Supplemental Material

Temporal Modulation of Differential Alternative Splicing in HaCaT Human Keratinocyte Cell Line Chronically Exposed to Arsenic for up to 28 Wk

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Additional File- Excel Document

Table S1. Comparison of STR profile of HaCaT cell line used in this study with Cellosaurus database*

Marker	Allele(s)	Cellosaurus HaCaT (CVCL_0038)
D3S1358	16	16
TH01	9.3	9.3
D21S11	28, 30.2	28, 30.2
D18S51	12	12
Penta E	7, 12	7, 12
D5S818	12	12
D13S317	10, 12	10, 12
D7S820	9, 11	9, 11
D16S539	9, 12	9, 12
CSF1PO	9, 11	9, 11
Penta D	11, 13	11, 13
vWA	16, 17	16, 17
D8S1179	14	14
TPOX	11, 12	11, 12
FGA	24	24
AMEL	X	X

*No mouse DNA was detected in any of the HaCaT samples.

Table S2. Primers and cycling conditions

Genes/Vectors	Sequence of forward and reverse primers	Annealing temperature, cycles
XRRA1	F: 5'-GGTTGTGTTTTCATCTTACCC-3' R: 5' CCACCAGAGGGTTGTTAT 3'	52°C, 36
SHC1	F: 5'-TAACCTAAGCTGGCAGTGGC-3' R: 5'-GAGTCCGGGTGTTGAAGTCC-3'	58°C, 36
SHC1 Nested	F: AGACTCCATGAGGCCCTGACGGAGT R: TTGACTGGAGGACCTCCACACAACCCATGTACT	68°C, 30
ELK4	F: 5'-ACTTGTGGAATGAGAGAAC-3' R: 5'-GGGACTTGCTTTAGTGTAA-3'	48°C, 36
TA cloning kit pCR vector	F: 5'-GTAAAACGACGGCCAG-3' R: 5'-CAGGAAACAGCTATGAC-3'	55°C, 30
PCR cloning kit pMiniT2.0 vector	F: 5'-ACCTGCCAACCAAAGCGAGAAC-3' R: 5'-TCAGGGTTATTGTCTCATGAGCG-3'	55°C, 30

Table S3. Characteristics of skipped exon events selected for RT-PCR validation

Gene	XRRA1	SHC1	ELK4
Included Isoform	XRRA1-202	Unannotated	Unannotated
Skipped Isoform	XRRA1-201	Isoform X12	Unannotated
Mean $\Delta\psi$	-0.382	0.345	0.621

XRRA1 annotation based on Ensembl, SHC1 annotation based on NCBI Ref Seq.

Table S4. List of antibodies and conditions used for immunoblotting

Molecule Probed	Gel %	Protein/lane (μg)	Primary Antibody Cat#; Supplier	Dilution	Incubation Conditions	Secondary Antibody (Cat#; Supplier)	Dilution	Incubation Conditions
SHC1	10	40	MAB7129; R&D Systems Minneapolis, MN	1:500	Overnight; 4 °C	Horse anti-mouse (7076; Cell Signaling Technology, Danvers, MA)	1:20000	1 h; Room temperature
XRRA1	7.5	20	ab102681; Abcam, Cambridge, MA	1:1000	Overnight; 4 °C	Goat anti-rabbit (7074; Cell Signaling Technology, Danvers, MA)	1:1000	1 h; Room temperature
XRRA1	7.5	20	ab235784; Abcam	1:1000	Overnight; 4 °C	Goat anti-rabbit (7074; Cell Signaling Technology, Danvers, MA)	1:1000	1 h; Room temperature
ELK4	15	10	14666-1-AP; Proteintech	1:500	Overnight; 4 °C	Goat anti-rabbit (7074; Cell Signaling Technology, Danvers, MA)	1:1000	1 h; Room temperature
Vinculin	10	40	13901; Cell Signaling Technology, Danvers, MA	1:1000	Overnight; 4 °C	Goat anti-rabbit (7074; Cell Signaling Technology, Danvers, MA)	1:1000	1 h; Room temperature
β-Actin	15	10	A5441; Millipore-Sigma, St. Louis, MO	1:5000	Overnight; 4 °C	Horse anti-mouse (7076; Cell Signaling Technology, Danvers, MA)	1:20000	1 h; Room temperature

Table S5. Differential alternative splicing events stratified by subtypes at different time points*

Splicing Category	7 weeks N (%)	19 weeks N (%)	28 weeks N (%)
Skipped Exon	1149 (65)	311 (50)	345 (37)
Mutually Excluded Exon	136 (8)	199 (32)	378 (41)
Alternative 5' Splice Site	99 (6)	43 (7)	43 (5)
Alternative 3' Splice Site	149 (8)	27 (4)	56 (6)
Retained Intron	246 (14)	46 (7)	105 (11)
Total	1779	626	927

*The subtype percentages (in parenthesis) presented in this table correspond to Figure 2B.

Table S6. Differential alternative splicing events stratified by locations in the gene at different time points*

Gene with Splicing Events	7 weeks N (%)	19 weeks N (%)	28 weeks N (%)
ORF Only	774 (61.1)	291 (56.1)	384 (54.5)
UTR Only	389 (30.7)	207 (39.9)	297 (42.2)
Both ORF and UTR	103 (8.2)	21 (4.0)	23 (3.3)
Total	1267	506	726

*The percentage of genes (in parenthesis) with events in ORF, UTR or both presented in this table correspond to Figure 2D.

Table S7. Observed $\Delta\psi$ values for the skipped exon events selected for RT-PCR validation*

Gene	Control	As^{3+} Treated
XRRA1	30.9±1.8	62.6±24.1
SHC1	17.6±10.0	5.1±3.4
ELK4	20.8±2.0	11.3±6.8

*The values presented in this table correspond to the densitometric analysis of RT-PCR events in Figure 3C.

Table S8. Sequence of predicted isoforms identified for XRRA1, SHC1 and ELK4 alternative splicing events

|	
XRRA1 included	GGTTGTGTTTTCATCTTACCCTGGATTTTCAACCTCAGAGACAACCAAGATATGTTCACTTCCTC CCATATTCGAGATCCTTCCTGTGAAGTCACTGAAGGCCAGGAACCAGACGCTGGCCCCACCCTTC CCAGAGCTGAGATACCTTAGCCTGGCCTACAACAAGATCGCAAAAGAGGATGCTGTCCTACCAGT AGCTCTCTTCCCATCTCTCTGCGAGTTCGTCTTCCATAACA
XRRA1 skipped	GGTTGTGTTTTCATCTTACCCTGGATTTTCAACCTCAGAGATCGCAAAAGAGGATGCTGTCCTAC CAGTAGCTCTCTTCCCATCTCTCTGCGAGTTCGTCTTTCATAACAACCCTCTGGTGG
SHC1 included	TTGACTGGAGGACCTCCACACAACCCATGTACTGAGGTCAGGAGTTCGAGGCTAGCCTAGCCAAC ATGGGGAAACCCCGTCTCTACTAAAAATACAAAAATTAGCCGGGCGTGGTGGTGGGCGCCTGTAA TCCCAGCTACTCAGGAGGCTGAGGCAGGAGAATCACTTGAACTCAGGAGGCGGAGGTTGCAGTGA GCCGAGATCGCACCATTGCACTCCAGCCTGGACGATAAGAGCGAAACTCCGTCAGGGCCTCATGG AGTCT
SHC1 skipped	TAACCTAAGCTGGCAGTGGCGTGATCCGGCACCAAATCGGCCCGCGGTGCGGTGCGGAGACTCCA TGAGGCCCTGTACATGGGTTGTGTGGAGGTCCTCCAGTCAATGCGTGCCCTGGACTTCAACACCC GGACTC
ELK4 included	ACTTGTGGAATGAGAGAACCGAGGAACGAAGAAACAGACATTCAACATGATTGCATTTGAATGAA TCCGATGTGCCTGATGGGTTAGATTTCCATATACAAGTAGCAGAGGAAAATCTGGGAACAGGGTG ATTCCACAGGATTCTGAAATGTCCAAATTCCTGAAAGGAAGATGAGGAAGAAAGTCCTACTTGTG TAACTGAGAAGCGTGTGGTGTTACACTAAAGCAAGTCCC
ELK4 skipped	ACTTGTGGAATGAGAGAACCGAGGAACGAAGAAACAGACATTCAACATGATTGCATTTGAAGTTA CACTAAAGCAAGTCCC

Figure S1
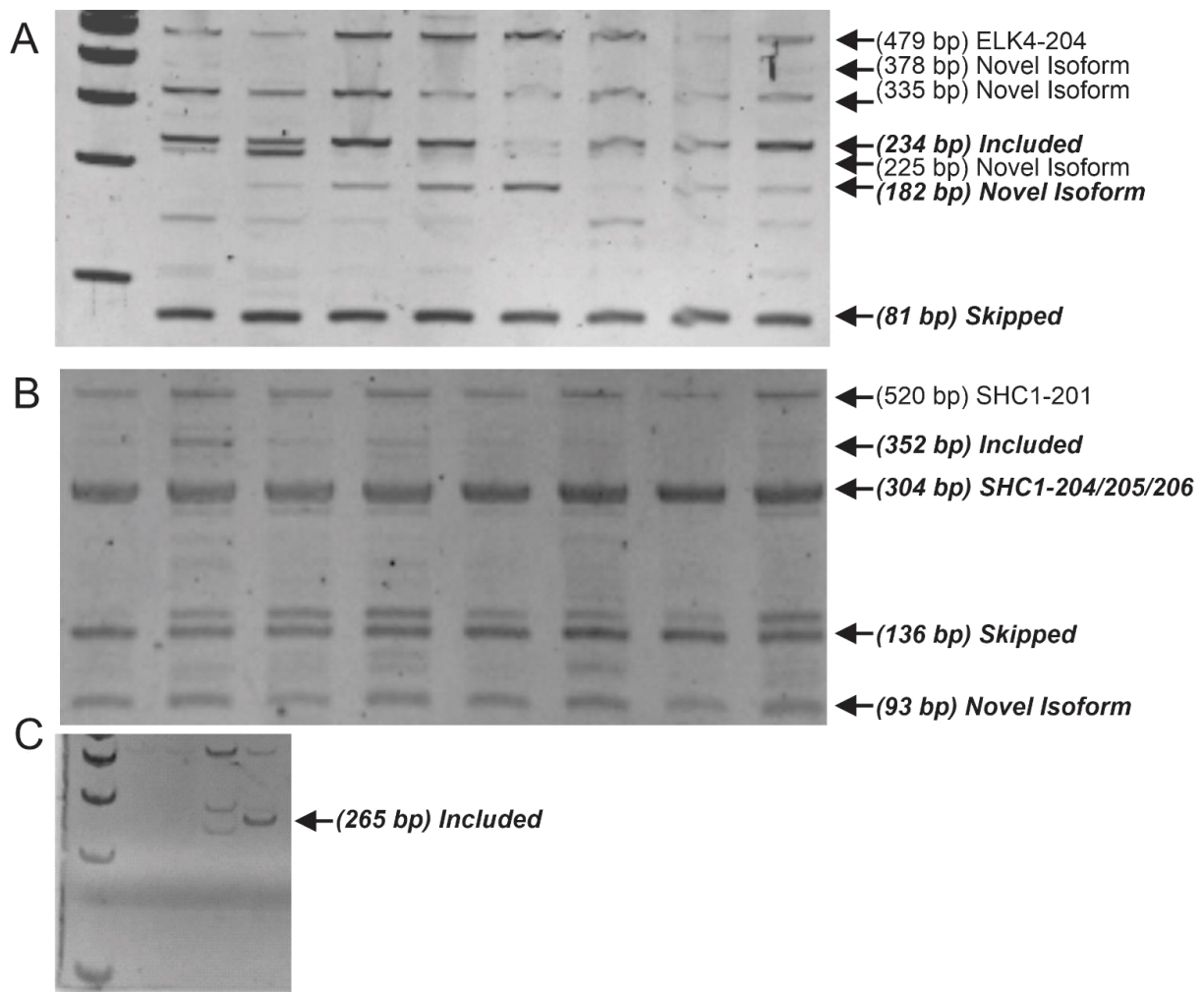
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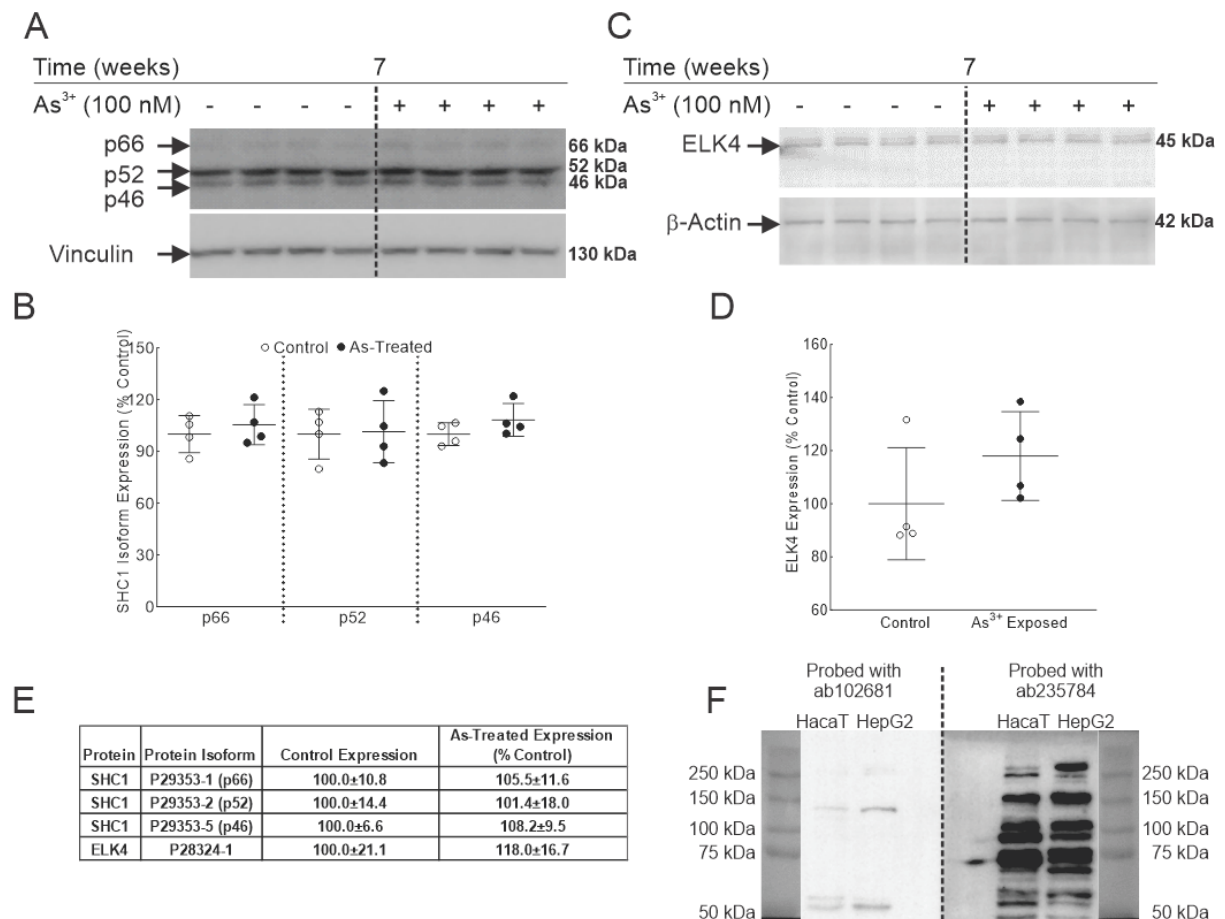
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