

CLEC14a-HSP70-1A interaction regulates HSP70-1A-induced angiogenesis

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Supplementary Figure legends

Supplementary Figure S1: Reactivity of commercial anti-HSP70-1A antibody to rhHSP70-1A. Following the separation of 0.1 μ g rhHSP70-1A by SDS-PAGE, the reactivity of the commercial anti-HSP70-1A antibody was measured via immunoblotting.

Supplementary Figure S2: Effect of rhHSP70-1A on HUVEC cell adhesion. (A) Representative fluorescent microscopic images of calcein AM-labeled HUVECs bound to HUVEC monolayers in the absence or presence of the indicated increasing concentrations of rhHSP70-1A (scale bar=400 μ m). (B) Fluorescence intensity of the calcein-AM labeled HUVECs is expressed as a bar graph. The data represent the mean $\pm$ SEM of triplicate measurements from two independent experiments; ** $P < 0.01$, *** $P < 0.001$.

Supplementary Figure S3: Measurement of the interaction between CLEC14a-CTLD-Fc-HRP and rhHSP70-1A or rhCLEC14a-ECD. A 96-well microtiter plate was coated with 0.1 μ g of rhHSP70-1A (A) or rhCLEC14a-ECD (B) per well, loaded with 1 μ g/well of CLEC14a-CTLD-Fc-HRP, and incubated for 2 h. Binding to the coated protein was assessed by ELISA. Data are presented as the mean $\pm$ SEM of triplicate measurements from two independent experiments; *** $P < 0.001$.

Supplementary Figure S4: Effect of the anti-CLEC14a-CTLD antibody on HSP70-1A-induced HUVEC tube formation and HSP70-1A-CLEC14a interaction. (A) Representative light microscopic images of tubes formed by HUVECs incubated for 8 h with 50 ng/ml rhHSP70-1A or 50 ng/ml rhHSP70-1A plus 20 μ g/ml control IgG or anti-CLEC14a-

CTLD antibody (scale bar=500 μ m). HUVECS incubated in the absence of rhHSP70-1A were used as controls (MOCK). **(B)** Quantitation of the total number of tube branches, expressed as a percent of control (MOCK) tube formation. **(C)** Wells of a 96-well plate were coated with 0.1 μ g of rhCLEC14a-ECD followed by the addition of 1 μ g of rhHSP70-1A. ELISA was performed in the absence (MOCK) or presence of 20 μ g/ml control IgG or anti-CLEC14a-CTLD antibody. The data represent the mean $\pm$ SD of triplicate experiments. *** P < 0.001.

Supplementary Figure S5: Effect of VER155008 or siRNA-mediated knockdown of CLEC14a on HSP70-1A-induced HUVEC tube formation. **(A)** Representative light microscopic images of tubes formed following treatment of HUVECs treated with scrambled- or CLEC14a siRNA. HUVECs were incubated for 8 h in the absence (MOCK) or presence of 20 nM VER155008, 50 ng/ml rhHSP70-1A or 50 ng/ml rhHSP70-1A plus 20 nM VER155008 (scale bar=500 μ m). **(B)** Quantitation of the total number of tube branches, expressed as a percent of control (MOCK) tube formation. The data represent the mean $\pm$ SD of triplicate experiments. * P < 0.05, ** P < 0.01, *** P < 0.001.

Supplementary Figure S6: Effect of the HSP70-1A-interacting region of CLEC14a-CTLD on EGM-induced HUVEC tube formation. **(A)** Representative light microscopic images of tubes formed by HUVECs incubated for 12 h in the absence (MOCK) or presence of EGM or EGM plus 20 μ g/ml CLEC14a-CTLD-Fc, F2-Fc, F5-Fc, or Fc (scale bar=500 μ m). **(B)** Quantitation of the total number of tube branches, expressed as a percent of control (MOCK) tube formation. The data represent the mean $\pm$ SEM of quadruplicate measurements from two independent experiments; *** P < 0.001.

Supplementary Figure S7: Full-length images of the cropped blots throughout this study.

(A) Immunoblot images depicting the expression of CLEC14a (left) or β -actin (right) from scrambled siRNA- or CLEC14a siRNA-treated HUVECs shown in Fig. 2B. (B) Immunoblot images depicting the co-immunoprecipitated HSP70-1A (left) or CLEC14a (right) shown in Fig. 2D. (C) Ponceau stained images depicting the amount of heavy and light chains of control antibody or anti-CLEC14a antibody shown in Fig. 2D. (D) Immunoblot images depicting the co-immunoprecipitated rhCLEC14a-ECD (left) or rhHSP70-1A (right) shown in Fig. 3A (left). (E). Ponceau stained images depicting the amount of heavy and light chains of control antibody or anti-CLEC14a antibody shown in Fig. 3A (left). (F) Immunoblot images depicting the co-immunoprecipitated rhCLEC14a-ECD (left) or rhHSP70-1A (right) shown in Fig. 3A (right). (G) Ponceau stained images depicting the amount of heavy and light chains of control antibody or anti-HSP70-1A antibody shown in Fig. 3A (right). (H) Immunoblot images depicting the amount of HSP70-1A bound to CLEC14a-CTLD-Fc or Fc fusion fragments (left) shown in the upper panel of Fig. 4B. Immunoblot images depicting the loaded amounts of CLEC14a-CTLD-Fc or Fc fusion fragments (right) shown in the lower panel of Fig. 4B. (I) Immunoblot images depicting the levels of phosphorylated ERK (left) or total ERK (right) shown in Fig. 6A.

Supplementary Table S1 Peptide sequences and masses from p70 by MALDI TOF

Peptide	Sequence ^a	M + H ⁺	
		Observed	Calculated ^b
		<i>Da</i>	
P1	LLQDFFN ⁺ GR (349-357)	1109.55	1108.56
P2	DAGVIAGLN ⁺ VLR (160-171)	1197.66	1196.68
P3	LVNHFVEEFK (237-246)	1261.65	1260.65
P4	LVNHFVEEFK ⁺ R (237-247)	1417.72	1416.75
P5	AQIHDLVLVGGSTR (329-342)	1464.77	1464.80
P6	TTPSYVAFTDTER (37-49)	1487.66	1486.69
P7	ATAGDTHLGGEDFDNR (221-236)	1675.69	1674.72
P8	HWP ⁺ FQVINDGDKPK (89-102)	1680.83	1679.84
P9	IINEPTAAAIAYGLDR (172-187)	1687.87	1686.89
P10	AAAIGIDLGT ⁺ TYSCVGVFQHGK (4-25)	2208.06	2207.10
P11	GVPQIEVTFDIDANGILNVTATDK (470-493)	2530.25	2529.29
P12	QTQIFT ⁺ TYSDNQPGLIQVYEGER (424-447)	2786.34	2785.35
P13	TLSSSTQASLEIDSLFEGIDFYTSITR (273-299)	2981.43	2980.45
P14	EIAEAYLGYPVTNAVITVPAYFNDSQR (129-155)	3001.48	3000.48

^a The matched peptides cover 37% (239 of 641 amino acids) of the proteins.

^b Monoisotopic mass.

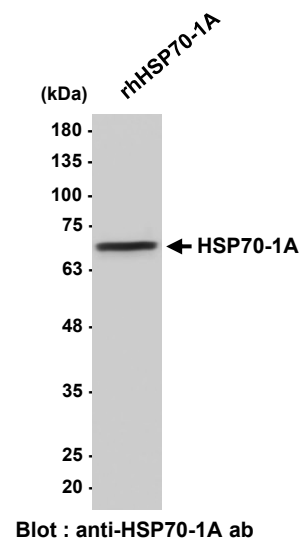
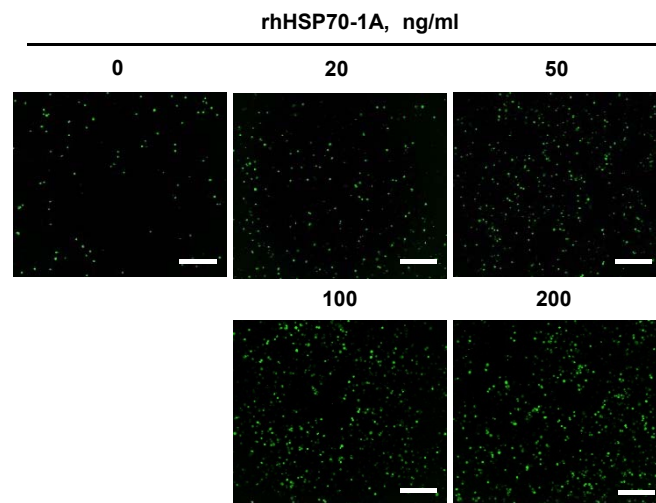
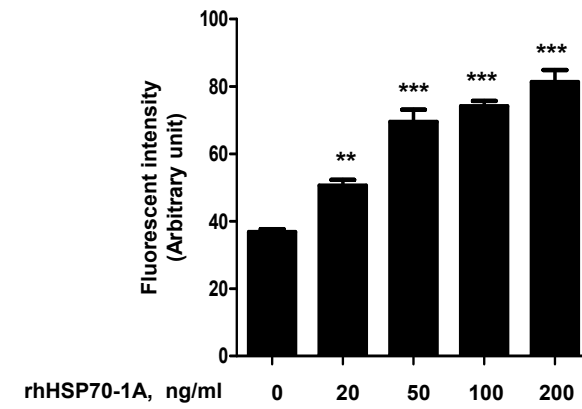


Fig. S1

A**B****Fig. S2**

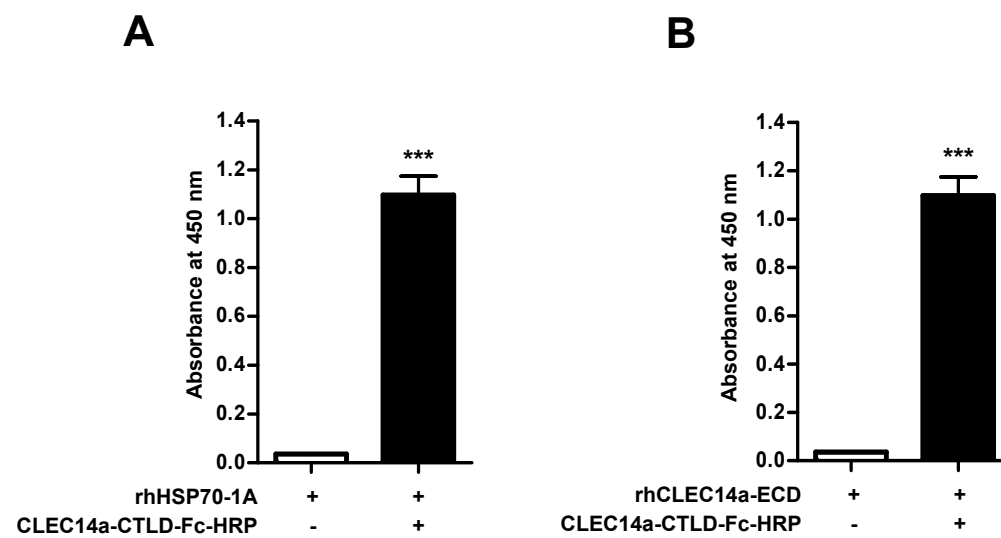


Fig. S3

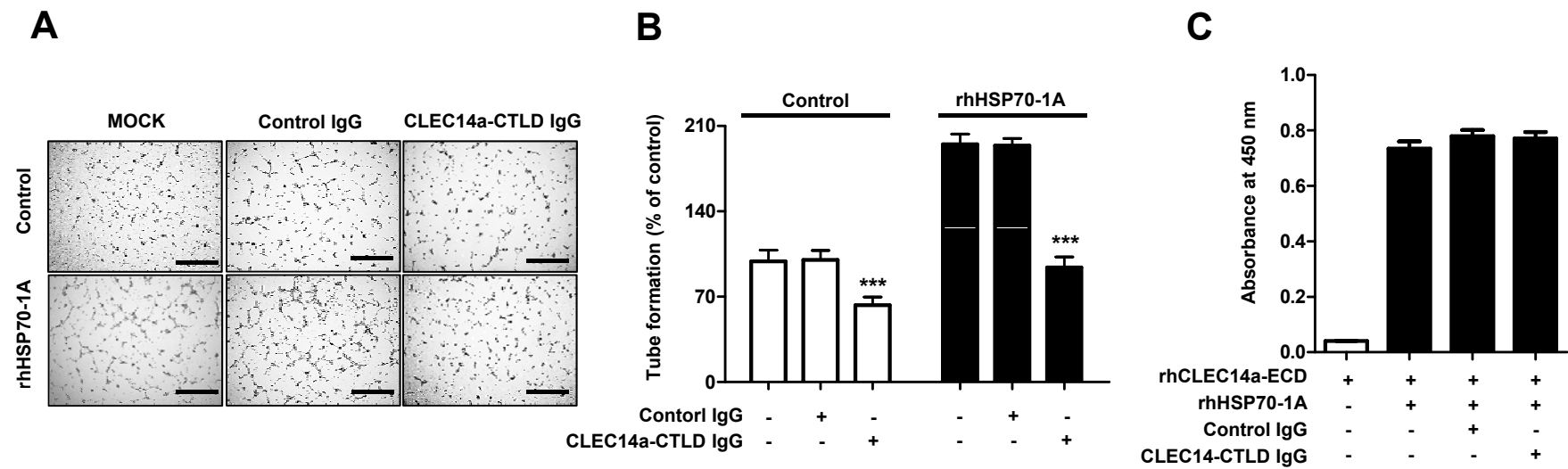


Fig. S4

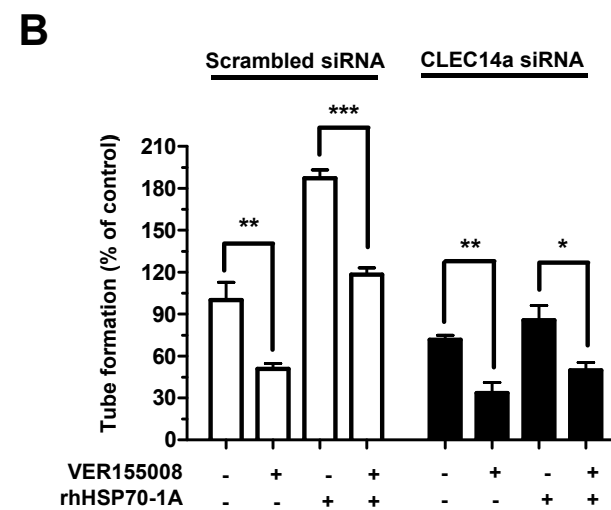
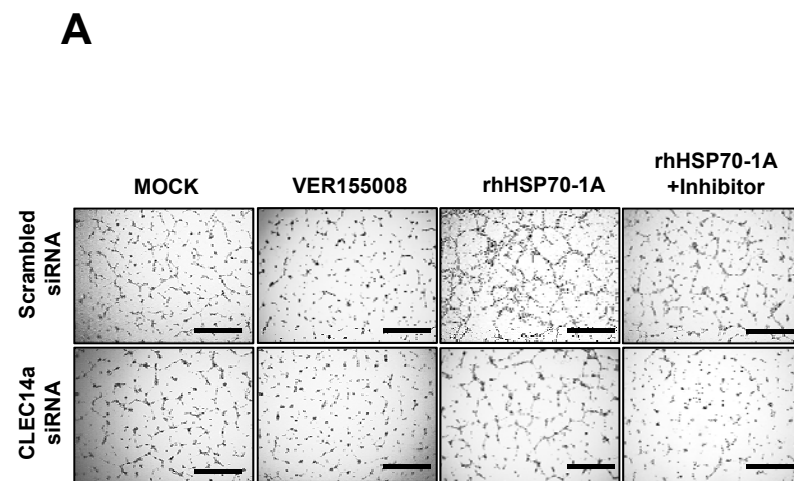
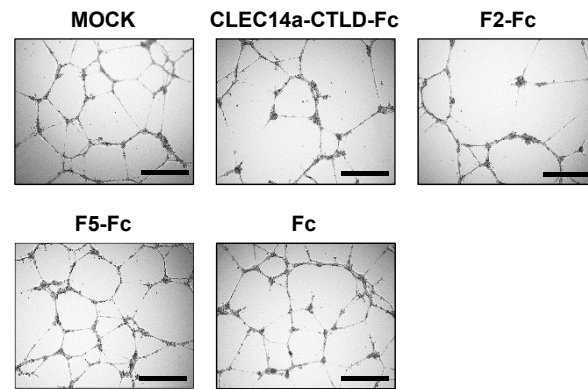
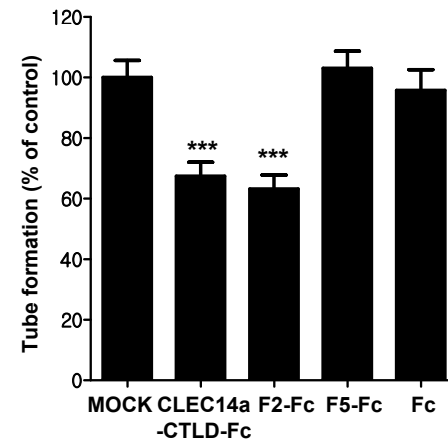


Fig. S5

A**B****Fig. S6**

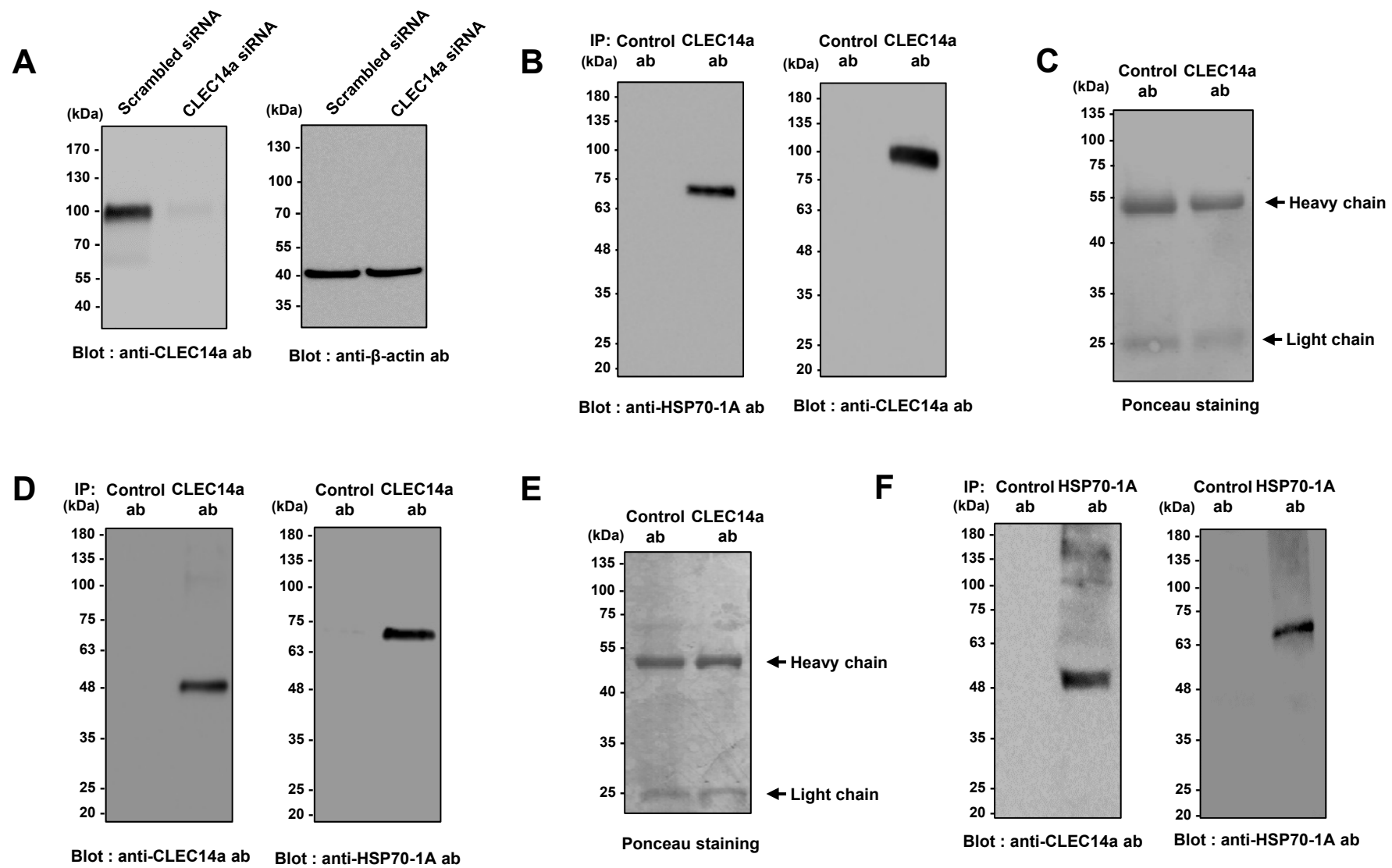


Fig. S7

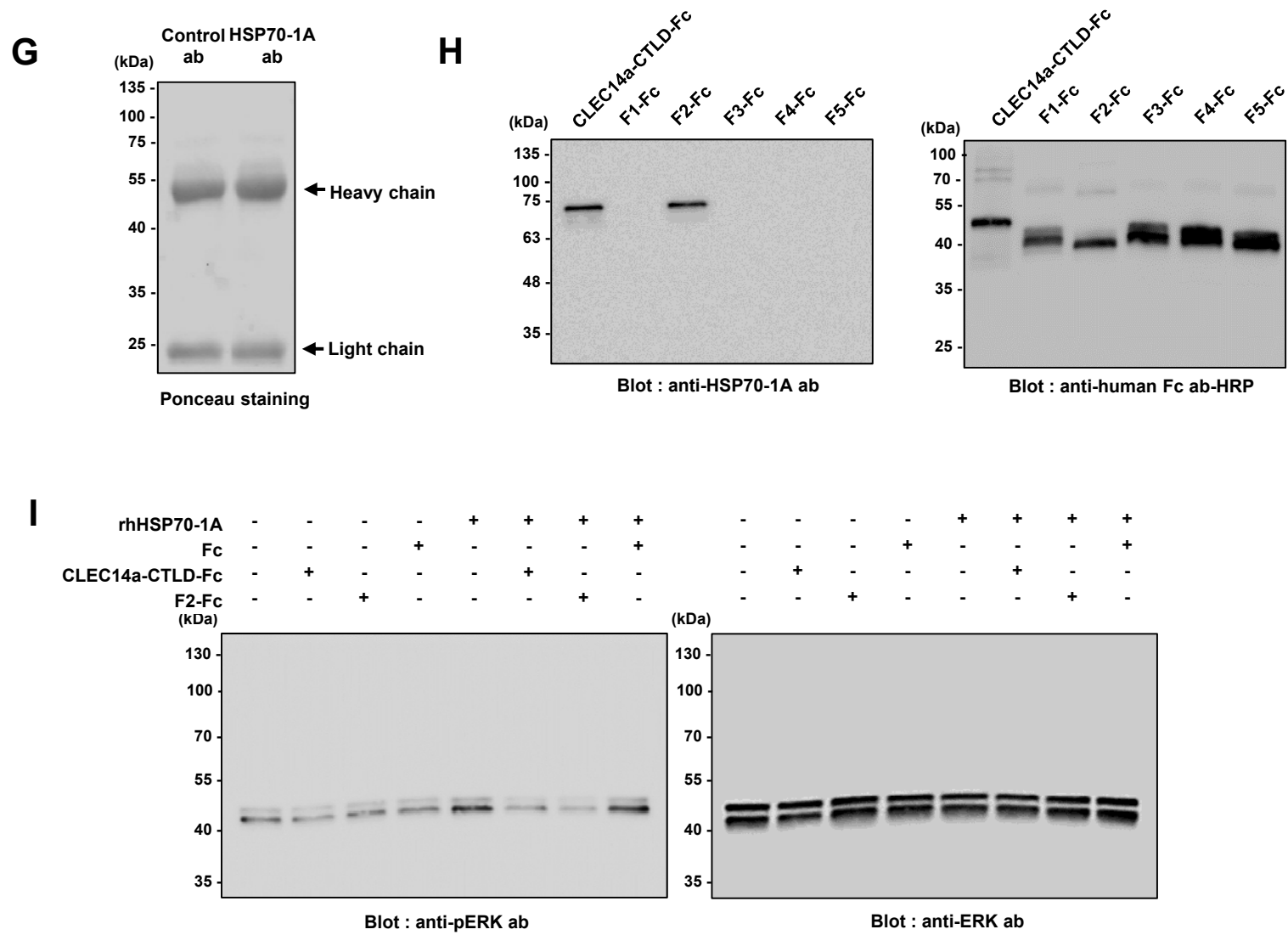


Fig. S7