Cell Reports, Volume *36*

Supplemental information

Identification of a class of non-conventional ER-stress-response-derived immunogenic peptides

Alessia Melacarne, Valentina Ferrari, Luca Tiraboschi, Michele Mishto, Juliane Liepe, Marina Aralla, Laura Marconato, Michela Lizier, Chiara Pozzi, Offer Zeira, Giuseppe Penna, and Maria Rescigno

SUPPLEMENTAL INFORMATION

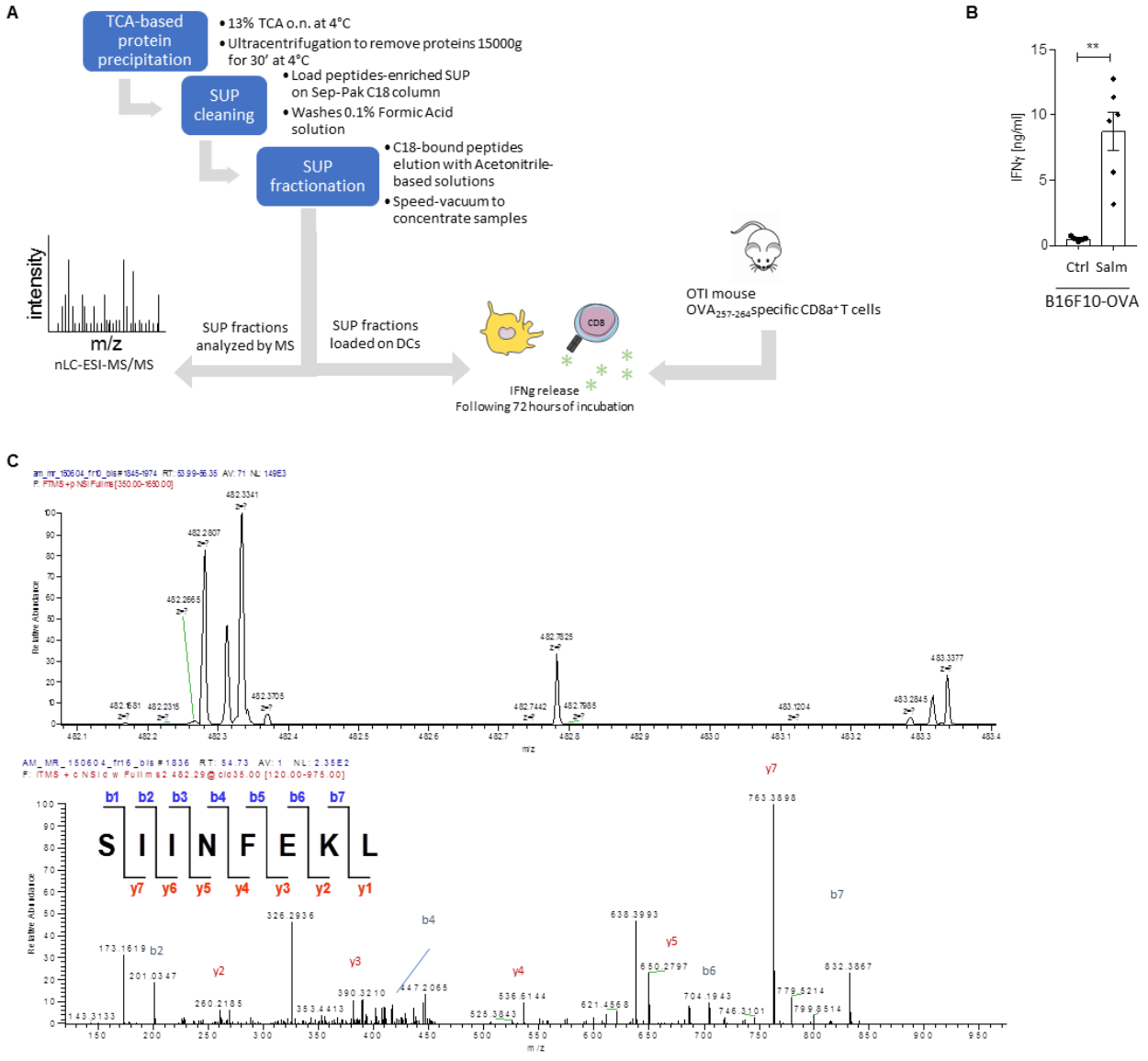

Figure S1. B16F10-OVA cells infected with *Salmonella* release peptides among which the immunogenic OVA-derived peptide SIINFEKL. (A) Pipeline to assess the release of $OVA_{257-264}$ peptide by B16F10-OVA cells upon *Salmonella* infection. Secretomes (SUP) were collected, proteins precipitated overnight at 4°C with trichloroacetic acid (TCA). Enriched peptides were desalted using Sep-Pak C18 and fractionated eluting them with Acetonitrile solution. Fractions were either loaded on DCs to test OVA-specific $CD8^+$ T cell

activation or underwent MS analysis. (B) ELISA quantification of IFN-γ released by $OVA_{257-264}$-specific OTI-CD8 (n>3). (C) MS analysis. $OVA_{257-264}$ (sequence: SIINFEKL) is mostly detected as double charge m/z=482.28 z=2. nLC-ESI-MS/MS spectrum of (m/z 482.28, z = +2) confirmed $OVA_{257-264}$ identity. See also Figure 1

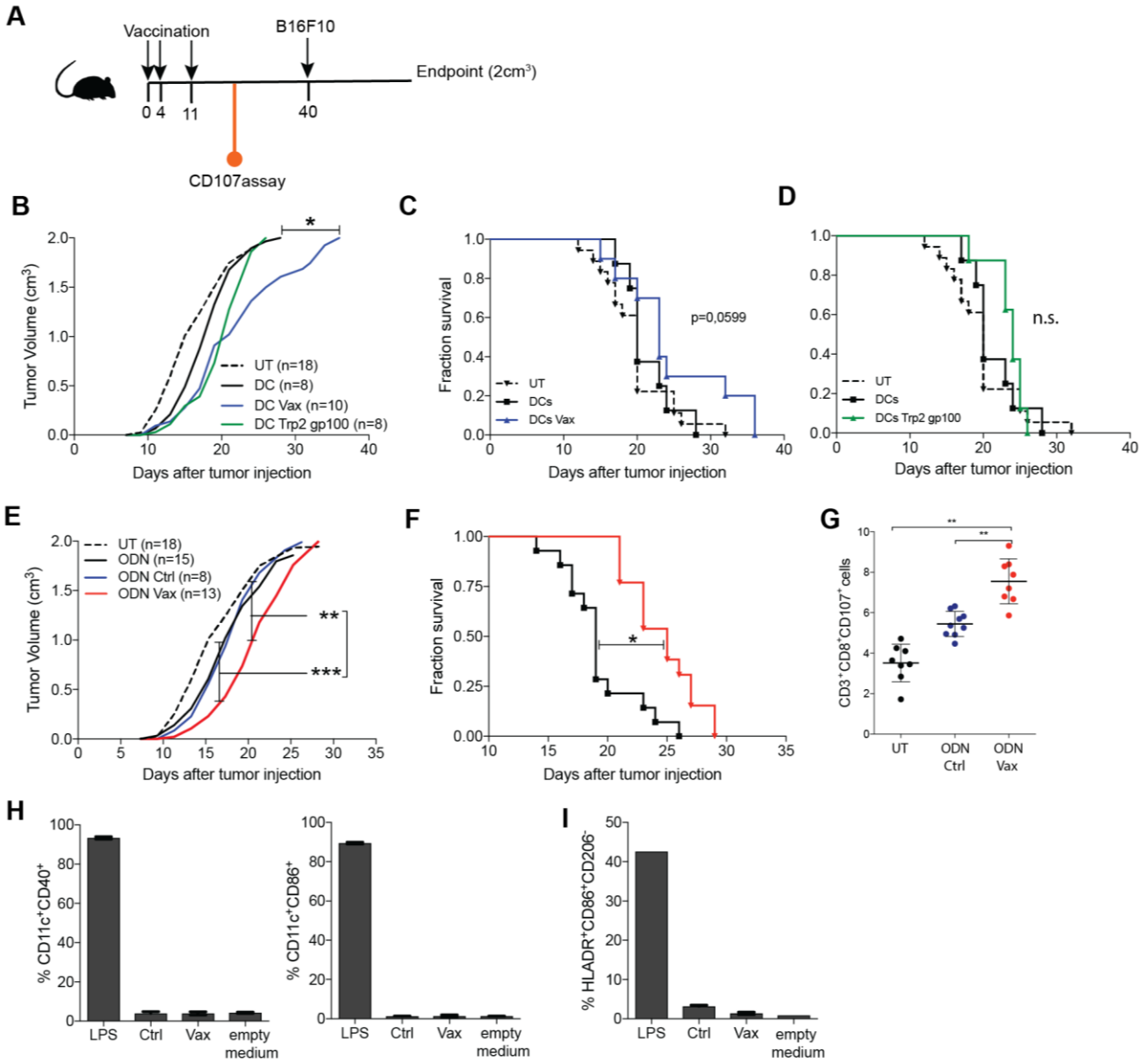

Figure S2 Peptides released by *Salmonella*-treated B16F10 cells induce an antitumor response in vivo either loaded on DCs or administered in combination with ODN1826. (A) Scheme of the immunization in vivo experiment. (B) Tumor growth and (C-D) Kaplan–Meier survival curves of mice immunized with DCs alone (DC), DCs loaded with melanoma antigens $Trp2_{180-188}$ $gp100_{25-33}$ (DC Trp2 gp100), and DCs loaded with peptides released by *Salmonella*-treated B16F10 cells (DC Vax); not immunized mice (untreated, UT) (n=6-12 mice per group). (E) Tumor growth and (F) Kaplan–Meier survival curves of mice immunized with ODN1826 alone (ODN), ODN combined with

secretome of *Salmonella*-infected B16F10 (ODN Vax), and secretome of untreated B16F10 cells (ODN Ctrl); mice not immunized (UT). Data are pooled from two independent experiments (n=5-9 mice per group). (G) Frequency of $CD3^+CD8^+CD107a^+$ (degranulating T cells) from PBMCs of immunized mice. Data of two pooled experiments are represented as mean ± SD using a scatter dot plot. (H-I) Frequency of (H) $CD11c^+CD40^+$ and $CD11c^+CD86^+$ murine DCs, and of (I) $HLADR^+CD86^+CD206^-$ primary human monocytes-derived DCs upon stimulation with LPS, with secretomes from *Salmonella*-treated B16F10 melanoma tumor cells (Vax), and with secretome from untreated cells (Ctrl). Data are represented as mean ± SD (n=2). Statistical analysis was evaluated using two-sided Mann-Whitney test (G,H,I), one-way ANOVA (B,E), or Log-rank Mantel-Cox test (C,D,F) **P*<0,05 ***P*<0.01. See also Figure 2.

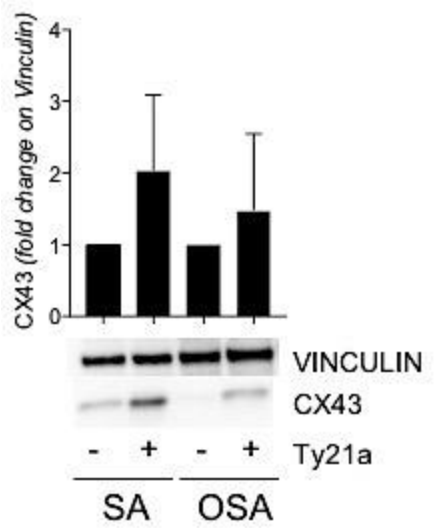

Figure S3 *Salmonella* infection of SA and OSA primary canine cells induce CX43 expression. CX43 and Vinculin expression in OSA19 and SA5 primary tumor cells tested by western blot after *Salmonella* infection (Ty21a). Data are pooled from 3 independent analysis. See also Figure 3.

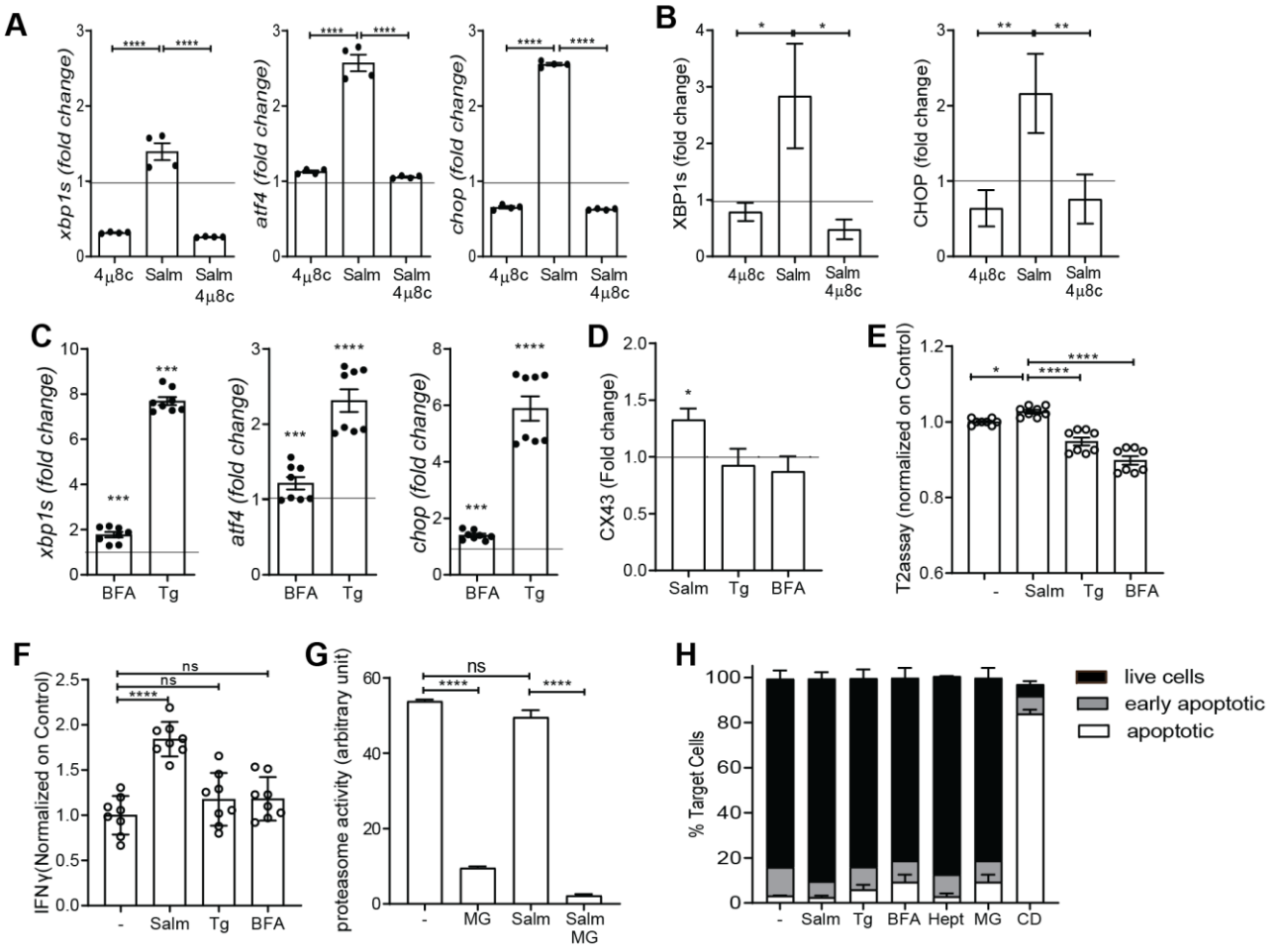

Figure S4 Both UPR exacerbation and hemichannels opening are necessary for peptide release. (A-B) Expression of UPR pathway at (A) gene and (B) protein level in 624-38 cells treated with 4μ8c with or without *Salmonella* (salm) infection. Data are normalized to (A) *Gapdh* or on (B) Vinculin and expressed as mean ± SEM of the fold change of the average expression. (C) Expression of xbp1s, atf4, chop at gene level in 624-38 melanoma cells treated with Tg and BFA. (D) CX43 protein expression in 624-38 treated with either *Salmonella* or thapsigargin (Tg) or BFA. (E) MFI of HLA-A*02:01 on T2-cells loaded with secretomes of 624-38 cells treated with either *Salmonella*, or Tg, or BFA. Data of three pooled experiments normalized on (-) are represented as mean ± SEM using a scatter dot plot. (F) ELISA quantification of IFN-γ released by CTL Vax upon stimulation with differently sourced secretomes. Data of three pooled experiments normalized on (-) are

represented as mean ± SEM using a scatter dot plot. (G) Chymotrypsin-like proteasome activity of 624-38 cell line. (-) untreated cells, MG132 (MG) treated cells. (H) Frequency of $Annexin^{-}PI^{-}$ (live), $Annexin^{+}PI^{-}$ (early-apoptotic), $Annexin^{+}PI^{+}$ (apoptotic) 624-38 tumor cells. Treated with MG, Tg, Hept, Salm, left untreated (-), with a lethal concentration of Tg: Dead-cells (CD). Statistical analysis was evaluated using two-sided Mann-Whitney test (A,B,E,F,G) or one-way ANOVA followed by multiple comparisons (Dunnet) (C,D). *P<0,05 **P<0.01. See also Figure 4.

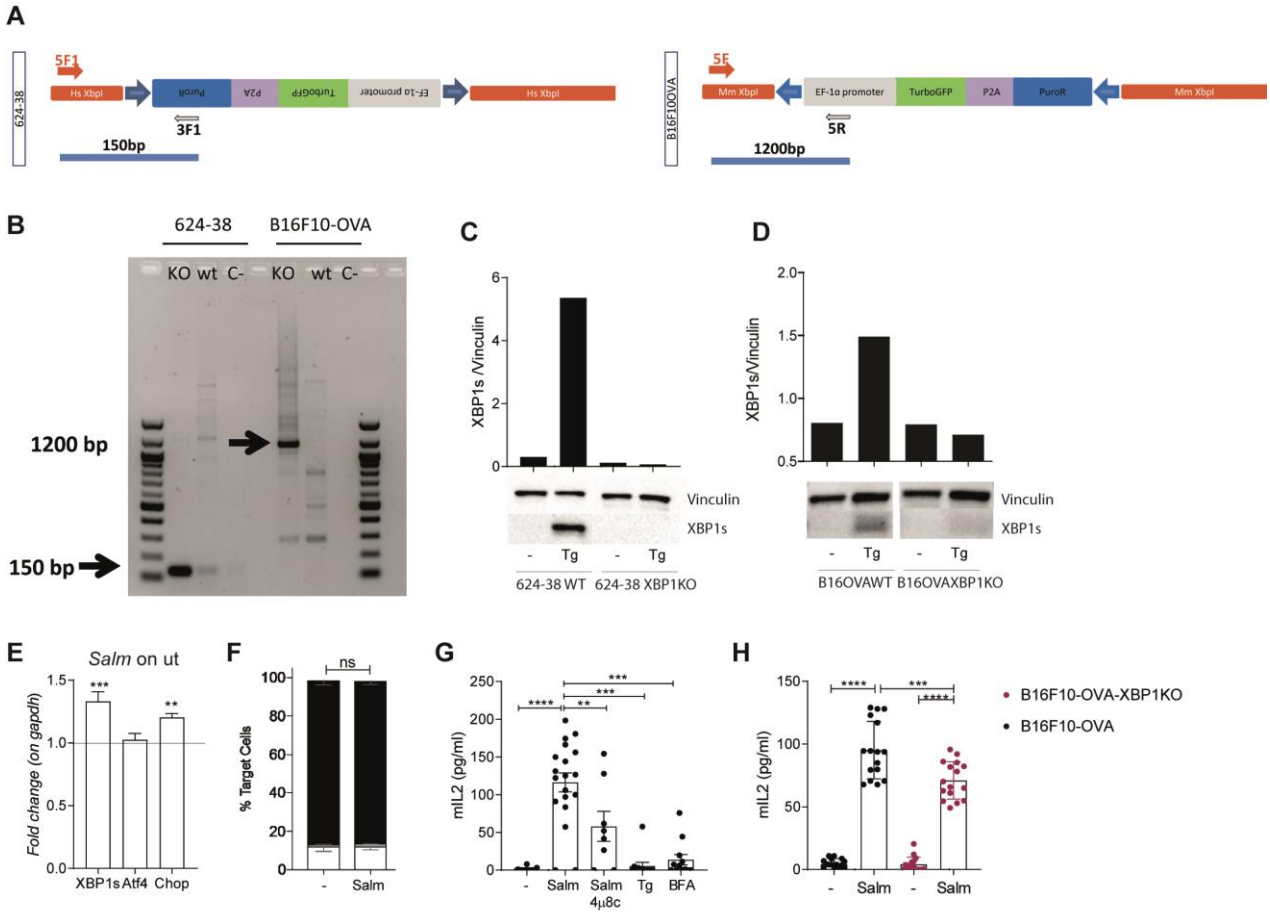

Figure S5. *Salmonella* infected murine melanoma cells release antigenic peptides upon induction of the ER-stress response 624-38 and B16F10-OVA XBP1KO cells. (A) Scheme of the PCR amplification strategy (B) PCR products analysis (C,D) Western blot quantification of XBP1s in (C) 624-38WT and in CRISPR/Cas9 Xbp1 knockout 624-38 (624-38 XBP1KO) cells and in (D) B16OVAWT and in CRISPR/Cas9 Xbp1 knockout B16OVA (B16OVAXBP1KO), treated for 4 hours with Tg or left untreated (-) (E-F) B16F10-OVA cells were infected with *Salmonella* (Salm) or left untreated (-). (E) Expression analysis of ER-stress genes. (F) Frequency of Annexin$^-$PI$^-$ (live), Annexin$^+$PI$^-$ (early-apoptotic), Annexin$^+$PI$^+$ (apoptotic) tumor cells. (G-H) ELISA quantification of mIL2 secretion by $OVA_{257-264}$-specific B3Z cells. (E-H) Data of three pooled experiments are represented as mean ± SEM using a (E) bar plot (G,H) scatter dot plot. Statistical analysis was evaluated using (E)

two-sided Mann-Whitney test, or (G,H) one-way ANOVA followed by multiple comparisons (Dunnet). *P<0,05 **P<0.01. See also Figure 4, S4, S6.

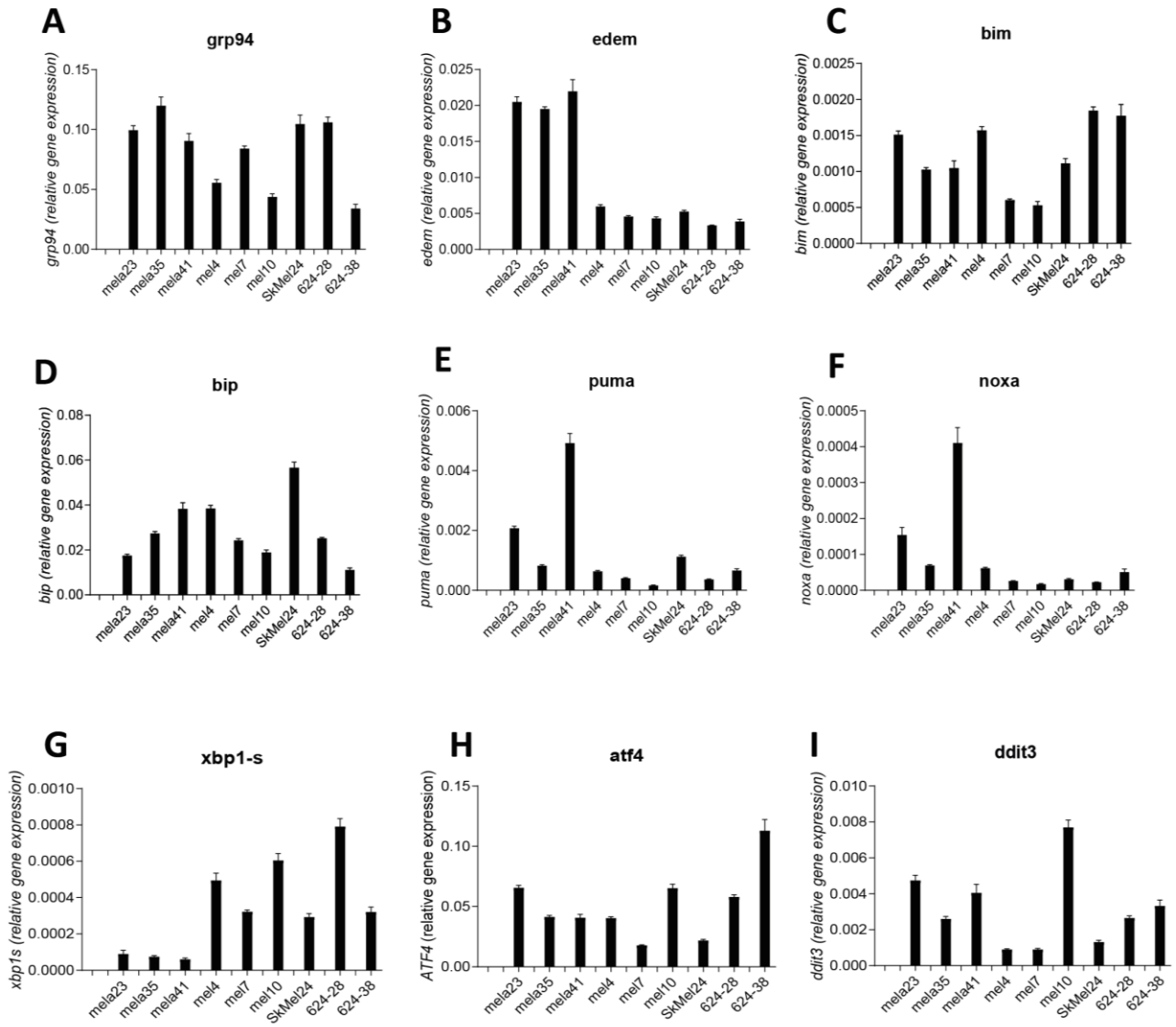

Figure S6. UPR-pathway is activated in melanoma cells and not in healthy melanocytes cells at steady state. Gene expression of UPR-related genes such as chaperones (grp94, edem, bip), pro-apoptotic mediators (bbc3, noxa, bim), and transcription factors (s-xbp1, atf4, ddit3) monitored in three melanocyte primary cells (mela23,35,41), three primary melanoma cells (mel4,7,10), and three melanoma cell lines (SkMel24, 624-28, 624-38). See also Figure 4, S4, S5.

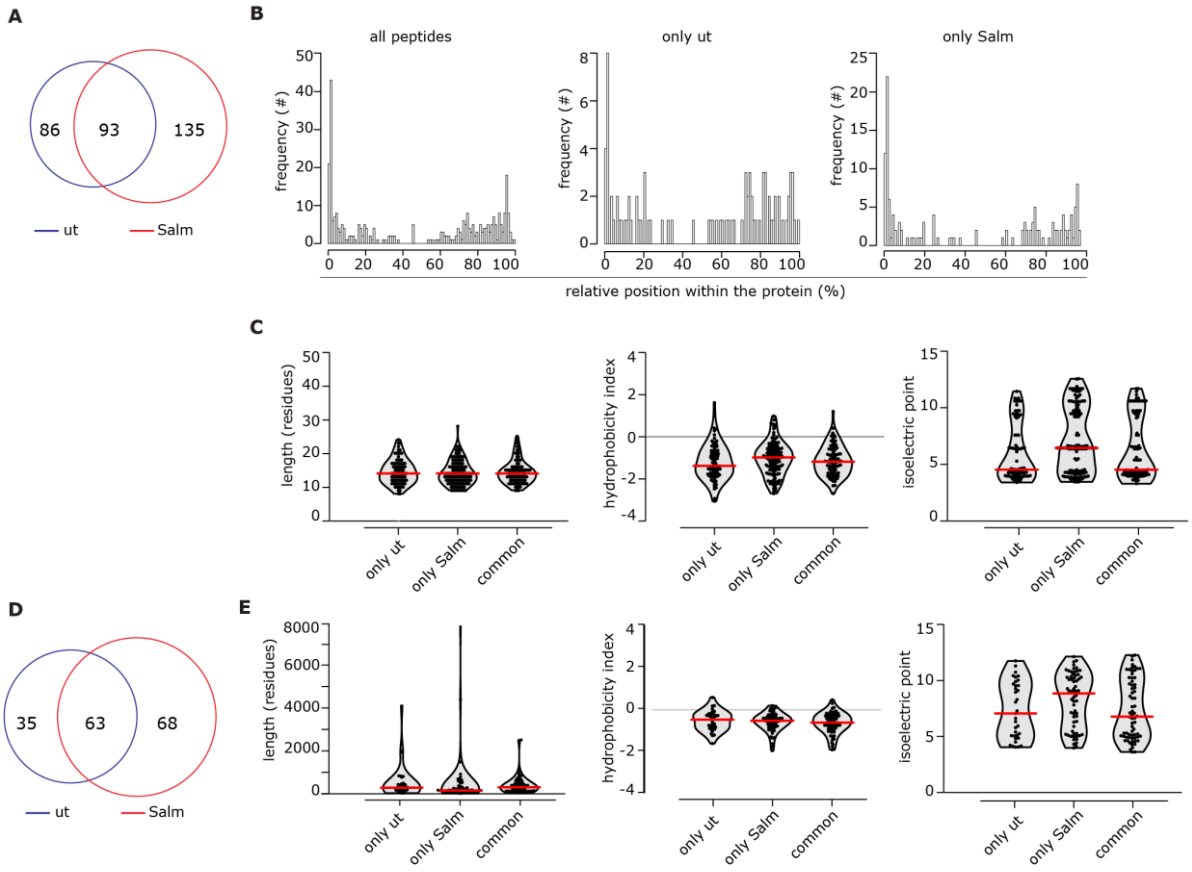

Figure S7. Characteristics of the 624-38 cell line secretomes. (A) Frequency of peptides identified only in the secretome of Salmonella-infected or not-infected 624-38 melanoma cell line or present in both. The peptides reported in the Venn diagram are unique peptides identified in at least one secretome. (B) Relative distribution of the secretome's peptides within the cognate protein sequence. (C) Distribution of length, hydrophobicity and isoelectric point of peptides identified. (D) Frequency of proteins having at least one peptide identified in the secretomes. (E) Distribution of length, hydrophobicity and isoelectric point of proteins having at least one peptide identified in the secretomes. In C and E, the violin plots indicate the frequency of the peptides/proteins, with all single peptides/proteins indicated as dots. Red lines indicate the median. See also Figure 5, 6.

Table S3. Primer list. See also Figure 4, S4-6**.**

	Forward primer	**Reverse primer**
Mm sXBP1 transcript variant 2	ctgagtccgaatcaggtgcag	gtccatgggaagatgttctgg
Mm usXBP1 transcript variant 1	cagcactcagactatgtgca	gtccatgggaagatgttctgg
Mm total XBP1	tggccgggtctgctgagtccg	gtccatgggaagatgttctgg
Mm activating transcription factor 4 (Atf4)	gggttctgtcttccactcca	aagcagcagagtcaggctttc
Mm DNA-damage inducible transcript 3 (Ddit3) CHOP	ccaccacacctgaaagcagaa	aggtgaaaggcagggactca
Hs sXBP1 transcript variant 2	ctgagtccgaatcaggtgcag	atccatggggagatgttctgg
Hs usXBP1 transcript variant 1	cagcactcagactacgtgca	atccatggggagatgttctgg
Hs total XBP1	tggccgggtctgctgagtccg	atccatggggagatgttctgg
Hs activating transcription factor 4 (Atf4)	gttctccagcgacaaggcta	atcctgcttgctgttgttgg
Hs DNA-damage inducible transcript 3 (Ddit3) CHOP	agaaccaggaaacggaaacaga	tctccttcatgcgctgcttt
5F1 (CRISPR/Cas9-XBP1-Human)	tctggagctatggtggtggt	
3F1 (CRISPR/Cas9-XBP1-Human)	gcaacctccccttctacgag	
5F (CRISPR/Cas9-XBP1-Mouse)	ctggaaatctggcctgagag	
3F (CRISPR/Cas9-XBP1-Mouse)	caggtggaagtaattcaaggcac	