Supplemental material

Turan et al., https://doi.org/10.1083/jcb.201801151

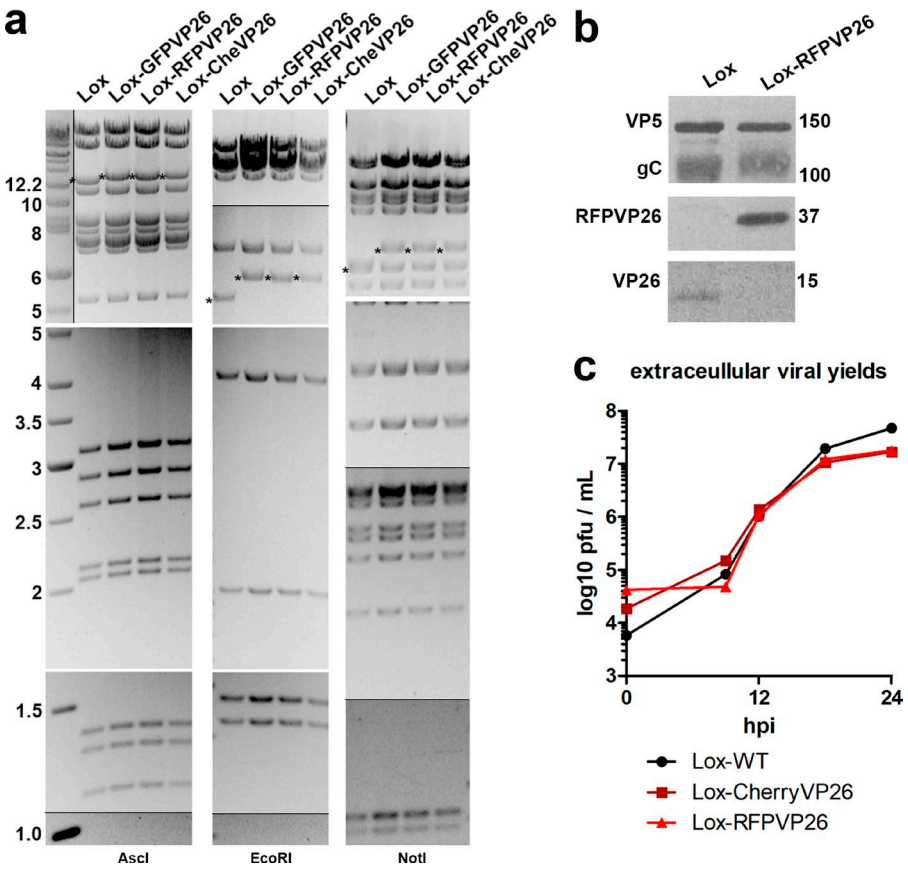

Figure S1. **Characterization of HSV1(17⁺)Lox-mRFPVP26. (a)** Agarose gel electrophoresis of restriction digests of pHSV1(17+)Lox, pHSV1(17⁺)Lox-EGFPVP26, HSV1(17⁺)Lox-mRFPVP26, and pHSV1(17⁺)Lox-CheVP26 with AscI, EcoRI, and NotI. All BACs showed the expected band shifts (*), which were calculated based on the published sequences of HSV1(17⁺) (GenBank accession number NC_001806). **(b)** Incorporation of RFP VP26 into mature extracellular viral particles was analyzed by immunoblotting. 6×10^7 or 1.3×10^7 PFUs per lane of HSV1(17⁺)Lox or HSV1(17⁺)Lox-mRFPVP26, respectively, were loaded onto a 6–18% gradient gel, transferred onto nitrocellulose, and probed with antibodies against anti-VP5, anti-gC, and anti-VP26. **(c)** For single-step growth kinetics, Vero cells were infected with 5 PFUs/ml HSV(17⁺)Lox, HSV1(17⁺)Lox-CherryVP26, or HSV1(17⁺)Lox-mRFPVP26, and secreted infectious virus at the indicated time points was titrated on Vero cells. This experiment was performed twice.

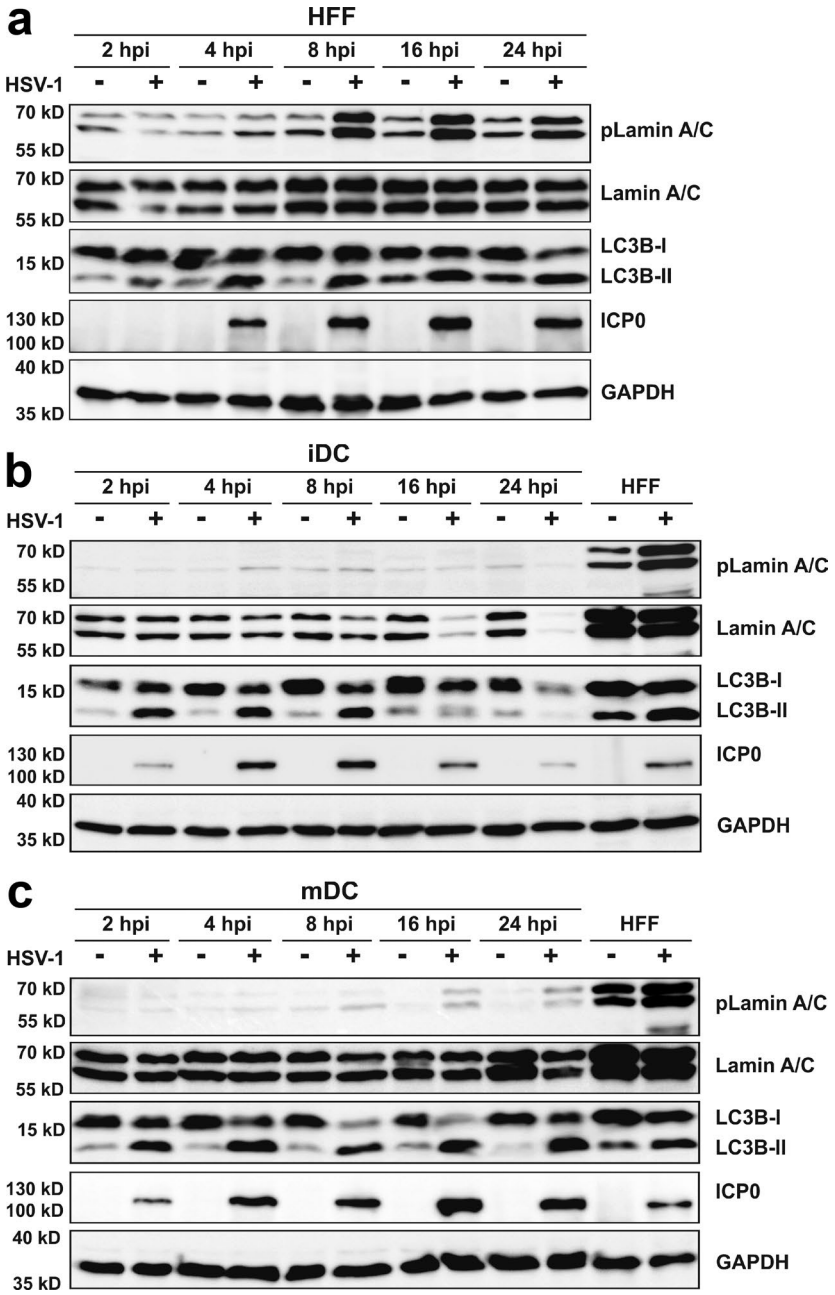

Figure S2. **Lack of lamin A/C phosphorylation in HSV-1–infected iDCs and mDCs. (a–c)** HFF (a), iDCs (b), and mDCs (c; 2×10^6) were mock- or HSV-1–infected and harvested at the indicated time points after infection. Cells were lysed and protein lysates were subjected to immunoblot analyses. Specific antibodies raised against phospho-lamin A/C (pLamin A/C), lamin A/C, LC3B, ICP0, or GAPDH (as an internal loading control) were used. Experiments were performed at least three times with cells from different healthy donors.

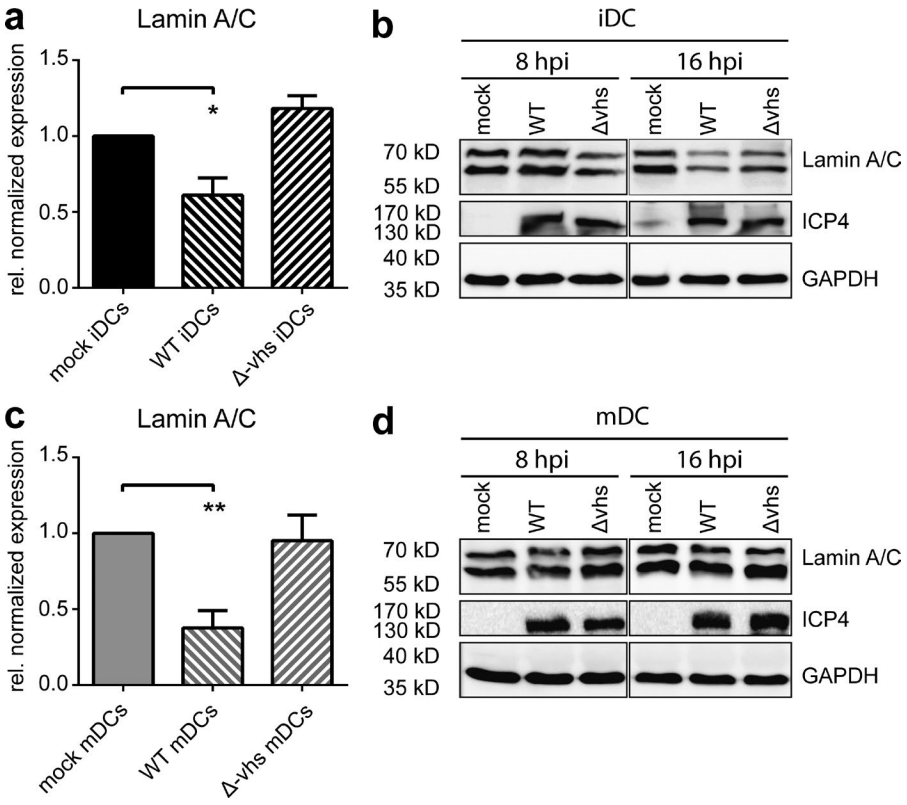

Figure S3. **Loss of lamin A/C protein levels is independent from its transcriptional regulation. (a–d)** iDCs (a and b) and mDCs (c and d) were mock-, HSV-1 WT–, or HSV-1 Δvhs–infected. For expression analyses of lamin A/C mRNA via quantitative real-time PCR, cells were harvested 8 hpi (a and c). For protein expression analyses of lamin A/C using immunoblotting, samples were analyzed 8 and 16 hpi (b and d). Specific antibodies raised against lamin A/C, ICP4, or GAPDH (as an internal loading control) were used. Error bars indicate SEM. Significant changes are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$). Experiments were performed at least three times with cells of different healthy donors.

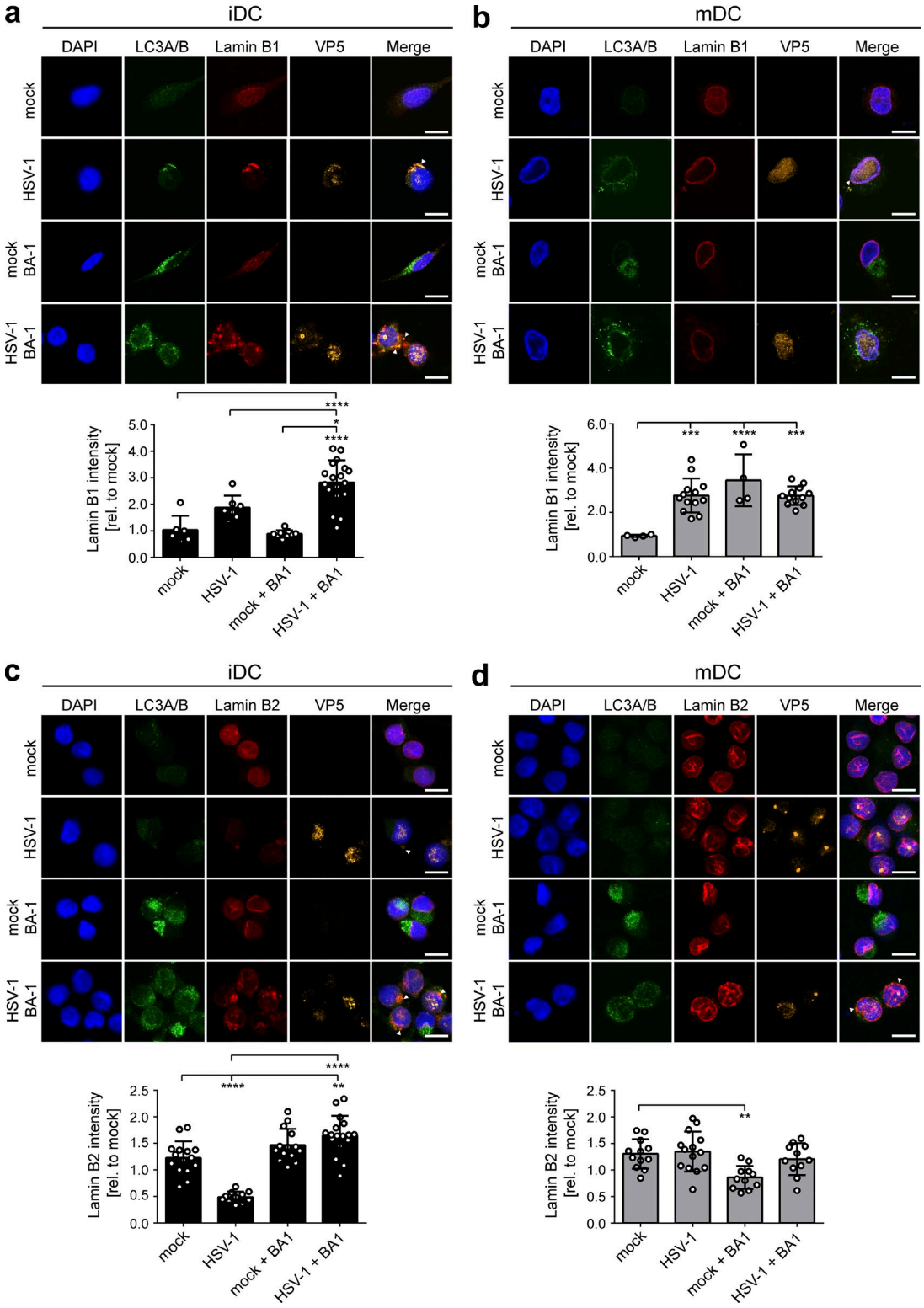

Figure S4. **Nuclear lamin proteins are cargos of autophagosomes in iDCs and mDCs. (a–d)** iDCs (a and c) and mDCs (b and d) were mock- or HSV-1–infected and treated with or without BA-1. After 24 h of incubation, cells were analyzed via immunofluorescence for expression of LC3A/B (green) and lamin B1 (a and b) or lamin B2 (red; c and d). VP5 (orange) indicates viral capsids. We quantified lamin B1 (a and b) and lamin B2 (c and d) immunofluorescence mean signal intensities. Values are shown relative to mock signal (lamin B1: iDC mock, $n = 6$; iDC HSV-1, $n = 7$; iDC mock + BA1, $n = 9$; iDC HSV-1 + BA1, $n = 18$; mDC mock, $n = 4$; mDC HSV-1, $n = 13$; mDC mock + BA1, $n = 4$; mDC HSV-1 + BA1, $n = 12$; lamin B2: iDC mock, $n = 15$; iDC HSV-1, $n = 11$; iDC mock + BA1, $n = 14$; iDC HSV-1 + BA1, $n = 17$; mDC mock, $n = 12$; mDC HSV-1, $n = 14$; mDC mock + BA1, $n = 11$; mDC HSV-1 + BA1, $n = 11$). Error bars indicate SD. Significant changes were analyzed using one-way ANOVA and Bonferroni multiple comparison post hoc tests and are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$). Nuclei were stained using DAPI (blue). Scale bars, 10 µm. Arrowheads indicate colocalization of LC3A/B and lamin proteins.

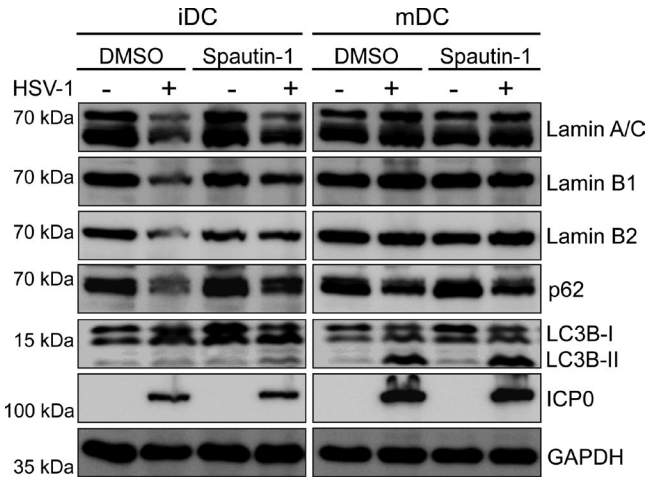

Figure S5. **Treatment with the autophagy inhibitor spautin-1 reduces lamin degradation in HSV-1–infected iDCs.** iDCs or mDCs (3×10^6) were pretreated with spautin-1 or DMSO for 1 h before infection. Subsequently, iDCs and mDCs were mock- or HSV-1–infected. Cells were harvested 18 hpi and subjected to Western blot analysis. Protein levels of lamin A/C, lamin B1, lamin B2, and LC3B were determined using Western blot analyses. Infection efficiency was checked by ICP0, and GAPDH was used as a loading control. The experiment was performed at least three times with cells of different healthy donors.

Table S1. **Antibodies used for immunoblot, immunofluorescence, and immunoprecipitation**

Antibody	Clone	Origin	Application	Supplier/reference
Anti-ARL8 A/B	H-8	Mouse	IB	Santa Cruz Biotechnology
Anti-GAPDH	6C5	Mouse	IB	Millipore
Anti-GAPDH	14C10	Rabbit	IB	Cell Signaling
Anti-gC	Polyclonal	Rabbit	IB	Eisenberg et al., 1987
Anti-ICP0	11060	Mouse	IB	Santa Cruz Biotechnology
Anti-ICP4	10F1	Mouse	IB	Santa Cruz Biotechnology
Anti-ICP5 (VP5)	3B6	Mouse	IB, IF	Santa Cruz Biotechnology
Anti-VP5	H1.4	Mouse	IB	Meridian Life Science
Anti-KIF1B	E-12	Mouse	IB	Santa Cruz Biotechnology
Anti-KIF2A	D-7	Mouse	IB	Santa Cruz Biotechnology
Anti-FIP200	D10D11	Rabbit	IB	Cell Signaling
Anti-lamin A/C	E-1	Mouse	IB	Santa Cruz Biotechnology
Anti-lamin A/C	2032S	Rabbit	IB, IF	Cell Signaling
Anti-lamin B1	A11	Mouse	IB	Santa Cruz Biotechnology
Anti-lamin B1	D4Q4Z	Rabbit	IB	Cell Signaling
Anti-lamin B1	Polyclonal	Rabbit	IF	Santa Cruz Biotechnology
Anti-lamin B2	F-8	Mouse	IB	Santa Cruz Biotechnology
Anti-lamin B2	D8P3U	Rabbit	IB, IF	Cell Signaling
Anti-LC3B	D11	Rabbit	IB, IP	Cell Signaling
Anti-LC3B	Polyclonal	Mouse	IP	Santa Cruz Biotechnology
Anti-mTOR	L27D4	Mouse	IB	Cell Signaling
Anti-p62	D5L76	Mouse	IB	Cell Signaling
Anti-p62	D5E2	Rabbit	IB	Cell Signaling
Anti-p-mTOR	D9C2	Rabbit	IB	Cell Signaling
Anti-VP26	Polyclonal	Rabbit	IB	Desai et al., 1998
Anti-mouse HRP	Polyclonal	Horse	IB	Cell Signaling
Anti-rabbit HRP	Polyclonal	Goat	IB	Cell Signaling
Anti-LAMP-1-Alexa Fluor 647	1D4B	Rat	IF	Santa Cruz Biotechnology
Anti-LC3A/B-Alexa Fluor 488	D3U4C	Rabbit	IF	Cell Signaling
Anti-rabbit Cy3	Poly4064	Donkey	IF	BioLegend
Anti-rabbit Alexa Fluor 568	Polyclonal	Goat	IF	Invitrogen
Anti-mouse Alexa Fluor 647	Polyclonal	Goat	IF	Invitrogen

IB, immunoblot; IF, immunofluorescence; IP, immunoprecipitation.

Table S2. **Raw data from mass spectrometry analysis of LC3B IP samples with three different donors**

Protein	p62/SQSTM1	Lamin A/C	Lamin B1	Lamin B2
UniProt accession number and name	Q13501 SQSTM_HUMAN	P02545 LMNA_HUMAN	E9PBF6 E9PBF6_HUMAN	Q03252 LMNB2_HUMAN
iDCs mock D1	7.98E5	1.27E5	5.15E5	4.6E4
iDCs mock D2	1.74E6	4.82E4	3.97E5	8.68E4
iDCs mock D3	1.82E6	2.37E5	8.17E5	5.74E4
iDCs HSV-1 D1	4.54E4	4.77E3	7.83E4	0.00E+00
iDCs HSV-1 D2	6.25E5	2.12E5	6.92E5	1.01E5
iDCs HSV-1 D3	1.38E5	3.04E4	5.94E4	4.55E3
mDCs mock D1	8.5E5	1.84E5	6.05E5	4.71E4
mDCs mock D2	1.53E6	9.68E4	7.5E4	3.95E4
mDCs mock D3	1.39E6	1.11E5	2.89E4	4.33E3
mDCs HSV-1 D1	4.03E5	1.49E5	6.54E5	4.95E4
mDCs HSV-1 D2	8.12E5	1.2E5	2.13E5	4.8E4
mDCs HSV-1 D3	2.9E5	1.15E5	2.33E5	9.7E3

D, donor.

Provided online is a supplemental ZIP file containing two custom-written macros. The custom-written macro for the quantification of HSV1-RFPVP26 signals located inside versus outside the nucleus is designated as MacroCapsidLocalization. The custom-written macro for the quantification of lamin and LC3B immunofluorescence signal intensities is designated as MacroLaminLC3B.

References

Desai, P., N.A. DeLuca, and S. Person. 1998. Herpes simplex virus type 1 VP26 is not essential for replication in cell culture but influences production of infectious virus in the nervous system of infected mice. *Virology.* 247:115–124. https://doi.org/10.1006/viro.1998.9230

Eisenberg, R.J., M. Ponce de Leon, H.M. Friedman, L.F. Fries, M.M. Frank, J.C. Hastings, and G.H. Cohen. 1987. Complement component C3b binds directly to purified glycoprotein C of herpes simplex virus types 1 and 2. *Microb. Pathog.* 3:423–435. https://doi.org/10.1016/0882-4010(87)90012-X