

## Product Datasheet

### Collagen Type IV Antibody (orb345354)

<b>Catalog Number</b>	orb345354
<b>Category</b>	Antibodies
<b>Description</b>	Collagen IV antibody
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Bovine, Human
<b>Form/Appearance</b>	Liquid (sterile filtered)
<b>Concentration</b>	1.18 mg/mL
<b>Buffer/Preservatives</b>	Preservative: 0.01% (w/v) Sodium Azide. Stabilizer: None; Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
<b>Purity</b>	This product has been prepared by immunoaffinity chromatography using immobilized antigens followed by extensive cross-adsorption against other collagens, human serum proteins and non-collagen extracellular matrix proteins to remove any unwanted specificities. Some class-specific anti-collagens may be specific for three-dimensional epitopes which may result in diminished reactivity with denatured collagen or formalin-fixed, paraffin embedded tissues. This antibody reacts with most mammalian Type IV collagens and has negligible cross-reactivity with Type I, II, III, V or VI collagens. Non-specific cross-reaction of anti-collagen antibodies with other human serum proteins or non-collagen extracellular matrix proteins is negligible.
<b>Immunogen</b>	Collagen Type IV from human and bovine placenta

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

<b>UniProt ID</b>	<b>P02462</b>
<b>Tested applications</b>	DOT, ELISA, IHC, IP, WB
<b>Dilution range</b>	ELISA: 1:5,000 - 1:50,000, IHC: 1:50 - 1:200, IP: 1:100, WB: 1:1,000 - 1:10,000
<b>Application notes</b>	Anti-Collagen Type IV has been tested by dot blot and IHC and is suitable for indirect trapping ELISA for quantitation of antigen in serum using a standard curve, immunoprecipitation, native (non-denaturing, non-dissociating) PAGE, immunohistochemistry, and western blotting for highly sensitive qualitative analysis.
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
<b>Dry Ice Shipping</b>	<b>Please note: This product requires shipment on dry ice. A dry ice surcharge will apply.</b>
<b>Note</b>	For research use only
<b>NCBI</b>	<b>NP_001290039.1</b>
<b>Expiration Date</b>	12 months from date of receipt.

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

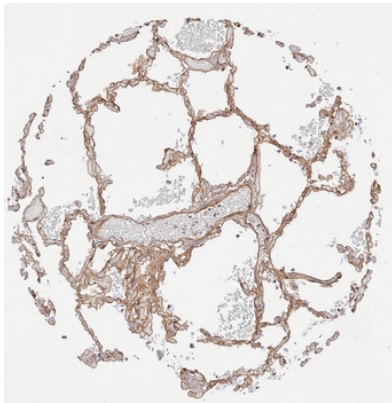
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

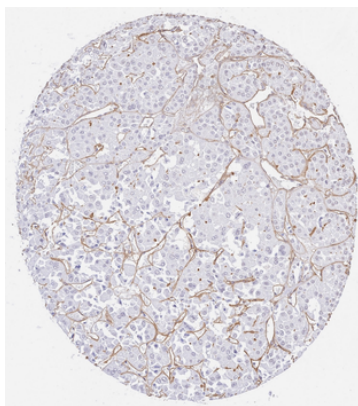
68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

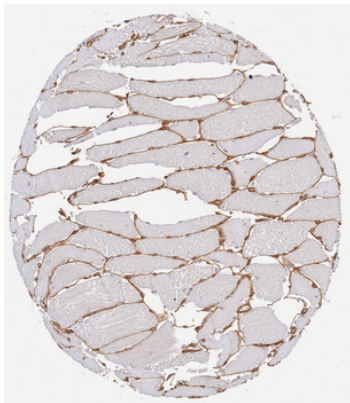
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)



Immunohistochemistry results of Rabbit Anti-Collagen Type IV Antibody. Tissue: human lung tissue. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type IV (p/n orb345352) at 1:15 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: basement membranes and vessels.



Immunohistochemistry results of Rabbit Anti-Collagen Type IV Antibody. Tissue: human renal oncocytoma. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type IV (p/n orb345352) at 1:15 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: dense collagen IV positive membranes surrounding tumor cell nests.



Immunohistochemistry results of Rabbit Anti-Collagen Type IV Antibody. Tissue: human skeletal muscle cells. Fixation: FFPE. Antigen Retrieval: HIER using Tris-EDTA-citrate buffer pH7.8 for 5 min. Blocking: Peroxidase-Blocking Solution for 10 min. Primary Antibody: Anti-Collagen Type IV (p/n orb345352) at 1:15 for 1 hr at 37 °C. Secondary Antibody: Dako REAL EnVision Detection Kit, Polymer-HRP, Rabbit/Mouse. Counterstain: Hematoxylin for 15 sec. Substrate: DAB-Chromogen, Rabbit/Mouse. Staining/Results: cells surrounded by collagen IV fibers.

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

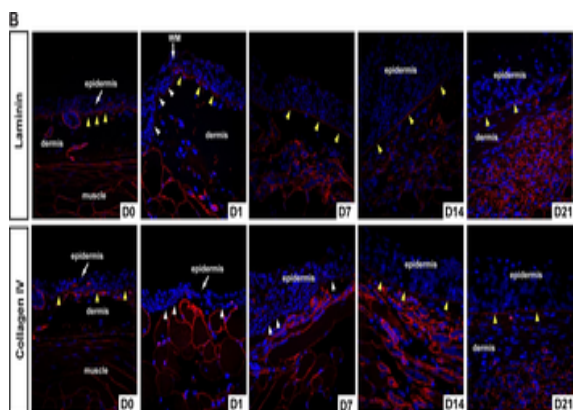
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

**Biorbyt LLC**

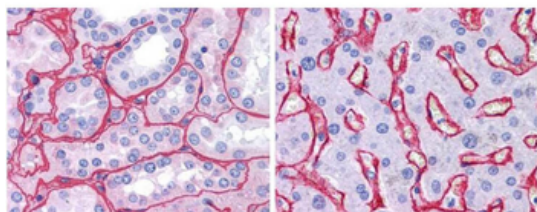
68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558



Lamina lucida and lamina densa regenerate before new ECM deposition. A) Histological examination of basement membrane (BM) regeneration in axolotls. The uninjured BM is visible as a thick blue-stained fibrous band (yellow arrows). An immature BM has begun to reform (yellow arrow D1) after re-epithelialization and is visible at the wound margin (WM) in contrast to the uninjured BM. The regenerated BM is visible at D47. Yellow arrows at D7 and D21 indicate reforming BM. B) Examination of lamina lucida (laminin) and lamina densa (collagen type IV) during basement membrane regeneration. The uninjured BM is positive for laminin and collagen type IV (yellow arrows) as are the basement membranes surrounding glands and muscle fibers. Following re-epithelialization the basal lamina of the epidermis is negative for laminin and collagen type IV (white arrows) and this is clearly evident at the wound margin (WM). Seven days post injury the BM stains strongly for laminin indicating reformation of the lamina lucida, while staining for collagen type IV is punctuated. The lamina densa is regenerated by D14 based on continuous collagen type IV staining and persists during dermal regeneration.



Biorbyt anti collagen IV antibody (1:400, 45 min RT) showed strong staining in FFPE sections of human kidney (Left) with strong red staining observed in glomeruli; and liver (Right) with strong staining in sinusoids. Staining for both tissues was consistent with a basement membrane distribution. Slides were steamed in 0.01 M sodium citrate buffer, pH6.0 at 99-100°C - 20 minutes for antigen retrieval.

#### Biorbyt Ltd.

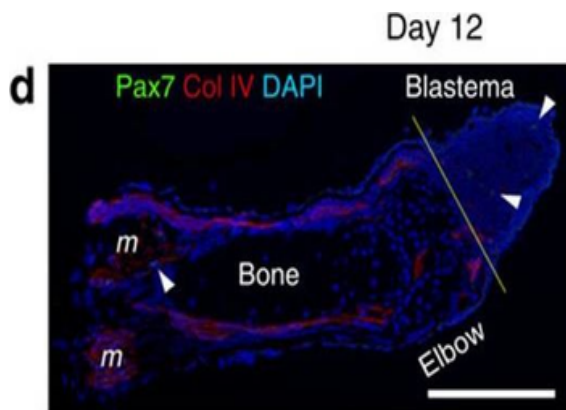
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

#### Biorbyt LLC

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558



SMFC tracking in larval newt limb regeneration. (a) Larva (3 months old). It has four limbs, as well as the gills and tail fin. Scale bar, 4 mm. (b) Monitoring of SMFCs (mCherry+) during limb regeneration (n = 6). mCherry was not detected in the regenerating part of the limb until ~30 days when the amputated limb had almost been recovered. Arrowheads: flexor muscle for digits. Scale bar, 1 mm. (c) Sections of regenerating limbs (n = 3 for each stage). SMFC-derived mCherry+ cells were not observed in the blastema. Lines: amputation site. m: muscle. Scale bar, 100  $\mu$ m. (d-f) Pax7 immunolabelling of regenerating limbs on day 12 (n = 3) and (g) day 15 (n = 3) after amputation. (d) On day 12, a few Pax7+ nuclei (arrowheads) were detected in blastema cells and in satellite cells along the muscle fibres. Col IV, collagen type IV immunoreactivity. DAPI (4, 6-diamidino-2-phenylindole), nuclei. Scale bar, 300  $\mu$ m. The Pax7+ nuclei pointed by arrowheads were enlarged in e and f, respectively. Scale bars, 100  $\mu$ m. (g) On day 15 when the regenerating part of the limb grew more distally, the number of Pax7+ nuclei (arrowheads) in the blastema was dramatically increased. Scale bar, 100  $\mu$ m. (h) Summary. In larval newts, MPCs, potentially satellite cells, were recruited for new muscle during limb regeneration, whereas SMFCs were not.

**Biorbyt Ltd.**

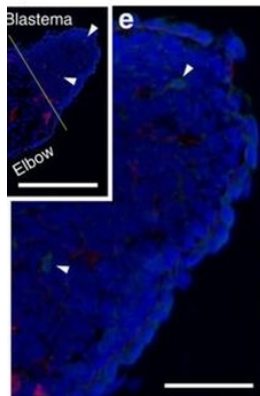
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)



SMFC tracking in larval newt limb regeneration. (a) Larva (3 months old). It has four limbs, as well as the gills and tail fin. Scale bar, 4 mm. (b) Monitoring of SMFCs (mCherry+) during limb regeneration (n = 6). mCherry was not detected in the regenerating part of the limb until ~30 days when the amputated limb had almost been recovered. Arrowheads: flexor muscle for digits. Scale bar, 1 mm. (c) Sections of regenerating limbs (n = 3 for each stage). SMFC-derived mCherry+ cells were not observed in the blastema. Lines: amputation site. m: muscle. Scale bar, 100  $\mu$ m. (d-f) Pax7 immunolabelling of regenerating limbs on day 12 (n = 3) and (g) day 15 (n = 3) after amputation. (d) On day 12, a few Pax7+ nuclei (arrowheads) were detected in blastema cells and in satellite cells along the muscle fibres. Col IV, collagen type IV immunoreactivity. DAPI (4, 6-diamidino-2-phenylindole), nuclei. Scale bar, 300  $\mu$ m. The Pax7+ nuclei pointed by arrowheads were enlarged in e and f, respectively. Scale bars, 100  $\mu$ m. (g) On day 15 when the regenerating part of the limb grew more distally, the number of Pax7+ nuclei (arrowheads) in the blastema was dramatically increased. Scale bar, 100  $\mu$ m. (h) Summary. In larval newts, MPCs, potentially satellite cells, were recruited for new muscle during limb regeneration, whereas SMFCs were not.

**Biorbyt Ltd.**

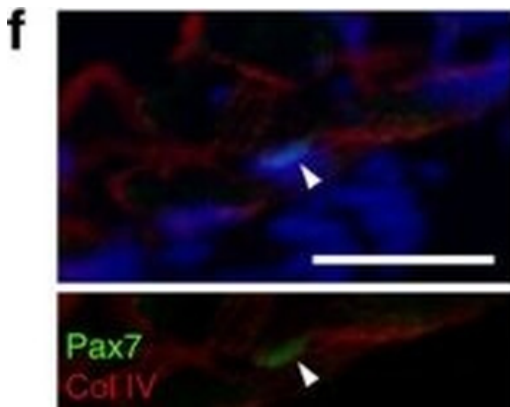
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)



SMFC tracking in larval newt limb regeneration. (a) Larva (3 months old). It has four limbs, as well as the gills and tail fin. Scale bar, 4 mm. (b) Monitoring of SMFCs (mCherry+) during limb regeneration (n = 6). mCherry was not detected in the regenerating part of the limb until ~30 days when the amputated limb had almost been recovered. Arrowheads: flexor muscle for digits. Scale bar, 1 mm. (c) Sections of regenerating limbs (n = 3 for each stage). SMFC-derived mCherry+ cells were not observed in the blastema. Lines: amputation site. m: muscle. Scale bar, 100  $\mu$ m. (d-f) Pax7 immunolabelling of regenerating limbs on day 12 (n = 3) and (g) day 15 (n = 3) after amputation. (d) On day 12, a few Pax7+ nuclei (arrowheads) were detected in blastema cells and in satellite cells along the muscle fibres. Col IV, collagen type IV immunoreactivity. DAPI (4, 6-diamidino-2-phenylindole), nuclei. Scale bar, 300  $\mu$ m. The Pax7+ nuclei pointed by arrowheads were enlarged in e and f, respectively. Scale bars, 100  $\mu$ m. (g) On day 15 when the regenerating part of the limb grew more distally, the number of Pax7+ nuclei (arrowheads) in the blastema was dramatically increased. Scale bar, 100  $\mu$ m. (h) Summary. In larval newts, MPCs, potentially satellite cells, were recruited for new muscle during limb regeneration, whereas SMFCs were not.

**Biorbyt Ltd.**

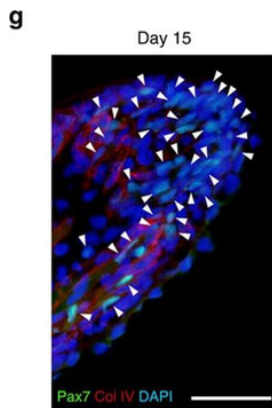
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558



SMFC tracking in larval newt limb regeneration. (a) Larva (3 months old). It has four limbs, as well as the gills and tail fin. Scale bar, 4 mm. (b) Monitoring of SMFCs (mCherry+) during limb regeneration (n = 6). mCherry was not detected in the regenerating part of the limb until ~30 days when the amputated limb had almost been recovered. Arrowheads: flexor muscle for digits. Scale bar, 1 mm. (c) Sections of regenerating limbs (n = 3 for each stage). SMFC-derived mCherry+ cells were not observed in the blastema. Lines: amputation site. m: muscle. Scale bar, 100  $\mu$ m. (d-f) Pax7 immunolabelling of regenerating limbs on day 12 (n = 3) and (g) day 15 (n = 3) after amputation. (d) On day 12, a few Pax7+ nuclei (arrowheads) were detected in blastema cells and in satellite cells along the muscle fibres. Col IV, collagen type IV immunoreactivity. DAPI (4, 6-diamidino-2-phenylindole), nuclei. Scale bar, 300  $\mu$ m. The Pax7+ nuclei pointed by arrowheads were enlarged in e and f, respectively. Scale bars, 100  $\mu$ m. (g) On day 15 when the regenerating part of the limb grew more distally, the number of Pax7+ nuclei (arrowheads) in the blastema was dramatically increased. Scale bar, 100  $\mu$ m. (h) Summary. In larval newts, MPCs, potentially satellite cells, were recruited for new muscle during limb regeneration, whereas SMFCs were not.

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)



SMFC tracking in metamorphosed newt limb regeneration. (a) Juvenile (16 months old). Scale bar, 15 mm. (b) Limb regeneration. Scale bar, 5 mm. (c-e) Tracking of SMFCs (mCherry+) (n = 2). This animal was a mosaic expressing EGFP in muscle only. mCherry+ fibres in the forearm were ~25% of total EGFP+ fibres. (c) On day 36 after amputation, fragments of muscle fibres (arrows) were observed in distal regions adjacent to the blastema. Scale bar, 100  $\mu$ m. (d) mCherry+ mononucleated cells (red arrowheads; enlarged in right-hand panels) and EGFP+ cells (green arrowheads) were observed in the blastema. Epi, epidermis. To-pro-3: nuclei. Scale bars, 50  $\mu$ m (left), 10  $\mu$ m (right-hand panels). (e) In the same limb, at day 96 after the second amputation in the upper arm (line), mCherry (arrows) and EGFP were observed only in muscle fibres. Scale bars, 1 mm (upper panel), 500  $\mu$ m (lower panels). (f-i) Pax7 immunolabelling of a regenerating limb on day 26 after amputation (n = 4). Pax7 immunoreactivity was not detected in the blastema. (f) Translucent image. Line: amputation site. (g) Merged fluorescence image. Col IV, collagen type IV immunoreactivity. To-pro-3: nuclei. Scale bar, 1 mm. (h) Enlargement of a region in the blastema and (i) a region proximal to the amputation site, enclosed by boxes in g. Scale bars, 250  $\mu$ m. Arrowheads in (i) Pax7+ nuclei. An example satellite cell (box) is enlarged in the right-hand panels (upper: Col VI/To-pro-3; lower: Col IV/Pax7). Scale bar, 50  $\mu$ m. (j) Summary. In metamorphosed newts, SMFCs were recruited for new muscle during limb regeneration, whereas MPCs such as satellite cells were not.

**Biorbyt Ltd.**

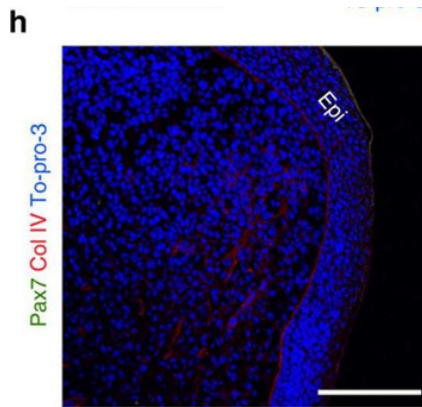
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)



SMFC tracking in metamorphosed newt limb regeneration. (a) Juvenile (16 months old). Scale bar, 15 mm. (b) Limb regeneration. Scale bar, 5 mm. (c-e) Tracking of SMFCs (mCherry+) (n = 2). This animal was a mosaic expressing EGFP in muscle only. mCherry+ fibres in the forearm were ~25% of total EGFP+ fibres. (c) On day 36 after amputation, fragments of muscle fibres (arrows) were observed in distal regions adjacent to the blastema. Scale bar, 100  $\mu$ m. (d) mCherry+ mononucleated cells (red arrowheads; enlarged in right-hand panels) and EGFP+ cells (green arrowheads) were observed in the blastema. Epi, epidermis. To-pro-3: nuclei. Scale bars, 50  $\mu$ m (left), 10  $\mu$ m (right-hand panels). (e) In the same limb, at day 96 after the second amputation in the upper arm (line), mCherry (arrows) and EGFP were observed only in muscle fibres. Scale bars, 1 mm (upper panel), 500  $\mu$ m (lower panels). (f-i) Pax7 immunolabelling of a regenerating limb on day 26 after amputation (n = 4). Pax7 immunoreactivity was not detected in the blastema. (f) Translucent image. Line: amputation site. (g) Merged fluorescence image. Col IV, collagen type IV immunoreactivity. To-pro-3: nuclei. Scale bar, 1 mm. (h) Enlargement of a region in the blastema and (i) a region proximal to the amputation site, enclosed by boxes in g. Scale bars, 250  $\mu$ m. Arrowheads in (i) Pax7+ nuclei. An example satellite cell (box) is enlarged in the right-hand panels (upper: Col VI/To-pro-3; lower: Col IV/Pax7). Scale bar, 50  $\mu$ m. (j) Summary. In metamorphosed newts, SMFCs were recruited for new muscle during limb regeneration, whereas MPCs such as satellite cells were not.

**Biorbyt Ltd.**

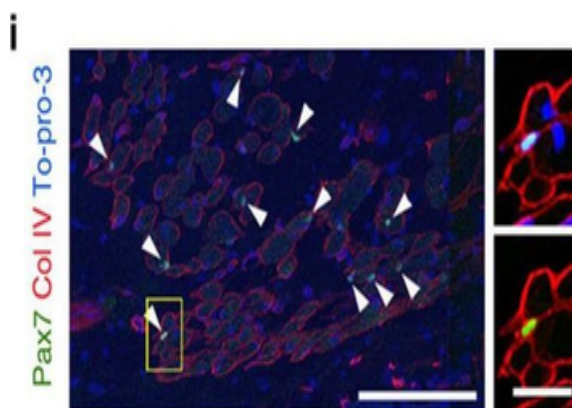
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558



SMFC tracking in metamorphosed newt limb regeneration. (a) Juvenile (16 months old). Scale bar, 15 mm. (b) Limb regeneration. Scale bar, 5 mm. (c-e) Tracking of SMFCs (mCherry+) (n = 2). This animal was a mosaic expressing EGFP in muscle only. mCherry+ fibres in the forearm were ~25% of total EGFP+ fibres. (c) On day 36 after amputation, fragments of muscle fibres (arrows) were observed in distal regions adjacent to the blastema. Scale bar, 100  $\mu$ m. (d) mCherry+ mononucleated cells (red arrowheads; enlarged in right-hand panels) and EGFP+ cells (green arrowheads) were observed in the blastema. Epi, epidermis. To-pro-3: nuclei. Scale bars, 50  $\mu$ m (left), 10  $\mu$ m (right-hand panels). (e) In the same limb, at day 96 after the second amputation in the upper arm (line), mCherry (arrows) and EGFP were observed only in muscle fibres. Scale bars, 1 mm (upper panel), 500  $\mu$ m (lower panels). (f-i) Pax7 immunolabelling of a regenerating limb on day 26 after amputation (n = 4). Pax7 immunoreactivity was not detected in the blastema. (f) Translucent image. Line: amputation site. (g) Merged fluorescence image. Col IV, collagen type IV immunoreactivity. To-pro-3: nuclei. Scale bar, 1 mm. (h) Enlargement of a region in the blastema and (i) a region proximal to the amputation site, enclosed by boxes in g. Scale bars, 250  $\mu$ m. Arrowheads in (i) Pax7+ nuclei. An example satellite cell (box) is enlarged in the right-hand panels (upper: Col VI/To-pro-3; lower: Col IV/Pax7). Scale bar, 50  $\mu$ m. (j) Summary. In metamorphosed newts, SMFCs were recruited for new muscle during limb regeneration, whereas MPCs such as satellite cells were not.

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558