

***In vitro* dipeptidyl peptidase IV inhibitory activity and *in situ* insulinotropic activity of milk  
and egg white protein digests**

Marta Santos-Hernández<sup>1,2</sup>, María Cermeño<sup>2</sup>, Isidra Recio<sup>1</sup>, Richard J. FitzGerald<sup>2\*</sup>

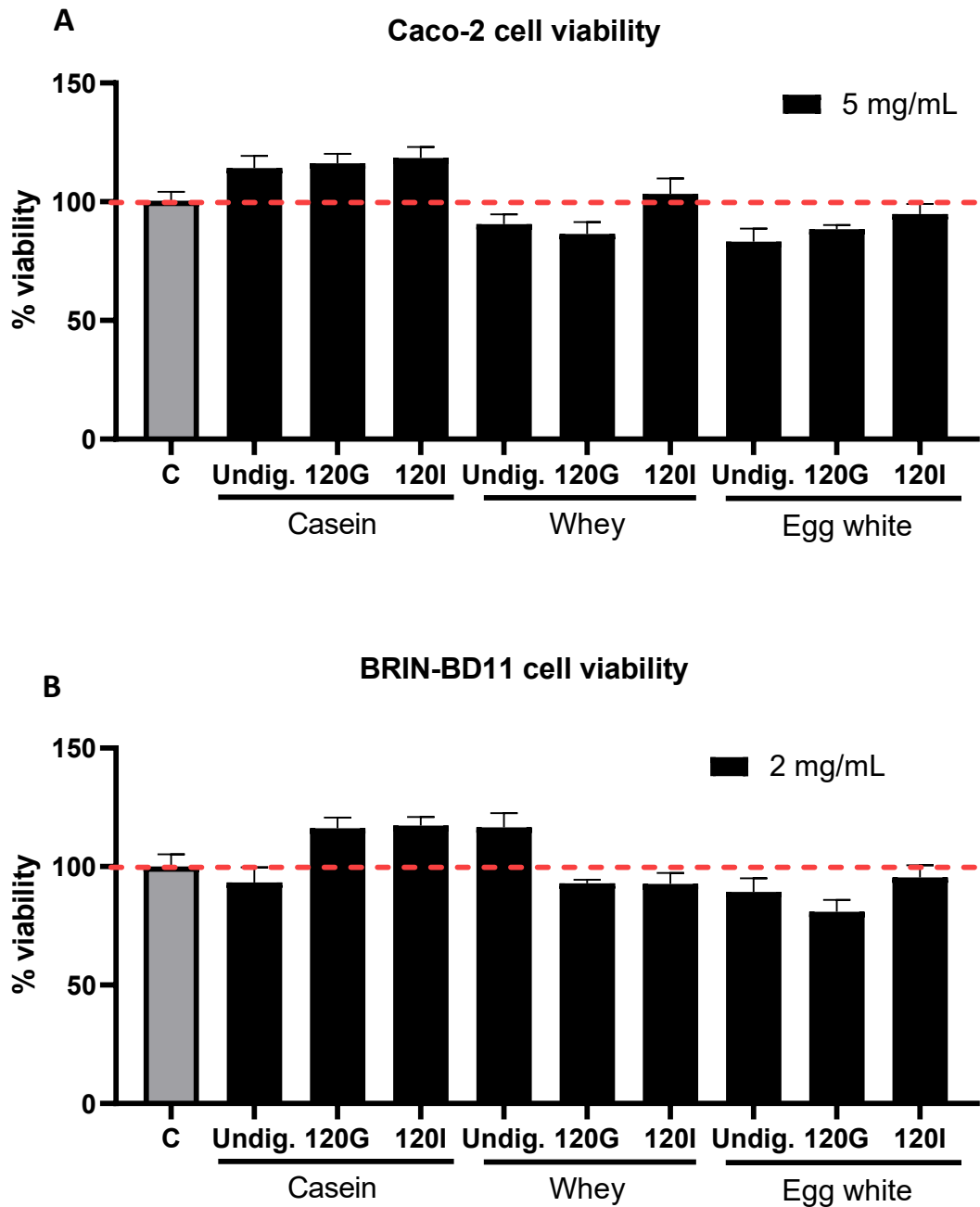
<sup>1</sup> Institute of Food Science Research, CIAL (CSIC-UAM), Nicolás Cabrera, 9, 28049 Madrid, Spain

<sup>2</sup> Department of Biological Sciences, University of Limerick, Castletroy, Limerick, Ireland

\*To whom correspondence should be addressed. E-mail: dick.fitzgerald@ul.ie

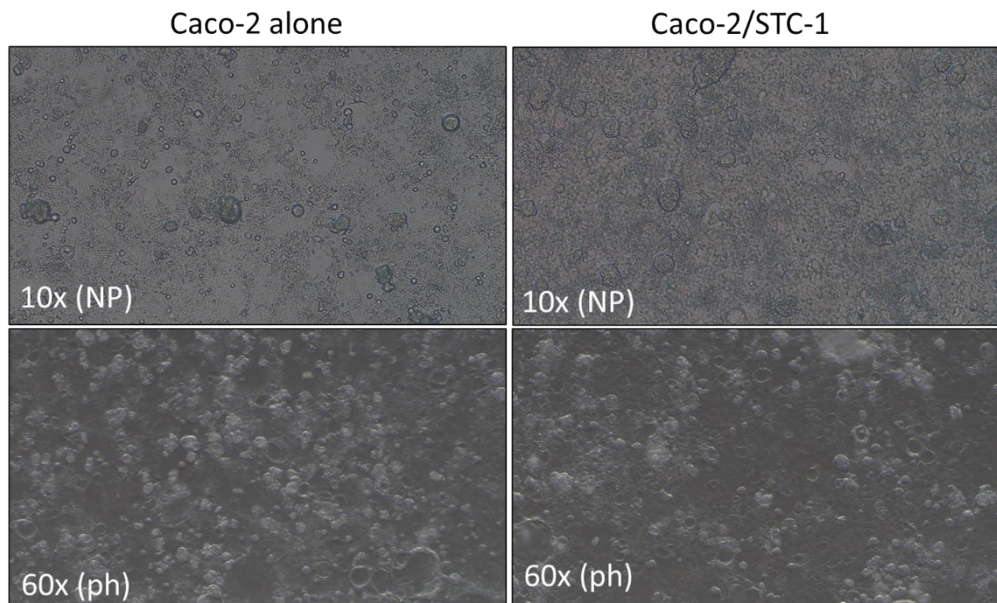
## 1. Cell viability

The methodology is described in the manuscript.



**Supplementary Figure 1:** Cell viability of milk and egg white protein gastric (120G) and intestinal (120I) digests, and undigested (Undig.) samples after 2 h incubation in (A) Caco-2 cell line or (B) BRIN-BD11 cells (mean  $\pm$  SEM, n=3). Control (C) was the buffer used for the dilution of the samples, Hanks' Balance Salt Solution (HBSS). There is no statistical significance in comparison with control (HBSS buffer, C) (one-way ANOVA with Tukey's post hoc test).

## 2. Micrographs of cell monolayers



**Supplementary Figure 2:** Micrographs of the Caco-2 cell (left) and Caco-2/STC-1 cell (right) monolayers following 23 days of culture.

### 3. TEER measurement values

**Supplementary Table 1:** Transepithelial resistance (TEER) values of the two-tiered transport model before and after the incubation of the gastric (120G), intestinal (120I) digests or undigested (Undig.) samples of egg white and whey protein. Only values of more than 700  $\Omega\cdot\text{cm}^{-2}$  were considered for the experiments.

		TEER ( $\Omega\cdot\text{cm}^{-2}$ )			
		Caco-2 / BRIN-BD11		Caco-2-STC-1 / BRIN-BD11	
		Before	After	Before	After
Control		1270	1170	1230	1060
		780	745	1050	850
		800	800		
Whey protein	W 120G 2mg/mL	1280	1150	1800	1520
		1190	1640	1930	1550
		1270	1170		
	W 120I 2mg/mL	1400	1200	1620	1460
		1300	1270	1600	1510
		1330	1450	1610	1460
Egg white	EW 120G 2mg/mL	1520	1440	1430	1030
		1640	1500	1330	1070
		1570	1550		
	EW 120I 2mg/mL	1560	1350	1460	1270
		1500	1360	1370	1550
		1650	1640	1300	1260
Whey protein	W U 2mg/mL	1660	1500		
		1740	1500		
		1650	1490		
	W U 0.2mg/mL	1430	1640		
		1410	1480		
	W 120G 0.2mg/mL	1670	1500		
1640		1500			
W 120I 0.2mg/mL	996	950			
	1080	910			
Egg white	EW U 2mg/mL	1060	730		
		1140	760		
	EW U 0.2mg/mL	980	800		
		1100	1080		
	EW 120G 0.2mg/mL	980	800		
		1380	1110		
		1290	1030		
		1160	1080		
EW 120I 0.2mg/mL	1280	960			
	1210	930			

## 1. Analysis by UPLC-tandem mass spectrometry

**Supplementary Table 2:** peptides identified in the basolateral fraction after 2h incubation with the intestinal digest (120I) of egg white (EW) or whey protein (W) in Caco-2 cell monolayers.

	<b>Protein</b>	<b>ID protein</b>	<b>Range</b>	<b>Sequence</b>
<b>EW 120I</b>	Ovalbumin	P01012	10 15	EFCFDV
			329 333	HAAHA
	Ovotransferrin	P02789	117 121	VKKGT
			235 245	NAPDQKDEYEL
			420 424	TAGVC
			509 513	PGSPP
			521 525	CQGSG
	Mucin-6	F1NBL0	84 92	NGIQIAPYG
			90 97	PYGRSVRL
			157 165	ALQKMDDPS
			258 267	SANQIYEECG
			314 325	CTLNGETYAPGD
			394 398	YEKSG
			485 490	LGLCGN
			507 511	EGTAS
820 824			SSTCN	
820 827			SSTCNLYG	
871 883			ICGKSGVTCSRSI	
930 937			NMTLIWNK	
1042 1048			RDSCGCD	
<b>W 120I</b>	$\beta$ -lactoglobulin	P02754	11 15	TCGAQ
			23 27	MKGLD
			52 56	SAPLR
	$\alpha_{s1}$ -casein	P02662	127 131	VPNSA