

## Supplemental Information

### An evolutionary transcriptomics approach links CD36 to membrane remodeling in replicative senescence

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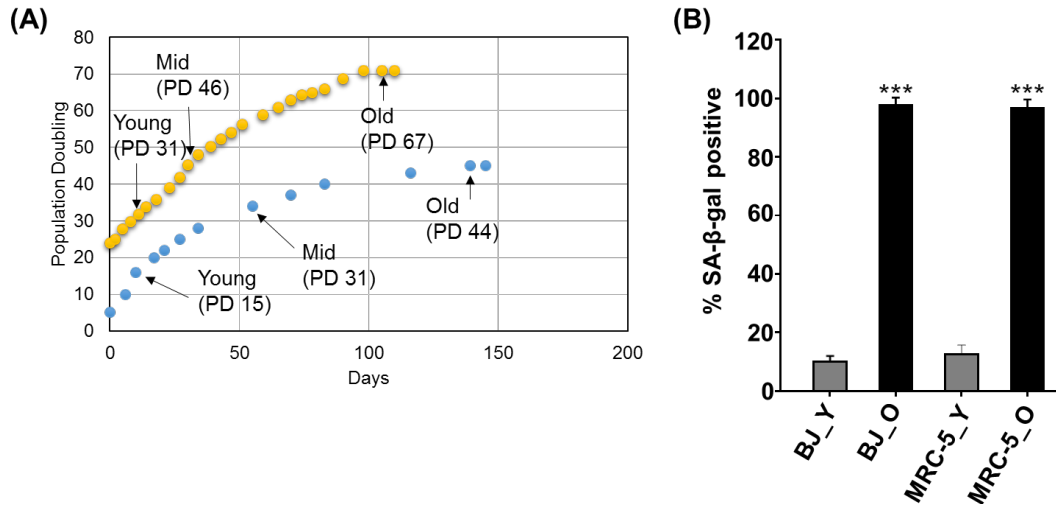
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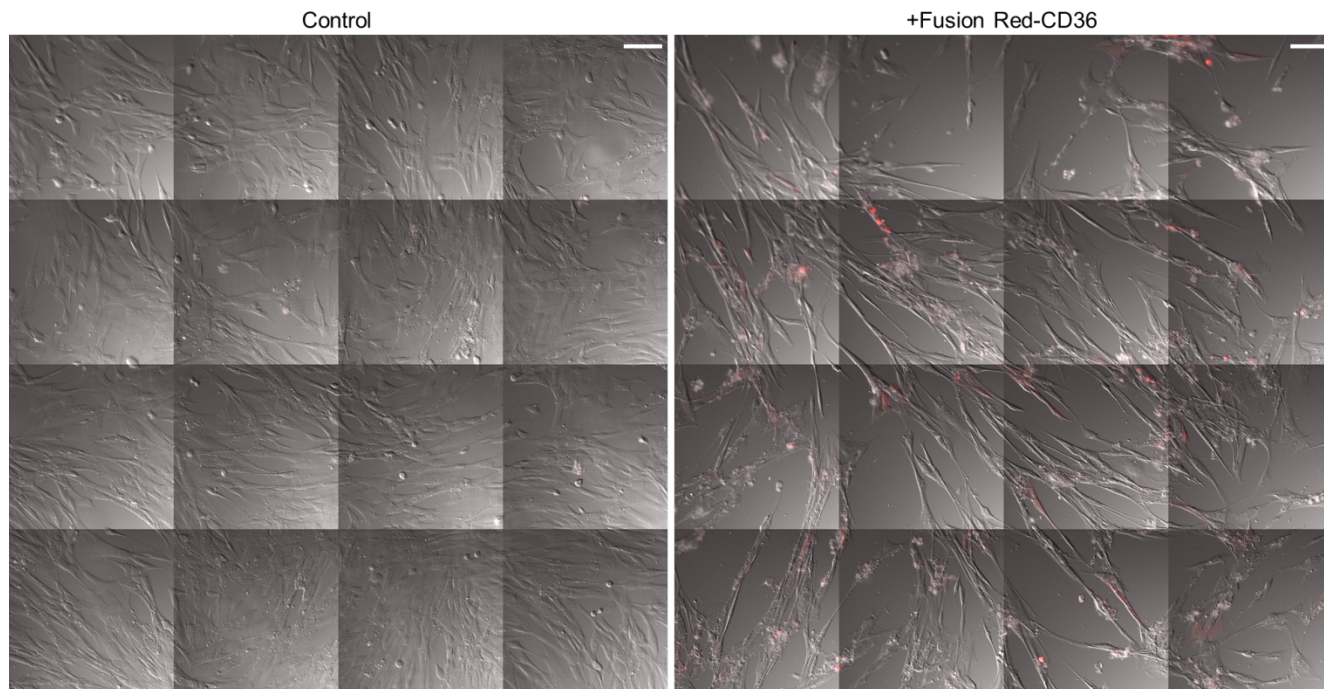
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**Figure S1.** Characterization of replicative senescence in BJ and MRC-5 cell lines. (A). BJ and MRC-5 cells were grown under standard culture conditions until they naturally reached their proliferative capacity. Yellow points: MRC-5 cell line, blue points: BJ cell line. For transcriptomics analysis pellets were collected at PD 31 as “young”, PD 46 as “mid-population” and PD 67 as “old” MRC-5. For BJ, PD 15 were collected as “young”, PD 31 as “mid-population” and PD 44 as “old”. The Y-axis represents the cumulative population doubling and the x-axis represents the days cells were maintained in culture. (B) Measurement of senescence associated β-galactosidase (SA-β-galactosidase) activity in BJ and MRC-5 cell lines during replicative senescence. A significant increase in SA-β-galactosidase positive cells was observed as the cells senesced. Data shown as means ± standard deviation, \*\*\*  $p < 0.001$ . Abbreviations, PD: population doubling; BJ\_Y: BJ young cells; BJ\_O, BJ old cells; MRC-5\_Y, MRC-5 young cells; MRC-5\_O, MRC-5 old cells.



**Figure S2.** Live MRC-5 cell line tile scan. Representative images of control and *CD36* transfected live young MRC-5 cells. As illustrated by the transient expression of FusionRed-CD36 (excitation wavelength = 580 nm), the transfection efficiency of young MRC-5 cells was ~10%. The tile scan was collected by adjoining single images. Scale bar = 100  $\mu\text{m}$ .

**Table S1.** Master table of evolutionary transcriptomics analyses for all protein coding genes analyzed in this study. This is provided as a separate excel spreadsheet. “GeneID” and “Gene” columns indicate the ENSEMBL ID and common names for each gene, respectively. “pLI”, “pRec”, “pNull” (columns C-E) values are measures of tolerance to loss of function, retrieved from The Exome Aggregation Consortium (ExAC), <http://exac.broadinstitute.org/>.<sup>1</sup> Columns F-I are the log<sub>2</sub> fold change and multiple hypotheses corrected *p*-value for the expression change from young to old in BJ and MRC-5 cell lines. Fold change was determined as “old expression” / “young expression” for each gene. “B.value” column shows the index of background selection between primate species. Values were retrieved from McVicker et al.<sup>2</sup> The “GWAS” column indicates whether or not these genes were associated in aging-related phenotypes (<https://www.ebi.ac.uk/gwas/>).

**Table S2.** Genes that showed significant (adjusted  $p < 0.0001$ ) fold change of expression between young and old populations in both BJ and MRC-5 cell lines. This is provided as a separate excel spreadsheet. “GeneID” and “Gene” columns indicate the ENSEMBL ID and common names for each gene, respectively. “Annotation” column shows the gene annotations. Columns D-E show the mean expression in BJ and MRC-5 cell lines. The “Direction” column indicates whether the gene was downregulated or upregulated during replicative senescence.

**Table S3.** Enriched GO categories of genes upregulated and downregulated in old cells. This is provided as a separate excel spreadsheet. Downregulated genes (columns A-O) and upregulated genes (columns T-AH) are listed. Gorilla was employed for the GO enrichment analysis (<http://cbl-gorilla.cs.technion.ac.il/>).<sup>3</sup> In Gorilla, given N (total number of all the genes), B (number of these genes associated with a particular GO term) and n (number of genes in the target dataset), the probability that b or more genes from the target set are associated with the given GO term was then calculated. “GO-term”, “Description”, *p*-value (Exact mHG *p*-value) and FDR *q*-value (*p*-value corrected for multiple testing using FDR) are also provided.

**Table S4** The input and results of evolutionary analyses. This is provided as a separate excel spreadsheet. Column A: the 23-species used for *CD36* evolutionary analysis. Column B: The four positively selected sites in the *CD36* gene. It describes the positively selected sites on human *CD36* gene, which is the probability that it has a  $w$  (nonsynonymous mutation rate/ synonymous mutation rate)  $>1$  (column C),  $p$ -value of likelihood test ( $*<0.05$ ,  $**<0.01$ ) and mean  $w \pm SE$  (columns D-E).

**Table S5.** Raw gene expression (relative read depth) of all the samples analyzed in this study. This is provided as a separate excel spreadsheet. A total of 18 samples were analyzed: triplicates for three time points for BJ and MRC-5 cell lines. BJ\_Y: young BJ; BJ\_M: mid-population BJ; BJ\_O: old BJ; MRC-5\_Y: young MRC-5; MRC5\_M: mid-population MRC-5; MRC-5\_O: old MRC-5.



**Table S6.** Abundances of lipids studied *via* comparative lipidomics are provided in a separate excel spreadsheet. Abbreviations: *m/z*: mass-to-charge ratio; P: proliferating; S: Senescent; SP\_ratio: Senescent / proliferating fold change; LPC: lysophosphatidylcholine; PC: phosphatidylcholine. Raw abundances are provided in columns C-J and relative abundances are provided in columns L-S. Relative abundances were calculated with respect to averaged abundances within lipid families. Abundance is the total ion count for a given ion. Each ion corresponds to a mass-to-charge ratio (*m/z*). SP ratio was determined as  $[\text{Abundance}_{\text{Senescent}}] / [\text{Abundance}_{\text{proliferating}}]$  for each lipid species. Total carbon number and the degree of unsaturation of the fatty acyl chains in the lipid species are listed, i.e. C38:4 correspond to 38 carbons and 4 double bonds. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  (*t-test*).

## Supplemental Experimental Procedures

**Cell culture and transfection conditions.** BJ cells were cultured in minimum essential medium (MEM) while MRC-5 cells were cultured in Eagle's MEM supplemented with 1.5 g/L sodium bicarbonate, non-essential amino acids, and sodium pyruvate. Growth medium was also supplemented with 2 mM L-glutamine, 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin. They were grown as monolayer culture at 37 °C and 5% CO<sub>2</sub> atmosphere under sterile conditions. Cells were routinely checked for mycoplasma contamination. The cumulative population doubling was calculated at each splitting using the formula  $\log_2(D/D_0)$ , where D is the density of cells during harvesting and D<sub>0</sub> is the density of cells at seeding. Transfections were performed using Lipofectamine 3000 according to the manufacturer's instructions. Briefly,  $0.30 \times 10^6$  cells were seeded in 6-well plates in medium supplemented with 10% (v/v) FBS. After 24 hours, DNA-lipid complex was added to the cells. The transient expression of FusionRed-CD36 was detected using a Leica DMI6000B inverted microscope equipped with a Leica-DFC3000G camera using the LAS AF software at an excitation wavelength of 580 nm 72 hours after transfection. After transfection, cells were used for imaging or stained for senescence associated  $\beta$ -galactosidase activity.

**Plasmid extraction.** FusionRed-CD36-C10 plasmid (#56104) was obtained from Addgene (deposited by the Davidson lab) as a bacterial stab and grown in 10mm x 20mm petri dishes overnight at 37 °C using the appropriate antibiotics. Single colonies were then picked and grown overnight in 50 mL culture using standard procedures. Cells were then pelleted by ultracentrifugation at 4 °C. Plasmid DNA was isolated using the E.Z.N.A.® FastFilter Plasmid Midi Kit (OMEGA Bio-Tek) following the manufacturer's instructions. Plasmid DNA concentration and integrity was measured using Thermo Nanodrop-1000 spectrophotometer. Plasmid DNA was then aliquoted into sterile microcentrifuge tubes and stored at -80 °C.

**Western blot analysis.** About  $0.50 \times 10^6$  cells were pelleted and lysed with mammalian protein extraction reagent containing a protease inhibitor cocktail. Cellular debris was removed by centrifugation and the supernatant was used to measure protein concentration by Bradford Assay. Equal amounts of proteins were resolved by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis and then transferred onto polyvinyl difluoride membranes using a wet transfer cell. Membranes were blocked in 5% non-fat dry milk in TBS-Tween (0.2% Tween in Tris-buffered saline) for 1 hour. Membranes were then washed four times (8 minutes each) in TBS-tween followed by incubation overnight at 4 °C with primary antibody. Antibodies were diluted in AbDil-tween according to manufacturer's instructions. Once the incubation with primary antibody was complete, membranes were washed four times (8 minutes each) in TBS-tween followed by incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody diluted in 5% non-fat dry milk in TBS-tween for 2 hours at room temperature. Once the incubation with secondary antibody was complete, membranes were washed in TBS-tween four times (8 minutes each) and developed using the Super Signal West Pico kit (ThermoFisher Scientific) according to manufacturer's guidelines.  $\alpha$ -tubulin was used as a loading control.

**Lipid extraction and analysis.** Preparation of lipid extracts, sample normalization, and liquid chromatography mass spectrometry data acquisition and analysis were performed as previously described.<sup>4</sup>

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