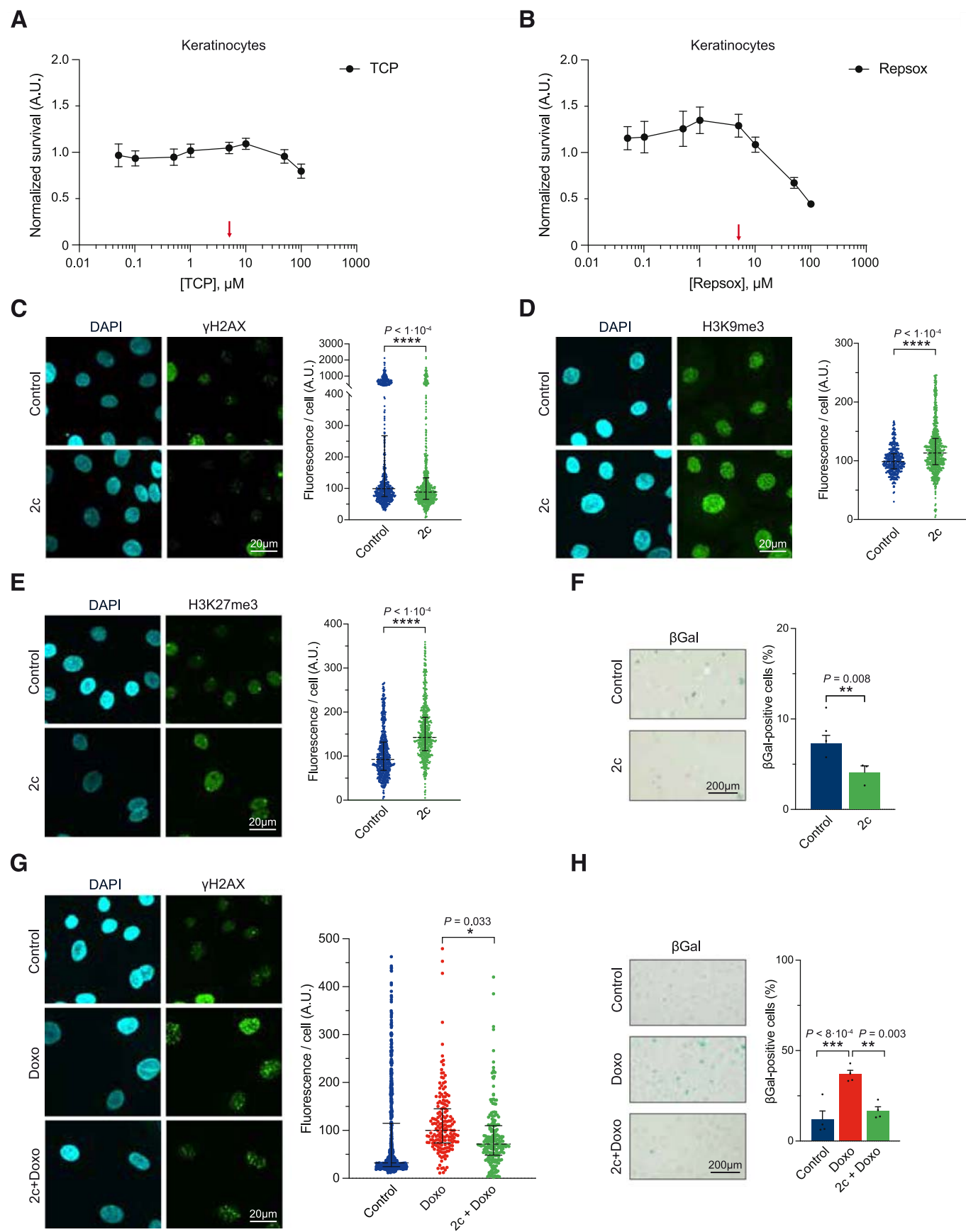
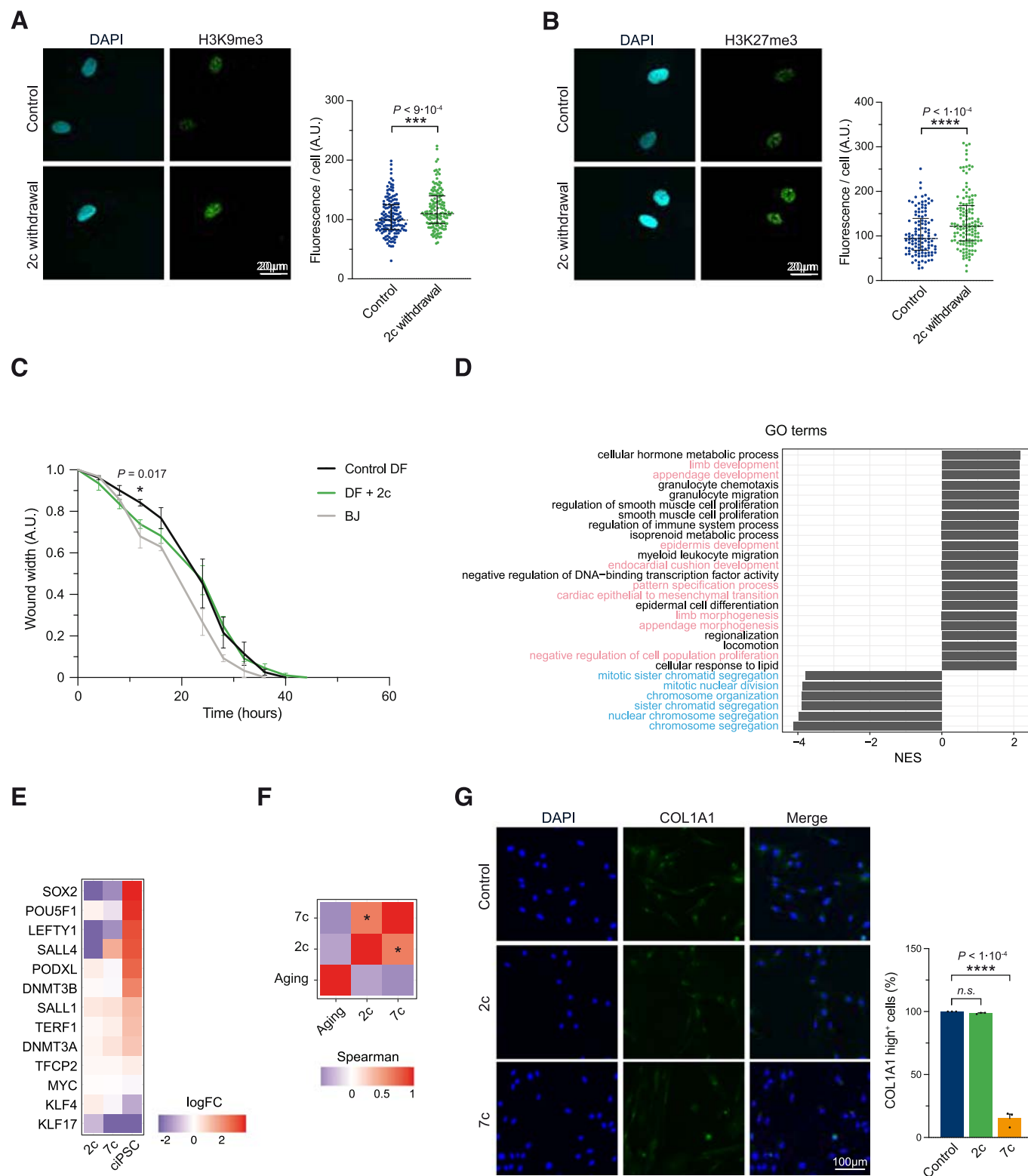


## Expanded View Figures

**Figure EV1. Optimized cocktail (2c) multiparameter rejuvenation of aging hallmarks are recapitulated in human keratinocytes.**

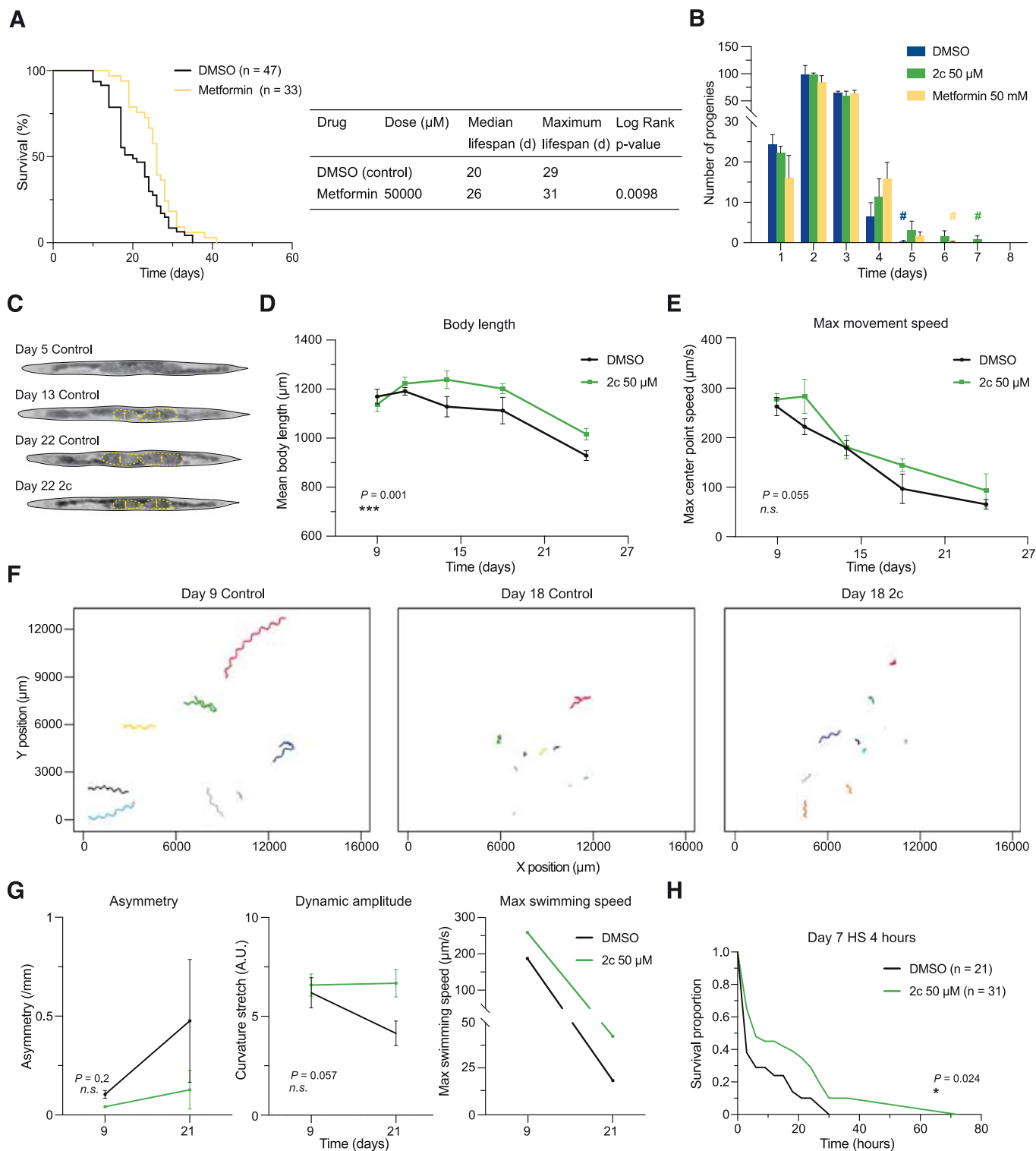
(A, B) MTS quantification of cell density following treatment of human epidermal keratinocyte with TCP (A) and Repsox (B). Red arrows indicate selected concentrations. (C) Immunofluorescence and quantification of  $\gamma$ H2AX following 2c treatment in keratinocytes (6 days, "6D"). (D, E) Immunofluorescence and quantification of H3K9me3 (D) and H3K27me3 (E) following 2c treatment (6D) in keratinocytes. (F) SA-beta-gal staining and quantification of senescence following 2c treatment (6D) in keratinocytes. (G, H) Immunofluorescence and quantification of  $\gamma$ H2AX (G) senescence-associated beta-galactosidase (SA-beta-gal) (H) following doxorubicin (100 nM) treatment in 2c pretreated keratinocytes (6D). Data were mean  $\pm$  SEM (A, B, F, H), median  $\pm$  IQR (C, E, G). (A–H)  $n \geq 3$ . Statistical significance was assessed by comparison to untreated control using paired two-tailed *t*-test (C–F), one-way ANOVA and Dunnett correction (G, H).





◀ **Figure EV2. Reduced 2c cocktail shows long-lasting effects upon treatment and no dedifferentiation.**

(A, B) Immunofluorescence and quantification of H3K9me3 (A) and H3K27me3 (B) upon 2c treatment for 6 days (6 days, “6D”) followed by 30 days chemical withdrawal. (C) Scratch assay analysis upon 2c treatment in adult dermal fibroblasts (DF, 6D) alongside a neonatal control (BJ). (D) Gene ontology (GO) terms overlap between 2c and 7c treatments, with developmental (in pink) and cell cycle (in blue) pathways highlighted. (E) Heatmap showing the expression pattern of differentially expressed genes associated with pluripotency following 2c and 7c treatment, and induction of chemical iPSC (ciPSC). (F) Heatmap of Spearman’s correlation of gene expression associated with aging, 2c and 7c. (G) Fluorescence detection and quantification of fibroblast identity marker COL1A1 following 2c and 7c treatments. Data were median  $\pm$  IQR (A, B), mean  $\pm$  SEM (C, E). (A–G)  $n \geq 3$ . Statistical significance was assessed by comparison to untreated control using paired two-tailed *t*-test (A, B), two-way ANOVA mixed-effects analysis and Geisser-Greenhouse correction (C), Spearman’s correlation test (F), one-way ANOVA and Dunnett correction (G). NES normalized enrichment scores, FC fold change.



◀ **Figure EV3. Treatment with 2c improves multiple healthspan parameters in *C. elegans*.**

(A) Survival of N2 *C. elegans* upon treatment with Metformin (50 mM). (B) Progeny production and reproductive span (denoted by #) of unmated hermaphrodite *C. elegans* upon treatment with 2c and Metformin. (C) Germline tumoral mass (denoted by t) development at young (day 5), middle (day 13), and old age (day 22) upon 2c treatment in linearized nematodes. (D) Mean body length of N2 *C. elegans* upon treatment with 2c at 50  $\mu$ M. (E) Maximum movement speed of N2 *C. elegans* upon treatment with 2c at 50  $\mu$ M. (F) 30-second movement map of N2 *C. elegans* at day 9 and day 18 upon treatment with 2c. (G) Mean swimming asymmetry, amplitude, and max speed of N2 *C. elegans* upon treatment with 2c at 50  $\mu$ M. (H) Thermotolerance of N2 *C. elegans* to 4 h HS at 7 days of adulthood upon 2c treatment. Data were mean  $\pm$  SEM (B, D, E, G). (B, D, E)  $n \geq 10$ , (G)  $n \geq 4$ . Number of animals and measures are detailed in Methods. Statistical significance was assessed by comparison to vehicle control using log-rank (Mantel-Cox) test (A), by comparison to vehicle control using two-way ANOVA mixed-effects analysis and Geisser-Greenhouse correction (D, E), paired two-tailed *t*-test for day 21 of adulthood (G), log-rank (Mantel-Cox) test (H). HS heat shock.