Supporting Information

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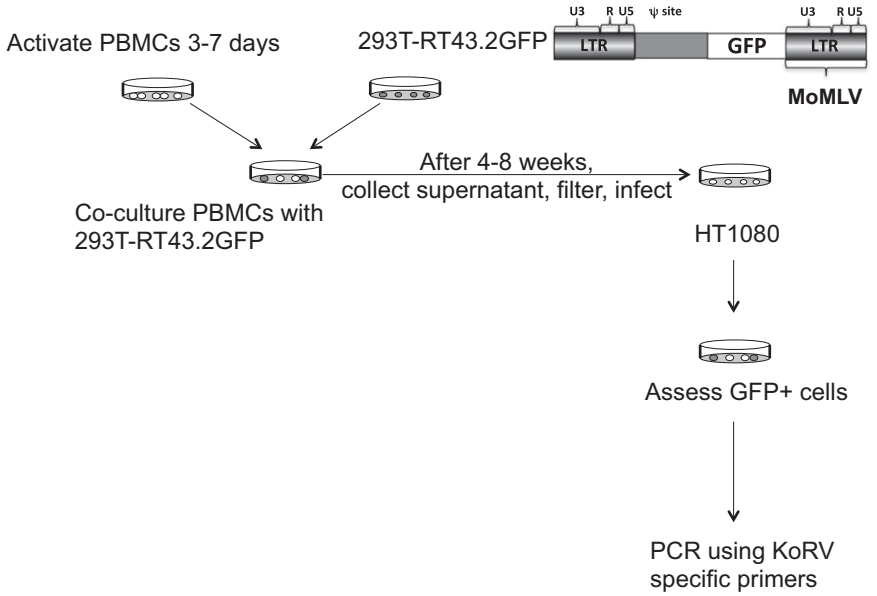

Fig. S1. Schematic summary of methods performed for viral marker rescue assay and infection of human HT1080 cells with koala endogenous retroviruses (KoRVs) from koala peripheral blood mononuclear cells (PBMCs). Experimental design of the coculture assay used to detect infectious KoRV subgroup A or B isolated from koala PBMCs. Gray circles correspond to cells expressing GFP. (*Upper Right*) Schematic representation of proviral form of RT43.2-GFP integrated in 293T cells (diagram not to scale). LTRs including U3, R, and U5 regions are derived from Moloney murine leukemia virus (MoMLV) and are highlighted in gray.

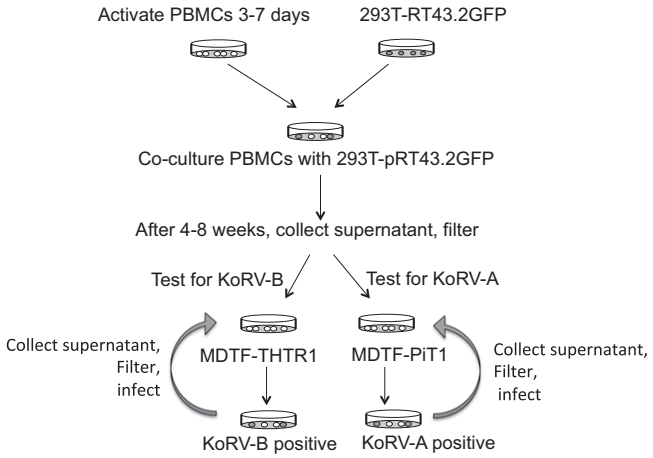

Fig. S2. Schematic representation of propagation of KoRV-A and KoRV-B in murine cells expressing phosphate transporter 1 (PiT1) or thiamine transporter (THTR1) receptors, respectively. Infectious KoRV-A and KoRV-B viruses obtained by coculturing 293T-GFP with koala PBMCs were used to infect and passage in mus dunni tail fibroblast (MDTF)-PiT1 or MDTF-THTR1 cells.

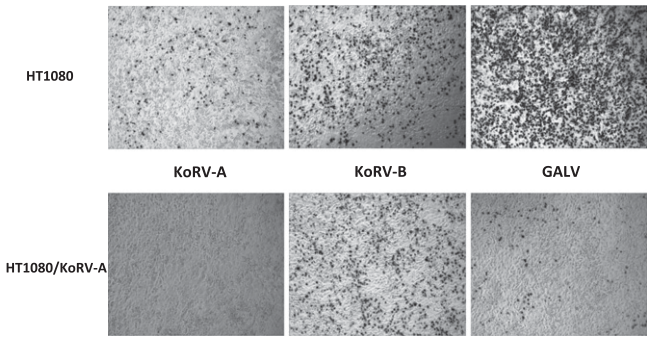

Fig. S3. KoRV-B uses a different receptor than that used by KoRV-A. HT1080 cells (*Upper*) and HT1080/KoRV-A cells (*Lower*) created by infecting HT1080 cells with the replication-competent retrovirus using KoRV-A env as described in Fig. 5*A* were infected with KoRVA, KoRVB, or Gibbon ape leukemia virus (GALV) enveloped retroviral vectors expressing nuclear localized β-gal as described in Fig. 5*B*. Positive infected cells are shown as black in the micrograph. Micrographs were taken with a 20× objective on a phase-contrast microscope.

Table S1. KoRV generic and type-specific real-time PCR assays

Region	Assay	Forward primer (5′ to 3′)	TaqMan probe (5′ to 3′)	Reverse primer (5′ to 3′)
env	KoRV generic	CACCCAGGCGTGCAGTTGACCA	AGGATCGGGGCCCACTACTACCGGTGGGGAG	TGGGAGGTCCTTGTYCTGCGAGGA
env	KoRV-A–specific	GCCAGGCCCCCTGATTCAA	AGCTACATCCCAGGGTTCCCCAAGTGATCTGATTATAAGCATG	GCACACGTAGAACTGGGACC
env	KoRV-B–specific	GCCAGAATCTCAACAGTCTGC	AGGTTCCATAGCTCGATTGCCTGACCAACCCTCTGCCGACCT	GGGACACACATAGAACTGAGATTG

env, envelope gene.