**A high-content screen reveals new small-molecule enhancers of Ras/Mapk signaling as probes for zebrafish heart development.**

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**Supplementary Data Legends:**

**Supplementary Data 1: PAINS analysis of primary confirmed hits.** Fifty visually confirmed primary screen hits were analyzed for predicted pan assay interference compounds (PAINS) by publicly available analysis engines (ZINC15, www.zinc15.docking.org); FAFDrugs4 (Free ADME-Tox Filtering Tool, http://fafdrugs3.mti.univ-paris-diderot.fr/); False Positive Remover (www.cbligand.org); accessed May 2018). Compounds were grouped by structural similarity. The last

column (cluster coverage) denotes the number of commercially available compounds that were repurchased from each cluster.

**Supplementary Data 2. Promiscuity analysis of primary confirmed hits.** Fifty visually confirmed primary screen hits were analyzed for predicted promiscuity using the Bioactivity data associative promiscuity pattern learning engine (Badapple); http://pasilla.health.unm.edu/tomcat/badapple/badapple; accessed June 2018). The scoring scheme is as follows: green, pScore 0-100, no indication of promiscuity; yellow, 100-300; weak indication of promiscuity; red, >300, strong indication of promiscuity; no color, unknown; no data. For each molecule, the highest scoring scaffold determines overall score.

**Table headings** are: mon_smi, compound structures; scaf_smi, scaffold structures; pScore, Badapple assigned promiscuity estimate (see scoring scheme above); Substances Tested, number of compounds containing scaffold substructure; Samples Tested, number of times substructure was tested; Assays Active, number of assays in which substructure was active; Assays Tested, number of assays substructure was tested; Percent Assays Active, calculated ratio of Assays Active/Assays Tested *100.

**Supplementary Data 3. LC-HRMS data for ST020101, ST011282, and ST00694.**

**Supplementary Data 4. Structure/identity confirmation and LC-HRMS of ST006994 analogs from the UPCMLD library.** For technical details please refer to the Materials and Methods Section.