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Supporting Material

**PULMONARY SURFACTANT PROTEIN SP-C COUNTERACTS THE
DELETERIOUS EFFECTS OF CHOLESTEROL ON THE ACTIVITY OF
SURFACTANT FILMS UNDER PHYSIOLOGICALLY RELEVANT
COMPRESSION-EXPANSION DYNAMICS**

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Supplementary Table 1.

Thermodynamic parameters of the main calorimetric transition of DPPC bilayers in the absence or presence of cholesterol and SP-C

	Cholesterol (% w/w)	ΔH (Kcal/mol)	T_m (°C)
DPPC	-	7.1 ± 0.06	41.4
	3	6.5 ± 0.13	40.7
	5	3.9 ± 0.13	43.7
	10	2.1 ± 0.05	40.3
	20	0.56 ± 0.15	41.4
DPPC/SP-C 2% w/w	-	6.4 ± 0.5	40.2
	3	5.1 ± 0.09	39.1
	5	3.9 ± 0.36	39.8
	10	2.02 ± 0.6	41.3
	20	0.89 ± 0.1	41
DPPC/SP-C 10% w/w	-	4.1 ± 0.05	41.2
	3	3.2 ± 0.03	41.5
	5	2.8 ± 0.6	41.6
	10	2.5 ± 0.7	41.0
	20	0.6 ± 0.3	41.0

Supplementary Table 2.

Thermodynamic parameters of the main calorimetric transition of DPPC:POPC:POPG bilayers in the absence or presence of cholesterol and SP-C.

	Cholesterol (% w/w)	ΔH (Kcal/mol)	T_m ($^{\circ}C$)	$\Delta T_{1/2}$ ($^{\circ}C$)
DPPC:POPC:POPG	-	3614 ± 12	29 ± 0.2	7.3 ± 0.05
	2	2310 ± 180	$22 \pm 0.1^*$, 29.6 ± 0.04	7 ± 0.2
	5	1700 ± 42	28 ± 0.1	10 ± 0.1
DPPC:POPC:POPG:SP-C 2%	-	2742 ± 140	27.6 ± 0.06	6 ± 0.2
	2	2172 ± 105	31 ± 0.06	8 ± 0.2
	5	1526 ± 85	27.5 ± 0.07	10 ± 0.2

* T_m for the first lipid transition observed in the thermogram.

Supplementary Table 3.

Parameters defining the surface behaviour of DPPC/POPC/POPG films, in the absence or presence of variable amounts of cholesterol and surfactant proteins SP-B, SP-C or an N-terminal synthetic SP-C peptide, upon quasi-static or dynamic compression-expansion cycling in the captive bubble surfactometer

	Cholesterol (% w/w)	Q-Stat, cycle 4			Dynamic, cycle 20		
		ν_{\min} (mN/m)	ν_{\max} (mN/m)	%compression*	ν_{\min} (mN/m)	ν_{\max} (mN/m)	%compression*
1% SP-C	-	16 ± 0.5	67 ± 1.4	80 ± 0.9	7 ± 0.4	72 ± 0.7	70 ± 2.1
	5	20 ± 1.2	61 ± 0.8	71 ± 1	20 ± 0.8	75 ± 1.4	64 ± 1.6
	10	21 ± 2	35 ± 1.1	71 ± 1.3	6 ± 0.7	73 ± 0.6	57 ± 2
2% SP-C	-	2 ± 0.3	57 ± 2.3	54 ± 0.4	3 ± 0.4	69 ± 0.9	58 ± 0.7
	5	9 ± 0.6	58 ± 1	60 ± 0.5	7 ± 0.5	66 ± 1	62 ± 0.9
	10	18 ± 1.3	61 ± 1.4	78 ± 1.1	4 ± 0.8	68 ± 2	74 ± 1.4
1% SP-B	-	2 ± 0.2	25 ± 0.7	22 ± 0.8	2 ± 0.3	34 ± 1	18 ± 0.7
	5	8 ± 0.4	64 ± 0.5	58 ± 0.9	8 ± 0.6	72 ± 0.8	51 ± 0.6
1% SP-B + 2% SP-C	5	3 ± 0.2	31 ± 0.4	28 ± 0.5	3 ± 0.3	42 ± 0.7	23 ± 0.4
2.5 mol Nt SPC + 1% SP-B	5	22 ± 1.8	71 ± 1.3	73 ± 1.2	16 ± 1.1	73 ± 2	79 ± 0.8

*% compression required to for the bubble to reach ν_{\min}

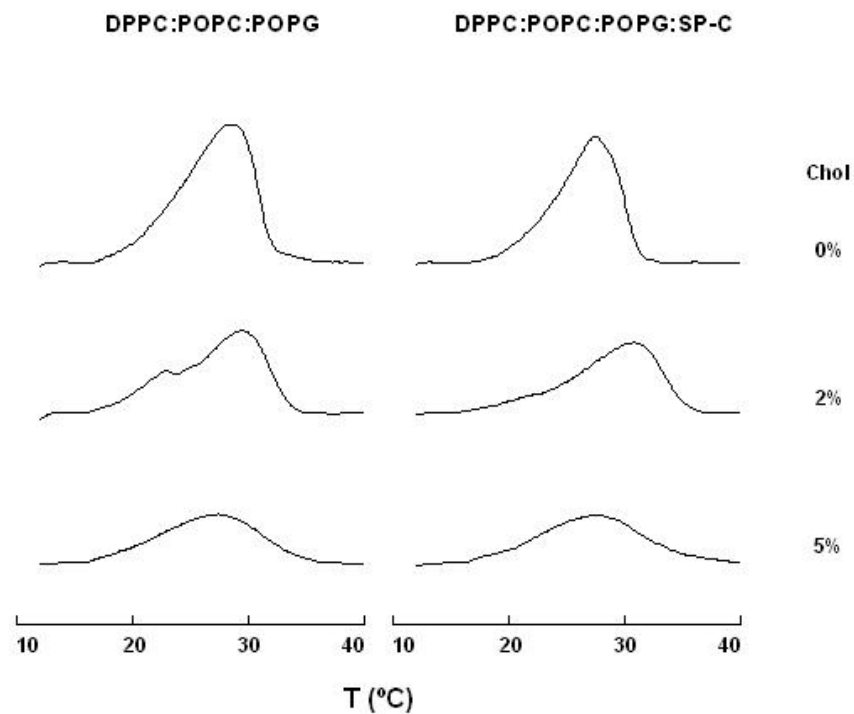


Figure S1.

Thermotropic behaviour of DPPC:POPC:POPG bilayers in the presence of Cholesterol and SP-C. Differential Scanning Calorimetry thermograms of DPPC:POPC:POPG multilamellar suspensions have been obtained in the absence (left) or in the presence (right) of 2% protein-to-phospholipid by weight of porcine SP-C, containing the indicated proportions of cholesterol (w/w with respect to phospholipid).

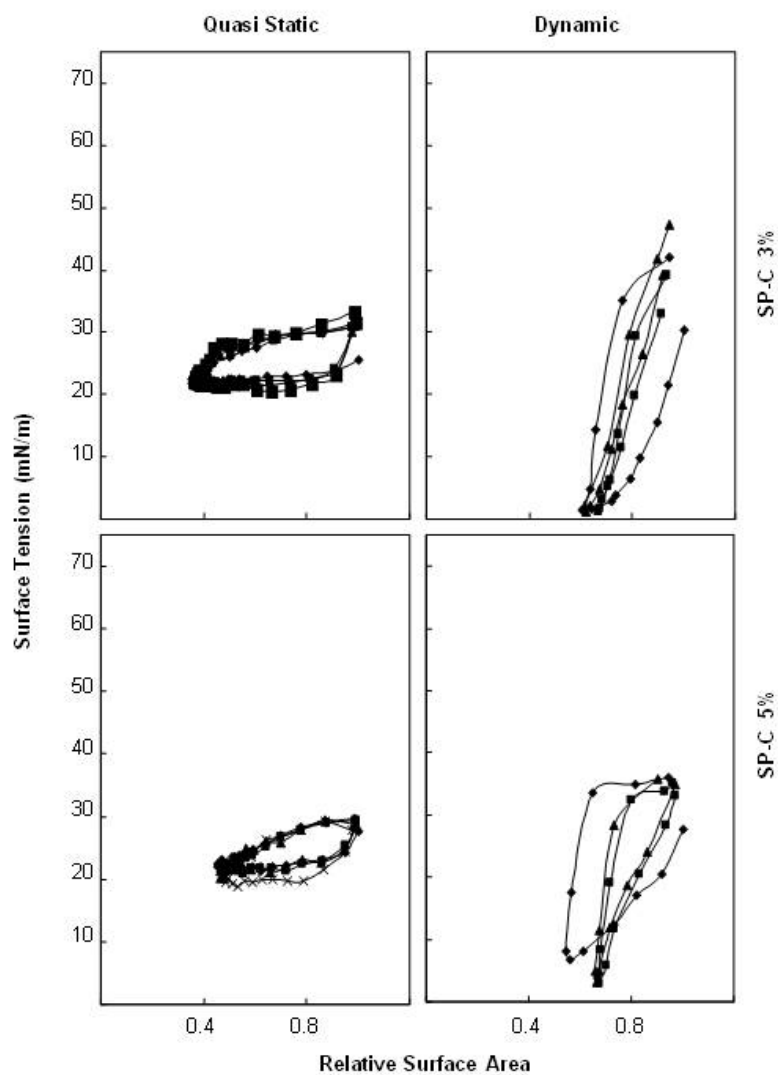


Figure S2
 Quasi-static (left panels) and dynamic (right panels) compression-expansion isotherms of DPPC/POPC/POPG (50:25:15, w/w/w) films in the presence of 5% cholesterol to phospholipid ratio by weight and 3% (upper panels) or 5% (lower panels) SP-C (protein to lipid, w/w). Quasi-static cycles 1st (◆), 2nd (■), 3rd (▲) and 4th (X) and dynamic cycles 1st (◆), 10th (■) and 20th (▲) are represented.