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Supplemental Table I. Verification of LIN9 knockdown by qPCR.

	shRNA	Average C_q GAPDH	Average C_q hLin9	ΔC_q hLin9 - GAPDH	ΔC_q Expression $2^{-\Delta C_q}$	Normalized ΔΔ C_q	%KD $(1-\Delta\Delta C_q)\times 100$
SW620 LacZ	non-targeting control	33.680	33.865	0.185	0.880	1.000	
	shRNA against Lin9	33.634	40.000	6.366	0.012	0.013	98%
SW620 hTRM9L	non-targeting control	33.763	35.741	1.978	0.254	1.000	
	shRNA against Lin9	33.999	40.000	6.001	0.016	0.061	93%

Supplemental Table II. tRNA modification levels and standard deviations in yeast cells expressing yTrm9, hALKBH8 and hTRM9L.

Compound Name	WT (average)	trm9D (average)	trm9D + yTRM9 (average)	trm9D + hTRM9L (average)	trm9D + hALKBH8 (average)	WT (stdev)	trm9D (stdev)	trm9D + yTRM9 (stdev)	trm9D + hTRM9L (stdev)	trm9D + hALKBH8 (stdev)
mcm5Um	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
cm5s2U	0.0	0.2	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0
ncm5Um	4.5	4.3	4.4	3.4	7.7	0.3	0.3	0.5	0.5	0.7
cm5U	0.0	1.3	0.0	0.8	0.8	0.0	0.1	0.0	0.1	0.0
ncm5U	12.2	15.6	11.0	15.6	18.8	1.1	1.6	1.0	1.4	0.1
m?G	1,382.8	1,718.6	1,957.8	2,681.8	3,214.3	114.5	127.4	220.5	143.4	42.4
ac4C	110.7	105.9	97.8	113.9	117.6	5.1	6.8	3.6	4.6	3.1
m1I	10.4	8.3	7.7	11.2	11.3	2.8	1.3	0.9	1.0	0.5
m1A	2,212.1	2,724.8	2,966.9	4,949.3	5,844.7	121.7	220.9	334.9	246.8	68.9
m5Um	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I	2,232.9	2,114.6	2,024.5	2,538.7	2,665.2	79.0	147.1	88.4	39.7	17.6
Um	19.5	21.5	25.0	24.5	28.9	0.7	0.8	1.2	2.2	1.8
Cm	472.3	552.7	628.7	950.3	919.3	21.6	30.1	67.5	20.5	16.9
m5U	202.5	212.9	211.4	256.7	308.2	18.9	12.7	19.6	13.3	5.1
m3C	368.0	435.1	439.6	591.7	657.6	28.7	28.1	31.5	46.0	10.3
m5C	1,016.9	1,025.8	1,037.2	1,644.9	1,815.8	89.9	40.2	103.5	60.6	10.2
D	45.5	46.7	48.6	58.4	68.6	7.8	8.7	2.3	1.7	2.5
Y	18.1	19.1	22.9	28.2	33.0	2.3	4.4	1.5	0.6	1.1
yW	22.8	16.7	13.8	24.0	18.5	4.4	0.8	1.1	2.5	0.7
t6A	100.8	83.7	75.9	88.1	98.2	21.3	5.2	2.1	12.7	11.5
i6A	90.1	66.0	51.2	46.1	76.4	14.4	5.6	8.9	6.0	9.2
mcm5s2U	27.1	0.0	20.8	0.0	3.9	4.0	0.0	2.4	0.0	0.5
mcm5U	18.2	0.0	16.1	0.0	5.1	1.6	0.0	1.6	0.0	0.4
m22G	1,798.0	1,699.9	1,721.8	2,957.3	4,193.7	208.2	82.9	205.3	412.7	478.9
m7G	1,854.1	2,040.8	2,610.9	4,340.1	5,620.3	170.9	162.9	416.8	326.5	182.9
m2G	1,077.0	997.6	1,016.4	1,708.9	2,426.1	111.8	48.9	131.8	253.8	130.8
Gm	827.6	837.7	1,008.1	1,334.2	1,623.8	14.7	27.3	92.7	121.4	84.9
Am	1,148.8	1,381.9	1,751.0	2,276.9	2,265.3	62.7	82.8	106.3	57.6	17.1

Supplemental Table III. tRNA modification log₂-fold changes and significance in yeast cells expressing yTrm9, hALKBH8 and hTRM9L.

Compound Name	log ₂ -fold change (WT / trm9D)	log ₂ -fold change (trm9D + yTRM9 / trm9D)	log ₂ -fold change (trm9D + hTRM9L / trm9D)	log ₂ -fold change (trm9D + hALKBH8 / trm9D)	P-value (WT to trm9D)	P-value (trm9D + yTRM9 to trm9D)	P-value (trm9D + hTRM9L to trm9D)	P-value (trm9D + hALKBH8 to trm9D)
mcm5Um	ND	ND	ND	ND	NA	NA	NA	NA
cm5s2U	-1.0	-1.0	0.7	0.1	0.00000	0.00000	0.00840	0.54303
ncm5Um	0.1	0.0	-0.4	0.8	0.42407	0.76193	0.04838	0.00137
cm5U	-1.0	-1.0	-0.6	-0.7	0.00002	0.00002	0.00560	0.00147
ncm5U	-0.4	-0.5	0.0	0.3	0.03928	0.01354	0.96450	0.02596
m?G	-0.3	0.2	0.6	0.9	0.02739	0.17912	0.00096	0.00004
ac4C	0.1	-0.1	0.1	0.2	0.38245	0.14287	0.16602	0.05364
m1I	0.3	-0.1	0.4	0.4	0.29355	0.58517	0.03474	0.01930
m1A	-0.3	0.1	0.9	1.1	0.02442	0.35505	0.00031	0.00002
m5Um	ND	ND	ND	ND	NA	NA	NA	NA
I	0.1	-0.1	0.3	0.3	0.28731	0.41478	0.00852	0.00300
Um	-0.1	0.2	0.2	0.4	0.02580	0.01542	0.09769	0.00300
Cm	-0.2	0.2	0.8	0.7	0.01977	0.15003	0.00005	0.00005
m5U	-0.1	0.0	0.3	0.5	0.47278	0.91728	0.01451	0.00027
m3C	-0.2	0.0	0.4	0.6	0.04453	0.86158	0.00732	0.00021
m5C	0.0	0.0	0.7	0.8	0.88334	0.86718	0.00012	0.00001
D	0.0	0.1	0.3	0.6	0.87123	0.72072	0.08278	0.01357
Y	-0.1	0.3	0.6	0.8	0.73591	0.23029	0.02447	0.00618
yW	0.4	-0.3	0.5	0.1	0.07946	0.02146	0.00855	0.04959
t6A	0.3	-0.1	0.1	0.2	0.24812	0.07360	0.61018	0.11962
i6A	0.4	-0.4	-0.5	0.2	0.05349	0.07216	0.01395	0.16927
mcm5s2U	1.0	0.8	0.0	0.1	0.00029	0.00012	1.00000	0.00014
mcm5U	1.0	0.9	0.0	0.3	0.00004	0.00006	1.00000	0.00002
m22G	0.1	0.0	1.4	1.3	0.49050	0.87221	0.00663	0.00089
m7G	-0.1	0.4	1.1	1.5	0.24261	0.09200	0.00040	0.00001
m2G	0.1	0.0	0.8	1.3	0.32228	0.82813	0.00887	0.00006
Gm	0.0	0.3	0.7	1.0	0.60151	0.03786	0.00230	0.00011
Am	-0.3	0.3	0.7	0.7	0.01774	0.00900	0.00010	0.00005

ND = not determined

grey shaded boxes represent wild-type normalized numbers, as the denominator was zero

Supplemental Table IV. tRNA modification levels and standard deviations in SW620-hTRM9L vs. SW620-LacZ cells (Untreated and Paromomycin, N = 3).

Compound Name	LacZ (average)	LacZ-Paro (average)	hTRM9L (average)	hTRM9L-Paro (average)	LacZ (stdev)	LacZ-Paro (stdev)	hTRM9L (stdev)	hTRM9L-Paro (stdev)
D	569,970.1	517,344.6	464,189.8	420,747.9	43,933.4	27,683.8	48,940.3	48,114.4
acp3U	34,614.6	33,686.5	33,466.8	33,578.5	2,359.1	1,005.5	1,166.2	2,651.9
m5C	4,549,514.7	4,748,438.6	5,043,406.5	4,983,707.4	32,342.2	63,886.0	334,606.8	144,479.8
Cm	1,042,166.6	1,171,780.4	1,180,874.1	1,431,514.6	17,823.3	156,384.7	66,226.8	280,419.7
m3C	2,341,851.2	2,287,157.2	2,360,371.4	2,434,123.8	110,837.4	54,867.2	38,961.8	204,487.1
I	52,030.4	56,180.5	61,835.6	69,141.5	1,906.9	1,383.1	1,856.0	6,059.2
m5U	197,387.7	187,846.5	189,700.6	194,766.8	7,503.7	6,907.0	2,538.0	16,604.7
ac4C	8,409,953.3	8,507,636.8	8,524,907.8	8,320,567.1	979,260.1	453,647.7	635,100.4	223,564.4
ncm5U	25,230.5	24,487.2	24,970.5	24,449.0	3,428.0	758.1	1,360.5	1,560.7
m7G	11,687,600.7	11,512,586.0	11,996,710.0	12,540,134.5	266,671.4	108,414.4	203,962.4	1,031,722.9
Um	23,354.1	24,219.3	24,817.8	28,556.0	667.0	1,589.8	1,768.1	4,089.7
m1A	25,505,705.9	24,652,977.2	22,417,268.8	20,203,837.8	184,646.2	680,195.6	963,848.5	2,517,777.4
mcm5U	1,596.9	1,375.6	1,520.1	1,832.5	38.1	14.9	81.4	169.3
Gm	774,407.5	932,879.6	1,060,966.8	1,361,785.2	26,289.3	159,815.8	125,765.1	311,387.3
m1G	5,462,700.9	5,396,492.3	5,834,001.2	5,964,107.5	253,297.6	198,883.4	165,837.2	740,883.6
ac4C	189,020.1	201,290.3	219,783.4	258,417.9	12,063.4	4,434.1	14,197.2	11,968.5
m2G	4,243,863.7	4,578,079.6	5,211,335.0	5,677,532.7	225,349.8	43,782.3	163,366.1	685,896.3
m5Um	15,522.0	15,666.8	16,290.7	16,460.3	405.7	267.6	479.0	2,034.2
15N5-dA	101,036.6	112,178.2	105,684.2	104,280.2	5,508.3	5,846.1	10,677.5	6,476.6
m22G	3,976,493.2	4,007,521.6	4,231,332.3	4,364,844.7	217,673.8	137,794.5	53,050.2	451,996.6
t6A	232,029.8	237,444.3	278,137.2	325,941.6	16,688.7	3,870.9	11,416.1	37,972.5
Am	409,079.2	649,943.9	642,903.9	1,212,295.6	85,495.0	446,896.5	256,783.3	930,758.6
OHyW	11,039.7	10,973.3	10,721.3	11,555.1	703.8	328.1	770.5	1,056.3
Am	21,695.8	22,957.7	22,312.1	21,296.0	1,928.8	2,015.1	1,862.8	1,294.8
i6A	53,323.1	51,671.9	56,880.5	57,183.7	3,454.0	5,906.5	2,849.7	4,376.0
mcm5s2U	4,333.1	3,178.4	2,845.2	2,573.2	1,856.0	134.1	266.8	151.2

Supplemental Table V. tRNA modification levels in SW620-hTRM9L vs. SW620-LacZ cells, log2-fold changes and significance (Untreated and Paromomycin, N = 3).

Compound Name	log2-fold change (hTRM9L to LacZ) untreated	log2-fold change (hTRM9L to lacZ) Paro	log2-fold change (LacZ Paro / LacZ)	log2-fold change (hTRM9L Paro / hTRM9L)	P-value (hTRM9L to LacZ) untreated	P-value (hTRM9L to lacZ) Paro	P-value (LacZ Paro / LacZ)	P-value (hTRM9L Paro / hTRM9L)
D	-0.3	-0.3	-0.1	-0.1	0.050	0.039	0.154	0.334
acp3U	0.0	0.0	0.0	0.0	0.293	0.951	0.565	0.950
m5C	0.1	0.1	0.1	0.0	0.057	0.061	0.009	0.791
Cm	0.2	0.3	0.2	0.3	0.029	0.234	0.227	0.206
m3C	0.0	0.1	0.0	0.0	0.420	0.296	0.486	0.573
I	0.2	0.3	0.1	0.2	0.022	0.023	0.038	0.117
m5U	-0.1	0.1	-0.1	0.0	0.137	0.542	0.180	0.629
ac4C	0.0	0.0	0.0	0.0	0.420	0.557	0.883	0.627
ncm5U	0.0	0.0	0.0	0.0	0.460	0.971	0.732	0.685
m7G	0.0	0.1	0.0	0.1	0.164	0.161	0.352	0.421
Um	0.1	0.2	0.1	0.2	0.176	0.162	0.434	0.220
m1A	-0.2	-0.3	0.0	-0.1	0.013	0.042	0.104	0.228
mcm5U	-0.1	0.4	-0.2	0.3	0.069	0.010	0.001	0.045
Gm	0.5	0.5	0.3	0.4	0.021	0.101	0.165	0.196
m1G	0.1	0.1	0.0	0.0	0.129	0.269	0.740	0.781
ac4C	0.2	0.4	0.1	0.2	0.022	0.001	0.174	0.023
m2G	0.3	0.3	0.1	0.1	0.015	0.050	0.065	0.316
m5Um	0.1	0.1	0.0	0.0	0.002	0.540	0.633	0.895
15N5-dA	0.1	-0.1	0.2	0.0	0.307	0.192	0.074	0.855
m22G	0.1	0.1	0.0	0.0	0.117	0.260	0.845	0.638
t6A	0.3	0.5	0.0	0.2	0.025	0.016	0.613	0.105
Am	0.7	0.9	0.7	0.9	0.152	0.399	0.411	0.365
OHyW	0.0	0.1	0.0	0.1	0.050	0.414	0.889	0.331
Am	0.0	-0.1	0.1	-0.1	0.335	0.296	0.477	0.481
i6A	0.1	0.1	0.0	0.0	0.046	0.264	0.697	0.925
mcm5s2U	-0.6	-0.3	-0.4	-0.1	0.130	0.007	0.343	0.199

Supplemental Figure Legends

Figure S1. (A) SW620 cells expressing either *LacZ* or *hTRM9L* were inoculated in nude mice and tumors were measured every 2 days, up to 11 days. Day 11 data for all tumors is shown. **(B)** Graphs showing quantitative PCR (qPCR) analysis of *hTRM9L* mRNA transcript levels normalized to *h36B4*. RNA was purified from *LacZ* and *Trm9* SW620 tumors grown on BALB/c nude mice (N = 10). Cells suspensions were prepared to a concentration of 375,000 cells in 100 μ l PBS++ and injected in two different sites per animal, in the neck and the flank. **(C)** CLUSTAL based amino acid alignment of *hTRM9L* homologs. The sequence conserved domain, representing motifs I-III and post motif I (Kahlor and Clarke, 2003; Katz et al., 2003), which define methyltransferase enzymes and are used to interact with SAM is marked by the diamond tipped line. We note that that there is a break in the sequence (....) with the last panel specific to the C-terminal region of *hTRM9L* and other homologs.

Figure S2. IHC staining results. For panels A to D tumor nodules of SW620-*LacZ* and SW620-*hTRM9L* expressing cells were harvested 2 to 5 days post-inoculation and stained for **(A)** caspase 3, **(B)** LC3, **(C)** phospho H3 **(D)** p21. **E.** Tumor nodules of SW620-GFP (grey bars) and SW620-*hTRM9L* (black bars) expressing cells were harvested 2 to 5 days post-inoculation and stained for acidic β -gal as in Figure 3B. **(F)** HP1 γ and H3K9me3 staining in SW620-GFP and SW620-*hTRM9L* cells.

Figure S3. CAM metastasis data and LIN9 protein levels. (A) *LacZ*- and *hTRM9*-SW620 cells do not differ in their intravasating capacity. GFP-SW620 overexpressing *hTRM9* or *LacZ* (1×10^6 cells) were inoculated onto CAMs. For details see (Kim et al., 1998) After 48 and 72 hrs, the CAM tissue on the opposite side of the embryo (lower CAM, where the cells can only arrive if they entered the vasculature) was analyzed by fluorescence microscopy to detect GFP-positive cells, which were counted in collagenase suspensions of this tissue, using a conventional hemocytometer. Note that there is no difference in the efficiency of intravasation between *LacZ*- and *hTRM9*-SW620 cells **(B)** Colonization of chick embryo livers was not affected by *hTRM9* gene in SW620 cells. The livers were collected from the same embryos described in A. After incubation with collagenase, DNA from these liver cell suspensions containing putative disseminated tumor cells were extracted using Extract-N-Amp kit. Then human-specific *Alu* expression was determined by quantitative PCR. Note that there is no difference in the detection of levels of *Alu*-sequence in the livers of embryos carrying *LacZ*- and

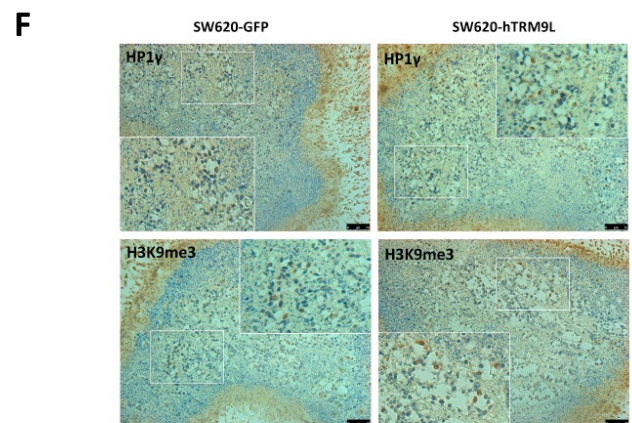
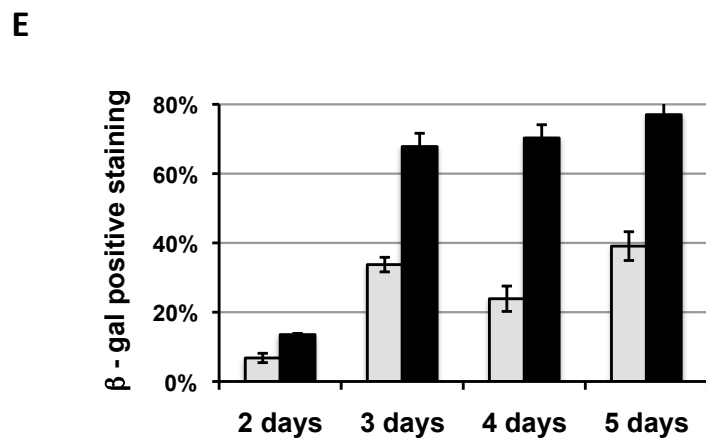
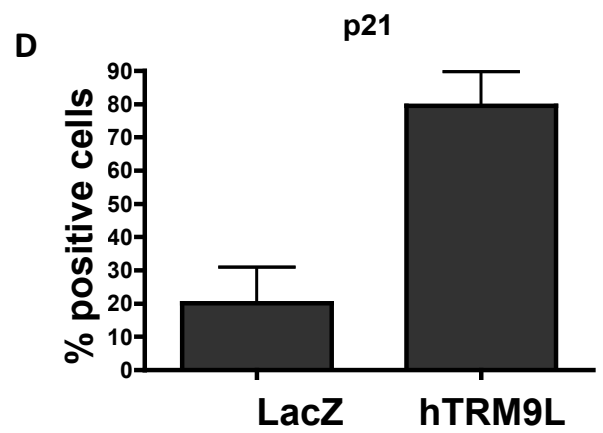
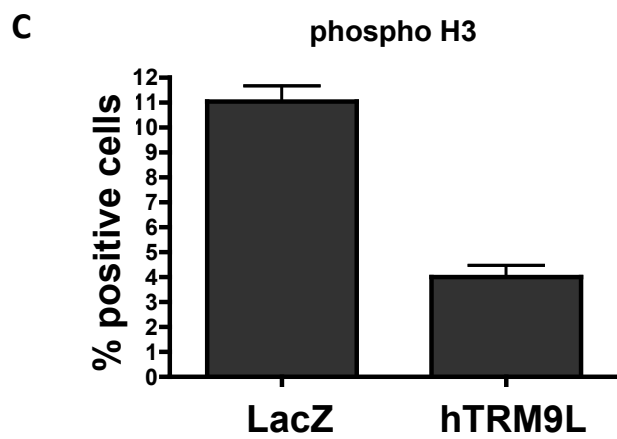
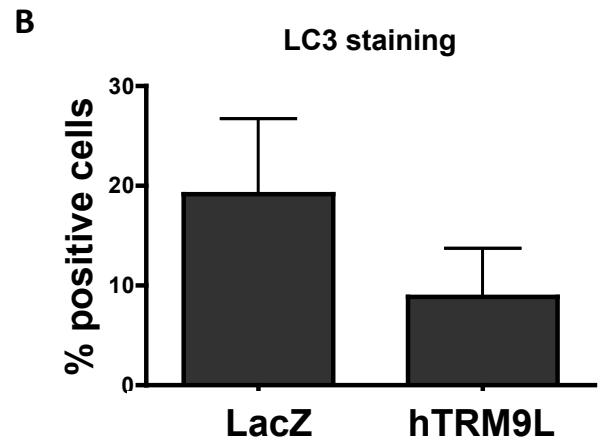
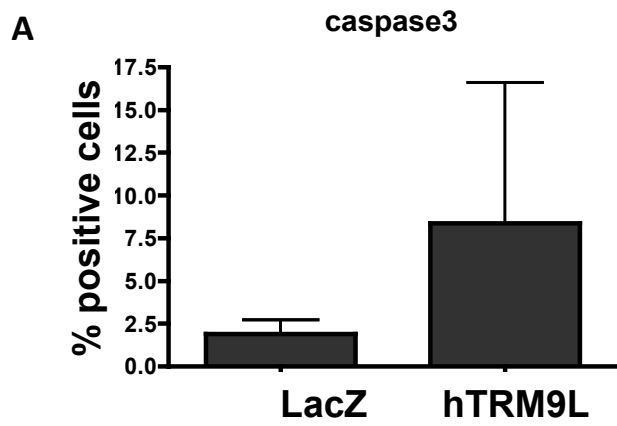
hTRM9-SW620 cells on the CAM. **(C)** LIN9 protein levels in SW620-LacZ and SW620-hTRM9L expressing cells were determined by immunoblotting. LIN9 bands were quantitated by densitometry and normalized to GAPDH to demonstrate a 1.8-fold increase in LIN9 in hTRM9L-proficient cells.

Figure S4. Quantitative analysis of GLUT1 levels. Tumor nodules of SW620-LacZ (black bar) and SW620-hTRM9L (white bar) expressing cells were harvested and stained for GLUT1 as in Figure 5E. The percentage of GLUT1 positive cells was determined and a T-test was used to determine significance.

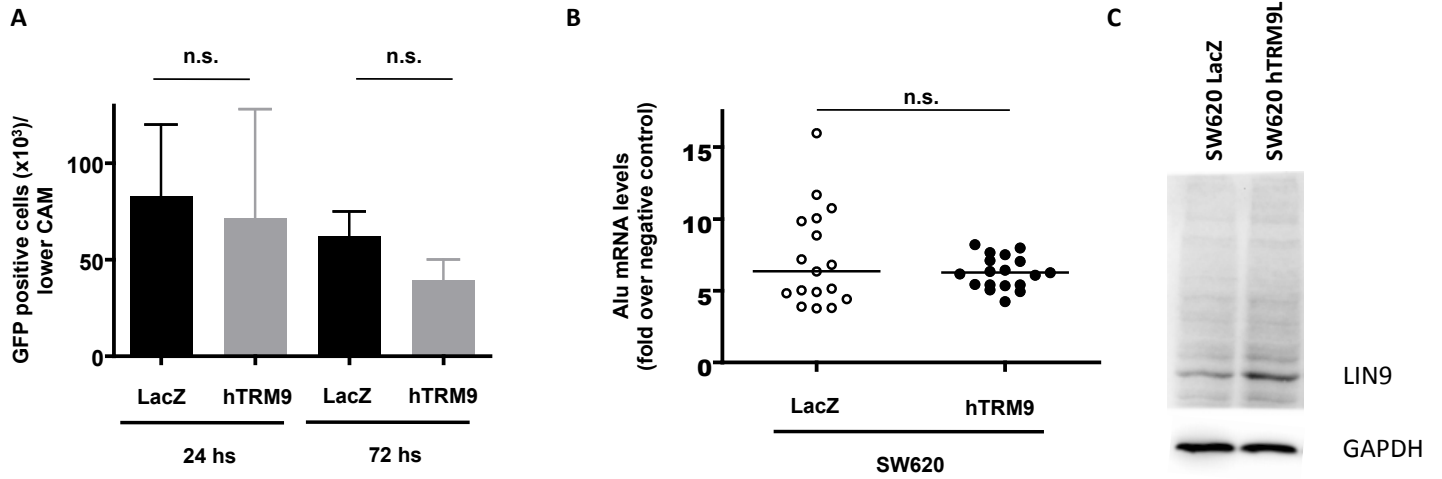
Figure S5. Cell viability of SW620-hTRM9L and SW480 after paromomycin treatment. SW480 (blue diamonds) and SW620-hTRM9L (red squares) data from Figure 6 were re-plotted to compare viability after paromomycin exposure.

Figure S6. Cell viability of SW620, HCT116 and HT29 cell lines after treatment with aminoglycoside antibiotics. **(A)** SW620-LacZ (blue diamonds) and SW620-hTRM9L (red squares) cells were exposed to gentamicin and the percent viability for each was determined by trypan blue staining 24 hours post exposure. **(B)** HT29-LacZ (blue triangles) and HT29-hTRM9L (red squares) cells were exposed to paromomycin and the percent viability for each was determined by trypan blue staining 24 hours post exposure. **(C)** HCT116-LacZ (blue diamonds) and HCT116-hTRM9L (red squares) cells were exposed to paromomycin and the percent viability for each was determined by trypan blue staining 24 hours post exposure.

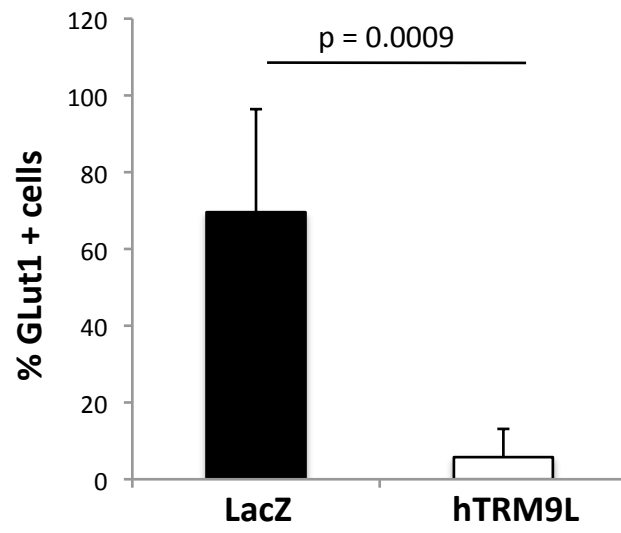
Supplemental Figure 2



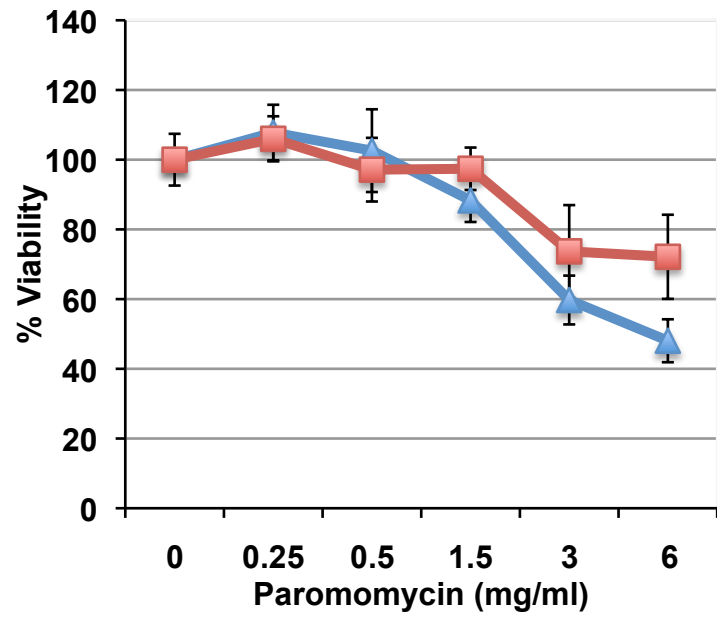
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

