Supplementary Material

Systematic estimation of biological age of *in vitro* cell culture systems by an age-associated marker panel

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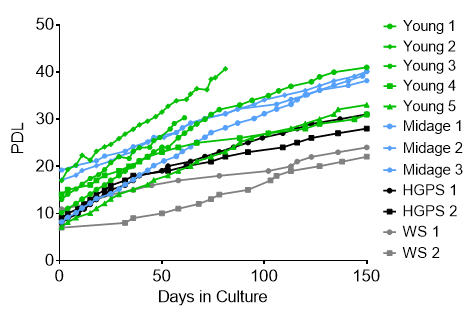
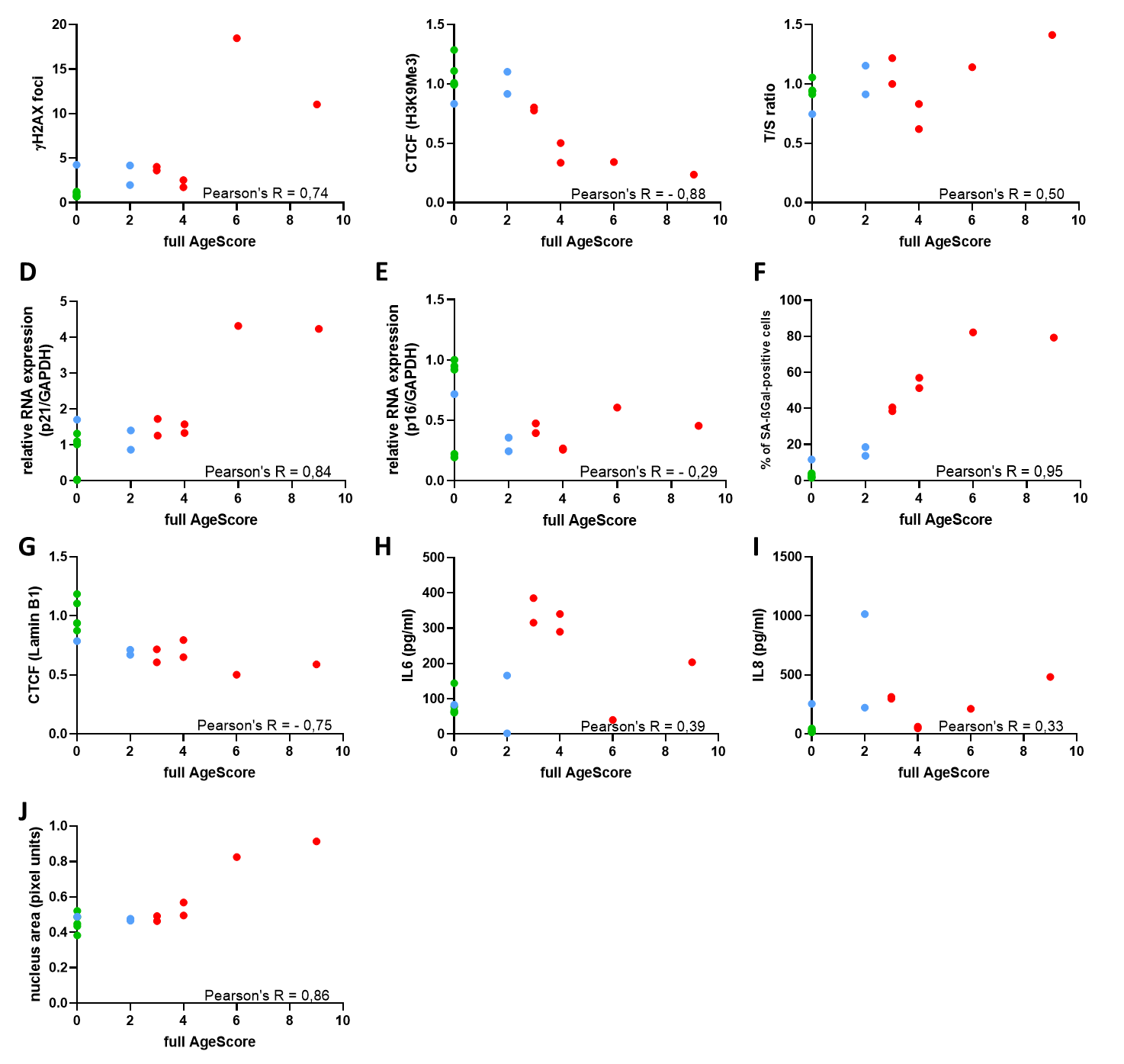
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# Supplementary Figures and Tables

## Supplementary Figures

**Supplementary Figure 1: Pearson’s Correlation of AgeScore and used age markers.** **(A - J)** Calculation of Pearson’s Correlation of each used age marker included in the here proposed AgeScore. The results display a very high correlation for the markers H3K9Me3 (B), p21 (D), nucleus size (J) and SA-ß-Gal and a high correlation for Lamin B1 (G) and H2AX (A). However, there is a moderate correlation for the telomere length (C) and a low correlation for p16 (E), IL6 (H), and IL8 (I).



**Supplementary Figure 2: Growth curve of Progeria Syndrome fibroblasts.** The replicative potential of human fibroblasts used for examining the AgeScore. Cells were cultured under stable conditions (37°C, 5% CO2) in DMEM medium (with 15% FBS, 1% Pen-Strep). PDL was observed over 150 days. Cells from donors of HGPS patients displayed no changes in PDL, whereas WS patient’s fibroblasts showed slower growth compared with the mean of age-matched controls.

## Supplementary Tables

**Table 1: Age markers and their detection methods**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Hallmark Category | Aging Hallmark | Example of age marker | Methods | Publications |
| Primary Hallmarks (causes of damage) | Genomic instability | Decrease of Lamin B1 expression | WB,  IF | (A. S. Wang et al. 2017), (Dreesen, Ong, et al. 2013; Dreesen, Chojnowski, et al. 2013),(Freund et al. 2012),(Shimi et al. 2011) |
| DNA damage accumulation | WB, IF | (Schumacher et al. 2021),(Mah, El-Osta, and Karagiannis 2010),(Sedelnikova et al. 2008),(Sedelnikova et al. 2004)(Redon et al. 2002) |
| Telomere Attrition | Telomere length | qPCR, TRF, TCA | (Liu et al. 2019);(Armanios et al. 2009);(Bodnar et al. 2016);(Allsopp et al. 1992) |
| Epigenetic Alterations | Histone Modification | WB, IF | (Zhang et al. 2016);(McCauley and Dang 2014);(Greer et al. 2010);(Siebold et al. 2010); |
| DNA Methylation | WB, IF | (Salameh, Bejaoui, and El Hajj 2020);(Sturm et al. 2019);(Horvath 2013);(Osorio et al. 2010);(Shumaker et al. 2006) |
| Transcriptional alteration | RNA-Sequencing | (Aramillo Irizar et al. 2018);(Harries et al. 2011);(Bahar et al. 2006)(Marthandan et al. 2016) |
| Loss of Proteostasis | Expression of heat shock proteins | WB, IF | (Koga, Kaushik, and Cuervo 2011);(Terry et al. 2006);(Wilhelmus et al. 2006);(Marini et al. 2004);(Cavinato et al. 2017) |
| Antagonistic Hallmarks (Responses to damage) | Deregulated Nutrient Sensing | Expression of proteins of AKT/mTOR/FOXO-pathways | WB, IF | (Barzilai et al. 2012); (Kenyon 2010) |
| Mitochondrial Dysfunction | ROS production | SOD/Catalase activity assay | (Damiani et al. 2018);(Green, Galluzzi, and Kroemer 2011);(Hekimi, Lapointe, and Wen 2011);(Mesquita et al. 2010);(Doonan et al. 2008) |
| Cellular Senescence | SA-β-Galactosidase | SA-β-Gal assay | (Kuilman et al. 2010);(C. Wang et al. 2009);(Dimri et al. 1995) |
| SASP | qPCR,  ELISA | (Basisty et al. 2020);(Acosta et al. 2013);(Coppé et al. 2008);(Collado, Blasco, and Serrano 2007); |
| cell cycle arrest | qPCR,  WB | (Ressler et al. 2006); (Krishnamurthy et al. 2004);(Serrano et al. 1997);(Hayflick and Moorhead 1961) |
| Integrative Hallmarks (Phenotype Inducer) | Stem Cell Exhaustion | cell amount | FACS | (Rera et al. 2011);(Rossi et al. 2007);(Sharpless and DePinho 2007); |
| Altered Intercellular Communication | e.g inflammaging | various | (Laplante and Sabatini 2012); (Salminen, Kaarniranta, and Kauppinen 2012);(Pont et al. 2012);(Durieux, Wolff, and Dillin 2011)(Lee et al. 2021) |

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