Supplementary information

M2-polarized macrophage contributes the neovasculogenesis, leading relapse of oral cancer following radiation

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Context of supplementary information

- 1. Supplementary Figures 1-5
- 2. Supplementary Table 1



Supplementary Figure S1.

The representative images of IHC for $CD11b^+$ myeloid cells in OSCC tumours before and after irradiation. HSC-3 cells (1×10^7 cells) and YCU-OR891 cells (3×10^6 cells) were s.c. implanted on the back of female BALB/c nude mice, and grown tumours were irradiated in a manner similar to the OSC-19 model. Tumour samples were stained with CD11b antibody.



Supplementary Figure S2.

Co-culture experiment of OSC-19-luc cells and BM-M1M φ s or BM-M2M φ s. OSC-19-luc cells (6×10³ cells) were co-cultured with BM-M1M φ s or BM-M2M φ s (1.5 or 6×10³ cells) in 24 well plates in quadruplicate. Bioluminescent signals were determined using the IVIS system on indicated date. Data shown are means ± SD.



Supplementary Figure S3.

Pattern diagram of the experimental design for co-injection of OSC-19 cells and BM-M1Mφs or BM-M2Mφs. OSC-19 cells were injected into non-IR or pre-IR sites alone or in the presence of BM-M1Mφs or BM-M2Mφs previously prepared *in vitro*.



Supplementary Figure S4.

Representative image of Matrigel Plug assay. BM-M2Mqs promoted tube-like

formation of CD31⁺ endothelial cells (red) in Matrigel plug.



Supplementary Figure S5.

FACS analysis of BMDCs and BM-Mφs. FACS analysis showed that CD11b⁺F4/80⁻ BMDCs harvested from BALB/c nude mice differentiated into CD11b⁺F4/80⁺ BM-Mφs by extended culture with M-CSF for 7 days. BMDC, bone marrow-derived cell; BM-Mφs, bone marrow-derived macrophages; M-CSF, macrophage colony-stimulating factor.

	Forward 5`⇒ 3`	Reverse 5` ⇒ 3`	Product length
IL-6	GACAAAGCCAGAGTCCTTCAGAGAG	CTAGGTTTGCCGAGTAGATCTC	228(bp)
iNOS	TTTGCTTCCATGCTAATGCGAAAG	GCTCTGTTGAGGTCTAAAGGCTCCG	600
Arg I	CAGAAGAATGGAAGAGTCAG	CAGATATGCAGGGAGTCACC	249
CD206	GCAAATGGAGCCGTCTGTGC	CTCGTGGATCTCCGTGACAC	300
MMR	AGCTACCATGGCATGAAGCAGAGA	ACCCATTCGAAGGCATTCCAGAGA	464
IL-13Rα2	CGCATTTGTCAGAGCATTGT	CCAAGCCCTCATACCAGAAA	216
GAPDH	TATGTCGTGGAGTCTACTGGT	GAGTTGTCATATTTCTCGT	149

Supplementary Table S1.

Primer sequences specification for RT-PCR.