Supplementary Data

Inhibition of apoptosis in human induced pluripotent stem cells during expansion in a defined culture using angiopoietin-1 derived peptide QHREDGS

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List of Antibodies:

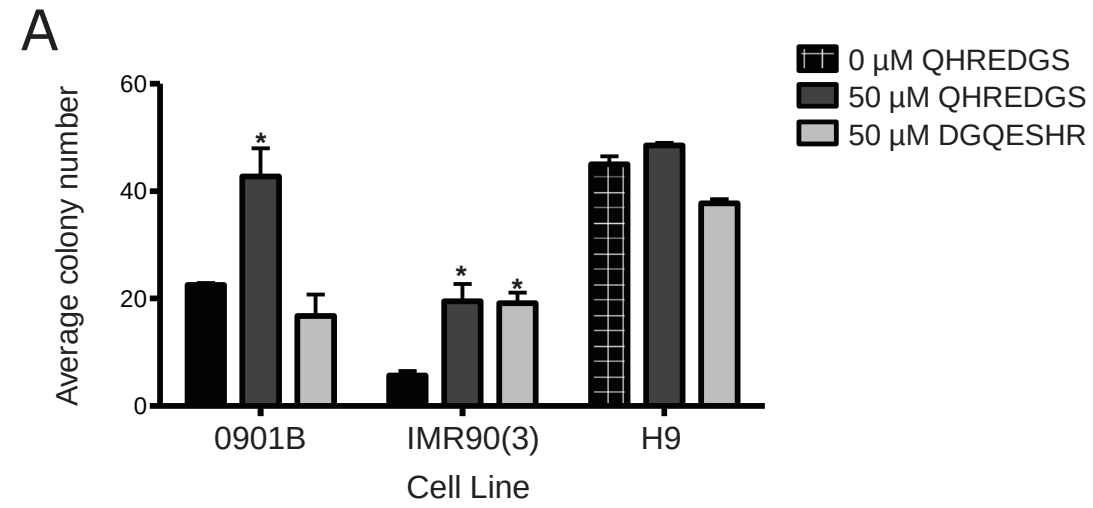
FACS analysis: Oct4 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, cat# sc-5279), SSEA-4 (Developmental Studies Hybridoma Bank, Iowa City, IA, cat# MC-813-70) and all secondary antibodies (Jackson ImmunoResearch, West Grove, PA).

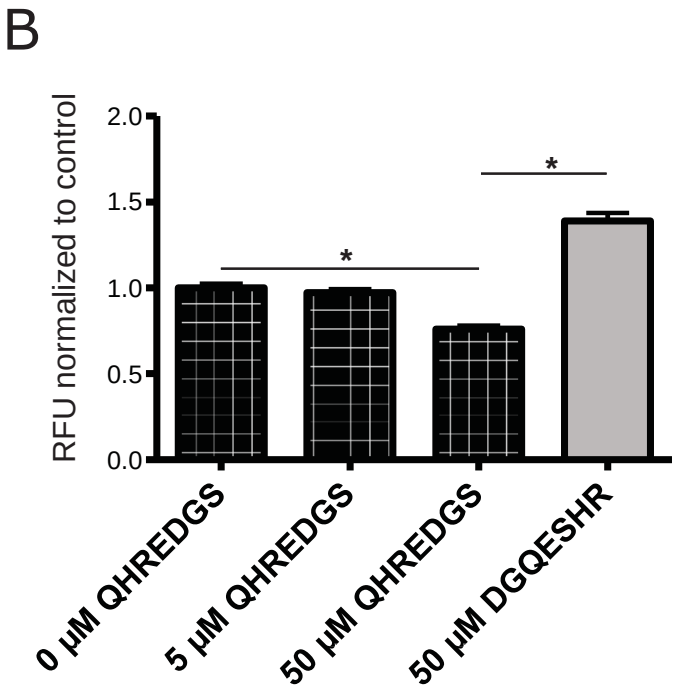
Immunostaining on Feeder Layers: SMA (Millipore, Temecula, CA, cat# CBL171), Gata6 (Santa Cruz Biotechnology Inc., sc-9055), and ß-III Tubulin (Life Technologies, Burlington, ON, cat# 480011), BrdU (AbD Serotec, Raleigh, NC, cat# MCA2060), Ki67 (Millipore, cat#AB9260) and all secondary antibodies (Jackson ImmunoResearch).

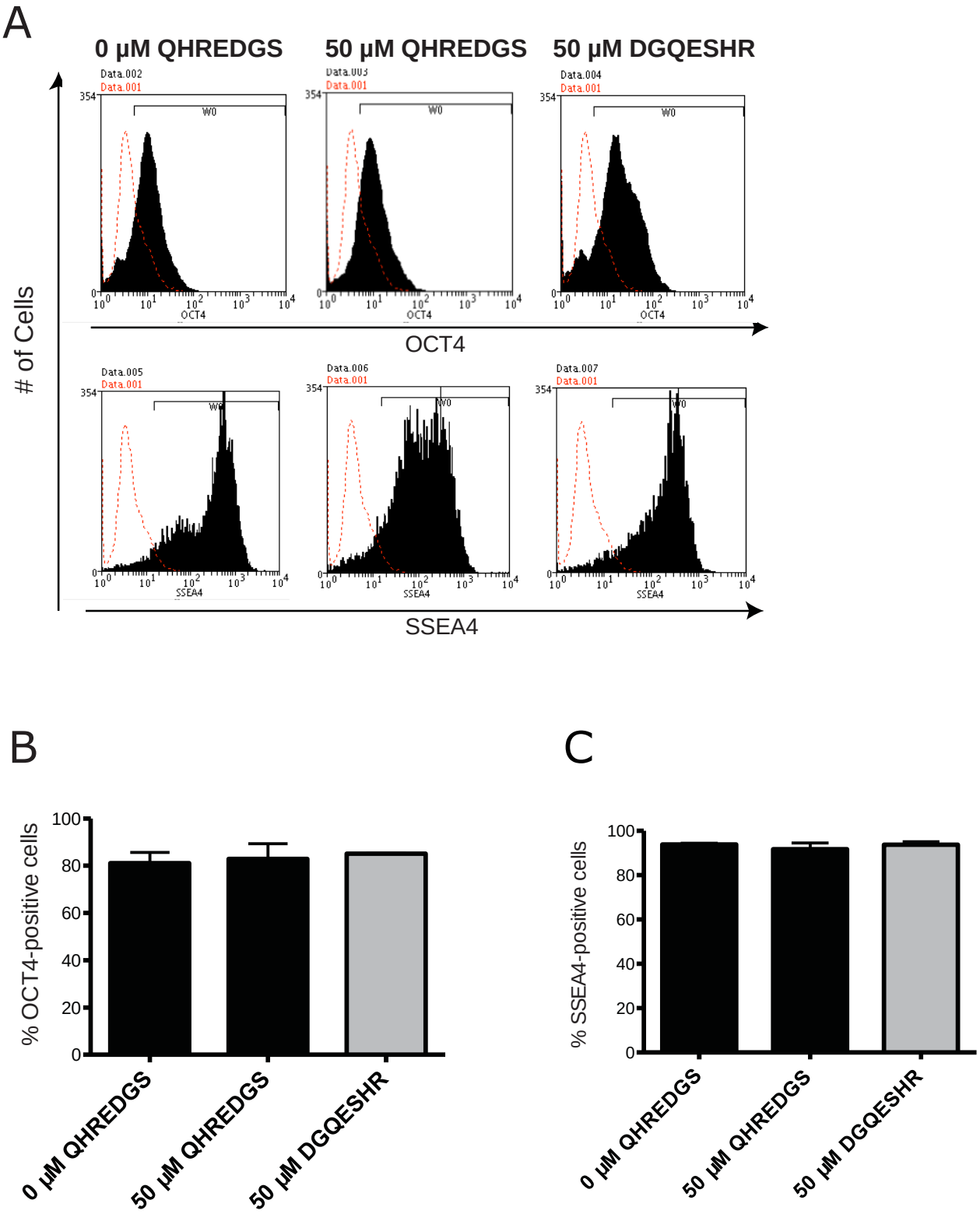
Immunostaining on Matrigel: All secondary antibody (Dako Canada, Inc., Burlington, ON).

Western blot: ILK (Cell Signaling Technology Inc., Danvers, MA, cat# 3856), phospho-AKT (Ser 473) (Cell Signaling Technology Inc., cat# 4060), AKT (Cell Signaling Technology Inc., cat# 2920), phospho-ERK1/2 (Thr202/Tyr204) (Cell Signaling Technology Inc., cat# 4370), ERK1/2 (Cell Signaling Technology Inc., cat# 4695) and all secondary antibodies (Dako Canada, Inc.).

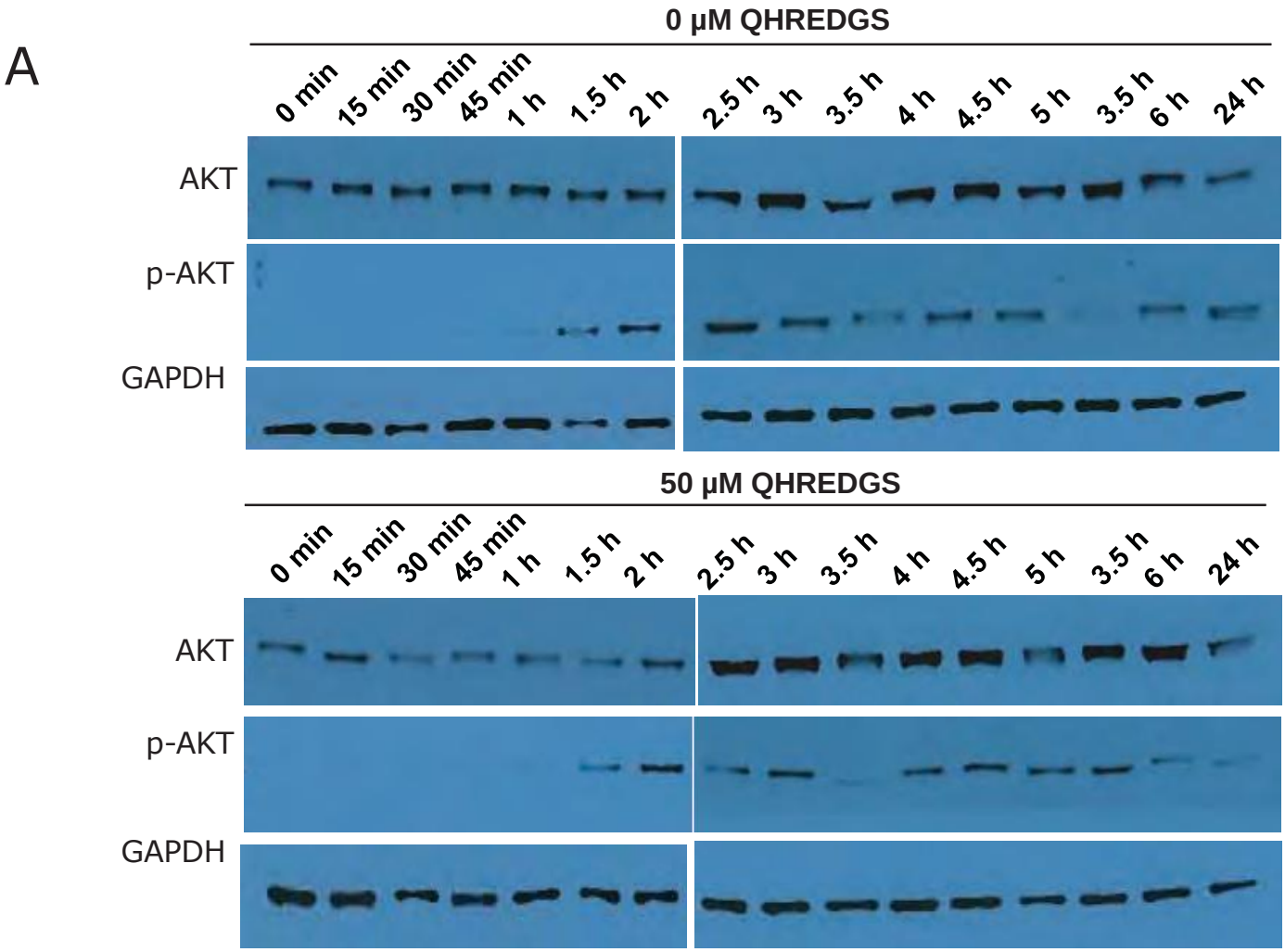
Adhesion Assay: CD29 (β_1-integrin) (BD Biosciences, Mississauga, CA, cat# MR2017).

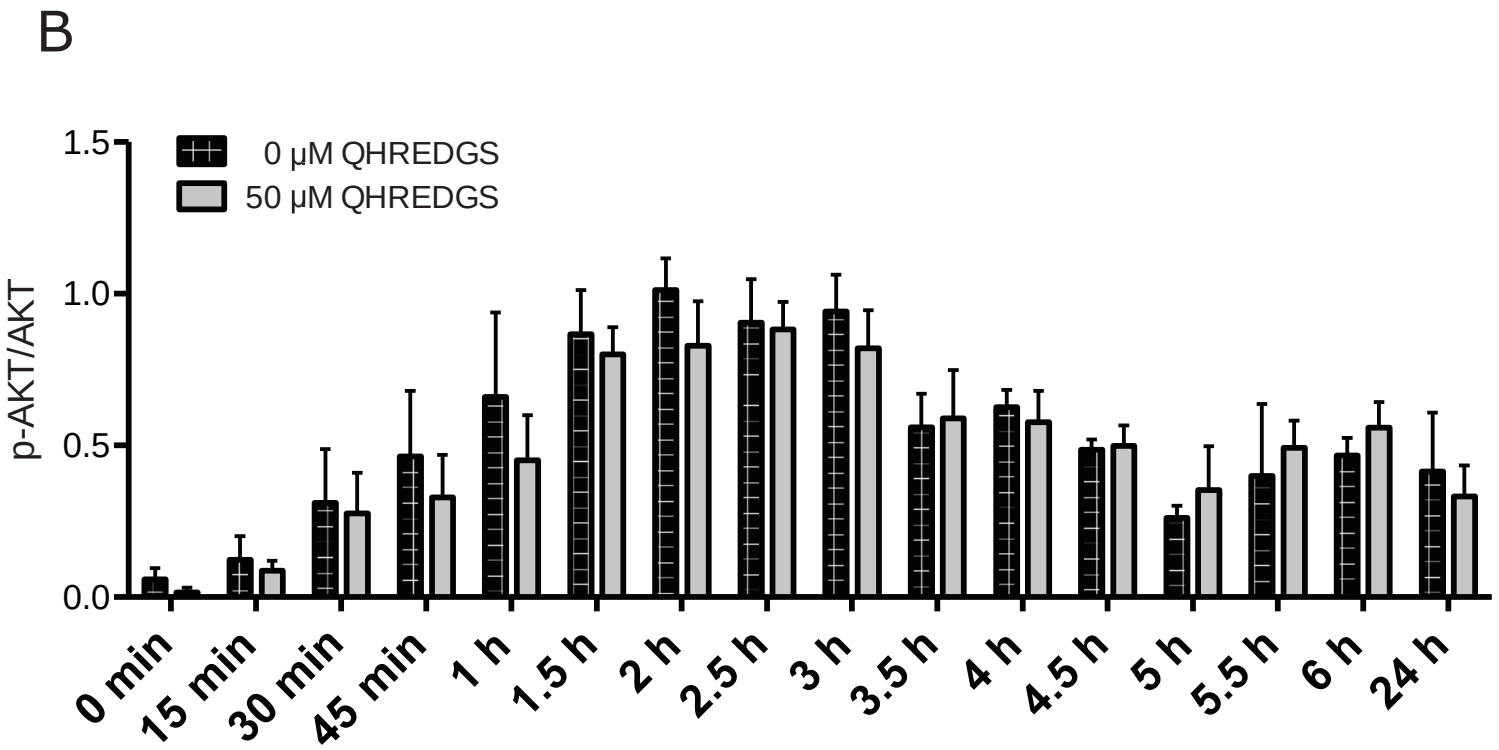

A
0 μM QHREDGS
50 μM QHREDGS
50 μM DGQESHR
Average colony number
60
40
20
0
*
*
*
0901B
IMR90(3)
H9
Cell Line


B
RFU normalized to control
2.0
1.5
1.0
0.5
0.0
*
*
0 μM QHREDGS
5 μM QHREDGS
50 μM QHREDGS
50 μM DGQESHR



Dang et al. SUPPLEMENTARY FIGURE 2





Dang et al. SUPPLEMENTARY FIGURE 3

Supplementary Figure 1: **QHREDGS increases hiPSC colony number and downregulates caspase-3/7 activity during single-cell dissociation in serum-free, feeder layer culture conditions.** [A] Human iPSCs were pre-treated for 5 passages with PBS alone (0μM QHREDGS; black bars), 50μM QHREDGS (dark grey bars) or 50μM DGQESHR (light grey bars), dissociated to single cells and plated at a low density, then cultured for 7 days in the presence of the treatments. Colony number was determined for various PSC lines—0901B, IMR90(3) and H9—7 days after single-cell dissociation. [B] 0901B hiPSCs were pre-treated for 5 passages with PBS alone (0μM QHREDGS), an increasing concentration of QHREDGS peptide or the scrambled peptide DGQESHR, dissociated to single cells and plated at a low density, then cultured for 7 days in the presence of the treatments. The cells were the assayed for caspase-3/7 activity. The relative fluorescence units (RFU) per cell is indicative of caspase-3/7 activity. Data presented are the mean ± SEM. *P* values are derived from Student's t-test, $P < 0.05$ considered significant (n=3).

Supplementary Figure 2: **QHREDGS treatment does not affect the pluripotency of 0901B hiPSCs *in vitro*.** [A-C] Flow cytometric analysis of PBS (0μM QHREDGS), 50μM QHREDGS and 50μM DGQESHR treated 0901B hiPSCs. [A] Representative Oct4 (top panels) and SSEA4 histograms (bottom panels). Red denotes secondary antibody only control and black denotes cell population positive for the designated antibody. [B-C] Quantification of Oct4+ [B] and SSEA4+ [C] cells. Data presented are the mean ± SEM. *P* values are derived from Student's t-test, $P < 0.05$ considered significant (n=3).

Supplementary Figure 3: **QHREDGS does not affect activation of AKT.** [A-B] BJ1D hiPSCs were single-cell dissociated and seeded onto Matrigel in the presence of PBS (0μM QHREDGS) or 50μM soluble QHREDGS. At various time points, cells were scraped and lysed. Cell lysates were analyzed by Western blot analysis using the denoted antibodies and the resultant bands were quantified by densitometry. [A] Representative images of Western blot membranes probed with denoted antibodies. [B] Quantification of Western blot analysis. Data presented are the average normalized phospho-AKT (p-AKT) concentration determined from the p-AKT:AKT ratio ± SEM (n=4).