

Supplementary Materials for

Isolation and micromass culturing of primary chicken chondroprogenitor cells for cartilage regeneration

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Stage-related external features of early-stage chicken embryos between Hamburger–Hamilton (HH) developmental stages 19–25

Materials and Methods

Chicken embryos of different stages were retrieved from eggs incubated for various durations (ranging from 68 to 115 hours), rinsed in calcium and magnesium free phosphate buffered saline (CMF–PBS), and photographed using a Leica S6 E LED 2500 microscope.

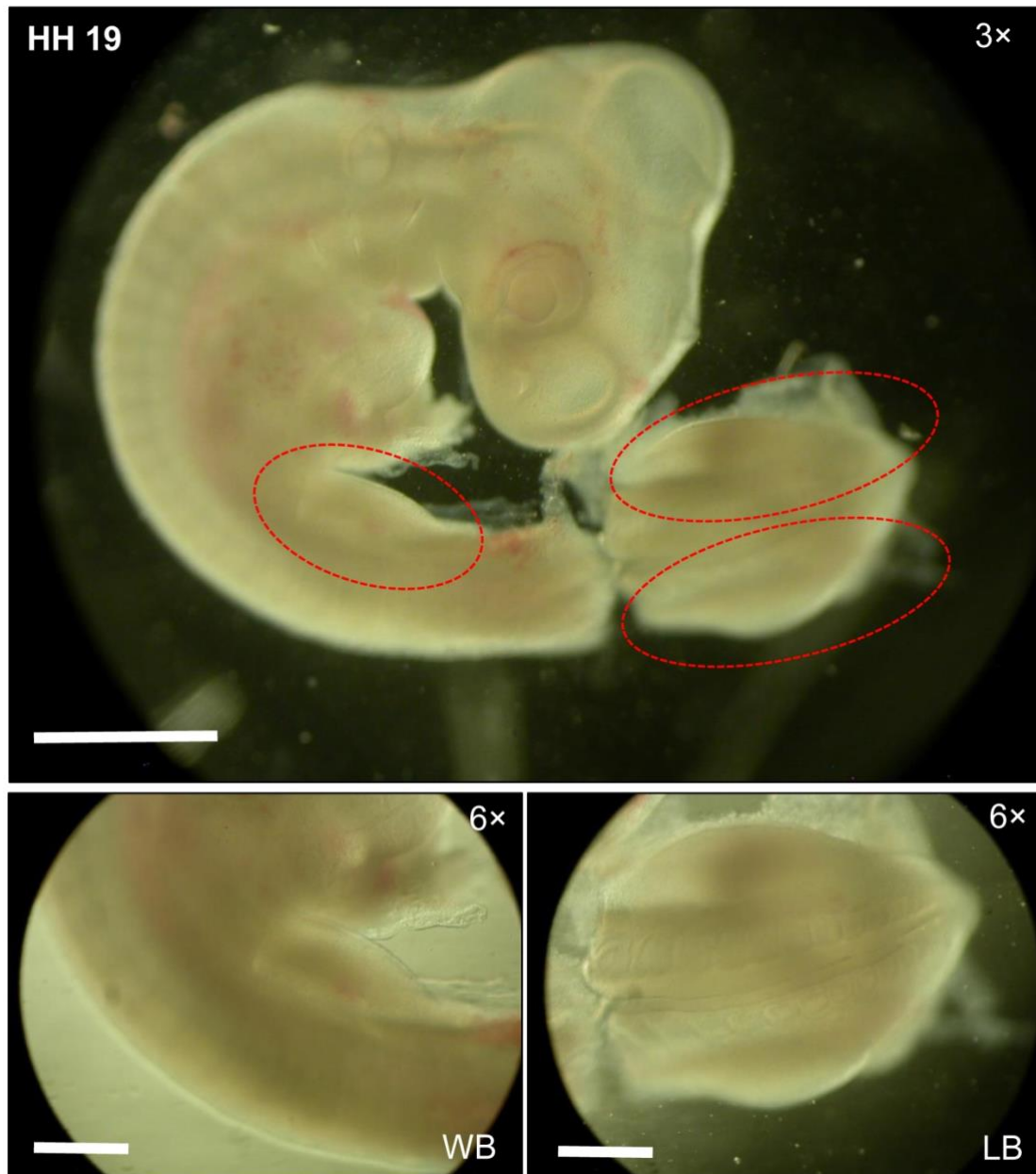


Figure S1. HH19. After 68–72 hours of incubation, the leg buds (LB; hindlimb) are larger than the wing buds (WB; forelimb). LB and WB are indicated by red dashed ovals. Somites are visible all over the back, while the tip of the tail is unsegmented and curves towards the head. The allantois appears at the angle of the tail curve but it is not yet vesicular. The eyes are unpigmented. Scale bars, 1 mm (3×), 500 μ m (6×). WB, wing bud; LB, leg bud.

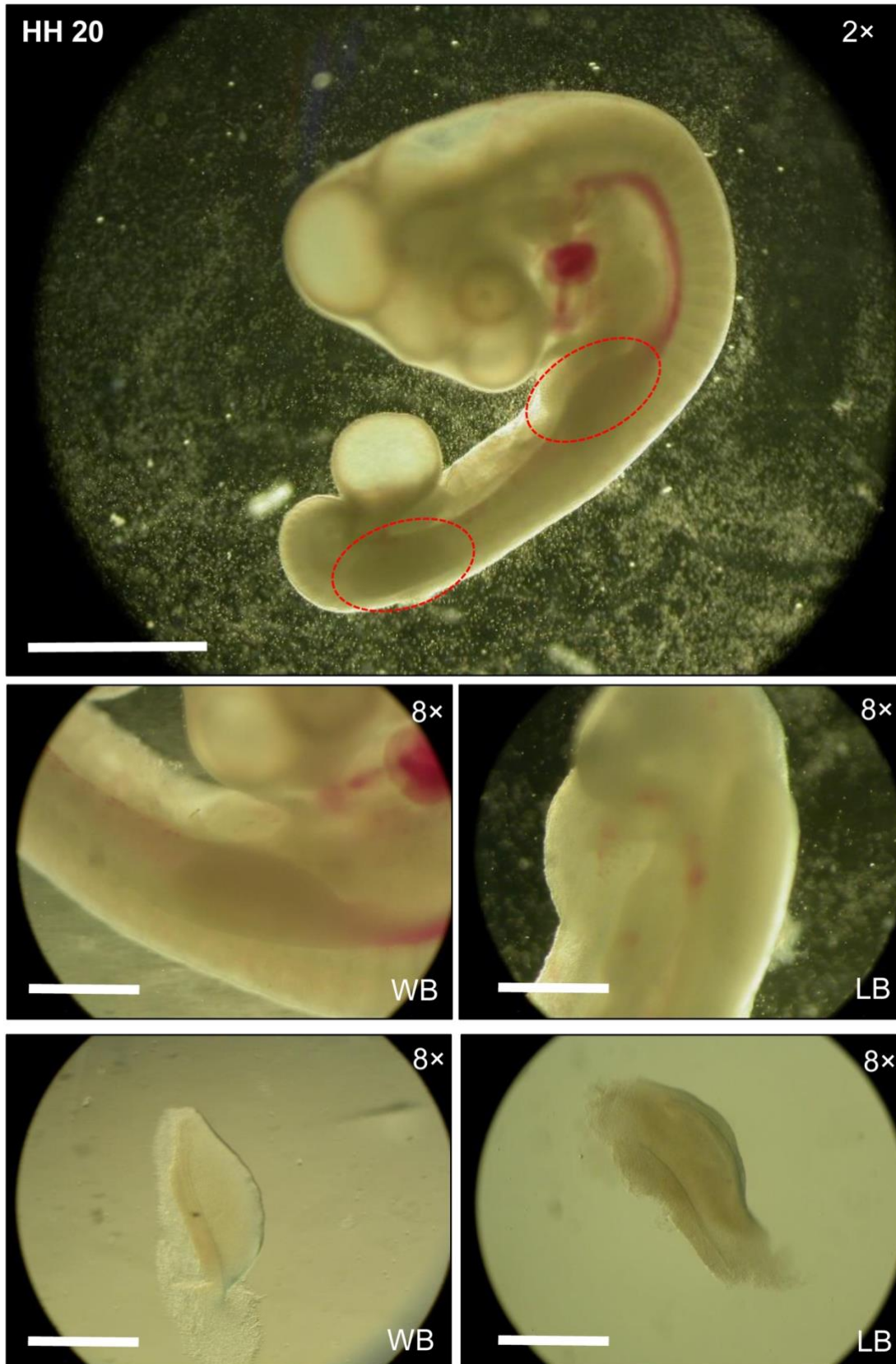


Figure S2. HH20. After 70–72 hours of incubation, the size of the limb buds has dramatically increased. From now on, LBs are consistently larger than WBs (marked by red dashed ovals) and become asymmetric. The tail is unsegmented and the allantois has become vesicular. Slight pigmentation appears in the eye. Scale bars, 2 mm (2×), 500 μ m (8×). WB, wing bud; LB, leg bud.

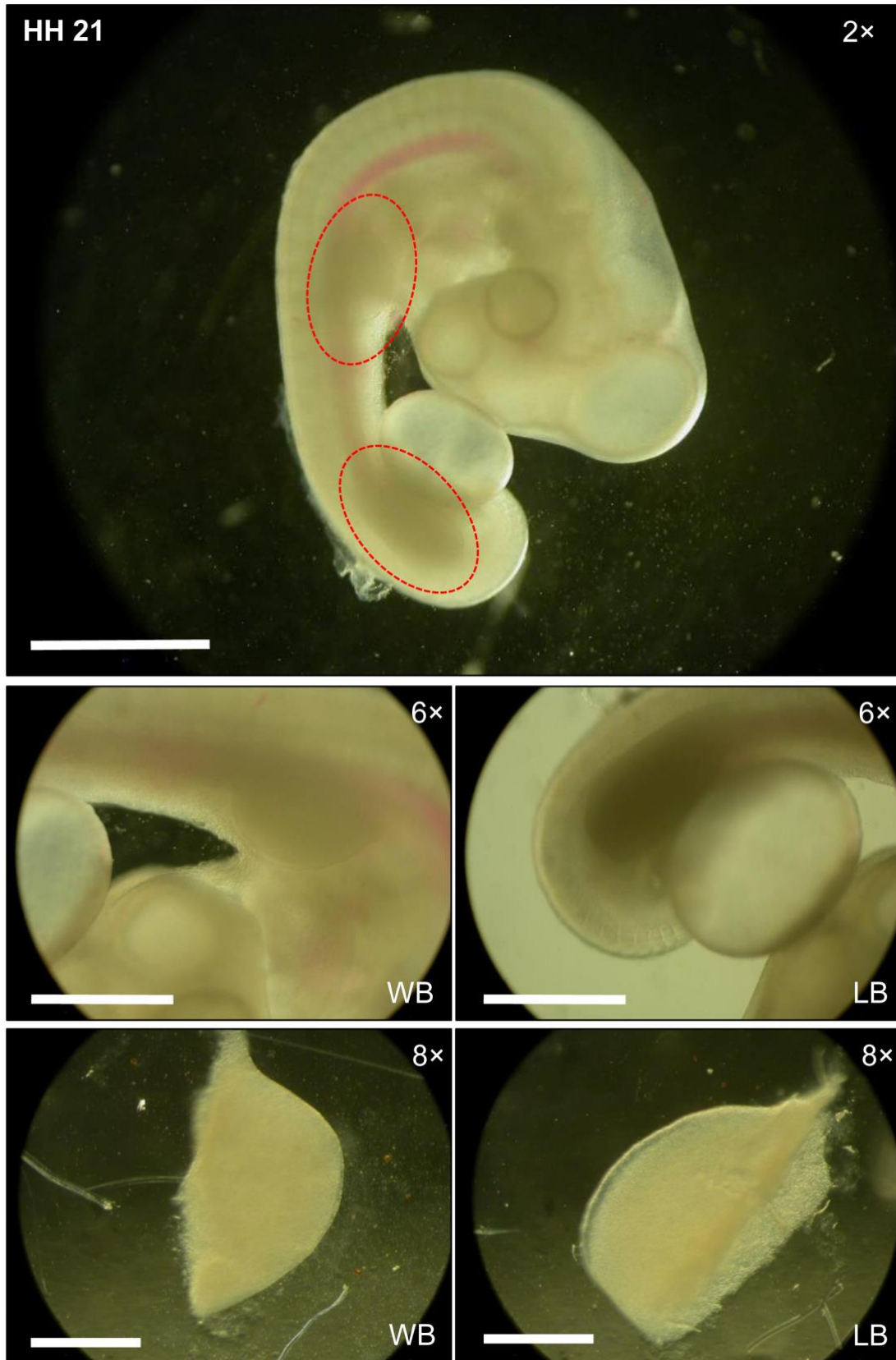


Figure S3. HH21. After 74 hours of incubation, both limb buds (marked by red dashed ovals) have become asymmetric. The distal border of the limb buds is sharper and more prominent than the proximal. The tip of the tail is still unsegmented. The size of the allantois varies, may reach the head. Eye pigmentation is getting more prominent, though still pale. Scale bars, 2 mm (2×), 1 mm (6×), 500 μ m (8×). WB, wing bud; LB, leg bud.

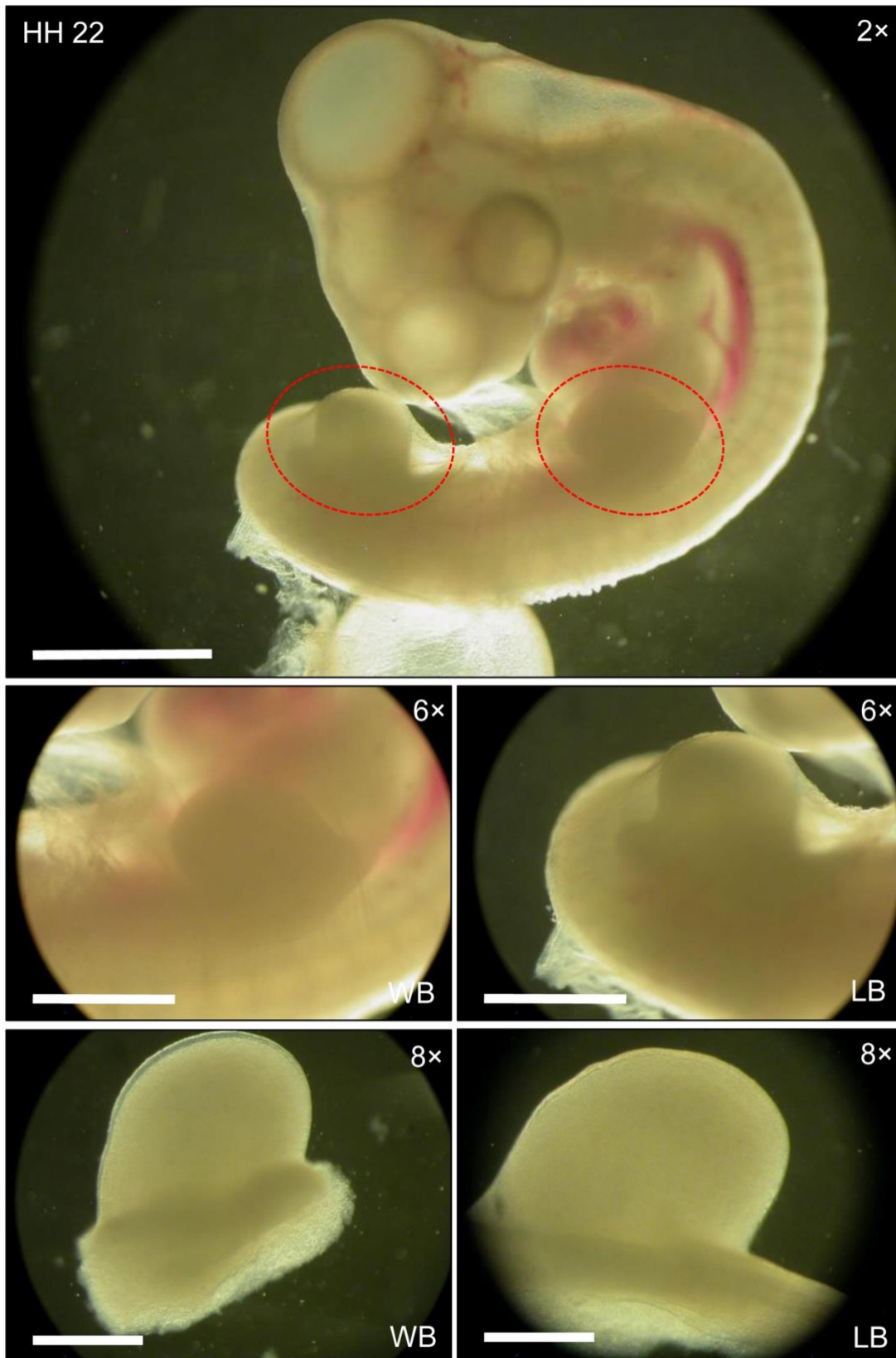


Figure S4. HH22. After 78 hours of incubation, somites (apparent segmentation) have reached the tail. The limb buds (marked by red dashed ovals) gradually enlarge and point caudally. The maxillary process is enlarged and the fourth branchial cleft is formed. Allantois might cover the forebrain. The periphery of the eye has become well defined by pigmentation. Scale bars, 2 mm (2x), 1 mm (6x), 500 μ m (8x). WB, wing bud; LB, leg bud.

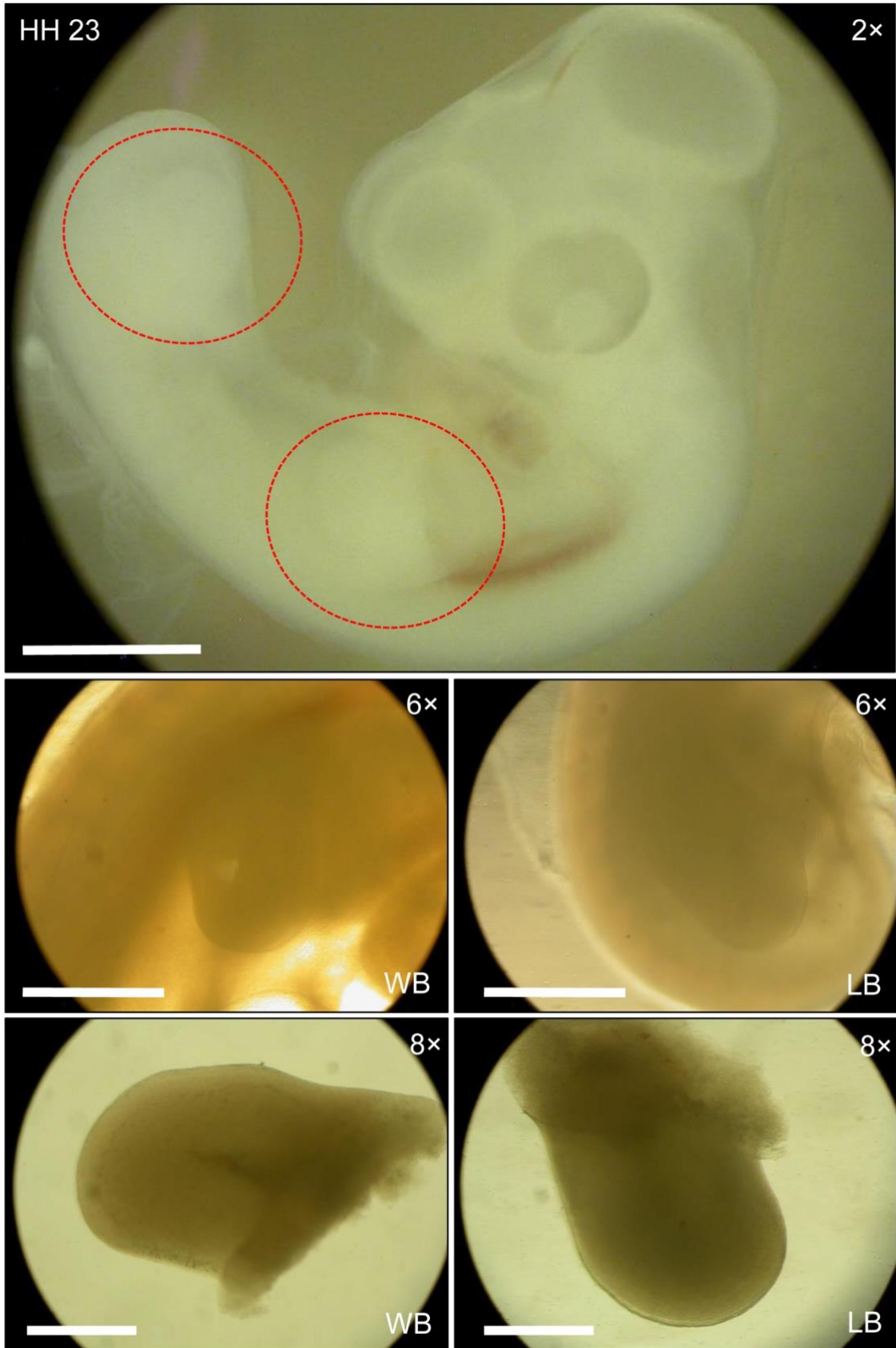


Figure S5. HH23. Just before 96 hours of incubation, the length vs. the width of the limb buds (marked by red dashed ovals) is approximately 1:1. Digital plates are not yet visible. Retinal pigmentation is increasingly prominent. Scale bars, 2 mm (2×), 1 mm (6×), 500 μm (8×). WB, wing bud; LB, leg bud.

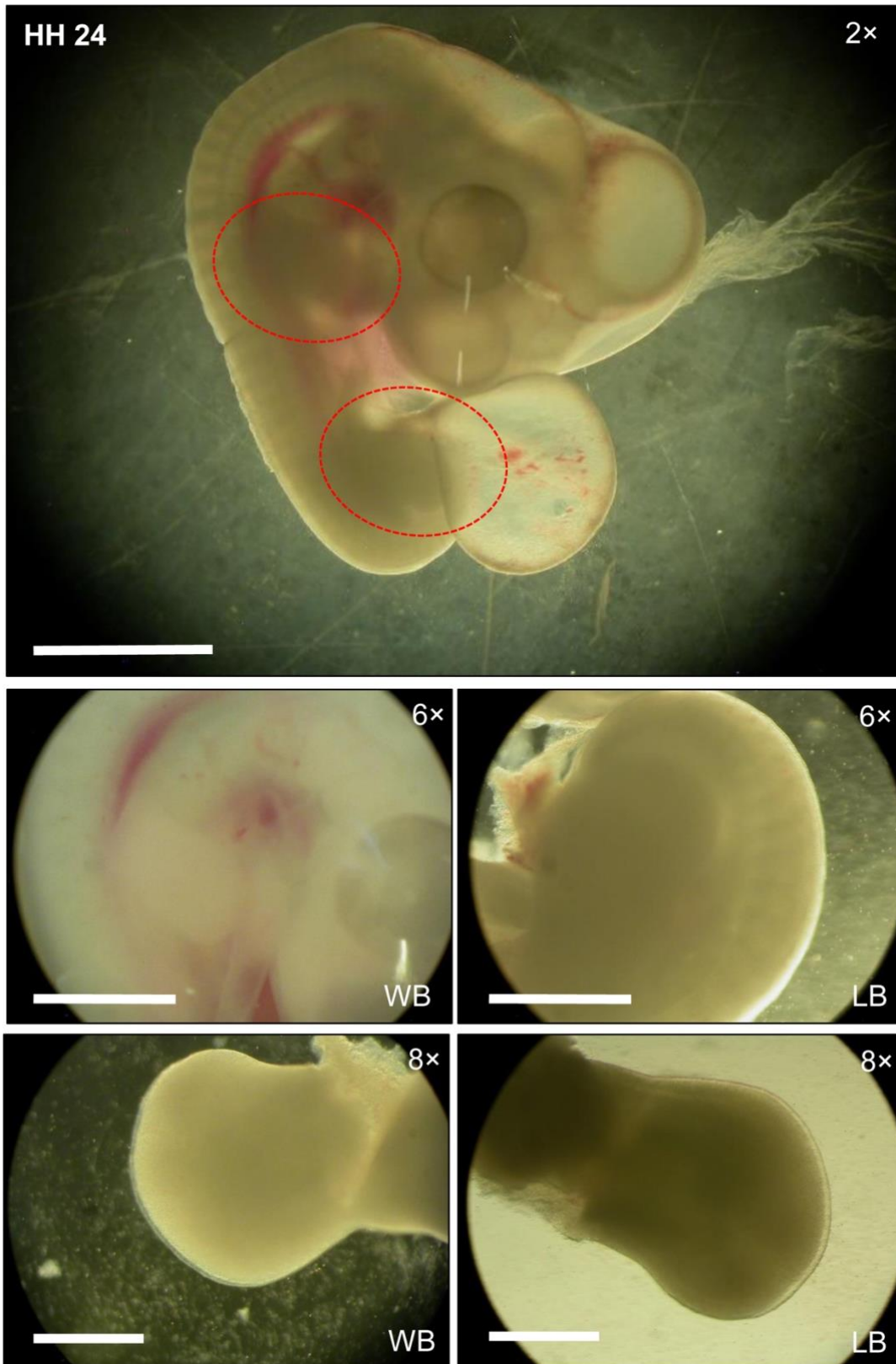


Figure S6. HH24. After 104 hours of incubation, the limb buds (marked by red dashed ovals) are longer than wider. Digital plates are not yet present at the WBs, but the toe plate is becoming distinct at the LBs. However, the digits are not yet demarcated. The second visceral arch is the most prominent, longer ventrally and wider than the mandibular process and all other arches. Scale bars, 2 mm (2 \times), 1 mm (6 \times), 500 μ m (8 \times). WB, wing bud; LB, leg bud.

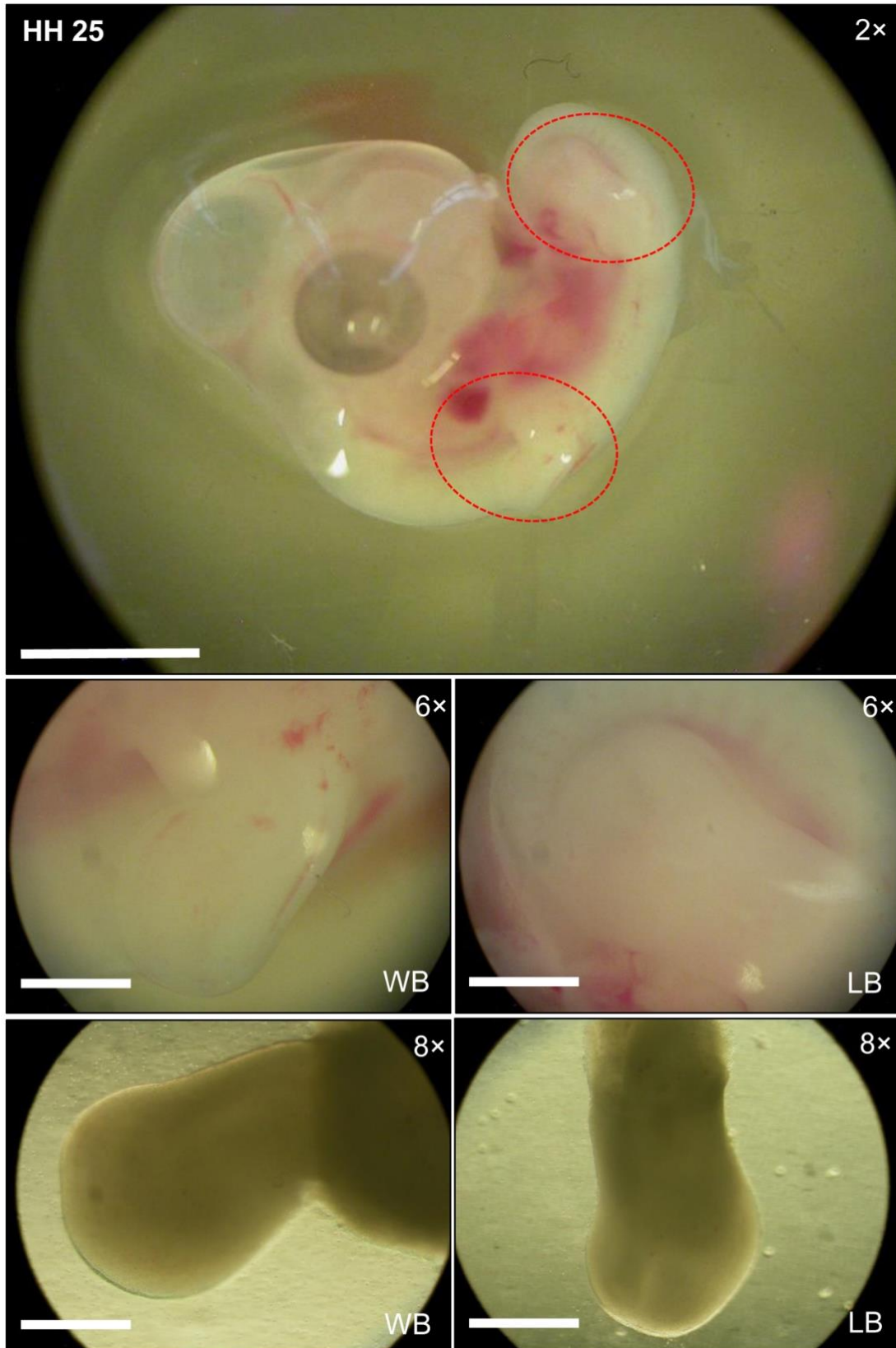


Figure S7. HH25. After 115 hours of incubation, cartilaginous templates of the radius and ulna are clearly visible in the forelimb primordium (limb buds are marked by red dashed ovals). The digital plate is visible, but not the individual digits. In the leg primordium, a shallow margin of the third toe is clearly delineated. Scale bars, 2 mm (2×), 1 mm (6×), 500 μ m (8×). WB, wing bud; LB, leg bud.

