Supplemental Data Legends

Supplemental Movie 1

Control showing that *P. aeruginosa* incubated in (0.9% NaCl) saline (no peptide control) for 15 min at 37 ^{o}C demonstrated active swimming motility. Phase-contrast, real-time, imaging of *P. aeruginosa* strain 6206 (10^8 cfu/ml) inoculated onto hTCEpi.

Supplemental Movie 2

A control showing that *P. aeruginosa* incubated with the scrambled version of cytokeratin 6A 19mer (100 μg/ml saline) for 15 min at 37 ^{o}C retained the capacity for active swimming motility similar to the no peptide control. Phase-contrast, real-time imaging of *P. aeruginosa* strain 6206 (10^8 cfu/ml) inoculated onto hTCEpi.

Supplemental Movie 3

Bacterial motility was almost completely abolished by incubation in synthetic cytokeratin 6A 19mer peptide (100 μg/ml saline) for 15 min at 37 ^{o}C. Some aggregates of bacteria were also observed. The hTCEpi appeared healthy. Phase-contrast real-time imaging of *P. aeruginosa* strain 6206 (10^8 cfu/ml) inoculated onto hTCEpi.

Supplemental Table 1

Sequences of keratin-derived peptides detected in the < 3 kDa lysate fraction of hTCEpi by mass spectrometry, (**A**) without trypsin digestion (**B**) with trypsin digestion.

Supplemental Table 2

Sequences of keratin-derived peptides detected in the 3-10 kDa lysate fraction of hTCEpi by mass spectrometry.

Supplemental Figure 1

TAMRA fluorochrome labeling of 19mer enhanced bactericidal activity against (**A**) *S. marcescens* and (**B**) *E. coli*, but lessened activity against (**C**) *S. pyogenes* (all $P < 0.005$ compared to 19mer). Activity against *S. pyogenes* remained statistically significant even with the TAMRA-tag ($P < 0.01$ compared to TAMRA alone).

Supplemental Figure 2

Sequence alignment of basic keratins K6A, K5, K7 and K8 showing glycine-rich repeats in the C-terminal region. Underlined sections indicate regions detected in hTCEpi lysates by mass spectrometry.

Supplemental Figure 3

Sequence alignment of acidic keratins K16, K15, K19 and K18 (complementary heterodimer with K6A, K5, K7 and K8 respectively) showing glycine-rich repeats in the N-terminal region. Underlined sections indicate regions detected in hTCEpi lysates by mass spectrometry.

Table 1.

Mass-spectrometric analysis of < 3 kDa and 3-10 kDa lysate fractions revealed that the majority of detected peptides were keratin-derived while cytokeratin 6A was common to all samples.

Lysate Fraction	Tryptic Digestion	Total no. of Proteins Detected	No. of Keratin Proteins Detected	Identity of Detected Keratins	Total no. of Peptides Detected	No. of K6A-derived Peptides Detected	No. of K6A (a.a. 515-559) Peptides Detected
<3 kDa	No	8	4	5, 6A, 15, 19	9	2	2
<3 kDa	Yes	1	1	6A	1	1	1
3-10 kDa	Yes	16	5	5, 6A, 14, 15, 19	82	24	20

Table 2.

Sequence and predicted structural characteristics of cytokeratin 6A-derived peptides. A 19-amino-acid peptide (a.a. 533-551) encompassing all five fragments (i.e. 18mer-N, a.a. 534-551; 18mer-C, a.a. 533-550; 17mer, a.a. 534-550; 14mer, a.a. 537-550; 13mer, a.a. 534-546) was predicted to be a cationic peptide. A 36-amino-acid variant representing a combined sequence of two detected fragments (a.a. 517-533 and a.a. 534-552) parted by a tryptic cut site, a 10-amino-acid variant representing the overlapping region between 14mer and 13mer, and an inactive scrambled 19-mer, were also assessed.

Peptide	**Sequence**	**Predicted Secondary Structure**	**Possible Transmembrane Helix**	**Predicted Hydrophobic Face**	**pI**	**Net Charge (pH 7.0)**	**Hydrophobic Moment (µH)**
19mer	RAIGGGLSSVGGGSSTIKY	Coil	Yes	Yes	9.99	+2	0.279
18mer-N	AIGGGLSSVGGGSSTIKY	Coil	Yes	Yes	8.63	+1	0.238
18mer-C	RAIGGGLSSVGGGSSTIK	Coil	Yes	Yes	11.0	+2	0.348
17mer	AIGGGLSSVGGGSSTIK	Coil	Yes	No	8.80	+1	0.309
14mer	GGLSSVGGGSSTIK	Coil	No	No	8.75	+1	0.252
13mer	AIGGLSSVGGGS	Coil	No	No	5.57	0	0.325
36mer	YGSGLGVGGGFSSSSGRAIGGGLSSVGGGSSTIKY	Coil	Yes	No	9.70	+2	0.219
10mer	GGLSSVGGGS	Coil	No	No	5.52	0	0.255
Scrambled 19mer	IRGSVTISGYSGGLKGSAG	Coil	No	Yes	9.99	+2	0.138

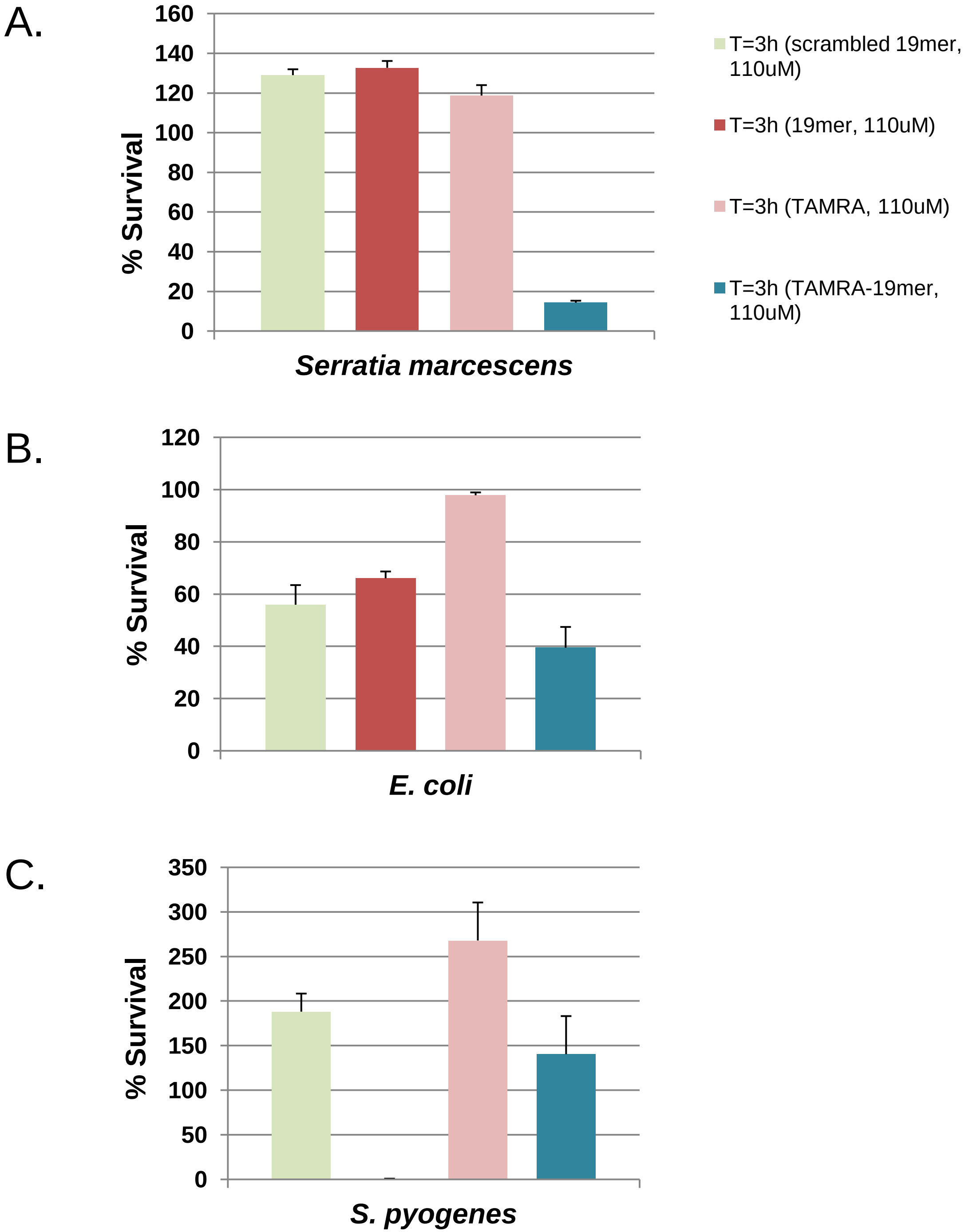
A.

Keratin	**Peptide**
K5A	L.GGGAGFGGGFGGP.G
K6A	Y.SYGSGLGVGGGFSS.S R.AIGGGLSSVGGGSSTIK.Y
K15	A.GGGGFGGGSLSGGGGSR.S
K19	F.GGLGGGSVRFGP.G

B.

Keratin	**Peptide**
K6A	R.AIGGGLSSVGGGSSTIK.Y

Keratin	Peptide
K5A (a.a. 30-47)	R.TSFTSVSRSGGGGGGGF.G R.TSFTSVSRSGGGGGGGFG.R
K5A (a.a. 553-576)	G.FSASSGRGLGVGFGSGGGSSSSVK.F A.SSGRGLGVGFGSGGGSSSSVK.F R.GLGVGFGSGGGSS.S R.GLGVGFGSGGGSSS.S
K6A (a.a. 31-68)	R.SGFSSVSVSR.S R.SRGSGGLGGACGGAGFGS.R R.GSGGLGGACGGAGFGS.R C.GGAGFGSRSLYGLGGSK.R
K6A (a.a. 515-559)	S.YSYGSGLGVGGGFSSSSGR.A Y.SYGSGLGVGGGFSSSSGR.A S.YGSGLGVGGGFSSSSGR.A Y.GSGLGVGGGFSSSSGR.A G.GGFSSSSGRAIGGGLSSVGGGSSTIK.Y G.FSSSSGRAIGGGLSSVGGGSSTIK.Y F.SSSSGRAIGGGLSSVGGGSSTIK.Y S.SSSGRAIGGGLSSVGGGSSTIK.Y S.SSGRAIGGGLSSVGGGSSTIK.Y S.SGRAIGGGLSSVGGGSSTIK.Y S.GRAIGGGLSSVGGGSSTIK.Y G.RAIGGGLSSVGGGSSTIK.Y R.AIGGGLSSVGGGS.S R.AIGGGLSSVGGGSS.T R.AIGGGLSSVGGGSSTIK.Y R.AIGGGLSSVGGGSSTIKYT.T I.GGGLSSVGGGSSTIK.Y G.GGLSSVGGGSSTIK.Y S.VGGGSSTIKYTTTSSSSR.K
K14 (a.a. 450-469)	R.TKVMDVHDGKVVSTHEQVLR.T K.VMDVHDGKVVS.T K.VMDVHDGKVVST.H K.VMDVHDGKVVSTHEQVLR.T D.VHDGKVVSTHEQVLR.T
K15 (a.a. 5-19)	T.FLQTSSSTFGGGSTR.G
K15 (a.a. 23-42)	S.LLAGGGGFGGGSLSGGGGSR.S L.LAGGGGFGGGSLSGGGGSR.S L.AGGGGFGGGSLSGGGGSR.S A.GGGGFGGGSLSGGGGSR.S G.GGGFGGGSLSGGGGSR.S
K19 (a.a. 8-24)	R.QSSATSSFGGLGGGSVR.F S.SATSSFGGLGGGSVR.F A.TSSFGGLGGGSVR.F


A.
160
140
120
100
80
60
40
20
0
% Survival
Serratia marcescens
T=3h (scrambled 19mer, 110uM)
T=3h (19mer, 110uM)
T=3h (TAMRA, 110uM)
T=3h (TAMRA-19mer, 110uM)
B.
120
100
80
60
40
20
0
% Survival
E. coli
C.
350
300
250
200
150
100
50
0
% Survival
S. pyogenes

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K6A       MASTSTTIRSHSSSRRGFSANSARLPGVSRSGFSSVSVSRSRGSGG-----LGGACGGAG
K5        -MSRQSSVSFRSGGSRSFSTASAITPSVSRTSFTSVSRSGGGGGGGFGRVSLAGACGVGG
K7        -----------------------MSIHFSSPVFTSRS----AAFSGRGAQVRLSSARPGG
K8        -----------------------MSIRVTQKSY------------------KVSTSGPRA
                                     .:   :                    .:.   .

K6A       FGSRSLYGLGGSKRISIGGGSCAISGGYGSRAGGSYGFGG-AGSGFGFGGGAGIGFGLGG
K5        YGSRSLYNLGGSKRISISTSGGSFRNRFGAGAGGGYGFGGGAGSGFGFGGGAGGGFGLGG
K7        LGSSSLYGLGASRPRV--------------------------------------------
K8        FSSRSYT-SGPGSRISSSSFS----------RVGSSN----------FRG----------
           .* *    * .

K6A       GAGLAGGFGGPGFPVCPPGGIQEVTVNQSLLTPLNLQIDPTIQRVRAEEREQIKTLNNKF
K5        GAGFGGGFGGPGFPVCPPGGIQEVTVNQSLLTPLNLQIDPSIQRVRTEEREQIKTLNNKF
K7        --AVRSAYGGP-----VGAGIREVTINQSLLAPLRLDADPSLQRVRQEESEQIKTLNNKF
K8        --GLGGYGGA----SGMGGITAVTVNQSLLSPLVLEVDPNIQAVRTQEKEQIKTLNNKF
            .. ..:**        .**  **:*****:** *: **.:* ** :* **********

K6A       ASFIDKVRFLEQQNKVLETKWTLLQEQGTKTVRQNLEPLFEQYINNLRRQLDSIVGERGR
K5        ASFIDKVRFLEQQNKVLDTKWTLLQEQGTKTVRQNLEPLFEQYINNLRRQLDSIVGERGR
K7        ASFIDKVRFLEQQNKLLETKWTLLQEQKSAK-SSRLPDIFEAQIAGLRGQLEALQVDGGR
K8        ASFIDKVRFLEQQNKMLETKWSLLQQQKT--ARSNMDNMFESYINNLRRQLETLGQEKLK
          ***************:*:***:***:* :    ..:  :**  *  ** **:::  :  :

K6A       LDSELRGMQDLVEDFKNKYEDEINKRTAAENEFVTLKKDVDAAYMNKVELQAKADTLTDE
K5        LDSELRNMQDLVEDFKNKYEDEINKRTTAENEFVMLKKDVDAAYMNKVELEAKVDALMDE
K7        LEAELRSMQDVVEDFKNKYEDEINHRTAAENEFVVLKKDVDAAYMSKVELEAKVDALNDE
K8        LEAELGNMQGLVEDFKNKYEDEINKRTEMENEFVLIKKDVDEAYMNKVELESRLEGLTDE
          *::**  ** :*************:**  ***** :***** ***.****::: : * **

K6A       INFLRALYDAELSQMQTHISDTSVVLSMDNNRNLDLDSIIAEVKAQYEEIAQRSRAEAES
K5        INFMKMFFDAELSQMQTHVSDTSVVLSMDNNRNLDLDSIIAEVKAQYEEIANRSRTEAES
K7        INFLRTLNETELTELQSQISDTSVVLSMDNSRSLDLDGIIAEVKAQYEEMAKCSRAEAEA
K8        INFLRQLYEEEIRELQSQISDTSVVLSMDNSRSLDMDSIIAEVKAQYEDIANRSRAEAES
          ***:: : : *: ::*:::***********.*.**:*.**********::*: **:***:

K6A       WYQTKYEELQVTAGRHGDDLRNTKQEIAEINRMIQRLRSEIDHVKKQCANLQAAIADAEQ
K5        WYQTKYEELQQTAGRHGDDLRNTKHEISEMNRMIQRLRAEIDNVKKQCANLQNAIADAEQ
K7        WYQTKFETLQAQAGKHGDDLRNTRNEISEMNRAIQRLQAEIDNIKNQRAKLEAAIAEAEE
K8        MYQIKYEELQSLAGKHGDDLRRTKTEISEMNRNISRLQAEIEGLKGQRASLEAAIADAEQ
           ** *:* **  **:******.*: **:*:** *.**::**: :* * *.*: ***:**:

K6A       RGEMALKDAKNKLEGLEDALQKAKQDLARLLKEYQELMNVKLALDVEIATYRKLLEGEEC
K5        RGELALKDARNKLAELEEALQKAKQDMARLLREYQELMNTKLALDVEIATYRKLLEGEEC
K7        RGELALKDARAKQEELEAALQRGKQDMARQLREYQELMSVKLALDIEIATYRKLLEGEES
K8        RGELAIKDANAKLSELEAALQRAKQDMARQLREYQELMNVKLALDIEIATYRKLLEGEES
          ***:*:***. *   ** ***:.***:** *:******..*****:*************.

K6A       RLNGEGVGQVNISVVQSTVSSGYGGASGVGSGLGLGG----------GSSYSY----GSG
K5        RLSGEGVGPVNISVVTSSVSSGYGSGSGYGGGLGGGLGGGLGGGLAGGSSGSYYSSSSGG
K7        RLAGDGVGAVNISVMNSTGGSS------SGGGIGLTLGGTMGSN-----ALSFSSSAG--
K8        RLESG---MQNMSIHTKT-TSG------YAGGLSSAYGGLTSP----GLSYSLGSSFGSG
          ** .      *:*:  .:  *.       ..*:.               : *     .

K6A       LGV-------GGGFSSSSG-RAIGGG-LSSVGGGSSTIKYTTTSSSSRKSYKH
K5        VGLGGGLSVGGSGFSASSG-RGLGVG-FGSGGGSSSSVKFVSTTSSSRKSFKS
K7        PGLLKAYSIRTASASRRSA-RD-------------------------------
K8        AGS--------SSFSRTSSSRAVVVKKIETRDG-----KLVSESSDV---LPK
           *         .. *  *. *
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```
K16      MTTCSRQFTSSSSMKGSCGIGGGIGGGSSRISSVLAGGSCRAPSTYGGGLSV-----SSR
K15      MTT-TFLQTSS------STFGGGSTRGGSLLA---GGGGFGGGSLSGGGGSRSISASSAR
K19      MTSYSYRQSSA-----TSSFG-GLGGGSVRFG---PGVAFRAPSIHGGSGGRGVSVSSAR
K18      ---MSF--T------TRSTFSTNYR----------SLGSVQAPSY------------GAR
             :   :        . :.                 .  . *             .:*

K16      FSSGGACGLGGGYGGGFSSSSSFGSGFGGGYGGGLGAGFGGGLAGFGGGFAGG-DGLLV
K15      FV---SSGSGGGYGGGMRV-----CGFGGGAGSVFGGGFGGGVGGGFGGGFGGGDGGLLS
K19      FV---SSSSSGAYGGGY------------------------------GG-VLTASDGLLA
K18      PVSSAASVYAGAGGSGSRISVSRSTSFRGGMGS-------GGLATGIAGGLAGM-GG--I
              :.  .*. *.*                               .* .     *

K16      GSEKVTMQNLNDRLASYLDKVRALEEANADLEVKIRDWYQRQRPS-EIKDYSPYFKTIED
K15      GNEKITMQNLNDRLASYLDKVRALEEANADLEVKIHDWYQKQTPTSPECDYSQYFKTIEE
K19      GNEKLTMQNLNDRLASYLDKVRALEAANGELEVKIRDWYQKQGPG-PSRDYSHYYTTIQD
K18      QNEKETMQSLNDRLASYLDRVRSLETENRRLESKIREHLEKKGPQ--VRDWSHYFKIIED
          .** ***.**********:**:**  *  ** **::  ::: *     *:* *:. *::

K16      LRNKIIAATIENAQPILQIDNARLAADDFRTKYEHELALRQTVEADVNGLRRVLDELTLA
K15      LRDKIMATTIDNSRVILEIDNARLAADDFRLKYENELALRQGVEADINGLRRVLDELTLA
K19      LRDKILGATIENSRIVLQIDNARLAADDFRTKFETEQALRMSVEADINGLRRVLDELTLA
K18      LRAQIFANTVDNARIVLQIDNARLAADDFRVKYETELAMRQSVENDIHGLRKVIDDTNIT
         ** :*:. *::*:: :*:************ *:* * *:*  ** *:.***:*:*: .::

K16      RTDLEMQIEGLKEELAYLRKNHEEEMLALRGQTGG-DVNVEMDAAPGVDLSRILNEMRDQ
K15      RTDLEMQIEGLNEELAYLKKNHEEEMKEFSSQLAG-QVNVEMDAAPGVDLTRVLAEMREQ
K19      RTDLEMQIEGLKEELAYLKKNHEEEISTLRGQVGG-QVSVEVDSAPGTDLAKILSDMRSQ
K18      RLQLETEIEALKEELLFMKKNHEEEVKGLQAQIASSGLTVEVDAPKSQDLAKIMADIRAQ
         * :** :**.*:*** :::******:  : .* ..  :.**:*:  . **:::: ::* *

K16      YEQMAEKNRRDAETWFLSKTEELNKEVASNSELVQSSRSEVTELRRVLQGLEIELQSQLS
K15      YEAMAEKNRRDVEAWFFSKTEELNKEVASNTEMIQTSKTEITDLRRTMQELEIELQSQLS
K19      YEVMAEQNRKDAEAWFTSRTEELNREVAGHTEQLQMSRSEVTDLRRTLQGLEIELQSQLS
K18      YDELARKNREELDKYWSQQIEESTTVVTTQSAEVGAAETTLTELRRTVQSLEIDLDSMRN
         *: :*.:**.: : :: .: ** .  *: .:  :  :.: :*:***.:* ***:*:*  .

K16      MKASLENSLEETKGRYCMQLSQIQGLIGSVEEQLAQLRCEMEQQSQEYQILLDVKTRLEQ
K15      MKAGLENSLAETECRYATQLQQIQGLIGGLEAQLSELRCEMEAQNQEYKMLLDIKTRLEQ
K19      MKAALEDTLAETEARFGAQLAHIQALISGIEAQLGDVRADSERQNQEYQRLMDIKSRLEQ
K18      LKASLENSLREVEARYALQMEQLNGILLHLESELAQTRAEGQRQAQEYEALLNIKVKLEA
         :**.**::* *.: *:  *: :::.::  :* :*.: *.: : * ***: *:::* :**

K16      EIATYRRLLEGEDAHLSSQQASGQSYSSR---EVFTSS-SSS-----SSRQTRP-ILKEQ
K15      EIATYRSLLEGQDAKMAGIGIREASSGGGGSSSNFHINVEESVDGQVVSSHKRE-I----
K19      EIATYRSLLEGQEDHYNNLSASKVL-----------------------------------
K18      EIATYRRLLEDGEDFNLGDALDS----SNSMQTIQKTTTRRIVDGKVVSETNDTKVLRH-
         ****** ***  :

K16      SSSSFSQGQSS
K15      -----------
K19      -----------
K18      -----------
```