

# Supplementary Material

## Sildenafil as a Candidate Drug for Alzheimer’s Disease: Real-World Patient Data Observation and Mechanistic Observations from Patient-Induced Pluripotent Stem Cell-Derived Neurons

**Supplementary Table 1.** Description of Clinformatics® data for pharmacoepidemiologic analysis.

	<b>Sildenafil</b>	<b>Bumetanide</b>	<b>Furosemide</b>	<b>Nifedipine</b>	<b>Spirolactone</b>
N	54,229	49,942	654,996	69,617	180,215
No. of AD	48	403	6,877	775	1,622
Female (%)	3.3	52.8	56.1	60.2	56.3
Mean age (s.d.)	71.7 (5.3)	77.3 (6.8)	76.7 (6.7)	75.2 (6.3)	76.0 (6.6)
Race (%)					
White	71.6	69.9	70.2	54.0	69.6
Black	9.6	12.5	11.5	19.0	12.5
Hispanic	12.7	10.9	10.4	17.9	10.2
Asian	2.5	1.9	2.5	3.8	2.3
Unknown	3.6	4.9	5.3	5.3	5.3
Disease comorbidities (%)					
Alcohol use disorder	3.1	5.2	4.0	3.2	6.3
Cancer	18.8	25.1	22.4	18.2	22.5
Cerebrovascular disease	15.6	40.9	31.2	33.3	33.4
Depression	11.8	27.5	22.3	20.8	24.4
Diabetes	30.8	59.7	45.4	50.6	49.6
Fall	5.8	25.5	17.8	14.9	19.2
Heart disease	17.0	72.4	46.0	32.6	60.0
Hypertension	73.1	95.7	90.3	94.0	93.5
Liver disease	8.6	18.0	13.0	12.6	19.6
Parkinson’s disease	1.1	1.9	1.8	1.2	1.5
Pulmonary disease	25.1	60.0	45.2	36.7	49.3
Renal disease	14.4	56.0	31.9	42.5	36.4
Traumatic brain injury	0.8	2.4	1.7	1.7	1.9

**Supplementary Table 2.** Description of MarketScan data for pharmacoepidemiologic analysis.

	<b>Sildenafil</b>	<b>Bumetanide</b>	<b>Furosemide</b>	<b>Nifedipine</b>	<b>Spirolactone</b>
N	116,412	56,815	759,141	95,031	189,256
No. of AD	93	863	15,598	1,595	2,442
Female (%)	2.0	53.7	55.9	55.1	56.1
Mean age (sd)	71.0 (5.8)	78.3 (8.7)	78.3 (8.8)	75.3 (8.2)	76.3 (8.5)
Geographics (%)					
Northeast	30.8	17.5	22.2	22.6	20.1
North central	18.9	39.3	30.3	28.9	31.0
South	29.9	33.4	33.0	34.1	33.0
West	19.4	9.1	13.9	13.8	15.3
Not available	1.0	0.6	0.5	0.6	0.6
Disease comorbidities (%)					
Hypertension	42.6	64.1	55.8	48.1	57.4
Myocardial Infarction	1.3	8.7	5.5	2.3	7.2
Congestive Heart Failure	3.9	42.6	23.4	7.6	33.1
Peripheral Vascular Disease	4.7	17.3	11.9	8.5	12.2
Cerebrovascular Disease	3.8	13.1	10.3	8.7	10.0
Dementia	0.2	2.5	2.3	1.2	1.8
Chronic Pulmonary Disease	8.2	29.4	20.5	9.9	20.9
Connective Tissue Disease- Rheumatic Disease	1.2	3.1	2.7	2.3	2.6
Peptic Ulcer Disease	0.3	1.3	0.9	0.5	1.0
Mild Liver Disease	1.5	3.8	2.8	1.6	6.3
Diabetes without complications	16.0	34.6	25.1	20.7	26.3
Diabetes with complications	4.3	15.9	9.2	8.2	9.8
Paraplegia and Hemiplegia	0.2	1.0	0.8	0.7	0.7
Renal Disease	4.1	26.0	11.9	14.2	12.7
Cancer	9.0	11.3	10.8	7.0	10.4
Moderate or Severe Liver Disease	0.2	0.9	0.5	0.2	2.0
Metastatic Carcinoma	0.5	1.9	2.3	0.9	2.2
AIDS/HIV	0.2	0.1	<0.1	0.1	0.1

**Supplementary Table 3.** Details of AD patient derived iPSC lines

<b>Given Name</b>	P1	P2	P10	P11
<b>Cell name, ID</b>	HVRDi001-A-1	UCSD241i-APP2-3	IUGB62.1	IUGB259
<b>Resource</b>	Wi Cell	Wi Cell	NCRAD	NCRAD
<b>Disease</b>	FAD	FAD	FAD	AD
<b>Sex</b>	Male	Female	Female	Male
<b>Mutation</b>	APPV717I	APP Duplication	APP Duplication	PSEN1 A246E c.737C>A
<b>Age (y)</b>	57	60	60	56
<b>APOE status (1/2)</b>	Unknown	2/2	3/3	3/3
<b>Origin</b>	Unknown	Unknown	Fibroblast	Fibroblast

**See separate Excel file for Supplementary Tables 4-8.**

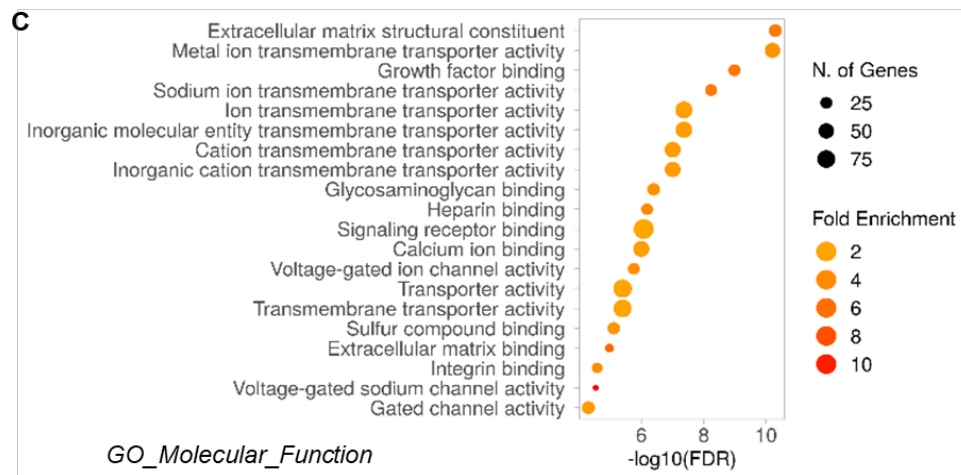
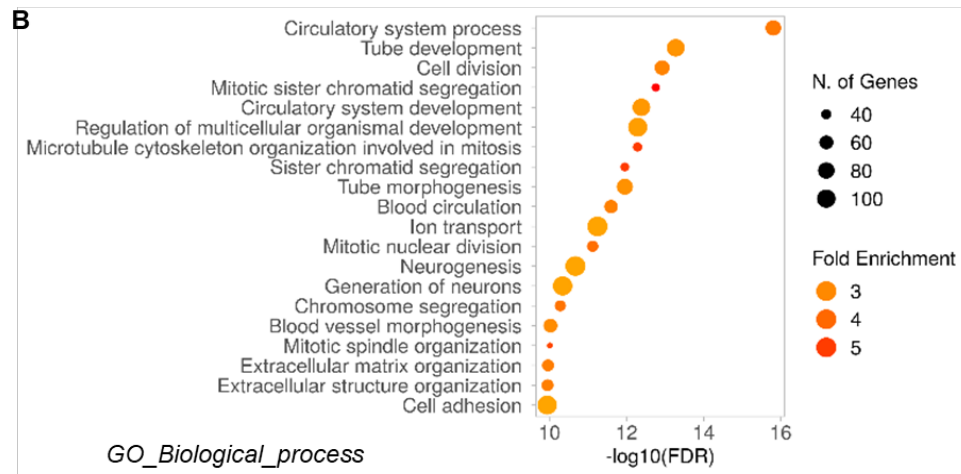
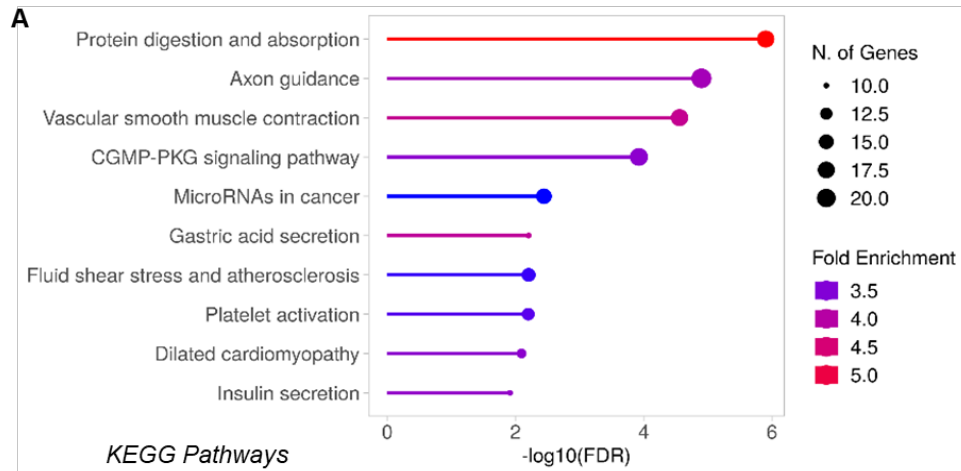
**Supplementary Table 4.** List of 817 differentially expressed genes (DEGs) between DMSO and 100  $\mu$ M sildenafil treated group.

**Supplementary Table 5.** Top enriched KEGG pathways and associated differentially regulated genes.

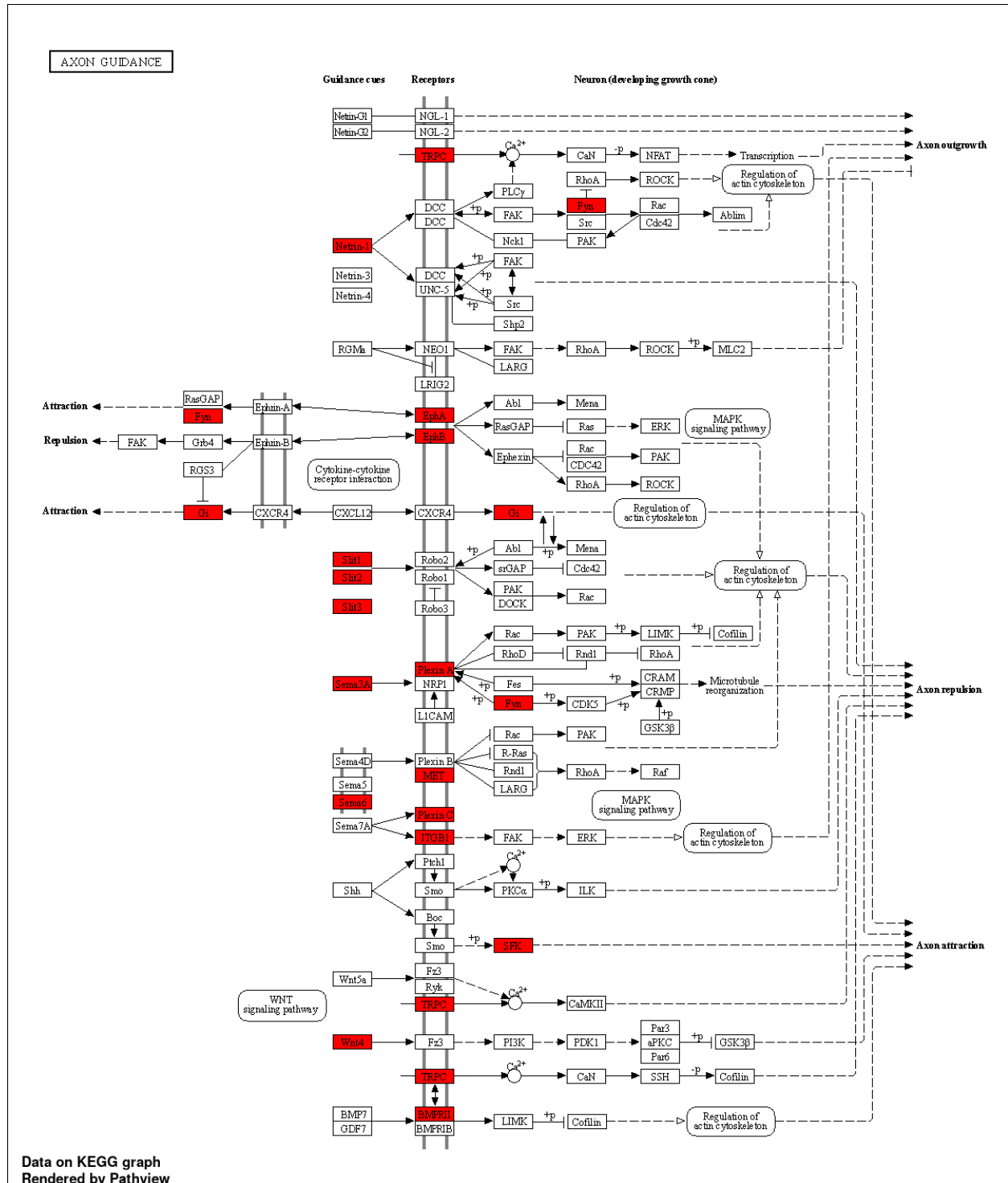
**Supplementary Table 6.** Significantly enriched PANTHER pathways and associated genes.

**Supplementary Table 7.** Top 20 biological processes functionally enriched in differentially regulated genes.

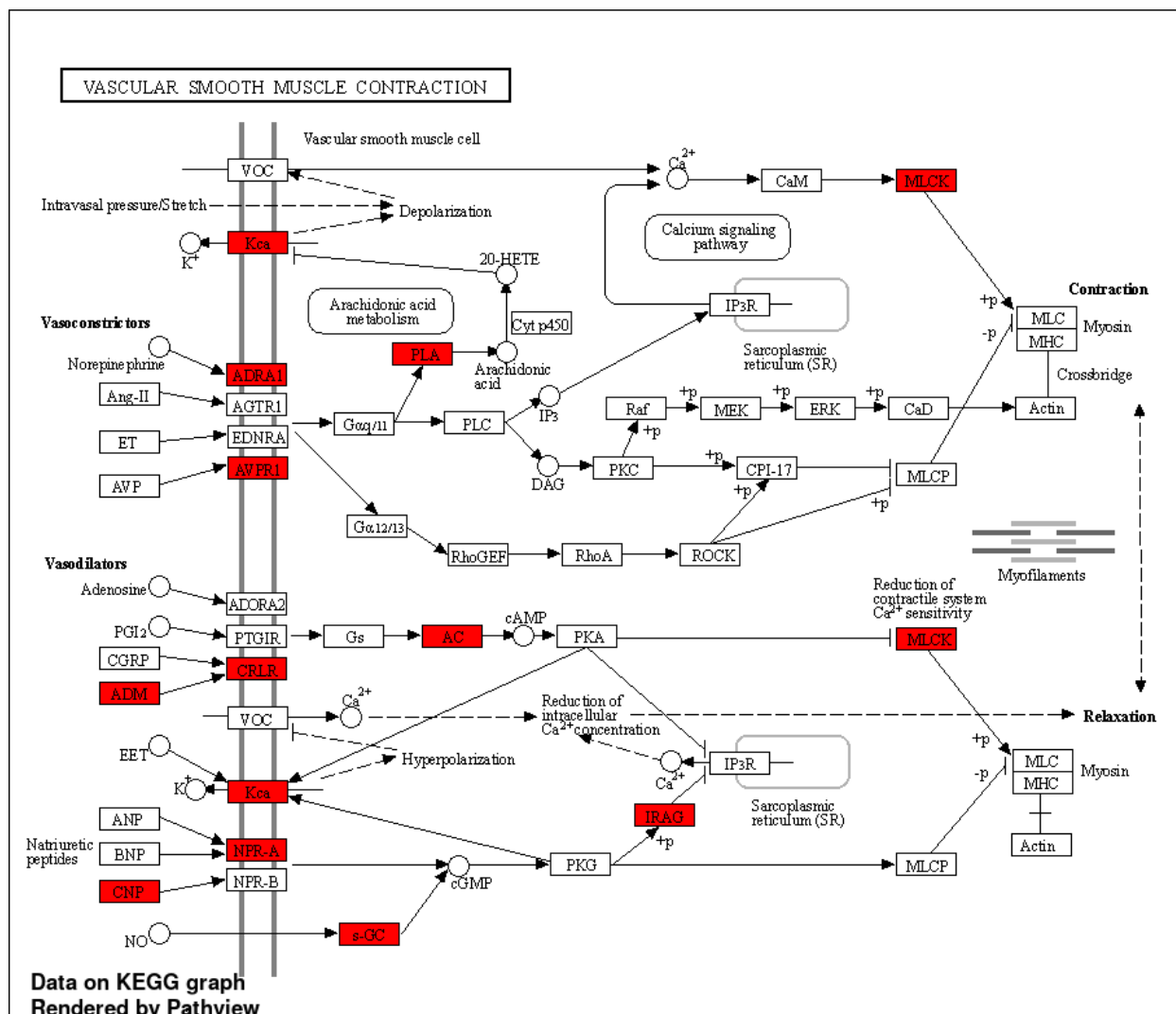
**Supplementary Table 8.** Top 20 molecular functions enriched among differentially regulated genes



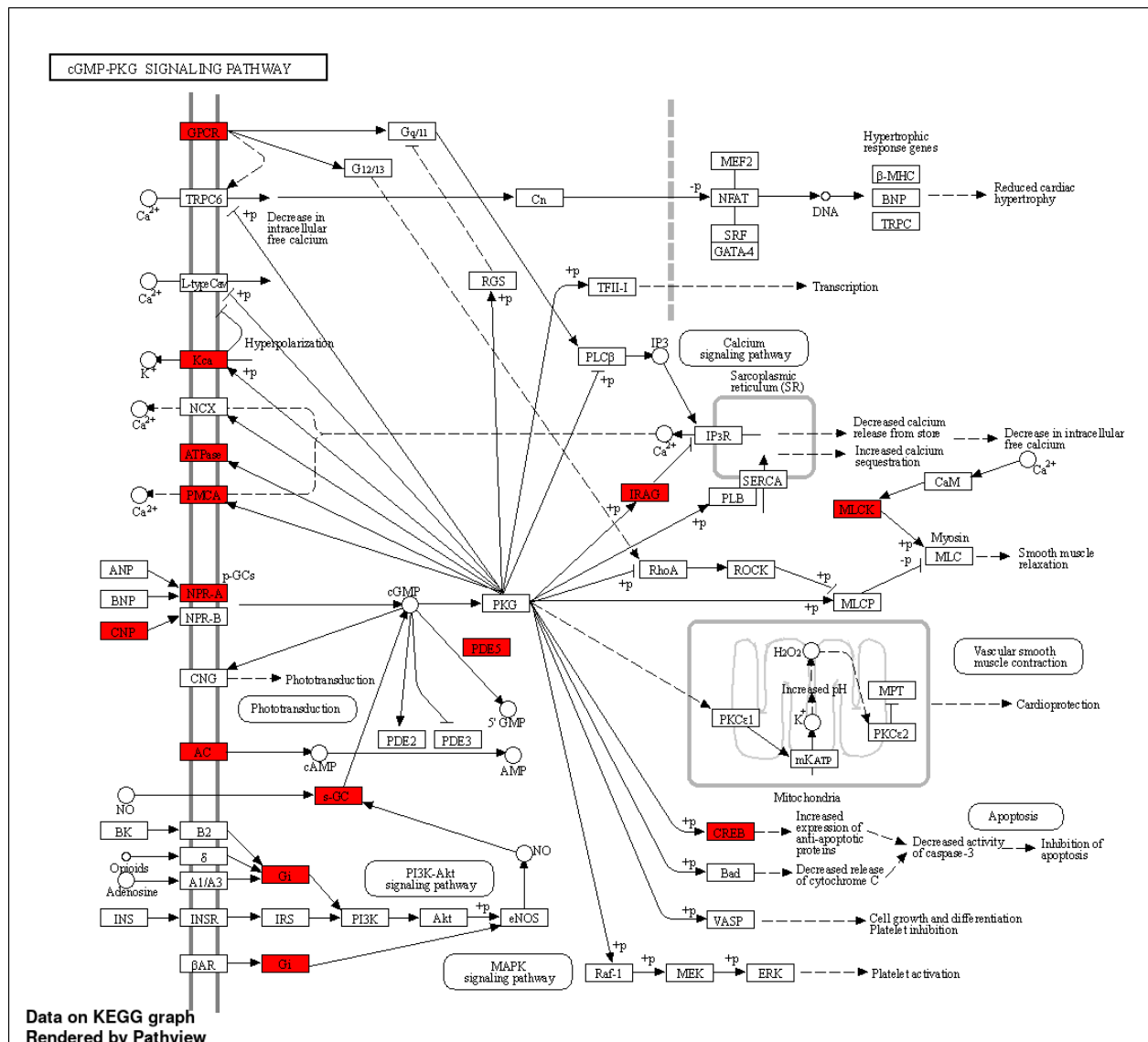
**Supplementary Figure 1.** Functional enrichment plot illustrating top 10 enriched KEGG pathways (A), 20 significant regulated biological processes (B), and top 20 molecular functions (C). x-axis in plots depict the enrichment FDR (*false discovery rate*), color-gradient represent the fold enrichment and bubble size indicates the number of genes in each category. Enrichment analysis has been performed and depicted utilizing the KEGG and ShinyGo v0.77.



**Supplementary Figure 2.** Graph depicts Axon guidance pathway using Pathview with highlighted differentially regulated genes between control and 100µM sildenafil treated group. This shows disrupted regulation of key gene families, i.e., EPHA, EPHB, TRPC, SEMA, Plexins.



**Supplementary Figure 3.** Graph shows vascular smooth muscle contraction pathway with marked dysregulated genes between control and 100  $\mu$ M sildenafil treated group.



**Supplementary Figure 4.** Graph showing cGMP-PKG signaling pathway components using Pathview. Differentially regulated genes like PDE5, CREB, MLCK, etc. between control and control and 100  $\mu$ M sildenafil treated group from the pathway are highlighted.