Nature Methods

Antiviral restriction factor transgenesis in the domestic cat

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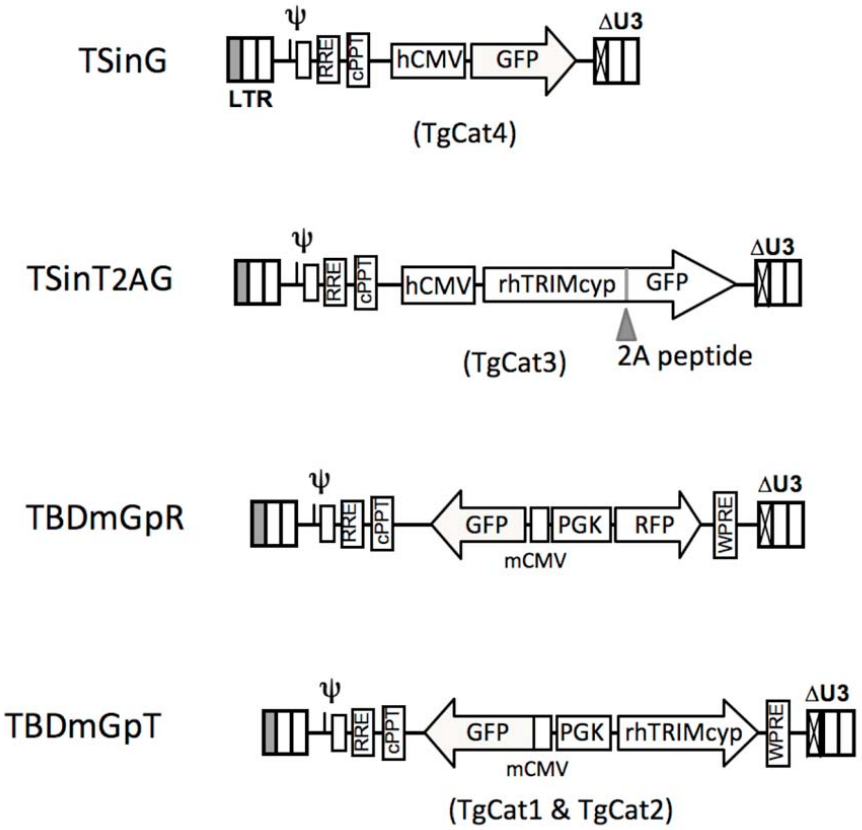

Supplementary Figure 1. Lentiviral vectors. TSinG and derivative TSinT2AG both utilize a standard 0.52 Kb internal hCMV promoter. For TSinT2AG, a porcine teschovirus 2A peptide (P2A) mediates expression of both proteins from a single precursor polypeptide. Proper co-translational cleavage at the 2A site was confirmed by immuoblotting. The other two vectors utilize a dual promoter having a minimal (0.16 Kb) hCMV core promoter (mCMV) and a PGK promoter. The four otherwise contain standard transfer vector elements: packaging signal, RRE, WPRE, cPPT-CTS (cPPT); all are U3-deleted (ΔU3). See Methods for further details.

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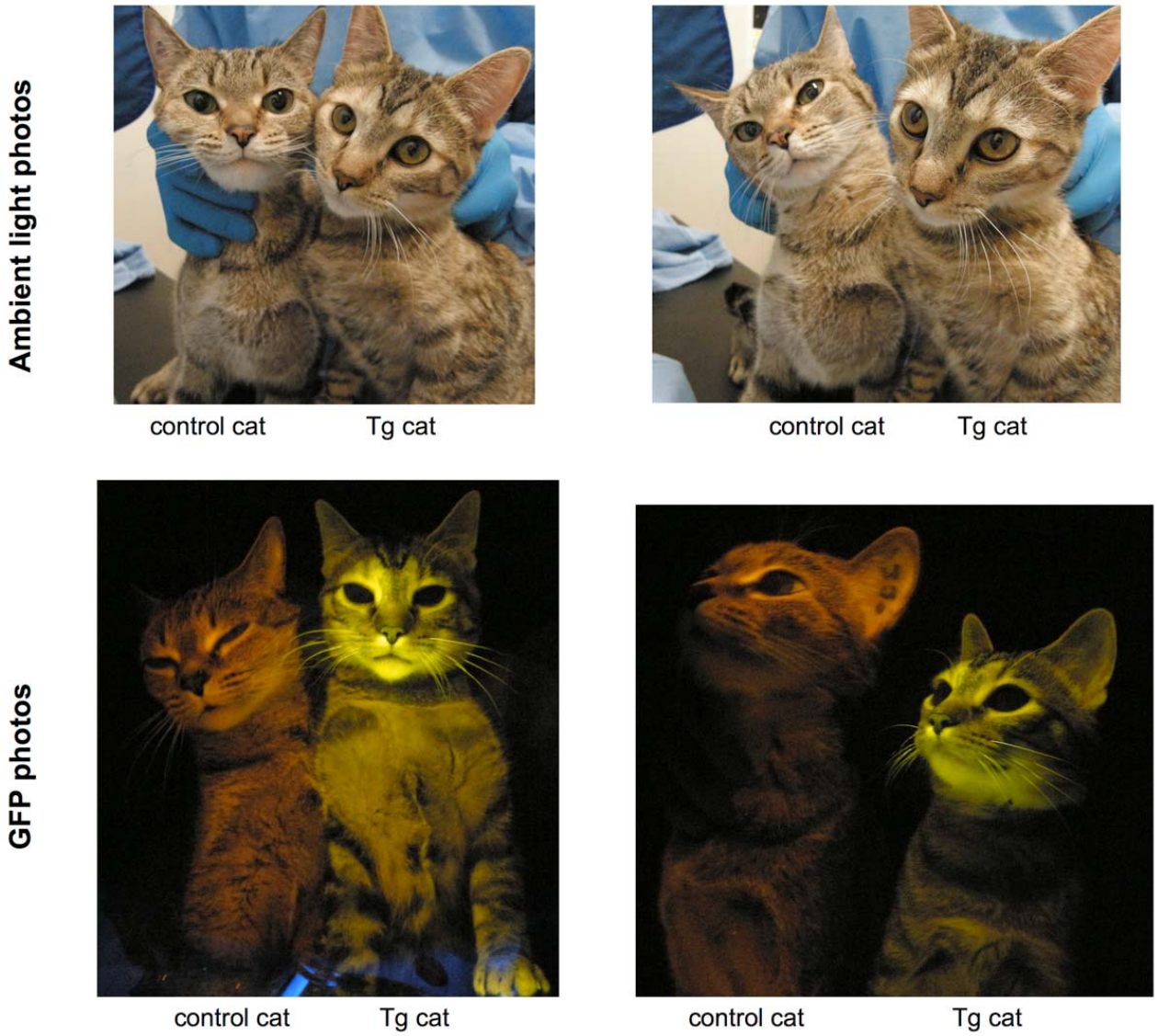
2a



Supplementary Figure 2. Additional animal images and radiographs (panels b-d on subsequent pages).
(a) 5.5 month images corresponding to Fig. 2A, with control and transgenic cat in the same frame.
(b) Simultaneous control cat images for day 2.
(c) Enlargements of the 30 day images in Fig. 2a.
(d) Films were taken on day 45 of a pregnancy that terminated in spontaneous miscarriage 6 days later (term is 61-63 days in this species).

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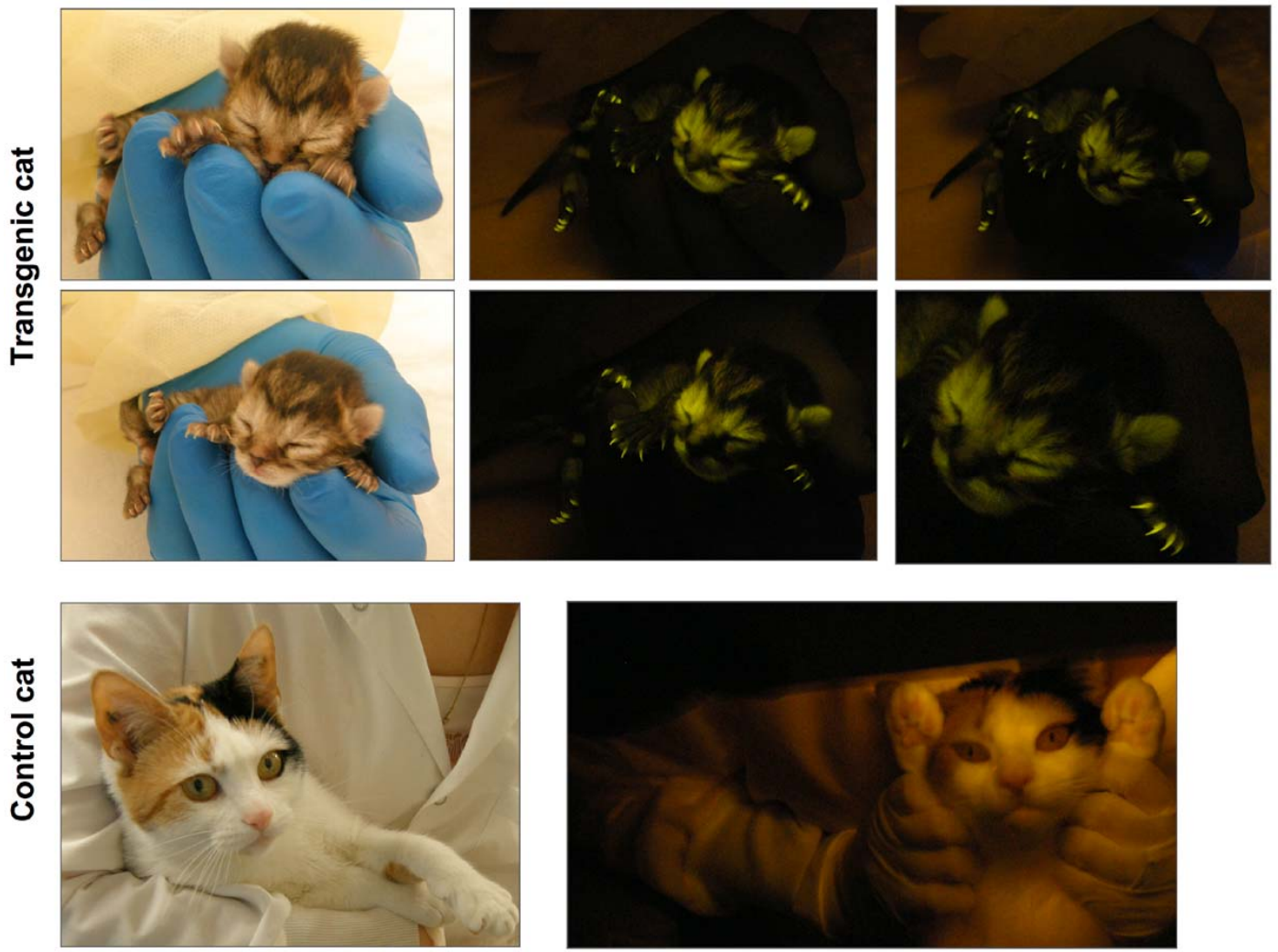
2b



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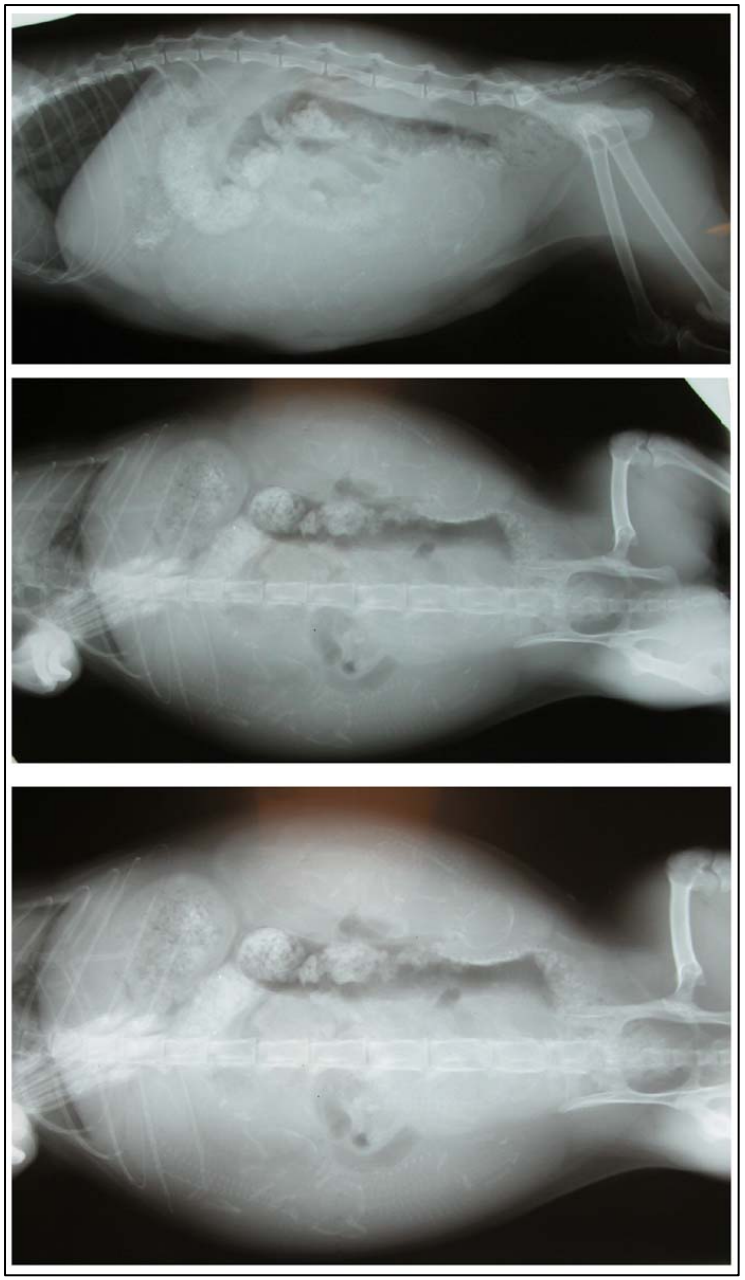
2c

2d



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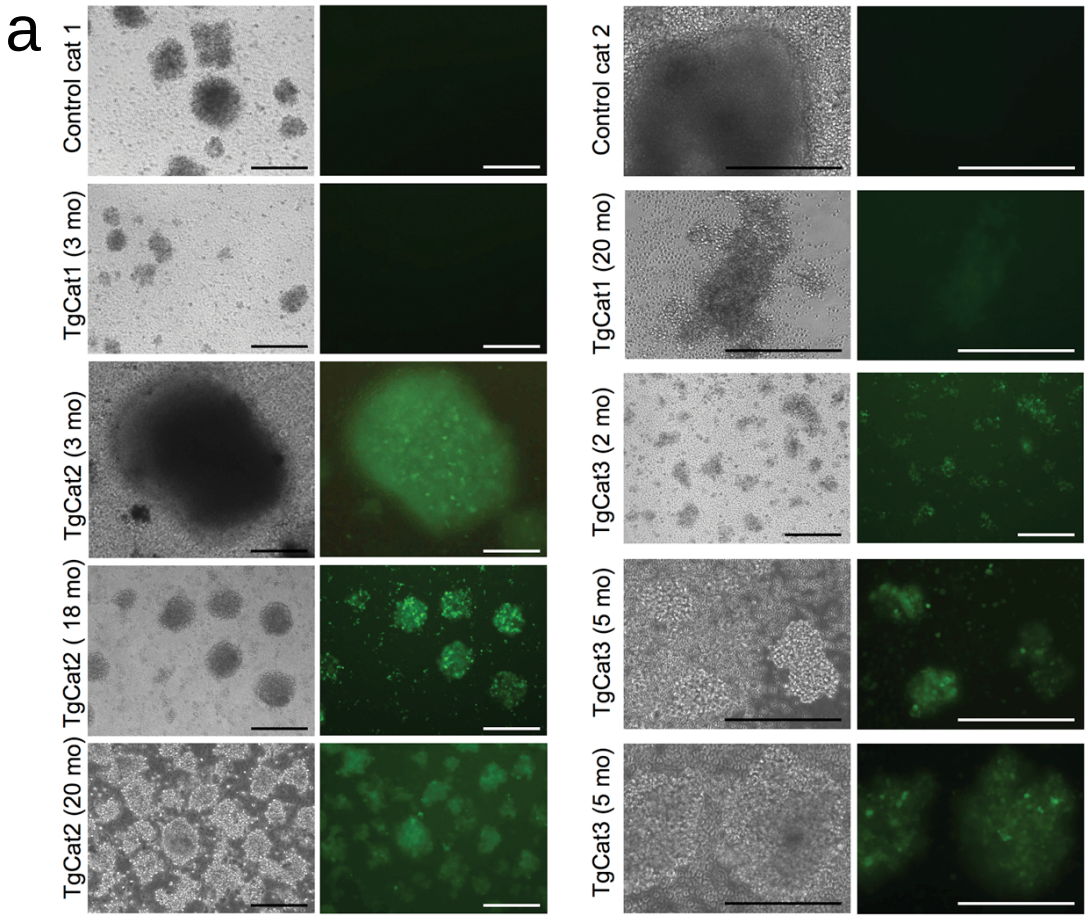

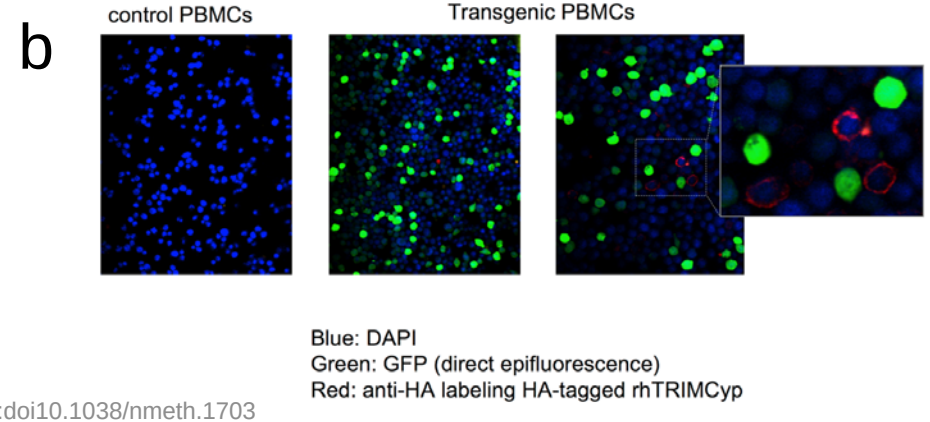

Supplementary Figure 3. GFP and rhTRIMcyp expression in PBMCs.
(a) Fluorescence microscopy of PBMCs. Animall ages are shown at left. Small and large size bars represent 500 and 200 μM respectively.
(b) rhTRIMCyp; cells are also imaged for GFP and nuclear DNA (DAPI). Immunofluorescence for HA-tagged rhTRIMCyp was done with rat anti-HA. Note that fewer cells expressed detectable TRIMCyp than GFP, and the two proteins were discordant to a large extent, suggesting the mCMV and PGK promoter elements in the vector are differentially active in subsets.

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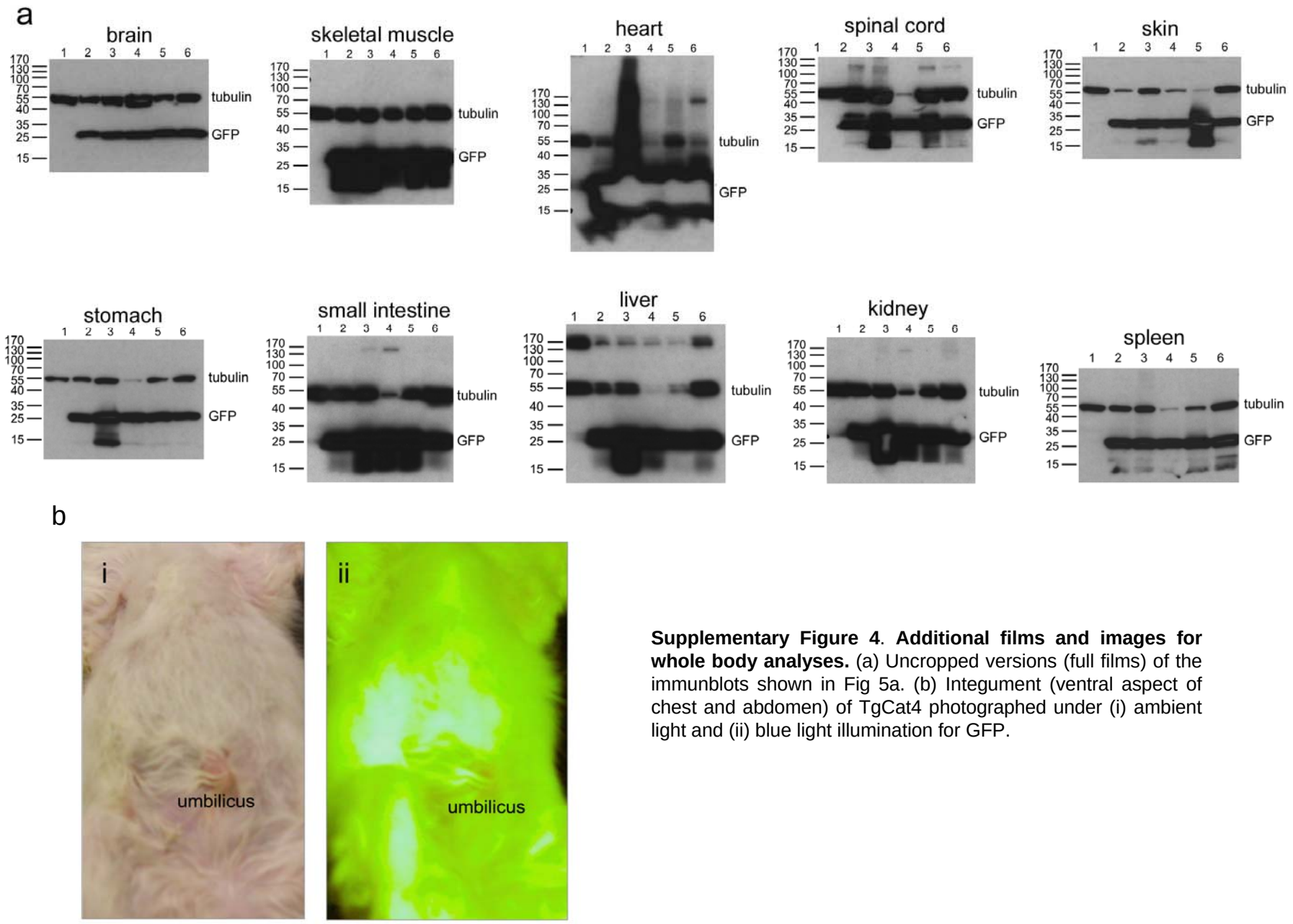

Supplementary Figure 4. **Additional films and images for whole body analyses.** (a) Uncropped versions (full films) of the immunblots shown in Fig 5a. (b) Integument (ventral aspect of chest and abdomen) of TgCat4 photographed under (i) ambient light and (ii) blue light illumination for GFP.

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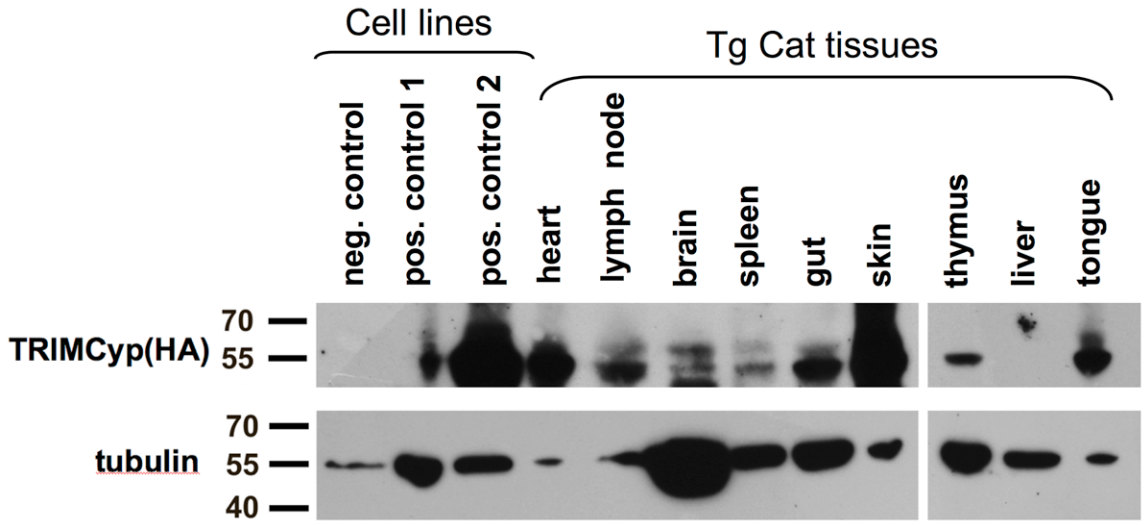

Supplementary Figure 5. rhTRIMCyp expression in solid organs. Immunoblotting with anti-HA was performed as in Fig. 3A.

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Supplementary Table 1. Domestic cat embryo cleavage and blastocyst rates, % transgene expression, effect of timing

Treatment	Number of oocytes	Cleavage rate (%)	Blastocyst rate (%)	Embryo GFP expression (%)	Mosaicism rate
None	112	65.2 ± 4.2	24.3 ± 4.7	0	
Pre-IVF lentiviral vector injection	130	59.3 ± 1.8	19.8 ± 6.4	84.0 ± 11.2	< 1%
Post-IVF lentiviral vector injection	165	60.7 ± 6.5	20.8 ± 6.4	78.8 ± 5.3	20-30%

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Supplementary Table 2. Transgenic embryo development with single vs. dual-gene vectors (all pre-IVF microinjections)

Lentiviral vector	Proteins or proteins encoded in vector	Promoter	Number of oocytes	Cleavage rate (%)	Blastocyst rate (%)	Embryo GFP expression (%)
TSinG	GFP	hCMV	109	62.4 ± 4.7	21.0 ± 2.38	84.5 ± 10.2
TBDmGpT	GFP, rhTrimcyp	mCMV↔PGK	204	61.6 ± 4.4	18.5 ± 5.3	88.3 ± 12.0
TBDmGpR	GFP, RFP	mCMV↔PGK	105	66.6 ±5.3	23.6 ± 4.7	92.7 ± 9.5
TSinT2AG	rhTRIMcyp-P2A-GFP	hCMV	nd	nd	nd	nd

n.d.: not done. hCMV: human cytomegalovirus immediate early gene promoter. mCMV: minimal hCMV promoter.

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Supplementary Table 3. Vector insertion sites

Host - vector junction 1 chrUn_ACBE01523911: 891-1,138 98.8% Identity

GGGTCTGTTCTTCCCATGATCTTCTTCCCCTGGGTACTTGATTCAGTGACTACTTTATAGGAGTTTAGCTG
AGTTTCCTTCAGTGATTTAGGTTTATGGTGAATGTCTTTAACGTTTATTTATTTTTGGGGGGACAGAGAGA
GACAGAGCATGAACGGGGGAGGGGCAGAGAGAGAGGGAGACACAGAATCGGAAACAGGCTCCAGGCTCAGA
GCCATCAGCCCAGAGCCTGACGCGGGGCTCGAACTTGGAAGGGCTAATTCACTCC

Host - vector junction 2 chrX: 127,955,725-127,956,886 91.3% Identity

GGGCATCATCAGCAGCACAGTCCTGTAAACGTTCTTGGATGTGGTTGGCACCCGGCCTTTGCTCGGTCAGA
GTGGGTCAAAGCGGTTCCTCAGAAGTGAGGGGTCAGGAAACACAGTCACATCTGAGATAAAAGGAGGGAGG
TGCTGCCTGGGCTTGGTCATGGACAGTGTAAAAGTGGGAATGGGTGGAAGCCAGAGACAAAGGATGGGTGC
GTGATTGCTGGTGGGGGAGAACAGAGTTCCGATACTAGAGACTGGGTAGCTGGGTGACATCATTTTTAATC
CTCCTGCGCGTGCGCGCAGGCACCCACAAGCACCACAACAATCCACCCCAGTAAGCTAAGAAGCACCTTCT
AGTGGAGAACGGAGCCTGAGCCAACCTCACTTTTCATGAACACCACAAGTCTCTCTGCCTGCTTAGTTCAT
GGACTATAAAGGGCTTCATAGTTTGACTTCTATGGGAAAATGATGTAATTTCAATTGTATTTCAGTTTGTT
CGCTGGCCCATCTATTAAATTTCTTTATCTCTTTTTTGTTTCTTTTCTTTTTCTTGAATACAGAAGATAAA
AAAAATATTTTTATTTTTAATTTTTATTAAAATATTTTGCTTTAATTTTTTCTACTTTATTTTTTACTTTT
TTGTAAATTTTATAAATTCTAATTTAATTCCATCATTTCATTTTATCCTATTTCATTGTATTCATTTTTCA
NATTTTCAAACATTTTACTTTTTTTATTCTTTTCTTACCTTTCCCTTTTAAATTTTTTTTAACATTTATTT
ATTTTTGAGACAGAGAGAGACAGAGCATGAATGGGGGAGGGGCAGAGAGAGAAGGAGACACAGAATCAGAA
ACAGTCTCCAGGCTCTGAGCTGTCAGCCCAGAGCCTGACGTGGGGCTCGAACTCATGGACCGCGAGATCGT
GACCTGGCTGAAGTCGGACGCTTAACCGACTGCGCCACCCAGGCGCCCCTACCTTTCCCTTTTTAATCTAA
TCTATAAAGCTCCTTTTGGAAGGGCTAATTCACTCC

Host - vector junction 3 chrD1: 2,438,470-2,438,540 99.0% Identity

CCAGTAAGCTAAGCAGCACCATCTAGTGGAGAACGGAGCCATTACACTAAGCCCCCTCGATCTTGGCCTGG
GCCAACCTCGATCTTCAGGAACACCACATGGAAGGGCTAATTCACTCC

Host - vector junction 4 chrB1: 21995843 21995941 100% Identity

CCAGTAAGCTAAGCAGCACCATCTAGTGGAGAACGGAGCCATTACACTAAGCCCCCTCGATCTTGGCCTGG
GCCAACCTCGATCTTCAGGAACACCACATGGAAGGGCTAATTCACTCC

Underlined sequences are the U3 terminus of the vector.