Supplementary Information

Dopamine D_5 receptor-mediated decreases in mitochondrial reactive oxygen species production are cAMP- and autophagy-dependent

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Running head: Dopamine D_5 receptor regulation of mitochondrial ROS

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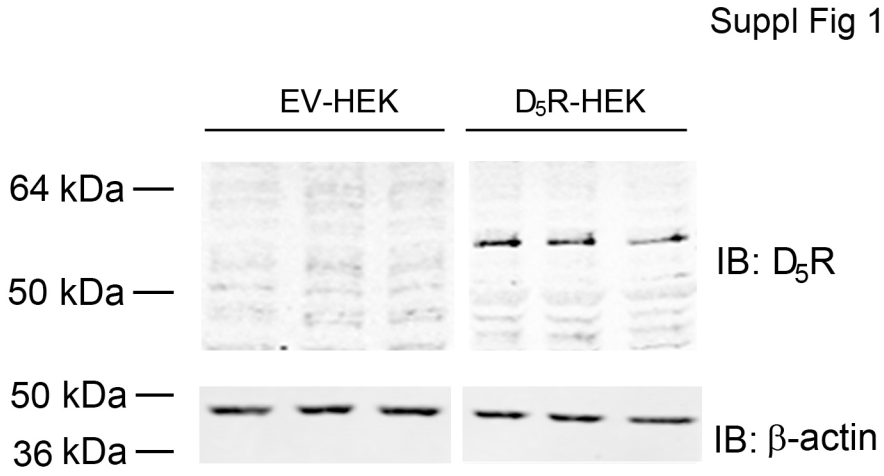
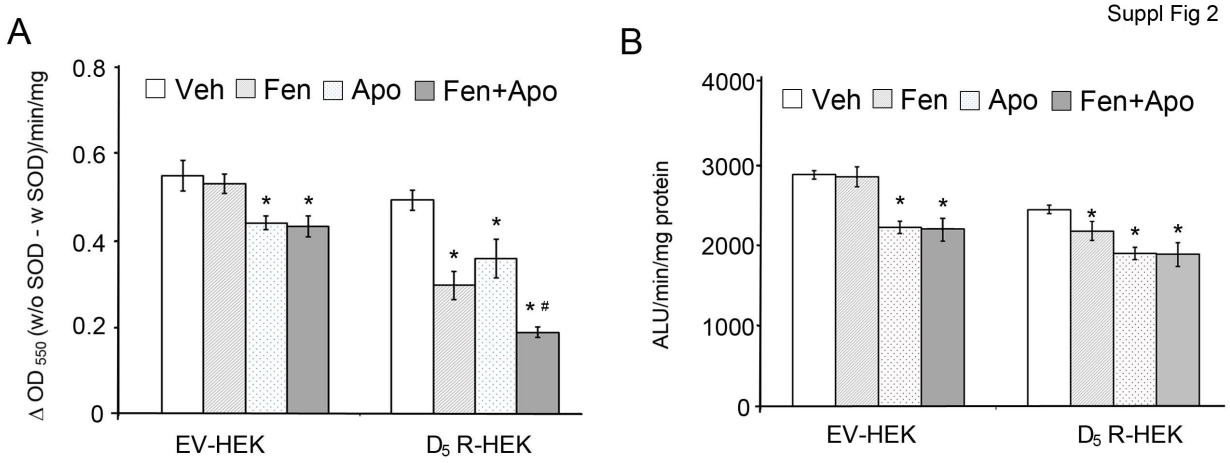
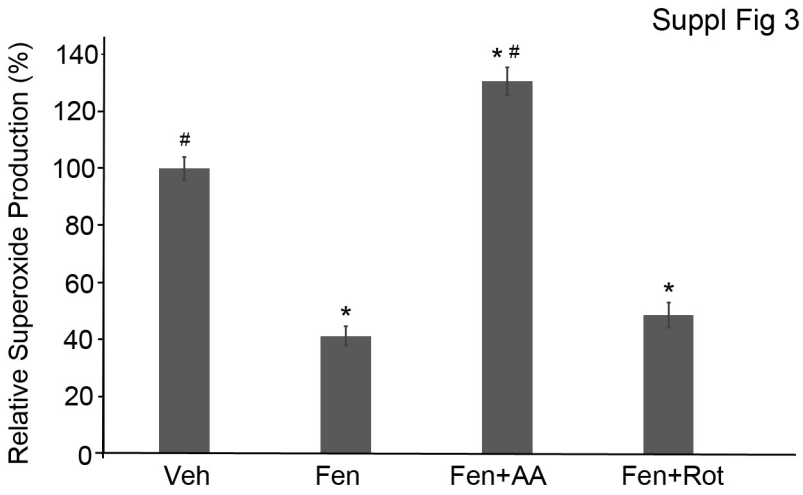


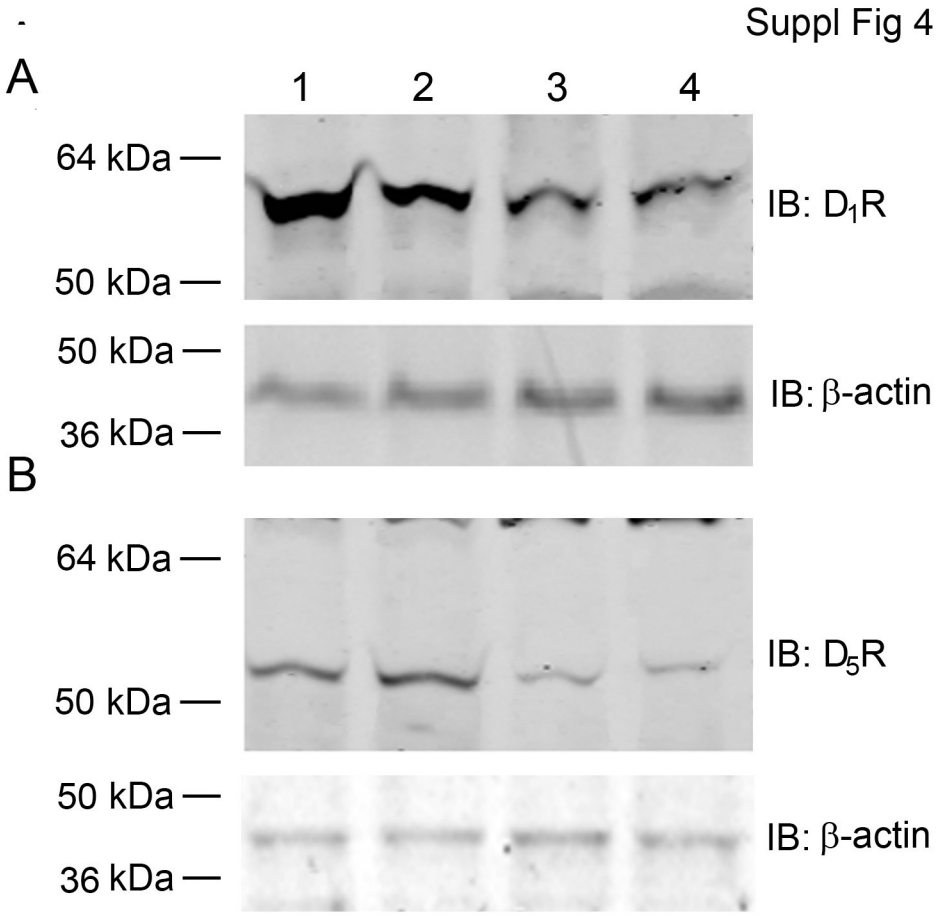
Supplementary Figure 1 D_5R protein overexpression in D_5R-HEK 293 cells. D_5R-HEK 293 cells were homogenized and D_5R protein was immunoblotted with specific anti-D_5R antibody. The β-actin protein level was used to measure the amount of sample loaded in each lane. EV-HEK, empty vector-transfected HEK293 cells; D_5R-HEK, D_5R-overexpressing HEK293 cells. One set of blots from 3 independent experiments is shown. The lanes for the EV-HEK and D_5R-HEK blot were run on the same gel but were noncontiguous.



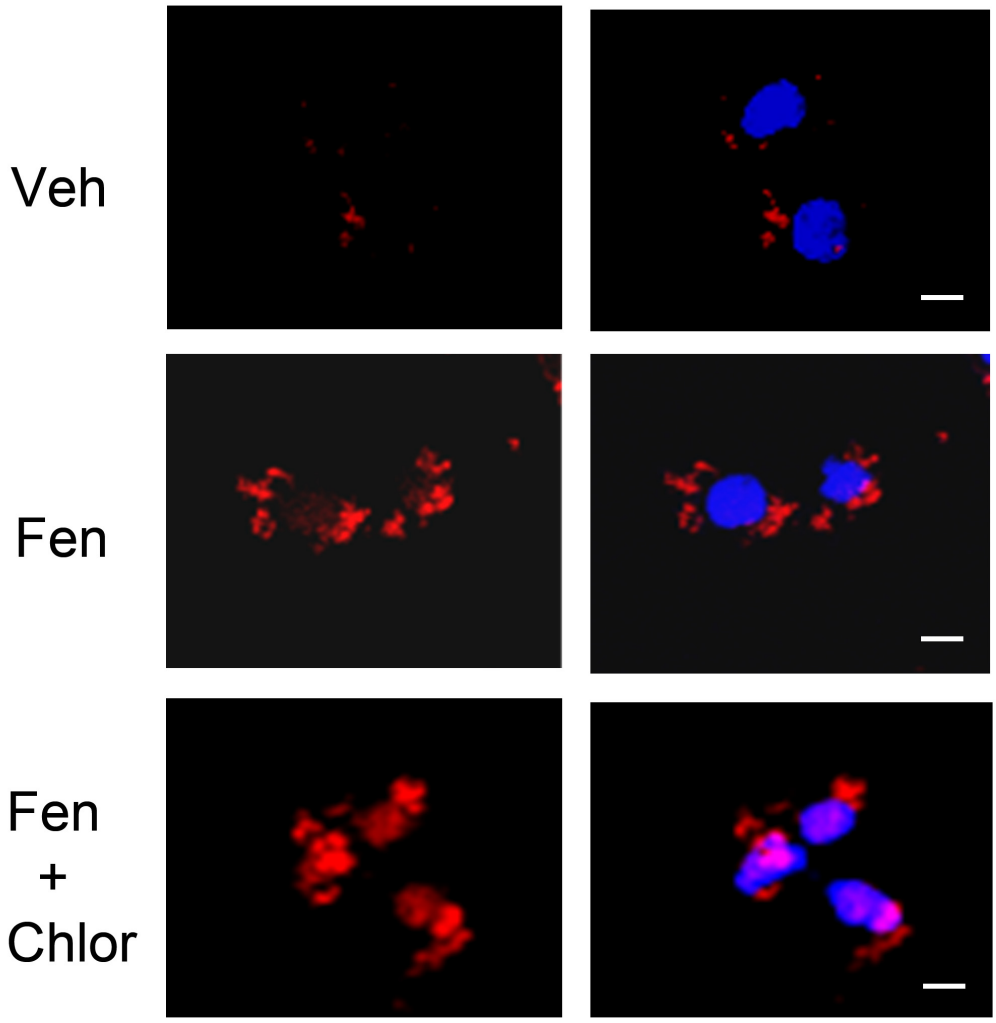
Supplementary Figure 2 ROS production in D_5R-HEK293 cells. **(A)** Superoxide production, per min per mg protein, is the difference between the OD_{550} in the absence (w/o) and presence (w) of superoxide dismutase (SOD). **(B)** NADPH oxidase activity is expressed as arbitrary light units (ALU) per min per mg protein. The D_5R-HEK293 cells were treated for 12 hr with Veh (vehicle), Fen (fenoldopam, D_1R/D_5R agonist, 1.0 μM), Apo (apocynin, NADPH inhibitor, 10 μM), or Fen+Apo. OD, optical density; EV-HEK, empty vector-transfected HEK293 cells; D_5R-HEK, D_5R-overexpressing HEK293 cells; n=4-8/group, $^{*}P<0.05$ *vs* Veh, $^{\#}P<0.05$ *vs* Apo.



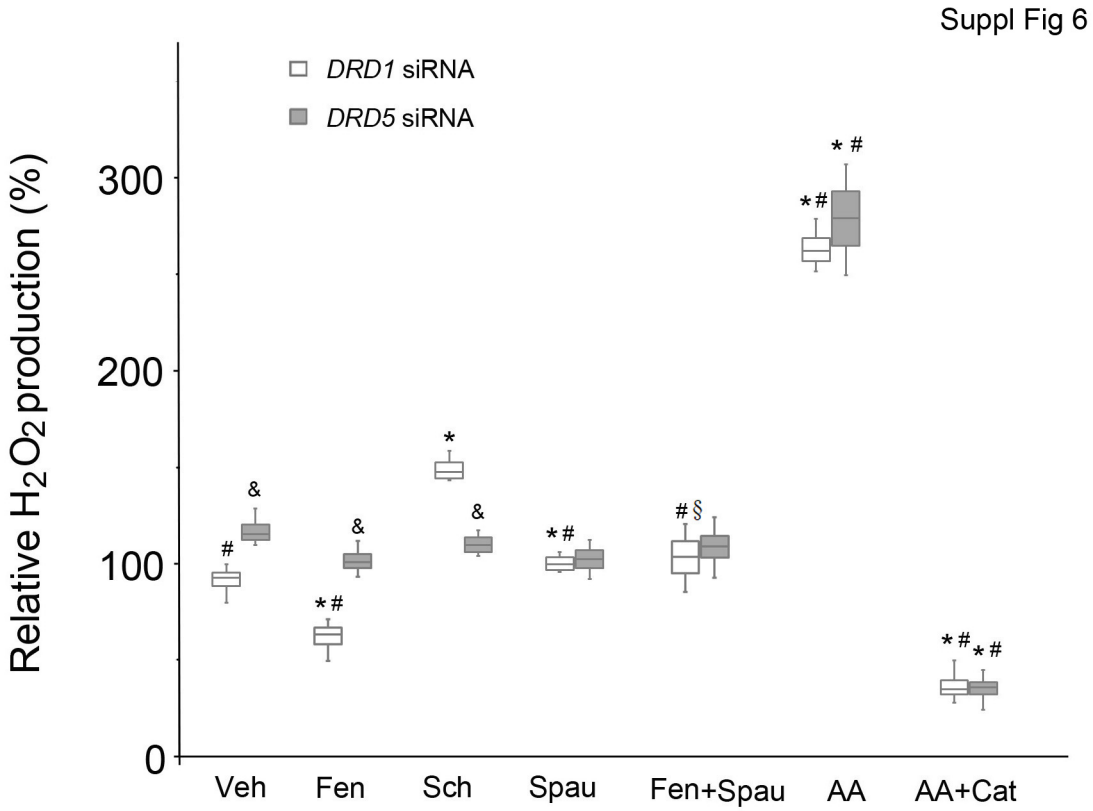
Supplementary Figure 3 Superoxide production in D_5R-HEK 293 cells. Superoxide production was measured by cytochrome C assay. Cells were treated for 20 min with Veh (vehicle), Fen (fenoldopam, D_5R agonist in the absence of D_1R, 1.0 μM), Fen+AA (antimycin A, 0.5 μM) or Fen+Rot (rotenone, 0.75 μM). Values were normalized with the values in Veh-treated cells. n=6/group, $^{*}P<0.05$ *vs* Veh, $^{\#}P<0.05$ *vs* Fen.



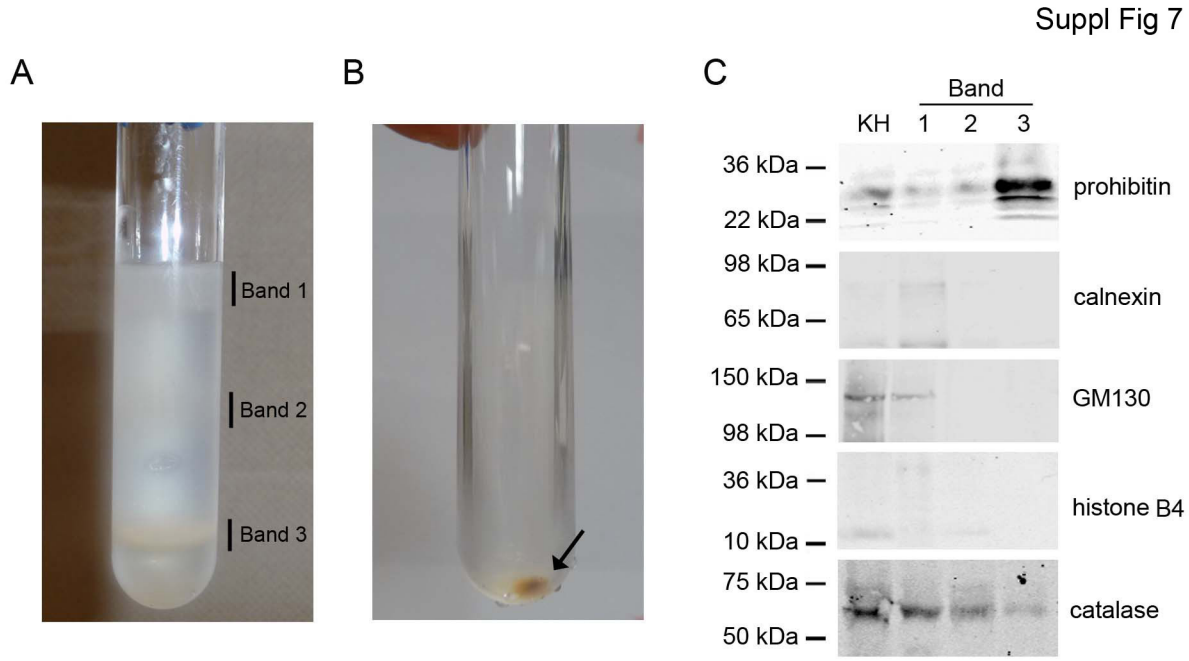
Supplementary Figure 4 Human D_1R (~60 kDa) and D_5R (~55 kDa) protein expression in human RPT cells with *DRD1* or *DRD5* gene silencing. Human RPT cells were transfected with either *DRD1* specific siRNA **(A)** or *DRD5* specific siRNA **(B)**. In lanes 1 and 2, the cells were transfected with control siRNA; in lanes 3 and 4, the cells were transfected with either *DRD1*- **(A)** or *DRD5*- **(B)** specific siRNA. Immunoblots show the decrease in protein expression with the silencing of the D_1-like receptors (*DRD1* and *DRD5*), by the receptor subtype-specific siRNA. The β-actin protein level was used to measure the amount of sample loaded in each lane.



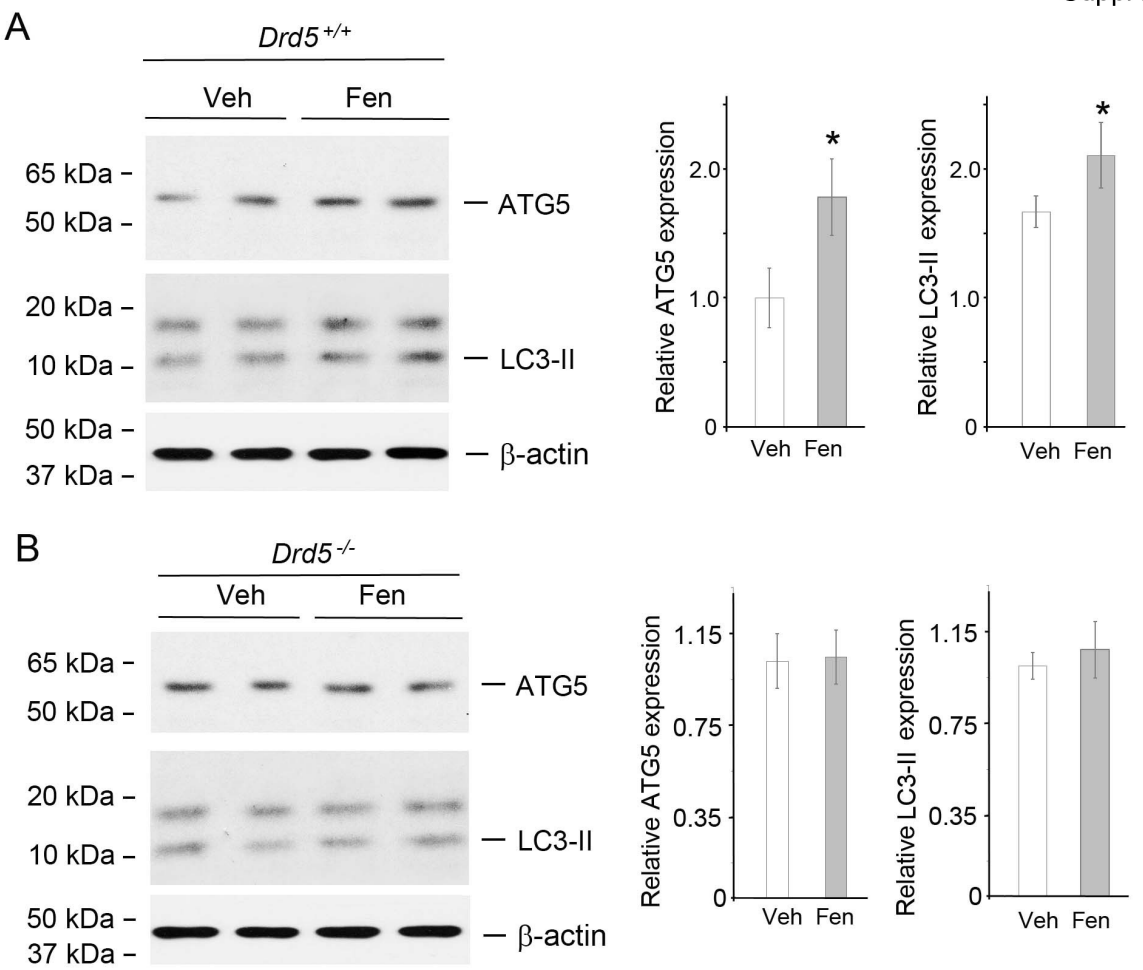
Supplementary Figure 5 D_5R-HEK293 cells were treated with Veh (vehicle), Fen (fenoldopam, 1.0 µM, 12hr), or Fen+Chlor (fenoldopam in the presence of 10 µM chloroquine, 12 hr) as indicated. Representative immunofluorescence images show endogenous LC3-II (red), a marker of autophagy. Blue, nuclei (DAPI). Scale bars, 20 µm. n=4 independent experiments.



Supplementary Figure 6 H_2O_2 production in mitochondria isolated from human RPT cells. The *DRD1* or *DRD5*-silenced human RPT cells were prepared identically to that described in Figure 5D. Veh (vehicle); Fen (fenoldopam, 1.0 μM, 12 hr); Sch (Sch23390, 1.0 μM, 12 hr); Spau (Spautin-1, autophagy inhibitor, 10 μM, 12 hr); AA (antimycin A, Qi site inhibitor of mitochondrial ETC Complex III, 1.0 μM, 2 hr); Cat (catalase, 10 μM, 30 min prior to AA treatment). n=4/group, $^{*}P<0.05$ *vs* Veh, $^{\#}P<0.05$ *vs* Sch, $^{\&}P<0.05$ *vs DRD1* siRNA, $^{\S}P<0.05$ *vs* Fen (due to space consideration in the figure, marker § is only labeled in the "Fen+Spau" group).



Supplementary Figure 7 Characterization of mitochondria purified from whole mouse kidneys by Percoll density gradient centrifugation. **(A)** Typical appearance of a centrifuge tube of Percoll density gradient centrifugation from kidney tissue is similar to that from cultured human RPT cells. Band 3 contains the purified mitochondria fraction. **(B)** Purified mitochondria pellet (arrow) from band 3 of **(A)**. **(C)** Equal protein samples (30 μg) of kidney homogenates (KH) and fractions from purified mitochondria were analyzed by immunoblotting with antibodies against markers for mitochondria (prohibitin), endoplasmic reticula (calnexin), Golgi bodies (GM130), nuclei (histone B4), and peroxisomes (catalase).



Supplementary Figure 8 Autophagy marker protein expression in the kidney cortices of *Drd5*$^{+/+}$ (**A**) and *Drd5*$^{-/-}$ (**B**) mice treated with Veh (vehicle) or Fen (fenoldopam, intraperitoneal injection, 1 mg/kg/day for 7 days). Immunoblots show increased protein expression of autophagy marker proteins ATG5 and LC3-II in the renal cortices from fenoldopam-treated *Drd5*$^{+/+}$, but not *Drd5*$^{-/-}$ mice. n= 4/group. $^{*}P<0.05$ vs Veh.