

1 **Title page**

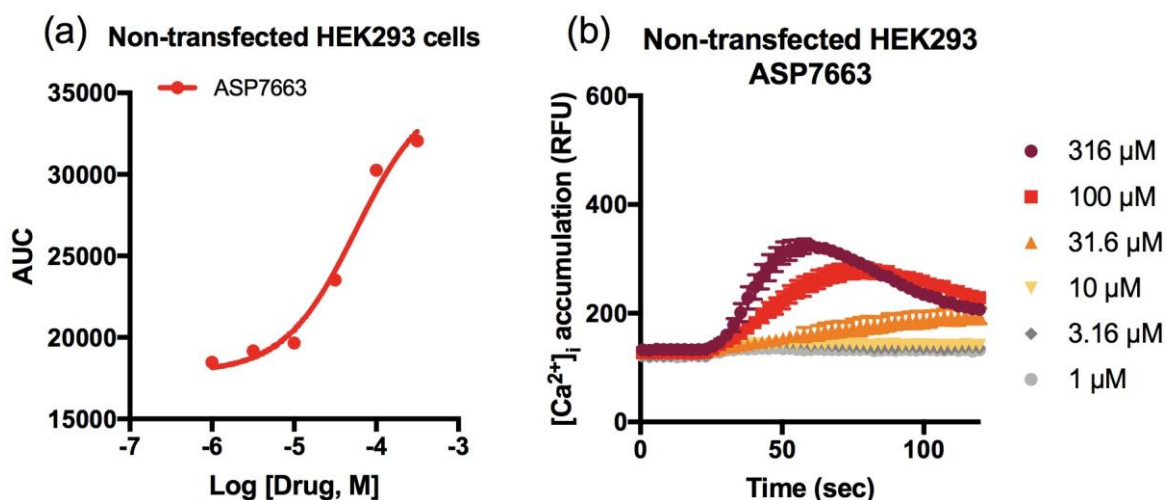
2 **Title:** A critical evaluation of TRPA1-mediated locomotor behavior in zebrafish as a
 3 screening tool for novel anti-nociceptive drug discovery

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 6 Yuk Fai Leung^{2,3,4,5,6}, Richard M. van Rijn^{1,3,4,5}

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Supplementary information

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11 **Supplemental Figure 1. Potential off-target effect of ASP7663 in non-transfected**

12 **HEK293 cells (a)** Dose-response curve of ASP7663 in non-transfected HEK293 cells

13 (ASP7663: $pEC_{50} = 4.27 \pm 0.03$, $n=3$). Relative Fluorescent Unit (RFU) of ASP7663 dose

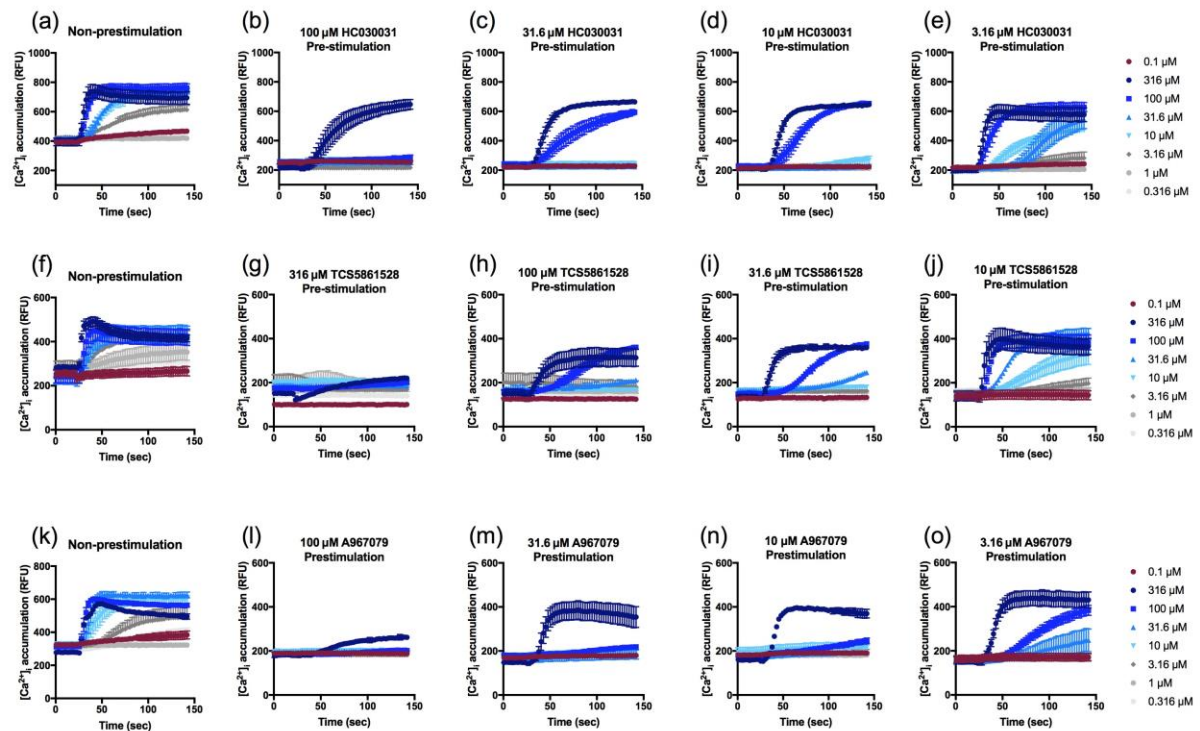
14 response calcium influx in non-transfected HEK293 (b). (a) was measured by area under the

15 curve (AUC) of the RFU.

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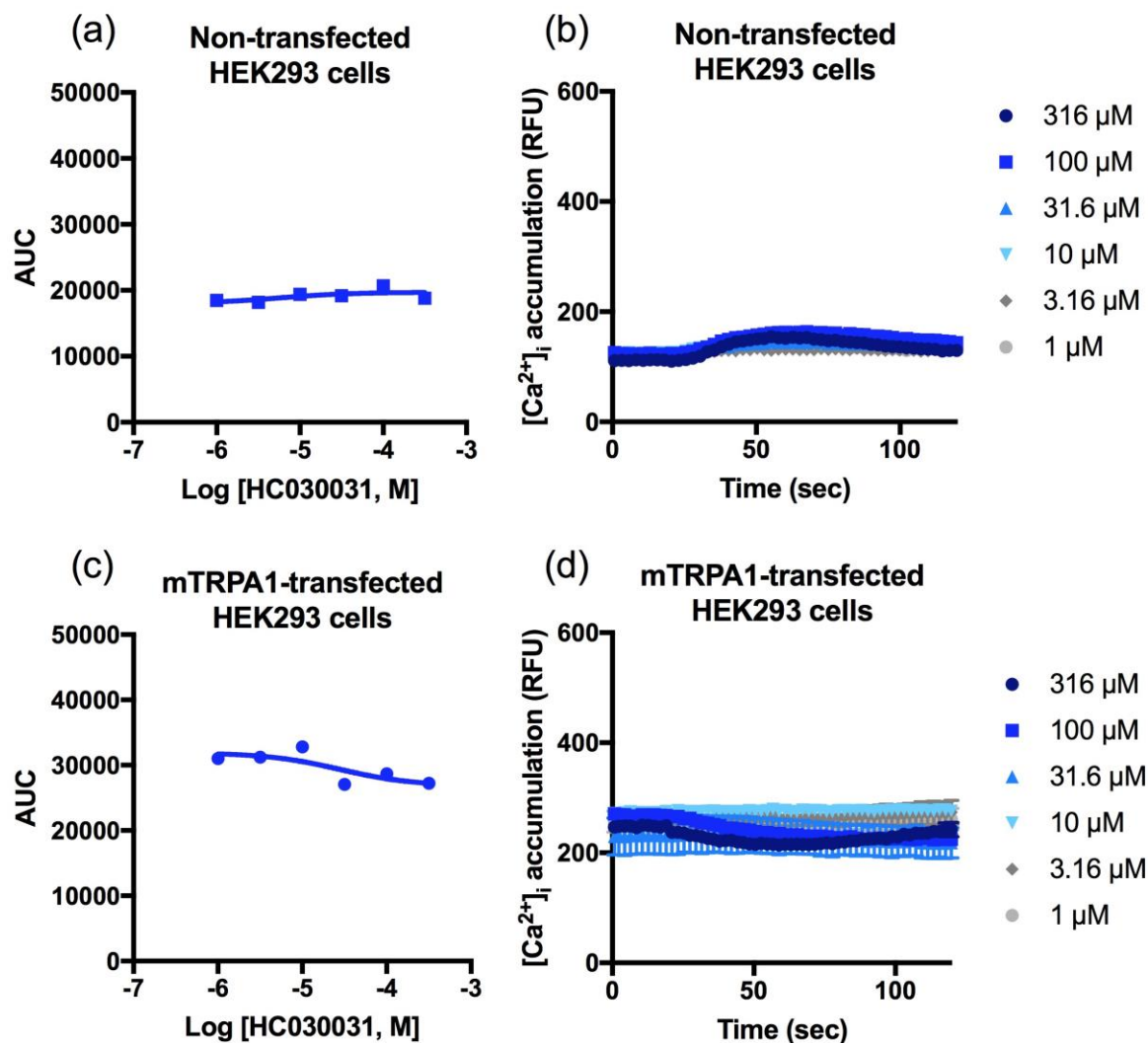
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2 **Supplemental Figure 2. Calcium influx to TRPA1 antagonists in HEK293 cells**3 **transiently expressing mTRPA1. (a, f, k) Intracellular calcium levels of HEK293 cells**4 **transiently expressing mTRPA1 to ASP7663 with half-log dilutions. (b-e) ASP7663 dose-**5 **response calcium influx to HC-030031 with half-log dilutions. (g-j) ASP7663 dose-response**6 **calcium influx to TCS-5861528 with half-log dilutions. ASP7663 dose-response calcium**7 **influx to A-967079 with half-log dilutions. The AUC of the RFU was further analyzed in**8 **Figure 2.**

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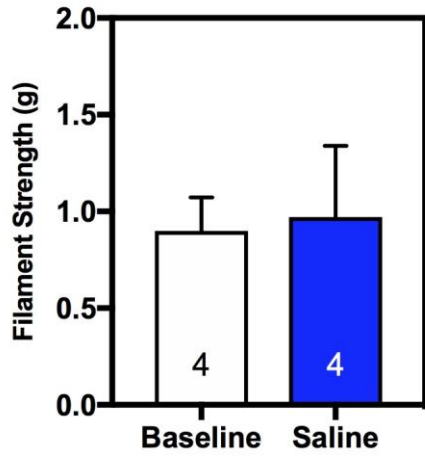


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2 **Supplemental Figure 3. Calcium influx to HC-030031 in non-transfected or mTRPA1**3 **transfected HEK293 cells. Dose response intracellular calcium levels of HC030031 in non-**4 **transfected HEK293 cells (a) and mTRPA1-transfected HEK293 cells (c). Relative**5 **Fluorescent Unit (RFU) of HC030031 dose response calcium influx in non-transfected**6 **HEK293 (b, d). (a, c) were measured by area under the curve (AUC) of the RFU. (mTRPA1:**7 **pIC₅₀ = 5.77 ± 0.21, n=4).**

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2 **Supplemental Figure 4. Mechanical hypersensitivity was not changed by saline injection**

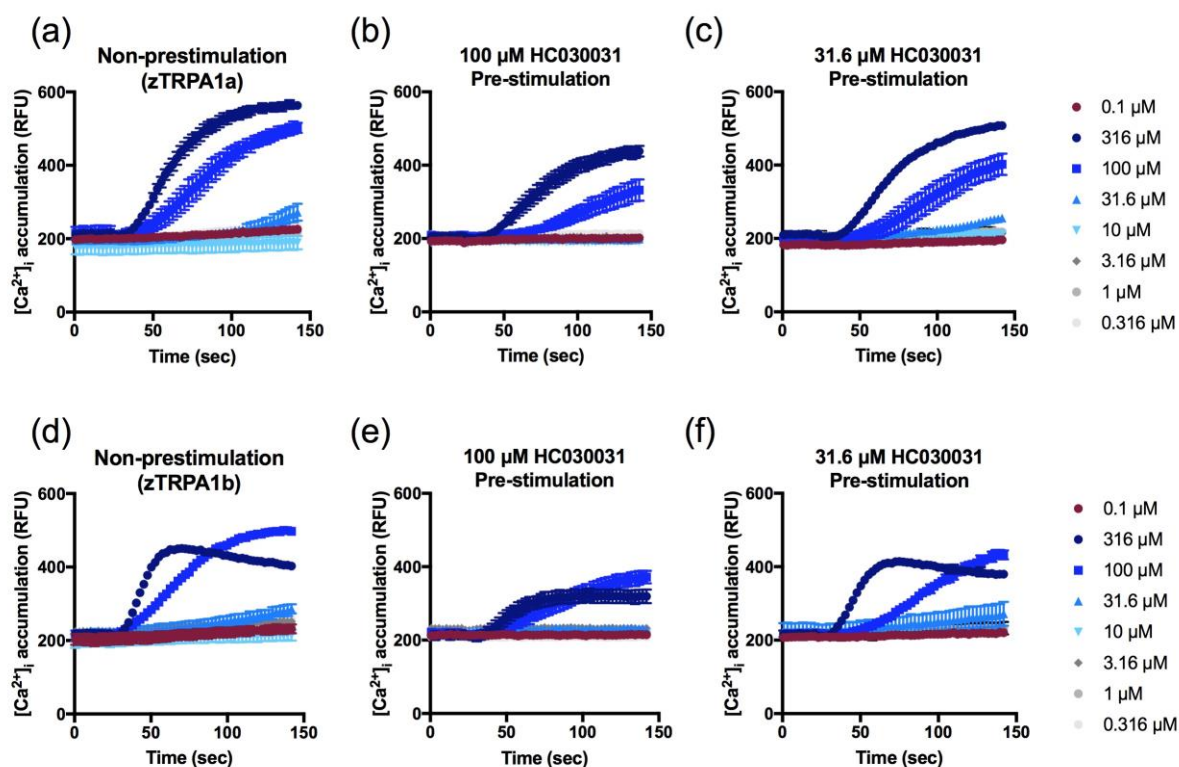
3 **in C57BL/6 mice.** Mechanical sensitivity was measured in C57BL/6 mice pre- and post-

4 saline administration (n=4 per treatment) in response to von Frey filament stimulation.

5 (Unpaired t-test, $p=0.8692$).

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2 **Supplemental Figure 5. Calcium influx to HC-030031 in HEK293 cells transiently**3 **expressing zTRPA1a and zTRPA1b. (a, b)** Intracellular calcium levels of HEK293 cells

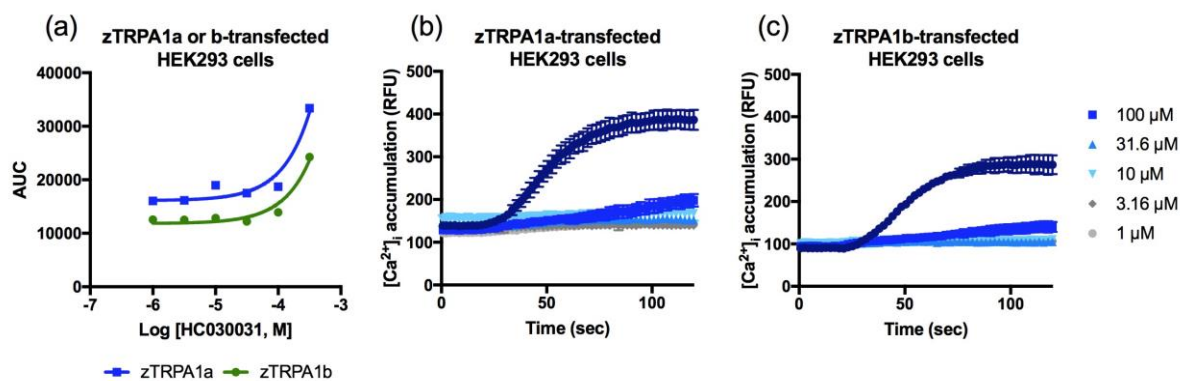
4 transiently expressing zTRPA1a and zTRPA1b respectively to ASP7663 with half-log

5 dilutions. **(b,c)** ASP7663 dose-response calcium influx in zTRPA1a to HC-030031 with a6 full-log dilution. **(e,f)** ASP7663 dose-response calcium influx in zTRPA1b to HC-030031

7 with a full-log dilution. The AUC of the RFU was further analyzed in Figure 4.

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2 **Supplemental Figure 6. HC030031 works as weak agonist at high concentration. (a)**

3 Dose-response curve of HC030031 in zTRPA1a-transfected, and zTRPA1b-transfected

4 (Green) HEK293 cells (zTRPA1a: $pEC_{50} = 2.85 \pm 0.19$, $n=4$; zTRPA1b: Non-determinant,5 $n=3$). Relative Fluorescent Unit (RFU) of HC030031 dose response calcium influx in

6 zTRPA1a-transfected (b), and zTRPA1b-transfected (c) HEK293 cells. (a) was measured by

7 area under the curve (AUC) of the RFU.

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