Supplemental Information for

Direct Efficient Cellular Transformation of Primary Rat Mesenchymal Precursor Cells by KSHV

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This file includes:

Supplemental Figures 1 to 9

Supplemental Figure 1. Genome copy numbers per cell in different passages of KMM cultures.

Supplemental Figure 2. Expression of LANA and ORF65 proteins in uninduced MM cells and MM cells induced with TPA for 48 h. (**A**) LANA. Scale bar: 10 μm. (**B**) ORF65. Scale bar: 50 μm. None of the MM cells expressed LANA and ORF65 proteins.

Supplemental Figure 3. Expression of KSHV transcripts in uninduced and TPA-induced KMM cells.

Supplemental Figure 4. Expression of cell surface markers in MM and KMM cells. MM and KMM cells were examined for the expression of vascular endothelial markers (β-catenin, VCAM-1, ICAM-1, VWF and VEGFR-1), and lymphatic endothelial markers (LYVE-1 and podoplanin). Scale bars: 50 μm.

Supplemental Figure 5. Regulation of cell growth and cell cycle of MM cells by KSHV. (**A**) KMM cells had shorter doubling time than MM cells indicated by faster CFSE dilution. Cells seeded at $2.5x10^5$ cells per T-25 flask were analyzed daily. (**B-C**) KSHV infection accelerated cell cycle progression. KMM cultures have more cells in S phase and less cells in G0G1 phase (**B**), and faster transition from G0G1 to S phase shown by more BrdU incorporation (**C**) than MM cells. Cells seeded at $2.5x10^5$ cells per T-25 flask were analyzed at day 1 post-seeding.

(**D**) KMM cells resisted cell cycle arrest in serum-free media. MM cells arrested in G0G1 phase at day 1 post-seeding in serum-free media while KMM cells resisted arrest with 20% remaining in S phase for up to day 4 post-seeding. Cell cycle was analyzed at day 2 post-seeding. (**E**) KMM cells incorporated BrdU at a faster rate than MM cells in serum-free media as shown at day 2 post-seeding. (**F**) KMM cells had higher saturation density than MM cells. Cells seeded at $2x10^5$ cells/well in 6-well plates and cultured with daily media change for 2 weeks were counted for total numbers of cells per well.

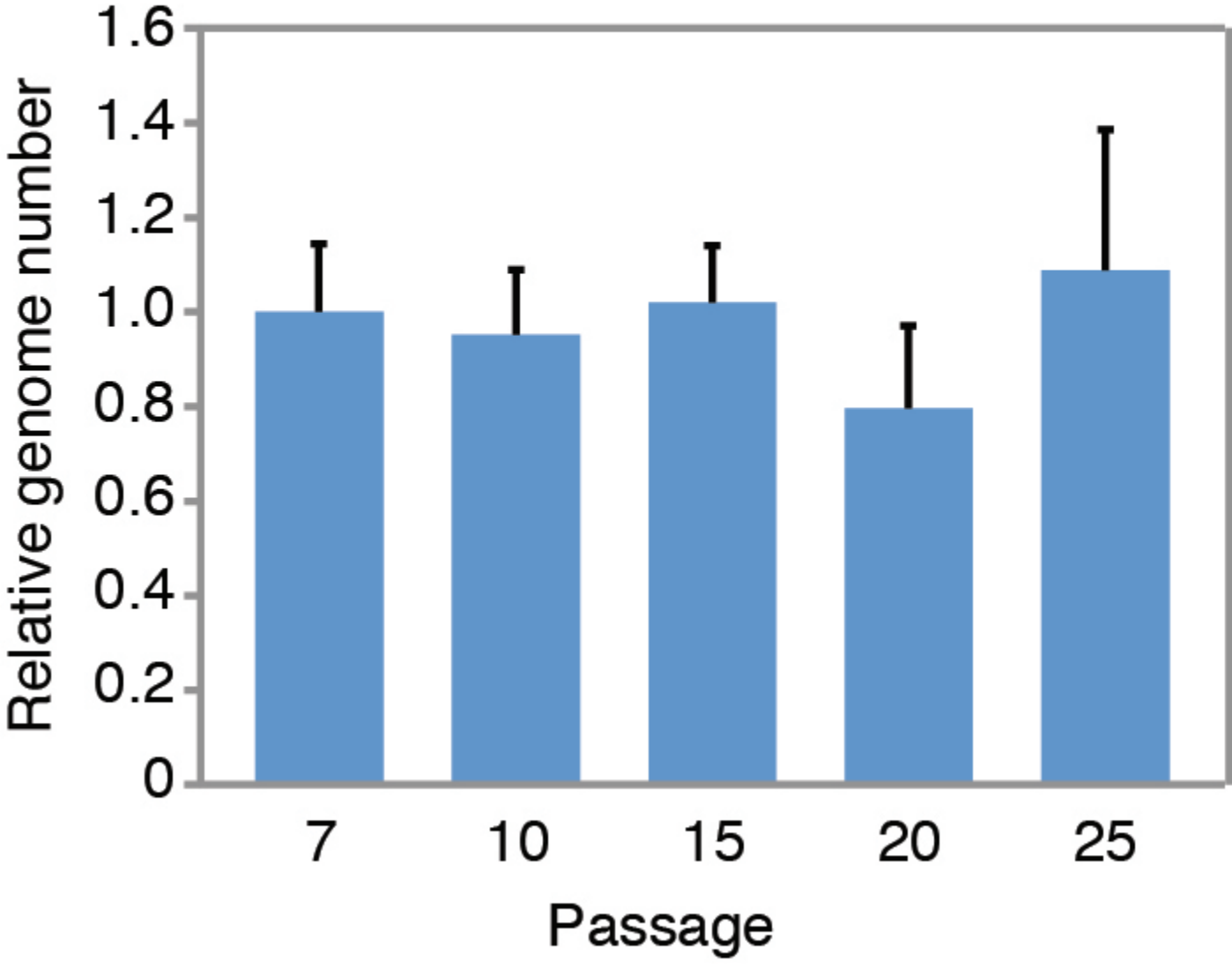
Supplemental Figure 6. Infection and cellular transformation of MM cells by KSHV derived from BCP-1 cells. (**A**) Morphology of uninfected cells (MM) and KSHV-infected cells (KMM) at day 1 and 3 post-seeding with the initial seeding at $5x10^4$ cells/well in 6 well plates. KMM cells grew substantially faster than MM cells. Scale bar: 50 μm. (**B**) Detection of KSHV latent protein LANA in KMM cells but not in MM cells. Scale bar: 20 μm. (C) KMM cells formed large colonies while MM cells did not form any colony in semisolid softagar media. Scale bar: 200 μm.

Supplemental Figure 7. Immunohistochemical staining of KMM tumors for H&E, vascular endothelial markers (VE-cadherin, ICAM-1 and VCAM-1), and lymphatic endothelial markers (podoplanin and PROX-1). Representative slit-like spaces were labeled with arrowheads while microvessels were labeled with arrows. Scale bars were 20 μm for all panels except for the H&E panel, which was 80 μm.

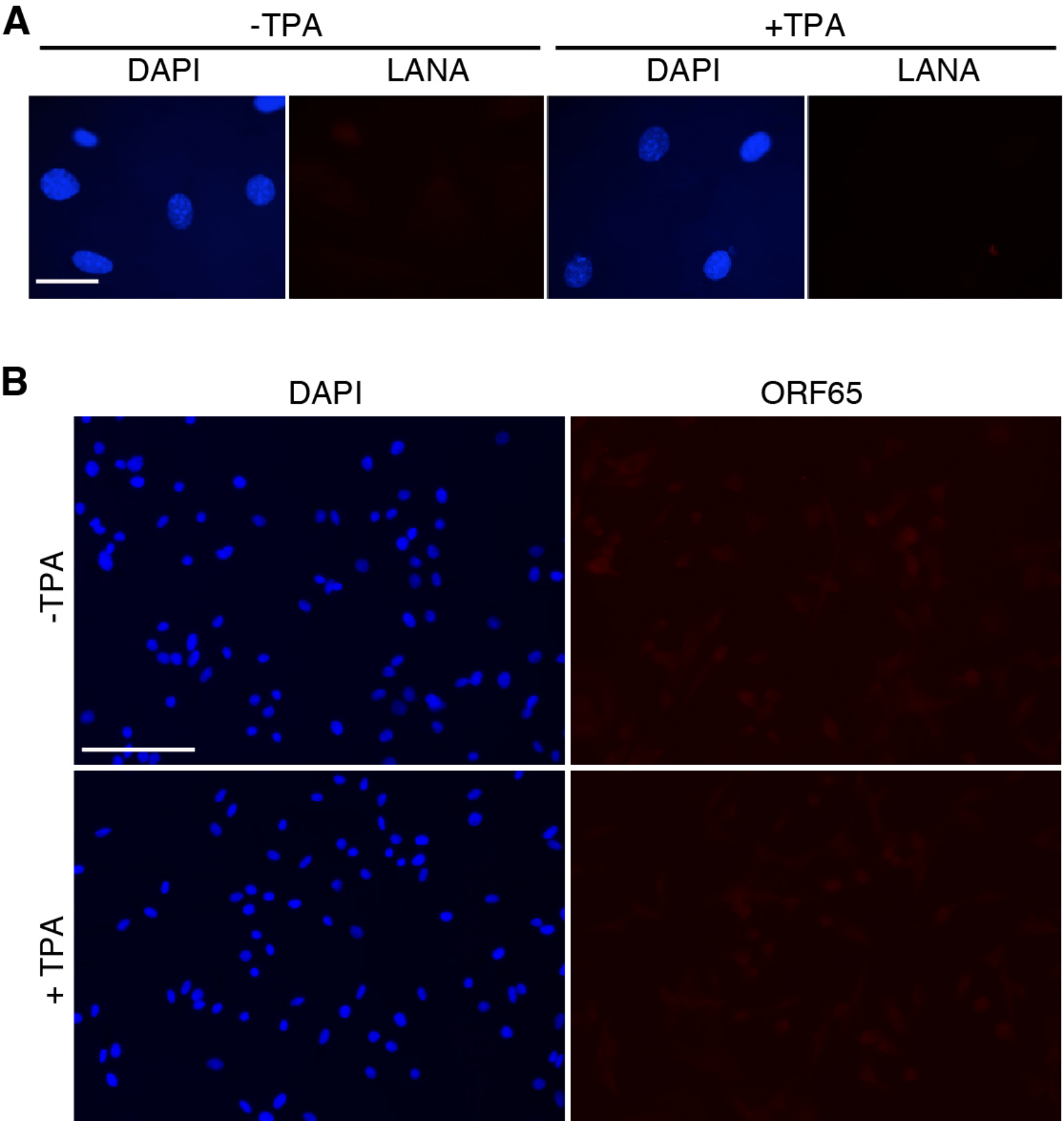
Supplemental Figure 8. Immunohistochemical staining of human KS tumors for H&E, Ki67, LANA, CD8, CD20, vascular endothelial markers (VE-cadherin, CD31, and VCAM-1), lymphatic endothelial markers (PROX-1, and VEGFR-3), and mesenchymal marker vimentin. Representative microvessels were labeled with arrows. Scale bar: 40 μm.

Supplemental Figure 9. Expression of KSHV genes in KMM tumors. Uninduced (U) and TPA-induced (I) KMM cells were used as controls with expression levels in uninduced KMM cells set as “1”.

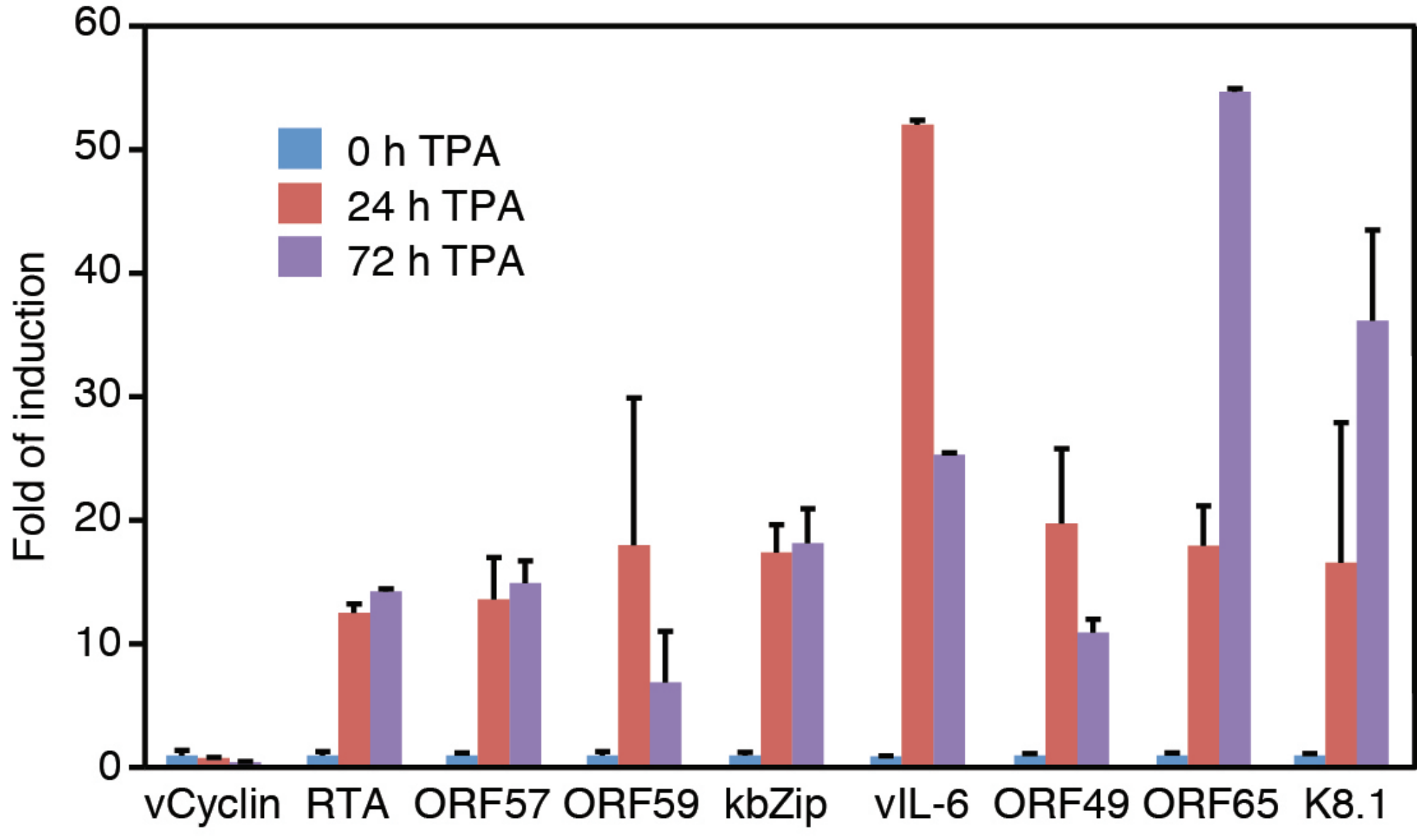
Supplemental Figure 1



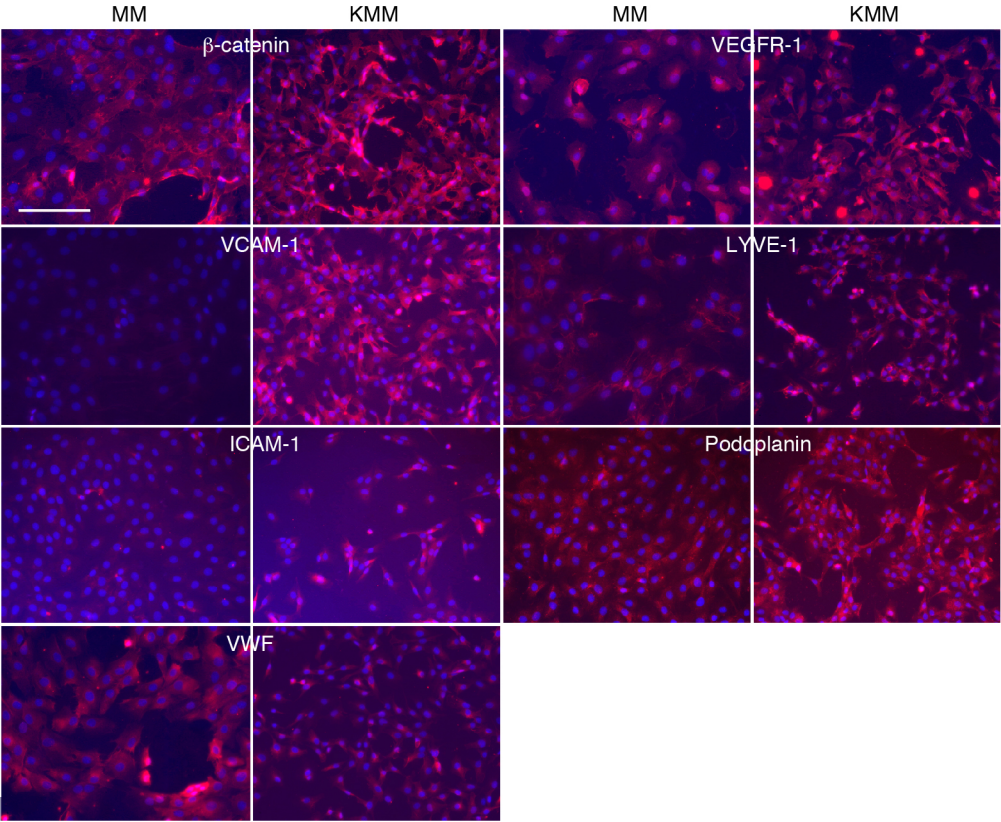
Supplemental Figure 2



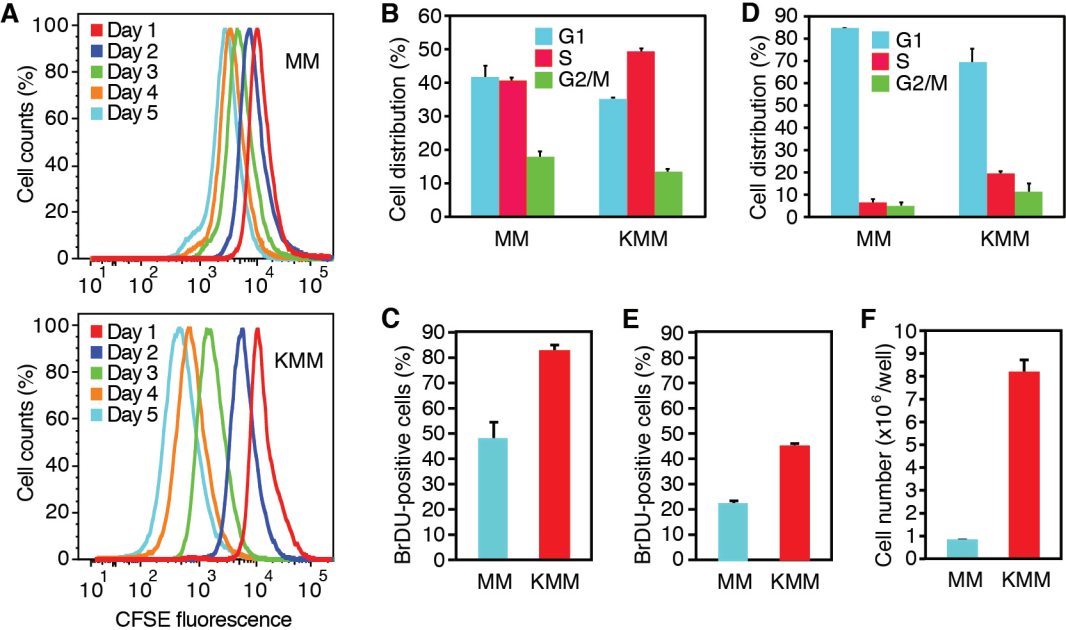
Supplemental Figure 3



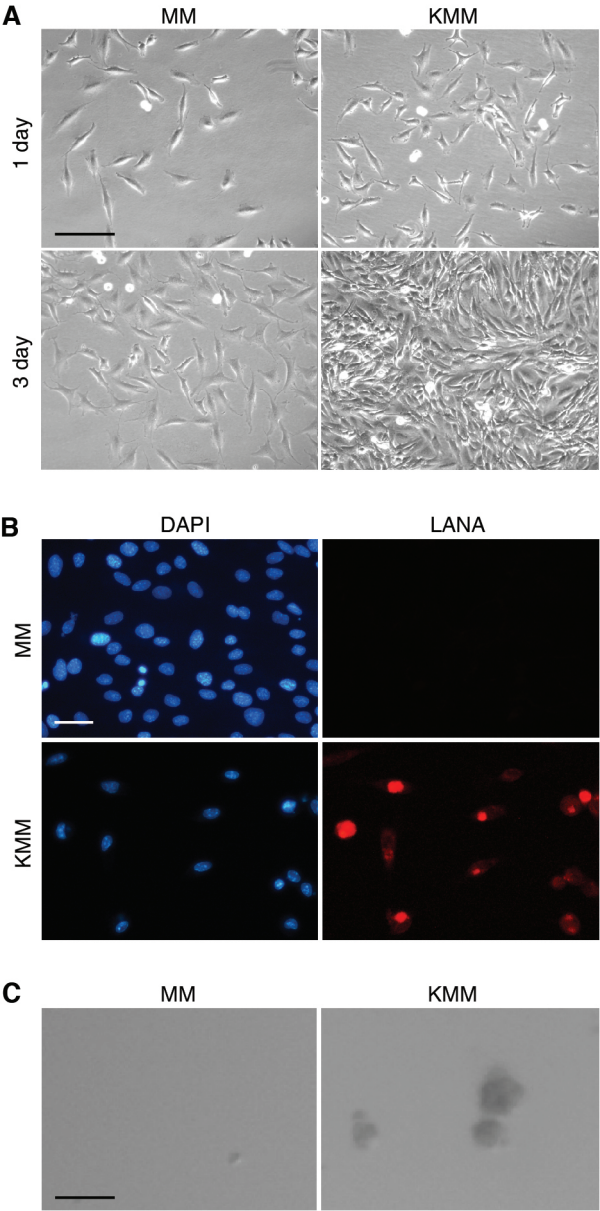
Supplemental Figure 4



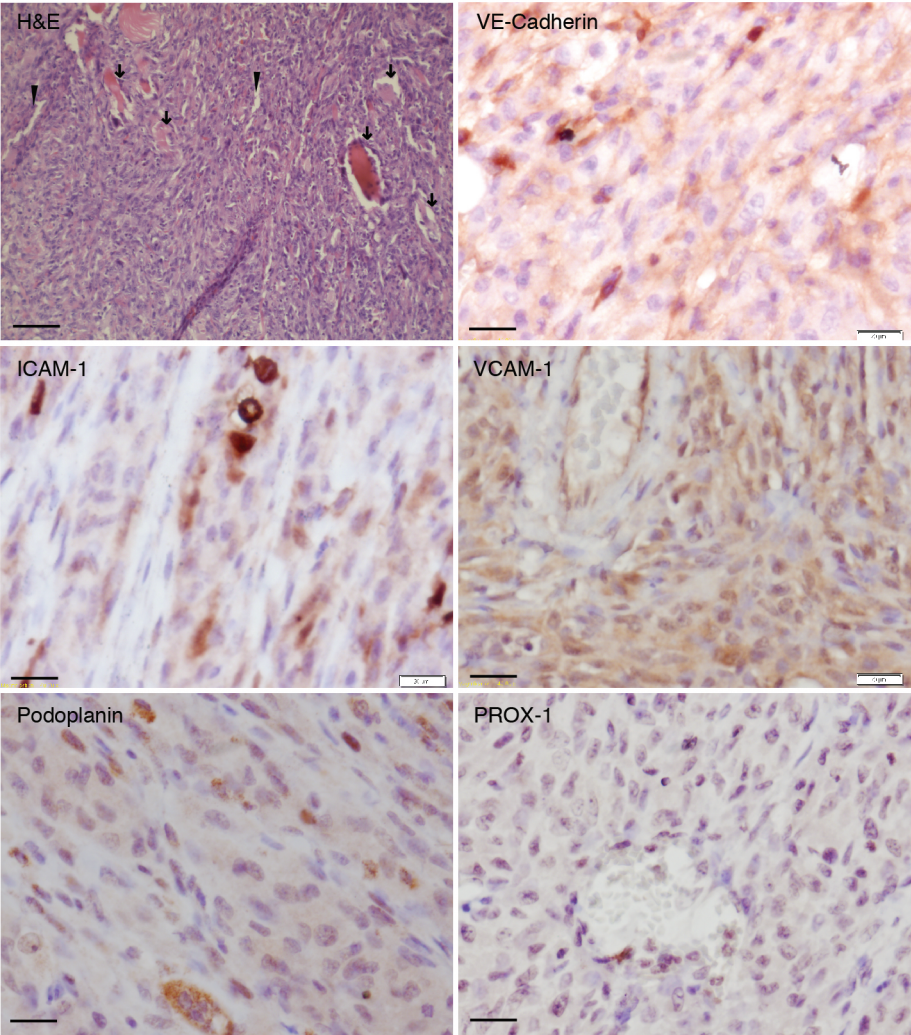
Supplemental Figure 5



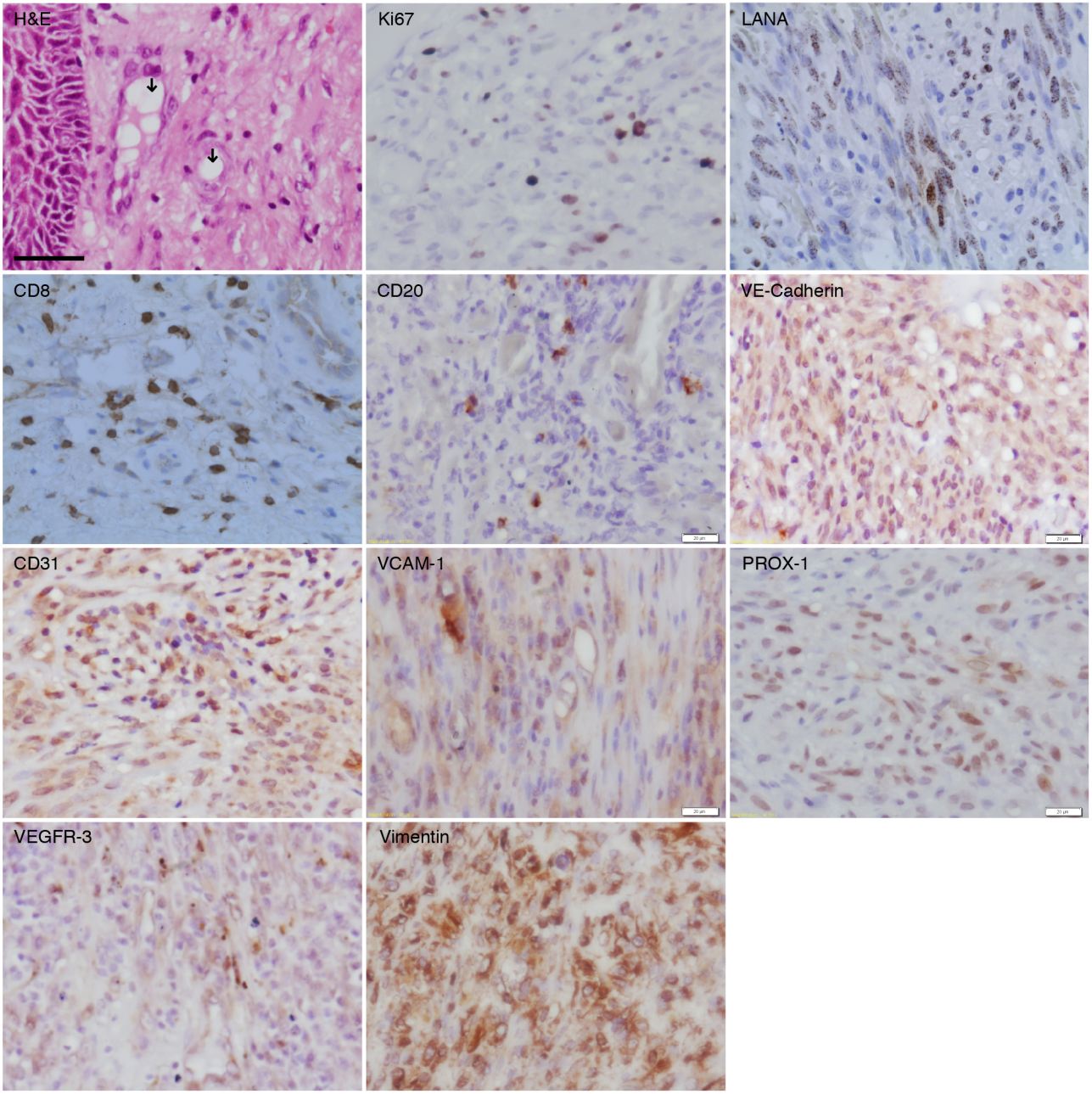
Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 9