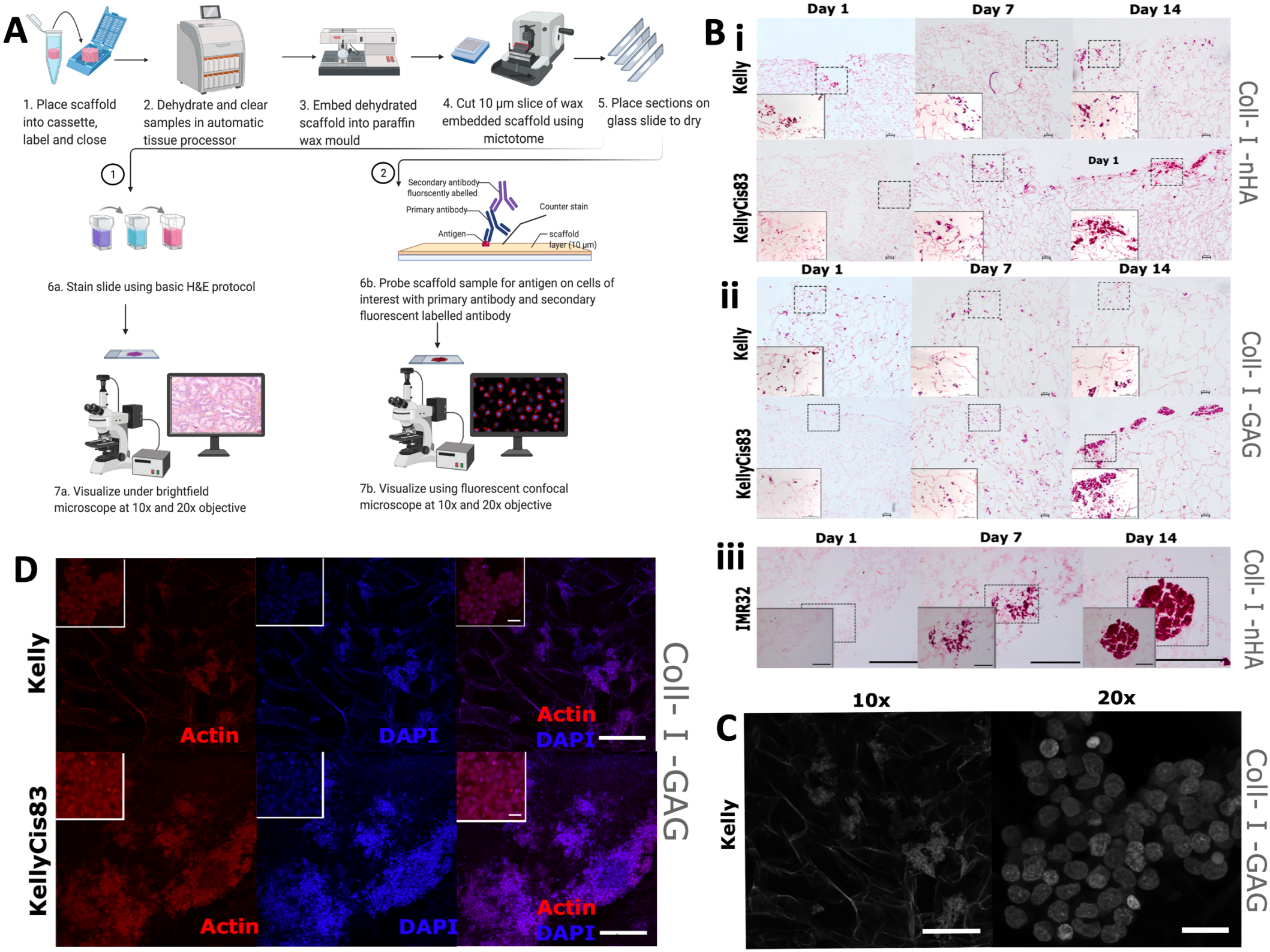
A
1. Place scaffold into cassette, label and close
2. Dehydrate and clear samples in automatic tissue processor
3. Embed dehydrated scaffold into paraffin wax mould
4. Cut 10 µm slice of wax embedded scaffold using mictotome
5. Place sections on glass slide to dry
1
2
Secondary antibody fluorscently abelled
Primary antibody
Antigen
Counter stain
scaffold layer (10 µm)
6a. Stain slide using basic H&E protocol
6b. Probe scaffold sample for antigen on cells of interest with primary antibody and secondary fluorescent labelled antibody
7a. Visualize under brightfield microscope at 10x and 20x objective
7b. Visualize using fluorescent confocal microscope at 10x and 20x objective
B
i
Day 1
Day 7
Day 14
Kelly
KellyCis83
Coll- I -nHA
ii
Coll- I -GAG
iii
IMR32
C
10x
20x
D
Actin
DAPI
Actin
DAPI