Nesterenko et al.:

Strategy to Replace Animal-Derived ECM by a Modular and Highly Defined Matrix

Supplementary Data

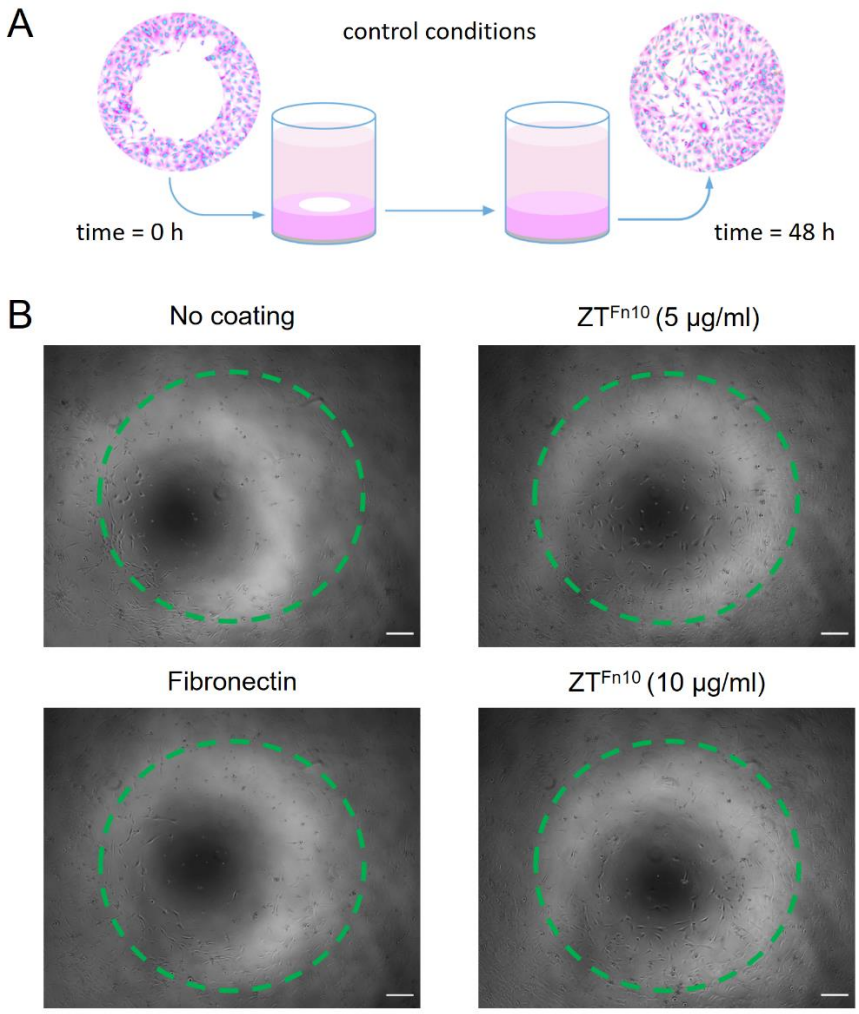

Fig. S1: Time lapse imaging of neural crest cell migration in the cMINC assay setup after 24 h
(A) Overview diagram to visualize processes and procedures of the MINC assay. To the left, the situation is shown for the bottom of a cell culture well directly after removal of the silicone stoppers. To the right, an example is given that illustrates to which extent cells populate the initially empty migration area within 48 h. The cylinders represent an entire cell culture well, with cells (dark magenta) forming initially a ring around the migration area. Light pink shading is meant to show the medium above the cells. (B) Exemplary single field images of NCC during time-lapse recording. The approximate location of the removed stopper is indicated by a green circle. The images are inserted as placeholders for video recordings of the last 24 h of migration. These time-lapse movies carry the real information, and are provided as movies (MS1-MS4[1]). (Scale bars: 100 µm).

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[1] doi:10.14573/altex.2003181s2; doi:10.14573/altex.2003181s3; doi:10.14573/altex.2003181s4; doi:10.14573/altex.2003181s5

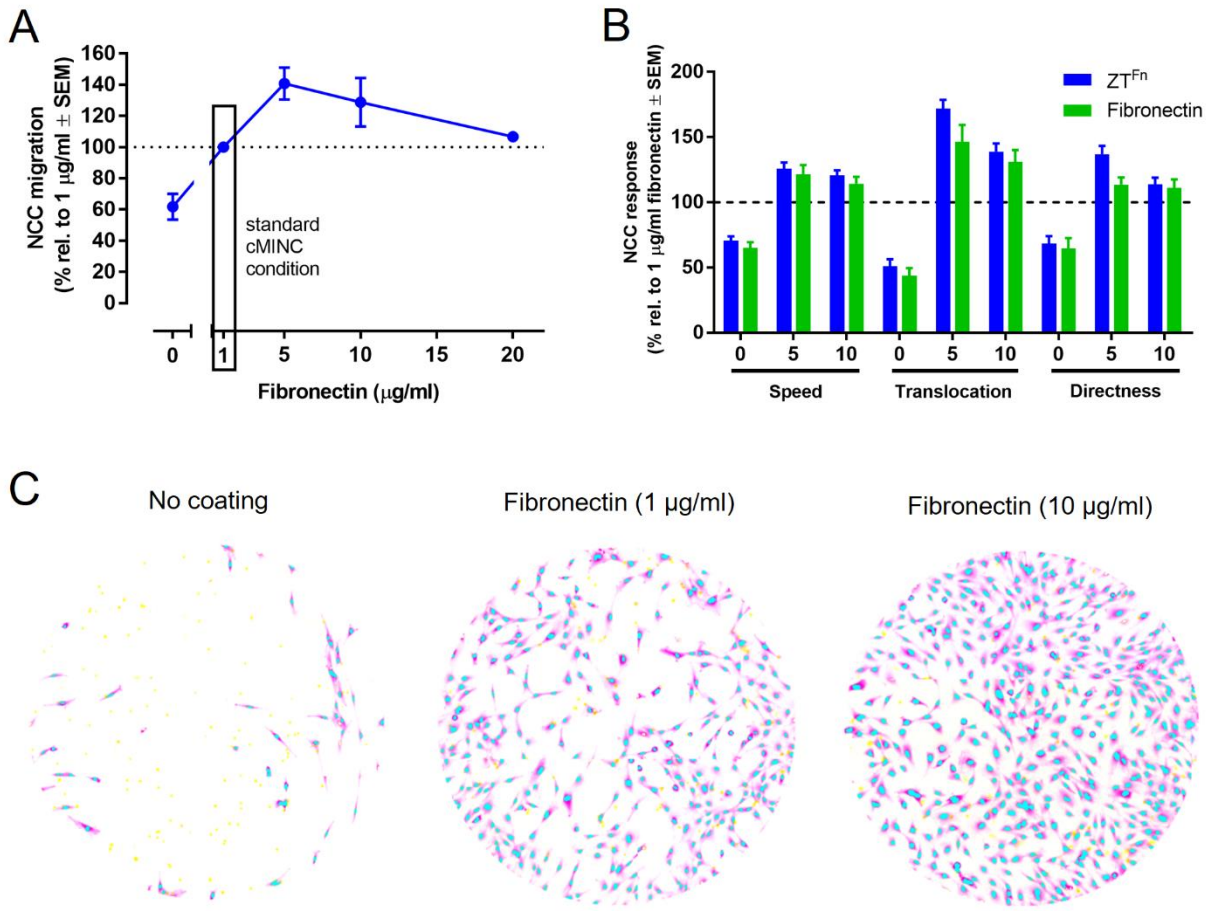

Fig. S2: Dependence of NCC migration on fibronectin concentrations in the standard cMINC
(A) Neural crest cell migration at 72 h after seeding on dishes coated with different fibronectin concentrations. Data are shown as means from three cell preparations. (B) Speed (distance along the track per time), cell translocation (Euclidean distance from start to endpoint of track) and directness (cell translocation/track length; i.e. "low degree of zig-zagging"), calculated from the trajectory of 20 cells from two cell preparations for ZT^{Fn} and for fibronectin from 20 cells of one cell preparation. (C) Exemplary fluorescence images of NCCs stained with H-33342 (nuclei, cyan) and calcein (cytosol, magenta) at the end of migration experiments on diverse fibronectin concentrations or no coating (as indicated). The diameter of each of the circular areas displayed was 2 mm.

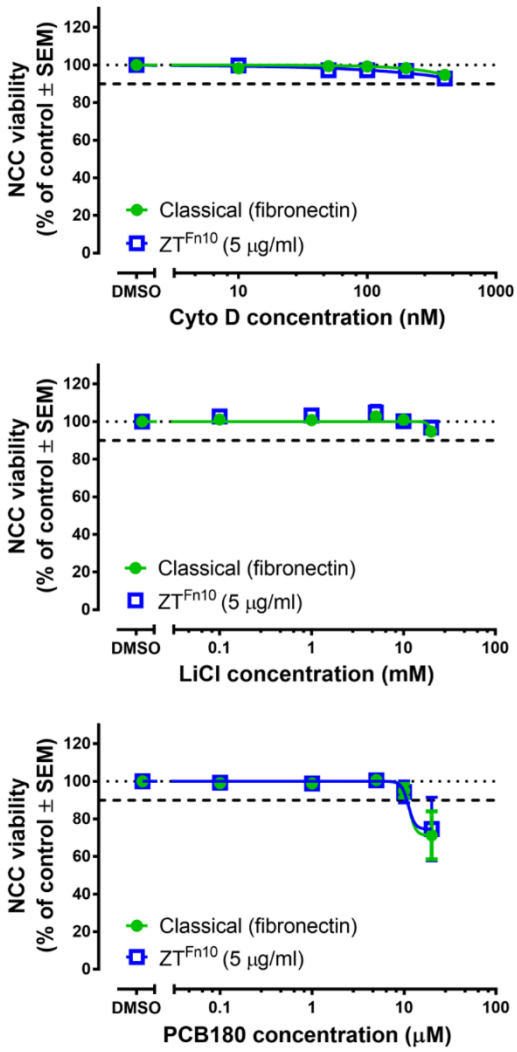

Fig. S3: NCC viability under toxicant exposure
NCC cells were plated under otherwise similar conditions on dishes coated with fibronectin or ZT^{Fn10}. They were exposed to cytochalasin D (CytoD), lithium chloride (LiCl) and polychlorinated biphenyl 180 (PCB180) for 24 h. Initial values (100%) correspond to untreated cells (control cell medium containing 0.1% DMSO as `negative control´). Cell viability data are all given relative to negative control values. Each data point shown is the mean ±SEM of three biological replicates (= different cell preparations). Dashed lines at 90% visualize the threshold value for impaired cell viability.