**SUPPLEMENTAL DATA**

Supplementary methods:

**Antibodies:**

In order to perform the western blot showed in Fig. S3, we used anti-CREB (1/1000, Cell Signaling) and anti-P-CREB (1/1000, Cell Signaling).

**Membrane extraction:**

Protein extracts from pituitary glands were prepared using 4 mice/genotype; tissues were homogenized in in 200 µl of extraction buffer (10 mM TrisHCl, 5 mM EDTA; pH 7.4). Homogenates were briefly centrifuged at 2000 rpm for ten minutes at 4°C. The supernatant was collected and the pellet was re-extracted in the same way. Supernatants from both extractions were pooled and centrifuged at 45000x g for 45 min at 4°C (Optima TLX Ultracentrifuge, Beckman Coulter). The obtained pellets were resuspended in 750 µl of extraction buffer followed by a second centrifugation in the same conditions. The resulting pellets were finally resuspended in 30 µl of 50 mM TrisHCl buffer (pH 7.4). Protein quantifications were performed by Bradford. 30 µg of proteins/genotype were analyzed by western blot using mouse monoclonal antibodies directed against D2R (1:4000), as previously described (Doi et al, Nat Neuroscience 2006). To control for the amount of proteins loaded, blots were also incubated with a membrane marker (pan-cadherin antibodies; Abcam; 1:1000).

**Legends**

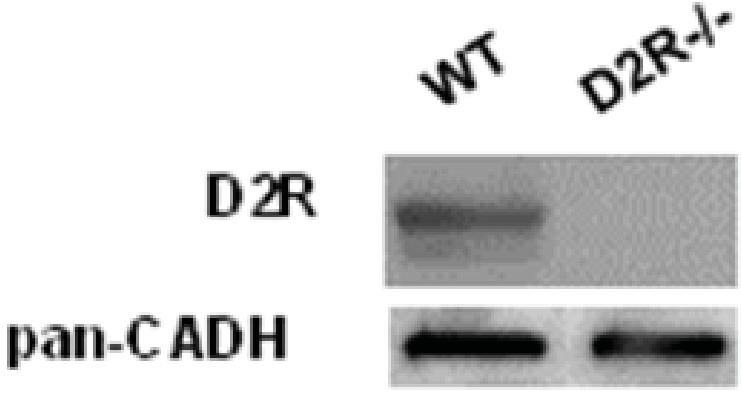
**Fig.S1: In vivo deletion of D2S or D2L in the pituitary gland leads to a similar amount of proteins immune-reactive to anti-D2R antibodies in membrane extracts.** Western blot analyses of membrane extracts from pituitary glands of WT, D2L-/- and D2S-/- mice show that deletion of either D2L in D2L-/- or D2S in D2S-/- pituitaries results into levels of D2 reactive bands similar to that of WT pituitaries. D2R antibodies detect a D2 specific bands at the approximate size of 90 kD. (A) Representative western blot of WT and D2R-/- pituitary extracts using mouse monoclonal D2R antibodies show the presence of a D2 specific band only in WT but not in D2R-/- extracts. Incubation of western blots with anti-pan-cadherin antibodies, a marker of membrane proteins, was used as internal control of loaded quantities of proteins. (B) Representative western blot of protein extracts from WT, D2L-/- and D2S-/- pituitaries incubated with antibodies directed against D2R and pan-cadherin as internal control. (C) Quantification of D2-specific band intensity in membrane extracts of WT, D2L-/- and D2S-/- pituitaries as shown in B), shows similar levels of D2-specific bands in all genotypes. Bars represent the ratio between values from D2R and pan-Cadherin specific bands quantified using Image J. Statistical analyses were performed by one-way ANOVA.

**Fig.S2: Absence of gender differences in the transduction pathways activated by either D2L or D2S**. Western blot analyses of pituitary extracts from 4-month-old WT, D2R-/-, D2L-/- and D2S-/- male mice showed that D2L or D2S affect the ERK and AKT pathways in males D2L-/- or D2S-/- pituitaries as they do in females. Bars represent the ratio between phospho-ERK 1/2/ ERK 1/2 (panel A) and phospho-AKT/AKT (panel B). At least 4 animals per genotype were used

and results analyzed by one-way ANOVA followed by Bonferroni's test (A: Genotype: $F_{(3, 16)}$= 40.57, B: Genotype: $F_{(3, 16)}$=32.35).

**Fig. S3: The level of CREB phosphorylation is not altered either by D2L or D2S ablation in the pituitary gland.** Comparative western blot analyses of CREB phosphorylation levels in 4-month-old WT, D2L-/- and D2S-/- pituitary extracts from mice treated either with saline or 20mg/kg haloperidol. Blots were analyzed using antibodies directed against total CREB and CREB phosphorylated on serine 133 (P-CREB). Bars represent the ratio between P-CREB/CREB levels obtained after quantifications. Statistical analyses were performed on at least 5 animals/genotype analyzed by two-way ANOVA followed by the appropriate post-hoc analysis. (Treatment: $F_{(5, 27)}$: 17.77). *$p<0.05$ vs respective genotype without HAL.

A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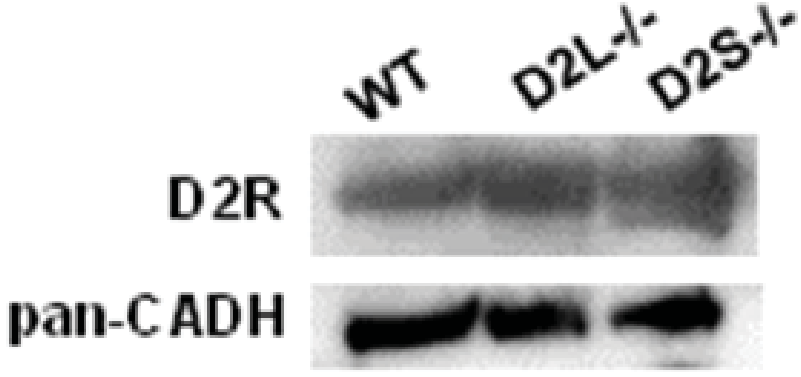
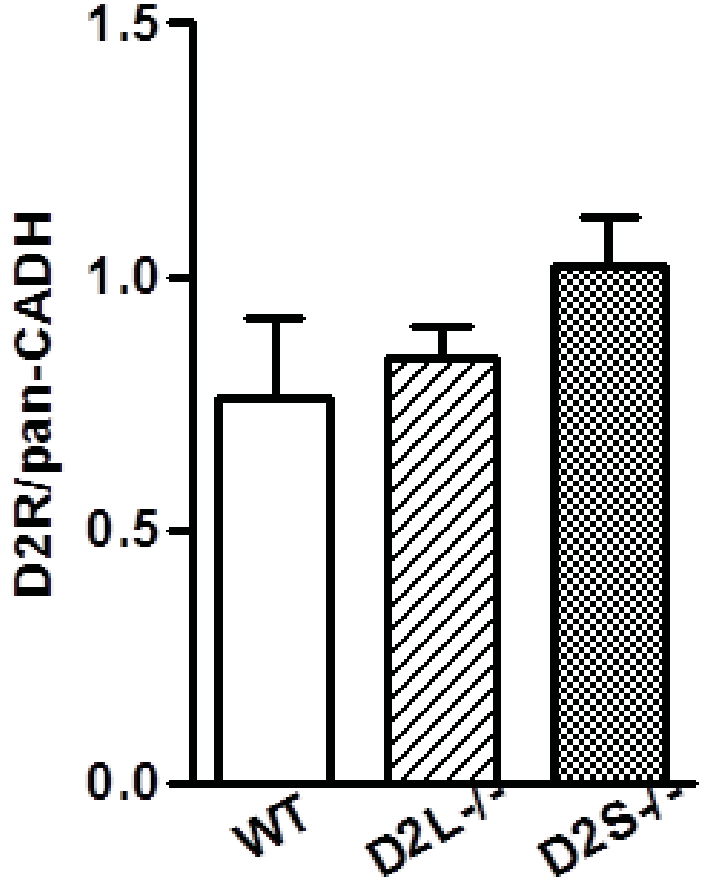
D2R-/-
D2R
pan-CADH

B


WT
D2L-/-
D2S-/-
D2R
pan-CADH

C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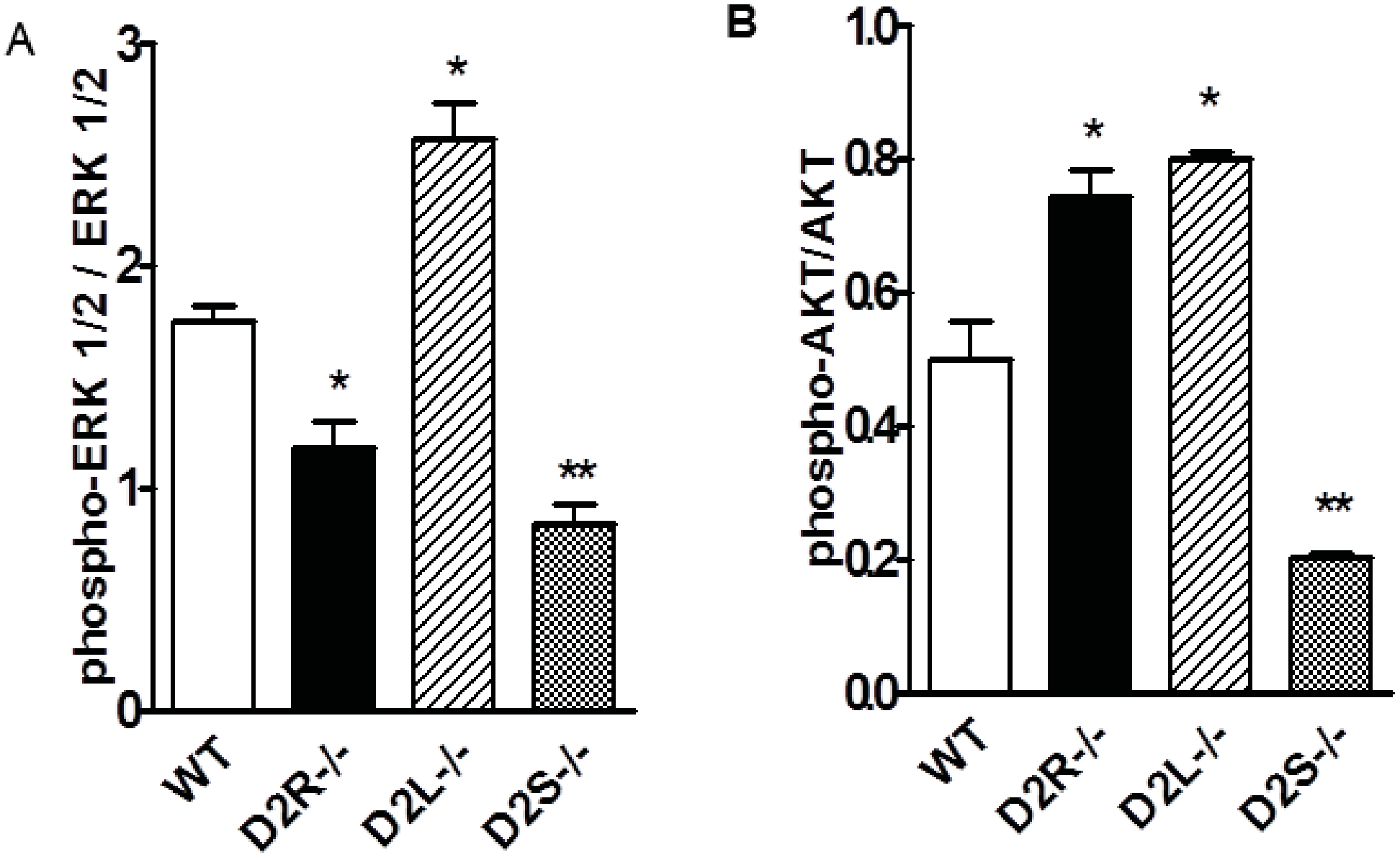
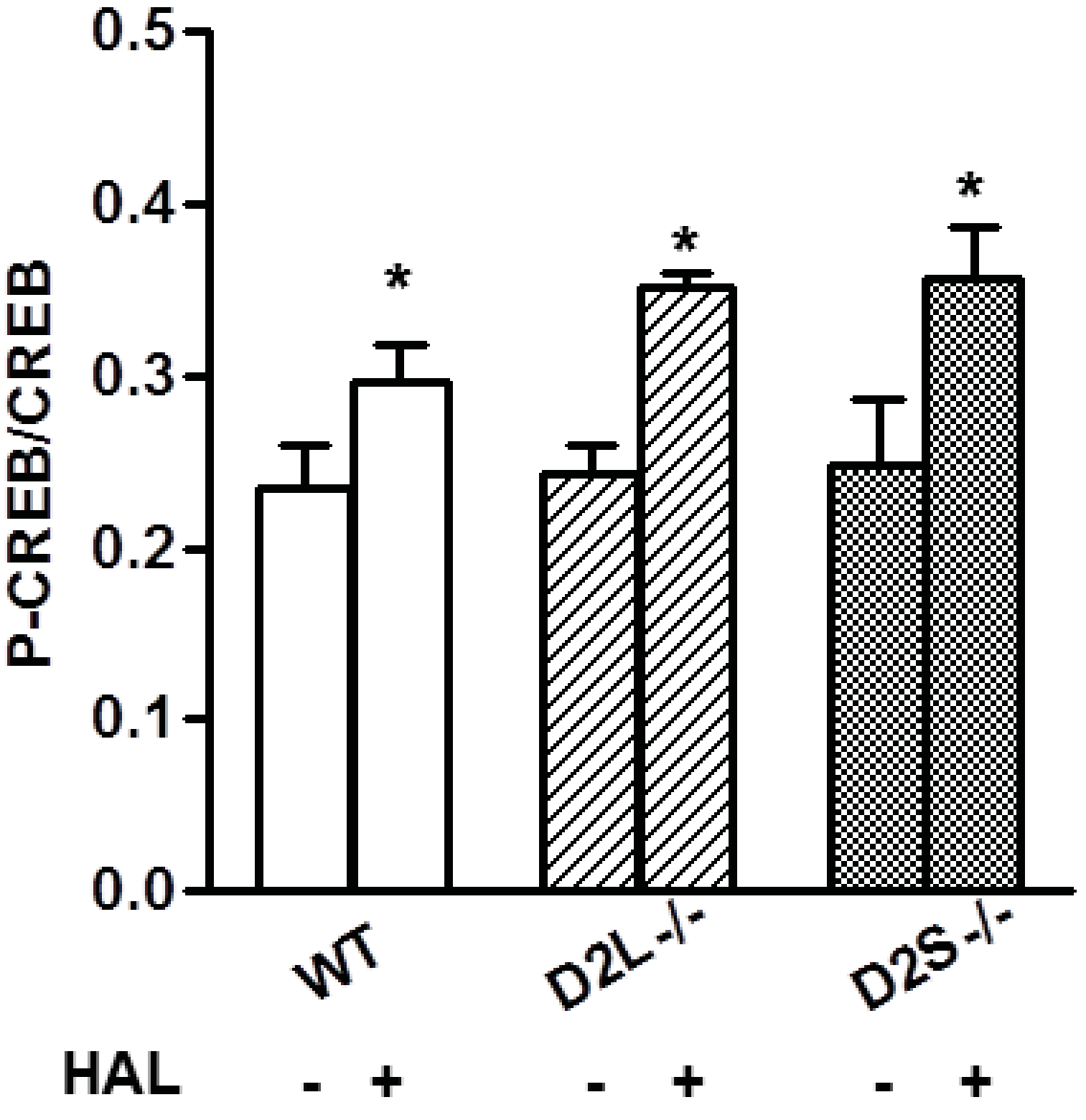
1.0
0.5
0.0
D2R/pan-CADH
WT
D2L-/-
D2S-/-


A
phospho-ERK 1/2 / ERK 1/2
0
1
2
3
*
*
**
WT
D2R-/-
D2L-/-
D2S-/-
B
phospho-AKT/AKT
0.0
0.2
0.4
0.6
0.8
1.0
*
*
**
WT
D2R-/-
D2L-/-
D2S-/-


0.5
0.4
0.3
0.2
0.1
0.0
P-CREB/CREB
*
*
*
WT
D2L-/-
D2S-/-
HAL
-
+
-
+
-
+