Supplemental Figure Legends

Figure S1. Immunofluorescence staining of GBM CSCs cultured in the presence of serum and absence of EGF and bFGF demonstrate positive expression of astrocytic marker glial fibrillary acidic protein (GFAP), neuronal class III β-tubulin (Tuj-1), neuronal-specific nuclear marker (NeuN), microtubule β-III tubulin, nerve cell intermediate filament (Nestin), and oligodendrocyte transcription factor 1 (Olig1). All images counterstained with DAPI and taken at 40x magnification.

Figure S2. Tumors formed from CSC-seeded PLG scaffold implantation exhibit clinical features of human GBM. A) B-mode cineloops of tumor cross-sections demonstrated multiple regions of blood flow, indicated by changing image contrast and later verified with color Doppler imaging. Scale bars as indicated on image. B-C) Pathological evaluation of H&E-stained tumor sections revealed frequent vascular hyperplasia (B, white arrows) and mitotic nuclei (C, white arrows) as observed in clinical GBM. Scale bars: 100μm. D) Tumors exhibit diffuse infiltration as indicated by invasive, multifocal tumor cell clusters (closed arrows) breaching the collagenous capsule formed around the tumor (open arrows); shown for two separate tumors. Scale bars 100μm.

Figure S3. Vascular characteristics of tumors formed from PLG scaffold implantation. A) Immunohistochemical staining for human (green) and mouse (red) $CD31^+$ cells demonstrate the anastomosis and co-participation of both cell types in perfused tumor vessel formation. Scale bars 20μm. B) H&E staining of tumors borne from CSC-seeded PLG scaffold implantation demonstrates that tumor vascularity (indicated by arrows) was similar between CSC+hCMEC and CSC alone. It is noteworthy that the average vessel diameter was larger with co-implantation of hCMECs with CSCs. Scale bars 100μm. C) Immunohistochemical analysis demonstrated co-localization of $Oct4^+$ (green) GBM CSCs with vascular structures formed by human $CD31^+$ (orange) cells. Scale bar 50μm.

Figure S4. CSC differentiation is impaired by 3-D hCMEC paracrine signaling cues. Conditioned media from 3-D cultured hCMECs (right) reduces CSC plate adherence and thus differentiation relative to media collected from 2-D cultured hCMECs (left) as suggested by representative brightfield images of CSCs cultured for 7 days in non-adherent flasks with the respective media. Scale bars 50μm.

Figure S5. Control experiments evaluating IL-8-induced chemotaxis and gene expression in CSCs. A) IL-8 promotes CSCs chemotaxis at concentrations relevant to conditioned media experiments (5ng/mL) as indicated by transwell migration assay. B) IL-8 expression was attenuated in CSCs following 3 days of treatment with 3-D EC conditioned media vs. basal control medium; however these differences were statistically insignificant. C) Transwell assays confirm that addition of IgG does not influence enhanced CSC migration towards IL-8 (50μg/mL). For all experiments, data are expressed as summary values from three experiments. *$p<0.05$; n.s., not significant.

Figure S6. Targeted knockdown of CXCR2 in GBM CSCs. A) CXCR2 expression in GBM CSCs transduced with lentiviruses encoding scrambled siRNA (control) or clones (36-40) targeting human CXCR2. Data are expressed as average of technical replicates and normalized to control CXCR2 expression. B) CXCR2 expression knockdown in CSCs previously transduced by lentiviral vectors expressing siRNA targeted against human CXCR2. Single lentiviral transduction (clone 38) caused a 59% knockdown in gene expression relative to scrambled siRNA transduction (control); transduction of three viral vectors (clones 36, 37, 38) induced a 82% knockdown. Data are expressed as average of technical replicates. C) Western blot analysis of siRNA control- or si-IL8-transduced CSC lysates confirming ~64% reduction in

CXCR2 protein with triple siRNA clone transduction. Inset, representative image of blot probed for CXCR2 and α-tubulin (loading control).

Figure S7. Tumor vascularization was not affected by siRNA-knockdown of IL-8 in CSCs. Extensive tumor vascularity was observed in tumors borne from siRNA-transduced CSCs, similar to that seen in tumors generated from native CSCs (see Figure S3B). Scale bars 100μm.