

# Supplementary Material

## Metformin and Glucose Concentration as Limiting Factors in Retinal Pigment Epithelial Cell Viability and Proliferation

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**Table S1.** hTERT RPE-1 cell viability time course measured with Cell Titter Blue assay

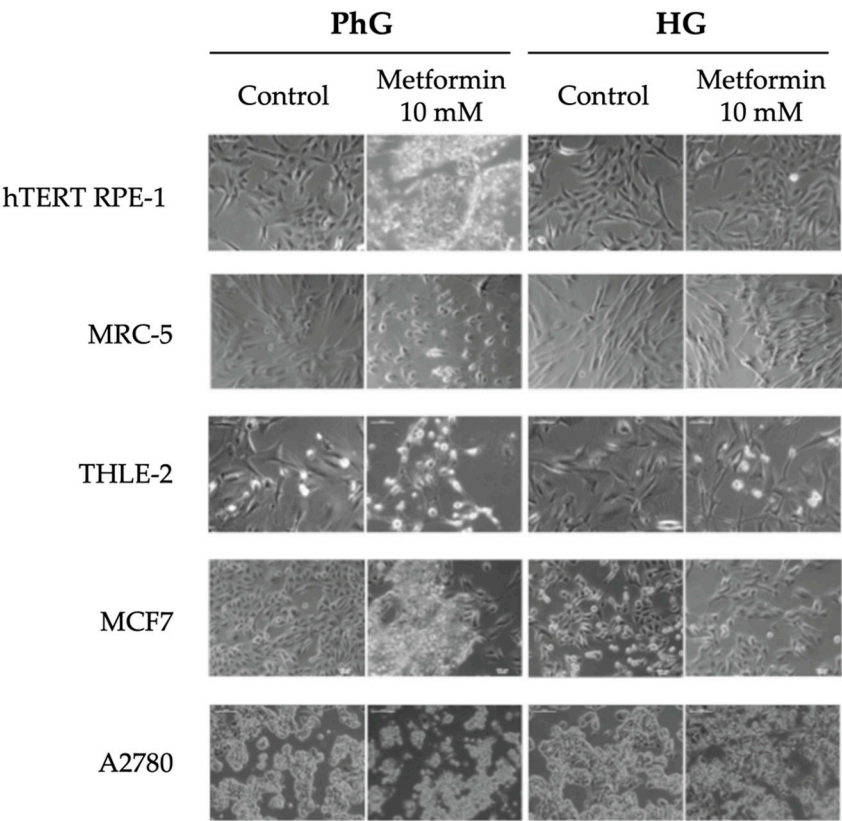
		N=1		N=2		N=3		MEAN	
		PhG	HG	PhG	HG	PhG	HG	PhG	HG
<b>0h</b>	DMEM	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Control	1.260	1.162	1.119	1.159	1.164	1.093	1.181	1.138
	1M	1.210	1.473	1.131	1.173	1.194	1.156	1.178	1.268
	10mM	1.255	1.226	1.133	1.100	1.167	1.199	1.185	1.175
	1mM	1.309	1.305	1.172	1.292	1.273	1.186	1.251	1.261
	50μM	1.233	1.333	1.083	1.329	1.342	1.206	1.219	1.289
<b>24h</b>	DMEM	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Control	4.053	3.988	2.804	3.921	2.934	2.354	3.264	3.421
	1M	1.198	1.248	1.239	1.218	1.237	1.254	1.225	1.240
	10mM	3.900	3.347	2.459	3.204	3.107	2.242	3.156	2.931
	1mM	3.844	4.006	3.209	4.675	3.061	2.265	3.372	3.648
	50μM	4.062	4.713	3.251	4.122	3.287	2.602	3.533	3.812
<b>48h</b>	DMEM	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Control	5.102	6.754	4.415	5.259	4.719	4.960	4.745	5.658
	1M	1.210	1.294	1.299	1.272	1.344	1.261	1.285	1.276
	10mM	3.251	6.817	2.471	5.492	2.408	5.018	2.710	5.776
	1mM	2.441	6.081	4.010	5.185	4.471	4.906	3.641	5.391
	50μM	4.909	7.409	4.051	4.969	4.424	5.202	4.461	5.860
<b>72h</b>	DMEM	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Control	5.388	7.544	7.057	13.817	6.368	8.549	6.271	9.970
	1M	1.054	1.273	1.381	1.268	1.398	1.320	1.278	1.287
	10mM	0.942	6.261	1.192	12.957	1.617	7.678	1.250	8.966
	1mM	5.485	6.797	2.959	13.448	2.713	7.604	3.719	9.283
	50μM	5.647	7.251	4.160	11.941	3.473	8.143	4.426	9.112

Absorbance measurements are normalized against DMEM (cultured in the same conditions but without cells). Absorbance measurements from triplicates at a wavelength of 590 nm

Supplementary methods

Cell line selection

In order to select the most appropriate cell line to assess the influence of glucose media concentration in the proliferative behaviour of different cell lines, five different cell lines were selected: hTERT RPE-1 (retinal epithelial cell line), MRC-5 (lung cell line), THLE-2 (liver cell line), MCF7 (breast cell line) and A2780 (ovarian cell line). Cells were treated with metformin and under two different glucose media concentration: high glucose media (HG; 450 mg/dl), and with physiological glucose media (PhG; 100 mg/dl). Metformin treatment concentration was established at 10 mM due to it being the most common metformin concentration used on literature. We could observe with microscopy images that treatment with 10mM metformin causes morphological changes under PhG conditions. Cells lose adherence to the plate, become rounded and decrease in size but continue to retain their refractive properties. On the contrary, under HG conditions the cells maintain a morphology like the control (Figure S1). Finally, only the hTERT RPE-1 cell line was selected to carry out all viability and proliferation analyses due to its greater relationship with diabetic pathology and treatment with metformin.



**Figure S1.** Cell culture (Magnification 10×) of different cell lines under two different glucose concentrations comparing control to treatment with 10 mM metformin. PhG: physiological glucose; HG: high glucose