Supplemental Information – Breitman et al.

MAPC conditioned medium

MAPC were plated at 1 x 10⁶ cells per well of a six well plate in 3 ml of MAPC medium (low-glucose DMEM (Life Technologies Invitrogen) supplemented with FBS (Atlas), ITS liquid medium supplement (Sigma), MCDB (Sigma), platelet-derived growth factor (R&D Systems), epidermal growth factor (R&D Systems), dexamethasone (Sigma), penicillin/streptomycin (Life Technologies Invitrogen), 2-Phospho-L-ascorbic acid (Sigma), and linoleic acid-albumin 9Sigma)) (BCM) and incubated for three days. The medium was refreshed with MAPC medium containing 10 ng/ml each of TNFα (Sigma-Aldrich, St Louis MO, USA), IL-1 β (Sigma-Aldrich, St Louis MO, USA), and IFN γ (Thermo Fisher Scientific Inc. Rockford IL USA) and incubated for three days. The conditioned medium was collected and spun at 600 x g for 8 minutes and transferred to new tubes. IFN γ antibody (Abcam, Cambridge MA, USA) was coupled to magnetic beads using the Dynabeads Antibody Coupling Kit (Life Technologies Corporation, Carlsbad CA, USA) according to manufacturer's instructions. To immune-deplete the conditioned medium of IFN γ the supernatant was incubated twice with antibody coupled beads at 4°C for one hour. The magnetic beads were removed and the protein eluted from the beads. The beads were rinsed with PBS and then added back to the conditioned medium for further incubation. The process was repeated for a total of five times. Complete removal of beads was confirmed and the conditioned medium was then aliquoted and frozen down at -80°C. The level of IFN γ remaining in the conditioned medium was guantified by ELISA (R&D Systems, Inc. Minneapolis, MN, USA) according to manufacturer's instructions. GMC was the same as CCM except that it was never used for cell culture and is therefore a control for CCM. Control medium (GMC)

consisted of MAPC growth medium supplemented with 10 ng/ml TNF α , 10 ng/ml IL-1 β , and an equivalent amount of IFN γ to that remaining in the IFN γ -depleted MAPC conditioned medium.