Supporting Information

AAQPR peptide from *Aspergillus oryzae*-fermented wheat peptone promotes the regenerative potential of dermal and epidermal layers of the skin in in vitro assays and clinical trials

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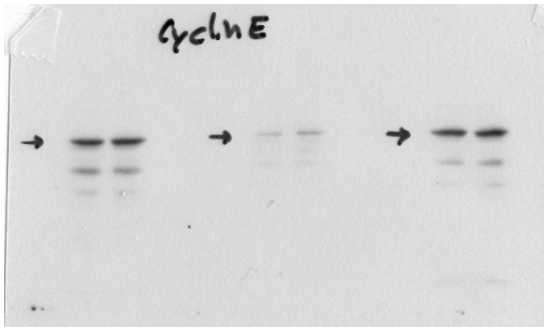
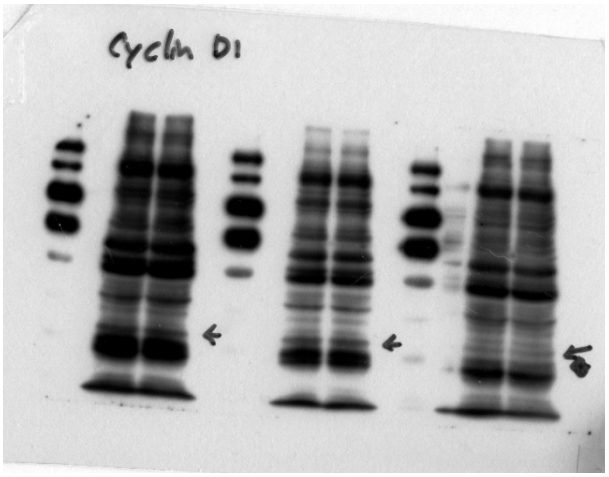
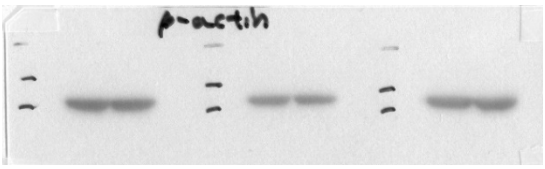
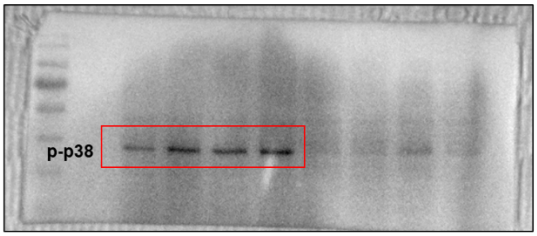






Supporting figures

Full blot of immunoblot in Fig. 1D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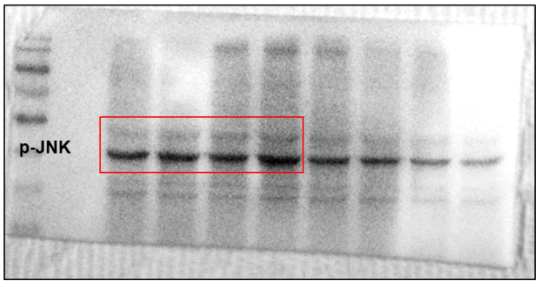
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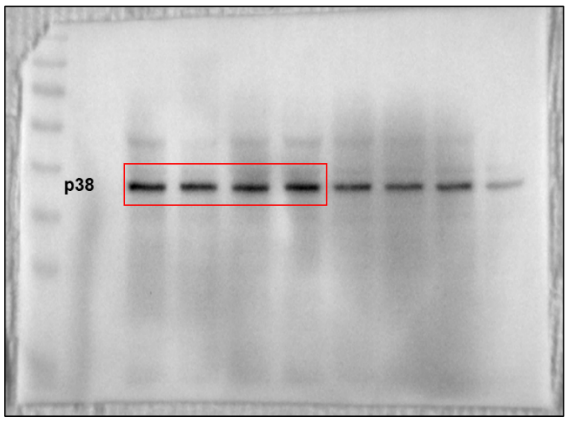
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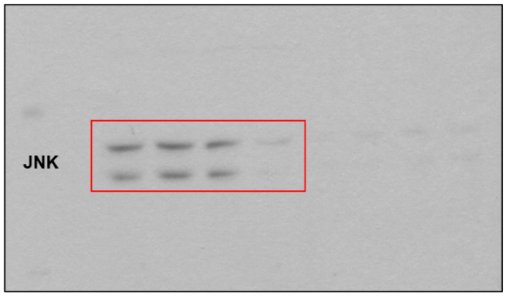
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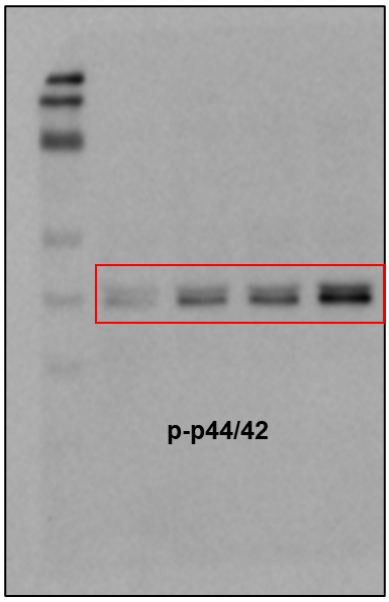
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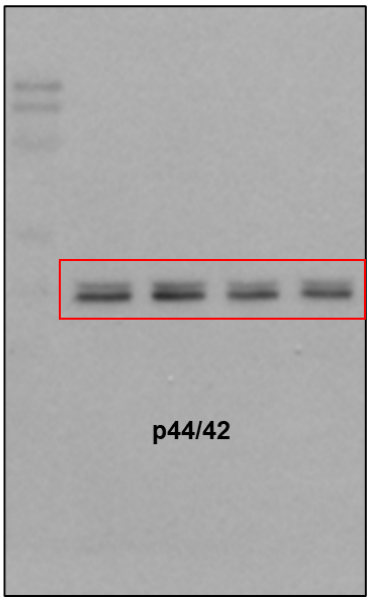
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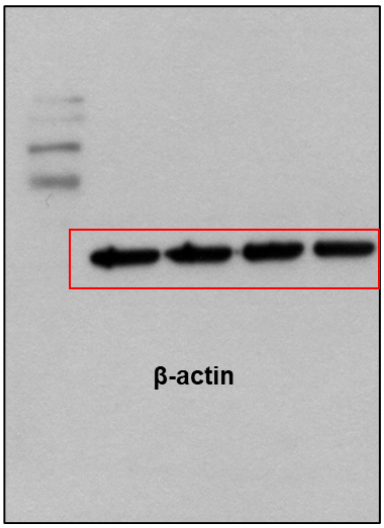
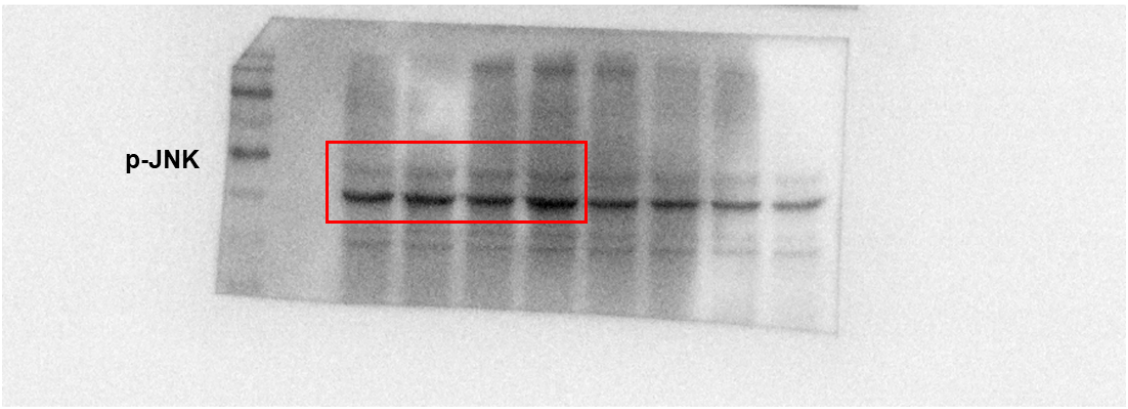
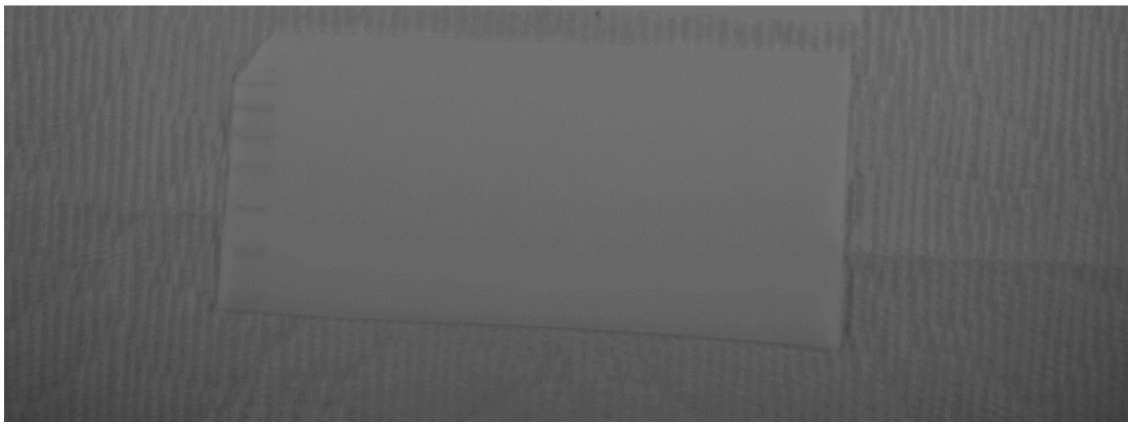

β-actin

1E

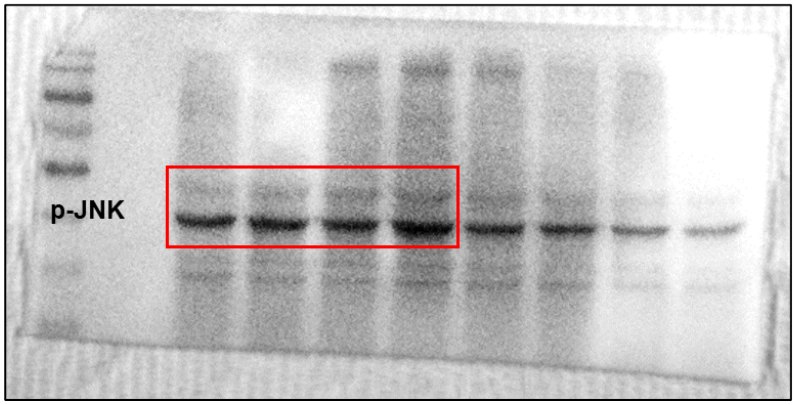
original image



marker image

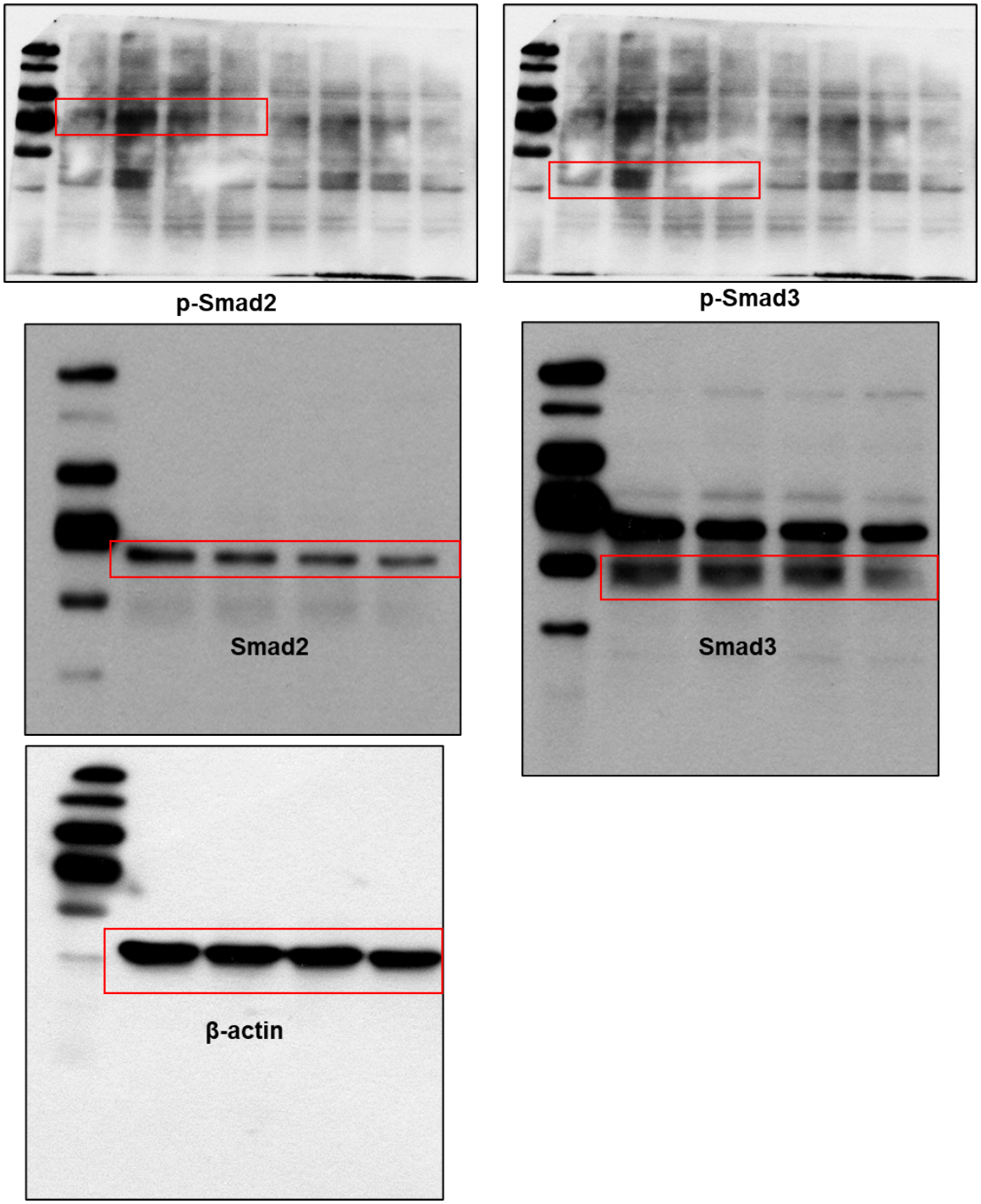


merge image



Supporting figures

Full blot of immunoblot in Fig. 1E



Supporting figures

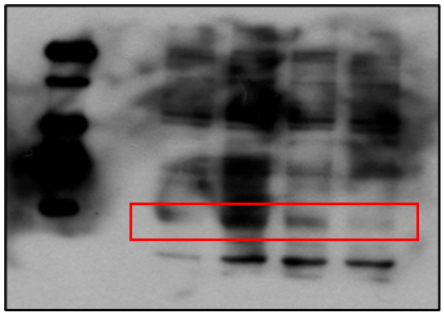
Full blot of immunoblot in Fig. 2B

Software Version	3.10.06
Experiment File Path:	
Protocol File Path:	
Plate Number	Plate 1
Date	2024-11-27
Time	오후 3:50:33
Reader Type:	Synergy HTX
Reader Serial Number:	151016E
Reading Type	Reader
Procedure Details	
Plate Type	96 WELL PLATE (Use plate lid)
Eject plate on completion	
Delay	0:10:00 (HH:MM:SS)
Read	Luminescence Endpoint
	A1..D4
	Integration Time: 0:01.00 (MM:SS.ss)
	Filter Set 1
	Emission: Hole
	Optics: Top, Gain: AutoScale
	Filter Set 2
	Emission: Hole
	Optics: Top, Gain: 100
	Read Speed: Normal, Delay: 100 msec
	Extended Dynamic Range
	Read Height: 4.5 mm
Automatic gain values	
Gain(Lum)	189
Results	
Actual Temperature:	25.2
Actual Temperature:	25.2

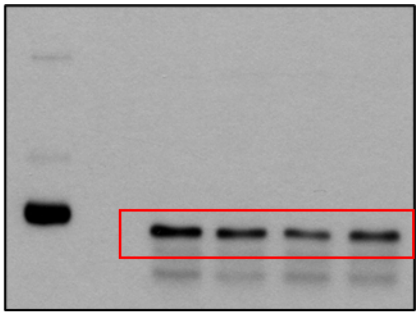
		1	2	3	4	5	6	7	8	9	10	11	12
control	A	4345	4660	4571									
PEP5	B	5063	4808	4909									
PEP5+SB	C	4661	5102	4806									
SB	D	4564	4695	4561									
	E												
	F												
	G												
	H												

Supporting figures

Raw data of cell viability in Fig. 2C



p-Smad 2



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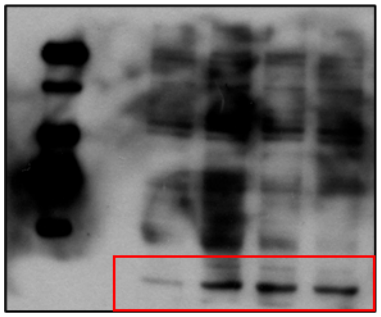
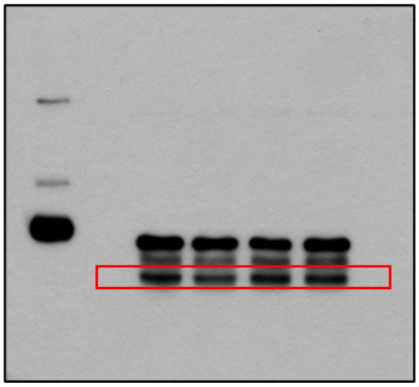
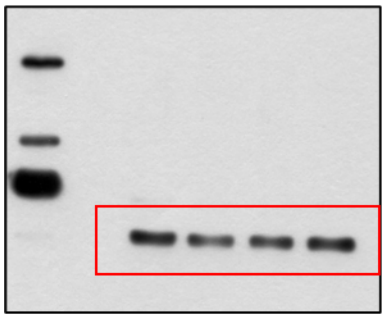


p-Smad 3



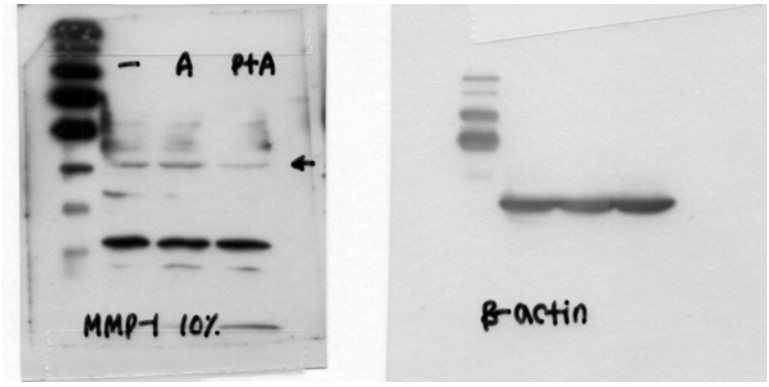
Smad 3



β-actin

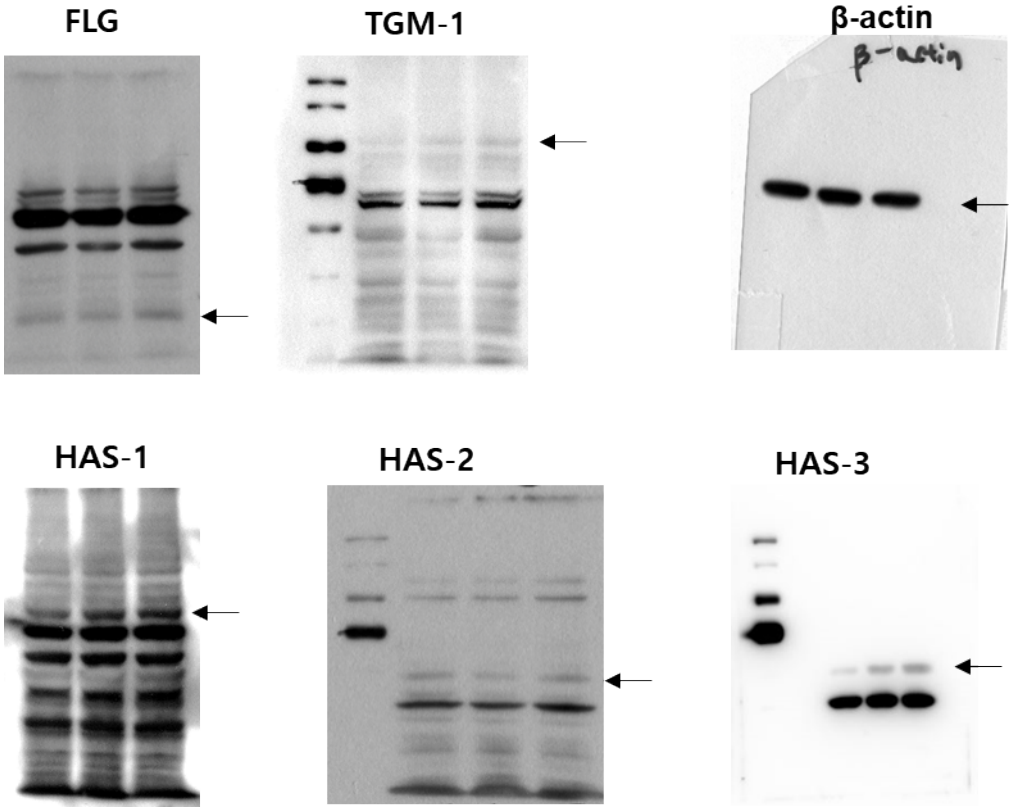
Supporting figures

Full blot of immunoblot in Fig. 2D



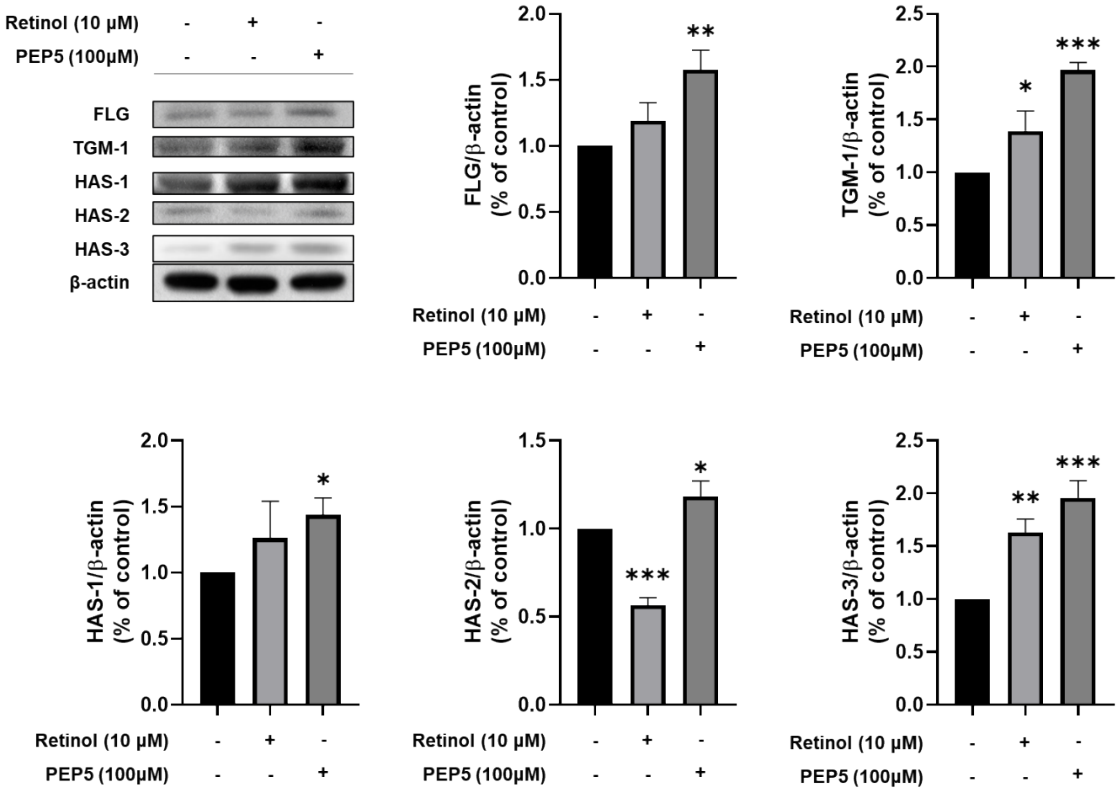
Supporting figures

Full blot of immunoblot in Fig. 5B



Supporting figures

Full blot of immunoblot in Fig. 6B

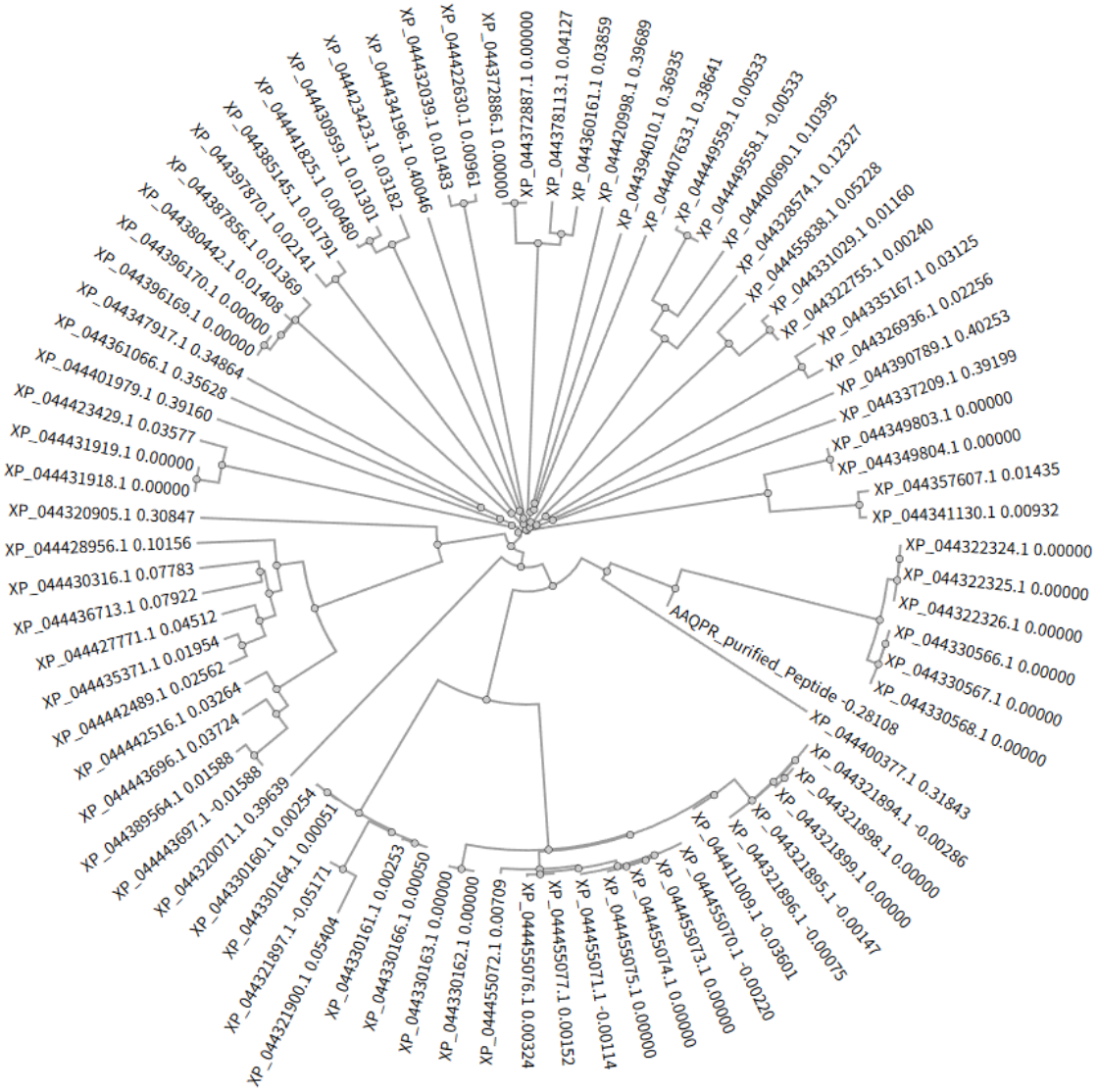


Supporting figures

Densitometric analysis of immunoblot in Fig. 6B

Supplementary Figure 1 and Table 3

To investigate the sequence relationships among bioactive peptides, a peptide library was constructed based on curated antioxidative peptide sequences obtained from the BIOPEP-UWM database.[1] The peptide sequences were aligned using Clustal Omega, which applies a progressive alignment strategy guided by a distance-based tree constructed via the mBed embedding method.[2] This approach enables efficient handling of large datasets by reducing the computational complexity to O(N log N), while maintaining high alignment accuracy through the use of profile-profile comparisons based on Hidden Markov Models. The resulting multiple sequence alignment was used to generate a phylogenetic tree in Newick format, from which peptides closely clustered with PEP5 (AAQPR) were identified and compared. Additionally, a motif similar to AAQPR was cross-referenced against known protein databases to evaluate its presence in natural sources, such as wheat-derived proteins, for biological relevance. Furthermore, protein sequences derived from *Triticum aestivum* (bread wheat, Taxonomy ID: 4565) were screened to identify entries containing the AAQPR motif. These proteins were then subjected to multiple sequence alignment and phylogenetic analysis alongside the functional peptide library to assess their evolutionary proximity to known antioxidative peptides.



Supplementary Fig. 1

Phylogenetic tree of *Triticum aestivum* proteins containing the AAQPR motif. Protein sequences derived from the *Triticum aestivum* genome (Taxonomy ID: 4565) were screened to identify entries containing the AAQPR peptide sequence. These proteins were aligned using Clustal Omega, and a phylogenetic tree was constructed to evaluate their evolutionary relationships.

Supporting Table

Supplementary Table 1

Item	Category	Frequency (number)	%
Number of subjects at onset	Male	19	100
	Female	0	0
Skin type	Dry	4	21.05
	Normal to dry	5	26.23
	Normal	4	21.05
	Normal to oily	5	26.32
	Oily	1	5.26
Skin moisture concentration	Very high	2	10.53
	Slightly high	1	5.26
	Moderate	6	31.58
	Slightly low	9	47.37
	Very low	1	5.26
Skin surface sebum	Very high	1	5.26
	Slightly high	3	15.79
	Moderate	8	42.11
	Slightly low	7	36.84
	Very low	0	0.00
Skin elasticity	No elasticity	7	36.84
	Moderate	9	47.37
	elasticity	3	15.79
Sleep time (average per day)	less than 5 hours	4	21.05
	5~8 hours	15	78.95
	More than 8 hours	0	0.0
Skin exposure to sunlight (hours)	less than 1 hours	11	57.89
	1~3 hours	7	38.84
	More than 3 hours	1	5.26
Smoking status (average per day)	Don’t smoke	19	100.00
	Less than 10 cigarettes	0	0.00
	More than10 cigarettes	0	0.00
	More than one pack	0	0.00
Does the skin feel easily irritated?	Yes	4	21.05
	No	15	78.95
Does the skin feel stinging within 30 min of product use?	Yes	0	0.00
	No	19	100.00
Have you experienced a side effect of cosmetics within the past 12 months?	Yes	0	0.00
	No	19	100.00

Supplementary Table 2

Reaction temperature	0 week	1 week	3 week	4 week
25℃	97.6 %	96.9 %	94.3 %	94.5 %
50℃	97.6 %	95.3 %	93.9 %	95.6 %

Supplementary Table 3

BIOPEP ID	**Name**	**Sequence**	**Chem. mass**	**Monois. mass**	**Activity**
8116	peptide derived from sardinelle by-products proteins (Sardinella aurita)	GAWA	403.4313	403.1850	antioxidative
9098	Antioxidative peptide	GLTSK	504.5761	504.2898	antioxidative
9364	Antioxidative peptide	DYK	424.4471	424.1951	antioxidative
9368	Antioxidative peptide	EQC	378.4016	378.1204	antioxidative
9436	Antioxidative peptide	IIAPPER	794.9360	794.4636	antioxidative
9439	Antioxidative peptide	KVEGDLK	787.8988	787.4425	antioxidative
10471	Antioxidative peptide	YF	328.3616	328.1418	antioxidative
10769	Antioxidative peptide	GVPY	434.4849	434.2158	antioxidative
10866	Antioxidative peptide	VNPESQQGSPR	1198.2402	1197.5720	antioxidative
10936	Antioxidative peptide	WAAWGIS~	788.8903	788.3958	antioxidative

Supplementary Table 4

Sample	Number of respondents	Degree of skin response		Average degreeof skin response*	Evaluation
		30min	24hr		
PEP5 Essence	0	0	0	0	No irritation
Control Essence	1	0	1	0.3906	Mild irritation
Distilled Water	0	0	0	0	No irritation

* Average degreeof skin response = sum of each skin reactivity) / 2

- Test subject : 32 people (39.0±11.36 years old)
- Test area: The back (except dorsal spine area)

Reference

1. P. Minkiewicz, A. Iwaniak and M. Darewicz, *International journal of molecular sciences*, 2019, **20**, 5978.

2. F. Sievers, A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert and J. Söding, *Molecular systems biology*, 2011, **7**, 539.