**Figure and legends:**

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**Figure S1. Generation and validation of the Nef transgenic mouse lines.** (**A**)Theschematic diagram shows the generation of the Nef transgenic (Nef-TG) mouse. HIV-Nef gene was cloned in the CAG-Lox-CAT vector. Four different founder mouse lines were generated. Founder mouse lines were bred with the wild-type mice and crossed with the alpha myosin heavy chain Cre (αMHC-Cre) mice to express Nef protein in the heart. (**B**) Western blot shows Nef protein expression in the heart of Nef-TG lines. GAPDH was used as a loading control. (**C**) The graph shows Nef protein quantification in Nef-TG mice's heart tissue. Data are presented with standard deviation. Statistical significances were calculated between the Nef TG mice lines (\*p ≤ 0.05, \*\*\* p ≤ 0.001, \*\*\*\* p ≤ 0.0001).

**Figure S2.** **Nef transgenic mice exhibit cardiac dysfunction at 24 weeks of age and heart failure at 48** **weeks of age.** Representative images show M-mode echocardiography of WT and TG-33 mice at (**A**) 24 and (**D**) 48 weeks of age. The images of the left ventricle were captured at the mid-papillary level (the parasternal short-axis view). Graphs show quantification of (**B**) fractional shortening and (**C**) volume of the left ventricle during systole (volume, s) of 24-week-old mice (n=30 WT (17 males, 13 females) and, 15 TG-33 (6 males, 9 females)), and (**E**) fractional shortening and (**F**) volume of the left ventricle during systole (volume, s) of 48-week-old mice (n=9 WT (7 males, 2 females) and 11 TG-33 (6 males, 5 females)). Data are presented with standard deviation. Statistical significances were calculated between WT and TG-33 mice (\*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*\* p ≤ 0.0001).

**Figure S3. Nef transgenic mice have a shortened life span.** Kaplan-Meier survival analysis shows that TG-33 mice have significantly shorter life spans than WT mice. (n=15 WT (7 males, 8 females) and 14 TG-33 (5 males, 9 females)). Statistical significance was calculated between WT and TG-33 mice (\*\*\* p ≤ 0.001).

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**Figure S4: Representative images show that Nef induces senescence in the cells.** (**A-B**) HeLa cells were transfected with plasmid DNA for 48 hours and then SA-βgal assay was performed. Cells were incubated in the staining solution for 48 hours and images were captured. (**C**) The graph shows the quantification of the SA-βgal positive cells (n=844 pShuttle cells and 849 Nef expressing cells). Experiments were repeated three times. Data are presented with standard deviation. Statistical significance was calculated between control and Nef expressing cells (\*\*\*\* p ≤ 0.0001).



**Figure S5**: **Representative microscopic images show that Nef causes accumulation of lipofuscin in the heart.** (**A**) Lipofuscin detection was performed in paraffin cardiac tissue sections by measuring the autofluorescence. DNA was stained with DAPI. (**B**) The graph shows counting of lipofuscin positive puncta (n=16 of 12-week-old WT mice (8 males, 8 females), 10 of 12-week-old TG-33 mice (4 males, 6 females), 11 of 24-week-old WT mice (7 males, 4 females), and 12 of 24-week-old TG-33 mice (4 males, 8 females)). Data are presented with standard deviation. Statistical significances were calculated between WT and TG-33 mice in different age group (\*p ≤ 0.05).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | WT (n=32) | Nef TG 12 (n=9) | Nef TG 22 (n=10) | Nef TG 33 (n=24) | Nef TG 56 (n=6) |
| LV DIAMs (mm) | 2.306±0.4971 | 2.572±0.6309 | 2.61±0.531 | 2.653±0.3155\*\* | 2.877±0.5356\*\* |
| LV DIAMd (mm) | 3.66±0.3538 | 3.685±0.5286 | 3.71±0.4953 | 3.857±0.3135\* | 3.925±0.3889 |
| LV VOLs (μl) | 19.71±9.932 | 26.2±16.2 | 26.44±11.3 | 26.49±8.082\*\* | 33.15±15.91\*\* |
| LV VOLd (μl) | 57.45±13.11 | 59.25±20.62 | 60±17.65 | 64.84±12.7\* | 67.78±16.25 |
| SV (ul) | 37.74±6.044 | 33.05±5.874\* | 33.56±8.148 | 42.75±10.73\* | 34.64±5.039 |
| EF (%) | 67.45±10.93 | 58.87±11.24\* | 57.78±10.15\* | 59.6±6.322\*\* | 53.02±11.05\*\* |
| FS (%) | 37.58±8.712 | 31±7.342\* | 30.25±6.927\* | 31.32±4.239\*\* | 27.17±6.551\*\* |
| CO (ml/min) | 16.32±2.956 | 15.03±2.734 | 13.89±4.204\* | 17.29±3.29 | 15.4±1.143 |
| LV mass (mg) | 118.1±22.49 | 121.8±22.26 | 133.3±21.3 | 123.6±27.6 | 143.7±26.91\* |
| LVAWs (mm) | 1.406±0.182 | 1.264±0.1788\* | 1.451±0.2017 | 1.319±0.2129 | 1.372±0.3011 |
| LVAWd (mm) | 0.9511±0.1362 | 0.8596±0.1148 | 1.002±0.1492 | 0.9037±0.1623 | 1.002±0.2632 |
| LVPWs (mm) | 1.279±0.2415 | 1.325±0.32 | 1.191±0.201 | 1.126±0.1454\*\* | 1.181±0.1246 |
| LVPWd (mm) | 0.8287±0.1877 | 0.9504±0.2286 | 0.9107±0.1741 | 0.816±0.0982 | 0.8838±0.06725 |
| HR (bpm) | 432±29.98 | 456.8±49.99 | 409.8±49.41 | 451.8±29.6\* | 449.1±43.52 |

**Table S1. Echocardiographic analyses of Nef transgenic mouse lines at 12 weeks of age.** LV DIAMs, systolic left ventricular diameter; LV DIAMd, diastolic left ventricular diameter; LV VOLs, left ventricular systolic volume; LV VOLd, left ventricular diastolic volume; SV, stroke volume; EF, ejection fraction; FS, fractional shortening; CO, cardiac output; LV mass, left ventricular mass; LVAWs, systolic left ventricular anterior wall thickness; LVAWd, diastolic left ventricular anterior wall thickness; LVPWs, systolic left ventricular posterior wall thickness; LVPWd, diastolic left ventricular posterior wall thickness; HR, heart rate. Data are presented with standard deviation. Statistical significances were calculated between WT and Nef TG mice (\*p ≤ 0.05, \*\*p ≤ 0.01).