Supplementary Materials

Table S1 to S5

Figure S1 to S8

References for Supplementary Materials

In:

Frequent Detection of Infectious Xenotropic Murine Leukemia Virus (XMLV) in Human Cultures Established from Mouse Xenografts

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Table S1. List of xenograft cell lines examined for murine leukemia virus detection

Cell line type	Cell lines[1]	Implants	Mice	Source: lab PI
NSCLC	H1703 xenograft	NCI-H1703 cells	SCID-NOD	R. Brekken
NSCLC	H1975 xenograft	NCI-H1975 cells	SCID-NOD	R. Brekken
NSCLC	H1993 xenograft	NCI-H1993 cells	SCID-NOD	R. Brekken
NSCLC	**1065met**	NCI-H1299 cells	nu/nu	B. Gao/J.Minna
NSCLC	LX7	Tumor	nu/nu	C. Rudin
NSCLC	LX14	Tumor	nu/nu	C. Rudin
NSCLC	LX31	Tumor	nu/nu	C. Rudin
SCLC	**NCI-N417**	Tumor	nu/nu	G. Bepler & A.Gazdar
SCLC	LX22	Tumor	nu/nu	C. Rudin
SCLC	LX33	Tumor	nu/nu	C. Rudin
SCLC	LX36	Tumor	nu/nu	C. Rudin
SCLC	**LX44**	Tumor	nu/nu	C. Rudin
SCLC	**LX47**	Tumor	nu/nu	C. Rudin
SCLC	LX48	Tumor	nu/nu	C. Rudin
Pancreas	PANC-1 xenograft	PANC-1 cells	nu/nu	R. Brekken
Pancreas	MIA PaCa-2 xenograft	MIA PaCa-2 cells	nu/nu	R. Brekken
Pancreas	XCL1	Tumor	nu/nu	A. Maitra
Pancreas	XCL2	Tumor	nu/nu	A. Maitra
Pancreas	XCL3	Tumor	nu/nu	A. Maitra
Pancreas	**CAK1**	Tumor	nu/nu	A. Maitra
Pancreas	Pa03C	Tumor	nu/nu	A. Maitra
Pancreas	Pa09C	Tumor	nu/nu	A. Maitra
Pancreas	Pa20C	Tumor	nu/nu	A. Maitra
Pancreas	Pa36C	Tumor	nu/nu	A. Maitra
Prostate	C4-2	LNCaP cells	nu/nu	JT Hsieh
Prostate	**LAPC-4**	Tumor	SCID	C. Sawyers & JT. Hsieh

1. The cell lines in bold were free of mouse DNA detection and positive for murine leukemia virus sequences. See Sup. References [1] for additional information on the pancreatic cell lines from A Maitra lab.

Table S2. List of non-xenograft cell lines examined for murine leukemia virus detection

Cell line type (no. of total lines)	Positive cell lines[1]	Negative cell lines	Source: Lab PI
NSCLC (49)	**NCI-H1155**	Calu3 PC9 NCI-H23 NCI-H522 NCI-H647 NCI-H650 NCI-H820 HCC827 NCI-H838 NCI-H920 HCC954 NCI-H969 DFC1024 DFC1032 NCI-H1264 NCI-1299 NCI-H1334 NCI-H1355 NCI-H1373 NCI-H1395 NCI-H1437 NCI-H1568 NCI-H1573 NCI-H1650 NCI-H1693 NCI-H1703 NCI-H1792 NCI-H1819 NCI-H1944 NCI-H1975 NCI-H1993 NCI-H2009 NCI-H2030 NCI-H2073 NCI-H2086 NCI-H2122 NCI-H2228 HCC2279 NCI-H2291 HCC2374 NCI-H2405 HCC2814 HCC2935 NCI-H3255 HCC4006 HCC4011 HCC4017 HCC4019	A.Gazdar
SCLC (54)	**NCI-H60 NCI-H182 NCI-H289 NCI-H1092 NCI-H1514**	HCC33 NCI-H69 NCI-H82 NCI-H128 NCI-H146 NCI-H187 NCI-H196 NCI-H209 NCI-H220 NCI-H345 NCI-H378 NCI-H510 NCI-H524 NCI-H526 NCI-H711 NCI-H738 NCI-H740 NCI-H748 NCI-H841 NCI-H865 NCI-H889 HCC970 NCI-H1045 NCI-H1048 NCI-H1105 NCI-1184 NCI-H1238 NCI-H1284 NCI-H1304 NCI-H1341 NCI-H1417 NCI-H1436 NCI-H1450 NCI-H1607 NCI-H1618 NCI-H1672 NCI-H1694 NCI-H1870 NCI-H1876 NCI-H1926 NCI-H1930 NCI-H1963 NCI-H1994 NCI-H2029 NCI-H2107 NCI-H2141 NCI-H2171 NCI-H2195 NCI-H2196	A.Gazdar
NSCLC (4)	**NCI-H460**	NCI-H358 H460TB10 H4606-05	C.Rudin
SCLC (4)	**NCI-H82 NCI-H146**	H82DS-06 H146CH-05	C.Rudin
Pancreas (9)		MIAPaCa-2 PANC-1 ASPC1 BXPC3 Panc8.13 Pa29C Pa21C Pa28C Pa16C	A.Maitra
Colon (1)	**RKO[2]**		A.Maitra
Pancreas (2)		MIAPaCa-2 PANC-1	R.Brekken
Prostate (7)	**PC3 PrEC2 LNCaP**	DU145 PZ-HPV-7 PrEC3 MDAPCa	JT.Hsieh

1. These cell lines in bold were detected positive for murine leukeima virus sequences in their late passage of cultures (see the Results).
2. The human colon carcinoma RKO culture from another source (Gazdar lab, originally from the American Type Culture Collection, Manassas, VA)(Sup. Ref. 2) was negative for XMLV and XMRV, implying XMRV contamination in the RKO culture from Maitra lab (see the Results).

Table S3. Primer sequences

Assays used	Primer Label	Sequence from 5'	Reference gene	Acc. No.	Location (nt)	Suppl. Ref.
MLV Sequencing	GagF1	GCGCCAGTCATCCGATAGACT	MTCR	X94150	1 to 21	3
MLV Sequencing	XS FM1_2 F	ACCTGGGAGGCTATTGCCTATGAA	MTCR	X94150	890 to 913	This report
MLV Sequencing	XS3	CGCCGAAACCGCGCCGCGCGT	MTCR	X94150	551 to 571	3
MLV Sequencing	XS4	TCGTCGCCCCGGACTGCCTTTCTG	MTCR	X94150	1507 to 1530	3
MLV Sequencing	XS FM2_3 F	TGCCTTAATCGAGTCTGTCCTCAC	MTCR	X94150	1396 to 1419	This report
MLV Sequencing	XS FM4_5 F	AGTTAAAGGCCCAGATCCACTTCG	MTCR	X94150	2528 to 2561	This report
MLV Sequencing	XS FM4_5 R	TCTTCCACCCGCTTGTTGACTTCT	MTCR	X94150	2962 to 2985	This report
MLV Sequencing	XS FM1_2 R1	GGTCAATGAGAGGTCCGCCATTAT	MTCR	X94150	1080 to 1103	This report
MLV Sequencing	VP62 2964F	TGCCCAACCCTTACAACCTCTTGA	XMRV VP62	EF185282.1	2964 to 2987	This report
MLV Sequencing	VP62 4263R	AGGCTTGGGTGAGTGCTATCAGTT	XMRV VP62	EF185282.1	4263 to 4286	This report
MLV Sequencing	Gag F2	GCAGCCCTGGGAGACGTC	XMRV VP62	EF185282.1	362 to 380	4
MLV Sequencing	Gag R2	CGGCGCGGTTTCGGCG	XMRV VP62	EF185282.1	520 to 536	4
MLV Sequencing	XS14	CATGGAAAGTCCAGCGTTCT	MTCR	X94150	5784 to 5803	3
MLV Sequencing	XS15	AGCTGCTCGAATTGTTTGGT	MTCR	X94150	7210 to 7229	3
XMRV qGag	GagQ2F2	AACCGTTTGTCTCTCCTAAACCC	XMRV, VP62	EF185282.1	900 to 922	5
XMRV qGag	GagQ2R2	GCAGGGTAAAGGGCAGATCG	XMRV, VP62	EF185282.1	980 to 999	5
XMRV qGag	Gag Q2P2	5'-FAM ACCGACAGCTCCCGTCCTCCCG BHQ-1	XMRV, VP62	EF185282.1	931 to 952	5
MLV qGPP	MLV Q4F4	GCCTGTCCAGGATCTGAGAG	MTCR	X94150	2944 to 2963	This report
MLV qGPP	MLV Q4R4	GAGGTTGTAAGGGTTGGGCA	MTCR	X94150	3000 to 3019	This report
MLV qGPP	MLV Q4P4	5'-FAM AAGTCAACAAGCGGGTGGAAGA BHQ-1	MTCR	X94150	2964 to 2985	This report
XMLV qEnv	MLV Q1F1	GGCAGGAGCCTCGGTACAA	MTCR	X94150	5868 to 5886	6
XMLV qEnv	MLV Q1R1	TGTCATTAGGTTGGTAACTCTCCAAGT	MTCR	X94150	5917 to 5943	6
XMLV qEnv	MLV Q1P1	5'-FAM TGACAGCCCTCACCAGGTCTTCAATG BHQ-1	MTCR	X94150	5889 to 5914	6
human GAPDH qPCR	hGAPDH gD F1	CCCCACACACATGCACTTACC	human GAPDH	NM_002046	chr12:6645040-6645061	7
human GAPDH qPCR	hGAPDH gD R1	CCTAGTCCCAGGGCTTTGATT	human GAPDH	NM_002046	chr12:6645096-6645115	7
human GAPDH qPCR	hGAPDH gD P1	5'-FAM AAAGAGCTAGGAAGGACAGGCAACTTGGC BHQ-1	human GAPDH	NM_002046	chr12:6645117-6645137	7
TM-PERT	MS2 RT F1	AACATGCTCGAGGGCCTTA	MS2 phage	NC_001417.2	1042 to 1060	8,9
TM-PERT	MS2 RT R1	GCCTTAGCAGTGCCCTGTCT	MS2 phage	NC_001417.2	1163 to 1181	8,9
TM-PERT	MS2 RT P1	5'-FAM CCCGTGGGATGCTCCTACATGTCA BHQ-1	MS2 phage	NC_001417.2	1066 to 1089	8,9
mouse GAPDH PCR	mGAPDH F1	AGTATGATGACATCAAGAAGG	mouse GAPDH*	NM_008084	chr6:125112397-125112414	10
mouse GAPDH PCR	mGAPDH R1	ATGGTATTCAAGAGAGTAGGG	mouse GAPDH*	NM_008084	chr6:125112892-125112913	10

. *: Multiple copies of mouse GAPDH peudogenes in mouse genome and mouse GAPDH PCR products (406 or 408 bp) without intron seqeneces were amplified by these primers (see Figure S2). MLV: murien leukemia virus; XMLV: xenotropic MLV; XMRV: XMLV-related virus; MTCR: murine type C retrovirus; TM-PERT: Taqman fluorogenic 5'-nulcease product-enhanced reverse transcriptase assay.

Table S4. Summary of nucleotide sequence differences between XMRV RKO isolate and XMRV VP62 viral genome and the corresponding sequences in other known XMRV strains

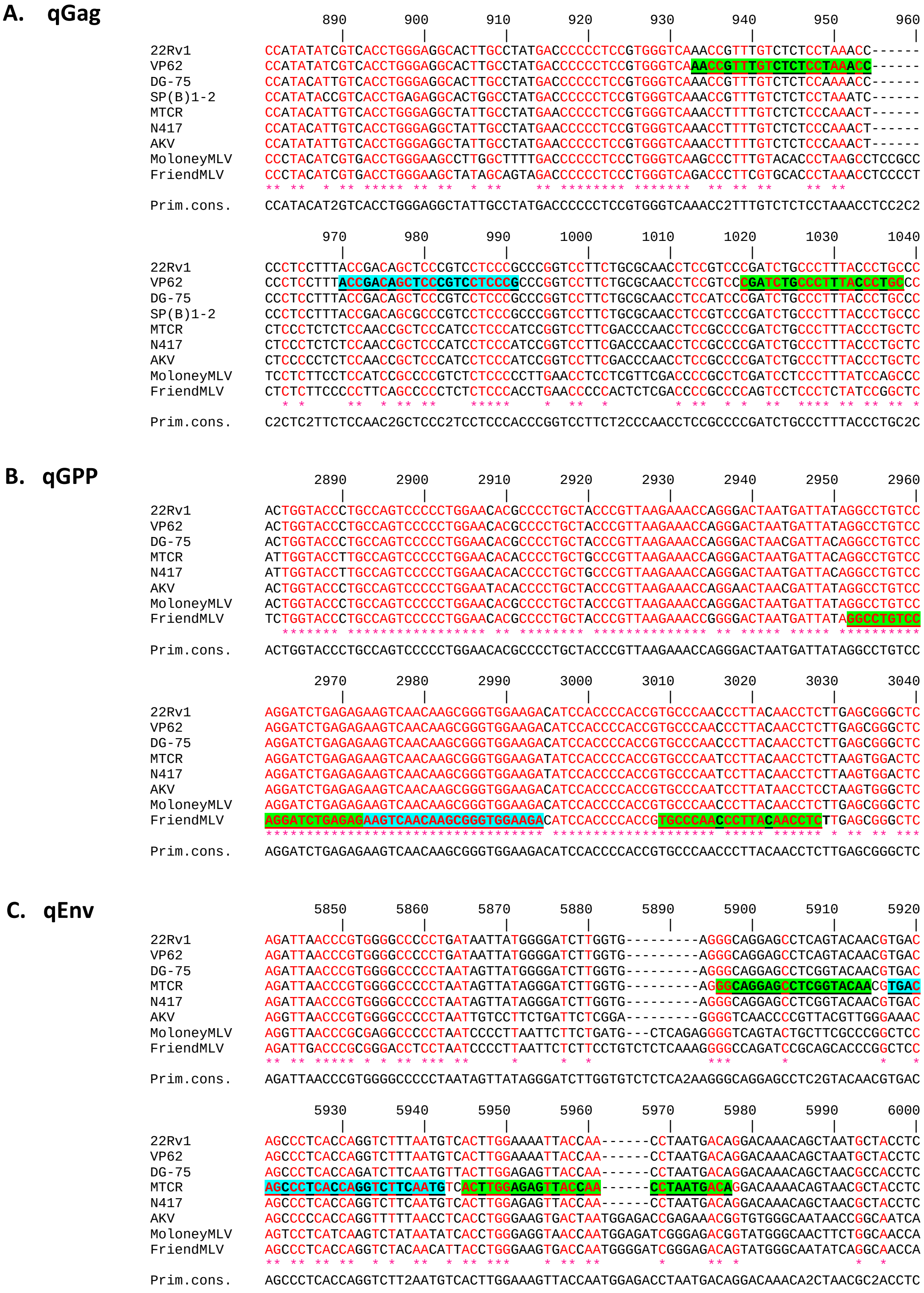
nt position	RKO[1] (1 to 8172 nt)	VP62 (5-8174 nt)	VP35 (1-8185 nt)	VP42 (1-8185 nt)	WPI-1106 (41-8147 nt)	WPI-1178 (41-8147 nt)	22Rv1 (1-8185 nt)
790	A	G	G	A	G	G	A
1104	T	C	C	C	C	C	C
1476	C	T	T	T	T	T	T
1824	G	A	A	G	A	A	G
4264	A	G	G	G	G	G	G
7699	T ins	-	T ins	T ins	-	T ins	T ins
7782	G ins	-	G ins	G ins	-	G ins	G ins
8112	G	C	C	C	C	C	C

1. The full provirus genome sequence of XMRV identified in the viral-positive RKO culture were the same as XMRV VP62 (>99.8% homology) except those nucleotide (nt) positions described in this Table. The RKO genomic DNA contains both XMRV provirus as well as minor variant form(s) of XMRV. The minor mutant forms of XMRV RKO (about 10-25% of XMRV provirus) contains G to A transition hypermuation in numerous sites (estimated 80-100 nt sites) cross XMRV RKO viral genome ,based on DNA sequencing analyses (see Materials and Methods). GeneBank: XMRV VP62 (EF185282.1), XMRV VP35 (DQ24241301), XMRV VP42 (DQ241302), WPI-1106 (GQ497344.1), WPI-1178 (GQ497343.1), and 22Rv1 (emb|FN692043.1), XMRV RKO (JF274252).

Table S5. Identifications of xenotropic murine leukemia viruses (XMLV) sequences in the cells infected with the XMLV-positive supernatants[1]

Cell Cultures	Input DNA (ng)	Ct of human qGAPDH	Ct of MLV qEnv	Ct of MLV qGag	Ct of MLV qGPP
LAPC-4	100	28.5	28.7	34.1	28.4
NCI-N417	100	27.8	21.1	Neg.	21.6
1065met	100	27.0	27.0	31.4	31.3
H1299_Unt d7	100	27.1	Neg.	Neg.	Neg.
H1299_Unt d14	100	27.2	Neg.	Neg.	Neg.
H1299_LAPC4 d7	100	27.4	28.9	Neg.	28.9
H1299_LAPC4 d14	100	27.0	27.1	Neg.	27.3
H1299_N417 d7	100	27.1	27.2	Neg.	27.5
H1299_N417 d14	100	26.7	27.8	Neg.	27.8
H1299_1065met d7	100	27.1	26.8	37.0	32.4
H1299_1065met d14	100	26.8	27.3	36.9	32.6
H69_Unt d14	100	28.1	Neg.	Neg.	Neg.
H69_LAPC4 d14	100	28.0	26.4	Neg.	26.2
H69_N417 d14	100	27.7	25.1	Neg.	25.1
H69_1065met d14	100	27.9	24.0	36.6	29.0
H1092_Unt d14	100	28.0	Neg.	Neg.	Neg.
H1092_LAPC4 d14	100	27.5	29.2	Neg.	29.2
H1092_N417 d14	100	27.6	27.4	Neg.	27.7
H1092_1065met d14	100	27.5	25.1	37.3	30.9
H460_Unt d18	100	28.6	Neg.	Neg.	Neg.
H460_LAPC4 d18	100	28.2	Neg.	Neg.	Neg.
H460_N417 d18	100	27.9	38.3	Neg.	38.5
H460_1065met d7	100	28.0	32.6	38.4	37.3
H460_1065met d18	100	27.7	31.5	38.2	36.1
HBEC3 Unt d18	100	27.4	Neg.	25.8	Neg.
HBEC3_LAPC4 d18	100	26.9	Neg.	26.1	Neg.
HBEC3_N417 d18	100	27.5	Neg.	25.8	Neg.
HBEC3_1065met d7	100	28.3	27.8	26.3	33.2
HBEC3 _1065met d18	100	27.4	26.2	25.1	32.5

1. Supernatant fluids from three xenograft XMLV-positive cultures (LAPC-4, NCI-N417 and 1065met)(Sup. Table S1) were used to infect four XMLV free lung cancer cultures (NSCLC lines NCI-H1299 and NCI-H460; SCLC lines NCI-H69 and NCI-H1092) and an immortalized human bronchial epithelial cell culture HBEC3KTR53 (HBEC3) (Sup. Ref. 11). The DNA after infections was isolated and examined by MLV qPCR with three viral probes and human GAPDH internal controls (see Materials/Methods and Sup. Table S3). The uninfected HBEC3 culture was positive for the *gag* region of MLV, presumably as a result of the retrovirus vectors for TERT and CDK4 used to immortalize the cells (Sup. Ref. 11). Unt: uninfected. d: day post-infection.



A. qGag

```
                     890       900       910       920       930       940       950       960
                       |         |         |         |         |         |         |         |
22Rv1         CCATATATCGTCACCTGGGAGGCACTTGCCTATGACCCCCCTCCGTGGGTCAAACCGTTTGTCTCTCCTAAACC------
VP62          CCATATATCGTCACCTGGGAGGCACTTGCCTATGACCCCCCTCCGTGGGTCAAACCGTTTGTCTCTCCTAAACC------
DG-75         CCATACATTGTCACCTGGGAGGCACTTGCCTATGACCCCCCTCCGTGGGTCAAACCGTTTGTCTCTCCAAAACC------
SP(B)1-2      CCATATACCGTCACCTGAGAGGCACTGGCCTATGACCCCCCTCCGTGGGTCAAACCGTTTGTCTCTCCTAAATC------
MTCR          CCATACATTGTCACCTGGGAGGCTATTGCCTATGAACCCCCTCCGTGGGTCAAACCTTTTGTCTCTCCCAAACT------
N417          CCATACATTGTCACCTGGGAGGCTATTGCCTATGAACCCCCTCCGTGGGTCAAACCTTTTGTCTCTCCCAAACT------
AKV           CCATATATTGTCACCTGGGAGGCTATTGCCTATGAACCCCCTCCGTGGGTCAAACCTTTTGTCTCTCCCAAACT------
MoloneyMLV    CCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCC
FriendMLV     CCCTACATCGTGACCTGGGAAGCTATAGCAGTAGACCCCCCTCCCTGGGTCAGACCCTTCGTGCACCCTAAACCTCCCCT
              ** **  * ** ***** ** **  * **    ** ******** *******  ** ** **    ** **

Prim.cons.    CCATACAT2GTCACCTGGGAGGCTATTGCCTATGACCCCCCTCCGTGGGTCAAACC2TTTGTCTCTCCTAAACCTCC2C2

                     970       980       990      1000      1010      1020      1030      1040
                       |         |         |         |         |         |         |         |
22Rv1         CCCTCCTTTACCGACAGCTCCCGTCCTCCCGCCCGGTCCTTCTGCGCAACCTCCGTCCCGATCTGCCCTTTACCCTGCCC
VP62          CCCTCCTTTACCGACAGCTCCCGTCCTCCCGCCCGGTCCTTCTGCGCAACCTCCGTCCCGATCTGCCCTTTACCCTGCCC
DG-75         CCCTCCTTTACCGACAGCTCCCGTCCTCCCGCCCGGTCCTTCTGCGCAACCTCCATCCCGATCTGCCCTTTACCCTGCCC
SP(B)1-2      CCCTCCTTTACCGACAGCGCCCGTCCTCCCGCCCGGTCCTTCTGCGCAACCTCCGTCCCGATCTGCCCTTTACCCTGCCC
MTCR          CTCCCTCTCTCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCTTTACCCTGCTC
N417          CTCCCTCTCTCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCTTTACCCTGCTC
AKV           CTCCCCCTCTCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCTTTACCCTGCTC
MoloneyMLV    TCCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCC
FriendMLV     CTCTCTTCCCCCTTCAGCCCCCTCTCTCCCACCTGAACCCCCACTCTCGACCCCGCCCCAGTCCTCCCTCTATCCGGCTC
                * *     **  * ** **    *****    *  **  *        * **  * *  ** **** ** ** ** *

Prim.cons.    C2CTC2TTCTCCAAC2GCTCCC2TCCTCCCACCCGGTCCTTCT2CCCAACCTCCGCCCCGATCTGCCCTTTACCCTGC2C
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B. qGPP

```
                    2890      2900      2910      2920      2930      2940      2950      2960
                       |         |         |         |         |         |         |         |
22Rv1         ACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAAACCAGGGACTAATGATTATAGGCCTGTCC
VP62          ACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAAACCAGGGACTAATGATTATAGGCCTGTCC
DG-75         ACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAAACCAGGGACTAACGATTACAGGCCTGTCC
MTCR          ATTGGTACCTTGCCAGTCCCCCTGGAACACACCCCTGCTGCCCGTTAAGAAACCAGGGACTAATGATTACAGGCCTGTCC
N417          ATTGGTACCTTGCCAGTCCCCCTGGAACACACCCCTGCTGCCCGTTAAGAAACCAGGGACTAATGATTACAGGCCTGTCC
AKV           ACTGGTACCCTGCCAGTCCCCCTGGAATACACCCCTGCTACCCGTTAAGAAACCAGGAACTAACGATTATAGGCCTGTCC
MoloneyMLV    ACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAAACCAGGGACTAATGATTATAGGCCTGTCC
FriendMLV     TCTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAAACCGGGGACTAATGATTATAGGCCTGTCC
                ******* ***************** ** ******** ************** ** ***** ***** **********

Prim.cons.    ACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAAACCAGGGACTAATGATTATAGGCCTGTCC

                    2970      2980      2990      3000      3010      3020      3030      3040
                       |         |         |         |         |         |         |         |
22Rv1         AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
VP62          AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
DG-75         AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
MTCR          AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGATATCCACCCCACCGTGCCCAATCCTTACAACCTCTTAAGTGGACTC
N417          AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGATATCCACCCCACCGTGCCCAATCCTTACAACCTCTTAAGTGGACTC
AKV           AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAATCCTTATAACCTCCTAAGTGGGCTC
MoloneyMLV    AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
FriendMLV     AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
              ********************************** ******************** ***** ****** * ** ** ***

Prim.cons.    AGGATCTGAGAGAAGTCAACAAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
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C. qEnv

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                    5850      5860      5870      5880      5890      5900      5910      5920
                       |         |         |         |         |         |         |         |
22Rv1         AGATTAACCCGTGGGGCCCCCTGATAATTATGGGGATCTTGGTG---------AGGGCAGGAGCCTCAGTACAACGTGAC
VP62          AGATTAACCCGTGGGGCCCCCTGATAATTATGGGGATCTTGGTG---------AGGGCAGGAGCCTCAGTACAACGTGAC
DG-75         AGATTAACCCGTGGGGCCCCCTAATAGTTATGGGGATCTTGGTG---------AGGGCAGGAGCCTCGGTACAACGTGAC
MTCR          AGATTAACCCGTGGGGCCCCCTAATAGTTATAGGGATCTTGGTG---------AGGGCAGGAGCCTCGGTACAACGTGAC
N417          AGATTAACCCGTGGGGCCCCCTAATAGTTATAGGGATCTTGGTG---------AGGGCAGGAGCCTCGGTACAACGTGAC
AKV           AGGTTAACCCGTGGGGCCCCCTAATTGTCCTTCTGATTCTCGGA---------GGGGTCAACCCCGTTACGTTGGGAAAC
MoloneyMLV    AGGTTAACCCGCGAGGCCCCCTAATCCCCTTAATTCTTCTGATG---CTCAGAGGGGTCAGTACTGCTTCGCCCGGCTCC
FriendMLV     AGATTGACCCGCGGGACCTCCTAATCCCCTTAATTCTCTTCCTGTCTCTCAAAGGGGCCAGATCCGCAGCACCCGGCTCC
              ** ** ***** * * ** *** **      *     *  *              ***      *           *   *

Prim.cons.    AGATTAACCCGTGGGGCCCCCTAATAGTTATAGGGATCTTGGTGTCTCTCA2AAGGGCAGGAGCCTC2GTACAACGTGAC

                    5930      5940      5950      5960      5970      5980      5990      6000
                       |         |         |         |         |         |         |         |
22Rv1         AGCCCTCACCAGGTCTTTAATGTCACTTGGAAAATTACCAA------CCTAATGACAGGACAAACAGCTAATGCTACCTC
VP62          AGCCCTCACCAGGTCTTTAATGTCACTTGGAAAATTACCAA------CCTAATGACAGGACAAACAGCTAATGCTACCTC
DG-75         AGCCCTCACCAGATCTTCAATGTTACTTGGAGAGTTACCAA------CCTAATGACAGGACAAACAGCTAACGCCACCTC
MTCR          AGCCCTCACCAGGTCTTCAATGTCACTTGGAGAGTTACCAA------CCTAATGACAGGACAAAACAGTAACGCTACCTC
N417          AGCCCTCACCAGGTCTTCAATGTCACTTGGAGAGTTACCAA------CCTAATGACAGGACAAACAGCTAACGCTACCTC
AKV           AGCCCCCACCAGGTTTTTAACCTCACCTGGGAAGTGACTAATGGAGACCGAGAAACGGTGTGGGCAATAACCGGCAATCA
MoloneyMLV    AGTCCTCATCAAGTCTATAATATCACCTGGGAGGTAACCAATGGAGATCGGGAGACGGTATGGGCAACTTCTGGCAACCA
FriendMLV     AGCCCTCACCAGGTCTACAACATTACCTGGGAAGTGACCAATGGGGATCGGGAGACAGTATGGGCAATATCAGGCAACCA
              ** ** ** **  * *  **  * ** ***    * ** **       *     ** *              *  *

Prim.cons.    AGCCCTCACCAGGTCTT2AATGTCACTTGGAAAGTTACCAATGGAGACCTAATGACAGGACAAACA2CTAACGC2ACCTC
```

Figure S1. Illustration of locations of the primers/probe for MLV qGag, qGPP and qEnv PCR. The representative XMRV viruses (22Rv1 and VP62), xenotropic MLV (SP(B) 1-2, DG-75, MTCR and N417), ecotropic MLV (AKV, MoloneyMLV and FriendMLV) are compared to the primers (bold, underlined, green highlight)/probes (bold, underline, blue highlight) sequences (see also Table S3) in Figure S1A., B. and C., for MLV qGag, qGPP and qEnv PCR, respectively. Nt no. refers to an approximate nt position in each viral genome. GeneBank Accession no.: XMRV 22Rv1 (emb|FN692043.1), XMRV VP62 (EF185282.1), MLV DG-75 (AF221065.1), MLV SP(B) 1-2 (AY349140.1), MTCR (X94150.1), MLV N417 (HQ246218), MLV AKV (J01998.1), Moloney MLV (AF033811.1) and Friend MLV (NC_001362.1).

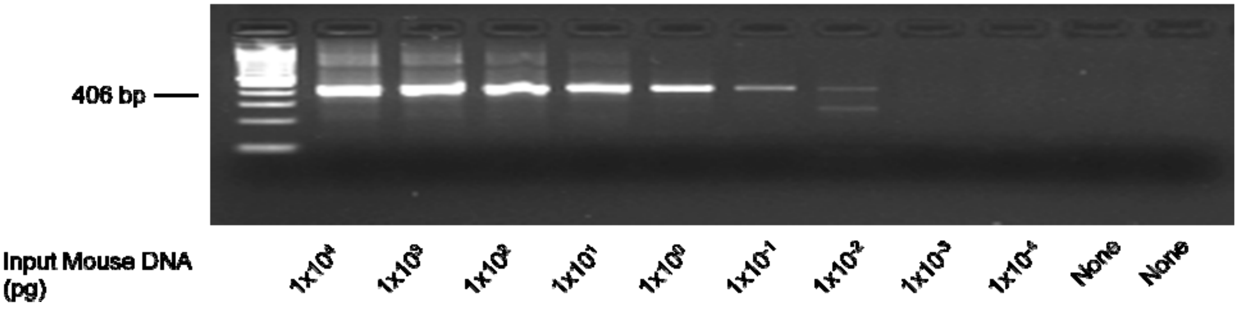


Figure S2. Determination of mouse DNA detection sensitivity of the PCR assay specific for mouse GAPDH. The PCR reaction contained mouse spleen DNA (ranging from 1 ng to 0.1 fg) plus salmon sperm carrier DNA (100 ng), or salmon sperm carrier DNA alone. The mouse DNA were detectable at amount of as low as 0.1 pg to 0.01 pg. The amplified 406 bp (or 408) bp PCR product were sequenced and confirmed to correspond to mouse GAPDH peudogene which is present with multiple copies in mouse genomes (see Materials/Methods and Table S3). Interestingly, the amplified PCR product of mouse genomic DNA was about 406 bp to 408 bp, which was differently from 506 bp as predicted from mouse GAPDH gene on chromosome 6 (GeneBank Accession no. NM_008084) (Figure 2). The reason for yielding a shorter PCR product appears due to the presence of numerous copies (>250) of mouse GAPDH pseudogene in many different chromosomes in mouse genome, for examples, the genes of Gene Accession no AL732526.8 (chromosome 2), NG_005467.5 (chromosome 5), NG_007762.3 (chromosome 8), NG_005468.5 (chromosome 7), NG_007811.1(chromosome 15) and NG_007228.1 (chromosome 4). The amplified PCR products from mouse spleen DNA were confirmed to match mouse GAPDH psedogene by DNA sequencing. Under our PCR conditions, the mouse GAPDH PCR is highly sensitive and able to detect 0.1 pg to 0.01 pg of mouse DNA.

```
LOCUS       1065met_XS1/GagR2      555 bp
ORIGIN
        1 TCCGATAGAC TGAGTCGCCC GGGTACCCGT GTATCCAATA AAGCCTTTTG CTGTTGCATC
       61 CGAATCGTGG TCTCGCTGAT CCTTGGGAGG GTCTCCTCAG AGTGATTGAC TGCCCAGCCT
      121 GGGGGTCTTT CATTTGGGGG CTCGTCCGGG ATTTGGAGAC CCCCGCCCAG GGACCACCGA
      181 CCCACCGTCG GGAGGTAAGC TGGCCAGCGA TCGTTTTGTC TCCGTCTCTG TCTTTGTGCG
      241 TGTGTGTGTG TGTGCCGGCA TCTACTTTTT GCGCCTGCGT CTGATTCTGT ACTAGTTAGC
      301 TAACTAGATC TGTATCTGGC GGCTCCGTGG AAGAACTGAC GAGTTCGTAT TCCCGACCGC
      361 AGCCCTGGGA GACGTCTCAG AGGCATCGGG GGCCCGCTGG GTGGCCCAAT CAGTAAGTCC
      421 GAGTCCTGAC CGATTCGGAC TATTTGGGGC CCCTCCTTTG TCGGAGGGGT ACGTGGTTCT
      481 TTTAGGAGAC GAGAGGTCCA AGCCCTCGCC GCCTCCATCT GAATTTTTGC TTTCGGTTTT
      541 TCGCCGAAAC CGCGC
//
```

```
LOCUS       1065met_XS3/XS4      831 bp
ORIGIN
        1 TTGGACCGCT TCTAAAAACA TGGGACAGAC CGTAACCACC CCTCTGAGTC TGACCCTAGA
       61 ACACTGGAAA GATGTCCAGC GCATCGCGTC CAATCAGTCC GTAGATGTCA AGAAGAGACG
      121 CTGGGTCACC TTCTGCTCTG CCGAGTGGCC AACTTTCGGT GTAGGGTGGC CACAAGATGG
      181 TACTTTTAAT TTGGACATTA TTCTACAGGT TAAATCTAAG GTGTTCTCTC CTGGTCCCCA
      241 CGGACACCCG GATCAGGTCC CATATATTGT CACCTGGGAG GCTATTGCCT ATGAACCCCC
      301 TCCGTGGGTC AAACCTTTTG TCTCTCCCAA ACTCTCCCCC TCTCCAACCG CTCCCATCCT
      361 CCCATCCGGT CCTTCGACCC AACCTCCGCC CCGATCTGCC CTTTACCCTG CTCTTACCCC
      421 CTCTATAAAA CCCAGACCTT CTAAACCTCA GGTTCTCTCC GATAATGGCG GACCTCTCAT
      481 TGACCTTCTC TCAGAAGACC CTCCGCCGTA CGGAGGACAG GGACTGTCCT CCTCGGACGG
      541 AGATGGCGAC AGAGAAGAGG CCACCTCCAC TTCTGAGATT CCTGCCCCCT CTCCCATAGT
      601 GTCTCGCCTG CGGGGCAAAA GAGACCCCCC CGCGGCAGAT TCCACCACCT CTCGGGCTTT
      661 CCCACTCCGT TTGGGGGGTA ATGGTCAGTT GCAGTACTGG CCGTTTTCCT CCTCTGATCT
      721 ATATAACTGG AAAAATAATA ATCCTTCCTT CTCTGAGGAT CCAGGTAAAC TGACTGCATT
      781 GATTGAATCC GTCCTCACCA CCCACCAGCC CACCTGGGAT GATTGCCAGC A
//
```

```
LOCUS       1065met_XS14/XS15     1338 bp
ORIGIN
        1 TAATAGTTAT GGGGATCTTG GTGAGGGCAG GAGCCTCGGT ACAACGTGAC AGCCCTCACC
       61 AGATCTTCAA TGTTACTTGG AGAGTTACCA ACCTAATGAC AGGACAAACA GCTAACGCCA
      121 CCTCCCTCCT GGGGACGATG ACAGACACCT TCCCTAAACT ATATTTTGAC CTGTGTGATT
      181 TAGTAGGAGA CTACTGGGAT GACCCAGAAC CCGATATTGG GGATGGTTGC CGCACTCCCG
      241 GGGGAAGAAG AAGGACAAGA CTGTATGACT TCTATGTTTG CCCCGGTCAT ACTGTACCAA
      301 TAGGGTGTGG AGGGCCGGGA GAGGGCTACT GTGGCAAATG GGGATGTGAG ACCACTGGAC
      361 AGGCATACTG GAAGCCATCA TCATCATGGG ACCTAATTTC CCTTAAGCGA GGAAACACTC
      421 CTAAGGATCA GGGCCCCTGT TATGATTCCT CGGTCTCCAG TGGCGTCCAG GGTGCCACAC
      481 CGGGGGGTCG ATGCAACCCC CTGGTCTTAG AATTCACTGA CGCGGGTAGA AAGGCCAGCT
      541 GGGATGCCCC CAAAGTTTGG GGACTAAGAC TCTATCGATC CACAGGGGCC GACCCGGTGA
      601 CCCGGTTCTC TTTGACCCGC CAGGTCCTCA ATGTAGGACC CCGCGTCCCC ATTGGGCCTA
      661 ATCCCGTGAT CACTGACCAG CTACCCCCAT CCCAACCCGT GCAGATCATG CTCCCCAGGC
      721 CTCCTCATCC TCCTCCTTCA GGCACGGTCT CTATGGTACC TGGGGCTCCC CCGCCTTCTC
      781 AACAACCTGG GACGGGAGAC AGGCTGCTAA ATCTGGTAGA AGGAGCCTAC CAAGCACTCA
      841 ACCTCACCAG TCCTGACAAA ACCCAAGAGT GCTGGTTGTG TCTGGTATCG GGACCCCCCT
      901 ACTACGAAGG GGTTGCCGTC CTAGGTACCT ACTCCAACCA TACCTCTGCC CCAGCTAACT
      961 GCTCCGTGGC CTCCCAACAC AAGCTGACCC TGTCCGAAGT AACCGGACAG GGACTCTGCG
     1021 TAGGAGCAGT TCCCAAAACC CATCAGGCCC TGTGTAATAC CACCCAGAAG ACGAGCGACG
     1081 GGTCCTACTA TCTGGCTGCT CCCGCCGGGA CCATCTGGGC TTGCAACACC GGGCTCACTC
     1141 CCTGCCTATC TACTACTGTA CTCAACCTCA CCACCGATTA CTGTGTCCTG GTTGAGCTCT
     1201 GGCCAAAGGT GACCTACCAC TCCCCTGGTT ATGTTTATGG CCAGTTTGAG AGAAAAACCA
     1261 AATATAAAAG AGAGCCGGTG TCATTAACTC TGGCCCTGCT GTTAGGAGGA CTTACTATGG
     1321 GCGGCATAGC TGCAGGAG
//
```

Figure S3. MLV-related sequences detected in 1065met lung cancer xenograft cell line. The sequencing data were obtained by sequencing of XS1/GagR2 (5’ end, 555 nts), XS3/XS4 (gag, 831 nts) and XS14/XS15 (env, 1338 nts) PCR products of 1065met DNA (see Table S3 for details). The sequence at the 5’ end and gag gene has 99% homology to AKV murine leukemia virus (GeneBank Accession no. J01998.1), and the sequence at env gene region has 99% homology to Murine xenotropic virus NZB (GeneBank Accession no EU33447.1).

```
                         10        20        30        40        50        60        70        80
                          |         |         |         |         |         |         |         |
H82_XS1_GagR2    ----------------------------TCTCGCTGATCCTTGGGAGGGTCTCCTCAGAGTGATTGACTGCCCAGCCTGG
H146_XS1_GagR2   ----------------------------TCTCGCTGATCCTTGGGAGGGTCTCCTCAGAGTGATTGACTGCCCAGCCTGG
LX47_XS1_GagR2   -CCTTTTGCTGTTGCATCCGAATCGTGGTCTCGCTGATCCTTGGGAGGGTCTCCTCAGAGTGATTGACTGCCCAGCTTGG
LX48_XS1_GagR2   CCTTTTGCTGTTTGCATCCGAAACGTGGCCTCGCTGTTCCTTGGGAGGGTCTCCTCAGAGTGATTGACTACCCAGCTCGG
                              ******* ******************************** ******  **

Prim.cons.       CC2TTT22222TTGCATCCGAA2CGTGGTCTCGCTGATCCTTGGGAGGGTCTCCTCAGAGTGATTGACTGCCCAGC2TGG

                         90       100       110       120       130       140       150       160
                          |         |         |         |         |         |         |         |
H82_XS1_GagR2    GGGTCTTTCATTTGGGGGCTCGTCCGGGATTTGGAGACCCCCGCCCAGGGACCACCGACCCACCGTCGGGAGGTAAGCTG
H146_XS1_GagR2   GGGTCTTTCATTTGGGGGCTCGTCCGGGATTTGGAGACCCCCGCCCAGGGACCACCGACCCACCGTCGGGAGGTAAGCTG
LX47_XS1_GagR2   GGGTCTTTCATTTGGGGGCTCGTCCGGGATTTGGAGACCCCCGCCCAGGGACCACCGACCCACCGTCGGGAGGTAAGCTG
LX48_XS1_GagR2   GGGTCTTTCATTTGGGGGCTCGTCCGGGATTTGGAGACCCCCGCCCAGGGACCACCGACCCACCGTCGGGAGGTAAGCTG
                 ********************************************************************************

Prim.cons.       GGGTCTTTCATTTGGGGGCTCGTCCGGGATTTGGAGACCCCCGCCCAGGGACCACCGACCCACCGTCGGGAGGTAAGCTG

                        170       180       190       200       210       220       230       240
                          |         |         |         |         |         |         |         |
H82_XS1_GagR2    GCCAGCGATCGTTTTGTCTCCGTCTCTGTCTTTGTGCGTGTGTGTGTGTGTGCCGGCACCTACTTTTTGCGCCTGCGTCT
H146_XS1_GagR2   GCCAGCGATCGTTTTGTCTCCGTCTCTGTCTTTGTGCGTGTGTGTGTGTGTGCCGGCACCTACTTTTTGCGCCTGCGTCT
LX47_XS1_GagR2   GCCAGCGATCGTTTTGTCTCCGTCTCTGTCTTTGTGCGTGTGTGTGTGTGTGCCGGCATCTACTTTTTGCGCCTGCGTCT
LX48_XS1_GagR2   GCCAGCGATCGTTTTGTCTCCGTCTCTGTCTTTGTGCGTGTGTGTGTGT--GCCGGCATCTACTTTTTGCGCCTGCGTCT
                 *************************************************  ******* *********************

Prim.cons.       GCCAGCGATCGTTTTGTCTCCGTCTCTGTCTTTGTGCGTGTGTGTGTGTGTGCCGGCA2CTACTTTTTGCGCCTGCGTCT

                        250       260       270       280       290       300       310       320
                          |         |         |         |         |         |         |         |
H82_XS1_GagR2    GATTCTGTACTAGTTAGCTAACTAGATCTGTATCTGGCGGCTCCGTGGAAGAACTGACGAGTTCGTATTCCCGACCGCAG
H146_XS1_GagR2   GATTCTGTACTAGTTAGCTAACTAGATCTGTATCTGGCGGCTCCGTGGAAGAACTGACGAGTTCGTATTCCCGACCGCAG
LX47_XS1_GagR2   GATTCTGTACTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGTGGAAGAACTGACGAGTTCGTATTCCCGACCGCAG
LX48_XS1_GagR2   GAATCTGTACTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGTGGAAGAACTGACGAGTTCGTATTCCCGACCGCAG
                 ** ************************************* ***************************************

Prim.cons.       GATTCTGTACTAGTTAGCTAACTAGATCTGTATCTGGCGG2TCCGTGGAAGAACTGACGAGTTCGTATTCCCGACCGCAG

                        330       340       350       360       370       380       390       400
                          |         |         |         |         |         |         |         |
H82_XS1_GagR2    CCCTGGGAGACGTCTCAGAGGCATCGGGGGCCCGCTGGGTGGCCCAATCAGTAAGTCCGAGTCCTGACCGATTCGGACTA
H146_XS1_GagR2   CCCTGGGAGACGTCTCAGAGGCATCGGGGGCCCGCTGGGTGGCCCAATCAGTAAGTCCGAGTCCTGACCGATTCGGACTA
LX47_XS1_GagR2   CCCTGGGAGACGTCTCAGAGGCATCGGGGGCCCGCTGGGTGGCCCAATCAGTAAGTCCGAGTCCTGACCGATTCGGACTA
LX48_XS1_GagR2   CCCTGGGAGACGTCTCAGAGGCATCGGGGGCCCGCTGGGTGGCCCAATCAGTAAGTCCGAGTCCTGACCGATTCGGACTA
                 ********************************************************************************

Prim.cons.       CCCTGGGAGACGTCTCAGAGGCATCGGGGGCCCGCTGGGTGGCCCAATCAGTAAGTCCGAGTCCTGACCGATTCGGACTA

                        410       420       430       440       450       460       470       480
                          |         |         |         |         |         |         |         |
H82_XS1_GagR2    TTTGGGGCCCCTCCTTTGTCGGAGGGGTACGTGGTTCTTTTAGGAGACGAGAGGTCCAAGCCCTCGCCGCCTCCATCTGA
H146_XS1_GagR2   TTTGGGGCCCCTCCTTTGTCGGAGGGGTACGTGGTTCTTTTAGGAGACGAGAGGTCCAAGCCCTCGCCGCCTCCATCTGA
LX47_XS1_GagR2   TTTGGGGCCCCTCCTTTGTCGGAGGGGTACGTGGTTCTTTTAGGAGACGAGAGGTCCAAGCCCTCGCCGCCTCCATCTGA
LX48_XS1_GagR2   TTTGGAGCCCCTCCTTTGTCGGAGGGGTACGTGGTTCTTTTAGGAGACGAGAGGTCCAAGCCCTCGCCGCCTCCATCTGA
                 ***** **************************************************************************

Prim.cons.       TTTGGGGCCCCTCCTTTGTCGGAGGGGTACGTGGTTCTTTTAGGAGACGAGAGGTCCAAGCCCTCGCCGCCTCCATCTGA

                        490       500
                          |         |
H82_XS1_GagR2    ATTTTTGCTTTCGGTTTTTCGC
H146_XS1_GagR2   ATTTTTGCTTTCGGTTTTTCGC
LX47_XS1_GagR2   ATTTTTGCTTTCGGTTTTTC--
LX48_XS1_GagR2   ATTTTTGCTTTCGGTTTTTC--
                 ********************

Prim.cons.       ATTTTTGCTTTCGGTTTTTCGC
```

Figure S4. Alignment analysis of the MLV-related sequences detected in LX47 and LX48 lung cancer xenograft lines and the contaminated NCI-H82 and NCI-H146 non-xenograft lines. The sequencing data were obtained by sequencing of XS1/GagR2 PCR products of each sample (see Table S1 S2 and S3 for details). The additional sequencing of XS3/XS4 PCR products showed the same sequences for viral positive NCI-H82 and NCI-H146. The LX47, NCI-H820 and NCI-H146 contain near identical MLV-related sequences which have 100% homology to Mus musculus endogenous ecotropic murine leukemia virus 1 (GeneBank Accession no. DQ366147.1) and 99% homology to AKV murine leukemia virus (GeneBank Accession no. J01998.1). The sequence at env gene region (XS14/XS15 PCR, not shown) were the same between LX47 and the positive NCI-H820 and has 99% homology to Murine xenotropic virus NZB (GeneBank Accession no EU33447.1). LX48 sequencing data is different from LX47 has 99% homology to Murine leukemia virus MCF1233 (GeneBank Accession no. MLU13766).

```
LOCUS       CAK1_XS3_XS4       903 bp
ORIGIN
        1 GTCATTTGTC GGTTCGTTAT TGTTTTGGAC CGTTTCTAAA AATATGGGAC AGACCGTAAC
       61 CACTCCTTTG AGTCTGACCC TAGAACACTG GGGAGACGTC CAGCGCATTG CGTCCAACCA
      121 GTCCGTGGAC GTCAAGAAGA GACGTTGGGT CACCTTCTGC TCTGCCGAGT GGCCAACTTT
      181 CGATGTGGGG TGGCCGCAAG ATGGTACTTT TAATTTGGAC ATTATTTTAC AGGTTAAATC
      241 TAAGGTGTTC TCTCCCGGTC CCCACGGACA CCCGGATCAG GTCCCATACA TTGTCACCTG
      301 GGAGGCACTT GCCTATGACC CCCCTCCGTG GGTCAAACCG TTTGTCTCTC CAAAACCCCC
      361 TCCTTTACCG ACAGCTCCCG TCCTCCCGCC CGGTCCTTCT GCGCAACCTC CGTCCCGATC
      421 TGCCCTTTAC CCTGCCCTTA CCCCCTCTAT AAAGACCAAA CCTCCTAAGC CCCAGGTTCT
      481 CCCTGATAAC GGCGGACCGC TCATTGACCT TCTCACAGAG GACCCCCCGC CGTACGGAGC
      541 ACAACCTTCC TCCTCTGCCA GAGGGAACGA TGAAGAAGAG GCGGCCGCCA CCTCCGAGGT
      601 TTCCCCCCCT TCTCCCATGG TGTCTCGACT GCGGGGAAGG AGGGACCCTC CCGCAGCGGA
      661 CTCCACCTCC TCCCAGGCAT TCCCACTCCG CATGGGGGGA GATGGCCAGC TTCAGTATTG
      721 GCCGTTTTCC TCCTCGGACT TATACAATTG GAAAAATAAT AACCCTTCCT TTTCTGAAGA
      781 CCCAGGTAAA TTGACGGCCT TGATTGAGTC CGTCCTCATC ACCCACCAGC CCACCTGGGA
      841 CGACTGTCAG CAGTTGTTGG GGACCCTGCT GACCGGAGAA GAAAAGCAGC GGGTGCTCCT
      901 AGA
//
```

```
LOCUS       CAK1_XS14_XS15     1352 bp
ORIGIN
        1 GTGGGGCCCC CTAATAGTTA TGGGGATCTT GGTGAGGGCA GGAGCCTCGG TACAACGTGA
       61 CAGCCCTCAC CAGATCTTCA ATGTTACTTG GAGAGTTACC AACCTAATGA CAGGACAAAC
      121 AGCTAACGCC ACCTCCCTCC TGGGGACGAT GACWGACACC TTCCCTAAAC TATATTTTGA
      181 CCTGTGTGAT TTAGTAGGAG ACTACTGGGA TGACCCAGAA CCCGATATTG GGGATGGTTG
      241 CCGCACTCCC GGGGGAAGAA GAAGGACAAG ACTGTATGAC TTCTATGTTT GCCCCGGTCA
      301 TACTGTACCA ATAGGGTGTG GAGGGCCGGG AGAGGGCTAC TGTGGCAAAT GGGGATGTGA
      361 RACCACTGGA CAGGCATACT GGAAGCCATC ATCATCATGG GACCTAATTT CCCTTAAGCG
      421 AGGAAACACT CCTAAGGATC AGGGCCCCTG TTATGATTCC TCGGTCTCCA GTGGCGTCCA
      481 GGGTGCCACA CCGGGGGGTC GATGCAACCC CCTGGTCTTA GAATTCACTG ACGCGGGTAR
      541 AAAGGCCAGC TGGGATGCCC CCAAAGTTTG GGGACTRAGA CTCTATCGAT CCACAGGGGC
      601 CGACCCGGTG ACCCGGTTCT CTTTGACCCG CCAGGTCCTC AATGTAGGAC CCCGCGTCCC
      661 CATTGGGCCT AATCCCGTGA TCACTGACCA GCTACCCCCA TCCCAACCCG TGCAGATCAT
      721 GCTCCCCAGG CCTCCTCATC CTCCTCCTTC AGRCACGGTC TCTATGGTAC CTGGGGCTCC
      781 CCCGCCTTCT CAACAACCTG GGACGGGAGA CAGGCTGCTA AATCTGGTAG AAGGAGCCTA
      841 CCAAGCACTC AACCTCACCA GTCCTGACAA AACCCAAGAG TGCTGGTTGT GTCTGGTATC
      901 GGGACCCCCC TACTACGAAG GGGTTGCCGT CCTAGGTACC TACTCCAACC ATACCTCTGC
      961 CCCAGCTAAC TGCTCCGTGG CCTCCCAACA CAAGCTGACC CTGTCCGAAG TGACCGGACA
     1021 GGGACTCTGC GTAGGAGCAG TTCCCAAAAC CCATCAGGCC CTGTGTAATA CCACCCAGAA
     1081 GACGAGCGAC GGGTCCTACT ATCTGGCTGC TCCCGCCGGG ACCATCTGGG CTTGCAACAC
     1141 CGGGCTCACT CCCTGCCTAT CTACTACTGT ACTCAACCTC ACCACCGATT ACTGTGTCCT
     1201 GGTTGAGCTC TGGCCAAAGG TGACCTACCA CTCCCCTGGT TATGTTTATG GCCAGTTTGA
     1261 GAAAAAAACC AAATATAAAA GGGAGCCGGT GTCATTAACT CTGGCCCTGC TGTTGGGAGG
     1321 ACTTACTATG GGCGGCATAG CTGCAGGAGT AG
//
```

Figure S5. MLV-related sequences detected in CAK1 pancreatic carcinoma xenograft cell line. The sequencing data were obtained by sequencing of XS1/GagR2 (5' end), XS3/XS4 (gag, 903 nts) and XS14/XS15 (env,1352 nts) PCR products of CAK1 DNA (see Sup. Table S3 for details). The sequence at the 5' end demonstrated wt and possible 1 nt del variant form at about 1:1 ratio. The gag gene and env gene sequencing data both have over 99% homology to DG-75 Murine leukemia virus (GeneBank Accession no AF221065.1).

```
                        10        20        30        40        50        60        70        80        90       100
                         |         |         |         |         |         |         |         |         |         |
WPI_1106        ----------------------------------------GTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
WPI_1178        ----------------------------------------GTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
VP35            GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
VP62            -----AGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
22Rv1           GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
VP42            GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
RKO             ---------------------GAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG
MTCR            GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAACGTGGCCTCGCTGTTCCTTGGGAG
                                                        *********************************** ************************

Prim.cons.      GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAG

                       110       120       130       140       150       160       170       180       190       200
                         |         |         |         |         |         |         |         |         |         |
WPI_1106        GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
WPI_1178        GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
VP35            GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
VP62            GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
22Rv1           GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
VP42            GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
RKO             GGTCTCCTCAGAGTRATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
MTCR            GGTCTCCTCTGAGTGATTGACTACCCGGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTTGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC
                ********* **************** ************************************* ***********************************

Prim.cons.      GGTCTCCTCAGAGTGATTGACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGCCCAGGGACCACCGACCCACCGTC

                       210       220       230       240       250       260       270       280       290       300
                         |         |         |         |         |         |         |         |         |         |
WPI_1106        GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
WPI_1178        GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
VP35            GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
VP62            GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
22Rv1           GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
VP42            GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
RKO             GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
MTCR            GGGAGGTAAGCTGGCCAGCGATCGTTTTGTCTCCGTCCCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTACTTTTTGCGCCTGCGTCTGAATCTGTA
                *********** **** ***************  *** *********************************** *  * *********************

Prim.cons.      GGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGTCTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA

                       310       320       330       340       350       360       370       380       390       400
                         |         |         |         |         |         |         |         |         |         |
WPI_1106        CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG
WPI_1178        CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG
VP35            CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCAGGGAGACGTCCCAGCGGCCTCGGGG
VP62            CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG
22Rv1           CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG
VP42            CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG
RKO             CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAARAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG
MTCR            CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGTGGAAGAACTGACGAGTTCGTATTCCCGACCGCAGCCCTGGGAGACGTCTCAGAGGCATCAGGG
                ************************************ *************************** ********* ********** *** *** ** ***

Prim.cons.      CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATTCCCGGCCGCAGCCCTGGGAGACGTCCCAGCGGCCTCGGGG

                       410       420       430       440       450       460       470       480       490       500
                         |         |         |         |         |         |         |         |         |         |
WPI_1106        GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTTTTTGGAG------------------------TGGCTTTGTTGGGGGACG
WPI_1178        GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTTTTTGGAG------------------------TGGCTTTGTTGGGGGACG
VP35            GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTCTTTGGAG------------------------TGGCTTTGTTGGGGGACG
VP62            GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTTTTTGGAG------------------------TGGCTTTGTTGGGGGACG
22Rv1           GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTTTTTGGAG------------------------TGGCTTTGTTGGGGGACG
VP42            GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTTTTTGGAG------------------------TGGCTTTGTTGGGGGACG
RKO             GCCCGTTTTGTGGCCCATTCTGTATCAGTTAA-CCTACCCGAGTCGGACTTTTTGGAG------------------------TGGCTTTGTTGGGGGACG
MTCR            GCCCGCTGGGTGGCCCGATCAGTAAGTCCGAGTCCTGACCGATTCGGACTATTTGGAGCCCCTCCTTTGTCGGAGGGGGACGTGGTTCTTTTAGGAGACG
                ***** *  *******  ** ***      *  ***  **** ******* *******                        *** * * ** ** ****

Prim.cons.      GCCCGTTTTGTGGCCCATTCTGTATCAGTTAATCCTACCCGAGTCGGACTTTTTGGAGCCCCTCCTTTGTCGGAGGGGGACGTGGCTTTGTTGGGGGACG

                       510       520       530       540       550
                         |         |         |         |         |
WPI_1106        AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
WPI_1178        AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
VP35            AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
VP62            AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
22Rv1           AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
VP42            AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
RKO             AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
MTCR            AGAGGTCCAAGCCCTCGCCGCCTCCATCTGAATTTTTGCTTTCGGTTTTT
                ****    *  * **  ***** ** ***********************

Prim.cons.      AGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTA
```

Figure S6. Comparison of sequences at 5' leader region of gag gene between XMRV RKO and the known XMRV strains and xenotropic MLV. XMRV RKO represented an isolate from the RKO cells (Maitra Lab) contaminated with XMRV viruses (see Sup.Table S2). The XMRV RKO sequence, which were obtained from PCR product of RKO genomic DNA using GagF1 and GagR2 primers (Table S3), contain XMRV 24-nt deletion feature as compared with a MTCR XMLV strain and >99.6% homology in 5' leader region of *gag* gene to all known XMRV strains (WPI-1106, WPI-1178,VP35, VP62 and 22Rv1).

```
                                970       980       990      1000      1010      1020
                                  |         |         |         |         |         |
MLV N417                 TCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCT
MTCR                     TCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCT
LAPC-4(2010)             -------------------------------------------CCGCCCCGATCTGCCCT
LAPC-4(1998)             TCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCT
                                                                    *****************

Prim.cons.               TCCAACCGCTCCCATCCTCCCATCCGGTCCTTCGACCCAACCTCCGCCCCGATCTGCCCT

                               1030      1040      1050      1060      1070      1080
                                  |         |         |         |         |         |
MLV N417                 TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTCTCTCCGA
MTCR                     TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTCTCTCCGA
LAPC-4 (2010)            TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTCTCTCCGA
LAPC-4 (1998)            TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTCTCTCCGA
                         ************************************************************

Prim.cons.               TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTCTCTCCGA

                               1090      1100      1110      1120      1130      1140
                                  |         |         |         |         |         |
MLV N417                 TAATGGCGGACCTCTCATTGACCTTCTCACAGAAGACCCTCCGCCGTACGGAGAACAGGG
MTCR                     TAATGGCGGACCTCTCATTGACCTTCTCACAGAAGACCCTCCGCCGTACGGAGAACAGGG
LAPC-4 (2010)            TAATGGCGGACCTCTCATTGACCTTCTCACAGAAGACCCTCCGCCGTACGGAGAACAGGG
LAPC-4 (1998)            TAATGGCGGACCTCTCATTGACCTTCTCACAGAAGACCCTCCGCCGTACGGAGAACAGGG
                         ************************************************************

Prim.cons.               TAATGGCGGACCTCTCATTGACCTTCTCACAGAAGACC2TCCGCCGTACGGAGAACAGGG

                               1150      1160      1170      1180      1190      1200
                                  |         |         |         |         |         |
MLV N417                 ACCGTCCTCCTCTGACGGAGATGGCGACAGAGAAGAGGCCACCTCCACTCCTGAGATTCC
MTCR                     ACCGTCCTCCTCTGACGGAGATGGCGACAGAGAAGAGGCCACCTCCACTCCTGAGATTCC
LAPC-4 (2010)            ACCGTCCTCCTCTGACGGAGATGGCGACAGAGAAGAGGCCACCTCCACTCCTGAGATTCC
LAPC-4 (1998)            ACCGTCCTCCTCTGACGGAGATGGCGACAGAGAAGAGGCCACCTCCACTCCTGAGATTCC
                         ************************************************************

Prim.cons.               ACCGTCCTCCTCTGACGGAGATGGCGACAGAGAAGAGGCCACCTCCACTCCTGAGATTCC

                               1210      1220      1230      1240      1250      1260
                                  |         |         |         |         |         |
MLV N417                 TGCCCCCTCTCCCATGGTGTCTCGCTTGCGGGGCAAAAGAGACCCCCCC-GCGGCAGTTT
MTCR                     TGCCCCCTCTCCCATGGTGTCTCGCTTGCGGGGCAAAAGAGACCCCCCCCGCGGCAGTTT
LAPC-4 (2010)            TGCCCCCTCTCCCATGGTGTCTCGCTTGCGGGGCAAAAGAGACCCCCCC-GCGGCAGTTT
LAPC-4 (1998)            TGCCCCCTCTCCCATGGTGTCTCGCTTGCGGGGCAAAAGAGACCCCCCC-GCGGCAGTTT
                         ************************************************* **********

Prim.cons.               TGCCCCCTCTCCCATGGTGTCTCGCTTGCGGGGCAAAAGAGACCCCCCCCGCGGCAGTTT

                               1270      1280      1290      1300      1310      1320
                                  |         |         |         |         |         |
MLV N417                 CCACCACCTCTCGGGCTTTCC-CACTCCGTTTGGGGGGTAATGGTCAGTTGCAGTACTGG
MTCR                     CCACCACCTCTCGGGCTTTCCGCACTCCGTTTGGGGGGTAATGGTCAGTTGCAGTACTGG
LAPC-4 (2010)            CCACCACCTCTCGGGCTTTCC-CACTCCGTTTGGGGGGTAATGGTCAGTTGCAGTACTGG
LAPC-4 (1998)            CCACCACCTCTCGGGCTTTCC-CACTCCGTTTGGGGGGTAATGGTCAGTTGCAGTACTGG
                         ********************* **************************************

Prim.cons.               CCACCACCTCTCGGGCTTTCCGCACTCCGTTTGGGGGGTAATGGTCAGTTGCAGTACTGG

                               1330      1340      1350      1360      1370      1380
                                  |         |         |         |         |         |
MLV N417                 CCG-TTTTCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTTCTCTGAAGA
MTCR                     CCGGTTTTCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTTCTCCGAAGA
LAPC-4 (2010)            CCG-TTTTCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTTCTCTGAAGA
LAPC-4 (1998)            CCG-TTTTCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTTCTCTGAAGA
                         *** ************************************************** *****

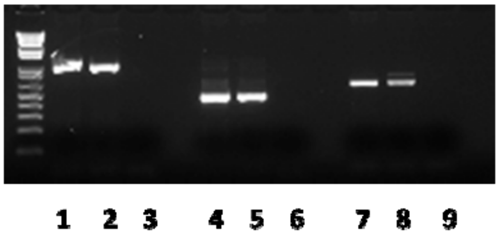
Prim.cons.               CCGGTTTTCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTTCTCTGAAGA
```

Figure S7A. Early passage (1998) and late passage (2010) of LAPC-4 cells contain the same MLV sequences identical to MLV N417 strain at gag gene region. Representative sequencing data which were obtained by sequencing of XS3/XS4 PCR products of LAPC-4 DNA (see Sup. Table S3 for details). nt positions referred to MTCR (Accession no. X94150.1). MLV N417: GeneBank Accession no. HQ246218.

```
nt

5821                       AATAGTTATA GGGATCTTGG TGAGGGCAGG AGCCTCGGTA
5881 CAACGTGACA GCCCTCACCA GGTCTTCAAT GTCACTTGGA GAGTTACCAA CCTAATGACA
5941 GGACAAACAG CTAACGCTAC CTCCCTCCTG GGGACGATGA CAGACACCTT CCCTAAACTA
6001 TATTTTGACT TGTGTGATTT AGTTGGAGAC CATTGGGATG ACCCAGAACC CGATATTGGA
6061 GATGGTTGCC GCTCTCCGGG GGGAAGAAAA AGGACAAGAC TGTATGACTT CTATGTTTGC
6121 CCCGGTCATA CTGTACCAAT AGGGTGTGGA GGGCCGGGAG AGGGCTACTG TGGCAAATGG
6181 GGATGTGAGA CCACTGGACA GGCATACTGG AAGCCATCAT CATCATGGGA CCTAATTTCC
6241 CTTAAGCGAG GAAACACTCC TAAGGATCAG GGCCCCTGTT ATGATTCCTC GGTCTCCAGT
6301 GGCGTCCAGG GTGCCACACC GGGGGGTCGA TGCAACCCCC TAGTCTTAGA ATTCACTGAC
6361 GCGGGTAAAA AGGCCAGCTG GGATGCCCCC AAAGTTTGGG GACTAAGACT CTACCGATCC
6421 ACGGGGGCCG ACCCGGTGAC CCGGTTCTCT TTGACCCGCC AGGTCCTCAA TGTAGGACCC
6481 CGCGTCCCCA TTGGGCCTAA TCCCGTGATC ACTGAACAGC TACCCCCCTC CCAACCCGTG
6541 CAGATCATGC TCCCCAGGCC TCCTCATCCT CCTCCTTCAG GCGCGGCCTC TATGGTGCCT
6601 GGGGCTCCCC CGCCTTCTCA ACAACCTGGG ACGGGGGACA GGCTGCTAAA CCTAGTAAAA
6661 GGAGCCTATC AAGCACTCAA CCTCACCAGT CCCGACAGAA CCCAAGAGTG CTGGCTGTGT
6721 CTGGTATCGG GACCCCCCTA CTACGAAGGG GTTGCCGTCC TAGGTACCTA CTCCAACCAT
6781 ACCTCTGCCC CAGCTAACTG CTCCGTGGCC TCCCAACACA AGCTGACCCT GTCCGAAGTG
6841 ACCGGGCAGG GACTCTGCGT AGGAGCAGTT CCCAAAACCC ATCAGGCCCT GTGTAATACC
6901 ACCCAGAAGG CGAGCGACGG GTCCTACTAT CTGGCTGCTC CCGCCGGGAC CATCTGGGCT
6961 TGCAACACCG GGCTCACTCC CTGCCTATCT ACCACTGTAC TCAACCTCAC CACCGATTAC
7021 TGTGTCCTGG TTGAGCTCTG GCCAAAGGTG ACCTACCACT CCCCTGGTTA TGTTTATGAC
7081 CAGTTTGAGA GAAAAACCAA ATATAAAAGA GAGCCGGTGT CATTAACTCT GGCCCTGCTG
7141 TTGGGAGGAC
```

Figure S7B. LAPC-4 cells contain the MLV sequences of 100% homology to MLV N417 strain at env gene region. The LAPC-4 env gene sequencing data of nt 5840 to 7150 was obtained by sequencing XS14/XS15 PCR products of LAPC-4 DNA (early passage) (see Sup. Table S3 for details), and has 100% homology to MLV N417 strain. The nt positions referred to MLV N417: GeneBank Accession no. HQ246218.



Lane 1: LAPC-4 1998, XS3_XS4, gag PCR
Lane 2: LAPC-4 2010, p138, XS3_XS4, gag PCR
Lane 3: water only ctrl
Lane 4: LAPC-4 1998, XS3_GagQ2R2, gag PCR
Lane 5: LAPC-4 2010, p138, XS3_XS4, gag PCR
Lane 6: water only ctrl
Lane 7: LAPC-4 1998, GagQ2F2_XS4, gag PCR
Lane 8: LAPC-4 2010, p138, GagQ2F2_XS4, gag PCR
Lane 9: water only ctrl

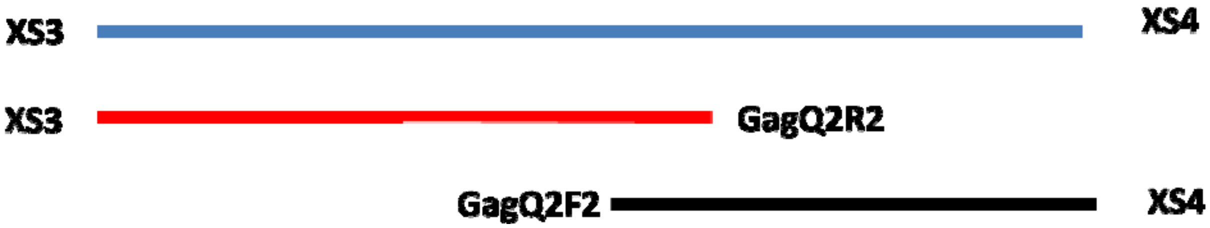


Figure S7C. Detection of MLV-related sequences of different strains in LAPC-4 by three over-lapping PCR reactions and sequencing. The DNA of early or late passage of LAPC-4 cells (see Materials/Methods) were positively amplified with the products of approximate expected size by three sets of overlapping primers (XS3/XS4, XS3/GagQ2R2 and GagQ2F2/XS4) as illustrated (not up to the scale) in this figure. The PCR products were sequenced and analyzed (Sup. Figs. S7D and E).

```
                          1030      1040      1050      1060      1070      1080
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTCTCTCCGA
LAPC_4_GagQ2F2/XS4  ----------------------------------------------------CCCTCCGA
                                                                        * ******

Prim.cons.          TTACCCTGCTCTTACCCCCTCTATAAAACCCAGACCTTCTAAACCTCAGGTTC2CTCCGA

                          1090      1100      1110      1120      1130      1140
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      TAATGGCGGACCTCTCATTGACCTTCTCACAGAAGACCCTCCGCCGTACGGAGAACAGGG
LAPC_4_GagQ2F2/XS4  --GCGGCGGCTCTC----CGGCCTCT--------GGCTCCCCGCCGTACGGAGCACA---
                        *****  ***     * ***          * * * ************* ***

Prim.cons.          TA22GGCGG22CTCTCAT2G2CCT22TCACAGAAG2C2C2CCGCCGTACGGAG2ACAGGG

                          1150      1160      1170      1180      1190      1200
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      ACCGTCCTCCTCTGACGGAGATGGCGACAGAGAAGAGGCCACCTCCACTCCTGAGATTCC
LAPC_4_GagQ2F2/XS4  ACCTTCCTCCTCTGCCAGGGAGAACAATGAAAAAGAGGCGGCCACCACCTCCGAGGTTTC
                    *** ********** * * **   * *   * *******  ** ****  * *** ** *

Prim.cons.          ACC2TCCTCCTCTG2C2G2GA222C2A222A2AAGAGGC22CC2CCAC22C2GAG2TT2C

                          1210      1220      1230      1240      1250      1260
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      TGCCCCCTCTCCCATGGTGTCTCGCTTGCGGGGCAAAAGAGACCCCCCCGCGGCAGTTTC
LAPC_4_GagQ2F2/XS4  CCCCCCTTCTCCCATGGTGTCTCGACTGCGGGGAAGGAGAGACCCTCCCGCAGCGGACTC
                      **** *****************  ******* *  ******** ***** ** *  **

Prim.cons.          22CCCC2TCTCCCATGGTGTCTCG22TGCGGGG2A22AGAGACCC2CCCGC2GC2G22TC

                          1270      1280      1290      1300      1310      1320
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      CACCACCTCTCGGGCTTTCCCACTCCGTTTGGGGGGTAATGGTCAGTTGCAGTACTGGCC
LAPC_4_GagQ2F2/XS4  CACCACCTCCCAGGCGTTCCCACTCCGCATGGGAGAAGATGGCCAGCTTCAGTACTGGCC
                    ********* * *** ***********  **** *   **** *** * ***********

Prim.cons.          CACCACCTC2C2GGC2TTCCCACTCCG22TGGG2G222ATGG2CAG2T2CAGTACTGGCC

                          1330      1340      1350      1360      1370      1380
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      GTTTTCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTTCTCTGAAGATCC
LAPC_4_GagQ2F2/XS4  GTTCTCCTCC-------------------------------CTTCCTTTTCTGAAGATCC
                    *** ******                               ******* ***********

Prim.cons.          GTT2TCCTCCTCGGATCTATATAACTGGAAAAATAATAACCCTTCCTT2TCTGAAGATCC

                          1390      1400      1410      1420      1430      1440
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      AGGTAAATTGACTGCCTTAATCGAGTCTGTCCTCACCACCCACCAGCCTACTTGGGATGA
LAPC_4_GagQ2F2/XS4  AGGTAAATTGACGGCCTTGATTGAGTCCGTCCTCATCACCCACCAGCCCACCCGGGACGA
                    ************ ***** ** ***** ******* ************ **  **** **

Prim.cons.          AGGTAAATTGAC2GCCTT2AT2GAGTC2GTCCTCA2CACCCACCAGCC2AC22GGGA2GA

                          1450      1460      1470      1480      1490      1500
                             |         |         |         |         |         |
LAPC_4_XS3/XS4      CTGTCAACAGTTGCTGGGGACTCTGCTGACAGGAGAAGAAAAGCAGCGGGTGCTCCTGGA
LAPC_4_GagQ2F2/XS4  CTG---------------------------------------------------------
                    ***

Prim.cons.          CTGTCAACAGTTGCTGGGGACTCTGCTGACAGGAGAAGAAAAGCAGCGGGTGCTCCTGGA
```

Figure S7D. LAPC-4 cells contain 2 different MLV-related sequences at gag gene region. Alignment analysis of the sequencing data which were obtained by sequencing of XS3/XS4 or GagQ2F2/XS4 PCR products of LAPC-4 DNA (see Sup. Fig. S5A, Table S3 for details). nt positions referred to MLV N417 (GeneBank Accession no. HQ246218).

```
                          1030      1040      1050      1060      1070      1080
                             |         |         |         |         |         |
SP(B)1-2            GCGTCCCTCCGAGCGGCGGCTCTCCGGCCTCTGGCTCCCCGCCGTACGGAGCACAACCTT
LAPC_4_GagQ2F2/XS4  ----CCCTCCGAGCGGCGGCTCTCCGGCCTCTGGCTCCCCGCCGTACGGAGCACAACCTT
                        ********************************************************

Prim.cons.          GCGTCCCTCCGAGCGGCGGCTCTCCGGCCTCTGGCTCCCCGCCGTACGGAGCACAACCTT

                          1090      1100      1110      1120      1130      1140
                             |         |         |         |         |         |
SP(B)1-2            CCTCCTCTGCCAGGGAGAACAATGAAAAAGAGGCGGCCACCACCTCCGAGGTTTCCCCCC
LAPC-4_GagQ2F2XS4   CCTCCTCTGCCAGGGAGAACAATGAAAAAGAGGCGGCCACCACCTCCGAGGTTTCCCCCC
                    ************************************************************

Prim.cons.          CCTCCTCTGCCAGGGAGAACAATGAAAAAGAGGCGGCCACCACCTCCGAGGTTTCCCCCC

                          1150      1160      1170      1180      1190      1200
                             |         |         |         |         |         |
SP(B)1-2            CTTCTCCCATGGTGTCTCGACTGCGGGGAAGGAGAGACCCTCCCGCAGCGGACTCCACCA
LAPC-4_GagQ2F2/XS4  CTTCTCCCATGGTGTCTCGACTGCGGGGAAGGAGAGACCCTCCCGCAGCGGACTCCACCA
                    ************************************************************

Prim.cons.          CTTCTCCCATGGTGTCTCGACTGCGGGGAAGGAGAGACCCTCCCGCAGCGGACTCCACCA

                          1210      1220      1230      1240      1250      1260
                             |         |         |         |         |         |
SP(B)1-2            CCTCCCAGGCGTTCCCACTCCGCATGGGAGAAGATGGCCAGCTTCAGTACTGGCCGTTCT
LAPC-4_GagQ2F2/XS4  CCTCCCAGGCGTTCCCACTCCGCATGGGAGAAGATGGCCAGCTTCAGTACTGGCCGTTCT
                    ************************************************************

Prim.cons.          CCTCCCAGGCGTTCCCACTCCGCATGGGAGAAGATGGCCAGCTTCAGTACTGGCCGTTCT

                          1270      1280      1290      1300      1310      1320
                             |         |         |         |         |         |
SP(B)1-2            CCTCCCTTCCTTTTCTGAAGATCCAGGTAAATTGACGGCCTTGATTGAGTCCGTCCTCAT
LAPC-4_GagQ2F2/XS4  CCTCCCTTCCTTTTCTGAAGATCCAGGTAAATTGACGGCCTTGATTGAGTCCGTCCTCAT
                    ************************************************************

Prim.cons.          CCTCCCTTCCTTTTCTGAAGATCCAGGTAAATTGACGGCCTTGATTGAGTCCGTCCTCAT

                          1330      1340      1350      1360      1370      1380
                             |         |         |         |         |         |
SP(B)1-2            CACCCACCAGCCCACCCGGGACGACTGTCAGCAGTTGTTGGGGACCCTGCTGACCGGAGA
LAPC-4_GagQ2F2/XS4  CACCCACCAGCCCACCCGGGACGACTG---------------------------------
                    ***************************

Prim.cons.          CACCCACCAGCCCACCCGGGACGACTGTCAGCAGTTGTTGGGGACCCTGCTGACCGGAGA
```

Figure S7E. LAPC-4 cells contain MLV-related sequences at gag gene region with 100 % homology to MLV SP(B) 1-2 isolate. The sequencing data which were obtained by sequencing of GagQ2F2/XS4 PCR products of LAPC-4 DNA (see Figs. S7A and B and Table S3 for details). nt positions referred to MLV SP(B) 1-2 (GeneBank Accession no. AY349140).

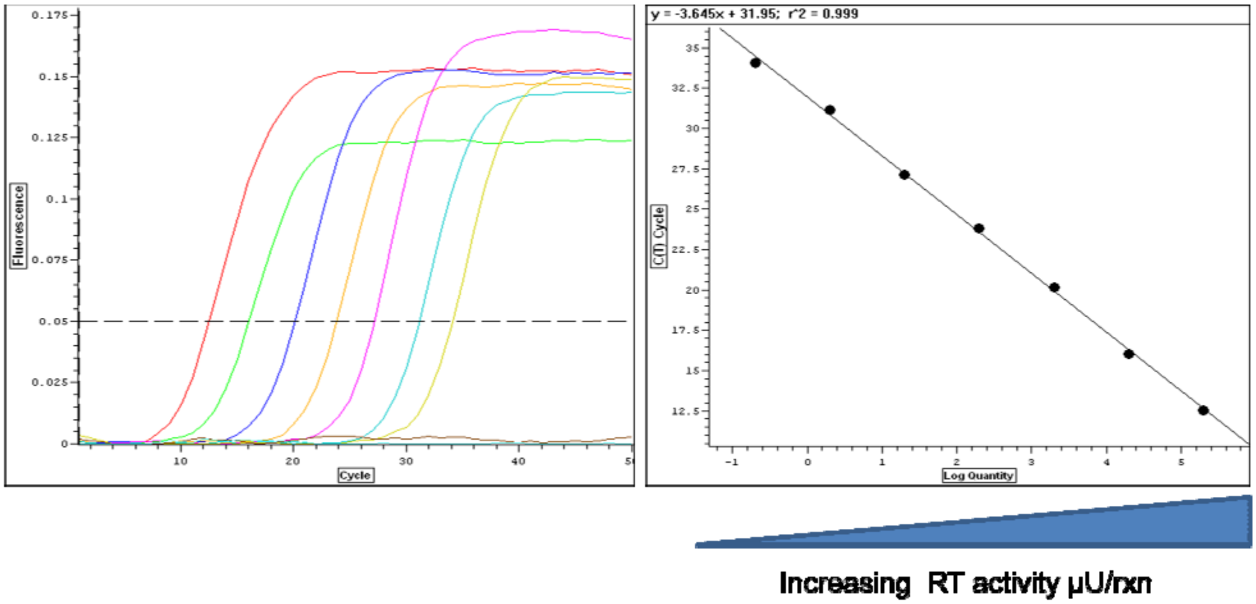


Figure S8. Validation of TM-PERT (<u>T</u>aqman fluorogenic 5'-nulcease <u>P</u>roduct-<u>E</u>nhanced <u>R</u>everse <u>T</u>ranscriptase) assay for measuring reverse transcriptase activity. See the Materials/Methods for the details. Titration range: 2×10^5µU/rxn to 0.02 µU/rxn. Buffer or water only: completely negative here. Rxn: react

References for Supplementary Materials

1. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008; 321:1801-6.
2. Brattain MG, Levine AE, Chakrabarty S, Yeoman LC, Willson JK, Long B. Heterogeneity of human colon carcinoma. Cancer Metastasis Rev 1984; 3:177-91.
3. Silverman RH, Nguyen C, Weight CJ, Klein EA. The human retrovirus XMRV in prostate cancer and chronic fatigue syndrome. Nat Rev Urol 2010; 7:392-402.
4. Hohn O, Krause H, Barbarotto P, Niederstadt L, Beimforde N, Denner J, et al. Lack of evidence for xenotropic murine leukemia virus-related virus(XMRV) in German prostate cancer patients. Retrovirology 2009; 6:92.
5. McCormick AL, Brown RH, Jr., Cudkowicz ME, Al-Chalabi A, Garson JA. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. Neurology 2008; 70:278-83.
6. Shi L, Chen Q, Norling LA, Lau AS, Krejci S, Xu Y. Real time quantitative PCR as a method to evaluate xenotropic murine leukemia virus removal during pharmaceutical protein purification. Biotechnol Bioeng 2004; 87:884-96.
7. Johnson KL, Dukes KA, Vidaver J, LeShane ES, Ramirez I, et al. (2004) Interlaboratory comparison of fetal male DNA detection from common maternal plasma samples by real-time PCR. Clin Chem 50: 516-521.
8. Brorson K, Xu Y, Swann PG, Hamilton E, Mustafa M, de Wit C, et al. Evaluation of a quantitative product-enhanced reverse transcriptase assay to monitor retrovirus in mAb cell-culture. Biologicals 2002; 30:15-26.
9. Brorson K, Swann PG, Lizzio E, Maudru T, Peden K, Stein KE. Use of a quantitative product-enhanced reverse transcriptase assay to monitor retrovirus levels in mAb cell-culture and downstream processing. Biotechnol Prog 2001; 17:188-96.
10. Peters SO, Bauermeister K, Simon JP, Branke B, Wagner T. Quantitative polymerase chain reaction-based assay with fluorogenic Y-chromosome specific probes to measure bone marrow chimerism in mice. J Immunol Methods 2002; 260:109-16.
11. Ramirez RD, Sheridan S, Girard L, Sato M, Kim Y, Pollack J, et al. Immortalization of human bronchial epithelial cells in the absence of viral oncoproteins. Cancer Res 2004; 64:9027-34.