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Supplemental Material

Arsenic Metabolism in Mice Carrying a *BORCS7/AS3MT* Locus Humanized by Syntenic Replacement

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Table of Contents

Table S1. Haplotype for selected single nucleotide polymorphisms (SNPs) of human *BORCS7-AS3MT* segment included in the humanized locus of Hs/Hs mice.

Table S2. Predesigned primers used in PCR reactions during the assembly of *Borcs5/As3mt* displacer and gene expression analyses.

Table S3. The pFloxxerX plasmid used during the assembly of *Borcs5/As3mt* displacer to derive the vector backbone carrying a ColE1 origin of replication and a spectinomycin gene.

Table S4. The pSfiI-JT15-neoJTZ17-SfiI vector used during the assembly of *Borcs5/As3mt* displacer as a source of the neomycin resistance gene.

Figure S1. Comparison of *AS3MT* and *As3mt* expression levels in adrenals and liver of the humanized (Hs/Hs) and wild type (WT/WT) mice and the WT/Hs heterozygotes: **A.** Relative expression of the human *AS3MT*; **B.** Relative expression of the mouse *As3mt*. 2 µg RNA was reverse transcribed and quantitative PCR was run using *AS3MT* (Hs00960526_q1), *As3mt* (Mm00491075_m1), and 18S gene expression assays (Applied Biosystems) and qPCRBIO Probe Blue Mix (low ROX, Genesee Scientific). For each tissue, expression level in the mice homozygous for the gene was assigned a value of 1 (Mean +SE, N=6 for adrenals, N=3 for liver).

Figure S2. Expression of *BORCS7-AS3MT* read-through transcripts in tissues of the humanized (Hs/Hs) and wild type (WT/WT) mice and the WT/Hs heterozygotes. **A.** Read-through transcript expression in testes, adrenal glands, and liver of Hs/Hs mice (N=1/tissue). **B.** Separation of cDNA from testes of WT/WT, WT/Hs and Hs/Hs mice on a 1.6% agarose gel (N=1/genotype): (a) 100 bp fragment, consistent with the previously described read-through transcript; (b) 220 bp fragment of a read-through transcript that has not been previously reported. **C.** Schematic structures of the unspliced *BORCS7* isoforms (lavender), *AS3MT* (blue) and the previously reported (orange) (Lu X et al. 2015) and novel (red) *BORCS7-AS3MT* read-through transcripts. Introns are indicated by thin lines, untranslated exonic sequence by medium lines, and exonic coding sequence by thick lines. The position of the stop codon is indicated for the top two *BORCS7* transcripts. The PCR used for quantification and sequencing of the *BORCS7-AS3MT* junction of the read-through transcripts is indicated at the bottom of the figure (green).

Figure S3. Concentrations of inorganic arsenic (iAs), methyl-arsenic (MAs) and dimethyl-arsenic (DMAs) ($\mu\text{g As/L}$) in urine of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs ($20 \mu\text{g As/kg}$ body weight). Mean (x), median (—), 25th and 75th percentiles (box), maximum and minimum (whiskers), and individual values including outliers are shown (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). MAs concentration was below limit of detection in 52 out of 54 urine samples collected from WT/WT mice during the 3 collection intervals; a value of $0 \mu\text{g As/L}$ was imputed for MAs concentrations in these samples. ^{a,b,c} Within each panel, statistically significant differences between strains and sexes are marked with different letters. (ANOVA with Student-Newman-Keuls post-test.).

Figure S4. Proportions of total arsenic (%tAs) represented by inorganic arsenic (%iAs), methyl-arsenic (%MAs) and dimethyl-arsenic (%DMAs) in urine of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs ($20 \mu\text{g As/kg}$ body weight). Mean +SD (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). MAs concentration was below limit of detection in 52 out of 54 urine samples collected from WT/WT mice during the 3 collection intervals; a value of $0 \mu\text{g As/L}$ was imputed for MAs. Within each panel, statistically significant differences between strains and sexes are marked with different letters: ^{a,b,c} for differences in %iAs, ^{d,e,f} for differences in %MAs, g,hi for differences in %DMAs. (ANOVA with Student-Newman-Keuls post-test.).

Figure S5. Concentrations of inorganic arsenic (iAs), methyl-arsenic (MAs) and dimethyl-arsenic (DMAs) ($\mu\text{g As/L}$) in feces of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs ($20 \mu\text{g As/kg}$ body weight). Mean (x), median (—), 25th and 75th percentiles (box), maximum and minimum (whiskers), and individual values including outliers are shown (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). DMAs concentration was below limit of detection in 48 out of 54 fecal samples collected during the 3 collection intervals from male and female Hs/Hs mice; a value of $0 \mu\text{g As/kg}$ was imputed for DMAs concentrations in these samples. ^{a,b} Within each panel, statistically significant differences between strains and sexes are marked with different letters. (ANOVA with Student-Newman-Keuls post-test.).

Figure S6. Proportions of total arsenic (%tAs) represented by inorganic arsenic (%iAs), methyl-arsenic (%MAs) and dimethyl-arsenic (%DMAs) in feces of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs (20 $\mu\text{g As/kg}$ body weight). Mean \pm SD (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). DMAs concentration was below limit of detection in 48 out of 54 fecal samples collected during the 3 collection intervals from male and female Hs/Hs mice; a value of 0 $\mu\text{g As/kg}$ was imputed for DMAs concentrations in these samples. Within each panel, statistically significant differences between strains and sexes are marked with different letters: ^{a,b,c} for differences in %iAs, ^{d,e,f,g} for differences in %MAs, ^{h,i,j} for differences in %DMAs. (ANOVA with Student-Newman-Keuls post-test.).

Figure S7. Proportions of total arsenic (%tAs) represented by inorganic arsenic (%iAs), methyl-arsenic (%MAs) and dimethyl-arsenic (%DMAs) in urine of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 4-week exposure to iAs in drinking water (400 $\mu\text{g As/L}$). Mean \pm SD (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). MAs concentration was below LOD in 70 out of 72 urine samples collected from WT/WT mice during the 4 collection intervals; a value of 0 $\mu\text{g As/L}$ was imputed for MAs concentrations in these samples. Within each panel, statistically significant differences between strains and sexes are marked with different letters: ^{a,b,c} %iAs, ^{d,e,f} %MAs, ^{g,h} %DMAs. (ANOVA with Student-Newman-Keuls post-test.).

Figure S8. Concentrations of total arsenic ($\mu\text{g As/kg}$) in livers and kidneys of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice after 4-week exposure to iAs in drinking water (400 $\mu\text{g As/L}$). Total arsenic was calculated as sum of inorganic arsenic, methyl-arsenic and dimethyl-arsenic. Mean (\bar{x}), median (—), 25th and 75th percentiles (box), maximum and minimum (whiskers), and individual values including outliers are shown (Mean \pm SD; N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). DMAs was below LOD in 16 out of 18 liver samples collected from Hs/Hs mice; a value of 0 $\mu\text{g As/kg}$ was imputed for DMAs concentrations in these samples. ^{a,b,c} Within each panel, statistically significant differences between strains and sexes are marked with different letters. (ANOVA with Student-Newman-Keuls post-test.).

References