SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

MATLAB script for automatic fibrotic area detection in picrosirius red staining


```matlab
clear %clear workspace
clc %clear command window

% Directory for the picrosirius red images (green channel)
filenames = dir('*.tif');

Resultlist = {'Filename','Area [pix]','Area fibrotic','% Fibrotic [%]'};
hl = 1; %headerlines in results list for output

for q = 1:numel(filenames)
    % Load image
    I = imread(filenames(q).name);
    Resultlist{q+hl,1} = filenames(q,1).name;

    if size(I,3) == 3 %Check if RGB image
        % If RGB image, take blue "3" channel. Change to "1" for red or "2"
        % for green.
        I = I(:,:,2);
    end

    % Takes the image complement
    I = imcomplement(I);

    % Remove background and fill in holes. May need to modify background
    % threshold number for imaging experiment (i.e., 55000, and 60)
    imgOrginal = I;
    img2 = I > 55000;
    deletesize = 300;
    img3 = bwareaopen(img2, deletesize);
    se = strel('disk',60);
    img3 = imclose(img3,se);
    [i,j] = size(img3);

    % Total cross-sectional area of heart
    sTotal = sum(sum(img3));
    Resultlist{q+hl,2} = sTotal;

    % Set background into original image
    for a = 1:i;
        for b = 1:j;
            if img3(a,b) == 0;
                I(a,b) = 0;
            end
        end
    end

    %Image average smoothening by (i)th times. Set to 4 to smoothen out
    %noise.
    I2 = I;
```

```matlab
    for i = 1:4
        h = fspecial('average');
        I2 = imfilter(I2,h);
    end

    %Hole-filling algorithm to fill in any hole (below threshold)regions in
    %nucleus
    I4 = imfill(I2,'holes');

    % Extract the nucleus from the original image to a black background
    [I5] = ExtractImage(I,I4);
    I5 = uint16(I5);

    %Intensity redistribution for I5
    A = max(max(I5));
    B = double(I5);
    C = double(A);
    I5 = (B/C)*65535;
    I5 = uint16(I5);

    % Set threshold for fibrotic area determination
    fibrotic = 1.05;
    thresh = max(max(I5))/fibrotic;
    bg = bwperim(img3);
    se2 = strel('disk',4);
    bg = imdilate(bg,se2);
    colI = I5 > thresh;
    colI = colI - bg;
    colI(colI < 0) = 0;
    imshow(colI)
    pTotal = sum(sum(colI));
    Resultlist{q+hl,3} = pTotal;
    PerFibrotic = (pTotal/sTotal)*100

    Resultlist{q+hl,4} = PerFibrotic;

end
```

Table S1. Echocardiographic Data for Male and Female, Vehicle and ISO-treated.

|	Females		Males			
	Vehicle	ISO	Vehicle	ISO (1 mg/kg)	ISO (2 mg/kg)	ISO (4 mg/kg)
	n=6	n=10	n=8	n=7	n=3	n=3
LVAW; d (cm)	0.11	0.169	0.128	0.16	0.141	0.147
SD	0.008	0.015	0.014	0.017	0.01	0.02
P		6.30E-07		0.00042	0.05144	0.03455
LVID; d (cm)	0.726	0.675	0.798	0.713	0.711	0.652
SD	0.058	0.031	0.022	0.061	0.059	0.056
P		0.023		0.0042	0.0132	0.0007
LVPW; d (cm)	0.125	0.166	0.134	0.166	0.159	0.15
SD	0.011	0.017	0.006	0.012	0.022	0.015
P		0.00012		6.25E-06	0.0046	0.0108
LVAW; s (cm)	0.183	0.264	0.204	0.276	0.274	0.267
SD	0.021	0.034	0.019	0.038	0.051	0.038
P		9.60E-05		0.0004	0.00583	0.00514
LVID; s (cm)	0.418	0.246	0.447	0.211	0.254	0.169
SD	0.052	0.06	0.066	0.054	0.118	0.085
P		3.80E-05		2.40E-06	0.00324	0.00013
LVPW; s (cm)	0.237	0.327	0.254	0.332	0.289	0.333
SD	0.029	0.038	0.036	0.032	0.044	0.043
P		0.00013		0.00029	0.095	0.00533
LV Vol; d (µl)	278.87	235.957	343.96	268.184	266.089	218.751
SD	50.467	24.85	21.462	53.434	49.585	42.591
P		0.0229		0.0047	0.014821	0.000983
LV Vol; s (µl)	79.025	23.492	93.799	16.078	29.074	10.955
SD	22.81	12.926	33.627	9.922	31.829	13.028
P		2.00E-05		2.40E-05	0.00858	0.00133
EF (%)	72.028	90.159	72.896	94.113	89.994	95.529
SD	4.442	4.991	8.776	3.5	9.5	4.649
P		3.54E-06		1.90E-05	0.009164	0.001074
FS (%)	42.58	63.571	44.006	70.49	64.773	74.616
SD	3.643	8.369	7.33	6.75	13.744	10.688
P		3.43E-05		3.10E-06	0.00415	0.000179
HR (bpm)	365.78	425.104	355.481	427.112	440.773	462.35
SD	44.889	15.05	25.286	26.856	46.88	23.741
P		0.0013		0.0001296	0.0023	0.00014758

Unpaired student's t-test applied comparing variable with vehicle in all cases.

Table S2. Echocardiographic Data for OVX females and CAS males.

|	OVX Females		CAS Males	
	Vehicle	ISO (4 mg/kg)	Vehicle	ISO (4 mg/kg)
	n=7	n=6	n=8	n=9
LVAW; d (cm)	0.119	0.175	0.12	0.161
SD	0.001	0.015	0.002	0.015
P		4.80E-07		9.40E-07
LVID; d (cm)	0.72	0.691	0.744	0.705
SD	0.033	0.044	0.053	0.06
P		0.1001		0.09266
LVPW; d (cm)	0.12	0.161	0.12	0.159
SD	0.002	0.007	0.001	0.014
P		8.52E-09		5.72E-07
LVAW; s (cm)	0.186	0.249	0.188	0.266
SD	0.018	0.041	0.012	0.023
P		0.00179		1.60E-07
LVID; s (cm)	0.393	0.319	0.381	0.262
SD	0.062	0.088	0.082	0.056
P		0.05228		0.0015
LVPW; s (cm)	0.193	0.244	0.193	0.269
SD	0.01	0.026	0.013	0.034
P		0.00023		1.40E-05
LV Vol; d (µl)	273.022	249.297	294.352	262.029
SD	28.801	35.704	47.105	51.403
P		0.1056		0.0992
LV Vol; s (µl)	69.144	44.884	66.141	26.798
SD	23.521	29.366	30.568	13.202
P		0.06302		0.00155
EF (%)	74.597	82.631	78.275	90.218
SD	9.005	9.56	7.685	3.337
P		0.0736		0.000351
FS (%)	45.457	54.097	49.141	63.139
SD	8.517	10.771	8.521	5.303
P		0.0671		0.000454
HR (bpm)	315.954	376.961	367.162	411.5
SD	29.694	42.042	34.237	46.746
P		0.0054		0.027

Unpaired student's t-test applied comparing variable with vehicle in all cases.

**Figure S1. Titration for ISO treatment to determine appropriate dose.**
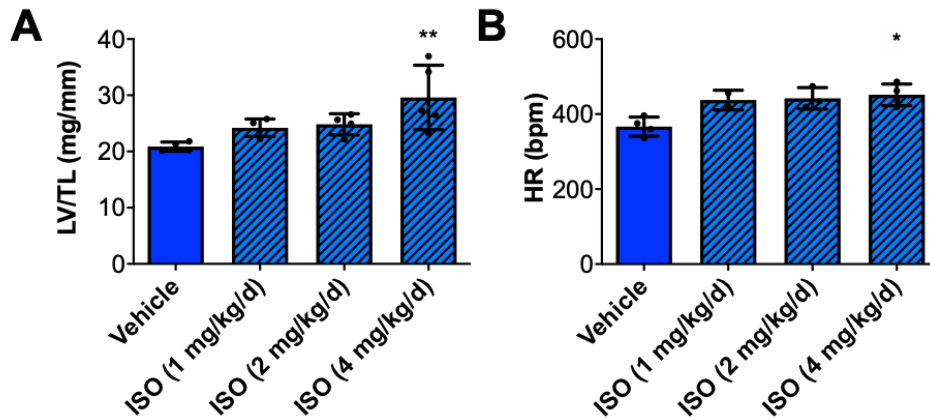
A) Cardiac hypertrophy measured by left ventricle to tibia length (LV/TL) in male rats treated with vehicle (n=4), 1 mg/kg/day ISO (n=4), 2 mg/kg/day ISO (n=4), 4 mg/kg/day ISO (n=4). **B)** Heart rate measured by echocardiography in male rats treated with vehicle (n=4), 1 mg/kg/day ISO (n=2), 2 mg/kg/day ISO (n=3), 4 mg/kg/day ISO (n=4). Kruskal-Wallis non-parametric test with Dunn's multiple comparison test applied. * $p < 0.05$, ** $p < 0.01$. All data reported as $\pm$ SEM.

**Figure S2. Percent survival of all male and female rats treated with ISO (4 mg/kg/day).**
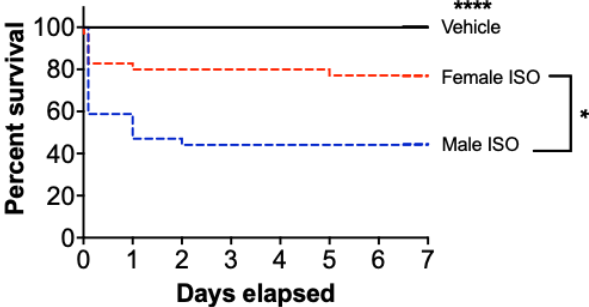
Male vehicle n=29, Male ISO n=93, Female vehicle n=33, Female ISO n=60. Gehan-Breslow-Wilcoxon method applied. * p<0.05, **** p<0.0001.

Figure S3. A) Percent survival OVX and CAS animals treated with ISO (4 mg/kg/day). CAS vehicle n=8, CAS ISO n=22, OVX vehicle n=8, OVX ISO n=22. Gehan-Breslow-Wilcoxon method applied. **B)** Percent survival male and female animals treated with ISO (4 mg/kg/day) that were either intact or gonadectimized. Intact male n=34, CAS male n=22, intact female n=35, OVX female n=22. Gehan-Breslow-Wilcoxon method applied. * p<0.05, ** p<0.01.
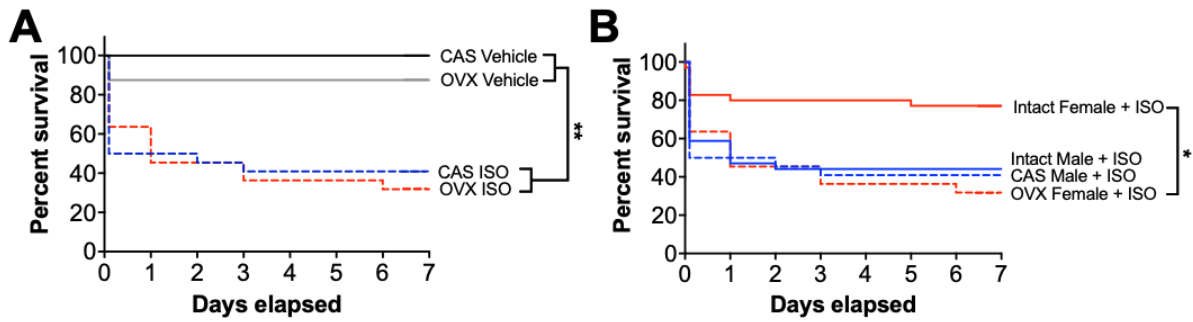
Figure S4. Gene expression of fibroblast and myofibroblast markers in CFs treated with vehicle or ISO.
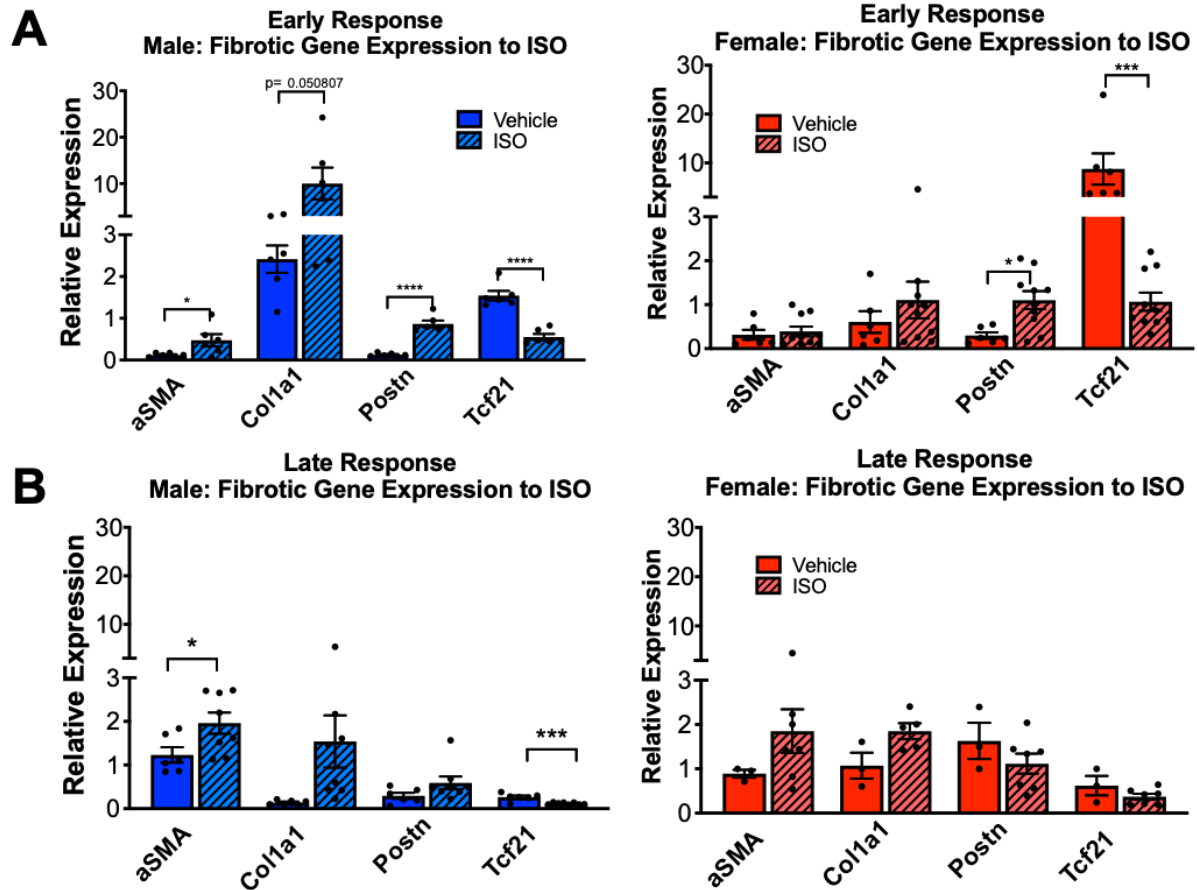
A) CFs from 3-day ISO-treated or vehicle-treated male (blue) or female (red) rats. αSMA, Col1a1, and Postn, and Tcf21 gene expression (normalized to TBP) was measured by qRT-PCR. Male Vehicle n = 6, Male ISO = 6. Female Vehicle n = 6, Female ISO n = 10. Unpaired student's t-test applied. **B)** CFs from 7-day ISO-treated or vehicle-treated male (blue) or female (red) rats. αSMA, Col1a1, and Postn, and Tcf21 gene expression (normalized to TBP) was measured by qRT- PCR. Male Vehicle n = 6, Male ISO = 8. Female Vehicle n = 3, Female ISO n = 7 (except Col1a1 n = 5). Unpaired student's t-test applied. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. All data reported as ± SEM.