SUPPLEMENTAL METHODS

Cell culture

Human EndoC-βH1/2, 293T and HepG2 cells were cultured as previously described (1, 2). Mouse NIH3T3 cells were cultured in F12/DMEM (50/50) medium (Invitrogen#31330-095) plus 10% FCS (Eurobio) and antibiotics. Human 22Rv1 (ATCC#CRL-2505) (a gift from V. Goffin, IRNEM, Paris, France), rat RIN-5F (ATCC#CRL-2058) and mouse βTC3 (a gift from D. Hanahan, ISREC, Lausanne, Switzerland) cells were cultured according to ATCC recommendations. βTC3 cells were cultured either on plastic or onto matrigel- and fibronectin-coated dishes. Mouse MIN6 and Rat INS-1E cells were cultured in the same medium as βTC3 and RIN-5F cells, respectively, supplemented in each case with sodium pyruvate (1 mM) (Life Technologies#11360-039), HEPES (10 mM) (Life Technologies#15630-080) and 2-mercaptoethanol (50 mM) (Sigma-Aldrich#M6250).

Immunocytochemistry

EndoC-βH1 or HepG2 cells were seeded in 8-well chamber slides (labtek#177402) and incubated overnight. mAb 13F25 or the isotype control mAb (directed against trinitrophenol) were preincubated with a Alexa Fluor 488-coupled donkey anti mouse IgG antibody (ImmunoResearch#715-545-151) for 30 min and then added on live cells in serum free media at 1ug/ml for 20 min. After incubation, cells were washed, fixed for 15 min. in 4% PFA. Pictures were obtained on an Axiovision fluorescence microscope. TNP and 13F25 are mouse monoclonal antibodies developed at Novo Nordisk.

IP and mass spectrometry

mAb 13F25 and isotype control Ab, were coupled to M270 tosylactivated magnetic beads (Invitrogen#14203) following manufacturers procedures. Lysates were prepared from one confluent T175 flask of EndoC-βH1 cells, which were lysed on ice in 1 ml NP-40

lysis buffer (Invitrogen#FNN0021A) with protease inhibitor cocktail (Roche#11836153001). For IP, Ab coupled beads were incubated with 1 ml cell lysate for 1 hr at 4ºC. After incubation the beads were washed five times in PBS with 0.1% BSA. Bound protein was eluted by incubation in glycine buffer at pH= 2.8. The eluted protein was treated with PNGAse F, separated by SDS-PAGE and visualized by staining with Coomassie brilliant blue (Thermo scientific Cat. 24615). For protein identification, bands were excised from the gel and subjected to in-gel digestion with trypsin as described (3). Digested peptides were acidified by addition of 2%ACN, 0.5% AcOH, 0.1%TFA and loaded on a stageTip as described (4). The StageTip was subsequently washed using; MeOH (100%); 60%ACN, 0.5% AcOH and 2%ACN, 0.5% AcOH, 0.1%TFA. After loading, the column was washed with 0.5% AcOH and the samples were eluted into a 96 well plate with 60% ACN, 0.5% AcOH. The samples were dried down by vacuum centrifugation and resuspended in 0.1% FA and 1% TFA. The samples were analyzed by LC-MS/MS on a Q-exactive instrument (Thermo Scientific, Bremen, Germany).

Primer sequences for genomic Bxv1 amplification

name	sequence	amplicon
P-ENVs	ATGGAAGGTCCAGCGTTCTC	1453pb
P-ENVas	CCTGGAGCTGCTCGAATTGT	
P0s	AATTCGAGCAGCTCCAGGCA	544pb
P0as	TCTTTCATTCCCCCCTCTTTCTGGA	
P1s	CCCGATCAGTTTGTGTTTGA	1236pb
P1as	GGAAGGTGTCTGTCATCGTC	
P2s	AAAACTGGGACCTTGGCGTCGG	1203pb
P2as	ACTCGTACCCCTGCCCCGATTT	
P3s	CGAAGCCAGACTGGGGATCA	1137pb
P3as	GGTTGCTTGACCAGTGCCTC	
P4s	ATCCATGTCGTTCATCTGGC	1200pb

P4as	TTGTAAGGATTGGGCACGGT	
P5s	CGCAAGATGGTACTTTTAAT	1203pb
P5as	CCTCTTTTTCCTCTGTTTCT	
P6s	TCGTCCGGGATTTGGAGACC	770pb
P6as	CGGAGGGGGTTCATAGGCAA	

Molecular identification of Bxv1 in EndoC-βH1/2 cells

Genomic DNA was extracted using the NucleoSpin® Tissue kit (Macherey-Nagel#740952.50) following manufacturer instructions. 5 ng of extracted genomic DNA was used for PCR amplification using GoTaq (Promega#M8291) recommended procedure and 35 amplification cycles composed of 1 min at 94°C, 1 min at 55 °C and 2 min at 72 °C. The resulting PCR products were either loaded on 1% agarose gel or extracted using Wizard PCR purification Kit (Promega#A9282) followed by Sanger sequencing on both stranded with each separated PCR primers.

Real time RT-PCR

Real time RT-PCR for expression of *Bxv1* transcripts was performed as previously described (1) using the following primers hybridizing the *Bxv1 env* sequence : Fw 5' gtatgacttctatgtttgcc 3'; Rev : 5' ccatgatgatgatggcttcc 3'. Expression of *Bxv1* transcripts in each population or cell line was calculated with the ΔΔCt method of relative quantification using the expression levels of *Cyclophilin A* (*CycA*) mRNA (of the relevant species) for normalization. To sequence the Env encoding cDNA fragment amplified from βTC3 and MIN6 cells, PCR were done with the same primers, using GoTaq and 30 ng of cDNA submitted to 40 cycles: 40 s at 95°C, 30 s at 60°C and 40 s at 72°C. The amplicons were purified and sequenced through standard procedures. EndoC-βH2-CRE and EndoC-βH2 cells have been transduced with a retrovector encoding CRE recombinase fused to GFP (pPRIHy-CRE-GFP), or with the control retrovector (pPRIHy),

respectively, then selected in hygromycin (50 µg/ml) (Invitrogen#10687-010). Analyses were performed 21 d after transduction. Withdrawal from cell cycle of EndoC-βH2-CRE cells was validated by measuring Ki67 mRNA expression (1).

***Virus production and quantification*.**

Virus quantification was performed by TexCell an independent viral safety company. Infectious murine ampho- or xenotropic retroviruses are routinely detected and quantified by a PG4 S+L- infectivity test (5). Briefly, media from a potentially infected cell line is transferred to *Mus dunni* cells allowing virus amplification. Then, media is tested for its ability to induce focus formation in the PG4 S+L- cell line originating from cat brain. For quantification, the end point dilution assay was used. Culture media of cell to be tested was collected after 7 d in culture. Media was diluted and each dilution was tested in 10 replicates for its ability to induce focus formation in PG4 S+L- cells. The lowest dilution which induced foci in a least half of the replicates was used to determine the TCID50 using the Spearman-Kärber formula as described elsewhere (6).

Propagation and trans-complementation assays

10^6 293T cells in 10 cm dishes were exposed for 4-6 h to 6-8ml of EndoC-βH1 conditioned medium (CM) in presence of polybrene (8 µg/ml) (Sigma-Aldrich#107689). For propagation assays, real time RT-PCR for *Bxv1* transcription was performed in 293T+EndoC-βH1 CM cells after 13 d and two passages to ensure that transcripts are endogenously produced. Except for this case, each population was cultured for 4 weeks between exposure to the contaminating medium and harvesting of RNA. MuLV-based retrovector trans-complementation assays were achieved by exposing 10^6 293T-TVA-Hy cells (293T cells transduced with the pPRIHy-TVA retrovector) to 6-8 ml of EndoC-βH1 CM with polybrene leading to 293T-TVA-Hy* cells. Naive 293T cells were next exposed to the CM of 293T-TVA-Hy* cells (6-8 ml with polybrene), cultured for 24-48

h, then selected in hygromycin (100-200 µg/ml) for 10-15 d. After 2 days in selection, cells were passaged once ($1/10^{e}$) to seed one Petri dish for fixation and coloration (paraformaldehyde 3%, Electron Microscopy Sciences#157145; crystal violet 0.05%, Sigma-Aldrich#V5265) and to another one for DNA and RNA extraction.

Xenotropic helper activity in mouse MIN6 and βTC3 and Rat INS-1E and RIN-5F cells was assessed under the same conditions after transduction of each cell line with the pPRIHy-TVA retrovector. RIN-5F cells were transduced with a VSV-G pseudoptyped retrovector, INS-1E, MIN6 and βTC3 cells with an ecotropic retrovector. Production of retrovectors was done as previously described (1). Each derived cell line was selected for at least 3 weeks in hygromycin (50-100 µg/ml) before testing its CM on naive 293T cells. For all these experiments, xenotropic helper activity in EndoC-βH2 cells also transduced with the pPRIHy-TVA retrovector was assessed in parallel to provide a positive control.

The xenotropic nature of the mobilized retrovector in EndoC-βH2 cells was tested under the same conditions in either parental NIH3T3 cells or NIHT3T3 cells stably transduced with the pLhXpr1SN retrovector (7; a gift from A.D. Miller, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA) encoding the human XPR1 (huXPR1) receptor for xenotropic and polytropic retrovirus (NIH3T3-huXPR1). huXPR1-transduced NIHT3T3 cells were selected in 200 µg/ml of geneticin (Invitrogen#10131027).

In all of the experiments described in this section, the CM were filtered on 0.45 µm (Dominique Dutscher#146561) just before their transfer to eliminate any contaminating cell.

Quantitative Bxv1 copy number determination

Genomic DNA from EndoC-βH1 and EndoC-βH2 cells were extracted as described above. For QPCR, two sets of primers were designed and used to amplify two distinct regions of *Bvx1* located in fragment P2 and P4 respectively (see figure 2B). Set-A: Bxv1-AF :

GTGACGACTGAAACCGAGGT Bxv1-AR : CTTGGGTGAGTGCGATCAGT located in fragment P2 and Set-B Bxv1-BF : ACTCTTTCCTCCATGTGCCG Bxv1-BR : CGAAGTGGATCTGGGCCTTT. Human Albumin was used as 2 copies per genome control and quantified using the following primers hAlbLC-R AAGCAGTGCACATCACATCAA and hAlbLC-F ACAAAGATGACAACCCAAACCT. To quantify copy number of *Bvx1* relative to human albumin, it is important to compensate for differences in primer efficacy. Therefore we perform 6 serial dilutions (1/4) of the genomic DNA and each dilution was probed with the 3 primer sets. The resulting Ct values were plotted according to the log of total DNA amount and linear regression was performed. For each primer set the regression coefficient was above 0.995. The extrapolated Ct values corresponding to the intercepts with the y axis were used for relative copy number determination.

Western blotting

Cell lysates were prepared by harvesting cells with 5mM EDTA in PBS, then spun down at 400g and lysed on ice in NP-40 lysis buffer (Invitrogen cat no FNN001) with protease inhibitor (Roche#11836153001). Lysates were cleared by centrifugation at 12.000 g. Total protein was measured using Pierce™ BCA protein Assay Kit (#23225). 25 µg of total protein was run unreduced on SDS-PAGE, and transferred to PVDF membrane. The primary antibodies were detected with a HRP conjugated goat anti-mouse IgG (Dako#P0447). Equivalent loading are validated using mouse monoclonal anti-β-

References

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Figure S1

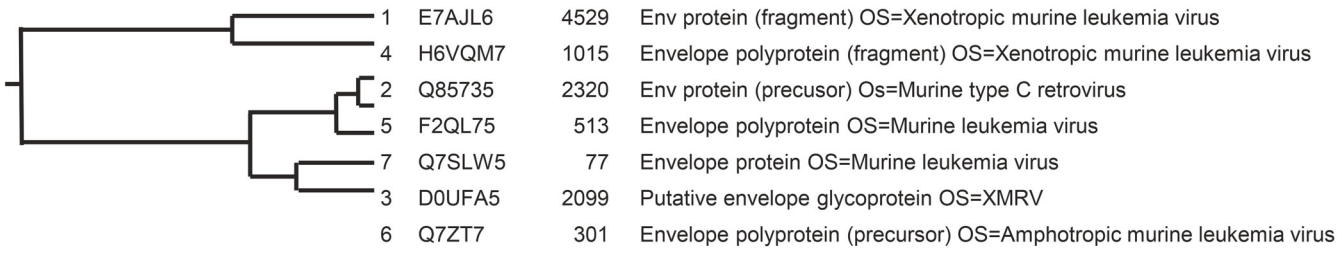

1 E7AJL6 4529 Env protein (fragment) OS=Xenotropic murine leukemia virus
4 H6VQM7 1015 Envelope polyprotein (fragment) OS=Xenotropic murine leukemia virus
2 Q85735 2320 Env protein (precusor) Os=Murine type C retrovirus
5 F2QL75 513 Envelope polyprotein OS=Murine leukemia virus
7 Q7SLW5 77 Envelope protein OS=Murine leukemia virus
3 D0UFA5 2099 Putative envelope glycoprotein OS=XMRV
6 Q7ZT7 301 Envelope polyprotein (precursor) OS=Amphotropic murine leukemia virus

Figure S2

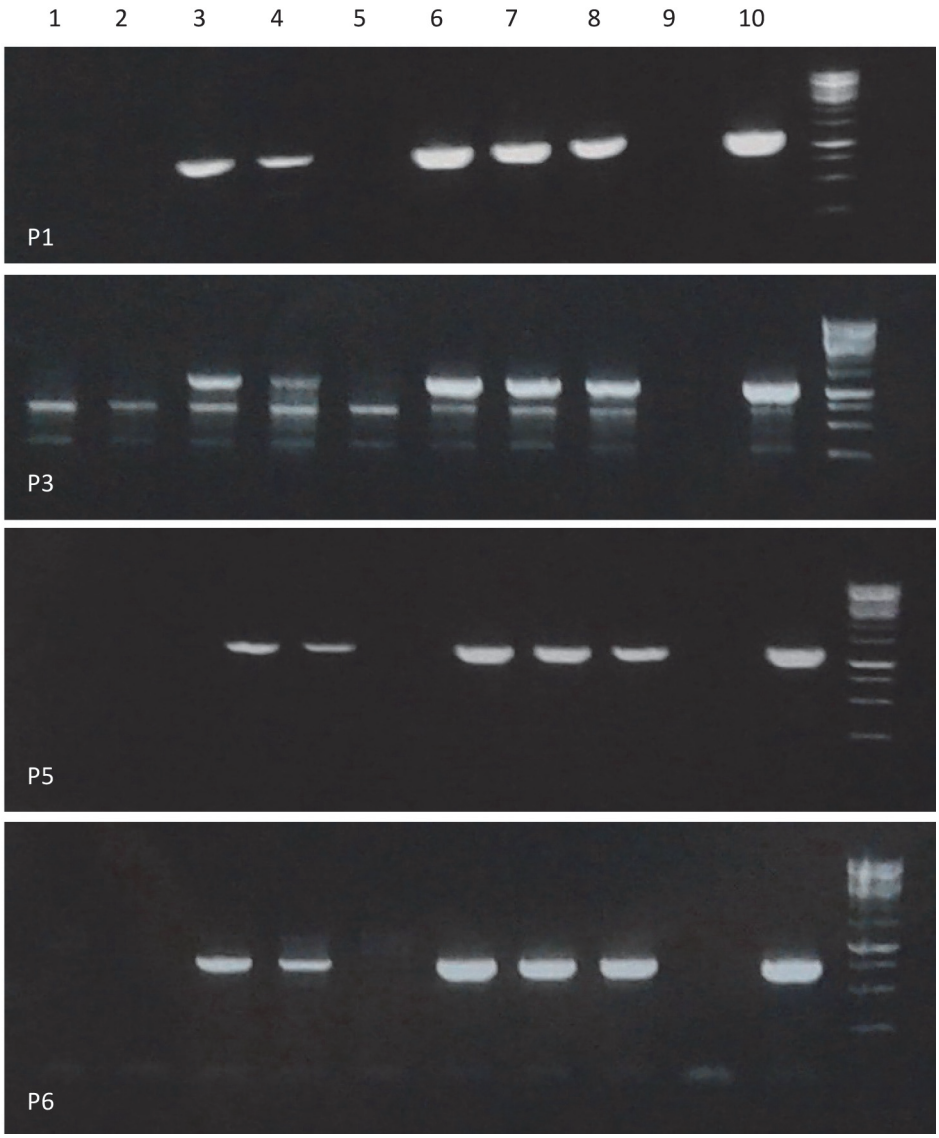


Figure S3

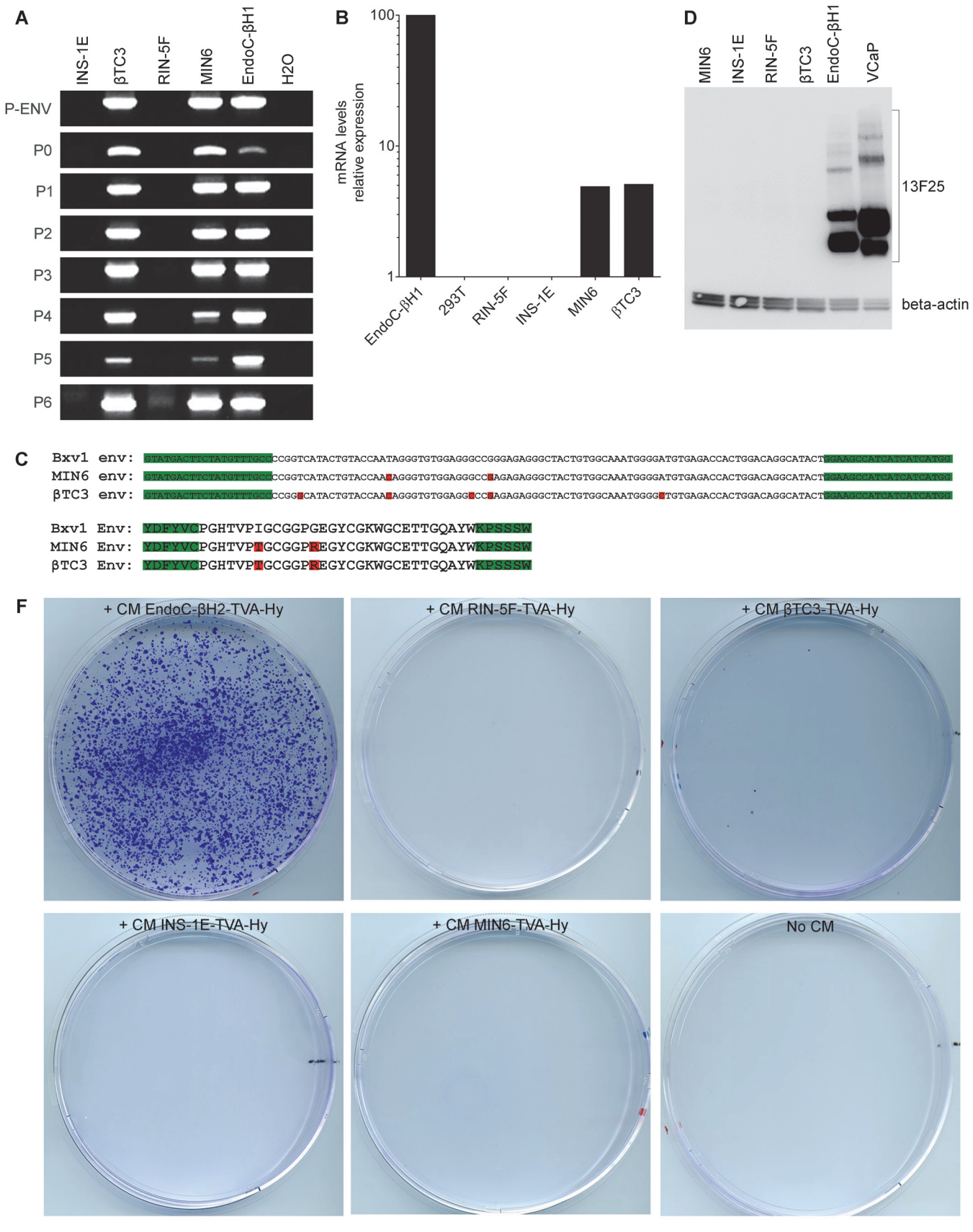

A
INS-1E
βTC3
RIN-5F
MIN6
EndoC-βH1
H2O
P-ENV
P0
P1
P2
P3
P4
P5
P6
B
100
10
1
mRNA levels
relative expression
EndoC-βH1
293T
RIN-5F
INS-1E
MIN6
βTC3
D
MIN6
INS-1E
RIN-5F
βTC3
EndoC-βH1
VCaP
13F25
beta-actin
C
Bxv1 env:
MIN6 env:
βTC3 env:
Bxv1 Env: YDFYVCPGHTVPIGCGGPGEGYCGKWGCETTGQAYWKPSSSW
MIN6 Env: YDFYVCPGHTVPTGCGGPREGYCGKWGCETTGQAYWKPSSSW
βTC3 Env: YDFYVCPGHTVPTGCGGPREGYCGKWGCETTGQAYWKPSSSW
F
+ CM EndoC-βH2-TVA-Hy
+ CM RIN-5F-TVA-Hy
+ CM βTC3-TVA-Hy
+ CM INS-1E-TVA-Hy
+ CM MIN6-TVA-Hy
No CM

Figure S1. Dendrogram of Mass Spectroscopy results.

Identified peptides were searched in the NCBInr database using MASCOT. The peptides mapped to a group of highly similar virus envelope proteins from xenotropic MuLVs.

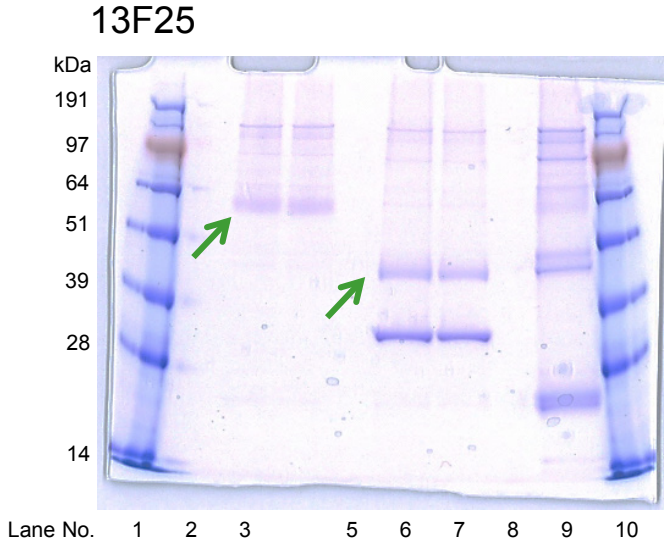
Figure S2. **PCR analyses for *Bxv1* genomic sequence.** The genomic DNA of the following cell populations were analyzed by PCR with the indicated primers to detect *Bxv1* sequences (see Figures 2B and 3A for primer and cell description). 1 : 293T; 2 : 293T-TVA-Hy; 3 : 293T-TVA-Hy*; 4 : 293T*; 5 : 293T; 6 : 293-T1; 7 : 293-T2; 8 : 293-T3; 9 : H_2O; 10 : EndoC-βH1. Each population was cultured 4 weeks between the exposure to the contaminating medium and the harvesting of genomic DNA.

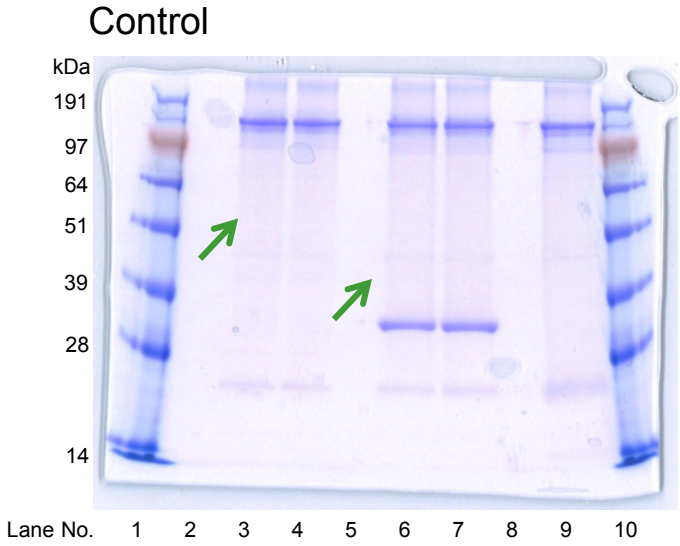
Figure S3. **Analysis of X-MuLVs presence and expression in rodent β cell lines.** Four commonly used rodent β cell lines, rat INS-1E and RIN-5F, and mouse MIN6 and βTC3, were analyzed for the presence and expression of *Bxv1* or closely related X-MuLVs. A) Genomic PCR with the same pair of primers as in Figure 2 (n= 3) . B) Real time RT-PCR with the same *env* primers as in Figure 3B (n=3). C) Sequence of partial Env encoding amplicon generated by RT-PCR in mouse MIN6 and βTC3 cells. The changes in nucleotide and amino-acid sequences compared to *Bxv1* are highlighted (blue); the sequence of the primers and corresponding peptides are also highlighted (green). D) Western blot analyses using the 13F25 antibody to detect xenotropic Env. Only the positive controls, EndoC-βH1 and VCaP cell lysates, show detectable expression (n=3). E) Helper assays. The four rodent cell lines were transduced with the MuLV-derived pPRiHy-TVA retrovector, and the conditioned medium (CM) of each derivative (RIN-5F-TVA-Hy, βTC3-TVA-Hy, INS-1E-TVA-Hy and MIN6-TVA-Hy) was added to 293T, which were then submitted to hygromycin selection, fixed and colored. For each derivative, helper assays have been carried out

at least three times and representative results are shown. In parallel, the CM of EndoC-βH2-TVA-Hy was systematically used as a positive control.

Uncut gels Figure 1B

Part 1 from Lysate



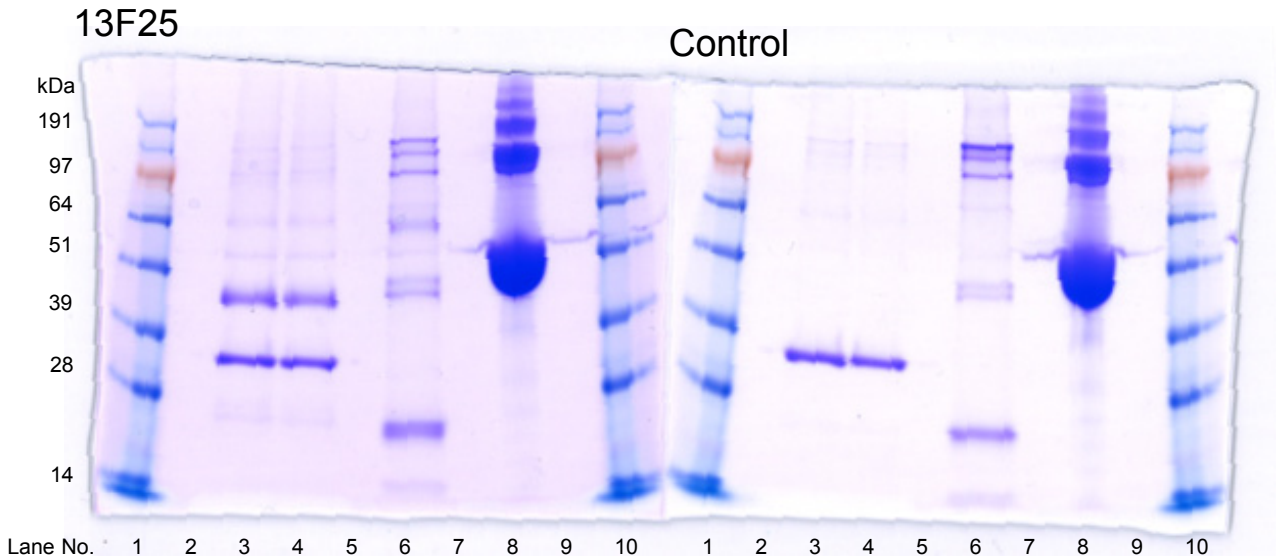


Lane	1	2	3	4	5	6	7	8	9	10
Sample	Seeblue Ladder	Empty	Non-treated	Non treated	Empty	PNGaseF	PNGAse F	Empty	SDS elution	Seeblue

All lanes are eluates from IP in EndoC-βH1 lysates with either 13F25 or control.
The eluates have been treated as indicated in the table.

Uncut gels Figure 1B

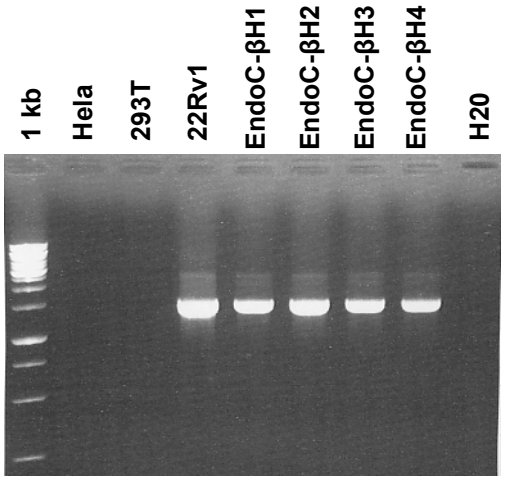
Part 2 from Media



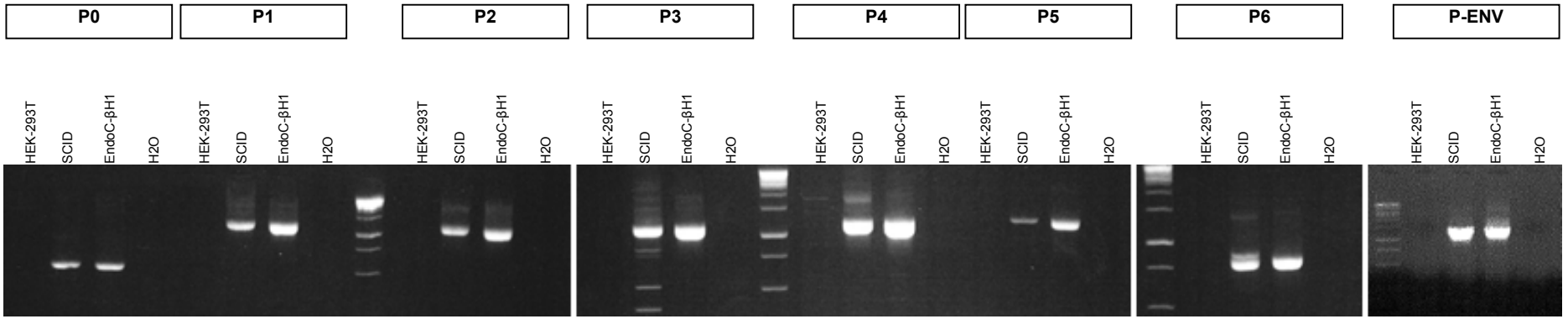
Lane	1	2	3	4	5	6	7	8	9	10
Sample	Seeblue Ladder	empty	PNGaseF	PNGase F	Empty	SDS elution	empty	Conditioned media	empty	Seeblue

All lanes are eluates from IP in EndoC-βH1 Media with either 13F25 or control.
The eluates have been treated as indicated in the table.

Uncut gels figure 2A



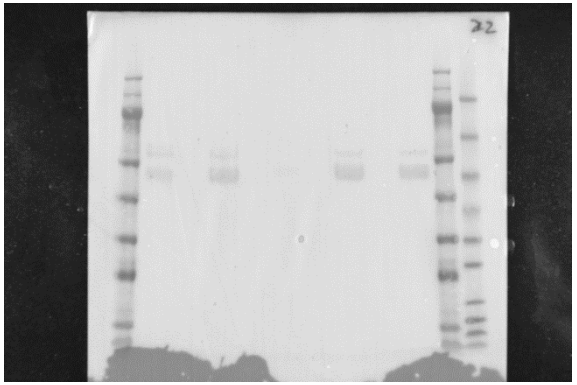
Uncut gels figure 2C



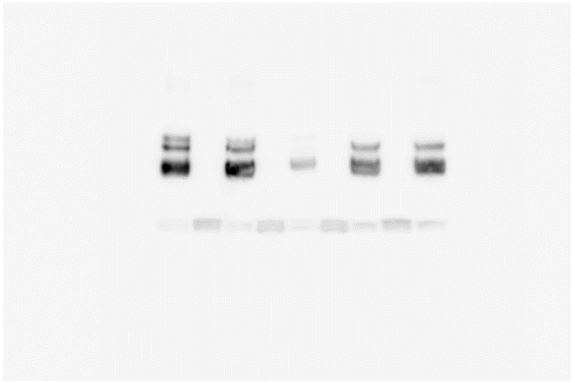
Uncut gels Figure 2D

Lane	1	2	3	4	5	6	7	8	9	10	11	12
Sample	BM	seeblue	Vcap2	Raw	Vcap2	Hep G2	EndoC non reduced	Hep G2	VCAP1	Raw	vcap1	seeblue

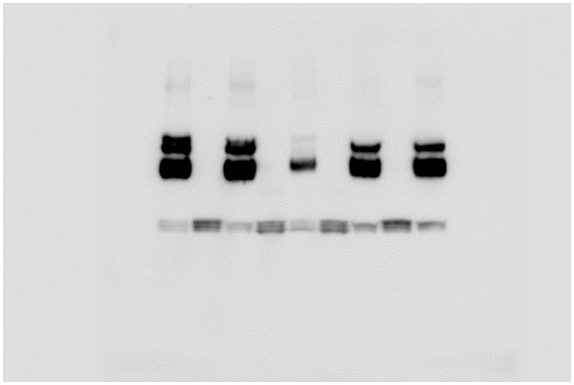
Picture of membrane



Short exposure



Long exposure



Uncut gels figure S3