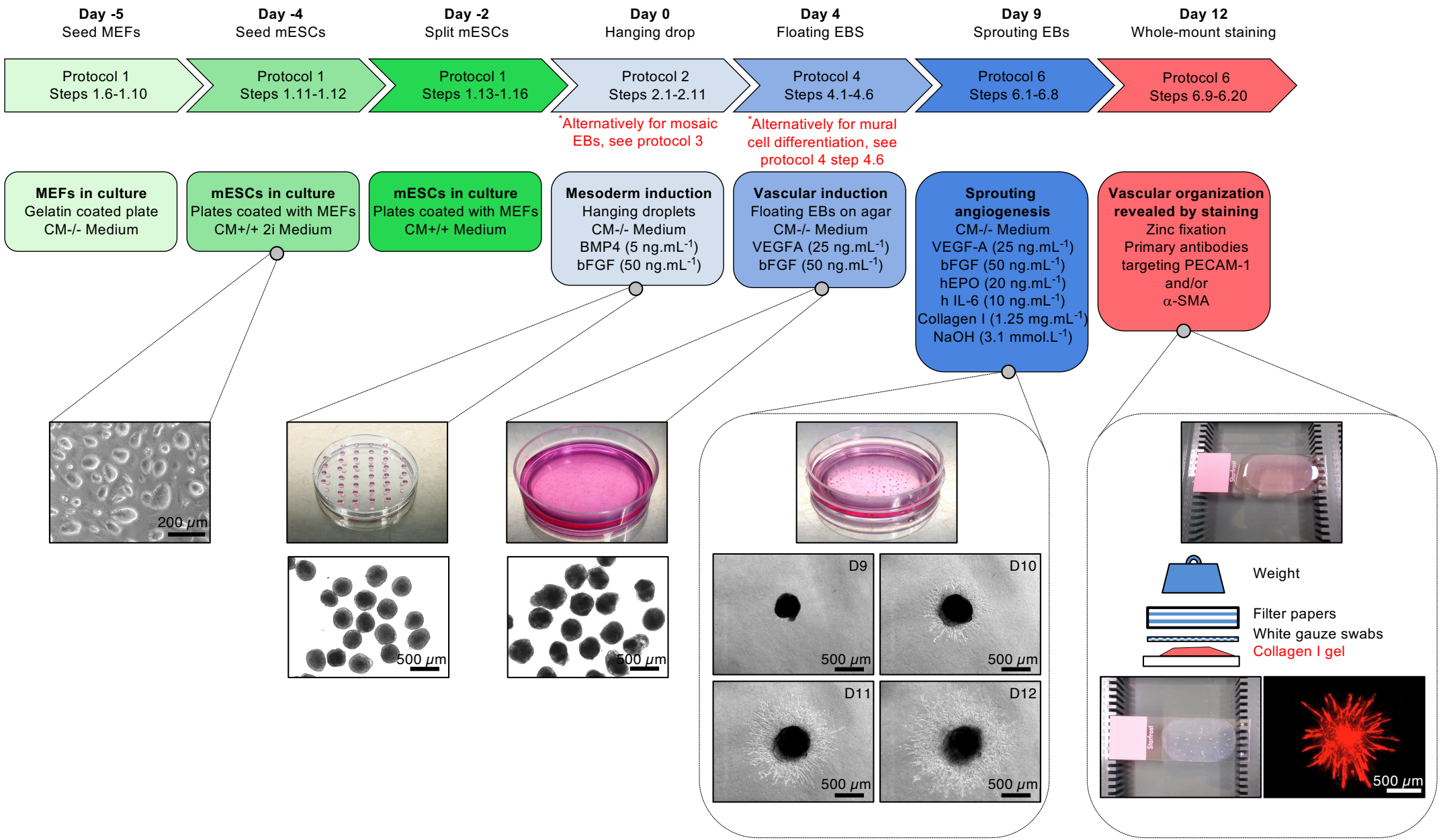
**Figure 1**


Day -5
Seed MEFs
Day -4
Seed mESCs
Day -2
Split mESCs
Day 0
Hanging drop
Day 4
Floating EBS
Day 9
Sprouting EBs
Day 12
Whole-mount staining
Protocol 1 Steps 1.6-1.10
Protocol 1 Steps 1.11-1.12
Protocol 1 Steps 1.13-1.16
Protocol 2 Steps 2.1-2.11
Protocol 4 Steps 4.1-4.6
Protocol 6 Steps 6.1-6.8
Protocol 6 Steps 6.9-6.20
*Alternatively for mosaic EBs, see protocol 3
*Alternatively for mural cell differentiation, see protocol 4 step 4.6
MEFs in culture
Gelatin coated plate
CM-/- Medium
mESCs in culture
Plates coated with MEFs
CM+/+ 2i Medium
mESCs in culture
Plates coated with MEFs
CM+/+ Medium
Mesoderm induction
Hanging droplets
CM-/- Medium
BMP4 (5 ng.mL-1)
bFGF (50 ng.mL-1)
Vascular induction
Floating EBs on agar
CM-/- Medium
VEGFA (25 ng.mL-1)
bFGF (50 ng.mL-1)
Sprouting angiogenesis
CM-/- Medium
VEGF-A (25 ng.mL-1)
bFGF (50 ng.mL-1)
hEPO (20 ng.mL-1)
h IL-6 (10 ng.mL-1)
Collagen I (1.25 mg.mL-1)
NaOH (3.1 mmol.L-1)
Vascular organization revealed by staining
Zinc fixation
Primary antibodies targeting PECAM-1 and/or α-SMA
200 µm
500 µm
500 µm
D9
D10
D11
D12
500 µm
Weight
Filter papers
White gauze swabs
Collagen I gel
500 µm