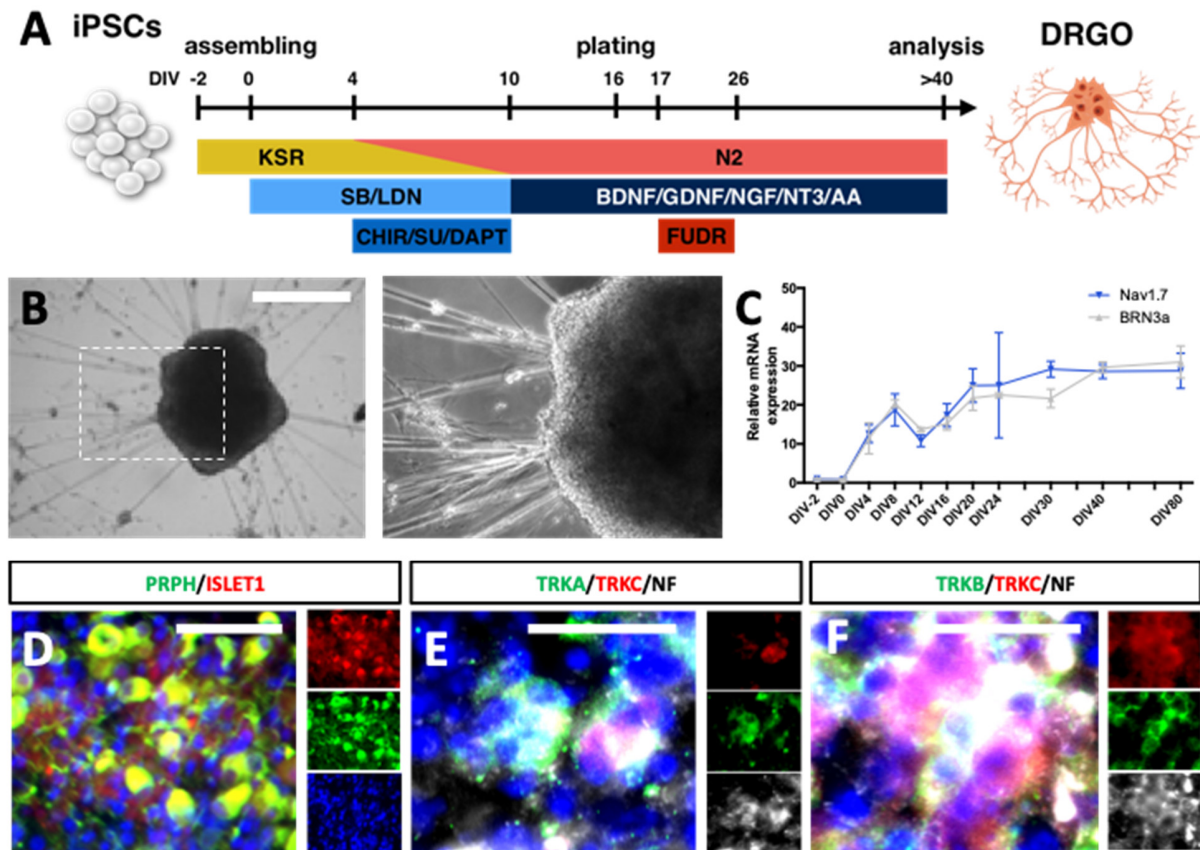
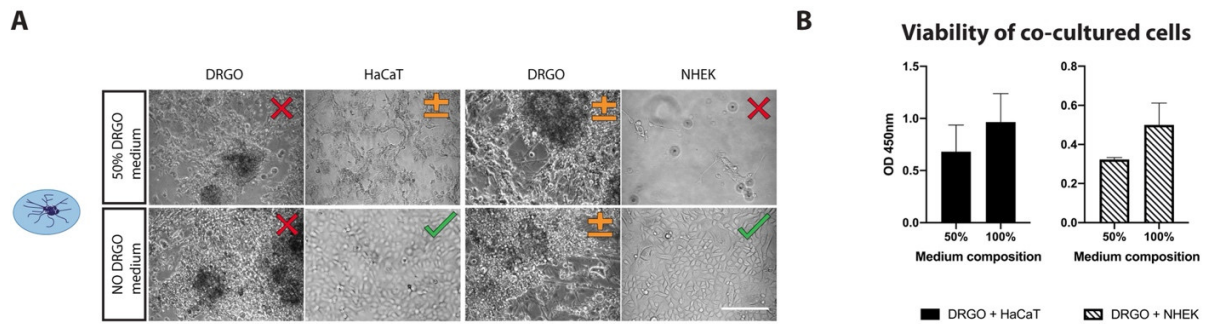


## SUPPLEMENTARY MATERIALS




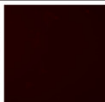


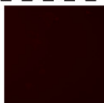





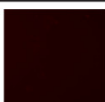
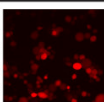
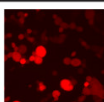
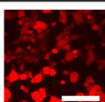


**Figure S1. iPSC-derived organoid resembling primary dorsal root ganglia (DRGOs).** (A) Illustration of the 3D culture system and sequential exposure to small molecules over time to obtain DRGOs. (B) Images of DRGO at DIV 40 with star-like web of neurites projections around the central mass and magnification. Scale bar, 500 $\mu$ m. (C) Quantitative analysis of BRN3A and NAV1.7 transcript levels during DRGO differentiation. Expression levels are normalized to actin. Mean  $\pm$  SD,  $n = 3$  independent experiments, 8-12 organoids/line/experiment. (D) Immunocytochemistry in DRGOs at DIV 40 for proteins localized along neuronal projections

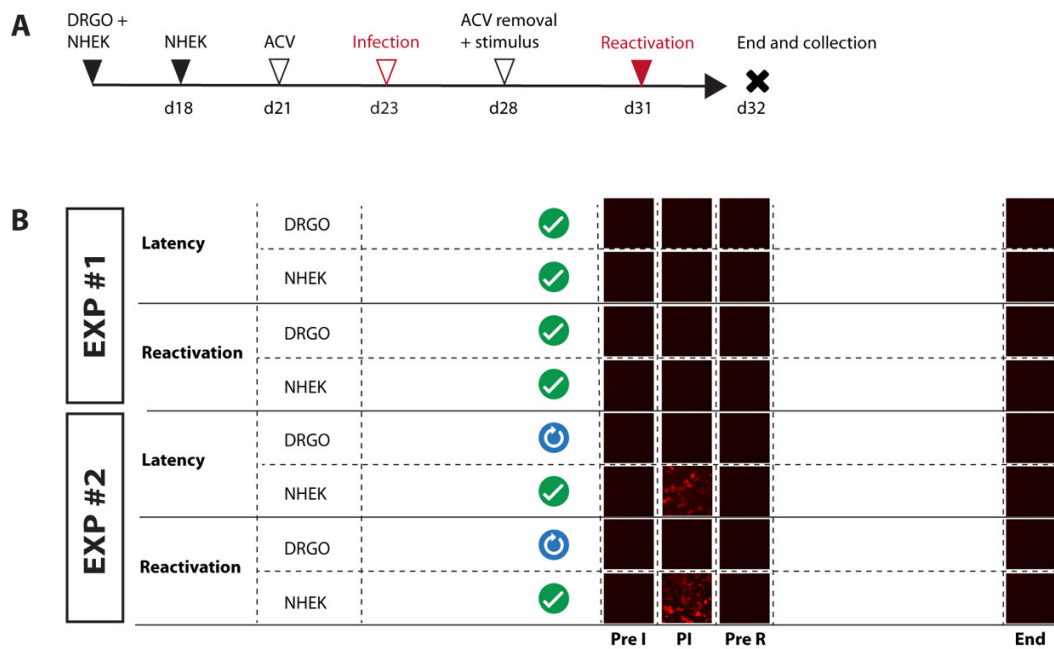
peripherin, PRPH and the sensory neuron-specific transcription factors ISL1. Scale bar, 50 $\mu$ m. (E) Immunocytochemistry on DIV 40 DRGOs of Neurotrophic receptor tyrosine kinases (NTRK) 1/2/3 combination distinguishing the nociceptive (TRKA+/TRKC-, red arrowheads). Scale bar, 10 $\mu$ m. (F) Immunocytochemistry to discriminate mechanoreceptive (TRKB+/TRKC+, yellow arrowheads) and proprioceptive (TRKB-/TRKC+, red arrowheads) neuronal subtypes. Scale bar, 10  $\mu$ m.



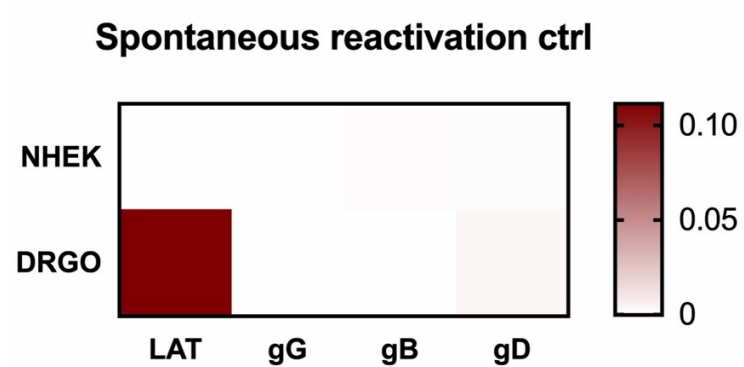
**Figure S2. Unpattern co-culture of organoids and keratinocytes.** (A) Bright-field microscopy images (20x magnification) of direct co-cultures of organoids (DRGOs) and both immortalized (HaCaT) and primary (NHEK) keratinocytes. Scale bar, 100  $\mu$ m. (B) Cell proliferation assay to test DRGOs, HaCaT and NHEK viability. Mean + SD. “X” indicates that culture conditions are detrimental for cell viability and morphology, “ $\pm$ ” indicates that viability of cells is around 50%, “ $\checkmark$ ” indicates that media allow to retain good cell morphology and viability

	ACV treatment	Pre I	PI	1h PI	24h PI
<b>Uninfected</b>	None				
	Pre + post infection				
	Post infection				
<b>Infected</b>	None				

**Figure S3. Microfluidics chip validation.** Chip supernatants collected at different time points tested on sensor cells for the presence of infective virus particles due to passive diffusion (lytic infection = red fluorescent signal). (Pre I = before infection; PI = post-infection; 1h PI = 1 h post-infection; 24h PI = 24 h post-infection). Scale bar, 500  $\mu\text{m}$ .



**Figure S4. Reactivation protocol in microfluidics chip.** (A) Schematic representation of latency establishment and reactivation protocol used in chip EXP #1 and #2. (B) Supernatants from different chambers and time points were tested on sensor cells as well (lytic infection = red fluorescent signal). Green tick indicates ACV addition to culture medium immediately after virus adsorption, blue arrow is for ACV delayed addition. (Pre I = 1 h before infection; PI = 1 h post-infection; Pre R = before reactivation, 5 DPI; End = end of the experiment, 8 DPI). Scale bar, 500  $\mu$ m, n = 6 independent experiments for each tested condition.



**Figure S5. Control of spontaneous reactivation.** Heatmap showing virus gene expression analysis of NHEK and DRGOs during latency without ACV addition to culture medium (dark red= high expression; white = no expression).