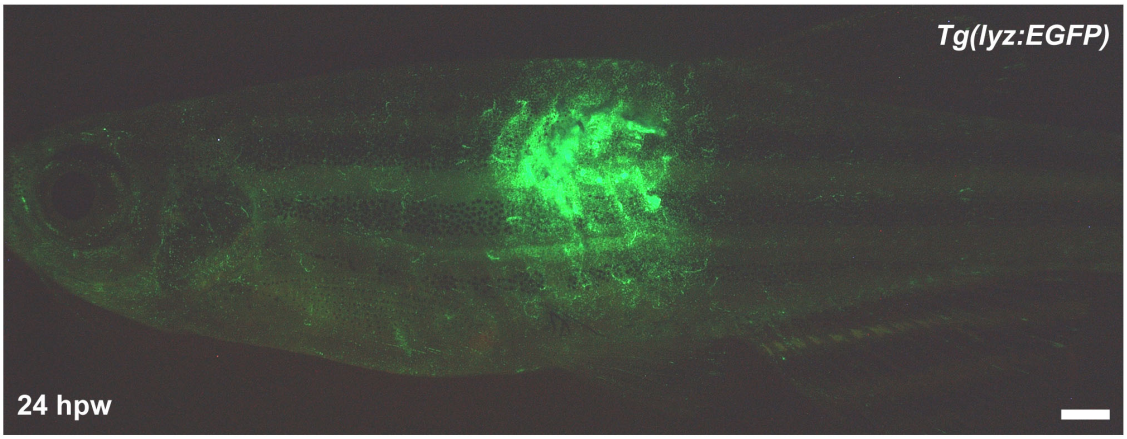
Supplementary Figure S1 - Structure of adult zebrafish skin

(a) Histological studies of adult zebrafish skin reveals overlapping scales wrapped by epidermis and separated from the underlying muscle (m) by a layer of subcutaneous adipocytes (adi). **(b)** At higher magnification PAS staining reveals a multi-layered epidermis wrapping the tip of a scale. The epidermis consists of specialized cells such as mucous cells (asterisks) embedded in keratinocytes, which are separated from the scale surface by a basement membrane (bm). **(c)** H&E staining demonstrates that the epidermis consists of approximately three layers of nucleated cells with an inner-most basal layer (bl in inset), one to two cell layers of intermediate cells (il in inset) and an outer layer (ol in inset) of flattened, superficial cells. **(d)** Immunofluorescent analysis of adult *Tg(krt4:GFP)gz7* transgenic fish reveals p63 expression (red) in the basal and intermediary keratinocytes and GFP expression (green) specifically in the outermost, superficial cells of the epidermis. Nuclei are stained with DAPI in blue. Arrowhead points to “hinge” region between two adjacent scales magnified in (f), arrowhead to dermal “tether” connecting the hinge region with inner-most dermal regions of the skin directly above the subcutaneous adipocytes. **(e, f)** Higher magnification shows the differential expression of p63 and GFP in the epidermis (e) and multiple p63-positive cells in the hinge region (f, arrowed in d,i). **(g,h)** *in situ* hybridization (g) and immunofluorescent analysis (h) reveals specific expression of *col1a1* / Col1 in dermal cells beneath the scale (g; inset with magnified view on tip of scale), at the border to the subcutaneous adipocytes (indicated with asterisks), and in the dermal tethers (indicated with arrowheads; arrows point to hinge regions). (i) Diagram summarizing the structure of the adult zebrafish skin. A key of the depicted structures is shown beneath the diagram.

Of note, dermal fibroblasts and epidermal cells display a differential distribution along the scales. Dermal fibroblasts are present at much higher density on the inner surface of the scales, while epidermis is confined to the distal regions of the scales, covering approximately three quarters of their outer surface, and one quarter of their inner surface.

Scale bars: a, b, c, f, inset in g and h = 50 μm, d and h = 200 μm, inset in c and e = 20 μm, g = 500 μm. Abbreviations: adi, adipocytes; bl, basal epidermal layer; bm, basement membrane; il, intermediary epidermal layer; m, muscle; ol, outer layer; sc, scale.

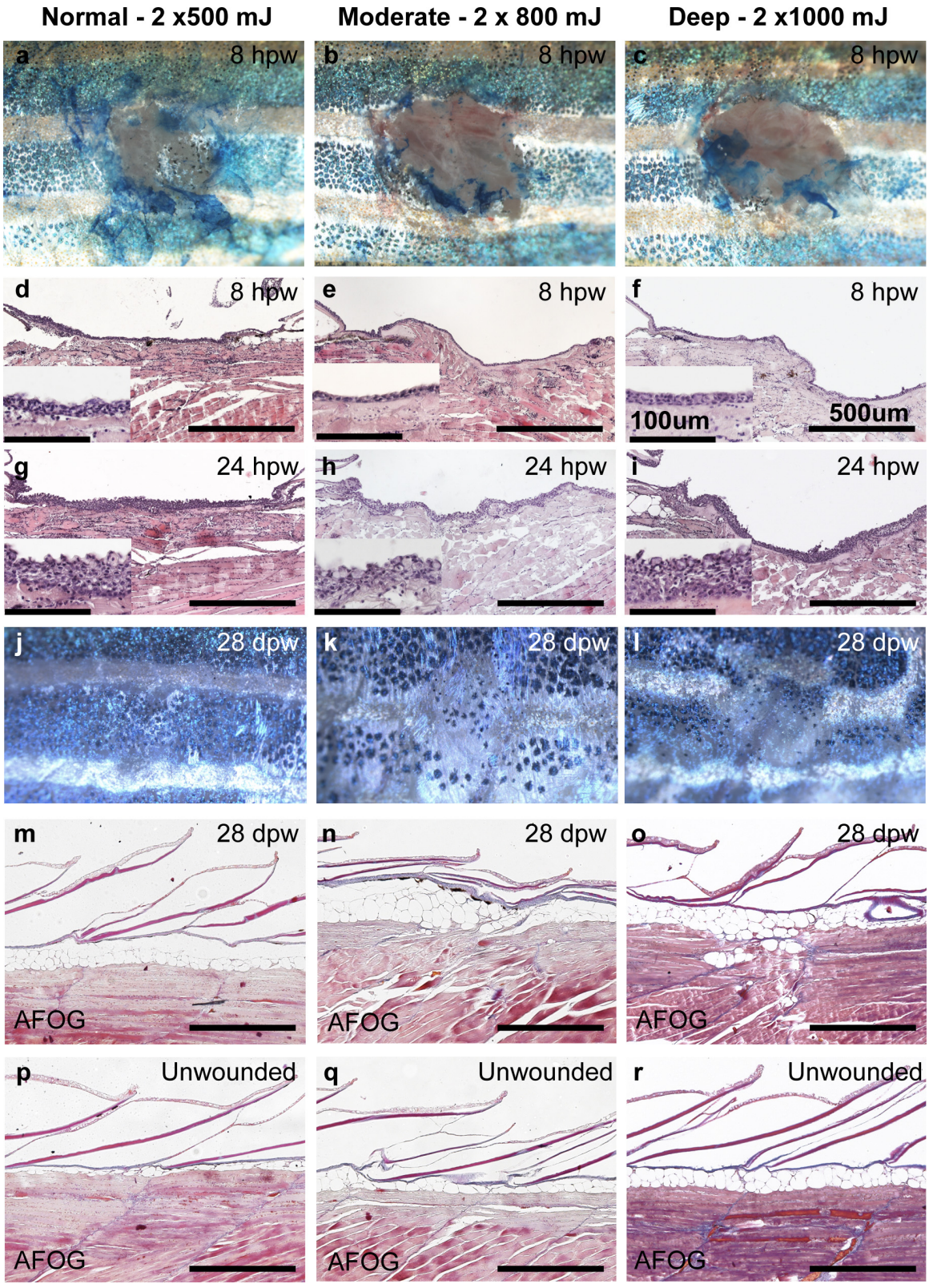
Supplementary Figure S2



Supplementary Figure S2 – Inflammation of skin wound

(**a**) Superficial view of an entire *Tg(lyz:GFP)* transgenic fish at 24 hpw demonstrates the extent of the inflammatory response to an introduced wound. Scale bar: 1mm.

Supplementary Figure S3



Supplementary Figure S3 - Wound depth does not affect re-epithelialization, re-stratification or the extent of scarring.

(**a-f**) Methylene blue (a, n=4/4; b, n=4/4; c, n=4/4) and histological analysis (d, n=3/3; e, n=3/3; f, n=3/3) at 8 hpw reveals a normal rate of re-epithelialization in all wound depths. Histological analysis at this time-point also reveals the extent of damage to the underlying muscle tissue in the deeper wounds (e and f). (**g-i**) Similar histological analysis at 24 hpw also demonstrates normal epidermal re-stratification in all cases (g, n=3/3; h, n=3/3; i, n=3/3). (**j-r**) At 28 dpw, whereas the site of wounding is difficult to distinguish following normal wounding (j; n=6/6), the wound site of deeper wounds is obvious (k, n=6/6; l, n=4/4). (**m-r**) Histological analysis with AFOG shows regeneration of epidermis, dermis, scales and adipocytes in all cases, however, regenerated scales from shallow wounds (m; n=3/3) appear normal (p-q), whereas scales regenerated from deeper wounds (n, n=3/3; o, n=3/3) appear malformed. Regeneration of the muscle tissue also seems to be impaired in deeper wounds (n,o). Scale bars: d-i, m-r = 500 μm, inset in d-i = 100 μm.