

TABLE E-1 Immunohistochemical Staining Protocols

Stain	VEGF	CD31	pSmad2/3	Follistatin
Fixation	Methanol, 5 min	Methanol, 5 min	Methanol, 5 min	Methanol, 5 min
Blocking reagent	10% horse serum (Invitrogen), 60 min	10% horse serum (Invitrogen), 60 min	10% horse serum (Invitrogen), 60 min	10% horse serum (Invitrogen), 60 min
Primary antibody	Rabbit anti-mouse VEGF (Abcam), 1:100 in 2.5% horse serum overnight at 4°C	Rat anti-mouse CD31 (BD Pharmingen), 1:100 in 2% horse serum overnight at 4°C	Goat anti-mouse pSmad2/3 (Santa Cruz Biotechnology), 1:300 in 2% horse serum overnight at 4°C	Goat anti-mouse follistatin (Santa Cruz Biotechnology), 1:100 in 2% horse serum overnight at 4°C
Secondary antibody	Anti-rabbit IgG-Alexa Fluor 594 (Molecular Probes), 1:300 in 2% horse serum for 60 min	Anti-rat IgG-Alexa Fluor 594 (Molecular Probes), 1:300 in 2% horse serum for 60 min	Anti-goat IgG-Alexa Fluor 488 (Molecular Probes), 1:300 in 2% horse serum for 60 min	Anti-goat IgG-Alexa Fluor 488 (Molecular Probes), 1:300 in 2% horse serum for 60 min

TABLE E-2 PCR Primers *

Primer	Sequence	NCBI Gene Access Number
mVegfa F	GTGTGCGCAGACAGTGCTCCA	NM_001025250
mVegfa R	AGGCAGCCCGCTCTCTTGCG	
mCD31 F	CTGCTCCGTCTCGGGCACAC	NM_001032378
mCD31 R	TGGATCGGTACCAGGCCGCT	
mFst F	GGATTCCAAGGTTGGCAGAGGTCG	NM_008046
mFst R	TGGCACACTCGCTGGCGTAT	
mAct F	CCACACCCGCCACCAAGTTCG	NM_007393
mAct R	TACAGCCCGGGGAGCATCGT	

*NCBI = National Center for Biotechnology Information, F = forward, and R = reverse.