem181b-ps	Pakap	Actl11
9530027J09Rik	4930505A04Rik	1110019D14Rik	Gm38509	Prr9
Krt33a	Nek11	Wfdc6b	Amer3	Gm16291
8030455M16Rik	Apol10a	Gpr150	Nps	4930546K05Rik
4930479D17Rik	4933427E11Rik	Gm5420	Epha10	Mir687
Sun3	Hist1h2bp	Mirlet7i	4930473A02Rik	Fgf4
Mir6970	C130060K24Rik	Tcaf3	1700125H20Rik	Gipr
Snord85	4930511A02Rik	H2-T23	Skint4	Skint7
Lrrc72	Zcchc13	Ankrd53	Gucy2d	Slc22a22
Prss52	Med9os	Cyp8b1	Sez6	4933433H22Rik
Kcnj13	Ccdc109b	Trim12a	Apoa2	Gabra3
C920006O11Rik	Cda	E130008D07Rik	Ces1f	4930412C18Rik
Cd200r3	Ttc9	Adgb		

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Table S3. Clustering of functional annotations enriched in FVB versus C57BL/6 as defined by DAVID analysis of differentially expressed genes (n = 75)

Annotation Cluster 1	Enrichment Score: 3.83	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Glycosylation site: N-linked (GlcNAc...)	25	5.70E-07	1.10E-04
UP_SEQ_FEATURE	Signal peptide	21	2.10E-05	9.90E-04
UP_KEYWORDS	Signal	25	3.00E-04	1.70E-02
UP_KEYWORDS	Glycoprotein	22	5.00E-04	1.90E-02
UP_KEYWORDS	Disulfide bond	18	2.40E-03	5.20E-02
UP_SEQ_FEATURE	Disulfide bond	15	2.40E-03	6.30E-02
Annotation Cluster 2	Enrichment Score: 3.63	Count	P_Value	Benjamini
INTERPRO	MHC classes I/II-like antigen recognition protein	6	1.20E-06	2.10E-04
KEGG_PATHWAY	Graft-versus-host disease	5	2.40E-06	1.10E-04
GOTERM_BP_DIRECT	Antigen processing and presentation of peptide antigen via MHC class I	5	2.40E-06	4.90E-04
KEGG_PATHWAY	Allograft rejection	5	3.20E-06	7.20E-05
INTERPRO	Immunoglobulin/major histocompatibility complex, conserved site	6	3.40E-06	3.00E-04
KEGG_PATHWAY	Type I diabetes mellitus	5	4.80E-06	7.20E-05
UP_KEYWORDS	MHC I	4	4.90E-06	5.60E-04
GOTERM_MF_DIRECT	Peptide antigen binding	5	6.40E-06	7.40E-04
SMART	IGc1	6	6.60E-06	2.40E-04
INTERPRO	MHC class I, alpha chain, alpha1/alpha2	5	6.70E-06	3.90E-04
INTERPRO	Immunoglobulin C1-set	6	8.00E-06	3.50E-04
KEGG_PATHWAY	Autoimmune thyroid disease	5	8.30E-06	9.30E-05
KEGG_PATHWAY	Cell adhesion molecules (CAMs)	6	9.70E-06	8.70E-05
GOTERM_CC_DIRECT	MHC class I protein complex	4	1.10E-05	7.80E-04
UP_SEQ_FEATURE	Region of interest: Alpha-3	4	1.20E-05	1.10E-03
KEGG_PATHWAY	Viral myocarditis	5	1.30E-05	9.50E-05
KEGG_PATHWAY	Antigen processing and presentation	5	1.50E-05	9.50E-05
UP_SEQ_FEATURE	Region of interest: Alpha-1	4	1.60E-05	1.00E-03
UP_SEQ_FEATURE	Region of interest: Alpha-2	4	1.60E-05	1.00E-03
INTERPRO	MHC class I-like antigen recognition	5	1.90E-05	6.50E-04
UP_SEQ_FEATURE	Domain: Ig-like C1-type	4	9.80E-05	3.70E-03
UP_SEQ_FEATURE	Region of interest: Connecting peptide	4	1.20E-04	3.60E-03
GOTERM_MF_DIRECT	TAP binding	3	2.00E-04	1.10E-02
KEGG_PATHWAY	Phagosome	5	2.80E-04	1.60E-03
GOTERM_MF_DIRECT	Beta-2-microglobulin binding	3	5.40E-04	2.10E-02
KEGG_PATHWAY	Herpes simplex infection	5	5.50E-04	2.80E-03
GOTERM_MF_DIRECT	T cell receptor binding	3	6.30E-04	1.80E-02
UP_KEYWORDS	Immunity	7	6.60E-04	1.80E-02
GOTERM_MF_DIRECT	Receptor binding	7	7.90E-04	1.80E-02
GOTERM_CC_DIRECT	Golgi medial cisterna	3	9.80E-04	1.80E-02
GOTERM_CC_DIRECT	Endoplasmic reticulum exit site	3	1.20E-03	1.80E-02
KEGG_PATHWAY	HTLV-I infection	5	1.60E-03	7.40E-03
INTERPRO	Immunoglobulin-like fold	10	2.70E-03	7.50E-02
INTERPRO	Immunoglobulin-like domain	9	3.30E-03	7.80E-02
KEGG_PATHWAY	Epstein-Barr virus infection	4	7.60E-03	3.10E-02
KEGG_PATHWAY	Viral carcinogenesis	4	9.20E-03	3.40E-02
KEGG_PATHWAY	Endocytosis	4	1.50E-02	5.20E-02
GOTERM_MF_DIRECT	Peptide binding	3	2.60E-02	4.00E-01
GOTERM_MF_DIRECT	Protein heterodimerization activity	4	1.60E-01	8.90E-01
GOTERM_BP_DIRECT	Immune response	3	1.60E-01	1.00E+00
GOTERM_CC_DIRECT	Golgi apparatus	6	2.30E-01	8.80E-01
GOTERM_CC_DIRECT	Cell surface	4	2.50E-01	8.80E-01
GOTERM_CC_DIRECT	Endoplasmic reticulum	6	3.00E-01	8.80E-01
GOTERM_MF_DIRECT	Poly(A) RNA binding	4	5.80E-01	1.00E+00

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Annotation Cluster 3		Enrichment Score: 3.48	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Signal peptide		21	2.10E-05	9.90E-04
GOTERM_CC_DIRECT	Extracellular region		15	2.00E-04	7.40E-03
GOTERM_CC_DIRECT	Extracellular space		13	6.40E-04	1.60E-02
UP_KEYWORDS	Secreted		12	4.30E-03	7.80E-02
Annotation Cluster 4		Enrichment Score: 0.93	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Transmembrane region		19	1.20E-02	2.50E-01
UP_SEQ_FEATURE	Topological domain: Extracellular		12	2.10E-02	3.50E-01
UP_SEQ_FEATURE	Topological domain: Cytoplasmic		13	4.60E-02	5.90E-01
UP_KEYWORDS	Transmembrane helix		24	1.20E-01	8.40E-01
UP_KEYWORDS	Transmembrane		24	1.30E-01	8.20E-01
GOTERM_CC_DIRECT	Integral component of membrane		23	2.30E-01	9.00E-01
UP_KEYWORDS	Membrane		27	2.50E-01	9.50E-01
GOTERM_CC_DIRECT	Plasma membrane		14	6.00E-01	9.90E-01
GOTERM_CC_DIRECT	Membrane		19	6.80E-01	1.00E+00
Annotation Cluster 5		Enrichment Score: 0.6	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Active site: Charge relay system		3	9.30E-02	7.80E-01
UP_KEYWORDS	Hydrolase		8	1.40E-01	8.20E-01
GOTERM_MF_DIRECT	Hydrolase activity		7	2.30E-01	9.50E-01
GOTERM_MF_DIRECT	Peptidase activity		3	4.10E-01	1.00E+00
UP_KEYWORDS	Protease		3	4.20E-01	9.90E-01
GOTERM_BP_DIRECT	Proteolysis		3	4.60E-01	1.00E+00
Annotation Cluster 6		Enrichment Score: 0.29	Count	P_Value	Benjamini
GOTERM_BP_DIRECT	Ion transport		3	4.60E-01	1.00E+00
UP_KEYWORDS	Ion transport		3	4.90E-01	9.90E-01
GOTERM_BP_DIRECT	Transport		6	5.40E-01	1.00E+00
UP_KEYWORDS	Transport		6	5.70E-01	1.00E+00
Annotation Cluster 7		Enrichment Score: 0.11	Count	P_Value	Benjamini
UP_KEYWORDS	Metal-binding		10	5.50E-01	1.00E+00
GOTERM_MF_DIRECT	Metal ion binding		7	9.10E-01	1.00E+00
UP_KEYWORDS	Zinc		4	9.30E-01	1.00E+00
Annotation Cluster 8		Enrichment Score: 0.05	Count	P_Value	Benjamini
GOTERM_CC_DIRECT	Intracellular		4	8.30E-01	1.00E+00
UP_KEYWORDS	Zinc		4	9.30E-01	1.00E+00
UP_KEYWORDS	Zinc-finger		3	9.30E-01	1.00E+00

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Table S4. Clustering of functional annotations enriched in C57BL/6 versus FVB as defined by DAVID analysis of differentially expressed genes (n=73)

Annotation Cluster 1	Enrichment Score: 1.17	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Signal peptide	15	3.00E-04	2.80E-02
UP_SEQ_FEATURE	Domain: Ig-like C1-type	3	1.70E-03	8.10E-02
UP_KEYWORDS	Signal	18	9.40E-03	6.10E-01
UP_SEQ_FEATURE	Topological domain: Extracellular	10	1.10E-02	3.10E-01
UP_SEQ_FEATURE	Transmembrane region	14	2.00E-02	3.30E-01
UP_SEQ_FEATURE	Disulfide bond	10	2.20E-02	3.00E-01
UP_KEYWORDS	Disulfide bond	12	5.80E-02	9.50E-01
GOTERM_CC_DIRECT	Integral component of membrane	19	9.90E-02	1.00E+00
UP_SEQ_FEATURE	Topological domain: Cytoplasmic	9	1.10E-01	8.00E-01
UP_SEQ_FEATURE	Glycosylation site: N-linked (GlcNAc...)	10	1.40E-01	8.50E-01
UP_KEYWORDS	Transmembrane helix	19	1.90E-01	1.00E+00
UP_KEYWORDS	Transmembrane	19	1.90E-01	1.00E+00
UP_KEYWORDS	Alternative splicing	14	2.00E-01	9.70E-01
INTERPRO	Immunoglobulin-like domain	4	2.80E-01	1.00E+00
INTERPRO	Immunoglobulin-like fold	4	3.80E-01	1.00E+00
GOTERM_CC_DIRECT	Membrane	16	4.10E-01	1.00E+00
UP_SEQ_FEATURE	Splice variant	10	4.20E-01	1.00E+00
UP_KEYWORDS	Glycoprotein	10	4.20E-01	1.00E+00
UP_KEYWORDS	Membrane	20	4.80E-01	1.00E+00
Annotation Cluster 2	Enrichment Score: 0.99	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Domain: Ig-like V-type	3	1.50E-02	3.00E-01
UP_KEYWORDS	Immunoglobulin domain	3	2.70E-01	9.90E-01
INTERPRO	Immunoglobulin-like domain	4	2.80E-01	1.00E+00
Annotation Cluster 3	Enrichment Score: 0.71	Count	P_Value	Benjamini
UP_SEQ_FEATURE	Topological domain: Extracellular	10	1.10E-02	3.10E-01
GOTERM_CC_DIRECT	Plasma membrane	9	7.90E-01	1.00E+00
UP_KEYWORDS	Cell membrane	7	8.30E-01	1.00E+00
Annotation Cluster 4	Enrichment Score: 0.38	Count	P_Value	Benjamini
GOTERM_BP_DIRECT	Ion transport	3	3.00E-01	1.00E+00
UP_KEYWORDS	Ion transport	3	3.80E-01	1.00E+00
GOTERM_BP_DIRECT	Transport	5	4.50E-01	1.00E+00
UP_KEYWORDS	Transport	5	5.80E-01	1.00E+00
Annotation Cluster 5	Enrichment Score: 0.3	Count	P_Value	Benjamini
INTERPRO	Protein kinase, catalytic domain	3	2.70E-01	1.00E+00
INTERPRO	Protein kinase-like domain	3	3.00E-01	1.00E+00
GOTERM_MF_DIRECT	Protein kinase activity	3	3.10E-01	1.00E+00
GOTERM_MF_DIRECT	Nucleotide binding	4	7.90E-01	1.00E+00
GOTERM_MF_DIRECT	ATP binding	3	8.40E-01	1.00E+00
UP_KEYWORDS	Nucleotide-binding	3	8.90E-01	1.00E+00
Annotation Cluster 6	Enrichment Score: 0.14	Count	P_Value	Benjamini
GOTERM_CC_DIRECT	Intracellular	5	3.90E-01	1.00E+00
UP_KEYWORDS	Zinc	4	8.30E-01	1.00E+00
UP_KEYWORDS	Zinc-finger	3	8.50E-01	1.00E+00
UP_KEYWORDS	Metal-binding	6	8.60E-01	1.00E+00
GOTERM_MF_DIRECT	Metal ion binding	6	8.70E-01	1.00E+00

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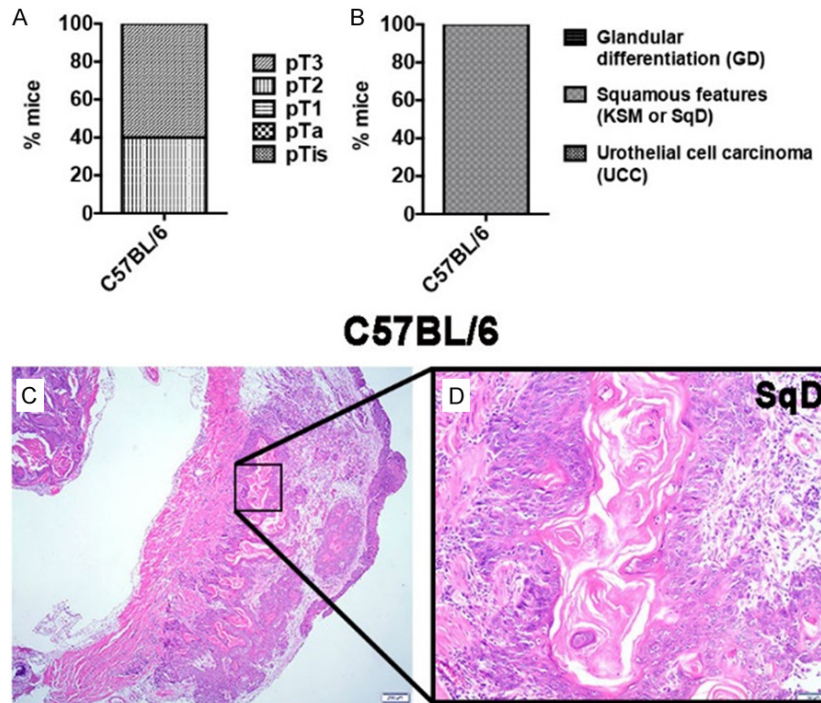


Figure S1. C57BL/6 Mice Develop Advanced Bladder Cancer with Squamous Morphologic Variants upon 20 Weeks of BBN. A. Quantification of percentage of C57BL/6 (n = 5) mice at different tumor stages pT2 (n = 2) and pT3 (n = 3) at 20 weeks of BBN exposure. B. Percentage of C57BL/6 (n = 5) mice per morphologic variant at 20 weeks of BBN exposure: all mice developed squamous elements. C. H&E of C57BL/6 bladders showing invasive UCC admixed with SqD in the presence of intracellular bridging and keratin deposition. D. Inset of C showing squamous differentiation.

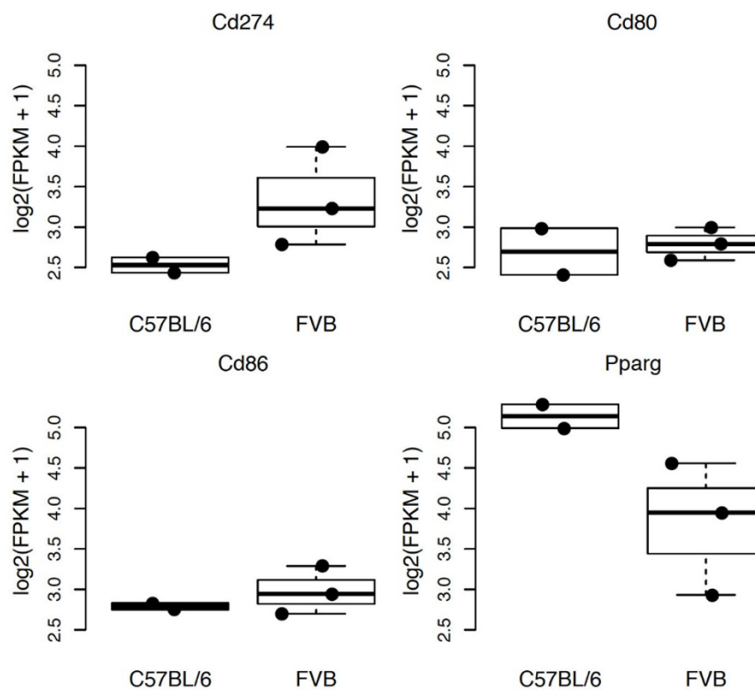


Figure S2. BBN-Induced Bladder Tumors in FVB Mice Exhibit an Increased Level of Transcripts of Cd274 (Pdl-1). Boxplot analysis of absolute expression of transcript levels of Cd274/Pdl-1, Cd80, Cd86 and Pparg in C57BL/6 and FVB bladders exposed to BBN for 12 weeks. Each dot represents one animal.

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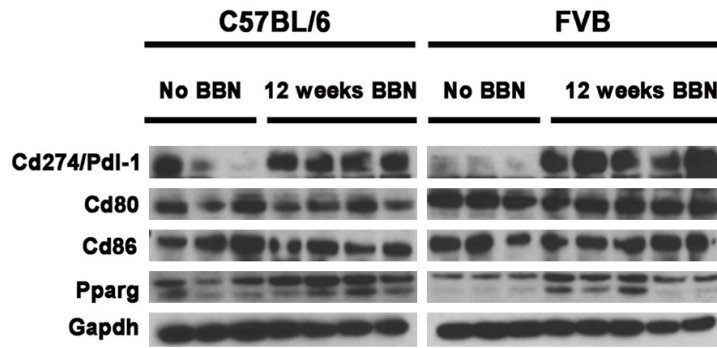


Figure S3. BBN Induces Inflammatory Response in C57BL/6 and FVB Mice. Western blot of the immune checkpoints (Cd274/PdL-1, Cd80 and Cd86), Pparg, and Gapdh loading control in normal urothelium (no BBN) versus tumors (12 weeks BBN) in C57BL/6 and FVB mice. Each lane represents one bladder: C57BL/6 no BBN (n = 3) and 12 weeks BBN (n = 4); and FVB no BBN (n = 3) and 12 weeks BBN (n = 5).