Supporting Information

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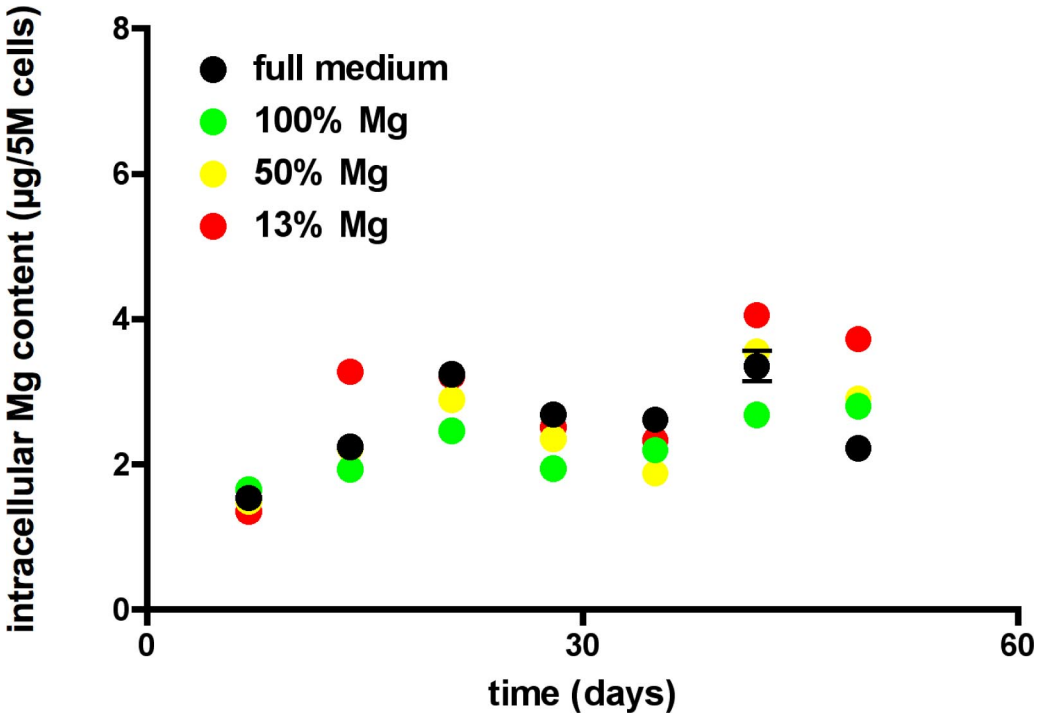

Fig. S1. Long-term exposure to magnesium-deficient conditions did not result in reduced intracellular magnesium content in primary IMR-90 cells. Cells were cultured in full medium (black) or magnesium-deficient media repleted to 13% (red), 50% (yellow), or 100% (green) of normal magnesium content in ambient oxygen. Intracellular magnesium content was measured by ICP in seven independent IMR-90 populations from early, mid, and late PD. Representative data shows mean ± SD ($n = 2$ replicate wells) magnesium content normalized to 5 million cells (5M) from a single population that was plotted as a function of PD. Intracellular magnesium levels ranged from 1 to 4 μg per 5M cells; assuming an average volume of 3,000 fl for young IMR-90 cells (1), this yields an intracellular magnesium concentration range of 3–11 mM. This finding is consistent with other reports of normal intracellular magnesium concentrations ranging between 5 and 20 mM for human and other mammalian cell types (2). There was an apparent increase in average total intracellular magnesium levels comparing early to late PD, as seen in a previous study (1). However, no consistent difference in total intracellular magnesium levels over time observed between full and magnesium-deficient conditions. Several other mammalian cell types have also been shown to maintain a normal intracellular magnesium level even when cultured in conditions with very high or very low extracellular magnesium (2, 3). If this is the case, these results suggest that IMR-90 cell populations in magnesium-deficient conditions were able to regulate magnesium transport to meet intracellular demand.

1. Killilea DW, Atamna H, Liao C, Ames BN (2003) Iron accumulation during cellular senescence in human fibroblasts *in vitro*. *Antioxid Redox Signal* 5:507–516.
2. Romani A, Marfella C, Scarpa A (1993) Regulation of magnesium uptake and release in the heart and in isolated ventricular myocytes. *Circ Res* 72:1139–1148.
3. Wolf FI, Torsello A, Fasanella S, Cittadini A (2003) Cell physiology of magnesium. *Mol Aspects Med* 24:11–26.

Table S1. Long-term exposure to magnesium-deficient conditions did not result in reduced cellular viability in primary IMR-90 cells

	Full medium			100% Mg			50% Mg			13% Mg			
PDL	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	ANOVA
20% O_2													
24–26	100.00	21.05	4	108.15	12.97	4	99.76	14.23	4	96.40	26.45	2	NS
35–37	100.00	14.84	3	110.26	22.68	3	113.85	11.62	3	103.59	21.00	3	NS
45–49	100.00	35.64	3	115.50	10.30	3	132.40	10.07	3	129.10	15.88	3	NS
47–51	100.00	26.18	3	92.30	14.40	3	128.00	17.92	3	115.70	12.44	3	NS
5% O_2													
25–30	100.00	11.18	3	119.51	9.68	3	132.11	9.47	3	119.11	19.14	3	NS
58–64	100.00	3.21	3	96.44	4.30	3	126.60	28.20	3	108.79	13.60	3	NS

Cells were cultured in full or magnesium-deficient media repleted to 13%, 50%, or 100% of normal magnesium content. For early toxicity studies, cells were incubated with 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 2–4 h at 37°C. The reaction was terminated and formazan crystals solubilized in 100% DMSO. Relative MTT reduction was measured by absorbance at 590 nm. MTT reduction in three independent IMR-90 populations from early, middle, and late PD maintained in different media conditions and oxygen tensions is indicated. Representative data shows mean ± SD (n = 2–4 replicate wells) of MTT reduction capacity normalized to control populations (full medium) as a function of PD. The differences in mean values within each PD group were determined not to be significant (NS; one-way ANOVA, Dunnett's multiple comparison post hoc test). For end-stage toxicity studies, cell suspension were incubated with 0.2% trypan blue in PBS for 5 min and then scored for trypan-blue exclusion by light microscopy. Trypan-blue exclusion paralleled MTT reduction results (data not shown).

Table S2. Long-term exposure to magnesium-deficient conditions did not result in reduced plating efficiency in primary IMR-90 cells

Day	Full medium	100% Mg	50% Mg	13% Mg	ANOVA
1	94	96	97	97	NS
2	93	93	94	92	NS
3	95	97	97	95	NS

Plating efficiency was determined by daily cell counts over 3 consecutive days for two independent IMR-90 populations maintained under varying media conditions at ambient oxygen tension. Representative data show percent of attached cells from total cells at 48, 72, and 96 h after plating as a function of media condition. The differences in mean values within each PD group were determined not to be significant (NS; one-way ANOVA, Dunnett's multiple comparison post hoc test).